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Solid-phase micro-extraction–gas chromatography–(tandem) mass spectrometry as a tool for pesticide residue analysis in water samples at high sensitivity and selectivity with confirmation capabilities

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Abstract

Gas chromatography–mass spectrometry (GC–MS) has been widely applied for pesticide monitoring because of its high sensitivity and specificity and for the potential of multi-residue and multi-class analysis. An analytical procedure was developed for the determination of pesticide multi-residues in water samples combining solid-phase micro-extraction (SPME) and gas chromatography–ion trap mass spectrometry. For SPME extraction a poly(dimethylsiloxane)–divinylbenzene coated fibre was selected whereas the mass spectrometer was operated under full scan, selected ion storage (SIS), μSIS (SIM) and MS–MS and the figures of merit compared. Quantitative and qualitative (confirmatory) capabilities of each operation mode are discussed. Using MS–MS, precision was typically below 10% and limits of detection (LODs) were improved by 1.3 to 20 times (to low- or sub-ppt levels) compared to μSIS, with the advantage of maintaining identification capabilities. The combination of selective extraction by SPME and highly selective determination by GC–MS–MS made possible ultra-selective and essentially error-free determination of pesticides in complex environmental samples. This aspect will be highlighted in the paper.

Keywords: Water analysis; Pesticides

1. Introduction

The contamination of the aquatic environment by organic pollutants, such as pesticides is a matter of great concern world-wide. In addition to effecting human health many pesticides released into the environment can also disrupt the normal endocrine function in a variety of aquatic life and wildlife [1,2].

In the last few decades, pesticides have been used on an increasingly wider scale throughout the world, although lately there is a tendency to slow down, or at least a motivation to use less harmful molecules. Pesticides are characterised by their diversity, their different physical and chemical properties and their low concentrations in real samples [3]. On account of the large number of active ingredients used, analytical procedures are required for the detection of the greatest number of compounds possible, with the fewest number of extraction and clean-up steps [3–5]. Because of the large-scale dilution these contaminants undergo in aquatic matrices, an enrichment procedure consisting on liquid–liquid extraction (LLE), solid-phase extraction (SPE), or more recently, solid-phase micro-extraction (SPME) is currently employed. SPME is a modern technique that consists on direct extraction of the analytes with the use of a small-diameter fused-silica fibre coated with an adequate polymeric stationary phase [6,7]. In recent years, SPME has gained widespread acceptance for the determination of a wide spectrum of analytes in various fields, such as environmental, forensic, pharmaceutical, food, beverage and flavour chemistry [8–10]. In the present study, a poly(dimethylsiloxane)–divinylbenzene (PDMS–DVB) SPME fibre was used and subsequent analysis was carried

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out by gas chromatography–mass spectrometry (GC–MS). MS is a very powerful tool for the identification and quantification of organic compounds in complex samples [1,3,5,11–14]. GC–MS operated under electron impact (EI) ionisation is certainly the most widely employed technique for these purposes [5]. A compound specific fragmentation pattern is thereby obtained comprising valuable information for elucidation of the identity of the solutes, which can be accomplished comparing their mass spectra with library-standard spectra (full scan or MS–MS). For quantification of priority pollutants, recording full scan spectra is advised and, in some countries, even required [11]. The identity of the analytical results must be firmly established prior to meaningful quantitative information can be extracted from the chromatograms i.e. without producing false positive results. Recently, a tendency has been observed towards the use of GC–MS (in the selected ion monitoring mode (SIM)) for quantitative purposes, in addition to the application as a confirmation technique following analysis by other GC detection systems such as electron-capture detection (ECD) and nitrogen–phosphorus detection (NPD). When operated in the scan mode, MS works as an universal detection method with only moderate sensitivity, however monitoring specific fragment ions from the analytes usually results in improved sensitivity, selectivity and accuracy in the determination [5,11,12].

Compared to single stage MS modes, tandem mass spectrometry (MS–MS) offers a higher degree of selectivity and sensitivity. MS–MS enables the analysis of pesticide trace levels in the presence of interfering compounds without losing identification capability due to a drastic reduction in the background [2,11,13–15]. Those analysis, in the past, which proved to be complicated are now made more straightforward by using MS–MS. Ion trap detectors are the most commonly used mass analysers to perform MS–MS once they have clear advantages in terms of cost, size, weight and pumping requirements [16].

The literature contains examples of the application of MS–MS to the determination of pesticide residues, with SPE enrichment or clean-up, in different matrices such as: waters [1,3,15], biological fluids [17–20] and vegetables [21]. This paper reports on the association of SPME with mass spectrometric detection and the merits of different operation modes will be compared. The aim of the present work was: (i) to develop a SPME-based procedure for screening purposes capable of qualitative (full scan) and quantitative (SIM) determination of organochlorine (OCPs), organophosphorous (OPPs), triazine, pyrethroid and other pesticides in groundwater samples; (ii) to develop a SPME–GC–MS–MS method based on pesticide occurrence in groundwater from an intensive horticulture area in Póvoa de Varzim in North Portugal. To our knowledge, only a few authors have explored the excellent selectivity and sensitivity that is recognisable to both SPME and MS–MS combined in a single technique.

2. Experimental

2.1. Chemicals and reagents

All pesticide analytical standards were supplied by Riedel-de Häen (Seelze, Germany). Exception noted for the organochlorine insecticides, which were purchased as a commercial mixture (EPA 608 Pesticide mix, 20 mg l^{-1}) from Supelco (Bellefonte, PA, USA), individual stock standard solutions were prepared by exact weighting of high-purity substances and dissolving them in an appropriate solvent as follows: pyrethroid insecticides were prepared in ethyl acetate, the organophosphorous insecticides and triazine herbicides were dissolved in methanol, individual solutions of some other pesticides were also made in methanol. Four separate mixtures by chemical group were then prepared in methanol containing 2.0 mg l^{-1} each individual pesticide, while hexachlorobenzene (HCB) and isodrin were added to the OCPs commercial mixture to obtain a stock solution of 100 μg l^{-1}, in methanol. Stock standard solutions were stored in a freezer whereas working standard solutions at concentrations ranging from 0.001 to 1.0 μg l^{-1} were prepared daily by appropriate dilution in ultrapure Milli-Q water (Millipore, Molsheim, France).

Ethyl acetate, methanol and *n*-hexane used in the handling of standards were of Pestanal and ChromaSolv grade from Riedel-de Häen, and SupraSolv grade from Merck (Darmstadt, Germany), respectively. Initial SPME optimisation involved pH adjustment using a Crison pH meter (Crison, Barcelona, Spain), and ionic strength corrections with NaCl analytical-reagent grade from Merck.

