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Enantiomer identification in the flavour and fragrance fields by "interactive" combination of linear retention indices from enantioselective gas chromatography and mass spectrometry

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ABSTRACT

This study describes the development of a gas chromatography–mass spectrometry (GC–MS) library to identify optically active compounds in the flavour and fragrance field using enantioselective GC with cyclodextrin derivatives (CDs) as chiral selectors in combination with MS. The library operates on the "interactive" combination of linear retention indices (I^T values) in parallel to MS spectra, so as to identify enantiomers reliably and to measure EE and/or ER unequivocally. Since MS is not a selective probe to discriminate optical isomers, mass spectra (or diagnostic ions in SIM mode) are used to locate the enantiomer(s) in the chromatogram, and I^T values to identify it(them) safely and reliably in particular in complex mixtures. The library has been built up through the following steps:

(a) Selection of CD derivatives able to cover a wide range of racemate separations. Four cyclodextrin derivatives were used: 2,6-di-*O*-methyl-3-*O*-pentyl-β-CD, 2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-β-CD, 2,3-di-*O*-ethyl-6-*O*-*tert*-butyldimethylsilyl-β-CD, and 2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-β-CD.
(b) Determination of the analytes' I^T values and evaluation of their stability and reliability at both intra- and inter-laboratory level.
(c) Determination of the range within which the I^T of an enantiomer has to fall to be correctly identified, i.e. determination of a common *retention index allowance* (RIA).
(d) Construction of the library, at the moment comprising the enantiomers of 134 racemates. A record has been attributed to each enantiomer including I^T values determined on the four CD coated columns, mass spectrum, IUPAC chemical name, CAS number, molecular weight, and, when separated, racemate enantiomer resolution on the CD investigated.

Some applications of the library are also reported.

1. Introduction

The interaction of a compound with a biological system has long been shown to be stereoselective. Enantiomer recognition and enantiomeric excess (EE) and/or ratio (ER) determination are a very important task in flavour and fragrance fields, so as (i) to define the correlation between chemical composition and organoleptic properties; (ii) to implement quality control and detect fraud or adulteration of "natural" samples; (iii) to determine the biosynthetic pathway when the formation of a compound is studied or to classify a sample; (iv) to determine the geographic origin of a "natural" sample.

Cyclodextrin derivatives (CDs) have truly represented a milestone in enantioselective gas chromatography (GC); being first introduced by Sybilska and Koscielski at the University of Warsaw in 1983 for packed columns [1] and applied to capillary columns in the almost contemporary works of Juvancz et al. [2] and Schurig and Nowotny [3]. Moreover, Schurig and co-workers first proposed diluting CD derivatives in moderately polar polysiloxane (OV-1701) to provide them with good chromatographic properties and a wider range of operative temperatures [4]. Since then, several groups have investigated CD derivatives as chiral selectors for enantioselective GC applications, and several hundreds of articles have been published dealing with the theory of chiral

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GC recognition with CDs, synthesis of new CD derivatives, their enantioselectivity and applications, many of them concerning the flavour and fragrance field [5–8].

Chiral recognition of marker compounds in complex real-world samples, as those in the flavour and fragrance field often are, generally requires a two-dimensional approach, because enantioselective GC may double the number of peaks of optically active analytes. This makes some parts of the total chromatograms even more complex and, as a consequence, increases the probability of interferences with a correct EE and/or ER determination. Two complementary but distinct approaches can therefore be adopted; the first and most popular one is to introduce a second dimension *in separation*. Chiral recognition is here generally carried out by conventional heart-cut GC–GC [9–12], where the first column, coated with a conventional phase, serves to locate the peak(s) of the optically active racemate(s), and the second column, coated with a CD stationary phase, separates its(their) enantiomers after online transfer through the heart-cut interface. When the number of components to be investigated is very high, comprehensive two-dimensional gas chromatography (GC × GC) can also be used but, in this case, column "geometry" must be inverted because of the high efficiency required by the columns coated with CDs in order to give reliable separations [13,14], the first column has to be coated with the enantioselective CD stationary phase while the second one "distributes" the peaks over the chromatographic plane [15,16]. The second approach involves the use of a second dimension *in identification*. In this case, the enantiomer is located and identified by MS detection (or very rarely FT-IR). Single- or multiple-ion monitoring-MS (SIM-MS) carried out after a careful choice of suitable diagnostic ions of the optically active marker(s) of the sample under investigation can be applied to "clean" the part of the chromatogram where the enantiomers elute, thus making correct EE and/or ER determination possible.

