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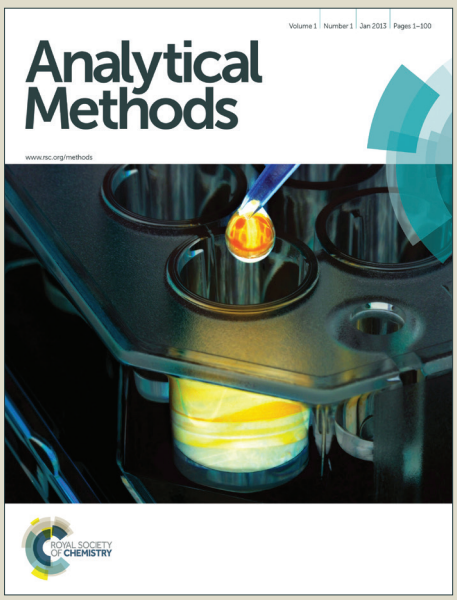

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Evaluation of dispersive and cartridge SPE clean-up procedures using the modified QuEChERS method for the analysis of pesticides in strawberries

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Abstract

A comparison of dispersive and cartridge solid-phase extraction, respectively d-SPE and c-SPE, for the clean-up step using the modified QuEChERS (quick, easy, cheap, effective, rugged and safe) method, followed by ultra high performance liquid chromatography coupled with diode array detection (UHPLC-DAD) was carried out for the determination of pesticide residues in strawberries. Dry ice was used for evaluation of different temperatures. The efficiency liquid-liquid partitioning step without salts was also evaluated. In the all experiments, the sorbent used in the clean-up step was primary secondary amine (PSA) and the evaluation of the sample preparation was made by the final aspect of extract, the chromatographic profile, the recovery, the amounts of coextractives in the matrix determined by gravimetric measurements and the matrix effect. A good clean-up procedure contributes to a longer life time for the chromatographic column and for the entire system and also leads to better detectability for the developed method. Results showed that all the sample preparation methods were efficient for multiresidue pesticide analysis in this complex matrix, in which analytes with different physicochemical characteristics at low concentrations are present. The recovery results were in the range 70-120% and the coefficients of variation were ≤ 20% for most of the pesticides, as recommended for these analyses. The clean-up of the strawberry extracts was more efficient with the use of d-SPE at ambient temperature, considering the higher recoveries of the pesticides and better clean-up by removal of interferences as well as being faster, easier and less expensive.

1 Introduction

The great demand for pesticide application, at all stages of production, is related to the need for high productivity of the crops and to improve the quality of foods due to control of pests and diseases. However, when there is noncompliance with good agricultural practices, there is growing concern for the health of the consumer and the farmer, the environmental impact and there is also the need to meet the requirements for export licenses related to the application of pesticides on foods.[1-3]

Strawberries are foods of great importance for high consumption, mainly *in nature*, they have diverse functional properties and are widely used in the food industries. In Brazil, strawberries are one of the crops most contaminated with unauthorized pesticides or above the maximum residue levels (MRLs).[4,5]

Multiresidue pesticide analysis in foods consists of sample preparation followed by instrumental determination for identification and quantification of the target analytes. The analytical determination for monitoring of pesticides in food samples is done most often by liquid chromatography (LC) and gas chromatography (GC).[6-8]

The development of new methods for sample preparation has become very important because they minimize or eliminate the interferences from complex food matrices and permit analyses of mixtures having different physicochemical properties at low concentrations. In addition to this, sample preparation often includes laborious steps that require time, generate large amounts of toxic waste and have high costs.[9-11]

In 2003, Anastassiades *et al*.,[12] with the objective of overcoming the practical limitations of methods of sample preparation applied for pesticide residues, introduced a new procedure called the QuEChERS method, which consists of organic solvent extraction followed by liquid-liquid partition with addition of salt to effect salting out and drying, and finally, a clean-up step by dispersive solid-phase extraction (d-SPE).[9,13,14] The modifications of the QuEChERS method, such as the AOAC Official Method with acetate buffer and the European Committee for Standardization (CEN) Standard Method EN 15662 with citrate buffer, were made to optimize the methods based on the physicochemical characteristics of the pesticides and the matrix composition.[15,16]

Other modifications may occur in other versions of the QuEChERS method such as the use of different organic solvents in the extraction step [12,15] and the use of different sorbents in the clean-up step to minimize the amount of matrix interferences.[17-21] Furthermore, the application of low temperature for reducing lipids and other nonpolar interferences that are frozen with water and also be able to separate a miscible solution of acetonitrile and water from one another by the use of dry ice.[12,17,22,23]

The procedure of dispersive clean-up in the QuEChERS method is simple, wherein the sample extract and the sorbent are stirred together favoring the clean-up step due to uniform distribution. After centrifugation to separate the phases, the supernatant, which is final extract of the sample, is removed.

In this procedure, the clean-up of interferences and reduction of waste water occur simultaneously without consuming time on other experimental steps. Water removal provides a final extract of less polarity, which facilitates the precipitation of polar interferences and allows direct injection of the final extract in the LC or GC equipment.[9,12] In addition, d-SPE has more interaction between the sample and the sorbent, which contributes to better recoveries, higher clean-up by removing the interferences and generates less waste due to the lower volume of organic solvent employed in the sample preparation.[10]

The cartridge solid-phase extraction (c-SPE) procedure, in contrast to d-SPE, includes many stages such as cartridge conditioning, sample loading, interference elution and analyte elution.[24,25] In this case, the analytes are collected in the effluent when they are eluted from the cartridge and the interferences are eluted first or remain on the cartridge.

