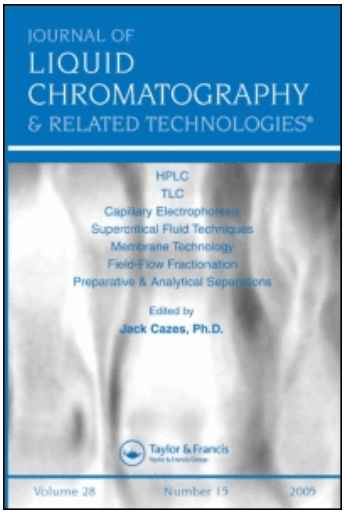

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Sherma, Joseph and Slobodien, Robert(1984) 'Determination of Chlorpyrifos and its Metabolite 3,5,6-Trichloro-2-Pyridinol in Tap Water and Bananas by Quantitative TLC on Preadsorbent Silica Gel', Journal of Liquid Chromatography & Related Technologies, 7: 14, 2735 — 2742
To link to this Article: DOI: 10.1080/01483918408067041
URL: http://dx.doi.org/10.1080/01483918408067041

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(14), 2735-2742 (1984)

DETERMINATION OF CHLORPYRIFOS AND ITS METABOLITE 3,5,6-TRICHLORO-2-PYRIDINOL IN TAP WATER AND BANANAS BY QUANTITATIVE TLC ON PREADSORBENT SILICA GEL

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ABSTRACT

Chlorpyrifos insecticide and its metabolite TCP were determined in tap water and banana samples by TLC of extracts on preadsorbent silica gel layers, detection with silver nitrate reagent, and densitometric scanning. Cleanup steps were required for the fruit sample extracts. Recovery of chlorpyrifos from tap water at 5 ppb averaged 87.5% and from banana at 0.05 ppm was 84.6%. Recovery of TCP from water at 5 ppb averaged 84.0% and from banana at 0.05 ppm was 86.8%. The sensitivity and precision of the method were shown to be adequate for routine residue analysis.

INTRODUCTION

Chlorpyrifos (Dursban) [0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl)-phosphorothioate] is an organophosphate pesticide that is used for the control of many insect pests in rice, vegetable and fruit crops, turf and ornamental plants, and animals (1, 2). Chlorpyrifos has been determined in bananas, peaches, and cottonseed by gas chromatography with a phosphorus selective flame photometric detector and in meat, milk, and lima and snap beans by electron-capture GC (2). The metabolite of chlorpyrifos (3,5,6-trichloro-2-pyridinol; TCP) was determined in bananas and meat by electron-capture GC as the trimethylsilyl derivative (2). HPLC on a cyano-bonded column was used to

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determine chlorpyrifos in polymeric pellet formulations, and a reversed phase ODS column was employed for the analysis of natural water after trace enrichment on a C_{18} Sep Pak cartridge (3).

Thin layer chromatography coupled with densitometric scanning has recognized advantages of high sample throughput, simplicity, versatility, and selectivity for the quantitative analysis of a wide variety of samples (4), including pesticide residues (5-7). With preadsorbent plates, sample application can be carried out rapidly, and the spotting area automatically produces sharp, narrow bar- or streak-shaped zones of constant size, even though different sample volumes are used. Accurate, precise, and sensitive densitometry requires that initial zones of samples and standards have small, uniform dimensions (8).

No studies on the TLC of chlorpyrifos have been reported. Described below are quantitative TLC determinations of this insecticide and its metabolite in tap water at 5 ppb and in banana pulp at 0.05 ppm. Preadsorbent silica gel layers are used for resolution and silver nitrate chromogenic reagent for detection prior to reflectance scanning. Water extracts were analyzed without clean-up while banana extracts required purification by solvent partitioning and column chromatography.

EXPERIMENTAL

Standard chlorpyrifos and 3,5,6-trichloro-2-pyridinol (TCP) were obtained from the Dow Chemical Co. (Midland, MI). Stock standard solutions were prepared in acetone at a concentration of 0.500 g/100 ml and diluted 1.00:100 to give solutions of 50.0 ng/µl.

Analyses were carried out on channeled 20 x 20 cm Analtech Uniplates containing a 48 mm high preadsorbent spotting area along the bottom below the analytical layer of silica gel G. The layer was scored into 19 channels 0.9 cm in width. The plates were prewashed with methylene chloride-methanol (1:1) and dried before use. The principles and practice of preadsorbent TLC were described in a recent manual (9).

