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GC–MS analysis of dichlobenil and its metabolites in groundwater

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Abstract

We have developed a new method for the determination of the widely used herbicide 2,6-dichlorobenzonitrile (dichlobenil) and its major metabolites 2,6-dichlorobenzamide (BAM) and 2,6-dichlorobenzoic acid (2,6-DCBA) in groundwater samples. The procedure is based on solid phase extraction (SPE) combined with a derivatization procedure before GC–MS analysis in order to quantify analytes simultaneously. This method can be used from regulatory laboratories for monitoring the presence of dichlobenil and its metabolites during testing groundwater samples quality.
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1. Introduction

Synthetic organic pesticides have been widely used for more than 40 years, and their use has helped boost food production worldwide and improved human and animal health. The use of pesticides had been based on the assumption that they are either retained in the upper soil or degraded by microrganisms, so they do not enter the groundwater. However, their success has not been without side effects, such as toxicity to non-target species, including humans, and persistent residues in soil and water. Several studies have quantified pesticides in water and soil but there are very few studies on their metabolites or degradation products, which can be more toxic than the pesticide itself [1,2]. Moreover the pesticides metabolites or degradation products can have different properties that enable them to reach environmental areas not reached by the pesticide itself. Thus, there is concern about contamination of drinking water sources and European regulation sets the limit for a single pesticide concentration at 0.1 and 0.5 $\mu g\,L^{-1}$ for all pesticides in water [3]. These limits are also applicable to compounds related to pesticides.

Pesticide metabolites may have major impact on groundwater quality [4–7], so it is important and timely to investigate them, especially new and promising active molecules that may be widely used.

In a qualitative screening with GC–MS of groundwater samples from the North of Italy, where groundwater is extensively used as drinking water, we found several anthropogenic compounds including 2,6-dichlorobenzamide (BAM). We therefore focused on the quantitative analysis of dichlobenil and its major metabolites: BAM and 2,6-dichlorobenzoic acid (2,6-DCBA).

Dichlobenil is a herbicide used to control weeds and grasses in agricultural, residential and industrial areas and to remove tree roots and inhibit their growth in sewers. Dichlobenil was first registered as a pesticide in the U.S. in 1964 and is still commercially available with common names such as Prefix, Barrier, Casoron, Dyclomec, Norosac. In Italy, dichlobenil was largely used until 1980, when it was partially replaced by glyphosate in agriculture and other uses. Actually dichlobenil is used mainly to control grasses in industrial area, car parks and railways and motorways sides.

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Dichlobenil is moderately volatile and dissipates by volatilisation in the environment (soil and surface water). Under conditions that reduce the potential for volatilisation, such as cooler climates, it is persistent and its main breakdown products in soil and plants are the metabolites BAM and 2,6-DCBA. Dichlobenil is strongly adsorbed to soil and sediments, which to some extent inhibits its entry into the groundwater, but the strong binding also prevents its degradation to BAM. Both dichlobenil and BAM can move to groundwater in coarse-texture soils low in organic matter and persist there, sometimes exceeding the levels of concern for groundwater quality [8–10]. Dichlobenil has consequently been banned in many countries since its discharge into groundwater has resulted in the widespread presence of BAM in drinking water in Europe and the United States.

Despite the fact that in Denmark the use of Prefix and Casoron was abandoned in 1997, the parent pesticide dichlobenil was still detected in many top-soils, and BAM can still leach from the residual pool of dichlobenil in the future. Danish EPA reported finding BAM in Danish groundwater [11] and it was also found in drinking water sources in Sweden [12], exceeding 0.1 $\mu g\,L^{-1}$.

A quantitative enzyme-linked immunoassay for the detection of BAM in water has been developed [13] and some studies have reported the toxicity of dichlobenil, chlorthiamide and BAM [14,15]. Although BAM groundwater contamination could become a problem in countries that had used dichlobenil as herbicide, there are not GC–MS studies simultaneously detecting dichlobenil and its metabolites. Here we describe an optimized and innovative derivatization-GC–MS method to determine all three compounds in a single analysis.

