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ARTICLE

Ultrasound-Assisted Emulsification Microextraction for Determination of 2,4,6-Trichloroanisole in Wine Samples by Gas Chromatography Tandem Mass Spectrometry

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A fast and effective microextraction technique is proposed for preconcentration of 2,4,6-trichloroanisole (2,4,6-TCA) from wine samples prior gas chromatography tandem mass spectrometric (GC−MS/MS) analysis. The proposed technique is based on ultrasonication (US) for favoring the emulsification phenomenon during the extraction stage. Several variables influencing the relative response of the target analyte were studied and optimized. Under optimal experimental conditions, 2,4,6-TCA was quantitatively extracted achieving enhancement factors (EF) $\geq$ 400 and limits of detection (LODs) 0.6−0.7 ng L^{-1} with relative standard deviations (RSDs) $\leq$11.3%, when 10 ng L^{-1} 2,4,6-TCA standard-wine sample blend was analyzed. The calibration graphs for white and red wine were linear within the range of 5−1000 ng L^{-1}, and estimation coefficients (r^2) were $\geq$0.9995. Validation of the methodology was carried out by standard addition method at two concentrations (10 and 50 ng L^{-1}) achieving recoveries >80% indicating satisfactory robustness of the method. The methodology was successfully applied for determination of 2,4,6-TCA in different wine samples.

KEYWORDS: Wine; cork taint; 2,4,6-trichloroanisole; ultrasound-assisted emulsification−microextraction; wine analysis; gas chromatography−mass spectrometry

INTRODUCTION

Haloanisoles are known to cause off-flavor in food and beverages. One of the major organoleptic defects in wine is cork taint, which is associated with a musty or moldy aroma of wine. Buser et al. first identified 2,4,6-trichloroanisole (TCA) as the main compound responsible for such odor (*1*). Chloroanisoles are produced as a consequence of biomethylation of halophenols by fungi present in cork. Occurrence of cork taint deteriorates the quality and acceptability of wines and causes significant financial loss to the wine industry. The taste and odor thresholds of TCA in wine are very low, but the concentration causing a defect is dependent on wine characteristics and composition. Moreover, only a fraction of the total TCA is releasable to contaminate wine. Although the perception threshold for TCA is higher than 0.03 ng L^{-1} (*2*, *3*), the TCA concentration considered to produce a defect in wine ranges from 10 to 40 ng L^{-1} (*4*).

Sample preparation plays an important role in the determination of TCA because of the complexity of the wine matrix and the low concentration of this analyte. Highly selective and sensitive analytical techniques are therefore required for the unequivocal identification and determination. In this way, capillary gas chromatography (GC) coupled with mass spectrometric (*5*−*7*), electron capture (*3*, *5*, *6*) or atomic emission detectors (*7*) are the most used techniques to analyze TCA in different matrixes. Since TCA concentration in wine is usually low, it is necessary to count on highly efficient preconcentration techniques for its estimation by gas chromatography (GC). Different sample preparation techniques have been proposed for TCA determination, which includes liquid−liquid extraction (LLE) (*1*), solid phase extraction (SPE) (*8*, *9*), pervaporation (*10*), solid phase microextraction (SPME) (*3*, *11*, *12*) and stir bar sorptive extraction (SBSE) (*13*, *14*).

In recent years, with the developing interest in miniaturization in analytical chemistry for solvent and sample savings, some newer miniaturized approaches to liquid extraction have been reported. Microextraction techniques are gaining importance since they are fast, simple, inexpensive, environmentally friendly and compatible with many analytical instruments (*15*). Solvent microextraction was first introduced by Jeannot and Cantwell (*16*), and it is based on analyte partition between a drop of organic solvent (extraction phase) and the aqueous sample bulk. Until recently, several different types of liquid-phase microextraction (LPME) have been developed including single drop microextraction (SDME) (*17*), hollow fiber LPME (*18*), and headspace LPME (*19*). Nevertheless, some drawbacks, such as instability of droplet and relatively low precision, are often reported (*20*).

