Magnesium oxide monograph modernization with ion chromatography

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Goal

To evaluate the ion chromatography methods for the Magnesium Oxide assay and the Limit of Calcium test in the proposed United States Pharmacopeia Magnesium Oxide monograph revision

Introduction

Magnesium is a naturally occurring mineral that is important for many processes in the body, such as regulating muscles, nerve function, and energy production. Magnesium oxide (MgO) is used as a supplement to maintain adequate magnesium levels in the body. MgO is also used as an antacid to treat indigestion, or as a laxative to relieve occasional constipation. It is an over-the-counter (OTC) drug product and typically available as chewable tablets and capsules.

The United States Pharmacopoeia (USP) monograph for MgO describes an assay based on titration. As part of monograph modernization efforts, the USP proposed a single ion chromatography (IC) method to replace the titration procedure used for the MgO assay and a wet chemical method used for the Limit of Calcium test.[1] This IC method was validated with a Thermo Scientific™ Dionex™ IonPac™ CS16 column (USP L84 packing). The same assay method was proposed in the USP monographs for MgO tablets and MgO capsules drug products.[2,3] All three monograph revisions proposed the same IC method and are scheduled to become official on June 1, 2021.

In this application note, we evaluated the proposed IC method for the MgO assay and Limit of Calcium test. Magnesium and calcium ions were separated on a Dionex IonPac CS16 column set followed by suppressed conductivity detection. Key performance parameters were evaluated, including separation, system suitability, linearity, limit of detection, and precision. Two MgO drug products, MgO tablets and MgO capsules, were analyzed and evaluated in accordance with the proposed USP monographs. The percentage of MgO and the limit of calcium results were compared with the proposed USP monographs' acceptance criteria.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-6000 HPIC system* including:
  - Dionex ICS-6000 DP Pump module
  - Dionex ICS-6000 EG Eluent Generator module with high-pressure degasser module
  - Dionex ICS-6000 Low Temperature DC Detector/ Chromatography module with two injection valves
  - CD Conductivity Detector
  - Tablet control
- Thermo Scientific™ Dionex™ AS-AP Autosampler with tray temperature control (P/N 074926). This AS-AP is equipped with a 250 µL syringe, (P/N 074306) and a 1.2 mL buffer loop (P/N 074989) installed.
- Thermo Scientific™ Dionex™ EGC 500 MSA Eluent Generator Cartridge (P/N 075779)
- Thermo Scientific™ Dionex™ CR-CTC 600 Continuously Regenerated Anion Trap Column (P/N 088663)
- Thermo Scientific™ Dionex™ CDRS 600 Cation Dynamically Regenerated Suppressor (4 mm, P/N 088668)
- Thermo Scientific™ Dionex™ AS-AP Autosampler Vials 10 mL (P/N 074228)

* This method can be run on any Thermo Scientific™ Dionex™ ion chromatography system with eluent generation and electrolytic suppression or any Dionex IC system with manually prepared eluent and chemical suppression.

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistance or better
- USP Magnesium Oxide Reference Standard (MgO RS) (Catalog number 1374281)
- USP Calcium Carbonate Reference Standard (Catalog number 1086403)
- Magnesium oxide tablets
- Magnesium oxide capsules

Preparation of standards and samples

Standard stock solution

3.3 mg/mL of USP MgO RS:
Accurately weigh 330 mg of USP MgO RS in a 100 mL volumetric flask. Add about 20 mL of 6 N hydrochloric acid and dissolve. Dilute with DI water to volume.

0.5 mg/mL USP calcium carbonate RS:
Accurately weigh 50 mg of USP calcium carbonate RS in a 100 mL volumetric flask. Add about 20 mL of 6 N hydrochloric acid and dissolve. Dilute with DI water to volume.

Standard solution

33 µg/mL USP MgO RS:
Dilute the stock standard (3.3 mg/mL of USP MgO RS) 100-fold to make 33 µg/mL USP MgO RS in DI water.

0.9 µg/mL USP calcium carbonate RS:
Measure 180 µL of 0.5 mg/mL USP calcium carbonate RS into a 100 mL volumetric flask. Dilute with diluent (0.02 N HCl) to volume.

System suitability solution

Dilute the above stock standards (USP MgO RS and USP calcium carbonate RS) 100-fold to make 33 µg/mL of USP MgO RS and 5 µg/mL of USP calcium carbonate RS in diluent (0.02 N HCl).

