

Multidimensional Gas Chromatography- A Powerful Tool for the Analysis of Multicomponent Mixtures

-Review-

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Abstract

The development of high resolution capillary columns and a large variety of different detectors led to a strong position of gas chromatography in instrumental analysis. Every effort has been made to solve sophisticated separation problems by column switching. Nowadays, several systems are commercially available for this purpose. The principle and the capabilities of multidimensional gas chromatography(MDGC) are illustrated by different applications in the field of modern flavor and essential oil research.

Key words: multidimensional gas chromatography, complex mixture analysis, flavor research, essential oils, chiral analysis

INTRODUCTION

The development of the capillary column technology brought about fundamental changes in the application of gas chromatography(GC) for the separation of complex mixtures. A huge variety of different detectors together with the development of high resolution capillary columns helped to bring GC in almost every analytical laboratory. Many chromatographic problems can be solved by standardized or "tailor made" GC columns. Complete analysis of multicomponent mixtures, however, has proven to be an elusive goal due to a variety of factors(1). Samples containing several hundred components are often cause difficulties because of incomplete separation and large dynamic ranges for detection. Some components with similar retention times may cause extremely long analysis time. In 1968 Deans reported for the first time on the column switching in gas chromatography(2). In the following years, the work of Schomburg and coworkers(3-5) played an important role in the development of modern column switching techniques.

THEORY OF MDGC

Standard setup of a GC instrument consists of injection system, column, and detection system. Application of two or more columns(e.g. polar and non-polar) to one injection system and to different detection systems(e.g. flame ionization detector(FID)), together

with flame photometric detector(FPD) is well known. The performance of this type of single stage GC system is limited by the separation characteristics of the column used. In case of multidimensional gas chromatography(MDGC), it is possible to combine different GC columns. The transfer of peaks in the effluent of the first column to a second column is the main operational mode in MDGC. Therefore, MDGC is a powerful tool to address a variety of different separation problems. The analysis of contaminated samples by highly sensitive detectors(e.g. electron capture detector(ECD)) causes less problems. Trace compounds can also be separated from complex mixtures. Deficient steps in sample cleanup can be compensated. Furthermore, enhanced peak purity helps to improve the precision of quantification methods.

In gas chromatography the dependance of resolution (R) on the column length(l) is described by $R \propto \sqrt{l}$. The improvement of selectivity, however, directly contributes to better resolution. Therefore, the first column is often used for a pre-separation, while the selectivity of the second column is chosen for a specific separation purpose.

The application of two columns of different polarity (e.g. first column nonpolar, second column polar) results in the excellent separation of many complex mixtures.

EFFLUENT TRANSFER

In MDGC the transfer of certain segments of the

initial complex chromatogram (precolumn) to a second column (main column) is also known as heart-cutting technique. Implementation of the heart-cutting can be performed by trapping or by direct transfer. In the trapping mode, the effluent is transferred to a cryogenically cooled trap. The final separation is achieved on the main column after heating up the trap. The focussing effect of the trapping technique has several advantages (6). Columns of different diameter and sample capacity can be used in the same system without any deficiency in chromatography. The need of automation has led to commercially available MDGC-instruments operating according to the principle of direct transfer. Considering the carrier gas control, MDGC-systems can be designed as an apparatus with a pressure controlled column switching device or with a flow controlled one (7). Both versions are operating with extremely low dead volume. The system design avoids any contact of the sample with switching valves. Furthermore, it is possible to reverse the carrier gas flow on the precolumn in order to elute high boiling matrix contaminations out of the system (backflush mode).

