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Analysis of anisoles in wines using pervaporation coupled to gas chromatography–mass spectrometry

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Abstract

Two procedures for the determination of 2,4,6-trichloroanisole, 2,6-dichloroanisole and 2,4,6-tribromoanisole in tainted wines have been developed. Both methods are based on pervaporation (PV) of the analytes and final determination by gas chromatography–ion-trap tandem mass spectrometry (GC–MS). In the Approach A, pervaporation was directly coupled to the GC–MS system (PV–GC–MS/MS) and in Approach B a solid-phase cryogenic trap-thermal desorption (CT–TD) device was connected to the pervaporator (PV–CT–TD–GC–MS/MS). Results show that last coupling present better sensitivity as well as precision. Detection limits (DLs) for 2,4,6-trichloroanisole were estimated to be 25.8 and 4.2 ng l^{-1} for Approaches A and B, respectively, when 10 ml of sample was analysed. Linear range of the calibration curves ranged from quantification limit to 15 ng for PV–GC–MS/MS and from quantification limit to 2 ng for PV–CT–TD–GC–MS/MS. Due to the low threshold odour concentration of these compounds in wine, Approach B is proposed as a reliable method for analytical quality control of this product.

Keywords: Anisoles; Wine; Pervaporation; Cork taint; Off-flavour

1. Introduction

Cork taint is the source of mouldy/musty off-odour in affected bottles, which is of great concern to the wine industry. Among the compounds responsible for the taint defect 2,4,6-trichloroanisole (TCA) is one of the principal contributors [1–3]. It has been found that fungi may biosynthesize TCA along with other chloroanisoles as a detoxification mechanism by methylation of chorophenols, which are derived from reactions between lignin breakdown products and chlorinated compounds, such as solutions used to bleach cork, chlorinated biocides and preservatives [1,2]. These substances may migrate into wine from the contaminated cork stoppers. A reasonable estimation of the corked bottles incidence ranged from 2.5 to 5% [4,5], which gives to the problem a great economical importance.

Some authors reported that TCA was present in 62% of the tainted wines they analysed [6]. However, some degradation products such as 2,6-dichloroanisole (DCA) [1] and brominated anisoles like 2,4,6-tribromoanisole (TBA) may also contribute to cork off-flavour. TBA is produced by *O*-methylation of its direct precursor 2,4,6-tribromophenol, which is present in the winery elements or atmosphere [7].

The human sensory threshold for these compounds in wine is in the range of 1.4–10.0 ng l^{-1} [6], concentrations which are beyond the sensitivity of most analytical systems without preconcentration step. Common analytical procedures to detect cork taint compounds, especially TCA, include liquid–liquid extraction [2,3,8] or solid phase extraction with a C_{18} cartridge [9] followed by reconcentration of the extract and direct injection into the standard GC-system. Supercritical fluid extraction (SFE) [10], Soxhlet and ultrasound-assisted extraction have also been used prior to the determination of TCA [2]. Solid-phase microextraction (SPME) appears as an alternative to these techniques, since

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it is a solvent-free technique, which saves preparation time, solvent use and cost [1,6,11–13]. Likewise, stir bar sorptive extraction (SBSE) has also been proposed as an alternative to SPME [14,15]. More recently, relationship between sensory and instrumental analysis of 2,4,6-trichloroanisole has been carried out [16].

Pervaporation (PV), a membrane-based separation technique, constitutes a reliable alternative to headspace analysis for the isolation and preconcentration of volatile compounds before their introduction in an instrumental analytical device for determination, especially gas chromatography [17]. This approach is based on the use of a pervaporation module and a manifold system to recover volatile analytes either from solid or liquid samples. The separation is achieved due to the different vapour pressure of the components introduced into the donor chamber (the lower part) but also by the selectivity of the membrane. Therefore, analytes extraction is integrated in the on-line system. In pervaporation, the analyte undergoes a phase change from solid or liquid to vapour, before permeating through a hydrophobic membrane. The two main joining forces that drive the process, evaporation and gas diffusion, provide a remarkable selectivity as well as simplicity, and the separation without sample–membrane contact avoids membrane clogging or damage [18].

