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Evaluation of automated clean-up for large scope pesticide multiresidue analysis by liquid chromatography coupled to mass spectrometry

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A B S T R A C T

Clean-up step is essential during the multiresidue sample preparation process to remove undesired matrix components that may cause analytical interferences or suppression effect. However, its application generally by specific sorbents entails time-consuming work producing low recoveries for some compounds. Moreover, it usually needs to be adapted to the different co-extractives from the matrix present in the samples by using different chemical sorbents increasing the number of validation procedures. Therefore, the development of a more efficient and automated and unified clean-up procedure means a significant time reduction and laboratory work with improved performance.

In this study, extracts from different matrices (tomato, orange, rice, avocado and black tea) were purified by manual dispersive clean-up (different procedures according to the matrix group) in parallel with an automated µSPE clean-up workflow, in both cases based on QuEChERS extraction. The latter procedure employed clean-up cartridges containing a mixture of sorbent materials (anhydrous MgSO4/PSA/C18/CarbonX) suitable for multiple matrices. All the samples were analysed by liquid chromatography mass spectrometry and the results obtained from both procedures have been compared in terms of the extract cleanness, performance, interferences, and sample workflow. At the levels studied, similar recoveries were achieved by both techniques (manual and automated) except for reactive compounds when PSA was used as the sorbent material producing low recoveries. However, the µSPE recoveries were between 70–120%. Furthermore, closer calibration line slopes were provided when µSPE was applied to the different matrix groups studied. It is important to note that up to 30% more samples per day can be analysed using an automated µSPE compared to the manual method (which requires shaking, centrifuging, then taking the supernatant and adding formic acid in ACN); it also provides good repeatability - an RSD (%) $<$ 10%. Consequently, this technique is a very useful option for routine analyses, greatly simplifying the work of muti-residue methods.

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1. Introduction

The use of pesticides in agriculture has made it possible to control pests and diseases, extend the half-life of plants and crops, and improve crop appearance (vegetables and cereals etc.); it has also led to increased food production. However, pesticide residues and/or pesticide degradation products can remain in food, and even accumulate, with such compounds reaching concentration levels that are high enough to be harmful both to humans and animals when ingested [1].

Fruit and vegetable analysis is key to assessing food safety for consumers [2–4]. To determine multiple pesticide residues, QuEChERS was introduced as a quick, easy, cheap, effective, rugged and safe multiclass, multiresidue analytical approach [5]. This technique involves the sample undergoing a salting-out acetonitrile (ACN) extraction/partitioning step and the extract being purified using dispersive solid-phase extraction (d-SPE) as a clean-up step [6]. The latter can be carried out (depending on the type of sample being analysed) by freezing out (for the removal of lipids, waxes,

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sugars and other matrix co-extractives with low solubility in ACN) and/or a mixture of the sorbents anhydrous $MgSO_4$ (to remove most of the undesirable water and to improve analyte partitioning) and primary secondary amine (PSA; to remove sugars and fatty acids, organic acids, lipids and some pigments) [7], with C18 and graphitized carbon black (GCB) [8] added, if required, to improve the removal of nonpolar matrices and chlorophyll. Alternatively, zirconia materials (Z-Sep) can be used to remove lipophilic matrix components [9]. When PSA is used in the clean-up step, the extract pH is in the 8 to 9 range, which endangers the stability of base-sensitive pesticides, so the pH value needs to be adjusted to around 5 by adding formic acid (5% in ACN) [10].

Automated laboratory workflows are attracting more interest than manual sample methodologies due to their increased robustness and repeatability [11–13]. Introducing automation to sample preparation using a clean-up based on micro-solid-phase extraction (µSPE) can significantly improve the performance and throughput of a multiresidue analysis method. This technique harmonises the clean-up step irrespective of the type of matrix and shortens the working time. Various works have been published on the basis of automated clean up but focused on GC-MS/MS [14–17,13] and for a limited scope of residues. In the case of LC-MS/MS one work has been published by Morris et al [18] using a zirconia-based sorbent with acceptable results.

To our knowledge the presented work is the first critical evaluation for large multiresidue LC-MS/MS method (243 pesticide residues) covering wide variety of fruit and vegetable groups (high water, high acid, high fat, and high starch) commodities in a comprehensive way.The effectiveness of automatic clean-up was compared with the results of dispersive clean-up, highlighting the cleanliness of the extracts, the sample workflow and the recovery of target compounds overcoming difficulties such as recoveries for acidic compounds.

