Assessment Of Fourteen Elemental Impurities In Doxapram Hydrochloride Drug Using Inductively Coupled Plasma Mass Spectrometry

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Abstract: The existence of elemental impurities in samples of pharmaceutical substances is a concern, not just due to significant toxicity, and also due to the possible impact on drug stability, shelf - life, or undesired side effects. Elemental impurities therefore need to be carefully monitored and regulated. Metal contents in doxapram hydrochloride API were assayed by inductively coupled plasma - mass spectrometry (ICP-MS). The doxapram hydrochloride active pharma ingredient (API) was analyzed for arsenic, cadmium, mercury, lead, vanadium, cobalt, nickel, copper, molybdenum, manganese, lithium, antimony, sodium and aluminum. The validation parameters like system suitability, specificity, linearity, LOD, LOQ, robustness, accuracy, precision, ruggedness and solution stability for the ICP-MS method was checked. Three batches of doxapram hydrochloride API was accessed by the validated ICP-MS method for the content of 14 selected elements. The method exhibited linearity in range of 0.425 - 3.40 ppb (cadmium), 1.25 - 10 ppb (lead and cobalt), 3.75 - 30 ppb (arsenic), 0.750 - 6.0 (mercury), 2.5 - 20 ppb (vanadium), 5.0 - 40.0 ppb (nickel), 22.5 - 180 ppb (antimony), 75 - 600 ppb (copper), 62.50 - 500 ppb (lithium), 375 - 3000 ppb (molybdenum) and 2500 - 20000 ppb (sodium, aluminium and manganese). The validation results are inside the limitations of criteria. All metals were found to be either below the detection limits or quantification limits except nickel in doxapram hydrochloride API. Analytical results of this investigation demonstrated that ICP-MS method validated is useful for monitoring the selected 14 metal impurities present in doxapram hydrochloride API.

Index Terms: Active pharmaceutical ingredients, Metal impurities, Inductively coupled plasma mass spectrometry, Doxapram hydrochloride, Validation

1 INTRODUCTION

Pharmaceuticals, recommended by traditional and modern medicines, are generally used by humans to prohibit and manage a number of diseases. Pharmaceuticals are required to be safe, effectual and should be of good quality as per World Health Organization [1]. Metals are important components in few pharmaceuticals (Eg. cis-platinum and carboplatin - anticancer therapy; barium sulfate - X-ray contrast agent). But in majority of pharmaceuticals, metals are not desired [2]. It is realized that a few metals are amazingly poisonous for human wellbeing, even at low quantities.Based on level of wellbeing concern, ICH Q3D proposed guidelines to classify metals [3].

- Class I: Metals in this class are extensively toxic (Eg. As, Cd, Hg, Pb).
- Class II: Metals in this class are pretty much poisonous relying upon the administration route. Class II is further divided in class II A (Eg. Co, Mo, Se, V) and class II B (Eg. Ag, Au, Ir, Os, Pd, Pt, Rh, Ru, Tl).
- Class III: Metals in this class are relatively small safety concern (Eg. Ba, Cr, Cu, Li, Ni, Sb, Sn).
- Class IV: Metals in this class have less innate toxicity. For these metals, permissible daily exposure is not established. (Eg. Al, B, Ca, Fe, K, Mg, Mn, Na, Zn and W).

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Metals could be presented in the active pharmaceutical ingredient as impurities by various sources like manufacturing equipment (Eg. reactors, pipes and other equipment), naturally derived plant/mineral raw materials, excipients or reagents used at the time of synthesis, metals used as catalysts and pharmaceutical packing materials degradation during storage [4]. Detection and evaluation of potential metal impurities in various phases of the manufacturing procedures of pharmaceuticals, particularly in the ultimate product, is important to counteract potential dangers to human wellbeing [5]. European Medicines Agency regularly update guidelines on specification limits for metal impurities residues in pharmaceuticals [6]. To evaluate potentially toxic metal elements, limit test is suggested by United States Pharmacopoeia [7], British Pharmacopoeia [8], European Pharmacopoeia [9] and Japanese Pharmacopoeia [10]. These methods are used in pharmaceutical quality control testing. Severe drawbacks of these methods are the lack of specificity, sensitivity and recovery to observe the actual levels of some metals properly. Few authors have proposed techniques for metal quantification in pharmaceuticals using atomic spectroscopy [11,12], wavelength dispersive X-ray fluorescence spectrometry [13], total reflectance X-ray fluorescence [14], laser induced breakdown spectrometry [15,16], Laser ablation inductively coupled plasma mass spectrometry [17], inductively coupled plasma mass spectrometry [18], flow injection inductively coupled plasma mass spectrometry [19], inductively coupled plasma optical emission spectrometry [20] and electrothermal vaporization inductively coupled plasma optical emission spectrometry [21]. Doxapram hydrochloride, described chemically as 1-ethyl-4- (2-morpholin-4-ylethyl)- 3,3-diphenyl-pyrrolidin-2-one, is an central and peripheral respiratory excitant [22,23]. Doxapram hydrochloride increases tidal volume and respiratory rate through its activity on carotid artery chemoreceptors. The current investigation is to develop and validate a method using inductively coupled plasma mass

