Bull. Environ. Contam. Toxicol. (1989) 43:17–21


Bulletin of Environmental Contamination and Toxicology

# Rapid and Simple Method for Identification of Glufosinate-ammonium Using Paper Chromatography

A. Suzuki and M. Kawana

Analytical Laboratory, Agricultural Divison, Hoechst Japan Limited, 3-2 Minamidai 1—chome, Kawagoe-shi, Saitama, 350 Japan

Glufosinate-ammonium (ammonium DL-homoalanine-4-yl(methyl)phosphinate) liquid (tradename BASTA liquid) developed by Hoechst AG, F. R. Germany, is a herbicide with a wide field of application. It is the ammonium salt of a phosphorus containing amino acid and therefore reacts with ninhydrin to produce the characteristic color-reaction. Taking note of this feature of glufosinate-ammonium, the authors have developed a rapid and easy method which makes possible the separation and identification of glufosinate-ammonium in samples taken from biological fluids (blood, urine, gastric-juice), even in the presence of other pesticides of similar chemical structure.

## MATERIALS AND METHODS

Glufosinate-ammonium (hereinafter called glufosinate), analytical standard (purity 99.5%), was supplied by Hoechst AG. Liquid formulation of glufosinate (content of active ingredient 18.5%), bialaphos (content of active ingredient 32.0%) and glyphosate (content of active ingredient 41.0%) were commercially available products. Acetic acid, trichloroacetic acid (TCA) and ninhydrin were reagent grade. Deionized water was used to prepare solutions. A ion-exchange cellulose filter paper (Whatman® DE-81) was used for paper chromatography.

A centrifuge, an oven, an atomizer (used with rubber bulbs), a microsyringe, and a chromatographic development chamber ( glass, 25(W) × 11(D) × 26(H) cm) were used in this study.

Commercially available glufosinate liquid was diluted 100 fold with deionized water and used as the standard solution. 1 mL of this solution contained about 1 mg of glufosinate.

Human urine and gastric-juice, plasma and serum from dogs were used as samples. To each 1 mL of the above four samples, 0.5 mL of the glufosinate standard solution was added and mixed (500 $\mu$g glufosinate / mL). Plasma and serum samples were submitted to a deproteinization process. (Deproteinization: to each 1 mL of plasma or serum, 1 mL of

Send reprint requests to A. Suzuki at the above address.

