Journal of Chromatography, 195 (1980) 113–117
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,771

DIRECT ANALYSIS OF THE HERBICIDE BARBAN IN WHEAT PRODUCTS BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

J. F. LAWRENCE*, L. G. PANOPIO and H. A. McLEOD
Food Research Division, Food Directorate, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2 (Canada)
(Received February 19th, 1980)

SUMMARY

Barban [4-chlorobut-2-ynyl N-(3-chlorophenyl)carbamate] is analysed as the intact herbicide in wheat products including flour, cereal and bread. Residues of the herbicide are extracted from the samples with methanol. An aliquot of the filtered extract is partitioned between water and dichloromethane. Following this, a Florisil column (3% deactivated) is used for clean-up. The eluted barban is then analyzed directly by liquid chromatography on a LiChrosorb RP-8 column with methanol–water (60:40) as the mobile phase. Recoveries over the range of 0.1–5.0 ppm were generally greater than 80%. Detection limit was estimated to be 0.02 ppm in the samples.

INTRODUCTION

Barban [4-chlorobut-2-ynyl N-(3-chlorophenyl)carbamate] has become of interest in Canada in recent years due to its great use in the western provinces for control of wild oats in wheat, barley and other grains. This herbicide, along with four others used for wild oat control, including benzoylprop-ethyl, bromoxynil octanoate, asulam and difenzoquat, accounted for about 50% of all pesticides used in this country in 1977. Essentially all of these herbicides were employed on grains in western Canada. Originally, barban was determined colorimetrically[1]. This method involved hydrolysis, steam distillation and subsequent reaction to form a colored product for quantitation, an approach not well suited for sample screening on a routine basis. Although the herbicide does not pass through a gas chromatograph without decomposition, two gas chromatographic (GC) methods have been published which made use of stable derivatives[2,3]. These methods also involved hydrolysis of barban to the chloroaniline followed by bromination or dinitrophenylation to increase sensitivity to electron-capture detection.

In the last few years, barban has been shown to pass readily through a liquid chromatographic (LC) system. Both reversed-phase[4–6] and normal-phase[4,5] separations have been employed. However, no applications of LC to the analysis of barban in samples have been reported. The present work describes an LC technique which

0021-9673/80/0000–0000/$02.25

has been applied to whole wheat products (cereal, flour and bread), and refined wheat products (flour and bread). The method was designed to be able to detect at least 0.1 ppm of barban, a level deemed necessary in light of its registration on a negligible residue basis in Canada. Also, it is intended that this method be incorporated into a multi-residue LC screening technique for all of the previously mentioned wild oat herbicides.

EXPERIMENTAL

Reagents

A stock solution of barban was prepared in glass-distilled analytical grade methanol at 1 mg/ml. Dilutions of this with methanol were made as required for preparation of the spiking solutions; while for standards, dilutions were made with mobile phase. All other solvents used for extraction and cleanup of the samples were pesticide or analytical-grade materials.

Liquid chromatography

A Waters Assoc. Model 6000A pump was used for solvent delivery. A Waters Model 450 variable-wavelength UV detector (flow cell volume, 8 μl) set at 238 nm and 0.01 absorbance units full scale (a.u.f.s.) was used for detection. The chromatography column was an Altex prepacked LiChrosorb RP-8 (10 μm) (250 × 3.2 mm I.D.). The mobile phase was methanol–water (60:40) at a flow-rate of 1.0 ml/min. Injections were made through a Valco syringe-loop injector. Injection volume was 25 μl.

