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Determination of polar pesticides by phase-transfer catalysed derivatization and negative-ion chemical ionization gas chromatography–mass spectrometry

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ABSTRACT

A single-step procedure for the extraction and derivatization of carboxylic pesticides from aqueous samples is described. The acidic compounds, including phenoxy herbicides, were extracted from aqueous samples using tetrahexylammonium hydrogensulphate as a catalyst and were subsequently derivatized with pentafluorobenzyl bromide to volatile stable and electron-capturing derivatives. Analysis was performed by gas chromatography with electron-capture detection combined with negative-ion chemical ionization mass spectrometry. The recovery of the compounds was 108 ± 8%. Detection limits were typically 0.05 μg/l, using mass unit resolution. Highly contaminated samples required increased resolution ($1000 \leq R_f \leq 4000$) for reduced chemical background and enhanced selectivity for detection at low-ppb levels. The method was applied to the determination of some phenoxycarboxylic pesticides in surface water and in blood samples.

INTRODUCTION

Sensitive methods are needed for the determination of a wide variety of pesticides in water in order to meet the present norms for drinking water as set by the US Environmental Protection Agency (EPA) and individual governments. In most methods chromatographic techniques are applied [1,2], but also immunoassays [3], *e.g.*, enzyme-linked immunosorbent assay, and electrochemical techniques, *e.g.*, polarography [4], are now being used for specific pesticides or special applications. The use of different detection techniques in conjunction with adequate isolation methods makes it possible to determine pesticides with a wide range of polarities and volatilities. The determination of polar pesticides, however, is complicated concerning both their isolation and their chromatographic separation and detection.

For the determination of polar pesticides, high-performance liquid chromatography (HPLC) appears to be the most appropriate technique. In recent years, numerous methods for the determination of polar pesticides have been published [5,6]. In most methods clean-up and concentration are achieved by solid-phase extraction (SPE) or column-switching techniques [7]. In addition, the coupled technique of LC with mass spectrometry (MS) has been used for confirmation and identification purposes [8]. Several interfaces have been described for coupling these two techniques [9,10], but the thermospray and particle beam interfaces have become the most popular. However, it was found that the behaviour and sensitivity of these techniques for different pesticides may vary widely. The behaviour appears to be mainly determined by the chemical and physical prop-

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erties of the analytes, but may also be dependent on instrumental and analytical conditions. In addition, LC–MS interfaces have restrictions concerning flow-rate and eluent additives (buffers, salts, etc). Further, LC is less useful for screening purposes than gas chromatography (GC), mainly because of its relatively low separation efficiency. GC may be the preferred technique for screening. The determination of polar compounds by GC, however, often necessitates prior chemical modification in order to increase their extraction and/or enhance their GC properties.

For polar pesticides, numerous isolation and derivatization techniques have been published [11]. Particularly isolation and SPE clean-up are often used for a wide range of pesticides, also because of the good possibility of automation for routine analysis.

For acidic herbicides, isolation from aqueous samples can be performed by liquid–liquid extraction or on C_{18} SPE columns, followed by alkylation (*e.g.*, methylation with diazomethane) and GC with flame ionization detection (FID) or electron-capture detection (ECD) [12]. The latter technique can only be used for the detection of compounds that have electron-capturing properties. Typical detection limits for such compounds are in the range 0.05–0.1 ppb in water, depending on the extent of concentration during sample preparation. However, the detection of non-electron-capturing compounds (*e.g.*, bentazone) can only be achieved by ECD after derivatization with a reagent having an electronegative function.

In this paper, we describe a method for the extraction and subsequent esterification of the acidic functional groups with pentafluorobenzyl bromide (PFB-Br). To ensure simplicity of the method for screening purposes (*i.e.*, rapid sample clean-up and the use of small, easy-to-handle sample sizes), we chose to extract and derivatize the compounds of interest simultaneously using phase-transfer catalysed (PTC) derivatization [13]. This technique has been applied previously in biochemical and pharmaceutical analysis, but has not been used frequently in environmental analysis [14,15].

