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ARTICLE

Multiresidue Determination and Uncertainty Analysis of 87 Pesticides in Mango by Liquid Chromatography−Tandem Mass Spectrometry

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A liquid chromatography−tandem mass spectrometry (LC-MS/MS)-based method was optimized and validated for the multiresidue analysis of 87 pesticides in mango at the ≤10 ng g^{-1} level. The method involves extraction of 10 g of homogenized mango samples (+10 mL of water + 1 g of sodium acetate + 10 g of sodium sulfate) with 10 mL of ethyl acetate; cleanup by dispersive solid-phase extraction with a combination of primary secondary amine (PSA, 50 mg), graphitized carbon black (GCB, 25 mg), and anhydrous sodium sulfate (150 mg); and final estimation by LC-MS/MS with multiple reaction monitoring. Direct analysis (no clean up) resulted in significant suppression in ionization of the majority of the test compounds over the electrospray ionization probe. However, clean up with the above combination of PSA + GCB reduced the matrix-induced signal suppressions significantly, and the signals in the cleaned extracts were nearly equivalent to the corresponding solvent standards. Substitution of PSA with florisil also gave equivalent clean up effects. The method was quite rugged as evident from a low Horwitz ratio (mostly <0.5) and low measurement uncertainties at 10 ng g^{-1}. The limit of quantification was <10 ng g^{-1} for all of the pesticides with recoveries within 70−120% for most pesticides even at 2.5 ng g^{-1}. The method offers a significantly effective, sensitive, cheaper, and safer alternative to the existing methods of multiresidue analysis.

KEYWORDS: Multiresidue analysis of pesticides; mango; liquid chromatography−tandem mass spectrometry (LC-MS/MS); validation; uncertainty analysis

INTRODUCTION

Mango is an important tropical fruit crop, consumed both as fresh fruit and after processing. The commercial cultivation of mango receives frequent application of a variety of contact and systemic pesticides throughout the cropping season. In India, at present, 54 pesticides are regularly monitored in exportable mangoes (*1*), which contain both the recommended chemicals as well as those chemicals the residues of which may appear from indirect sources (e.g., soil, contaminated agro-inputs, drift from adjoining crop fields, etc). This list of pesticides for monitoring is expanding with the introduction of new and safer pesticides for pest and disease management and that is the reason why a comprehensive residue monitoring program requires monitoring of as many pesticides as possible.

When the Government of India initiated the export of mangoes to Japan in 2007, we found the absence of an official multiresidue method for pesticide analysis in mango. Hence, the Indian residue testing laboratories had to initially adopt the official Japanese method (*2*) to comply with the statutory import requirements of Japan, despite the time-consuming and complicated nature of the method. In our earlier efforts, we modified the ethyl acetate-based multiresidue method of Mol et al. (*3*) and reported single laboratory validation (SLV) in different fruits like grape (*4*), pomegranate, apple, and orange (*5*). In continuation of these endeavors, being the National Referral Laboratory (NRL), we took up the need-based project to develop a simple multiresidue analysis method for mango with due consideration to its unique and typical nature of the matrix. Our strategic endeavor was initiated with an ethyl acetate extraction followed by analysis on liquid chromatography−tandem mass spectrometry (LC-MS/MS) after solvent exchange to methanol−water without any cleanup. However, this resulted in a high degree of matrix-induced signal suppressions for the majority of the test compounds over the ESI (electrospray ionization) probe of the LC-MS/MS, and there were several interfering coeluting signals from the matrix, rendering the residue monitoring ambiguous and uncertain. The high degree of matrix influence could possibly be attributed to rich contents of carotenoid pigments (*6*, *7*) in mango (up to 130 μg g^{-1} in Alphonso variety), which are fat-soluble compounds and thus gets partially coextracted in ethyl acetate. Besides, mango is rich in sugar and also contains a variety of saturated, monounsaturated, and polyunsaturated fatty acids, which may interfere in LC-MS/MS analysis if coextracted and

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coeluted. The matrix influence remained similar when we applied the validated method of Mol et al. (*3*) or the acetonitrile-based AOAC-approved QuEChERS (quick, easy, cheap, effective, rugged, and safe) technique of Lehotay (*8*). Thus, we understood the need of giving special attention to improve the sample preparation technique for mango with special reference to cleanup for removal of coextractives before injection into LC-MS/MS.

In this paper, we report the optimization and SLV of a simple multiresidue analysis method for mangoes based on ethyl acetate extraction, cleanup by dispersive solid-phase extraction (DSPE), and analysis by LC-MS/MS−multiple reaction monitoring (MRM). The performance of the method was evaluated against the Japanese technique (*2*), the method of Mol et al. (*3*), and Lehotay (*8*) with simultaneous assessment of global uncertainties at the lowest official MRL of 10 ng g^{-1}. Furthermore, the reproducibility of the method was assessed among six accredited laboratories of India through a small-scale interlaboratory proficiency test (PT) program.

MATERIALS AND METHODS

Selection of Pesticides and Matrix. A total of 87 pesticides were considered for this study, which includes 33 compounds out of the 54 regularly monitored chemicals. The rest of the 21 regularly monitored compounds could not be included in this study, as these are typically gas chromatography amenable compounds (e.g., DDT, aldrin, heptachlor, chlorpyriphos, permethrin, etc.) and will be reported separately. The list includes all of the pesticides, which may appear in mangoes through direct and indirect sources (**Table 1**). The new pesticides, which are currently under development, were also included. For method optimization, raw (green skin, at the onset of maturity) and ripe Alphonso mangoes (golden yellow skin) were collected from an organic farm, which did not receive any application of the test pesticides. Alphonso is the major commercial variety of India, which contributes more than 40% of the total mango export. The seeds of the mangoes, being nonedible, were removed and not considered for analysis.

Reagents and Materials. Certified reference standards of all of the test pesticides were of > 98% purity and purchased from the Ehrenstorfer GmbH (Augsburg, Germany). All of the solvents, namely, ethyl acetate, acetonitrile, methanol, and water, were of high-performance liquid chromatography (HPLC) grade and purchased from Merck India Ltd. The DSPE sorbents viz. primary secondary amine (PSA), florisil, octadecyl silane (C18), and graphitized carbon black (GCB) were received from United Chemical Technology (Bristol, PA). The other reagents, namely, formic acid, ammonium formate, sodium acetate, acetic acid, anhydrous sodium sulfate, magnesium sulfate, and sodium chloride, were of analytical reagent grade and purchased from the Merck India Ltd. Sodium and magnesium sulfate were activated by heating at 650 °C for 4 h before use and kept in desiccators.

Preparation of Standard Solutions. The stock solutions of the individual pesticide standards were prepared by accurately weighing 10 mg (±0.1 mg) of each analyte in volumetric flasks (certified "A" class) and dissolving in 10 mL of methanol. These were stored in dark vials in a refrigerator at 4 °C. An intermediate mixture of 10 mg L^{-1} was prepared by mixing the appropriate quantities of the individual stock solutions followed by requisite volume make up with methanol. A working standard mixture of 1 mg L^{-1} was prepared by diluting the intermediate stock standard solution, from which the calibration standards - (1−50 ng L^{-1}) were prepared by serial dilution with methanol−water (1:1, v/v).

Standardization of Sample Preparation Technique. *Sample Size for Extraction*. To decide the sample size for extraction, fresh fruits (2 kg, without stone) were treated with the pesticide mixture at 10 ng g^{-1} level. The fruits (with peel) were chopped into about 1 cm^2 size pieces and macerated thoroughly in a blender (Model GX_7, Bajaj India Limited). From this macerated mass, 200 g of sample was drawn in separate sets for fine crushing and homogenization (DIAX 900 homogenizer with 18F shaft; Heidolph, Germany). The extent of homogeneity was evaluated by analyzing 10 random portions (10 and 20 g drawn separately) of the homogenized mass by LC-MS/MS. The sets of data for 10 and 20 g over three different days were compiled and statistically evaluated by Student's *t* test. The relative standard deviations (RSDs) for each data set were further compared with the results obtained through the other validated methods (*2*, *3*, *8*).

Sample Preparation. The samples were extracted using ethyl acetate at a 1:1 ratio. Ten grams of homogenized sample was drawn in a 50 mL centrifuge tube, and to it 10 mL of water, 0.5−1 g of sodium acetate, and 10 g of sodium sulfate (anhydrous) were added and mixed thoroughly by vortexing for 1 min. (For a sample size of 20 g, the corresponding quantities of the solvents and reagents were doubled.) This mixture was homogenized for 2 min at 15000 rpm and then centrifuged at 3000 rpm for 5 min for phase separation. An aliquot of 4 mL was drawn from the upper ethyl acetate phase and placed in a 15 mL polypropylene vial for cleanup.

