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Application News

Anion Suppressor Ion Chromatograph HIC-ESP

Simultaneous Analysis of Nitrate and Nitrite Ions in Pharmaceutical Additives

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User Benefits

- Nitrite and nitrate ions contained in pharmaceutical additives can be determined with simple pretreatment procedure.
- High-sensitivity analysis on the order of µg/L can be performed using UV detector.
- Employed instrument setup can be applied to multipurpose anion analysis using suppressor-based ion chromatography.

■ Introduction

Since 2018, efforts have been underway in various countries to reduce the risk of nitrosamines contamination, originated by the detection of nitrosamines, a carcinogenic substance, in pharmaceutical products in Japan and other countries and following recall or discontinuation of certain pharmaceutical products.

Nitrosamines are known to be formed by the reaction of amines with nitrite. It has been reported that nitrous acid added for the purpose of inactivating azide compounds used in synthetic process and nitrites presenting as impurities in reagents and excipients raise a risk for the formation of nitrosamines. For this reason, the US FDA guidance[1)] and EP monograph[2)] also refer to the analysis of nitrite as an impurity.

■ Analytical Conditions

A suppressor type ion chromatograph HIC-ESP with a UV detector and Shim-pack™ IC-SA3 analytical column were employed for this study. Fig. 1 shows the flow path diagram and Table 1 shows the analysis conditions. The UV detector was used for the high sensitivity trace level determination of nitrite and nitrate ions in pharmaceutical additives.

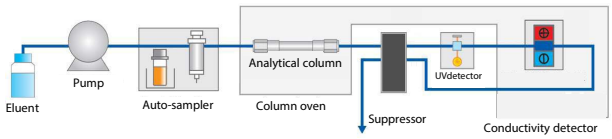


Fig.1 Flow Pass Diagram of Suppressor Type Ion Chromatography HIC-ESP

Table1 Analytical Conditions

System	: HIC-ESP＋SPD-40
Column	: Shim-pack IC-SA3*1
	(250 mm×4.0 mm I.D., 5 µm)
	: Shim-pack IC-SA3(G)*2
	(10 mm × 4.6 mm I.D., 5 µm)
Mobile Phase	: 3.6 mmol/L Sodium Carbonate
Flow Rate	: 0.8 mL/min
Column Temp.	: 45 ℃
Injection Vol.	: 50 µL
Detection	: UV 210 nm

*1 P/N : 228-41600-91, *2 P/N : 228-41600-92

■ Analysis of Standard Solutions

Fig. 2 shows a chromatogram at the lowest level of the calibration curve (0.002 mg/L each for nitrite and nitrate ions), and Fig. 3 shows a six-level linear calibration curve from 0.002 to 0.1 mg/L. The coefficients of determination were all larger than 0.9998, showing good linearities.

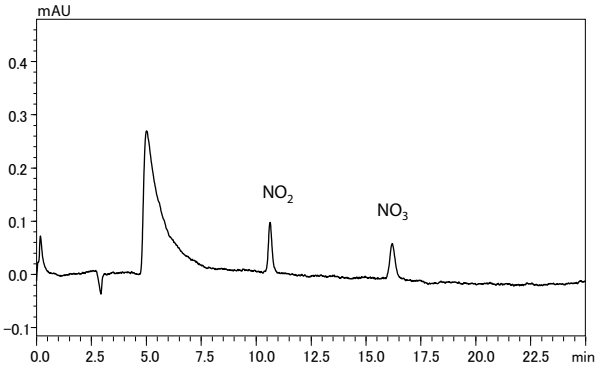
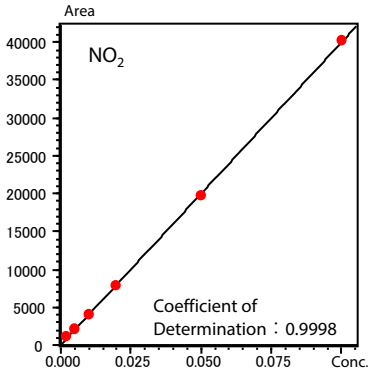


Fig.2 Chromatogram of Standard Nitrate and Nitrite Ions (Each 0.002 mg/L)



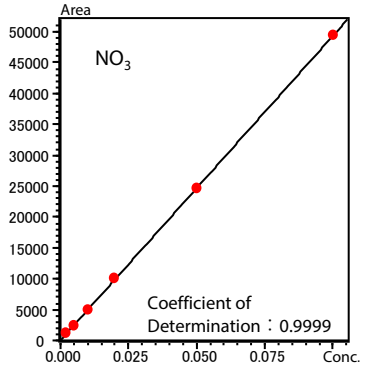


Fig.3 Calibration Curves for Nitrate and Nitrite Ions (Each 0.002-0.1 mg/L)

■ Applications

Polyvinyl Alcohol

10 mL of ultrapure water was added to 0.1 g of sample and heated to 60 ℃ to dissolve. Obtained chromatogram is shown in Fig. 4.