PTC derivatization uses a complexing agent (commonly a tetraalkylammonium compound) which transports the compound of interest from the aqueous sample into the organic layer, where the derivatization occurs (Fig. 1). The derivatization of the compounds is based on the alkylation by an alkyl halide of the carboxylic moiety to the corresponding ester. To achieve maximum sensitivity, some constraints with respect to the derivatization reagent are necessary: (1) the reagent must be fairly stable towards hydrolysis because of the presence of a two-phase system; and (2) for sensitive measurements, compounds with electron-capturing groups are preferred to accomplish electron-capture negative ion chemical ionization MS (EC-NICI-MS) detection; consequently, a halogen-rich reagent has to be used to introduce the electron-capturing moiety into all of the derivatives. The use of perfluorobenzyl bromide satisfies these conditions and, in addition, yields stable and volatile derivatives.

In addition to the method development, the application of the method to the determination of some polar herbicides in different water samples and in human blood is described.

EXPERIMENTAL

Chemicals and reagents

Before use, demineralized water was passed through a Milli-Q reagent water system (Milli-

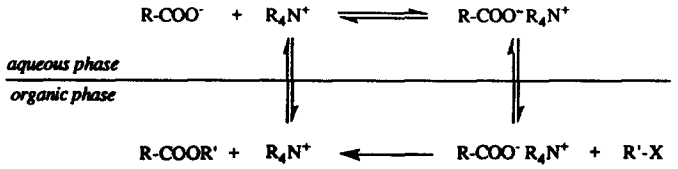

Fig. 1. Scheme of the phase-transfer alkylation of a carboxylic acid (RCOOH) with an alkyl halide (R'X) in the presence of a tetraalkylammonium catalyst ($R_4N^+X^-$).

pore, Milford, MA, USA), fitted with one Super-C carbon cartridge, two Ion-Ex cartridges and an Organex-Q cartridge.

The structures of the compounds studied are shown in Fig. 2. Dalapon-sodium (2,2-dichloropropionic acid, sodium salt) was obtained from Riedel-de Haën (Seelze, Germany), dicamba (3,6-dichloro-*o*-anisic acid), endothal-sodium (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid, disodium salt), MCPA (4-chloro-*o*-tolyloxyacetic acid), MCPB [4-(4-chloro-*o*-tolyloxy)butyric acid], mecoprop [MCPP; (*R*,*S*)-2-(4-chloro-*o*-tolyloxy)propionic acid], 2,4,5-T [(2,4,5-trichlorophenoxy)acetic acid] and bentazone [3-isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide] from Dr. S. Ehrenstorfer (Augsburg, Germany), dikegulac-sodium (2,3:4,6-di-O-isopropylidene-α-L-*xylo*-2-hexulofuranosonic acid, sodium salt) from Schmidt (Amsterdam, Netherlands) and 2,3,6-TBA (2,3,6-trichlorobenzoic acid) from the National Physical Laboratory (Teddington, Middlesex, UK). Tetrahexylammonium hydrogensulphate was obtained from Fluka (Buchs, Switzerland), pentafluorobenzyl bromide and triethylamine from Pierce (Rockford, IL, USA), acetonitrile from Rathburn (Walkerburn, UK) and sodium dihydrogenphosphate monohydrate, anhydrous dipotassium hydrogenphosphate, orthophosphoric acid (85%), fuming hydrochloric acid (37%), dichloromethane and *n*-hexane from Merck (Darmstadt, Germany). Anhydrous sodium sulphate (Baker, Deventer, Netherlands) was heated at 450°C for at least 4 h before use.

A 10 m*M* solution of tetrahexylammonium hydrogensulphate in dichloromethane was prepared freshly each week. Phosphate buffer (pH 7.4) was made by dissolving 14.96 g of