2. Material and methods

2.1. Reagents and materials

High purity standards of the 243 pesticides and the internal standards were obtained from Sigma-Aldrich (Steinheim, Germany) and LGC (Teddington, UK), and stored at -30°C. From these individual standards, 10 mg L^{-1} stock dilutions were prepared in opaque screw-capped bottles and stored at -20°C in the dark.

Prior to testing, a 1 mg L^{-1} intermediate dilution of all the pesticides was prepared from the stock dilutions and stored for a maximum of one week at -20°C in the dark. The 243 pesticides evaluated are listed in Supplementary Material Table S1. The reagents used for the extraction were: QuEChERS salts (anhydrous magnesium sulphate, sodium chloride, sodium hydrogen citrate sesquihydrate and sodium citrate tribasic dihydrate) (Sigma-Aldrich (Steinheim, Germany)). The magnesium sulphate (purity ≥ 98%) was supplied by Honeywell/FlukaTM (Seelze, Germany), the Z-Sep and calcium chloride by Sigma-Aldrich (St. Louis, MO, USA), and the µSPE-GCQuE1-45 (µSPE cartridges for the PAL® system containing a 20/12/12/1 ratio of anhydrous $MgSO_4$/PSA/C18/CarbonX, respectively) by PAL® SYSTEM (Switzerland). The solvents used were: water, Optima™ LC/MS grade (Fisher Chemical (Geel, Belgium)), acetonitrile (ACN) HPLC grade (purity ≥ 99.9%) from Honeywell/Riedel-de Haën (Seelze, Germany), ACN LC/MS grade (purity ≥ 99.9%) from Honeywell/Riedel-de Haën (Seelze, Germany), formic acid (98%) from Fluka Analytical (Steinheim, Germany), ammonium formate from Sigma-Aldrich (Steinheim, Germany) and methanol from Fluka Analytical (Steinheim, Germany). The Pierce™ Triple Quadrupole Calibration Solution was provided by Thermo Fisher Scientific (Waltham, MA, USA). The automated process for the extract clean-up step was carried out using a PAL® RTC X-Y-Z autosampler (CTC analytics, Zwingen, Switzerland). Blank samples (tomato, orange, avocado, rice and black tea) and real samples were purchased from local shops in Almeria and analysed prior to testing so as to check there were no positive results for the pesticides under study.

2.2. Manual sample treatment

The samples were extracted using the citrate-buffered CEN QuEChERS method [19]. In brief, following homogenisation, 10 g of each matrix (tomato, orange and avocado), 5 g of rice and 2 g of black tea were individually weighed in 50 mL PTFE centrifuge tubes. For the dry matrices (rice and black tea), 10 mL of water was added, and the tubes were shaken manually for 3 seconds. Then, 10 mL of acetonitrile was added to all the matrices and the samples were shaken in an automatic axial extractor (AGYTAX®, Cirta Lab. S.L., Spain) for 6 min. Afterwards, 4 g of magnesium sulphate, 1 g of sodium chloride, 1 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate were added, and the samples were again shaken in the AGYTAX for 6 min. Each extract was then centrifuged at 3700 rpm for 5 min. At this point, an aliquot of the supernatant was taken for the µSPE experiments as a sample of the raw extract.

The dispersive SPE clean-up step was adapted to each matrix, as described below: *Tomato and orange*: 5 mL of the supernatant was transferred to a 15 mL PTFE centrifuge tube containing 750 mg of anhydrous magnesium sulphate and 125 mg of PSA and vortexed for 30 sec. Subsequently, the tubes were centrifuged at 3700 rpm for 5 min and finally the supernatant was transferred to a 4-mL vial to which 10 µL of formic acid solution in acetonitrile (5% volume) was added for each mL of extract. *Rice* [20]: 8 mL of the supernatant was transferred to a 15-mL PTFE centrifuge tube, which was placed in a box with dry ice for 20 min (the freezing-out step). The next step was to centrifuge (3700 rpm for 5 min) and the same dSPE clean-up step as described above for tomato and orange was carried out. *Avocado*: 5 mL of the supernatant was transferred to a 15 mL PTFE centrifuge tube containing 175 mg of Z-Sep and mixed for 30 seconds. Next, the tubes were centrifuged at 3700 rpm for 5 min and the supernatant was transferred to a 4-mL vial. Finally, for *black tea*: 5 mL of the supernatant was added in a 15 mL PTFE centrifuge tube with 250 mg of calcium chloride and 125 mg of PSA and then mixed for 30 seconds [21]. The tubes were centrifuged (3700 rpm for 5 min) and the supernatant was transferred to a 4-mL vial with 10 µL of formic acid solution in acetonitrile (5% volume) per mL.

