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Optimization of stir bar sorptive extraction applied to the determination of pesticides in vinegars

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Abstract

Stir bar sorptive extraction (SBSE) has been evaluated for analysing pesticides in vinegar. The extraction analytical conditions have been optimised using a two-level factorial design expanded further to a central composite design. After optimization, the proposed analytical conditions are: sample volume 40 mL, sampling time 150 min, and stirring speed 1000 rpm. On the basis of the results, it was decided not to add NaCl. The SBSE procedure developed shows detection limits and linear ranges adequate for analysing this type of compound, giving recoveries close to 100%. The repeatability and reproducibility values obtained were lower than 18 and 23%, respectively. The method was applied to a variety of commercial vinegars. SBSE is a very simple, solvent-free, and fast technique with high sensitivities.
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Keywords: Stir bar sorptive extraction; Vinegar; Pesticides; Two-level factorial design; Central composite design

1. Introduction

In spite of the trends towards adopting more ecological approaches in agriculture, it is still necessary to employ phytosanitary products in order to ensure both the required quality and quantity of very many crops.

Pesticides are used on agricultural products such as grapes and wine grapes, and consequently some of these compounds can be carried through the production process and into the finished wine [1] or wine vinegar.

For every common pest there exists a wide range of pesticides, of different chemical families, each with a different form of action [2]. Many products are recommended for the control of pests and diseases of the grapevine, and in one or other way, these may persist throughout the various stages of production, into the finished vinegar.

Most authorized insecticides and acaricides belong to the group of organophosphorous compounds, and these are also used mixed with oils for treatments applied during winter; most of the fungicides used belong to the families of thiocarbamates, phthalimides, benzimidazoles and dicarboximides. Among the herbicides, the most commonly used are triazines and phenylureas [3].

When a phytosanitary treatment is applied a certain amount of the substance remains on the plant, and it is called pesticide deposit. This quantity of pesticide diminishes progressively over time, until the grapes are harvested, when only a small residue can be found. The most problematic pesticides are those applied close to the harvest such as the antibotrytics [1].

The appearance of pesticide residues in wine, and therefore in the final vinegar, depends on several factors, as many as the number of stages comprising the production process [4].

Pesticide residues are not directly legislated in wine, but are generally regulated through the various national standards for foodstuffs, as a maximum residue limit (MRL) on the viniferous grapes. Today there is a trend to lower the MRL to be separate strictly for wine. The legislation is reducing the maximum permitted quantity of pesticides, with a clear intention to reach “zero tolerance” [5,6]. This trend has also been observed in vinegar and an increasing number of products are appearing on the market with the label: “ecological product”.

Another factor is the aromatic quality of the wine, and therefore of the vinegar, which can be modified by the presence of

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pesticide residues during the fermentation [7–9]. The presence of pesticide residues and their degradation products may also influence negatively the stability of the finished wine, producing colloidal haze [10].

Therefore it is considered of commercial interest to possess reliable and sensitive methods for determining the levels of phytosanitary products in vinegars, not only because of the adverse effect their occurrence could have on the organoleptic characteristics of the vinegar [11], or because of the risk to public health that a large quantity of these residues could represent, but also for its implication in obtaining the denomination of “ecological vinegar”.

Numerous analytical methods have been developed for the determination of pesticides in wines by gas chromatography (GC) or liquid chromatography (LC). Generally prior to the chromatographic separation, a sample treatment is required [12–17]. Most of these are time and solvent consuming. Solvent-free sample preparation techniques based on sorptive extraction have been demonstrated to be good alternatives to traditional methods such as liquid extraction [18].

Stir bar sorptive extraction (SBSE) is a recently-developed technique [19–22] in which a stir bar coated with 50–300 μL of polydimethylsiloxane (PDMS) is employed to extract analytes from a variety of matrices. The extraction mechanism is similar to that of solid phase microextraction (SPME) based on PDMS sorption [23]. A magnetic stirring bar is added to the sample to promote the transfer of analytes to the polymer coating and, after a predetermined extraction period, the analytes are thermally desorbed in the GC injector.

The advantage of SBSE is the much higher mass of PDMS available, which results in high recoveries and higher sample capacity [24]. The applications developed with SBSE have shown low detection limits and good repeatability [25–27], which confirm the great potential of this technique.

Some previous applications of this technique to the analysis of pesticides in wine can be found [28,29], but it has never been applied to vinegar samples.

The present paper describes the optimization and validation of a stir bar sorptive extraction and thermal desorption procedure coupled to capillary gas chromatography-mass spectrometry for the determination of pesticide residues in vinegar.

