UNIVERSIDADE FEDERAL DE SÃO CARLOS
CENTRO DE CIÊNCIAS EXATAS E DE TECNOLOGIA
DEPARTAMENTO DE QUÍMICA
PROGRAMA DE PÓS-GRADUAÇÃO EM QUÍMICA

# DETERMINAÇÃO DE IMPUREZAS INORGÂNICAS EM MEDICAMENTOS: AVALIAÇÃO DE PREPARO DE AMOSTRA E MEDIDAS ESPECTROANALÍTICAS

**Fernanda Costa Pinheiro***

Tese apresentada como parte dos requisitos para obtenção do título de DOUTOR EM CIÊNCIAS, área de concentração: QUÍMICA ANALÍTICA.

**Orientador: Prof. Dr. Joaquim de Araújo Nóbrega**

***Bolsista CNPq**: processos nº 141634/2017-0 e 202722/2019-8

**São Carlos - SP**
**2021**

**UNIVERSIDADE FEDERAL DE SÃO CARLOS**

Centro de Ciências Exatas e de Tecnologia
Programa de Pós-Graduação em Química

## Folha de Aprovação

Defesa de Tese de Doutorado da candidata Fernanda Costa Pinheiro, realizada em 16/06/2021.

### Comissão Julgadora:

Prof. Dr. Joaquim de Araújo Nóbrega (UFSCar)

Profa. Dra. Márcia Foster Mesko (UFPel)

Profa. Dra. Clésia Cristina Nascentes (UFMG)

Prof. Dr. George Luís Donati (WFU)

Profa. Dra. Ana Rita de Araujo Nogueira (EMBRAPA)

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.
O Relatório de Defesa assinado pelos membros da Comissão Julgadora encontra-se arquivado junto ao Programa de Pós-Graduação em Química.

# DEDICATÓRIA

*Dedico este trabalho aos meus pais Antonio e Leonida por todo amor …*

*…. e a Ariane Isis Barros pela valiosa amizade e inspiradora parceria.*

## AGRADECIMENTOS

A priori gostaria de dirigir meus agradecimentos a todos que se dedicaram a ler esta tese. Ela é fruto de um bonito caminho de aprendizados e resiliência que percorri durante meus felizes anos de Doutorado.

Gostaria de expressar meus sinceros agradecimentos ao Prof. Dr. Joaquim de Araújo Nóbrega. Com sua excelente orientação e dedicação, o senhor se fez um grande mestre em minha vida profissional e pessoal. Estendo meu cordial cumprimento ao Prof. Dr. Edenir Rodrigues Pereira Filho, à Dra. Ana Rita Araújo Nogueira e à Dra. Lucimar Lopes Fialho pela oportunidade de participar do Grupo de Análise Instrumental Aplicada. Obrigada por serem inspiradores exemplos de pesquisadores e coordenarem o GAIA com tamanha afeição.

Também compartilho meus calorosos agradecimentos aos colegas do Grupo de Análise Instrumental Aplicada pela maravilhosa convivência nos laboratórios e pelas amizades as quais almejo conservar por toda a vida. Em sete curtos anos de GAIA conheci pessoas incríveis e aprendi muito com cada uma delas.

Al profesor Dr. Antonio Canals, agradezco por la oportunidad, la confianza y la orientación que he tenido durante la realización de las prácticas del Doctorado en el extranjero y a todos los del grupo de Espectroscopia Atómica y Química Analítica en Condiciones Extremas por la agradable bienvenida. En especial, extiendo mis sinceros agradecimientos al Dr. Miguel A. Aguirre por la amistad y por la gran dedicación en los trabajos desarrollados durante mi tiempo en la muy hermosa Universidad de Alicante. Estoy inmensamente agradecida a todas las personas maravillosas que han hecho mis seis meses en San Vicente del Raspeig, Alicante, España ¡Inolvidables!

Agradeço à Universidade Federal de São Carlos, ao Departamento de Química e à Embrapa Pecuária Sudeste pelas estruturas fornecidas. Nesses locais tive a sorte de conhecer professores/pesquisadores fantásticos aos quais agradeço por todo conhecimento compartilhado.

E estendo meus agradecimentos:

Ao Programa de Pós-graduação em Química, secretárias e Coordenação do PPGQ por todo suporte.

Às empresas Agilent Technologies, Analítica e Milestone pela parceria em instrumentação analítica.

À editora Elsevier pela autorização do uso dos artigos publicados e apresentados no corpo desta tese.

Aos membros da banca avaliadora pelas estimadas contribuições.

Aos coautores dos artigos apresentados pela parceria e valiosas contribuições: Dr. Diego V. Babos, Prof. Dr. Antonio H. Canals, Prof. Dr. Edenir R. Pereira-Filho e Prof. Dr Joaquim A. Nóbrega. Em especial, meu sincero e caloroso agradecimento aos coautores Dra. Ariane I. Barros e Dr. Miguel Á. Aguirre. Ambos são pesquisadores incríveis e tive grande sorte de trabalhar/aprender muito com vocês durante meu doutorado.

E aos órgãos de fomento CNPq e CAPES. O presente trabalho foi realizado com apoio do CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brasil (bolsas concedidas no país , processo nº 141634/2017-0 e no exterior, processo nº 202722/2019-8) e com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES, código de financiamento 001.

Finalmente, dirijo meus sinceros votos de gratidão aos meus amados pais e querida irmã por todo amor e constante apoio e por todos os amigos que estiveram presentes e por todos os alegres momentos compartilhados durante esta etapa da minha formação.

*"O quão feliz é uma pessoa depende da profundidade de sua gratidão."*
*(John Miller)*

# LIST OF ACRONYMS

| | |
|---|---|
| **$^1$H NMR** | Proton nuclear magnetic resonance |
| **8-HQ** | 8-Hydroxyquinoline |
| **AAS** | Atomic absorption spectrometry |
| **ADT** | Aerosol dilution technique |
| **ANVISA** | Agência Nacional de Vigilância Sanitária |
| **API** | Active pharmaceutical ingredient |
| **BEC** | Background equivalent concentration |
| **BP** | British Pharmacopeia |
| **$C_{analyte}$** | Analyte concentration |
| **CCD** | Central composite design |
| **CCT** | Collision cell technology |
| **CH-AD** | Digestion in closed vessel using conventional heating |
| **CRM** | Certified reference material |
| **DES** | Deep eutectic solvents |
| **DDTC** | Diethyldithiocarbamate |
| **DF** | Dilution factor |
| **DLLME** | Dispersive liquid-liquid microextraction |
| **DLLME-ICP OES** | Dispersive liquid-liquid microextraction inductively coupled plasma optical emission spectrometry |
| **DMSO** | Dimethyl sulfoxide |
| **DRC-ICP-MS** | Dynamic reaction cell inductively coupled plasma mass spectrometry |
| **DSC** | Differential scanning calorimetry |
| **EC** | External standard calibration |
| **EDTA** | Ethylenediamine tetra acetic acid |
| **EF** | Enrichment factor |
| **EMA** | European Medicines Agency |
| **EP** | European Pharmacopoeia |
| **ETAAS** | Electrothermal atomic absorption spectroscopy |
| **ETV-DRC-ICP-MS** | Electrothermal vaporization dynamic reaction cell inductively coupled plasma mass spectrometry |

| | |
|---|---|
| **ETV-ICP OES** | Electrothermal vaporization inductively coupled plasma optical emission spectrometry |
| **FAAS** | Flame atomic absorption spectrometry |
| **FDA** | Food and Drug Administration |
| **FI-CVG-ICP-MS** | Flow injection cold vapor generation inductively coupled plasma mass spectrometry |
| **FI-ICP-MS** | Flow injection inductively coupled plasma mass spectrometry |
| **FT-IR** | Fourier transform infrared |
| **GF AAS** | Graphite furnace atomic absorption spectrometry |
| **HBA** | Hydrogen bond acceptor |
| **HBD** | Hydrogen bond donor |
| **HMI** | High matrix introduction |
| **HR-CS GFAAS** | High resolution continuum source graphite furnace atomic absorption spectrometry |
| **IARC** | International Agency for Research on Cancer |
| **ICH** | International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use |
| **ICH Q3D** | Guideline for elemental impurities Step 4 version |
| **ICH Q3D(R1)** | Guideline for elemental impurities Step 5 version |
| **ICP OES** | Inductively coupled plasma optical emission spectrometry |
| **ICP-MS** | Inductively coupled plasma mass spectrometry |
| **IP** | Indian Pharmacopoeia |
| **IS** | Internal standardization |
| **IUPAC** | International Union of Pure and Applied Chemistry |
| **JP** | Japanese Pharmacopoeia |
| **KED** | Kinetic energy discrimination |
| **LA-ICP-MS** | Laser ablation inductively coupled plasma mass spectrometry |
| **LIBS** | Laser-induced breakdown spectroscopy |
| **LLE** | Liquid-liquid extraction |
| **LPME** | Liquid-phase microextraction |
| **LOD** | Limit of detection |
| **LOQ** | Limit of quantification |
| **MDD** | Maximum daily dose |
| **MEC** | Multi-energy calibration |

| | |
|---|---|
| **MICal** | Multi-isotope calibration |
| **MIP OES** | Microwave-induced plasma optical emission spectrometry |
| **m/z** | Mass/charge ratio |
| **MW-AD** | Microwave-assisted digestion in closed vessel |
| **NA** | Not applicable |
| **NaDDTC** | Sodium diethyldithiocarbamate |
| **NIST** | National Institute of Standard and Technology |
| **OD** | Overall desirability |
| **OP SA** | One-point standard addition |
| **ORS** | Octopole reaction system |
| **PDE** | Permitted daily exposure |
| **PFA** | Perfluoro alkoxy alkanes |
| **$R^2$** | Linear correlation coefficient |
| **RA** | Residual acidity |
| **RF** | Radio frequency |
| **RSD** | Relative standard deviation |
| **SA** | Standard additions |
| **SBR** | Signal-to-background ratio |
| **SDA** | Standard dilution analysis |
| **SE** | Standard error |
| **SI** | Signal intensity |
| **SRC** | Single reaction chamber |
| **TDS** | Total dissolved solids |
| **TGA** | Therapeutic goods administration |
| **USP** | United States Pharmacopoeia |
| **UV-Vis** | Ultraviolet-visible spectrophotometry |

# LIST OF TABLES

TABLE 1.1 Classification according ICH Q3D and concentration limits of elemental impurities in drug and pharmaceutical products set by USP (µg $g^{-1}$, considering maximum daily dose of 10 g) and ANVISA (µg $g^{-1}$)…………………………..………………………….... 08
TABLE 1.2 Selected studies for determination of elemental impurities in drugs and pharmaceuticals…………………………..………… 12
TABLE 1.3 Selected studies for microwave-assisted digestion of dietary supplements aiming elemental determination …………………….. 20
TABLE 1.4 Selected procedures for preconcentration of As, Cd, Co, Hg, Ni, Pb, and V prior to measurements using spectroanalytical methods……………………………………………………..…….. 25
TABLE 3.1 Operating parameters used in iCAP6000 ICP OES and Agilent 7800 Quadrupole ICP-MS……………………………..……….. 43
TABLE 3.2 Class, oral permissible daily exposures and the *J* values obtained considering MDD of 10 g $day^{-1}$ [20,21]………………………………….. 48
TABLE 3.3 Parameters analytical performance for Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V in drugs samples digested in three sample preparation procedure by ICP-MS using *HMI* mode………………..………… 54
TABLE 3.4 Determination of As, Ba, Cd, Co, Cu, Cr, Li, Mo, Ni, Pb, Sb, Se, Sn, Tl, and V (µg $g^{-1}$, mean ± standard deviation, n = 3) in drug samples (A–I) and one sample of dietary supplement (DS) digested in each sample digestion procedures by ICP-MS...…….. 58
TABLE 3.5 Parameters analytical performance for Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V in drugs samples by ICP OES…………………..…………… 63
TABLE 3.6 Recoveries and relative standard deviation (%) obtained for the spiked in digested drug samples (A-I) according to the *J* value by ICP OES (n = 3)……………………………………………..…… 65
TABLE 3.7 Recoveries and relative standard deviation (%) obtained for the spiked in digested drug samples (A-I) according to the *J* value by ICP-MS (n = 3)…………………………………………….……… 68

TABLE 3.8 Operating parameters used in Agilent 720-ES ICP OES………… 77
TABLE 3.9 Experimental variables and levels of the Plackett-Burman design. 80
TABLE 3.10 Experimental variables and levels of the central composite design……………………………………………………………… 82
TABLE 3.11 Analytical figures of merit for Cd, Hg and Pb determination using DLLME-ICP OES and conventional ICP OES analysis…………… 84
TABLE 3.12 Quantification limits for Cd, Hg and Pb of the ICP OES methods used for elemental impurities determination in pharmaceutical samples……………………………………………………………… 86
TABLE 3.13 Found concentrations (mean ± standard deviation, μg $L^{-1}$, n = 3) and recovery values in parenthesis (mean ± RSD, %) obtained for the spiked in digested drug samples (A-H) according to the *J* value using DLLME-ICP OES……………………………………….. 88
TABLE 4.1 Function, active principle and excipients for the liquid drug samples analyzed………………………………………………..… 96
TABLE 4.2 Instrumental parameters for ICP OES determinations……….….….. 97
TABLE 4.3 Evaluation of calibration methods used for Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V determination by ICP OES in liquid drug samples (A-D) 10-fold diluted. Recovery (relative standard deviation, n = 3) for addition level of 0.10 mg $L^{-1}$…………………………………...……... 105
TABLE 4.4 ANOVA table obtained by calculating the regression model for arsenic……………………………………………………………….. 110
TABLE 4.5 Analytical performance parameters for the determination of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V in liquid drug samples by ICP OES using the calibration methods EC, IS, SA and OP SA………………….... 112
TABLE 4.6 Operating parameters used in Agilent 720-ES ICP OES………… 119
TABLE 4.7 Active principle, function, indication and maximum daily dose (MDD) for the oral and parenteral drug samples analyzed……….. 121
TABLE 4.8 Class [20,21], PDE 21 and *J* values (μg $L^{-1}$) for Cd, Co, Hg, Ni, Pb, and V for oral and parenteral drug samples……………...……... 122

TABLE 4.9 Analytical figures of merit for Cd, Co, Hg, Ni, Pb, and V determination in oral and parenteral drug samples using DES-based DLLME-ICP OES and direct ICP OES analysis……………. 129
TABLE 4.10 Recoveries and relative standard deviation (%, n = 3) obtained for the spiked in oral (LA-LB) and parenteral (PA-PC) drug samples at two different levels: 0.5*J* and 1.5*J* (i.e., spike in μg $L^{-1}$) using DES-based DLLME-ICP OES……………………………… 132
TABLE 4.11 Comparison of analytical characteristic of the proposed method with some published method using a DES for metal liquid-phase microextraction in aqueous samples………………………………. 134
TABLE 5.1 Microwave-assisted digestion of sport supplement samples: matrix of experiments (based on a modified Doehlert factorial design) showing the variables evaluated for optimizing $H_2O_2$ volume and $HNO_3$ concentration……………………………..…….. 144
TABLE 5.2 Overall desirability (OD) considering the factorial design responses: residual acidity (RA), dissolved carbon content and analyte recoveries for As, Cd and Pb by ICP-MS using EC, IS, OP SA and MICal…………………...……………..……………….. 148
TABLE 5.3 Analytical performance parameters for As, Cd, Hg and Pb in sport supplement samples by ICP-MS using EC, IS, MICal and OP SA as calibration methods…..……………..………………….. 151
TABLE 5.4 Recoveries and relative standard deviations (%) for spikes in digested sport supplement samples (S1 to S5) according to the *J* values by ICP-MS (n = 3) using EC, IS, OP SA and MICal as calibration methods…………………………………………………. 152
TABLE 5.5 Determination of As, Cd, Hg and Pb (μg $g^{-1}$, mean ± standard deviation, n = 3) in sport supplement samples (S1 to S10) by ICP-MS using IS, OP SA and MICal as calibration methods……... 152

# LIST OF FIGURES

FIGURE 1.1 Sample preparation of pharmaceutical products according to the USP General Chapter <233> [23]. Adapted from BALARAM, V. (2016) [2]………...………………………………………………….…. 06

FIGURE 1.2 Potential contamination sources of elemental impurities in drugs and pharmaceutical on the basis of the ICH Q3D guidance [20]. Adapted from BALARAM, V. (2016) [2]………..…………………… 09

FIGURE 1.3 Scheme of the DLLME procedure for preconcentration of metals.. 22

FIGURE 1.4 System representation of ICP-MS with aerosol dilution. Adapted from BARROS, et al. (2018) [115]..……..……………………………... 27

FIGURE 1.5 Linear model for (a) IS, (b) MICal, (c) SA and (d) OP SA curves. (a) $C_{analyte}$ on the x-axis and the ratio standard signal/internal standard signal on the y-axis. (b) MICal calibration plot using six isotopes. (c) SA calibration plot using four additional points ($x_1$-$x_4$). (d) OP SA calibration plot using one additional point ($x_1$). (c, d) $x_0$ represents the sample without any standard addition with extrapolation represented by broken line…………………………… 31

FIGURE 1.6 System representation of CCT and KED to suppress polyatomic interferences caused by $^{35}Cl^{16}O^+$, $^{40}Ar^{12}C^+$ and $^{40}Ar^{35}Cl^+$ in the determination of $^{51}V^+$, $^{52}Cr^+$ and $^{75}As^+$, respectively……………….. 33

FIGURE 3.1 Sample C. (a) $3HNO_3$:1HCl v $v^{-1}$ (220 °C); (b) $HNO_3$ 7.0 mol $L^{-1}$ (220 °C); (c) $HNO_3$ 2.0 mol $L^{-1}$ (220 °C). Total digestion only when using procedure (a)………………………………………….. 49

FIGURE 3.2 Sample H. (a) $3HNO_3$:1HCl v $v^{-1}$ (240 °C); (b) $3HNO_3$:1HCl v $v^{-1}$ (220 °C); (c) $HNO_3$ 7.0 mol $L^{-1}$ (220 °C); (d) $HNO_3$ 2.0 mol $L^{-1}$ (220 °C). Residual solids in all tested sample preparation procedures……………………………………………………….... 50

FIGURE 3.3 Sample E. (a) $3HNO_3$:1HCl v $v^{-1}$ (220 °C); (b) $HNO_3$ 7.0 mol $L^{-1}$ (220 °C); (c) $HNO_3$ 2.0 mol $L^{-1}$ (220 °C). Total digestion in all tested sample preparation procedures……………………………… 50

FIGURE 3.4 Dissolved carbon content in samples digested (A-DS) for each sample preparation by ICP OES (mg $L^{-1}$, mean ± standard deviations, n = 3). (■) 3$HNO_3$:1HCl v $v^{-1}$ (240 °C); (■) 3$HNO_3$:1HCl v $v^{-1}$ (220 °C); (□) $HNO_3$ 7.0 mol $L^{-1}$ (220 °C); (■) $HNO_3$ 2.0 mol $L^{-1}$ (180 °C)................................................ 51
FIGURE 3.5 (a) Percentage recoveries for addition 0.10 mg $L^{-1}$ in standard solutions by ICP OES. (■) 0.14 mol $L^{-1}$ $HNO_3$; (■) 0.25% m $v^{-1}$ carbon; (□) 0.50% m $v^{-1}$ carbon; (■) 1.0% m $v^{-1}$ carbon. (b) Percentage recoveries for addition 0.10 mg $L^{-1}$ in sample digested using 2.0 mol $L^{-1}$ $HNO_3$ with and without IS by ICP OES. (■) Without IS; (□) With IS................................................ 52
FIGURE 3.6 Scheme of the DLLME procedure for preconcentration of Cd, Hg and Pb in drug samples................................................ 79
FIGURE 3.7 Pareto charts obtained in the screening study of the experimental variables affecting the DLLME of (a) Cd, (b) Hg and (c) Pb. (■) significant effect; (□) insignificant effect. Analyte concentration of 500 µg $L^{-1}$................................................ 81
FIGURE 3.8 Response surface from central composite design for (a) Cd, (b) Hg and (c) Pb. Analyte concentration of 500 µg $L^{-1}$.................. 82
FIGURE 4.1 Dissolved organic carbon in samples (A-D) for each sample preparation procedure (mg $L^{-1}$, mean ± standard deviations, n = 3). (■) A; (■) B; (□) C; (■) D. MW-AD means microwave-assisted digested using 7.0 and 2.0 mol $L^{-1}$ $HNO_3$. ............................. 99
FIGURE 4.2 Signal intensities for (a) As, (b) Au, (c) Hg, (d) Pt, (e) Sb and (f) Se in standard solutions containing increasing concentrations of carbon. (■) Internal standard signal. (□) Analyte signal; (▶) Ratio of the analyte signal/internal standard signal.......................... 101
FIGURE 4.3 Linear model for SA (a) and OP SA curve (b) for Hg determination in drug sample A 10-fold diluted. (a) x0 = 0.10, x1 = 0.20 and x2 = 0.40 mg $L^{-1}$ of Hg; (b) x0 = 0.10 and x1 = 0.40 mg $L^{-1}$ of Hg................................................ 103

FIGURE 4.4 Percentage recoveries for addition 0.10 mg $L^{-1}$ in samples (a) A; (b) B; (c) C and (d) D; 20-fold diluted with and without internal standard by ICP OES. (■) Without internal standard; (□) With internal standard.......................................................... 104
FIGURE 4.5 Percentage recoveries for addition 0.10 mg $L^{-1}$ in sample D with and without internal standard by ICP OES. (a) Digested in 7.0 mol $L^{-1}$ $HNO_3$; (b) Digested in 2.0 mol $L^{-1}$ $HNO_3$. (■) Without internal standard; (□) With internal standard............................ 108
FIGURE 4.6 Schematic representation of the DES-based DLLME procedure for preconcentration of Cd, Co, Hg, Ni, Pb, and V in parenteral and oral drug samples....................................................... 122
FIGURE 4.7 FT- IR spectra of pure DL-menthol, pure decanoic acid and DES (i.e., DL-menthol and decanoic acid (2:1 molar ratio) mixture....... 123
FIGURE 4.8 $^{1}H$ NMR spectra of (a) pure DL-menthol; (b) pure decanoic acid; (c) DL-menthol:decanoic acid (2:1) mixture; (d) pure 8-HQ; (e) DL-menthol:decanoic acid (2:1) mixture and 8-HQ..................... 124
FIGURE 4.9 Phase diagram for DL-menthol:decanoic acid eutectic mixture..... 125
FIGURE 4.10 Pareto charts obtained in the screening study of the main factors affecting the DLLME of (a) Cd, (b) Co, (c) Hg, (d) Ni, (e) Pb and (f) V. (■) Significant effect; (■) Non-significant effect. Bars to the right indicate a positive effect and bars to the left indicate a negative effect. Analyte concentration of 100 μg $L^{-1}$.................. 126
FIGURE 4.11 Response surface from central composite design for (a) Cd, (b) Co, (c) Hg, (d) Ni, (e) Pb, and (f) V. Analyte concentration of 100 μg $L^{-1}$..................................................................... 127
FIGURE 5.1 Schematic representation of the general procedure used in recovery experiments using the calibration methods MICal and OP SA according to 0.5*J* values........................................... 146
FIGURE 5.2 Linear model for multi-isotope calibration for (a) Cd, (b) Hg and (c) Pb in sport supplement sample (S1) with 1.5*J* addition level.... 154

FIGURE 5.3 One-point standard addition curve for (a) $^{75}As^{+}$, (b) $^{112}Cd^{+}$, (c) $^{202}Hg^{+}$ and (d) $^{208}Pb^{+}$ in sport supplement sample (S1) with 1.5*J* addition level. In (a) and (c) x1 = 11.2 and x2 = 22.5 µg $L^{-1}$; (b) x1 = 3.75 and x2 = 7.50 µg $L^{-1}$, and (d) x1 = 7.50 and x2 = 15.0 µg $L^{-1}$……………..……………………………………………………... 154

# RESUMO

DETERMINAÇÃO DE IMPUREZAS INORGÂNICAS EM MEDICAMENTOS: AVALIAÇÃO DE PREPARO DE AMOSTRA E MEDIDAS ESPECTROANALÍTICAS. Esta tese visou o desenvolvimento de procedimentos menos agressivos de preparo de amostra com ênfase na digestão assistida por radiação micro-ondas para a determinação de impurezas elementares em produtos farmacêuticos por métodos espectroanalíticos baseados em plasma de argônio seguindo os requisitos dos Capítulos 232, 233 e 2232 da Farmacopeia Norte Americana. Descreve-se no Capítulo 3 um procedimento de preparo de amostras de medicamentos assistido por radiação micro-ondas usando solução de ácido nítrico diluído seguido pela determinação de 24 elementos por espectrometria de emissão óptica com plasma acoplado indutivamente (ICP OES) e espectrometria de massas com plasma acoplado indutivamente (ICP-MS). Nesse capítulo também é apresentado um procedimento envolvendo a microextração líquido-líquido dispersiva de Cd, Hg e Pb de amostras de medicamentos previamente digeridas para subsequente determinação por ICP OES. Descreve-se no Capítulo 4 um procedimento "dilute-and-shoot" para determinação de 23 elementos em medicamentos líquidos por ICP OES e um procedimento de microextração líquido-líquido dispersiva de Cd, Co, Hg, Ni, Pb e V de amostras de medicamentos líquidos para subsequente determinação por ICP OES. Por sua vez, descreve-se no Capítulo 5 um procedimento de preparo de amostras assistido por radiação micro-ondas de suplementos esportivos usando ácido nítrico diluído seguido pela determinação de As, Cd, Hg e Pb por ICP-MS. Em todos os três capítulos anteriormente mencionados, estratégias de calibração, i.e. adição de padrão, padronização interna, calibração multi-isótopos e adição de padrão de um único ponto; bem como estratégias instrumentais, i.e. diluição de aerossol e tecnologia de cela de colisão para medições por ICP-MS, foram aplicadas. Essas estratégias instrumentais e de calibração foram essenciais para corrigir interferências espectrais e não espectrais viabilizando a determinação exata de impurezas elementares em amostras farmacêuticas na forma sólida (comprimidos e pílulas), líquida (soluções orais e medicamentos parenterais) e em suplementos esportivos por métodos baseados em ICP.

# ABSTRACT

DETERMINATION OF INORGANIC IMPURITIES IN MEDICINES: EVALUATION OF SAMPLE PREPARATION AND SPECTROANALYTICAL METHODS. Green sample preparation procedures were developed in this dissertation for determination of elemental impurities in drugs and dietary supplements by argon-based plasma spectroanalytical methods according to Chapters 232, 233 and 2232 from the United States Pharmacopeia. Chapter 3 describes the development of a procedure for microwave-assisted sample preparation of medicines using diluted nitric acid solution followed by determination of 24 elements using inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS). A dispersive liquid-liquid microextraction procedure for determination of Cd, Hg, and Pb in drug samples by ICP OES is also presented. A dilute-and-shoot procedure for determination of 23 elements in liquid pharmaceutical samples by ICP OES is presented in Chapter 4 as well as a procedure for dispersive liquid-liquid microextraction of Cd, Co, Hg, Ni, Pb, and V in liquid drug samples for subsequent measurements by ICP OES. Chapter 5 describes the development of microwave-assisted sample preparation procedure of sport supplements using dilute nitric acid solution followed by determination of As, Cd, Hg and Pb using ICP-MS. In all chapters previously mentioned, calibration strategies, such as standard additions, internal standardization, multi-isotope calibration and one-point standard addition, are evaluated for correction of matrix effects as well as instrumental strategies based on aerosol dilution and collision cell technology for ICP-MS measurements. These strategies were essential to correct for spectral and non-spectral interferences, enabling the accurate determination of elemental impurities in solid pharmaceutical samples (tablets and pills), liquid drugs (oral solutions and parenteral drugs) and sport supplements by ICP-based methods.

## SUMMARY

**1 - CHAPTER 1 - INTRODUCTION** ........ **1**
1.1 - Introduction ........ 2
1.2 - Elemental impurities: regulatory bodies update ........ 3
1.3 - Elemental impurities in drugs and pharmaceuticals ........ 8
1.4 - Sample preparation of drugs and pharmaceuticals ........ 10
1.4.1 - Dispersive liquid-liquid microextraction ........ 21
1.6 - Determination of elemental impurities by ICP-based methods: instrumental strategies and calibration methods ........ 26
1.6.1 - Matrix effects and calibration methods ........ 28
1.6.2 - Spectral interferences in ICP-MS and collision cell technology ........ 32
**2 - CHAPTER 2 - GOALS** ........ **35**
2.1 - Goals ........ 36
2.2 - Specific Goals ........ 36
**3 - CHAPTER 3 - PHARMACEUTICAL SAMPLES IN SOLID DOSAGE FORM** ........ **37**
3.1 - Microwave-assisted sample preparation of medicines for determination of elemental impurities in compliance with United States Pharmacopeia: How simple can it be? ........ 38
3.1.1 - Abstract ........ 38
3.1.2 - Graphical abstract ........ 39
3.1.3 - Introduction ........ 39
3.1.4 - Experimental ........ 42
*3.1.4.1 - Instrumentation ........ 42*
*3.1.4.2 - Samples and microwave-assisted sample preparation ........ 44*
*3.1.4.3 - Reagents and standard solutions ........ 45*
*3.1.4.4 - Evaluation of accuracies obtained by ICP OES and ICP-MS according to USP requirements ........ 46*
3.1.5 - Results and discussion ........ 48
*3.1.5.1 - Evaluation of the sample digestion procedures - Dissolved organic carbon contents and matrix effects ........ 48*
*3.1.5.2 - Analytical performance of ICP-MS ........ 53*
*3.1.5.3 - Accuracy of the procedure according to USP requirements ........ 62*
3.1.6 - Conclusions ........ 70

3.2 - Dispersive liquid–liquid microextraction of Cd, Hg and Pb from medicines prior to ICP OES determination according to the United States Pharmacopeia ... 72
3.2.1 - Abstract ... 72
3.2.3 - Introduction ... 73
3.2.4 - Experimental ... 75
*3.2.4.1 - Reagents and standard solutions ... 75*
*3.2.4.2 - Instrumentation ... 76*
*3.2.4.3 - Samples and sample preparation ... 77*
*3.2.4.4 - Dispersive liquid–liquid microextraction procedure ... 78*
*3.2.4.5 - Evaluation of accuracy according to USP requirements ... 79*
3.2.5 - Results and discussion ... 80
*3.2.5.1 - Reagents Optimization of dispersive liquid–liquid microextraction ... 80*
*3.2.5.2 - Analytical performance for DLLME-ICP OES method according to the USP requirements ... 83*
3.2.6 - Conclusions ... 88
**4 - CHAPTER 4 - PHARMACEUTICAL SAMPLES IN LIQUID DOSAGE FORM ... 91**
4.1 - Evaluation of dilute-and-shoot procedure for determination of inorganic impurities in liquid pharmaceutical samples by ICP OES ... 92
4.1.1 - Abstract ... 92
4.1.2 - Graphical Abstract ... 93
4.1.3 - Introduction ... 93
4.1.4 - Experimental ... 96
*4.1.4.1 - Samples and sample preparation ... 96*
*4.1.4.2 - Instrumentation ... 97*
*4.1.4.3 - Reagents and standard solutions ... 97*
4.1.5 - Results and Discussion ... 99
*4.1.5.1 - Dilute-and-shoot procedure and matrix effects ... 99*
*4.1.5.2 - Analytical performance for each calibration method ... 102*
*4.1.5.3 - Methods accuracy ... 103*
*4.1.5.4 - Limits of detection and concentrations limits based on J values ... 109*
*4.1.5.5 - Determination of inorganic impurities in liquid drug samples ... 113*
4.1.6 - Conclusion ... 113

4.2 - A green dispersive liquid-liquid microextraction based on deep eutectic solvent for elemental impurities determination in oral and parenteral drugs by inductively coupled plasma optical emission spectrometry ............................... 115
4.2.1 - Abstract.............................................................................................. 115
4.2.3 - Introduction ........................................................................................ 116
4.2.4 - Experimental ....................................................................................... 118
*4.2.4.1 - Instrumentation ..................................................................................... 118*
*4.2.4.2 - Synthesis of hydrophobic DES........................................................ 119*
*4.2.4.3 - Reagents and standard solutions.................................................... 120*
*4.2.4.4 - Samples and sample preparation ................................................... 120*
*4.2.4.5 - Dispersive liquid–liquid microextraction procedure .......................... 121*
*4.2.4.6 - Addition and recovery tests according to USP requirements ........... 122*
4.2.5 - Results and discussion ...................................................................... 123
*4.2.5.1 - Characterization of hydrophobic DES ............................................. 123*
*4.2.5.2 - Optimization of dispersive liquid–liquid microextraction .................. 125*
*4.2.5.3 - Analytical performance for DES-based DLLME-ICP OES method... 128*
*4.2.5.4 - Addition and recovery tests according to USP requirements ........... 131*
*4.2.5.5 - Comparison with other hydrophobic DES-based LPME procedures.131*
4.2.6 - Conclusions ........................................................................................ 133
**5 - CHAPTER 5 - DIETARY SUPPLEMENT SAMPLES................................................................ 137**
5.1 - Microwave-assisted digestion using dilute nitric acid solution and investigation of calibration strategies for determination of As, Cd, Hg and Pb in dietary supplements using ICP-MS ................................................................. 138
5.1.1 - Abstract.............................................................................................. 138
5.1.2 - Graphical Abstract ............................................................................. 139
5.1.3 - Introduction ........................................................................................ 139
5.1.4 - Experimental ....................................................................................... 141
*5.1.4.1 - Instrumentation ..................................................................................... 141*
*5.1.4.2 - Samples, standards and reagents .................................................. 142*
*5.1.4.3 - Microwave-assisted sample preparation: Doehlert design ............... 143*
*5.1.4.4 - Addition and recovery tests according to USP requirements ........... 145*
5.1.5 - Results and discussion ...................................................................... 146
*5.1.5.1 - Optimization of the microwave-assisted digestion procedure .......... 146*
*5.1.5.2 - Analytical performance and accuracy according to USP…………….149*

*5.1.5.3 - Determination of As, Cd, Hg and Pb in dietary supplements ........... 155*
5.1.6 - Conclusions ........................................................................... 157
**6 - CHAPTER 6 – GENERAL CONCLUSIONS ........................................................ 159**
6.1 - General conclusions.......................................................................... 160
**7 - CHAPTER 7 - REFERENCES .............................................................................. 163**
7.1 - References......................................................................................... 164

# 1 - CHAPTER 1

# INTRODUCTION

## 1.1 - Introduction

Safety and efficacy of pharmaceutical drugs are fundamental issues in the pharmaceutical area. It is important to monitor concentrations of inorganic impurities for quality assurance and control of medicines because some elements may cause unwanted pharmacological–toxicological effects. Consequently, new elemental impurities regulations for pharmaceutical products are often published in order to modernize and standardize the elementary analysis of drugs, excipients and active pharmaceutical ingredients (APIs) [1–4].

Sampling and sample preparation are critical steps to the success of elemental determination [1,3–5]. For this, microwave-assisted digestion in closed vessels has clear advantages over traditional acid digestion using conventional heating in terms of better recoveries of volatile elements, less contamination, lower volume of reagents, as well as in reproducibility and a safer working environment [5]. In the determination of several elemental impurities recommended by Pharmacopoeias, argon-based plasma spectroanalytical methods, i.e. inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS), have been found to provide multi-element determination capabilities, high sample throughput, high sensitivity, accuracy, robustness and low detection limits [6–8].

This study is a response to the demand for straightforward sample preparation procedures as well as the need to apply more sensitive and specific instrumental methods for elemental analysis of pharmaceuticals. The development and feasibility of simplified sample preparation procedures based on microwave-assisted digestion using dilute nitric acid solution for different types of drugs (pills, tablets, oral solutions and parenteral drugs) and dietary supplements were investigated. Additionally, the performances of ICP OES and ICP-MS were evaluated, using different instrumental strategies: type of nebulizer, aerosol dilution and collision cell technology as well as calibration methods to correct the matrix and spectral interferences for simultaneous determination of 24 elemental impurities.

## 1.2 - Elemental impurities: regulatory bodies update

The United States Pharmacopoeia (USP) sets standards for the identity, strength, quality and purity of medicines, food ingredients and dietary supplements that are manufactured, distributed and consumed worldwide [9]. For many years the USP General Chapter 231 Heavy Metals [10] regulated the determination of elemental impurities in pharmaceutical samples using sulfide precipitation and evaluation by comparative visual examination. Although simple and inexpensive, the Chapter 231 recommendations provided the determination of a reduced number of elements without quantitative and element-specific information using many samples and toxic reagents.

In 2010, the USP proposed three new General Chapters on elemental impurities to replace Chapter 231 [10]. Chapters 232 (Elementary Impurities - Limits [11]), 233 (Element Impurities - Procedures [12]) and 2232 (Elemental Contaminants in Dietary Supplements [13]) were approved by the Expert Committee in April 2012 and became official in February 2013 [14]. In 2015 [15], the USP announced plans to publish these new chapters only after the harmonization of the General Chapter 232 with the final version of the harmonized guideline on elemental impurities (ICH Q3D Step 4) [16] of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) [17].

The ICH brings together both regulatory authorities and the pharmaceutical industry to discuss scientific and technical aspects of pharmaceuticals and to develop ICH guidelines that meet greater worldwide demands considering safety, effectivity and high quality of medicines [17]. The ICH Q3D guideline on elemental impurities applies to newly introduced drug products and new drug products containing existing drug substances. In short, there are three parts of this guideline: the evaluation of the toxicity data for potential elemental impurities; the establishment of Permitted Daily Exposures (PDEs) for each element of toxicological concern; and application of a risk-based approach to control elemental impurities in drug products [16].

The new version of Chapter 232 harmonized with ICH Q3D [15] was published in the Pharmacopeial Forum in May 2016 [18] and became official in

December 2017 [19], implemented together with the two other General Chapters in January 2018. A new version of the ICH guideline on elemental impurities, ICH Q3D(R1) step 5 [20], was transmitted to the Committee for Human Medicinal Products (CHMP) on 26 April 2018 and adopted by the CHMP on 22 March 2019. The most recent version of Chapter 232 [21] specifies limits for the amounts of 24 elemental impurities in drug products. The general chapter is divided in six sections:

(i) introduction;
(ii) speciation;
(iii) routes of exposure;
(iv) drug products;
(v) drug substance and excipients; and
(vi) analytical testing.

The introduction section defines elemental impurities, their contamination sources and products which do not apply in this chapter. The section on speciation states that only As and Hg need to be reported via a procedure that quantifies the different forms, and only in cases where the limits are exceeded in a total-As and total-Hg procedure, i.e. 1.5 and 3.0 µg $g^{-1}$, considering a maximum daily dose of 10 g. The arsenic limit is based on the inorganic (most toxic) form. On the other hand, as the methyl mercury form (most toxic) is rarely an issue for pharmaceuticals, the limit for Hg is established assuming the most common Hg inorganic form.

The routes of exposure section describe the limits of elemental impurity toxicities and their bioavailability based on chronic exposure. The extent of exposure is determined for each elemental impurity of interest for three routes of administration: oral, parenteral, and inhalational. These permitted daily exposures (PDE values) are separated into three classes based on the toxicity and probability of occurrence in drugs. The elements from Class 1 (As, Cd, Hg, and Pb) are toxic to humans and have limited or no use in the manufacture of pharmaceuticals. Their presence in drug products typically comes from the non-drug materials commonly associated with the manufacturing process, such as excipients and packaging. Class 2 elements are further divided into sub-classes 2A (Co, Ni, and V) and 2B (Ag, Au, Ir, Os, Pd, Pt, Rh, Ru, Se, and Tl). Class 2A elements are relatively common in drug

products and thus must be evaluated for all potential sources of toxicity. On the other hand, Class 2B elements are unlikely to be present in drug products and they may be excluded from the risk assessment unless they are intentionally added during the manufacture of excipients or other components of the drug product. Class 3 elements, i.e. Ba, Cr, Cu, Li, Mo, Sb, and Sn, have relatively low toxicities when administered via oral route [20]. Similarly, Chapter 2232 [22] specifies the toxicity limits for As, Cd, Hg, and Pb also considering the PDE values for these elements.

The drug products section describes elemental impurities and their toxicity limits for 24 elements considering the PDE values according to indicated routes of administration. The drug substance and excipients section provides examples of concentration limits calculated for components of drug products, considering the PDE values and a maximum daily dose of 10 g $day^{-1}$. Finally, the analytical testing section presents recommendations for using Chapter 233 when testing is carried out to demonstrate compliance. Analogously, Chapter 233 [23] is divided in four sections:

(i) introduction;
(ii) compendial procedures 1 i.e. ICP OES and 2 i.e. ICP-MS;
(iii) limit procedures;
(iv) quantitative procedures.

The topic of compendial procedures describes sample preparation and two analytical procedures. The procedure 1 uses ICP OES and procedure 2 uses ICP-MS for the evaluation of the levels of the elemental impurities. For sample preparation, the chapter mentions three options of general procedures, (i) direct dissolution in aqueous solution; (ii) direct dissolution in organic solution; and (iii) indirect solution, e.g. total metal extraction or closed vessel digestion. For samples that must be digested, this chapter recommends closed vessel digestion because this sample preparation procedure minimizes losses of volatile impurities.

Additionally, the sample preparation topic suggests that the use of any concentrated acid may be appropriate, among the following were cited: concentrated ultra-pure nitric, sulfuric, hydrochloric, or hydrofluoric acids or aqua regia (mixture of concentrated hydrochloric and nitric acids, typically at ratios of $3HCl:1HNO_3$ or $4HCl:1HNO_3$ v $v^{-1}$). Each one, however, has inherent

safety risks, requiring appropriate safety precautions. Figure 1.1 presents strategies for sample preparation of pharmaceutical products as recommended by Chapter 233.

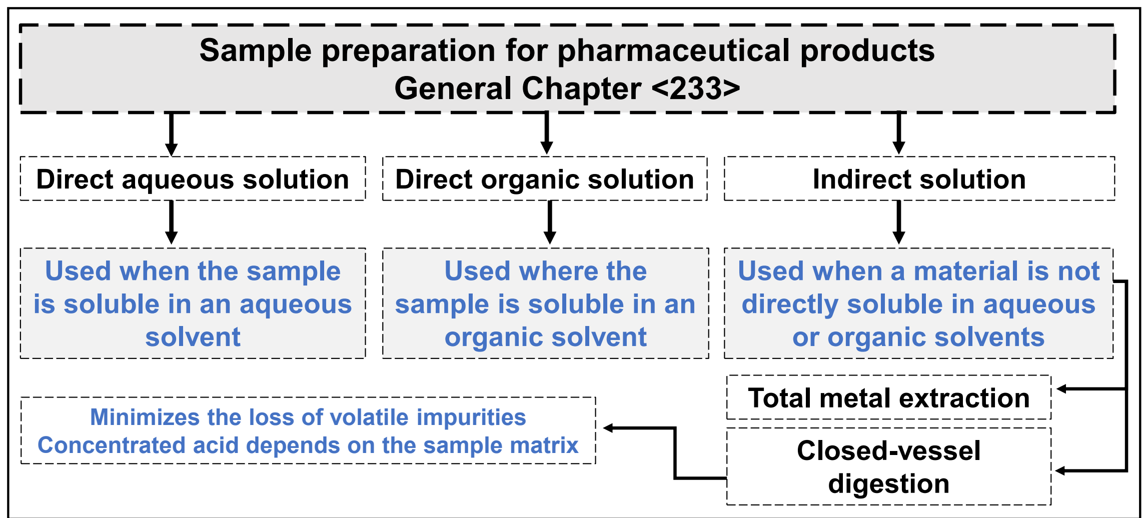



FIGURE 1.1 - Sample preparation of pharmaceutical products according to the USP General Chapter 233 [23]. Adapted from BALARAM, V. (2016) [2].

Before using procedures 1 (ICP OES) and 2 (ICP-MS) it is recommended that the analyst verify the appropriate procedure for the instrument and sample used by following the alternative procedure validation requirements. Measurements using both ICP-based methods must be performed according to manufacturer's suggestions for operational conditions and emission lines (ICP OES) and isotopes (ICP-MS). In addition, the compendial procedures topic also indicate that appropriate strategies must be taken to correct for matrix-induced interferences for both instrumental methods.

The validation parameters for the acceptability of alternative limit procedures include detectability, repeatability and specificity. The quantitative procedures section defines the validation parameters for the acceptability of alternative quantitative procedures considering accuracy, precision, specificity, limit of quantitation, range, and linearity. According to Chapter 233 [23], measurement of at least three calibration standards in the range between blank and at least 1.5*J* for each target element are recommended for linearity. For all previously mentioned cases, *J* values are concentration limits calculated by dividing the permissible daily exposures value (PDE) for each element by the

maximum daily dose (MDD) of the drug and multiplied by the dilution factor (DF) adopted in the analytical procedure. So, the *J* value, also called the target limit, is the concentration of the element(s) in m $m^{-1}$ of interest at the target limit, appropriately diluted for the working range of the instrument.

For method precision, repeatability should be estimated from independent measurements of 6 samples spiked with 0.5*J* and 1.5*J* for each target element. Intermediate precision must be evaluated by analysis of 12 samples spiked with 1*J* for each target element. There is no specific recommendation for calculating LOQs, but LOQs values ≤0.3*J* are suggested as acceptance criteria. Finally, accuracy must be evaluated by addition and recovery experiments with acceptable recoveries ranging from 70 to 150% of the spiked value added before any sample preparation steps, i.e. digestion or solubilization, in concentrations ranging from 0.5*J* to 1.5*J* values for each target element, considering up to 20% of repeatability.

Other reputable international authorities, such as the United States Food and Drug Administration (FDA), European Medicines Agency (EMA) Therapeutic Goods Administration (TGA) as well as other regulatory bodies, e.g. the European Pharmacopoeia (EP), the British Pharmacopeia (BP), the Japanese Pharmacopoeia (JP) and the Indian Pharmacopoeia (IP), strongly advise that element contamination in pharmaceutical products should be evaluated [2,24]. The EMA replaced the guidelines on the specification limits for residues of metal catalysts and metal reagents [25] by ICH Q3D guidelines [16]. Compared to the USP Chapter 232 target elements [21], the EMA guideline [25] did not include the elements As, Cd, Hg, and Pb. These country-specific guidelines may not be entirely consistent with the ICH Q3D guidelines, and in this case, the regional implementation guidance prevails.

Two methods for the determination of heavy metals in drug products are recommended by the Brazilian Pharmacopoeia of the Agência Nacional de Vigilância Sanitária (ANVISA): the first is a limit test for formation of solid particles of sulfides, and the second, elemental determination by atomic spectrometry [26]. For sample preparation, according to the 5th edition of the regulation effective since 2010, there is no need for prior decomposition of samples with water-soluble components; these can be analyzed directly after dissolution. However, if the sample is not soluble in water or other solvent

compatible with the atomic spectrometric method, two procedures are recommended: wet digestion in a closed system and microwave-assisted combustion in pressurized vessels [26]. Table 1.1 shows the concentration limits of elemental impurities in drug and pharmaceutical products set by the USP General Chapter 232 harmonized with ICH Q3D(R1) [20] and Brazilian Pharmacopoeia, ANVISA [26] according the drug categories: oral, parenteral and inhalation.

TABLE 1.1 - Classification according ICH Q3D and concentration limits of elemental impurities in drug and pharmaceutical products set by USP (μg $g^{-1}$, considering maximum daily dose of 10 g) and ANVISA (μg $g^{-1}$).

| Element | ICH Q3D Class | Oral exposure | | Parenteral exposure | | Inhalation exposure | |
|---|---|---|---|---|---|---|---|
| | | USP | ANVISA | USP | ANVISA | USP | ANVISA |
| Cd | 1 | 0.5 | 0.5 | 0.2 | 0.05 | 0.2 | NA |
| Pb | 1 | 0.5 | 1 | 0.5 | 0.1 | 0.5 | NA |
| As | 1 | 1.5 | 1.5 | 1.5 | 15 | 0.2 | NA |
| Hg | 1 | 3 | 1.5 | 0.3 | 0.15 | 0.1 | NA |
| Co | 2A | 5 | NA | 0.5 | NA | 0.3 | NA |
| V | 2A | 10 | 25 | 1 | 2.5 | 0.1 | NA |
| Ni | 2A | 20 | 25 | 2 | 2.5 | 0.5 | NA |
| Tl | 2B | 0.8 | NA | 0.8 | NA | 0.8 | NA |
| Au | 2B | 10 | NA | 10 | NA | 0.1 | NA |
| Os | 2B | 10 | <10 | 1 | <10 | 0.1 | NA |
| Ir | 2B | 10 | <10 | 1 | <10 | 0.1 | NA |
| Pd | 2B | 10 | 10 | 1 | 1 | 0.1 | NA |
| Pt | 2B | 10 | 10 | 1 | 1 | 0.1 | NA |
| Rh | 2B | 10 | <10 | 1 | <10 | 0.1 | NA |
| Ru | 2B | 10 | <10 | 1 | <10 | 0.1 | NA |
| Ag | 2B | 15 | NA | 1 | NA | 0.7 | NA |
| Se | 2B | 15 | NA | 8 | NA | 13 | NA |
| Li | 3 | 55 | NA | 25 | NA | 2.5 | NA |
| Sb | 3 | 120 | NA | 9 | NA | 2 | NA |
| Ba | 3 | 140 | NA | 70 | NA | 30 | NA |
| Cu | 3 | 300 | 250 | 30 | 25 | 3 | NA |
| Mo | 3 | 300 | 25 | 150 | 2.5 | 1 | NA |
| Sn | 3 | 600 | NA | 60 | NA | 6 | NA |
| Cr | 3 | 1100 | 25 | 1100 | 2.5 | 3.0 | NA |

## 1.3 - Elemental impurities in drugs and pharmaceuticals

"Elemental impurities include catalysts and environmental contaminants that may be present in drug substances, excipients, or drug products. These impurities may occur naturally, be added intentionally, or be

introduced inadvertently (e.g. by interactions with processing equipment and the container–closure system)" [21]

During the synthesis of drugs and pharmaceuticals, elemental impurities can stem from different sources and phases, including solvents, raw materials, APIs, excipients, reagents, catalysts, electrodes, reaction vessels, plumbing and other equipment used. Because of this, monitoring elemental impurities is an important activity for both reaction intermediates and final drug substances [1–3,21,27,28]. The potential contamination sources of elemental impurities are schematically shown in a fishbone diagram in Figure 1.2.

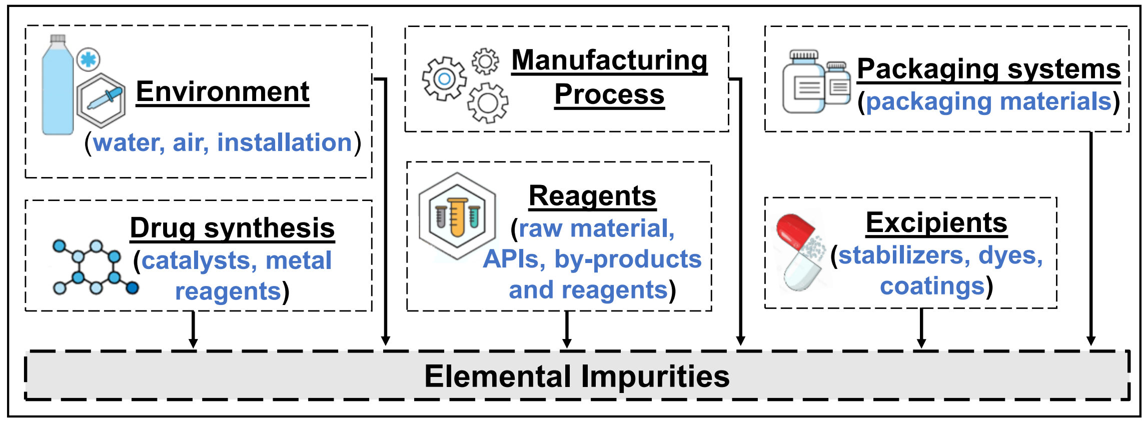



FIGURE 1.2 - Potential contamination sources of elemental impurities in drugs and pharmaceutical on the basis of the ICH Q3D guidance [20]. Adapted from BALARAM, V. (2016) [2].

For synthesis of pharmaceuticals, some catalysts (e.g. Ir, Os, Pd, Pt, Rh, and Ru) or metals and metalloids (e.g. Ag, Au, Ba, Li, and Pt) may be incorporated in the drug intermediate reaction process [2,27]. In addition, during the formulation and drug production, manufacturing equipment commonly contain Hastelloy (alloy formed by Ni, Cr, Fe and Mo), stainless steel and glass materials because of their superior chemical resistance. Common elements found in those machines (e.g. Co, Cr, Cu, Mo, Ni, and V) under extreme/corrosive reaction conditions (as high temperature and low/high pH) may suffer leaching [3,4,8,29].

Even at low concentration levels, metals such as Cd, Cr, Hg, and Pb pose a serious health risk when used for pharmaceutical purposes [2,28,29]. According to the International Agency for Research on Cancer (IARC) [30], As

and Cd, in their inorganic forms, are considered carcinogenic to humans. Inorganic Hg and Pb are not classified as carcinogenic, however they may cause toxicological and hematopoietic effects, i.e. renal effects and skin diseases caused by Hg and complications on neurological, reproductive, immune, cardiovascular and renal systems caused by Pb [28–30]. On the other hand, Co, Cu, Mo, and Se are classified as essential elements for human body, but in high concentrations they are also harmful to health.

In most cases, target elements included in commercial pharmaceutical samples are below the respective limits of quantification of the analytical method. This pattern was observed in several studies [31–39]. In addition, a database with elemental impurities excipient [28] confirms that elemental impurity concentrations in excipients are generally low. Trace concentrations of As, Ba, Cd, Co, Cu, Cr, Li, Mo, Ni, Pb, Sb, Se, Sn, Tl, and V were determined in nine drug samples for continuous use and one dietary supplement sample [40] and in seven omeprazole drug samples from different commercial brands [41]. The concentrations of these elements present in all samples, however, were lower than the limits proposed by USP Chapter 232 [21].

In the analysis of dietary supplements (e.g. botanicals, multivitamins, creatine, and sport supplements), the elemental impurities As, Cd, Hg, and Pb are also present in low concentrations [42–45]. A great variability of concentration ranges for As, Cd, Hg, and Pb were determined in 95 dietary supplements [46]. An analysis of 45 widely used pharmaceutical products evidenced only that six products had Pb concentrations higher than 100 μg $kg^{-1}$ [47]. Thus, because of these typically low concentrations of elemental impurities present in drug and pharmaceuticals, it is essential to develop highly sensitive and selective methods for elemental determination in pharmaceutical substances in order to ensure the safety and efficacy of drugs intended for human consumption is needed.

## 1.4 - Sample preparation of drugs and pharmaceuticals

Sample preparation is a critical step prior to accurate instrumental analysis [1,3–5]. Drugs, excipients, APIs and other pharmaceuticals in general are

complex with diverse matrices. Therefore, appropriate pharmaceutical sample preparation procedures for ICP-based methods and also for other spectroanalytical techniques measurements need to be carefully evaluated [1–4]. According to the literature, sample preparation for drugs and pharmaceuticals involves simple dissolution of samples in aqueous solutions of mineral acids [31,32,37,48–52] or organic solvents [39,53–55], ultrasound-assisted extraction [56], or other more sophisticated procedures for sample decomposition, such as digestion in closed vessel using conventional heating [52,57–59], microwave-induced combustion [33,60–62], microwave-assisted digestions in closed vessels [34–38,40,41,61,63–77], microwave-assisted vapor-phase systems [66] and wet digestion using an ultra-high-pressure chamber assisted by microwave irradiation [78,79]. Table 1.2 shows selected sample preparation procedures applied for the determination of elemental impurities in drugs and pharmaceuticals reported in the literature over the 21 years from 2000 to 2021.

