



## Nitrite and Nitrate Analysis

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Nitrite and nitrate are 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. Nitrite levels in food could also be produced by reduction of nitrate to nitrite during processing.

AOAC Official method 993.03<sup>1</sup> 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<sup>2</sup> 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.

### METHOD

#### EQUIPMENT

- LC with a binary pump
- UV/VIS detector
- Pickering Laboratories single reagent Pinnacle PCX post-column derivatization unit
- Pickering Laboratories anion exchange column, 4.6 X 150mm (Cat. No. 0755150)

#### REAGENTS

Sodium acetate  
Vanadium(III)chloride  
N-(1-Naphthyl)ethylenediamine dihydrochloride  
m-Nitro aniline  
20%(v/v) Hydrochloric acid

## Simultaneous Determination of Nitrite and Nitrate in Baby Foods

#### LC CONDITIONS

LC COLUMN TEMPERATURE: 40°C  
SAMPLE INJECTION VOLUME: 10mL  
LC FLOW RATE: 1mL/min  
MOBILE PHASE: 15mM Sodium acetate

#### POST-COLUMN CONDITIONS

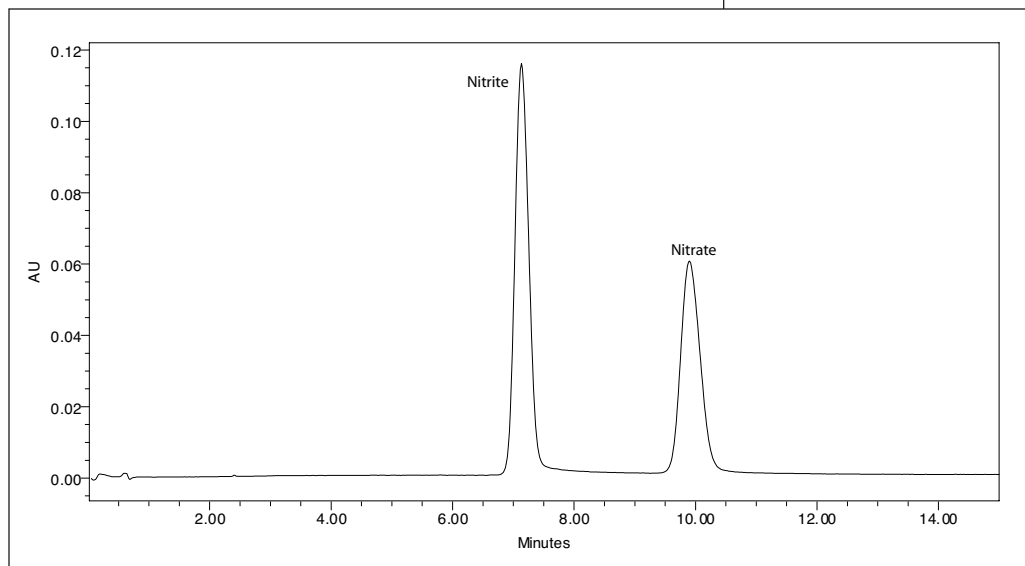
REACTOR VOLUME: 0.5mL  
REACTOR TEMPERATURE: 100°C  
REAGENT FLOW RATE: 0.1mL/min  
  
REAGENT: (i) 1% Vanadium(III)chloride in 20% HCl  
(ii) 1% m-Nitro aniline in 20%HCl  
(iii) 1% N-(1-Naphthyl) ethylenediamine dihydrochloride in 20% HCl  
Mix 50mL of (i) and (ii), and 1.25mL of (iii) and dilute to 250mL using 20%HCl  
  
DETECTOR: UV/VIS,  $\lambda_{max} = 535nm$

### Sample Preparation

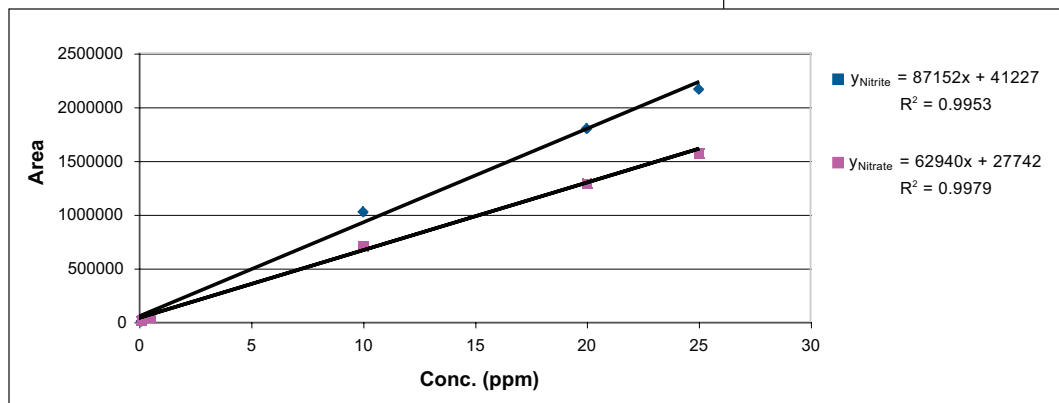
To 5g of baby food in a 50mL centrifuge tube add 25mL of 50-60°C water (for vegetables) or 15mM Sodium acetate (for fruits) and shake for 10min. Add 12.5mL of acetonitrile and make up the volume to 50mL using water (for vegetables) and sodium acetate (for fruits). Centrifuge the mixture for 15mins at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

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## 20ppm Nitrate and Nitrite Standard



## Calibration Curve for Nitrite and Nitrate



## Recovery Data

Sample	Spiked Conc. (mg/kg)		Calc. Conc. (mg/kg)		Recovery (%)	
	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate
Sweet Potato	500	500	573	580	115	116
	2500	2500	2714	2667	109	107
Pears	500	500	544	550	109	110
	2500	2500	2808	2710	112	108
Apple Sauce	500	500	565	546	113	109
	2500	2500	2839	2657	114	106

### NOTES:

Post-column reagent solutions are stored in plastic or Teflon containers.  
 All solutions are filtered through 0.45 $\mu$  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.

### ACKNOWLEDGMENTS:

John A. Casanova, Food and Drug Administration, 60 8th Street, Atlanta, GA 30309

### REFERENCES:

AOAC- Official Methods of Analysis of AOAC International (2000) 17th Ed., Section 50.1.11. Use of Griess Reagents Containing Vanadium (III) for the Post-Column Derivatization and Simultaneous Determination of Nitrite and Nitrite in Baby Food. Casanova, John A.; Gross, Lois K.; McMullen, Sarah E.; Schenck, Frank, JAOAC International, 2006; 89(2), 447-451.