PICKERING LABORATORIES

Simultaneous Determination of Nitrite and Nitrate in Processed foods

Nitrite either alone or in combination with nitrate is added to food to preserve the color and taste and to prevent foods from becoming rancid. They are also used in food for their anti-microbial properties. Higher levels in vegetables and leafy greens are possible from the use of nitrate fertilizers and/or livestock manure.

Nitrate can be reduced to nitrite at certain physiological conditions in the human body. Nitrite however can oxidize Fe (II) in hemoglobin to methemoglobin, an 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. Nitrite levels in food could also be produced by reduction of nitrate to nitrite during processing.

The AOAC Official Method 993.03[1] for the analysis of nitrate involves reduction using spongy Cadmium which is toxic and carcinogenic. The USFDA improved on this method by using Vanadium (III) chloride and heat[2] 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 535 nm. Pickering Laboratories Inc. has further improved this method by substituting the corrosive and volatile hydrochloric acid with methane sulfonic acid.

Method

Analytical Conditions

IC System: ICS 900 or equivalent IC system (Thermo Scientific)

Analytical Column: IonPac AS9-HC, 4 x 250 mm (Thermo Scientific)

Flow Rate: 1 mL/min

Column Temperature: 30 °C

Mobile Phase: 9.0 mM Sodium Carbonate

Injection Volume: 20 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 0.5 mL

ReactorTemperature: 100 °C

Reagent Flow Rate: 0.1 mL/min

Detection: UV/VIS, 535 nm

Reagent : Mix 50 mL of (i) and (ii) and 1.25 mL of (iii) in 250 mL volumetric flask. Bring to volume with 20/80 Methanesulfonic Acid / Water.

(i) 1% Vanadium (III) Chloride in 20/80 Methanesulfonic Acid / Water.

(ii) 1% m-Nitro Aniline in 20/80 Methanesulfonic Acid / Water.

(iii) 1% N-(1-Naphthyl)ethylenediamine Dihydrochloride in 20/80 Methanesulfonic Acid / Water.

Extraction Procedure

Fruits and Vegetables

To 5 g of baby food in a 50 mL centrifuge tube add 25 mL of 50-60 °C water (for vegetables) or 15 mM 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 mins at 5000 rpm. Filter the supernatant through a 0.45 µ 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 µ nylon filter and dilute to fall within the linear range.

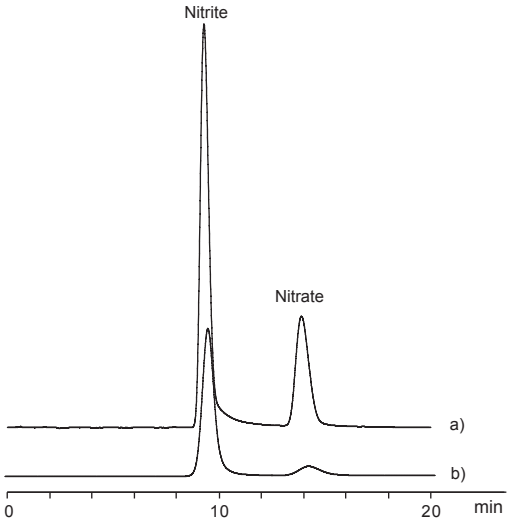
Notes

Post-column reagent solutions are stored in plastic or Teflon containers.

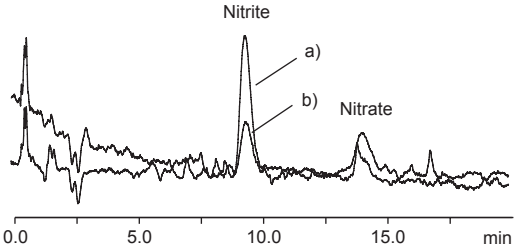
All solutions are filtered through 0.45 µ nylon filter before use.

Nitrate/Nitrite standards should be checked prior to use for oxidation.

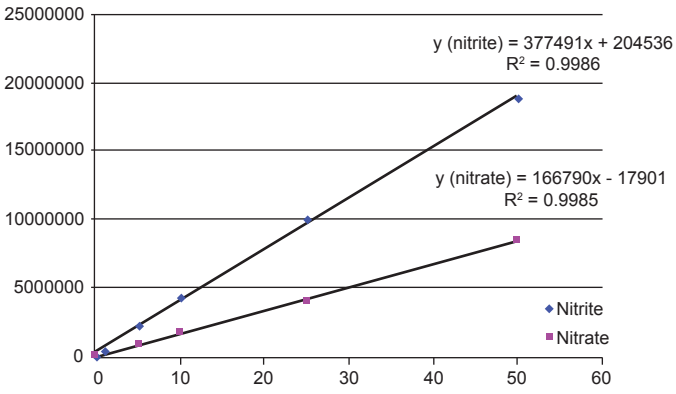
Sample pH should be checked to determine the choice of extraction solution since acidic pH facilitates the conversion of nitrite to nitrate.



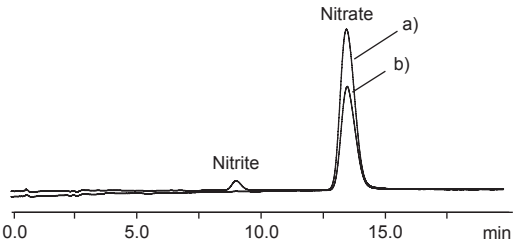
10 ppm nitrite-nitrate standard using (a) 20 % methane-sulfonic acid , (b) 20 % hydrochloric acid



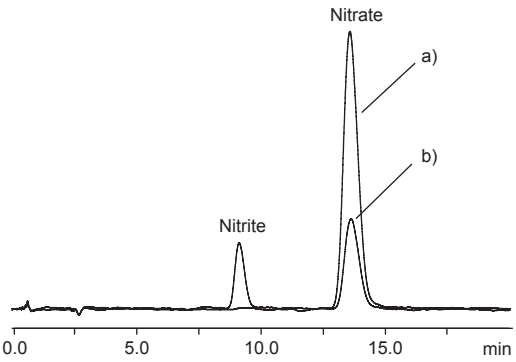
(a) 0.05 ppm, (b) 0.01 ppm nitrite-nitrate standard



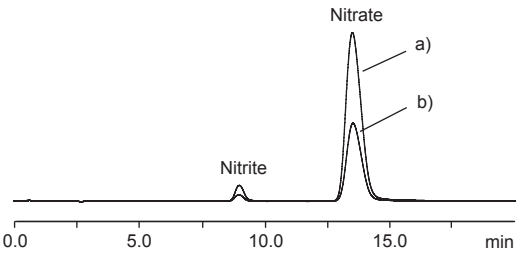
Calibration Curve for Nitrite-Nitrate (0.05 ppm – 50 ppm)



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

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

Acknowledgments

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References

1. 1. AOAC- Official Methods of Analysis of AOAC International (2000) 17th Ed., Section 50.1.11.

2. 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.