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DETERMINATION OF ELEMENTAL IMPURITIES IN CLOMIPRAMINE HYDROCHLORIDE CAPSULES USP 25MG, 50MG AND 75MG BY INDUCTIVE COUPLED PLASMA MASS SPECTROMETRY (ICP-MS).

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Abstract:

Determination of elemental impurities "Cadmium, Lead, Arsenic, Mercury, Cobalt, Vanadium and Nickel" in Clomipramine Hydrochloride capsules USP by Inductive coupled plasma mass spectrometer (ICP-MS). The Microwave digester system, Nitric acid and Hydrogen peroxide were used for sample preparation. specified limit of elemental impurities in Clomipramine Hydrochloride Capsule USP (25mg, 50mg, 75mg,) are taken from ICHQ3D. The validation parameters and acceptance criteria were carried out in accordance with USP general chapter <232), <233> and ICHQ3D. During validation specific, precise, linear, accurate, rugged and LOD-LOQ parameters were performed. Range of the validated method is LOQ to 200% of specification level for each elemental Impurities. The limit of detection and limit of quantification are 8.33% and 25% of specification level for each elemental impurity. The validated method was effectively useful to determine the elemental impurities in Clomipramine Hydrochloride capsules USP

***Keywords**: Inductive coupled plasma mass spectrometer (ICP-MS), Clomipramine Hydrochloride Capsule USP, Elemental impurities, ICH guideline.*

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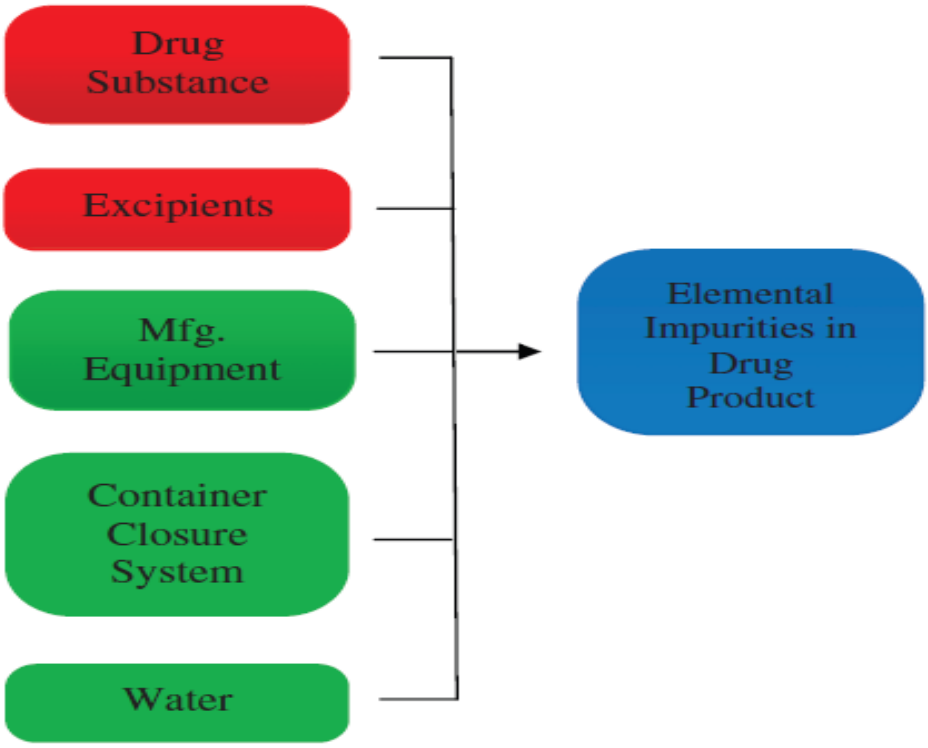
INTRODUCTION:

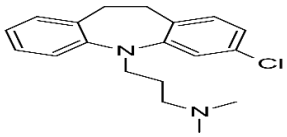
Clomipramine, is used for the treatment of obsessive–compulsive disorder, panic disorder, major depressive disorder, Chronic pain and premature ejaculation. Elemental impurities in drug products may arise from several sources; they may be residual catalysts that were added intentionally in synthesis 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. Based on the toxicity (PED), the elemental impurities were classified into three classes. Class 1: The elements, As, Cd, Hg, and Pb, are human toxicants that have limited or no use in the manufacture of pharmaceuticals. Class 2: Elements in this class are generally considered as route-dependent human toxicants. Class 2 elements are further divided in sub-classes 2A and 2B based on their relative probability of occurrence in the drug product. Class 3: The elements in this class have relatively low toxicities by the oral route of administration (> 500 µg/day) but may require consideration in the risk assessment for inhalation and parenteral routes. For oral routes of administration, unless these elements are intentionally added, they do not need to be considered during the risk assessment. Several acute and chronic toxic effects of heavy metals affect different body organs. Gastrointestinal and kidney dysfunction, nervous system disorders, skin lesions, vascular damage, immune system dysfunction, birth defects, and cancer.

The presence of trace levels of elemental impurities is of special concern to global regulators. As a result, USFDA and other regulatory agencies have taken steps to address the issue of elemental impurities in pharmaceuticals products. Detection and quantification of these trace level elemental impurities in active pharmaceutical ingredient and drug products can be challenging and necessitates the use of advanced and sensitive tools to meet regulatory requirements.



Impurity guidelines have been developed by international Conference on Harmonization (ICH). ICH M7 (R1) assessment and control of mutagenic impurities in pharmaceuticals to limit potential carcinogenic risk. Depending on their potential risk to human health. ICH Q3D is currently published and will include elements and limits for heavy metal impurities. The elemental impurities considered unsafe at specific level. The limit for elemental impurities with an understood toxicity can be calculated based upon the know PDE. Clomipramine Hydrochloride Capsule use as **Oral Dosage and as per ICHQ3D**, class 1 (Pb, Cd, As, Hg) and class 2A (Co, V, Ni) elements are evaluated in this paper.

