APPLICATION NOTE

As of: April, 2008

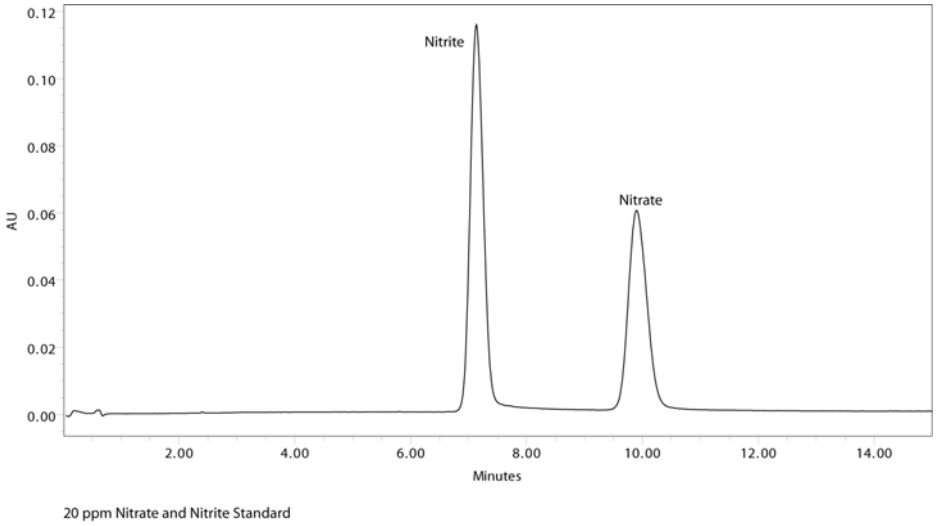
NITRITE AND NITRATE IN BABY FOOD AND PROCESSED MEAT

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 could 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 met hemoglobin, and Fe(III) product. The oxidized product is incapable of binding molecular oxygen and high concentrations of met hemoglobin 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.

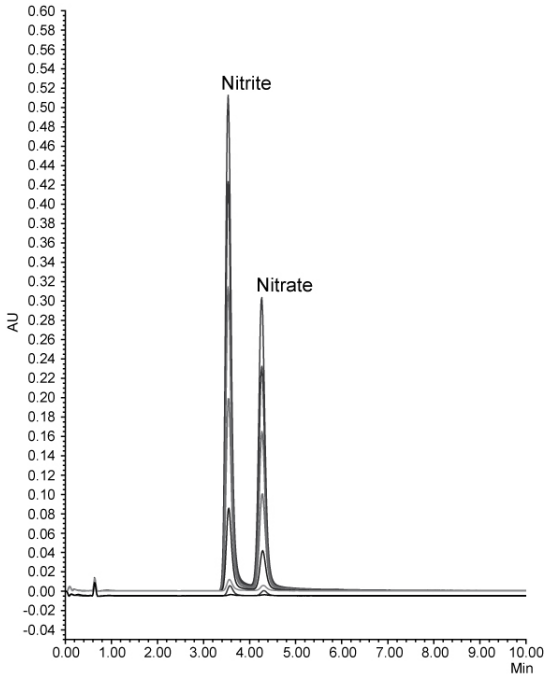
Description of Method

AOAC Official method 993.03 for the analysis of nitrate involves reduction using spongy cadmium which is toxic and carcinogenic. 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.

