

Development and Validation of an Inductively Coupled Plasma Mass Spectrometry Method for Estimation of Elemental Impurities in Calcium Acetate Active Pharmaceutical Ingredient

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Chawla *et al.*: Elemental Impurities Estimation in Calcium Acetate

A sensitive and selective method has been developed and validated for the simultaneous quantitative estimation of aluminum, magnesium, potassium, strontium and sodium in calcium acetate active pharmaceutical ingredient by inductively coupled plasma mass spectrometry. Inductively coupled plasma mass spectrometry is an advanced technique which is capable of analyzing multiple elements simultaneously with high selectivity, sensitivity and much lower detection limit. The test sample was prepared with microwave assisted acid digestion and introduced into optimized instrumental parameters for use of a quadrupole based Agilent 7800 inductively coupled plasma mass spectrometry instrument. Scandium is used as an internal standard in the study. The developed method was validated in terms of specificity, linearity, accuracy, precision, range, limit of detection, limit of quantitation and ruggedness. The results for recoveries of all elements were found between 85.3 to 103.9 %. The relative standard deviation for precision was within 15 %. Calibration plots were linear. The low relative standard deviation values and high recoveries of the method confirm the suitability of the method.

Key words: Calcium acetate, inductively coupled plasma mass spectrometry, microwave digestion, method validation

Calcium acetate is a calcium salt of acetic acid. Calcium ethanolate is the systematic name and its standard name is calcium acetate^[1]. Calcium acetate is used to prevent high blood phosphate levels (called hyperphosphatemia) in patients who are on dialysis due to severe kidney disease. Dialysis lowers phosphate level from the blood, but it is difficult to remove enough quantity to keep the blood phosphate levels balanced. Decreasing phosphate levels in blood can help to keep the bones strong, prevent unsafe buildup of minerals in the body and prevent the risk of heart disease and strokes that result from high blood phosphate levels. Calcium acetate is a natural mineral that works by holding on to phosphate from the regular diet, so that phosphate can pass out of the body^[2]. Calcium acetate is official in United States Pharmacopoeia^[3] and European Pharmacopoeia^[4], the monographs states to determine aluminum (Al), magnesium (Mg), potassium (K), strontium (Sr) and sodium (Na) in calcium acetate active pharmaceutical ingredient (API) with

specification given in Table 1. The analytical methods given in the monographs are fluorescence spectroscopy and color comparison methods for aluminum, atomic absorption spectrometry at 285.2 nm for magnesium, atomic absorption spectrometry at 766.5 nm for potassium, atomic absorption spectrometry at 460.7 nm for strontium and atomic absorption spectrometry at 589.0 nm for sodium. Hence for determination of stated elements, 5 different methods are required to use. The literature survey revealed that few methods are reported for determination of calcium in serum by atomic absorption spectroscopy^[5,6]. Calcium determination by flame photometry is also reported^[7,8]. Some methods are reported estimation of calcium by inductively

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TABLE 1: SPECIFICATIONS

Element	In percentage (%) (Not more than)	In ppm (Not more than)
Aluminum (Al)	0.0002	2
Magnesium (Mg)	0.05	500
Potassium (K)	0.05	500
Strontium (Sr)	0.05	500
Sodium (Na)	0.5	5000

coupled plasma-mass spectroscopy (ICP-MS)^[9,10]. It was observed that there is no method available for quantitative estimation of stated 5 elements in calcium acetate API by ICP-MS. The purpose of this research article is to develop and validate a single method for simultaneous determination of stated 5 elements in calcium acetate API by ICP-MS.

MATERIALS AND METHODS

Materials:

Aluminum (1000 mg/l), magnesium (1000 mg/l), potassium (1000 mg/l), strontium (1000 mg/l), sodium (1000 mg/l) and scandium (1000 mg/l) inductively coupled plasma (ICP) standards were procured from Merck Millipore, India. Trace metal grade nitric acid (67 %) was procured from Fisher Scientific, India. De-ionized water used in standard and sample preparation was produced by Millipore Milli-Q water purification system. Calcium acetate samples were received as gift samples from Suven Life Sciences Limited, Hyderabad, India.

Instrument and method:

The method was developed and validated on Agilent Technologies, USA, 7800 series ICP-MS equipped with data acquisition and processing software Mass hunter. The samples were digested using a CEM Mars 5 microwave accelerated reaction system (MARS). The ICP-MS parameters were optimized and optimized conditions are shown in Table 2.