Chemical Structure of Clomipramine:

Clomipramine**: ($C_{19}H_{23}ClN_2$)** ***Molecular weight:*** 314.86 g·mol^{-1}	

Experimental Methodology:

Instrumentation

Inductive coupled plasma mass spectrometry (ICP-MS) with Auto sampler (Perkin Elmer -2000B). All the weighing in the experiments was done with Mettler toledo electronic balance (Mettler Toledo / XSE 205) capable of measuring with an accuracy of 0.01 mg and during solution preparations Eppendorf Micropipettes (research plus) were used.

Chemicals and Reagents: Following chemicals and standards were used during the validation studies:

Sr. No.	Name of the material	Grade	Make
1.	Nitric Acid	Super pure	CarloErba
2.	Hydrogen peroxide	OPTIMA	Fisher Chemical
3.	Water	LC-MS	Fisher Chemical
4.	Cadmium	ICP/MS	Inorganic Ventures
5.	Lead	ICP/MS	Inorganic Ventures
6.	Arsenic	ICP/MS	Inorganic Ventures
7.	Mercury	ICP/MS	Inorganic Ventures
8.	Cobalt	ICP/MS	Inorganic Ventures
9.	Vanadium	ICP/MS	Inorganic Ventures
10.	Nickel	ICP/MS	Inorganic Ventures
11.	Scandium	ICP/MS	Inorganic Ventures
12.	Yttrium	ICP/MS	Inorganic Ventures
13.	Bismuth	ICP/MS	Inorganic Ventures
14.	Gold	ICP/MS	Inorganic Ventures

Instrument Conditions for ICP/MS: The instrumental conditions are given below:

Sr. No.	Instrument Parameter	Condition / Input
1.	Instrument	Perkin Elmer NexION 2000 B
2.	Carrier Gas	Argon
3.	Timing Parameters	Sweeps / Reading: 40 Readings/Replicate : 1 Number of Replicates : 3
4.	Mode	KED, Cell Gas 4.5 for all Selected elements
5.	Set Internal Standard	Sc For :-V, Co, & Ni
		Y For :- As & Cd
		Bi For :- Hg & Pb
6.	Sample unit	ppb
7.	Standard Unit	ppb
8.	Dwell time	50.0 ms : For- All element
9.	Processing Parameter	Default input
10.	Equation Parameters	Default input
11.	Calibration Parameters	Mass of analyte, Mass
12.	Curve type	Linear thru Zero
13.	Sampling Device	Auto sampler

Mass values for Elemental standard and Internal standard as mentioned below.

Mass of Elements Standard						
V	**Co**	**Ni**	**As**	**Cd**	**Hg**	**Pb**
51	59	60	75	111	202	208

Mass of Internal standard		
Sc	**Y**	**Bi**
45	89	209

Sampling Parameters:

	Time (Sec)	Speed (+ / - rpm)
Sample flush	120	-42
Read Delay	35	-35
Analysis	-	-35
Wash	120	-42

Preparation of diluent: Transfer 30.0 mL of Concentrated Nitric acid into a 500 mL volumetric flask containing about 200 mL of LC-MS grade water. Dilute to volume with LC-MS grade water and mix thoroughly.

Preparation Internal standard stock solution: Transfer 0.1 mL of each Scandium, Yttrium and Bismuth standard solution (about 1000 ppm) into a 50 mL volumetric flask and make up the volume with diluent and mix thoroughly.

Preparation of standard stock solutions: Transfer 150µl of Arsenic standard solution, 50µl of Cadmium standard solution, 50µl of Lead standard solution, 300µl of Mercury standard solution and 500µl of Cobalt standard solution into a 10mL volumetric flask and dilute the volume with diluent. Label this solution as **standard stock solutions-1.** Now Transfer 125µl of Nickel standard solution, 62.5µl of Vanadium standard solution and 625µl of standard stock solutions-1 into a 25mL volumetric flask and dilute the volume with diluent. Label this solution as **standard stock solutions-2.**

Preparation of Calibration standard solutions.

Name of element	Concentration (in ppb)	Final Volume (mL)	Calibration standard					Specification Limit (ppm)
			Std-1	Std-2	Std-3	Std-4	Std-5	
			Add volume of standard stock Solution -2 (mL)					
			0.125	0.250	0.500	0.750	1.00	
			Level-25%	Level-50%	Level-100%	Level-150%	Level-200%	
Cd		Up to 25mL	1.25	2.5	5	7.5	10	NMT 0.5 ppm
Pb			1.25	2.5	5	7.5	10	NMT 0.5 ppm
As			3.75	7.5	15	22.5	30	NMT 1.5 ppm
Hg			7.5	15	30	45	60	NMT 3 ppm
Co			12.5	25	50	75	100	NMT 5 ppm
V			25	50	100	150	200	NMT 10 ppm
Ni			50	100	200	300	400	NMT 20 ppm

Add 50µl of Gold standard stock solution (1000ppm) and 125µl internal standard stock Solution to each level of calibration standard before make up to the volume with diluent.

Preparation Blank solution: Accurtaely Pipette and transfer 250µl of internal standard stock solution and 100µl gold standard stock solution into a 50 mL volumetric flask. Make up the volume with diluent and mix thoroughly.

Preparation sample Blank solution: Prepare as same as sample solution preparation without adding sample.

