International Journal of Pharmaceutical Sciences and Drug Research, 2023;15(6):770-779

Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

A Quantitative Approach for the Determination of Elemental Impurities in Zinc Orotate Dihydrate Drug Substance by ICP-MS Method

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ARTICLE INFO

Article history:

Received: 02 September, 2023

Revised: 11 October, 2023

Accepted: 16 October, 2023

Published: 30 November, 2023

Keywords:

Elemental impurities, Inductively coupled plasma mass spectrometry, Validation, Zinc orotate dihydrate.

DOI:

10.25004/IJPSDR.2023.150611

ABSTRACT

These days elemental impurities are commonly present in active pharmaceutical ingredients, raw materials, during the synthesis of compounds, drug excipients, finished products, equipment, containers and closures. Inductively coupled plasma mass spectrometry (ICP-MS) is one of the advanced techniques to analyze elemental impurities in drug substances. An ICP-MS method was developed and validated for testing 17 elements, namely, V, Co, Ni, As, Se, Ru, Rh, Pd, Ag, Cd, Os, Ir, Pt, Au, Hg, Tl, and Pb in zinc orotate dihydrate. The samples were analyzed after diluting with concentrated nitric acid and concentrated hydrochloric acid. Li, Y, Tl, Co and Ce were assigned tuning solutions to correct the baseline drift and matrix interference. The RF power was 1550 W, RF matching was 1.80 V, sample depth was 8.0 mm, nebulizer gas flow was 1.01 L/min, nebulizer pump flow was 0.10 rps, spray chamber temperature 2°C, He flow rate was 4.3 mL/min and the energy discrimination rate was 3.0 V. All 17 elements exhibited excellent linearity in their testing range, with a coefficient of determination ≥ 0.9996. The limits of detection of the 17 elements were within the range of 0.0004 to 0.00411 ppm. The intra- and inter-day precision (relative standard deviation) was $< 6.4\%$. The recoveries of the spiked standard for all elements were 88.5 to 108.2%. Among the 17 elements of the zinc orotate dihydrate, the measured results of all the 17 elements were within the specified range, and the results of all the elements were also satisfactory. The developed method was simple, rapid, and effective. This method can be a powerful tool and imperative technology for the quantification of compounds in drug substances and pharmaceutical industries.

INTRODUCTION

Elemental contaminants may be present in drug formulations that may arise from various products; they may be residual impurities that may be added deliberately in chemical synthesis or may be present as contaminants.[1] These elemental impurities may be produced by interactions with instruments/equipment or packaging systems or by being present in compositions of the drug substances.[2] Since elemental contaminants do not produce any therapeutic activity, their levels in the drug products should be present within the lowest possible limits. [3] In some cases, they may also be produced through acceleration by degradation of drug products. Inductively coupled plasma mass spectrometry (ICP-MS) is an analytical technique that can be used to measure elements at trace levels in pharmaceutical products.[4] The most significant application of ICP-MS is its multi-element analysis, which allows multiple substances to be analyzed simultaneously in a single run. [5] Elemental impurities can be categorized into three classes, class 1, class 2A and 2B. The class 1 elements include, As, Cd, Hg, and Pb, are human toxic elements that have no use or limited use in the manufacture of pharmaceutical preparations. They may be present in drug products which are typically obtained from commonly used materials such as excipients.[6]

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The class 2A elements are: Co, Ni and V. Class 2A elements have high access of extinction to drug formulations/ products and thus require risk assessment across all possible sources of elemental impurities and routes of administration.[7]

Class 2B elements have a lower accessibility of existence in the drug products related to their decreased abundance and reduced potential to be co-existence with other materials. As a result, they may be eliminated from the risk assessment unless they are deliberately added during the manufacture of active pharmaceutical ingredients, excipients or other components of the drug formulations/product. The elemental impurities present in class 2B include Ag, Au, Ir, Os, Pd, Pt, Rh, Ru, Se and Tl.[8]

Plants and animals utilize orotic acid mineral salts, referred to as orotates, to produce DNA and RNA. Orotate salts easily pass through cell membranes because they are neutrally charged.[9] Higher concentrations are produced as a result of the mineral salts being transported by orotate into cells and tissues.[10] Thymus atrophy, cardiovascular disease, diabetes, hypogonadism, low testosterone levels, low white blood cell counts, osteoporosis and decreased immunity are the symptoms of zinc deficiency and can be treated with zinc orotate.[11] A deficiency of zinc has been associated to thymus atrophy, a decrease in white blood cell count, and an increased susceptibility to infection. Zinc is also essential for immunological function.[12] Numerous risk factors, such as hypertension, hypertriglyceridemia, and insulin resistance (syndrome X), have been associated with zinc deficiency, including an increased prevalence of diabetes and coronary artery disease. The chemical name of Zinc orotate dihydrate (Fig. 1) is (2+) bis (2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate) dihydrate.[13]

Since the existence of diverse chromophores, heterocycles and heterocyclic derivatives continue to function as versatile molecules for the synthesis of numerous natural products.[14,15] Pyrimidine carboxylic acid, also known as orotic acid, is found in a wide range of naturally occurring products, including milk whey, and acts as an intermediate in the biosynthesis of pyrimidine, which is a necessary component of DNA and RNA.[16,17] Furthermore, orotic acid can boost cardiac output and aid in heart failure rehabilitation.[17,18] It can also act as a grostimulant in mammals and may help with calcium, magnesium, and other critical nutrient absorption.[19,20]

Orotic acid has also been shown to lower bilirubin levels in infants and to be effective in the treatment of gout.[21] Many orotic acid analogs exhibit remarkable antitumor and antimicrobial activities.[22] Some of them also serve as enzyme inhibitors, thereby attracting the attention of chemists and molecular biologists. Numerous analogs of orotic acid display impressive anti-tumor and anti-microbial properties. Chemists and molecular biologists are interested in them since some of them also act as enzyme inhibitors.[23]