Analytical pervaporation has been proposed for speciation analysis of mercury (Me_2Hg, Et_2Hg, MeHgCl) [17], selenium (Me_2Se, Me_2Se_2, Et_2Se_2) [19] and tin ($MeSnCl_3$, Me_2SnCl_2, Me_3SnCl) [20] in soil and sewage sludge. The technique has also been employed for the analysis of volatile organic compounds [21] and pesticides in soils [22]. In food analysis, pervaporation is a good alternative for monitoring urea and ammonia in wines [23], determination of total and volatile acidity in wines [24], trimethylamine in fish [25] and selective determination of pectinesterase activity in fruits [26]. The versatility of this system allows the coupling with an on-line preconcentration step such as solid-phase cryogenic trap-thermal desorption (PV–CT–TD), which can be performed when necessary. Therefore, PV–CT–TD constitutes a quick, reliable and accurate tool for the analysis of volatile compounds in complex matrices, especially in food. The approach is very useful when it is coupled with some powerful instrumental device compatible with on-line gas–vapour sample introduction, namely gas chromatography.

In the present work, a method based on the use of pervaporation for the analysis of TCA and other anisoles in wine has been developed. The use of CT–TD preconcentration is also considered and compared with single PV. After the isolation of anisoles from wines, gas chromatography with mass spectrometry was used for the determination of these analytes. Main aims were taken on method recovery, accuracy, simplicity and sample throughput, in order to perform quality control monitoring in bottled wine. The optimized method was applied to determine TCA on Spanish tainted wines.

2. Experimental

2.1. Standard solutions and reagents

Milli-Q water (Millipore, Waford, UK) was used for aqueous solutions and intermediate standard solutions. Ethanol absolute (ROMIL-SpS) was obtained from Teknokroma (Barcelona, Spain).

2,6-Dichloroanisole (97%), 2,4,6-trichloroanisole (99%), 2,4,6-tribromoanisole (99%) and lindane (97%) (used as internal standard [27]) were purchased from Aldrich (Steinheim, Germany). Stock solutions were prepared at 514, 512, 514 and 720 $\mu g\,l^{-1}$ in 12% (v/v) ethanol/water for 2,6-dichloroanisole, 2,4,6-trichloroanisole, 2,4,6-tribromoanisole and lindane, respectively. Intermediate solutions were prepared by dissolving appropriate volumes of stocks in 12% (v/v) ethanol/water.

All standard solutions were stored in the dark at 4 °C until analysis.

2.2. Instrumentation

Two couplings were assayed: Approach A, without preconcentration (Fig. 1) and Approach B, with a preconcentration step (Fig. 2). The instrumental coupling for anisoles analysis consisted of a high-pressure injection valve (Rheodyne, USA), the unit of pervaporation (home-made device), a gas chromatography–mass spectrometry (GC–MS) system (Varian Iberica, Barcelona, Spain) and a purge and trap system for preconcentration.

The pervaporation device consists of two chambers: a lower compartment where the sample is placed and an upper compartment where a carrier gas collects the volatile analytes. Both modules are separated by a hydrophobic membrane (PTFE membrane, 1.5 mm thick, 40 mm diameter, Trace Biotech AG, Braunschweig, Germany) placed on a support. The volume of the lower chamber can be selected by

