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DETERMINATION OF TOXIC HEAVY METALS IN CHOLIC ACID USING QUADRUPOLE INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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ABSTRACT

Objective: The information on the presence of toxic heavy metals in pharmaceutical starting materials and finished product is very crucial from the viewpoint of human life and its hazardous impact on the worldwide environment. The present work deals with the detailed quantification of the toxic heavy metals, namely, V, Co, Ni, Cd, Hg, Pb, and As, present in colic acid using quadrupole inductively coupled plasma mass spectrometry (Q-ICPMS) with prior microwave-assisted digestion. Moreover, the preliminary characterization of commercially available cholic acid by FT-IR, NMR (1H and 13C). SEM-EDAX has also been carried out.

Methods: Cholic acid of synthesis grade, Nitric acid (65%) AR. grade, ethylene diamine tetra acetic acid sodium salt AR grade, and certified reference metal stock standard solutions (1000 mg/L) of multiple elements prepared in 2–3% HNO3 of analytical grade were purchased from Merck (Darmstadt, Germany). All the samples were treated with nitric acid and microwave-assisted digestion. For the accurate determination of the elemental amount, various digested solutions and post-digestion diluents were tested. The linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) of the analytical technique were evaluated in accordance with the United States Pharmacopoeia 233 standard.

Results and Discussion: The Q-ICPMS-based analytical method was validated for specificity, LOD, LOQ, linearity, accuracy, precision, and uncertainty. The estimated detection limits of the toxic heavy metals in cholic acid were in the range $2-180 \mu g/L$. The quantification limits were in the range of $1.5-60 \mu g/L$. Mean recoveries±standard deviations at different spiking levels were in the range $75.3\pm2.1-104.9\pm8.5\%$. The coefficients of variation were in the range of 0.5-8.1%.

Conclusion: The precision of the analytical method, in terms of relative standard deviation, was below 1.95%. The uncertainty in the quantification of all the validated elements was found to be $\leq 1.70\%$ for Sample 1.

Keywords: Cholic acid, Metal impurities, Heavy metals, Quadrupole inductively coupled plasma mass spectrometry, Analytical method development and validation. Microwave acid digestion.

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INTRODUCTION

Cholic acid is a primary bile acid. Bile acids are biological compounds belonging to the steroidal family generated in humans (liver) and the majority of animals [1,2]. Lipid-rich diet may be the source of the accumulation of toxic elements, such as Zn, Cu, Cd, Mn, and Ni in the liver, that produces bile acids. As a result of the accumulation of toxic heavy metals in the liver, the enzymatic activity is inhibited and the metabolic pathways are altered. Moreover, the presence of such toxic heavy metals increases the risk of tumor formation [3].

Monitoring and quantification of the presence of toxic heavy metals in the liver or the byproducts of the liver like those of bile acids is a necessity from a health perspective. Though a web of science search with the keywords, namely, cholic acid and toxic heavy metals shows four results, none of them match with either the objective or the outcome of the present study. The aim of this work is to have the complete information on the amount of toxic heavy metals present in cholic acid-containing drugs which are consumed by humans in everyday life and to ensure whether it is under the permissible limit set by the United States Pharmacopeia (USP) 233 standard.

Harmful effects of the presence of toxic heavy metals, such as vanadium (V), nickel (Ni), cadmium (Cd), mercury (Hg), lead (Pb), and arsenic (As) in water, food, drugs, and environment are well known and mankind is no stranger to bearing the heat of such contaminants and this needs no elaborate introduction [4-12]. Pharmaceutical regulatory

agencies have set the permitted levels of heavy metals in medication, which are consistently monitored using limit tests. These tests confirm that no inorganic impurities are introduced into the medications during any of the manufacturing phases. The USP, the British pharmacopeia, the European pharmacopeia, and the Japanese pharmacopeia are all jointly monitoring the total metal impurity contents in pharmaceutical products. However, the procedures adopted are non-specific, insensitive, and time-consuming, needing improvement in accuracy excepting the few new legislations namely USP 232 and 233. Thus, very sensitive and selective procedures are urgently needed for determining trace toxic heavy metals in pharmaceutical compounds, not only to meet the demanding regulatory criteria but also to ensure the safety and efficacy of medication intended for human consumption [13].

In Quadrupole inductively coupled plasma mass spectrometry (Q-ICP-MS), the energy source, namely, plasma is advantageous over other energy sources, such as flame ionization, because it allows ionization to occur in a chemically inert environment, preventing oxide formation and the ionization is more complete. Q-ICP-MS analysis of toxic heavy metals is superior to other methods such as atomic absorption spectrometry, X-ray fluorescence spectrometry, and ICP optical emission spectrometry owing to exceptionally low detection limits for a large range of elements. Some components can be measured to the billionth of a trillionth of a trillion [14]. Many researchers have previously applied this sophisticated analytical technique of Q-ICP-MS for bioanalytic purposes successfully [15-17].

In addition to Q-ICP-MS of the toxic heavy metals in cholic acid, for comparison, ICP-OES analysis was carried out. Moreover, cholic acid commercial sample was further systematically characterized using SEM-EDAX to know the purity of the sample Fig. 1.

The goal of this study is thus to develop a fast, effective, simple, and accurate method of cholic acid sample preparation in conjunction with Q-ICP-MS to accurately determination of above mentioned toxic heavy metal impurities in oral medicinal products in a single test.

METHODS

Materials and solutions

Cholic acid of synthesis grade used in the study is procured from Suvidhinath laboratories. Nitric acid (65%), ethylene diamine tetra acetic acid sodium salt AR grade, and certified reference metal stock standard solutions (1000 mg/L) of V, Co, Ni, Cd, Hg, Pb, and As prepared in 2-3% HNO, of analytical grade were purchased from Merck (Darmstadt, Germany). Deionized water was prepared using a Milli-O plus water purification system from Millipore (Bedford, MA, USA). Yttrium standard for ICP TraceCERT® (1000 mg/L Y in nitric acid), bismuth standard for ICP TraceCERT® (1000 mg/L Bi in nitric acid), nitric acid ≥69.0%, TraceSELECT™ for trace analysis from Honeywell were used for the study. All the autosampler vials, centrifuge tubes, and plastic bottles, were cleaned by soaking in 20% v/v HNO3 analytical grade reagent for 4 h, followed by rinsing with deionized Milli-Q water thrice. Element impurities according to ICH Q3D, Standard 1 (containing 15 ppm of Arsenic (As), 5 ppm each of Lead (Pb) and Cadmium (Cd), 30 ppm of Mercury (Hg), 50 ppm of Cobalt (Co), 100 ppm of Vanadium (V), 200 ppm of Nickel (Ni) and three other elements i.e., 150 ppm each of Selenium (Se) and Silver (Ag) and 8 ppm of Thallium (Tl) multi-standard were procured from Sigma-Aldrich.

Sample preparation

Weighed accurately about 100 mg of cholic acid commercial sample into a 15 mL calibrated plastic tube. Transferred 90 mL of (65%) $\rm HNO_3$ into 3000 mL volumetric flask containing 1000 mL of deionized water mixed well and diluted up to the mark with water, and shaken well. Added 3 mL of concentrated nitric acid to the sample in the sample tube and allowed the sample to digest with intermittent shaking. After sample digestion, when the sample became clear and no more fumes of nitric acid were evolved from the sample tube, the content is made up to 10 mL mark with water.

Microwave digestion

There are open and closed-vessel approaches to microwave-assisted digestion. A closed vessel method is appropriate for a majority of pharmaceutical applications. Digestion was performed using Mth 2018–001, STD 75 manufactured by PerkinElmer 16 position unit size microwave digestion system. Weighed accurately 0.2 g sample into 10 mL volumetric flask and mixed it with 7.0 mL conc. $\rm HNO_3$. Transferred into the digester vessel and selected the digestion method as above and digested the sample. Cooled to the room temperature and transferred into 10 mL volumetric flask and made up with purified water. Pipetted out 5.0 mL into 10 mL volumetric flask and dilute up to the mark with deionized water. Details of sample digestion were given in Table S1.

Standard stock solutions for calibration

Standard stock solutions for calibration were prepared by taking 1.0 mL of elemental impurities according to ICH Q3D standard, namely, 1 mL of a standard containing 100 ppm of Vanadium (V), 50 ppm of Cobalt (Co), 200 ppm of Nickel (Ni), 5 ppm of Cadmium (Cd), 30 ppm of Mercury (Hg), 5 ppm of Lead (Pb) and 15 ppm of Arsenic (As) (Table S2). The standard stock solutions were then diluted to 20 mL with 2% nitric acid. Then, these stock solutions were further diluted to make different levels of standards for calibration (Table 1).

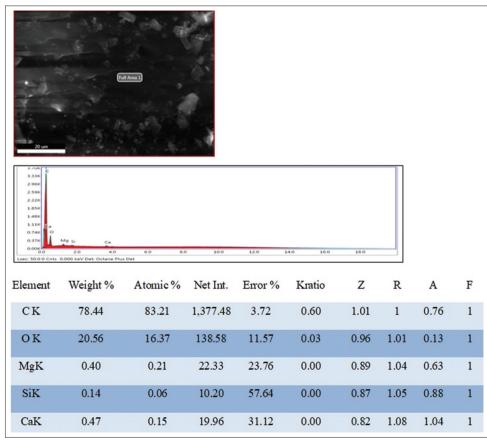


Fig. 1: SEM-EDAX analysis of cholic acid (commercial sample)

Table 1: Dilution of standards for calibration

Solution	Volume of standard	Make up	Concen	tration (µg,	/L)				
name (%)	stock solution (mL)	volume (mL)	v	Со	Ni	Cd	Hg	Pb	As
LOQ (30)	0.3	50	30	15.	60	1.5	9.0	1.5	4.5
50	0.5	50	50	25	100	2.5	15	2.5	7.5
80	0.8	50	80	40	160	4.0	24	4.0	12
100	1.0	50	100	50	200	5.0	30	5.0	15
120	1.2	50	120	60	240	6.0	36	6.0	18
200	2.0	50	200	100	400	10	60	10	30

LOQ: Limit of quantification

Spiked sample solution

Weighed accurately about 100 mg of sample into 15 mL calibrated plastic tube. The amount of standard stock solution 2 to be added is specified in Table 2. Added 3 mL of conc. $\mathrm{HNO_3}$ and allowed the sample to digest with intermittent shaking. After sample digestion, when the sample become clear and all the fumes of nitric acid ceased to evolve from the sample tube, the digestion was made up to the mark with deionized water.

