Simultaneous analysis of endosulfan, chlorpyrifos, and their metabolites in natural soil and water samples using gas chromatography-tandem mass spectrometry

Manoj K. Tiwari · Saumyen Guha

Abstract Analysis of endosulfan, chlorpyrifos, and their nonpolar metabolites in extracts from environmental aqueous and soil samples was performed using a gas chromatography-tandem mass spectrometry (GC–MS/MS) technique. Full-scan GC–MS analysis showed poor sensitivity for some of the metabolites (endodiol and endosulfan ether). A multisegment MS/MS method was developed and MS/MS parameter isolation time, excitation time, excitation voltage, and maximum excitation energy were optimized for chosen precursor ions to enhance selectivity and sensitivity of the analysis. The use of MS/MS with optimized parameters quantified analytes with significantly higher accuracy, and detection limits were lowered to ~1/6th compared with the full-scan method. Co-eluting compounds, chlorpyrifos and chlorpyrifos oxon, were also analyzed successfully in the MS/MS mode by choosing exclusive precursor ions. Analysis of soil and water phase samples from contaminated soil slurry bioreactors showed that the MS/MS method could provide more reliable estimates of these pesticide and metabolites (especially those present in low concentrations) by annulling interferences from soil organic matter.

Introduction

Analysis of pesticide residues and their toxic transformation products in natural soil and water samples is increasingly becoming a subject of scientific attention. Several multiresidue analytical methods are available for detecting a large number of pesticides in a single sample (Fernández Moreno et al. 2008; Lu 2010; Lian et al. 2010); however, the analysis of metabolites has received limited attention. A number of metabolites of various pesticides were shown to have toxicity comparable to, or at times higher than, their parent compound [like chlorpyrifos oxon is nearly 1,000 times more toxic than chlorpyrifos (Duirk and Collette 2006)]. Therefore, monitoring of the residues of toxic metabolites is equally important for characterization of soil and water samples. Over 60 % of registered pesticides and some of their metabolites are amenable to gas chromatography–mass spectrometry (GC–MS) without any derivatization, making it the most widely used technique in trace analysis of pesticides (Geerdink et al. 2002; Lin et al. 2007; Alder et al. 2006;

M. K. Tiwari (✉) · S. Guha
Department of Civil Engineering,
Indian Institute of Technology Kanpur,
Kanpur 208016, India
e-mail: mktiwari@iitk.ac.in

Fernández Moreno et al. 2008; Raina 2011). Thermally stable nature of the majority of pesticides makes them suitable for the GC technique (Sauret et al. 2000), and ability of the MS to identify compounds based on their mass spectrum makes it the most informative detector.

In the case of complex environmental matrices, many pesticides exhibit low intensity for molecular ion relative to other interfering compounds. As a result, mass spectra and resulting molecular structure of target compounds may not be very specific (Lin et al. 2007; Béguin et al. 2006; Pyle et al. 1998). The following are the two major problems encountered: (1) the molecular ion being too low in abundance to be observed and (2) co-elution of other compounds of interest or from background matrix that cannot be distinguished in the mass spectra. The first case is common for the compounds present in substantially lower concentrations compared with other compounds in the same sample. Simultaneous analysis of parent pesticides and their metabolites often encounters such condition because of large differences in concentrations of parent compounds and metabolites, especially at early stages of transformation. The second problem of co-eluting peaks may arise with similar (or closely related) structured compounds that could be difficult to separate in GC columns. Analytical laboratories are perpetually attempting to overcome these issues by improvising techniques to reduce background signal, and improve selectivity and sensitivity of the analysis.

Earlier, GC–MS in a selected ion monitoring (SIM) mode was used to increase sensitivity, but it enhances risk of false positives especially for samples with high background noise (Raina 2011; Arrebola et al. 2001; Leung et al. 1998). With development of ion trap instruments, GC-tandem MS (GC–MS^n) was successfully demonstrated to exclude interferences in the background matrix by filtering out all eluted ions except the chosen daughter ion from the analyte of interest (Béguin et al. 2006; Wong et al. 2010). This also allows analysis of co-eluting compounds without chromatographic resolution by choosing mutually exclusive daughter ions for secondary fragmentation. The reduced background noise results in relatively higher resolution and sensitivity of measurement, compared with the full-scan MS analysis (Lin et al. 2007; Rashid et al. 2010). Superior selectivity and sensitivity of GC–MS/MS compared with GC–MS have been reported for several compounds of

environmental interests (Pyle et al. 1998; Sauret et al. 2000; Raina and Hall 2008).

The sensitivity of the GC–MS/MS depends on precursor ion isolation time, excitation voltage, excitation time, excitation energy for dissociation of the chosen ion, and detection of the product ions (Mandalakis et al. 2001; Béguin et al. 2006; Sinha 2010). Therefore, these parameters in the instrument settings must be optimized in order to attain lower detection limits. A few studies have attempted the optimization of the instrumental parameters of GC–MS/MS for pesticides, polychlorinated biphenyls (PCBs), and dioxins by exploring influence of individual parameters on the sensitivity in the detection of product ions (Mandalakis et al. 2001; Béguin et al. 2006; Sinha et al. 2011). Ideal operating conditions vary with the compounds and hence, instrument parameters should be optimized separately for each target compound.

In this paper, we discuss the development of a GC–MS/MS method for the sensitive analysis of two among the most commonly used agricultural pesticides, endosulfan and chlorpyrifos, along with their nonpolar transformation products. The residue of these pesticides and their degradation products in soil and water matrices vary from a few parts per billion to parts per million levels and is considered toxic to human health (Sutherland et al. 2004; Weber et al. 2010; Racke 1993; Shunthirasingham et al. 2010; Bempah and Donkor 2011). These two pesticides can also be present together in the same soil due to their various common applications or due to drift during spray application on adjacent land. Therefore, sensitive analytical methods are required to precisely detect and quantify these pesticides as well as their metabolites in complex environmental samples. The reported method in this paper was developed in order to analyze the soil and water sample contaminated with both these pesticides and their nonpolar metabolites. Test samples from experiments on biodegradation of endosulfan and chlorpyrifos by soil microorganisms were used to evaluate the efficacy of the MS/MS analysis over full-scan MS analysis.

