Identification of Flavor Components in Perfumes by Headspace Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry

ARTICLE *in* JOURNAL OF CHEMICAL EDUCATION · DECEMBER 2001

Impact Factor: 1.11 · DOI: 10.1021/ed079p98

3 AUTHORS, INCLUDING:

Gerd Eduard Knupp
Hochschule Bonn-Rhein-Sieg
44 PUBLICATIONS **238** CITATIONS
SEE PROFILE

Peter Kusch
Hochschule Bonn-Rhein-Sieg
17 PUBLICATIONS **125** CITATIONS
SEE PROFILE

Identification of Flavor Components in Perfumes by Headspace Solid-Phase Microextraction and Gas Chromatography–Mass Spectrometry

Gerd Knupp, Peter Kusch,* and Michael Neugebauer

Fachhochschule Bonn-Rhein-Sieg, University of Applied Sciences, Fachbereich Biologie, Chemie und Werkstofftechnik, D-53359 Rheinbach, Germany; **peter.kusch@fh-bonn-rhein-sieg.de*

In analyses of flavor and fragrance compounds, sample preparation usually involves concentrating the analytes of interest using headspace, purge and trap, liquid–liquid extraction, solid-phase extraction (SPE), or simultaneous distillation/extraction techniques. These methods have various drawbacks, including long preparation time and excessive use of organic solvents.

Solid-phase microextraction (SPME), an adsorption/desorption technique developed by Pawliszyn (*1–8*) at the University of Waterloo (Ontario, Canada) eliminates the need of solvents or complicated apparatus for concentrating volatile or nonvolatile compounds in liquid samples or headspace.

The SPME process has two steps: partitioning of analytes between the coating and the sample matrix, and desorption of concentrated extracts into an analytical instrument (gas chromatograph [GC] or gas chromatograph–mass spectrometer [GC–MS] system) (*8*). In the first step, the coated fiber is exposed to the sample or its headspace, which causes the target analytes to partition between the sample matrix and the coating. The fiber, bearing the concentrated analytes, is then transferred to the GC or GC–MS for desorption, whereupon separation and quantitation of extracts can take place. SPME provides linear results over a wide concentration range, often down to parts per trillion (*7*).

The SPME device consists of a 1–2 cm length of fused silica fiber, coated on the outer surface with a stationary phase and bonded to a stainless steel plunger, and a holder that looks like a modified microliter syringe (*5–7*). Organic analytes adsorb to the phase coating the fiber. The holder protects the phase-coated fiber and controls exposure of the fiber for sample adsorption and desorption. After sample adsorption the SPME device is introduced into the gas chromatograph injector, where the adsorbed analytes are thermally desorbed and delivered to a GC column. Desorption of an analyte from an SPME fiber depends on the boiling point of the analyte, the thickness of the coating on the fiber, and the temperature of the injector port. The SPME device is compatible with any packed column or capillary gas chromatograph–mass spectrometer system, and can be used with split/splitless or direct/packed injector (*8*). Recently, a new SPME fiber with a dual coating of divinylbenzene (DVB) and Carboxen materials, each suspended in polydimethylsiloxane (PDMS), has been developed (*9*). The two-phase-coated fiber would be ideal for the extraction of a broad range of molecular weight compounds—for example, flavor analytes or solvents.

This paper describes an experiment for the identification of flavor components in perfume Original Eau de Cologne (which is famous in Germany and throughout Europe) by headspace-SPME (HS-SPME) followed by GC–MS. It was developed for second-year chemistry students and performed in the instrumental analysis course.

Experimental Procedure

Samples

Samples of the commercially available perfumes 4711 Original Eau de Cologne (Muelhens, Cologne, Germany) and Echt Kölnisch Wasser (Jünger & Gebhardt, Cologne, Germany) were used in our investigations.

