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Simultaneous determination of pesticides from soils: a comparison between QuEChERS extraction and Dutch mini-Luke extraction methods†

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The expanding nature of the agricultural sector has fuelled the intensification of plant protection products usage, including pesticides. These pesticides may persist in soils, necessitating their accurate determination in a variety of soil types. However, due to their complex nature, the effective extraction of pesticide residues from soil matrices can present challenges to pesticide detection and quantification. This research compared two well-known extraction methods, QuEChERS and Dutch mini-Luke, by assessing their specificity, sensitivity, accuracy, precision and reproducibility in extracting seven distinct pesticides with a range of chemico-physical characteristics from Irish soils. The HPLC-UV conditions were optimised to separate the seven pesticides, and it was shown that both extraction methods successfully extracted neonicotinoids with recovery values ranging between 85 and 115%. Fluroxypyr and prothioconazole could not be efficiently extracted using QuEChERS, however, the recovery values of both the analytes ranged between 59 and 117% using Dutch mini-Luke. Furthermore, with the exception of prothioconazole using Dutch mini-Luke, both extraction methods resulted in reproducibility and precision values below or equal to 20%. Lastly, Dutch mini-Luke is noted to have a lower matrix effect than QuEChERS, except for prothioconazole. The comparison results showed that Dutch mini-Luke resulted in superior method sensitivity, better recovery, and lower matrix effect towards most investigated analytes and was the only extraction technique that successfully extracted all pesticides analysed in soil matrices.

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1. Introduction

Pesticide usage in the agriculture sector is prevalent as plant protection products are considered integral to increasing agricultural productivity and food production.[1,2] It is estimated that in 2019, the global usage of pesticides is approximately 2 million tonnes annually, with an approximate increase of 75% the following year.[3] In Europe alone, pesticide sales volume was 333 418 tonnes in 2019.[4] It is projected that in 2050, the world population will increase to between 9.4 and 10.1 billion people, and arable land use and pesticide application will likely increase accordingly. With extensive and increasing agrochemical use and increased prevalence of crop pests and diseases in combination with inappropriate pesticide use, there exists considerable potential for environmental pollution. The unintended fate of those polluting pesticide compounds occurs through numerous simultaneous transfers, including spray drift,[5,6] surface runoff,[7–9] volatilisation,[10,11] degradation and leaching.[12–14] Even if pesticide transfer in the environment does not occur during the application of the chemicals, some studies show that pesticides can give rise to contamination through non-direct processes, such as the generation of airborne clouds of dust during the sowing of pesticide coated seeds,[15–17] or during litter breakdown, where pesticide residues, systemically persistent in the plant material are released into the environment.[18,19] As most applied chemicals end up settling on the soil, pesticide residues can get deposited and persist in the soil layers.[20,21] The persistence of pesticide residue depends on how strongly they adsorb to the available soil sorbents, and there are typically three available soil sorbents; soil organic matter, metal-(oxyhydrl)oxide, and clay.[22–24] Clay and organic matter make up the major constituents of soil,[22,25,26] and are associated with numerous functional groups, such as carbonyl, amino, imidazole, sulfonic, sulfhydryl, carboxyl, inorganic hydroxyl, and siloxane ditrigonal cavities, which increases the affinity of pesticide residues to chemically adsorb to soil components.[13] Once adsorbed, these pesticide residues are usually excluded from further degradation or transport through the environment.[20,21] However, fluctuations of biotic and abiotic factors in the soil environment could induce desorption of the adsorbed pesticide back into the environment.[27–29] Following their

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desorption, these pesticide residues can be transported around the environment through multiple routes.

In order for thorough assessments of the levels of chemical residues and their persistence in the soil to be conducted, reproducible and robust methods and protocols for extracting and identifying pesticides are required. However, pesticide residue extraction from the soil remains challenging, and the quantification of persisting pesticide residues is difficult due to the complex interactions between the soil sorbents and the sorptive pesticide compounds.[10,30] A number of extraction methods have proven effective in extracting pesticides from soils, namely single-drop and liquid microextraction,[31] supercritical and pressurised liquid extraction (PLE),[32] microwave-assisted extraction (MAE),[33] solid-phase microextraction (SPME),[34] sorptive-phase extraction,[35] hollow-fibre membrane solvent microextraction,[36] ultrasonic solvent extraction (USE),[37] and Soxhlet extraction.[38] However, these methods have associated disadvantages, including considerable time requirements in their set up, excessive solvent consumption or limited success for specific compounds.[39] The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, which significantly simplified pesticide extraction, was welcomed by modern analytical labs to overcome these issues. Anastassiades *et al.* first developed the QuEChERS extraction method in 2003 to analyse pesticide residues in food products,[40] but since then, it has been adapted to extract pesticides from many other matrices.[41–46] The QuEChERS method is not the only economised version of the previously described extraction methods, but it also has more environmentally friendly procedures that align with current green chemistry and analytical ethics. In addition, this method allows for large sample throughput and consistent reproducibility with high recoveries of the broad spectrum of compounds.[39] QuEChERS extraction only requires three steps to obtain pesticide residue extracts; partitioning, salting-out, and clean-up.[40,47,48]

One of the main challenges associated with the analytical analysis of environmental samples, particularly soils, is the interference encountered in the form of matrix effects. Based on the European Guidelines document (SANTE),[49] the matrix effect can be unfamiliar for the analytical system adopted, inconsistent in presence and intensity, and may not be obvious. As it affects method selectivity and sensitivity, in the form of enhancement of suppression effect on the detection system response, eliminating as much as possible matrix effects during pesticide residue analysis is critical. A complex matrix like soil requires robust clean-up steps to remove any co-eluting compounds, which are often quite numerous in the soil matrix, whilst still ensuring a reproducible and effective extraction of analytes of interest. The clean-up steps in QuEChERS extraction involve dispersive solid-phase extractions (d-SPE), involving three of the most widely used solid sorbents; primary and secondary amine (PSA), C_{18}, and graphitised carbon black (GCB).[40] Usage of these solid sorbents aids the removal of contaminants and prevents unwanted co-extractants from the matrix, where the addition of PSA enhances the removal of polar compounds, such as sugars, organic acids, and fatty acids; C_{18} removes lipids, sterols, and other non-polar compounds; and GCB removes pigments.[40,50] Even though these clean-up components are crucial, QuEChERS does not have one fixed procedure for eliminating matrix effects from different matrices. Usage of d-SPE cleaning components in any QuEChERS extraction requires additional optimisation study. An inclusion of d-SPE during clean-up steps requires consideration of their suitability to the analytes of interest,[39,51,52] the particular matrix,[40,53] quantity of d-SPE required,[54,55] the combinations of d-SPE to be used,[39,56] and standing time for the mixture of d-SPE components in sample extracts.[57] Only then can the most appropriate clean-up procedure that maximises the efficiency of pesticide residue recovery be selected.

