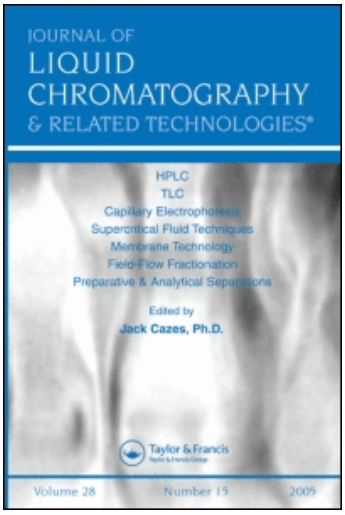

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

THIN LAYER CHROMATOGRAPHY ANALYSIS OF DIALKYL PHOSPHATE DEGRADATION PRODUCTS OF ORGANOPHOSPHATE PESTICIDES ON C-18 CHEMICALLY BONDED PLATES

Joseph Sherma[a]; Hasan D. Harnett[a]; Anant V. Jain[b]
[a] Department of Chemistry, Lafayette College, Easton, PA, U.S.A. [b] Athens Diagnostic Laboratory, College of Veterinary Medicine, University of Georgia, Athens, GA, U.S.A.

Online publication date: 13 January 2005

To cite this Article Sherma, Joseph , Harnett, Hasan D. and Jain, Anant V.(1999) 'THIN LAYER CHROMATOGRAPHY ANALYSIS OF DIALKYL PHOSPHATE DEGRADATION PRODUCTS OF ORGANOPHOSPHATE PESTICIDES ON C-18 CHEMICALLY BONDED PLATES', Journal of Liquid Chromatography & Related Technologies, 22: 1, 137 — 141
To link to this Article: DOI: 10.1081/JLC-100101649
URL: http://dx.doi.org/10.1081/JLC-100101649

J. LIQ. CHROM. & REL. TECHNOL., 22(1), 137–141 (1999)

THIN LAYER CHROMATOGRAPHY ANALYSIS OF DIALKYL PHOSPHATE DEGRADATION PRODUCTS OF ORGANOPHOSPHATE PESTICIDES ON C-18 CHEMICALLY BONDED PLATES

Joseph Sherma,[1] Hasan D. Harnett,[1] Anant V. Jain[2]

[1] Department of Chemistry
Lafayette College
Easton, PA 18042, USA

[2] Athens Diagnostic Laboratory
College of Veterinary Medicine
University of Georgia
Athens, GA 30602, USA

ABSTRACT

Methods are described for the separation, detection, and quantification of the thio (PS-containing) dialkyl phosphates O,O-dimethyl phosphorothionate (DMTP), O,O-dimethyl phosphorodithioate (DMDTP), O,O-diethyl phosphorothionate (DETP), and O,O-diethyl phosphorodithioate (DEDTP) by thin layer chromatography. The compounds were separated on C_{18} chemically bonded silica gel plates and detected with TCQ chromogenic reagent; calibration curves were prepared by densitometric scanning of a range of standard aliquots.

The procedures can be used for qualitative confirmation of metabolite identity and for quantification of the compounds in samples directly spotted or prepared by existing procedures. To our knowledge, this is the first publication reporting TLC systems useful for the analysis of these thio alkyl degradation products.

INTRODUCTION

Dialkyl phosphates are degradation products of organophosphate (OP) pesticides. Their determination in urine can provide an assessment of mammalian exposure to the parent pesticides, and analysis of environmental samples can locate contamination by the pesticides. The usual method of analysis of dialkyl phosphates involves gas chromatography, utilizing a phosphorus-specific flame photometric detector after derivatization with pentafluorobenzyl bromide to form the corresponding esters.[1] A qualitative thin layer chromatography (TLC) method using silica gel layers and 4-p-nitrobenzylpyridine/tetraethylpentamine and o-tolidine detection reagents was reported[2] for determination of OP pesticides and O,O-dimethyl phosphate, which contain a P=O group, in urine, beverages, and environmental water. No publications have been found describing the TLC of dialkyl phosphates with P=S groups. This paper reports the results of studies on the separation and selective detection of the four most important dialkyl phosphates containing P=S groups (DMTP, DMDTP, DETP, DEDTP) and demonstrates the potential of TLC for quantification of the compounds by scanning densitometry.