Experimental

Pesticide/metabolite standards

Certified standards of α-endosulfan (99.6 %), β-endosulfan (99.9 %), endosulfan sulfate (97.7 %),

endodiol (99.9 %), endosulfan ether (99.9 %), endosulfan lactone (99.9 %), and chlorpyrifos (99.2 %) from Sigma-Aldrich, USA, and chlorpyrifos-oxon (99.9 %) from Dr. Ehrenstorfer GmbH were used as external standards. The 2,4,5,6-tretrachloro-*m*-xylene (99.9 %) from Sigma-Aldrich, USA, was used as the internal standard (IS) at a concentration of 200 ppb. Solvent used to prepare stock and working solutions was high performance liquid chromatography (HPLC)-grade *n*-hexane (99 %) from E. Merck Limited, India.

Samples for analysis

Standard mix

A mixed standards solution prepared for separation consisted of (in micrograms per liter): α-endosulfan, 500; β-endosulfan, 200; endosulfan sulfate, 100; endodiol, 100; endosulfan ether, 100; endosulfan lactone, 100; chlorpyrifos, 200; and chlorpyrifos oxon, 200.

Test samples

The test samples were sourced from a lab-scale batch soil slurry (1:3 w/w soil–water ratio) reactor setup with contaminated agricultural soil and indigenous soil bacteria in aerobic environment for studying biodegradation of endosulfan and chlorpyrifos. Initial concentration of pesticides on soil was 6.57 ± 0.34 mg/kg endosulfan and 11.07 ± 0.94 mg/kg chlorpyrifos, which were in the range of typical concentrations reported in the environmental samples (Racke 1993; Weber et al. 2010). The concentration of pesticides and metabolites was anticipated to vary at different stages of biodegradation. The soil and water test samples contaminated with the target pesticides and their metabolites were obtained at day 2 (initial stage), day 7 (middle stage), and day 15 (termination stage) of the slurry reactor. The pesticides and metabolites were extracted in ethyl acetate from water samples using liquid–liquid extraction with 1:6 (v/v) sample-to-solvent ratio (Tiwari and Guha 2012). The extraction from soil samples was performed using solid–liquid extraction in ethyl acetate with a 1:5 (w/v) soil-to-solvent ratio (Khodadoust et al. 1999).

Analysis

A trace GC coupled with an ion trap MS detector (PolarisQ, Thermo Finnigan, USA) equipped with a programmable Split/Splitless Injector and TriPlus Autosampler was used for measurements. Three columns DB-5 ms, DB-XLB, and DB-35 ms (Agilent Technologies, India), each with length of 30 m, internal diameter of 0.25 mm, and film thickness of 0.25 μm, were tested as stationary phase for separation. The results were evaluated using XCalibur software and the MS libraries, NIST 2.0 and Wiley 7.

GC–MS was operated under the following conditions: carrier gas (helium) flow of 1 mL/min, injection volume of 1 μL (splitless mode), injector temperature of 250 °C, ion source temperature of 280 °C, auxiliary line temperature of 240 °C, electron energy of 70 eV, emission current of 250 mA, and automatic gain control target value of 50. The oven temperature program (selected to provide better separation of analytes) was as follows: start at 140 °C, increase to 180 °C at 8 °C/min, hold for 1 min, increase to 250 °C at 4 °C/min, and hold for 1 min. The detection was made in full-scan electron ionization (EI) mode as well as multisegment MS/MS mode. The MS signals were acquired after the first 5–6 min in order to protect the unnecessary contamination of the filament from the solvent vapors eluting within the first few minutes as recommended by the instrument's manufacturer. Full-scan EI mass spectra were recorded by scanning the ions with mass-to-charge ratio (m/z) from 50 to 500. Retention time (RT) of the compounds (Table 1) served as the basis for selecting time segments for MS/MS analysis. The MS/MS parameters for the each segment (Table 2) were optimized to produce the highest peak area and signal-to-noise (S/N) ratio of the target compounds' peak with the chosen precursor ion, as described later in "MS/MS analysis".

Results

Separation of the compounds

Separation of analytes in the *standard mix* was compared in full-scan mode for differences in RT and sharpness of the peaks on all three columns (DB-5 ms, DB-XLB, and DB-35 ms) with several oven temperature programs. All three columns were able

Table 1 Average retention time (RT) for the various compounds analyzed using DB-5 ms, DB-35 ms, and DB-XLB columns with same operating conditions and final oven temperature program (given in "Analysis"). The *values in parenthesis* are the difference in the RT of successively eluting compounds

Compounds	Retention time (RT) (min)		
	DB-5 ms	DB-35 ms	DB-XLB
2,4,5,6-Tetrachloro-*m*-xylene	6.22	5.84	6.71
Endosulfan ether	8.27 (+2.05)	7.01 (+1.17)	9.71 (+3.00)
Chlorpyrifos/chlorpyrifos oxon[a]	10.79 (+2.52)	8.32 (+1.31)	13.04 (+3.33)
Endodiol	11.78 (+0.99)	8.95 (+0.63)	14.54 (+1.50)
Endosulfan lactone	12.42 (+0.64)	9.50 (+0.55)	15.37 (+0.83)
Alpha Endosulfan	13.25 (+0.83)	10.25 (+0.75)	16.41 (+1.04)
Beta Endosulfan	15.83 (+2.58)	12.46 (+2.21)	19.90 (+3.49)
Endosulfan sulfate	17.30 (+1.47)	13.70 (+1.24)	21.73 (+1.83)

[a] Chlorpyrifos and chlorpyrifos oxon appeared at nearly the same RT and were not separated on any of the three columns

to separate all the analytes in the *standard mix*, barring chlorpyrifos and chlorpyrifos oxon. Chlorpyrifos and its metabolite, chlorpyrifos oxon, appeared at nearly the same RT, and a better separation could not be obtained even after numerous variations in the oven temperature program and carrier gas flow rate on all three columns. Although, gaps in the RT of successive eluting compounds increased at slower rates of temperature ramping in the GC oven, the sharpness of the peaks reduced, making the base of the peaks wider. Therefore, the rate of oven temperature ramping was adjusted to a level ensuring sharp peaks and significant gaps in the RT of successive eluting compounds.