Compared to d-SPE based clean-up of QuEChERS, dissolution using liquid/liquid extraction methods employed through Dutch mini-Luke[58,59] is one of the oldest and most effective means of reducing matrix effects. Despite liquid/liquid partitioning disadvantages, namely the higher volumes of solvents and waste, the Dutch mini-Luke extraction provides relatively cleaner extracts even without additional clean-up step.[58,60] Additionally, as Dutch mini-Luke uses a combination of acetone/petroleum ether/dichloromethane (v/v/v 1/1/1), it presents a lower co-extractive concentration than acetonitrile and ethyl acetate. Lower concentrations of co-extractives result in fewer contaminants being introduced to the instrument systems.[61] Given that there is no requirement for clean-up step optimisation, Dutch mini-Luke represents a robust extraction method that can be successfully employed on multiple matrices effectively without the additional modification.

We present here a systematic comparison of the QuEChERS and Dutch mini-Luke extraction methods for the quantification of multiple classes of pesticides from blank soils (defined in Section 2.3) through fortified recovery experiments using High-Performance Liquid Chromatography (HPLC) coupled with ultraviolet detection. The extraction methods were fully validated and evaluated based on extraction efficiency, limits of detection and quantification, and pesticide recoveries. The target analytes which included five insecticides, one herbicide, and one fungicide, were selected based on their abundance in pesticide usage records for Ireland. To our knowledge, a comparison between QuEChERS and the Dutch mini-Luke pesticide extraction method for soil matrix has not previously been reported. The comparison provided the most effective way for a single mixed extraction and analysis of widely used pesticides in soil.

2. Materials and methods

2.1 Reagents and materials

HPLC grade acetonitrile (MeCN), HPLC grade methanol (MeOH), HPLC grade dichloromethane (DCM), HPLC grade acetone, HPLC grade ethyl acetate, reagent grade MeOH, formic acid 98%, acetic acid (HAc) 100%, ammonium formate, anhydrous sodium sulphate, citrate salt extraction tube (sodium chloride: 1 g, sodium citrate dibasic sesquihydrate: 0.5 g, sodium citrate tribasic dihydrate: 1 g), primary-secondary amine (PSA), anhydrous magnesium sulphate ($MgSO_4$), and the certified reference standards, all of >97% purity, of

acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam, fluroxypyr, prothioconazole and the internal standard of triphenyl phosphate were purchased from Sigma-Aldrich (Ireland). A 25 kg pack of sand of 50–70 mesh particle size was obtained from Lennox (Ireland). HPLC grade petroleum ether was obtained from Fisher Scientific (Ireland). Millipore Millex syringe filters with hydrophilic PTFE membrane (pore size 0.22 μm and 20 mm diameter) and 1.5 mL autosampler vials were purchased from Sigma-Aldrich. Ultrapure water, deionised to a resistance of <18 MOhm, used throughout the study was generated using ELGA Purelab Ultra SC MK2 (ELGA, UK).

2.2. Preparation of standard solutions

Individual stock standard solutions (1000 ng μL^{-1}) of acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam, fluroxypyr, prothioconazole and the internal standard of triphenyl phosphate (TPP) were prepared monthly in HPLC grade acetonitrile and stored glass vials in −20 °C. The working standard solutions (10 ng μL^{-1}) were prepared by diluting in 25% HPLC grade MeOH in ultrapure water.

2.3. Collection of blank soil samples

Blank soil samples were collected from the Dublin City University (DCU) community garden, a small-scale pesticide use free, biologically intensive vegetable farm. The same extraction methods were performed on the blank soil samples to ensure there was no potential interference.

2.4. Soil sample preparation

The surface soil samples were collected at 15 cm to 20 cm depths. The collected blank soil samples were air-dried for 24 h at room temperature, ground and sieved through a 1 mm mesh to remove any plant roots, rocks, *etc.* Prepared soil samples were then stored in zip-lock bags at −4 °C until analysis.

2.5. Extractions

2.5.1. QuEChERS extraction. Five grams of each soil sample were weighed into 50 mL PTFE centrifuge tubes and spiked at the required fortification level of pesticide standard solution. Then the centrifuge tube was hand-shaken for homogeneous mixing of the pesticide standard and the soil and left standing for 45 min in a fume hood. After 45 min, 5 mL of deionised water was added to the mixture to hydrate the soil, followed by 10 mL acetonitrile with 1% acetic acid. The mixture then was shaken vigorously for 1 min and sonicated for the next 10 min. Following sonication, the citrate salt mixture (sodium chloride: 1 g, sodium citrate dibasic sesquihydrate: 0.5 g, sodium citrate tribasic dihydrate: 1 g) was added into the centrifuge tube. The centrifuge tube was then vortexed for 1 min before being centrifuged at 4000 rpm for 10 min. Nine mL of supernatant was transferred to a 15 mL PTFE centrifuge tube containing 300 mg PSA and 900 mg $MgSO_4$. The extract was vortexed for 1 min, followed by 10 min centrifugation at 4000 rpm. 5.0 mL of supernatant was then transferred into a silanised glass vial and then concentrated to dryness under a gentle stream of nitrogen. The concentrated extract was then reconstituted in 500 μL of mobile phase solution. Finally, the extract was filtered through a 0.22 μm hydrophilic PTFE syringe filter into an autosampler vial for HPLC-UV analysis.

2.5.2. Dutch mini-Luke extraction. 15 g of blank soil samples were weighed into 250 mL PTFE centrifuge tubes and spiked at the required fortification level of pesticide standard solution. Subsequently, 15 mL of deionised water was added and shaken vigorously for 1 min, followed by adding of 30 mL of 1% acetic acid in acetone, and the mixture was then homogenised using IKA Ultra-Turrax T-25 homogeniser for 30 s at 1500 rpm. 30 mL petroleum ether and 30 mL dichloromethane were added to the homogenate, and the sample mixture was homogenised again using the homogeniser for 30 s at 1500 rpm to induce phase separation. After centrifugation at 4000 rpm for 10 min, 60 mL of the obtained supernatant was carefully transferred into a 100 mL conical flask. The extracts were evaporated to reduce the volume before being transferred to a 10 mL volumetric flask made up to volume with ethyl acetate. Following that, 0.5 mL of the ethyl acetate was then diluted into 10 mL volumetric flask topped up with methanol. Finally, 1 mL of methanol extract then filtered through 0.22 μm hydrophilic PTFE syringe filter into an autosampler vial for HPLC-UV analysis.