Instrumentation

Toxic heavy metal impurities in the cholic acid sample were determined by Agilent Technologies 5110 ICP-MS. The quantity of heavy metals, namely, V, Co, Ni, Cd, Hg, Pd, and As, were determined by Q-ICP-MS an iCAP RQ ICP-MS (Thermo Fisher Scientific) using QtegraTM software equipped with Q Cell Collision Reaction Cell, RAPID lens, with a quartz spray chamber, glass concentric nebulizer, online internal standard (ISTD) addition kit, and exchangeable skimmer cones. Optimization of Q-ICP-MS is important because the flow rates of the nebulizer gas and makeup gas should be adjusted to ensure the stability of the plasma. Q-ICP-MS was allowed to stabilize for 1 h and the performance was optimized based on radio frequency power, auto tune function in the control software, and A tune B solution, the quadrupole ion deflector voltages were optimized stepwise to find the settings that maximize signal intensity over the mass range, as well as for the mass calibration of Li, Co, In, Ba, Ce, Bi, and U, sampling depth, argon flow rate, collision cell gas flow rate, lens voltage, sample uptake rate. The instrument was kept in KED mode for the analysis of V, Co, Ni, Cd, Hg, Pd, and As.

Criteria for validating the analytical method

For method validation, several criteria such as linear dynamic range, method linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and measurement of uncertainty were investigated and evaluated. In compliance with ICH Q2 (R1), Q-ICP-MS was used to validate the analytical method for the quantification of V, Co, Ni, Cd, Hg, Pd and As.

Q-ICP-MS analysis

Six replicate readings of 30 sweeps over the analyte mass range with a dwell time of 40 μ s for each mass per sweep were used in a typical method of analysis. Sample aspiration followed by rinsing with 2% HNO_3 was done for 60 s. For running the instrument in KED mode, 4.34 mL min-1 of He gas was used.

Characterization of Cholic acid

Fourier transform infrared spectra of the samples were recorded at room temperature on a Perkin Elmer, U.S.A, spectrometer: (Model: Spectrum GX). The background due to air was measured, the adsorbent was added to KBr, and the sample was scanned 32 times over a frequency range of $400-4000~\rm cm^{-1}$. $^1{\rm H}$ and $^{13}{\rm C}$ NMR spectra were obtained using a Bruker, Model: $400~\rm MHz$ FTNMR, Avance III Spectrophotometer, and [CDCl $_3$] acetone was used as a solvent. The chemical shifts are reported in ppm with respect to the TMS internal reference. SEM-EDAX analysis of the samples was carried out on Philips, Netherlands Model: ESEM EDAX XL-30 after depositing a gold coating. The voltage was 30 keV, and the field electron source was scanned at a resolution of 2 nm.

Table 2: Dilution for spiked sample preparation

Level of spiked sample preparation (%)	Amount of calibration standard stock solution to be added (mL)
LOQ (30%)	0.3
100%	1.0
150%	1.5

LOQ: Limit of quantification

RESULTS AND DISCUSSION

Internal standard for the detection of toxic heavy metals

While using Q-ICP-MS for elemental analysis, selecting an appropriate internal standard is critical. This would have a significant impact on the accuracy and precision of the results.

Optimization of operation parameters of Q-ICP-MS

Various optimized Q-ICP-MS parameters were reported (Table 3).

Method validation

In analytical chemistry, method validation is one of the technical aspects of the overall quality assurance scheme. Selectivity and specificity are determined by the element chosen and the corresponding possible potential interferences. It is always about "the extent to which the approach may be employed to determine the specific analytes in mixtures or matrices without interference from other components that behave similarly [18]. The selectivity of the current approach was investigated using primary isotopes of each element, ⁵¹V, ⁵⁹Co, ⁶⁰Ni, ¹¹¹Cd, ²⁰²Hg, ²⁰⁸Pb, and ⁷⁵As. A validation study was conducted to determine and prove the method's reliability. Some analytical characteristics were used to validate the approach.

Estimated LOD

The lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified, is known as the LOD. It is a limit test that determines whether an analyte is above or below a certain threshold from the calibration function using Equation (1) [19].

$$LOD = \frac{3.3\sigma}{S} \tag{1}$$

where, σ is standard deviation S is slope derived from the calibration curve

The estimated LODs were found to be 0.01, 0.01, 0.18, 0.002, 0.02, 0.02, and 0.10 $\mu g/L$ for V, Co, Ni, Cd, Hg, Pd, and As, respectively. The minimum practical concentrations of tested elements in the analyzed samples, which can be determined with acceptable accuracy, were performed by analyzing 3 replicates at 30 $\mu g/L$ for V, at 15 $\mu g/L$ for Co. The estimated LODs were found to be 0.01, 0.01, 0.18, 0.002, 0.02, 0.02, and 0.10 $\mu g/L$ for V, Co, Ni, Cd, Hg, Pd, and as, respectively. The minimum practical concentrations of tested elements in the analyzed samples, which can be determined with acceptable accuracy, were performed by analyzing 3 replicates at 30 $\mu g/L$ for V, at 15 $\mu g/L$ for Co, at 60 $\mu g/L$ for Ni, at 1.5 $\mu g/L$ for

Cd, at 9.0 $\mu g/L$ for Hg, at 1.5 $\mu g/L$ for Pb, and at 4.5 $\mu g/L$ for As. The results were reported in Table 4.

Estimated LOO

The lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the method's stated operational circumstances is known as the LOQ. The noise-to-signal ratio for LOQ should be 1:10. The estimated LOQs were found to be 30, 15, 60, 1.5, 9.0, 1.5, and 4.5, μ g/L for V, Co, Ni, Cd, Hg, Pb, and As, respectively. The results are reported in Table 4 [20].

Table 3: Typical Q-ICP-MS instrument parameters for the analytical method

Parameter	Setting
RF ^a power (W)	1600
RF matching (V)	1.80
Sampling depth (mm)	4.6
Carrier gas (L min ⁻¹)	1.02
Spray chamber temperature (°C)	2
Nebulizer pump (revolutions per second, rps)	0.1
Extract (V)	3.7
Einzel 1,3 (V)	-100
Einzel 2 (V)	22
Cell entrance (V)	-50
Cell exit (V)	-42
Plate bias (V)	-43
QPb bias (V)	-4.6
OctPc RF (V)	190
OctP bias (V)	-7.0

^aRF: Radiofrequency; ^bQP: Quadrupole; ^cOctP: Octupole

Method linearity

The linearity of a test process is its ability (within a certain range) to deliver results that are directly proportional to the concentration of analyte in the sample, according to the CPMP guidelines [21]. If the value of the calibration curve coefficient of determination (R2) is higher than 0.995, the quantification result will be accurate as analytical response will be linear over certain concentration ranges. The method linearity was investigated over a specific working range from different concentrations of reference standards.

Linearity of the calibration curves

The dynamic linear range was found to be linear from 30 to 150 μ g/L for 51 V, 15–75 μ g/L for 59 Co, 60–300 μ g/L for 60 Ni, 1.5–7.5 μ g/L for 111 Cd, 9–45 μ g/L for 202 Hg and 1.5–12.5 208 Pb and 4.5–22.5 μ g/L for 75 As (Fig. 1) for Sample 1.

Method linearity

The method linearity was checked using seven different levels of samples at 0, 30, 50, 100, 150, 200, and 250 $\mu g/L$ for 51 V, 0, 15, 25, 50, 75, 100, 125 $\mu g/L$ for 59 Co, 0, 60, 100, 200, 300, 400, 500 $\mu g/L$ for 60 Ni, 0, 1.5, 2.5, 5, 7.5, 10, 12.5 $\mu g/L$ for 111 Cd, 208 Pb, 0, 9, 15, 30, 45, 60, 75 $\mu g/L$ for 202 Hg, and 0, 4.5, 7.5, 15, 22.5, 30, 37.5 $\mu g/L$ for 75 As. The method linearity was found to be linear from LOQ values up to 30, 15, 60, 1.5, 9, 1.5, and 4.5 $\mu g/L$ for 51 V, 59 Co, 60 Ni, 111 Cd, 202 Hg, 208 Pb, and 75 As (Fig. 2) for Sample-1.