Diluent preparation (Diluted nitric acid):

Dilute 40.0 ml of nitric acid to 1000 ml with Milli-Q water.

Standard stock preparation:

Preparation of 0.5 parts per million (ppm) of aluminum standard (Stock-A solution): Transfer 0.1 ml of 1000 ppm standard of aluminum into a 10 ml volumetric flask and make up to volume with diluent. This is 10 ppm solution, from this solution transfer

0.5 ml into a 10 ml volumetric flask and make up to volume with diluent.

Preparation of 125.0 ppm each of magnesium, potassium and strontium standard (Stock-B solution): Transfer 1.25 ml each of 1000 ppm standard of magnesium, potassium and strontium into a 10 ml volumetric flask and make up to volume with diluent.

Preparation of 500 ppm of sodium standard (Stock-C solution): Transfer 5.0 ml of 1000 ppm of sodium standard into a 10 ml volumetric flask and make up to volume with diluent.

Preparation of 10.0 ppm of scandium (Stock-D solution/internal standard): Transfer 1.0 ml of 1000 ppm of scandium standard into a 10 ml volumetric

TABLE 2: OPTIMIZED ICP-MS CONDITIONS OF THE DEVELOPED METHOD

Contents	Optimized ICP-MS conditions
RF power	1550 Watts
Plasma gas flow	15 l/min
Spray chamber temperature	2°
Peak pattern	3 points
Helium gas flow	4.3 ml/min
Integration time	0.3 s
Tune mode	Helium gas
Number of replicates	3
Mass of aluminum	27 (Analyte)
Mass of magnesium	24 (Analyte)
Mass of potassium	39 (Analyte)
Mass of strontium	88 (Analyte)
Mass of sodium	23 (Analyte)
Mass of scandium	45 (Internal standard)
Before acquisition	
Uptake speed (Nebulizer pump)	0.30 rounds/s
Uptake time	60 s
Stabilization time	45 s
After acquisition (probe rinse)	
Rinse speed (Nebulizer pump)	0.30 rounds/s
Rinse at rinse port (sample)	10 s
Rinse at rinse port (standard)	10 s
Rinse vial 1 (4 % nitric acid)	1
Rinse speed (Nebulizer pump)	0.30 rounds/s
Rinse at rinse vial (step 1)	20 s
Rinse at rinse port (step 1)	20 s
Rinse vial 2 (2 % nitric acid)	2
Rinse speed (Nebulizer pump)	0.30 rounds/s
Rinse at rinse vial (step 2)	20 s
Rinse at rinse port (step 2)	20 s
Rinse vial 3 (milli-Q water)	3
Rinse speed (Nebulizer pump)	0.30 rounds/s
Rinse at rinse vial (step 3)	20 s
Rinse at rinse port (step 3)	20 s

Note: RF-Radio frequency, °-temperature in degree centigrade

flask and make up to volume with diluent. This is 100 ppm solution, from this solution transfer 1.0 ml into a 10 ml volumetric flask and make up to volume with diluent.

Preparation of linearity standard solutions:

Calibration blank: Transfer 0.1 ml of stock-D solution into 10 ml volumetric flask and make up to volume with diluent.

Calibration standard solution-1 (Aluminum 0.005 ppm, magnesium 1.25 ppm, potassium 1.25 ppm, strontium 1.25 ppm and sodium 12.5 ppm): Transfer 0.1 ml of stock-A solution, 0.1 ml of stock-B solution, 0.25 ml of stock-C solution and 0.1 ml of stock-D solution into 10 ml volumetric flask and make up to volume with diluent.

Calibration standard solution-2 (Aluminum 0.01 ppm, magnesium 2.5 ppm, potassium 2.5 ppm, strontium 2.5 ppm and sodium 25.0 ppm): Transfer 0.2 ml of stock-A solution, 0.2 ml of stock-B solution, 0.5 ml of stock-C solution and 0.1 ml of stock-D solution into 10 ml volumetric flask and make up to volume with diluent.

