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Journal of Chromatography A, 1061 (2004) 85–91
JOURNAL OF CHROMATOGRAPHY A
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Purge-and-trap preconcentration system coupled to capillary gas chromatography with atomic emission detection for 2,4,6-trichloroanisole determination in cork stoppers and wines

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Received 12 July 2004; received in revised form 28 October 2004; accepted 4 November 2004

Abstract

A method based on solvent extraction and purge-and-trap capillary gas chromatography with atomic emission detection (PT-GC–AED) for the determination of 2,4,6-trichloroanisole (TCA) in wines and cork stoppers was optimized and evaluated. TCA was previously extracted from the samples in pentane and the preconcentrated extract was reconstituted in water before being injected into the chromatograph by means of the PT system. Element-specific detection and quantification was carried out by monitoring the chlorine (479 nm) emission line. Two different calibration graphs were used to quantify TCA in the cork or the wine samples, owing to the interference produced by the ethanol content in the wines. Detection limits of 25 pg g^{-1} and 5 ng l^{-1} were obtained for corks and wines, respectively. The method provided recoveries from spiked samples ranging from 88.5 to 102.3%, confirming the reliability of the procedure and its suitability for routine monitoring.
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Keywords: 2,4,6-Trichloroanisole; Cork; Wine; Purge-and-trap; Gas chromatography–atomic emission detection

1. Introduction

Cork taint is an organoleptic defect in wines. The determination of 2,4,6-trichloroanisole (TCA), which was first reported to be the main compound responsible for the cork taint [1], is an important task in the wine industry, since TCA has a great influence on the acceptance or rejection of wines by the consumer. Indeed, this off-odor problem [2–4] causes economic losses every year in the wine industry. Although olfactory and taste thresholds for TCA range from 0.03 to 50 ng l^{-1} [5–7], depending on the age and the variety of the wine and the sensitivity and training of the judge, the TCA concentration considered as a defect in wine ranges from 10 to 40 ng l^{-1} [7,8].

The source of TCA in wines is usually related to the cork stoppers, where it is synthesised by several microorganisms as a detoxification mechanism in order to remove chlorophenols from their environment [9]. A portion of the TCA present in the cork can contaminate wine [8]. Nevertheless, the TCA found in wines may also come from other sources, such as fungicides, biocides, herbicides, wood preservatives containing 2,4,6-trichlorophenol in the wood used to make barrels or from the use of hypochlorite as a cork bleaching agent [10,11]. In all cases, the fungal methylation of chlorophenols is implied.

Electronic “noses” such as headspace-mass spectrometry (HS-MS) systems, have been used for determining TCA in wines, the detection limits thus achieved being higher than the sensory threshold [12]. Gas chromatography with mass spectrometry (GC–MS) under selected ion monitoring conditions [4–6,10,13–20] and electron-capture detection (GC–ECD) [4,7,11,21] are the most commonly used techniques for this

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doi:10.1016/j.chroma.2004.11.005

purpose, since they offer low detection limits. As far as we know, gas chromatography with atomic emission detection (AED) has not been yet used for determining TCA in wines and cork stoppers. AED is a selective and sensitive detection method and is easier to operate than mass spectrometry methods, and the resulting chromatograms can be interpreted by semi-skilled analysts. On the contrary, a disadvantage of AED is the high cost of maintenance.

The literature reports different extraction and preconcentration techniques used in the analysis of TCA in wines and cork stoppers, such as the classical solvent extraction [4,13,20,21], solid phase extraction (SPE) [16], solid phase microextraction (SPME) [5,6,17], headspace solid-phase microextraction (HS-SPME) [7,11], stir bar sorptive extraction (SBSE) [15,18,19] and supercritical fluid extraction (SFE) [10]. Solvent extraction is the most practical way for industrial use [21], since it is within the possibilities of most laboratories because of its simplicity. Nevertheless, when solvent extraction is applied, a clean-up step [3,13,21] is usually necessary prior to GC analysis, although such a step can be avoided by preconcentrating by means of a purge-and-trap (PT) system. Problems arising from the extraction and preconcentration steps and the lack of reference materials add importance to the search for new methods to determine TCA in wine and corks. The procedure here proposed involves the extraction of TCA using a solvent extraction with pentane, which can be done at minimum cost and with very simple sample manipulation. The analyte is then concentrated with a PT system.

