Journal of Chromatography A, 1381 (2015) 210–218

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Determination of 2-, 3-, 4-methylpentanoic and cyclohexanecarboxylic acids in wine: Development of a selective method based on solid phase extraction and gas chromatography-negative chemical ionization mass spectrometry and its application to different wines and alcoholic beverages

Elisa Gracia-Moreno*, Ricardo Lopez, Vicente Ferreira

Laboratorio de análisis del aroma y enología, Departamento de Química Analítica, Facultad de Ciencias, Aragon Institute of Engineering Research (I3A), Universidad de Zaragoza, E-50009 Zaragoza, Spain

ARTICLE INFO

Article history:
Received 3 October 2014
Received in revised form
19 December 2014
Accepted 23 December 2014
Available online 31 December 2014

Keywords:
2-, 3- and 4-Methylpentanoic acids
Cyclohexanecarboxylic acid
Wine
SPE
GC-NCI-MS
Selective isolation

ABSTRACT

A method to analyse 2-methylpentanoic, 3-methylpentanoic and 4-methylpentanoic acids as well as cyclohexanecarboxylic acid has been developed and applied to wine and other alcoholic beverages. Selective isolation with solid phase extraction, derivatization with 2,3,4,5,6-pentafluorobenzyl bromide at room temperature for 30 minutes, and further analysis by gas chromatography-mass spectrometry in negative chemical ionization mode provides detection limits between 0.4 and 2.4 ng/L. Good linearity up to 3.6 μg/L, satisfactory reproducibility (RSD < 10%) and signal recovery of around 100% represent a robust method of analysis. Concentration data of these analytes in wine and other alcoholic beverages are reported for the first time. The levels found ranged from the method detection limits to 2630 ng/L, 2040 ng/L and 3810 ng/L for 2-, 3- and 4-methylpentanoic acids, respectively, and to 1780 ng/L for cyclohexanecarboxylic acid. There are significant differences depending on the type of wine or beverage. Distilled beverages, beer and aged wines have higher contents in methylpentanoic and cyclohexanecarboxylic acids.

1. Introduction

Fatty acids are essential in living organisms as components of cellular membranes and as energy reservoirs in the form of triacylglycerols. They can be classified into long- and short-chain as well as into straight- and branched-chain fatty acids. In wine, short-chain fatty acids (SCFAs) are relevant because they are related to unpleasant aromas such as rancid, butter, cheese and sweat [1].

On the other hand, the esterification of fatty acids in the presence of ethanol produces their corresponding ethyl esters [2]. This has been amply studied because of the aromatic importance of ethyl esters in the overall aroma of wine [3–5]. Their fruity descriptors contribute to a positive balance in the aroma. A different behaviour during ageing has been found for esters of branched fatty acids and those of linear fatty acids. The first group increases in concentration during ageing, whereas the second one decreases [2]. Thus, short-chain branched fatty acids could act as reservoirs of fruity aromas to be developed during ageing.

In the last decade, Campo et al. identified four novel esters in wine as responsible for powerful strawberry aromas: 2-, 3-, and 4-methylpentanoate ethyl esters and cyclohexanecarboxylate ethyl ester [6,7]. The same authors reported a connection between ageing of the samples and a higher content of the esters, and postulated that the origin of these ethyl esters could be the esterification of their corresponding acids [8]. These results suggest the plausibility of finding 2-, 3- and 4-methylpentanoic and cyclohexanecarboxylic acids in wine. To the best of our knowledge, none of the four analytes has yet been analysed in grape wine. However, the presence of 2- and 4-methylpentanoic acids, as well as 4-methylpentanoate and cyclohexanecarboxylate ethyl esters, has already been described in Chinese liquors made from mixtures of cereals [9,10]. 4-Methylpentanoic acid has also been determined in rice wine [11] and 2-methylpentanoic acid has been identified in some commercially available yeast derivatives added to wine [12,13]. Finding these acids in wine would be the first step towards eventually proving or refuting the hypothesis

* Corresponding author. Tel.: +34 876553330; fax: +34 976761292.
E-mail address: elisagm@unizar.es (E. Gracia-Moreno).

http://dx.doi.org/10.1016/j.chroma.2014.12.074
0021-9673/

that the origin of the corresponding ethyl esters is esterification.

The ratio between acid and ethyl ester concentrations ranges from two up to ten for branched and linear acids [14]. Assuming a similar behaviour for the methylpentanoic and cyclohexanecarboxylic acids, the predictable concentrations of the acids studied in this paper could be expected to be higher than those obtained for their corresponding ethyl esters. Following this hypothesis, and taking into account the concentration of the ethyl esters obtained in [8,14,15], we could expect concentrations to be a few μg/L in the case of 4-methylpentanoic acid. For the rest of the acids, ng/L levels could be expected. In particular, low ng/L concentrations are expected for cyclohexanecarboxylic acid.

The sample preparation methods used to analyse methylpentanoic acids in other matrices have been based on the extraction of large quantities of brew or fish sauce with different sorbents (Tenax or Porapack Q) in classic columns [16,17], solid–liquid extraction from tobacco leaves in an acidified medium [18] or HS-Tenax extraction in the case of dry fermented sausages [19]. In the case of Chinese liquors, liquid–liquid extraction with diethyl ether and further fractionation into acidic, basic and neutral fractions was used [9]. However, no quantitative data were provided with this method. The analysis and detection of the extracts in the aforementioned cases was carried out by gas chromatography (GC). The columns used for the isolation of the analytes were polar in most cases [16–18] with the exception of [19] in which an apolar column was used. As for the detection, flame ionic detector (GC-FID) [16–18] and mass spectrometric detection in electronic impact mode and (GC-MS-EI) [16,17,19] were used. Fan et al. used both types of column and carried out the identification of compounds with an olfatometric detector (GC-O-FID) and GC-MS-EI [9].