Preparation sample solution:

Take 1capsule for the analysis of Clomipramine Hydrochloride Capsule USP 75mg,

Take 1capsule for the analysis of Clomipramine Hydrochloride Capsule USP 50mg,

Take 3capsule for the analysis of Clomipramine Hydrochloride Capsule USP 25mg.

Weigh the capsules and transferred in to sample digestion vessel, add 2.0 mL Hydrogen peroxide, 4.0 mL Nitric acid and gold standard stock solution#. keep solution at least 30 min for pre-digestion. Then close the digestion vessel and place in to microwave sample digestion system, digest the sample by using Sample digestion program for Microwave Digestion System.

#For Clomipramine Hydrochloride Capsule USP 75mg: Add 90µl gold standard stock solution.

#For Clomipramine Hydrochloride Capsule USP 50mg: Add 60µl gold standard stock solution.

#For Clomipramine Hydrochloride Capsule USP 25mg: Add 100µl gold standard stock solution.

After completion of digestion cycle, open the digestion vessel slowly after cool down up to the room temperature and the remove gases, then transfer digested sample in 50 mL Centrifuge Tube.

For Clomipramine Hydrochloride Capsule USP 75mg: Add 225µl internal standard stock solution. dilute to volume with LC-MS grade water (Some milky solution may appear) make Up to 45mL mark.

For Clomipramine Hydrochloride Capsule USP 50mg: Add 150µl internal standard stock solution. dilute to volume with LC-MS grade water (Some milky solution may appear) make Up to 30mL mark.

For Clomipramine Hydrochloride Capsule USP 25mg: Add 250µl internal standard stock solution. dilute to volume with LC-MS grade water (Some milky solution may appear) make Up to 50mL mark.

Centrifuge the sample solution at 5000 RPM for 10 min. After complete cycle of centrifuge take the supernatant (Clear solution) part of sample solution and filter through 0.45µm PTFE syringe filter and filtered solution shall be use for analysis.

Microwave Digestion program:

Temperature [°C]	Pressure [bar]	Ramp (Min.)	Hold	Power [%]
150	30	2	5	70
190	30	2	10	80
210	30	2	15	90

System suitability acceptance criteria:

Correlation coefficient of calibration standard solution should not be less than 0.995 for each element.

% drift variation of standard check Solution-After and before should be within ± 20% for each element.

% Drift Calculation:

$$\%\mathbf{Drift} = \left(\frac{\mathbf{A}}{\mathbf{B}}\mathbf{X}\ \mathbf{100}\right) - \mathbf{100}$$

A= Concentration(ppb) of 150% level calibration Standard After sample introduction.

B=Concentration(ppb) of 150% level calibration Standard Before sample introduction

Concentration of the analyte in the sample:

Conc of elemental in sample (ppm) = $\frac{(C_S - C_{RB})}{1000} \times DF$

CS = Observed concentration in ppb of individual elemental impurity in sample.

CRB = Observed concentration in ppb of individual elemental impurity in Sample blank.

DF = Dilution factor {Ratio of Diluent Volume (mL) /Sample wt. (g)}.

VALIDATION OF ELEMENTAL IMPURITIES BY ICP-MS METHOD

Specificity:

Specificity was determined by aspirating 10 replicate blanks, LOD solution and evaluated for obtained intensity at the specified mass no. of each element in blanks solution and compared with LOD solution. The response of analyte in blank at particular mass should not be more than the response of analyte in LOD level standard solution at particular mass

Sr. No.	Blank	**Intensity of elements in LOD standard solution**						
		Vanadium	**Cobalt**	**Nickel**	**Arsenic**	**Cadmium**	**Mercury**	**Lead**
		40762	63827	73423	725	1037	14311	17598
		Intensity of elements in blank solution						
1.	Replicate-1	8	12	47	3	4	160	205
2.	Replicate-2	8	14	44	3	3	137	200
3.	Replicate-3	7	15	48	3	4	124	201
4.	Replicate-4	8	13	55	2	5	111	197
5.	Replicate-5	9	11	53	3	2	102	202
6.	Replicate-6	8	12	49	3	4	98	208
7.	Replicate-7	6	12	52	3	4	99	202
8.	Replicate-8	7	11	49	5	4	78	202
9.	Replicate-9	8	10	45	3	4	75	200
10.	Replicate-10	10	10	54	4	4	80	212

The response of analyte in blank at particular mass found less than the response of analyte in LOD level standard solution at particular mass.

Limit of detection and limit of quantitation (LOD & LOQ) determination:

Limit of detection (LOD) was determined through prediction linearity from 25% to 200% concentration of each elemental impurity with respect to specification limit by using regression plot of intensity of elements against concentration of standard.

Plotted a graph of response of analyte (at Y-axis) versus Concentration (at X-axis). Measured the residual standard deviation of response and slope through regression technique from the linearity data. Calculate the limit of detection and limit of quantitation by using the following formula.

Calculate the limit of detection by using the following formula.

$$\text{LOD (in ppb)} = \frac{3.3 \times \text{Residual standard deviation of response (STEYX)}}{\text{Slope}}$$

Calculate the limit of quantitation by using the following formula.

$$\text{LOQ (in ppb)} = \frac{10 \times \text{Residual standard deviation of response (STEYX)}}{\text{Slope}}$$

Name of Element	LOD (Std-Conc.)	LOD (w.r.t. sample)	LOQ (Std-Conc.)	LOQ (w.r.t. sample)
	ppm			
Vanadium	0.00833	0.83	0.02500	2.50
Cobalt	0.00417	0.42	0.01250	1.25
Nickel	0.01667	1.67	0.05000	5.00
Arsenic	0.00125	0.13	0.00375	0.38
Cadmium	0.00042	0.04	0.00125	0.13
Mercury	0.00250	0.25	0.00750	0.75
Lead	0.00042	0.04	0.00125	0.13

LOD precision:

Precision of LOD was established by six replicate injections of concentration equivalent to LOD as determined in determination of limit of detection and the relative standard deviation was calculated for each element.
The average net intensity/ratio at LOD level should be significant above the blank net intensity/ratio in all six replicates of LOD level standard solution and the percent relative standard deviation of each element for LOD concentration shall not be more than 33%.