FLOW CONTROLLED MDGC-SYSTEMS

The commercially available multidimensional Gerstel MCS system (Gerstel Germany) can be used for analytical as well as for preparative purposes. The system is provided with a mass flow controlled switching device whereby the different flows are electronically set by digital mass flow controllers. After the chromatographic separation on precolumn and main column, single peaks can be transferred to a cooled trapping unit (8). The successful adaptation of the trapping system for the isolation of material in the microgram range has been demonstrated by Werkhoff and coworkers (9). The authors reported the isolation of various flavor compounds (e.g. *trans*- α -ionone from costus root oil, *cis*- and *trans*-2,4,5-trimethyl-5-hydroxy-3-thiazoline and 2-isobutyl-4,5-dimethyl-3-thiazoline from yeast extracts) using the fully automated MCS system for preparative capillary gas chromatography including a modified trapping device. For large sample capacity the system was equipped with a thick film precolumn (5m DB-1/df=5 μ m) and a 30m main column (DB-1/df=1.5 μ m). For the isolation of the different thiazolines, the instrument was run several days (55 cycles), and

then material was collected for different $^1\text{H-NMR}$ pulse experiments.

PRESSURE CONTROLLED MDGC-SYSTEMS

Schomburg and coworkers (10) developed the principle of a pressure controlled MDGC-system, which has been called "Live-column switching system". The main part of this setup is a coupling union (Live-T-piece), which contains a coupling capillary made of platinum and iridium. The whole system operates in a GC with two ovens (Sichromat 2-8, Siemens, Germany), which enables different temperature programs for pre- and main column.

Furthermore, there are also other types of MDGC-systems available. In the MCSS-system (Moving-Column-Stream-Switching) the front end of the second column can be moved into the effluent stream of the first column in order to transfer single peaks or peak groups (11). The two-dimensional switching system, called MUSIC (Multiple Switching Intelligent Controller) developed by Chrompack in the Netherlands, is equipped with a trapping device for the transfer of fractions from the first column to the second column (12).

HYPHENATED MDGC-SYSTEMS

In MDGC simple detection systems, such as flame ionization detection (FID), are suitably used and the ideal detector should be selective, sensitive and structurally informative. Therefore the coupling to mass spectrometry (MDGC-MS) (13,14), to infrared spectrometry (MDGC-IR) and to both spectroscopic techniques (MDGC-IR-MS) (15) are the closest approach to this idea.

Fig. 1 shows the coupling of the pressure controlled double oven GC Sichromat 2-8 (Siemens, Germany) to an Ion-Trap mass spectrometer (Finnigan, USA). The instrument is equipped with a non-chiral precolumn and a chiral main column. Pre- and main column are connected by a Live-T-piece, which allows a stream of effluent to a monitor detector (FID 1) or to the main column. Restriction capillary is connected to a second Live-T-piece so the effluent of the chiral column can be transferred to the MS unit. The interfacing to the MS-detector consists of a transferline and an open-split interface. This MDGC-MS system is equipped

