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Short communication

Dynamic headspace coupled to perevaporation for the analysis of anisoles in wine by gas chromatography–ion-trap tandem mass spectrometry

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Abstract

Off-flavours in wines are mainly due to the presence of 2,4,6-trichloroanisole and other haloanisoles. The purpose of this study was to develop a method based on the coupling of dynamic headspace and perevaporation to GC–MS–MS to attain better analyte sensitivity and selectivity. The approach has been applied to the analysis of 2,6-dichloroanisole, 2,4,6-trichloroanisole and 2,4,6-tribromoanisole in various wines. For these compounds that cause taste and odour problems, the method was linear from the quantification limit to 3 ng for all the analytes with recoveries greater than 80% and satisfactory precision. Detection limits were as low as 2–36 ng l^{-1}.

Keywords: Anisoles; Wine; Pervaporation; Perevaporation; Headspace; Hygroscopic membrane; Off-flavour; Musty compounds; Carbofrit®

1. Introduction

The majority of cork-related taints are the so-called "musty" taints, principally due to volatile organic compounds such as geosmin and, especially, chloroanisoles, of which 2,4,6-trichloroanisole (TCA) is very well known. TCA is not the only one that produces this effect, but the most commonly off-flavour compound occurring in this type of sample. Some authors reported that TCA was present in 62% of the tainted wines they analysed [1], which affects a significative percentage of the European bottled wines [2], and has important economical consequences.

Several approaches have been proposed for the extraction of anisoles from wine, such as liquid–liquid extraction (LLE) [3] and solid-phase extraction [4]. Solvent-less solid-phase microextraction (SPME) has also been applied to isolate TCA from wines [1,5] and cork [6]. Anticó and co-workers [2] evaluated soxhlet, ultrasound assisted and shake-flask extraction as extraction methods from cork stoppers. More recently, the use of stir bar sorptive extraction (SBSE) [7,8] and pervaporation (PV) [9,10] have been reported for the same purpose.

The approach proposed in the present study, dynamic headspace followed by perevaporation (DHS-PEV), is a hybrid of pervaporation [9,10] and dynamic headspace, since evaporation and gas diffusion through the membrane occur as two separate steps. It represents a modification of the pervaporation manifold on the basis of the distant position and behaviour of the membrane. In PV, evaporation and gas diffusion through the hydrophobic membrane provide a selective transport of the analytes to the analytical instrument. This increases selectivity, and creates simplicity. The DHS-PEV approach consists of three steps (Fig. 1): (i) sample treatment by heating under stirring inside the sealed cell until equilibrium is reached between the liquid and vapour phases, (ii) subsequent on-line analyte transport with an inert gas through a high-pressure injection valve to a hygroscopic perevaporation membrane where the matrix (mainly water) is eliminated, (iii) subsequent transport to a injector packed with Carbofrit® (CPL) and analysis in a GC–MS system.

In this study, the perevaporation membrane is used to eliminate the wine matrix to get a selective introduction of the analytes into the chromatograph. The analytes and sample matrix

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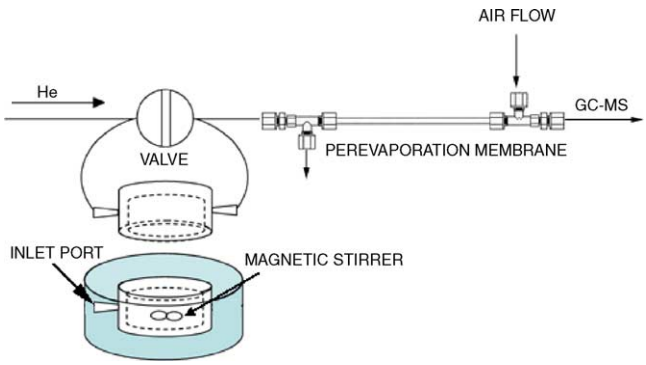

Fig. 1. Scheme of coupling device.

change their passage direction from PV (the analyte passes through the hydrophobic membrane) to DHS-PEV (the matrix passes through the hygroscopic membrane). The use of a high-flow countercurrent auxiliary gas (air) favours complete matrix elimination and prevents the presence of water in the analyte flow, which constitutes a drawback of pervaporation and traditional dynamic headspace.