Sr. No.	Blank	**Intensity of elements in blank solution**						
		Vanadium	**Cobalt**	**Nickel**	**Arsenic**	**Cadmium**	**Mercury**	**Lead**
		5	**6**	**57**	**2**	**3**	**65**	**55**
		Intensity of elements in standard solution						
1.	Replicate-1	44029	70520	78997	759	1077	14693	18314
2.	Replicate-2	43847	69231	78925	784	1084	14717	18460
3.	Replicate-3	43845	69387	78817	741	1093	14677	18303
4.	Replicate-4	43748	68848	79072	759	1097	14621	18404
5.	Replicate-5	43807	69478	77996	766	1089	14680	18381
6.	Replicate-6	43696	69687	78672	757	1087	14709	18448
Avg. intensity		43829	69525	78747	761	1088	14683	18385

The net intensity at LOD level standard solution was observed significantly above from the blank net intensity in all six replicates of LOD level standard solution.

The percent relative standard deviation of each element for LOD concentration are given below and %RSD of each element for LOD level standard concentration are within acceptance criteria.

Sr. No.	LOD Concentration (ppb)						
	V	Co	Ni	As	Cd	Hg	Pb
1	8.198	4.115	16.363	1.280	0.421	2.561	0.426
2	8.236	4.075	16.492	1.307	0.418	2.543	0.426
3	8.320	4.126	16.642	1.242	0.425	2.559	0.426
4	8.197	4.042	16.481	1.286	0.431	2.537	0.426
5	8.161	4.056	16.166	1.284	0.423	2.531	0.423
6	8.211	4.104	16.447	1.267	0.421	2.551	0.427
Average	8.221	4.086	16.432	1.278	0.423	2.547	0.426
SD	0.054	0.034	0.159	0.022	0.004	0.012	0.001
% RSD	**0.7**	**0.8**	**1.0**	**1.7**	**1.1**	**0.5**	**0.3**

LOQ precision:

Precision of LOQ was established by six replicate injections of concentration equivalent to LOQ as determined in determination of limit of quantitation and relative standard deviation was calculated. The percent relative standard deviation of each element for LOQ concentration not more than 20%.
The calculated LOQ shall be less than 50% of the specification limit. If the calculated LOQ is higher than LOQ shall be considered at lower side and shall be established based on accuracy study. If acceptable accuracy is not achieved at calculated LOQ level than higher concentration shall be considered for LOQ, till acceptable accuracy is not achieved.

Sr. No.	LOQ Concentration (ppb)						
	V	Co	Ni	As	Cd	Hg	Pb
1	24.078	11.985	47.865	3.724	1.228	7.367	1.235
2	24.295	12.050	48.434	3.695	1.208	7.375	1.241
3	24.186	11.948	48.038	3.679	1.225	7.406	1.243
4	24.354	12.000	48.331	3.716	1.228	7.419	1.228
5	24.286	11.967	47.955	3.671	1.231	7.405	1.240
6	24.190	12.041	47.812	3.800	1.246	7.467	1.249
Average	24.232	11.999	48.073	3.714	1.228	7.407	1.239
SD	0.099	0.040	0.254	0.047	0.012	0.036	0.007
% RSD	**0.4**	**0.3**	**0.5**	**1.3**	**1.0**	**0.5**	**0.6**

The percent relative standard deviation of each element for LOQ level concentration are within acceptance criteria.
Accuracy at LOQ level:

Accuracy study at LOQ level was performed by spiking, known amount of LOQ standard stock solution at established LOQ level of elements into the sample. Accuracy solutions were prepared in triplicate for LOQ concentration level as per procedure defined in methodology and known amount of LOQ standard stock solution was spiked.

The % recovery of known analyte element is calculated against the actual quantity added for each element. The Accuracy of spiked samples at predicted LOQ level should be within 50.0 to 150.0%. The % Recovery at LOQ level is found within specified acceptance criteria

Accuracy at LOQ level	Vanadium	Cobalt	Nickel	Arsenic	Cadmium	Mercury	Lead
Preparation -1	106.8	105.6	101.2	96.5	100.0	101.9	106.4
Preparation -2	105.3	103.3	100.4	96.5	98.4	101.6	104.8
Preparation -3	105.8	104.5	101.1	96.0	97.6	101.1	104.8
Avg. % recovery	106.0	104.5	100.9	96.3	98.7	101.5	105.3

System Precision:

System precision was determined by aspirating six replicates of standard solution (at specification level) as per methodology. The percent relative standard deviation was calculated from intensity for each element in six replicates of standard solution at specification level and system suitability also calculated. The acceptance criteria for system suitability is given in methodology and the acceptance criteria for system precision is the % RSD for average net intensity/ratio of intensities of six replicate aspiration of standard preparation should not be more than 20.

