Determination of assay of Calcium Carbonate and its Alkali salts & Magnesium content by Ion Chromatography

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ABSTRACT: *Calcium carbonate has wide applications in the pharmaceutical industry as an excipient and also as an active ingredient; and in food industry as a major dietary supplement. An analytical method was developed and validated for estimation of calcium and its impurities of magnesium and other Alkaline Metals in calcium carbonate. The developed method was simple, accurate, reproducible, and sensitive for the estimation of calcium and its related substances in calcium carbonate. Calcium, magnesium and alkaline metals ions were separated using ion chromatography technique using isocratic elution with a flow rate of 0.64 mL/min. The linearity of method has been tested in the range of 1.0µg/ml to 25.0µg/ml of calcium and correlation coefficient (R2) was 0.9997. The Limits of Detection and Quantification have been established for calcium(2.0µg/L&6.0µg/L), magnesium(1.0µg/L &3.0µg/ml), potassium (2.0µg/L & 6.0µg/L), ammonium (1.0µg/L & 4.0µg/L), sodium (1.0µg/L & 3.0µg/ml), and lithium (1.0µg/L & 3.0µg/L), respectively. Hence, the validated method is easy to adapt for regular analysis for assay and content of alkali salts and magnesium by Ion Chromatography.*

Key Words: *Calcium carbonate, Calcium, Magnesium, Alkali Salts, Ion chromatography, Suppressed Conductivity.*

I. Introduction

Calcium carbonate is widely used in pharmaceuticals both as an active ingredient as well as excipient. FDA has approved the use of calcium carbonate(800 mg) in combination with magnesium hydroxide(165 mg) and famotidine(10 mg) for the treatment of heartburn associated with acid indigestion and sour stomach. Famotidine works by decreasing the amount of acid production whereas calcium and magnesium neutralize the acid present in the stomach. Currently, orally chewable tablets of this combination manufactured by two companies, 'Jonhson and Johnson consumer Inc.' and 'Perrigo R and D co' are granted approval to market as over the counter medication in the US.[1]

Calcium is well known for its role in maintaining strong bones and teeth, blood clotting, nerve conduction and muscle contraction. It is found in many foods, dairy and soy products and it is best to meet the needs by food sources. But for those who find it difficult to meet the required intake, calcium supplements are helpful. They are present in different doses as per age groups(eg. 800 mg for 4-8 years; 1000 mg for 19-50 years) and also for conditions like pregnancy and lactation(1000-3000 mg). Calcium carbonate and calcium citrate are the optimal forms of calcium supplements. Calcium carbonate provides 40% elemental calcium whereas calcium citrate provides 20% ie. A 600 mg calcium carbonate tablet will provide for 240 mg of elemental calcium whereas only 120 mg of elemental calcium will be available with the same strength of calcium citrate tablet. Since fewer pills are needed, calcium carbonate is the preferred supplement.[2]

The FDA is not authorized to review dietary supplements before they enter the market. Anyway it has expressed in 21 CFR 101.36(b)(2)(ii) that the heaviness of calcium must be recorded, as opposed to the heaviness of the calcium carbonate, the source segment, in the "Supplement Facts" board and Announcement of net proportion of substances be demonstrated in whichever weight/measure (in grams/kilograms) or calculating it as per 21 CFR 101.105. It is accordingly, the makers and distributers duty to guarantee that their items are protected and contain the portion as referenced on name. Likewise, to stay away from lethality, it is instructed to not surpass 2500 mg with respect to essential calcium every day and a USP Pharmacopeia image on the mark implies that it is free of lead and different impurities. It is thus of vital importance to have a method that will be able to accurately determine the amount of calcium and its impurities from calcium carbonate.[3][4]

As an excipient, calcium carbonate is widely used as an abrasive in toothpastes and has the ability to neutralize plaque acids. By using scanning electron microscopy, it is confirmed that it stays retained in

plaque, and a further potential benefit includes acting as a calcium reservoir that helps to reduce softening of enamel[5]

The USP-NF monograph of Calcium carbonate includes a titrimetric method for its assay, end point of which is based on visual inspection. Ion chromatography provides an accurate quantification of calcium carbonate in terms of the number of calcium ions present in the sample. Also, a limit test for the presence of magnesium and alkaline metals salts is included in the test for related substances. It makes use of hazardous chemicals like hydrochloric acid, sodium hydroxide and sulfuric acid along with an ignition test which makes it a lengthy and tedious procedure. Ion chromatography is an accurate and sensitive technique which provides a better estimation of individual alkaline metal present in the sample.[6]

Thus, present study, involves developing a method which is convenient, rapid, accurate and precise for determining the assay of calcium carbonate along with estimation of its related substances, magnesium and alkaline metals salts using Ion Chromatography with suppressed conductivity detection. Column utilized is IonPac CS16 which is a cation exchange column that allows determination of group 1 and group 2 cations, alkanol amines and methylamines.