Cleanup. Different clean up strategies were tried, which included DSPE with PSA, C-18, florisil, GCB, and their combinations in different proportions in addition to 150 mg of anhydrous sodium sulfate. All of these chemicals were selected, keeping in view their specific target matrix components for removal from the extract. In each case, 4 mL of ethyl acetate extract was taken in a centrifuge tube containing a cleanup mixture comprised of 50 mg of PSA, 25 mg of GCB, and 150 mg of Na_2SO_4 and centrifuged at 10000 rpm. The cleaned extract (3 mL) was further drawn in a fresh test tube, and to it, 200 μL of 10% diethylene glycol (in methanol) was added (as keeper) and mixed thoroughly by vortexing. This mixture was subsequently evaporated to near dryness under a gentle stream of nitrogen in a low-volume concentrator (TurboVap LV; Caliper Life Sciences, Russelsheim, Germany) at 35 °C. The residues were dissolved in 1 mL of methanol + 1 mL of 0.1% acetic acid by vortexing (30 s), followed by sonication (1 min). This solution was analyzed by LC-MS/MS after filtering through a 0.2 μm polyvinylidene fluoride (PVDF) membrane filter.

Japanese Method. The official Japanese technique (*2*) was tested simultaneously for comparison. In brief, the method involved extraction of 20 g of sample (+ 20 mL of water, with standing for 15 min) with 50 mL of acetonitrile by homogenization. The sample residue was washed with 20 mL of acetonitrile twice and added to the extract. The volume of the combined extract was adjusted to 100 mL. From this, 20 mL of extract was drawn and cleaned by liquid−liquid partitioning after adding 10 g of sodium chloride and 20 mL of 0.5 mol/L phosphate buffer (pH 7.0). The aqueous layer was discarded, and the acetonitrile layer was dried through sodium sulfate and then evaporated to dryness at ≤35 °C. The residues were reconstituted in a 2 mL mixture of acetonitrile and toluene (3:1). The sample cleanup was performed on a preconditioned graphite carbon/aminopropylsilanized silica gel-layered minicolumn (500 mg/500 mg) and eluted with 20 mL of acetonitrile/toluene (3:1). The entire effluent was collected, evaporated to dryness at ≤35 °C, and redissolved in 4 mL of methanol to inject into the LC-MS/MS system.

Validated Method of Mol et al. (*3*). In brief, the method involved extraction of 25 g samples with 40 mL of ethyl acetate (+ 25 g of Na_2SO_4) after adding phosphate buffer (4 mol L^{-1}, 2 mL) of pH 7.0 by homogenization and centrifugation. The extract was directly analyzed by LC-MS/MS without any cleanup (*3*).

AOAC-Approved Lehotay's Method. The homogenized mango samples (15 g) were extracted with 15 mL of acetonitrile. Phase separation was accomplished by adding 6 g of anhydrous magnesium sulfate and 1.5 g of sodium acetate. The acetonitrile phase (1 mL of upper layer) was cleaned by DSPE with 50 mg of PSA + 150 mg of anhydrous magnesium sulfate followed by direct analysis by LC-MS/MS (*8*).

Determination. An Agilent 1200 series HPLC system hyphenated to an API 4000 Q-Trap (Applied Biosystems, MDS Sciex, Canada) mass spectrometer (MS) was used with Analyst software (version 1.4.2). The ESI interface was set at positive polarity. The HPLC separation was performed by injecting 10 μL through an autosampler on a Purosphere STAR RP-18e (150 mm × 4.6 mm × 5 μm, Merck, Germany) column maintained at 35 °C. The mobile phase was composed of (A) methanol/water (20:80, v/v) and (B) methanol/water (90:10, v/v) with both having 5 mM ammonium formate; flow rate, 1 mL min^{-1} with split; gradient: 0−1.0 min, 20% B; 1−8 min, 20−100% B; 8−15 min, 100% B; 15−16 min, 100−20% B; and 16−20 min, 20% B. The MS parameters included the

Table 1. Overview of the LC-MS/MS Multiresidue Monitoring of the Test Pesticides[a]

sr. no.	pesticide (class[b])	RT (min)	Q1	Q2	Q3	LOD (ng/g)	LOQ (ng/g)	R^2
1	acephate (I)	2.35	184	143	125	2.0	5.0	0.9979
2	acetamiprid (IV)	6.74	223	126	56	0.3	1.0	0.9993
3	alachlor (XIX)	11.21	270	238	147, 132, 162	1.0	2.5	0.9982
4	atrazine (VII)	10.16	216	174	104, 96	0.3	1.0	0.9992
5	azinphos-methyl (I)	10.49	318	160	132	1.0	2.5	0.9940
6	azoxystrobin (V)	10.24	404	372	344	0.1	0.25	0.9982
7	benalaxyl (VIII)	11.40	326	208	148	0.4	1	0.9996
8	bitertanol (II)	11.52	338	269	70	1.0	2.5	0.9985
9	buprofezin (XVII)	12.18	306	201	116	0.3	1.0	0.9998
10	butachlor (XIX)	12.37	312	238	162, 91	1.5	5.0	0.9992
11	carbendazim (XI)	8.17	192	160	132	0.75	2	0.9993
12	carbaryl (III)	9.44	202	145	127	0.3	1.0	0.9988
13	carbofuran (III)	8.95	222	165	123	0.3	1.0	0.9963
14	carbofuran-3-OH (III)	6.35	238	163	181, 107	0.5	1.5	0.9970
15	clothianidin (IV)	5.44	250	169	132	1.0	2.5	0.9992
16	cymoxanil (X)	7.35	199	111	128	1.0	2.5	0.9971
17	demeton-S-methyl (I)	9.18	231	89	155, 61	1.0	2.5	0.9968
18	demeton-S-methyl sulfone (I)	3.53	263	169	121	1.0	2.5	0.9984
19	diazinon (I)	11.58	305	169	153, 97	0.3	1.0	0.9997
20	dichlofluanid (XX)	11.00	333	224	123	1.5	5.0	0.9963
21	dichlorvos (I)	9.07	221	109	127	2.0	5.0	0.9898
22	difenoconazole (II)	11.70	406	337	251	0.3	1.0	0.9993
23	diflubenzuron (XXIII)	11.39	311	158	141	0.75	2.0	0.9985
24	dimethoate (I)	6.84	230	199	125	0.2	0.5	0.9981
25	dimethomorph (XV)	6.40	388	301	165	0.3	1.0	0.9993
26	disodium methylarsonate (DMSA) (XXI)	8.20	201	137	92	0.3	1.0	0.9947
27	diniconazole (II)	10.50	326	159	70	2.0	5.0	0.9982
28	emamectin benzoate (VI)	13.06	886.5	158	82.3	2.0	5.0	0.9992
29	ethion (I)	12.26	385	199	171	0.75	2.0	0.9991
30	etrimfos (I)	11.64	293	125	265, 79	1.0	2.5	0.9993
31	famoxadone (XXIV)	11.28	392	311	238, 93	2	5.0	0.9889
32	fenamidone (XIII)	10.42	312	236	92	0.3	1.0	0.9991
33	fenarimol (II)	11.02	331	268	81	1.0	2.5	0.9968
34	fenobucarb (III)	10.40	208	95	152	0.3	1.0	0.9981
35	fenpyroximate (XIV)	13.10	422	366	135, 138	0.3	1.0	0.9992
36	fenthion (I)	11.63	279	247	169, 105	1.0	2.5	0.9972
37	flufenoxuron (XVII)	12.31	489	158	141, 113	2.0	5.0	0.9881
38	flusilazole (II)	11.08	316	165	247	0.7	2.0	0.9993
39	forchlorfenuron (IX)	10.22	248	129	155	1	2.5	0.9976
40	hexaconazole (II)	11.58	314	70	159	1.0	2.5	0.9979
41	imazalil (XIII)	11.48	297	159	201	1.0	2.5	0.9991
42	imidacloprid (IV)	5.69	256	209	175	1.0	2.5	0.9988
43	indoxacarb (III)	11.45	528	203	249, 56	2.0	5.0	0.9982
44	iprovalicarb (III)	10.80	321	203	186, 119	0.3	1.0	0.9992
45	isoprothiolane (XII)	10.64	291	231	189, 145	0.3	1.0	0.9992
46	isoproturon (IX)	10.00	207	72	165	0.3	1.0	0.9997
47	iprobenfos /kitazin (I)	11.23	289	91	205	0.3	1.0	0.9968
48	kresoxim methyl (V)	11.26	314	267	206, 116	2	5.0	0.9917
49	malathion (I)	10.71	331	127	285, 99	0.3	1.0	0.9994
50	malaoxon (I)	8.87	315	127	99	0.3	1.0	0.9959
51	mandipropamid (XVI)	10.32	412	328	356, 125	0.3	1.0	0.9985
52	metalaxyl (VIII)	9.84	280	192	220, 160	0.3	1.0	0.9987
53	methamidophos (I)	2.25	142	94	125	2.0	5.0	0.9956
54	methidathion (I)	10.35	303	145	85	0.3	1.0	0.9988
55	methomyl (III)	3.76	163	106	88	1.0	2.5	0.9982
56	metribuzin (XXII)	9.14	215	187	84	2.0	5.0	0.9888
57	mevinphos (I)	6.40	225	193	127	1.0	2.5	0.9986
58	monocrotophos (I)	4.04	224	127	98	0.3	1.0	0.9983
59	myclobutanil (II)	10.70	289	70	125	1.0	2.5	0.9985
60	omethoate (I)	2.45	214	125	109, 183	1.0	2.5	0.9979
61	oxydemeton methyl (I)	3.10	247	169	229, 109	0.3	1.0	0.9969
62	paraxon methyl (I)	8.40	248	202	231, 127	1.0	2.5	0.9910
63	penconazole (II)	11.42	284	159	70	1.0	2.5	0.9984
64	pendimethalin (XXV)	12.88	282	212	194, 71	2.0	5.0	0.9938
65	phenthoate (I)	11.12	321	163	275, 247	0.3	1.0	0.9990
66	phosalone (I)	11.59	368	182	138, 111	1.0	2.5	0.9993
67	phosmet (I)	10.49	318	160	77	1.0	2.5	0.9940
68	phosphamidon (I)	8.11	300	174	127	0.75	2.0	0.9903