System Suitability				
Element	Correlation coefficient		% drift variation	
	Acceptance Criteria	Result	Acceptance Criteria	Result
Vanadium	NLT 0.995	1.000	±20	0.5
Cobalt		1.000		1.2
Nickel		1.000		0.8
Arsenic		1.000		-2.2
Cadmium		1.000		0.3
Mercury		1.000		0.0
Lead		1.000		-0.2

Standard solution	System precision (intensity of standard solution)						
	V	Co	Ni	As	Cd	Hg	Pb
Replicate-1	452520	709498	794574	7629	10933	153868	195141
Replicate-2	447657	704818	794306	7486	10961	153310	192877
Replicate-3	446922	702236	789735	7435	10753	152142	191613
Replicate-4	446306	699827	794111	7515	10908	153018	193959
Replicate-5	442611	699836	787873	7514	10913	152197	191575
Replicate-6	440308	699155	790246	7462	10825	151819	192113
Average	446054.000	702561.667	791807.500	7506.833	10882.167	152725.667	192879.667
SD	4244.207	3995.569	2878.054	67.301	77.934	796.747	1426.392
%RSD	**1.0**	**0.6**	**0.4**	**0.9**	**0.7**	**0.5**	**0.7**

The system suitability and percent relative standard deviation (%RSD) for average intensities of six replicate of standard solution for each element found within acceptance criteria.

Method Precision:

Method precision was determined by analyzing six spiked sample preparations as per methodology representing a single batch and percent relative standard deviation (%RSD) of the results for each element were calculated. The % RSD for results of six spiked sample preparation for method precision should not be more than 20 % for each element.

Sr. No.	Method Precision Results (in ppm)						
	V	Co	Ni	As	Cd	Hg	Pb
1	10.153	4.907	19.400	1.411	0.480	3.070	0.539
2	10.106	4.956	19.596	1.436	0.492	3.094	0.539
3	10.211	4.976	19.646	1.429	0.488	3.101	0.547
4	10.039	4.907	19.284	1.438	0.485	3.078	0.539
5	10.091	4.897	19.268	1.425	0.484	3.022	0.533
6	9.948	4.826	19.140	1.423	0.479	3.003	0.527
Average	10.091	4.912	19.389	1.427	0.485	3.061	0.537
SD	0.091	0.052	0.198	0.010	0.005	0.040	0.007
% RSD	**0.9**	**1.1**	**1.0**	**0.7**	**1.0**	**1.3**	**1.3**

The % RSD of obtained results of six spiked samples preparation for method precision found within acceptance criteria.

Intermediate Precision:

Intermediate precision was determined by analyzing six spiked sample preparations as per protocol and method representing a single batch by different analyst on different day and percent relative standard deviation (%RSD) of the results for each element were calculated. The % RSD for results of six spiked sample preparation for method precision should not be more than 20 % for each element and cumulative % RSD for results of 12 spiked sample preparation for method precision and intermediate precision should not be more than 20% for each element.

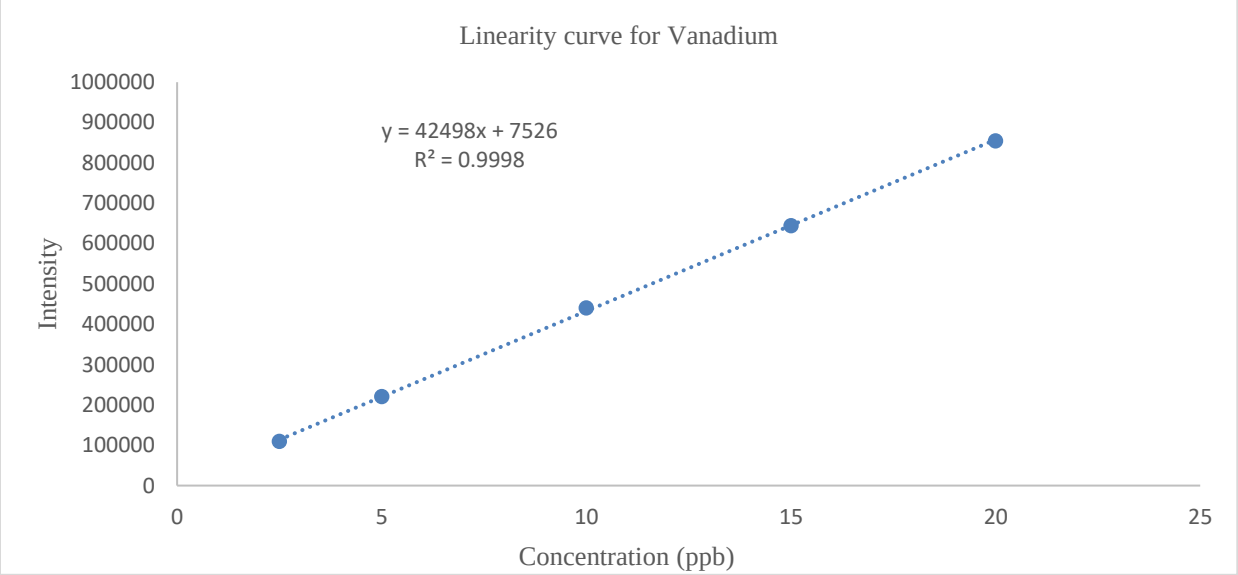
Sr. No.	Intermediate Precision Results (in ppm)						
	V	Co	Ni	As	Cd	Hg	Pb
1	10.530	5.189	20.304	1.387	0.499	3.036	0.549
2	10.152	4.977	19.308	1.358	0.478	2.923	0.534
3	10.569	5.157	19.955	1.463	0.487	3.000	0.553
4	10.503	5.154	19.968	1.467	0.486	2.964	0.553
5	10.506	5.117	19.862	1.399	0.485	2.985	0.559
6	10.537	5.114	19.826	1.441	0.481	2.974	0.549
Average	10.466	5.118	19.871	1.419	0.486	2.980	0.550
SD	0.156	0.075	0.323	0.044	0.007	0.038	0.008
% RSD	**1.5**	**1.5**	**1.6**	**3.1**	**1.5**	**1.3**	**1.5**

The % RSD for results of six spiked sample preparation for intermediate precision found within acceptance criteria for each element and the cumulative % RSD for results of 12 spiked sample preparation for method precision and intermediate found within acceptance criteria for each element.

