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Validation and uncertainty analysis of a multiresidue method for 42 pesticides in made tea, tea infusion and spent leaves using ethyl acetate extraction and liquid chromatography–tandem mass spectrometry

Bappaditya Kanrar, Sudeb Mandal, Anjan Bhattacharyya*

Export Testing Laboratory, Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur 741252, West Bengal, India

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ABSTRACT

A rapid, specific and sensitive multiresidue method to determine 42 pesticides in made tea, tea infusion and spent leaves has been developed and validated for the routine analysis by liquid chromatography–tandem mass spectrometry (LC–MS/MS). The method was reproducible (Horwitz ratio (HorRat) <0.5 at 50 ng/g) and validated by the analysis of sample spiked at 50 and 100 ng/g in made tea, tea infusion and spent leaves. The samples were extracted with ethyl acetate + cyclohexane (9:1; v/v), and the extracts were cleaned up by dispersive solid phase extraction with primary secondary amine sorbent + graphitized carbon black + Florisil. The recoveries of all the pesticides were between 70% and 120% with a relative standard deviation of less than 15% and correlation coefficient for each pesticide was $R^2 \geq 0.99$. The matrix effect on signal of respective compounds was measured by comparing matrix-matched calibration standards with those in solvent-only. The limits of quantitation (LOQ) met the requirements of the maximum residue limits (MRLs) for pesticides in tea as recommended by the European Union.

1. Introduction

Tea is a popular beverage throughout the world and valued for its specific aroma and flavor as well as health-promoting properties [1]. However, the contamination of pesticide multiresidues in tea is a potential threat to the health of tea drinkers. In recent years, a number of research works have dealt with the behavior of different pesticides in tea focusing on the influence of various manufacturing processes on the residues in made tea, and their transfer potential from made tea to infusion [2,3]. Residue levels of many pesticides in made tea and in its infusion have also been reported [4–8].

Trace-level multiresidue analysis of pesticides in tea has become important because of the increasingly stringent regulatory requirements of the EU agencies and other tea-importing countries [9]. In general, pesticide residue analysis is carried out in a sequence of steps, viz. extraction of target compounds from sample matrix, cleanup and pre-concentration, followed by chromatographic analysis [10,11]. Nowadays, the pesticide residue analysis methods have been widely developed to analyze multiresidues in fresh vegetables, fruit, water, honey, etc. [12]. However, since tea samples contain complex components including pigments, alkaloids and polyphenols, etc. the analysis of pesticide multiresidue in tea is usually difficult owing to matrix interference and complicated extraction procedures. Wu et al. [13] proposed extraction of tea with acetonitrile, cleanup through amino cartridges and subsequent identification and quantification of 19 carbamate pesticides by HPLC. Pang et al. [14] proposed the use of cyclohexane + ethyl acetate (1 + 1) for the extraction of pesticides from animal tissues, GPC for cleanup, and gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the determination of 660 pesticide residues. Several methods have already been reported to analyze multiresidue in different tea matrices by GC–MS/MS [15,16].

Literature survey reveals lack of a suitable cost effective multiresidue method for trace-level quantification of pesticide residues in tea matrix by LC–MS/MS. Mastovska and Lehotay [17] evaluated and compared the suitability of six organic solvents for pesticide residue analysis and stability of multi-class pesticides and identified acetonitrile as the most suitable extraction solvent for a variety of matrices. Ethyl acetate is equally acceptable as extraction solvent for different products [18,19], since it does not pose limitations in terms of lipid co-extractives.

The aim of this paper was to optimize and validate a multiresidue analysis method based on ethyl acetate + cyclohexane

* Corresponding author. Tel.: +91 33 25827139; fax: +91 33 25828460.
E-mail address: anjan_84@rediffmail.com (A. Bhattacharyya).

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extraction followed by simultaneous determination of 42 pesticides in tea by LC–MS/MS with good selectivity, high sensitivity and a wide application scope.

2. Experimental

2.1. Apparatus

An Alliance 2695 Separations Module (Waters, Milford, MA, USA) was coupled to a Micromass Quattro Micro triple-quadrupole mass spectrometer (Micromass, Manchester, UK) using electrospray ionization in the positive ion (ES+) and negative ion (ESI−) mode. MassLynx $V_{4.1}$ software was used for instrument control and QuanLynx for data analysis. Samples were evaporated by using a Turbo Vap LV instrument from Caliper Life Science (Hopkinton, MA, USA). The extracts were centrifuged by using a high speed refrigerated centrifuge, Model Avanti J-30I (Beckman coulter, USA). The rotor heads were suitable for holding eight 50 mL (JA-30.50 T_1) and eighteen 10 mL (JA-21) samples. A top-loading balance with digital display (Sartorius, CP 225D, Germany) was used to weigh the samples and powder reagents. For both the extraction and dispersive-SPE cleanup steps, 50 mL and 10 mL fluorinated ethylene propylene (FEP) centrifuge tubes (Nalgene, Rochester, NY) respectively were employed. Standard 1.8 mL dark glass autosampler vials were used to contain final extracts. Homogenizer (Polytron, PT-MR-3100, Kinemetica AG, Switzerland) and Incubator Shaker (ZHWY-200D; Zhicheng, China) were also used for sample preparation.

2.2. Reagents

Pesticide reference standards (purity > 98%) were obtained from Sigma–Aldrich/Riedel-de-Haen/Supelco. Residue analysis grade organic solvents: Acetonitrile (MeCN), Ethyl Acetate (EA) obtained from JT Baker (Phillipsburg, USA) were used. Purified water prepared by using Milli-Q (Millipore, Bedford, MA) water purification system. Analytical reagent grade anhydrous sodium sulfate (Na_2SO_4) and sodium chloride (NaCl) were purchased from Merck India Ltd. (Mumbai, India). The Na_2SO_4 were heated in a muffle furnace at 400–450 °C for 5 h before use and kept in desiccator. Triphenylphosphate (TPP) was obtained from Sigma–Aldrich and used as internal standard (ISTD). A working ISTD concentration of 10 μg/mL in methanol was prepared and added to the test sample during sample preparation. An appropriate dilution of this ISTD to 1 μg/mL with methanol was also prepared and used for the preparation of the matrix-matched calibration standards. Primary secondary amine (PSA; 40 μm particle size), Bondesil C_{18} (ODS) were obtained from Varian (Harbor City, CA), graphitized carbon black (GCB) from United Chemical Technology (Bellefonte, PA). Florisil (60–100 mesh; Acros, Belgium), Bond Elute Amino (–NH_2; Varian, USA), Silica (Si; 60–120 mesh; Qualigens, Mumbai) were used.

2.3. Tea samples and fortifications

Made tea was purchased from Bio garden of Makaibari Tea and Trading Co. (P) Ltd., Kurseong, Darjeeling, India. This was used in fortification experiments and as matrix blanks for matrix-matched calibration standards.

