IJPSR (2023), Volume 14, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 December 2022; received in revised form, 13 February 2023; accepted 29 May 2023; published 01 August 2023

DETERMINATION OF ELEMENTAL IMPURITIES IN ESLICARBAZEPINE ACETATE A PARTIAL SEIZURE DRUG BY USING ICP-MS

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Keywords:

Eslicarbazepine Acetate (ESA), ICP-MS, Heavy metals, Genotoxic Impurities

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ABSTRACT: Eslicarbazepine acetate is a chemical compound that has found medical use as a partial seizure drug. Monitoring of heavy metals (As, Mn, Cd, Cr, V, Mo, Cu, and Sb) in-process, intermediates, and final drug substances is an important activity in the pharmaceutical industry because of the potential for toxicity and the risk of serious health hazards even at very low doses. This research article established and validated an easy and rapid analytical method for determining eight heavy metals in Eslicarbazepine acetate. Drugs must first be processed through acid digestion using microwave radiation before being analyzed for these elemental impurities. The % RSD of the LOQ solution was found to be < 20.0 for all the elements. The analytical method seems linear, with a correlation coefficient > 0.99. Eslicarbazepine acetate sample amounts spiked at three different concentrations, resulting in acceptable percentage recoveries of heavy metal ions ranging from 70% to 150%. Individually, precision and robustness for all conditions met the acceptable limits. Heavy metal analysis by ICP-MS is a powerful technique; therefore, the present method is selective, sensitive, accurate, linear, precise, and robust. It can also be used for routine quantitatively determining eight elemental impurities in Eslicarbazepine acetate.

INTRODUCTION: Eslicarbazepine Prodrug to Eslicarbazepine being acetate (ESA) is likewise an oxcarbazepine active metabolite. (S)-10-acetoxy-10, 11-dihydro-5H-dibenz [b,f] azepine5-carboxamide is its chemical name **Fig. 1**. Due to structure change, it is shown to increase effectiveness and safety and to prevent the generation of a harmful epoxide metabolite. It can be employed to treat trigeminal neuralgia and bipolar disorder.

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.14(8).3892-04

This article can be accessed online on
www.ijpsr.com

DOI link: http://doi.org/10.13040/IJPSR.0975-8232.14(8).3892-04

Eslicarbazepine Acetate was administered to patients who had partial-onset seizures that are resistant to testing, and it was discovered to be effective and well-tolerated [1-5]. The introduction of different metal contaminants into drug products, bulk drug compounds, and their intermediates, including using electrodes, reaction containers, and other synthesis-related equipment, can occur in several ways.

Most notably, metals can be added by using catalysts at various stages of the manufacture of various drugs. Concepts of monitoring and accurate assessments of concentrations for toxic and hazardous elements are required to develop risk assessment and sustainable development. Very few methods become available in the literature such as FT-IR [6], HPLC [7-11], HPTLC [12], LC-MS/MS [13], for the assessment of ESA and their related organic impurities. Possibly harmful substances have ill effects on the human body. These may be mainly monitored to reduce uncontrolled anthropogenic activities and protect public health [14-15]. To quantify these heavy metal ions for this study, sensitive and precise analytical techniques must be employed. Among the modern analytical spectral techniques, ICP-MS was found to be selective, sensitive, and rapid for determining these genotoxicimpurities. A microwave oven is a commonly used technique in the pharmaceutical industry for liberating heavy metal ions from organic matrices, which has good repeatability and the least loss of target elements. According to published reports for the determination of heavy metals, very few of trace elements related methods by ICP-MS were available, which are in plants [16], cereals [17], breast milk [18], dietary supplements [19], solid waste [20], rice [21], *etc* [22-34].

No ICP-MSmethod was applied for the assessment of heavy metal ions like Cadmium (Cd), Arsenic (As), Chromium (Cr), Vanadium (V), Molybdenum (Mo), Manganese (Mn), Copper (Cu) and antimony (Sb) in Eslicarbazepine Acetate. At present, our research study aims to develop an ICP-MS method for the quantification of genotoxic heavy metal ions in Eslicarbazepine acetate and validate the method.

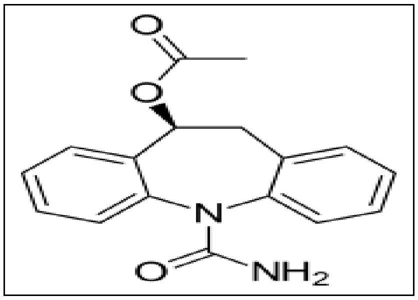

FIG. 1: STRUCTURE OF ESA

EXPERIMENTAL: ICP-MS instrumentation setup for elemental impurity determination: Heavy metal ion determination in this study was accomplished by employing an Agilent 7800 Tandem ICP-MS (ICP-MS/MS, make: Agilent Technologies from Japan) equipped with nickel cones. **Table 1** summarizes the tuning settings and operating parameters for detecting eight elemental impurities in Eslicarbazepine acetate.