The recovery studies were carried out at two spiking levels, 0.05 and 0.01 mg kg^{-1}. The blank matrices were spiked with the 243 pesticides. Each concentration/matrix combination was extracted five times with the corresponding QuEChERS method and the dSPE clean-up step.

The injection vial was prepared by the following procedure: a 100 µL aliquot of the extract was diluted with 50 µL of acetonitrile and 400 µL of ultrapure water containing dimethoate-d6 as quality control injection standard. Calibration points were prepared with a 100 µL aliquot of the blank extract, 50 µL of pesticide mix at the corresponding calibration level and 400 µL of ultrapure water containing dimethoate-d6 (quality control injection standard). To check that all injections have been performed correctly, the area of injection standard should be withing 60–140% of the mean injection standard.

2.3. Automated sample treatment

The µSPE cartridges (p/n µSPE-GCQuE1-45 provided by CTC Analytics) were employed to perform an automated clean-up procedure which was then compared to the manual dispersive-clean-

Table 1
Automatic μSPE program step duration.

Time (mm:ss)	Steps
0:20	Required tool selected
	Syringe wash (3 cycles)
2:00	Load 100 μL AcN
2:30	Condition μSPE cartridge
3:30	Load 200 μL of sample (3 strokes)
4:00	Elution cartridge step with sample
6:00	Syringe wash (3 cycles)
6:30	3 strokes with elution solvent (ACN 5% formic acid)
6:50	Load 100 μL and elution step
8:00	Syringe wash (3 cycles)
8:24	Required tool is selected
9:00	Syringe wash (2 cycles)
9:40	Load sample
10:00	1 μL was injected
13:28	Final GC

up methods. The cartridges comprised 45 mg of sorbent with the following composition (weight percentage): 20/12/12/1 anhydrous $MgSO_4$/PSA/C18/CarbonX, respectively. Only one type of cartridge was applied to all the matrices, unlike the manual dispersive-clean-up methods (Supplementary Material Table S2). To prepare the injection sample in the automated μSPE vial, 200 μL of raw extract sample was mixed with 50 μL of acetonitrile. Calibration points were prepared with 200 μL of this extract and 50 μL of the acetonitrile solution of the pesticide.

The following sample workflow was optimized based on the method developed by Lehotay et al. [14]: first, the cartridges were preconditioned with 100 μL of acetonitrile prior to sample loading. Then, 200 μL of each raw extract sample was loaded into the cartridge at 100 μL/sec and the clean extract was collected in a 2-mL vial with a pre-cut septum cap. Subsequently, the cartridges were eluted with 100 μL of acetonitrile (5% formic acid). The automatic μSPE programme duration is shown in Table 1, where at 8:00 min, a 100 μL aliquot of the total automated μSPE extract was mixed with 400 μL of ultrapure water containing dimethoate-d_6 (the injection standard) and injected into the LC-MS/MS.

2.4. Analysis by LC-QqQ-MS/MS

For the LC separation, Thermo Scientific™ Transced™ DUO LX-2 (Thermo Scientific™, Germering, Germany) was used. Mobile phase A was 98% water and 2% methanol whereas mobile phase B was 98% methanol and 2% water; both mobile phases contained 5 mM of ammonium formate and 0.1% formic acid. For the separation, an Accucore C18 column (2.1 mm in diameter, 100 mm in length, with a 2.6 μm particle size) was used. The column was thermostatted at 30 °C. Supplementary Material Table S3 presents the gradient applied and information about the data windows. The autosampler was thermostatted at 10°C and the injection volume was 2.5 μL.

A TSQ Altis (Thermo Scientific, San Jose, USA) mass spectrometer equipped with an Opta Max NG ion source was used. The parameters were described by Diaz-Galiano et al. [22]. The optimised parameter values and the SRM transitions selected in the analytical method are shown in Supplementary Material Table S1. TraceFinder 4.1 software (Thermo Scientific, San Jose, USA) was used for the qualitative and quantitative analysis. Automatic detection and quantification were followed up by manual verification.