2.2. SPME extraction procedure

All SPME fibres (Supelco, Bellefonte, USA) used for sample preparation were new at the beginning of the study and were conditioned according to supplier's instructions. Manual operation of the SPME technique was performed using 4 ml amber glass vials, manual SPME holder and a Variomag agitation plate (Monotherm, Munich, Germany) with temperature control. The extraction procedure adopted for this study consisted on the following: 3 ml-aliquots of the samples were extracted by immersion of a 60 μm PDMS–DVB coated fibre during 60 min; sample agitation was employed at the maximum agitation rate (around 900 rpm) and extraction temperature kept at 60 °C; neither pH adjustment nor ionic strength correction were needed.

2.3. Gas chromatographic analysis

Chromatographic analyses were carried out in a Varian 3400 CX (Walnut Creek, CA, USA) gas chromatograph equipped with a SPI/1078 temperature-programmable injector and a CPSil-8 CB low bleed MS capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness) from Chrompack (Middelburg, The Netherlands). The split/splitless injection

port was maintained in split-less mode for 5 min, the lapse of time for SPME fibre desorption and set at a fixed temperature of 250 °C.

High-purity helium (99.9999%) at a flow rate of 1.0 ml/min (150 °C oven temperature) was used as the carrier gas and also as the collision gas at the ion trap chamber for MS–MS experiments. Samples were analysed using the following oven temperature programme: initial temperature 80 °C (held for 2 min), increased by 15 °C min^{-1} to 190 °C (held for 4 min), increased by 10 °C min^{-1} to 230 °C (held for 5 min) and, finally, increased by 10 °C min^{-1} to 290 °C and held at this temperature for 6 min (3 min in the case of MS–MS method).

2.4. Mass spectrometry instrumental conditions

A Saturn 2000 ion trap mass spectrometer from Varian Instruments (Sunnyvale, CA, USA) operated in the EI mode was used for full scan, selected ion storage (SIS), μSIS (similar to SIM) and MS–MS experiments. The manifold, trap and transfer line temperatures were set at 50, 200 and 240 °C, respectively. The analyses were performed with a filament–multiplier delay of 5 min (11 min for MS–MS) and acquisition was performed in the range *m/z* 35–430, with a background mass of *m/z* 45. The emission current of the ionisation filament was set at 50 μA generating electrons with 70 eV energy and the axial modulation amplitude voltage was 3.7 V. The mass spectrometer was calibrated frequently to perfluortributylamine (PFTBA) through an auto tune process. The electron multiplier voltage was established by auto tune at around 2000 V (+300 V offset above the adjusted value). The automatic gain control (AGC) was turned on in order to achieve the maximum sensitivity by completely filling the trap with target ions. Nevertheless, its adjustment depends on the operation mode because the correct number of ions present simultaneously in the trap may be different [8]. To avoid phenomena of charge repulsion or ion molecule reactions induced by the ion trap saturation, the target counts (target total ion chromatogram TIC) were limited to: 65 000 in full scan MS, 15 000 in SIS and μSIS, and 5000 in MS–MS. Instrument control and mass spectrometry data were managed by a personal computer running the Saturn GC–MS WorkStation software (version 5.52).

3. Results and discussion

3.1. SPME details

Most of the papers published until now dealing with pesticide multi-residue methods use SPE as the extraction procedure [22–24]. Although this wide application of SPE to the analysis of environmental water samples, which is also the recommended technique by the regulatory agencies in the EU and USA, losses of analyte or matrix interferences are frequently mentioned [25]. In addition to obvious advantages in terms of simplicity, rapidity, low cost, automation and solvent-free properties, SPME overcomes problems related to matrix effects and elution of impurities generated by extraction materials [6,7,10,25]. In a previous work by the same authors, an effective analytical protocol was established based on SPME extraction and coupled electron-capture detection–thermionic specific detection (ECD–TSD) [9]. The same extraction protocol was adopted here in association with MS detection, which should give equivalent sensitivity but also allowing the unequivocal confirmation of the identity of the pesticides. As a result of the quite different relative sensitivities between detectors towards the groups of pesticides studied, the extraction conditions were checked with this detection system (data not shown). However, no relevant discrepancies were found to entail the use of a different set of SPME conditions. In comparison with the previous method, a few more compounds were included which can now be analysed together. Briefly, a PDMS–DVB fibre was chosen with regard to its intermediate polarity properties that proved to be especially suited for the simultaneous analysis of the target analytes. 3 ml-aliquots of the samples were directly extracted, without any pH or ionic strength correction, by immersion of a 60 μm PDMS–DVB fibre during 60 min; a vigorous sample agitation was employed and the temperature controlled at 60 °C. Further details can be found elsewhere [9].

3.2. Single MS analysis

An extent list of pesticides including the most currently used, former persistent compounds and degradation products belonging to the chemical classes mentioned in Section 2.1 were selected for a screening method based on single stage MS (see Table 1). Despite the well-known selectivity of SPME procedure, MS total ion chromatograms (TICs) were characterised by the appearance of some peaks in the blank, mainly from impurities of the fibres, glass material or septa. The selection of characteristic fragment ions for integration avoided such possible interferences and allowed to obtain acceptable reagent and field blanks. As it is well accepted, MS in full scan mode lacks sensitivity when compared to conventional selective detection methods like ECD or NPD. Indeed, its major utility is related to identity confirmation and structural elucidation rather than for quantitative purposes. Even so, most of the times this approach showed adequate sensitivity for pesticide residue analyses, on account of the 0.1 μg l^{-1} limit established by the European Union (EU) legislation for drinking waters. The limits of detection (LODs) for each pesticide were determined as the lowest concentration of a compound giving a response with a signal-to-noise ratio (S/N) of 3 and the limits of quantitation (LOQs) giving a S/N ratio of 10, computed from the analysis of a low concentration standard (0.1 μg l^{-1}). Retention time data, quantitation ions and LODs are summarised in Table 1. Positive results in real samples at defined retention times would be confirmed later by comparing their

Table 1
Pesticides included in the SPME–GC–MS methods (full scan, SIS and μSIS), respective retention times (t_R) and quantitation ions (m/z) used for calculations