The proposed DHS-PEV-CPL-GC–MS method has been used for the analysis of 2,6-dichloroanisole (DCA), 2,4,6-trichloroanisole and 2,4,6-tribromoanisole (TBA). Results were compared with those obtained with other methods used to analyse anisoles in wine [9,10].

2. Materials and methods

2.1. Standard solutions and reagents

All reagents and standard solutions used in the present study were described elsewhere [9].

2.2. Instrumentation and procedures

2.2.1. Instrumentation

The instrumental set-up consists of a high-pressure injection valve (Rheodyne, Rohnert Park, CA, USA), a laboratory-made evaporation chamber, a hygroscopic membrane drying tube 30 cm × 1.3 mm o.d. (Perma Pure, Toms River, NJ, USA) and a gas chromatography–ion-trap-mass spectrometer (Varian Ibérica, Barcelona, Spain).

The evaporation module consisted of a lower compartment, where the sample was placed, and an upper compartment in which the carrier gas collected the volatile analytes. The volume of the chambers could be varied by putting spacers between the compartments. The two chambers were aligned using two metallic bars. The whole module was placed between two aluminium supports and four long screws closed the system tightly. This module was used in other studies as a pervaporation chamber [9,10], but in the present study the membrane is removed.

The perevaporation membrane consists of a single Nafion tube housed in a flexible plastic tube shell. Sample gas flows within the Nafion tube while matrix (water) is absorbed into the Nafion membrane tube walls and is removed. An air stream is used as dry purge gas within the shell which flows countercurrent to the sample and removes moisture permeating the membrane. The scheme of the coupling device is shown in Fig. 1.

2.2.2. Analysis of anisoles by DHS-PEV-CPL-GC–MS

A 5-ml aliquot of wine was injected in the evaporation module using a hypodermic needle. Then it was placed in a water bath at 95 °C and mechanically stirred for 15 min. Once a headspace had been created above the sample and phase equilibrium had been reached, the high-pressure valve was switched, with a He stream (60 ml min^{-1}) driving the analytes from the upper chamber to the perevaporation membrane where matrix was eliminated by the air stream (180 ml min^{-1}) surrounding the internal concentric tube which transport the analytes. The perevaporation outlet was directly coupled to the GC injector packed with Carbofrit® and was kept inside the injector port for 5 min. In this time interval, dynamic headspace, perevaporation and preconcentration occur simultaneously.

GC–ion-trap MS–MS and several pervaporation approaches for analysis of anisoles were described elsewhere [9]. Pervaporation outlet was directly coupled to a split–splitless injector with a Carbofrit® packed liner: initial temperature 50 °C ramped to 310 °C at 200 °C min^{-1}. Split valve was opened at 20:1 ratio from initial time to 0.01 min, then closed during 1 min, and finally opened to 1:50 ratio. The analytes were separated in a capillary column with a VF-5 ms stationary phase and dimensions: 30 m × 0.25 mm i.d., 0.25 μm film thickness (Factor Four CPSIL-8, Varian Iberica). The carrier gas was helium at 1 ml min^{-1}. The oven was set at 45 °C for 2 min, subsequently increased to 265 °C at 12 °C min^{-1}, and finally held at 265 °C for 1 min.

The spectrometer operated in MS–MS mode with the following settings: emission current at 80 μA, scan time 0.6 s/scan. The overall run time was splitted in five segments: 80–190 (*m*/*z*) in the second segment (9.00–11.30 min); 90–210 (*m*/*z*) in the third segment (11.30–14.00 min); 120–360 (*m*/*z*) in the fourth segment (14.00–15.80 min) and 80–360 (*m*/*z*) in the fifth segment (15.80–21.33 min). Precursor ions were isolated using 3 amu isolation window and subjected to collision-induced dissociation (CID).

