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Optimisation of stir bar sorptive extraction for the analysis of volatile phenols in wines☆

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Abstract

An easy, fast and reliable analytical method is proposed for the determination of the concentration of volatile phenols (ethyl- and vinylphenols) in wines. The novel stir bar sorptive extraction (SBSE) technique is employed, following a simple and fast procedure that allows 15 samples to be extracted simultaneously using very small sample volume. Extracts are desorbed in a thermodesorption system (TDS) coupled on-line to a gas chromatograph–mass spectrometry system. The SBSE offers better recovery and linear regression coefficient (r^2) for the four volatile phenols than solid-phase extraction (SPE). The mass spectrometric detection in selected ion monitoring mode contributes to the lower detection limit and good sensibility obtained with this method.

Keywords: Stir bar sorptive extraction; Extraction methods; Wine; Food analysis; Phenols; Volatile organic compounds

1. Introduction

Aroma, in addition to being one of the most important identity signals of a wine, is an indicator of its quality. The organoleptic analysis of wine is extremely useful for enologists, because it can provide information about the quality of grapes employed, the enological practices applied in the production of the wine, and possible diseases or other alterations suffered.

Organoleptic alterations, often described as pricked, medicinal or animal odours in the wine, negatively affect its quality, and they are caused by the presence in the wine of volatile phenols, specifically the ethylphenols (4-ethylphenol and 4-ethylguaiacol) and the vinylphenols (4-vinylphenol and 4-vinylguaiacol) [1]. These compounds are produced by enzymatic decarboxylation and reduction of the cinnamic acids, *trans*-*p*-coumaric and *trans*-ferulic [2,3]. It is known that yeasts of the *Brettanomyces* genus can produce this enzymatic transformation [4,5] and these are responsible for many unpleasant odours detected in beer, cider, red wines and fino sherry wines [5–7].

Volatile phenols are usually analysed by gas chromatography, with a prior extraction treatment of the sample being required. Traditionally, liquid–liquid extraction methods were employed [8,9], but nowadays easier and more selective extraction methods have been applied, such as solid-phase extraction (SPE) [10,11] or solid-phase microextraction (SPME) [12].

Research into new adsorbent materials for SPE and SPME has made these techniques more selective towards the compound studied, so offering cleaner and more concentrated extracts. It allows the analysis to be "tuned" to particular minority compounds in complex samples, such as wine, because clearer chromatograms and lower detection limits can be obtained.

Recently, a new extraction technique for aqueous samples, called stir bar sorptive extraction (SBSE), has appeared [13], in which a magnetic rod encapsulated in a glass jacket and coated with polydimethylsiloxane (PDMS), known as a twister, is employed. The extraction takes

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place while the aqueous sample is being stirred with this rod, for a given time. Later, the stir bar is thermally desorbed on-line with capillary GC–MS. SBSE has been applied to the analysis of contaminants in wine such as pesticides [14], 2,4,6-trichloroanisole [15] and fungicides [16].

In this study, a new method for the analysis of volatile phenols in wine by SBSE, in combination with thermal desorption and on-line capillary GC–MS, is proposed. SBSE has been optimised to obtain selectively the best extraction of volatile phenols with the minimum interference from other substances, thus giving chromatograms that are as clean as possible. Finally, this new method has been applied to the analysis of different types of wine.

2. Experimental

2.1. Reagents, standards and samples

Standards of 4-ethylguaiacol, 4-ethylphenol, 4-vinylguaiacol and 4-vinylphenol were acquired from Sigma–Aldrich (St. Louis, MO, USA). The 3,4-dimethylphenol, employed as internal standard, also was supplied by Sigma–Aldrich. The ethanol, of chromatographic quality, and the tartaric acid were from Merck (Darmstadt, Germany). The water employed was previously purified in a Milli-Q system (Millipore, Bedford, MA, USA).

The method was applied to the analysis of two red wines, a white table wine and two fino sherry wines, all of them supplied by Bodegas Osborne and Cía. (El Puerto de Sta. María, Cádiz, Spain).

