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Determination of fragrance allergens in cosmetics by size-exclusion chromatography followed by gas chromatography–mass spectrometry

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Abstract

A method using size-exclusion chromatography (SEC) combined with gas chromatography–mass spectrometry (GC–MS) was developed for the quantitation of 24 restricted allergenic fragrance compounds in cosmetic samples. To achieve reproducible results fragrance calibration has to be performed with propyl acetate as a solvent containing a constant proportion of matrix components. With the exception of hydroxycitronellal ($66 \pm 5\%$) all compounds showed good recovery rates in the range of 90–120%. The mean accuracy (relative error) was $1 \pm 10\%$ for all 24 compounds in five spiked creams (10 mg/kg per allergen) and $8 \pm 34\%$ in a reference sample (4–15 mg/kg). The biggest benefit compared to other methods is the flexible clean up with SEC which allows the determination of a large range of compounds in difficult matrices with GC–MS.

Keywords: Allergen; Cosmetics; Fragrances; SEC; GC–MS

1. Introduction

In the European Union and next year also in Switzerland legal restrictions limit the use of 26 fragrance ingredients suspected of causing skin reactions [1,2]. These regulations require an indication of the presence of potential fragrance allergens in cosmetic products if a limit of 0.01% (100 mg/kg) for rinse-off and 0.001% (10 mg/kg) for leave-on products is exceeded. Hence a routine analytical method is required to ensure that the regulations are observed by producers and importers. Previously, some procedures for the detection of such allergens in aqueous/ethanolic products were developed [3–6] which could be used for market survey purposes. In most cases sample preparation of liquid products consists of a single dilution step which allows an accurate determination of the substances of interest by GC–MS. There is no need for a special sample clean up because the performance of the GC system is rarely hampered by other constituents of the sample. Occasional problems with interfering compounds can be met by choosing selective columns, optimised temperature programs and specific ion monitoring [5,6]. In contrast, the identification of allergenic fragrance compound traces in cream samples demand a much higher effort in extraction and clean up procedures than in liquid ones. The extremely complex fatty matrix can affect the stability of the GC–MS performance and cause interference with the peaks of interest. To enhance the reproducibility of the results and to enable the monitoring of contents in the range of 0.001% in difficult matrices, a clean up must be capable of removing unwanted matrix components from the target substances. This is a difficult task because the regulated fragrance compounds represent a broad range of chemical classes (e.g. alcohols, alkanes, esters, aldehydes and ketones) which also applies to most matrix constituents. Although headspace and many other methods for the isolation of fragrance ingredients have been developed [7], no single method is available for the determination of the 26 regulated compounds at low levels. A promising method using silica columns for sample preparation of creams and lotions only covers 10 compounds [3]. Several other methods concerning 12 allergenic compounds with a very low concentration level exist which either use solid phase extraction, a microdistiller or simultaneous distillation–extraction. However, results were unsatisfactory owing to low recovery rates [8].

Another approach to solve the separation problems may be the use of size-exclusion chromatography (SEC), also called gel permeation chromatography. In contrast to the above mentioned methods, SEC separates primarily by molecular size and not by

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different chemical characteristics of the solutes. For example, SEC was successfully applied to the isolation of small molecules of interest (<1000 Da) from larger matrix compounds in the case of migrants from food packaging materials [9].

In this study, a clean up method using SEC was developed for the sample preparation of cosmetics followed by GC–MS analysis. Determination of 24 of the 26 restricted fragrance compounds was possible, 2 are natural extracts (oak moss and tree moss) unsuitable for GC–MS.

2. Experimental

2.1. Chemicals

The organic solvents acetone and propyl acetate (analytical grade or higher) were obtained from Merck (Germany) and Aldrich (USA), respectively. The pigment Foron Rubin S-2 GFL (disperse red 167.1, CAS 61968-52-3, MW = 505.9) from Clariant (Muttenz, Switzerland) was used as internal marker for SEC (SECM).

2.2. Standards

Reference compounds, their suppliers and parameters for identification and quantitation are indicated in Table 1. Purities of the reference compounds are at least 98%.

$^2H_8$ naphthalene (Nap-d8, 99.0%, CAS 1146-65-2, CIL, Andover, MA, USA) and hexachlorobenzene (HCB, 99.5%, CAS 118-74-1, Ehrenstorfer, Augsburg, Germany) were used as internal standards for determination and quality control. Ions used for quantitation were 136 and 284, respectively.

