



Application Note AN-S-400

Assay of nitrite in sodium nitrite

Column equivalency study according to U.S. Pharmacopeia

Cyanide, a toxic substance, may accidentally be ingested or inhaled as volatile hydrogen cyanide. Even small amounts of cyanide are lethal as it quickly blocks the cellular respiration process. In severe cases of cyanide poisoning, sodium nitrite is used along with sodium thiosulfate for treatment [1]. Sodium nitrite (NaNO_2) is believed to exert a therapeutic effect by reacting with hemoglobin to form methemoglobin. Methemoglobin has a high affinity for cyanide, and this complex helps safely remove the toxin from the body [2]. Sodium nitrite is listed in the WHO Model Lists of Essential Medicines [3]. Pharmaceuticals require strict quality control.

Therefore, it is necessary to determine impurities as well as the content of active ingredients. The U.S. Pharmacopeia (USP) **monograph Sodium Nitrite** describes the analytical method to determine the main component nitrite (NO_2^-) and the anionic impurity nitrate (NO_3^-) simultaneously with ion chromatography (IC) [4]. This Application Note describes the nitrite IC assay with the Metrosep A Supp 4 column and suppressed conductivity detection. Nitrite and nitrate in sodium nitrate are determined within one analysis run. The column equivalency study was in cooperation with the USP according to the USP General Chapter <621> [5].

SAMPLES AND STANDARDS

To prepare sample solutions, commercially available sodium nitrite salts were diluted in ultrapure water. The final nominal concentration for samples was 0.12 mg/mL nitrite.

A single point calibration was created with 0.12 mg/mL nitrite as prepared from a USP sodium nitrite reference standard (CAS RM® 7632-00-0).

EXPERIMENTAL

The samples were automatically injected with the 889 IC Sample Center, which guarantees fast and precise injections (Figure 1). They were subsequently

analyzed with a 940 Professional IC Vario using the method parameters given in the respective USP monograph (Table 1).



Figure 1. Instrumental setup including a 940 Professional IC Vario ONE/SeS/PP, IC Conductivity Detector (L) and the 889 IC Sample Center (R).

Table 1. IC method parameters as per USP monograph «Sodium Nitrite» [4].

Column with L105 packing	Metrosep A Supp 4 - 250/4.0
Eluent	2.7 mmol/L sodium carbonate 0.3 mmol/L sodium bicarbonate
Flow rate	1.5 mL/min
Column temp.	ambient
Injection volume	25 µL
Detection	Conductivity with sequential suppression

Anionic components were isocratically separated on a Metrosep A Supp 4 - 250/4.0 column, which contains packing material L105. The conductivity signal was detected after sequential suppression. Nitrite and nitrate eluted in less than five minutes. However,

according to the USP requirements the total running time needs to be set to at least four times the nitrite retention time. For the column equivalency study, system suitability (e.g., repeatability, tailing factors) and sample recoveries were evaluated (**Table 2**).

RESULTS

Nitrite and nitrate were quantified in dissolved sodium nitrite salts (**Figure 2**). The determination of the main component nitrite and the impurity nitrate in sodium nitrite was conducted according to USP General Chapter <621>, Chromatography [5]. A column equivalency study was performed, and all acceptance criteria (e.g., repeatability, resolution, tailing factor, and accuracy) were fulfilled. The

Metrosep A Supp 4 - 250/4.0 column was efficient (4000 theoretical plates), and nitrite eluted as a symmetric peak (tailing factor 1.06) with high repeatability (0.08% relative standard deviation for the nitrite peak area in the standard solution). The resolution between the nitrite and nitrate peaks was 4.2. In all analyzed samples, recoveries for nitrite were 101% and the nitrate content was <0.2% (**Table 2**).