2. Experimental

2.1. Chemicals

Dichlobenil (97%), BAM (97%) and 2,6-DCBA (97%) were purchased from Aldrich (Steinheim, Germany). As internal standards, 3,5-dichlorobenzonitrile (97%) and 2,4-dichlorobenzoic acid (98%) were also purchased from Aldrich and 2,4-dichlorobenzamide (98%) was supplied by Lancaster (Morecambe, Lancashire, UK). For qualitative analysis 2-chlorobenzothiazole (99%), as internal standard, was purchased from Aldrich. All materials were handled in accordance with current material safety data sheets. Ethyl acetate, methanol and acetone of pesticide residue analysis grade were supplied by Carlo Erba Reagenti (Rodano, Italy). For derivatization *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) was supplied by Pierce (Rockford, USA). Hydrochloric acid (37%, v/v) was supplied by Merck (Darmstadt, Germany).

Stock standard solutions of each compound (1 $mg\,mL^{-1}$) were prepared by weight in acetone. A mixed standard solution of dichlobenil, BAM and 2,6-DCBA was prepared at 10 $ng\,\mu L^{-1}$. An internal standard solution with 3,5-dichlorobenzonitrile, 2,4-dichlorobenzamide and 2,4-dichlorobenzoic acid was also prepared at the same concentration. All solutions were stored in the dark at 4 °C.

2.2. Groundwater samples

Groundwater samples were collected in Lombardy (Northern Italy) by the Regional Agency for the Environmental Protection, ARPA, during the year 2003 and analysed within 24 h.

2.3. Solid phase extraction (SPE)

Samples were collected in Pyrex glass containers and stored at 4 °C before analysis. Extraction was carried out as soon as possible, but always within 24 h after collection. The pH before extraction was in the range 6–6.5. For qualitative analysis aqueous samples (250 mL) were subjected to SPE with 3 mL disposable LiChrolut EN cartridges, 200 mg (Merck). The cartridges were conditioned just before use by washing with 6 mL of ethyl acetate/methanol 1:1 and 3 mL

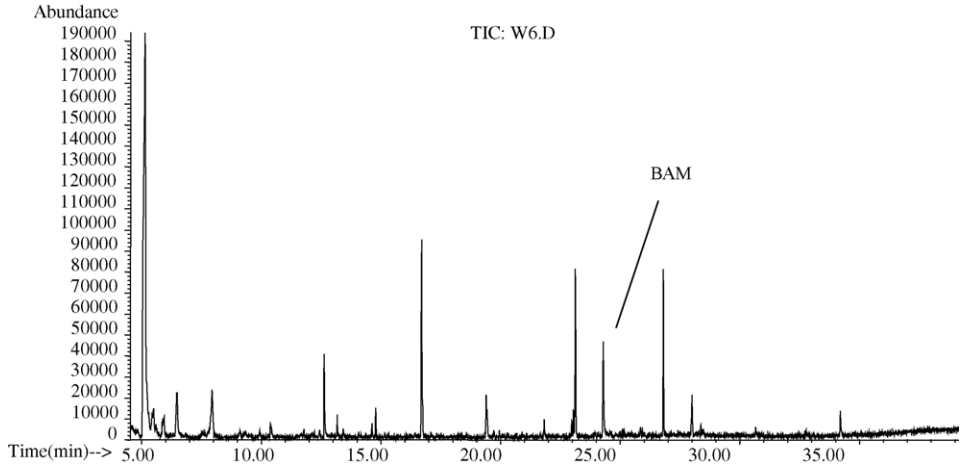

Fig. 1. Total ion chromatogram for qualitative analysis of a groundwater sample.

of water. Before extraction, water samples were spiked with 300 ng of 2-chlorobenzothiazole as internal standard. After drying the solid phase using a vacuum pump, cartridges were eluted with 6 mL of ethyl acetate/methanol 1:1. Eluates were concentrated to 50 μL under a stream of nitrogen and 2 μL of the concentrate were injected into the GC/MS apparatus.

For quantitative analysis we used 3 mL disposable Oasis HLB cartridges, 60 mg (Waters, MA, USA). The cartridges were conditioned just before use by washing with 3 mL of methanol and 3 mL of water; 250 mL of the water sample at pH 4 (see Section 3.2.1) were spiked before extraction with 500 ng of each of the three internal standards. After extraction cartridges were eluted with 3 mL of acetone and concentrated to 50 μL under a stream of nitrogen to avoid volatilisation and loss of dichlobenil. Then 20 μL of the concentrated sample was added to 30 μL of *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) for derivatization in covered glass vessels at 60 °C for 30 min. Two microliters of the derivatized samples were injected into the GC/MS apparatus.

The same procedure was used for the calibration points.