Very recently, a novel microextraction technique, viz., ultrasound-assisted emulsification−microextraction (USAEME), has been developed by Regueiro et al. (*21*). USAEME is based on the

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use of ultrasonic (US) radiation for accelerating the emulsification phenomenon. During the sonication stage, the solution becomes turbid due to the dispersion of fine extraction solvent droplets into the aqueous bulk. The emulsification phenomenon favors the mass-transfer process of the analytes from the aqueous bulk into the organic phase. This leads to an increase in the extraction efficiency of the technique in a minimum of time (*22*, *23*). By combining the benefit of microextraction and ultrasound radiation, it is possible to establish an efficient preconcentration technique for determining analytes at trace concentration levels as reported for extraction of synthetic musk fragrances, phthalate esters, lindane and polybrominated diphenyl ethers in aqueous samples prior to their determination by GC–MS (*21*, *24*).

To our knowledge, this paper reports the application of the USAEME procedure for extraction and preconcentration of TCA from wine for the first time. Several factors including the nature of extraction solvent and its volume, extraction time, extraction temperature, influence of matrix modifiers and tandem mass spectrometric (MS/MS) parameters were studied and optimized over the relative response of TCA by using USAEME–GC–MS/MS. The analytical performance was evaluated in terms of enhancement factor (EF), limit of detection (LOD), matrix effect, repeatability (relative standard deviation, RSD) and linear working range. Moreover, the technique was applied in different red and white wine samples and the robustness of the method was evaluated in terms of recovery factors (RF%).

MATERIALS AND METHODS

Reagents. The certified reference standard of 2,4,6-TCA (99%, solid crystal form) was purchased from Sigma-Aldrich (Steinheim, Germany). The internal standard (IS) 2,2′,4,4′- tetrabromodiphenyl ether (BDE-47) was purchased from Accustandard (New Haven, CT). Stock solutions of both were prepared in methanol (MeOH) at a concentration level of 1000 mg L^{-1} and stored in amber colored bottles at −20 °C. The working standard solutions were prepared afresh daily in MeOH and stored at 4 °C. The solvents, viz., ethanol, methanol, chloroform and trichloroethene, were purchased from Merck (Darmstadt, Germany), and carbon tetrachloride was purchased from Sigma-Aldrich. Sodium chloride, hydrochloric acid, potassium chloride, sodium tetraborate, potassium phosphate, sodium phosphate, acetic acid and sodium acetate were all from Merck. The buffer solutions were prepared in ultrapure water, and the final concentrations were as follows: hydrochloric acid (0.05 mol L^{-1}, pH 2.0), acetate (0.1 mol L^{-1}, pH 4.0), phosphate (0.05 mol L^{-1}, pH 7.0), tetraborate (0.013 mol L^{-1}, pH 8.0), tetraborate (0.02 mol L^{-1}, pH 10) and phosphate (0.025 mol L^{-1}, pH 12.0). Ultrapure water (18MΩ cm) was obtained from a Milli-Q water purification system (Millipore, Paris, France). All reagents were of analytical grade or higher purity.

Sampling and Sample Preparation. For method optimization, two samples of wine blend were prepared: one with white wine by mixing 750 mL of different white wines of five grape varieties (Chardonay, Sauvignon Blanc, Semillon, Viognier and Pinot Grigio) and the second one prepared with red wine by mixing 750 mL of grape varieties Malbec, Cabernet Sauvignon, Merlot, Tempranillo and Syrah. Four different wine samples (two white) were obtained from commercial sources. The white wine samples corresponded to Torrontes and Chardonnay grape varieties, and the red wines were Malbec and Cabernet Sauvignon. The Malbec grape variety corresponded to an oak-aged wine, and others were young wines. To ensure the absence of TCA in the wine sample blend, the wine blend was analyzed with an optimized SPME–GC–MS/MS methodology and none of them reported detectable TCA concentration. Wine samples were stored in sterile dark bottles at 4 °C and analyzed within 12 h. All samples were analyzed in triplicate with the proposed USAEME–GC–MS/MS methodology.

Analytical Method. A 40 kHz and 600 W ultrasonication (US) bath with temperature control (Test Lab, Buenos Aires, Argentina) was used for assisting the emulsification process of the microextraction technique. The volume of extraction phase was measured using a 25, 50, and 250 μL Hamilton syringe (Reno, NV). Injections into the GC–MS were made by using a 5 μL Hamilton syringe. Centrifugation of samples was performed at 3500 rpm (1852.2*g*). GC–MS analyses were performed on a Varian 3900 GC equipped with an ion trap mass detector Varian Saturn 2000 (Varian Inc., Walnut Creek, CA). The system was operated by Saturn GC–MS WorkStation v6.4.1 software. The GC column used was VF-5MS (25 m × 0.25 mm, 0.25 μm film thickness; Varian, Lake Forest, CA). The temperature program was as follows: 70 °C, held for 2 min; increased at the rate of 20 °C $\min^{-1}$ to 150 °C, held for 1 min, rating 20 °C $\min^{-1}$ to a final temperature of 280 °C and held for 7 min. The total run time was 20.5 min. Helium (purity 99.999%) was used as a carrier gas at a flow rate of 1.0 mL $\min^{-1}$. The injector temperature was set at 280 °C, and the injections were performed in the splitless mode.

