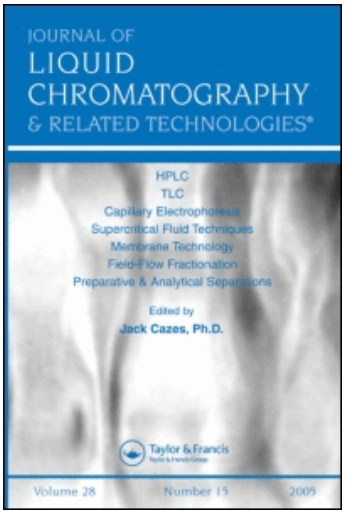

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Simultaneous Determination of Warfarin, Sulphaquinoxaline and Fenitrothion in Wheat-Based Rodenticide Baits by High Pressure Liquid Chromatography

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SIMULTANEOUS DETERMINATION OF WARFARIN, SULPHAQUINOXALINE AND FENITROTHION IN WHEAT-BASED RODENTICIDE BAITS BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

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ABSTRACT

The simultaneous determination of warfarin, sulphaquinoxaline and fenitrothion in wheat-based rodenticides is achieved by extracting the three components from the bait with dimethylformamide followed by an isocratic, high-pressure liquid chromatographic separation using a reverse-phase RP-8 column and 0.005 M pentane sulphonic acid in methanol:water (60:40) as eluent. The three components are detected at 280 nm after separation. Recoveries in the concentration range investigated were fenitrothion 97.2%, warfarin 97.8% and sulphaquinoxaline 96.9%.

INTRODUCTION

Commercial grain baits containing warfarin, (3-[acetonyl-benzyl]-4-hydroxycoumarin) and sulphaquinoxaline (2-[p-aminobenzenesulphonamido] quinoxaline) are frequently used for the control of

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rodent infestations. The addition of fenitrothion (0,0-dimethyl O-[3-methyl-4-nitrophonyl] phosphorothioate) helps to control insects that attack and infest the bait.

Several methods have been published for the determination of warfarin including thin layer chromatography (TLC) followed by ultra violet (UV) detection at 305 nm (1), electron capture gas chromatography (2), and extraction with 1% pyrophosphate solution followed by UV determination at 308 nm (3). In addition several high-pressure liquid chromatographic (HPLC) methods have also appeared (3,4,5.). The determination of sulphaquinoxaline in various matrices has also received considerable attention. Published methods include diazotization followed by coupling in the presence of zirconium and measurement of the coloured complex at 550 nm (6), underivatised UV determination at 350 nm (7,8), and HPLC (5).

Methods used for the determination of fenitrothion include infra-red analysis (9), gas chromatography (10, 11, 12) and HPLC (13, 14, 15).

Although the article by Trujillo (5) describes the determination of both warfarin and sulphaquinoxaline by HPLC, no information was found for the simultaneous determination of the three components

warfarin, sulphaquinoxaline and fenitrothion. In addition Trujillo's article refers to the analysis of rodenticide concentrates and not to the baits themselves.

The need for a quick and specific method for the determination of all three components in rodenticide baits led to the HPLC method described here, which involves extraction of the bait with dimethylformamide (DMF) followed by HPLC on an RP-8 reverse-phase column using methanol:water (60:40) containing pentane sulphonic acid, and detection at 280 nm.

EXPERIMENTAL

Apparatus

A Waters Model 6000A pump, U6K injector and Model 450 variable wavelength UV detector (Waters Associates, Sydney, Australia) were used. The column used was a Brownlee Laboratories RP-8, (10μ), 25 cm x 4.5 mm (i.d.) reverse-phase column (Activon Scientific Services, Granville, Australia). The detector was coupled to a Curken 250-1 recorder (Varian Pty. Ltd., Sydney, Australia) and injections were made with a Hamilton 25μl syringe.

Reagents and Standards

Fenitrothion 99.5% and sulphaquinoxaline 99.9% (Cooper Australia Ltd.).

Warfarin 99.0% (Chemoswed A.B., Sweden).
Methanol HPLC grade (Burdick and Jackson, from Alltech Associates, Sydney Australia).
A 0.25M solution of 1-pentanesulphonic acid in glacial acetic acid (from Waters Associates, Sydney, Australia).

