Detection of pesticides in popular medicinal herbs: a modified QuEChERS and gas chromatography–mass spectrometry based approach

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Abstract Although a lot of work has been conducted in the pesticide residues analysis in food matrices but the quality determination (with respect to major contaminants such as pesticide residues) in medicinal plant matrices has a long way to go. Hence a study was conducted to determine pesticide residues in four medicinal herbs, which are highly traded commodities in international market. Samples were extracted and cleaned up by modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach and analyzed by gas chromatograph–mass spectrometer (GC–MS). The method was subjected to thorough validation procedure in-terms of accuracy, precision, limits of quantification (LOQ), matrix effect, linearity and uncertainty analysis. The mean recoveries for most of the pesticides were in the range of 70–120% with RSD <20% and measurement uncertainties were less than 20% for all the compounds at spiking level of 0.5 mg kg⁻¹ in all the matrices. The limits of quantification (LOQ) ranged from 0.01 to 0.069 mg kg⁻¹. The proposed method was successfully applied to determine pesticide residues in 52 commercial market samples obtained from different locations in India.

Keywords Pesticides residues · Medicinal herbs · Gas chromatography–mass spectrometry · QuEChERS · Method validation

Introduction

According to a World Health Organization (WHO) report, around 80% of the world population relies on traditional medicines, largely based on plants (Tripathy et al. 2015). So, equal importance should be given to the safety of these herbs as it is given to the other foods. Although efforts are being made to establish the basic parameters to guarantee their quality and safety, yet lack of appropriate methods for determining specific contaminants and residues in medicinal herbs are a major challenge. Reports are available for the presence of pesticides in herbal drugs worldwide (Tripathy et al. 2015). Though most of the earlier studies focus on the determination of organochlorine pesticides (s) in medicinal herbs (Sun et al. 2007), but recently workers have reported multi pesticide residues (including fungicides, herbicides) in medicinal herbs (Tong et al. 2014). Because the growing demand of medicinal herbs requires increased agricultural production and for sustained and extensive agricultural practices needs the use of chemicals including fertilizers and number of pesticides. The detection of multi pesticide residues in medicinal herbs may also be due to the cross contamination from the cultivation sites where pesticides are applied or during storage or transportation.

Most of the conventional methodologies for pesticide residue analysis of medicinal plants, such as European Pharmacopeia (EP) procedure are costly, time consuming and require large sample size and higher quantities of hazardous solvents. New trends in pesticides residue analysis are focussing on the miniaturization of the sample preparation methodology, leading to the development of faster, cost-effective, and environmental friendly procedures, such as QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) for routine use in laboratories

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(Anastassiades et al. 2003). QuEChERS approach is often considered as the method of choice for sample preparation before analysis by gas and liquid chromatography coupled to mass spectrometry, e.g. GC/MS, LC/MS (Du et al. 2011; Chen et al. 2013). This technique not only provides qualitative and quantitative determination but also is very important in confirmation of the residues as the pesticides determination in medicinal herbs of unidentified pesticide treatment history is a difficult task, because it involves the identification and quantification of several hundred possible single compounds or combinations in the presence of complex matrices. Although India has a rich biodiversity of medicinal herbs, there are few reports on the development of standard and validated methods for assessing their quality (Rai et al. 2008; Tripathy et al. 2016). Some of the popular and economically important medicinal plants grown and traded in India are *Withania somnifera* (Ashwagandha), *Plantago ovata* (Isabgol), *Cassia angustifolia* (Senna) and *Andrographis paniculata* (Kalmegh). Therefore, an urgent need was realized to develop a standard method for the determination of multi class pesticides in the popular medicinal herbs. With this background in mind, a modified QuEChERS based method was studied for the determination of 11 multi-class pesticides in the economic parts of the medicinal plants, viz., the roots of ashwagandha, seeds of isabgol, leaves of senna and aerial part (herbage) of kalmegh followed by GC–MS analysis.

Materials and methods

Chemicals and reagents

All the pesticide standards were of >98% purity and purchased from Sigma Aldrich, Germany. Chromatography grade solvents (n-hexane, acetonitrile, toluene and water) and analytical grade reagents (anhydrous magnesium sulphate (MgSO_4) and sodium sulfate) were purchased from Merck India Ltd., Mumbai, India. Primary secondary amine (PSA, 40 μm , Bondesil) and C18 sorbent was purchased from Agilent Technologies, the USA.

Sample materials

Roots of ashwagandha (*W. somnifera* L.), isabgol (*P. ovata*) seed with husk, senna (*C. angustifolia* L.) leaves, and aerial part (herbage) of kalmegh (*A. paniculata*) were obtained from the field of ICAR-Directorate of Medicinal and Aromatic Plants Research (DMAPR), Anand, Gujarat, India with no history of pesticide use and used as a control sample. These samples were used as blanks, fortified samples for recovery assays and matrix-matched standards for calibration in the comparison of methods. About 1 kg

portion of the economic part of each herb was air-dried, cut and grounded. Samples were stored at 4 °C. The market samples (Total 52 samples: ashwagandha (n = 14), senna (n = 13), isabgol (n = 20) and kalmegh (n = 5)) were procured from the Agricultural Produce Marketing Cooperation (APMC) wholesale/retail markets of Anand and Unjha (Gujarat), Mandsaur (Madhya Pradesh), Delhi and Udaipur (Rajasthan) in India.

