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Development, Validation and Application of an Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) Method to Determine Cobalt in Apixaban

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Abstract: ***Elemental impurities in drug products /substances from several sources are often observed; they may be residues or impurities from catalyst required during synthesis or from material of construction (e.g., through interactions with processing equipment's or container/closure systems or by being present in components of the drug substance (Figure No.1). From January 1, 2018 a new guideline ICH Q3D, regarding elemental impurities in drug products became effective published by International Conference on Harmonization. In this article, a case study is represented by developing a selective and highly sensitive Inductive Coupled Plasma Mass Spectrometry (ICP-MS)method for the determination of Elemental impurity in Apixabandrug substance. Determination of Cobalt content in Apixaban by Inductively Coupled Plasma Mass Spectrometer (ICP- MS) is carried out in the present work. A method is developed and study was performed to support the method precision, accuracy and Linearity. Specifically, three representative batches of drug substance Apixabanwere analyzed and the results were interpreted. The method was found suitable for the intended purpose. The linearity for all the standards was established from 30% to 150% of the concentration range (correlation coefficients were found more the 0.99). Similarly the accuracy at 30% concentration level was estimated and recoveries were between 70% and 130%(%w/w). The precision of the same preparation was also found within the acceptablelimit.***

Keywords: Cobalt content, Elemental Impurities, Apixaban and InductivelyCoupled Plasma Mass spectrometer (ICP-MS)

1. Introduction

The presence of elemental impurities in pharmaceutical samples is a concern, not only because some of them are toxic, but also they may cause unwanted side-effects, or may adversely affect drug stability and shelf-life. As a result, elemental impurities must be monitored and controlled in raw materials used for drug manufacturing, intermediates, active pharmaceutical ingredients (APIs), excipients (stabilizers, fillers, binders, colors, flavors, coatings, and so forth), and in final drug products. Elemental impurities have toxicological risks to patients without providing any therapeutic benefit to the consumer. During study of some cases, it was observed that, the elemental impurities were identified in the pharmaceutical drugs[2],[3],[4],[5], which had no therapeutic benefit to the patients / human but had severe adverse effect. Based on such reported incidences, the international conference of harmonization has come up with a new guideline ICH Q3D, where in control of every element as per its toxicity has been recommended. These elements are further classified based on their toxicity and Class 1 & 2A elements, which are more toxic, needs to be controlled, either by means of risk assessment or a routine quality control check. As per ICH Q3D, for the oral route of administration, the risk assessment should evaluate the possibility for inclusion of class 1 and class 2A elemental impurities in the drug product. So we have done a case study as a risk assessment of class 1 and class 2A elements, including Mo and Cr which were considered due to possibility of their presence in the material of constructs (MOC) of equipment used for the manufacturing of drug substance, Apixaban.

Apixaban is an oral anticoagulant and direct inhibitor of factor Xa which is used to decrease the risk of venous thromboses, systemic embolization and stroke in patients with atrial fibrillation, and lower the risk of deep vein thrombosis and pulmonary embolus after knee or hip replacement surgery. Apixaban has been linked to a low rate of serum aminotransferase elevations during therapy and to rare instances of clinically apparent liver injury. [6, 7, 8]

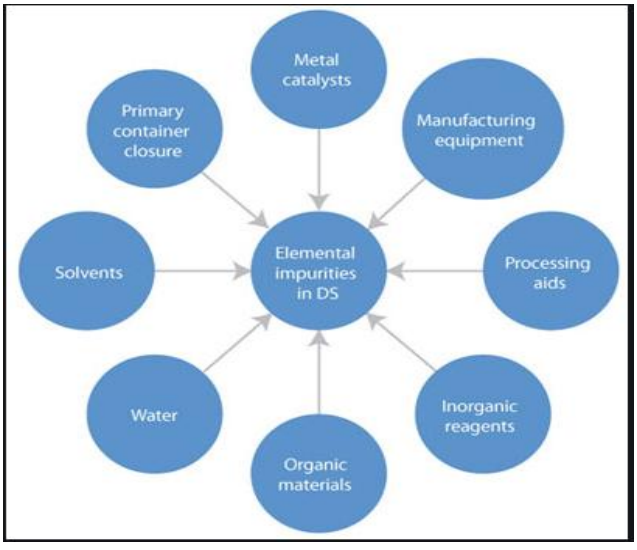

Figure 1: Sources of elemental impurities.[1]

The chemical name for Apixaban is 1-(4-methoxyphenyl) -7-oxo-6-[4-(2-oxopiperidin-1-yl) phenyl]-4, 5-dihydropyrazolo [3,4-c] pyridine-3- carboxamide. Its molecular formula is C25H25N5O4, which corresponds to a

molecular weight of 459.5 g/mol.

