Multiresidue Analysis of Pesticides in Three Indian Soils: Method Development and Validation Using Gas Chromatography Tandem Mass Spectrometry

Arijita Bhattacharyya

National Research Centre for Grapes

Anita Pardeshi

National Research Centre for Grapes

Apurva Nerpagar

National Research Centre for Grapes

Kaushik Banerjee (✉ kbgrape@yahoo.com)

National Research Centre for Grapes <https://orcid.org/0000-0002-5026-2370>

Research Article

Keywords: Multiresidue analysis, pesticide residues, Indian soils, GC-MS/MS, QuEChERS

Posted Date: January 19th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1217458/v1>

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Abstract

The paper aimed to develop an effective multiresidue extraction method in three major soil types, namely i) new alluvial soil (NAS); ii) red lateritic soil (RS) and iii) black soil (BS) in the Indian subcontinent for determining the residues of pesticides from various chemical classes. To establish an effective pre-treatment process, an ethyl acetate-based extraction method with a freezing out cleanup step was employed. The analysis was performed using gas chromatography-tandem mass spectrometry (GC-MS/MS). The validation experiments were performed on 220 GC-amenable pesticides by spiking all soil samples. The linearity of the calibration curves was satisfactory in matrix-matched standards and yielded the coefficients of determination (R^2) ≥ 0.99 for approximately 98% of the target analytes in all matrices. At 10 ng/g, the recoveries of the spiked pesticides were in the range of 70–120%, with associated precision-RSD values $\leq 20\%$ for approximately 85%, 88.6% and 89% of the compounds for BS, RS and NAS respectively. At the higher spiking levels of 20 and 50 ng/g, average recoveries and RSDs readily met the validation criteria of all the studied pesticides. Overall, the method holds potential as a suitable procedure for multiresidue analysis in soils for safety evaluation and risk assessment purposes.

Highlights

- A multiresidue method is reported for simultaneous analysis of multiclass pesticides in soil
- The soil types represented the major crop growing areas of the Indian subcontinent
- Sample preparation involved extraction with ethyl acetate and cleanup by a freezing step
- The residues were estimated by gas chromatography tandem mass spectrometry
- The method sensitivity, accuracy and precision complied with the SANTE/12682/2019 guidelines

Introduction

Soil, a non-renewable natural component of our ecology, is the foundation of successful agriculture and human civilization. In modern agricultural practices, the application of pesticides has been unavoidable to increase crop productivity and quality. Pesticides are not only detrimental to human and animal health but also to the environment (EC 2007; Mondal et al. 2020; Asensio-Ramos et al. 2010). And, the widespread and indiscriminate use of pesticides has become a global concern with regard to food safety. Hence, it is of high interest to monitor residues of these pesticides in the soil to protect human health and the environment. For this, there is an interest in industrial and governmental sectors to develop accurate, easy, precise and cost-effective analytical methods.

In the past two decades, researchers have noted that the physico-chemical properties of soil and the nature of pesticides determine the types of interaction (persistence, fate, environmental behaviour and pollution risk) once a compound is introduced into soil (Andreu et al. 2004; Kah et al. 2007; Ahumada et al. 2013). Soil is a heterogeneous complex matrix consisting of organic (humus 10–15%, lipids, carbohydrates, lignin, flavonoids, pigments, resins and fulvic acids) and inorganic (variable percentage of sand, silt, and clay) components (Łozowicka et al. 2017). Because of the polar, non-polar and ionic activities of pesticides, they are often retained in the soil (Mondal et al. 2020), making residue analysis difficult. Besides, a pesticide is likely to be exposed to chemical as well as microbial degradation, producing metabolites of variable toxicological significance. Hence, a comprehensive risk assessment of a soil will necessitate analysis of these metabolites in conjunction with precursor molecules, complicating multiresidue analysis even more.

Over the past decade, many researchers have studied conventional and modern sample preparation methods for the determination of pesticide residues in soil (Naeeni et al. 2011; Rouvière et al. 2012; Guo and Lee 2013; Pastor-Belda et al. 2015; Ukalska-Jaruga et al. 2020). Several other communications have also been published on the application of QuEChERS-based methods in determining pesticides in soil matrices using GC-MS/MS (Yu et al. 2016; Jo et al. 2021), LC-MS/MS (Caldas et al. 2011; Chen et al. 2019; Zaidon et al. 2019) and LC-QTOF/MS (Caria et al. 2021). For instance, Asensio-Ramos (2010) proposed a modified version of the QuEChERS method in three different soils (forestal, ornamental and agricultural, pH 4.2–5.9) by GC-MS/MS, in which they targeted only a few pesticides (majorly organophosphates with one thiadiazine). In another study, Leyva-Morales reported a pressurised liquid extraction (using dichloromethane: acetone, 50:50, v/v) method by GC coupled with an electron capture detector, a pulsed flame photometric detector and a thermionic specific detector (Leyva-Morales 2015). However, this method not only needed more time but also additional solvents, thus making it exceedingly expensive. The method also did not involve mass spectrometry based confirmation. Łozowicka et al. reported a QuEChERS method in a type of Polish soil (pH- 6.6, organic matter- 1.45%, clay content- 2.43%) that allowed them to analyse a wide range of compounds (216 pesticides), but it required a lengthy chromatographic run time of 41.88 min (Łozowicka et al. 2017). Thus, all of the reported studies have one or some more of the following drawbacks: the use of limited scope of analytes (Jo et al. 2021; Rouvière et al. 2012; Yu et al. 2016), high-priced and difficult analytical steps involved (Leyva-Morales 2015; Caria et al. 2021) or needed longer time for analysis (Łozowicka et al. 2017; Fernandes et al. 2013). To develop a method, we first tried a modified QuEChERS-based multiresidue method reported by Łozowicka et al. which studied a wide range of pesticides (Łozowicka et al. 2017). However, this method required a longer chromatographic runtime. We also recorded poor recoveries of several target pesticides with this method. Through literature surveys we concluded that there are scarcely any studies on the quantification and estimation of health risks associated with pesticide residues in Indian soils. All these knowledge gaps necessitate a new method, and we aimed to develop an effective multiresidue extraction method in three major agricultural soils (Balasubhramuniam 2017) of the Indian subcontinent. The performance of the method was verified by determining 220 GC-amenable pesticides from various chemical classes in three types of soils, namely new alluvial soil (NAS), red lateritic soil (RS) and black soil (BS). These soils were chosen because they have texturally diverse physico-chemical properties and are also located in prime crop-growing areas. The method was thoroughly validated in terms of confirmatory identification and quantification. Overall, the method demonstrated the potential to be used as a suitable procedure for multiresidue analysis of pesticides in soils across laboratories for monitoring and risk assessment purposes.

Experimental

Pesticide selection

A total of 220 pesticides (GC amenable) from various chemical classes were chosen as they are widely relevant in today's agriculture and also demonstrate a range of physico-chemical properties (e.g., organochlorines, organophosphates, synthetic pyrethroids, heterocyclics and also their metabolites). Among them, some were reported as soil pollutants (Lozowicka et al. 2017) while others are banned or of restricted use, thus necessitating frequent monitoring [Indian Council of Agriculture Research (ICAR), Central Insecticides Board and Registration Committee (CIB & RC), http://ppqs.gov.in/sites/default/files/list_of_pesticides_which_are_banned_refused_registration_and_restricted_in_use_as_on_30.06.2020.pdf] (Supplementary Table 1).

1.2. Selection of test matrices, blank sample collection and sample preparation

For the method development and validation, the following pesticide-free soil samples were collected from three different agro-climatic zones: new alluvial soil (NAS) from the University Research Station, Bidhan Chandra Krishi Viswavidyalaya (BCKV) (Mohanpur, district- Nadia, West Bengal), ii) red and lateritic soil (RS) from the Regional Research Station, BCKV (Jhargram, district- Midnapore, West Bengal) and iii) black soil (BS) from agricultural land (Pune, Maharashtra State). The physico-chemical properties of these soils are mentioned in Table 1.

