Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1143 (2007) 8–15
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Quantitative determination of wine highly volatile sulfur compounds by using automated headspace solid-phase microextraction and gas chromatography-pulsed flame photometric detection Critical study and optimization of a new procedure

Ricardo López *, Ana Cristina Lapeña, Juan Cacho, Vicente Ferreira

Department of Analytical Chemistry, Faculty of Sciences, Universidad de Zaragoza, 50009 Zaragoza, Spain

Received 17 October 2006; received in revised form 15 December 2006; accepted 18 December 2006
Available online 21 December 2006

Abstract

The quantitative determination of wine volatile sulfur compounds by automated headspace solid-phase microextraction (HS-SPME) with a carboxen-polydimethylsiloxane (CAR-PDMS) fiber and subsequent gas chromatography-pulsed flame photometric detection (GC-PFPD) has been evaluated. The direct extraction of the sulfur compounds in 5 ml of wine has been found to suffer from matrix effects and short linear ranges, problems which could not be solved by the use of different internal standards or by multiple headspace SPME. These problems were attributed to saturation of the fiber and to competitive effects between analytes, internal standards and other wine volatiles. Another problem was the oxidation of analytes during the procedure. The reduction in sample volume by a factor 50 (0.1 ml diluted with water or brine) brought about a reduction in the amount of sulfur compounds taken in the fiber by a factor just 3.3. Consequently, a new procedure has been proposed. In a sealed vial containing 4.9 ml of saturated NaCl brine, the air is thoroughly displaced with nitrogen, and the wine (0.1 ml) and the internal standards (0.02 ml) are further introduced with a syringe through the vial septum. This sample is extracted at 35 °C for 20 min. This procedure makes a satisfactory determination possible of hydrogen sulfide, methanethiol, ethanethiol, dimethyl sulfide, diethyl sulfide and dimethyl disulfide. The linear dynamic ranges cover the normal ranges of occurrence of these analytes in wine with typical r^2 between 0.9823 and 0.9980. Reproducibility in real samples ranges from 10 to 20% and repeatability is better than 10% in most cases. The method accuracy is satisfactory, with errors below 20% for hydrogen sulfide and mostly below 10% for the other compounds. The proposed method has been applied to the analysis of 34 Spanish wines.

Keywords: Volatile sulfur compounds; Thiols; Sulfides; Disulfides; Wine; Aroma; Headspace; SPME; Pulsed flame photometric detection; Gas chromatography; Automated methods

1. Introduction

Wine contains a significant number of volatile sulfur compounds which can exert a large influence on its flavor and aroma. Some of these sulfur compounds are highly volatile molecules with boiling points below 50 °C. The compounds in this, category belong to two different but related chemical families: thiols, such as hydrogen sulfide (H_2S), methanethiol (MeSH) and ethanethiol (EtSH); sulfides, such as dimethyl sulfide (DMS); disulfides, such as dimethyl disulfide (DMDS). Their characteristics odors range from rotten eggs to cooked cabbage and are associated with the reduction off-flavors found in some wines. DMS constitutes a particular and ambiguous case, since different reports suggest that it could positively contribute to the flavor of some aromatic wines [1] or even that it could be an "odor enhancer" of the fruity notes of some high quality red wines [2]. The main source of these compounds in wine is yeast metabolism; these compounds are formed at higher levels in the fermentation of musts with low levels of assimilable nitrogen However, the formation mechanisms are not completely understood [3]. Other sources of volatile sulfur compounds include non-enzymatic pathways such as photochemical and thermal reactions [4].

As sulfur off-flavors are a major concern for the wine industry, much research has been devoted both to understand and control the formation of these compounds and to develop

* Corresponding author. Tel.: +34 976761290; fax: +34 976761292.
E-mail address: riclopez@unizar.es (R. López).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.12.053

analytical method for their quantification and control. The analytical methods for the quantitative determination of volatile sulfur compounds in wine was the subject of a relatively recent review [4]. Due to their volatility, the preferred analytical technique for their determination is gas chromatography (GC) coupled with sulfur specific detectors. However, and given the low concentrations at which these compounds are usually found in wine, different pre-concentration and/or isolation steps are required before the chromatographic analysis. Different sample preparation strategies have been proposed, such as liquid–liquid extraction with organic solvents [5], static headspace sampling [6–9], large volume headspace sampling [10], dynamic headspace [11] and more recently SPME [2,12–15]. However, the analysis of those highly volatile sulfur compounds is a challenging task not only because their low concentration, but because of their low chemical stability. These compounds are specially sensitive to oxidants and can be easily oxidized to different products [16]. Small quantities of oxidants, light or metals can cause significant losses of analytes during the different steps of the analysis. For instance, the formation in the course of the analysis [14] or during the chromatographic injection [17,18], of DMDS from MeSH or of dimethyl sulfoxide from DMS, is well documented in the literature [17,18].

