

METHOD FOR THE ANALYSIS OF N-NITROSOGLYPHOSATE , (NNG) (IMPURITY- GLYPHOSATE)

1. Principle of Method

A one millilitre injection of sample is made into the HPLC system. The NNG is separated from other compounds on a strong anion exchange (SAX) column. The effluent from the HPLC system is then sent into a Griess post column reactor where NNG reacts with HBr to form nitrosyl cation. Nitrosyl cation then reacts with N-(1-Naphthyl)ethylenediamine and sulphanilamide to form a purple azo dye which is detected at 550 nm.

2. Safety

Hydrobromic acid and hydrochloric acids are very corrosive. Sulfamic acid, sodium hydroxide, and hydrogen peroxide are also corrosive. Avoid any contact with the skin.

All solutions should be made in a fume hood. Proper gloves are recommended when handling these chemicals.

3. Range and Sensitivity

Range

This method has been validated in the range of 200 - 400 ppb NNG in glyphosate wetcake. The standard curve range is 10 – 200 ppb NNG.

Sensitivity

The sensitivity of the method is 1 mV/ppb NNG.

4. Interferences

Other N- nitroso compounds and nitrate ion give response with the Griess post column reactor. These interferences should be removed by the analytical method.

There are no known interferences for this method, however nitrate ion will react with glyphosate to form NNG. All glassware and equipment must be rinsed with sulfamic acid to remove any nitrite ions. A solution of sodium hydroxide/hydrogen peroxide is added to the samples and standards to help prevent the formation of NNG.

5. Precision and accuracy

Precision

The pooled coefficient of variation for glyphosate wetcake is 0.014 and the correlation coefficient is 0.9991.

Accuracy

The average recovery for glyphosate wetcake is 93%.

6. Advantages and disadvantages

Advantages

This method, using HPLC/post-column reactor methodology, gives a procedure that is sensitive to NNG at the parts per billion level. The large injection volume gives the needed sensitivity without the need for any concentration steps.

Disadvantages

The use of the post column reactor adds to the complexity of the method and the analysis time.

7. Apparatus

Equipment

Dupont 8800 Pump Module.

Sample injector – Waters Intelligent Sample Processor (WISP) 710B.

Technicon Proportioning Pump III.

Technicon single Channel Colorimeter Equipped with 2.0 X 50 mm Flow Cell and 550 nm filters

Technicon Oil Bath Cartridge Kit, Type A.

Electronic Filter- Spectrum 1021 Filter and Amplifier.

Strip Chart Recorder, 0 – 100 mV span.

Millipore Solvent Filtering Apparatus _ Type GS, 0.22 micron Filters.

Monsanto Chromatography Data System – A Computer Data handling System.

Technicon Mixing Coils and Tees

HPLC column, Whatman Partisil 10 SAX, 25 cm X 4.6 mm I. D.

Pump Tubing

Orange – Orange Silicon, 0.42 mL/min, Fisher Catalog Number 116-0497-090.

Orange – White, PVC, 1.00 mL/min, Fisher Catalog Number 14-190-75.

Gray – Gray, PVC, 1.00 mL/min, Fisher Catalog number 14-190-80

Standard Laboratory Glassware.

System Auto-Zero (optional), P.J. Cobert Catalog Number AZ_1436.

HPLC Operating Conditions

HPLC Pump Flow Rate: 1.5 mL/min

Sample Injecton Size: 0.100 mL.

Run Time(WISP): 5 min.

Run Time(MCDS): 20 min.

Detection Wavelength: 550 nm.

Detector settings:

DAMP-NORMAL

Std. Cal. – 6.00

Output – Telemetry Plug (5 volts Full Scale)

Spectrum Filter Settings:

Cutoff Frequency – 0.01
Attenuation – 1.0
Post-Column Reactor Oil Bath – 94 deg. C.

8. Reagents

Chemicals

Sulfamic Acid(Fisher A-295)
Sodium Hydroxide, 2.5 N (Fisher, SO-414)
Hydrogen Peroxide, 30% (Fisher, H-325)
Ammonium Phosphate Monobasic (Fisher, A-684)
Methanol, HPLC Grade (Fisher, A-452)
N-(1-Naphthyl)ethylenediamine Dihydrochloride (NED). (Eastman,4835)
Hydrobromic Acid, 48% (Fisher,A-140)
Sulfanilamide (Fisher, 0-4525)
Hydrochloric acid, 12 N (Fisher, A-144)
Phosphoric acid, 85% (Fisher, A-242)
Brij 35, 30% (Fisher, Cs-285-2)
High Temperature Bath Oil (Fisher, 0-2)

HPLC Mobile Phase

Mix 20 g ammonium phosphate monobasic into 2.0 liter deionized water. Add 400 mL methanol and bring total volume to 4.0 liters with deionized water. Adjust pH to 2.1 with 85% phosphoric acid, filter and degas mobile phase through a 0.22 micron filter.

