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Analytical Methods

Stereodifferentiation of some chiral aroma compounds in wine using solid phase microextraction and multidimensional gas chromatography

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ABSTRACT

The stereodifferentiation of some chiral components of importance for the character or intensity of wine aroma was achieved with headspace solid phase microextraction and multidimensional gas chromatography coupled to mass spectrometry. Chiral evaluation of linalool and 2,3-butanediol is performed in less than 75 min (overall analysis time) using a polydimethylsiloxane/divinylbenzene fibre during the sample concentration step and permethylated β-cyclodextrin as the chiral stationary phase in the main column of the multidimensional system. Enantiomeric excesses of linalool and percentage values of 2,3-butanediol stereoisomers were determined in three different white wines.

1. Introduction

Delicate and balanced aroma is widely recognised as a valuable characteristic of high-class wines. Thus, wine quality evaluation demands the reliable analysis of aroma compounds, gas chromatography being usually the technique of choice in the separation step. However, wine aroma is a very complex mixture in which certain relevant compounds are often chromatographically overlapped by other components which may disturb the separation and, eventually, may avoid satisfactory resolutions to be obtained. Moreover, although the influence of stereochemical aspects has been occasionally underlined in quality assurance of food and beverages (Ekborg-Ott & Armstrong, 1997; Luan, Mosandl, Gubesch, & Wüst, 2006; Romano et al., 2000), it is easy to realise that chiral separations and enantiomeric ratio assessments are not usually considered in the available literature when analysing wine aroma. This is probably due to the fact that enantiomeric resolutions of chiral compounds that are of importance for the character or intensity of wine aroma may be especially difficult as two chromatographic signals, for each stereochemical centre, can be observed when performing the analysis in a chiral column of adequate enantioselectivity (Schurig, 2002; Subramanian, 2001).

Precisely due to the difficulty of achieving satisfactory separations in complex mixtures, there is often required a substantial increase in the resolving power of unidimensional chromatographic techniques conventionally used. In this respect, Multidimensional Gas Chromatography (MDGC) allows a two-dimensional operation to be accomplished using two coupled columns in such a way that narrow cuts containing unresolved compounds can be selected in the precolumn and subsequently transferred to the second column, namely the main column (Bertsch, 1999; Deans, 1981; Herraiz, Reglero, Herraiz, & Loyola, 1990; Schomburg, 1995).

On the other hand, the choice of the concentration procedure most suitable to enrich the sample prior to the chromatographic analysis itself is considered as one of the most critical steps when performing an analytical method. For that reason, several authors have proposed very different sample preparation approaches to analyse volatile compounds occurring in complex matrices (Bicchi, Cordero, Liberto, Sgorbini, & Rubiolo, 2008; David & Sandra, 2007; Durán, Natera, Castro, & García-Barroso, 2006; Salinas, Zalacain, Pardo, & Alonso, 2004) and, particularly, in wine aroma (Blanch, Reglero, & Herraiz, 1995,1996; Campo, Cacho, & Ferreira, 2007; Villén, Señoráns, Reglero, & Herraiz, 1995; Zalacain, Marín, Alonso, & Salinas, 2007).

In this respect, Headspace Solid Phase Microextraction (HS-SPME) (Pawliszyn, 1995; Zhang & Pawliszyn, 1993) has proven its versatility and ease of use for volatile analysis in complex matrices of quite different characteristics, also including wine aroma (de la Calle García, Magnaghi, Reichenbächer, & Danzer, 1996; Kalua & Boss, 2008; Pozo-Bayón, Pueyo, Martín-Álvarez, & Polo, 2001; Setkova, Risticevic, & Pawliszyn, 2007). As reported by these authors, using this technique the sample preparation step is rapidly performed via the extraction and concentration on the SPME fibre of volatile compounds which subsequently are thermally desorbed and then introduced into the chromatographic system.

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So far, the usefulness of multidimensional chromatographic approaches to assess enantiomeric ratios of chiral compounds occurring in wine aroma has been occasionally reported (Bouchilloux et al., 2000; Darriet et al., 2001; Fernandes, Relva, Gomes da Silva, & Costa Freitas, 2003) although a relatively complicated sample isolation step, which includes the use of organic solvents as well as of different fractionation systems, is usually demanded. Therefore the combination of a rapid and solvent free alternative for sample preparation with a multidimensional separation technique seems to be of potential interest when dealing with complex wine aroma in which special attention must be paid to the possible presence of relevant chiral components hidden behind interferences.

The aim of this work was to evaluate the possibility of performing a rapid and reliable stereodifferentiation of relevant chiral compounds without requiring the use of organic solvents during the enrichment step. Specifically, there was intended the use of SPME combined with MDGC-MS analysis to determine the enantiomeric ratios of some chiral compounds occurring in wine aroma.

2. Materials and methods

2.1. Samples and materials

Linalool and 2,3-butanediol standards used for stereoisomer identification purposes were obtained from Fluka (Buchs, Switzerland). The three white wines analysed in this study were purchased in the commercial market. Wine 1 was elaborated from Verdejo grapes and Wine 2 from both Verdejo and Viura grapes while the variety used for Wine 3 was unspecified in the label.

