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Commentary

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Development of a method for the elemental impurity analysis in oral drugs according to USP and ICHQ3D standards by HR-ICP-AES

Abstract

An elemental analysis with HR-ICP-AES were performed on seven elemental impurities in 37 oral drugs samples of three different active pharmaceutical ingredients (APIs): paracetamol, ibuprofen and phloroglucinol. For paracetamol, 14 samples defined as nine generic tablets and five APIs. For ibuprofen 11 samples were used including seven generics tablets and four pure APIs. 12 phloroglucinol's samples were processed: nine generic tablets and three pure APIs. A comparative study between digestion with conventional heating in open vessel and digestion with microwave radiation in clossed vessel was carried out. Results showed that closed system digestion with microwave radation was more appropriate for HR-ICP-AES analysis. The Hg analysis was performed using a direct mercury analyzer. An HR-ICP-AES for screening elemental impurities in pharmaceutical samples has been developed in accordance with requirements of established USP <232/233> chapters and ICHQ3D guidelines. The HR-ICP-AES methodology covering the determination of Cd, Pb, As, Co, V and Ni was successfully validated in terms of linearity, accuracy, sepcificity, precision, intermediate precision and limits of detection and quantification. The proposed method was for quality control in pharmaceutical industries.

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Jihene Dabloun,[a] Fathi Safta,[1] Najet Chaabene,[2] Kawthar Zribi,[1] Houyem Abderrazak[2]

[1]Laboratory of chemical, Galenic and pharmacological development of medicine, Faculty of pharmacy of Monastir, Tunisia

[2]Laboratory of Useful Materials, National Institute for Research and Physical Chemical Analysis, Technopole Sidi thabet, 2020, Ariana, Tunisia

Correspondence: Jihene Dabloun, Laboratory of chemical, Galenic and pharmacological development of medicine, Faculty of pharmacy of Monastir, Tunisia, Tel +216 97 090 433, Email Dabloun.jihen@gmail.com

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Introduction

Impurities are the unwanted substances present with the active pharmaceutical ingredient (API) or their product which may lower its quality and efficiency. According to the International Council of Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), the impurity can be defined as any substance in the drug product (DP) that is not the API or the excipient.[1] One set of impurities that present potentially high risk in terms of reactivity and safety impact are those impurities, organic or inorganic, whose chemical formula include elements from the following series in the periodic table: transition metals, metalloids, other metals and lanthanides and actinides. Impurities that contain such elements have been termed elemental impurities.[2] Some elemental impurities are toxic even when present at trace concentration level. In pharmaceuticals, contamination by elemental impurities may occur by use of raw materials, reagents, excipients, the catalysts involved in the APIs synthesis, by interaction with equipment, containers and surfaces during drug production which can generate unwanted and unknown pharmacological or/and toxicological effects.[3] The former United States Pharmacopeia (USP) method for monitoring inorganic contaminants in pharmaceutical samples as defined in general chapter <231> is over a 100 year-old colorimetric test. This method, known as the "heavy metals limit test", is based on precipitation of 10 sulfide-forming elements (Ag, As, Bi, Cd, Cu, Hg, Mo, Pb, and Sn), in a reaction with reagent such as thioacetamide. The resulting colored precipitate is compared visually to a reference of 10 µg/g Pb to determine compliance with the heavy metal limit. Besides the obvious potential variability associated with a subjective visual comparison, USP <231> is a limit test based on the sum of 10 elements, and thus does not give individual concentrations for each element.[4] Over the past years, industry consortia, pharmacopoeias and regulators developed a more effective approach to the control of elemental impurities, leading to a replacement of existing wet chemical and colorimetric test USP <231>. The USP, in parallel with the ICH, has published new standards for measuring and controlling elemental impurities in pharmaceuticals and their ingredients. The ICH method is defined in the "Guideline for Elemental impurities" (Q3D), which has been in effect since June 2016 for new marketing authorization applications and was implemented in December 2017 for previously authorized medicinal products. Several authorities aligned their specific chapters to the content of ICH Q3D, such as the new USP General Chapters <232> (Elemental Impurities-limits) and <233> (Elemental Impurities –Procedures), which were implemented in January 2018.[5] The evaluation of element and route specific toxicological data resulted in permitted daily exposures (PDEs). The PDE is a limit for an elemental impurity in a pharmaceutical product per daily consumption and is dependent on oral, parenteral and inhalational routes of administration. 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 this equation: J= $J = \frac{PDE\left(\frac{\mu g}{day}\right)}{MDD\left(\frac{g}{day}\right) X\, D\dot{F}}$

Based on their toxicity (PDE) and likelihood of occurrence in the DP, the elements included in ICH Q3D were divided into three classes. There are 24 elemental impurities of potential concern identified by both USP <232> and ICH Q3D. Table 1 lists their PDEs by route of administration and classification according to risk based upon toxicity and likelihood of occurrence.

Class 1 is assigned to metals that are highly toxic to humans and consequently should have limited or no use in the manufacture of pharmaceuticals. Class 2 elements are considered as route-dependent human toxicants and are further divided in two sub-classes 2A and 2B, based on their relative likelihood of occurrence in the DP. The class

2A should always be evaluated in a risk assessment. Class 2B have reduced risk of occurrence and can only be included if intentionally added to the process. Class 3 regroups elements with a relatively low toxicity by the oral route of administration (high PDEs, generally > 500 µg/day) but it could be necessary to consider those in the risk assessment for inhalation and parental routes of administration. Elements that are not included in class 3 are with low inherent toxicity.[5] Prior to the development of USP <233>, Wang et al.[6] and Lewen et al.[7] had proposed and demonstrated that ICP-MS could be used as a rapid screening technique for heavy metals in pharmaceutical compounds and materials. Thus and with Lewen's involvement on the USP Expert Committee developing <232> and <233> led to the inclusion of both ICP-AES and ICP-MS as the reference methods. Therefore, ICP-MS and ICP-AES have seen greater use within the pharmaceutical industry in more recent years.[8-10] The methods of sample preparation described in the pharmacopoeias often involve several steps, low throughput, a high risk of contamination or loss, and require the use of several reagents.[11,12] The treatment of a substance with oxidant reagents conventionally heated or by means of microwave radiation is the commonest approach for matrix digestion to obtain a solution containing analytes and components from the matrix partially or completely oxidized. The approach is known as "wet digestion" and is applied for almost all matrices.[13] Wet digestion can be performed in open or closed systems.[14] Digests with oxidizing acid may produce interferences resulting in enhancement or suppression of analytical signal for some element in ICP.[15,16] Regarding the acidity of digests, it is well known that the nebulization of concentrated nitric acid solutions can cause severe signal suppression of analytical signal in ICP.[17-20] In this sense, it is important to point out that the development of an effective wet digestion method for APIs can be considered a challenge in chemistry. A common known problem is the adsorption of Hg on the surface of the tubes, spray chamber and nebulizer, which accumulates in the ICP sample introduction system. This memory effect causes a gradual increase of the Hg signal, non-linear calibration and a long wash-out time.[21] The aim of this work is to develop an HR-ICP-AES method for the quantification of USP <232> and ICH Q3D target elements (class 1 and 2a) in DP and APIs for oral rout and to analyze Hg in DP and APIs using direct mercury analyzer (DMA). A comparative study between digestion with conventional heating in an open vessel and digestion with microwave radiation was also performed.

