



Application Note AN-S-402

Nitrite in hydroxypropyl methylcellulose

Ion chromatography method with automated sample preconcentration, matrix elimination, and UV/VIS detection

The FDA has recently issued some guidance for managing nitrosamine impurities in pharmaceutical products [1]. Even in trace quantities, the presence of carcinogenic nitrosamine in medications risks patients' safety. Controlling the nitrite concentration in pharmaceutical products and processes can help to prevent nitrosamine formation. For this reason, the determination of nitrite in pharmaceutical products and their raw materials with sensitive analytical methods is essential. Often, dimethylamine is used to synthesize different

medications. Under acidic conditions, it reacts with nitrite, forming nitrosamines [2]. This is also the case for the production of hydroxypropyl methylcellulose (Hypromellose), a common excipient. This Application Note covers the determination of nitrite in hydroxypropyl methylcellulose with ion chromatography (IC) using a Metrosep A Supp 10 column and direct UV/VIS detection at 215 nm. Sample preparation is performed with the Metrohm intelligent Pre-Concentration Technique with Matrix Elimination (MiPCT-ME).

SAMPLES AND STANDARDS

Hydroxypropyl methylcellulose (Hypromellose) was received as a powder from a pharmaceutical company. A 0.1 g sample portion was accurately weighed and transferred into a clean 10 mL volumetric flask containing 5.0 mL of ultrapure water (UPW). The content was dissolved using a vortex mixer for approximately 20 minutes and the flask was

filled up to the mark with UPW. The prepared sample solution was filtered through a 0.2 µm syringe filter and kept in a sample processor under closed conditions prior to analysis. A single-point calibration was used with 4 µg/L NO₂⁻ prepared from a 1000 mg/L NIST certified standard (Sigma TraceCERT No. 67276).

EXPERIMENTAL

The sample was analyzed with a chromatographic separation technique as described in USP <621> [3] (Figure 1). A MiPCT-ME setup was used in conjunction with the method parameters in Table 1. A 2 mL sample was preconcentrated on a Metrosep A PCC 2 HC/4.0, and the matrix was eliminated with 3 mL of ultrapure water. After injection, the anionic components were isocratically separated within 45 minutes on a Metrosep A Supp 10 - 250/4.0 column. The UV/VIS detector signal was recorded at 215 nm. Confirmation of the method accuracy was done with a spiking study. The sample was spiked with a nitrite standard at two concentration levels (1.0 µg/L and 4 µg/L), and the recovery values were evaluated.



Figure 1. Instrumental setup including a 940 Professional IC Vario (center), 947 Professional UV/VIS Detector Vario SW (top center), 858 Professional Sample Processor (right), and MiPCT-ME, performed with the Metrosep A PCC 2 HC/4.0 and a Dosino (left).

Table 1. IC method parameters for the determination of nitrite impurities in hydroxypropyl methylcellulose (Hypromellose).

Column	Metrosep A Supp 10 - 250/4.0
Eluent	5.0 mmol/L sodium carbonate 5.0 mmol/L sodium hydroxide
Flow rate	1.0 mL/min
Column temp.	45 °C
Injection volume	2 mL (preconcentration volume)
Detection	UV/VIS detection at 215 nm

Nitrite was quantified in hydroxypropyl methylcellulose (**Figure 2**). The method was sensitive enough to quantify trace levels of nitrite

present in the sample matrix. A two-level spiking study confirmed the method accuracy, achieving recoveries between 80 and 120%.

