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ASSESSMENT OF ARSENIC, MERCURY, CADMIUM, LEAD, AND PALLADIUM IN ROPIVACAINE HYDROCHLORIDE MONOHYDRATE BY AN INDUCTIVELY COUPLED PLASMA MASS SPECTROMETER (ICPMS)

Ashish Kumar Pal and Raja Sundararajan✉
Department of Pharmaceutical Analysis, GITAM Institute of Pharmacy,
Gandhi Institute of Technology and Management (GITAM) (Deemed to be University),
Visakhapatnam, Pin code: 530 045, Andhra Pradesh (State), India
✉Corresponding Author: sraja61@gmail.com

ABSTRACT

Ropivacaine hydrochloride monohydrate is prolonged acting amide local anesthesia. In its guidelines, the International Conference on Harmonization notes that elemental inorganic contaminants may enter active pharmaceutical ingredients and drug formulations from various causes. Arsenic, mercury, cadmium, mercury, and lead are examples of class 1 elements, which occur as inorganic contaminants that have hazardous effects on the human body. During the synthesis of ropivacaine hydrochloride monohydrate, palladium assisted as a catalyst. A simple, reliable, and reproducible inductively-coupled plasma mass-spectrometric (ICP-MS) method for assessing trace metals in ropivacaine hydrochloride monohydrate by open vessel acid digestion method was established and validated. All of the elements' limits were set by ICH Q3D.

Keywords: Ropivacaine Hydrochloride Monohydrate, Trace Metal Assessment, Inductively-Coupled Plasma Mass-Spectrometry (ICP-MS), Open Vessel Acid Digestion Method, ICH Q3D

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INTRODUCTION

In its guidelines, the International Conference on Harmonization notes that elemental inorganic contaminants may enter active pharmaceutical ingredients and drug formulations from a variety of causes. The end-user is not therapeutically benefited from these elemental contaminants.[1] It damages the typical physiological system. The allowed daily exposure (PDE) to these elemental contaminants must therefore be assessed, quantified, and calculated. PDE is the highest permissible level of elemental impurity that can be eaten daily from drug items without having any negative effects. Arsenic, mercury, cadmium, and lead are examples of class 1 elements, which are inorganic contaminants that have hazardous consequences for the body.[1] Recent studies have suggested that palladium toxicity can cause the potential collapse of mitochondrial membranes and reduce the cellular glutathione level.[2] In its quality guideline, the international council for harmonization guidelines, ICH Q3D (R1)[3] categorizes palladium under category class-2B. It means that palladium has less probability of occurrence in drugs due to low abundance and low potential to co-isolate with other materials. However, when they are intentionally added during the synthesis of drugs, they must quantify. Hence, the risk assessment has to be done compulsorily. Ropivacaine is used in acute pain management for postoperative and labor pain control.[4] Palladium is used as a catalyst in the synthesis of ropivacaine hydrochloride monohydrate (Fig-1). Palladium is required as a catalyst for the synthesis of Ropivacaine hydrochloride monohydrate. It is required for hydrodehalogenation of 2-butyl-4-chloro-5-formylimidazole to 2-substituted 5-formylimidazole which is an intermediate for ropivacaine hydrochloride monohydrate. The amount of palladium used ranges from 5% to 15% based on the weight of 2-butyl-4-chloro-5-formylimidazole.[5] There is a high risk that the finished product will contain significant levels of palladium, an elemental contaminant that can be hazardous to the body. Ropivacaine hydrochloride monohydrate is a well-known anesthetic, and substantial study has already been done using various analytical techniques to assay estimate ropivacaine hydrochloride monohydrate and its organic impurities. Using different plates of thin layer chromatography and cyclodextrins at varying temperatures, a new, cost-effective stereoselective thin layer chromatography (TLC) coupled with densitometry for

