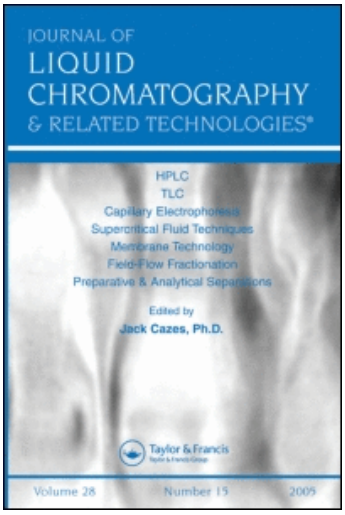

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Determination of Clorsulon Residues in Milk Using a Solid-Phase Extraction Cleanup and Liquid Chromatographic Determination

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DETERMINATION OF CLORSULON RESIDUES IN MILK USING A SOLID-PHASE EXTRACTION CLEANUP AND LIQUID CHROMATOGRAPHIC DETERMINATION

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ABSTRACT

A method for the isolation and liquid chromatographic determination of the antiparasitic drug clorsulon in milk is presented. Milk samples are deproteinized and the supernatant is purified by solid-phase extraction (SPE). The purified extracts are quantified by HPLC analysis with UV detection. The overall recovery of clorsulon from 30 control milk samples spiked at 25-200 ppb was 77.0% (C.V.= 5.3%). This method, a liquid-liquid extraction method, and a matrix solid phase dispersion (MSPD) method for the determination of clorsulon in milk gave comparable values for milk samples with incurred clorsulon residues. However, the SPE method uses less solvent and is more rapid than the liquid-liquid extraction method and is more sensitive than the MSPD method.

INTRODUCTION

Clorsulon (4-amino-6-trichloroethenylbenzene-1,3-disulfonamide) is approved for the treatment of immature and adult

liver fluke (*Fasciola hepatica*) infestation in cattle (1). While clorsulon is accepted for use in beef cattle, its use in female dairy cattle of breeding age is prohibited (2). Chiu et al (3) found that the depletion half-life of clorsulon in milk was 0.84 days and that the unchanged drug was the major residue component.

Very few methods are reported in the literature for the determination of clorsulon in milk. Chiu et al (3) used batch isolation with carbonic anhydrase-Sepharose 4B affinity agarose gel to extract clorsulon from milk. Schenck et al (4) used matrix solid phase dispersion (MSPD), mixing milk with 40 μm C-18, packing the homogenate into a column and then eluting with solvent. Liquid-liquid extraction has been used for the determination of clorsulon in milk (5). This method unfortunately is very time consuming and prone to emulsion formation.

Reverse-phase solid-phase extraction (SPE), passing an aqueous liquid through a column containing silica chemically bonded with a polymeric lipophilic phase, has been widely used to extract drug and pesticide residues from aqueous solutions. Milk cannot be passed directly through an SPE column because the milk solids will clog the column. We have developed an SPE procedure for determining clorsulon in milk. After precipitation of the milk solids, clorsulon is adsorbed and eluted from a cyclohexyl (CH) SPE column. Clorsulon in the resulting eluate is determined by HPLC with UV detection at 265 nm.

MATERIALS AND METHODS

Reagents and Materials

(a) Solvents- Obtained from commercial sources; highest purity available and used without further purification.

(b) Water- Distilled and passed through a Milli-Q Water Purification System (Waters Corp., Milford MA).

(c) Analytical standard- Clorsulon, Lot # L-631,529-000U055 (Merck Sharpe and Dohme Research Laboratories, Rahway, NJ).

(d) Stock standard solutions- Weigh 5 mg clorsulon into a 100 mL volumetric flask. Add ca. 30 mL water and use ultrasound to completely dissolve. Add water to volume.

(e) Intermediate standard solution (5.0 μg/mL)- Pipette 10.0 mL of stock standard solution into a 100 mL volumetric flask. Dilute to volume with water.

(f) HPLC working standards- Prepare dilutions of the 5.0 μg/mL intermediate standard solution in HPLC mobile phase to obtain standards ranging from 0.02-0.125 μg/mL. Store all standard solutions at 4°C.

(g) Solid-phase extraction- Mega Bond Elut cyclohexyl (CH) SPE columns, 6 mL size, 1.0 g; Bond Elut adapters and 30 mL size solvent reservoirs (Varian Sample Preparation Products, Harbor City, CA).

(h) Syringe filters- Acrodisc CR, 0.2 micron disposable PTFE syringe filters (Gelman Sciences, Ann Arbor, MI).