2. Experimental

2.1. Chemicals

2,4,6-Trichloroanisole (99%), in solid crystal form, was supplied by Aldrich (Steinheim, Germany). A 1000 mg l^{-1} standard solution was prepared in methanol (LabScan, Dublin, Ireland) and kept at −20 °C, at which temperature it remains stable for 4 years. Working standard solutions were prepared daily and stored at 4 °C in the refrigerator. Pentane was obtained from LabScan. Sulphuric acid (95–97%, m/v) and sodium chloride were provided by Merck (Darmstadt, Germany) and Panreac (Barcelona, Spain), respectively.

The plasma gas and carrier gas used for GC was helium. The reagent gas for the AED was oxygen. Nitrogen was used for purging the AED system. All the gases were supplied by Air Liquide (Madrid, Spain).

2.2. Instrumentation

The purge-and-trap sample enrichment system was a Tekmar Dohrmann 3100 model (Agilent, Waldbronn, Germany) controlled by Teklink (2.02 Version) software. The purging vessel was a 5 ml glass U-tube with frit sparger 1/2 in. top fit. This was rinsed with the sample before each experiment, and further rinsed with deionized Milli-Q water after each analysis. The purge vessel was thermostated at 25 °C, using a lab-made system. The analyte was purged out from 5 ml of aqueous solution with helium at a flow-rate of 40 ml min^{-1} for 10 min and maintaining the trap temperature at 35 °C. The TCA was directed to a trap column (30.5 cm × 0.312 cm o.d.) coated with Carbopak B, Carboxen 1000 and Carboxen 1001. The purge-and-trap system included a moisture control module (MCM). After concentration, the TCA was desorbed from the trap at 240 °C by opening the valves for 5 min. Once the analyte had been desorbed, a bake step was programmed at 260 °C for 8 min, to avoid possible memory effects. The purge-and-trap system was directly coupled to the gas chromatograph in a direct split interface (DSI) configuration (split ratio, 2.6:1), set at 250 °C in order to avoid analyte condensation during analysis. The end of the transfer line was directly inserted into the split injector of the GC.

An Agilent 6890 gas chromatograph was directly coupled by a transfer line to a G2350A microwave-induced plasma atomic emission detector (Agilent). Updated G2070AA ChemStation application with the G2360AA GC–AED software was used to control and automate many features of the GC and AED systems, and for data acquisition and treatment. The chromatograph was fitted with a 30 m × 0.32 mm i.d. HP-5, 5% phenylmethyl polysiloxane capillary column from Agilent with a 0.25 μm film thickness. The initial column temperature was set at 45 °C for 4 min, increasing to 150 °C at 30 °C min^{-1} and holding for 1 min. In this way, TCA was eluted with a retention time of 7.8 min. Helium was used as the carrier gas at 4 ml min^{-1}, in the constant-flow mode. The helium make-up flow-rate was set at 40 ml min^{-1}, which was measured with the window purge gas flow on. Solvent venting was switched on immediately after starting the desorption step in the purge-and-trap system and switched off 6 min later. The spectrometer was purged with nitrogen at a flow rate of 2.5 l min^{-1}. Oxygen at 20 psi was used as reagent gas. Filter and backamount adjustment in the AED were set according to Agilent default specifications. The element analyzed and its emission line was chlorine at 479.45 nm.

An IKA A11 grinder (IKA, Staufen, Germany) and an ultrasonic probe processor UP 200H (Dr. Hielscher, Germany) were used for treating the cork samples. A Büchi vacuum V-500 rotatory evaporator R-200 coupled to a Büchi heating bath B-490 (Switzerland) was used to concentrate the sample extracts.

2.3. Samples and extraction procedures

A series of red, white and rosé wines were obtained from commercial sources, to make up a total of 15 different samples. Wine samples were stored in sterile glass jars at 4 °C until analysis. The corresponding cork stoppers were separately ground, weighed and placed in a 50 ml polyethylene closed flask before storing at −20 °C until analysis. The cork samples were agglomerates, which have been proved to release some of their endogenous TCA into the wines to a greater ex-

tent than natural corks [16]. For the optimization procedure unused cork stoppers were purchased.