Method accuracy

In the context of an analytical method, according to ICH guidelines, accuracy "is sometimes termed as trueness." The trueness of an analytical procedure reflects the closeness of agreement between the value that is either accepted as a conventional true value or an accepted

Table 4: Estimated LODs, practical LOQs, and maximum permissible limits (number of replicates=06) for Sample-1

Element	Estimated values		Practical val	ues	CV%	Maximum p	ermissible	limits (µg/L)
	Standard deviation (SD)	LOD (µg/L)	LOQ (µg/L)	Mean concentration±SD		Egyptian	EU	WHO
V	0.004482	0.01346	30	30.9±0.32	1.03	-	-	-
Co	0.003981	0.005803	15	15.2±0.29	1.90	-	-	-
Ni	0.03359	0.1795	60	59.8±1.31	2.19	20	20	70
Cd	0.003963	0.001525	1.5	1.6±0.07	4.57	3	5	3
Hg	0.004465	0.02271	9.0	9.7±0.35	3.61	1	1	6
Pb	0.004448	0.02066	1.5	1.5±0.01	0.51	10	10	10
As	0.008723	0.09737	4.5	4.7±0.32	6.86	10	10	10

LOQ: Limit of quantification, LOD: Limit of detection

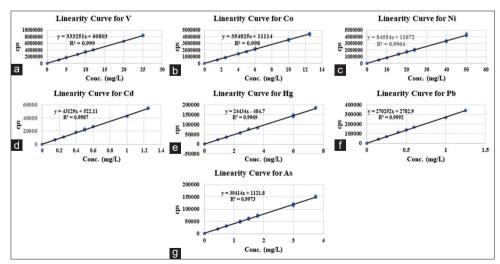


Fig. 2: Method linearity (a) V (3.0–25.0 mg/L), (b) Co (1.5–12.5 mg/L), (c) Ni (6.0–50.0 mg/L), (d) Cd (0.15–1.25 mg/L), (e) Hg (0.9–7.5 mg/L, (f) Pb (0.15–1.25 mg/L), and (g) As (0.45-3.75 mg/L)

reference value with the observed value. Therefore, accuracy is an expression of both trueness and precision since both of these influence the result [22]. Accuracy can be measured by spiking the sample matrix with a known concentration of analyte standard and analyzing the sample using the "method to be validated [23].

Precision study - repeatability and reproducibility

The precision of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling. According to ICH, precision may be considered at three levels: repeatability, intermediate precision, and reproducibility. The precision was calculated in terms of relative standard deviation (RSD) using Equation 2 and a single estimation of precision uncertainty was calculated using Equation 3, respectively.

$$RSD = (S * 100)/x$$
 (2)

Table 5: Linear regression analysis for Sample-1

Element	Linear range (mg/L)	Slope	Intercept	Determination coefficient
V	0.3-25.0	333251	60803	0.9990
Co	1.5-12.5	354825	11114	0.9980
Ni	6.0 - 50.0	84554	12672	0.9964
Cd	0.15 - 1.25	43129	522.11	0.9987
Hg	0.9 - 7.5	24434	-484.70	0.9969
Pb	0.15 - 1.25	270252	2783	0.9992
As	0.45-3.75	39414	1122	0.9973

Where.

RSD = Relative standard deviation

S = Standard deviation

x = Mean of the data

Uncertainty (u) =
$$\sqrt{\sum \left(x_{i^{-\mu}}\right)^2/(n^*(n-1))}$$
 (3)

Where,

 $x_i = i^{th}$ reading in the data set

 μ = Mean of the data set

n = Number of readings in the data set

The results of the repeatability test expressed as RSD were found to be 2.74%, 2.46%, 1.95%, 5.02%, 3.45%, 2.56%, and 2.64%, for $^{\rm 111}{\rm Cd},$ $^{\rm 202}{\rm Hg},$ $^{\rm 208}{\rm Pb},$ $^{\rm 75}{\rm As},$ $^{\rm 51}{\rm V},$ $^{\rm 59}{\rm Co},$ and $^{\rm 60}{\rm Ni},$ respectively. Results of the linear regression analysis are reported in Table 5.

Estimation of measurement uncertainty

According to EURACHEM/CITAC GUIDE CG4, the term uncertainty (of measurement) is defined as "A parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand." There are various contributing factors such as weighing of samples, sample and standard preparation, instrumental measurements, linearity measurement, laboratory repeatability, and reproducibility. The expanded uncertainty was measured by multiplying the combined uncertainty, by using a coverage factor (k) of 2, at confidence level of 95%. The measurement uncertainties expressed as expanded uncertainties were estimated to be 13.3, 2.8, 16.7, 0.6, 4.1, 0.6, and 1.8, for V, Co, Ni, Cd, Hg, Pd, and As,

Table 6: Uncertainty tests of sample -1

Element	Result (mg/L)	Standard deviation	Sample size	Confidence interval	Uncertainty	Results±Uncertainty (mg/L)
V	8.967	0.285	6	95	0.232	8.967±0.232
Co	4.498	0.097	6	95	0.079	4.498±0.079
Ni	19.504	0.471	6	95	0.385	19.504±0.385
Cd	0.516	0.011	6	95	0.009	0.516±0.009
Hg	3.037	0.058	6	95	0.047	3.037±0.047
Pb	1.195	0.022	6	95	0.018	1.195±0.018
As	2.130	0.104	6	95	0.085	2.130±0.085

Table 7: Comparison for uncertainty statistics in three different commercial samples of cholic acid

Element	Result (mg/L)	Standard deviation	Sample size	confidence Interval	Uncertainty	Results±Uncertainty (mg/L)
Sample-1						
V	8.967	0.285	6	95	0.232	8.967±0.232
Co	4.498	0.097	6	95	0.079	4.498±0.079
Ni	19.504	0.471	6	95	0.385	19.504±0.385
Cd	0.516	0.011	6	95	0.009	0.516±0.009
Hg	3.037	0.058	6	95	0.047	3.037±0.047
Pb	1.195	0.022	6	95	0.018	1.195±0.018
As	2.13	0.104	6	95	0.085	2.130±0.085
Sample-2						
V	9.074	0.400	6	95	0.327	9.074±0.327
Co	4.8518	0.035	6	95	0.086	4.852±0.086
Ni	20.239	0.209	6	95	0.336	20.239±0.336
Cd	0.5088	0.007	6	95	0.029	0.509±0.029
Hg	3.2107	0.051	6	95	0.037	3.211±0.037
Pb	1.2863	0.007	6	95	0.029	1.286±0.029
As	2.1908	0.022	6	95	0.014	2.191±0.014
Sample-3						
V	9.8695	0.167	6	95	0.264	9.87±0.264
Co	4.9748	0.035	6	95	0.063	4.975±0.063
Ni	19.648	0.209	6	95	0.301	19.648±0.301
Cd	0.5658	0.007	6	95	0.029	0.566±0.029
Hg	3.2527	0.051	6	95	0.021	3.253±0.021
Pb	1.3343	0.007	6	95	0.024	1.334±0.024
As	2.4686	0.022	6	95	0.086	2.469±0.086

respectively. The results on the uncertainty of each element in cholic acid are presented in Table 6. The study was extended to three more commercial samples of cholic acid and similar results were obtained as shown in Table 7.

Bias study (recovery test)

The spiking levels used for the recovery test were at 1.5, 5 and 7.5 $\mu g/L$ for $^{111}\text{Cd},\,^{208}\text{Pb},\,4.5,\,15,\,$ and 22.5 $\mu g/L$ for $^{75}\text{As},\,9,\,30,\,$ and 45 $\mu g/L$ for $^{202}\text{Hg},\,15,\,50$ and 75 $\mu g/L$ for $^{59}\text{Co},\,60,\,200$ and 300 $\mu g/L$ for ^{60}Ni and 30, 100 and 150 $\mu g/L$ for $^{51}\text{V}.$ The mean recoveries±standard deviations at different levels varied between 75.3±2.1% and 104.9±8.5% with coefficient of variation expressed as RSD ranged from 0.5% to 8.1%. Freshmen attending analytical chemistry courses are referred to the elegant review on ICP-MS technique by Wilschefski and Baxter.

CONCLUSION

The measurement of heavy metals in cholic acid using a microwave-aided acid digestion process and Q-ICP-MS analysis was satisfactorily confirmed in this study. For trace metal analysis of cholic acid, it is considered to be a good, reliable, and rapid approach. The accuracy of the present method is (30–150% of the target value) and precision value (n=6) successfully achieved the criteria defined by WHO, AOAC, USFDA, ICH, and USP 232/233. The proposed method was effectively applied for the routine analysis of heavy metals in cholic acid. LOD, LOQ, linearity, repeatability, reproducibility, accuracy, and precision have all been successfully assessed using the validated method. The suggested validated method is highly simple, quick, easy, cost-effective, and reliable, making it ideal for quantification of these hazardous metals in regular laboratory analysis.

AUTHORS CONTRIBUTIONS

Thakar Meet Kumar: Performed experiments and collection of data. Sheth Jateen: Calculation of results in Excel format. Indra Neel Pulidindi: Given some suggestions. Suthar Vaishali: Made figures and wrote rough draft. Sharma Pankaj: Conceptualization, Methodology, Formal analysis, Writing an original draft, Supervision, Writing-review, and editing.

CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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DETERMINATION OF TOXIC HEAVY METALS IN CHOLIC ACID USING QUADRUPOLE INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (Q-ICP-MS)

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ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Table S1. Conditions of microwave digestion of cholic acid samples

Digestion conditions	Temperature (T) (°C)	Pressure (P) (Bar)	Ramp (°C/min)	Hold Time (min)	Percentage (%)
1	170	30	5	2	80
2	200	30	2	20	90
3	50	20	2	5	0

Table S2. Impurities classification and specification limit.