The mass spectrometer was operated in electron ionization (EI) mode (70 eV). The manifold and ion trap temperatures were set at 40 and 220 °C, respectively. The analysis was performed with filament multiplier delay of 6 min. The automatic gain control (AGC) was activated with an AGC target of 10000 counts. The filament emission current was 25 μA. The MS/MS process was carried out by collision induced dissociation (CID). The electron multiplier voltage was 1700 V (+150 V offset above the autotuning process). The peak identification was based on the base peak and the isotopic pattern of the 2,4,6-TCA. Specific ions were selected for 2,4,6-TCA, and the base ion was selected as a quantitative ion. Quantification was carried out using m/z 195 and 197 for 2,4,6-TCA and m/z 324, 326, and 328 for the internal standard BDE-47.

Data Analysis. For optimization of variables, the assays for each point were carried out in triplicate, and the mean and standard deviation were calculated and plotted. In the recovery study, the samples were analyzed in triplicate and the results expressed as follows:

$$\bar{x} \pm \frac{t \cdot \mathrm{SD}}{\sqrt{n}}$$

with a 95% confidence interval.

Ultrasound-Assisted Emulsification–Microextraction Procedure. A 5 mL wine sample was placed in a 10 mL glass-centrifuge tube, and to it 400 μL of 6.15 mol L^{-1} NaCl and 500 μL of 0.013 mol L^{-1} tetraborate (pH 8) buffer were added. Then, 25 μL of trichloroethene as extraction solvent was added and mixed up. The resulting emulsion was sonicated for 5 min at 20 °C. During the sonication process, a cloudy state was observed due to the dispersion of fine trichloroethene droplets into the sample. The emulsion was centrifuged at 3500 rpm (1852.2*g*) for 2 min in order to disrupt the emulsion and separate both phases. After centrifugation, the extraction solvent remained at the bottom of the conical tube with a volume of 10 μL. 1 μL of the resulting organic phase was removed from the bottom of the centrifuge tube and injected into the GC–MS/MS.

RESULTS AND DISCUSSION

The efficiency of the USAEME technique was found to be affected by several variables, including type of extraction solvent and its extraction solvent volume, sample salting out effect, sample acidity, extraction time and temperature as well as centrifugation time. The above-mentioned variables were optimized by modifying each factor at a time while keeping the remaining ones constant. 5 mL of wine sample blend (both red and white) containing 0.5 μg L^{-1} of TCA was used to perform the assays in triplicate. The relative chromatographic peak area was used to evaluate the influence of those variables on the extraction efficiency of USAEME technique.

Optimization of MS/MS Method. The MS/MS method was optimized in two steps. The first step involved the isolation of precursor ion, which in the second step was dissociated to characteristic daughter ion on collision with helium gas. The isolated parent ion was chosen so as to ensure the highest sensitivity and selectivity. The CID parameters were optimized to generate MS/MS spectra with relative abundance of the surviving precursor ion within 10–30%. The multiple reaction monitoring (MRM) transition 212 > 197 (a loss of CH_3) was used for quantification since it could provide highest sensitivity in terms of signal-to-noise ratio. The next most abundant MRM 195 > 167 was selected for confirmation. The abundance ratio of these two MRMs was used for unambiguous analysis in an unknown sample within ±20% tolerance range at LOQ level. The excitation storage level was optimized by using the Varian software tool "q calculator". The CID voltage was strategically optimized in two steps using the automated method development software (AMD) feature. During the first step, the CID was increased in steps of 1 V to identify a gross voltage range for further optimization. Once a good spectrum was obtained, a narrow voltage stepping of 0.2 V was used to identify the best parameter value to obtain the optimum MS/MS spectrum. The excitation time was set constant at 20 ms.