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separation and quantification of R (+) and S (-) ropivacaine was developed.[6] Various authors[7-10] have reported on the quantification of ropivacaine hydrochloride monohydrate in drug substances and drug products using high-performance liquid chromatography (HPLC). Because ropivacaine hydrochloride monohydrate is chiral, chiral chromatography estimation in bulk drugs and pharmaceutical formulations has been described.[11] Capillary electrophoresis has been utilized by a few authors to estimate the purity of the enantiomer of ropivacaine hydrochloride monohydrate.[12-13] The measurement of ropivacaine in biological models was done by means of gas chromatography (GC) and mass spectrometry (MS).[14] The measurement of ropivacaine hydrochloride and its associated impurity-A was also reported using a quantitative NMR approach.[15] Several methods for determining ropivacaine hydrochloride monohydrate in blood samples have been developed, including the hyphenated method of liquid chromatography along with mass-spectrometry.[16-17] Arsenic, mercury, cadmium, lead, and palladium contaminants in ropivacaine hydrochloride monohydrate drug material were not determined using any analytical approach. This research aimed to develop and validate an ICP-MS method for assessing elemental contaminants such as arsenic, mercury, cadmium, lead, and palladium in ropivacaine hydrochloride monohydrate using an open sample digestion procedure.

EXPERIMENTAL

Reagents

From SS Pharma, Guntur, a sample of ropivacaine hydrochloride monohydrate was received as a gift. Merck produced ICP standards (1000 mg/l) and they have approved reference materials (CRM). Merck and Milli-Q water provided the nitric acid (Analytical Grade) and hydrochloric acid (Analytical Grade).

Instrument and Equipment

Thermo-iCAP-Qc scientific type ICP-MS was used for the study and was run in both without-gas mode and with-gas mode. The balance was made by Metrohm. Royal Scientific RSW 127 make Hot plate was used.

Sample Preparation - Developmental Trials and Optimization

The solubility investigation was the first phase in the technique development process. The sample was discovered to be insoluble in water. As a result, open digestion with concentrated ultra-pure acids was considered. A sample of around 0.5g was obtained, and various quantities of hydrochloric acid (concentrated) along with nitric acid (concentrated) were included, then boiled at various temperatures combined with time periods over a hot plate. The preparation of the sample below demonstrated full digestion and was completed based on the extent of sample matrix digestion.

Preparation of Sample Solution

0.5g of ropivacaine hydrochloride monohydrate sample was accurately weighed and removed into a beaker of volume 100 ml. 5.0 ml nitric acid (concentrated) was added up to this and mixed well. Further, carefully added 5.0 ml of hydrochloride acid (concentrated) and subjected to heating on a hot-plate apparatus. In order to dilute the leftover solution to the diluent mark after digestion, it was moved to a 50 ml volumetric flask.

Preparation of Blank Solution

Concentrated nitric acid (5.0 ml) was carefully added up to 5.0 ml of hydrochloric acid (concentrated) of volume 5 ml into a 100 ml beaker. This mixture was subjected to heating on a hot plate for 10 min. After being chilled, this blank solution was moved to a volumetric flask of volume 50 ml and diluted to fill the remainder of the space.

Diluent Preparation: Nitric acid (1%) was made in the necessary quantity.

Choice of Isotopes, the Method of Measurement, and the Designation of Internal Standards

In order to reduce interferences caused by free radicals generated during the ionization stage, it is critical to choose an appropriate elemental isotope and measurement mode. This method development started with a preliminary scan of all the elements' measurable, accessible isotopes, followed by measurements both in without-gas and with-gas mode. Internal standards are chosen by taking into account the analyte's mass number as closely as possible. These internal standards were introduced to the above-prepared solutions

and tested in STD mode and KED mode. All sample solutions included gold as a stabilizer to prevent mercury from reacting with other elements. The assignment of internal standards was completed by examining the compatibility of various internal standards with elements and their recovery in STD and KED modes. Table-1 contains the finalized isotopes and their measurement modes, which are the best fit and have been confirmed and recorded.

