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 µg/L. The quantification limits were in the range of 1.5–60 µg/L. Mean recoveries±standard deviations at different spiking levels were in the range 75.3±2.1–104.9±8.5%. 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 ≤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.

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_3 of analytical grade were purchased from Merck (Darmstadt, Germany). Deionized water was prepared using a Milli-Q 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 HNO_3 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%) 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. 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).

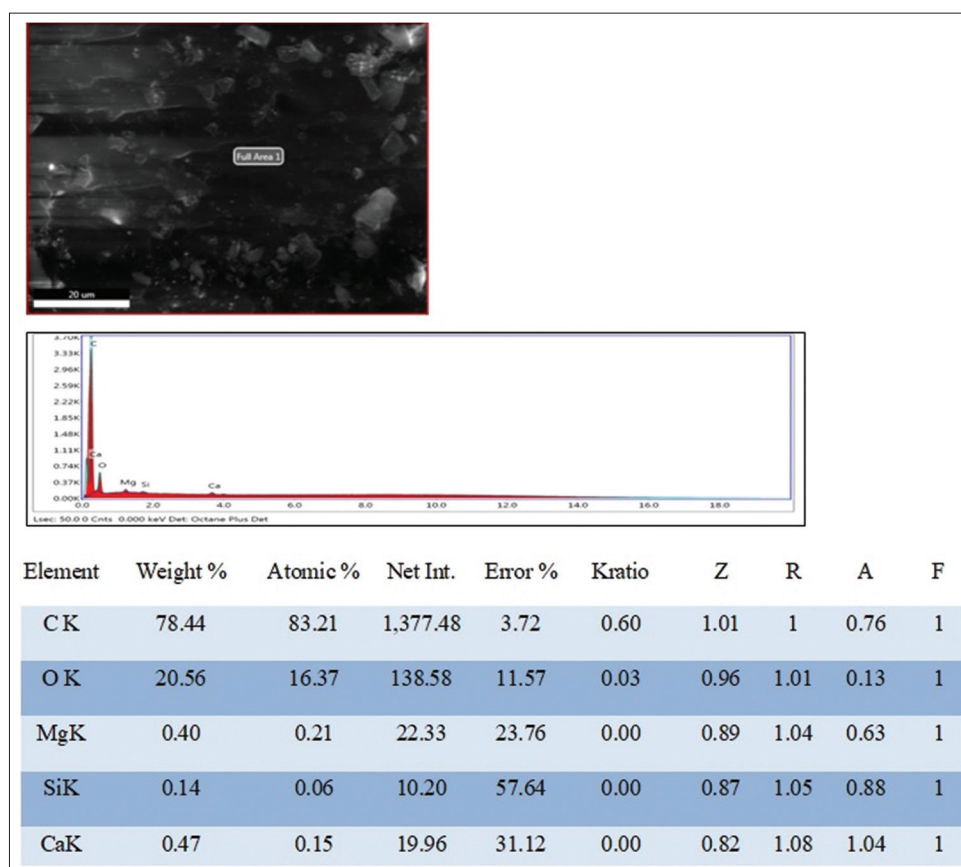| Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	R	A	F
C K	78.44	83.21	1,377.48	3.72	0.60	1.01	1	0.76	1
O K	20.56	16.37	138.58	11.57	0.03	0.96	1.01	0.13	1
MgK	0.40	0.21	22.33	23.76	0.00	0.89	1.04	0.63	1
SiK	0.14	0.06	10.20	57.64	0.00	0.87	1.05	0.88	1
CaK	0.47	0.15	19.96	31.12	0.00	0.82	1.08	1.04	1

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

Table 1: Dilution of standards for calibration

Solution name (%)	Volume of standard stock solution (mL)	Make up volume (mL)	Concentration (μg/L)						
			V	Co	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. 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 μs 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 cm⁻¹. ¹H and ¹³C NMR spectra were obtained using a Bruker, Model: 400 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 μg/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 μg/L for V, at 15 μg/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 μg/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 μg/L for V, at 15 μg/L for Co, at 60 μg/L for Ni, at 1.5 μg/L for

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

Estimated LOQ

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, µg/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
QP[b] bias (V)	−4.6
OctP[c] RF (V)	190
OctP bias (V)	−7.0