Gas Chromatography–Mass Spectrometry

A ThermoQuest Trace 200 GC (ThermoQuest CE Instruments, Milan, Italy) interfaced to a ThermoQuest/Finnigan Voyager quadrupole mass spectrometer (ThermoQuest/Finnigan MassLab Group, Manchester, UK) with a ThermoQuest Xcalibur data system, the *NIST 98* spectral library, and a CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland) were used. The column was a DB-5MS (J&W Scientific, Folsom, CA) cross-linked fused-silica capillary column (60 m, 0.25 mm i.d.) coated with a 0.25-μm film of poly(5%-diphenyl-95%-dimethylsiloxane). The temperature of the split/splitless injector was 250 °C and the split ratio was 1:10. Mass spectra and reconstructed chromatograms (total ion current, TIC) were obtained by automatic scanning in the mass range *m/z* 30–430 amu. The GC–MS data were acquired after elution of ethanol at 9 min. Identification of compounds was carried out by comparison of retention times and mass spectra of standards (when available), study of the MS spectra, and comparison with members of the *NIST 98* spectral library.

Solid-Phase Microextraction

A 2-cm dual-layered SPME fiber coated with highly cross-linked 50/30-μm divinylbenzene/Carboxen, each suspended in polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, Bellefonte, PA), was used for the headspace extraction of flavors from the perfumes. The syringe device (Supelco) with the new fiber was conditioned before use at 270 °C for 4 hours in a GC injector.

Two-and-one-half milliliters of the sample sealed in a 20-mL glass vial with silicone septa and cap closure was placed for 2 min in the heating block of the CombiPAL autosampler equilibrated at 80 °C. The septum of the vial was pierced with the needle of the SPME device and the fiber was exposed approximately 2 cm above the sample for 30 s. Then the fiber was drawn back into the needle and the device was pulled

out of the vial. The needle of the SPME device was introduced to the injector of the GC–MS system for 5 s, during which the analytes were thermally desorbed at 250 °C, and the GC–MS run was started.

Hazards

There are no significant hazards.

Results and Discussion

An important ingredient in many perfumes, also in Original Eau de Cologne, is bergamot oil. This essential oil is produced from the fruit-peels of *Citrus aurantium* L. subsp. *bergamia* (Risso et Poit.) almost exclusively in the Italian province of Calabria, and more recently also in the Ivory Coast, Brazil, and Argentina. The main components of bergamot oil are (*R*)-(+)-limonene, (*R*)-(–)-linalool, and (*R*)-(–)-linalyl acetate (*10*). Other essential oils used in Original Eau de Cologne are neroli oil, lavandin oil, rose oil, rosemary oil, and sage and thyme oils (*11*).

Our investigations were performed with the headspace sampling and the solid-phase microextraction technique (HS/SPME) to protect the GC–MS column from water. Figure 1 shows a typical total ion current GC–MS chromatogram obtained from the headspace of the Original Eau de Cologne by SPME using dual-layered divinylbenzene–Carboxene–polydimethylsiloxane fiber. This fiber was selected because it could retain both volatile and semivolatile analytes. The split ratio of 1:10 for the split/splitless injector was necessary to protect the GC–MS column from overloading and to achieve an optimal separation and peak symmetry. The retention data, the boiling points, and the summarizing of the identification results are given in Table 1.

Students obtained and printed out the total ion chromatogram (TIC) and the mass spectrum corresponding to each of the peaks in their chromatogram. The compounds were identified by comparison of their mass spectra with those given in the *NIST 98* spectral library. The mass spectra of the major peaks in Figure 1, such as limonene (16.10 min), linalool (17.89 min), and linalyl acetate (21.97 min) were also interpreted. The students had to identify the molecular ions and were asked to write down the most important fragmentation reactions.

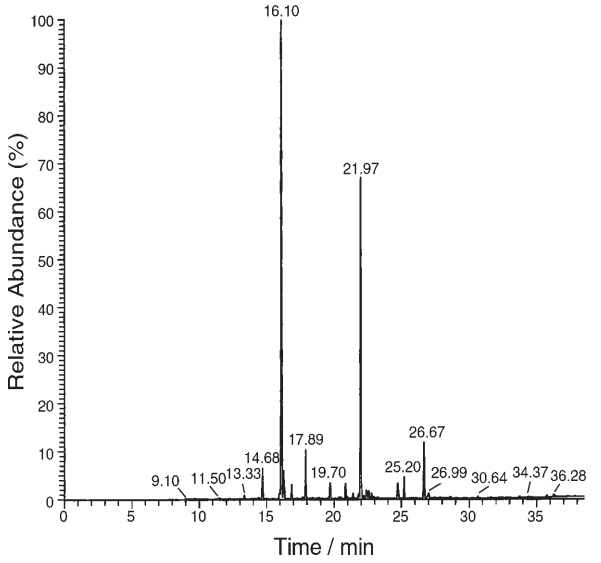

Figure 1. Total ion chromatogram (TIC) of the Original Eau de Cologne perfume obtained by the headspace SPME using DVB/Carboxene/PDMS fiber. The peak identities are shown in Table 1.