Effect of Extraction Solvent. For evaluation, the extraction solvents were selected on the basis of their higher density with respect to wine, affinity to 2,4,6-TCA and compatibility with GC. On the basis of these considerations, three organic solvents, viz., chloroform, carbon tetrachloride and trichloroethene, were initially assayed for the USAEME of TCA. The density of these solvents is higher than 1 g mL^{-1}, and they have different polarities (chloroform, 4.1; carbon tetrachloride, 1.6; and trichloroethene, 1.0). All these solvents were able to form an emulsion during sonication, leading to a biphasic system after centrifuging the solution. As demonstrated in **Figure 1**, trichloroethene showed a higher relative response than chloroform and carbon tetrachloride. The conversion of naturally occurring compounds into 2,4,6-TCA by using halogenated solvent was verified by analyzing a blend of real wine samples free of the target analyte. 2,4,6-TCA was not detected in the blend of wine samples; therefore it was assumed that the solvent is not able to produce a detectable 2,4,6-TCA amount under working conditions. Hence, trichloroethene was selected as the extraction solvent for further studies.

Effect of Extraction Solvent Volume. To examine the extraction solvent volume to be added in order to obtain the highest extraction efficiency and the highest relative response of the TCA, different extraction solvent volumes were assayed within the range of 25−300 μL. The extraction procedure was the one described above. The resulting organic-phase volume was measured by using a glass syringe. Greater relative response for TCA was obtained when 25 μL of trichloroethene was used to carry out the microextraction (**Figure 2**). When the volume of trichloroethene was increased from 25 to 300 μL, the resulting organic phase volume increased, but the relative response decreased due to a dilution effect of the analyte in this phase. The volumes of extraction solvent smaller than 25 μL led to a biphasic system with a very small organic phase. This rendered the precise measurement of the resulting organic phase volume difficult, which also affected the reproducibility of the microextraction technique. Therefore, the volume of trichloroethene was optimized at 25 μL for further studies in order to obtain higher relative responses and lower detection limit.

Effect of Salting out and Extraction Temperature. Ionic strength could affect the affinity of the analytes toward the extraction phase, in addition to the extraction solvent solubility and the sample viscosity. The mentioned aspects alter the emulsification phenomena conditioning the mass transfer process of the analyte from the sample bulk into the extraction phase microvolume. Additionally, changes in viscosity of the medium affected the US effect. As the viscosity of the medium increases, the ultrasound waves are absorbed and dispersed as calorific energy; thus, the cavitation process is withdrawn and the organic phase is not able to be dispersed in the form of fine droplets (*25*). Therefore, emulsion can drastically minimize, diminishing the efficiency of the mass transfer process and consequently affecting the extraction efficiency of the technique (*21*). In view of these considerations, the salting out study was carried out by adding different volumes of sodium chloride (6.15 mol L^{-1}) to the extraction system within the range of 0.00−2.8 mL. The extraction procedure was the one described above. The results are presented in **Figure 3a**. The best relative responses were observed in the volume range of 140−700 μL of sodium chloride. At higher volumes of NaCl, the solubility of extraction solvent decreased, increasing the extraction phase volume and diminishing the relative response of the analyte due to dilution effect. Therefore, 400 μL of 6.15 mol L^{-1} NaCl was chosen as optimum working conditions for further studies.

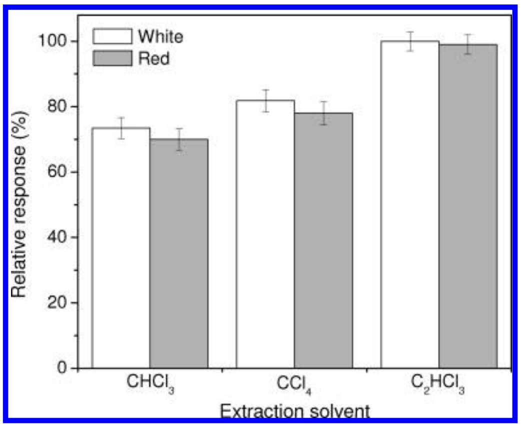

Figure 1. Extraction solvent effect on the relative response of 2,4,6-TCA. Extraction conditions: sample volume, 5 mL; extraction solvent volume, 50 μL; extraction time, 5 min; centrifugation time, 2 min; extraction temperature, 20 °C. 2,4,6-TCA concentration: 0.5 μg L^{-1}.