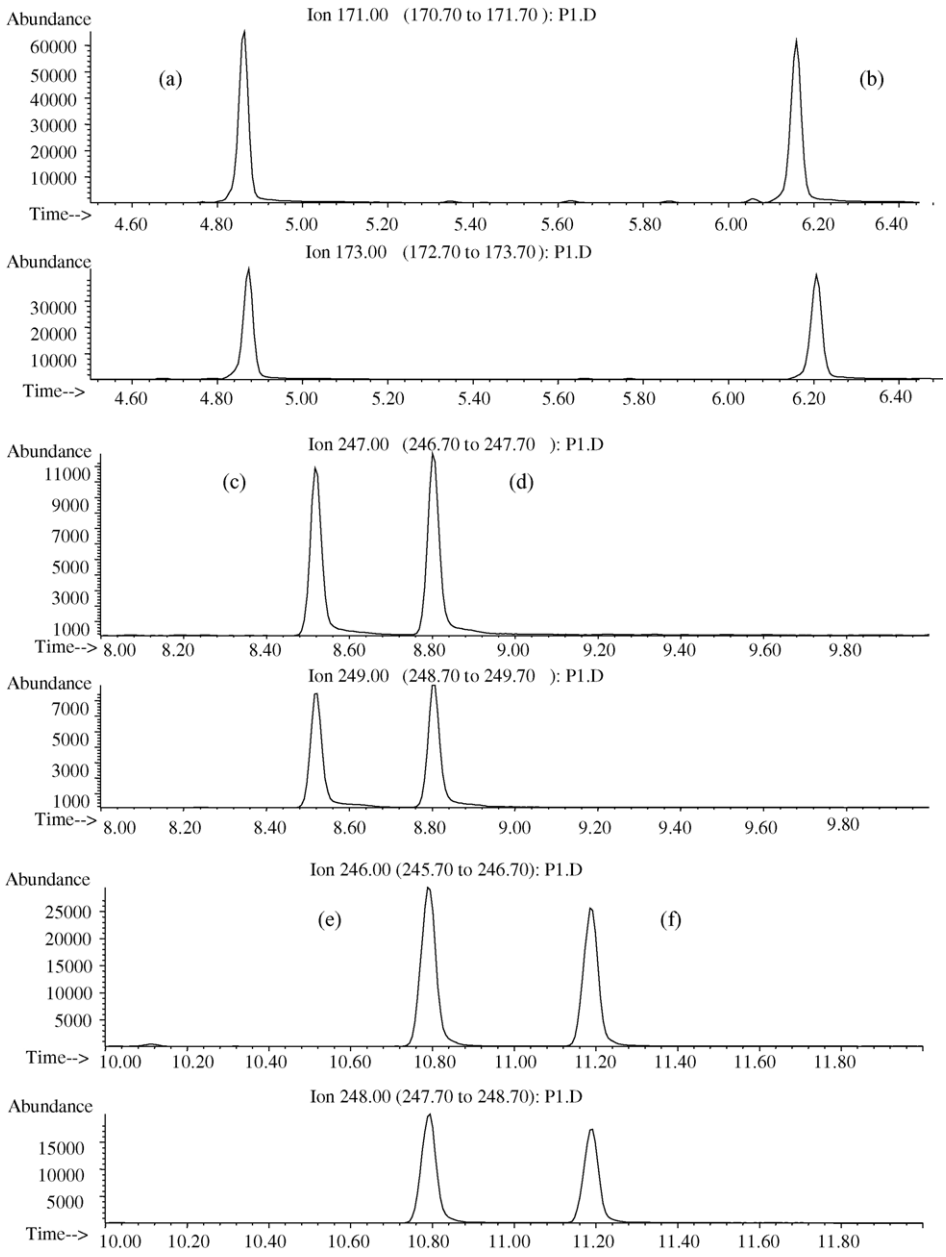

Fig. 2. Ion chromatograms for a standard 1:1 solution of 2,6-dichlorobenzonitrile (peak b: 6.2 min), 2,6-dichlorobenzoic acid (peak c: 8.5 min), 2,6-dichlorobenzamide-BAM (peak f: 11.2 min) and the internal standards 3,5-dichlorobenzonitrile (peak a: 4.9 min), 2,4-dichlorobenzoic acid (peak d: 8.8 min) and 2,4-dichlorobenzamide (peak e: 10.8 min).

