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# Gas Chromatographic/Mass Spectrometric Method for Analysis of Chlorophenoxy Acid Herbicides: MCPB and MCPA in Peas

Matt J. HENGEL, Charles R. MOURER and Takayuki SHIBAMOTO*

University of California, Davis
(One Shields Avenue, Davis, California 95616, USA)

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**Summary**

An analytical method for the determination of 2-methyl-4-chlorophenoxybutyric acid (MCPB) and its primary metabolite 2-methyl-4-chlorophenoxyacetic acid (MCPA) residues in peas was developed utilizing liquid-liquid partitioning, derivatization of acids with diazomethane, Florisil® column cleanup, and gas chromatography/mass spectrometry. Method validation recoveries from various pea matrixes (peas without pods, peas with pods, and dry peas) ranged from 69-108% for MCPB and 60-94% for MCPA, over four levels of fortification (0.01, 0.10, 0.50, and 1.0 ppm). Control and MCPB-treated pea samples collected from six IR-4 (USDA, Interregional Research Project No. 4, Minor Use Pesticide Registration Program) field sites had residue levels less than 0.01 ppm, except for one that had 0.017 ppm. The method was validated down to the limit of quantitation at 0.01 ppm and to the limit of detection at 0.0045 ppm.

**Key words:** Diazomethane, GC/MS, MCPB, MCPA, Herbicides, Peas

## INTRODUCTION

MCPB (2-methyl-4-chlorophenoxybutyric acid) has been used as a post-emergence control of annual and perennial broad-leaved weeds, especially Canadian Thistle in clovers, cereals, sainfoin, and peas since 1954[1,2].

Analysis of MCPB and its metabolite MCPA (2-methyl-4-chlorophenoxyacetic acid) by gas chromatography (GC) is difficult due to their high polarity and low volatility. Therefore, formation of more volatile derivatives such as alkyl esters[3] is required for GC analysis. In order to apply a highly sensitive GC detector, such as an electron capture detector (ECD), some halogenated derivatives were also prepared[4,5]. These methods are satisfactory when the analytical matrix is simple like water and soil. However, if a matrix is more complex (*e.g.*, foods, plant leaves, and animal tissues), it requires an appropriate cleanup process and a high-resolution GC column.

In the present study; a new selective and sensitive analytical method for MCPB and its primary metabolite, MCPA, in pea samples has been developed. The new method involves a liquid-liquid partitioning, derivatization with diazomethane, Florisil® cleanup, and a gas chromatograph (GC) equipped with a fused silica capillary column and interfaced to a mass selective detection (MSD).

## EXPERIMENTAL

### *Materials*

MCPB (99.4%) and MCPA (99.6%) were acquired

*Author to whom all correspondence should be addressed

from Rhône-Poulenc, Essex, England. Diazald® (N-methyl-N-nitroso-*p*-toluenesulfonamide, 99%) was bought from Aldrich Chemical Co., Phillipsburg, NJ. Florisil® (silica based, 60/100 mesh) was obtained from Floridin Co., Berkeley Spring, WV.

Standard solutions of MCPB and MCPA (1.0 mg/mℓ) were prepared by adding 100 mg each of MCPB and MCPA to acetone in 100 mℓ volumetric flasks. MCPB and MCPA fortification solutions (100, 50, and 10 mg/mℓ) were prepared by adding 10, 5, and 1 mℓ of the MCPB or MCPB standard solution to acetone in 100 mℓ volumetric flasks. All solutions were stored at -20℃ until use.

### *Preparation of Diazomethane*

Diazomethane was prepared according to a method previously reported[6]. Ethanol (95%, 25 mℓ) and an 8 mℓ aqueous solution of potassium hydroxide (5 g) were mixed in a 100 mℓ distillation flask. The solution was heated in a water bath to 65℃, and then 200 mℓ of diethyl ether containing 21.5 g of Diazald® (0.1 mole) was added through the dropping funnel. An additional 40 mℓ of diethyl ether was slowly added. The reaction solution was distilled until the distillate became colorless. The final concentration of the diazomethane solution was 15 mg/mℓ. The solution was stored in a -20℃.