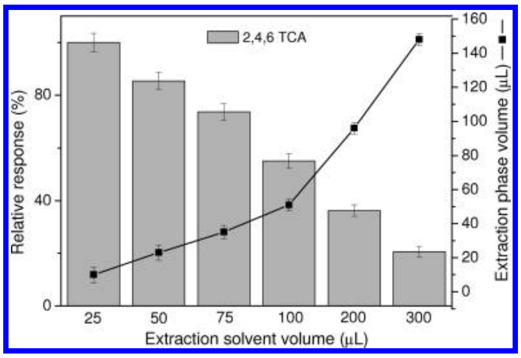

Figure 2. Correlation between the added trichloroethene, extraction phase volume and relative response of 2,4,6-TCA. Extraction conditions: sample volume, 5 mL; extraction solvent, trichloroethene; extraction time, 5 min; centrifugation time, 2 min; extraction temperature, 20 °C. 2,4,6-TCA concentration: 0.5 μg L^{-1}.

Extraction temperature is also an important parameter that affects the extraction procedure of the LPME, since it affects kinetics of the mass transfer process. Additionally, it concerns the analyte and the solubility of organic solvent in wine as well as the emulsification phenomenon. Therefore, it was found important to study this variable in the perspective of the microextraction technique. The temperature study was carried out within the temperature range of 5−75 °C (**Figure 3b**). At low temperatures (< 20 °C), the relative responses were low. Too low temperature increased the viscosity of trichloroethene affecting the emulsification phenomenon and in turn the mass transfer process. In the temperature range of 20−55 °C, the emulsification could be achieved easily and stayed as such during the whole extraction time. At a temperature above 45 °C, the relative response decreased because the solubility of TCA in wine was favored.

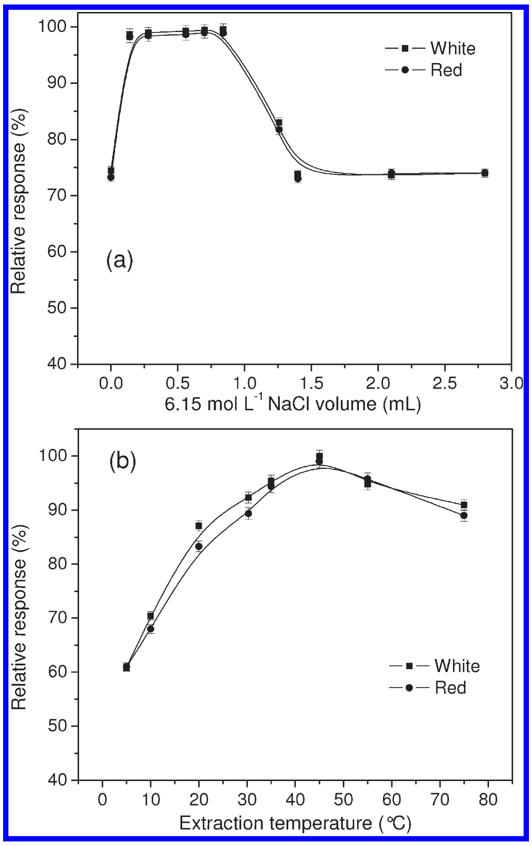

Figure 3. (**a**) Effect of ionic strength on the relative response for 2,4,6-TCA. (**b**) Extraction temperature effect on the relative response for 2,4,6-TCA. Extraction conditions: sample volume, 5 mL; extraction solvent volume, 25 μL trichloroethene; extraction time, 5 min; centrifugation time, 2 min. 2,4,6-TCA concentration: 0.5 μg L^{-1}.

It is interesting to point out that the relative responses of TCA obtained under the optimum conditions of salting out (addition of 400 μL of 6.15 mol L^{-1} NaCl and room temperature; 20 °C) and extraction temperature (45 °C without salt addition) were compared. The comparison was made by using a two-sample *t* test at the 95% confidence level, and it was observed that there are not significant differences between both optimized conditions. On the other hand, both optimum conditions were combined (addition of 400 μL of 6.15 mol L^{-1} NaCl and extraction at 45 °C). The results showed no significant differences compared with each of the optimum individual conditions. Therefore, in order to make the microextraction technique simpler and faster, the working conditions selected for further studies were addition of 400 μL of 6.15 mol L^{-1} sodium chloride and room temperature (20 °C).

