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# Identification, quantitation and method validation for the analysis of suspected allergens in fragrances by comprehensive two-dimensional gas chromatography coupled with quadrupole mass spectrometry and with flame ionization detection

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**Abstract**

The first part of this study first aims at validating a method to identify suspected allergens limited by EU regulations in fragrances by comprehensive two-dimensional gas chromatography (GC × GC) coupled with rapid scanning quadrupole MS (GC × GC-qMS). The effectiveness of the quadrupole MS operating at different scanning speed (1000 and 11,111 amu/s) was evaluated in identifying (full scan mode acquisition – TIC) and in quantifying (single ion monitoring – SIM) the target analytes in complex mixtures. In full scan acquisition mode the mass range was reduced to 40–240 amu to increase the scan acquisition rate while in SIM mode the influence of different dwell-times (40, 10 and 5 ms) was tested. The number of scans for each single modulated chromatographic GC × GC peak and the total number of scans for the 2D peak, together with half height peak width (referred to apex) of each allergen in the standard mixture in both TIC and SIM modes were determined. Moreover, the match quality of the spectra obtained by GC/MS at 1000 and 11,111 amu/s and by GC × GC–MS at 11,111 amu/s were compared and the occurrence of spectral skewing verified. In the second part of this work quantitative methods by GC × GC-SIM/qMS and GC × GC-FID were validated on the basis of Eurachem/CITAC protocols through which the following performance parameters were determined: confirmation of identity, selectivity and specificity, limit of detection (LOD), limit of quantitation (LOQ), linearity (working and linear range), precision and accuracy and uncertainty. Suspected allergens were spiked in a concentration range between 2 and 25 ppm (μg/mL) on a Test fragrance taken as a reference, while 1,4 dibromo-benzene and 4,4′-dibromodiphenyl were used as internal standards.


*Keywords:* GC × GC-FID; GC × GC-qMS; Rapid scanning quadrupole-MS coupling; Method validation; Volatile allergens analyses; Quantification; Fragrances

## 1. Introduction

The directive 2003/15/EC of the European Parliament and of the Council of 27/02/2003 regulates cosmetic products taking into account the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) intended for consumers opinions [1] and asserts that some cosmetic components have been identified as an important cause of contact-allergy reaction in fragrance-sensitive consumers. For those substances responsible, or suspected to be responsible, of causing allergic reactions their use had to be limited and/or strictly regulated. A list of the suspected allergens is reported in Table 1, the maximum residue limit for "leave-on" and "rinse-off" cosmetic products is fixed at 0.001% and 0.01%, respectively.

Regulatory requirements such as those mentioned above require reliable reference analytical methods suitable to be applied for routine quality control.

This study first aims to evaluate how effective can be GC × GC coupled to conventional quadrupole mass spectrometry (qMS) operating in fast scanning for target analysis (identification and quantitation) of suspected allergens in fragrances, and, second to validate a quantitative analytical method suitable to achieve the residue level set by E.U. legislation.

Few articles concerning analytical methods dealing with quantitation of allergens are available in the literature; Rastogi [2] first proposed a method where 11 of the 24 allergens were

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Table 1
List of chemical names, CAS numbers, purity index (%) and *m/z* target ions used for SIM acquisition mode of the analytes under study

| Name [CAS registry no.] | Purity (%) | SIM *m/z* ions |
|---|---|---|
| Amylcinnamic alcohol [101-85-9] | 92.0 + 4.5 *Z* + *E* | **133**, 115, 204 |
| Amylcinnamic aldehyde [122-40-7] | 93.7 + 4.7 *Z* + *E* | **202**, 201, 129 |
| Anisyl alcohol [105-13-5] | 97.2 | **138**, 137, 109 |
| Benzyl alcohol [100-51-6] | 97.7 | **108**, 79, 107 |
| Benzyl benzoate [120-51-4] | >99.9 | **105**, 212, 194 |
| Benzyl cinnamate [103-41-3] | 98.3 | **131**, 192, 193 |
| Benzyl salicylate [118-58-1] | 99.6 | **91**, 228, 65 |
| Cinnamic alcohol [104-54-1] | 98.4 | **92**, 134, 115 |
| Cinnamic aldehyde [104-55-2] | 1.8 + 93.63 *Z* + *E* | **131**, 132, 103 |
| Citral [5392-40-5] | 37.3 − 62.6 *Z* + *E* | Neral: **69**, 94, 109<br>Geranial: **69**, 84, 94 |
| Citronellol [106-22-9] | 99.2 | **69**, 95, 81 |
| Coumarine [91-64-5] | 98.5 | **146**, 118, 89 |
| Estragole [140-67-0] | 98.9 | **148**, 147, 117 |
| Eugenol [97-53-0] | 99.7 | **164**, 103, 149 |
| Farnesol [106-28-5] | 45.9 + 53.6<br>ZE + EE | **69**, 93, 81 |
| Geraniol [106-24-1] | 95.5 | **69**, 123, 93 |
| Hexylcinnamic aldehyde [101-86-0] | 94.0 + 4.0 Z + E | **216**, 215, 129 |
| Hydroxycitronellal [107-75-75] | 97.9 | **59**, 71, 43 |
| Isoeugenol [97-54-1] | 7.8 + 92.2 Z + E | **164**, 149, 131 |
| Butylphenyl methylpropional [80-54-6] | 2.4 + 96.5 Z + E | **189**, 147, 204 |
| Limonene [5989-27-5] | 97.1 | **68**, 93, 67 |
| Linalool [78-70-6] | 97.9 | **93**, 71, 121 |
| Hydroxyisohexyl-3-cyclohexene carboxaldehyde [31906-04-4] | 27.5 + 72.5<br>(3-)*c* + (4-)*c* | **136**, 192, 149 |
| Methyl 2-nonynoate [111-80-8] | 99.9 | **79**, 137, 123 |
| Methyl 2-octynoate [111-12-6] | 97.6 | **95**, 123, 79 |
| Methyleugenol [93-15-2] | 99.3 | **178**, 163, 147 |
| Phenylacetaldehyde [122-78-1] | 98.2 | **91**, 120, 92 |
| α-Isomethylionone [127-51-5] | 85.7 | **135**, 206, 150 |
| 1,4-Dibromobenzene | 100.0 | **236**, 234, 238 |
| 4,4′-Dibromobiphenyl | 98.7 | **312**, 310, 314 |