Forty milliliters of pentane were added to the ground cork and the mixture was sonicated for 2 min (60% amplitude) by means of a probe directly immersed into the solution. The supernatant was filtered through filter paper and the sample residue submitted to a second extraction stage by adding 40 ml of pentane and sonicating for 1 min. The combined filtered extracts obtained were concentrated to almost dryness using a rotatory vacuum evaporator at 25 °C and 700 mbar. The extract obtained was reconstituted in 10 ml of an aqueous solution containing 3 M sodium chloride and 0.15 M sulphuric acid, 5 ml of which was submitted to the purge-and-trap system.

For wines, a sample of 25 ml was extracted twice with 10 ml of pentane by manual shaking for 10 min in a separation funnel. The two organic phases were separated from the sample using a phase separator paper (Albet 302 SF, 15 cm diameter). The organic phases were collected in a flask and concentrated almost to dryness in the rotatory vacuum evaporator, after which the residue obtained was treated in the same way as the cork samples. In both cases (corks and wines), extreme care was taken to prevent the extracts from completely drying out as TCA is easily lost.

2.4. Recovery assays

Since no reference materials are available for the validation of the method, spiked samples were prepared. Ten unused cork stoppers, preliminary submitted to the entire extraction and analysis procedure in order to verify the absence of TCA, were used as blank matrix. TCA was added in the 0.75–5 ng g^{-1} concentration range. Samples were vigorously shaken to homogenize the mixture and then submitted to the extraction procedure described above. Three replicates were analyzed at each fortification level.

For wine samples, 100 μl volumes of methanolic standard solutions were added to 25 ml of the sample, the spike ranging between 50 and 250 ng l^{-1}. The fortification procedure was applied to three different wine samples at four concentration levels and three replicates were analyzed in each case.

3. Results and discussion

3.1. Optimization of the method

3.1.1. Chromatographic and AED parameters

Because only one analyte was considered, a high helium flow rate of 4 ml min^{-1} could be used. Flow-rate values higher than that selected are not recommended because problems in the stationary phase begin to appear. Under the selected temperature program, TCA was eluted once the desorption time had finished, without overlapping with the water peak. No effect was observed on the signal when the injection temperature was varied between 240 and 300 °C, and so 250 °C was selected.

Once the PT and chromatographic parameters had been optimized, the detector operating conditions were studied to obtain the highest degree of sensitivity. Oxygen was used since it prevents the accumulation of carbonaceous residues on the wall of the discharge tube. When the oxygen pressure was varied between 15 and 30 psi, a slight decrease in sensitivity was observed. A 20 psi pressure was adopted, lower pressures being not recommended because of the above mentioned carbonaceous deposition effect. The helium make-up gas flow was varied between 30 and 60 ml min^{-1}. Maximum peak areas were obtained between 30 and 40 ml min^{-1}, while sensitivity decreased for at higher flow-rates. The selected value was, 40 ml min^{-1} which provided the maximum peak height and lowest peak width. The transfer line and the cavity temperatures were set at 250 °C, following the manufacturer's recommendations of using similar temperatures for both. No differences were observed in varying these temperatures between 225 and 285 °C.

Fig. 1A shows the chromatogram obtained for a standard solution of TCA under the selected conditions.

3.1.2. Optimization of the purge-and-trap system

The evaluation of the effectiveness of the PT system was made by comparing peak areas obtained by either PT