Effect of Buffering. The addition of buffers to the wine sample blend tended to regulate the sample pH, which is conditioned by the matrix composition of the sample. Thus, it was found interesting to study this effect by adding different buffers to the wine blend and carry out the microextraciton of the 2,4,6-TCA. Six different buffers, including hydrochloric acid (pH 2), acetate (pH 4), phosphate (pH 7), tetraborate (pH 8), tetraborate (pH 10) and phosphate (pH 12), were evaluated. The extraction procedure was the one described above. For potassium chloride (pH 2), acetate (pH 4), phosphate (pH 7), tetraborate (pH 10) and phosphate (pH 12) buffers, low relative responses were obtained (**Figure 4**). Furthermore, it was observed that the relative responses were lower by using acid buffers than by using neutral and basic buffers. However, with tetraborate (pH 8) buffer, TCA showed the highest relative response. At lower sample pH, stronger TCA−matrix interaction was observed due to the protonated forms of acidic substances regularly found in wine matrix (*3*). In order to get higher relative responses and lower matrix effects, 500 μL of 0.013 mol L^{-1} tetraborate buffer was used for the following studies.

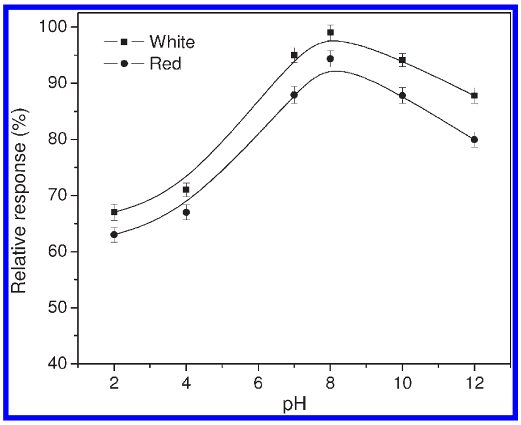

Figure 4. Effect of pH on the relative response of 2,4,6-TCA. Extraction conditions: sample volume, 5 mL; 400 μL 6.15 mol L^{-1} NaCl; extraction time, 5 min; centrifugation time, 2 min; extraction temperature, 20 °C. 2,4,6-TCA concentration: 0.5 μg L^{-1}.

Effect of Extraction and Centrifugation Time. Extraction time is also an important variable in USAEME procedure. It plays an important role in the emulsification and mass transfer phenomena, influencing the extraction efficiency of the TCA, and thus its relative response. The extraction time interval was defined as the time elapsed between trichloroethene addition and the end of the sonication stage. To determine the influence of the extraction time, it was varied within the range of 1 to 15 min. The extraction procedure was the one described earlier. It was observed that by increasing the extraction time, the relative response could be increased, reaching the maximum value at 4 min; after which, it remained constant (data not shown). Therefore, 5 min sonication time was chosen as working conditions for further studies.

Centrifugation was required to break down the emulsion and accelerate the phase separation process. In this way, different centrifugation times of 2−15 min were evaluated. Similar results were achieved in the whole time frame studied (data not shown); thus the minimum time (2 min) was selected as the centrifugation time necessary to get a satisfactory biphasic system.

Analytical Performance. Two calibration curves were made under optimized conditions: (1) the white wine sample blend and (2) with the red wine sample blend. Extraction conditions were as described in the section Ultrasound-Assisted Emulsification−Microextraction Procedure. The obtained enhancement factors for a sample volume of 5 mL were 425 and 400 for white and red wines, respectively. EF was calculated as the ratio between the initial wine sample blend volume and the resulting trichloroethene one after the USAEME technique considering the obtained recovery of each sample blend. The LODs of the analyte corresponding to the preconcentration of a 5 mL sample blend spiked at 10 ng L^{-1}, calculated as three times the signal-to-noise ratio (S/N = 3), were 0.6 ng L^{-1} and 0.7 ng L^{-1} for white and red wines, respectively. The precision of USAEME−GC−MS/MS was evaluated over six replicates (95% confidence interval), resulting in RSD values of 10.5% and 11.3% for white and red wines. The calibration curves showed a satisfactory linearity within the concentration range of 5−1000 ng L^{-1}. Furthermore, the coefficients of estimation (r^2) were 0.9995 and 0.9997 for white and red wines, correspondingly. In order to validate the analytical methodology, a recovery study of TCA at

Table 1. Recovery Study of 2,4,6-TCA in Different Commercial Grape Variety Wine Samples[a]