TABLE 1.2 - Selected studies for determination of elemental impurities in drugs and pharmaceuticals.

| Sample | Analyte | Sample mass (mg) | Sample preparation solution and reagents | Analytical method | Reference |
|---|---|---|---|---|---|
| Dissolution in water | | | | | |
| Acetylsalicylic acid and L-serine | As, Cd, Cr, Cu, Fe, Hg, Ir, Mn, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, V, Zn | 100 | 10 mL of 1% v $v^{-1}$ $HNO_3$ + 0.15% v $v^{-1}$ HCl | FI-ICP-MS | 31 |
| Liquid drugs | Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, V | NA | 10-fold dilution in 0.14 mol $L^{-1}$ $HNO_3$ | ICP OES | 32 (Chapter 4.1) |
| Pharmaceutical excipients | As, Cd, Cu, Cr, Fe, Hg, Ir, Mn, Mo, Ni, Os, Pd, Pt, Rh, Ru, V, Zn | 100 | 0.009 mM $KBrO_3$ in 1% v $v^{-1}$ $HNO_3$ + 1% v $v^{-1}$ HCl | ICP-MS | 37 |
| Parenteral solutions | (1) As, Cd, Mo, Pb<br>(2) Cr, Mn, Ni, V<br>(3) Hg | NA | Diluted in 5% v $v^{-1}$ $HNO_3$ | (1) ICP-MS<br>(2) DRC-ICP-MS<br>(3) FI-CVG-ICP-MS | 48 |
| Enalapril maleate, calcium folinate and levodopa | Pd, Pt, Rh | 100 | 100 mL of 0.3 mol $L^{-1}$ $HNO_3$ | ICP-MS | 49 |
| Drug substances, intermediate and raw materials | 69 elements | 10 | 10 mL of 80% v $v^{-1}$ of $HNO_3$ | ICP-MS | 50 |
| Liquid drugs | Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, V | NA | 50-fold dilution in 0.14 mol $L^{-1}$ $HNO_3$ | ICP-MS | 51 |
| Eye drops and mouthwash | Al, Cr, Co, Cu, Fe, Mn, Ni, Zn | NA | 4-fold dilution in 0.14 mol $L^{-1}$ $HNO_3$ | MIP OES | 52 |
| Dissolution in organic solvents | | | | | |
| Several APIs | As, Se, Sn, Sb, Pd, Cd, In, Pt, Pb, Bi, Hg, Ru, Mo | 25 | 2-butoxyethanol: water solution (25:75 v $v^{-1}$) | ICP-MS | 39 |
| Several APIs | Pd | 20 to 500 | 20 g diethylene glycol monoethyl ether + 200 mg thioacetamide | ICP-MS | 53 |
| Several APIs | Al, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pd, Pt, Rh, Ru, W, Zn, Zr | 10 | 5 mL of 2% EDTA solution prepared in N,N-dimethylformamide | ICP OES | 54 |

TABLE 1.2 - Selected studies for determination of elemental impurities in drugs and pharmaceuticals (continuation).

| Sample | Analyte | Sample mass (mg) | Sample preparation solution and reagents | Analytical method | Reference |
|---|---|---|---|---|---|
| Dissolution in organic solvents | | | | | |
| Several APIs | Cr, Pd, Rh | NA | Dissolution in ethanol and the use of solid adsorbents for screening test | FI-ICP-MS | 55 |
| Ultrasound-assisted automated extraction | | | | | |
| Several APIs | Ag, As, Bi, Cd, Cu, Hg, Mo, Pb, Sb, Sn | 100 | 9 mL of 2HCl:98DMSO v $v^{-1}$ | ICP OES | 56 |
| Microwave-induced combustion | | | | | |
| Several APIs | Br, I | 500 | 20 bar $O_2$; diluted solution: 50 mmol $L^{-1}$ of $(NH_4)_2CO_3$ | ICP-MS | 33 |
| Acetylsalicylic acid tablets | As, Cd, Cr, Cu, Hg, Ir, Mn, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, V | 400 to 700 | 20 bar $O_2$; diluted solution: 5 mL of 20% v $v^{-1}$ $HNO_3$ | ICP-MS | 60 |
| Carbamazepine, amitriptyline hydrochloride and imipramine hydrochloride | As, Cd, Hg, Pb | 500 | 20 bar $O_2$; diluted solution: 6 mL of 7 mol $L^{-1}$ $HNO_3$ | ICP-MS | 61 |
| Several APIs | As, Cd, Cr, Cu, Mn, Mo, Ni, Pb, Pd, Pt, Rh, Ru, V | (1) 2500 (2) 125 | (1) Solid sampling: sample + Freon as modifier (2) 20 bar $O_2$; diluted solution: 6 mL of 14 mol $L^{-1}$ $HNO_3$ | (1) ETV-ICP OES (2) ICP-MS | 62 |
| Digestion in closed vessel using conventional heating | | | | | |
| Antibiotic tablets | Ag, Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, Mg, Mn, Ni, Pd, Pb, Se, Zn | 100 to 300 | 5 mL of 14 mol $L^{-1}$ $HNO_3$ + 1 mL of $H_2O_2$ 30% v $v^{-1}$ | ICP OES | 57 |
| Several API tablets | Al, B, Cr, Cu, Fe, Mg, Mn, Pb, Ti, Zn | 100 to 300 | 5 mL of 14 mol $L^{-1}$ $HNO_3$ + 1 mL of $H_2O_2$ 30% v $v^{-1}$ | ICP OES | 58 |
| Drugs (pills and tablets) | Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Ti, Zn | 100 | 5 mL of 14 mol $L^{-1}$ $HNO_3$ + (not specific) $H_2O_2$ 30% v $v^{-1}$ | ICP-MS | 59 |
| Children’s cough syrup | Al, Cr, Co, Cu, Fe, Mn, Ni, Zn | 5 mL* | 1.5 mL of 14 mol $L^{-1}$ $HNO_3$ + 3 mL of $H_2O_2$ | MIP OES | 52 |

TABLE 1.2 - Selected studies for determination of elemental impurities in drugs and pharmaceuticals (continuation).

| Sample | Analyte | Sample mass (mg) | Sample preparation solution and reagents | Analytical method | Reference |
|---|---|---|---|---|---|
| Microwave-assisted digestion in closed vessel | | | | | |
| Lu tablets | As, Cd, Cu, Cr, Fe, Hg, Mn, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, V, Zn | 450 | 12 mL of $3HNO_3$:1HCl v $v^{-1}$ | ICP OES | 34 |
| Liquid drugs | Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, V | 1 mL* | 7 mL of (1) 7 mol $L^{-1}$ $HNO_3$ (2) 2 mol $L^{-1}$ $HNO_3$ | ICP OES | 32 |
| Levodopa, primaquine diphosphate, propranolol hydrochloride and sulfamethoxazole | (1) Cd, Ir, Mn, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru (2) Cr, Cu (3) As, Hg | 250 to 500 | 6 mL of (1) $3HNO_3$:1HCl v $v^{-1}$ (2)(3) 14 mol $L^{-1}$ $HNO_3$ | (1) ICP-MS (2) DRC-ICP-MS (3) FI-CVG-ICP-MS | 35 |
| Drugs (pills and tablets) | As, Cd, Hg, Pb | 100 to 500 | 5 mL of $3HNO_3$:1HCl v $v^{-1}$ | ICP OES; ICP-MS | 36 |
| Liquid drugs | Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, V | 200 | 7 mL of 14 mol $L^{-1}$ $HNO_3$ + 2 mL of HCl + 1 mL of $H_2O_2$ 30% v $v^{-1}$ | ICP OES | 38 |
| Drugs (pills and tablets) | Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, V | 500 | 7 mL of (1) 2 mol $L^{-1}$ $HNO_3$; (2) 3HNO3:1HCl v v-1 | (1) ICP OES (2) ICP-MS | 40 (Chapter 3.1) |
| Omeprazole tablets | Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, V | 500 | 7 mL of (1) 2 mol $L^{-1}$ $HNO_3$ (2) $3HNO_3$:1HCl v v-1 | ICP-MS | 41 |
| Carbamazepine, amitriptyline hydrochloride and imipramine hydrochloride | As, Cd, Hg, Pb | 500 | 6 mL of 14 mol $L^{-1}$ $HNO_3$ | ICP-MS | 61 |
| Acyclovir ointment and its constituents | As, Cu, Cr, Ni, Pb, V | 200 | 6 mL of 7 mol $L^{-1}$ $HNO_3$ + 2 mL of $H_2O_2$ 30% v $v^{-1}$ | ICP-MS | 77 |

* Unit in mL.

TABLE 1.2 - Selected studies for determination of elemental impurities in drugs and pharmaceuticals (continuation).

| Sample | Analyte | Sample mass (mg) | Sample preparation solution and reagents | Analytical method | Reference |
|---|---|---|---|---|---|
| Microwave-assisted digestion in closed vessel | | | | | |
| Several APIs | Ag, As, Au, Bi, Cd, Cu, Hg, Mo, Pb, Pd, Pt, Ru, Sb, Sn, V | 100 to 500 | 5 mL of 14 mol $L^{-1}$ $HNO_3$ | ICP-MS | 63 |
| Levetiracetam | Ag, Au, As, Bi, Cd, Cr, Cu, Fe, Hg, Ir, Mn, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, Sb, Sn, V, Zn | 1000 | 15 mL of 14 mol $L^{-1}$ $HNO_3$ + 2 mL of $H_2O_2$ 30% v $v^{-1}$ | ICP OES | 64 |
| Several APIs and finished products (tablets) | (1) Cd, Co, Ir, Mn, Mo, Ni, Os, Pb, Pd, Pt, Ru, Rh, Sn, Sb, V; (2) Cu, Fe, Zn | (1)(2) 400 | 2 mL of $H_2O$ + 4 mL of $1HNO_3$:1HCl | (1) ICP-MS (2) ICP OES | 65 |
| Pharmaceutical raw materials | Ca, Cr, Cu, Fe, Mg, Mn, Mo, P, Se, Zn | 200 to 600 | 6 mL of 14 mol $L^{-1}$ $HNO_3$ | ICP-MS; ET AAS | 66 |
| Pharmaceutical excipients | Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, V | 100 | 2 mL of 14 mol $L^{-1}$ $HNO_3$ + 2 mL of HCl + 1 mL $H_2O_2$ 30% v $v^{-1}$ | ICP-MS | 68 |
| Vitamin E | Cr, Ni, Sn, Pb | 100 | 5 mL of $HNO_3$ + 0.5 mL of $H_2O_2$ 30% v $v^{-1}$ | ICP-MS | 70 |
| Methadone hydrochloride oral solution | (1) As, Cd, Co, Hg, Ni, Pb, V; (2) As, Pb | 5 mL* | 8 mL of 5.3 mol $L^{-1}$ $HNO_3$ | (1) ICP OES (2) ICP-MS | 71 |
| Raw materials, excipients, APIs and drugs | As, Ag, Au, Cd, Co, Hg, Ir, Ni, Os, Pb, Pd, Pt, Rh, Ru, Se, Tl, V | (1) 100 (2) 50 | (1) Solid sampling: pelletized sample (2) 4 mL of 14 mol $L^{-1}$ $HNO_3$ + 10 mL of $HClO_4$ + 0.25 mL of HCl | (1) LA-ICP-MS (2) ICP-MS | 72 |
| One API | Pd | 100 | 4 mL of 14 mol $L^{-1}$ $HNO_3$ + 1 mL of $HClO_4$ | ICP-MS | 73 |

* Unit in mL.

TABLE 1.2 - Selected studies for determination of elemental impurities in drugs and pharmaceuticals (continuation).

| Sample | Analyte | Sample mass (mg) | Sample preparation solution and reagents | Analytical method | Reference |
|---|---|---|---|---|---|
| Microwave-assisted digestion in closed vessel | | | | | |
| Antihypertensive tablets | Cd, Cr, Mo, Pb, Pd, Pt | (1) 100<br>(2) 200 | (1) Solid sampling (slurries): 10 mL water + 1% m $v^{-1}$ of APDC + 0.025% m $v^{-1}$ of 8-HQ; (2) 3 mL 14 mol $L^{-1}$ $HNO_3$ + 1 mL of HCl. After cooling: 2 mL of $H_2SO_4$ + 1 mL of $HNO_3$ + 1 mL of HCl | (1) ETV-DRC-ICP-MS<br>(2) DRC-ICP-MS | 74 |
| Arbidol | Cd, Co, Cu, Mn, Ni, Pb | (1)(2) 100 | (1) Solid sampling: pelletized sample (2) 6 mL of 14 mol $L^{-1}$ $HNO_3$ + 2 mL of $H_2O_2$ 30% v $v^{-1}$ | (1) LA-ICP-MS<br>(2) ICP-MS | 75 |
| Microwave-assisted cloud point extraction | | | | | |
| Enalapril and ramipril tablets | Pd, Pt, Rh | 200 | 40 mL of 0.5% v $v^{-1}$ Triton X-100 + 2-mercaptobenzothiazole | ICP-MS | 69 |
| Wet digestion using an ultra-high-pressure chamber assisted by microwave radiation | | | | | |
| Fingolimod | Cu, Fe, Ni, Pb, Pd, Zn | 200 | 4 mL of 14 mol $L^{-1}$ $HNO_3$ + 0.2 mL $HClO_4$ | ICP OES | 78 |
| Microcrystalline cellulose | Os | 100 | 3 mL of 14 mol $L^{-1}$ $HNO_3$; After digestion: 0.5% v $v^{-1}$ of acetic acid containing 0.01 mol $L^{-1}$ thiourea + 0.1 g $L^{-1}$ of ascorbic acid | ICP-MS | 79 |

According to the General Chapter 233 [23], direct aqueous or organic dilution/dissolution can be adopted when the sample is soluble in an aqueous or organic solvent, respectively. When a material is not directly soluble in aqueous or organic solvents, a digestion procedure is recommended. In this sense, the sample form (liquid or solid samples), is extremely relevant for the choice of an appropriate sample preparation procedure. For liquid samples, e.g. eye drops, syrups and oral solutions, although all samples are in liquid form, differences of viscosity are highly significant for the adoption of a simple aqueous or organic sample dissolution or a sample digestion procedure. For solid samples, e.g. tablets and pills, even using a digestion procedure, the choice of digestion solution (type of concentrated or diluted acid or acid mixtures) is extremely relevant, mainly for distinct types of medicines which present diverse types of excipients in their formulations and, consequently, can present different behavior, e.g. total or partial digestion, even when submitted to the same sample digestion conditions.

Sample dissolution in aqueous media or organic solvents is a simple and time saving preparation procedure; however, it requires a careful matrix interference evaluation. Considering liquid pharmaceutical products, components used for parenteral nutrition solutions were diluted in $HNO_3$ 1% v $v^{-1}$ for determination of elemental impurities using ICP-MS [48]. Similarly, liquid medicines were 10-fold [32] and 50-fold [51] diluted in 1% v $v^{-1}$ $HNO_3$ for determination of elemental impurities using ICP OES [32] and using ICP-MS [51], respectively. Similar procedures were applied for analysis of solid pharmaceutical and APIs, two drug samples were diluted in 1% v $v^{-1}$ $HNO_3$ and 0.15% v $v^{-1}$ HCl to determine eighteen elemental impurities by flow injection combined with ICP-MS [31]. Samples of APIs were dissolved in diethylene glycol monoethyl ether for Pd determination using ICP-MS [53] and in 2% v $v^{-1}$ EDTA solution prepared in N, N-dimethylformamide for determination of fifteen elements using ICP OES [54]. The main limitation of dilution methods is the low solubility of APIs [1,3,61].

Methods based on microwave-induced combustion were applied for digestion of APIs and subsequent determination of Br and I [33] and As, Cd, Hg, and Pb [61] using ICP-MS. In this system, the combustion is started by microwave radiation and the complete digestion of samples occurs in oxygen

pressurized closed quartz vessels. Thus, since oxygen is the main reagent for sample digestion, concentrated acids are avoided and, consequently, contamination risks are reduced. As demonstrated, only diluted acid or alkaline solutions are necessary for analyte absorption [33,60,61].

Closed-vessel digestion has clear advantages compared to conventional acid digestion using open systems for decomposition of pharmaceutical sample matrices resulting in better recoveries of volatile elements, lower contamination, lower volume of reagents, better reproducibility, and a relatively safer operation [1,3]. Most pharmaceutical products, APIs or related products are hard to digest even under extreme temperature and pressure conditions. Therefore, depending on the matrix, analytes, and/or digestion system, different acids e.g. $HNO_3$, HCl, HF, $HClO_4$, $H_3PO_4$, and $H_2SO_4$ and/or mixtures of them should be used [1,5].

Some drugs and pharmaceuticals surveyed were microwave-assisted digested using concentrated $HNO_3$ [31,35,37,61,63,66] or its mixtures with $H_2O_2$ [64,70,75], and with HCl in different ratios [65] or using aqua regia, i.e. 3HCl:1$HNO_3$ v $v^{-1}$ [80] or inverse aqua regia, i.e. 3$HNO_3$:1HCl v $v^{-1}$ [34–36,40,41]. More complex digestion mixtures have been employed, i.e. $HNO_3$ with HCl and $H_2O_2$ [38,68], $HNO_3$ with $H_2SO_4$ [74,76], $HNO_3$ with HCl and $HClO_4$ [72], $HNO_3$ with $HClO_4$ [73], or even $HNO_3$ with HCl, $H_2O_2$ and HF [81] for complete solubilization of matrices containing silicon dioxide, titanium dioxide, or talc. Three digestion solutions (concentrated $HNO_3$, aqua regia, and inverse aqua regia) were evaluated for digestion of APIs [35]. Masses of 500 mg of APIs were efficiently digested using only $HNO_3$ allowing the determination of As, Cd, Cr, Cu, Hg, Mo, Ni, Pb, and V using ICP-MS. Inverse aqua regia was suitable for digestion of sample masses up to 250 mg for the determination of Ir, Pd, Pt, Rh, and Ru [35]. Suitable digestion procedures of pharmaceuticals using inverse aqua regia, i.e. 3$HNO_3$:1HCl v $v^{-1}$ have also been reported [34–37,39,82].

On the other hand, although necessary for sample matrices hard to digest, sample digestion performed with concentrated acids should be handled with care because of their high vapor pressure and/or corrosive properties. Nevertheless, these problems are minimized when using microwave-assisted digestion in closed vessels. The presence of oxygen in the gas phase inside the closed digestion vessel and the temperature gradient

between gas and liquid phases along the vessel allow the nitric acid regeneration and, consequently, the use of lower concentrations of nitric acid or even the use of less aggressive acids without losing the efficiency of digestion. The use of dilute acids could be considered as an alternative to a rapid increase of pressure inside closed reaction vessels, resulting even lower volume of reagents and waste [5].

Microwave-assisted sample digestion using dilute nitric acids was successfully used with organic samples [5]. Since most pharmaceutical products are hard to digest, however, remaining solids are expected when using dilute acid solutions. Microwave-assisted acid digestion using 7.0 mol $L^{-1}$ $HNO_3$ and $H_2O_2$ was proposed by Gonzalez et al. [77] for complete digestions of acyclovir ointment and its constituents. On the other hand, Pinheiro et al. [40] evaluated three solutions, i.e. inverse aqua regia, 7.0, and 2.0 mol $L^{-1}$ $HNO_3$ for microwave-assisted digestion of nine drug samples. The proposed digestion procedures using 7.0 and 2.0 mol $L^{-1}$ $HNO_3$ were suitable, even when remaining solids were present in the digests. Partial digestion using 2.0 mol $L^{-1}$ $HNO_3$ was also used for microwave-assisted digestion of seven omeprazole drug samples [41].

Microwave-assisted digestion of dietary supplements has been reported using several means of oxidizing acids and mixtures [83], e.g. concentrated $HNO_3$ [47,84], mixtures of $HNO_3$ with HCl [43], with $H_2O_2$ [43,85,86], with $H_2O_2$ and HCl [44], and with HCl and HF [87]. Microwave-assisted digestion of dietary supplements using dilute nitric acid solutions was used for sports supplements [42] and medicinal plants [88] to determine As, Cd, Hg, and Pb using ICP-MS according to the USP Chapter 2232. In addition, mixtures of diluted $HNO_3$ with $H_2O_2$ [89] or pressurized with oxygen gas [90] were used as oxidizing agents for dietary supplements digestion. Table 1.3 shows selected procedures based on microwave-assisted digestion of dietary supplements for determination of elements in dietary supplements reported in the literature.

TABLE 1.3 - Selected studies for microwave-assisted digestion of dietary supplements aiming elemental determination.

| Sample | Analyte | Sample mass (mg) | Sample preparation | Analytical method | Reference |
|---|---|---|---|---|---|
| Sports supplements | As, Cd, Hg, Pb | 200 | 5 mL of 3.75 mol $L^{-1}$ $HNO_3$ | ICP-MS | 42 (Chapter 5.1) |
| Dietary supplements and botanicals | Na, Mg, Al, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Cd, Ba, Hg, K, Pb | 100 to 500 | 8 mL of 14 mol $L^{-1}$ $HNO_3$ + 3 mL HCl | ICP-MS | 43 |
| Multivitamin dietary supplements | Cr, Cu, Fe, Mn, Se | 250 | 4 mL of 14 mol $L^{-1}$ $HNO_3$ + 1.5 mL of HF + 1 mL of $H_2O_2$ 30% v $v^{-1}$ | HR-CS GFAAS | 44 |
| Creatine supplements | As, Cd, Hg, Pb | 500 | 3 mL of 14 mol $L^{-1}$ $HNO_3$ + 0.5 mL 30% v $v^{-1}$ $H_2O_2$ | ICP-MS | 45 |
| Pharmaceutical products and dietary supplements | Pb | 200 | 5 mL of 14 mol $L^{-1}$ $HNO_3$ | ICP-MS | 47 |
| Multivitamin and multimineral supplements | (1) As, Cd, Pb, REEs, Ti, Au, Pt, Pd; (2) Na, K, Ca, Mg, P, Cu, Fe, Mn, Zn, Cr, Ni, V | 200 | 5 mL of 14 mol $L^{-1}$ $HNO_3$ + 0.5 mL 30% v $v^{-1}$ $H_2O_2$ or + 0.5 mL of HF | (1) ICP-MS (2) ICP OES | 85 |
| Multivitamin and multimineral supplements | As, Bi, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Se, V, Zn | 500 | 7 mL of 14 mol $L^{-1}$ $HNO_3$ + 3 mL of 30 % v $v^{-1}$ $H_2O_2$ | ICP OES | 86 |
| Dietary supplements | (1) Cu, Zn; (2) Cd, Pb | 350 | 4 mL of 14 mol $L^{-1}$ $HNO_3$ + 4 mL of HF + 1 mL of HCl | (1) AAS (2) GF AAS | 87 |
| Medicinal plants | (1) As, Cd, Pb; (2) Hg | 500 | 6 mL of 4 mol $L^{-1}$ $HNO_3$ | (1) ICP-MS (2) FI-CVG-ICP-MS | 88 |
| Dietary supplements | Ca, Cd, Cr, Cu, Fe, Mg, Pb, Zn | 250 | 5 mL of 2 mol $L^{-1}$ $HNO_3$ + 3 mL 30% v $v^{-1}$ $H_2O_2$ | ICP OES | 89 |
| Sports supplements | Ca, Cu, Fe, K, Mg, Mn, Na, Zn, P, S | 1350 | 6 mL of 5 mol $L^{-1}$ $HNO_3$; 5 bar of $O_2$ pressure | ICP OES | 90 |

### 1.4.1 - Dispersive liquid-liquid microextraction

As previously described, an effective sample preparation procedure is crucial for accurate determination of elements using argon-based plasma spectroanalytical methods. Moreover, when the analytical instrument is not sensitive enough for direct analyte quantification at trace/ultra-trace levels, a specific procedure entailing an effective separation or pre-concentration methodology prior to quantification step is also needed. For this, the main goals of sample preparation based on extraction and microextraction techniques are isolation and/or preconcentration of analytes aiming at the elimination of the sample matrix and also the enrichment of the analytes to levels within the limits of detection of the analytical instrument [91,92].

Extraction/preconcentration techniques are considered relatively time consuming, due to the many steps of sample manipulation. Significant progress based on new approaches, materials and techniques has been made to overcome this disadvantage. Alternatively, liquid-liquid extraction (LLE) overcomes many drawbacks of other sample pretreatments as well as having low costs, ease of use and, mainly, the reduction of processing time. Liquid-phase microextraction (LPME) can be defined as a miniaturization of LLE technique since the volume of the extractant phase is equal or below 100 µL [92,93]. This provides a very significative advantage, since miniaturization implies in higher enrichment factors using extremely low solvent volumes, and consequently, reduced residues generation, making LPME into an environmentally friendly preconcentration technique.

Several LPME approaches have been suggested for the preconcentration of metals prior to their determination with instruments appropriate for detecting metals. Particularly dispersive liquid-liquid microextraction (DLLME) has been extensively used, with the following advantages:

(i) simplicity of operation;
(ii) low sample volume;
(iii) low cost;
(iv) short extraction times;
(v) environment friendliness;

(vi) versatility for coupling with many analytical techniques for determination of the analytes at trace and ultra-trace levels;

(vii) a high enrichment factors using an extremely low quantity of extractant solvent [91–93].

DLLME was proposed for the first time in 2006 by Rezaee and coworkers [94]. This microextraction technique is based on a ternary component solvent system in which a water-immiscible organic solvent is dispersed in fine drops into the aqueous sample with the aid of an organic disperser agent. A cloudy solution is then formed because of the cosolvency of the dispersant with the other two phases, leading to a great contact surface area. Finally, the phases are separated by centrifugation, and the enriched organic phase that sedimented in the bottom of the centrifuge tube is collected and directly analyzed or diluted in a suitable dispersive solvent prior to analysis. Figure 1.5 shows a general scheme for a DLLME procedure.

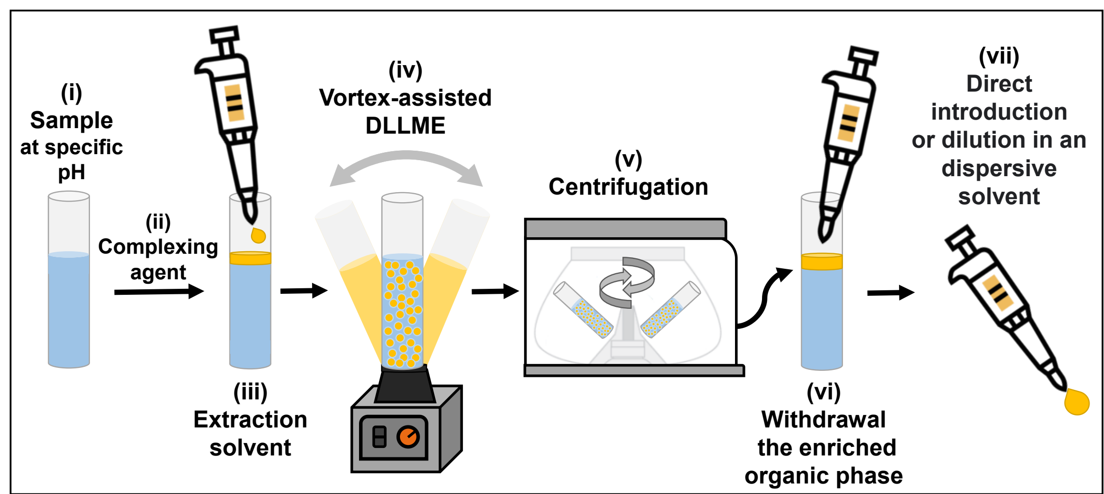



FIGURE 1.3 - Scheme of DLLME procedure for preconcentration of metals.

For DLLME application, some critical parameters must be optimized for a successful analyte extraction, such as sample amount, extraction and centrifugation time, pH, volume and types of disperser and extraction solvents and even salt addition [95,96]. Due to low solubility in water and ability to form a turbid stable solution, organic solvents, the following may be used as extraction solvents: carbon tetrachloride, chloroform, toluene, tetrachloroethylene, tetrachloroethane, chlorobenzene, dichloromethane, and

dichloroethane. In order to overcome some of these DLLME limitations, the use of extracts from green solvents, e.g. supramolecular solvents, deep eutectic solvents and switchable solvents, have been used in LPME aiming to develop green preconcentration methods [97]. The choice of a suitable dispersive solvent is also important. Ethanol, methanol, 2-propanol, acetone and acetonitrile are commonly used as disperser solvents. Several authors have reported that the optimization of these parameters can be easily obtained by applying multivariate optimization design [95,96].

Despite its simple procedure, classical DLLME suffers from some limitations that are under continuous revision, such as:

(i) harmful organic solvents, denser than water, are used as extractants;
(ii) the organic rich phase requires a dispersant solvent that competes with the extractant solvent for the analyte, thereby reducing extraction efficiency;
(iii) centrifugation is necessary to separate phases after microextraction. In addition, DLLME is not appropriate for the direct extraction of analytes from solid food samples, requiring sample preparation steps prior to analysis [92,93].

Dispersive LLME technique can be combined with spectrochemical methods, such as ETAAS, FAAS, ICP OES, ICP-MS, LIBS and Ultraviolet-Visible (UV-vis) spectrophotometry. Due to the low sample volume obtained after microextraction procedure, the organic phase is usually diluted in another miscible organic solvent prior to conventional liquid sample introduction using pneumatic nebulization [98–100]. Even considering lower dispersive solvent volume, the organic phase dilution might deteriorate the enhancement factor achieved for DLLME and, additionally, considering ICP-based methods, the introduction of organic matrices into the argon plasma may cause carbon deposits on the torch as well as severe matrix effects [101]. In order to address these challenges, the combined use of multinebulizer-based systems and ICP OES analysis has been successfully applied for the analysis of samples with high organic contents. This system allows the simultaneous introduction of organic and aqueous solutions into the plasma, thus reducing carbon deposits and also correcting for matrix effects [102–107].

According to USP requirements [21,23] and ICH Q3D(R1) [20], the elements in class 1 (As, Cd, Hg, and Pb) and class 2A (Co, Ni, and V) must be reported in all pharmaceutical products. Assuming that any other elemental impurities are identified as contributions from the manufacturing process, the other 17 elements are not required to be investigated. Due to *their* toxicities, lower target-limits are recommended for these elements. In comparison to class 1, higher target-limits for drugs administered via oral route are recommended for class 2A. But for parenteral drugs, the target-limits for Co, Ni, and V are 10-times lower and, along with As, Cd, Hg, and Pb, have target-limits ranging from 2 to 20 $\mu g\ mL^{-1}$ [20,21,23].

Therefore, due to these low levels, equivalent to 0.5*J* and 1.5*J* for the above-mentioned target elements [21,23], most proposed ICP methods for elemental impurities determination are based on ICP-MS analysis [1,2,4]. In order to reach sufficient sensitivity to determine these elements in drug samples using other spectroanalytical methods, a preconcentration step prior to measurement needs to be applied [91,92]. Consequently, some studies have indicated other DLLME procedures that could be applied for preconcentration of As, Cd, Co, Hg, Ni, Pb, and V in numerous samples prior to measurements using different analytical methods. Table 1.4 shows several DLLME procedures used to determine these elements.

TABLE 1.4 - Selected procedures for preconcentration of As, Cd, Co, Hg, Ni, Pb, and V prior to measurements using spectroanalytical methods.

| Sample | Analyte | Reagent | LOD | Analytical method | Reference |
|---|---|---|---|---|---|
| Mussels, rice, red wine and chocolate | As, Cd, Pb | Complexing reagent: 1-Pyrrolidinecarbodithioic acid; extraction solvent: tetrahydrofuran/1-decanol; alcohol as dilution solvents | As: 2.4 μg $L^{-1}$<br>Cd: 0.6 μg $L^{-1}$<br>Pb: 1.6 μg $L^{-1}$ | ICP OES | 108 |
| Fish oil | Cd, Ni, Pb | Extraction agent: nitric acid; dispersant agent: n-propanol | Cd: 0.12 μg $kg^{-1}$<br>Ni: 0.11 μg $kg^{-1}$<br>Pb: 0.58 μg $kg^{-1}$ | ICP OES | 109 |
| Spinach and lettuce | Co, Ni | Complexing reagent: 1-(2-pyridylazo)-2-naphthol; extraction solvent: the ionic liquid 1-hexyl-3-methylimidazolium bis(tri-fluoromethylsulfonyl)-imide | Co: 0.65 μg $L^{-1}$<br>Ni: 0.32 μg $L^{-1}$ | UV-Vis | 110 |
| Sugar | As, Cd | Complexing reagent: ammonium pyrrolidinedithiocarbamate; Extraction solvent: acetone; carbon tetrachloride as dispersive solvent | As: 0.21 ng $g^{-1}$<br>Cd: 0.060 ng $g^{-1}$ | ICP-MS | 111 |
| Spice, vegetable and fruit | Ni, Pb | Extraction solvent e: 1-butyl-3-methylimidazolium hexafluorophosphate and carbon tetrachloride as dispersant solvent | Ni: 0.49 μg $L^{-1}$<br>Pb: 0.95 μg $L^{-1}$ | FAAS | 112 |
| Drugs (pills and tablets) | Cd, Hg, Pb | Complexing reagent: sodium diethyldithiocarbamate; extraction solvent: toluene; no dispersive solvent | Cd: 0.08 μg $L^{-1}$<br>Hg: 0.6 μg $L^{-1}$<br>Pb: 0.5 μg $L^{-1}$ | ICP OES | 113 (Chapter 3.2) |
| Liquid drugs | Cd, Co, Hg, Ni, Pb, V | Complexing reagent: 8-hydroxyquinoline; extraction solvent: deep eutectic solvent; no dispersive solvent | Cd: 0.05 μg $L^{-1}$<br>Co: 0.6 μg $L^{-1}$<br>Hg: 0.8 μg $L^{-1}$<br>Ni: 0.9 μg $L^{-1}$<br>Pb: 0.5 μg $L^{-1}$<br>V: 0.8 μg $L^{-1}$ | ICP OES | 114 (Chapter 4.2) |

An analytical method for simultaneous DLLME of Cd, Hg, and Pb from drug samples prior to measurements by ICP OES was developed by Pinheiro et al. [113]. This was the first report applying an extraction/preconcentration procedure for drug samples for determination of elemental impurities in accordance with ICH guidelines and USP recommendations. When compared to conventional ICP OES analysis, DLLME improved limits of quantitation (LOQs) ca. 40-fold for all analytes. Consequently, suitable sensitivity within USP requirements for determination of Cd, Hg, and Pb using ICP OES was achieved using DLLME before microwave-assisted digestion of samples using dilute nitric acid solution. An analytical method for simultaneous DLLME of Cd, Co, Hg, Ni, Pb, and V from liquid drug samples (oral and parenteral drugs) was also developed by these same authors [114]

## 1.5 - Determination of elemental impurities by ICP-based methods: instrumental strategies and calibration methods

As well as sample preparation procedures, the choice of one analytical method for elemental impurities determination in pharmaceutical samples is clearly an important aspect to be evaluated. Considering the limits proposed by the Chapters 232 [21] and 2232 [22] for twenty-four and four analytes, respectively, the ICP OES and ICP-MS processes are attractive because they provide multi-elemental analysis with high sensitivity, accuracy, robustness and low limits of detection (LODs), in typical ranges of mg $L^{-1}$ for ICP OES and µg $L^{-1}$ for ICP-MS. Usually, determinations using both analytical methods, i.e. ICP OES and ICP-MS, require appropriate sample dilution considering total dissolved solids (TDS) contents below 1.0 and 0.2% m $v^{-1}$, and residual acidities (RA) below 10 and 1% v $v^{-1}$,, respectively [6–8].

Some sample introduction systems able to deal with high solid contents permit the introduction of solutions with TDS up to 20% m $v^{-1}$ when using ICP OES [6]. Babington-type nebulizers are suitable for nebulizing solutions containing high salts contents. The sample solution flows along a V-shaped groove, then a gas jet emerges from a capillary hole in the middle of this groove

and disrupts the solution flow, which enables nebulization without blocking. However, due to the high matrix amounts introduced, the effects on sample nebulization, aerosol transport, quartz torch, and plasma properties should be carefully assessed [6].

On the other hand, to overcome these limitations posed by ICP-MS, some instruments are equipped with an aerosol dilution system, also named as High Matrix Introduction (HMI) or Aerosol Dilution Technique (ADT). This modern technology uses auto-optimization of aerosol dilution by a flow of argon gas between the spray chamber and the torch to further improve matrix tolerance, reducing both aerosol density and water vapor loading in the plasma. Thus, the aerosol entering the plasma contains less solvent, avoiding a pronounced decrease of plasma energy and, consequently, leading to lower oxides formation. Moreover, this eliminates possible contaminations associated with manual dilution, saves time and reduces waste compared to liquid dilution [8,115]. Figure 1.4 presents a scheme for ICP-MS with aerosol dilution system.

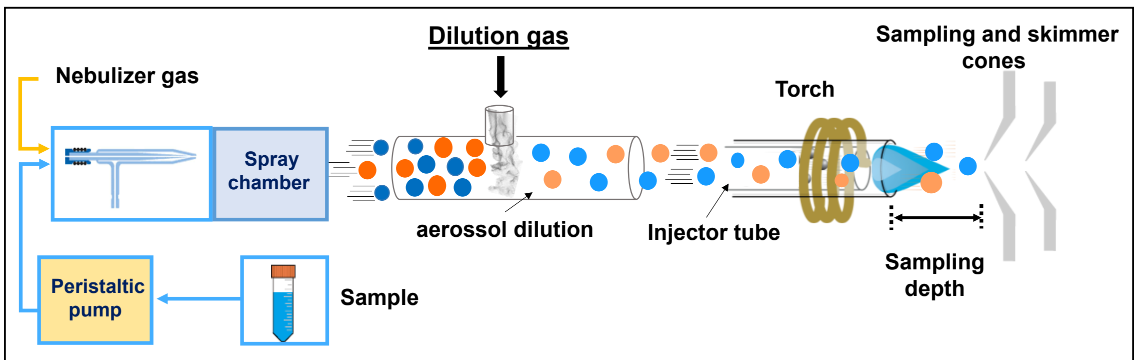



FIGURE 1.4 - System representation of ICP-MS with aerosol dilution. Adapted from BARROS, et al. (2018) [115].

Due to the low TDS and RA required for ICP-MS measurements, the dilution factor of sample solution (ratio between final volume to the sample mass) is usually high. For pharmaceutical samples the dilution factor can range between 160 and 1000-fold [36,59,61,66]. Considering these matrices, ICP-MS with aerosol dilution system has been reported to be used for elemental analysis of digests of medicines [40,41] and dietary supplements [42] and for liquid drugs [51]. The digests were diluted 100 and 200-fold, implying total dissolved solids of 1% [40,41] and 0.5% m $v^{-1}$ [42], respectively. Liquid drugs were diluted 10 to 50-fold [51]. Using

an ICP-MS without this device would require a final sample dilution of 5 to 100-fold higher. Considering both ICP-based methods, lower dilution factors i.e. ranging from 12 to 100-fold dilution, were used for analysis of pharmaceutical samples by ICP OES [32,34,40,57,64,65] and ICP-MS [31,34,35,37,40,41,65,68,77]. It is important to highlight that the lower dilution factor implies in higher sensitivity.

In addition to drawbacks associated with traditional dilution strategies, argon-based plasma spectroanalytical methods are susceptible to spectral and non-spectral interferences. Non-spectral interferences also known as matrix effects can be associated with transport and nebulization processes and/or energetic effects in the argon plasma. The difference in viscosities between the sample and aqueous standard solutions may cause transport interferences. Additionally, solutions with high concentrations of easily ionized elements, mainly Na and K, produce free electrons and affect plasma conditions. Similarly, elevated carbon concentrations may cause changes in the plasma characteristics and, consequently, in distribution of species in the argon plasma due to increase of analytical signals caused by charge transfer reactions between $C^+$ and some elements in the plasma, for example As, Au, Cd, Hg, Ir, Se, Sb, Pb, and Pt [101,116]. On the other hand, spectral interferences are associated with analytical signal overlap.

### 1.5.1 - Matrix effects and calibration methods

Generally, analytical calibration curves are essential for quantitative determinations using spectroanalytical methods. The traditional external standard calibration method (EC) is widely applied for determinations involving simple matrices. Physical and chemical differences among samples and reference solutions, however, can cause severe matrix effects and, consequently produce inaccurate results [117,118]. A variety of calibration methods have been used to correct matrix effects for elemental determination in drugs and pharmaceutical products using ICP OES and ICP-MS, such as standard additions (SA) [32], standard dilution analysis (SDA) [52], multi-isotope calibration (MICal) [42], one-point standard addition (OP SA) [32,42] and internal standardization (IS) [31,32,37,39,40,42,53,57,65–68].

For EC, the calibration curve is built by plotting the analyte concentration on the x-axis with the signal intensity (SI) on the y-axis; the analyte concentration in the sample ($C_{analyte}$) is obtained using the relationship $C_{analyte}$ = (SI - b)/a, where (b) is the intercept of the regression line and (a) is the slope of straight line. In a traditional SA calibration, the analyte is added to the sample in increasing concentrations, thus, the analytical curve construction is made in the sample medium, which corrects matrix effects. This procedure implies perfect matrix matching, but it is time-consuming [32,117,118]. The IS calibration curve is built using the same EC approach; however, the IS calibration curve is built by plotting the analyte concentration on the x-axis with the ratio analyte signal / internal standard signal on the y-axis. The internal standard, an element not present in any sample, is added in a constant concentration to all solutions of blanks, standards and samples. Thus eventual degradation of analytical performance due to an instrument drift and variation in sample introduction efficiency is compensated [118].

On the other hand, for the processes involved in using MICal and OP SA methods, only two standards are used per sample. As both solutions contain the sample, no matrix effect is expected [118]. The MICal method uses the instrument response from a single analyte concentration recorded at multiple points on the spectrum for calibration. The calibration plot is built with signals recorded for solution 1 and solution 2 on the x-axis and y-axis, respectively, thus, each point in the calibration plot corresponds to a different isotope. Consider the following functional relationships for solution 1 (S1) and solution 2 (S2) (equations 1 and 2, respectively), where $S(x_1)_{Sam+Std}$ and $S(x_1)_{Sam}$ are the instrument responses for a given ($x_1$) analytical signal source (i.e., wavelength, isotope or molecular ion); m is a proportionality constant; and $C(A)_{Sam}$ and $C(A)_{Std}$ are the analyte concentrations in the sample and in the standard solution added to S1 [118,119].

$$S(x_1)_{Sam+Std} = m\,[C(A)_{Sam} + C(A)_{Std}] \qquad \text{Equation (1)}$$

$$S(x_1)_{Sam} = m\,C(A)_{Sam} \qquad \text{Equation (2)}$$

By plotting $S(x_1)_{Sam+Std}$ (from S1) on the x-axis, and $S(x_1)_{Sam}$ (from S2) on the y-axis, with multiple signal sources of the same analyte

corresponding to different points on the calibration graph, the slope of the linear regression model will can be calculated and the analyte concentration in the sample may be easily determined which leads to equation (3) [118,119].

$$C(A)Sam = \frac{\text{Slope x C(A)Std}}{(1 - \text{Slope})} \qquad \text{Equation (3)}$$

For SA, at least four calibration points are needed, (i.e. $x_0$ and $x_1$-$x_4$), where ($x_0$) is a point without any analyte added and ($x_1$-$x_4$) are solutions with increasing concentrations of analyte. So, the $C_{analyte}$ is obtained by extrapolation of the x axis at y = 0, (i.e. $C_{analyte}$ = b/a). The OP SA strategy follows the same principle of SA, however, only two standard solutions are used to obtain the analytical curve [32,42,118]. So the $C_{analyte}$ is also obtained by extrapolation of the x axis at y = 0, but using only two calibrations points, (i.e. $x_0$ and $x_1$). For OP SA and SA, the accuracy is evaluated based on the standard error (SE), according to equation (4) [120,121]:

$$SE = \sqrt{\frac{\sum_i^n (y_i - \hat{y})^2}{n-1}} \qquad \text{Equation (4)}$$

where $y_i$ is the analyte reference concentration, $\hat{y}$ is the concentration determined by calibration strategy, and $n$ is the number of samples analyzed.

In addition, for OP SA, the linearity is tested applying the test F by the calculation of the ratio $F_{experimental}/F_{tabulated}$. When the calculated ratio is ≥10, it demonstrate that the variances are statistically different (the quadratic mean of the regression is statistically different when compared with the quadratic mean of the residues) and the model can be considered linear [120,121]. Figure 1.5 represents illustrative images for calibration plots for IS, MICal, SA, and OP SA.

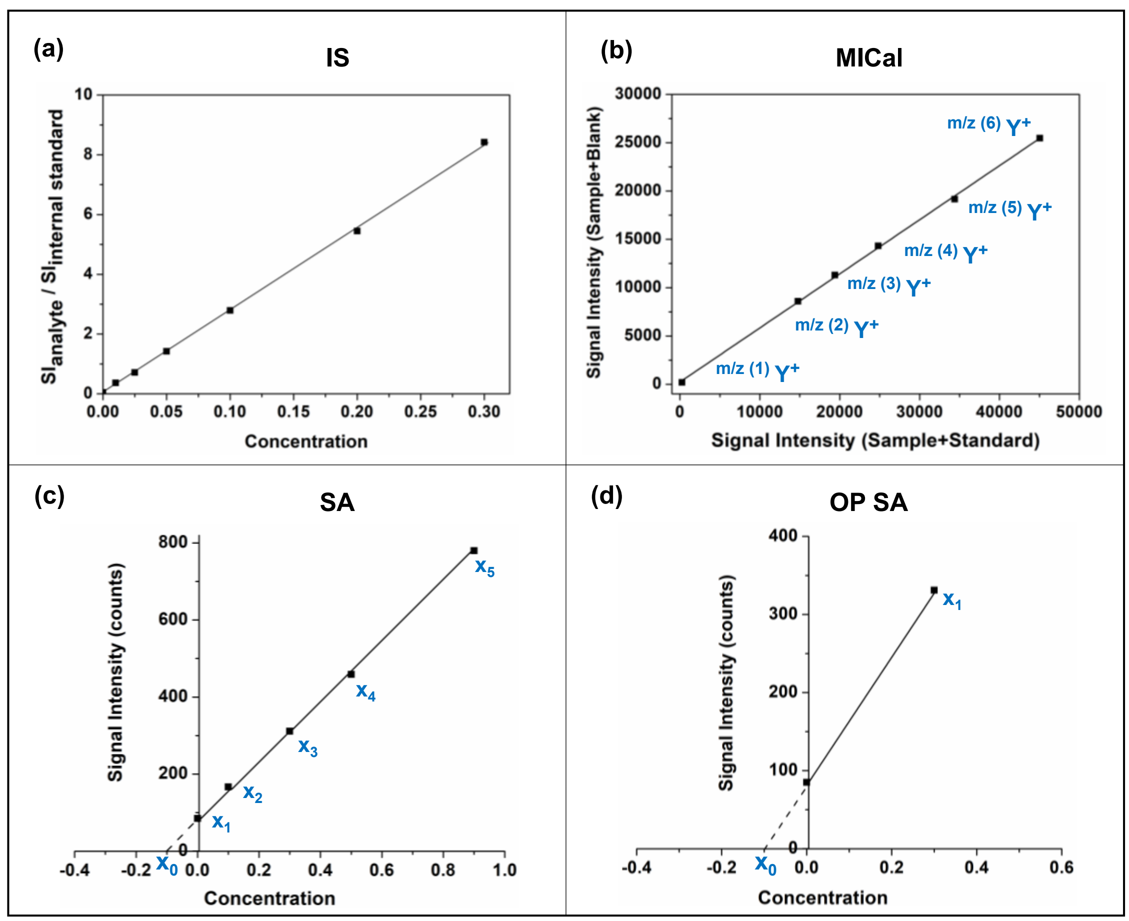



FIGURE 1.5 - Linear model for **(a)** IS, **(b)** MICal, **(c)** SA and **(d)** OP SA curves. **(a)** $C_{analyte}$ on the x-axis and the ratio standard signal/internal standard signal on the y-axis. **(b)** MICal calibration plot using six isotopes. **(c)** SA calibration plot using four additional points ($x_1$-$x_4$). **(d)** OP SA calibration plot using one additional point ($x_1$). **(c, d)** $x_0$ represents the sample without any standard addition with extrapolation represented by broken line.

Internal standardization is another well-established calibration method frequently applied for elemental determination in pharmaceuticals. Yttrium has been used as internal standard for determination of several elements in medicines [40], liquid drugs [32] and samples of cosmetics and pharmaceuticals [58] when using ICP OES. Measurements performed by ICP-MS generally require the use of more than one internal standard for all determined isotopes. Some elements have been reported as internal standard for elemental determination in drugs and pharmaceuticals, e.g. Y [37,42,65,67,68], In [31,37,53,67–69], Sc [37,65,67,68], Bi, Ho, Lu, Re, Rh, Tb, and Te [37,65,67,68]. Even elements which

currently are included in the more recent version of Chapter 232 [21], e.g. Co [66], Li, and Tl [37] have been used as internal standards.

### 1.5.2 - Spectral interferences in ICP-MS and collision cell technology

Considering its high sensitivity, ICP-MS offers many benefits to laboratories performing determination of trace elements; a major disadvantage of its use, however, is the occurrence of mass interferences, also called as spectral interferences, caused by atomic or polyatomic species having approximately the same mass/charge ratio of analytes considering typical resolution of quadrupole mass spectrometers [7,8].

Due to the large number of analytes required by USP Chapter 232 [21] isotopic interference effects on ICP-MS measurements must be carefully evaluated [1,8]. Most APIs are organic substances and may contain elements such as chlorine, nitrogen, oxygen and sulfur in their composition. Therefore, monitoring of isotopes $^{51}V^+$, $^{52}Cr^+$, $^{58}Ni^+$, $^{63}Cu^+$, $^{65}Cu^+$, and $^{75}As^+$, can be directly affected by polyatomic ions formed with Cl, C, and Ar, e.g. $^{38}Ar^{13}C^+$ and $^{35}Cl^{16}O^+$ interfering on $^{51}V^+$; $^{35}Cl^{16}OH^+$ and $^{40}Ar^{12}C^+$ on $^{52}Cr^+$; $^{38}Ar^{40}Ar^+$ and $^{38}Ar^{40}Ca^+$ on $^{78}Se^+$; and $^{23}Na^{35}Cl^+$, $^{12}C^{16}O^{35}Cl^+$, $^{12}C^{18}O^{35}Cl$ and $^{40}Ar^{35}Cl^+$ interfering on $^{58}Ni^+$, $^{63}Cu^+$, $^{65}Cu^+$ and $^{75}As^+$, respectively. In addition, polyatomic species formed between the analytes and plasma constituents can directly affect the determination of other target-analytes, e.g. $^{59}Co^{16}O^+$ on $^{75}As^+$; $^{60}Ni^{16}O^+$ on $^{76}Se^+$; $^{40}Ar^{63}Cu^+$ on $^{103}Rh^+$; $^{95}Mo^{16}O^+$ on $^{111}Cd^+$; $^{98}Mo^{16}O^+$ on $^{114}Cd^+$; $^{190}Pt^{16}O^+$ on $^{206}Pb$; $^{191}Ir^{16}O^+$ on $^{207}Pb$ and $^{192}Os^{16}O^+$ on $^{208}Pb$ [7,8].

In order to correct polyatomic interferences in ICP-MS measurements, collision cell technology (CCT) features a cell placed before the mass spectrometer. The cell can be filled with an inert gas, usually He, that collides preferentially with polyatomic species, thus with larger diameter than the analyte. So, the interfering species/ions with low energies are rejected by kinetic energy discrimination (KED), and the analyte ions, which have a higher kinetic energy, are transmitted to the mass analyzer and detected free of analytical signal overlap. It provides effective correction of polyatomic interferences, eliminating the need for reactive cell gases in routine analysis [7,8].

Figure 1.6 presents a scheme for ICP-MS with collision cell and discrimination by kinetic energy.

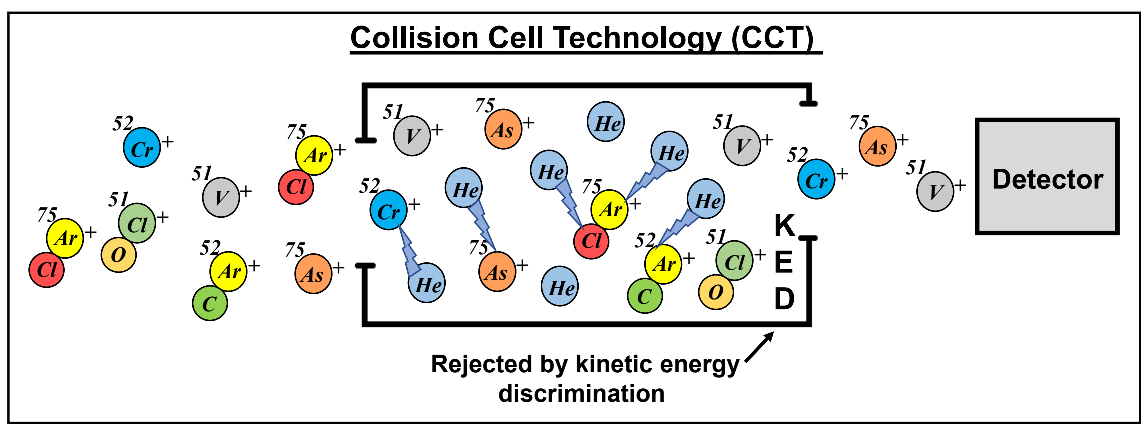



FIGURE 1.6 - System representation of CCT and KED to suppress the polyatomic interferences $^{35}Cl^{16}O^+$, $^{40}Ar^{12}C^+$ and $^{40}Ar^{35}Cl^+$ in the determination of $^{51}V^+$, $^{52}Cr^+$ and $^{75}As^+$, respectively.

For analysis using CCT and KED, measurements are sequentially performed in both acquisition modes, i.e. *standard mode* (without collision with He gas) and *collision mode*. Generally, the sensitivity is lower when using the KED-based collision cell, however, this limitation is not critical based on the extreme sensitivity of ICP-MS. Despite its advantages, the collision gas mode cannot be used for doubly charged interfering species. In addition to polyatomic and doubly charged interferences, it is also important to verify isotopic interferences on major abundance isotopes [7,8]. For determination of elemental impurities, some cases should be careful evaluated, e.g. isotopic interference of $^{96}Ru^+$ on $^{96}Mo^+$, $^{102}Ru^+$ on $^{102}Pd^+$, $^{106}Cd^+$ on $^{106}Pd^+$, $^{108}Cd^+$ on $^{108}Pd^+$, $^{112}Sn^+$ on $^{112}Cd^+$,$^{114}Sn^+$ on $^{114}Cd^+$, and $^{130}Ba^{2+}$ on $^{65}Cu^+$. In these cases, when the analyte is not monoisotopic, more than one isotope can be evaluated.

# 2 - CHAPTER 2

# GOALS

## 2.1 - Goals

This dissertation aimed to develop analytical methods to determine elemental impurities in drugs and pharmaceutical products using argon-based plasma spectroanalytical methods. The main goals were the development of sample preparation procedures for pharmaceuticals based on microwave-assisted digestion, acid dilution and dispersive liquid-liquid microextraction using dilute acid solutions and/or a minimum volume of reagents.