Following the standard methodology of sampling, the soil samples were collected from the upper layer (between 15 and 20 cm of depth). The samples were air-dried, ground, passed through a 2 mm sieve, subsampled by the usual method of quartering and stored in zip-lock bags at -20 °C. The sample collection, preservation and storage were based on the US EPA Method 1699. The physico-chemical properties of the soil samples were tested by the following methods: the hydrometer method for measuring the soil texture (Gee and Bauder 1986); the soil pH was measured after adding deionised water (1 + 2.5 by weight) (Jackson 1973) and the Walkley and Black wet oxidation method was used for determining the organic carbon content of the soil (Nelson and Sommers 1982).

Chemical reagents and materials

The certified reference standards of $\geq 98\%$ purity were purchased from Sigma-Aldrich (Steinheim, Germany) and Dr Ehrenstorfer GmbH (Augsburg, Germany). The HPLC grade ethyl acetate was obtained from J.T. Baker (CenterValley, USA). Acetic acid and anhydrous sodium sulfate (Na_2SO_4) were purchased from Merck (Bengaluru, India). The dispersive solid phase extraction (dsPE) sorbents [e.g. primary secondary amine (PSA) and C_{18} (end-capped)] were procured from Agilent Technologies (Santa Clara, CA, USA). The polytetrafluoroethylene (PTFE) syringe filters (0.22 μm) were sourced from Chromatopak Analytical Instrumentation Pvt. Ltd. (Mumbai, India). The HPLC grade water was generated using a Sartorius water purification system (Gottingen, Germany).

Apparatus

At different stages of sample preparation, the following apparatus were used: a precision analytical balance (Adair Datt, ADGR 202, Mumbai, India), a pH meter (Thermo Orion, Chelmsford, MA, USA), an orbital shaker (SciGenics Instruments, Mumbai, India), vortex mixer (Genie 2T, Imperial Biomedicals, Mumbai, India), an ultrasonic bath (Oscar Electronics, Mumbai, India), a low-volume concentrator (TurboVap LV; Caliper Life Sciences, Russelsheim, Germany), a high speed refrigerated centrifuge (Kubota 6500, Kubota Corporation, Tokyo, Japan) and a micro-centrifuge (Dlab Instruments, Hyderabad, India).

Preparation of standards

The primary stock solutions of the individual pesticide standards were prepared by weighing 10 (± 0.01) mg of each analyte in a volumetric flask (certified class 'A') and dissolving in 10 mL of ethyl acetate solvent and were stored in the dark at -20 ± 2 °C. The intermediate standard mixture of 1 $\mu\text{g/mL}$ was prepared using appropriate dilution and stored in the dark at -20 ± 2 °C. During the entire study, the stability of the standards was monitored as per the SANTE guidelines (SANTE/12682/2019). By means of serial dilution of the intermediate mixture with ethyl acetate, the calibration standards (0.002-0.05 $\mu\text{g/mL}$) were prepared. For the matrix-matched standards preparation, the appropriate volume of pesticide-spiked mixture was added to the blank matrix extract.

Instrumental parameters for GC-MS/MS analysis

The sample analysis was performed using a gas chromatograph (GC 2010, Shimadzu Corporation, Kyoto, Japan) equipped with an AOC-20i auto-injector and hyphenated to a triple quadrupole tandem mass spectrometer (TQ8040, Shimadzu Corporation, Kyoto, Japan). Analytical separation of the target analytes was performed by using an Rxi®-5Sil MS (30 m $\times$ 0.25 mm, 0.25- μm film thickness) column from Restek Corporation (Bellefonte, USA). In the splitless mode, 1 μL volume was injected. At a constant flow rate of 1.2 mL/min, ultra-pure grade helium was used as the carrier gas, while argon was used as the collision gas. The oven temperature was initially set to 90 °C (1 min), then gradually increased to 130 °C (3.71 min) at a rate of 35 °C/min, then to 240 °C (1 min) at a rate of 10 °C/min and finally, from 15 °C to 290 °C (4 min). This resulted in a total run time of 21.31 min. The temperatures of the transfer line and the ion source were kept constant at 290 and 230 °C respectively. The detector voltage was set at 0.70 kV. The solvent delay time was 1.5 min. The data acquisition, which started at 2 min, was performed in the scan mode and multiple reaction monitoring (MRM) mode. In the MRM mode, the compound-specific m/z helped in the selective identification and quantification of each pesticide. To control the instrument and acquire and process data, the GC-MS LabSolutions® software (Version 4.45 SU2, Japan) was used. The method precision (repeatability) was checked as per the SANTE/12682/2019 guidelines.

Optimisation of sample preparation

In order to optimise an effective extraction method, we used our previous ethyl acetate-based extraction method on fruits and vegetables (Banerjee et al. 2007, 2012) with modifications. In our quest to develop a single concurrent extraction protocol, the sample preparation method was first optimised in the BS matrix and validated, and then extended to the other two soils, namely RS and NAS. BS has more clay and more organic carbon, both of which interact with

pesticides and hold them through adsorption and absorption, and this could make the extraction relatively more difficult. For this, we decided to optimise the method in BS first. When BS demonstrated adequate recoveries, the remainder of the matrices were anticipated to follow suit. All experiments were carried out after homogenising the soils and passing them through a 2 mm sieve.

Sample size optimisation

To determine the effect of the sample size on the sensitivity, accuracy and precision, three different sample sizes (2, 5 and 10 g) were selected. The samples were spiked at 0.1 mg/kg of the test analytes and extracted with 10 mL of distilled water and 10 mL of ethyl acetate in separate batches (n=6).

Shaking versus sonication

The effects of shaking and sonication were separately evaluated by keeping the samples on a shaker at a speed of 250 times/min for 10, 20 and 30 min. In a parallel experiment, the samples were sonicated for 10, 20 and 30 min. The recovery results were comparatively evaluated.

Cleanup optimisation

Freezing

To optimise the freezing temperature, a portion of the organic layer (2 mL) of each tube was pipetted out and kept in a 15 mL centrifuge tube at -20 °C (for 30 min and 60 min) and -80 °C (for 10 min and 20 min). The results were evaluated in terms of recoveries and matrix effects.

Application of adsorbents in dSPE cleanup

The dSPE cleanup was evaluated using the following sorbent combinations: Cleanup 1 (50 mg PSA, 150 mg Na₂SO₄), Cleanup 2 (25 mg PSA, 25 mg C₁₈ and 150 mg Na₂SO₄) and Cleanup 3 (25 mg PSA, 50 mg C₁₈ and 150 mg Na₂SO₄). Each tube was vigorously vortexed for 1 min before being centrifuged at 5600 ×g for 5 min at 4 °C. The combined effect of the dSPE cleanup and freezing was also evaluated.

Optimising the method

For the recovery experiments, the pre-spiked soil was equilibrated for 20 min at room temperature. For extraction, a sample (5 g) was placed in a 50 mL polypropylene centrifuge tube and vortexed for 30 s. Afterwards, 10 mL of distilled water was added to hydrate the matrix and handshaken for 30 s. To it, 10 mL of ethyl acetate was added, followed by 10 g anhydrous Na₂SO₄. With no further delay, the resulting mixture was vigorously hand-shaken for 30 s and vortexed for 2 min. The tubes were shaken at 250 times/min on a mechanical shaker for 20 min, followed by centrifugation at 2800 ×g for 10 min. The clear supernatant was taken into a 15 mL centrifuge tube and held at -80 °C for 20 min. After the phase separation, 2 mL of the organic extract was taken into another centrifuge tube, which was centrifuged at 11200 ×g for 5 min. The sample was filtered through a 0.22 µm PTFE syringe filter and kept in an autosampler vial. Finally, the sample was injected into the GC-MS/MS.

