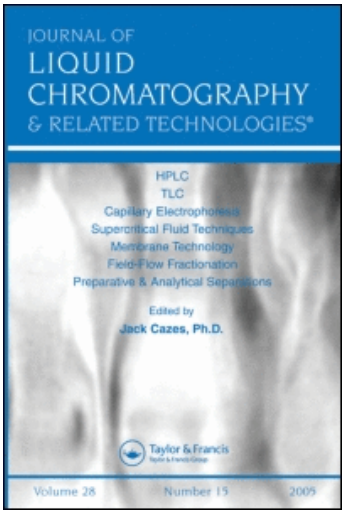

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MATRIX SOLID PHASE DISPERSION (MSPD) ISOLATION AND LIQUID CHROMATOGRAPHIC DETERMINATION OF CLORSULON IN MILK

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ABSTRACT

A method for the isolation and liquid chromatographic determination of the anti-parasitic drug clorsulon in milk is presented. Milk samples (0.5 g) were blended with 2 grams of C-18 (octadecylsilyl-derivatized silica column packing material). A column was made from the C-18/milk matrix and first washed with hexane. The clorsulon was then eluted from this column with ethyl ether. The ether eluate was cleaned up by Florisil solid phase extraction, and clorsulon was subsequently determined by liquid chromatography with UV detection. The overall recovery of clorsulon from milk spiked at 50-200 ppm was 93.0 % with a coefficient of variation of 5.6 %. The MSPD method and a solid phase extraction method used by the FDA gave comparable values for milk containing incurred clorsulon residues.

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). Regulatory guidelines for the use of clorsulon in beef cattle as well as withdrawal times for treated animals prior to slaughter have been established to ensure that beef does not contain residues that exceed the legal tolerance (2). Clorsulon 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.

The extraction of drug residues from complex biological matrices can be a time consuming and labor intensive task. Chiu et al. (3) used batch isolation with carbonic anhydrase-Sepharose 4B affinity agarose gel to extract clorsulon from milk. The method used by the Food and Drug Administration (FDA) entails deproteination of the milk followed by solid phase extraction cleanup (4). No other methods for the analysis of clorsulon residues in milk have been reported in the literature.

Barker et al (5) have demonstrated that biological matrices can be homogenized with C-18 (octadecylsilyl-derivatized silica column packing material), the resulting mixture packed in a column, and various residues then selectively eluted. This method, called matrix solid phase dispersion (MSPD), eliminates the need for the tedious homogenization and centrifugation steps found in classical isolation techniques, while reducing both analytical time and the amount of solvents used. The MSPD method has been successfully used for the isolation of sulfonamides (6), benzimidazoles (7), chlorsulfuron (8), chloramphenicol (9), furazolidone (10) and tetracyclines (11) in milk.

This paper describes the use of MSPD for the extraction of clorsulon from milk followed by HPLC-UV determination. This method was used to analyze milk containing both spiked and incurred clorsulon residues.

MATERIALS AND METHODS

Reagents and Materials

(a) Solvents- Acetonitrile and methanol (glass distilled-suitable for spectrophotometry and liquid chromatography) and hexane (EM Science, Gibbstown, NJ); ethyl ether, high purity solvent containing 2% ethanol as a preservative (Burdick and Jackson, Muskegon MI).

(b) Water- Distilled and deionized by a Milli-Q Water 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, and add water to volume. Dilute 10 mL of this solution to 100 mL with water to prepare a 5.0 μg/mL solution. Dilute 10 mL of this solution to 50 mL with water to prepare a 1.0 μg/mL solution

(e) HPLC standards- Prepare dilutions of the 1.0 μg/mL aqueous standard solution in 30% acetonitrile/water to obtain standards ranging from 0.02-0.125 μg/mL. Store all standard solutions at 4°C.

(f) 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.

(g) Solid phase extraction cartridges- Supelclean LC-Florisil, 6 mL size, containing 1 g sorbent (Supelco Inc., Bellefonte PA).

(h) MSPD column material- Bondesil, 40 μm octadecylsilyl derivatized silica (Analytichem International, Harbor City, CA).

(i) Filters- Acrodisc-CR, 0.45 μm PTFE syringe filters (Gelman Sciences, Ann Arbor, MI).

(h) Milk- Raw milk was obtained from dairies as part of the FDA pesticide surveillance program. Milk containing incurred clorsulon residues was obtained from Merck Sharpe and Dohme Research Laboratories. Milk samples were stored at -70° C.