### *Collection of Field Samples*

A total of 35 pea samples (18 treated and 17 untreated controls) were collected from the IR-4 field trial sites in California (3), Idaho (4), New York (4), Oregon (4), Washington (12), and Wisconsin (8) and transferred to our laboratory in a frozen state. The rate of the pesticide application was 1.5 lbs (active ingredient)/acre. Upon arrival, the samples were stored in a -20℃ until analysis. See Table 1 for field trial information.

### *Preparation of GC Calibration Curve*

Standard stock solution of MCPB or MCPA (5 mℓ) was derivatized with diazomethane. A GC standard curve was obtained using 200, 100, 50, and 25 pg/mℓ solutions prepared from the derivatized stock solutions.

Recovery efficiencies and quantitative analysis of MCPB and MCPA were determined using a standard GC curve. Injections were performed in duplicate. Chemstation® v. A.02.00 software was used to determine peak area, while Microsoft® Excel® v. 7.0 was used to calculate the standard curve, average peak area, and concentration.

### *Sample Preparation*

Peas without pods, peas with pods, and dry peas (300 g each) were chopped with equal amounts of dry ice using a Hobart food chopper. Each chopped sample was stored in a one-quart jar and a lined lid was loosely closed on top to allow the dry ice to dissipate during storage at -20℃.

A 50 g aliquot of fresh peas (25 g dried peas) was weighed into a 200 mℓ centrifuge bottle (for dried

Table 1 Field trial information

| Sample # | Field location | Sampling date | Crop matrix of pea samples | PHI[1] (days) |
|---|---|---|---|---|
| 1 | Chualar, California | July 28, 1994 | dried | 61 |
| 2 | Kimberly, Idaho | July 27, 1994 | dried | 62 |
| 3 | Freeville, New York | July 18, 1994 | with pods | 35 |
| 4 | Aurora, Oregon | July 8, 1994 | without pods | 46 |
| 5 | Arlington, Wisconsin | June 27, 1994 | without pods | 31 |
| 6 | Arlington, Wisconsin | July 11, 1994 | dried | 28 |
| 7 | Prosser, Washington | July 6, 1994 | without pods | 29 |
| 8 | Prosser, Washington | July 26, 1994 | without pods | 64 |

[1]Post-harvest interval: number of days between pesticide application and crop harvest.

peas, 50 mℓ of distilled water was added and left overnight prior to extraction). Samples for the recovery tests were fortified at this point. Acetone (100 mℓ) was added to the centrifuge bottle and the sample was homogenized using an Ultra-Turrax T25 (Janke & Kunkel, West Germany) for 2 min. The homogenized sample was centrifuged at 1600 rpm for 5 minutes and the supernatant solution was transferred to a 400 mℓ storage bottle. The pea pellet was re-homogenized with 100 mℓ of an acetone/water (1/1, v/v) solution for 2 min and centrifuged at 1600 rpm for 5 min. The resulting material was also decanted into a 400 mℓ storage bottle.

Half of the total sample solution (25 g aliquot, 12.5 g dried peas) and 1 mℓ of concentrated hydrochloric acid was mixed in a 250 mℓ separatory funnel and briefly swirled. The sample solution was extracted with 50 mℓ of petroleum ether. The extract was transferred into a 500 mℓ round-bottom flask through 10 g of anhydrous sodium sulfate. The aqueous fraction was extracted twice with 50 mℓ portions of petroleum ether. Each extract was pooled through anhydrous sodium sulfate into the 500 mℓ round-bottom flask. The pooled extracts were concentrated under vacuum using a rotary evaporator at 30℃ to complete dryness. To each dried sample, 2 mℓ of diazomethane solution was added. The sample was swirled and allowed to stand for 10 minutes at room temperature. After reaction, 5 mℓ of hexane was added to the sample and then the solvent was removed by a rotary evaporator to near dryness. After concentration, 5 mℓ of hexane was added to the sample. The samples were cleaned using a Florisil® column.