Peak no.	Pesticides	t_R (min)	Quantitaion ion (m/z)	SIS, μSIS segment	Full scan LOD ($\mu g\,l^{-1}$)	SIS, LOD ($\mu g\,l^{-1}$)	μSIS			
							LOD ($\mu g\,l^{-1}$)	Repeatability (% R.S.D.), $n = 6$	Intermediate precision (% R.S.D.), $n = 20$	Determination coefficient (r^2)
1	Dichlorvos	7.298	109	1	0.078	0.020	0.032	13.5	15.1	0.958
2	DEA	11.932	172	2	1.006	0.236	0.146	8.5	8.2	0.991
3	HCB	12.920	284	3	0.005	0.002	0.001	18.7	19.8	0.990
4	Dimethoate	13.115	87	3	12.414	12.857	5.263	11.7	10.1	0.848
5	Simazine	13.295	201	4	0.131	0.182	0.200	15.6	28.5	0.870
6	Atrazine	13.432	200	4	0.010	0.008	0.006	8.9	9.4	0.993
7	Propazine	13.535	214	4	0.006	0.005	0.005	5.6	11.1	0.994
8	Terbuthylazine	13.908	214	4	0.004	0.002	0.004	4.0	9.6	0.994
9	Lindane	13.934	181	4	0.005	0.002	0.001	5.1	9.1	0.992
10	Diazinon	14.017	179	4	0.003	0.002	0.002	13.3	13.0	0.998
11	Propyzamide	14.019	173	4	0.005	0.004	0.004	7.6	17.2	0.984
12	Fonofos	14.123	109	4	0.003	0.002	0.002	10.9	13.0	0.993
13	Metribuzin	15.715	198	5	0.032	0.038	0.045	7.8	11.9	0.995
14	Parathion-methyl	15.890	263	5	0.006	0.006	0.012	5.5	10.5	0.996
15	Alachlor	15.895	188	5	0.005	0.004	0.003	6.8	11.3	0.992
16	Simetryn	16.024	213	5	0.014	0.011	0.008	13.9	17.9	0.890
17	Heptachlor	16.224	272	6	0.010	0.004	0.002	12.4	20.0	0.983
18	Fenitrothion	16.631	260	6	0.005	0.003	0.001	11.6	11.0	0.997
19	Malathion	16.822	127	6	0.030	0.021	0.019	11.4	17.0	0.981
20	Chlorpyrifos	17.059	314	6	0.004	0.001	0.002	12.1	19.4	0.962
21	Aldrin	17.289	66	7	0.014	0.012	0.120	35.3	26.4	0.864
22	Parathion-ethyl	17.299	139	7	0.014	0.016	0.014	15.9	21.1	0.988
23	Chlorfenvinphos E	18.006	267	7	0.027	0.025	0.005	12.8	19.9	0.981
24	Pendimethalin	18.054	252	7	0.002	0.002	0.003	9.2	25.7	0.994
25	Isodrin	17.187	193	7	0.018	0.010	0.012	17.3	21.4	0.964
26	Chlorfenvinphos Z	18.356	267	7	0.068	0.045	0.009	9.1	11.6	0.981
27	Heptachlor epoxide	18.475	353	7	0.001	0.0006	0.001	12.1	11.7	0.995
28	Procymidone	18.729	96	8	0.005	0.003	0.006	8.8	11.1	0.994
29	γ-Chlordane	19.275	373	8	0.003	0.002	0.0006	17.6	22.1	0.997
30	Tetrachlorvinphos	19.304	329	8	0.058	0.020	0.010	10.5	12.1	0.998
31	Endosulfan I	19.792	241	9	0.010	0.006	0.004	10.8	11.7	0.991
32	Fenamiphos	19.823	303	9	0.036	0.030	0.012	8.1	10.2	0.991
33	4,4′- DDE	20.558	318	10	0.001	0.0009	0.0007	15.3	26.0	0.983
34	Dieldrin	20.898	79	10	0.009	0.009	0.010	11.5	14.8	0.988
35	Endrin	21.872	81	10	0.051	0.060	0.019	14.6	18.8	0.984
36	Endosulfan II	22.395	195	11	0.018	0.008	0.005	11.2	11.9	0.991
37	4,4′-DDD	22.537	235	11	0.002	0.002	0.001	7.3	17.1	0.994
38	Endrin aldehyde	23.074	345	12	0.009	0.012	0.014	10.6	18.1	0.984
39	Endos. sulphate	24.067	272	12	0.005	0.003	0.001	4.9	11.5	0.997
40	4,4′-DDT	24.184	235	12	0.013	0.012	0.002	14.7	24.6	0.979
41	Azinphos-methyl	27.263	160	13	0.194	0.053	0.057	21.9	26.1	0.996
42	λ-Cyhalothrin	27.513	181	13	0.015	0.006	0.008	34.5	34.2	0.983
43	α-Cypermethrin	30.570	181	14	0.067	0.029	0.037	20.6	26.3	0.977
44	Deltamethrin	34.041	172 + 174 + 181	14	0.375	0.338	0.170	31.5	31.5	0.975

Quantitation ions are valid for the three operation modes except lindane with m/z 183 and azinphos-methyl with m/z 132 in the SIS mode, and dimethoate with m/z 85 + 86, lindane with m/z 183, procymidone with m/z 95 and azinphos-methyl with m/z 132 in the μSIS mode. Time segments are applicable for SIS and μSIS methods (see also Table 2). LODs are given for the three operation modes under single MS whereas other validation parameters are also given for the μSIS approach.

measured mass spectra with reference spectra included in the US National Institute of Standards and Technology (NIST) database, requiring a minimum spectral fit of 700. Not always, this could be attained around the LOQ value due to high background signal, unless background correction was applied. This procedure was most useful for identification of unknown compounds, not included in the present method, that were detected in real samples such as: metolachlor, metalaxyl, benalaxyl, pirimicarb, quinalphos and prometryn.

Through a technique called selected ion storage (SIS), ion trap mass spectrometers are able to enrich the sample ions relative to the unwanted matrix ions by storing the former and ejecting the later from the trap. This allows for a much cleaner spectrum and a considerable increase in sensitivity. In practice, this is accomplished defining time segments throughout the chromatographic run containing the most appropriate mass ranges to be stored. Since the most typical parts of each particular spectrum can be collected in a narrow scan range this technique also allows to obtain a library-search spectrum. The time segments and SIS mass ranges are presented in Table 2 whereas LODs calculated as S/N 3, as described above, are presented in Table 1. Exception noted for dimethoate, simazine, metribuzin, parathion-ethyl, endrin and endrin aldehyde, the LODs ranged from equal to 4.3 times lower than those obtained under the full scan acquisition, with an average improvement of 1.7. Despite combining an enhanced sensitivity while maintaining some degree of qualitative information, this technique is labour intensive to settle the mass ranges that best match the spectra of the analytes included in a SIS segment.