3. Results and discussion

3.1. Automated μSPE workflow optimization

Recent studies on the automation of the clean-up stage with μSPE use different workflows, the main differences being the addition of the cartridge conditioning step and the cartridge elution step. Morris et al. [18] conditioned with 150 μL of elution solvent loaded with 150 μL of sample and eluted with 150 μL of solvent while Lehotay et al. [14] simply loaded the cartridge with 300 μL of sample. Cartridge conditioning step was evaluated by calculation of relative standard deviation (RSD) of tomato blank raw extract spiked at 0.01 mg L^{-1} of pesticide mix where an aliquot was loaded (200 μL) into the cartridge without condition the step. The same experiment was carried out with conditioning the cartridge with 100 μL of acetonitrile. Both methods were evaluated in triplicate. RSD values were in general higher. For some compounds, such as dodine (RSD 45%), fenazaquin (RSD 34%), fenbendazole (RSD (%) 24%), and tolfenpyrad RSD (%) where in case of a previous conditioning the step was <20% in all cases. This lack of reproducibility probably due to channelling effects means the interest of previous conditioning for a more robust results.

Moreover, the following six methods were evaluated, considering the cartridge conditioning step indispensable for maintaining a constant volume of eluted sample. For Method 1 (M1), the cartridges were pre-conditioned with 100 μL of ACN prior to sample loading. Then, 200 μL of raw extract sample was loaded into the cartridge. For Method 2 (M2), the raw extract sample was acidified with ACN (5% formic acid) in the same proportion as for the dSPE method (10 μL per mL of extract) followed by M1 as the next step. For Method 3 (M3), the cartridges were pre-conditioned with 100 μL of acetonitrile prior to sample loading. Then, 200 μL of each raw extract sample was loaded into the cartridge at 100 μL/sec and the clean extract was collected in a 2 mL vial with a pre-cut septum cap. Subsequently, the cartridges were eluted with 100 μL of acetonitrile. Methods 4 (M4), 5 (M5) and 6 (M6) all had the conditioning step with 100 μL acetonitrile prior to sample loading, 200 μL of sample volume and an elution step with acetonitrile (5% formic acid), where the volumes evaluated were 100, 200 and 600 μL, respectively.

Acidic compounds (fluazifop, haloxyfop and quizalofop) were not detected by M1, M2 and M3 because they reacted with the PSA by ion pairs. To overcome this, an elution step was carried out with acetonitrile (5% formic acid). An evaluation was made of the elution solvent amount needed to break the ion-pair interaction (M4, M5, M6). M4 was the optimized automated μSPE method workflow since recoveries were between 80–120% (Table 2) and the sample dilution was the lowest, thus avoiding possible sensitivity issues (Supplementary Material (Fig. S1)).

3.2. TICs and extract appearance

To compare the Total Ion Current (TIC), dSPE and μSPE blank extracts were injected in *full-scan* mode. The automated μSPE dilution factor was considered when preparing the dSPE vials. As depicted in the TIC chromatograms (Supplementary Material (Fig. S2)), the baseline obtained with the μSPE method is similar to that of the dSPE manual method for tomato, orange, rice, and black tea. The greatest difference was observed in avocado (Fig. 1) where the μSPE baseline was lower than the dSPE baseline. The maximum integrated area of the avocado TIC was 1.34×10^9 for the μSPE blank extract; however, for dSPE blank extract, it was 7.31×10^9. The comparison of these profiles could lead to inferences when dSPE is used. Fig. 2 shows how the pesticide metamitron is affected when employing dSPE clean-up. Moreover, the cartridge-derived components were evaluated to determine how the cartridge salts might affect the analysis. CTC Analytics (Switzerland) supplied us with unfilled cartridges through which the solvent (acetonitrile) was passed; then, an aliquot of this was compared with another filled with μSPE salts - very similar baselines were obtained.

Table 2
Recoveries obtained with different automated µSPE workflows.

COMPOUND	Chemical Structure	RECOVERIES OF TOMATO SAMPLE AT 0.01 mg L^{-1} (%)					
		M1	M2	M3	M4	M5	M6
FLUAZIFOP		ND	ND	ND	119	77	77
HALOXYFOP		ND	ND	ND	112	79	85
QUIZALOFOP		ND	ND	ND	84	70	85
FLAZASULFURON		ND	ND	ND	104	78	72
ORTHOSULFAMURON		ND	ND	ND	103	77	81
OXASULFURON		ND	ND	ND	110	80	74