TABLE 1: ICP-MS INSTRUMENT OPERATING CONDITIONS AND PARAMETERS

S. no.	Parameters	Method Condition
	ICP-MS- Plasma Condition	
01	-RF Power	:-1550 W
02	-RF Matching	:-1.80 V
03	-Sample depth	:-8.0 mm
04	-Nebulizer Gas flow	:-1.01 (L/min)
05	-Nebulizer pump speed	:-0.10 rps
06	-Spray Chamber Temperature	:-20C
	Octopole Condition	
07	Helium flow	ON
08	Helium gas flow	4.3 (mL /min)
09	Energy Discrimination	3.0 V
	Acquisition Parameters	
10	Acq Mode	Spectrum
11	Peak Pattern	3 Points
12	Replicates	3
13	Sweeps/Replicate	100
14	Integration Time/Mass (sec)	0.0900 sec
	Auto Sampler Settings **Pre-Run**	
15	Uptake speed (Nebulizer Pump) Pump)	0.3 rps
16	Uptake Time	45 sec
17	Stabilize	55 sec
	Post Run- (Probe Rinse)	
18	-Rinse Speed (Nebulizer Pump)	0.3 rps
19	-Rinse at Rinse Port (Sample)	10 sec
20	(Std.)	10 sec
Post Run (Rinse)		**Rinse Vial 1**

21	-Rinse Speed (Nebulizer Pump)		0.1 raps
22	-Rinse at Rinse	Vial (Step 1)	60 sec
23		Port (Step 1)	10 sec
Post Run (Rinse)			**Rinse Vial 2**
24	-Rinse Speed (Nebulizer Pump)		0.1 raps
25	-Rinse at Rinse @Vial (Step 2)		30 sec

Samples and Reagents used for ICP-MS/MS analysis: ICP-MS/MS analysis was executed by employing trace metal grade concentrated Nitric acid (HNO_3 :Fluka), Cadmium (Cd), Arsenic (As), Chromium (Cr), Vanadium (V), Molybdenum (Mo), Manganese (Mn), Copper (Cu) and antimony (Sb), as well as Tuning Solution (Agilent), Water (Milli-Q) and Hydrogen Peroxide (Merck AR grade). All solution preparations were carried out in a fume hood. Chemicals, reagents, and standards of their grades are summarized in **Table 2.**

TABLE 2: CHEMICALS, REAGENTS AND STANDARD

Standard/Reagent	Grade
Conc. Nitric acid	Trace metal/ Equivalent
Cadmium, Arsenic, Vanadium, Chromium, Manganese , Molybdenum, Copper and antimony – against standard 1000 µg/mL	ICP grade/ Equivalent
Scandium, Yttrium, Germanium and Indium– against standard 1000 µg/mL (ISTD)	ICP grade/ Equivalent
Tuning Solution	Agilent
Milli-Q Water	Milli Q / Equivalent

Nitric acid is utilized as a blank reagent solution as well as a washing/rinsing solution for an ICP-MS measurement since it has the simplest spectra of all the acids and the lowest background levels when compared to pure water. The sensitive measurement of metals at ppb (1 ppb = 10^{-9} g ml^{-1}) or ppt (1ppt = 10^{-12} g ml^{-1}) levels in sample solutions generally necessitate a high-purity reagent. As a result, selecting an appropriate nitric acid grade (ICP grade, make: Fluka, Batch No. J2120) was critical for the elemental analysis.

Preparation of Solutions: Diluent preparation (2% v/v HNO3): Conc. nitric acid (20 mL) should be added to 200 milliliters of demineralized water in a 1.0 L volumetric flask to make up the remaining volume.

Making Standard Stock Solutions:

Preparation of Standard Stock Solutions -A: Transfer 0.25 mL of Cd standard solutions at 1000 ppm into volumetric flasks or 10 mL polypropylene tubes, then add diluent to produce the volume.

Preparation of Standard Stock Solution-B: Transfer 10 mL polypropylene tubes or volumetric flasks with the following contents:

1.0 mL of above standard stock solution-A, 0.075 mL of As, and 0.5 mL of V of 1000 ppm standard solution. Fill the remaining space with diluent.

Preparation of Standard Stock Solution-C: In 10 mL polypropylene tubes or volumetric flasks, transfer 1.0 mL of the aforementioned stock solution-B, 0.6 mL of the Sb and 0.75 mL of the Mn from the 1000 ppm standard solution. Fill the remaining space with diluent.

Transfer 1.0 mL of above standard stock solution-C, 0.15 mL of Mo, 0.15 mL of Cu, and 0.55 mL of Cr into 10 mL polypropylene tubes or volumetric flasks, and then dilute to the required volume.

Making the internal standard solution (10 ppm) of scandium, yttrium, germanium and indium: Transfer 0.5 ml of a standard solution containing 1000 ppm of scandium, yttrium, germanium and indium into a 50 ml volumetric flask or polypropylene tube, dilute to the proper level and aspirate into an ICP-MS.

RESULTS AND DISCUSSIONS: To validate the analytical procedures for the quantitative determination of the heavy metals Cadmium, Arsenic, Vanadium, Chromium, Manganese, Molybdenum, Copper, and Antimony in Eslicarbazepine Acetate, the following factors have been considered and assessed: applicability, specificity, range, linearity, the minimum detection limit, repeatability, the limit of quantification, accuracy, and robustness.

