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Enantiomeric analysis of limonene and carvone by direct introduction of aromatic plants into multidimensional gas chromatography

C. Barba [a], R.M. Toledano [b], G. Santa-María [a], M. Herraiz [a,*], R.M. Martínez [a]

[a] Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN), Consejo Superior de Investigaciones Científicas (CSIC), c/Juan de la Cierva 3, 28006 Madrid, Spain
[b] Escuela Técnica Superior de Ingenieros Agrónomos, Universidad de Castilla-La Mancha (UCLM), Campus Universitario s/n, 02071 Albacete, Spain

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ABSTRACT

Analysis of chiral compounds in complex mixtures is achieved by multidimensional gas chromatography using heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin stationary phase as the main column of the system to separate specific selected cuts containing components unresolved in the first dimension. The proposed procedure allows rapid analysis of both solid and liquid matrices by direct introduction, into the programmed temperature vaporizer (PTV) of a gas chromatograph, of either the plant material or the essential oil, respectively. A comparison between enantiomeric excesses data obtained, from plant leaves (or plant seeds) and the corresponding essential oils, by direct injection (i.e., without sample pretreatment or concentration step) into the multidimensional system is also included. Reported data demonstrate that no racemization occurs during analysis as identical enantiomeric excesses are obtained in both cases for specific chiral compounds.

1. Introduction

Reliable analysis of mixtures containing numerous compounds from various chemical classes demand sufficient resolutions of specific pairs of components. However, this can be difficult to accomplish even if stationary phases with adequate selectivities as well as columns with the required separation efficiencies, in terms of theoretical plate numbers, are used. Thus, high-resolution and/or high-performance analyses are to be performed specially in those cases in which maximum resolutions are required (e.g., for the separation of compounds with similar structures and, specifically, for achieving enantiomeric resolution of optical isomers).

Generally speaking, the analysis of complex mixtures cannot be accomplished by a single chromatographic separation (i.e., in a one-dimensional system), even when careful optimization of chromatographic parameters has been performed. Particularly, mixtures of compounds covering a wide range of concentrations usually require successive chromatograms to be run in order to adjust the sample size demanded for each compound of interest while also controlling overloading due to major components occurring in concentrated samples.

As previously reported [1,2], very often selected fractions of the eluate resulting from a pre-separation can be analyzed, without losing relevant information, instead of analyzing the total mixture. Moreover, the fact that the main separation can be carried out undisturbed by peak overlapping is an interesting advantage concerning the reliability of the compound identification. Thus, by combining two columns of different polarities and selectivities, two sets of retention data can be finally obtained from a single sample introduction into the system [3–6].

In this respect, the use of a double column system may allow the selective removal of some disturbing components in such a way that they are prevented from entering the main separation. Actually, only a sharp cutting of significant peaks are selected and subsequently allowed to enter the second column in which the chromatographic resolution of the target compounds can be lastly achieved [7–11].

Specifically, multidimensional gas chromatography (MDGC) meets a number of the well-known requirements that involves the analysis of chiral compounds in complex mixtures as demonstrated when analyzing different real-life samples [12–17].

On the other hand, the interest of developing reliable analysis of natural plant components due to their extensive use as raw materials in the agro-food, pharmaceutical and cosmetic industries is widely recognized. The complexity of the sample very often demands sample preparation and concentration steps to be performed prior to the beginning of the chromatographic analysis itself. To this aim, the use of different techniques such as liquid–liquid extraction, steam distillation, headspace sampling, high-pressure solvent extraction, supercritical fluid extraction as well as other solvent free methods (e.g., solid phase microextraction and stir bar sorptive extraction) has been proposed [18–21].

* Corresponding author. Tel.: +34 91 258 7535; fax: +34 91 564 4853.
E-mail address: mherraiz@ictan.csic.es (M. Herraiz).

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Nevertheless, these steps may be a source of errors as well as artifacts and, occasionally, the initial composition of the sample can be altered due to rearrangement reactions resulting from reactive components. This aspect is particularly relevant when aiming the enantiomeric resolution of chiral compounds as, under some experimental conditions applied during sample preparation, racemization can be brought about, and consequently, unreliable enantiomeric ratios will be eventually established.

Previous work has shown the possibility of analyzing volatile compounds in solid matrices by direct introduction into the programmed temperature vaporizer (PTV) of a one-dimensional GC system [22] but risks of column overloading and overlapped peaks could not easily be prevented. Also, the use of adsorbent materials to trap (as well as to concentrate) the target compounds has been earlier proposed although a preparation step was required prior to the chromatographic separation in order to sweep the analytes from the matrix and, subsequently, to retain them onto a suitable material placed inside the injector [23,24].

The aim of this work was to evaluate the usefulness of on-line coupled MDGC–MS to perform the chiral analysis of both solid and liquid matrices by direct introduction (i.e., without any kind of sample handling), into the PTV of the chromatograph housing the pre-column, of either solid plant material or the essential oils obtained thereof.

