Development and Validation of an Analytical Method for the Estimation of Elemental Impurities in Choline Bitartrate

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Abstract

Choline, a water-soluble vitamin-like essential nutrient, can affect diseases such as liver disease, atherosclerosis and neurological disorders[1]. Although the liver can produce small amounts of choline, choline needs to be obtained through the diet to avoid deficiencies[2]. In addition to natural sources of choline like meat, poultry, fish, dairy products, and eggs, it can also be availed through chemical produced choline supplements like Choline bitartrate. Elemental impurities in drug products may arise from several sources; they may be residual catalysts that were added intentionally in synthesis or may be present as impurities (e.g., through interactions with processing equipment or container/closure systems or by being present in components of the drug product). Because elemental impurities do not provide any therapeutic benefit to the patient, their levels in the drug product should be controlled within acceptable limits[3]. Therefore, it is important to control elemental impurities like cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As), Vanadium (V), Cobalt (Co) and Nickel (Ni) present in Choline bitartrate by using inductively coupled plasma mass spectrometry (ICPMS). The aim of this work is to optimize and validate a quick and easy method to determine and quantify above elemental impurities. Method showed that the estimated quantification limits varying between 0.15µg/g and 6.0µg/g, recoveries within 70-150% and relative standard deviation below 20%. This will help us to quantify and control elemental impurities in Choline bitartrate.

Keywords

Choline bitartrate, Elemental Impurities, ICPMS

Introduction

Choline was officially recognized as an essential nutrient by the Institute of Medicine in 1998[4]. Choline is required to make the phospholipids phosphatidylcholine, lysophosphatidylcholine, choline plasmalogen, and sphingomyelin – essential components for all membranes[5]. It plays important roles in brain and memory development in the fetus and appears to decrease the risk of the development of neural tube defects[6,7]. Choline intakes for children, adults, and pregnant women are often far below the adequate intake levels[8]. Especially during pregnancy and lactation choline consumption is very important. During these periods the demand for choline is high. Dietary sources of choline include eggs, beef, fish, pork, liver, soybean, and wheat germ[9]. Choline bitartrate is choline combined with a salt of tartaric acid. It has a much better absorption rate than pure choline, which is an essential human nutrient.

Impurities in pharmaceuticals are the unwanted chemicals that remain with the active pharmaceutical ingredients (APIs), or develop during formulation, or upon aging of both API and formulated APIs to medicines. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products[10]. Impurities can be classified into three categories namely Organic impurities (process- and drug-related), Inorganic impurities (reagents, catalyst, filter aids related), and Residual solvents (used as vehicle in processes)[11].

Elemental impurities are basically inorganic impurities. They are classified in three classes, i.e. Class 1 (highly toxic elemental impurities), Class 2 (2A & 2B) (elemental impurities with relatively lower toxicity), and Class 3 (elemental impurities with lower toxicity). They do not provide any therapeutic benefit to the patient and they need to be controlled below their allowable concentration which are calculated based on their PDE (Permissible Daily Exposure) limits (established based on toxicity studies performed for an individual elements). There are three risk-based approaches namely Option 1, 2 (includes sub-

options 2a and 2b) and 3 for establishment of allowable concentration of elemental impurities in samples based on route of administration.[3]

PDE values are derived from toxicity studies and are route dependent. Three routes of administration have been provided for establishment of PDE values namely oral (drugs which are taken orally), parenteral (drugs which are taken intravenous) and inhalation (drugs which are taken through inhalation).

Further Option 1 provides common allowable concentration for each component of the drug product (final formulation e.g. tablet, capsule, injection, nasal spray, etc.) like API (active pharmaceutical ingredient), excipients like sweetener, binder, etc. considering 10 gm maximum daily intake of the drug product. Option 2a provides common allowable concentration for each component of the drug product considering drug specific maximum daily intake. Option 2b provides specific daily intake for individual component of the drug product based on maximum daily intake of the drug product.

As choline bitartrate is taken orally, considering Option 1 allowable concentration for each of the elements shall be as per Table 1.

For determination and quantification of elemental impurities, there are several analytical techniques inclusive of classical method of analysis (colorimetry heavy metal tests), instrumental methods – AAS (Atomic Absorption Spectrometer), ICPMS (Inductively coupled plasma mass spectrometry), ICP-OES (Inductively coupled plasma optical emission spectrometry), XRF, etc.