2.2. Preparation of the samples

A sample of 25 ml of wine, to which 125 μl of 1000 mg/l solution of 3,4-dimethylphenol had been added as internal standard, was diluted 1:4 with water and an aliquot of 15 ml was poured into a headspace vial of 20 ml. A twister coated with PDMS was stirred in this sample for 60 min at a speed of 900 rpm. After sampling, the twister was rinsed in distilled water and water droplets were removed with tissue paper. For thermal desorption (TD), the stir bar was put into a glass tube of 187 mm length, 6 mm o.d. and 4 mm i.d., that is placed in the tray of the TDS-2.

2.3. Instrumentation and conditions

The analyses were performed using a TDS-2 thermodesorption unit mounted on a 6890 Agilent GC system, which is coupled to an Agilent 5973 mass spectrometric detector (Agilent Technologies, Little Falls, DE, USA).

The analytes were cryofocused in a programmed temperature vaporizing injector (PTV) (CIS-3, Gerstel) at –100 °C with cryogenic nitrogen prior to injection. An empty baffled liner was used in the PTV. Then the analytes were thermally desorbed at the TDS-2 in splitless mode, ramping from 20 to 280 °C at a rate of 60 °C/min, and the upper temperature was held for 5 min.

A split injection (ratio 1:30) was employed by ramping the PTV from 100 to 250 °C at a rate of 600 °C/min. Capillary GC analyses were performed on a DB-WAX column (60 m × 0.25 mm i.d., 0.50 μm film thickness) (Agilent Technologies) with helium as carrier gas at an initial flow of 0.6 ml/min.

The oven temperature program was: 60 °C for 5 min, then raised to 100 °C (held for 10 min) at a rate of 3 °C/min, to 160 °C (held for 30 min) at a rate of 5 °C/min, to 180 °C (held for 80 min) at a rate of 2 °C/min, and to 230 °C (held for 5 min) at a rate of 2 °C/min. The mass spectrometric detection was performed in the selected ion monitoring mode with a dwell time of 100 ms for all compounds. Table 1 shows the retention times and mass fragments of the volatile phenols used.

Table 1
Retention times and MS fragments of volatile phenols using the proposed method

Compound	Retention time (min)	Quantitative fragments (*m*/*z*)
4-Ethylguaiacol	81.62	137 + 152
4-Ethylphenol	98.69	107 + 122
4-Vinylguaiacol	101.65	150 + 135
3,4-Dimethylphenol (I.S.)	105.70	107 + 122
4-Vinylphenol	141.51	120 + 91

2.4. Validation of the method

The calibration curves were prepared for each volatile phenol from a stock solution with the four volatile phenols in ethanol at 1000 mg/l, by dilution in a hydro-alcoholic solution (15% ethanol and 3 g/l tartaric acid) to different concentrations between 0.05 and 1 mg/l for 4-ethylphenol and between 0.5 and 5 mg/l for the other three volatile phenols. The ethanol concentration chosen for calibration curves was 15% because it is the greater level found of this compound in the group of wine samples studied.

These hydro-alcoholic solutions of four volatile phenols were then subjected to the previously described SBSE process, with the prior addition of the internal standard, as performed with any normal sample. Their extracts were desorbed and analysed by GC–MS using the method already explained, and the peak area results obtained were used to construct the calibration curves, representing for each volatile phenol the area relative to the internal standard against the different concentrations.

From each calibration curve, the regression coefficient (r^2), linearity and other analytical characteristics were calculated according to García et al. [17]. The detection limit was determined as the addition of the origin ordenate to three times the standard deviation.

The recovery study was performed by spiking a red wine with the four volatile phenols at different concentrations, the same as those used for the calibration curves.

The repeatability of the method was evaluated by processing five replicates of a red wine, following the SBSE procedure described previously and later thermally desorbed and analyzed by GC–MS.

