Original Article

Analysis of ICH-Q3D Endorsed Elemental Impurities in Macrolide Antibiotics by ICP-MS

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ABSTRACT
Aim: The objective of this study was to determine the amount of twenty-three elemental impurities in Azithromycin API. A rapid, selective, precise, accurate and sensitive approach for detecting elemental impurities in Azithromycin API was developed and validated using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). **Background:** Conventional analytical methods used in pharmacopoeia's are less sensitive and accurate to detect heavy metal impurities. Concerning this issue, a new inductively coupled plasma mass spectrometry-based method was developed and validated as a different choice to investigate the Elemental impurities in an Azithromycin API sample. **Materials and Methods:** In the present research work, test samples were prepared with microwave supported acid digestion. **Results:** After a sequence of trails, the mixture of nitric acid, hydrochloric acid, and hydrogen peroxide (4 mL: 1 mL: 0.5 mL) with the accepted recovery range was chosen for the Azithromycin API's digestion. **Conclusion:** According to ICH and USP 233 guidelines, the proposed technique was successfully validated using ICP-MS for linearity, precision, accuracy, LOD, LOQ, and ruggedness.

Keywords: Azithromycin API, Elemental Impurities, Inductively coupled plasma mass spectrometry, Method Development, ICH Guidelines.

INTRODUCTION

Azithromycin is an oral azalide antibiotic, which is a sub-class of macrolide antibiotics.[1] Gram-positive and Gram-negative bacteria that cause respiratory infections, primarily, are the pathogens can be treated with Azithromycin. The antibiotic azithromycin prevents bacteria from generating several proteins necessary for their survival. Azithromycin is quickly absorbed and distributed throughout the body, eventually concentrating in cells.[2] Recently impurities in pharmaceutical products are getting more attention by researchers due to their adverse effects. Whatever the nature of impurities, it should be minimised as per regulatory bodies' prescribed limits. To improve the therapeutic property and minimize the toxicity of pharmaceutical products, the detection of impurities is the key step. So far, API related impurities and

degradation products of API are the most analysed impurities in the research area, whereas process related impurities are less explored. Heavy metal impurities present in pharmaceutical products lead to toxic effects on patients. These metallic impurities can enter into pharmaceutical products through various processes or products, such as formulation ingredients, catalysts, process equipment, reagents, solvents, containers and closures. Methods prescribed in official pharmacopoeias to detect heavy metals are semi-quantitative analysis. Limitations in official methods are compounds that can be identified by visual inspection. This colour comparison method does not have accuracy, specificity, and sensitivity in metal determination.[3] Instrumental method of analysis will be the effective and alternative method of analysis to determine the heavy metal contents. Inductively Coupled Mass Spectroscopy (ICP-MS) is the best tool to determine the multi elemental analysis. The significance of ICP-MS detection limits is in parts per trillion.[4] ICP-MS is an advanced analytical technique for assessing elements in biological fluids at trace levels.[5] ICP-MS technique was also used to analyse the heavy metals in honeybee venom.[6]

The ICP-MS method was used to compare heavy metal residues in vegetable and oil samples.[7] Unani formulation was also analysed by using ICP-MS.[8]

Heavy metals in river water reference material, natural water and soil were analysed using the ICP-MS technique.[9-11] ICP-MS gives extensive details of the heavy metal's compounds than the conventional official methods prescribed in Pharmacopoeia's.[12,13] Concerning the threat of heavy metal toxicity, a rapid, robust, sensitive, and selective method should be developed to estimate the metal content in pharmaceuticals. As a result, the research work aims to develop a feasible method for determining elemental impurities content in Azithromycin API such as Iridium (Ir), Barium (Ba), Cadmium (Cd), Arsenic (As), Platinum (Pt), Mercury (Hg), Cobalt (Co), Nickel (Ni), Gold (Au), Vanadium (V), Palladium (Pd), Rhodium (Rh), Lithium (Li), Selenium (Se), Silver (Ag), Ruthenium (Ru), Molybdenum (Mo), Antimony (Sb), Copper (Cu), Tin (Sn), Chromium (Cr), Lead (Pb), and Thallium (Tl), in Azithromycin API and validate the method by inductive coupled plasma mass spectrometry.