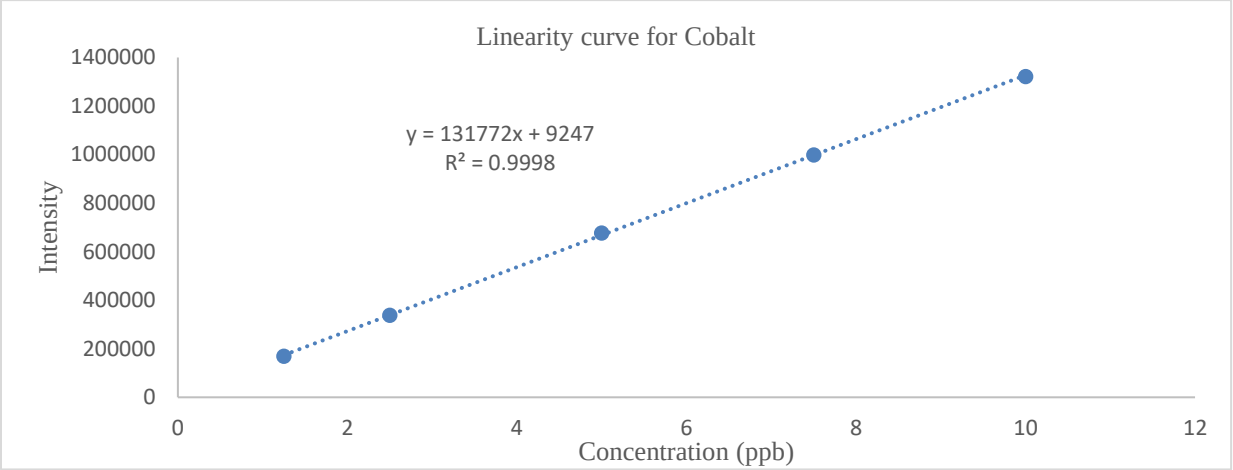
Linearity:

Linearity study was performed by aspirating five standard solutions in the range of LOQ to 200% of specification limit.
Linearity solutions are prepared as per procedure define in methodology (calibration curve). A Linearity curve was plotted
Plotted a graph from response of analyte (at Y-axis) versus Concentration of linearity standards (at X-axis) and calculate the Squared correlation coefficient, slope and y-intercept through regression technique from the linearity data. The Squared Correlation coefficient should not be less than 0.990.

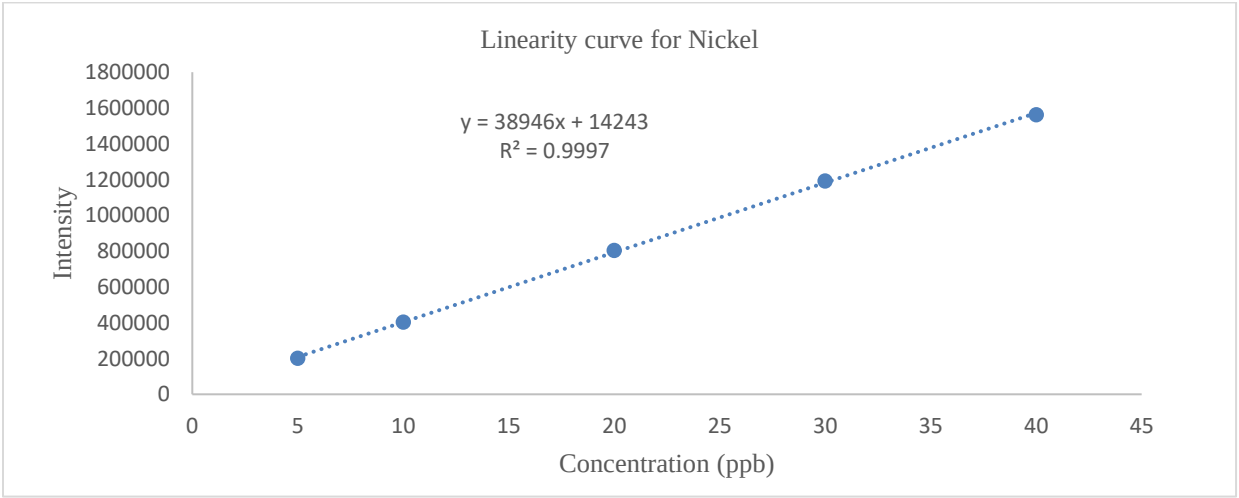
Vanadium			
Sr. No.	**Linearity Conc. level**	**Concentration (ppb)**	**Intensity**
1	LOQ (25%) Level	2.500	109356
2	50% Level	5.000	220294
3	100% Level	10.000	440095
4	150% Level	15.000	644424
5	200% Level	20.000	854595



