Journal of Chromatography A, 1399 (2015) 18–24

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Evaluation of three headspace sorptive extraction coatings for the determination of volatile terpenes in honey using gas chromatography–mass spectrometry

J.I. Cacho, N. Campillo, P. Viñas, M. Hernández-Córdoba*

Department of Analytical Chemistry, Faculty of Chemistry, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, E-30100 Murcia, Spain

ARTICLE INFO

Article history:
Received 13 March 2015
Received in revised form 20 April 2015
Accepted 21 April 2015
Available online 28 April 2015

Keywords:
Monoterpenes
Honey
Headspace sorptive extraction (HSSE)
Gas chromatography–mass spectrometry (GC–MS)

ABSTRACT

Headspace sorptive extraction (HSSE) was used to preconcentrate seven monoterpenes (eucalyptol, linalool, menthol, geraniol, carvacrol, thymol and eugenol) for separation by gas chromatography and mass spectrometry (GC–MS). Three commercially available coatings for the stir bars, namely Polydimethylsiloxane (PDMS), polyacrilate (PA) and Ethylene glycol-silicone (EG-Silicone), were tested, and the influential parameters both in the adsorption and the thermal desorption steps were optimized. PDMS provided the best sensitivity for linalool, geraniol, menthol and eucalyptol, whereas EG-Silicone was best for extracting the phenolic monoterpenes studied. Considering the average obtained slopes from all compounds, PDMS pointed as the best option, and the analytical characteristics for the HSSE-TD-GC–MS method using this coating were obtained. Quantification of the samples was carried out by matrix-matched calibration using a synthetic honey. Detection limits ranged between 0.007 and 0.032 ng g^{-1}, depending on the compound. Twelve honey samples of different floral origins were analyzed using the HSSE-GC–MS method, the analytes being detected at concentrations up to 64 ng g^{-1}.

1. Introduction

Headspace sorptive extraction (HSSE) is a stir bar sorptive extraction (SBSE) derived technique [1], in which the stir bar coated with a polymeric extracting phase is exposed to the headspace of the sample vial, trapping the volatile analytes. Thermo-desorption of the retained compounds in a specific injector composed of a thermal desorption unit (TDU) and a programmed temperature vaporizing (PTV) injector is the best option when the analytes are to be submitted to gas chromatography (GC) separation. Due to the larger amount of extracting phase than when solid-phase microextraction (SPME) is used, HSSE provides higher recoveries and sensitivities [2]; moreover, the robustness of the stir bar assembly facilitates its application.

Unlike SPME, for which a wide range of extracting phases is available, PDMS has long been the only choice in HSSE. Polar compounds, with octanol-water partition coefficients ($K_{o/w}$) lower than 1000, usually show poor recovery when PDMS is used as extracting phase, due to the non-polar nature of this polymer [2]. In these cases, a derivatization step is usually needed in order to improve $K_{o/w}$ values and thus extraction efficiency. The importance of some hydrophilic species, such as polar pesticides, alcohols, esters or phenolic compounds, has led to the development of stir bars coated with polar friendly coatings [3].

In this sense, different in-house procedures for stir bar coatings based on sol-gel technology, monolithic materials, new materials such as PDMS/polypyrrole, and more selective materials based on restricted access materials and molecular imprinted polymers have been developed and evaluated [4]. Nevertheless, difficulty in the preparation, a lack of stability, high bleeding rates and the low extraction efficiencies are just some of the disadvantages of these coatings. In addition, these materials are usually degraded at high temperature, and are therefore unstable for thermal desorption, requiring liquid desorption, which takes longer and is less effective. Recently, stir bars coated with polar materials, such as polyacrylate (PA) or ethylene glycol-silicone (EG-Silicone) have become available commercially. EG-Silicone and PA coatings have been applied for the determination of pharmaceuticals [5] and personal care products [6] in waters, volatiles from vegetable matrices [3] and cork taint-related compounds in wine [7]. Considering the obtained results, these coatings might be expected to be useful for the determination of other volatile polar compounds, which is why their

* Corresponding author. Tel.: +34 868 887406; fax: +34 868 887682.
E-mail address: hcordoba@um.es (M. Hernández-Córdoba).

http://dx.doi.org/10.1016/j.chroma.2015.04.041
0021-9673/

application for the HSSE preconcentration of volatile monoterpenes from honey is evaluated herein.

Honey volatile compounds comprise a complex mixture of different chemical families, originated from various biosynthetic pathways, including terpenes, norisoprenoids, and other compounds, that are responsible for characteristics taste, smell and flavor of honey [8]. The volatile composition of honey is determined by origin of the nectar and processing and storage conditions, and is similar to that of many plants natural essential oils. Since flowers of many plant species contain significant amounts of these compounds, the sucking of nectar by bees leads to their incorporation into honey [9]. Essential oils have been used for thousands of years as pharmaceuticals, and, recently, a great interest has been developed in the potential use of some of their components for the treatment of diseases. The essential oils present in honey have been identified as responsible for some of its biological and health related values [10].