MATERIALS AND METHODS

Instruments and Chemicals used

ICP-MS Perkin elmer Nexion, Digester - Titan MPS and Anton Paar. ICH Specified Elemental Impurities Standards from Inorganic ventures, Eppendorf tubes from electronic pipettes, Elementals free grade of Nitric acid, Hydrochloric acid, Hydrogen peroxide and Water from Merck, and Azithromycin API from DNR Pharma, Chennai.

Sampling Devices

Peristaltic pump control: No, Sample flush time: 120, Sample flush speed: -35, Read delay time: 30, Read delay and analysis speed: - 18.00, Wash time: 170, Wash speed: -35.00.

Timing Parameters

Sweep reading: 30, Readings / replicate: 1, replicates: 3, Dwell time: 50.0 ms, Masscal file: Default, Mode: Helium KED, Conditions file: Default.

Plasma condition

RF Power: 1500W, Carrier gas flow: 1.03 L/min, Helium flow: On, Helium Flow rate: 4.3 mL/min.

Digester Condition

The temperature gradually increased from 50°C to 100°C to 170°C and then decreased to 50°C. Pressure is constantly maintained at 35 (bar). Hold time (in minutes) for each temperature: 50°C (10), 100°C (10), 170°C (15), and 50°C (5).

Solvent Selection

After various trials, the solvent mixture of Nitric acid: Hydrochloric acid: Hydrogen peroxide (4 mL: 1 mL: 0.5 mL) was selected for the study. To adjust the selenium recovery result, nitric acid and hydrogen peroxide solvent volumes increased. All the results were found in the acceptance criteria. Hence, the solvent combination Nitric acid: Hydrochloric acid: Hydrogen peroxide (4 mL: 1 mL: 0.5 mL) was selected for validation. Since it showed maximum recovery of elemental impurities in the digestion process. There is no acid corrosion of the digesting vessel PTFE walls with hydrogen peroxide, no formation of insoluble salts with an acid anion, and no alteration of the sample matrix by an acid. Because of its high oxidation power, just a minimal amount of hydrogen peroxide is required to create concentrated sample solutions.

Method Validation

Linearity

Linearity of the method was performed by diluting individual elemental impurities at different concentration levels from 25% (LOQ) to 200%. The prepared solutions were analyzed and calculated. The results of the correlation coefficient of the proposed method should not be less than 0.99.

Precision

The Precision of the developed method can be measured by using individual target materials of a concentration of 25% to 150%. As per the ICH guidelines, six individual measurements of samples can be made quickly and under the same analytical conditions. The Percentage relative standard deviation of the target material should be less than 20%.

Recovery

The known concentration of standard added with the test sample in the level from 25% (LOQ) to 150% of the impurity's specification level. 0.100 gm of the sample was transferred into digestion vessels and it was treated with Nitric acid, Hydrochloric acid and Hydrogen peroxide in the ratio of (4:1:0.5). Kept on the bench top for 5 min to initiate pre-digestion of a sample. Consecutively, digestion vessels are loaded into a digester. Blank alone and sample without addition of standard were executed. After completion of the successive digestion processes, samples were diluted to 50 mL of water. After the treatment, processed samples were spiked by standards. The recovery should be within the limit of 70%-150% at all levels.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The solution for the estimation of LOQ was prepared at a concentration of 25% of the specification limit. Six injections of LOQ Level reference solutions were made to assess precision. % Relative Standard Deviation of intensity from LOQ solutions

should not exceed 20%. LOD solution which is three times less concentration than LOQ solution. LOD level standard solution was prepared consecutively injected 3 times into ICP-MS.

Specificity

Sample blank and Calibration blank intensity ought to be lower than the intensity from the LOD level.

Ruggedness

Six individual sample preparations were processed on different days by various analysts. The percentage Relative Standard Deviation NMT 25 should not be exceeded in results from method precision and intermediate precision.