Sample analysis

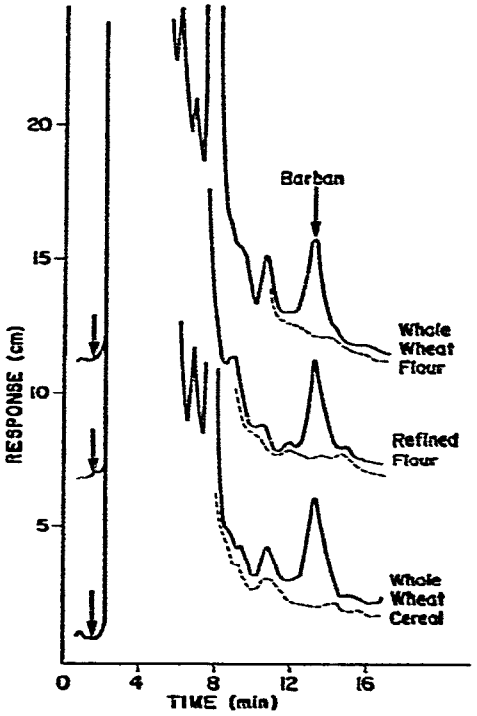
The samples consisted of locally purchased whole wheat cereal, whole wheat and refined flour, and bread samples. A 25-g sample was blended with 70 ml of methanol for 2 min in a Sorval homogeniser at medium speed (setting 5). The homogenate was filtered under reduced pressure through a 150-ml medium-porosity sintered glass funnel. The filter cake was then rinsed with 30 ml of methanol which was combined with the original filtrate, transferred and made up to exactly 100 ml. A 20-ml aliquot (representing 5.0 g sample) was added to a 500-ml separatory funnel containing 275 ml of distilled water and 25 ml saturated sodium chloride solution. This mixture was extracted with three successive 40-ml portions of methylene chloride. The combined extracts were dried by passing through a filter funnel containing a plug of glass wool and about 5 g anhydrous sodium sulfate. The funnel was rinsed with 25 ml methylene chloride and the combined methylene chloride was reduced to an oily residue by rotary vacuum evaporation at 30°C in a 250-ml round bottom flask. The residue was dissolved in 2 ml hexane for column chromatography.

The clean-up column consisted of 10 g, 3% deactivated Florisil (prepared by adding 3 g water to 97 g of Florisil activated at 300°C for 5 h) in a glass column (1.5 cm I.D.) with Teflon® stopcock. The column was rinsed with hexane before use. The sample residue was placed on the column and allowed to percolate into the Florisil. The column was then washed in succession with 100 ml of 30% methylene chloride–hexane and 15 ml of 15% acetone–hexane. These were discarded. Barban was then eluted immediately with a further 30 ml of 15% acetone–hexane. The eluate

was evaporated at 30°C under reduced pressure to an oily residue and dissolved in an appropriate volume of methanol for LC analysis. Spiked samples were prepared by adding a volume of the spiking solution to the wheat product in the homogenizer jar, mixing, then adding the 70 ml of methanol for blending. The remaining procedure was identical to that described above.

RESULTS AND DISCUSSION

Figs. 1 and 2 show chromatographic results obtained at a 0.1 ppm spiking level for the types of samples examined. This level was easily detected in the wheat products. The detection limit was estimated to be 0.02 ppm. Table I lists recoveries obtained at various spiking levels for the commodities studied.



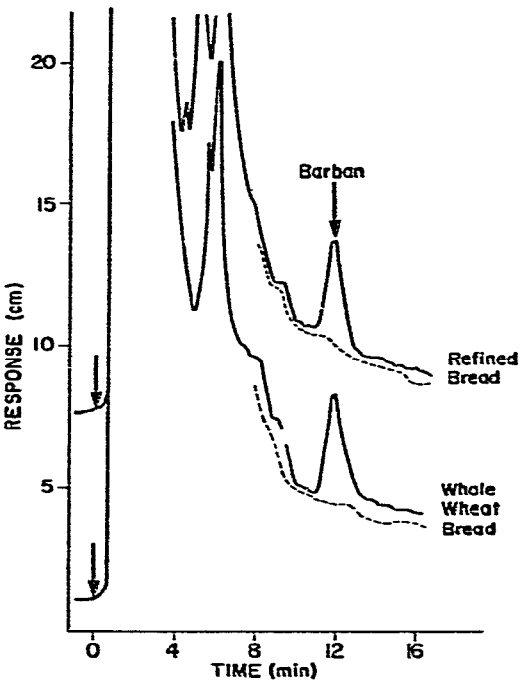


Fig. 1. Chromatograms of whole wheat and refined flour, and whole wheat cereal, spiked at 0.1 ppm. with barban. LC conditions are as described in the text. 250 mg of equivalent sample injected (25 ng barban). Dashed lines indicate sample blank.