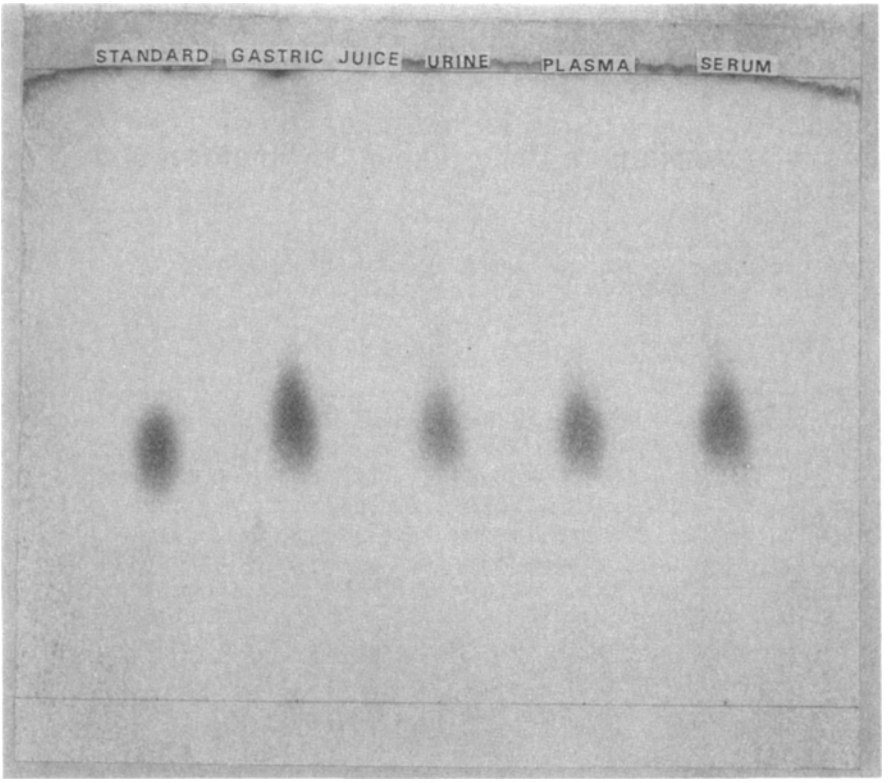


Figure 1. Identification of glufosinate in biological fluids

5% TCA aqueous solution was added. The mixture was centrifuged at 3,000 rpm for 10 min. The supernatant was used as sample. The deproteinization was done at room temperature.) Urine and gastric-juice samples were used without deproteinization. The plasma, serum, urine and gastric-juice samples were spotted in that order at even intervals beginning from the right edge of the ion-exchange cellulose filter paper (20 × 20 cm, hereinafter called filter paper) 1.5 cm from the lower edge of the filter paper. The glufosinate standard solution was spotted lastly as control. For each of the samples and the standard solution, the quantity applied was 10 $\mu$L. Microsyringe was used for application. After application, the filter paper was air-dried.

10% aqueous acetic acid was used as the developing solvent mixture. The atmosphere of the chromatographic development chamber was saturated with the vapours of the solvents prior to the experiment. The filter paper was placed in the chamber carefully so that 5 mm of its lower edge was immersed into the solvent without touching the walls of the chamber. The chamber was closed airtight, and the development was allowed to proceed at room temperature. When the solvent front advanced 15 cm over the starting line where the samples had been applied, the filter paper was taken out from the chamber and dried in an oven at 100 ℃.

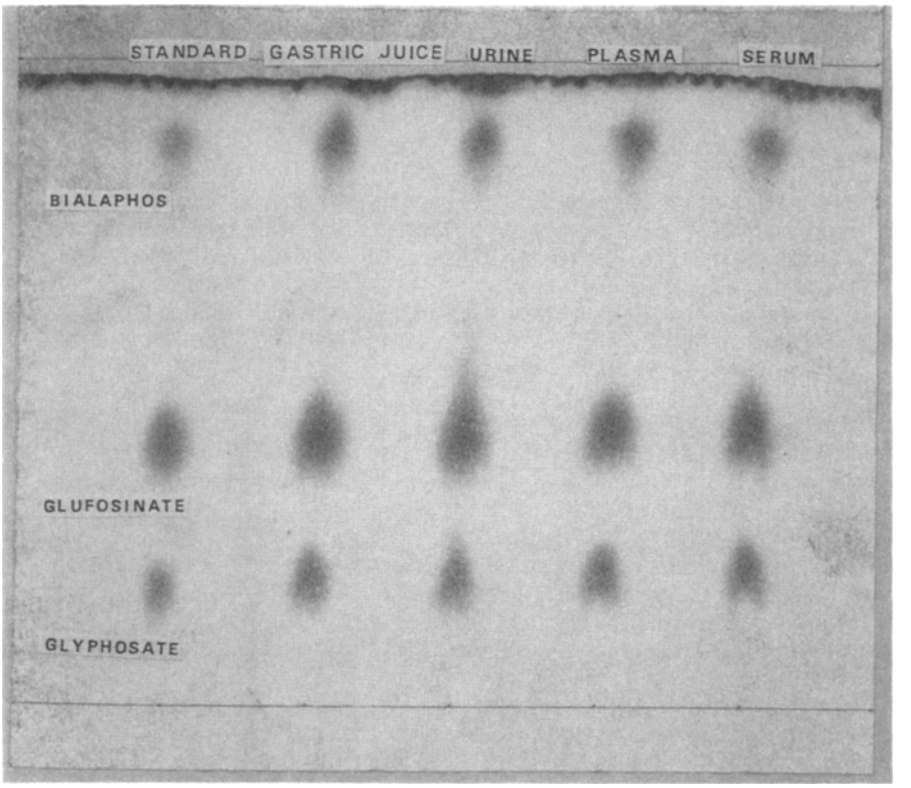


Figure 2. Identification of glufosinate, bialaphos and glyphosate in biologcial fluids

After drying, 1% ninhydrin aqueous solution was sprayed uniformly onto the filter paper by using an atomizer. The filter paper was put into the oven at 100 ℃ for 5 min for color development. Sites of the spots of the four samples were compared with that of glufosinate standard solution.