Calibration standards

Dilute volumes of the 3.3 mg/mL USP MgO RS stock solution and 0.5 mg/mL USP calcium carbonate RS stock solution listed below in a 100 mL volumetric flask with diluent to prepare the mixed calibration standards of magnesium and calcium (Table 1).

Table 1. Preparation of calibration standards

	MgO conc. (µg/mL)	Stock volume added (µL)	Calcium carbonate conc. (µg/mL)	Stock volume added (µL)	Diluent* (mL) up to
Cal std 1	5.00	152	0.25	50.0	100
Cal std 2	10.0	303	0.50	100	100
Cal std 3	25.0	758	1.00	200	100
Cal std 4	50.0	1515	2.50	500	100
Cal std 5	100	3030	5.00	1000	100

* 0.02 N HCl

Sample stock solution

MgO tablet stock sample (3.3 mg/mL):

Step 1: Crush 30 tablets in a blender to a fine powder.

Step 2: Weigh 597.13 mg and dissolve in 20 mL of 6 N HCl.

Step 3: Heat to boiling and maintain for 10 min with continuous stirring.

Step 4: Cool and transfer to a 100 mL volumetric flask containing 10 mL DI water.

Step 5: Bring to volume with DI water and filter using 0.45 µm filter.

MgO capsule stock sample (3.3 mg/mL):

Step 1: Empty 30 capsule shells and place the powder in a container.

Step 2: Weigh 380.18 mg and dissolve in 20 mL of 6N HCl.

Follow steps 3, 4, and 5 as described above for the MgO tablet stock sample (3.3 mg/mL).

Sample solution

Dilute the stock sample 100-fold with DI water to make a 33 µg/mL sample solution.

Spike recovery experiment

Unspiked sample:

Accurately weigh 190 mg of the MgO capsule powder sample in a 50 mL volumetric flask and add 10 mL 6 N HCl. Heat to boiling and maintain for 10 min with continuous stirring. Cool and transfer to a 50 mL volumetric flask containing 5 mL DI water. Bring to volume with DI water and filter using 0.45 µm filter. Then dilute 100-fold with DI water to make a 33 µg/mL sample solution.

Spiked samples**:**

Accurately weigh 190 mg of MgO capsule powder sample in a 50 mL volumetric flask, add 37.5 mg of USP MgO RS and 2.5 mL of 0.5 mg/mL of USP calcium carbonate stock solution and dissolve in 10 mL 6 N HCl. Heat to boiling and maintain for 10 min with continuous stirring. Cool and transfer to a 50 mL volumetric flask containing 5 mL DI water. Bring to volume with DI water and filter using a 0.45 µm filter. Then dilute 100-fold with DI water to make spiked sample 1. To make spiked sample 2, follow the same procedure substituting the 37.5 mg USP MgO RS with 75 mg USP MgO RS.

Table 2. Chromatography conditions (method in the proposed USP monograph)

Parameter	Value
System	Dionex ICS-6000
Columns	Dionex IonPac CS16, Analytical, 5 × 250 mm (P/N 079805) Dionex IonPac CG16, Guard, 5 × 50 mm (P/N 057574)
Eluent	48 mM MSA
Eluent source	Dionex EGC 500 MSA Eluent Generator Cartridge with CR-CTC 600 trap column
Flow rate	1 mL/min
Column temp.	40 °C
Autosampler temp.	10 °C
Injection volume	10 µL
Detection	Suppressed conductivity
Suppressor	Dionex CDRS 600 Suppressor (4 mm), recycle mode, 4.0 V, constant voltage mode
Run time	18 min (NLT 2 times the retention time of magnesium)

Results and discussion

The assay in the proposed USP MgO monograph revision describes a Dionex IonPac CG16 guard column and a Dionex IonPac CS16 analytical column with L84 packing for the separation of magnesium. The Dionex IonPac CS16 column is a high capacity, weak cation exchanger functionalized with carboxylic acid groups.[4] Figure 1 displays the chromatographic profile of standard solution (33 µg/mL of USP MgO RS) using the eluent condition described in the proposed USP monograph revision (Table 2). The eluent was generated electrolytically using a Dionex EGC 500 MSA cartridge. The retention time (RT) for magnesium is 8.46 min. The revised monograph states that magnesium elutes at approximately 8.4 min.[1] The separation is followed by suppressed conductivity detection.

