

## Application Note

### ► Determination of Nitrite and Nitrate in Fruit Juices by UV Detection



Category	Food
Matrix	Fruit Juice
Method	HPLC
Keywords	Ion pair chromatography, fruit juice, inorganic anions
Analytes	Nitrate, Nitrite
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#### Summary

A method for the determination of Nitrite and Nitrate in parallel by UV detection is described. The analysis of Nitrite and Nitrate in fruit juices can be carried by ion pair chromatography on RP C18 phases using various counter ions.

#### Introduction

Simultaneous determination of Nitrite and Nitrate is proposed for monitoring threshold levels of these additives in foods. The acute toxicity of Nitrate is in this instance of only peripheral importance. The primary health hazard lies rather in the rapid reduction of Nitrate to Nitrite which leads to the production of carcinogenic Nitrosamine when Nitrite reacts with secondary amines in the stomach. In addition, Nitrite is also known to cause methemoglobinemia (oxygen deficiency) to occur in infants. In order to provide a way to monitor the legal recommended limits of these additives in foods, a rapid and cost-effective HPLC method is presented here.

#### Experimental Sample Preparation

Commercially available fruit and vegetable juices were investigated after treatment with the Carrez I and II solutions in a ratio of 50:1. Once precipitated, the sample was centrifuged and the supernatant was removed. In order to remove potentially interfering substances, the sample can be further cleaned on an SPE anion exchange cartridge. The filtrate can then be used directly for HPLC analysis.

Carrez Solution I: Solution of potassium hexacyanoferrate (II) in water, p.A.  $c = 150$  g/L

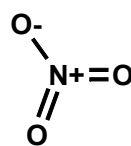
Carrez Solution II: Solution of zinc sulfate in water, p.A.,  $c = 300$  g/L

SPE method: condition SAX cartridges with 2 ml MeOH and 4 ml water, load 1 ml sample, wash with 3 x 1 ml water, elute with 2 ml 0.5 M NaCl solution

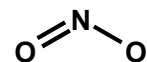
#### Experimental Preparation of Standard Solution

Calibration solutions were prepared from Nitrite and Nitrate standards in the range of 0.1 ppm to 25 ppm. The nitrate concentration range was extended to 200 ppm.

## Chemical Structures



Nitrate



Nitrite

## Method Parameters

<b>Column</b>	Eurosil Bioselect 300-5 C18, 120 x 4 mm
<b>Eluent A</b>	0.01 M n-octylamine, set to pH 4
<b>Eluent B</b>	5 mM tetrabutylammonium hydrogen sulphate pH 6.5
<b>Flow rate</b>	1.0 ml/min
<b>Injection volume</b>	5 µl
<b>Column temperature</b>	40 °C
<b>Detection</b>	UV at 210 nm

## Results

Since both ions are typically present in markedly different concentrations, their simultaneous determination using a conductivity detector under a set sensitivity range is not always possible. Herein lays the strength of this method with ion pair chromatography and UV detection. With this technique, both ions can be detected down to a concentration of 0.1 ppm. This value represents the limit of quantitation for both ions. The use of two different ion pair compounds as eluents allows for adjustment of coeluting peaks and ensures reliable results. Linear calibration curves for the standards run with both eluents showed regression coefficients (r<sup>2</sup>) of 0.9998 or better. The recovery rate of both ions is not influenced by the Carrez precipitation procedure: 95 – 98 %, without the SPE step. Inclusion of the SPE step only reduces the recovery rate slightly, however the sensitivity is reduced by a factor of 2 through dilution in the elution step (1 ml loaded sample / 2 ml extraction volume). As it had been expected, nitrite ion concentrations below the 0.1 ppm limit of quantitation were found in the fruit and vegetable juices examined. Manufacturers of such juices must not allow Nitrite concentrations to exceed 0.1 ppm in their products. Nitrate values varied widely among the vegetable juices tested. While the Nitrate value for one brand of carrot juice was 140 ppm, another brand of red beet juice gave a nitrate value of approximately 850 ppm, well outside of the calibration range.