[a]RF: Radiofrequency; [b]QP: Quadrupole; [c]OctP: 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 (R^2) 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 µg/L for [51]V, 15–75 µg/L for [59]Co, 60–300 µg/L for [60]Ni, 1.5–7.5 µg/L for [111]Cd, 9–45 µg/L for [202]Hg and 1.5–12.5 [208]Pb and 4.5–22.5 µg/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 µg/L for [51]V, 0, 15, 25, 50, 75, 100, 125 µg/L for [59]Co, 0, 60, 100, 200, 300, 400, 500 µg/L for [60]Ni, 0, 1.5, 2.5, 5, 7.5, 10, 12.5 µg/L for [111]Cd, [208]Pb, 0, 9, 15, 30, 45, 60, 75 µg/L for [202]Hg, and 0, 4.5, 7.5, 15, 22.5, 30, 37.5 µg/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 µg/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 values		CV%	Maximum permissible 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
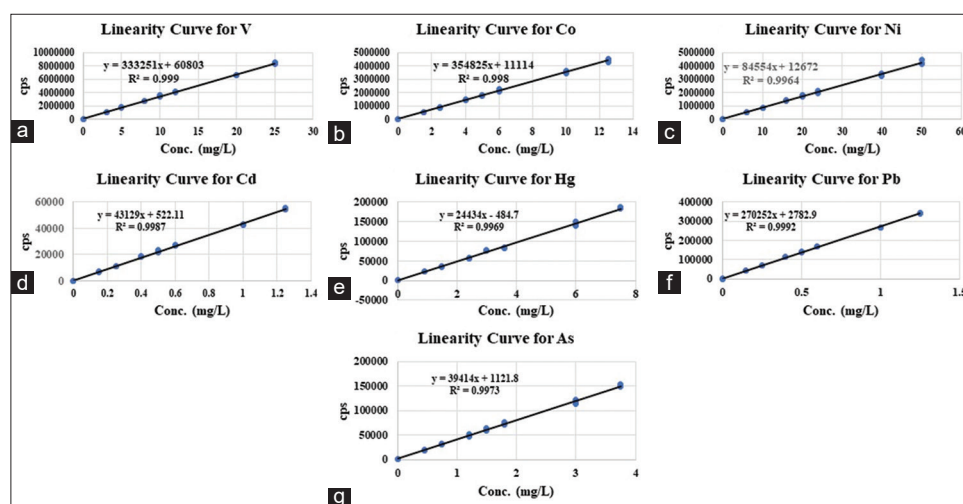
Fig. 2: Method linearity (a) V (3.0–25.0 mg/L), (b) Co (1.5–12.5 mg/L), (c) Ni (6.0–50.0 mg/L), (d) Cd (0.15–1.25 mg/L), (e) Hg (0.9–7.5 mg/L, (f) Pb (0.15–1.25 mg/L), and (g) As (0.45–3.75 mg/L)

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.

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

Where,
RSD = Relative standard deviation
S = Standard deviation
x = Mean of the data

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

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 [111]Cd, [202]Hg, [208]Pb, [75]As, [51]V, [59]Co, and [60]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 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

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 µg/L for [111]Cd, [208]Pb, 4.5, 15, and 22.5 µg/L for [75]As, 9, 30, and 45 µg/L for [202]Hg, 15, 50 and 75 µg/L for [59]Co, 60, 200 and 300 µg/L for [60]Ni and 30, 100 and 150 µg/L for [51]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
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.