spectrometry technique to quantify fourteen elements (arsenic, cadmium, mercury, lead, vanadium, cobalt, nickel, copper, molybdenum, manganese, lithium, antimony, sodium and aluminum) in doxapram hydrochloride raw material. The sensitivity, selectivity and multi element analysis capabilities of inductively coupled plasma mass spectrometry technique allowed us to use this technique for the quantification of fourteen elements in doxapram hydrochloride.

2 EXPERIMENTAL SECTION

2.1 Materials

Cadmium, mercury, lead, vanadium, cobalt, nickel, copper, molybdenum, manganese, lithium, antimony, gold and sodium (each element with concentration 1000 ppm) were procured from Spexertificate (Metuchen, NJ, US). The elements arsenic and aluminum with concentration 1000 ppm each were obtained from Chem lab (Zedelgem, Belgium) and Inorganic Venture (Christiansburg, VA, USA), respectively. Analytical reagent grade nitric acid and hydrogen peroxide were acquired from Fisher scientific (Waltham, MA, USA) and Merck KGaA (Darmastadt, Germany), respectively. Milli-Q-Water used throughout the investigation was from Millipore water system (Merck Life Science Private Limited, Mumbai, India). Doxapram hydrochloride was kindly provided by GVK Biosciences private limited (Hyderabad, India).

2.2 Instrumentation

- Inductively coupled plasma mass spectrometry (Agilent, Model:7800, CA, USA)
- Analytical Balance (Sartorius, Model: Secura225D/10IN, Sartorius AG Weender Landstrasse)
- Microwave reaction system (Antonpaar, Multiwave pro, Telangana, India)
- The data was acquired using computer-based Mass Hunter software version 4.4 (Agilent Technologies, USA).

2.3 Inductively coupled plasma mass spectrometry operating conditions

The appropriate instrumental conditions are shown in Table 1 and 2.

TABLE 1
OPERATING CONDITIONS FOR THE MULTIELEMENT ANALYSIS

Acquisition parameters		*Tune conditions*	
Parameter	*Value*	*Parameter*	*Value*
Acquition Mode	*Spectrum*	*Radio frequency Power*	*1550 W*
Carrier Gas	*Argon*	*Radio frequency Matching*	*1.75 V*
Cone	*Nickel*	*Sample depth*	*10.0 mm*
Peak Pattern	*3 Points*	*Nebulizer Gas flow*	*1.00 L/min*
Replicates	*3*	*Option Gas*	*0.0 %*
Quick Scan	*ON*	*Nebulizer pump*	*0.10 rps*
Stabilization time	*10 sec*	*Spray chamber temperature*	*2°C*
Sweeps/Replicates	*100*	*Gas Switch*	*Dilution gas*
Number of masses	*14*	*Helium gas flow*	*4.3 ml/min*
Tune mode	*Helium (He)*	*Measurement units*	*CPS*

TABLE 2
MASS NUMBER AND INTEGRATION TIME OF THE SELECTED ELEMENTS

Element	*Mass number*	*Integration time/mass (sec)*
Lithium	*7*	*0.30*
Sodium	*23*	*0.30*
Aluminum	*27*	*0.30*
Vanadium	*51*	*0.30*
Manganese	*55*	*0.30*
Cobalt	*59*	*0.30*
Nickel	*60*	*0.30*
Copper	*63*	*0.30*
Arsenic	*75*	*0.30*
Molybdenum	*95*	*0.30*
Cadmium	*111*	*0.30*
Antimony	*121*	*0.30*
Mercury	*202*	*0.30*
Lead	*208*	*0.30*

2.4 Solutions preparation

2.4.1 Diluent

Forty ml of pure nitric acid was transferred to a 1000 ml volumetric flask with 200 ml of Milli-Q-Water. Mixed well and made to the volume with the similar solvent.