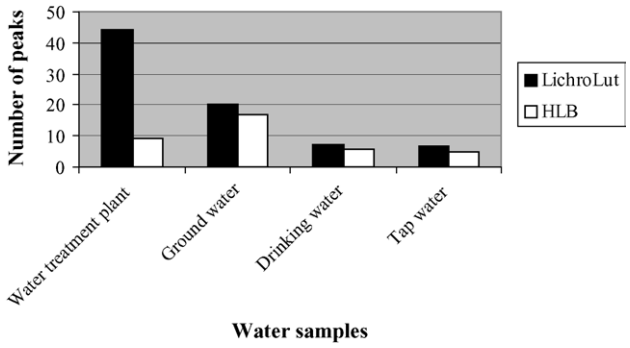

Fig. 3. Number of peaks in the GC–MS chromatograms after extraction with LichroLut and HLB Oasis cartridge in different water samples.

2.4. GC–MS analysis

The GC–MS system was a HP 5890 (II) gas chromatograph interfaced with a HP 5971 quadrupole mass-selective detector (Hewlett-Packard, Palo Alto, CA, USA). An SGE BPX5 (Analytical Technology, Brugherio, Milan, Italy) 30 m × 0.25 mm capillary column was used; the film thickness of the stationary phase was 0.25 μm. The injector temperature was 250 °C. The carrier gas was helium with a purity of 99.999% and inlet pressure 80 kPa. Analyses were done in the splitless mode. The GC automatic injector was an Hewlett Packard 7673, operated in the EI mode with an electron energy of 70 eV and a source temperature of 280 °C. Detection was in SCAN mode for qualitative analysis,