Calibration standard solution-3 (Aluminum 0.02 ppm, magnesium 5.0 ppm, potassium 5.0 ppm, strontium 5.0 ppm and sodium 50.0 ppm): Transfer 0.4 ml of stock-A solution, 0.4 ml of stock-B solution, 1.0 ml of stock-C solution and 0.1 ml of stock-D solution into 10 ml volumetric flask and make up to volume with diluent.

Calibration standard solution-4 (Aluminum 0.03 ppm, magnesium 7.5 ppm, potassium 7.5 ppm, strontium 7.5 ppm and sodium 75.0 ppm): Transfer 0.6 ml of stock-A solution, 0.6 ml of stock-B solution, 1.5 ml of stock-C solution and 0.1 ml of stock-D solution into 10 ml volumetric flask and make up to volume with diluent.

Calibration standard solution-5 (Aluminum 0.04 ppm, magnesium 10.0 ppm, potassium 10.0 ppm, strontium 10.0 ppm and sodium 100.0 ppm): Transfer 0.8 ml of stock-A solution, 0.8 ml of stock-B solution, 2.0 ml of stock-C solution and 0.1 ml of stock-D solution into 10 ml volumetric flask and make up to volume with diluent.

Calibration standard solution-6 (Aluminum 0.05 ppm, magnesium 12.5 ppm, potassium 12.5 ppm, strontium 12.5 ppm and sodium 125.0 ppm): Transfer 1.0 ml of stock-A solution, 1.0 ml of stock-B

solution, 2.5 ml of stock-C solution and 0.1 ml of stock-D solution into 10 ml volumetric flask and make up to volume with diluent.

Preparation of standard check solution (Aluminum 0.02 ppm, magnesium 5.0 ppm, potassium 5.0 ppm, strontium 5.0 ppm and sodium 50.0 ppm): Transfer 0.4 ml of stock-A solution, 0.4 ml of stock-B solution, 1.0 ml of stock-C solution and 0.1 ml of stock-D solution into 10 ml volumetric flask and make up to volume with diluent.

Test sample preparation:

Weigh and transfer about 100 mg of calcium acetate API test sample into a Teflon digestion tube and transfer 0.1 ml of stock-D solution, add 1 ml of nitric acid and 2 ml of diluent. Fix the tube in microwave digester and start the microwaving cycle with 1200 W power, 150° temperature, 200 psi pressure, ramp 20 min and hold time 15 min. After completion of digestion cycle, carefully transfer the solution into 10 ml volumetric flask. Rinse the tube with 2 ml of diluent and transfer into volumetric flask, make up to volume with diluent.

Sample blank preparation:

Proceed as directed in test sample preparation without taking calcium acetate test API.

Procedure: Aspirate one time each of calibration standard solutions and develop a calibration curve and standard check solution of Al, Mg, K, Sr and Na. Aspirate sample blank and sample preparation into the instrument and calculate the Al, Mg, K, Sr and Na content respectively.

Calculations: Sample content (in %)=Instrument reading (in ppm)/Sample weight (in g)×10/10 000

Sample content (in ppm)=Instrument reading (in ppm)/Sample weight (in g)×10

Acceptance criteria for system suitability:

Correlation coefficient for aluminum, magnesium, potassium, strontium and sodium should be not less than 0.99 from calibration standard solutions.

Concentration of aluminum, magnesium, potassium, strontium and sodium in standard check solution should not vary by ±20 % of actual concentration.

Method validation:

The developed method was validated as per International Conference on Harmonization (ICH) Q2 (R1)

guidelines^[11], United States Pharmacopoeia general chapter <233>^[12] and United States Pharmacopoeia general chapter <1225>^[13].

System suitability: System suitability was checked as per the developed method and accordance with United States Pharmacopoeia general chapter <233>^[12]. Aspirated one time each of calibration standard solution 1 to 6 and standard check solution into ICP-MS instrument and evaluated the correlation coefficient and concentration in standard check for aluminum, magnesium, potassium, strontium and sodium as per the system suitability acceptance criteria.

Specificity: The procedure must be able to unequivocally assess each target element in the presence of components that may be expected to be present, including matrix components. Blank interference was performed to establish the non-interference or extent of interference of blank in estimation of elements by ICP-MS, as per the developed procedure, aspirated blank and standard solutions. Blank interference was evaluated by measuring percentage (%) interference of 10 replicate injections of blank injections response, with respect to calibration standard solution-1 response. Being the method is by mass spectrometry and each element is analyzed by its individual respective mass weight, the interference of elements within the method and other elements present in blank and matrix is negligible. The acceptance criteria were set as the interference of calibration blank of aluminum, magnesium, potassium, strontium and sodium should be not more than 20 % response (counts per seconds (cps)) of the calibration standard solution-1.