Preparation of standard solutions

The mixed stock solutions (100 $\mu\text{g mL}^{-1}$) and working standard solutions (0.05–2.5 $\mu\text{g mL}^{-1}$) of different pesticides were prepared in HPLC grade n-hexane/toluene (1:1v/v) and stored at –20 °C (± 1 °C).

Extraction

Extraction was carried out according to a modified version of the QuEChERS method (Anastassiades et al. 2007). To prepare sample extracts for GC–MS analysis, two grams of the homogenised dry herb sample was weighed into a 50 mL centrifuge tube and analyte solution were added (except for the matrix matched calibration, in which analytes were added after the QuEChERS procedure). Samples and standards were carefully mixed and left at room temperature for at least 15 min before the extraction procedure. The samples were dissolved in 13 mL of Milli-Q water, extracted with 15 mL of 1% acetic acid in acetonitrile, vortexed for 30 s and kept in deep freezer (–20 °C) for 30 min. A buffer-salt mixture (6 g anhydrous MgSO_4 + 1.5 g of sodium sulfate) was added for phase separation and pesticide partitioning. The samples were then centrifuged at 3500 rpm for 2 min. The supernatant (2 mL) was concentrated under gentle stream of nitrogen (15 Ψ) in the Turbovap LV (Rapid Vap Labconco, Kansas City, The USA) at 40 °C and reconstituted in 2 mL of n-hexane for analysis in the GC–MS without cleanup or subjected to cleanup with the sorbents as detailed below.

Cleanup

Dispersive solid phase extraction (d-SPE) cleanup with PSA or C18 was compared with no-cleanup (no sorbent) in terms of analyte recovery and interference from the study matrices. After centrifugation at 3500 rpm for 2 min as described in the extraction, 6 mL of supernatant was transferred to 15 mL centrifuge tubes containing 300 mg of PSA or C18 along with MgSO_4 (0.9 g). The extract was vortexed and centrifuged for 2 min at 2500 rpm. The supernatant (2 mL) was transferred to turbo tube and concentrated to near dryness under a gentle stream of nitrogen in the Turbovap LV as described earlier. The

residues were reconstituted in 2 mL of n-hexane for multi pesticide residue analysis in GC–MS.

Apparatus and GC–MS conditions

For the detection and quantification of pesticide residues in the medicinal herbs, samples were analysed on GC/MS (Focus–Polaris Q) bench top ion trap mass spectrometer detector in Electron Ionization mode and Thermo triplus autosampler. GC separation was performed on a DB-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm) with the following operating conditions; initial oven temperature 120 °C, held for 3 min, then a 5 °C min^{−1} ramp to 290 °C and held for 10 min; carrier gas He constant flow @ 1.0 mL min^{−1}, injection volume 1 µl (splitless), the temperature for Inlet, Ion source and MS transfer line was 250; 230 and 290 °C, respectively; Total run time was 47 min. Selective ions monitored for the different pesticides are given in Table 1.

Method validation parameters

The performance of the analytical method was assessed as per the single laboratory validation approach (European Commission. Document SANCO/10684/2009; Thompson et al. 2002) The method performance was evaluated by

considering the validation parameters viz., linearity, limit of quantification (LOQ), matrix effect, accuracy and precision. All the analyses were performed using the same blank samples.

Linearity

Calibration curves for all of the target analytes were drawn by plotting the peak area against the concentration of the corresponding calibration standards at five calibration levels ranging between 0.05 and 2.5 µg mL^{−1}.

Sensitivity

Limit of quantification (LOQ) of each analyte was determined by considering a signal-to-noise ratio of 10 at the lowest fortified level of the respective pesticides in the different herb matrices.

Matrix effects

Matrix effect (ME) in terms of signal suppression or enhancement due to the co-elution of matrix components was evaluated by post-extraction spiking and compared with the solvent standard. The matrix effect was evaluated by spiking pesticide free medicinal herb samples with the

Table 1 GC–MS parameters, linear equations, linear ranges, regression coefficients (R^2) and LOQ for different pesticides