II. Experiment

Reagents and Chemicals

All chemicals used for preparation of reagents, standards and mobile phase were of analytical grade. Ultrapure deionized water (18.2 MΩ cm, Milli-Q system) was used forthe preparation of mobile phase, standards and samples.Calcium Chloride dehydrate (Merck). Magnesium chloride (Merck), lithium hydroxide monohydrate (Spectrochem), sodium chloride (Sigma Aldrich), potassium sulphate (Merck), ammonium chloride (Merck), Calcium Carbonate (Merck) were used to prepare standard and sample solutions.

Apparatus

The equipment used was Thermo Scientific DionexIntegrion HPIC system having AS-AP Autosampler with a 25µL loop, IonPacCS16 column (4 x 250mm) and its guard (4 x 50mm) was used. CDRS600, 4mm suppressor at constant voltage was utilized throughout the sequence. Software used for data acquisition was Thermo Fisher Scientific DionexChromeleon (version: 7.20). Chromatograms were monitored simultaneously during analysis.

Preparation of eluent and diluent

- Preparation of eluent: Dionex EGC 500 MSA Eluent Generator Cartridge was used for isocratic eluent generation of 30 mMMethanesulfonic Acid (MSA). Similarly, 30mM MSA can also be prepared and used for isocratic elution for analysis.
- Preparation of diluent: 30 mM solution of MSA was prepared as a diluent for standard and sample injections.

Preparation of standard solution

- Preparation of standard stock solution: 0.18g of calcium chloride dihydrate was dissolved in 50 mL of diluent which resulted in a solution of strength 500 µg/ml.
- From this 500µg/ml standard calcium solution, 1.0, 2.5, 5.0, 10.0, 15.0, and 25.0µg/mlof calcium was prepared for the linearity study, and 6 standards of 10µg/ml were prepared for the precision.
- Similarly, 5.0µg/ml each mixture of Lithium, Sodium, Ammonium, Potassium and Magnesium was prepared and used for analysis for content of alkali salts and magnesium.

Sample preparation

For assay of Calcium carbonate sample: About 0.18g of calcium carbonate (Merck) was dissolved in 50 mL of diluent which resulted in a solution of strength 500 µg/ml. 200 µL was taken from the above solution and was further diluted to 10 ml with diluent.

For alkali salts and magnesium content in Calcium carbonate sample: 62.5 mg of USP reference standard was dissolved in 50 mL of diluent which resulted in a solution of strength 500 µg/ml.

An Autosampler (Dionex AS-AP) was used to inject standard solution containing calcium, magnesium and other alkaline metals ions into the ion chromatography system. Subsequently, the standard solution in the sample loop was transferred onto the separator column, on which calcium, and its related substances, magnesium and alkaline metals ions were separated. A sequence containing the blank, standards, samples and recovery samples were run and results were then interpreted.

Selection of chromatographic condition

Optimized chromatographic conditions:
Column: IonPac CS16 column(4µ) (4 x 250mm) and its guard(4µ) (4 x 50mm)
Eluent: 30 mMMethanesulfonicAcid (MSA)
Flow rate: 0.64 mL/min
Injection volume: 10 µl
Detector: conductivity
Suppressor: CDRS600, 4mm
Suppressor Mode: Constant Voltage
Run time: 30 minutes for standard and 50minutes for sample.

III. Result and discussion

Linearity and Range:

The response of calcium was linear over the range of 1.0 to 25.0µg/ml. Calibration curve fits well and that is significantly linear having correlation coefficient of 0.9997. Each standard injection was repeated thrice. Therefore, number of calibration points (n) for linearity study was 18.