Validation of the developed method

The method validation parameters such as accuracy, precision, linearity, specificity, ruggedness and matrix effect were evaluated as described in the SANTE/12682/2019 guidelines. These analytical performance parameters were evaluated by analysing all three types of soils, each spiked at three concentrations (10, 20 and 50 ng/g). Each experiment was performed in six replicates.

The linearity of the calibration curves was studied at a concentration that ranged between 2 and 40 ng/g. The limit of quantification (LOQ) for each pesticide was determined as the lowest spiked concentration that could be quantified with satisfactory recoveries (70–120%) and precision (an RSD of less than 20%). The accuracy was estimated by recovery experiments (fortified at three-levels such as LOQ, 2×LOQ and 5×LOQ). The results were calculated using matrix-matched calibration standards, which were injected at the beginning as well as the end of every batch. The precision was expressed as within-the-laboratory repeatability in terms of the relative standard deviation (RSD). By involving three different analysts, the recovery experiments were conducted on three different days, showing intra-laboratory reproducibility.

The matrix effect (ME) was evaluated as signal suppression (%) or enhancement (%). The ME was determined by comparing the peak areas of the matrix-matched standards (peak areas of the post-extraction spike) with the corresponding peak areas of standards in solvent at 0.02 µg/mL. According to Ferrer et al. the ME was classified into three categories as follows: soft (<20%), medium (20-50%) and strong matrix (more than 50%) (Ferrer et al. 2011). The ruggedness of the method was confirmed by analysing a quality check/control (QC) sample (at 50 ng/g) in every batch. In order to ensure the accuracy and precision of test results, analytical quality control criteria were applied. Each batch of samples was accompanied by the analysis of matrix blanks, spiked samples and matrix-matched calibration standards bracketing the real samples. One of the quality assurance measures was evaluated by confirming the reproducibility of analytical results. This was performed by the repeated determination of the spiked soil samples and the analysis of different analytical portions on different days and by employing different analysts. The results were evaluated with reference to the SANTE guidelines.

Method comparison

The method performance was compared to that of Łozowicka et al. method (Łozowicka et al. 2017) (discussed later in the result section).

Method application in real-world samples

Pesticide residues were evaluated in 25 field samples taken from various farms in Maharashtra and West Bengal states. Six replicates of each positive sample were examined and the precision-RSDs were estimated.

Results And Discussion

Because of the complexity of soil matrices, careful sample preparation was required to obtain a repeatable and sensitive multiresidue analysis. As mentioned earlier, the extraction efficiency of ethyl acetate was equivalent in comparison to other water-miscible solvents in different matrices.

Sample size optimisation

The sample size was optimised to ensure satisfactory recoveries. In practice, it is common to add a volume of water to dry samples prior to the extraction step (Rutkowska et al. 2018; Zhou et al. 2012). Next, the effect of sample size on method performance was evaluated. Almost all of the analytes provided an RSD of < 20% for a 2 g sample. The ME for 85% of the test analytes was less than 20%. In addition, the initial residue was diluted, which lowered the quantification limit. This made the analysis of the incurred residues of the compounds difficult. For sample sizes of 5 g and 10 g, the precision-RSDs were satisfactory (<20%) for most of the analytes. But, when compared to a 5 g sample size, the ME was higher for approximately 22% of the compounds (including dichlorvos, atrazine, fenitrothion, profenofos, propargite, p,p'-DDT and pretilachlor) for a 10 g sample. As a result, 5 g was determined to be the best sample size for further research. The results of the sample size optimisation of selected pesticides (including early, mid and late eluting ones from different chemical classes) are presented in **Figure 1a** and **1b**.

Shaking versus sonication

When compared to sonication, shaking provided a better recovery percentage. As shown, 20 min of shaking time offered a better recovery (10-15%) (**Figure 2**) in comparison to the shaking time of 10 min. But, when this time was increased from 20 to 30 min, no significant changes in the recovery were observed. Thus, a shaking time of 20 min was chosen.

Optimisation of cleanup

Without cleanup, 13% of the analytes showed recoveries higher than 130%, while 26% of them exhibited a significant ME (above 50%). This indicated that the cleaning up of the soil extract was necessary to minimise the ME.

Optimisation of freezing temperature

The main fraction of the soil matrix is humus, which mainly comprises organic acids and their conjugate bases, and 20% of soil humas occurs in the form of lipids (Kononova 1966; Morrison 1969). Because of their high solubility in ethyl acetate, these lipids might get co-extracted with the target pesticide residues. By putting the extracts at -80 °C for 20 min, the GC-MS full scan mode analysis revealed that a large extent of matrix-derived compounds were reduced (**Supplementary Figure 1**). A longer span of freezing (20 min, 30 min and 1 hour, at -20 °C) affected the recoveries of some of the organochlorine pesticides in BS. The successful recovery of 86.7% of the tested pesticides demonstrated the effectiveness of this cleanup step. The ion ratio (the qualitative to quantitative MRMs, %) deviations (with the matrix matched standards) of some compounds (e.g. dichlorvos and parathion) did not comply with the accepted criteria (<30%) due to higher matrix effects, resulting in their false negative detection. Such false detection issues were resolved by performing the freezing step (**Supplementary Figure 2a, 2b**). Additionally, the peak shape of a number of compounds (e.g. chlorothalonil, oxyfluorfen, chlorfenapyr and fipronil) was improved with the freezing step (**Supplementary Figures 3a, 3b, 3c, 3d**).

dSPE cleanup optimisation

A separate cleanup experiment with different dSPE sorbents was also evaluated. The Cleanup 1 strategy included only PSA, which increased the pH of the BS extract (pH-7.8). As seen, satisfactory results were obtained for only 71% of the target analytes. The recoveries were unsatisfactory for the remaining analytes (mostly the base sensitive compounds), keeping their recoveries between 60-70% and 120-140%. The precision-RSDs were mostly < 20%. A lower recovery for a few herbicides (e.g. fluzifop-p-butyl, clomazone, propanil) might be due to their reaction with the dSPE sorbents (Lehotay 2007; Niell et al. 2009). Hence, for Cleanup 2, the amount of PSA was reduced and combined with C₁₈. This provided satisfactory results for 84.8% of the analytes. The Cleanup 3, which had an enhanced amount of C₁₈, was effective in binding and removing the starch and sugar co-extractives, also reported earlier by Caldas et al. 2011. The results were comparable to Cleanup 2 and Cleanup 3. Therefore, we chose Cleanup 2 as the optimum sorbent combination for the dSPE cleanup.

Combined effect of freezing and dSPE cleanup

When the effectiveness of Cleanup 2 was estimated, almost 83.3% of the analytes demonstrated a recovery range of 70-120%, which was much lower than the percentage of the analytes obtained only in the case of the freezing step without dSPE cleanup. For example, a significant number of compounds, for example, chlorfenapyr, tetrachlorvinphos, triazophos and tricyclazole, suffered from poor recoveries of <30% when the cleanup sorbents were used. The results showed that freezing at -80 °C for 20 min (without a dSPE cleanup) recovered the highest number of compounds (**Figure 3a**) within the acceptable range of 70-120%. The use of freezing plus dSPE cleanup did not have a significant effect on the recovery of pesticides from the extracts. The absence of a dSPE cleanup step is also observed in the study by Rutkowska et al. 2018 for dry herb samples, although Caldas et al. obtained better results for multiclass pesticides in the soil samples under paddy cultivation (Caldas et al. 2011). In another study on herbicides in soil, Zhou et al. noted even a better recovery for pyrazosulfuran ethyl with a normal extraction over PSA or C₁₈ (Zhou et al. 2012). The recovery data of some compounds including allidochlor, dichlobenil, chlorthalonil, biphenyl, ethion, mevinphos, paclobutrazole, phorate, profenofos, tau- fluralinate, trans-chlordane, tricyclazole and 2,3,5,6 tetrachloro aniline supporting this fact are presented in **Figure 3b**. Compared to the extraction method using the dSPE cleanup step, this method was more cost-effective as it

involved fewer solvents and reagents (excluding expensive phase PSA and C₁₈). Besides, the current study was less laborious as it did not require a partitioning method or the weighing of cleanup reagents.