Pesticides	Rt (mins)	Mol wt.	Selective ions monitored	Linear equation	Linear range (µg mL ^{−1})	R^2	LOQ (mg kg ^{−1})			
α-HCH	14.83	288	181, 183, 290, 260	$y = 30425x - 2800$	0.05–2.5	0.993	Ashwagandha	Isabgol	Senna	Kalmegh
Atrazine	15.95	215	68, 173, 200, 215	$y = 43086x + 49894$	0.05–2.5	0.979	0.015	0.01	0.018	0.01
γ-HCH	16.16	288	181, 183, 290, 260	$y = 39444x + 12320$	0.05–2.5	0.988	0.032	0.02	0.039	0.041
Chlorothalonil	17.36	266	266, 264, 268, 270	$y = 34593x + 19016$	0.05–2.5	0.988	0.01	0.01	0.02	0.023
Chlorpyrifos	20.60	349	314, 199, 197, 258, 260, 286, 316	$y = 55302x + 27993$	0.05–2.5	0.993	0.04	0.032	0.049	0.034
Pendimethalin	21.83	281	281, 252, 220	$y = 55874x + 24324$	0.05–2.5	0.994	0.024	0.02	0.02	0.028
α-Endosulfan	23.12	404	241, 195, 207, 209, 193, 206	$y = 67107x + 46616$	0.05–2.5	0.996	0.031	0.029	0.036	0.028
Profenofos	23.96	372	339, 139, 337, 338, 267, 208, 97	$y = 27909x + 18023$	0.05–2.5	0.996	0.038	0.037	0.046	0.041
β-Endosulfan	25.24	404	241, 207, 209, 193, 232, 159	$y = 48907x + 41094$	0.05–2.5	0.996	0.049	0.053	0.069	0.051
Endosulfan sulphate	26.8	420	272, 274, 239, 253, 208, 288, 193	$y = 37223x + 17395$	0.05–2.5	0.995	0.037	0.041	0.05	0.048
Trifloxystrobin	27.05	408	116, 131, 222	$y = 55636x + 31268$	0.05–2.5	0.995	0.026	0.021	0.036	0.029

pesticide mixture at 0.5 mg kg^{-1} level. The peak area response of individual pesticides in solvent was compared with that of the corresponding response in the matrix-matched standard at the same concentration level. A negative and positive value of ME (%) indicates matrix-induced signal suppressions or enhancement, respectively.

The matrix effect was estimated by the following method:

$$\text{Matrix effect (ME, \%)} = (A_2 - A_1) \times 100/A_1,$$

where, A_1 is the average area of the pesticide standard in pure solvent and A_2 is the average area of the pesticide standard in the extracts of the pesticide-free samples.

Accuracy and precision of the method

The accuracy was determined as % recovery at three different fortification levels (0.1 , 0.5 and 2 mg kg^{-1}). Precision was evaluated in terms of repeatability at three different fortification levels, with three replicates at each level on the same day (intra-day precision), and reproducibility (inter-day precision) was calculated with three replicates at each of the three concentration levels on three different days. The numerical values of precision were expressed by relative standard deviation of replicate measurements of the analytes, using the equation

$$\text{RSD} = (\text{SD}/\text{mean}) \times 100,$$

where SD = standard deviation of replicates.

Determination of uncertainties

Combined uncertainty was determined for all the pesticides at 0.5 mg kg^{-1} spiked level as per the statistical procedure of the EURACHEM/CITAC Guide CG 4. Five individual sources of uncertainty were taken into account which are 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) were evaluated for all the pesticides in all the matrices.

The combined uncertainty (U) was calculated as $U = (U_1^2 + U_2^2 + U_3^2 + U_4^2 + U_5^2)^{1/2}$ and reported as expanded uncertainty ($2U$) which is twice the value of the combined uncertainty at 95% confidence level.

Results and discussion

Selection of the sample extraction method

Determination of residual pesticides in medicinal herbs is a difficult task since it contains various pigments and

secondary metabolites. For sample preparation, the current widely used quick, easy, cheap, effective, rugged and safe (QuEChERS) strategy was employed as this is a very flexible approach and offers many options for analysis depending on the range of pesticides and matrices being tested. After a careful review of the bibliography in this study, we chose to use acetonitrile because of its effectiveness for polar and non-polar pesticides from a diverse range of matrices and also give high recoveries of a wide polarity range of pesticides (Anastassiades et al. 2003; Maštovská and Lehotay, 2004). The physicochemical and practical advantages of acetonitrile over other solvent like ethyl acetate in pesticide residue analyses have already been discussed in detail by earlier workers (Anastassiades et al. 2003; Maštovská and Lehotay, 2004; Lehotay et al. 2010; Ramasubramanian et al. 2014). Also, we lowered the sample amount (to 2 g) and added water (13 mL) before proceeding with the acetonitrile extraction to enable the extraction solvent to thoroughly penetrate better in the dry plant tissues and ensure complete transfer of the analytes from naturally contaminated samples (Lacina et al. 2012). So for further study, modified QuEChERS-based approach with acetonitrile as an extraction solvent was used.

Optimization of clean up procedure

Routine analysis of pesticide residues in complex matrices requires adequate cleanup of the extract with the proper choice of sorbent(s) which is critical for reduction of co-extractives in order to enhance sensitivity (with less noise) and facilitates identification and quantification of low concentration of pesticides. The QuEChERS technique inherently involves a dispersive-solid phase extraction (dispersive-SPE) step, in which the sorbents like primary secondary amine (PSA) is used primarily to remove the organic acids and other polar matrix compounds while C18 is used for its strong affinity for fats and lipids. The use of graphitized carbon black (GCB) was avoided as it adsorbs pesticides with planar functionality leading to unsatisfactory recoveries of a number of pesticides susceptible to this adsorption (Walorczyk, 2014; Walorczyk et al. 2015). So, PSA and C18 were selected for the clean-up of samples at 0.5 mg kg^{-1} spiked level after solvent extraction to investigate the influence on recovery in four matrices.