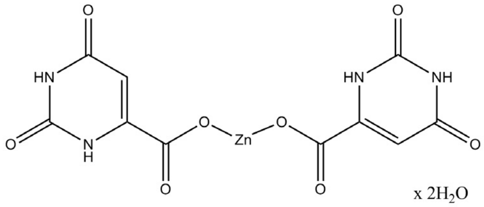


Fig. 1: Structure of zinc orotate dihydrate

Orotic acid formulated coordination network of formula [Zn (HOr)·$3H_2O$]$_n$ (1) prepared by reactions of orotic acid potassium salt [K(H_2Or)] accompanied by metal salts Zn $(OAc)_2$·$2H_2O$ at room temperature.[24] Preparation, X-ray crystal structure, thermal behavior, and IR spectroscopic studies of cis-diaquabis(1,10-phenanthroline) zinc (II) diorotate hydrate are described.[25] Zinc orotate ($ZnOr_2$), which is a new kind of poly (vinyl chloride) (PVC) stabilizer, is prepared in this work through the precipitation method, and its impact on the thermal stability of PVC is measured by thermogravimetric analysis (TG), Congo red test, and discoloration test.[26] There are certain advantages of ICP-MS in the current scenario in pharmaceutical research, this method has excellent detection limits, this method can estimate the total amount of an element in a sample. ICP-MS is a reliable and precise technique for determining chemical binding, elemental composition, and oxidation states. It is also crucial for assessing toxicity in water quality control, pharmaceutical and medical analysis, environmental analysis, and the food and beverage industry. Excellent limits of detection in trace analysis, multiple-element analysis for concurrent determining almost all the elements in the periodic table, fast and effective, semi-quantitative analysis can be determined, ICP-MS can calculate isotopic ratios and perform analysis *via* isotope dilution, samples that are liquid, solid, or gaseous can be analyzed and it requires only a small amount of sample for analysis.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals utilized for the study were procured from standard suppliers: such as conc. nitric acid (69%) obtained from Honey well trace analysis, conc. hydrochloric acid (35%) procured from Fisher Scientific Trace Analysis, and tuning solutions used were procured from Inorganic Ventures ICP-MS grade. The standards such as arsenic, cadmium, cobalt, iridium, lead, mercury, nickel, osmium, palladium, platinum, rhodium, ruthenium, selenium, silver, thallium, vanadium, were procured from Inorganic Venture and Gold standard belonging to Acc standard.

Instruments Used

The instruments and equipment used for the study were an Agilent ICP-MS 7800 series (Software Mass Hunter), an analytical balance radwag AS 82/220.X2, micropipette

Table 1: Instrument parameters

Parameters	*Method condition*	*Parameters*	*Method condition*
Plasma condition		*Octopole condition*	
Rf power	1550 W	Energy discrimination	3.0 V
Rf matching	1.80 V	Acquisition parameters	
Sample depth	8.0 mm	Acq mode	Spectrum
Nebulizer gas flow	1.01 L/min	Peak pattern	3 points
Nebulizer pump	0.10 rps	Replicates	3
Spray chamber temperature	2°C	Sweeps/Replicate	100
Mode	He	Integration time/ Mass (sec)	0.0999 sec
He flow	on	Number of Masses	17
He flow rate	4.3 mL/min		

Table 2: Tuning solution

Standard solution	1 ppb mixture of Li, Y, Tl, Co and Ce tuning solute
Expected m/z	7, 89, 205, 58 and 140
RSD limit	NMT 15% (Li, Y, Tl / Co)

brand 20, 200, 1000 µL and a microwave digester milestone ETHOSUP 17092516. Table 1 represents instrument parameters Table 2 represents tuning solutions.

Preparation of Diluent (2% v/v HNO_3 and HCL)

The diluents 20 mL of conc. nitric acid (69%) and conc. hydrochloric acid (35%) was transferred into a 1000 mL volumetric flask, and the flask previously rendered with 500 mL of purified water and the volume was adjusted up to the mark with purified water.

Preparation of Standard Mix Stock Solution

The required volumes of each element 1000 ppm concentration were pipetted out and transferred into a 50 mL volumetric flask and the made up to the volume with diluent and mixed well. The required volumes of each element 1000 ppm concentration were pipetted out and transferred into a 50 mL volumetric flask and the made up to the volume with diluent and mixed well.

Preparation of Standard Linearity Level Solutions

The required volumes of standards were pipetted out and transferred individually into a separate five 10 mL volumetric flask and the volume was adjusted up to the mark with diluent and mixed well. The concentration was mentioned respectively in ppb and labeled as calibration standard level -2 to 6.

Preparation of Sample Solution

The sample of about 0.2 g was weighed accurately and transferred into a clean and dried microwave digestion 5 mL capacity sample insert vessels and 2.0 mL of concentrated nitric acid and 1-mL concentrated hydrochloric acid were added, and the vessels were closed and kept for pre-digestion for 30 minutes on bench top, then the vessels were kept inside the microwave digester following program conditions.

Condition	Temperature (°C)	Hold (min)	Power (W)
Ramp	190	0–15	900
Constant	180	20	800

After completion of microwave digestion, the vessels were cooled at room temperature and the sample solution was transferred into 25 mL volumetric flasks, the vessels were washed with a portion of 10 mL purified water and transferred to above volumetric flasks then the volume was made up to the mark with purified water and mixed well.

RESULTS AND DISCUSSION

System Suitability

A system suitability study was performed to interpret the system precision, blank, and calibration. Standard solutions were prepared as per the test method and aspirated into ICP-MS system. The system suitability parameters were calculated and observed to be within the acceptable range.

Specificity

Specificity was performed using the blank, sample blank, calibration standard solutions and unspiked test solution as per the method of analysis were prepared and aspirated into ICP-MS system. Elements response was evaluated and the interference of blank and sample blank in each element abundance was calculated.

Determination of LoD and LoQ

The detection limit and quantitation limit were performed based on the residual standard deviation method by aspirating six levels (5, 10, 25, 50, 75 and 100%) with respect to the target level. The concentration of LoD and LoQ of the solution was derived by using the formula and the results were evaluated in Table 4. Mass spectrum was evaluated for the limit of detection and limit of quantitation level. The LoD level was confirmed by aspirating the solution in triplicate and the precision was determined at limit of quantitation level by injecting six times a solution of spiked standard with the concentration at LoQ level and the relative standard deviation of peak response was calculated. The limit of detection and limit of and quantitation value results are summarized in Table 3 and LoD and LoQ are represented in Fig. 2.

