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Short communication

# Determination of chlorophenoxy herbicides in waters by capillary gas chromatography with ion trap detection

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**Abstract**

A method for the determination of 4-chloro-2-methylphenoxyacetic acid (MCPA), 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in water samples is reported. Methyl esterification with boron trifluoride–methanol ($BF_3$–$CH_3OH$) and mass spectrometric detection in the electron ionization mode (EI–MS) have been evaluated. The use of mass spectrometry detection places less pressure on the need for a very efficient clean-up procedure. Further, herbicides positively identified in samples are automatically confirmed without resorting to a mass spectrometer. In this procedure we can avoid the clean-up procedure and the use of anhydrous sodium sulfate as a drying agent. The water samples were acidified to pH<1 and the herbicides were extracted with methylene chloride. After methylation with boron trifluoride, the esters were determined by gas chromatography–ion trap spectrometry with a SE-54 fused-silica capillary column, over the mass range 60–430 amu. The method was successfully validated for herbicide concentration as low as 0.04 μg/l for MCPA and 2,4-D and 0.05 μg/l for 2,4,5-T. Recoveries from water samples spiked with the 3 herbicides at 0.05 to 0.25 μg/l ranged from 63.5 to 92.0%.

*Keywords:* Pesticides; MCPA; 2,4-D; 2,4,5-T

## 1. Introduction

Pollution of surface waters by pesticides is a problem of increasing environmental concern which should be regularly monitored. During studies of the extent of possible pesticide contamination of surface waters in Baixo Mondego Valley, Portugal, it was necessary to monitor the chlorophenoxy herbicides in natural waters.

The most extensive use of these herbicides in water, is in the production of rice, and one could expected water bodies within the treated areas to become contaminated. Thus, analysis of samples originating from agricultural drainage is important since this data gives valuable information about the general risk of surface water pollution.

The interest in and apprehension of phenoxy herbicide residues in the environment are undoubtedly related to their extensive use, their toxicity, as well as the presence of the extremely toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as a side product in the production of 2,4,5-T [1–3].

Effective water pollution control requires analytical methodology that allows correct identification and measurement of the low concentration of these compounds in water samples [4].

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There are still many requests for analysis of these compounds, particularly for samples originating from agricultural drainage; irrespective of their formulation they are determined as free acids. This partly results from the assumption that ester hydrolysis generally occurs rapidly in an aquatic environment, and thus only the acidic form need to be considered [5,6]. For this reason prior hydrolysis was omitted in this method.

The acidic herbicides were determinated in water samples by GC–ECD after their conversion to methyl esters or as pentafluorobenzyl derivatives [7].

Traditionally, esterification to methyl esters has been the method of choice for the chlorophenoxy acids. However, because the electron capture response of monochlorinated herbicides is particularly weak, it is necessary to use their pentaflurobenzyl esters (PFB) [8]. A major disadvantage of the PFB procedure is the large amount of interfering substances present when the extract is analyzed by ECD necessitating a clean-up procedure for the extracts. Some clean-up procedures require lengthy liquid–liquid partition steps and suffer from possible loss of analytes during the transfer processes [9]. Another problem associated with the PFB approach lies in the experimental conditions necessary for derivatization (up to 5 h at room temperature) [10].

The procedures for the determination of chlorinated herbicides proposed by the EPA, method 615 [11] and method 8150 [12], use diazomethane for methylation of the free acid herbicides. This reagent is known to be toxic and explosive. Method 8150 states that microcoulombic detection is preferred to electron capture detection for the methylated herbicides. Method 615 is a more recent one than method 8150: the determination is made by GC using an electron capture detector and it is recommended that gas chromatography–mass spectrometry (GC–MS) techniques be employed to support qualitative compound identifications.