Method validation

All the tested pesticides could be selectively estimated in a single GC run of 21.31 min. Dichlorvos was the earliest compound (t_R = 4.44 min), while fenvalerate (t_R = 21.04 min) was the last one in the chromatogram. The GC programme provided chromatographic separation of the pyrethroid isomers and facilitated their accurate quantification as observed for cyfluthrin [alpha-HCH (t_R = 9.303 min), beta-HCH (t_R = 9.918 min), gamma-HCH (t_R = 9.985 min) and delta-HCH (t_R = 10.584 min), cyfluthrin-I (t_R = 19.117 min), cyfluthrin-II (t_R = 19.234 min), cyfluthrin-III (t_R = 19.307 min) and cyfluthrin-IV (t_R = 19.357 min)] and cypermethrin [cypermethrin-I (t_R = 19.501 min), cypermethrin-II (t_R = 19.633 min), cypermethrin-III (t_R = 19.705 min) and cypermethrin-IV (t_R = 19.762 min)]. The quantitative results in three soil matrices are presented in **Table 2**. The validation of this optimised analytical method was evaluated by different parameters including linearity (expressed as R^2), LOQ, ME, accuracy (expressed as recovery) and precision (expressed as RSD) (**SANTE/12682/2019**).

A good linearity ($R^2 \geq 0.99$) was obtained in the calibration curve for all compounds over a concentration range of 2-40 ng/g. A slightly lower R^2 value (~ 0.98) was noted for fipronil (BS), hexachlorobenzene (NAS), cypermethrin (RS) and permethrin (RS). In the case of BS, a considerable ME was mainly observed for phenylureas (e.g. linuron), aromatic ethers (e.g. oxyfluorfen), polychlorobenzene (e.g. hexachlorobenzene), monosubstituted with nitro- (e.g. quintozone) and dinitrile (e.g. chlorothalonil) group, the mid-eluting organophosphates (e.g. phoratesulfide, tolcofos methyl chlorfenvinphos etc.) and late-eluting synthetic pyrethroids (e.g. cypermethrin etc.) compounds (**Supplementary Figure 4**).

According to the findings, 69% of the compounds had a lower ME ($\pm 20\%$) in BS, while 59 pesticides showed a medium ME (ranged from 21 to 49%). Among all, only 9 pesticides including tolcofos methyl, phorate sulfide, linuron, 4th peak of cypermethrin eluting at 19.762 min, delta-HCH, endosulfan sulfate, etridazole, oxyfluorfen and parathion showed a stronger ME of $\geq 50\%$. Tolcofos methyl showed the greatest ME value of 78.39%. In the RS soil type, 83% of pesticides showed a lower ME; 33 pesticides had values ranging from ± 21 to $\pm 49\%$. Four pesticides including alpha-endosulfan, chlorothalonil, fenvalerate and tetradifon exhibited signal enhancements of $>50\%$. Tolcofos methyl showed the greatest ME value of 78.4% in RS. A signal suppression was observed only for (E)-chlorfenvinfos in BS as well as RS. Whereas in NAS, 90% of compounds showed no ME. A moderate ME was observed for 17 compounds excluding chlorpyrifos, alpha-endosulfan and oxyfluorfen, which had a higher ME. The ME of some selected pesticides is presented in **Supplementary Figure 4**.

The ME was lowered by applying the freezing step. A considerable ME of the same or different compounds was also observed previously in soil matrices. For instance, **Fernandes et al. 2013** reported high MEs for 12 pesticides, which included alpha- and beta-HCH, HCB, o,p-DDT, bupirimate, chlorpyrifos, fludioxonil, malathion, methiocarb and pendimetaline. In another study, **Asensio-Ramos et al.** reported a significant ME for 11 pesticides, namely buprofezin, chlorpyrifos, chlorpyrifos-methyl, diazinon, dimethoate, ethoprophos, fenitrothion, malaoxon, malathion and phosmet (**Asensio-Ramos et al. 2010**). According to **Łozowicka et al.** positive MEs (signal enhancement) were reported for alpha-endosulfan, beta-endosulfan, endosulfan sulfate, dichlorvos, methamidophos, oxyfluorfen and oxamyl, whereas negative MEs (signal suppression) were observed in the cases of bupirimate, dichlobenil, etaconazole, propham and trifloxystrobin (**Łozowicka et al. 2017**). Therefore, to correct the recoveries, in our study, the matrix-matched calibration graphs (soil type-specific) were used for quantification. This approach adjusted the accuracies within the acceptable range of 70-120%.

At the lowest fortification level of 10 ng/g, 85% of the analytes demonstrated satisfactory method performance (recoveries ranged within 70-120% with a $<20\%$ RSD). The LOQ for these compounds was set at 10 ng/g. At this LOQ level of 10 ng/g, 11 chemicals, including p,p'-DDT, p,p'-DDD, p,p'-DDE, o,p'-DDE, o,p'-DDT, hexachloro-exo-epoxide, cypermethrin-1, aldrin and others, showed a recovery of between 60 and 69% and a reasonable precision RSD of 10%. For example, transfluthrin, trans nonachlor, trans permethrin, linuron, fluchloralin and the third isomeric peak of cypermethrin (eluting at 19.703) showed around 60% of recoveries. The precision RSD in each case was $< 20\%$. The only exception was trans-nonachlor, which had a poor precision-RSD at 10 ng/g (above 20%). At 20 ng/g, however, a satisfactory result was achieved for all these pesticides. Only a handful of compounds, for instance, fenitrothion, propoxur, tau-fluvalinate and azinphos ethyl, showed recoveries of more than 120%, although their precision-RSDs were $<10\%$. At the fortification level of 20 ng/g, all these analytes complied with the method performance criteria (**Table 2**).

In a previous study, certain compounds, for example, aldrin, tolyfluanid, dichlofluanid, dieldrin, fipronil, propargite, profenofos, chlorothalonil, p,p'-DDT, lambda-cyhalothrin, pyriproxyfen, diazinon, oxadiazon and edifenphos, demonstrated poor recoveries (**Walorczyk et al. 2012**). In our earlier study conducted on animal feed, a poor recovery was also experienced for these compounds (**Kumar et al. 2020**). However, the recoveries and precision of these compounds were satisfactory in this study (**highlighted in bold, Table 2**). Our results showed non-detection of allethrin, flucythrinate, phenothrin, tetramethrin, cycloate, endrin and paraoxon methyl in BS. Some compounds did not meet the identification criteria of the ion ratio matching (within $\pm 30\%$), especially at lower concentrations where isobaric interferences from co-eluting matrix compounds might alter the value and result in non-detection (**Walorczyk et al. 2012**) (**Figure 4a**). Another reason for false negatives in the case of some compounds (e.g. paraoxon methyl, cycloate and endrin) might be because of their recovery loss in the sample preparation stage. The degradation of cycloate, a thiocarbamate ester, might have occurred due to alkaline hydrolysis. For paraoxon methyl, the breakdown could have been due to the oxidation of mixed mineral oxides present in the soil matrix (**Zimmermann et al. 2013**).