50 mg of analytical grade glufosinate was dissolved in deionized water. The solution volume was adjusted to 100 mL. The solution was further diluted with deionized water in order to prepare solutions of different concentrations of glufosinate. Aliquots of these solutions were added to plasma, serum, urine and gastric-juice. Using these samples, the identification limit for glufosinate was determined according to the method described for the identification of glufosinate.

Bialaphos (Tachibana et al. 1986) and glyphosate (Henriet et al. 1985) are compounds having a similar chemical structure to glufosinate. The above method was applied to distinguish glufosinate from the other two pesticides and to identify the three pesticides simultaneously. Equal amounts of 100 fold diluted solutions of bialaphos and glyphosate liquid formulations and the standard solution of glufosinate were mixed

to obtain the standard mixture. To 1 mL each of plasma, serum, urine and gastric-juice, 0.5 mL of the standard mixture was added. Further procedure was the same as described for the identification of glufosinate.

## RESULTS AND DISCUSSION

On the chromatogram (Figure 1), the spots of 5 $\mu$g of glufosinate contained in the plasma, serum, urine and gastric-juice were detected at the same height as that of glufosinate standard solution. The identification of glufosinate was easy, because there were no interfering spots derived from coextractives. The time required for the whole procedure was about 1 hr for urine and gastric-juice samples and about 1.5 hr for plasma and serum samples.

In all samples of serum, plasma, urine and gastric-juice, 2 $\mu$g of glufosinate was identified as the minimum detectable amount on the chromatogram after development when 50 $\mu$L of each sample ( 40 $\mu$g glufosinate / mL) was spotted.

In all samples of serum, plasma, urine and gastric-juice, glufosinate, bialaphos and glyphosate were distinguished from each other and identified simultaneously (Figure 2). Table 1 shows the Rf values of bialaphos, glufosinate and glyphosate in each of the biological fluids.

In summary, the method described above allowed identification of glufosinate with usually available equipments and simple procedures in a very short time. An amount of 2 $\mu$g of glufosinate was sufficient to be detected. The method also enabled us to distinguish glufosinate from bialaphos and glyphosate. Therefore, the method can be regarded as a useful method to identify water soluble glufosinate easily, selectively and quickly.

Table 1. Rf values of bialaphos, glufosinate and glyphosate contained in biological fluids

| | Bialaphos | Glufosinate | Glyphosate |
|---|---|---|---|
| Standard solution | 0.86 | 0.39 | 0.18 |
| Gastric-juice | 0.86 | 0.41 | 0.19 |
| Urine | 0 86 | 0.42 | 0.20 |
| Serum | 0 86 | 0.42 | 0.20 |
| Plasma | 0 86 | 0.42 | 0.20 |

Rf value of bialaphos : 0.86
Rf value of glufosinate: 0.39 - 0.42
Rf value of glyphosate : 0.18 - 0.20

Acknowledgments. The authors wish to thank Dr. M. Schwalbe-Fehl of Hoechst AG for her helpful advice and Dr. H. Stuebler of Hoechst Japan Limited for his support.

REFERENCES

Henriet J, Martijn A, Povlsen HH (1985) CIPAC Handbook 1C. Collaborative International Pesticides Analytical Council Limited, Hertfordshire, England

Tachibana K, Kaneko K (1986) Development of a new herbicide, Bialaphos Journal of Pesticide Science 11:297-304

Received September 15, 1988; accepted January 16, 1989.