In the Blue Book method [13], the herbicides were reacted with pentaflurobenzyl bromide to form the corresponding esters, which were determined by capillary column gas chromatography with electron capture detection. However, the reactive nature of the reagent and the strong electron capture properties of the derivatives cause the method to be often subject to unwanted interferences and poor base lines. The interferences are often unpredictable and are often not offset by the inherently very low limit of detection of the method. As a result, GC–MS operated in the multiple ion detection mode to determine the methylated esters of the herbicides has been introduced as a confirmatory procedure. In fact, at present this is often the preferred method. Interference effects with this approach are negligible and limits of detection of 0.05 μg/l have been achieved.

In this study, an alternative procedure for the esterification with boron trifluoride–methanol ($BF_3$–$CH_3OH$) and mass spectrometry detection in electron ionization mode (EI–MS) was investigated.

The method was applied to MCPA, 2,4-D and 2,4,5-T in surface water samples collected from 24 stations along the paddy field located at Baixo Mondego Valley, Portugal, during the cultivation season.

## 2. Experimental

### 2.1. Reagents

Each batch was tested for any impurities before use. Dichloromethane, acetone and isooctane, pesticide grade solvents, were purchase from Carlo Erba (Italy). Hexane (capillary analysed), sulfuric acid (analysed 95–97%) were obtained from J.T. Baker (Netherlands). Boron trifluoride–methanol 14% was purchased from Sigma–Aldrich. Sodium sulfate pesticide grade was obtained from Merck (Germany). A solution at 2% (w/v) was prepared. Water was purified by distillation and passage through Milli-Q system (Millipore). The first 100 ml of distillate was discarded [14]. Anthracene $d_{10}$ was acquired from Alltech Associates (USA). Herbicide acid standards – analytical grade standards – were obtained from Dr. Ehrenstorfer (Germany): MCPA 99.9%; 2,4-D 99.9% and 2,4,5-T 99.6%. Individual stock standards solutions were prepared in 100 ml hexane, containing 100 mg neat standard corrected to 100% purity. The final working standard solutions were prepared in hexane at 0.05, 0.10, 0.25, 0.50 and 1.00 μg/ml. Calibration standards were prepared by derivatizing these solutions with boron trifluoride–

methanol. Stock standards solutions were stored at 4°C protected from light. Stock standards solutions were checked frequently for degradation or evaporation, especially immediately before preparing the calibration standards. Ashless filter-papers Whatman No 41 were used. Note: to remove any possible interferents, the filter-papers and cotton were Soxhlet-cleaned with acetone for 8 h, dried in a ventilation hood and stored by wrapping in aluminium foil [15]. All glassware was soaked in concentrated acid–dichromate solution, washed thoroughly with a solution of Extran MA 03 (Merck, Germany) (10% v/v), rinsed with distilled water and dried at 80°C, then rinsed with pesticide grade acetone and/or hexane immediately prior to use [16].

### 2.2. Apparatus and chromatographic conditions

A Perkin Elmer Model 8500 gas chromatograph coupled to an ion trap detector (ITD -Finnigan Mat 800) in electron impact mode (EI) was used. The gas chromatograph was fitted with a 30 m×0.25 mm I.D., SE-54 capillary column with a film thickness of 0.25 μm (J&W Scientific). The linear velocity of helium (carrier gas) was 32 $cm^{-1}$ at 200°C and a column head pressure of 15 psi. Injector temperature was 280°C. Samples were injected in the splitless mode with a 1-min purge off. Column temperature program: initial 70°C for 1 min, program 10°C/min to 190°C, hold 2 min, program 5°C/min to 250°C, hold 10 min. The GC to ITD interface: capillary direct interface. The mass spectrometric conditions were as follows: electron energy, 70 eV; electron multiplier voltage, 1800 V; transfer line temperature, 270°C. The ITD was optimised with the automatic set-up program in the Automatic Gain Control (AGC) software and produced mass spectrum that met calibration compound (perfluorotributylamine) performance criteria. Tune values used to set the resolution at *m/z* 69, 131, 264 and 502, in the spectrum of perfluorotributylamine were: 50, 95, 130 and 200 at a "B" value of 5000. The computer system connected to the mass spectrometer must allow continuous acquisition and storage of all mass spectra obtained, throughout the complete chromatographic program. Mass spectra were normalized after background subtraction.

