



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**).

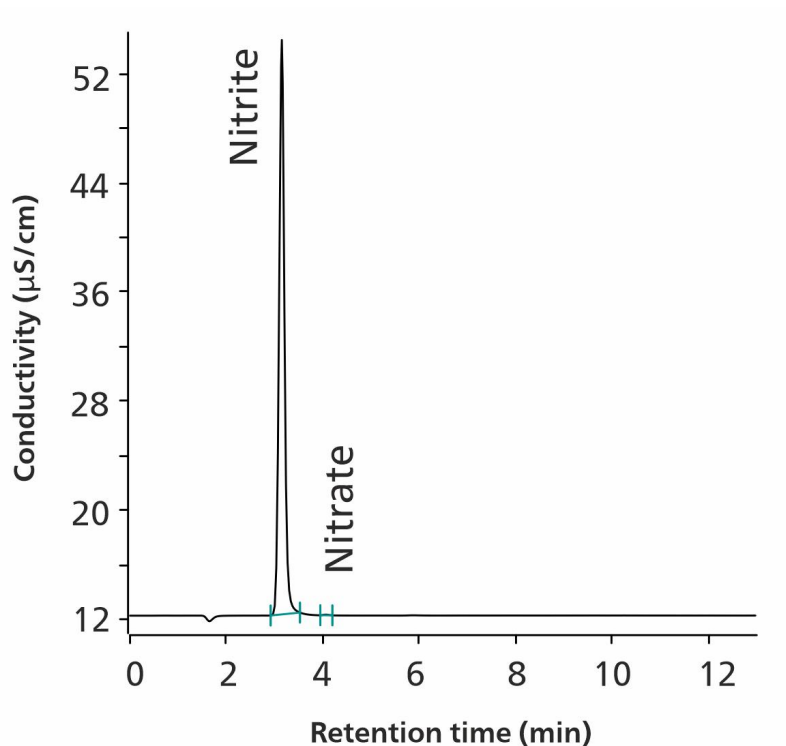


Figure 2. Chromatogram showing the analysis of nitrite and traces of nitrate in a sodium nitrite sample solution containing 0.121 mg/mL nitrite (100.8% recovery).

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. <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.

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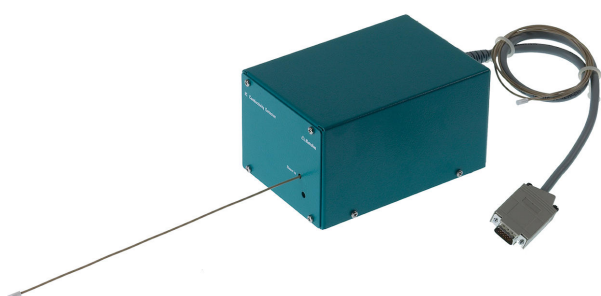
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CONFIGURATION



Metrosep A Supp 4 - 250/4.0

Metrosep A Supp 4 - 250/4.0 是固的色柱,具有十分秀的分性能。分段包含聚乙醇粒,季基,直径 9 μm 。此能保高定性,与可通内置板的微粒相比来允差更大。Metrosep A Supp 4 - 250/4.0 具有中等子交能力;硫酸将于 12.5 分之后淋洗出来。通此分柱可到的基数高于 Metrosep Anion Dual 2 - 75/4.6。A Supp 4 - 250/4.0 特用于水分析中的所有常工作。



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)。