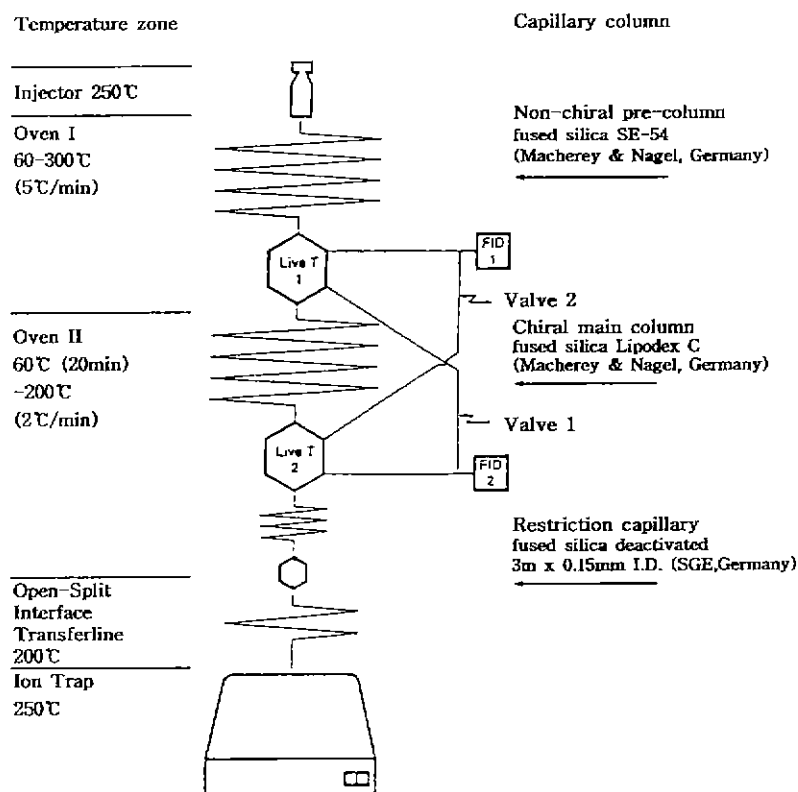


Fig. 1. Multidimensional gas chromatography-mass spectrometry (MDGC-MS); Coupling of a double oven GC (Sichromat 2-8, Siemens, Germany) to an Ion Trap mass spectrometer (ITD-800, Finnigan, San Jose, USA). Oven 1 is equipped with a non-chiral pre-column. Oven 2 is equipped with a chiral main column(16).

with three detectors and two separation columns all together.

Nitz et al.(17) reported the first application of MDGC coupled on-line to isotope ratio mass spectrometry (MDGC-IRMS). This special system allows the determination of the ratio of ^{13}C to ^{12}C in different enantiomers, which is also known as enantioselective stable isotope ratio analysis(enantio-SIRA).

MDGC IN FLAVOR RESEARCH

In the last years, MDGC techniques were essential for the progress in flavor and essential oil research. In particular, the analysis of chiral flavor compounds using enantioselective MDGC with non-chiral precolumn and chiral main column started a systematic evaluation of enantiomeric ratios in natural products. Chiral flavor and fragrance components of natural origin generally have a characteristic distribution of enantiomers, which often differ not only in their qualitative organo-

leptic properties but also in their threshold values(18-21).

2-Methylbutyric acid ethyl ester(2-MBE) is an important component of apple flavor. Mosandl and co-workers(22,23) showed that 2-methylbutyric acid and the corresponding esters are present in natural products at high optical purity favoring the (S)-con-figured enantiomer. Fig. 2 illustrates the enantioselective MDGC analysis of 2-MBE in apple aroma(24). After pre-separation on a non-chiral precolumn 2-MBE is transferred to a chiral main column. Therefore, the peak for 2-MBE does not appear in the chromatogram of the pre-separation(Fig. 2 middle). The stereodifferentiation of 2-MBE reveals a racemic distribution. The picture on the right side shows the high optical purity of the (S)-enantiomer in apples. This finding clearly indicates that this apple aroma contains 2-MBE coming from sources other than apples.

Fig. 3 shows the chiroselective analysis of *cis*- and *trans*- α -irone, which are components of iris root oil (25). *Trans*- α -irone is responsible for a typical and

