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Optimization of two-dimensional gas chromatography time-of-flight mass spectrometry for separation and estimation of the residues of 160 pesticides and 25 persistent organic pollutants in grape and wine

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ABSTRACT

Two-dimensional gas chromatography (GC × GC) coupled with time-of-flight mass spectrometric (TOFMS) method was optimized for simultaneous analysis of 160 pesticides, 12 dioxin-like polychlorinated biphenyls (PCBs), 12 polyaromatic hydrocarbons (PAHs) and bisphenol A in grape and wine. GC × GC–TOFMS could separate all the 185 analytes within 38 min with >85% NIST library-based mass spectral confirmations. The matrix effect quantified as the ratio of the slope of matrix-matched to solvent calibrations was within 0.5–1.5 for most analytes. LOQ of most of the analytes was ≤10 μg/L with nine exceptions having LOQs of 12.5–25 μg/L. Recoveries ranged between 70 and 120% with <20% expanded uncertainties for 151 and 148 compounds in grape and wine, respectively, with intra-laboratory Horwitz ratio <0.2 for all analytes. The method was evaluated in the incurred grape samples where residues of cypermethrin, permethrin, chlorpyriphos, metalaxyl and etophenprox were detected at below MRL.

1. Introduction

Owing to the wide array of agro-inputs in modern agriculture as well as exposure to environmental pollutants, food commodities are often contaminated with the residues of toxic and harmful chemicals. In addition to pesticides, commodities such as grapes and wines may get contaminated by persistent environmental contaminants. In case of wine, due to the presence of some lipophilic components, the corks might attract environmental pollutants which in turn might contaminate the wines. In case of grapes, atmospheric deposition on the surface may also lead to contamination. In international trade, consumer safety is regulated through stringent norms of maximum residue limits (MRLs), which shows a decreasing trend over recent years in most countries especially the European Union, where the lowest MRL in agricultural commodities has been set at ≤0.01 mg/kg [1]. Unambiguous identification and quantification of contaminant residues in food at such trace levels demands use of selective sample preparation techniques coupled with highly sensitive and sophisticated instrumentation methods. Numerous methods of sample preparation for analysis of the residues of pesticide and environmental contaminants in grape and wine are reported in literature [2–8]. During multiresidue analysis of natural matrices wide range of compounds of matrix origin, interfere with the detection and quantification of the target compounds leading to false positive and negative detections influencing regulatory decisions. In an earlier study we showed the unique nature of grape and wine matrices, for which separate sample preparation techniques were developed for multiresidue analysis by GC–TOFMS [3,4].

To ensure the desired level of analytical selectivity and sensitivity by GC–MS, the regulatory and commercial testing laboratories usually adopt selective techniques like tandem mass spectrometry (MS/MS) or selected ion monitoring (SIM) to get better sensitivity as compared to full-scan techniques. Due to wide diversity in

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the natural and indirect sources of contamination in food, target-oriented residue monitoring by MS/MS or SIM often fails to provide holistic assessment of the contamination status of any food sample.

Residue monitoring by unit-resolution GC–MS can be suitably performed either on a quadrupole or time-of-flight (TOF) instrument. For a complex mixture of analytes, a TOFMS, when coupled with a fast GC system, can perform simultaneous analysis of a large number of compounds within a reasonably short time period with sufficient accuracy, which is otherwise not possible with slower, scanning type mass detectors (MS) like quadrupoles or ion traps. In case of multiresidue screening by GC–MS full-scan the separation of a large component mixture is often a challenging task due to limited peak capacity and the resulting co-elution of interfering matrix compounds. Since peak deconvolution algorithms require sufficient sampling to resolve complex peaks, correct identification of residues at trace levels becomes less likely as more compounds co-elute at a single location in the chromatogram. Such analytical problems can however be resolved with comprehensive two-dimensional gas chromatography (GC × GC) [9].

Although a number of papers described the separation efficiency of GC × GC–TOFMS [10–15], the literature evaluating the quantitative performance of this technique in agricultural products of international trade significance like grape and wine is rather limited. The present study was therefore designed to optimize a GC × GC method for simultaneous analysis of 185 compounds including 160 pesticides and 25 environmental organic pollutants in grape and wine to facilitate holistic measurement of sample. In addition, method validation in grape and wine was also carried out through single laboratory validation approach.

2. Experimental

2.1. Chemicals

Certified reference standards of the test analytes were of >98% purity and purchased from Ehrenstorfer (Augsburg, Germany). Ethyl acetate was of specially dried residue analysis grade from Thomas Baker (Mumbai, India). Primary secondary amine (PSA, 40 μm, Bondesil) sorbent was purchased from Varian (Palo Alto, USA). Florisil was purchased from Waters (Milford, Massachusetts). Anhydrous sodium sulfate of analytical reagent grade was purchased from Merck (Mumbai, India), and further purified by heating at 650 °C for 4 h before use and kept in desiccators.

2.2. Selection of pesticides and contaminants

The selected pesticides (160) included all the GC-amenable chemicals registered in Indian agriculture, which are currently available in market [16]. Twenty-five environmental contaminants selected for the study included 12 dioxin-like polychlorinated biphenyls (PCBs), bisphenol A and 12 polyaromatic hydrocarbons (PAHs). The names of the test chemicals are presented in Table 1.

2.3. Apparatus

Pegasus IV GC × GC–TOFMS system (Leco, St. Joseph, MI, USA) including an Agilent 6890N GC system (Agilent Technologies, USA) and equipped with a CTC Combipal (CTC Analytics, Switzerland) autosampler was used for analysis. Dry nitrogen gas (INOX Air Product, Mumbai, India), liquid nitrogen and compressed air were provided for modulation. Ultra-pure grade helium (Brin's Oxygen Company, Kolkata, India) was used as the carrier gas. Other equipment used in this project included high-speed homogenizer (DIAX-900, Heidolph, Germany), low-volume concentrator (TurboVap LV; Caliper Life Sciences, Russelsheim, Germany), non-refrigerated centrifuge (Eltek, Mumbai, India), refrigerated centrifuge (Kubota, Japan) and a microcentrifuge (Microfuge Pico, Kendro D-37520, Osterode, Germany).

2.4. Standard preparation

Stock solutions (1000 μg/mL) of individual standards were separately prepared by dissolving 25 mg each in 25 mL ethyl acetate. A standard mixture was prepared by mixing each of the 185 test chemicals in appropriate proportions to achieve a working standard mixture of 20 μg/mL. The calibration standards of concentration 5, 10, 20, 50, 100, 250 and 500 μg/L were prepared by successive dilutions of the above working standard mix with ethyl acetate.

2.5. Calibration

The calibration graphs (seven points) for all the compounds were obtained by plotting the individual peak areas against the concentration of the corresponding calibration standards in ethyl acetate. Matrix-matched standards at the same concentrations were simultaneously prepared using organically grown fresh untreated green seedless grapes (variety: Thompson Seedless) and wine (variety: Cabernet Sauvignon). The matrix extracts were at first analyzed by GC–MS and LC–MS/MS (liquid chromatography–tandem mass spectrometry) to confirm the absence of the test analytes in them before spiking for method development studies. For each analyte, the mass fragment (m/z) with maximum signal-to-noise ratio (S/N) was selected as the quantification ion (Table 1) and its peak area for different concentrations was used to construct the calibration graph. To minimize error in quantification in samples, matrix-matched standards were used. The limit of detection (LOD) of the test compounds was set at S/N of 3 in a blank, whereas, the limits of quantification (LOQs) were set to a signal with S/N of 10. To evaluate the matrix influence in terms of suppression or enhancement of analyte signals, the slopes of the matrix calibration graph for each analyte was divided by its corresponding solvent standard and the ratios compared.