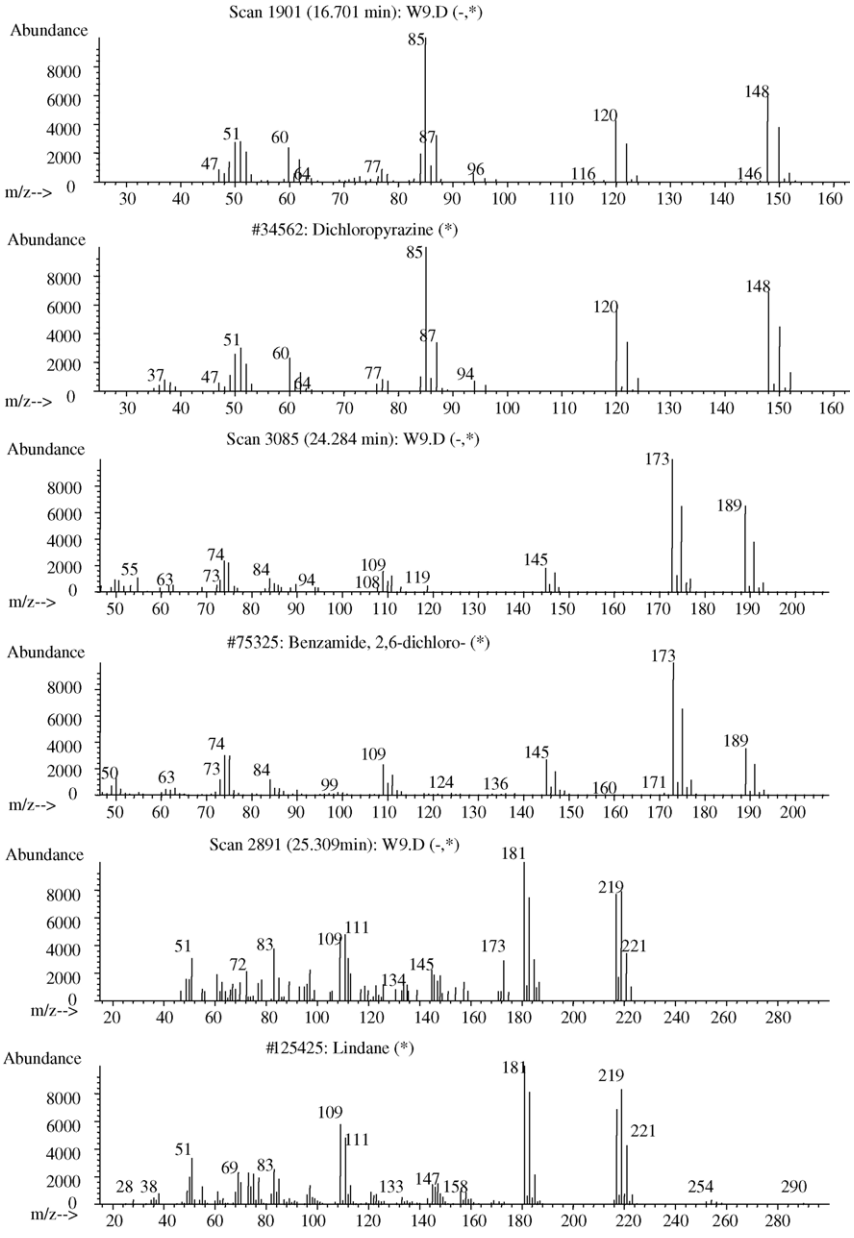

Fig. 4. Identification of some compounds found in qualitative analysis of a groundwater sample, using the instrument library (NIST 98).

Table 1
Retention time (RT) and selected ions for dichlobenil and its metabolites BAM and 2,6-DCBA

Compound	RT (min)	Ions
2,6-Dichlorobenzonitrile	6.20	171–173
2,6-Dichlorobenzoic acid	8.50	247–249
2,6-Dichlorobenzamide	11.20	246–248

with a mass scan range of 50–500 μm, and in selected ion monitoring (SIM) mode for quantitative analysis, measuring selected peak areas, using the internal-standard method.

For qualitative analysis the column was held at 35 °C for 4 min, ramped at 8 °C min^{-1} to 300 °C and held for 2 min. Fig. 1 shows a qualitative chromatogram of a real groundwater sample.

For quantitative analysis the column was operated with the oven temperature 120 °C for 1 min, ramped at 8 °C min^{-1} to 200 °C, then up to 300 °C at 15 °C min^{-1}, with a final isotherm of 3 min at 300 °C. The selected ions and retention times for each compound are reported in Table 1.

A quantitative chromatogram of 4 ng of a mix of standards is shown in Fig. 2.

These chromatographic conditions gave a good peak shape and optimal separation between the analytes and between each analyte and its internal standard.

We also considered the possibility of simultaneous HPLC-MS analysis of the three compounds but after some trials we did not do it because the pesticide was not detected, 2,6-DCBA was analysed in negative mode and BAM in positive mode, so to quantify the metabolites we had to inject the same sample twice, doubling the analysis time. The best solution was derivatization with GC–MS.

3. Results and discussion

3.1. Qualitative analysis

Qualitative screening was done with SPE-GC–MS, without derivatization, which gave information about a wide range of compounds in groundwater samples. We extracted the water with Lichrolut for identification because a comparison with Waters Oasis HLB showed that for more complex samples, like water from the treatment plant, it extracted more compounds (Fig. 3).

Table 2
Recoveries (%) at different pH values

Compound	pH 2	pH 4	pH 6
3,5-Dichlorobenzonitrile	44	52	99
2,6-Dichlorobenzonitrile	42	67	98
2,6-Dichlorobenzoic acid	81	80	9
2,4-Dichlorobenzoic acid	98	99	0
2,4-Dichlorobenzamide	73	74	24
2,6-Dichlorobenzamide	82	76	19

This is in agreement with previous reports of superior extraction with Lichrolut in the case of dirty samples [16,17]. Identification was based on the comparison of the mass spectrum for the compounds with those in the instrument library (NIST 98). The main compounds in groundwater samples were BAM, 2,5-dichloropyrazine and lindane (see Fig. 4), confirmed by injecting their reference standards. Blank samples were glass-bottled drinking water.

3.2. Quantitative analysis

3.2.1. Derivatization, SPE and recoveries

For quantitative measurements we used a derivatization procedure for GC–MS analysis to improve the gas chromatographic behavior of the amide and the acid compounds. BSTFA was used as derivatization reagent [18]. Dichlobenil did not react with BSTFA so it cannot be derivatized, 2,6-DCBA derivatized more easily than BAM, which needed a higher temperature and longer time. We ran several studies in order to optimize each step of the derivatization procedure. We finally used 30 μL of BSTFA because amide compounds reached more than 65% derivatization and acid compounds more than 80%. There are no real differences between 30 and 40 μL and this is a compromise to avoid dilution of the