Experimental

Reagent and materials

Concentrated nitric acid 65%, concentrated hydrochloric acid 37%, hydrofluoric acid 40% and hydrogen peroxide 30% were purchased from Sigma-aldrich, and used throughout this work to prepare standards and samples. Ultrapure water with a resistivity of 18.2 Ω.cm used in the experiments was prepared using the Milli-Q millipore water purification system. Standard solutions for elemental analysis (Cd, Pb, As, Hg, Co, V and Ni) were prepared by diluting commercially available, NIST traceable single element 1000 mgL^{-1} stock solution. Spike solutions for recovery assessment were also prepared from these stock solutions. Six generic oral paracetamol drugs (500 mg) were used for analytical validation. Screening of the elemental impurities (EIs) was carried out on 37 samples of three different active pharmaceutical ingredients (APIs): paracetamol, ibuprofen and phloroglucinol. (Table 2)

Table 1 Established permitted daily exposures (PDEs) for elemental impurities

	Class	Oral PDE µg/day	Parenteral PDE µg/day	Inhalation PDE µg/day
Cd	1	5	2	2
Pb	1	5	5	5
As	1	15	15	2
Hg	1	30	3	1
Co	2A	50	5	3
V	2A	100	10	1
Ni	2A	200	20	5
Tl	2B	8	8	8
Au	2B	100	100	1
Pd	2B	100	10	1
Ir	2B	100	10	1
Os	2B	100	10	1
Rh	2B	100	10	1
Ru	2B	100	10	1
Se	2B	150	80	130
Ag	2B	150	10	7
Pt	2B	100	10	1
Li	3	550	250	25
Sb	3	1200	90	20
Ba	3	1400	700	300
Mo	3	300	1500	10
Cu	3	3000	300	30
Sn	3	6000	600	60
Cr	3	11000	1100	3

Table 2 Samples used for the screening of the elemental impurities

	Tablets	Active Pharmaceutical Ingredient
Paracetamol	9	5
Ibuprofen	7	4
Phlorglucinol	9	3

For paracetamol 14 samples defined as: 9 tablets each containing 500 mg and 5 APIs pure, one of which were synthesized by a green chemistry process in the chemistry laboratory of Faculty of Pharmacy of Monastir. For Ibuprofen 11 samples were used including, 7 generic tablets each containing 400 mg and four pure APIs powder. 12 samples of phloroglucinol were processed: 9 generic tablets each containing 80 mg of API and three APIs pure powder. All the generic tablets are fabricated by Tunisian industries.

Instrumentation

Samples were digested using Start-D Milestone microwave. This system is equipped with temperature and pressure sensors. An ICP-OES, PlasmaQuant PQ 9000 Elite analytic Jena, was used throughout the experiments for elemental analyses. The instrumental parameters are listed in table 3.

Table 3 ICP-AES operating parameters

Parameter	Setting
Plasma RF Power (W)	1200
Plasma Gaz Flow (L/min)	12.00
Nebulizer Gaz Flow (L/min)	0.50
Auxiliary Gas Flow (L/min)	0.50
Pump Rate (ml/min)	2.20

Instrument control and data analysis were carried by Aspect PQ software. The open-system mineralization was done with Labtech ED54 hot block. DMA-80 milestone implements an Atomic Absorption Spectrophotometric technique designed for the analysis of very low-level mercury in solid and liquid samples without any further treatment. The DMA-80 is equipped with a sample changer that allows the automatic passage of solid and liquid samples in stainless steel nacelles of known capacity.

Sample preparation and optimization

First, a wet open-system mineralization was carried out in order to test the behavior of the drugs studied and to determine the most appropriate operating conditions. For each API, few DP samples were selected based on whether they contain talc or silica as an excipient since they are refractory substances which decompose under the action of hydrofluoric acid.[22] The protocol used was based on studies by Pluhacek et al.,[23] Pinheiro et al.[24] and Menoutis et al.[25] and on sample composition. Two different mineralization protocols were carried out, the first one for samples containing talc (paracetamol DP6, paracetamol DP8, ibuprofen DP2 and DP7) and the second for samples that do not contain talc (paracetamol DP1, Phloroglucinol DP1 and phloroglucinol DP 6). The first protocol is to weigh 0.4 g of DP as a powder to which 4 ml of nitric acid was added in a 50 ml volumetric flask which were then heated to 130°C in the heating block under the hood for one hour. Then 1 ml of hydrochloric acid and 2 ml of hydrofluoric acid were added. The preparation is maintained under the same temperature conditions until the total dissolution of the matrix. After cooling to room temperature, ultrapure water was added to reach a final volume of 30 ml. The second protocol consists of weighing 0.4 g of DP in a 50 ml volumetric flask as a powder to which 6 ml of nitric acid was added. A temperature of 130°C was applied through the heating block for 20 minutes. 2 ml of hydrochloric acid and 2 ml of hydrogen peroxide were added. Heating is continued until the sample is completely dissolved. After cooling to room temperature, ultrapure water was added to reach a final volume of 30ml. Following this preliminary digestion, some matrices were not completely mineralized. Paracetamol and phloroglucinol's samples were more sensitive to the nitric acid oxidation. Another protocol has been established to treat paracetamol and phloroglucinol API and DP samples and ibuprofen API. In a 50 ml volumetric flask, 0.4 g of the substance was weighed and then 4.0 ml nitric acid and 2.0 ml hydrogen peroxide were added. After 10 minutes, 4.0 ml nitric acid were added. The samples were digested under a temperature of 130°C in the hot block until no visible particles remained. The acid extract was then recovered in a 30 ml tube in which ultrapure water was added to adjust the final volume to 30 ml. For ibuprofen DP samples, 8.0 ml of nitric acid in a 50 ml volumetric flask was added to 0.2 g of pulverized DP after one hour of heating in the 130°C heating block, 2.0 ml of hydrofluoric acid and 2.0 ml of hydrogen peroxide, then the same steps were continued. Samples 1, 2, 6 and 7 had a solid residue indicating incomplete mineralization. To optimize the mineralization, we treated 0.2 g of pulverized sample with 7.0 ml of nitric acid, 3.0 ml of hydrofluoric acid, 2.0 ml of hydrogen peroxide and 1 ml of hydrochloric acid, while following the same steps mentioned above. For microwave digestion, the oral drugs were prepared in accordance according to USP <233>. For paracetamol and phloroglucinol DP and API, and ibuprofen API, 0.4 g of the oral drug was weighed in a microwave digestion container. Pre-digestion began with the addition of 4.0 ml of nitric acid with a conservative addition of 2.0 ml of hydrogen peroxide in each container and leave the reaction to stand for 10 minutes. Then, a supplement of 4.0 ml of nitric acid was added. Each container was then assembled and carefully sealed. The samples were digested by microwave in a closed container allowing the sample to decompose under high temperature and pressure. A modified microwave digestion program was used using a two-step microwave program consisting of a 5-minute ramp at 120°C and 40 bars with an 8-minute wait. The samples were left to cool for 20 minutes before ventilating and opening the digestion containers. The digested samples were transferred quantitatively to 50 ml volumetric flasks in which ultrapure water was added to obtain a final volume of 30 ml. For ibuprofen API samples the same steps were performed, 2.0 ml fluorhydric acid was added among the reagents used. Some matrices have not been fully mineralized. The optimization consisted in modifying the microwave program by increasing the temperature and duration of the mineralization.