Made tea (5 g) was infused in 150 mL of boiled water. After 3 min of brewing, the hot aqueous extract was filtered, cooled which is considered as tea infusion sample. The matrices used for residue study were the tea infusion, spent leaves left in the filter and made tea (processed tea shoots).

In recovery studies, 1.0 mg/L working standard mixture solution at desired concentration was added to each 1 g blank sample of made tea, spent leaves and 10 mL blank tea infusion. The tube containing fortified sample was vortexed for 30 s and left standing for 2 h to distribute pesticides evenly and given time to interact with the matrix before extraction.

2.4. Selection of pesticides

We have selected 42 pesticides considering the pesticide use pattern mainly in Indian tea garden. Among the 42 LC amenable pesticides include triazinylsulfonylurea, neonicotinoid, organo phosphate, carbamate, triazole, aryloxyphenoxypropionic, dinitroaniline sulfonamide, pyrimidinyloxybenzoic acid, conazole, chitin synthesis inhibitor, and macrocyclic compounds, e.g. spinosad and avermectin group of compounds. The detail LC/MS parameters are presented in Table 1.

2.5. Preparation of standard solution

Stock solutions of the individual pesticide standards were prepared by accurately weighing 10 + 0.01 mg of each of the 42 pesticides for LC/MS (Table 1) in volumetric flasks (certified "A" class) and dissolving each in 10 mL methanol. This stock solution was stored in dark vials in a refrigerator at 4 °C. An intermediate stock standard mixture of 10 mg/L was prepared by mixing appropriate quantities of the individual stock solution and diluted accordingly. A working standard mixture of 1.0 mg/L was prepared by diluting the intermediate stock standard solution, from which the calibration standards within the range 1–200 ng/mL were prepared by serial dilution with methanol:water (1:1; v/v).

2.6. Dispersive-SPE cleanup

In this study using the dispersive solid phase extraction (d-SPE) approach, we compared the following combination of different sorbents, PSA, –NH_2, Florisil, GCB, Si and ODS to get better analyte recovery and less matrix interference in tea infusion, spent leaves and made tea. Combinations were (i) no sorbent; (ii) 25 mg PSA; (iii) 25 mg PSA and 25 mg GCB; (iv) 25 mg PSA, 25 mg GCB and 25 mg Florisil; (v) 25 mg PSA, 25 mg GCB and 25 mg NH_2; (vi) 25 mg PSA, 25 mg GCB and 25 mg ODS; (vii) 25 mg PSA, 25 mg GCB and 25 mg Si; (viii) 25 mg PSA, 15 mg GCB and 25 mg Florisil; (ix) 25 mg PSA, 20 mg GCB and 25 mg Florisil; (x) 25 mg PSA, 30 mg GCB and 25 mg Florisil; (xi) 25 mg PSA, 35 mg GCB and 25 mg Florisil; (xii) 25 mg PSA, 40 mg GCB and 25 mg Florisil; and (xiii) 25 mg PSA, 50 mg GCB and 25 mg Florisil. In addition to the above combination, 150 mg Na_2SO_4 per mL extract was also used in every case. The above cleanup experiments with dispersive solid phase sorbent were done with 2 mL of organic phase extract.

2.7. Extraction and cleanup procedure

Tea samples (Made tea and spent leaves: 1 g) were taken in a 50 mL FEP centrifuge tubes and mixed with distilled water (10 mL), 100 μL internal standard of 10 μg/mL concentration, 10 mL ethyl acetate + cyclohexane (9:1) plus 1 g NaCl by vortexing 30 s followed by blending for 1 min at 15,000 rpm (27,200 rcf) in a polytron homogenizer. The homogenized samples were then centrifuged at 3500 rpm (1500 rcf) for 5 min.

For the dispersive solid phase extraction, an aliquot of 2 mL supernatant was transferred into a 10 mL FEP centrifuge tube prefilled with PSA, GCB and Florisil each 25 mg and 300 mg Na_2SO_4. The mixture was vortexed for 30 s and centrifuged at 6000 rpm (4400 rcf) for 10 min. For LC–MS/MS analysis, one aliquot of 1 mL was transferred from the supernatant to a 15 mL disposable glass tube. After addition of 200 μL 10% diethylene glycol in methanol as a keeper, the contents of the tube were mixed thoroughly in

vortex mixer for 30 s, the mixture was evaporated to near dryness under a gentle stream of nitrogen in a low volume concentrator at 35 °C. The residues were then dissolved in 1 mL mixture of LC mobile phase solvent A and B. The mixture was then vortexed and filtered through 0.2 μm polyvinylidene fluoride membrane filter paper. The filtrate was then injected to LC–MS/MS instrument.

From the prepared tea infusion (described above), a 10 mL aliquot (cooled to room temperature) was transferred to a 50 mL FEP centrifuge tubes. The pesticides were extracted with 10 mL ethyl acetate + cyclohexane (9:1) plus 1 g NaCl. The spent leaves were taken after drying with filter paper and residues were extracted by similar procedure as followed in made tea. The cleanup procedure for tea infusion was also similar to that of the made tea.

2.8. LC/MS analyses

The residue analyses were performed by liquid chromatography–tandem mass spectrometry. The HPLC separation was performed by injecting 20 μL via autosampler on a Symmetry C_{18} (5 μm; 2.1 × 100 mm) column (Waters, USA) at the flow rate of 0.3 mL/min. Two injections are required to analyze for all the pesticides. Table 1 lists the compounds along with their reten-

Table 1
Overview of the LC–MS/MS multiresidue monitoring of the test pesticides.