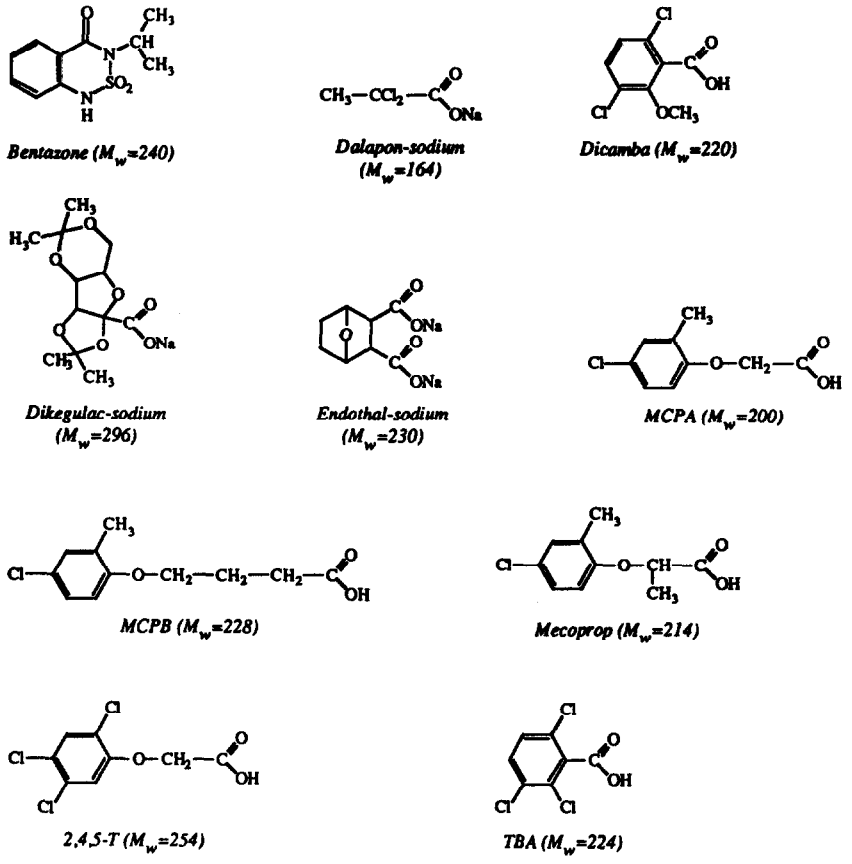

Fig. 2. Structures of model compounds studied. M_w = Molecular mass.

$Na_2HPO_4 \cdot 2H_2O$ and 2.16 g of KH_2PO_4 in 100 ml of demineralized water.

Standard solutions of the individual pesticides were prepared at a concentration of 1 mg/ml in demineralized water. A pesticide standard mixture with a final concentration of 0.5 ng/ml of each pesticide in demineralized water was prepared from the individual standard solutions and stored at 4°C.

Derivatization

Simultaneous extraction and derivatization of the compounds was carried out in test-tubes with PTFE-lined screw-caps. To the water sample or the pesticide standard mixture (9 ml) were added 1 ml of phosphate buffer (pH 7.4), 3 ml of tetrahexylammonium hydrogensulphate in dichloromethane (10 m*M*) and 20 μl of pentafluorobenzyl bromide. The mixture was shaken vigorously for 50 min in a horizontal position with *ca.* 250 strokes/min. The reaction was stopped by the addition of 350 μl of 6 *M* HCl. After phase separation, the organic layer was transferred into a clean tube, dried with anhydrous sodium sulphate and subsequently evaporated to dryness with a small flow of nitrogen at room temperature. Finally, the residue was dissolved in 200 μl of *n*-hexane and 2 μl of the extract was subjected to GC–MS analysis.

Recoveries were calculated by comparison of the yield of the aqueous derivatization with that of a direct anhydrous derivatization. This direct derivatization was carried out on dry residues in 50 μl of acetonitrile, to which 10 μl of pentafluorobenzyl bromide and 10 μl of triethylamine were added. After incubation for 10 min at room temperature, the derivatives were isolated by adding 0.5 ml of 0.1 *M* HCl and 3 ml of ethyl acetate. After extraction, 2 μl of the organic layer were subjected to GC–MS analysis.

Gas chromatography–mass spectrometry

Experiments were performed on a Finnigan 4500 GC–MS system. GC separations were carried out on a CP Sil 19CB fused-silica column (25 m × 0.25 mm I.D., 0.25 μm film thickness) (Chrompack, Middelburg, Netherlands), connected to a split–splitless capillary injector. The injector and transfer line temperatures were 260°C and 2 μl of the final extract were injected in the splitless mode. The oven temperature was programmed from 70°C (held for 1 min) to 220°C at 15°C/min, followed by an increase to 260°C at 5°C/min, the final temperature being maintained for 10 min.