Preparation of standards

Working standards were prepared from the stock solution 1000mgL^{-1} (Cd, Pb, As, Hg, Co, V, Ni) by serial dilution with 1.0 % of nitric acid. These standards cover the range of 0.5 – 2.0 of the Target Limit (J-Value) called for in USP <233>.

Results and discussion

Mineralization

In order to be able to explain the behavior of the different drugs, we based ourselves on two points. The first is the chemical structure of the three active pharmaceutical ingredients shown in Figure 1.

Paracetamol (Figure 1a) and phloroglucinol (Figure 1c) include an aromatic ring in their structure with a -OH group known for its activating power. According to Wurfels et al.[26] this group facilitates the oxidation of the aromatic rings by nitric acid. This increases the

ease of mineralization of paracetamol and phloroglucinol samples. It has also been demonstrated by Wurfels et al.[26] that the presence of carbohydrates in the reaction medium improves nitric acid mineralization performance. The carbohydrate fraction in the sample is completely decomposed by hydrolysis with NO_2 formation. This compound will be used to quantitatively degrade other substances in the reaction medium. This explains the effectiveness of the mineralization obtained for paracetamol and phloroglucinol DP, as they contain carbohydrates in their formula in the form of lactose, cellulose, starch and saccharose. The four ibuprofen APIs come from four different pharmaceutical industries and therefore probably from different suppliers. Although it is the same active ingredient (Figure 1b) processed under the same treatment conditions, the mineralization was different. For API 1 and API 2 a residue remained testifying that the mineralization was partial and for API 3 and API 4 the mineralization was complete and, hence, a clear solution was obtained. At the end of this observation, a series of tests to explain the difference observed during the mineralization was carried out. The four ibuprofen APIs were characterized by infrared. The Fourier Transform Infrared (FTIR) spectrum of each sample is recorded at room temperature in the range of 400-4000 cm^{-1}. The different spectra are presented in Figure 2.

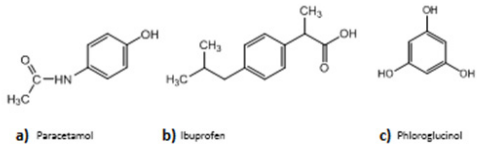

Figure 1 Structures of paracetamol, ibuprofen and phlorglucinol.

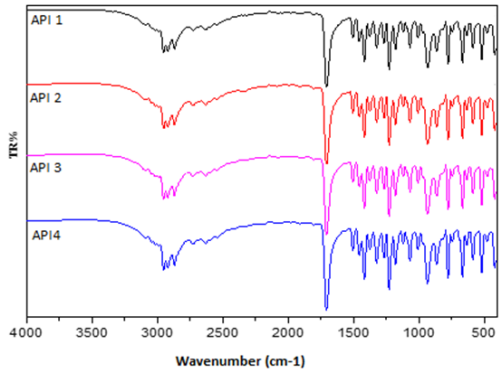

Figure 2 Infrared spectra of ibuprofen API.

The comparison with the spectra of ibuprofen found in the literature allowed us to index our spectrum.[27] Table 4 summarizes the characteristic peaks of pure ibuprofen as well as the vibration types of the corresponding bonds.

Table 4 Infrared absorption bands characteristic of pure ibuprofen

Connection and type of vibration	Wavenumber v (cm^{-1})
Aromatic C–H Elongation Vibration	3090
CH_3 Antisymmetric Strain Vibration	2955
C=O (COOH) elongation vibration	1720
Cyclic C–C Strain Vibration	1509
C–C–O–H Strain/distortion Vibration	1420
	1269
C–O elongation vibration (COOH) and O–H distortion vibration	1230S
	1183
O–H Distortion vibration out of plane (acid dimer)	935

The four IRTF spectra are superimposed, which can be concluded that the API analyzed by infrared are pure ibuprofen. The four samples were analyzed using X-ray diffraction spectroscopy (XRD). We obtained the four diffractograms presented in the Figure 3.

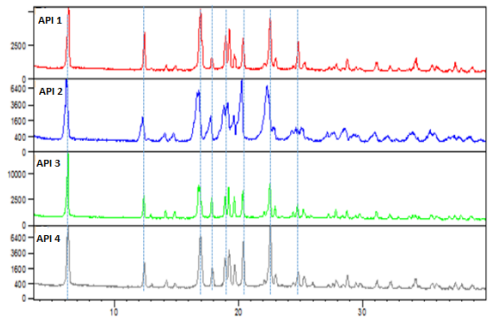

Figure 3 X-ray diffractograms of ibuprofen active pharmaceutical ingredient.

The XRD diagrams show that ibuprofen has a crystalline structure (Figure 3), as indicated by the sharpness of the diffraction peaks. The characteristic peaks are located in the 2Θ positions: 6.1°, 12.3°, 16.6°, 20.2° and 22.4°. These results are similar to those found in the literature.[28]

A Differential Scanning Calorimetric Analysis (DSC) was performed for the four ibuprofen APIs to assess their purity. The thermograms (Figure 4) obtained show that the melting point of the analyzed substances is 77°C.[29]

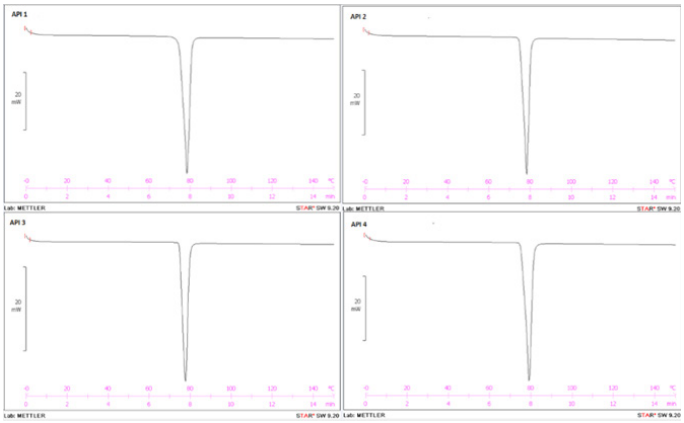

Figure 4 Thermograms of ibuprofen active pharmaceutical ingredients.

The XRD diagrams and thermograms were similar for the different ibuprofen APIs and, thus, could not explain the difference between the results of the acid mineralization of the four API. On the other hand, ibuprofen is a chiral molecule; therefore, we determined the angle of rotation of the four APIs (Table 5). The mean of the rotation angle for Ibuprofen API 1 and API 2 was 0.00. This shows that API 1 and API 2 are racemic mixture. The mean of rotation angle for ibuprofen API 3 was -0.03 and for ibuprofen API 4 was -0.05, this reveals that there is more dextrogyres molecules than levogyres ones.