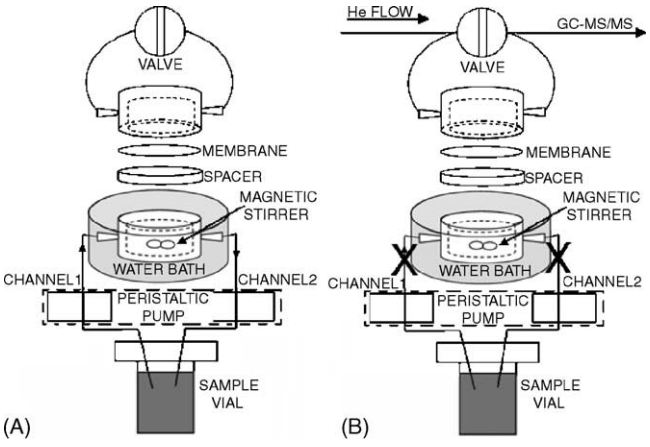

Fig. 1. Pervaporation home-made device without preconcentration step. (A) Recirculation using the peristaltic pump and pervaporation. (B) Analytes sweeping to the GC–MS. In this later stage, the peristaltic pump is stopped which closes both inlet and outlet of the lower chamber.

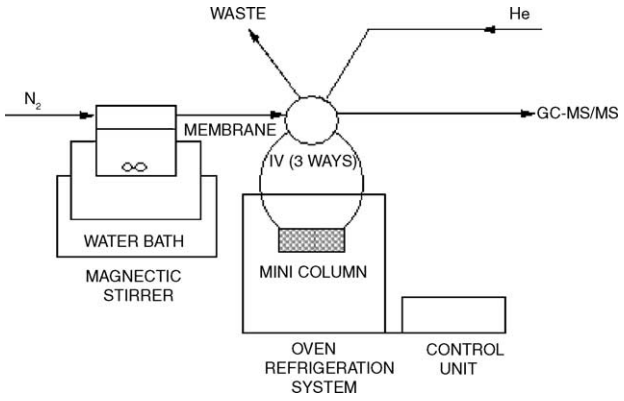

Fig. 2. Scheme of instrumental coupling with preconcentration approach.

putting spacers between the membrane support and the corresponding compartment. The two chambers were aligned with the membrane support using two metallic bars. The whole module was placed between two aluminium supports and four long screws to close the system tightly. A Minipuls-3 peristaltic pump (Gilson, France) was used for liquid sample introduction.

The purge and trap system for preconcentration was fitted with different sorbents. Type B (65.2% Tenax, 34.8% and Silica Gel), type E (4% Chromosorb, 32% Tenax, 32% Silica Gel and 32% Charcoal), type G (4.1% Chromosorb and 95.9% Tenax) and type K (58.8% Carbopack B, 35.3% Carboxem 1000 and 5.9% Carboxem 1001) sorbents from Supelco (Bellefonte, PA, USA), were assayed to retain the analytes.

Volatile compounds were analysed using a Varian Model 3800 gas chromatograph paired with a Saturn 2000 ion-trap mass spectrometry detector (Varian, Sunnyvale, CA, USA). The gas chromatograph was fitted with a fused-silica 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).

2.3. Procedures

2.3.1. Chromatographic analysis

The carrier gas was helium at a flow-rate of 1 ml min^{-1}. Pervaporation outlet was directly coupled to a split–splitless injector, operated in the splitless mode (splitless time: 60 s) at 260 °C. The temperature of the GC–MS transfer line was 280 °C. The oven temperature program started at 45 °C for 2 min, subsequently increased to 265 °C at 12 °C min^{-1} and finally hold at 265 °C for 1 min.

Full scan electron impact ionization data were acquired under the following conditions: solvent delay 9 min, 70 eV electron impact energy, emission current 30 μA, scan time 1 s $scan^{-1}$, manifold and trap temperatures 50 and 200 °C, respectively. The automatic gain control was switched on with a target fixed at 20000 counts. The overall run time consisted of 9 min of delay and one segment from 9 to 21.33 min scanning the following range (*m*/*z*): 90–400.