3. Results and discussion

3.1. Optimisation of the extraction method

This method was optimised by testing several values for all the parameters involved. First, the dilutions of the sample tested were 1:4, 1:10, 2:10 and 5:10. Then, the effect of

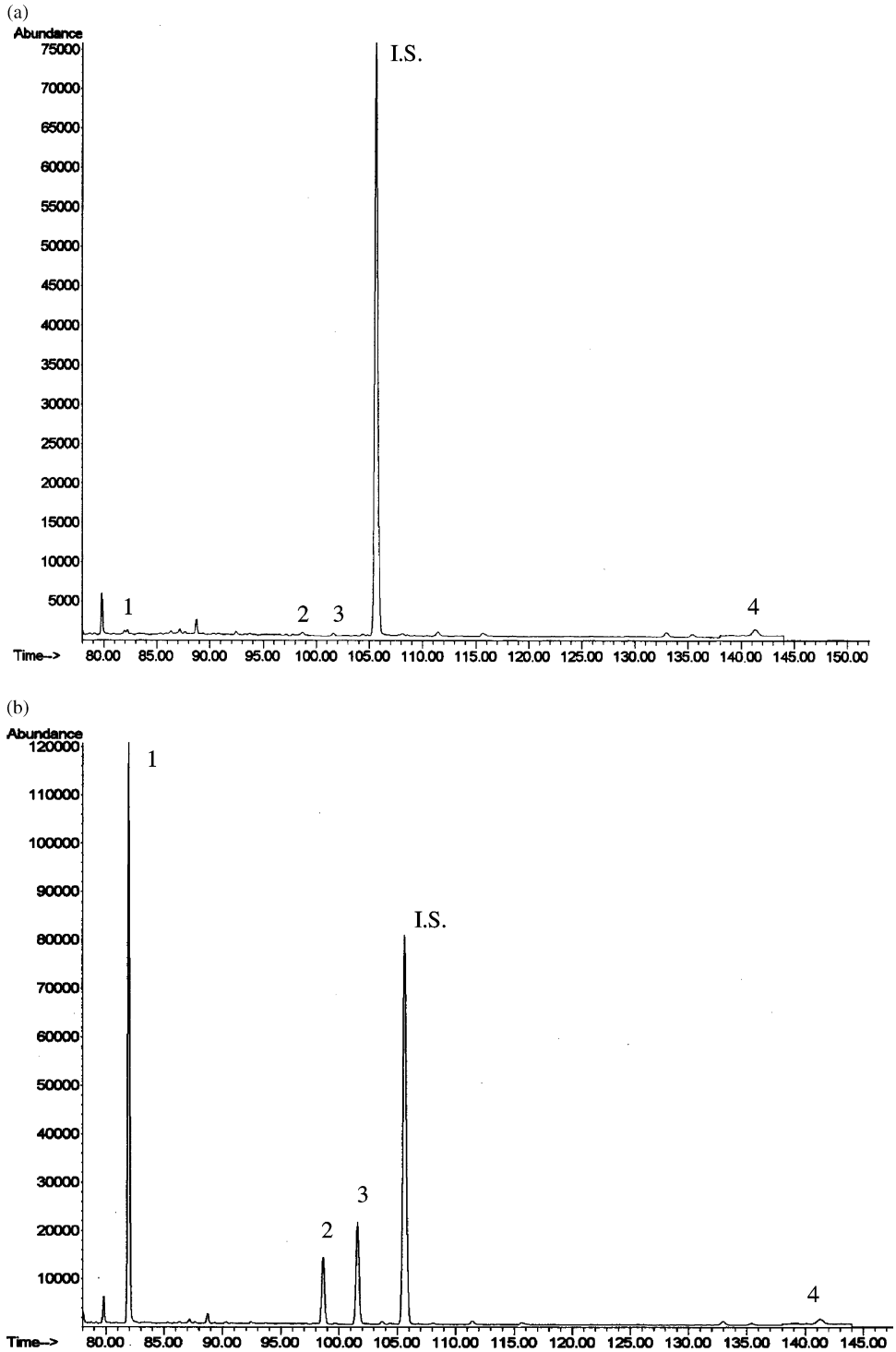

Fig. 1. Chromatograms of the SBSE extracts of (a) a red wine and (b) the same red wine spiked with the four volatile phenols. 1: 4-ethylguaiacol, 2: 4-ethylphenol, 3: 4-vinylguaiacol, 4: 4-vinylphenol. Time scale in min.