Table 5 Angle of rotation of ibuprofen active pharmaceutical ingredient (n=2)

Sample	Rotation angle (1)	Rotation angle (2)	Mean
API 1	0.01	-0.01	0.00
API 2	0.01	-0.01	0.00
API 3	-0.08	0.05	-0.03
API 4	-0.01	-0.04	-0.05

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After an acid mineralization carried out in closed system, a complete dissolution was obtained for API 3 and API 4 but an incomplete dissolution was observed for API 1 and API 2 under the same operating conditions. From the analyses performed (IRTF, XRD and DSC) we can conclude that the four ibuprofen APIs are pure. The rotation angles are different for ibuprofen APIs, therefore, we assume that chirality has an impact on the difference observed when samples are subjected to high temperatures and pressure.

Linearity test

To evaluate the linearity of the method, a series of multi-element standards prepared at concentrations of 0.0J, 0.5J, 1.0J and 2.0J (Table 6) in 1% nitric acid, were analyzed. Linear regression using the peak emission intensity counts of the standards as y-axis and the concentration of the standards as x-axis provided correlation coefficients $r^2 > 0.995$ (Table 6) for the six evaluated elemental impurities. The results clearly displayed the linearity of the instrument response as function of the concentration range studied.

Accuracy and specificity

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 of 0.5J to 1.5J of the J-value for each target element before digestion in order to check accuracy of the developed analytical procedure. The results reported in Table 7 show that the recovery rates for the analyzed elements are ranging between 70.70 % and 146.60%.

Table 6 J-Values in accordance with Oral PDEs at a maximum dose ≤ 4g and related calibration standard

	Concentration limit (µg/g) based on maximum daily dose of ≤ 4g/day	0.5J (µg/L) based upon sample dilution of 0.4g/30ml	1.0J (µg/L) based upon sample dilution of 0.4g/30ml	1.5J (µg/L) based upon sample dilution of 0.4g/30ml	2J (µg/L) based upon sample dilution of 0.4g/30ml	R2
Cd	1.25	8	16	25	35	0.996
Pb	1.25	8	16	25	35	0.998
As	3.75	25	50	75	100	0.997
Co	12.50	80	170	250	350	0.999
V	25.00	160	350	500	670	0.999
Ni	50.00	300	670	1000	1350	0.999

Standards concentration were adjusted to ± 4% of the Target Limit

Table 7 Recovery rate of the elemental impurities

	wavelength (nm)	Concentration of spiked element (µg/L)	Mean (n=3)	SD	(%)	RSD
Cd(S1)	226.502	8	9.25	0.80	(115.63)	8.65
Cd(S2)	226.502	16	17.35	0.36	(108.42)	2.07
Cd(S3)	226.502	25	27.27	2.22	(109.09)	9.97
Pb(S1)	220.353	8	11.34	0.26	(141.75)	2.29
Pb(S2)	220.353	16	23.46	0.25	(146.60)	1.07
Pb(S3)	220.353	25	36.13	0.80	(144.53)	2.21
As(S1)	193.698	25	34.03	1.25	(136.13)	3.67
As(S2)	193.698	50	70.18	1.43	(140.37)	2.04
As(S3)	193.698	75	104.20	1.04	(138.93)	1.00
Co(S1)	228.615	80	83.41	1.81	(104.26)	2.17
Co(S2)	228.615	170	172.72	2.54	(101.60)	1.47
Co(S3)	228.615	250	251.64	1.60	(100.66)	0.64
V (S1)	309.311	160	113.13	0.77	(70.71)	0.68
V (S2)	309.311	350	247.46	1.66	(70.70)	0.67
V (S3)	309.311	500	411.79	2.36	(82.36)	0.57
Ni(S1)	231.604	300	304.13	1.04	(101.38)	0.34
Ni(S2)	231.604	670	677.46	1.06	(101.11)	0.16
Ni(S3)	231.604	1000	1005.40	2.71	(100.54)	0.27

S1. S2. S3: spiked element

Precision

The precision of the method was evaluated by preparing six spiked samples at concentration 0.5J. 1.0J and 1.5J. The results shown in Figure 5 revealed that the highest % RSD is 18.15% which is lower than the limit required by USP <233> (20.00%).

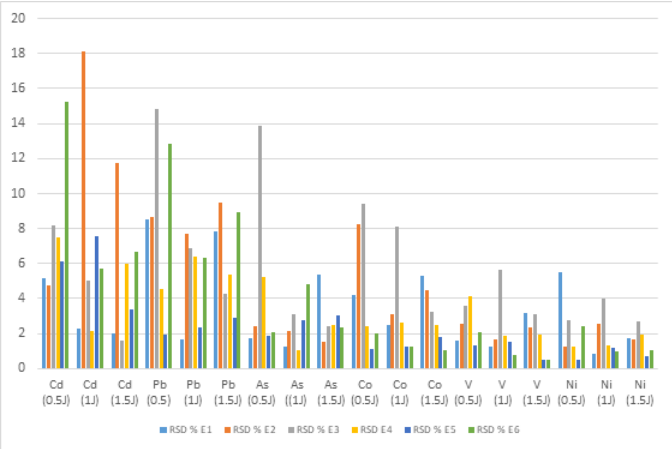

Figure 5 RSD percentage of precision.

Intermediate precision

Intermediate precision has been performed by repeating the same analysis as for repeatability but operating on different days. Figure 6 illustrates the obtained results, indicating that the highest RSD is 24.19 % which is inferior to the limit imposed by the USP <233> (25.00%).

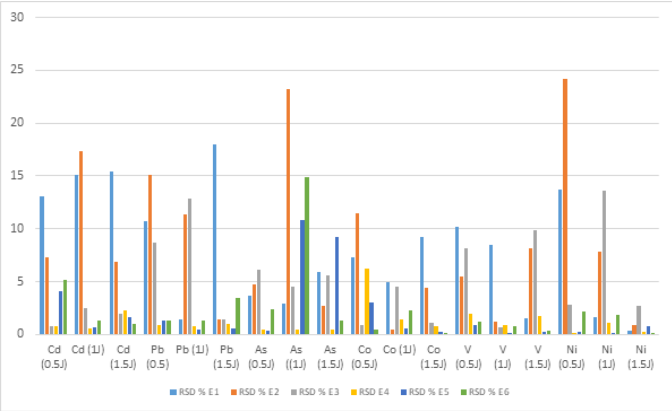

Figure 6 RSD percentage of intermediate precision.

Limits of detection and quantification

The limits of detection (LOD) and quantification (LOQ) were calculated, respectively, by using the signal-to-background ratio and relative standard deviation for 10 measurements of blank solutions. The LOD is calculated as three times the standard deviation, and the LOQ is calculated as 10 times the standard deviation. The results shown in Table 8 reveal that the LODs and LOQs for the six elemental impurities are below 0.5J and 1.0J, respectively. The limits of quantification obtained in our study are slightly lower than the limits found in the literature.[25,30,31]

Table 8 LOD and LOQ of target Elemental Impurities

	LOD (µg/L)	LOQ (µg/L)
Cd	0.15	0.31
Pb	0.14	0.31
As	0.63	0.68
Co	1.41	2.98
V	1.19	2.45
Ni	11.81	15.68