### 2.3. Sample extraction, derivatization and concentration procedure

Duplicate surface water samples (2.5 l) were collected in brown glass bottles and extracted within 24 h of collection. Prior to extraction the entire water sample was filtered. The water sample was shaken in the sampling container and then poured into a separation funnel. The sample, after pH adjustment with concentrated $H_2SO_4$ until pH<1 (pH paper), was extracted vigorously three times with 100 ml of methylene chloride for 2 min. The extracts were collected into a 500-ml round-bottom flask. Residual water was eliminated by submitting the extracts to a freezing process and filtration through filter-papers with cotton. The combined dichloromethane extracts were then concentrated to approximately 5 ml by using a rotary vacuum evaporator (35°C), transferred to a 15-ml conical centrifuge tube and further concentrated to ca. 2 ml under a gentle stream of nitrogen. Then, the residue was esterified with 2 ml of $BF_3$–methanol mixture for 15 min at 70°C in a water bath (centrifuge tube must be tightly stoppered and intermittent swirling of the tube contents must be employed during esterification). The tube contents were cooled unstopped and after adding 2 ml of 2% $Na_2SO_4$ solution and 10 ml of hexane, they were shaken vigorously and centrifuged for 5 min at approximately 1800 *g*. Isooctane (Keeper) (1 ml) was added to the *n*-hexane extracts which were concentrated to just 0.5 ml under a gentle stream of nitrogen. Anthracene $d_{10}$, used as internal standard, was added to the sample extract at 1 mg per ml of injection volume and mixed thoroughly immediately before injection.

## 3. Results and discussion

### 3.1. Chromatography and detection

The usefulness of the SE-54 column is clearly shown by the adequate resolution of all three herbicide methylated esters in the total ion chromatogram, produced by scanning over the mass range *m/z* 60–430 amu (Fig. 1).

The presence of these compounds in samples was confirmed by comparison of their GC retention times

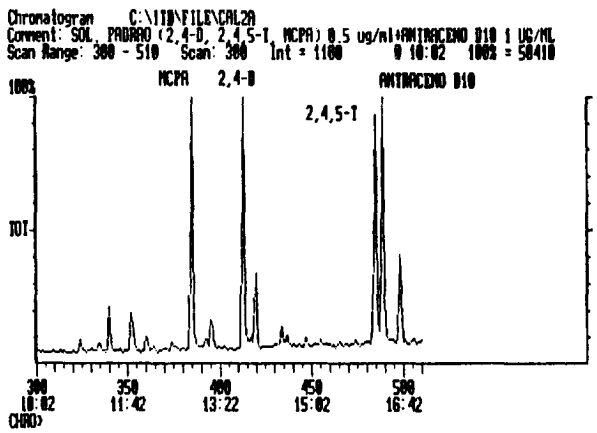


Fig. 1. Total ion chromatogram of methyl esters of the herbicide standards with added anthracene $d_{10}$.

and ion trap mass spectra with those of calibration standards analyzed under identical conditions and by additional library searches in NBS Library. Ion trap mass spectra of the methyl esters of the calibration standards of MCPA, 2,4-D, 2,4,5-T and anthracene-$d_{10}$ at 1 ng, are shown in Fig. 2. The methyl esters of these compounds yield abundant molecular ions which together with the chlorine isotope abundance ratios permit their easy identification. The derivatives of 2,4-D and 2,4,5-T (with a chlorine atom in the *ortho* position) exhibit a base peak corresponding to the loss of this chlorine atom. The methyl esters of MCPA with a methyl substituent on the benzene ring, show abundant ions at $m/z$ 125 (chlorotropylium ion) [17].

### 3.2. Quantification

We used as internal standard deuterated anthracene not normally found in water. A constant amount of the internal standard is added to calibration standards just before injection. The peak measurements used were based on the extracted ion current profiles for the specific characteristic ion of each calibration standard and internal standard, i.e.: MCPA, $m/z$ ranged from 214–216; 2,4-D, $m/z$ ranged from 199–201; 2,4,5-T, $m/z$ ranged from 233–234; anthracene $d_{10}$, $m/z$ 188 [18–20].