Sample preparation, mainly the clean-up step, can contribute positively to the analytical determination, by providing extracts with less interferences that avoid damages and reduce maintenance of the chromatographic system, while also increasing the useful life of columns, as well as, in the detection is often better detectability with higher signal/noise ratio and less chance of false positives that are assigned to the matrix effect.[6,26,27]

The aim of this study was to evaluate the technique of d-SPE as a function of temperature variation (ambient and low), removal of the salts for the salting-out effect, a drying step and the use of buffering agents in the liquid-liquid partitioning step. Furthermore, the dispersive clean-up was compared with the use of a cartridge. The clean-up techniques followed a modified QuEChERS method, based on the European Committee for Standardization (CEN) Standard Method EN-15662 version, and was applied for determination of multiresidue pesticides in strawberries by UHPLC-DAD. In this study, the evaluation and comparison of the sample preparation methods were based on the final aspect of extracts, the chromatographic profile, the recovery of pesticides, the amount of interferences that were determined by gravimetric measurements and the matrix effect.

2 Experimental

2.1 Reagentes and materials

The reagents and solvents were of analytical grade or HPLC grade, respectively. Acetonitrile and methanol were from Tedia (Fairfield, NJ, USA), toluene from J.T. Baker (Ecatepec, Mexico), formic acid from Synth (Diadema, Brazil), sodium chloride from Ecibra (São Paulo, Brazil), anhydrous magnesium sulphate from Vetec (Rio de Janeiro, Brazil), sodium citrate tribasic dihydrate and sodium hydrogencitrate sesquihydrate from Sigma-Aldrich (St. Louis, MO, USA), Bondesil PSA (40 µm) from Varian (Palo Alto, CA, USA) and Supelclean™ PSA SPE Tube (200mg/3mL) from Supelco

(Bellefonte, PA, USA). Ultrapure water was obtained from a Milli-Q system from Millipore (Bedford, MA, USA) with 18.2 MΩ cm^{-1} conductivity.

The analytes were selected based on pesticides with values of the Maximum Residue Limits (MRLs) established by ANVISA (Agência Nacional de Vigilância Sanitária) of Brazil.[5] Some of these pesticides have limits established by other international regulatory agencies such as the Codex Alimentarius and EU Pesticides database.[28,29] The selection of pesticides was also based on the pesticides having chromophore groups that would allow their detection in the UV region, due to the use of the DAD detector. The pesticide standards were purchased from Chem Service (West Chester, PA, USA), Sigma-Aldrich (Buchs, Switzerland) and Pestanal (Steinheim, Germany). All pesticide standards presented purities higher than 97%.

The individual stock solutions were prepared at 1.0 mg mL^{-1} by dissolution in methanol, except for simazine (0.2 mg mL^{-1}) because of its low solubility. Appropriate dilutions from the stock solutions were carried out to prepare working solutions that consisted of mixtures of all the pesticides in methanol. All solutions were stored at -20 ºC.

2.2 Equipments

Pesticide residue determinations were performed using an ACQUITY UPLC® coupled with ACQUITY UPLC® Photodiode Array Detector (Waters, Milford, MA, USA). The chromatographic separations were carried out with an Acquity UPLC® BEH C18 (50 mm x 2.1 mm i.d., 1.7 µm) analytical column coupled to a Van Guard™ BEH C18 (5 mm x 2.1 mm i.d., 1.7 µm) guard column, both from Waters, which were maintained at 30 ºC. The mobile phase consisted of 0.1% of formic acid in water (solvent A) and methanol (solvent B). Gradient elution was applied at a flow rate of 0.12 mL min^{-1} as follows: initial conditions of 30% B, increased linearly to 95% B, returning to the initial conditions at 10 min with re-equilibration to the initial conditions in 3 min. The detector operates within a range between 190 and 500 nm, but each pesticide had its selected wavelength according to the maximum absorption and sample selectivity. The injection volume was 1.9 µL. Instrument control and data analysis were performed using Empower 2.0 software from Waters.

Figure 1 displays a chromatogram of the separation of pesticides in methanol obtained with the above chromatographic conditions.

Figure 1

The substance group, partition coefficient, retention time and wavelength of pesticides are presented in Table 1.[30]

Table 1

Sample preparation was made using a model CP 225 D analytical balance (Sartorius, Goettingen, Germany) or a model A-250 analytical balance (Fisher Scientific, Loughborough, UK), a model AP 56 vortex (Phoenix, Araraquara, Brazil), a model Rotofix 32A centrifuge (Hettich Zentrifugen, Tuttlingen**,** Germany) and a model Visiprep ™ SPE vacuum manifold for 12 samples (Supelco, Bellefonte, Pennsylvania, USA).

2.3 Sample preparation

Pesticide-free strawberries produced by organic agriculture were purchased from a market of organic products in Campinas, Brazil.

