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Accurate Determination of 2,4,6-Trichloroanisole in Wines at Low Parts Per Trillion by Solid-Phase Microextraction Followed by GC-ECD

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A headspace solid-phase microextraction (HS-SPME) procedure at 30 °C with a 100 μm PDMS fiber of a saturated NaCl solution stirred at 1100 rpm combined to GC-ECD for the 2,4,6-trichloroanisol (TCA) determination in wines has been developed. Due to the matrix complexity and ethanol absorption into the fiber, the internal standard selection was crucial to obtain unbiased results. Thus, matrix effects were observed when analyzing different types of Spanish wines (white, early, and vintage red wines) spiked with TCA at low concentration levels (i.e., <40 ng L^{-1}). In contrast, the use of 2,4,6-tribromoanisole (TBA) as internal standard overcame these matrix effects, whereas the use of 2,4,6-trichlorophenyl ethyl ether led to inconsistent results. The developed HS-SPME-GC-ECD methodology reaches a limit of quantitation for TCA in wine within 2.9−18 ng L^{-1}, with a relative standard deviation of 2.5−13.4%, depending on the TCA concentration level and wine characteristics. This analytical method is comparable to the existing methodologies based on HS-SPME followed by GC-MS in terms of accuracy, precision, length of determination, and length of quantification; however, analysis cost is reduced.

KEYWORDS: SPME-headspace; TCA; GC-ECD; internal standard selection; wine; matrix effect; musty odor

INTRODUCTION

A serious and highly costly problem related to a moldy-musty off-flavor called "cork taint" or "moldy taint" is raising the attention of wine and cork industries. Buser et al. (*1*) were the first authors to report the correlation of cork taint in wine with the presence of 2,4,6-trichloroanisole (TCA). However, not all the TCA present in wine can arise from cork, other factors, such us transportation, storage, and handling can increase the TCA concentration in the final product (*2*). Moreover, not all TCA present in the cork can contaminate wine, because only a fraction of the total TCA is releasable (*3*).

TCA possess very low taste and odor (T&O) thresholds in wine, but the concentration to determine a defect is dependent on wine characteristics and composition. Although the perception level for TCA is in the range of 0.03−10.0 ng L^{-1} (*4*, *7*), the TCA concentration considered to produce a defect in wine is higher, ranging from 10 to 40 ng L^{-1} (*2*). This sensory threshold can be affected by different factors, such as wine type (white, red, etc.), and it is closely related to the sensor capacity of the expert taster. Moreover, the number of organoleptic assays that the expert taster can do per day is limited. All these factors show that there is a great deal of uncertainty surrounding the setting of sensory limit values. In addition, not only TCA can contribute to give an unpleasant odor/flavor to wine. The presence of other compounds, such as guaiacol, 1-octen-3-ona, 1-octen-3-ol, geosmine, and 2-methylisoborneol can also produce T&O problems to wine, but TCA is one of the most common occurrences (*2*).

Different analytical methodologies have been developed to determine TCA presence in corks and/or wine (*4*). Because of its volatility, gas chromatography (GC) is the most suitable technique for TCA determination, coupled to a mass spectrometer detector (MSD) at selective ion monitoring mode (*7*−*9*, *13*−*15*) or to an electron capture detector (ECD) (*16*). However, due to low target TCA concentration level in wine, a preconcentration step is required. Liquid−liquid extraction (LLE) (*5*, *17*) and solid-phase extraction (SPE) with different sorbents, such as C_{18} (*11*), Ambersorb 512 (*18*), silica gel/ENVI−Carb (*19*), have been tested. The drawbacks of these methodologies are the use of solvents, matrix dependent recoveries in cases of complex matrixes such wines, and poor selectivity. Solid-phase microextraction (SPME), because is a solventless, cost-effective, and provides high sensitivity, can be an alternative to conventional extraction procedures. Although headspace solid-phase microextraction (HS-SPME) has been successfully applied to TCA analysis in water and wines (*7*−*9*, *13*−*15*), a detailed study

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on the matrix effects in the TCA determination in wines has not been carried out yet, being of primary interest for its application in routine basis.