2.2. Solid phase microextraction

Although experimental conditions used for the SPME procedure were initially set as suggested by other authors (Kalua & Boss, 2008; Pozo-Bayón et al., 2001) for the analysis of achiral compounds occurring in wine aroma, slight variations were introduced in the present work with a view to the specific requirements of the proposed stereodifferentiation of the target chiral compounds.

Specifically, a Supelco (Bellefonte, PA, USA) SPME fibre holder and a 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) coated fused-silica fibre were employed to retain the compounds of interest. According to the recommendations of the manufacturer, prior to its use the fibre was conditioned for 30 min in the GC injector port maintained at 250 °C. Wine samples were not subjected to any kind of pretreatment prior to SPME. As ethanol concentration in the sample has been reported to have a particularly important effect on the response of different compounds and, on the other hand, diluting wine with water negligibly alters, at least for some volatile components, the partitioning between the sample and the headspace (Kalua & Boss, 2008), a 3.4 ml volume of water and 1.6 ml of the wine was directly placed into a 12 ml vial that was sealed with plastic film after having added a 2 g weight of NaCl.

In all cases the fibre was exposed to the headspace of the sample during the so-called stabilization time (i.e., 10 min) at 40 °C and then the extraction properly said was performed by operating the fibre in the headspace mode (i.e., HS-SPME) during 10 min (at 40 °C). To facilitate the release of the investigated compounds into the headspace, constant sample stirring was applied over the whole experimental run. Upon completion of the extraction step, the target compounds were thermally desorbed into the GC injector (splitless mode) at 250 °C for 2 min and subsequently analysed by MDGC-MS as explained below.

2.3. MDGC-MS analysis of the extracts obtained by HS-SPME

The MDGC system consisted of two independent Varian (Palo Alto, CA, USA) gas chromatographs (model CP-3800) in which two columns, namely precolumn and main column, were housed. Both columns were serially coupled through a Deans based switching system (Deans, 1981) and a transfer line, which was maintained at 180 °C throughout the experimentation. Sampling introduction (achieved, as already mentioned, by thermal desorption of the solutes previously retained onto the SPME fibre) was performed using the programmed temperature vaporiser (PTV injector) of the gas chromatograph in which the precolumn is placed. The preseparation was carried out on a 30 m × 0.25 mm I.D. fused-silica capillary column coated with a 0.25 μm layer of ZB-Wax (Micron Analítica, S.A., Madrid, Spain). The oven temperature was initially kept at 40 °C and successively increased up to 95 °C (4 °C/min), 120 °C (2 °C/min) and 230 °C (4 °C/min). The final temperature was maintained for 10 min. Those cuts containing the target compounds were then transferred into the second dimension (main column) and analysed on a 25 m × 0.25 mm I.D. fused-silica capillary column coated with a 0.25 μm film thickness of permethylated β-cyclodextrin (Chirasil-β-Dex, Varian, Middelburg, The Netherlands) by increasing its temperature from 50 °C (15 min) to 70 °C (1 °C/min) then to 140 °C (2 °C/min) and finally up to 200 °C (4 °C/min). In both dimensions, helium served as the carrier gas at an approximate head pressure of 30 psig in the precolumn and 24 psig in the main column.

Separations achieved in the precolumn were monitorised using an FID detector (operated at 250 °C) while the main column was connected to a Saturn 2000 ion-trap mass spectrometer (Varian). Data acquisition was performed using a Star Toolbar system (Varian).

The target compounds were identified by matching their GC retention times (in both the precolumn and the main column) with those obtained from authentic standards analysed under the same experimental conditions. Moreover, mass spectra recorded from the standard compounds were also compared with those provided by the US National Institute of Standards and Technology (NIST) library. For the MS, the electron multiplier was set to 1850 V and ionisation was accomplished by electron impact (EI). The temperatures of the transfer line, the manifold and the trap were fixed at 180, 120 and 220 °C, respectively. The recorded spectra covered the range from 40 to 650 m/z.

Under the experimental conditions applied in the overall analysis, acceptable blanks (i.e., runs made with no sample injected to clean out any impurities that might have accumulated in the column) were obtained for the complete procedure between consecutive runs.

3. Results and discussion

The starting point of this research was the realisation that the large array of chemical and enzymatic reactions which, through the winemaking process, contributes to the characteristic volatile composition of the final product, result in such a complex sample that the reliable stereodifferentiation of the occurring chiral compounds implies a quite difficult task.

Thus, we considered essential for successful performance of our work the combined use of efficient analytical techniques suitable to achieve the required enrichment of the target stereoisomers as well as its subsequent separation and identification. Specifically, these requirements imply that the enrichment step previous to the gas chromatography operation must preserve the characteristic enantiomeric excesses of the target compounds (i.e., experimental conditions avoiding the racemisation of chiral components