The development of an appropriate sample preparation is one of the most important but laborious steps in the determination of pesticide residues in foods. The modified QuEChERS method based on the European Committee for Standardization (CEN) Standard Method EN-15662 was used for extraction of pesticides from strawberries.[14-16]

A portion of 10 g of homogenized strawberry samples was placed in a polypropylene centrifuge tube (50 mL), and 10 mL acetonitrile was added to the tube (extraction step). The mixture was stirred for 1 min by mechanical agitation in a vortex. Afterwards, 4 g of anhydrous magnesium sulfate, 1 g sodium chloride, 1 g sodium citrate tribasic dihydrate and 0.5 g sodium hydrogencitrate sesquihydrate were added to this mixture and the tube was vigorously shaken in vortex for 1 min (liquid-liquid partitioning step). After centrifugation at 5,000 g for 15 min, the organic layer was transferred into a polypropylene centrifuge tube (15 mL) containing 150 mg anhydrous magnesium sulfate and 25 mg of PSA sorbent per mL of extract (d-SPE clean-up step). The mixture was shaken in vortex for 1 min and centrifuged for 5 min. After the clean-up step, 10 µL 5 % formic acid in acetonitrile was added per mL of supernatant (acidification step). Subsenquently, the solvent was evaporated under a nitrogen stream. The residue was redissolved in 1 mL methanol and a volume of 1,9 µL was injected into the UHPLC–DAD system.

Evaluation of the dispersive clean-up step was made at ambient and low temperatures with or without salts in liquid-liquid partitioning step.[14-16,22] The same procedure at ambient temperature was performed in c-SPE clean-up, but the cartridge of PSA sorbent was conditioned with a solvent mixture of acetonitrile and toluene (3:1 v/v).[31-33] The scheme of the sample preparation procedures shown in Figure 2.

Figure 2

Temperatures below zero on a mixture of two solvents may reduce their entropies and allow them to separate.[22] In the liquid-liquid partition of the QuEChERS method, the freezing the water will separate it from the MeCN layer. In the d-SPE experiment, at low temperature, a major disadvantage

is that the freezing of the sample requires time. However the use of dry ice allows freezing of the solution in a few minutes.

2.3.1 Evaluation of sample preparation

The clean-up step has an influence on several parameters, such as recovery, selectivity, amount of coextractives and matrix effect. This later parameter influences both on the performance of the chromatographic instrument and the detection system.

The PSA is a silica-based material, which is chemically bonded to ethylenediamine-*N*-propyl. The PSA sorbent has a structure with both primary and secondary amines, which acts as weak anion exchanger.[9,34,35] The sorbent is capable of removing sugars, organic acids and anthocyanins, which are pigments in the composition of strawberries. The performances of four modifications of the QuEChERS method were evaluated according to some parameters.[13,15,21,36-38]

2.3.1.1 Physical aspect of final extract

The physical aspect of final extract of samples consisted in visual evaluation of its color, intensity and transparency. These characteristics are related to the presence of matrix interferences that are co-extracted and which were not removed in the clean-up step.

2.3.1.2 Recovery

The recovery was determined by comparing the analytical response of samples spiked with pesticides before (blank sample) and after (final extract) of the sample preparation using the modified QuEChERS method. Recovery (% RE) was calculated with the following formula:

$$\% \text{ RE} = \frac{\text{Area of pre\_extraction spike}}{\text{Area of post\_extraction spike}} \text{ x } 100$$

2.3.1.3 Amount of matrix coextractives during sample preparation

The sample extracts were divided in two parts, no clean-up and clean-up, and then the solvent was evaporated and the residue was weighed to evaluate the percentage of interferences remaining in the extract after the clean-up step of strawberry extracts.[13,15,21,36,37] The experiment had the purpose of estimating the efficiency of the clean-up during sample preparation, using either d-SPE or c-SPE, by gravimetric measurements of the amounts of coextracted interferences that were not removed from the final extract.

2.3.1.4 Matrix effect

The matrix effect was used to evaluate the presence of interferences that coeluted with the target analyte in the chromatographic run. It can influence, mainly, the recovery and the selectivity of the analytical determination. The matrix effect (% ME) was calculated by the ratio of the analytical response of the final extract spiked with the mixture of the pesticides and the solution of standard mixture in solvent at the same concentration, as follows:[22,26,38]

$$\% \text{ ME} = \left(\frac{\text{Area of post\_extraction spike}}{\text{Area of standard}} - 1\right) \text{x } 100$$

A ME equal to 0% indicates there is no matrix effect, while values negative or positive indicate suppression or enhancement signal by the matrix components.[22,36]

After evaluating the all parameters mentioned above, the best sample preparation can be selected mainly due to the recovery and efficiency of clean-up for multiresidue pesticides in strawberries determined by UHPLC-DAD.

3 Results and discussion

For selecting one of four procedures using the modified QuEChERS method based on the CEN-15662 version, several parameters were tested for a more detailed evaluation depending on the extraction of pesticides and the level of clean-up of the strawberry extracts. The performances of sample preparation procedures were evaluated with strawberry samples spiked with 250 ng g^{-1} of pesticides. Finally, the mean of the results in triplicate were used for evaluation. The results are described below.

3.1 Coloration of the final extract

At first, there was a visual observation to evaluate the degree of clean-up that depends on the sample preparation applied to the samples. The final extracts of strawberries showed differences in color, ranging from light yellow for d-SPE at both ambient and low temperatures, to darker yellow for c-SPE and to orange for d-SPE without salts in the liquid-liquid partitioning step. All final extracts of strawberries showed few or no particles, but the color of the extract cleaned by d-SPE without salts is due to higher amount of the anthocyanin pigments remaining after this sample preparation procedure.