(i) Mobile phase- Dissolve 1.36 g monobasic potassium phosphate in 1.0 L water. Adjust to pH 7.0 with potassium hydroxide and filter. Mix 750 mL of this buffer with 250 mL acetonitrile and degas.

(j) Milk- Control raw milk was obtained from various dairies in the mid-atlantic states. Milk containing incurred clorsulon residues was obtained from Merck Sharpe and Dohme Research Laboratories. Milk samples were stored at -70° C.

(k) Hydroxylamine hydrochloride solution- Dissolve 10 g of ACS reagent grade hydroxylamine hydrochloride in 50 mL of water. Prepare fresh daily.

Apparatus

(a) Sorvall SS-4 manual superspeed centrifuge with type SS-34 head (DuPont Co., Wilmington, DE) or equivalent.

(b) Polypropylene centrifuge tubes (50 mL size) with plastic screw caps (Corning Glass, Corning, NY).

(c) Rotating shaker apparatus- (Arthur H. Thomas Inc.).

(d) Solid-phase extraction vacuum manifold- (Supelco Inc., Bellefonte, PA).

(e) Vortex Mixer- (Thomas Scientific, Swedesboro, NJ).

(f) Liquid chromatographic system- Series 410 LC pump and ISS-100 autosampler (Perkin Elmer Corp., Norwalk, CT);, Model 383A programmable absorbance detector (Applied Biosystems Inc., Ramsey, NJ); Econosphere C-18 HPLC column, 3 μm, 15.0 cm x 4.6 mm id. (Alltech Associates, Deerfield, IL). Injection volume, 50 μL; mobile phase flow rate, 1.0 mL/min.; column temperature, ambient; detector wavelength, 265 nm; sensitivity, 0.005 - 0.010 AUFS.

Extraction and Cleanup

Pipette 6.0 g milk into a 50-mL polypropylene centrifuge tube. For spike recoveries add a suitable aliquot of the 5.0 μg/mL intermediate standard solution to the milk, mix by inverting three times and allow to equilibrate for one minute. Add 6.0 mL hydroxylamine hydrochloride solution and vortex. Add 4.0 mL methanol and cap the centrifuge tube. Place tube on a rotating shaker, set at 180 rpm and shake for 30 minutes. Centrifuge at 8000 rpm for 30 minutes.

Prepare a CH solid-phase extraction column by washing with two 3-mL portions of methanol followed by two 3-mL portions of water. Attach a solvent reservoir to the SPE column and attach the column to the SPE vacuum manifold.

Carefully remove 10.0 mL of the supernatant from the polypropylene centrifuge tube and add to column reservoir. Elute the supernatant through the SPE column at a flow rate of ≤ 2 drops per second, discarding the eluate. Wash reservoir and SPE column two times with 4 mL water, discarding the eluate. Elute the clorsulon from the SPE column with two 4-mL portions of

acetonitrile, collecting the eluates in a disposable glass culture tube. Evaporate the acetonitrile eluate to dryness by heating at 60° C and with the aid of a stream of nitrogen. Add 1.0 mL mobile phase to the culture tube and vortex for 5 seconds to dissolve the residue. Filter through a PTFE syringe filter.

Inject 50 μL of each of the standard and sample solutions into the HPLC and measure the peak heights. Derive the concentration of clorsulon in the sample extract solution by comparing its peak height to the least squares regression of the standard curve responses.

Calculations

Calculate the concentration of clorsulon in the milk samples as follows:

$$\text{Clorsulon } (\mu g/mL) = C \times (W_1/W_2) \times (V_1/V_2)$$

C = concentration of clorsulon (μg/mL) in sample extract as determined by HPLC from standard curve.

W_1 = total volume of milk, hydroxylamine hydrochloride solution and methanol (16 mL).

W_2 = initial sample volume (6.0 mL).

V_1 = volume final extract dissolved in before HPLC analysis (1.0 mL).

V_2 = volume of supernatant taken through SPE cleanup (10.0 mL).

RESULTS AND DISCUSSION

Representative chromatograms resulting from the analysis of blank and 100 ppb fortified milk samples are shown in figures 1 (A) and 1 (B) respectively. The clorsulon is eluted in ca. 13.5 minutes with no interfering peaks from the milk matrix. Table 1 shows the percent recoveries and coefficients of variation for clorsulon recovered from raw milk samples spiked between 25 and 200 ppb. The limit of detection is ca. 5 ppb (S/N=4).