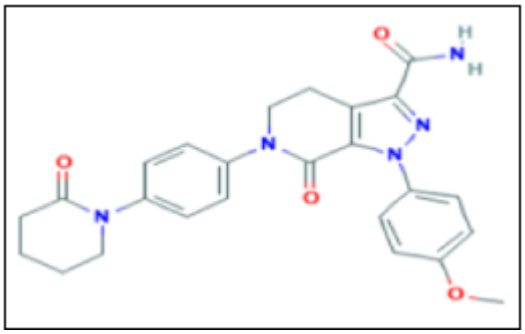

Figure 2: Structure of Apixaban.[5]

2. Experimental

Chemical and reagents

Nitric Acid (69%) was purchased from Sinopharm while Cobalt single element standard solution (ICPMS grade) were purchased from National Nonferrous Metal Electronic Materials Analysis and Testing Centeras per the availability. Water used for the preparation of solution was from Milli-Q water purification system (Merck).

Instrumentation

ICP system is equipped with single quadruple mass spectrometer (ICP-MS Perkin Elmer (NexION 2000)) along with Syngitix^{TM}version 2.2 software. Microwave Reaction System of CEM (Mars 6) was used for the sample digestion. Analytical balance used was of Sartorius make with model ME 235 P.

Microwave Reaction Conditions

Microwave digestion of blank, test sample and spike test solution was carried out at as per below table.

Table 1: Microwave Reaction Conditions

Microwave Digestion Apparatus	CEM MARS6			
Control type	Ramp To Temperature			
Sample type	Organic			
Full tank temperature monitoring system	Open，＜260℃			
Digestion type	Xpress plus			
Heating program	Heating rate /min	Hold time /min	temperature /℃	power /w
	15	8	130	1600
	5	8	160	1600
	5	5	185	1600

Table 2: ICP-MS Conditions

ICP-MS	Perkin Elmer NexION 2000
Timing	Number of scans：20 Times；Read times：1 Times；repeat times：3 Times；Analysis element：Co；Scan Mode:Peak hopping; Dwell time：50ms；RPa: 0.012;RPq: 0.25
Calibration	Sample unit：μg/L；Reference unit：μg/L；Reference substance concentration：3,6,15,30,36,45
Report	Performance Check

Preparation of solutions

Nitric acid in water was used as a diluent for all the preparations and also taken as blank.

Linearity /Standard solution and sample preparation

Sample analysis were carried out in accordance with the requirements described inChinese Pharmacopoeia 2015 Edition Four General Principles 0412and United States Pharmacopeia (USP <233> Elemental Impurities Procedures.[10-11] The linearity solutions of standard ranging from 30 to 150% of Target Limit (Refer Table No. 1) from the ready stock of 1000ppm available. Internal standardization was applied, using Yttrium (Y) as an internal standards at 1 μg·L-1 respectively.

Test samples as such and spiked test samples at 30% level (6 Preparations) was prepared of concentration as per Target limits for the 9 elements specified in ICH Q3D / USP <232> by performing microwave digestion. Filtered the solution with 0.45 u syringe filter and used the filtrate for analysis. Run the Linearity and then test solutions, spiked test solutions followed by Bracketing standards.

Table 3: Target limits for the cobalt specified in ICH Q3D / USP <232>.