ND: Not detected.
M1: Cartridges were pre-conditioned with 100 µL of ACN prior to sample loading. Then, 200 µL of raw extract sample was loaded into the cartridge. M2: Raw extract sample was acidified with ACN (5% formic acid) in the same proportion as for the dSPE method (10 µL per mL of extract) followed by M1 as the next step. M3: Cartridges were pre-conditioned with 100 µL of acetonitrile prior to sample loading. Then, 200 µL of each raw extract sample was loaded. Subsequently, the cartridges were eluted with 100 µL of acetonitrile. M4: Conditioning step with 100 µL acetonitrile prior to sample loading, 200 µL of sample volume and an elution step with 100 µL acetonitrile (5% formic acid). M5: Conditioning step with 100 µL acetonitrile prior to sample loading, 200 µL of sample volume and an elution step with 200 µL acetonitrile (5% formic acid). M6: Conditioning step with 100 µL acetonitrile prior to sample loading, 200 µL of sample volume and an elution step with 600 µL acetonitrile (5% formic acid)

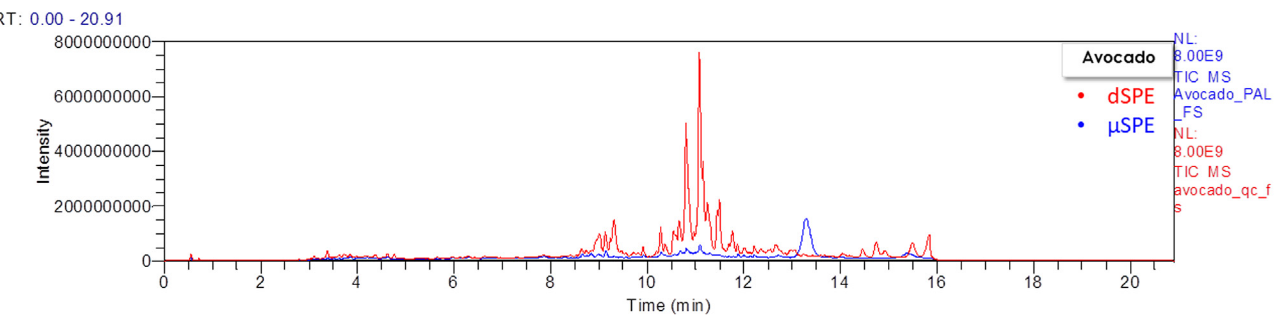

Fig. 1. TIC chromatograms obtained with a LC-MS/MS instrument for the blank extracts from the dSPE method (dark red color) and µSPE method (blue color) of avocado sample.

Remarkable differences were found when comparing the extract appearance. Fig. 3 presents three vials: citrate QuEChERS raw extract without the clean-up step, the final extract after manual dSPE clean-up, and the final extract after automated µSPE clean-up. Only minor differences can be observed between the raw extract and the dSPE extract. In contrast, all the µSPE extracts were clear and colourless. This is relevant because increased cleanliness improves the functioning of the LC/MS system, increases the lifetime of the column and helps to maintain the ion source, thus avoiding possible interfering signals.

In addition, a spinach sample was analysed because of its high chlorophyll content, which makes it particularly eye-catching Supplementary Material (Fig. S3). The recovery experiment, which was performed by spiking the spinach sample at 0.05 mg kg^{-1}, showed similar results but avoided pigment accumulation on the analytical column and minimized the instrument's downtime. Moreover,