Objective of this work is to quantify multiple elemental impurities in pharmaceuticals at very low level concentrations. Based on this requirement ICPMS and ICPOES are best suits. Both these instruments can analyze and quantify multiple elements at a time. Out of these two instruments, ICPMS instrument can quantify elements at lower levels compared to ICP-OES. Therefore, ICPMS instrument was selected for the optimization and validation of an analytical method for estimation of elemental impurities in Choline Bitartrate.

This study aims to optimize and validate method for determination of elemental impurities of Class 1 and Class 2A (as per ICH Q3D) on ICPMS instrument which shall be utilized for routine evaluation of elemental impurities in Choline bitartrate.

Validation of an analytical method ensures results are authentic and the method is fit for its intended purpose of quantifying elemental impurities. By quantifying elemental impurities in drug, we can know the levels of elemental impurities in drug substance and further the control the same through removing the sources of elemental impurities in the synthetic reaction of drug preparation.

Materials and Methods

Reagents and materials

Nitric acid (65%), Hydrochloric acid (37%) and certified reference metal stock for 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 other 3 elements i.e., 150 ppm each of Selenium (Se) and Silver (Ag) and 8 ppm of Thallium (Tl). 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, gold standard for ICP TraceCERT®, 1000mg/L Au in hydrochloric acid, nitric acid ≥69.0%, TraceSELECT™ for trace analysis from Honeywell were used for the study. All the autosampler vials and glassware's, centrifuge tubes, plastic bottles, were cleaned by soaking in 20% v/v HNO_3 analytical grade reagent for 4 h, then after rinsing three times with deionized Milli-Q water.

In analytical chemistry, sample preparation refers to the ways in which a sample is treated prior to its analysis.

Sample preparation

250 mg of sample was taken in 50 mL polyethylene tube and 0.2 mL of 1000 mg/L gold standard solution was added and then make up to 25 mL with 2% Nitric acid to make 10mg/mL sample solution.

In analytical chemistry, a calibration curve plotted by injecting different level standards of known concentration, is a general method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known

concentration. Set of standards with known concentration used for plotting calibration curve are known as calibration standards.

Calibration standard preparation

For calibration standard stock solution preparation, 0.5 mL of elemental 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) was diluted to 20mL with 2% nitric acid. Then 0.2 mL of 1000mg/L Gold standard solution was added to each of them, and this stock solution was further diluted as per Table 2 to make different level calibration standards:

Spike sample is a sample to which known concentrations of specific analytes (components under testing, here elemental impurities) have been added in such a manner as to minimize the change in the matrix of the original sample.

Spiked sample preparation

250 mg of sample was taken in 50 mL polyethylene tube, added amount of calibration standard stock solution as per Table 3, 0.2 mL of gold standard solution, 0.04 mL of 5 ppm internal standard stock solution and make up to 25 mL with 2% nitric acid to make respective level spiked sample solution.

ICPMS is an instrument which can identify and quantify elemental impurities based on their masses. For optimum response under the testing conditions, instrument needed to be optimized.

ICP-MS instruments optimization

The quantities of heavy metals were determined by inductively coupled plasma quadrupole mass spectrometry (ICP-MS) an iCAP RQ ICP-MS (Thermo Fisher Scientific) using Qtegra software equipped with Q Cell Collision Reaction Cell, RAPID Lens, with a quartz spray chamber, glass concentric nebulizer, online internal standard (ISTD) addition kit, exchangeable skimmer cones. The optimization of ICP-MS is important because the nebulizer gas and make up gas flows should be adjusted to ensure plasma stability. The ICP-MS was allowed to stabilize for 1 h and the performance was optimized based on radio frequency (RF) power, autotune function in the control software and A tune B solution, the quadrupole ion deflector

voltages were optimized to step wise find the settings that maximize signal intensity over the mass range, as well as 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 in KED mode for analysis of Cd, Hg, Pb, As, V, Co and Ni.

Method validation is the process to check suitability of an analytical method for its intended purpose i.e. an analytical method is capable of quantifying elemental impurities at the level provided in the methodology.

Method validation criteria

Several parameters have been considered and evaluated for the method validation like linear dynamic range (LDR), method linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ). Analytical method validation of ICP-MS for analysis of Cd, Hg, Pb, As, V, Co and Ni was applied in accordance with ICH Q2(R1).

