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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CLASS-I ELEMENTAL IMPURITIES IN PROPOFOL EMULSION USING ICP-MS

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ABSTRACT
Elemental impurities are the traces of metals that can be found in finished drug products which may enter through various sources like excipients, and active pharmaceutical ingredients etc., their toxicity is to be estimated as they may pose serious toxicological effects to patients. The aim of this work is to estimate Class-I elements (Cd, Pb, As, Hg) simultaneously in Propofol 10 mg/mL Emulsion for Injection or Infusion using modern technique Inductively coupled plasma mass spectrometry, employing microwave-assisted digestion of sample. The developed method was validated for various parameters according to ICHQ2(R1) to prove that the developed method is fit for the required purpose and can be adaptable regularly. Validation results showed that the method was linear over the range of LOQ to 250%, with a good linearity correlation factor of R≥0.99, the method was found to be robust against the deliberate changes in sample preparation and uptake speed.
Keywords: Elemental Impurities, Simultaneous Estimation, Inductively Coupled Plasma Mass Spectrometry, Microwave Digestion, Analytical Method Validation.
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INTRODUCTION

Propofol (PFL), is a pharmacological compound that exerts its effects intravenously and acts as a brief-acting general anesthetic.[1] Chemically PFL(2,6-di(propan-2-yl) phenol) is an alkyl phenol derivative.[2] PFL exerts its influence by reducing the entry of calcium ions through calcium channels located at the trans sarcolemma, as well as decreasing potassium currents as a result of its negative ionotropic effect.[3] Elemental impurities are the traces of metals that can be found in finished drug products. According to ICHQ3D R1 guidelines elemental impurities are classified into three classes. Class I elements (Cd, Pb, As, Hg) are highly toxic and they come from mined excipients. Hence, they should be assessed for all routes of administration.[4,5] The USP 232 chapter describes the elemental impurities, and their permitted levels based on toxicity.[6] Modern instrumentation techniques and sample preparation techniques, estimation of elemental impurities have been described in chapter USP233.[7,8] ICP-MS is the most sensitive technique compared to other techniques like limit tests, Atomic absorption spectrometry, flame photometry, etc., ICP-MS overcomes is the limitations of other ICP techniques and helps in the detection of lower concentrations i.e., ppb to ppt levels.[9] The United States Pharmacopeia (USP) has recommended a number of modifications to the methodology for determining elemental impurities in active medicinal components (APIs). Like preparing the sample by microwave-assisted digestion method for clear sample preparation and easy identification of these elements.[10]

This technique also has more advantages like reduced contamination, better reproducibility with lesser use of acids, and good recovery of volatile elements.[11] Thorough literature review reports state that there are fewer published results reporting that there is less work done on the estimation of elemental impurities in pharmaceutical drug products by using the latest equipment. The main objective of this work is to estimate class-I elements i.e., Cd, Pb, As, and Hg in propofol emulsion.

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EXPERIMENTAL

Apparatus

A high-resolution ICPMS, Agilent-7800 equipped with Mass Hunter workstation software was used for all mass measurements. Analytical Balance of Mettler Toledo-XS 205 DU make was used for weighing of the samples and a Microwave digester of Anton paar make was used for the digestion of sample.

Preparation of Solutions

Diluent Preparation

Transferred 4.500 mL of Nitric acid and 0.500 mL of Hydrochloric acid into a 50 mL centrifuge tube containing 25 mL of Type-I water and dilute to 50 mL with Type-I water.
Standard stock solutions, sample blank solutions, and samples were prepared.

Preparation of Sample Blank

Transferred 4.500 mL of Nitric acid, 0.500 mL of Hydrochloric acid, and 2.000 mL of Hydrogen peroxide into the digestion vessel. Performed microwave digestion. After completion of digestion, allow the digestion vessel to come to room temperature, and remove the microwave digestion vessel. Opened cool digestion vessel, and transferred the solution into a 50 mL centrifuge tube. Added 0.500 mL of Standard Stock-B solution into the above centrifuge tube, vortexed then diluted to 50 mL with Type-I water.