Apparatus

(a) Empty syringe barrels (reservoirs), 8 mL size, frits and Bond Elut adapters (Analytichem International).

(b) Agate mortar and pestle- 75 mm. od. (Thomas Scientific Co.).

(c) SPE vacuum manifold- (Supelco Inc.).

(d) Liquid chromatographic system- Series 410 LC pump and ISS-100 autosampler (injection volume 200 μL) (Perkin Elmer Corp, Norwalk, CT); Model 383A UV-visible detector (ABI-Kratos Inc, Ramsey, NJ) set at 265 nm. Octadecylsilyl (ODS) derivatized silica column (3 μm, 15.0 cm x 4.6 mm id., Econosphere, Alltech Associates, Deerfield IL), solvent flow rate 1.0 mL/min, column temperature ambient.

Extraction Procedure

Weigh 2 g of C-18 into a mortar. Place 0.5 g milk on the C-18. For spike recoveries inject aqueous stock standard solution into the milk using a HPLC syringe and allow to equilibrate for 1 minute. Blend the milk with the C-18 using a pestle until a homogeneous mixture is obtained. Transfer the resulting C-18/milk matrix to an empty syringe barrel (reservoir) fitted with a frit. Compress the matrix using a syringe plunger and then place the column on the vacuum manifold. Wash the column with 3 mL hexane, using ca 2" Hg vacuum. When all the hexane appears to be eluted, increase the vacuum to maximum for 5 seconds. Discard the hexane eluate. Remove the C-18/milk column from the vacuum manifold.

Attach a Florisil solid phase extraction cartridge which has been washed with ethyl ether below the milk/C-18 column. Place the tandem columns on the vacuum manifold. Elute the tandem columns with three 3 mL aliquots of ethyl ether using ca 2" Hg vacuum, collecting the eluates in a glass culture tube. Remove the milk/C-18 column and elute the Florisil with an additional 2 mL of ether. Evaporate the ether eluate to dryness under nitrogen at 40° C. Add exactly 1.0 mL mobile phase to the dry residue and vortex mix to dissolve the residue. Filter the sample extract through a disposable PTFE syringe filter.

Inject 200 μL aliquots of each standard solution and sample filtrate onto the HPLC.

RESULTS

Representative chromatograms of extracts of clorsulon-spiked and blank raw milk are shown in figures 1a and 1b. Table 1 lists the fortification levels, percent recoveries and coefficients of variation of clorsulon isolated from spiked raw milk. Table 2 illustrates that the MSPD method and a method using deproteination and solid phase extraction yielded comparable results for milk samples containing incurred clorsulon residues.

DISCUSSION

The isolation of clorsulon from milk using matrix solid phase dispersion (MSPD) rapidly yields extracts with minimal interfering co-extractants. When milk is mixed with C-18 it is dispersed over a large surface area, exposing the entire sample to the extraction process. Although the volume of extracting solvents is small (< 15 mL), the process can be envisioned as an exhaustive extraction whereby a large volume of solvent is passed over an extremely thin layer of sample. Elution of the milk/C-18 complex with hexane

