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Collaborative validation of the quantification method for suspected allergens and test of an automated data treatment

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ABSTRACT

Previous publications investigated different data treatment strategies for quantification of volatile suspected allergens by GC/MS. This publication presents the validation results obtained on "ready to inject" samples under reproducibility conditions following inter-laboratory ring-testing. The approach is based on the monitoring of three selected ions per analyte using two different GC capillary columns. To aid the analysts a decisional tree is used for guidance during the interpretation of the analytical results. The method is evaluated using a fragrance oil concentrate spiked with all suspected allergens to mimic the difficulty of a real sample extract or perfume oil. At the concentrations of 10 and 100 mg/kg, imposed by Directive 76/768/EEC for labeling of leave-on and rinse-off cosmetics, the mean bias is +14% and −4%, respectively. The method is linear for all analytes, and the prediction intervals for each analyte have been determined. To speed up the analyst's task, an automated data treatment is also proposed. The method mean bias is slightly shifted towards negative values, but the method prediction intervals are close to that resulting from the decisional tree.

1. Introduction

Directive 2003/15/EC amending Council Directive 76/768/EEC relating to cosmetic products, regulates the obligation to inform consumers of the presence of potentially allergenic fragrance substances identified as likely to cause allergenic reactions in cosmetic products [1]. It requires that these substances must be listed in the ingredients of cosmetic products if they exceed 0.001% "in leave-on products" or 0.01% in "rinse-off products". Among the list of 26 regulated compounds, 24 are volatile and can be analyzed by gas-chromatography. Quantification represents a challenge, due to the complex composition of fragrance oils: taking into account all constituents, their isomers, and the constituents of natural ingredients used in a formulation, more than 100 GC peak responses can be detected in a fragrance oil. This problem has given rise to many publications and most of them involve the use of GC/MS using electron impact mode (EI) [2–7], and in few cases chemical ionization mode (CI) [8]. Fast GC, interfaced to quadrupole MS has also been investigated [9]. As co-elutions are frequent, either the selectivity of the detector can be increased by using tandem mass spectrometry [10], or the separation resolution is enhanced using comprehensive two-dimensional GC (GC × GC) [11–13]. The "targeted mode" of GC × GC has also been proposed [14].

Additional analytical techniques have been investigated to quantify the suspected allergens. Comprehensive GC × GC with a flame ionization detector (FID) was shown not to be very selective in avoid overlapping peaks, and to unambiguously assign the target analytes in complex fragrance oils [11]. Using HPLC diode array detection, the low resolution of LC columns compared to capillary GC columns is questionable, as many peaks overlap during analysis

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of the 24 analytes [15]. GC/FTIR was applied to the quantification in various cosmetic products, but only 10 suspected allergens were quantified [16]. A recent paper reports the use of electrokinetic chromatography in a microemulsion phase, and using UV detection [17]. Good resolutions and linearities were achieved but the method could not determine all of the 24 compounds and was only applied to rinse-off cosmetics.

The papers dedicated to the quantification of suspected allergens in real consumer products are variants of the initial paper of the International Fragrance Association (IFRA) [2]: Niederer et al. proposed a size-exclusion clean-up to remove the non-volatiles from cosmetic products [4]. To analyze fragranced baby bathwater, Lamas et al. recovered the volatiles using solid-phase microextraction (SPME) [6]. The same SPME technique was also applied in the evaluation of perfumes [18].

The data analysis strategy varies strongly from one publication to another. When quantifying target compounds in a complex mixture, it is advisable to check the identity of analytes. When a monodimensional detector is used, this identification is not feasible and so, a risk of confusion with the other perfume or cosmetic ingredients exists [15,17]. When using an MS detector and only a few mass fragments, the identity should be assessed using qualifiers and ion ratios between the qualifiers and the quantifier [19]. However, this precaution does not overcome the over-estimation of co-eluted peaks. To avoid this problem, some authors prefer the scan mode to allow the selection of another quantification ion that does not appear in the overlapping peak [3,9]. Unfortunately, the sensitivity resulting from ions extracted from a full scan does not reach that of ions monitored in SIM mode, and the GC-peak area reproducibility is significantly greater with the latter. To minimize the over-evaluation caused by a co-elution, we previously proposed a "play-down strategy": it consists in successively considering each of the three ions monitored in SIM as the quantifier, and using the two other ions as qualifiers. Selecting the minimum amount from the six values resulting from an analysis two different GC columns, in combination with a computerised peak recognition, was shown to be efficient [2].