A typical separation obtained in each column for the final oven temperature program is shown in Table 1. The DB-XLB followed by DB-5 ms provided larger differences in the RT of the analytes (Table 1) and visually sharper peaks compared with DB-35 ms. Therefore, all subsequent analyses were made using the DB-XLB column in full-scan as well as MS/MS modes.

Full-scan analysis

Full-scan raw chromatogram showing separation of the compounds in the *standard mix* analyzed using DB-XLB is shown in Fig. 1. Some additional peaks of impurities also appeared and were identified as column bleeds in the form of various siloxane compounds (Fialkov et al. 2007). Recorded mass spectrum

Table 2 The optimum set of MS/MS parameters for all the compounds producing the highest analytical sensitivity

Compound	Segment		Isolation parameters		Excitation parameters		
	Start time (min)	End time (min)	Precursor ion (*m/z*)	Time (ms)	Voltage (V)	Time (ms)	Energy (*q* value)
Endosulfan ether	9	11	69	40	0.8	60	0.45
Chlorpyrifos oxon	11	14	314	30	3	60	0.45
Chlorpyrifos	11	14	298	30	3	60	0.45
Endodiol	14	15	143	60	0.6	10	0.45
Endosulfan lactone	15	16	277	40	2	60	0.45
α- and β-endosulfan[a]	16	21	239	40	1.5	60	0.45
Endosulfan sulfate	21	24.5	272	40	0.8	60	0.45

[a] α- and β-endosulfan were analyzed in the same segment for the precursor ion *m/z* 239 at RT 16.41 and 19.90 min, respectively

Fig. 1 A full-scan chromatogram showing the separation of the compounds in the *standard mix*. The peaks of chlorpyrifos and chlorpyrifos oxon merged at nearly the same RT (13.02–13.04 min)
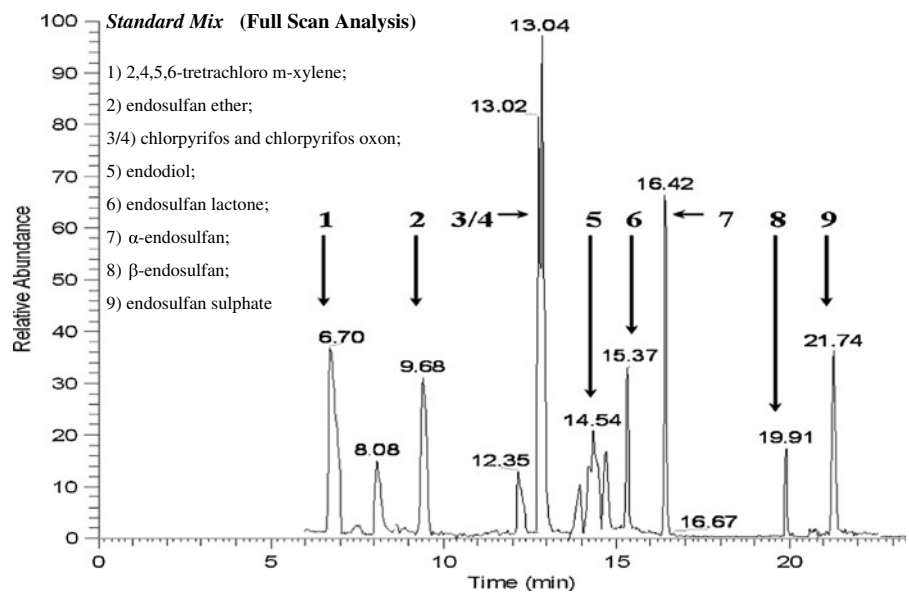
of all the analytes matched well with respective mass spectrum of compounds from the NIST 2.0 as well as Wiley 7 MS libraries.

Validation for the method included linearity check of calibration curves, and precision and accuracy studies of the analytes. The five-point standard calibration curves for quantification of the compounds were prepared by plotting their concentration (in the range of 50–500 μg/L) in the mixed standard solutions analyzed in triplicate, with the *Response* (peak area obtained for analyte / peak area obtained for IS). Since the full-scan method was not able to separate chlorpyrifos and chlorpyrifos oxon, single standards (along with IS) of these analytes were injected independently and the obtained peak area was used to estimate the *Response*. The standard calibration curves for the compounds in the selected concentration range were linear. Regression slope and coefficient of regression (r^2) for all the compounds are shown in Table 3.

The analytes were quantified in the standard mix in triplicate. Accuracy was estimated as percent recovery, while precision of the assay was determined as percent of coefficient of variation (%CV), i.e., the percentage of the ratio of standard deviation to the average concentration (Berthouex and Brown 2002). Accuracy and precision for chlorpyrifos and chlorpyrifos oxon were estimated using single standards of chlorpyrifos and

chlorpyrifos oxon of 200 μg/L concentrations. With the exception of endodiol and endosulfan ether, all other analytes were estimated with over 90 % accuracy (Table 3). Recoveries were comparable with existing reports for the analysis of various pesticides using GC–MS (Cho et al. 2008; Yang et al. 2011).