## 2.2 - Specific Goals

- To develop simplified sample preparation procedures using dilute nitric acid solution for different types of drugs and dietary supplements;

- To evaluate the performance of ICP OES and ICP-MS as well as instrumental strategies and calibration methods to correct matrix and spectral interferences;

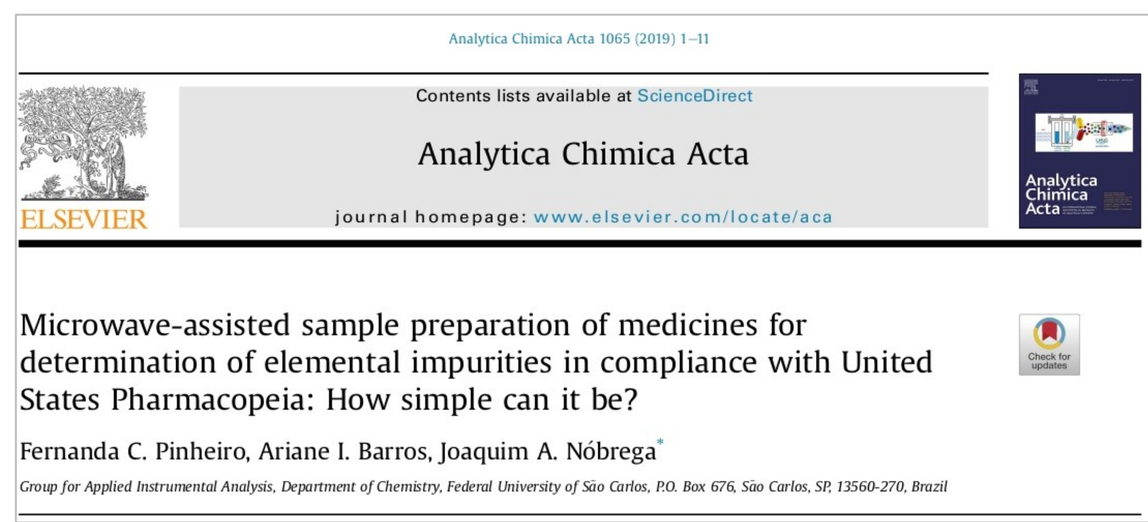
- To simultaneously determine 24 elemental impurities in drugs and As, Cd, Hg, and Pb in dietary supplements according to ICH requirements and USP Chapters 232, 233, and 2232;

- To develop sample preparation procedures based on DLLME for extraction/preconcentration of elemental impurities in order to improve the limit of quantification for ICP OES analysis.

# 3 - CHAPTER 3

# PHARMACEUTICAL SAMPLES IN SOLID DOSAGE FORM

## 3.1 - Microwave-assisted sample preparation of medicines for determination of elemental impurities in compliance with United States Pharmacopeia: How simple can it be?


Analytica Chimica Acta 1065 (2019) 1–11

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Microwave-assisted sample preparation of medicines for determination of elemental impurities in compliance with United States Pharmacopeia: How simple can it be?

Fernanda C. Pinheiro, Ariane I. Barros, Joaquim A. Nóbrega*

Group for Applied Instrumental Analysis, Department of Chemistry, Federal University of São Carlos, P.O. Box 676, São Carlos, SP, 13560-270, Brazil

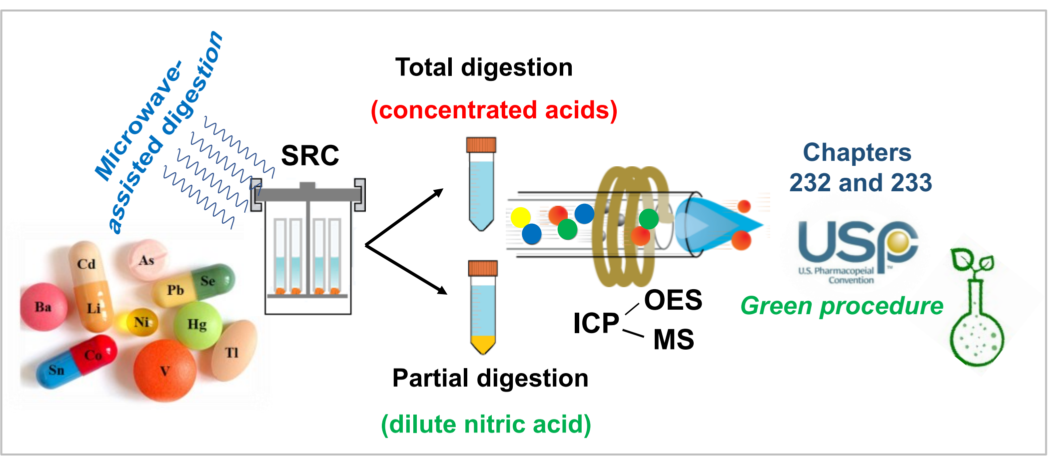
### 3.1.1 - Abstract

This work proposed a procedure for microwave-assisted sample preparation of medicines using diluted nitric acid followed by determination of elemental impurities using inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS) according to the United States Pharmacopeia Chapters 232 and 233. Three solutions, i.e. inverse aqua regia, 7.0, and 2.0 mol $L^{-1}$ $HNO_3$, were evaluated for microwave-assisted digestion of nine drugs samples. The applicability of each digestion procedure was assessed by comparison of analyte concentrations determined using total (reference procedure) and partial digestions (proposed procedure) as well as by determining dissolved carbon content and evaluating matrix effects. There were none significant differences at a 95% confidence level among the concentrations determined applying reference and proposed procedures. Internal standardization (ICP OES) and aerosol dilution (ICP-MS) were applied for minimization and correction of matrix effects. Addition and recovery experiments were performed according to oral

*Group for Applied Instrumental Analysis, Department of Chemistry, Federal University of São Carlos, P.O. Box 676, São Carlos, SP, 13560-270, Brazil.*
**Corresponding author: djan@ufscar.br*
*DOI: https://doi.org/10.1016/j.aca.2019.03.016*

permissible daily exposures values specific for each element and each sample was spiked with element concentrations of 0.5*J* and 1.5*J* in order to check accuracies for 24 analytes. Recoveries ranged from 70 to 138% for ICP OES and from 72 to 128% for ICP-MS, for all elements but Os. All analytes were below the respective limits of quantification when applying all sample preparation procedures, except As, Ba, Co, Cu, Cr, Mo, Ni, Pb, Sb, Sn, Tl and V, however the determined concentrations for these elements were lower than the limits proposed by Chapter 232.

### 3.1.2 - Graphical abstract




### 3.1.3 - Introduction

Some inorganic impurities are toxic even when present at trace concentration levels. In pharmaceuticals, contamination by elemental impurities may occur by use of raw materials, reagents and excipients (As, Cd, Hg and Pb), catalysts (Ir, Os, Pd, Pt, Rh, Ru and W) used in active pharmaceutical ingredients (APIs) synthesis, and by drugs interaction with equipment, containers and surfaces during drug production [1,3,34,35,61] which can generate unwanted and unknown pharmacological–toxicological effects [122].

In 2010, the United States Pharmacopoeia (USP) published two new chapters on elemental impurities: Chapter 232 [21] and 233 [23] replacing Chapter 231. In 2015, USP announced plans to establish the new chapters only in 2018 aiming at a harmonization with the final version of the Elemental Impurities Guidelines of the International Council for Harmonization of Technical

Requirements for Pharmaceuticals for Human Use (ICH Q3D) [15,16]. The new version of Chapter 232 was published in the Pharmacopeial Forum in May 2016 [18] and became official in December 2017. Chapter 232 [21] specifies elemental impurities and their toxicity limits for 24 elements considering the oral permissible daily exposures values (PDEs) of different drug categories (oral, parenteral and inhalation), whereas Chapter 233 [23] describes sample preparation strategies, standardization solutions and accuracy for elemental determinations by ICP OES or ICP-MS.

The concentration limits (known as *J* values) defined by USP is calculated by dividing the PDE for each element value by the maximum daily dose (MDD) of the drug and multiplied by the dilution factor (DF) adopted in the analytical procedure, as shown in equation (1):

$$J = \frac{PDE(\frac{\mu g}{day})}{\mathrm{MDD}\left(\frac{\mathrm{g}}{\mathrm{day}}\right) \mathrm{x\ DF}} \qquad \text{Equation (1)}$$

The accuracy is evaluated by addition and recovery experiments with acceptable recoveries ranging from 70 to 150% with concentrations from 0.5*J* to 1.5*J* values. The acceptable analytical procedures for sample preparation include direct dissolution in aqueous solution, direct dissolution in organic solution, total metal extraction or closed vessel digestion (for materials not directly soluble in aqueous or organic solvents) or even minimum preparation (for liquids or alternative procedures that allow the examination of solvable samples). About the use of closed vessel digestion, Chapter 233 mentions that the use of any concentrated acid may be appropriate, but each one introduces inherent safety risks [23].

Sample preparation is a critical step considered key to the success of analysis [3]. Sample preparation for pharmaceutical products embraces procedures as simple as dissolution either in diluted acid [31] or in organic solvents [53,54], microwave-induced combustion [33,61] and microwave-assisted digestion in closed vessels [34–37,39]. Generally, the main limitation for application of simple dissolution methods is the low solubility of APIs [61]. Müller et al. [35] evaluated three solutions (concentrated $HNO_3$, aqua regia and inverse aqua regia) for digestion of six APIs aiming the determination of 14 elements using

single reaction chamber digestion and ICP-MS. In the optimized conditions, concentrated $HNO_3$ was applied for As, Cd, Cr, Cu, Hg, Mo, Ni, Pb, and V determination and inverse aqua regia was adopted for Ir, Os, Pd, Pt, Rh and Ru determination. Microwave-assisted digestion in inverse aqua regia solution was also used to tablets [34], drug samples [36] and excipients [37].

Sample digestion performed with concentrated acids should be handled with care because of their high vapor pressure and/or corrosive properties. Nevertheless, these problems are minimized when dilute acids are used in microwave-assisted digestion procedures. Microwave-assisted digestion in closed vessels has clear advantages compared to traditional acid digestion using conventional heating in terms of better recoveries of volatile elements, lower contamination, lower volume of reagents, better reproducibility and a better working environment. In this context, the use of dilute acids could be considered as an alternative to improve occupational health and safety, preventing fast increase of pressure inside closed reaction vessels [123].

Argon-based plasma spectroanalytical methods are robust and reliable instrumental strategies. Considering the 24 elements required by Chapter 232 [21], ICP OES and ICP-MS provide multielemental analysis, high sample throughput, high sensitivity, accuracy, robustness and low detection limits. However, sample dilution is often required in analyses by ICP OES and ICP-MS to keep the total dissolved solids (TDS) contents below 1.0 and 0.2% m $v^{-1}$, respectively [6–8]. To overcome these limitations in measurements using ICP OES, the introduction of samples with about 5% m $v^{-1}$ of TDS is possible when employing sample introduction systems able to deal with high solid contents [6], but effects on quartz torch and analyte signals must be considered. For ICP-MS, some instruments are equipped with an aerosol dilution system, which introduces a flow of argon gas between the spray chamber and the torch to promote aerosol dilution, reducing both aerosol density and water vapor loading in the plasma [8]. Also named as High Matrix Introduction (HMI), this system enables the direct analysis of samples containing high TDS, avoiding a pronounced decrease of plasma energy and oxides formation, because less solvent and matrix enter the plasma [8,115].

On the other hand, the direct introduction of complex samples with high TDS can induce severe matrix effects and spectral interferences. Matrix

matching, standard additions method and internal standardization can be good strategies to matrix effect correction [118]. In internal standardization, the intensity ratio between the spectral lines of the element and of the internal standard is used. It is expected that internal standard acts as a control throughout the sample processing from nebulization, transport and plasma processes. Thus, the ratio between analyte and internal standard intensities correct for possible fluctuations occurring during the analysis and matrix effects can be compensated [6,115].

In this context, due to the demand for straightforward sample preparation methods for pharmaceuticals elemental analysis, this study aimed to develop microwave-assisted digestion procedures using dilute nitric acid solution. The hypothesis here tested is that partial digestions are efficient for quantitative recoveries of all target elements; i.e. in other words eventual presence of residual solids does not imply that analytes are trapped and consequently the analysis of the supernatant solution is generally enough for full recoveries of analytes. Additionally, it was evaluated the performance of HMI using ICP-MS and internal standardization using ICP OES for determination of 24 elemental impurities in nine different types of commonly used drugs according to the new USP requirements. Effect of residual carbon concentration and correction strategies to matrix and spectral interferences were studied in order to improve accuracy and precision of the analytical procedure.

### 3.1.4 - Experimental

*3.1.4.1. Instrumentation*

Experiments were performed using an iCAP6000 ICP OES (Thermo Fisher Scientific, Waltham, MA, USA) operated in axial viewing modes and an Agilent 7800 Quadrupole ICP-MS (Agilent Technologies, Tokyo, JHS, Japan) operated in *No gas*, *He* and *HMI* acquisition mode. *No gas* mode means not using the collision cell, and *He* mode means when the collision cell is pressurized with pure He (99.999%, White Martins-Praxair, Sertãozinho, SP, Brazil). *HMI* mode implies that aerosol was diluted with argon at the optimized HMI gas flow rate of 0.62 L $min^{-1}$ and carrier gas flow rate of 0.40 L $min^{-1}$, thus

1.02 L $min^{-1}$ of total flow rate [115]. Introduction of samples containing high solids contents were performed using V-Groove and MiraMist nebulizers in ICP OES and ICP-MS, respectively. Argon (99.999%, White Martins-Praxair) was used in all measurements in both equipments. Nitrogen (99.9%, White Martins-Praxair) was used for pressurization of the UltraWave microwave oven. Plasma operating conditions used in ICP OES and ICP-MS are shown in Table 3.1.

TABLE 3.1 - Operating parameters used in iCAP6000 ICP OES and Agilent 7800 Quadrupole ICP-MS.

| Instrument parameter | ICP OES | ICP-MS |
|---|---|---|
| RF applied power (kW) | 1.20 | 1.55 |
| Plasma gas flow rate (L $min^{-1}$) | 12 | 15 |
| Auxiliary gas flow rate (L $min^{-1}$) | 0.50 | 1.0 |
| Carrier gas flow rate (L $min^{-1}$) | 0.70 | 1.02 |
| Carrier gas flow rate in *HMI mode* (L $min^{-1}$) | NA | 0.40 |
| HMI gas flow rate (L $min^{-1}$) | NA | 0.62 |
| Sampling depth (mm) | NA | 8.0 |
| He flow rate in collision cell (mL $min^{-1}$) | NA | 4.5 |
| Integration time (s) | 15 | 3.0 |
| Nebulizer | V-Groove | Mira-Mist |
| Spray chamber | Cyclonic | Double pass |
| Number of replicates | 3 | 3 |
| Analytes | Emission line (nm) | Isotope (m/z) |
| Ag | 328.068; 338.289 | 109 |
| As | 189.042 | 75 |
| Au | 242.795; 267.595 | 197 |
| Ba | 455.403; 493.409 | 137; 138 |
| Cd | 226.502; 228.802 | 111;112;114 |
| Co | 228.616; 238.892 | 59 |
| Cr | 283.563; 267.716 | 52;53 |
| Cu | 324.754; 327.396 | 63;65 |

TABLE 3.1 - Operating parameters used in iCAP6000 ICP OES and Agilent 7800 Quadrupole ICP-MS (continuation).

| Analytes | Emission line (nm) | Isotope (m/z) |
|---|---|---|
| Hg | 184.950 | 200;202 |
| Ir | 212.681; 224.268 | 191;193 |
| Li | 670.784; 610.362 | 7 |
| Mo | 203.845; 202.030 | 95;96;98 |
| Ni | 221.647; 231.604 | 58;60 |
| Os | 228.226 | 190;192 |
| Pb | 220.353 | 206;208 |
| Pd | 324.270; 340.458 | 106;108 |
| Pt | 203.646; 214.423 | 194;195 |
| Rh | 343.489; 369.236 | 103 |
| Ru | 240.272; 267.876 | 102;104 |
| Sb | 206.833; 217.581 | 121;123 |
| Se | 196.090 | 76;78;82 |
| Sn | 189.989; 283.999 | 118;120 |
| Tl | 190.856; 276.787 | 203;205 |
| V | 292.402; 309.310 | 51 |
| Internal Standard | Y 371.030 | NA |

NA: not applicable.

*3.1.4.2. Samples and microwave-assisted sample preparation*

Nine drug samples (A-I) in tablets form (oral administration route) were analyzed: A) Sodium dipyrone, isometheptene mucate and anhydrous caffeine used as analgesic; B) levothyroxine sodium, used for thyroid treatment; C) sodium dipyrone, used as analgesic; D) orfenadrine citrate, monoidratated dipirone and caffeine anidra, used as muscle relaxant and analgesic; E) metformin hydrochloride, used for diabetes treatment; F) diclofenac sodium, paracetamol, carisoprodol and caffeine, used for rheumatism treatment; G) losartan potassium, used for hypertension treatment; H) gestodene and ethinylestradiol, used as contraceptive; I) omeprazole, used for benign (gastric

or duodenal) peptic ulcers treatment and one sample of dietary supplement (DS). All analyzed samples were purchased in local pharmacies in São Carlos, SP, Brazil.

All samples were ground and homogenized using pestle and mortar. Masses of approximately 500 mg were accurately weighed directly in the perfluoroalkoxy alkanes (PFA) digestion vessels and microwave-assisted digested in triplicate using a single reaction chamber oven (UltraWave™, Milestone, Sorisole, Italy). Volumes of 7 mL of different acid solutions were applied: 1) 7.0 mol $L^{-1}$ $HNO_3$; 2) 2.0 mol $L^{-1}$ $HNO_3$ and 3) inverse aqua regia $3HNO_3$:1HCl v $v^{-1}$ as reference procedure [34–37,39]. Volumes of 150 mL of water and 5 mL of concentrated nitric acid were inserted into the single reaction chamber (SRC) and the chamber was pressurized with nitrogen gas to 40 bar. The microwave heating program was applied as follows: (1) 2.5 min to reach 140 °C, (2) 2.5 min hold at 140 °C, (3) 2.5 min to reach 180 °C, (4) 2.5 min hold at 180 °C, (5) 10 min to reach 220 ºC and (6) 10 min hold at 220 °C. For samples not completely digested using inverse aqua regia, masses of approximately 100 mg were digested using the same condition at the maximum temperature of 240 °C.

Subsequently, digests were diluted to 50.0 mL with distilled-deionized water and an aliquot of each solution was appropriately diluted with deionized water, followed by quantification using ICP OES and ICP-MS, except for all samples digested with 2.0 mol $L^{-1}$ $HNO_3$ (final dilution of 100-fold and a solids content of 1.0% m $v^{-1}$). These digests were centrifuged for 3 min at 6000 rpm for sedimentation of residual solids.

*3.1.4.3. Reagents and standard solutions*

Experiments were performed using $HNO_3$ (Synth, Diadema, SP, Brazil) purified in a sub-boiling distillation apparatus Distillacid$^{TM}$ BSB-939-IR (Berghof, Eningen, Germany), HCl (Qhemis, São Paulo, SP, Brazil) purified in a sub-boiling distillation system (Milestone, Sorisole, Italy) for inverted aqua regia preparation, and ultrapure water, resistivity higher than 18.2 MΩ cm, (Milli-Q®, Millipore, Bedford, MA, USA). Standard solutions used for ICP OES and ICP-MS calibrations and for addition and recovery experiments were prepared by

dilution of 1000 mg $L^{-1}$ of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V (Qhemis, São Paulo, SP, Brazil) in 0.14 mol $L^{-1}$ $HNO_3$ medium, as well as the internal standard evaluated: Bi, Ga, Ge, and Y.

For ICP-MS measurements, the concentrations of the analytical solutions used for calibration were 0, 0.05, 0.1, 0.5, 1.0, 5.0, 10, 25 and 50 µg $L^{-1}$ in 0.49 mol $L^{-1}$ $HNO_3$ medium, 0.14 mol $L^{-1}$ $HNO_3$ medium and inverse aqua regia diluted 300-fold (acid concentration compatible with the dilution performed for each digestion procedure) for all elements. Addition and recovery experiments were performed at two addition levels (1.0 and 5.0 µg $L^{-1}$). Clean up of the sample introduction system with 0.060 mol $L^{-1}$ HCl after calibration was required for avoiding memory effects for Hg [124]. For ICP OES measurements, the concentrations of the analytical solutions used for calibration were 0, 0.010, 0.025, 0.050, 0.10, 0.20, 0.30 and 0.50 mg $L^{-1}$ in 0.14 mol $L^{-1}$ $HNO_3$ medium for all elements. To correct for matrix effects ISs were added at 0.10 mg $L^{-1}$ to analytical calibration solutions, analytical blanks and samples.

The dissolved organic carbon concentration was determined in all digest solutions. Carbon was determined by ICP OES using the atomic emission line 193.090 nm and dehydrated oxalic acid (Mallinckrodt Chemicals, St. Louis, MO, USA) was used as the carbon source for preparing calibrating analytical solutions. Carbon effects were investigated by determination of all analytes in solutions prepared in 0.14 mol $L^{-1}$ $HNO_3$ and containing increasing concentrations of carbon: 0.25, 0.50 and 1.0% m $v^{-1}$. Matrix effects were evaluated using addition and recovery tests (0.10 and 0.30 mg $L^{-1}$) for samples digested using 2.0 mol $L^{-1}$ $HNO_3$. Spikes were added before microwave-assisted digestion.

#### *3.1.4.4. Evaluation of accuracies obtained by ICP OES and ICP-MS according to USP requirements*

Addition and recovery experiments taking into account *J* values were performed in 2.0 mol $L^{-1}$ $HNO_3$ by using ICP OES and ICP-MS measurements [23]. According to the USP 233 analytical procedures must

demonstrate accurate spike recoveries between 70 and 150% of the spiked value for the mean of 3 samples spiked at concentrations ranging from 50 to 150% of the *J* value for each target element. *J* values were calculated according to PDE value specific for each element, divided by MDD and multiplied by DF required by the elemental determination method. For example, for Cd and Pb which PDE specific to oral administration is 5.0 µg $day^{-1}$, considering MDD of 10 g $day^{-1}$, the *J* value will be 0.50 µg $g^{-1}$. Thereby, considering DF 100-fold dilution adopted for sample preparation, the concentration added for addition and recovery experiments was 5.0 µg $L^{-1}$.

Samples were spiked before microwave-assisted digestion with concentrations of 0.5*J* and 1.5*J* in order to check the accuracy of the developed analytical procedure. Added concentrations for each analyte can be observed in Table 3.2. The concentrations of the solutions used for obtaining analytical calibration curves were 0.1*J*, 0.25*J*, 0.5*J*, 1.0*J*, 1.5*J* and 2.0*J* in 0.14 mol $L^{-1}$ $HNO_3$ medium for all elements.

TABLE 3.2 - Class, oral permissible daily exposures and the *J* values obtained considering MDD of 10 g $day^{-1}$ [20,21].

| Element | Class[a] | PDE | *J* Valor | Accuracy[b] | |
|---|---|---|---|---|---|
| | | μg $day^{-1}$ | μg $g^{-1}$ | 0.5*J* (μg $g^{-1}$) | 1.5*J* (μg $g^{-1}$) |
| Ag | 2B | $1.5x10^2$ | 15 | 7.5 | 22 |
| As | 1 | 15 | 1.5 | 0.75 | 2.2 |
| Au | 2B | $1.0 x10^2$ | 10 | 5.0 | 15 |
| Ba | 3 | $1.4 x10^3$ | $1.4x10^2$ | 70 | $2.1x10^2$ |
| Cd | 1 | 5 | 0.50 | 0.25 | 0.75 |
| Co | 2A | 50 | 5.0 | 2.5 | 7.5 |
| Cr | 3 | $1.1 x10^4$ | $1.1x10^3$ | $5.5x10^2$ | $1.6x10^3$ |
| Cu | 3 | $3.0 x10^3$ | $3.0x10^2$ | $1.5x10^2$ | $4.5x10^2$ |
| Hg | 1 | 30 | 3.0 | 1.5 | 4.5 |
| Ir | 2B | $1.0 x10^2$ | 10 | 5.0 | 15 |
| Li | 3 | $5.5 x10^2$ | 55 | 27 | 82 |
| Mo | 3 | $3.0 x10^3$ | $3.0x10^2$ | $1.5x10^2$ | $4.5x10^2$ |
| Ni | 2A | $2.0 x10^2$ | 20 | 10 | 30 |
| Os | 2B | $1.0 x10^2$ | 10 | 5.0 | 15 |
| Pb | 1 | 5.0 | 0.50 | 0.25 | 0.75 |
| Pd | 2B | $1.0x10^2$ | 10 | 5.0 | 15 |
| Pt | 2B | $1.0x10^2$ | 10 | 5.0 | 15 |
| Rh | 2B | $1.0x10^2$ | 10 | 5.0 | 15 |
| Ru | 2B | $1.0x10^2$ | 10 | 5.0 | 15 |
| Sb | 3 | $1.2x10^3$ | $1.2x10^2$ | 60 | $1.8x10^2$ |
| Se | 2B | $1.5x10^2$ | 15 | 7.5 | 22 |
| Sn | 3 | $6.0x10^3$ | $6.0x10^2$ | $3.0x10^2$ | $9.0x10^2$ |
| Tl | 2B | 8.0 | 0.80 | 0.40 | 1.2 |
| V | 2A | $1.0x10^2$ | 10 | 5.0 | 15 |

[a] Class according ICH [9]; [b] Concentration added in addition and recovery experiments.

Taking into account that the concentration of elements in the drugs sample usually are low [36], accuracies were also evaluated in lower levels 0.10 and 0.30 mg $L^{-1}$ for ICP OES and 1.0 and 5.0 μg $L^{-1}$ for ICP-MS as well as analytical performance parameters.

### 3.1.5 - Results and discussion

*3.1.5.1. Evaluation of the sample digestion procedures - Dissolved organic carbon contents and matrix effects*

When compared to other digestion solutions, the analytical procedure using only nitric acid is attractive, even when remaining solid residues are present, because the addition of HCl may lead to the formation of interfering species in ICP-MS and the use of $H_2O_2$ may imply in the addition of contaminants due to the relatively poor purity of analytical grade reagent [3,8,123]. Thus, the feasibility of microwave-assisted digestions using only dilute nitric acid solution was evaluated by comparison with an adopted reference procedure, i.e. microwave-assisted digestion with inverse aqua regia.

When using the reference procedure, complete digestion was obtained for samples B, C (Figure 3.1) and E at 220 ºC. However, for samples F, I and DS (dietary supplement sample) complete digestion was only obtained applying 240 °C as maximum temperature and by decreasing sample masses to 100 mg. For samples B, C and E, a complete digestion was also obtained for digestions using 7.0 mol $L^{-1}$ nitric acid at 220 °C. However, residual solids were observed for samples A, D, G and H (Figure 3.2) for all sample preparation procedures tested. On the other hand, for most samples digested using 2.0 mol $L^{-1}$ $HNO_3$ the digested presented a yellow dark color and solid residues remained as suspended particles. Complete digestion with dilute nitric acid solution was only obtained for samples B and E (Figure 3.3).

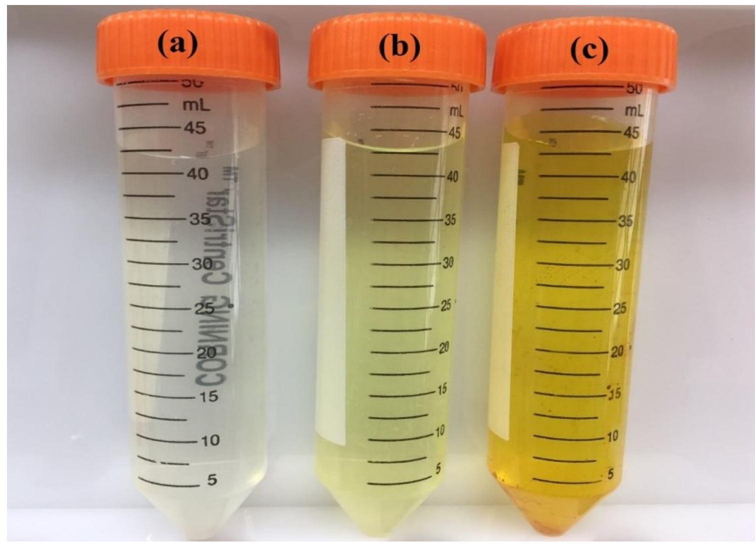



FIGURE 3.1 - Sample C. **(a)** $3HNO_3$:$1HCl$ v $v^{-1}$ (220 °C); **(b)** $HNO_3$ 7.0 mol $L^{-1}$ (220 °C); **(c)** $HNO_3$ 2.0 mol $L^{-1}$ (220 °C). Total digestion only when using procedure **(a)**.

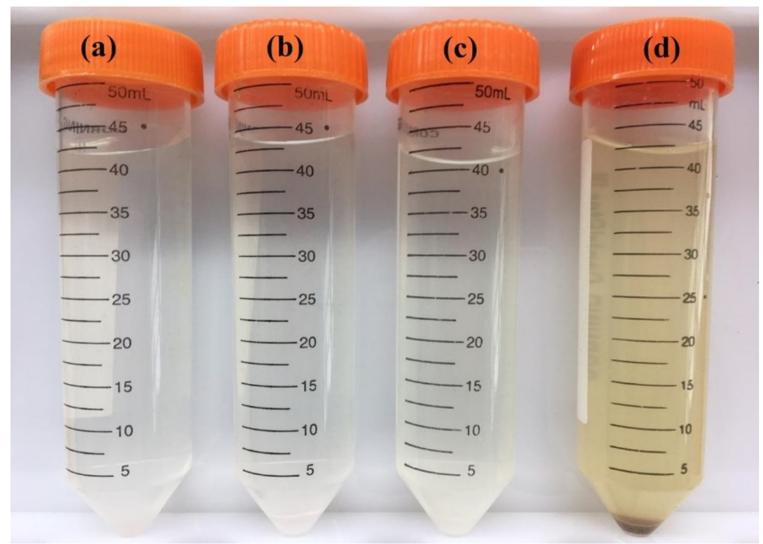



FIGURE 3.2 - Sample H. **(a)** $3HNO_3$:1HCl v $v^{-1}$ (240 °C); **(b)** $3HNO_3$:1HCl v $v^{-1}$ (220 °C); **(c)** $HNO_3$ 7.0 mol $L^{-1}$ (220 °C); **(d)** $HNO_3$ 2.0 mol $L^{-1}$ (220 °C). Residual solids in all tested sample preparation procedures.

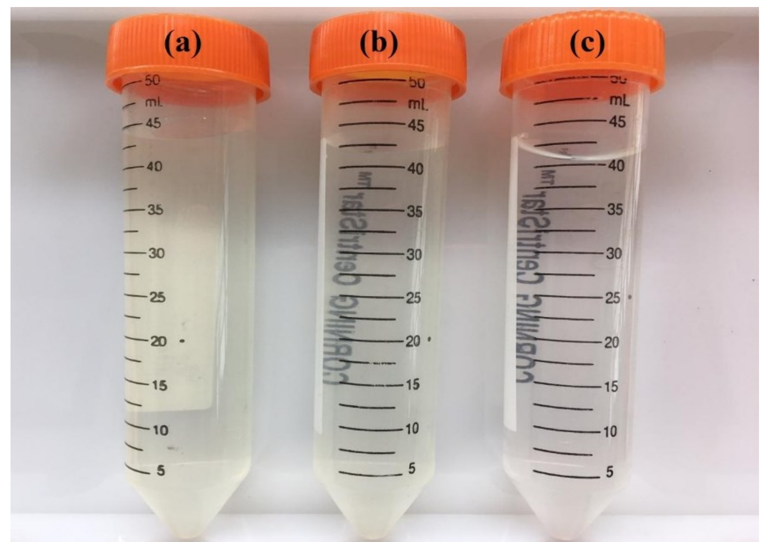



FIGURE 3.3 - Sample E. **(a)** $3HNO_3$:1HCl v $v^{-1}$ (220 °C); **(b)** $HNO_3$ 7.0 mol $L^{-1}$ (220 °C); **(c)** $HNO_3$ 2.0 mol $L^{-1}$ (220 °C). Total digestion in all tested sample preparation procedures.

The main problems related to partial digestion are the possibility of analytes trapping in the remaining solids and also effects caused by the dissolved organic carbon in ICP OES and ICP-MS measurements. Thus, ICP OES was applied for determining dissolved organic carbon concentrations in digests in order to evaluate the efficiency of the sample decomposition using different acid solutions and heating temperatures (Figure 3.4).

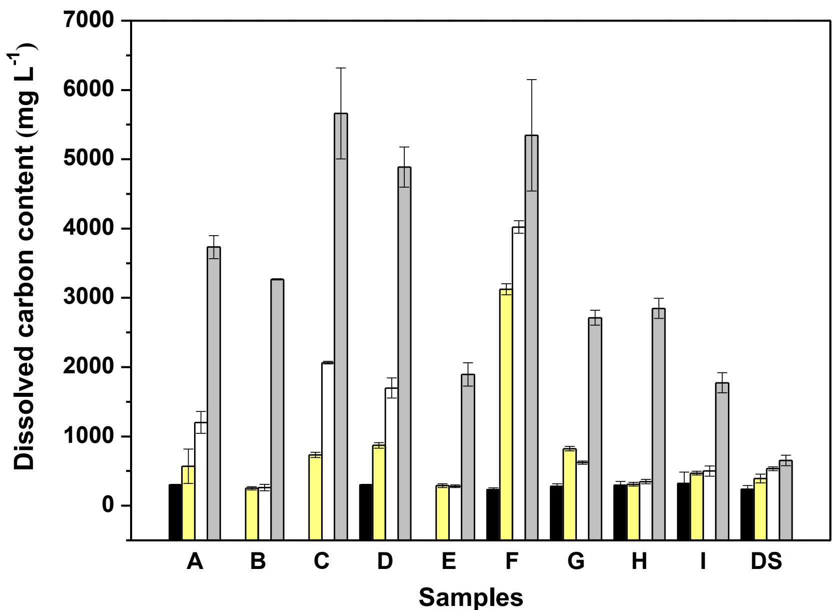



FIGURE 3.4 - Dissolved carbon content in samples digested (A-DS) for each sample preparation by ICP OES (mg $L^{-1}$, mean ± standard deviations, n = 3). (■) 3$HNO_3$:1HCl v $v^{-1}$ (240 °C); (■) 3$HNO_3$:1HCl v $v^{-1}$ (220 °C); (□) $HNO_3$ 7.0 mol $L^{-1}$ (220 °C); (■) $HNO_3$ 2.0 mol $L^{-1}$ (180 °C).

As expected, dissolved organic carbon concentrations were higher for samples digested using only dilute nitric acid solution. Solutions with high carbon concentrations may cause changes in the plasma characteristics and consequently in species distribution in the argon plasma. It also causes an increase in the analytical signal for elements with high-energy ionization by transfer of charge between $C^+$ and the respective element [2,35,101,116]. Thus, we evaluated the effects caused by dissolved carbon on determination of analytes. Addition and recovery experiments in two levels were made using standard solutions with different concentrations of carbon (oxalic acid) and using digested sample with 2.0 mol $L^{-1}$ $HNO_3$.

For all carbon levels tested, recoveries ranged from 81 to 114%, except to Se and Sn (Figure 3.5). On the other hand, for samples digested using 2.0 mol $L^{-1}$ $HNO_3$, recoveries without IS ranged from 115 to 190%, except for Tl (104%) and Sn (71%). To correct for carbon effects different internal standards were evaluated and best recoveries obtained ranged from 85 to 124% using Y as internal standard, as shown in Figure 3.5.

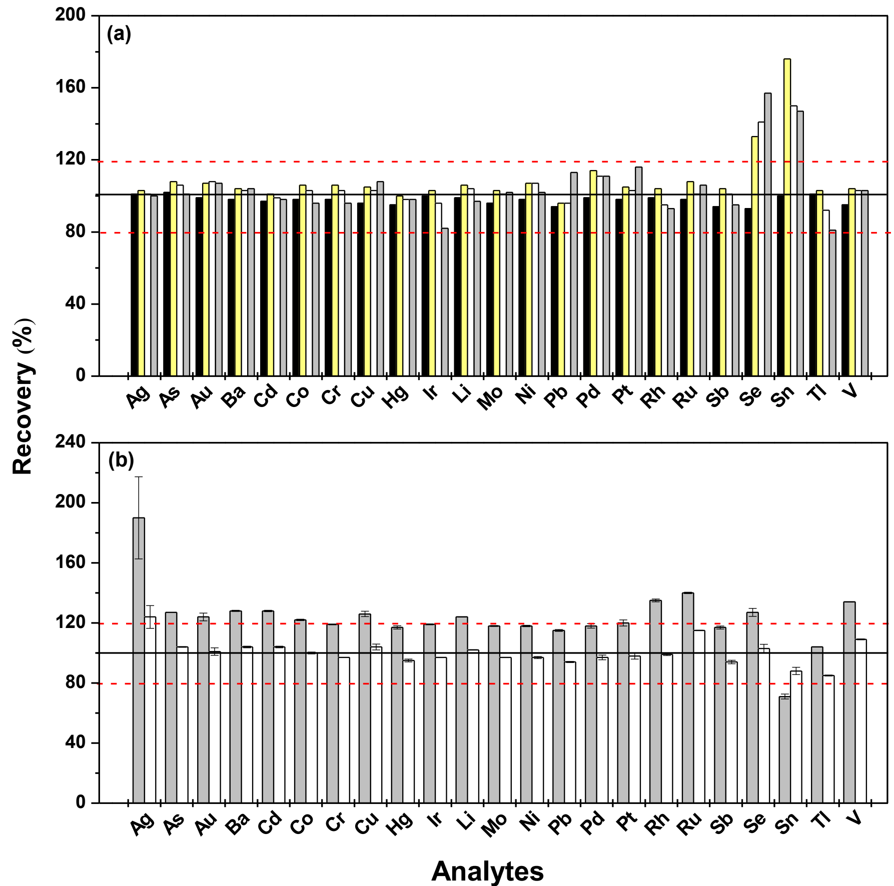



FIGURE 3.5 - **(a)** Percentage recoveries for addition 0.10 mg $L^{-1}$ in standard solutions by ICP OES. (■) 0.14 mol $L^{-1}$ $HNO_3$; (■) 0.25% m $v^{-1}$ carbon; (□) 0.50% m $v^{-1}$ carbon; (■) 1.0% m $v^{-1}$ carbon. **(b)** Percentage recoveries for addition 0.10 mg $L^{-1}$ in sample digested using 2.0 mol $L^{-1}$ $HNO_3$ with and without IS by ICP OES. (■) Without IS; (□) With IS.

Among the internal standard evaluated, Y presented better results for most analytes. Yttrium was also used as internal standard for As, Cd, V, Cr, Ni, Cu, Mo, Ru, Rh and Pd for analysis of two excipients by ICP-MS and Tl was used for Os, Ir, Pt, Pb and Hg [37]. Commonly used internal standard, such as Pt, Rh and Pd, were not evaluated because they were analytes of interest as well as Tl, once Tl was incorporated in Chapter 232 after harmonization with ICH [20]. Without using internal standard, recoveries with positive errors (>120%) were observed for most analytes. The concentrations of dissolved organic carbon in the digestion samples were lower than 0.70% m $v^{-1}$ (Figure 3.4), thereby, matrix effects observed were not due to the carbon, since satisfactory recoveries were obtained in the standards solutions with carbon concentration until 1.0% m $v^{-1}$

(Figure 3.5). Probably, internal standardization led to more accurate recoveries due to correction of matrix effects associated with transport, nebulization, and/or energetic effects in the argon plasma [6,7].

*3.1.5.2. Analytical performance of ICP-MS*

The two tested digestion procedures using 2.0 and 7.0 mol $L^{-1}$ $HNO_3$ were evaluated by determining the studied analytes by ICP-MS. Determined concentrations were compared with those concentrations determined using the adopted reference procedure with inverse aqua regia [34–37,39]. Before the analysis of digests, analytical performance of ICP-MS was evaluated for each sample preparation procedure. As previously mentioned in the Experimental section, for each procedure the analytical solutions were matrix matched with digests considering residual acid concentrations.

Limits of detection (LOD) and quantification (LOQ) were calculated considering background equivalent concentration (BEC), signal-to-background ratio (SBR) and relative standard deviations (RSD) for 10 measurements of blank solutions [125]. The isotopes, mode of acquisition, linear correlation coefficient, slopes of analytical curves and LOQs obtained for all analytes are shown for each sample preparation procedure in Table 3.3.

TABLE 3.3 - Parameters analytical performance for Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V in drugs samples digested in three sample preparation procedure by ICP-MS using *HMI* mode.

| Isotope | Acquisition mode | Sample preparation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $3HNO_3$:1HCl v $v^{-1}$ (240 °C)[a] | | | (220 °C)[b] | $HNO_3$ 7.0 mol $L^{-1}$ (220 °C)[b] | | | $HNO_3$ 2.0 mol $L^{-1}$ (220 °C)[b] | | |
| | | Slope | $R^2$ | LOQ µg $g^{-1}$ | LOQ µg $g^{-1}$ | Slope | $R^2$ | LOQ µg $g^{-1}$ | Slope | $R^2$ | LOQ µg $g^{-1}$ |
| $^{7}$Li | *No gas-HMI* | 1.4x10$^{3}$ | 0.9998 | 0.42 | 0.083 | 1.9 | 0.9998 | 0.023 | 1.8 x10$^{4}$ | 0.9998 | 0.0030 |
| $^{51}$V | *He-HMI* | 1.0 x10$^{3}$ | 0.9998 | 0.28 | 0.057 | 6.4 x10$^{2}$ | 0.9998 | 0.0070 | 1.0 x10$^{3}$ | 0.9998 | 0.0030 |
| $^{52}$Cr | *He-HMI* | 1.5 x10$^{3}$ | 0.9998 | 0.23 | 0.047 | 9.5 x10$^{2}$ | 0.9998 | 0.032 | 1.5 x10$^{3}$ | 0.9998 | 0.010 |
| $^{58}$Ni | *No gas-HMI* | NA | NA | NA | NA | 1.6 x10$^{4}$ | 1.000 | 0.020 | 1.5 x10$^{4}$ | 0.9998 | 0.013 |
| $^{58}$Ni | *He-HMI* | 2.0 x10$^{3}$ | 0.9998 | 0.32 | 0.063 | NA | NA | NA | NA | NA | NA |
| $^{59}$Co | *No gas-HMI* | 2.2 x10$^{4}$ | 0.9998 | 0.033 | 0.0070 | 2.9 x10$^{4}$ | 0.9998 | 0.013 | 2.8 x10$^{4}$ | 0.9998 | 0.0010 |
| $^{63}$Cu | *He-HMI* | 2.6 x10$^{3}$ | 0.9998 | 0.72 | 0.14 | 1.6 x10$^{4}$ | 1.000 | 0.013 | 1.5 x10$^{4}$ | 0.9998 | 0.0030 |
| $^{75}$As | *He-HMI* | 1.3 x10$^{2}$ | 0.9998 | 0.62 | 0.12 | 6.8 x10$^{2}$ | 0.9990 | 0.068 | 1.2 x10$^{2}$ | 0.9998 | 0.020 |
| $^{78}$Se | *He-HMI* | 5.6 | 0.9960 | 6.2 | 1.2 | 3.6 | 0.9990 | 0.71 | 6.1 | 0.9976 | 0.60 |
| $^{98}$Mo | *No gas-HMI* | 5.9 x10$^{3}$ | 1.000 | 0.47 | 0.093 | 6.1 x10$^{3}$ | 0.9998 | 0.0030 | 5.5 x10$^{3}$ | 1.000 | 0.0030 |
| $^{102}$Ru | *No gas-HMI* | 1.4 x10$^{4}$ | 0.9998 | 0.033 | 0.0070 | 1.6 x10$^{4}$ | 0.9998 | 0.0010 | 1.6 x10$^{4}$ | 0.9998 | 0.0010 |
| $^{103}$Rh | *He-HMI* | 4.1 x10$^{4}$ | 0.9998 | 0.033 | 0.0070 | 4.7 x10$^{4}$ | 0.9996 | 0.0010 | 4.6 x10$^{4}$ | 0.9998 | 0.0010 |
| $^{107}$Ag | *No gas-HM* | 1.6 x10$^{4}$ | 0.9996 | 0.033 | 0.0080 | 1.6 x10$^{4}$ | 0.9994 | 0.0070 | 1.7 x10$^{4}$ | 0.9974 | 0.0030 |
| $^{108}$Pd | *No gas-HMI* | 1.1 x10$^{4}$ | 0.9996 | 0.65 | 0.13 | 1.0 x10$^{4}$ | 0.9990 | 0.0010 | 1.2 x10$^{4}$ | 0.9996 | 0.0030 |
| $^{112}$Cd | *No gas-HMI* | 3.6 x10$^{3}$ | 0.9998 | 0.017 | 0.0030 | 4.0 x10$^{3}$ | 0.9998 | 0.0010 | 4.0 x10$^{3}$ | 0.9998 | 0.0010 |
| $^{120}$Sn | *No gas-HMI* | 1.5 x10$^{4}$ | 0.9998 | 0.083 | 0.020 | 1.7 x10$^{4}$ | 0.9998 | 0.0070 | 1.7 x10$^{4}$ | 0.9998 | 0.0030 |
| $^{123}$Sb | *No gas-HMI* | 1.2 x10$^{4}$ | 0.9998 | 0.033 | 0.0070 | 1.3 x10$^{4}$ | 0.9998 | 0.0030 | 1.3 x10$^{4}$ | 1.000 | 0.0010 |
| $^{138}$Ba | *No gas-HMI* | 3.5 x10$^{4}$ | 0.9994 | 0.22 | 0.039 | 3.7 x10$^{4}$ | 0.9994 | 0.027 | 3.6 x10$^{4}$ | 0.9998 | 0.0030 |

[a] Sample masses digested of 100 mg; [b] Sample masses digested of 500 mg; NA: not applicable.

TABLE 3.3 - Parameters analytical performance for Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V in drugs samples digested in three sample preparation procedure by ICP-MS using *HMI* mode (continuation).

| Isotope | Acquisition mode | Sample preparation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $3HNO_3$:1HCl v $v^{-1}$ | | | | $HNO_3$ 7.0 mol $L^{-1}$ | | | $HNO_3$ 2.0 mol $L^{-1}$ | | |
| | | (240 °C)[a] | | | (220 °C)[b] | (220 °C)[b] | | | (220 °C)[b] | | |
| | | Slope | $R^2$ | LOQ µg $g^{-1}$ | LOQ µg $g^{-1}$ | Slope | $R^2$ | LOQ µg $g^{-1}$ | Slope | $R^2$ | LOQ µg $g^{-1}$ |
| $^{190}Os$ | *No gas-HMI* | 3.4 $x10^7$ | 0.9998 | 0.50 | 0.10 | 7.0 $x10^7$ | 0.9996 | 0.14 | 7.1 $x10^7$ | 0.9994 | 0.10 |
| $^{193}Ir$ | *No gas-HMI* | 3.0 $x10^4$ | 0.9998 | 0.13 | 0.027 | 2.8 $x10^7$ | 0.9998 | 0.0020 | 2.9 $x10^4$ | 0.9998 | 0.0010 |
| $^{195}Pt$ | *No gas-HMI* | 1.1 $x10^4$ | 0.9998 | 0.58 | 0.12 | 1.0 $x10^4$ | 0.9998 | 0.10 | 1.1 $x10^4$ | 0.9998 | 0.013 |
| $^{197}Au$ | *No gas-HMI* | 1.8 $x10^4$ | 0.9998 | 0.17 | 0.033 | 1.5 $x10^4$ | 0.9791 | 0.0030 | 1.8 $x10^4$ | 0.9990 | 0.0030 |
| $^{202}Hg$ | *No gas-HMI* | 5.4 $x10^3$ | 0.9996 | 0.23 | 0.047 | 5.0 $x10^3$ | 0.9998 | 0.010 | 5.0 $x10^3$ | 0.9998 | 0.013 |
| $^{205}Tl$ | *No gas-HMI* | 3.5 $x10^4$ | 0.9998 | 0.0080 | 0.0020 | 3.2 $x10^4$ | 0.9998 | 0.013 | 3.3 $x10^4$ | 0.9998 | 0.0010 |
| $^{208}Pb$ | *No gas-HMI* | 2.5 $x10^4$ | 0.9998 | 0.083 | 0.017 | 2.3 $x10^4$ | 1.000 | 0.010 | 3.4 $x10^4$ | 0.9998 | 0.010 |

[a] Sample masses digested of 100 mg; [b] Sample masses digested of 500 mg; NA: not applicable.

These parameters were selected using addition and recovery experiments in two lower addition levels. For ICP-MS, internal standardization was not needed to improve recoveries as required for ICP OES. Probably this effect can be explained by matrix effects minimization provided by the HMI system, since aerosol dilution with argon led to lower matrix concentrations entering in the plasma [8,115].

Polyatomic species formed with chloride and carbon were expected because HCl was present in the analytical procedure using inverse aqua regia and also in dilute nitric acid medium because of higher dissolved organic carbon contents, as well as possible constituent elements from components of the drugs, APIs and excipients [2,34,61,122]. Müller et al. [35] verified that Cl concentrations above 100 mg $L^{-1}$ caused positive errors in As, Cr and V determinations in APIs, and carbon concentrations higher than 250 mg $L^{-1}$ interfered in the determination of $^{52}Cr^{+}$ [35,126].

Sample preparation using inverse aqua regia presented higher LOQs than the procedures using dilute nitric acid, except to $^{59}Co^{+}$, $^{190}Os^{+}$ and $^{205}Tl^{+}$ using 7.0 mol $L^{-1}$ $HNO_3$ procedure. The Octopole Reaction System (ORS) was used with *He* in collision mode for correcting for spectral interferences. For all sample preparation procedures, quantitative recoveries were obtained for $^{51}V^{+}$, $^{52}Cr^{+}$, $^{63}Cu^{+}$, $^{75}As^{+}$, $^{78}Se^{+}$ and $^{103}Rh^{+}$ only when using *He* mode. For $^{58}Ni^{+}$ determination in inverse aqua regia medium, *He* mode was chosen due to lower LOQ than *No gas* mode.

For these isotopes, recoveries higher than 120% were obtained in standard mode, probably due to spectral interferences caused by polyatomic species. The isotopes $^{51}V^{+}$, $^{52}Cr^{+}$, $^{58}Ni^{+}$, $^{63}Cu^{+}$ and $^{75}As^{+}$, are directly affected by polyatomic ions formed with chloride: $^{35}Cl^{16}O^{+}$, $^{35}Cl^{16}OH^{+}$, $^{23}Na^{35}Cl^{+}$, $^{12}C^{16}O^{35}Cl^{+}$ and $^{40}Ar^{35}Cl^{+}$, respectively, with carbon: $^{38}Ar^{13}C^{+}$ and $^{40}Ar^{12}C^{+}$ interfering in $^{51}V^{+}$ and $^{52}Cr^{+}$, respectively, and with argon: $^{38}Ar^{40}Ar^{+}$ and $^{38}Ar^{40}Ca^{+}$ in $^{78}Se^{+}$ and $^{40}Ar^{63}Cu^{+}$ in $^{103}Rh^{+}$.

Due to the large number of analytes required by USP Chapter 232 [21] it is also important to verify isotopic interferences effects on the greater abundance isotopes, for example: isotopic interference of $^{96}Ru^{+}$ on $^{96}Mo^{+}$; $^{102}Ru^{+}$ on $^{102}Pd^{+}$; $^{106}Cd^{+}$ on $^{106}Pd^{+}$; $^{108}Cd^{+}$ on $^{108}Pd^{+}$; $^{112}Sn^{+}$ on $^{112}Cd^{+}$; $^{114}Sn^{+}$ on $^{114}Cd^{+}$, $^{130}Ba^{2+}$ on $^{65}Cu^{+}$, as well as the possible formation of polyatomic species

between the analytes and plasma constituents: $^{59}Co^{16}O^+$ on $^{75}As^+$; $^{60}Ni^{16}O^+$ on $^{76}Se^+$; $^{95}Mo^{16}O^+$ on $^{111}Cd^+$; $^{98}Mo^{16}O^+$ on $^{114}Cd^+$; $^{190}Pt^{16}O^+$ on $^{206}Pb$ and $^{191}Ir^{16}O^+$ on $^{207}Pb$. However, quantitative recoveries were obtained for most analytes, except for $^{51}V^+$, $^{52}Cr^+$, $^{63}Cu^+$, $^{75}As^+$, $^{78}Se^+$ and $^{103}Rh^+$ without adopting collision *mode* operation, inferring that there were no spectral interferences for these isotopes. Two or three isotopes were measured for each analyte for evaluating eventual isotopic interferences. The isotopes studied did no show significant differences in recoveries, consequently the most abundant isotope was selected for further measurements.

HMI system allows the introduction of samples with TDS around 3% m $v^{-1}$ and residual acidity around 5% v $v^{-1}$ [8,115] providing conditions for minimum dilution of digests. Table 3.4 shows analytes determined using each sample digestion procedure.

TABLE 3.4 - Determination of As, Ba, Cd, Co, Cu, Cr, Li, Mo, Ni, Pb, Sb, Se, Sn, Tl, and V (μg $g^{-1}$, mean ± standard deviation, n = 3) in drug samples (A–I) and one sample of dietary supplement (DS) digested in each sample digestion procedures by ICP-MS.

| Isotope | Sample | Sample preparation | | | |
|---|---|---|---|---|---|
| | | $3HNO_3$:1HCl v $v^{-1}$ (240 °C) [a] | $3HNO_3$:1HCl v $v^{-1}$ (220 °C) [b] | $HNO_3$ 7.0 mol $L^{-1}$ (220 °C) [b] | $HNO_3$ 2.0 mol $L^{-1}$ (220 °C) [b] |
| $^{7}Li$ | DS | <0.42 | <0.083 | 0.045 ± 0.009 | 0.045 ± 0.007 |
| $^{51}V$ | A | 0.97 ± 0.04 | 0.73 ± 0.04 | 0.88 ± 0.05 | 0.95 ± 0.03 |
| | B | NA | <0.057 | 0.025 ± 0.003 | 0.021 ± 0.003 |
| | C | NA | <0.057 | 0.059 ± 0.005 | 0.056 ± 0.002 |
| | E | NA | <0.057 | 0.049 ± 0.004 | 0.042 ± 0.001 |
| | F | <0.28 | <0.057 | 0.039 ± 0.007 | 0.040 ± 0.003 |
| | G | <0.28 | 0.07 ± 0.01 | 0.070 ± 0.009 | 0.07 ± 0.01 |
| | H | <0.28 | <0.057 | 0.053 ± 0.004 | 0.056 ± 0.001 |
| | I | <0.28 | 0.201 ± 0.007 | 0.27 ± 0.01 | 0.236 ± 0.006 |
| | DS | 10 ± 1 | 11.0 ± 0.8 | 11.0 ± 0.7 | 11 ± 1 |
| $^{52}Cr$ | A | 0.66 ± 0.03 | 0.50 ± 0.01 | 0.531 ± 0.007 | 0.58 ± 0.01 |
| | B | NA | 0.06 ± 0.03 | 0.04 ± 0.01 | 0.060 ± 0.003 |
| | C | NA | 0.11 ± 0.02 | 0.08 ± 0.01 | 0.09 ± 0.01 |
| | D | <0.23 | 0.12 ± 0.02 | 0.14 ± 0.01 | 0.136 ± 0.007 |
| | E | NA | <0.047 | 0.04 ± 0.02 | 0.03 ± 0.01 |
| | F | <0.23 | 0.122 ± 0.007 | 0.19 ± 0.04 | 0.135 ± 0.006 |
| | G | <0.23 | 0.118 ± 0.008 | 0.10 ± 0.02 | 0.12 ± 0.02 |
| | H | <0.23 | 0.14 ± 0.01 | 0.20 ± 0.01 | 0.12 ± 0.02 |
| | I | 0.3 ± 0.1 | 0.35 ± 0.01 | 0.38 ± 0.03 | 0.304 ± 0.004 |
| | DS | 53 ± 2 | 53 ± 2 | 46 ± 8 | 53 ± 1 |

[a] Sample masses digested of 100 mg; [b] Sample masses digested of 500 mg; NA: not applicable for samples completely digested using $3HNO_3$:1HCl v $v^{-1}$ at 220 °C.