EXPERIMENTAL

Preparation of Standards

The following dialkyl phosphate compounds were supplied by the EPA Pesticide Repository (Research Triangle Park, NC): O,O-dimethyl phosphate (DMP); O,O-diethyl phosphate (DEP); O,O-dimethyl phosphorothionate (DMTP); O,O-dimethyl phosphorodithioate (DMDTP); O,O-diethyl phosphorothionate (DETP); and O,O-diethyl phosphorodithioate (DEDTP). DMP and DEP were received as barium salts and the other standards as potassium salts. Individual stock standard solutions at 1000 ppm were prepared by dissolving appropriate weights in methanol-acetonitrile (1:1) for DMP and DEP and in acetonitrile for the others. TLC standards, 100 ppm (100 ng/μL), were prepared by dilution of the stock standards with acetonitrile.

Thin Layer Chromatography

TLC was performed on 20 x 10 cm Whatman (Clifton, NJ) LKC18D channeled, pre-adsorbent chemically bonded silica gel reversed phase plates that were pre-developed with dichloromethane-methanol (1:1). Standard and sample solutions were applied in 2.50-10.0 μL aliquots on the pre-adsorbent areas of adjacent lanes using a Drummond (Broomall, PA) 10 μL digital microdispenser The initial zones were dried with warm air from a hair dryer prior to insertion into the tank. Ascending development was carried out for a distance of 16 cm beyond the pre-adsorbent-silica gel interface in a paper-lined Camag (Wilmington, NC) twin-trough tank using methanol-0.50 M NaCl (48:52) or tetrahydrofuran (THF)-0.50 M NaCl (40:60) as the mobile phase. Development required ca. 30 min.

After development, the plate was dried with a hair dryer, and zones were detected by dipping into a 5% methanolic solution of magnesium chloride, air drying, dipping into a hexane solution of 0.3% N,2,6-trichloro-benzoquinoneimine (TCQ), and heating in an oven at 110°C until maximum contrast between the pink spots produced by the thio (PS-containing) compounds and the white plate background was achieved (10-15 min).[3]

For determination of calibration curves, the standard zones were scanned at 440 nm with a Shimadzu (Columbia, MD) Model 930 densitometer in the single beam-single wavelength visible reflectance mode using a slit size of 6 mm (height) x 0.4 mm (width). This wavelength was used earlier for solid phase extraction-TLC densitometric quantification of OP pesticides in water on silica gel plates after detection with TCQ reagent.[3] The calibration curve was calculated from the zone areas and weights (ng) using a PC linear regression program.

RESULTS AND DISCUSSION

A large number of two- and three-component mobile phases were tested for the separation and detection of the DEDTP, DETP, DMDTP, and DMTP on silica gel and chemically bonded C_{18} reversed phase layers. No solvent system was found to separate the compounds on silica gel, but development on C_{18} layers with methanol-0.50 M NaCl (48:52) provided separation with respective R_f values of 0.59, 0.68, 0.87, and 0.93.

The separation of DMDTP and DMTP was improved by development with THF-0.50 M NaCl (40:60); respective R_f values with this mobile phase were 0.65, 0.85, 0.85, and 0.95.

TCQ detected the four separated thio alkyl phosphates as compact pink zones with a detection limit of 200 ng. Other chromogenic reagents studied included 0.01% Rhodamine B, iodine vapor, 0.5% palladium chloride, palladium chloride plus iodine, and 4-(p-nitrobenzyl)pyridine-tetraethylene-pentamine, but none of these produced zones and were as sensitive or stable as TCQ. DEP or DMP were not detected by any of these reagents. The described TLC method, based on the use of C_{18} layers and TCQ reagent, is, therefore, selective for compounds containing P=S groups in the presence of P=O compounds. OP pesticides with P=S groups, which would also be detected by the TCQ reagent,[3] are less polar than the phosphate metabolites and would be located at the bottom of the reversed phase layer.