The chromatographic glass column (10 cm × 1.5 cm) packed with 3 g of Florisil®, which was activated at 150℃ for 12 h, was prewashed with 10 mℓ of hexane. After the sample was loaded onto the column, the compounds were eluted with 20 mℓ of an ethyl acetate/hexane (5/95, v/v) solution. After all the developing solvent was eluted, the sample volume was adjusted such that the response would fall within the standard curve on the GC/MSD.

### *Analysis of Samples*

Analysis of samples was conducted with a Hewlett-Packard (HP) 6890 gas chromatograph equipped with a 30 m × 0.25 mm i.d. (df = 0.25 μm) HP-5 Trace capillary column (HP, Avondale, PA) and interfaced to a HP 5972 mass selective detector in selected ion monitoring (SIM) mode. The MS ionization voltage was 70 eV. The MS fragment ions (m/z) monitored for MCPA were 214 and 216, and those for MCPB were 142 and 242. The injector and detector of the GC were operated at 285 and 280℃, respectively. An HP Model 6890 series autoinjector was used to inject 2 μℓ (set for fast injection) of sample in splitless mode. The oven temperature was programmed from 80℃ to 145℃ at 8℃/min and then programmed again at 35℃/min to 280℃ and held for 15 min. Helium was used as the carrier gas at 2.3 mℓ/min.

### *Storage Stability Study*

A storage stability study on MCPB and MCPA was conducted using three untreated samples of peas without pods, peas with pods, and dried peas. The testing samples were fortified with 1 ppm of MCPB and MCPA, and stored at -20℃ until analysis. The stored samples were analyzed after all the field samples were analyzed.

## RESULTS AND DISCUSSION

There are only a few reports on the determination of MCPA and MCPB in complex matrix systems such as foods and animal tissues. Concentrations as low as 0.1 mg/ℓ of MCPA and 0.2 mg/ℓ MCPB in a simple water sample were reported by using esters of 1-bromomethyl-2,3,4,5,6-pentafluorobenzene and a packed column GC with an ECD[5].

Later, a simultaneous determination of residues of MCPA and MCPB in soil was achieved by using identical derivatives and a GC system[7]. However, there have been very few reports on MCPA and MCPB analysis using a GC/MS method until recently. For example, MCPA spiked in soil at the level of 0.003 ppm was satisfactorily analyzed after derivatization with diazomethane; the quantitative analysis was done by a capillary column GC/MS[8]. More recently, 30 acidic herbicides and related compounds, including MPCA and MPCB, were determined in a simple water sample[9]. The herbicides were derivatized with pentafluorobenzyl bromide to form their pentafluorobenzylic derivatives; these were subsequently recovered from water using a reversed-phase $C_{18}$-adsorbent. The determination of less than 1 pg of the recovered herbicides was achieved using GC/MS.

In the present study, MPCA and MPCB in considerably complex matrixes of pea samples were satis-

factorily determined. The limit of quantitation for MPCB and MPCA was 0.01 ppm. The minimum detectable value for MPCB and MPCA was 0.0045 ppm. Table 2 shows the results of recovery tests on MPCB and MPCA for all three pea matrixes. Recoveries for MCPB in peas without pods, peas with pods, and dry peas ranged from 69-108%, 87-100%, and 81-103%, respectively. Recoveries for MCPA in peas without pods, peas with pods, and dry peas ranged 63-94%, 72-86%, and 60-79%, respectively.