Another alternative to improve by a significant factor the LODs in MS analyses is to perform μSIS (similar to SIM) although with the expense of qualitative information. Table 1 shows the LODs (S/N of 3) obtained by monitoring the corresponding quantitation ion (1 *m/z* window) of each pesticide in μSIS mode. With exception for simazine, metribuzin, parathion-methyl, aldrin, pendimethalin, procymidone, dieldrin and endrin aldehyde, the LODs were lowered up to 7.5 times (2.6 times on average), regarding those in full scan mode. These values were later confirmed analysing standards of decreasing concentrations down to $0.001\,\mu g\,l^{-1}$. Only those for dichlorvos, malathion, endrin and endrin aldehyde proved to be a little higher than the ones theoretically calculated.

As these were the lowest detection limits that we could achieve for pesticide analysis in water samples processed according to the described SPME–GC–MS procedure, the validation of the method included also an estimation of the repeatability and intermediate precision at $0.1\,\mu g\,l^{-1}$ concentration level (see Table 1). With a few exceptions, repeatability as within-day RSD of peak areas was always below 20%. The values obtained for aldrin and azinphos-methyl can be explained by their LODs being close to the tested concentration whereas the pyrethroids group typically show higher variation between analyses. Pyrethoids are detected as a pair of chromatographic peaks and the values presented here are reported for the most intense one, although for quantitation purposes both should be considered. The intermediate precision calculated as between-day RSD of peak areas in three consecutive days can be considered acceptable, as it was generally lower than 25% (average value of 17%). The poorer values obtained for aldrin, simazine and pyrethroids can be explained by the same reasons discussed above whereas for pendimethalin and 4,4′-DDE it seems that the extraction performance of the SPME fibre was less consistent during time.

Since this method was intended for quantitative purposes, calibration curves were constructed over 6 concentration levels (three replicates each): 0.001, 0.01, 0.05, 0.1, 0.5 and $1.0\,\mu g\,l^{-1}$ (10 times higher for dimethoate and desethylatrazine DEA). The determination coefficients (r^2) are presented in Table 1. In summary, for most of the compounds the calibration graphs were good ($r^2 > 0.990$), however for 12 of them the values can only be considered acceptable ($r^2 > 0.980$) while for 9 compounds they were considered

Table 2
Instrumental conditions for operation of the mass spectrometer in selected ion storage (SIS) mode: SIS time ranges, SIS mass ranges and number of compounds monitored

SIS segments	Time ranges (min)	Number of peaks	SIS mass ranges (*m/z*)
1	6.00–9.00	1	109–110, 185–185
2	11.00–12.10	1	172–174, 187–187
3	12.10–13.20	2	87–93, 142–142, 282–288
4	13.20–14.50	8	109–110, 137–137, 172–184, 200–202, 214–219
5	14.50–16.10	4	109–109, 188–188, 198–200, 213–214, 262–264
6	16.10–17.15	4	97–100, 124–127, 260–278, 314–316
7	17.15–18.60	7	65–67, 137–139, 193–195, 252–278, 352–354
8	18.60–19.50	3	95–97, 109–109, 283–283, 329–331, 371–375
9	19.50–20.30	2	154–159, 195–197, 237–243, 302–304
10	20.30–22.00	3	78–82, 246–246, 277–281, 316–318
11	22.00–22.90	2	159–165, 195–197, 234–241
12	22.90–24.40	3	165–165, 229–236, 270–274, 345–345, 387–387
13	26.50–29.00	2	130–132, 159–161, 180–182, 197–197
14	29.00–34.33	2	170–174, 180–182

poor ($r^2 > 0.848$). The authors consider that these values are mostly affected by some less precision of the method for certain compounds, as can be observed in Table 1, rather than a lack of linearity. The inclusion of a great number of compounds to be monitored under MS may have produced a negative effect in the precision of the technique.

The overall evaluation of SPME–GC–MS performance characteristics indicates that this method is appropriate for pesticide residue analysis in ground and drinking waters, on account of the 0.1 $\mu g\,l^{-1}$ regulatory limit. Except for DEA, dimethoate and deltamethrin, which cannot be analysed at the required levels, the method showed adequate sensitivity, selectivity and precision. In general, as it has been reported by other authors, the technique is a bit less sensitive and precise compared to others employing selective detectors, however it has several advantages as discussed before. Fig. 1 presents chromatograms of a pesticide mixture and a contaminated groundwater sample analysed by SPME–GC–MS in the μSIS mode.

3.3. Tandem MS analysis

Recent improvements in commercial ion trap tandem MS equipment are encouraging their introduction into research and routine environmental laboratories [5]. In fact, application of MS–MS as a tool for unequivocal confirmation of pesticides in environmental samples is only in the beginning. Monitoring secondary fragmentation products enables better discrimination from matrix interferences than single MS [3]. In MS–MS, a specific ion (parent ion) formed by EI or chemical ionisation is isolated in the ion trap and subsequently undergoes fragmentation increasing its collisions with the helium molecules, that acts as carrier and collision gas [26]. By excluding all ions from the trap except the selected parent ion, a drastic reduction in the background of the sample is achieved. Only the parent ion is fragmented into characteristic product ions and MS–MS spectrum is obtained by scanning the mass spectrometer in the normal manner.

An MS–MS method was established based on the known occurrence of certain pesticides in groundwater samples from Póvoa de Varzim, in North Portugal, due to contamination from intensive horticulture and greenhouse farming (see Table 3).

MS–MS requires: (i) good selection of the parent ion, (ii) efficient ion isolation and storage and (iii) optimum collision induced dissociation (CID) conditions.

(i) The selection of the parent ion was accomplished from a compromise of the following two considerations: it should have the highest possible *m/z* value, good selectivity (high *m/z* ions are less prone to interferences) and the highest ion intensity in the single MS spectrum, good sensitivity (detection limits are highly dependent on the abundance of the parent ion). Whenever possible, selecting the molecular ion can give rise to an MS–MS spectrum matching a standard electron impact spectrum.

(ii) The isolation efficiency of the parent ion depends on the radiofrequency (RF)-storage voltage applied, which is related to the trapping field that stabilises the parent ion. The RF storage level for each analyte was calculated using the ion trap MS–MS tool kit software considering the *m/z* ratio of the parent ion and a "*q*" value of 0.4. A 2-*m/z*-isolation window was used.