ICPMS analysis

The MS-method used 3 replicate readings of 30 sweeps over the analyte mass-range with a dwell time of 40µs for each mass per sweep. Sample aspiration followed by rinse program with 2% HNO_3 kept for 60 s. For running the instrument in KED-mode, 4.34 mL min-1 of He gas was used.

Results and Discussion

Optimization of ICP-MS parameters

It is important to extract and keep elemental impurities are kept suspended in solution using concentrated acids and are transported through Peristaltic pump into ICPMS instrument. Peristaltic pump is known for its flow characteristic which is not smooth and consistent causing slight variation in response in terms of counts observed in ICPMS analysis. On the other hand, careful study of the matrix effect in ICPMS showed that, in all cases studied, the magnitude of the signal suppression or enhancement depends in a regular way on the mass number. Hence, accurate correction for non-spectral interferences is only possible using an internal standard with mass number close to that of the analyte element(s). It is also shown that using an internal standard with mass number close to that of the analyte improves the precision. To obtain

optimal precision and accuracy, the internal standard should be selected as close in mass number as possible to that of the analyte element(s). When a number of elements over a considerable mass range are to be determined, several internal standards have to be used[12].

Based on this fact for analysis of seven elements out of which 5 elements having mass in the range 51-111 whereas 2 other elements having mass in the range 202-209. Based on this fact two different internal standards - Yttrium Y^{89} internal standard is used for elements in the mass range from 51-111 and Bismuth Bi^{209} internal standard is used for elements in the mass range 202-208[12].

Mercury (Hg) analysis can be combined with the typical multi-element analysis performed by ICP-MS, but this presents several challenges:

a) Hg has a very high first ionization potential (10.44eV) which means that it is only about 4% ionized in the plasma. This leads to low sensitivity for Hg, as only ions (not atoms) are measured by ICP-MS.
b) Hg has a high number of naturally occurring isotopes (7), all less than 30% abundant. Since the total element concentration is divided among many separate isotopes, the number of ions (and therefore the sensitivity) is lower for each individual isotope.
c) Hg is very easily lost from aqueous samples by adsorption to the walls of the container and sample introduction components resulting in low recovery and extended washout times. Hg is also volatile and can be lost if samples are digested in open vessels.

Based on above facts, to improve sensitivity and recovery for mercury analysis is a challenging job and it requires, Samples preparation to be done using closed vessel/closed vessel digestion. The plasma must be operated at the highest possible temperature to maximize ionization of the Hg atoms. Appropriate sample preparation and preservation chemistry must be used to ensure stability of Hg. Considering all above facts, sample preparation done in a closed vessel. Maximum RF power 1600W was used to generate plasma. Further to stabilize Hg in solution Researchers at the National Exposure Research Laboratory have investigated the reliability of 2% HNO_3 as a preservative for mercury and reported that mercury was lost within a few days. The use of 2% HNO_3 containing 1 mg/L $AuCl_3$ was recommended. It was claimed that mercury standard solutions preserved in this mixture were stable for several years. Allibone and Chen have also reported the addition of a trace amount of gold chloride to 1–2%

nitric acid in order to preserve mercury for the simultaneous determination of mercury and other elements by ICP-MS.[9,13]

For accurate and correct results various instrumental parameters like torch alignment, the nebulizer gas and make up gas flows, kinetic energy discrimination (KED), lens and mass calibration and resolution, detector voltage, dual detector should be optimized to achieve maximum instrumental performance. The plasma stability and position are most important parameter since this determine the sensitivity of instrument. To ensure this, the nebulizer gas and make up gas flows had to be adjusted. This instrumental requirement has been achieved by adjusting torch position and tuning for reduced oxide and doubly charged ion formation with a standard tuning solution containing 10 ng/g of ^{7}Li, ^{89}Y, ^{140}Ce and ^{205}Tl in 2% HNO_3. Optimized ICPMS instrument parameters are given in Table 4.

Method validation

Specificity

Specificity is the ability to assess unequivocally the analyte (component/ impurity under analysis) in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc.[14]. Primary isotopes of each element, ^{111}Cd, ^{202}Hg, ^{208}Pb, ^{75}As, ^{51}V, ^{59}Co and ^{60}Ni were used in the present study to investigate the selectivity of the present method. Specificity has been verified by confirming recovery of the elements while quantified considering above isotope masses.