2. Experimental

2.1. Vinegar samples

A commercial Sherry vinegar sample spiked with the selected pesticides was used to optimize the extraction conditions and to determine some performance characteristics (recovery and precision). After optimization and validation, several commercial vinegars were analyzed.

2.2. Chemicals and reagents

Pesticides, comprising pyrimethanil, flufenoxuron, chlorpyriphos-methyl, vinclozolin, metalaxyl, fenitrothion, malathion, dicofol, chlorpyriphos, cyprodinil, triadimenol, procymidon, hexythiazox, fludioxonil, iprodion, benalaxyl, fenhexamid were supplied by Sigma–Aldrich (PESTANAL, Riedel-de Haën, Seelze, Germany). A global stock standard solution was prepared by accurately weighing 5–10 mg of each individual pesticide standard into a 50 mL volumetric flask, dissolving with acetone and diluting to volume with ethanol. Working solutions used in calibration process, were prepared by diluting different amounts of the global standard solution in a synthetic vinegar solution (2 g/L of tartaric acid, 80 g/L of acetic acid, 1 g/L ethyl acetate, and 10 mL/L of ethanol, in Milli-Q water).

All these solutions were stored at 4 °C.

Heptachlor epoxide, supplied by Sigma–Aldrich, was employed as internal standard. NaCl was purchased from Scharlau (Barcelona, Spain).

2.3. Sample preparation

The extractions were carried out with 20 mm × 0.5 mm (length × film thickness) PDMS commercial stir bars, supplied by Gerstel (Mülheim a/d Ruhr, Germany). After optimization, and for each SBSE analysis, 40 mL of sample (natural and synthetic vinegar) were pipetted and placed into a 100 mL Erlenmeyer flask. Each sample was spiked with 40 μL of a solution of heptachlor epoxide (3.42 mg/L in acetone). The Erlenmeyer flask was placed on a 15 position magnetic stirrer (Mülheim a/d Ruhr, Germany). The stir bar was stirred at 1000 rpm at 25 °C for 150 min. After removal from the vinegar sample, the stir bar was placed for a few seconds in distilled water and gently dried with a lint-free tissue. Then, it was transferred into a glass thermal desorption tube and then thermal desorption was carried out.

After each analysis and in order to remove completely all the possible pesticide residues that the stir bar may retain, a cleaning up procedure was performed (300 °C during 15 min). No measurable signal corresponding to any pesticide was detected after this.

2.4. Apparatus

The coated stir bars were thermally desorbed using a commercial TDS-2 thermal desorption unit (Gerstel) connected to a programmed-temperature vaporisation (PTV) injector CIS-4 (Gerstel) by a heated transfer line. The PTV injector was installed in an Agilent 6890 GC-5973 MS system (Agilent Technologies, Palo Alto, CA, USA). An empty baffled liner was used in the PTV system. The thermodesorption unit was equipped with a MPS 2L autosampler (Gerstel) capable of handling the program for 98 coated stir bars. The desorption temperature was programmed from 30 to 300 °C (held for 10 min) at 60 °C min^{-1} under a helium flow (75 mL/min) and the desorbed analytes were cryofocused in the PTV system with liquid nitrogen at −150 °C. Finally, the PTV system was programmed from −150 to 300 °C (held for 5 min) at 10 °C s^{-1} for analysis by GC-MS. Capillary GC-MS analyses in the electron impact mode were performed on an Agilent 6890 GC-5973N MS system (Agilent, Little Falls, DE, USA), equipped with a HP-5 capillary column (J&W Scientific, Folsom, CA, USA), 30 m × 0.25 mm i.d., with a 0.25 μm

Table 1
Pesticides studied

Compound	Retention time (min)	Monitored ions[a] (m/z)
Pyrimethanil	13.19	198, 199, 200
Flufenoxuron	14.15	305, 126, 307
Chlorpyriphos-methyl	15.26	286, 288, 125
Vinclozolin	15.34	212, 198, 285, 187
Metalaxyl	15.94	206, 249, 160
Fenitrothion	16.61	125, 277, 109, 260
Malathion	17.33	173, 127, 125, 93
Dicofol	17.61	139, 250, 111
Chlorpyriphos	17.69	197, 199, 314, 97
Cyprodinil	18.97	224, 225, 210
Triadimenol	19.95	112, 168, 128
Procymidon	20.17	96, 283, 285, 67
Hexythiazox	20.33	156, 155, 227, 184
Fludioxonil	22.57	248, 127, 154, 182
Iprodion[b]	24.31	187, 244, 189
Benalaxyl	25.45	148, 91, 206
Fenhexamid	25.55	97, 177, 55