Preparation of Sample

Weighed about 0.1000 g of sample and transferred it into the digestion vessel. Added 4.500 mL of Nitric acid, 0.500 mL of Hydrochloric acid, and 2.000 mL of Hydrogen peroxide into the digestion vessel. Performed microwave digestion. After completion of digestion, cooled the digestion vessel to room temperature, and removed the microwave digestion vessel. Opened the cooled digestion vessel, and transferred the solution into a 50 mL centrifuge tube. Added 0.500 mL of Standard Stock-B solution into the above centrifuge tube, vortexed then diluted to 50 mL with Type-I water. Selected the method& stabilized the system. Aspirated six replicates of system precision solution and calculated the % Relative Standard Deviation for the ratio of analyte response to ISTD response from six replicate aspirations of system precision solution.

Method Development

A method was developed based on the nature of the drug, matrix, and composition which is required for the complete digestion of the drug in suitable acids. For the proper and complete digestion of the sample Microwave digestion method was carried out and the results of this third trial were found to be within the acceptance criteria (Table-1).

Method Validation

The developed method was validated according to ICHQ2(R1) on various parameters like system suitability, specificity, Limit of Detection (LOD), Limit of Quantification (LOQ), Linearity, Precision, Accuracy, Linearity Range, and Stability of analytical solution to prove that the developed method is fit for the required purpose and can be adaptable regularly.[9,10,11]

System Suitability

To evaluate the system suitability, aspirated diluent, six replicates of system precision solution, calibration blank, calibration standards, and standard check solution. Calculated the % RSD for six replicate aspirations and % variation of the standard check. The correlation coefficient of the calibration curve is obtained through software. System suitability solutions were prepared the same as those of the standard stock solutions.

Specificity

Blank Interference

Specificity was established by studying interference of calibration blank and recovery study at the LOQ level to prove that there is no interference from the matrix. Prepared calibration blank and aspirated it ten times.

Preparation of Calibration Blank
4.500 mL of Nitric acid, 0.500 mL of Hydrochloric acid, and 0.500 mL of standard stock-B solution were transferred into 50 mL of centrifuge tube containing 25 mL of Type-I water and vortexed then diluted to 50 mL with Type-I water by using the dispenser.

Limit of Detection (LOD) and Limit of Quantification (LOQ)
The sample blank was aspirated ten times and the results obtained are shown in Table-2. The LOD and LOQ concentrations were predicted based on the standard deviation of the Ratio of analyte response to ISTD response in sample blank of ten aspirations and slope of the calibration curve.

Linearity
Linearity solutions in the range of LOQ to 250% of Specification level were prepared and the Correlation coefficient for a standard using the respective value of the ratio of analyte response to ISTD response versus concentration.

Precision
System Precision
System precision was evaluated by aspirating diluent, six replicates of system precision solution, calibration blank, calibration standards, and standard check solution. calculated the % RSD for six replicate aspirations and % variation of the standard check. The results obtained are shown in Table-3.

Method Precision
Prepared and aspirated six individual Samples and Spike Samples at the specification level. Spike Sample at specification level was prepared by weighing about 0.1000g of sample and transferred into the digestion vessel. To this 4.500 mL of Nitric acid, 0.500 mL of Hydrochloric acid, 2.000 mL of Hydrogen peroxide, and 2.000 mL of Standard Stock-A solution were added in the digestion vessel. Performed microwave digestion. After completion of digestion, vessels were cooled to room temperature, transferred the solution into a 50 mL centrifuge tube. To this 0.500 mL of Standard Stock-B solution was added, vortexed then diluted to 50 mL with Type-I water.

Intermediate Precision
Intermediate precision was performed by different analysts on different days with different instruments available. Prepared and aspirated six individual samples at different specification levels.

Accuracy
To demonstrate the accuracy of the analytical method, prepared such samples, and spiked samples at different levels i.e., LOQ, 50%, 100%, and 150% in triplicate. Calculated the mean % recovery at each level for the analyte.