Sample	Sample wt. (g)	Conc (ppb)	cps	Int. Std. cps	cps ratio	Calculation based on cps ratio				Calculation based on cps			
						Result (ppm)	Amount Recovered	% Recovery	Mean % Recovery	Result (ppm)	Amount Recovered	% Recovery	Mean % Recovery
1) Accuracy in the quantification of V													
As such-1	0.1019	0	84842.57686	1355290.6	0.062601022	0.070790916				0.272372312			
As such-2	0.1019	0	87126.67839	1371981.5	0.063504266	0.077517107				0.275739853			
As such-3	0.1019	0	85818.70966	1361540.6	0.063030592	0.073665419				0.273973865			
LOQ spiked-1	0.10342	3	1113350.843	1404841.6	0.792509876	3.053972971	2.979981823	99.3	96.7	2.94967305	2.675644374	89.1	86.5
LOQ spiked-2	0.1034	3	1088767.484	1424294	0.764426083	2.983221131	2.909229983	96.9		2.847058445	2.573029768	85.7	
LOQ spiked-3	0.1034	3	1058588.344	1403762	0.754108135	2.895639249	2.821648102	94.0		2.80914836	2.535119684	84.5	
100% spiked-1	0.1018	10	3262920.367	1406230	2.320331928	9.43881321	9.364822062	93.6	90.5	8.698346616	8.424317939	84.2	81.7
100% spiked-2	0.1022	10	3114073.807	1387849.2	2.243812805	8.964836061	8.890844913	88.9		8.379855425	8.105826748	81.0	
100% spiked-3	0.1016	10	3096700.06	1404991.6	2.20407016	8.966464969	8.892473821	88.9		8.280733796	8.006705119	80.0	
120% spiked-1	0.1028	12	3741974.319	1371792.4	2.727799279	10.74535682	10.67136567	88.9	90.1	10.1195821	9.845553422	82.0	83.3
120% spiked-2	0.1028	12	3744931.194	1377556.2	2.718532423	10.75398795	10.67999681	88.9		10.0853352	9.811306525	81.7	
120% spiked-3	0.1028	12	3880542.13	1355419.6	2.862982157	11.14983695	11.0758458	92.2		10.61916839	10.34513971	86.2	
2) Accuracy in the quantification of Co													
As such-1	0.1019	0	3620.59626	1355290.6	0.002671454	-0.020072502				0.083950702			
As such-2	0.1019	0	3930.673464	1371981.5	0.002864961	-0.01986742				0.084628559			
As such-3	0.1019	0	3440.551401	1361540.6	0.002526955	-0.02122297				0.083444521			
LOQ spiked-1	0.10342	1.5	512752.297	1404841.6	0.364989403	1.367009183	1.387614319	92.5	92.6	1.333264923	1.249256995	83.2	82.9
LOQ spiked-2	0.1034	1.5	515262.0236	1424294	0.361766618	1.374114147	1.394719284	92.9		1.322397145	1.238389218	82.5	
LOQ spiked-3	0.1034	1.5	511364.0158	1403762	0.364281136	1.363489672	1.384094808	92.2		1.331077735	1.247069808	83.1	
100% spiked-1	0.1018	5	1664934.767	1406230	1.183970451	4.578529227	4.599134364	91.9	92.0	4.226195667	4.14218774	82.8	83.3
100% spiked-2	0.1022	5	1674632.317	1387849.2	1.206638529	4.587351508	4.607956644	92.1		4.288828108	4.204820181	84.0	