Volumes of standards between 1.00 and 20.0 µl (100-1000 ng) were applied by spotting the preadsorbent areas of the lanes using a 25 µl Drummond Dialamatic microdispenser. Application was confined to an area 3 mm below the layer interface and 5 mm above the bottom edge of the plate. The spots were allowed to air dry completely after application.

Plates were developed for a distance of 10 cm beyond the layer interface in a paper lined, saturated (10 min.) rectangular glass TLC chamber with hexane-chloroform (80:20) for chlorpyrifos or hexane-acetone-methanol-glacial acetic acid (60:30:10:0.2) for TCP. Chromatograms were air dried in a fume hood and then dipped for 20 seconds in silver nitrate detection reagent prepared from 8 ml of $AgNO_3$ stock solution (20 g/100 ml water) + 200 ml acetone + 20 ml H_2O + 12 ml concentrated ammonium hydroxide. The NH_4OH is added last with stirring to avoid cloudiness. The dipped plate was dried in a dark hood for 30 minutes and then irradiated for 20-30 minutes by a Hanovia 679A 176W germicidal lamp (UV source) placed 40 cm above the layer and set at 4 amps.

Zones were scanned with a Kontes Model 800 fiber optics scanner in the single beam transmission mode using the B head (8 mm light beam length) and the white phosphor (440 nm peak, 300 nm band width). Peaks were drawn and areas reported by a Hewlett-Packard Model 3390A recorder/integrator coupled to the scanner. All zones were scanned twice, and calibration curves were calculated from the average peak areas by a linear regression program run on a Commodore 64 minicomputer.

Actual analyses were demonstrated using fortified pesticide-free tap water and banana samples. The 50.0 µg/ml standard solutions of chlorpyrifos and TCP were diluted 1.00:10.0 to prepare spiking solutions of 5.00 µg/ml. One ml of chlorpyrifos spiking solution was added to 1.00 liter of tap water, which was shaken vigorously for 5 minutes. The resultant sample contained 5.00 ppb of the pesticide. The water sample was placed into a 2 liter separatory funnel and extracted with 50 ml of hexane. The (lower) water phase was drained into a second separatory funnel, and the hexane layer was dried by

passage through Whatman phase separation (PS) paper and collected in a 250 ml beaker. The extraction of the water was repeated twice and the hexane extracts combined in the beaker. The hexane was evaporated to 3-5 ml under a stream of nitrogen gas on a warm hot-plate, transferred quantitatively by rinsing with hexane to a 12 ml calibrated centrifuge tube, and evaporated to 0.500 ml. Thirty µl of extract (representing 300 ng if recovery is 100%) was spotted along with bracketing standards (100-1000 ng) and an extract from blank (unfortified) water on the same plate. After development, detection, and scanning, the amount of pesticide in the sample was calculated by interpolation of the average of the sample zone areas, minus the blank area, if any, from the calibration curve using a Commodore 64 computer program. The recovery was calculated by comparison to the theoretical 300 ng amount. The spiking and extraction of TCP were performed in the same way except that 2 ml of conc. HCl and 170 g of benzene-washed NaCl were added to the 1 liter water sample, and three 50 ml portions of benzene were used in place of hexane.

To demonstrate use of the TLC method with a sample requiring cleanup, banana pulp was spiked at 0.05 ppm and analyzed by the procedure described as chlorpyrifos Method I in Volume II of the FDA Pesticide Analytical Manual (2). The fortified sample (10.0 g) was extracted in a blender with acetone, the extract filtered, and the filtrate evaporated. The residue was partitioned into hexane and cleaned up by elution with hexane through a 15% water deactivated silica gel column. The eluate was further purified by elution with acetonitrile-benzene (1:1) through a charcoal-MgO-Celite (1:2:4 w/w) column, as described in the FDA PAM, Volume I (10). The eluate was evaporated to dryness, taken up in 50.0 µl of acetone, and a 30.0 µl aliquot was spotted with bracketing standards for TLC. The theoretical value was 300 ng for 100% recovery. Banana pulp spiked with TCP at 0.05 ppm (10.0 g) was analyzed by chlorpyrifos Method III in Volume II of the FDA PAM (2). The fortified sample was extracted with methanol and the extract chromatographed on an acidic alumina column (activated

at 130° C with conc. HCl-H_2O (40:60) as eluent. Eluted TCP was partitioned into benzene, followed by sodium bicarbonate partioning, acidification with HCl, and partitioning into benzene. The solution was concentrated to 50.0 µl and a 30.0 µl aliquot was analyzed by TLC. Again, the theoretical value was 300 ng.