Digestion process assessment

The 37 samples analyzed underwent two different digestion ways: the first one using microwave and the second procedure by heating block. The comparison was based on the EI content found in each sample. For every sample, the analysis covered all the six elemental impurities. For paracetamol samples (Table 9), the samples 1, 2, 3, 4, 6, 9, 11, 12 and 14 contain cadmium levels below the LOQ after microwave treatment, while after heating with hot block system; it reaches levels above the same limit. For samples 5, 7 and 13 the LOQ is reached after both mineralizations, however the levels of cadmium are higher after a heating block treatment. For sample 8 the level of Cd is higher after the microwave treatment. Sample 10 does not reach the LOQ regardless of the type of mineralization performed. Sample 4 has a lead content lower than the LOQ after microwave treatment, whereas, after heating with hot block system it is quantifiable. Samples 1, 2, 4 and 5 have a lead level exceeding the LOQ after microwave treatment, while it is not reached after hot block treatment. Samples 3, 6 and 7 have higher lead levels after a heating block treatment, whereas samples 8, 9, 10, 11, 12, 13 and 14 have significantly higher lead levels after microwave treatment. For arsenic, no sample exceeds the LOQ regardless of the treatment used. For cobalt, all samples have levels below the LOQ regardless of the type of mineralization excepted for samples 13 and 14 that have quantifiable levels only after a heating block treatment. All paracetamol samples have vanadium levels above the LOQ except for sample 14. Samples 1, 4, 11 and 13 have higher levels of vanadium after a heating block treatment, while samples 2, 3, 5, 6, 7, 8, 9, 10 and 12 have higher levels of vanadium after a microwave treatment. All paracetamol samples show nickel levels that do not exceed the LOQ after a heating block system treatment but are quantifiable after microwave treatment, except for sample 6 which has a much higher nickel level after microwave treatment (4.851 µg/g) compared to heating block system (0.218 µg/g).

Ibuprofen samples (Table 10) have cadmium levels that not exceed the LOQ after both ways of mineralization except for samples 2 and 3. The LOQ is reached only after a hot block mineralization. Samples 1, 3 and 6 have lead levels above the LOQ after a microwave treatment, but after a heating block treatment, this limit is not reached. However, sample 2 has higher lead level above the LOQ after a heating block treatment but this limit is not reached after a microwave treatment. All remaining samples have a lead level that not reaches the LOQ regardless the type of mineralization. All ibuprofen samples do not exceed the LOQ of arsenic for both mineralization treatments. Samples 2, 5 and 6 of ibuprofen have higher levels of cobalt after treatment with heating block. Samples 3 and 7 have higher levels of cobalt after a microwave digestion. Sample 8 have cobalt level that exceeds the LOQ after microwave treatment, whereas it does not reach this limit after a heating block system treatment. Only, sample 2 have higher amount of vanadium after a hot block treatment, samples 4, 6 and 8 have level of vanadium higher after a microwave treatment. For nickel, samples 1, 4, 5, 10 and 11 have higher levels after a hot block mineralization and samples 2, 3, 7, 8 and 9 are higher after a microwave treatment.

Samples 1, 4, 5, 6 and 7 of phloroglucinol (Table 11) have higher cadmium levels after a heating block mineralization, while for all the rest of the samples microwave mineralization allows higher cadmium levels. Sample 3 have equal concentration of cadmium regardless the type of mineralization. Samples 3, 4, 5, 6 and 7 have higher lead levels after heating block treatment, whereas samples 1, 2, 8, 9, 10, 11 and 12 have higher lead levels after microwave digestion. No sample of

phloroglucinol has a value that exceeds the LOQ of arsenic and cobalt except for sample 8 which has a content that exceeds this limit merely after microwave treatment. For vanadium and nickel all samples have higher levels after microwave treatment.

Table 9 Elemental impurity concentrations for paracetamol samples after both types of mineralization

	Cd (µg/g)	Pb (µg/g)	As (µg/g)	Co (µg/g)	V (µg/g)	Ni (µg/g)
S1 M	**< LOQ**	**0.029**	< LOQ	<LOQ	**0.104**	**0.049**
S1 H	**0.012**	**<LOQ**	< LOQ	<LOQ	**0.266**	**<LOQ**
S2 M	**< LOQ**	**0.545**	< LOQ	<LOQ	**0.328**	**0.207**
S2 H	**0.011**	**<LOQ**	< LOQ	<LOQ	**0.294**	**<LOQ**
S3 M	**< LOQ**	**0.402**	< LOQ	<LOQ	**0.112**	**0.024**
S3 H	**0.009**	**0.692**	< LOQ	<LOQ	**0.050**	**<LOQ**
S4 M	**< LOQ**	**0.395**	< LOQ	<LOQ	**0.166**	**0.123**
S4 H	**0.032**	**<LOQ**	< LOQ	<LOQ	**0.266**	**<LOQ**
S5 M	**0.008**	**0.254**	< LOQ	<LOQ	**0.181**	**0.966**
S5 H	**0.011**	**<LOQ**	< LOQ	<LOQ	**0.138**	**<LOQ**
S6 M	**< LOQ**	**0.008**	< LOQ	<LOQ	**6.976**	**4.851**
S6 H	**0.017**	**0.072**	< LOQ	<LOQ	**1.620**	**0.218**
S7 M	**0.005**	**0.144**	< LOQ	<LOQ	**0.376**	**0.223**
S7 H	**0.025**	**0.412**	< LOQ	<LOQ	**0.199**	**<LOQ**
S8 M	**0.010**	**0.135**	< LOQ	<LOQ	**6.531**	**3.003**
S8 H	**0.008**	**0.064**	< LOQ	<LOQ	**0.061**	**<LOQ**
S9 M	**<LOQ**	**0.386**	< LOQ	<LOQ	**0.111**	**0.036**
S9 H	**0.019**	**0.091**	< LOQ	<LOQ	**0.067**	**<LOQ**
S10 M	< LOQ	**0.671**	< LOQ	<LOQ	**0.399**	**0.039**
S10 H	< LOQ	**0.176**	< LOQ	<LOQ	**0.287**	**<LOQ**
S11 M	**< LOQ**	**0.723**	< LOQ	<LOQ	**0.073**	**1.341**
S11 H	**0.018**	**0.124**	< LOQ	<LOQ	**0.135**	**<LOQ**
S12 M	**< LOQ**	**0.550**	< LOQ	<LOQ	**0.087**	**1.115**
S12 H	**0.017**	**0.020**	< LOQ	<LOQ	**0.075**	**<LOQ**
S13 M	**0.006**	**0.817**	< LOQ	**<LOQ**	**0.113**	**1.113**
S13 H	**0.014**	**0.053**	< LOQ	**0.052**	**0.136**	**<LOQ**
S14M	**< LOQ**	**0.549**	< LOQ	**<LOQ**	<LOQ	**0.199**
S14 H	**0.028**	**0.077**	< LOQ	**0.316**	<LOQ	**<LOQ**

M: microwave; H: hotblock

Table 10 Elemental impurity concentrations for ibuprofen samples after both ways of mineralization