Name of Element	Class	Specification Limit (μg/L)
V	2A	10
Co	2A	5
Ni	2A	20
Cd	1	0.5
Hg	1	3.0
Hg Pb	1	0.5
As	1	1.5

Table S3. Accuracy/recovery test of toxic heavy metals (V, Co, Ni, Cd, Hg, Pd, As) from Q-ICP-MS analysis. Calculation based on cps ratio Calculation based on cps

J. Accuration for the quantification of V assetz J. Accuration of J. Accuration of J. Accuration of J. Accurat	Sample	Sample wt. (g)	Conc (ppb)	cbs	Int. Std. cps	cps ratio	Result (ppm)	Amount Recovered	% Recovery	Mean % Recovery	Result (ppm)	Amount Recovered	% Recovery	Mean % Recovery
8712.66.889 1375.681. 1275.2590. 0.047.257.381. 1275.2591. 8712.66.889 1371.681. 0.0635042.6 0.075.171.0 0.027.373.865. 1275.645.77 8812.66.889 1371.681. 0.0635042.6 0.0635042.6 0.0635042.6 0.0505042.6 0.0535042.6 <td>1) Accuracy in the</td> <td>quantificat</td> <td>ion of V</td> <td></td> <td>1</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2</td> <td></td> <td></td> <td></td>	1) Accuracy in the	quantificat	ion of V		1						1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			
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36250.367 1407762	LOQ spiked-2	0.1034	3	1088767.484	1424294	0.764426083	2.983221131	2.909229983	6.96		2.847058445	2.573029768	85.7	
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1661582.263 1404901.6 118262789 4.57824255 4.5884769 91.9 4.229798096 4.145790169 1992073.564 137756.2 1.482168392 5.430850245 5.271284493 878 4.929605504 4.445790169 1962073.554 137756.2 1.398385305 5.23067935 5.271284493 878 4.9296050504 4.484597077 2046064.188 1355419.6 1.509543014 5.599472035 93.3 5.315582714 5.231574787 23680.471.1 1355590.6 0.017472575 0.127761056 0.132643687 0.529003508 0.542003578 5.231574787 24551.45472 1361540.6 0.017811775 0.134388682 0.441245333 90.6 91.4 5.24691184 4.890550718 249992.1019 1404841.6 0.35590699 5.572446044 92.7 5.445691184 4.890550718 240908.5021 1402402 0.35590699 5.554746044 92.7 5.4450911 4.880550718 240908.5021 140232 0.35880626 0.226446044 92.7 5.54450704 4	100% spiked-2	0.1022	2	1674632.317	1387849.2	1.206638529	4.587351508	4.607956644	92.1		4.288828108	4.204820181	84.0	
1922073.564 137192.4 1.452168992 5.43145382 90.6 5.11635865 5.032350723 1922073.564 1377556.2 1.398385305 5.250679357 5.271284493 87.8 90.6 5.11635865 5.032350723 2046064.188 1355290.6 1.509543014 5.578866899 5.599472035 93.3 4.929605604 4.845597677 23680.417.13 1355290.6 0.017472575 0.12761056 0.1276449 0.1276449 0.1276464 0.12764484 0.1276464 0.12764484 0.0176464 0.1276464 0.1276464 0.1276464 0.1276464 0.1276464 0.12764644 0.0174841.1 0.01764841.4 0.01764941.4 0.01764941.4 0.01764941.4 0.01764941.4 0.01764941.4 0.01764941.4 0.01764941.4 0.01764041.4 0.01764041.4 0.01764041.4 0.01764041.4 0.01764041.4 0.01764041.4 0.01764041.4 0.01764041.4 0.01764041.4 0.01764041.4 0.01764041.4 0.01764041.4 0.01764041.4 0.01764041.4 0.01764041.4 0.01766041.4 0.01766041.4 0.01766041.4 0.01766041.4 0	100% spiked-3	0.1016	2	1661582.263	1404991.6	1.182627899	4.578242553	4.59884769	91.9		4.229798096	4.145790169	82.9	
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236804.1713 1355419.6 1.509543014 5.578866899 5.599472035 9.33 5.315582714 5.231574787 236804.1713 1355290.6 0.017472575 0.127761056 6.53840105 0.53840105 24510.1.056 1371981.5 0.0178664 0.132643687 6.542003578 0.542003578 24551.45472 1361540.6 0.017811775 0.134388682 6.54136714 90.6 91.4 5.429691814 4890550718 249992.1019 1404841.6 0.35590639 5.572843122 5.44124533 90.6 91.4 5.429691814 4890550718 499992.1019 1404841.6 0.35590639 5.57284422 5.54746044 90.6 5.429691814 4890550718 49998.202 140230 1.25160353 2.02888602 2.022690845 10.11 98.3 18.74462798 18.0559285 1696093.257 1404991.6 1.20719103 19.59595533 19.46435752 92.9 20.98884901 20.44970791 195234.502 1377954.4 1.42173442 2.22205663 2.21604888 92.9<	120% spiked-2	0.1028	9	1926354.346	1377556.2	1.398385305	5.250679357	5.271284493	87.8		4.929605604	4.845597677	80.7	
23680.41713 1355290.6 0.017472575 0.127761056 0.132643687 0.53840105 0.53840105 24101.1056 1371981.5 0.01756664 0.132643687 0.53840105 0.53840105 2425.145472 1361540.6 0.017811775 0.134388682 0.542003578 0.542003578 499992.1019 1404841.6 0.35590639 5.572843142 5.44124533 90.6 91.4 5.429691814 4890550718 499992.1019 1404841.6 0.35590639 5.572843142 5.44124533 90.6 91.4 5.429691814 4890550718 499908.5082 142224 0.356986881 5.572964923 5.44136714 90.6 5.359493131 4820352036 510695.344 14063762 1.25604353 2.056444044 92.7 5.545493131 4820352036 1696093.257 1404991.6 1.20719103 19.59595533 19.46435752 97.3 18.744629 17.5947226 195224.56.2 1371792.4 1.421734442 22.29205663 22.1604588 9.0 18.0744821 17.5347342	120% spiked-3	0.1028	9		1355419.6	1.509543014	5.578866899	5.599472035	93.3		5.315582714	5.231574787	87.1	
2360.41713 1355290.6 0.017472575 0.13764056 0.132643687 0.537018659 24101.1056 1371981.5 0.0175664 0.132643687 0.0175664 0.132643687 0.537018659 2425.145472 1361540.6 0.017781175 0.13488682 5.572843142 5.44124533 90.6 91.4 5.420991814 4.890550718 499998.1091 1404841.6 0.355996881 5.572964923 5.44136714 90.6 91.4 5.49691814 4.890550718 510695.344 140230 1.25516035 2.035850626 2.022690845 101.1 98.3 18.746279 18.2054868 1691073.777 1387849.2 1.2071913 19.2512626 96.4 92.7 5.545134081 5.05992985 1691073.777 1387849.2 1.20719103 19.5559533 19.44435552 97.3 18.0744821 17.53534101 1950324.530.5 1.207192.4 1.42179442 2.22205663 22.16045882 92.3 92.9 20.9884991 17.53534101 1992433.05 1355419.6 1.449975125	3)Accuracy in the	quantificati	on of Ni											
24101.1056 1371981.5 0.01756664 0.132843687 24251.45472 1361540.6 0.017811775 0.13488682 0.542003578 0.542003578 24251.45472 1361540.6 0.017811775 0.134388682 0.441245333 90.6 91.4 5.429691814 4.890550718 499908.5082 1424294 0.35590639 5.572843142 5.41246714 90.6 5.359493131 4.820352036 510695.344 1403762 0.363804793 5.696343853 5.564746044 92.7 5.359493131 4.820352036 510695.344 1406376 0.21888248 19.222828662 20.22690845 101.1 98.3 18.74462798 18.20549688 1691073.77 1387849.2 1.20719103 19.59595533 19.46435752 97.3 92.9 20.98884901 20.44970791 1950324.502 1371792.4 1.421734442 22.22205663 22.16045882 92.3 92.9 20.98884901 20.44970791 1944821.221 1377556.2 1.411790837 22.22874317 22.6449045 94.3 21.691593	As such-1	0.1019	0	23680.41713	1355290.6	0.017472575	0.127761056				0.537018659			
2425145472 1361540.6 0.017811775 0.134388682 0.542003578 0.542003578 499992.1019 1404841.6 0.35590639 5.572843142 5.441245333 90.6 91.4 5.429691814 4.890550718 499992.1019 1404841.6 0.35590639 5.572843142 5.441245333 90.6 91.4 5.429691814 4.890550718 510695.344 1403762 0.363804793 5.696343853 5.54136044 92.7 5.545134081 5.005992985 176504.143 1406230 1.255160353 20.325690845 101.1 98.3 18.134681 18.05496888 1696093.257 1404991.6 1.20719103 19.59595533 19.46435752 97.3 18.0744821 17.53534101 1950324.502 1371792.4 1.421734442 22.22205663 22.16045882 92.3 92.9 20.98884901 20.4495726 1952433.095 1355419.6 1.469975125 22.77650231 22.6449045 94.3 21.69159333 21.15245224 196244821.221 1377556.2 1.449946 92.3	As such-2	0.1019	0	24101.1056	1371981.5	0.01756664	0.132643687				0.53840105			