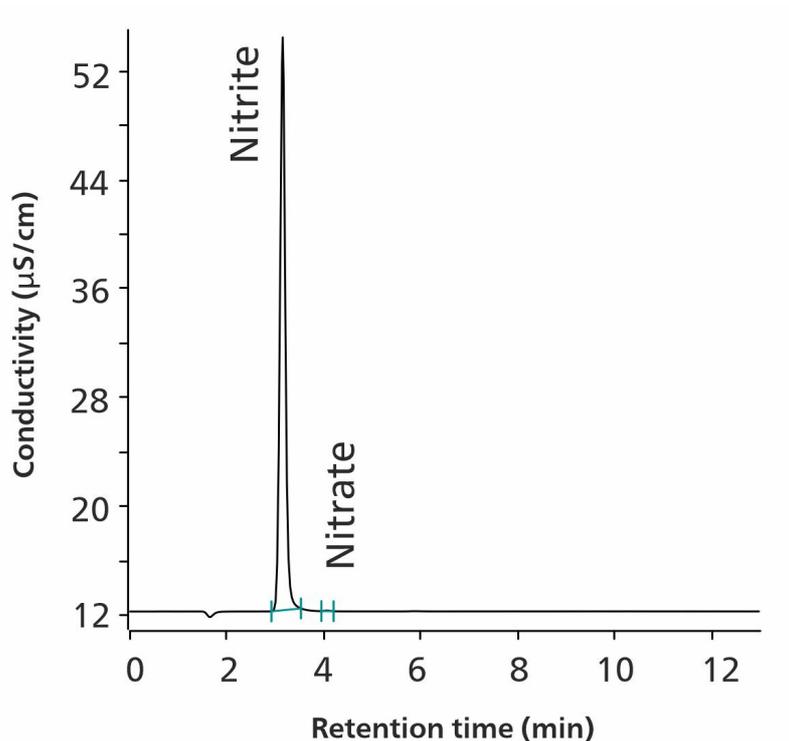


Figure 2. Chromatogram showing the analysis of nitrite and traces of nitrate in a sodium nitrite sample solution containing 0.121 mg/mL nitrite (100.8% recovery).

Table 2. Selected performance characteristics.

Performance characteristics	Acceptance criteria	Results
Tailing factor	Tailing factors (asymmetry) for the phosphate peak is NMT 2.0	1.06
Column efficiency	NLT 3000 theoretical plates	4000
Repeatability	Relative standard deviation for the nitrite peak area in the standard solution is NMT 1.5% for five replicates	0.08 %
Resolution	Resolution between nitrite and nitrate peak in sample solution	4.2
Accuracy	Average % recovery should be 98.0–102.0% of the manufacturer's CoA value	100.8 %
Impurity	Limit of sodium nitrate NMT 0.4%	0.2%

CONCLUSION

The presented IC method for the determination of nitrite and nitrate in sodium nitrite with the Metrosep A Supp 4 column (packing material L105) is officially included into the USP. Robustness and reliability of the method was demonstrated within the column

equivalency study following the guidelines of the USP General Chapter <621> [5]. The setup is suitable to quantify nitrite and the impurity nitrate in sodium according to USP requirements.

REFERENCES

- [1] Bebarta, V. S.; Brittain, M.; Chan, A.; et al. Sodium Nitrite and Sodium Thiosulfate Are Effective Against Acute Cyanide Poisoning When Administered by Intramuscular Injection. *Annals of Emergency Medicine* **2017**, *69* (6), 718-725.e4. <https://doi.org/10.1016/j.annemergmed.2016.09.034>.
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[3] *eEML - Electronic Essential Medicines List*. <https://list.essentialmeds.org/> (accessed 2022-10-28).

[4] U.S. Pharmacopeia. USP-NF Sodium Nitrite. *Monograph*. https://doi.org/10.31003/USPNF_M76880_03_01.

[5] *621 Chromatography*. https://doi.org/10.31003/USPNF_M99380_01_01.

Internal reference: AW IC IN6-2181-062021

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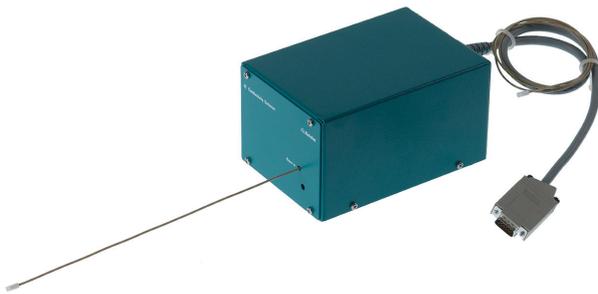
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CONFIGURATION



Metrosep A Supp 4 - 250/4.0

The Metrosep A Supp 4 - 250/4.0 is an extremely robust column with very good separation properties. The separation phase is comprised of polyvinyl alcohol particles with quaternary ammonium groups and a diameter of 9 μm . This structure guarantees great stability and a greater tolerance to very small particles that could pass through the integrated filter pad. The Metrosep A Supp 4 - 250/4.0 has a medium ion exchange capacity; sulfate elutes after 12.5 minutes. The plate numbers that can be achieved with this separation column is higher than those for the Metrosep Anion Dual 2 - 75/4.6. The A Supp 4 - 250/4.0 is particularly suitable for all routine tasks in water analysis.



IC Conductivity Detector

Compact and intelligent high performance conductivity detector for intelligent IC instruments. Outstanding temperature stability, the complete signal processing within the protected detector block and the latest generation of DSP – Digital Signal Processing – guarantee the highest precision of the measurement. No change of measuring ranges (not even automatic ones) is required, due to the dynamic working range.