Analytical sensitivity of the method was evaluated statistically (Corley 2003) by estimating the limit of detection (L_D) and the limit of quantification (L_Q). Negative controls (pure *n*-hexane and ethyl acetate) showed no detectable peaks for any of the compounds in the chromatograms. Samples containing 5–50 ppb of pure compounds were analyzed and the standard deviations (σ) of 10–20 replicates were estimated. The L_D and L_Q were estimated as 3.29σ and 10σ, respectively (Armbruster and Pry 2008). The estimated L_D values ranged between 3.1 and 14.8 μg/L and L_Q were obtained in the range of 9.4–44.9 μg/L for full-scan GC–MS analysis (Table 3).

Lesser accuracy and higher L_Q values for some of the compounds (endodiol and endosulfan ether) indicated that low level quantification of these analytes may not be trustworthy. Therefore, it was required to increase the sensitivity of the method towards these analytes. This, in addition to the inability of the method in quantifying chlorpyrifos and chlorpyrifos oxon from the mixed samples, warranted detection to be switched in the MS/MS mode.

Table 3 Accuracy, precision, and detection and quantification limits of the analytes for the GC–MS full-scan and GC–MS/MS analysis methods

Compound	Calibration details Slope response/(mg/L) (r^2 in parenthesis)		Accuracy and precision Measured conc. in standard mix (µg/L)		Accuracy (% recovery)		Precision (% coff. of variance)		Limit of detection (L_D) and limit of quantification (L_Q) Measured std. deviation (σ) (µg/L)		L_D (µg/L)		L_Q (µg/L)	
	Full scan	MS/MS	Full scan	MS/MS	Full scan	MS/MS	Full scan	MS/MS	Full scan	MS/MS	Full scan	MS/MS	Full scan	MS/MS
α-Endosulfan	2.153 (0.96)	0.149 (0.99)	472.6±23.7	491.2±9.6	94.5	98.2	5.01	1.95	1.396	0.365	4.6	1.2	14.0	3.6
β-Endosulfan	2.402 (0.97)	0.090 (0.96)	186.8±15.1	202.5±5.5	93.4	101.2	8.08	2.72	1.112	0.279	3.73	0.9	11.1	2.8
Endosulfan sulfate	1.704 (0.94)	0.113 (0.99)	94.6±6.0	98.5±0.8	94.6	98.5	6.34	0.81	1.907	0.403	6.3	1.3	19.1	4.0
Endodiol	0.841 (0.88)	0.029 (0.99)	76.8±16.4	92.7±8.2	76.8	92.7	21.35	8.85	4.491	0.842	14.8	2.8	44.9	8.4
Endosulfan ether	1.586 (0.92)	0.036 (0.99)	87.2±11.5	95.5±4.2	87.2	95.5	13.19	4.40	2.402	0.560	7.9	1.8	24.0	5.6
Endosulfan lactone	1.569 (0.96)	0.034 (0.98)	91.5±7.2	97.8±2.0	91.5	97.8	7.87	2.04	0.940	0.256	3.1	0.8	9.4	2.6
Chlorpyrifos[a]	3.121 (0.94)	0.266 (0.99)	180.1±17.5	198.4±5.0	90.1	99.2	9.72	2.52	1.104	0.152	3.4	0.5	11.0	1.5
Chlorpyrifos oxon[a]	3.645 (0.96)	0.149 (0.99)	190.4±14.0	188.1±6.4	95.2	94.1	7.35	3.40	1.926	0.337	6.3	1.1	19.3	3.4

[a] Since chlorpyrifos and chlorpyrifos oxon were not separated in the full-scan method, their quantification parameters for the full-scan method were estimated using the single standards of chlorpyrifos and chlorpyrifos oxon independently

MS/MS analysis

Selection of the MS/MS parameters

In order to achieve the highest sensitivity for each compound, the MS/MS parameters were optimized for the individual analytes in their time segment (Table 2). The m/z of the ion with the highest relative abundance in the mass spectrum was selected as the precursor ion for each compound. Isolation width of the precursor ion was reduced to the lowest possible value to improve the specificity by ensuring the least interference from the nontarget ions (Mandalakis et al. 2001). The other MS/MS parameters (isolation time, excitation voltage, excitation time, and maximum excitation energy) were optimized to produce the highest sensitivity (peak area and S/N ratio). This exercise was performed for two to four largest precursor ions (major base peaks) in the mass spectrum of each compound. These parameters were varied within the entire range suggested by the manufacturer and supported by the literature (Mandalakis et al. 2001).

The maximum excitation energy (q value) could be adjusted to only three preset options, i.e., low (0.225), medium (0.3), and high (0.45). However, higher sensitivity was recorded at the highest q value for all the analytes owing to their stable chlorinated carbon ring structure (March 1997; Mandalakis et al. 2001). An illustrative example for the effect of q values for 239 m/z precursor ion of α-endosulfan is shown in Fig. 2a. Similar trends were obtained for other analytes as well. Therefore, maximum excitation energy was set at "High" for all the analytes (Table 2).