The method performance was evaluated in the remaining soils, namely NAS and RS. As illustrated in **Figure 4b** and **Figure 4c**, a few compounds, for instance, boromophos ethyl, chlorobenzilate, hexachlorobenzene, propanil, myclobutanil and nitrofen in NAS and bromofenvinphos, cis-permethrin, etofenprox, phenothrin and tetramethrin in RS, were not detected. In the NAS matrix, 89% of pesticides satisfied the method validation criteria laid out in the SANTE/12682/2019 guidelines, with the associated recoveries within 70-120% and precision RSD $\leq 20\%$ at the LOQ of 10 ng/g. Among all, only prochloraz and ethalfluralin suffered from a poor recovery of $<60\%$, with an associated precision RSD $\leq 20\%$. However, at a 20 ng/g level, their recoveries were satisfactory.

In RS, which is also an acidic matrix, this proportion was decreased to ~88.63%. Both beta-endosulfan (a sulfite ester) and EPN (a phosphonic ester) suffered from poor recoveries even in this acidic soil at 10 and 20 ng/g. Nevertheless, the recoveries were obtained in the range of 70-120% for the majority of pesticide-matrix combinations. In all matrices, a similar group of problematic compounds was observed with recovery values ranging from 60 to 130% with a consistent $\leq 20\%$ RSD (**Table 2, highlighted in bold, Supplementary Figure 5**).

When calculating against different calibration curves, agreeable quantification results were obtained, which clearly indicated the satisfactory reproducibility and ruggedness of this method (**Supplementary Table 2**). The repeatability (intra-day precision) and reproducibility (inter-day precision) RSD values were $< 20\%$ for all the target analytes. Besides, 30 continuous injections of the post-extraction spiked samples at 50 ng/g provided the RSD values $< 7\%$ for all the target compounds, indicating good instrument repeatability (**Supplementary Table 2**).

Method throughput

Following GC-MS/MS analysis, four batches of the extracted samples (48 in number) could be acquired in a 24-hour cycle. This facilitated a high throughput residue analysis. For all the target compounds in all matrices, the output of this method was also economically viable with regard to time and productivity.

Applications in real-life situations

In the real soil samples, six different pesticides viz, dichlorvos, biphenyl, diphenylamine, anthraquinone, tricyclazole and chlorpyrifos were detected (**Supplementary Table 3**). A repeat-extraction did not result in any additional recovery of residues. In all cases, the precision-RSDs were highly satisfactory (RSD, $< 10\%$, $n=6$).

Comparison with other methods

As previously stated, the ethyl acetate-based method is widely used in pesticide residue analysis, particularly for vegetables and fruits. However, no previous applications of this method have been reported on soil. So, in this study, 220 pesticides were analysed in three different types of soil using a GC-MS/MS instrument in a single chromatographic run time of 21.31 minutes. The previous works required a longer run time (e.g. 41.88 min or 25 min), had a limited number of pesticides (e.g. 34, 58, 123 and 216 compounds) in scope or matrices (specific soil, no wider pH range). In comparison to the QuEChERS method, the ethyl acetate extraction provided superior recoveries for the traditionally low-recovery compounds, as described in section 3.4. However, in our study, the absence of a dSPE cleanup step might have resulted in better recoveries. When some compounds were extracted with acetonitrile as done in the QuEChERS method, the results pertaining to chlorobenzenes (RSD ranged between 26 and 41%), chlorophenols (RSD ranged between 21-35%) and HCH isomers (RSDs for alpha, beta, delta, gamma were 25, 31, 37 and 29% respectively) were less repeatable. These observations were also supported by an earlier study by Rouviere et al. (Rouviere et al. 2012). However, the ethyl acetate-based method here showed satisfactory and consistent recovery of the above-mentioned compounds with satisfactory precision RSDs of $\leq 20\%$ (**Table 2**) in all the three soil matrices.

Conclusions

In this study, the conventional ethyl acetate-based extraction method in three different types of Indian soils was applied, although with certain modifications. The various steps that affect the extraction were optimised for 220 GC amenable pesticides (different chemical classes). The developed method was validated in three texturally different soils (covering a wide range of pH between 5.5 and 7.8).

In all of the soil matrices, the method provided satisfactory performance. Clean extracts were obtained using the optimised approach, which did not require any dispersive SPE cleanup. The cost of analysis was reduced because PSA was not necessary. However, it is worthwhile to devote time to optimising analytical processes in order to improve technique performance. The majority of the target compounds met the validation criteria, with recoveries ranging from 70 to 120% and RSDs less than or equal to 20%. Additionally, the chromatographic technique produced well-defined and well-shaped peaks in a short amount of time. As the method demonstrated high precision for incurred residues, it appears fit for purpose and hence is recommended for regulatory soil testing purposes across laboratories.

Declarations

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent for publish

Not applicable.

Author contributions

AB and KB contributed to the study conception and design, and drafted the manuscript. AB performed sample preparation and data analysis. AP and AN performed the GC-MS/MS analysis. All authors read and approved the final manuscript.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing interests

The authors declare no competing and conflict of interest.

Availability of data and material

All data are available with the institute repository.

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Tables

Table 1. Physico-chemical characteristics of the soils

Physico-chemical properties	RS	NAS	BS
Soil order	Aridisol	Mollisol	Vertisol
Location	Jhargram	Mohanpur	Pune
pH	5.45	7.02	7.8
Organic Carbon (%)	0.64	1.00	0.67
Sand (%)	54	12	16
Silt (%)	16	65	24
Clay (%)	30	23	60

Table 2
Method validation results [Recovery (Rec %) and Precision (RSD %)]

Sr. No.	Compound Name	RT (min)	Black Soil		New Alluvial Soil				
			10 ng/kg		20 ng/kg		10 ng/kg		20 ng/kg
			Rec %	RSD	Rec %	RSD	Rec %	RSD	RSD
1	(E)-Chlorfenvinphos	12.762	110.00	18.99	120.00	16.48	84.25	15.11	9.5
2	(Z)-Chlorfenvinphos	12.839	92.03	16.93	109.37	3.05	107.78	0.98	9.5
3	1,1-Dichloro-2,2-bis(4-ethylphenyl)ethane	14.632	75.65	11.24	88.74	4.04	66.70	8.57	6.5
4	2,3,5,6-Tetrachloroaniline	8.519	75.74	19.74	81.93	3.11	94.06	7.20	8.5
5	2,4'-Methoxychlor	15.914	74.50	4.52	80.88	5.38	103.47	19.86	9.5
6	2-Phenylphenol	7.365	82.51	9.28	94.87	1.09	88.00	6.67	8.5
7	3,4-Dichloroaniline	6.444	85.25	19.21	77.90	2.98	83.36	6.50	8.5
8	4 Bromo 2 Chlorophenol	4.787	85.40	9.19	97.84	2.96	96.62	6.42	9.5
9	4 chloro 3 methyl phenol	4.996	96.85	7.79	103.46	1.90	96.36	11.63	9.5
10	4,4'-Dichlorobenzophenone	12.347	86.37	7.78	113.96	4.16	93.81	8.25	9.5
11	4,4'-methoxychlor olefin	15.547	73.12	11.17	101.34	3.07	84.67	14.47	8.5
12	Acetochlor	11.043	89.41	17.16	103.25	2.64	88.27	19.44	9.5
13	Acrinathrin-2	17.866	76.72	12.80	114.81	2.57	115.22	6.79	8.5
14	Alachlor	11.221	96.34	5.19	98.48	3.81	103.30	5.98	8.5
15	Aldrin	12.135	62.23	7.71	77.18	5.66	83.47	4.16	8.5
16	Allethrin-1,2	12.909	ND	-	-	-	77.20	8.41	7.5
17	Allethrin-3,4 (Bioallethrin)	12.878	ND	-	-	-	139.75	6.07	9.5
18	Allidochlor	4.887	103.00	2.41	108.16	1.76	99.01	0.92	8.5
19	alpha-HCH	9.303	81.33	4.99	101.52	6.40	95.63	5.91	8.5
20	alpha-Endosulfan	13.661	81.67	14.40	86.38	15.11	111.85	15.14	1.5
21	Anthraquinone	12.174	95.84	19.51	103.80	9.40	93.45	7.62	8.5
22	Atrazine	9.755	108.77	2.56	111.74	4.29	102.18	2.25	8.5
23	Azinphos-ethyl	18.116	112.80	7.64	122.76	3.58	80.65	4.45	7.5
24	Azinphos-methyl	16.747	72.83	15.57	120.94	7.43	119.47	6.39	9.5
25	Bendiocarb	8.866	111.45	10.50	133.17	5.35	134.79	0.94	9.5
26	Benfluralin	8.825	71.50	15.40	102.23	9.76	89.73	3.88	7.5
27	beta-HCH	9.918	76.41	3.69	103.20	4.14	101.00	2.46	7.5
28	beta-Endosulfan	14.852	85.93	17.28	71.17	19.10	116.32	13.23	1.5
29	Biphenyl	5.946	81.91	3.10	90.14	3.12	89.48	3.90	
30	Bromfenvinfos-methyl	12.869	86.04	13.83	132.81	9.77	85.40	9.64	
31	Bromfenvinphos	13.647	76.90	9.39	99.78	13.11	95.30	15.61	
32	Bromophos	12.457	76.09	7.61	95.61	8.50	82.41	2.48	
33	Bromophos-ethyl	13.254	73.55	13.81	105.96	6.04	ND	-	
34	Bromopropylate	16.833	78.48	9.79	96.55	1.47	72.28	8.03	
35	Bupirimate	14.165	91.87	3.26	93.55	6.23	83.45	5.11	
36	Carbophenothion	15.576	72.62	13.54	75.99	6.77	75.99	13.19	
37	Carfentrazone-ethyl	15.454	91.87	12.10	93.59	3.01	94.95	10.26	