Table 3: Detection limit and quantitation limit values

Element name	*Correlation coefficient*	*Observed LoD in ppm*	*Observed LoQ in ppm*	*Acceptance criteria*
Vanadium (V)	0.99996	0.00093	0.00282	LoQ - NMT 30% of Specification limit in ppm
Cobalt (Co)	0.99998	0.00038	0.00116	
Nickel (Ni)	0.99995	0.00222	0.00673	
Arsenic (As)	0.99976	0.00037	0.00111	
Selenium (Se)	0.99991	0.00227	0.00689	
Ruthenium (Ru)	0.99998	0.00076	0.00230	
Rhodium (Rh)	0.99999	0.00053	0.00161	
Palladium (Pd)	0.99997	0.00085	0.00257	
Silver (Ag)	0.99991	0.00226	0.00686	
Cadmium (Cd)	0.99998	0.00004	0.00011	
Osmium (Os)	0.99999	0.00054	0.00164	
Iridium (Ir)	0.99997	0.00084	0.00255	
Platinum (Pt)	0.99993	0.00135	0.00409	
Gold (Au)	0.99932	0.00411	0.01245	
Mercury (Hg)	0.99997	0.00024	0.00073	
Thallium (Tl)	0.99961	0.00025	0.00075	
Lead (Pb)	0.99991	0.00008	0.00023	

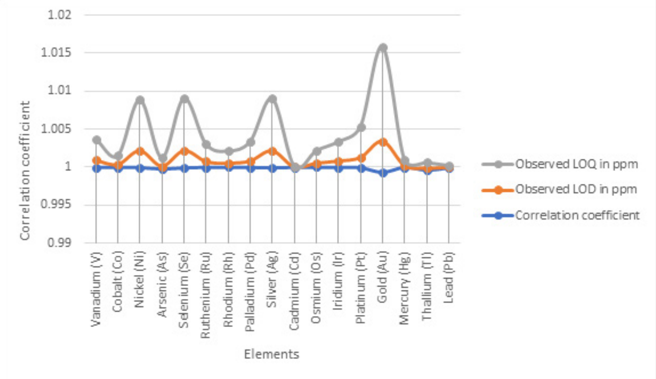


Fig. 2: Represents LoD and LoQ of zinc orotate dihydrate

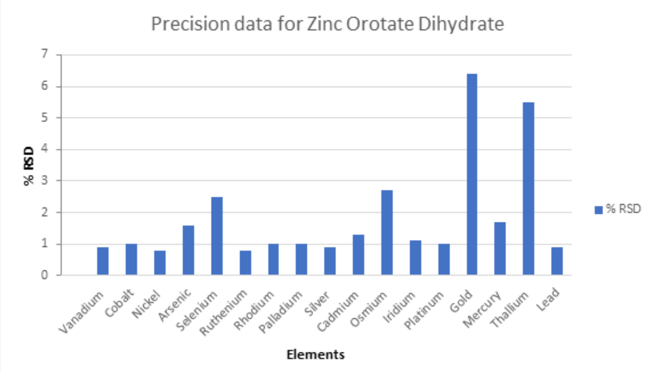


Fig. 3: Method precision of zinc orotate dihydrate

Method precision

The method precision of test method was analyzed by evaluating six spiked test samples and aspirated into ICP-MS system. The content of elemental impurities was calculated in a sample. The RSD of six sample preparations of each %elemental impurity were perceived to be within the desired limit. The precision data for zinc orotate dihydrate are summarized in Table 4 and method precision is represented in Fig. 3.

Linearity

The linearity of test method was evaluated by preparing the standard solutions of LoQ, 25, 50, 100, 200, and 300% of the intended concentration and evaluated as per the method. The correlation coefficient was observed to be within the specified limit. The linearity results are summarized in Table 5 and linearity curve is shown in Fig. 4.

Ruggedness

The intermediate precision of test method was evaluated by analysing six spiked samples and aspirated into the ICP-MS system. The study was performed on different day and with different analyst. The content of elemental impurities in sample was calculated. The RSD of six sample preparation of each %elemental impurity were observed to be within the specified limit. The ruggedness results are summarized in below Table 6 and ruggedness is represented in Fig 5.

Accuracy/Recovery

The accuracy of the method was evaluated by recovering the element from spiked samples. Samples were prepared by spiking the element class 1, class 2A and 2B with sample at different levels ranging from LoQ to 300% of the target concentration of known standards. The sample solutions were prepared in triplicate at LoQ, 100, 200 and 300%

Table 4: Precision data for zinc orotate dihydrate

Element name	*Recovered concentration in %*							*%RSD*
	Met. precision-1	*Met. precision-2*	*Met. precision-3*	*Met. precision-4*	*Met. precision-5*	*Met. Precision-6*	*Average*	
vanadium	110.0	111.5	111.4	110.4	111.6	112.7	111.3	0.9
Cobalt	100.6	102.4	102.6	100.9	102.3	103.1	102.0	1.0
Nickel	100.4	101.5	101.7	100.8	102.0	102.6	101.5	0.8
Arsenic	97.2	94.8	97.7	97.0	95.3	98.9	96.8	1.6
Selenium	100.6	99.8	98.9	95.6	102.7	101.9	99.9	2.5
Ruthenium	99.9	101.3	101.6	100.3	101.9	101.8	101.1	0.8
Rhodium	99.0	100.6	100.7	100.1	101.5	101.8	100.6	1.0
Palladium	98.8	101.3	101.5	100.5	101.3	101.5	100.8	1.0
Silver	93.2	95.1	95.1	94.2	94.8	95.8	94.7	0.9
Cadmium	95.9	97.7	98.6	98.8	99.7	98.5	98.2	1.3
Osmium	101.5	108.3	108.1	105.8	108.7	109.2	106.9	2.7
Iridium	101.1	103.4	103.6	102.6	103.6	104.4	103.1	1.1
Platinum	99.2	101.2	101.6	100.7	101.5	102.3	101.1	1.0
Gold	97.1	115.1	115.4	109.9	115.0	113.4	111.0	6.4
Mercury	97.0	101.3	101.6	101.1	100.3	101	100.4	1.7
Thallium	98.6	113.5	114.4	107.6	113.6	110.4	109.7	5.5
Lead	92.0	92.7	93.6	93.6	93.6	94.4	93.3	0.9

Table 5: Linearity data of zinc orotate dihydrate

Element name	*Correlation coefficient*	*Squared correlation Coefficient (r^2) for linearity levels*	*% Variation of bracketing standard solution*	
			Before linearity levels	*After linearity levels*
Vanadium	0.99809	0.994	10	4
Cobalt	0.99996	0.999	0	4
Nickel	0.99992	0.999	1	5
Arsenic	0.99989	0.999	5	8
Selenium	0.99993	0.999	2	3
Ruthenium	0.99997	0.999	0	6
Rhodium	0.99992	0.999	3	1
Palladium	0.99997	0.999	1	5
Silver	0.99894	0.997	3	1
Cadmium	0.99989	0.999	4	2
Osmium	0.99993	0.999	2	9
Iridium	0.99995	0.999	4	0
Platinum	0.99996	0.999	4	0
Gold	0.99999	1.000	1	12
Mercury	0.99957	0.998	3	2
Thallium	0.99903	0.997	2	11
Lead	0.99994	0.999	3	2