Range
The range of the analytical method was determined from the data of precision, accuracy, and linearity.

Stability of Analytical Solution
Sample blank, samples spiked at the Specification level, were kept on a benchtop for 24 hours, and freshly prepared sample blank and samples spiked at each specification level were aspirated to establish the stability of solutions, results obtained were shown in Table-4.

Robustness
The robustness of the analytical method was established by demonstrating its reliability against deliberate changes in Nitric acid, Hydrochloric acid, and Hydrogen peroxide volumes used for sample preparation and deliberate changes in instrument method, and the results obtained were shown in Table-5 and Table-6.

RESULTS AND DISCUSSION

Instrument readings of method development. Mean recovery should be between 70-150% at each level. As the % recovery of the spiked samples is within the limits, the method developed was considered optimized.

Table-1: Results of Method Development

		Cd	Pb	As	Hg
Sample weight(g)		1	1	1	1
Dilution(ml)		50	50	50	50
Concentration of solution(ppb)	S. B	0.001	0.011	0.011	0.019
	SP-1	0.002	0.019	0.026	0.009
	SP-2	0.003	0.018	0.015	0.015
	LOQ-1	0.098	0.259	0.793	0.161
	LOQ-2	0.101	0.269	0.775	0.157
	Spec-1	0.425	1.042	3.222	0.625
	Spec-2	0.421	1.054	3.127	0.625
% Recovery	LOQ-1	96	98.4	102.9	94.7
	LOQ-2	98	102.4	100.5	92
	Spec-1	105.5	103	106.7	101
	Spec-2	104.5	104.2	103.5	101

System Suitability

The relative standard deviation for the ratio of analyte response to ISTD response for six replicates of system precision solution should be NMT 15.0% for each analyte, the correlation coefficient of calibration curve should be ≥ 0.99 for each analyte and the concentration of each analyte in standard check solution should be ± 20.0 % of actual concentration.

Specificity

There was an interference in the calibration blank at the mass of each analyte, but it was found lower than the calibration standard solution-1 response, and the mean % recovery at the LOQ level was found within acceptance criteria, which proves that the method is specific.

Table-2: Results of Prediction of LOD and LOQ

	Sample blank (Ratio of analyte response to ISTD Response)			
Name of the Elements	Cd	Pb	As	Hg
Mean	0.00000	0.0010	0.00010	0.00010
Standard deviation	0.00000	0.0000	0.00000	0.00000
Slope (From Calibration curve)	0.01577	0.0462	0.00515	0.00962

Linearity

The results of Linearity met the acceptance criteria, the method was found linear in the range of LOQ to 250% of the Specification level.

Precision

The relative standard deviation for the ratio of analyte response to ISTD response for six replicates of system precision solution was NMT 15.0% for each analyte, and the % RSD is within the limit. The % RSD of each analyte content from the six preparations of the Sample at Specification level solutions was not more than 20.0, and the cumulative % RSD of each analyte content in twelve preparations (Method precision and Intermediate precision) was not more than 25.0. The results were found within the acceptance criteria, this indicates the method is precise.

Table-3: Results of System Precision

	Ratio of analyte response to ISTD response			
Name of the Elements	Cd	Pb	As	Hg
Mean	0.0062	0.0434	0.0148	0.0056
Standard deviation	0.00005	0.00019	0.00015	0.00008
% RSD	0.8	0.4	1	1.4
Slope	0.01577	0.04624	0.00515	0.00962
Correlation coefficient (r-value)	0.9997	0.9998	0.9997	0.9997

Accuracy

The mean % recovery value at each level was in between 70.0 and 150.0 for each analyte.

