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Multiresidue Analysis of 50 Pesticides in Grape, Pomegranate, and Mango by Gas Chromatography−Ion Trap Mass Spectrometry

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A selective and sensitive multiresidue analysis method is reported for simultaneous determination of 50 pesticides of different chemical classes in three commercially important fruits of different nature viz. grape, pomegranate, and mango. The sample preparation method involves extraction of a 10 g sample with 10 mL of ethyl acetate; cleanup by dispersive solid phase extraction with primary secondary amine (PSA, 25 mg) for grape and PSA + graphitized carbon black (25 + 5 mg) for pomegranate and mango; and determination by gas chromatography−ion trap mass spectrometry through multiple reaction monitoring (MRM). Sample preparation under acidified (pH 4) and cold (<4 °C) conditions, use of PTV-large volume injection (20 μL) through multibaffled liner and chromatographic separation on a short 10 m VF-5MS capillary column gave a satisfactory response for all of the analytes including relatively unstable compounds such as captan, captafol, folpet, endrine, and iprodione within 31.8 min. The limit of quantification (LOQ) of most of the compounds was $\leq$10 ng g^{-1} except for captan, captafol, and folpet, where the LOQ was $\leq$20 ng g^{-1}. For each analyte, the unique and most abundant MRM was selected for quantification, and the next most abundant for confirmation, with their abundance ratio being used for unambiguous identification of any detected pesticide in samples within 20% tolerance range at the LOQ level. Use of matrix-matched standards could minimize the matrix effect, which was lowest in grape, followed by pomegranate and mango. Recoveries ranged within 70−120% at 10, 20, and 50 ng g^{-1} in all three matrixes with associated relative standard deviations <20% (n = 6). The method could be successfully applied to the screening of 100 farm samples for compliance to EU maximum residue limits.

KEYWORDS: Multiresidue analysis of pesticides; grape; pomegranate; mango; gas chromatography−tandem mass spectrometry (GC-MS/MS); validation

INTRODUCTION

Grape, pomegranate, and mango are nutritionally important fruit crops of international trade significance and consumed both as fresh and processed products. The cultivation of these crops in tropical Indian climate experiences infestation by a wide range of pests and diseases causing reduction in yield as well as deterioration in quality of the fruits. To prevent such losses, a variety of contact and systemic pesticides are applied frequently during the cultivation of these crops, the residues of which may accumulate at toxic level in/on the fruits at the stage of harvest. Besides, residues of some other nonrecommended and restricted use pesticides may also appear in fruits from indirect sources such as drift from adjoining crop fields, contaminated agro-inputs, etc. Thus, monitoring of pesticide residues in these fruits becomes essential to ensure food safety to the consumers, especially in a situation where the regulations are becoming more and more stringent in most countries.

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In India, the residue monitoring program is implemented for grape, pomegranate, and mango to regulate and monitor as much as 97 pesticides in exportable fruits (*1*−*3*), which includes the recommended, banned, and restricted use pesticides appearing in fruits from direct and indirect sources. Full scan GC-MS analysis is commonly used for the screening of samples for compliance to maximum residue limits (MRL), as this technique provides both quantitative as well as library-matching-based qualitative information. But analysis in full scan mode often fails to provide the desired level of sensitivity and selectivity and suffers from unacceptably high matrix interferences. To overcome these problems, gas chromatography−tandem mass spectrometry (GC-MS/MS) appears as a powerful technique because of its capability to exclude spectral interferences by separating coeluting compounds on the basis of compound-specific target-oriented multiple reaction monitoring (MRM) transitions. Tandem mass spectrometry (MS/MS) can be accomplished both by using triple quadrupole or ion trap systems, and out of these two, the ion trap mass spectrometers are more popular because of the relatively lower cost of the instrument and the ability to perform MS^n analysis (*4*−*7*). It can perform full scan and MS/MS in a single

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run, while the external ion source avoids direct contamination and minimizes the space charge effect. However, we observed difficulty with ion trap when the number of overlapping target analytes exceeds four to five. Thus, chromatographic separation plays a very important role in multiresidue analysis involving an ion trap GC-MS system.

In our previous efforts (*8*−*10*), we reported methods for the determination of multiclass pesticide residues in different fruit commodities using liquid chromatography−tandem mass spectrometry. But, the GC amenable pesticides could not be considered in our earlier studies. It was, therefore, essential to develop and validate a GC-based method for the selective analysis of pesticides in grape, mango, and pomegranate. Special focus was given to improve the precision and accuracy in analyzing relatively unstable problematic compounds such as captan, captafol, folpet, etc., which are prone to degradation during sample preparation as well as GC-MS determination (*11*−*13*) and are not amenable to LC-MS analysis.

MATERIALS AND METHODS

Selection of Pesticides and Matrix. A total of 50 pesticides were selected for this study, which included 19 organochlorine (including isomers), 12 organophosphorus, 9 synthetic pyrethroids (including isomers), 4 dicarboximide, 1 *N*-phenylsulfamide, 1 phenyl pyrazole, 1 dithiolane, 1 substituted thiadiazinanone, 1 nitrophenyl ether, and 1 triazole compound. These pesticides included recommended, restricted, and banned chemicals that are amenable for GC analysis and require frequent monitoring. Three important tropical fruits, viz., grape (variety: Thompson Seedless), mango (variety: Alphonso), and pomegranate (variety: Ganesh), that constitute a major share to the total export of fresh horticultural produce from India were considered for the study. Organically grown mature fruits were collected and screened to confirm an absence of any residues before using in method development and validation studies.

Reagents and Materials. Certified reference standards (**Table 1**) of the test pesticides (>98% purity) were purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany). Residue analysis grade (dried) ethyl acetate and sodium acetate were from Thomas Baker (Mumbai, India). Primary secondary amine (PSA, 40 μm, Bondesil) and graphitized carbon black (GCB) were procured from United Chemical Technology (Bristol, PA, USA). Anhydrous sodium sulfate (analytical reagent grade) was purchased from Merck (Mumbai, India) and activated by heating at 450 °C for 6 h and kept in desiccators.

Preparation of Standard Solutions. The stock solutions of the individual pesticide standards were prepared by accurately weighing 10 (±0.1) mg of each analyte in volumetric flasks (certified A class) and dissolving in 10 (±0.1) g of ethyl acetate. These were stored in dark vials at 4 °C. A working standard mixture of 1 mg L^{-1} was prepared by appropriate dilution of the stock solution, from which the calibration standards (5−250 ng mL^{-1}) were prepared by serial dilution with ethyl acetate.