2.4.2 Standard blank

0.05 ml of gold standard solution (1000 ppm) was transferred to a 10 ml volumetric flask and made to the volume with diluent.

2.4.3 Wash blank

Two ml of pure nitric acid solution was transferred to a 10 ml volumetric flask and made to the volume with Milli-Q-water.

2.4.4 Multielement stock solution

The standard elements solution (1000 ppm) was diluted appropriately with diluent to prepare a multielement stock solution I with concentration 0.045 ppm (cadmium), 0.1250 ppm (lead and cobalt), 0.3750 ppm (arsenic), 0.0750 ppm (mercury), 0.25 ppm (vanadium), 0.50 ppm (nickel), 2.25 ppm (antimony), 7.50 ppm (copper), 6.25 ppm (lithium) and 37.50 ppm (molybdenum). Sodium, manganese and aluminum standard element solution (1000 ppm) was diluted appropriately with diluent to prepare a multielement stock solution II with concentration 250 ppm of sodium, manganese and aluminum.

2.4.5 Calibration standard solutions

Five calibration standard solutions of 25%, 50%, 100%, 150% and 200% level concentration were prepared by diluting multielement stock solution I, II, gold standard solution aptly with diluent. The process of dilution and

concentration of calibration standard solutions are summarized in Table 3 and 4.

TABLE 3
PREPARATION OF FIVE CONCENTRATION LEVELS OF CALIBRATION SOLUTION

Cali-bration solution	*Multi element stock solution I (ml)*	*Multi element stock solution II (ml)*	*Gold standard solution (ml)*	*Volume made with diluent (ml)*
1 (25% level)	*0.1*	*0.1*	*0.5*	*10*
2 (50% level)	*0.2*	*0.2*	*0.5*	*10*
3 (100% level)	*0.4*	*0.4*	*0.5*	*10*
4 (150% level)	*0.6*	*0.6*	*0.5*	*10*
5 (200% level)	*0.8*	*0.8*	*0.5*	*10*

TABLE 4
CONCENTRATION OF SELECTED ELEMENTS IN FIVE CALIBRATION SOLUTIONS

Element	*Concentration (ppb) of element in calibration solution at level*				
	25%	*50%*	*100%*	*150%*	*200%*
Cadmium	*0.425*	*0.85*	*1.70*	*2.55*	*3.40*
Lead	*1.25*	*2.50*	*5.00*	*7.50*	*10.00*
Cobalt	*1.25*	*2.50*	*5.00*	*7.50*	*10.00*
Arsenic	*3.75*	*7.50*	*15.00*	*22.50*	*30.00*
Mercury	*0.75*	*1.50*	*3.00*	*4.50*	*6.00*
Vanadium	*2.50*	*5.00*	*10.00*	*15.00*	*20.00*
Nickel	*5.00*	*10.00*	*20.00*	*30.00*	*40.00*
Antimony	*22.50*	*45.00*	*90.00*	*135.00*	*180.00*
Copper	*75.00*	*150.00*	*300.00*	*450.00*	*600.00*
Lithium	*62.50*	*125.00*	*250.00*	*375.00*	*500.00*
Molybdenum	*375.00*	*750.00*	*1500.00*	*2250.00*	*3000.00*
Sodium	*2500.00*	*5000.00*	*10000.00*	*15000.00*	*20000.00*
Aluminum	*2500.00*	*5000.00*	*10000.00*	*15000.00*	*20000.00*
Manganese	*2500.00*	*5000.00*	*10000.00*	*15000.00*	*20000.00*

2.4.6 Standard check solution
Calibration solution at 150% level concentration was used as check solution.

2.4.7 Specification level concentration solution (10%)
1.0 ml of 100% level calibration standard solution and 0.05 ml of gold standard solution (1000 ppm) were transferred to 10 ml volumetric flask. The mixture was diluted to 10 ml with Milli-Q-Water and mixed well.