100% spiked-3	0.1016	5	1661582.263	1404991.6	1.182627899	4.578242553	4.59884769	91.9		4.229798096	4.145790169	82.9	
120% spiked-1	0.1028	6	1992073.564	1371792.4	1.452168392	5.430850245	5.451455382	90.8	90.6	5.11635865	5.032350723	83.8	83.9
120% spiked-2	0.1028	6	1926354.346	1377556.2	1.398385305	5.250679357	5.271284493	87.8		4.929605604	4.845597677	80.7	
120% spiked-3	0.1028	6	2046064.188	1355419.6	1.509543014	5.578866899	5.599472035	93.3		5.315582714	5.231574787	87.1	
3) Accuracy in the quantification of Ni													
As such-1	0.1019	0	23680.41713	1355290.6	0.017472575	0.127761056				0.537018659			
As such-2	0.1019	0	24101.1056	1371981.5	0.01756664	0.132643687				0.53840105			
As such-3	0.1019	0	24251.45472	1361540.6	0.017811775	0.134388682				0.542003578			
LOQ spiked-1	0.10342	6	499992.1019	1404841.6	0.35590639	5.572843142	5.441245333	90.6	91.4	5.429691814	4.890550718	81.5	81.8
LOQ spiked-2	0.1034	6	499908.5082	1424294	0.350986881	5.572964923	5.441367114	90.6		5.359493131	4.820352036	80.3	
LOQ spiked-3	0.1034	6	510695.344	1403762	0.363804793	5.696343853	5.564746044	92.7		5.545134081	5.005992985	83.4	
100% spiked-1	0.1018	20	1765044.143	1406230	1.255160353	20.35850626	20.22690845	101.1	98.3	18.74462798	18.20548688	91.0	88.9
100% spiked-2	0.1022	20	1691073.777	1387849.2	1.218485248	19.42282407	19.29122626	96.4		18.1338637	17.5947226	87.9	
100% spiked-3	0.1016	20	1696093.257	1404991.6	1.20719103	19.59595533	19.46435752	97.3		18.0744821	17.53534101	87.6	
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	86.0
120% spiked-2	0.1028	24	1944821.221	1377556.2	1.411790837	22.22874317	22.09714536	92.0		20.84399592	20.30485482	84.6	
120% spiked-3	0.1028	24	1992433.095	1355419.6	1.469975125	22.77650231	22.6449045	94.3		21.69159333	21.15245224	88.1	
4) Accuracy in the quantification of Cd													
As such-1	0.1019	0	130.1355718	1355290.6	0.0000960	-0.008919108				0.001908381			
As such-2	0.1019	0	140.1459446	1371981.5	0.0001021	-0.008691331				0.002084912			
As such-3	0.1019	0	100.1037279	1361540.6	0.0000735	-0.009602458				0.001260291			
LOQ spiked-1	0.10342	0.15	7049.056517	1404841.6	0.0050177	0.146332338	0.155403303	103.6	104.3	0.141572772	0.139821577	93.2	93.4
LOQ spiked-2	0.1034	0.15	7369.681097	1424294	0.0051743	0.15355035	0.162621315	108.4		0.1460453	0.144294105	96.2	
LOQ spiked-3	0.1034	0.15	6858.822337	1403762	0.0048860	0.142094817	0.151165783	100.8		0.137862552	0.136111357	90.7	

