

## NITRITE AND NITRATE IN BABY FOOD AND PROCESSED MEAT



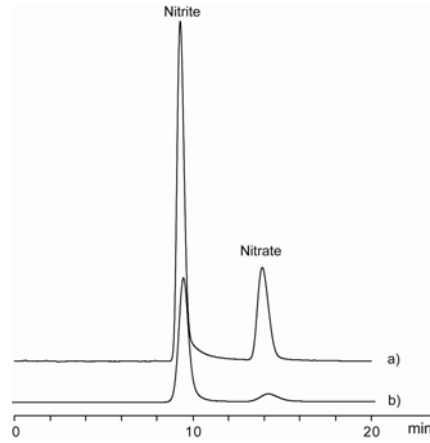
Nitrite and nitrate are added to food for their anti-microbial properties: to preserve the color and taste, and to prevent food from becoming rancid. Higher levels in vegetables and leafy greens are possible from the use of nitrate fertilizers and/or livestock manure. Nitrite levels in food can also be produced by reduction of nitrate to nitrite during processing.

Nitrate can be reduced to nitrite at certain physiological conditions in the human body. Nitrite however can oxidize Fe(II) in hemoglobin to methemoglobin, and Fe(III) product. The oxidized product is incapable of binding molecular oxygen and high concentrations of methemoglobin can result in methemoglobinemia especially in infants. Nitrite can also react with secondary amines present in food products or in the digestive system to form nitrosamines, a class of carcinogenic compounds.

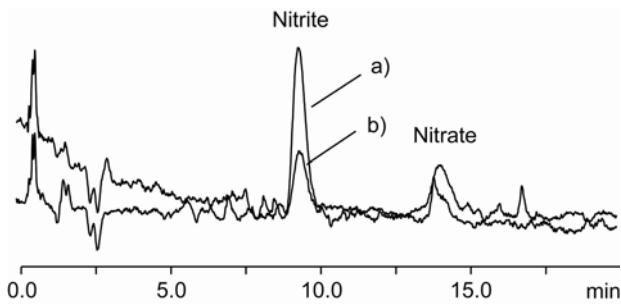
### Description of Method

The AOAC Official method 993.03 for the analysis of nitrate involves reduction using spongy cadmium which is toxic and carcinogenic. The FDA improved on this method by using vanadium(III) chloride and heat for the post-column reduction of nitrate to nitrite. Nitrite reacts with this modified Griess reagent to produce a red chromophore with maximal absorbance at 535nm. Pickering Laboratories Inc. has further improved this method by substituting the corrosive and volatile hydrochloric acid with methanesulfonic acid.

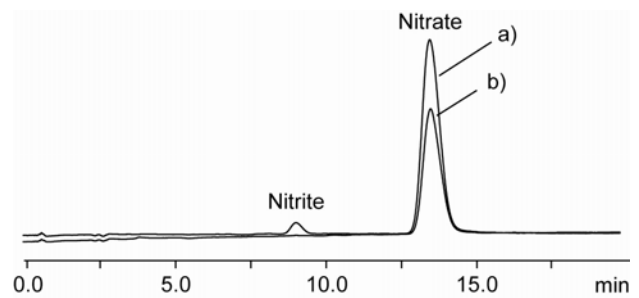
## Chromatograms



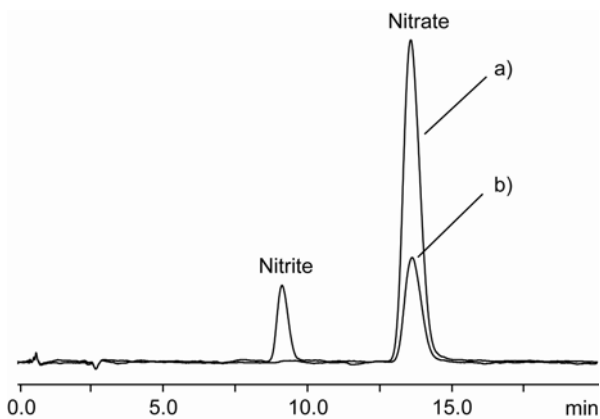
10 ppm nitrite-nitrate standard using  
(a) 20 % methanesulfonic acid, (b) 20 % hydrochloric acid



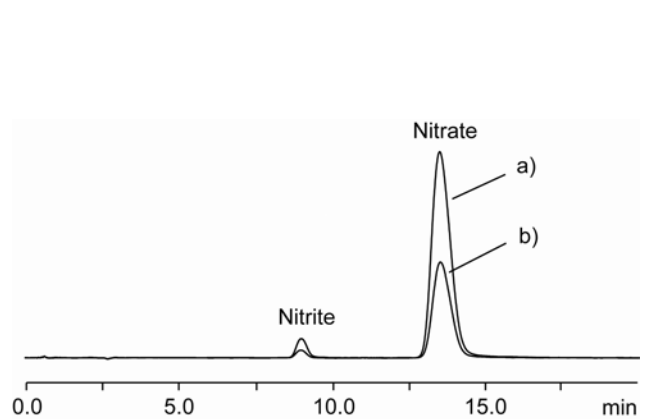
(a) 0.05 ppm,  
(b) 0.01 ppm nitrite-nitrate standard



Banana (baby food): (a) spiked with  
100 ppm nitrite-nitrate solution, (b) blank



Carrot (baby food): (a) spiked with  
100 ppm nitrite-nitrate solution, (b) blank