Sr. No.	Compound Name	RT (min)	Black Soil				New Alluvial Soil			
			10 ng/kg		20 ng/kg		10 ng/kg		20 ng/kg	
			Rec %	RSD	Rec %	RSD	Rec %	RSD	R	
38	Chlorbenside	13.339	75.64	3.70	73.37	1.37	74.42	8.91		
39	Chlorfenapyr	14.409	93.44	19.92	120.30	5.82	86.62	9.44		
40	Chlorfenson	13.837	77.63	4.69	96.28	2.78	140.79	11.19		
41	Chlorobenzilate	14.813	119.68	3.62	118.50	5.59	ND	-		
42	Chlorothalonil	10.162	119.80	18.26	128.00	9.76	97.96	4.46		
43	Chlorpropham	8.765	80.24	4.39	91.22	5.09	95.12	9.05		
44	Chlorpyrifos	12.008	71.73	7.63	104.87	12.36	99.57	4.74		
45	Chlorpyrifos-methyl	11.088	95.54	16.68	96.80	2.77	100.63	8.00		
46	Chlorthal-dimethyl	12.116	84.91	17.88	113.75	4.07	100.51	11.07		
47	Chlorthiophos-2	15.027	76.49	6.59	73.24	2.60	87.61	11.29		
48	Chlorthiophos-3	15.029	71.25	15.76	78.04	7.80	90.48	2.25		
49	Chlozolate	12.800	63.22	19.20	70.45	9.84	97.23	7.35		
50	cis-Chlordane	13.362	61.62	14.33	97.92	7.69	94.69	9.86	105.00	
51	cis-Nonachlor	15.080	71.40	17.69	79.60	7.10	80.62	5.57	87.82	
52	cis-Permethrine	17.135	79.40	18.50	88.30	8.92	102.55	9.28	87.25	
53	Clomazone	9.801	92.90	5.65	90.89	3.74	109.32	10.40	112.93	
54	Coumaphos	18.719	86.87	19.22	119.77	2.24	97.47	11.03	87.16	
55	Cycloate	8.543	ND	-	-	-	87.17	2.59	84.22	
56	Cyfluthrin-1	19.117	64.99	18.30	96.09	2.15	106.97	5.61	70.35	
57	Cyfluthrin-2	19.234	77.38	11.69	111.86	6.24	92.17	12.51	73.75	
58	Cyfluthrin-3	19.307	67.06	15.90	119.61	2.33	74.92	14.84	61.57	
59	Cyfluthrin-4	19.357	83.24	19.86	120.28	8.99	78.37	6.52	72.45	
60	Cypermethrin-1	19.501	67.67	9.25	92.73	8.54	71.96	19.72	62.28	
61	Cypermethrin-2	19.633	67.61	14.07	109.49	8.61	97.34	11.86	91.93	
62	Cypermethrin-3	19.705	50.21	14.00	70.58	14.00	76.09	0.53	79.07	
63	Cypermethrin-4	19.762	83.81	4.37	104.21	4.61	76.79	8.23	71.47	
64	Cyprodinil	12.709	90.55	11.53	93.09	3.72	84.87	15.95	81.01	
65	DDMU	13.371	76.52	11.53	97.77	1.09	62.41	19.31	77.02	
66	delta-HCH	10.584	89.48	18.98	98.40	11.70	119.12	18.21	66.54	
67	Di-allate-1	9.122	78.49	5.40	94.03	8.18	85.06	3.71	79.10	
68	Di-allate-2	9.314	62.15	16.64	72.75	12.13	89.35	2.90	82.41	
69	Diazinon	10.081	77.27	17.92	88.86	14.95	80.34	6.36	82.26	
70	Dichlobenil	5.492	87.83	4.39	96.10	1.14	94.00	3.13	94.61	
71	Dichlofluanid	11.849	90.93	8.54	113.94	6.73	115.20	15.95	93.85	
72	Dichlorvos	4.440	91.55	0.94	117.00	2.85	87.14	7.51	80.34	
73	Dicloran	9.556	76.21	19.13	73.24	19.24	71.30	9.90	80.63	
74	Dicofol deg. (DCBP)	14.813	71.87	1.35	86.66	10.54	89.57	19.98	81.25	
75	Dieldrin	14.227	91.08	20.67	112.13	13.12	85.27	7.59	82.83	
76	Dimethachlor	10.970	87.58	7.48	88.84	6.50	108.64	2.67	99.39	
77	Diphenamid	12.454	104.56	5.35	102.46	1.28	70.03	14.27	73.75	