Specificity: The capacity of a method to test an analyte specifically or selectively in the presence of components (such as Eslicarbazepine Acetate, Cd, As, V, Cr, Mn, Mo, Cu and Sb) that may be anticipated to be present in the sample is known as specificity. Specificity was confirmed by examining the interference in the ICP-MS with the blank and standard solutions. Since there was less than 3.0% detected interference between the test blank and the blank, the approach is specific.

Limits of Quantitation (LOQ) and Detection (LOD): The lowest concentration or amount of an analyte (Cd, As, V, Cr, Mn, Mo, Cu and Sb) that can be measured with a tolerable degree of statistical certainty is known as the minimum detection limit. The limit of quantification, or LOQ, is the lowest concentration of the analyte that can be identified with a respectable level of accuracy, precision, and repeatability. To establish the LOQ value, predict the first calibration standard as (i.e., 25% specification level) LOQ and establish the LOD value as two times lower than LOQ value. For the establishment of LOD and LOQ values by employing of the new analytical approach of ICP-MS, analyze blank solution, LOD solution, and six injections of LOQ solution (*i.e.,* 25% specification level) in ICP-MS. According to test concentration, the LOD and LOQ for the element were determined to be 0.06 ppm and 0.13ppm for Cadmium (Cd), 0.11 ppm and 0.38 ppm for Arsenic (As), 1.25ppm and 2.5 ppm for Vanadium (V), 83.33 ppm and 275 ppm for Chromium (Cr), 11.36 ppm and 37.5 ppm for Manganese (Mn), 22.73 ppm and 75 ppm for Molybdenum (Mo), 22.7 ppm and 75.2 ppm for Copper (Cu) and 15ppm and 30ppmfor Antimony (Sb) **Table 3.**

TABLE 3: LOD AND LOQ DETERMINATION

S. no.	Element Name	LOD (ppb)	LOQ (ppb)	LOD (ppm) with respect to sample concentration (ppm)	LOQ (ppm) with respect to sample concentration (ppm)	Blank response (cps)	LOD solution response (cps)
1	Cadmium (Cd)	0.125	0.25	0.06	0.125	4.81	698.18
2	Arsenic (As)	0.23	0.75	0.11	0.38	18.52	1337.28
3	Chromium (Cr)	166.67	550	83.33	275	1292.83	6108034.45
4	Vanadium (V)	2.5	5	1.25	2.5	108.52	42258.02
5	Molybdenum (Mo)	45.45	150	22.73	75	66.68	926859.48
6	Manganese (Mn)	22.73	75	11.36	37.5	3034.02	517065.34
7	Copper (Cu)	45.46	150	22.76	75.2	66.91	926926.21
8	Antimony (Sb)	30	60	15	30	1112.65	1872086.2

Precision at the LOQ: The LOQ precisions were assessed using six replicates of the LOQ concentration to calculate the % RSD. The obtained %RSD of the element was 1.30% for Cadmium (Cd), 1.36% for Arsenic (As), 0.60% for Vanadium (V), 0.62% for Chromium (Cr), 0.45% for Manganese (Mn), 0.73 % for Molybdenum (Mo), 0.18% for Copper (Cu) and 1.31% for Antimony (Sb) **Table 4.**

TABLE 4: PRECISION AT LIMIT OF QUANTITATION

Element Name	LOQ Precision							
	Cd	As	V	Cr	Mn	Mo	Cu	Sb
Aspiration-1	2894.34	3508.3	129874.53	126389.14	1520721.2	2615761.5	2615851.7	1320721.9
Aspiration-2	2897.6	3630.56	129971	127914.23	1532705.7	2654360	2619179.3	1342705.3
Aspiration-3	2922.91	3612.09	129534.88	128115.4	1531608.7	2638877.6	2625985.7	1341652.4
Aspiration-4	2918.87	3571.21	129490.09	128026.74	1534567.3	2664062.6	2626958.2	1306254.3
Aspiration-5	2826.58	3545.38	130340.08	126991.83	1538832.1	2661437.2	2624522.6	1307532.7
Aspiration-6	2856.24	3524.31	131581.73	128504.65	1539976.8	2664887.6	2627687.8	1306546.5
Mean	2886.09	3565.31	130132.05	127657	1533068.6	2649897.7	2623364.2	1320902.2
SD	37.59	48.6	775.35	797.68	6897.96	19313.94	4770.23	17351.48
% RSD	1.3	1.36	0.6	0.62	0.45	0.73	0.18	1.31

Linearity and Range: Calibration solutions were used to determine the calibration curves, y = ax + b. (y is the signal intensity, and x is the know concentration of the given analyte in the calibration solution). Five specified varied concentrations of each element standard were run under optimal working conditions, CPS was recorded, and a calibration curve was plotted spanning the range from LOQ to 200% to assess the linearity of the ICP-MS method. The squared correlation coefficient was found to be 1.00 for Cadmium (Cd), 1.00 for Arsenic (As), 1.00 for Vanadium(V), 1.00 for Chromium (Cr), 1.00 for Manganese (Mn), 1.00 for Molybdenum (Mo),1.00 for Copper(Co) and 1.00 for Antimony (Sb). The correlation coefficients for each element (Cd, As, V, Cr, Mn, Mo, Cu and Sb) in Eslicarbazepine Acetate were R ≥0.99 and therefore met the requirement. These results demonstrated the linearity of this ICP-MS method over the specified range of Eslicarbazepine Acetate; their corresponding results are given in **Table 5** to **Table 12,** and graphs are depicted **Fig. 2** to **Fig. 9.**