2.4.8 Sample solution
Weighed & transferred 0.10044 gm, 0.09974 gm and 0.10020 gm of doxapram hydrochloride was precisely weighed and transferred into three individual digestion vessels. To these vessels, 2.0 ml of nitric acid, 1.0 ml of hydrogen peroxide and 0.05 ml gold standard solution (1000 ppm) were added. The mixture was mixed thoroughly and digested by microwave sample digester (conditions used: power – 1500 W; temperature – 200 ^{o}C; ramp time – 20 min; holding time – 50 min). Following digestion, the sample volume was made up to 10 ml with Milli-Q-Water in a 10 ml volumetric flask. The sample solution was filtered using 0.45 µ PVDF membrane syringe filter.

2.4.9 Sample blank
Prepared as explained in section “sample solution” without doxapram hydrochloride.

2.5.10 Spiked sample solution
This solution was prepared at four concentration levels (25%, 50%, 100% and 150%). Doxapram hydrochloride (0.1 gm) was accurately weighed and transferred to the digestion vessel. Nitric acid (2 ml), hydrogen peroxide (1.0 ml), multielement stock solution I (0.1 ml for 25%, 0.2ml for 50%, 0.4 ml for 100% and 0.6 ml for 150%), multielement stock solution II (0.1 ml for 25%, 0.2 ml for 50%, 0.4 ml for 100% and 0.6 ml for 150%) and gold standard solution (0.05 ml, 1000 ppm) were added. The mixture was mixed thoroughly and digested by microwave sample digester (conditions used: power – 1500 W; temperature – 200 ^{o}C; ramp time – 20 min; holding time – 50 min). Following digestion, the sample volume was made up to 10 ml with Milli-Q-Water in a 10 ml volumetric flask and filtered using 0.45 µ PVDF membrane syringe filter.

2.6. Calibration curves of selected elements
The linearity of selected elements was established by analyzing five calibration standard solutions of 25%, 50%, 100%, 150% and 200% level by aspirating into ICP-MS system. The operating conditions given in Table 1 were followed. The response of all the solutions was recorded as Counts per Second (CPS). The calibration curves were drawn by plotting CPS versus concentration of element. The quantities of unknown samples were determined by the equation given below, using regression analysis of calibration standards: $y = m x + c$. Where, y = analyte count, x = concentration of analyte, m = slope of the calibration curve and c = y-axis intercept value. Correlation coefficient and unknown concentration values generated from the instrument software were used for calculation and reporting.

2.7 Analysis of selected elements in doxapram hydrochloride
The sample solution as prepared in section “sample solution” was analyzed by aspirating into ICP-MS system. The operating conditions shown in Table 1 were followed. The content of 14 selected elements was determined using respective calibration curves or regression equations.

3 RESULTS AND DISCUSSION

3.1. Optimization of conditions
For all measurements, an Agilent 7800 ICP-MS system was used. The instrument was equipped with standard nickel sampling, glass nebulizer, skimmer cones, quartz spray chamber and a 2.5 mm id injector. The ICP-MS system also has a standard helium (He) mode cell gas line which efficiently removes the most common polyatomic interferences (He gas flow is 4.3 ml). During optimization of digestion process, the order of addition of HNO_3, H_2O_2, multielement stock solution I, II and gold standard solution were investigated. The trails were done with doxapram

hydrochloride sample solution (0.1 gm), 25% and 100% spiked doxapram hydrochloride samples at 100% level of elements specification limit. In all trails, the accuracy of selected elements at 25% and 100% levels were determined. The accuracy of all elements are inside the acceptance criteria using the below procedure. 2.0 ml of HNO_3, 1.0 ml of H_2O_2, 0.05 ml of gold (1000 ppm) standard solution, 0.1 ml (for 25%) or 0.4 ml (for 100%) of multielement stock solution I, II were added into digestion vessels. Digest the sample by using microwave digestion conditions like power – 1500 W, temperature – 200 ^oC, ramp time – 20 min and holding time – 50 min. After completion of digestion, solution was made up to 10 ml with Milli-Q-Water followed by filtration with 0.45µ PVDF membrane syringe filter.

3.2 Method validation

The method validation was completed considering the attributes for consideration while assessing any analytical method developed for the investigation of APIs as per the ICH guideline.