Sl. no.	Pesticide (class[a])	Mobile phase	RT (min)	Q	Q_1	CV (V)	CE (V)	Q_2	CV (V)	CE (V)	LOQ[x] (ng/g)	LOQ[y] (ng/g)	LOQ[z] (ng/g)	MRL[*] (mg/kg)
1.	2,4-D (XXI)	MF 1	2.67	217.05	58.43	31	14	53.28	31	5	45	47	50	0.1[b]
2.	Abamectin (XXIV)	MF 2	3.38	873.92	68.65	38	76	113.01	38	55	39	46	50	0.02
3.	Acephate (III)	MF 1	1.22	183.95	142.90	19	11	94.4	19	25	10	12	15	0.05
4.	Acetamiprid (II)	MF 1	4.23	223.12	125.90	33	18	55.40	33	11	20	22	25	0.1
5.	Bifenox (XII)	MF 1	7.59	342.07	310.10	26	08	108.9	26	56	40	43	48	0.05
6.	Bispyribac sodium (XIV)	MF 1	4.90	431.14	118.80	33	19	413.1	33	18	10	13	15	
7.	Carbendazim (VII)	MF 1	4.52	192.09	160	29	17	132	29	30	4	7	10	0.1[c]
8.	Carbofuran (IV)	MF 1	4.84	222.10	122.9	28	21	165.10	28	12	5	6	10	0.05
9.	Carbosulfan (IV)	MF 1	3.22	381.26	160.10	28	14	75.60	28	35	30	37	40	0.1
10.	Carfentrazone ethyl (X)	MF 1	6.42	412.05	346.10	39	23	366.10	39	16	16	19	20	0.02
11.	Chlorfluazuron (XXII)	MF 1	2.77	541.94	141.01	38	52	384.99	38	21	40	46	50	
12.	Clothianidin (II)	MF 1	4.13	250.05	112.8	24	25	169	24	13	8	10	13	0.1[d]
13.	Cyhalofop butyl (XI)	MF 1	9.33	358	119.96	27	26	91.9	27	41	40	46	50	0.05
14.	Cymoxanil (V)	MF 1	4.39	199.16	110.80	20	22	82.60	20	24	30	32	35	0.05
15.	Diclosulam (XX)	MF 1	4.69	405.98	160.98	33	27	89.70	33	77	10	12	15	
16.	Diflubenzuron (XIX)	MF 1	6.42	311.08	140.90	27	44	112.77	27	75	25	31	35	0.1
17.	Dimethoate (III)	MF 1	4.30	230.04	124.8	20	22	199.0	20	10	25	27	33	0.05[e]
18.	Emamectin benzoate (XXIV)	MF 2	2.71	886.97	81.86	55	79	126.04	55	51	5	6	10	
19.	Flonicamid (II)	MF 1	3.67	230	174.10	36	18	147.90	36	28	40	43	45	0.05
20.	Haloxyfop (XI)	MF 1	7.73	376.07	316.10	36	17	64.40	36	65	15	17	21	0.05
21.	Hexythiazox (IX)	MF 1	5.68	353.11	115.5	29	40	168.1	29	16	16	21	25	0.05
22.	Imidacloprid (II)	MF 1	4.08	255.06	175.1	25	15	209.20	25	15	8	8	10	0.05
23.	Malathion (III)	MF 1	5.68	330.97	126.90	22	12	98.6	22	23	30	33	37	0.5
24.	Metalaxyl (VI)	MF 1	5.11	280.10	220.30	24	14	192.2	24	17	6	8	12	0.1[f]
25.	Methomyl (IV)	MF 1	3.58	163	87.6	17	7	121.9	17	5	40	46	50	0.1[g]
26.	Monocrotophos (III)	MF 1	3.85	224.10	126.9	23	15	193.10	23	8	7	8	10	0.01
27.	Monolinuron (XVIII)	MF 1	5.07	215.07	126	28	16	98.80	28	35	7	10	10	0.01
28.	Myclobutanil (XVII)	MF 1	5.81	289.15	124.90	39	31	67.50	39	18	25	29	31	0.05
29.	Oryzalin (XIII)	MF 1	6.04	347.16	198.10	31	35	305.20	31	13	17	18	20	0.02
30.	Penoxsulam (XVI)	MF 1	4.45	484.04	195.10	53	30	124	53	53	10	12	15	0.02
31.	Prochloraz (VIII)	MF 1	7.24	376.06	84.7	23	22	308.10	23	11	15	20	23	0.1
32.	Profenofos (III)	MF 1	10.09	374.94	305	26	21	347	26	11	40	43	49	0.1
33.	Propaquizafop (XI)	MF 1	4.87	444.17	99.80	33	18	55.51	33	19	15	17	23	0.05
34.	Propiconazole (X)	MF 1	6.96	342.1	68.60	45	18	159.0	45	37	15	16	20	0.1
35.	Quinalphos (III)	MF 1	6.57	299.07	96.70	25	28	163.10	25	28	25	29	31	0.1
36.	Quizalofop ethyl (XI)	MF 1	10.45	373.11	299.15	38	18	90.73	38	28	30	33	37	0.05
37.	Spinosyn A (XXIII)	MF 2	4.00	732.82	142.17	43	30	68.78	43	77	5	7	9	0.05[h]
38.	Spinosyn D (XXIII)	MF 2	4.70	746.89	142.18	43	28	686.78	43	79	7	9	12	0.05[h]
39.	Thiacloprid (XV)	MF 1	4.39	253.05	98.70	35	41	89.80	35	44	40	17	50	0.05
40.	Thiamethoxam (II)	MF 1	3.72	292.04	181.00	24	22	131.90	24	24	20	22	26	0.1[d]
41.	Thiaphanate methyl (IV)	MF 1	4.75	343.11	151.10	26	22	92.60	26	48	11	15	19	0.1
42.	TPP (internal standard) (III)	MF 1 & MF 2	6.70 & 1.39	327.04	152.17	52	34	76.62	52	39				
43.	Triasulfuron (I)	MF 1	0.75	401.82	141.0	33	21	55.80	33	49	10	12	15	0.1

RT: retention time; Q: protonated parent ion; Q_1: quantifier ion; Q_2: second transition; CV: cone voltage; and CE: collision energy.

[a] Pesticide class designations: I: Triazinylsulfonylurea; II: Neonicotinoid; III: Organo Phosphate; IV: carbamate; V: Aliphatic nitrogen; VI: Benzenoid; VII: Benzimidazole; VIII: Amide; IX: Thiazolidine; X: Triazole; XI: Aryloxyphenoxypropionic; XII: Nitrophenyl ether; XIII: Dinitroaniline sulfonamide; XIV: Pyrimidinyloxybenzoic acid; XV: Pyridylmethylamine; XVI: Sulfonamide; XVII: Conazole; XVIII: Phenylurea; XIX: Benzamide; XX: Sulfonanilide; XXI: Phenoxyacetic; XXII: Chitin synthesis inhibitor; XXIII: insecticide spinosad; and XXIV: Avermectin.

[b] Sum of 2,4-D and its ester.

[c] Sum of Benomyl and Carbendazim.

[d] Sum of Thiamethoxam and Clothianidin.

[e] Sum of Dimethoate and Omethoate.

[f] Sum of Metalaxyl and Metalaxyl-M.

[g] Sum of Methomyl and Thiodicarb.

[h] Sum of Spinosyn A and Spinosyn D.

[x] LOQ in tea infusion.

[y] LOQ in spent leaves.

[z] LOQ in made tea.

[*] MRL established by EU (Regulation (EC) No 396/2005) updated on 20/05/2009.

tion times (RTs), mobile phase method used in analysis (labeled as MF 1 and MF 2) and quantifier as well as qualifier ions. The mobile phase (MF 1) was composed of (A) methanol/water 10/90 (v/v) with 5 mM ammonium acetate and (B) methanol/water 90/10 (v/v) with 5 mM ammonium acetate; gradient 0–1.0 min/90–5% A, 1–2 min/5% A, 2–18 min/5–90% A, 18–20 min/90% A. For analysis of four macromolecules, the samples were run with 100% solvent B for 8 min separately (MF 2). Selection and tuning of transitions as well as analyte-dependent parameters were performed by direct infusion of each of the pesticides in methanol at a concentration of 1 mg/L. Only 2,4-D was detected using electro spray ionization in the negative ion mode. The optimized MS instrument parameters includes: capillary voltage, 1.20 kV; cone voltage, 20 V; source temperature, 120 °C; desolvation temperature, 350 °C; desolvation gas flow, 650 L/h nitrogen; cone gas flow, 25 L/h; argon collision gas pressure to $3.5e^{-3}$ psi for MS/MS. Estimation of the residues was performed by multiple reaction monitoring (MRM) with two mass transition for each test pesticides with dwell time 0.1 s.