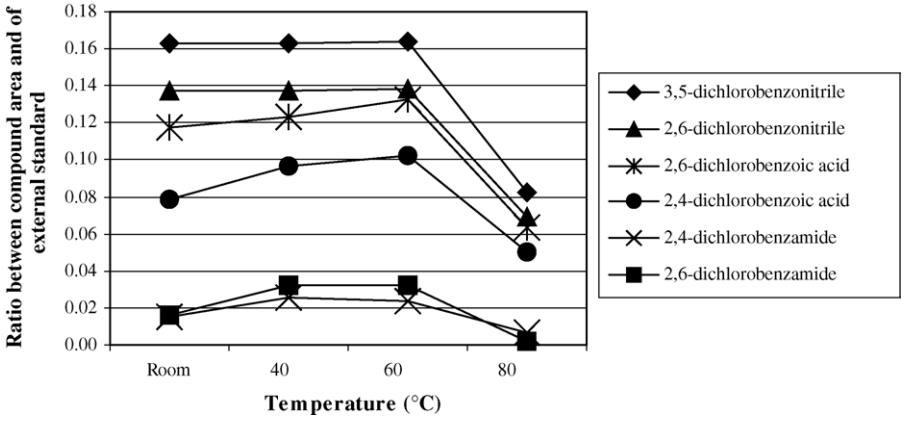

Fig. 5. Derivatization in relation to temperature.