Table 1. Continued

sr. no.	pesticide (class[b])	RT (min)	Q1	Q2	Q3	LOD (ng/g)	LOQ (ng/g)	R^2
69	profenophos (I)	12.15	373	303	344, 207	0.75	2.0	0.9990
70	propargite (XVIII)	12.47	368	231	175	0.4	1.5	0.9993
71	propiconazole (II)	11.47	342	159	69	1.0	2.5	0.9966
72	pyraclostrobin (V)	11.54	388	194	163, 296	0.1	0.3	0.9997
73	quinalphos (I)	11.63	299	147	163, 243	0.3	1.0	0.9996
74	simazine (VII)	9.34	202	132	124, 96	1.0	2.5	0.9966
75	spinosyn A (VI)	13.99	732	142	99	2.0	5.0	0.9960
76	spinosyn D (VI)	14.10	746	142	99	2.0	5.0	0.9988
77	tebuconazole (II)	11.34	308	70	125	1.0	2.5	0.9981
78	temefos (I)	12.07	467	419	341, 125	2.0	5.0	0.9993
79	tetraconazole (II)	10.82	372	70	169	1.0	2.5	0.9970
80	thiamethoxam (IV)	4.00	292	211	132	1.0	2.5	0.9980
81	thiacloprid (IV)	7.52	253	126	186	0.3	1.0	0.9998
82	thiodicarb (II)	9.55	355	88	193, 163	0.3	1.0	0.9987
83	thiometon (I)	9.87	247	89	61	3.3	10.0	0.9896
84	triazophos (I)	10.90	314	162	119	0.2	0.5	0.9998
85	triadimefon (II)	10.75	294	197	69	0.3	1.0	0.9990
86	triadimenol (II)	10.83	296	70	227	1.0	2.5	0.9991
87	trifloxystrobin (V)	11.63	409	186	206, 116	0.1	0.25	0.9999
88	triphenyl phosphate (IS) (I)		327	215	152, 77, 51			0.9963

[a] RT, retention time (min); Q, $[M + H]^+$; Q2, quantifier daughter ion; and Q3, qualifier daughter ion. [b] Pesticide class designations: I, organophosphorus; II, triazole; III, carbamate; IV, neonicotinoid; V, strobilurin; VI, macrocyclic lactone; VII, triazine; VIII, acylamino acid; IX, urea; X, aliphatic nitrogen; XI, benzimidazole; XII, dithiolane; XIII, imidazole; XIV, pyrazole; XV, morpholine; XVI, amide; XVII, chitin synthesis inhibitor; XVIII, sulfite ester; XIX, chloroacetanilide; XX, sulfamide; XXI, arsenical; XXII, triazinone; XXIII, benzoylphenylurea; XXIV, dicarboximide; and XXV, dinitroaniline.

following: ion spray voltage, 5500 V; nebulizer gas, 30 psi; curtain gas, 25 psi; heater gas, 60 psi; and ion source temperature, 450 °C.

During optimization of the MS method, the target ion with the highest relative intensity in full scan was initially selected, and its fragmentation was done with the help of collision energy in the form of nitrogen gas. The most abundant and stable fragment ion was selected for the quantification, whereas the next abundant ion(s) were used for the confirmation. For each ion, different voltages were applied to achieve the highest stable signal. Analysis was done in MRM mode. For unknown samples, the ratio of the quantifier and confirmatory MRMs was used for unambiguous identification of residues within the ±10% tolerance range. The MRM transitions are presented in **Table 1**.

The LCMS/MS analysis gave satisfactory performance for all 87 pesticides in terms of peak shape, linearity, and sensitivity. Matrix-matched calibration was used for quantification of the residues to avoid over- or underestimations. Furthermore, to minimize errors in estimation, triphenylphosphate was used as an internal standard (IS) at a concentration of 10 ng mL^{-1} of methanol.

To account the cleanup effect, β-carotene was estimated (*9*) by HPLC-UV on a C30 column (*10*) (250 mm × 4.6 mm, 5 μm). The mobile phase was constituted of methanol (A)−MTBE (methyl *tert*-butyl ether) with a gradient programming of 0−3 min, 90% A; 3−8 min, 90−5% A; 8−10 min, 5% A; 10−12 min, 5−90% A; and 12−18 min, 90% A, with a flow rate of 1 mL min^{-1}. Quantification was done at 452 nm with the signal at a retention time (RT) = 9.75 min.

Method Performance. The analytical method was validated as per the SLV approach (*11*, *12*). The performance of the method was evaluated considering different validation parameters that include the following items.

Calibration Range. The calibration curves for all of the compounds in pure solvent and matrix were obtained by plotting the peak area against the concentration of the corresponding calibration standards at five calibration levels ranging between 1 and 50 ng g^{-1}.

Sensitivity. The limit of detection (LOD) was determined by considering a signal-to-noise ratio of 3 with reference to the background noise obtained from blank sample, whereas the limits of quantification (LOQ) were determined by considering a signal-to-noise ratio of 10.

Precision. The precision in the conditions of repeatability (three different analysts prepared six samples each on a single day) and intermediate precision (three different analysts prepared six samples each on six different days) were determined separately for a standard concentration of 10 ng g^{-1} of all of the analytes. The Horwitz ratio (HorRat) pertaining to intralaboratory precision, which indicates the acceptability of a method with respect to precision (*13*), was calculated for all of the pesticides in the following way:

$$\text{HorRat} = \text{RSD}/\text{PRSD} \quad (1)$$

where PRSD is the predicted RSD = $2C^{-0.15}$ and where C is the concentration expressed as a mass fraction (10 ng g^{-1} = 10×10^{-9}).

Accuracy−Recovery Experiments. Organically grown mangoes (which did not receive any treatment of the test pesticides) were used as blanks. The recovery experiments were carried out on fresh untreated mangoes by fortifying the samples (10 g) in six replicates with a pesticide mixture separately at four concentration levels, that is, 2.5, 5, 10, and 25 ng g^{-1}. The recoveries [recovery (%) = (peak area of pre-extraction spike × 100/peak area of post extraction spike)] obtained were compared with the results of the Japanese method at the 10 ng g^{-1} level, which is the lowest MRL as per the European Commission guidelines (*14*).

Matrix Effect. The matrix effect was assessed by employing matrix-matched standards prepared in a similar fashion as for solvent standards using a matrix extract of the untreated mangoes. The matrix effect (ME %) was evaluated by following equation: ME % = (peak area of post extraction spike × 100/peak area of solvent standard).

Measurement Uncertainty. Global uncertainty was determined for all of the pesticides at the level of 10 ng g^{-1} as per the statistical procedure of the EURACHEM/CITAC Guide CG 4 (*15*). Five individual sources of uncertainty were taken into account viz. uncertainty associated with the calibration graph ($U1$), day-wise uncertainty associated with precision ($U2$), analyst-wise uncertainty associated with precision ($U3$), day-wise uncertainty associated with accuracy/bias ($U4$), and analyst-wise uncertainty associated with accuracy/bias ($U5$). The global uncertainty (U) was calculated as $U = (U_1^2 + U_2^2 + U_3^2 + U_4^2 + U_5^2)^{1/2}$ and was reported as expanded uncertainty, which is twice the value of the global uncertainty.