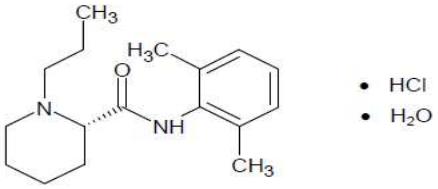

Fig-1: Structure of Ropivacaine Hydrochloride Monohydrate

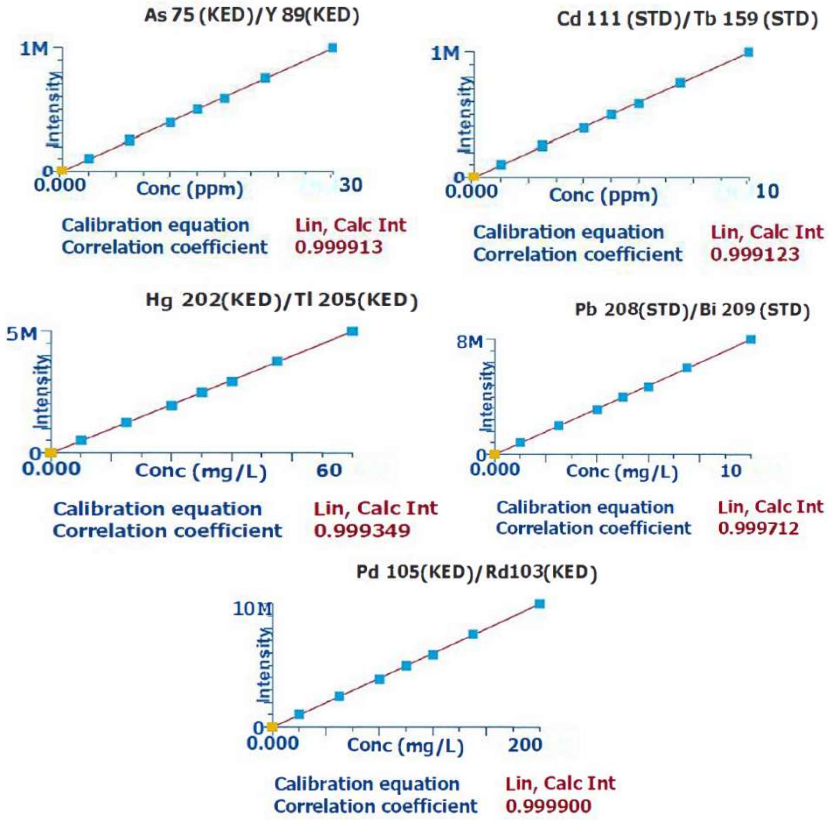

Fig-2: Linearity Graph Displaying each Element's Correlation Coefficient after Being Rectified by its Corresponding Internal Standard

Table-1: Choice of Mass Isotopes and Measurement Mode

Name of the Elements	Acquisition Measurement Mode	Mass Number (m / e)	Internal Standard Assigned	Acquisition Measurement Mode	Mass Number (m / e)
Arsenic (As)	KED	75	Yttrium (Y)	KED	89
Cadmium (Cd)	STD	111	Terbium (Tb)	STD	159
Mercury (Hg)	KED	202	Thallium (Tl)	KED	205
Lead (Pb)	STD	208	Bismuth (Bi)	STD	209
Palladium (Pd)	KED	105	Rhodium (Rd)	KED	103
Gold (Au) - Used as Stabilizer for Mercury					

System Suitability Linear Standard Solutions

For all elements, the J value represents the instrument's working concentration range. The value- 'J' of all elements was determined by dividing the element's specified limit by the sample dilution factor. Table-2

explains the element specification limit, while Table-3 explains the working system suitability linear standard concentration. 0.5'J' level solution was made by mixing 0.5ml of standard linear stock containing all elements with 1.0ml of finalized linear internal standard stock, which was then made up to 50ml with the diluent (1% nitric acid. 'J' level aliquots were prepared by means of diluting 1.0 ml of linear internal standard stock to 50ml with diluent and adding 1.0ml of linear standard stock containing all the constituents. 1.5 level J aliquots were made by means of diluting 1.0ml of linear internal standard stock to 50ml with diluent and adding 1.5ml of linear standard stock containing all the components.