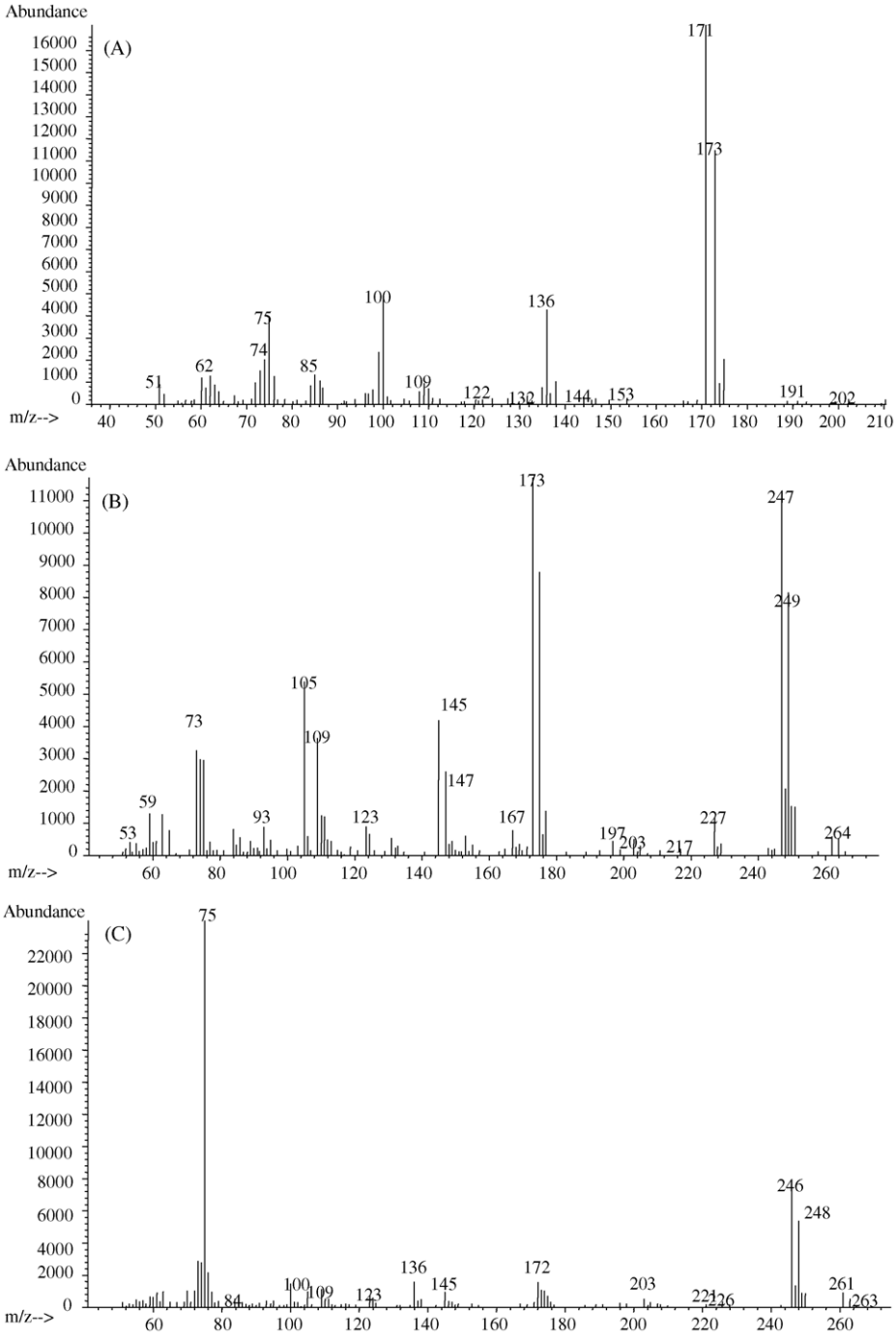

Fig. 6. Mass spectra of the quantified compounds: (A) 2,6-dichlorobenzonitrile, (B) 2,6-dichlorobenzoic acid and (C) 2,6-dichlorobenzamide after derivatization with BSTFA.

Table 3
Limit of quantification (LOQ) and limit of detection (LOD) for each compound

Compound	Method		Instrumental	
	LOQ (1:9) (ng L^{-1})	LOD (1:3) (ng L^{-1})	LOQ (1:9) (ng L^{-1})	LOD (1:3) (ng L^{-1})
2,6-Dichlorobenzonitrile	51	17	45	15
2,6-Dichlorobenzoic acid	39	13	30	10
2,6-Dichlorobenzamide	60	20	48	16

Table 4
Qualitative analysis and compounds identified in groundwater samples

Compounds	RT (min)	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13	W14	W15	W16	W17
Propanedioic acid, 2,2-dimethyl-, (methylthio)-methyl ester	4.57		X															
Tetrachloroethylene	7.41															X		
Benzene, 1-ethyl-2-methyl	11.56														X	X		
Benzaldehyde	11.81										X	X						
Benzene, 1,2,3-trimethyl	12.36														X			
Propanitrile, 2,2′-azobis(2-methyl)	13.64									X								
Unknown 91[a]	16.26														X			
Dichloropyrazine	16.70	X	X	X	X	X	X	X		X	X					X		
Unknown 55-56-120	16.86	X																
Phenol, *p-tert*-butyl	17.99									X								
Unknown 56-71-83-98	18.85									X								
Propanoic acid, 2-methyl-2-ethyl-3-hydroxyhexyl ester	19.23									X								
1,2-Benzenedicarboxaldehyde	19.43		X	X	X	X	X	X										
Pentadecane	21.05									X								
Unknown 77-91-121-163	21.47														X			
1*H*-Benzotriazole	21.58																X	
Unknown 55-99-111	23.23												X					
Unknown 236-251	23.78									X								
Benzamide, 2,6-dichloro	24.28	X	X	X	X	X	X			X	X							
Methyl tetradecanoate	24.90														X			
Lindane	25.31									X								
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	26.88														X			
Unknown 143-227-242	30.55													X				
Benzyl butyl phthalate	32.46		X															
Phenol, 2,2-methylenebis(6-(1,1-dimethylethyl)-4-methyl	33.06		X															
Squalene	36.73	X													X	X	X	X

RT: retention time.
[a] For unknown compounds we indicated the main ions.

extracted sample. We also studied how derivatization varied with time and temperature. The best results were obtained with derivatization at 60 °C for 30 min. From 20 to 50 min the relative standard deviation (R.S.D.) of the derivatization was lower than 15%, so finally we chose 30 min as an appropriate analysis time. From room temperature to 60 °C the analytical response, as the ratio between the peak area of the compounds and of an external standard, 2-chlorobenzothiazole, rose for all compounds except the nitriles that cannot be derivatized, but at a higher temperature (80 °C) the ratio dropped and the R.S.D. increased (Fig. 5).

The first step in the extraction method was the selection of the SPE cartridge. After comparing Lichrolut EN and Oasis HLB we decided to use Oasis HLB for quantitative analysis as it gave higher recoveries for all three compounds [7]. To improve recoveries we also studied the influence of the pH; we acidified samples to pH 2 and 4 with HCl (37%, v/v) and compared the recoveries with those obtained at pH 6–6.5 (original pH of the water samples). Different behavior was observed for the different analytes. We selected pH 4 as a compromise (Table 2).

3.2.2. Compounds mass spectra

Fig. 6 shows the GC/MS mass spectrum of each quantified analyte after derivatization with BSTFA. 2,6-Dichlorobenzonitrile cannot be derivatized.