The uncertainty values for each pesticide are reported as relative uncertainties in **Table 3**. Here, $U_1 = (s/b_1)[(1/p) + (1/n) + \{(c_0 - \acute{c})^2/s_{xx}\}]^{1/2}$, where s is the standard deviation of the

residuals of the calibration curve, b_1 is the slope of the calibration curve, p is the number of measurements of the unknown, n is the number of points used to form the calibration curve, c_0 is the calculated concentration of the analyte from the calibration curve, $\acute{c}$ is the average of all of the standards used to make the calibration curve, and s_{xx} is calculated as follows: $s_{xx} = \sum(c_j - \acute{c})^2$, where $j = 1, 2, ..., n$. c_j is the concentration of each calibration standard used to build up the calibration curve. $U_2 = s_1/n^{1/2}$, where s_1 is the standard deviation of the results obtained from a single analyst on different days and n is the number of assays. $U_3 = s_2/n^{1/2}$, where s_2 is the standard deviation of the results obtained from different analysts on a particular day and n is the number of assays. $U_4 = s_1(\eta)/n^{1/2}$, where $s_1(\eta)$ is the standard deviation of the percentage recoveries obtained from a single analyst on different days and n is the number of assays. $U_5 = s_2(\eta)/n^{1/2}$, where $s_2(\eta)$ is the standard deviation of the percentage recoveries obtained from different analysts on a particular day and n is the number of assays.

Interlaboratory Method Validation. After the SLV was accomplished, the method was subjected to a small-scale interlaboratory comparison to assess reproducibility of the method. Six commercial testing laboratories of India viz. Vimta Laboratories Ltd. (Hyderabad), SGS India Ltd. (Chennai), Reliable Analytical Laboratories (Mumbai), Geochem Laboratory (Mumbai), Shriram Institute of Industrial Research (Bangalore), and Insecticide Residue Testing Laboratory (Pune) participated. All of these laboratories are accredited to the quality system as per the ISO/IEC 17025:2005 (*16*) and other national certifying bodies. The laboratories were provided with the mango matrix, reference standard solutions, and the standard operating procedure to complete the validation process in all respects within 1 month of time. We scrutinized their validation records thoroughly, and once those were found to be satisfactory, the PT round was organized among them. For the PT, the laboratories were provided with the working standard solutions for solvent-based and matrix-matched calibrations. A blank and a fortified mango sample were supplied to all of the laboratories in a common meeting. The fortified unknown samples were spiked with six pesticides, the identity of which was kept undisclosed to the laboratories. The laboratories were asked to analyze the PT sample in three replicates to identify and quantify the fortified compounds and submit the results within the next 4 days, along with the relevant documents. The results were compiled, and Z scores were calculated using the formula Z = [(reported result − assigned value)/standard deviation]. The assigned value of each pesticide was the average of all of the laboratory results specific to each pesticide (n = 18; six laboratories × three replicates).

RESULTS AND DISCUSSION

Standardization of Sample Preparation. Cutting the mangoes into small pieces of about 1 cm^2 area improved the extent of homogenization as observed from less than 5% RSD for all of the test compounds in the replicated study. It also improved the recovery as a result of the increase in surface contact between the sample and the solvent during extraction. An addition of water to the mango homogenate (especially for raw mangoes) before extraction improved the phase separation, and recovery of the ethyl acetate phase also increased from around 6 (without water addition) to 9 mL when 10 mL of solvent was used for extraction. Sodium acetate was added to adjust the pH of mango matrix at a range optimum for the stability of the test analytes. At the onset of maturity, the pH of the raw mangoes was in the range of 2.5 to 2.8, which increased to around 4.5 on the addition of 1 g of sodium acetate. For moderately mature mangoes, the addition of 0.5 g of sodium acetate was adequate to adjust the pH at the desired level of 4.5−5. The addition of sodium acetate improved the stability of most of the compounds during sample preparation and while waiting in the autosampler before injection.

A second step homogenization of 200 g of subsample improved the precision of analysis. We compared the effect of two-step homogenization with one-step homogenization (10 g samples were directly drawn after homogenizing 2 kg of mango sample and then analyzed in 10 replicates). The two-step homogenization improved the overall precision of analysis with RSD of < 5%, whereas the one-step homogenization resulted > 12% RSD at the 10 ng g^{-1} residue level.

The sample size of 10 and 20 g gave equivalent results, and the recoveries for the sample−solvent ratio of 1:1 and 1:2 were statistically similar. Thus, we decided the smaller sample size to minimize the solvent usage and in this way, the required sample and solvent quantities could be significantly reduced when compared to the Japanese method (*2*) as well as the method reported by Mol et al. (*3*).

Evaluation of Matrix Influence. The matrix-induced suppression in target signals was prominent for a large number of pesticides, which possibly occurred as a result of suppressions in the ionization process at the ESI probe due to coeluted matrix compounds. The slopes of the matrix-matched calibration equations were significantly different to pure solvent-based calibrations at a 95% level of statistical confidence. An overall signal suppression by 25−80% was observed for most of the compounds. For certain pesticides, the interfering matrix compounds with the same MRM transitions were observed that establish the need of chromatographic separation during LC-MS/MS analysis. For example, in the case of diazinon, there was 50% suppression in signal height and also there were coeluting matrix components of the matching MRMs of 305.0/153.0 and 305.0/97.0, which were suppressing the diazinon signal significantly. However, on cleanup with 50 mg of PSA + 25 mg of GCB + 150 mg of Na_2SO_4, the coeluent could be removed significantly with a concomitant increase in the signal-to-noise ratio (S/N) of diazinon by around 2 times at the 10 ng g^{-1} level. Similarly, for dimethoate (230.0/199.0), acephate (184.0/143.0), omethoate (214.0/125.0), and quinalphos (299.0/147.0), the S/N at 10 ng g^{-1} enhanced significantly as a result of cleanup with PSA + GCB and became equivalent to the S/N of the corresponding solvent standards. A similar cleanup effect was observed with florisil (50 mg) + GCB (25 mg).

A strong coelution of a matrix compound resulted in a broad peak of the quantifier MRM of demeton-S-methyl sulfone (263.0/169.0) with around a 2 min peak width, resulting in bad peak shape with poor calibration. The use of DSPE with PSA (or florisil) + GCB (50 + 25 mg) improved the peak shape and reduced the peak width to less than 1 min as a result of removal of the coeluting interfering compound. An increase in the quantities of PSA or florisil and GCB or changing their proportion did not improve the extent of cleanup and instead resulted in adsorption loss of residues. The use of 25 mg of GCB was optimum to remove the color of the matrix, but an excess quantity (> 25 mg for 4 mL of ethyl acetate extract) affected the recovery of several pesticides viz. carbendazim, phosalone, paraoxon-methyl, diflubenzuron, forchlorfenuron, imazalil, emamectin benzoate, and spinosad due to surface adsorption on GCB. The comparative effect of different cleanup strategies is presented in **Figure 1** for selected pesticides.

For oxydemeton-methyl, cleanup with PSA + C18 (50 mg each) increased the peak area and S/N by more than two times as compared to the uncleaned extract, but the peak area decreased

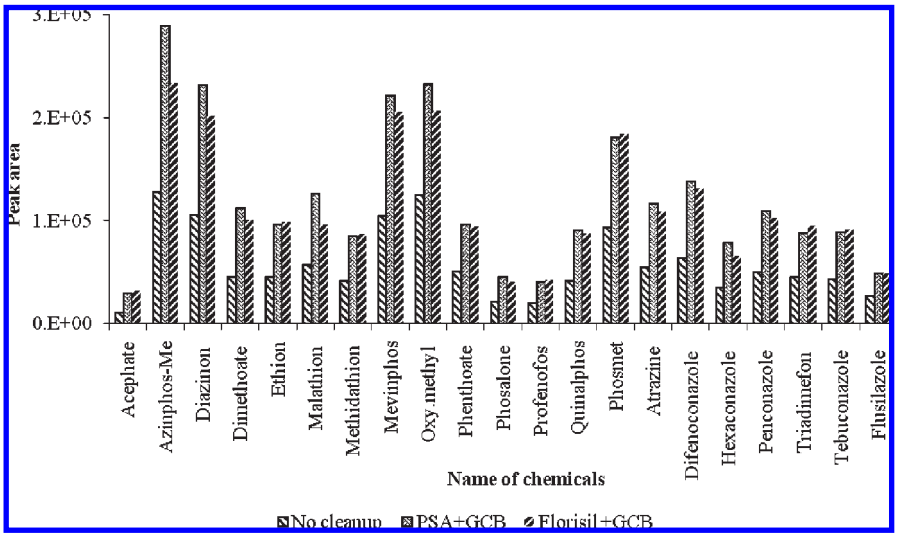

Figure 1. Effect of different cleanup strategies on the peak area of selected pesticides at 10 ng g^{-1}.