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5.10695.344 1403762 0.363804793 5.696434853 5.564746044 92.7 5.545134081 5.005992985 1765044.143 1406230 1.255160353 20.35850626 20.22690845 101.1 98.3 18.74462798 18.20548688 1691073.77 1387849.2 1.218485248 19.42282407 19.2912262 96.4 18.1338637 17.594722 1696093.27 1.20719103 19.5595533 19.46435752 97.3 18.0744821 17.5534101 19503.24.502 1.371752.4 1.421774442 22.22905663 22.10645882 92.9 20.98884901 20.44970791 1994821.221 1377556.2 1.4117790837 22.22874317 22.09714536 92.0 20.98884901 20.44970791 1992433.095 1355419.6 1.469975125 22.77650231 22.6449045 94.3 21.6915933 21.15245224 130.1355718 1355290.6 0.0000960 -0.008919108 20.008919108 0.00020084912 0.0002084912 0.0002084912 0.0002084912 0.00020084912 0.0002084912 0.0002084912	LOQ spiked-2	0.1034	9	499908.5082	1424294	0.350986881	5.572964923	5.441367114	90.6		5.359493131	4.820352036	80.3	
1765044.14314062301.25516035320.3289062620.22690845101.198.318.746279818.20486881691073.771387849.21.21848524819.4228240719.2912262696.418.13863717.5947221696093.2771387849.21.2071910319.595953319.4643575297.392.920.9888490120.448211950324.5021.371792.41.42173444222.2920566322.1604588292.392.920.8888490120.44821194821.2211.377556.21.41179083722.2287431722.0971453692.020.8888490120.48554821992433.0951.355419.61.46997512522.7765023122.644904594.321.6915933321.15245224130.13557181.355290.60.0009960-0.0089191080.002096024580.0020849120.002084912100.10357791.464841.60.00501770.1463323380.155403303103.6104429440150.14429440157369.681997140.37620.142048170.142048170.14242944050.1378625570.1344294105	LOQ spiked-3	0.1034	9	510695.344	1403762	0.363804793	5.696343853	5.564746044	92.7	0	5.545134081	5.005992985	83.4	0
1691073.777 1387849.2 1.218485248 1942282407 19.29122626 96.4 18.1338637 17.5947226 1696093.257 1404991.6 1.20719103 19.59595533 19.46435752 97.3 18.0744821 17.53534101 1950324.502 1371792.4 1.421734442 22.29205663 22.16045882 92.9 20.98884901 20.449070791 1944821.221 1377556.2 1.411790837 22.2874317 22.09714536 92.0 20.84399592 20.30485482 1992433.095 1355419.6 1.469975125 22.77650231 22.6449045 94.3 21.69159333 21.15245524 130.1355718 1355290.6 0.0000960 -0.0086919108 20.002084912 0.002084912 0.002084912 140.1459446 1371981.5 0.0000735 -0.009602458 0.146332338 0.155403303 103.6 104.3 0.141572772 0.144294105 7049.056517 14404841.6 0.005177 0.142621315 10.84 0.1442944 0.1442944 0.1424294 0.1442944105 0.1442944105 858.82337	100% spiked-1	0.1018	07	1/65044.143	1406230	1.255160353	70.35850626	70.72690845	101.1	98.3	18./4462/98	18.20548688	91.0	88.9
1696093.257 1404991.6 1.20719103 19,59595533 19,40435752 97.3 18,0744821 17,53534101 1950324.502 1371792.4 1,421734442 22,22050563 22,16045882 92.9 20,9884901 20,4970791 194821.221 1377556.2 1,411790837 22,22874317 22,09714536 92.0 20,884399592 20,30485482 1992433.095 1355419.6 1,469975125 22,77650231 22,6449045 94.3 21,69159333 21,15245524 130.1355718 1355290.6 0.0000960 -0.0086919108 0.0012084912 0.002084912 0.002084912 140.1459446 1371981.5 0.0000735 -0.009602458 0.146332338 0.155403303 103.6 104.3 0.141572772 0.139821577 7049.056517 14404841.6 0.0050177 0.146332338 0.152621315 108.4 0.1460453 0.144294105 7859.681097 1403362 0.14165783 10.08 0.13761357 0.1341357	100% spiked-2	0.1022	20	1691073.777	1387849.2	1.218485248	19.42282407	19.29122626	96.4		18.1338637	17.5947226	87.9	0.98
1950324.502 1371792.4 1.421734442 22.29205663 22.16045882 92.3 92.9 20.98884901 20.44970791 194821.221 1377556.2 1.411790837 22.22874317 22.09714536 92.0 20.84399592 20.30485482 1992433.095 1355419.6 1.469975125 22.77650231 22.6449045 94.3 21.6915933 21.15245224 130.1355718 1355290.6 0.0000960 -0.0086919108 0.002084912 0.002084912 140.1459446 1371981.5 0.0001021 -0.009602458 0.0050458 0.044532338 0.155403303 103.6 104.3 0.141572772 0.139821577 7049.056517 1404841.6 0.005177 0.146332338 0.152621315 10.84 0.1460453 0.144294105 6858.82237 1403762 0.048860 0.14204817 0.15116578 10.08 0.137611357	100% spiked-3	0.1016	70	1696093.257	1404991.6	1.20719103	19.59595533	19.46435752	97.3		18.0744821	17.53534101	9.78	
1944821.221 1377556.2 1.411790837 22.22874317 22.09714536 92.0 20.84399592 20.30485482 1992433.095 1355419.6 1.469975125 22.77650231 22.6449045 94.3 21.69159333 21.15245224 130.1355718 1355290.6 0.0000960 -0.0086919108 0.002084912 0.002084912 140.1459446 1371981.5 0.00000735 -0.009602458 0.001260291 7049.056517 1404841.6 0.0050177 0.146332338 0.155403303 103.6 104.3 0.141572772 0.139821577 7369.681097 1424294 0.0051743 0.14555035 0.162621315 100.8 0.137862552 0.13411357 6858.822337 1403762 0.0048860 0.14204817 0.1413578 0.004204105	120% spiked-1	0.1028	24	1950324.502	1371792.4	1.421734442	22.29205663	22.16045882	92.3	92.9	20.98884901	20.44970791	85.2	
1992433.095 1355419.6 1.469975125 22.77650231 22.6449045 94.3 21.69159333 21.15245224 130.1355718 1355290.6 0.0000960 -0.0086919108 0.002084912 0.002084912 140.1459446 1371981.5 0.00000735 -0.009602458 0.001260291 100.1055577 1404841.6 0.0050177 0.146332338 0.155403303 103.6 104.3 0.141572772 0.139821577 7369.681097 1424294 0.0051743 0.155403303 100.8 0.1469453 0.144294105 6858822337 1403762 0.0048860 0.142094817 0.151165783 10.08 0.137862552 0.1378111357	120% spiked-2	0.1028	24	1944821.221	1377556.2	1.411790837	22.22874317	22.09714536	92.0		20.84399592	20.30485482	84.6	
130.1355718 1355290.6 0.0000960 -0.008919108 0.002084912 140.1459446 1371981.5 0.0001021 -0.008691331 0.002084912 100.1037279 1361540.6 0.0000735 -0.009602458 0.0155403303 103.6 104.3 0.141572772 0.139821577 7049.056517 1404841.6 0.0051743 0.1555035 0.162621315 108.4 0.1460453 0.144294105 6858.82237 1403762 0.0048860 0.14204817 0.15165783 10.08 0.137862552 0.13411357	120% spiked-3	0.1028	. 24		1355419.6	1.469975125	22.77650231	22.6449045	94.3		21.69159333	21.15245224	88.1	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	4) Accuracy in the	quantincat	ion or C		7 0000		0000000				100000			
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	As such-1	0.1019	0	130.1355/18	1355290.6	0.0000960	-0.008919108				0.001908381			
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	As such-2	0.1019	0 0	140.1459446	13/1981.5	0.0001021	-0.008691331				0.002084912			
.1 0.10342 0.15 7049,030301 1404641.0 0.00301// 0.146322336 0.133403303 103.0 104.3 0.146041.0 0.13966213// 2 0.1034 0.15 6858 822337 1424294 0.00051743 0.1557635 0.162621315 1084 0.15 6858 822337 1403762 0.0048860 0.14504817 0.15167583 0.137862552 0.136141357	AS SUCIL-5	0.1019) (7040.057279	1301340.0	0.0000/33	-0.009002430	0.157402202	1036	0 7 0 7	0.001260291	7771000110	7 7 7	7 60
.z 0.1034 0.15 6858822337 1403762 0.0048860 0.142094817 0.151165783 1008 0.137862552 0.136111357	LOQ spiked-1	0.10342	0.15 0.15	7369 681097	1404641.0	0.0050177	0.140332330	0.155405505	108.4	104.3	0.1413/2//2	0.1396213//	7.5.6 96.7	73.4
	LOQ spined-2	0.1034	0.15	6858822337	1424294	0.0031/43	0.142094817	0.151165783	100.4		0.137862552	0.136111357	2.06	

