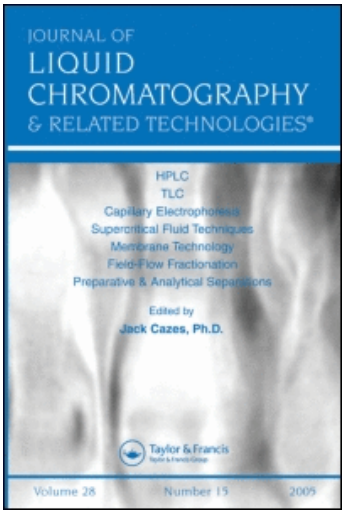

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Determination of Pentachlorophenol Residues in Tallow by Quantitative TLC

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DETERMINATION OF PENTACHLOROPHENOL RESIDUES IN TALLOW BY QUANTITATIVE TLC

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ABSTRACT

A TLC method is described for screening and quantification of PCP in tallow. PCP is extracted and cleaned up by alumina column chromatography, and is determined by preadsorbent silica gel TLC with silver nitrate detection and densitometric scanning. The detection limit of the analysis was 0.50 ppm in tallow. Recoveries varied from 97.9 to 103% over the fortification range of 1 to 20 ppm. The CV of the analysis was 6.2%.

INTRODUCTION

Pentachlorophenol (PCP) has been found to be a contaminant in tallow used as an ingredient in animal feed, posing a toxicological threat to livestock and, ultimetly, the consumer (1). A method using automated gel permeation and Florisil column chromatography cleanup, methyl ether derivatization, and capillary column gas chromatography with electron capture detection was reported for analysis of

tallow for PCP residues (1). A quantitative TLC method was described (2) for determining PCP in wooden containers and urine. This method involved conventional solvent extraction, K_2HPO_4-treated HP layers, an automated spray-on sample applicator, and fluorescence quench detection. In this paper, we describe a simple solid phase extraction (SPE)/TLC method for screening or quantifying PCP residues in tallow, using unimpregnated silica gel plates, manual sample application, and silver nitrate reagent for selective detection.

EXPERIMENTAL

Standard Solutions

PCP standard was obtained from the Aldrich Chemical Co. (catalog # P260-4). A 1.00 mg/ml stock solution was prepared in toluene, and a TLC standard solution with 0.100 ug/ul concentration was prepared by an exact 1:10 dilution of an aliquot of stock solution with toluene. Volumes ranging from 1.0-15.0 ul (0.10-1.50 ug of PCP) were spotted to prepare the TLC calibration curve on each plate. A spiking solution was prepared by dissolving 10.0 mg of PCP in 50.0 ml of ethyl acetate (200 ug/ml), and this was diluted 1:10 to produce a 20.0 ug/ml solution.

Thin Layer Chromatography

Analyses were performed on 20 x 20 cm Analtech silica gel GF Uniplates with preadsorbent and channels (catalog #

32911), which were predeveloped with methylene chloride-methanol (1:1) and allowed to air dry before spotting. Initial zones of samples and standards were applied to the preadsorbent using a 25 ul Drummond digital microdispenser.

Development was carried out in a paper-lined, vapor-saturated rectangular glass tank with the mobile phase hexane-acetone-methanol-glacial acetic acid (35:10:5:0.1 v/v) followed by drying with a hair drier (low heat) under a hood to remove the solvent. PCP zones were detected by dipping the plate into a solution of 20% aqueous $AgNO_3$-acetone-deionized water-conc. ammonium hydroxide (8:20:20:12), drying the plate in a dark hood for 10-15 minutes, further drying with the hair drier, and exposure to a germicidal UV lamp as described earlier (3). Standard and sample zones were scanned immediately with a Kontes Model 800 fiber optics densitometer as previously described (4), a calibration equation was calculated by linear regression of the area and weight data of the standards, the amount of PCP represented by the areas of sample zone scans was interpolated from the calibration curve, and recovery was calculated by comparing the analytical results to the theoretical spike concentration.