	Cd (µg/g)	Pb (µg/g)	As (µg/g)	Co (µg/g)	V (µg/g)	Ni (µg/g)
S1 M	< LOQ	**0.013**	< LOQ	< LOQ	< LOQ	**< LOQ**
S1 H	< LOQ	**< LOQ**	< LOQ	< LOQ	< LOQ	**27.796**
S2 M	**< LOQ**	**< LOQ**	< LOQ	**0.115**	**1.022**	**10.638**
S2 H	**0.041**	**0.198**	< LOQ	**1.286**	**1.477**	**5.559**
S3 M	**< LOQ**	**0.139**	< LOQ	**7.494**	< LOQ	**< LOQ**
S3 H	**0.100**	**< LOQ**	< LOQ	**5.909**	< LOQ	**19.993**
S4 M	< LOQ	< LOQ	< LOQ	< LOQ	**2.766**	**< LOQ**
S4 H	< LOQ	< LOQ	< LOQ	< LOQ	**1.047**	**2.980**
S5 M	< LOQ	< LOQ	< LOQ	**9.421**	< LOQ	**4.413**
S5 H	< LOQ	< LOQ	< LOQ	**10.173**	< LOQ	**5.617**
S6 M	< LOQ	**0.034**	< LOQ	**0.027**	**0.029**	< LOQ
S6 H	< LOQ	**< LOQ**	< LOQ	**0.214**	**< LOQ**	< LOQ
S7 M	< LOQ	< LOQ	< LOQ	**5.496**	< LOQ	**56.046**
S7 H	< LOQ	< LOQ	< LOQ	**3.827**	< LOQ	**< LOQ**
S8 M	< LOQ	< LOQ	< LOQ	**5.199**	**2.396**	**1.502**
S8 H	< LOQ	< LOQ	< LOQ	**< LOQ**	**< LOQ**	**0.089**
S9 M	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**2.583**
S9 H	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**
S10 M	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**
S10 H	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**0.016**
S11 M	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**< LOQ**
S11 H	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	**0.202**

M: microwave; H: hotblock

Table 11 Elemental impurity concentrations for phloroglucinol samples after both types of mineralization

	Cd (µg/g)	Pb (µg/g)	As (µg/g)	Co (µg/g)	V (µg/g)	Ni (µg/g)
S1 M	**0.032**	**3.424**	< LOQ	< LOQ	**0.666**	**1.151**
S1 H	**0.350**	**0.166**	< LOQ	< LOQ	**0.156**	**0.522**
S2 M	**0.029**	**1.464**	< LOQ	< LOQ	**0.771**	**2.329**
S2 H	**<LOQ**	**0.237**	< LOQ	< LOQ	**0.171**	**0.024**
S3 M	**0.020**	**1.150**	< LOQ	< LOQ	**0.636**	**1.890**
S3 H	**0.020**	**1.362**	< LOQ	< LOQ	**0.239**	**< LOQ**
S4 M	**0.004**	**0.411**	< LOQ	< LOQ	**0.354**	**1.435**
S4 H	**0.080**	**3.004**	< LOQ	< LOQ	**0.345**	**0.165**
S5 M	**0.009**	**1.032**	< LOQ	< LOQ	**0.727**	**1.732**
S5 H	**0.161**	**6.330**	< LOQ	< LOQ	**0.576**	**0.651**
S6 M	**0.024**	**0.913**	< LOQ	< LOQ	**0.692**	**1.994**
S6 H	**0.187**	**10.080**	< LOQ	< LOQ	**0.390**	**0.717**
S7 M	**0.014**	**0.933**	< LOQ	< LOQ	**1.161**	**1.202**
S7 H	**0.217**	**11.575**	< LOQ	< LOQ	**0.184**	**< LOQ**
S8 M	**0.025**	**0.800**	< LOQ	**0.110**	**0.548**	**3.231**
S8 H	**0.002**	**< LOQ**	< LOQ	**< LOQ**	**0.139**	**< LOQ**
S9 M	**0.018**	**0.798**	< LOQ	< LOQ	< LOQ	**1.715**
S9 H	**0.007**	**0.408**	< LOQ	< LOQ	< LOQ	**0.150**
S10 M	**0.022**	**1.180**	< LOQ	< LOQ	< LOQ	**2.271**
S10 H	**< LOQ**	**0.815**	< LOQ	< LOQ	< LOQ	**0.027**
S11 M	**0.015**	**0.705**	< LOQ	< LOQ	**0.362**	**1.097**
S11 H	**< LOQ**	**0.001**	< LOQ	< LOQ	**< LOQ**	**< LOQ**
S12 M	**0.016**	**0.830**	< LOQ	< LOQ	**0.352**	**1.173**
S12 H	**< LOQ**	**< LOQ**	< LOQ	< LOQ	**< LOQ**	**0.088**

M: microwave; H: hotblock

For the digestion with microwave irradiation in closed system, 50% of the elements do not exceed the LOQ, while in open system, 59% of the elements do not exceed this same limit. For 65% of EIs the digestion in closed system allows to find higher contents, while open-system digestion allows achieving higher levels for 34% of elemental impurities. 1% of elemental impurities have equal levels regardless of the type of digestion performed. Closed-system digestion with microwave radiation is therefore better suited for the solution of elemental impurities in the trace concentration levels; it offers the advantage of a more stable digestion with less loss. The use of closed vessels is advantageous because it is possible to use higher digestion temperature without dryness of acid or analyte losses. Therefore, the degradation of organic compounds is possible contributing to better digestion efficiency. This result is consistent with those found in the literature.[14,32,33]

Screening elemental impurities in pharmaceutical samples

The validated method was applied for the screening of the target elemental impurities in 37 samples for three APIs: paracetamol, ibuprofen and phloroglucinol. For this, the highest concentration of EI was chosen. The Maximum Permitted Concentration (µg/g) (MPC) reflects the maximum amount of impurity allowed per gram of medication so that there are no adverse effects for the patient. A control threshold (CT) has been established by the guideline ICH Q3D for each EI, which is equivalent to 30% of the corresponding MPC.

DP of paracetamol 2,3,4,7 and 9 (Table 12) contain Pb levels that exceed the ICH Q3D CT, however they remain below the MPC. All paracetamol APIs have a Pb content that exceeds the CT but remains below the MPC according to ICH Q3D (Table 12). This may explain the high levels of Pb found in some DPs. The paracetamol API 3 was synthesized in the chemistry laboratory at Faculty of Pharmacy of Monastir by a green chemistry process. All of the EIs it contains have levels similar to those found in other paracetamol APIs samples. The synthesis by a green chemistry process does not appear to have an impact on EIs. For ibuprofen samples (Table 13), the measured concentration of tested EI are below the CT except for DP7 that have nickel level upper than the CT but under the MPC according to ICH Q3D.

DP of Phloroglucinol 2, 3, 8 and 9 have Pb levels that exceed the CT but do not exceed the MPC. Phloroglucinol's DPs 1, 4, 5, 6 and 7 have Pb levels above the MPC and do not comply with ICH Q3D. Phloroglucinol APIs 1 and 3 (Table 14) have Pb levels that exceed the CT, which could explain the high level of Pb found in phloroglucninol DPs. Several hypotheses could explain this phenomenon. The batch selected for these APIs is not representative of the drug (only one batch was tested for each drug) and may reflect an exceptional situation. This is insufficient because the analysis must be carried out on three different batches in order to cover the intrinsic variations related to the manufacturing process, the equipment used and the various suppliers of packaging articles and raw materials.

