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 m syringe filter and kept in a sample processor under closed conditions prior to analysis.

A single-point calibration was used with 4 g/L NO_2^- 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 g/L and 4 g/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

RESULTS

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%.

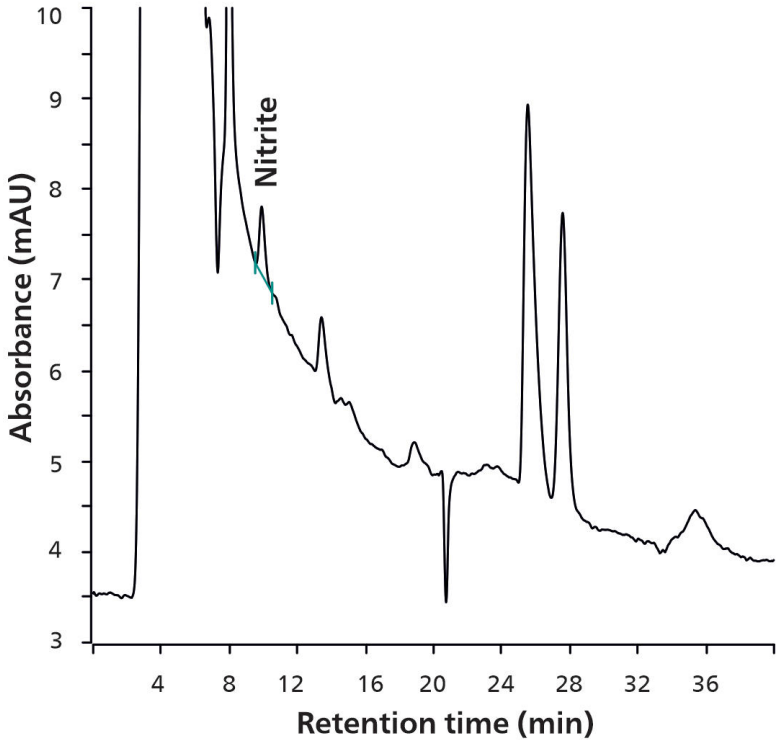

Figure 2. Chromatogram for 288 µg/kg nitrite in a hydroxypropyl methylcellulose sample.

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.

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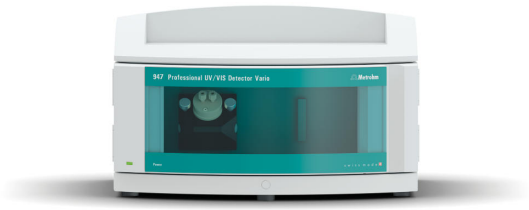
CONFIGURATION

940 Professional IC Vario ONE/SeS/PP

The 940 Professional IC Vario ONE/SeS/PP is the intelligent IC instrument with **sequential suppression** and a **peristaltic pump** for suppressor regeneration. The instrument can be used with any separation and detection methods.

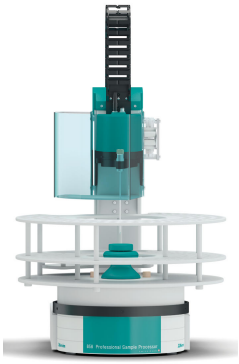
Typical areas of application:

- Anion or cation determinations with sequential suppression and conductivity detection
- Trace analysis for anions or cations
- Online monitoring for anions or cations



947 Professional UV/VIS Detector Vario SW

As an intelligent, single-wavelength detector, the 947 Professional UV/VIS Detector Vario SW permits a secure and reliable quantification of substances active in the ultraviolet or visible range. One wavelength can be selected.



858 Professional Sample Processor – Pump

The 858 Professional Sample Processor – Pump processes samples from 500 µL to 500 mL. The sample transfer takes place either with the installed bidirectional two-channel peristaltic pump or with an 800 Dosino.

Metrosep A Supp 10 - 250/4.0

The Metrosep A Supp 10 - 250/4.0 separation column is based on a high-capacity polystyrene-divinylbenzene copolymer with a particle size of only 4.6 µm. The longest column of the A Supp 10 product range offers the greatest selectivity and flexibility. Utilization of the MSM-HC is particularly recommended with longer chromatogram duration. Changes in temperature, flow and composition of the eluent also enable a wide variety of separations of anions on this separation column.

The Metrosep A Supp 10 - 250/4.0 has a very high capacity. It is suitable for samples with high ionic strength, for complex separation tasks and for analyzing samples in which great differences in concentration between the individual components are present.

Metrosep A PCC 2 HC/4.0

For anion preconcentration and matrix elimination. The enlargement of the packing bed increases the capacity of the two preconcentration columns completely made of PEEK. The high capacity is required primarily when matrix effects might cause an overloading of the preconcentration column or when samples with high ionic strength are to be analyzed.