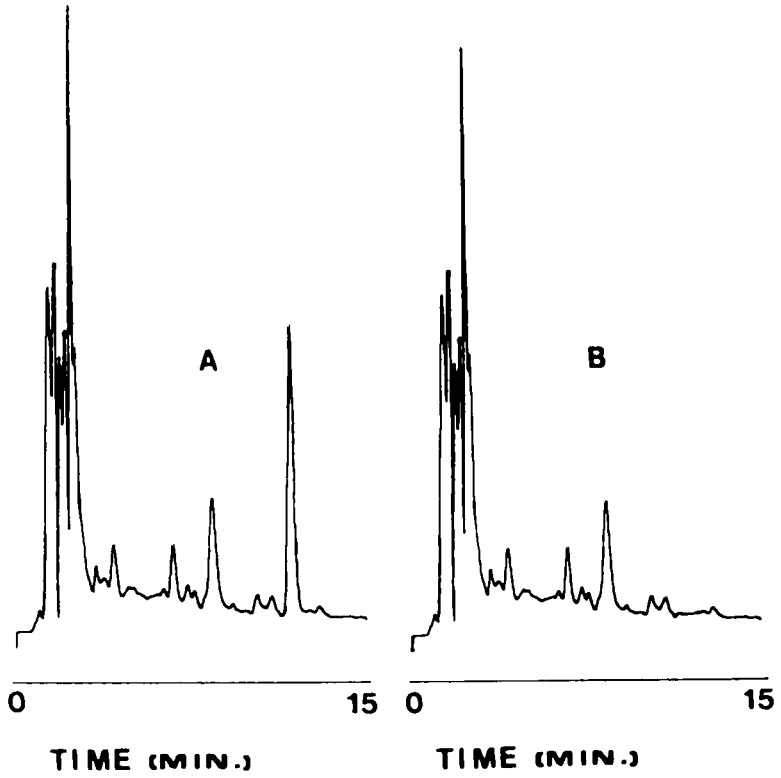

FIGURE 1. Representative cromatograms of (A) control milk spiked with 75 ppb clorsulon, and (B) control milk.

TABLE 1

Recoveries of Clorsulon from Fortified Raw Milk

Fortification (ng/g)	Recovery, %[a]	CV, %
50	92.5	6.7
75	95.3	3.7
100	92.2	3.4
150	96.6	2.7
200	88.8	7.9

[a] 5 determinations at each level

TABLE 2

Recoveries of Incurred Clorsulon Residues from Milk

Method	Clorsulon Found (ng/g)
MSPD	286, 270, 280
FDA (4)	271, 282, 288

removes the lipids from the complex. Clorsulon is then eluted from the complex with a more polar solvent. While ethyl ether, ethyl acetate, or acetonitrile can elute the clorsulon from the milk/C-18 complex, the latter two solvents also elute many co-extractants, visible in the eluate as an opaque white residue. The ether eluate appears clear but still contains contaminants which are apparent during the HPLC analysis. More of the co-extracted interfering materials are removed by Florisil SPE.

The MSPD method eliminates many of the problems associated with classical isolation techniques. The method uses small quantities of solvent and has a minimal number of steps. The short analytical time and the use of small quantities of solvents make this method attractive for the isolation of clorsulon from milk.

REFERENCES

1. "Compound Evaluation and Analytical Capability, 1988 National Residue Program Plan, Sec. 5.8." USDA, FSIS, Science Program, Washington D.C. 20250.

2. Code of Federal Regulations 1990, Title 21, Sections 520.462 and 556.163.

3. Chiu, S.L., Baylis, F.P., Taub, R., Green, M., Halley, B.A. and Bodden, R.M. Depletion of [^{14}C] clorsulon in Cow's milk. J. Agric. Food Chem., 37, 819, 1989.

4. Schenck, F.J., Wagner, R. and Bargo, W. Comparison of two methods for the extraction and determination of clorsulon residues in milk: solid phase extraction vs liquid-liquid extraction. Laboratory Information Bulletin #3554, Food and Drug Administration, Division of Field Science, 5600 Fishers Lane, Rockville MD.

5. Barker, S.A., Long, A.R. and Short, C.R. Isolation of drug residues from tissues by solid phase dispersion. J. Chromatogr., 475, 353, 1989.

6. Long, A.R., Short, C.R. and Barker, S.A. Mulltiresidue method for the isolation and liquid chromatographic determination of sulfonamides in milk. J. Chromatogr., 502, 87, 1990.

7. Long, A.R., Hsieh, L.C., Marlbrough, M.S., Short, C.R. and Barker, S.A. Mulltiresidue method for the isolation and liquid chromatographic determination of seven bezimidazole anthelminthics in milk. J. Assoc. Off. Anal. Chem., 72, 739, 1989.

8. Long, A.R., Hsieh, L.C., Marlbrough, M.S., Short, C.R. and Barker, S.A. Method for the isolation and gas chromatographic determination of chlorsulfuron in milk. J. Assoc. Off. Anal. Chem., 72, 813, 1989.

9. Long, A.R., Hsieh, L.C., Bello, A.C., Short, C.R. and Barker, S.A. Determination of chloramphenicol in milk. J. Agric. Food Chem., 38, 427, 1990.

10. Long, A.R., Hsieh, L.C., Marlbrough, M.S., Short, C.R. and Barker, S.A. Method for the isolation and liquid chromatographic determnination of furazolidone in milk. J. Agric. Food Chem., 38, 430, 1990.

11. Long, A.R., Hsieh, L.C., Marlbrough, M.S., Short, C.R. and Barker, S.A. Matrix solid phase dispersion (MSPD) isolation and liquid chromatographic determination of oxytetracycline, tetracycline and chlortetracycline in milk. J. Assoc. Off. Anal. Chem., 38, 379, 1990.