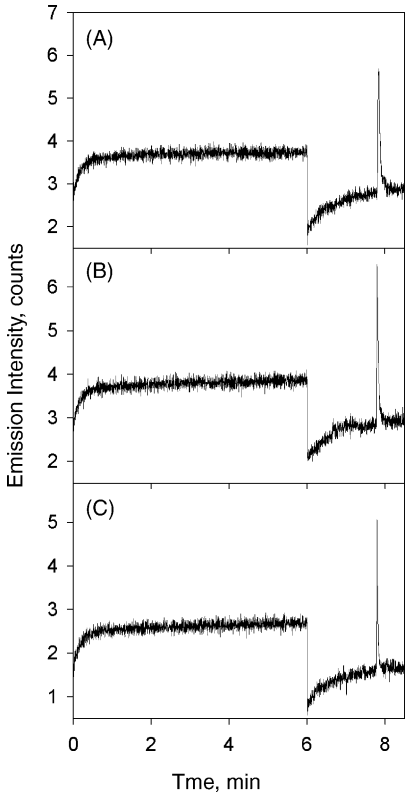

Fig. 1. PT-GC–AED chromatograms obtained from: (A) an aqueous standard solution of TCA (0.25 ng ml^{-1}) in the presence of sulphuric acid and sodium chloride in the selected concentrations; (B) a spiked cork stopper sample (0.7 ng g^{-1}) and (C) a spiked red wine sample (100 ng l^{-1}).

injections or direct injections of standard solutions (considering the same mass of compound injected in the column in both cases). All experiments were carried out in duplicate.

Since the partitioning of volatile substances, such as TCA, between liquid and gas phases is governed by the compound's volatility and solubility [7], the effect of sodium chloride, as a salting-out agent, on the purging step was checked, fixing the instrumental PT parameters at 10 min purge time, 35 °C purging temperature, 6 min desorption time and 230 °C desorption temperature. Generally, an increase in ionic strength lowers the solubility of neutral molecules in the water and improves transfer to the gas phase, although in some cases, a salting-in effect is observed at high salt concentrations. Indeed, by varying the salt concentration between 0 and 1 M, the purge efficiency increased and then remained constant up to 2 M NaCl. Higher salt concentrations decreased the transfer of TCA to the gas phase and, at the same time, increased the risk of salt precipitation in the PT system. Selecting a 1 M sodium chloride concentration, the effect of acids in the solution to be purged was studied by adding hydrochloric acid or sulphuric acid. In both cases, an increase in the acid concentration increased the purge efficiency. At the neutral pH TCA is largely in its ionic form, when the pH is lowered the acid–base equilibrium of the analyte shifts significantly towards the neutral form, increasing its purge efficiency. A 0.15 M sulphuric acid concentration was chosen since, as shown in Fig. 2, this provided the best sensitivity. The effect of the salt concentration was then re-studied between 0 and 4 M and, as can be seen in Fig. 2, purge efficiency increased up to 3 M NaCl and then remained constant, so that a therefore 3 M sodium chloride concentration was finally selected.

The purge gas flow-rate was set at 40 ml min^{-1}, in accordance with the manufacturer's recommendations. The total amount of TCA purged was substantially enhanced by increasing the purging time from 8 to 10 min, with a desorption cycle of 6 min at 230 °C and a purge temperature of 35 °C.

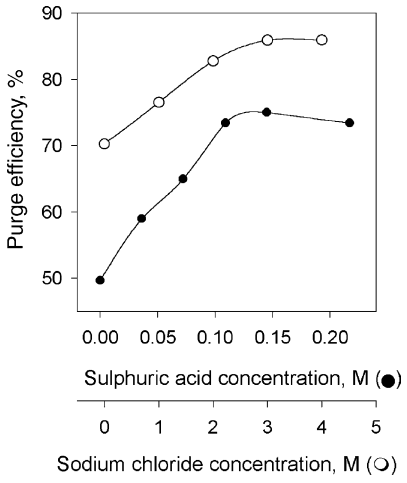

Fig. 2. Effect of sulphuric acid and sodium chloride concentrations on the purge efficiency of trichloroanisole (1 ng ml^{-1}).

Further increases in the purging time led to a decrease in the purging efficiency, as can be observed from Fig. 3A, where the influence of this parameter is expressed by reference to the maximum purge efficiency obtained with the system used, i.e. in the conditions finally selected. A purge time of 10 min was chosen. Indeed, longer purge times decreased the signals because the helium itself causes stripping of the trapped TCA, and at the same time the vapour water entering the trap increases.

To study the influence of temperature on purge efficiency, the purge vessel temperature was varied from ambient to 90 °C. As can be seen from Fig. 3B, purge efficiencies did not vary significantly between 25 and 40 °C. Higher temperatures led to a decrease in the purge efficiency, probably due to the amount of water entering the trap and so all the experiments were carried at ambient temperature. The trap temperature during the purge step did not affect the results obtained when varied between 30 and 40 °C, and 35 °C was used. The desorption time was varied between 3 and 8 min, and was finally fixed at 5 min, which provided the highest sensitivity (Fig. 3C). The influence of the temperature of the trap during the desorption cycle was checked by increasing it from 210 to 250 °C. Fig. 3D shows that maximum sensitivity was attained at 240 °C. When the transfer line temperature was varied in the 200–250 °C range, sensitivity increased up to 240 °C and then slightly decreased, and so 240 °C was adopted.

