Application Note AN-C-198

醋酸囊中的醋酸定

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

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.

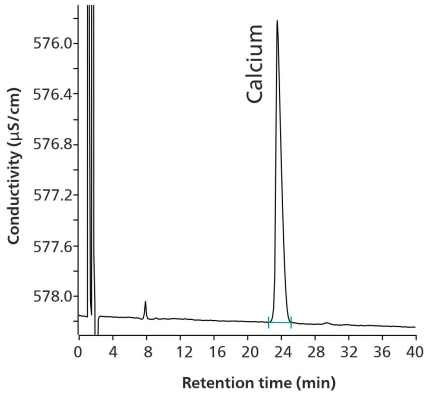


Figure 2. Chromatogram of a calcium peak from a calcium acetate capsule sample, containing 0.082 mg/mL calcium acetate (102% recovery).

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

1. Biruete, A.; Gallant, K. M. H.; Lindemann, S. R.; et al. Phosphate Binders and Nonphosphate Effects in the Gastrointestinal Tract. *Journal of Renal Nutrition* **2020**, *30* (1), 4–10. DOI:10.1053/j.jrn.2019.01.004
2. Sekar, A.; Kaur, T.; Nally, J. V.; et al. Phosphorus Binders: The New and the Old, and How to Choose. *CCJM* **2018**, *85* (8), 629–638. DOI:10.3949/ccjm.85a.17054
3. U.S. Pharmacopeia. USP-NF Calcium Acetate Capsules. *Monograph*. DOI:10.31003/USPNF_M11403_08_01
4. *<621> Chromatography, General Chapter; U.S.* Pharmacopeia/National Formulary: Rockville, MD.
5. *〈1225〉 Validation of Compendial Procedures*; General Chapter; U.S. Pharmacopeia/National Formulary: Rockville, MD. DOI:10.31003/USPNF_M99945_04_01

CONTACT

瑞士万通中国
北京市海淀区上地路1号院
1号楼7702
100085 北京
marketing@metrohm.com.cn

CONFIGURATION

Metrosep C 6 - 150/4.0

C 6 Metrosep C 6 - 150/4.0

930 Compact IC Flex Oven/Deg

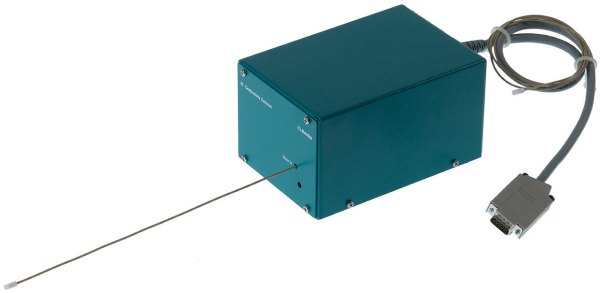
930 Compact IC Flex Oven/Deg Compact IC ,

:

- ,
- UV-VIS

919 IC Autosampler plus

919 IC Autosampler plus ,



IC Conductivity Detector

用于智能型子色的智能型高性能器。不凡的温度定性,受保的器端子板内的整个信号理程以及新一代的DSP(数字式信号理)均能保量的准性。功于工作范,无需行范更(也不是自行)。