### 3.3. Calibration and recovery

The calibration graphs for the standards MCPA, 2,4-D and 2,4,5-T (Fig. 3) were obtained by plotting the ratio of the peak areas of the calibration standards and the internal standard, against their concentration ratio between 0.05 and 1.00 μg/ml, i.e. at 0.05; 0.10; 0.25; 0.50 and 1.00 μg/ml respectively. The plots were linear with a mean correlation coefficient of 0.998 for MCPA and 2,4-D and 0.993 for 2,4,5-T.

Experiments with five spiked surface water samples showed that recoveries were better than 80% for all the three herbicides at both levels (0.05 and 0.25 μg/l). The coefficient of variation (C.V.) was between 5.7 and 11.0%, indicating good reproducibility of the method. The detection limit for this method of the studied herbicides, based on 2.5 l of sample concentrated to 0.5 ml and injection of 1 μl, was 0.04 μg/l for MCPA and 2,4-D and 0.05 μg/l for 2,4,5-T. The results show that the accuracy, sensitivity and reproducibility of the method are adequate for the determination of these residues in water. Low background interferences in natural waters easily permitted the limits of detection initially achieved.

### 3.4. Application to actual samples

The proposed method was applied to surface waters. These waters were found to contain MCPA at levels far below the allowed tolerances, this concentration ranged from 0.062 to 0.196 μg/l. Fig. 4 shows an example of a full scan chromatogram for a sample. The Baixo Mondego Valley is a typical rice

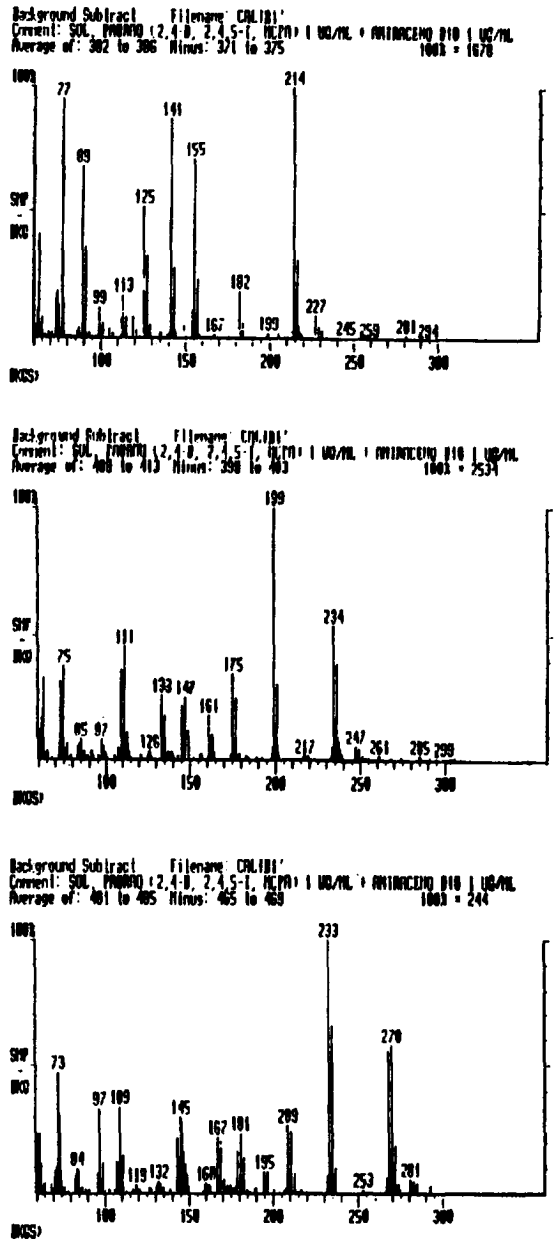

Fig. 2. Ion trap mass spectra of the methyl esters of MCPA, 2,4-D and 2,4,5-T.

cultivation area where MCPA is applied in large amounts during spring and summer. According to Soderquist and Crosby [21] the disappearance of MCPA in water was due primarily to biological and chemical breakdown and not to dilution. With the inherent limitations of applying this data to other situations, it seems that irrigation water in a rice field treated with MCPA should contain negligible amounts within 14 days of application.

