



Application Note AN-C-198

Calcium acetate assay in calcium acetate capsules

Method validation according to the U.S. Pharmacopeia (USP)

Calcium acetate functions as a phosphate binder in the gastrointestinal tract, helping to lower high phosphate levels in individuals with kidney disease who are receiving dialysis treatment [1,2]. To meet the stringent quality standards for pharmaceutical products, manufacturers and laboratories must use validated methods from the United States Pharmacopeia – National Formulary (USP-NF). Previously, such methods included titration or liquid chromatography (LC) with UV detection. As part of their modernization efforts, the USP has updated the calcium monograph to include ion chromatographic (IC) analysis, which is more straightforward and sensitive than previous methods. For the calcium

acetate assay, the USP specifies ion chromatography using a cation-exchange column with L76 column material and non-suppressed conductivity detection to quantify the amount of calcium ions in calcium acetate capsules [3].

The current IC method employs a Metrosep C 6 - 150/4.0 column (L76) to separate calcium from other ions in calcium acetate capsules. This method has been validated according to USP General Chapters <621> Chromatography [4] and <1225> Validation of Compendial Procedures [5]. All acceptance criteria for the calcium acetate assay in the USP monograph «Calcium Acetate Capsules» are met.

SAMPLE AND SAMPLE PREPARATION

The standard solution nominally contains 0.08 mg/mL of USP Calcium Acetate Reference Standard (Cat# 1086334) in water. It is prepared by accurately weighing 80.0 mg of USP Calcium Acetate Reference Standard and transferring it into a clean 1000 mL volumetric flask. It is dissolved and made up to the mark with ultrapure water (UPW).

For sample stock solutions nominally containing 6.7 mg/mL of calcium acetate, an appropriate portion of the contents of at least 20 capsules are transferred into a 2000 mL volumetric flask. UPW is added to

about 40% of the final volume of the volumetric flask, and the solution is then sonicated for 30 minutes with intermittent shaking. Afterwards, the solution is made up to the mark with UPW and filtered through 0.2 µm filter paper.

For sample solutions nominally containing 0.08 mg/mL of calcium acetate, 5.97 mL of sample stock solution are transferred into a clean 500 mL volumetric flask. This is diluted and made up to the mark with UPW. All solutions are sonicated for 5 minutes before injection.

EXPERIMENTAL

Samples are directly injected into the IC with a 919 IC Autosampler plus (Figure 1).

Cations are separated using a Metrosep C 6 -150/4.0 column (L76) and detected with non-suppressed conductivity (Table 1). The run time was 40 minutes which complies with the USP requirements of 1.5 times the retention time of the calcium peak (here: 24 minutes).

A one-point calibration with the standard solution of 0.08 mg/mL of USP Calcium Acetate was used for quantification. Samples were evaluated as triplicates. Repeatability studies are done with 6-fold injections.



Figure 1. Instrumental setup including a 930 Compact IC Flex Oven/Deg and a 919 IC Autosampler plus.

Table 1. Parameters for the IC method as per USP Monograph «Calcium Acetate Capsules» [3].

Column with L76 packing	Metrosep C 6 - 150/4.0
Eluent	0.75 mmol/L dipicolinic acid + 1.7 mmol/L nitric acid
Flow rate	0.9 mL/min
Temperature	35 °C
Injection volume	10 µL
Detection	Direct conductivity

RESULTS

IC method parameters followed the requirements of the USP assay for calcium acetate (**Table 2**). Chromatograms did not show any interferences or contaminations, and the calcium peak eluted after 24 minutes (**Figure 2**).

All results for validation testing were within the specified USP requirements. Column efficiency was exceptional with >5900 theoretical plates. Relative standard deviation for a 6-fold standard injection was 0.4% (USP requirement <2.0%). The measured amount of calcium acetate in the capsules correlated well with the labeled amount of calcium acetate, e.g., the calculated percentage was 102.6% of the labeled content (90.0–110.0% is acceptable) (**Table 2**). Thus, the IC method was suitable to determine calcium in calcium acetate capsules.

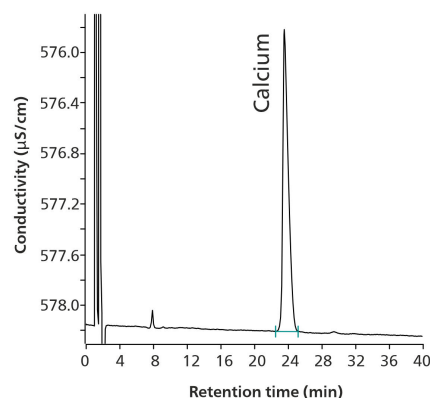


Figure 2. Chromatogram of a calcium peak from a calcium acetate capsule sample, containing 0.082 mg/mL calcium acetate (102% recovery).

Table 2. Exemplary results and USP requirements from the IC method validation for calcium acetate in calcium acetate capsules as per USP [3].

Parameter	Result	USP requirement
Theoretical plates	5909	NLT 1000
RSD % (n = 6)	0.406%	NMT 2.0%
Percentage of labeled amount	102.6%	90.0–110.0%

CONCLUSION

According to the USP monograph for calcium acetate capsules [3], the assay for calcium acetate involves determining calcium content using ion chromatography (IC) on a separation column with L76 packing material (here: Metrosep C 6). The validation results met all requirements of the monograph and

adhered to the guidelines specified in USP General Chapters <621> Chromatography and <1225> Validation of Compendial Procedures [4,5]. The described IC method is appropriate for quantifying calcium in calcium acetate capsules.

REFERENCES

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5. 1225 Validation of Compendial Procedures; General Chapter; U.S. Pharmacopeia/National Formulary: Rockville, MD.
[DOI:10.31003/USPNF_M99945_04_01](https://doi.org/10.31003/USPNF_M99945_04_01)

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CONFIGURATION



Metrosep C 6 - 150/4.0

Le matériau haute capacité de la C 6 fait de la colonne de séparation Metrosep C 6 - 150/4,0 la solution optimale pour la séparation des cations standard à des concentrations très différentes avec des temps de rétention raisonnables. Les eaux potables présentant de faibles teneurs en ammonium peuvent être déterminées à l'aide de cette colonne.



930 Compact IC Flex Oven/Deg

Le 930 Compact IC Flex Oven/Deg est un appareil CI compact intelligent avec un **four à colonne**, **sans suppression** et avec un **dégazeur** intégré. L'appareil peut être utilisé avec n'importe quelles méthodes de séparation et de détection.

Domaines d'application typiques :

- Déterminations d'anions et de cations sans suppression avec détection de conductivité
- Applications simples avec UV/VIS ou détection ampérométrique



919 IC Autosampler plus

Le 919 IC Autosampler plus satisfait les exigences des laboratoires ayant un nombre moyen d'échantillons à traiter. Il permet l'automatisation de tous les systèmes de chromatographie ionique de Metrohm.



IC Conductivity Detector

Détecteur de conductivité haute performance compact et intelligent destiné aux appareils CI intelligents. Excellente constance de la température, tout le traitement du signal au sein du bloc de détecteur protégé et DSP – Digital Signal Processing – de la dernière génération garantissent une précision de mesure optimale. Grâce à la plage de travail dynamique, aucun changement de plage n'est nécessaire (même automatique).