Sample Preparation. The laboratory sample unit was 2 kg for grape (only berry) and 10 fruits each for pomegranate and mango. The samples were cooled to nearly 0 °C by storing in a deep freezer (−20 °C) for 30 min. Grape (berries only) was directly blended, while for pomegranate (with rind) and mango (without stone), the fruits were chopped into small pieces (around 1 cm^2) before blending. The blended samples (200 g) were further homogenized at high speed (15000 rpm) for 1 min, and from this,10 g was transferred into a 50 mL centrifuge tube. The pH of the crushed samples was adjusted to around 4 by adding 0.5% glacial acetic acid (v/v) and then extracted with 10 mL of ethyl acetate (precooled to around 4 °C), in the presence of 10 g of sodium sulfate and 5 mL of ice-cold water. The mixture was then homogenized at 15000 rpm for 2 min followed by centrifugation at 3000 rpm for 5 min at −10 °C. An aliquot of 1 mL was drawn from the upper ethyl acetate layer into a 2 mL Eppendorf tube containing 25 mg of PSA for grapes and 25 mg of PSA + 5 mg of GCB for pomegranate and mango. The Eppendorf tube was vortexed for 1 min and then centrifuged at 10000 rpm for 5 min. This solution was analyzed by GC-MS/MS after filtering through a 0.2 μm polytetrafluoroethylene (PTFE) membrane filter.

GC-MS/MS Analysis. A TRACE GC Ultra gas chromatograph hyphenated to a Polaris Q ion trap mass spectrometer (Thermo Fisher Scientific, Austin, TX, USA) controlled by using Xcalibur 1.2 software was used for the determination of residues. The system included a TriPlus AS auto sampler that was run on Basic injection mode with an injection depth of 20 mm and an injection speed set at 2 μL s^{-1} to inject 20 μL through deactivated multibaffled glass liner (6 baffles, 120 mm length × 2 mm ID). A Varian VF-5MS (Palo Alto, USA) capillary column (5% phenyl 95% dimethylpolysiloxane; 10 m × 0.15 mm, 0.15 μm film thickness) was used for analysis. Ultrapure grade helium (BOC India Limited, Kolkata) was used as the carrier gas at 1 mL min^{-1} flow.

The GC oven temperature was programmed from an initial temperature of 80 °C (2 min hold), ramped at 25 °C min^{-1} up to 150 °C, then ramped at 4 °C min^{-1} up to 220 °C, and finally at 10 °C min^{-1} to 285 °C with holding for 3 min. This program resulted in a total run time of 31.80 min.

The programmed temperature vaporizer−large volume injection (PTV-LVI) process consisted of four distinct phases, viz., injection, evaporation, analyte transfer to column, and cleaning phases. Each phase was optimized in tandem to maximize the analyte transfer to the column. At injection phase, the PTV injector was held for 0.15 min at 67 °C with pressure at 50 kPa and gas (helium) flow rate of 20 mL min^{-1}. At the evaporation phase, the pressure was maintained at 50 kPa with temperature ramped at 10 °C s^{-1} up to 87 °C (0.3 min hold). The stop purge time was set at 1.5 min. During the transfer phase, we increased the pressure up to 250 kPa and ramped the temperature at 14.5 °C s^{-1} up to 285 °C (1.5 min hold). In the cleaning phase, the temperature was ramped to 290 °C and held for 10 min with helium flow maintained at 50 mL min^{-1}. The split flow and solvent valve temperature were maintained at 50 mL min^{-1} and 100 °C, respectively. The split valve was closed during the analyte transfer phase, and in rest of the period, it remained open.

The other optimized parameters included a transfer line temperature of 285 °C, an ion source of 230 °C, and microscan and maximum ion times set at 1 and 25 ms, respectively. The damping gas flow was set at 0.6 mL min^{-1}, and emission current was 250 μA. The compound specific MS/MS parameters are presented in **Table 1**.

Method Validation. The analytical method was validated as per the single laboratory validation approach (*14*, *15*). The performance of the method was evaluated considering the following validation parameters.

Linearity. The calibration curves for all of the compounds in pure solvent and individual matrix were obtained by plotting the peak area against the concentration of the corresponding calibration standards at five calibration levels ranging between 5 and 100 ng mL^{-1}.

Sensitivity. Limit of detection (LOD) was determined by considering a signal-to-noise ratio (S/N) of 3 with reference to the background noise obtained from the blank sample, whereas the limit of quantification (LOQ) was determined by considering an S/N of 10 for the quantifier MRM and 3:1 for qualifier MRM.

Recovery and Repeatability. The recovery experiments were carried out on fresh untreated fruits by fortifying the samples (10 g) in six replicates with the pesticide mixture separately at three concentration levels, i.e., 10, 20, and 50 ng g^{-1}, and extracting by the method described above. The quantification of recovery samples was done using the calibration obtained from matrix-matched standards.

Matrix Effect. The matrix effect (ME %) was evaluated by the following equation:

$$\text{ME}\% = \frac{\text{peak area of post extraction spike}}{\text{peak area of solvent standard}} \times 100$$

Values of ME above 110% indicate enhancement of ionization, and values lower than 90% indicate suppression of ionization.

Evaluation of the Method for Screening Farm and Incurred Samples. The optimized method was applied to the screening of 50 grape and 25 each of pomegranate and mango samples collected at random from commercial farms located in peninsular India, which is the major cropping area of these fruits. The method was also evaluated on incurred samples of two grape and one each of pomegranate and mango (N = 4 for each) fruits.