The objective of this work has been to develop a cost-effective, accurate, and fast analytical methodology for the determination of TCA in wines at low ng L^{-1} concentration levels. Therefore, a HS-SPME followed by GC-ECD procedure has been developed, with emphasis on the variables affecting the matrix effects. Different internal standards (IS) have been evaluated to match the TCA behavior in the HS-SPME from ethanol/water (12% v/v) and real matrixes (i.e., white wine, vintage red wine, and early red wine). Temperature and pH as key variables affecting matrix−analyte interaction have been tested. Furthermore, the experimental distribution constants of the selected IS and TCA were measured in ethanol/water (12%) to evaluate their affinity for the PDMS fiber.

MATERIALS AND METHODS

Materials. The following reagents were obtained from Sigma-Aldrich Chemie (Steinheim, Germany): 2,4,6-trichlorophenol (98%); 2,4,6-trichloroanisole (TCA, 99%); 2,4,6-tribromoanisole (TBA, 99%); and 1,3,5-trichlorobenzene (TCB, 99%). The 2,4,6-trichlorophenylethyl ether (TCPEE) was prepared as described below. Water (HPLC grade), toluene, ethyl acetate, hexane, acetone, and methanol were obtained from Merck for trace organic grade (Darmstadt, Germany). Sodium chloride (for analysis) and absolute ethanol were obtained from Carlo Erba (Milan, Italy). K_2CO_3 anhydride was obtained from Panreac Química (Barcelona, Spain). Ethyl iodide (>99%) and 2,3,6-trichlorotoluene (TCT, 87.6%) were obtained from Fluka Chemie GmbH (Buchs, Switzerland). Silica gel was obtained from SDS (Marseille, France). Poly(dimethylsiloxane) (PDMS, 100 μm) fiber was obtained from Supelco (Bellefonte, PA).

TCA, TCPEE, TCT, TCB, and TBA stock standard solutions were diluted in toluene to prepare a working standard solution of 1883 μg g^{-1}, 1725 μg g^{-1}, 2344 μg g^{-1}, 2000 μg g^{-1}, and 1490 μg g^{-1}, respectively. Standard working solutions of TCA (8.4 and 42.2 ng g^{-1}), TCPEE (24.5 ng g^{-1}), and TBA (29.8 ng g^{-1}) were prepared by diluting stock standard solution in methanol. Stock and working solutions were stored at 4 °C in the dark. Different (white, red early, and red vintage) commercial Spanish wines (13 samples) were analyzed.

2,4,6-Trichlorophenylethyl Ether (TCPEE) Synthesis. To a suspension of anhydrous K_2CO_3 (700 mg) in dry acetone (5 mL), 2,4,6-trichlorophenol (200 mg, 1.01 mmol) and iodoethane (160 μL, 312 mg, 2 mmol) were sequentially added. After stirring the mixture for 24 h at room temperature (c.a. 25 °C), the solid was filtered off, and the filtrate was evaporated under slight vacuum. The residue (310 mg) was purified by column chromatography over silica gel (30 g, 40−63 μm, 60 Å), eluting with hexane/ethyl ether (80:20) to obtain the target compound as a slightly yellow solid (155 mg, 68% of the theoretical yield) with purity better than 99.9% by GC-ECD. In addition, the compound identity was confirmed by proton ^{1}H NMR (δ, $CDCl_3$) 7.30 (s, 2H), 4.09 (q, J = 7 Hz,2H), 1.46 (t, J = 7 Hz,3H) ppm and ^{13}C NMR (δ, $CDCl_3$) 150.65 (s), 130.18 (s), 129.24 (s), 128,68 (d), 69.81 (t), 15.42 (q) ppm. For the synthesis of the internal standard, thin-layer chromatography (TLC) was performed on 0.25-mm, precoated silica gel 60 F254, aluminum sheets (Merck, Darmstadt, Germany). ^{1}H and ^{13}C NMR spectra were obtained with a Varian Unity-300 (Varian Inc., Palo Alto, CA) spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). Theoretical calculations regarding pK_a values were carried out using the ACD/pK_a 1.2 software (Advanced Chemistry Development Inc., Toronto, ON) at the Department of Analytical Chemistry (University of Lund, Sweden).