RESULTS

The correlation coefficient (R^2) for the proposed concentration for twenty-three elements was found to be 0.9994 to 1.000 using five calibration levels: 25%, 50%, 100%, 150%, and 200%. The results of linearity data are depicted in Table 1. The method's repeatability is the result of it running under comparable conditions for a short period of time. Precision was achieved using six independent

Table 1: Linearity Results.

Elements	Correlation Coefficient
Cadmium (Cd)	0.999
Lead (Pb)	1.000
Arsenic (As)	1.000
Mercury (Hg)	1.000
Cobalt (Co)	1.000
Vanadium (V)	1.000
Nickel (Ni)	1.000
Thallium (Tl)	1.000
Gold (Au)	1.000
Palladium (Pd)	1.000
Iridium (Ir)	1.000
Rhodium (Rh)	1.000
Ruthenium (Ru)	1.000
Selenium (Se)	1.000
Silver (Ag)	1.000
Platinum (Pt)	1.000
Lithium (Li)	1.000
Antimony (Sb)	1.000
Barium (Ba)	1.000
Copper (Cu)	1.000
Molybdenum (Mo)	1.000
Tin (Sn)	1.000
Chromium (Cr)	1.000

samples spiked with elemental impurities at the specification level (100%). The % Relative Standard Deviation for each elemental impurity content was found less than 20. The precision results are shown in Table 2. The recoveries at 25%, 50%, 100% and 150% level concentrations were calculated. The average percentage recovery for all 23 elements ranged from 88.70 to 104.94%. Table 3 displays the accuracy results. The LOQ and LOD values are shown in Table 4. Specificity of the developed method shown in Table 5 and the Ruggedness results are mentioned in Table 6.

DISCUSSION

Linearity studies for each elemental impurity were achieved by using the concentration range from 25% to 200%. Precision is achieved on a single level with a relative standard deviation less than 20%. The proposed approach's recoveries fell within the acceptable range of 70% to 150%, indicating that the method was accurate. The LOQ and LOD values indicate, respectively,

Table 2: Precision Results.

Elements	Average Content of Analyte in ppm (*n*=6)	% Relative Standard Deviation
Cadmium (Cd)	0.488	0.88
Lead (Pb)	0.522	0.83
Arsenic (As)	1.378	1.48
Mercury (Hg)	2.972	1.44
Cobalt (Co)	4.534	0.75
Vanadium (V)	9.156	2.36
Nickel (Ni)	19.438	1.12
Thallium (Tl)	0.837	0.78
Gold (Au)	9.963	0.83
Palladium (Pd)	9.302	0.59
Iridium (Ir)	10.500	0.61
Rhodium (Rh)	8.980	0.63
Ruthenium (Ru)	9.453	0.73
Selenium (Se)	14.089	0.36
Silver (Ag)	15.565	1.10
Platinum (Pt)	9.921	1.44
Lithium (Li)	52.192	1.33
Antimony (Sb)	126.082	0.74
Barium (Ba)	139.891	0.70
Copper (Cu)	288.179	1.39
Molybdenum (Mo)	273.976	0.70
Tin (Sn)	617.055	0.74
Chromium (Cr)	1023.285	0.72

Table 3: Accuracy Results.