Table 2. Mean Recovery (±RSD), HorRat, and Matrix Effect of the Test Pesticides

		recovery % (mean ± RSD)					
sr. no.	pesticide name	I[a]	II[a]	III[a]	IV[a]	HorRat (10 ng g^{-1})	ME (%)[b]
1	acephate	ND[c]	82 (±24)	78 (±10)	78 (9)	0.32	−76.8
2	acetamiprid	86 (±9)	91 (±7)	87 (±7)	85 (±4)	0.22	−4.3
3	alachlor	88 (±18)	98 (±11)	94 (±5)	98 (±7)	0.16	−38
4	atrazine	80 (±13)	96 (±6)	89 (±7)	91 (±6)	0.22	−38
5	azinphos-methyl	83 (±29)	87 (±21)	84 (±12)	88 (±9)	0.38	−15.1
6	azoxystrobin	93 (±26)	85 (±8)	80 (±5)	75 (±7)	0.16	−12.6
7	benalaxyl	91 (±5)	84 (±7)	79 (±6)	73 (±6)	0.19	−32.4
8	bitertanol	78 (±17)	76 (±19)	71 (±9)	77 (±10)	0.28	−18.5
9	buprofezin	73 (±12)	70 (±6)	78 (±11)	75 (±9)	0.35	−7.8
10	butachlor	69 (±23)	63 (±20)	73 (±12)	72 (±10)	0.38	−51
11	carbaryl	96 (±11)	97 (±9)	93 (±5)	93 (±4)	0.16	−61.1
12	carbendazim	71 (±16)	85 (±10)	81 (±6)	83 (±5)	0.19	−14.7
13	carbofuran	95 (±12)	117 (±17)	90 (±6)	91 (±5)	0.19	−9.6
14	carbofuran-3-OH	104 (±12)	98 (±9)	89 (±6)	87 (±8)	0.19	−8.9
15	clothianidin	81 (±32)	95 (±17)	85 (±9)	81 (±8)	0.28	−30
16	cymoxanil	94 (±20)	105 (±13)	102 (±8)	91 (±7)	0.25	−21.3
17	demeton-S-methyl	128 (±58)	118 (±35)	116 (±18)	96 (±12)	0.57	−33.7
18	demeton-S-methyl sulfone	90 (±7)	89 (±7)	87 (±7)	86 (±8)	0.22	−25.4
19	diazinon	81 (±13)	81 (±9)	83 (±8)	88 (±5)	0.25	−28.5
20	dichlofluanid	81 (±43)	92 (±20)	88 (±11)	89 (±10)	0.35	−36.4
21	dichlorvos	42 (±84)	45 (±51)	53 (±28)	52 (25)	0.88	−29.1
22	difenconazole	72 (±15)	74 (±11)	71 (±6)	76 (±7)	0.19	−28.9
23	diflubenzuron	78 (±20)	85 (±15)	92 (±14)	98 (±10)	0.44	−45
24	dimethoate	89 (±11)	85 (±9)	86 (±8)	90 (±4)	0.25	−10.3
25	dimethomorph	81 (±9)	86 (±7)	77 (±7)	83 (±6)	0.22	−13.9
26	diniconazole	91 (±19)	84 (±16)	76 (±12)	79 (±9)	0.38	−34.6
27	DMSA	81 (±13)	80 (±8)	85 (±7)	80 (±7)	0.22	−26.4
28	emamectin benzoate	75 (±22)	73 (±12)	75 (±10)	76 (±8)	0.32	−25.1
29	ethion	60 (±24)	67 (±12)	66 (±7)	64 (±7)	0.22	−56.4
30	etrimphos	91 (±32)	75 (±28)	77 (±16)	79 (±10)	0.50	−29.5
31	famoxadone	ND	85 (±22)	96 (±13)	92 (±12)	0.41	−33.4
32	fenamidone	86 (±10)	84 (±9)	79 (±8)	80 (±9)	0.25	−15.9
33	fenarimol	69 (±32)	89 (±24)	75 (±16)	71 (±11)	0.50	−18.1
34	fenobucarb	94 (±10)	92 (±9)	81 (±8)	74 (±6)	0.25	−27
35	fenpyroximate	65 (±15)	53 (±24)	69 (±17)	70 (±11)	0.54	−53.3
36	fenthion	71 (±26)	75 (±14)	77 (±16)	69 (±10)	0.50	−42.8
37	flufenoxuron	ND	83 (±25)	84 (±17)	89 (±15)	0.54	−43.3
38	flusilazole	99 (±27)	88 (±21)	77 (±12)	77 (±7)	0.38	−31.9
39	forchlorfenuron	92 (±11)	82 (±13)	80 (±8)	77 (±6)	0.25	−24.6
40	hexaconazole	79 (±14)	79 (±13)	75 (±6)	71 (±7)	0.19	−31.6
41	imazalil	90 (±9)	90 (±11)	84 (±7)	80 (±6)	0.22	−17.7
42	imidacloprid	99 (±18)	89 (±8)	88 (±9)	82 (±6)	0.28	−26.8
43	indoxacarb	60 (±33)	59 (±21)	58 (±12)	62 (±12)	0.38	−41.9

Table 2. Continued

sr. no.	pesticide name	recovery % (mean ± RSD) I[a]	II[a]	III[a]	IV[a]	HorRat (10 ng g^{-1})	ME (%)[b]
44	iprovalicarb	93 (±7)	89 (±5)	79 (±8)	73 (±6)	0.25	−22.8
45	isoprothiolane	84 (±9)	93 (±16)	82 (±5)	75 (±7)	0.16	−21.7
46	isoproturon	95 (±6)	94 (±6)	86 (±4)	78 (±5)	0.13	−15.8
47	iprobenfos/kitazin	85 (±20)	89 (±15)	87 (±16)	92 (±10)	0.50	−21.8
48	kresoxim methyl	114 (±30)	89 (±17)	119 (±16)	100 (±19)	0.50	−18.2
49	malaoxon	85 (±9)	89 (±7)	85 (±9)	80 (±6)	0.28	−13.8
50	malathion	95 (±11)	93 (±10)	85 (±6)	78 (±10)	0.19	−13.8
51	mandipropamid	95 (±18)	99 (±13)	97 (±14)	98 (±8)	0.44	−24.6
52	metalaxyl	89 (±13)	90 (±6)	84 (±6)	77 (±3)	0.19	−9.1
53	methamidophos	95 (±23)	73 (±14)	73 (±15)	87 (±13)	0.47	−87.6
54	methidathion	103 (±13)	106 (±9)	99 (±5)	85 (±8)	0.16	−72.5
55	methomyl	101 (±6)	98 (±7)	89 (±6)	84 (±5)	0.19	−37.9
56	metribuzin	79 (±33)	98 (±15)	95 (±14)	85 (±10)	0.44	−21.5
57	mevinphos	103 (±12)	92 (±7)	87 (±5)	85 (±7)	0.16	−13.6
58	monocrotophos	98 (12)	92 (11)	86 (8)	89 (6)	0.25	−13.9
59	myclobutanil	98 (±15)	94 (±15)	82 (±6)	86 (±6)	0.19	−21.3
60	omethoate	90 (±16)	83 (±10)	78 (±11)	74 (±5)	0.35	−62.4
61	oxydemeton methyl	87 (±8)	83 (±7)	81 (±8)	74 (v7)	0.25	−3.8
62	paraoxon methyl	43 (±87)	57 (±42)	75 (±15)	80 (±10)	0.47	−33.3
63	penconazole	81 (±14)	86 (±9)	82 (±7)	73 (±5)	0.22	−27.4
64	pendimethalin	ND	75 (±18)	74 (±12)	77 (±10)	0.38	−35
65	phenthoate	74 (±12)	74 (±10)	76 (±8)	78 (±8)	0.25	−50.4
66	phosalone	70 (±22)	68 (±14)	72 (±8)	66 (±8)	0.25	−38.8
67	phosmet	92 (±26)	94 (±17)	88 (±19)	92 (±19)	0.60	−23.8
68	phosphamidon	88 (±12)	94 (±15)	89 (±12)	83 (±9)	0.38	−11.2
69	profenophos	76 (±36)	79 (±18)	73 (±12)	75 (±8)	0.38	−33.3
70	propargite	60 (±23)	61 (±13)	61 (±14)	62 (±12)	0.44	−63.3
71	propiconazole	88 (±15)	82 (±14)	81 (±11)	72 (±9)	0.35	−19.6
72	pyraclostrobin	70 (±8)	70 (±6)	76 (±8)	81 (±7)	0.25	−27.4
73	quinalphos	71 (±29)	82 (±15)	77 (±9)	77 (±7)	0.28	−25.1
74	simazine	65 (±30)	61 (±18)	79 (±18)	78 (±16)	0.57	−29.5
75	spinosyn A	70 (±13)	75 (±19)	71 (±11)	73 (±10)	0.35	−36.3
76	spinosyn D	86 (±15)	82 (±11)	78 (±10)	71 (±6)	0.32	−42.7
77	tebuconazole	78 (±19)	80 (±13)	77 (±8)	72 (±9)	0.25	−29.7
78	temephos	40 (±41)	44 (±32)	42 (±27)	44 (±15)	0.85	−69.7
79	tetraconazole	82 (±29)	82 (±17)	80 (±11)	77 (±7)	0.35	−22.4
80	thiacloprid	94 (±13)	95 (±11)	85 (±4)	88 (±3)	0.13	−26.5
81	thiamethoxam	96 (±17)	95 (±15)	90 (±8)	83 (±6)	0.25	−51.8
82	thiodicarb	55 (±6)	60 (±4)	56 (±7)	60 (±4)	0.22	−15.2
83	thiometon	118 (±42)	124 (±30)	98 (±22)	93 (±20)	0.69	−55.6
84	triadimefon	79 (±21)	78 (±17)	76 (±8)	80 (±8)	0.25	−28.3
85	triadimenol	80 (±24)	81 (±10)	81 (±8)	88 (±6)	0.25	−14.4
86	triazophos	83 (±9)	85 (±5)	80 (±7)	84 (±9)	0.22	−19.4
87	trifloxystrobin	80 (±11)	74 (±7)	82 (±9)	87 (±6)	0.28	−32.3