The mass spectrometer was operated in the negative-ion chemical ionization mode with methane as moderator gas [source pressure 0.30 Torr (1 Torr = 133.3 Pa)] and an electron energy of 70 eV. The source and manifold temperatures were 140 and 90°C, respectively. Multiple ion detection (MID) was performed at the *m/z*

TABLE I

PESTICIDES INVESTIGATED AND THE PROMINENT FRAGMENTS OF THE CORRESPONDING PENTAFLUOROBENZYL ESTERS WITH THE RELATIVE INTENSITIES UNDER EC-NICI CONDITIONS

No.	Compound	Molecular mass as PFB derivative	Main ions (*m/z*)[a]
1	Bentazone	420	239.0490 (100), 241.0470 (5.8)
2	Dalapon	322	140.9510 (100), 142.9481 (64)
3	Dicamba	400	218.9616 (100), 220.9587 (65)
4	Dikegulac	454	273.0974 (100)
5	Endothal	546	199.0606 (100)
6	MCPA	380	199.0162 (100), 201.0135 (33)
7	MCPB	408	227.0475 (100), 229.0449 (33)
8	Mecoprop	394	213.0318 (100), 215.0292 (33)
9	2,4,5-T	434	252.9226 (100), 254.9197 (97)
10	TBA	404	222.9120 (100), 224.9091 (97)

[a] Relative intensities (%) in parentheses.

values listed in Table I. For identification purposes, full-scan spectra were acquired in both electron impact and positive-ion CI modes (1 scan/s).

High-resolution EC-NICI experiments ($1000 \leq R_f \leq 4000$) were carried out on a Finnigan MAT-95 GC–MS system. The GC parameters were as described above. The mass spectrometer was operated in the MID mode with perfluorokerosene (PFK) as reference gas. Methane was used as moderator gas at a source pressure of 0.15 Torr.

RESULTS

Derivatization

The most important parameters affecting the reaction kinetics are the pH of the aqueous phase, the polarity and solvatation ability of the organic layer and the concentration of the counter ion.

Fogelquist *et al.* [16] showed that at high pH (>9) a significant increase in the amount of by-products occurred, which could hamper the determination of the compounds of interest. At pH 7.4 the derivatization was complete within 50 min, without the formation of excess by-products interfering the EC-NICI-MS measurements at low levels. However, the extracts were not clean enough for direct GC–ECD measurements at trace levels, owing to the lack of selectivity of this detection method. Allender [17] described a method for an additional SPE clean-up of PFB extracts on Florisil for analysis by GC–ECD of the PFB esters of some chlorophenoxy herbicides.

The PTC derivatization of the pesticides was quantitative when compared with the anhydrous derivatization. The overall recovery of the PTC derivatization was 108 ± 8% (Table II).

GC–MS analysis

As shown in Fig. 3, the PFB derivatives of acidic pesticides possess favourable GC properties. Although co-elution occurred for some compounds, no interference was found when MID was performed. EI- and positive-ion CI measurements were carried out to confirm the identity of the derivatives. Negative-ion CI mass spectra contained intense fragment ions at $[M - 181]^-$ for most of the derivatives investigated (Fig. 4). After ionization, the esters were subjected to a unique tailor-made fragmentation to yield the neutral 2,3,4,5,6-pentafluorobenzyl radicals and $[M - 181]^-$ as the highly specific and diagnostic carboxylate anions [18]. For the doubly derivatized (aliphatic) pesticide endothal, the fragmentation pattern was different: rearrangement of the substituents occurred on both carboxylic moieties, yielding a monomethyl ester

TABLE II

COMPARISON OF THE YIELD OF THE AQUEOUS PHASE-TRANSFER CATALYSED DERIVATIZATION WITH THAT OF THE DIRECT ANHYDROUS DERIVATIZATION METHOD