The remaining parameters, excitation time, isolation time, and excitation voltage, were varied within the possible ranges of 0–100 ms, 2–64 ms, and 0–60 V, respectively. For each precursor ion, a three-factor multilevel experimental design (Berthouex and Brown 2002) was constructed by simultaneously varying these parameters. The number of experiments was different for different analytes (precursor ion) depending on proximity to the maxima and nature of the response curve (Berthouex and Brown 2002). Variation of each parameter around the optimum, while keeping the other parameters constant at the optimum level, sheds some light on topography of the multidimensional space. A typical example of the effect of the variations in these parameters on the
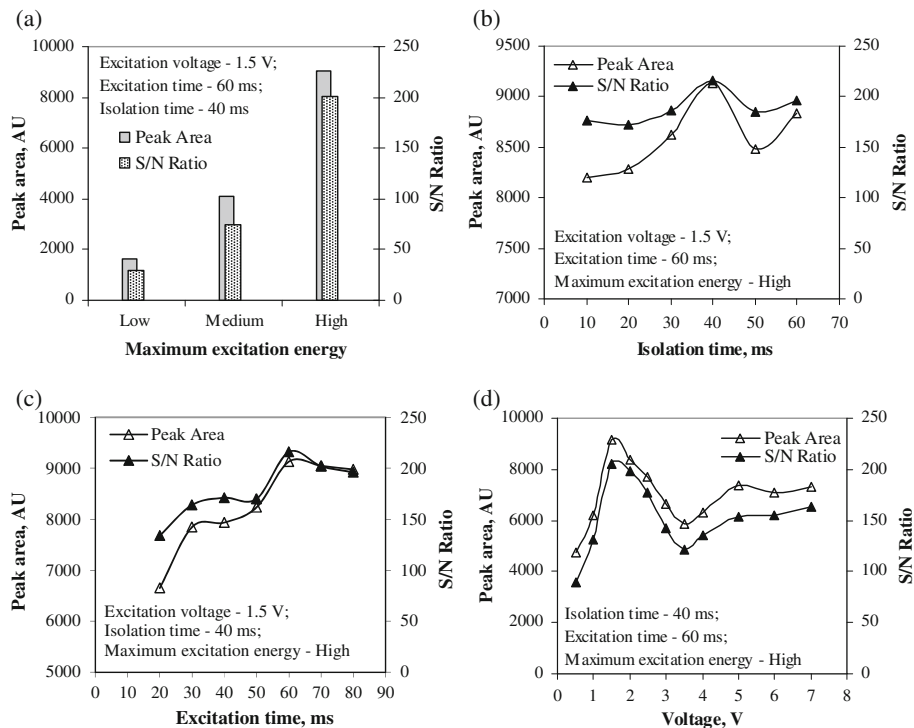
Fig. 2 The variation in chromatographic responses (area and S/N ratio) for the 239 m/z precursor ion of α- and β-endosulfan with the variations in MS/MS parameters **a** maximum excitation energy, **b** isolation time, **c** excitation time, and **d** excitation voltage

sensitivity of detection for 239 m/z precursor ion of α-endosulfan is illustrated in Fig. 2b–d. Similar responses were obtained for other compounds as well. The optimum values of the three parameters resulting in the highest peak area and S/N ratio for all the analytes of interest are listed in Table 2.

Analysis of co-eluting compounds

In the full-scan mode analysis, chlorpyrifos and chlorpyrifos oxon peaks appeared at approximately the same RT (the difference was statistically insignificant) and was not separated by varying the oven temperature programs, carrier flow rates, and columns. Therefore, in the MS/MS mode, these two were analyzed at the same RT by choosing mutually exclusive precursor ions in two different injections.

Mass spectrum of chlorpyrifos and chlorpyrifos oxon obtained through injecting their single standards in full-scan mode showed that the 314 m/z base peak of chlorpyrifos was absent in the mass spectrum of chlorpyrifos oxon, whereas 298 m/z base peak of chlorpyrifos oxon was absent in the mass spectrum of chlorpyrifos. This

was also confirmed with the mass spectrum of chlorpyrifos and chlorpyrifos oxon sourced from NIST 2.0 Standard MS Library. Since these base peaks were mutually exclusive, chlorpyrifos oxon would not interfere in the chlorpyrifos analysis with 314 m/z as precursor ion in MS/MS mode. Similarly, chlorpyrifos oxon could be analyzed with 298 m/z as precursor ion, without interference from chlorpyrifos. Therefore, these daughter ions were selected as precursor ions for the MS/MS analysis of the respective compounds in the same time segment. The MS/MS parameters were selected for these precursor ions, similar to that described earlier, by injecting single standards of the analytes. Composite samples were injected twice, first with the MS/MS parameter settings for chlorpyrifos and then for chlorpyrifos oxon. The interference was assessed by analyzing the 200 μg/L pure chlorpyrifos standard, 200 μg/L pure chlorpyrifos oxon standard, and a mixed standard with 200 μg/L concentration of each, in triplicate. Differences in the mean of the *Response* obtained for the pure compounds with the mean of the *Response* obtained from mixed sample analysis were not statistically significant (at 95 % confidence level) for either

compound. This indicated that the MS/MS method can be used for quantification of chlorpyrifos and chlorpyrifos oxon in the same sample.

Method validation and detection/quantification limits

The *standard mix* analyzed in MS/MS mode produced noise-free clean peaks for each analyte in their time segment. The mixed standard of all the analytes in the range of 5–500 μg/L was analyzed in triplicate for plotting the five-point standard calibration curves. The standard calibration curve for all the compounds was linear with $r^2 \geq 0.96$ (Table 3). Accuracy, precision, L_D, and L_Q values for the MS/MS method were obtained similar to the full-scan method and are listed in Table 3. The MS/MS method produced recoveries over 92.7 % for all the analytes. The estimated accuracy and precision of the assay for all the analytes were in acceptable range and comparable with the literature (Pinho et al. 2010; Singh et al. 2009). The higher accuracy and lower %CV for all the analytes indicated that the MS/MS method would be more accurate for the measurement of these analytes, compared with full-scan method.

Higher order fragmentation

Similar to the MS/MS fragmentation of the chosen precursor ion from the full-scan mass spectra of the analyte, it is possible to choose a precursor ion from the MS/MS spectra and carry out its further fragmentation (MS^n). The instrument used in this study (PolarisQ) allowed carrying out the subsequent fragmentations up to MS^5. However, MS/MS led to clean chromatograms and very good quantification for the target compounds, and further fragmentation (MS^3) did not lead to any significant improvement in detection and quantification.