Element	59Co
Class	2A
Target limit J (μg·g-1) Limit in ppm	5

3. Results and Interpretation

Specificity

The response values of the limit solution (30 μg / L), diluent, and blank solution were measured by ICP-MS, and the results were as follows:

Table 4: Specific experiment results

Solution	Limit solution	5% of limit solution	diluent	Blank solution
Response Values (cps)	82543.500	4127.175	48.200	66.0
	80547.878	4027.394	38.333	37.667
	70649.079	3532.454	25.667	18.667

The results show that under the conditions of the instrument, the response values of the diluent and the blank solution are less than 5% of the limit solution, indicating that the method has good specificity.

Linearity

Six standard solutions were analyzed by ICP-MS. The

response values was plotted on the ordinate and the concentration was plotted on the abscissa. The linear regression equation and correlation coefficient r were calculated. The results are shown in the table below and Figure 3.

Table 5: Linearity results

Response values (cps)	Concentration (μg/L)	Linear equation
6949.04	3.00	y=2371.8x-152.13 r=1.000
13994.9	6.00	
35702.63	15.05	
70649.08	29.95	
86349.68	36.29	
105438.89	44.62	

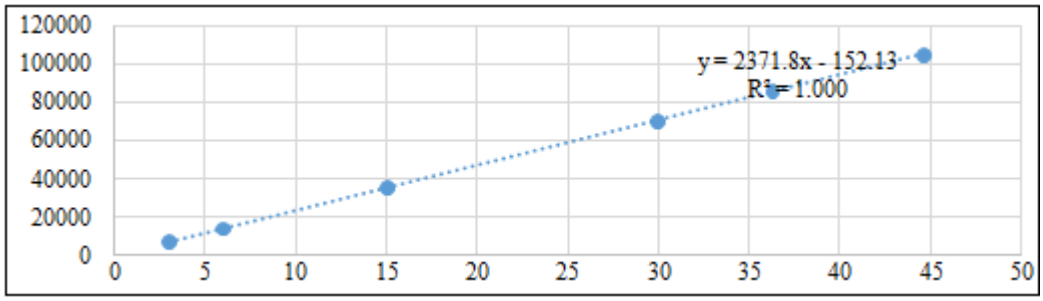

Figure 3: Standard curve

The experimental results show that the linear relationship is good in the range of 3.00μg / L ～ 44.62μg / L. The linear equation is y = 2371.8x-152.13, r = 1.000; the standard deviation of the calculated intercept is δ= 208.6, and the slope S = 2371.8.

Limit of Detection:
According to the formula LOD = 3.3δ / S, the detection limit concentration was 0.29 μg / L.

Limit of Quantitation:
According to the formula LQD = 10δ / S, the quantitation limit concentration was 0.88 μg / L.

Accuracy
Base solution: Accurately weigh 0.3g of Apixaban, place it in a digestion tank, add 12ml of nitric acid and heat at 120 ° C for 20min for pre-digestion, then cover the inner lid, tighten the jacket, external cover, and place in Microwave Digestion Apparatus for digestion. After that, remove the tank and heat at 160 ° C until the red-brown steam is exhausted, and concentrate to 0.5 ~ 1ml. Use a diluent to transfer to a 50ml volumetric flask and dilute to the mark. Shake well.

20% accuracy solution: Precisely measures 0.6ml of Co element standard stock solution, put it in a digestion tank, and heat it at 160 ℃ for 5min. accurately weigh 0.3 g of Apixaban, place it in above digestion tank, and prepared according to the preparation method of the base solution (3 samples prepared simultaneously).

50% accuracy solution: Precisely measures 1.5ml of Co element standard stock solution, put it in a digestion tank, and heat it at 160 ℃ for 5min. accurately weigh 0.3 g of Apixaban, place it in above digestion tank, and prepared according to the preparation method of the base solution. (3 samples prepared simultaneously).

100% accuracy solution: Precisely measure 3ml of Co element standard stock solution, put it in a digestion tank, and heat it at 160 ℃ for 5min. Accurately weigh 0.3 g of Apixaban, place it in above digestion tank, and prepared according to the preparation method of the base solution. (3 samples prepared simultaneously).

150% accuracy solution: Precisely measures 4.5ml of Co element standard stock solution, put it in a digestion tank, and heat it at 160 ℃ for 5min. accurately weigh 0.3 g of Apixaban, place it in above digestion tank, and prepared according to the preparation method of the base solution. (3 samples prepared simultaneously).

