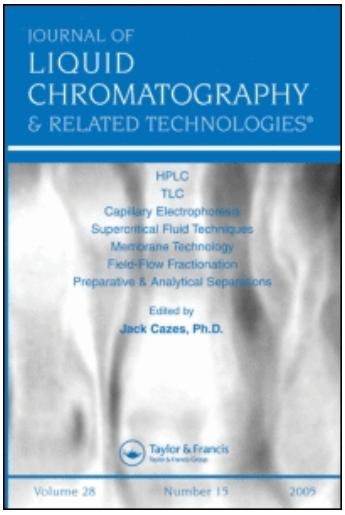

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QUANTITATION OF THE TRIAZINE HERBICIDES ATRAZINE AND SIMAZINE IN WATER BY THIN LAYER CHROMATOGRAPHY WITH DENSITOMETRY

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ABSTRACT

A TLC-densitometric method for determination of the triazine herbicides atrazine and simazine in natural and tap water is described. Separation was carried out on silica gel G, followed by detection with silver nitrate and UV exposure. Alumina column cleanup was required for tap water. Recovery of samples fortified at 10 ppb was >80%, and the relative standard deviation was better than 7.5%.

INTRODUCTION

One of the earliest applications of TLC-densitometry to pesticides was the analysis of triazine herbicides detected by fluorescence quenching. However, the optimum sensitivity for quantitation was reported to be as high as 6 μg per spot, and only standards rather than real samples were studied (1). The TLC and HPTLC of 14 triazines were later investigated, but again noncharacteristic

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fluorescence quench detection was employed, and no real samples were analyzed (2). The purpose of this paper is to report a thin layer densitometric procedure for residues of the important triazine herbicides atrazine and simazine in water based on detection with Cl-selective silver nitrate detection reagent.

EXPERIMENTAL

s-Triazine herbicide standards were obtained from the Agricultural Division of Ciba-Geigy Corporation, Greensboro, NC: atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine); simazine [2-chloro-4,6-bis-(ethylamino)-s-triazine]; and the nonchlorinated compounds dipropetryne, prometon, and ametryn.

Stock solutions (1.0 mg/ml) were prepared in ethyl acetate for atrazine, chloroform for simazine, and acetone for the other compounds. Quantitative 1:10 dilution with the respective solvents gave 100 ng/µl standard solutions for spotting with a Drummond Dialamatic micropipet (1-10 µl variable volume) or Drummond microcap pipets. Application of larger volumes was made by repeated spotting of 1 or 2 µl volumes with drying in between. All sample and standard initial zones were kept as small and uniform as possible. Pesticide grade solvents were used throughout this study. All pesticide solutions were refrigerated when not in use and were stable for at least one month.

The detection reagent was prepared by mixing, immediately before use, 216 ml of silver nitrate stock solution (17 g $AgNO_3$ dissolved in 25 ml of distilled water, made up to one liter with acetone, stored in a brown glass bottle in the dark, and made fresh each week), 20 ml of distilled water, and 14 ml of 6 M ammonium hydroxide. Plates were preimpregnated by dipping for

several seconds into the reagent contained in a Thomas-Mitchell dip tank. The plate was air dried in a dark cabinet, and the dip tank was rinsed immediately to prevent damage from silver nitrate.

Whatman K-4 silica gel G plates, 20 x 20 cm, were predeveloped with chloroform-methanol (1:1 v/v) and thoroughly air dried before impregnation with $AgNO_3$.

Spotted, preimpregnated plates were developed for a distance of 10 cm in an unsaturated rectangular glass TLC tank inside a dark cabinet to preclude darkening of the layer. The mobile phase was chloroform-acetone (9:1 v/v).

The chromatogram was air dried in a dark cabinet and then exposed to UV light from a Hanovia 679A 176W germicidal lamp for 10 minutes. The lamp required 30 minutes for warmup and was operated at 4 amps. The layer was placed 24 cm below the lamp for uniform irradiation, and the entire assembly was kept in a hood to exhaust ozone. Black spots of atrazine and simazine began to form after irradiation for about 4 minutes and reached maximum contrast with a very light grey background in about 10 minutes.

The spots were scanned in the direction of solvent development with a Kontes Chromaflex fiber optics densitometer equipped with a baseline corrector. The visible wavelengths emitted by the longwave UV source were employed for the double beam measurement. Operating parameters were scan speed 6 cm/minute; recorder settings 250 inches/hour and 0.025 V; baseline corrector settings 10 mV input - 4 drift - 4 suppression - 20 mV output. See reference (3) and the manufacturer's manual for details of operation.