Recoveries of all the pesticides were in the range of 64.5–96.4%, 61.0–92.5%, 53.4–89.2% and 59.2–91.1% in ashwagandha, isabgol, senna and kalmegh, respectively when the samples were processed without any cleanup (Table 2). Among the two sorbents tested, PSA was superior to C18 in all the matrices and it was also higher as compared to the no-clean method. The recoveries of most of the pesticides (except atrazine, chlorothalonil, α -endosulfan, β -endosulfan and endosulfan sulfate) decreased

Table 2 Cleanup capabilities of sorbents in different medicinal herbs fortified with 0.5 mg kg⁻¹ of the pesticides

Medicinal herbs	Sorbent	Pesticides										
		α -HCH	Atrazine	γ -HCH	Chlorothalonil	Chlorpyrifos	Pendimethalin	α -Endosulfan	Profenofos	β -Endosulfan	Endosulfan sulphate	Trifloxystrobin
Ashwagandha	No cleanup	94.4 $\pm$ 10.9	77.3 $\pm$ 9.4	96.4 $\pm$ 7.7	68.0 $\pm$ 10.5	76.9 $\pm$ 11.5	87.5 $\pm$ 8.1	74.1 $\pm$ 6.8	64.5 $\pm$ 8.8	72.2 $\pm$ 18.4	69.8 $\pm$ 6.4	68.1 $\pm$ 7.7
	PSA	107.3 $\pm$ 2.0	82.8 $\pm$ 5.4	115.5 $\pm$ 5.2	73.0 $\pm$ 7.2	83.7 $\pm$ 3.7	96.9 $\pm$ 1.7	83.4 $\pm$ 7.2	70.6 $\pm$ 4.8	80.4 $\pm$ 4.7	72.8 $\pm$ 5.6	74.0 $\pm$ 6.1
	C18	81.8 $\pm$ 12.2	74.7 $\pm$ 19.3	81.3 $\pm$ 5.4	70.6 $\pm$ 6.7	71.0 $\pm$ 12.0	73.6 $\pm$ 15.7	82.2 $\pm$ 13.1	59.0 $\pm$ 19.1	84.2 $\pm$ 15.1	73.3 $\pm$ 10.9	55.4 $\pm$ 9.4
Isabgol	No cleanup	92.5 $\pm$ 16.0	67.5 $\pm$ 12.7	91.9 $\pm$ 12.5	64.1 $\pm$ 11.4	71.9 $\pm$ 15.9	71.4 $\pm$ 18.3	77.0 $\pm$ 14.3	63.0 $\pm$ 18.9	77.9 $\pm$ 11.9	71.8 $\pm$ 12.0	61.0 $\pm$ 7.1
	PSA	112.3 $\pm$ 3.1	82.3 $\pm$ 5.1	119.7 $\pm$ 5.6	74.3 $\pm$ 10.2	85.7 $\pm$ 4.9	80.7 $\pm$ 11.2	83.3 $\pm$ 17.2	65.3 $\pm$ 14.5	85.3 $\pm$ 14.0	75.7 $\pm$ 8.6	64.3 $\pm$ 19.0
	C18 $\pm$	83.9 $\pm$ 10.2	69.5 $\pm$ 6.3	78.6 $\pm$ 12.2	73.0 $\pm$ 10.0	61.1 $\pm$ 7.8	63.1 $\pm$ 7.7	81.4 $\pm$ 3.3	53.4 $\pm$ 6.2	82.7 $\pm$ 16.7	74.0 $\pm$ 12.0	51.4 $\pm$ 18.1
Senna	No cleanup	89.2 $\pm$ 15.6	61.6 $\pm$ 13.8	87.6 $\pm$ 9.9	64.9 $\pm$ 12.8	74.4 $\pm$ 12.2	75.4 $\pm$ 9.5	63.3 $\pm$ 15.5	70.5 $\pm$ 2.1	71.2 $\pm$ 17.8	72.3 $\pm$ 4.2	53.4 $\pm$ 9.7
	PSA	97.2 $\pm$ 7.7	73.5 $\pm$ 13.6	91.0 $\pm$ 3.0	71.7 $\pm$ 15.5	83.4 $\pm$ 6.5	79.1 $\pm$ 5.9	71.8 $\pm$ 9.2	68.7 $\pm$ 8.6	72.1 $\pm$ 9.4	76.0 $\pm$ 10.2	66.0 $\pm$ 11.2
	C18	78.1 $\pm$ 14.3	71.3 $\pm$ 16.6	79.6 $\pm$ 10.6	69.0 $\pm$ 8.6	62.4 $\pm$ 12.2	61.6 $\pm$ 18.0	69.8 $\pm$ 16.4	43.1 $\pm$ 13.4	72.3 $\pm$ 6.0	75.5 $\pm$ 11.8	36.5 $\pm$ 12.7
Kalmegh	No cleanup	91.1 $\pm$ 6.7	63.3 $\pm$ 16.9	88.6 $\pm$ 16.5	61.9 $\pm$ 6.2	69.9 $\pm$ 9.3	79.3 $\pm$ 14.0	68.2 $\pm$ 11.6	65.5 $\pm$ 6.0	73.9 $\pm$ 15.0	81.2 $\pm$ 111.9	59.2 $\pm$ 3.7
	PSA	100.2 $\pm$ 4.4	76.7 $\pm$ 8.8	98.3 $\pm$ 11.2	77.0 $\pm$ 6.7	85.4 $\pm$ 10.0	85.9 $\pm$ 10.5	79.4 $\pm$ 17.6	72.5 $\pm$ 5.1	78.9 $\pm$ 7.5	87.9 $\pm$ 3.8	71.4 $\pm$ 12.7
	C18	83.9 $\pm$ 16.5	76.6 $\pm$ 14.6	81.2 $\pm$ 10.8	71.0 $\pm$ 10.7	64.4 $\pm$ 6.5	66.9 $\pm$ 7.6	78.3 $\pm$ 12.5	58.3 $\pm$ 11.3	77.3 $\pm$ 6.8	86.6 $\pm$ 8.1	46.8 $\pm$ 16.8