Take the accuracy solution, the base solution and the blank solution, and analyze them by injection. The recovery rate is calculated according to the following formula. The results are shown in Table 7.

$$\text{recovery rate} = \frac{\text{Measurements} - \text{Original amount}}{\text{Adding amount}} \times 100\%$$

Table 6: Accuracy results

Accuracy solution		Actual amount μg/L	Amount added μg/L	Measurements μg/L	Recovery rate /%	RSD %
20%	1	Not detected	6.0	6.96	116.0	4.19
	2		6.0	7.11	118.5	
	3		6.0	6.56	109.3	
50%	1		15.0	16.23	108.2	0.68
	2		15.0	16.23	108.2	
	3		15.0	16.04	106.9	
100%	1		30.0	33.72	112.4	2.58
	2		30.0	32.29	107.6	
	3		30.0	33.37	111.2	
	4		30.0	34.45	114.8	
	5		30.0	34.61	115.4	
	6		30.0	34.30	114.3	
150%	1		45.0	51.66	114.8	1.02
	2		45.0	52.60	116.9	
	3		45.0	52.57	116.8	

The results showed that the % Recovery of 15 solutions were between 70% and130%, and the %RSD were not more than 15%, indicating that the accuracy of the Methodis good.

Precision

Repeatability:
Standard solution for testing: 100% accuracy solution. (6 replicates)It can be seen from the six 100% accuracy solutions under "Accuracy" that the RSD is2.58% <15%, indicating that the method has good repeatability.

Intermediate Precision:
Taken the same batch of this product, at different times, different analysts, perform experiments according to the repeatability requirements of 6.6.1, and calculated 6 solutions prepared by both Analyst and 12 solutions prepared by two Analysts. The results are shown in the table below.

Table 7: Intermediate precision Results

	Analyst1	Analyst2
Concentration μg/L	33.72	33.14
	32.29	33.88
	33.37	32.64
	34.45	32.58
	34.61	33.03
	34.30	32.61
% RSD	2.58%	1.52%
	2.39%	

The results showed that the RSDs of 6 solutions prepared by each analyst confirm the repeatability requirements; the RSDs of 12 solutions of two analysts were 2.39% <15%. This shows that the method has good intermediate precision.

Range
Based on the results of linearity, precision, and accuracy, the range of the cobalt detection method was determined to be 3.00 μg / L -52.60 μg / L (approximately 20% -150% of the limit concentration).

Solution stability
Take 30μg / L standard solution and a 100% accuracy solution at room temperature, injected and analyzed at 0, 0.5h, 1h, 2h, and 3h respectively, and compared the response with 0h to calculate the change in response. The results are shown in the table below.

Table 8: Solution stability results

Time / Response value（cps）	0h	0.5h	1h	2h	3h
30μg / L standard solution	71424.5	72166.2	75557.6	79054.2	78385.1
Response value change	-	1.04%	5.79%	10.68%	9.75%
100% accuracy solution	78500.8	80663.2	88187.5	92167.3	95087.1
Response value change	-	2.75%	12.34%	17.41%	21.13%

As can be seen from the table above, the response values of the two solutions were increases with time. The 30 μg / L standard solution is left for 3 hours and the change rate of the response value for 0 hours does not exceed 15%; The 100% accuracy solution is more than 15% for 2 hours. Therefore, it is determined that the standard solution is stable for 3 hours, and the spiked solution is stable for 1 hour.

Durability:
Take the 100% accuracy solution to calculate the cobalt content at heating temperature 150℃ and 170℃, and compare it with 160 ℃ to calculate the relative average deviation. The results are as follows:

Table 9: Durability structure results

	150℃	160℃	170℃
Cobalt content (μg/L)	31.27	33.79	30.74
Relative average deviation	3.87%	-	4.73%

The experimental results shows that the relative average deviation of the heating temperature at 150 ℃, 170 ℃ and 160 ℃ is less than 15%, so the method has better durability in the range of heating temperature of 160±10 ℃.

Batch Analysis
Six different batches of Apixaban were prepared according to the "basic solution" method (2 replicates), and then tested by ICP-MS. The results are shown in the table below.