2.6. HPLC-UV analysis

The HPLC-UV analysis was carried out using a Shimadzu SPD-20A Prominence HPLC system coupled with a UV-Vis detector (Tokyo, Japan), set at a wavelength of 254 nm. The chromatographic separation was performed using an XBridge UPLC BEH column (4.6 × 100 mm i.d., 3.5 μL 3.9 × 5 mm). The analytes were separated using a gradient of 5 mM ammonium formate with 0.1% formic acid in ultrapure water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). The mobile phase gradient was as follows: 10% B from 0 to 0.5 min; a gradient increase to 98% B in 28 min; composition maintained at 98% B for 4 min, followed by returning to the starting mobile phase and re-equilibration time for 4 min. The flow rate used was kept constant at 0.5 mL min^{-1}. The injection volume was 10 μL.

2.7 Parameters of method validation and comparison

2.7.1. Specificity. Validation of method specificity was carried out by analysing blank soil samples ($n = 3$) to determine whether any interference was occurring for any of the targeted analytes.

2.7.2. Linearity. Pesticide standards were injected individually at seven concentrations incrementally. The lowest point of concentration for each analyte was the method quantification limit (MQL). The linearity of each of the targeted analytes was measured based on their response in the solvent and soil calibration ranges (matrix-matched calibration).

2.7.3. Accuracy (recovery%) and precision (RSD%). Recovery and precision assessment were carried out by fortifying blank samples at three concentration levels, MQL, five times

MQL and ten times MQL, for three replicates. Recoveries between 70–120% with RSD% lower than 20% were considered satisfactory.[49] Recoveries of the three concentrations were further evaluated using multiple unpaired *t*-tests of an analyte between both extraction methods to determine significant differences between the means of an analyte's recoveries. The method precision was validated in terms of reproducibility and repeatability, represented by the relative standard deviation (RSD%).

2.7.4. Method detection limit (MDL) and method quantification limit (MQL). Method sensitivity was evaluated by MDL and MQL. MDL was considered acceptable when the signal to noise ratio (S/N) was ≥3. MQL was deemed satisfactory when quantified with acceptable accuracy with the lowest fortification level when recoveries are between 70–120%, with RSD% lower than 20%. The significant difference of a respective analyte's MDL and MQL in both extraction methods was evaluated using multiple unpaired *t*-tests.

2.7.5. Matrix effect (%). Matrix effect (ME%) was calculated to assess the influence of co-extracted compounds from the soil on analytical signals. ME% were calculated based on eqn (1), comparing the slopes in the matrix (S_m) (blank extracts) calibration solutions and the pure solvent (S_s) (in acetonitrile) calibration solutions.

$$\text{ME}\ (\%) = ((S_m/S_s) - 1) \times 100\% \quad (1)$$

2.7.6. Quality control. Quality control was carried out by using TPP as an internal standard for each batch of sample analysis and fortified before each extraction to reach a concentration of 1 ng μL^{-1} in the final extract.

3. Results and discussion

3.1. Comparison of the procedural blanks for the two methods

A series of procedural blank sand and blank soil samples for the two extraction methods were performed to check for potential contamination or co-elutants respective to each extraction procedure. The inclusion of blank sand samples in each extraction method was to assess if any co-elutants resulted from the extraction components themselves rather than from the soil matrix. These checks were performed to confirm no co-elutants from the blank samples and the extraction components eluted at the same retention time as the targeted analytes (Fig. S1 and S2†). Overall, the specificity of the method was confirmed, with no other contamination observed at the same retention time as the targeted compounds from all the blanks using the two extraction methods.

3.2. Comparison of method detection limit (MDL) and method quantification limit (MQL)

The linearity of the calibration curves was evaluated using seven procedural calibration points (ranging between 0.1 to 3 ng μL^{-1}), which were performed by spiking the blank samples before extraction. Correlation coefficients (r^2) were evaluated for both methods (Tables 1 and 2). For the QuEChERS extraction, the r^2 value for most analytes ranged between 0.901 and 0.939 in blank sand samples and between 0.914 and 0.961 in soil samples (Table 1). For both blank samples, the linearity of the fluroxypyr and prothioconazole could not be determined due to a failure to detect both analytes through the procedural calibration line. For the Dutch mini-Luke extraction, all targeted analytes were successfully resolved using a procedural calibration line, with r^2 values ranging between 0.938 and 0.991 and 0.934 and 0.992 for the sand and soil samples respectively (Table 2). Although the r^2 ranges obtained for both QuEChERS and Dutch mini-Luke were acceptable and allowed for the accurate determination of MDL and MQL in both substrates, the failure to identify fluroxypyr and prothioconazole from the QuEChERS extracted samples highlights a limitation with this method in comparison to DML.

The sensitivity of each extraction method was assessed in terms of MDL and MQL, which were estimated based on the standard deviation of the response and slope of the constructed procedural calibration line. The QuEChERS extraction was only successful for determining the MDL and MQL for neonicotinoids in blank sand and soil samples. In the blank sand samples, QuEChERS extraction resulted in MDL values QuEChERS extracted blank sand samples ranged between 0.31

Table 1 QuEChERS range, correlation coefficient (r^2), MDL and MQL of targeted pesticides through procedural calibration line[a]

	Solvent				Sand				Soil			
Pesticides	Range (ng μL^{-1})	r^2	MDL (ng μL^{-1})	MQL (ng μL^{-1})	Range (ng μL^{-1})	r^2	MDL (ng μL^{-1})	MQL (ng μL^{-1})	Range (ng μL^{-1})	r^2	MDL (ng μL^{-1})	MQL (ng μL^{-1})
Acetamiprid	0.05–3	0.999	0.13	0.39	0.1–3	0.917	0.31	0.95	0.1–1.5	0.961	0.56	1.7
Clothianidin	0.05–3	0.999	0.13	0.38	0.1–3	0.901	0.32	0.97	0.1–3	0.914	0.85	2.58
Fluroxypyr	0.1–3	0.999	0.22	0.68	ND	ND	ND	ND	ND	ND	ND	ND
Imidacloprid	0.05–3	0.999	0.18	0.54	0.1–3	0.934	0.31	0.95	0.1–0.7	0.942	0.69	2.08
Prothioconazole	0.05–3	0.999	0.15	0.44	ND	ND	ND	ND	ND	ND	ND	ND
Thiacloprid	0.05–3	0.999	0.13	0.39	0.1–3	0.919	0.36	1.08	0.1–1.5	0.927	0.78	2.35
Thiamethoxam	0.05–3	0.991	0.15	0.46	0.1–3	0.939	0.31	0.95	0.5–3	0.915	0.85	2.56

[a] Not detected (ND).

