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Gas chromatographic quantification of aliphatic aldehydes in freshly distilled Calvados and Cognac using 3-methylbenzothiazolin-2-one hydrazone as derivative agent

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Abstract

A new precise and sensitive method was used for the quantification of aliphatic aldehydes from C_5 to C_{11} in highly ethanolic beverages such as freshly distilled spirits. Carbonyl compounds were derivatized using 3-methylbenzothiazolin-2-one hydrazone (MBTH) and then separated and detected by gas chromatography–mass spectrometry (GC–MS). Selective mass spectrometric detection of molecular ions of derivatives was performed to obtain a good sensibility (0.2–1.2 $\mu g\,l^{-1}$) and a good selectivity. For a concentration of 20 $\mu g\,l^{-1}$, relative standard deviations were lower than 10% except for heaviest compounds (decanal and undecanal) where RSD were between 11 and 13%. The concentrations of aliphatic aldehydes were determined in nine samples of freshly distilled Calvados and two samples of freshly distilled Cognac with highest concentrations reported for 3-methylbutanal (from 170 to 1220 $\mu g\,l^{-1}$ in Calvados and from 1540 to 5500 $\mu g\,l^{-1}$ in Cognac). 3-Methylbutanal and hexanal, due to their low detection thresholds, could be important olfactive markers of these two products. Less than 1h30 is required to quantify the nine studied aliphatic aldehydes in freshly distilled spirits.

Keywords: Calvados; Cognac; Aliphatic aldehydes; Gas chromatography–mass spectrometry; MBTH; Derivatization; Beverage analysis

1. Introduction

Carbonyl compounds are frequently quantified in natural and food products because of their odorant or toxic properties. Their occurrence in freshly distilled Calvados and Cognac was already determined in a previous work [1] and it showed that numerous aliphatic aldehydes were present in such spirits. However, they were not quantified whereas some of them are known to have a real olfactive impact [2,3]. Various methods were employed for the quantification of aldehydes in natural or food products. These carbonyl compounds are generally derivatized in order to be easily detected and then quantified. The most popular method is using the transformation of 2,4-dinitrophenylhydrazine (2,4-DNPH) in 2,4-dinitrophenylhydrazones. This derivative agent was firstly used for the identification of carbonyl compounds with separation and detection by thin layer chromatography [4]. However, separation of derivatives was rapidly performed with more accurate techniques like gas chromatography [5] or liquid chromatography [6,7]. LC was also coupled with mass spectrometry (MS) for the detection of a deuterated derivative of 2,4-DNPH [8]. Taking advantage of the specific reaction between hydrazines and carbonyl compounds, many reagents like 2,4,6-trichlorophenylhydrazine [9], 4-dimethylamino-6-(4-methoxy-1-naphthyl)-1,3,5-triazine-2-hydrazine (DMNTH) [10] and 2-nitrophenylhydrazine (2-NPH) [11] have been selected for the quantification of aldehydes and ketones. The detection of the corresponding hydrazones was performed by GC–ECD or LC coupled with mass spectrometry. Another hydrazine, the pentafluorophenylhydrazine (PFPH) was chosen using GC coupled with detectors such as ECD, MS, flame ionisation (FID) or nitrogen-phosphorus (NPD) [12]. PFPH was also adsorbed on a solid-phase microextraction fiber (SPME) for the analysis of carbonyl compounds by GC [13]. The other most common derivatizing agents are *o*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBOA) [14–16], 2-aminoethanethiol (cysteamine) [17,18] which are, respectively converted in hydrox-

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imes and in thiazolidines by reaction with aldehydes and ketones. PFBOA derivatives can be easily detected using electron capture detection after GC separation because of the presence of five fluor atoms.

Only a few works were aimed at quantifying aliphatic aldehydes in highly ethanolic beverages. To our knowledge, authors systematically chose to use PFBOA as derivatizing agent to quantify aliphatic aldehydes from C_2 (ethanal) to C_7 (heptanal) in beer [19], unsaturated aldehydes also in beer [20] and finally low weight aliphatic aldehydes from C_1 (methanal) to C_6 (hexanal) in spirits like vodka [21].

In this study we chose to take advantage of the specific reaction between carbonyl compounds and 3-methylbenzothiazolin-2-one hydrazone (MBTH) in order to quantify aliphatic aldehydes from C_5 (pentanal) to C_{11} (undecanal) in two French freshly distilled spirits: Calvados and Cognac.