Table S3. (Continued)

100% spiked-1 0.1018 0.5 100% spiked-2 0.1022 0.5 100% spiked-3 0.1016 0.5 120% spiked-1 0.1028 0.6 120% spiked-3 0.1028 0.6 120% spiked-3 0.1028 0.6 120% spiked-3 0.1028 0.6 As such-1 0.1019 0 As such-2 0.1019 0 As such-3 0.1019 0 LOQ spiked-1 0.10342 0.9 LOQ spiked-2 0.1034 0.9 LOQ spiked-1 0.1038 3.6 100% spiked-1 0.1018 3 100% spiked-1 0.1018 3.6 120% spiked-1 0.1028 3.6 120% spiked-2 0.1028 3.6 120% spiked-1 0.1028 3.6 120% spiked-1 0.1038 0.1 As such-1 0.1019 0 As such-2 0.1019 0 As such-3 0.1019 0 LOQ spiked-2 0.1034 0.15 LOQ spiked-2 0.1034 0.15 LOQ spiked-2 0.1034 0.15 LOQ spiked-2 0.1018 0.5	0.55 0.66 0.66 0.06 0.09 0.09 0.09 0.09 3.36 3.36 0.09 0.09 0.09 0.09 0.09 0.09 0.09	23627.92 23531.11 23263.61333 26352.3485 24657.64353 25710.55924 6361.783 6081.58 6051.666 23041.11 22961.07 74766.46 75966.35 75966.35 83600.18 885774.09	1406230 1387849.2 1387849.2 1371792.4 1377556.2 1355419.6 10267124 10194306 10335583 10267124 10194306 1033583 10293788 10293788 10293788 1029378 10335541 10330564 10317603	0.0168023 0.0169551 0.0165578 0.0192102 0.0178996 0.0189687 0.000585 0.000244 0.0002244 0.002244 0.002244 0.002244 0.002344 0.002344 0.002344 0.007343 0.007313 0.007313	0.526269663 0.518991659 0.518991659 0.582599654 0.54437567 0.568124131 0.274983 0.263729 0.263729 0.263729 0.931007 0.889565 0.928019 3.025368 3.05587 3.05587	0.535340629 0.531084501 0.528062624 0.59167062 0.553446636 0.577195096 0.577195096 0.663927 0.663927 0.660939 2.758288 2.758288	107.1 106.2 105.6 98.6 92.2 96.2 73.7 69.1 73.7 69.1 73.4	106.3 95.7 72.1	0.483633435 0.486128572 0.477521922 0.54768317 0.510259788 0.540788744 0.196491 0.186584 0.181813	0.48188224 0.484377377 0.475770727 0.545931975 0.508508594 0.539037549	96.4 96.9 95.2 91.0	96.1
100% spiked-1 0.1018 100% spiked-2 0.1022 100% spiked-3 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1019 As such-1 0.1019 As such-2 0.1019 As such-3 0.1019 As such-3 0.1019 LOQ spiked-1 0.1034 LOQ spiked-1 0.1024 LOQ spiked-2 0.1024 100% spiked-1 0.1028 120% spiked-2 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1028 120% spiked-1 0.1028 120% spiked-1 0.1034 120% spiked-1 0.1038 120% spiked-2 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1038 120% spiked-1 0.1019 As such-1 0.1019 As such-2 0.1034 LOQ spiked-1 0.10342 LOQ spiked-2 0.1034 100% spiked-1 0.1034 100% spiked-1 0.1018	0.5 0.5 0.6 0.6 0.0 0.0 0.9 0.9 3.3 3.6 0.9 0.9 0.9 0.9	23527.92 23531.11 23263.61333 26352.3485 2657.6.353 25710.55924 6081.58 6081.58 6051.66 23041.11 21989.56 22961.07 74766.46 75966.35 75966.35 83600.18 885774.09			0.526269663 0.522013536 0.518991659 0.582599654 0.54437567 0.274983 0.263729 0.263729 0.262528 0.931007 0.889565 0.928019 3.025368 3.061578 3.056587	0.535.340.629 0.531084501 0.528062624 0.59167062 0.553446636 0.577195096 0.663927 0.663927 0.663928 2.758288 2.758288	106.2 106.2 106.2 98.6 92.2 96.2 73.7 69.1 73.4 91.9	95.7	0.485033435 0.486128572 0.477521922 0.54768317 0.510259788 0.540788744 0.196491 0.186584 0.181813	0.48188224 0.484377377 0.475770727 0.508508594 0.539037549	96.4 96.9 95.2 91.0	96.1
100% spiked-2 0.1024 100% spiked-3 0.1016 120% spiked-3 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1019 As such-3 0.1019 As such-3 0.1019 LOQ spiked-1 0.1034 LOQ spiked-1 0.1034 100% spiked-2 0.1024 100% spiked-2 0.1022 100% spiked-2 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1028 120% spiked-1 0.1028 120% spiked-1 0.1028 120% spiked-1 0.1028 120% spiked-2 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1038 100% spiked-3 0.1038 100% spiked-1 0.1019 As such-1 0.1019 As such-1 0.10342 LOQ spiked-3 0.1034 100% spiked-2 0.1034 100% spiked-1 0.1034 100% spiked-1 0.1034	0.5 0.6 0.6 0.0 0.0 0.0 0.9 0.9 3.3 3.6 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0	23263.111 23263.61333 24657.64353 25710.55924 6361.783 6081.58 6051.666 23041.11 21989.56 22961.07 74766.46 75966.35 75966.35 83600.18 885774.09			0.558124131 0.558124131 0.568124131 0.274983 0.263729 0.263729 0.262528 0.931007 0.989565 0.928019 3.025368 3.061578 3.056587	0.528062624 0.528062624 0.553446636 0.577195096 0.663927 0.663927 0.622485 0.660939 2.758288 2.758288	100.2 100.6 98.6 92.2 96.2 73.7 69.1 73.4 91.9	95.7 72.1	0.477521922 0.5477521922 0.540788744 0.540788744 0.196491 0.186584 0.181813	0.539037549	95.2 95.2 91.0	1
120% spiked-1 0.1028 120% spiked-2 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1019 As such-3 0.1019 As such-3 0.1019 LOQ spiked-1 0.1034 LOQ spiked-1 0.1034 LOQ spiked-2 0.1024 100% spiked-1 0.1028 120% spiked-2 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1028 120% spiked-1 0.1028 120% spiked-1 0.1028 120% spiked-2 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1034 LOQ spiked-1 0.10342 LOQ spiked-3 0.1019 As such-1 0.10342 LOQ spiked-3 0.1034 LOQ spiked-1 0.1034 LOQ spiked-1 0.1034 100% spiked-2 0.1034 100% spiked-1 0.1018	0.6 0.6 0.6 0.0 0.9 0.9 0.9 3.3 3.6 0.9 0.0 0.9 0.0 0.0 0.0 0.0 0.0 0.0 0.0	24657.64353 24657.64353 25710.55924 6361.783 6081.58 6051.666 23041.11 21989.56 22961.07 74766.46 75966.35 75966.35 83600.18 836774.09			0.582599654 0.568124131 0.274983 0.263729 0.263729 0.262528 0.931007 0.889565 0.928019 3.025368 3.061578 3.056587 3.377631	0.59167062 0.553446636 0.577195096 0.663927 0.622485 0.660939 2.758288 2.758288	98.6 92.2 96.2 96.2 73.7 69.1 73.4 91.9	95.7 72.1	0.540788744 0.540788744 0.196491 0.186584 0.181813	0.539037549	91.0	1
120% spiked-3 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1019 As such-2 0.1019 As such-3 0.1019 LOQ spiked-1 0.1034 LOQ spiked-2 0.1034 LOQ spiked-2 0.1024 LOQ spiked-1 0.1028 120% spiked-1 0.1028 120% spiked-1 0.1028 120% spiked-2 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1028 120% spiked-1 0.1038 120% spiked-2 0.1028 120% spiked-3 0.1038 120% spiked-3 0.1019 As such-1 0.1019 As such-1 0.10342 LOQ spiked-1 0.10342 LOQ spiked-1 0.10342 LOQ spiked-2 0.1034 100% spiked-1 0.1034	0.6 0.6 0.6 0.0 0.9 0.9 0.9 3.3 3.6 0.9 0.0 0.9 0.0 0.0 0.0 0.0 0.0 0.0 0.0	24657.64353 25710.55924 6361.783 6081.58 6051.666 23041.11 21989.56 22961.07 74766.46 75966.35 75966.35 83600.18 836774.09			0.568124131 0.568124131 0.274983 0.263729 0.262528 0.931007 0.889565 0.928019 3.025368 3.061578 3.056587	0.55344663 0.577195096 0.663927 0.622485 0.660939 2.758288 2.758288	92.2 96.2 96.2 73.7 69.1 73.4 91.9	72.1	0.540788744 0.540788744 0.196491 0.186584 0.181813	0.539037549		х х х
120% spiked-3 5) Accuracy in the quantification As such-1 As such-2 Col. 0.1019 As such-3 Col. 0.1019 As such-3 Col. 0.1019 LOQ spiked-1 CoQ spiked-1 CoQ spiked-2 CoQ spiked-2 CoQ spiked-3 Col. 0.1024 CoQ spiked-1 CoQ spiked-1 CoQ spiked-1 CoQ spiked-1 CoQ spiked-2 Col. 0.1028 Col. 0.1038 Col. 0.1034 Col.	0.6	25710.55924 6361.783 6081.58 6051.666 23041.11 21989.56 22961.07 74766.46 75966.35 75966.35 83600.18 885774.09			0.568124131 0.274983 0.263729 0.262528 0.931007 0.889565 0.928019 3.025368 3.061578 3.056587 3.347631	0.577195096 0.663927 0.660939 2.758288 2.758288	96.2 73.7 69.1 73.4 91.9 93.1	72.1	0.540788744 0.196491 0.186584 0.181813 0.881271	0.539037549	84.8	
5) Accuracy in the quantification As such-1 As such-2 O.1019 As such-3 O.1019 LOQ spiked-1 LOQ spiked-1 LOQ spiked-2 LOQ spiked-2 LOQ spiked-2 LOQ spiked-3 O.1034 LOQ spiked-3 O.1028 100% spiked-1 O.00% spiked-1 O.00% spiked-1 C.00% spiked-1 C.100% spiked-1 C.100% spiked-1 C.100% spiked-2 C.1028 C.1034 C.1028 C.1028 C.1034 C.1028 C.1028 C.1028 C.1028 C.1028 C.1028 C.1028	on of Hg 0 0 0 0 0 0.9 0.9 0.9 3.3 3.6 3.6 0.0 on of Pb 0	6361.783 6081.58 6051.666 23041.11 22961.07 74766.46 75966.35 75966.35 83600.18 85774.09			0.274983 0.263729 0.262528 0.931007 0.889565 0.928019 3.025368 3.061578 3.056587 3.3747631	0.663927 0.622485 0.660939 2.758288 2.754498	73.7 69.1 73.4 91.9 93.1	72.1	0.196491 0.186584 0.181813 0.881271		868	
