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The gas-liquid chromatographic determination of 2-hydroxy-*s*-triazines*

In many biological systems, the 2-chloro-*s*-triazine herbicides give rise to the 2-hydroxy analogs as major metabolites. The latter compounds—which are also the products of photochemical decomposition[1]—are usually determined by TLC or spectrophotometry. Though gas-liquid chromatography has been employed successfully for the analysis of the parent triazines[2-7], their products of hydrolysis have not been amenable to this technique. The only report we found in the literature concerned the formation of TMS derivatives for identification purposes by MONTGOMERY *et al.*[8].

This study describes our search for a quantitative derivatization for several 2-hydroxy-*s*-triazines as well as the purification and analysis of soil and corn samples for Hydroxysimazine as a model compound

Derivatization

Several common silylation reagents were evaluated; of these, BSTFA [bis(trimethylsilyl)trifluoroacetamide)[9] gave the best results. When solvents such as acetonitrile or acetone were used, the reactions gave rise to three peaks. As may be expected, the fastest eluting peaks became more prominent with an increase in the temperature and/or an increase in the time of reaction. However, when BSTFA was used neat and at exactly controlled conditions, a single peak with very small amounts of side products was obtained for the 2-hydroxy analogs of Simazine, Atrazine, and Propazine. The trimethylsilylation was usually performed in a teflon-sealed, closed vial (Corning No. 2826) at 150° for 15 min, similar to a technique used by GEHRKE and co-workers[10]. Note: This reaction should always be carried out behind a safety shield with due caution.

Either these screw-cap vials or sealed capillaries can serve as a reaction vessel. As a sideline, the latter technique has been used to determine rather minute amounts of standards, as follows: A solution containing 2-hydroxy-*s*-triazine—and anthracene as an internal standard—was transferred into a pyrex capillary, the solvent evaporated, BSTFA added, the capillary sealed and kept at 150° for 15 min, then broken open and most of the reaction mixture injected into the gas chromatograph. One of the calibration curves obtained with the hydrogen flame detector is shown in Fig. 1; Hydroxyatrazine and Hydroxypropazine yield similar plots.

The trimethylsilylation of standards in screw-cap vials was conducted as described below in the analysis of a corn sample. Linear calibration curves were obtained again for all hydroxy-*s*-triazines mentioned.

Gas-liquid chromatography

Two columns have performed well with both the 2-chloro-*s*-triazine parent herbicides and the TMS derivatives of their 2-hydroxy analogs: 0.5% neopentylglycol

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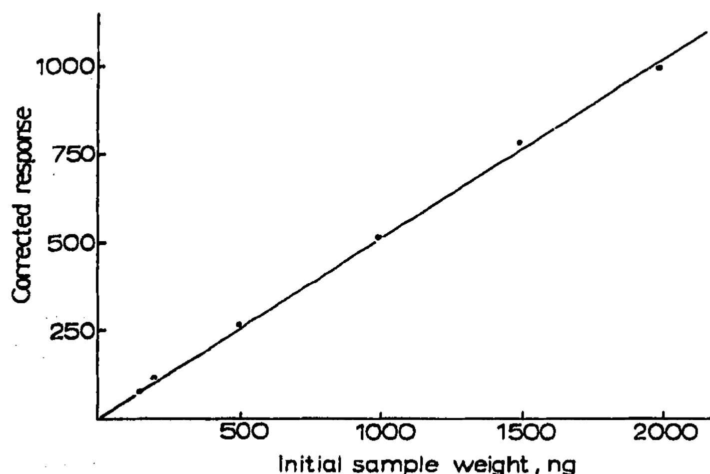

Fig. 1. Standard curve for Hydroxysimazine.

sebacate on 80/100 Chromosorb G-HP and 10% OV-17 on Chromosorb W-HP. Of these, the phenylmethylpolysiloxane OV-17 showed better stability and was used for all following experiments.

Analysis of corn samples

The following procedure uses modifications of the extraction method by PLAISTED AND THORNTON[11] and the purification steps reviewed by KNÜSLI[12]. Stem and leaves of a corn plant were frozen in liquid nitrogen and ground in a Waring blender. The material was spiked with various amounts of Hydroxysimazine, 1 g of each sample weighed into a flask, and 25 ml of methanol added. After ultrasonic stirring for 15 min, the liquid was drawn off with a pipet, the extraction repeated with fresh solvent, and the combined extracts taken to dryness on a rotary evaporator. The residue was redissolved in several (6–8 ml) portions of chloroform by ultrasonic stirring, and introduced into a dry, acidic alumina column (Brockman activity 1, Fisher Scientific Co.). Background material was eluted with more chloroform followed by 10 ml of 50% methanol in chloroform. Finally, the Hydroxysimazine was eluted with 25 ml of methanol and the collected fraction taken to dryness on the rotary evaporator. A column of the same size, 150 × 9 mm I.D., was packed with Silica Gel Grade 923, 100/200 mesh (Fisher Scientific Co.) in methanol, and the methanol eluted from the column with chloroform, thereby changing the appearance of the column from opaque white to colorless translucent. After the elution of the methanol and back-flushing with chloroform to remove residual air bubbles, the residue was introduced in the same manner as described for the alumina column. Some more interfering co-extractants were removed by chloroform and 5 ml of 5% methanol in chloroform; then the Hydroxysimazine was eluted with 10 ml of 25% methanol in chloroform and collected in a culture tube while the solvent was evaporated simultaneously by a stream of nitrogen. After evaporating to dryness, 0.1 ml of BSTFA was added, the tube sealed and placed in a 150° oil bath for 15 min, removed, and allowed to cool. The reaction products were chromatographed at 190° on a 6 ft. × 3 mm I.D. pyrex U-tube filled with 10% OV-17 on 80/100 mesh Chromosorb W-HP, and detected by flame ionization.

