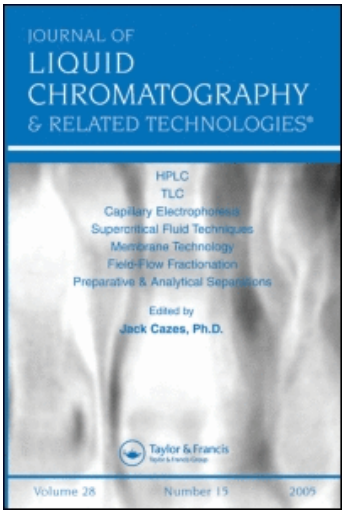

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Fogarty, Andrew M. , Traina, Samuel J. and Tuovinen, Olli H.(1994) 'Determination of Dicamba by Reverse-Phase HPLC', Journal of Liquid Chromatography & Related Technologies, 17: 12, 2667 — 2674
To link to this Article: DOI: 10.1080/10826079408013406
URL: http://dx.doi.org/10.1080/10826079408013406

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(12), 2667-2674 (1994)

DETERMINATION OF DICAMBA BY REVERSE-PHASE HPLC

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ABSTRACT

Reverse-phase HPLC methodology was developed for resolution and quantitative analysis of dicamba (3,6-dichloro-2-methoxybenzoic acid) with a C-18 column and acetonitrile-phosphate buffer as the mobile phase under isocratic conditions. This solvent system was also suitable for mixtures containing dicamba, 2,4-dichlorophenoxyacetic acid (2,4-D), and 2-(2-methyl-4-chlorophenoxy)propionic acid (MCPP) and allowed the resolution of these herbicides with the respective retention times of 4.5, 7.1, and 9.5 min. UV-spectroscopy was unsuitable for the resolution of mixtures of dicamba, 2,4-D, and MCPP due to overlapping absorption spectra.

INTRODUCTION

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a commonly used pre- and post-emergence herbicide with major application in the lawn care industry. Dicamba is readily water-soluble and residues are relatively mobile in the

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environment. The degradation of this herbicide in soil is believed to be primarily mediated by microorganisms (1). Dicamba has been resolved and quantified by UV-spectroscopy, GC, and HPLC. TLC methodology is also available (2). Confirmation of dicamba by capillary SFC/MS and HPLC/MS techniques has been reported (3, 4). While UV-spectroscopy offers a quick and simple method for quantifying dicamba, its specificity is subject to interference from breakdown products. Traditionally, dicamba has been analyzed by GC (5-7). However, this approach is limited due to potential problems of thermal destruction associated with GC analysis. HPLC methodology alleviates this potential problem of thermal alteration or destruction of test compounds. Normal-phase HPLC has been used to resolve dicamba because the compound is relatively polar and does not generally adsorb strongly to a reverse-phase support (8). With tetrabutylammonium phosphate as an ion pair reagent, good resolution of dicamba has been achieved on a C-18 reverse-phase column (9, 10). An HPLC system utilizing a C-18 column and a gradient mobile phase was successfully used by Kim et al. (11) for dicamba and several phenoxyalkanoic herbicides. Previous studies have shown variable success with reverse-phase HPLC for dicamba and other herbicides depending on the solvent system (12).

In the present work, an analytical method based on isocratic HPLC was developed for dicamba that could also resolve it in mixture with the phenoxy-herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-(2-methyl-4-chlorophenoxy)propionic acid (MCPP).

EXPERIMENTAL

Materials and Reagents

Analytical-grade (>99.9% purity) and technical-grade (86.8% purity) dicamba were obtained courtesy of Sandoz Crop Protection (Des Plaines, IL). Analytical-grade 2,4-D (98.7% purity) and MCPP (98.0% purity) were obtained from Dow Chemical Company (Midland, MI). HPLC-grade distilled water and chemicals were used in the preparation of analytical standards and mobile phase.

Demineralized double distilled water was used for preparing the dicamba standard solutions for UV-spectroscopy.

HPLC Methodology

The HPLC system was composed of an Altex model 100A solvent delivery pump (Berkeley, CA) and a Hitachi 100-40 UV-detector (Danbury, CT) fitted with an Altex spectrophotometer flow cell set at 229 nm (range, 0.2; time constant, 0.3). The retention data and peak areas were recorded and analyzed by a Hewlett-Packard H3396 A integrator (Avondale, PA) which had the following settings: attenuation, 32; peak width, 0.2; threshold, 1.0; and area rejection 100. The HPLC column used was a commercially available Phenomenex ODS (4.6 mm x 150 mm) with Spherisorb packing consisting of a 5.0 μm pore size (Torrance, CA). The mobile phase was 40% acetonitrile-60% phosphate buffer (6.0 g K_2HPO_4 and 3.0 ml conc. H_3PO_4 l^{-1}, pH 3.0) (13). Stock solutions for HPLC analysis were prepared in 0.05 M NaOH and the working standards were diluted in 25 ml of 0.5 M NaOH containing 4.0 ml glacial acetic acid. All standards were filtered through a 0.45 μm Acrodisc LC25 filter (Gelman Sciences, Ann Arbor, MI) before injection (100 μl) into HPLC. Analyses were carried out under isocratic conditions at a flow rate of 1 ml min^{-1} and chart speed of 0.5 cm min^{-1}.