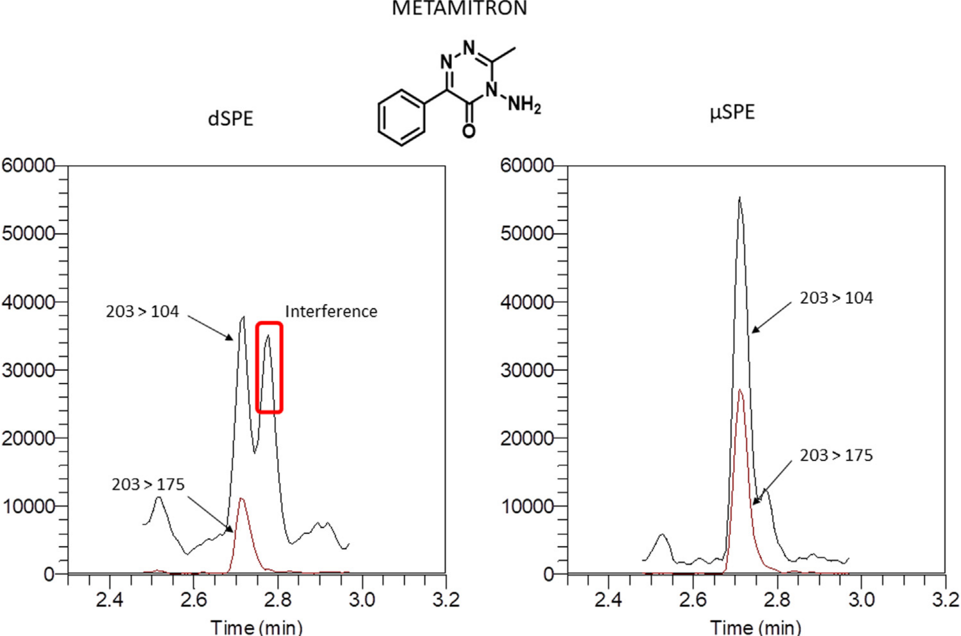

Fig. 2. Blank avocado sample spiked at 0.01 mg kg^{-1} with pesticide mix.

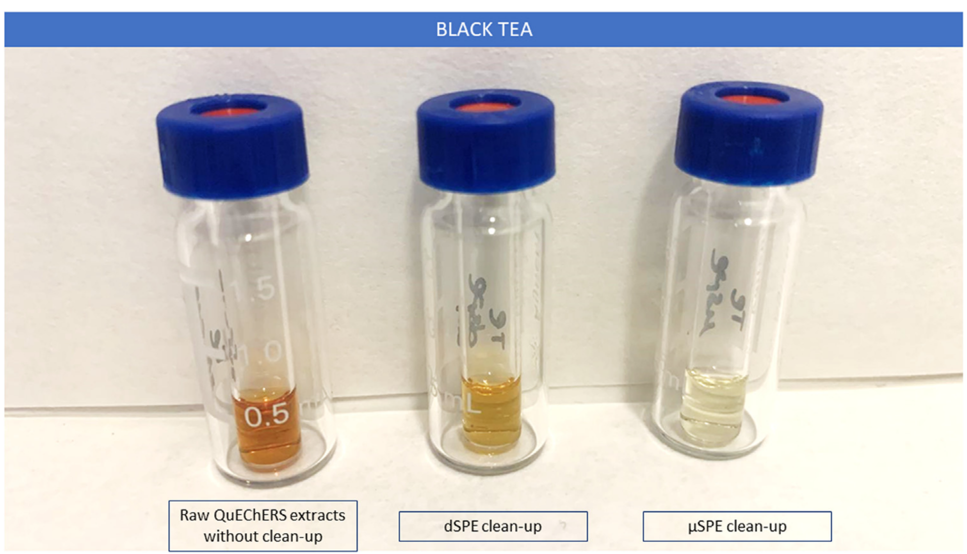

Fig. 3. Extracts of black tea. From left to right: raw QuEChERS extracts without clean-up, QC extract (dispersive clean-up) and µSPE extract (cartridges).

a blank spinach sample was analysed, both by dSPE and by the automated µSPE methods in full-scan mode the µSPE baseline was significantly lower than that of the dSPE Supplementary Material (Fig. S4).

3.3. Method validation

3.3.1. Linearity and matrix effect

Method validation was performed according to SANTE/11312/2021 [23]. Five sets of calibration curves were prepared using extracts of blank samples from each of the five matrices (tomato, orange, rice, avocado, and black tea). Linearity was determined for the 243 compounds in the 0.005 mg L^{-1} -0.2 mg L^{-1} range with five calibration levels (0.005 mg L^{-1}, 0.01 mg L^{-1}, 0.02 mg L^{-1}, 0.1 and 0.2 mg L^{-1}). Deviation of the back-calculated concentration from the true concentration was $<$ 20% for each level studied.