(iii) The non-resonant mode of excitation gave enough cleavage energy to obtain good quality secondary spectra. The optimisation of the excitation amplitude voltage for each pesticide was carried out experimentally using the automated method development (AMD) option included in the MS–MS tool kit software. The optimum value for this parameter was reached when a secondary spectrum with multiple and intense product ions was obtained while keeping the parent ion intensity around 10%. Information on the selected parent ion, excitation storage voltage and collision-induced dissociation CID amplitude voltage for each individual pesticide, arranged in time segments, is shown in Table 3. It should be noted that, slightly different CID conditions were selected for the endosulfan isomers, which allowed the confirmation of the identity of each one by library-search. For MS–MS a good chromatographic resolution of the solutes is desirable however, even when this is not possible, the technique may be useful through a variation called multiple reaction monitoring (MRM). MRM allows the resolution of overlapped signals applying different CID conditions that commutate in a short period of time. After acquisition, separate plotting of the experimental data obtained for each pesticide offers a selective chromatogram where quantitation and identification can be conducted [20]. In the established method the entire chromatogram was split into 13 MS–MS segments and 2 MRM segments for the analyte pairs lindane/propyzamide and metolachlor/chlorpyrifos. Fig. 2 presents the MS–MS spectra of alachlor, propyzamide, chlorfenvinphos and endosulfan II. MS–MS spectra of the pesticides obtained in our optimum instrumental conditions were stored in a home-built EI–MS–MS library. Positive analyte identification in real samples required a spectral fit >700 relative to those spectra stored in the home built library. Table 3 presents data on the product ions in MS–MS spectra and respective relative ion intensities.

To confirm that the analytical method has performance capabilities compatible with its use in pesticide monitoring, method validation was carried out afterwards, calculating the detection limits and precision of the entire analytical procedure. At this point of the discussion should be noted that, for the majority of analytes the extracted ion chromatograms (EICs) monitoring the respective quantitation ions had

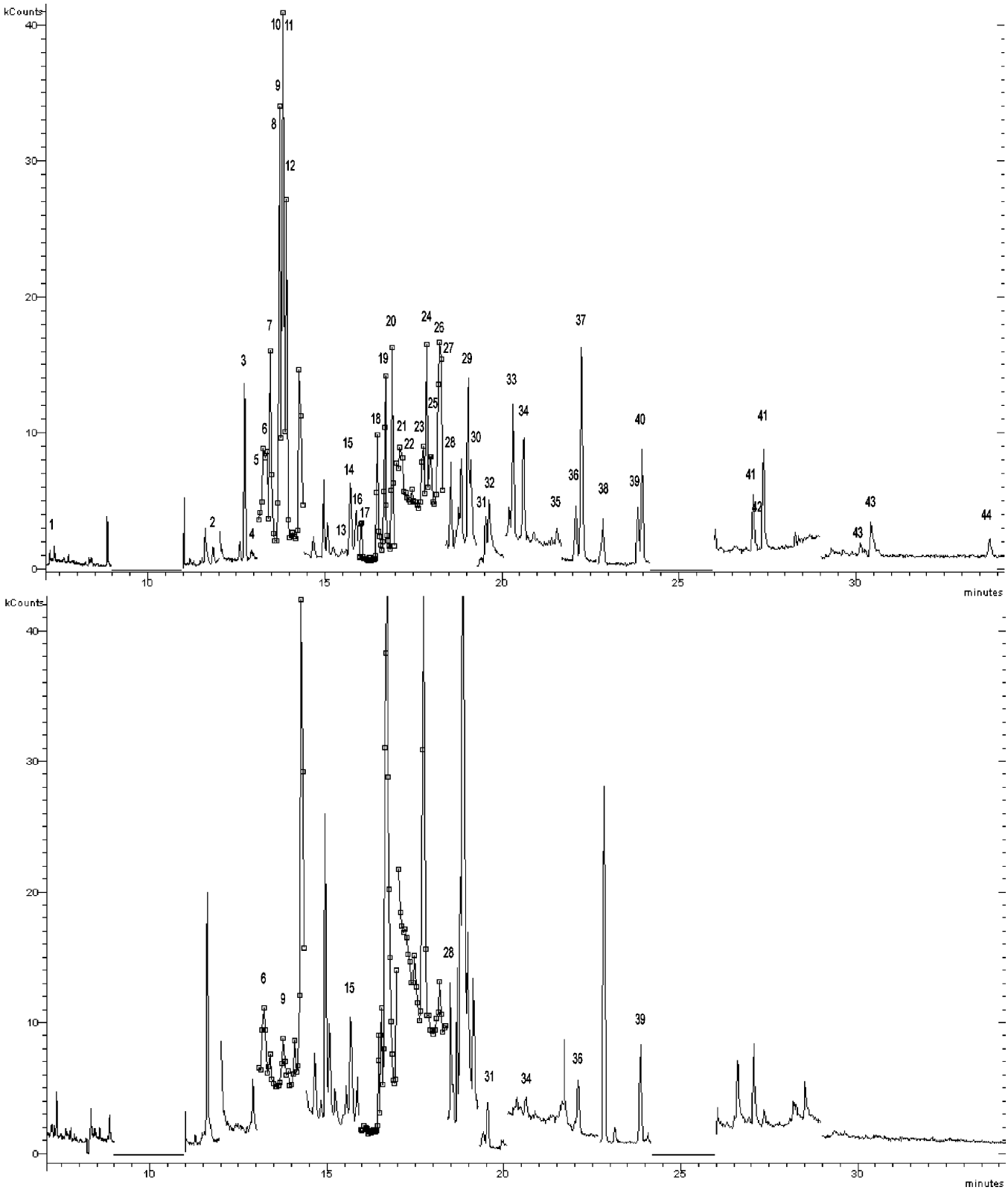

Fig. 1. GC–MS total ion chromatograms obtained under μSIS acquisition after SPME extraction of a 0.1 $\mu g\,l^{-1}$ spiked sample (above) and a contaminated groundwater sample (below) containing the following pesticide concentrations: atrazine 0.043 $\mu g\,l^{-1}$, lindane 0.006 $\mu g\,l^{-1}$, alachlor 0.020 $\mu g\,l^{-1}$, procymidone 0.068 $\mu g\,l^{-1}$, endosulfan I 0.115 $\mu g\,l^{-1}$, dieldrin not quantitated, endosulfan II 0.108 $\mu g\,l^{-1}$ and ensosulfan sulphate 0.597 $\mu g\,l^{-1}$. For peak assignment see Table 1.