In general, most GC–MS software takes insufficient account of the identification potential of GC, because the identification power of mass spectrometry when used as detector for GC is considered to be, and very often is, exhaustive. Retention indices (I_s) are the most reliable and effective tool for analyte identification by GC data. They were first introduced by Kovats for isothermal analysis [17] and then by Van den Dool and Kratz for temperature programmed analysis [18], the latter being better known as linear retention indices (I^T values). Most GC–MS software packages do not include I^T values as identification criterion, and only some of them report I^T values in the library as "blind or inactive" data appearing in the legend of each proposed identification record, making them only useful for further or additional confirmation. On the contrary, the "interactive" use of I^T values (i.e. their use as an active identification parameter) can be highly effective since it provides a second "independent" tool to identify a compound, operating actively and simultaneously in parallel to MS spectra. Moreover, I^T values are based on a *chemical property* of an analyte of a completely different nature compared to MS, which can orthogonally and synergically be combined with its MS fragmentation pattern, i.e. its chromatographic interaction with a given chromatographic separation system or better with a given stationary phase.

Mass spectrometry is well known to be unable to discriminate between optical isomers, not being a selective chiral probe in this sense, and therefore giving indistinguishable spectra. As a consequence it cannot be used alone to determine which enantiomer is present in a sample, or to establish the predominant one or to measure its EE and/or ER. In enantioselective GC–MS, a given optical isomer can only unequivocally be identified through its I^T obtained with a column coated with a chiral selector suitable to separate it from its enantiomer. In the chiral recognition of optically active isomers in a complex mixture, the two identification parameters (i.e. I^T values and MS spectrum) must therefore be combined but, unlike conventional GC–MS analysis, mass spectra (or diagnostic ion monitoring) are used to locate the two enantiomers in the chromatogram, and I^T values for their identification.

This study deals with the development of a MS library specific for the identification of optically active compounds in the flavour and fragrance field using "interactive" I^T values in parallel to MS spectra, so as to identify enantiomers reliably and, when necessary, to enable the measurement of EE and/or ER unequivocally.

2. Experimental

2.1. Racemate standards and essential oils

Analyses of 134 racemate standards and pure enantiomers solubilised in cyclohexane at a concentration of 100 ppm were carried out. The enantiomeric recognition of the marker compounds characteristic of commercially available essential oils (e.o.) and extracts was also carried out, in particular balm lemon, bergamot, boronia, cornmint, lavender, lemon, peppermint, and rosemary e.o.s.; apple flavour and apricot, peach and coconut headspace sampled by solid-phase microextraction (SPME) were analysed.

2.2. Enantioselective GC columns

The library has been built on the basis of I^T values obtained on four columns (25 m × 0.25 mm I.D., d_f: 0.25 μm) coated with four cyclodextrin derivatives as chiral selectors diluted at 30% in PS-086:

2,6-di-*O*-methyl-3-*O*-pentyl-β-CD
(2,6DM3PEN-β-CD) [19,20]
2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-β-CD
(2,3DM6TBDMS-β-CD) [21]
2,3-di-*O*-ethyl-6-*O*-*tert*-butyldimethylsilyl-β-CD
(2,3DE6TBDMS-β-CD) [22]
2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-β-CD
(2,3DA6TBDMS-β-CD) [21]

All columns were from MEGA (Legnano, Italy). Their performance were periodically tested through the Grob test [23,24] and a laboratory-made chiral test [25] consisting of limonene, 2-octanol, camphor, isobornyl acetate, linalyl acetate, 2-methyl-(3*Z*)-hexenyl butyrate, menthol, hydroxycitronellal, γ-decalactone and δ-decalactone racemates.

2.3. Enantioselective GC–MS conditions

A Shimadzu QP2010 GC–MS system was used and results were elaborated with the Shimadzu GCMS Solution 2.51 software (Shimadzu, Milan, Italy).

GC conditions: injection mode, split; split ratio, 1:20 for standard solutions, 1:50 for essential oils; injection volume, 1 μl. Temperatures: injection, 220 °C; transfer line, 230 °C; ion source: 200 °C; temperature programme: from 50 to 220 °C at 2 °C/min (if not specified otherwise). Carrier gas: He; flow rate: 1.0 ml/min.

2.4. Library setting-up

The library was created on the basis of the analysis of the 134 racemate standards and pure enantiomers, on each column, recording their mass spectra, calculated I^T values and enantiomer resolution (R). The I^T determination was carried out by injecting an homologous series of *n*-alkanes containing 17 *n*-hydrocarbons (C_9–C_{25}) purchased from Supelco (Bellefonte, PA, USA), each at