Table 3 shows the results of storage stability test on MPCB and MPCA. Recoveries for MCPB stored over 850 days ranged from 86-91%, 83-84%, and 77-85% for peas without pods, peas with pods, and dry peas, respectively. Recoveries for MCPA stored over 850 days ranged 74-78%, 71-72%, and 68-77% for peas without pods, peas with pods, and dry peas, respectively. Results from the storage stability study suggests that MCPB and MCPA have little or no breakdown under extended storage times in -20℃.

The results obtained in the present study are comparable to the method reported by Manley[10] who utilized gel permeation chromatography for sample cleanup followed by derivatization with pentafluorobenzyl bromide. Alternatively, we chose to use a simpler method of Florisil® cleanup which uses much less solvent and requires less time to run multiple samples. In addition, diazomethane was used as the derivatizing agent because of the rapid methylation of MCPB and MCPA, and the methylated compounds eluted earlier from the capillary

Table 2 Results of recovery efficiency tests

| Sample matrix | Amount of fortification (ppm) | Recovery of MCPB (%) | Recovery of MCPA (%) |
|---|---|---|---|
| peas without pods | 0.01 | 101 ± 5 (n = 5)[1] | 87 ± 4 (n = 5) |
| peas without pods | 0.10 | 80 ± 12 (n = 5) | 69 ± 9 (n = 5) |
| peas without pods | 0.50 | 88 ± 5 (n = 3) | 78 ± 3 (n = 3) |
| peas with pods | 0.01 | 94 ± 6 (n = 4) | 78 ± 3 (n = 4) |
| peas with pods | 0.10 | 94 ± 5 (n = 4) | 85 ± 1 (n = 4) |
| peas with pods | 0.50 | 90 ± 1 (n = 3) | 82 ± 2 (n = 3) |
| dry peas | 0.01 | 92 ± 7 (n = 5) | 69 ± 6 (n = 5) |
| dry peas | 0.10 | 88 ± 3 (n = 4) | 76 ± 3 (n = 4) |
| dry peas | 0.50 | 85 ± 4 (n = 3) | 76 ± 3 (n = 3) |
| dry peas | 1.00 | 88 ± 1 (n = 3) | 75 ± 5 (n = 3) |

[1]Values are mean ± standard deviation; n is number of duplications.

Table 3 Results of storage stability tests on MCPB and MCPA

| | MPCB | | MPCA | |
|---|---|---|---|---|
| sample matrix | storage time (days) | recovery (%) | storage time (days) | recovery (%) |
| peas without pods | 895 | 89 ± 3[1] | 895 | 77 ± 2 |
| peas with pods | 895 | 84 ± 1 | 895 | 71 ± 1 |
| dry peas | 852 | 80 ± 5 | 852 | 71 ± 5 |

[1]Values are mean ± standard deviation (n = 3).

column than the majority of the matrix compounds in the extracted pea samples. Even though the GC/MS with selected-ion monitoring method is highly specific, it is ideal to obtain minimal chromatographic interference.

Pea samples treated with MCPB and control samples from the trial fields had no residues (MCPB or MCPA) above the limit of quantitation at 0.01 ppm, except for one dry peas sample obtained from Idaho which contained 0.017 ppm of MCPA.

The USDA IR-4 (United State Department of Agriculture Inter Regional Research Project No. 4, Minor Use Pesticide Registration Program)[11] has undertaken to study the minor use registration of MCPB on peas for control of broadleaf weeds. The present study was conducted in part as an IR-4 project. Given the recent passage of the Food Quality Protection Act (FQPA) in the U.S., the United States Environmental Protection Agency (USEPA) has pushed pesticide tolerances lower with time, thus pushing laboratories to create faster methods with greater sensitivity. In order to maintain the tolerances set by the EPA (0.1 ppm for MCPB and MCPA), it is required that the magnitude of the residue in or on the commodity be determined according to the USEPA Guidelines 171-174 and Subdivision O[12]. The new method developed in the present study for determination of MCPB and MCPA satisfies these requirements.

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