Some of the above-cited papers were submitted to a partial or extensive validation. The fast-GC/MS method was only tested for its limit of quantification (LOQ) and relative standard deviations (RSD) of GC peak areas using a standard solution [9]. The SPME-GC/ion-trap-MS and the GC × GC/MS methods were tested for linearity, precision and LOQ [6,13]. However in the three cases the LOQs were determined from blank samples, which is not representative of the complexity of a real perfume sample for which a baseline free of peaks is an exception. The IFRA method based on a GC/MS determination using the above-mentioned play-down strategy, was evaluated for its reproducibility in different fragrance concentrates, using 10–15 compounds among the 24 suspected allergens, but the precision and the LOD were not determined under reproducibility conditions [20].

Since these 24 volatile allergens are now regulated in the European Union, a working group was created by a European Committee for Standardisation (CEN), to select and validate a method for use as a European standard. However, the analysis of suspected allergens in consumer products requires two steps: isolation from a representative sample matrix that is compatible with GC/MS analysis, and quantification of the analytes from the sample extract. The alcoholic fragrances of fine perfumery and the fragrance oil concentrates produced by the fragrance industry are usually directly compatible with this second step. Therefore, this work only reports the validation of the later performed by the CEN working group, *i.e.* the GC/MS quantification of ready-to-inject samples. The consumer products requiring a sample preparation will be considered in another publication. The present approach starts from the IFRA method based on GC/MS in selected-ion monitoring mode (SIM) [2], as such equipment is available in most laboratories dealing with the analysis of these types of analytes. In addition, the risk of over-estimation was evaluated thanks to a theoretical calculation estimating the probability that an analyte co-elutes with another fragrance constituent exhibiting isobaric fragments [20]. The objective of this work is the validation of the method under reproducibility conditions and the investigation of an automated data treatment procedure to reduce the analyst's time on this task.

2. Experimental

2.1. Materials

Suspected allergens were supplied by Firmenich, with the following purities: limonene: 97.6%, linalool: 99.8%, methyl 2-octynoate: 99.5%, citronellol: 99.2%, citral quantified as the sum of its two isomers, neral: 37.1%; geranial: 62.9%, cinnamaldehyde: 98.6%, anisyl alcohol: 100%, hydroxycitronellal: 98.4%, cinnamic alcohol: 98.7%, eugenol: 100%, coumarine: 100%, isoeugenol: 96.0%, α-amylcinnamic alcohol: 42.0% (+ benzylheptanol), benzyl alcohol: 99.7%, α-amylcinnamaldehyde: 99.5%, α-hexylcinnamaldehyde: 91.7% (sum of both isomers), benzyl benzoate: 100%, benzyl cinnamate: 100%, farnesol: 100% (quantified using its two main isomers: 50.9% and 42.4%), geraniol: 99.4%, lilial®: 98.3%, lyral®: 81.3%, alpha isomethylionone: 87.6%, benzyl salicylate 100%. All participants were supplied with the suspected allergen analytes from the same batches to minimize the variability inherent in different suppliers, the final results were corrected for purity. The spiking of samples was completed using the same batches. The internal standards, 1,4-dibromobenzene and 4,4′-dibromobiphenyl, had a purity of 98% and were supplied by Aldrich (Steinheim, Germany).

The above-listed analytes were spiked at concentration levels between 10 and 100 mg/kg in a perfume made of volatile constituents representing about 150 GC peaks (ingredients + isomers + impurities). This non-spiked perfume was included in the ring test to check that it did not contain any of the 24 analytes. Neral and geranial were calibrated and quantified separately, but only their sum was taken into account, in agreement with the European Directive.

2.2. Standard solutions

The ring-test protocol required preparation of a stock standard solution of all analytes at a concentration of 5 g/l in an inert and non-volatile solvent (methyl pivalate, *o*-cresol-free *o*-fluorotoluene). This was stored for less than 1 month in the absence of light at a temperature below −18 °C. Alternatively, two different stock standard solutions were prepared, one for carbonyl compounds, another for non-carbonyl compounds and store in the dark below + 4 °C for a maximum of 2 months.

The internal standard solution of 1–4 dibromobenzene and 4-4′-dibromobiphenyl, was prepared at 1 g/l in the same solvent as the stock standard solutions and stored at about +4 °C for a maximum of 2 months. From the stock standard solution(s), calibration standards were prepared. Each containing 10 mg/l of internal standards, and 1, 5, 10, 20, 30 or 40 mg/l of analytes.

2.3. GC/MS

The collaborative validation was performed by the organizations/companies that are listed in the authors of this paper, except LGC. Prodarom's experimental results were provided by Robertet SA (Grasse, F). Chanel was represented by two contributing laboratories. The MS instruments used in this ring test are listed in Table 1.