Compound	PTC[a] ($n = 2$)	Direct[a] ($n = 3$)	Recovery of PTC method (direct = 100%)
Bentazone	4.66	4.61	101
Dalapon	8.69	6.99	124
Dicamba	4.53	4.33	105
Dikegulac	1.73	1.71	101
Endothal	1.12	0.96	117
MCPA	4.11	4.01	102
MCPB	1.99	1.81	110
Mecoprop	5.11	4.94	103
2,4,5-T	1.44	1.34	108
TBA	3.29	3.12	105
		Mean:	108 ± 8

[a] Response of the derivatized compound *versus* that of an injection standard added to the final extract (*i.e.* tetrachloro-*p*-phthalic acid dimethylester in *n*-hexane).

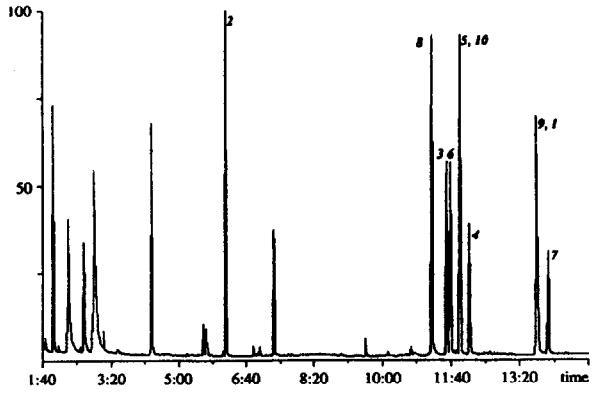

Fig. 3. Reconstructed ion chromatogram of a derivatized carboxylic pesticide mixture. Numbers on the peaks refer to Table I. Time in min:s.

(via proton transfer) in conjunction with a singly charged carboxylate anion $[M-347]^-$.

In the absence of an additional clean-up, the detection limit was mainly determined by the chemical noise introduced from both the sample and the derivatization procedure. In drinking water and relatively clean surface water samples, the selectivity of the method was high enough for detection and identification based on retention times and isotope ratios, permitting determinations at low-μg/l levels ($\geq$0.05 μg/l). The calibration graphs for the individual pesticides were straight and passed through the origin. An example is shown in Fig. 5 for dikegulac. Results of the determination of some polar acidic pesticides in samples from different origins are given in Table III. Groundwater, spiked at a level of *ca.* 0.2 μg/l of each pesticide, showed recoveries of *ca.* 106%. In polluted surface water, no significant difference was found in the results with or without removal of the solids by means of centrifugation prior to the PTC derivatization.

Further, the method was tested on whole blood samples from a subject suspected to have been exposed to mecoprop. After removal of the