Table 6: Ruggedness values for zinc orotate dihydrate

Element name	*Recovered concentration in %*							*% RSD*
	Int. Precision-1	*Int. Precision-2*	*Int. Precision-3*	*Int. Precision-4*	*Int. Precision-5*	*Int. Precision-6*	*Average*	
Vanadium	102.6	101.6	100.1	100.5	98.2	97	100.0	2.1
Cobalt	96.1	96.2	93.9	94.2	91.8	91	93.9	2.3
Nickel	96.4	95.4	93.2	93.7	91.2	90.4	93.4	2.5
Arsenic	92.9	96.7	89.5	91.3	89.5	89.7	91.6	3.1
Selenium	93.2	94.2	91.6	95.2	93.1	92.2	93.3	1.4
Ruthenium	96.4	96.3	93.9	94.5	91.3	91.3	94.0	2.4
Rhodium	97.5	97.1	95	95	93.1	92.1	95.0	2.2
Palladium	93.4	93.2	92.2	92.1	89	88.4	91.4	2.4
Silver	95.2	94.8	93	94.6	118.8	117.7	102.4	12.1
Cadmium	92	90.1	93.7	91.2	87.8	89.6	90.7	2.3
Osmium	92.1	93.4	93	95	87.4	86.4	91.2	3.8
Iridium	99.3	98.7	97.9	99.3	94.7	94.5	97.4	2.3
Platinum	98.6	98.3	97	97.6	93.8	93.6	96.5	5.6
Gold	83.8	96.3	98.3	96.5	96.3	94.6	94.3	5.6
Mercury	95.9	96.4	95.1	94.2	92.3	92.2	94.4	1.9
Thallium	84	94.7	97.3	95.7	99.4	98.2	94.9	5.9
Lead	90.1	91.2	89.3	88.7	91.9	94.3	90.9	2.2

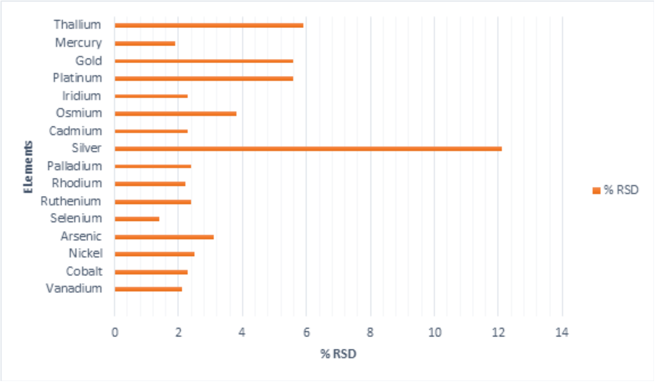


Fig. 4: Represents ruggedness for zinc orotate dihydrate

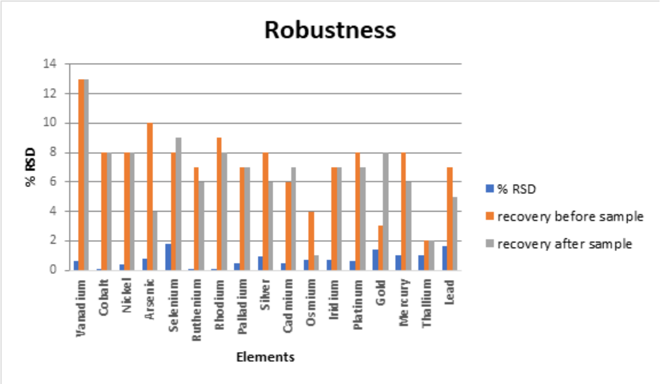


Fig. 5: Robustness for zinc orotate dihydrate

spiked levels and subtract the content from the unspiked sample. The accuracy results are summarized in Table 7.

Robustness

- *Effect of variation in stabilization time*

The robustness of the analytical method was established by its reliability against deliberate changes in instrumental conditions and sample preparation. The test samples were prepared spiked at specification level and analyzed as per the method of analysis by changing the following conditions like, variation in stabilization time (± 10%). The system suitability parameters were evaluated and the % RSD of the content of each elemental impurities in sample were calculated as per the above variant test method. The robustness results are summarized in Table 8 and robustness is represented in Figs 6-9.

DISCUSSION

The present research work focused on precise and rugged ICP-MS method development and validation of 17 elemental impurities, i.e., class 1 impurities Cd, Pb, As, Hg, class 2A Co, V, Ni and class 2B T1, Se, Ag, Au, Pd, Ir, Os, Rh, Ru, and Pt in zinc orotate dihydrate drug substance. For the analysis of zinc orotate dihydrate, drug substance sample digestion was done using nitric acid and sulfuric acid. Among the elemental impurities detected in zinc orotate dihydrate drug substance samples, elemental impurities in class 1, impurities in class 2A and 2B, according to the

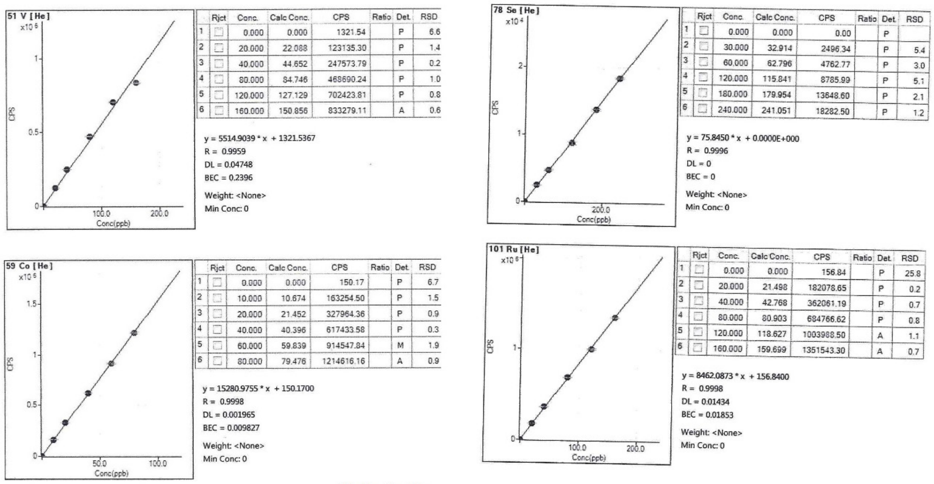
Table 7: Accuracy values for zinc orotate dihydrate