Residues of 2,4-D and 2,4,5-T were not detected in the 24 samples subjected to analysis.

## 4. Discussion

The extraction with dichloromethane produced good recoveries, and since this solvent has a much higher dielectric constant and is much less soluble in water than ethyl ether and ethyl acetate, we could avoid the use of anhydrous sodium sulphate [22,23]. The use of anhydrous sodium sulphate, even acidified, as described by Goerlitz and Lamar [24], was not incorporated into this procedure for drying of the sample extract. The use of this drying agent led to erratic recoveries of acidic herbicides [25] and was a source of interferences of laboratory origin [26]. For a dichloromethane extract, freezing and filtering through a small plug of cotton will remove all the water from the extract [23]. Furthermore, dichloromethane has a higher density than water and it can be easily separated. Since we have used a GC coupled to an ion trap detector, it was not necessary to use the PFB procedure to increase the sensitivity of monochlorinated herbicides towards electron capture detection in GC. Furthermore, PFB reaction solutions should be concentrated only to 10 ml rather than 0.5 ml because of the poor blanks. This is reflected in less sensitive detection [27]. In fact, "dirty" GC–ECD chromatograms of MCPA air samples forced Crosby et al. [28] to abandon PFB derivatization and revert to diazomethane derivatization.

Gurka [29] discovered that the PFB ester of 2,4-D coeluted from a GC (DB-5 capillary) column with a non-herbicide by-product that survived the clean-up procedure. Accordingly, the work on the 2,4-D PFB ester was suspended.

As a result of a quantitative comparison of esterification techniques for 2,4-D, Horner et al. [30] observed that the reagent of choice for producing a high yield of gas chromatographically pure esters in the shortest time was the $BF_3$–$CH_3OH$ reagent. In our laboratory, we investigated the possibility of extending this derivatization procedure to MCPA and 2,4,5-T and we observed that the esterification