Elements	Average % Recovery (*n*=3)			
	25%	50%	100%	150%
Cadmium (Cd)	96.80	96.93	97.63	98.27
Lead (Pb)	104.53	102.27	102.67	103.16
Arsenic (As)	98.84	98.00	98.56	99.23
Mercury (Hg)	90.19	89.76	90.61	89.36
Cobalt (Co)	90.55	89.43	91.38	90.91
Vanadium (V)	95.43	96.29	96.47	94.26
Nickel (Ni)	104.83	104.75	104.58	104.94
Thallium (Tl)	100.07	100.40	99.67	99.30
Gold (Au)	93.21	93.73	93.08	94.59
Palladium (Pd)	104.36	103.75	105.01	103.17
Iridium (Ir)	89.20	90.89	89.82	88.70
Rhodium (Rh)	93.27	93.92	94.53	93.27
Ruthenium (Ru)	95.07	94.98	94.82	94.09
Selenium (Se)	102.76	103.49	103.76	104.85
Silver (Ag)	99.67	99.86	99.20	99.76
Platinum (Pt)	94.83	95.62	94.89	94.40
Lithium (Li)	105.38	103.90	105.07	103.73
Antimony (Sb)	99.40	100.05	99.74	100.64
Barium (Ba)	95.18	95.14	96.06	95.36
Copper (Cu)	91.29	90.78	91.33	91.25
Molybdenum (Mo)	101.80	101.67	102.84	102.25
Tin (Sn)	95.29	93.11	92.09	93.73
Chromium (Cr)	91.63	92.94	93.00	94.32

Table 4: LOD and LOQ Results.

Elements	LOQ in ppm (*n*=6)	Average % Relative Standard Deviation for LOQ (*n*=6)	LOD in ppm (*n*=3)
Cadmium (Cd)	0.122	0.28	0.039
Lead (Pb)	0.115	0.39	0.036
Arsenic (As)	0.333	1.07	0.071
Mercury (Hg)	0.758	0.36	0.249
Cobalt (Co)	1.262	0.38	0.416
Vanadium (V)	2.301	0.25	0.645
Nickel (Ni)	5.070	0.90	1.674
Thallium (Tl)	0.198	0.85	0.066
Gold (Au)	2.511	1.03	0.831
Palladium (Pd)	2.530	1.02	0.839
Iridium (Ir)	2.514	1.01	0.810
Rhodium (Rh)	2.506	1.08	0.828
Ruthenium (Ru)	2.522	0.81	0.833

Elements	LOQ in ppm (*n*=6)	Average % Relative Standard Deviation for LOQ (*n*=6)	LOD in ppm (*n*=3)
Selenium (Se)	3.567	1.02	1.018
Silver (Ag)	3.799	0.65	1.255
Platinum (Pt)	2.520	0.71	0.833
Lithium (Li)	13.865	0.77	4.574
Antimony (Sb)	30.154	0.99	9.922
Barium (Ba)	35.287	0.75	11.571
Copper (Cu)	75.167	0.76	24.868
Molybdenum (Mo)	75.397	0.72	24.806
Tin (Sn)	150.064	0.15	49.169
Chromium (Cr)	280.051	1.09	92.086

Table 5: Specificity Results.

Elements	Calibration blank Intensity	Sample Blank Intensity	LOD Intensity (*n*=3)
Cadmium (Cd)	3.56	3.33	83.55
Lead (Pb)	105.23	145.77	1590.34
Arsenic (As)	23.32	8.44	28.99
Mercury (Hg)	17.21	63.11	2027.37
Cobalt (Co)	8.56	5.55	5691.05
Vanadium (V)	650.98	185.55	2451.39
Nickel (Ni)	23.54	19.55	7035.37
Thallium (Tl)	9.43	18.44	4562.92
Gold (Au)	56.22	388.22	35224.19
Palladium (Pd)	6.43	80.34	10416.10
Iridium (Ir)	2200.43	2060.81	65876.50
Rhodium (Rh)	376.43	468.45	37127.60
Ruthenium (Ru)	6.11	72.34	12051.78
Selenium (Se)	7.32	10.35	30.30
Silver (Ag)	77.23	28.44	27229.55
Platinum (Pt)	3.45	3.11	27330.66
Lithium (Li)	9.32	6.22	3438.48
Antimony (Sb)	457.54	401.33	40585.78
Barium (Ba)	2100.32	2177.67	159543.04
Copper (Cu)	1151.34	970.47	298059.49
Molybdenum (Mo)	300.36	399.53	246743.83
Tin (Sn)	2634.57	242.00	238117.32
Chromium (Cr)	267.54	224.00	531978.82

Table 6: Ruggedness Results.