Linear and branched short chain fatty acids have been analysed in wine by different methods such as liquid–liquid extraction with different solvents [20], solid phase extraction (SPE) [21] and solid phase micro-extraction (SPME) [22]. However, the expected low amount of the target acids in this study requires a method able to provide a good pre-concentration of the sample that can be provided by SPE. Furthermore, the use of the acid properties of the analytes can help with the pre-concentration and cleaning of the samples. Acid and basic properties of the analytes have been used in the past to improve the selectivity of the isolation: ionic or mixed-mode sorbents, selective elution or both [23,24]. The bad chromatographic properties of the acids and their poor detectability in MS are addressed with a derivatization method.

The objectives of this paper are the development and validation of a method to analyse the three above-mentioned methylpentanoic acids and cyclohexanecarboxylic acid at the ng/L level, as well as to provide the first data relating to the four analytes in a variety of wines and other beverages.

2. Materials and methods

2.1. Reagents and standards

The standards of 2-methylpentanoic acid (2MePc), 3-methylpentanoic (3MePc) acid, 4-methylpentanoic acid (4MePc), cyclohexanecarboxylic acid and 2-ethylbutanoic (2EtBc) acid were supplied by Aldrich (Steinheim, Germany) with purity higher than 96% in all cases. 2,3,4,5,6-Pentafluorobenzyl bromide (PFBBr) and tetrabutylamonium chloride (NBu_4Cl) (>97%) were also obtained from Aldrich.

The solvents used were Unisolv quality hexane (Hx), Lichrosolv quality ethanol, Suprasolv quality methanol (MeOH) and dichloromethane (DCM), and diethyl ether, all supplied by Merck (Darmstadt, Germany). Toluene 99.5% was supplied by Panreac (Barcelona, Spain). Pure water was obtained from a milli-Q purification system (Millipore, Bedford, MA, USA).

The sorbents used were: Oasis MAX (60 mg, 3 mL reservoir) supplied by Waters (Milford, USA), and LiChrolut EN resins both pre-packed (200 mg, 3 mL reservoirs) and in-house packed (50 mg in 1 mL reservoir) obtained from Merck. SPE was performed with the help of a Vac Elut 20 system supplied by Varian (Sunnyvale, CA, USA). Silica-gel 60 was obtained from Merck.

Standard solutions of the acids were prepared in hexane to avoid esterification. Those used to spike, either wine or synthetic wine, were prepared in ethanol prior to spiking.

2.2. Wines and alcoholic beverages samples

Two commercial Spanish young red wines were used for the development of the method. Additionally, twenty-one samples were analysed, including red and white wines with diverse degrees of ageing, and other alcoholic beverages such as beer, whisky and brandy. Detailed information about the samples can be found in the supplementary content (Table 1).

2.3. SPE method development

2.3.1. Sorbent selection and breakthrough volumes

Mixed-mode anionic Oasis MAX sorbent (60 mg, 3 mL reservoir) was conditioned with 2 mL DCM, 2 mL MeOH and 4 mL hydroalcoholic solution (12% ethanol). Synthetic wine was spiked with 1.6 mg/L of the acids studied and its pH was adjusted to 7.0 prior to the loading of the cartridges. Vacuum suction was not applied in this particular experiment to avoid losses of the non-retained analytes due to their volatility. The percolated solutions (10 mL fractions up to 100 mL) were collected and the pH readjusted to 2.7. The solutions were then analysed with the method described in [21]. Lichrolut EN sorbent (200 mg, 3 mL reservoirs) conditioned with 4 mL DCM, 4 mL MeOH and 4 mL hydroalcoholic solution (12% ethanol) was used to analyse the samples. After loading the samples under vacuum suction, 1 mL of milli-Q water was used to clean the cartridges. The sorbent was dried under nitrogen and the analytes were eluted with 1.6 mL of DCM.

Generic hydrophobic LiChrolut EN sorbent (200 mg, 3 mL reservoirs) was also studied. Conditioning was done with 4 mL DCM, 4 mL MeOH and 4 mL hydroalcoholic solution (12% ethanol). A young red wine spiked in this case with the analytes in a concentration of 5 mg/L was loaded without vacuum suction. Different fractions (10 mL each) up to 100 mL of the percolated solution were recovered and analysed as described above. Ten milliliter of the spiked wine was analysed following the same procedure as with the percolated fractions and was used as a reference to calculate the breakthrough volumes.

2.3.2. Removal of interferences and matrix compounds

Fifty milliliter of a young red wine from Rioja spiked with 5 mg/L of the analytes was loaded into a 200 mg LiChrolut EN cartridge. Five fractions (1 mL each) of a 40% MeOH solution in milli-Q water buffered at pH 3 with H_3PO_4/NaH_2PO_4, were used to clean the cartridge without vacuum suction. The percolated solutions were analysed as in [21].

2.3.3. Optimization of the elution strategy

Five LiChrolut EN cartridges conditioned as aforementioned were loaded with 50 mL each of a young red wine from Rioja spiked with 5 mg/L of the analytes. Five solutions of milli-Q water buffered at pH 7.0 with NaH_2PO_4/Na_2HPO_4, containing different percentages of MeOH (5, 15, 25, 35, and 40) were prepared and used to elute a different cartridge each (4 fractions of 5 mL). The 20 recovered eluates were each supplemented with 2 mL of a 0.625 M tartaric