2.9. Preparation of matrix-matched calibration standards

For calibration in LC/MS, seven concentration levels (1, 5, 10, 20, 50, 100 and 200 ng/g) were prepared. For matrix-matched calibration standards the supernatant after cleanup following the above procedure was used as the matrix solvent at the sample-solvent ratio of 1:1. For calibration in fortification experiments, matrix-matched standards were prepared by adding the appropriate volumes of the pesticide standards mixture, ISTD and analyte protectant solutions to each blank extract.

2.10. Method validation

The analytical method was validated as per the single laboratory validation approach [20]. The performance of the method was evaluated considering different validation parameters that include the following items.

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 seven calibration levels ranging between 1 and 200 ng/g.

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 irrespective of the matrices, made tea, tea infusion and spent leaves by using matrix-matched standards.

2.11. Precision

The precision in the conditions of repeatability (two different analysts' prepared six samples each on a single day) and intermediate precision (two different analysts prepared six samples each on six different days) were determined separately for a standard concentration of 50 ng/g in made tea and spent leaves and 50 ng/mL in tea infusion of all of the analytes. The Horwitz ratio (HorRat) pertaining to intra-laboratory precision, which indicates the acceptability of a method with respect to precision [21,22], was calculated for all of the pesticides in the following way:

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

where RSD is the relative standard deviation and PRSD is the predicted RSD = $2C^{-0.15}$ and where C is the concentration expressed as a mass fraction (50 ng/g = 50×10^{-9}).

2.12. Accuracy-recovery experiments

Made tea obtained from a bio garden (which did not receive any treatment of the test pesticides) was used as blanks. The recovery experiments were carried out on fresh untreated made tea, tea infusion and spent leaves by fortifying the samples in six replicates with a pesticide mixture separately at two concentration levels, i.e. 50 and 100 ng/g in made tea and spent leaves and 50 and 100 ng/mL in tea infusion. The results are reported in Table 2.

2.13. Matrix effect

The matrix effect (ME) was assessed by employing matrix-matched standards. The slope of the calibration graph based on the matrix-matched standards of made tea, tea infusion and spent leaves was compared with the slope of the pure solvent-based calibration graph. A higher slope of the matrix calibration indicates matrix-induced signal enhancement, whereas, a lower slope represents signal suppression. The matrix effect (ME%) was evaluated by the following equation:

$$\text{ME, \%} = \frac{(\text{peak area of matrix standard} - \text{peak area of solvent standard}) \times 100}{\text{peak area of solvent standard}}$$

In view of the above equation, the negative and positive values of the ME signify matrix-induced suppression and enhancement, respectively. Furthermore, in order to minimize any errors in estimation, TPP (10 μg/mL, in methanol) was used as an internal standard which also normalizes the calibration slope in matrix-matched as well as solvent-based calibration.

2.14. Measurement uncertainty

Global uncertainty was determined for all of the pesticides at the level of 50 ng/g as per the statistical procedure of the EURACHEM/CITAC Guide CG 4 [23] in the same way as reported by Banerjee et al. [24]. Five individual sources of uncertainty were taken into account, viz. uncertainty associated with the calibration graph (U_1), day wise uncertainty associated with precision (U_2), analyst wise uncertainty associated with precision (U_3), day wise uncertainty associated with accuracy/bias (U_4), and analyst wise uncertainty associated with accuracy/bias (U_5). 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.

3. Results and discussion

3.1. Selection of extraction solvent

Three organic solvents and solvent mixture, viz. ethyl acetate, acetonitrile and ethyl acetate + cyclohexane were evaluated for their extraction efficiency. The extraction efficiency of ethyl acetate + cyclohexane in the ratio of 9:1. 8:2, 7:3, 6:4 and 1:1 (v/v) were also compared.

The results of the Student's *t*-test performed on the comparative recoveries obtained by using the above solvents showed that the recoveries of all the pesticides were statistically on par at 95% level of confidence (Fig. 1). In case of ethyl acetate, the recoveries of polar pesticides like acephate were more than 65% with less polar pesticides like carbamates having more than 70% recovery when quantified with matrix-matched standards but abamectin with only 40% recovery. With acetonitrile extraction, however, the recoveries of all the pesticides were above 70%. But with ethyl acetate + cyclohexane (9:1) the recoveries of all the pesticides including acephate, carbamate and abamectin were more than 65% with good precision when quantified with matrix-matched calibra-

Table 2
Recovery % (RSD)[a], HorRat and matrix effect of test pesticides from spent leaves, made tea and tea infusion.

