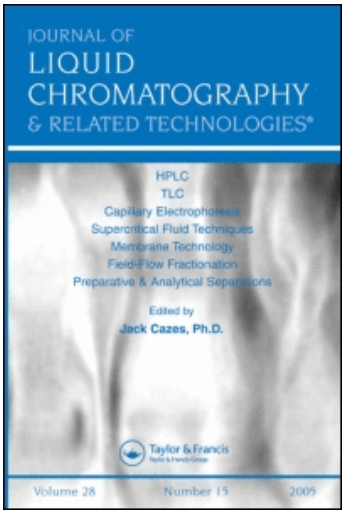

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DETERMINATION OF WARFARIN AND SULFAQUINOXALINE IN RODENTICIDE CONCENTRATES BY HPLC

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ABSTRACT

Warfarin and sulfaquinoxaline are active ingredients in formulated rodenticide concentrates. They are solvent-extracted and, after injection into a liquid chromatograph, a simple buffered mobile phase is used to elute warfarin as a paired ion and sulfaquinoxaline as an ion-suppressed non-ionic species by reverse phase chromatography. A variable wavelength ultraviolet detector is used for detection and external standard calibration is used to quantitate each of the two analytes.

INTRODUCTION

The active ingredients used in the manufacture of certain rodenticide concentrates are warfarin, an anticoagulant, and sulfaquinoxaline, a synergist. The concentrates are ordinarily supplied to bait formulators in concentrations of 0.5% for each active compound with cornstarch used as an inert diluent. Verification of the active contents of the concentrate prior to shipment is of concern to both manufacturer and formulator.

Methods exist for analyzing the warfarin in rodenticides by ultraviolet (UV) spectrophotometric techniques (1) and by high-

pressure liquid chromatography (HPLC) (2). Sulfaquinoxaline could possibly be analyzed by visible spectrophotometric techniques after a tedious extraction and complexation procedure (3). Known methods of analysis for a rapid and simultaneous quantitation of both warfarin and sulfaquinoxaline were not available and attempted quantitation of warfarin by UV techniques showed interference from the co-extraction of sulfaquinoxaline.

A method of separation of the two ingredients was expected through the use of HPLC. Warfarin and sulfaquinoxaline were separated by use of a buffered mobile phase at pH 7.5 on a C_{18} reverse phase column. Quantitation was accomplished by external standard calibration using peak areas and UV detection at 287 nm.

MATERIALS

Reagents

Methanol, LiChrosolv MC/B Manufacturing Chemists, Inc. (Cincinnati, Ohio).

Water, LC grade purified with Milli-Q System, Millipore Corporation (Bedford, Massachusetts).

PIC Reagent A, Waters Associates, Inc. (Milford, Massachusetts).

Reference Standards

Warfarin, Velsicol Chemical Corporation (Chicago, Illinois).

Sulfaquinoxaline, Pfaltz & Bauer, Inc. (Stamford, Connecticut).

Apparatus

Liquid Chromatograph, Model 601, Perkin-Elmer Corporation (Norwalk, Connecticut).

Injector, Model 7105, Reodyne (Berkeley, California).

Detector, Model LC-55, Perkin-Elmer Corporation (Norwalk, Connecticut).

Chart Recorder, OmniScribe Model 35249-15, Houston Instruments (Austin, Texas).

Data System, 3352B Laboratory Data System, Hewlett-Packard (Avondale, Pennsylvania).

Operational Amplifier, Model 1021A, Spectrum Scientific Corporation (Newark, Delaware).

Guard Column, Part No. 84550, Waters Associates (Milford, Massachusetts).

Guard Column Packing, Bondapak C_{18}/Corasil, Waters Associates (Milford, Massachusetts).

Analytical Column, Partisil-10 ODS 25 cm x 4.6 mm, Whatman, Inc. (Clifton, New Jersey).

Centrifuge, Model 2K, Damon/IEC Division (Needham Heights, Massachusetts).

Shaker, Model S-500, Kraft Apparatus, Inc. (Mineola, New York).

Filter Apparatus, Part No. XX1504700, Millipore Corporation (Bedford, Massachusetts).

Syringe Filter Holder, Swinny, Cat. No. 4310, Gelman Instrument Company (Ann Arbor, Michigan).

Filters, Cat. No. LSWP01300 (5 μm), LSWP04700 (5 μm), FHUP04700 (0.5 μm). Millipore Corporation (Bedford, Massachusetts).

EXPERIMENTAL

Mobile Phase

Because warfarin is a weak acid and sulfaquinoxaline is a weak base, a combination ion-pairing and ionic-suppression technique was chosen for separating the two active ingredients after extraction (4). Suppliers have readily available mobile phase modifying reagents that can be rapidly prepared before analysis. PIC A, a commercially prepared tetrabutylammonium phosphate (TBAP) ion-pairing reagent buffered at pH 7.5, is used to pair warfarin, while sulfaquinoxaline, at pH 7.5, is in its non-ionic form. Conversely, PIC B (Waters Associates, Inc.), an alkyl sulfonate buffered at pH 3.5, could be used to pair sulfaquinoxaline and suppress warfarin to its non-ionic form. However, since PIC A reagent was already being used in our laboratory for pairing chlorinated phenoxy herbicides, we chose this reagent for the rodenticide work.