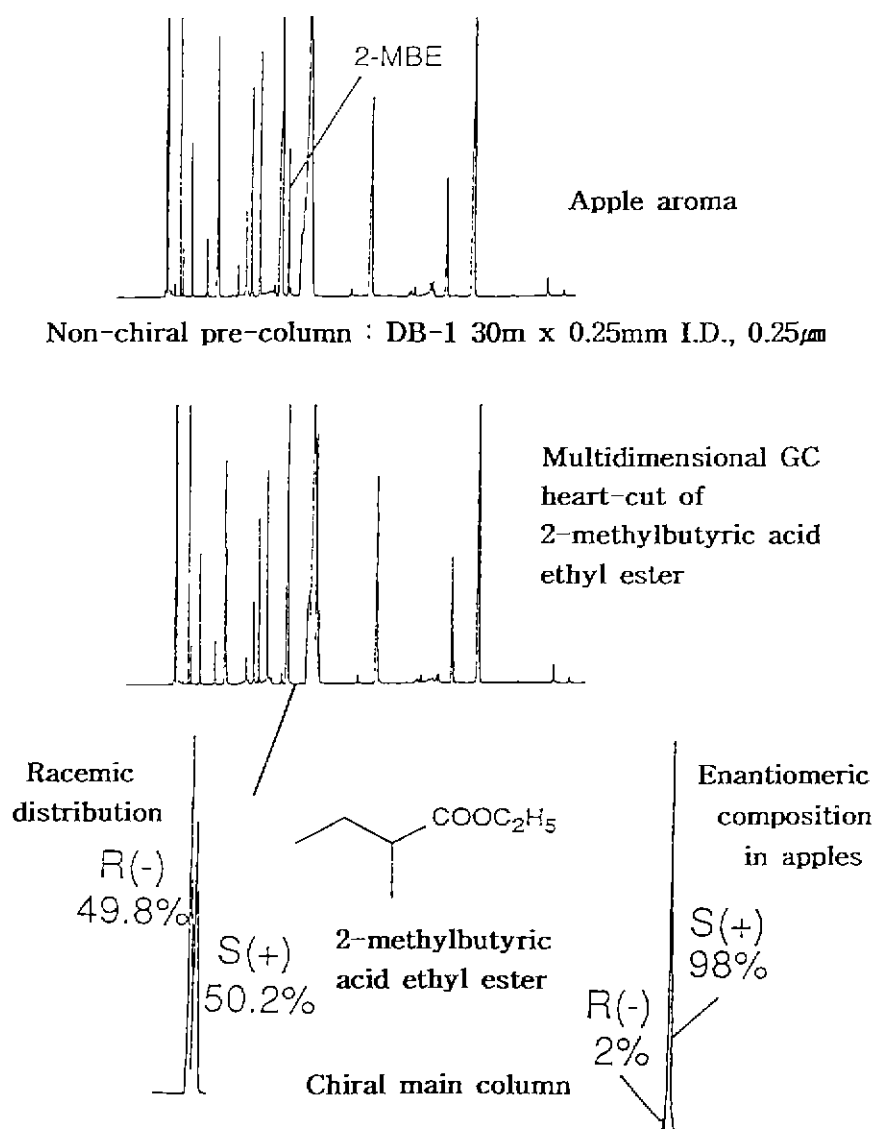


Fig. 2. Enantioselective MDGC analysis of 2-methylbutyric acid ethyl ester(2-MBE) in apple aroma; pre-separation on a non-chiral pre-column(DB-1, 30 \times 0.25mm I.D., 0.25 μ m).

Heart-cut of 2-MBE to a chiral main column. Chiral analysis on a heptakis-(2,3,6-tri-O-methyl)- β -cyclodextrin column 1A. racemic 2-MBE, 1B: High optical purity of S(+)-2-MBE in an apple aroma(24)

pleasant odor impression while the sensory description of *cis*-irone determines weaker woody notes. Using the MDGC-technique both compounds were transferred from a polar precolumn to a chiral main column. The enantiospecific analysis of both isomers revealed a specific enantiomeric distribution(26). The dextrorotatory form of *trans*- and *cis*- α -irone dominates in this essential oil.

In flavor chemistry lactones play an important role. 3-Methyl-4-octanolide(see Fig. 4) has been reported to possess a woody odor. Suomalainen and Nykaenen

(27) were the first to identify the lactone in blended Scotch whisky. Masuda and Nishimura(28) showed that this compound, also known as whisky lactone, originates from oak. It has been detected in almost all beverages which have undergone maturation in oak barrels. Guenther and Mosandl(29) showed 3-methyl-4-octanolide to be present in oakwood as the (3S, 4S)-*cis* and the (3S, 4R)-*trans* form. Fig. 4 illustrates the GC separation of volatile flavor compounds in whisky aroma. Werkhoff and coworkers(30) isolated all 4 stereoisomers of racemic *cis*- and *trans*-whisky lactone by