TABLE 3.4 - Determination of As, Ba, Cd, Co, Cu, Cr, Li, Mo, Ni, Pb, Sb, Se, Sn, Tl, and V ($\mu g\ g^{-1}$, mean ± standard deviation, n = 3) in drug samples (A–I) and one sample of dietary supplement (DS) digested in each sample digestion procedures by ICP-MS (continuation).

| Isotope | Sample | Sample preparation: $3HNO_3$:1HCl v $v^{-1}$ (240 °C) [a] | $3HNO_3$:1HCl v $v^{-1}$ (220 °C) [b] | $HNO_3$ 7.0 mol $L^{-1}$ (220 °C) [b] | $HNO_3$ 2.0 mol $L^{-1}$ (220 °C) [b] |
|---|---|---|---|---|---|
| $^{58}Ni$ | A | 0.35 ± 0.02 | 0.44 ± 0.08 | 0.451 ± 0.002 | 0.36 ± 0.02 |
| | B | NA | <0.063 | 0.04 ± 0.01 | 0.037 ± 0.007 |
| | C | NA | 0.12 ± 0.02 | 0.114 ± 0.003 | 0.118 ± 0.007 |
| | D | <0.30 | 0.08 ± 0.05 | 0.160 ± 0.006 | 0.085 ± 0.009 |
| | E | NA | <0.063 | 0.054 ± 0.005 | 0.059 ± 0.009 |
| | F | <0.30 | 0.222 ± 0.009 | 0.16 ± 0.08 | 0.18 ± 0.01 |
| | G | <0.30 | 0.104 ± 0.008 | 0.082 ± 0.006 | 0.096 ± 0.007 |
| | H | <0.30 | 0.15 ± 0.05 | 0.21 ± 0.02 | 0.20 ± 0.03 |
| | I | 0.5 ± 0.2 | 0.31 ± 0.02 | 0.38 ± 0.03 | 0.35 ± 0.05 |
| | DS | 8.0 ± 0.6 | 8.5 ± 0.8 | 8.0 ± 0.2 | 8.7 ± 0.6 |
| $^{59}Co$ | A | 0.29 ± 0.02 | 0.224 ± 0.008 | 0.252 ± 0.003 | 0.237 ± 0.006 |
| | C | NA | 0.011 ± 0.001 | <0.013 | 0.011 ± 0.001 |
| | D | <0.033 | 0.03 ± 0.01 | 0.035 ± 0.003 | 0.041 ± 0.001 |
| | I | <0.033 | 0.026 ± 0.001 | 0.021 ± 0.001 | 0.020 ± 0.001 |
| | DS | 4.5 ± 0.3 | 4.6 ± 0.2 | 4.2 ± 0.6 | 4.6 ± 0.2 |

[a] Sample masses digested of 100 mg; [b] Sample masses digested of 500 mg; NA: not applicable for samples completely digested using $3HNO_3$:1HCl v $v^{-1}$ at 220 °C.

TABLE 3.4 - Determination of As, Ba, Cd, Co, Cu, Cr, Li, Mo, Ni, Pb, Sb, Se, Sn, Tl, and V (μg $g^{-1}$, mean ± standard deviation, n = 3) in drug samples (A–I) and one sample of dietary supplement (DS) digested in each sample digestion procedures by ICP-MS (continuation).

| Isotope | Sample | Sample preparation | | | |
|---|---|---|---|---|---|
| | | $3HNO_3$:1HCl v $v^{-1}$ (240 °C) [a] | $3HNO_3$:1HCl v $v^{-1}$ (220 °C) [b] | $HNO_3$ 7.0 mol $L^{-1}$ (220 °C) [b] | $HNO_3$ 2.0 mol $L^{-1}$ (220 °C) [b] |
| $^{63}Cu$ | A | <0.72 | <0.14 | 0.14 ± 0.06 | 0.19 ± 0.01 |
| | B | NA | <0.14 | 0.16 ± 0.04 | 0.14 ± 0.01 |
| | C | NA | <0.14 | 0.09 ± 0.05 | 0.08 ± 0.04 |
| | D | <0.72 | <0.14 | 0.110 ± 0.007 | 0.12 ± 0.02 |
| | F | <0.72 | <0.14 | 0.04 ± 0.07 | 0.05 ± 0.04 |
| | G | <0.72 | <0.14 | 0.03 ± 0.01 | 0.04 ± 0.01 |
| | H | <0.72 | <0.14 | 0.138 ± 0.001 | 0.19 ± 0.03 |
| | I | <0.72 | <0.14 | 0.12 ± 0.01 | 0.14 ± 0.02 |
| | DS | 353 ± 5 | 349 ± 6 | 343 ± 7 | 354 ± 8 |
| $^{78}Se$ | DS | 20 ± 3 | 22 ± 1 | 18 ± 2 | 19 ± 1 |
| $^{75}As$ | I | <0.62 | <0.12 | 0.08 ± 0.01 | 0.079 ± 0.006 |
| | DS | <0.62 | <0.12 | 0.10 ± 0.04 | 0.11 ± 0.02 |
| $^{98}Mo$ | A | <0.46 | <0.093 | 0.016 ± 0.001 | 0.013 ± 0.001 |
| | C | NA | 0.10 ± 0.06 | 0.129 ± 0.002 | 0.154 ± 0.003 |
| | D | <0.46 | <0.093 | 0.029 ± 0.001 | 0.033 ± 0.001 |
| | E | NA | 0.14 ± 0.01 | 0.12 ± 0.03 | 0.2 ± 0.1 |
| | F | <0.46 | 0.174 ± 0.007 | 0.16 ± 0.03 | 0.181 ± 0.003 |
| | G | <0.46 | <0.093 | 0.03 ± 0.01 | 0.047 ± 0.001 |
| | H | <0.46 | <0.093 | 0.020 ± 0.001 | 0.024 ± 0.001 |
| | I | <0.46 | <0.093 | 0.062 ± 0.002 | 0.060 ± 0.003 |
| | DS | 80 ± 2 | 80 ± 2 | 78 ± 6 | 80 ± 2 |

[a] Sample masses digested of 100 mg; [b] Sample masses digested of 500 mg; NA: not applicable for samples completely digested using $3HNO_3$:1HCl v $v^{-1}$ at 220 °C.

TABLE 3.4 - Determination of As, Ba, Cd, Co, Cu, Cr, Li, Mo, Ni, Pb, Sb, Se, Sn, Tl, and V (μg $g^{-1}$, mean ± standard deviation, n = 3) in drug samples (A–I) and one sample of dietary supplement (DS) digested in each sample digestion procedures by ICP-MS (continuation).

| Isotope | Sample | Sample preparation | | | |
|---|---|---|---|---|---|
| | | $3HNO_3$:1HCl v $v^{-1}$ (240 °C) [a] | $3HNO_3$:1HCl v $v^{-1}$ (220 °C) [b] | $HNO_3$ 7.0 mol $L^{-1}$ (220 °C) [b] | $HNO_3$ 2.0 mol $L^{-1}$ (220 °C) [b] |
| $^{112}$Cd | DS | 0.32 ± 0.06 | 0.38 ± 0.01 | 0.37 ± 0.09 | 0.38 ± 0.03 |
| $^{120}$Sn | B | NA | <0.020 | 0.02 ± 0.02 | 0.02 ± 0.01 |
| | G | <0.083 | 0.032 ± 0.004 | 0.035 ± 0.006 | 0.032 ± 0.008 |
| $^{123}$Sb | C | NA | 0.009 ± 0.001 | 0.009 ± 0.002 | 0.011 ± 0.003 |
| | I | 0.12 ± 0.02 | 0.125 ± 0.001 | 0.08 ± 0.04 | 0.122 ± 0.001 |
| $^{138}$Ba | A | 1.4 ± 0.2 | 1.1 ± 0.1 | 1.1 ± 0.6 | 1.3 ± 0.1 |
| | B | NA | 0.12 ± 0.01 | 0.11 ± 0.03 | 0.12 ± 0.01 |
| | C | NA | 0.23 ± 0.02 | 0.22 ± 0.02 | 0.26 ± 0.01 |
| | F | 0.17 ± 0.07 | 0.19 ± 0.04 | 0.15 ± 0.02 | 0.179 ± 0.008 |
| | G | 0.20 ± 0.04 | 0.20 ± 0.02 | 0.22 ± 0.03 | 0.24 ± 0.02 |
| | H | 0.70 ± 0.02 | 0.73 ± 0.01 | 0.64 ± 0.02 | 0.71 ± 0.01 |
| | I | 0.71 ± 0.02 | 0.65 ± 0.04 | 0.72 ± 0.08 | 0.72 ± 0.03 |
| | DS | 1.2 ± 0.1 | 1.3 ± 0.3 | 1.2 ± 0.1 | 1.2 ± 0.1 |
| $^{205}$Tl | J | 0.016 ± 0.002 | 0.014 ± 0.001 | 0.014 ± 0.001 | 0.016 ± 0.001 |
| $^{208}$Pb | B | <0.083 | <0.017 | 0.022 ± 0.006 | 0.029 ± 0.008 |
| | I | 3.4 ± 0.3 | 3.3 ± 0.1 | 3.5 ± 0.7 | 3.7 ± 0.1 |
| | DS | 0.14 ± 0.01 | 0.12 ± 0.01 | 0.18 ± 0.02 | 0.140 ± 0.01 |

[a] Sample masses digested of 100 mg; [b] Sample masses digested of 500 mg; NA: not applicable for samples completely digested using $3HNO_3$:1HCl v $v^{-1}$ at 220 °C.

All analytes were below respective LOQs for all sample preparation procedures, except for elements shown in Table 3.4. This pattern was also observed in previous studies with commercial pharmaceutical samples [31,34–37]. On the other hand, the concentrations of As, Ba, Cd, Co, Cu, Cr, Li, Mo, Ni, Pb, Sb, Se, Sn, Tl and V determined in drug samples and one dietary supplement sample in the two sample preparation procedures using dilute nitric acid did not present significant differences (t-paired test with 95% of confidence) with the concentrations determined using the reference method (total digestion). Consequently, it may be inferred that the proposed digestion procedures are applicable even when remaining solids are present in the digests.

For some samples the analyte concentrations determined in the procedures using 7.0 and 2.0 mol $L^{-1}$ $HNO_3$ could not be compared with the concentrations determined using the reference method due to the higher LOQs (showed in Table 3.3) obtained for this at the minimum temperature of 220 °C or even at the maximum temperature of 240 °C, since the reduction of sample mass from 500 to 100 mg implied in LOQs 5 times higher.

The efficiency of digestion was also evaluated using a dietary supplement sample due to the matrix similarity and because it enabled the determination of 13 elements established by Chapter 232 in higher concentrations [21]. USP proposes limits (1.5, 0.5, 1.5 and 1.0 µg $g^{-1}$) for elemental contaminants (As, Cd, Hg and Pb, respectively) in dietary supplements and dietary ingredients according to the Chapter 2232 [22]. For the dietary supplement analyzed, concentrations of As, Cd, Hg and Pb were lower than the specified limits. The concentrations of As, Ba, Co, Cu, Cr, Mo, Ni, Pb, Sb, Sn and V determined in all drug samples were also lower than the limits established by Chapter 232 (Table 3.2).

*3.1.5.3. Accuracy of the procedure according to USP requirements*

As the proposed sample preparation strategies were validated by comparison with the reference procedure [34–37,39] both analytical methods, i.e., ICP OES and ICP-MS, were evaluated in terms of accuracy and sensitivity in compliance with USP requirements. Since no appropriated certified reference materials (CRMs) for pharmaceuticals are available for elemental analysis, the

accuracy of the method was evaluated by spike experiments at levels equivalent to 0.5*J* and 1.5*J* for each target element and the repeatability was demonstrated by a precision lower than 10% RSD for all samples [23]. The ICP OES analytical performance is shown in Table 3.5.

TABLE 3.5 - Parameters analytical performance for Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Os, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V in drugs samples by ICP OES.

| Analyte (nm) | $R^2$ | LOQ µg $g^{-1}$ |
|---|---|---|
| Ag (328.068) | 0.9996 | 2.6 |
| As (189.042) | 0.9996 | 2.7 |
| Au (267.595) | 0.9994 | 5.7 |
| Ba (493.409) | 1.000 | 0.19 |
| Cd (226.502) | 0.9998 | 0.54 |
| Co (228.616) | 0.9996 | 0.50 |
| Cr (267.716) | 1.000 | 0.36 |
| Cu (324.754) | 0.9996 | 1.1 |
| Hg (184.950) | 0.9994 | 2.1 |
| Ir (224.268) | 0.9998 | 0.17 |
| Li (670.784) | 0.9996 | 0.40 |
| Mo (202.030) | 0.9998 | 3.0 |
| Ni (231.604) | 0.9998 | 0.99 |
| Os (228.226) | 0.9956 | 1.0 |
| Pb (220.353) | 0.9996 | 3.9 |
| Pd (340.458) | 0.9998 | 2.1 |
| Pt (203.646) | 0.9998 | 9.1 |
| Rh (369.236) | 0.9998 | 7.3 |
| Ru (267.876) | 0.9998 | 3.7 |
| Sb (217.581) | 0.9998 | 6.0 |
| Se (196.090) | 0.9996 | 5.5 |
| Sn (189.989) | 0.9994 | 2.0 |
| Tl (190.856) | 0.9976 | 1.4 |
| V (292.402) | 0.9994 | 1.9 |

The LOQs obtained for As, Pb and Tl were higher than 1.5*J* considering the MDD (10 g $day^{-1}$), for Au, Cd, Hg, Pt and Rh were higher than 0.5*J*. Recoveries were evaluated in both instruments using a tailored multielemental standard solution (showed in Table 3.2). For determination by ICP OES (Table 3.6), no dilution of the samples was required. However, for

determination by ICP-MS (Table 3.7), dilutions of 1:50 v $v^{-1}$ were required for concentrations of added analytes higher than 0.55 mg $L^{-1}$ (in ascending order: Li, Sb, Ba, Cu, Mo, Sn and Cr). It is important to highlight that MDD for most drugs is lower than 10 g $day^{-1}$ hence the 0.5*J* and 1.5*J* would be higher and the LOQs obtained would be suitable to meet USP requirements. The MDD of 10 g $day^{-1}$ was used to obtain the minimal *J* value's that can be determined. The LOQs obtained for As, Au, Cd, Hg, Pt and Rh are suitable to follow USP requirements using drugs with MDD until 4.0 g $day^{-1}$ and for Pb drugs with MDD until 0.60 g $day^{-1}$.

TABLE 3.6 - Recoveries and relative standard deviation (%) obtained for the spiked in digested drug samples (A-I) according to the $J$ value by ICP OES (n = 3).

| Analyte (nm) | $J$ Addition[a] | Samples | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I |
| Ag (328.068) | 0.5$J$ | 81 (1) | 46 (9) | 83 (1) | 82 (2) | 152 (2) | 170.0 (0.9) | 90 (2) | 85 (2) | 80 (1) |
| | 1.5$J$ | 77 (5) | 65.2 (0.2) | 74 (5) | 55 (5) | 112.1 (0.2) | 76.5 (0.2) | 106.5 (0.3) | 75 (6) | 74 (5) |
| As (189.042) | 0.5$J$ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| | 1.5$J$ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| Au (267.595) | 0.5$J$ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| | 1.5$J$ | 106 (4) | 123 (2) | 94.5 (0.4) | 136 (2) | 103.0 (0.7) | 111.0 (0.8) | 103.0 (0.4) | 106 (2) | 94.1 (0.4) |
| Ba (493.409) | 0.5$J$ | 74 (5) | 110 (6) | 112 (1) | 82 (4) | 107 (2) | 104 (1) | 94 (4) | 74 (3) | 102 (1) |
| | 1.5$J$ | 104 (5) | 107 (1) | 102 (1) | 85 (4) | 96 (8) | 104.0 (0.1) | 101 (5) | 95 (4) | 101 (1) |
| Cd (226.502) | 0.5$J$ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| | 1.5$J$ | 116 (3) | 120.1 (0.2) | 111.0 (0.2) | 118 (4) | 120 (7) | 127 (1) | 105 (2) | 110 (4) | 110.0 (0.2) |
| Co (228.616) | 0.5$J$ | 100 (2) | 104 (6) | 111.0 (0.2) | 116 (4) | 112 (7) | 117 (1) | 117 (8) | 106 (4) | 102.0 (0.2) |
| | 1.5$J$ | 105 (3) | 105.5 (0.2) | 117.1 (0.6) | 118 (5) | 113 (6) | 123 (2) | 111 (2) | 118 (5) | 119.1 (0.6) |
| Cr (267.716) | 0.5$J$ | 106 (2) | 106 (3) | 113 (1) | 119 (2) | 123 (8) | 125 (4) | 128 (2) | 111 (2) | 118 (1) |
| | 1.5$J$ | 109 (1) | 105 (2) | 95 (2) | 121 (5) | 110 (3) | 100 (1) | 100 (5) | 121 (4) | 95 (2) |
| Cu (324.754) | 0.5$J$ | 101 (1) | 106 (3) | 112.0 (0.7) | 110.2 (0.6) | 110 (3) | 109 (2) | 102 (4) | 111.0 (0.6) | 102.2 (0.7) |
| | 1.5$J$ | 111 (2) | 105 (2) | 115 (1) | 113 (5) | 100 (8) | 109.1 (0.3) | 113 (3) | 103 (5) | 105 (1) |
| Hg (184.950) | 0.5$J$ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| | 1.5$J$ | 140 (1) | 126 (1) | 131 (1) | 138 (8) | 163.1 (0.2) | 165 (9) | 153 (9) | 128 (8) | 131 (1) |
| Ir (224.268) | 0.5$J$ | 99.5 (0.6) | 106 (5) | 113.0 (0.3) | 119 (4) | 123 (9) | 125 (3) | 128 (2) | 119 (4) | 113.0 (0.3) |
| | 1.5$J$ | 101 (2) | 103 (1) | 119 (1) | 121 (5) | 121 (4) | 132 (2) | 122 (8) | 122 (5) | 119 (1) |

[a] $J$ values are presented in Table 3.2.

TABLE 3.6 - Recoveries and relative standard deviation (%) obtained for the spiked in digested drug samples (A-I) according to the *J* value by ICP OES (n = 3) (continuation).

| Analyte (nm) | *J* Addition[a] | Samples | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I |
| Li (670.784) | 0.5*J* | 109 (2) | 126 (7) | 135.5 (0.5) | 110 (1) | 78 (4) | 79 (1) | 73 (2) | 110 (1) | 135.1 (0.5) |
| | 1.5*J* | 109 (3) | 84 (2) | 136 (3) | 113 (5) | 75 (5) | 76.0 (0.7) | 73 (3) | 113 (5) | 106 (3) |
| Mo (202.030) | 0.5*J* | 99 (2) | 104 (5) | 103.0 (0.4) | 109 (4) | 108 (9) | 108 (4) | 106 (1) | 109 (4) | 103.0 (0.4) |
| | 1.5*J* | 103 (2) | 105 (2) | 110 (2) | 113 (5) | 108 (9) | 115 (1) | 101 (3) | 113 (5) | 110 (2) |
| Ni (231.604) | 0.5*J* | 99.5 (0.8) | 103 (5) | 107.1 (0.5) | 113 (4) | 113 (9) | 116 (3) | 115 (2) | 112 (1) | 107.5 (0.5) |
| | 1.5*J* | 102 (2) | 101 (2) | 113 (1) | 115 (6) | 111 (5) | 121 (2) | 109 (6) | 105 (6) | 113 (1) |
| Pb (220.353) | 0.5*J* | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| | 1.5*J* | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| Pd (340.458) | 0.5*J* | 121 (2) | 97 (5) | 118 (1) | 124 (10) | 111 (4) | 125 (9) | 87 (2) | 124 (1) | 118 (14) |
| | 1.5*J* | 95 (3) | 119 (2) | 120 (4) | 136 (5) | 107 (9) | 121 (2) | 88 (2) | 126 (6) | 130 (4) |
| Pt (203.646) | 0.5*J* | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| | 1.5*J* | 116 (4) | 116 (2) | 127.0 (0.6) | 126 (4) | 109 (8) | 133 (3) | 117 (5) | 116 (3) | 127.1 (0.6) |
| Rh (369.236) | 0.5*J* | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| | 1.5*J* | 129 (3) | 122.0 (0.2) | 118 (2) | 109 (6) | 112 (10) | 104 (1) | 126 (7) | 109 (6) | 118 (2) |
| Ru (267.876) | 0.5*J* | 96 (2) | 117 (1) | 96 (2) | 115.0 (0.3) | 94 (1) | 116 (2) | 114 (4) | 105 (1) | 96 (2) |
| | 1.5*J* | 85 (2) | 116 (2) | 130 (2) | 116 (4) | 95 (5) | 130 (4) | 126.0 (0.7) | 116 (4) | 120 (2) |
| Sb (217.581) | 0.5*J* | 103.0 (0.2) | 105 (3) | 109.0 (0.4) | 114 (3) | 113 (5) | 113 (3) | 113 (3) | 104 (4) | 109.0 (0.4) |
| | 1.5*J* | 107 (2) | 107 (2) | 114 (1) | 116 (5) | 110 (8) | 118 (1) | 110 (3) | 116 (5) | 114 (2) |
| Se (196.090) | 0.5*J* | 113 (5) | 108 (3) | 95 (2) | 121 (6) | 110 (5) | 100 (9) | 100 (5) | 122 (6) | 105 (2) |
| | 1.5*J* | 120 (2) | 118 (1) | 125 (3) | 122 (1) | 110 (9) | 124 (1) | 117 (8) | 122 (9) | 125 (3) |

[a] *J* values are presented in Table 3.2.

TABLE 3.6 - Recoveries and relative standard deviation (%) obtained for the spiked in digested drug samples (A-I) according to the *J* value by ICP OES (n = 3) (continuation).

| Analyte (nm) | *J* Addition[a] | Samples | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I |
| Sn (189.989) | 0.5*J* | 101 (2) | 105 (6) | 91 (5) | 119 (2) | 118 (10) | 118 (4) | 118.0 (0.8) | 109 (3) | 91 (5) |
| | 1.5*J* | 106 (2) | 107 (2) | 85 (2) | 122 (5) | 118 (4) | 126 (2) | 113 (5) | 119 (5) | 85 (2) |
| Tl (190.856) | 0.5*J* | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| | 1.5*J* | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| V (292.402) | 0.5*J* | 77 (4) | 72 (8) | 71 (11) | 73 (4) | 65 (2) | 114 (1) | 59.5 (0.6) | 73 (4) | 81 (9) |
| | 1.5*J* | 83 (4) | 70 (6) | 75 (5) | 70 (4) | 66 (3) | 71 (2) | 56 (3) | 70 (4) | 75 (5) |

[a] *J* values are presented in Table 3.2.

TABLE 3.7 - Recoveries and relative standard deviation (%) obtained for the spiked in digested drug samples (A-I) according to the $J$ value by ICP-MS (n = 3).

| Isotope | $J$ Addition[a] | Samples | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I |
| $^{7}Li$ | 0.5$J$ | 98 (4) | 95.1 (0.5) | 99 (8) | 103.0 (0.1) | 106 (3) | 111 (3) | 98 (2) | 99 (1) | 106 (3) |
| | 1.5$J$ | 105 (5) | 105.2 (0.2) | 107 (2) | 107.0 (0.2) | 109 (2) | 112 (2) | 110 (4) | 107 (2) | 109 (2) |
| $^{51}V$ | 0.5$J$ | 98 (2) | 91 (1) | 103.1 (0.5) | 105 (4) | 80 (2) | 97 (3) | 93.0 (0.1) | 103.4 (0.5) | 80 (2) |
| | 1.5$J$ | 103 (2) | 83 (2) | 97 (8) | 117 (2) | 91 (2) | 94 (11) | 95 (7) | 97 (8) | 91 (2) |
| $^{52}Cr$ | 0.5$J$ | 105 (2) | 107.0 (0.1) | 102 (2) | 107 (2) | 103 (2) | 107 (2) | 107 (2) | 100 (9) | 103 (2) |
| | 1.5$J$ | 113 (4) | 114 (3) | 109 (7) | 118 (2) | 110 (1) | 118 (2) | 114.0 (0.2) | 109 (7) | 110 (1) |
| $^{58}Ni$ | 0.5$J$ | 107.1 (0.5) | 105 (4) | 100 (9) | 109 (1) | 108 (2) | 110 (1) | 117 (9) | 101 (2) | 108 (2) |
| | 1.5$J$ | 109 (3) | 109.4 (0.7) | 107 (2) | 113 (0.9) | 114.0 (0.6) | 115 (1) | 118 (5) | 107 (2) | 114.0 (0.6) |
| $^{59}Co$ | 0.5$J$ | 103 (3) | 107.1 (0.9) | 99 (4) | 102 (3) | 115 (4) | 97 (2) | 92 (2) | 99 (4) | 115 (4) |
| | 1.5$J$ | 106.3 (0.7) | 108 (2) | 93 (2) | 114.0 (0.6) | 127 (2) | 94 (1) | 98 (3) | 93 (2) | 107 (2) |
| $^{63}Cu$ | 0.5$J$ | 109 (3) | 110.0 (0.3) | 107 (8) | 116.4 (0.3) | 116 (2) | 118 (2) | 114.2 (0.6) | 107 (8) | 116 (2) |
| | 1.5$J$ | 111 (4) | 113.1 (0.6) | 112 (3) | 118.1 (0.8) | 119 (2) | 119 (2) | 121 (4) | 112 (3) | 119 (2) |
| $^{75}As$ | 0.5$J$ | 109 (5) | 123 (3) | 105 (3) | 110 (3) | 108 (8) | 125 (3) | 111 (4) | 105 (3) | 108 (8) |
| | 1.5$J$ | 110 (5) | 115 (1) | 111 (10) | 124 (3) | 109 (2) | 125 (10) | 110 (2) | 111 (1) | 109 (10) |
| $^{78}Se$ | 0.5$J$ | 123 (4) | 110 (3) | 120 (2) | 127 (6) | 128 (1) | 106 (2) | 116 (2) | 120 (2) | 128 (1) |
| | 1.5$J$ | 121.0 (0.8) | 111 (2) | 124 (7) | 105 (1) | 120 (7) | 102.2 (0.8) | 124.1 (0.3) | 121 (7) | 120 (7) |
| $^{98}Mo$ | 0.5$J$ | 89 (4) | 102 (12) | 86 (2) | 89 (1) | 96 (4) | 72 (10) | 85 (10) | 96 (2) | 96 (4) |
| | 1.5$J$ | 93 (6) | 89 (10) | 95.5 (0.5) | 93 (2) | 103.4 (0.4) | 83.7 (0.8) | 96 (13) | 95.8 (0.5) | 101.5 (0.4) |
| $^{102}Ru$ | 0.5$J$ | 98 (14) | 96 (3) | 102 (1) | 104 (5) | 90 (3) | 106 (3) | 96.7 (0.1) | 102 (1) | 86 (3) |
| | 1.5$J$ | 103 (2) | 82 (3) | 91 (8) | 116.2 (0.2) | 104 (2) | 93 (12) | 98 (8) | 91 (8) | 90 (2) |
| $^{103}Rh$ | 0.5$J$ | 96 (13) | 93 (3) | 99 (2) | 103 (4) | 95 (2) | 103.2 (0.2) | 94.6 (0.7) | 99 (2) | 106 (2) |
| | 1.5$J$ | 102 (2) | 86 (3) | 95 (9) | 115 (2) | 99 (2) | 101 (11) | 97 (8) | 95 (9) | 94 (2) |
| $^{107}Ag$ | 0.5$J$ | 99 (18) | 96.5 (0.3) | 106 (1) | 107 (8) | 92 (1) | 113 (1) | 97.7 (0.5) | 116 (1) | 92 (2) |
| | 1.5$J$ | 104 (1) | 73.8 (0.1) | 93 (10) | 122 (3) | 107 (2) | 109 (12) | 100 (7) | 93 (1) | 107 (2) |

[a] $J$ values are presented in Table 3.2.

TABLE 3.7 - Recoveries and relative standard deviation (%) obtained for the spiked in digested drug samples (A-I) according to the *J* value by ICP-MS (n = 3) (continuation).

| Isotope | *J* Addition[a] | Samples | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I |
| $^{108}Pd$ | 0.5*J* | 98 (2) | 108 (1) | 113 (1) | 117 (7) | 98 (1) | 121 (4) | 73 (5) | 111 (1) | 98 (1) |
| | 1.5*J* | 111.7 (0.3) | 94 (3) | 106 (9) | 120.1 (0.6) | 114 (2) | 99 (1) | 92.7 (0.5) | 106 (9) | 114 (2) |
| $^{111}Cd$ | 0.5*J* | 102 (3) | 90 (1) | 103 (5) | 105 (5) | 103 (5) | 99 (1) | 105 (2) | 103 (6) | 104 (5) |
| | 1.5*J* | 104.7 (0.8) | 104 (2) | 105 (4) | 118.0 (0.3) | 117 (3) | 103.1 (0.6) | 109 (3) | 105 (4) | 117 (3) |
| $^{120}Sn$ | 0.5*J* | 98 (3) | 74 (10) | 82 (8) | 97 (3) | 87 (11) | 83 (11) | 89 (5) | 80 (9) | 87 (11) |
| | 1.5*J* | 106 (6) | 81 (5) | 84 (9) | 109 (2) | 102 (6) | 94 (2) | 107 (3) | 84 (9) | 102 (6) |
| $^{123}Sb$ | 0.5*J* | 106 (2) | 106 (2) | 104 (6) | 110.5 (0.1) | 110.0 (0.5) | 112 (1) | 109.1 (0.8) | 104 (6) | 110.1 (0.5) |
| | 1.5*J* | 108 (5) | 106.2 (0.4) | 109 (3) | 111 (1) | 114 (1) | 113 (1) | 115 (5) | 109 (3) | 114 (1) |
| $^{138}Ba$ | 0.5*J* | 109 (3) | 110 (2) | 108 (8) | 115.5 (0.2) | 114 (1) | 116 (1) | 113 (2) | 108 (7) | 114 (1) |
| | 1.5*J* | 111 (5) | 113.1 (0.5) | 113 (2) | 116 (1) | 116 (1) | 117 (2) | 117 (4) | 113 (2) | 116 (1) |
| $^{193}Ir$ | 0.5*J* | 97 (15) | 95 (5) | 100.1 (0.8) | 98 (5) | 81 (3) | 106 (2) | 94.5 (0.2) | 100.1 (0.8) | 81 (1) |
| | 1.5*J* | 103 (2) | 87 (1) | 94 (9) | 110.4 (0.5) | 97 (2) | 103 (11) | 96 (6) | 94 (9) | 97 (2) |
| $^{195}Pt$ | 0.5*J* | 100 (13) | 100 (6) | 103 (2) | 101 (5) | 83 (3) | 108 (1) | 100 (1) | 103 (2) | 83 (2) |
| | 1.5*J* | 105 (1) | 90 (2) | 95 (9) | 112.2 (0.6) | 98 (2) | 106 (13) | 102 (5) | 95 (9) | 98 (2) |
| $^{197}Au$ | 0.5*J* | 91 (12) | 96 (3) | 102 (1) | 100 (4) | 96 (2) | 106 (3) | 91 (3) | 102.5 (0.5) | 96 (3) |
| | 1.5*J* | 101.4 (0.5) | 87 (5) | 94 (8) | 112.2 (0.3) | 104 (15) | 103 (11) | 87.5 (0.8) | 94 (8) | 104 (11) |
| $^{202}Hg$ | 0.5*J* | 102 (16) | 91 (1) | 112.7 (0.3) | 120 (9) | 73 (1) | 113 (2) | 98 (2) | 112.5 (0.3) | 75 (1) |
| | 1.5*J* | 114 (1) | 87 (2) | 99 (10) | 119.8 (0.1) | 97 (18) | 108 (10) | 100 (6) | 99 (10) | 98 (18) |
| $^{205}Tl$ | 0.5*J* | 104 (4) | 108 (2) | 107 (5) | 101 (3) | 107 (2) | 102 (2) | 97 (1) | 107 (5) | 107 (2) |
| | 1.5*J* | 107.1 (0.9) | 107.5 (0.9) | 112 (1) | 118 (1) | 105 (4) | 98.5 (0.4) | 91 (4) | 112 (1) | 93 (4) |
| $^{208}Pb$ | 0.5*J* | 98 (8) | 86.7 (0.2) | 84 (5) | 107 (7) | 91 (3) | 83 (5) | 92 (5) | 85 (11) | 90 (3) |
| | 1.5*J* | 95 (4) | 94 (5) | 82 (8) | 107 (3) | 105 (2) | 110 (3) | 98 (4) | 96 (2) | 104 (4) |

[a] *J* values are presented in Table 3.2.

In most cases, recoveries ranged from 72 to 128% for ICP-MS and from 70 to 138% for ICP OES. For ICP OES, the 1.5*J* for Au, Cd, Hg and Rh were higher than respective LOQs, but close to these values, leading to recoveries higher than 120% for some samples. Silver recoveries lower than 70% were obtained for samples B and D and higher than 150% for samples E and F. For V, recoveries lower than 70% were obtained for samples E and G. These unsatisfactory recoveries obtained for ICP OES may be related to instrumental determination errors or matrix effects, since recoveries obtained for ICP-MS for all samples ranged from 73 to 122% for silver and from 80 to 117% for vanadium.

All recoveries for Os were lower than 60%. Probably lower recoveries were related to the formation of $OsO_4$ in acid nitric medium, which is volatile and toxic. Quantitative Os determination was only reported when using a procedure of oxidizing distillation and recovery of $OsO_4$ in geological samples digested in aqua regia [127] or still using complexing agents and stabilization of nitric acid digests with a reagent mixture containing acetic acid, thiourea and ascorbic acid [79]. Except for Os, accurate determinations were observed at lower concentration levels (0.10 and 0.30 mg $L^{-1}$ for ICP OES and 1.0 and 5.0 µg $L^{-1}$ for ICP-MS) and also by spike experiments at levels equivalent to 0.5*J* and 1.5*J* for each target elements described in Chapter 232, based on acceptable recoveries established from 70 to 150% of the *J* value [23].

### 3.1.6 - Conclusions

Digests obtained using dilute nitric acid solution presented higher dissolved organic carbon, however these carbon concentrations did not cause matrix effects. Probably the observed matrix effects were originated from differences in viscosities among digests and standards solutions and these were corrected using internal standardization for ICP OES measurements and minimized using HMI for ICP-MS measurements. For ICP OES, the LOQs obtained for As, Pb and Tl were higher than 1.5*J* and higher than 0.5*J* for Au, Cd, Hg, Pt and Rh. For ICP-MS, collision cell mode was effective for overcoming polyatomic interferences on $^{51}V^+$, $^{52}Cr^+$, $^{63}Cu^+$, $^{75}As^+$, $^{78}Se^+$ and $^{103}Rh^+$ determinations and LOQs were compatible with USP requirements. The

microwave-assisted sample preparation here proposed using dilute nitric acid solution is simple and can be seen as an alternative to sample preparation using inverse aqua regia for further determination of 23 elements (except Os) by ICP OES and ICP-MS. This ease-of-use procedure can help the pharmaceutical industry in the quality control of the drugs but its applicability must be further demonstrated for other samples.

## 3.2 - Dispersive liquid–liquid microextraction of Cd, Hg and Pb from medicines prior to ICP OES determination according to the United States Pharmacopeia

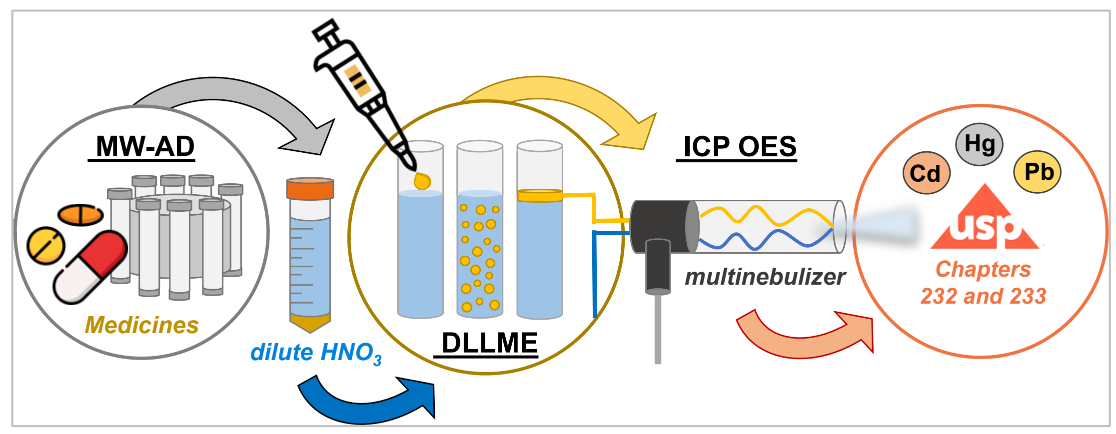
Fernanda C. Pinheiro,[a,b] Miguel Ángel Aguirre,[b] Joaquim A. Nóbrega[a] and Antonio Canals[b*]

### 3.2.1 - Abstract

A simple, sensitive and matrix effect free analytical method for simultaneous determination of Cd, Hg and Pb in drug samples (i.e., commercial dosage tablets) by inductively coupled plasma optical emission spectrometry (ICP OES) has been developed. According to the United States Pharmacopoeia (USP) Chapter 232, those metals are considered elemental impurities from class 1 and they must be assessed in pharmaceutical production as well as in quality control evaluation. In order to increase the sensitivity of the analysis, a dispersive liquid-liquid microextraction (DLLME) was performed and seven factors affecting analyte extraction were optimized by multivariate analysis. The microvolume of analyte enriched phase was directly introduced into the plasma using a multinebulizer, providing a high enrichment factor. When compared to conventional ICP OES analysis, DLLME improves limits of quantitation (LOQ) values on average 40-fold for all analytes. Consequently, LOQ values were significantly lower than their permissible daily exposures for oral drugs. Accuracy was evaluated by addition and recovery experiments following USP recommendations in eight commercial drug samples. Recovery and RSD values were within the range of 90-108% and 1-9%, respectively.

[a] Group for Applied Instrumental Analysis, Department of Chemistry, Federal University of São Carlos, P.O. Box 676, São Carlos, SP, 13560-270, Brazil.
[b] Department of Analytical Chemistry and Food Sciences, University Institute of Materials, Faculty of Science, University of Alicante, P.O. Box 99, 03080, Alicante, Spain.
*Corresponding author: a.canals@ua.es

### 3.2.2 - Graphical abstract




### 3.2.3 - Introduction

In the pharmaceutical field, safety and efficacy of medicines are fundamental issues. On this matter, the monitoring of elemental impurities provide assurance of the quality of pharmaceuticals products since some elements can possess unwanted pharmacological–toxicological effects [1–4]. For this purpose, two guidelines have been recently recommended by the United States Pharmacopeia (USP): (i) Chapter 232, Elemental Impurities Limits [21], and (ii) Chapter 233, Elemental Impurities Procedures [23]. Chapter 232 specifies 24 elemental impurities and their toxicity limits considering the oral permissible daily exposure (PDE) values of three drug categories (i.e. oral, parenteral and inhalation drugs) [21]. Chapter 233 describes analytical procedures for elemental determination using two spectroanalytical methods: inductively coupled plasma optical emission spectrometry (ICP OES) or inductively coupled plasma mass spectrometry (ICP-MS) [23].

Although the drafting process of these two chapters started in 2010, a new version of the general Chapter 232 in a strict compliance with the International Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Q3DR1) guideline [17,20] was published in 2016 [18], became official one year later [19] and was only implemented in 2018. According to ICH and USP requirements [20,21,23] the PDE values for elemental impurities (target elements) are grouped into four main categories: class 1 (Cd, Pb, As, Hg), class 2A (Co, V, Ni), class 2B (Tl, Au, Pd, Ir, Os, Rh, Ru, Se, Ag, Pt), and class 3 (Li, Sb, Ba, Mo, Cu, Sn, Cr). These

categories are based on the toxicity of target elements, their likelihood of occurrence and route of administration. Chapter 232 [21] and ICH [20] also provide guidance on which of those 24 elemental impurities must be tested for.

The elements from class 1 are considered toxic to humans and have limited or no use in the manufacture of pharmaceuticals. Their presence in pharmaceuticals typically comes from commonly raw materials and must be evaluated in all finished pharmaceutical products and in potential sources of contamination, for instance active pharmaceutical ingredients and excipients [1,2,4,20]. Even at low concentration levels, the heavy metals Cd, Hg and Pb pose a serious health risk when used for pharmaceutical purposes [2,20,28,29]. Cadmium in their inorganic forms are considered carcinogenic to humans [30] and, although Hg and Pb are not classified as carcinogenic, these elements may cause severe toxicological and hematopoietic effects [28–30]. Due to their high toxicity, low PDE values are recommended for these target elements [21,23].

ICP based methods enable fast multi-elemental analysis with high sensitivity, accuracy and robustness [6–8]. On one hand, considering the low PDE values recommended for the above-mentioned elements, the majority of the proposed ICP based methods for elemental impurities determination are focused on ICP-MS analysis [1,2,4,8]. On the other hand, ICP OES should be considered a suitable analytical method for this purpose since its higher availability, in contrast to the higher instrumentation cost of ICP-MS.

In order to reach enough sensitivity for determination of these elements in drug samples using ICP OES, a preconcentration step prior to measurement could be used [91,128]. On this regard, preconcentration approaches based on microextraction techniques, particularly dispersive liquid-liquid microextraction (DLLME), have been extensively used since their advantages, including simplicity, speed, ease of use, low cost and high enrichment factors using an extremely low extractant solvent volume [91–93]. Traditional DLLME involves the use of a mixture of solvents (i.e., extractant and disperser solvents) which are injected into the aqueous sample forming a cloudy solution. The dispersion of extraction solvent accelerates the analyte extraction and after a centrifugation step is possible to collect an aliquot of the enriched extractant [92,93,129]. In order to eliminate the disperser solvent and to enhance the extractant phase dispersion, vortex-assisted DLLME has been employed [92]. The use of

vortex agitation to disrupt the extractant phase reduces the consumption of organic solvents, because the use of the disperser solvent is not needed.

After the DLLME procedure, the low extractant solvent volume is generally dissolved in another miscible organic solvent before the introduction of extract using pneumatic nebulization, nevertheless, this step can deteriorate the enrichment factor achieved during the preconcentration. Moreover, the introduction of organic matrices into the argon plasma can cause severe matrix effects and also the formation of carbon deposits on the plasma torch. In order to address these challenges, a multinebulizer has been successfully used for the simultaneous introduction of organic and aqueous solutions for preventing the formation of carbon deposits [103,105–107,130]. This novel multinebulizer incorporates two independent liquid inlets into a single nebulization body with a common nebulization gas inlet and a unique outlet orifice allowing that two liquids, miscible or immiscible, be mixed at the tip of the nebulizer [130]. Hence, a microvolume of analyte enriched extract (without further dilution) and aqueous solution can be simultaneously introduced into the plasma by independent channels, reducing carbon deposits on the torch without decreasing the enrichment factor.

To our knowledge, this is the first report which an extraction methodology is applied for drug samples to elemental impurities determination in accordance with ICH guidelines and USP chapters. It is well-known that ICP-MS afford suitable sensitivity for the ultra-trace determination of the elemental impurities. However, given the larger number of laboratories that already employ ICP OES, this study aimed to develop a simple DLLME procedure for the simultaneous preconcentration of Cd, Hg, and Pb in drug samples for subsequent measurement by ICP OES.

### 3.2.4 - Experimental

*3.2.4.1. Reagents and standard solutions*

To minimize contamination all laboratory glassware were kept in 10% v $v^{-1}$ nitric acid solution for 24 h and then washed with ultrapure water before use. Experiments were performed using concentrated high purity grade $HNO_3$ (Merck, Darmstadt, Germany) and ultrapure water, resistivity higher than

18.2 MΩ cm, (Millipak-40 Filter Unit 0.22 mm NPT, Bedford, MA, USA). Sodium diethyldithiocarbamate (DDTC, 99%, Sigma-Aldrich, Steinheim, Germany) was used as complexing agent. Buffer solutions were prepared by dissolving the appropriate amount of sodium acetate (Panreac Químicas S.A., Castellar del Vallés, Spain) at pH 4 and 6 and sodium phosphate (Scharlau, Barcelona, Spain) at pH 9. Toluene (99.9%, Sigma Aldrich) and 1-octanol (99.9%, Sigma Aldrich) were used as extracting solvent. Analytical reference solutions used for ICP OES calibrations and for addition and recovery experiments were prepared by appropriate dilutions of 1000 mg $L^{-1}$ of Cd, Hg, and Pb (High Purity Standards, Charleston, SC, USA) in 0.14 mol $L^{-1}$ $HNO_3$ medium.

*3.2.4.2. Instrumentation*

A pHmeter (Crison Instrument, Barcelona, Spain) with a combined glass electrode was used for pH measurements. A centrifuge (model 2690/5, Nahita Centrifuges, Beriain, Spain) was used to accelerate the phase separation. Experiments were performed using an Agilent 720-ES inductively coupled plasma optical emission spectrometer (Agilent Technologies, Melbourne, Australia) operating in axial viewing mode. Argon (99.9992%, Carburos Metálicos S.A, Barcelona, Spain) was used in all measurements. Plasma operating conditions used in ICP OES are shown in Table 3.8.

TABLE 3.8 - Operating parameters used in Agilent 720-ES ICP OES.

| Instrument parameter | Value |
|---|---|
| RF applied power (kW) | 1.2 |
| Plasma gas flow rate (L $min^{-1}$) | 15 |
| Auxiliary gas flow rate (L $min^{-1}$) | 1.5 |
| Nebulizer gas flow rate (L $min^{-1}$) | 0.75 |
| Organic extract uptake rate (µL $min^{-1}$) | 50 |
| Aqueous solution uptake rate (µL $min^{-1}$) | 200 |
| Nebulizer | MultiNeb® |
| Spray chamber | Cyclonic spray chamber |
| Number of replicates | 3 |
| Analytes | Emission line (nm) |
| Cd | 226.502 II |
| Hg | 253.652 I |
| Pb | 220.353 II |

I: Atomic line; II: Ionic line.

Introduction of extract (i.e., analyte enriched phase) were performed using a multinebulizer (MultiNeb®, Ingeniatrics, Seville, Spain) [130]. This multinebulization device is an advanced version of another previous prototypes already described [131]. It presents two independent liquid inlets and two different types of peristaltic tubes were used depending on the solution introduced. In the liquid inlet where the analyte enriched phase was introduced, a peristaltic tube compatible with most petroleum-based products (F-4040-A, id. 0.25 mm, Ismatec, Switzerland) was used. In the other one where an ultrapure water was continuously pumped, a Tygon® peristaltic tubes (R-3607, id. 0.76 mm, Ismatec) was employed.

*3.2.4.3. Samples and sample preparation*

Eight drug samples in solid dosage form (A-H) were analyzed: A) metformin hydrochloride, used for diabetes treatment; B) losartan potassium, used for hypertension treatment; C) orfenadrine citrate, monoidratated dipirone and caffeine anidra, used as muscle relaxant and analgesic; D) sodium

dipyrone, used as analgesic; E) nimesulide, used as anti-inflammatory; F) omeprazole, used for benign (gastric or duodenal) peptic ulcers treatment; G) levothyroxine sodium, used for thyroid treatment; and H) diclofenac sodium, paracetamol, carisoprodol and caffeine, used for rheumatism treatment. All analyzed samples were classificated as oral administration route and were purchased in local pharmacies in São Carlos, São Paulo, Brazil and in San Vicente del Raspeig, Alicante, Spain.

Sample preparation for drugs in solid dosage form was performed based on previously proposed works for microwave-assisted sample digestion [40,41]. All samples were ground and homogenized using pestle and mortar and masses of approximately 500 mg were microwave-assisted digested in triplicate using a volume of 7 mL of 2 mol $L^{-1}$ $HNO_3$. An Ethos 1 microwave oven (Milestone, Sorisole, Italy) was used. The heating program was applied in two steps: (1) 15 min to reach 220 °C, (2) 15 min at 220 °C, and (3) an additional 15-min cooling step. A maximum 1.5 kW of microwave power was applied. Subsequently, digests were diluted to 25 mL with distilled-deionized water (final dilution of 50-fold) after adjusting the pH. The samples not completely digested were centrifuged for 2 min at 3000 rpm for sedimentation of residual solids.

*3.2.4.4. Dispersive liquid–liquid microextraction procedure*

A 8.0 mL aliquot of the digested sample, at pH 6 and DDTC concentration of 1.0% m $v^{-1}$, was transferred to 10-mL glass tubes. Then, 100 µL of the extractant solvent (i.e. toluene) was added, and the mixture was shaken using a vortex shaker for 3 min. After shaking, the solution was centrifuged at 3000 rpm for 2 min to separate the two phases, with the analyte enriched phase at the top of the solution. Eighty microliters of the organic phase was collected from the glass tube using a micropipette and directly inserted into the ICP OES without further dilution. A schematic representation of the general DLLME procedure is presented in Figure 3.6. During the optimization, standard solutions containing 500 µg $L^{-1}$ of Cd, Hg and Pb were used. NemrodW statistical software (NemrodW® v.2007/2010, LPRAI, Marseille, France) was used to construct the experimental designs and evaluate the results.

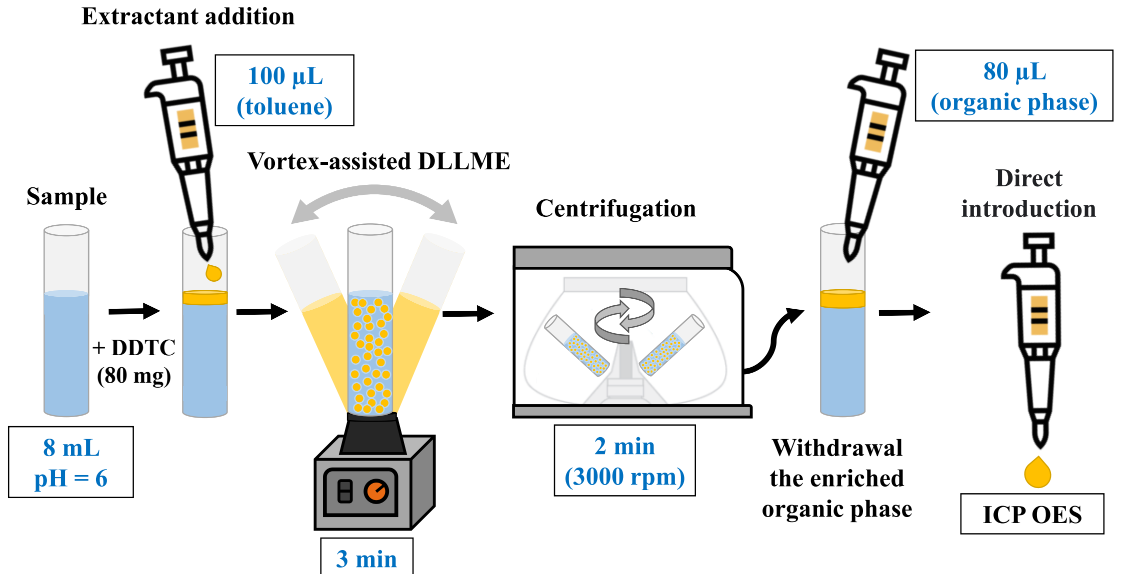



FIGURE 3.6 - Scheme of the DLLME procedure for preconcentration of Cd, Hg and Pb in drug samples.

### *3.2.4.5. Evaluation of accuracy according to USP requirements*

According to the USP Chapter 233 accuracy must be evaluated by addition and recovery experiments with acceptable recoveries ranging from 70 to 150% of the spiked value at concentrations ranging from 0.5*J* to 1.5*J* values for each target element, considering up to 20% of repeatability [21,23]. In this case, the *J* value (also named target limit) is the concentration of the element(s) in µg $g^{-1}$ of interest at the target limit, appropriately diluted to the working range of the instrument. Thus, *J* values were calculated according to oral PDE values specific for each target element (i.e. 5.0, 30 and 5.0 µg $day^{-1}$ for Cd, Hg and Pb, respectively) divided by the maximum daily dose (MDD) and the dilution factor (DF), as shown in Equation (1):

$$J = \frac{\mathrm{PDE}\left(\frac{\mu g}{day}\right)}{\mathrm{MDD}\left(\frac{g}{day}\right) \mathrm{x\ DF}} \qquad \text{Equation (1)}$$

The MDD ranged from 0.23 to 10 g $day^{-1}$ for all samples analyzed. For that, the MDD of 10 g $day^{-1}$ was adopted for all samples to obtain the minimal *J* value that can be determined. In this work, therefore, considering the MDD of 10 g $day^{-1}$ and the DF of 50 (i.e. 500.0 mg of sample in 25.00 mL), the

added concentrations (i.e. 0.5*J* to 1.5*J* values) were 5.0 and 15 μg $L^{-1}$ for Cd and Pb; and 30 and 90 μg $L^{-1}$ for Hg.

### 3.2.5 - Results and discussion

*3.2.5.1. Reagents Optimization of dispersive liquid–liquid microextraction*

The multivariate optimization of the DLLME procedure was proceeded into two complementary steps: (i) a Plackett-Burman design was employed as screening approach to identify between significant and non-significant factors, followed by (ii) a central composite design (CCD) to obtain optimal values for the significant factors. In both steps, the experiments were randomly performed in order to nullify the effect of extraneous or nuisance factors. Seven factors at two levels were evaluated on the Plackett-Burman design. The DLLME variables investigated and their low (-) and high (+) levels are described in Table 3.9. The results of the Plackett-Burman design are visualized using Pareto charts of the standardized effect in Figure 3.7.

TABLE 3.9 - Experimental variables and levels of the Plackett-Burman design.

| Factor | Level | |
|---|---|---|
| | Low (-) | High (+) |
| Extractant solvent | 1-octanol | toluene |
| Extractant solvent volume (μL) | 100 | 200 |
| Sample pH | 4 | 9 |
| DDTC concentration (% m $v^{-1}$) | 0.5 | 1 |
| Extraction time (min) | 1 | 3 |
| Centrifugation time (min) | 2 | 4 |
| Centrifuge speed (rpm) | 2000 | 3000 |

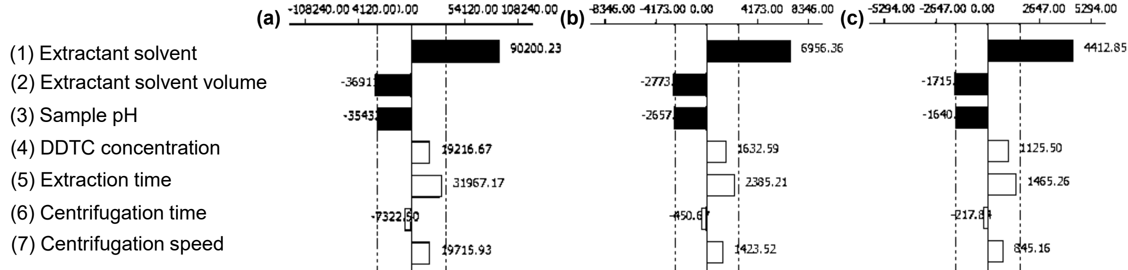



FIGURE 3.7 - Pareto charts obtained in the screening study of the experimental variables affecting the DLLME of **(a)** Cd, **(b)** Hg and **(c)** Pb. (■) significant effect on DLLME procedure; (□) insignificant effect on DLLME procedure. Analyte concentration of 500 µg $L^{-1}$.

In the Pareto charts (Figure 3.7 a-c) the black bars indicate variables presenting a significant effect on DLLME procedure, while non-significant factors are indicated by white bars. The bars to the right indicate a positive effect, i.e., favorable condition at higher values of that factor, while the opposite effect is indicated by bars to the left. In general, DLLME was favored when using toluene as extractant solvent at high values of DDTC concentration, extraction time and centrifuge speed and low centrifugation time. Unfortunately, the use of 1-octanol does not satisfy the threshold limit established by the USP (data not shown), and therefore, toluene was used as extractant solvent. Only the factors (1) extractant solvent; (2) extractant solvent volume; and (3) sample pH showed a significant effect on the Plackett-Burman experiment.

Due to pH influence on the complexation step, its evaluation is indispensable in metal extraction procedures [132]. In case of DDTC, pH values below 3.95 favors the protonated form of DDTC (pKa = 3.95 [133]), therefore limiting chelate formation. Moreover, high pH values could also have a negative effect on extraction, since analytes can form hydroxides decreasing the amount extracted. In turn, the extractant solvent volume infers directly in the enrichment factor of the analytes [92,93]. By increasing the extractant solvent volume to a certain degree, the extraction efficiency is increased. However, further increases could cause a dilution effect, resulting in a decrease in the enrichment factor.

In order to optimize both significant factors, a CCD was performed. Both factors were investigated at five levels as described in Table 3.10 and the response surfaces obtained for the different elements are shown in Figure 3.8.