Table 2 Dutch mini Luke range, correlation coefficient (r^2), MDL and MQL of targeted pesticides through procedural calibration line

	Solvent				Sand				Soil			
Pesticides	Range (ng μL^{-1})	r^2	MDL (ng μL^{-1})	MQL (ng μL^{-1})	Range (ng μL^{-1})	r^2	MDL (ng μL^{-1})	MQL (ng μL^{-1})	Range (ng μL^{-1})	r^2	MDL (ng μL^{-1})	MQL (ng μL^{-1})
Acetamiprid	0.05–3	0.999	0.13	0.39	0.05–3	0.962	0.37	1.13	0.05–2	0.961	0.32	0.97
Clothianidin	0.05–3	0.999	0.13	0.38	0.1–3	0.985	0.26	0.80	0.05–3	0.992	0.20	0.60
Fluroxypyr	0.1–3	0.999	0.22	0.68	0.05–3	0.983	0.25	0.74	0.05–1.5	0.935	0.42	1.26
Imidacloprid	0.05–3	0.999	0.18	0.54	0.05–2	0.946	0.44	1.34	0.05–1.7	0.939	0.40	1.22
Prothioconazole	0.05–3	0.999	0.15	0.44	0.05–2	0.938	0.48	1.46	0.5–1.7	0.934	0.42	1.26
Thiacloprid	0.05–3	0.999	0.13	0.39	0.05–3	0.977	0.29	0.87	0.05–3	0.952	0.42	1.27
Thiamethoxam	0.05–3	0.999	0.15	0.46	0.05–3	0.991	0.18	0.53	0.05–3	0.978	0.28	0.85

to 0.36 ng μL^{-1}, while the MQL ranged from 0.95 to 1.08 ng μL^{-1}. In the blank soil samples, QuEChERS resulted in MDL values ranging between 0.56 and 0.85 ng μL^{-1} and MQL values ranging between 1.7 and 2.58 ng μL^{-1}. On the other hand, Dutch mini-Luke extractions provided complete information on MDL and MQL for all the targeted analytes. In the blank sand samples, the MDL ranged between 0.18 to 0.48 ng μL^{-1} and the MQL ranged between 0.53 to 1.46 ng μL^{-1}, whereas in blank soil samples, the MDL of analytes ranged between 0.20 to 0.42 ng μL^{-1} and the MQL ranged between 0.60 to 1.27 ng μL^{-1}.

When MDL and MQL values were compared, it was determined that Dutch mini-Luke allows for the detection and quantification at lower pesticide concentration values than the QuEChERS method (Fig. 1 and 2). Even though Dutch mini-Luke's MDL values for most analytes are observed to be lower, only the analytes acetamiprid and thiamethoxam result in significant difference (p-value<0.01). On the other hand, the MQL value for all the neonicotinoids were observed to be significantly different, with either p-value less than 0.01 or 0.001. As MDL and MQL are estimated through the procedural calibration line, the value obtained could broadly vary from one method to another as it known to be affected by interference from the matrix.[62–64] In the specificity study, analysis of sand blank extract using QuEChERS extraction still shows the presence of co-elutants, while Dutch mini-Luke displays a baseline that indicates low to no presence of co-elutants. Considering that sand blanks are not expected to have any possible elutants, it is possible that the elutants could be from the QuEChERS extraction component themselves. Hence, the lower MDL and MQL from Dutch mini-Luke ultimately translate to better method sensitivity.

3.3. Comparison of QuEChERS and Dutch mini Luke recoveries

Recovery efficiency is a critical property of any extraction method as it signifies the method's accuracy and performance. The recovery experiments were performed by fortifying blank soil and sand samples at three levels, corresponding to low, medium, and high concentrations of pesticide analytes in the soil, based on the MQL values calculated from the linearity. Pesticide recovery was reported as a percentage of the spiked

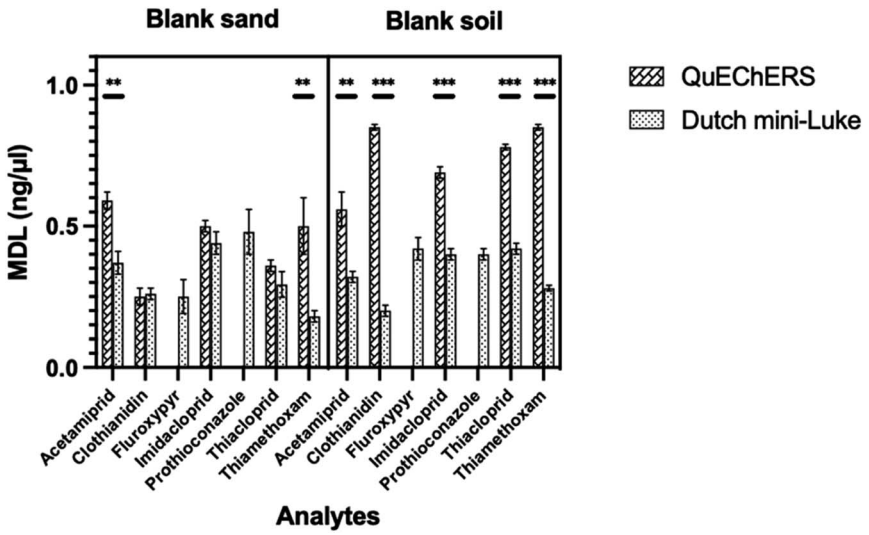

Fig. 1 Comparison of MDL values obtained through QuEChERS and DML using blank sand and blank soil samples. Asterisks show statistical significance (**p < 0.01, ***p < 0.001).

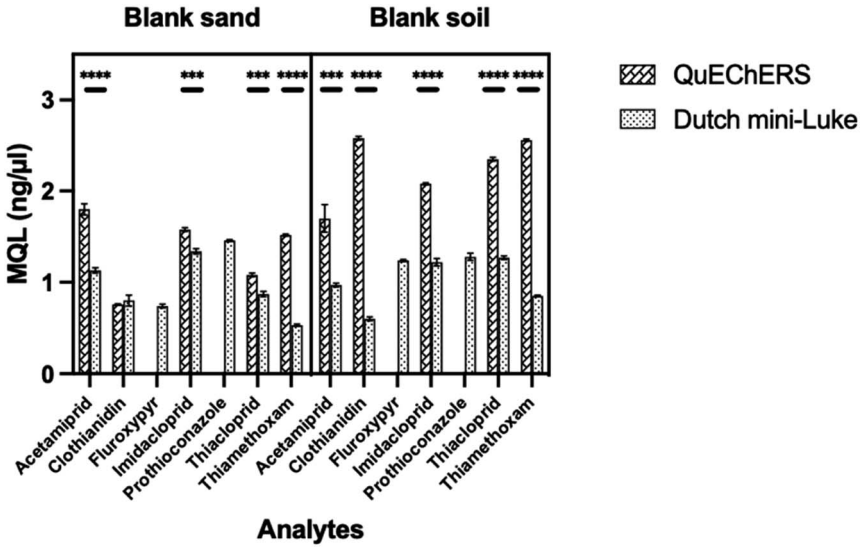

Fig. 2 Comparison of MQL values obtained through QuEChERS and DML using blank sand and blank soil samples. Asterisks show statistical significance (***$p < 0.001$, ****$p < 0.0001$).