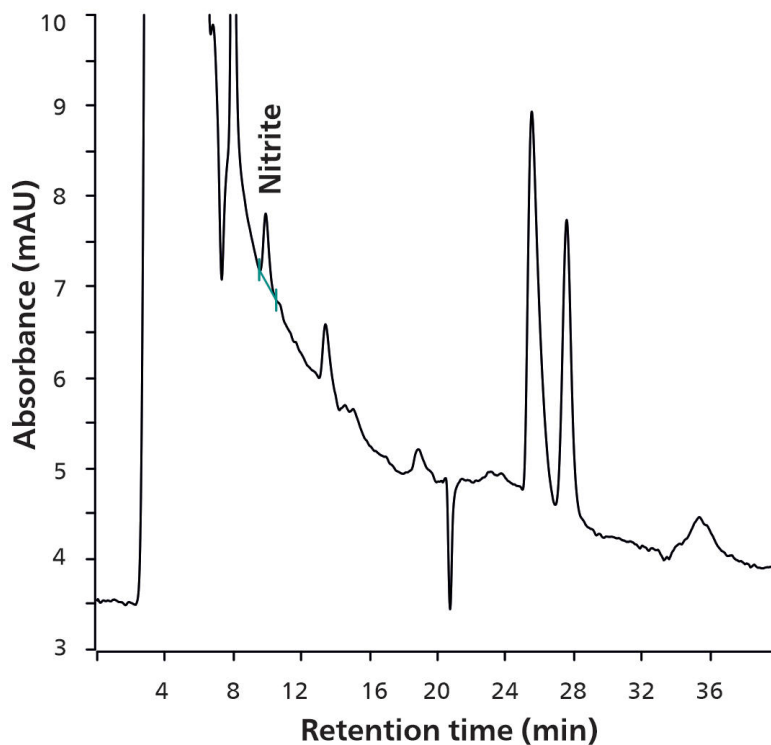


Figure 2. Chromatogram for 288 $\mu\text{g/kg}$ nitrite in a hydroxypropyl methylcellulose sample.

CONCLUSION

Quantification of nitrite in hydroxypropyl methylcellulose according to USP <621> is possible with the presented IC method. Preconcentration of the sample offers higher sensitivity for the accurate determination of trace quantities of nitrite. Inline matrix elimination removes the interfering sample matrix before injection, further improving results.

Separation of nitrite from other matrix components was achieved on the Metrosep A Supp 10 column. Method accuracy was confirmed by spiking studies. This IC method is suitable for quality control of the impurity nitrite in pharmaceutical manufacturing processes involving the excipient hydroxypropyl methylcellulose.

REFERENCES

1. U.S. Department of Health and Human Services Food and Drug Administration; Center for Drug Evaluation and Research (CDER). Control of Nitrosamine Impurities in Human Drugs - Guidance for Industry. *Pharmaceutical Quality/Manufacturing Standards/ Current Good Manufacturing Practice (CGMP)* **2021**.
2. U.S. Pharmacopeia. USP-NF Nitrosamine Impurities. *General chapter*. https://doi.org/10.31003/USPNF_M15715_02_01.
3. *621 Chromatography*. https://doi.org/10.31003/USPNF_M99380_01_01.

CONTACT

Metrohm Suisse SA
Industriestrasse 13
4800 Zofingen

info@metrohm.ch

CONFIGURATION



940 Professional IC Vario ONE/SeS/PP

Le 940 Professional IC Vario ONE/SeS/PP est l'appareil CI intelligent avec **suppression séquentielle** et **pompe péristaltique** pour la régénération du supprimeur. L'appareil peut être utilisé avec n'importe quelles méthodes de séparation et de détection.

Domaines d'application typiques :

- Déterminations d'anions ou de cations avec suppression séquentielle et détection de conductivité
- Analyse de traces pour les anions ou les cations
- Surveillance en ligne pour les anions ou les cations



947 Professional UV/VIS Detector Vario SW

Le 947 Professional UV/VIS Detector Vario SW, un détecteur intelligent à longueur d'onde unique, permet une quantification sûre et fiable des substances actives dans le domaine ultraviolet ou visible. Une longueur d'onde peut être sélectionnée.



858 Professional Sample Processor – Pump

Le 858 Professional Sample Processor – Pump traite des échantillons de 500 µL à 500 mL. Le transfert des échantillons s'opère soit au moyen de la pompe péristaltique bidirectionnelle à deux voies intégrée soit par un 800 Dosino.



Metrosep A Supp 10 - 250/4,0

La colonne de séparation Metrosep A Supp 10 - 250/4,0 est basée sur un copolymère haute capacité de polystyrène-divinylbenzène dont la dimension des particules est de seulement 4,6 µm. Cette colonne, la plus longue de la famille A Supp 10, offre la sélectivité et la flexibilité les plus grandes. L'utilisation du module MSM-HC est spécialement recommandée lorsque les durées de chromatogrammes sont longues. Sur cette colonne également, la possibilité de modifier la température, le débit et la composition de l'éluant permet les séparations d'anions les plus diverses.

La colonne Metrosep A Supp 10 - 250/4,0 possède une très grande capacité. Elle convient aux échantillons à force ionique élevée, aux tâches de séparation complexes et aux analyses d'échantillons dont les divers composants présentent des concentrations très différentes.



Metrosep A PCC 2 HC/4.0

Pour la préconcentration des anions et l'élimination de la matrice. L'agrandissement du lit de matériau permet d'augmenter la capacité des deux colonnes de préconcentration totalement fabriquées en PEEK. Cette haute capacité est surtout requise lorsque les effets de matrice pourraient provoquer une surcharge de la colonne de préconcentration ou que des échantillons de force ionique élevée doivent être analysés.