RESULTS AND DISCUSSION

Sample Preparation. Ethyl acetate was found to be a suitable solvent for all three low-fat, sugar-rich test commodities because

Table 1. MS/MS Parameters and Ion Ratios (%) for the Test Compounds[a]

name	start time (min)	RT	target MRM (T)	width	voltage	Q	scan range	qualifier MRM-I (Q1)	qualifier MRM-II (Q2)	ion ratio (%) (Q1 × 100)/T (mean ± RSD)	ion ratio (%) (Q2 × 100)/T (mean ± RSD)
Dichlorvos (1)	4.0	4.51	185 > 93	5	1.5	0.225	83−195	185 > 109		17.7 (±8.4)	
4-Br,2-Cl-Phenol (1)	4.0	4.93	208 > 172	5	2	0.3	120−215	208 > 170		23.0 (±18.6)	
Phorate (2)	9.0	9.33	231 > 203	3	1.5	0.3	165−240	231 > 175		44.2 (±7.9)	
α-HCH (2)	9.0	9.50	219 > 183	5	2	0.3	140−230	219 > 181	219 > 145	94.9 (±2.8)	6.9 (±10.1)
β-HCH (3)	9.99	10.43	219 > 183	5	2	0.45	140−230	219 > 181	219 > 145	85.8 (±5.3)	12.3 (±9.2)
γ-HCH (3)	9.99	10.56	219 > 183	5	2	0.45	140−230	219 > 181	219 > 145	99.0 (±5.7)	7.2 (±4.7)
Diazinon (3)	9.99	10.97	304 > 179	3	2	0.45	152−310	304 > 195	304 > 162	9.7 (±10.1)	5.2 (±18.3)
Chlorothalonil (4)	11.04	11.14	266 > 231	5	2.5	0.45	225−270	266 > 229		99.8 (±1.7)	
δ-HCH (5)	11.36	11.62	219 > 183	5	2	0.45	140−230	219 > 181	219 > 145	87.7 (±3.0)	14.1 (±3.4)
Chlorpyriphos-methyl (6)	12.07	12.55	286 > 271	5	2	0.3	230−290	286 > 273	286 > 241	68.5 (±6.5)	11.2 (±11.0)
Parathion-methyl (7)	12.22	12.81	263 > 246	5	1.5	0.3	100−270	263 > 136	263 > 109	22.7 (±5.4)	13.1 (±9.3)
Heptachlor (7)	12.22	12.94	272 > 237	5	2.5	0.3	225−280	272 > 235	272 > 239	60.2 (±4.1)	48.6 (±6.2)
Fenitrothion (8)	13.33	13.78	277 > 260	5	2.0	0.3	120−280	277 > 125		25.9 (±7.8)	
Diclofluanid (8)	13.33	13.95	224 > 123	5	2.0	0.3	115−230	224 > 124	224 > 189	6.8 (±8.1)	4.6 (±11.1)
Aldrin (9)	14.05	14.22	263 > 191	5	2.2	0.45	185−270	263 > 193	263 > 228	90.6 (±4.2)	37.3 (±5.8)
Chlorpyriphos-ethyl (9)	14.05	14.34	314 > 258	5	2	0.3	250−325	314 > 286	314 > 260	92.6 (±3.7)	56.9 (±6.8)
Parathion-ethyl (10)	14.41	14.67	155 > 125	5	2.0	0.45	115−165	291 > 263	291 > 274	67.0 (±12.7)	23.1 (±13.3)
Fipronil (11)	15.30	16.05	367 > 245	5	2.5	0.3	205−375	367 > 255	367 > 257	72.8 (±4.6)	52.5 (±5.6)
Chlorfenvinphos (11)	15.30	16.12	323 > 267	5	2.4	0.45	260−330	323 > 269		62.8 (±8.5)	
Captan (12)	16.18	16.32	79 > 79	5	0.0	0.225	70−270	264 > 236	264 > 238	21.6 (±5.6)	13.4 (±6.8)
Folpet (12)	16.18	16.50	260 > 232	5	2.0	0.3	125−265	260 > 130	260 > 200	27.6 (±5.6)	97.6 (±3.6)
cis-chlordane (13)	16.60	16.71	373 > 266	5	2.5	0.45	255−380	373 > 264	373 > 301	93.0 (±4.0)	41.1 (±4.1)
2,4-DDE (13)	16.60	16.93	318 > 246	5	2.5	0.45	235−325	318 > 248	318 > 281	60.8 (±2.4)	17.4 (±2.0)
α-Endosulfan (14)	17.06	17.15	241 > 206	5	2.5	0.45	165−250	241 > 204	241 > 170	86.4 (±3.7)	46.5 (±6.3)
trans-Chlordane (14)	17.06	17.16	373 > 266	5	2	0.3	255−380	373 > 264	373 > 301	94.6 (±3.7)	80.6 (±5.7)
Isoprothiolane (15)	17.69	18.17	189 > 145	3	2	0.3	115−195				
Profenophos (15)	17.69	18.21	337 > 267	5	2	0.3	260−345	337 > 309	337 > 295	42.9 (±3.7)	23.6 (±5.7)
Dieldrin (15)	17.69	18.29	263 > 193	5	2.5	0.45	185−270	263 > 191	263 > 228	86.9 (±11.3)	30.9 (±18.9)
4,4-DDE (15)	17.69	18.31	318 > 246	5	2.5	0.45	235−325	318 > 248	318 > 283	61.6 (±1.7)	13.2 (±4.2)
Buprofezin (16)	18.47	18.69	249 > 193	3	1.8	0.45	185−255	249 > 192		38.1 (±13.7)	
Flusilazole (16)	18.47	18.75	233 > 165	3	2	0.45	130−240	233 > 152	233 > 183	48.7 (±4.2)	15.2 (±10.7)
Oxyfluorfen (16)	18.47	18.86	300 > 223	5	2.5	0.45	210−310	300 > 252	300 > 280	25.9 (±9.3)	15.9 (±10.9)
Endrin (17)	18.92	19.08	263 > 193	5	2.6	0.45	185−270	263 > 191	263 > 229	70.5 (±11.3)	15.7 (±35.5)
β-Endosulfan (18)	19.35	19.62	241 > 206	5	2	0.3	165−245	241 > 170	241 > 204	65.2 (±10.2)	60.8 (±6.6)
2,4-DDT (19)	19.86	20.07	235 > 165	5	1.6	0.225	155−240	235 > 199	235 > 200	36.5 (±13.7)	30.1 (±9.7)
Endosulfan-sulfate (20)	20.68	21.28	272 > 237	5	2.5	0.3	230−280	272 > 235	272 > 239	58.5 (±4.4)	51.9 (±4.5)
4,4-DDT (21)	21.44	21.59	235 > 165	5	1.7	0.3	155−240	235 > 199	235 > 200	31.8 (±3.4)	38.6 (±6.4)
Captafol (22)	22.04	22.46	79 > 79	5	0.0	0.225	78−320	313 > 278	313 > 276	27.5 (±12.2)	23.4 (±15.0)
Iprodione (23)	22.99	23.46	314 > 245	5	2.5	0.3	240−320	314 > 247	314 > 271	52.0 (±10.4)	33.0 (±3.2)
Dicofol (24)	23.67	23.84	139 > 111	5	2	0.45	100−145	139 > 113	251 > 215*	30.7 (±9.7)	49.5 (±7.0)
Phosalone (25)	24.20	24.57	182 > 111	3	1.5	0.3	100−190	182 > 138	182 > 102	52.8 (±2.6)	15.4 (±6.5)
λ-cyhalothrin (26)	24.94	25.29	181 > 152	5	2.5	0.45	117−191	181 > 151	181 > 127	25.4 (±7.6)	4.9 (±16.7)
Permethrin I (27)	25.81	26.28	183 > 168	5	2.0	0.3	140−190	183 > 165	183 > 153	95.9 (±9.8)	58.1 (±6.7)
Permethrin II (27)	25.81	26.47	183 > 168	5	2.0	0.3	140−190	183 > 165	183 > 153	99.9 (±3.2)	52.6 (±7.4)
Cyfluthrin (28)	26.78	27.02−27.30	165 > 127	5	2.0	0.225	81−175	165 > 91	165 > 129	25.2 (±19.8)	20.1 (±17.91)
Cypermethrin (28)	26.78	27.40−27.67	165 > 127	5	2.0	0.225	81−175	165 > 91	165 > 129	17.4 (±9.8)	21.0 (±16.47)
Ethofenprox (28)	26.78	27.76	163 > 135	3	2.0	0.3	100−173	163 > 107		15.3 (±9.9)	
Fenvalerate (29)	28.12	28.43	225 > 147	5	2.5	0.225	110−235	225 > 119		67.0 (±11.2)	
Esfenvalerate (29)	28.12	28.68	225 > 147	5	2.5	0.225	110−235	225 > 119		68.3 (±14.0)	
Deltamethrin (30)	29.03	29.33	253 > 172	5	2.5	0.225	164−263	253 > 174		93.0 (±6.2)	