Pesticides	Level of fortification											
	Spent leaves (ng/g)				Made tea (ng/g)				Tea infusion (ng/mL)			
	50	100	HorRat[b]	ME (%)[c]	50	100	HorRat[b]	ME (%)[c]	50	100	HorRat[b]	ME (%)[c]
2,4-D	71 (12)	69 (10)	0.49	−64	70 (10)	70 (10)	0.42	−78	73 (12)	71 (11)	0.48	−60
Abamectin	68 (12)	67 (11)	0.49	−78	66 (12)	68 (6)	0.50	−86	69 (9)	66 (8)	0.36	−68
Acephate	69 (11)	68 (8)	0.43	19	68 (12)	67 (12)	0.49	25	70 (12)	67 (7)	0.48	9
Acetamiprid	89 (12)	79 (6)	0.46	−49	88 (10)	80 (7)	0.41	−63	86 (10)	83 (7)	0.41	−45
Bifenox	91 (10)	85 (6)	0.40	−54	95 (10)	85 (4)	0.40	−64	96 (7)	87 (7)	0.30	−43
Bispyribac sodium	72 (5)	75 (6)	0.19	−59	74 (5)	75 (4)	0.21	−66	75 (5)	76 (5)	0.20	−49
Carbendazim	86 (9)	89 (7)	0.35	−6	85 (9)	86 (8)	0.36	−11	88 (11)	85 (13)	0.44	−7
Carbofuran	93 (3)	90 (5)	0.13	25	94 (4)	90 (5)	0.16	35	97 (2)	92 (2)	0.10	17
Carbosulfan	81 (7)	80 (3)	0.29	−56	84 (8)	82 (5)	0.31	−66	86 (5)	83 (3)	0.22	−47
Carfentrazone ethyl	89 (5)	86 (3)	0.21	−41	91 (2)	88 (3)	0.07	−59	93 (3)	88 (2)	0.12	−37
Chlorfluazuron	77 (9)	78 (6)	0.38	−59	76 (11)	80 (7)	0.45	−72	79 (8)	83 (11)	0.33	−50
Clothianidin	73 (5)	78 (4)	0.18	9	75 (4)	79 (5)	0.18	11	74 (5)	80 (6)	0.20	6
Cyhalofop butyl	86 (4)	81 (5)	0.17	−49	88 (4)	83 (4)	0.17	−56	91 (4)	81 (4)	0.15	−39
Cymoxanil	89 (7)	96 (3)	0.29	−48	91 (7)	94 (7)	0.27	−43	92 (5)	98 (2)	0.22	−34
Diclosulam	81 (12)	83 (9)	0.47	−58	80 (5)	81 (9)	0.22	−65	81 (10)	81 (8)	0.39	−50
Diflubenzuron	79 (8)	77 (13)	0.33	−38	82 (3)	79 (12)	0.12	−36	84 (5)	75 (4)	0.19	−31
Dimethoate	85 (7)	89 (8)	0.27	53	86 (6)	93 (4)	0.26	76	87 (6)	90 (3)	0.24	25
Emamectin benzoate	88 (9)	86 (5)	0.35	−71	87 (8)	83 (4)	0.32	−75	92 (9)	89 (9)	0.37	−59
Flonicamid	77 (7)	80 (4)	0.29	−27	81 (4)	81 (4)	0.16	−33	83 (3)	85 (12)	0.13	−18
Haloxyfop	92 (6)	82 (4)	0.23	−38	92 (5)	84 (4)	0.21	−49	95 (4)	88 (4)	0.16	−30
Hexythiazox	86 (10)	99 (6)	0.42	−33	89 (11)	101 (6)	0.44	−32	93 (5)	102 (6)	0.21	−20
Imidacloprid	94 (7)	98 (3)	0.28	−36	96 (7)	99 (4)	0.28	−34	98 (6)	98 (8)	0.23	−21
Malathion	86 (9)	79 (5)	0.35	−21	89 (5)	81 (5)	0.22	−33	93 (3)	83 (3)	0.12	−19
Metalaxyl	89 (5)	81 (5)	0.21	−43	90 (4)	83 (4)	0.17	−55	92 (2)	84 (4)	0.10	−32
Methomyl	73 (7)	74 (3)	0.28	−53	73 (7)	77 (5)	0.28	−69	74 (4)	78 (5)	0.14	−42
Monocrotophos	94 (3)	90 (3)	0.11	−49	95 (4)	94 (4)	0.16	−57	97 (4)	93 (6)	0.15	−32
Monolinuron	77 (4)	73 (3)	0.18	−43	80 (5)	74 (3)	0.21	−49	82 (3)	76 (6)	0.14	−37
Myclobutanil	71 (5)	74 (3)	0.22	−11	72 (7)	77 (6)	0.28	−19	74 (6)	77 (6)	0.23	−8
Oryzalin	90 (5)	87 (4)	0.20	−50	92 (2)	89 (3)	0.10	−49	94 (3)	91 (6)	0.12	−43
Penoxsulam	71 (7)	75 (4)	0.28	−33	72 (6)	74 (4)	0.24	−39	75 (5)	74 (4)	0.20	−21
Prochloraz	78 (5)	80 (4)	0.20	−37	82 (4)	81 (5)	0.17	−48	84 (4)	83 (3)	0.16	−26
Profenofos	82 (9)	90 (5)	0.36	9	87 (8)	92 (5)	0.34	17	88 (8)	93 (4)	0.34	11
Propaquizafop	86 (6)	81 (4)	0.26	8	87 (5)	85 (4)	0.22	11	89 (5)	82 (9)	0.19	6
Propiconazole	84 (7)	97 (6)	0.30	−5	85 (7)	99 (6)	0.29	−6	88 (3)	99 (7)	0.13	−7
Quinalphos	87 (9)	101 (10)	0.38	8	89 (9)	103 (9)	0.35	8	92 (5)	105 (7)	0.20	5
Quizalofop ethyl	88 (7)	85 (8)	0.30	10	89 (7)	87 (6)	0.29	13	92 (4)	84 (7)	0.16	6
Spinosyn A	91 (5)	90 (7)	0.20	−71	92 (7)	87 (3)	0.30	−77	94 (6)	91 (7)	0.24	−64
Spinosyn D	91 (9)	84 (9)	0.35	−69	88 (10)	85 (10)	0.40	−77	87 (11)	85 (7)	0.45	−61
Thiacloprid	70 (7)	75 (4)	0.28	−41	71 (5)	77 (6)	0.19	−46	73 (4)	76 (7)	0.15	−33
Thiamethoxam	83 (11)	92 (8)	0.46	−67	87 (11)	89 (8)	0.45	−76	92 (10)	92 (6)	0.39	−51
Thiaphanate methyl	80 (5)	80 (4)	0.22	−18	81 (5)	81 (4)	0.20	−21	83 (4)	82 (4)	0.16	−12
Triasulfuron	81 (8)	89 (4)	0.34	−42	83 (9)	91 (7)	0.35	−56	84 (8)	90 (6)	0.32	−33

[a] $n = 6$.
[b] HorRat at 50 ng/g for made tea and spent leaves and 50 ng/mL for tea infusion.
[c] ME (%) pertains to matrix-induced signal suppression ("−"sign) or enhancement.

tions. Precision in terms of HorRat (single laboratory) at 50 ng/g level was less than 0.5 for all the pesticides (Table 2), indicating satisfactory repeatability and ruggedness of the methodology. Increase in cyclohexane portion in the extracting solvent mixture (ethyl acetate + cyclohexane; 8:2, 7:3, 6:4 and 1:1; v/v) results in significant increase in the recovery percentage of abamectin but decrease in the acephate. A relatively less recovery of 2,4-D was found irrespective of any extracting solvent.

From this study it clearly revealed that mixture of ethyl acetate + cyclohexane (9:1; v/v) gave higher recovery percentage than other solvent or solvent mixtures used for extraction. Although, acetonitrile gave higher (>70%) recoveries but ethyl acetate is not only economically cheaper but also toxicologically safer than acetonitrile and that is why it is very much appropriate in selecting solvent mixture (ethyl acetate + cyclohexane 9:1; v/v) for extraction of a matrix like tea.

3.2. Comparison of shaking versus blending versus vortexing

The extractability of polar and non-polar residues was assessed through comparison of shaking versus blending versus vortexing to achieve the best initial extraction step to be followed for made tea and spent leaves. Most MRMs for pesticides in tea use blender during extraction [15,25] but Gupta and Shanker [26] validated and implemented a shaking procedure for tea. From our results it is revealed that blending gave better recovery for most of the pesticides compared to vortexing and shaking based methods (Fig. 2). Thus, we adopted homogenization procedure by blending for extraction of residues from made tea and spent leaves.

3.3. Comparison of different SPE sorbents by LC–MS/MS analysis

Tea matrix contains high amount of polyphenols, methyl xanthines such as caffeine, purines and also different phenolic acids [27]. The main aim of cleanup step was to remove those co-extractives as much as possible from the extract by using different sorbents. The most commonly used sorbents include weak ion exchange (PSA or –NH2), GCB, SAX, and/or ODS SPE cartridges [28–32].