Table 1
MS/MS parameters for the analysis of anisoles

Anisole compound	Precursor ion	Product ion	CID parameters	
			Store level (*m*/*z*)	Amplitude (V)
2,6-Dichloroanisole	176	133	80	69
2,4,6-Trichloroanisole	195	167	90	78
2,4,6-Tribromoanisole	344	329 + 301	120	97
Lindane	183	148 + 146 + 109	80	72

2.3.1.1. MS–MS mode. In order to improve sensitivity and selectivity, the option MS–MS was used. When the mass detector is operated in MS–MS mode, emission current was fixed at 80 μA and scan time 0.6 s/scan. To program the isolation of precursor ions for every compounds along the chromatographic run, the overall run time was split into five segments scanning the following ranges (*m*/*z*): 80–190 in the second segment (9.00–11.30 min); 90–210 in the third segment (11.30–14.00 min); 120–360 in the fourth segment (14.00–15.80 min) and 80–360 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). MS–MS parameters are shown in Table 1. Automated method development toolkit software was used to optimise the CID parameters (low mass cutoff and CID voltage) to obtain maximum sensitivity. The excitation storage level was selected at the minimum value that allowed the dissociation of the precursor ion. High CID energies were required due to the stable nature of the selected precursor ions.

2.3.2. Pervaporation and analysis without solid-phase preconcentration (Approach A)

A peristaltic pump was used for liquid sample introduction into the pervaporation device. 9 ml of red or white wine were placed in a 10 ml glass vial sealed with a silicone septum. An additional aliquot of 1 ml of wine was introduced in the lower chamber of the pervaporation module. A spacer was placed below the membrane in order to create a headspace above the liquid sample. Two Teflon tubes were connected to the sample containing vial through the septum. One of them, was attached to the inlet port of the pervaporation device (lower chamber) and the other one, was connected to the outlet port to allow the recirculation of the sample. Sample was flushed through the tubes by the peristaltic pump at 5 ml min^{-1}. Pervaporation device was placed in a water bath at 85 °C and sample was submitted to pervaporation process for 5 min. Then, the peristaltic pump was stopped which closes both the inlet and outlet of the lower chamber. Finally, the high-pressure valve was switched and a He stream (60 ml min^{-1}) drove the pervaporated analytes to the chromatograph. A scheme of this approach is shown in Fig. 1.

2.3.3. Pervaporation and analysis with solid-phase preconcentration (Approach B)

A U-shaped stainless steel minicolumn (25 cm length × 1 mm i.d.) packed with sorbent type K was placed in an oven–refrigeration system connected to the injection valve. After sample pervaporation and recirculation, nitrogen was used as carrier gas to sweep the analytes to the minicolumn at a flow rate of 60 ml min^{-1} during 5 min. The minicolumn was immersed in an ice water bath (about 0 °C) for sorption. In the desorption step, the minicolumn was placed in an oven at 210 °C and a helium stream at 80 ml min^{-1} swept the analytes to the gas chromatograph. A scheme of this approach is shown in Fig. 2.

3. Results and discussion

3.1. Optimization of pervaporation variables

3.1.1. Approach A (without solid-phase preconcentration)

The most important variable affecting pervaporation process is the temperature, which enhances the releasing of the compounds from the matrix when it is increased. This fact also enhances sensitivity. Table 2 shows the relative peak areas of the compounds under study when the temperature was assayed from 60 to 90 °C. The highest signal for most analytes were obtained at 85 °C, and the precision expressed as standard deviation for this temperature was also better, for this reason this value was selected as optimum for further experiments.

The preconcentration time of the analytes in the static gas volume of the headspace was also optimized. Longer time had a positive effect on analytes pervaporation from matrix. Pervaporation time was studied from 3 to 15 min at a constant flow rate of 5 ml min^{-1} in the lower chamber. The analytical signal increased from 3 to 5 min but levelled off for longer intervals. Therefore, 5 min was chosen as optimum.

Table 2
Optimization of temperature and sweeping gas flow (Approach A), values of relative area[a] ± S.D.