[a] Segment numbers are given in parentheses.

of the limited solubility of carbohydrates in ethyl acetate. Sample preparation at <4 °C at the adjusted matrix pH of nearly 4.0 offered stability to all of the 50 compounds, especially the organophosphates and phthalimides, as evident from their satisfactory recoveries, and this observation is in agreement with the literature (*12*). The breakdown of the problematic compounds (such as captan and captafol) during sample preparation could be significantly minimized by extracting at low temperature. High speed homogenization of precooled samples did not increase the system temperature above 10 °C, which was very useful in maintaining the stability of the phthalimides such as captan. Similarly, addition of 5 mL of ice-cold water to the sample before homogenization helped to compensate for the heat generated during homogenization and resulted in superior phase separation. The low temperature processing of samples under acidic pH also minimized the degradation of dicofol to 4,4′-dichlorobenzophenone during extraction. Dispersive SPE with 25 mg of PSA was effective in removing sugary and fatty coextractives, and the use of 5 mg of GCB was enough to render the extracts nearly colorless, which enhanced the life of the GC liner, column, and filament. This sorbent combination did not have any adverse effect on pesticide recoveries, and there were no requirements to add toluene for recovering the compounds with planar structure such as chlorothalonil.

Chromatographic Method. During chromatographic method optimization, we standardized the PTV-LVI method and investigated the influence of the type of liner, column length, and chromatographic conditions on sensitivity (S/N) in response to every possible combination of different parameters to attain the highest S/N for all of the test analytes.

Optimization of PTV-LVI Parameters. The injection phase temperature was set at 10 °C lower than the boiling point (bp) of ethyl acetate to prevent any evaporation loss of the analytes. At the evaporation phase, the temperature was set at 10 °C above the solvent bp to selectively remove ethyl acetate as the temperature of 87 °C was much lower than the temperature required for elution of the lowest boiling analyte, viz., dichlorvos (around 120 °C). At the transfer phase, the temperature was increased to 285 °C, which is higher than the bp of the heaviest analyte, i.e., deltamethrin, but it was not too high to transfer heavier matrix compounds on to the column. In the cleaning phase, the split valve was open, and the temperature was kept above the transfer phase temperature to ensure the effective removal of the unwanted coeluted matrix substances from the system prior to the next injection.

Selection of the Liner. The effects of different deactivated glass liners (sintered and multibaffled) used in the PTV injector on the response of the test analytes were investigated. The peak shapes and S/N of the test analytes were compared with special emphasis to improve the analysis of problematic compounds such as captan, captafol, folpet, endrin, dicofol, and iprodione. The empty glass liners were not considered for the optimization experiment as they did not allow large volume injection, and in splitless mode, we could not achieve sensitivity of many of the analytes at below 50 ng g^{-1}. Similarly, the gooseneck liner, although achieving a better response (with minimum breakdown of captan and captafol) in splitless injection mode (2 μL), could not be used because of incompatibility with PTV-LVI. The sintered glass liner (120 mm length × 2 mm ID) was not suitable for captan, captafol, and folpet, which degraded to their corresponding phthalimide (e.g., 1,2,3,6-tetrahydrophthalimide for captan), and dicofol degraded to 4,4′-dichlorobenzophenone. Since these degradation products are not part of the EU official residue definition of these pesticides, monitoring of degradation products could not serve our purpose. The S/N for iprodione and endrine was also low, which could be due to the possible interaction of such chemicals with the active sites in sintered glass liners. In the case of the deactivated multibaffled glass liner (120 mm × 2 mm, 6 baffles), all pesticides showed good recovery at 10 ng g^{-1} including captan, captafol, and folpet. The peak shape and S/N of iprodione and endrine also improved significantly. In the case of large volume injection, the multibaffled liner allows adequate surface area for effective solvent evaporation and thus helps in achieving good peak shape without splitting or distortion. The injection volume of 20 μL at slow and controlled speed ensured the effective transfer of analytes to the liner and then to the capillary column.