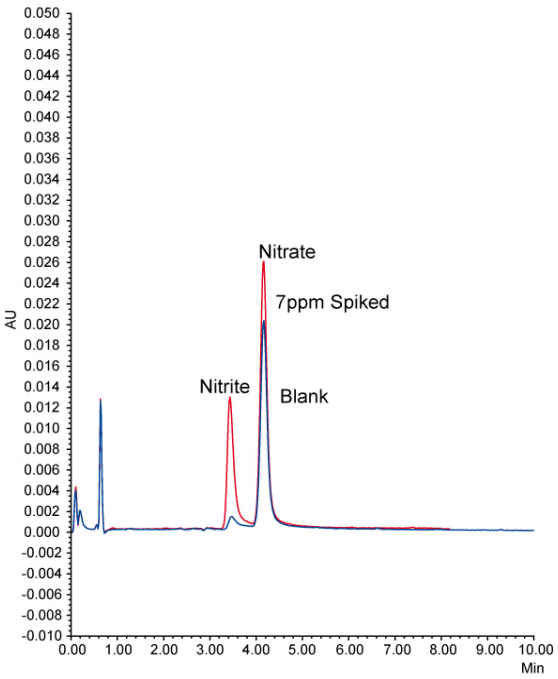
Chromatogram



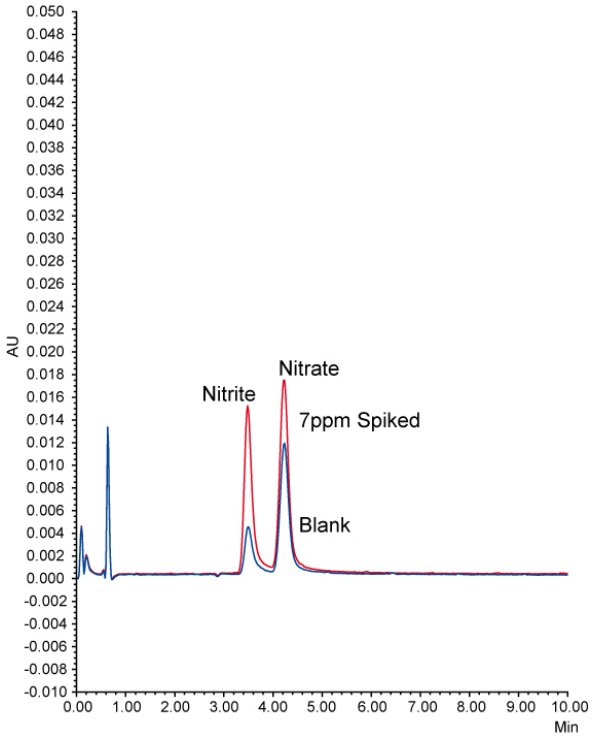
Chromatogram of a nitrite and nitrate standard at 20 ppm



Nitrite Nitrate standard (0.1 ppm – 25 ppm)



Beef franks



Pastrami

HPLC Conditions and Derivatization Parameters

HPLC	
Operation Mode	Isocratic
Eluant	15 mM sodium acetate
Degassing	Helium- or vacuum-degassed
HPLC Column	Pickering anion exchange column, 4.6 x 150 mm
Column Oven	40 °C
Flow Rate	1.0 mL/min
Post-Column Derivatization	
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 % HCl, 50 mL of 1% *m*-nitroaniline in 20 % HCl and 1.25 mL of 1 % N-(1-naphthyl) ethylenediamine dihydrochloride in 20 % HCl. Dilute to 250 mL using 20 % HCl.
Reagent Flow	0.1 mL/min
Detection	
Detection Type	UV/VIS detection
UV/VIS	535 nm
Flowcell	Analytic; pressure stable up to 7 bar

Sample Preparation

Baby Food
To 5 g of baby food in a 50 mL centrifuge tube add 25 mL of 50-60 ^{o}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 ^{o}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.

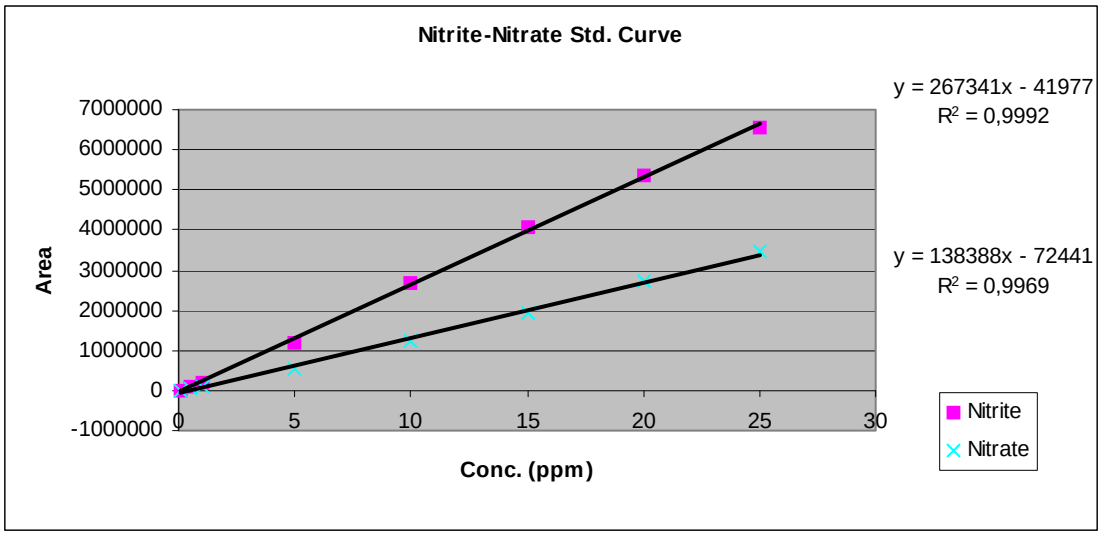
Notes
1) Post-column reagent solutions are stored in plastic or Teflon containers.
2) All solutions are filtered through 0.45µm nylon filter before use.
3) Nitrate/Nitrite standards should be checked prior to use for oxidation.
4) Sample pH should be checked to determine the choice of extraction solution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

Analytical Data

Recovery Data

Sample	Spiked Conc. (ppm)		Cal.Conc. (ppm)		% Recovery	
	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate
Sweet Potato	50	50	57.3	58.0	115	116
	250	250	271.4	266.7	109	107
Pears	50	50	54.4	55.0	109	110
	250	250	280.8	271.0	112	108
Apple Sauce	50	50	56.5	54.6	113	109
	250	250	283.9	265.7	114	106
Beef Franks	7	7	4.57	6.06	91	87
	10	10	7.61	9.03	76	90
	50	50	45.50	56.72	91	113
Pastrami	7	7	6.59	5.61	94	80
	10	10	9.11	11.21	91	112
	50	50	45.31	43.44	91	87

Calibration Curve



Literature

1. AOAC- Official Methods of Analysis of AOAC International (2000) 17th 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 8th Street, Atlanta, GA 30309.

Order Information

Order Number	Description
1153-1022	PINNACLE PCX; single-pump, 500 µL reactor
0755150	Anion exchange column, 4.6 x 150 mm