Instruments and Apparatus. The SPME holding device was purchased from Supelco (Barcelona, Spain). GC-ECD determination of TCA was carried out using an HRGC 5300 Mega series chromatograph (Carlo Erba Instruments, Milan, Italy) with an ECD 800 (Fisons Instruments, Milan, Italy) at 310 °C of body temperature, pulse amplitude of 50 V, current of 1 nA, and pulse width of 1μs. A Tracer Meta X5 (30 m, 0.25 mm i.d.) coated with a 0.25 μm film thickness (Teknokroma, Sant Cugat, Spain) column was used. The gas chromatographic conditions were as follows: the initial oven temperature was 70 °C for 2.0 min, then programmed from 70 to 250 °C at 10 °C min^{-1}, to a final holding time of 1.0 min. The detector temperature was 280 °C. The injector temperature was 260 °C at the splitless mode (2 min), and desorption time for the SPME fiber was 5 min. Ethanol extraction profile was determined using a GC-FID (split ratio 1/20). Data were acquired by a Nelson-PE interface with a sampling frequency of 100 Hz and processed with a PC computer using PE 2600 software.

SPME Procedure. Before the initial analysis, the PDMS fiber (100 μm) was conditioned for 60 min at 250 °C. After the conditioning process, a fiber blank was run to confirm fiber cleanness. Samples for method development were prepared by adding 25 mL of wine or ethanol/water (12% v/v) into a 40-mL glass vial, sealed with a PTFE septum, and 270 mm × 90 mm magnetic stirring bar at 1100 rpm were used. The extraction temperature was controlled by a water bath system, maintained at constant temperature (30 °C). Microliter volume of working standard solution of analytes was spiked into a vial to obtain the following concentrations: TCPEE 18.8 ng L^{-1}, TBA 23.5 ng L^{-1}, and TCA 0.1−150 ng L^{-1}. HS-SPME was performed, avoiding any direct contact with the sample. The sorption time profile was performed, exposing the fiber in the HS sample for 1, 5, 10, 15, 20, 30, and 40 min (TCB = 250 ng L^{-1}, TCT = 189.6 ng L^{-1}, TCA = 133.3 ng L^{-1}, TCPEE = 60.19 ng L^{-1}, TBA = 75.3 ng L^{-1}).

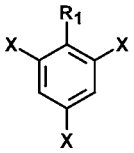
Desorption times were evaluated at 2, 5, and 15 min. NaCl addition was evaluated at 0, 25, 50, and 100% of the saturated concentration at 25 °C. The linearity was evaluated from 0.1 to 150 ppt (ng L^{-1}) for TCA. Detection (LOD) and quantitation (LOQ) limits were calculated from low concentration value of the calibration curves, by considering the peak area corresponding from three (LOD = 3σ) to 10 (LOQ = 10σ) times the signal-to-noise ratio of a procedural blank.

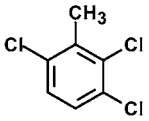
According to previous reports, the selected fiber was PDMS 100 μm (*7*, *8*, *16*). Salt addition, high stirring rate, and mild temperatures (20−30 °C) are relevant variables affecting to the TCA extraction (*8*) in wine. Thus, the HS-SPME extraction conditions adopted were as follows: stirring rate 1100 rpm, saturated with NaCl, extraction temperature 30 °C, sample volume 25 mL (40 mL of vial sample), extraction time 20 min, and desorption time 2 min at splitless mode. The sample was allowed five minutes of equilibration time before the SPME analysis. No carry-over was detected, and low concentration levels (very low ng L^{-1}) were able to be determined.

RESULTS AND DISCUSSION

Internal Standard Evaluation. The use of IS for SPME is strongly recommended to improve the accuracy and precision of the analytical procedure (*20*). Obviously, perdeuterated surrogates are the best option, because physicochemical properties are closely related to those of the analytes. However, deuterated surrogates are very expensive, and MS is mandatory when deuterated IS's are employed. In the case of TCA, its synthesis (*7*) or its purchase is demanded. MS is a highly sensitive and selective detector, especially when selective ion monitoring (SIM) mode is employed, but ECD can compete or even improve MS in terms of sensitivity and cost-effective analysis. IS selection for ECD has to be a halogenated compound with similar behavior to TCA. In this work, four different halogenated compounds as IS have been tested: TCB, TCT, TBA, and TCPEE (see **Table 1**). These compounds exhibit high response factors in the ECD; however, their behavior by HS-SPME-ECD, using the PDMS 100 μm fiber, was different, regarding the matrix effect. As shown in **Figure 1**, TCB and TCT reach the equilibrium conditions in 10−15 min, but when the extraction time is increased, the TCB and TCT extracted amount decreases. This effect can be explained by the chemical properties of TCB/TCT and the matrix evaluated. TCB and TCT are nonpolar compounds and possess a high affinity for the PDMS fiber. However, because of the high amount of ethanol present in wine (i.e., 11−14%), a small fraction of ethanol