Elements	Average Content of Analyte in ppm (*n*=12)	% Relative Standard Deviation (*n*=12)
Cadmium (Cd)	0.483	1.43
Lead (Pb)	0.505	3.67
Arsenic (As)	1.353	2.38
Mercury (Hg)	2.889	3.29
Cobalt (Co)	4.528	0.74
Vanadium (V)	8.998	2.89
Nickel (Ni)	19.271	1.39
Thallium (Tl)	0.829	1.22
Gold (Au)	9.912	0.96
Palladium (Pd)	9.206	1.21
Iridium (Ir)	10.444	0.82
Rhodium (Rh)	8.968	0.64
Ruthenium (Ru)	9.422	0.78
Selenium (Se)	13.784	2.33
Silver (Ag)	15.624	1.09
Platinum (Pt)	9.787	1.98
Lithium (Li)	51.371	2.10
Antimony (Sb)	124.312	1.64
Barium (Ba)	139.439	0.77
Copper (Cu)	287.926	1.36
Molybdenum (Mo)	269.097	2.01
Tin (Sn)	606.789	1.91
Chromium (Cr)	1015.799	1.02

the lowest concentrations of the analyte that can be accurately quantified and the lowest concentrations of the analyte that can be detected. The Intensity of the blank solution is lesser than the intensity of LOD solution showing specificity of the developed method. The overall outcomes from method precision and intermediate precision were found to be less than 25% of the Relative Standard Deviation showing the ruggedness of the developed method. All the results comply with ICH Q2R1 and ICH Q3D guidelines.[14-16] Iridium (Ir), Barium (Ba), Cadmium (Cd), Arsenic (As), Platinum (Pt), Mercury (Hg), Cobalt(Co), Nickel (Ni), Gold (Au), Vanadium (V), Palladium (Pd), Rhodium (Rh), Lithium (Li), Selenium (Se), Silver (Ag), Ruthenium (Ru), Molybdenum (Mo), Antimony (Sb), Copper (Cu), Tin (Sn), Chromium (Cr), Lead (Pb), and Thallium (Tl) can be analyzed at trace levels in a single run. The method has been developed without using any internal standards. The Results obtained for the proposed method obeyed the ICH analytical method validation guidelines and USP 233. It can be an effective alternative method of analysis to estimate the elemental impurities in azithromycin API

CONCLUSION

An ICP-MS technique has been created for the sensitive, selective, precise and accurate determination of elemental impurities in azithromycin API. The proposed method is useful for determining the elemental impurities such as Iridium (Ir), Barium (Ba), Cadmium (Cd), Arsenic (As), Platinum (Pt), Mercury (Hg), Cobalt (Co), Nickel (Ni), Gold (Au), Vanadium (V), Palladium (Pd), Rhodium (Rh), Lithium (Li), Selenium (Se), Silver (Ag), Ruthenium (Ru), Molybdenum (Mo), Antimony (Sb), Copper (Cu), Tin (Sn), Chromium (Cr), Lead (Pb), and Thallium (Tl), at trace levels in a single run. The method has been developed without using any internal standards. The Results obtained for the proposed method obeyed the ICH analytical method validation guidelines and USP 233. It can be an effective alternative method of analysis to estimate the elemental impurities in azithromycin API.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ICP-MS: Inductively Coupled Plasma Mass Spectrometry; **ppm:** Parts Per Million; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **NMT:** Not More Than; **ICH:** International Conference on Harmonization; **API:** Active Pharmaceutical Ingredients; **USP:** United States Pharmacopoeia; **gm:** Gram.

SUMMARY

The proposed work explored the analysis of ICH-Q3D elemental impurities in Azithromycin API. The results obtained from the given research work will be useful for the routine analysis of Azithromycin API to estimate the twenty-three elemental impurities in a single run without the addition of internal standards.

REFERENCES

1. Miguel L, Barbas C. LC determination of impurities in azithromycin tablets. J Pharm Biomed Anal. 2003;33(2):211-7. doi: 10.1016/s0731-7085(03)00258-9, PMID 12972086.

