Analytica Chimica Acta 660 (2010) 197–205

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Comparison of extraction techniques and mass spectrometric ionization modes in the analysis of wine volatile carbonyls

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ARTICLE INFO

Article history:
Received 1 August 2009
Received in revised form
23 September 2009
Accepted 26 September 2009
Available online 1 October 2009

Keywords:
Carbonyls
Trichloroanisol
Solid phase extraction (SPE)
Solid phase microextraction (SPME)
Negative chemical ionization (NCI)
Wine

ABSTRACT

This work presents a comparative study of the analytical characteristics of two methods for the analysis of carbonyl compounds in wine, both based on the derivatization with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA). In the first method derivatives are formed in the solid phase extraction (SPE) cartridge in which the analytes have been previously isolated, while in the second method derivatives are formed in a solid phase microextraction (SPME) fibre saturated with vapors of the reagent and exposed to the sample headspace. In both cases detection has been carried out by electron impact (EI) or negative chemical ionization (NCI) mass spectrometry. The possibility of determining haloanisols simultaneously has been also considered.

The method based on SPE presents, in general, better analytical properties than the SPME one. Although linearity was satisfactory for both methods ($R^2 > 0.99$), repeatability of the SPE method (RSD < 10%) was better than that obtained with SPME (9% < RSD < 20%). Detection limits obtained with EI are better for the SPE method except for trihaloanisols, while with NCI detection limits for both strategies are comparable, although the SPME strategy presents worse results for ketones and methional. Detection limits are always lower with NCI, being the improvement most notable for SPME.

Recovery experiments show that in the case of SPE, uncertainties are lower than 12% in all cases, while with the SPME method the imprecision plus the existence of matrix effects make the global uncertainty to be higher than 15%.

1. Introduction

Carbonyl compounds are compounds of interest due to their aromatic [1], environmental, biological and technological relevance [2–6]. The presence of carbonyl compounds is associated with oxidation and fermentation processes in food and drinks [1], with lipid peroxidation in biological systems [7], with olefin ozonolysis, and with photochemical hydrocarbon reactions in the atmosphere [8]. The direct determination of carbonyls in complex matrixes is difficult due to the reactivity of the carbonyl group, particularly aldehyde, to many chromatographic phases, and to the low specificity of their mass spectra [9–10]. In the case of wine, these difficulties are aggravated by interactions with matrix components and by the major presence of carbonyls such as acetaldehyde (more than 300 mg L^{-1}) and pyruvic acid (more than 500 mg L^{-1}) [1,11–14]. For these reasons, the strategies for determining carbonyls usually are based on derivatization of the carbonyl group [6,9,10,15–19]. The most common strategy is derivatization with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA). The oximes formed with PFBHA have relatively specific mass spectra and high sensitivity in different detection systems, such as electron capture detection (ECD), electron impact mass spectrometry (EI-MS), and negative-ion chemical ionization mass spectrometry (NCI-MS) [4,16,18]. Other reagents have been used, such as 2,4-dinitrophenylhydrazine, cysteamine, and pentafluorophenyl-hydrazine, in the determination of carbonylic compounds [7,10,13,14,16,18].

Other aroma-related compounds closely associated with wine defects are the haloanisols. These compounds have a strong impact on wine quality [20] and their presence is related to microbiological contamination arising mainly from the cork. From a functional point of view, these compounds are similar to the pentafluorobenzyl-oximes, which makes that they could be determined using the same detection system used for the PFBHA-derivatives of the carbonyls, and hence, that “a priori” the simultaneous determination of carbonyls and haloanisols should be possible.

The methods most often used for the analysis of carbonyls in wine are solid phase extraction (SPE) [16–17] and solid phase microextraction (SPME) [18,21,22], in both cases using derivatiza-

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doi:10.1016/j.aca.2009.09.041

tion with PFBHA. In the case of SPE, Ferreira et al. [16] proposed a method of analysis in which derivatization is performed directly in the same solid phase extraction cartridge in which the compound was extracted. This method allows quantitative analysis of the aldehydes sensorially most significant in wine. In the case of SPME, Wang et al. [18] developed a general strategy for the analysis of carbonyl compounds based on the simultaneous headspace extraction-derivatization on a SPME fibre. The method was applied to the fully automated determination of wine carbonyls. The same strategy was used by Schmarr et al. [21,22] to exhaustively characterize the volatile carbonyls present in wine by using bidimensional gas chromatography and mass spectrometry. One drawback of both methods for the determination of certain carbonyl compounds is the ubiquity of some of the compounds in blanks. The haloanisols have also been determined in wine with both SPE [23,24] and SPME [20,25] methods, fundamentally using GC–MS as the quantification technique.

The comparison of SPE and SPME strategies is not easy at present. On the one hand, SPME has a series of obvious advantages, such as ease of automatization, simple management, and the absence of any need for organic solvents. As a result of these advantages, SPME is gaining ground with respect to other strategies that also are consolidated but require more manual labor and better knowledge of the functioning of chromatographic systems, such as SPE. Nevertheless, the greater tendency of SPME to give matrix-dependent signals in complex systems [26–28] and the problems associated with irregular fibre behavior [29] should be seriously considered and weighed when comparing the two techniques.

For this reason, the primary objective of the present study was to make an in-depth comparison of the analytical characteristics of the SPE-based and SPME-based strategies in the simultaneous determination of carbonyl compounds and haloanisols in a complex sample, such as wine. The comparative study will also include a comparison of the modes of ionization, electron impact (EI) versus negative-ion chemical ionization (NCI).