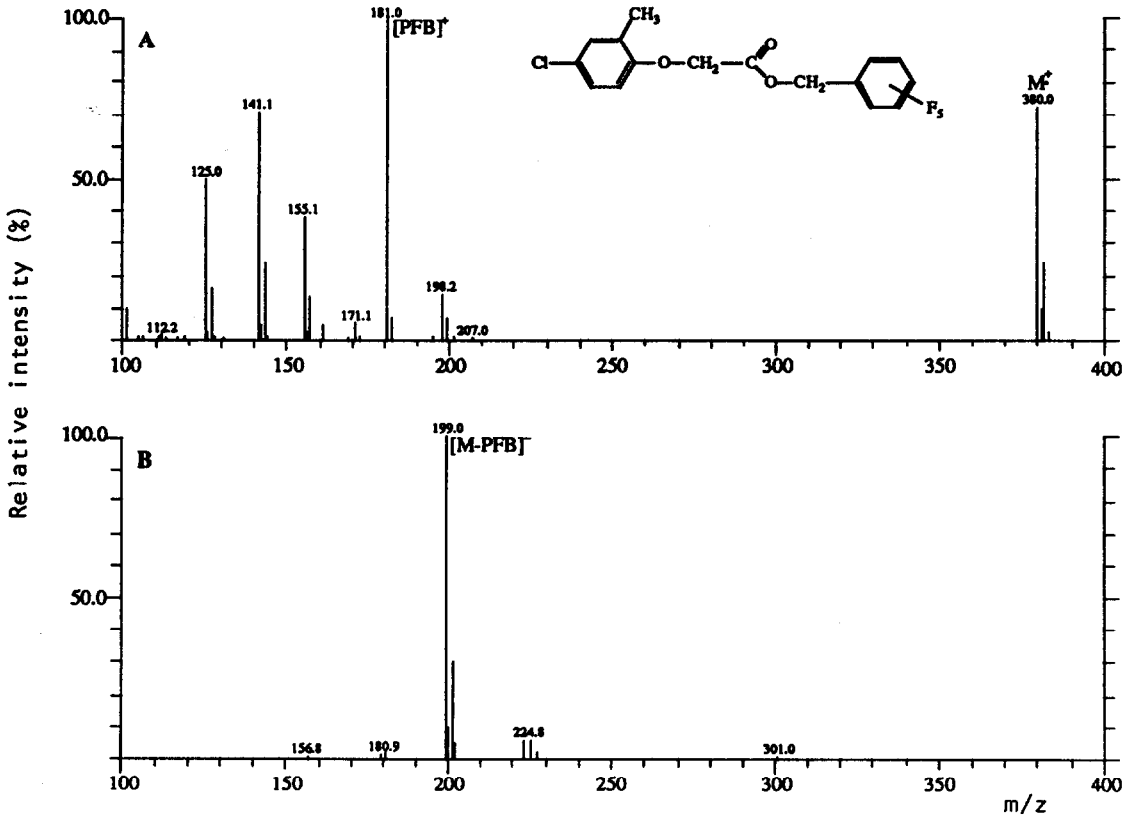

Fig. 4. (A) EI and (B) NICI mass spectra of the PFB derivative of MCPA.

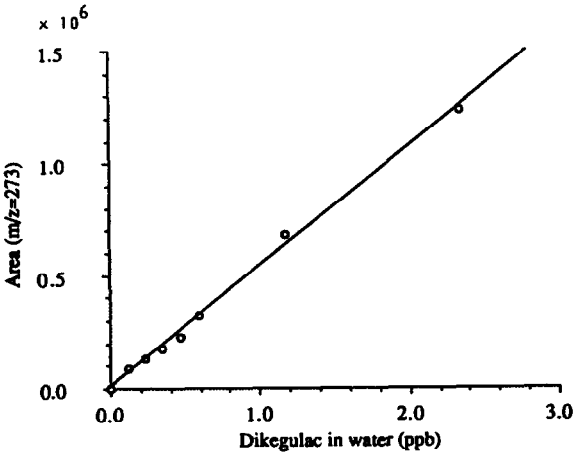

Fig. 5. Calibration graph for dikegulac in water using PTC derivatization with selected ion monitoring at m/z 273 (*i.e.*, $[M - PFB]^-$). ppb = μg/l.

red blood cells by centrifugation, 400 μl of the clear supernatant were diluted with water and subjected to the PTC derivatization, followed by GC–MS on a quadrupole instrument. No mecoprop could be found in the sample, but the addition of mecoprop to the sample before the centrifugation step at a level of 1.2 mg/l resulted in a high response for the mecoprop–PFB derivative. Under these conditions, the limit of detection for mecoprop in blood was found to be *ca.* 7 μg/l at a signal-to-noise ratio of 10. Obviously, the PTC derivatization was suitable for the determination of acidic pesticides without further sample clean-up for difficult samples such as human blood.

Increased resolution MS analysis

Although mass unit resolution analysis on a quadrupole instrument gave sufficient selectivity for most samples, increased selectivity was required for low-level analyses of dirty samples. Addition of mecoprop at the 0.1 μg/l level to such a sample (ground water), showed no identifiable peaks above the background at unit resolution (Fig. 6). At a resolution of 1000 the background was significantly reduced, but the measured isotope ratio from chlorine (theoretical ratio 33%, measured 5.4%) was indicative of a peak impurity. Elimination of the interfering chemical background was achieved at a resolving power of 4000, with a moderate decrease in sensitivity.

TABLE III

LEVELS OF SOME ACIDIC PESTICIDES IN SURFACE WATER

All measurements were performed at mass unit resolution.