2.6. Sample preparation

Grape samples (2 kg, berry only) fortified with the analyte mixture were blended under ambient conditions. A portion of the blended sample (200 g) was further homogenized at high speed and 10 g sample was drawn from it, for final analysis. The samples were extracted with 10 mL ethyl acetate (+10 g anhydrous sodium sulfate) and processed as per the method described earlier [3]. The final extract (1 mL) was cleaned by dispersive solid phase extraction (DSPE) with PSA (25 mg), centrifuged at 10,000 rpm for 2 min and the supernatant was filtered through 0.2 μm polytetrafluoroethylene membrane filter and directly injected to the GC–MS system. This resulted in concentration of 1 g matrix/mL sample.

Wine samples (2 L, filtered through Whatman 1 filter paper) spiked with the analyte mixture were shaken under ambient conditions. From the homogenized sample, a further portion of 250 mL was drawn and homogenized thoroughly. From this mixture, 10 mL was drawn and extraction carried out according to earlier described method [4].

2.7. Method validation

Method validation was carried out as per the single laboratory validation approach of Thompson et al. [17]. The recovery experiments were carried out on organically grown fresh pre-tested grapes and wine by fortifying the samples (10 g and 10 mL, respectively, for grape and wine) in ten replicates with analyte mixture at 10, 25 and 50 μg/L level. All the treated samples were analyzed

Table 1
Validation results for grape and wine,.

Sr. no.	Analyte	*m/z*	2-D-RT (s, s)	Recovery (%) ± SD, *n* = 10					
				Grape			Wine		
				10 μg/kg	25 μg/kg	50 μg/kg	10 μg/L	25 μg/L	50 μg/L
1	Naphthalene	128	450, 0.940	89 ± 2	84 ± 2	86 ± 1	83 ± 2	86 ± 3	84 ± 1
2	Diflubenzuron (as *p*-chloroaniline)	127	455, 1.080	102 ± 5	106 ± 3	107 ± 4	103 ± 2	106 ± 4	102 ± 3
3	Isoproturon (as 4-isopropylphenylisocyanate)	146	460, 0.880	90 ± 3	92 ± 2	96 ± 2	95 ± 2	96 ± 2	96 ± 1
4	Dichlorvos	109	465, 1.000	86 ± 3	84 ± 2	94 ± 3	96 ± 4	98 ± 2	97 ± 3
5	Methamidophos	94	465, 1.220	–	76 ± 7	79 ± 5	–	79 ± 4	79 ± 3
6	4-Bromo-2-chlorophenol	208	495, 1.080	98 ± 4	100 ± 3	100 ± 1	95 ± 3	95 ± 2	98 ± 1
7	Diuron (as 3,4-dichlorophenylisocyanate)	124	515, 1.080	73 ± 6	74 ± 6	78 ± 2	79 ± 5	77 ± 4	72 ± 4
8	Sulfosulfuron	154	520, 1.220	75 ± 6	76 ± 5	74 ± 3	78 ± 6	74 ± 5	76 ± 2
9	Metoxuron (as 3-chloro-4-methoxyphenylisocyanate)	168	575, 1.320	105 ± 2	101 ± 2	102 ± 1	98 ± 3	104 ± 2	102 ± 2
10	*cis*-Mevinphos	127	575, 1.800	92 ± 1	94 ± 1	96 ± 1	93 ± 3	97 ± 2	98 ± 2
11	*trans*-Mevinphos	127	580, 1.480	90 ± 2	92 ± 2	91 ± 2	91 ± 2	93 ± 1	92 ± 1
12	Acephate	136	600, 1.880	–	95 ± 3	97 ± 2	–	94 ± 4	94 ± 3
13	Acynephthylene	152	635, 1.720	90 ± 6	93 ± 3	94 ± 2	85 ± 7	89 ± 5	92 ± 3
14	Acynephthene	154	660, 1.820	92 ± 3	95 ± 4	95 ± 3	94 ± 3	95 ± 3	99 ± 2
15	Fenbucarb	121	745, 2.380	84 ± 3	86 ± 2	84 ± 2	85 ± 3	84 ± 2	85 ± 1
16	Propoxur	110	750, 2.640	90 ± 2	94 ± 2	96 ± 1	94 ± 3	96 ± 3	94 ± 2
17	Omethoate	156	750, 3.260	–	79 ± 5	76 ± 8	–	71 ± 9	79 ± 6
18	Fluorene	166	765, 2.340	89 ± 3	92 ± 3	89 ± 2	83 ± 5	87 ± 3	84 ± 3
19	Demeton-S-methyl	88	775, 2.840	–	125 ± 7	130 ± 6	–	132 ± 10	130 ± 7
20	Monocrotophos	127	840, 2.760	82 ± 2	84 ± 2	86 ± 1	87 ± 4	86 ± 3	82 ± 2
21	Phorate	75	850, 2.040	95 ± 3	97 ± 2	98 ± 1	94 ± 3	98 ± 3	100 ± 1
22	Methabenzthiazuron	136	860, 2.500	75 ± 10	74 ± 9	75 ± 11	72 ± 11	74 ± 9	76 ± 9
23	α-Hexachlorocyclohexane	181	885, 1.920	96 ± 2	96 ± 2	97 ± 1	99 ± 2	95 ± 2	94 ± 2
24	Carbofuran	164	905, 2.180	107 ± 5	103 ± 4	102 ± 5	94 ± 4	99 ± 3	100 ± 3