3.2 Evaluation of the chromatographic profile

The LC using UHPLC-DAD allows qualitative and/or quantitative analysis. Analyses of chromatographic profiles at the same wavelength, obtained by injection of the blank sample after different methods of sample preparation, can provide an indication of the amount of interferences not removed after the clean-up step. Emphasizing that a selected wavelength may facilitate the detectability of the analyte, however, it may not be sufficiently selective if it also provides greater absorption for the interferences in the sample. In multiresidue analyses that include several pesticides at low concentrations, one should be concerned about the selectivity of the method so as not to generate false-positive results, and also that the method developed can reach the limits of quantification required for analytical determinations.

The chromatographic profiles obtained from blank samples using the modified QuEChERS method after clean-up step with d-SPE, at ambient or low temperatures, using c-SPE or d-SPE without salts in blank samples of strawberries can be seen in Figure 3.

Figure 3

At the start of the chromatographic run there is a large peak in the sample preparation by d-SPE without salts. Analyzing the majority of peaks that have retention times of 1 to 5 minutes in the chromatogram of Figure 3, it is found that the use of low temperature (with the aid of dry-ice) has not contributed to an increased removal of interferences, but the clean-up employing a cartridge showed lower intensities of analytical signal in this range. For the range of 5 to 14 minutes, it can be seen that all procedures extracted practically the same amount of interferences. Therefore, analyzing the chromatographic profile it can be concluded that there was a greater removal of interferences with the use of the PSA cartridge for clean-up of strawberry extracts.

3.3 Recovery

Figure 4 shows the values of recovery (%) with their CV (%), indicated by the error bars, obtained in the extraction of pesticides in strawberries.

Figure 4

It is observed that all pesticides, except chlorpyrifos in sample preparation employing c-SPE, showed recovery values between 70-120% and CV <20%, according to the recommended guidelines for the analysis of pesticide residues in foods.[39,40] Chlorpyrifos belongs to the class of organophosphates and has a significant partition coefficient (Kow = 4.7). When the analyte has a high partition coefficient it shows that there is a high concentration of chlorpyrifos in the organic phase compared to the aqueous phase, or greater transfer of the analyte to the nonpolar phase in relation to

the polar phase. In the sample preparation procedure employing c-SPE, the cartridge was conditioned with acetonitrile:toluene (3:1 v:v). This solution decreases the polarity due to the apolar character of the toluene solvent, thus this analyte may have been retained in the cartridge in the sample application step, and its recovery value is lower due to loss of analyte. However, recovery values out of the range of 70-120% can be accepted, if the CV is <20%.[39] The CV value of chlorpyrifos was 1.9%, indicating that it is in the acceptable range, consequently, the method has precision for analytical determination of all studied pesticides.

Analyzing Figure 4, it is observed that the sample preparation using d-SPE, at ambient and low temperatures, showed higher recoveries compared to c-SPE and d-SPE without salts methods. However, any of four modifications of the QuEChERS method could be applied for strawberries within the required criteria. The recovery values demonstrate the versatility of QuEChERS for a complex sample with pesticides of diverse physicochemical characteristics at low concentrations, which enables variations in the method without affecting the efficiency of extraction in sample preparation.

3.4 Matrix interferences by gravimetric measurements

For the analysis of extracts, with and without clean-up, the amount of interferences that remained after the use of different procedures for clean-up in strawberries was evaluated. The results are shown in Table 2.

Table 2

For the analysis of Table 2, the sample preparation by d-SPE at ambient temperature showed the best performance of clean-up, because the percentage of matrix interferences in the final extract was significantly lower than those obtained using the other sample preparation procedures. It was found that the four procedures have not significantly affected the recovery values for the pesticides, which were presented and discussed in item 3.3. However, sample preparation can greatly influence in the amounts of coextracted matrix components, since the clean-up employing cartridges (c-SPE) showed approximately triple the amount of interferences that have not been removed in relation to the d-SPE at ambient temperature. The two dispersive clean-ups, with addition of dry ice, had similar performances in amount of residual interferences.

The comparison between the chromatographic profile and the amount of matrix interferences show lower intensity in the chromatographic peak in contrast to larger amount of waste after cartridge clean-up step. It can be concluded that, at the wavelength employed in the analyses of these samples, a greater quantity of interferences with low absorptivity was not removed in clean-up by the cartridges

and remained in the final extract, as was confirmed by the amount of residual interferences found in gravimetric measurements.

The development of a sample preparation with less residual interferences will contribute positively in aspects involving detectability and selectivity of the method and also lead to less chromatographic maintenance due to injecting lower amounts of interferences in each analytical run.

3.5 Matrix Effect

The matrix effect should be evaluated in any method development, because it can generate errors in the analytical determination due to the presence of matrix interferences.[38,41] There is major concern and studies with evaluation of the matrix effect in liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses, mainly with electrospray ionization, have been reported.[26,27,38,41] However, we can also evaluate and compare this effect with a DAD detector to identify the influence of the clean-up step.

There are some ways to minimize the ME,[27,38] but in experiments involving a DAD detector, for example, use of calibration curves with matrix-matched standards, improvements in the clean-up step, changes in the blank matrix, modification of the wavelength or optimization of the chromatographic separation by use of different analytical columns or gradient elution are usually examined.

Figure 5 shows the matrix effect of pesticides determined by UHPLC-DAD in strawberry samples with different clean-up procedures using the modified QuEChERS methods.