TABLE 3.10 - Factor and levels of the central composite design.

| Experimental variable | Level | | | Star points (α = 1.41) | |
|---|---|---|---|---|---|
| | Low (-) | Central (0) | High (+) | -α | +α |
| Extractant solvent volume (μL) | 115 | 150 | 185 | 100 | 200 |
| Sample pH | 4.7 | 6.5 | 8.3 | 4.0 | 9.0 |

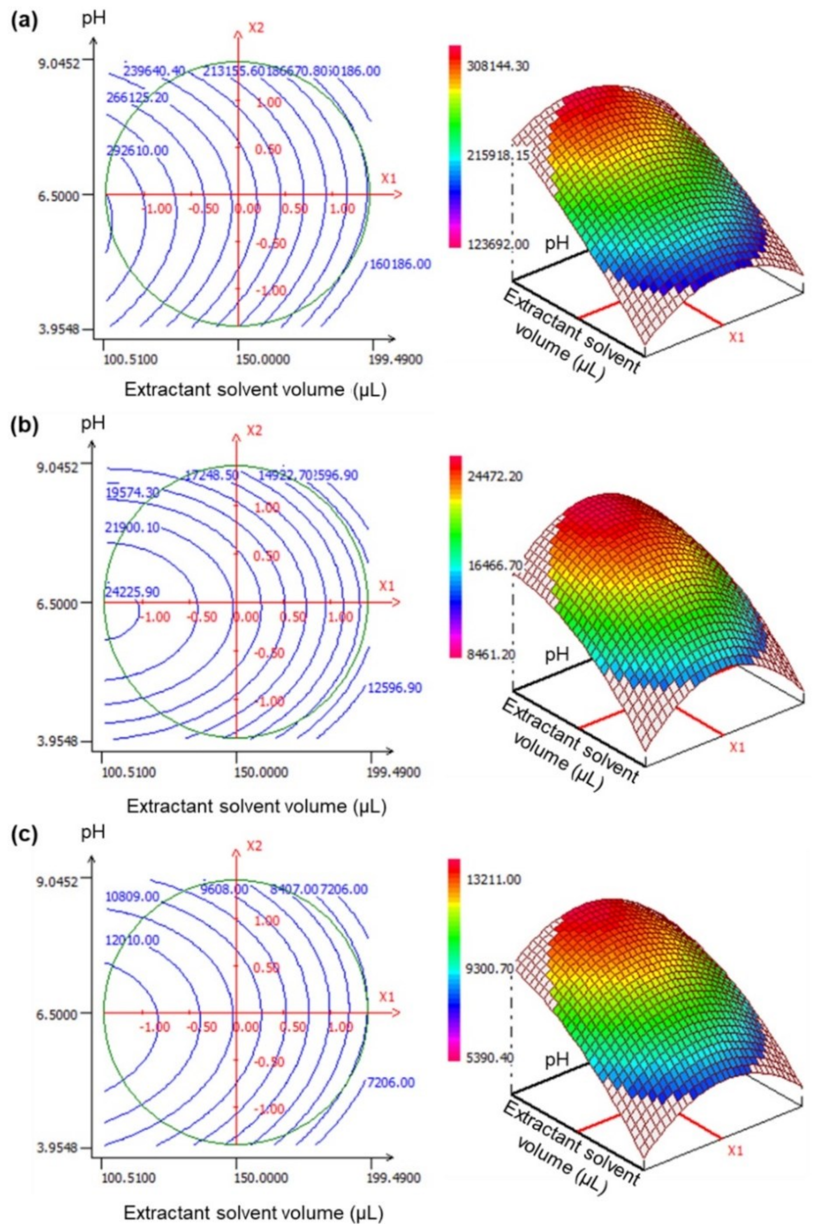



FIGURE 3.8 - Response surface from central composite design for **(a)** Cd, **(b)** Hg and **(c)** Pb. Analyte concentration of 500 μg $L^{-1}$.

Optimum conditions for extraction of the different elements were obtained using the lowest level of extractant solvent volume and pH value at 6.1, 6.3 and 5.2 for Cd, Hg and Pb, respectively. On that basis pH 6.0 (average of those values) and an extractant solvent volume of 100 µL were selected as the most favorable conditions for all analytes. In summary, the optimized conditions for simultaneous DLLME of Cd, Hg and Pb were: DDTC concentration 1.0% m $v^{-1}$, 100 µL of toluene as extractant solvent, pH 6.0, extraction time of 3 min, centrifugation time of 2 min and a centrifugation speed of 3000 rpm.

*3.2.5.2. Analytical performance for DLLME-ICP OES method according to the USP requirements*

The proposed microextraction procedure provided a significant increase in sensitivity for all elements. Table 3.11 summarizes the analytical figures of merit of the developed DLLME-ICP OES method and conventional ICP OES analysis for determination of Cd, Hg and Pb in drug samples. Coupling DLLME to ICP OES is particularly challenging due to spectral and non-spectral interferences caused by organic solvents [91–93]. On this regard, in the multinebulization device used, water is continuously introduced into the plasma. This advantage over conventional nebulization system facilitates the introduction of organic solvents that are not so compatible with plasma, avoiding the need of continuous cleaning of torch and injector tube [103,130].

TABLE 3.11 - Analytical figures of merit for Cd, Hg and Pb determination using DLLME-ICP OES and conventional ICP OES analysis.

| | Emission line (nm) | | |
|---|---|---|---|
| | Cd (226.502) | Hg (253.652) | Pb (220.353) |
| ICP OES | | | |
| Linear range (μg $L^{-1}$) | 1000 - 5000 | 1000 - 5000 | 1000 - 5000 |
| Sensitivity (cps L $μg^{-1}$)[a] | 10.85 ± 0.14 | 0.99 ± 0.02 | 0.455 ± 0.006 |
| r[b] | 0.9994 | 0.9994 | 0.9990 |
| LOQ (μg $L^{-1}$) | 12 | 70 | 66 |
| DLLME-ICP OES | | | |
| Working range (μg $L^{-1}$) | 2.50 - 120 | 2.50 - 120 | 2.50 - 120 |
| r[b] | 0.9996 | 0.9994 | 0.9980 |
| Sensitivity (cps L $μg^{-1}$)[a] | 734 ± 11 | 54.4 ± 1.0 | 32.6 ± 0.7 |
| EF[c] | 68 ± 2 | 55 ± 2 | 72 ± 2 |
| LOQ (μg $L^{-1}$) | 0.3 | 1.8 | 1.6 |
| USP LOQ ≤0.3*J* (μg $L^{-1}$) | ≤3 | ≤18 | ≤3 |
| Repeatability 0.5*J* (RSD%)[d] | 1.6 | 5 | 4 |
| Repeatability 1.5*J* (RSD%)[e] | 4 | 3 | 4 |

[a] Slope ± standard deviation; [b] Correlation coefficient (seven calibration points); [c] Enrichment factor ± expanded uncertainty. Calculated as slope ratio between calibration curves with and without DLLME; [d] Mean value for six replicate analyses of spiked solution with 5.0, 30 and 5.0 μg $L^{-1}$ of Cd, Hg and Pb, respectively; [e] Mean value for six replicate analyses of spiked solution with 15, 90 and 15 μg $L^{-1}$ of Cd, Hg and Pb, respectively.

#### *3.2.5.2.1. Linearity, sensitivity and precision*

Two calibration curves were performed: (i) conventional ICP OES analysis (i.e., without DLLME procedure) using six calibration points with working range from 1.0 to 5.0 mg $L^{-1}$ for all analytes, and (ii) DLLME-ICP OES using seven calibration points with working range from 2.5 to 120 μg $L^{-1}$ for Cd, Hg and Pb. According to the USP Chapter 233, measurement of at least three calibration standards in the working range between 0.3*J* and at least 1.5*J* for each target element are recommended [23]. In case of the developed analytical method, the working range was set from 0.25*J* to 2.0*J* for simultaneous determination of Cd, Hg and Pb. The correlation coefficients (r) obtained for all DLLME-ICP OES calibration curves ranged from 0.9980 to 0.9996, showing good linearity. The enrichment factor (EF) values for each analyte were

calculated as the ratio between sensitivity values with and without DLLME procedure. High EF values were obtained, ranging from 55 to 72.

The repeatability was estimated from six independent measurements of samples spiked at 0.5*J* and 1.5*J* of each target element. The relative standard deviations obtained were ranged from 1.6 to 5%. Obtained repeatability values were significantly lower than 20% of RSD stated by the USP Chapter 233 for repeatability [23].

#### *3.2.5.2.2. Limits of detection and quantification*

Limits of detection (LOD) and quantification (LOQ) were calculated according to IUPAC recommendations considering three times and ten times standard deviation of 10 measurements from blank solutions at 99% confidence level [134]. The LOQ values for conventional ICP OES analysis were all higher than the target-limits. In turn, the LOQ values for Cd, Hg and Pb using DLLME are 36, 33 and 6-times, respectively, lower than their respective *J* values. This means a LOQ improvement on average 24-fold. Following USP recommendations, LOQ values ≤0.3*J* are suggested as acceptance criteria [23]. Once LOQ values achieved for Cd, Hg and Pb were 11, 10 and 2-times lower than their 0.3*J*, respectively, it may be inferred that the proposed DLLME-ICP OES method is suitable to meet USP requirements even using drugs in tablets form with MDD higher than 10 g $day^{-1}$.

Taking into account the low target limits for elements from class 1 [20,21], Table 3.12 summarizes analytical methodology previously reported for Cd, Hg and Pb determination in pharmaceutical samples using ICP OES. Considering MDD of 10 g $day^{-1}$, the LOQ values obtained for Cd [40,57,64], Hg [40] and Pb [36,40,57,64] were higher than 0.3*J* (i.e. LOQ established by USP). As it can be noted, none of the aforementioned analytical methods meet the USP requirements for these three analytes at the same time. There are only two analytical methods with comparable LOQ values [34,38].

TABLE 3.12 - Quantification limits for Cd, Hg and Pb of the ICP OES methods used for elemental impurities determination in pharmaceutical samples.

| Pharmaceutical sample | Sample mass (mg) | Sample preparation procedure | Sample preparation details | DF[a] method | Quantification limit ($\mu g\ L^{-1}$) | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | Cd | Hg | Pb | |
| Pills and tablets | 500 | MW-AD[b] | 7 mL of 2 mol $L^{-1}$ $HNO_3$; final digest volume of 50 mL | 100 | 5.4 | 21 | 39 | 40 |
| Antibiotic tablets | 200 | CH-AD[c] | 5 mL of 14 mol $L^{-1}$ $HNO_3$ + 1 mL of $H_2O_2$ 30% v $v^{-1}$; final digest volume of 25 mL | 125 | 4.2 | NA | 64 | 57 |
| Pills and tablets | 100 | MW-AD[b] | 5 mL of $3HNO_3$:1HCl v $v^{-1}$; final digest volume of 50 mL | 500 | 2.6 | 10 | 114 | 36 |
| Levetiracetam | 1000 | MW-AD[b] | 15 mL of 14 mol $L^{-1}$ $HNO_3$ + 2 mL of $H_2O_2$ 30% v $v^{-1}$; final digest volume of 25 mL | 25 | 16 | 16 | 4 | 64 |
| Lu tablets | 450 | MW-AD[b] | 12 mL of $3HNO_3$:1HCl v $v^{-1}$; final digest volume of 13 mL | 29 | 0.32 | 1.55 | 0.70 | 34 |
| Aspirin and Lisinopril | 200 | MW-AD[b] | 7 mL of 14 mol $L^{-1}$ $HNO_3$ + 2 mL of HCl + 1 mL of $H_2O_2$ 30% v $v^{-1}$; final digest volume of 50 mL | 250 | 0.4 | 1.2 | 0.7 | 38 |
| Pills and tablets | 500 | MW-AD[b] + DLLME | 7 mL of 2 mol $L^{-1}$ $HNO_3$; final digest volume of 25 mL | 50 | 0.3 | 1.8 | 1.6 | This work |

[a] Dilution factor, considering sample mass, final digest volume and further sample dilutions before analysis; [b] Microwave-assisted digestion in closed vessel; [c] Conventional heating-assisted digestion in closed vessel; NA: Not applicable.

In the first work [34], the low LOQ values were achieved using a dilution factor lower than 30-fold. Generally for conventional sample introduction by pneumatic nebulization using ICP OES, maximum total dissolved solids recommended is lower than 1% m $v^{-1}$ [6]. In addition, the low dilution factor can induce severe matrix effects, and therefore, effects on aerosol transport and plasma properties should be carefully assessed [6]. In the second study [38], the authors used an ultrasonic nebulizer with a relative high sample consumption (i.e. 1.9 mL $min^{-1}$), being the main disadvantage the high cost of the ultrasonic nebulizer.

*3.2.5.2.3. Accuracy*

The trueness was evaluated by addition and recovery experiments performed taking into account *J* values as USP Chapter 233 recommendation [23]. All samples were spiked at levels equivalent to 0.5*J* and 1.5*J* for Cd, Hg and Pb in order to check the trueness of the method (Table 3.13). All analytes were below their respective LOQ values for all samples analyzed. This pattern was also observed in previous studies with commercial drug samples in solid dosage form [34,36,38,40,41,57]. Consequently, all samples are within the limits recommended by the USP Chapter 232 taking into account the maximum daily dose of each medicine as indicated in the package insert, i.e., lower than 10 g $day^{-1}$ for tablets drugs. Recovery values ranged from 90 to 108% were observed by spike experiments at both levels based on acceptable recoveries established from 70 to 150% [23]. No matrix effects were observed for DLLME-ICP OES measurements and the repeatability was demonstrated by a precision ≤9% RSD (n = 3) considering all samples.

TABLE 3.13 - Found concentrations (mean ± standard deviation, µg $L^{-1}$, n = 3) and recovery values in parenthesis (mean ± RSD, %) obtained for the spiked in digested drug samples (A-H) according to the *J* value using DLLME-ICP OES.

| Sample | Added concentration | Found concentration | | |
|---|---|---|---|---|
| | | Cd | Hg | Pb |
| A | 0.5*J* | 5.0 ± 0.2 (98 ± 2) | 31 ± 2 (102 ± 5) | 5.0 ± 0.3 (99 ± 6) |
| | 1.5*J* | 14.9 ± 0.7 (98 ± 4) | 95 ± 3 (105 ± 4) | 15.3 ± 0.9 (102 ± 6) |
| B | 0.5*J* | 5.2 ± 0.1 (99 ± 4) | 30.3 ± 0.6 (101 ± 2) | 4.6 ± 0.2 (91 ± 4) |
| | 1.5*J* | 14.9 ± 0.7 (97 ± 6) | 87 ± 3 (97 ± 3) | 14 ± 1 (91 ± 8) |
| C | 0.5*J* | 5.1 ± 0.3 (98 ± 2) | 30 ± 2 (99 ± 8) | 4.5 ± 0.3 (90 ± 6) |
| | 1.5*J* | 14.3 ± 0.9 (94 ± 7) | 88 ± 7 (98 ± 7) | 13.7 ± 0.8 (91 ± 5) |
| D | 0.5*J* | 5.0 ± 0.3 (100 ± 6) | 29 ± 2 (95 ± 6) | 5.2 ± 0.3 (103 ± 7) |
| | 1.5*J* | 15 ± 1 (100 ± 9) | 85 ± 4 (95 ± 4) | 15 ± 1 (101 ± 8) |
| E | 0.5*J* | 4.8 ± 0.3 (97 ± 6) | 32 ± 1 (105 ± 4) | 5.40 ± 0.09 (108 ± 2) |
| | 1.5*J* | 16 ± 1 (103 ± 7) | 90 ± 7 (100 ± 8) | 15 ± 1 (102 ± 9) |
| F | 0.5*J* | 5.0 ± 0.3 (99 ± 6) | 30 ± 2 (100 ± 7) | 5.0 ± 0.2 (101 ± 4) |
| | 1.5*J* | 15 ± 1 (98 ± 6) | 93 ± 6 (104 ± 6) | 14.9 ± 0.6 (99 ± 4) |
| G | 0.5*J* | 4.8 ± 0.1 (96 ± 3) | 31 ± 2 (103 ± 6) | 4.8 ± 0.2 (95 ± 3) |
| | 1.5*J* | 16.0 ± 0.1 (106 ± 1) | 96 ± 2 (107 ± 3) | 14.7 ± 0.8 (98 ± 5) |
| H | 0.5*J* | 5.13 ± 0.06 (103 ± 1) | 29 ± 2 (98 ± 8) | 5.0 ± 0.3 (99 ± 5) |
| | 1.5*J* | 15.4 ± 0.9 (103 ± 6) | 88 ± 5 (97 ± 5) | 14.7 ± 0.2 (98 ± 2) |

0.5*J*: Spiked digest with 5.0, 30 and 5.0 µg $L^{-1}$ of Cd, Hg and Pb, respectively; 1.5*J*: Spiked digest with 15, 90 and 15 µg $L^{-1}$ of Cd, Hg and Pb, respectively.

### 3.2.6 - Conclusions

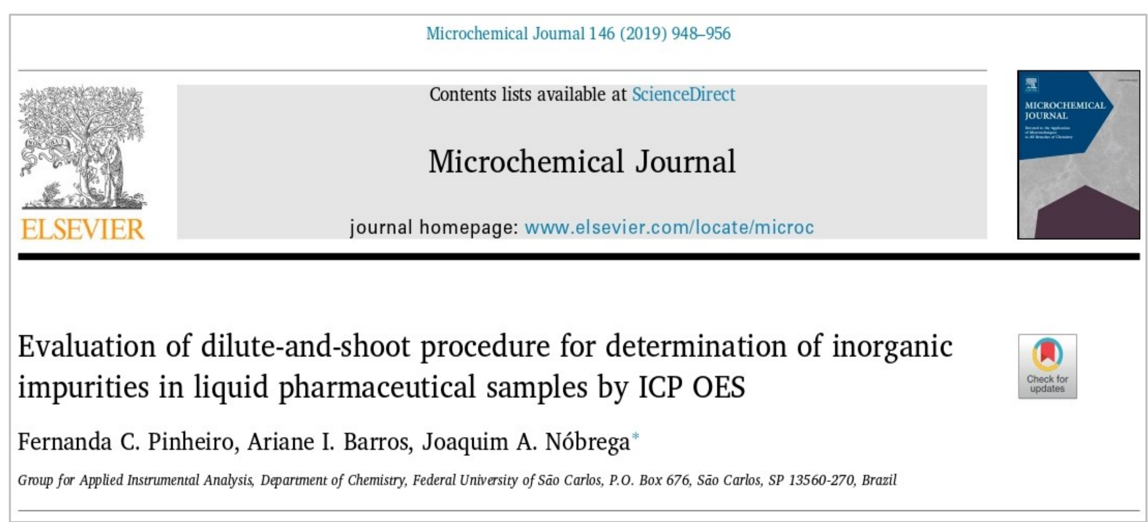
The developed DLLME procedure combined with ICP OES was successfully applied to the simultaneous extraction/preconcentration of Cd, Hg and Pb for trace determination of the above-mentioned elements using ICP OES after a microwave-assisted acid digestion of drug samples using dilute nitric acid. Analytical performance was well validated in the terms of linearity, LOQ, repeatability, and accuracy in accordance with the USP Chapter 233. Pharmaceutical sample preparation using dilute nitric acid solutions provide safer operation and reduced acid consumption. Posteriorly, DLLME affords high enrichment factors, simplicity and sustainability once reagents requirements and waste generation are extremely minimized. When compared with conventional ICP OES analysis, DLLME-ICP OES affords a significant increase of sensitivity showing an enrichment factor on average of 65-fold.

Consequently, considering the benefits of direct analysis of organic phase using a multinebulization based system and the appropriate multivariate optimization of DLLME, suitable sensitivity to follow USP requirements for determination of Cd, Hg and Pb using ICP OES was achieved. Therefore, DLLME-ICP OES methods can be seen as a promising alternative for trace elemental analysis in drug samples according to ICH guidelines and USP chapters.

# 4 - CHAPTER 4

# PHARMACEUTICAL SAMPLES IN LIQUID DOSAGE FORM

## 4.1 - Evaluation of dilute-and-shoot procedure for determination of inorganic impurities in liquid pharmaceutical samples by ICP OES


Microchemical Journal 146 (2019) 948–956

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Evaluation of dilute-and-shoot procedure for determination of inorganic impurities in liquid pharmaceutical samples by ICP OES

Fernanda C. Pinheiro, Ariane I. Barros, Joaquim A. Nóbrega*

*Group for Applied Instrumental Analysis, Department of Chemistry, Federal University of São Carlos, P.O. Box 676, São Carlos, SP 13560-270, Brazil*

### 4.1.1 - Abstract

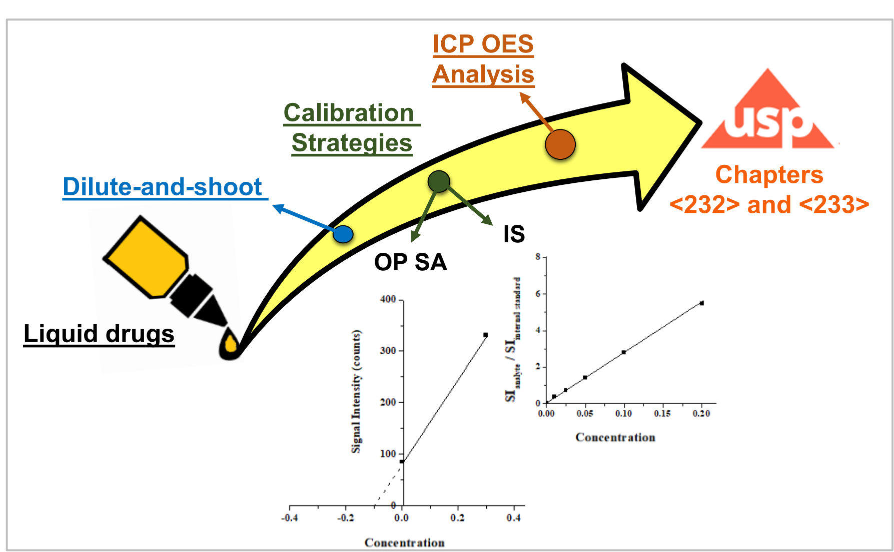
This study evaluated a dilute-and-shoot procedure for determination of 23 elemental impurities in liquid pharmaceutical samples by inductively coupled plasma optical emission spectrometry (ICP OES). Two dilution factors were tested for analysis of four liquid drugs (10-fold and 20-fold dilution in 0.14 mol $L^{-1}$ $HNO_3$). Microwave-assisted digestion using 7.0 and 2.0 mol $L^{-1}$ $HNO_3$ was used for comparison purposes. The accuracy and precision were evaluated by addition-recovery experiments and satisfactory recoveries were obtained only when matrix effects were corrected for applying internal standardization (IS) or one-point standard addition (OP SA) calibration methods. Bismuth, Ge and Y were evaluated as internal standards and recoveries ranged from 86 to 116% when the best internal standard was employed for each analyte. For OP SA, recoveries varied from 78 to 119%. The relative standard deviations for all elements and samples were lower than 9.0% for both calibration methods applied. The LODs obtained for IS and OP SA were lower than the lower level of addition suggested by the Chapter 233,

---

*Group for Applied Instrumental Analysis, Department of Chemistry, Federal University of São Carlos, P.O. Box 676, São Carlos, SP, 13560-270, Brazil.*
**Corresponding author: djan@ufscar.br*
*DOI: https://doi.org/10.1016/j.microc.2019.02.021*

except for Pb and Tl, and all samples are within the limits recommended by USP considering the maximum daily dose of each liquid drug and the diluted factor adopted in the analytical procedure. The tailored calibration methods were essential to correct for matrix effects enabling application of dilute-and-shoot procedure for samples 10-fold diluted and making feasible the elemental impurities analysis of liquid drugs by ICP OES.

### 4.1.2 - Graphical Abstract




### 4.1.3 - Introduction

After a century of the Chapter 231 validity, which regulated the determination of elemental impurities in pharmaceutical samples using sulfide precipitation and evaluation of the staining resulting from the suspension, the United States Pharmacopoeia (USP) has established two news Chapters, 232 [21] and 233 [23]. These chapters proposed analytical procedures for determination of 24 elemental impurities by either inductively coupled plasma optical emission spectrometry (ICP OES) or inductively coupled plasma mass spectrometry (ICP-MS). It is well known how some of these elements are also critical when present in foods, such as fishes [135]. On the other hand, ICP OES and ICP-MS are largely used for trace analysis and even combination of both instrumental methods was already demonstrated and recently re-evaluated [136].

Sample preparation methods of pharmaceutical products can include simple dissolution in acids [31] or organic solvents [53,54], and procedures

generally used for active pharmaceutical ingredients (APIs) and solid drugs (pills and tablets), such as microwave-induced combustion [61,137] and microwave-assisted digestions [34–38,61] for elemental determination or, when needed, for speciation analysis of toxic elements [138]. Dilute-and-shoot procedures are interesting for fast routine analysis [139], however, for elemental determination in complex samples by instrumental methods based on plasma, the direct introduction of only diluted samples must be carefully evaluated due to the possibility of matrix effects associated with nebulization, transport and plasma energy. Differences among complex samples properties and standard solutions used for calibration, for instance, viscosities, dissolved carbon compounds, main matrix constituents, such as high concentrations of easily ionizable elements, can cause severe transport or spectral interferences [6,140].

For complex matrices analysis, external standard calibration (EC) method may not be effective due to the physical and chemical differences among samples and reference solutions. Some calibration methods alternatives to EC can be used to correct for matrix effects [118], such as standard additions (SA) [141], internal standardization (IS) [37,142], standard dilution analysis (SDA) [52], multi-energy calibration (MEC) [143] and one-point standard addition (OP SA) [144,145].

For MEC and SDA only two calibrations solutions are needed per sample. In the MEC method, the instrument response at several wavelengths is monitored for each analyte [143,146]. On the other hand, SDA is a novel calibration strategy based on combining the methods of IS and SA to simultaneously correct for matrix effects and signal fluctuations [52,118]. Although both methods are effective for matrix effects correction, a minor difficulty associated to these calibrations strategies is data processing. Probably, the implementation of data processing software to automatically calculate the analyte concentration in the sample would contribute to increase the adoption of both strategies in routine analysis.

In SA calibration, the analyte is added to the sample in increasing concentrations, thus, standard solutions are prepared in the sample medium correcting for matrix effects [118,141]. Usually more than four standard solutions are used to analytical curve construction. Thereby, the use of multi-point SA calibration can be a time-consuming method not interesting for analysis of a

high number of samples. This problem can be avoided employing OP SA calibration, since only two standards are used per sample. Proposed by Zhu and Chiba (2012) [120], OP SA calibration was used for elemental analysis by ICP-MS combining gravimetric standard additions method with internal standardization. The analyte concentration is determined considering the signal and mass of analyte using appropriate equation proposed by authors. This calibration strategy was also used to determine As in seawater [144] and Sb in natural waters [145] by photochemical vapor generation ICP-MS. There are no reports in the literature about matrix effects correction using OP SA method for ICP OES analysis.

Other well established calibration method is the IS. In this, the calibration curve is plotting correlating the standard concentrations with the ratio standard signal/internal standard signal. It is expected that internal standard controls the sample processing during the analyses correcting for possible fluctuations [118,147]. So, a constant concentration of internal standard is added to all samples, standard solutions and analytical blanks and, preferably, the selected internal standard would present chemical and physical properties similar to the analyte. Thereby, the selected internal standard must not be present in the original samples. In determinations by ICP OES, the use of Y as internal standard is commonly reported in the literature [37,142,148–151]. Yttrium was used as internal standard for determination of As, Cd, V, Cr, Ni, Cu, Mo, Ru, Rh, and Pd in two excipients by ICP-MS and Tl was used as internal standard for Os, Ir, Pt, Pb, and Hg [37]. However, after harmonization with International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) [20], Tl was incorporated as analyte in the Chapter 232 [21].

Therefore, considering the need of simplifying drug analysis aiming routine determinations this work evaluated the application of a dilute-and-shoot approach associated with IS or OP SA for determination of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V in liquid drugs. The applicability of the developed procedure was assessed by comparison of analyte determinations using as reference microwave-assisted digestion as well as by determining dissolved organic carbon and evaluation of carbon effects on plasma signals.

### 4.1.4 - Experimental

*4.1.4.1. Samples and sample preparation*

Four drug samples marketed in liquid form (oral administration route) were analyzed (Table 4.1).

TABLE 4.1 - Function, active principle and excipients for the liquid drug samples analyzed.

| Sample | Function | Active principle | Excipient |
| --- | --- | --- | --- |
| A | hepatic metabolic disorders | choline citrate, betaine and racemetionine | sorbitol, sodium saccharin dihydrate, quinoline yellow, methylparaben, propylparaben, pineapple aroma and water |
| B | antipyretic and antipyretic | dipyrone monohydrate | sodium phosphate monobasic, sodium phosphate dibasic, sodium saccharin and water |
| C | antiallergic | dexchlorpheniramine maleate | sucrose, ethyl alcohol, orange flavor, sodium citrate, sodium chloride, menthol, methylparaben, propylparaben, propylene glycol, sorbitol and water |
| D | analgesic | paracetamol | citric acid, sodium benzoate, sodium cyclamate, sodium saccharin, sodium metabisulphate, macrogol 400, caramel flavor, yellow colorant and water |

Two dilution factors were evaluated using 0.14 mol $L^{-1}$ $HNO_3$ as diluent: (1) 10-fold and (2) 20-fold diluted. For comparison purposes two sample preparation methods were applied (1) microwave-assisted acid digestion in 2.0 mol $L^{-1}$ $HNO_3$ and (2) microwave-assisted acid digestion in 7.0 mol $L^{-1}$ $HNO_3$. For samples digestion, approximately 1.0 mL were placed in the teflon-perfluoroalkoxy alkanes (PFA) digestion vessels and microwave-assisted digested in triplicate (UltraWave, Milestone, Sorisole, Italy) in 7.0 mL of both nitric acid concentrations. Subsequently, digests were diluted to 40.0 mL with distilled-deionized water. Volumes of 150 mL of water and 5 mL of concentrated nitric acid were inserted into the single reaction chamber (SRC) and the chamber was pressurized with nitrogen gas to 40 bar. The microwave heating program was applied as follows: (1) 2.5 min to reach 140 °C, (2) 2.5 min hold at 140 °C, (3) 2.5 min to reach 180 °C, (4) 2.5 min hold at 180 °C, (5) 10 min to reach 220 ºC and (6) 10 min hold at 220 °C.

*4.1.4.2. Instrumentation*

Elemental analysis was performed using an iCAP 6000 ICP OES (Thermo Fisher Scientific, EUA) operated in axial view. Argon (99.996%, White Martins-Praxair, Sertãozinho, SP, Brazil) was used in all measurements. A *V-Groove* nebulizer was used aiming the introduction of samples with high solids contents. Plasma operating conditions are described in Table 4.2.

TABLE 4.2 - Instrumental parameters for ICP OES determinations.

| Instrument parameter | Operating condition |
|---|---|
| RF applied power (kW) | 1.2 |
| Plasma gas flow rate (L $min^{-1}$) | 12 |
| Auxiliary gas flow rate (L $min^{-1}$) | 0.50 |
| Carrier gas flow rate (L $min^{-1}$) | 0.70 |
| Integration time (s) | 15 |
| Sample introduction flow rate (mL $min^{-1}$) | 1.0 |
| Nebulizer | V-Groove |
| Spray chamber | Cyclonic |
| Number of replicates | 3 |

| Element | Emission line (nm) | Element | Emission line (nm) | Element | Emission line (nm) |
|---|---|---|---|---|---|
| Ag | 328.068 | Hg | 184.950 | Pt | 214.423 |
| As | 189.042 | Ga | 294.363 | Rh | 343.489 |
| Au | 242.795 | Ge | 265.118 | Ru | 267.876 |
| Ba | 455.403 | Ir | 224.268 | Sb | 217.581 |
| Bi | 223.061 | Li | 670.784 | Se | 196.090 |
| Cd | 226.502 | Mo | 202.030 | Sn | 189.989 |
| Co | 228.616 | Ni | 221.647 | Tl | 190.856 |
| Cr | 357.869 | Pb | 220.353 | V | 292.402 |
| Cu | 324.754 | Pd | 340.458 | Y | 371.030 |

*4.1.4.3. Reagents and standard solutions*

Experiments were performed using $HNO_3$ (Synth. Diadema, SP, Brazil) purified in a sub-boiling distillation system Distillacid™ BSB-939-IR (Berghof, Eningen, Germany) and ultrapure water, resistivity >18.2 MΩ cm,

(Milli-Q®, Millipore, Bedford, MA, USA). Standard solutions used for ICP OES calibration and for addition and recovery experiments were prepared by dilution of 1000 mg $L^{-1}$ of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V (Qhemis, São Paulo, SP, Brazil) in 0.14 mol $L^{-1}$ $HNO_3$ medium, as well as the internal standards evaluated: Bi, Ge and Y.

For IS method, the concentrations of the solutions used for analytical calibration curve for all elements were 0, 0.010, 0.025, 0.050, 0.10, 0.20, 0.30 and 0.50 mg $L^{-1}$ prepared in 0.14 mol $L^{-1}$ $HNO_3$ medium and 0.10 mg $L^{-1}$ of each internal standard was added to each solution, analytical blank and diluted and digested samples. The accuracy and precision of the methods were evaluated by addition and recovery experiments in two concentration levels: 0.10 and 0.30 mg $L^{-1}$. Spikes were added before microwave-assisted digestion.

For OP SA method, calibration curves were obtained using two calibration standards for each sample. Standard 1 is composed of sample + blank and Standard 2 is composed of sample + standard addition [120,144,145]. Thus, Standard 1 was composed of sample 10 or 20-fold diluted in 0.14 mol $L^{-1}$ $HNO_3$ and Standard 2 contained sample 10 or 20-fold diluted and 0.10 mg $L^{-1}$ of all analytes. The blank was used in the Standard 1 to adjust with the standard addition volume of the Standard 2. Accuracies were evaluated by addition and recovery experiments in concentrations of 0.10 and 0.20 mg $L^{-1}$ and three concentrations of standard addition were evaluated for each level. Thereby, for level 0.10 mg $L^{-1}$ Standard 1 was composed of sample 10 or 20-fold diluted in 0.14 mol $L^{-1}$ $HNO_3$ and all analytes in the concentration of 0.10 mg $L^{-1}$ and the Standard 2 contained sample 10 or 20-fold diluted and all analytes in the concentrations evaluated (0.20, 0.40 and 0.60 mg $L^{-1}$). For the concentration 0.20 mg $L^{-1}$ Standard 1 was composed of sample 10 or 20-fold diluted in 0.14 mol $L^{-1}$ $HNO_3$ and all analytes at 0.20 mg $L^{-1}$ and the Standard 2 contained sample 10 or 20-fold diluted and all analytes in the concentrations evaluated (0.40, 0.60 and 1.0 mg $L^{-1}$).

The dissolved organic carbon concentration was determined in all digests and diluted solutions. Carbon was determined using the atomic emission line 193.090 nm and dehydrated oxalic acid (Mallinckrodt Chemicals, St. Louis, MO, USA) was used as the carbon source for preparing calibrating analytical solutions. Carbon effects were also investigated by determination of

all analytes in standard solutions containing increasing concentrations of carbon: 0.50, 1.0, 2.0 and 3.0 % m $v^{-1}$.

## 4.1.5 - Results and Discussion

*4.1.5.1. Dilute-and-shoot procedure and matrix effects*

For analysis of liquid pharmaceutical samples, application of a dilute-and-shoot strategy is interesting for routine analysis. However, taking into account the solution characteristics for elemental determination by ICP OES, some important aspects must be evaluated, such as residual acidity (RA), total dissolved solids (TDS) and dissolved organic carbon for avoiding matrix effects and also for avoiding wear of equipment [6]. Thus, the dilute-and-shoot procedure was applied for liquid drug samples 10 and 20-fold diluted and microwave-assisted digested using 7.0 and 2.0 mol $L^{-1}$ $HNO_3$. Dissolved organic carbon was determined in all samples (Figure 4.1).

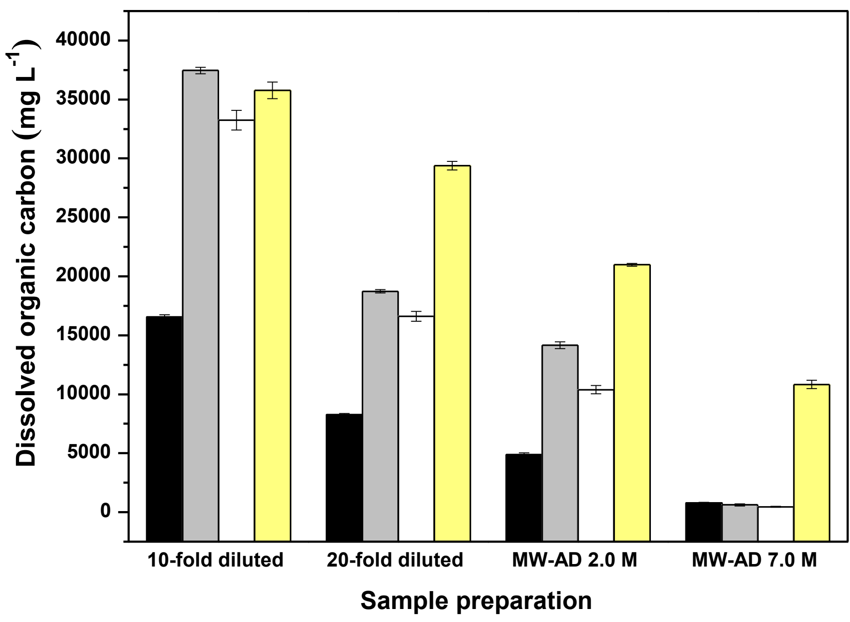



FIGURE 4.1 - Dissolved organic carbon in samples (A-D) for each sample preparation procedure (mg $L^{-1}$, mean ± standard deviations, n = 3). (■) A; (■) B; (□) C; (■) D. MW-AD means microwave-assisted digested using 7.0 and 2.0 mol $L^{-1}$ $HNO_3$.

As expected, dissolved organic carbon concentrations were significantly higher for only diluted samples when compared with microwave-assisted digested samples. Additionally, the four samples analyzed have distinct concentrations of dissolved organic carbon since they contain different APIs and excipients (Table 4.1), characterizing complex and different matrices.

Solutions with high carbon concentrations may cause changes in the plasma characteristics and, consequently, in distribution of species in the argon plasma. Grindlay et al. [101] showed that sensitivities for As, Au, Hg, Sb, and Se are higher for carbon-containing solutions than for solutions without carbon. Besides the changes in the plasma characteristics, the authors related the matrix effects for these elements to increase of analytical signals caused by charge transfer reactions between $C^+$ and the respective element in the plasma. Other elements, such as Cd, Pb, Ir and Pt, could also be involved in carbon-based charge transfer reactions [101,116].

Thus, carbon effects were evaluated monitoring the signal intensities for all analytes in standard solutions containing increasing concentrations of carbon. As expected, increments of signal intensities were observed for As, Au, Sb, Se, and Pt, however, this was not observed for Hg neither for the other analytes (Figure 4.2). For As, Au, Sb, Se, and Pt the signal intensities were normalized when using IS for all concentrations of carbon evaluated.

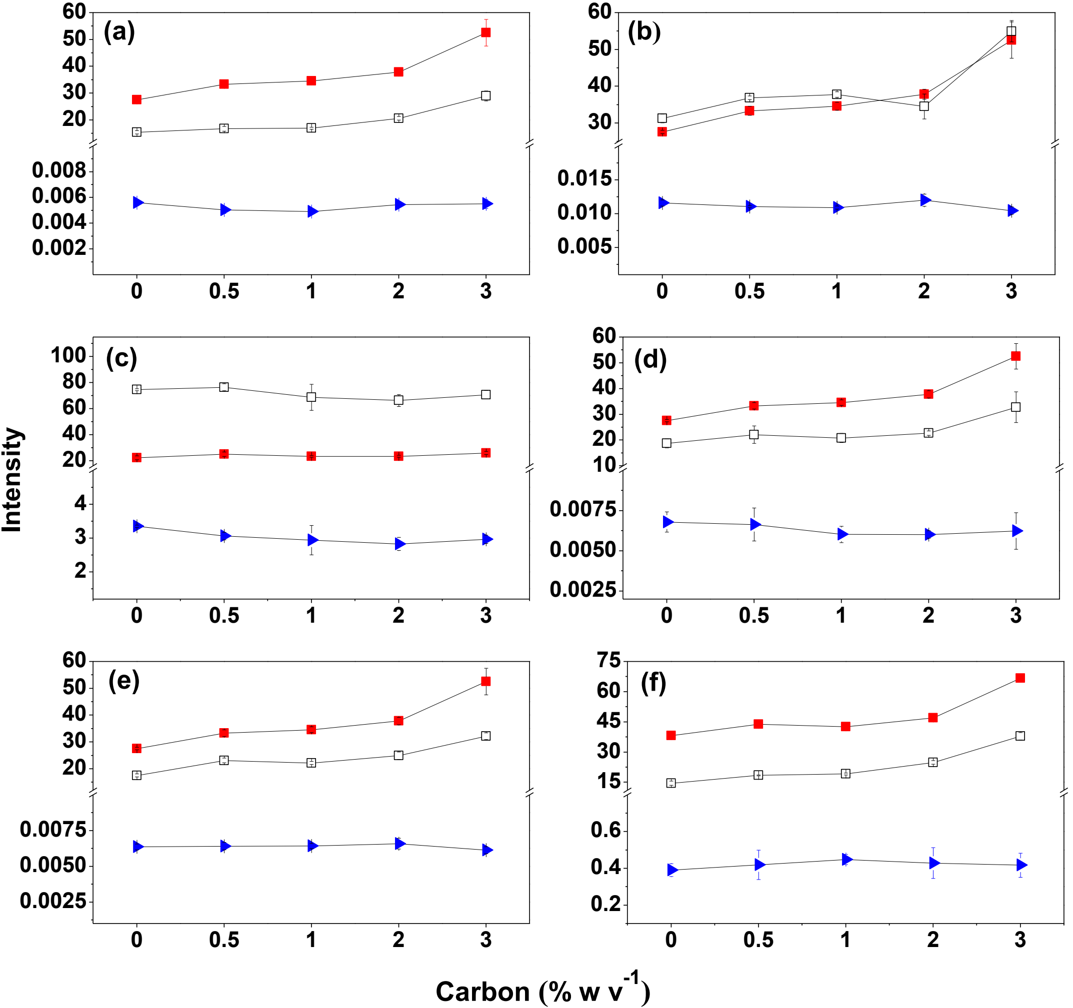



FIGURE 4.2 - Signal intensities for **(a)** As, **(b)** Au, **(c)** Hg, **(d)** Pt, **(e)** Sb, and **(f)** Se in standard solutions containing increasing concentrations of carbon. (■) Internal standard signal. (□) Analyte signal; (▶) Ratio of the analyte signal/internal standard signal.

Bismuth, Ga, Ge and Y were evaluated as internal standards for 23 analytes. Bismuth was the best internal standard for Hg, Mo and Rh; and Ge was the best one for Pd, Se, Sn and Tl. For Ag, As, Au, Ba, Cd, Co, Cr, Cu, Ir, Li, Ni, Pb, Pt, Ru, Sb, and V the best internal standard was Y.

Another effective alternative to correct for matrix effects is the SA method, however, the amount of sample consumed and preparation time are drawbacks associated with this method because more than four addition points are needed to obtain analytical calibration curve for each sample and analyte [118,141]. These disadvantages can be avoided using OP SA calibration, since only two standards are used for sample. However, for proper accuracy of this

method, the concentration of the addition point must be evaluated because the added standard concentration cannot be too higher compared to the analyte concentration [120,152,153]. Thus, OP SA also was evaluated in the study to correct for matrix effects. For comparison purposes, EC and SA were evaluated.

*4.1.5.2. Analytical performance for each calibration method*

The main figures of merit for evaluating analytical performance (accuracy, linear correlation coefficient and standard error) were calculated for all calibration methods evaluated. For EC, the calibration curve was built by plotting the analyte concentration on the x-axis with the signal intensity (SI) on the y-axis and the analyte concentration in the sample ($C_{analyte}$) is obtained using the relationship $C_{analyte}$ = (SI - b)/a, where (b) is the intercept of the regression line and (a) is the slope of straight line.

The IS calibration curve was built using the same approach, however with the ratio analyte signal/internal standard signal on the y-axis. However, for SA calibration, the $C_{analyte}$ was obtained by extrapolation of the x axis at y = 0, (i.e. $C_{analyte}$ = b/a) and for OP SA, the $C_{analyte}$ was also obtained by extrapolation of the x axis at y = 0, however only two calibrations points, (i.e. x0 and x1) were used. For SA, at least four calibration points are needed, (i.e. x0 and x1-x4), where (x0) is a point without any analyte added and (x1-x4) are additions points with increasing concentrations [118,153], as shown in Figure 4.3 for Hg determination.

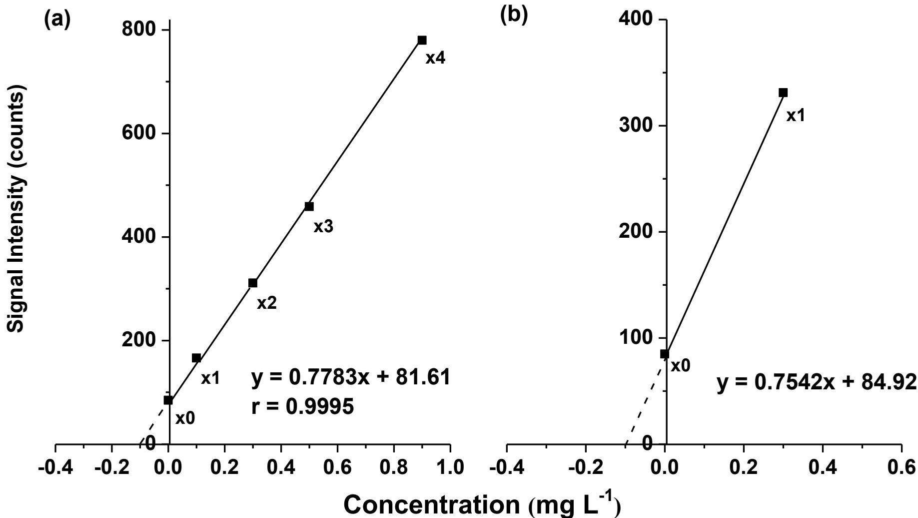



FIGURE 4.3 - Linear model for SA **(a)** and OP SA curve **(b)** for Hg determination in drug sample A 10-fold diluted. **(a)** x0 = 0.10, x1 = 0.20 and x2 = 0.40 mg $L^{-1}$ of Hg. **(b)** x0 = 0.10 and x1 = 0.40 mg $L^{-1}$ of Hg.

*4.1.5.3. Methods accuracy*

For evaluation of the methods accuracy, addition and recovery experiments in two concentration levels were applied in digested samples using 7.0 and 2.0 mol $L^{-1}$ $HNO_3$ and samples 10 and 20-fold diluted in 0.14 mol $L^{-1}$ $HNO_3$ using EC, IS, SA and OP SA. For determinations using EC, recoveries lower than 80% were observed for most analytes for samples A, B and C and positive errors (recoveries >120%) for sample D 10-fold diluted (Table 4.3) were observed. However, when using the IS method, best recoveries were obtained ranging from 91 to 116% for sample A; 81 to 107% for sample B; 89 to 118% for sample C; and 87 to 115% for sample D. The same behavior was observed for samples 20-fold diluted (Figure 4.4).

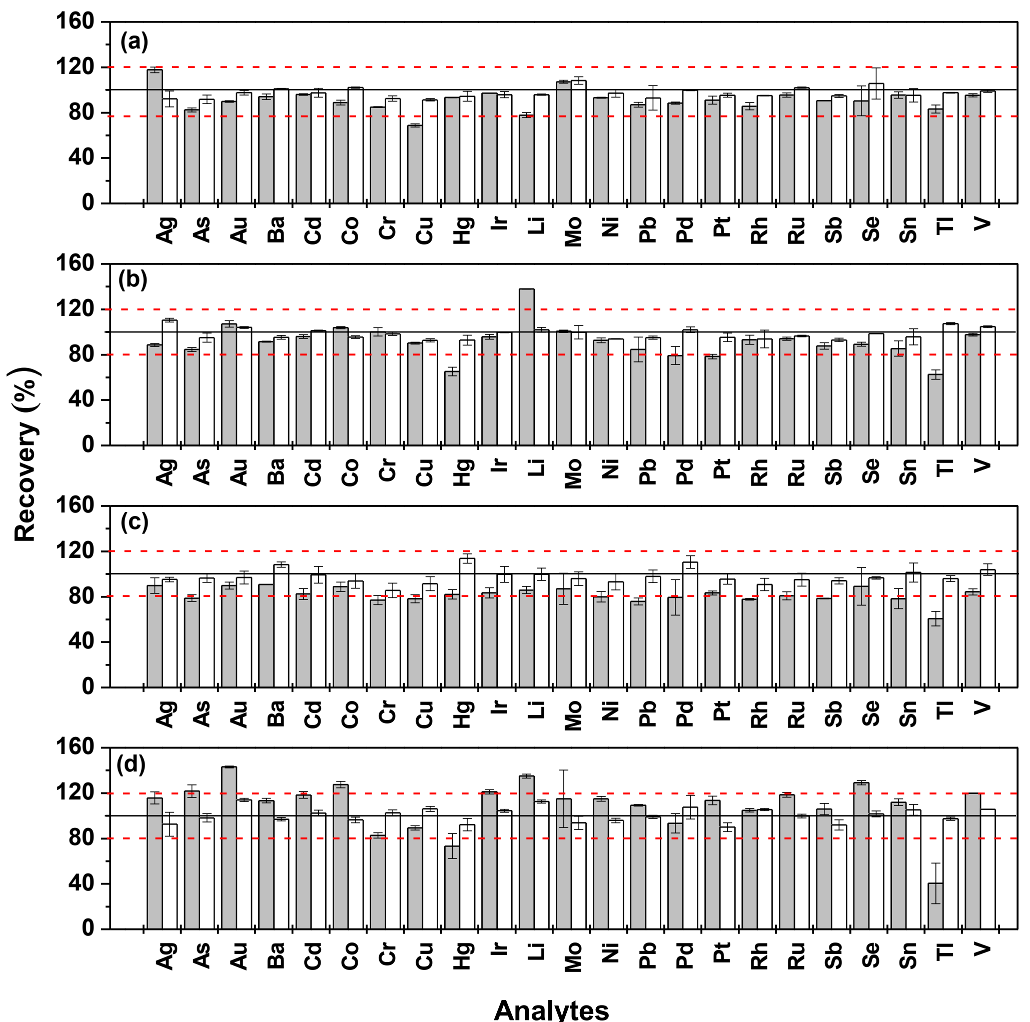



FIGURE 4.4 - Percentage recoveries for addition 0.10 mg $L^{-1}$ in samples **(a)** A; **(b)** B; **(c)** C and **(d)** D; 20-fold diluted with and without internal standard by ICP OES. (■) Without internal standard; (□) With internal standard.

TABLE 4.3 - Evaluation of calibration methods used for Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V determination by ICP OES in liquid drug samples (A-D) 10-fold diluted. Recovery (relative standard deviation, n = 3) for addition level of 0.10 mg $L^{-1}$.

| Analyte | IS | Samples | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | | | | B | | | | C | | | | D | | | |
| | | EC | IS | SA | OP SA | EC | IS | SA | OP SA | EC | IS | SA | OP SA | EC | IS | SA | OP SA |
| Ag | Y | 92 | 102.1 | 125 | 108 | 81 | 108.1 | 94 | 104 | 91 | 99 | 65 | 78 | 140 | 104 | 144 | 92 |
| | | (2) | (0.8) | (6) | (8) | (1) | (0.3) | (4) | (8) | (7) | (7) | (7) | (9) | (4) | (2) | (6) | (6) |
| As | Y | 130 | 98.9 | 93 | 91 | 132 | 98 | 92 | 97 | 152 | 96 | 97 | 101 | 141 | 109 | 93.9 | 84 |
| | | (2) | (0.2) | (4) | (6) | (4) | (6) | (4) | (7) | (3) | (1) | (4) | (2) | (5) | (3) | (0.4) | (2) |
| Au | Y | 81 | 108 | 97 | 107 | 125 | 99 | 103 | 112 | 83 | 95 | 64 | 94 | 135 | 98 | 97 | 96 |
| | | (6) | (2) | (4) | (7) | (2) | (1) | (1) | (6) | (1) | (2) | (8) | (4) | (2) | (2) | (4) | (5) |
| Ba | Y | 81 | 92 | 97 | 94 | 85 | 96 | 103 | 98 | 78 | 96 | 102 | 97 | 121 | 98 | 97 | 94 |
| | | (2) | (3) | (4) | (4) | (2) | (5) | (2) | (5) | (3) | (4) | (3) | (7) | (5) | (4) | (2) | (2) |
| Cd | Y | 84 | 100 | 97.7 | 95 | 87 | 99 | 101 | 98 | 82 | 102.1 | 99 | 97 | 125 | 102 | 97 | 96 |
| | | (4) | (1) | (0.7) | (4) | (2) | (5) | (1) | (4) | (1) | (0.2) | (2) | (7) | (3) | (1) | (2) | (3) |
| Co | Y | 90 | 100 | 96 | 96 | 95 | 95 | 100 | 98 | 87.4 | 95.7 | 99 | 97 | 133 | 95 | 97 | 95 |
| | | (4) | (1) | (1) | (2) | (2) | (5) | (1) | (4) | (0.4) | (0.4) | (2) | (7) | (3) | (1) | (1) | (3) |
| Cr | Y | 81.9 | 111 | 97 | 94 | 97 | 103 | 95 | 97 | 79 | 92 | 102 | 99 | 119 | 92 | 98 | 96 |
| | | (0.2) | (5) | (3) | (2) | (3) | (5) | (10) | (5) | (4) | (5) | (6) | (7) | (5) | (3) | (2) | (3) |
| Cu | Y | 83.7 | 103 | 97 | 93 | 84 | 94 | 96 | 98 | 78 | 94 | 103 | 98 | 119 | 95 | 97 | 94 |
| | | (0.2) | (5) | (4) | (4) | (2) | (5) | (10) | (4) | (2) | (3) | (3) | (7) | (5) | (3) | (2) | (2) |
| Hg | Bi | 81 | 92 | 92 | 95 | 60 | 81 | 95 | 99 | 83 | 90 | 98 | 95 | 120 | 89 | 66 | 107 |
| | | (2) | (6) | (2) | (4) | (4) | (4) | (9) | (2) | (1) | (8) | (1) | (7) | (2) | (8) | (2) | (6) |

* SE: Standard error.