The result of the Student's *t*-test performed on the comparative recoveries obtained by using the combination no. (iv) 25 mg PSA,

Table 3
Individual and global uncertainties[a] for each pesticide in spent leaves, made tea and tea infusion.

Pesticides	Spent leaves							Made tea							Tea infusion						
	CC	Precision		Bias		GU	EU	CC	Precision		Bias		GU	EU	CC	Precision		Bias		GU	EU
	(U_1)	U_2	U_3	U_4	U_5	(U)	($2U$)	(U_1)	U_2	U_3	U_4	U_5	(U)	($2U$)	(U_1)	U_2	U_3	U_4	U_5	(U)	($2U$)
2,4-D	7.2	2.1	1.9	3.5	3.7	9.3	18.5	6.7	1.8	1.7	4.1	3.9	9.1	18.2	5.6	2.2	1.8	4.3	4.1	8.7	17.3
Abamectin	5.9	2.5	2.4	4.8	4.7	9.6	19.2	5.5	1.6	1.4	4.9	4.8	9.1	18.1	5.3	1.1	1.2	5.1	5.3	9.2	18.4
Acephate	7.2	2.2	2.1	3.8	3.5	9.4	18.7	7.7	1.5	1.4	4.2	4.1	10	19.8	6.1	1.7	1.4	3.9	3.5	8.3	16.7
Acetamiprid	6.1	1.3	1.2	3.9	3.7	8.3	16.6	5.6	1.4	1.3	4.1	4.3	8.4	16.8	5.1	1.1	0.9	4.1	3.8	7.7	15.4
Bifenox	1.7	0.3	0.2	1.5	1.7	2.9	5.7	3.7	1.3	1.2	2.5	2.3	5.3	10.7	3.3	1.2	0.9	4.1	3.3	6.4	12.8
Bispyribac sodium	3.7	1.8	1.7	2.1	1.6	5.2	10.4	4.2	1.5	1.4	3.1	3	6.4	12.7	3.7	1.2	0.8	2.9	3.1	5.8	11.6
Carbendazim	4.7	2.0	1.9	5.4	5.1	9.2	18.4	5.1	1.7	1.5	4.2	4.1	8.1	16.2	5.3	1.1	0.9	4.3	4.1	8.1	16.2
Carbofuran	3.8	1.7	1.5	2.5	2.3	5.6	11.2	3.5	1.6	1.4	4.3	4.2	7.3	14.6	4.1	1.9	1.7	3.9	3.7	7.2	14.5
Carbosulfan	4.9	1.7	1.5	3.4	3.3	7.2	14.4	5.2	1.3	1.2	4.1	4.0	7.9	15.9	5.1	1.5	1.3	3.7	3.4	7.4	14.9
Carfentrazone ethyl	1.9	0.8	0.7	1.4	1.2	2.9	5.7	2.7	0.9	1.1	2.4	2.1	4.4	8.8	2.3	0.9	0.3	1.7	1.3	3.3	6.6
Chlorfluazuron	6.4	2.8	2.5	3.7	3.4	9.0	17.9	5.8	2.3	2.2	3.9	3.8	8.6	17.1	5.3	1.6	1.2	4.2	2.7	7.6	15.1
Clothianidin	3.7	1.7	1.8	2.2	2.1	5.4	10.8	4.2	2.1	2.0	3.2	3.1	6.8	13.6	3.8	1.6	1.4	3.3	3.1	6.3	12.6
Cyhalofop butyl	2.2	0.7	0.6	1.6	1.5	3.4	6.5	2.7	1.1	1.0	2.2	2.1	4.3	8.7	2.3	1.2	1.0	2.3	1.9	4.1	8.2
Cymoxanil	1.3	0.2	0.2	2.4	1.7	3.2	6.5	1.7	0.6	0.4	2.1	2	3.4	6.9	1.3	0.9	0.5	1.9	1.7	3.0	6.1
Diclosulam	6.9	2.5	2.2	4.2	4.1	9.7	19.3	6.4	2.1	2.0	4.7	4.6	9.6	19.3	6.1	1.7	1.3	3.4	5.2	9.0	17.9
Diflubenzuron	3.2	1.5	1.3	2.4	2.3	5.0	10.1	3.4	1.3	1.2	2.7	2.6	5.4	10.7	2.8	1.1	0.8	2.3	2.7	4.7	9.4
Dimethoate	1.5	0.4	0.3	2.8	2.4	4.0	8.0	1.9	0.9	0.8	2.7	2.6	4.4	8.7	2.3	0.7	0.9	2.3	2.2	4.1	8.2
Emamectin Benzoate	1.6	0.3	0.2	1.4	1.3	2.5	5.0	2.1	0.5	0.6	1.7	0.6	2.9	5.8	1.9	0.6	0.3	1.5	1.1	2.7	5.5
Flonicamid	3.6	1.8	1.7	2.2	2.1	5.3	10.7	3.9	1.7	1.6	2.6	2.5	5.8	11.6	3.3	1.1	0.9	2.2	2.1	4.7	9.4
Haloxyfop	2.8	0.9	0.8	1.7	1.6	3.8	7.7	3.1	1.3	1.1	2.5	2.3	4.9	9.8	3.3	1.3	0.9	2.1	1.7	4.6	9.1
Hexythiazox	2.5	1.4	1.3	2.8	2.6	4.9	9.9	2.8	1.3	1.4	3.2	3.1	5.6	11.2	2.6	1.1	0.9	3.3	3.2	5.5	10.9
Imidacloprid	1.4	0.3	0.2	1.9	1.6	2.9	5.8	2.4	0.8	0.7	2.1	2.2	4.0	8.0	2.6	0.9	0.8	1.9	1.8	3.9	7.8
Malathion	4.3	1.6	1.5	2.1	2.3	5.7	11.5	5.3	1.2	1.3	3.9	3.7	7.8	15.5	4.7	0.9	1.1	3.2	3.0	6.6	13.2
Metalaxyl	1.9	0.3	0.2	1.4	1.3	2.7	5.4	1.8	1.1	0.9	2.3	2.2	3.9	7.9	1.9	1.2	0.9	1.9	1.7	3.5	7.0
Methomyl	1.4	0.5	0.4	2.7	2.6	4.1	8.1	1.8	0.9	1.1	2.4	2.3	4.0	8.1	1.6	0.5	0.2	2.1	2.2	3.5	7.0
Monocrotophos	2.2	0.3	0.2	2.6	2.4	4.2	8.4	2.7	0.7	0.6	1.6	1.7	3.7	7.4	1.9	0.9	0.7	2.1	2	3.7	7.3
Monolinuron	2.1	0.2	0.2	1.7	1.8	3.3	6.5	2.2	0.6	0.9	2.1	2.3	4.0	7.9	2.5	0.9	0.7	1.9	1.4	3.6	7.2
Myclobutanil	4.2	1.3	1.2	2.2	2.1	5.5	11.0	3.7	1.2	1.1	2.4	2.3	5.2	10.5	3.9	1.3	1.1	2.7	2.3	5.5	11.1
Oryzalin	3.2	1.2	0.9	4.2	4.1	6.9	13.7	4.2	1.1	0.9	5.1	5.2	8.5	17.1	2.9	0.9	0.5	3.2	2.9	5.3	10.6
Penoxsulam	1.4	0.2	0.2	1.9	1.8	3.0	6.0	2.6	0.7	1.2	2.9	2.7	4.9	9.9	2.3	0.9	0.5	2.2	1.7	3.8	7.5
Prochloraz	2.1	0.4	0.3	1.6	1.5	3.1	6.2	2.4	0.9	1.1	2.1	1.9	4.0	8.0	1.9	0.7	0.5	2.1	1.8	3.5	6.9
Profenofos	3.3	0.3	0.2	1.2	1.3	3.8	7.5	4.1	1.1	0.9	2.2	2.1	5.3	10.6	3.7	1.2	0.9	3.2	3.1	6.0	12.0
Propaquizafop	3.6	1.3	1.2	2.2	1.8	4.9	9.8	3.4	0.9	1.1	2.7	2.6	5.3	10.5	3.3	1	0.9	2.3	2.1	4.7	9.5
Propiconazole	1.9	0.2	0.2	2.1	1.8	3.4	6.7	2.9	1.4	1.3	2.3	2.2	4.7	9.4	2.2	1.2	0.9	3.1	2.8	5.0	9.9
Quinalphos	1.8	0.7	0.5	1.4	1.3	2.8	5.5	1.9	1.1	1.2	2.1	2.0	3.8	7.7	1.6	0.9	0.6	2.1	1.7	3.3	6.6
Quizalofop ethyl	2.7	0.5	0.4	1.7	1.5	3.6	7.2	4.1	1.5	1.3	2.7	2.6	5.9	11.8	3.7	1.6	1.1	2.9	2.6	5.7	11.4
Spinosyn A	1.2	0.4	0.3	2.1	1.6	2.9	5.9	1.7	0.6	0.9	1.9	1.8	3.3	6.6	2.1	0.8	0.4	1.7	1.5	3.2	6.4
Spinosyn D	1.3	0.3	0.2	1.8	1.4	2.7	5.3	1.5	0.4	0.3	1.9	1.8	3.1	6.1	1.4	0.9	0.4	2.1	1.7	3.2	6.4
Thiacloprid	2.0	0.6	0.7	1.4	1.1	2.8	5.7	1.8	0.8	1.1	2.4	2.3	4.0	8.0	2.1	0.9	0.6	1.8	1.5	3.3	6.7
Thiamethoxam	5.1	2.8	2.6	5.3	5.2	9.8	19.6	5.4	1.8	1.7	3.3	3.2	7.5	15.0	4.7	1.1	0.9	3.2	3.1	6.6	13.3
Thiophenate methyl	2.7	0.4	0.3	1.3	1.2	3.3	6.5	2.9	0.5	0.9	1.7	1.8	3.9	7.9	3.1	0.8	0.6	1.9	1.7	4.1	8.3
Triasulfuron	2.6	0.9	0.7	3.1	2.9	5.1	10.2	2.9	1.6	1.5	2.3	2.2	4.8	9.7	2.4	0.6	0.5	2.1	2.0	3.8	7.7