Name of the elements	*LoQ level recovered conc. in ppm*			*100% level recovered conc. in ppm*		
	1	*2*	*3*	*1*	*2*	*3*
Vanadium (V)	0.0032	0.0032	0.0031	0.0851	0.0839	0.0834
Cobalt (Co)	0.0013	0.0013	0.0013	0.0400	0.0394	0.0394
Nickel (Ni)	0.0076	0.0073	0.0072	0.1608	0.1580	0.1567
Arsenic (As)	0.0011	0.0011	0.0012	0.0115	0.0114	0.0112
Selenium (Se)	0.0065	0.0070	0.0069	0.1137	0.1156	0.1137
Ruthenium (Ru)	0.0023	0.0023	0.0023	0.0797	0.0788	0.0782
Rhodium (Rh)	0.0017	0.0016	0.0016	0.0806	0.0798	0.0789
Palladium (Pd)	0.0025	0.0025	0.0024	0.0787	0.0778	0.0769
Silver (Ag)	0.0067	0.0068	0.0065	0.1247	0.1231	0.1222
Cadmium (Cd)	0.0001	0.0001	0.0001	0.0038	0.0038	0.0040
Osmium (Os)	0.0017	0.0017	0.0017	0.0816	0.0804	0.0807
Iridium (Ir)	0.0027	0.0027	0.0027	0.0837	0.0841	0.0821
Platinum (Pt)	0.0040	0.0040	0.0039	0.0819	0.0815	0.0810
Gold (Au)	0.0128	0.0121	0.0127	0.0797	0.0785	0.0788
Mercury (Hg)	0.0008	0.0007	0.0007	0.0245	0.0239	0.0243
Thallium (Tl)	0.0008	0.0008	0.0008	0.0063	0.0063	0.0063
Lead (Pb)	0.0002	0.0002	0.0002	0.0040	0.0039	0.0039

Table 8: Robustness values for zinc orotate dihydrate

Robustness – Actual condition							
Element name	*Correlation coefficient*	*Robust-1 % recovered conc.*	*Robust-2 % recovered conc.*	*Average*	*% RSD*	*% Variation of Bkt. std solution recovery before sample*	*% Variation of Bkt. std solution recovery after sample*
Vanadium	0.9903	112.6	111.7	112.2	0.6	13	13
Cobalt	0.9981	107.0	106.8	106.9	0.1	8	8
Nickel	0.9988	107.3	106.7	107.0	0.4	8	8
Arsenic	0.9991	107.8	106.6	107.2	0.8	10	4
Selenium	0.9989	105.6	108.3	107.0	1.8	8	9
Ruthenium	0.9987	106.7	106.8	106.8	0.1	7	6
Rhodium	0.9987	107.1	107.2	107.2	0.1	9	8
Palladium	0.9993	105.4	106.1	105.8	0.5	7	7
Silver	0.9969	105.7	104.3	105.0	0.9	8	6
Cadmium	0.9987	107.1	107.9	107.5	0.5	6	7
Osmium	0.9997	107.9	108.9	108.4	0.7	4	1
Iridium	0.9986	107.4	106.4	106.9	0.7	7	7
Platinum	0.9989	106.8	105.9	106.4	0.6	8	7
Gold	0.9991	107.2	109.4	108.3	1.4	3	8
Mercury	0.9986	108.0	106.5	107.3	1.0	8	6
Thallium	0.9991	106.4	107.9	107.2	1.0	2	2
Lead	0.9985	104.8	102.5	103.7	1.6	7	5

elemental impurities' classification based on toxicity from ICH guidelines. Till date, no ICP-MS method was reported for the concurrent quantification of elemental impurities in zinc orotate dihydrate drug substance. So, an attempt was made to develop simple, rapid ICP-MS method, and it was validated with precision, specificity, linearity, ruggedness, robustness, accuracy, LoD and LoQ consecutively. Estimated concentrations of these elements



51 V [He]

	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.000	0.000	1321.54		P	6.6
2	☐	20.000	22.088	123135.30		P	1.4
3	☐	40.000	44.652	247573.79		P	0.2
4	☐	80.000	84.746	468690.24		P	1.0
5	☐	120.000	127.129	702423.81		P	0.8
6	☐	160.000	150.856	833279.11		A	0.6

y = 5514.9039 * x + 1321.5367
R = 0.9959
DL = 0.04748
BEC = 0.2396
Weight: <None>
Min Conc: 0

78 Se [He]

	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.000	0.000	0.00		P	
2	☐	30.000	32.914	2496.34		P	5.4
3	☐	60.000	62.796	4762.77		P	3.0
4	☐	120.000	115.841	8785.99		P	5.1
5	☐	180.000	179.954	13648.60		P	2.1
6	☐	240.000	241.051	18282.50		P	1.2

y = 75.8450 * x + 0.0000E+000
R = 0.9996
DL = 0
BEC = 0
Weight: <None>
Min Conc: 0

59 Co [He]

	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.000	0.000	150.17		P	6.7
2	☐	10.000	10.674	163254.50		P	1.5
3	☐	20.000	21.452	327964.36		P	0.9
4	☐	40.000	40.396	617433.58		P	0.3
5	☐	60.000	59.839	914547.84		M	1.9
6	☐	80.000	79.476	1214616.16		A	0.9

y = 15280.9755 * x + 150.1700
R = 0.9998
DL = 0.001965
BEC = 0.009827
Weight: <None>
Min Conc: 0

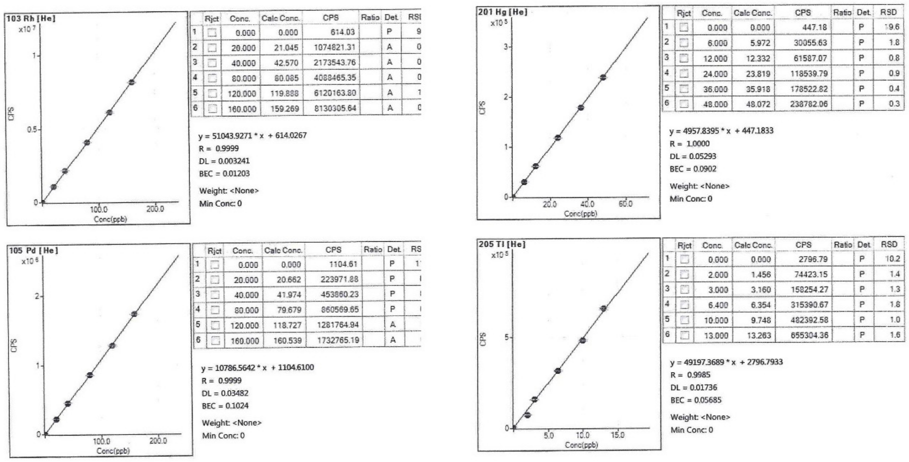
101 Ru [He]

	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.000	0.000	156.84		P	25.8
2	☐	20.000	21.498	182078.65		P	0.2
3	☐	40.000	42.768	362061.19		P	0.7
4	☐	80.000	80.903	684766.62		P	0.8
5	☐	120.000	118.627	1003988.50		A	1.1
6	☐	160.000	159.699	1351543.30		A	0.7

y = 8462.0873 * x + 156.8400
R = 0.9998
DL = 0.01434
BEC = 0.01853
Weight: <None>
Min Conc: 0

