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Comparison of Liquid and Gas Chromatography for the Determination of Bromoxynil Octanoate and Benzoylprop Ethyl in Wheat Products

James F. Lawrence,* Luz G. Panopio, and Harry A. McLeod

Wheat products, including whole grain wheat, shredded wheat cereal, whole wheat flour and bread, and refined white flour and bread were blended with methanol to extract bromoxynil octanoate [[(3,5-dibromo-4-octanoyl)oxy]benzonitrile] and benzoylprop ethyl [ethyl *N*-benzoyl-*N*-(3,4-dichlorophenyl)-2-aminopropionate] from the samples. An aliquot of the extract was partitioned between methylene chloride and water. The organic extract was reduced to a small volume and passed through a 3% deactivated Florisil column. The fraction containing the herbicides was analyzed by both gas (GC) and liquid (LC) chromatography. Detection limits in the samples were about 0.05 ppm by LC and about 0.005 ppm by GC. Recoveries were generally higher than 80% by both LC and GC at 0.1 ppm or greater.

Statistics released by the Canadian government in 1977 (Statistics Canada) indicated that wild oat herbicides [including bromoxynil octanoate [[(3,5-dibromo-4-octanoyl)oxy]benzonitrile], benzoylprop ethyl [ethyl *N*-benzoyl-*N*-(3,4-dichlorophenyl)-2-aminopropionate], barban, difenzoquat, and asulam] accounted for about 50% by weight of all pesticides sold in Canada in 1976. Essentially all (99.9%) were used in western Canada. As a result of this great usage, a need has arisen to monitor cereal grains, particularly wheat, for residues of these herbicides. At the present time no routine method exists which can adequately screen for them. Of the five herbicides, only bromoxynil octanoate (Helfant, 1979) and benzoylprop ethyl (Wright and Mathews, 1976) pass through a gas chromatograph (GC) without derivatization. Little or no work has been carried out on the analysis of the remaining three by GC after derivatization. Barban has been analyzed by GC after conversion to 3-chloro-2,4,6-tribromoaniline (Harris and Whiteoak, 1972). Recently, a GC method involving hydrolysis and derivatization has been reported for asulam (Bardalaye et al., 1979). No GC method has been reported for difenzoquat although it is possible that the sodium borohydride method for diquat reported by King (1978) might be suitable.

An alternative approach to GC is LC where most organic compounds can be separated by some means without recourse to derivatization. In fact, derivatization in LC is usually employed to improve detectability and not chromatographic behavior (Lawrence, 1979; Jupille, 1979). The wild oat herbicides mentioned above have adequate UV absorbance so that such derivatization becomes unnecessary. Thus, it should be possible to analyze all five herbicides directly by LC at levels of 0.1 ppm or greater. This

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detection level (0.1 ppm) is necessary since the compounds are registered in Canada on a negligible residue basis. Some LC work has already been reported for barban (Hoodless et al., 1978) and benzoylprop ethyl (Lawrence, 1976). An LC method for paraquat (Paschal et al., 1979) may be suitable with modification for difenzoquat determination.

The purpose of the present work is twofold. First, it presents results of our efforts toward developing LC methodology suitable for the direct routine monitoring of bromoxynil octanoate and benzoylprop ethyl at levels of 0.1 ppm or greater. This is to be included in a wider plan for the direct LC analysis of all five wild oat herbicides. Second, a comparison of LC is made with GC for residue analysis where possible (that is, for those compounds which pass through a GC without derivatization). In such cases GC can be used to confirm results obtained by LC especially near the LC detection limit. In the present work this is shown to be very useful.

EXPERIMENTAL SECTION

Liquid Chromatography. A Waters Associates Model 6000A pump was used for solvent delivery. A Waters Model 450 variable wavelength UV detector (8-μL cell volume) set at 228 nm and 0.01 absorbance unit full-scale (AUFS) was used for detection of the herbicides. The chromatography was carried out on an Altex prepacked Lichrosorb RP-8 (10 μm) 25 cm × 3.2 mm (i.d.) column. The mobile phase consisted of either 65 or 75% methanol in distilled water. Samples were injected via a Valco syringe-loop injector. Sample volume was 25 μL throughout.