Conclusion

The experiment developed for identification of Original Eau de Cologne shows that headspace SPME in combination with capillary GC–MS is a powerful tool for the study of flavor compounds in perfumes.

The students learn the common and systematic names of typical flavor substances. They learn about and get experience in modern sophisticated SPME and GC–MS techniques. The interpretation of the mass spectra for different classes of chemical substances such as cyclic aliphatic hydrocarbons,

Table 1. Flavor Components in Original Eau de Cologne Identified by HS-SPME and GC–MS

t_R/min	bp/°C	M_r	Identification	
			Common Name	IUPAC Name
14.68	163–164	136.23	β-Pinene	6,6-Dimethyl-2-methylen-bicyclo[3.1.1]heptane
16.10	176–177	136.23	*d*-Limonene	4-Isopropenyl-1-methyl-1-cyclohexene
16.26	176–177	154.24	1,8-Cineole	1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane
16.85	183	136.23	γ-Terpinene	1-Isopropyl-4-methyl-1,4-cyclohexadiene
17.89	198–200	154.24	Linalool	3,7-Dimethyl-1,6-octadien-3-ol
19.70	205	154.24	*cis*-Pinan-2-ol	(1*RS*,2*RS*,5*RS*)-2,6,6-trimethyl-bicyclo[3.1.1]heptan-2-ol
20.85	217–218	154.24	(+)-α-Terpineol	(*R*)-2-(4-Methyl-3-cyclohexenyl)isopropanol
21.97	220	196.28	Linalyl acetate	3,7-Dimethyl-1,6-octadien-3-yl acetate
22.56	228	152.23	*cis*-Citral	3,7-Dimethyl-2,6-octadienal
24.72	229–230	154.24	*cis*-Geraniol	(*Z*)-3,7-Dimethyl-2,6-octadien-1-ol
25.20	134[a]	196.28	Neryl acetate	(*Z*)-3,7-Dimethyl-2,6-octadien-1-yl acetate
26.67	Oil	208.30	Nopyl acetate	6,6-Dimethyl-bicyclo[3.1.1]hept-2-ene-2-ethyl acetate

[a]At 3.4 kPa.

aliphatic alcohols, and aliphatic esters gives them an understanding of the processes that take place in a mass spectrometer. Use of the mass spectral database *NIST 98* allows them to estimate advantages and limitations of this tool.

[W]Supplemental Material

A more detailed version of this paper is available in this issue of *JCE Online*. It includes further background information, a materials and equipment list, instructions for students, and electron impact mass spectra and their interpretation.

Literature Cited

1. Arthur, C. L.; Pawliszyn, J. *Anal. Chem.* **1990**, *62*, 2145.
2. Zhang, Z.; Pawliszyn, J. *Anal. Chem.* **1993**, *65*, 1843.
3. Pawliszyn, J. *J. Chromatogr. Sci.* **1993**, *31*, 31.
4. Yang, M. J.; Pawliszyn J. *LC-GC* **1996**, *14*, 364.
5. Yang, M. J.; Orton, M. L.; Pawliszyn, J. *J. Chem. Educ.* **1997**, *9*, 1130.
6. Pawliszyn, J. *Solid Phase Microextraction: Theory and Practice*; Wiley-VCH: New York, 1997.
7. Supelco Bulletin 869; Supelco Inc.: Bellefonte, PA, 1997.
8. Supelco Bulletin 923A; Supelco Inc.: Bellefonte, PA, 1999.
9. Haag, I. *Labor Praxis* **1999**, *23* (3), 30.
10. König, W. A.; Fricke, Ch.; Saritas, Y.; Momeni, B., Hohenfeld, B. *J. High Resol. Chromatogr.* **1997**, *20*, 55.
11. *Roempp Chemie Lexikon*; Falbe, J.: Regitz, M., Eds.; Thieme: New York, 1995.