TABLE 5: LINEARITY RESULTS OF CADMIUM IN ESA

Level	Conc. (ppb) w.r.to sample dilution	Corrected cps
LOQ (25%)	0.25	2848.81
50%	0.5	5616.73
75%	0.75	8374.02
100%	1	11372.87
125%	1.25	13326.75
150%	1.5	16794.09
200%	2	22377
Correlation coefficient (r)	1	1
Intercept	32.37	32.37
Slope	11101.19	11101.19

TABLE 6: LINEARITY RESULTS AND GRAPH OF ARSENIC IN ESA

Level	Conc. (ppb) w.r.to sample dilution	Corrected cps
LOQ (25%)	0.75	3819.56
50%	1.5	6958.29
75%	2.25	10709.2
100%	3	14413.2
125%	3.75	17001.6
150%	4.5	21003.7
200%	6	27460.3
Correlation coefficient (r)	1	1
Intercept	422.41	422.41
Slope	4524.55	4524.55

TABLE 7: LINEARITY RESULTS AND GRAPH OF VANADIUM IN ESA

Level	Conc. (ppb) w.r.to sample dilution	Corrected cps
LOQ (25%)	5	131163
50%	10	251736
75%	15	390609
100%	20	521024
125%	25	631786
150%	30	765108
200%	40	1022871
Correlation coefficient (r)	1	1
Intercept	3467.34	3467.34
Slope	25448.5	25448.5

TABLE 8: LINEARITY RESULTS AND GRAPH OF CHROMIUM IN ESA

Level	Conc. (ppb)w.r.to sample dilution	Corrected cps
LOQ (25%)	550	18051870.77
50%	1100	34061080.85
75%	1650	53153428.73
100%	2200	71150752.28
125%	2750	85779962.79
150%	3300	104446255.8
200%	4400	139870840.9
Correlation coefficient (r)	1	1
Intercept	291143.75	291143.75
Slope	31628.6	31628.6

TABLE 9: LINEARITY RESULTS AND GRAPH OF MANGANESE IN ESA

Level	Conc. (ppb) w.r.to sample dilution	Corrected cps
LOQ (25%)	75	1563883.87
50%	150	2939443.54
75%	225	4547146.06
100%	300	6089761.43
125%	375	7304042.53
150%	450	8884231.63
200%	600	11912444.72
correlation coefficient (r)	1	1
intercept	65015.6	65015.6
slope	19671.7	19671.7

TABLE 10: LINEARITY RESULT AND GRAPH OF MOLYBDENUM IN ESA

Level	Conc. (ppb)w.r.to sample dilution	Corrected cps
LOQ (25%)	150	2651904.42
50%	300	5023661.21
75%	450	7759953.4
100%	600	10396238.2
125%	750	12696967.5
150%	900	15313929.6
200%	1200	20637281.7
correlation coefficient (r)	1	1
intercept	6145.64	6145.64
slope	17111.93	17111.93

TABLE 11: LINEARITY RESULTS AND GRAPH OF COPPER IN ESA

Level	Conc. (ppb)w.r.to sample dilution	Corrected cps
LOQ (25%)	150	2689290.42
50%	300	5053061.21
75%	450	7868974.40
100%	600	10409254.88
125%	750	12696967.51
150%	900	15247187.61
200%	1200	20684645.12
correlation coefficient (r)	1.0000	1.0000
intercept	59853.14	59853.14
slope	17064.46	17064.46

TABLE 12: LINEARITY RESULTS AND GRAPH OF ANTIMONY IN ESA

Level	Conc. (ppb)w.r.to sample dilution	Corrected cps
LOQ (25%)	60	3797549.95
50%	120	8021429.82
75%	180	12153608.91
100%	240	17011038.96
125%	300	21094143.75
150%	360	25194308.65
200%	480	33524032.85
correlation coefficient (r)	1.0000	1.0000
intercept	-420717.40	-420717.40
slope	28446.24	28446.24