Estimated LOD

The term LOD is defined as the lowest concentration at which the instrument can detect but not quantify and the noise to signal ratio for LOD should be 1:3. LOD was estimated from the calibration function using Eq. (1)[14].

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

Where

σ is standard deviation

S is slope derived from calibration curve

The estimated LODs were found to be 0.25, 0.82, 0.16, 0.58, 3.05, 1.66 and 7.23 µg/ L for Cd, Hg, Pb, As, V, Co and Ni 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 1.5 µg/L for Cd and Pb, at 4.5 µg/L for As, at 9.0 µg/L for Hg, at 15 µg/L for Co, at 30 µg/L for V and at 60 µg/L for Ni. The results are reported in Table 5.

Practical LOQ

The term LOQ is defined as the lowest concentration at which the instrument can detect and quantify. Control Threshold values as per ICH Q3D (R1) para 5.6 considered for verification as LOQ values and based on successful verification of precision, linearity (from LOQ) and recovery at LOQ level, below values considered as LOQ. The noise to signal ratio for LOQ should be 1:10. The estimated LOQs were found to be 1.5, 9.0, 1.5, 4.5, 30, 15 and 60 µg/ L for Cd, Hg, Pb, As, V, Co and Ni respectively. The results are reported in Table 5.

Method Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.[14].

The method linearity was checked using seven different levels of samples at 0, 0.15, 0.25, 0.5, 0.75, 1.0, 1.25 mg/L for ^{111}Cd, ^{208}Pb, 0, 0.45, 0.75, 1.5, 2.25, 3.0, 3.75 mg/L for ^{75}As, 0, 0.9, 1.5, 3.0, 4.5, 6.0, 7.5 mg/L for ^{202}Hg, 0, 1.5, 2.5, 5.0, 7.5, 10.0, 12.5 mg/L for ^{59}Co, 0, 6.0, 10.0, 20.0, 30.0, 40.0, 50.0 mg/L for ^{60}Ni and 0, 3.0, 5.0, 10.0, 15.0, 20.0, 25.0 mg/L for ^{51}V. The method linearity was found to be linear from LOQ values up to 1.25 mg/L for ^{111}Cd, ^{208}Pb, 3.75 mg/L for ^{75}As, 7.5 mg/L for ^{202}Hg, 12.5 mg/L for ^{59}Co, 50 mg/L for ^{60}Ni and 25 mg/L for ^{51}V (Table 5).

Method accuracy

In the context of an analytical method, according to ICH guidelines, accuracy ‘is sometimes termed trueness’. The trueness of an analytical procedure reflects the closeness of agreement between the value that is either accepted as conventional true value or an accepted reference value with the observed value. Therefore, accuracy is an expression of both trueness and precision since both these influence the result[14]. Accuracy can be measured by spiking the

sample matrix with a known concentration of analyte standard and analysing the sample using the "method to be validated." The results of the accuracy are presented in Table 6.

Precision study: repeatability and reproducibility

Precision of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings[15]. According to ICH[14], precision may be considered at three levels: repeatability, intermediate precision, and reproducibility. The precision was calculated in terms of relative standard deviation (RSD) by Eq. 2 and single estimation of precision uncertainty is calculated by Eq. 3, respectively[16].

$$RSD = \frac{S \times 100}{\bar{x}}$$

Where,

RSD = Relative standard deviation

S = Standard deviation

$\bar{x}$ = Mean of the data

The results of repeatability test expressed as RSD were found to be 0.88%, 0.68%, 0.98%, 0.64%, 0.84%, 1.00% and 1.43% for ^{51}V, ^{59}Co, ^{60}Ni, ^{75}As, ^{111}Cd, ^{202}Hg and ^{208}Pb respectively. The results of the precision study are presented in Table 7 and Linear regression analysis results are reported in Table 8.