Figure 5

Some studies consider that values below ± 20% are not significant or are soft matrix effects.[42,43] Therefore, this work considered that the analytical determination of the analytes has no matrix effect if in this range. Dispersive clean-up, at ambient or low temperatures, showed similar matrix effects, where approximately 64% of pesticides analyzed had no matrix effect. The use of the cartridges showed to be the most adequate for all pesticides, except atrazine, by providing lower values of the matrix effect. Liquid-liquid partitioning without salts showed that 36% of the pesticides analyzed had no matrix effect, in other words, the addition of dry ice was not as efficient as the salts of partitioning step and reduces removal of the interferences that co-eluted with the analytes.

Figure 5 shows that simazine pesticide presented an enhancement signal in all sample preparations. Commonly, this behavior may be related to the large amount of interferences that coeluted at the start of chromatographic run. Positive values of ME can produce false negative results by blinding the chromatographic peak of interest or false positive results due to matrix components which are considered as analyte.

In the analysis using the DAD detector there is a requirement to perform a more complete analytical separation for identification and quantification. Therefore, the analytes must be better separated from interferences than when using a mass spectrometer (MS) detector. In this aspect, the MS detector has an advantage because it allows quantification and identification by the use of multiple reaction monitoring (MRM), which permits analytical determinations even when interferences occur at the retention time of the target analyte. This makes the development of method more rapid, because less time is needed for sample preparation due to the higher selectivity of the detector and with DAD detection development of a gradient elution to improve chromatographic separation is often required in analyses.

4 Conclusion

The analysis of pesticide residues in foods includes sample preparation and analytical determination of analytes. Most of the studies of sample preparation methods only evaluate the recovery values as a parameter of efficiency, selectivity and detectability for development of an analytical method. For the present study other parameters were also evaluated such as the final aspect of extract, the chromatographic profile, the recovery values , the amounts of coextractives in the matrix determined by gravimetric measurements and the matrix effect. The four procedures studied could be applied for the analysis of multiresidue pesticides with different physicochemical characteristics, at low concentrations, in a complex matrix such as strawberries containing anthocyanin pigments, sugars and organic acids, because the recovery results were in the range of 70-120% and the CV <20% for most of the pesticides.

The c-SPE procedure showed a lower matrix effect and a better chromatographic profile compared to dispersive clean-up. The addition of dry ice to decrease the temperature and to replace the salts in liquid-liquid partitioning was not efficient. On the other hand, d-SPE at ambient temperature was more efficient by providing higher recoveries, extracts with clearer physical aspect and lower percentages of coextractives in the final extract. Also, it is cheaper, faster, easier, uses a lower volume of extraction solvent and produces less waste compared to the other procedures studied in this work.

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Figure Captions

Fig. 1 Chromatogram of separation of 1.5 μg mL^{-1} pesticides measured at 254 nm. Column: Acquity UPLC ®BEH C18 (50 mm, 2.1 mm i.d., 1.7 μm particles); injection volume: 1.9 μL; flow rate: 0.12 mL min^{-1}; mobile phase A: 0.1% formic acid in water and mobile phase B: methanol. Gradient program: 70% A from the start ramped to 95% B over the course of 10 min. Compound identification: (1) simazine, (2) carbaryl, (3) atrazine, (4) azoxystrobin, (5) fludioxonil, (6) procymidone, (7) diflubenzuron, (8) difenoconazole, (9) chlorpyrifos, (10) fenazaquin and (11) abamectin.

Fig. 2 Scheme of the modified QuEChERS method for d-SPE and c-SPE clean-ups for strawberries determined by UHPLC-PDA.

Fig. 3 Chromatograms for blank samples (no fortification) obtained for different clean-up procedures with the modified QuEChERS method for strawberries. Chromatographic conditions in Figure 1.

Fig. 4 Recoveries (%) and CV (%) obtained for the different clean-up procedures with the modified QuEChERS methods, spiked at 250 ng g^{-1} in strawberries, determined by UHPLC-DAD.

Fig. 5 Matrix effect (%) of pesticides in strawberries with different clean-up procedures using the modified QuEChERS methods determined by UHPLC-DAD.

Tables

Table 1 Summary of the experimental parameters for the pesticides

Pesticides	Substance group	Partition coefficient (log Kow) *	Retention time (min)	Wavelength (nm)
Abamectin	Avermectin	4.4	10.2	244.7
Atrazine	Triazine	2.5	5.4	222.1
Azoxystrobin	Strobilurin	2.5	5.7	246.5
Carbaryl	Carbamate	1.9	5.0	221.5
Chlorpyrifos	Organophosphorus	4.7	8.7	229.4
Difenoconazole	Triazole	4.2	7.5	244.1
Diflubenzuron	Benzoylurea	3.9	6.7	257.6
Fenazaquin	Quinazoline	5.5	9.8	217.2
Fludioxonil	Phenylpyrrole	4.1	6.0	265.5
Procymidone	Dicarboximide	3.1	6.5	210.0
Simazine	Triazine	2.1	4.8	222.1

* Octanol-water partition coefficient

Table 2 Amount of coextractives in strawberries after the clean-up step using the modified QuEChERS methods

Clean-up procedures	% Coextractives
d-SPE	11
d-SPE (dry ice)	24
d-SPE (dry ice and no salts)	26
c-SPE	30