Table S3. (*Continued*)

Sample	Sample wt. (g)	Conc (ppb)	cps	Int. Std. cps	cps ratio	Result (ppm)	Amount Recovered	% Recovery	Mean % Recovery	Result (ppm)	Amount Recovered	% Recovery	Mean % Recovery
100% spiked-1	0.1018	0.5	23627.92	1406230	0.0168023	0.526269663	0.535340629	107.1	106.3	0.483633435	0.48188224	96.4	96.1
100% spiked-2	0.1022	0.5	23531.11	1387849.2	0.0169551	0.522013536	0.531084501	106.2		0.486128572	0.484377377	96.9	
100% spiked-3	0.1016	0.5	23263.61333	1404991.6	0.0165578	0.518991659	0.528062624	105.6		0.477521922	0.475770727	95.2	
120% spiked-1	0.1028	0.6	26352.3485	1371792.4	0.0192102	0.582599654	0.59167062	98.6	95.7	0.54768317	0.545931975	91.0	88.5
120% spiked-2	0.1028	0.6	24657.64353	1377556.2	0.0178996	0.54437567	0.553446636	92.2		0.510259788	0.508508594	84.8	
120% spiked-3	0.1028	0.6	25710.55924	1355419.6	0.0189687	0.568124131	0.577195096	96.2		0.540788744	0.539037549	89.8	
5) Accuracy in the quantification of Hg													
As such-1	0.1019	0	6361.783	10267124	0.00062	0.274983				0.196491			
As such-2	0.1019	0	6081.58	10194306	0.000597	0.263729				0.186584			
As such-3	0.1019	0	6051.666	10336583	0.000585	0.262528				0.181813			
LOQ spiked-1	0.10342	0.9	23041.11	10267124	0.002244	0.931007	0.663927	73.7	72.1	0.881271	0.692975	76.9	75.3
LOQ spiked-2	0.1034	0.9	21989.56	10194306	0.002157	0.889565	0.622485	69.1		0.844556	0.65626	72.9	
LOQ spiked-3	0.1034	0.9	22961.07	10336583	0.002221	0.928019	0.660939	73.4		0.871779	0.683483	75.9	
100% spiked-1	0.1018	3	74766.46	10293788	0.007263	3.025368	2.758288	91.9	92.7	3.053693	2.865397	95.5	96.2
100% spiked-2	0.1022	3	75966.35	10301726	0.007374	3.061578	2.794498	93.1		3.089236	2.90094	96.6	
100% spiked-3	0.1016	3	75393.62	10309664	0.007313	3.056587	2.789507	92.9		3.081096	2.8928	96.4	
120% spiked-1	0.1028	3.6	83600.18	10317603	0.008103	3.347631	3.080551	85.5	86.2	3.381456	3.19316	88.6	89.2
120% spiked-2	0.1028	3.6	85774.09	10325541	0.008307	3.43418	3.1671	87.9		3.468461	3.280165	91.1	
120% spiked-3	0.1028	3.6	83076.38	10333479	0.00804	3.326777	3.059697	84.9		3.354568	3.166272	87.9	
6) Accuracy in the quantification of Pb													
As such-1	0.1019	0	193738.0744	10267123.5	0.018869752	0.693406866				0.707248952			
As such-2	0.1019	0	193948.3042	10194305.8	0.019025161	0.694170264				0.71328656			
As such-3	0.1019	0	191196.1575	10336583	0.018497037	0.68417652				0.692768998			
LOQ spiked-1	0.10342	0.15	235010.382	10267123.5	0.022889603	0.830883155	0.140298605	93.5	88.4	0.850729887	0.146295051	97.5	92.2
LOQ spiked-2	0.1034	0.15	232562.7585	10194305.8	0.022813006	0.822284861	0.131700311	87.8		0.847961813	0.143526976	95.6	
LOQ spiked-3	0.1034	0.15	230879.8647	10336583	0.022336188	0.816262497	0.1256677947	83.7		0.829706213	0.125271376	83.5	
100% spiked-1	0.1018	0.5	329014.9	10293787.9	0.031962471	1.185795053	0.495210503	99.0	99.1	1.217094536	0.5126597	102.5	102.4
100% spiked-2	0.1022	0.5	326491.8567	10301726.13	0.031692927	1.172019059	0.481434509	96.2		1.20188993	0.497455094	99.4	
100% spiked-3	0.1016	0.5	332611.2967	10309664.36	0.032262088	1.201227286	0.510642736	102.1		1.231164842	0.526730006	105.3	
120% spiked-1	0.1028	0.6	346201.5147	10317602.59	0.033554453	1.236122661	0.545538111	90.9	89.1	1.266562009	0.562127172	93.6	91.6
120% spiked-2	0.1028	0.6	341993.7914	10325540.81	0.033121151	1.220977118	0.530392568	88.3		1.249875602	0.545440765	90.9	
120% spiked-3	0.1028	0.6	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													
As such-1	0.1019	0	18112.29	1355290.6	0.013364138	0.423035064				0.426382909			
As such-2	0.1019	0	17331.15	1371981.5	0.012632204	0.403585945				0.403313918			
As such-3	0.1019	0	18062.3	1361540.6	0.013266075	0.421790394				0.423292193			
LOQ spiked-1	0.10342	0.45	35756.27	1404841.6	0.025452172	0.849667426	0.433530292	96.3	104.8	0.795505777	0.37784277	83.9	90.8
LOQ spiked-2	0.1034	0.45	37310.45	1424294	0.026619575	0.887966972	0.471829838	104.8		0.818755671	0.401092665	89.1	
LOQ spiked-3	0.1034	0.45	38875.5	1403762	0.027693797	0.926368892	0.510231757	113.3		0.865285974	0.447622968	99.4	
100% spiked-1	0.1018	1.5	94358.08	1406230	0.067100033	2.323712013	1.907574879	127.1	116.7	2.122106427	1.704443421	113.6	104.8
100% spiked-2	0.1022	1.5	87272.44	1387849.2	0.06288323	2.138714171	1.722577036	114.8		1.981286186	1.563623179	104.2	
100% spiked-3	0.1016	1.5	82815.21	1404991.6	0.058943562	2.040038881	1.623901747	108.2		1.868450129	1.450787123	96.7	
120% spiked-1	0.1028	1.8	94952	1371792.4	0.069217471	2.315765992	1.899628858	105.5	106.1	2.167616201	1.749953195	97.2	98.1
120% spiked-2	0.1028	1.8	91659.1	1377556.2	0.066537467	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.