EI levels above the safety limit may be justified, for drugs whose benefits are greater than the risk, or for drugs whose intake does not exceed one month. In this context, usually taking these medications is punctual they are used to relieve pain, to decrease fever[34-37] or as symptomatic treatment of spasms.[38,39] Besides, in adults on average, only 5-10% of the ingested dose is absorbed.[40,41] At the end of exposure, the elimination kinetics of lead is polyphasic: after a single exposure, the first period has a very short half-life (30 minutes to a

few hours), it corresponds to a distribution phase. During the second period, the half-decay time of Pb is about 30-40 days.[42] Mercury was analyzed by DMA, the results (Table 15) show that mercury levels are well below the CT.

Table 12 Results of elemental impurities for 14 paracetamol samples screening

	Cd (µg/g)	Pb (µg/g)	As (µg/g)	Co (µg/g)	V (µg/g)	Ni (µg/g)
MPC	1.250	1.250	3.750	12.500	25.000	50.000
CT	0.370	0.370	1.120	3.750	7.500	15.000
DP 1	0.012	0.029	< LOQ	< LOQ	0.266	0.049
DP 2	0.011	**0.545**	< LOQ	< LOQ	0.328	0.207
DP 3	0.009	**0.692**	< LOQ	< LOQ	0.112	0.024
DP 4	0.032	**0.395**	< LOQ	< LOQ	0.266	0.123
DP 5	0.011	0.254	< LOQ	< LOQ	0.181	0.966
DP 6	0.017	0.072	< LOQ	< LOQ	6.976	4.851
DP 7	0.025	**0.412**	< LOQ	< LOQ	0.376	0.223
DP 8	0.010	0.135	< LOQ	< LOQ	6.531	3.003
DP 9	0.019	**0.386**	< LOQ	< LOQ	0.111	0.036
API 1	< LOQ	**0.671**	< LOQ	< LOQ	0.399	0.039
API 2	0.018	**0.723**	< LOQ	< LOQ	0.135	1.341
API 3	0.017	**0.550**	< LOQ	< LOQ	0.087	1.115
API 4	0.014	**0.817**	< LOQ	0.052	0.136	1.113
API 5	0.028	**0.549**	< LOQ	0.316	< LOQ	0.199

MPC: Maximum Permitted Concentration CT: Control threshold

Table 13 Results of elemental impurities for screening 11 ibuprofen samples

	Cd (µg/g)	Pb (µg/g)	As (µg/g)	Co (µg/g)	V (µg/g)	Ni (µg/g)
MPC	4.170	4.170	12.500	41.670	83.330	166.670
CT	1.250	1.250	3.750	12.500	25.000	50.000
DP 1	< LOQ	0.013	< LOQ	< LOQ	< LOQ	27.796
DP 2	0.041	0.198	< LOQ	1.286	1.477	10.638
DP 3	0.100	0.139	< LOQ	7.494	< LOQ	5.559
DP 4	< LOQ	< LOQ	< LOQ	< LOQ	2.766	19.993
DP 5	< LOQ	< LOQ	< LOQ	10.173	< LOQ	4.413
DP 6	< LOQ	0.034	< LOQ	0.214	0.029	< LOQ
DP 7	< LOQ	< LOQ	< LOQ	5.496	< LOQ	**56.046**
API 1	< LOQ	< LOQ	< LOQ	5.199	2.396	1.502
API 2	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	2.583
API 3	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.016
API 4	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.202

MPC: maximum permitted concentration; CT: control threshold

Table 14 Results of elemental impurities for screening 12 phloroglucinol samples

	Cd (µg/g)	Pb (µg/g)	As (µg/g)	Co (µg/g)	V (µg/g)	Ni (µg/g)
MPC	2.400	2.400	7.200	24.000	48.000	96.000
CT	0.720	0.720	2.160	7.200	14.400	28.800
DP 1	0.350	**3.424**	< LOQ	< LOQ	0.666	1.151
DP 2	0.029	**1.464**	< LOQ	< LOQ	0.771	2.329
DP 3	0.020	**1.362**	< LOQ	< LOQ	0.636	1.890
DP 4	0.080	**3.004**	< LOQ	< LOQ	0.354	1.435
DP 5	0.161	**6.330**	< LOQ	< LOQ	0.727	1.732
DP 6	0.187	**10.080**	< LOQ	< LOQ	0.692	1.994
DP 7	0.217	**11.575**	< LOQ	< LOQ	1.161	1.202
DP 8	0.025	**0.800**	< LOQ	0.110	0.548	3.231
DP 9	0.018	**0.798**	< LOQ	< LOQ	< LOQ	1.715
API 1	0.022	**1.180**	< LOQ	< LOQ	< LOQ	2.271
API 2	0.015	0.705	< LOQ	< LOQ	0.362	1.097
API 3	0.016	**0.830**	< LOQ	< LOQ	0.352	1.173

MPC: maximum permitted concentration; CT: control threshold

Table 15 Results for mercury in 37 pharmaceutical samples

	Paracetamol	Ibuprofen	Phloroglucinol
	MPC - CT (µg/g)	MPC - CT (µg/g)	MPC - CT (µg/g)
	7.50 – 2.25	25.00 – 7.50	62.50 - 18.75
DP 1	0.00005	0.00556	0.00115
DP 2	0.00021	0.00704	0.00233
DP 3	0.00002	0.00435	0.00189
DP 4	0.00012	0.00458	0.00144
DP 5	0.00097	0.00561	0.00173
DP 6	0.00485	0.00607	0.00199
DP 7	0.00022	0.01593	0.00120
DP 8	0.00300		0.00323
DP 9	0.00004		0.00172
API 1	0.00004	0.00150	0.00227
API 2	0.00134	0.00258	0.00110
API 3	0.00112	0.00087	0.00117
API 4	0.00111	0.00109	
API 5	0.00020		

MPC: maximum permitted concentration; CT: control threshold

Conclusion

Drug quality assurance is a major concern for the patient and pharmaceutical industries. The elemental impurities are some of contaminants that can affect the quality of pharmaceuticals. They are today a major relevant topic, especially with the appearance of the ICH Q3D directive. In this context, several analytical methods for the analysis of elemental impurities have been developed to meet these requirements, for example HR-ICP-AES analysis after acid digestion. As part of this study, several protocols have been established to ensure proper mineralization. Optimizations were performed in order to select the most appropriate protocol that leads to a total dissolution of each analyzed sample. Once the analyses carried out, several observations were noted: acid mineralization depends closely on the chemical structure of the active ingredient and the components, especially the excipients present in the final product. The mineralization of ibuprofen API in closed system gave different responses. After conducting a series of analyses to characterize them, including FTIR, DSC, DRX and the angle of rotation, it was concluded that the ibuprofen APIs were pure and that the chirality could have an impact on the behavior of a molecule when it is subject to high temperatures and pressure. All samples included in this study underwent two ways of digestion in parallel, one in an open system and the second in a closed system. A comparison between these two types of mineralization was made based on the level of elemental impurities found in each sample. It was noted that wet digestion with microwave irradiation and closed vessels was found to be better than the digestion with conventional heating in open vessel. Then, an HR-ICP-AES method for quantification of elemental impurities in oral drugs according to the new chapters <232> Elemental Impurities – Limits and <233> Elemental Impurities – Procedures in compliance with ICH Q3D has been performed. The Validation of the method showed that the USP requirements to linearity, precision, accuracy, specificity, LOD and LOQ were met for all six impurities. The memory effect of Hg was handled by using DMA-80 which is very sensitive and can detect Hg in matrix in the order of µg/kg. The screening of 37 pharmaceutical samples showed that some have lead concentration above the target limit which can be harmful for human use especially for chronic treatment.