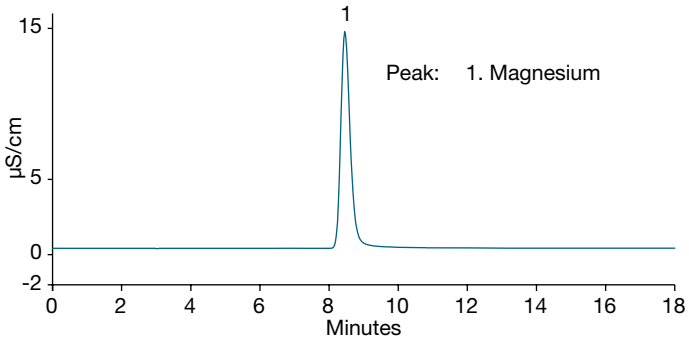

Figure 1. Chromatogram of 33 µg/mL of USP MgO RS

System suitability requirements

In the proposed USP monograph revision for MgO, three system suitability requirements are specified. The first requirement is that the resolution between the magnesium and calcium ions for the system suitability standard solution is not less than (NLT) 3.0. Figure 2 displays the separation of the system suitability solution (33 µg/mL of USP MgO RS and 5 µg/mL of USP calcium carbonate RS in 0.02 N HCl) and shows this resolution requirement is met (Rs = 5.04). The other two requirements are that the magnesium peak tailing factor for a 33 µg/mL standard solution of USP MgO RS is not more than (NMT) 2.0 and the relative standard deviation (RSD) for replicate injections of the same standard solution is NMT 0.73%. The RSDs of the retention time (RT), peak area, and peak height were determined from six replicate injections of the 33 µg/mL of USP MgO RS standard solution. System suitability requirements were tested using two sets of Dionex IonPac CS16-5 mm columns from different lots.

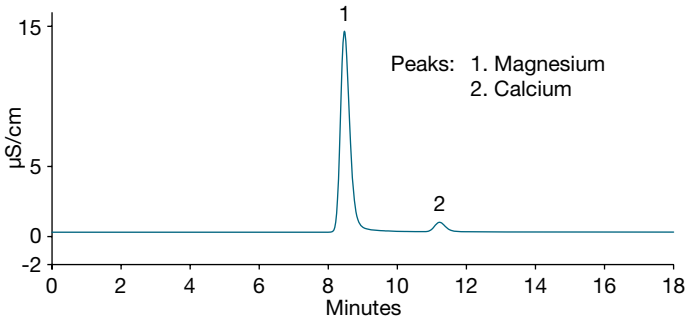

Figure 2. Chromatogram of a system suitability standard (33 µg/mL of USP MgO RS and 5 µg/mL of USP calcium carbonate RS)

Figure 3 displays chromatograms of the system suitability standard on the two columns. The RTs of the magnesium and calcium peaks on two columns were found to differ by 0.05% and 0.80%, respectively. Table 3 shows that the USP requirements for resolution, tailing factor, and repeatability are met with both columns.