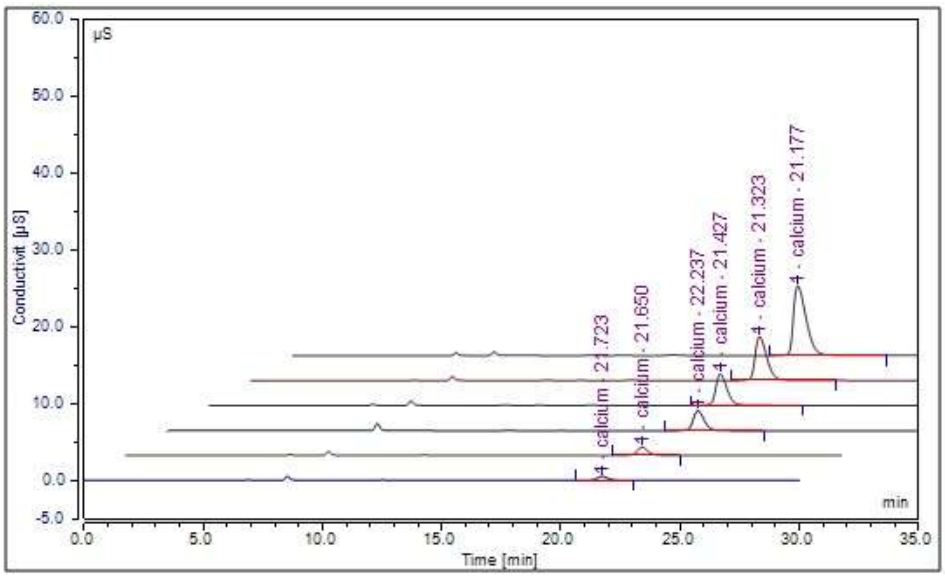

Fig 1a: Overlaid linearity plot of calcium standard (1.0, 2.5, 5.0, 10.0, 15.0 and 25.0µg/ml)

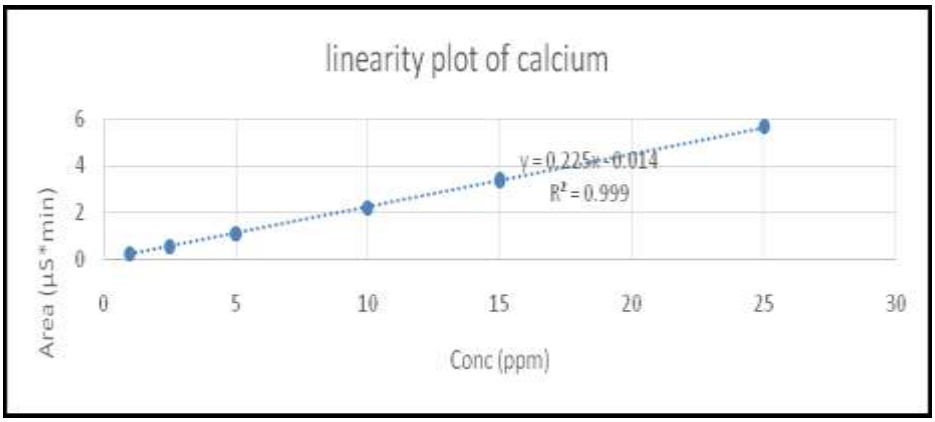

Fig 1b: linearity plot of calcium

Table 1: Linearity data of calcium

Analyte	Points	Correlation coefficient	Offset	Slope
Calcium	18	0.9997	-0.0143	0.2255

Specificity:

Method specificity was done with injection of Calcium (10µg/ml). Its chromatogram was shown in figure 5. It was also done for magnesium and alkaline metals salts (5 µg/ml) as shown in fig 3.