2. Materials and methods

2.1. Materials

Plants of *Mentha piperita* L. (*Mentha spicata x Mentha aquatica*, chemotype menthone) and seeds from *Carum carvi* L. (caraway, chemotype estragole) coming from wild plants grown in Murcia (Spain) were collected and dried. Essential oils were obtained, using a Clevenger-type system, by hydrodistillation for 3 h of aerial parts from *M. piperita* and seeds from *C. carvi* and subsequently dried with anhydrous sodium sulfate, thoroughly shaking and standing until the supernatant oil had become clear, and kept in amber vials at 4 °C until starting their chromatographic analysis. *M. piperita* and *C. carvi* were selected because of the availability of both the plant material and the essential oil obtained thereof with the guarantee of knowing for certain their origin and traceability.

For identification purposes, a test solution containing (*R*)-limonene, (*S*)-limonene, (*R*)-carvone and (*S*)-carvone was used. To establish the enantiomeric composition of limonene and carvone in both *M. piperita* and *C. carvi*, two different approaches were considered (i.e., direct introduction into the MDGC system described below of a plant material as well as of the corresponding essential oil resulting from the same plant).

2.2. Chromatographic columns

Column 1: 30 m × 0.25 mm i.d. fused-silica capillary column coated with a 0.25 μm layer of 5% phenyl-95% polydimethylsiloxane (ZB-Wax, Micron Analítica, S.A., Madrid, Spain).
Column 2: 30 m × 0.25 mm i.d. fused-silica capillary column having a 0.25 μm film thickness of heptakis-(2,3,6-tri-*O*-methyl)-*β*-cyclodextrin, (Chirasil-*β*-Dex, Varian, Middelburg, The Netherlands).

2.3. Direct introduction of the solid material

0.8 mg sample weight of dried and crushed leaves or seeds from the plant material was introduced without any pre-treatment into the glass liner (54 mm × 3.4 mm i.d. × 5 mm o.d.) of the PTV injector between two small plugs of deactivated glass wool. The glass liner was placed into the injector, kept at 40 °C, after having interrupted carrier gas circulation. Once carrier gas flow was established again, the chromatographic analysis was performed by thermal desorption and subsequent transfer of the material to the capillary column by increasing (at approximately 200 °C/min) the injector temperature up to 250 °C. The end temperature was maintained for 5 min and the PTV was operated in the split mode, 10:1 being the split ratio. After completing the thermal desorption step, the sample was analyzed using either GC–FID with the ZB-Wax column described in Section 2.2 or MDGC–MS with the ZB-Wax and the heptakis-(2,3,6-tri-*O*-methyl)-*β*-cyclodextrin as the pre-column and the main column, respectively (see Section 2.2 for further details). In all cases, the flame ionization detector (FID) used was set at 250 °C.

2.4. MDGC–MS analysis of plants and essential oils obtained thereof

Direct introduction of plants as well as of the essential oils obtained from them were performed under the following conditions: the MDGC equipment consisted of two independent gas chromatographs (Varian, model CP-3800, Palo Alto, CA, USA) housing two columns, namely pre-column and main column which were serially coupled through a Deans (Varian) based switching system, and a transfer line kept at 180 °C throughout the experimentation. This pneumatic flow switching with a valveless Deans switch system was used to transfer the selected analytes (contained in the so-called heart-cut) from one column to the other because it eliminates the problems associated with the use of mechanical valves (e.g., dead volumes, sample adsorption and bad resistance to high temperatures). However, pressure adjustments were required before being operational as the flow switching during the transfer time is achieved by pressure-directed changes in flow from an auxiliary electronic pressure control (EPC) module. In this case, flow direction control was obtained through a solenoid valve so that flow eluting from the first column could be directed either to a detector (FID) or to the main column, upon elution of the selected cut containing the target compounds. This Deans switch system could be applied at high temperatures because it uses flow channels with no valves or rotor faces, so that sample components are not in contact with any moving part. Moreover, a system of flows between and in the columns could also be established for the reversal of the eluent direction after peaks of interest were detected. This backflushing protects the columns from degradation and contamination and also allows column changes and injector maintenance without loss of vacuum in the MS detector.

The pre-separation was performed using the ZB-Wax column mentioned in Section 2.2. The oven program temperature was started at 60 °C (1 min), increased at 4 °C/min up to 150 °C (5 min), and finally raised from 150 to 200 °C at 5 °C/min (20 min). The selected cuts were transferred into the main column (i.e., to the second dimension) and analyzed using heptakis-(2,3,6-tri-*O*-methyl)-*β*-cyclodextrin as stationary phase (column 2 in Section 2.2). The oven temperature was initially set at 50 °C (15 min) and then successively raised to 70 °C (1 °C/min), to 140 °C (2 °C/min) and finally to 200 °C (4 °C/min). In both dimensions, helium served as the carrier gas at an approximate head pressure of 30 psig in the pre-column and 24 psig in the main column. In all cases, a 0.1 μL volume of essential oil (without previous dilution) was injected into the MDGC system.

Separations achieved in the pre-column were monitorised using an FID detector (operated at 250 °C) while the main column was connected to a Saturn 2000 ion-trap mass spectrometer (Varian). Data acquisition was carried out using a Star Toolbar system (Varian). The target compounds were identified by matching the GC retention times observed in both dimensions with those