concentration. Both blank soil and sand samples were fortified at the same concentration levels to be comparable. Based on the SANTE guidelines, acceptance criteria of the validation parameters of the method should have an average recovery in the range of 70–120% with RSD% less or equal to 20%.[49] Three different fortification concentrations, the MQL, five times MQL, and ten times MQL, were chosen for each extraction method. The fortification at these three concentrations levels gives a complete evaluation of the method's robustness in efficiently recovering all the targeted analytes over a range of concentrations.

Evaluation of the extraction efficiencies achieved using QuEChERS shows that the recovery percentage from the blank sand matrix had a slightly higher or similar recovery percentage than the recovery in blank soil samples. Recovery of the neonicotinoids from the blank sand and blank soil samples were deemed satisfactory, with recovery values of 85 to 111% (Fig. 3). However, the analytes fluroxypyr and prothioconazole were not detected at any of the three fortified levels. Comparing the recovery of analytes in the blank soil, at MQL level, Dutch mini-Luke's extraction method is observed to have significantly higher recovery percentages for all the neonicotinoids compared to QuEChERS, where the multiple unpaired *t*-tests depict comparison *p*-value is either between 0.05 or 0.01. Meanwhile, the fortification at five times MQL level in blank soil, only acetamiprid and thiamethoxam were noted to have significantly different recovery percentages ($p < 0.05$), and Dutch mini-Luke performed consistently better in the recovery of neonicotinoids from blank soil samples at ten times MQL, with clothianidin, imidacloprid, thiacloprid, and thiamethoxam noted to have significant difference of either $p < 0.05$ or $p < 0.01$ (Fig. 3).

The failure of QuEChERS to extract detectable fluroxypyr and prothioconazole from the blank soil samples could be due to the presence of organic matter and clay components. The amounts of organic matter and clay in soil matrices are directly

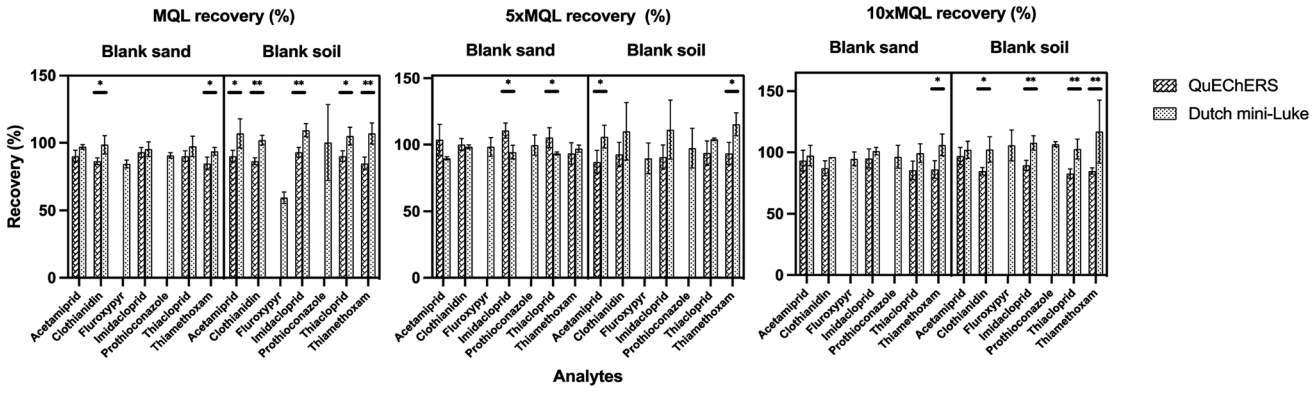

Fig. 3 Comparison of recovery efficiencies using three different fortification concentrations extracted from blank sand and soil samples. Asterisks show statistical significance (*$p < 0.05$, **$p < 0.01$).

Table 3 Recoveries and RSD% of the seven targeted pesticides fortified at MQL, five times MQL, and ten times MQL concentrations in blank sand and soil samples using QuEChERS and Dutch mini Luke extractions[a40,58,59]

		QuEChERS, % recovery ± RSD%		Dutch mini-Luke, % recovery ± RSD%	
Pesticides	Fortification level	Blank sand	Blank soil	Blank sand	Blank soil
Acetamiprid	MQL	90 ± 4.9	90 ± 4.9	97 ± 1.4	107 ± 9.8
	5 × MQL	105 ± 11.1	87 ± 9.9	90 ± 1.5	106 ± 8.1
	10 × MQL	93 ± 9.3	97 ± 7.0	101 ± 3.1	108 ± 5.8
Clothianidin	MQL	86 ± 2.6	86 ± 2.6	99 ± 6.7	102 ± 3.4
	5 × MQL	100 ± 4.9	92 ± 9.6	99 ± 1.4	110 ± 19.6
	10 × MQL	87 ± 7.3	85 ± 3.9	96 ± 0.3	102 ± 10.3
Fluroxypyr	MQL	ND	ND	84 ± 2.8	59 ± 6.8
	5 × MQL	ND	ND	99 ± 7.1	90 ± 12.5
	10 × MQL	ND	ND	99 ± 7.6	103 ± 8.0
Imidacloprid	MQL	93 ± 3.6	93 ± 3.6	95 ± 5.9	109 ± 4.5
	5 × MQL	111 ± 5.1	91 ± 10.1	94 ± 5.8	111 ± 20.3
	10 × MQL	95 ± 8.4	89 ± 4.3	95 ± 5.8	106 ± 11.9
Prothioconazole	MQL	ND	ND	91 ± 1.9	100 ± 27.8
	5 × MQL	ND	ND	99 ± 7.4	97 ± 15.5
	10 × MQL	ND	ND	106 ± 8.3	117 ± 21.8
Thiacloprid	MQL	90 ± 4.6	90 ± 4.6	97 ± 8.1	105 ± 6.1
	5 × MQL	105 ± 7.2	94 ± 9.9	93 ± 1.2	104 ± 1.0
	10 × MQL	85 ± 8.9	83 ± 4.2	96 ± 9.1	107 ± 1.9
Thiamethoxam	MQL	85 ± 5.4	85 ± 5.4	94 ± 3.0	107 ± 7.7
	5 × MQL	93 ± 8.5	93 ± 9.5	97 ± 2.6	115 ± 7.3
	10 × MQL	86 ± 8.6	85 ± 3.3	97 ± 8.3	102 ± 7.0