Retention times and monitored ions for each one.
[a] The first one of each compound is its quantifying ion, the rest are their qualifying ions.
[b] Degradation product: (3,5-dichlorophenyl)hydantoin.

coating. The carrier gas was helium at a flow rate of 1.0 mL/min. The GC oven was programmed as follows: held at 70 °C for 2.5 min, then ramped at 25 °C min^{-1} to 150 °C. Then it was raised to 200 °C at 3 °C min^{-1}, and to 300 °C at 8 °C min^{-1}, then held for 10 min. The mass detector operated in EI+ mode at 70 eV. Selected ion monitoring mode, choosing for each compound one quantifying ion and two or three qualifying ions, was employed. The studied pesticides with their retention times and their selected ions are shown in Table 1.

The signal was recorded and processed with a RTL pesticides library supplied by Agilent Technologies (Palo Alto, CA, USA). Peak identification was carried out by analogy of mass spectra and confirmed by retention indices of standards.

Quantitative data from the identified compounds were obtained by measuring the relative molecular ion peak area in relation to that of heptachlor epoxide, the internal standard. Iprodione showed low thermal stability and was measured through its degradation product (3,5-dichlorophenyl) hydantoin [28].

2.5. Experimental design

The Statgraphics Statistical Computer Package “Statgraphics Plus 5.0” for Windows 98 was used for data treatment.

A sequential exploration of the response, which was carried out in two stages, was selected. In the first stage, in order to establish the relative influence of the factors and their interactions on the total chromatographic area obtained, related to the selected quantifying ions, four factors were selected: time of extraction, sample volume, stirring speed and ionic strength effect.

Therefore a factorial design of 2^4 was chosen (16 experiments, in duplicate, undertaken in random order to avoid the effects of lurking variables). The values corresponding to the high (+), and low (−) points for each factor are shown in Table 2.

Table 2
Factor levels for the extraction condition optimization

Factor	Low (−)	High (+)	Centre	Axial (−α)	Axial (+α)
Sample volume (mL)	10	40	25	5.7	44.3
NaCl (M)	2.0	4.0	1.0	1.71	4.28
Extraction time (min)	20	150	85	1.3	167
Stirring speed (rpm)	500	1500			

In the second phase, this two-level factorial design was expanded to a star design. A central composite design (CCD, with $\alpha = 2.0796$) was obtained, since the centres of the two separate designs were coincidental. Table 2 lists the values given to each factor.

3. Results and discussion

3.1. Previous study

Baltussen et al. [19] found that the total amount of semivolatile compounds extracted by SBSE depends on the phase ratio between sample volume and volume of PDMS sorbent. So, before optimizing the extraction conditions, a study was carried out to determine the most appropriate twister (stir bar). A sample of commercial vinegar spiked with the pesticides studied was extracted with four PDMS twisters of different lengths (10 mm or 20 mm) and thicknesses (0.5 mm or 1.0 mm). On the basis of the results (data not shown), the 0.5 mm × 20 mm PDMS twister was selected. This provided a greater chromatographic area for all pesticides without exceeding the column capacity.

3.2. Extraction condition optimization

Time of extraction, sample volume, stirring speed and the addition of different amounts of NaCl were evaluated to reach the best overall analytical conditions. The factor extraction temperature has a significant influence on the SBSE efficiency, but a high temperature reduces the lifetime of the PDMS phase [30], so in this work, the extraction temperature was set at 25 °C.

Total chromatographic area of the quantifying ions corresponding to all the pesticides studied was selected as the experimental response for optimizing.

In this study, the desorption condition optimization (desorption time and temperature, helium flow and cryofocusing temperature) was not considered taking into account that in a previous work [27] we found that they had only a modest effect on chromatographic signals.

3.2.1. Screening by a 2^4 factorial design

The initial screening design served to detect those variables that have the most influence on the experimental response.