Application to real-sample analysis

Soil and water phase extracts of test samples were analyzed in full-scan as well as MS/MS mode. Concentrations of various compounds in the soil and water extracts of day 2, day 7, and day 15 samples estimated using MS/MS as well as full-scan method are shown in Table 4. In the full-scan mode, both soil and water phase extracts had more interference from natural hydrophobic organic matters compared with the standard

mix. Such impurities were reported to produce interferences in the GC–MS chromatogram for PCBs and pesticides extracted from soil matrix (Raina and Hall 2008; Alder et al. 2006; Fialkov et al. 2007). These impurities led to unwanted peaks in the full-scan chromatogram as illustrated in the example chromatogram from the full-scan analysis of the soil phase extract of day 7 sample (Fig. 3).

The same set of samples analyzed in MS/MS mode produced noise-free clean peaks for each analyte in their time segment. For example, the MS/MS chromatogram of the soil phase extract of day 7 sample (for which full-scan chromatogram is shown in Fig. 3) is shown in Fig. 4. Due to differences in chromatographic responses for various precursor ions, the chromatogram was analyzed segmentwise for better visualization. Chlorpyrifos and chlorpyrifos oxon shown in the segment 11–14 min in Fig. 4 were analyzed in two separate injections. Further fragmentation of the chosen precursor ions for analytes evidently filtered out all the noise and interferences due to natural organic matter and column bleed that were observed in the full-scan chromatogram (Fig. 4).

Discussion

The separation of a mixture of compounds on GC depends on various factors including polarity of the stationary phase. In the present study, a fused silica-based DB-XLB column recommended (by the manufacturer) for the analysis of pesticides, herbicides, PCBs, and polycyclic aromatic hydrocarbons (PAHs) provided better separation compared with DB-5 ms and DB-35 ms. Better separation with DB-XLB indicated greater differences in the holdup times for these analytes on fused silica compared with low (5 %; DB-5 ms) and high (35 %, DB-35 ms) phenyl content stationary phases.

Analysis in the full-scan mode was able to detect the target compounds, but sensitivity towards the compounds present in smaller concentrations was low resulting in lesser accuracy and higher L_Q values for these compounds (Table 3). This, added with the method's failure in analyzing co-eluting chlorpyrifos and chlorpyrifos oxon, warranted the analysis to be performed in MS/MS mode. The MS/MS parameters (i.e., isolation width, isolation time, excitation voltage, excitation time, and maximum excitation energy)

Table 4 Analysis of the compounds in the test samples (sourced from various stages of soil slurry reactors setup for the biodegradation of these contaminants) using the GC-MS/MS and full-scale analysis methods. Ratio of recovery (RR) was obtained as the ratio of concentrations measured using full-scan method to that using MS/MS method, as the true concentrations (unknown) of the compounds were cancelled out when calculating the ratio of recoveries

Compound	Day 2			Day 7			Day 15		
	MS/MS	Full scan	Ratio of recovery	MS/MS	Full scan	Ratio of recovery	MS/MS	Full scan	Ratio of recovery
Soil phase concentration (µg/kg soil)									
α-Endosulfan	2,893.8±244.2	2,657.7±305.2	0.92	2,087.9±85.5	1,986.2±185.4	0.95	1,461.1±20.3	1,326.8±68.5	0.91
β-Endosulfan	1,200.7±101.7	1,139.6±126.2	0.95	647±32.6	618.6±38.0	0.96	431.4±16.8	398.8±20.2	0.93
Endosulfan sulfate	99.7±6.1	67.6±8.5	0.68	223.0±15.3	177.7±25.6	0.80	432.5±15.3	490.7±32.4	1.13
Endodiol	70.0±9.9	ND	–	105.8±10.0	72.2±20.1	0.68	194.0±19.7	140.8±27.4	0.73
Endosulfan ether	ND	ND	–	144.1±11.0	120.0±15.0	0.83	223.6±24.8	171.5±24.1	0.77
Endosulfan lactone	ND	ND	–	100.1±8.4	78.5±11.4	0.79	203.4±15.4	185.6±12.6	0.91
Chlorpyrifos	7,634.7±125.0	NM	–	6,694.2±95.9	NM	–	4,926.9±44.4	NM	–
Chlorpyrifos oxon	154.9±1.8	NM	–	476.4±28.9	NM	–	520.3±59.2	NM	–
Water phase concentration (µg/L)									
α-Endosulfan	109.9±4.1	89.5±6.8	0.82	102.9±12.2	101.7±15.2	0.93	93.6±9.6	93.6±6.9	1.00
β-Endosulfan	48.8±3.89	40.7±4.5	0.83	44.8±6.7	36.6±7.4	0.82	40.7±5.0	44.8±4.3	1.10
Endosulfan sulfate	38.1±7.0	29.6±11.2	0.78	112.4±12.4	101.5±16.4	0.90	100.7±0.8	88.8±2.6	0.88
Endodiol	46.0±3.1	25.3±7.0	0.55	81.7±13.5	57.8±18.5	0.71	81.6±12.8	68.5±17.5	0.84
Endosulfan ether	ND	ND	–	85.0±8.0	68.6±8.9	0.81	61.0±2.8	51.4±10.2	0.84
Endosulfan lactone	ND	ND	–	41.9±1.8	35.7±7.0	0.85	131.9±5.9	114.2±10.8	0.87
Chlorpyrifos	391.9±3.2	NM	–	326.2±9.3	NM	–	265.3±4.6	NM	–
Chlorpyrifos oxon	23.6±1.4	NM	–	66.9±2.6	NM	–	97.3±3.9	NM	–