[a] ND: not detected. RSD%: relative standard deviation.

proportional to the adsorption of pesticide analytes.[65–67] Fluroxypyr and prothioconazole have log K_{OW} values of 2.20 (ref. 68 and 69) and 4.05 (ref. 70 and 71) respectively, which indicates their greater affinity to organic matter. Therefore, it can be assumed that both residues are adsorbed strongly to the organic matter or clay components in the blank soil samples as soon as they are spiked. Hence, sample preparation remains a crucial step in any extraction method, which presents a challenge for the QuEChERS extraction to trigger the desorption of pesticide analytes from soil constituents. In addition, soil is a complex matrix that requires extra attention on the clean-up step during an extraction. Hence, the clean-up step in QuEChERS extraction on soil matrix is essential for removing any co-extractants that might also have been extracted. As much as dispersive SPE (d-SPE), utilising PSA, is crucial for the matrix clean-up step, it can inhibit the recovery of analytes. Sack *et al.* had shown that PSA inclusion during sample clean-up during acidic pesticide analysis increases the loss of free acids.[52] The results of this study support this finding as we failed to quantify fluroxypyr and prothioconazole using QuEChERS extraction, and it seems that using d-SPE comes with a trade-off between obtaining a cleaner extract and comprehensive analyte recovery.

In contrast to QuEChERS, the Dutch mini-Luke extraction successfully extracted fluroxypyr and prothioconazole with recovery efficiency values between 59 to 117% and good recovery values (between 102 and 115%) were obtained for the neonicotinoids. The Dutch mini-Luke extraction method also has better recovery efficiencies across all the targeted analytes in comparison to QuEChERS (Fig. 3). Even though, under the SANTE guidelines, fluroxypyr extracted using Dutch mini-Luke with MQL fortification concentration level gives a lower percentage (59%) than the acceptance criteria (70–120%), it still outperforms QuEChERS where fluroxypyr was not quantified at all. Compared to QuEChERS, Dutch mini-Luke has additional advantages. Dutch mini-Luke not only includes mechanical energy through high rpm homogeniser, but it also provides chemical energy, with the inclusion of higher organic solvents such as acetone, dichloromethane, and petroleum ether. Mechanical grinding in immiscible organic solvents breaks the soil constituents into much smaller particles, exposing more extensive surface area for extraction, which helps to expose the adsorbed pesticide analytes in the humic substances or the inter-crystalline layers of clay minerals, subsequently breaking their bonds and improving partitioning into the organic phase.[72]

Furthermore, fluroxypyr and prothioconazole with pK_a values of 2.94,[73] and 6.9,[74] respectively, are most stable at low pH values. With this in mind, both of these analytes are expected to be extracted most efficiently using acidified solvents.[75] While salt is required to extract polar analytes, such as neonicotinoids, it inhibits the extraction of fluroxypyr and prothioconazole. QuEChERS cannot offer flexible modification towards the pH of extraction solvents without sacrificing the overall performance of multi-class pesticide extraction. However, even when using acidified solvents, such as 1% acetic acid in acetonitrile (as used in this study), fluroxypyr and prothioconazole were still not extracted using QuEChERS. The usage of PSA during

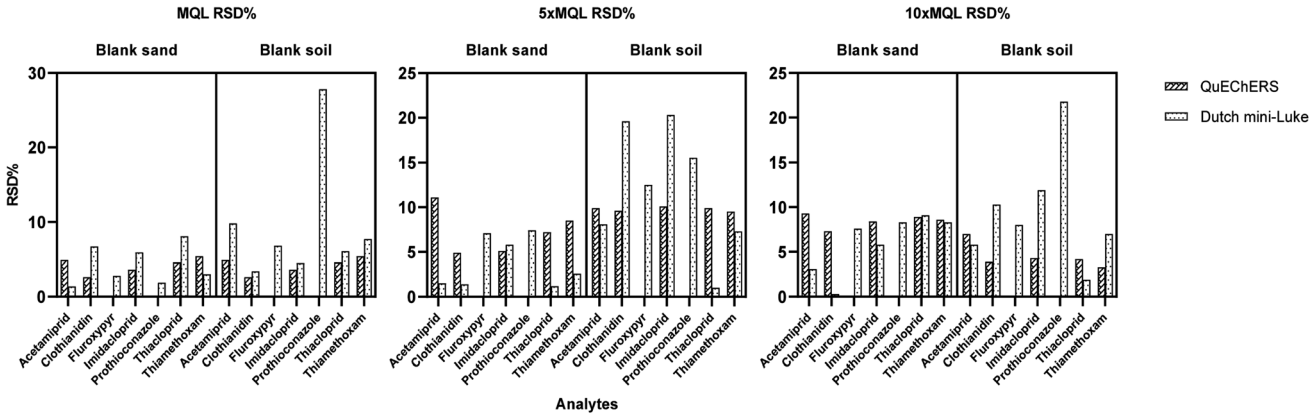

Fig. 4 Comparison of RSD% value obtained using three different fortification concentrations extracted from blank sand and soil samples.

QuEChERS d-SPE clean-up is crucial as PSA helps to efficient removal of sugars, pigments, and organic acids.[76]

However, as Lehotay *et al.* had shown, the usage of the PSA clean-up step during extraction decreases the acidity of the extracts by 2–3 pH units.[75] This drastic change in pH could lead to loss of fluroxypyr and prothioconazole through degradation or be retained, as PSA interacts with both labile acidic analytes. This reasoning is supported by published studies demonstrating that the recovery of acidic analytes' improves when PSA is not used for clean-up.[44,77,78]

3.4. Comparison of QuEChERS and Dutch mini Luke reproducibility

Due to the failure to quantify fluroxypyr and prothioconazole using QuEChERS, the RSD% of both these analytes were unsuccessfully measured. QuEChERS provided good reproducibility for the neonicotinoids with values ranging from 2.6 to 10.1%, below the RSD% acceptance threshold of 20% (Table 3). On the other hand, the RSD% percentage of all the targeted analytes was successfully measured using Dutch mini-Luke, with values less than or equal to 20%, except for prothioconazole, where there was a higher variability observed for the fortification level of MQL and ten times MQL. To fully interpret the reason behind this variability, the RSD% percentages of prothioconazole extracted using Dutch mini-Luke were compared in the blank sand and blank soil extracts, where high variability was only observed in the prothioconazole fortified soil sample (Fig. 4). The higher than acceptable RSD% value could have resulted from their unpredictable behaviour toward the organic matter components in the soil. This explanation is supported by assessment of the matrix effect, which for prothioconazole was more affected by soil matrix components than for the other analytes (Fig. 5).