GC Oven Program Optimization. The GC oven program was optimized with the objective to chromatographically separate all of the test compounds with good peak shape, minimum matrix interferences, and increased sensitivity (S/N). The initial temperature was set at 80 °C, which resulted in symmetrical peak shapes and higher S/N of early eluting compounds such as dichlorvos and 4-bromo-2-chlorophenol (metabolite of profenophos). A start temperature below the boiling point of ethyl acetate did not improve separation and unnecessarily increased the run time. A fast temperature ramping at the rate of 25 °C min^{-1} from 80 to 150 °C substantially reduced the relatively large retention time (RT) gap between 4-bromo-2-chlorophenol and the next compound phorate. The oven temperature was then increased up to 220 °C at the rate of 4 °C min^{-1} to allow separation of closely eluting sets of peaks such as (chlorpyriphos methyl, parathion methyl, and heptachlor) and (chlorfenvinphos and fipronil). A ramping rate of 10 °C min^{-1} to 285 °C was helpful to attain symmetrical peak shape and higher S/N for iprodione and phosalone, and holding this temperature for 3 min helped the separation of late eluting synthetic pyrethroids without loss of resolution among cyfluthrin, cypermethrin, and ethofenprox. Despite trying different temperature programs and ramping rates, it was difficult to chromatographically separate closely eluting pesticides such as isoprothiolane, profenophos, dieldrin and 4,4′-DDE (Mix I), buprofezin, flusilazole and oxyfluorfen (Mix II), and *trans*-chlordane and α-endosulphan (Mix III). While examining in GC-MS full scan mode (50−500 Da), these closely eluting compounds interfered with each other's analysis and resulted in poor mass spectral purity. Hence, their identification was uncertain. In MS/MS mode, such ambiguity in separation related identification could be completely resolved (**Figure 1**) because of compound-specific selective MRM transitions.

Column Length. Use of a 10 m capillary column significantly minimized the breakdown of problematic compounds, viz., captan, captafol, folpet, profenophos, iprodione, and dicofol, during GC separation against the conventional 30 m column, where we recorded nearly 20−30% degradation relative to the 10 m column. A short VF 5MS column with superior end-capping reduced the residence time of the analytes within the column system and thus minimized their chemical interaction with active sites of the stationary phase.

MS/MS Method Optimization. The MS/MS method was optimized in three steps, viz., parent (precursor) ion isolation, ion excitation, and dissociation into product ion, to scan within a particular mass range (*16*, *17*). Before MS/MS optimization, the retention time (RT) window (segment) was fixed for each compound by referring to the full scan chromatogram and RT of individual compounds. In the first step, the compound-specific precursor ions were selected from the full scan spectrum, which mostly included the base peak except for HCH (base peak m/z 181) and DDE (base peak m/z 246) isomers (**Table 1**), for which the precursor ion with m/z 219 and 318, respectively, gave significantly better selectivity and higher S/N. In the case of captan and captafol, the precursor ion (m/z 79) was directly monitored without any breakdown (voltage = 0) to ensure maximum S/N. As these compounds were chromatographically well separated, there was no ambiguity in their identification. Furthermore, the confirmatory MRM for captan and captafol are selective and different.

The precursor ion width was kept at 5 amu for compounds containing isotopic atoms such as Cl or Br (e.g., chlorinated hydrocarbons, pyrethroids, etc.) and 3 amu for nonisotopic compounds. Precursor ion isolation time was optimized by varying between 2 to 20 at 2 ms intervals for individual compounds. While optimizing this parameter, the excitation voltage was kept at zero, and the excitation energy (q value) was kept at medium level, i.e., 0.3 for each compound. The isolation time was selected to correspond with the highest peak area and S/N. The excitation energy (q), which is required to stabilize a precursor ion during excitation process, was selected by comparing the S/N at three different levels, viz., low (0.225), medium (0.3), and high (0.45), for individual precursor ions. For dichlorvos and 2,4-DDT, the excitation energy was kept low (0.225) to avoid losses of precursor and/or product ion from the trap during excitation. The precursor ions of HCH isomers, aldrin, and isomers of chlordane and DDE required the highest excitation energy (q = 0.45), whereas for the rest of the compounds, the q value was set at

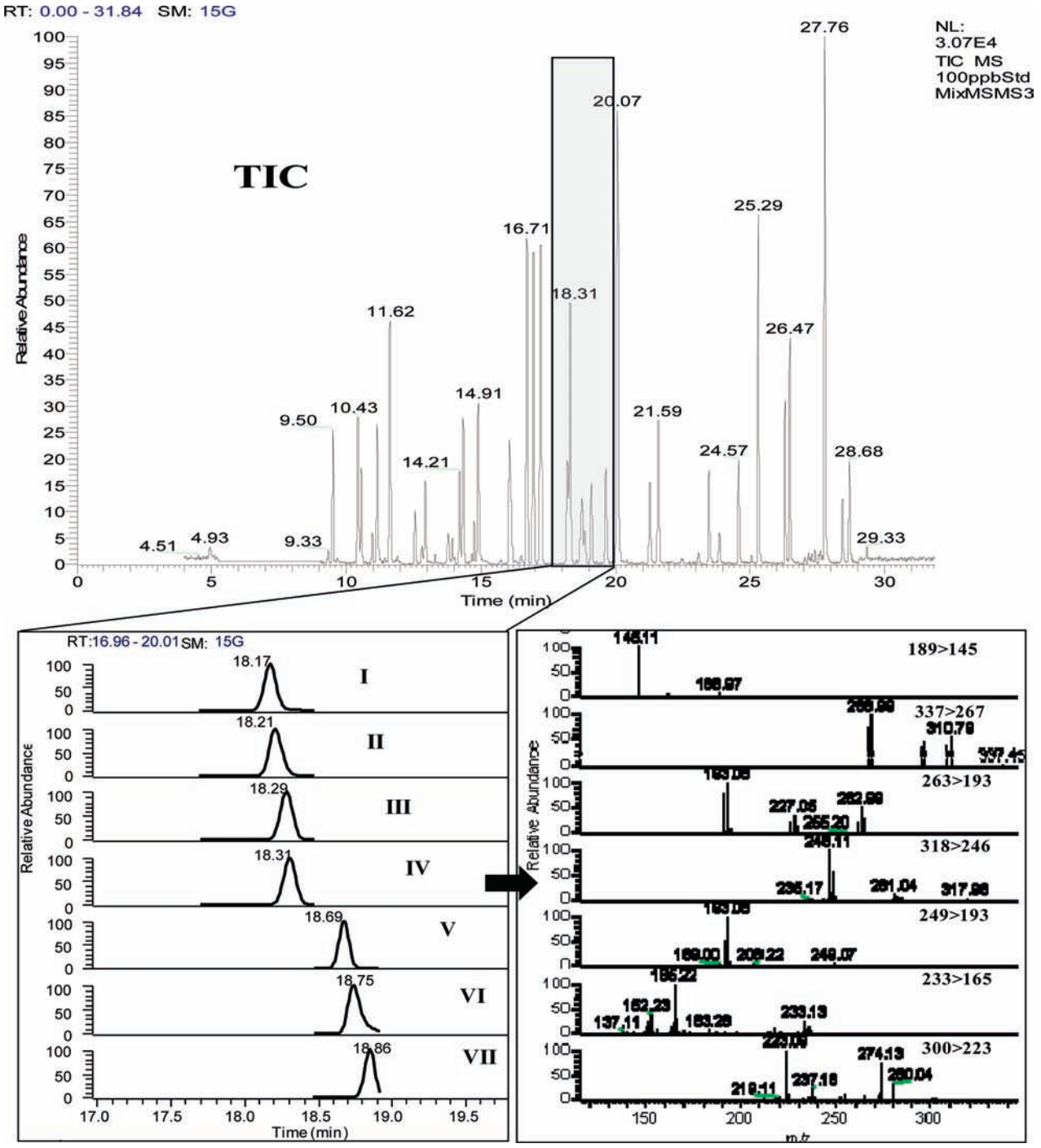

Figure 1. GC-MS/MS multiresidue chromatogram at 100 ng g^{-1} and separation of six closely eluted pesticides.