25	Thiometon	88	905, 2.180	88 ± 6	87 ± 5	86 ± 3	89 ± 7	86 ± 6	87 ± 6
26	Dimethoate	87	905, 2.300	89 ± 6	82 ± 4	87 ± 3	87 ± 6	82 ± 4	83 ± 4
27	Atrazine	200	915, 1.822	77 ± 1	77 ± 1	78 ± 1	70 ± 3	71 ± 3	70 ± 2
28	Fluchloralin	63	935, 1.320	73 ± 11	74 ± 10	75 ± 8	79 ± 10	81 ± 11	79 ± 8
29	Diazinon	137	935, 1.540	89 ± 3	97 ± 2	93 ± 2	83 ± 2	82 ± 3	81 ± 4
30	*cis*-Phosphamidon	127	945, 1.800	93 ± 5	98 ± 3	94 ± 3	97 ± 4	98 ± 4	96 ± 6
31	β-Hexachlorocyclohexane	181	945, 1.840	95 ± 2	95 ± 2	96 ± 1	92 ± 3	94 ± 3	96 ± 2
32	γ-Hexachlorocyclohexane	183	950, 1.760	90 ± 4	90 ± 1	90 ± 3	90 ± 4	90 ± 3	90 ± 2
33	Pyremethanil	198	965, 1.660	105 ± 3	107 ± 3	106 ± 2	101 ± 4	103 ± 2	102 ± 2
34	Etrimphos	181	970, 1.540	93 ± 3	93 ± 4	92 ± 2	95 ± 3	96 ± 3	98 ± 2
35	Chlordene	66	980, 1.520	89 ± 4	87 ± 5	88 ± 5	84 ± 5	82 ± 7	83 ± 4
36	Paraoxon methyl	109	980, 1.780	90 ± 3	94 ± 5	96 ± 4	92 ± 8	98 ± 5	91 ± 4
37	Flufenoxuron	126	985, 1.400	78 ± 3	74 ± 6	75 ± 2	76 ± 5	74 ± 2	73 ± 1
38	Phenanthrene	178	985, 1.660	89 ± 2	92 ± 2	90 ± 2	94 ± 2	93 ± 2	95 ± 2
39	Chlorothalonil	266	985, 1.780	93 ± 3	99 ± 4	100 ± 2	98 ± 3	95 ± 6	98 ± 3
40	Kitazin	91	990, 1.520	88 ± 2	90 ± 2	94 ± 1	89 ± 2	92 ± 2	90 ± 1
41	Anthracene	178	995, 1.620	85 ± 3	88 ± 2	90 ± 2	84 ± 7	86 ± 5	92 ± 3
42	Fenchlorphos-oxon	269	1005, 1.620	111 ± 5	112 ± 3	112 ± 4	110 ± 3	113 ± 2	113 ± 1
43	*trans*-Phosphamidon	127	1010, 1.680	80 ± 3	80 ± 2	82 ± 3	82 ± 3	83 ± 2	84 ± 3
44	δ-Hexachlorocyclohexane	183	1010, 1.720	96 ± 3	98 ± 2	99 ± 1	95 ± 3	92 ± 3	93 ± 3
45	Spiroxamine:1	100	1025, 1.200	103 ± 4	105 ± 4	105 ± 3	101 ± 5	103 ± 2	102 ± 4
46	Malaoxon	127	1025, 1.680	96 ± 6	94 ± 5	97 ± 2	97 ± 4	96 ± 6	98 ± 4
47	Chloropyriphos-methyl	286	1030, 1.740	79 ± 5	79 ± 4	81 ± 2	78 ± 4	79 ± 5	78 ± 2
48	Vinclozoline	124	1035, 1.580	98 ± 4	102 ± 3	99 ± 2	98 ± 2	96 ± 3	99 ± 2
49	Propanil	161	1035, 1.780	86 ± 4	92 ± 3	91 ± 4	85 ± 4	87 ± 3	89 ± 3
50	Alachlor	160	1040, 1.620	92 ± 3	94 ± 2	96 ± 1	91 ± 4	93 ± 3	95 ± 3
51	Metribuzine	198	1040, 1.940	69 ± 5	68 ± 6	69 ± 3	72 ± 5	71 ± 3	74 ± 2
52	Methyl parathion	109	1045, 1.860	87 ± 2	88 ± 3	88 ± 1	86 ± 4	88 ± 5	85 ± 4
53	Carbofuran-3-OH	137	1045, 2.140	106 ± 4	104 ± 5	105 ± 3	102 ± 3	104 ± 5	104 ± 5
54	Metalaxyl	132	1050, 1.780	95 ± 4	96 ± 2	98 ± 2	92 ± 5	94 ± 4	96 ± 2
55	Phenchlorphos	285	1060, 1.760	89 ± 4	85 ± 2	87 ± 1	88 ± 3	90 ± 2	92 ± 2
56	Carbaril	144	1065, 1.060	90 ± 3	92 ± 5	94 ± 2	97 ± 5	99 ± 4	101 ± 2
57	Heptachlor	100	1065, 1.640	91 ± 3	92 ± 3	91 ± 1	93 ± 4	95 ± 2	93 ± 1
58	Pirimiphos-methyl	276	1065, 1.760	98 ± 4	95 ± 5	98 ± 3	96 ± 4	92 ± 3	98 ± 2
59	Spiroxamine:2	100	1070, 1.282	98 ± 3	97 ± 3	96 ± 4	96 ± 2	96 ± 3	94 ± 2
60	Demeton-S-methyl sulfone	169	1080, 2.420	–	135 ± 11	132 ± 9	–	135 ± 10	136 ± 8
61	Malathion	127	1085, 1.880	89 ± 8	87 ± 6	89 ± 5	85 ± 3	82 ± 7	90 ± 3
62	Phenitrothion	277	1085, 2.000	100 ± 5	103 ± 4	102 ± 3	102 ± 2	104 ± 3	106 ± 2
63	Linuron	61	1100, 1.980	68 ± 8	67 ± 5	69 ± 5	64 ± 8	67 ± 8	69 ± 5
64	Dichlofluanid	123	1100, 2.080	103 ± 4	104 ± 5	106 ± 2	101 ± 5	108 ± 4	108 ± 2
65	Chlorpyriphos-ethyl	199	1105, 1.900	101 ± 7	103 ± 5	107 ± 4	105 ± 4	103 ± 2	104 ± 2
66	Tetraconazole	336	1120, 1.800	98 ± 5	96 ± 3	98 ± 4	89 ± 5	92 ± 3	93 ± 2
67	Phenthion	278	1120, 2.200	101 ± 3	104 ± 2	102 ± 2	100 ± 3	102 ± 4	104 ± 2
68	Triadimefon	208	1125, 1.900	72 ± 4	73 ± 5	76 ± 2	77 ± 5	74 ± 3	74 ± 3
69	Parathion	291	1125, 2.000	104 ± 7	103 ± 5	103 ± 3	104 ± 6	108 ± 6	103 ± 7
70	Aldrin	66	1130, 1.890	88 ± 5	89 ± 5	91 ± 4	88 ± 4	90 ± 4	93 ± 4