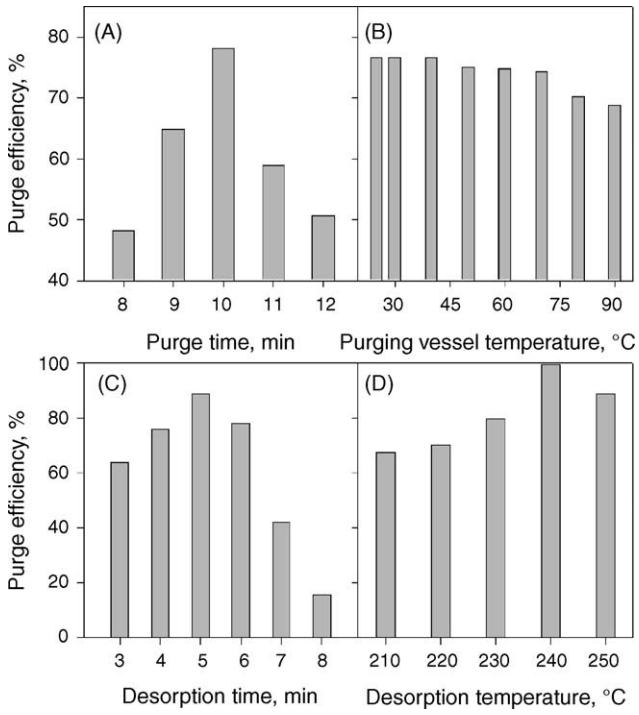

Fig. 3. Influence of: (A) purge time; (B) purging vessel temperature; (C) desorption time and (D) desorption temperature on the purge efficiency of 1 ng ml^{-1} trichloroanisole in the presence of 0.15 M sulphuric acid and 3 M sodium chloride.

3.1.3. Optimization of the extraction procedures

To optimize the extraction procedure for cork stoppers, every unit was separately ground and fortified by adding 200 μl of a 50 ng ml^{-1} TCA methanolic solution. Taking into account that the mean mass of each cork stopper was 4 g this means a 2.5 ng g^{-1} level.

Preliminary experiments were carried out using pentane and mixtures with different proportions of diethyl ether as the organic solvent, extracting twice with 40 ml of the organic phase with magnetic stirring for 60 and 30 min. The need for two extraction steps was previously checked, and was in agreement with the results obtained by other authors [21]. A third extraction step proved unnecessary. As shown in Fig. 4A, pentane provided higher TCA recoveries from the solid matrix than any of the different mixtures with diethyl ether, and was therefore selected for the extraction treatment. The solvent volume in the first extraction step was studied, assaying volumes of 40, 60 and 80 ml, and keeping the other experimental conditions constant. Increasing the extraction volume decreased the recoveries obtained (Fig. 4B), probably due to the longer time needed to evaporate the pentane, which would have led to losses in TCA. Volumes lower than 40 ml proved unviable owing to the physical characteristics of the sample.

Stirring times of 30, 60 and 90 min were assayed for the first extraction, and best results were obtained at 60 min. To shorten the duration of the extraction procedure, an ultrasound probe was used. The need for two extraction steps was again evident even when sonicating the sample, and so different times were assayed for the first extraction step, maintaining 1 min for the second extraction. As can be seen from Fig. 4C, recovery values of nearly 90% were attained with a total treatment time of 3 min of ultrasounds. Different times of between 30 s and 4 min for the second extraction did not affect the results obtained. Therefore, 2 and 1 min were adopted for the first and second extraction steps, respectively. Extending the sonication time, large losses in recovery were observed probably due to the fact that, although the extraction vessel was maintained in an icebath, there is local overheating inside the vessel, which produces TCA volatilization. No differences were observed when varying the amplitude of the probe, so a 60% amplitude value was adopted. It is interesting to note that although ultrasounds have previously been used for TCA extraction in cork stoppers [21], they have not been used in conjunction with a probe and we achieved a considerable saving of time (3 min as opposed to 30 min). Moreover, very good recovery values were obtained here compared with those obtained using an ultrasonic bath [21].