3.2.1 Specificity

Specificity of a method is the capacity to evaluate plainly the analyte within the sight of components which might be relied upon to be available. This was checked by aspirating a standard blank and10% specification level standard solution into the ICP-MS system. The CPS values for standard blank and10% specification level standard solution were determined (Table 5). The response observed for standard blank is less than 10% specification limit level concentration response for all the elements. This indicated specificity of the method.

TABLE 5
SPECIFICITY RESULTS OF THE METHOD

Element	*CPS of standard blank*	*CPS of 10% level standard solution*
Lithium	*377*	*14386*
Sodium	*309258*	*2978023*
Aluminum	*726*	*566999*
Vanadium	*268*	*13368*
Manganese	*736*	*10425123*
Cobalt	*61*	*14865*
Nickel	*366*	*15740*
Copper	*1887*	*617637*
Arsenic	*23*	*3168*
Molybdenum	*1256*	*1513603*
Cadmium	*32*	*1346*
Antimony	*41*	*106386*
Mercury	*371*	*2243*
Lead	*1073*	*20209*

3.2.2 Linearity and range

Linearity of the method is expressed in terms of variance around the slope of regression line, calculated in accordance with established mathematical relationship between the test results obtained from the analysis of impurity. Five different concentrations from 25%, 50%, 100%, 150% and 200% of working level concentration for standard solutions including standard blank was prepared as per methodology and analyzed with ICP-MS method. The response at each concentration was recorded and linear regression line was plotted (CPS of element vs. concentration of element in ppb). The correlation coefficients were calculated. The data of linear regression the calibration plots of elements indicated a good linear relationship between CPS of element and concentration of element over a wide range (Table 6). The correlation coefficient values of element's regression equation indicated high significance.

TABLE 6
LINEARITY, LOD AND LOQ RESULTS OF SELECTED ELEMENTS

Element	*Linearity (ppb)*	*y = m x + c*	*Correlation coefficient*
Lithium	*62.50 – 500*	*y = 632.5958 x + 417.80*	*0.9996*
Sodium	*2500 - 20000*	*y =2622.39 x + 348844.41*	*0.9997*
Aluminum	*2500 - 20000*	*y =529.58 x + 10941.19*	*0.9996*
Vanadium	*2.5 - 20*	*y =12775.10 x + 1361.23*	*0.9996*
Manganese	*2500 - 20000*	*y =9469.90 x + 631.15*	*0.9997*
Cobalt	*1.25 - 10*	*y =28312.97 x + 236.67*	*0.9995*
Nickel	*5.0 - 40*	*y =7510.28 x + 338.90*	*0.9994*
Copper	*75 - 600*	*y =20074.30 x + 13290.24*	*0.9992*
Arsenic	*3.75 - 30*	*y =2021.96 x+ 68.89*	*0.9993*
Molybdenum	*375 - 3000*	*y =9544.14 x + 123.33*	*0.9997*
Cadmium	*0.425 - 3.40*	*y =7879.67 x + 68.89*	*0.9996*
Antimony	*22.5 - 180*	*y =11143.34 x + 43.33*	*0.9994*
Mercury	*0.750 - 6*	*y =6445.69 x + 700.05*	*0.9997*
Lead	*1.25 - 10*	*y =32545.31 x + 2794.87*	*0.9997*

3.2.3 Limit of detection (LOD) and quantification (LOQ)

The approach adopted to determine detection limit and quantification limit was based on linearity and blank solution. For this, ten standard blank injections were made into ICP-MS system. Measured the standard deviation of ten standard blank CPS. Using standard deviation and slope of linearity curve, limit of detection and limit of quantitation for each element was calculated as follows:

LOD (ppb) = 3.3 × standard deviation for ten blank CPS/ slope of linearity curve.

LOQ (ppb) = 10 × standard deviation for ten blank CPS/ slope of linearity curve.

The obtained LOD and LOQ values established were very low and not practically achievable with the instrument sensitivity. Hence, considered the proposed LOD and LOQ values. To determine the proposed LOD and LOQ values, 8% (LOD solution) and 25% (LOQ solution) specification level multielement standard solutions were made and analyzed six times by the ICP-MS method. For LOD evaluation, CPS values of elements in 8% specification level standard solution was compared with CPS values of standard blank solution. The CPS response at LOD level observed is sufficiently higher than the response of standard blank for all the elements. For LOQ evaluation, relative standard deviation values for six CPS values of

elements at LOQ level (25%) were determined and found well within the acceptance criteria (≤ 20%). The results are presented in Table 7.