3. Results and discussion

3.1. Optimisation the DHS-PEV-CPL variables

The variables, which have to be optimised in this approach, are sweeping and dry gases flows, temperature, and time for headspace, perevaporation and Carbofrit® preconcentration. The temperature in the module was optimised from 60 to 95 °C, and finally temperature was fixed at 95 °C. This affected a two- to three-fold increase in peak area for the analytes, with the largest effect for TBA. The time needed to

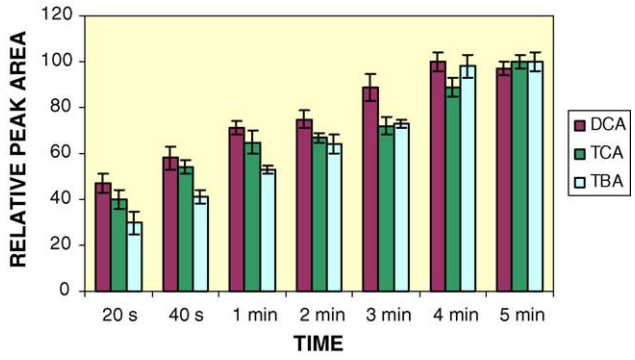

Fig. 2. Optimisation of the preconcentration time in the injector. Values of relative peak area ± standard deviation (%). Relative peak area = (peak area/maximum peak area) × 100.

reach equilibrium between phases is another important parameter which has to be optimised since longer time improves sensitivity. At the optimised temperature, this parameter was fixed at 15 min in order to attain a compromise between sensitivity and time of analysis.

In relation with the carrier gas flow, it was observed that the analytes signals were improved by high values. The He flow rate was varied from 10 to 60 ml min^{-1}, which effected an about two-fold increase of the peak areas for all the analytes. A flow of 60 ml min^{-1} was therefore selected for all further work; this was especially beneficial in the case of DCA.

A preconcentration step based on the use of Carbofrit® into the GC injector port [9] was carried out before the chromatographic run. This device improves the inertness of the injector port referred to the standard quartz wool commonly used. This injection technique increases the sensitivity with a simultaneous reduction of the background noise. The results of the optimisation of this parameter from 20 s to 5 min are collected in Fig. 2; a 5 min time was selected for all further work.

3.2. Performance of the method

When the complete procedure was applied to 10 solutions of DCA, TCA (20 ng l^{-1} each) and TBA (250 ng l^{-1}) in 12% (v/v) ethanol in water, the relative standard deviations (R.S.D.) were 6, 5 and 8% for DCA, TCA and TBA, respectively. Linear calibration curves with R^2 of 0.996–0.999 were obtained from the quantification limit to 3 ng for all analytes. The instrumental detection limits (3σ) were 2, 3 and 36 ng l^{-1} for DCA, TCA and TBA, respectively (confidence level 99.5%). The procedure was applied to 5 ml of wine.

3.3. Analysis of anisoles in wine

Informal sensory analyses were done at the laboratory to detect tainted wines. The results of the analysis of the affected wines by the proposed method are summarised in Table 1.