Table-2: All Elemental Impurities Specification Limit and Instrument Working Limit (J-Value)

Name of Element	Spec. Limit (as per ICH Q3D / USP <232> Concentration limits µg/g or ppm	J-Value (as per sample dilution factor) in µg/l or ppb
Arsenic (As)	1.5	15
Cadmium (Cd)	0.5	5
Mercury (Hg)	3.0	30
Lead (Pb)	0.5	5
Palladium (Pd)	10.0	100

Instrument Parameters

ICP- MS Instrument Parameters

The nebulizer pump speed was adjusted to 0.3 rps, the flow of helium gas was set to 4.3 ml/min, the power of radio frequency was 1550 W, and the integration time was 0.5 seconds. The carrier gas flow is - 1.0 l/min, the flow of makeup gas is - 0.l l/min, the flow of dilution gas is - 0.1 l/min, and the nebulizer pump speed is - 0.l rps. The uptake time – was 45 seconds, the uptake speed - 0.40 rps prior to sampling, and the stabilization time - 50 seconds. The conditions like "the rinse speed were 0.40 rps, rinse on rinse port (sample) was 30 secs, rinse on rinse port (STD) was 30 secs, rinse speed was 0.40 rps, rinse on rinse vial (step1) was 30 secs, rinse on rinse port (step1) was 30 secs, rinse speed was 0.40 rps, rinse on rinse vial (step2) was 30 secs, rinse on rinse port (step2) was 30 secs, rinse speed was 0.3 rps, rinse on rinse vial (step3) was 30 secs, rinse on rinse port (step3) was 30 secs, executive pre-emptive rinse was set on, pre-emptive time was 35 secs and terminate a rinse step at the end of Acquisition was Off" were maintained after acquisition (probe rinse),

Specificity

Blank Solution Preparation

The blank matrix solutions were prepared by mixing acids equivalent to the standard and sample solution matrix, introduced into ICP-MS, and checked for specificity.

Individual Element Impurity Standard Solution Preparation

For all inorganic elemental impurities, unique standard solutions containing only one element at a time were prepared solution at the specification level i.e., 'J', and was analyzed.

Preparation of A Mixture of Elemental Impurities in A Standard Aliquot at Level 'J'

A standard all-in-one solution consisting of a variety of each element was generated at the specification level (J- value Level) and then introduced into ICP-MS.

Preparation of A Sample Solution Laced with Impurities of Elements

A sample blank solution and an unspiked sample were made and introduced into the ICP-MS. By spiking all components to the specified level, a spiked sample solution was prepared (J- value Level). In specificity, the response of elements in a standard solution containing a mixture of elements compared to spiked sample solution and all elemental impurities % recovery for the sample were compared.

Precision of Method

ICP-MS was used to analyze the precision of the method using six replicates of spiked J-level samples and another single unspiked sample for sample recovery corrections.

Accuracy
The trace metals were spiked into the sample in the span starting from 50%, 100%, and 150% of J to prepare a spiked sample in triplicate at each level. Another single unspiked sample was prepared and introduced into the ICP-MS.

Linearity
Over the range starting from 25% to a maximum range of 200% of the J value concentrations, the linearity of each trace metal was calculated. To create a calibration curve, calibration standards were made from dilutions of the standard stock solution.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)
Using the standard linear solution injection in the ICP-MS, LOD, and LOQ were calculated. The LOD for each element is automatically provided by the system. Using the formula, the LOQ may be determined from the LOD results.