Stability of Analytical solution

Table-4: Results of Stability of Analytical Solution

Name of the Element	Time	Content w.r.t sample (ppm)			
		Cd	Pb	As	Hg
The sample spiked at the specification level	Fresh	0.198	0.501	1.516	0.301
	27 Hrs	0.187	0.47	1.447	0.285
% Variation		-5.6	-6.2	-4.6	-5.3

Robustness

Table-5: Results of system precision of Uptake speed at (+30%): ~0.45 rps

Name of the element	Ratio of analyte response to ISTD response			
	Cd	Pb	As	Hg
Mean	0.0088	0.0706	0.0237	0.009
Standard Deviation	0.00017	0.00047	0.0006	0.00008
%RSD	1.9	0.7	2.5	0.9

Table-6: Results of system precision of Uptake speed at (-30%): ~0.25 rps

Aspiration	Ratio of analyte response to ISTD response			
	Cd	Pb	As	Hg
Mean	0.0087	0.071	0.0241	0.009
Standard Deviation	0.00031	0.0004	0.0005	0.00011
%RSD	3.6	0.6	2.1	1.2

Percentage recovery value should be between 70.0 to 150.0 for each analyte. The results obtained were within the acceptance criteria; this indicates the robustness of the analytical method against the deliberate changes in sample preparation and uptake speed.

CONCLUSION

A new method has been developed for the simultaneous estimation of Class-I Elemental Impurities (EI's) Cd, Pb, As, Hg by ICP-MS employing microwave-induced digestion in Propofol 10 mg/mL Emulsion for Injection or Infusion as previously there is no method developed for this particular drug. The developed method has been validated according to ICHQ2(R1) guidelines for various parameters proving that the developed method is fit for the required purpose and can be adaptable regularly. By validating all these parameters, it was found that the developed method is system-suitable, specific, linear, accurate, precise, robust, and can be used for the regular analysis of class-I elemental impurities in propofol emulsion. Results show that the method is linear over the range of LOQ to 250%, with a good linearity correlation factor of $R^2 > 0.99$, the method was found to be robust against the deliberate changes in sample preparation and uptake speed. ICP-MS has been proven a promising tool for the estimation of elemental impurities at very low concentrations. Thus, the method proposed was considered to be suitable for the simultaneous estimation of Class-I elements in propofol emulsion.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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REFERENCES

1. S. M. Omar, F. Ibrahim, A. Ismail, *Saudi Pharmaceutical Journal*, **28(3)**, 349(2020), https://doi.org/10.1016/j.jsps.2020.01.016
2. https://pubchem.ncbi.nlm.nih.gov/compound/Propofol
3. N. Hatakeyama, F. Sakuraya, N. Matsuda, J. Kimura, H. Kinoshita, O. Kemmotsu, *et al. Journal of Pharmacological Science*,**110(3)**, 334(2009), https://doi.org/10.1254/jphs.09060FP
4. International council for harmonisation of technical requirements for pharmaceuticals for human use. ICH Guideline Q3D for Elemental Impurities. Available at https//www.ema.europa.eu/en/documents/scientific-guideline/international-conference harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-32
5. Elemental Impurities in Drug Products Guidance for Industry. US Food and Drug Administration Guidance for Industry. Available at https//www.fda.gov/media/135956/download
6. USP<232>Elemental impurities-Limits, supplement-1
7. USP<233>Elemental impurities-Procedures, supplement-2
8. A. L. H. Muller, J. S. S. Oliveira, P. A. Mello, E. I. Muller, E. M. M. Flores, *Talanta*, **136**, 161(2015), https://doi.org/10.1016/j.talanta.2014.12.023
9. J. S. Barin, P. A. Mello, M. F. Mesko, F.A. Duarte, E. M. M. Flores, *Analytical and Bioanalytical Chemistry*, **408**, 4547(2016), https://doi.org/10.1007/s00216-016-9471-6
10. C. S. da Silva, F. C. Pinheiro,C. D. B. do Amaral, J. A. Nóbrega, *Spectrochim Acta-Part B*,**138**, 14(2017), https://doi.org/10.1016/j.sab.2017.10.004
11. F. C. Pinheiro, A. I. Barros, J. A. Nóbrega, *Analitica Chimica Acta,* **1,** 1065(2019), https://doi.org/10.1016/j.aca.2019.03.016

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