Sample	Sample wt. (g)	Conc (ppb)	cps	Int. Std. cps	cps ratio	Calculation based on cps ratio				Calculation based on cps			
						Result (ppm)	Mean Result (ppm)	SD	% RSD	Result (ppm)	Mean Result (ppm)	SD	% RSD
1) Precision in the quantification of V													
As such-1	0.1019	0	84842.57686	1355290.6	0.062601022	0.07	0.07	0.003	4.56	0.27	0.27	0.002	0.61
As such-2	0.1019	0	87126.67839	1371981.5	0.063504266	0.08				0.28			
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	3096700.06	1404991.6	2.20407016	8.97				8.28			
100% spiked-4	0.1025	10	3025864.33	1410965.1	2.144535205	8.68				7.99			
100% spiked-5	0.1031	10	3040183.287	1420922.6	2.139584019	8.67				7.92			
100% spiked-6	0.1008	10	3110912.56	1405752	2.21298818	9.08				8.38			
2) Precision in the quantification of Co													
As such-1	0.1019	0	3620.59626	1355290.6	0.002271454	-0.02	-0.02	0.001	-3.33	0.08	0.08	0.001	0.71
As such-2	0.1019	0	3930.673464	1371981.5	0.002864961	-0.02				0.08			
As such-3	0.1019	0	3440.551401	1361540.6	0.002526955	-0.02				0.08			
100% spiked-1	0.1018	5	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	5	1674632.317	1387849.2	1.206638529	4.59				4.29			
100% spiked-3	0.1016	5	1661582.263	1404991.6	1.182627899	4.58				4.23			
100% spiked-4	0.1025	5	1607591.59	1410965.1	1.139356027	4.39				4.04			
100% spiked-5	0.1031	5	1647933.727	1420922.6	1.15976319	4.47				4.09			
100% spiked-6	0.1008	5	1578386.173	1405752	1.122805568	4.38				4.05			
3) Precision in the quantification of Ni													
As such-1	0.1019	0	23680.41713	1355290.6	0.017472575	0.13	0.13	0.003	2.61	0.54	0.54	0.003	0.48
As such-2	0.1019	0	24101.1056	1371981.5	0.01756664	0.13				0.54			
As such-3	0.1019	0	24251.45472	1361540.6	0.017811775	0.13				0.54			
100% spiked-1	0.1018	20	1765044.143	1406230	1.255160353	20.36	19.504	0.471	2.42	18.74	17.98	0.474	2.64
100% spiked-2	0.1022	20	1691073.777	1387849.2	1.218485248	19.42				18.13			
100% spiked-3	0.1016	20	1696093.257	1404991.6	1.20719103	19.60				18.07			
100% spiked-4	0.1025	20	1658666.853	1410965.1	1.175554841	18.99				17.45			
100% spiked-5	0.1031	20	1685167.79	1420922.6	1.185967336	19.19				17.50			
100% spiked-6	0.1008	20	1671783.257	1405752	1.1892448	19.47				17.95			
4) Precision in the quantification of Cd													
As such-1	0.1019	0	130.1355718	1355290.6	9.60204E-05	-0.01	-0.01	0.000	-5.23	0.00	0.00	0.000	24.79
As such-2	0.1019	0	140.1459446	1371981.5	0.000102149	-0.01				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				0.48			
100% spiked-5	0.1031	0.5	22725.55667	1420922.6	0.015993522	0.50				0.46			
100% spiked-6	0.1008	0.5	23330.5	1405752	0.016596455	0.52				0.48			
5) Precision in the quantification of Hg													
As such-1	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			

(Contd....)

Table S4. *(Continued)*

Sample	Sample wt. (g)	Conc (ppb)	cps	Int. Std. cps	cps ratio	Calculation based on cps ratio				Calculation based on cps			
						Result (ppm)	Mean Result (ppm)	SD	% RSD	Result (ppm)	Mean Result (ppm)	SD	% RSD
100% spiked-1	0.1018	3	74766.46	112226846.7	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	112234982.6	0.006761591	3.06				2.83			
100% spiked-3	0.1016	3	75393.62333	112298447.3	0.006672919	3.06				2.81			
100% spiked-4	0.1025	3	76167.69667	114483387.4	0.00663286	3.06				2.76			
100% spiked-5	0.1031	3	73215.23	114446110.9	0.006396516	2.93				2.65			
100% spiked-6	0.1008	3	75624.92667	113325018.6	0.006677687	3.09				2.83			
6) Precision in the quantification of Pb													
As such-1	0.1019	0	193738.0744	102671123.5	0.018869752	0.69	0.69	0.006	0.81	0.71	0.70	0.011	1.50
As such-2	0.1019	0	193948.3042	101943305.8	0.019025161	0.69				0.71			
As such-3	0.1019	0	191196.1575	10336583	0.018497037	0.68				0.69			
100% spiked-1	0.1018	0.5	329014.9	112226846.7	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	112234982.6	0.02906029	1.17				1.10			
100% spiked-3	0.1016	0.5	332611.2967	112298447.3	0.029438673	1.20				1.12			
100% spiked-4	0.1025	0.5	333765.2767	114483387.4	0.029065054	1.19				1.10			
100% spiked-5	0.1031	0.5	332678.45	114446110.9	0.029064759	1.18				1.09			
100% spiked-6	0.1008	0.5	339113.0467	113325018.6	0.029943708	1.23				1.15			
7) Precision in the quantification of As													
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			