2. Experimental

2.1. Materials

Isobutyraldehyde 99%, 2-methylbutanal 95%, 3-methylbutanal (isovaleraldehyde) 97%, (*E*)-2-hexenal 98%, (*E*)-2-octenal 98%, (*E*)-2-nonenal 97%, phenylacetaldehyde >90%, methional, 2-methylpentanal 98% and hexachlorobenzene 99% were purchased from Aldrich–Spain (Madrid, Spain). (*E*)-2-heptenal 98%, 2,4-dichloroanisol (>97%, DCA), 2,4,6-tricholoroanisol (99%, TCA), 2,3,6-tricholoroanisol (99%, 2,3,6-TCA), 2,4,6-tribromoanisol (99%, TBA) and O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (99%, PFBHA), were purchased from Fluka-Spain (Madrid). 1-octen-3-one was supplied by Lancaster Synthesis 97% (Eastgate, England). 3-Methyl-2,4-nonadione >97% was a gift from Takasago International Chemicals-Europe (Murcia-Spain).

Dichloromethane (HPLC quality) was from Fisher Chemicals (Leicester, UK), methanol (HPLC grade), *n*-hexane Unisolv for traze analysis, and diethylether Pro Analyst were supplied by Merck (Darmstadt, Germany), *n*-pentane for GC-analysis >99% was purchased from Fluka. Absolute ethanol and sodium hydrogencarbonate, both ARG quality, were from Panreac (Barcelona, Spain), sulfuric acid (95–97%, synthesis grade) was from Scharlau (Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

LiChrolut-EN resins (styrene–vinylbenzene, divinylbenzene polymer), prepacked in a 200 mg cartridge (3 mL total volume) were obtained from Merck. PDMS–DVB (65 μm) SPME fibres were purchased from Supelco-Spain (Madrid, Spain).

Semiautomated solid phase extraction was carried out with a VAC ELUT 20 station system from Varian (Walnut Creek, CA, USA).

Wines for the validation study were four reds and four whites with alcoholic degrees comprised between 11.0% and 14.5% (v/v) and pHs ranging from 3.2 to 3.8; all of them were dry table wines with ages between 1 and 5 years old.

2.2. Methods

2.2.1. Solid phase extraction (SPE)

The method proposed by Ferreira et al. [16] was used. Ten milliliters of wine, containing 20 $\mu g\,L^{-1}$ of 2-methylpentanal and 5 $\mu g\,L^{-1}$ of 2,6-dichloroanisol as surrogated standards, were loaded onto a 200 mg LiChrolut-EN solid phase extraction cartridge (previously conditioned with 4 mL of dichloromethane, 4 mL of methanol and 4 mL of a 12% ethanol (v/v) aqueous solution). Acetaldehyde and some other major wine carbonyl compounds were removed by cleanup with 10 mL of an aqueous solution containing 1% $NaHCO_3$. Carbonyls retained in the cartridge were directly derivatized by passing 2 mL of an aqueous solution of PFBHA (5 $mg\,mL^{-1}$), and letting the cartridge imbibed in the reagent for 15 min at room temperature (25 °C). Excess of reagent was removed with 10 mL of 0.05 M sulfuric acid. Derivatized analytes were finally eluted with 2 mL of hexane–10% of diethylether, containing 300 $\mu g\,L^{-1}$ of hexachlorobenzene in the case of SPE-EI analysis or 500 $\mu g\,L^{-1}$ of 2,3,6-trichloroanisol in the case of SPE-NCI analysis, as internal standards. This volume was collected in a 2 mL autosampler vial and analyzed by injecting 2 μL of the extract in the chromatographic system: GC-EI-MS or GC-NCI-MS.

2.2.2. Solid phase microextraction (SPME)

The general strategy proposed by Wang et al. [30] and Vesely et al. [31] has been applied after some modifications described below. According to the method proposed by Wang 250 μL of wine are added to a 20 mL standard headspace vial that contained 5 mL of brine; then 30 μL of a solution of 2,3-DCA and 2-methylpentanal (500 $\mu g\,L^{-1}$) were added into the vial as internal standards. The PDMS/DVB SPME fibre was then placed in the headspace of the PFBHA solution (500 μL of the PFBHA 6 $\mu g\,mL^{-1}$ solution in 10 mL of deionized water) for 15 min at 50 °C. The SPME fibre loaded with PFBHA was then exposed to the headspace of the sample for 20 min at 50 °C. In both cases agitation speed was 500 rpm. Finally, the fibre containing the PFB-oximes is desorbed directly in the injection port of the chromatographic system for their determination by GC-EI-MS or GC-NCI-MS. Total automation of the procedure was achieved using a CTC CombiPal autosampler (Zwingen, Switzerland), which was programmed using the CycleComposer with macroeditor software and equipped with sample trays, a temperature controlled agitator tray and a fibre-conditioning device.

2.3. GC–MS conditions

The apparatus was a Shimadzu QP-2010 gas chromatograph with a quadrupole mass spectrometric detection system. The injector was a standard split/splitless.

In the case of SPE, splitless mode injection was used at a temperature of 250 °C with a pulse of pressure of 467 kPa during the 1.5 min splitless time. The carrier gas was He at a constant linear velocity of 35 $cm\,s^{-1}$ (≈0.62 $mL\,min^{-1}$) during the run. The flow during the splitless time (1.5 min) was 2.69 $mL\,min^{-1}$. The column was a Factor Four capillary column VF-35MS from Varian, 20 m × 0.15 mm I.D., with 0.15 μm film thickness. The chromatographic oven was held at 45 °C for 2 min, then raised to 200 °C at 10 °C min^{-1}, then to 320 °C at 10 °C min^{-1} and finally the temperature was held at 320 °C for 3 min. The temperature of the ion source was 220 °C and the interface was kept at 250 °C.