Accuracy/Recovery

The spiking levels used for recovery test were at 0.15, 0.5 and 0.75 mg/L for ^{111}Cd, ^{208}Pb, 0.45, 1.5 and 2.25 mg/L for ^{75}As, 0.9, 3.0 and 4.5 mg/L for ^{202}Hg, 1.5, 5.0 and 7.5 mg/L for ^{59}Co, 6.0, 20.0 and 30.0 mg/L for ^{60}Ni and 3.0, 10.0 and 15.0 mg/L for ^{51}V. The mean recoveries ± standard deviations at different levels varied between 91.0% and 137.7%[17,18]. The results of recovery test are presented in Table 6.

Conclusion

Based on literature, a novel analytical method (which is not published in any literature) for simultaneous determination of low level elemental impurities namely Cd, Hg, Pb, As, V, Co, and Ni (at levels as suggested in ICH guidelines) in Choline Bitartrate has been optimized and validated on ICP-MS instrument. The validated method has been successfully evaluated in terms of LOD, LOQ, linearity, repeatability, reproducibility, accuracy, and precision. It meets all the acceptance criteria as given in USP General chapter <233>. The validated method is very simple, quick, easy, cost effective and reliable. Therefore, it can be very useful for pharmaceutical industries (manufacturing or formulating Choline Bitartrate for end use) to quantify toxic metals in routine laboratories analysis. Low level quantification of elemental impurities further enables us to control these impurities in Choline bitartrate drug and thereby enhancing safety and efficacy of the drug.