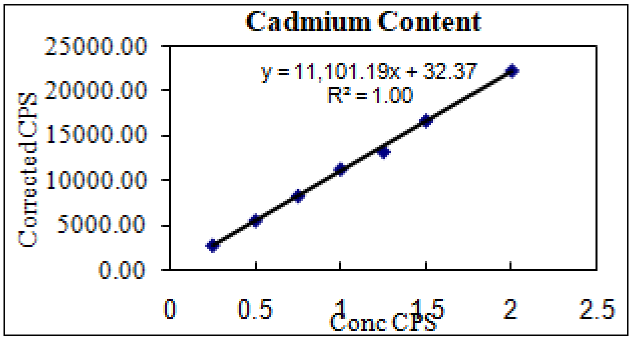

FIG. 2: LINEARITY GRAPH FOR CADMIUM IN ESA

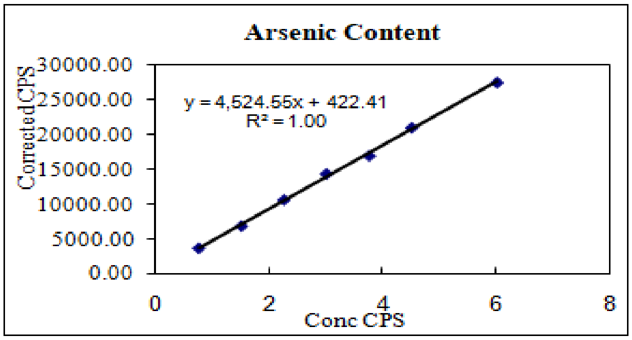

FIG. 3: LINEARITY GRAPH FOR ARSENIC IN ESA

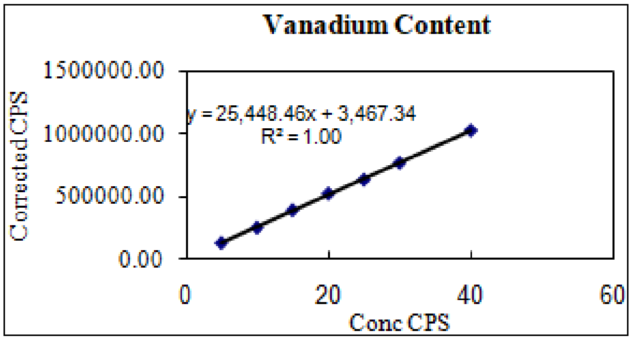

FIG. 4: LINEARITY GRAPH FOR VANADIUM IN ESA

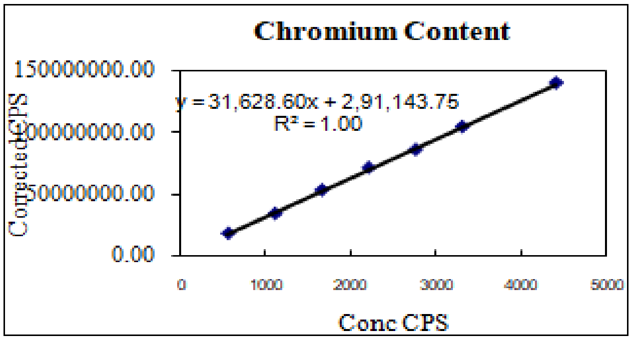

FIG. 5: LINEARITY GRAPH FOR CHROMIUM IN ESA

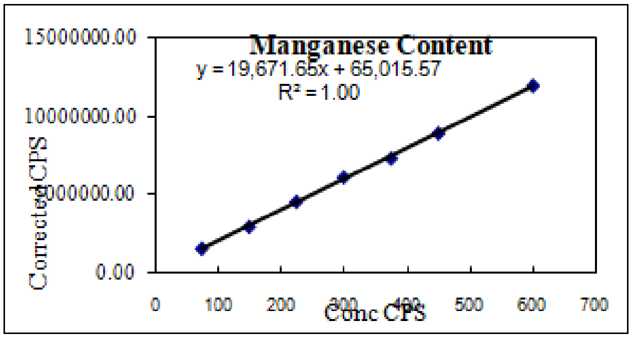

FIG. 6: LINEARITY GRAPH FOR MANGANESE IN ESA

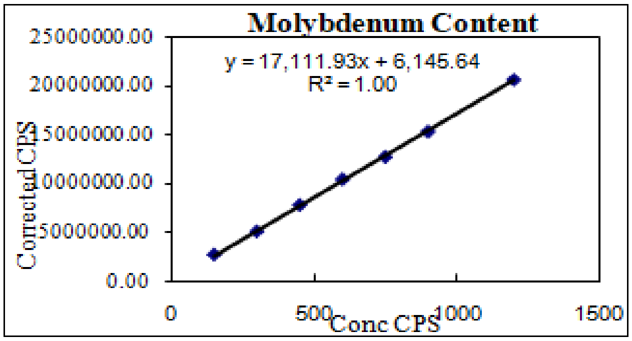

FIG. 7: LINEARITY GRAPH FOR MOLYBDENUM IN ESA

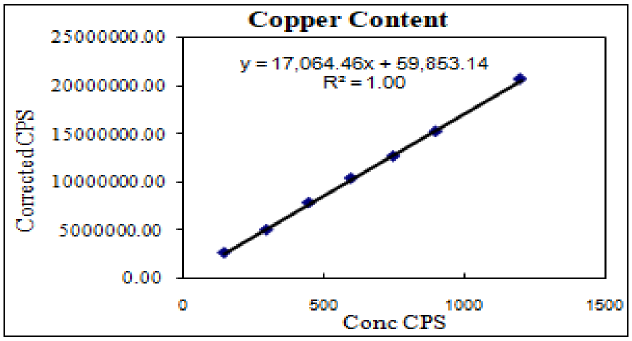

FIG. 8: LINEARITY GRAPH FOR COPPER IN ESA

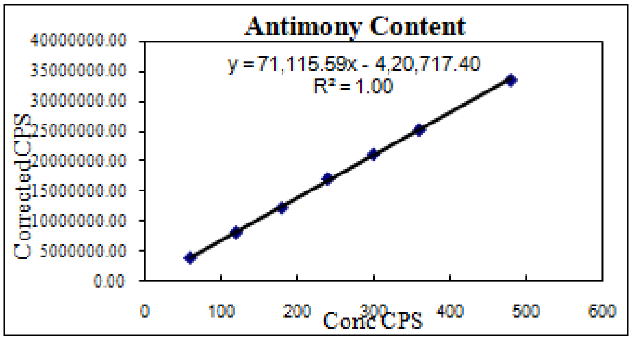

FIG. 9: LINEARITY GRAPH FOR ANTIMONY IN ESA

Precision: To assess the precision, the %RSD was calculated. To determine the analytical instrument system precision or whether the instrument can consistently reproduce the measurement, the aspirated diluent, the system's precision solution, the calibration standards, the calibration blank, and the standard check solution. Calculate the RSD percentage as well as the variance percentage for six replicate aspirations. Software is utilized to determine the calibration curve's correlation coefficient. For each element Cd, As, V, Cr, Mn, Mo, Co, and Sb, the percentage RSD for 6 replicates of the system precision should not be greater than 15.0. The precision results are represented in **Table 13.**

TABLE 13: PRECISION RESULTS OF EACH ELEMENT IN ESLICARBAZEPINE ACETATE

Element Name	System Precision							
	Cadmium	Arsenic	Vanadium	Chromium	Manganese	Molybdenum	Copper	Antimony
Aspiration-1	11005.86	13041.08	482153.87	66011931.3	5588497.35	9859552.26	9662608.42	5142957.65
Aspiration-2	10846.43	13300.77	477664.53	65681772.53	5536241.39	9707742.68	9707742.68	5012321.19
Aspiration-3	10772.38	13712.49	487956.43	67027159.1	5649944.29	9695933.1	9859001.6	5147961.89
Aspiration-4	10802.07	13590.02	489067.7	66914097.6	5656066.05	9804153.52	9817862.01	5056857.05
Aspiration-5	11161.71	13152.45	484284.49	66323137	5592482	9752313.94	9824207.6	5152385.3
Aspiration-6	10661.16	13337.71	484100.2	66527824	5653581	9901504.36	9809665	5050548.5
Mean	10874.9	13355.8	484204.5	66414320.3	5612802	9786866.6	9780181.2	5093838.6
SD	179.84	255.28	4116.34	518669.33	48537.41	83002.7	76825.54	61084.34
% RSD	1.65	1.91	0.85	0.78	0.86	0.85	0.79	1.2