Table 1. Physicochemical Properties of the Compounds Evaluated





2,4,6-TCA, X=Cl; R_1=OCH_3
2,4,6-TBA, X=Br; R_1= OCH_3
2,4,6-TCPEE, X=Cl, R_1=OCH_2CH_3
1,3,5-TCB, X=Cl, R_1=H

2,3,6-TCT

compound	abbrev	solubility[a] (g L^{-1})[b]	log p[a]	log K' (RSD%)[c]
2,4,6-trichloroanisole	TCA	5.4×10^{-3}	3.97 ± 0.34	3.93 (3.5)
2,4,6-trichlorophenyl ethyl ether	TCPEE	22×10^{-3}	4.5 ± 0.34	4.33 (2.4)
2,4,6-tribromoanisole	TBA	32×10^{-3}	4.14 ± 0.49	4.03 (2.3)
1,3,5-trichlorobenzene	TCB	16×10^{-3}	4.04 ± 0.31	ND[d]
2,3,6-trichlorotoluene	TCT	4.8×10^{-3}	4.5 ± 0.32	ND

[a] Calculated at the Analytical Chemistry Department (Lund, Sweden) Advanced Chemistry Development log P program. [b] Solubility at 25 °C in water. [c] $n = 8$, experimental condition in the text. [d] ND = not determined.

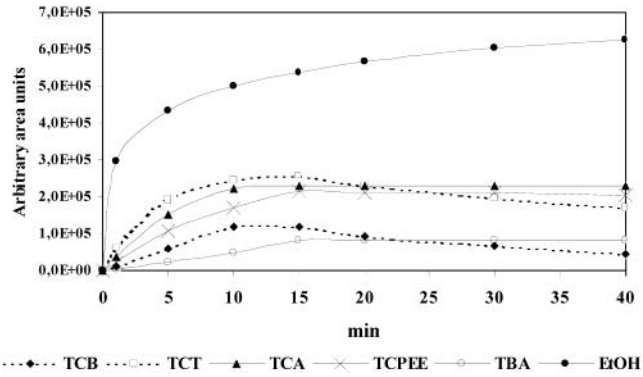


Figure 1. Extraction time profile of TCA, TCB, TCT, TCPEE, TBA and ethanol in ethanol/water (12% v/v). PDMS fiber (100 μm), extraction temperature 30 °C, NaCl saturated, sample volume 20 mL, vial volume 40 mL, and stirring rate 1100 rpm.

present in the matrix is absorbed into the PDMS affecting the TCB and TCT extraction time profiles and diminishing the amount extracted with time (see **Figure 1**). The influence of ethanol concentration on the SPME extraction of TCA has been evaluated previously (*16*). When ethanol concentration is increased, TCA recovery decreases. The use of a suitable internal standard as TBA or TCPEE compensates this effect; however, neither TCT nor TCB is not able to.

This behavior prompted us on a replacement of TCB and TCT as IS, because of their effect of worsening the precision of the analytical methodology. Thus, we had proven that the extraction time profile of TCPEE and TBA was very similar to TCA. Both compounds reached the equilibrium conditions at 15 min, and the extraction time profile was not affected by the presence of ethanol in the matrix (see **Figure 1**). Moreover, as is shown in **Figure 2**, a high chromatographic resolution was obtained, and the total GC run can be accomplished in less than 20 min. Therefore, TCPEE and TBA appeared as suitable internal standards for TCA determination by HS-SPME-GC combined either to ECD or MS even for samples with high ethanol content such as wines.