RESULTS AND DISCUSSION

The R_F value of chlorpyrifos was 0.58 using hexane-chloroform (80:20) in a saturated chamber. TCP had an R_F of 0.24 in this mobile phase, which was below the optimum range of 0.3-0.7 recommended for accurate and precise densitometric quantification (9). Therefore, hexane-acetone-methanol-glacial acetic acid (60:30:10:0.2), which gave an R_F of 0.52 for TCP and 0.90 for chlorpyrifos, was used for determination of TCP. The zones were in the form of sharp, narrow streaks across the lanes with both mobile phases. The concentration of acetic acid was very critical in the TCP mobile phase and may require variation between 0.1 and 0.3 ml with different layers and temperature/humidity conditions to obtain an R_F value in the 0.3-0.7 range.

Silica gel G was chosen because polymer bound layers gave a dark background when sprayed with $AgNO_3$ detection reagent and exposed to UV light (11). The pesticide and metabolite were detected as dark brown zones on a white or yellow-white background. Zones should be scanned immediately after detection, if possible, but plates could be stored up to 2 hours in the absence of light without significant darkening of the background. The maximum contrast between the zones and the background was obtained after an irradiation period of 20-30 minutes. The sensitivity limits were approximately 100 ng for chlorpyrifos and 25-50 ng for TCP. Other reagents for detection of phosphate pesticides (TCQ + $MgCl_2$), phenols (diazotized p-nitroaniline, diazotized sulfanilic acid, potassium ferricyanide), and organochlorines (o-tolidine) either gave no reaction or less satisfactory sensitivity or contrast between the zones and background with the compounds.

FIGURE 1. Typical densitometer scans of 200 ng (A) and 400 ng (C) standard chlorpyrifos zones and fortified tap water extract (B) chromatographed on the same layer. The sample represented 86.7% recovery (300 ng theoretical) when its area was interpolated from the calibration curve calculated from all standards chromatographed in parallel with the sample. An attenuation setting of X5 was used.

Calibration curves between 100 and 1000 ng for chlorpyrifos and 50 and 1000 ng for TCP typically had linearity (R) values of > 0.99. To correct for variations in slope and intercept values, bracketing standards always were chromatographed on the same plate with samples. Duplicate scan areas of a given zone usually agreed within 1%. Precision of the TLC was tested by developing, detecting, and scanning eight 500 ng samples of TCP applied to adjacent lanes on a single plate. The coefficient of variation (RSD) of the peak areas was 4.0%, which is acceptable reproducibility for densitometry in trace analysis.

Recoveries from tap water using the procedures described above were 77.8%, 91.3%, 94.0%, and 86.7% for four separate samples fortified with 5.00 ppb of chlorpyrifos. Figure 1 illustrates a typical scan of 200 and 400 ng standard zones and the sample zone from the latter analysis. Recoveries of TCP from tap water fortified at the same concentration were 87.2% and 80.8% for two samples. Figure 2 shows a scan of a 300 ng standard TCP zone and an extract zone with a theoretical value of 300 ng. A small zone usually appeared in the blank at the R_F value of TCP, the area of which was subtracted from the sample area before calculation of the percentage recovery.

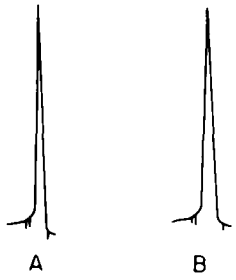

FIGURE 2. Densitometer scans of 300 ng TCP standard (A) and fortified tap water extract (B) zones representing 80.8% recovery (300 ng theoretical) at attenuation X7.

Since the combined selectivity of the TLC separation and the detection reagent allowed the tap water samples to be analyzed without cleanup, banana pulp was chosen to demonstrate application of the method to a more complex sample. The extraction and cleanup procedures described in detail in the FDA PAM (2) and outlined above for determination by FPD-GC were used with an additional carbon column cleanup step (11). No extraneous zones that interfered with scanning of the chlorpyrifos zone were present on the chromatogram of the final acetone solution. Recovery from a sample fortified at 0.05 ppm was 84.6%. The methanol extraction and alumina column and solvent partition cleanup procedures described for the electron capture GC determination ot TCP (2) provided a sample of adequate purity for TLC quantification. Recovery of a sample fortified at 0.05 ppm was 86.8%. Blank values were minimal in both cases. These accuracy (recovery) values obtained for water and banana are well within the range considered acceptable for residue analysis at the low ppb and ppm level, and the recoveries from banana samples compared favorably with those reported by the RPA (2) for determination by GC.

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