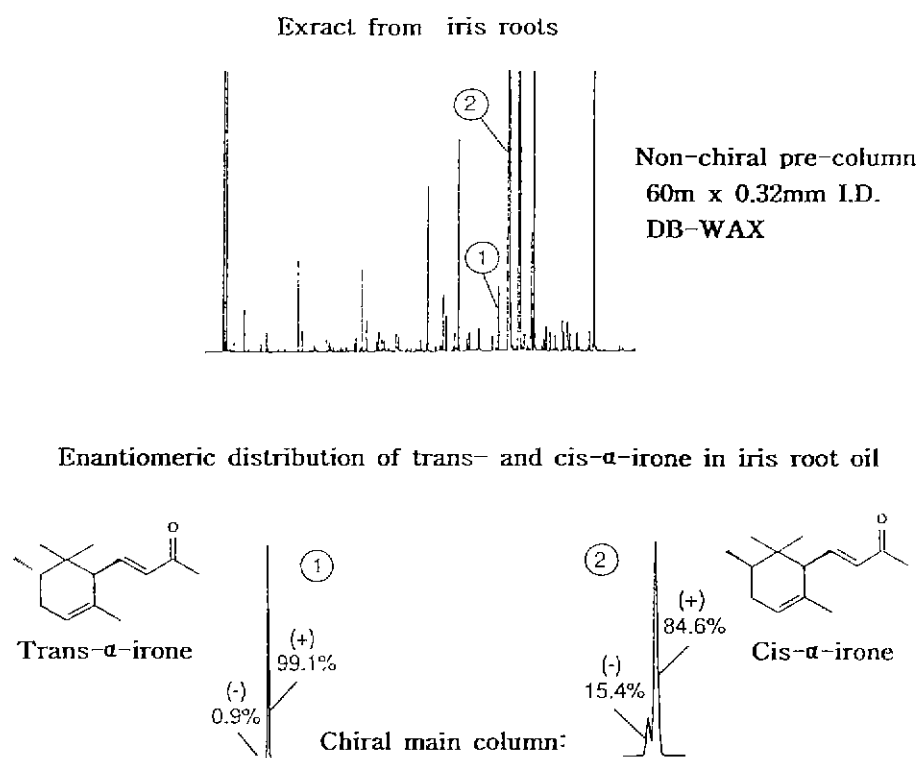


Fig. 3. Chirospecific analysis of *trans*- and *cis*- α -irone from iris roots oil; pre-separation on a non-chiral pre-column (DB-WAX, 60m \times 0.32mm I.D./0.25 μ m).

Heart-cut of *trans*- and *cis*- α -irone on two different chiral main columns (column 1: heptakis-(2,3,6-tri-O-methyl)- β -cyclodextrin; column 2: octakis-(3-O-butyl-2,6-di-O-pentyl)- γ -cyclodextrin)(25).

Table 1. Sensory description of racemic and optically pure *trans*- and *cis*-whisky lactone(30,31)

Compound	Odor impression(1% in ethyl alcohol)
<i>trans</i> -whisky lactone	lactone-like, herbaceous, hay, massoi-lactone, celery, spicy, slightly lovage-like, jasmine-like
<i>cis</i> -whisky lactone	sweet, lactone-like, cinnamon-like, jasmine lactone, fatty, coumarin-like
<i>trans</i> -(3S, 4R)	lactone-like, coconut-like, herbaceous, woody, spicy, hay, celery, lovage-like, massoi oil character, cis-jasmine-like, very strong odor impression
<i>trans</i> -(3R, 4S)	sweet, lactone-like, fruity, flowery, hay, peach, jasmine lactone character, coconut-like, celery, herbaceous, strong odor impression
<i>cis</i> -(3R, 4R)	sweet, smooth, lactone-like, hay, coumarin-like, weak coconut character, moderate odor impression
<i>cis</i> -(3S, 4S)	sweet, cinnamon-like, smooth, lactone-like, fatty, herbaceous, very weak coconut-like, massoilactone- /cis-jasmine-like, moderate odor impression

preparative chromatography on a chiral octakis-(3-O-butyl-2,6-di-O-pentyl)- γ -cyclodextrin-thickfilm column. The sensory description of the racemic *trans*- and *cis*- whisky lactone and of the optically pure en-

antiomers(Table 1) revealed different organoleptic properties. This example demonstrates characteristic sensory differences between mirror image isomers, which clearly indicates the importance of enantioselective MDGC in