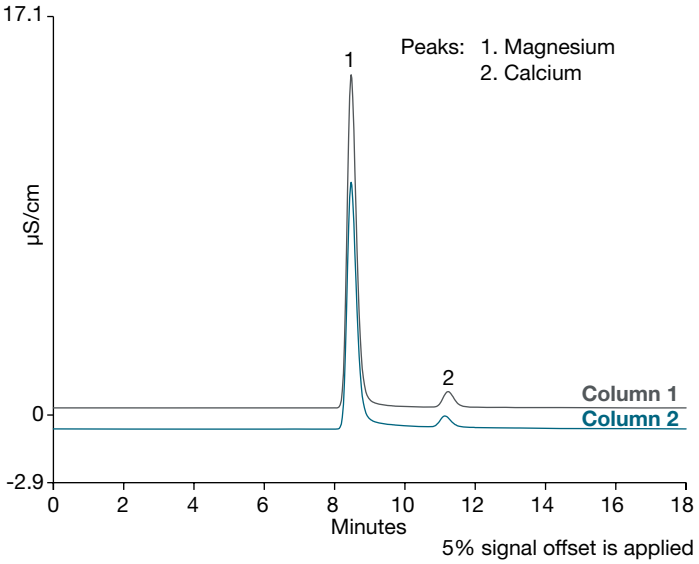

Figure 3. Chromatograms of a system suitability standard on each of two columns

Table 3. System suitability - MgO assay

Requirement			Value	
			Column 1	Column 2
System suitability solution	Relative RT of Mg^{2+} and Ca^{2+}	1 and 1.30	1 and 1.33	1 and 1.32
	Resolution	NLT 3.0	5.04	4.72
33 µg/mL USP MgO RS standard	Tailing factor	NMT 2.0	1.32	1.40
	RT RSD	NMT 0.73%	0.05	0.06
	Peak area RSD	NMT 0.73%	0.19	0.31
	Peak height RSD	NMT 0.73%	0.18	0.23

Calibration

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the USP General Chapter <1225> guidelines[5] recommend a minimum of five concentrations to establish linearity in an assay. For a drug substance or finished product, the minimum specified range is from 80% to 120% of the test concentration for method accuracy validation testing. Method linearity was evaluated by constructing calibration curves using five concentrations of USP MgO RS as listed in Table 1. Figure 4 displays the chromatograms of five calibration standards. The calibration plot of peak area versus concentration was fit using linear regression. The calibration curves for both magnesium and calcium are shown in Figure 5.

Limits of detection (LOD) and quantitation (LOQ)

To determine the LOD and LOQ for calcium, the baseline noise (N) was first determined by measuring the peak-to-peak noise in a representative one-minute segment of the baseline where no peaks elute but close to the calcium peak. The signal (S) was determined from the average peak height of seven injections of 0.01 µg/mL USP calcium carbonate RS standard. The LOD and LOQ were calculated as 3 × S/N and 10 × S/N, respectively. Table 4 lists the LOD and LOQ values of calcium.