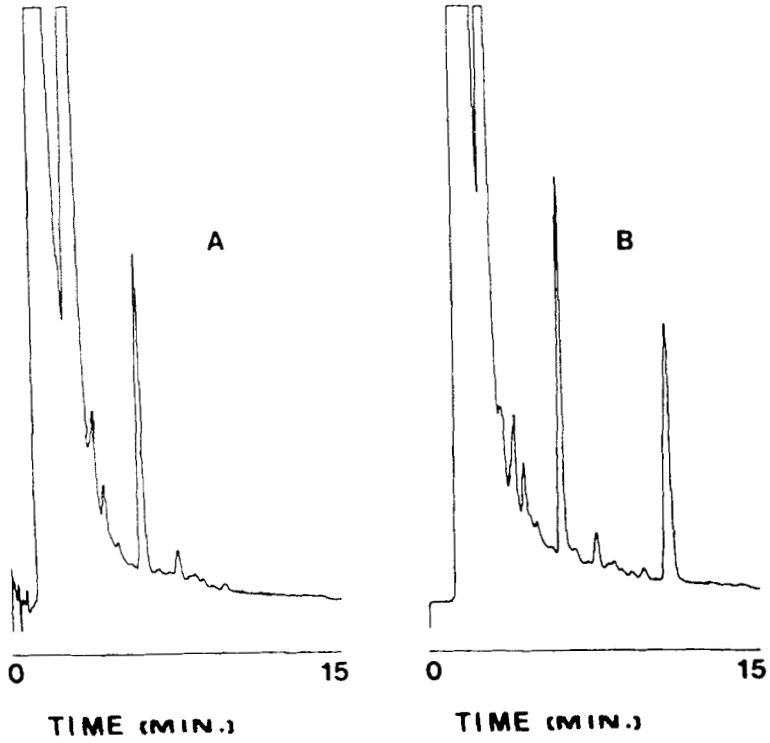

FIGURE 1. Representative chromatograms of (A) control milk, and (B) control milk spiked with 100 ppb clorsulon.

TABLE 1. Recoveries of clorsulon from fortified raw milk.

Fortification, ppb	Recovery, %[a]	C.V., %
25	79.4	7.0
50	76.5	5.9
75	77.5	3.2
100	76.1	7.4
150	78.8	3.1
200	73.7	0.8

[a] n=5

TABLE 2. Incurred clorsulon residues in raw milk as determined by the solid phase extraction (SPE) method, liquid-liquid extraction [LLE] (5) and matrix solid phase dispersion [MSPD] (4).

Milk #	mean ppb clorsulon found (% C.V.) [b]		
	SPE	LLE	MSPD
1	257 (2.0)	274 (3.3)	279 (2.9)
2	288 (3.5)	271 (6.7)	

[b] n=3

In an effort to determine at what point the clorsulon was being lost, milk supernatants were spiked with clorsulon after the deproteinization and centrifugation steps. The average recovery of clorsulon from the spiked supernatants was 93% compared to 77% when the milk was spiked before deproteinization. This data indicates that the loss of clorsulon occurred during the deproteinization step.

Chiu (3) noted that loss of clorsulon during extraction from milk may occur. He attributed this loss to conjugation of clorsulon with endogenous acetaldehyde. The use of hydroxylamine hydrochloride to prevent the interaction between clorsulon and endogenous liver tissue aldehydes has been reported (6).

We added hydroxylamine hydrochloride to the milk to help prevent interference from endogenous aldehydes and to make the milk weakly acidic, facilitating the precipitation of milk proteins. We found the average clorsulon recoveries to be slightly lower when the milk was acidified with 0.1 N HCl.

Methanol is then added to the milk and the milk solids are removed by centrifugation. A portion of the supernatant is eluted through a prepared cyclohexyl SPE column which will retain the clorsulon while the more polar milk components are eluted. Clorsulon is subsequently eluted from the SPE column with acetonitrile. The eluate is evaporated to dryness and the residue is dissolved in mobile phase, filtered and injected into the HPLC.

Milk samples containing incurred clorsulon residues were analyzed by the SPE method, a liquid-liquid extraction method (5) and an MSPD method (4). Table 2 shows that the three methods gave comparable results for milk containing incurred clorsulon residues.

The advantages of the SPE method described include use of small volumes of solvent, minimal generation of hazardous waste and elimination of many of the time consuming solvent extraction and evaporation steps found in traditional liquid-liquid extraction methods and greater sensitivity than the MSPD method.

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