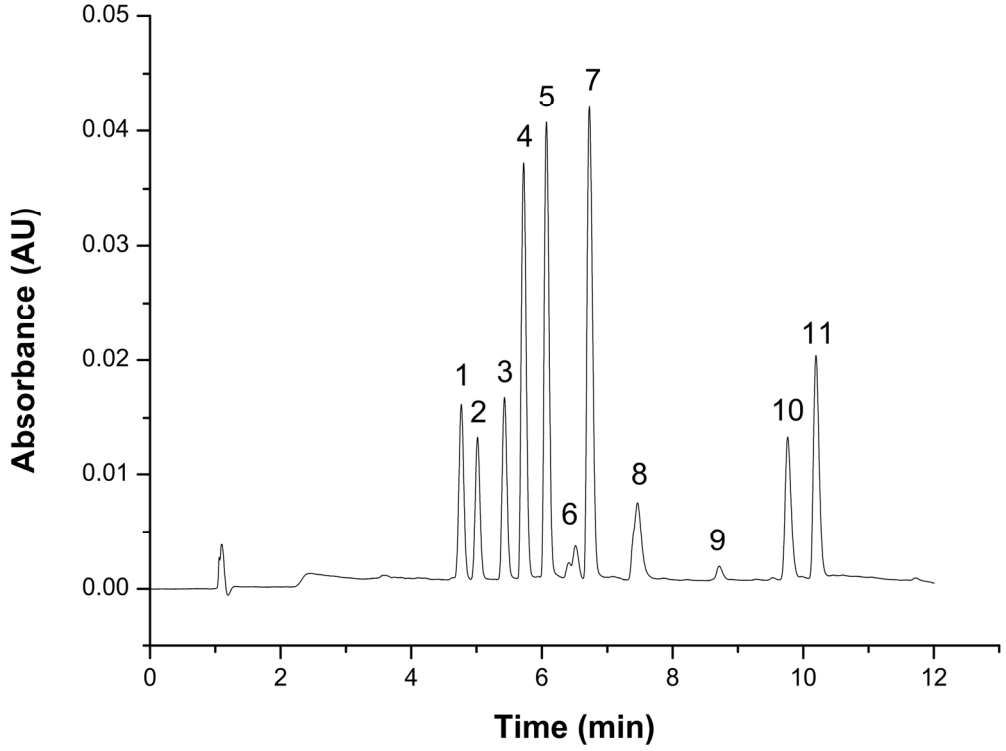
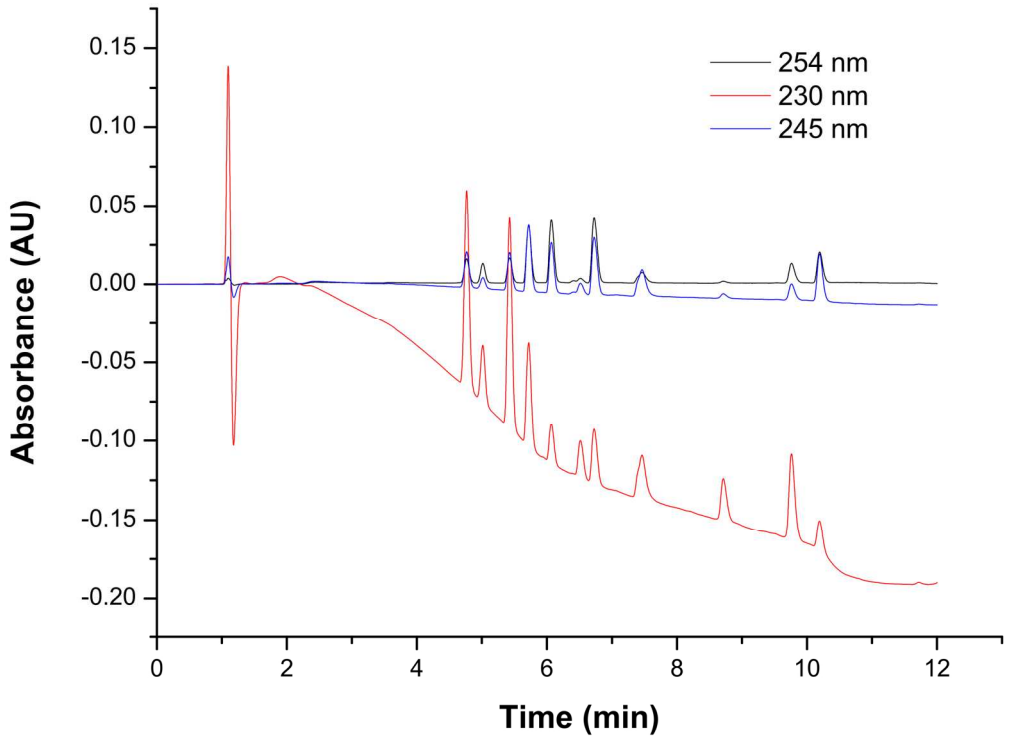


Fig. 1 Chromatogram of separation of 1.5 µg mL-1 pesticides measured at 254 nm. Column: Acquity UPLC ®BEH C18 (50 mm, 2.1 mm i.d., 1.7 µm particles); injection volume: 1.9 µL; flow rate: 0.12 mL min-1; mobile phase A: 0.1% formic acid in water and mobile phase B: methanol. Gradient program: 70% A from the start ramped to 95% B over the course of 10 min. Compound identification: (1) simazine, (2) carbaryl, (3) atrazine, (4) azoxystrobin, (5) fludioxonil, (6) procymidone, (7) diflubenzuron, (8) difenoconazole, (9) chlorpyrifos, (10) fenazaquin and (11) abamectin.