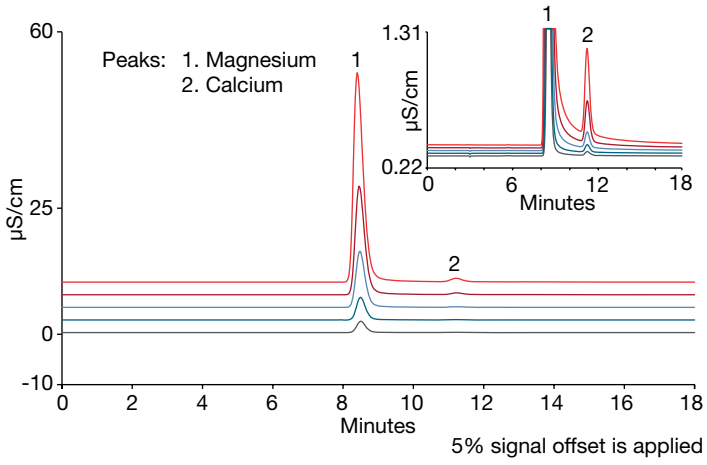

Figure 4. Chromatograms of five calibration standards

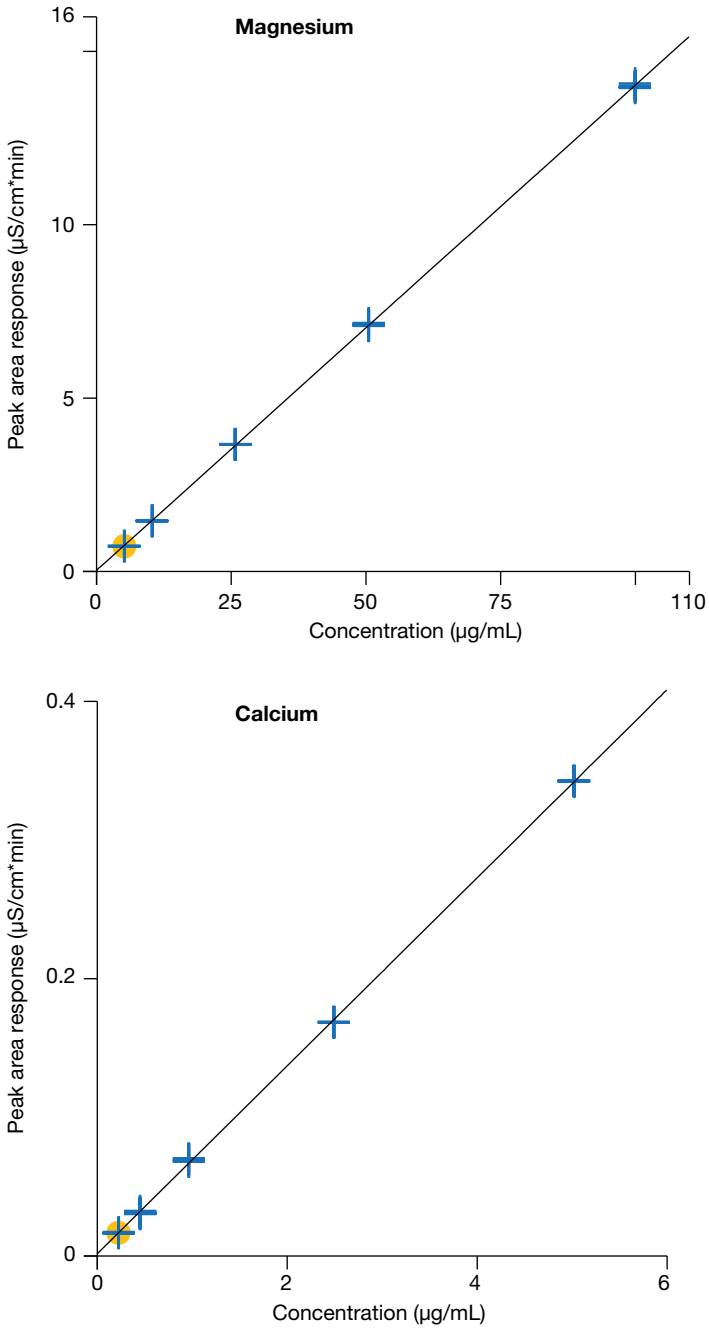

Figure 5. Calibration curves of magnesium and calcium

Table 4. Method calibration, LOD, and LOQ

	Coefficient of determination (r^2)	LOD (µg/L)	LOQ (µg/L)
Magnesium	0.9999	n.a.	n.a.
Calcium	0.9998	2.89	9.65

Robustness study

Following the guidelines of USP Physical Tests, <621> Chromatography[6], the robustness of this method was evaluated by examining RT, resolution, and peak asymmetry after imposing small variations (±10%) in procedural parameters (e.g., flow rate, eluent concentration, and column temperature). The system suitability standard solution was injected in triplicate for each condition for three consecutive days. The same procedure was applied to another column set from a different lot.

The variations tested were:

- Flow rate at 0.90 mL/min, 1.00 mL/min, and 1.10 mL/min
- Column temperature at 36 °C, 40 °C, and 44 °C
- Eluent concentration at 43.2 mM, 48 mM, 52.8 mM MSA

Table 5 summarizes the method robustness study results for the IC assay method in the proposed USP MgO monograph revision. These results indicate that the method is robust and suitable for MgO assay.

Sample analysis

Two commercial MgO drug products, MgO tablets and MgO capsules, were purchased and tested using the proposed monograph assay method. Figure 6 displays the chromatograms of tablet and capsule samples prepared according to the proposed USP monograph revision assay. The samples were prepared as specified, using 30 tablets or capsules, such that the nominal concentration of MgO in the sample was 33 µg/mL.

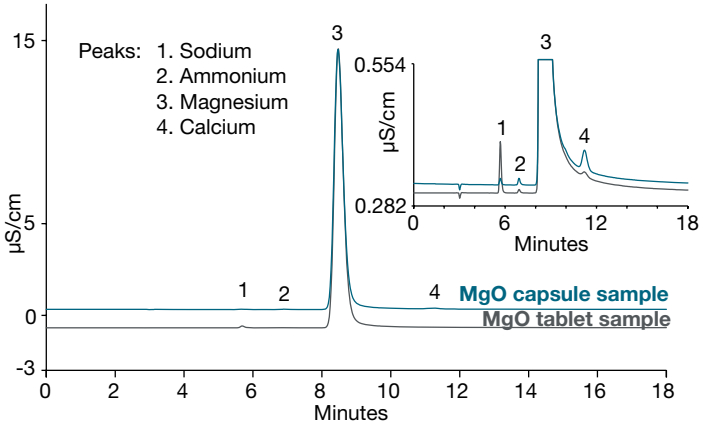

Figure 6. Chromatograms of MgO tablet and capsule samples

The percentage of the labeled amount of MgO in the tablet or capsule was calculated according to the formula below:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of magnesium from the *Sample solution*

r_S = peak response of magnesium from the *Standard solution*

C_S = concentration of USP MgO RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of MgO in the *Sample solution* (µg/mL)

According to the USP acceptance criterium, the tablet or capsule should contain NLT 90.0% and NMT 110.0% of the labeled amount of MgO. Table 6 lists the assay results.