Table 1 (*Continued*)

Sr. no.	Analyte	*m/z*	2-D-RT (s, s)	Recovery (%) ± SD, *n* = 10					
				Grape			Wine		
				10 μg/kg	25 μg/kg	50 μg/kg	10 μg/L	25 μg/L	50 μg/L
71	*cis*-Chlorfenvinphos	267	1160, 2.120	104 ± 5	106 ± 5	106 ± 6	98 ± 7	99 ± 5	97 ± 6
72	Fipronil	367	1165, 1.800	89 ± 2	84 ± 3	87 ± 3	92 ± 1	96 ± 3	95 ± 2
73	Pendimethalin	252	1170, 2.120	86 ± 4	88 ± 3	88 ± 2	89 ± 2	87 ± 2	88 ± 2
74	Bioallethrin	123	1175, 1.820	85 ± 3	82 ± 3	84 ± 1	87 ± 3	88 ± 4	86 ± 3
75	Cyprodinil	224	1180, 2.360	99 ± 4	95 ± 4	95 ± 3	62 ± 1	64 ± 5	62 ± 1
76	Penconazole	159	1185, 2.260	100 ± 2	102 ± 2	103 ± 2	94 ± 3	93 ± 3	99 ± 1
77	*trans*-Chlorfenvinphos	267	1185, 2.320	89 ± 4	91 ± 6	94 ± 3	95 ± 3	92 ± 6	97 ± 2
78	Trifloxystrobin acid metabolite	116	1195, 2.200	115 ± 7	118 ± 6	117 ± 5	116 ± 6	117 ± 6	118 ± 4
79	Phenthoate	121	1200, 2.640	100 ± 5	102 ± 5	103 ± 4	102 ± 3	103 ± 4	104 ± 2
80	Prallethrin	123	1205, 2.040	96 ± 3	94 ± 5	99 ± 2	97 ± 3	96 ± 3	98 ± 2
81	Quinalphos	146	1205, 2.580	116 ± 3	117 ± 4	119 ± 2	95 ± 3	98 ± 4	99 ± 3
82	Triadimenol:1	112	1210, 2.260	108 ± 5	106 ± 4	107 ± 5	103 ± 6	107 ± 8	106 ± 6
83	Epoxyheptachlor	183	1215, 2.440	82 ± 6	84 ± 4	86 ± 3	89 ± 5	87 ± 3	90 ± 2
84	Procymidone	96	1215, 2.480	90 ± 2	95 ± 2	96 ± 1	89 ± 3	92 ± 2	93 ± 2
85	Triadimenol:2	112	1230, 2.360	101 ± 3	104 ± 5	106 ± 2	99 ± 5	98 ± 6	102 ± 5
86	Captan	79	1235, 3.160	–	87 ± 4	90 ± 6	–	89 ± 8	87 ± 3
87	Butachlor	160	1240, 2.240	105 ± 3	107 ± 3	103 ± 2	98 ± 4	93 ± 5	104 ± 3
88	Fluoranthene	202	1245, 2.860	85 ± 5	89 ± 4	87 ± 3	82 ± 6	83 ± 4	86 ± 4
89	Methidathion	145	1245, 3.260	86 ± 1	91 ± 2	93 ± 1	93 ± 2	92 ± 3	94 ± 1
90	*cis*-Chlordane	373	1250, 1.500	97 ± 3	98 ± 2	95 ± 4	95 ± 3	97 ± 6	92 ± 4
91	*o,p*-DDE	246	1250, 2.620	97 ± 1	98 ± 1	97 ± 1	98 ± 3	98 ± 2	99 ± 3
92	Folpet	260	1250, 3.280	75 ± 5	76 ± 3	78 ± 3	78 ± 5	76 ± 4	75 ± 4
93	Thiabendazole	174	1255, 3.540	–	83 ± 6	87 ± 3	–	84 ± 3	86 ± 3
94	Vamidothion	87	1260, 3.569	–	79 ± 10	79 ± 8	–	76 ± 11	72 ± 9
95	Paclobutrazole	125	1265, 2.460	89 ± 5	87 ± 8	86 ± 5	84 ± 6	83 ± 5	87 ± 5
96	*trans*-Chlordane	373	1285, 1.600	96 ± 2	96 ± 3	94 ± 2	95 ± 7	95 ± 5	93 ± 5
97	α-Endosulfan	241	1290, 2.740	88 ± 4	90 ± 32	91 ± 4	95 ± 3	99 ± 4	100 ± 2
98	Imazalil	173	1300, 2.939	–	82 ± 3	84 ± 2	–	85 ± 3	84 ± 3
99	Oxadiazon	175	1305, 2.500	105 ± 6	105 ± 3	104 ± 5	98 ± 6	96 ± 5	99 ± 3
100	Hexaconazole	83	1305, 2.760	104 ± 3	103 ± 3	104 ± 2	102 ± 5	102 ± 3	101 ± 2
101	Pyrene	202	1310, 3.280	98 ± 3	94 ± 2	95 ± 2	96 ± 3	92 ± 3	96 ± 2
102	Isoprothiolane	118	1310, 3.640	96 ± 7	98 ± 5	99 ± 4	97 ± 5	99 ± 3	98 ± 3
103	Profenofos	208	1315, 2.860	110 ± 4	105 ± 3	103 ± 3	110 ± 3	107 ± 8	97 ± 4
104	Oxyfluorfen	252	1320, 2.440	104 ± 3	95 ± 3	96 ± 2	97 ± 4	102 ± 4	104 ± 4
105	*p,p*-DDE	246	1330, 2.620	99 ± 1	99 ± 1	99 ± 1	94 ± 3	96 ± 2	96 ± 3
106	Kresoxim-methyl	116	1330, 3.160	96 ± 7	94 ± 5	96 ± 3	94 ± 3	95 ± 2	99 ± 2
107	Buprofezin	105	1335, 2.620	96 ± 2	97 ± 3	98 ± 2	84 ± 1	83 ± 2	85 ± 1
108	Flusilazole	233	1335, 2.700	106 ± 5	102 ± 4	105 ± 3	109 ± 4	107 ± 3	103 ± 1
109	Bisphenol A	213	1335, 3.380	119 ± 8	105 ± 5	104 ± 3	95 ± 6	97 ± 3	99 ± 5
110	PCB IUPAC No. 077	290	1340, 2.660	101 ± 2	102 ± 2	101 ± 1	96 ± 2	99 ± 2	95 ± 2
111	Myclobutanil	179	1340, 2.860	85 ± 4	85 ± 4	86 ± 2	84 ± 3	82 ± 2	82 ± 2
112	*o,p*-DDD	235	1350, 2.780	81 ± 8	84 ± 6	83 ± 2	99 ± 5	98 ± 5	93 ± 4
113	Dieldrin	79	1360, 2.640	103 ± 2	101 ± 2	100 ± 1	99 ± 2	101 ± 3	102 ± 3
114	PCB IUPAC No. 081	290	1365, 2.620	103 ± 2	102 ± 2	103 ± 1	96 ± 3	99 ± 3	94 ± 3
115	PCB IUPAC No. 114	326	1405, 2.460	99 ± 2	98 ± 2	99 ± 1	96 ± 3	99 ± 3	95 ± 4
116	Ethion	231	1410, 2.600	104 ± 6	102 ± 5	104 ± 3	96 ± 3	100 ± 2	100 ± 2
117	Endrin	263	1410, 2.680	91 ± 4	94 ± 3	92 ± 5	95 ± 4	96 ± 5	93 ± 3
118	PCB IUPAC No. 118	326	1415, 2.420	98 ± 2	98 ± 2	99 ± 1	96 ± 3	98 ± 6	96 ± 4
119	PCB IUPAC No. 105	326	1435, 2.480	103 ± 2	105 ± 2	102 ± 1	99 ± 3	98 ± 3	97 ± 2
120	*o,p*-DDT	235	1435, 2.520	100 ± 3	100 ± 2	100 ± 2	99 ± 3	99 ± 2	100 ± 2
121	β-Endosulfan	195	1435, 2.720	88 ± 5	92 ± 3	95 ± 4	98 ± 4	98 ± 3	99 ± 4
122	Trifloxystrobin	116	1455, 2.420	120 ± 5	121 ± 4	118 ± 3	118 ± 6	124 ± 6	113 ± 5
123	Triazophos	161	1455, 3.060	100 ± 6	103 ± 7	100 ± 3	107 ± 2	102 ± 3	104 ± 2
124	PCB IUPAC No. 126	326	1470, 2.520	104 ± 3	105 ± 3	106 ± 1	95 ± 3	99 ± 2	98 ± 4
125	Benalaxyl	148	1470, 2.640	101 ± 2	101 ± 2	102 ± 1	85 ± 3	88 ± 6	95 ± 4
126	*cis*-Propiconazole	259	1485, 2.460	100 ± 5	101 ± 4	102 ± 3	96 ± 5	101 ± 3	102 ± 3
127	Flupicolide	209	1495, 2.382	82 ± 4	86 ± 2	87 ± 1	87 ± 5	86 ± 4	83 ± 2
128	*trans*-Propiconazole	259	1495, 2.441	104 ± 3	106 ± 3	102 ± 3	104 ± 5	102 ± 4	106 ± 2
129	Ediphenphos	109	1495, 2.980	83 ± 5	87 ± 3	88 ± 4	104 ± 3	102 ± 4	104 ± 2
130	*p,p*-DDT	235	1505, 2.416	90 ± 4	98 ± 5	99 ± 5	98 ± 2	99 ± 4	98 ± 3
131	Endosulfan sulfate	272	1510, 2.700	106 ± 3	106 ± 4	104 ± 2	105 ± 5	104 ± 3	101 ± 2
132	Propargite	135	1520, 2.220	80 ± 5	82 ± 3	86 ± 6	85 ± 3	86 ± 3	84 ± 2
133	Tebuconazole	125	1535, 2.380	104 ± 3	105 ± 2	106 ± 2	109 ± 3	105 ± 3	101 ± 2
134	Triphenyl phosphate	325	1535, 2.800	107 ± 8	109 ± 6	108 ± 3	98 ± 6	102 ± 5	106 ± 3
135	PCB IUPAC No. 123	326	1540, 2.212	104 ± 3	102 ± 1	103 ± 1	98 ± 3	99 ± 3	98 ± 2
136	Biphenthrin	181	1565, 2.000	95 ± 3	95 ± 3	97 ± 1	97 ± 4	98 ± 5	95 ± 4
137	PCB IUPAC No. 157	360	1565, 2.260	103 ± 3	103 ± 2	104 ± 1	96 ± 3	99 ± 3	96 ± 3
138	Captafol	79	1565, 2.940	84 ± 5	88 ± 5	80 ± 2	83 ± 3	87 ± 5	88 ± 4
139	Oxycarboxin	175	1565, 3.420	76 ± 3	78 ± 5	74 ± 3	76 ± 5	76 ± 2	73 ± 2
140	Iprodione	314	1570, 2.483	–	68 ± 5	68 ± 5	–	68 ± 5	69 ± 5
141	Tetramethrin	164	1585, 2.400	117 ± 10	115 ± 8	118 ± 6	120 ± 11	118 ± 8	116 ± 8
142	Fenpropathrin	181	1595, 2.260	118 ± 3	121 ± 4	120 ± 1	108 ± 2	109 ± 4	105 ± 4