medium 0.3 (**Table 1**). The excitation voltage was optimized by varying within 0.5 to 2.5 V with increments of 0.5 V and comparing the corresponding S/N. Finally, the duration of application of the excitation RF voltage to end-cap electrodes (excitation voltage time) was optimized within the range of 5 to 21 ms with increments of 2 ms to achieve the highest sensitivity. For all of the test compounds, the characteristic product ion with highest intensity was used for quantification. The next most intense product ion was used for confirmation. To ensure the highest S/N, the product ion scan range was kept as narrow as possible (**Table 1**). The ratio of the confirmatory to quantitative MRM transitions was used for unequivocal identification of detected pesticides in real world samples within ± 20% tolerance range at LOQ.

Evaluation of Matrix Influence. In general, the matrix effect (ME) was relatively more prominent at lower calibration levels. Among the three test commodities, ME was minimum in grape for most of the compounds except the cases of signal enhancements for chlorpyriphos-ethyl, profenophos, buprofezin, and heptachlor to the extent of 30(±3), 39(±2), 54(±5), and 35(±3)%, respectively, at 20 ng g^{-1} (n = 6). In pomegranate and mango, the reasons for relatively higher ME could be due to coextraction of different fat-soluble pigments (polyphenols), fatty acids, etc. in ethyl acetate.

In pomegranate, the matrix effects were characterized by suppressions in analytical signal (S/N) for all of the organochlorine pesticides except for chlorothalonil where signal enhancement was up to 22(±4) %. For organophosphates, the

Table 2. Recovery and LOQ of the Test Compounds in Grape, Pomegranate, and Mango at Different Fortification Levels