During the last years, HS-SPME has been gaining acceptance as the technique of choice for the analysis of highly volatile sulfur compounds in wine. The first report on the quantitative determination of these compounds in wine using this technique dates from 1998 [13]. In that work polydimethylsiloxane (PDMS) and polyacrylate (PA) fibers were used, but in any subsequent report [11,12,15,20,21] a carboxen-polydimethylsiloxane (CAR-PDMS) fiber is chosen, as this fiber has the strongest affinity for low-molecular-weight sulfur compounds. However, the use of these fibers is not exempted of risk, since the final amount of analytes sorbed in the CAR-PDMS fiber may depend not only on the concentration of analytes in the sample but also on the levels in that particular sample of some unspecified third party compounds, as it has been demonstrated by Murray [19]. A likely consequence of such dependence is that the analytical response will be extremely dependent on the matrix composition, which ultimately may render impossible or impractical the correct calibration of the method. As it can be easily understood, such matrix effects are directly related to the amount of third party extractable compounds, and those, in turn, are related to the overall volume of sample introduced in the system. This volume has been successively decreased in a parallel manner to the increase in sensitivity of the detectors. In the first methods 25 ml of wine were sampled [12,13,20]; by 2002 the volumes were 10 or 15 ml [11,21], and a more recent procedure propose just 5 ml as optimal wine sample volume [15]. Such reduction in the sample volume is due to the high sensitivity of the pulsed flame photometric detection (PFPD) [23], which is cheaper and simpler than the alternatives sulfur chemiluminescence detection (SCD) or atomic emission detection (AED). In spite of these reductions in volume, matrix effects are still evident and different internal standards [12,14,15] or calibration strategies [12,20] are used in order to overcome or at least minimize the problem. However, and in spite of the claims of a recent report [15], such problems may not be completely controlled.

The main aim of the present study is to evaluate the analytical performance of a method based on the automated HS-SPME sampling of small volumes of wine with CAR-PDMS fibers and subsequent GC-PFPD for the quantitative determination of wine highly volatile sulfur compounds and to develop a new procedure less sensitive to matrix effects.

2. Materials and methods

2.1. Reagents and standards

Ethanol and methanol of LiChrosolv quality were from Merck (Darmstadt, Germany) and pure water was obtained from a Milli-Q purification system (Millipore, Billerica, MA, USA). Sodium sulfide, methanethiol, ethanethiol, propanethiol, dimethyl sulfide, diethyl sulfide, dimethyl disulfide, ethylmethyl sulfide, ethanedial, 5-(hydroxymethyl)-2-fufural and acetaldehyde were supplied by Sigma–Aldrich (St. Louis, MO, USA). Sodium chloride and L-tartaric acid were supplied by Panreac (Barcelona, Spain).

To preserve integrity of sulfur standards all solutions and samples were prepared in sealed vials protected from light, with solvents and vials purged with nitrogen, and always kept at low temperatures (−20 °C for methanolic solutions and 5 °C for aqueous solutions). H_2S was generated by adding aqueous solutions of known concentration of Na_2S to wines or synthetic wines.

Synthetic wine samples were prepared with a 12% (v/v) ethanolic solution containing 3.5 g/l of tartaric acid at pH adjusted to 3.5 with 2 M NaOH. NaCl brine solutions were prepared dissolving 44 or 175 g of NaCl in 500 ml of water (9 and 35%, w/v, respectively).

2.2. SPME equipment and conditions

Headspace sampling of sulfur compounds was carried out with a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) equipped with an 85 μm CAR-PDMS SPME fiber (Supelco, Bellefonte, PA, USA). Samples were incubated for 5 min at 35 °C and then extracted for 20 min at the same temperature. The extraction was performed with agitation at 250 rpm in cycles of 8 s on and 2 s off. Twenty milliliter vials were used for headspace sampling. Desorption took place in the injection port at 300 °C for 7 min.

2.3. Gas chromatography

All analyses were carried out using a Varian CP-3800 gas chromatograph fitted with a PFPD system (Walnut Creek, CA, USA). The column was a DB-WAXetr from J&W (Folsom, CA, USA), 30 m × 0.32 mm I.D. with 1 μm film thickness. The temperature program was as follows: 35 °C for 3 min, then raised at 10 °C/min up to 100 °C and then raised at 20 °C/min up to 220 °C. Injector temperature, 300 °C. Detector temperature, 300 °C. Carrier gas was hydrogen at a constant flow rate of 2 ml/min.