As a consequence of coeluting sample components, the analyte signal may be enhanced or suppressed, compared to the signal from the same analyte, when injected into the solvent [24]. To evaluate the percentage of matrix effects (ME) for each analyte, the slope of the calibration curves prepared in solvent at the same concentration levels were used, and the ME levels were determined by comparing the solvent and the matrix-matched calibration curves in terms of slope ratios (Eq. (1))

$$ME = \left(\frac{Slope_{matrix}}{Slope_{\ solvent}} - 1 \right) \times 100 \tag{1}$$

Signal suppression would occur if the percentage of the difference between these slopes were negative. If it were positive, it would indicate signal enhancement. Ion suppression is a far more common phenomenon, especially with electrospray ionization [25]. The matrix effects were classified into different categories based on the value of this percentage. A strong matrix effect would oc-

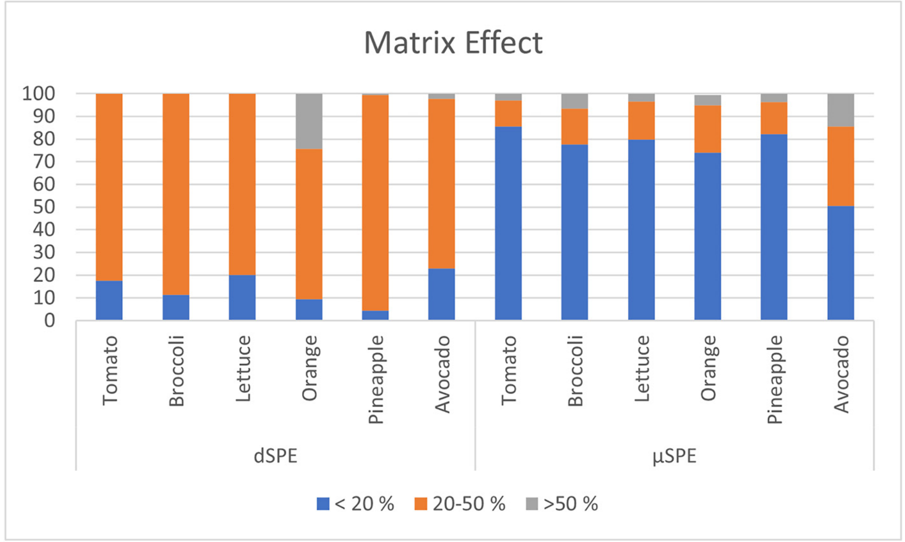

Fig. 4. Percentage of compounds with different ranges of matrix effect. Evaluated for tomato, broccoli, lettuce, orange, pineapple, grapefruit and avocado.

cur when the absolute matrix effect was > 50%, a medium matrix effect when the value was between 20–50%, and a low or negligible matrix effect when it was < 20%.

Solvent calibration curves to evaluate the dSPE and the automated µSPE clean-up step were carried out in acetonitrile spiked at each concentration level. The presence of the matrix effect for more than 50 percent of the studied compounds was considered low (ME < 20%) when using automated µSPE. In contrast, when dSPE was used, more than 60 percent of the compounds presented a moderate matrix effect (Fig. 4). The cleaner extracts obtained by automated µSPE - as mentioned in the previous section - also justifies the lower matrix effect obtained since there are lower interferents and so lower ion suppression than with dSPE.

Matrix-matched calibration is the most widely used approach to account for this matrix effect phenomenon and to avoid incorrect quantification [26]. Six compounds (acephate, metolcarb, metalaxyl, malathion, metolachlor and buprofezin; ordered in terms of increasing retention time) were selected to compare the linearity of both methods Supplementary Material (Fig. S5).

For all the cases studied, the calibration curves had very close slopes offering good quantitative results for the different commodities; when µSPE was employed for the clean-up, a single calibration curve was used. The total amount of injected sample for the dSPE method was 0.5 mg whereas for the µSPE method, it was 0.33 mg. Injecting less matrix should result in less equipment maintenance and a lower signal suppression effect.

3.3.2. Recoveries

The recoveries for each matrix were studied at two recovery levels (10and 50 µg kg^{-1}) with five replicates for each level. The results are shown in Supplementary Material: Tables S4–S8 together with the RSD (%) values. In general, very similar results were obtained for each method, although, in some cases, they were slightly higher for µSPE.

Supplementary Material (Fig. S6) shows the number of compounds in the different recovery ranges for orange. For the vast majority of compounds, the results obtained were very similar because the extraction step is the same, despite using only one unified cartridge type for the automated clean-up rather than specific salts for each matrix group. Furthermore, the matrix effect was lower in µSPE.