Determination of Experimental K'_{TCA}, K'_{TBA} and K'_{TCPEE} Values. At equilibrium conditions with PDMS 100 μm fiber, it

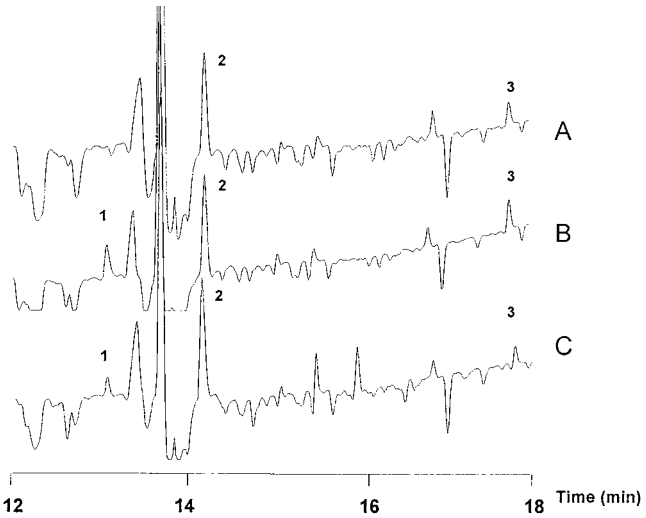


Figure 2. HS-SPME-GC-ECD chromatograms of wine matrixes (A: blank) with low/high (B/C) TCA interaction. Spiked level 20 ng L^{-1} of TCA (1), 40 ng L^{-1} TCPEE (2) and 25 ng L^{-1} of TBA (3).

is possible to calculate the distribution constant (K) of the TCA and TCPEE at the experimental condition established, with the following equation (*20*):

$$K' = \frac{nV_s}{V_f(C_oV_s - n)}$$

where the fiber volume (V_f) is 3.57×10^{-4} mL, the initial concentration (C_o) 50 ng L^{-1}, sample volume (V_s) is 25 mL (ethanol/water 12% v/v, NaCl saturated) and the amount extracted into the fiber (n) was determined by direct injection (GC-ECD) of the calibration curve standards of TCA, TBA, and TCPEE. The log experimental K' ($\log K'_{TCA,TBA\&TCPEE}$) values were 3.93 with an RSD of 3.5% ($n = 8$) for TCA, 4.03 with an RSD of 2.3% ($n = 8$) for TBA and 4.33 with an RSD of 2.4% ($n = 8$) for TCPEE (see **Table 1**). The $\log K'_{TCA,TBA\&TCPEE}$ values show a high affinity of these analytes for the PDMS 100 μm fiber. More than 15% of the total TCA, TCB, and TCPEE present in the sample were extracted into the fiber, allowing very low trace level TCA analysis in wine. TCPEE shows a higher experimental K' value than TCA. The introduction of a methylene group in the TCPEE decreases the polarity of this compound compared to TCA, and this effect is reflected on its log K' value.

Variables Affected by the Matrix Effect. TCPEE and TBA exhibited a similar HS-SPME behavior to that of TCA in ethanol/water (12%v/v). However, a broad spectrum of compounds occurring in wine can interact with TCA. To test TCPEE and TBA as internal standard candidates for TCA analysis in wines, 13 different Spanish wines, free of TCA, were spiked at the 20 ng L^{-1} level and analyzed using the external calibration (EC) and the internal calibration (IC) methods. The TCA calibration curve was prepared in ethanol/water (12% v/v to mimic the wine matrix). From the results shown in **Figure 3**, it can be observed that every wine exhibited a different behavior during TCA analysis, independent of the wine nature (white, early red, and vintage red wine). Each wine is unique, and this effect is reflected in the results shown in **Figure 3**. The use of internal standard is mandatory to improve accuracy and precision. Thus, when the EC method was applied, low TCA recoveries were determined, assuming all the samples analyzed as a whole (4.0 ng L^{-1}, RSD 80.2%). Due to the intrinsic TCA properties, we assumed that at low concentrations, most of the