Recorder chart peaks were photocopied, and the copies were cut out and weighed. Calibration curves were plotted and peak weight in grams times attenuation setting versus micrograms of herbicide spotted.

A 500 ml natural water spiked with 10 ppb (1 µg/100 ml) atrazine sample was extracted three times by shaking 3 minutes with 25 ml portions of chloroform in a 1-liter separatory funnel. The extracts were combined, dried by filtering through Whatman phase separating paper, and evaporated to near dryness in a 125 ml Kuderna-Danish concentrator (Kontes) fitted with a 1 ml concentrator tube calibrated to 0.1 ml units (Kontes). This assembly was connected to a vacuum rotary evaporator (Brinkmann-Buchi Rotavapor-R), and evaporation was assisted with a warm air gun. The concentrator flask and joint were rinsed with chloroform, and the solution in the tube was evaporated just to dryness under nitrogen gas. The residue was dissolved in 100 µl of chloroform, and a 10 µl aliquot was spotted (equivalent to 500 ng if recovery was 100%). Three or four bracketing standards were spotted on the same $AgNO_3$ preimpregnated plate. The amount of pesticide in the sample was interpolated from the calibration curve constructed from the peak areas of the standard spots, and percentage recovery was calculated by taking into consideration the aliquot of the spiked sample spotted.

Easton, PA, tap water required an alumina column (4) cleanup step for successful analysis of simazine added at a fortification level of 10 ppb. The extraction was carried out as above to the point of evaporation of the combined extracts to dryness. The residue was dissolved in 10 ml of carbon tetrachloride and transferred to a 18 mm i.d. x 200 mm glass column dry packed with 25 g of aluminum oxide activity V (330 g of Woelm grade I super, W 200 basic plus 70 ml of water are mixed and allowed to stand overnight in a closed bottle). The sample tube was rinsed with an additional 10 ml of CCl_4, which was also transferred to the column.

When the sample and wash just entered the column, elution was carried out with 80 ml of CCl_4 (discard) and 125 ml of CCl_4-ethyl ether (95:5 v/v). One hundred ml is used to elute atrazine. The latter eluate fraction, containing the simazine (or atrazine) was evaporated just to dryness in a Kuderna-Danish apparatus attached to a rotary evaporator (5), the residue in the concentrator tube was dissolved in 100 µl of $CHCl_3$, and 10 µl was spotted for TLC.

RESULTS AND DISCUSSION

The detection system described was chosen after testing many different formulations of silver nitrate, applied by predipping, postdipping, and spraying. Reagents composed of acidic potassium permanganate plus o-tolidine (6); brilliant green followed by exposure to bromine vapor (7); bromocresol green (8); and 4-(p-nitrobenzyl)pyridine followed by tetraethylenepentamine (9) were also evaluated and found to be inferior in terms of sensitivity, stability of spots, and/or reproducibility of detection compared to silver nitrate. The sensitivity of the recommended silver nitrate detection method for practical quantitation by densitometry was 100 ng, although amounts below this were often visually detectable. The presence of phenoxyethanol in the $AgNO_3$ reagent caused darkening of the upper half of the chromatogram.

The Kontes automatic applicator designed by Getz (10) was unsuitable for spotting solutions because the large volume of solution (1 ml) disturbed the $AgNO_3$ impregnation and caused streaking of the spots. Manual application was made instead with a Drummond Dialamatic microdispenser or microcap disposable capillary pipets, which have been shown to have the highest precision for manual sample application (11).

Whatman K4 silica gel G provided the best contrast between the dark spots and the plate background. Analtech silica gel G gave similar results, but the spot-background contrast was not consistently as high. Whatman linear K5 and reversed phase layers both gave brown-black backgrounds with the $AgNO_3$ detection system and were not useable.

The R_F values of the oval-shaped atrazine and simazine spots in chloroform-acetone (9:1 v/v) were 0.64 and 0.49, respectively. This solvent was chosen for the densitometric quantitation of the individual compounds because of the tight zones produced after development and the ideal location (12) near the center of the plate. The R_F values were affected by the amount of NH_4OH in the $AgNO_3$ dipping solution, more base leading to higher R_F values. The difference between R_F values was practically identical for all proportions of ammonia tested, however. If both herbicides are present in the same extract and greater resolution between them is required, toluene-acetone (85:15 v/v) in a saturated chamber provides respective R_F values of 0.68 and 0.58 for atrazine and simazine (13), while carbon tetrachloride-nitromethane (1:1 v/v) gives 0.60 and 0.42, respectively (7). The other triazines tested did not interfere with the determination of atrazine and simazine because, being nonchlorinated, they did not give a dark spot with the $AgNO_3$ detection system.