Table 3
Operation conditions of the mass spectrometer in order to perform MS–MS: parent ion isolation, collision–induced dissociation (CID) and product ions obtained under the selected instrumental conditions, on distinct time segments

Pesticides	MS–MS segments (min)	Main EI fragment ions *m/z* and (abundance, %)	Parent ion (*m/z*)	Excitation storage level (*m/z*)	CID excitation amplitude (V)	Product ions *m/z* and (relative abundances,%)
DEA	11.00–12.20	187(30), 172(100), 68(26)	172	75.7	85	136(21), 109(32), 100(100)
Atrazine	12.20–13.60	215(64), 200(100), 58(33)	215 (M)	143	98	200 (100), 172 (20)
Lindane	13.60–15.20[a]	219(55), 181(100), 111(33)	183	80.5	76	148(100), 109(58)
Propyzamide		254(35), 173(100), 157(39)	173	76.1	75	145(39), 109(100)
Alachlor	15.20–16.20	188(100), 160(84), 45(44)	188	82.8	66	160(100), 132(98)
Metolachlor	16.20–17.12[a]	238(70), 162(100), 146(12)	162	71.3	68	132(100), 117(60)
Chlorpyrifos		314(100), 197(61), 97(66)	314	138.5	84	286(50), 258(100)
Parathion-ethyl	17.12–17.50	291(100), 109(98), 97(63)	291 (M)	81	58	125(34), 114(100), 109(52)
Pendimethalin	17.50–18.16	252(100), 191(21), 162(20)	252	111.1	72	208(100), 191(65), 162(62)
Chlorfenvinphos Z	18.16–18.60	323(58), 295(38), 267(100)	267	117.7	98	203(46), 159(100)
Procymidone	18.60–19.50	283(60), 96(100), 67(77)	283 (M)	124.3	94	255(100)
Endosulfan I	19.50–20.50	241(99), 195(100), 160(79)	241	106.2	99	206(94), 170(100)
Dieldrin	20.50–21.50	277(34), 263(24), 79(100)	277	122.1	94	241(100), 239(43)
Endosulfan II	21.50–22.70	241(85), 195(100), 160(95)	241	80	83	206(41), 170(100), 136(32)
Endosulfan sulphate	22.70–24.40	387(72), 272(100), 239(50)	272	119.9	98	237(100)
λ-Cyhalothrin	26.80–28–20	197(81), 181(100), 141(50)	197	86.7	70	161(11), 141(100)
α-Cypermethrin	28.20–31.33	181(89), 163(100), 127(99)	181	79.7	90	152(100)

CID time was 20 ms except for endosulfan I and endosulfan sulphate 30 ms. CID fragmentation was achieved with non-resonant wave forms for all compounds. M indicates molecular ion.
[a] Multiple reaction monitoring segments.

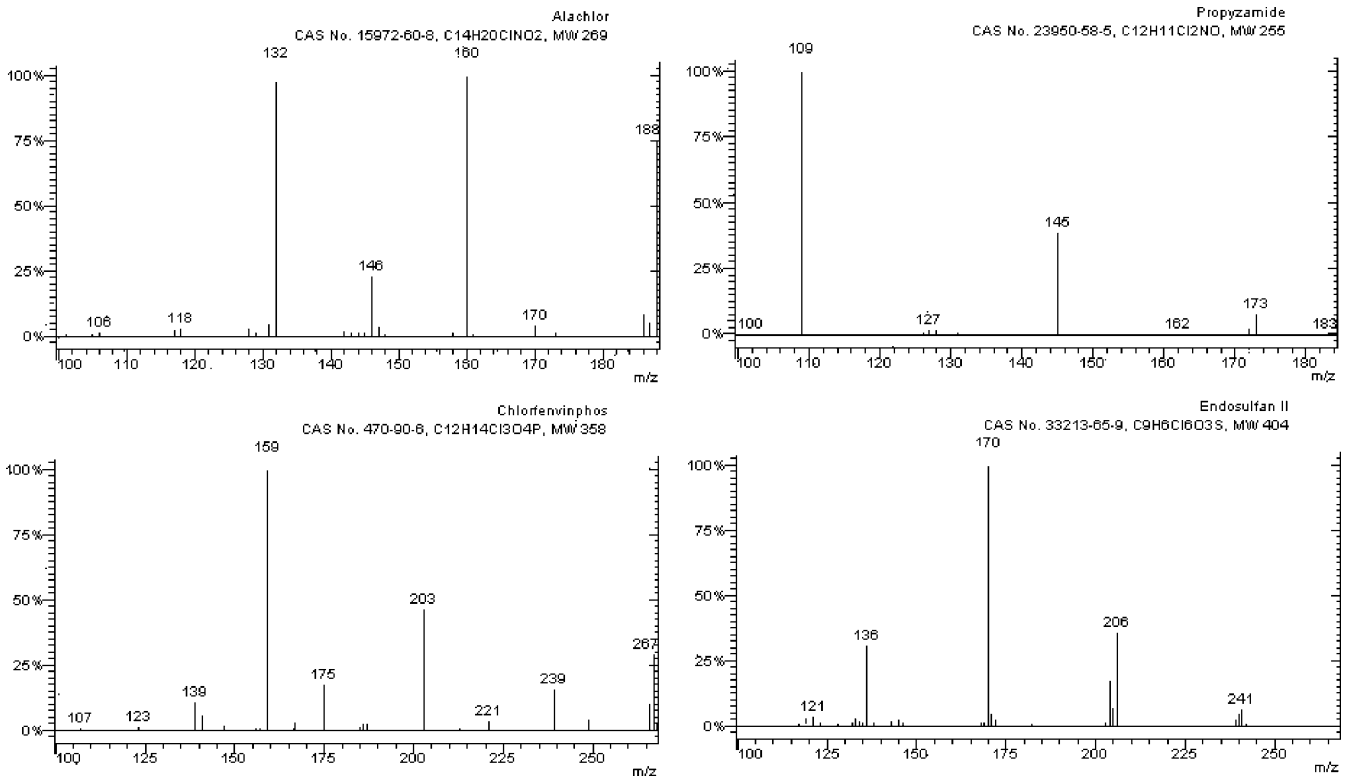

Fig. 2. MS–MS spectra for alachlor, propyzamide, chlorfenvinphos and endosulfan II showing their typical fragmentation patterns in our experimental conditions.