Table 1 (*Continued*)

Sr. no.	Analyte	*m/z*	2-D-RT (s, s)	Recovery (%) ± SD, *n* = 10					
				Grape			Wine		
				10 μg/kg	25 μg/kg	50 μg/kg	10 μg/L	25 μg/L	50 μg/L
143	Phosmet	160	1600, 3.160	101 ± 5	103 ± 2	102 ± 2	100 ± 3	102 ± 2	100 ± 1
144	PCB IUPAC No. 167	360	1605, 2.400	98 ± 1	98 ± 1	99 ± 1	98 ± 3	98 ± 2	98 ± 2
145	PCB IUPAC No. 169	360	1615, 2.320	100 ± 3	101 ± 3	102 ± 2	96 ± 3	94 ± 3	91 ± 2
146	Phenothrin	123	1620, 1.900	85 ± 4	86 ± 3	84 ± 4	86 ± 5	87 ± 4	87 ± 3
147	Dicofol	139	1620, 2.480	99 ± 4	97 ± 4	95 ± 5	103 ± 4	105 ± 3	104 ± 5
148	Fenazaquin	145	1625, 2.385	86 ± 3	82 ± 4	83 ± 5	84 ± 4	83 ± 5	89 ± 2
149	Benz(a)anthracene	228	1625, 2.780	91 ± 3	95 ± 2	96 ± 3	87 ± 5	89 ± 3	90 ± 3
150	Chrysene	228	1635, 2.780	92 ± 4	91 ± 3	95 ± 4	94 ± 3	93 ± 2	92 ± 3
151	Phosalone	182	1655, 2.780	112 ± 8	115 ± 7	113 ± 4	117 ± 9	118 ± 5	117 ± 6
152	λ-Cyhalothrin	181	1660, 2.200	109 ± 5	104 ± 6	102 ± 4	104 ± 4	106 ± 3	102 ± 5
153	PCB IUPAC No. 156	360	1670, 2.500	100 ± 2	100 ± 2	101 ± 1	95 ± 4	98 ± 2	94 ± 2
154	Azinphos-methyl	132	1675, 3.580	121 ± 9	121 ± 6	124 ± 8	110 ± 1	106 ± 2	91 ± 7
155	Diafenthiuron	284	1705, 2.820	76 ± 7	78 ± 5	78 ± 3	75 ± 5	74 ± 6	78 ± 3
156	Fenarimol	139	1715, 3.320	104 ± 5	106 ± 5	108 ± 3	92 ± 5	93 ± 5	91 ± 4
157	PCB IUPAC No. 189	394	1720, 2.520	98 ± 2	98 ± 2	99 ± 2	99 ± 3	82 ± 3	99 ± 3
158	Oryzalin	275	1740, 3.480	–	76 ± 6	73 ± 6	–	74 ± 9	72 ± 6
159	*cis*-Permethrin	183	1755, 3.060	103 ± 4	106 ± 2	101 ± 2	101 ± 4	99 ± 4	101 ± 3
160	Bitertanol	170	1755, 3.180	106 ± 3	103 ± 4	102 ± 2	98 ± 6	99 ± 2	94 ± 3
161	*trans*-Permethrin	183	1765, 3.120	103 ± 5	101 ± 4	103 ± 2	101 ± 5	98 ± 5	96 ± 5
162	Cyfluthrin:1	163	1810, 3.320	77 ± 7	78 ± 5	84 ± 5	85 ± 4	88 ± 3	89 ± 7
163	Cyfluthrin:2	163	1820, 3.340	82 ± 5	84 ± 6	85 ± 4	82 ± 4	82 ± 4	87 ± 3
164	Cyfluthrin:3	163	1830, 3.400	79 ± 5	84 ± 4	82 ± 4	85 ± 6	84 ± 5	84 ± 3
165	Cyfluthrin:4	163	1840, 3.740	86 ± 4	84 ± 5	88 ± 4	89 ± 5	82 ± 3	84 ± 4
166	Cypermethrin:1	163	1850, 3.740	110 ± 4	106 ± 7	106 ± 4	109 ± 5	101 ± 2	107 ± 4
167	Flucythrinate:1	157	1865, 3.680	129 ± 12	125 ± 10	125 ± 8	124 ± 9	125 ± 8	124 ± 8
168	Cypermethrin:2	163	1865, 3.860	102 ± 5	104 ± 5	104 ± 5	104 ± 5	106 ± 7	106 ± 4
169	Cypermethrin:3	163	1875, 3.860	97 ± 5	98 ± 4	101 ± 3	102 ± 5	99 ± 4	101 ± 5
170	Cypermethrin:4	163	1885, 3.860	98 ± 6	104 ± 4	103 ± 3	105 ± 7	103 ± 5	102 ± 5
171	Benzo(b)fluoroathene	252	1885, 4.700	83 ± 5	88 ± 6	91 ± 3	89 ± 6	86 ± 5	89 ± 3
172	Flucythrinate:2	199	1890, 3.868	120 ± 9	119 ± 8	121 ± 9	115 ± 8	118 ± 8	121 ± 6
173	Etofenprox	163	1895, 4.160	115 ± 4	112 ± 4	114 ± 2	112 ± 4	116 ± 2	115 ± 4
174	Fluvalinate:1	250	1980, 4.060	95 ± 2	96 ± 2	98 ± 1	98 ± 2	97 ± 3	94 ± 1
175	Fenvalerate	125	1985, 4.800	93 ± 4	91 ± 2	96 ± 3	95 ± 4	92 ± 3	94 ± 5
176	Fluvalinate:2	250	1990, 4.140	92 ± 4	96 ± 2	97 ± 1	93 ± 4	96 ± 2	99 ± 1
177	Benzo(a)pyrene	252	1995, 0.940	85 ± 7	84 ± 4	89 ± 4	85 ± 6	86 ± 4	88 ± 5
178	Esfenvalerate	125	2020, 0.060	98 ± 4	97 ± 3	99 ± 2	96 ± 2	98 ± 2	100 ± 3
179	Indoxacarb	150	2075, 0.659	76 ± 4	78 ± 2	77 ± 2	79 ± 3	78 ± 4	79 ± 2
180	Difenoconazole:1	265	2085, 1.390	–	74 ± 6	79 ± 5	–	72 ± 5	76 ± 4
181	Difenoconazole:2	265	2095, 1.480	76 ± 4	79 ± 5	73 ± 4	79 ± 4	72 ± 6	79 ± 4
182	Deltamethrin	253	2125, 1.200	–	87 ± 4	88 ± 5	–	88 ± 5	82 ± 5
183	Azoxystrobin	344	2170, 4.762	–	82 ± 5	88 ± 3	–	79 ± 4	82 ± ±3
184	Dimethomorph-1	301	2200, 3.220	–	83 ± 6	87 ± 5	–	87 ± 6	89 ± 5
185	Dimethomorph-2	301	2265, 4.010	–	73 ± 6	71 ± 2	–	84 ± 6	73 ± 4