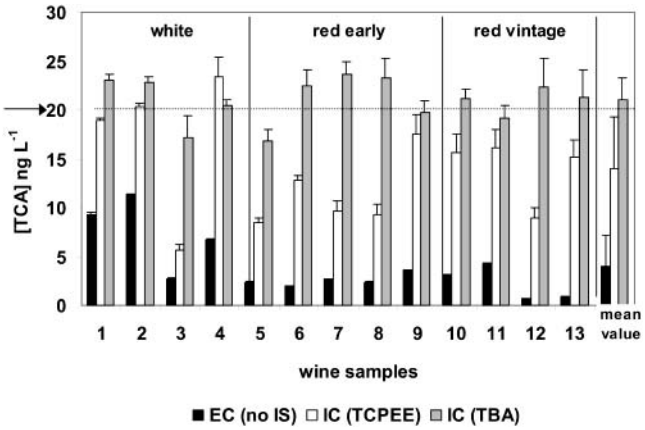


Figure 3. TCA determination of different commercial Spanish wines (white, red early, and red vintage wines) at 20 ng L^{-1} spiked level by EC and IC, employing TCPEE and TBA as IS. TCA mean value concentration, assuming the total samples analyzed as wine matrixes by EC and IC. The analyses were performed in triplicate.

TCA is strongly interacting with the matrix (wine) components. It could explain the low recoveries achieved by the HS-SPME of TCB and TCT, probably because they do not interact with the wine matrix, as TCA does. Thus, when TCB and TCT were used as IS, the TCA determination results were similar to those obtained by using the EC method. By using TCPEE as IS in the IC method, the accuracy was improved, but the experimental value obtained still underestimated the target (14 ng L^{-1}, RSD 37%). In contrast, the use of TBA as IS afforded the best results (21 ng L^{-1}, RSD 10%). Although initially TCPEE seemed to be an ideal candidate as IS in HS-SPME-GC-ECD due to the slight physicochemical and structural differences (methoxy vs ethoxy group) between TCA and TCPEE (see **Table 1**), such a subtle variation is the only argument to explain the disparity observed in the results obtained. Thus, TBA with a methoxy group in the molecular structure is a much better IS candidate. We can speculate that TCPEE interacts with the matrix to a lower extent than TCA, leading to a drop of the method accuracy during the TCA determination. TCPEE could be used as IS, but calibration curves had to be prepared using the same wine as a solvent or to perform the standard addition; however, it is evident that analysis time would be increased.

Employing an adequate IS, matrix effect can be compensated, but it cannot be avoided. Different attempts have been carried out to reduce the TCA−wine interactions and to increase the TCA recoveries. Changes in pH and extraction temperature could potentially avoid the TCA matrix effect. However, our results were not satisfactory. Increase of the extraction temperature (i.e. 40 °C) dramatically worsened the LOQ, because of a decrease in the K' value. Changes in pH improved the TCA determination (pH range 7−9) in some wines analyzed but failed in others. At low pHs (i.e., 1−2) it was observed that the TCA−matrix interaction was stronger than at neutral−basic pH, probably due to an increase in matrix hydrophobicity due to the protonated forms of acidic substances. Matrix effect was always more evident at low TCA concentration values. Wine 4 and wine 5 (**Figure 3**), which were characteristic of wines exhibiting low and high matrix effects, respectively, were spiked at different TCA concentration levels, as shown in **Table 2**. At higher TCA concentration values, the method accuracy improved when TCPEE was used as IS, demonstrating a clear evidence of matrix effect.

TCA determination by HS-SPME-GC-ECD at different matrixes (water/ethanol and three different wines) has been evaluated. TCA external calibration curves of each matrix are shown in **Figure 4A**. The calibration curves (with a correlation coefficient of $r = 0.98-0.99$) reflect a different sensitivity (slope) for each matrix evaluated for TCA determination. The sensitivity decreased from ethanol/water (12% v/v) to vintage red wine. This trend is closely related to the complexity of the matrix. Ethanol/water (12% v/v) was the simplest matrix evaluated, and no interaction with TCA was expected if compared to wine samples. White and early red wines showed similar matrix effect based on their TCA-HS-SPME behavior; however, small sensitivity differences were appreciated. Vintage red wine was the most complex matrix evaluated, showing the highest matrix effect dependence for TCA determination. This effect shows the need to perform a calibration curve depending on the matrix evaluated, when external calibration is carried out. This drawback is critical, when different wine samples are analyzed during TCA quality control, increasing the analysis cost and time. TCA determination by HS-SPME is a matrix-effect-dependent analytical procedure, but the use of a suitable internal standard can minimize or avoid this effect. In **Figure 4B** is shown the internal calibration curve for all the matrixes evaluated ($r > 0.99$). Thus, a single calibration curve can be used, avoiding the necessity to perform a new calibration curve each time a new matrix is evaluated. Employing an adequate internal standard such as TBA, the matrix effect is minimized in TCA-HS-SPME. Some authors have suggested building a calibration curve by using a mixture of different red wines from

