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Comparative study of two chromatographic methods for quantifying 2,4,6-trichloranisole in wines

M. Riu, M. Mestres, O. Busto, J. Guasch *

Departament de Química Analítica i Química Orgànica, Unitat d'Enologia del CeRTA, Facultat d'Enologia de Tarragona, Universitat Rovira i Virgili, C/ Marcel·lí Domingo, s/n 43007 Tarragona, Catalonia, Spain

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Abstract

Here we present the validation and the comparative study of two chromatographic methods for quantifying 2,4,6-trichloroanisole (TCA) in wines (red, rosé and white wines). The first method involves headspace solid-phase microextraction and gas chromatography with electron-capture detection (ECD). The evaluation of the performance parameters shows limit of detection of 0.3 ng l^{-1}, limit of quantification of 1.0 ng l^{-1}, recoveries around 100% and repeatability of 10%. The second one implies a headspace solid-phase microextraction and gas chromatography with mass spectrometric detection. The performance parameters of this second method are limit of detection of 0.2 ng l^{-1}, limit of quantification of 0.8 ng l^{-1} and repeatability of 10.1%. From the comparative study we can state that both methods provide similar results and the differences between them are the better sensitivity of the GC-ECD method and the very shorter chromatogram running time of the GC–MS method. The two methods are able to quantify TCA below the sensorial threshold in red, rosé and white wines using just a calibration graph, thus they could be a very good tool for quality control in wineries.

Keywords: 2,4,6-Trichloroanisole; Musty taint; Comparative study; Solid-phase microextraction; Electron capture detector; Mass spectrometer detector

1. Introduction

The quality of wine is highly dependent on their flavor. So, it is very important to avoid the presence of compounds which could give organoleptic defects to wine. One of them is the *musty taint*, which has classically been known as *cork* taint [1]. The main responsible for this organoleptic defect is 2,4,6-trichloroanisole (TCA) [2], although other compounds can also be involved such as other chloroanisoles, guaiacol, 1-octen-3-one, 1-octen-3-ol, 2-methylisoborneol [3] and even 2,4,6-tribromoanisole [4].

The most common technique for analyzing TCA in wines is gas chromatography (GC) coupled with either electron-capture (ECD) [5–9] or mass spectrometry (MS) detection [11–20], although the atomic emission detector has also been recently used [21]. However, these techniques are not sensitive enough to detect the low threshold perception of TCA in wines (between 1 and 50 ng l^{-1} depending on the wine and the sensitivity and training of the judge) [9,10,22]. Therefore, the analyte first needs to be extracted and concentrated, so it is essential to choose a suitable technique in order to obtain good results. Classical methods of extraction, such as liquid–liquid extraction [13] or solid-phase extraction (SPE) [14] have been used to quantify TCA in alcoholic beverages. The main disadvantages of these techniques are that they require large volumes of organic solvent, are time consuming and prone to lose analytes. Recently, other extraction techniques have been also used but, according to the literature, they present some problems as well; for instance, the purge and trap technique requires a previously solvent extraction [21] and pervaporation [16,17] provides high limits of quantification for TCA.

Regarding the solid-phase microextraction (SPME), it carries out the extraction and concentration of the analytes in just one step, it is easier to handle and it does not need any organic solvent [3,5,12,18,20]. Some techniques related with SPME have been used, as multiple solid-phase microextraction [19] or stir bar sorptive extraction [15]. However, the first one requires multiple extractions that imply long times of analyses, and, the second one, requires a special device to make possible both desorption

* Corresponding author. Tel.: +34 977 55 84 96; fax: +34 977 55 84 46.
E-mail address: qaenol@urv.net (J. Guasch).

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and injection of the analytes on the GC. Thus, taking into account all these disadvantages, the SPME is presented as the most suitable technique to extract and concentrate TCA present in wine.

The purpose of this paper was to compare the results obtained with two different chromatographic methods for quantifying TCA in wines by using the two most widely used detectors. The first method (SPME-GC-ECD) can be described as a classical gas chromatography coupled with electron capture detection that uses a polar chromatographic column to separate TCA peak from other wine aromas. The second method (SPME-GC-MS) is also a gas chromatography but coupled with mass spectrometry detector in selected-ion monitoring (SIM) and using a non polar column. Due to the specificity the SIM mode provides, it was not necessary a total chromatographic separation which results in a shortening of the running time.