Mobile Phase

The mobile phase was prepared by adding one vial of the 1-pentanesulphonic acid solution to 400 ml of distilled water and making to 1000 ml with HPLC grade methanol. The solution was then degassed by vacuum.

Preparation of Standard

A stock solution was prepared by dissolving 0.0126 g of fenitrothion, 0.4620 g warfarin and 0.2217 g of sulphaquinoxaline in 100 ml DMF. A 10 ml aliquot of this stock solution was transferred to a 100 ml volumetric flask and made to volume with DMF. This final analytical standard contained 0.00126% (12.6 ppm) fenitrothion, 0.04620% (462 ppm) warfarin and 0.02217% (221.7 ppm) sulphaquinoxaline.

Preparation of Baits

Using a mixture of 60% wheat and 40% cornflour, three samples of bait were prepared containing fenitrothion, warfarin and sulphaquinoxaline in the concentrations shown in Table I.

TABLE 1

Rodent Bait Samples Prepared and Analysed

Component (ppm) in Wheat/Cornflour Mix

Sample	Fenitrothion	Warfarin	Sulphaquinoxaline
1	10	440	200
2	12	460	220
3	14	480	240

Extraction Procedure

Approximately 20.0 g of each bait was accurately weighed and transferred to a 500 ml stoppered conical flask. After addition of approximately 150 ml of DMF the flask was stoppered and shaken for 1 hour by means of a mechanical shaker. At the end of 1 hour the contents of the flask were filtered into a 200 ml volumetric flask, the residue washed several times with small portions of DMF and the washings added to the flask. The solution was then made to 200 ml with DMF. A portion of the solution was then filtered through a 5 μm teflon filter by means of a syringe filter. This procedure was performed in duplicate for each sample.

The same extraction procedure was used on a single sample of untreated wheat/cornflour mixture.

Chromatography

With a flow rate of 2 ml/min, the detector set at 280 nm and 0.1 AUFS and the recorder at 0.5 cm/min, duplicate 25 µl injections including the blank were made and the average peak height of each duplicate determined. By comparing the average height of each sample injection with that of the standard, the amount of warfarin, sulphaquinoxaline and fenitrothion in the original bait was calculated.

Results and Discussion

There are no interfering co-extractives when DMF is used to extract fenitrothion, warfarin and sulphaquinoxaline from wheat/cornflour based rodent baits, as can be seen from the chromatogram of the blank extract. (Fig. 1). The chromatogram of the standard solution of fenitrothion, warfarin and sulphaquinoxaline (Feg. 2) shows that, using the chromatographic conditions described, good separation of the three components is achieved within 9 minutes. As shown in Table 2, the average recovery of the three components in the concentration range investigated is fenitrothion 97.2%, warfarin 97.8% and sulphaquinoxaline 96.9%. Fig. 3 is the resulting chromatogram of a bait manufactured under actual "production conditions" which has been extracted and chromatographed as described.

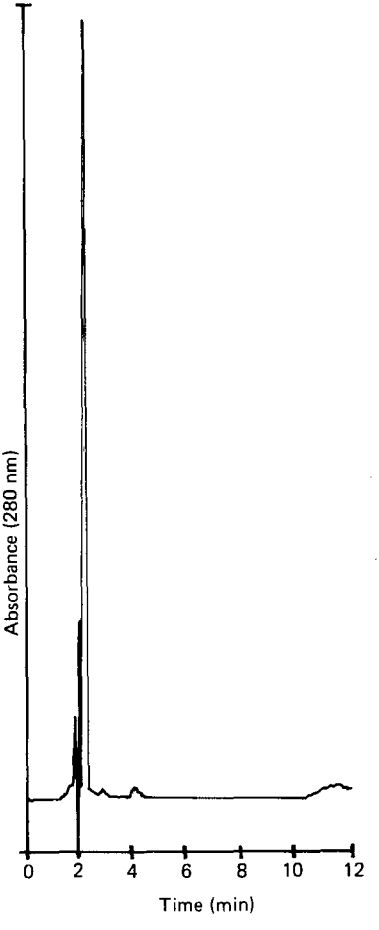

FIGURE 1. Chromatogram of untreated wheat/cornflour mixture, 25 ul injection (blank). Extraction and chromatographic conditions as described under Experimental.