Accuracy/ Recovery Study: The study on the accuracy of the method used to be decided by doping the respective concentration solution of element in test preparation and determining the content of 8 elemental impurities from test preparation. This can be expressed as percentage recovery R [%]. Accuracy was obtained by reading the pure sample and three known concentrations (LOQ, 50%, 100% and 150 %) of samples. The accuracy of the method is determined by spiking the sample with Cd, As, V, Cr, Mn, Mo, Cu and Sb in Eslicarbazepine Acetate elements at LOQ, 50%, 100% and 150% of the specification level concentration as well as recovery studies were carried out. We evaluated the % Rec obtained; the results were within the range of 95.5% to 102.7% for Cadmium, 96.8% to 106% for Arsenic, 95.5% to 104.5% for Chromium, 96.2% to 105.5% for Vanadium, 97% to 107.9% for Manganese, 95.3% to 104.5% for Molybdenum, 98.5% to 103.8% for Copper, 98.9% to 100.4% for Antimony. The obtained % recovery was well within the limit of 70% to 150%, and accuracy results were presented in **Table 14.**

TABLE 14: ACCURACY / RECOVERY STUDY OF EACH ELEMENT IN ESLICARBAZEPINE ACETATE

Cadmium												
Levels		LOQ			50%			100%			150%	
Preparations	1	2	3	1	2	3	1	2	3	1	2	3
Obtained concentration (ppm)	0.13	0.13	0.13	0.25	0.24	0.24	0.48	0.49	0.47	0.71	0.7	0.73
Amount addeded (ppm)		0.125			0.25			0.5			0.75	
% Recovery	102.5	104.2	101.5	99.9	98	96.1	95.7	98.3	95	95.3	93.2	97.9
Avg % Recovery		102.7			98			96.3			95.5	
Arsenic												
Levels		LOQ			50%			100%			150%	
Preparations	1	2	3	1	2	3	1	2	3	1	2	3
Obtained concentration (ppm)	0.38	0.41	0.4	0.74	0.74	0.72	1.44	1.49	1.5	2.14	2.19	2.2
Amount addeded (ppm)		0.375			0.75			1.5			2.25	
% Recovery	101.2	108.9	107.7	99.1	99.2	95.7	96.1	99.3	99.8	95.3	97.2	98
Avg % Recovery		106			98			98.4			96.8	
Vanadium												
Levels		LOQ			50%			100%			150%	
Preparations	1	2	3	1	2	3	1	2	3	1	2	3
Obtained concentration (ppm)	2.63	2.64	2.65	4.87	4.94	4.86	9.74	9.74	9.75	14.31	14.39	14.58
Amount addeded (ppm)		2.5			5			10			15	
% Recovery	105.2	105.4	105.8	97.5	98.9	97.2	97.4	97.4	97.5	95.4	95.9	97.2
Avg % Recovery		105.5			97.8			97.4			96.2	
Cadmium												
Levels		LOQ			50%			100%		150%		
Preparations	1	2	3	1	2	3	1	2	3	1	2	3
Obtained concentration (ppm)	0.13	0.13	0.13	0.25	0.24	0.24	0.48	0.49	0.47	0.71	0.7	0.73