		recovery in grape (±RSD%, n = 6)				recovery in pomegranate (±RSD%, n = 6)				recovery in mango (±RSD%, n = 6)			
Sr no.	Name	10 ng/g	20 ng/g	50 ng/g	LOQ ng/g	10 ng/g	20 ng/g	50 ng/g	LOQ ng/g	10 ng/g	20 ng/g	50 ng/g	LOQ ng/g
1.	Dichlorvos	85 (±18)	77 (±12)	71 (±3)	5.5	108 (±11)	99 (±7)	102 (±1)	4.5	93 (±7)	87 (±8)	76 (±6)	6.5
2.	4-Bromo-2-Chloro Phenol	77 (±10)	99 (±9)	107 (±11)	9.5	84 (±14)	84 (±8)	100 (±8)	10.0	95 (±18)	82 (±9)	93 (±7)	8.0
3.	Phorate	83 (±12)	93 (±4)	89 (±2)	5.2	91 (±10)	81 (±4)	100 (±7)	6.2	102 (±8)	84 (±5)	89 (±8)	5.9
4.	α-HCH	95 (±8)	97 (±7)	96 (±3)	5.5	79 (±8)	88 (±11)	95 (±6)	5.0	95 (±8)	82 (±7)	96 (±4)	5.3
5.	Diazinon	86 (±15)	88 (±11)	88 (±4)	6.4	82 (±10	81 (±4)	100 (±7)	6.5	88 (±9)	87 (±2)	89 (±1)	7.5
6.	β-HCH	107 (±15)	111 (±7)	108 (±6)	5.8	78 (±7)	97 (±8)	105 (±6)	5.6	86 (±9)	81 (±3)	99 (±4)	5.5
7.	γ-HCH	93 (±7)	97 (±5)	104 (±2)	5.5	72 (±12)	91 (±6)	94 (±9)	6.5	87 (±10)	83 (±9)	89 (±7)	5.0
8.	Chlorothalonil	97 (±8)	95 (±5)	91 (±9)	8.0	82 (±10	84 (±16)	86 (±9)	6.5	79 (±11)	82 (±6)	89 (±4)	8.5
9.	δ-HCH	91 (±14)	94 (±8)	91 (±7)	5.5	79 (±9)	82 (±4)	90 (±6)	5.3	78 (±7)	76 (±5)	83 (±3)	5.0
10.	Chlorpyriphos-methyl	82 (±8)	77 (±5)	76 (±5)	5.0	75 (±10)	96 (±8)	95 (±7)	6.5	79 (±9)	76 (±6)	88 (±5)	5.0
11.	Parathion-methyl	99 (±6)	79 (±10)	73 (±8)	5.5	85 (±11)	80 (±13)	100 (±8)	6.5	70 (±12)	75 (±15)	86 (±9)	5.6
12.	Heptachlor	102 (±14)	112 (±9)	114 (±4)	5.3	92 (±13)	102 (±5)	95 (±3)	6.3	99 (±9)	87 (±9)	103 (±3)	6.5
13.	Diclofluanid	85 (±12)	82 (±10)	88 (±5)	6.2	75 (±12)	72 (±10)	78 (±5)	7.2	86 (±12)	88 (±10)	85 (±5)	6.5
14.	Fenitrothion	86 (±15)	89 (±9)	85 (±4)	6.2	84 (±16)	83 (±10)	90 (±4)	6.8	80 (±14)	86 (±9)	81 (±3)	7.6
15.	Chlorpyriphos-ethyl	76 (±8)	83 (±7)	76 (±3)	6.5	76 (±6)	89 (±2)	90 (±3)	5.3	87 (±8)	83 (±6)	82 (±4)	5.2
16.	Aldrin	102 (±4)	98 (±8)	101 (±5)	5.0	82 (±17)	110 (±16)	88 (±9)	5.0	76 (±10)	76 (±5)	78 (±8)	5.3
17.	Parathion-ethyl	81 (±12)	77 (±10)	75 (±7)	9.0	85 (±3)	96 (±10)	89 (±9)	6.0	86 (±18)	80 (±15)	88 (±7)	8.0
18.	Captan		71 (±13)	75 (±8)	18.9		76 (±12)	79 (±10)	19.1		70 (±16)	78 (±6)	18.5
19.	Chlorfenvinphos	79 (±14)	83 (±4)	79 (±3)	6.0	78 (±11)	84 (±10)	83 (±6)	6.2	74 (±7)	78 (±7)	90 (±4)	6.4
20.	Fipronil	100 (±16)	106 (±11)	108 (±8)	6.1	73 (±8)	73 (±11)	78 (±6)	10.0	99 (±7)	94 (±8)	96 (±6)	9.0
21.	Folpet		73 (±12)	75 (±9)	19.3		76 (±13)	80 (±8)	15.0		80 (±11)	76 (±5)	17.0
22.	*cis*-chlordane	100 (±4)	98 (±7)	104 (±3)	6.5	89 (±11)	78 (±4)	88 (±7)	6.0	82 (±5)	86 (±10)	94 (±5)	6.5
23.	2,4-DDE	99 (±10)	101 (±3)	101 (±2)	5.0	82 (±4)	77 (±4)	85 (±5)	6.5	88 (±6)	80 (±8)	93 (±3)	5.4
24.	*trans*-Chlordane	85 (±12)	100 (±5)	104 (±3)	5.1	78 (±7)	78 (±8)	85 (±9)	5.5	86 (±9)	84 (±10)	90 (±4)	5.0
25.	α-Endosulfan	82 (±12)	103 (±7)	110 (±8)	6.5	76 (±15)	70 (±8)	78 (±9)	7.4	110 (±16)	93 (±10)	81 (±7)	6.4
26.	Profenophos	90 (±10)	95 (±6)	84 (±4)	5.0	79 (±5)	74 (±7)	84 (±4)	5.0	79 (±12)	70 (±5)	70 (±7)	5.2
27.	Isoprothiolane	75 (±14)	93 (±8)	89 (±9)	7.2	76 (±15)	70 (±8)	78 (±9)	10.0	72 (±5)	77 (±6)	78 (±3)	7.5
28.	4,4′-DDE	99 (±6)	99 (±4)	98 (±4)	9.5	77 (±8)	70 (±7)	75 (±7)	10.0	85 (±10)	78 (±10)	93 (±6)	6.5
29.	Dieldrin	100 (±10)	101 (±12)	111 (±7)	5.7	73 (±14)	94 (±10)	86 (±8)	8.5	87 (±8)	85 (±2)	89 (±2	8.5
30.	Buprofezin	117 (±8)	113 (±7)	106 (±3)	5.3	70 (±4)	76 (±4)	84 (±1)	6.3	76 (±12)	82 (±8)	91 (±10)	5.0
31.	Oxyfluorfen	86 (±15)	93 (±5)	100 (±5)	6.5	75 (±5)	71 (±9)	93 (±4)	6.2	98 (±20)	78 (±18)	97 (±10)	7.3
32.	2,4-DDT	106 (±6)	108 (±9)	107 (±4)	5.2	70 (±17)	74 (±5)	70 (±7)	8.3	83 (±9)	73 (±3)	79 (±3)	7.0
33.	Flusilazole	95 (±13)	95 (±9)	103 (±7)	7.5	75 (±10)	81 (±9)	85 (±7)	6.8	77 (±13)	76 (±11)	85 (±5)	5.5
34.	Endrin	74 (±5)	105 (±16)	109 (±7)	9.5	72 (±19)	73 (±6)	83 (±5)	9.6	83 (±9)	73 (±3)	79 (±3)	8.5
35.	β-Endosulfan	82 (±12)	110 (±12)	109 (±8)	5.2	75 (±15)	70 (±14)	87 (±9)	5.8	94 (±14)	76 (±3)	84 (±1)	6.2
36.	4,4-DDT	94 (±15.6)	101 (±2.0)	89 (±0.3)	5.0	70 (±8)	71 (±7)	79 (±5)	5.4	76 (±11)	90 (±10)	102 (±6)	5.0
37.	Endosulfan-sulfate	91 (±11)	88 (±13)	92 (±6)	6.6	77 (±13)	91 (±11)	87 (±10)	7.8	89 (±9)	82 (±6)	94 (±7)	6.5
38.	Captafol		71 (±13)	75 (±8)	18.9		76 (±12)	79 (±10)	20		70 (±16)	78 (±6)	18.5
39.	Dicofol	81 (±12)	78 (±4)	80 (±4)	7.3	91 (±8)	96 (±7)	97 (±8)	6.0	72 (±10)	75 (±12)	90 (±6)	8.5
40.	Iprodione	88 (±9)	88 (±8)	85 (±7)	6.5	88 (±14)	97 (±7)	90 (±4)	6.7	95 (±6)	74 (±7)	98 (±4)	6.5
41.	Phosalone	70 (±14)	74 (±5)	77 (±4)	5.5	101 (±6)	87 (±4)	109 (±5)	5.6	71 (±4)	74 (±12)	98 (±5)	6.2
42.	λ-cyhalothrin	81 (±13)	96 (±4)	97 (±3)	8.2	79 (±12)	81 (±8)	84 (±5)	9.4	95 (±10)	91 (±3)	94 (±4)	8.4
43.	Permethrin I	71 (±12)	78 (±3)	81 (±2)	5.8	70 (±11)	74 (±5)	85 (±2)	8.6	80 (±11)	82 (±5)	85 (±2)	7.6
44.	Permethrin II	86 (±16)	88 (±9)	85 (±5)	5.6	95 (±17)	99 (±5)	102 (±3)	8.4	98 (±17)	95 (±5)	99 (±3)	8.4
45.	Cyfluthrin	72 (±14)	76 (±13)	80 (±8)	9.3	75 (±16)	73 (±12)	77 (±10)	8.4	70 (±12)	78 (±10)	77 (±9)	8.4
46.	Cypermethrin	80 (±16)	98 (±12)	102 (±8)	7.5	90 (±18)	96 (±15)	99 (±12)	6.9	90 (±18)	88 (±15)	82 (±11)	8.9
47.	Ethofenprox	85 (±8)	90 (±4)	87 (±3)	5.6	92 (±9)	92 (±7)	97 (±4)	5.2	88 (±8)	80 (±7)	85 (±5)	5.4
48.	Fenvalerate	99 (±15)	96 (±11)	102 (±6)	9.2	96 (±11)	93 (±9)	105 (±3)	8.8	89 (±11)	86 (±9)	99 (±3)	8.8
49.	Esfenvalerate	89 (±12)	88 (±10)	98 (±6)	9.4	99 (±15)	98 (±12)	95 (±8)	8.2	99 (±15)	98 (±12)	95 (±8)	8.2
50.	Deltamethrin	75 (±16)	78 (±9)	80 (±8)	8.5	72 (±18)	75 (±9)	80 (±6)	8.3	75 (±17)	74 (±11)	81 (±8)	9.3