Table 5. Robustness of the proposed USP method performed using a system suitability standard (n=9)

		Column 1						Column 2					
		RT		Resolution		Asymmetry		RT		Resolution		Asymmetry	
Parameter		Avg	% Diff.	Avg	% Diff.	Avg	% Diff.	Avg	% Diff.	Avg	% Diff.	Avg	% Diff.
No change		8.457	0	5.03	0	1.32	0.0	8.461	0.0	4.72	0.0	1.42	0
Eluent	-10%	9.803	15.9	5.56	10.5	1.36	3.0	9.797	15.8	5.22	10.6	1.62	14.1
	+10%	7.471	-11.7	4.59	-8.7	1.31	-0.8	7.481	-11.6	4.22	-10.6	1.41	-0.7
Flow rate	-10%	9.392	11.1	5.32	5.8	1.35	2.3	9.398	11.1	4.99	5.7	1.63	14.8
	+10%	7.742	-8.5	4.87	-3.2	1.30	-1.5	7.700	-9.0	4.52	-4.2	1.39	-2.1
Column temp.	-10%	8.487	0.4	5.04	0.2	1.31	-0.8	8.490	0.3	4.72	0.0	1.38	-2.8
	+10%	8.493	0.4	5.02	-0.2	1.35	2.3	8.477	0.2	4.59	-2.8	1.54	8.5

Table 6. Assay results

Sample	r_u	r_s	C_s	C_u	Result (acceptable range: 90–110%)
MgO tablet	5.05	4.75	33.0	33.0	106
MgO capsule	4.93	4.75	33.0	33.0	104

Sample recovery

Method accuracy was evaluated by measuring recoveries of magnesium and calcium at two concentrations. The unspiked and spiked samples were prepared as described in the sample preparation section. The recovery percentages were calculated according to formula below:

$$Recovery\ \% = \frac{(C\ spiked\ sample - C\ unspiked\ sample)}{(C\ analyte\ added)} \times 100$$

Figure 7 shows the representative chromatograms of unspiked and spiked capsule sample at two levels. The recovery of two spiked levels in both the samples was in the range of 90 to 110% (Table 7).

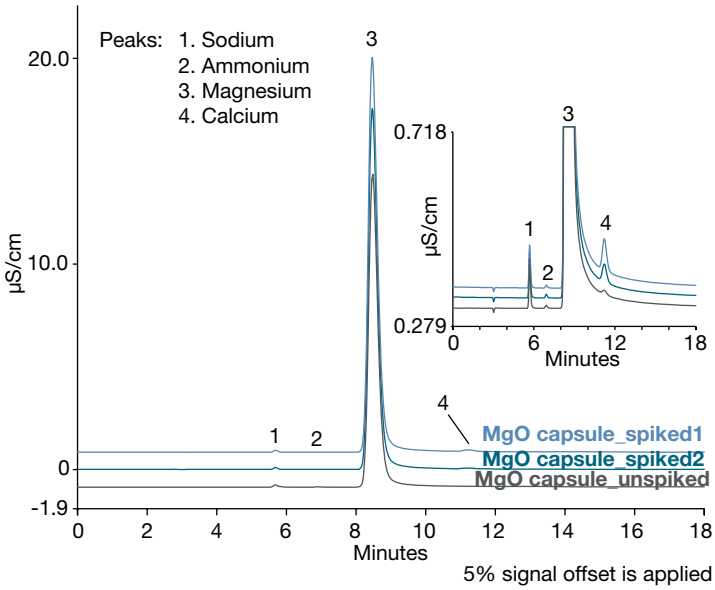

Figure 7. Chromatograms of unspiked and spiked MgO capsule sample at two levels

Table 7. Recovery study (n=3)