Sample	Concentration (μg/l)						
	Bentazone	Dalapon	Dicamba	Dikegulac	MCPA	Mecoprop	2,4-D
Ground water 1		0.15	0.05		0.05	0.36	<0.05
Ground water 2		0.14	0.05		0.05	2.19	<0.05
Ground water 3		0.20	0.05		0.06	0.07	<0.03
Ground water 4		0.16	<0.05		0.08	0.06	0.06
Ground water (add.)[a]		0.22(0.21)	0.20(0.18)		0.21(0.19)	0.22(0.19)	0.19(0.20)
River water 1				0.31			
River water 2				0.36			
River water 3				<0.05			
Drinking water 1[b]	688				790	523	853
Drinking water 2[b]	793				87	<0.05	107
Drinking water 3[b]	570				805	783	690
Drinking water 4[b]	549				<0.05	<0.05	<0.05

[a] Measured values of blank ground water with addition of *ca.* 0.2 μg/l (in parentheses) of the pesticides.

[b] Drinking water samples contained additions of several pesticides, used to investigate the behaviour of organic micropollutants during slow sand filtration.

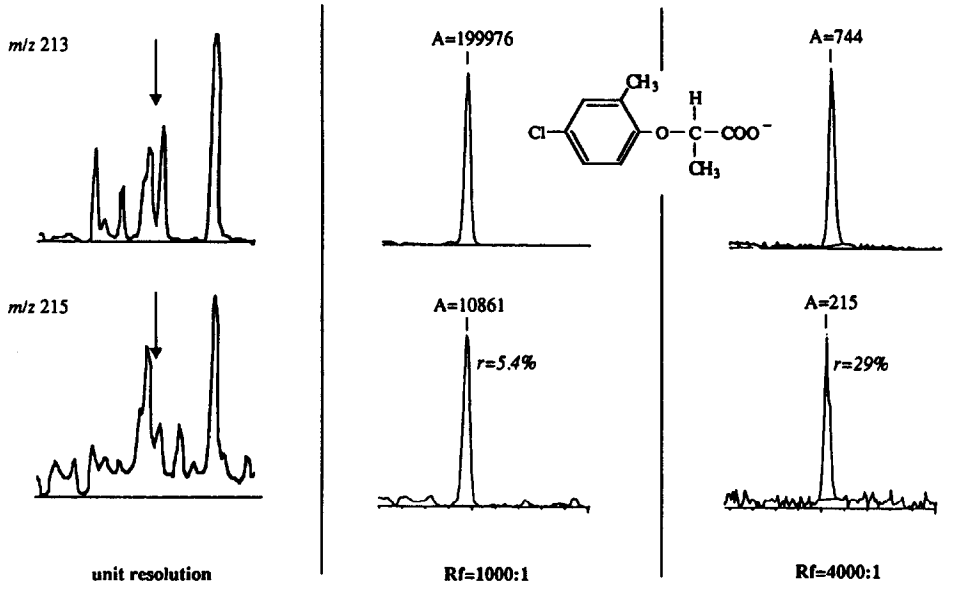

Fig. 6. Influence of increased mass resolution measurements of mecoprop at 0.1 μg/l in a dirty ground water sample.

DISCUSSION

PTC derivatization was found to be a rapid and reliable method for the determination of polar acidic pesticides using GC techniques. For (phenoxy-)carboxylic pesticides, the PTC derivatization technique was found to be suitable for screening purposes. Although it is not possible to use this method with an ECD system without any further sample clean-up, MS detection provides sufficient selectivity and sensitivity in the negative-ion CI mode. An electron-capturing functionality is introduced into the compound by derivatization; hence the presence of an electron-capturing group in the original pesticide is not a prerequisite.

Confirmation and determination down to the 0.05 μg/l level were easily achieved on a quadrupole instrument with mass unit resolution. However, increased resolution may be necessary when high chemical background levels are observed (*e.g.*, with dirty samples).

Future research will be focused on the applicability of this screening method to other (phenoxy-)carboxylic pesticides and the application of the two-phase system derivatization technique to pesticides containing other classes of functional groups (*e.g.*, OH or NH/NH_2 groups).

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