	DCA	TCA	TBA	γ-HCH
T (°C)				
60	67 ± 3.6	58 ± 5.3	45 ± 5.1	48 ± 2.1
65	75 ± 2.3	67 ± 4.1	58 ± 4.7	61 ± 2.5
70	89 ± 2.7	69 ± 3.8	71 ± 4.5	63 ± 1.3
75	99 ± 3.6	84 ± 2.9	82 ± 5.2	82 ± 3.5
80	100 ± 2.2	98 ± 2.1	97 ± 3.9	95 ± 2.6
85	98 ± 3.8	100 ± 1.0	100 ± 2.1	100 ± 1.2
90	90 ± 6.6	97 ± 5.2	96 ± 5.0	99 ± 4.4
He flow (ml min^{-1})				
10	62 ± 3.2	57 ± 2.3	46 ± 2.5	48 ± 4.9
20	85 ± 4.1	68 ± 3.5	54 ± 2.2	63 ± 5.1
30	83 ± 3.7	75 ± 2.1	59 ± 2.8	83 ± 6.9
40	97 ± 5.6	71 ± 3.2	57 ± 3.5	80 ± 2.8
50	99 ± 5.3	86 ± 5.2	92 ±4.6	99 ± 3.5
60	100 ± 3.2	96 ± 2.5	100 ± 3.8	100 ± 3.3
70	98 ± 4.6	100 ± 3.1	99 ± 4.2	97 ± 3.7

[a] Relative peak area = (peak area/maximun peak area) × 100.

Table 3
Comparison of recoveries with different sorbents (CT–TD)

Sorbent	Recovery (%)		
	TCA	DCA	TBA
Type B	63.2	32.5	76.9
Type E	35.4	<DL	40.6
Type G	26.1	<DL	54.7
Type K	100.6	97.2	99.4

DL: detection limit.

In order to improve sensitivity, large volumes of sample (10 ml) were introduced in the pervaporation device using a peristaltic pump. The sample flow rate was optimised in the range 2–5 ml min^{-1}. Amount of 2 ml min^{-1} is the lowest flow that allows the whole sample volume to pass one time through the lower chamber. When flow rate higher than 5 ml min^{-1} were used, sample splashes the membrane surface. Therefore, 5 ml min^{-1} was chosen as the optimum since it allowed the sample to pass two and a half times through the chamber, maintaining the dynamic equilibrium.

The use of a high carrier gas flow (He) improves the signal due to the higher amount of analyte introduced in the chromatograph. Helium flow values ranging from 10 to 70 ml min^{-1} were tested (Table 2). The best results of peak areas were obtained from 60 to 70 ml min^{-1} (at atmospheric pressure) with no important differences. Consequently, 60 ml min^{-1} was chosen in further experiments. Higher flow rates were not assayed due to the limitations of the chromatograph injector dynamic for higher flow values.

3.1.2. Approach B (with solid-phase preconcentration)

The key variables of this coupling are similar to that optimised in Approach A, but others such as type of sorbent for the retention of the anisoles, preconcentration unit temperature for retention and desorption, and carrier gas flow for sorption (N_2) and desorption (He), have to be considered.

Several types of sorbents were studied for analytes (Types E, G, B and K) in a preliminary study (Table 3). Type K was chosen since it was the only one that provided quantitative sorption and subsequent desorption of the analytes without peak tailing. The minicolumn was prepared in stainless steel tubing and placed in the loop of the injection valve. First of all, sample was submitted to pervaporation with recirculation for 5 min at 85 °C. After that, and when the peristaltic pump had been stopped, the preconcentration step was carried out. In the load position of the valve, a nitrogen stream (60 ml min^{-1}) passed through the pervaporation device and drove the analytes to the sorbent trap (in clockwise direction). The non-retained compounds from the matrix were driven to waste. Longer sweeping time increases the amount of analytes loaded in the trap due to the additional effect of the carrier gas on the volume of sample contained in the lower chamber when the pump is stopped. Finally, sweeping time was fixed at 5 min as a compromise between sample

Table 4
Optimization of temperature in the preconcentration step (Approach B), values of relative area[a] ± S.D.