TABLE 4.3 - Evaluation of calibration methods used for Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V determination by ICP OES in liquid drug samples (A-D) 10-fold diluted. Recovery (relative standard deviation, n = 3) for addition level of 0.10 mg $L^{-1}$ (continuation).

| Analyte | IS | Samples | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | | | | B | | | | C | | | | D | | | |
| | | EC | IS | SA | OP SA | EC | IS | SA | OP SA | EC | IS | SA | OP SA | EC | IS | SA | OP SA |
| Ir | Y | 85 | 101 | 94 | 100 | 87 | 98 | 97 | 98 | 82.9 | 101.2 | 93 | 101 | 126 | 103.9 | 86 | 93 |
| | | (4) | (1) | (2) | (4) | (1) | (3) | (5) | (3) | (0.3) | (0.7) | (3) | (9) | (2) | (0.3) | (5) | (3) |
| Li | Y | 80 | 110 | 93 | 95 | 143 | 106 | 104 | 100 | 83.9 | 100 | 103 | 98 | 147 | 116 | 98 | 97 |
| | | (2) | (5) | (3) | (2) | (2) | (5) | (1) | (3) | (0.3) | (4) | (3) | (5) | (5) | (3) | (1) | (3) |
| Mo | Bi | 82 | 100 | 93 | 96 | 92 | 99 | 97 | 95 | 81 | 88.9 | 97 | 97 | 123 | 87 | 96 | 92 |
| | | (5) | (1) | (1) | (2) | (2) | (1) | (3) | (3) | (6) | (0.3) | (3) | (9) | (8) | (9) | (1) | (3) |
| Ni | Y | 81 | 100 | 95 | 95 | 85 | 93 | 99 | 101 | 79.9 | 95.9 | 97 | 96 | 121 | 96.9 | 93 | 94 |
| | | (4) | (2) | (2) | (3) | (3) | (5) | (2) | (1) | (0.3) | (0.3) | (3) | (8) | (3) | (0.9) | (4) | (3) |
| Pb | Y | 79 | 91 | 94 | 95 | 77 | 94 | 99 | 97 | 78.7 | 105 | 97 | 96 | 112 | 98 | 91 | 93 |
| | | (3) | (3) | (2) | (6) | (1) | (3) | (3) | (3) | (0.3) | (7) | (4) | (9) | (4) | (3) | (3) | (6) |
| Pd | Ge | 101 | 100.1 | 98 | 104 | 65 | 102 | 91 | 101 | 85 | 119.1 | 99 | 103 | 86 | 108 | 99.8 | 88 |
| | | (4) | (0.7) | (6) | (6) | (5) | (9) | (9) | (7) | (6) | (0.3) | (3) | (9) | (2) | (4) | (0.2) | (2) |
| Pt | Y | 86 | 92 | 97 | 96 | 68 | 87 | 91 | 109 | 83.4 | 96 | 87 | 94 | 127 | 94.1 | 89 | 88 |
| | | (1) | (3) | (6) | (7) | (3) | (2) | (8) | (4) | (0.3) | (3) | (2) | (9) | (3) | (0.9) | (2) | (2) |
| Rh | Bi | 83 | 94 | 80 | 86 | 93 | 94 | 90 | 84 | 79.8 | 91 | 87 | 96 | 121 | 106.1 | 69 | 81 |
| | | (4) | (7) | (1) | (9) | (4) | (8) | (4) | (6) | (0.3) | (5) | (7) | (7) | (6) | (0.9) | (5) | (7) |
| Ru | Y | 85 | 95 | 94 | 91 | 85 | 95 | 102 | 99 | 81.9 | 99 | 102 | 101 | 121 | 97.7 | 92 | 93 |
| | | (3) | (4) | (3) | (2) | (1) | (2) | (2) | (5) | (0.3) | (5) | (5) | (9) | (2) | (0.2) | (4) | (4) |

* SE: Standard error.

TABLE 4.3 - Evaluation of calibration methods used for Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V determination by ICP OES in liquid drug samples (A-D) 10-fold diluted. Recovery (relative standard deviation, n = 3) for addition level of 0.10 mg $L^{-1}$ (continuation).

| Analyte | IS | Samples | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | | | | B | | | | C | | | | D | | | |
| | | EC | IS | SA | OP SA | EC | IS | SA | OP SA | EC | IS | SA | OP SA | EC | IS | SA | OP SA |
| Sb | Y | 86 (5) | 91 (5) | 92 (3) | 97 (4) | 84 (2) | 97 (4) | 95 (1) | 95 (6) | 80.8 (0.3) | 100 (3) | 100 (6) | 109 (7) | 112 (1) | 92 (2) | 97 (2) | 94 (7) |
| Se | Ge | 80 (10) | 116 (1) | 94 (4) | 99 (9) | 75 (5) | 80 (8) | 93 (6) | 119 (8) | 84 (4) | 95.5 (0.3) | 98 (4) | 91 (6) | 135 (10) | 103 (7) | 86 (4) | 80 (8) |
| Sn | Ge | 86 (5) | 100 (1) | 89.8 (0.4) | 94 (2) | 97 (8) | 98 (7) | 99 (5) | 95 (6) | 76.8 (0.3) | 105 (3) | 99 (10) | 102 (7) | 117 (10) | 111 (4) | 89 (10) | 84 (4) |
| Tl | Ge | 78.9 (0.9) | 102.1 (0.8) | <SE* | <SE* | 74 (2) | 87.5 (0.5) | <SE* | <SE* | 69.9 (0.3) | 91 (4) | <SE* | <SE* | 84 (10) | 86 (5) | <SE* | <SE* |
| V | Y | 81 (3) | 96 (5) | 95 (2) | 93 (2) | 88 (2) | 103 (4) | 102 (2) | 99 (4) | 82.9 (0.3) | 105 (5) | 102 (3) | 98 (8) | 128 (5) | 107 (3) | 95 (3) | 93 (2) |

* SE: Standard error.

Due to the large matrix differences among the samples, it can be observed different behaviors for some recoveries of analytes without using internal standard. For instance, for samples 10-fold diluted, Ag, As and Co showed recoveries higher than 120% only for sample D, for Li the same was observed for samples B and D, however, positive errors were observed for Au in all samples. On the other hand, recovery was lower than 60% for Hg in sample B, and for Tl recoveries were lower than 80% for all samples. Matrix effects were also observed for sample D digested in both nitric acid concentrations (Figure 4.5).

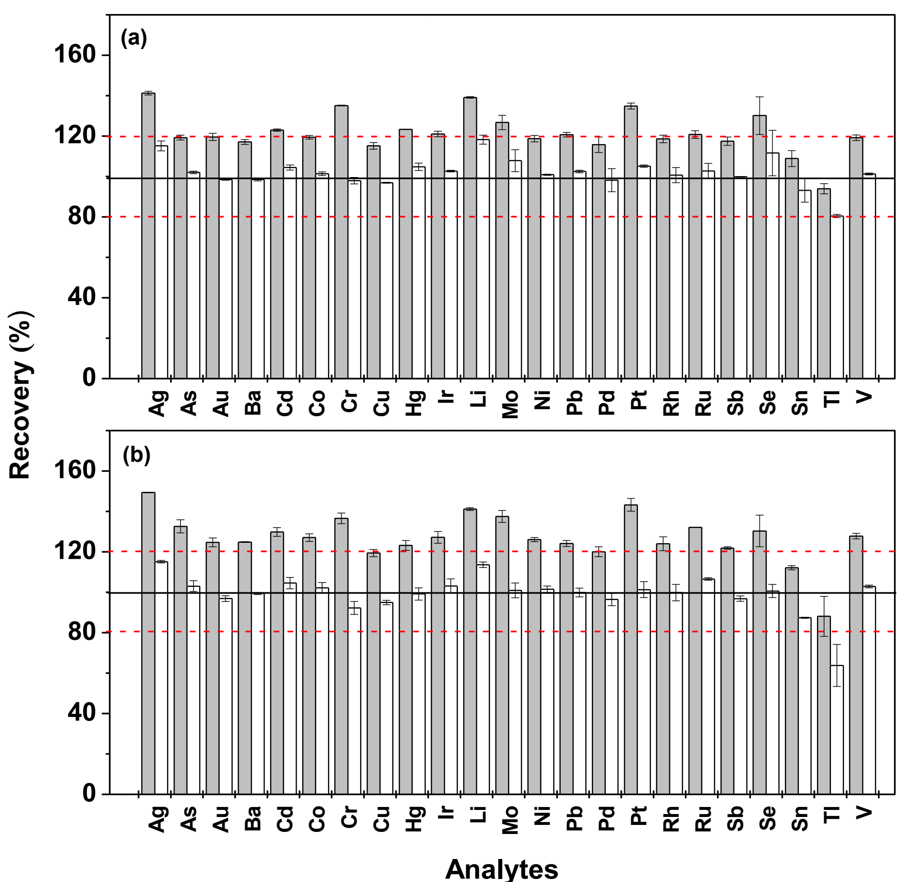



FIGURE 4.5 - Percentage recoveries for addition 0.10 mg $L^{-1}$ in sample D with and without internal standard by ICP OES. **(a)** Digested in 7.0 mol $L^{-1}$ $HNO_3$; **(b)** Digested in 2.0 mol $L^{-1}$ $HNO_3$. (■) Without internal standard; (□) With internal standard.

Positive errors (recoveries >115%) were obtained for all analytes in samples digested using 7.0 mol $L^{-1}$ $HNO_3$ and recoveries higher than 120%

were obtained in samples digested using 2.0 mol $L^{-1}$ $HNO_3$, except for Cu, Sn and Tl in both cases. When using internal standard best recoveries were obtained ranging from 93 to 115% for samples digested using 7.0 mol $L^{-1}$ $HNO_3$ and ranging from 92 to 115% samples digested using 2.0 mol $L^{-1}$ $HNO_3$. In addition to carbon effects, probably the observed matrix effects were originated by differences in viscosities for digests, diluted samples, and standards solutions. The use of IS led to more accurate recoveries due to correction of matrix effects associated with transport, nebulization, and/or energetic effects in the argon plasma [6,142].

For OP SA, accuracies were evaluated by addition and recovery experiments in two concentration levels (0.10 and 0.20 mg $L^{-1}$) and three concentrations of addition point concentrations were evaluated for each level: 0.20; 0.40 and 0.60 mg $L^{-1}$ for addition level 0.10 mg $L^{-1}$ and 0.40; 0.60 and 1.0 mg $L^{-1}$ for addition level 0.20 mg $L^{-1}$. For both levels of addition, the three addition point concentrations were effective to obtain satisfactory recoveries (80 to 120 %), showing that good results can be obtained when adding concentrations equivalent to twice the analyte concentrations. Table 4.3 shows the recoveries obtained for addition level of 0.10 mg $L^{-1}$ for all samples in each calibration method. Using a t-paired test with 95% of confidence, the recoveries obtained for Ag in all samples were better using OP SA than SA (For sample D, better recoveries were obtained using OP SA than SA for Hg and Rh. Recoveries were similar for others analytes and samples.

*4.1.5.4. Limits of detection and concentrations limits based on J values*

For EC and IS, limits of detection (LOD) and quantification (LOQ) were calculated considering background equivalent concentration (BEC), signal-to-background ratio (SBR) and relative standard deviation (RSD) for 10 measurements of blank solutions [125]. For OP SA and SA, the accuracy was evaluated based on the standard error (SE), according to equation (1):

$$SE = \sqrt{\frac{\sum_i^n (y_i - \hat{y})^2}{n-1}} \qquad \text{Equation (1)}$$

where $y_i$ is the analyte reference concentration (from the lower addition level value), $\hat{y}$ is the concentration determined by calibration strategy, and *n* is the number of samples analyzed (n = 4). For OP SA, the linearity was tested applying the test F, and in this case the ratio $F_{experimental}$ / $F_{tabulated}$ was calculated. Table 4.4 present ANOVA table obtained by calculating the regression model for arsenic. The ratio $F_{experimental}$ / $F_{tabulated}$ was 2236741.

TABLE 4.4 - ANOVA obtained by calculating the regression model for arsenic.

| | Degrees of freedom | Quadratic sum (QS) | Square mean (SM) | F calculated | F significance | F tabulated |
|---|---|---|---|---|---|---|
| Regression | 1 | 13205 | 13205 | 9960 | $6x10^{-8}$ | 0.004 |
| Residue | 4 | 5 | 1 | | | |
| Total | 5 | 13210 | | | | |

The $F_{calculated}$ value is related to mean of the square of regression (MQR) and mean of the square of residues (MQr), Ideally, the ratio between MQR and MQr should be high to affirm that the calculated model has an adequate statistical condition, allowing its use for predictions. The calculation of this ratio represents the variance, in which the values of F calculated and F tabulated are compared [154]. This ratio ≥10 demonstrated that the variances are statistically different (the quadratic mean of the regression is statistically different when compared with the quadratic mean of the residues) and the model can be considered linear [120,121,155].

The concentrations limits (known as *J* values) defined by Chapter 232 and 233 [21,23] is calculated by dividing the permissible daily exposures value (PDE) for each element by the maximum daily dose (MDD) of the drug and multiplied by the dilution factor (DF) adopted in the analytical procedure, as shown in equation (2):

$$J = \frac{PDE(\frac{\mu g}{day})}{\mathrm{MDD}\left(\frac{\mathrm{mL}}{\mathrm{day}}\right)\mathrm{x\ DF}} \qquad \text{Equation (2)}$$

According to the Chapter 233 [23] the accuracy must be evaluated by addition and recovery experiments with concentrations from 0.5*J* to 1.5*J* values. Thus, for evaluating if the respective LODs obtained for each calibration

method are adequate in terms of sensitivities, it was calculated the lower level of addition 0.5*J* considering 10-fold dilution (lower dilution adopted in this procedure) and MDD of 30 mL $day^{-1}$ (MDD for sample A). The specific MDD for other samples are 8 mL $day^{-1}$ for sample B, 20 mL $day^{-1}$ for sample C and 4.3 mL $day^{-1}$ for sample D. Higher MDD was chosen because it led to lower *J* values, and consequently, higher strictness to evaluate sensitivities. Table 4.5 shows the PDE values specific to oral administration, the addition level 0.5*J*, limit of detection, linear correlation coefficient, standard error and ratio $F_{experimental}$ / $F_{tabulated}$ obtained for determination of all analytes using each calibration method.

TABLE 4.5 - Analytical performance parameters for the determination of Ag, As, Au, Ba, Cd, Co, Cr, Cu, Hg, Ir, Li, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Se, Sn, Tl, and V in liquid drug samples by ICP OES using the calibration methods EC, IS, SA and OP SA.

| Analyte (nm) | PDE (µg dia$^{-1}$) | 0.5*J* addition (µg L$^{-1}$)[a] | LOQ (µg L$^{-1}$) | | | $R^2$ | | Standard error (µg L$^{-1}$) | | ratio $F_{experimental}$ / $F_{tabulated}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | EC_D[c] | EC | IS | EC | IS | SA | OP SA | OP SA |
| Ag (328.068) | 150 | 250 | 9 | 12 | 11 | 0.9982 | 0.9991 | 43 | 27 | 1979 - 57323 |
| As (189.042) | 15 | 25 | 18 | 9 | 10 | 0.9969 | 0.9997 | 8 | 10 | 180846 - 6295551 |
| Au (242.795) | 100 | 167 | 37 | 43 | 57 | 0.8994 | 0.9909 | 21 | 8 | 46485 - 902266 |
| Ba (455.403) | 1400 | 2.3[b] | 0,7 | 2 | 3 | 0.9973 | 1.0000 | 3 | 5 | 128974 - 4691952 |
| Cd (226.502) | 5 | 8 | 1 | 0.7 | 1 | 0.9968 | 0.9997 | 3 | 3 | 213855 - 2558852 |
| Co (228.616) | 50 | 83 | 3 | 2 | 3 | 0.9981 | 0.9996 | 3 | 4 | 229547 - 2926340 |
| Cr (357.869) | 11000 | 18.3[b] | 30 | 13 | 15 | 0.9957 | 0.9995 | 4 | 3 | 99779 - 2416358 |
| Cu (324.754) | 3000 | 5[b] | 14 | 4 | 4 | 0.9974 | 0.9996 | 4 | 4 | 119394 - 7320281 |
| Hg (184.950) | 30 | 50 | 7 | 6 | 7 | 0.9977 | 0.9991 | 20 | 12 | 211974 - 1643246 |
| Ir (224.268) | 100 | 167 | 13 | 7 | 10 | 0.9969 | 0.9997 | 10 | 5 | 168441 - 2749456 |
| Li (670.784) | 550 | 917 | 2 | 0.7 | 1 | 0.9962 | 0.9998 | 5 | 3 | 115832 - 6977673 |
| Mo (202.030) | 3000 | 5[b] | 5 | 14 | 14 | 0.9894 | 0.9991 | 6 | 6 | 197914 - 1962392 |
| Ni (221.647) | 200 | 333 | 9 | 5 | 6 | 0.9975 | 0.9995 | 5 | 4 | 226272 - 2925675 |
| Pb (220.353) | 5 | 8 | 24 | 16 | 18 | 0.9956 | 0.9991 | 7 | 4 | 273123 - 2904947 |
| Pd (340.458) | 100 | 167 | 23 | 19 | 21 | 0.9997 | 0.9994 | 5 | 10 | 93015 - 2468047 |
| Pt (214.423) | 100 | 167 | 50 | 19 | 23 | 0.9981 | 0.9994 | 11 | 10 | 210479 - 6883870 |
| Rh (343.489) | 100 | 167 | 40 | 32 | 43 | 0.9933 | 0.9992 | 23 | 17 | 86304 - 1753669 |
| Ru (267.876) | 100 | 167 | 22 | 15 | 19 | 0.9978 | 0.9989 | 6 | 6 | 123226 - 3145053 |
| Sb (217.581) | 1200 | 2[b] | 40 | 21 | 28 | 0.9970 | 0.9994 | 6 | 7 | 230038 - 897978 |
| Se (196.090) | 150 | 250 | 60 | 40 | 43 | 0.9949 | 0.9990 | 10 | 18 | 236189 - 1018746 |
| Sn (283.997) | 6000 | 10[b] | 53 | 20 | 33 | 0.9956 | 0.9979 | 9 | 10 | 268225 - 830041 |
| Tl (190.856) | 8 | 13 | 26 | 14 | 22 | 0.9982 | 0.9998 | 113 | 104 | 278019 - 2639911 |
| V (292.402) | 100 | 167 | 7 | 5 | 7 | 0.9968 | 0.9998 | 5 | 5 | 113599 - 5545457 |

[a] Based in the higher MDD for all drugs, 30 mL day$^{-1}$; [b] Values in mg L$^{-1}$; [c] LOQ for sample digested determinations by external standard calibration.

The analyte LOQs obtained for EC and IS and the standard errors calculated for SA and OP SA were lower than the lower level of addition suggested by the Chapter 233, considering the dilution factor of 10-fold adopted in the analytical procedure, except for Pb and Tl. For Pb, the 0.5$J$ value is 8.00 µg $L^{-1}$ and the LOQs obtained for EC and IS were 15.7 and 18.0 µg $L^{-1}$, respectively. However, the standard errors calculated for SA and OP SA are lower, 6.60 and 4.10 µg $L^{-1}$, respectively. For Tl, with 13 µg $L^{-1}$ as 0.5$J$ value, the LOQs were 14.3 and 21.6 µg $L^{-1}$ for EC and IS, and the standard errors were 113 and 104 µg $L^{-1}$ for SA and OP SA, respectively. The high standard errors for Tl can be explained due to the recovery <70% obtained for samples C and D for SA and OP SA methods. However, for the addition level of 0.20 mg $L^{-1}$ all recoveries ranged from 80 to 110%.

*4.1.5.5. Determination of inorganic impurities in liquid drug samples*

All analytes were below the respective LODs for the calibration methods EC and IS and below the respective standard errors for SA and OP SA methods for all drug samples in both sample preparation procedures (microwave-assisted digestion and nitric acid dilution). Consequently, all samples are within the limits suggested by USP taking into account the maximum daily dose of each liquid drug indicated in the package insert.

### 4.1.6 - Conclusion

The dilute-and-shoot procedure is a simple strategy, less expensive and faster than the traditional sample preparation procedure using microwave-assisted digestion. Calibration strategies as IS and OP SA were effective to correct for matrix effects and allowed the adoption of the dilute-and-shoot procedure for determination of 23 elemental impurities in liquid pharmaceutical samples by ICP OES. The LOQs and standard errors obtained for IS and OP SA, respectively, were suitable to meet USP requirements considering the adopted factor of dilution and the specific MDD for each drug. The low dilution factor of the procedure led to higher $J$ values, allowing suitable LOQs using ICP OES. Consequently, this procedure can be easily used for

pharmaceutical laboratories to control elemental impurities contamination in liquid drugs.

## 4.2 - A green dispersive liquid-liquid microextraction based on deep eutectic solvent for elemental impurities determination in oral and parenteral drugs by inductively coupled plasma optical emission spectrometry

Fernanda C. Pinheiro,[a,b] Miguel Ángel Aguirre,[b] Joaquim A. Nóbrega,[a] Nerea González-Gallardo,[c] Diego J. Ramón[c] and Antonio Canals[b*]

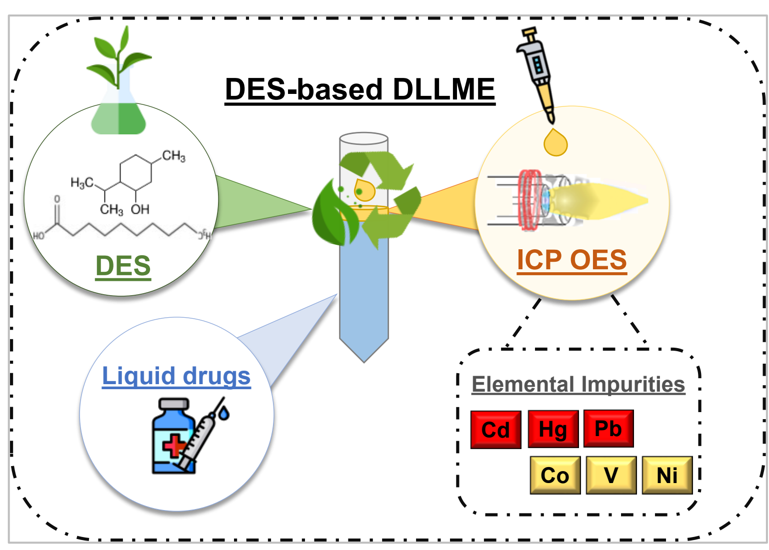
### 4.2.1 - Abstract

A simple, fast, sensitive and green pretreatment method for the determination of Cd, Co, Hg, Ni, Pb, and V in oral and parenteral drug samples using inductively coupled plasma optical emission spectrometry (ICP OES) has been developed. According to United States Pharmacopoeia (USP), those metals must be reported in all pharmaceutical products for quality control evaluation (i.e., elemental impurities from classes 1 and 2A of USP Chapter 232). To improve the analytical capabilities of ICP OES, a dispersive liquid-liquid microextraction (DLLME) was performed using a safe, cheap and biodegradable deep eutectic solvent (DES) as extractant solvent (a mixture of 1:2 molar ratio of decanoic acid and menthol). Seven parameters affecting the microextraction efficiency were carefully optimized by multivariate analysis. Under optimized conditions, the DES-based DLLME procedure improved limit of quantitation (LOQ) values on range from 22 to 85-fold for all analytes and afforded an enrichment factors on average 60-times higher than those obtained to direct ICP OES analysis. Consequently, LOQ values for Cd, Co, Hg, Ni, Pb, and V were on average 10 and 21-times lower than target limits recommended for drugs from parenteral and oral route of administration, respectively. Accuracy was evaluated by addition and recovery experiments following USP recommendations for four oral drug samples in liquid dosage form and three

[a] Group for Applied Instrumental Analysis, Department of Chemistry, Federal University of São Carlos, P.O. Box 676, São Carlos, SP, 13560-270, Brazil.
[b] Department of Analytical Chemistry and Food Sciences, University Institute of Materials, Faculty of Science, University of Alicante, P.O. Box 99, 03080, Alicante, Spain.
[c] Department of Organic Chemistry and Institute of Organic Synthesis (ISO), Faculty of Sciences, University of Alicante, PO Box 99, 03080 Alicante, Spain.
*Corresponding author: a.canals@ua.es

parenteral drugs. Recovery and RSD values were within the range of 91-109% and 1-6%, respectively. All analytes were below the respective LOQ values, hence, lower than the limits proposed by USP Chapter 232.

### 4.2.2 - Graphical Abstract




### 4.2.3 - Introduction

The presence of elemental impurities in drug products can potentially have adverse health effects and therefore must be carefully monitored. According to International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) [20] and USP Chapter 232 [21], permissible daily exposures (PDE) values for elemental impurities are stablished for pharmaceuticals from three routes of administration (i.e., oral, parenteral, and inhalational). These target elements are also grouped into four main categories based on the toxicity and their likelihood of occurrence: class 1 (As, Cd, Hg, Pb), class 2A (Co, Ni, V), class 2B (Ag, Au, Ir, Os, Pd, Rh, Pt, Ru, Se, Tl), and class 3 (Ba, Cu, Cr, Li, Mo, Sb, Sn). Moreover, inductively coupled plasma techniques associated with an optical emission detector (ICP OES) or mass spectrometer (ICP-MS) are described by USP Chapter 233 [23] as analytical procedures for target elements determinations.

Class 1 elements are extremely toxic to humans whereas class 2A elements have relatively high probability of occurrence in pharmaceuticals. Thus, both classes must be evaluated in all potential sources of contamination.

On the other hand, classes 2B and 3 show lower toxicity and a reduced probability of occurrence in pharmaceuticals, so they may be excluded from the risk assessment unless they are intentionally added during the manufacture of excipients or other components of drug product [4,20,21]. Considering the target-limits recommended for classes 1 and 2A, higher PDE values for Co, Ni and V are suggested for drugs administered via oral route. Nonetheless, for parenteral medications, the PDEs for the above-mentioned elements are 10-times lower and, along with elements from class 1, range from 2 to 20 μg $day^{-1}$ [21].

An effective sample preparation procedure is crucial to accurate elemental determination in complex matrices using spectroanalytical techniques [2,3]. For this reason, several sample preparation for pharmaceutical products (e.g., drugs, excipients, and active pharmaceutical ingredients) have been developed for elemental impurities determination by ICP-based methods [1,4]. These procedures significantly depends on the dosage form of the drug (i.e., tablet, pill or liquid) and include different sample preparations like dilution with diluted acid solution [32,48,51] or with organic solvents [39,55], microwave-induced combustion [33,61], digestion in closed vessel using conventional heating [57], microwave-assisted digestion in closed vessels [35–37,40], among others. Moreover, based on the target-limits from USP Chapter 232, when analytical instrument is not sensitive enough for direct analyte quantification at trace/ultra-trace levels, a specific procedure entailing an effective extraction/preconcentration methodology prior to quantification is also required [91].

Accordingly, dispersive liquid-liquid microextraction (DLLME) is a successful extraction technique in which a water-immiscible solvent form a cloudy solution when injected into an aqueous sample, and after centrifugation, the extracting solvent containing the analytes are separated from the aqueous phase enabling high pre-concentration factor [92]. This miniaturized and solvent-minimized sample preparation has gained increasing research interest since their advantages, including simplicity of operation, high speed, high extraction efficiency with matrix effect free, low cost, and minimum requirements for sample and organic solvents [92,93,156]. In order to enhance the extractant phase dispersion, vortex-assisted DLLME has been developed [92].

The main obstacle for DLLME is the suitable selection of an extraction solvent considering its effectiveness, availability, cheapness and which meets

the green principles [157,158]. On this regard, deep eutectic solvents (DESs) have surged as one of the most promising alternatives to the use of toxic organic solvents [156,159,160]. The first DES application in metal liquid phase microextraction (LPME) was employed to the extraction of cadmium and lead in edible oils [161]. Thereafter, the combination of DESs with DLLME was rapidly developed, but is still seldom applied to elemental detection techniques [156]. DESs are defined as mixtures of two or more safe, cheap, renewable and biodegradable components. Its synthesize is carried out between hydrogen bond acceptors (HBAs) like quaternary ammonium salts, and hydrogen bond donors (HBDs) such as phenols, amines, carboxylic acids, or alcohols [92,156,160,162]. They are also known as cheap analogues of ionic liquids since their advantages, including low toxicity, high thermal stability, ease of synthesis and low cost [160,162].

In view of the above, this study aimed to develop a simple, fast and green sample preparation procedure based on DLLME using a synthesized DES (decanoic acid and menthol 1:2 molar ratio) for the simultaneous extraction and preconcentration of Cd, Co, Hg, Ni, Pb, and V at trace levels from oral and parenteral drug samples for subsequent measurement by ICP OES. Before DLLME, all drug samples were only diluted in dilute nitric acid solution. In order to increase the sensitivity of the ICP OES for determination of these elements following USP requirements, parameters affecting the extraction efficiency were carefully optimized by multivariate analysis.

### 4.2.4 - Experimental

#### *4.2.4.1. Instrumentation*

Experiments were performed using an Agilent 720-ES inductively coupled plasma optical emission spectrometer (Agilent Technologies, Melbourne, Australia) operating in axial viewing mode. Argon (99.9992%, Carburos Metálicos, Barcelona, Spain) was used in all measurements. Plasma operating conditions used in ICP OES are shown in Table 4.6.

TABLE 4.6 - Operating parameters used in Agilent 720-ES ICP OES.

| Instrument parameter | Value |
|---|---|
| RF applied power (kW) | 1.2 |
| Plasma gas flow rate (L $min^{-1}$) | 15 |
| Auxiliary gas flow rate (L $min^{-1}$) | 1.5 |
| Nebulizer gas flow rate (L $min^{-1}$) | 0.75 |
| Organic extract uptake rate (µL $min^{-1}$) | 50 |
| Nebulizer | OneNeb® |
| Spray chamber | Cyclonic spray chamber |
| Number of replicates | 3 |
| **Analytes** | **Emission line (nm)** |
| Cd | 226.502 II |
| Co | 238.892 II |
| Hg | 253.652 I |
| Ni | 216.555 II |
| Pb | 220.353 II |
| V | 311.837 II |

I: Atomic line; II: Ionic line.

A centrifuge (model 2690/5, Nahita Centrifuges, Beriain, Spain) was used to accelerate the phase separation and a pHmeter (Crison Instrument, Barcelona, Spain) with a combined glass electrode was used for pH measurements. NemrodW statistical software (NemrodW® v.2007/2010, LPRAI, Marseille, France) was used to construct the experimental designs and evaluate the results. For the characterization of hydrophobic DES, infrared spectra were measured on a Jasco FT/IR-4100 Fourier Transform Infrared (FT-IR) spectrometer. Proton nuclear magnetic resonance ($^1H$ NMR) spectra were recorded on Bruker AC-300 (300 MHz) or AC-400 (400 MHz) NMR spectrometers in proton coupled mode. Differential Scanning Calorimetry (DSC) analyses were performed on a Mettler Toledo equipment, model TGA/SDTA851e/LF/1600. In DSC, the samples were continuously purged with 50 mL $min^{-1}$ of nitrogen. About 6 mg of each compound was crimped in an aluminum standard melting pot and analyzed under nitrogen atmosphere by heating (5 ºC $min^{-1}$) and cooling (5 ºC $min^{-1}$) cycles between -10 and 100 ºC.

*4.2.4.2. Synthesis of hydrophobic DES*

For the synthesis of hydrophobic DES, DL-menthol (purity ≥ 98%) provided by Alfa-Aesar™ (Tewksbury, MA, United State) was used as hydrogen bond acceptor (HBA) for the DES. Decanoic acid (purity ≥ 98%) provided by Sigma-Aldrich was employed as a hydrogen bond donor (HBD). Reagents were used without any further purification. The hydrophobic DES formed by decanoic acid and DL-menthol was synthesized by simply mixing decanoic acid (1 mol) with DL-menthol (2 mol) at 60 ºC under argon atmosphere, stirring the mixture until a clear and homogenous liquid was formed (usually 30 min).

*4.2.4.3. Reagents and standard solutions*

Experiments were performed using concentrated high purity grade $HNO_3$ (Merck, Darmstadt, Germany) and ultrapure water, resistivity higher than 18.2 MΩ cm, (Millipak-40 Filter Unit 0.22 mm NPT, Bedford, MA, USA). Complexing agent (8-Hydroxyquinoline (8-HQ), purity ≥ 98%, Sigma-Aldrich, Steinheim, Germany) solution of 16% m $v^{-1}$ was prepared by dissolving the appropriate amount of reagent in ethanol (99.9%, AppliChem, Darmstadt, Germany) and acetic acid glacial (99.8%, Scharlau Chemie, Barcelona, Spain) at a ratio of 4:1 v $v^{-1}$. Analytical reference solutions used for ICP OES calibrations and for addition and recovery experiments were prepared by appropriate dilutions of 1000 mg $L^{-1}$ of Cd, Co, Hg, Ni, Pb, and V (High Purity Standards, Charleston, SC, USA) in 0.07 mol $L^{-1}$ $HNO_3$ medium. The concentrations of the analytical solutions used for calibration for conventional ICP OES analysis were 0, 0.5, 1, 1.5, 2, 3, and 4 mg $L^{-1}$ for all analytes and 0, 5, 15, 30, 60, 125, and 250 µg $L^{-1}$ of Cd, Co, Hg, Ni, Pb, and V for DLLME-ICP OES. To minimize contamination all laboratory glassware were kept in 10% v $v^{-1}$ nitric acid solution for 24 h before use.

*4.2.4.4. Samples and sample preparation*

Three oral drug samples (OA-OC) and three parenteral drug samples (PA-PC) in liquid dosage form were analyzed. More details about these samples are presented in Table 4.7. The maximum permissible daily dose (MDD) for each drug was consulted on the package leaflet. These drugs are

intended to be used orally or parentally for different disorders and are accessible to population without prescription. All analyzed samples were purchased in local pharmacies in San Vicente del Raspeig, Alicante, Spain. Before DLLME, oral and parenteral drug samples were 10-fold diluted with distilled-deionized water after adjusting pH.

TABLE 4.7 - Active principle, function, indication and maximum daily dose (MDD) for oral and parenteral drug samples analyzed.

| Drug samples | Active principle | Function | Indication | MDD (mL day$^{-1}$) |
|---|---|---|---|---|
| OA | ibuprofen | anti-inflammatory | 10-12 years, 30-40 kg | 40 |
| OB | paracetamol | analgesic and antipyretic | 9-10 years, 25-32 kg | 19.2 |
| OC | metamizol magnesium | analgesic and antipyretic | ≥15 years, >53 kg | 10 |
| PA | metamizol magnesia | analgesic and antipyretic | ≥15 years, >53 kg | 12.4 |
| PB | diclofenac sodic | anti-inflammatory | ≥18 years | 9 |
| PC | dexketoprofen | anti-inflammatory | ≥18 years | 6 |

*4.2.4.5. Dispersive liquid–liquid microextraction procedure*

A volume of 8.0 mL of the 10-fold diluted sample, at pH 3.4 and 8-HQ concentration of 1.0 % m v$^{-1}$, was transferred to 10-mL glass tubes. Then, 70 µL of the DES extractant solvent was added, and the mixture was shaken using a vortex shaker for 3 min. After shaking, the solution was centrifuged at 3000 rpm for 4 min to separate the organic and aqueous phases. Fifty microliters of the organic extract (at the top of the solution) was collected from the glass tube using a micropipette and directly inserted into the ICP OES without furthermore dilution. Figure 4.6 shows a schematic representation of the general optimized DES-based DLLME procedure. During the optimization, standard solutions containing 100 µg L$^{-1}$ of all analytes were used.

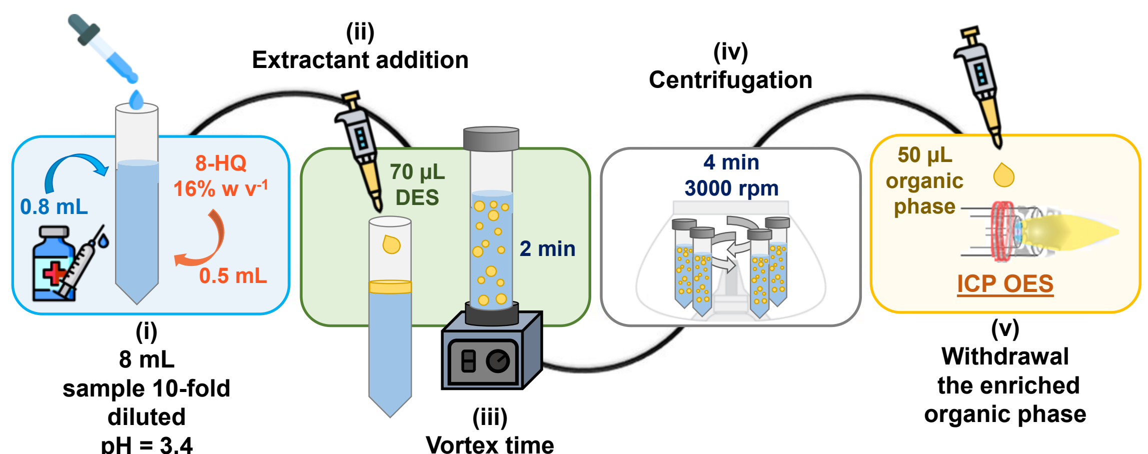



FIGURE 4.6 - Schematic representation of the DES-based DLLME procedure for preconcentration of Cd, Co, Hg, Ni, Pb, and V in parenteral and oral drug samples.

*4.2.4.6. Addition and recovery tests according to USP requirements*

Addition and recovery experiments were performed according to *J* values (expressed in µg $L^{-1}$), which were calculated based on the specific PDE value for each element (in µg $day^{-1}$) considering oral or parenteral route of administration, divided by the MDD (in mL $day^{-1}$) and the dilution factor (DF) adopted during sample preparation [21,23] as follows *J* = PDE / MDD x DF. Table 4.8 shows PDE and *J* values for all analytes for oral and parenteral drug samples considering the specific MDD of each medication, as indicated in the package insert (Table 4.7), and DF of 10-fold.

TABLE 4.8 - Class [20,21], PDE [21] and *J* values (µg $L^{-1}$) for Cd, Co, Hg, Ni, Pb, and V for oral and parenteral drug samples.

| | | Oral drug samples | | | | Parenteral drug samples | | | |
|---|---|---|---|---|---|---|---|---|---|
| Analyte | Class | PDE (µg $day^{-1}$) | OA | OB | OC | PDE (µg $day^{-1}$) | PA | PB | PC |
| Cd | 1 | 5 | 13 | 26 | 50 | 2 | 16 | 22 | 33 |
| Pb | 1 | 5 | 13 | 26 | 50 | 5 | 40 | 56 | 83 |
| Hg | 1 | 30 | 75 | 156 | 300 | 3 | 24 | 33 | 50 |
| Co | 2A | 50 | 125 | 260 | 500 | 5 | 40 | 56 | 83 |
| V | 2A | 100 | 250 | 521 | 1000 | 10 | 81 | 111 | 167 |
| Ni | 2A | 200 | 500 | 1042 | 2000 | 20 | 161 | 222 | 333 |

According to *J* values for each analyte, all drug samples were spiked in triplicate with concentrations of 0.5*J* and 1.5*J* in order to verify the trueness of the DES-based DLLME procedure. Considering oral drug samples, due to *J* values are higher than the proposed working range (i.e., 5.0-250 µg $L^{-1}$), 0.5*J* and 1.5*J* values for Hg, Co, V and Ni were 5, 10, 10 and 20-times divided, respectively. For parenteral drugs, only 0.5*J* and 1.5*J* values for Ni were divided by 2.

### 4.2.5 - Results and discussion

*4.2.5.1. Characterization of hydrophobic DES*

To confirm the structure of DES, FT-IR spectra of pure DL-menthol, pure decanoic acid, and DES were examined and results are presented in Figure 4.7.

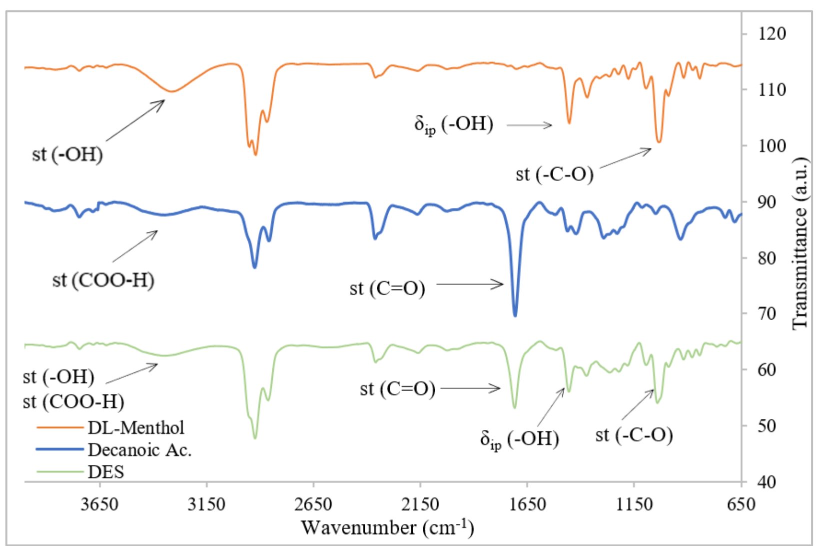



FIGURE 4.7 - FT- IR spectra of pure DL-menthol, pure decanoic acid and DES (i.e., DL-menthol and decanoic acid (2:1 molar ratio) mixture.

In the spectrum of pure DL-menthol, absorptions corresponding to the tension and flexion -OH (3309, 1454 $cm^{-1}$, respectively) and the absorption corresponding to the tension C-O (1029 $cm^{-1}$) were observed. In the spectrum of pure decanoic acid, the COO-H and C=O vibrations were positioned at 3351 and 1727 $cm^{-1}$, respectively. All these characteristic peaks were also found in

DES FT-IR spectrum at the same position, demonstrating that the DES is comprised of DL-menthol and decanoic acid.

Regarding to $^{1}H$ NMR experiments on DES, it was possible to see a clear interaction between the alcohol substituent (R-OH) of the DL-menthol and the proton of the decanoic acid (R'-$CO_2H$), since a significant shift in the signals of both was observed in comparison with pure starting materials (Figure 4.8, compare a, b and c). These results indicated the successful synthesis of the hydrophobic DES. In order to see whether the 8-hydroxyquinoline (8-HQ) influenced in the DES structure, several $^{1}H$ NMR experiments were carried out. As it was expected for a compound with hydrogen donor capacity, an interaction between the DES and the 8-HQ was detected since a shift and a change in the shape of the signal of the DES were observed (Figure 4.8, compare c, d and e). In case of the alcohol substituent (R-OH) of the 8-HQ, the signal is overlapped with signals corresponding to the aromatic protons (Figure 4.8 d).

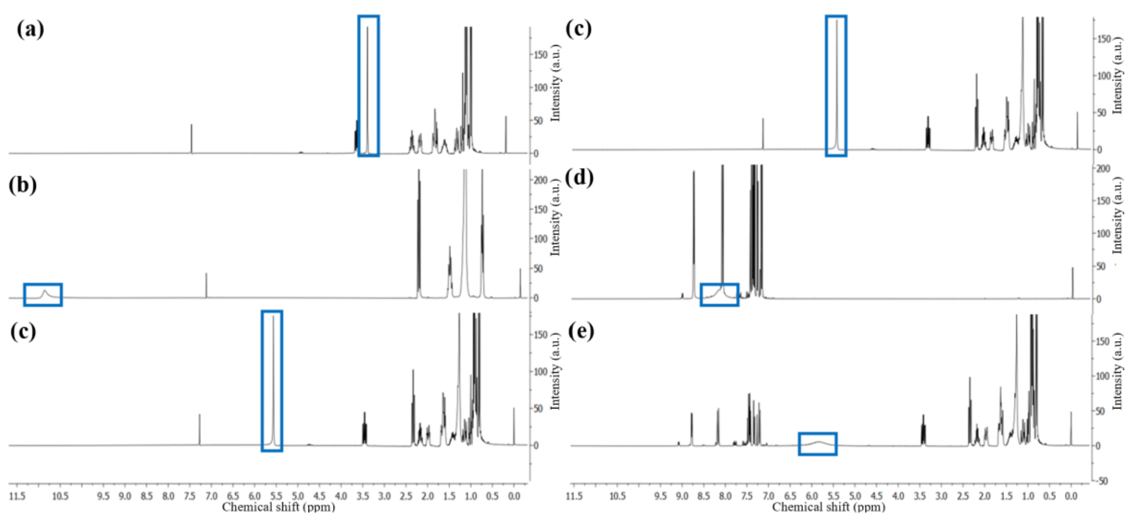



Figure 4.8 - $^{1}H$ NMR spectra of **(a)** pure DL-menthol; **(b)** pure decanoic acid; **(c)** DL-menthol:decanoic acid (2:1) mixture; **(d)** pure 8-HQ; and **(e)** DL-menthol:decanoic acid (2:1) mixture and 8-HQ.

Regarding to DSC experimenters, several samples with different proportion of DL-menthol and decanoic acid were prepared by simple mixing the two components and grinding them until a homogeneous mixture was obtained. Those samples were analyzed by DSC. With the melting point of each

one, a phase diagram was plotted, showing a eutectic point for a molar ratio 2:1 DL-menthol:decanoic acid (Figure 4.9).

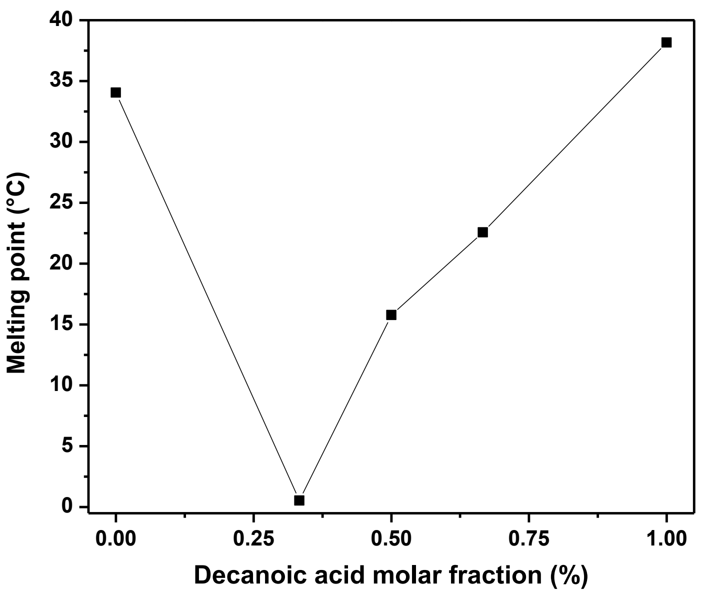



FIGURE 4.9 - Phase diagram for DL-menthol:decanoic acid eutectic mixture.

*4.2.5.2. Optimization of dispersive liquid–liquid microextraction*

Due to the several factors affecting the DLLME procedure, the application of multivariate optimization designs help to determine the best model of the relationship between them, as well as the optimal experimental conditions, mainly considering the simultaneous determination of different analytes [96]. Thus, the multivariate optimization of the DES-DLLME procedure was performed using a Plackett-Burman design for screening approach to identify between significant and non-significant factors followed by a central composite design (CCD) to obtain optimal values for the significant factors.

The seven DLLME factors evaluated on the Plackett-Burman design and their low (-) and high (+) levels, respectively, were (i) DES volume (50 and 100 µL); (ii) sample pH (2 and 4); (iii) 8-HQ concentration (0.50 and 1.0 % m $v^{-1}$); (iv) extraction time (1 and 3 min); (v) centrifugation time (2 and 4 min); (vi) centrifuge speed (2000 and 3000 rpm); and (vii) ionic strength, NaCl concentration (0 and 5% m $v^{-1}$). Pareto charts of the standardized effect show the results of the Plackett-Burman design for different elements (Figure 4.10).

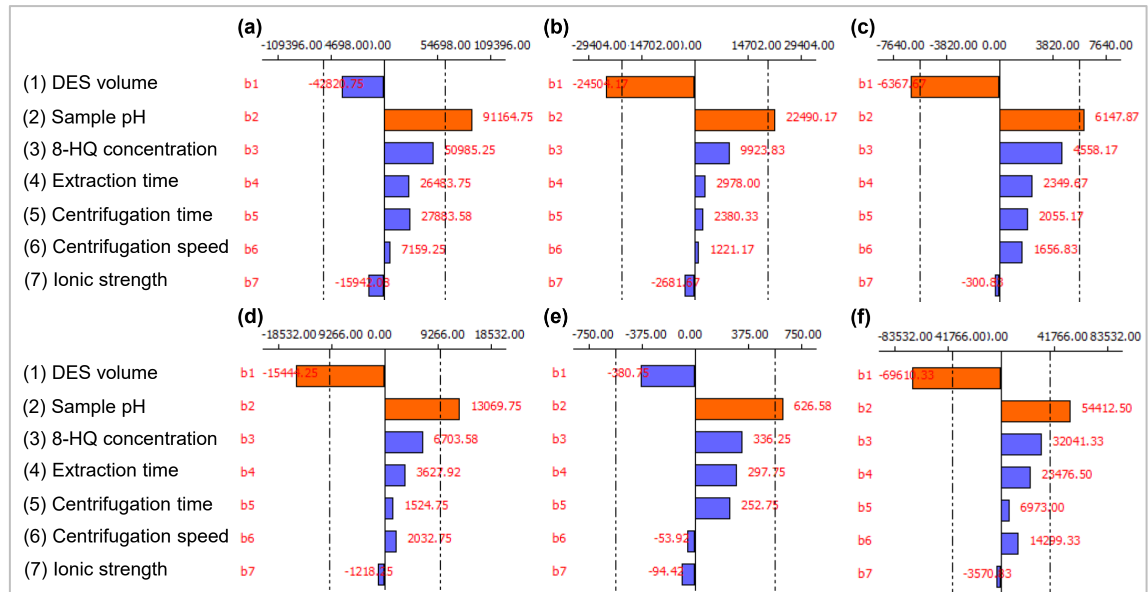



FIGURE 4.10 - Pareto charts obtained in the screening study of the main factors affecting the DLLME of **(a)** Cd, **(b)** Co, **(c)** Hg, **(d)** Ni, **(e)** Pb and **(f)** V. (■) Significant effect; (■) Non-significant effect. Bars to the right indicate a positive effect and bars to the left indicate a negative effect. Analyte concentration of 100 µg $L^{-1}$.

Considering all analytes, DLLME was favored without adding NaCl (i.e., negative effect) and at high levels (i.e. positive effects) of 8-HQ concentration, extraction time, centrifuge time and centrifuge speed, except to Pb for centrifuge speed factor (Figure 4.10 e). All these factors showed a non-significant effect on DLLME of all analytes. Additionally, the factors (i) DES volume (for Co, Hg, Ni, and V) and (ii) sample pH (for all analytes) showed a significant effect on signal intensities. Generally, the sample pH and extractant solvent volume are factors extremely significative for metal extraction procedures [92,96] because the pH has direct influence on the complexation step and the extractant solvent volume infers directly in the enrichment factor of analytes [92,93,160].

Therefore, a central composite design (CCD) was performed to optimize DES volume and sample pH. The different level values chosen in the CCD were: (i) DES volume (50, 57, 75, 93, and 100 µL), and (ii) sample pH (2, 2.3, 3, 3.7, and 4). The response surfaces obtained are shown in Figure 4.11.

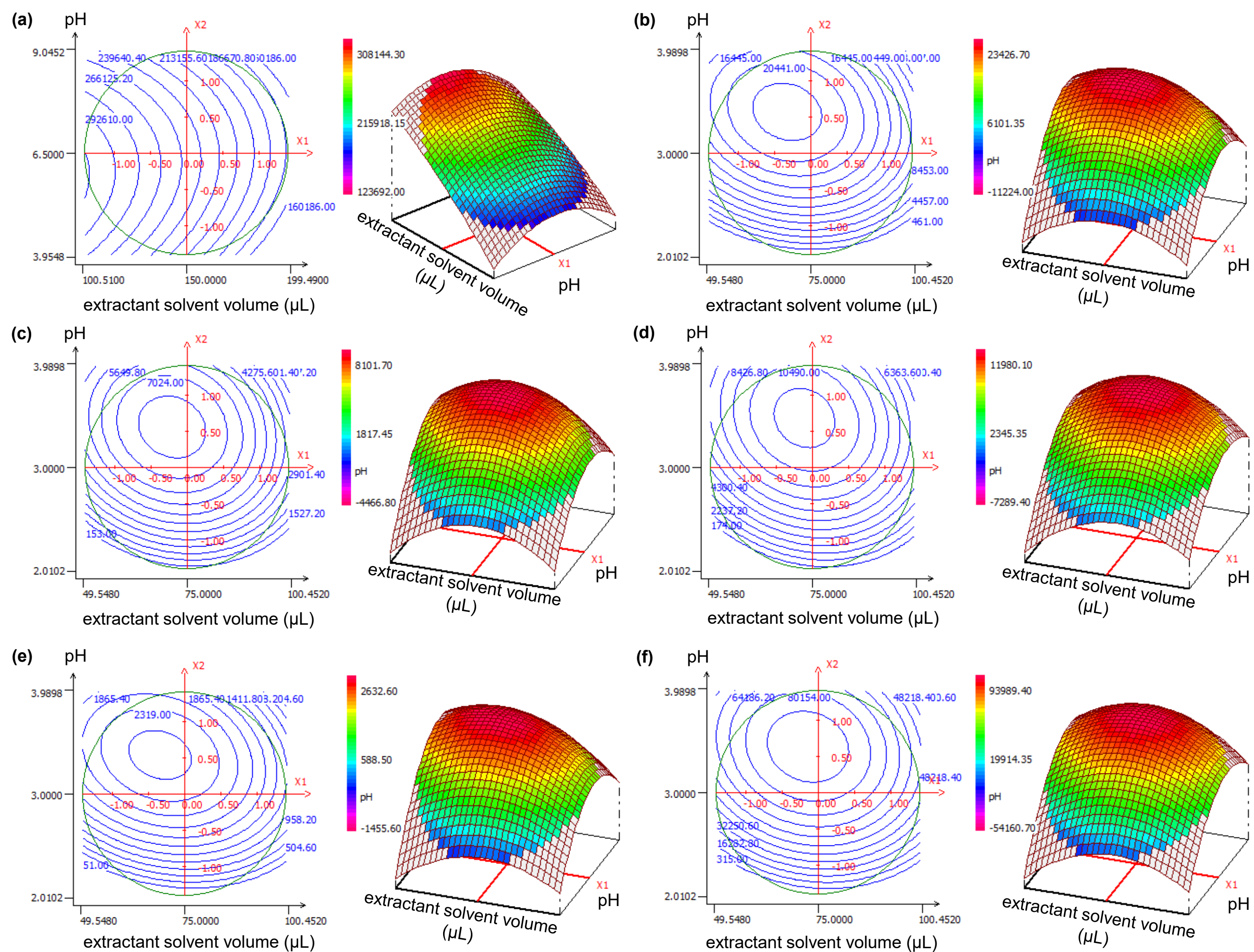



FIGURE 4.11 - Response surface from central composite design for **(a)** Cd, **(b)** Co, **(c)** Hg, **(d)** Ni, **(e)** Pb, and **(f)** V. Analyte concentration of 100 μg $L^{-1}$.

The optimized DES volume and sample pH for extraction of different analytes were: 66 µL and 3.3 for Cd, 69 µL and 3.4 for Co, 71 µL and 3.4 for Hg, 73 µL and 3.5 for Ni, 69 µL and 3.4 for Pb, 72 µL and 3.5 for V. As no significant differences in optimum sample pH and the DES volume for each element were obtained, the average of those values (i.e., DES volume of 70 µL and pH at 3.4) were selected as the most favorable conditions for all analytes. Therefore, the optimized conditions for simultaneous extraction of Cd, Co, Hg, Ni, Pb, and V were: 3.4 of sample pH, 8-HQ concentration of 1.0 % m $v^{-1}$, 70 µL of DES as extractant solvent, vortex time of 3 min, centrifugation time of 4 min and centrifugation speed of 3000 rpm.

*4.2.5.3. Analytical performance for DES-based DLLME-ICP OES method*

Table 4.9 summarizes the analytical figures of merit obtained by developed DES-based DLLME-ICP OES method and direct ICP OES analysis for determination of Cd, Co, Hg, Ni, Pb, and V in oral and parenteral drug samples. The enrichment factors (EF) were defined as the ratio of the calibration curve slope with and without the preconcentration procedure. The correlation coefficients (r) obtained for all DLLME-ICP OES calibration curves ranged from 0.9985 to 0.9996 and EF values ranged from 22 to 86, showing good linearity and significant increase in sensitivity for all analytes.