CC: Calibration curve; GU: Global uncertainty; EU: Expanded uncertainty; U_2: day wise uncertainty associated with precision; U_3: analyst wise uncertainty associated with precision; U_4: day wise uncertainty associated with accuracy/bias; and U_5: analyst wise uncertainty associated with accuracy/bias.

[a] Calculated at 50 ng/g and expressed as '%'.

25 mg GCB and 25 mg Florisil and (vi) 25 mg PSA, 25 mg GCB and 25 mg ODS gave the statistically on per better result at 95% level of confidence (Fig. 3). But looking at the high price of ODS, Florisil is the best option as sorbent in our opinion. So the combination of PSA, GCB and Florisil is an excellent cleanup sorbent for removal of a variety of co-extractives.

3.4. Method validation

All the 42 pesticides could be analyzed by two chromatographic runs of 28 (20 + 8) min (Fig. 4). All the pesticides could be detectable at 50 ng/mL or even at lower level with the instrumental condition used in this experiment as indicated by the LOQ. Linearity of the calibration curve was established for all the pesticides. The correlation coefficient (R^2) of the calibration curve, both pure solvent-based as well as matrix-matched was ≥0.99 for most of the compounds. LOQ for all the test pesticides (Table 1) are below the maximum residue limit (MRL) values of the respective compounds in tea as fixed by the EU [9]. 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. Response enhancement due to matrix effect was also observed for some pesticides, viz. carbofuran, clothianidin, dimethoate, profenofos, propaquizafop, quinalphos and quizalofop ethyl. The slopes of the matrix-matched calibration equations were significantly different to pure solvent-based calibrations at a 95% level of statistical confidence for each of the tea matrices. An overall signal suppression by 5–86% as well as signal enhancement by 5–25% was observed irrespective of tea matrices (Table 2). However, it is not the true measurement of ion suppression or enhancement effect but a relative one to TPP in the different matrices. The matrix effect was prominent for the polar (acephate, acetamiprid and thiamethoxam) and macromolecule (abamectin, spinosyn A, spinosyn D and emamectin benzoate) compounds, and the extent varies for three matrices. The highest signal suppression (86%) was observed in case of abamectin. Considering the variable matrix influences for different compounds in mixture, the matrix-matched calibrations were used for respective matrix based quantification purposes to avoid any over or under-estimation of residues. Our method is quite satisfactory as the relative standard deviation is less than 15% for each compound analyzed in six replicates.

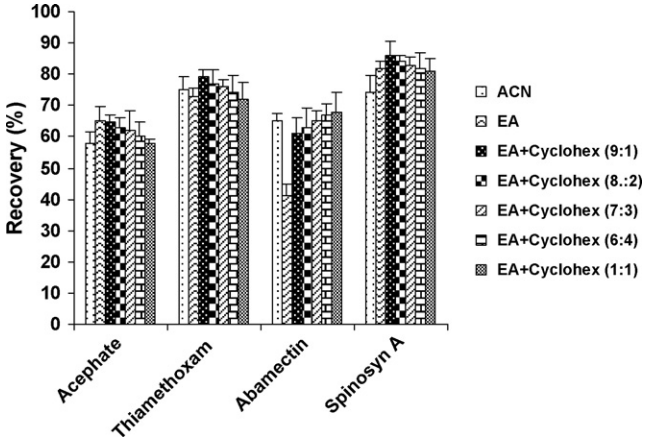

Fig. 1. Comparison of extraction capabilities of different solvent systems (10 mL) in the final method from made tea (1 g) spiked at 100 ng/g ($n = 6$). Error bars signify standard deviation.