3.5. Comparison of QuEChERS and Dutch mini Luke matrix effect

Matrix matched calibration was carried out by reconstituting increasing pesticide concentrations in dried sand and blank soil extracts. Matrix effect (ME) was calculated by comparing the slopes of the calibration curves of standards in solvent and samples. MEs with values between +20% and −20% are considered to represent low matrix effects, values between +20% and +50% represent a medium matrix effect, and values less than −50% or higher than +50% represent high matrix effects.[79,80] In the blank sand sample, QuEChERS extraction displayed a low matrix effect consistently across most analytes, except for prothioconazole, which was not detected in both blank sand and blank soil. The highest matrix effect using QuEChERS was exhibited by imidacloprid with 19%, and the lowest is fluroxypyr with 11%. However, in QuEChERS's blank soil extract, fluroxypyr shows a high matrix effect with a value of 291% (Fig. 5). The failure to detect prothioconazole and the high matrix effect value for fluroxypyr are examples of matrix effects signal suppression (loss in response) and signal enhancement (increase in response). Lin *et al.* also reported signal suppression for prothioconazole but could eliminate the matrix effects by performing calibration using an external matrix-matched standard.[81]

Additionally, Kaczyński *et al.* noted that the inclusion of PSA in the clean-up step not only failed to provide the expected recovery range for fluroxypyr from a soil matrix but also the matrix effect could not be reduced.[44] In their study, the inclusion of PSA had enhanced the signal of fluroxypyr by 47.2%, compared to not including a clean-up step at all.[44] Due to these matrix effects, signal suppression of prothioconazole could lead to a false-negative measurement,[82] whereas an enhanced signal for fluroxypyr could lead to a false-positive measurement.[83,84] An ever-present issue with pesticide quantification from complicated matrices such as soil is the presence of co-eluting compounds that negatively affect the extraction method's precision, sensitivity, and accuracy.[85] In the matrix effect experiment, all the analytes are reconstituted in the dried blank extracts, so failure to detect them is not due to failure to extract them. The specific mechanism of matrix effects is uncertain, although it is thought to arise from competition between an analyte and undetectable matrix components that co-elute.[86] A number of factors can produce signal suppression or enhancement, but they are mainly caused by endogenic compounds already present in the sample and remain in the

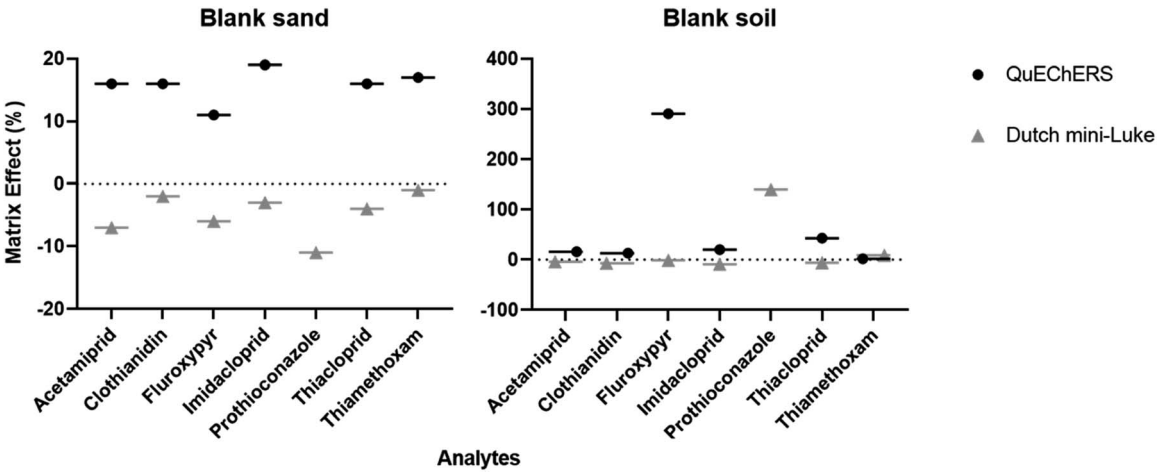

Fig. 5 Comparison of matrix effect using a matrix-matched calibration curve with blank sand and blank soil samples.

Table 4 Comparison of additional parameters for the QuEChERS and Dutch mini-Luke extractions

Parameters	QuEChERS extraction	Dutch mini-Luke extraction
Sample weight	5 g	15 g
Extraction time (a batch of four)	1 hour	3 hours
Extraction steps to analysis	Eight steps (extraction, salting-out, phase partitioning, centrifugation, clean-up, second centrifugation, concentrating, reconstitution)	Six steps (extraction, phase partitioning, centrifugation, concentrating, dissolutions into ethyl acetate, dissolutions into MeOH)
Sequential or simultaneous procedure	Two sequential extraction containers	Four sequential containers
Solvent usage	15 mL (deionised water and acetonitrile)	105 mL (deionised water, acetone, DCM, and petroleum ether)

extract after sample preparation or extraction. Endogenic compounds can be ionic compounds, such as inorganic electrolytes or salts, polar compounds, such as amines, carbohydrates, lipids, peptides or urea.[83] As discussed earlier, soil organic matter and PSA are the main affecting factors for analytes with the QuEChERS extraction method. Even though it is hard to determine which factor plays a more prominent role, the inclusion of a blank sand matrix in the comparison experiments can represent the suppression or enhancement effect presented by the components used during the extraction procedure. The suppression of prothioconazole's signal is an indication that the QuEChERS extraction components, namely PSA, are interfering with the quantification of this base-sensitive analyte.[52,75,77]

Even though the ME values for all the analytes in the Dutch mini-Luke blank sand were observed to be negative in value (Fig. 5), they still represent low matrix effects. The analytes were reconstituted in dried blank extracts, the only possible factor in the blank sand extract that suppresses the analytes' signal would be the carry-over from the Dutch mini-Luke extraction components. However, as the analyte's signal suppression values range between −1 and −11%, based on the SANTE guidance document, it is classified as subtle interference, and the extraction method does not require additional modification for sample analysis.[49] In addition, prothioconazole was successfully quantified using Dutch mini-Luke, although a high matrix effect of 140% was observed. The low matrix effects in the blank sand matrix extract allow us to conclude that the high matrix effect for prothioconazole is not due to any of the Dutch mini-Luke extraction components. Therefore, the high matrix effect for prothioconazole is most likely due to the soil matrix itself, with a strong possibility it is caused by the soil organic matter.