V, Co, Se, Ru

Fig. 6: Calibration data for vanadium, cobalt, selenium and ruthenium



103 Rh [He]

	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RS
1	☐	0.000	0.000	614.03		P	9
2	☐	20.000	21.045	1074821.31		A	0
3	☐	40.000	42.570	2173543.76		A	0
4	☐	80.000	80.085	4088465.35		A	0
5	☐	120.000	119.888	6120163.80		A	1
6	☐	160.000	159.269	8130305.64		A	0

y = 51043.9271 * x + 614.0267
R = 0.9999
DL = 0.003241
BEC = 0.01203
Weight: <None>
Min Conc: 0

201 Hg [He]

	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.000	0.000	447.18		P	19.6
2	☐	6.000	5.972	30055.63		P	1.8
3	☐	12.000	12.332	61587.07		P	0.8
4	☐	24.000	23.819	118539.79		P	0.9
5	☐	36.000	35.918	178522.82		P	0.4
6	☐	48.000	48.072	238782.06		P	0.3

y = 4957.8395 * x + 447.1833
R = 1.0000
DL = 0.05293
BEC = 0.0902
Weight: <None>
Min Conc: 0

105 Pd [He]

	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RS
1	☐	0.000	0.000	1104.61		P	1
2	☐	20.000	20.662	223971.88		P	
3	☐	40.000	41.974	453860.23		P	
4	☐	80.000	79.679	860569.65		P	
5	☐	120.000	118.727	1281764.94		A	
6	☐	160.000	160.539	1732765.19		A	

y = 10786.5642 * x + 1104.6100
R = 0.9999
DL = 0.03482
BEC = 0.1024
Weight: <None>
Min Conc: 0

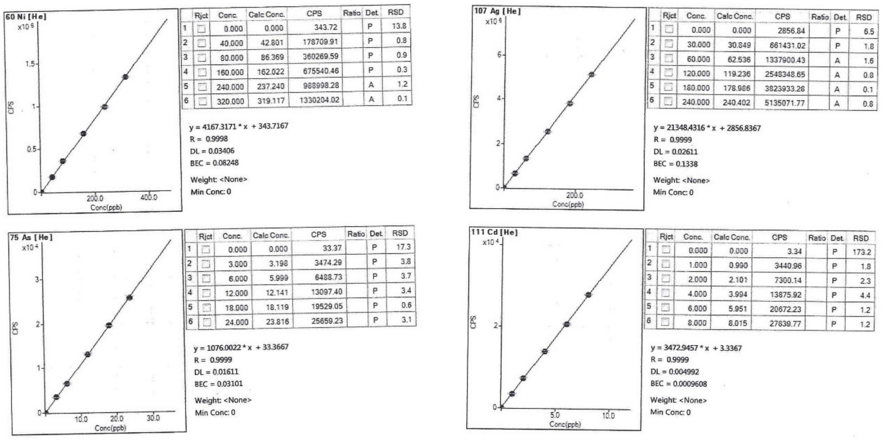
205 Tl [He]

	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.000	0.000	2796.79		P	10.2
2	☐	2.000	1.456	74423.15		P	1.4
3	☐	3.000	3.160	158254.27		P	1.3
4	☐	6.400	6.354	315390.67		P	1.8
5	☐	10.000	9.748	482392.58		P	1.0
6	☐	13.000	13.263	655304.36		P	1.6

y = 49197.3689 * x + 2796.7933
R = 0.9985
DL = 0.01736
BEC = 0.05685
Weight: <None>
Min Conc: 0

Rh, Pd, Hg, Ti

Fig. 7: Calibration data for rhodium, paladium, mercury and titanium



60 Ni [He]

	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.000	0.000	343.72		P	13.8
2	☐	40.000	42.801	178709.91		P	0.8
3	☐	80.000	86.369	360269.59		P	0.9
4	☐	160.000	162.022	675540.46		P	0.3
5	☐	240.000	237.240	988998.28		A	1.2
6	☐	320.000	319.117	1330204.02		A	0.1

y = 4167.3171 * x + 343.7167
R = 0.9998
DL = 0.03406
BEC = 0.08248
Weight: <None>
Min Conc: 0

107 Ag [He]

	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.000	0.000	2856.84		P	6.5
2	☐	30.000	30.849	661431.02		P	1.8
3	☐	60.000	62.536	1337900.43		A	1.6
4	☐	120.000	119.236	2548348.65		A	0.8
5	☐	180.000	178.986	3823933.28		A	0.1
6	☐	240.000	240.402	5135071.77		A	0.8

y = 21348.4316 * x + 2856.8367
R = 0.9999
DL = 0.02611
BEC = 0.1338
Weight: <None>
Min Conc: 0

75 As [He]

	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.000	0.000	33.37		P	17.3
2	☐	3.000	3.198	3474.29		P	3.8
3	☐	6.000	5.999	6488.73		P	3.7
4	☐	12.000	12.141	13097.40		P	3.4
5	☐	18.000	18.119	19529.05		P	0.6
6	☐	24.000	23.816	25659.23		P	3.1

y = 1076.0022 * x + 33.3667
R = 0.9999
DL = 0.01611
BEC = 0.03101
Weight: <None>
Min Conc: 0

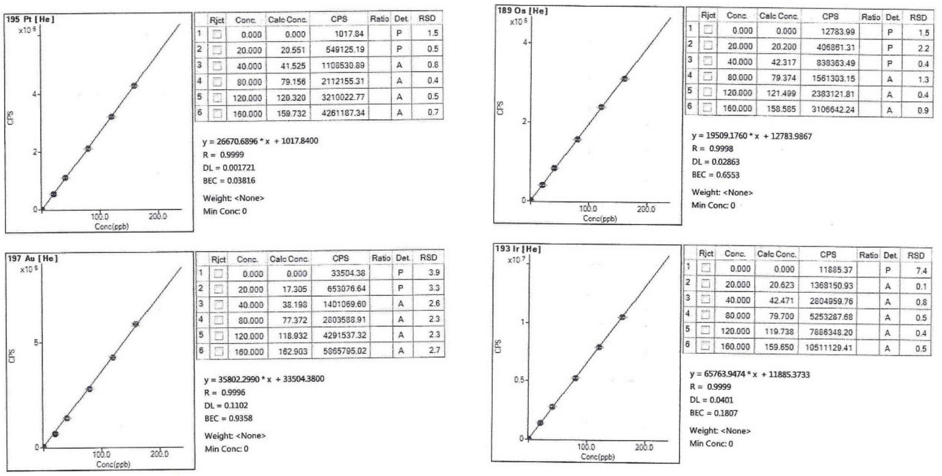
111 Cd [He]