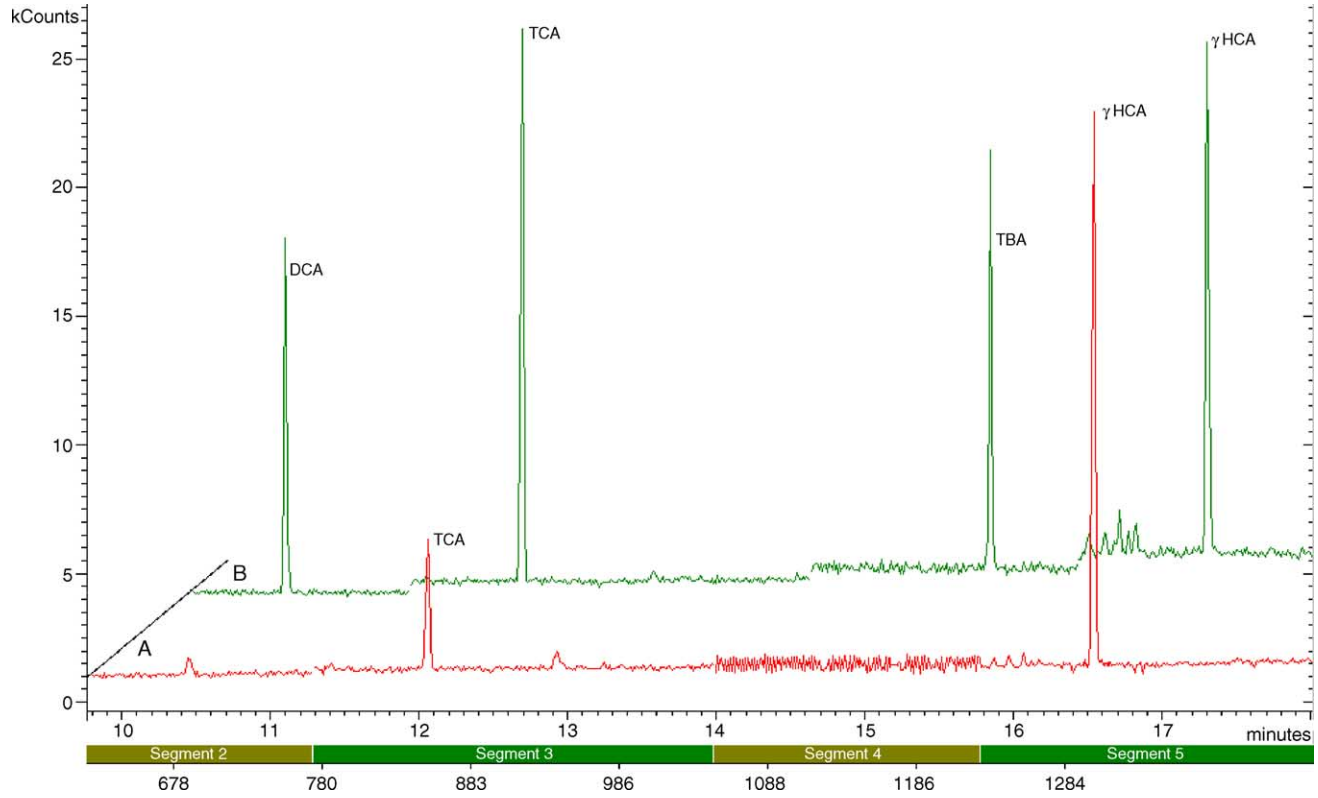

Fig. 3. Chromatograms obtained from an unspiked (A) and spiked (B) wine. Using spikes of 20 ng l^{-1} for DCA and TCA, 250 ng l^{-1} for TBA and 50 ng l^{-1} for the internal standard (lindane, γ-HCA).

Table 1
Recovery of TCA from wine ($n = 3$)

Wine	TCA concentration $\bar{X} \pm \sigma$ (ng l^{-1})	Mean recovery (%) of 20 ng l^{-1} spike
La Mancha (red wine)	10.0 ± 0.7	113
Rioja (red wine)	109 ± 6	116
Rioja (white wine)	28.3 ± 1.3	88
Valdepeñas (red wine)	<DL	98
Condado de Huelva (white wine)	<DL	102

DL: detection limit.

