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CATALYST FOR SUCCESS

NITRITE AND NITRATE ANALYSIS

SIMULTANEOUS DETERMINATION OF NITRITE AND NITRATE IN BABY FOODS

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[1] 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[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 Hydrochloric Acid with Methanesulfonic Acid.

METHOD

Equipment

- LC with a binary pump
- UV/VIS detector
- Pickering Laboratories single reagent Pinnacle PCX post-column derivatization unit (1153-1021 - 120 V, 1153-1022 – 240 V)
- Thermo Scientific Ion Pac™ AS9-HC Column (Cat. No. 051786) or AS22 (Cat. No. 064137)

Chemicals

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

LC Conditions

Sample Injection Volume: 10 µL

Column Temperature: 50 °C

LC Flow Rate: 1 mL/min

Mobile Phase: 1.8 mM Na_2CO_3 / 1.7 mM $NaHCO_3$
for AS9 - HC Column
or
4.5 mM Na_2CO_3 / 1.4 mM $NaHCO_3$
for AS22 Column

Post-Column Conditions

Reactor Volume: 0.5 mL

Reactor Temperature: 100 °C

Reagent Flow Rate: 0.1 mL/min

Reagent: (i) 1 % Vanadium (III) chloride in 20 % Methanesulfonic Acid

(ii) 1 % *m*-Nitro aniline in 20 % Methanesulfonic Acid

(iii) 1 % N-(1-Naphthyl)ethylenediamine

dihydrochloride in 20 % Methanesulfonic Acid

Mix 50 mL of (i) and (ii), and 1.25 mL of (iii) and dilute to 250 mL using 20 % Methanesulfonic Acid

Detection: UV/VIS, λ_{max} = 535 nm

Sample Preparation

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) or Sodium acetate (for fruits). Centrifuge the mixture for 15 mins at 5000 rpm. Filter the supernatant through a 0.45 µm nylon filter and dilute to fall within the linear range.

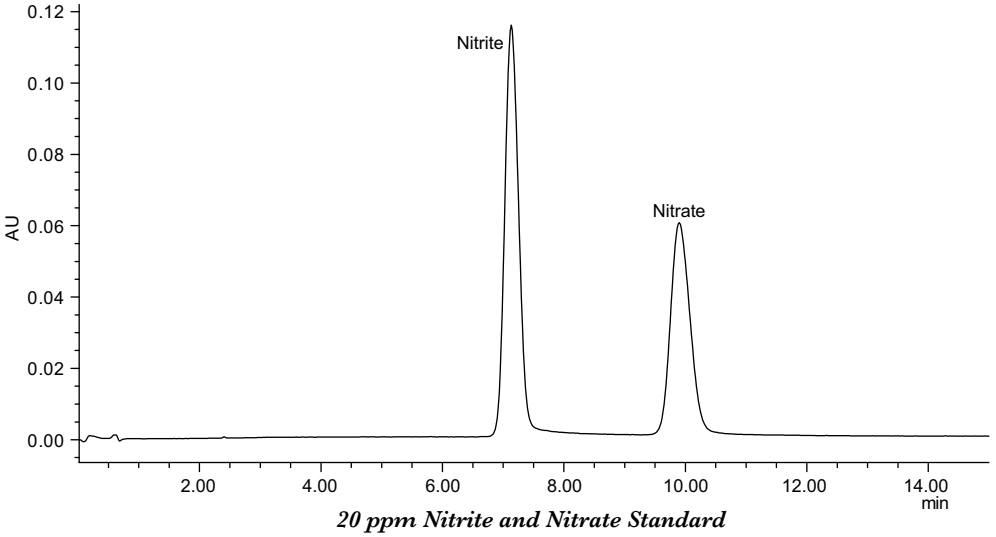
NOTES

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

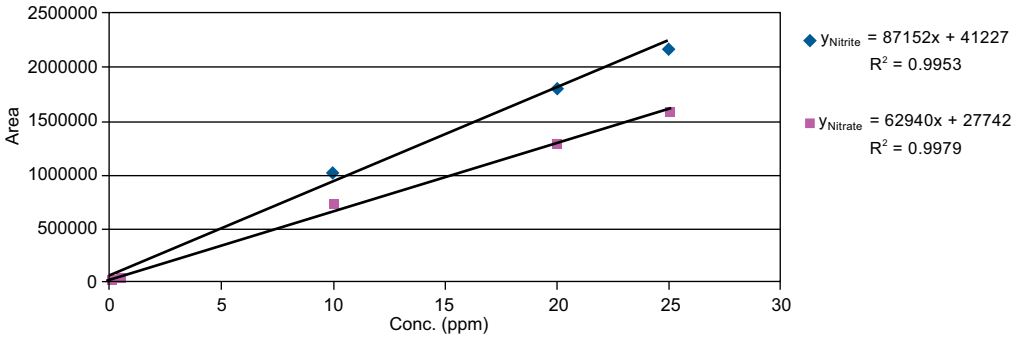
All solutions are filtered through 0.45 µm nylon filter before use.

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.



20 ppm Nitrite and Nitrate Standard



Calibration Curve for Nitrite and Nitrate

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

ACKNOWLEDGMENTS
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REFERENCES

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.*

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