As such-1 As such-2 As such-3 Such-3 LOQ spiked-1 LOQ spiked-2 LOQ spiked-2 LOQ spiked-3 LOQ spiked-3 LOQ spiked-1 LOQ spiked-3 LOOS spiked-1 LOOS spiked-1 LOOS spiked-1 LOOS spiked-3 LOOS spiked-1 LOOQ spiked-1 LOOQ spiked-1 LOOQ spiked-1 LOOS spiked-3 LOOS spiked-3 LOOS spiked-3 LOOS spiked-1 LOOS spiked-2 LOOS spiked-3	0 0 0 0.9 0.9 0.9 3.6 3.6 0 0 0 Pb	6361.783 6081.58 6051.666 23041.11 21989.56 22961.07 74766.46 75966.35 75966.35 83600.18 85774.09			0.274983 0.263729 0.262528 0.931007 0.889565 0.928019 3.025368 3.061578 3.056587 3.347631	0.663927 0.622485 0.660939 2.758288 2.754498	73.7 69.1 73.4 91.9 93.1	72.1	0.196491 0.186584 0.181813 0.881271			
As such-2 As such-3 As such-3 LOQ spiked-1 LOQ spiked-2 LOQ spiked-3 LOQ spiked-3 LOQ spiked-3 LOQ spiked-1 LOOS spiked-1 LOOS spiked-2 LOOS spiked-2 LOOS spiked-2 LOOS spiked-2 LOOS spiked-3 LOOS spiked-1 LOOS spiked-1 LOOS spiked-1 LOOS spiked-1 LOOS spiked-1 LOOS spiked-1 LOOS spiked-2 LOOS spiked-2 LOOS spiked-1 LOOS spiked-1 LOOS spiked-1 LOOS spiked-1 LOOS spiked-1 LOOS spiked-1 LOOS spiked-2 LOOS spiked-2 LOOS spiked-2 LOOS spiked-2 LOOS spiked-2 LOOS spiked-2 LOOS spiked-1	0 0.9 0.9 0.9 3 3 3 3.6 3.6 0 on of Pb 0	6081.58 6051.666 23041.11 21989.56 22961.07 74766.46 75966.35 75393.62 83600.18 85774.09			0.263729 0.262528 0.931007 0.889565 0.928019 3.025368 3.061578 3.3656587 3.37631	0.663927 0.622485 0.660939 2.758288 2.794498	73.7 69.1 73.4 91.9 93.1	72.1	0.186584 0.181813 0.881271			
As such-3 1.00 spiked-1 1.00 spiked-2 1.00 spiked-3 1.00% spiked-3 1.00% spiked-1 1.00% spiked-1 1.00% spiked-1 1.00% spiked-2 1.00% spiked-3 1.00% spiked-1 1.00% spiked-1 1.00% spiked-1 1.00% spiked-1 1.00% spiked-1 1.00% spiked-1 1.00% spiked-2 1.00% spiked-1 1.00% spiked-1 1.00% spiked-2 1.00% spiked-1 1.00% spiked-2 1.00% spiked-1 1.00% spiked-2 1.00% spiked-2 1.00% spiked-2 1.00% spiked-2 1.00% spiked-2 1.00% spiked-2	0 0.9 0.9 3 3 3 3.6 3.6 0 on of Pb 0	6051.666 23041.11 21989.56 22961.07 74766.46 75966.35 75393.62 83600.18 85774.09			0.262528 0.931007 0.889565 0.928019 3.025368 3.061578 3.056587 3.3747631	0.663927 0.622485 0.660939 2.758288 2.794498	73.7 69.1 73.4 91.9 93.1	72.1	0.181813 0.881271			
LOQ spiked-1 0.10342 LOQ spiked-2 0.1034 LOQ spiked-3 0.1034 100% spiked-1 0.1018 100% spiked-2 0.1022 100% spiked-1 0.1028 120% spiked-3 0.1028 120% spiked-3 0.1028 6) Accuracy in the quantificati As such-1 0.1019 As such-2 0.1019 As such-2 0.1019 LOQ spiked-1 0.1034 LOQ spiked-2 0.1034 LOQ spiked-2 0.1034 LOQ spiked-2 0.1034 100% spiked-2 0.1034	0.9 0.9 0.9 3 3 3.6 3.6 on of Pb 0	23041.11 21989.56 22961.07 74766.46 75966.35 75393.62 885774.09 83076.38			0.931007 0.889565 0.928019 3.025368 3.061578 3.056587 3.347631	0.663927 0.622485 0.660939 2.758288 2.794498	73.7 69.1 73.4 91.9 93.1	72.1	0.881271			
LOQ spiked-2 0.1034 LOQ spiked-3 0.1034 100% spiked-1 0.1018 100% spiked-2 0.1022 100% spiked-3 0.1028 120% spiked-1 0.1028 120% spiked-1 0.1028 6) Accuracy in the quantificati As such-1 0.1019 As such-2 0.1019 As such-2 0.1019 LOQ spiked-1 0.1034 LOQ spiked-1 0.1034 LOQ spiked-2 0.1034 LOQ spiked-2 0.1034 LOQ spiked-2 0.1034 100% spiked-2 0.1028	0.9 0.9 3 3 3.6 3.6 on of Pb 0	21989.56 22961.07 74766.46 75966.35 75393.62 83600.18 85774.09 83076.38			0.928019 0.928019 3.025368 3.061578 3.056587 3.347631	0.622485 0.660939 2.758288 2.794498	69.1 73.4 91.9 93.1	92.7	7117700	0.692975	6.92	75.3
LOQ spiked-3 0.1034 100% spiked-1 0.1018 100% spiked-2 0.1022 100% spiked-3 0.1016 120% spiked-1 0.1028 120% spiked-2 0.1028 120% spiked-3 0.1028 6) Accuracy in the quantificati As such-1 0.1019 As such-2 0.1019 As such-3 0.1019 LOQ spiked-1 0.1034 LOQ spiked-1 0.1034 100% spiked-1 0.1034 100% spiked-1 0.1034	0.9 3 3 3 3.6 3.6 on of Pb 0	22961.07 74766.46 75966.35 75393.62 83600.18 85774.09			0.928019 3.025368 3.061578 3.056587 3.347631	0.660939 2.758288 2.794498	73.4 91.9 93.1	92.7	0.844556	0.65626	72.9	
100% spiked-1 0.1018 100% spiked-2 0.1022 100% spiked-3 0.1016 120% spiked-1 0.1028 120% spiked-2 0.1028 120% spiked-3 0.1028 6) Accuracy in the quantificati As such-1 0.1019 As such-2 0.1019 As such-3 0.1019 LOQ spiked-1 0.1034 LOQ spiked-2 0.1034 100% spiked-1 0.1018 100% spiked-1 0.1018	3 3 3.6 3.6 0.00 of Pb	74766.46 75966.35 75393.62 83600.18 85774.09 83076.38			3.025368 3.061578 3.056587 3.347631	2.758288 2.794498	91.9 93.1	92.7	0.871779	0.683483	75.9	
100% spiked-2 0.1022 100% spiked-3 0.1016 120% spiked-1 0.1028 120% spiked-2 0.1028 120% spiked-3 0.1028 6) Accuracy in the quantificati As such-1 0.1019 As such-3 0.1019 As such-3 0.1019 LOQ spiked-1 0.1034 LOQ spiked-2 0.1034 100% spiked-1 0.1018 100% spiked-2 0.1034	3 3 3.6 3.6 on of Pb 0	75966.35 75393.62 83600.18 85774.09 83076.38			3.061578 3.056587 3.347631	2.794498	93.1	;	3.053693	2.865397	95.5	96.2
100% spiked-3 0.1016 120% spiked-1 0.1028 120% spiked-2 0.1028 120% spiked-3 0.1028 6) Accuracy in the quantification of second of such-1 0.1019 As such-3 0.1019 As such-3 0.1019 LOQ spiked-1 0.1034 LOQ spiked-2 0.1034 100% spiked-1 0.1034 100% spiked-1 0.1038 100% spiked-2 0.1022	3 3.6 3.6 3.6 on of Pb 0	75393.62 83600.18 85774.09 83076.38			3.056587 3.347631				3.089236	2.90094	9.96	
120% spiked-1 0.1028 120% spiked-2 0.1028 120% spiked-3 0.1028 6) Accuracy in the quantificati. As such-1 0.1019 As such-3 0.1019 As such-3 0.1019 LOQ spiked-1 0.1034 LOQ spiked-2 0.1034 LOQ spiked-2 0.1034 100% spiked-1 0.1018	3.6 3.6 3.6 on of Pb 0	83600.18 85774.09 83076.38			3.347631	2.789507	92.9		3.081096	2.8928	96.4	
120% spiked-2 0.1028 120% spiked-3 0.1028 6) Accuracy in the quantification of the such-1 0.1019 As such-2 0.1019 As such-3 0.1019 LOQ spiked-1 0.10342 LOQ spiked-2 0.1034 LOQ spiked-2 0.1034 100% spiked-1 0.1018 100% spiked-1 0.1018	3.6 3.6 on of Pb 0	85774.09 83076.38			017070	3.080551	85,5	86.2	3.381456	3,19316	98.6	89.2
120% spiked-3 0.1028 6) Accuracy in the quantification As such-1 0.1019 As such-2 0.1019 As such-3 0.1019 LOQ spiked-1 0.10342 LOQ spiked-2 0.1034 LOQ spiked-3 0.1034 100% spiked-1 0.1018 100% spiked-2 0.1028	3.6 on of Pb 0 0	83076.38			0.40410	3.1671	87.9		3.468461	3.280165	91.1	
6) Accuracy in the quantification As such-1 As such-2 As such-3 LOQ spiked-1 LOQ spiked-2 LOQ spiked-2 LOQ spiked-3 LOQ spiked-3 LOQ spiked-1 LOOW spiked-1 LOOW spiked-1 LOOW spiked-1 LOOW spiked-2 LOOW spiked-3	on of Pb 0 0				3.326777	3.059697	84.9		3.354568	3.166272	87.9	
As such-1 0.1019 As such-2 0.1019 As such-3 0.1019 LOQ spiked-1 0.10342 LOQ spiked-2 0.1034 LOQ spiked-3 0.1034 100% spiked-1 0.1018	0											
:d-1 :d-2 :d-3 ked-1 ked-2	0	193738 0744	10267123 5	0.018869752	0.693406866				0.707248952			
:d-1 :d-2 :d-3 :ked-1 :ked-2	•				0.694170264				0.71328656			
7 7	0				0.68417652				0.692768998			
7.7	0 15		L		0.830883155	0 140298605	93.5	88 4	0.850729887	0 146295051	975	92.2
7 7	0.15	LC			0.822284861	0.131700311	87.8		0.847961813	0.143526976	95.6	1
7 7	0.15				0.816262497	0.125677947	83.7		0.829706213	0.125271376	83.5	
	0.5		6		1.185795053	0.495210503	0.66	99.1	1.217094536	0.5126597	102.5	102.4
	0.5	267	3	131692927	1.172019059	0.481434509	96.2		1.20188993	0.497455094	99.4	
100% spiked-3 0.1016	0.5			32262088	1.201227286	0.510642736	102.1		1.231164842	0.526730006	105.3	
120% spiked-1 0.1028	9.0	346201.5147	10317602.59	0.033554453	1.236122661	0.545538111	6.06	89.1	1.266562009	0.562127172	93.6	91.6
	9.0			0.033121151	1.220977118	0.530392568	88.3		1.249875602	0.545440765	6.06	
120% spiked-3 0.1028	9.0	341153.3415	10333479.04	0.033014374	1.21795195	0.5273674	87.8		1.245763659	0.541328822	90.2	
7) Accuracy in the quantification of As	on of As											
	0				0.423035064				0.426382909			
	0	വ			0.403585945				0.403313918			
	0				0.421790394				0.423292193			
	0.45		9:	7	0.849667426	0.433530292	96.3	104.8	0.795505777	0.37784277	83.9	8.06
	0.45	2			0.887966972	0.471829838	104.8		0.818755671	0.401092665	89.1	
	0.45				0.926368892	0.510231757	113.3		0.865285974	0.447622968	99.4	
	1.5			67100033	2.323712013	1.907574879	127.1	116.7	2.122106427	1.704443421	113.6	104.8
	1.5				2.138714171	1.722577036	114.8		1.981286186	1.563623179	104.2	
	1.5	21		58943562	2.040038881	1.623901747	108.2		1.868450129	1.450787123	2.96	
	1.8				2.315765992	1.899628858	105.5	106.1	2.167616201	1.749953195	97.2	98.1
	1.8				2.234495913	1.818358779	101.0		2.0838877	1.666224694	92.5	
120% spiked-3 0.1028	1.8	99433.14	1355419.6	0.073359674	2.426362306	2.010225172	111.6		2.297026655	1.879363648	104.4	