[a] I, 2.5 ng/g; II, 5 ng/g; III, 10 ng/g; and IV, 25 ng/g. [b] ME (%) pertains to matrix-induced signal suppressions in LC-(ESI)-MS/MS when the extracts were directly analyzed after solvent exchange without any cleanup. The "−" sign indicates signal suppressions. [c] ND, not detected.

by 15% when C18 was substituted with 25 mg of GCB, indicating adsorption of this chemical on the surface of GCB. In the case of spinosyn A and D, the peak area remained unchanged on cleanup with PSA + GCB, but the S/N reduced by nearly 60% when the extract was cleaned with PSA + C18, which might have occurred as a result of adsorption of this pesticide on C18 sorbent. For some azole derivatives like myclobutanil, tebuconazole, triadimefon, and tetraconazole and organophosphorus pesticides like acephate, ethion, methidathion, profenophos, temephos, and phosmet, the recoveries increased by nearly 20% when DSPE cleanup was performed with 50 mg of PSA + 25 mg of GCB. In general, the recoveries of all of the test pesticides were within the range of 70−120% at all four levels of fortifications except for selected compounds like acephate, famoxadone, flufenoxuron, and pendimethalin (**Table 2**), which were not detectable at 2.5 ng g^{-1} level. The addition of sodium acetate during extraction significantly improved the recoveries, which could be due to its buffering as well as salting out effects during phase separation. The recoveries of the nonpolar pesticide temephos were low (40−44%) at all of the fortification levels on account of its limited extraction in ethyl acetate, which is in agreement with our results reported earlier for grapes (*4*). The poor recovery and high RSD for dichlorvos could be due to its volatile and unstable nature. For some compounds, the RSD of recovery was >30% (**Table 2**) at the fortification levels of 2.5 and 5 ng g^{-1}, but at 10 and 25 ng g^{-1} levels, their RSDs were within 20% except for dichlorvos, temephos, and thiometon, where RSDs were within 20−30%. The HorRat of most of the compounds was within 0.5 (**Table 2**), indicating satisfactory intralaboratory precision. The highest HorRat was for dichlorvos (0.88), which could be attributed to its relatively less repeatable signal because of its volatile nature.

The results received from the six participating laboratories, which validated the current method as per our instructions, are similar to our results. The small-scale PT results were satisfactory

Table 3. Results of Individual and Global Uncertainties for Each Pesticide

		uncertainty components (expressed as relative measures, calculated at 10.0 ng g^{-1})						
			precision		accuracy/bias			
sr. no.	name of pesticides (group designation[a] in Roman numerals)	calibration curve (U_1)	U_2	U_3	U_4	U_5	global uncertainty (U)	expanded uncertainty (2U)
1	acephate (I)	0.0182	0.0022	0.0026	0.0214	0.0262	0.039	0.077
2	acetamiprid (I)	0.0173	0.0013	0.0013	0.0127	0.0132	0.025	0.051
3	alachlor (I)	0.0190	0.0022	0.0024	0.0201	0.0210	0.035	0.070
4	atrazine (I)	0.0165	0.0015	0.0017	0.0126	0.0155	0.026	0.052
5	azinphos-methyl (IV)	0.0164	0.0087	0.0019	0.0869	0.0190	0.091	0.182
6	azoxystrobin (I)	0.0170	0.0016	0.0016	0.0164	0.0159	0.029	0.057
7	benalaxyl (I)	0.0183	0.0019	0.0022	0.0189	0.0225	0.035	0.069
8	bitertanol (II)	0.0180	0.0025	0.0030	0.0254	0.0292	0.043	0.086
9	buprofezin (III)	0.0177	0.0020	0.0065	0.0198	0.0647	0.070	0.141
10	butachlor (IV)	0.0233	0.0052	0.0079	0.0522	0.0785	0.098	0.195
11	carbaryl (I)	0.0173	0.0013	0.0011	0.0130	0.0109	0.024	0.049
12	carbendazim (I)	0.0165	0.0016	0.0015	0.0161	0.0151	0.028	0.055
13	carbofuran (I)	0.0193	0.0015	0.0015	0.0151	0.0148	0.029	0.057
14	carbofuran-3-OH (I)	0.0202	0.0023	0.0020	0.0228	0.0212	0.037	0.074
15	clothianidin (I)	0.0203	0.0020	0.0017	0.0200	0.0169	0.033	0.066
16	cymoxanil (I)	0.0171	0.0023	0.0019	0.0229	0.0185	0.034	0.068
17	demeton-S-methyl (III)	0.0256	0.0049	0.0042	0.0495	0.0416	0.070	0.140
18	demeton-S-methyl sulfone (I)	0.0193	0.0016	0.0013	0.0157	0.0134	0.028	0.057
19	diazinon (I)	0.0203	0.0020	0.0017	0.0200	0.0170	0.033	0.067
20	dichlofluanid (II)	0.0216	0.0028	0.0034	0.0282	0.0335	0.049	0.098
21	dichlorvos (III)	0.0212	0.0063	0.0040	0.0630	0.0396	0.078	0.155
22	difenoconazole (II)	0.0175	0.0022	0.0029	0.0224	0.0290	0.041	0.082
23	diflubenzuron (II)	0.0156	0.0032	0.0029	0.0326	0.0303	0.047	0.095
24	dimethoate (I)	0.0179	0.0014	0.0019	0.0175	0.0191	0.032	0.063
25	dimethomorph (I)	0.0186	0.0019	0.0020	0.0187	0.0196	0.033	0.066
26	diniconazole (II)	0.0171	0.0031	0.0029	0.0308	0.0198	0.041	0.081
27	DMSA (II)	0.0171	0.0031	0.0028	0.0308	0.0198	0.041	0.081
28	emamectin benzoate (II)	0.0238	0.0031	0.0028	0.0305	0.0305	0.049	0.099
29	ethion (II)	0.0183	0.0041	0.0032	0.0410	0.0323	0.056	0.111
30	etrimfos (II)	0.0176	0.0038	0.0033	0.0375	0.0333	0.053	0.107
31	famoxadone (II)	0.0210	0.0036	0.0039	0.0365	0.0405	0.059	0.117
32	fenamidone (I)	0.0183	0.0013	0.0019	0.0134	0.0193	0.030	0.060
33	fenarimol (II)	0.0215	0.0029	0.0029	0.0293	0.0284	0.046	0.093
34	fenobucarb (I)	0.0183	0.0015	0.0013	0.0150	0.0130	0.027	0.054
35	fenpyroximate (IV)	0.0244	0.0049	0.0063	0.0497	0.0635	0.085	0.169
36	fenthion (III)	0.0159	0.0058	0.0041	0.0586	0.0411	0.074	0.147
37	flufenoxuron (IV)	0.0268	0.0065	0.0049	0.0648	0.0476	0.085	0.170
38	flusilazole (II)	0.0165	0.0027	0.0029	0.0276	0.0292	0.044	0.087
39	forchlorfenuron (I)	0.0178	0.0020	0.0009	0.0202	0.0097	0.029	0.057
40	hexaconazole (I)	0.0153	0.0017	0.0023	0.0178	0.0238	0.034	0.067
41	imazalil (I)	0.0166	0.0018	0.0020	0.0179	0.0120	0.027	0.055
42	imidacloprid (II)	0.0218	0.0027	0.0029	0.0270	0.0274	0.044	0.089
43	indoxacarb (II)	0.0158	0.0040	0.0035	0.0404	0.0354	0.056	0.112
44	iprobenfos (I)	0.0155	0.0013	0.0019	0.0136	0.0194	0.028	0.057
45	iprovalicarb (I)	0.0171	0.0015	0.0013	0.0154	0.0134	0.027	0.053
46	isoprothiolane (I)	0.0166	0.0013	0.0020	0.0128	0.0203	0.029	0.059
47	isoproturon (I)	0.0170	0.0015	0.0013	0.0152	0.0132	0.026	0.053
48	kresoxim methyl (IV)	0.0253	0.0063	0.0052	0.0628	0.0522	0.086	0.172
49	malaoxon (I)	0.0189	0.0018	0.0013	0.0177	0.0131	0.029	0.058
50	malathion (I)	0.0198	0.0014	0.0027	0.0141	0.0276	0.037	0.074
51	mandipropamid (II)	0.0145	0.0020	0.0034	0.0205	0.0350	0.043	0.087
52	metalaxyl (I)	0.0206	0.0015	0.0013	0.0150	0.0132	0.029	0.058
53	methamidophos (II)	0.0184	0.0031	0.0031	0.0310	0.0319	0.048	0.097
54	methidathion (I)	0.0185	0.0017	0.0022	0.0173	0.0223	0.034	0.068
55	methomyl (I)	0.0203	0.0014	0.0014	0.0143	0.0143	0.029	0.057
56	metribuzin (II)	0.0216	0.0024	0.0023	0.0242	0.0232	0.040	0.080
57	mevinphos (I)	0.0228	0.0021	0.0019	0.0214	0.0196	0.037	0.074
58	monocrotophos (I)	0.0207	0.0013	0.0015	0.0131	0.0149	0.029	0.057
59	myclobutanil (I)	0.0216	0.0016	0.0016	0.0164	0.0162	0.032	0.063
60	omethoate (I)	0.0216	0.0016	0.0016	0.0164	0.0161	0.032	0.063
61	oxydemeton methyl (I)	0.0240	0.0031	0.0013	0.0310	0.0013	0.039	0.079
62	paraoxon methyl (II)	0.0212	0.0031	0.0026	0.0313	0.0263	0.046	0.092
63	penconazole (II)	0.0228	0.0017	0.0028	0.0167	0.0278	0.040	0.080