Gas Chromatography. A Tracor Model MT 220 gas chromatograph equipped with a ^{63}Ni electron capture detector was employed. Two chromatographic systems were studied. A glass column, 1.8 m × 4 mm (i.d.), packed with 6% OV-210 + 4% SE-30 on Chromosorb W (AW) was used at 225 °C and a nitrogen gas flow rate of 60 mL/min. The second system consisted of a 1.2 m × 2 mm (i.d.) glass column packed with 3% OV-1 on Chromosorb W (AW) at 175 °C and a gas flow rate of 50 mL/min.

Reagents. All solvents used for the sample extractions and liquid chromatography were distilled in glass. Stock solutions of benzoylprop ethyl [ethyl *N*-benzoyl-*N*-(3,4-dichlorophenyl)-2-aminopropionate] and bromoxynil octanoate [[(3,5-dibromo-4-octanoyl)oxy]benzonitrile] were prepared in ethanol at 1.0 mg/mL. For spiking purposes and for LC standards, these were appropriately diluted with methanol. Standards for GC were prepared by dilution with hexane.

The wheat products studied were locally purchased commodities of whole wheat cereal, flour, and bread. In addition, samples of refined flour and bread were included.

Sample Extraction. A 25-g sample was blended with 100 mL of methanol in a Sorvall Omni Mixer at medium speed (setting 5) for 2 min. The homogenate was filtered under vacuum through a medium porosity sintered glass funnel into a 250-mL flask. The filtrate was transferred and made up to exactly 100 mL with methanol. A 20-mL aliquot (equivalent to 5 g of sample) was added to a 500-mL separatory funnel containing 275 mL of water and 25 mL of saturated NaCl solution. The mixture was extracted with three successive 40-mL portions of methylene chloride. The combined organic extracts were dried by passing through a conical funnel containing ca. 3 g of anhydrous Na_2SO_4 and then evaporated to an oily residue by using a rotary vacuum evaporator at 40 °C. The residue was dissolved in 2 mL of hexane for column chromatographic cleanup.

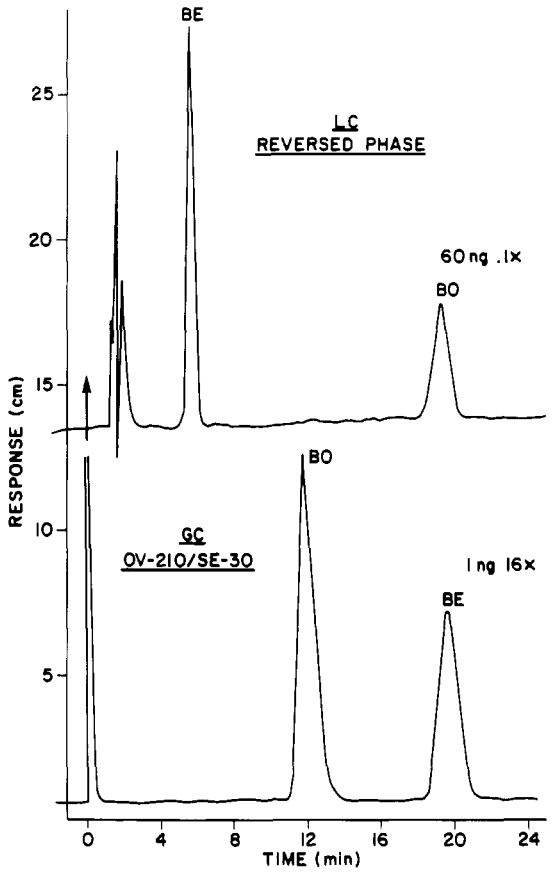

Figure 1. Chromatograms obtained by LC and GC for benzoylprop ethyl (BE) and bromoxynil octanoate (BO). 60 ng of each was injected for LC (0.01 AUFS) and 1.0 ng of each for GC (attenuation 16×). Conditions were as described in the text.