Amount addeded (ppm)		0.125			0.25			0.5			0.75	
% Recovery	102.5	104.2	101.5	99.9	98	96.1	95.7	98.3	95	95.3	93.2	97.9
Avg % Recovery		102.7			98			96.3			95.5	
Chromium												
Levels		LOQ			50%			100%			150%	
Preparations	1	2	3	1	2	3	1	2	3	1	2	3
Obtained concentration (ppm)	285.78	286.78	289.24	538.88	545.94	535.7	1073.4	1080	1071	1562.5	1576.2	1586.9
Amount addeded (ppm)		275			550			1100			1650	
% Recovery	103.9	104.3	105.2	98	99.3	97.4	97.6	98.2	97.4	94.7	95.5	96.2
Avg % Recovery		104.5			98.2			97.7			95.5	
Manganese												
Levels		LOQ			50%			100%			150%	
Preparations	1	2	3	1	2	3	1	2	3	1	2	3
Obtained concentration (ppm)	40.46	40.43	40.55	75.45	76.15	74.82	148.66	149.9	149	216.56	218.74	219.56
Amount addeded (ppm)		37.5			75			150			225	
% Recovery	107.9	107.8	108.1	100.6	101.5	99.8	99.1	99.9	99.3	96.2	97.2	97.6
Avg % Recovery		107.9			100.6			99.5			97	
Molybdenum												
Levels		LOQ			50%			100%			150%	
Preparations	1	2	3	1	2	3	1	2	3	1	2	3
Obtained concentration (ppm)	77.4	78.8	79	146.49	148.55	146.41	291.03	291.2	290.7	426.06	428.14	432.96
Amount addeded (ppm)		75			150			300			450	
% Recovery	103.2	105.1	105.3	97.7	99	97.6	97	97.1	96.9	94.7	95.1	96.2
Avg % Recovery		104.5			98.1			97			95.3	
Copper												
Levels		LOQ			50%			100%			150%	
Preparations	1	2	3	1	2	3	1	2	3	1	2	3
Obtained concentration (ppm)	76.4	78.2	79.1	147.9	149.5	146.9	298.3	293.2	295.7	456.6	448.14	439.96
Amount addeded (ppm)		75			150			300			450	
% Recovery	101.8	104.2	105.4	98.6	99.6	97.9	99.4	97.7	98.5	101.4	99.58	99.76
Avg % Recovery		103.8			98.7			98.5			100.2	
Antimony												
Levels		LOQ			50%			100%			150%	
Preparations	1	2	3	1	2	3	1	2	3	1	2	3
Obtained concentration (ppm)	15.2	14.61	15.4	29.1	30.5	29.5	59.3	59.1	60.9	90.7	90.2	89.4

Amount addeded (ppm)		15			30			60			90	
% Recovery	101.3	97.4	102.6	97	101.6	98.3	98.8	98.5	101.5	100.7	100.2	99.3
Avg % Recovery		100.4			98.9			99.6			100.06	

Robustness Study: The robustness study of these elements (Cd, As, V, Cr, Mn, Mo, Cu and Sb) in Eslicarbazepine Acetate was administered by varying the instrument parameter of ICP-MS and discovered the content material of heavy metals. By proving the analytical method's reliability in the event of anticipated changes in volume: %, its robustness will be established. Each analyte's recovery value should range from 70.0% to 150%. Integration time changes: % RSD cannot exceed 15.0% for every given condition. As the recovery results obtained from the study were within the acceptable limit of recovery Cadmium, Arsenic, Vanadium, Chromium, Manganese, Molybdenum, Copper, and Antimony for preparations of Sample Spiked with 100% Levels of which were well within the acceptance criteria. The % RSD for Arsenic (As), Cadmium (Cd), Chromium (Cr), Vanadium (V), Molybdenum (Mo), Manganese (Mn), Copper (Cu), and Antimony (Sb), for six injections of solutions at 25% (+10% and -10%) level which were well within the acceptance criteria. **Table 15** and **Table 16** display the element's obtained results.