Precision: Precision of the development method was evaluated by aspirating 6 independent samples of material under test (taken from the same lot) spiked with reference materials for the target elements at the specification level. As per the test method, prepared and aspirated 6 independent preparations of 100 % spiked sample solutions and evaluated the precision by calculating target element content and percentage relative standard deviation (% RSD) for 6 preparations of spiked sample solutions. Intermediate precision (IP) of the method was also evaluated using different analyst on different day by aspirating 6 independent preparations of 100 % spiked sample solutions prepared as same for precision. The acceptance criteria for individual precision % RSD was not more than 20.0 and for 12 preparation results was not more than 25.0.

Accuracy (Recovery): The accuracy of an analytical procedure is the closeness of test results obtained by

that procedure to the true value. Recovery study was performed to evaluate the accuracy of the method by spiking method. Recovery study was done by spiking the target elements into test sample in the concentration of 25 %, 50 %, 100 %, 150 % and 250 % level of the proposed concentration. The test samples were prepared in triplicate for 50 %, 100 % and 150 % level and 6 preparations for 25 % and 200 %. Aspirated the prepared spiked samples in the proposed experimental conditions and percentage (%) recoveries of target elements were calculated for all the levels. The acceptance criterion for % recovery for aluminum, magnesium, potassium, strontium and sodium should be 70 % to 150 % for each individual spike level and % RSD for 6 recovery results at 25 % and 250 % was not more than 15.0.

Limit of detection (LOD) and limit of quantitation (LOQ): LOD is the lowest amount of analyte in sample that can be detected, but not necessarily quantifiable and LOQ is the lowest amount of analyte that can be quantitated with acceptable accuracy, precision, under the stated experimental conditions. The LOD and LOQ were established by the standard deviation (SD) of the response and the slope method using calibration curve. The LOD and LOQ were determined by aspirating known concentrations of aluminum, magnesium, potassium, strontium and sodium elements from 10 % to 100 % of the target concentration under the stated experimental conditions. The LOD and LOQ were calculated using the formula based on the SD of the response and the slope. LOD and LOQ were calculated by using equations, $LOD=3.3 \times SD/S$ and $LOQ=10 \times SD/S$, where SD=standard deviation, S=slope of the calibration curve.

Linearity: Linearity of the method was established for aluminum, magnesium, potassium, strontium and sodium elements from 25 % to 250 % of the proposed concentration using 6 calibration levels (25, 50, 100, 150, 200 and 250 %). The reference standards were used to prepare calibration levels. The calibration curves were plotted for all elements as concentration of level verses response. The result of linearity was evaluated by regression analysis.

Solution stability: Solution stability was established for calibration standards and test sample preparations. Bench-top (ambient temperature) stability was established by aspirating standards and test sample spiked at 100 % level at regular interval for 2 d. Solution stability was established by calculating similarity factor

for standard against fresh standard and percentage element content difference for test sample from initial value. The acceptance criteria for solution stability were considered $\pm 20\%$ relative of initial concentration/content.

RESULTS AND DISCUSSION

System suitability of the method was evaluated by means of correlation coefficient of calibration standard solution and concentration in standard check for aluminum, magnesium, potassium, strontium and sodium. The system suitability results were found within the acceptance criteria. The results are presented in Table 3.

The specificity was performed to check blank interference and evaluated the percentage interference for aluminum, magnesium, potassium, strontium and sodium. The result shows it meets the criteria of not more than 20 % response (cps) interference of the calibration standard solution-1. The results are presented in Table 4.