Table 2. Accuracy and Precision Results from the Analysis of Two Wine Samples (high/low matrix interaction) Spiked at Different TCA Levels Using Two Different Internal Standards ($n = 5$ at each level)

wine matrix interaction	internal standard	spiked level (ng L^{-1})	measured mean value (ng L^{-1})	recovery (%)	RSD (%)
low	TCPEE	10.1	10.1	100	1.4
		76.0	73.5	97	2.5
	TBA	20.0	22.5	113	2.5
		66.7	69.3	104	1.9
high	TCPEE	20.0	5.6	28	11.5
		66.7	48.4	72	3.8
		200	191.8	96	1.5
	TBA	20.0	16.6	83	13.4
		66.7	71.8	108	3.3
		200	208.5	104	3.1

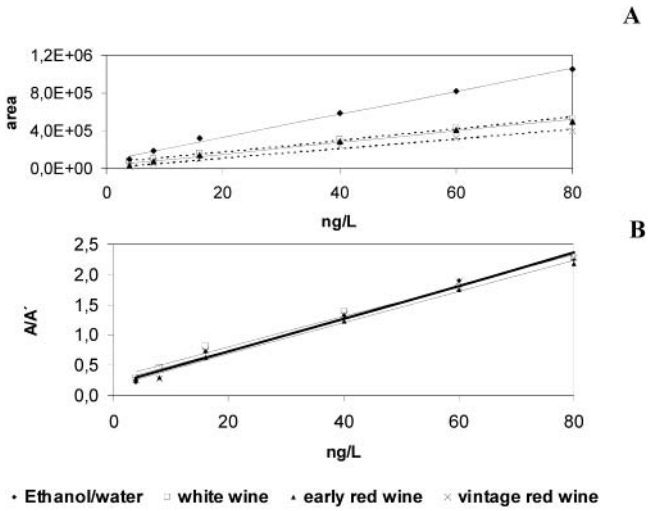


Figure 4. External (A) and Internal (B) calibration curves of TCA in different matrixes (ethanol/water (12% v/v), white wine, red early wine, and red vintage wine). TBA is used as IS.

different origins and ethanol contents, to have a "representative" wine matrix sample when internal calibration (using TCT as IS) is developed (*16*, *21*). However, to our knowledge and according to our experience, each wine sample is unique, and therefore, analysis reliability could be questioned.

Partitioning of volatile substances such as TCA between liquid and gas phase is mainly governed by the aroma compound volatility and solubility. However, these physicochemical properties could be influenced by other wine constituents present in the medium, such as polysaccharides, proteins and polyphenols. Wine phenolic compounds originating from grapes encompass several structural groups, such as anthocyanins, proanthocyanidins, etc. (*22*). Moreover, aging in oak barrels promotes the extraction of low molecular weight phenolic compounds, mainly ellagitannins, into wine (*23*). Wine polyphenols have attracted much attention, because of their ability to interact with proteins as well as aroma substances such as isoamyl acetate, ethyl acetate, ethyl hexanoate, and benzaldehyde (*22*). Processes involving polyphenol aggregation could lead to a significant loss of aroma compounds through intermolecular interactions. Traditionally, wine samples are divided into two well differentiated matrixes, white and red, when TCA determinations have been developed by HS-SPME (*16*). White wine is assumed to be a matrix with low TCA interaction, and red vintage wine is asumed to be a matrix with high matrix effect. Following the same approach, red early wine is an intermediate matrix. This wine matrix classification is based on their polyphenol contents. However, our results show that the approach regarding wines as whites or reds (low/high polyphenol contents) might be too simple. Both white and early red wines can exhibit a higher TCA−matrix interaction (low TCA recoveries) than red vintage wine, as shown in **Figure 3**. Although the content of red wine polyphenolic substances is higher than that for white wines, their concentration level is at the same range (0.5−3.0 g/L), considering that it is 10^8 times higher than the expected TCA concentration levels found in wines. Thus, it is probably more the nature of these polyphenolic compounds that affects TCA recoveries than their concentration in wine.