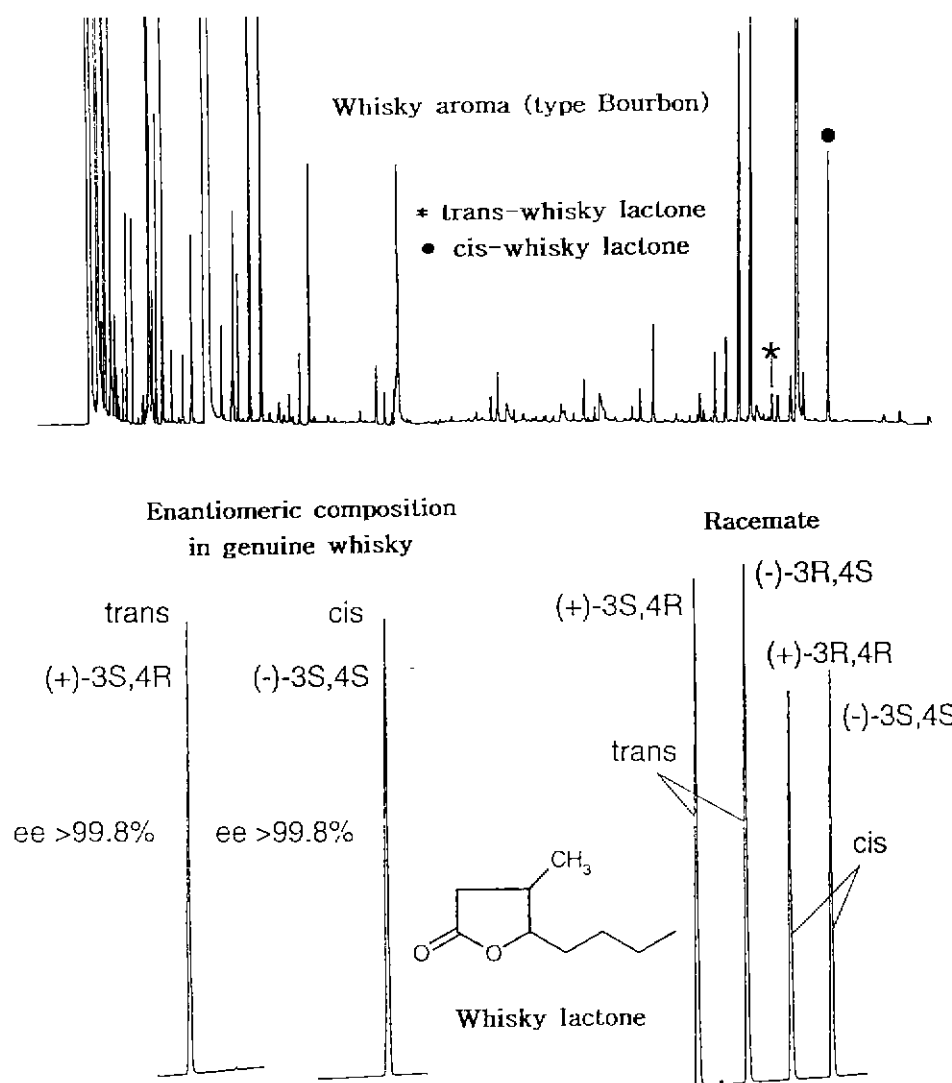


Fig. 4. a. GC-Analysis(DB-WAX ; J&W Scientific ; Folsom, CA, USA) of volatile flavor compounds in whisky aroma(type Bourbon).

b. Enantiomeric composition of *trans*- and *cis*-whisky lactone in whisky. Enantiomeric excess(ee) of the (+)-3S, 4R *trans*-isomer and (-)-3S, 4R *cis*-isomer higher than 99%.

In comparison chirospecific analysis of the racemate. Column 30m octakis-(3-O-butyl-2,6-di-O-pentyl)- γ -cyclodextrin(30).

flavor and essential oil research.

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