To demonstrate the potential of the system for quantitative analysis, calibration curves between 250 and 1000 ng were determined for DETP and DEDTP using the methanol-NaCl mobile phase and DMDTP with THF-NaCl mobile phase. The curve for each compound had unique slope and intercept values that were quite consistent from plate to plate and linearity correlation coefficients of 0.98 or higher. For quantitative analysis of samples, standards would be chromatographed on each plate with samples to correct for variations in slope and intercept values of the calibration plots.

The described TLC system would be useful for separation, identification, and quantification of the analytes in any sample that is sufficiently pure and concentrated to produce discrete, regular zones within the calibration range specified. This could include samples that can be directly spotted on the layer, such as was reported earlier for determination of amino acids in blood and urine;[4] p-aminobenzoic acid in urine;[5] cholesterol in human saliva, blood, seminal fluid, and rat bile;[6] cinnamyl anthranilate in cosmetic products;[7] and caffeine,[8] aspartame,[9] organic acid preservatives,[10] and sugars[11] in beverages, among many other analyses. Alternatively, extraction and cleanup by existing methodology, such as addition of acetonitrile and azeotropic distillation of the metabolites from urine[1] or extraction from water, urine, and beverages with acetonitrile-diethyl ether (1:1) after adding NaCl and HCl,[2] might be used to prepare samples for TLC analysis. Assuming a 1 mL sample extracted by either of these methods, a 500 μL reconstitution volume, and 10 μL spotting volume, concentrations of 25 ppm could be determined (500 ng theoretical weight of in the sample zone for 100% recovery); with a 10 mL sample, the concentration under the same conditions would be reduced to 2.5 ppm. The sensitivity of the TLC method is not as low as the previously reported gas chromatographic methods[1,2] but, in some cases of OP pesticide exposure, the metabolite concentration in urine during at least the first or second day could be high enough for the TLC method to be applied or a high concentration of metabolite might be present in an environmental water sample as a result of a pesticide

spill. Unlike GC, the TLC method does not require derivatization prior to analysis, it is specific for thio alkyl phosphates, and it is convenient and simple and has high sample throughput as a result of the ability to apply multiple samples on a single plate. An internal standard is not required as in GC analysis because external standards are applied with samples to generate a calibration curve on each plate.

REFERENCES

1. S. J. Reid, R. R. Watts *J. Anal. Tocicol*., **5**, 126-132 (1981).

2. M. Katagi, H. Tsuchihashi, S. Handa, H. Jinmori, K. Otsuki. *Jap. J. Toxicol. Environ. Health*, **39**, 459-468 (1993).

3. J. Sherma, W. Bretschneider. *J. Liq. Chromatogr*., **13**, 1983-1989 (1990).

4. H. K. Berry. in *Quantitative Thin Layer Chromatography.* J. C. Touchstone, ed. New York: Wiley-Interscience (1973), pp. 113-129.

5. J. Sherma, L. Dorflinger. *Am. Lab*., **8**(1), 63-66 (1976).

6. J. C. Touchstone, G. J. Hansen, C. M. Zelop, J. Sherma. in *Advances in Thin Layer Chromatography-Clinical and Environmental Applications.* J. C. Touchstone, ed. New York: Wiley-Interscience (1982), pp. 219-228.

7. J. Sherma, M. Pilgrim. *J. Planar Chromatogr*., **1**, 360-361 (1988).

8. J. Sherma, R. L. Miller, Jr. *Am. Lab*., **16**(2), 126-127 (1984).

9. J. Sherma, S. Chapin, J. M. Follweiler. *Am. Lab*., **17**(3), 131-133 (1985).

10. S. H. Khan, M. P. Murawski, J. Sherma. *J. Liq. Chromatogr*., **17**, 855-865 (1994).

11. J. Sherma, D. L. Zulick. *Acta Chromatogr*., **6**, 7-12 (1996).

Received April 15, 1998
Accepted June 20, 1998
Manuscript 4817-TLC