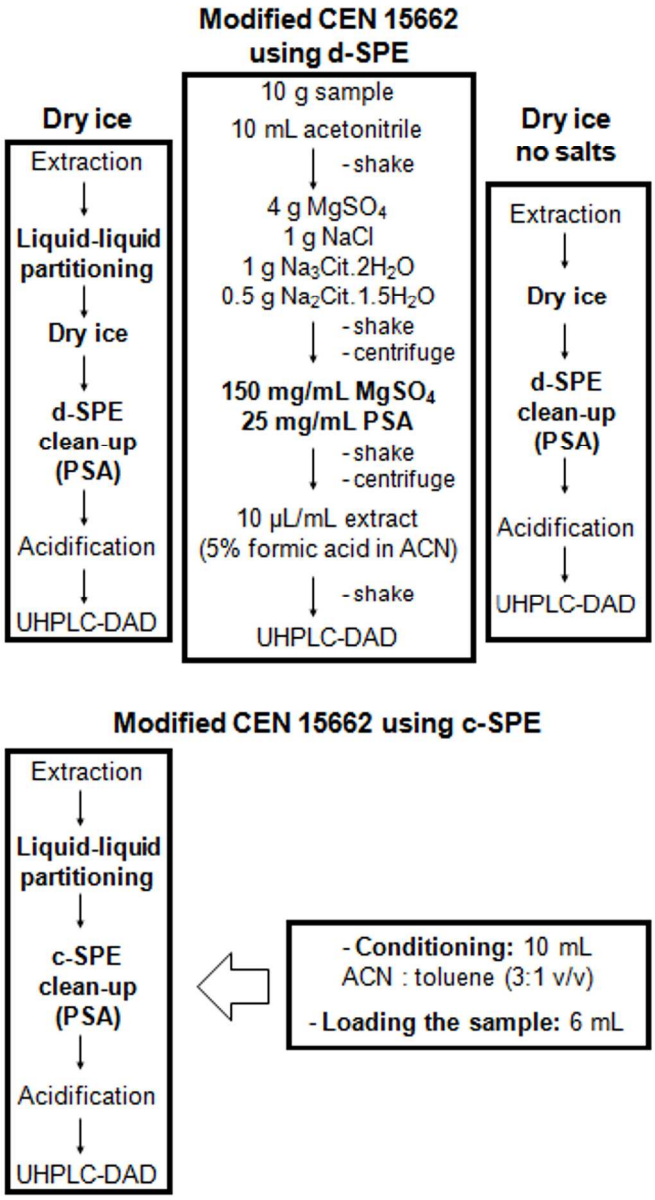
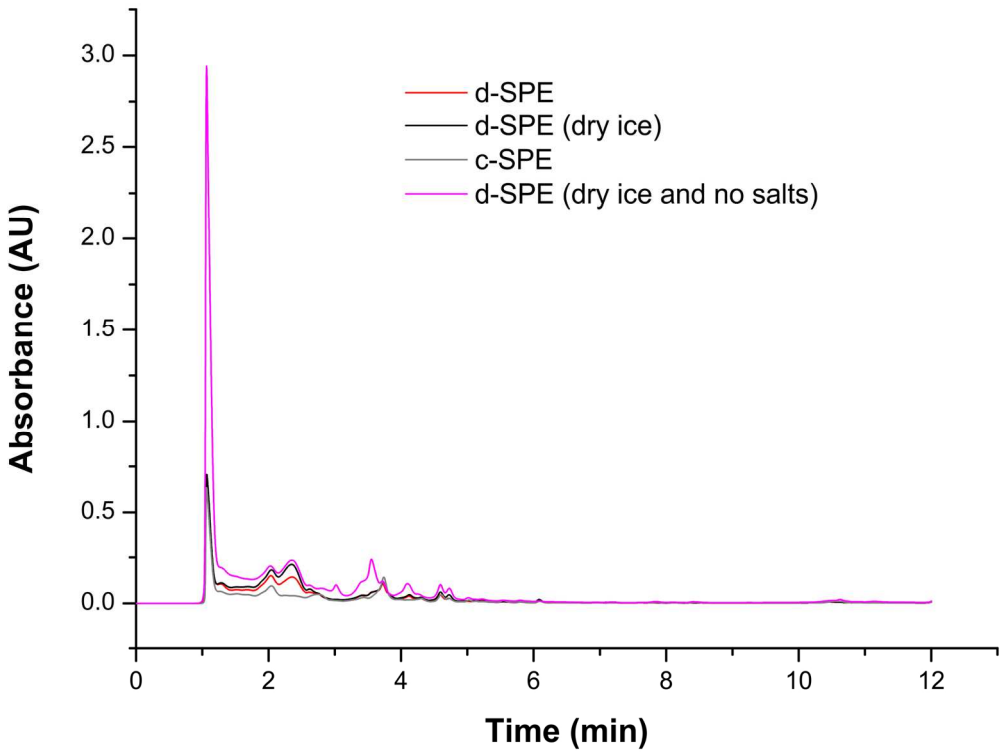


Fig. 2 Scheme of the modified QuEChERS method for d-SPE and c-SPE clean-ups for strawberries determined by UHPLC-PDA.