MBTH was used for the first time as an analytical tool in 1967 by Sawicki et al. [22]. These authors developed a colorimetric test in order to estimate and detect the presence of carbonyl compounds. This test was applied to the determination of various kinds of compounds like total aliphatic aldehydes [23], phenols [24], reducing sugars [25] and antidepressant drugs [26]. It enables to quantify the whole compounds, which possess a carbonyl function. Moreover, separation and detection of MBTH derivatives (azines) was performed using HPLC in order to selectively quantify low weight aliphatic aldehydes from methanal to butanal [27]. In a previous work we used MBTH to determine the concentration of acrolein [28] in cider and Calvados. Azines produced from acrolein were separated by GC and were detected using a nitrogen phosphorus detector. Compared to other methods, MBTH derivation of carbonyl compounds can be achieved at room temperature. This can avoid the unwanted reaction of acetalisation, which may occur with an excess of ethanol.

For the quantification of aliphatic aldehydes, the corresponding azines will be separated by gas chromatography. Due to its sensibility and to the opportunity of selecting a particular ion, the mass spectrometer can be considered as an universal detector for carbonyl compounds derivatives and it will be consequently used in this study.

2. Experimental

2.1. Samples

Nine samples of freshly distilled Calvados were studied with four selected from the vintage 2000 (labelled Calvados 1-00; 2-00; 3-00 and 4-00) and five from the vintage 2001 (labelled Calvados 1-01; 2-01; 3-01; 4-01 and 5-01). Two samples of freshly distilled Cognac from the vintage 2001 (labelled Cognac 1-01 and 2-01) were also analyzed. Samples were selected by producers themselves as "good quality" spirits.

2.2. Solutions and reagents

- A stock solution containing nine aliphatic aldehydes was prepared: 250 mg of 3-methylbutanal, 2-methylbutanal, pentanal, hexanal, heptanal, octanal, nonanal, decanal and undecanal were added in 250 ml of pure hexane. Solutions containing the nine aliphatic aldehydes at 10^{-2} (A), 10^{-3} (B) and 10^{-4} (C) g l^{-1} were then prepared in hexane by successive dilutions. Standard solutions at 100, 200, 400, 600, 800, 1000, 2000, 4000, 6000, 8000 and finally 10,000 μg l^{-1} in hexane were prepared from solutions A, B and C in order to build the calibration curve. Hexane of HPLC grade and each aliphatic aldehydes of highest purity were purchased from ACROS Organics (Noisy Le Grand, France). Solutions were stored in a freezer (−18 °C) for a maximum period of 4 weeks.
- MBTH solution was prepared using 0.216 g of MBTH as chlorhydrate salt (from Sigma-Aldrich Chimie SARL, St Quentin Fallavier, France) in 100 ml of HCl (0.1 M). A phosphate buffer solution was prepared by adding 4.30 g of Na_2HPO_4 and 10.46 g of NaH_2PO_4 in 100 ml of ultra pure water. These solutions were stored in a refrigerator (+4 °C) for a maximum period of 4 weeks.
- In order to control aldehyde quantities in standards solutions and samples an internal standard solution was prepared. It was composed of 2,2-dimethylpropanal (2,2-DMP) at 4 mg l^{-1} in hexane.

2.3. Derivation of aldehydes using MBTH

0.5 ml of solution containing the aliphatic aldehydes in hexane and 0.5 ml of the internal standard solution (2,2-DMP at 4 mg l^{-1} in hexane) are placed in a 50 ml-conical flask. 2 ml of the MBTH solution, 2 ml of the phosphate buffer solution and 25 ml of an ethanol/water (70/30) mixture are then added. Reaction is led at room temperature under magnetic stirring during 45 min. The mixture is then poured in a separatory funnel and the upper layer is recovered in a test tube and stored in a refrigerator before analysis in gas chromatography–mass spectrometry (GC–MS).

For sample preparation, the 0.5 ml of the solution containing aldehydes is replaced by 0.5 ml of pure hexane and the 25 ml ethanol/water mixture is replaced by 25 ml of freshly distilled Calvados or Cognac. At that point it is important to note that volumes of standard solutions are 0.5 ml whereas volumes of samples are 25 ml. As a consequence, if the concentration of an aldehyde is 1 mg l^{-1} in a standard solution, its concentration will be equivalent to 20 μg l^{-1} in a sample. For each sample, another analysis was operated using 1 ml of each sample diluted in 25 ml of an ethanol/water 70/30 mixture in order to quantify compounds, which concentrations may be over range.

2.4. Gas chromatography–mass spectrometry

GC–MS analyses were carried out on a Varian 3800 gas chromatography interfaced with a Saturn 2000 mass spectrometer equipped with an ion trap analyser. Separations were performed using a 30 m × 0.25 mm (i.d.) capillary column, coated with a 0.15 μm film of ZB-Wax stationary phase (100% polyethyleneglycol from Phenomenex, Torrance, CA) equivalent to DB-Wax or Carbowax 20M. Helium was used as a carrier gas with a 1 ml/min flow rate. For the analysis of the extracts the oven program temperature used was 150–260 °C at a rate of 15 °C/min,