PICKERING LABORATORIES
GUARANTEED CHEMISTRY

NITRITE AND NITRATE ANALYSIS

SIMULTANEOUS DETERMINATION OF RESIDUAL NITRITE AND NITRATE IN PROCESSED MEATS

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Nitrite and nitrate are added to processed meats for their antimicrobial properties. 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.

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.

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 150 mm

Reagents

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

LC Conditions

LC Column Temperature: 40 °C

Sample Injection Volume: 10 mL

LC Flow Rate: 1 mL/min

Mobile Phase: 15 mM Sodium acetate

Post-column Conditions

Reagent Flow Rate: 0.1 mL/min

Reactor Volume: 0.5 mL

Reactor Temperature: 100 °C

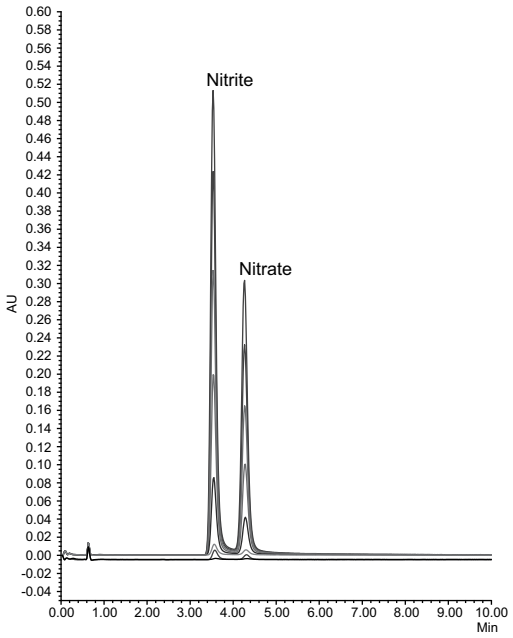
Post-Column Reagent:
Mix 50mL of 1 % Vanadium(III)chloride in 20 % HCl, 50mL of 1 % m-Nitroaniline in 20 % HCl and 1.25 mL of 1 % N-(1-Naphthyl)ethylenediamine dihydrochloride in 20 % HCl .

Detector: UV/VIS, λ_{max} = 535 nm

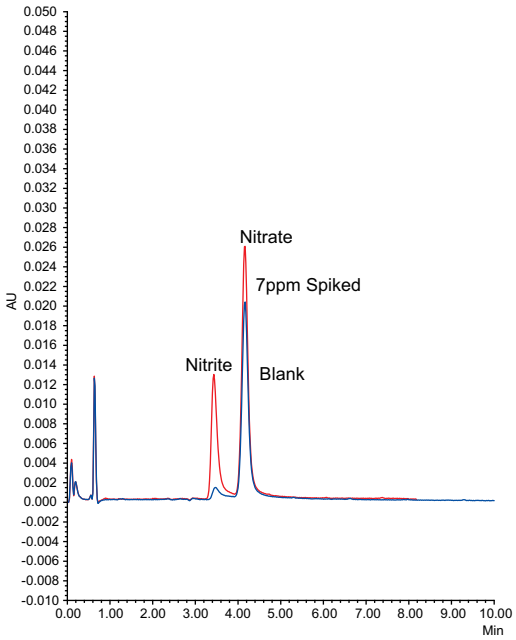
Sample Preparation

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 m nylon filter and dilute to fall within the linear range.

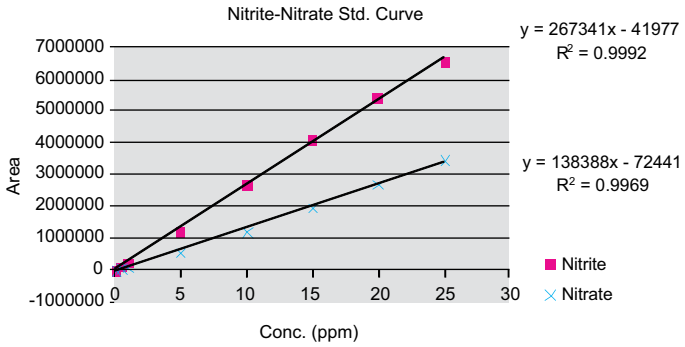
Nitrite-Nitrate Standard (0.1 ppm – 25 ppm)



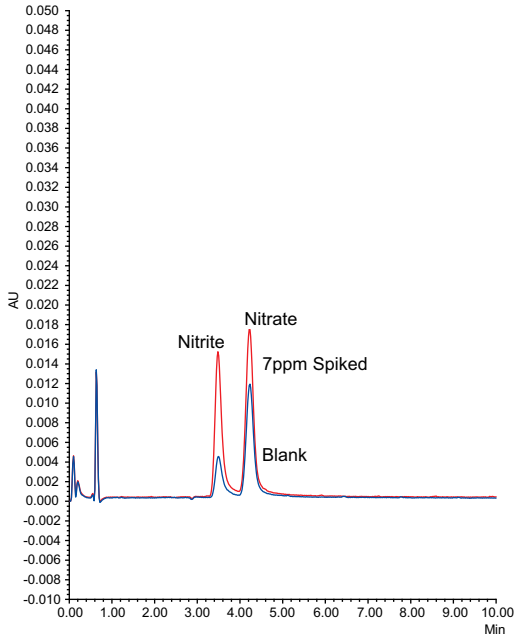
Beef Franks



Standard Curve for Nitrite-Nitrate (0.1 ppm – 25 ppm)



Pastrami



RECOVERY DATA						
SAMPLE	**SPIKED CONC. (PPM)**		**CAL. CONC. (PPM)**		**RECOVERY (%)**	
	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate
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

NOTES

Post-column reagent solutions are stored in plastic or containers. All solutions are filtered through 0.45 µ nylon filter before use.
Nitrate/Nitrite standards should be checked prior to use for oxidation.

ACKNOWLEDGMENTS

John A. Casanova, Food and Drug Administration, 60 8th Street, Atlanta, GA 30309 for his valuable suggestions regarding the modified method.

REFERENCES

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.

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