Table 3. Continued

		uncertainty components (expressed as relative measures, calculated at 10.0 ng g^{-1})						
			precision		accuracy/bias			
sr. no.	name of pesticides (group designation[a] in Roman numerals)	calibration curve (U_1)	U_2	U_3	U_4	U_5	global uncertainty (U)	expanded uncertainty (2U)
64	pendimethalin (III)	0.0268	0.0045	0.0060	0.0449	0.0589	0.079	0.158
65	phenthoate (II)	0.0238	0.0022	0.0027	0.0221	0.0272	0.043	0.085
66	phosalone (II)	0.0199	0.0026	0.0031	0.0258	0.0308	0.045	0.090
67	phosmet (IV)	0.0164	0.0087	0.0019	0.0869	0.0190	0.091	0.182
68	phosphamidon (I)	0.0208	0.0016	0.0015	0.0161	0.0153	0.031	0.061
69	profenophos (II)	0.0219	0.0028	0.0034	0.0276	0.0344	0.049	0.099
70	propargite (III)	0.0212	0.0051	0.0036	0.0512	0.0365	0.067	0.133
71	propiconazole (I)	0.0224	0.0016	0.0027	0.0156	0.0266	0.038	0.076
72	pyraclostrobin (II)	0.0162	0.0122	0.0028	0.0220	0.0277	0.041	0.082
73	quinalphos (II)	0.0218	0.0025	0.0036	0.0223	0.0362	0.048	0.096
74	simazine (III)	0.0232	0.0041	0.0039	0.0409	0.0391	0.061	0.123
75	spinosyn A (III)	0.0193	0.0042	0.0050	0.0426	0.0502	0.069	0.138
76	spinosyn D (II)	0.0181	0.0042	0.0036	0.0424	0.0362	0.059	0.118
77	tebuconazole (III)	0.0240	0.0044	0.0043	0.0437	0.0434	0.066	0.133
78	temephos (IV)	0.0244	0.0064	0.0046	0.0638	0.0456	0.083	0.165
79	tetraconazole (II)	0.0183	0.0026	0.0028	0.0263	0.0286	0.043	0.086
80	thiacloprid (I)	0.0158	0.0016	0.0011	0.0164	0.0114	0.026	0.051
81	thiamethoxam (I)	0.0242	0.0016	0.0015	0.0158	0.0154	0.033	0.066
82	thiodicarb (II)	0.0158	0.0036	0.0010	0.0362	0.0100	0.041	0.082
83	thiometon (IV)	0.0540	0.0097	0.0130	0.1000	0.1302	0.174	0.347
84	triadimefon (I)	0.0168	0.0025	0.0024	0.0249	0.0237	0.038	0.077
85	triadimenol (I)	0.0205	0.0023	0.0016	0.0233	0.0159	0.035	0.070
86	triazophos (I)	0.0184	0.0012	0.0020	0.0119	0.0203	0.030	0.060
87	trifloxystrobin (II)	0.0193	0.0028	0.0033	0.0276	0.0333	0.048	0.095

[a] Refer to the text in the Results and Discussion.

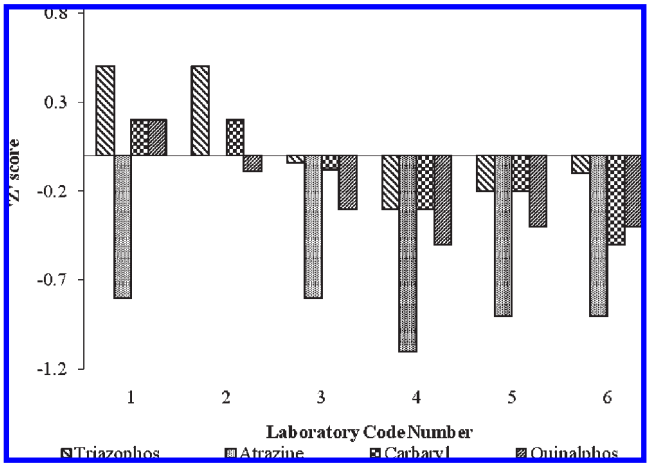

Figure 2. *Z* scores of the test pesticides in the PT sample for six participating laboratories.

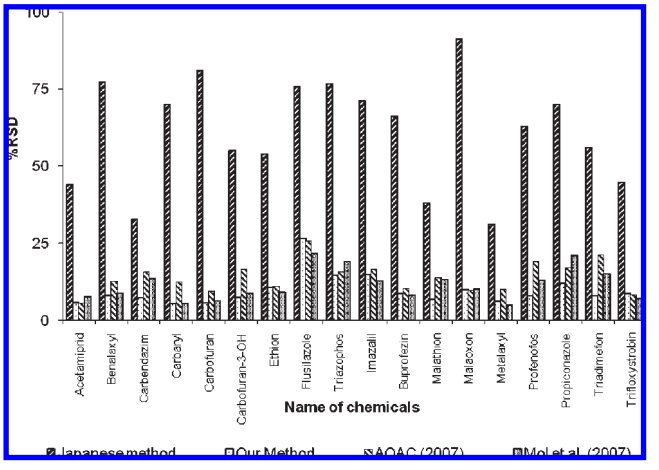

Figure 3. Comparison of different sample preparation methods for selected pesticides at 10 ng g^{-1}.

with the *Z* score of all of the participating laboratories being within +2 and −2 (**Figure 2**). Each laboratory could identify all of the target compounds, and their results were close to the true values. None of the participating laboratories reported any inconveniences in adopting this method or criticized its performance. We plan to do large-scale interlaboratory validation in our future endeavor.

The cleanup effect could be attributed to the removal of fatty acids and sugars by PSA, whereas GCB was effective in removing carotenoids and any other plant pigments. Because β-carotene is the chief carotenoid compound in mango, its concentration in uncleaned and cleaned extracts was compared by HPLC to assess the cleanup effect. PSA alone could not remove any β-carotene as observed by HPLC analysis. However, DSPE with 25 mg of GCB could remove more than 90% β-carotene from the ethyl acetate extract. An increase in GCB to 50 mg could completely remove the carotenoids, but it affected the recovery of several compounds. The addition of C18 sorbent did not result in any significant improvement in recoveries and hence was not considered. Substitution of PSA with florisil gave a nearly equivalent cleanup effect for the majority of the pesticides. This indicates that along with the carotenoids, the fatty acids might be the major coextractives. The cleanup effect rendered by PSA or florisil was evaluated by exploring into the coextracted matrix peaks of mono- and polyunsaturated fatty acids by gas chromatography−time-of-flight mass spectrometry (GC-TOFMS), and the results showed significant removal of these interfering compounds upon cleanup. In general, the matrix