The data obtained were evaluated by ANOVA at the 5% significance level. These results are shown in bar chart format (Fig. 1). Extraction time, sample volume and NaCl concentration were significant parameters. Extraction time was the most

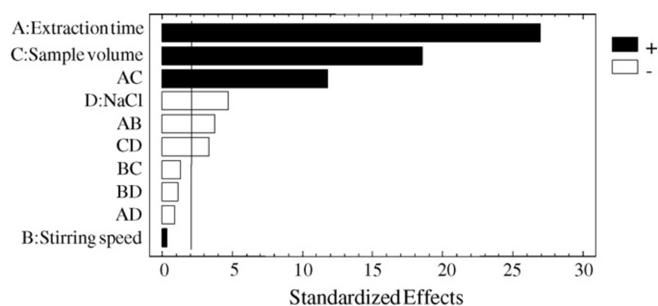

Fig. 1. Pareto chart of the main effects in the factorial 2^4 design for the pesticides studied.

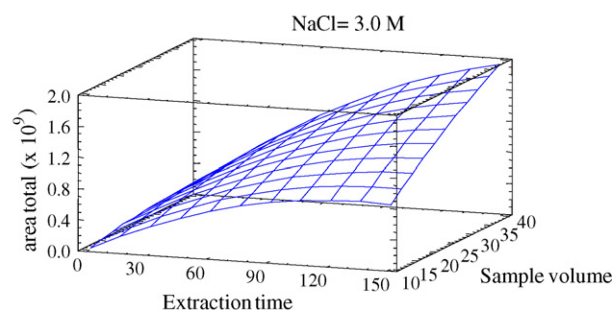

Fig. 2. Estimated response surface obtained using the central composite design by plotting extraction time vs. sample volume.

influential variable. All the significant factors affected with a positive sign with the exception of NaCl concentration.

As can be seen in Fig. 1, the SBSE efficiency is also affected by the interrelated variables. The interaction between the factors extraction time and sample volume appears to be the most significant interaction statistically, with a positive sign. The interactions between extraction time-stirring speed and sample volume-NaCl were also significant (negative sign).

3.2.2. Optimization by a central composite design

Since stirring speed was not shown to be a significant parameter, in this study we decided to eliminate this factor. It was set at 1000 rpm. For the central composite design (CCD), the three parameters utilised were: extraction time, sample volume and NaCl. The axial values for these parameters are located on a sphere surrounding the two-level factorial design (Table 2).

After the CCD, as expected from the screening experiments, the extraction time and the sample volume appeared, with a positive sign, as statistically significant parameters (Table 3). Leon et al. [20] observed that for apolar compounds ($\log K_{o/w} > 3.5$), higher volume samples increased the chromatographic responses, whereas for polar analytes, increased volume samples had a little effect on the signals obtained.

In relation to extraction time, long extraction times by SBSE are normally required to reach the equilibrium [20,24]. Therefore, it is impractical to use the full capacity of the extraction phase in many applications. Good precision and reproducibility are obtained when the conditions are strictly controlled.

Among the two factor interactions, and based on the *p*-values and *F*-ratios of the ANOVA data (Table 3) the extraction time–sample volume interaction was statistically the most significant.

The response surface plot obtained by plotting extraction time versus sample volume is showed in Fig. 2. As can be seen, a long sampling time (150 min) produces the extraction of a higher amount of pesticides when a high sample volume is used. For short extraction times the use of a higher sample volume, lightly increases the total chromatographic area.

In relation to the NaCl addition, it was not statistically significant for the central composite design whereas for the initial screening design, it had a negative influence on the experimental response. Taking these findings into account, a study with and without NaCl addition was carried out. A spiked vinegar sample was extracted five times with NaCl added (1.71 M), and five times without addition. The results showed that there were no statistical differences between the two types of samples for the most of the pesticides considered.

In addition, several authors have already described [20,24] that the recovery for more hydrophilic solutes ($\log K_{o/w} < 3.5$) dramatically increased on increasing concentration of NaCl; however, the recovery for more hydrophobic solutes ($\log K_{o/w} > 5$) considerably decreased. In our case, the pesticides studied exhibit a $\log K_{o/w} > 3$, so they are quite apolar and the addition of NaCl should decrease their recoveries.

On the other hand, the salt may damage the stir bar when it is not completely dissolved, so we decided to eliminate this parameter.

To sum up, after evaluation of the main factors and their interactions, the best conditions for extracting the pesticides of vinegar were: sample volume 40 mL, sampling time 150 min and stirring speed 1000 rpm without NaCl addition.

Table 3
Main effects and interactions in the central composite design

Effect	*F* ratio	*p*-Value
A: Extraction time	673.12	0.0000[a]
B: Sample volume	338.03	0.0000[a]
C: NaCl	0.26	0.6147
AA	50.70	0.0000[a]
AB	82.94	0.0000[a]
AC	0.01	0.9417
BB	5.07	0.0352[a]
BC	0.85	0.3657
CC	0.32	0.5792

[a] Values are significant at $p < 0.05$.