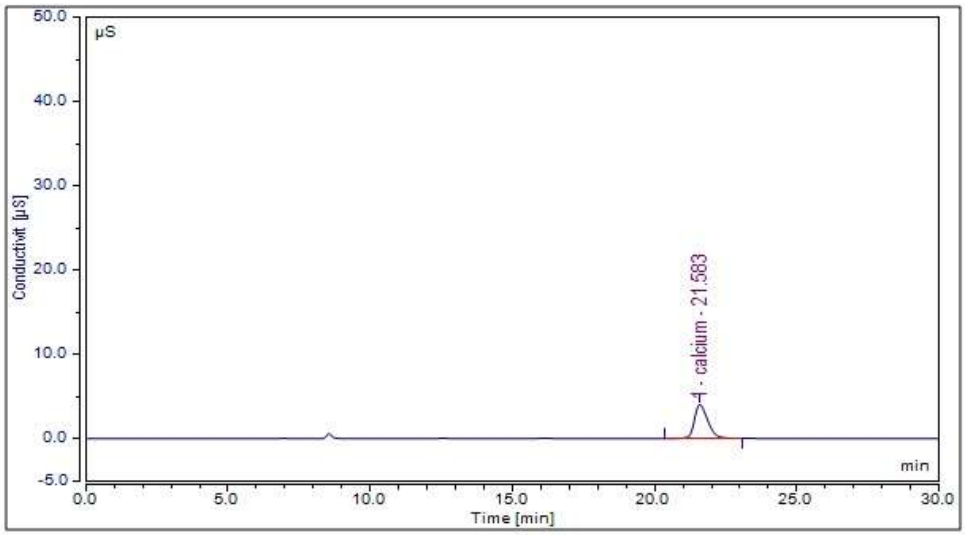

Fig 2: Specificity chromatogram for calcium (10 µg/ml)

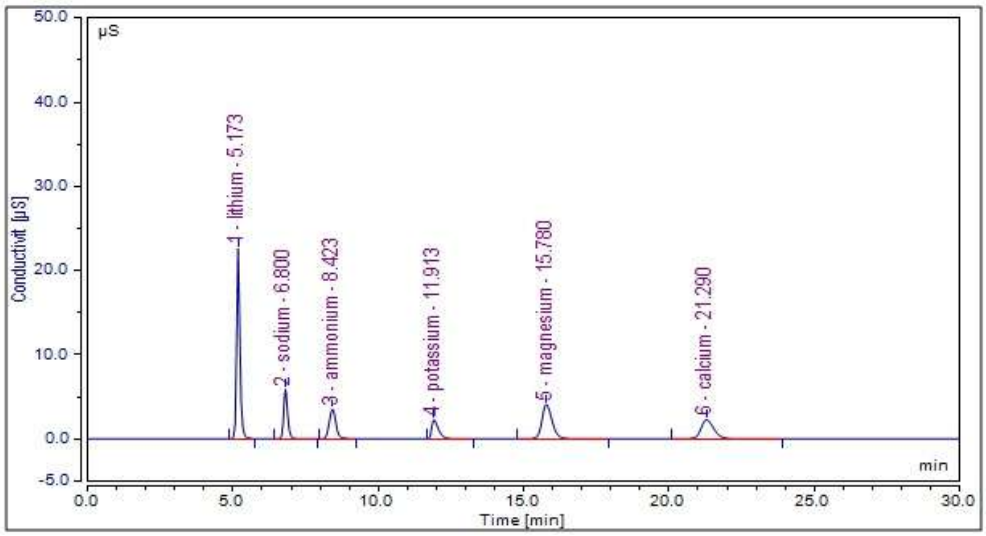

Fig 3: Specificity chromatogram for magnesium and alkaline metals (5 µg/ml)

Precision:

Replicate injections of mixture of calcium, magnesium and other alkaline metals of lithium, sodium, ammonium and potassium were done and their percent relative standard deviation for peak area calculated. Table 2 shows results for its precision study.

Table 2: Precision data for calcium, magnesium and other alkaline metals.

Analyte	Amount, µg/ml	*% RSD (n =10)*
Calcium	**5**	1.91
Magnesium	5	2.11
Lithium	5	1.71
Sodium	5	1.52
Ammonium	5	1.30
Potassium	5	1.40

Sample results

For assay of calcium in calcium carbonate sample,

Precision:

Ten replicate injections of sample was done and its percentage Related Standard Deviation was calculated (%RSD). Table 3 shows results of precision in sample.

Table 3: Precision data for calcium carbonate sample

Sample	*B.No.*	*%RSD*
Calcium Carbonate	MJ2 K520146	2.18%

Accuracy:

Percentage recovery was determined by spiking standard calcium carbonate at three levels (50,100 and 150%) in calcium carbonate sample (B.No. MJ2 K520146)

Table 4: Accuracy data for calcium carbonate sample (B. No. MJ2 K520146)

Recovery Level	*Spiked Concentration*	*Amount Added µg/ml*	*Amount Recovered µg/ml*	*% Recovery ± Std.Dev.*
1	50%	5	4.90	98.079% *±0.015*
2	100%	10	9.89	98.88% *±0.047*
3	150%	15	15.01	100.02% *±0.69*

The developed method was specific, accurate, precise, and linear over the range of 1 to 25 µg/ml of calcium. It was thus used to perform the assay of calcium carbonate from calcium carbonate sample.