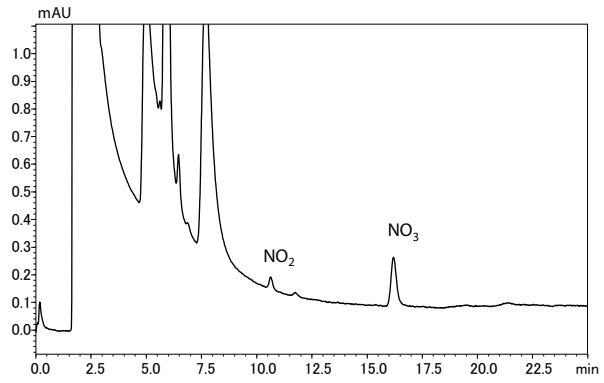


Fig.4 Chromatogram of Nitrate and Nitrite Ions in Polyvinyl alcohol

Silicic Acid Anhydride

5 mL of ultrapure water was added to 0.5 g of sample, and it was shaken with a shaker for 10 min, then centrifuged (2500 rpm, 10 min). obtained supernatant was subjected to analysis. Obtained chromatogram is shown in Fig. 5.

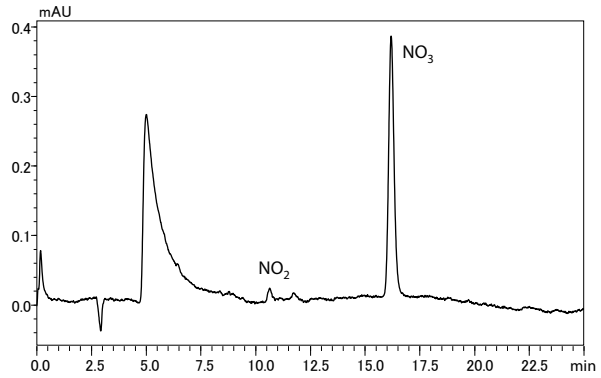


Fig.5 Chromatogram of Nitrate and Nitrite Ions in Silicic Acid Anhydride

Sodium Stearyl Fumarate

5 mL of ultrapure water was added to 0.2 g of sample, and it was shaken with a shaker for 10 min, then centrifuged (2500 rpm, 10 min). Obtained supernatant was filtered with a membrane filter (pore size 0.2 µm) for ion chromatography then subjected to analysis. Obtained chromatogram is shown in Fig. 6.

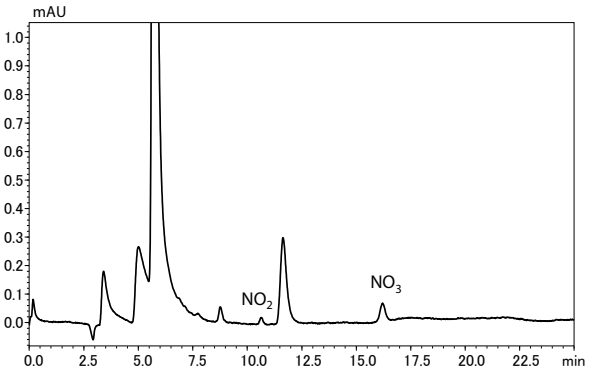


Fig.6 Chromatogram of Nitrate and Nitrite ions in Sodium Stearyl Fumarate

Determination results and recoveries

Resulting concentrations and recovery rates are shown in Table 2. Recovery rates were calculated by adding a standard nitrate and nitrite compounds to respective samples. and the results were good enough for ranging from 95% to 100% for both nitrite and nitrate ions.

■ Conclusion

A suppressor type ion chromatograph HIC-ESP was connected to a UV detector for high sensitivity analysis of nitrite and nitrate ions in pharmaceutical additives. Trace amounts of nitrite and nitrate ions were detected in all samples, and satisfactory recovery rates of more than 95% was obtained.

Many samples can be handled by dissolving or extracting them into water as a pretreatment for this method. A simple pretreatment enables high sensitivity analysis.

Table 2 Resulting concentrations and recovery rates

Sample	Sample amount g	Sample volume mL	Added standard mg/L		Resulting conc. mg/L		Recovery rate*3 %		Content rate µg/g	
			NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-
Polyvinyl alcohol	0.100	10	–	–	0.001*4	0.006	100	95	0.1	0.6
	0.100	10	0.02	0.02	0.021	0.025				
Silicic acid anhydride	0.496	5	–	–	0.001*4	0.013	95	100	0.01	0.13
	0.499	5	0.02	0.02	0.020	0.033				
Sodium stearyl fumarate	0.200	5	–	–	0.001*4	0.002	95	100	0.03	0.05
	0.200	5	0.02	0.02	0.020	0.022				

*3 Recovery rate=(concentration after addition-concentration before addition)/additional amount x 100
*4 Obtained through extrapolation of calibration curve

＜References＞

1) FDA Control of Nitrosamine Impurities in Human Drugs
https://www.fda.gov/regulatory-information/search-fda-guidance-documents/control-nitrosamine-impurities-human-drugs
（Confirmed on Sep. 26th, 2023）

2) European Pharmaceutical Review
https://www.europeanpharmaceuticalreview.com/news/178501/ph-eur-adopts-revised-general-monographs-after-adding-para-on-n-nitrosamines/ （Confirmed on Sep. 26th, 2023）

01-00643-EN First Edition: Nov. 2023

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