Limit of Quantitation (ppb) = 3.3 x Limit of detection

System Suitability
Linear standard aliquots concerning the J concentration and blank solution were made and introduced into ICP-MS.

Trace Metal Estimation in Samples
Standard aliquots and drug substance samples of ropivacaine hydrochloride monohydrate were aspirated under appropriate technique conditions. The standard slope was used to compute the elemental concentration in the sample.

RESULTS AND DISCUSSION

Evaluating the response of trace metals in the blank with their corresponding individual standard aliquots to the intensity in the J-level standard solution of elements, the method's specificity for all elements was demonstrated. This demonstrated the element's mass homogeneity (Table-4.1). Furthermore, at J, the concentrations of all elemental impurities in the spiked solution of the sample were similar to those in the standard mixture solution. For each element, spike recovery ranged from 70% to 150 percent (Table-4.2).
The preciseness of the newly developed method was established by evaluating the relative standard deviation (percent RSD) for six replicate spiked sample results for all elemental impurities at J-level spiked samples. The percent RSD was less than 5% for all elements (Table-5). This demonstrates how consistent the results are across preparations and for each ingredient. This demonstrates how comparable the results are for each ingredient across various preparations. The spiked sample recovery was between 70% and 150 percent for the spiked preparations at 50 percent, 100 percent, and 150 percent spiked levels, demonstrating accuracy. At each level of spiking, the standard calibration curve computed the recovery of all elements. All components showed recovery between 90 to 120 percent at each level. The computed findings were close to the real values because they fell within pre-established acceptance standards (Table-6). The method's linearity and range were shown to be between 25 and 200 percent of the J level. The correlation coefficient (R) for every element line was not less than 0.99 (Table-7, Fig.-2). Using linearity standards that were injected, the linearity and range of each ingredient were determined. The results demonstrate that each element's approach was linear over the designated concentration range. According to USP 232 and 233, validation parameters for analytical procedures were established. To ensure the accuracy of the data acquired in an ICP-MS, the suitability of the system parameter was run and verified before beginning any validation parameters. The optimal response is given by the correlation coefficient, and the drift (%) shows that the instrument's plasma settings don't change over time. The correlation coefficient between the linear standard solution and each component was over 0.99 (Table-9.1). The difference between the standard and recheck solutions (both at 1.5 J level) for each element was not more than 20 (Table-9.2). The method's sensitivity was illustrated by the validation parameter - limit of detection, and the method parameter limit of quantitation indicates the smallest quantity the method can calculate consistently. The calibration curve of each element was used to calculate the detection limit. The formula was used to compute and report the limit of quantitation based on these limits of detection values (Table-8). Furthermore, ropivacaine hydrochloride monohydrate sample analysis was performed using a newly constructed and verified ICP-

MS, and the results for all element was within acceptable limits (Table-10). In a commercial ropivacaine hydrochloride monohydrate sample, a unique, simple, rapid, and dependable ICP-MS approach was established to identify arsenic, mercury, cadmium, and lead, as well as palladium.

Table-3: Concentration (in ppb) of all Elemental Impurities in Linear Standard Solutions

J- LEVEL	As (75)	Cd (114)	Hg (202)	Pb (208)	Pd (105)	Y (89)	Tb (159)	Tl (205)	Bi (209)	Rd (103)	Au (197)
0.5 J conc (ppb)	7.5	2.5	15	2.5	50	5	5	5	5	5	10
J conc (ppb)	15	5	30	5	100	5	5	5	5	5	10
1.5 J conc (ppb)	22.5	7.5	45	7.5	150	5	5	5	5	5	10

Table-4.1: Specificity Outcomes Comparing Standard in Mixed Concentration with Individual Standard Concentration

Element Name	Conc. in Cal. Blank (ppb)	Conc. in Individual Standard at J (ppb)	Conc. in Mixture Standard at J (ppb)
As	0.000	15.101	15.462
Cd	0.001	5.062	5.075
Hg	0.008	30.012	30.079
Pb	0.010	5.065	4.990
Pd	0.001	100.156	101.034