Sample	Base amount (µg/mL)		Spiked amount (µg/mL)	Measured (µg/mL)	Recovery (%)
MgO tablet	Magnesium	33.4	7.50	50.0	102
			15.0	46.8	89.4
	Calcium	0.354	0.25	0.613	104
			0.50	0.853	99.9
MgO capsule	Magnesium	32.7	7.50	41.1	111
			15.0	47.9	101
	Calcium	0.548	0.25	0.810	106
			0.50	1.00	90.3

Limit of Calcium

In the proposed USP monograph revision for MgO, the atomic absorption procedure for the “Limit of Calcium” test is replaced with an IC procedure the same as that proposed for the MgO assay. Figure 8 displays a chromatogram of 0.9 µg/mL calcium carbonate RS standard using the eluent condition described in the proposed USP monograph revision (Table 1). The RT for calcium is 11.26 min. According to the proposed monograph revision, calcium elutes at approximately 11 min.

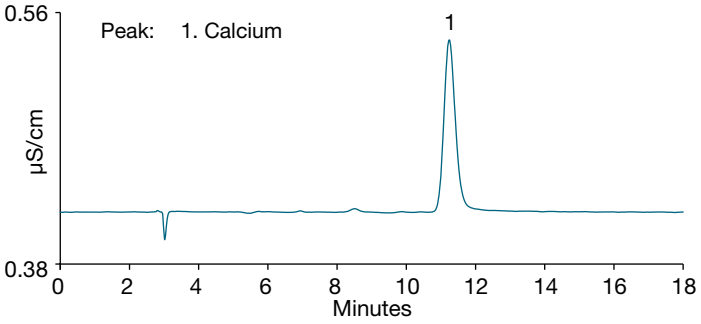

Figure 8. Chromatogram of 0.9 µg/mL of USP calcium carbonate RS in diluent

The suitability requirements for the Limit of Calcium test are the same as the MgO assay. The first requirement is that the resolution between the magnesium and calcium ions for the system suitability standard solution is NLT 3.0. The other two requirements are that the calcium peak tailing factor for a 0.9 µg/mL USP calcium carbonate RS standard solution is NMT 2.0 and that the RSD for replicate injections of 0.9 µg/mL USP calcium carbonate RS standard solution is NMT 5%. The RSDs of the RT, peak area, and peak height were determined from six replicate injections of the 0.9 µg/mL USP calcium carbonate RS standard solution. Table 8 shows that the USP requirements for resolution, tailing factor, and repeatability are met with both columns.

USP acceptance criterium

The peak response of calcium from the sample solution does not exceed that of the standard solution, which translates to NMT 1.1%. All three samples passed for the Limit of Calcium test (Table 9).

Conclusion

In this application note, we demonstrated that the IC method in the proposed USP Magnesium Oxide monograph revision can be successfully executed with a Dionex IonPac CS16 column using a Thermo Scientific Dionex IC system. Two commercial MgO drug products, MgO tablets and MgO capsules, were tested, and both were found to contain MgO within 90 to 110% as specified in the proposed USP monograph revisions for these two drug products. We also demonstrated that the same IC method can be successfully used for the Limit of Calcium test in the proposed MgO monograph revision. The separation, linearity, reproducibility, and sensitivity were found to meet or exceed the proposed USP Magnesium Oxide monograph revision performance requirements.

Table 8. System suitability requirements - Limit of Calcium

Requirement		USP criteria	Measured	
			Column 1	Column 2
System suitability solution	Relative RT of Mg^{2+} and Ca^{2+}	1 and 1.30	1 and 1.33	1 and 1.32
	Resolution	NLT 3.0	5.04	4.72
0.9 µg/mL of USP calcium carbonate RS	Tailing factor	NMT 2.0	1.20	1.21
	RT RSD	NMT 5%	0.04	0.02
	Peak area RSD		0.31	0.42
	Peak height RSD		0.34	0.32

Table 9. Limit of Calcium

		Calcium peak area response (µS/cm · min)	Result (limit - NMT 1.1%)
Standard	0.9 µg/mL calcium carbonate RS	0.0444	n. a
Sample 1	33 µg/mL MgO RS	0.0185	passed
Sample 2	33 µg/mL MgO tablet	0.0259	passed
Sample 3	33 µg/mL MgO capsule	0.0380	passed

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