significantly when C18 was used. Use of C18 decreases the recoveries of α -HCH, γ -HCH, chlorpyrifos, pendimethalin, profenofos and trifloxystrobin up to 13.4, 13.8, 16.0, 18.2, 38.9 and 31.6% respectively (Table 2). Although Lehotay et al. (2010) has shown that the addition of C18 along with PSA had no adverse effect on the recoveries of analytes. But in our study we have found decreased recoveries of some of the analytes in C18-based cleanup as compared to the no-cleanup. Such loss in recovery may be due to the non-polar nature of these compounds. Johnson, 2012 reported reduced recoveries of some strongly non-polar polyaromatic hydrocarbons with C18 clean-up. Ramasubramanian et al. (2014) also found lower recoveries of fipronil and its metabolites in sugarcane juices with increased amounts of C18 from 10 to 50 mg g⁻¹ of matrix. As the PSA-based cleanup showed higher recoveries compared to the no-cleanup procedure, the former has been chosen to optimize the extraction procedure.

Method validation parameters

Linearity and sensitivity

The calibration curves were obtained using the linear least square regression procedure of the peak area versus the concentration of different pesticides. The linearity for different pesticides, in the working standard solutions of five concentration levels, ranged between 0.05 and 2.5 $\mu\text{g mL}^{-1}$ while the correlation coefficient (R^2) >0.979 (Table 1).

Method sensitivity was evaluated in terms of limit of quantification (LOQ) and it was established at the lowest fortified level in each matrix that yielded an S/N ratio of 10:1. The LOQ of the pesticides in ashwagandha, isabgol,

senna and kalmegh were in the range of 0.01–0.049, 0.01–0.053, 0.018–0.069 and 0.01–0.051 mg kg⁻¹, respectively (Table 1). A representative chromatogram (Fig. 1a, b) showed no interference from matrix of ashwagandha.

Evaluation of matrix effect

Matrix components, which are unavoidably present in the samples, may adversely affect the identification/quantification of analytes by the instrument. For this, European Commission residues analysis criteria require matrix-matched standards if the matrix effect (ME) exceeds the threshold limit of 20% (European Commission Document SANCO/10684/2009). The ME was evaluated by spiking untreated herb samples with the pesticide mixture at spiking level 0.5 mg kg⁻¹. As shown in Fig. 2, values of MEs for most of the pesticides in different matrices were pesticide and matrix specific. Though the values were between –20 and +20% for most of the pesticides, considered as no ME, but it was prominent for a few pesticides, viz., atrazine, chlorothalonil, chlorpyrifos, endosulfan sulphate in ashwagandha matrix, chlorpyrifos, endosulfan sulphate in isabgol matrix; chlorothalonil, chlorpyrifos, profenofos, β -endosulfan, endosulfan sulphate and trifloxystrobin in senna matrix; profenofos, endosulfan sulphate in kalmegh matrix. Among the four matrices senna leaves and ashwagandha roots were found to have maximum suppression/enhancement effect. Among the pesticides, signal of chlorothalonil, chlorpyrifos, profenofos, β -endosulfan and endosulfan sulphate were remarkably affected by the different matrices. Though the matrix blank shows no co-extractives from medicinal herbs, still matrix suppression was found for some compounds. This may be due to the

Fig. 1 Representative GC–MS chromatogram of ashwagandha control (a) and matrix-matched standard (b)

Fig. 2 Matrix effects of different pesticides in pesticide free medicinal herbs matrices analysed by GC–MS

fact that the ME not only associated with samples but also the pesticides. Because the pesticides with sulfate, phosphate, hydroxyl, azoles, amino, carboxyl groups are the most susceptible type of analytes to ME. So, to overcome the variable ME, the quantification was done using matrix-matched standards.

Accuracy-recovery and precision of the method

The accuracy was determined as % recovery at three different spiking levels (0.1, 0.5 and 2 mg kg⁻¹). The recovery of pesticides from ashwagandha roots at 0.1 mg kg⁻¹ of spiking level ranged between 73.5 (endosulfan sulphate)–114.7% (γ-HCH) with the exception of chlorothalonil, profenofos, and trifloxystrobin (Table 3). Recovery ranged from 70.6 (profenofos)–115.5% (γ-HCH) and 70.3 (trifloxystrobin)–108.5% (α-HCH) at 0.5 and 2 mg kg⁻¹ levels, respectively (Table 3). Thus, the optimized method is appropriate (acceptable range 70–120%) for the determination of most of the pesticides in ashwagandha root matrix. The recoveries of pesticides from isabgol matrix, ranged from 72.0 (profenofos)–113.7% (α-HCH and γ-HCH); 74.3 (chlorothalonil)–119.7% (γ-HCH) and 73.6 (chlorothalonil)–112.5% (γ-HCH) at 0.1, 0.5 and 2 mg kg⁻¹ levels, respectively. Profenofos recovery at 0.5 and 2 mg kg⁻¹ of spiking level and trifloxystrobin recovery at all spiking level were lower than the acceptable range (Table 3).