TABLE 3: SYSTEM SUITABILITY RESULTS

System suitability parameter	Element	Result
Correlation coefficient (r)	Aluminum	0.9999
	Magnesium	1.0000
	Potassium	0.9999
	Strontium	0.9999
	Sodium	1.0000
Concentration variation in standard check solution	Aluminum	1.65
	Magnesium	-0.55
	Potassium	1.95
	Strontium	-0.30
	Sodium	-1.85

Precision was evaluated by aspirating 6 independent samples spiked with reference materials for the target elements at the specification level (i.e. 100 %). % RSD of aluminum, magnesium, potassium, strontium and sodium (n=6) was found to be 3.1, 1.7, 1.6, 1.4 and 0.5 respectively. For IP, it was found to be 2.8, 2.2, 2.4, 2.0 and 0.7 respectively. % RSD of precision and IP (n=12) was found to be 2.8, 2.0, 2.1, 2.4 and 0.6 respectively. The results were found within acceptance criteria for precision of the developed method. The results of precision are compiled in Table 5.

The accuracy was evaluated by calculating the recoveries at 25 %, 50 %, 100 %, 150 % and 250 % level of the proposed concentration. The individual recoveries for all 5 elements at all levels were found between 85.3 to 103.9 % and the mean percentage (%) recoveries were found between 91.2 to 100.8 %. % RSD for 6 recovery results (precision) at 25 % and 250 % were found between 1.0 to 7.6. The recoveries were found within the acceptance criteria and method found to be accurate. The results of accuracy are presented in Table 6 and Table 7.

The LOD and LOQ were determined by aspirating known concentrations of aluminum, magnesium, potassium, strontium and sodium elements from 10 % to 100 % of the target concentration and the LOQ were found to be 0.31 ppm, 1.56 ppm, 1.83 ppm, 0.12 ppm and 3.65 ppm with respect to test concentration respectively. The determined LOQ values are much below than the 25 % of the targeted concentrations. The LOD and LOQ values are presented in Table 8.

Linearity of the method was established for aluminum, magnesium, potassium, strontium and sodium elements

TABLE 4: SPECIFICITY-CALIBRATION BLANK INTERFERENCE

Aspiration	Calibration blank response (cps)				
	Al	Mg	K	Sr	Na
Calibration blank-1	15.56	276.68	335.69	114.45	1881.22
Calibration blank-2	25.56	488.92	335.35	2058.17	2083.22
Calibration blank-3	34.44	307.79	327.74	130.00	1662.71
Calibration blank-4	30.00	348.91	325.19	116.67	1610.32
Calibration blank-5	27.78	452.25	334.62	193.34	1659.09
Calibration blank-6	10.00	461.13	331.88	132.23	1595.30
Calibration blank-7	21.11	500.03	341.87	122.23	1613.15
Calibration blank-8	16.67	494.47	330.15	130.01	1604.60
Calibration blank-9	21.11	568.92	327.49	128.89	1562.59
Calibration blank-10	17.78	604.48	320.68	134.45	1528.56
Mean (n=10)	22.00	450.36	33102.07	326.04	16802.48
Calibration standard solution-1	2518.90	285816.37	262155.87	3317994.57	5515151.45
20 % response (cps) of calibration standard solution-1	503.78	57163.27	52431.17	663598.91	1103030.29

Note: Al-Aluminum; Mg-Magnesium; K-Potassium; Sr-Strontium and Na-Sodium

TABLE 5: PRECISION RESULTS

Spiked test sample	Content (%)									
	MP	IP	MP	IP	MP	IP	MP	IP	MP	IP
	Al	Al	Mg	Mg	K	K	Sr	Sr	Na	Na
01	0.0001924	0.0002019	0.049	0.047	0.047	0.048	0.053	0.051	0.482	0.490
02	0.0001872	0.0001989	0.048	0.049	0.048	0.047	0.054	0.052	0.488	0.486
03	0.0001979	0.0001939	0.050	0.050	0.046	0.049	0.053	0.05	0.489	0.492
04	0.0002021	0.0001892	0.049	0.048	0.047	0.046	0.052	0.051	0.486	0.488
05	0.0001996	0.0002031	0.050	0.049	0.047	0.048	0.054	0.052	0.484	0.483
06	0.0002029	0.0001927	0.050	0.048	0.048	0.049	0.053	0.053	0.487	0.484
Mean (n=6)	0.0001970	0.0001966	0.049	0.049	0.047	0.048	0.053	0.052	0.486	0.487
% RSD (n=6)	3.1	2.8	1.7	2.2	1.6	2.4	1.4	2.0	0.5	0.7
% RSD (n=12)	2.8		2.0		2.1		2.4		0.6	