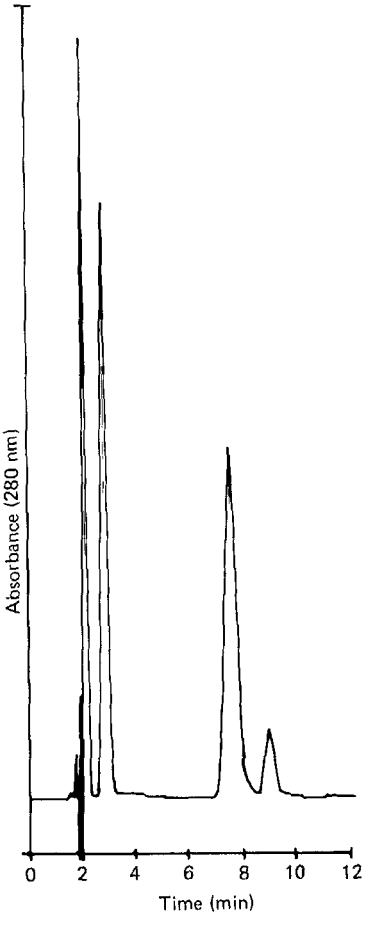

FIGURE 2. Chromatogram of standard analytical working solution. A, sulphaquinoxaline (221 ppm); B, warfarin (462 ppm); C, fenitrothion (12.6 ppm). Injection volume, 25 ul. Retention times: sulphaquinoxaline, 2.5 min; warfarin, 7.7 min; fenitrothion, 9.0 min. Chromatographic conditions as described in Experimental section.

TABLE 2

Recovery of Fenitrothion, Warfarin and Sulphaquinoxaline from Wheat/Cornflour Rodent Baits

Fenitrothion			Warfarin			Sulphaquinoxaline		
Added (ppm)	Found[(a)] (ppm)	Recovery %	Added (ppm)	Found (ppm)	Recovery %	Added (ppm)	Found (ppm)	Recovery %
10.0	9.8	98.0	440	431	98.0	200	191	95.5
10.0	9.6	96.0	440	432	98.2	200	195	97.5
12.0	11.8	98.3	460	451	98.0	220	213	96.8
12.0	11.3	94.2	460	449	97.6	220	213	96.8
14.0	13.9	99.3	480	470	97.9	240	236	98.3
14.0	13.6	97.1	480	466	97.1	240	231	96.3
Mean Recovery: 97.2%			Mean Recovery: 97.8%			Mean Recovery: 96.9%		

(a) As determined from average of two injections.

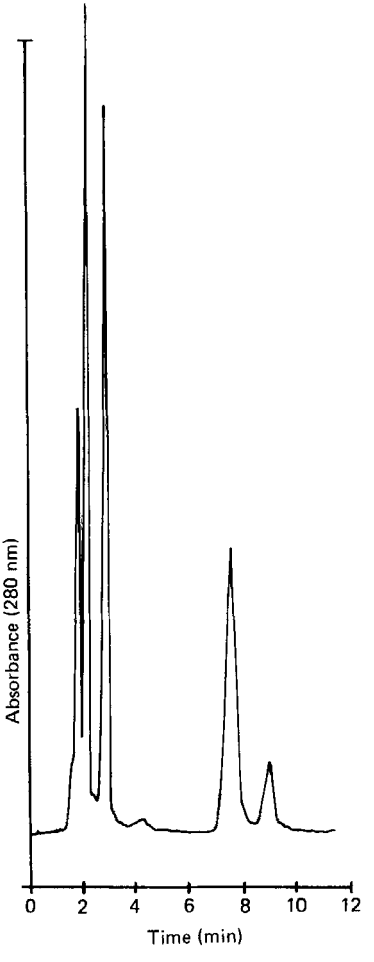

FIGURE 3. Typical chromatogram of extract of bait manufactured under actual "Production Conditions". Extraction and chromatographic conditions as described in Experimental section.