Sr. No.	Compound Name	RT (min)	Black Soil				New Alluvial Soil			
			10 ng/kg		20 ng/kg		10 ng/kg		20 ng/kg	
			Rec %	RSD	Rec %	RSD	Rec %	RSD	R	
78	Diphenylamine	8.479	95.40	3.78	77.21	3.64	85.36	6.88	85.84	
79	Disulfoton	9.980	79.98	17.96	72.02	18.82	144.46	10.71	112.44	
80	Edifenphos	15.639	87.01	18.85	123.60	0.34	87.57	10.70	83.86	
81	Endosulfan ether	10.883	90.63	16.47	106.17	20.54	100.28	15.70	94.55	
82	Endosulfan sulfate	15.782	75.60	15.53	78.41	14.25	110.72	8.81	104.90	
83	Endrin	14.710	ND	-	-	-	108.35	8.36	88.28	
84	Endrin aldehyde	15.045	113.42	14.75	71.48	43.64	90.83	2.62	81.24	
85	EPN	15.729	87.50	14.60	89.23	18.90	101.11	7.63	79.13	
86	Ethalfuralin	8.611	88.41	18.21	75.20	10.03	58.45	11.72	71.79	
87	Ethion	14.961	78.80	8.76	96.35	0.61	82.32	5.67	76.68	
88	Etofenprox	18.025	75.40	12.25	79.60	14.62	101.80	2.89	86.66	
89	Etridiazole	6.565	80.39	7.78	95.27	4.67	90.51	3.20	71.94	
90	Etrimfos	10.424	79.99	13.92	108.53	3.06	106.73	13.12	91.56	
91	Fenamiphos	13.683	119.49	13.16	104.55	18.26	79.71	0.34	78.64	
92	Fenarimol	18.024	74.19	10.81	98.15	4.82	86.22	5.76	82.72	
93	Fenchlorphos	11.430	85.60	8.08	113.25	2.50	99.87	1.24	115.17	
94	Fenitrothion	11.692	106.38	15.86	124.20	4.19	94.31	15.06	85.70	
95	Fenpropathrin	16.703	106.08	13.08	102.54	4.51	80.92	2.24	82.38	
96	Fenson	12.444	72.18	2.25	93.88	1.80	80.91	2.39	79.44	
97	Fenthion	12.083	84.42	11.33	89.16	5.19	80.96	14.83	83.44	
98	Fenvalerate-1	20.724	67.64	9.63	101.56	9.22	77.48	9.13	80.44	
99	Fenvalerate-2 (Esfenvalerate)	21.046	61.29	8.52	82.64	6.42	90.78	13.98	60.44	
100	Fipronil	12.798	70.18	16.67	74.56	20.23	61.39	13.28	73.44	
101	Fipronil sulfide	12.579	71.80	5.79	113.02	4.18	97.17	15.42	77.44	
102	Fluazifop-P-butyl	14.547	71.18	19.88	77.66	9.47	81.28	4.62	83.44	
103	Fluchloralin	10.156	58.81	13.59	86.54	14.28	94.31	12.52	70.44	
104	Flucythrinate-1	18.050	ND	-	-	-	83.32	15.42	70.44	
105	Fludioxonil	13.910	90.43	0.80	101.93	2.62	98.17	8.96	90.44	
106	Fluquinconazole	18.742	79.00	3.86	98.81	1.02	95.59	10.64	73.44	
107	Fluridone	20.273	101.66	9.09	90.84	5.23	116.23	15.28	101.44	
108	Flusilazole	14.179	73.46	19.31	80.88	9.74	87.36	3.27	83.44	
109	Flutolanil	13.766	74.02	3.90	87.01	1.33	60.97	6.59	60.44	
110	Flutriafol	13.692	102.69	11.33	100.15	4.29	77.22	10.01	83.44	
111	Fonofos	10.095	78.59	13.28	71.94	6.83	93.85	6.27	90.44	
112	gamma-HCH (Lindane)	9.985	100.87	3.37	110.51	2.43	76.20	17.35	80.44	
113	Heptachlor	10.883	75.53	17.57	91.66	11.62	88.70	5.67	93.44	
114	Heptachlor-exo-epoxide	12.901	60.37	9.10	105.07	9.62	83.32	18.22	70.44	
115	Hexachlorobenzene	9.386	110.25	7.74	107.64	17.54	ND	-	-	
116	Hexazinone	15.908	72.40	18.06	74.21	13.77	107.08	20.41	101.44	
117	Iodofenphos	13.814	76.79	5.83	115.49	3.54	89.14	11.25	83.44	

Sr. No.	Compound Name	RT (min)	Black Soil				New Alluvial Soil			
			10 ng/kg		20 ng/kg		10 ng/kg		20 ng/kg	
			Rec %	RSD	Rec %	RSD	Rec %	RSD	R	
118	Isazofos	10.338	72.04	17.27	88.38	1.90	112.25	5.48	90	
119	Isodrin	12.710	73.52	14.29	103.17	6.27	123.40	16.66	100	
120	Isopropalin	12.506	63.11	16.50	89.08	2.56	89.04	18.42	80	
121	lambda-Cyhalothrin	17.725	85.90	19.16	98.61	4.92	92.43	1.40	80	
122	Lenacil	15.780	96.89	4.74	90.46	6.51	119.11	8.54	80	
123	Leptophos	16.564	71.07	18.69	78.26	12.58	73.94	11.29	70	
124	Linuron	11.861	48.53	12.98	71.30	17.46	103.96	3.16	80	
125	Malathion	11.833	95.48	6.84	112.04	5.30	81.55	8.28	60	
126	Metalaxyl (Mefenoxam)	11.348	111.29	13.01	105.93	8.87	88.92	8.20	90	
127	Metazachlor	12.716	109.87	6.93	110.69	7.69	110.13	9.10	90	
128	Methacrifos	6.987	92.46	2.75	95.19	2.30	102.70	9.33	90	
129	Methoxychlor	16.074	88.05	10.25	110.38	6.19	71.23	12.62	80	
130	Metolachlor (S-Metolachlor)	11.975	102.04	12.99	110.44	2.08	125.28	19.75	100	
131	Mevinphos-1	6.226	99.72	2.81	105.00	1.80	112.18	10.48	90	
132	Mevinphos-2	6.226	99.72	2.81	105.00	1.80	112.18	10.48	90	
133	MGK 264-1	12.466	82.49	19.63	91.64	13.64	98.91	5.64	90	
134	MGK 264-2	12.712	86.39	12.48	71.38	7.97	90.43	4.90	90	
135	Mirex	17.921	78.83	7.81	92.77	1.85	76.36	13.09	70	
136	Myclobutanil	14.167	91.65	7.96	95.07	3.92	ND	-	-	
137	N-(2,4-dimethylphenyl) formamide	6.923	104.42	4.02	98.36	1.42	111.87	3.19	90	
138	Nitralin	16.136	71.10	16.57	80.64	8.18	80.11	6.36	60	
139	Nitrofen	14.643	65.46	8.53	92.20	2.27	ND	-	-	
140	Norflurazon	15.620	86.92	9.27	83.26	7.48	76.14	5.73	80	
141	o,p'-DDD	14.247	71.39	1.26	93.66	2.86	114.92	5.35	90	
142	o,p'-DDE	13.374	61.72	4.19	94.15	3.62	93.40	6.24	80	
143	o,p'-DDT	15.052	63.04	3.11	98.94	2.11	90.83	2.62	80	
144	Oxadiazon	14.013	79.07	18.34	87.81	4.41	102.37	5.63	80	
145	Oxyfluorfen	14.151	71.38	12.24	99.36	6.78	115.48	12.56	90	
146	p,p'-DDD	15.052	63.18	3.49	98.94	2.11	133.27	9.26	100	
147	p,p'-DDE	14.071	61.53	8.08	97.30	2.92	85.72	8.57	80	
148	p,p'-DDT	15.829	67.20	3.94	82.91	3.61	101.46	14.44	80	
149	Paclobutrazol	13.468	90.02	16.53	92.76	1.32	82.55	5.58	80	
150	paraoxon methyl	10.305	ND	-	-	-	116.90	8.22	80	
151	Parathion	12.161	97.29	14.65	103.02	11.19	139.85	5.66	80	
152	Parathion-methyl	11.206	89.93	14.37	110.98	1.99	70.20	7.12	80	
153	Pebulate	6.629	86.95	7.30	85.16	4.81	88.11	5.17	80	
154	Penconazole	12.802	83.52	8.65	92.27	5.70	100.62	11.04	90	
155	Pendimethalin	12.659	84.48	9.92	83.55	9.37	62.29	16.18	70	
156	Pentachloroaniline	10.883	90.78	12.15	75.53	19.56	92.00	17.32	80	
157	Pentachloroanisole	9.483	84.24	8.74	105.31	5.44	74.59	7.66	80	

