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Short Communication

Determination of gamma-butyrolactone (GBL) in foods by SBSE-TD/GC/MS

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Abstract

4-hydroxybutyric acid lactone, or gamma-butyrolactone (GBL), is a molecule included in the FEMA (Flavor Extract Manufacturers Association) list of flavouring substances (No. 3291) and used as natural-identical substance in flavouring formulation. Recently, the use of GBL has been subjected to restrictions by FDA because, together with the conversion derivative GHB (gamma-hydroxybutyric acid), it has been recognized and classified as psychotropic. In 1999 the Italian law included GBL in the list of psychotropic substances and more recently in the Table of substances inducing psychic and physical addiction. However, GBL has been detected in several natural products. Under a toxicological point of view the difference between natural and natural-identical GBL in food is not relevant. In any case, the evaluation of the total content of GBL in many foods contributes to evaluate the consumption ratio and the aim of this paper is to evidence the possibility of detecting GBL with a high sensitivity method also in complex matrices with analytical procedure characterized by high recovery. This paper proposes a new, simplified, sensitive and selective method (SBSE-TD/GC/MS) useful to detect and quantify the GBL in foods. The work shows, as an application of this method, the detection of this molecule in the very complex matrix of a confectionery product on the international market. The reliability of the method was verified by recovery and repeatability tests. Recovery average values result between 85–92% (CV% = 8.2–5.3) for the concentration range 1–10 mg/l and between 92–95% (CV% = 6.5–4.2) for the concentration range 10–25 mg/l.

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1. Introduction

GBL (4-hydroxybutyric acid lactone or gamma-butyrolactone, FEMA No. 3291) is a small four carbon molecule ($C_4H_6O_2$, CAS No. 96-48-0) which has a faint, sweet-aromatic odour (Arctander, 1969) and for its buttery flavour (Dufossé, Latrasse, & Spinnler, 1994) is often used in flavourings formulation. It is also included in the “Register of flavouring substances” adopted by the European Union in 1996 (Decision 217/EC, 1999) and in its last revision (Decision 113/EC, 2002).

GBL has been identified in various natural food matrices and foodstuffs. For various natural matrices containing GBL the bibliography reports quantitative data, as for example in table and sherry wines (2.8–24.7 mg/l) (Etievant, 1991), roasted coffee (4.7 mg/kg) (Stofberg & Stoffelsma, 1981), soy beans (1370 mg/kg) and mung beans (1857 mg/kg) (Lee & Shibamoto, 2000). It has also been identified in tea and cocoa (Van Straten & Maarse, 1983), and some tropical fruits (Wan, Stevenson, Chen, & Melton, 1999; Paniandy, Chane-Ming, & Pieribattesti, 2000).

In 1990 the US FDA banned this compound and in 1999 asked the companies that manufacture products containing GBL to voluntarily recall them (US Department of Health and Human Services and Administration, 1999).

In 1999 the Italian law included GBL, and GHB (4-hydroxybutyric acid), in the list of drugs and psychotropic substances (Decreto, 1999); successively in 2002 GBL and GHB have been included in “Table IV” of drugs and psychotropic substances, namely the table including substances of therapeutic usage having an actual risk of inducing psychic or physical addiction (Decreto, 2002).

This work aims to confirm the possibility of using the SBSE/TD system to evidence small amounts of GBL (natural or added) with high sensitivity and without the use of extraction procedures which can induce loss of extraction yields. The aim of the work is not the differentiation of natural and/or added GBL, because under a toxicological point of view the difference does not exist. Therefore the problem of differentiation is absolutely not relevant.

The analytical system proposed—SBSE (Stir Bar Sorptive Extraction) associated with the TD/GC/MS system—can give more satisfactory results to determine various specific compounds at trace level with high sensitivity and a high recovery.

The SBSE method has been successfully applied to evaluate other trace compounds (David, Sandra, Hoffman, Harms, & Nietzsche, 2001; Heiden, Kolahgar, & Hoffman, 2001; Hoffman, Sponholz, David, & Sandra, 2000; Hoffman & Heiden, 2000; Ochiai et al., 2002; Sandra, Baltussen, David, & Hoffman, 2000; Sandra et al., 2001; Tateo & Bononi, 2003a, b; Tredoux, Lauer, Heideman, & Sandra, 2000; Vercauteren et al., 2001). The work shows, as an application of this method, the detection of this molecule in the very complex matrix of a confectionery product on the international market.

2. Materials and methods

2.1. Materials

A confectionery product sold on the international market made up of a chocolate shell filled with a complex matrix was chosen as an application example of the suggested analytical

technique. The method is described through the analysis of the liquid-phase filling containing an aqueous-alcoholic cherry-flavoured sugary syrup.

For the evaluation of recovery and to prepare standard solutions for calibration, GBL standard (>99%) from Sigma-Aldrich (Milan, Italy) has been used.

2.2. Apparatus and principles of use

The extraction was performed by a particular stir bar known as Twister consisting of a magnetic bar sealed inside a glass tube 12 mm long and coated with PDMS (polydimethylsiloxane) with a thickness of 0.5 mm (Gerstel, Muellheim a/d Ruhr, Germany).