Column Chromatography. Ten grams of 3% deactivated Florisil (prepared by adding 3 g of H_2O to 97 g of Florisil which has been activated by heating at 300 °C for about 5 h) was placed in a glass column (1.5 cm i.d.) and washed with 40 mL of hexane. The sample in hexane was added to the top of the column and permitted to percolate into the Florisil. The column was then washed with 100 mL of 30% methylene chloride in hexane which was discarded. Following this, 15 mL of 15% acetone in hexane was added and the eluate discarded. The herbicides were then eluted with an additional 30 mL of 15% acetone in hexane. This fraction was evaporated almost to dryness and then dissolved in 1 mL of methanol for LC analysis or 1 mL of hexane for GC analysis.

RESULTS AND DISCUSSION

Figure 1 compares chromatographic results obtained by LC and GC for standards of the two herbicides. The LC chromatogram was obtained isocratically with a mobile phase composition of 75% methanol/water at 1.0 mL/min. The GC results were obtained under the conditions described under Experimental Section for the OV-210/SE-30 column. The response expressed on the basis of signal to noise ratio indicated that GC was about 100-fold more sensitive than LC for both herbicides. Table I lists the retention times of the two compounds determined on the various systems examined. The system using 75% methanol/water was employed for all LC determinations of bromoxynil octanoate. The same mobile phase was used for benzoylprop ethyl analyses at 0.5 ppm or greater. However, at 0.1 ppm 65% methanol frequently was required for this herbicide in order to improve sample background. In the GC analyses, both columns functioned

Table I. Retention Times of Bromoxynil Octanoate and Benzoylprop Ethyl[a]

	retention time, min			
	LC		GC	
compd	75% MeOH/ H_2O	65% MeOH/ H_2O	OV-1	OV-210/ SE-30
bromoxynil octanoate	19.2	>40.0[b]	5.8	11.8
benzoylprop ethyl	5.6	15.0	10.3	19.5

[a] Chromatography conditions were as described under Experimental Section. [b] The peak did not appear at 40 min.

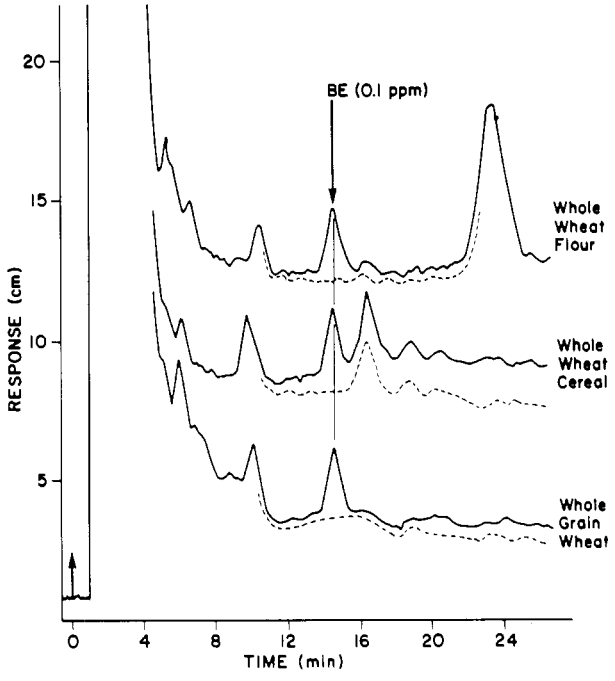

Figure 2. LC chromatograms of three wheat samples containing benzoylprop ethyl (BE) at 0.1 ppm. The mobile phase consisted of 65% methanol/water. Detector sensitivity, 0.01 AUFS. All other conditions were as described under Experimental Section. 250 mg of equivalent sample was injected. Dashed line indicates sample blank.

well for the determination of benzoylprop ethyl at levels well below 0.1 ppm in all samples studied. However, under the conditions described for the OV-1 column, an interfering sample peak was present at a retention time corresponding to bromoxynil octanoate and equivalent to between 0.05 and 0.2 ppm of bromoxynil octanoate. The same samples analyzed on the OV-210/SE-30 column did not show the presence of this peak, and therefore it was considered the column of choice.