NED/HBr Solution

Dissolve 4.35 g N-(1-naphthyl)ethylenediamine dihydrochloride in 400 mL deionized water. Add 500 mL 48% HBr and bring volume to 1.0 L with deionized water.

Sulfanilamide Solution

To 2.0 liters deionized water, add 400 mL concentrated HCl. Add 40.0 g sulphanilamide and 135 mL 30% Brij 35. Bring volume to 4.0 liters with deionized water.

Sulfamic Acid Solution

Dissolve 20 g sulfamic acid in 1.0 L deionized water.

9. Calibration and Standardization

NOTE

All glassware must be rinsed with the sulfamic acid solution and then with copious amounts of deionized water prior to use. For each volume of sulfamic acid solution used, use an equal volume of deionized water for each rinse. Nalgene sample bottles must also be washed and rinsed before using.

Preparation of Standards

Standards and samples are prepared and diluted on a weight per weight basis. Measure and record weight to the proper significant figure.

Stock Solutions

A 1000 ppm NNG stock solution is prepared by weighing 0.10000 ± 0.00001 g of analytical grade NNG into a 100 mL volumetric flask and diluting to 100.00 ± 1.00 deionized water.

A 5.000 ppm NNG working stock solution is made by weighing 0.5000 ± 0.0010 g of the 1000 ppm nitrite stock solution into a 100 mL volumetric flask and diluting to 100.00 ± 1.00 g. This working solution should be made fresh weekly along with all standard solutions.

Standards

Standards in the range of 10 – 200 ppb NNG are prepared by the appropriate dilutions of the 5.000 ppm nitrite standard into 50.00 ± 1.00 g deionized water.

Weight 5.000 ppm NNG Stock Solution	NNG concentration
0.1000 g	10 ppb
0.2000 g	20 ppb
0.5000 g	50 ppb
1.0000 g	100 ppb
2.0000 g	200 ppb

Calibration

A series of external standards in the range of 0 – 200 ppb NNG are prepared and analyzed. The height of the NNG peak is measured and a calibration curve is prepared.

10. Procedure

Cleaning of Equipment

All glassware and nalgene bottles must be rinsed with the sulfamic acid solution and then with copious amounts of deionized water. This should remove any trace amounts of nitrite and any other contaminants.

Collection and Shipping of Samples

Samples should be collected in brown nalgene bottles that have been washed with sulfamic acid and rinsed with deionized water. Failure to use sulfamic acid and deionized water washed bottles will compromise sample integrity.

Sample Preparation

Samples and standards are prepared and diluted on a weight per weight basis. Measure and record weights to the proper significant figure.

Wetcake

Weigh out 0.4000 ± 0.100 g glyphosate wetcake into a clean sample bottle. Add 0.85 mL 2.5 N NaOH/0.3% hydrogen peroxide and dilute to $10.00 \pm$ g with deionized water.

Analysis of prepared Samples

Start all reagents and mobile phase flowing. Once a good baseline is obtained, start injections, alternating samples and standards throughout the analysis. Measure the height of the NNG peak for standards and samples. Prepare a calibration curve. For wetcake samples find the amount of NNG in the sample from the calibration curve and the equation in section 11.

11. Calculations

The concentration of NNG in the sample is calculated using the weight of the sample (sample weight), the total weight of the prepared sample (total weight), and the amount of NNG injected which is found using the calibration curve (ppb NNG injection). The equation is :

$$\text{ppb NNG} = \frac{(\text{total weight})(\text{ppb NNG injection})}{(\text{sample weight})}$$

12. Discussion

The retention of NNG onto the column is reduced over a period of time. The amount of salt in the mobile phase can be reduced to maintain the same retention time. A precolumn filter and/or guard column may also help to increase column lifetime.

Analysis of other types of samples for NNG may also be possible. Spike recoveries will help to determine if quantitation is correct. It is also recommended that about 10% of the samples analyzed be spiked with NNG so that good quality control can be insured.

13. References

C. A. Pelezo, Fayetteville Standard Analytical Method Number F-62-84. Revision 1, July, 1984.

D.J. Augustin and F.Y. Triebe. Monsanto Analytical Method, ANMET Number 80, March, 1984.