2. Material and methods

2.1. Chemicals and reagents

TCA ([87-4-1], 99%) was supplied by Sigma–Aldrich (Madrid, Spain). The internal standard (I.S.) used was 2,3,6-trichlorotoluene (TCT [2077-46-5], 97%), which was supplied by Fluka (Buchs, Switzerland). An individual stock solution of 1000 mg l^{-1} of each compound was prepared in ethanol and stored at 4 °C. From these stock solutions we prepared standard solutions of 100, 10 and 1 μg l^{-1} by diluting with ethanol. They were also stored at 4 °C. Working solutions used in further studies were prepared by diluting different amounts of the standard solutions in either synthetic or real wine (red, rosé and white wine). All these working solutions were freshly prepared.

To validate the method we prepared two kinds of synthetic wine in order to get a matrix for emulating wine: the first one was called "synthetic wine" and the second one "complex synthetic wine". The synthetic wine was prepared by diluting 3.5 g l^{-1} of L-(+)-tartaric acid and 120 ml l^{-1} of ethanol in Milli-Q grade water. Finally, the pH was adjusted to 3.5 using NaOH. Regarding to the complex synthetic wine, it was made in order to obtain another matrix much more similar to real wine. With this purpose we added some of the main wine volatiles [25] to the synthetic wine: methanol (125 mg l^{-1}), ethanal (75 mg l^{-1}), ethyl acetate (100 mg l^{-1}), isoamyl acetate (10 mg l^{-1}), 3-methyl-1-butanol (200 mg l^{-1}), 2-methyl-1-butanol (50 mg l^{-1}) and potassium metabisulphite (275 mg l^{-1}), all of which had a purity of over 98% and were supplied by Aldrich.

2.2. SPME fibers

The manual SPME device and 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (1 cm) fibers used in this study were purchased from Supelco (Bellefonte, PA, USA). These were conditioned before use according to the supplier's instructions.

2.3. Headspace (HS)-SPME procedure

To prepare the samples, 20 ml of commercial, synthetic or complex synthetic wine were placed into a 50 ml vial (43 mm × 73 mm), with a magnetic stirring and a suitable amount of NaCl to get a saturated sample. The vials were tightly capped with a PTFE-faced silicone septum and placed in a thermostatic bath. SPME was carried out under constant magnetic stirring (500 rpm). Before the extraction step, samples were equilibrated for 30 min at 40 °C. Then, the fiber was exposed to the headspace over the sample for 70 min at the same temperature (40 °C). Finally, the fiber was removed from the sample headspace and inserted into the injection port of the gas chromatograph for the thermal desorption of the analytes at 270 °C for 1 min in splitless mode.

Although the ruggedness of the fibers has highly improved in the last years, to carry out the different studies and experiments we used different fibers in order to consider their response variability [3,6].

2.4. Samples

To carry out the different studies, we used 45 different wines (red, rosé and white) from different origins (different Spanish regions) which is representative of the degree of variability of wines within Spain.

For the validation procedure it was necessary to get wines absolutely free of TCA. However, sometimes, these were difficult to obtain so, when needed, we applied an SPE method to completely eliminate native TCA. The C_{18} cartridges of SPE (500 mg) used were supplied by Varian (Harbor City, USA). These were first conditioned with 2 ml of ethyl acetate, 2 ml of absolute ethanol and 2 ml of 10% (v/v) ethanol in Milli-Q grade water. Then, the cartridge was ready to pass an aliquot of 20 ml of wine [14].

2.5. Statistical analysis

We used the ULC (univariate linear calibration) [26] computer program to calculate the slope and intercept of the calibration curve, the determination coefficient (r^2) and the standard errors of the coefficients via linear least-squares regression. We also used ULC and linear least-squares regression to calculate the analysis of variance to determine the linearity of the calibration curves and compare their slopes. The analysis of the variance to determine the repeatability and intermediate precision was calculated by the statistical package supplied in Excel®. The comparisons of the slope and intercept using linear regression with errors in both axes (BLS) [23] were calculated using home-made subroutines (Matlab for Microsoft Windows ver. 5.3, the Mathworks, Natick, MA, USA).

2.6. Instrumental analysis

2.6.1. GC-ECD

A Hewlett-Packard (HP, Palo Alto, CA, USA) 5890 series II gas chromatograph equipped with a HP ECD system was used. The injection and thermal desorption of the analytes inside the GC injector port were made in the splitless mode for 1 min at 270 °C. Separation was made using a Chrompack (Middel-