The most notable differences were observed for acidic compounds such as quizalofop, fluazifop and haloxyfop. For these, recoveries were lower than 22% in tomato, 43% in orange and were not detected at all in the rice matrix when using the dSPE method; this occurred when the dispersive clean-up step included PSA due to the ion-pairs between the molecules and the secondary amine. When using µSPE, the recovery values were between 80% – 120% as a result of automatically performing the elution step on the cartridge with acetonitrile (5% formic acid), during which the molecular interactions with PSA were broken Supplementary Material (Table S9)

Another group of compounds for which the µSPE method had a clear advantage over dSPE were the pyrimidinylsulfonylureas (flazasulfuron, orthosulfamuron, and oxasulfuron). Recoveries were < 30% for rice with dSPE whereas, with µSPE, they were in the 80–105% range. Detecting these compounds at low concentrations in a matrix with a high protein content is a big challenge when PSA is used in the clean-up step because their weak acidity can be adsorbed by the secondary amine [27] Supplementary Material (Table S10).

3.5. µSPE-cartridge retention factor

To assess if any analytes were partially retained in the cartridge, a blank matrix extract (that did not undergo a clean-up step) was fortified with 0.01 mg L^{-1} of the pesticide mix. An aliquot was passed through the µSPE cartridge, and another aliquot was diluted with ACN to account for the automated µSPE dilution. Finally, injection vials prepared with a 5 fold dilution of ultrapure water containing dimethoate-d$_6$ (the injection standard) were injected into the LC-MS/MS. Eq. (2) was used to compare the area for each matrix in order to calculate a theorical µSPE-cartridge retention factor (Supplementary Material (Table S11).

$$\text{Area Accuracy (\%)} : \frac{\overline{\text{Area without clean} - \text{up}} - \overline{\text{Area with } \mu\text{SPE}}}{\overline{\text{Area without clean} - \text{up}}} \times 100 \quad (2)$$

The study was carried out with five injected replicates subjected to µSPE clean-up and five replicates that were not. Acceptable experimental variability was studied using the internal injection standard (dimethoate-d6), for which the variation obtained was less than 20%.

Analyte-cartridge interaction is compensated for by subjecting the matrix calibration curve to the µSPE cartridges without negative sensitivity effects. Automation means that submitting the cal-

ibration curve to the clean-up is not such a tedious and time-consuming step; in contrast, if this were carried out using dSPE, the analysis time would be 30% longer.

3.6. Analysis of real samples

Thirteen real samples were bought at a local shop in Almeria and then analysed by citrate QuEChERS, employing either the dSPE or the µSPE clean-up steps. The results were compared and the difference between the reported concentrations was less than 20% (Supplementary Material (Table S12). Considering an uncertainty value of 50% [28], values above the European Union Maximum Residue Level (EU MRL) were detected for imazalil (EU MRL: 4 mg kg^{-1}), fluazifop (EU MRL: 0.01 mg kg^{-1}), and propiconazole (EU MRL: 0.01 mg kg^{-1}) in orange.

Furthermore, a proficiency test on lemon material [29] was analysed using the automated µSPE clean-up method, obtaining Z score values lower than ± 1.2 in all cases (Supplementary Material (Table S13).

4. Conclusions

The use of automated µSPE clean-up reduces the laboratory workflow and allows increased sample throughput in routine analysis by 30%. Moreover, as only a single clean-up is employed equally for all commodities, a simplify multiresidue method is obtained with the important benefits with method application. Very high homogeneity is typically obtained in the calibration curves avoiding typical quantitation errors. Instrument maintenance is also positively affected because cleaner extracts are obtained and so the lifespan of certain instrument parts (such as the ion source and columns) increase.

In general, the µSPE method provides recoveries that are very similar to those obtained with manual clean-up because the extraction step is the same for both approaches. Of great interest is that some compounds are positively affected when using µSPE cartridges, such as acidic compounds (fluazifop, haloxyfop and quizalofop), where, in general, recoveries < 50% are obtained when manual dispersive clean-up is used (they are retained by the PSA sorbent). The automated clean-up step can attain recoveries from 70% -120% for them because a final elution step of the cartridge (with acidified acetonitrile) was included. In addition, some compounds, such as sulfonylureas (flazasulfuron, orthosulfamuron, oxasulfuron) have better recoveries when using the automatic method with certain commodities (e.g., rice) because this method is based on chromatography and it is therefore more effective at avoiding inconvenient trapping processes. In conclusion, automatic µSPE avoids the qualitative and quantitative errors that are produced when dSPE is applied.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Lorena Manzano Sánchez: Conceptualization, Methodology, Validation, Resources, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Florencia Jesús:** Methodology, Validation, Investigation. **Carmen Ferrer:** Conceptualization, Methodology. **M. Mar Gómez-Ramos:** Writing – original draft. **Amadeo Fernández-Alba:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2023.463906.

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