We conducted recovery studies for 42 pesticides fortified at 50 and 100 ng/g levels in spent leaves and made tea and 50 and 100 ng/mL in tea infusion. The results (Table 2) of recovery experiment in different tea matrices gave satisfactory recovery percentage with a range 66–105% except 2,4-D, acephate and abamectin which had poor recoveries due to their limited solubility in ethyl acetate and huge matrix related interference. Some of the pesticides have the same parent ion, i.e. Propiconazole (m/z 342.1) and Bifenox (m/z 342.07); Prochloraz (m/z 376.06) and Haloxyfop (m/z 376.07); Flonicamid (m/z 230) and Dimethoate (m/z 230.04) but their respective quantifier transition as well as RTs are different. HorRat of all the analytes calculated at 50 ng/g (made tea and spent leaves) and 50 ng/mL (tea infusion) level of fortification was below 0.5 in spent leaves, made tea as well as tea infusion. Thus, the method provided satisfactory level of intra-laboratory precision and accuracy.

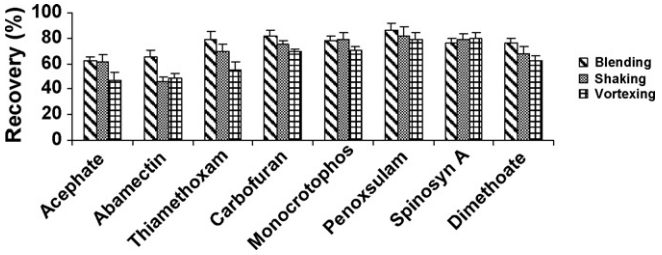

Fig. 2. Extraction capabilities of blending over shaking and vortexing in selected pesticides after 2 h of spiking of the pesticide mixture at 100 ng/g in the made tea using 10 mL ethyl acetate:cyclohexane (9:1) as extracting solvent ($n = 6$). Error bars signify standard deviation.

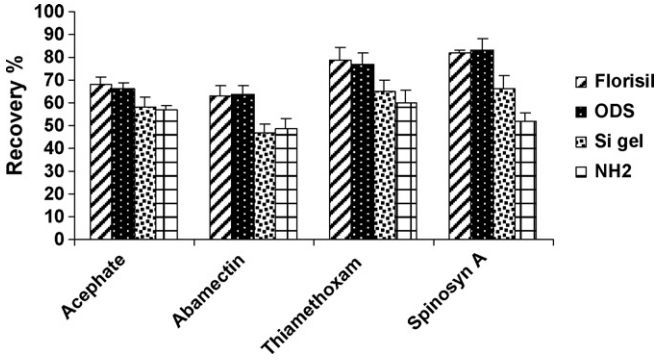

Fig. 3. Cleanup capabilities of different d-SPE sorbents (25 mg) when used with 25 mg PSA and 25 mg GCB in the final method from made tea when spiked at 100 ng/g ($n = 6$). Error bars signify standard deviation.

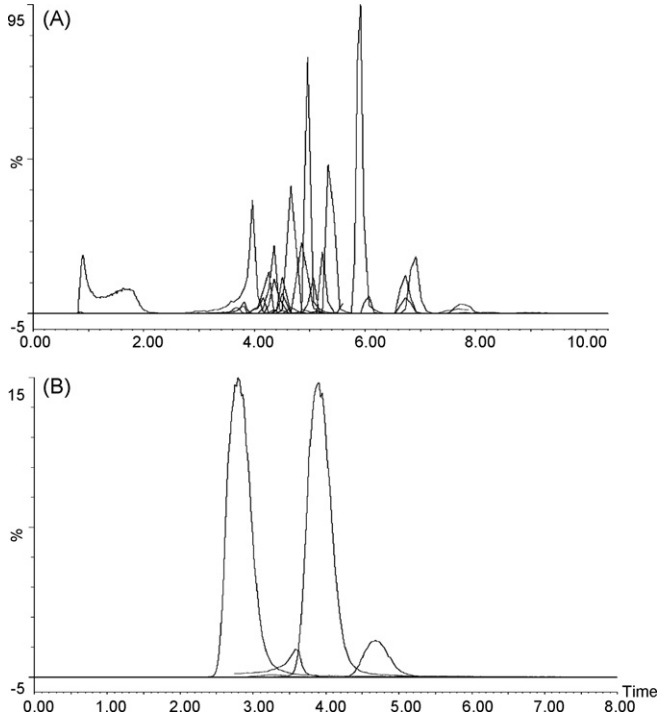

Fig. 4. LC–MS/MS chromatogram with quantifier ion, A: 38 pesticides, B: 4 pesticides in tea infusion matrix at 100 ng/mL.

3.5. Measurement of uncertainty analyses

The total uncertainty was evaluated assuming that all the contributions were independent of each other. A coverage factor of 2 was decided at a confidence level of 95% to evaluate the expanded uncertainty at 50 ng/mL of fortification (Table 3). On the basis of expanded uncertainties, the test pesticides could be classified into three groups: group I (up to 10%), group II (10–15%) and group III (15–20%). In case of spent leaves 24 pesticides could be graded as group I, 10 as group II and 8 as group III. In case of made tea and tea infusion 20 and 23 pesticides respectively were graded as group I, 11 and 12 as group II, and 11 and 7 as group III. All the pesticides, irrespective of matrices the group I pesticides had low uncertainties associated with bias (mostly below 3%), group II pesticides had higher uncertainties associated with bias (ranging around 3% and above) as compared to group I. It can therefore be concluded that the method selected for sample preparation and analysis is efficient enough and suitable for determination of pesticide residues belonging to these groups. Group III pesticides, although had low uncertainties associated with precision ($\leq$3%) but the uncertainty in bias (ranging around 5% and above) along with uncertainties associated with calibration curve (mostly above 5%) contributed hugely towards the total and in turn expanded uncertainty, which is in conformity with relatively high standard deviation, poor recovery of around 70% and higher LOQ values for 2,4-D, abamectin, acephate, carbendazim, oryzalin and thiamethoxam. This might have occurred due to instability or incomplete extraction. Special attention is required in improving the recoveries of these compounds in future endeavour.

4. Conclusion

The multiresidue analysis method proposed and validated in this work using dispersive-SPE–LC–MS/MS for sensitive identification and determined 42 pesticides in tea samples within 28 min. The extraction process using a mixture of ethyl acetate + cyclohexane (9:1; v/v) proved to be the optimal method for extracting multi-class pesticides from tea samples. With d-SPE cleanup by PSA + GCB + Florisil provided high cleanup efficiency and low matrix effects thus enabling adaptation of this sensitive and selective method for routine multiresidue analysis of pesticides in tea matrices with satisfactory recovery (66–105%). The method offers low cost of analysis as well as low level of measurement uncertainty ($\leq$20%), indicating suitability to the requirements of the International standards.

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