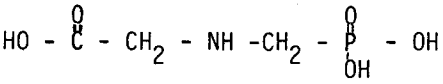
Determination of Glyphosate (N-phosphonomethyl glycine) Using an Amino Acid Analyzer

by GEORG EKSTROM
The National Swedish Institute for Plant Protection, Solna, Sweden
and SVEN JOHANSSON
The National Swedish Laboratory for Agricultural Chemistry, Uppsala, Sweden

Glyphosate is the common name of the active compound in the herbicidal formulation Roundup (trade mark of Monsanto Company). The manufacturer's GC method is quite tedious because of the many steps in the derivatisation technique which is necessary to render the compound volatile. The relationship with the common amino acid glycine gave a hint that this substance might also give a coloured complex with ninhydrin (1, 2, 3-triketohydrindene hydrate) and therefore could be determined with a colorimeter after separation from interfering substances on an ion exchange column.



Glyphosate (N-phosphonomethyl glycine) MW 169.1

Materials and methods

Standard solutions were prepared from Roundup which contains 360 grammes of glyphosate per litre in the form of isopropylamine salt. All dilutions were made with citrate buffer pH 2.2 to obtain concentrations from 0.2 to 1.0 micromole per ml.

Citrate buffer pH 2.2 (sodium ion conc. 0.20 M)
Citrate buffer pH 3.28 (sodium ion conc. 0.198 M)
Ninhydrin reagent (in acetate buffer pH 5.83)
Sodium hydroxide solution 0.2 M (for regeneration of the column)

Buffers and reagents were prepared according to EAKER (1968) and Beckman's Instruction Manual.
A Beckman model 121 automatic amino acid analyzer was used. Half a milliliter of the standard solution was injected.

The analyzer was fitted with a 690x9 mm glass column packed to a height of 550 mm with Beckman spherical cation exchange resin type M 82. The pH 3.28 citrate buffer was used as eluting solvent. The light absorption of the ninhydrin-glyphosate complex was measured in a 2.2 mm cuvette at 570 nm. The elution time for glyphosate is 20 minutes (cysteic acid position) and the elution volume is 23 ml. There will be no interference from the common amino acids in a pretein hydrolysate. The run time including regeneration and equilibration of the column is roughly 1 hour.

Bulletin of Environmental Contamination & Toxicology, Vol. 14, No. 3 © 1975 by Springer-Verlag New York Inc.

Results and discussion

The table gives optical densities (O.D.) as measured on the logarithmic strip chart and peak areas from the electronic integrator printout.

TABLE

conc. (micromoles ml)	conc. (microgrammes ml)	O.D.	peak area
0.2	33.8	0.138	10622
0.4	67.6	0.310	24022
0.6	101	0.460	34929
0.8	135	0.670	51071
1.0	169	0.860	63883

When either the optical densities or peak areas were plotted against the concentrations a linear relationship was obtained. Correlation coefficients = 0.99.
The minimum detectable amount is roughly 1 nanomole of glyphosate. The smallest amount that can be quantitated in a reproducible way is rouhgly 20 nanomoles or 3.4 microgrammes of glyphosate.
Work in progress is directed to the extraction and cleanup steps.

References

EAKER, D.: The Determination of Free and Protein-Bound Amine Acids, Symposium on "Evaluation of Novel Protein Products", Stockholm, Sept. 9-11, 1968.

Unichrom Amino Acid Analyzer. Instruction Manual. Beckman Instruments, Inc.

Determination of N-phosphonomethyl glycine and of its major metabolite, amino methylphosphonic acid. Monsanto Co. Private communication.