Note: MP-Method precision; IP-Intermediate precision; Al-Aluminum; Mg-Magnesium; K-Potassium; Sr-Strontium; Na-Sodium; % RSD-% Relative standard deviation, (n=6) is number of test samples=6; (n=12) is number of test samples=12

TABLE 6: ACCURACY RESULTS

Accuracy	Aluminum		Magnesium		Potassium		Strontium		Sodium	
	R (%)	Mean (%)	R (%)	Mean (%)	R (%)	Mean (%)	R (%)	Mean (%)	R (%)	Mean (%)
25 % sample-1	103.5		88.7		92.4		88.5		93.4	
25 % sample-2	93.3		96.6		91.4		93.2		96.8	
25 % sample-3	85.3	93.2	91.5	91.3	87.3	91.2	90.8	92.0	94.7	96.2
25 % sample-4	96.4	(n=6)	93.9	(n=6)	96.2	(n=6)	92.7	(n=6)	97.9	(n=6)
25 % sample-5	89.6		90.7		88.4		91.3		98.3	
25 % sample-6	91.1		86.5		91.4		95.4		95.9	
50 % sample-1	94.5		98.2		96.4		95.6		98.5	
50 % sample-2	98.7	96.7	96.3	97.5	94.6	95.4	96.5	96.5	99.7	98.4
50 % sample-3	96.8	(n=3)	97.9	(n=3)	95.2	(n=3)	97.5	(n=3)	96.9	(n=3)
100 % sample-1	97.3		99.3		97.2		99.4		100.5	
100 % sample-2	98.1	97.7	98.6	98.5	99.1	98.2	100.5	99.4	103.1	100.7
100 % sample-3	97.7	(n=3)	97.7	(n=3)	98.2	(n=3)	98.4	(n=3)	98.4	(n=3)
150 % sample-1	99.3		99.8		102.1		103.9		103.1	
150 % sample-2	98.6	98.6	103.0	100.5	100.3	100.3	98.3	100.5	99.5	100.3
150 % sample-3	97.9	(n=3)	98.6	(n=3)	98.4	(n=3)	99.4	(n=3)	98.2	(n=3)
250 % sample-1	100.9		99.0		100.5		102.8		102.4	
250 % sample-2	99.7		102.4		99.1		101.5		101.3	
250 % sample-3	99.4	99.3	100.3	100.5	97.3	100.1	99.1	99.7	99.8	100.8
250 % sample-4	98.7	(n=6)	98.5	(n=6)	101.5	(n=6)	98.3	(n=6)	97.9	(n=6)
250 % sample-5	98.2		99.9		103.5		97.2		102.8	
250 % sample-6	98.9		102.7		98.6		99.4		100.8	

Note: R=Recovery

from 25 % to 250 % of the proposed concentration using 6 calibration levels (25, 50, 100, 150, 200 and 250 %) and the correlation coefficient (r^2) were found to be 0.9997, 0.9998, 0.9997, 0.9997 and 0.9997 respectively. The linearity data is presented in Table 9.

Solution stability of calibration standards and test sample preparations were established on bench-top

(ambient temperature) and were found stable for 2 d. The acceptance criteria of ± 20 % relative of initial concentration/content were found within the limit.

A single method was developed for simultaneous determination of aluminum, magnesium, potassium, strontium and sodium elements in calcium acetate API and validated. The proposed method is innovative,

TABLE 7: PRECISION AT 25 % AND 250 % RESULTS

Precision	Aluminum		Magnesium		Potassium		Strontium		Sodium	
	R (%)	Mean (%) (n=6)	R (%)	Mean (%) (n=6)	R (%)	Mean (%) (n=6)	R (%)	Mean (%) (n=6)	R (%)	Mean (%) (n=6)
25 % sample-1	103.5		88.7		92.4		88.5		93.4	
25 % sample-2	93.3		96.6		91.4		93.2		96.8	
25 % sample-3	85.3		91.5		87.3		90.8		94.7	
25 % sample-4	96.4	93.2	93.9	91.3	96.2	91.2	92.7	92.0	97.9	96.2
25 % sample-5	89.6		90.7		88.4		91.3		98.3	
25 % sample-6	91.1		86.5		91.4		95.4		95.9	
% RSD	6.7		4.0		3.5		2.6		2.0	
250 % sample-1	100.9		99.0		100.5		102.8		102.4	
250 % sample-2	99.7		102.4		99.1		101.5		101.3	
250 % sample-3	99.4		100.3		97.3		99.1		99.8	
250 % sample-4	98.7	99.3	98.5	100.5	101.5	100.1	98.3	99.7	97.9	100.8
250 % sample-5	98.2		99.9		103.5		97.2		102.8	
250 % sample-6	98.9		102.7		98.6		99.4		100.8	
% RSD	1.0		1.7		2.2		2.1		1.8	