Ion at *m*/*z* 171 is the molecular ion. Loss of one and two Cl produced the fragments at *m*/*z* 136 and 100 respectively and further the loss of CN produced the fragment at *m*/*z* 75.

BAM and 2,6-DCBA derivatized and their *m*/*z* were a result of the reaction between the molecule and the BSTFA reactive group. The molecular weight of the compound increased after derivatization due to the linkage of the $Si(CH_3)_3$ group from BSTFA.

For 2,6-DCBA, the loss of CH_3 produced the fragment *m*/*z* 247, the fragment *m*/*z* 173 was originated by the loss of $OSi(CH_3)_2$, the subsequent loss of CO produced the fragment at *m*/*z* 145 and finally the loss of the two Cl generated the fragment at *m*/*z* 75. The fragments at *m*/*z* 105 and 73 are produced by the fragments C_6H_5CO and $Si(CH_3)_3$, respectively.

For BAM, the loss of CH_3 produced the fragment at *m*/*z* 246, the fragment *m*/*z* 172 is originated by the loss of $NH_2Si(CH_3)_2$, the subsequent loss of CO produced the fragment at *m*/*z* 145 and finally the loss of the two Cl generated the fragment at *m*/*z* 75. Additionally this ion probably has contribution for the $NH_3Si(CH_3)_2$ group.

3.2.3. Limit of detection (LOD)

The limits of detection (LOD) and quantification (LOQ) of the analytes were experimentally obtained on a signal-to-noise basis of 3:1 and 9:1, respectively. LOQ for all compounds was below the limit set by European regulations (0.1 μg L^{-1}). Table 3 shows instrumental and methodological LOD and LOQ.

3.2.4. Calibration and linearity

For calibration we prepared standard aqueous solutions of all three compounds in the range of 0.01–15 μg L^{-1} and extracted three replicates of each point of the calibration curve by SPE according to the procedure described above.

Calibration curves were computed by measuring the most abundant ions for each compound (GC–MS); linearity was good for all analytes up to 10 μg L^{-1} (coefficient of correlation > 0.998). Quantification was done using: 3,5-dichlorobenzonitrile, 2,4-dichlorobenzoic acid and 2,4-dichlorobenzamide respectively as internal standards for dichlobenil, 2,6-DCBA and BAM.

We studied the reproducibility of the method by extracting the same sample spiked with 50 ng of each compound five times and calculating the R.S.D., which was below 20% for each analyte.

3.3. Qualitative and quantitative results

We used the method to analyse groundwater samples from several wells of a big city in Northern Italy. Qualitative and quantitative results are reported in Tables 4 and 5.

From the qualitative screening resulted that one well (W9) showed the higher number of compounds (9) whereas other wells contained fewer compounds. Some compounds such as BAM and dichloropyrazine were found in almost the 50% of analysed wells and these analytes can be considered widespread contaminants in this area.

The parent pesticide dichlobenil was never found but we always found the main metabolite, BAM, and in one case (W5) also 2,6-DCBA was measurable. In all wells but one, the BAM concentration exceeded 0.1 μg L^{-1}. The concentration range was between 0.15 and 3.1 μg L^{-1}.

Table 5
Quantitative analysis and concentrations (mg L^{-1}) of pollutants in groundwater samples

Samples	2,6-Dichloro benzonitrile	2,6-Dichloro benzoic acid	2,6-Dichloro benzamide
W1	<0.051	<0.039	1.962
W2	<0.051	<0.039	0.158
W3	<0.051	<0.039	2.281
W4	<0.051	<0.039	1.290
W5	<0.051	0.049	1.984
W6	<0.051	<0.039	3.110
W7	<0.051	<0.039	0.148
W8	<0.051	<0.039	1.097
W9	<0.051	<0.039	1.460
W10	<0.051	<0.039	1.708
W11	<0.051	<0.039	0.179
W12	<0.051	<0.039	0.640
W13	<0.051	<0.039	0.817
W14	<0.051	<0.039	<0.060
W15	<0.051	<0.039	0.368
W16	<0.051	<0.039	0.196
W17	<0.051	<0.039	0.245

4. Conclusion

We have developed a new procedure to simultaneously determine the presence of dichlobenil and its metabolites BAM and 2,6-DCBA in water and used it to analyse groundwater samples. In many groundwater samples BAM exceeded the level of 0.1 $\mu g\,L^{-1}$ established by European Regulations in force in Italy. This confirms the importance of testing groundwater not only for pesticides but also for metabolites.

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