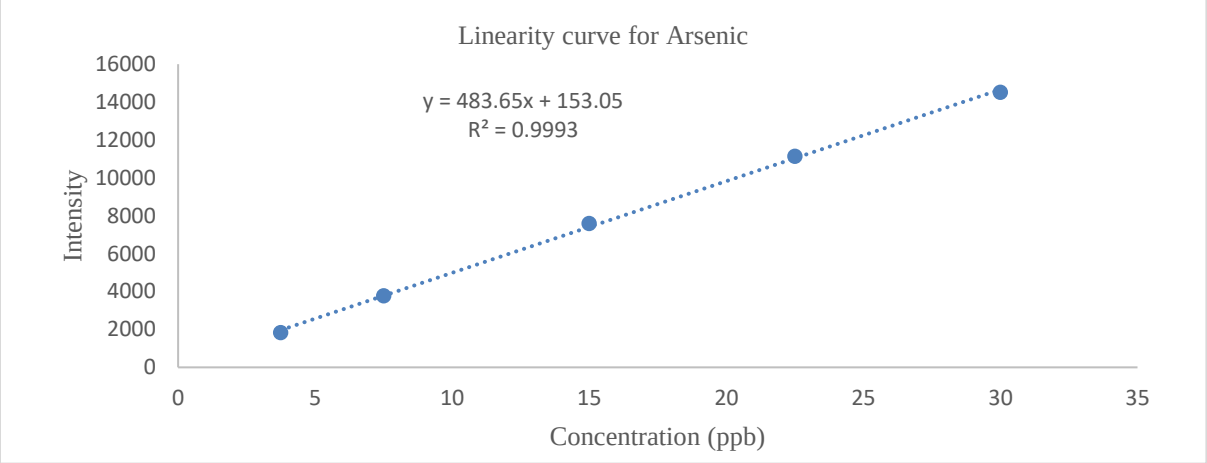
Cobalt			
Sr. No.	**Linearity Conc. level**	**Concentration (ppb)**	**Intensity**
1	LOQ (25%) Level	1.250	168700
2	50% Level	2.500	338168
3	100% Level	5.000	677449
4	150% Level	7.500	998796
5	200% Level	10.000	1322136



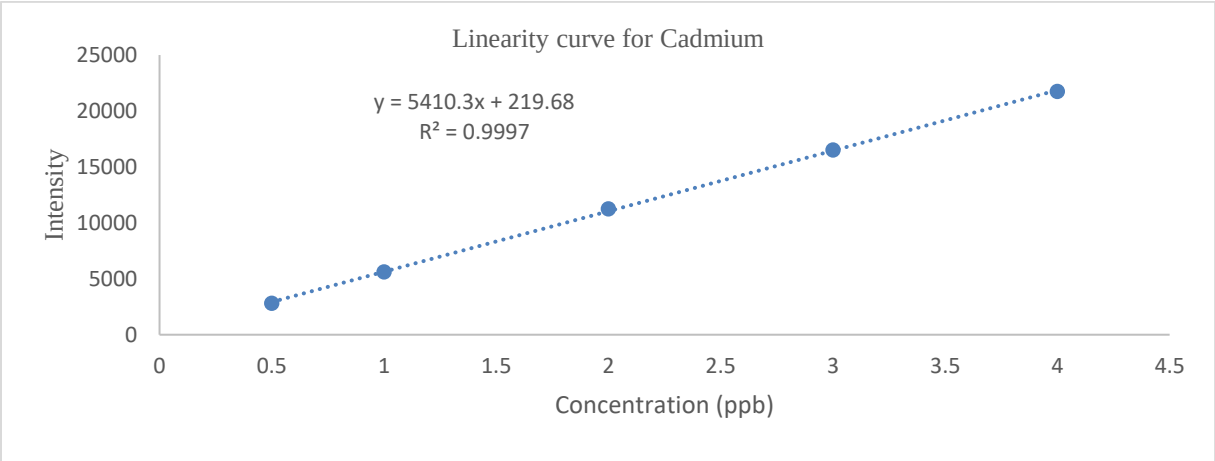
Nickel			
Sr. No.	**Linearity Conc. level**	**Concentration (ppb)**	**Intensity**
1	LOQ (25%) Level	5.000	200869
2	50% Level	10.000	403514
3	100% Level	20.000	803131
4	150% Level	30.000	1191679
5	200% Level	40.000	1561405



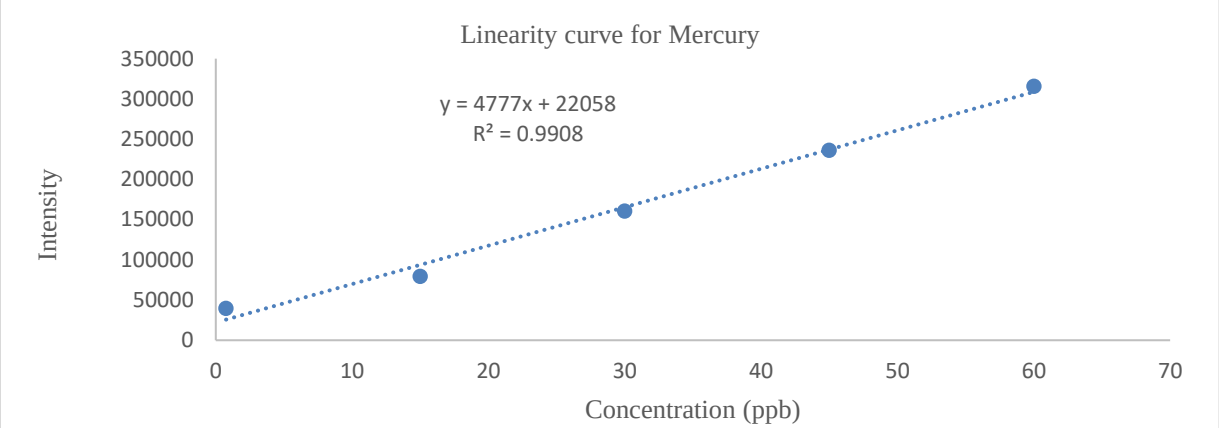
Arsenic			
Sr. No.	**Linearity Conc. level**	**Concentration (ppb)**	**Intensity**
1	LOQ (25%) Level	3.750	1840
2	50% Level	7.500	3776
3	100% Level	15.000	7584
4	150% Level	22.500	11140
5	200% Level	30.000	14513