TABLE 4.9 - Analytical figures of merit for Cd, Co, Hg, Ni, Pb, and V determination in oral and parenteral drug samples using DES-based DLLME-ICP OES and direct ICP OES analysis.

| | Emission line (nm) | | | | | |
|---|---|---|---|---|---|---|
| | Cd (226.502) | Co (238.892) | Hg (253.652) | Ni (216.555) | Pb (220.353) | V (311.837) |
| ICP OES | | | | | | |
| Linear range (µg $L^{-1}$) | 500-4000 | 500-4000 | 500-4000 | 500-4000 | 500-4000 | 500-4000 |
| r[a] | 0.9989 | 0.9992 | 0.9982 | 0.9982 | 0.9990 | 0.9989 |
| Sensitivity (cps L µg$^{-1}$)[b] | 6.4 ± 0.2 | 4.1 ± 0.1 | 0.93 ± 0.03 | 1.84 ± 0.05 | 0.60 ± 0.01 | 15.1 ± 0.4 |
| LOQ (µg $L^{-1}$) | 17 | 87 | 183 | 237 | 88 | 103 |
| DLLME-ICP OES | | | | | | |
| Working range (µg $L^{-1}$) | 5.0-250 | 5.0-250 | 5.0-250 | 5.0-250 | 5.0-250 | 5.0-250 |
| r[c] | 0.9985 | 0.9992 | 0.9990 | 0.9993 | 0.9996 | 0.9990 |
| Sensitivity (cps L µg$^{-1}$)[a] | 546 ± 9 | 214 ± 3 | 74 ± 1 | 142 ± 2 | 13.4 ± 0.1 | 677 ± 16 |
| EF[d] | 86 ± 2 | 52 ± 2 | 79 ± 3 | 77 ± 3 | 22.2 ± 0.7 | 45 ± 2 |
| LOQ (µg $L^{-1}$) | 0.2 | 2 | 3 | 3 | 4 | 3 |
| USP LOQ (µg $L^{-1}$)[e] | ≤5 | ≤12 | ≤7 | ≤48 | ≤12 | ≤24 |
| Repeatability 0.5*J* (RSD%)[f] | 6 | 5 | 5 | 6 | 6 | 3 |
| Repeatability 1.5*J* (RSD%)[g] | 3 | 6 | 4 | 4 | 3 | 3 |

[a] Correlation coefficient (five calibration points); [b] Slope ± standard deviation; [c] Correlation coefficient (seven calibration points); [d] Enrichment factor ± expanded uncertainty. Calculated as slope ratio between calibration curves with and without DLLME. [e] LOQ values ≤0.3*J* considering the sample with lower *J* values (i.e., parenteral drug sample PA); [f] Mean value for six replicate analyses of spiked solution with 8.0 µg $L^{-1}$ of all analytes; [g] Mean value for six replicate analyses of spiked solution with 24 µg $L^{-1}$ of all analytes.

Limits of detection (LOD) and quantification (LOQ) were calculated according to Eurachem guidelines [163] considering the analyte concentration corresponding to the obtained standard deviation (i.e., determined by 10 consecutive measurements of the blank) at low levels multiplied by a factor k. The IUPAC default value for k is 10 for LOQ and 3 for LOD. Following USP Chapter 233, LOQ values ≤0.3*J* are suggested as acceptance criteria once accuracy must be demonstrated at lower spiked concentrations of 0.5*J* for each target element [23]. In this context, the LOQ values for direct ICP OES analysis were all higher than 0.3*J* for all elements for parenteral drug samples. Due to the higher PDE values recommended for oral route of administration, the LOQ values obtained for oral samples using ICP OES without DLLME were higher than 0.3*J* for all elements for sample OA; for Cd, Co, Hg and Pb for sample OB; and for Cd, Hg and Pb for sample OC.

Limits of quantification values for Cd, Co, Hg, Ni, and V using DES-based DLLME are 21, 20, 8, 50 and 28-times, respectively, lower than their respective 0.3*J* for sample OA (i.e., lower *J* values among all oral drug samples analyzed) and 26, 6, 3, 16, 3 and 9-times lower than their respective 0.3*J* values for Cd, Co, Hg, Ni, Pb and V, respectively, for sample PA (i.e., lower *J* values among all parenteral drug samples analyzed). Consequently, it may be inferred that the LOQ values obtained for DES-based DLLME of Cd, Co, Hg, Ni and V are suitable to meet USP requirements even using oral liquid drugs with MDD higher than 40 mL $day^{-1}$ and for Pb with MDD until 40 mL $day^{-1}$. For parenteral route of administration, considering the simultaneous determination of six analytes, the LOQ values obtained for DLLME of Hg and Pb are suitable to meet USP requirements using parenteral drugs with MDD until 30 mL $day^{-1}$.

The repeatability was estimated from six independent measurements of sample spiked at 8.0 and 24 µg $L^{-1}$ of all analytes. These values were selected considering the sample with lower 0.5*J* and 1.5*J* values among all elements (i.e., parenteral drug sample PA). Repeatability ranged from 3 to 6%, values significantly lower than 20% of RSD stated by USP Chapter 233 [23].

*4.2.5.4. Addition and recovery tests according to USP requirements*

All analytes were below their respective LOQ values for all oral and parenteral drug samples analyzed, hence, the analyzed samples are within the limits suggested by USP Chapter 232 [21] taking into account the MDD of each parenteral and oral drug. According to the USP Chapter 233 [23] analytical procedures must demonstrate accurate spike recoveries between 70 and 150% of the spiked value at concentrations ranging from 0.5*J* to 1.5*J* of the value for each target element. Consequently, the samples were spiked at levels equivalent to 0.5*J* and 1.5*J* for all analytes in order to check the trueness of DES-based DLLME-ICP OES method (Table 4.10).

In order to group all analytes in a unique analytical working range, some addition levels (i.e., 0.5*J* and 1.5*J*) for some analytes were properly divided as previously mentioned in Section 2.6. The specific addition values for each analyte and sample were also presented in Table 5. Recoveries ranging from 91 to 109% were observed by spike experiments at both levels and the repeatability was demonstrated by a precision ≤6% RSD considering all oral and parenteral drug samples.

TABLE 4.10 - Recoveries and relative standard deviation (%, n = 3) obtained for the spiked in oral (LA-LB) and parenteral (PA-PC) drug samples at two different levels: 0.5*J* and 1.5*J* (i.e., spike in µg $L^{-1}$) using DES-based DLLME-ICP OES.

| Analyte | Sample | Spike | Recovery | Analyte | Sample | Spiked | Recovery | Analyte | Sample | Spike | Recovery |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cd | OA | 6 | 106 (3) | Co | OA | 6 | 98 (5) | Hg | OA | 8 | 91 (3) |
| | | 19 | 100 (6) | | | 19 | 97 (4) | | | 23 | 95 (4) |
| | OB | 13 | 103 (5) | | OB | 13 | 101 (5) | | OB | 16 | 101 (6) |
| | | 39 | 105 (4) | | | 39 | 100 (5) | | | 47 | 95 (3) |
| | OC | 25 | 93 (4) | | OC | 25 | 93 (4) | | OC | 30 | 95 (1) |
| | | 75 | 97 (4) | | | 75 | 100 (3) | | | 90 | 96 (5) |
| | PA | 8 | 92 (2) | | PA | 20 | 92 (6) | | PA | 12 | 92 (1) |
| | | 24 | 92 (5) | | | 60 | 99 (6) | | | 36 | 97 (2) |
| | PB | 11 | 90 (4) | | PB | 28 | 98 (3) | | PB | 17 | 102 (2) |
| | | 33 | 93 (5) | | | 83 | 104 (1) | | | 50 | 109 (3) |
| | PC | 17 | 95 (2) | | PC | 42 | 99 (5) | | PC | 25 | 101 (5) |
| | | 50 | 100 (1) | | | 125 | 103 (6) | | | 75 | 110 (4) |
| Ni | OA | 13 | 96 (5) | Pb | OA | 6 | 93 (3) | V | OA | 13 | 104 (1) |
| | | 38 | 108 (2) | | | 19 | 107 (3) | | | 38 | 95 (5) |
| | OB | 26 | 100 (4) | | OB | 13 | 98 (2) | | OB | 26 | 95 (6) |
| | | 78 | 98 (5) | | | 39 | 97 (6) | | | 78 | 101 (6) |
| | OC | 50 | 96 (2) | | OC | 25 | 96 (2) | | OC | 50 | 100 (1) |
| | | 150 | 99 (5) | | | 75 | 96 (5) | | | 150 | 96 (2) |
| | PA | 40 | 94 (4) | | PA | 20 | 98 (3) | | PA | 40 | 94 (3) |
| | | 121 | 107 (6) | | | 60 | 105 (2) | | | 121 | 98 (6) |
| | PB | 56 | 91 (2) | | PB | 28 | 100 (4) | | PB | 56 | 98 (5) |
| | | 167 | 106 (2) | | | 83 | 92 (2) | | | 167 | 106 (1) |
| | PC | 83 | 107 (2) | | PC | 42 | 105 (1) | | PC | 83 | 106 (2) |
| | | 250 | 93 (4) | | | 125 | 97 (2) | | | 250 | 99 (2) |

*4.2.5.5. Comparison with other hydrophobic DES-based LPME procedures*

According to our knowledge, this is the first report which a LPME procedure using a DES as extractant solvent is applied for elemental determination in drug samples. Hence, a comparison of the developed DES-based DLLME-ICP OES method with previously reported methods using DES-based liquid-phase microextraction procedures for aqueous samples is shown in Table 4.11. A small volume of DES, i.e. lower than 100 µL, low extraction time and no disperser solvent are advantageous analytical characteristics of the developed method. In contrast to some reported methods that using ice bath for DES solidification [164,165], in the proposed DES-based DLLME procedure the organic extract is directly collected from the glass tube and immediately analyzed without furthermore sample preparation steps.

TABLE 4.11 - Comparison of analytical characteristic of the proposed method with some published method using a DES for metal liquid-phase microextraction in aqueous samples.

| Sample (amount, mL) | Analytes | Sample treatment | DES (molar ratio; amount, µL) | Extraction time (min) | Analytical technique | LOD (µg $L^{-1}$) | EF[a] or PF[b] | Ref[c] |
|---|---|---|---|---|---|---|---|---|
| Human blood (10) | Hg (II) | VA-DLLME[d] | 1-octyl-3-methylimidazolium chloride and 1-undecanol (1:2; 55) | <13 | ETAAS[e] | 0.1 | 112 | 164 |
| Food and water (30) | Pb (II) | AA-LPME[f] | ChCl[g]:phenol (1:4; 600) with 800 µL of THF[h] | 9 | ETAAS[e] | $0.6x10^{-3}$ | 60 | 166 |
| Food and water (50) | Cd | UA-LPME[i] | ChCl[g]:phenol (1:4; 500) with 600 µL of THF[h] | 8 | ETAAS[e] | $0.2x10^{-4}$ | 100 | 167 |
| Black tea, water and urine (20) | Cd, Cu, Ni, Pb | AA-LL-ELLME[j] | ChCl[g]:TNO[l] (1:2 with TEA[m] 1:1; 100) | 4 | FAAS[n] | 0.3-1 | 67-69 | 168 |
| Milk (5) | Cd, Cu, Pb | DLLME[o] | Menthol:sorbitol:mandelic acid (1:2:1; 100) | 7 | FAAS[n] | 0.4 | - | 165 |
| Liquid drugs (8) | Cd, Co, Hg, Ni, Pb, V | DLLME[o] | DL-menthol and decanoic acid (2:1; 70) | 7 | ICP OES[p] | 0.05-1 | 22-86 | This work |

[a] Enrichment factor; [b] Preconcentration factor; [c] Reference; [d] Vortex assisted dispersive liquid−liquid microextraction; [e] Electrothermal atomic absorption spectrometry; [f] Air-agitated liquid–phase microextraction; [g] Choline chloride; [h] Tetrahydrofuran; [i] Ultrasonic assisted-liquid phase microextraction; [j] Air-assisted ligandless emulsification liquid–liquid microextraction; [l] 5,6,7,8-Tetrahydro-5,5,8,8-tetramethylnaphthalen-2-ol; [m] Triethylamine; [n] Flame atomic absorption spectrometry; [o] Dispersive liquid-liquid microextraction; [p] Inductively coupled plasma optical emission spectrometry.

Considering all elements, the limit of detection of DES-based DLLME-ICP OES method by using only 8 mL of aqueous sample is better or similar to other methods. It is noted that for ETAAS methods [166,167], a higher sensitivity and enrichment factor were achieved, but these are monoelemental methods and the microextraction procedure was proposed just for one element at a time, i.e. Cd [167] and Pb [166]. In this sense, other important feature of the develop DES-based DLLME-ICP OES method are the relatively high number of analytes. Except to the method proposed by Zounr, et al. [167], univariate optimization was used for obtain the optimized sample treatment, which is a more difficult way to determine the optimal experimental conditions for simultaneous extraction of different elements.

### 4.2.6 - Conclusions

The developed sample pretreatment procedure based on DLLME of Cd, Co, Hg, Ni, Pb and V from oral and parenteral drug samples prior to their determination by ICP OES is simple, faster and meets the green principles since it includes the application of a reduced volume of an DES as extractant solvent. DES-based DLLME-ICP OES method affords enrichment factors on average 60-fold in comparison with conventional ICP OES analysis, consequently, the results was proved to be sensitive and reliable enough to follow USP requirements for determination of above-mentioned elements in drugs in liquid dosage form considering target-limits for oral and parenteral route of administration. While ICP-MS achieved suitable sensitivity for elemental ultratrace determination, the synergetic combination of DLLME and ICP OES can be considered an affordable option for trace elemental determination in medicines.

# 5 - CHAPTER 5

# DIETARY SUPPLEMENT SAMPLES

## 5.1 - Microwave-assisted digestion using dilute nitric acid solution and investigation of calibration strategies for determination of As, Cd, Hg and Pb in dietary supplements using ICP-MS

Journal of Pharmaceutical and Biomedical Analysis 174 (2019) 471–478

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Microwave-assisted digestion using dilute nitric acid solution and investigation of calibration strategies for determination of As, Cd, Hg and Pb in dietary supplements using ICP-MS

Fernanda Costa Pinheiro, Diego Victor Babos, Ariane Isis Barros, Edenir Rodrigues Pereira-Filho, Joaquim Araújo Nóbrega*

*Group for Applied Instrumental Analysis, Department of Chemistry, Federal University of São Carlos, P.O. Box 676, São Carlos, SP, 13560-270, Brazil*

### 5.1.1 - Abstract

This study proposes an analytical procedure for microwave-assisted sample preparation of dietary supplements for athletes using dilute nitric acid solution followed by determination of elemental impurities (As, Cd, Hg and Pb) by inductively coupled plasma mass spectrometry (ICP-MS) according to the United States Pharmacopeia Chapters 2232 and 233. Calibration strategies as internal standardization (IS), multi-isotope calibration (MICal), and one-point standard addition (OP SA) were applied for correction of matrix effects. The optimization of the sample preparation procedure was performed using a Doehlert experimental design based on overall desirability results (residual acidity, dissolved organic carbon and recoveries reached for certified reference material of Typical Diet) for each calibration method evaluated. Accuracy was also evaluated by recovery experiments according to the

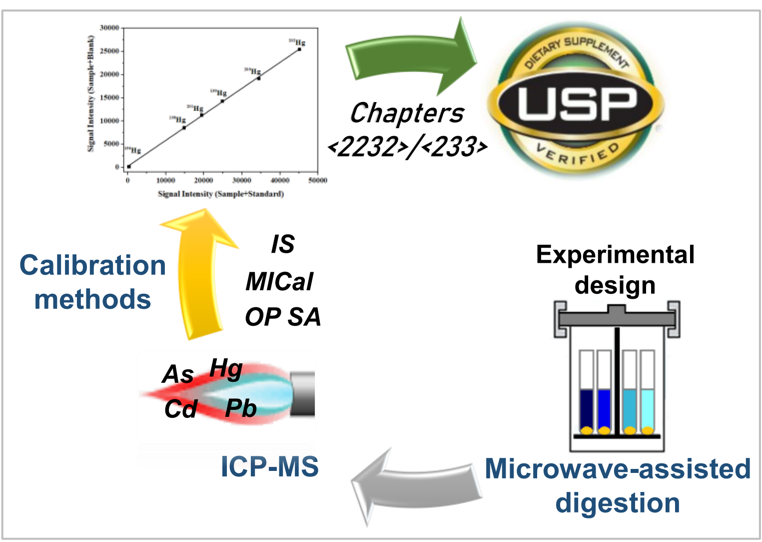
*Group for Applied Instrumental Analysis, Department of Chemistry, Federal University of São Carlos, P.O. Box 676, São Carlos, SP, 13560-270, Brazil.*

*[*]Corresponding author: djan@ufscar.br*

*DOI: https://doi.org/10.1016/j.jpba.2019.06.018*

permissible daily exposure specific for each element and samples were spiked with element concentrations of 0.5*J* and 1.5*J* in order to check accuracies for As, Cd, Hg and Pb. Recoveries ranged from 82 to 120% using IS, 90 to 125% using MICal, 88 to 120% using OP SA and the repeatability was demonstrated by a precision lower than 10% RSD. Ten samples of dietary sport supplements were analyzed using the three calibration methods evaluated and the concentrations of As, Cd and Pb determined in eight samples were lower than the limits established by the Chapter 2232.

### 5.1.2 - Graphical Abstract




### 5.1.3 - Introduction

The consumption of dietary supplements is widely spread to increase the daily intake of essential vitamins and nutrients. People use supplements for seeking to compensate for diets, medical conditions and for high sports performance associated with the gain of muscle mass. Among the several types of dietary supplements, sport supplements are commonly used by athletes and people who practice bodybuilding exercises. Generally, they are derived from whey, egg and soy [169,170].

In the United States of America, the Food and Drug Administration (FDA) is responsible for regulating dietary supplements and ensure their identity, purity, strength, and composition [169]. In Brazil, the Resolution RDC no. 18/2010 [171] establishes the classification and composition requirements in

dietary supplements for athletes. Considering inorganic impurities, the United States Pharmacopeia (USP) proposed three new general chapters on elemental impurities, Chapter 232 Elementary Impurities, Limits [21], Chapter 233 Element Impurities, Procedures [21] and Chapter 2232 Elemental Contaminants in Dietary Supplements [22]. Chapter 2232 proposes limits based on the permissible daily exposure (PDE) for As, Cd, Hg and Pb [22]. In turn, the Chapter 233 describes performance-based methods for elemental determinations using either inductively coupled plasma optical emission spectrometry (ICP OES) or inductively coupled-plasma mass spectrometry (ICP-MS) [21].

Sampling and sample preparation are critical steps in most chemical analysis procedures. Thus, the combination of microwave-assisted acid digestion and closed vessels allows the use of elevated temperatures without losses of reagents and analytes and minimizes the risks of contamination. Several possibilities of oxidizing acids and mixtures have been reported to digest dietary supplements [83], such as concentrated $HNO_3$ [47,84], mixtures of $HNO_3$ and HCl [43], $HNO_3$, HCl and HF [44,87] and concentrated $HNO_3$ and $H_2O_2$ [45,85,86]. Mixtures of diluted $HNO_3$ and $H_2O_2$ [89] and pressurized $O_2$ [90] were used as oxidizing agents to dietary supplements digestion. Microwave-assisted digestion using only dilute $HNO_3$ was used for medicinal plant samples, however, there is no report in the literature using only dilute $HNO_3$ solutions for digestion of sport supplements. Therefore, the use of dilute acids could be considered as an alternative to safer operation in microwave-assisted digestion procedures. The procedure using only nitric acid is attractive since the use of concentrated acids, such as HCl, may lead to the formation of interfering species in ICP-MS and the use of other reagents may imply in the addition of contaminants. On the other hand, it is well known that high quality reagents are more expensive [3,8].

Considering the limits proposed by Chapter 2232 [22], ICP-MS provides multi-elemental analysis with high sensitivity, accuracy, robustness and low limits of detection (LODs). However, analyses by ICP-MS require appropriate sample dilution aiming suitable total dissolved solids contents (TDS) and residual acidity (RA) [7,8]. To overcome these limitations, some instruments are equipped with an aerosol dilution system, also named as High Matrix Introduction (HMI), which introduces a flow of argon gas between the spray

chamber and the torch to promote an aerosol dilution. This system enables the direct analysis of samples containing high TDS and RA, reducing both aerosol density and water vapor loading into the plasma, furthermore, eliminates possible contaminations associated with manual dilution, saves time and reduces waste volume [8,115].

In addition to the drawbacks associated with traditional dilution strategy, ICP-MS determination is susceptible to spectral and non-spectral interferences due to possibility of matrix effects associated with nebulization, transport and plasma energetic effects, as differences in the viscosity of the matrix of sample and spatial charge effects. Some calibration methods as internal standardization (IS) [115,118], multi-isotope calibration (MICal) [119] and one-point standard addition (OP SA) [32,144] can be good calibration strategies to correct for matrix effects as recently discussed for ICP-MS [118].

In this context, the present study developed a microwave-assisted digestion procedure using dilute nitric acid solutions for determination of As, Cd, Hg and Pb in ten sport supplements by ICP-MS. Correction strategies for spectral interferences using collision cell technology and non-spectral interferences using HMI system and calibration methods were evaluated in order to improve accuracy and precision of the analytical procedure. The optimization of the sample preparation procedure was performed using Doehlert experimental design [121] based on overall desirability results for each calibration method adopted. Accuracy was evaluated by recovery experiments according to the Chapter 233 of USP and also by the use of certified reference material of Typical Diet.

### 5.1.4 - Experimental

*5.1.4.1. Instrumentation*

Experiments were performed using an Agilent 7800 Quadrupole ICP-MS (Agilent Technologies, Tokyo, JHS, Japan) operated in *no gas-HMI* and *He-HMI* acquisition mode. *HMI* mode implies that aerosol was diluted with argon at the optimized HMI gas flow rate of 0.62 L min$^{-1}$ and carrier gas flow rate of 0.40 L min$^{-1}$, thus 1.02 L min$^{-1}$ of total flow rate [115]. The *no gas* mode means the collision cell is not used, and *He* mode means when the collision cell

is pressurized with pure He (99.999%, White Martins-Praxair, Sertãozinho, SP, Brazil). Carbon was determined by an iCAP6000 ICP OES (Thermo Fisher Scientific, Waltham, MA, USA) operated in axial viewing mode. Argon (99.999%, White Martins-Praxair) was used in all measurements in both instruments. Plasma operating conditions used in ICP OES and ICP-MS were previous established by Pinheiro, et al. [40]. The isotopes monitored for EC, IS and OP SA methods were $^{75}As^{+}$, $^{112}Cd^{+}$, $^{202}Hg^{+}$ and $^{208}Pb^{+}$. The isotopes monitored as internal standard were $^{69}Ga^{+}$, $^{70}Ge^{+}$, $^{89}Y^{+}$ and $^{104}Pd^{+}$. The isotopes monitored for MICal method were $^{111}Cd^{+}$, $^{112}Cd^{+}$, $^{113}Cd^{+}$, $^{114}Cd^{+}$; $^{116}Cd^{+}$, $^{196}Hg^{+}$, $^{198}Hg^{+}$, $^{199}Hg^{+}$, $^{200}Hg^{+}$, $^{201}Hg^{+}$, $^{202}Hg^{+}$, $^{206}Pb^{+}$, $^{207}Pb^{+}$ and $^{208}Pb^{+}$.

*5.1.4.2. Samples, standards and reagents*

Ten sport supplements of type whey protein of different brands were analyzed and coded as S1 to S10. The percentage range of proteins, carbohydrates and fats according supplement labels are from 34 to 77, from 8 to 45 and from 2 to 7% m $m^{-1}$, respectively. All analyzed samples were purchased in local pharmacies in São Carlos, SP, Brazil. The Certified Reference Material (CRM) NIST 1548a Typical Diet (National Institute of Standard and Technology, Gaithersburg, MD, USA) was used for the optimization of the microwave-assisted digestion procedure. There is no certified reference material for As, Cd, Hg, and Pb determination in dietary supplements. Typical diet was chosen as certified reference material in this study because it is a mixture of several food items and also due to the presence of proteins, carbohydrates and fats in its composition.

All aqueous solutions were prepared with analytical-grade reagents and ultrapure water, resistivity higher than 18.2 MΩ cm, (Milli-Q®, Millipore, Bedford, MA, USA). Experiments were performed using $HNO_3$ (Synth, Diadema, SP, Brazil) purified in a sub-boiling distillation apparatus Distillacid™ BSB-939-IR (Berghof, Eningen, Germany) and hydrogen peroxide 30% (v $v^{-1}$) (Synth, Diadema, SP, Brazil). Standard solutions used for ICP-MS calibrations were prepared by dilution of 1000 mg $L^{-1}$ of As, Cd, Hg and Pb (Qhemis, São Paulo, SP, Brazil) in 0.14 mol $L^{-1}$ $HNO_3$ medium, as well as the internal standards evaluated: Ga, Ge, Pd and Y. The analytical solutions for calibration

contained from 0.050 to 20 µg $L^{-1}$ of each analyte and the internal standards were added at 5.0 µg $L^{-1}$ to analytical calibration solutions, analytical blanks and samples. Clean up of the sample introduction system with 0.06 mol $L^{-1}$ HCl after calibration was required for avoiding memory effects for Hg [124].

For MICal method, calibration curves were obtained using two solutions for each sample [118,119]. Solution 1 was composed of 50% v $v^{-1}$ of the CRM digested and 50% v $v^{-1}$ of a standard solution containing Cd, Hg and Pb at 1.4, 0.20 and 1.8 µg $L^{-1}$, respectively, prepared in 3.4% v $v^{-1}$ $HNO_3$. These concentrations represent two times the value of the certified concentrations in CRM. Solution 2 contained 50% v $v^{-1}$ CRM digested and 50% v $v^{-1}$ of analytical blank, i.e. $HNO_3$ 3.4% v $v^{-1}$. Accuracies were evaluated by certified concentrations recoveries.

For OP SA, calibration curves were also obtained using the same Solution 1 and Solution 2 used for MICal [32,118,144], however Solution 1 was composed of 50% v $v^{-1}$ of the digested CRM and 50% v $v^{-1}$ of a standard solution containing As, Cd, Hg and Pb at 8.0, 1.4, 0.20 and 1.8 µg $L^{-1}$. For evaluate the best concentrations for standard additions, Solution 1 was also composed of 50% v $v^{-1}$ of the digested CRM and 50% v $v^{-1}$ of a standard solution containing As, Cd, Hg and Pb at 12, 2.1, 0.30 and 2.6 µg $L^{-1}$, respectively, prepared in 3.4% v $v^{-1}$ $HNO_3$. These concentrations represent three-fold the values of the certified concentrations in the CRM.

The dissolved organic carbon concentration and residual acidity was determined in all digest solutions. Carbon was determined by ICP OES using the atomic emission line 193.090 nm and dehydrated oxalic acid (Mallinckrodt Chemicals, St. Louis, MO, USA) was used as the carbon source for preparing calibrating analytical solutions. Residual acidity was determined by acid-base titration using 0.9417 mol $L^{-1}$ NaOH as titrant and phenolphthalein as indicator.

*5.1.4.3. Microwave-assisted sample preparation: Doehlert experimental design*

Masses of approximately 200 mg of CRM 1548a Typical Diet were accurately weighed directly in the perfluoroalkoxy alkanes (PFA) digestion vessels and microwave-assisted digested using a single reaction chamber oven

(UltraWave™, Milestone, Sorisole, Italy). Volumes of 5 mL of $HNO_3$ concentrations in three different levels and several volumes of $H_2O_2$ were applied. A modified Doehlert factorial design in three levels for the variable $HNO_3$ concentration and four levels for the variable volume of $H_2O_2$ was used for optimization of the sample preparation, totalizing 12 experiment + 12 blanks. The effects of $HNO_3$ concentration were evaluated from 0.50 (lower level, -0.866) to 7.0 mol $L^{-1}$ (higher level, +0.866) and volume of $H_2O_2$ between 0 (lower level, -1) and 3.0 mL (higher level, +1). In addition, experiments in the intermediate conditions, used to calculate the experimental error, were developed using 3.75 mol $L^{-1}$ (central point, 0) $HNO_3$ and 1.75 mL (intermediate condition, 0.167) of $H_2O_2$. More details about the experimental design are shown in Table 5.1.

TABLE 5.1 - Microwave-assisted digestion of sport supplement samples: matrix of experiments (based on a modified Doehlert factorial design) showing the variables evaluated for optimizing $H_2O_2$ volume and HNO3 concentration.

| Experiment | $H_2O_2$ (mL) | | $HNO_3$ (mol $L^{-1}$) | |
|---|---|---|---|---|
| | real | coded | real | coded |
| 1 | 3.00 | 1 | 3.75 | 0 |
| 2 | 0.500 | -0.667 | 3.75 | 0 |
| 3 | 3.00 | 1 | 7.00 | 0.866 |
| 4 | 0.500 | -0.667 | 7.00 | 0.866 |
| 5 | 3.00 | 1 | 0.500 | -0.866 |
| 6 | 1.75 | 0.167 | 7.00 | 0.866 |
| 7 | 1.75 | 0.167 | 0.500 | -0.866 |
| 8 | 0 | -1 | 7.00 | 0.866 |
| 9 | 0 | -1 | 3.75 | 0 |
| 10* | 1.75 | 0.167 | 3.75 | 0 |
| 11* | 1.75 | 0.167 | 3.75 | 0 |
| 12* | 1.75 | 0.167 | 3.75 | 0 |

* Central point.

Volumes of 150 mL of water and 5.0 mL of concentrated $HNO_3$ were inserted into the single reaction chamber (SRC) and the chamber was pressurized with nitrogen gas (99.9%, White Martins-Praxair) to 40 bar. The microwave heating program was applied as follows: (1) 2.5 min to reach 180 °C, (2) 2.5 min hold at 180 °C, (3) 2.5 min to reach 210 °C, (4) 2.5 min hold at 210 °C, (5) 10 min to reach 220 ºC and (6) 10 min hold at 220 °C. Subsequently, digests were diluted to 20.0 mL with distilled-deionized water and aliquot of

each solution was appropriately 2-fold diluted, followed by quantification by ICP-MS using four calibration methods: (1) external standard calibration (EC), (2) IS, (3) MICal, and (4) OP SA.

Afterwards, using the best conditions established for digestion of Typical Diet, masses of approximately 200 mg of all samples were accurately weighed and microwave-assisted digested in triplicate using 3.75 mol $L^{-1}$ $HNO_3$. Subsequently, digests were diluted to 20 mL with distilled-deionized water and aliquot of each solution was appropriately 2-fold diluted (final dilution of 200-fold, TDS of 0.50% m $v^{-1}$ and RA of 3.4%) followed by quantification using ICP-MS in each calibration methods previously cited.

*5.1.4.4. Addition and recovery tests according to USP requirements*

Recovery experiments were performed according to *J* values, which were calculated according to specific PDE value (15 for As; 5.0 for Cd; 15 for Hg and 10 µg $day^{-1}$ for Pb) [22], divided by the maximum permissible daily dose of supplement (MDD) and multiplied by the dilution factor adopted in the analytical procedure (DF), as shown in equation (1):

$$J = \frac{PDE(\frac{\mu g}{day})}{\mathrm{MDD}\left(\frac{\mathrm{g}}{\mathrm{day}}\right) \mathrm{x\ DF}} \qquad \text{Equation (1)}$$

According to the USP 233 [23] analytical procedures must demonstrate accurate spike recoveries between 70 and 150% of the spiked value for the mean of 3 samples spiked at concentrations ranging from 50 to 150% of the *J* value for each target element. Consequently, samples were spiked before microwave-assisted digestion with concentrations of 0.5*J* and 1.5*J* in order to verify the accuracy of the developed analytical procedure. Considering a MDD of 10 g day, $^{-1}$ the added concentrations for each analyte were 0.25 and 0.75 µg $g^{-1}$ for Cd, 0.50 and 1.5 µg $g^{-1}$ for Pb and 0.75 and 2.2 µg $L^{-1}$ for As and Hg. Considering the DF of 200-fold used in the procedure, the added concentrations for each analyte were 1.25 and 3.75 µg $L^{-1}$ for Cd, 2.50 and 7.50 µg $L^{-1}$ for Pb and 3.75 and 11.2 µg $L^{-1}$ for As and Hg.

For recovery experiments using MICal, Solution 1 was composed of 50% v $v^{-1}$ of the sample digested spiked at 0.5*J* and 1.5*J* and 50% v $v^{-1}$ of a standard solution containing Cd, Hg and Pb at 7.50, 22.5 and 15.0 µg $L^{-1}$, respectively, prepared in 3.4% v $v^{-1}$ $HNO_3$. These concentrations represent 2-fold the 1.5*J* values. Solution 2 contained 50% v $v^{-1}$ sample digested and 50% v $v^{-1}$ of analytical blank, i.e. $HNO_3$ 3.4% v $v^{-1}$. For recovery experiments using OP SA, Solutions 1 and 2 were the same used for MICal, however Solution 1 was composed of 50% v $v^{-1}$ of the sample digested spiked at 0.5*J* and 1.5*J* and 50% v $v^{-1}$ of a standard solution containing As, Cd, Hg and Pb at 22.5, 7.50, 22.5 and 15.0 µg $L^{-1}$, respectively. Figure 5.1 shows a schematic representation of the recovery experiments using MICal and OP SA according to 0.5*J* values.

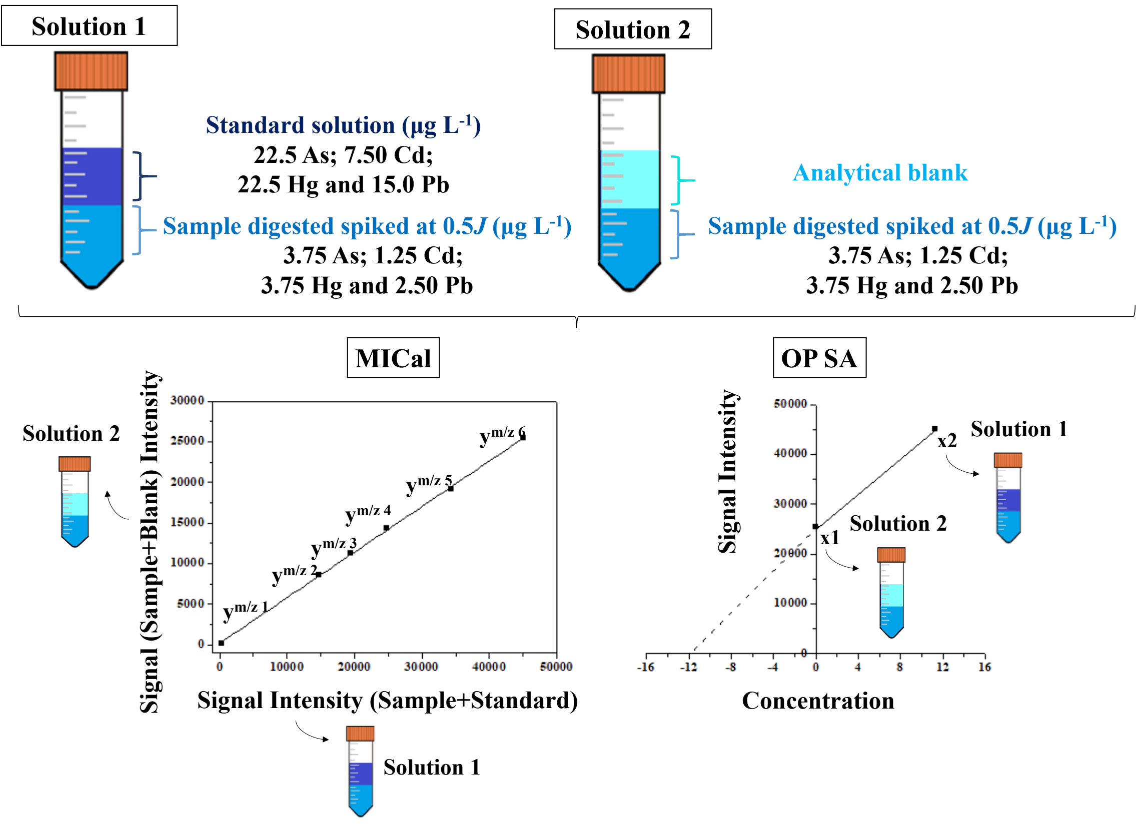



FIGURE 5.1 - Schematic representation of the general procedure used in recovery experiments using the calibration methods MICal and OP SA according to 0.5*J* values.

## 5.1.5 - Results and discussion

*5.1.5.1. Optimization of the microwave-assisted digestion procedure*

Experimental conditions for microwave-assisted digestion of CRM Typical Diet ($HNO_3$ concentration and volume of $H_2O_2$) were optimized using a modified Doehlert factorial design for each acid mixture. Twelve experiments were performed to establish an optimal condition employing the minimum acid concentration and lower volume of $H_2O_2$. Residual acidity, dissolved organic carbon concentrations and recoveries obtained for As, Cd and Pb using the calibrations methods: EC, IS, OP SA and MICal (except for As) were used as factorial design responses. Mercury recoveries were not used because the informative concentration (not certified) in CRM (0.005 $\mu g\ g^{-1}$) was below the respective limit of quantification (LOQ).

In each experiment, the individual desirability value was calculated as a function of the minimum acid concentration and lower volume of $H_2O_2$ [121,172]. This mathematical approach converts each response into an individual desirability (di) value, coded from 0 (undesired response) to 1 (desired response). In this case, (di = 1) corresponds to a desired response (analyte recoveries ranging from 80 to 120%, low RA and low dissolved organic carbon concentration), while (di = 0) represents a response outside the acceptable range (recoveries outside the range from 80 to 120%, high values of both RA and dissolved organic carbon concentration). Therefore, the individual desirability of each analyte was combined into a single response the overall desirability (OD) [172] through the geometric mean.

The OD was calculated from recoveries obtained using different calibration methods in order to evaluate possible matrix effects in experiments using low acid concentrations. The goal was to establish the optimal condition considering low RA (once minimum sample dilution is required) and dissolved organic carbon concentration that is easily corrected by calibration strategies. In short, a more dilute $HNO_3$ solution could be suitable for accurate determinations even in digests with higher carbon concentrations when proper calibration strategy was adopted. Table 5.2 shows the overall desirability obtained for all experiments in each calibration method evaluated.

TABLE 5.2 - Overall desirability (OD) considering the factorial design responses: residual acidity (RA), dissolved carbon content and analyte recoveries for As, Cd and Pb by ICP-MS using EC, IS, OP SA and MICal.

| Experiment | Factorial design responses and OD | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | RA (%) | Carbon (mg $L^{-1}$) | Recovery (%) for EC | | | OD | Recovery (%) for IS* | | | OD | Recovery (%) for OP SA | | | OD | Recovery (%) for MICal | | | OD |
| | | | As | Cd | Pb | EC | As | Cd | Pb | IS | As | Cd | Pb | OP SA | As | Cd | Pb | MCal |
| 1 | 2.3 | 268 | 87 | 87 | 46 | 0.59 | 101 | 90 | 51 | 0.59 | 80 | 106 | 102 | 0.79 | NA | 80 | 78 | 0.69 |
| 2 | 1.4 | 64 | 96 | 82 | 85 | 0.88 | 90 | 85 | 85 | 0.88 | 88 | 114 | 75 | 0.78 | NA | 90 | 54 | 0.60 |
| 3 | 5.4 | 81 | 105 | 91 | 101 | 0.73 | 107 | 94 | 106 | 0.73 | 109 | 90 | 113 | 0.73 | NA | 92 | 88 | 0.66 |
| 4 | 4.4 | 32 | 110 | 82 | 136 | 0.60 | 80 | 85 | 138 | 0.60 | 116 | 91 | 118 | 0.80 | NA | 80 | 97 | 0.75 |
| 5 | 0.67 | 225 | 144 | 85 | 93 | 0.71 | 107 | 87 | 85 | 0.89 | 126 | 93 | 95 | 0.83 | NA | 90 | 73 | 0.71 |
| 6 | 5.2 | 103 | 128 | 92 | 82 | 0.72 | 85 | 95 | 81 | 0.77 | 135 | 98 | 90 | 0.57 | NA | 88 | 78 | 0.66 |
| 7 | 0.68 | 459 | 154 | 102 | 103 | 0.60 | 92 | 105 | 90 | 0.80 | 127 | 91 | 87 | 0.71 | NA | 98 | 76 | 0.65 |
| 8 | 4.0 | 37 | 111 | 96 | 66 | 0.60 | 110 | 98 | 66 | 0.60 | 127 | 95 | 90 | 0.70 | NA | 97 | 87 | 0.75 |
| 9 | 1.6 | 70 | 135 | 105 | 111 | 0.69 | 112 | 108 | 100 | 0.91 | 86 | 98 | 114 | 0.89 | NA | 101 | 95 | 0.86 |
| 10 | 2.7 | 114 | 109 | 93 | 123 | 0.66 | 87 | 96 | 173 | 0.66 | 105 | 127 | 89 | 0.66 | NA | 111 | 97 | 0.83 |
| 11 | 2.7 | 112 | 108 | 96 | 105 | 0.86 | 100 | 99 | 109 | 0.86 | 107 | 88 | 98 | 0.86 | NA | 94 | 75 | 0.70 |
| 12 | 2.7 | 184 | 127 | 82 | 125 | 0.79 | 97 | 85 | 102 | 0.83 | 113 | 83 | 97 | 0.83 | NA | 84 | 87 | 0.79 |

* Yttrium was the best internal standard for As, Cd and Pb; NA: not applicable.

It was not possible to propose a multivariate model since all coefficients were not significant at 95% of confidence level. Thus, the best experimental conditions were chosen according to the higher OD. For EC method, the highest OD value (0.88) was observed for experiment 2 (digestion using 3.75 mol $L^{-1}$ $HNO_3$ and 0.5 mL of $H_2O_2$). Considering the experiments using 7.00 mol $L^{-1}$ $HNO_3$, lower values of OD was observed probably due to higher RA, once the recoveries for the experiments 3 and 6 ranged from 80 to 120% and dissolved organic carbon concentration for these were not so different compared to the experiments using 3.75 mol $L^{-1}$ $HNO_3$. On the other hand, the highest overall desirabilities (0.91, 0.89 and 0.86) values were obtained for the experiment 9 (digestion using only 3.75 mol $L^{-1}$ $HNO_3$) when using the calibration methods IS, OP SA and MICal, respectively.

For elemental determinations in trace concentrations, the analytical procedure using only $HNO_3$ is attractive because the use of $H_2O_2$ may imply in the addition of contaminants due to the relatively poor purity of analytical grade reagent [3,8]. Thus, 3.75 mol $L^{-1}$ $HNO_3$ was chosen as optimal condition for digestion of all sport supplements samples, since good recoveries were obtained for all analytes using the calibration strategies and both experiments (2 and 9) led to similar concentrations of dissolved organic carbon (64 and 70 mg $L^{-1}$) respectively.

*5.1.5.2. Analytical performance and accuracy according to USP requirements*

Sample dilution is required in analyses by ICP-MS to keep the total dissolved solids contents below 0.1% m $v^{-1}$ [7,8]. However, HMI system provides conditions for minimum dilution of digests allowing the introduction of samples with TDS around 3% m $v^{-1}$ and residual acidity around 5% v $v^{-1}$ [8,115]. Thereby, HMI mode enabled a lower dilution factor in the analytical procedure, once the final dilution of samples (200-fold) implied in a TDS of 0.50% m $m^{-1}$and RA of 3.4% v $v^{-1}$ and for analysis by ICP-MS in standard mode (without HMI system) would be necessary a final dilution 5-fold higher.

The limits of detection (LODs) and quantification (LOQs) were calculated for EC and IS according to IUPAC's recommendations considering three times and 10 times standard deviation of 10 measurements from blank

solutions and 10 estimated concentrations obtained from the blank solutions for MICal. For the LODs calculation using MICal [118,119], ten calibration curves were built. Solution 2 was composed of 50% v $v^{-1}$ digested blank plus 50% v $v^{-1}$ blank, i.e. 3.4% v $v^{-1}$ $HNO_3$, and Solution 1 composed of 50% v $v^{-1}$ digest blank plus 50% v $v^{-1}$ of a standard solution containing Cd, Hg and Pb at 7.50, 22.5 and 15.0 µg $L^{-1}$.

For OP SA, the accuracy was evaluated based on the standard error (SE), according to equation (2):

$$SE = \sqrt{\frac{\sum_i^n (y_i - \hat{y})^2}{n-1}} \qquad \text{Equation (2)}$$

where $y_i$ is the analyte reference concentration (from the addition level 0.5*J* value), $\hat{y}$ is the concentration determined by OP SA calibration and *n* is the number of samples analyzed in recovery experiments (n = 5). The linearity was tested applying the test F (the ratio $F_{experimental}$ / $F_{tabulated}$ was calculated) [120,121]. This ratio ≥10 demonstrated that the variances are statistically different (the mean of square of the regression is statistically different when compared with the mean of square of the residues) and the model can be considered linear [120,121,155].

Table 5.3 shows linear determination coefficient ($R^2$), slopes of analytical curves and LOQs obtained for all analytes determined using EC, IS and MICal, even SE and ratio $F_{experimental}$ / $F_{tabulated}$ obtained for determinations using OP SA calibration. All LODs and SE were lower than the addition levels suggested by Chapter 233 for all analytes.

TABLE 5.3 - Analytical performance parameters for As, Cd, Hg and Pb in sport supplement samples by ICP-MS using EC, IS, MICal and OP SA as calibration methods.

| Analyte | As | Cd | Hg | Pb |
|---|---|---|---|---|
| Acquisition mode | *He-HMI* | *No gas-HMI* | *No gas-HMI* | *No gas-HMI* |
| PDE (µg day$^{-1}$) | 15 | 5.0 | 15 | 10 |
| 0.5*J* Addition (µg g$^{-1}$) | 1.5 | 0.50 | 1.5 | 1.0 |
| 1.5*J* Addition (µg g$^{-1}$) | 2.2 | 0.75 | 2.2 | 1.5 |
| Calibration method | | EC | | |
| Slope | 103 | 1606 | 2145 | 9072 |
| $R^2$ | 0.9998 | 0.9997 | 0.9995 | 0.9991 |
| LOQ (µg g$^{-1}$) | 0.034 | 4.8x10$^{-3}$ | 0.33 | 0.038 |
| Calibration method | | IS | | |
| IS | Y | Y | Ge | Ge |
| $R^2$ | 0.9999 | 0.9999 | 0.9993 | 0.9992 |
| LOQ (µg g$^{-1}$) | 0.019 | 5.1x10$^{-3}$ | 0.30 | 0.038 |
| Calibration method | | MICal | | |
| LOQ (µg g$^{-1}$) | NA | 5.3x10$^{-3}$ | 0.37 | 0.026 |
| Calibration method | | OP SA | | |
| SE (µg g$^{-1}$) | 0.029 | 4.8x10$^{-3}$ | 0.20 | 0.013 |
| $F_{experimental}$ / $F_{tabulated}$ | 59939-869815 | 38424-1731958 | 9890-214219 | 11615-1755519 |

Satisfactory recoveries were obtained for Cd, Hg and Pb adopting *No gas* mode, inferring that there were no spectral interferences for the isotopes $^{112}Cd^+$, $^{202}Hg^+$ and $^{208}Pb^+$ monitored using EC, IS and OP SA, neither for the isotopes monitored for Cd ($^{111}Cd^+$, $^{112}Cd^+$, $^{113}Cd^+$, $^{114}Cd^+$ and $^{116}Cd^+$), Hg ($^{196}Hg^+$, $^{198}Hg^+$, $^{199}Hg^+$, $^{200}Hg^+$, $^{201}Hg^+$ and $^{202}Hg^+$) and Pb ($^{206}Pb^+$, $^{207}Pb^+$ and $^{208}Pb^+$) using MICal. For MICal, the isotopes $^{106}Cd^+$, $^{108}Cd^+$ and $^{110}Cd^+$ were not monitored for Cd due to isotopic interferences caused by $^{106}Pd^+$, $^{108}Pd^+$ and $^{110}Pd^+$ respectively, once Pd was evaluated as internal standard, as well as the isotopes $^{204}Hg^+$ and $^{204}Pb^+$. Satisfactory recoveries (lower than 130%) were obtained for $^{75}As^+$ using IS and OP SA only when using *He* mode, probably due to spectral interferences caused by polyatomic species such as $^{40}Ar^{35}Cl^+$, $^{59}Co^{16}O^+$; $^{23}Na^{12}C\ ^{40}Ar^+$ and $^{12}C\ ^{31}P^{16}O_2{}^+$, formed with common ions to plasma and other elements that can be present in the dietary supplement compositions. MICal was not applied to the monoisotopic ($^{75}As^+$).

In addition to the Typical Diet CRM, the accuracy of the developed analytical procedure was also evaluated by recovery experiments (Table 5.4) at levels equivalent to 0.5*J* and 1.5*J* for each target elements described in Chapter 2232 [22]. Accurate determinations were observed at both *J* levels based on acceptable recoveries established from 70 to 150% of the *J* value [23]. Recoveries using EC ranged from 69 to 85% for As, 70 to 99% for Cd, 84 to 138% for Hg and 57 to104% for Pb. Similarly, recoveries for all analytes ranged from 82 to 120% using IS, 90 to 125% using MICal and 88 to 120% using OP SA (except for Hg in sample DS1). The repeatability was demonstrated by a precision lower or equal 10% RSD for all samples.

TABLE 5.4 - Recoveries and relative standard deviations (%) for spikes in digested sport supplement samples (S1 to S5) according to the *J* values by ICP-MS (n = 3) using EC, IS, OP SA and MICal as calibration methods.

| Isotope | *J* addition | EC | | | | |
|---|---|---|---|---|---|---|
| | | S1 | S2 | S3 | S4 | S5 |
| $^{75}As^{+}$ | 0.5*J* | 84 (7) | 82 (12) | 76 (7) | 69 (2) | 78 (6) |
| | 1.5*J* | 84 (7) | 85 (2) | 76(2) | 75 (2) | 78 (6) |
| $^{112}Cd^{+}$ | 0.5*J* | 93 (2) | 95 (6) | 88 (5) | 99 (9) | 70 (2) |
| | 1.5*J* | 91 (1) | 93 (1) | 87 (2) | 86 (2) | 82 (3) |
| $^{202}Hg^{+}$ | 0.5*J* | 138 (9) | 125 (14) | 99 (7) | 115 (6) | 124 (14) |
| | 1.5*J* | 99 (9) | 84 (7) | 95 (10) | 92 (1) | 94 (2) |
| $^{208}Pb^{+}$ | 0.5*J* | 80 (11) | 69 (3) | 100 (4) | 103 (2) | 104 (2) |
| | 1.5*J* | 74 (3) | 57 (14) | 99 (1) | 100 (1) | 102 (1) |

| Isotope | *J* addition | IS | | | | |
|---|---|---|---|---|---|---|
| | | S1 | S2 | S3 | S4 | S5 |
| $^{75}As^{+}$ | 0.5*J* | 97 (6) | 101 (2) | 110 (4) | 91 (2) | 100 (7) |
| | 1.5*J* | 103 (8) | 102 (3) | 94 (5) | 98 (2) | 98 (6) |
| $^{112}Cd^{+}$ | 0.5*J* | 104 (2) | 108 (6) | 118 (2) | 120 (2) | 87 (1) |
| | 1.5*J* | 104 (7) | 107.1 (0.5) | 99 (2) | 106 (1) | 98 (2) |
| $^{202}Hg^{+}$ | 0.5*J* | 104 (3) | 106 (2) | 118 (2) | 102 (6) | 103 (10) |
| | 1.5*J* | 104 (7) | 90 (6) | 98 (2) | 106 (3) | 104 (5) |
| $^{208}Pb^{+}$ | 0.5*J* | 89 (10) | 82 (2) | 115 (9) | 115 (1) | 118 (6) |
| | 1.5*J* | 84 (2) | 110 (9) | 101 (1) | 113 (5) | 113 (2) |

TABLE 5.4 - Recoveries and relative standard deviations (%) for spikes in digested sport supplement samples (S1 to S5) according to the *J* values by ICP-MS (n = 3) using EC, IS, OP SA and MICal as calibration methods (continuation).

| Isotope | *J* addition | OP SA | | | | |
|---|---|---|---|---|---|---|
| | | S1 | S2 | S3 | S4 | S5 |
| $^{75}As^{+}$ | 0.5*J* | 95 (8) | 108 (9) | 98 (6) | 98 (1) | 94 (1) |
| | 1.5*J* | 108 (2) | 108 (2) | 106 (6) | 103 (2) | 101 (3) |
| $^{112}Cd^{+}$ | 0.5*J* | 105 (6) | 102 (2) | 105 (2) | 94 (6) | 101 (8) |
| | 1.5*J* | 101 (4) | 104 (6) | 92 (1) | 102 (7) | 100 (1) |
| $^{202}Hg^{+}$ | 0.5*J* | 127 (5) | 91 (8) | 112 (4) | 101 (9) | 120 (2) |
| | 1.5*J* | 124 (2) | 93 (7) | 88 (4) | 108 (4) | 107 (8) |
| $^{208}Pb^{+}$ | 0.5*J* | 107 (10) | 103 (3) | 99 (3) | 102 (3) | 104 (1) |
| | 1.5*J* | 114 (10) | 102 (8) | 98 (8) | 104 (5) | 100 (2) |
| **Analyte** | ***J* addition** | **MICal** | | | | |
| | | **S1** | **S2** | **S3** | **S4** | **S5** |
| Cd | 0.5*J* | 104 (2) | 102 (2) | 93 (3) | 103 (4) | 96 (3) |
| | 1.5*J* | 103 (4) | 103 (2) | 90 (2) | 94 (7) | 100 (3) |
| Hg | 0.5*J* | 115 (4) | 93 (6) | 114 (7) | 122 (6) | 102 (9) |
| | 1.5*J* | 125 (5) | 92 (7) | 104 (9) | 107 (7) | 109 (10) |
| Pb | 0.5*J* | 101 (10) | 106 (4) | 107 (2) | 102 (1) | 113 (4) |
| | 1.5*J* | 101 (7) | 101 (9) | 97 (5) | 104 (1) | 104 (3) |

For IS method, the best internal standard was evaluated by recoveries of certified concentrations for Typical Diet and by addition-recovery experiments to five samples (Table 5.4). Yttrium was the best internal standard for all analytes, but satisfactory recoveries were also obtained when using $^{104}Pd^{+}$ as internal standard for $^{75}As^{+}$, $^{69}Ga^{+}$ for $^{208}Pb^{+}$, and $^{70}Ge^{+}$ for $^{202}Hg^{+}$.

For OP SA and MICal methods, the addition point concentration used in Solution 1 (twice the 1.5*J* value) was effective to obtain satisfactory recoveries. The standard concentration added is a significant parameter to be evaluated because if it is too smaller or too bigger compared to the analyte concentration, the calibration method may become ineffective [118]. MICal calibration plots and linear model for OP SA curves are depicted in Figures 5.2 and 5.3, respectively. As showed in Figure 5.2, five isotopes were monitored for Cd, six for Hg and three for Pb, and good determination coefficients were obtained in all cases. For OP SA, the range of ratios $F_{experimental}$ / $F_{tabulated}$ (showed in Table 5.3) calculated considering all samples demonstrated that the

variances are statistically different and the model can be considered linear for all analytes.

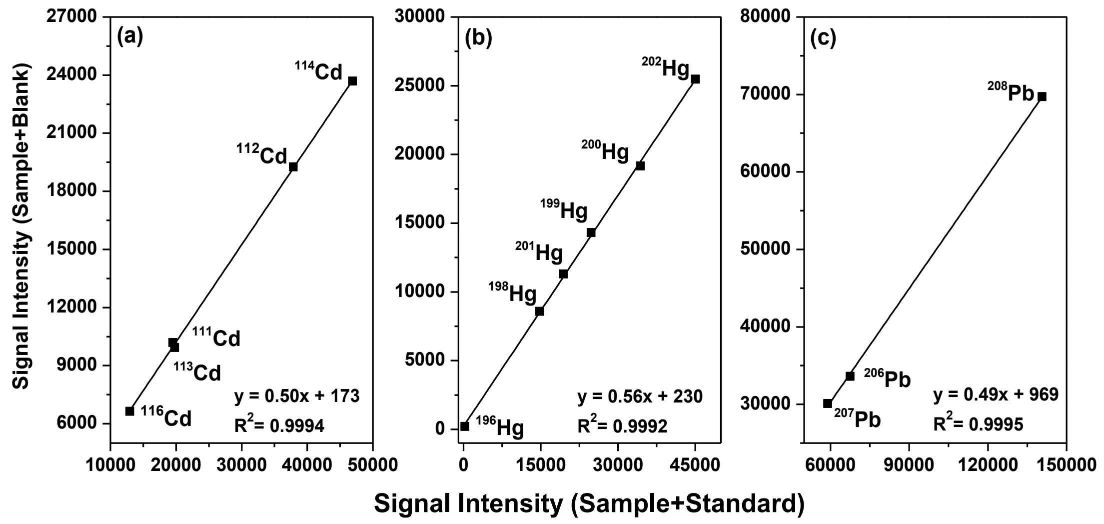



Figure 5.2 - Linear model for multi-isotope calibration for **(a)** Cd, **(b)** Hg and **(c)** Pb in sport supplement sample (S1) with 1.5*J* addition level.