Table 5: Assay results for calcium carbonate sample

Sample	*B.No.*	*Assay %*
Calcium Carbonate	MJ2 K520146	99.09%

For limit of magnesium and alkaline metals salts,

Specificity for magnesium, lithium, sodium, ammonium, potassium, and calcium was performed as seen in figure 2.Limit of Detection (LOD)and Limit of Quantification (LOQ) standards were injected and its data was shown in table 6.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

Limit of detection was determined for analytes whose signal to noise ratio is greater than or equal to 3 and Limit of quantification was determined for analytes whose signal to noise ratio is greater than or equal to 10. Following table 6 shows LOD and LOQ for Lithium, Sodium, Ammonium, Potassium, Magnesium and Calcium.

Table 6: LOD and LOQ data of calcium, magnesium and other alkaline metals.

Analyte	***LOD, µg/L (ppb)***	***LOQ, µg/L (ppb)***
Calcium	**2**	**6**
Magnesium	**1**	**3**
Lithium	**1**	**3**
Sodium	**1**	**3**
Ammonium	**1**	**4**
Potassium	**2**	**6**

As per USP monograph of calcium carbonate, acceptance criteria for these Alkaline Metals is NMT 1%. A solution containing 500 µg/ml of calcium was injected to determine the level of magnesium and other alkali metals in calcium carbonate sample (B. No. MJ2 K520146)

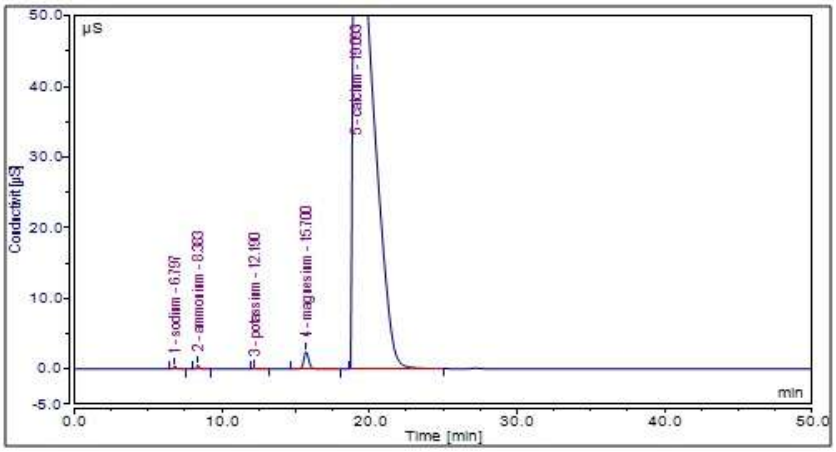

Fig 4:Calcium carbonate sample(B. No. MJ2 K520146) chromatogram for limit of magnesium and other Alkaline Metals.

Table 7:Routine sample analysis results for magnesium and other Alkaline Metals content in Calcium carbonate sample(B. No. MJ2 K520146).

Analyte in Sample	*Amount (%)*
Magnesium	0.23
Lithium	N.D.
Sodium	0.03
Ammonium	0.04
Potassium	0.01
Total	**0.32**

The percentage of magnesium and other alkaline metals account for 0.32% which is well within the USP monograph guideline limit of NMT 1.0%.

Intraday analysis of Samples was done for seven consecutive days for which they are passing its label claim limit and percentage of magnesium and alkali salts observed was also similar. Similarly, under given analytical conditions, different lots of columns were tried for which final results obtained are same for calcium assay and percentage of magnesium and alkali salts content.

IV. Conclusion

Determination of calcium content in calcium carbonate is a very important when used as an antacid, to ensure it is safe and effective for patients. Also, calcium carbonate supplements are consumed by a variety of masses to make up for required calcium intake. Ion Chromatography with suppressed conductivity detection gives specific, sensitive and precise method for estimation of calcium, magnesium and other Alkaline Metals in calcium carbonate without any pretreatment. This technique is cost-effective with respect to analysis required for keeping a check on the limits of magnesium and other Alkaline Metals as provided by USP and other regulatory bodies. This method can also be useful for checking assay of calcium from these samples.

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