ND not detected (below detection limit), *NM* not measured (chlorpyrifos and chlorpyrifos oxon could not be measured using full-scan method)
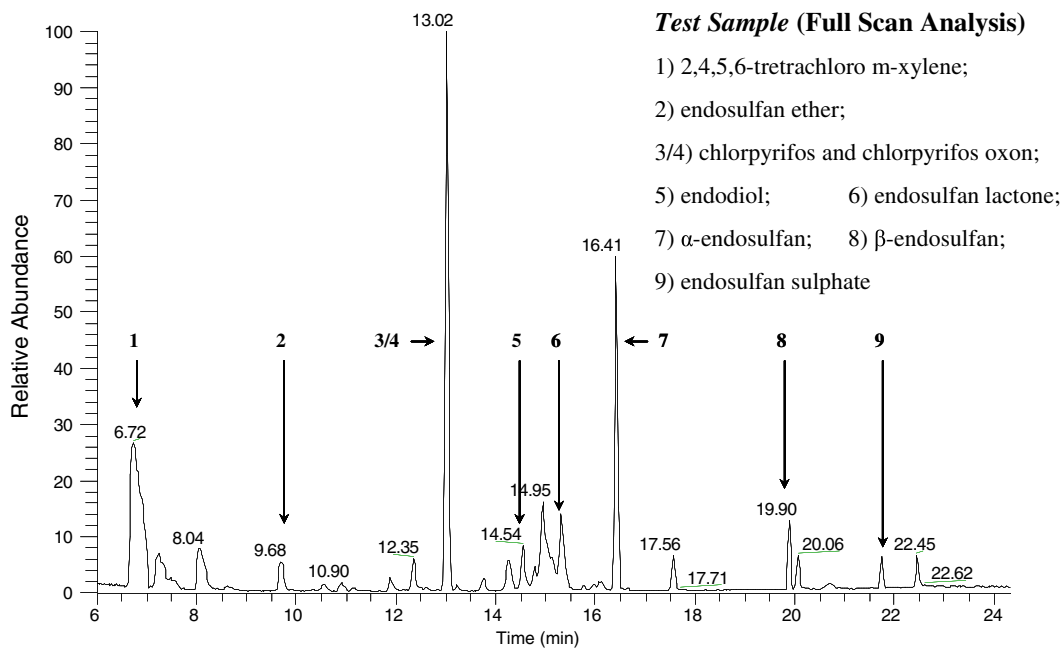
Fig. 3 A full-scan chromatogram (6–24 min) showing the separation of the compounds in the soil phase extract of day 7 sample sourced from the soil slurry reactor. The peaks of chlorpyrifos and chlorpyrifos oxon merged at nearly the same RT (~13.02 min)

primarily depend on the precursor ion and, therefore, vary with compounds. The theoretical background of the effects of these parameters is available in the operation manual of the instrument as well as elsewhere in the literature (March 1997). With an optimized set of MS/MS parameters (providing the highest peak area and S/N ratio for each analyte), MS/MS analysis produced noise-free chromatographs and significantly (95 % confidence limit) lowered the L_D and L_Q values compared with the full-scan mode (Table 3). About 1–2 order of magnitude lower (than ours) L_D (6–19 ng/L) and L_Q (26–68 ng/L) for endosulfan and some of its metabolites with a GC–MS/MS method was reported by Arrebola et al. (2001). However, these limits were calculated statistically using standards of 10 and 30 ppb with only five replicates (Arrebola et al. 2001). We computed the L_D and L_Q using standards of concentration of 5–50 μg/L with 10–20 replicates. The L_D values for the MS/MS method estimated in the present study were either lower or comparable to nearly all other reports for these and other pesticides with various detectors, such as electron capture detector (ECD), 0.8–10 μg/L (Castro et al. 2002; Pinho et al. 2010; Ramesh and Ravi 2002); MS in full-scan or SIM mode, 1–80 μg/L (Singh et al. 2009; Cho et al.

2008; Wong et al. 2010); and MS/MS mode, 0.1–6 μg/L (Sinha et al. 2011; Rashid et al. 2010; Wong et al. 2010). Nearly an order of magnitude lower L_Q indicated that all the compounds could be reliably quantified at a much lower concentration in the MS/MS mode compared with the full-scan mode. Among the target compounds, endodiol and endosulfan ether had relatively higher L_D and L_Q compared with other compounds in both full-scan as well as MS/MS modes (Table 3). Note that the GC–MS column and settings were targeted for the analysis of nonpolar compounds. Therefore, the method may have been comparatively less sensitive for endodiol and endosulfan ether due to their relatively more polar nature compared with others compounds (Sutherland et al. 2000).

A set of soil and water phase test samples analyzed in full-scan and MS/MS modes demonstrated the significant improvement in the analytical sensitivity of the method by reduction in the background noise. Similar improvements have been demonstrated for a wide range of analytes including pesticides, PCBs, dioxins, and biological compounds in the natural samples when analyzed in the MS/MS mode (Pyle et al. 1998; Raina and Hall 2008; Sinha et al. 2011; Rashid et al. 2010).