Method Evaluation. Once the HS-SPME-GC-ECD procedure for TCA determination was optimized, the analytical parameters (LOD, LOQ, accuracy, precision, linearity, reproducibility, and repeatability) were evaluated. The linearity of the response was checked in the range of 0.1 to 150 ng L^{-1} for TCA, showing a linearity range (internal standard calibration) with an excellent correlation coefficient ($r > 0.995$). LOD, LOQ, and precision values are affected, depending on the wine characteristic (high/low TCA interaction, low/high TCA recoveries) analyzed. **Figure 2** shows a chromatogram with different TCA responses at two very different wine samples (low/high matrix interaction). **Table 3** shows the LOQ and LOD for TCA in ethanol/water (12% v/v) and in two well-characterized wines, depending on TCA−matrix effect (the lowest and the highest TCA−matrix interaction among the 13 commercial Spanish wines evaluated). All matrixes evaluated were used as solvent for calibration curves, and TCA no trace was found in the procedural blank. However, noise level is increased when wine samples are evaluated (matrix complexity) and different TCA levels are obtained (**Table 3**). This fact is reflected on the LOD's and LOQ's reached.

Ethanol/water (12% v/v) matrix allows the lowest LOD (0.5 ng L^{-1}) among the matrixes evaluated. Releasable TCA is defined as the concentration of TCA in a cork soak after it reaches equilibrium. Only a small portion of the cork's TCA is transferred to a soak solution (less than 1%). Therefore, ethanol/water (12% v/v) can be used to determine the releasable TCA in cork, instead of wine, and then to apply the analytical methodology developed, improving their determination.

Table 3. LOD and LOQ for TCA Determination (ng L^{-1}) with the Methodology Developed and Comparison with Other Analytical Methodologies

matrix	analytical methodology	LOD	LOQ	RSD%	ref
ethanol/water 12% v/v	HS-SPME-ECD	0.15	0.5	1.5	present work
wine (low interaction)	HS-SPME-ECD	1.0	2.9	2.5	present work
wine (high interaction)	HS-SPME-ECD	5.4	18.0	13.4	present work
white varietal wine	HS-SPME-MSD		5.0	13	7
wine not specified	LLE, GC-MSD		2–5	5–8	24
white wine	HS-SPME-ECD	1.0	4.0	7.8	16
red wine	HS-SPME-ECD	1.0	8.0	6.1	16

Two well-characterized wine samples were spiked with TCA at two concentration levels (20 and 67 ng L^{-1}) and, applying the analytical procedure developed, the within-day assay repeatability RSD value ($n = 5$) found was 2.5 and 1.9% for the wine with low TCA-matrix interaction and 13.4 and 3.3% for the one with high TCA−matrix interaction. The interday repeatability RSD ($n = 10$) values were at the same range. TCA determination by HS-SPME-GC-ECD improves the analytical parameters in terms of precision (RSD%), LOD, and LOQ compared to their equivalent methodology employing MSD (*7*) as is shown in **Table 3**. HS-SPME-GC-ECD avoids exhaustive extraction, cleanup step, solvents, etc., significantly diminishing the total analysis time, compared to other conventional methods (*1*, *12*, *24*). The same PDMS 100 μm fiber was used to perform more than 100 analyses without any significant damage.

In summary, HS-SPME-GC is a suitable technique for TCA determination in wines, provided that an appropriate internal standard is used. Among the compounds evaluated, only TBA exhibited a satisfactory performance as internal standard, because its HS-SPME behavior is similar to that of TCA in the wine samples evaluated. LOQ and RSD depend on the wine evaluated (high/low TCA−matrix interaction) and are independent of wine class (white/red wine), with a range of 2.9−18 ng L^{-1} and 2.5−13.4%, respectively. Although mass spectrometry is the detector selected for most of the TCA determination in wines, our work demonstrate that a selective, low cost, and reliable detector such as ECD can be used, providing similar accuracy, precision, LOD's, and LOQ's, but significantly reducing the total analysis cost.

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