Table S4. Precision test of toxic heavy metals (V, Co, Ni, Cd, Hg, Pd, As) from Q-ICP-MS analysis.

			- 4	ada maa	•		•				ode we means were and		
						Result (ppm)	Mean Result (ppm)	SD	% RSD	Result (ppm)	Mean Result (ppm)	SD	% RSD
1) Precision in the quantification of V	uantification of V	,	20277 57000	1255200 6	0.0020200	0.07	0.07	6000	7 1 7	700	0.37	2000	170
As such-1	0.1019	0 0	87126.67839	1371981.5	0.063504266	0.08	0.07	0.003	4:30	0.28	0.27	0.002	0.01
As such-3	0.1019	0	85818.70966	1361540.6	0.063030592	0.07				0.27			
100% spiked-1	0.1018	10	3262920.367	1406230	2.320331928	9.44	8.967	0.285	3.17	8.70	8.27	0.286	3.45
100% spiked-2	0.1022	10	3114073.807	1387849.2	2.243812805	8.96				8.38			
100% spiked-3	0.1016	10	3096/00.06	1404991.6	2.2040/016	9.97				87.8			
100% spiked-4	0.1023	10	3023604.33	1420923.1	2.144333203	0.00				7 92			
100% spiked-6	0.1008	10	3110912.56	1405752	2.21298818	9.08				8:38			
Э	quantification of Co	0,											
As such-1	0.1019	0	3620.59626	1355290.6	0.002671454	-0.02	-0.02	0.001	-3.33	80.0	80.0	0.001	0.71
As such-2	0.1019	0	3930.673464	1371981.5	0.002864961	-0.02				80.0			
As such-3	0.1019	0	3440.551401	1361540.6	0.002526955	-0.02				80.0			
100% spiked-1	0.1018	ا کا	1664934.767	1406230	1.183970451	4.58	4.498	0.097	2.15	4.23	4.15	0.106	2.56
100% spiked-2	0.1022	Λι	16/4632.31/	138/849.2	1.206638529	4.59				4.29			
100% spiked-3	0.1016	Λι	1661582.263	1404991.6	1.182627899	4.58				4.23			
100% spiked-4	0.1025	Λι	1607591.59	1410965.1	1.139356027	4.39				4.04			
100% spiked-5	0.1031	Λι	164/933./2/	1420922.6	1.159/6319	4.47				4.09			
100% spiked-6	0.1008		15/8386.1/3	1405/52	1.122805568	4.38				4.05			
Aggingh 11 mie c	0.1010		22500 41712	1255200 6	0.017477575	0.10	0.13	0000	7.61	Z 1	7 1 0	000	0 7 0
As such-1	0.1019	0 0	24101 1056	1355290.6	0.01/4/25/5	0.13	0.13	0.003	7.01	0.54	0.54	0.003	0.48
As sucil-2	0.1019	0	24101.1030	12615406	0.01/30004	0.13				0.04			
48 Sucil-3	0.1019	20	176501.43472	1301340.0	1.255160252	20.26	10 504	0.471	2 4.2	10.04	17 00	0.474	261
100 % spiked-1	0.1010	20	1691073777	1387849 2	1.233100333	19.30	10000	1 /1:0	1.1	18.13	17:70	1.5	10.1
100% spiked-2	0.1022	20	1606002 257	1707017.2	1.210403240	10.60				10.07			
100% spiked-3	0.1010	20	1650666 053	1404991.0	1.20/17103	19.00				17.45			
100% spiked-4	0.1025	07	1658666.855	1410965.1 1420022 6	1.1/5554841	18.99				17.45			
100% spiled 6	0.1031	20	1671792 257	1420922.0	1.103707330	10.17				17.05			
100 / Spinea- 0 Stroop	o.routification of C	24 5	107:00/1/01	20/10011	01177011	17:11				67.71			
As curch-1	0.1019	, c	130 1355718	13552906	9 60204E-05	-0.01	-0.01	0000	-5 23	0.00	000	0000	24.79
As such-2	0.1019	0 0	140.1459446	1371981.5	0.000102149	-0.01	100		1	0.00			
As such-3	0.1019	0	100,1037279	1361540.6	7.35224E-05	-0.01				0.00			
100% spiked-1	0.1018	0.5	23627.92	1406230	0.016802315	0.53	0.516	0.011	2.20	0.48	0.47	0.013	2.74
100% spiked-2	0.1022	0.5	23531.11	1387849.2	0.016955091	0.52				0.49			
100% spiked-3	0.1016	0.5	23263.61333	1404991.6	0.016557831	0.52				0.48			
100% spiked-4	0.1025	0.5	22805.77333	1410965.1	0.016163244	0.50				0.46			
100% spiked-5	0.1031	0.5	22725.55667	1420922.6	0.015993522	0.50				0.45			
100% spiked-6	0.1008	0.5	23330.5	1405752	0.016596455	0.52				0.48			
5) Precision in the o	quantification of Hg	Hg											
	0.1019	0	6361.783352	10267123.5	0.000619627	0.27	0.27	0.007	2.57	0.20	0.19	0.007	3.98
As such-2	0.1019	0	6081.58011	10194305.8	0.000596566	0.26				0.19			
As such-3	0.1019	0	6051.66617	10336583	0.000585461	0.26				0.18			

Table S4. (Continued)

Sample	Sample wt. (g) Conc (ppb)		cps	Int. Std. cps	cps ratio	Calculat	Calculation based on cps ratio			Calculati	Calculation based on cps		
						Result	Mean Result (ppm)	SD	% RSD	Result	Mean Result (ppm)	SD	% RSD
						(mdd)				(mdd)			
100% spiked-1	0.1018	3	74766.46	11226846.7	0.006659614	3.03	3.037	0.058	1.92	2.79	2.78	0.068	2.46
100% spiked-2	0.1022	3	75966.35333	11234982.6	0.006761591	3.06				2.83			
100% spiked-3	0.1016	3	75393.62333	11298447.3	0.006672919	3.06				2.81			
100% spiked-4	0.1025	3	76167.69667	11483387.4	0.00663286	3.06				2.76			
100% spiked-5	0.1031	3	73215.23	11446110.9	0.006396516	2.93				2.65			
100% spiked-6	0.1008	3	75624.92667	11325018.6	0.006677687	3.09				2.83			
6) Precision in the quantification of Pb	quantification of P	p											
As such-1	0.1019	0	193738.0744	10267123.5	0.018869752	69.0	69.0	900.0	0.81	0.71	0.70	0.011	1.50
As such-2	0.1019	0	193948.3042	10194305.8	0.019025161	69.0				0.71			
As such-3	0.1019	0	191196.1575	10336583	0.018497037	89.0				69.0			
100% spiked-1	0.1018	0.5	329014.9	11226846.7	0.029306083	1.19	1.195	0.022	1.81	1.11	1.11	0.022	1.95
100% spiked-2	0.1022	0.5	326491.8567	11234982.6	0.02906029	1.17				1.10			
100% spiked-3	0.1016	0.5	332611.2967	11298447.3	0.029438673	1.20				1.12			
100% spiked-4	0.1025	0.5	333765.2767	11483387.4	0.029065054	1.19				1.10			
100% spiked-5	0.1031	0.5	332678.45	11446110.9	0.029064759	1.18				1.09			
100% spiked-6	0.1008	0.5	339113.0467	11325018.6	0.029943708	1.23				1.15			
7) Precision in the) Precision in the quantification of As	S											
As such-1	0.1019	0	18112.29	1355290.6	0.013364138	0.42	0.42	0.011	2.62	0.43	0.42	0.013	3.00
As such-2	0.1019	0	17331.15	1371981.5	0.012632204	0.40				0.40			
As such-3	0.1019	0	18062.3	1361540.6	0.013266075	0.42				0.42			
100% spiked-1	0.1018	1.5	94358.08	1406230	0.067100033	2.32	2.130	0.104	4.87	2.12	1.95	0.098	5.02
100% spiked-2	0.1022	1.5	87272.44	1387849.2	0.06288323	2.14				1.98			
100% spiked-3	0.1016	1.5	82815.21	1404991.6	0.058943562	2.04				1.87			
100% spiked-4	0.1025	1.5	87187.7	1410965.1	0.061792953	2.13				1.94			
100% spiked-5	0.1031	1.5	84163.11	1420922.6	0.059231312	2.04				1.85			
100% spiked-6	0.1008	1.5	84734	1405752	0.060276635	2.10				1.93			