2. Al-Rimawi F, Kharoaf M. Analysis of azithromycin and its related compounds by RP-HPLC with UV detection. J Chromatogr Sci. 2010;48(2):86-90. doi: 10.1093/chrom sci/48.2.86, PMID 20109282.

3. Krishna Murty ASR, Kulshresta UC, Nageswara Rao T, Kumar Talluri MVN. Determination of heavy metals in selected drug substances by inductively coupled plasma – mass spectrometry. Indian J Chem Technol. 2005;12(2):229-31.

4. Al-Hakkani MF. Guideline of inductively coupled plasma mass spectrometry ICP–MS: fundamentals, practices, determination of the limits, quality control, and method validation parameters. SN Appl Sci. 2019;1(7):791. doi: 10.1007/s42452-019-0825-5.

5. Wilschefski SC, Baxter MR. Inductively coupled plasma mass spectrometry: introduction to analytical aspects. Australian Association for Clinical Biochemistry and Laboratory Medicine. 2019;40(3):115-33.

6. Kokot ZJ, Matysiak J. Inductively coupled plasma mass spectrometry determination of metals in honeybee venom. J Pharm Biomed Anal. 2008;48(3):955-9. doi: 10.1016/ j.jpba.2008.05.033, PMID 18617350.

7. Bakkali K, Martos NR, Souhail B, Ballesteros E. Determination of heavy metal content in vegetables and oils from Spain and Morocco by inductively coupled plasma mass spectrometry. Anal Lett. 2012;45(8):907-19. doi: 10.1080/00032719.2012.655658.

8. Ali MA, Hamiduddin H H, Zaigham M, Nafees S, Ikram M, Jahangeer G. Comparative physicochemical analysis of Kushta-E-Murdarsang prepared by classical and muffle furness method. J Young Pharm. 2021;13(3):205-10. doi: 10.5530/jyp.2021.13.43.

9. Beauchemin D, McLaren JW, Mykytiuk AP, Berman SS. Determination of trace metals in a river water reference material by inductively coupled plasma mass spectrometry. Anal Chem. 1987;59(5):778-83. doi: 10.1021/ac00132a021.

10. Didukh-Shadrina SL, Losev VN, Samoilo A, Trofimchuk AK, Nesterenko PN. Determination of metals in natural waters by inductively coupled plasma optical emission spectroscopy after preconcentration on silica sequentially coated with layers of Polyhexamethylene guanidinium and sulphonated nitrosonaphthols. Int J Anal Chem. 2019;2019(3):1467631. doi: 10.1155/2019/1467631, PMID 31354824.

11. Nyika J, Onyari E. Olumana Dinka M, Bhardwaj Mishra S. A comparison of reproducibility of inductively coupled spectrometric techniques in soil metal analyses. Air Soil Water Res. 2020;12(1):1-10.

12. Lewen N, Mathew S, Schenkenberger M, Raglione T. A rapid ICP-MS screen for heavy metals in pharmaceutical compounds. J Pharm Biomed Anal. 2004;35(4):739-52. doi: 10.1016/j.jpba.2004.02.023, PMID 15193718.

13. Chawla RK, Gudhanti SNKR, Kulandaivelu U, Panda SP, Alavala RR. Development and validation of an inductively coupled plasma mass spectrometry method for estimation of elemental impurities in calcium acetate active pharmaceutical ingredient. Indian J Pharm Sci. 2021;83(4):830-7. doi: 10.36468/pharmaceutical-sciences.834.

14. ICH harmonised Guideline. Guideline for elemental impurities. Eur Med Agency. 2019;Q3D:(R1)

15. ICH harmonized tripartite guideline, validation of analytical procedures: text and methodology. Q2R1. Eur Med Agency. 2005.

16. Ravisankar M, Alexandar S, Senthilkumar R, Kumar M, Venkateswarlu BS. Validation of the Inductively Coupled Plasma Mass Spectrometry (KED-MODE) Method for Estimation of Class 1 Elemental impurities in Flavoxate hydrochloride – API. J Pharm Neg Results. 2022;13(9):2887-91.