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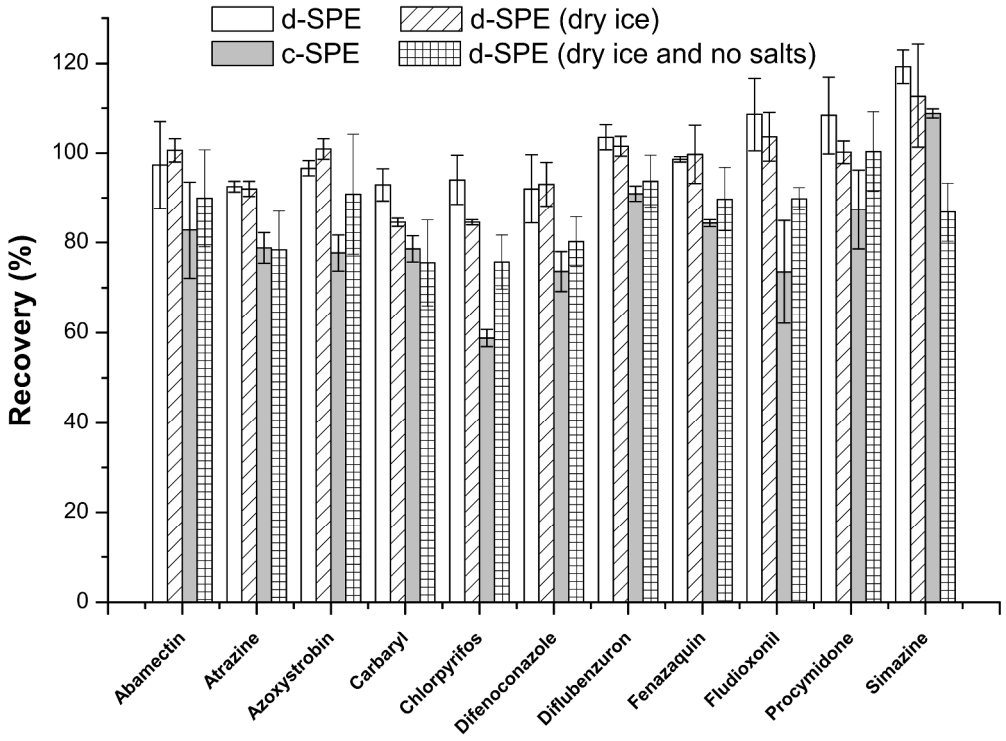

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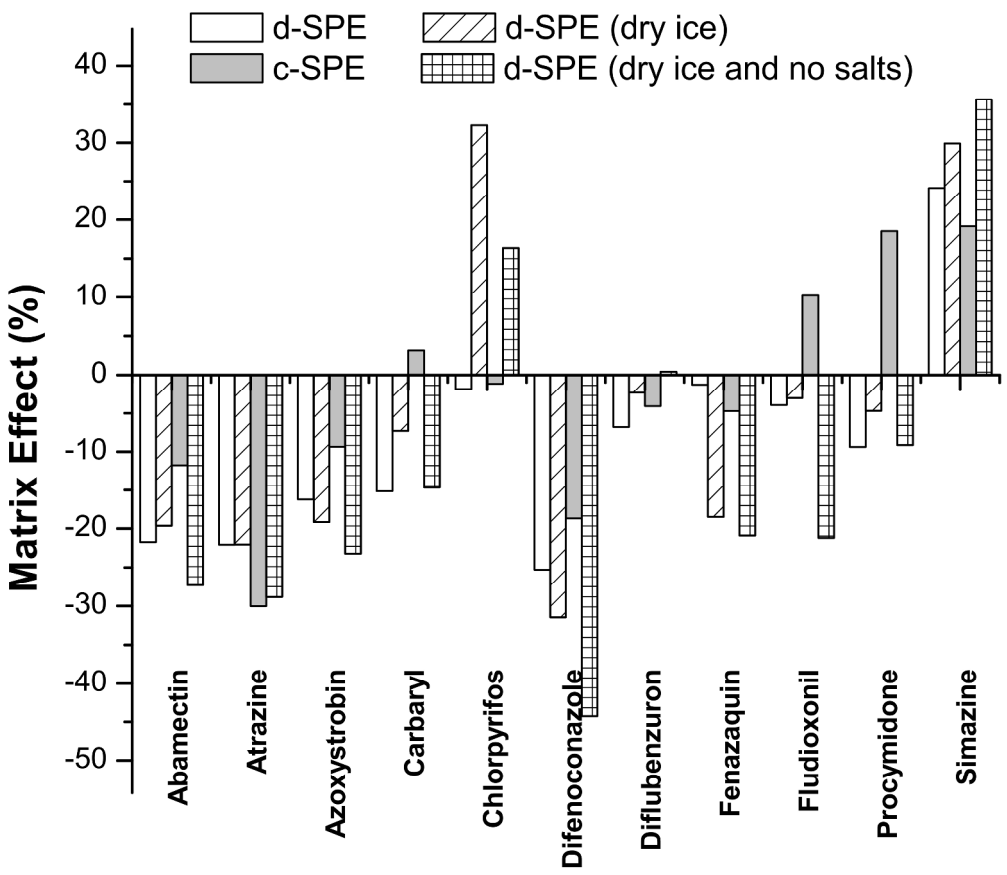

Fig. 5 Matrix effect (%) of pesticides in strawberries with different clean-up procedures using the modified QuEChERS methods determined by UHPLC-DAD.