immediately. Quantification in the recovery samples was performed by external calibration using matrix-matched standards.

2.8. GC × GC–TOFMS conditions

The GC × GC separation was performed by injecting 2 μL (splitless) on a DB-5MS capillary column (5% phenyl polysilphenylene-siloxane; 30 m × 0.25 mm, 0.25 μm) connected in series to a Varian V-17 capillary column (50% phenyl, 50% dimethylpolysiloxane; 1 m × 0.10 mm, 0.10 μm) as the secondary column. Helium was used as the carrier at the corrected constant flow rate of 1.5 mL/min. The injector port was set at 250 °C. A gooseneck splitless liner (78.5 mm × 6.5 mm, 4 mm) from Restek Corporation (PA, USA) was used. Transfer line temperature was maintained at 305 °C. Electron impact ionization was achieved at 70 eV and the ion source temperature was set at 240 °C. The mass spectrum of perfluorotributylamine was used to tune the mass spectrometer. The detector voltage was set at −1750 V and the data acquisition was carried out within the mass range of 50–550 *m/z* at acquisition rate of 250 spectra/s at 2-D mode. The optimized oven program for GC × GC–TOFMS is described in Section 3.1.

2.9. Data processing and quantification

ChromaTOF® 3.34 software was used for data processing. The chromatograms were processed with baseline offset 0.5 (computation through the middle of noise) [3], peak find with S/N of the quantifier ion at least 3, peak width 0.1 s for GC × GC. Minimum similarity match to NIST library spectra (v. 2.0 from the National Institute of Standards and Technology, NIST, Gaithersburg, Maryland, USA) was set at 600. The threshold for automatically combining second dimension peaks based on spectral match was set to 700. Quantification was done on the basis of single diagnostic ion (Table 1) and the peak assignments, integration and summation peak slice areas were automatically performed by the software. Quantifications on the basis of seven-point calibrations were also manually checked and corrected for any errors. The time taken to process a single GC × GC–TOFMS file for quantification was around 32 min. After the initial processing, it took around 10 min to build the calibration curves. Once the calibration curve is constructed for each compound, then based on this calibration it took around 15 min to process any file for quantification.

2.10. Validation data analysis and statistical calculations

2.10.1. Precision

The precision in the conditions of repeatability (three analysts prepared six samples each on a single day) and intermediate precision (three analysts prepared six samples each on six different days) was determined separately at fortification level of 10 μg/kg grapes and 10 μg/L wine, for all the analytes. Horwitz ratio (HorRat) [18,19], was calculated for all test compounds in the following way:

$$\text{HorRat} = \frac{\text{RSD}}{\text{Prsd}}$$

where RSD stands for relative standard deviation and Prsd is the predicted relative standard deviation. $\text{Prsd} = 2C^{-0.15}$, where C is the concentration expressed as mass fraction ($10\ \mu g/L = 10 \times 10^{-9}$). Validation data were further tested by identification and determination of uncertainty sources in measurement as described in Section 2.10.3.

2.10.2. Accuracy–recovery studies

The accuracy in terms of percent recovery (Table 1) was calculated with the following equation:

$$\text{Recovery}(\%) = \frac{\text{peak area of pre-extraction spike}}{\text{peak area of post-extraction spike}} \times 100$$

2.10.3. Assessment of global uncertainty

The global uncertainty was assessed as per the statistical procedure described in EURACHEM/CITAC Guide CG 4 [20] in the same way as reported earlier [2,4]. Uncertainty associated with the calibration graph (U_1), day-wise uncertainty associated with precision (U_2), analyst-wise uncertainty associated with precision (U_3), day-wise uncertainty associated with accuracy/bias (U_4), and analyst-wise uncertainty associated with accuracy/bias (U_5) was evaluated for all the test compounds in both grapes and wines. The global uncertainty (U) was calculated as

$$U = \sqrt{U_1^2 + U_2^2 U_3^2 + U_4^2 + U_5^2}$$

and reported as expanded uncertainty which is twice the value of the global uncertainty. The uncertainty values for each analyte are reported as relative uncertainties. Relative uncertainty stands for the ratio of uncertainty value at a given concentration to the concentration at which the uncertainty is calculated.

3. Results and discussions

3.1. Optimization of GC × GC programs

Before optimization of the GC × GC program the ion source temperature was optimized to achieve highest S/N. For step-wise increase in ion source temperature from 150 to 250 °C, an increase in S/N by a factor of 20–35% was observed, which is in agreement with literature [21]. An ion source temperature of 240 °C was found optimum for the whole range of test analytes. The carrier gas flow rate was optimized at 1.5 mL/min to achieve faster separation and improve the accuracy in analyzing problematic compounds such as captan, captafol, dichlofluanid that are prone to degradation during GC analysis. The peak areas, peak heights and S/N of these compounds decreased significantly at flow rates lower than 1.5 mL/min. For example, at flow rate of less than 1 mL/min, more than 90% of captan was degraded to tetrahydrophthalimide, which probably occurred due to degradation in the injector and/or column due to long residence time.

Several temperature programs were attempted to optimize the separation of the mixture initially with solvent standards and subsequently with matrix-matched standards at constant helium flow rate of 1.5 mL/min. Lowering of ramping rate resulted in broader peaks of some compounds, which in turn reduced peak resolution rendering identifications at trace level difficult and ambiguous. For wine samples, major matrix interfering compounds were fatty acids, which were left even after clean up with PSA. Typical peaks of fatty acids like butyric, myristic, palmitic acid, etc. had considerable tailing that masked signals from analytes of interest. In addition, these interfering compounds had fragment ions (m/z) common to the target analytes, which could lead to over-estimation or false negatives. Similar co-elutions from natural products in grape also masked target analyte signals.