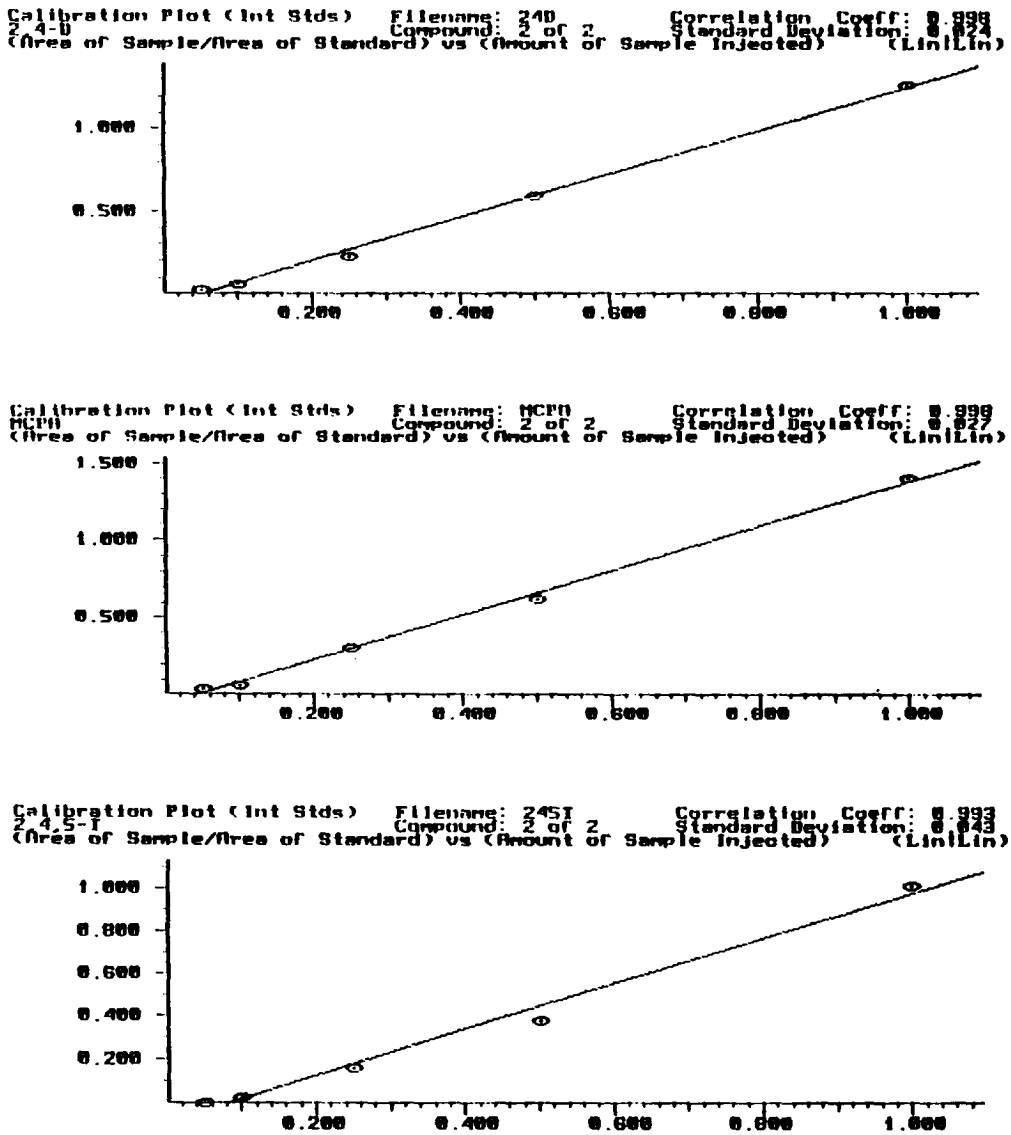


Fig. 3. Calibration graphs of 2,4-D, MCPA and 2,4,5-T.

procedure was efficient, simple, safe and gave a good response in the GC–MS system for all the herbicides. This procedure introduced the least contamination and allowed concentration of the extract to 0.5 ml even in the absence of clean-up [29].

The advantage of gas chromatography coupled to an ITD, compared with ECD, is that the selectivity of the ITD allows quantification of the herbicide irrespective of extraneous peaks present at the same retention time [31–33]. The use of ITD places less pressure on the need for a very efficient clean-up procedure and since we produced clean blanks the clean-up procedure was eliminated. Furthermore, using deuterated anthracene as an internal standard and mass spectrometric analysis, no purification of water extracts is required.

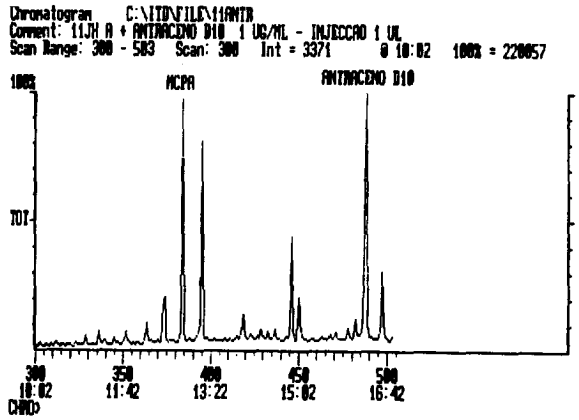


Fig. 4. Contaminated sample with MCPA.

As a result, gas chromatography linked to ion trap mass spectrometry to determine the methylated esters of the herbicides, is the preferred method. Interference effects with this approach are negligible at very low limits of detection and sensitivity is high [34].

The identity of the species to be quantified is confirmed by the ion profile as well as the retention time on the gas chromatographic column, thus avoiding the double gas chromatographic runs for peak identification found in many standard GC methods.

The proposed method has adequate sensitivity, accuracy and selectivity, and water samples can be analyzed within a reasonable time at a reasonable cost in the laboratory.

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