Chromatographic separation of the three components is based on a combination of ion-pairing and ion-suppression. The pentanesulphonic acid solution, as purchased, is buffered at pH 3.5 under which conditions sulphaquinoxaline, being a weak base, forms an ion-pair with pentanesulphonic acid and the ionisation of the weak acid warfarin is suppressed. Fenitrothion is essentially non-polar at pH 3.5. By means of this technique the retention and separation of the three compounds is achieved by reversed-phase chromatography.

CONCLUSION

The method described here for the extraction and analysis of rodent baits based on a wheat/cornflour mixture containing fenitrothion, warfarin and sulphaquinoxaline is rapid, reproducible and accurate with an average recovery of 97.2% fenitrothion, 97.8% warfarin and 96.9% sulphaquinoxaline. The extraction procedure described does not extract any component from the wheat/cornflour mixture that may interfere with the determination of the three components of interest.

REFERENCES

1. Fishwick, F.B. and Taylor, A., The Determination of Warfarin in Animal Relicta, Analyst, 92, 192, 1967.

2. Fessman, J., Quantitation of non-halogenated compounds by electron-capture gas chromatography, J. Chromatogr, 1980, 184(3); Chromatogr Ref, 24(3), 313.

3. "EPA" Manual of Chemical Methods for Pesticides and Devices." Assoc. Off. Anal. Chem, Bontoyal W.R., Chairman, Edit, Comm., Washington, D.C. 1976.

4. Billings, T.J., Hanks, A.R. and Colvin, B.M., Determination of Warfarin by High Performance Liquid Chromatography, J. Assoc. Off. Anal. Chem., 59, 1104, 1976.

5. Trujillo W.A., Determination of Warfarin and Sulfaquinoxaline in Rodenticide Concentrates by HPLC, J. Liq. Chromatogr, 3(8), 1219, 1980.

6. Szalkowski, C.R., Determination of sulfaquinoxaline in Feeds: Collaborative Study, J. Assoc. Off. Anal. Chem., 56(3), 758, 1973.

7. Cieri, U.R., Determination of Sulfaquinoxaline, Sulfathiazole, Sulfamerazine and Sulfamethazine in Feed Concentrates or Premixes. J. Assoc. Off. Anal. Chem., 56(6), 1475, 1973.

8. Cieri, U.R., Collaborative Study of the Determination of Sulfaquinoxaline, Sulfathiazole, Sulfamerazine, and Sulfamethazine in Feed Concentrates and Premixes. J. Assoc. Off. Anal. Chem., 57(2), 345, 1974.

9. Delves, R.B., and Williams, V.P., The Analysis of the Organophosphorus Pesticide Fenitrothion, by an Infrared Method, Analyst, 91, 779, 1966.

10. Desmarchelier, J., Bengston, M., Connell, M., Minett, W., Moore, B., Phillips, M., Snelson, J., Sticka, R., and Tucker, K., A Collaborative Study of Residues on Wheat of Chlorpyrifos-methyl, Fenitrothion, Malathion, Methacrifos and Perimiphos-methyl, Pestic. Sci. 8, 473, 1977.

11. Coburn, J.A., and Chan, A.S.Y., Confirmation of Pesticide Residue Identity. VIII Organophosphorus Pesticides. J. Assoc. Off. Anal. Chem., 57(6), 1272, 1974.

12. Grift, N., and Lockhart, W.L., Gas-Liquid Chromatographic Determination of Fenitrothion in Fish, Water and Sediment. J. Assoc. Off. Anal. Chem., 57(6), 1282, 1974.

13. Marshall, W.D., and Greenhalgh, R., Determination of Impurities in Technical Fenitrothion by Liquid Chromatography. Pestic. Sci. 5, 781, 1974.

14. Cochrane, W.P., Lanouette, M., Greenhalgh, R., Quantitation of the Contaminants in Technical Fenitrothion by Reverse Phase High Pressure Liquid Chromatography. J. Assoc. Off. Anal. Chem., 62(6), 1222, 1979.

15. Perez, R.L., Determination of Fenitrothion, Bioresmethrin and Piperonyl Butoxide in Aerosol Concentrates by High Performance Liquid Chromatography. J. Chromatogr., in press.