UV-Spectroscopy

The characteristic wavelengths of maximum absorption and the concentrations of dicamba, MCPP, and 2,4-D in aqueous solutions were determined by UV-spectroscopy (Varian 2200, Palo Alto, CA). Standard stock solutions of MCPP and 2,4-D were prepared in 0.05 M NaOH.

RESULTS AND DISCUSSION

The analytical-grade and technical-grade dicamba had a maximum absorbance at 274 nm and 276.5 nm, respectively (Figure 1). Standard curves for dicamba

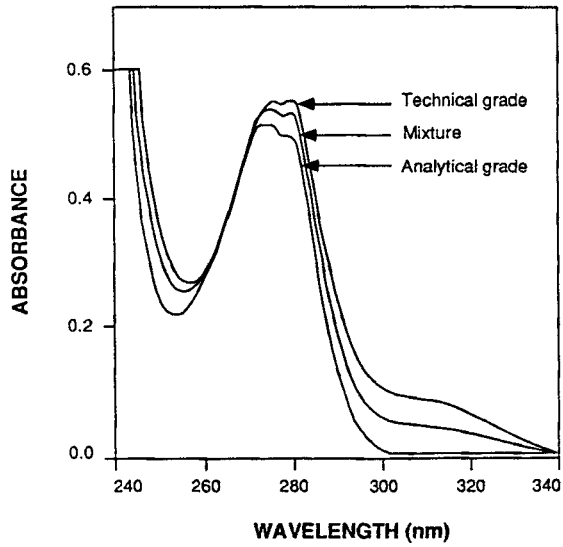

FIGURE 1. UV-spectral scans of standard aqueous solutions containing 250 mg l^{-1} of analytical-grade or technical-grade dicamba, and a mixture of 125 mg l^{-1} of both analytical-grade and technical-grade dicamba.

at these wavelengths displayed linearity between 1 and 1000 mg l^{-1}. The linear regression equations describing the standard curves for the technical- and analytical-grade dicamba were $y = 0.00239 x + 0.0484$ ($r = 0.995$) and $y = 0.00242 x + 0.017$ ($r = 0.998$), respectively ($y = A_{274}$, x = mg l^{-1}). Upon storage at 4°C and -20°C for four weeks, the losses of dicamba were 1% and 30%, respectively. Less than 0.1% dicamba was lost upon autoclaving at 121°C for 15 min.

MCPP and 2,4-D had peaks of maximum absorption at 279 and 283 nm, respectively (Figure 2A). UV-spectroscopic analysis of a mixture of dicamba, MCPP, and 2,4-D produced a broad peak in the 280 nm range in which the peaks overlapped (Figure 2B), and peaks of maximum absorption at either 274, 279, or 283 nm could not be discerned. Several other aromatic herbicides have wavelengths of maximum absorption in the same range which further limits the use of UV-spectroscopy in multiherbicide residue analysis.

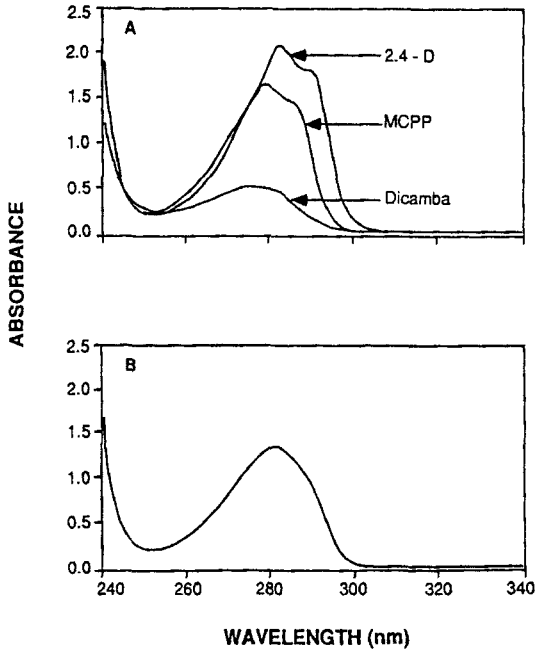

FIGURE 2. UV-spectral scans of standard aqueous solutions containing herbicides as follows. **A**, 250 mg l^{-1} of analytical-grade dicamba, 2,4-D, or MCPP; **B**, a mixture of analytical-grade dicamba, 2,4-D, and MCPP (83.3 mg l^{-1} each).