Monoterpenes are isoprene-derived chemicals that are common in essentials oils obtained from plants. These compounds have a volatile nature and strong odor, being widely used in perfumery industry. They form the major volatile fraction in flower organs, where they attract pollinators, acting as both guide and stimulating agents. Monoterpenes include species like linalool (common floral scent in *Freesia* plants) or geraniol (from geranium and roses). In addition, these compounds have other interesting properties, since they act as wound healing [11], analgesic [12], anti-inflammatory [13], antibacterial and antifungal [14] agents. The frequent intake of honey has been claimed to provide benefits for human health as result of its monoterpene content among many other components [8].

Monoterpenes are usually analyzed using GC and mass spectrometry (MS) coupled to different extraction and preconcentration techniques. In this sense, different methodologies have been used for the extraction of the volatile fraction of honey [15], such as dynamic headspace [16,17], purge and trap [18–20] and SPME [21–26]. These extraction techniques have demonstrated to be more efficient than classical ones, such as hydrodistillation or simultaneous distillation extraction, which use heat that can affect the composition of the volatile fraction [8].

In the present study, the use of HSSE, through three commercially available extraction coatings, is evaluated for the determination of seven volatile monoterpenes, namely eucalyptol (EUC), linalool (LIN), menthol (MEN), geraniol (GER), carvacrol (CAR), thymol (THY) and eugenol (EUG), in honey using GC–MS.

2. Experimental

2.1. Chemicals

Eucalyptol (EUC), linalool (LIN), menthol (MEN), geraniol (GER), carvacrol (CAR), thymol (THY), eugenol (EUG) and 3-methyl-4-chlorophenol (3M4CP) were supplied by Sigma (St. Louis, MO, USA). Stock solutions of these compounds were prepared by dilution with pure acetone (Lab-Scan, Dublin, Ireland) and kept at 4 °C in dark bottles sealed with PTFE/silicone caps. Working standard solutions were prepared daily by dilution with water. Sodium chloride was obtained from Sigma. Helium was supplied by Air Liquide (Madrid, Spain).

2.2. Instrumentation

Commercial stir bars coated with a 0.5 mm layer thickness of PDMS (24 μL), EG-Silicone (32 μL) and PA (25 μL) were obtained from Gerstel (Mullheim an der Ruhr, Germany). Stir bars were conditioned prior to use according to the instructions of the supplier.

Table 1
Experimental conditions of the TD-GC–MS procedure.

Thermal desorption unit	
Mode	Splitless
Temperature programme	75–250 °C at 350 °C min^{-1}, held 7.5 min
Desorption flow	50 mL min^{-1}
Cooled injector system	
Mode	Solvent venting
Liner	Packed silanized glass wool, 2 mm i.d.
Temperature programme	15–275 °C (5 min) at 650 °C min^{-1}
GC–MS	
Capillary column	HP-5MS, 5% diphenyl-95% dimethylpolysiloxane (30 m × 0.25 mm, 0.25 μm)
Carrier gas	Helium, 1 mL min^{-1}
Oven programme	60° C, held 0.5 min 60–115 °C at 10 °C min^{-1} 115–155 °C at 20 °C min^{-1}, held 1.5 min 155–235 °C at 40 °C min^{-1}, held 0.5
Transfer line temperature	300 °C
Quadrupole temperature	150 °C
Ion source temperature	230 °C
Ionization	Electron-impact mode (70 eV)

In order to control the temperature during the extraction step, a laboratory-made heating system, constructed in the Central Laboratory Service of the University of Murcia and consisting of a drilled block provided with an electronic temperature control system, was used. An RH-KT/C magnetic stirrer (IKA, Staufen, Germany) was used for stirring the sample solutions.

The sample introduction system was composed of a thermal desorption unit (TDU-2) equipped with a multipurpose autosampler (MPS-2) and a programmed temperature vaporization (PTV) cooled injector system (CIS-4), from Gerstel. The optimized experimental conditions used for the sample introduction system are summarized in Table 1. GC analyses were performed on an Agilent 6890 N (Agilent, Waldbronn, Germany) gas chromatograph coupled to an Agilent 5973 quadrupole mass selective spectrometer equipped with an inert ion source. Under the selected conditions (Table 1), the analytes eluted with retention times (Table 2) between 5.3 and 10.2 min, corresponding to EUC and EUG, respectively. The compounds were quantified in the selected ion monitoring (SIM) mode in order to improve the detection limits using different ions (Table 2). The identification of the compounds was confirmed by injection of pure standards and comparison of the retention times and full MS-spectra.

2.3. Samples and analytical procedure

Samples of honeys from different geographical and botanical origin, including orange blossom, rosemary, eucalyptus, thyme, lavender, heather, chesnut and multifloral, were obtained from local supermarkets. In order to simulate as closely as possible the characteristics of a typical honey sample during the optimization procedure, a synthetic honey was elaborated. This mixture contained 30% (w/w) glucose, 50% (w/w) fructose and 20% (w/w) water [27], and was heated to 50 °C to achieve the complete dissolution of the sugars.

Two grams of honey were weighed into a 15 mL glass vial and dissolved in 2 mL acetate/acetic (0.2 M) buffer solution. 3M4CP was added as internal standard, at a concentration of 5 ng g^{-1}. HSSE extraction was carried out by exposing the coated stir bar to the vial headspace, while the sample was stirred at 900 rpm and heated at 50 °C for 2 h until extraction equilibrium was reached. After analyte extraction into the polymeric phase, the stir bar was rinsed with pure water, dried with a lint-free tissue and placed in a desorption tube for analysis.