TABLE 7
LINEARITY, LOD AND LOQ RESULTS OF SELECTED ELEMENTS

Element	Calculated		Proposed	
	LOD (ppb)	LOQ (ppb)	LOD (ppm)	LOQ (ppm)
Lithium	0.194	0.587	2.0	6.25
Sodium	0.173	0.524	80	250
Aluminum	19.597	59.384	80	250
Vanadium	3.023	9.16	0.080	0.25
Manganese	26.419	80.059	80	250
Cobalt	4.433	13.435	0.040	0.125
Nickel	1.143	3.465	0.160	0.50
Copper	3.506	10.624	2.40	7.50
Arsenic	1.047	3.172	1.2	3.75
Molybdenum	12.127	36.75	12	37.50
Cadmium	3.442	10.429	0.014	0.043
Antimony	4.813	14.584	0.720	2.250
Mercury	0.589	1.784	0.024	0.075
Lead	15.045	45.591	0.040	0.125

3.2.4 Method precision

To demonstrate the method precision (repeatability), six spiked doxapram hydrochloride samples at 100% level of specification limit for respective elements were aspirated into the ICP-MS system. The % RSD for the content of each element was determined (Table 8). The %RSD values were less than 20% (acceptance limit), indicated the preciseness of the method.

3.2.5 Intermediate precision

Intermediate precision (ruggedness) expresses within laboratory variations such as different days and different analysts. The method ruggedness was checked by analyzing selected elements content in six spiked doxapram hydrochloride samples at 100% level of specification limit for respective elements by the ICP-MS method. The % RSD for the content of each selected element was calculated (Table 8). The %RSD values were less than 20% (acceptance limit), indicated the method ruggedness.

TABLE 8
REPEATABILITY AND RUGGEDNESS DATA OF THE METHOD

Element	Content of element (ppm)*	Method precision %RSD	Content of element (ppm)*	Inter-mediate precision %RSD
Lithium	24.567	1.3	26.172	1.2
Sodium	967.552	3.5	1039.194	2.1
Aluminum	966.155	2.2	1063.235	1.8
Vanadium	0.984	1.2	1.01	2.2
Manganese	984.628	2.2	1079.25	1.2
Cobalt	0.491	1.1	0.527	1.6
Nickel	1.943	4.2	1.838	2.9
Copper	29.683	6.3	31.317	1.6
Arsenic	1.572	2.0	1.735	1.9
Molybdenum	154.803	1.2	165.422	1.2
Cadmium	0.167	1.5	0.178	1.8
Antimony	7.937	2.1	9.061	1.4
Mercury	0.279	1.3	0.228	2.0
Lead	0.506	2.9	0.581	2.7

**Average of six values*

3.2.6 Accuracy

The accuracy of method is determined by analyzing three times doxapram hydrochloride samples spiked with elements at 25%, 50%, 100%, 150% of specification level concentration by the ICP-MS method. The mean percent recovery at each level was calculated. The mean percent recoveries for all the selected elements at each level were in the range 70% - 150% (acceptance limit), Table 9. Hence, proved the method's accuracy.

TABLE 9
PERCENT RECOVERY OF ELEMENTS

Element	Recovery percent of elements at			
	25%*	50%*	100%*	150%*
Lithium	99.3	101.1	98.5	101.1
Sodium	97.7	99.2	98.6	99.3
Aluminum	99.6	99.7	97.9	100.1
Vanadium	95.7	99.3	98.9	102.1
Manganese	101.1	101.4	99.6	102.1
Cobalt	97.3	99.6	98.2	101.4
Nickel	86.1	94.2	95.2	99.9
Copper	100.8	101.7	99.2	101.9
Arsenic	106.7	105.7	105.7	108.8
Molybdenum	102.2	104.2	103.4	107.1
Cadmium	103.5	99.6	98.0	101.3
Antimony	84.0	89.1	87.9	97.5
Mercury	77.8	89.1	93.7	97.3
Lead	96.3	92.5	99.9	104.2

**Average of three values*

3.2.7 Robustness

Robustness of the analytical method was established by demonstrating the reliability against deliberate changes in nitric acid and hydrogen peroxide volumes used for sample preparation. Robustness was evaluated by computing the percent variation for elements content (ppm). For this, spiked doxapram hydrochloride sample solutions at 100% specification limit for respective elements was analyzed by the ICP-MS method using following conditions:

- Condition 1: -10% change in volume (nitric acid volume - 1.8 ml; hydrogen peroxide volume - 0.9 ml)
- Condition 2: +10% change in volume (nitric acid volume – 2.2 ml; hydrogen peroxide volume – 1.1 ml)

The percent content variation of average value for selected elements are found within ±20% (Table 10). Therefore, the method's robustness was demonstrated.