TABLE 15: ROBUSTNESS STUDY RESULTS (CHANGE IN VOLUMES ±10%)

S. no.	Name	Sample preparation		Corrected sample Conc. in (ppb)	Analyte content in (ppm)	% of Recovery
1	Cadmium:	Sample spiked @ specification level	- (Ideal)	1.98	0.98	97.5
			- (+10%)	1.98	0.97	96.9
			- (-10%)	1.97	0.97	96.8
2	Arsenic:	Sample spiked @ specification level	- (Ideal)	5.98	2.95	98.2
			- (+10%)	5.99	2.93	97.8
			- (-10%)	5.97	2.93	97.7
3	Chromium:	Sample spiked @ specification level	- (Ideal)	440.92	217.22	98.7
			- (+10%)	440.1	215.48	97.9
			- (-10%)	441.18	216.44	98.4
4	Vanadium	Sample spiked @ specification level	- (Ideal)	39.99	19.70	98.5
			- (+10%)	39.78	19.48	97.4
			- (-10%)	39.76	19.50	97.5
5	Manganese	Sample spiked @ specification level	- (Ideal)	599.92	295.5	98.5
			- (+10%)	597.52	292.56	97.5
			- (-10%)	598.98	293.85	97.9
6	Molybdenum:	Sample spiked @ specification level	- (Ideal)	1202.12	592.23	98.7
			- (+10%)	1201.14	588.10	98.0
			- (-10%)	1203.47	590.47	98.4
7	Copper	Sample spiked @ specification level	- (Ideal)	1210.48	596.35	99.4
			- (+10%)	1213.34	594.08	99.0
			- (-10%)	1220.24	598.63	99.8
8	Antimony	Sample spiked @ specification level	- (Ideal)	481.22	237.08	98.8
			- (+10%)	482.3	236.16	98.4
			- (-10%)	483.1	237.0	98.7

TABLE 16: ROBUSTNESS STUDY RESULTS (CHANGE IN INTEGRATION TIME ± 10%)

Integration +10%	Response (cps)							
	Cd	As	Cr	V	Mn	Mo	Cu	Sb
1	2912.2	3529.9	126387.2	129899.4	1520721.5	2611562.2	2501568.8	1490723.5
2	2791.8	3442.4	122451.4	127652.86	1552642.2	2626587.1	2552512.6	1479625.1
3	2898.5	3602.7	125414.52	128191.62	1584562.9	2591568.8	2603456.5	1468526.7
4	2853.8	3544.5	125576.72	126892.95	1516483.6	2589912.6	2554400.3	1457428.3
5	2846.9	3498.9	126121.2	127857.12	1548404.3	2579715.9	2605344.1	1446329.9
6	2940.1	3577.3	125227.2	128812.74	1530325.4	2529419.2	2556288.0	1435231.5
Mean	2873.88	3532.60	125196.37	128217.78	1542189.98	2588127.65	2562261.72	1462977.50

SD	53.5	57.2	1414.3	1038.0	25321.2	33349.0	38546.8	20763.2
%RSD	1.86	1.62	1.13	0.81	1.64	1.29	1.50	1.42
Integration -10%	**Response (cps)**							
	Cd	**As**	**Cr**	**V**	**Mn**	**Mo**	**Cu**	**Sb**
1	2807.7	3485.3	123943.3	124034.6	1570721.5	2580902.7	2554245.3	1457428.3
2	2850.8	3463.7	124487.8	124990.3	1592642.2	2630606.0	2605394.1	1446329.9
3	2884.0	3442.1	125032.2	125945.9	1584562.9	2580309.3	2556213.0	1435231.5
4	2853.8	3520.5	125576.7	126901.5	1576483.6	2630012.6	2573898.5	1424133.1
5	2846.9	3498.9	126121.2	127857.1	1598404.3	2579715.9	2574842.3	1413034.7
6	2940.1	3577.3	125227.3	128812.7	1561225.4	2629419.2	2575786.1	1401936.3
Mean	2863.88	3497.93	125064.75	126423.69	1580673.32	2605160.98	2573396.55	1429682.30
SD	44.6	47.5	774.1	1787.8	13911.0	27228.8	18406.2	20763.2
%RSD	1.56	1.36	0.62	1.41	0.88	1.05	0.72	1.45

CONCLUSION: An ICP-MS method has been developed for the selective, sensitive, linear, precise, robust, and accurate determination of Cadmium (Cd), Arsenic (As), Manganese (Mn), Vanadium (V), Molybdenum (Mo), Chromium (Cr), Copper (Cu) and Antimony (Sb) in Eslicarbazepine acetate.

This method produced high precision (%RSD less than 15.0 for each element), accuracy (% average recovery of each element at each level lay between 70% and 150%), linearity (r^2 result was more than 0.99 for all the elements), LOD (the LOD response was higher than the blank response for all the elements), and LOQ (the % RSD of the analyte response for six replicates of the LOQ solution was less than 20.0 for all the elements). The samples were prepared using a simple digestion method; however, no matrix interferences were found. Considering what the sample analysis revealed, it can be concluded that Eslicarbazepine acetate complies with the regulations on the maximum residue limits established by the various regulatory agencies because these harmful metals are well below the permitted limit.

ACKNOWLEDGMENT: The authors are thankful to the Professor. T. Siva Rao from Andhra University for providing the necessary facilities to carry out the present research work.

CONFLICTS OF INTEREST: No conflict of interest from the authors.

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How to cite this article:
Satish G, Rao TS, Srinivas RK, Suman P, Sridhar B and Divya G: Determination of elemental impurities in eslicarbazepine acetate a partial seizure drug by using ICP-MS. Int J Pharm Sci& Res 2023; 14(8): 3892-04. doi: 10.13040/IJPSR.0975-8232.14(8).3892-04.