The single beam, reference head mode with the long-wave ultraviolet source provides the most sensitive determination (greatest peak area per μg of compound) employing the Kontes densitometer. This mode can only be used, however, if all sample and standard spots are no wider than the 5 mm length of the reference head beam. Since this was not always the case, double beam scan-

ning with the larger head was employed for greatest accuracy and precision with adequate sensitivity.

A time study was performed by scanning one developed and detected 500 ng zone of atrazine every 10 minutes for one hour. No significant change in peak area occurred over this period. Chromatograms were thereafter routinely scanned within 5 to 10 minutes of the appearance of maximum spot-background contrast under the germicidal lamp.

The reproducibility of the determination was tested by spotting six 500 ng spots of atrazine with the Dialamatic dispenser on a single plate, followed by development, visualization, and scanning. The relative standard deviation of the peak areas was a very acceptable 4.5%.

A typical calibration curve for atrazine is illustrated in Figure 1. Computer analysis of the data points

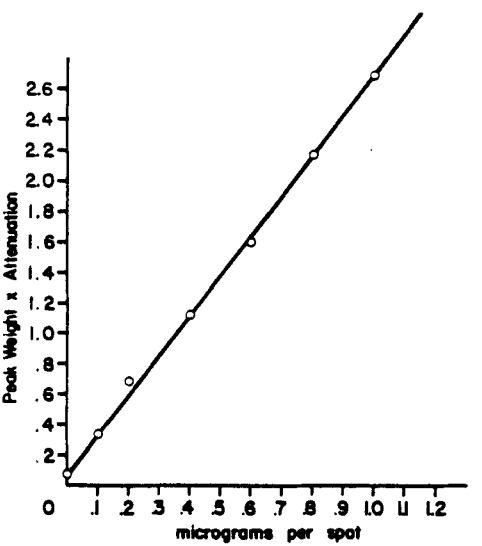

Figure 1. Calibration curve for 100-1000 ng spots of atrazine scanned in the double beam, long UV mode of the Kontes densitometer.

gave a slope of 2.63, y-intercept 0.064, and linearity index 0.995. The calibration curve for simazine was similar, and curves for both compounds were quite consistent from plate to plate. However, three standards were applied along with actual samples on each plate to definitely establish the calibration curve to be used for each determination.

To demonstrate the efficacy of the proposed analytical method, natural water fortified to 10 ppb with atrazine and tap water containing the same level of simazine were analyzed as described in the Experimental Section. Quadruplicate analyses of the same natural water sample (no cleanup) gave an average recovery of atrazine of 86% and a relative standard deviation of 5.3%. Figure 2 shows typical scans of a natural water extract and bracketing standards. Quadruplicate analyses of the tap water sample (alumina column cleanup) gave an average

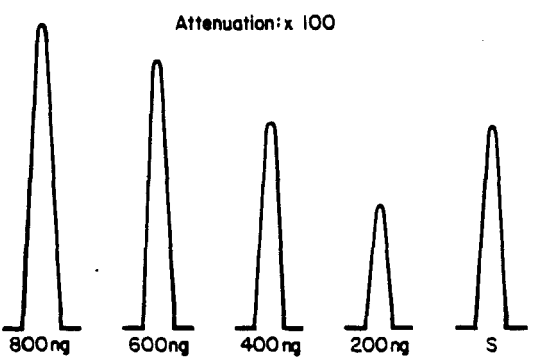

Figure 2. Densitometer scans of 200, 400, 600, and 800 ng standards and a sample (S) from a single chromatographic plate. The weight of S read from the constructed calibration curve corresponded to 82.0% of the theoretical amount in the spotted aliquot of the fortified sample extract.

recovery of simazine of 83% and an RSD of 7.5%. Both of these accuracy and precision results are within generally acceptable levels for residue analysis at the ppb level. The alumina column cleanup procedure allows complete (> 98%, as proven by TLC analysis of column fractions) recovery of both atrazine and simazine. The cleanup on the column would undoubtedly be adequate for sample matrices other than tap water, e.g., crop extracts and soil (4). Substrate blanks analyzed in parallel with the spiked water samples proved that no extraneous zones were present near enough to the location of the herbicides to interfere with densitometric evaluation.

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