3.3. Performance characteristics

3.3.1. Calibration and linearity

Seven levels of concentration were tested in triplicate; these concentrations covered the concentration ranges expected for the various pesticide compounds in vinegars.

The [pesticide/internal standard] molecular ion peak area ratio for the identified pesticide was used for each compound. The range of linearity studied for each compound appears in Table 4. The correlation coefficients obtained for each compound (Table 4) were good ($r^2 > 0.99$). This was also corroborated by the "on-line linearity (LOL) = 100 − RSD(*b*)", with

Table 4
Characteristics of the calibration curves

Compound	Linear range (μg/L)	Regression coefficient	Linearity (LOL, %)	Slope	Intercept
Pyrimethanil	0.54–540	0.9999	99.71	0.0079	−0.0047
Flufenoxuron	1.08–540	0.9994	99.11	0.0871	−0.0199
Chlorpyriphos-methyl	0.51–510	0.9991	99.28	0.3367	−0.8937
Vinclozolin	0.46–460	0.9996	99.46	0.0499	0.0368
Metalaxyl	1.05–1050	0.9965	98.36	0.0002	0.0017
Fenitrothion	0.5–100	0.9943	97.64	0.0194	−0.0267
Malathion	0.8–160	0.9940	96.81	0.0074	−0.0177
Dicofol	0.47–470	0.9992	99.37	0.3112	−0.5738
Chlorpyriphos	0.43–430	0.9994	99.30	0.2806	0.4549
Cyprodinil	0.55–550	0.9996	99.40	0.0085	−0.0032
Triadimenol	1.01–202	0.9933	97.80	0.0007	−0.0025
Procymidon	0.49–490	0.9981	99.05	0.0208	−0.0356
Hexythiazox	1–100	0.9957	97.67	0.1207	−0.2630
Fludioxonil	0.65–130	0.9931	97.07	0.0095	0.0049
Iprodion[a]	1.09–218	0.9927	97.24	0.0018	0.0074
Benalaxyl	0.48–96	0.9974	98.32	0.0425	0.0429
Fenhexamid	0.9–900	0.9996	99.38	0.0008	0.0180

[a] Degradation product: (3,5-dichlorophenyl)hydantoin.

values higher than 97% (Table 4). RSD(b) is the relative standard deviation of the slope (expressed as a percentage).

3.3.2. *Detection and quantitation limits and recovery*

Detection and quantitation limits were calculated from the calibration curves constructed for each pesticide, using the Alamin Computer Program [31].

The limits of detection (three times the relative standard deviation of the analytical blank values calculated from the calibration curve) and quantitation (10 times the relative standard deviation of the analytical blank values calculated from the calibration curve) obtained (Table 5) are low enough to determine these compounds in real vinegar samples.

The technique of standard additions was used to check the accuracy of this method. A sample of representative vinegar was taken as the matrix and known quantities of the global standard solution were added at seven levels and in triplicate. The slopes of the lines thus obtained for each of the pesticides were compared with the corresponding slopes obtained in the calibration with standards (t criterion). In general, no significant differences were found between them at $p < 0.05$.

Table 5 gives the data for the recovery of each pesticide, determined by the slope of the line plotting the concentration found against the concentration expected. Good recoveries have been obtained, with values ranging from 91 to 110%.

3.3.3. *Repeatability and reproducibility*

The repeatability and reproducibility have been evaluated by means of five series of five extractions of a commercial sherry wine vinegar spiked with the selected pesticides performed using five different twisters.

Table 5
Performance characteristic

Compound	Detection limit (LOD, μg/L)	Quantitation limit (LOQ, μg/L)	Recovery (%)	Repeatability (RSD, %)	Reproducibility (RSD, %)
Pyrimethanil	0.18	0.60	109.39	4.09	7.80
Flufenoxuron	0.19	0.63	102.59	4.36	22.22
Chlorpyriphos-methyl	0.23	0.77	93.20	5.91	2.06
Vinclozolin	0.16	0.53	97.34	1.77	3.37
Metalaxyl	0.81	2.7	101.78	5.78	16.99
Fenitrothion	0.34	1.13	109.81	10.31	7.93
Malathion	0.65	2.16	107.72	8.09	6.38
Dicofol	0.19	0.63	99.58	2.94	4.39
Chlorpyriphos	0.15	0.50	97.45	4.56	4.19
Cyprodinil	0.25	0.83	109.11	5.09	9.10
Triadimenol	0.64	2.13	96.27	17.16	14.40
Procymidon	0.30	1.00	99.81	6.23	6.61
Hexythiazox	0.14	0.46	92.93	8.58	4.34
Fludioxonil	0.60	2.00	91.69	6.94	8.49
Iprodion[a]	0.77	2.56	94.65	10.11	4.76
Benalaxyl	0.13	0.43	99.16	8.46	16.47
Fenhexamid	0.26	0.86	109.32	16.26	8.55

[a] Degradation product: (3,5-dichlorophenyl)hydantoin.