GC × GC optimization was similar to the approach of Hoh et al. [21]. Before optimization of GC × GC parameters, the transfer and detection efficiency of the modulation process was evaluated. The peak areas of the analytes from GC analysis were compared to that of GC × GC analysis keeping the GC and MS parameters unchanged. The modulation period was set at 4 s with hot-pulse duration of 1 s. Peak areas of analytes from GC × GC analysis were nearly 90% of those from GC analysis. Increased losses were observed for compounds that showed significant peak tailing in both solvent as well as matrix-matched standards, e.g. methamidophos, acephate, thiometon, etc. Simple addition of modulation to the GC program was not enough to separately identify all 185 analytes. Uniform separation of the analytes was not observed and most analytes eluted in bunches or groups clustered at specific regions. Several combinations of the GC × GC oven program were attempted to separate the close and co-eluting peaks at uniform ramping rate of 10 °C/min. The primary oven was programmed differently from the secondary oven since the secondary oven is equipped to handle only four-step ramping. The primary oven temperature program was set at 100 °C (2 min hold) increased to 190 °C at 15 °C/min (5 min hold); to 200 °C at 10 °C/min (0 min hold); to 242 °C at 15 °C/min (5 min hold); and finally to 285 °C at 10 °C/min (12 min hold). The secondary oven temperature program was set at 106 °C (2 min hold); increased to 195 °C at 15 °C/min (5 min hold); to 250 °C at 15 °C/min (5 min hold); and finally to 305 °C at 10 °C/min (11 min hold). This resulted in a total run time of 38 min and complete separation of all the analytes could be achieved. The relatively short run time not only increased the laboratory output, but also considerably saved the consumption of liquid nitrogen used in the modulation process. The hold of 5 min at 190 °C enabled complete separation of the matrix compounds from the analyte peaks. Following the optimization of the oven program, the modulation period was varied from 2 to 8 s, and its effect on the separation and sensitivity of analysis was explored. The optimization was done considering two factors: (a) comparative increase in S/N and (b) preserving the analyte separation. Optimal results were obtained with modulation period set at 5 s. Losses in sensitivity and co-elutions owing to modulation could be avoided and hence all the 185 analytes could be detected with considerable resolution and S/N. The hot-pulse duration was optimized next to increase the sensitivity of analysis. The duration of the hot-pulse affected the height of the maximum peak also known as the "base slice" in GC × GC. We therefore checked the maximum peak height and S/N of each analyte with respect to changes in hot-pulse duration, focusing especially on analytes with higher LODs. It was found that when the hot-pulse time was kept at 1.80 s, cool time between stages at 0.70 s, with modulation period of 5 s, the necessary sensitivity of detection (10–50 μg/L) was attained for most analytes. Similarly, the modulator temperature offset was optimized by checking its effect on the S/N ratio of the analytes. The modulator temperature offset was set at 25–50 °C in steps of 5 °C and optimized at 35 °C. In an earlier study Dalluge et al. [11] reported the separation of 58 pesticides in food samples by

GC × GC in 50 min run time, while Zrostlikova et al. [10] reported the separation of 20 pesticides in apple and peach extracts. Others studies on comprehensive GC × GC with nitrogen–phosphorus detection has been reported for the separation and quantitation of fungicides in vegetable samples [23]. Rapid scanning quadrupole mass spectrometric methodology [24] for the analysis of 92 pesticides contained in red grape-fruit extracts has also been reported. The current method however encompasses a wider range of analytes in a shorter run time and improved sensitivity in comparison to the reported methods [10,22–24]. The ambiguity in estimating deltamethrin owing to its late elution [10] could also be resolved by the current method.

3.2. Conversion of urea derivatives in GC injection port

Most urea derivatives could not be detected as intact compounds. Individual injections of each urea-based pesticide were done to generate their specific spectrum for possible matching with the available NIST library for identification purposes. The compounds like diuron, isoproturon and metoxuron converted to their respective isocyanates and identified in those forms [22]. Diuron was converted to 3,4-dichlorophenylisocyanate; isoproturon to 4-isopropylphenylisocyante and metoxuron to 3-chloro-4-methoxyphenylisocyanate. This indicated that the $-N(CH_3)_2$ group of these compounds was more labile to thermal cleavage in the GC injection port. The breakdown products from diflubenzuron were *p*-chloroaniline and 2,6-difluorobenzamide. The peak of *p*-chloroaniline was used to identify and quantify diflubenzuron. In the present study, all these breakdown products were specific to the respective analytes and were absent in blank matrix. However, 2,6-difluorobenzamide is the degradation product of several other benzyolurea derivatives (e.g. teflubenzuron, hexaflumuron, etc.). It is therefore required to confirm the presence of the parent compound by LC–MS/MS analysis. Other urea derivatives could be identified as such, after confirmation of the parent compound. For cross verification, the mixture of these 185 test compounds was examined by LC–MS/MS, but no degradation could be identified, indicating that degradation or hydrolysis did not occur in the test mixture and it occurred only after injection in to the GC system. For real samples, the signals from incurred metabolites and metabolites formed after GC injection would combine in a single peak, and were therefore expressed in terms of concentration of the parent compound.

3.3. Co-elution of analytes could be resolved by GC × GC

Diazinon (*m*/*z* 137) and fluchloralin (*m*/*z* 63) elute so closely that the deconvolution algorithm failed to separate the two compounds at analyte concentrations below 25 μg/L because a mixed mass spectrum was obtained. Similar observations were noted for pyremethanil, etrimphos; penconazole and *trans*-chlorfenvinphos and could be resolved by GC × GC.

3.4. Masking of target analytes by matrix compounds and its solution over 2-D in wine

Despite the necessary clean up, some fractions of co-eluting matrix compounds were retained that had a number of fragment ions (*m*/*z*) common with the target analytes and resulted in overestimation of the target residues and false positive or negative detections.

3.4.1. Matrix interferences in wine

Triazophos and a matrix component, viz. ethyl hexyl cinnamate had common *m*/*z* 161 and 162. At higher analyte concentrations, the effect of the matrix component could not be identified since the software was not able to deconvolute the spectra of the matrix compound from triazophos. However, there was an enhancement of peak area when the signal from *m*/*z* 161 was considered as the quantifier ion for triazophos. At lower analyte concentrations (<50 μg/L), the effect of this matrix component was pronounced and the triazophos peak was completely masked and could not be detected. This incongruity could be solved by GC × GC analysis where the peaks of triazophos and ethyl hexyl cinnamate could be baseline separated. Similar masking noted for flupicolide by matrix compound butyric acid in wine samples could also be resolved by GC × GC.

3.4.2. Matrix interferences in grape

Grape has a number of natural compounds that affected the analysis of several compounds through co-elution and signal enhancement. γ-Tocopherol (identified on NIST library matching) is a natural compound in grape that interferes with the estimation of the pesticide indoxacarb at analyte concentration above 250 μg/L. Although Vitamin E and γ-tocopherol could be removed by DSPE with graphitized carbon black, we avoided the treatment owing to recovery losses of target compounds such as chlorothalonil. At lower concentrations (<50 μg/L), the relatively weaker signal of indoxacarb was completely masked by the strong response from γ-tocopherol and it was not possible to identify indoxacarb. Similarly, for the two isomers of dimethomorph, the detection of the first isomer was considerably affected by the co-eluting matrix compound Vitamin E (α-tocopherol), which had a common fragment *m*/*z* of 165. Analysis by GC × GC could separate them in space leading to unambiguous detection and quantification.