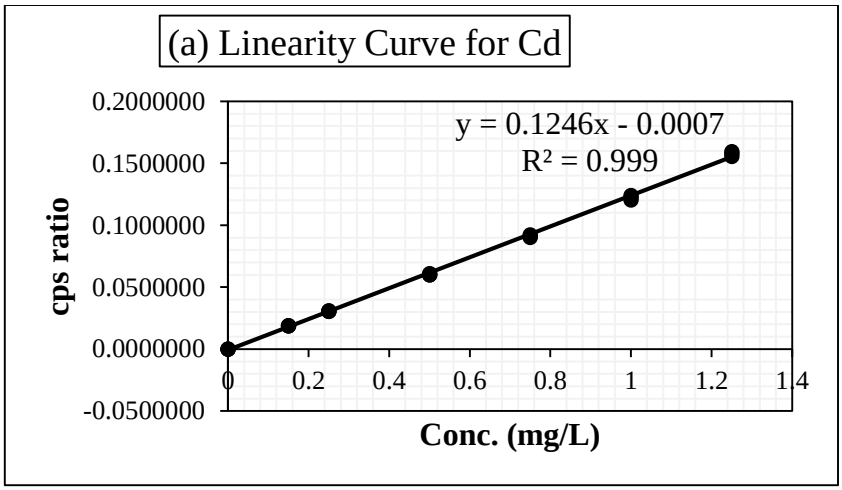

Figure 1: Linearity Curve for Cd

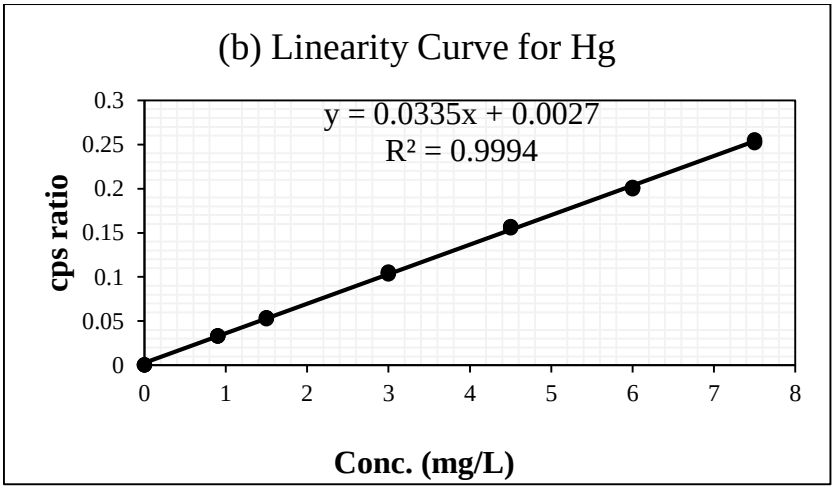

Figure 2: Linearity Curve for Hg

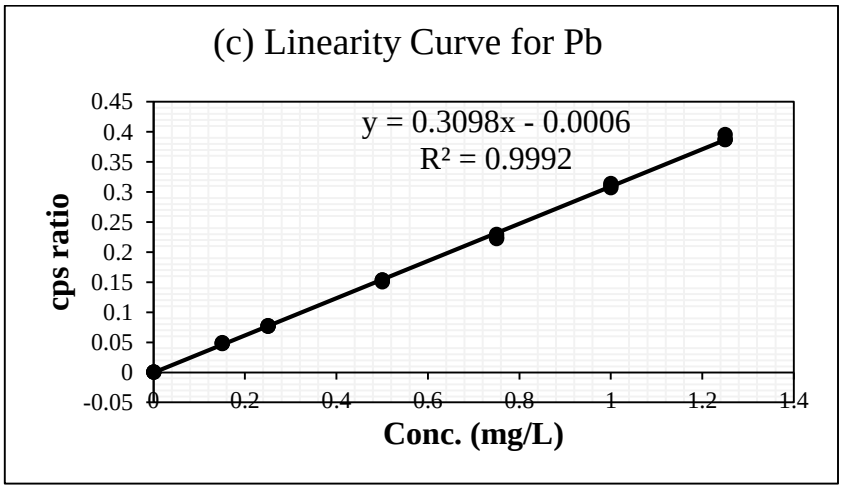

Figure 3: Linearity Curve for Pb

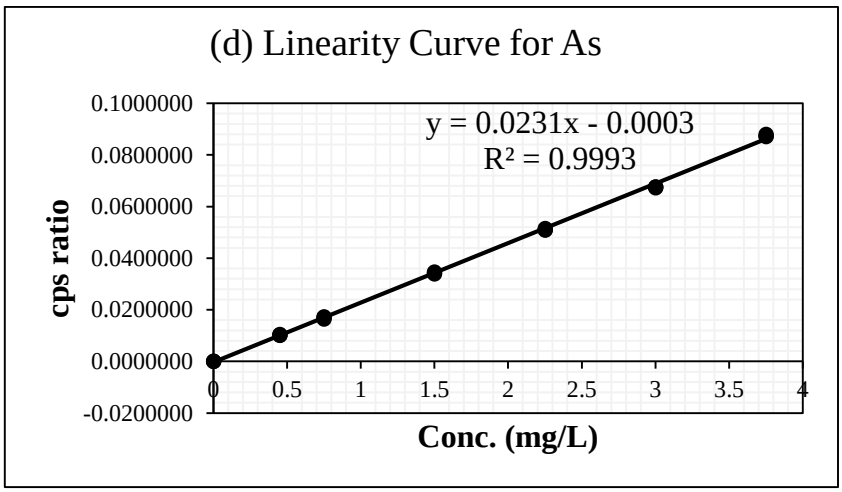

Figure 4: Linearity Curve for As

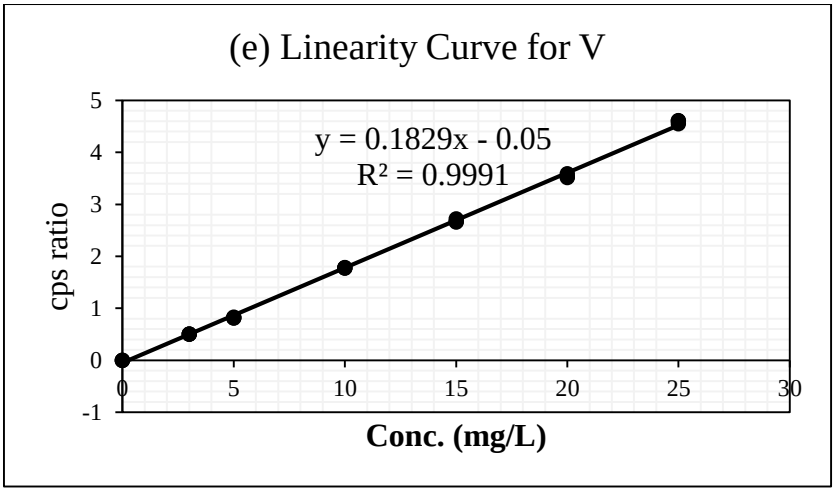

Figure 5: Linearity Curve for V

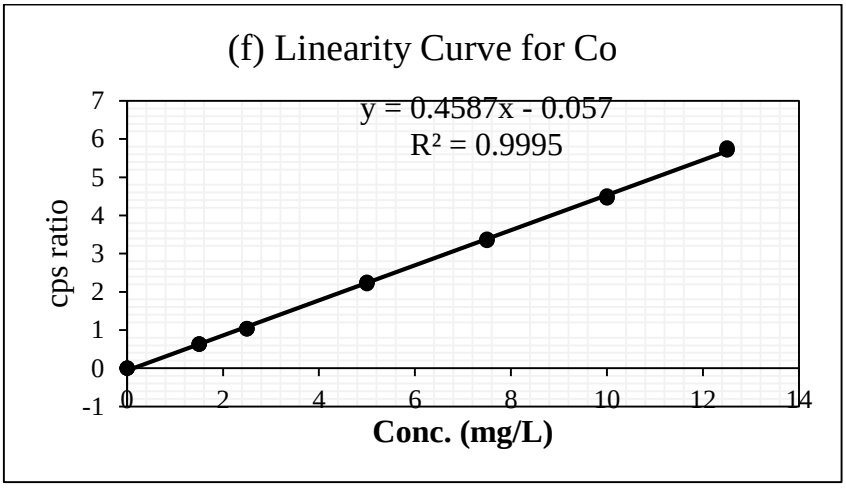

Figure 6: Linearity Curve for Co

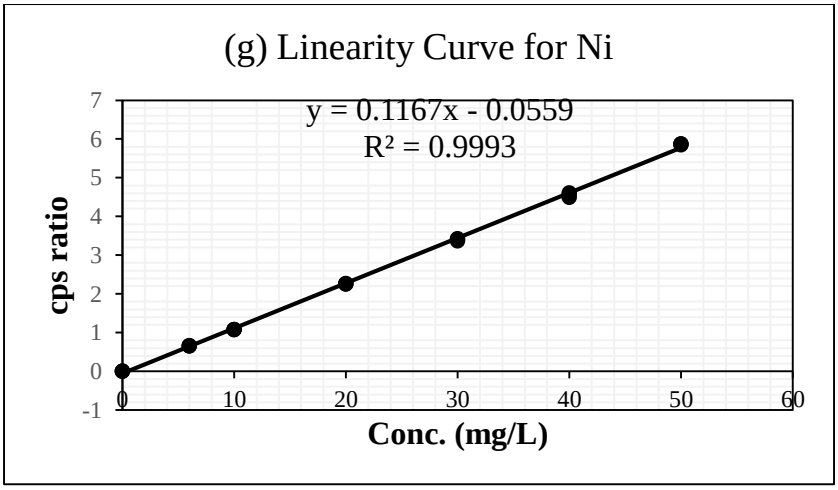

Figure 7: Linearity Curve for Ni

Element	Class (as per ICH Q3D)	Allowable Concentration (mg/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 1: Allowable Concentration of Elements of Toxicological Concern

Level	Amount of calibration standard stock solution to be added (mL)	Final volume (mL) makes up with 2% nitric acid	Calibration standard Concentration (µg/L)						
			Pb	Cd	As	Hg	Co	V	Ni
0%	0.0	25	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30%	0.3	25	1.5	1.5	4.5	9.0	15.0	30.0	60.0
50%	0.5	25	2.5	2.5	7.5	15.0	25.0	50.0	100.0