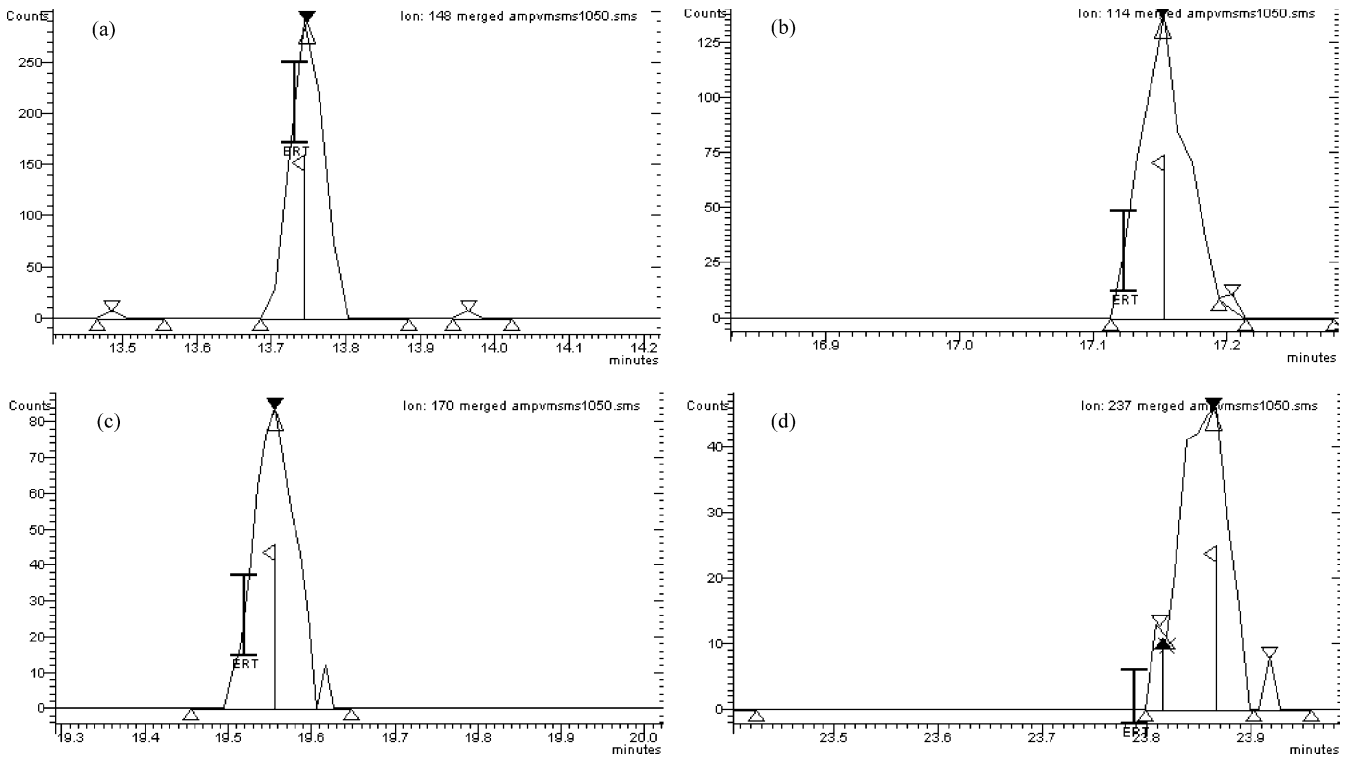

Fig. 3. Analysis of a 0.01 $\mu g\,l^{-1}$ aqueous solution of the following pesticides: (a) lindane, (b) parathion-ethyl, (c) endosulfan I and (d) endosulfan sulphate by SPME–GC–MS–MS. Extracted ion chromatograms monitoring the quantitation ions of these pesticides and showing low baseline noise.