Table 10: Batch Analysis sample measurement results

sample		Response value (cps)	Solution concentration (μg/L)
Limit solution (30μg/L)		70614.1	29.64
Detection limit		690.6	0.29
160901	Spl-1	34.0	Below LOD
	Spl-2	45.7	
160902	Spl-1	53.7	

	Spl-2	39.3	
160903	Spl-1	57.3	
	Spl-2	56.3	
1912H01(Y)	Spl-1	54.3	
	Spl-2	28.0	
1912H01(Y)	Spl-1	45.7	
	Spl-2	17.3	
1912H01(Y)	Spl-1	122.0	
	Spl-2	91.7	

As can be seen from the above table, the response value of cobalt in the sample is far below the detection limit.

Verification Summary

The specificity, detection limit, limit of quantitation, accuracy, precision, linearity, solution stability, and durability of the method were verified to ensure that this method can accurately and reliably detect cobalt in Apixaban.

Table 11: Verification summary

Validation content	Validation results
Specificity	Under the conditions of this instrument, the method was used to detect the cobalt content, and the response values of the diluent and the blank solution were less than 5% of the limit solution, indicating that the method has good specificity.
Linearity& Range	1）The linear relationship is good in the range of 3.00μg / L ～ 44.62μg / L. The linear equation is y = 2371.8x-152.13, and r = 1.000. 2）According to the results of linearity, precision, and accuracy, the determination range of cobalt detection method is 3.00 μg / L -52.60 μg / L (approximately 20% to 150% of the limit concentration)
Detection limit, limit of Quantitation	According to the formula LOD = 3.3δ / S, the detection limit concentration was 0.29 μg / L. According to the formula LOQ = 10δ / S, the quantitation limit concentration was 0.88 μg / L.
Accuracy	The experimental results show that the recovery rates of 15 accuracy solutions are all between 70% and 130%, and the RSD are not more than 15%, indicating that the accuracy of this method is good.
Precision	1）Six 100% accuracy solutions show that the RSD is 2.58% <15%, which indicates that the repeatability of the method is good. 2）The RSD of 6 solutions prepared by each researcher conform the repeatability requirements; the RSD of 12 solutions of two researchers was 2.39% <15%. This shows that the method has good intermediate precision.
Solution Stability	The standard solution is stable within 3 hours, and the spiked solution is stable within 1 hour.
Durability	The experimental results show that the method has better durability in the range of 160 ± 10 ℃.
Multiple batch sample measurements	The response value of cobalt in the sample is far below the detection limit.

4. Conclusion

If the total elemental impurity level from all sources in the drug substances is expected to be consistently less than 30% of the PDE, then additional controls are not required, provided the applicant has appropriately assessed the data and demonstrated adequate controls on elemental impurities.[9] Based on ICH Q3D, a case study for risk assessment was performed to determine the probability of presence of cobaltcontent in the Apixabanand to establish the appropriate controls to ensure the quality of the drugsubstance.

Hence the method for determination of cobalt content was developed on very selective and high sensitive technique of ICPMS. The suitability of the method was then established by; mean of Lineartiy, Specificity, Spike precision, Accuracy and Batch Analysis of Apixaban. As per USP <232> AND <233> the Linearity is to be checked from 50% to 150% of limit level. But for risk assessment approach to control elemental impurities in line with USP general chapters <232> and <233> and ICH Q3D seems workable by setting ranges from 20% to 150% of limit level as provided in this article. This also includes the exclusion criteria for routine check as proposed by European Directorate for the Quality of Medicines European(EDQM), Medicines Evaluation Agency (EMEA) or European Medicines Agency (EMA). Since the results obtained are well within the acceptable limits as per ICH Q3D, USP and even less than 30% of the PDE, hence the drug substance is safe and risk free and the routine control for elemental impurities is notrequired.

This is an apt case study to demonstrate the risk assessment approach, which can be employed in case of drug product / substance to comply with regulatory norms and requirements. This case can be taken as an example to decide the approach for risk assessment and elemental impurities control can be extrapolated to variousproducts.

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6. Declaration

This manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose, but we do respectfully request that review our manuscript. If you feel that the manuscript is appropriate for your journal please consider for review and publication.

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