		10 ng L^{-1} spiked		50 ng L^{-1} spiked	
sample	level found	found[b]	recovery[c]	found[b]	recovery[c]
Torrontes	nd[d]	8.3 ± 2.2	83 ± 4	43.1 ± 11.2	86 ± 5
Chardonnay	nd[d]	8.5 ± 2.3	85 ± 5	44.3 ± 11.5	88 ± 6
Malbec	nd[d]	8.0 ± 2.2	80 ± 7	41.2 ± 11.6	82 ± 6
Cabernet Sauvignon	nd[d]	8.1 ± 2.3	81 ± 6	42.3 ± 11.8	84 ± 5

[a] Extraction conditions: sample volume, 5 mL; 400 μL of 6.15 mol L^{-1} sodium chloride; 500 μL of 0.013 mol L^{-1} tetraborate buffer pH 8; extraction solvent, 25 μL of trichloroethene; extraction time, 5 min; centrifugation time, 2 min; extraction temperature, 20 °C. [b] Results expressed as ± $[t \cdot SD \cdot (n^{-1/2})$; $n = 3$; 95% confidence interval; ng L^{-1}. [c] [(Found-Base)/Added] × 100. [d] Not detectable.

Table 2. Determination of 2,4,6-TCA in Wine Samples by Using Different Analytical Techniques

method	LOD (ng L^{-1})	RSD (%)	extraction time (min)	refs
SPME−GC−MS/MS[a]	2.50	5.2	60	*11*
SBSE−GC−MS/MS[b]	0.03	13.3	60	*14*
HS−SDME−GC−ECD[c]	8.10	12.4	25	*6*
USAEME−GC−MS/MS	0.70	10.9	5	this work

[a] Solid-phase microextraction and gas chromatography−tandem mass spectroscopy. [b] Stir bar sorptive extraction and gas chromatography−tandem mass spectrometry. [c] Headspace single-drop microextraction and gas chromatography−electron capture detection.

two different concentration levels (10 and 50 ng L^{-1}) was carried out over different grape varieties of wines. The study led to recoveries of ≥80% and RSD % < 7% (**Table 1**). These results are in agreement with the recovery values obtained for other authors by applying different extraction techniques. Considering the complexity of wine matrix, the achieved recoveries showed satisfactory robustness of the proposed methodology.

Application of the Method to Real Samples. USAEME−GC−MS/MS was applied for the determination of TCA in four different wines, including red and white wines from different grape varieties of Mendoza Province, Argentina. Sample and recovery results were carried out by triplicate (**Table 1**). The original TCA content prior to spiking was below the detection limit of the proposed methodology. TCA external calibration curves of each matrix showed different sensitivity (slope) for each matrix evaluated for TCA determination. White wines showed a relatively lower matrix effect than red wines, however, small differences in the technique sensitivity were appreciated. This trend could be closely related to the complexity of the matrix. This effect shows the need to use matrix-matched calibration curve specific for white and red wine, when external calibration is carried out. To avoid this quantification technique for each different kind of white and red matrix, it is also possible to build the calibration curve by using a spiked sample blend of different white and red wines from different origins (*26*, *27*). In this way, a matrix of white and red wine as representative as possible was obtained as described above.

Comparison of USAEME−GC−MS/MS with Other Analytical Methodologies. The analytical performance of USAEME−GC−MS/MS for TCA determination in wine samples was compared with other previously reported analytical methodologies (**Table 2**). The LODs were lower than SPME−GC−MS/MS and HS−SDME−ECD. Only SBSE−GC−MS/MS showed lower LODs than USAEME−GC−MS/MS, although the extraction time was higher and it required special approaches and instruments. For USAEME simple and inexpensive equipment is required. Moreover, the extraction equilibrium is established within a few minutes in comparison to other methodologies.

The application of the proposed analytical methodology based on USAEME proved to be effective for the determination of TCA in wine by GC−MS/MS at concentrations considered to produce a defect. Under optimized working conditions, high EF were obtained allowing a detection limit on the order of low ng L^{-1} to be reached with an acceptable precision, suitable for real world applications. An important aspect of the proposed methodology is the low organic solvent consumption, which turns it into a low cost and environmentally friendly technique. All these results establish that USAEME is a sensitive, rapid and reproducible technique, which could be successfully applied to the analysis of real commercial white or red wines of different grape varieties.

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