Note: R-Recovery; % RSD-% Relative standard deviation

TABLE 8: LOD AND LOQ

Element	In ppm, with respect to test concentration	
	LOD	LOQ
Aluminum (Al)	0.10	0.31
Magnesium (Mg)	0.51	1.56
Potassium (K)	0.60	1.83
Strontium (Sr)	0.04	0.12
Sodium (Na)	1.20	3.65

TABLE 9: LINEARITY

Linearity	Aluminum	Magnesium	Potassium	Strontium	Sodium
	Response (cps)	Response (cps)	Response (cps)	Response (cps)	Response (cps)
Linearity level-1 (25 %)	2400.28	337012.15	337166.43	4156070.98	6562823.03
Linearity level-2 (50 %)	4690.92	672397.66	637765.24	8224560.02	13081520.64
Linearity level-3 (100 %)	9093.13	1337954.73	1239875.34	16433392.67	26083381.00
Linearity level-4 (150 %)	13840.30	2043741.97	1943350.14	25267466.57	39438009.69
Linearity level-5 (200 %)	18327.54	2809319.89	2596602.72	34741596.70	54786090.85
Linearity level-6 (250 %)	23482.87	3470405.05	3208190.71	43546251.02	67554352.88
Slope	4651.98	28066268.94	25715479.14	348576772.2	54356746.49
Y-intercept	-45.11	-34141.29	-5335.54	-654029.99	-696562.75
Correlation coefficient (r)	0.9997	0.9998	0.9997	0.9997	0.9997

selective, simple, accurate and rugged which can be used for routine analysis in quality control and research laboratory.

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Conflict of interests:

The authors declare no conflict of interest.

REFERENCES

1. Calcium acetate. Wikipedia: The free encyclopedia; 2020.
2. Calcium acetate. WebMD; 2020.
3. The United States Pharmacopeia 2018: USP 41; The national formulary: NF 36. Official monographs, calcium acetate. The

- United States Pharmacopeial Convention 2018. p. 627-9.
- The European Pharmacopoeia 9.0, Council of Europe (2017), Official monographs, calcium acetate 2017. p. 1910-1.
 - Sudhakar K, Sujatha M, Babu SR, Padmavathi P, Reddy PP. Serum calcium levels in patients with essential hypertension and their first degree relatives. *Indian J Clin Biochem* 2004;19(1):21-3.
 - Maqueda C, Morillo E. Determination of calcium by atomic-absorption spectrometry in samples dissolved by acid mixtures. *Fresenius J Anal Chem* 1990;338(3):253-4.
 - Baker RW. The determination of calcium in serum by flame photometry. *Biochem J* 1955;59(4):566-71.
 - The simple Flame Photometric determination of calcium. Bibby Scientific, Protocol: P05-011A; 2020.
 - Chen Z, Griffin IJ, Kriseman YL, Liang LK, Abrams SA. Inductively coupled plasma mass spectrometric analysis of calcium isotopes in human serum: a low-sample-volume acid-equilibration method. *Clin Chem* 2003;49(12):2050-5.
 - Lee A, Yang V, Hsu J, Wu E, Shih R, Mizobuchi K. Ultratrace measurement of calcium in ultrapure water using the Agilent 8800 triple quadrupole ICP-MS. Agilent Technologies, Tokyo, Japan; 2012.
 - International Conference on Harmonization, Q2 (R1). Validation of analytical procedure: Text and Methodology. Step-4 Consensus Guideline, International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. Geneva 1994.
 - The United States Pharmacopoeia 41, the National Formulary 36. General Chapter <233> Elemental Impurities—Procedures. United States Pharmacopeia Convention; 2018.
 - The United States Pharmacopoeia 41, the National Formulary 36. General Chapter <1225> Validation of Compensial Procedures, United States Pharmacopeia Convention; 2018.
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