100%	1.0	25	5.0	5.0	15.0	30.0	50.0	100.0	200.0
150%	1.5	25	7.5	7.5	22.5	45.0	75.0	150.0	300.0
200%	2.0	25	10.0	10.0	30.0	60.0	100.0	200.0	400.0
250%	2.5	25	12.5	12.5	37.5	75.0	125.0	250.0	500.0

Table 2: Calibration Standard 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

Table 3: Spiked Sample Preparation

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 (ºC)	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

Table 4: ICPMS Instrument Parameters

[a] RF: Radiofrequency; [b] QP: Quadrupole; [c] OctP: Octapole

Element	Estimated LOD values			Practical LOQ values			CV%	Maximum permissible limits (mg/L) ICH Q3D
	Standard Deviation (S)	µg/L	mg/L	µg/L	mg/L	Mean Conc.± S		
Cd	1187	0.25	0.02	1.5	0.15	0.16 ± 0.001	1.11	0.5
Hg	8824	0.82	0.08	9.0	0.90	0.94 ± 0.002	2.17	3.0
Pb	16135	0.16	0.02	1.5	0.15	0.16 ± 0.003	3.00	0.5
As	514	0.58	0.06	4.5	0.45	0.46 ± 0.003	3.50	1.5
V	21401	3.05	0.31	30	3.00	3.01 ± 0.024	23.58	10.0
Co	29246	1.66	0.17	15	1.50	1.50 ± 0.010	10.28	5.0
Ni	32412	7.23	0.72	60	6.00	6.10 ± 0.023	23.49	20.0