TABLE 10
ROBUSTNESS INFORMATION ABOUT THE METHOD

Element	Content of element (ppm) analyzed under			% variation in Y	% variation in Z
	X	Y	Z		
Lithium	24.567	26.179	26.515	6.6	7.9
Sodium	967.552	1032.581	1035.241	6.7	7.0
Aluminum	966.155	1052.581	1071.489	8.9	10.9
Vanadium	0.984	1.001	1.015	1.7	3.2

Manganese	*984.628*	*1073.441*	*1093.31*	*9.0*	*11.0*
Cobalt	*0.491*	*0.525*	*0.533*	*6.9*	*8.6*
Nickel	*1.943*	*2.160*	*2.109*	*11.2*	*8.5*
Copper	*29.683*	*30.945*	*31.453*	*4.3*	*6.0*
Arsenic	*1.572*	*1.731*	*1.737*	*10.1*	*10.5*
Molybdenum	*154.803*	*165.301*	*168.371*	*6.8*	*8.8*
Cadmium	*0.167*	*0.179*	*0.180*	*7.2*	*7.8*
Antimony	*7.937*	*8.979*	*9.087*	*13.1*	*14.5*
Mercury	*0.279*	*0.227*	*0.228*	*-18.6*	*-18.3*
Lead	*0.506*	*0.553*	*0.570*	*9.3*	*12.6*

*X-optimized condition; Y – condition 1; Z – condition 2; *Average of five values*

3.2.8 Solution stability

The solution stability check is done to determine the period of usability or reusability (for maximum of 48 hr) of the spiked sample preparations with results of similar quality as for freshly prepared solutions. For this, spiked doxapram hydrochloride sample solutions at 100% specification limit for corresponding elements was analyzed by the ICP-MS method at initial and after 24 hr. The percent content variation of selected elements average value for 24 hr when compared to the selected elements average value at initial hour was found to be within ±20% for all elements (except mercury, whose value is nearer to ±20%), Table 11. The prepared sample solutions should therefore be injected into the analysis system as soon as possible after preparation to ensure that the ICP - MS captures maximum elemental content in the sample during analysis as per solution stability data.

TABLE 11
STABILITY INFORMATION ABOUT SAMPLES

Elements	Content of element (ppm) analyzed at		
	Initial hour*	After 24 hr	Percent variation
Lithium	24.639	35.855	4.9
Sodium	997.038	1044.265	4.7
Aluminum	981.472	1066.637	8.7
Vanadium	0.991	1.004	1.3
Manganese	997.512	1083.512	8.6
Cobalt	0.517	0.551	6.6
Nickel	2.441	2.714	11.2
Copper	29.823	31.870	6.9
Arsenic	1.590	1.699	6.9
Molybdenum	155.181	166.092	7.0
Cadmium	0.167	0.178	6.6
Antimony	7.913	8.985	13.5
Mercury	0.281	0.228	-18.9
Lead	0.535	0.606	13.3

*Average of five values

3.2.9 System suitability

System suitability check is done to prove that system is working perfectly before the analysis on ICP-MS system. System suitability check is required to be done before determination of specificity, linearity, LOD, LOQ, precision, accuracy, robustness and batch analysis of doxapram hydrochloride. For this, standard blank and calibration solutions were aspirated into the ICP-MS system. Determine the CPS values of all elements. Plotted a graph of standard CPS of element against element concentration (ppb). The correlation coefficient and percent drift value for 150% level standard solution were determined. In all situations, correlation coefficient value was greater than 0.99 and percent drift value was within ±20%. The achieved values are within the criteria of acceptance. The suitability of the system for analyzing the content of 14 elements in doxapram hydrochloride was thus demonstrated.