Compared to d-SPE clean-up of QuEChERS, Dutch mini-Luke employs a more straightforward means of reducing or eliminating the matrix effect through sample dilution. The main advantage of using sample dilution to reduce or eliminate the matrix effect is that it introduces less matrix load into the

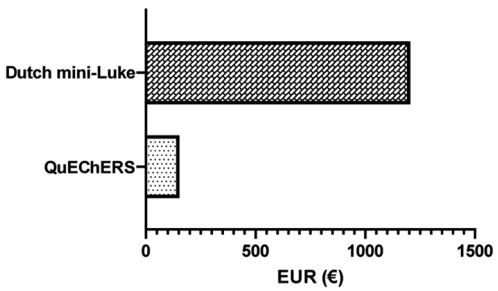

Fig. 6 Comparison of equipment costs required for QuEChERS and Dutch mini-Luke extraction methods.

Table 5 Summary of the method validation parameters comparison between QuEChERS and Dutch mini-Luke

	QuEChERS		Dutch mini-Luke	
Method validation parameters	Blank sand	Blank soil	Blank sand	Blank soil
Method detection limit (MDL) (ng μL^{-1})	0.31–0.36	0.56–0.85	0.18–0.48	0.20–0.42
Method quantification limit (MQL) (ng μL^{-1})	0.95–1.08	1.7–2.58	0.53–1.46	0.60–1.27
Accuracy (recovery%)	85–111	83–97	84–106	59–117
Precision (RSD%)	2.6–11.1	2.6–10.1	0.3–9.1	1.0–27.8
Matrix effect (%)	11–19	−11 to −1	2–291	−9–140

chromatographic system with every injection. Ferrer *et al.* discussed how in the analysis of a multi-residue method, the sample extract injection would be similar to the amount of the matrix injection, that is, 1 g of sample per mL.[87] In contrast, this study's Dutch mini-Luke extraction method has a sample dilution factor of 1/20. This means that 1 g of sample extract injection would only introduce the chromatographic system of 0.05 g of matrix load. This translates to better sensitivity and does not require additional extraction components that could compromise the quantitative analysis of the targeted analytes. With reduced levels of matrix components being injected into the analytical system, the life of sensitive equipment can be prolonged.[86–88]

3.6. Other parameters for comparison

In addition to the validation parameters described above, a number of additional parameters were considered, such as sample weight requirement, extraction time, number of extraction steps and procedures, and volume of solvent usage (Table 4).

The selection of extraction parameters, general characteristics and solvent requirements was based on established protocols, as reported in the literature and standard operating procedures (SOPs) in place in governmental bodies.[40,58] Both methods vary considerably and there are advantages to using each method. For example, QuEChERS requires lower amounts of starting sample, lower volumes of solvent, fewer extraction steps, and a more rapid method overall. Due to these differences, the extraction conditions are not directly comparable between these two extraction methods.

The potential shortcomings of Dutch mini-Luke include longer times and higher costs in comparison to QuEChERS. Fig. 6 depicts the estimated cost for the dedicated equipment required for both extraction methods. The high cost for employing Dutch mini-Luke extraction comes in the form of homogeniser, and its disperser tool, where during the time of writing this article, the total cost for both of the equipment comes to a total of €3690.50.[89,90] QuEChERS does not require a specific tool to assist in the extraction, and it only requires a vortex mixer to ensure thorough mixing of the extraction components. During the time of purchase in 2019, the vortex mixer cost €153.13.[91] In addition, the use of higher volumes of organic solvents, namely acetone, dichloromethane, and petroleum ether, for Dutch mini-Luke extractions also presents a certain degree of risk to the user and additional waste handling requirements. When using these extraction solvents, the user must practise extra vigilance when handling and changing between solvents in various steps.

Selected examples of the health risks associated with each solvent include: acetone – can cause severe eye irritation and specific target organ toxicity with a single exposure (Category 3),[92] dichloromethane – suspected of causing cancer,[93] petroleum ether – can cause specific target organ toxicity with repeated exposure (Category 2) and can be fatal if swallowed or enters the airway.[94]

These health risks can be avoided if the user is attentive during every extraction while following recommended exposure controls, using the required personal protective equipment (PPE), and disposed in accordance with the national and local regulations. On the other hand, acetonitrile would be the only non-polar solvent used in QuEChERS extraction, where it can be toxic when in contact with skin, causes serious eye irritation and harmful if inhaled or swallowed, and can be avoided with proper use of PPE and cautiousness was practised. Moreover, as Dutch mini-Luke requires the usage of additional tools, namely a homogeniser and its attachment, a certain level of technical training is required before the tools can be used efficiently during extraction. For these reasons, carrying out Dutch mini-Luke extractions requires a higher user skill level in comparison to QuEChERS.

4. Conclusion

Even though the QuEChERS extraction method complements recent trends toward "green" pesticide extraction techniques by providing faster, more straightforward, and cost-efficient approaches, it is not always suitable for determining pesticides belonging to certain chemical groups. To explore this further, we compared QuEChERS and the traditional Dutch mini-Luke and assessed their extraction efficiencies for seven pesticide analytes representing a number of different chemical groups of insecticide, herbicide, and fungicide. Tables 5 and S1† summarise our findings, demonstrating that all targeted analytes could be successfully recovered from both blank sand and soil samples, with good recovery (59–117%), except fluroxypyr at MQL fortification level with 59%. As for the repeatability of Dutch mini-Luke, all analytes had an RSD% value lower than or equal to 20%, except for prothioconazole at MQL and 10 × MQL fortification with 27.8% and 21.8%, respectively.

On the other hand, the QuEChERS extraction method had a satisfactory recovery for all the fortified neonicotinoids with percentages ranging between 85 to 111% and RSD% values of 2.6 to 10.1%. However, QuEChERS could recover neither fluroxypyr nor prothioconazole in any blank samples or at any fortification level. Compared to QuEChERS, Dutch mini-Luke does present analytical advantages, where it offers better sensitivity in the form of lower MDL and MQL, better recovery, and lower matrix effects in relation to most analytes. Hence, Dutch mini-Luke was determined to be the preferred extraction method for a single mixed analysis of neonicotinoids, triazoles and synthetic auxin pesticides from soil samples.

Author contributions

Mathavan Vickneswaran: investigation, formal analysis, visualisation and writing – original draft; James C. Carolan: funding acquisition, supervision and writing – review & editing; Blánaid White: conceptualisation, funding acquisition, supervision, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

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