Pesticide recoveries in senna leaf matrix (Table 3) were 73.6 (β-endosulfan)–98.4% (α-HCH); 71.7 (chlorothalonil)–97.2% (α-HCH) and 74.8 (β-endosulfan)–107.2% (α-HCH) at 0.1, 0.5 and 2 mg kg⁻¹ levels, respectively. However, there were exceptions such as profenofos and trifloxystrobin at all the spiking levels; endosulfan sulphate at 0.1 mg kg⁻¹ and chlorothalonil at 0.1 and 2.0 mg kg⁻¹

levels showed the recovery below the acceptable range. Recoveries of pesticides from kalmegh matrix ranged from 74.3 (endosulfan sulphate) to 114.0% (pendimethalin); from 71.4 (trifloxystrobin) to 100.2% (α-HCH) and 70.7 (chlorothalonil) to 109.9% (α-HCH) at 0.1, 0.5 and 2 mg kg⁻¹ spiking levels, respectively, with the exceptions of chlorothalonil, profenofos and trifloxystrobin at 0.1 mg kg⁻¹ level.

The precision of the method was determined by repeatability and reproducibility studies, expressed by RSD. The repeatability RSD_r (intra-day precision) was measured by comparing standard deviation of the % recovery of pesticides run on the same day and it was three alternate days for reproducibility RSD_R (inter-day precision). Intraday precision was lower than 20, 19, 18 and 15% for ashwagandha, isabgol, senna and kalmegh, at three concentration levels (0.1, 0.5 and 2 mg kg⁻¹). Only profenofos shows >26% RSD_r in kalmegh matrix (Table 3). The interday precision was lower than 17, 20, 16 and 20% (Table 3) for ashwagandha, isabgol, senna and kalmegh, respectively, with exception of trifloxystrobin (>32%) in kalmegh matrix.

Estimation of measurement uncertainty

There can be many potential sources of uncertainties arising from individual steps of the described multi-residue method, the total uncertainty was evaluated taking five main independent uncertainties arising out of, GC–MS calibration graph and day-wise and analyst-wise uncertainty associated with precision and accuracy/bias.

The expanded uncertainty of the pesticides could be categorized into three ranges viz., (a) <10% (b) 10–15% and (c) 15–20% (Table 4). In case of ashwagandha, 9 pesticides out of the total of 11 pesticides fall in the range

Table 3 Recovery and precision (%) of different pesticides from fortified samples of ashwagandha, isabgol, senna and kalmegh