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Conflicts of Interest

None.

References

1. Pokar D, Rajput N, Sengupta P. Industrial approaches and consideration of clinical relevance in setting impurity level specification for drug substances and drug products. *Int J Pharm.* 2020;576: 119018.
2. Jiang Y, Xia JP, Yang JH, et al. Guidelines and strategy of the International Conference of Harmonization (ICH) and its member states to overcome existing impurity control problems for antibiotics in China. *Chin J of Nat Med.* 2015;13:498–506.
3. FC. Pinheiro, AI. Barros, JA. Nóbrega, 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.* 2019;1065:1–11.
4. Chahrour O. Malone J, Collins M. et al. Development and validation of an ICP–MS method for the determination of elemental impurities in TP–6076 active pharmaceutical ingredient (API) according to USP □232□/□233□. *J Pharm Biomed Anal.* 2017;145:84–90.
5. Tabersky D, Woelfle M, Ruess JA, et al. Recent Regulatory Trends in Pharmaceutical Manufacturing and their Impact on the Industry. *Chimia (Aarau).* 2018;72(3):146–150.
6. Wang T, Wu J, Hartman R, et al. A multi-element ICP-MS survey method as an alternative to the heavy metals limit test for pharmaceutical materials. *J Pharm Biomed Anal.* 2000;23:867–890.
7. Lewen N, Mathew S, Schenkenberger M, et al. A rapid ICP-MS screen for heavy metals in pharmaceutical compounds. *J Pharm Biomed Anal.* 2004;35(4):739–752.
8. Beauchemin D. Inductively coupled plasma mass spectrometry. *Anal Chem.* 2006;78(12):4111–4136.
9. Huang J, Hu X, Zhang J, et al, The application of inductively coupled plasma mass spectrometry in pharmaceutical and biomedical analysis. *J Pharm Biomed Anal.* 2006;40(2):227–234.

10. Nageswara Rao R, MVN Talluri K. 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.* 2007;43(1):1–13.

11. Flores EMM. *Microwave-assisted sample preparation for trace element determination*. *Newnes*, 2014;415.

12. Mester Z, Sturgeon RE. *Sample preparation for trace element analysis.* Elsevier. 2003;1339.

13. Flores EMM, Barin JS, Paniz JNG, et al. Microwave-assisted sample combustion: a technique for sample preparation in trace element determination. *Anal Chem.* 2004;76(13):3525–3529.

14. Barin JS, Mello PA, Mesko MF. Determination of elemental impurities in pharmaceutical products and related matrices by ICP-based methods: a review. *Anal Bioanal Chem*. 2016;408(17):4547–4566.

15. Reed NM, Cairns RO, Hutton RC, et al. Characterization of polyatomic ion interferences in inductively coupled plasma mass spectrometry using a high resolution mass spectrometer. *J Anal At Spectrom.* 1994;9:881–896.

16. Grindlay G, Mora J, Loos–Vollebregt Md, et al. A systematic study on the influence of carbon on the behavior of hard-to-ionize elements in inductively coupled plasma-mass spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy*. 2013;86:42–49.

17. Hu Z, Gao S, Hu S, H. et al. Suppression of interferences for direct determination of arsenic in geological samples by inductively coupled plasma mass spectrometry. *J Anal At Spectrom.* 2005; 20:1263–1269.

18. Guo W, Hu S, Zhang J, et al. Reduction of acid effects on trace element determination in food samples by CH4 mixed plasma-DRC-MS. *Talanta.* 2012;91:60–64.

19. Stewart II, Olesik JW. Steady state acid effects in ICP-MS. *Anal At Spectrom*. 1998;13:1313–1320.

20. Stewart II, Olesik JW. Transient acid effects in inductively coupled plasma optical emission spectrometry and inductively coupled plasma mass spectrometry. J Anal At Spectrom. 1998;13: 1249–1256.

21. Støving C, Jensen H, Gammelgaard B, et al. 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.* 2013;84:209–214.

22. La validation de méthode en spectrométrie d'émission optique à source plasma de l'échantilon au résultat, EDP science, France, 2017.

23. 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. *Analytica Chimica Acta.* 2019; 1078:1–7.

24. Pinheiro FC, Barros AI, Nóbrega JA. Elemental impurities analysis in name-brand and generic omeprazole drug samples. *Heliyon*. 2020;6(2).

25. 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*. 2018;152:12–16.

26. Wurfels M, Jackwerth E. *Analytica Chimica Acta,* 1989;226:31–41.

27. Bannach G, Arcaro R, Ferroni DC, et al. Thermoanalytical study of some anti-inflammatory analgesic agents. *Journal of Thermal Analysis and Calorimetry*. 2010;102:163–170.

28. Otsuka Y, Ito A, Matsumura S, et al. Quantification of Pharmaceutical Compounds Based on Powder X-Ray Diffraction with Chemometrics. *Chem Pharml Bull (Tokyo)*. 2016;64:1129–1135.

29. Ren F, Su J, Xiong H, et al. Characterization of ibuprofen microparticle and improvement of the dissolution. *Pharm Dev Technol.* 2017;22(1):63–68.

30. Fischer L, Zipfel B, Koellensperger G, et al. 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*. 2014;95:121–129.

31. Balaram V. Microwave plasma atomic emission spectrometry (MP-AES) and its applications – A critical review. *Microchemical Journal.* 2020;159:105483.

32. Zachariadis G, Sahanidou E. Analytical performance of a fast multi-element method for titanium and trace elements determination in cosmetics and pharmaceuticals by ICP-AES. *Open Chemistry*. 2011;9:213–217.

33. Zachariadis GA, Michos CE. 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*. 2007;43(3):951–958.

34. Bertolini A, Ferrari A, Ottani S, et al. Paracetamol: new vistas of an old drug. CNS Drug Rev. 2006;12(3-4):250–275.

35. What dose of paracetamol for older people?. *Drug Ther Bull.* 2018;56(6):69–72.

36. Kantor TG. Ibuprofen. *Ann Intern Med*. 1979;91(6):877–882.

37. Aycock DG. Ibuprofen. *Am Pharm*. 1991;NS31:46–49.

38. Gavilánez Buñay TC, Colareda GA, Ragone MI, et al. Intestinal, urinary and uterine antispasmodic effects of isoespintanol, metabolite from Oxandra xylopioides leaves. Phytomedicine. 2018;51(1):20–28.

39. Lacroix C. Hurault–Delarue S. Kessler C. et al. First epidemiologic data about phloroglucinol exposure during first trimester of pregnancy. *Gynecol Obstet Fertil.* 2011;39:694–697.

40. Alexander FW. The uptake of lead by children in differing environments. *Environmental Health Perspectives.* 1974;7:155–159.

41. Ziegler EE, Edwards BB, Jensen RL, et al. Absorption and retention of lead by infants. *Pediatr Res*. 1978;12(1):29–34.

42. Garnier R. Toxicité du plomb et de ses dérivés Toxicity of lead and lead compounds. *EMC – Toxicologie–Pathologie.* 2005;2(2):67–88.