Table 6
Pesticide residues (μg/L) found in commercial vinegars

Compound	Sherry vinegars		Non-sherry wine vinegars		Apple vinegars		Balsamic vinegars	
	1	2[a]	3	4	5	6	7	8[a]
Pyrimethanil	<D.L.	<D.L.	n.d.	n.d.	n.d.	n.d.	22.28	2.91
Flufenoxuron	n.d.	n.d.	<D.L.	<D.L.	n.d.	n.d.	n.d.	<D.L.
Chlorpyriphos-methyl	<D.L.	<D.L.	<D.L.	<D.L.	<D.L.	<D.L.	<D.L.	<D.L.
Vinclozolin	n.d.	<D.L.	<D.L.	<D.L.	<D.L.	<D.L.	n.d.	<D.L.
Metalaxyl	n.d.	n.d.	186.03	n.d.	n.d.	n.d.	146.21	32.82
Fenitrothion	<D.L.	n.d.	n.d.	n.d.	<D.L.	n.d.	n.d.	n.d.
Malathion	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Dicofol	<D.L.	<D.L.	<D.L.	n.d.	n.d.	n.d.	n.d.	n.d.
Chlorpyriphos	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<D.L.	n.d.
Cyprodinil	n.d.	<D.L.	<D.L.	<D.L.	<D.L.	2.95	8.90	3.43
Triadimenol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	29.87	n.d.
Procymidon	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.50	3.61
Hexythiazox	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fludioxonil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.62	n.d.
Iprodion[b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	31.71	n.d.
Benalaxyl	n.d.	n.d.	n.d.	n.d.	n.d.	<D.L.	n.d.	n.d.
Fenhexamid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	31.27	n.d.

n.d.: not detected; <D.L.: under detection limit.
[a] Aged vinegars.
[b] Degradation product: (3,5-dichlorophenyl)hydantoin.

The corresponding relative standard deviations (RSD) were calculated (Table 5). The RSD obtained for each twister ranges between 1.77 and 17.16%. The inter-twister precision showed RSD values similar to intra-twister precision (2.06–22.22%). In general, RSD values lower than 10% were obtained, confirming the high reproducibility of this technique.

3.4. Determination of pesticide residues in vinegars

This analytical method was used to analyse commercial vinegar samples from different raw materials (sherry and non-sherry wine vinegars, apple vinegars and balsamic vinegars) and different periods of ageing in wood. Each sample was analysed in triplicate.

The mean results obtained are shown in Table 6. In the bibliography no data are available concerning concentrations of pesticides in vinegars. Cyprodinil and fludioxonil were found in concentrations ranging from 0.9 to 28.6 μg/L in Galician white wines [32]. Scarponi and Martinetti [33] determined these same fungicides in Italian white and rosé wines at levels of 30 μg/L for cyprodinil and 34 μg/L for fludioxonil. Iprodione and vinclozolin were found in several wines analysed by Pietschmann et al. [34]. In the case of Italian white and red wines, about 57% of the wines studied were positive for one or more pesticides usually used on grapes [35].

In our case, most of the pesticides were not detected or their concentrations were lower than the calculated quantitation limits. The highest concentrations for several pesticides were found in balsamic vinegars, ranging from 146.21 μg/L for metalaxyl to 2.91 μg/L for pyrimethanil. As can be seen, only small amounts of the pesticides used on the grapes and/or apples appear in commercial vinegars due, possibly, to their degradation and precipitation during fermentation and clarification processes [36].

4. Conclusions

The conditions for the analysis of pesticide residues in vinegars using SBSE-TD-GC-MS have been optimized by means of a statistical approach. Under the optimized conditions used in this study, SBSE can be considered an appropriate technique for the analysis of this type of compounds in vinegars. It is a very simple, solvent-free and fast technique. The detection and quantitation limits, and the recoveries obtained are adequate for the quantification of these compounds in vinegars, and especially for monitoring ecological products in which the use of pesticides is not permitted.

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