*m/z* target ion in bold.

identified by GC/MS and quantified by GC/FID in a separate chromatographic run. The same author described a revised procedure where the allergens were quantified through extracted ions from GC/MS data acquired in full scan mode [3]. Unfortunately, this approach gives results highly inaccurate, as shown by Gilbert [4], and makes the reported quantitative data unreliable. On the basis of these considerations, Ellendt et al. [5] introduced a quantitative analytical method by GC/MS operating in Selected Ion Monitoring (SIM) mode, whose scopes were extended and the results consolidated by Chaintreau et al. [6]. With this method the quantitation of fragrance compounds suspected to cause skin reactions was carried out by GC/MS operating in SIM mode taking at least three selected ions (one target ion and two qualifiers) for each component both to quantify and to confirm the analyte identity on the basis of the quality values referred to target ions area ratios of a reference standard. This GC/MS method has been taken as a reference, in terms of potentials, limits and performance parameters for the present work in which the second analytical dimension, i.e. mass spectrometry, was replaced by a second chromatographic dimension, orthogonal to the first one (i.e. with a different stationary phase), suitable to separate the target analytes from the other components of the fragrance. Very recently Kinani et al. [7] discussed the possibility to quantify suspected allergens in fragrances by GC-SIM/qMS, GC-Ion Trap MS/MS and GC-Triple Quad MS/MS. The use of GC-FTIR has been proposed by Jiang et al. [8]. Although attractive, this method suffers of a severe lack of sensitivity.

Comprehensive two-dimensional gas chromatography (GC × GC) is a valuable analytical tool for the analysis of flavour and fragrance samples [9–19] and a lot of work has already been done in this field. Recently Adahchour et al. [17] discussed in depth potentials and benefits of the rapid scanning quadrupole mass spectrometer as a detector for GC × GC for trace-analysis of flavours, allergens and PCBs. The authors' aim was not to discuss an optimization procedure albeit they mention limit of detection and linearity.

To the best of authors' knowledge, up to now, it has not been described a GC × GC validated method suitable to identify and to quantify the 24 suspected allergens in fragrances with a given accuracy, precision and limits supported by a suitable number of data (limit of quantitation (LOQ) and limit of determination (LOD)).

On the basis of the results obtained up to now in allergen analysis by other groups, an analytical strategy able to assess a good level of confidence of the results from its application was designed. The reliability of GC × GC coupled with fast-scanning quadrupole mass spectrometry (GC × GC-qMS) to identify target analytes was evaluated in agreement with regulatory rules concerning the Quality in Analytical Chemistry [20–23] through (i) the quality match index obtained by application of the NIST algorithm in spectral research and (ii) by monitoring the spectral skewing, which is a consequence of the changes in molecular fragment concentration in the ion source during the fast peak elution. The validity of the quantitative results obtained both by GC × GC-qMS and GC × GC-FID was demonstrated through several method performance parameters such as linearity in the working range, repeatability precision, selectivity, specificity and accuracy/trueness.

## 2. Experimental

### 2.1. Samples

Pure standard samples of α-isomethyl-ionone, amylcinnamic alcohol, amylcinnamic aldehyde, anisyl alcohol, benzyl alcohol, benzyl benzoate, benzyl cinnamate, benzyl salicylate, butyl-phenyl methylpropional, cinnamic alcohol, cinnamic aldehyde, citral, citronellol, coumarine, estragole, eugenol, farnesol, geraniol, hexylcinnamic aldehydes, hydroxycitronellal, isoeugenol, limonene, linalool, hydroxyisohexyl-3-cyclohexene carboxaldehyde (Lyral), methyl-2-nonynoate, methyl-2-octynoate, methyleugenol, phenylacetaldehyde, 1,4-dibromobenzene (ISTD-1), 4,4′-dibromodiphenyl (ISTD-2) were supplied by Robertet S.A. (Grasse Cédex, France). It should be noted that estragole and phenylacetaldehyde are not