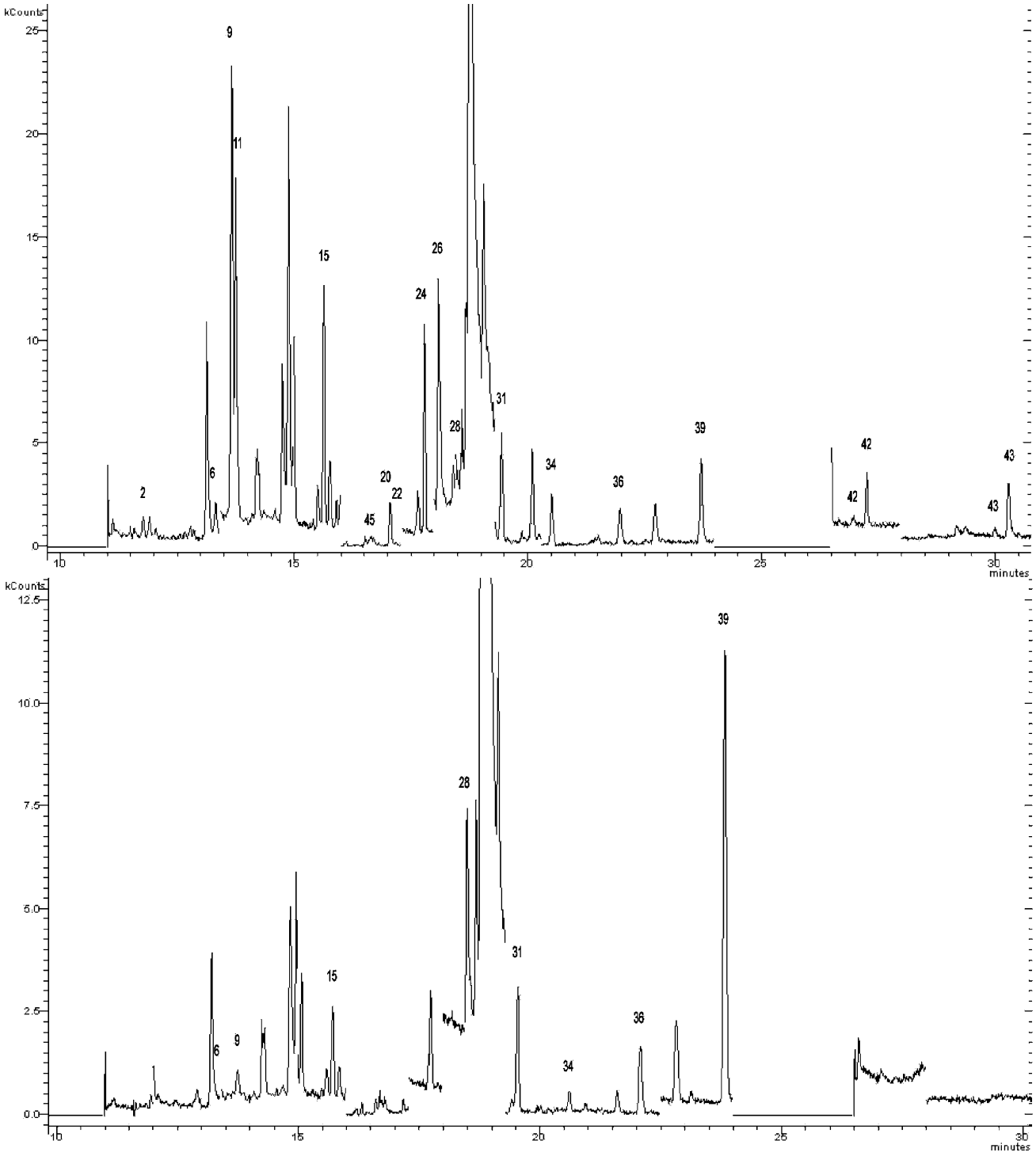

Fig. 4. Tandem MS chromatogram representative from a 0.1 $\mu g\,l^{-1}$ pesticide standard solution obtained by GC–MS–MS after SPME (above). Tandem MS chromatogram resulting from the analysis of the same contaminated groundwater sample as in Fig. 1 (below). For peak assignment refer to Table 4.

complete absence or extremely low baseline noise in the vicinity of target retention times, both for reagent and field blanks. Fig. 3 presents EICs for lindane, parathion-ethyl, endosulfan I and endosulfan sulphate at 0.01 $\mu g\,l^{-1}$ concentration level showing what has been described. In this situation, detection limits were determined as the lowest concentration giving a chromatographic peak for the quantitation ion that met the following criteria: peak width higher than 4 scans; maintaining two typical fragments in the spectrum; do not exist in the blank or a S/N of 3. Repeatability as within-day R.S.D. and intermediate precision as between-day R.S.D. of peak areas were also calculated for

Table 4
Pesticides included in the SPME–GC–MS–MS method and respective performance parameters in terms of sensitivity, precision and calibration

Peak No.	Pesticides	Quantitation. ion (*m/z*)	LOD ($\mu g\,l^{-1}$)	Repeatability (% R.S.D.), $n = 9$	Intermediate precision (% R.S.D.), $n = 20$	Determination coefficient (r^2)
2	DEA	100	0.040	11.7	15.4	0.991
6	Atrazine	200	0.004	3.7	5.8	0.994
9	Lindane	148	0.0002	7.1	8.1	0.993
11	Propyzamide	109	0.0005	4.6	5.2	0.996
15	Alachlor	160	0.0005	5.6	7.0	0.998
45	Metolachlor	132	0.002	2.9	8.9	0.997
20	Chlorpyrifos	258	0.001	9.9	12.8	0.995
22	Parathion-ethyl	114	0.001	9.2	10.0	0.993
24	Pendimethalin	208	0.002	8.2	10.5	0.995
26	Chlorfenvinphos Z	159	0.002	8.6	9.7	0.997
28	Procymidone	255	0.004	8.5	9.7	0.992
31	Endosulfan I	170	0.0008	5.8	9.8	0.997
34	Dieldrin	241	0.0005	7.7	9.3	0.994
36	Endosulfan II	170	0.001	7.8	8.7	0.999
39	Endos. Sulphate	237	0.0005	6.3	8.5	0.995
42	λ-Cyhalothrin	141	0.002	26.9	24.3	0.985
43	α-Cypermethrin	152	0.004	25.5	28.9	0.989

Note that peak number 45 corresponding to metolachlor at 16.880 min was only analysed by MS–MS.

a $0.1\,\mu g\,l^{-1}$ pesticide solution. As can be seen in Table 4, the LODs obtained were very low, some of them below $1.0\,ng\,l^{-1}$ and the rest below $10\,ng\,l^{-1}$ (except DEA). The precision was also very good typically below 10%. Method calibration was conducted in the same manner as reported before and good determination coefficients ($r^2 > 0.991$) were obtained. MS–MS chromatograms of a $0.1\,\mu g\,l^{-1}$ standard solution and a contaminated sample are presented in Fig. 4. Better LODs and precision values were obtained using GC–MS–MS rather than GC–MS for all pesticides, likewise it has been reported by other authors [3,15]. Gains in sensitivity up to 10-fold and LODs below $1.0\,ng\,l^{-1}$ using MS–MS, were also reported [1,3]. The SPME–GC–MS–MS method presented here lowered by a factor of 1.3–20.7 the detection limits obtained in single MS (μSIS mode). SPME-tandem MS allowed to attain a sensitivity comparable to SPE methods, which represent the great majority of applications on this field, with additional advantages.

It is clear from the chromatographic data in Figs. 1 and 4 that the analysis of contaminated samples is much easier using tandem MS, which allows quantitation of lower concentration levels, readily confirmable in the same analysis at LOQ level, which is unlikely with single MS. Furthermore, the SPME–GC–MS–MS method is as sensitive and precise when compared with the previously published SPME–GC–ECD/TSD. Depending on the purpose, screening or monitoring, single MS can also be chosen.

4. Conclusions

Multi residue analysis is the commonest way of determining pesticides. We have carried out experiments on mass spectrometric determination of pesticide residues in water samples after solid-phase micro-extraction and successful methods were established. The PDMS–DVB coating proved to be efficient on the extraction of about 40 pesticides and, thus, suitable for multi residue analysis. Subsequently, the GC–MS technique was selected due to its high selectivity i.e. high discriminating power among analytes and between these and matrix interferences. Used for monitoring or screening purposes a single MS method in the μSIS mode showed adequate sensitivity, selectivity and precision for pesticide analysis, regarding the $0.1\,\mu g\,l^{-1}$ EU limit. However, its improved sensitivity was accomplished on the expense of qualitative data and thus for confirmation of positive results a second analysis in full scan mode should be conducted. Additionally, in certain circumstances only concentrations well above the LOQ could be confirmed due to the presence of high background in the spectra. The GC–SIS–MS approach didn't prove much useful. For a set of pesticides currently found as contaminants in groundwater samples from Póvoa de Varzim also an SPME–GC–MS–MS method was developed. This approach allowed important improvements in selectivity and sensitivity and thus in identification and quantification capabilities for low traces of pesticides in water samples. Background noise and interferences were almost completely eliminated and clean secondary spectra permitted identifications with high certainty. Analysis of an extent list of analytes under MS–MS is not a limitation however it's difficult to implement and manage.

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