	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.000	0.000	3.34		P	173.2
2	☐	1.000	0.990	3440.96		P	1.8
3	☐	2.000	2.101	7300.14		P	2.3
4	☐	4.000	3.994	13875.92		P	4.4
5	☐	6.000	5.951	20672.23		P	1.2
6	☐	8.000	8.015	27839.77		P	1.2

y = 3472.9457 * x + 3.3367
R = 0.9999
DL = 0.004992
BEC = 0.0009608
Weight: <None>
Min Conc: 0

Ni, As, Ag, Cd

Fig. 8: Calibration data of nickel, arsenic, silver and cadmium



	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.000	0.000	1017.84		P	1.5
2	☐	20.000	20.551	549125.19		P	0.5
3	☐	40.000	41.525	1108530.89		A	0.8
4	☐	80.000	79.156	2112155.31		A	0.4
5	☐	120.000	120.320	3210022.77		A	0.5
6	☐	160.000	159.732	4261187.34		A	0.7

y = 26670.6896 * x + 1017.8400
R = 0.9999
DL = 0.001721
BEC = 0.03816

Weight: <None>
Min Conc: 0

	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.000	0.000	12783.99		P	1.5
2	☐	20.000	20.200	406861.31		P	2.2
3	☐	40.000	42.317	838363.49		P	0.4
4	☐	80.000	79.374	1561303.15		A	1.3
5	☐	120.000	121.499	2383121.81		A	0.4
6	☐	160.000	158.585	3106642.24		A	0.9

y = 19509.1760 * x + 12783.9867
R = 0.9998
DL = 0.02863
BEC = 0.6553

Weight: <None>
Min Conc: 0

	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.000	0.000	33504.38		P	3.9
2	☐	20.000	17.305	653076.64		P	3.3
3	☐	40.000	38.198	1401069.60		A	2.6
4	☐	80.000	77.372	2803588.91		A	2.3
5	☐	120.000	118.932	4291537.32		A	2.3
6	☐	160.000	162.903	5865795.02		A	2.7

y = 35802.2990 * x + 33504.3800
R = 0.9996
DL = 0.1102
BEC = 0.9358

Weight: <None>
Min Conc: 0

	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.000	0.000	11885.37		P	7.4
2	☐	20.000	20.623	1368150.93		A	0.1
3	☐	40.000	42.471	2804959.76		A	0.8
4	☐	80.000	79.700	5253287.68		A	0.5
5	☐	120.000	119.738	7886348.20		A	0.4
6	☐	160.000	159.650	10511129.41		A	0.5

y = 65763.9474 * x + 11885.3733
R = 0.9999
DL = 0.0401
BEC = 0.1807

Weight: <None>
Min Conc: 0

Pt, Au, Os, Ir

Fig. 9: Calibration data for platinum, gold, osmium, iridium

in drug substance samples were lower than the limits established by the chapter 232.

The correlation coefficient of the calibration curve should be ≥ 0.99 for each analyte. From the linearity data, it was concluded that the method was linear. In the system suitability study, the concentration of each analyte in the bracketing standard should not vary by ± 20% of the actual concentration. The correlation coefficient of the calibration curve for each analyte was found to be > 0.99. From the presented data it was observed that system was suitable. Specificity study demonstrates the concentration of each analyte in the bracketing standard was within the acceptance criteria. (± 20% of actual concentration) from the available data it was concluded that the method is specific. The quantitation limit for each analyte should not be more than 30% of the specification limit. The response of the detection limit solution for each element was found to be consistent. %RSD of class – 1, class – 2A and 2B elements response at LoQ level was found to be within 20%. Based on the above data, it is concluded that the LoD and LoQ value for each elemental impurity and reported values are precise. %RSD of the content of each elemental impurities in six samples were found to be less than 20%. The correlation coefficient for each analyte was found to be > 0.99. The residual sum of square, the intercept and the slope of the regression line were reported. Based on the linearity, precision and accuracy data, the range of the test method is from LoQ to 300% of the target concentration. The %RSD of the content of each elemental impurities in six samples were found to be below 20%. The cumulative %RSD for the residue of class 1, class 2A and 2B elements in twelve preparations (i.e. method and intermediate precision) for each analyte was found to be less than 25%. From the above data it was concluded that the method was precise and rugged. The mean %recovery for each analyte at each level was found to be within 70 to 130%. From the above data, it was concluded that the method was accurate. The %RSD of the content of each elemental impurities in duplicate spiked samples were found to be below 20%. From the data, it was concluded that method was robust and precise.

All the validation parameters such as system suitability, specificity, linearity, precision, LoD and LoQ, accuracy and robustness compiled with the acceptance limits according to USP and ICH guidelines. In the true sense, the daily maximum dose for zinc orotate dihydrate and thus the risk is very little; therefore, the limits established considering this maximum daily dose may be elevated.

CONCLUSION

This method is exceptional because it was simple, rapid, sensitive and precise. Substantial testing has shown that an analytical method for elemental impurities that complies with USP 232 and 233 requirement and reliable in terms of its accuracy, linearity, specificity, repeatability, ruggedness and solution stability. An ICP-MS method was developed for the concurrent determination of 17 elements in zinc orotate dihydrate. It can rapidly and sensitively detect 17 elements in zinc orotate dihydrate simultaneously, with minimal sample consumption. Sample pretreatment was simple, only requiring the use of a mixed diluent for dilution. The detection of each sample could be completed within a short time, which is suitable for tracking a large number of samples. In order to ensure the stability of the substance, nitric acid and hydrochloric acid were used together. As a result, a reliable ICP-MS method was developed and validated for the concurrent identification of inorganic elements in such complex mixtures, the suggested sample treatment was not only successful but also compatible with this method.

ACKNOWLEDGMENTS

Authors express their sincere gratitude to Seven Hills College of Pharmacy, Tirupati, for continuous motivation, support, and guidance for research activity and for providing all required facilities to accomplish the entitled work.