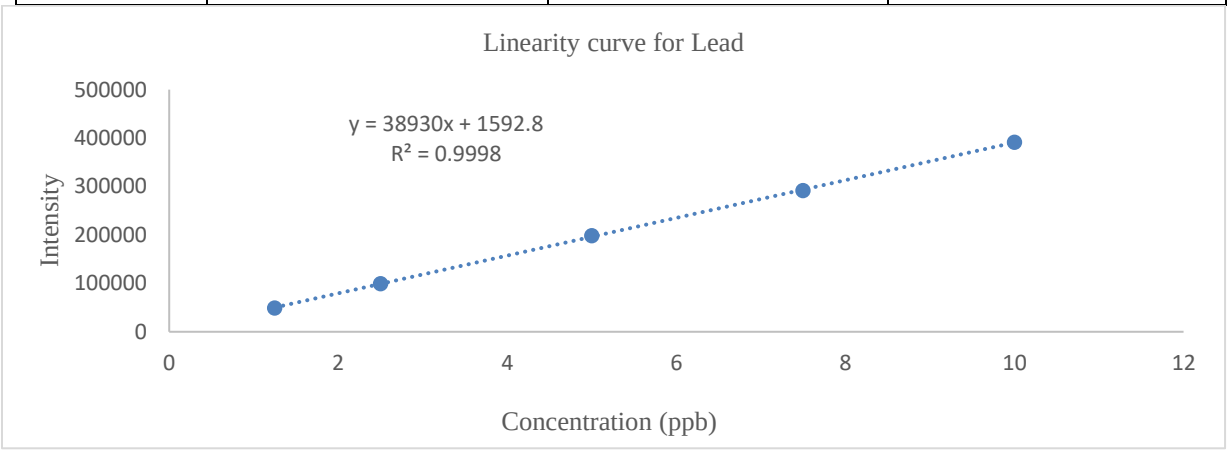
Cadmium			
Sr. No.	**Linearity Conc. level**	**Concentration (ppb)**	**Intensity**
1	LOQ (25%) Level	0.500	2803
2	50% Level	1.000	5620
3	100% Level	2.000	11240
4	150% Level	3.000	16508
5	200% Level	4.000	21736



Mercury			
Sr. No.	**Linearity Conc. level**	**Concentration (ppb)**	**Intensity**
1	LOQ (25%) Level	0.750	39399
2	50% Level	15.00	79266
3	100% Level	30.00	160362
4	150% Level	45.00	236031
5	200% Level	60.000	315363



Lead			
Sr. No.	**Linearity Conc. level**	**Concentration (ppb)**	**Intensity**
1	LOQ (25%) Level	1.250	49142
2	50% Level	2.500	99211
3	100% Level	5.000	198792
4	150% Level	7.500	291498
5	200% Level	10.000	391244



Accuracy (Recovery):

Accuracy study is performed by spiking of known amount of analyte element in to the sample at LOQ level, specification level and 150% of specification level. Accuracy solutions were prepared in triplicate for each concentration level as per procedure defined in methodology.

The % recovery of known analyte element is calculated against the actual quantity added for each solution. The % Accuracy shall be between 70.0 to 150.0 for specification level and 150.0% of specification level and 50.0 to 150.0 % for LOQ level.

Accuracy at Specification level							
Sample Preparation	**Vanadium**	**Cobalt**	**Nickel**	**Arsenic**	**Cadmium**	**Mercury**	**Lead**
Preparation -1	104.8	103.7	101.1	92.3	99.8	97.8	106.6
Preparation -2	101.1	99.4	96.2	90.3	95.6	97.4	103.6
Preparation -3	105.2	103.0	99.4	97.3	97.4	100.0	107.4
Avg. % recovery	**103.7**	**102.0**	**98.9**	**93.3**	**97.6**	**98.4**	**105.9**
Accuracy at 150% of Specification level							
Preparation -1	102.4	99.7	95.8	90.8	94.1	97.0	106.0
Preparation -2	103.8	100.3	97.6	92.5	94.5	98.2	109.3
Preparation -3	99.9	96.3	94.2	89.3	91.3	95.6	103.7
Avg. % recovery	**102.0**	**98.8**	**95.9**	**90.9**	**93.3**	**96.9**	**106.3**

Range:
Based on the data obtained for Precision, Linearity and Accuracy, the analytical method is found suitable in the range of LOQ to 150% of specification limit.

DISCUSSION:

An Inductive coupled plasma mass spectrometry (ICP-MS) method involves demonstrating specificity, which is the ability of the method to accurately measure the all elemental impurities as per **ICHQ3D for Oral Dosage** in the presence of all potential sample components. The Inductive coupled plasma mass spectrometry (ICP-MS) system was suitable for the determination of elemental impurities "Cadmium, Lead, Arsenic, Mercury, Cobalt, Vanadium and Nickel" determination in Clomipramine Hydrochloride capsules USP 25mg, 50mg and 75mg. The developed method was performed for specificity, LOD and LOQ determination, LOD-LOQ precision, method precision, intermediate precision, linearity, Accuracy and range.

CONCLUSION:

Only few methods were reported for the determination of elemental impurities by Inductive coupled plasma mass spectrometry (ICP-MS) in drug product. Based on the above results it can be concluded that method for elemental impurities "Cadmium, Lead, Arsenic, Mercury, Cobalt, Vanadium and Nickel" determination in Clomipramine Hydrochloride capsules USP 25mg, 50mg and 75mg by ICP-MS is Specific, Precise, Linear and Accurate. Hence method for the determination of elemental impurities in Clomipramine Hydrochloride capsules USP 25mg, 50mg & 75mg by ICP/MS is successfully validated and the results of all validation parameters are found well within the acceptance criteria.

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