T (°C)	DCA	TCA	TBA	γ-HCH
25	65 ± 6.5	76 ± 6.5	75 ± 3.8	70 ± 4.1
0	98 ± 4.2	100 ± 4.6	100 ± 6.1	100 ± 5.5
−50	100 ± 3.2	99 ± 5.1	95 ± 4.6	97 ± 4.3

[a] Relative peak area = (peak area/maximum peak area) × 100.

throughput and sensitivity. The flow control was performed with a needle valve placed in the tubing system, which regulated the gas flow from the nitrogen cylinder. In order to optimise the retention temperature in the preconcentration minicolumn, three different cryogenic mixtures were assayed (Table 4): dry ice–ethanol (about −50 °C), ice–water (about 0 °C) and room temperature (about 25 °C). The results were unaffected by the trap temperature in the first two cases, in which the same peak areas were obtained. When preconcentration was performed at room temperature peaks areas were lower. Finally, the trap temperature was fixed at 0 °C. In this position of the valve, helium flow passed directly to GC–MS.

In the inject position of the valve, the helium stream passed through the sorbent minicolumn in a counterclockwise direction driving analytes to the GC–MS. The flow was optimised from 70 to 90 ml min^{-1} (measured at the minicolumn outlet) and finally, 80 ml min^{-1} was selected. Desorption temperature in the minicolumn was also considered and better recoveries for all the analytes were observed when it was increased. This parameter was ranged from 150 to 250 °C, and optimum results were achieved at 210 °C that was chosen for further experiments. Recoveries at this temperature were in the order of 100% for all the analytes, which was confirmed by repeating the desorption process to assure that the second run provided a blank signal. A desorption/injection time of 5 s was enough to desorb all the analytes quantitatively, and it allowed all the desorbed compounds to be flushed from the loop to the chromatographic column with the selected helium flow rate. The chromatogram obtained using optimum conditions is shown in Fig. 3.

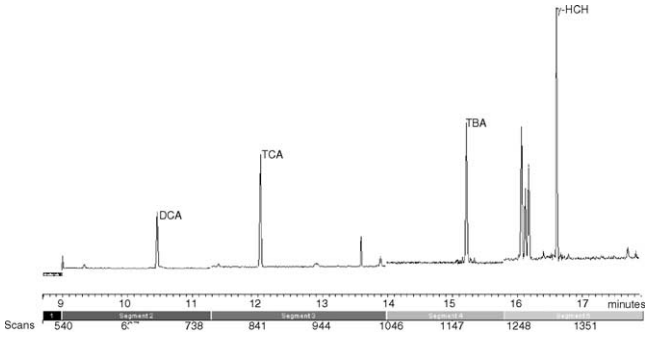

Fig. 3. Chromatogram obtained from red wine spiked with 405.5, 404.9, 405.9 and 563.3 pg of DCA, TCA, TBA and lindane, respectively. The retention times were 10.5, 12.1, 15.2 and 16.6 min, respectively (Approach B).

Table 5
Features of Approaches A and B

Analyte	DL (pg)	%R.S.D.	R^2
Approach A			
DCA	409	11.9	0.995
TCA	258	6.2	0.996
TBA	183	8.5	0.998
Approach B			
DCA	38.2	8.5	0.997
TCA	42.0	6.3	0.999
TBA	45.7	4.7	0.998

DL: detection limit; R.S.D.: relative standard deviation; R^2: coefficient of determination.

3.2. Methods performance, validation and comparison

Results obtained with both approaches are summarized in Table 5. For both procedures the relative standard deviation (%R.S.D.) was obtained for 10 sequential injections of the analytes and the internal standard at 500 ng/l in 12% (v/v) ethanol/water mixture. Linear calibration curves were obtained from quantification limits to 5 ng (Approach A) and 2 ng (Approach B) for all the analytes.