Spiking level mg kg ⁻¹	0.1			0.5			2.0		
Pesticides	Recovery	RSD _r	RSD _R	Recovery	RSD _r	RSD _R	Recovery	RSD _r	RSD _R
Ashwagandha									
α -HCH	103.7	5.1	7.5	107.3	2.0	15.4	108.5	7.9	11.8
Atrazine	78.3	3.7	7.1	82.8	5.4	7.6	71.6	15.4	16.1
γ -HCH	114.7	2.7	4.1	115.5	5.2	6.2	97.3	7.6	14.6
Chlorothalonil	69.7	12.6	11.3	73.0	7.2	11.8	72.2	19.2	13.1
Chlorpyrifos	81.1	6.9	7.0	83.7	3.7	7.2	98.3	12.2	16.2
Pendimethalin	95.0	3.8	10.6	96.9	1.7	7.5	92.1	8.7	10.6
α -endosulfan	87.3	7.7	16.6	83.4	7.2	14.6	82.6	5.5	11.2
Profenofos	66.2	9.9	10.1	70.6	4.8	7.7	64.4	11.6	10.8
β -endosulfan	83.1	7.0	6.2	80.4	4.7	9.2	85.5	13.7	13.0
Endosulfan sulphate	73.5	4.7	11.8	72.8	5.6	11.6	81.7	14.8	8.9
Trifloxystrobin	67.2	4.1	9.1	74.0	6.1	12.1	70.3	17.8	12.0
Isabgol									
α -HCH	113.7	6.7	14.6	112.3	3.1	5.3	88.8	10.7	12.2
Atrazine	80.7	7.0	9.0	82.3	5.1	9.4	77.6	14.0	16.1
γ -HCH	113.7	9.3	6.4	119.7	5.6	11.8	112.5	6.3	6.7
Chlorothalonil	74.3	8.8	7.0	74.3	10.2	6.6	73.6	9.7	15.0
Chlorpyrifos	89.3	7.6	5.9	85.7	4.9	10.7	97.0	8.7	8.4
Pendimethalin	109.0	2.4	7.9	80.7	11.2	12.5	92.3	13.9	11.6
α -endosulfan	87.7	4.7	3.2	83.3	17.2	12.8	88.0	9.6	8.3
Profenofos	72.0	6.4	7.3	65.3	14.5	11.6	65.5	17.1	18.3
β -endosulfan	85.7	9.9	6.7	85.3	14.0	9.5	86.7	7.9	13.7
Endosulfan sulphate	74.0	11.1	13.2	75.7	8.6	7.2	85.5	6.9	19.1
Trifloxystrobin	68.0	10.3	7.1	64.3	19.0	14.1	67.0	19.0	13.2
Senna									
α -HCH	98.4	12.6	10.1	97.2	7.7	7.2	107.2	9.5	7.9
Atrazine	77.7	14.5	12.4	73.5	13.6	11.2	76.9	11.8	12.7
γ -HCH	87.3	11.7	15.3	91.0	3.0	8.9	98.5	17.6	12.3
Chlorothalonil	65.2	12.9	12.5	71.7	15.5	12.8	65.9	16.1	11.8
Chlorpyrifos	83.8	6.7	10.7	83.4	6.5	6.3	88.9	5.7	11.5
Pendimethalin	93.3	3.6	7.6	79.1	5.9	12.9	78.9	14.0	13.6
α -endosulfan	76.3	9.7	11.8	71.8	9.2	10.6	74.8	10.2	10.4
Profenofos	63.5	8.3	7.6	68.7	8.6	12.2	66.5	13.9	15.2
β -endosulfan	73.6	7.7	12.8	72.1	9.4	12.0	74.8	10.1	7.5
Endosulfan sulphate	68.4	13.1	13.9	76.0	10.2	8.9	79.3	12.5	10.1
Trifloxystrobin	56.6	14.3	14.2	66.0	11.2	15.0	69.9	1.8	15.4
Kalmegh									
α -HCH	91.0	13.7	14.7	100.2	4.4	11.4	109.9	6.6	8.8
Atrazine	79.0	14.7	13.3	76.7	6.7	19.3	73.1	5.1	12.1
γ -HCH	89.3	11.4	16.8	98.3	11.0	7.2	105.0	13.2	13.3
Chlorothalonil	68.8	11.7	10.6	77.0	5.2	10.8	70.7	9.3	12.5
Chlorpyrifos	86.9	10.3	9.3	85.4	8.6	16.5	79.7	4.6	13.9
Pendimethalin	114.0	7.6	17.9	85.9	9.0	15.4	89.3	11.1	9.3
α -endosulfan	89.5	10.8	13.0	79.4	14.0	14.3	76.9	7.8	12.3
Profenofos	68.3	26.8	17.4	72.5	3.7	10.5	72.0	10.7	12.3
β -endosulfan	79.9	6.6	9.0	78.9	5.9	15.8	81.8	8.2	15.0
Endosulfan sulphate	74.3	10.8	11.5	87.9	3.4	17.9	71.0	12.7	16.3
Trifloxystrobin	51.2	7.9	32.5	71.4	9.1	15.4	71.6	5.7	14.4

Table 4 Results of individual and global uncertainties for each pesticide calculated at 0.5 mg kg⁻¹ spiked level in different medicinal herbs