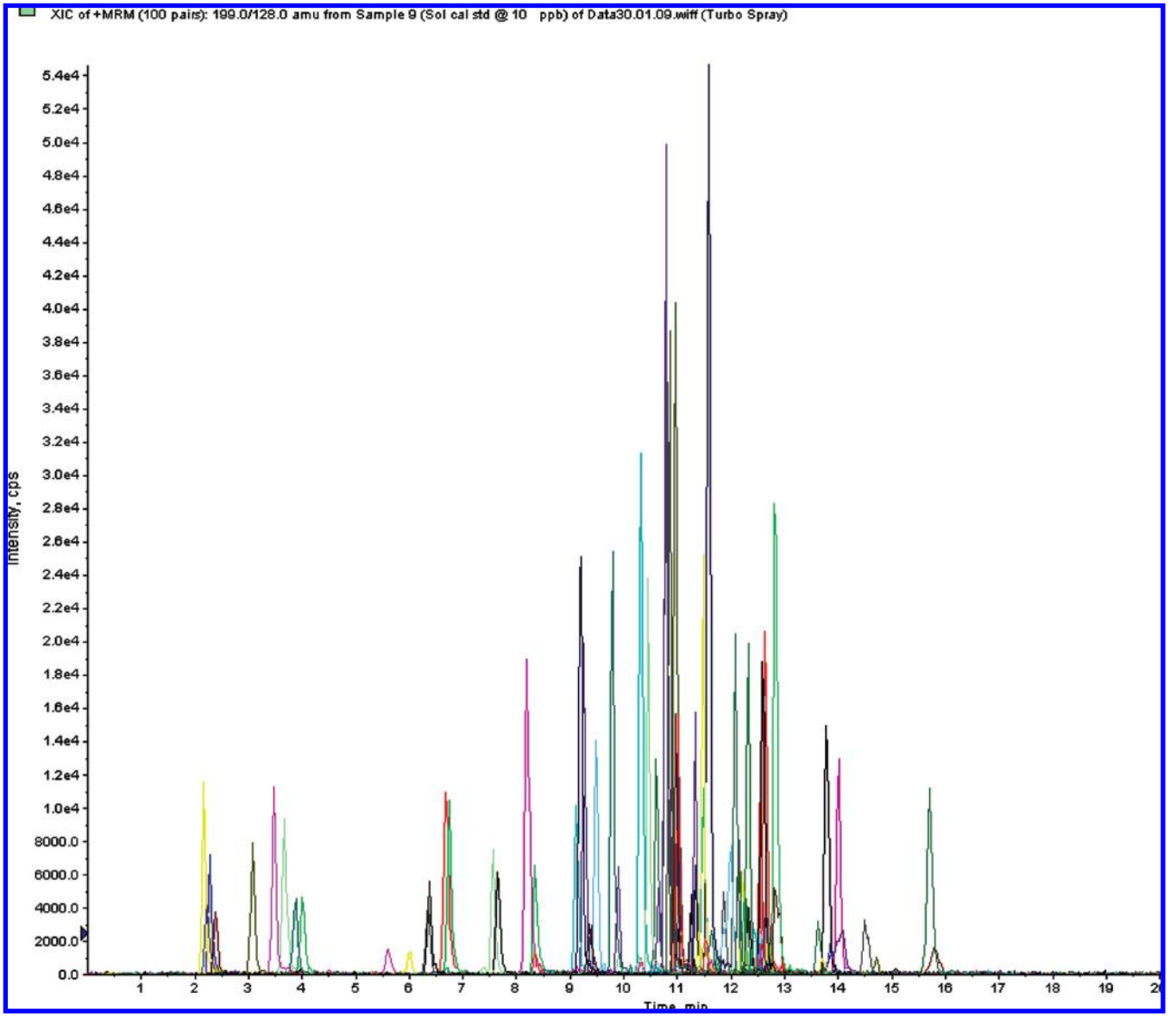

Figure 4. LC-MS/MS chromatogram of 87 pesticides in mango at 10 ng g^{-1}.

influence was less for ethyl acetate when compared to acetonitrile extraction, which might be on account of less solubility of sugars and other polar matrix components in ethyl acetate.

The Japanese method (*2*) involved multistep sample preparation, which caused losses in recoveries and, in turn, poor repeatability. The validation data generated at the NRL as well as the participating nominated laboratories indicate a high RSD in the range of 60−70% or even higher at 10 and 25 ng g^{-1} levels for a large proportion of the test compounds. In comparison, the corresponding RSDs in the current method were in general less than 10%. A comparative assessment for selected compounds is presented in **Figure 3**, which clearly establishes superiority of the current method over the Japanese method. The performance of the method was, however, statistically similar to the method of Mol et al. (*3*) or Lehotay (*8*) as per the Student's *t* test. The use of ethyl acetate was also economically cheaper and toxicologically safer than acetonitrile used in the Japanese or Lehotay's method and thus found to be more appropriate for extraction of a matrix like mangoes, which contains high sugar and less fat.

Method Performance/Fitness for Purpose. All of the 87 pesticides could be analyzed by a single chromatographic run within 20 min (**Figure 4**). The dwell time of 10 ms was found to be optimum for all of the compounds to achieve good peak shape having at least 15 data points across a peak at the 10 ng g^{-1} level. The linearity of the calibration curve was established for all of the pesticides. The correlation coefficient (R^2) of the calibration curve was > 0.99 (**Table 1**). For matrix-matched calibration, too, the R^2 was > 0.99 for most of the compounds. The LODs and LOQs for all of the compounds are presented in **Table 1**.

Measurement Uncertainty of Analyses. The global uncertainty of the test pesticides varied until 10%, with the exception of thiometon (17.4%). On the basis of the global uncertainty values, the test pesticides could be classified into four groups: group I (U < 4%), group II ($U \sim 4-6\%$), group III ($U \sim 6-8\%$), and group IV (U > 10%). Forty-two compounds could be graded as group I, 28 as group II, 9 as group III, and 8 as group IV.

The compounds classified under group I had lower uncertainties associated with precision (< 0.3% for both U_2 and U_3) as well as the accuracy/bias (< 3% for both U_4 and U_5). This suggests that the method gave repeatable and reliable results for these 42 compounds, without any major loss of residues during sample preparation. Compounds belonging to group II had lower uncertainties of precision (< 0.3% for both U_2 and U_3), similar to group I; however, uncertainties relating to accuracy/bias were

higher (2.5−4% for both U_4 and U_5) than that of group I analytes. Therefore, repeatability in their recoveries was affected, which could be due to loss during extraction and drying steps.

The group III pesticides consisted of simazine, tebuconazole, propargite, spinosyn A, demeton-S-methyl, buprofezin, fenthion, dichlorvos, and pendimethalin. Uncertainties in precision for these compounds were low (0.4−1.0%); however, the uncertainty in bias (in each case being within 4−5%) contributed considerably toward the global uncertainty. This is in conformity to relatively high RSD and poor recoveries (**Table 2**) for these analytes. A similar trend was observed for group IV pesticides consisting of temephos, fenpyroximate, flufenoxuron, kresoxim methyl, azinphos methyl, phosmet, butachlor, and thiometon. Thus, the method exhibited relatively poor performance for these compounds because of relatively larger uncertainties in bias, which might have occurred due to relative instability or incomplete extraction of these compounds. However, because the uncertainty level was within 10% for most of the compounds, the method performance could be considered satisfactory for the whole range of the test pesticides.

Economics of Analysis. The total input cost of analysis (solvents and reagents only) for one sample was INR 38, which is equivalent to little less than 1 U.S. dollar (USD). It provided an overall savings to the tune of around 90% per sample as compared to the Japanese technique, which requires around 9 USD to process a single sample. As per our estimate, in 8 working hours, one laboratory chemist could process around 20 samples up to the stage of ready-to-inject condition for LC-MS/MS analysis. Such output is comparable to the literature methods (*3*, *8*). On the contrary, by the Japanese method, it was not possible to prepare more than 5−6 samples/person/day. Hence, the current method increases the overall turnover of a testing laboratory significantly and thus has promise to be adopted in regular residue testing on mangoes. The current method is also relatively safer to the analysts due to nominal exposure to organic solvent.

Final Method of Sample Preparation. On the basis of the above results, the final method of sample preparation involves extraction of 10 g of sample (+10 mL of water + 1 g of Na-acetate) with 10 mL of ethyl acetate (+10 g of Na_2SO_4) by homogenization and centrifugation. A 4 mL amount of ethyl acetate extract was cleaned by DSPE with 50 mg of PSA + 25 mg of GCB + 150 g of sodium sulfate and finally analyzed by LC-MS/MS MRM after solvent exchange to 2 mL of methanol:water (1:1) with 0.1% acetic acid.

The method gives distinct advantages over the related techniques of multiresidue analysis by minimizing the sample size and volume of ethyl acetate as the extracting solvent in spite of ensuring satisfactory precision and accuracy at a residue level as low as 2.5 ng g^{-1}. The method is sufficiently rugged and recommended for the compounds with low measurement uncertainties (groups I and II) and should be applicable for the group III and IV compounds with special attention. The cleanup strategies could effectively minimize matrix influence and reduce the chances of false detections and over- or underestimations. The method reduces the cost of analysis and also offers a low level of measurement uncertainty, indicating suitability for regulatory and monitoring purposes.

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Supporting Information Available: MS/MS optimization parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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