enhancement in S/N at 20 ng g^{-1} was recorded for all compounds except for phosalone where S/N was suppressed by 46(±6) %. For other pesticides such as isoprothiolane (+38%), buprofezin (+45%), and flusilazole (+20%), enhancement in S/N was evident in pomegranate. In mango, at 20 ng g^{-1}, an overall enhancement in S/N was observed for a majority of the compounds, except for dicofol where S/N was suppressed by around 24(±2) %. The matrix induced signal enhancements were within 30−40% for chlorothalonil, chlorpyriphos methyl, and flusilazole; within 50−60% for chlorfenvinphos and profenophos; 60−70% for parathion methyl and parathion ethyl; and 75% for dichlorvos at 20 ng g^{-1}. The matrix effects at 10 ng g^{-1}were statistically on par to that at 20 ng g^{-1}, while at higher concentration levels, e.g., 50 ng g^{-1}, the matrix effects were comparatively less.

The differences in matrix effects among the test commodities could be due to the different biochemical compositions and influence of coextracted matrix-selective compounds on the ionization process of the test analytes.

Method Performance/Fitness for Purpose. The method worked well in estimating the test compounds in all three matrixes. The MS/MS method could resolve the problem of coelution of the compounds that are difficult to separate chromatographically (**Figure 1**) in full scan mode. Linearity of the calibration curve was established for all of the pesticides with the correlation coefficient (R^2) > 0.99 for solvent as well as matrix standards in all of the

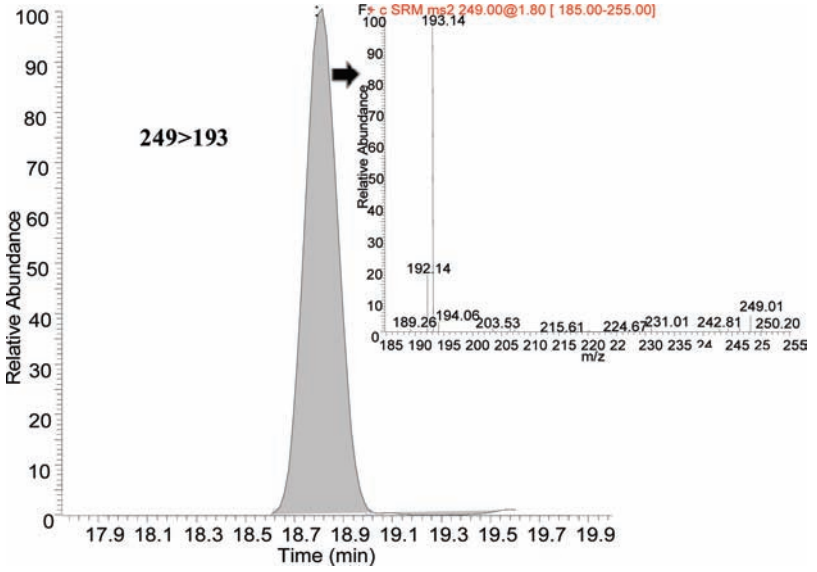

Figure 2. Identification of buprofezin residues in incurred grape sample at 0.03 mg kg^{-1}.

three commodities tested. The LOQ of all of the compounds (**Table 2**) were lower than the harmonized MRL of the European Union (*18*). The recovery of all of the compounds was found to be 70−120% with the RSDs below 20% for all of the three matrixes tested (**Table 2**).

Internal Quality Criteria. In order to ensure quality results in routine analysis, several quality control criteria were implemented. Analysis of the blank sample extract ensured minimization of false positives. A typical chromatographic batch started with duplicate injections of solvent blank and matrix blank. Then matrix-matched calibration standards (five levels) were run, which was followed by injection of a recovery sample (pre-extraction spike at 20 ng g^{-1}) and a solvent blank. The samples were analyzed in five consecutive injections, followed by one injection of pre-extraction spike at 20 ng g^{-1}, solvent blank, and then next five samples. The blank samples spiked at concentration within linearity range ensured the extraction efficiency, which was accepted within 70−120% recoveries. All of the samples with positive detections were reanalyzed by injecting twice repetitively to avoid any false detection.

Evaluation of the Method for Screening Farm and Incurred Samples. In two grape samples, the residues of the fungicide, viz., flusilazole, and insecticide, viz., buprofezin, were detected at 0.02 and 0.03 mg kg^{-1} levels, respectively, which were much below their EU-MRL of 0.05 and 1.0 mg kg^{-1}, respectively (*18*). In one pomegranate sample, chlorpyriphos (0.02 mg kg^{-1}) was detected, which was less than the EU-MRL of 0.05 mg kg^{-1}.

The detections of the above pesticides were confirmed on the basis of their qualifier to target MRM ratio within 20% tolerance range of the corresponding matrix-matched standard (*15*). The same positive samples when analyzed in GC-MS at full scan mode could only provide qualitative detection (although with uncertainty) with matching to the NIST mass spectral library to the extent of only 60%. In full scan mode, quantification was not possible because of poor peak shape and uncertain peak area. This establishes superior selectivity and sensitivity of target-oriented tandem mass spectrometry in analyzing agricultural samples for pesticide residues at trace level. All of the mango samples were free from the residues of the target compounds.

We identified buprofezin residues in one incurred grape sample at the concentration of 0.03 ± 0.005 mg kg^{-1} (**Figure 2**). In the incurred samples of pomegranate and mango, no pesticides were detected.

The optimized GC-MS/MS method reported here is proven to be efficient as well as robust and has the potentiality for routine application in monitoring the MRL compliance of a wide range of commodity−pesticide combinations. Use of 10 m columns could save 12.2 min of chromatographic run time in comparison to the 44 min run time required for the 30 m columns. Thus, in a 24 h time cycle, a commercial laboratory can accommodate at least 13 more injections into GC-MS that in turn increased the laboratory output significantly. The different extents of the matrix effect in different fruits calls for future investigations on the influence of specific biochemical constituents on the ionization and mass fragmentation of target pesticides.

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