3.2.1. Methods comparison

Two experimental approaches based on the use of pervaporation have been tested in the analysis of anisoles from 12% (v/v) ethanol/water solutions. Pervaporation is a suitable pretreatment for volatile analytes, such as anisoles, which can be quantitatively separated from solid or liquid matrices. When low levels of analytes have to be quantified, a preconcentration step is mandatory after PV extraction to make possible the analysis. In this work, PV and PV–CT–TD have been tested and compared. Table 5 shows the analytical features for these methods when they are applied to the analysis of anisoles from ethanol/water solutions. Detection limits are markedly reduced when the preconcentration step is performed. Pervaporation shows lower sensitivity, especially for DCA, with detection limit about 10-fold higher than that for PV–CT–TD. For TCA using PV, the detection limit is about six-fold higher than that for PV–CT–TD. Consequently, the use of preconcentration is necessary for the analysis of anisoles in tainted wine due to their low threshold odour concentrations.

3.3. Application to anisoles analysis in wine

Applicability of Approach B to evaluate the anisoles level in natural tainted wines has been tested. Occurrence of taint defect was detected by sensory trial. Additional recovery studies were carried out in these samples after TCA spike. Table 6 shows the results obtained, with standard deviations ranging from 1.1 to 4.0 ng/l. The averaged recovery in the spike experiments was 102.2% over a wide concentration range.

Table 6
Recovery trial for TCA spiked into "tainted" wines (Approach B)

Wine sample	Initial measured concentration of TCA[a], $\overline{X} \pm \sigma$ (ng l^{-1})	Measured concentration after 20 ng/l spike[a], $\overline{X} \pm \sigma$ (ng l^{-1})	Mean recovery of spike (%)
Navarra (red wine)	<DL	21.4 ± 1.4	107
Rioja (red wine)	59.4 ± 3.8	80.1 ± 4.0	104
Rioja (white wine)	38.2 ± 2.4	55.3 ± 3.2	86
Jumilla (red wine)	17.4 ± 1.1	36.8 ± 2.2	97
Condado de Huelva (white wine)	<DL	23.6 ± 1.5	118

DL: detection limit.
[a] Average of three replicates.

4. Conclusions

The coupling of the pervaporation cell to the chromatographic system works similarly to a headspace device; however, noteworthy advantages are obtained in comparison with both static and dynamic headspace sampling. The small air gap into the lower chamber requires very small amounts of analytes to reach the equilibrium with the vapour phase; therefore a short equilibration time is necessary. A further advantage is represented by the PTFE membrane, which avoids the passage of water vapour from this type of matrices to the chromatograph. This fact constitutes a common drawback when headspace sampling is used.

Pervaporation is a useful alternative to the existing methods for the analysis of TCA and other anisoles in wine. Generally, on-line preconcentration is necessary due to their low threshold odour concentrations in wines. The combination of PV with a cryogenic trap-thermal desorption device fitted to the GC–MS, provides a sensitive and precise method for the analysis of TCA in these matrices. Therefore, PV–CT–TD can be used for routine analysis of TCA in wines to monitor the incidence and causes of cork taint. A drawback of PV–CT–TD–GC–MS/MS is the limited sample throughput, therefore, forthcoming studies based on the use of faster preconcentration devices such as packed GC liners connected to the pervaporation cell will be carried out.

On the other hand, quantification limits are comparable with those reported in the literature for solid-phase microextraction using either mass spectrometry or electron capture detector, which range from 2.9 to 18 ng l^{-1} for TCA [6,11,12].

Precision of PV–CT–TD for TCA is 6.3% (expressed as %R.S.D.), which compares favourably with that of SPME, established around 10% [6,11,13], at the same level. Finally, the method proposed (PV–CT–TD–GC–MS/MS) shows good linearity over a wide range of concentrations (from the quantification limit to 200 ng l^{-1} for TCA, when the procedure is applied to 10 ml of wine) with a coefficient of determination (R^2) of 0.999. These values are similar to those reported in the literature for the analysis of TCA in wine using SPME coupled to GC–MS [6].

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