3.3 Determination of 14 selected elements in doxapram hydrochloride

The selected elements are determined in three batches (DOX300216, DX003D16 and DX004D16) of doxopram hydrochloride using the described ICP-MS conditions. All the elements are found to be either below the detection limit and quantification limit, except the nickel (Table 12).

TABLE 12
ASSAY OF SELECTED 14 ELEMENTS CONTENT IN DOXAPRAM HYDROCHLORIDE

Element	*Batch Number*	*Weight of sample (gm)*	*Content obtained (ppm)*	*Specification limit (ppm)*	*Remarks*
Lithium	*DOX300216*	*0.10061*	*0.0*	*≤ 25*	*BDL*
	DX003D16	*0.10049*	*0.0*		*BDL*
	DX004D16	*0.10064*	*0.0*		*BDL*
Sodium	*DOX300216*	*0.10061*	*11.3*	*≤1000*	*BDL*
	DX003D16	*0.10049*	*11.5*		*BDL*
	DX004D16	*0.10064*	*14.3*		*BDL*
Aluminum	*DOX300216*	*0.10061*	*2.7*	*≤1000*	*BDL*
	DX003D16	*0.10049*	*2.8*		*BDL*
	DX004D16	*0.10064*	*4.0*		*BDL*
Manganese	*DOX300216*	*0.10061*	*1.5*	*≤1000*	*BDL*
	DX003D16	*0.10049*	*1.5*		*BDL*
	DX004D16	*0.10064*	*1.4*		*BDL*
Vanadium	*DOX300216*	*0.10061*	*0.0*	*≤1.0*	*BDL*
	DX003D16	*0.10049*	*0.0*		*BDL*
	DX004D16	*0.10064*	*0.0*		*BDL*
Cobalt	*DOX300216*	*0.10061*	*0.0*	*≤0.5*	*BDL*
	DX003D16	*0.10049*	*0.0*		*BDL*
	DX004D16	*0.10064*	*0.0*		*BDL*
Nickel	*DOX300216*	*0.10061*	*0.6*	*≤2.0*	*0.6*
	DX003D16	*0.10049*	*0.6*		*0.6*
	DX004D16	*0.10064*	*2.0*		*2.0*
Copper	*DOX300216*	*0.10061*	*0.6*	*≤30*	*BDL*
	DX003D16	*0.10049*	*0.6*		*BDL*

Element	*Batch Number*	*Weight of sample (gm)*	*Content obtained (ppm)*	*Specification limit (ppm)*	*Remarks*
Arsenic	*DX004D16*	*0.10064*	*0.8*	*≤1.5*	*BDL*
	DOX300216	*0.10061*	*0.0*		*BDL*
	DX003D16	*0.10049*	*0.0*		*BDL*
Molybdenum	*DX004D16*	*0.10064*	*0.0*	*≤150*	*BDL*
	DOX300216	*0.10061*	*0.0*		*BDL*
	DX003D16	*0.10049*	*0.1*		*BDL*
Cadmium	*DX004D16*	*0.10064*	*0.1*	*≤0.17*	*BDL*
	DOX300216	*0.10061*	*0.0*		*BDL*
	DX003D16	*0.10049*	*0.0*		*BDL*
Antimony	*DX004D16*	*0.10064*	*0.0*	*≤9.0*	*BDL*
	DOX300216	*0.10061*	*0.0*		*BDL*
	DX003D16	*0.10049*	*0.0*		*BDL*
Mercury	*DX004D16*	*0.10064*	*0.0*	*≤0.3*	*BDL*
	DOX300216	*0.10061*	*0.0*		*BDL*
	DX003D16	*0.10049*	*0.0*		*BDL*
Lead	*DX004D16*	*0.10064*	*0.1*	*≤0.5*	*BLQ*

BDL – below detection limit; BLQ – below limit of quantification

4 CONCLUSION

An analytical method for determining arsenic, cadmium, mercury, lead, vanadium, cobalt, nickel, copper, molybdenum, manganese, lithium, antimony, sodium and aluminum content in doxapram hydrochloride by ICP-MS is proposed and validated for its system suitability, specificity, determination and confirmation of LOD, LOQ, method precision, ruggedness, linearity, robustness, solution stability and accuracy parameters. The obtained results in all the validation parameters were within the approval criteria. It was therefore concluded that this method is appropriate for its intended use.

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6 CONFLICTS OF INTEREST

Authors declare none

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