REFERENCES

1. Wilschefski SC, Baxter MR. Inductively Coupled Plasma Mass Spectrometry: Introduction to Analytical Aspects. *Clin Biochem Rev.* 2019;40(3):115-133. doi:10.33176/AACB-19-00024
2. Clases D, Gonzalez de Vega R. Facets of ICP-MS and their potential in the medical sciences-Part 1: fundamentals, stand-alone and hyphenated techniques. *Anal Bioanal Chem.* 2022;414(25):7337-7361. doi:10.1007/s00216-022-04259-1
3. Tang, X., Li, B., Lu, J. *et al.* Gold determination in soil by ICP-MS: comparison of sample pretreatment methods. *J Anal Sci Technol* 2020; 11, 45. https://doi.org/10.1186/s40543-020-00245-3
4. Bulska E, Wagner B. Quantitative aspects of inductively coupled plasma mass spectrometry. *Philos Trans A Math Phys Eng Sci.* 2016;374(2079):20150369. doi:10.1098/rsta.2015.0369
5. Mazarakioti EC, Zotos A, Thomatou AA, Kontogeorgos A, Patakas A, Ladavos A. Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), a Useful Tool in Authenticity of Agricultural Products' and Foods' Origin. *Foods*. 2022;11(22):3705. doi:10.3390/foods11223705
6. O. Chahrour et al., "Development and validation of an ICP-MS method for the determination of elemental impurities in TP-6076 active pharmaceutical ingredient (API) according to USP ◎232◎/◎233◎," J. Pharm. Biomed. Anal., 2017; 145, 84–90.
7. United States Pharmacopeia Ad hoc advisory panel on inorganic impurities and heavy metals and USP staff—stimuli to the revision process: general chapter on inorganic impurities: heavy metals, 2008.
8. The United States Pharmacopeia Convention, The United States pharmacopeia, Chapter 232: Elemental Impurities – Limits, 2013.
9. Agh F, Hasani M, Khazdouz M, Amiri F, Heshmati J, Aryaeian N. The Effect of Zinc Supplementation on Circulating Levels of Brain-Derived Neurotrophic Factor (BDNF): A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Int J Prev Med.* 2022; 13:117. doi: 10.4103/ijpvm.IJPVM_478_20
10. Wegmüller R, Tay F, Zeder C, Brnic M, Hurrell RF. Zinc absorption by young adults from supplemental zinc citrate is comparable with that from zinc gluconate and higher than from zinc oxide. *J Nutr.* 2014;144(2):132-136. doi:10.3945/jn.113.181487
11. Löffler M, Carrey EA, Zameitat E. Orotate (orotic acid): An essential and versatile molecule. *Nucleosides Nucleotides Nucleic Acids.* 2016;35(10-12):566-577. doi:10.1080/15257770.2016.1147580
12. Rosenfeldt FL. Metabolic supplementation with orotic acid and magnesium orotate. *Cardiovasc Drugs Ther.* 1998;12 Suppl 2:147-152. doi:10.1023/a:1007732131887
13. Brugger D, Windisch WM. Strategies and challenges to increase the precision in feeding zinc to monogastric livestock. Anim Nutr. 2017;3(2):103-108. doi: 10.1016/j.aninu.2017.03.002.
14. Resano M, Aramendía M, García-Ruiz E, Bazo A, Bolea-Fernandez E, Vanhaecke F. Living in a transient world: ICP-MS reinvented *via* time-resolved analysis for monitoring single events. *Chem Sci.* 2022;13(16):4436-4473. doi:10.1039/d1sc05452j
15. Kucukguzel SG, Rollas S, Kucukguzel I, Kiraz M. Synthesis and antimycobacterial activity of some coupling products from 4-aminobenzoic acid hydrazones. Eur J Med Chem.; 2009; 34:1093–1100.
16. Dogan HN, Duran A, Rollas S, Sener G, Uysal MK, Gülen D. Synthesis of new 2,5-disubstituted-1,3,4-thiadiazoles and preliminary evaluation of anticonvulsant and antimicrobial activities. *Bioorg Med Chem.* 2002;10(9):2893-2898. doi:10.1016/s0968-0896(02)00143-8
17. Bari A, Amer A, Syed SA, Azmat K, Al-obaid A. A facile one pot synthesis and anticancer evaluation of novel substituted 1,2-dihydropyridine and 1,2,3,4-tetrahydropyrimidine analogues. J Heterocycl Chem.; 2016; 53(2):377–82. DOI: 10.1002/jhet.2400
18. Syed, Saeed Ali, Mahmood, Amer, Alfayez, Musaad. "Synthesis of orotic acid derivatives and their effects on stem cell proliferation" *Open Chemistry*, 2020; 18 (1): 620-627. https://doi.org/10.1515/chem-2020-0096
19. Hill CL, Weeks MS, Schinazi RF. Anti-HIV-1 activity, toxicity, and stability studies of representative structural families of polyoxometalates. J Med Chem. 1990; 33(10):2767-72. doi: 10.1021/jm00172a014.
20. Matunas R, Lai AJ, Lee C. Iridium-catalyzed selective N-allylation of hydrazines. Tetrahedron. 2005; 61:6298–308. DOI: 10.1016/j.tet.2005.03.105
21. Bari A, Syed SA, Hashmi IA. Synthetic studies on the synthesis of some new heterocyclic compounds derived from 3-formylchromones. Chem Heterocyclic Compd. 2014; 49(12):1723–30. DOI: 10.1007/s10593-014-1424-4
22. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD. Multilineage potential of adult human mesenchymal stem cells. Science. 1999; 2;284(5411):143-7. doi: 10.1126/science.284.5411.143.
23. Hong CI, Piantadosi C, Irvin JL. Potential anticancer agents. VI. 5-Substituted pyrimidine-6-carboxaldehydes. J Pharm Sci. 1970;59(11):1637-45. doi: 10.1002/jps.2600591120.
24. Siddiqui, K.A., Bharati, A.K. and Lama, P. Zinc-orotate coordination polymer: synthesis, thermogravimetric analysis and luminescence properties. *SN Appl. Sci.* 2020; **2**, 392. https://doi.org/10.1007/s42452-020-2197-2
25. Yeşilel O, Uçar İ, Bulut A, Ölmez H, Büyükgüngör O. Synthesis, Crystal Structure, Spectral and Thermal Characterization of cis-Diaquabis(1,10-phenanthroline) zinc (II) Diorotate Hydrate, cis- [Zn (H2O)2(phen)2] (H2Or)2 · (H2O)2.125. Zeitschrift für Naturforschung B. 2006;61(2): 147-152. https://doi.orgssss/10.1515/znb-2006-0205
26. Ye, Feng, Qiufeng Ye, Haihua Zhan, Yeqian Ge, Xiaotao Ma, Yingying Xu, Synthesis and Study of Zinc Orotate and Its Synergistic Effect with Commercial Stabilizers for Stabilizing Poly (Vinyl Chloride). *Polymers*. 2019; 11(2):194. https://doi.org/10.3390/polym11020194

HOW TO CITE THIS ARTICLE: Sivagami B, Gurupriya G, Chandrasekar R, Niranjan BM. A Quantitative Approach for the Determination of Elemental Impurities in Zinc Orotate Dihydrate Drug Substance by ICP-MS Method. Int. J. Pharm. Sci. Drug Res. 2023;15(6):770-779. **DOI:** 10.25004/IJPSDR.2023.150611