Table 2
Analytical characteristics of the method

	4-Ethylguaiacol	4-Ethylphenol	4-Vinylguaiacol	4-Vinylphenol
Concentration range (mg/l)	0.57–5.79	0.05–1.08	0.52–5.19	0.52–5.15
r^2	0.9995	1.00	0.9999	0.9964
Linearity curve (%)	98.40	99.72	99.25	95.77
Detection limit (mg/l)	0.159	0.006	0.067	0.373
Quantitation limit (mg/l)	0.529	0.021	0.223	1.244
Analytical sensitivity	0.0648	0.0024	0.0273	0.1524
R.S.D. (%)	4.67	3.77	–	–
Recovery (%)	97	101	89	89

Table 3
Concentrations (μg/l) of volatile phenols in different types of wine

	4-Ethylguaiacol	4-Ethylphenol	4-Vinylguaiacol	4-Vinylphenol
White wine	42	n.d.[a]	94	367
Red wine-1	50	76	49	730
Red wine-2	65	84	54	4385
Red wine-3	42	7	53	1803
Fino sherry wine-1	263	226	55	41
Fino sherry wine-2	296	232	55	n.d.[a]

[a] n.d.: not detected.

the sample volume used for the extraction was analyzed, with 10, 15 and 20 ml of diluted wine being tested. Another variable considered was the time of extraction, and times of 45, 60 and 180 min were applied. Agitation speeds of 600, 900, 1200 and 1500 rpm, and the effect of ionic force by addition of NaCl (0.10 g) to the sample prior to extraction, are other variables that have been tested.

From all these tests, the optimal values selected for this extraction method were the following: dilution of the sample, 1:4; sample volume used for extraction, 15 ml of diluted wine; time of extraction, 60 min; agitation speed, 900 rpm and no addition of NaCl.

Fig. 1 shows the chromatograms of a red wine extract obtained using this SBSE extraction method under the optimal conditions. The coupling of the extraction technique with gas chromatography and mass spectrometry detection in single ion monitoring (SIM) mode makes this technique a powerful tool for the analysis of particularly complex samples, as is the case with wine. Other valuable features are the need for only a very small quantity of sample, and the possibility of simultaneously performing as many extractions as the number of twisters available. Further, the utilisation of MS detection in the SIM mode makes this an especially selective technique.

3.2. Validation of the analytical method

Table 2 shows the analytical properties of the calibration curves obtained for all the phenols studied. The highest detection limit was 373 μg/l for 4-vinylphenol and the lowest was 6 μg/l for 4-ethylphenol. Therefore the limits of detection LODs were between 10 and 100 times better than the resulting LODs obtained by the authors previously [11]. Moreover, sensitivity was also increased dramatically (Table 2).

It can also be seen in Table 2 that the lowest recovery for spiked phenols was 89%. Hence recoveries were also much higher than those obtained in the method developed previously [11].

The slopes obtained from the standard addition experiment done for the recovery study were very similar to those corresponding to the calibration curves. So, it can be concluded that no matrix effect exists.

The good repeatability of this method can be deduced from the low R.S.D. values of for 4-ethylguaiacol and 4-ethylphenol. None of the vinylphenols were detected in the wine used, so the R.S.D. were not calculated.

3.3. Application to wines

Finally, the new method was applied to wines. A white table wine, three red wines and two sherry wines were analyzed to check the applicability of the method developed to different kind of samples. Table 3 shows the results for these wines. It can be seen that levels of phenols in these samples were extremely low. Specifically, the compound used for detecting the Brettanomyces contamination, i.e. 4-ethylphenol, was found only in very low concentration because the samples selected were all high quality, healthy wines.

4. Conclusions

An easy, fast and reliable analytical method is proposed for the determination of the concentration of volatile phenols

(ethyl- and vinylphenols) in wines. The novel SBSE technique is employed, following a simple and fast procedure that allows 15 samples to be extracted simultaneously using very small sample volume. The mass spectrometric detection in selected ion monitoring mode contributes to the lower detection limit and good sensitivity obtained with this method.

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