Corned Beef: (a) spiked with 50 ppm nitrite and  
500 ppm nitrate solution, (b) blank

# APPLICATION NOTE

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## HPLC Conditions and Derivatization Parameters

<b>HPLC</b>	
Operation Mode	Isocratic
Eluant	0.15 M sodium acetate
Degassing	Helium- or vacuum-degassed
HPLC Column	Pickering anion exchange column, 4.6 x 150 mm
Column Oven	50 °C
Flow Rate	1.0 mL/min
Injection Volume	10 µL
<b>Post-Column Derivatization</b>	
Pinnacle PCX	Single-pump; 500 µL reactor
Reactor Volume	500 µL
Reactor Temperature	100 °C
Reagent	Mix 50 mL of 1% vanadium-(III) chloride in 20 % methanesulfonic acid, 50 mL of 1% <i>m</i> -nitroaniline in 20 % methanesulfonic acid and 1.25 mL of 1 % N-(1-naphthyl) ethylenediamine dihydrochloride in 20 % methanesulfonic acid. Dilute to 250 mL using 20 % methanesulfonic acid.
Reagent Flow	0.1 mL/min
<b>Detection</b>	
Detection Type	UV/VIS detection
UV/VIS	535 nm
Flowcell	Analytic; pressure stable up to 7 bar

# APPLICATION NOTE

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## Sample Preparation

### Baby Food

To 5 g of baby food in a 50 mL centrifuge tube add 25 mL of 50-60 °C water (for vegetables) or 15mM sodium acetate (for fruits) and shake for 10 min. Add 12.5 mL of acetonitrile and make up the volume to 50 mL using water (for vegetables) and sodium acetate (for fruits). Centrifuge the mixture for 15 min at 5000 rpm. Filter the supernatant through a 0.45 µm nylon filter and dilute to fall within the linear range.

### Processed Meat

To 5 g of homogenized processed meat in a blender add 25 mL of 50-60 °C water and blend for 2 min. Add 25 mL of acetonitrile and blend for an additional 2 min. Transfer into a beaker and make up the volume to 100 mL using warm water. Filter the mixture using Whatman filter paper. Filter further through a 0.45 µm nylon filter and dilute to fall within the linear range.

### Notes

- 1) Post-column reagent solutions are stored in plastic or Teflon containers.
- 2) All solutions are filtered through 0.45µm nylon filter before use.
- 3) Nitrate/Nitrite standards should be checked prior to use for oxidation.
- 4) Sample pH should be checked to determine the choice of extraction solution since acidic pH facilitates the conversion of nitrite to nitrate.

## Analytical Data

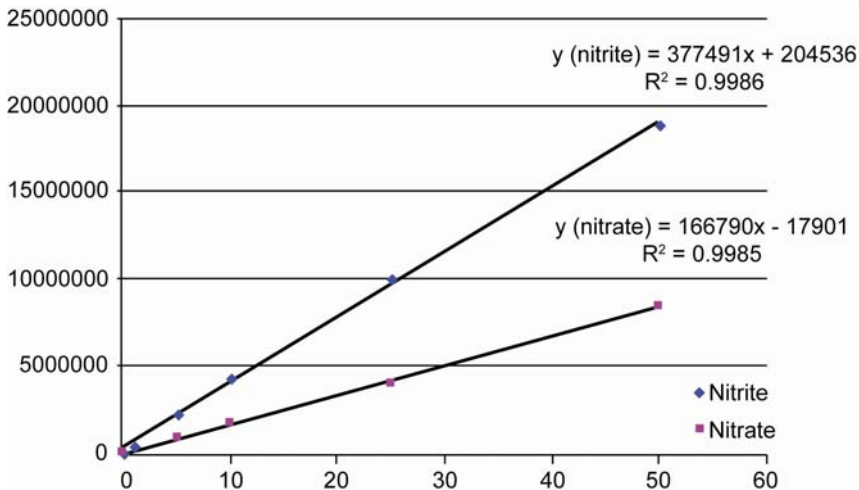
### Recovery Data

Sample	Spiked Conc. (ppm)	Cal. Conc. (ppm)	Recovery %	Spiked Conc. (ppm)	Cal. Conc. (ppm)	Recovery %
	Nitrite			Nitrate		
<b>Baby Food</b>						
Carrot	100	92.25	92	100	79.3	79
Banana	100	94.6	95	100	102.5	103
<b>Processed Food</b>						
Corned Beef	50	54.4	109	500	478.9	96

# APPLICATION NOTE

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## Calibration Curve



Calibration Curve for Nitrite-Nitrate (0.05 ppm – 50 ppm)

## Literature

1. AOAC- Official Methods of Analysis of AOAC International (2000) 17<sup>th</sup> Ed., Section 50.1.11.
2. John A. Casanova, Lois K. Gross, Sarah E. McMullen and Frank Schenck. (2006) J.AOAC Int. Vol. 89, No. 2, 447 – 451.
3. Use of Griess Reagents Containing Vanadium(III) for the Post-Column Derivatization and Simultaneous Determination of Nitrite and Nitrite in Baby Food, John A. Casanova, Lois K. Gross, Sarah E. McMullen and Frank Schenck, Food and Drug Administration, 60 8<sup>th</sup> Street, Atlanta, GA 30309.

## Order Information

Order Number	Description
1153-1022	PINNACLE PCX; single-pump, 500 µL reactor
0785150	Anion exchange column, 4.6 x 150 mm