Only some wines contain detectable levels of TCA. DCA and TBA were undetected. Additional recovery trials were done by adding a 20 ng l^{-1} spike of TCA to red and white wines (Table 1). The recoveries ranged from 88 to 116% over a wide concentration range. The averaged recovery in this experiment was 103.4%.

Fig. 3 shows two chromatograms obtained from an unspiked (Fig. 3A) and spiked (Fig. 3B) wine. Using spikes of 20 ng l^{-1} for DCA and TCA, 250 ng l^{-1} for TBA and 50 ng l^{-1} for the internal standard (lindane, γ-HCH).

3.4. Comparison of DHS-PEV-CPL-GC–MS and related techniques

In this section, the DHS-PEV-CPL-GC–MS coupling will be compared with different approaches based on pervaporation which are summed up as follows: (i) pervaporation without preconcentration (PV-GC–MS), (ii) pervaporation with preconcentration carried out into a minicolumn fitted with a sorbent type K (58.8% Carbopack B, 35.3% Carboxem 1000 and 5.9% Carboxem 1001) (PV-CT-TD-GC–MS), and (iii) pervaporation with preconcentration by using a Carbofrit® packed liner into the injector port of the gas chromatograph (PV-CPL-GC–MS). These approaches were previously tested and reported elsewhere [9,10].

Due to the low threshold odour concentrations for these compounds in wine, a preconcentration step is generally required. Detection limits for DHS-PEV-CPL-GC–MS were two-fold lower than those obtained with PV-CT-TD-GC–MS. Using PV-CPL-GC–MS, detection limits were lightly higher. On the other hand, precision (R.S.D. = 2% for TCA) and time of analysis (25 min) for PV-CPL-GC–MS is better than that for DHS-PEV-GC–MS (40 min).

The use of a tubular membrane in the proposed approach represent an innovative aspect that allows better separation of the analytes due to the dynamic character of the dry purge gas flowing countercurrent, which also avoids membrane saturation. In addition, the distant position of the membrane respect to the heater source avoids its damage.

Several drawback can be pointed out in relation to other widely techniques used for anisole isolation from wines. SPME exhibits problem in the recovery associated to the relatively small amount of sorbent available on the fibre [11]. This problem has been solved with SBSE [11]. Table 2 shows comparatively the performance of the proposed methodology and pervaporation versus SPME and SBSE. As it can be deduced SBSE exhibits the best sensitivity (limit of quantification, LOQ = 1 ng l^{-1}), however the results correspond to experiments performed on a synthetic wine and quantification limit are 200-fold higher when this procedure is applied to real samples. Similar results are reached with SPME, although in this case the matrix effect decrease due to the small amount of sorbent in the fibre. The same fact also affects in the precision. The methods based on pervaporation or DHS-PEV involves the elimination of the matrix before the sorption step. As we can see in Table 1, TCA was quantified with the proposed method in a wine at 10 ng l^{-1} and the precision as %R.S.D. in this experiment ranged from 5 to 7% ($n = 3$; calculated from the data in Table 1).

Table 2
Comparison of methods for analysis of TCA in wine

Analytical methodology	QL (ng l^{-1})	R.S.D. (%)	References
SPME-GC–MS	3–18	1.5–13 ($n = 10$)	[1,5,12]
SBSE-GC–MS	1–206	2–4 ($n = 3$)	[7]
DHS-PEV-CPL-GC–MS	10	5 ($n = 10$)	Present work
PV-CPL-GC–MS	18	2 ($n = 10$)	[9]
PV-CT-TD-GC–MS	14	6 ($n = 10$)	[10]

QL: quantification limit; R.S.D.: relative standard deviation.

4. Conclusions

To our knowledge, the method described is the only one that applies perevaporation for direct sample introduction into a gas chromatograph. The results obtained in the present work and in previous reports related to pervaporation, show that these systems are versatile and allow the coupling with different preconcentration devices necessary in sample pretreatment. The proposed method reached satisfactory results for anisoles analysis in wine which have low threshold odour concentrations.

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