Prior to use, the stir bar was conditioned under a flow of helium in a glass desorption tube (178 mm × 6 mm OD, 4 mm ID) of the thermal desorption unit (TDS-2, Gerstel) installed on a GC-MS (HP 5890/5971A, Hewlett Packard, Little Falls, CA).

The stir bar, removed from the desorption tube, was placed in a vial where the sorption of analytes was realized (see *Extraction procedures* below).

The GC separations were performed with a polar column SupelcoWax-10 (30 m × 0.25 mm ID, 0.25 μm film; Supelco, Bellefonte, PA). The GC-MS is provided with a cooling injection system (CIS-3—Gerstel) able to cryofocus the analytes desorbed from the stir bar, maintaining a temperature of −50°C during the desorption time utilising liquid nitrogen.

A schematic overview of the apparatus is shown in Fig. 1.

2.3. Extraction procedure and TD/GC/MS operating conditions

The stir bar was placed in a vial containing 5 ml of the filling mechanically withdrawn from 6 to 8 sample units. The vial was crimped with a Teflon-coated silicone rubber septum. The stir bar was stirred for 120 min at room temperature. It was then removed from the sample and washed with a small volume of deionized water and dried on a paper cloth. The stir bar was then positioned in the desorption tube and the analysis performed as described below.

For the thermal desorption unit (TDS-2) the parameters were set as follow: temperature was programmed from 20°C to 240°C (held for 20 min) at 60°C/min; carrier gas was helium at 50 kPa at constant pressure; desorption in splitless mode; transfer line was at 280°C.

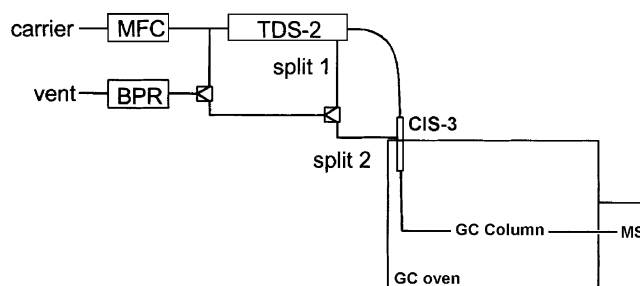


Fig. 1. Schematic overview of the TDS-/GC-MS system. MFC: mass flow controller; BPR: back pressure regulator; TDS-2: thermal desorption system; CIS-3 (cooling injection system).

The injection device (CIS-3) PTV was set in split mode (split ratio: 1/20); the injection temperature was programmed from -50°C to 240°C (held for 2 min) at 12°C/s .

For the GC-MS analysis the following parameters were set: oven temperature was programmed from 50°C (10 min) to 200°C at 2°C/min ; carrier gas was helium at 50 kPa constant pressure. The mass spectrometer was operated in multiple ion detection (MID) mode with electronic impact ionization (ionization voltage 70 eV). For MID two ions were monitored (m/z 56 and m/z 86).

3. Results and discussions

In the case of SBSE application on alcohol–aqueous matrices the quantification of organic compounds is usually done using standard addition. Considering that the slopes of the standard addition curves for various organic compounds, included GBL, result to be constant for very similar matrices having the same alcoholic degree, the same sugar concentration and same pH, it follows that the calibration can be done by spiking blank reference matrices.

For the purpose of confirming the identity of GBL in the samples, relative ion intensities (ions m/z 56 and 86) were calculated for standard solutions as well as for the matrices. All peaks of GBL identified were confirmed by spiking.

The reliability of the method was verified by recovery and repeatability tests.

Recoveries of added GBL were evaluated analysing samples spiked with GBL standard in two concentration range, the first between 1–10 mg/l and the second between 10–25 mg/l. Recovery experiments were performed in triplicate and the average values were between 85–92% ($\text{CV}\% = 8.2\text{--}5.3$) for the concentration range 1–10 mg/l and between 92–95% ($\text{CV}\% = 6.5\text{--}4.2$) for the concentration range between 10–25 mg/l.

When operating the mass spectrometer in ion monitoring mode, the limit of detection for GBL was in the order of 0.05 mg/l (LOD) and the limit of quantitation was assumed to be 0.15 mg/l