The optimization of the procedure for wine samples was carried out with red wine. When purging TCA directly from wine samples to which sodium chloride and sulphuric acid had been added, a problem arose with the ethanol contained in the samples, which blocked the trap, and so liquid–liquid extraction was assayed to isolate the analyte. Preliminary experiments were carried out using 10 ml of a standard solution, which was extracted twice with 10 ml of organic solvent by shaking manually for 5 min. The extracts were combined, evaporated to almost dryness and reconstituted in an aqueous solution containing sodium salt and sulphuric acid. Hexane, ethyl acetate, diethyl ether and pentane, separately and mixed in different proportions, were assayed. The best extraction percentages (65–96%) when a 12% (v/v) ethanol accompanied the analyte in the aqueous phase, were obtained with pentane and pentane–diethyl ether mixtures. A 10 min shaking time proved appropriate for maximum extraction and, consequently, a double extraction with pentane for 10 min was adopted.

Wine volumes between 10 and 50 ml were tested in an attempt to increase the sensitivity of the method. Sample volumes higher than 25 ml could not be used because of the abundant foam which appeared during the purging step. Since white and rosé wines showed a similar behaviour, a 25 ml sample volume, which was finally reconstituted in 10 ml of aqueous phase, was adopted.

It should be noted that the optimization of both the purge-and-trap system and extraction steps were carried out by a single-variable-at-a-time method. Taking into account the diversity of parameters, factor interactions could exist, but they were not considered in this work.

3.2. Analytical characteristics of the method

Two different calibration graphs were used to quantify TCA in the cork or the wine samples, owing to the interfer-

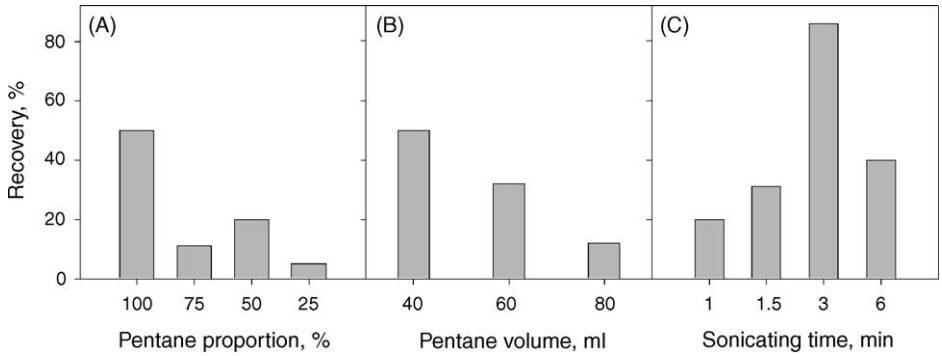

Fig. 4. Effect of: (A) proportion of pentane in the organic solvent mixture pentane–diethyl ether, (B) pentane volume and (C) sonicating time on the extraction percentage of TCA for a spiked cork stopper at a concentration of 2.5 ng g^{-1}.

Table 1
Calibration parameters

Calibration	Slope ± S.D.[a] (ml ng^{-1})	Ordinate ± S.D.[a]	Linearity (ng l^{-1})	Detection limit (ng l^{-1})	R.S.D.[b] (%)
Aqueous	23.631 ± 0.212	−0.935 ± 0.106	50–2000	10	2.8 (300)
Ethanol/water 12% (v/v)	14.674 ± 0.215	0.736 ± 0.102	75–2500	12.5	6.5 (100), 5.2 (350)

[a] Mean ± standard deviation (n = 3).
[b] Values in brackets are TCA concentrations in ng l^{-1}.

ence produced by the ethanol content in the wine. For quantification purposes in the case of the cork stoppers, calibration was carried out by preparing standards at seven TCA concentrations, 5 ml of each standard being purged and measured. Two replicates for each calibration level were carried out. Because of the matrix effect observed for the wine samples due to the ethanol content, calibration was in this case made by using aqueous standards containing 12% (v/v) ethanol submitted to the extraction procedure described for wines. Since the interference of ethanol did not appear for cork stoppers, in this case aqueous standards were prepared in the absence of ethanol and directly submitted to the purge step.