Table-4.2: Comparison of the Standard's Concentration in the Mixture and the Sample's Spiking Solution Yields Data for Specificity

Element Name	Conc in mixture Standard at J (ppb)	Conc. in the spiked sample (Spiked at J) (ppb)	% Recovery
As	15.462	15.143	98
Cd	5.075	5.175	102
Hg	30.079	30.142	100
Pb	4.990	5.021	101
Pd	101.034	100.256	99

Table-5: Information on Method Precision that Displays Each Element's Percentage Relative Standard Deviation at Spiked Samples at the 100 Percent (J) Level

Element Name	Mean Conc. (µg/g or ppm)	% RSD
As	1.5	2
Cd	0.5	1
Hg	2.9	3
Pb	0.5	2
Pd	10.1	3

Table-6: Accuracy Data Indicating the Average Recovery of Each Element in Samples of Ropivacaine Hydrochloride Monohydrate at 50 Percent, 100 Percent, and 150 Percent Spiked Levels

Element Name	Spiked level	Average Recovery (%)
As	50% spiked samples	98
	100% spiked samples	101
	150% spiked samples	97
Cd	50% spiked samples	102
	100% spiked samples	98
	150% spiked samples	99
Hg	50% spiked samples	92
	100% spiked samples	97
	150% spiked samples	95

Pb	50% spiked samples	103
	100% spiked samples	102
	150% spiked samples	99
Pd	50% spiked samples	100
	100% spiked samples	102
	150% spiked samples	99

Table-7: Results of Linearity Displaying Each Element's Correlation Coefficient

Correlation coefficient of each Element	
Target Element Name	Linearity
As	0.999913
Cd	0.999123
Hg	0.999349
Pb	0.999712
Pd	0.999900

Table-8: Values for the Limit of Detection and the Limit of Quantitation

Element Name	LOQ Conc. (ppb)	LOQ Conc. (ppb)
As	0.0140	0.0462
Cd	0.0106	0.0350
Hg	0.0050	0.0165
Pb	0.0030	0.0099
Pd	0.0120	0.0396

Table-9.1: Results of the System Suitability Test Displaying the Correlation Coefficient Value before Beginning the Validation Parameters

Correlation Co-efficient of each element obtained when performing validation parameters		
Element Name	Method Precision, Accuracy	Linearity, Specificity
As	0.99	0.99
Cd	1.00	0.99
Hg	1.00	1.00
Pb	0.99	1.00
Pd	0.99	0.99

Table-9.2: System Appropriateness Displaying Percent Drift Results Prior to Beginning Validation Parameters

% Drift of each element obtained when performing validation parameters		
Target Element Name	Method Precision, Accuracy	Linearity, Specificity
As	2	2
Cd	0	1
Hg	1	2
Pb	2	2
Pd	1	0

Table-10: Analytical Sample Results Displaying the Number of Elemental Impurities

Parameter	Results		Acceptance Criteria
Ropivacaine hydrochloride monohydrate sample	Target Element Name	Concentration µg/g or ppm	Concentration limits µg/g or ppm
	As	0.01	1.5
	Cd	0.00	0.5
	Hg	0.05	3
	Pb	0.02	0.5
	Pd	0.01	10

CONCLUSION

Ropivacaine hydrochloride monohydrate is a commonly used prolonged-acting amide local anesthesia. Trace elements like arsenic, mercury, cadmium, lead, and palladium should be measured. A new

inductively-coupled plasma mass-spectrometric technique for assessment of arsenic, mercury, cadmium, lead, and palladium trace metals in ropivacaine hydrochloride monohydrate was established and validated, complying with ICH as well as USP guidelines. This new method may be used as a sensitive, common, and precise technique for elemental impurities estimation in ropivacaine hydrochloride monohydrate.

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