Sr. No.	Compound Name	RT (min)	Black Soil				New Alluvial Soil			
			10 ng/kg		20 ng/kg		10 ng/kg		20 ng/kg	
			Rec %	RSD	Rec %	RSD	Rec %	RSD	Rec %	RSD
158	Pentachlorobenzene	7.377	79.69	9.28	104.46	2.31	93.41	4.11	84.46	8.46
159	Pentachlorobenzonitrile	9.964	90.94	16.12	105.10	4.97	90.26	4.92	90.26	9.03
160	Pentachlorothioanisole	11.857	75.04	17.62	95.74	15.19	100.34	7.92	88.34	8.34
161	Phenothrin-1	16.864	ND	-	-	-	109.10	4.87	80.10	8.10
162	Phorate	9.139	78.30	18.09	71.22	20.03	97.29	10.31	97.29	9.73
163	phorate sulfide	11.900	119.80	17.31	127.23	4.14	100.60	11.84	97.60	9.76
164	phorate sulfone	12.024	100.34	2.91	121.59	5.56	92.65	12.40	92.65	9.27
165	Piperonyl butoxide	16.203	89.66	8.82	105.47	3.32	92.74	4.97	92.74	9.27
166	Pirimiphos ethyl	12.383	80.51	11.64	82.59	5.60	84.18	9.48	84.18	8.42
167	Pirimiphos-methyl	11.608	102.10	7.07	90.89	2.38	96.28	12.07	86.28	8.63
168	Pretilachlor	13.875	85.10	12.99	98.86	3.91	107.23	14.09	110.23	11.02
169	Prochloraz	18.797	80.53	6.39	98.73	7.24	58.67	13.09	70.67	7.07
170	Procymidone	13.072	70.79	10.81	84.19	2.76	87.12	3.02	87.12	8.71
171	Prodiamine	11.670	61.53	18.04	115.91	12.32	90.89	9.38	80.89	8.09
172	Profenofos	13.968	105.80	15.65	130.61	8.16	80.14	10.41	80.14	8.01
173	Profluralin	9.880	64.49	13.97	103.64	3.63	94.48	2.00	74.48	7.45
174	Propachlor	8.233	98.15	1.67	97.40	4.15	117.68	6.13	90.68	9.07
175	Propanil	11.084	76.15	9.60	88.66	17.70	ND	-	-	-
176	Propargite-1	16.120	113.10	16.90	127.80	6.55	96.86	7.31	76.86	7.69
177	Propargite-2	16.120	98.23	19.70	125.40	7.31	93.39	13.21	83.39	8.34
178	Propisochlor	11.306	77.55	8.92	91.01	8.77	88.38	4.07	88.38	8.84
179	Propoxur	8.210	97.81	12.18	131.19	3.32	88.28	2.03	90.28	9.03
180	Propyzamide	10.116	96.88	7.67	107.32	3.99	87.04	8.35	87.04	8.70
181	Prothiofos	13.865	75.30	7.78	91.67	6.23	98.46	11.62	110.46	11.05
182	Pyraclofos	18.236	71.34	12.45	102.91	9.78	85.95	14.17	97.95	9.79
183	Pyrazophos	17.936	97.08	8.81	104.40	3.17	83.93	14.46	73.93	7.39
184	Pyridaben	18.747	76.41	6.81	90.59	2.49	62.75	5.04	62.75	6.27
185	Pyridaphenthion	16.567	107.13	5.88	98.21	2.95	87.70	8.89	87.70	8.77
186	Pyrimethanil	10.247	85.19	5.52	79.41	6.16	89.65	16.53	97.65	9.77
187	Pyriproxyfen	17.584	85.12	9.86	104.03	4.95	99.98	15.38	89.98	8.99
188	Quinalphos	12.982	98.22	7.49	111.18	6.57	131.31	19.70	111.31	11.13
189	Quintozene	9.874	120.00	13.11	104.72	6.29	140.22	12.15	60.22	6.02
190	Resmethrin-1	15.967	71.69	18.60	74.62	3.98	101.10	16.96	97.10	9.71
191	Resmethrin-2 (Bioresmethrin)	15.997	83.63	10.29	75.83	1.41	78.83	2.65	83.83	8.38
192	Sulfotep	8.861	78.55	16.81	90.27	7.15	82.63	3.26	72.63	7.27
193	Sulprofos	15.336	78.99	18.90	75.00	15.88	78.74	17.31	80.74	8.07
194	tau-Fluvalinate-1	20.883	70.86	8.75	127.54	3.49	96.89	10.63	76.89	7.69
195	tau-Fluvalinate-2	20.992	74.57	16.46	117.31	4.23	84.21	6.42	74.21	7.42
196	Tebuconazole	16.124	83.86	3.88	88.13	7.11	97.79	8.43	97.79	9.77
197	Tebufenpyrad	17.038	86.62	12.38	94.95	3.44	119.85	13.26	119.85	11.99

Sr. No.	Compound Name	RT (min)	Black Soil				New Alluvial Soil			
			10 ng/kg		20 ng/kg		10 ng/kg		20 ng/kg	
			Rec %	RSD	Rec %	RSD	Rec %	RSD	Rec %	RSD
198	Tecnazene	8.113	70.96	4.87	79.35	5.01	78.14	17.32	85.14	8.51
199	Tefluthrin	10.332	72.88	10.08	95.77	0.18	96.37	8.86	96.37	9.01
200	Terbacil	10.444	116.11	9.61	107.35	8.34	79.78	7.83	79.78	7.83
201	Terbufos	9.986	96.59	16.03	92.05	11.28	100.66	13.55	99.66	9.96
202	Terbutylazine	10.025	110.45	16.25	112.26	1.39	71.23	2.59	71.23	9.01
203	Tetrachlorvinphos	13.373	94.32	12.52	91.83	11.50	87.77	4.36	87.77	7.77
204	Tetradifon	16.432	71.96	18.22	71.43	22.45	90.82	4.39	90.82	7.77
205	Tetramethrin-1	15.933	ND	-	-	-	110.05	2.79	110.05	5.01
206	THPI (Tetrahydrophthalimide)	7.055	86.76	7.63	95.85	7.89	93.32	2.98	93.32	8.51
207	Tolclofos-methyl	11.236	99.86	15.87	111.16	9.18	102.99	3.01	102.99	9.01
208	Tolyfluanid	12.832	87.90	12.22	120.84	1.92	81.07	12.27	81.07	9.01
209	trans-Chlordane	13.621	70.53	13.39	94.33	3.27	117.22	21.41	117.22	8.51
210	Transfluthrin	11.233	52.58	14.57	92.06	3.79	82.81	6.40	82.81	8.51
211	trans-Nonachlor	13.705	49.90	22.12	70.75	18.89	80.62	5.48	80.62	8.51
212	trans-Permethrine	17.306	49.78	12.07	88.13	6.49	89.39	5.79	89.39	8.51
213	Triadimefon	12.196	109.15	12.47	105.20	10.58	75.23	11.79	75.23	7.77
214	Triadimenol-1	13.083	94.38	11.49	95.97	3.99	119.39	13.31	119.39	1.01
215	Tri-allate	10.489	79.96	7.17	96.47	5.11	73.92	12.91	73.92	8.51
216	Triazophos	15.305	77.38	12.55	100.06	7.59	83.06	12.49	83.06	9.01
217	Tricyclazole	14.060	70.16	16.12	92.80	2.66	118.21	3.65	118.21	8.51
218	Triflumizole	13.077	90.91	17.85	103.09	13.81	81.50	13.27	81.50	7.77
219	Trifluralin	8.769	70.99	7.86	99.04	3.75	92.50	5.60	92.50	8.51
220	Vinclozolin	11.172	70.19	8.67	89.82	3.28	74.79	15.65	74.79	8.51

Figures

Fig. 1a

Fig. 1b

Figure 1

1a. Sample size optimisation in BS (pesticides with recovery %)

1b. Sample size optimisation in BS (pesticides with RSD %)

Figure 2

Shaking versus sonication (pesticides with average recovery %)

Fig. 3a

Fig. 3b

Figure 3

3a. Per cent of compounds recovered with satisfactory accuracy for different cleanup procedures

3b. Average recovery (%) data of selected pesticides in BS for different cleanup procedures

Fig. 4a.

Fig. 4b

Fig. 4c

Figure 4

4a. False detection of cycloate in BS

4b. False detection of propanil in NAS

4c. False detection of bromofenvinphos in RS

Supplementary Files

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