Peak areas were used for calibration purposes in both cases. Table 1 shows the characteristics of both calibrations, that showed correlation coefficients of 0.9998. The detection limits calculated using signal-to-noise ratios of 3 are shown in Table 1. Detection limits of 25 pg g^{-1} and 5 ng l^{-1} were obtained for cork stoppers and wines, respectively. The quantification limits calculated using signal-to-noise ratios of 10 were 110 pg g^{-1} and 17 ng l^{-1} for cork stoppers and wines, respectively. The repeatability of the proposed method for cork samples was demonstrated by repetitive analyses, calculating the average relative standard deviation (R.S.D.) for 10 successive injections of a standard solution of 300 ng l^{-1}. Standard solutions of 100 and 350 ng l^{-1}, which would correspond to cork taints of increasing olfactory severity, were submitted 10 times to the optimized procedure proposed for wine samples. The results obtained were used to evaluate the precision of the method and are shown in Table 1.

3.3. Real samples and recovery studies

The optimized method for the analysis of cork stopper samples was applied to 15 samples and TCA was detected in two of them, but below the quantification limit (110 pg g^{-1}). It is worth nothing that the detection limit obtained for cork samples was low enough to detect TCA in corks pulled from wines which were described as tainted by experienced judges, since the TCA concentrations detected in such corks ranged from 10 ng g^{-1} to 2.1 μg g^{-1} [4,10]. As expected, no TCA was detected in any of the commercial wines analyzed.

The recoveries from spiked corks and wine samples varied from 88.5 to 102.3% with an average recovery ± S.D. (n = 51) of 96.0 ± 6.8%, as can be seen from Table 2. Fig. 1B and C shows the chromatograms obtained for a spiked wine and a spiked cork stopper, respectively.

Table 2
Mean recovery efficiencies and R.S.D. obtained in fortified samples

Sample	Spike level[a]	Found level[a,b]	Recovery[b] (%)	R.S.D. (%)
Cork stopper	0.75	0.67 ± 0.04	88.9 ± 5.7	6.4
	1.25	1.16 ± 0.03	92.9 ± 2.4	2.6
	1.75	1.68 ± 0.17	96.1 ± 9.9	10.3
	2.25	2.03 ± 0.23	90.5 ± 10.4	11.5
	5	4.78 ± 0.27	95.6 ± 5.4	5.6
Red wine	50	49.6 ± 2.3	99.2 ± 4.5	4.5
	150	140.6 ± 15.5	93.8 ± 10.3	11.0
	200	203.3 ± 9.4	101.7 ± 4.7	4.5
	250	243.3 ± 23.5	97.3 ± 9.4	9.7
White wine	50	47.7 ± 1.3	95.5 ± 2.7	2.8
	150	132.8 ± 6.6	88.5 ± 4.4	5.0
	200	186.7 ± 11.8	93.3 ± 5.9	6.3
	250	247.3 ± 32.0	98.9 ± 12.8	12.9
Rosé wine	50	50.1 ± 1.1	100.2 ± 2.3	2.3
	150	153.1 ± 15.5	102.1 ± 10.3	10.1
	200	191.7 ± 7.1	95.8 ± 3.5	3.6
	250	255.7 ± 16.5	102.3 ± 6.6	6.4

[a] ng g^{-1} for corks and ng l^{-1} for wines.
[b] Mean ± standard deviation (n = 3).

4. Conclusion

The combination PT-GC–AED described in this paper represents a sensitive procedure for the determination of TCA in cork stoppers and wines. The ultrasonic treatment for corks substantially reduces the treatment time. The analytical characteristics and recovery data prove the reliability of the procedures, which make them suitable for the monitoring of TCA in the samples studied.

Acknowledgements

The authors are grateful to the Spanish MEC (Project BQU2003-01731) for financial support. N. Aguinaga acknowledges a fellowship from MEC. Partial finantial support from Fundacion Seneca (CARM, Spain, Project PB/35/FS/02) is also acknowledged.

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