The mobile phase was prepared by adding PIC A to methanol and to water. The modified methanol was passed through a 0.5 μm Teflon filter using a filter apparatus; the modified water was passed through a 5.0 μm Teflon filter. The larger pore size filter was used for water to significantly reduce the filtering time. The methanol and water were then placed into their

appropriate reservoirs and the chromatographic system was allowed to equilibrate at 1.5 ml/min (2000 psi) with a modified methanol/water ratio of 35/65.

Standard Preparation

A stock solution of warfarin was prepared by dissolving 100 mg reference standard (adjusted for purity) in 50 ml methanol. A stock solution of sulfaquinoxaline was prepared by dissolving 25 mg reference standard (adjusted for purity) in 50 ml methanol. Preparation of more highly concentrated solutions of sulfaquinoxaline should not be attempted because the solubility of sulfaquinoxaline is near its limit when prepared as suggested.

Aliquots of each stock solution, 5 ml of warfarin, and 20 ml of sulfaquinoxaline, were pipetted into a 50 ml volumetric flask and diluted to volume with methanol. Replicate 8 µl aliquots (1.6 µg on column) were injected into the liquid chromatograph for calibration purposes.

Sample Preparation

A 2 g portion of the 0.5% warfarin/0.5% sulfaquinoxaline concentrate was weighed into a 4 oz bottle. A 50 ml volume of methanol was pipetted into the bottle, which was capped with a polyethylene lined closure; the contents were then mixed on a wrist action shaker for 1 h. The sample was removed from the shaker and centrifuged at 1000 rpm for 15 min. A portion of the solvent was carefully removed and placed into a syringe fitted with a Swinny adaptor containing a 5 µm Teflon filter. Replicate 8 µl aliquots (320 µg on column) of the filtered sample were injected into the liquid chromatograph.

Columns

A guard column packed with 37-50 μm particle size C_{18} was used between the injector and the analytical column to ensure column life. The C_{18} bonded phase analytical column provided baseline separation of the two components of interest in less than 10 min (Figure 1). Injection volumes larger than 15 μl of either standard or sample caused peak distortion of the sulfaquinoxaline.

Detection

UV absorbance maxima were found to be 308 nm for warfarin and 252 nm for sulfaquinoxaline. An intermediate wavelength of 287 nm was chosen to allow equivalent response and simultaneous analysis. The relative response of warfarin to sulfaquinoxaline at 287 nm with a variable wavelength detector was found to be

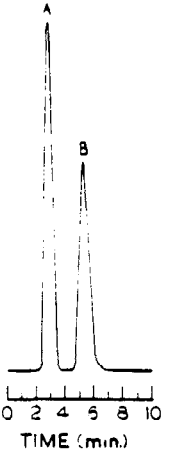

FIGURE 1. HPLC Chromatogram: (A) Sulfaquinoxaline, (B) Warfarin.

1.00:1.03 by area for 1.6 µg each injected on column. For equal peak height response, the wavelength could be adjusted accordingly for a 1:1 response, if so required. A chart recorder (50 mv, 0.05 AUFS) was used to obtain chromatographic documentation.

The UV detector used in this study was found to be incompatible with the laboratory data system if simultaneous chart recording and computer integration were to be accomplished. However, the compatibility problem was solved by placing an operational amplifier between the analog-to-digital converter of the data system and a secondary signal output of the UV detector.

Calculations

Calculations were performed using the following equation:

$$\% \text{ Warfarin in SPL} = \frac{\text{Area Warfarin in SPL}}{\text{Area Warfarin in STD}} \times \frac{\text{WT Warfarin STD INJ µg}}{\text{WT SPL INJ µg}} \times 100$$

Where SPL = sample

STD = standard

WT = weight

INJ = injected

The same calculations were used to determine the quantity of sulfaquinoxaline in the sample.

RESULTS

Repetitive analysis of a concentrate, theoretically containing 0.5% warfarin and 0.5% sulfaquinoxaline, showed a presence of 0.51 ± 0.01% warfarin and 0.49 ± 0.01% sulfaquinoxaline. Repeated injections from a sample showed much less variation, ± 0.004% and ± 0.003% respectively.

CONCLUSIONS

The method reported here is rapid, repeatable, and accurate for the analysis of warfarin and sulfaquinoxaline in rodenticide concentrates containing an inert cornstarch diluent. Further investigations could be made into the use of alkyl sulfonates as mobile phase modifiers rather than buffered TBAP used in this study. In either case, the chromatographic parameters could be of advantage in the analysis of cereal baits, provided an efficient method of extraction were used.

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