Reverse-phase HPLC, with a C-18 column and 40:60 acetonitrile-phosphate buffer as the mobile phase (flow rate 1 ml min^{-1}), was able to resolve analytical-grade dicamba at concentrations < 1 mg l^{-1}, with a single peak eluting at 4.5 min (Figure 3). This resolution under isocratic conditions compares favorably with results reported by Kim et al. (11) using a gradient mobile phase (Table 1). Thus, the data demonstrate that both isocratic and gradient analyses yield comparable results. This isocratic mobile phase has been previously used for resolution of 2,4-D and MCPP (13). The peak of technical-grade dicamba had identical retention time of 4.5 min, but a minor peak also eluted at 6.6 min. The identity of this impurity is unknown at this time. Standard curves based on

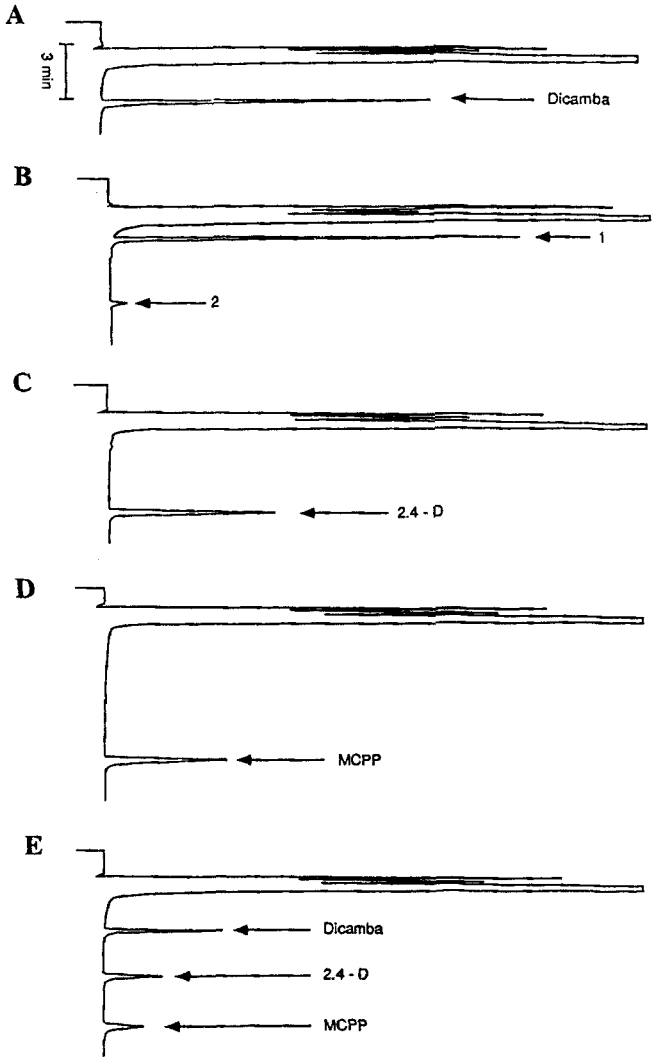

FIGURE 3. Reverse-phase HPLC chromatograms of (**A**) 10 mg l^{-1} of analytical-grade dicamba; (**B**) 10 mg l^{-1} of technical-grade dicamba; (**C**) 10 mg l^{-1} of 2,4-D; (**D**) 10 mg l^{-1} of MCPP; (**E**) a mixture of analytical-grade dicamba, 2,4-D, and MCPP (3.3 mg l^{-1} each). The retention times were 4.5 min for dicamba, 7.1 min for 2,4-D, and 9.5 min for MCPP.

TABLE 1. Comparison of HPLC retention times.

Analyte	Retention time (min)	
	Present work Isocratic; 40:60 Acetonitrile-phosphate buffer 15 cm column, 4.6 mm i.d.	Kim et al. (11) Gradient; Water:acetonitrile:acetic acid 10 cm column, 2.1 mm i.d.
Dicamba	4.5	4.5
2,4-D	7.1	7.4
MCPP	9.5	9.6

integrated peak areas displayed linearity at least up to 70 mg dicamba l^{-1}. The linear regression equations describing the standard curves for the technical- and analytical-grade dicamba were $y = 0.889 x + 1.736$ ($r = 0.997$) and $y = 1.29 x - 2.05$ ($r = 0.995$), respectively ($y = A_{229}$, x = mg l^{-1}).

A mixture of herbicides containing 3.3 mg each of dicamba, 2,4-D, and MCPP l^{-1} was successfully resolved by HPLC analysis using the same conditions as for dicamba alone (Figure 3). The retention times were 4.5, 7.1, and 9.5 min for dicamba, 2,4-D, and MCPP, respectively.

The HPLC system could thus be used to determine dicamba at concentrations of <1 mg l^{-1}. The resolution of dicamba from 2,4-D and MCPP at low concentrations was also accomplished using a acetonitrile-phosphate buffer solvent system that was compatible for all three compounds. Although dicamba, 2,4-D, and MCPP each have characteristic wavelengths of maximum absorption, UV-spectroscopy was unsuitable for the resolution of dicamba, 2,4-D, and MCPP in a mixture due to their overlapping absorption spectra.

ACKNOWLEDGMENTS

Partial support was received from the Robert H. Edgerley Environmental Toxicology Fund (A.M.F.).

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Received: January 6, 1994
Accepted: January 31, 1994