2.3. Instruments and accessories

Analytical balance (AE 200, Mettler Toledo, Nänikon, Switzerland), ultra sonic bath (Branson 3510, Danbury, CT, USA), vortex (Huber & Co, Reinach, Switzerland), teflon syringe filters, 0.45 μm (Schmidlin, Neuheim, Switzerland).

SEC system consisting of a low pressure mixing gradient pump (Waters 600 MS, Milford, CT, USA), an autosampler (Waters 600 MS), a photodiode array detector (Waters PDA 996), a sampling injector (Gilson 233 XL, Villiers le Belle, France) and Millenium software. Semi preparative column: Phenogel (polystyrene/divinylbenzene, Part-Nr. 00H-0441-KO, Phenomenex, Torrance, CA, USA), 5 μm, 300 × 7.8 mm × 25 cm, 50 Å; SEC conditions: acetone isocratic 100% (0–30 min); flow rate 0.8 ml/min, UV 515 nm for SECM and UV 350 nm for matrix; fractions: routinely 10.5–16.9 min for all 24 compounds (total fragrance fraction) or for individual ones as shown in Table 1.

GC–MS system (PolarisQ, Thermo Finnigan, Austin, TX, USA) with a PAL autosampler (CTC Analytics, Zwingen,

Table 1
Fragrance compound standards and parameters for determination (quant. mass: ions used for quantitation, fraction SEC: fraction time for selective SEC-clean up, tR: retention time GC, LQ: limit of quantitation in real sample)

Name	CAS no.	Supplier	Quant. mass	tR (min)	Fraction SEC (min)	Accuracy (%)	LQ (mg/kg)
Amylcinnamic alcohol	101-85-9	Aldrich	115 + 129	32.1	12.0–13.0	3	3
Amylcinnamic aldehyde	122-40-7	Aldrich	115 + 129	29.7	13.5–14.5	−2	3
Anisyl alcohol	105-13-5	Aldrich	137 + 138	14.3	11.5–13.0	14	4
Benzyl alcohol	100-51-6	Fluka	107 + 108	9.7	11.5–13.0	17	3
Benzyl benzoate	120-51-4	Aldrich	105 + 194	35.1	15.0–16.9	1	4
Benzyl cinnamate	103-41-3	Aldrich	193	38.9	15.5–16.9	−16	3
Benzyl salicylate	118-58-1	Acros	91	36.4	15.0–16.0	0	3
Butylphenyl methylpropional (Lilial)	80-54-6	Givaudan	189	21.5	11.5–13.5	−9	3
Cinnamic alcohol	104-54-1	Fluka	92	14.7	12.5–13.0	18	4
Cinnamic aldehyde	104-55-2	Fluka	131	14.1	13.5–14.5	2	3
Citral[a]	5392-40-5	Merck	79 109 + 137	12.0 12.4	12.5–13.0	−5	3
Citronellol	106-22-9	Acros	67	11.2	11.0–12.0	0	3
Coumarine	91-64-5	Sigma	118	22.1	14.0–15.5	17	3
Eugenol	97-53-0	Fluka	164	15.4	11.5–13.0	2	3
Farnesol[a]	4602-84-0	Aldrich	81	29.9 31.5	11.5–12.5	15	5
Geraniol	106-24-1	Aldrich	93 + 123	11.8	11.5–12.5	1	3
Hexylcinnamic aldehyde	101-86-0	Aldrich	129	33.6	13.5–14.5	−1	4
Hydroxycitronellal	107-75-5	Sigma	71	12.8	10.5–11.5	2	4
Hydroxyisohexyl-3-cyclohexene carboxaldehyde (Lyral)[a]	31906-04-4	IFF	79	31.4 31.7	11.0–12.0	−15	3
Isoeugenol[a]	97-54-1	Aldrich	164	17.3 18.8	12.5–13.5	6	3
Limonene	5989-27-5	Aldrich	93	8.7	14.5–16.0	−20	2
Linalool	78-70-6	Aldrich	93	9.5	11.0–12.0	6	3
Methyl-2-octynoat	111-12-6	Aldrich	95 + 123	11.5	12.0–12.5	0	3
α-Isomethylionone[a]	127-51-5	Pfalz & Bauer	135 121	17.7 19.5	12.5–13.5	−6	3

[a] At least two isomers, which are determined routinely.