Medicinal herbs	Uncertainties	Pesticides										Trifloxystrobin
		α -HCH	Atrazine	γ -HCH	Chlorothalonil	Chlorpyrifos	Pendimethalin	α -Endosulfan	Profenofos	β -Endosulfan	Endosulfan sulphate	
Ashwagandha	U1	1.09	2.68	1.51	2.31	1.89	2.58	1.99	2.89	1.85	2.65	3.26
	U2	1.43	3.90	1.72	3.77	2.62	3.44	2.15	3.75	4.47	3.76	3.51
	U3	5.13	2.54	2.07	3.93	2.39	2.51	4.85	2.55	3.06	3.88	4.02
	U4	1.57	3.14	2.00	2.99	2.21	2.99	1.86	2.57	3.92	2.60	2.34
	U5	5.28	1.97	2.42	2.68	2.05	2.42	4.28	1.70	2.43	2.98	2.82
	U	7.74	6.52	4.41	7.15	5.03	6.29	7.34	6.19	7.35	7.20	7.25
	2U	15.47	13.05	8.81	14.30	10.05	12.58	14.69	12.39	14.70	14.40	14.51
	U1	1.09	2.68	1.51	2.31	1.89	2.58	1.99	2.89	1.85	2.65	3.26
	U2	3.60	3.25	1.75	3.79	3.27	4.36	4.21	3.59	5.22	3.59	4.47
	U3	1.76	3.12	3.93	2.20	3.56	4.17	4.26	3.87	3.17	2.40	4.70
Isabgol	U4	1.57	3.14	2.00	2.99	2.21	2.99	1.86	2.57	3.92	2.60	2.34
	U5	5.28	1.97	2.42	2.68	2.05	2.42	4.28	1.70	2.43	2.98	2.82
	U	6.90	6.42	5.54	6.38	6.00	7.60	7.85	6.76	7.87	6.43	8.13
	2U	13.80	12.84	11.08	12.76	12.01	15.21	15.70	13.51	15.74	12.86	16.26
	U1	1.09	2.68	1.51	2.31	1.89	2.58	1.99	2.89	1.85	2.65	3.26
	U2	3.04	3.61	5.47	4.13	4.34	4.62	2.85	3.18	4.68	4.08	4.09
	U3	2.38	3.73	2.97	4.26	2.10	4.29	3.52	4.08	3.99	2.96	5.01
	U4	3.28	2.57	5.23	2.76	3.59	3.25	2.09	2.10	3.54	3.24	2.50
	U5	2.33	2.52	2.73	3.02	1.81	3.54	2.66	2.68	2.95	2.17	3.00
	U	5.68	6.86	8.71	7.56	6.56	8.33	6.00	6.83	7.91	6.91	8.23
Senna	2U	11.36	13.71	17.42	15.13	13.11	16.67	11.99	13.66	15.81	13.81	16.45
	U1	1.09	2.68	1.51	2.31	1.89	2.58	1.99	2.89	1.85	2.65	3.26
	U2	3.16	3.79	3.33	4.01	3.19	3.70	4.50	3.31	4.38	2.41	3.04
	U3	3.79	6.42	2.41	3.59	5.49	5.14	4.75	3.49	5.27	5.97	5.15
	U4	3.45	2.82	3.61	2.94	2.76	3.29	3.58	2.30	3.71	2.05	2.15
	U5	3.79	5.19	2.31	2.56	5.32	4.14	4.22	2.51	4.88	5.14	3.55
	U	7.20	9.89	6.13	7.04	8.93	8.64	8.80	6.57	9.38	8.90	7.97
	2U	14.40	19.77	12.26	14.07	17.86	17.29	17.60	13.13	18.76	17.80	15.95
	U1	1.09	2.68	1.51	2.31	1.89	2.58	1.99	2.89	1.85	2.65	3.26
	U2	3.16	3.79	3.33	4.01	3.19	3.70	4.50	3.31	4.38	2.41	3.04
Kalmegh	U3	3.79	6.42	2.41	3.59	5.49	5.14	4.75	3.49	5.27	5.97	5.15
	U4	3.45	2.82	3.61	2.94	2.76	3.29	3.58	2.30	3.71	2.05	2.15
	U5	3.79	5.19	2.31	2.56	5.32	4.14	4.22	2.51	4.88	5.14	3.55
	U	7.20	9.89	6.13	7.04	8.93	8.64	8.80	6.57	9.38	8.90	7.97
	2U	14.40	19.77	12.26	14.07	17.86	17.29	17.60	13.13	18.76	17.80	15.95
	U1	1.09	2.68	1.51	2.31	1.89	2.58	1.99	2.89	1.85	2.65	3.26
	U2	3.16	3.79	3.33	4.01	3.19	3.70	4.50	3.31	4.38	2.41	3.04
	U3	3.79	6.42	2.41	3.59	5.49	5.14	4.75	3.49	5.27	5.97	5.15
	U4	3.45	2.82	3.61	2.94	2.76	3.29	3.58	2.30	3.71	2.05	2.15
	U5	3.79	5.19	2.31	2.56	5.32	4.14	4.22	2.51	4.88	5.14	3.55

(b), 1 pesticide each fall in the range (a) and (c) range. In case of isabgol, 7 out of 11 pesticides have uncertainty 10–15%, and rest 4 has more than 15% of uncertainty. In case of senna, 6 pesticides fall in the range (b) followed by 5 in the range of (c). In case of kalmegh, 7 pesticides out of the total of 11 pesticides fall in the range (c) and rest 4 in the range of (b). Therefore it can be assumed that for the multi-residue method adopted, most of the pesticides falling in the range (b) for different matrices discussed. The uncertainty associated with precision accuracy/bias for the pesticides belonging to the range (c) has contributed to their relatively higher values of expanded uncertainties. The uncertainty associated with the calibration graph (U1) was the minor contributor. The relatively high value of uncertainty of trifloxystrobin is in conformity with its poor recovery which was <70% in most of the matrices which showing that the method exhibited relatively poor performance for this compound.

Analysis of market samples

To evaluate the effectiveness and applicability of the developed method in measuring trace levels of the studied pesticides, the developed method was applied to the analysis of a total of 52 samples collected from five different Agricultural Produce Marketing Corporations/Wholesale markets of Gujarat, Rajasthan, Madhya Pradesh and Delhi in India and were analysed for the presence of the studied pesticides. Traces of chlorpyrifos (an organophosphorous pesticide) residues were detected in two samples of isabgol, one obtained from Udaipur and the other obtained from Delhi but the level was below the Limit of Quantification (LOQ). None of the other samples were found to contain any other pesticide residues. The results obtained using the described preparation method for these real samples were accurate and precise. The proposed method was suitable for the analysis of the studied pesticides in medicinal herb samples.

Conclusion

Determination of the pesticides in herbs is essential to assess the safety to the consumers and avoid the chronic toxicity related to the long-term use. Development of simple and sensitive analytical methods would be quite useful in developing regulatory guidelines for the management of pesticide residues in herbal products. Considering this, a rapid and sensitive method has been proposed for the extraction of multiclass pesticides in different popular medicinal herbs in India and the method standardized in the present study will also be of immense use in

monitoring market samples of medicinal herbs for ensuring food quality and safety the consumers worldwide.

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Compliance with ethical standards

Conflict of interest No potential conflict of interest was reported by the authors.

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