Figures 2 and 3 compare chromatograms of three sample analyses obtained by LC for benzoylprop ethyl and bromoxynil octanoate, respectively, at a spiking level of 0.1 ppm. Both compounds were detected with similar sensitivity in the commodities examined. Few differences were observed between the whole wheat and refined flour or bread. The refined samples were slightly cleaner; however, the detection limits were similar, being about 0.05 ppm for both types of products.

Figure 4 compares results of three analyses made by GC using the OV-210/SE-30 column. Both bromoxynil octanoate and benzoylprop ethyl were easily detected at the 0.1-ppm level in all of the products studied. Detection limits were estimated to be about 10-fold better in the

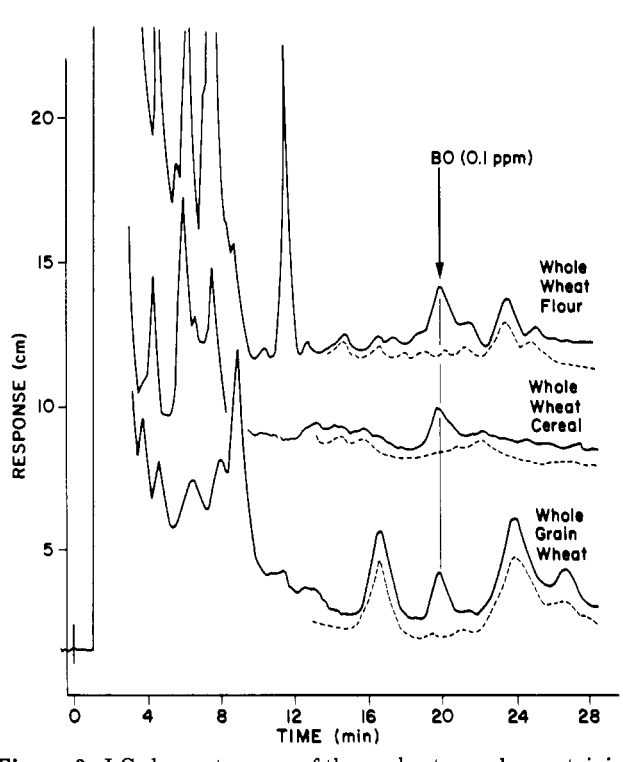

Figure 3. LC chromatograms of three wheat samples containing bromoxynil octanoate (BO) at 0.1 ppm. All conditions were as described under Experimental Section. 250 mg of equivalent sample was injected. Dashed line indicates sample blank.

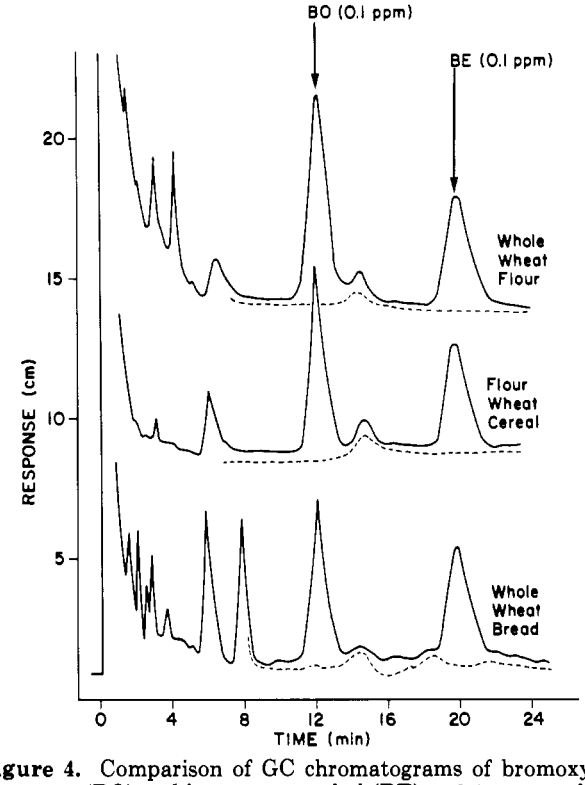

Figure 4. Comparison of GC chromatograms of bromoxynil octanoate (BO) and benzoyprop ethyl (BE) at 0.1 ppm each in three wheat products. GC conditions were as described under Experimental Section. Detector attenuation, 16×. 5 mg of equivalent sample was injected. Dashed line indicates sample blank.