Matrix enhancement was noted for cypermethrin, endosulfan sulfate, flucythrinate, oryzalin, paclobutrazole, phorate, spiroxamine, and sulfosulfuron while there were suppressions in signal for azoxystrobin, buprofezin, captan, cyfluthrin, esfenvalerate, folpet, iprodione, linuron and thiabendazole. Hence, for quantification purposes matrix standards were preferred to solvent standards.

3.5. Method accuracy and precision

Intra-laboratory precision in terms of HorRat of the analytes calculated at 10 μg/L level of fortification were below 0.2, with average recoveries ranging from 70 to 120% in both grape and wine. For analytes with higher LOQ, the HorRat was calculated at 25 μg/L level of fortification. In addition to the Horwitz equation, measurement uncertainties were identified and estimated according to the GUM approach [25] which is consistent with ISO 17025 [26]. The details are described in the following section.

3.6. Estimation of measurement uncertainty

Uncertainty of measurement for most analytes was estimated at 10 μg/L level of fortification except for difenconazole, dimethomorph, oryzalin, acephate, indoxacarb, thiabendazole, deltamethrin, omethoate and vamidothion that had LOQ > 10 μg/L and hence their uncertainty analysis was carried out at 25 μg/L. Based on the expanded uncertainty values, the analytes could be broadly classified into four groups in both grape and wine.

Group I: Expanded uncertainty up to 10%: 116 compounds in grape and 101 in wine.
Group II: Expanded uncertainty 10–20%: 35 compounds in grape and 47 in wine.
Group III: Expanded uncertainty 20–50%: 21 compounds in grape and 26 in wine.

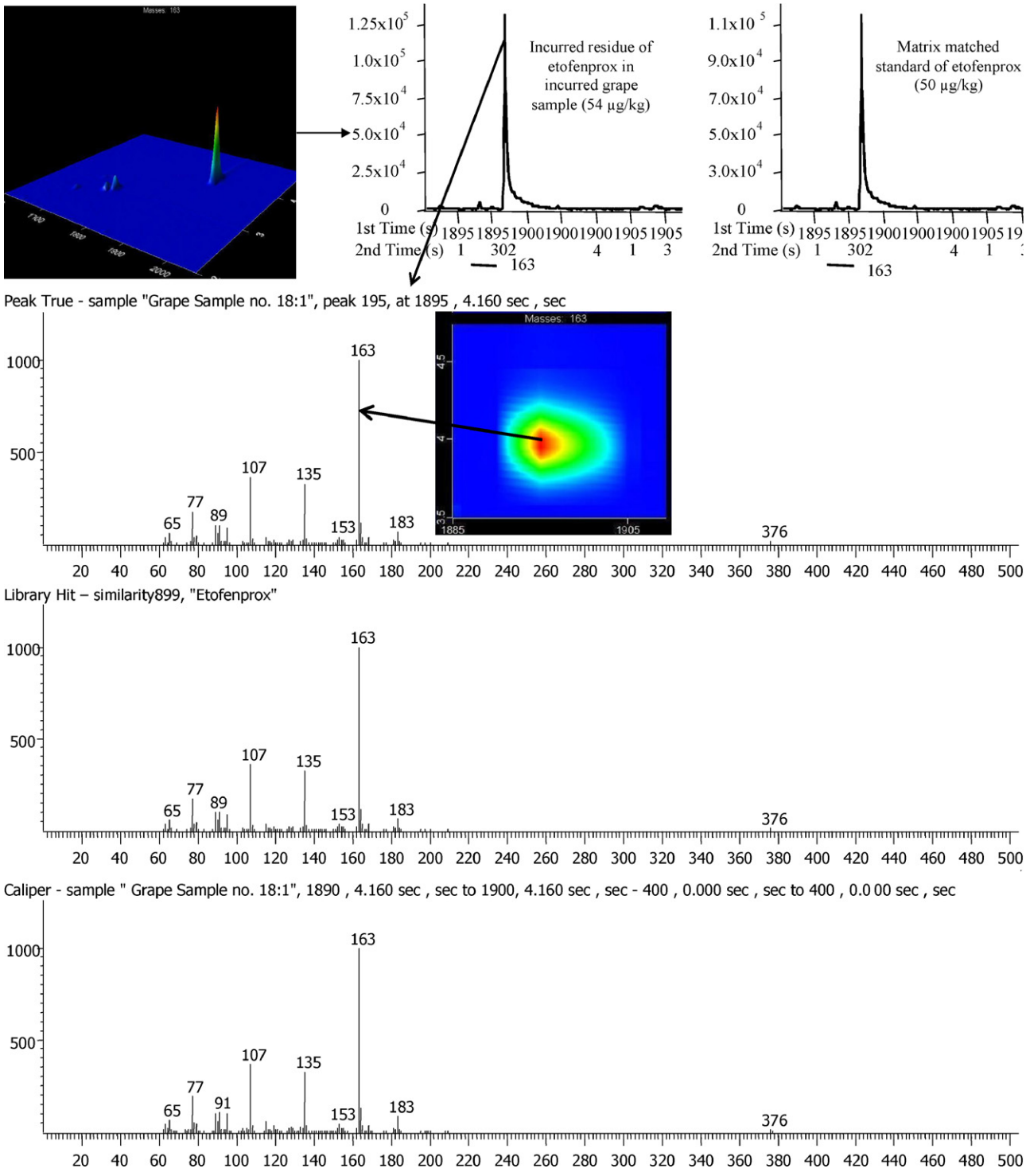

Fig. 1. Residues of etophenprox in the incurred grape sample.

Group IV: Expanded uncertainty more than 50%: 13 compounds in grape and 11 in wine.

Most analytes could therefore be estimated with ≤20% uncertainties in grape (151 no.) and wine (148 no.). Analytes belonging to Group III were mostly having low response and co-elution problems which caused variable response during analysis. Analytes belonging to group IV included cypermethrin, oryzalin, iprodione, indoxacarb, methamidophos, monocrotophos, dimethoate, thiabendazole and the isomers of diemethomorph and difenconazole, which had higher detection limits owing to poor and broader peak shapes, low response and considerable peak tailing. Thus, the reason for higher uncertainties of measurement for these Group III and IV analytes are understandable and hence their analysis requires extra precaution. LOQ of the entire test analytes were at or below the prescribed MRL [1] and reliable confirmation of analyte identity was possible even for typically troublesome pesticides such as polar organophosphates and synthetic pyrethroids.

3.7. Application of the optimized method to real samples

3.7.1. Screening of farm and incurred samples of grape and wine

A total number of 60 grape and 50 wine samples collected from farm-gates and local markets of Pune district, India were screened for pesticide residues by this optimized method. In two grape samples, the residues of cypermethrin and permethrin were detected and the concentrations were 45 and 25 μg/kg, which were much below their harmonized EU-MRL of 0.5 mg/kg. Other compounds detected were chlorpyriphos (15 μg/kg), metalaxyl (25 μg/kg) and etophenprox (54 μg/kg, Fig. 1).

4. Conclusions

GC × GC–TOFMS provided distinct advantages for the multiresidue analysis of 185 compounds in grape and wine in terms of sensitivity, precision, accuracy of analysis and laboratory efficiency. GC × GC offered good separation of all the analytes in space within 38 min run time. The possibility of false negatives is reduced with the lower detection limits offered by GC × GC and the well-resolved peaks allowing for improved identification capability (greater than 85% correct matches of the target compounds against the NIST library). The technique therefore has great potential in regulatory system for resolving conflicting results from different laboratories and issues related to false positives and negatives.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.04.003.

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