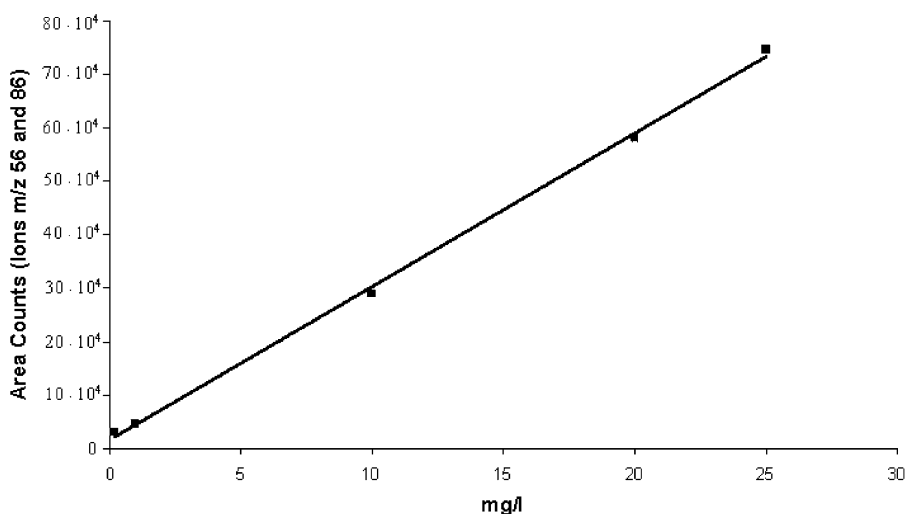


Fig. 2. Calibration curve obtained by standard GBL between concentration 0.1–25 mg/l ($R^2 = 0.9988$).

(LOQ). Linearity was tested ($R^2 = 0.9986$) in a concentration range between 0.1 and 25.0 mg/l using the area counts corresponding to the sum of the ions m/z 56 and 86.

The calibration curve (Fig. 2) was made using standard solutions prepared at the same sugar concentration (45% w/w), alcoholic degree (30% v/v) and pH (4.4) of the analysed syrup.

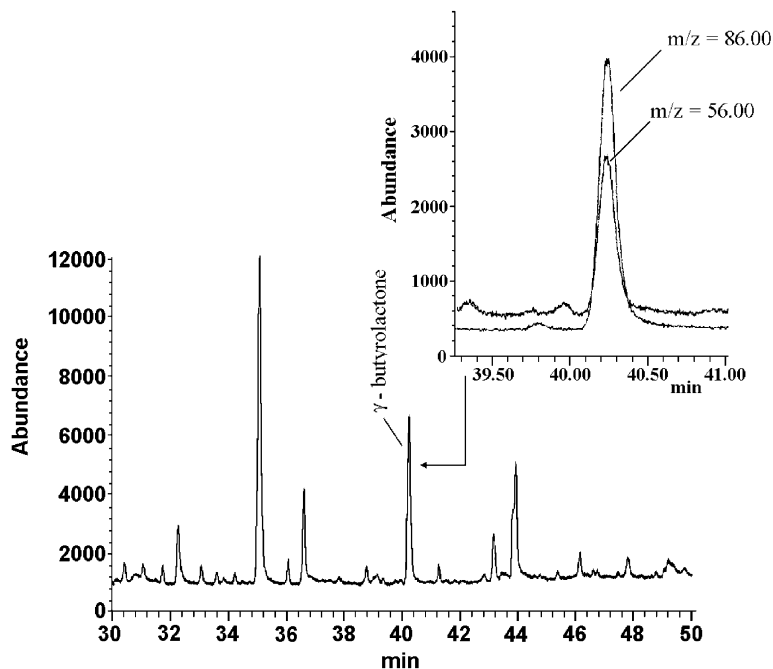


Fig. 3. GC/MS-MID trace (56 and 86 m/z) of a cherry-flavoured sugary syrup containing 15.3 mg/l of GBL. GBL produces a clear and well detectable signal.

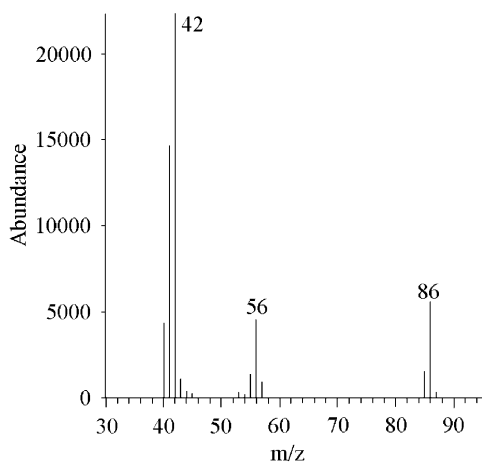


Fig. 4. MS spectrum produced by standard GBL in scan mode.

Fig. 3 shows the chromatogram obtained from the analysis of the sample described in the “Materials and Methods” section. In the same figure the traces of the ions at m/z 56 and 86 are shown for the peak eluted at 40.22 min: the ratio between these ions and the retention time clearly identify the peak as GBL, confirmed also by spiking. A scan spectrum obtained from a standard solution of GBL is reported for comparison in Fig. 4.

In this way the application of the SBSE method has been successful to identify and quantify the GBL molecule, poorly detectable by other extraction and analysis methods.

The calibration curve allows to define a value for the GBL content in the analysed sample: in that way we were able to set a value of 15.3 mg/l for the GBL in the filling of the confectionery product taken into consideration (Fossati, 2003).

If the composition of the sample matrix may by any chance justify the presence and the spontaneous conversion of GHB to GBL, the evaluation of total GBL content, detected as is, constitutes in any case an index useful for toxicological consideration.

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