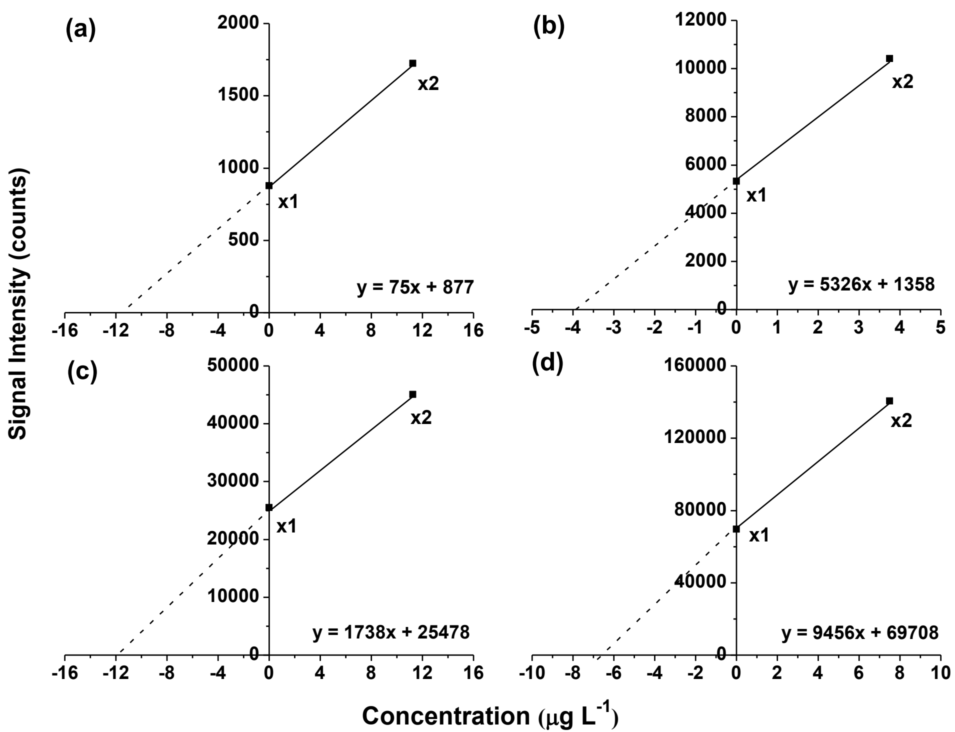



Figure 5.3 - One-point standard addition curve for **(a)** $^{75}As^{+}$ **(b)** $^{112}Cd^{+}$, **(c)** $^{202}Hg^{+}$ and **(d)** $^{208}Pb^{+}$ in sport supplement sample (S1) with 1.5*J* addition level. In **(a)** and **(c)** x1 = 11.2 and x2 = 22.5 μg L$^{-1}$; **(b)** x1 = 3.75 and x2 = 7.50 μg L$^{-1}$, and **(d)** x1 = 7.50 and x2 = 15.0 μg L$^{-1}$.

*5.1.5.3. Determination of As, Cd, Hg and Pb in dietary supplements*

Table 5.5 shows the concentrations of As, Cd, Hg and Pb determined in ten sport supplements using the three calibration methods evaluated (IS, MICal and OP SA). All analytes were below the respective LODs and LOQs, except As in sample S8, Cd in samples S1, S3, S5, S6, S7, S9 and S10 and Pb in samples S3, S5 and S9. For these three samples, the concentrations of Pb were only determined using MICal and OP SA due to the lower LOQs when compared to IS. The analyte concentrations determined in samples did not present significant differences (t-paired test with 95% of confidence) between all calibration methods evaluated. In addition, all sport supplements analyzed presented concentrations below the maximum allowed limits recommended by Chapter 2232 [22].

TABLE 5.5 - Determination of As, Cd, Hg and Pb ($\mu g\ g^{-1}$, mean ± standard deviation, n = 3) in sport supplement samples (S1 to S10) by ICP-MS using IS, OP SA and MICal as calibration methods.

| Sample | As | | | Cd | | | Hg | | | Pb | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IS | MICal | OP SA | IS | MICal | OP SA | IS | MICal | OP SA | IS | MICal | OP SA |
| S1 | <0.020 | NA | <0.030 | 0.019 ± 0.009 | 0.030 ± 0.003 | 0.026 ± 0.004 | <0.090 | <0.12 | <0.20 | <0.012 | <0.0080 | <0.013 |
| S2 | <0.0060 | NA | <0.030 | <1.5x10$^{-3}$ | <1.6x10$^{-3}$ | <4.5x10$^{-3}$ | <0.090 | <0.12 | <0.20 | <0.012 | <0.0080 | <0.013 |
| S3 | <0.0060 | NA | <0.030 | 0.012 ± 0.001 | 0.012 ± 0.002 | 0.011 ± 0.001 | <0.299 | <0.37 | <0.20 | <0.038 | 0.042 ± 0.006 | 0.041 ± 0.001 |
| S4 | <0.0060 | NA | <0.030 | <1.5x10$^{-3}$ | <1.6x10$^{-3}$ | <4.5x10$^{-3}$ | <0.090 | <0.12 | <0.20 | <0.038 | <0.026 | <0.013 |
| S5 | <0.0060 | NA | <0.030 | 0.018 ± 0.004 | 0.025 ± 0.005 | 0.025 ± 0.007 | <0.090 | <0.12 | <0.20 | <0.038 | 0.037 ± 0.001 | 0.035 ± 0.005 |
| S6 | <0.0060 | NA | <0.030 | 0.008 ± 0.001 | 0.009 ± 0.001 | 0.008 ± 0.001 | <0.090 | <0.12 | <0.20 | <0.012 | <0.0080 | <0.013 |
| S7 | <0.020 | NA | <0.030 | 0.019 ± 0.003 | 0.02 ± 0.01 | 0.020 ± 0.004 | <0.090 | <0.12 | <0.20 | <0.012 | <0.0080 | <0.013 |
| S8 | 0.035 ± 0.008 | NA | 0.032 ± 0.004 | <5.1x10$^{-3}$ | <5.3x10$^{-3}$ | <4.5x10$^{-3}$ | <0.090 | <0.12 | <0.20 | <0.012 | <0.0080 | <0.013 |
| S9 | <0.020 | NA | <0.030 | 0.017 ± 0.002 | 0.011 ± 0.004 | 0.012 ± 0.004 | <0.30 | <0.37 | <0.20 | <0.038 | 0.035 ± 0.003 | 0.032 ± 0.003 |
| S10 | <0.0060 | NA | <0.030 | 0.012 ± 0.003 | 0.014 ± 0.002 | 0.012 ± 0.003 | <0.090 | <0.12 | <0.20 | <0.012 | <0.0080 | <0.013 |

### 5.1.6 - Conclusions

An adequate microwave-assisted sample preparation for dietary sport supplement samples using only dilute nitric acid solution (3.75 mol $L^{-1}$) was proposed. A modified Doehlert factorial design enabled to optimize the concentration of the acid solution used for sample digestions according to the adopted calibration strategy. Due to the magnitude of matrix effects on analytes determination, a mild optimal digestion condition can be used when combined with proper calibration methods, such as IS, MICal and OP SA, which correct for matrix effects on ICP-MS measurements. In addition, collision cell mode was effective for overcoming polyatomic interferences on $^{75}As^{+}$. There were no significant differences among the LODs obtained for As, Cd, Hg and Pb in each calibration strategy evaluated. In addition, LODs (IS and MICal) and SE (OP SA) were lower than the addition levels suggested by the USP Chapter 2232 for all analytes. It is important to emphasize that for sample preparation of dietary supplements with different compositions it can be necessary to evaluate other acid mixtures. Nevertheless, this procedure is attractive for quality control of sport supplements without using concentrated acids and critical digestion conditions.

# 6 - CHAPTER 6

# General conclusions

## 6.1 - General conclusions

It is well known that sample preparation is a critical step for the success of analysis. Generally, pharmaceutical samples present a wide range of different compositions, e.g. excipients, flavoring agents, stabilizers, among others. These complex matrices make the sample preparation a challenging step. This dissertation presents alternative green sample preparation procedures for drugs and pharmaceuticals based on microwave-assisted digestion, acid dilution and dispersive liquid-liquid microextraction to determine elemental impurities using argon-based plasma spectroanalytical methods.

It was here demonstrated that microwave-assisted sample preparation of drugs and dietary supplements using dilute nitric acid solution can be seen as an alternative to the use of aggressive acid solutions and critical conditions of digestion for further determination of elemental impurities using ICP OES and/or ICP-MS. Alternatively, a simple, inexpensive and faster dilute-and-shoot procedure for liquid drugs analysis by ICP OES was also presented. These procedures meet the green chemistry principles because only dilute nitric acid solution was used for sample preparation.

This dissertation also demonstrates the use of calibration methods, such as multi-isotope calibration (MICal), one-point standard addition (OP SA) and internal standardization (IS), for correction of matrix effects in digests and for applying dilute-and-shoot procedure prior to ICP OES and ICP-MS measurements. In addition, instrumental strategies based on aerosol dilution (HMI) and collision cell technology (CCT-KED) for ICP-MS measurements and different sample introduction systems, e.g. V-shaped groove nebulizer (used for high solid contents) and a multinebulizer (used for the simultaneous introduction of organic and aqueous solutions into the argon plasma), for ICP OES measurements were also employed to correct for spectral and non-spectral interferences making feasible the accurate determination of elemental impurities by both ICP-based methods.

In order to improve the analytical capabilities of ICP OES, sample pretreatment procedures based on DLLME have been developed and successfully applied to the simultaneous extraction/preconcentration of elemental impurities for trace elemental determination using ICP OES.

Extraction techniques based on DLLME meet green recommendations because they use only a few microliters of an organic extractant solvent. Moreover, research on a DLLME procedure using a deep eutectic solvent (green synthesized solvent) was also presented. Compared to ICP-MS, the availability of ICP OES instruments is higher. Therefore, DLLME-ICP OES methods can be seen as a promising alternative for trace elemental analysis in drug samples.

Considering ICP-MS analytical performance, this is the most suitable analytical method for elemental analysis of pharmaceutical products according to the new USP requirements. ICP-MS can be applied to determine trace concentrations of all target-elements recommended by USP Chapter 232. On the other hand, ICP OES, which provides easier operation and higher sample throughput, can be typically used for elemental analysis of medicines with low daily dose requirements and/or for sample preparation procedures not requiring very high dilutions. Alternatively, as demonstrated here, ICP OES can be combined with some extraction/preconcentration techniques to provide suitable sensitivity for trace elemental determination.

# 7 - CHAPTER 7

# REFERENCES

## 7.1 - References

1. BARIN, J. S.; MELLO, P. A.; MESKO, M. F.; DUARTE, F. A. & FLORES, E. M. M. "Determination of elemental impurities in pharmaceutical products and related matrices by ICP-based methods: a review". Anal. Bioanal. Chem., **408**: 4547-4566, 2016.

2. BALARAM, V. "Recent advances in the determination of elemental impurities in pharmaceuticals - Status, challenges and moving frontiers". TrAC Trends Anal. Chem., **80**: 83-95, 2016.

3. LEWEN, N. "Preparation of pharmaceutical samples for elemental impurities analysis: some potential approaches". Spectroscopy, **31**: 36-43, 2016.

4. POHL, P.; BIELAWSKA-POHL, A. DZIMITROWICZ, A.; JAMROZ, P. & WELNA, M. "Impact and practicability of recently introduced requirements on elemental impurities". TrAC Trends Anal. Chem.,**101**: 43-55, 2018.

5. BIZZI, C. A.; PEDROTTI, M. F.; SILVA, J. S.; BARIN, J. S.; NÓBREGA, J. A. & FLORES, E. M. M. "Microwave-assisted digestion methods: Towards greener approaches for plasma-based analytical techniques". J. Anal. At. Spectrom., **32**: 1448-1466, 2017.

6. NÖLTE, J. ICP Emission Spectrometry: A Practical Guide. First ed., Wiley-VCH, New York, 2003.

7. THOMAS, R. J. Practical Guide to ICP-MS. Third ed., Boca Raton: CRC Press, New York, 2013.

8. THOMAS, R. J. Measuring Elemental Impurities in Pharmaceuticals: A Practical Guide. First ed., Boca Raton: CRC Press, New York, 2018.

9. USP (2020). The United States Pharmacopeia. *Empowering a healthy tomorrow*. <https://www.usp.org/>. Accessed in April, 2021.

10. The United States Pharmacopeia. The National Formulary, USP 37/NF 32, The United States Pharmacopeial Convention, Rockville, Chapter 231, Heavy elements, 2014.

11. The United States Pharmacopeia Convention, The United States Pharmacopeia, Chapter 232: Elemental Impurities - Limits, Revision Bulletin, Official February 1, 2013.

12. The United States Pharmacopeia Convention, The United States Pharmacopeia, Chapter 233: Elemental Impurities - Procedures, Revision Bulletin, Official February 1, 2013.

13. The United States Pharmacopeia Convention. The United States Pharmacopeia. Chapter 2232: Elemental Contaminants in Dietary Supplements. Pharmacopeia Forum - PF 38(3), May-June, 2012.

14. USP (2020). The United States Pharmacopeia. *Elemental Impurities Updates*. <https://www.usp.org/chemical-medicines/elemental-impurities-updates>. Accessed in April, 2021.

15. The United States Pharmacopeia Convention, The United States Pharmacopeia, Chapter 232: Elemental Impurities – Limits, Forum 42(2), March- April, 2016.

16. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Guideline for Elemental Impurities, Q3D Step 4, December 16, 2014.

17. ICH (2020). The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. *Harmonisation for better health*. <https://www.ich.org/>. Accessed in April 2021.

18. The United States Pharmacopeia Convention, The United States Pharmacopeia, Chapter 232: Elemental Impurities - Limits, USP 39 – NF 34, Official May 1, 2016.

19. The United States Pharmacopeia Convention, The United States Pharmacopeia, Chapter 232: Elemental Impurities - Limits, USP 40 – NF 35, First Supplement, Official December 1, 2017.

20. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Guideline for Elemental Impurities, ICH Q3D(R1) Step 5, Geneva, Switzerland, March 28, 2019.

21. The United States Pharmacopeia, Chapter <232> Elemental Impurities Limits, U.S. Pharmacopeia National Formulary 2019: UPS42-NF37, United States Pharmacopeia Convention, 2019.

22. The United States Pharmacopeia, Chapter <2233> Elemental Contaminants in Dietary Supplements, U.S. Pharmacopeia National Formulary 2019: UPS42-NF37, United States Pharmacopeia Convention, 2019.

23. The United States Pharmacopeia, Chapter <233> Elemental Impurities Procedures, U.S. Pharmacopeia National Formulary 2019: UPS42-NF37, United States Pharmacopeia Convention, 2019.

24. EUR (2020). Council of European. *Update on the ph. eur. policy on elemental impurities*. <https://www.edqm.eu/en/news/ph-eur-launches-public-consultation-general-chapter-2420-determination-elemental-impurities>. Accessed in April 2021.

25. European Medicines Agency, Guideline on the specification limits for residues of metal catalysts or metal reagents EMEA/CHMP/SWP/4446/2000, 2008.

26. Brazilian Pharmacopeia, 5th Ed., Chapter 5.3.2.3 Limit test for heavy metals, the assay method. National Sanitary Surveillance Agency, 2010.

27. HOLM, R. & ELDER, D. P. "Analytical advances in pharmaceutical impurity profiling". Eur. J. Pharm. Scienc., **87**: 118-135, 2016.

28. BOETZEL, R.; CESZLAK, A.; DAY, C.; DRUMM, P.; BEJAR, J. G.; GLENNON, J.; HARRIS, L.; HEGHES, C. I.; HORGA, R.; JACOBS, P. L.; KEURENTJES, W. J. T. M.; KING, F.; LEE, C. W.; LEWEN, N.; MARCHANT, C. A.; MARIS, F. A.; NYE, W.; POWELL, S.; ROCKSTROH, H.; RUTTER, L.; SCHWEITZER, M.; SHANNON, E.; SMALLSHAW, L.; TEASDALE, A.; THOMPSON, S. & WILKINSON, D. "An elemental impurities excipient database: a viable tool for ICH Q3D drug product risk assessment". J. Pharm. Sci., **107**: 2335-2340, 2018.

29. FLLSZAR, K. A.; WALKER, D. & ALLAIN, L. "Profiling of metal ions leached from pharmaceutical packaging materials". PDA J. Pharma. Sci. Technol., **60**: 337-342, 2016.

30. IARC (2020). The International Agency for Research on Cancer. *IARC monographs on the evaluation of carcinogenic risks to humans*. <http://monographs.iarc.fr/ENG/Monographs/vol100C/mono100C.pdf>.
Accessed in April, 2021.

31. FISCHER, L.; ZIPFEL, B.; KOELLENSPERGER, G.; KOVAC, J.; BILZ, S.; KUNKEL, A.; VENZAGO, C. & HANN, S. "Flow injection combined with ICP-MS for accurate high throughput analysis of elemental impurities in pharmaceutical products according to USP <232>/<233>". J. Pharm. Biomed. Anal., **95**: 121-129, 2014.

32. PINHEIRO, F. C.; BARROS, A. I. & NÓBREGA, J. A. "Evaluation of dilute-and-shoot procedure for determination of inorganic impurities in liquid pharmaceutical samples by ICP OES". Microchem. J., **146**: 948-956, 2019.

33. MULLER, A. L. H.; MELLO, P. A.; MESKO, M. F.; DUARTE, F. A.; DRESSLER, V. L.; MULLER, E. I. & FLORES, E. M. M. "Bromine and iodine determination in active pharmaceutical ingredients by ICP-MS". J. Anal. At. Spectrom., **27**: 1889-1894, 2012.

34. STØVING, C.; JENSEN, H.; GAMMELGAARD, B. & STURUP, S. "Development and validation of an ICP-OES method for quantitation of elemental impurities in tablets according to coming US pharmacopeia chapters". J. Pharm. Biomed. Anal., **84**: 209-214, 2013.

35. MULLER, A. L. H.; OLIVEIRA, J. S. S.; MELLO, P. A.; MULLER, E. I. & FLORES, E. M. M. “Study and determination of elemental impurities by ICP-MS in active pharmaceutical ingredients using single reaction chamber digestion in compliance with USP requirements”. Talanta, **136**: 161-169, 2013.

36. SILVA, C. S.; PINHEIRO, F. C.; AMARAL, C. D. B. & NÓBREGA, J. A. “Determination of As, Cd, Hg and Pb in continuous use drugs and excipients by plasma-based techniques in compliance with the United States Pharmacopeia requirements”. Spectrochim. Acta Part B., **138**:14-17, 2017.

37. VAN HOECKE, K.; CATRY, C. & VANHAECKE, F. “Optimization of sample preparation and a quadrupole ICP-MS measurement protocol for the determination of elemental impurities in pharmaceutical substances in compliance with USP guidelines”. J. Anal. At. Spectrom., **27**: 1909-1919, 2012.

38. MENOUTIS, J.; PARISI, A. & VERMA, N. “Study of the use of axial viewed inductively coupled plasma atomic emission spectrometry with ultrasonic nebulization for the determination of select elemental impurities in oral drug products”. J. Pharm. Biomed. Anal., **152**: 12-16, 2018.

39. LEWEN, N.; MATHEW, S.; SCHENKENBERGER, M. & RAGLIONE, T. “A rapid ICP-MS screen for heavy metals in pharmaceutical compounds”. J. Pharm. Biomed. Anal., **35**: 739-752, 2004.

40. PINHEIRO, F. C.; BARROS, A. I. & NÓBREGA, J. A. “Microwave-assisted sample preparation of medicines for determination of elemental impurities in compliance with United States Pharmacopeia: How simple can it be?”. Anal. Chim. Acta, **1065**: 1-11, 2019.

41. PINHEIRO, F. C.; BARROS, A. I. & NÓBREGA, J. A. “Elemental impurities analysis in name-brand and generic omeprazole drug samples”. Heliyon, **6**: e03359, 2020.

42. PINHEIRO, F. C.; BABOS, D. V.; BARROS, A. I.; PEREIRA-FILHO, E. R. & NÓBREGA, J. A. “Microwave-assisted digestion using dilute nitric acid solution and investigation of calibration strategies for determination of As, Cd, Hg and Pb in dietary supplements using ICP-MS”. J. Pharm. Biomed. Anal., **174**: 471-478, 2019.

43. AVULA, B.; WANG, I.; SMILLIE, T. J.; DUZGOREN-AYDIN, N. S. & KHAN, I. A. “Quantitative determination of multiple elements in botanicals and dietary supplements using ICP-MS”. J. Agric. Food Chem., **58**: 8887-8894, 2010.

44. KRAWCZYK, M. “Determination of macro and trace elements in multivitamin dietary supplements by high–resolution continuum source graphite furnace atomic absorption spectrometry with slurry sampling”. J. Pharm. Biomed. Anal., **88**: 377-384, 2014.

45. MORET, S.; PREVARIN, A. & TUBARO, F. “Levels of creatine, organic contaminants and heavy metals in creatine dietary supplements”. Food Chem., **126**: 1232-1238, 2011.

46. DOLAN, S. P.; NORTRUP, D. A.; BOLGER, M. P. & CAPAR, S. G. “Analysis of dietary supplements for arsenic, cadmium, mercury, and lead using inductively coupled plasma mass spectrometry”. J. Agric. Food Chem., **51**: 1307-1312, 2003.

47. KAUFFMAN, J. F.; WESTENBERGER, B. J.; ROBERTSON, J. D.; GUTHRIE, J.; JACOBS, A. & CUMMINS, S. K. “Lead in pharmaceutical products and dietary supplements”. Regul. Toxicol. Pharmacol., **48**: 128-134, 2007.

48. ANTES, F. G.; MESKO, M. F.; BARIN, J. S.; MOREIRA, C. M.; FLORES, E. M. M. & DRESSLER, V. L. “Development of multi-elemental method for quality control of parenteral component solutions using ICP-MS”. Microchem. J., **98**: 144-149, 2011.

49. LÁSZTITY, A.; KELKÓ-LÉVAI, A.; VARGA, I.; ZIH-PERÉNYI, K. & BERTALAN, E. “Development of atomic spectrometric methods for trace metal analysis of pharmaceuticals”. Microchem. J., **73**: 59-63, 2002.

50. WANG, T. B.; WU, J.; HARTMAN, R.; JIA, X. & EGAN, R. S. “A multi element ICP-MS survey method as an alternative to the heavy metals limit test for pharmaceutical materials”. J. Pharm. Biomed. Anal., **23**: 867-890, 2000.

51. LORENÇATTO, R.; CARVALHO, G. G. A. & OLIVEIRA, P. V. “Direct analysis of liquid drugs by inductively coupled plasma mass spectrometry using aerosol dilution”. J. Anal. At. Spectrom., **35**: 2179-2190, 2020.

52. ALTHOFF, G. A.; WILLIAMS, C. B.; MCSWEENEY, T.; GONÇALVES, D. A. & DONATI, G. L. “Microwave-induced plasma optical emission spectrometry (MIP OES) and standard dilution analysis to determine trace elements in pharmaceutical samples”. Appl Spectrosc., **71**: 2692-2698, 2017.

53. AL-AMMAR, A. S. & NORTHINGTON, J. “Accuracy improvement in the determination of palladium in pharmaceuticals by eliminating volatility error when using ICP-MS coupled with direct introduction of sample dissolved in organic solvents”. J. Anal. At. Spectrom., **26**: 1531-1533, 2011.

54. TU, Q.; WANG, T. B. & ANTONUCCI, V. “High-efficiency sample preparation with dimethylformamide for multi-element determination in pharmaceutical materials by ICP-AES”. J. Pharm. Biomed. Anal., **52**: 311-315, 2010.

55. TU, Q.; WANG, T. B. & WELCH, C. J. “High-throughput metal screening in pharmaceutical samples by ICP-MS with automated flow injection using a modified HPLC configuration”. J. Pharm. Biomed. Anal., **51**: 90-955, 2010.

56. SIMS, J.; SMITH, A.; PATEL, D.; BATCHELOR, R. & CARREIRA, J. “Automated sample preparation for ICP analysis of active pharmaceutical ingredients and intermediates”. J. Lab. Autom., **16**: 377-380, 2011.

57. ZACHARIADIS, G. A. & MICHOS, C. E. “Development of a slurry introduction method for multi-element analysis of antibiotics by inductively coupled plasma atomic emission spectrometry using various types of spray chamber and nebulizer configurations”. J. Pharm. Biomed. Anal., **43**: 951-958, 2007.

58. ZACHARIADIS, G. A. & SAHANIDOU, E. “Analytical performance of a fast multi element method for titanium and trace elements determination in cosmetics and pharmaceuticals by ICP-AES”. Cent. Eur. J. Chem., **9**: 213-217, 2011.

59. KRISHNA MURTY, A. S. R.; KULSHRESTA, U. C.; NAGESWARA RAO, T. & KUMAR TALLURI, M. V. N. “Determination of heavy metals is selected drug substances by inductively coupled plasma e mass spectrometry”. Indian J. Chem. Technol., **12**: 229-231, 2005.

60. KNAM, K. N.; ISENSEE, R.; INFANTINO, G.; PUTYERA, K. & WANG, X. W. “Microwave-induced combustion for ICP-MS: a generic approach to trace elemental analyses of pharmaceutical products”. Spectroscopy, **26**: 36-41, 2011.

61. BARIN, J. S.; TISCHER, B.; PICOLOTO, R. S.; ANTES, F. G.; DA SILVA, F. E. B.; PAULA, F. R. & FLORES, E. M. M. “Determination of toxic elements in tricyclic active pharmaceutical ingredients by ICP-MS: a critical study of digestion methods”. J. Anal. At. Spectrom., **29**: 352-358, 2014.

62. KACZALA, S.; COSTA, A. B.; POSSELT, E. L.; BARIN, J. S.; FLORES, E. M. M. & DRESSLER, V. L. “Element determination in pharmaceuticals using direct solid analysis-electrothermal vaporization inductively coupled plasma optical emission spectrometry”. J Braz. Chem. Soc., **26**: 475-83, 2015.

63. NIEMELA, M.; KOLA, H.; EILOLA, K. & PERAMAKI, P. “Development of analytical methods for the determination of sub-ppm concentrations of palladium and iron in methotrexate”. J. Pharm. Biomed. Anal., **35**: 433-439, 2004.

64. GONZALEZ, M. H.; DA SILVA, C. S.; AMARAL, C. D. B.; BIANCHI, S. R.; DE OLIVEIRA, L. H. B.; COELHO, J. S.; OLIVEIRA, A. & NOGUEIRA, A. R. A. “Determination of elemental impurities in acyclovir ointment and raw materials using microwave acid digestion (MWAD) and ICP-MS”. J. Braz. Chem. Soc., **28**: 98-105, 2017.

65. PLUHACEK, T.; HANZAL, J.; HENDRYCH, J. & MILDE, D. “Microwave-assisted digestion using nitric acid for heavy metals and sulfated ash testing in active pharmaceutical ingredients”. Pharmazie, **71**: 177-180, 2016.

66. RAGHURAM, P.; SOMA RAJU, I. V. & SRIRAMULU, J. “Heavy metals testing in active pharmaceutical ingredients: An alternative approach”. Pharmazie, **65**: 15-18, 2010.

67. WOLLEIN, U.; BAUER, B.; HABERNEGG, R. & SCHRAMEK, N. “Potential metal impurities in active pharmaceutical substances and finished medicinal products - A market surveillance study”. Eur. J. Pharm. Sci., **77**: 100-105, 2015.

68. SOŁTYK, K.; ŁOZAK, A.; OSTAPCZUK, P. & FIJAŁEK, Z. “Determination of chromium and selected elements in multimineral and multivitamin preparations and in pharmaceutical raw material”. J. Pharm. Biomed. Anal., **32**: 425-432, 2003.

69. JIN, C. “Clean chemistry for elemental impurities analysis of pharmaceuticals in compliance with USP 232”. AAPS PharmSciTech, **17**: 1141-1149, 2016.

70. LI, G.; SCHONEKER, D.; ULMAN, K. L.; STURM, J. J.; THACKERY, L. M. & KAUFFMAN, J. F. “Elemental impurities in pharmaceutical excipients”. J. Pharm. Sci., **104**: 4197-4206, 2015.

71. SIMITCHIEV, K.; STEFANOVA, V.; KMETOV, V.; ANDREEV, G.; KOVACHEV, N. & CANALS, A. “Microwave-assisted cloud point extraction of Rh, Pd and Pt with 2-mercaptobenzothiazole as preconcentration procedure prior to ICP-MS analysis of pharmaceutical products”. J. Anal. At. Spectrom., **23**: 717-726, 2008.

72. PONCE DE LEON, C. A.; MONTES-BAYAN, M. & CARUSO, J. A. “Trace element determination in vitamin E using ICP-MS”. Anal. Bioanal. Chem., **374**: 230-234, 2002.

73. JANCHEVSKA, K.; STAFILOV, T.; MEMED-SEJFULAH, S.; BOGDANOSKA, M.; UGARKOVIC S. & PETRUSHEVSKI, G. “ICH Q3D based elemental impurities study in liquid pharmaceutical dosage form with high daily intake – comparative analysis by ICP-OES and ICPMS”. Drug Dev. Ind. Pharm., **46**: 456-461, 2020.

74. PLUHÁČEK, T.; RUČKA, M. & MAIER, V. “A direct LA-ICP-MS screening of elemental impurities in pharmaceutical products in compliance with USP and ICH-Q3D”. Anal. Chim. Acta, **1078**: 1-7, 2019.

75. MILDE, D.; PLUHÁČEK, T.; KUBA, M.; SOUČKOVÁ, J. & DA SILVA, R. J. N. B. “Measurement uncertainty evaluation from correlated validation data: Determination of elemental impurities in pharmaceutical products by ICP-MS”. Talanta, **220**: 121386, 2020.

76. LIN, M. L. & JIANG, S. J. “Determination of trace Cr, Mo, Pd, Cd, Pt and Pb in drug tablets by ultrasonic slurry sampling electrothermal vaporization inductively coupled plasma mass spectrometry”. J. Anal. At. Spectrom., **26**: 1813-1818, 2011.

77. RUDOVICA, V.; VIKSNA, A. & ACTINS, A. “Application of LA-ICP-MS as a rapid tool for analysis of elemental impurities in active pharmaceutical ingredients”. J Pharm. Biomed. Anal., **91**: 119-122, 2014.

78. DASH, K.; VENKATESWARLU, G.; THANGAVEL, S.; RAO, S. V. & CHAURASIA, S. C. “Ultraviolet photolysis assisted mineralization and determination of trace levels of Cr, Cd, Cu, Sn, and Pb in isosulfan blue by ICP-MS”. Microchem. J., **98**: 312-316, 2011.

79. VENZAGO, C.; POPP, M.; KOVAC, J. & KUNKEL, A. “Pharmacopeial requirements for elemental impurities: a novel approach to the trace determination of osmium by oxidative pressure vessel sample digestion and measurement using inductively coupled plasma mass spectrometry (ICP-MS) after complexation and stabilization”. J. Anal. At. Spectrom., **28**:1125-1129, 2013.

80. GU, X.; ZHU, S.; YAN, L.; CHENG, L.; ZHU, P. & ZHENG, J. “Development of sample preparation method for accurate analysis of 24 elemental impurities in oral drug products by ICP-MS according to USP/ICH guidelines”. J. Anal. At. Spectrom., **36**: 512-517, 2021.

81. CROSS, A. “Elemental impurities regulations view from a CRO”. The Reading Science Centre, 1-9, 2016.

82. GARCIA, M. O. Preparo de amostras e análise elementar de produtos farmacêuticos. São Carlos, Programa de Pós-Graduação em Química - UFSCar, 2016. Dissertação de mestrado, 130 p.

83. SMICHOWSKI, P. & LONDONIO, A. “The role of analytical techniques in the determination of metals and metalloids in dietary supplements: A review”. Microchem. J., **136**: 113-120, 2018.

84. VAN DER VOET, G. B.; SARAFANOV, A.; TODOROV, T. I.; CENTENO, J. A.; JONAS, W. B.; IVES, J. A. & MULLICK, F. G. “Clinical and analytical toxicology of dietary supplements: A case study and a review of the literature”. Biol. Trace Elem. Res., **125**:1-12, 2008.

85. KREJČOVÁ, A.; LUDVÍKOVÁ, I.; ČERNOHORSKÝ, T. & POUZAR, M. “Elemental analysis of nutritional preparations by inductively coupled plasma mass and optical emission spectrometry”. Food Chem., **132**: 588-596, 2012.

86. MARRERO, J.; REBAGLIATI, R. J; LEIVA, E.; LONDONIO, A. & SMICHOWSKI, P. “Inductively coupled plasma optical emission spectrometric determination of fifteen elements in dietary supplements: are the concentrations declared in the labels accurate?”. Microchem. J., **108**: 81-86, 2013.

87. GARCÍA–RICO, L.; LEYVA–PEREZ, J. & JARA–MARINI, M.E. “Content and daily intake of copper, zinc, lead, cadmium, and mercury from dietary supplements in Mexico”. Food Chem. Toxicol., **45**: 1599-1605, 2007.

88. MULLER, A. L. H.; MULLER, E. I.; BARIN, J. S. & FLORES, E. M. M. "Microwave-assisted digestion using diluted acids for toxic element determination in medicinal plants by ICP-MS in compliance with United States pharmacopeia requirements". Anal. Methods, **7**: 5218-5225, 2015.

89. AUGUSTO, A. S.; CASTRO, J. P.; SPERANÇA, M. A. & PEREIRA-FILHO, E. R. "Combination of multi-energy calibration (MEC) and laser-induced breakdown spectroscopy (LIBS) for dietary supplements analysis and determination of Ca, Mg and K". J. Braz. Chem. Soc., **30**: 804-812, 2019.

90. PEREIRA, R. M.; CRIZEL, M. G.; NOVO, D. R.; DOS SANTOS, C. M. M. & MESKO, M. F. "Multitechnique determination of metals and non-metals in sports supplements after microwave-assisted digestion using diluted acid". Microchem. J., **145**: 235-241, 2019.

91. LEMOS, V. A.; OLIVEIRA, R. V.; W. N. L.; MENEZES, R. M.; SANTOS, L. B. & FERREIRA, S. L. C. "Liquid phase microextraction associated with flow injection systems for the spectrometric determination of trace elements". TrAC Trends Anal. Chem.,**110**: 357-366, 2019.

92. AGUIRRE, M. A.; BAILE, P.; VIDAL, L. & CANALS, A. "Metal applications of liquid-phase microextraction". TrAC Trends Anal. Chem., **112**: 241-247, 2019.

93. KOKOSA, J. M. "Advances in solvent-microextraction techniques". TrAC Trends Anal. Chem., **43**: 2-13, 2013.

94. REZAEE, M.; ASSADI, Y.; HOSSEINI, M-R. M.; AGHAEE, E.; AHMADI, F. & BERIJANI, S. "Determination of organic compounds in water using dispersive liquid-liquid microextraction". J. Chromatogr. A, **1116**: 1-9, 2006.

95. VIÑAS, P.; CAMPILLO, N.; LÓPEZ-GARCÍA, I. & HERNÁNDEZ-CÓRDOBA, M. "Dispersive liquid–liquid microextraction in food analysis". A critical review. Anal. Bioanal. Chem., **406**: 2067-2099, 2014.

96. MOUSAVI, L.; TAMIJI, Z. & KHOSHAYAND, M. R. "Applications and opportunities of experimental design for the dispersive liquid–liquid microextraction method - A review". Talanta, **190**: 335-356, 2018.

97. AN, J.; TRUJILLO-RODRÍGUEZ, M. J.; PINO, V. & ANDERSON, J. L. "Non-conventional solvents in liquid phase microextraction and aqueous biphasic systems". J. Chromatogr. A, **1500**: 1-23, 2017.

98. OVIEDO, J. A.; FIALHO, L. L. & NOBREGA, J. A. "Determination of molybdenum in plants by vortex-assisted emulsification solidified floating organic drop microextraction and flame atomic absorption spectrometry". Spectrochim. Acta Part B, **86B**: 142-145, 2013.

99. REZAEE, M.; YAMINI, Y.; KHANCHI, A.; FARAJI, M. & SALEH, A. “A simple and rapid new dispersive liquid–liquid microextraction based on solidification of floating organic drop combined with inductively coupled plasma-optical emission spectrometry for preconcentration and determination of aluminum in water samples”. J. Hazard. Mater., **178**: 766-770, 2010.

100. RANJBAR, L.; YAMINI, Y.; SALEH, A.; SEIDI, S. & FARAJI, M. “Ionic liquid based dispersive liquid-liquid microextraction combined with ICP-OES for the determination of trace quantities of cobalt, copper, manganese, nickel and zinc in environmental water samples”. Microchim. Acta, **177**: 119-127, 2012.

101. GRINDLAY, G.; GRAS, L.; MORA, J. & DE LOOS-VOLLEBREGT, M. T. C. “Carbon-, sulfur-, and phosphorus-based charge transfer reactions in inductively coupled plasma–atomic emission spectrometry”. Spectrochim. Acta Part B, **115**: 8-15, 2016.

102. AGUIRRE, M. A.; FIALHO, L. L.; NÓBREGA, J. A.; HIDALGO, M. & CANALS, A. “Compensation of inorganic acid interferences in ICP-OES and ICP-MS using a Flow Blurring® multinebulizer”. J. Anal. At. Spectrom., **29**: 1218-1228, 2014.

103. BARROS, J. A. V. A.; AGUIRRE, M. A.; KOVACHEV, N.; CANALS, A. & NÓBREGA, J. A. “Vortex-assisted dispersive liquid-liquid microextraction for the determination of molybdenum in plants by inductively coupled plasma optical emission spectrometry”. Anal. Methods, **8**: 810-815, 2016.

104. PEREIRA, C. D.; AGUIRRE, M. A.; NÓBREGA, J. A.; HIDALGO, M. & CANALS, A. “Correction of matrix effects for As and Se in ICP OES using a Flow Blurring® multiple nebulizer”. J. Anal. At. Spectrom., **27**: 2132-2137, 2012.

105. AGUIRRE, M. A.; KOVACHEV, N.; HIDALGO M. & CANALS, A. “Analysis of biodiesel and oil samples by on-line calibration using a Flow Blurring® multinebulizer in ICP OES without oxygen addition”. J. Anal. At. Spectrom., **27**: 2102-2110, 2012.

106. OROZCO, F. D. A.; KOVACHEV, N.; AGUIRRE, M. A.; DOMINI, C. E.; FERNÁNDEZ-BAND B. S. & CANALS, A. “Analysis of metals and phosphorus in biodiesel B100 from different feedstock using a Flow Blurring® multinebulizer in inductively coupled plasma-optical emission spectrometry”. Anal. Chim. Acta, **827**: 15-21, 2014.

107. GARCÍA, M.; AGUIRRE, M. A. & CANALS, A. “Determination of As, Se, and Hg in fuel samples by in-chamber chemical vapor generation ICP OES using a Flow Blurring® multinebulizer”. Anal. Bioanal. Chem., **409**: 5481-5490, 2017.

108. MARTÍNEZ-RUBIO, D.; GRINDLAY, G.; LLAVER, M.; WUILLOUD R. G. & MORA, J. "Development of preconcentration strategies for the simultaneous ultratrace determination of As, Cd and Pb in foods by ICP-OES: knotted-reactor vs. dispersive liquid–liquid microextraction†". J. Anal. At. Spectrom., **35**: 933-942, 2020.

109. KALSCHNE, D. L.; CRISTIANE, C.; BARIN, J. S.; PICOLOTO, R. S.; LEITE, O. D. & FLORES, E. L. M. "Reversed-phase dispersive liquid-liquid microextraction (RP-DLLME) as a green sample preparation method for multielement determination in fish oil by ICP-OES". Food Anal. Methods, **13**: 230-237, 2020.

110. KHANI, R. & SHEMIRANI, F. "Simultaneous determination of trace amounts of cobalt and nickel in water and food samples using a combination of partial least squares method and dispersive liquid-liquid microextraction based on ionic liquid". Food Anal. Methods, **6**: 386-394, 2013.

111. SEEGER, T. S.; VECCHIA, P. D.; MACHADO, E. Q.; REINKE, K.; MESKO, M. F. & DUARTE, F. A. "Feasibility of DLLME for the extraction and preconcentration of as and cd in sugar for further determination by ICP-MS". J. Braz. Chem. Soc., **28**: 1691-1697, 2017.

112. JALBANI, N. & SOYLAK, M. "Ligandless ultrasonic-assisted and ionic liquid-based dispersive liquid–liquid microextraction of copper, nickel and lead in different food samples". Food Chem., **167**: 433-437, 2015.

113. PINHEIRO, F. C.; AGUIRRE, M. A.; NÓBREGA, J. A. & CANALS, A. "Dispersive liquid–liquid microextraction of Cd, Hg and Pb from medicines prior to ICP OES determination according to the United States Pharmacopeia". Chapter 3, section 3.2 of this document.

114. PINHEIRO, F. C.; AGUIRRE, M. A.; NÓBREGA, J. A.; GONZÁLEZ-GALLARDO, N.; RAMÓN, D. J. & CANALS, A. "A dispersive liquid-liquid microextraction based on deep eutectic solvent for elemental impurities determination in oral and parenteral drugs by inductively coupled plasma optical emission spectrometry". Chapter 4, section 4.2 of this document.

115. BARROS, A. I.; PINHEIRO, F. C.; AMARAL, C. D. B.; LORENÇATTO, R. & NÓBREGA, J. A. "Aerosol dilution as a simple strategy for analysis of complex samples by ICP-MS". Talanta, **178**: 805-810, 2018.

116. GRINDLAY, G.; MORA, J.; DE LOOS-VOLLEBREGT, M. T. C. & VANHAECKE, F. "A systematic study on the influence of carbon on the behavior of hard-to-ionize elements in inductively coupled plasma–mass spectrometry". Spectrochim. Acta Part B, **86**: 42-49, 2013.

117. NAGESWARA RAO, R. & KUMAR TALLURI, M. V. N. "An overview of recent applications of inductively coupled plasma-mass spectrometry (ICP-MS) in determination of inorganic impurities in drugs and pharmaceuticals". J. Pharm. Biomed. Anal., **43**: 1-13, 2007.

118. CARTER, J. A.; BARROS, A. I.; NÓBREGA, J. A. & DONATI. G.L. “Traditional calibration methods in atomic spectrometry and new calibration strategies for inductively coupled plasma mass spectrometry”. Front. Chem., **504**: 1-25, 2018.

119. VIRGILIO, A.; NÓBREGA, J. A. & DONATI, G. L. “Multi-isotope calibration for inductively coupled plasma mass spectrometry”. Anal. Bioanal. Chem., **410**: 1157-1162, 2018.
120. ZHU, Y. & CHIBA, K. “Internal standard method coupled with a gravimetric standard addition method for elemental measurements by ICP-MS”. J. Anal. At. Spectrom., **27**: 1000-1006, 2012.

121. PEREIRA, F. M. V. & PEREIRA-FILHO, E. R. “Application of free computational program in experimental design: A tutorial”. Quim. Nova, **41**: 1061-1071, 2018.

122. RESANO, M.; FLÓREZ, M. D. R.; QUERALT, I. & MARGUÍ, E. “Determination of palladium, platinum and rhodium in used automobile catalysts and active pharmaceutical ingredients using high-resolution continuum source graphite furnace atomic absorption spectrometry and direct solid sample analysis”. Spectrochim. Acta Part B, **105**: 38-46, 2015.

123. ARAÚJO, G. C. L.; GONZALEZ, M. H.; FERREIRA, A. G.; NOGUEIRA, A. R. A. & NÓBREGA, J. A. “Effect of acid concentration on closed-vessel microwave-assisted digestion of plant materials”. Spectrochim. Acta Part B, **57**: 2121-2132, 2002.

124. AMARAL, C. D. B.; AMAIS, R. S.; FIALHO, L. L.; SCHIAVO, D.; AMORIM, T.; NOGUEIRA, A. R. A.; ROCHA, F. R. P. & NÓBREGA, J. A. “A novel strategy to determine As, Cr, Hg and v in drinking water by ICP-MS/MS”. Anal. Methods, **7**: 1215-1220, 2015.

125. THOMSEN, V.; SCHATZLEIN, D. & MERCURO, D. “Limits of detection in spectroscopy”. Spectroscopy, **18**: 112-114, 2003.

126. WILTSCHE, H.; WINKLER, M. & TIRK, P. “Matrix effects of carbon and bromine in inductively coupled plasma optical emission spectrometry”. J. Anal. At. Spectrom., **30**: 2223-2234, 2015.

127. COHEN, A. S. & WATERS, F. G. “Separation of osmium from geological materials by solvent extraction for analysis by thermal ionization mass spectrometry”. Anal. Chim. Acta, **332**: 269-275, 1996.

128. PEREIRA, M. G. & ARRUDA, M. A. Z. “Trends in preconcentration procedures for metal determination using atomic spectrometry techniques”. Microchim. Acta, **141**: 115-131, 2003.

129. YAN, H. & WANG, H. "Recent development and applications of dispersive liquid-liquid microextraction". J. Chromatogr. A, **1295**: 1-15, 2013.

130. GARCÍA, M.; AGUIRRE, M. A. & CANALS, A. "A new multinebulizer for spectrochemical analysis: Wear metal determination in used lubricating oils by on-line standard dilution analysis (SDA) using inductively coupled plasma optical emission spectrometry (ICP OES)". J. Anal. At. Spectrom., **35**: 265-272, 2020.

131. KOVACHEV, N.; ALMAGRO, B.; AGUIRRE, M. A.; HIDALGO, M.; GAÑÁN-CALVO, A. M. & CANALS, A. "Development and characterization of a Flow Focusing multi nebulization system for sample introduction in ICP-based spectrometric techniques". J. Anal. At. Spectrom., **24**: 1213-1221, 2009.

132. DADFARNIA, S. & HAJI SHABANI, A. M. "Recent development in liquid phase microextraction for determination of trace level concentration of metals - A review". Anal. Chim. Acta, **658**: 107-119, 2010.

133. FUJINAGA, K.; SEIKE, Y. & OKUMURA, M. "Solvent extraction of transition metal ions by an in situ extractant formation method. Diethyldithiocarbamate-forming system". Anal. Sci., **13**: 225-230, 1997.

134. COMMITTEE, A.M. "Recommendations for the definition, estimation and use of the detection limit". Analyst, **112**: 199-204, 1987.

135. GRIBOFF, J.; WUNDERLIN, D. A. & MONFERRAN, M. V. "Metals, As and Se determination by inductively coupled plasma-mass spectrometry (ICP-MS) in edible fish collected from three eutrophic reservoirs. Their consumption represents a risk for human health?". Microchem. J., **130**: 236-244, 2017.

136. DENG, Y.; WU, X.; TIAN, Y.; ZOU, Z.; HOU, X. & JIANG, X. "Sharing one ICP source for simultaneous elemental analysis by ICP-MS/OES: Some unique instrumental capabilities". Microchem. J., **132**: 401-405, 2017.

137. NAM, K. H.; ISENSEE, R.; INFANTINO, G.; PUTYERA, K. & WANG, X. W. "Microwave-induced combustion for ICP-MS: a generic approach to trace elemental analyses of pharmaceutical products". Spectroscopy, **26**: 36-41, 2011.

138. YU, H.; DU, H.; WU, L.; LI, R.; SUN, Q. & HOU, X. "Trace arsenic speciation analysis of bones by high performance liquid chromatography-inductively coupled plasma mass spectrometry". Microchem. J., **141**: 176-180, 2018.

139. AMAIS, R. S.; DONATI, G. L.; SCHIAVO, D. & NÓBREGA, J. A. "A simple dilute-and-shoot procedure for Si determination in diesel and biodiesel by microwave-induced plasma optical emission spectrometry". Microchem. J., **106**: 318-322, 2013.

140. MACHADO, R. C.; AMARAL, C. D. B.; SCHIAVO, D.; NÓBREGA, J. A. & NOGUEIRA, A. R. A. “Complex samples and spectral interferences in ICP-MS: Evaluation of tandem mass spectrometry for interference-free determination of cadmium, tin and platinum group elements”. Microchem. J., **130**: 271-275, 2017.

141. KOŚCIELNIAK, P. & WIECZOREK, M. “Univariate analytical calibration methods and procedures. A review”. Anal. Chim. Acta, **944**: 14-28, 2016.

142. SCHEFFLER, G. L. & POZEBON, D. “Internal standardization in axially viewed inductively coupled plasma optical emission spectrometry (ICP OES) combined with pneumatic nebulization and aerosol desolvation”. Anal. Methods, **5**: 4371-4377, 2013.

143. VIRGILIO, A.; GONÇALVES, D. A.; MCSWEENEY, T.; GOMES NETO, J. A.; NÓBREGA, J. A. & DONATI, G. L. “Multi-energy calibration applied to atomic spectrometry”. Anal. Chim. Acta, **982**: 31-36, 2017.

144. GAO, Y.; STURGEON, R. E.; MESTER, Z.; HOU, X. & YANG, L. “Multivariate optimization of photochemical vapor generation for direct determination of arsenic in seawater by inductively coupled plasma mass spectrometry”. Anal. Chim. Acta, **901**: 34-40, 2015.

145. GAO, Y.; STURGEON, R. E.; MESTER, Z.; HOU, X.; ZHENG, C. & YANG, L. “Direct determination of trace antimony in natural waters by photochemical vapor generation ICPMS: Method optimization and comparison of quantitation strategies”. Anal. Chem., **87**: 7996-8004, 2015.

146. MACHADO, R. C.; SILVA, A. B. S.; DONATI, G. L. & NOGUEIRA, A. R. A. “Multi-energy calibration as a strategy for elemental analysis of fertilizers by microwave-induced plasma optical emission spectrometry”. J. Anal. At. Spectrom., **33**: 1168-1172, 2018.

147. BELCHAMBER, R. M. & HORLICK, G. “Correlation study of internal standardization in ICP atomic emission spectrometry”. Spectrochim. Acta Part B, **37B**: 1037-1046, 1982.

148. ROBERTI, J.; BATISTONI, D. A. & GARAVAGLIA, R. N. “Matrix effects in the analysis of biological matrices by axial view”. Spectrochim. Acta Part B, **57**: 1925-1938, 2002.

149. DE SOUSA, R. A.; SILVA, J. C. J.; BACCAN, N. & CADORE, S. “Determination of metals in bottled coconut water using an inductively coupled plasma optical emission spectrometer”. J. Food Compos. Anal., **18**: 399-408, 2005.

150. Zhang, X.; Li, H. & Yang, Y. “Determination of impurities in highly pure platinum by inductively coupled plasma-atomic emission spectrometry”. Talanta, **42**: 1959-1963, 1995.

151. DE SOUZA, R. M.; LEOCÁDIO, L. G. & DA SILVEIRA, C. L. P. “ICP OES simultaneous determination of Ca, Cu, Fe, Mg, Mn, Na, and P in biodiesel by axial and radial inductively coupled plasma-optical emission spectrometry”. Anal. Lett., **41**: 1615-1622, 2008.

152. ANDRADE, J. M.; TERÁN-BAAMONDE, J.; SOTO-FERREIRO, R. M. & CARLOSENA, A. “Interpolation in the standard additions method”. Anal. Chim. Acta, **780**: 13-19, 2013.

153. BADER, M. “A systematic approach to standard addition methods in instrumental analysis”. J. Chem. Educ., **57**: 703-706, 1980.

154. HILÁRIO, F. F.; CASTRO, J. P.; BARROS, T. E. & PEREIRA-FILHO, E. R. “Planejamento de misturas e visualização da região ótima com planilhas no excel: um tutorial”. Quim. Nova, 1-8, 2021.

155. MACHADO, R. C.; SILVA, A. B. S.; ANDRADE, D. F.; PINHEIRO, F. C.; WILLIAMS, C. B.; DONATI, G. L.; NOGUEIRA, A. R. A. & E. R. PEREIRA-FILHO. “Alternative approaches applied to inductively coupled plasma techniques: multi-flow and two-flow calibration”. J. Braz. Chem. Soc., **32** 842-851, 2021.

156. LI, G. & ROW, K. H. “Utilization of deep eutectic solvents in dispersive liquid-liquid micro-extraction”. TrAC Trends Anal. Chem., **120**: 115651, 2019.

157. GAŁUSZKA, A.; MIGASZEWSKI, Z. & NAMIEŚNIK, J. “The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices”. TrAC Trends Anal. Chem., **50**: 78-84, 2013.

158. ANASTAS, P. & EGHBALI, N. “Green Chemistry: Principles and Practice”. Chem. Soc. Rev., **39**: 301-312, 2010.

159. CUNHA, S. C. & FERNANDES, J. O. “Extraction techniques with deep eutectic solvents”. TrAC Trends Anal. Chem., **105**: 225-239, 2018.

160. SANTANA-MAYOR, A; RODRÍGUEZ-RAMOS, R.; HERRERA-HERRERA, A. V.; SOCAS-RODRÍGUEZ, B. & RODRÍGUEZ-DELGADO, M. A. “Deep eutectic solvents. The new generation of green solvents in analytical chemistry”. TrAC Trends Anal. Chem., **134**: 116108, 2021.

161. KARIMI, M.; DADFARNIA, S.; SHABANI, A. M. H.; TAMADDON, F. & AZADI, D. “Deep eutectic liquid organic salt as a new solvent for liquid-phase microextraction and its application in ligandless extraction and preconcentration of lead and cadmium in edible oils”. Talanta, **144**: 648-654, 2015.

162. KUDŁAK, B.; OWCZAREK, K. & NAMIEŚNIK, J. “Selected issues related to the toxicity of ionic liquids and deep eutectic solvents - a review”. Environ. Sci. Pollut. Res., **22**: 11975-11992, 2015.

163. MAGNUSSON, B. & ORNEMARK, U. Eurachem Guide: the Fitness for Purpose of Analytical Methods - A Laboratory Guide to Method Validation and Related Topics. Second ed., 2014.

164. AKRAMIPOUR, R.; GOLPAYEGANI, M. R.; GHEINI, S. & FATTAHI, N. "Speciation of organic/inorganic mercury and total mercury in blood samples using vortex assisted dispersive liquid-liquid microextraction based on the freezing of deep eutectic solvent followed by GFAAS". Talanta, **186**: 17-23, 2018.

165. SOROURADDIN, S. M.; FARAJZADEH, M. A. & DASTOORI, H. "Development of a dispersive liquid-liquid microextraction method based on a ternary deep eutectic solvent as chelating agent and extraction solvent for preconcentration of heavy metals from milk samples". Talanta, **208**: 120485, 2019.

166. ZOUNR, R. A.; TUZEN, M.; DELIGONUL, N. & KHUHAWAR, M. Y. "A highly selective and sensitive ultrasonic assisted dispersive liquid phase microextraction based on deep eutectic solvent for determination of cadmium in food and water samples prior to electrothermal atomic absorption spectrometry." Food Chem., **253**: 277-283, 2018.

167. ZOUNR, R. A.; TUZEN, M. & KHUHAWAR, M. Y. "A simple and green deep eutectic solvent based air assisted liquid phase microextraction for separation, preconcentration and determination of lead in water and food samples by graphite furnace atomic absorption spectrometry". J. Mol. Liq., **259**: 220-226, 2018.

168. EZODDIN, M.; LAMEI, N.; SIAMI, F.; ABDI, K. & KARIMI, M. A. "Deep Eutectic Solvent Based Air Assisted Ligandless Emulsification Liquid–Liquid Microextraction for Preconcentration of Some Heavy Metals in Biological and Environmental Samples". Bull. Environ. Contam. Toxicol., **101**: 814-819, 2018.

169. FDA (2015). Food and Drug Administration. *Dietary Supplements*. <http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm050803.htm>. Accessed in April 2021.

170. NIH (2018). Department of Health & Human Services U.S, National Institutes of Health. *Dietary supplements*. <https://ods.od.nih.gov/>. Accessed in April 2021.

171. Resolution RDC no 18. April 27th, 2010. Provides for food for athletes. National Sanitary Surveillance Agency, 2010.

172. DERRINGER, G. & SUICH, R. "Simultaneous optimization of several response variables". J. Qual. Technol., **12**: 214-219, 1980.