Table 5: Data sheet for Estimated LOD & Practical LOQ

Sample	%Recovery						
	V	**Co**	**Ni**	**As***	**Cd**	**Hg**	**Pb**
LOQ spiked-1	92.8	93.0	92.5	130.2	100.7	99.2	94.3
LOQ spiked-2	92.7	92.5	93.8	134.8	101.4	98.4	94.0
LOQ spiked-3	94.5	92.1	94.4	134.9	100.4	98.8	93.6
100% spiked-1	101.8	101.8	100.4	137.5	103.5	102.6	97.3
100% spiked-2	99.8	99.8	99.1	136.6	102.2	101.8	96.0
100% spiked-3	102.1	102.3	101.2	139.0	103.1	102.6	94.4
150% spiked-1	97.0	97.0	96.3	130.3	97.5	98.1	91.0
150% spiked-2	96.2	95.7	95.9	129.2	98.0	97.9	90.9
150% spiked-3	97.1	97.0	95.4	133.7	98.3	98.0	90.9
	Mean %Recovery						
LOQ Level	93.3	92.5	93.6	133.3	100.8	98.8	94.0
100% Level	101.2	101.3	100.2	137.7	102.9	102.3	95.9
150% Level	96.8	96.6	95.9	131.1	97.9	98.0	91.0

Table 6: Data sheet for Recovery

Element	Result (mg/L)												
	As such Sample-1	**As such Sample-2**	**As such Sample-3**	**Mean**	**Spiked Sample-1**	**Spiked Sample-2**	**Spiked Sample-3**	**Spiked Sample-4**	**Spiked Sample-5**	**Spiked Sample-6**	**Mean**	**SD**	**RSD**
V	0.3	0.3	0.3	**0.3**	9.4	9.4	9.5	9.5	9.5	9.6	**9.5**	**0.08**	**0.88**
Co	0.1	0.1	0.1	**0.1**	4.7	4.7	4.7	4.7	4.7	4.7	**4.7**	**0.03**	**0.68**
Ni	0.5	0.5	0.5	**0.5**	18.5	18.5	18.7	18.5	18.5	19.0	**18.6**	**0.18**	**0.98**
As	0.0	0.0	0.0	**0.0**	2.1	2.1	2.1	2.1	2.1	2.1	**2.1**	**0.01**	**0.64**
Cd	0.0	0.0	0.0	**0.0**	0.5	0.5	0.5	0.5	0.5	0.5	**0.5**	**0.00**	**0.84**
Hg	ND	ND	ND	**ND**	2.9	2.9	2.9	2.8	2.9	2.9	**2.9**	**0.03**	**1.00**
Pb	0.0	0.0	0.0	**0.0**	0.5	0.5	0.5	0.5	0.5	0.5	**0.5**	**0.01**	**1.43**

Table 7: Data sheet for Repeatability/ Precision

Element	Linear range (mg/L)	Slope	Intercept	Determination coefficient
Cd	0.15 – 1.25	0.0125	-0.0007	0.9990
Hg	0.90 – 7.50	0.0033	0.0027	0.9994
Pb	0.15 – 1.25	0.0310	-0.0006	0.9992
As	0.45 – 3.75	0.0023	-0.0003	0.9993
V	3.00 – 25.00	0.0183	-0.0500	0.9991
Co	1.50 – 12.50	0.0459	-0.0570	0.9995
Ni	6.00 – 50.00	0.0117	-0.0559	0.9993

Table 8: Data sheet for linearity

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Glossary

LOD- The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOQ- The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

ICPMS- Inductively coupled plasma Mass Spectrometer is an instrument to identify and quantify elemental impurities based on their mass to charge ratio.

ICPOES- Inductively coupled plasma Optical Emission Spectroscopy is an instrument to identify and quantify elemental impurities based on their spectral lines.