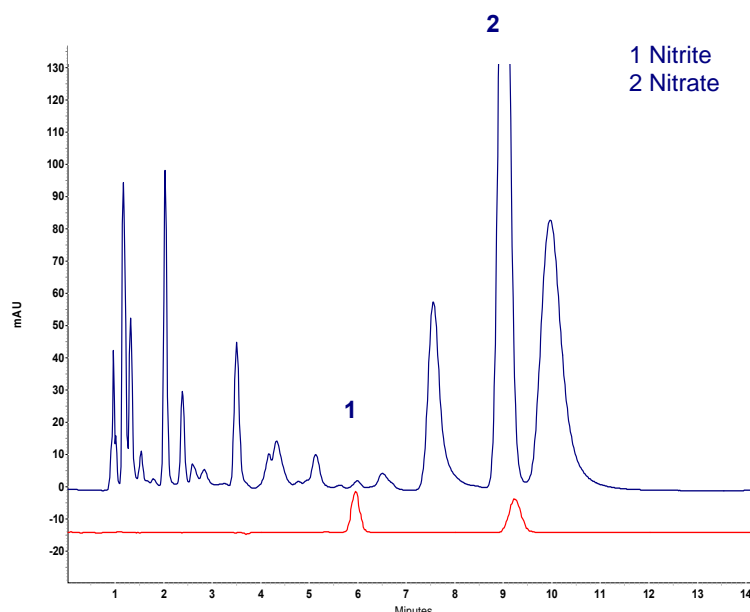
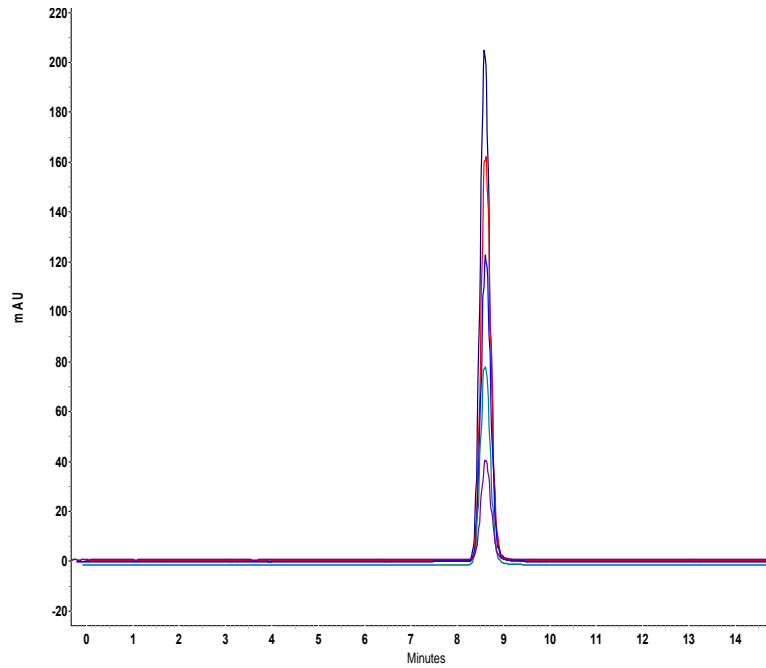
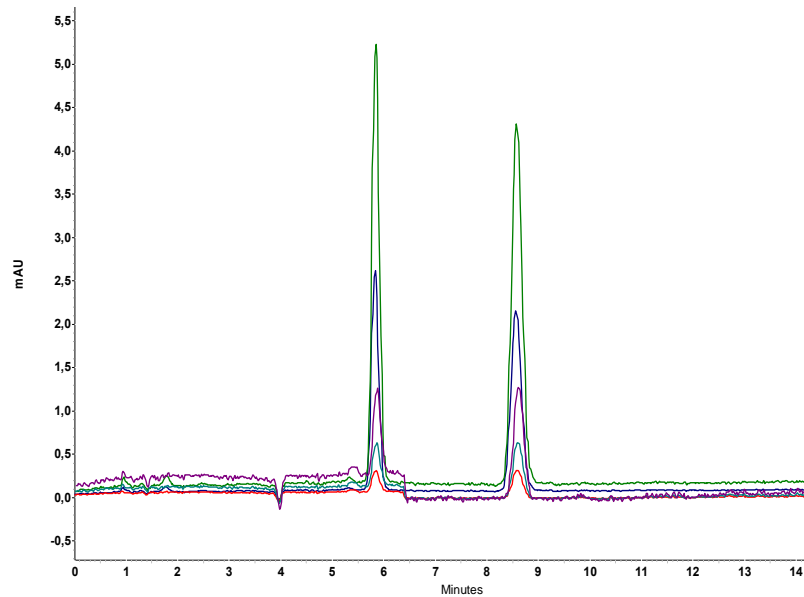


Fig. 1

Chromatogram of carrot juice (blue) overlaid with 1 ppm standard (red)



**Fig. 2**  
Overlaid Nitrate standard solutions (10 – 200 ppm)



**Fig. 3**  
Overlaid Nitrite/Nitrate standard solutions (0.1 – 5 ppm)

Substance	$t_R$ (min)	Area	ppm	LOD (ppm)
Nitrate	8.63	2877790	140	0.1
Nitrite	5.86	12487	<0.1	0.1

### Method Performance

<b>Limit of detection</b>	ppm range (S/N = 3)
<b>Linearity (r<sup>2</sup>)</b>	0.9998-0.999999
<b>Linearity range</b>	1 to 200 ppm for Nitrate; 0.1 to 5 ppm for Nitrite
<b>Retention time precision*</b>	< 2 % RSD
<b>Peak area precision*</b>	< 2 % RSD

\*repeatability calculated over 5 replicate runs

### Conclusion

A fast separation of Nitrate and Nitrite with excellent peak symmetry is easily accomplished by ion pair chromatography using a reversed phase C18 column and Smartline HPLC. High amount of Nitrate values in fruit and vegetable juices in parallel to low amount of Nitrite can be analyzed with precision better than 2 % RSD.

### References

[1] B. Luckas Z. Anal. Chem., 318, 428-433 (1984)

### Physical Properties of recommended Column



Eurosil Bioselect 300 has been specifically developed for the determination and purification of peptides, proteins and oligonucleotides. Eurosil Bioselect sorbent is characterized by loadability and a long lifetime. Due to the pore size of 300 Å and lower density of C18 chain the equilibration time for ion pair chromatography is lower comparing to other C18 phases with smaller pore sizes.

<b>Stationary phase</b>	Eurosil Bioselect 300-5 C18
<b>USP code</b>	L1
<b>Pore size</b>	300 Å
<b>Particle size</b>	5 µm
<b>Form</b>	spherical
<b>Surface area</b>	100 m <sup>2</sup> /g
<b>% C</b>	6
<b>Endcapping</b>	yes
<b>Dimensions</b>	120 x 4 mm
<b>Order number</b>	11DHK181EBJ

### Recommended Instrumentation



This application requires an isocratic HPLC system equipped with degasser, autosampler, column oven, and UV detector. Other configurations are also available. Please contact KNAUER to configure a system that's perfect for your needs.

Description	Order No.
Smartline Pump 1000, incl. 10 ml pump head	A50303
Smartline Manager 5000 with degasser	A5316
Autosampler 3900	A1508
Smartline Column Oven 4000	A5300
Smartline UV Detector 2500	A5140
10 mm Flow Cell	A4061
ChromGate Software	A1493

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