Fig. 2. Chromatograms of whole wheat and refined (white) bread spiked with barban at 0.1 ppm. Details as in Fig. 1.

The sample solutions were dissolved in methanol for analysis instead of the mobile phase because the oily residue from the clean-up would not dissolve in the latter. Methanol had no adverse effect on the chromatography of barban under the conditions described above. At the 0.1 ppm level it was necessary to dissolve the residue in 0.5 ml methanol. This resulted in a clouded solution which could not be

TABLE I

RECOVERIES OF BARBAN IN WHEAT SAMPLES

Spiking level (ppm)	*Recovery (%)*				
	Cereal	*Whole flour*	*Refined flour*	*Whole bread*	*Refined bread*
5.0	102, 95	97, 91	—	—	—
1.0	79, 72	102, 97	—	—	—
0.5	83, 75	85, 82	—	—	—
0.1	85, 79	82, 81	85, 82	85, 81	86, 85

cleared by centrifugation. Passing the solution through a Millipore filter improved this in most cases. Repeated injections of these solutions caused no problems in the analyses.

A detector wavelength of 238 nm was chosen even though 206 nm has been shown to be more than twice as sensitive ($\varepsilon \approx 41{,}000$)[5]. Such a low wavelength is not generally useful for residue analysis due to the absorption in that region by sample coextractives. Also, for practical purposes the selection of solvents for the mobile phase becomes limited due to absorption in the low 200 nm range, either by the solvent itself or by trace impurities.

The reversed-phase system employed in this work performed well throughout the investigation. It is probable that adsorption chromatography on silica gel would also be satisfactory. One advantage of an adsorption system would be that the sample extract would dissolve easily in hexane (or mobile phase) and remain stable for long periods of time. Although barban was found to be stable in the aqueous mobile phase used, we have observed for other pesticides that storage in the presence of methanol or water can cause significant degradation, a phenomenon not observed when hexane is used.

The extraction and clean-up procedure proved to be satisfactory for all samples analyzed. At levels of 0.5 ppm or greater, direct methylene chloride extraction of the wheat products followed directly by column clean-up was possible. However, the methanol extraction followed by the partition step was required for low levels of barban. This latter approach was adopted for routine analysis.

We consider column clean-up to be an integral part of LC methods just as it is in GC analysis of pesticide residues. It serves primarily to remove sample coextractives which would interfere in the detection of the barban or other pesticides. However, in LC, column clean-up also serves to remove any very polar substances that have passed through the partition step which might irreversibly bind to the LC column causing deterioration. This is more critical in adsorption than reversed-phase chromatography. Nevertheless, cleaner samples will undoubtedly help prolong column life in any type of chromatographic system. Also, as long as some type of column clean-up is employed before LC analysis there is no need of guard columns, something the authors have never found to be necessary even for residue analysis in complex samples such as foodstuffs. Such LC methods may be easily integrated with GC residue analysis programs since most GC methods for pesticides include column clean-up as standard practice.

CONCLUSION

An LC method has been developed for the direct analysis of barban residues in wheat products down to levels of 0.1 ppm or less. The approach makes use of standard analytical techniques commonly employed for GC including solvent extraction, partition and column clean-up. The method involves no steam distillation apparatus or derivatization reactions that are necessary for colorimetry or GC. It may be incorporated into routine pesticide monitoring programs with little difficulty.

REFERENCES

1 J. R. Riden and T. R. Hopkins, *J. Agr. Food Chem.*, 10 (1962) 455.
2 I. C. Cohen and B. B. Wheals, *J. Chromatogr.*, 43 (1969) 233.
3 R. J. Harris and R. J. Whiteoak, *Analyst (London)*, 97 (1972) 294.
4 A. D. Thurston, Jr., *Environmental Protection Technology Series, EPA-R2-72-079*, U.S. EPA, Corvallis, OR, 1972.
5 C. M. Sparacino and J. W. Hines, *J. Chromatogr. Sci.*, 14 (1979) 549.
6 R. A. Hoodless, J. A. Sidwell, J. C. Skinner and R. D. Treble, *J. Chromatogr.*, 166 (1978) 279.