Sample Preparation

Samples were prepared for TLC by SPE with an alumina column. Alcoa F-20 basic alumina (Thomas Scientific) (200 g) was washed in a 60 ml fritted glass funnel with 500 ml of

isopropanol, dried for 3 hours at 130°C, and stored in a desiccator. A 20 cm x 2 cm (od) glass drying tube was plugged with 1 cm of glass wool, on top of which was poured 5 g of the alumina and a 2 cm layer of fine sand.

The method was tested using 3 tallow samples [Montfort 129-164 (I), Corenco 649-186 (II), and "edible tallow" (III), all obtained from Dr. Daniel Schwartz, USDA Eastern Regional Research Center, Philadelphia, PA] spiked with PCP. Tallow (10.0 g) melted at 45°C was weighed into a 250 ml beaker and dissolved in 100 ml of methylene chloride. To prepare 20.0, 10.0, and 1.0 ppm fortifications, 1.00 ml and 0.50 ml of the 200 ug/ml spiking solution and 0.50 ml of the 20.0 ug/ml solution were added, respectively. The spiked solution was poured into the column, and an additional 100 ml of methylene chloride was used to wash the beaker and the walls of the tube as it was passed through the column. When the last of the methylene chloride had entered the sand (do not allow the column to go dry), the column was washed with 50 ml of ethyl acetate, and then PCP was eluted with 30 ml of methanol collected in a stoppered, graduated 50 ml centrifuge tube. The eluate was concentrated to a definite volume using a stream of nitrogen with the tube in a hot water bath, and a 40.0 ul aliquot was spotted on a plate with 0.10, 0.20, 0.40, 0.80, and 1.50 ug standard zones. For the 20.0 ppm spike, the solution was concentrated to 10.0 ml, and the theoretical value for 100% recovery was 0.80 ug. For the 10.0 ppm and 1.0

ppm spikes, the volumes and theoretical weights were 10.0 ml/0.40 ug and 1.0 ml/0.40 ug, respectively.

RESULTS AND DISCUSSION

More than 40 mobile phases were tested, and the best one for producing a compact PCP zone with an R_f value near the optimum center region of the plate and a light plate background was hexane-acetone-methanol-glacial acetic acid (35:10:5:0.1). The R_f value was 0.65 with this solvent mixture. Many different chromogenic and fluorogenic detection reagents and several variations of silver nitrate were evaluated for visualization of PCP, and the silver nitrate formulation described above gave the best combination of sensitivity and reliability. PCP was detected as a dark brown-grey zone on a light tan background. The minimum visual detection limit for PCP was about 50 ng, and calibration curves consistently had linearity coefficients of 0.98-0.99 between 0.10 and 1.50 ug.

Each of the tallows was analyzed after spiking at 20, 10, and 1 ppm. The respective percentage recoveries for tallow I were 99.0, 98.2, and 99.1; for tallow II, 101, 103, and 102; and for tallow III, 100, 97.9, and 103. Unspiked samples were analyzed in each case, and no PCP was found. To determine reproducibility of the method, sample I spiked at 10 ppm was analyzed three additional times, and the coefficient of variation for the 4 analyses was 6.2 %, which is acceptable for quantitative TLC residue analysis.

Sample chromatograms contained no additional zones that interfered with scanning of PCP, attesting to the selectivity of the alumina extraction/cleanup method and the silver nitrate detection reagent. Samples containing interfering impurities that are not eluted from the alumina column by methylene chloride or ethyl acetate but are removed from the column with methanol can be further cleaned up by eluting with 20% methanol in ethyl acetate prior to methanol. This solvent will eliminate certain impurities that are less polar than PCP, but will not elute PCP. This additional cleanup prior to PCP elution with methanol was not required with the three tallow samples used in this study. The majority of free fatty acids present in the tallow is apparently retained on the column after elution with methanol.

Since the visual detection level (ca. 50 ng) is lower than the minimum concentration for reproducible scanning (ca. 100 ng), the TLC method is primarily useful as a qualitative screen down to 0.50 ppm or for quantification of residues down to 1 ppm. Below these concentrations, the more sensitive method based on GC with electron capture detection (1), which was validated over the fortification range of 0.005-0.05 ppm, is recommended. The TLC method has high sample throughput, since up to 12 samples can be analyzed along with the 5 standards on a single plate.

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