presence of sample coextractives than the LC method (about 0.005 ppm), although the absolute detector sensitivity to the compounds was about 100-fold better by GC. As found in the LC analyses, the differences between refined and whole wheat products were superficial and did

Table II. Recovery Studies

herbicide	concn, ppm	% recovered from whole grain wheat	whole wheat flour	whole wheat cereal
LC				
benzoylprop ethyl	5.0	91	91	98
	1.0	82	97	83
	0.5	92	86	83
	0.1	78	65	75
bromoxynil octanoate	5.0	92	81	95
	1.0	97	106	108
	0.5	82	98	111
	0.1	75	83	78
GC[a]				
benzoylprop ethyl	0.1	78, 88	82, 83	90, 91
bromoxynil octanoate	0.1	59, 59	87, 89	83, 71

[a] GC was carried out on the OV-210/SE-30 column.

not alter the detectability of the two herbicides.

Table II lists some recoveries through the extraction procedure with analysis by both LC and GC. The extraction method produced comparable recoveries for both compounds in refined flour and the two types of bread. Although extensive recovery studies were not performed, the data suggests that the method has good potential for routine screening of these two compounds.

The described extraction procedure has been shown to be useful for the determination of benzoylprop ethyl and bromoxynil octanoate in various types of wheat samples by both LC and GC at levels as low as 0.1 ppm. The GC method proved to be superior to LC with a potential of detecting the herbicides in the low part per billion range. However, GC is not suitable for the direct determination of other wild oat herbicides such as asulam or barban; thus, it does not have the potential of LC for screening all five of these compounds without chemical derivatization. The use of the GC analysis as a confirmation of LC results is dramatically illustrated in Figures 2–4. The small peaks of bromoxynil octanoate and benzoylprop ethyl in the LC chromatograms appear as large well-defined peaks when the same extracts are subjected to GC analysis.

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Some Antifungal Components of Diseased Blue Ribbon Iris Bulbs

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Methanolic extracts of tunic-free *Penicillium*-infected Blue Ribbon iris bulbs from 30 °C storage possessed antifungal properties when bioassayed with *Penicillium corymbiferum* and *Fusarium oxysporum* from iris, while comparable disease-free bulbs possessed none. Thin-layer chromatographic (TLC) fractions of the diseased bulb extract, after treatment with diazomethane, were examined by gas–liquid chromatography (GLC) and by gas chromatography–mass spectroscopy (GC–MS). Identified in the esterified active fraction were methyl benzoate, methyl salicylate, methyl cinnamate, coumarin, methyl caprate, and other compounds. Details of extraction, purification, and identification are reported.

It has been recognized that plants are able to synthesize a variety of antifungal substances. Some are present in plants in a nontoxic form until metabolized by the invading organism; others are produced in response to injury or to invasion by microorganisms and may occur at low levels in plant tissues or be completely absent in a healthy plant (Deverall, 1972; Kuc, 1972a,b; Ingham, 1972). It was with this in mind that diseased bulbs were selected from a badly infected set of *Penicillium*-susceptible Blue Ribbon iris bulbs stored at 30 °C and subsequently frozen. Methanolic extracts prepared from these bulbs were biologically active against the bioassay fungi, but it was not determined specifically whether the activity arose from the host or pathogen or from interaction of the two. Methanolic extracts repeatedly prepared from healthy bulbs without tunic produced no biological response except fungal growth enhancement. Thus, the active material was subjected to further examination by TLC, GLC, and GC–MS to determine the cause of this antifungal activity. This paper reports the results of that study.

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EXPERIMENTAL SECTION

Preparation of the Sample. *Penicillium*-infected Blue Ribbon iris bulbs from 30 °C growth-retarding storage were selected after freezing and removal of the tunic which was retained for a separate study. The bulbs were passed through a Toledo food chopper (Model 4120) into a polyethylene bag and mixed while frozen. An 0.69-kg amount of the chopped and still frozen sample was weighed

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