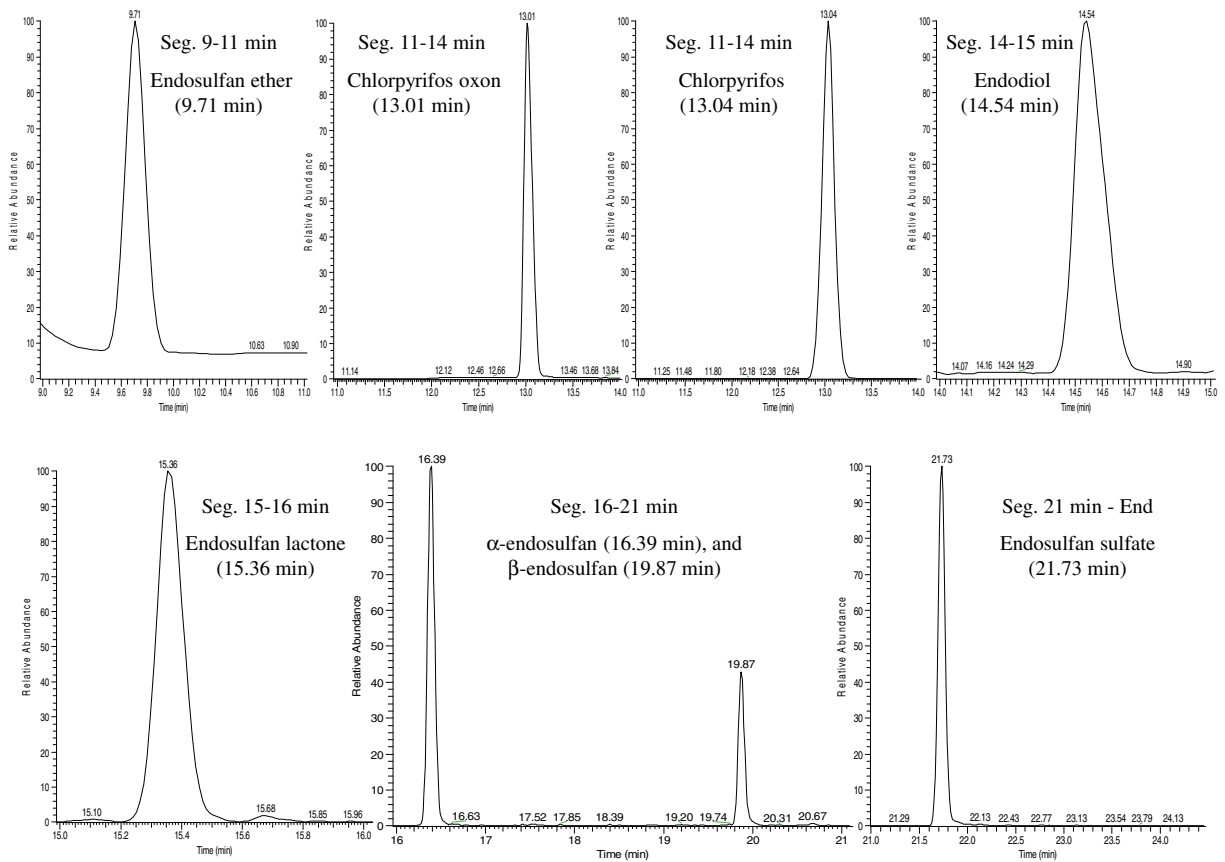Since the test samples originated from a soil slurry reactor of a biodegradation experiment, the level of

Fig. 4 The segmentwise chromatograph obtained for the soil phase extract of day 7 sample sourced from the soil slurry reactor and analyzed in MS/MS mode

concentrations of parent pesticides and various metabolites were likely to be different within and across the samples. Similar characteristics are expected in the natural samples of food, plants, aquatic organisms, field soils, and waters. Day 2 samples were representative of initial stage in the degradation process and, hence, likely to hold significantly lower concentration of metabolites than parent pesticides as observed for day 2 samples (Table 4). With the progress of degradation experiments, the concentration of metabolites was likely to increase while that of parent pesticides decreases in the sample (day 7, Table 4), and by the termination stage of the experiment, significant buildup of metabolites was expected in the samples (day 15, Table 4). The concentrations estimated in the water extracts were lower compared with the corresponding soil extracts. In the water phase, the concentrations were limited by aqueous solubility and sorption–desorption kinetics/equilibrium of the contaminants.

Differences in the level of contaminants resulted in substantial (several orders of magnitude) differences in

the peak area of some compounds appearing in the same chromatograph when analyzed in full-scan mode (Fig. 3). The natural field samples are also likely to have similar characteristics. Expectedly, effects of the background interference were more prominent on the quantification of smaller-sized peaks of the metabolites (lower S/N ratio) compared with the larger peaks of the parent pesticides. As a result, the quantification in the full-scan mode was relatively less sensitive to the compounds that were present in a smaller amount. The errors in the estimation were higher for relatively polar metabolites endodiol and endosulfan ether due to less sensitivity of the methods towards these compounds.

Ratios of recoveries (RR) obtained using full-scan method to that obtained using MS/MS method were over 0.9 for parent isomers (with few exceptions for water extracts), while that for metabolites were relatively lower, especially when present in low concentrations (Table 4). However, RR for metabolites increased with the increase in their concentrations (Table 4), indicating

enhancement in the accuracy of the quantification with the full-scan method. This indicated that a reliable estimate of these compounds could be obtained using the full-scan method only when the contaminants are present in high concentration. However, the MS/MS method would be more apt for reliable quantification of low levels of these analytes in natural samples. For example, the MS/MS method was able to quantify 70 μg/kg of endodiol in the soil (~14 μg/L in the extraction solvent) that was not detected with the full-scan method.

Conclusions

A GC–MS method was developed for the simultaneous analysis of endosulfan, chlorpyrifos, and their nonpolar metabolites. The DB-XLB column, used as a stationary phase, resulted in better separation of analytes than DB-5 ms and DB-35 ms. Chlorpyrifos and chlorpyrifos oxon were not separated on any of the columns with several tested temperature programs. The detection done in full-scan mode had lower sensitivity especially for the compounds present in low concentrations. A segmentwise MS/MS method with optimized parameters for the selected precursor ions helped to eliminate interferences typically present in a natural environmental sample and provided higher accuracy and sensitivity compared to analysis in the full-scan mode. Minimum detection limits for all the compounds were approximately sixfold lower in the MS/MS mode compared to those achieved in the full-scan mode. Chlorpyrifos and chlorpyrifos oxon (not separated clearly in the GC column) were also quantified independently by selecting mutually exclusive precursor ions in the same time window for the MS/MS analysis.

The MS/MS method could be more helpful in analyzing natural and laboratory soil or water samples contaminated with these pesticides, which are expected to be rich in background noise and likely to have different levels of concentrations for principle compounds and metabolites. The protocol outlined here can be used to develop methods for similar systems often encountered for other organic compounds.

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