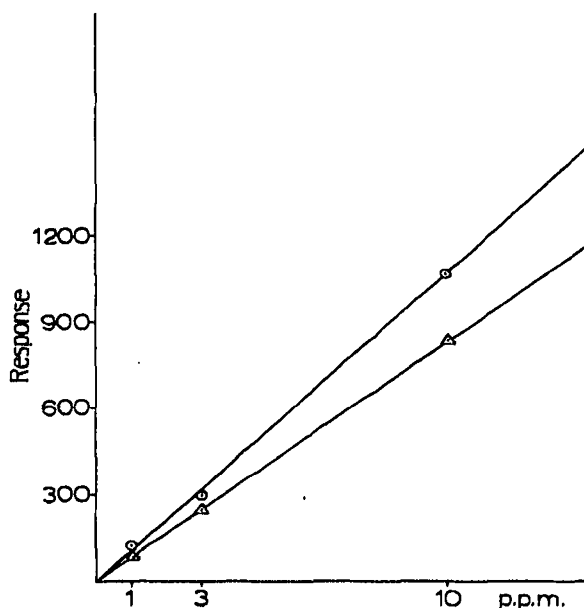

Fig. 2. Comparison of a standard curve of 2-Hydroxyatrazine to the recovery of the standard from corn. ⊙ = standard (single injection), △ = sample (average of three injections).

Analysis of soil samples

Menfro silt loam, a soil of medium organic content, was spiked with 1, 3, and 10 p.p.m. of Hydroxyatrazine. The soil samples were extracted with a mixture of conc. ammonium hydroxide–water–acetonitrile (4:10:86). The extract was taken to dryness on a rotary evaporator and processed as described above.

Results and discussion

The linear calibration curves which had been obtained for standards, promised a quantitative and sensitive method for the GLC determination of 2-hydroxy-s-

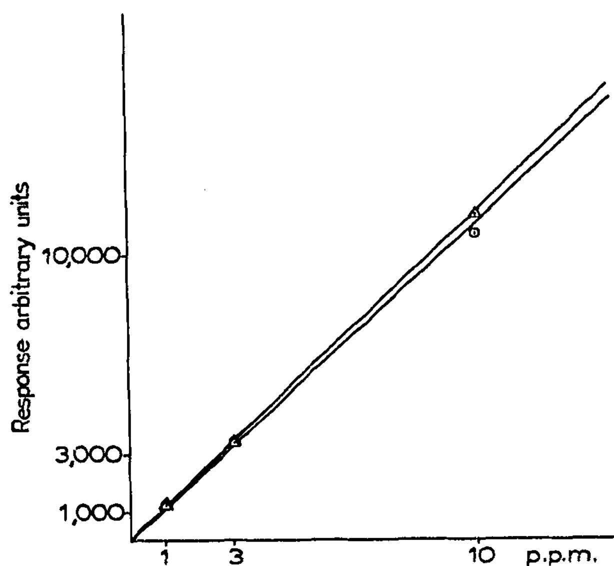

Fig. 3. Comparison of a standard of 2-Hydroxyatrazine to the recovery of the standard from Menfro silt loam. △ = standard (single injection), ⊙ = sample (average of three injections).

triazines as their trimethylsilyl derivatives. The determination of Hydroxyatrazine in spiked corn and soil samples, however, showed a minimum detectable level of not better than 1 p.p.m. due to the interference from co-extractants. Many components in the sample are apparently derivatized by BSTFA and the double column chromatographic cleanup does not remove them completely. Furthermore, the purification procedure is severely limited by the general low solubility of the hydroxytriazines. Fig. 2 shows a comparison of a standard curve to the recovery of the standard from corn and Fig. 3 shows the same for Menfro silt loam. In both figures, the sample data are averages of at least three chromatographic runs.

The 1 p.p.m. minimum detectable limit disqualifies the developed procedures for most residue analysis problems. They can be valuable, though, for certain metabolism studies, for confirmation of results obtained by other methods, and the like. If improved purification methods can be developed for biological extracts, the GLC determination of 2-hydroxy-*s*-triazines as trimethylsilyl derivatives should prove a significant improvement over existing methods.

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