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The use of shape-selective stationary phases in GC. Part 1: Cyclodextrin phases

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ABSTRACT - Cyclodextrin phases are one of the most interesting geometry selective stationary phase in GC. They are outstanding in their ability to separate isomeric and enantiomeric compounds. Two examples of the separation of cyclic compounds on cyclodextrins are given. The combination of chiral and achiral phases in series coupled columns is a useful tool for complicate separation and identification problems.

INTRODUCTION

During the last few years the interest in analysis of isomeric compounds of complex mixtures (flavours, fragrances, foods, biological and environmental samples) has been steadily growing. But the use of GC stationary phases on the basis of classical interaction forces offers only limited possibilities for the separation of isomeric compounds with similar properties, even if capillary columns with a high efficiency are used. This requires the study of the separation properties and the potential of so-called shape-selective stationary phases.
It is not easy to give a proper definition of shape-selective stationary phases. In this paper in a simplified way we assume that by using this type of stationary phases the geometry of the molecules which have to be separated is the predominant factor for the retention and the separation, too.

TABLE 1. Shape-selective stationary phases in GC

GC Phase	Retention mechanism	Separation according to
Nonspecific non-porous adsorbents	Nonspecific interaction	Geometry of molecules
Liquid crystals	Mixed adsorption and partition	Ratio of length to width
modified cyclodextrins	hydrophobic and H-bonding interaction	Enantioselective host guest interaction/inclusion effects
Metal complexes	Complexation-GC	Geometry of formed molecular complexes
Optically active polysiloxanes	H-bonding interaction	Functional groups of the molecules

The advantage of shape-selective stationary phases is to achieve a separation of "problematic compounds", e.g. isomers or enantiomers (compounds with very similar properties), but the application of these shape-selective stationary phases can be connected with some problems and difficulties:

- The possibility of coelution of separated isomers or enantiomers with other components which frequently occurs in very complex mixtures.
- The peak overlapping well be, nevertheless, shifted to some other point of the chromatogram.
- The lack of retention data available in the literature prevents the use of a retention index concept as an identification tool.
- It is difficult to make a statement about the retention of unknown compounds because of the limited knowledge about the retention mechanism and separation characteristics.

SEPARATION ON CYCLODEXTRINS

Cyclodextrins are cyclic oligosaccharides, containing glucose units which are linked through a 1.4 bonding in a rigid chair conformation. α-cyclodextrin consists of 6, β-cyclodextrin of 7, γ-cyclodextrin of 8 glucose units. These cyclic molecules form a truncated cone opened on both sides. The secondary 2- and 3-hydroxy groups of the glucose units are located at the larger opening. The primary hydroxy group in 6-position which is able to rotate is located at the smaller opening of the cavitiy.

The interior of the cavity contains only methylene groups and glycosidic oxygen bridges. It is, therefore, rather hydrophobic with a high electron density. The three cyclodextrines differ in the inner diameter of the cavity, while their hight is the same. The cyclodextrins are able to form reversible inclusion complexes. In the case of chiral substances, diasteromere associates of a different stability will result (ref. 1).
Therefore, the cyclodextrins have become increasingly important as stationary phases for the separation of isomers - both positional and geometrical isomers - and especially of enantiomers in gas and liquid chromatography.
In capillary GC exclusively modified cyclodextrins are used as stationary phases, i.e.,

- Peralkylated cyclodextrins (permethyl or perpentyl)
- Cyclodextrins which are partly alkylated and/or acylated

We studied the retention of different substances on commercially available capillary columns coated with permethylated α- and β-cyclodextrin, respectively. In both columns the cyclodextrin is dissolved in a weak polar polysiloxane (OV-1701).

The separation of the isomeric methylcyclohexanols depend on the position of the methylene group: Whereas the cis- and the trans-2-methylcyclohexanols could be separated into the enantiomers, for the 3-methylcyclohexanols only a partly enantiomer separation for the trans-isomer could be achieved by using a column with hexakis(2.3.6-tri-o-methyl)-α-cyclodextrin as stationary phase. It should be noted that by using β-cyclodextrin we could not get any separation although it was described in literature (ref. 2).

An interesting fact is the influence of the ring size of investigated molecules on the retention. In Fig. 1 we compared the retention of 2-methylcyclohexanol with that of 2-methylcycloheptanol and of 2-methylcyclooctanol. In each case there exist 2 chiral isomers, i.e. 4 enantiomers. It is well known that the cyclohexane chair has a more rigid structure, whereas the cycloheptane ring is more flexible. The cyclooctane ring

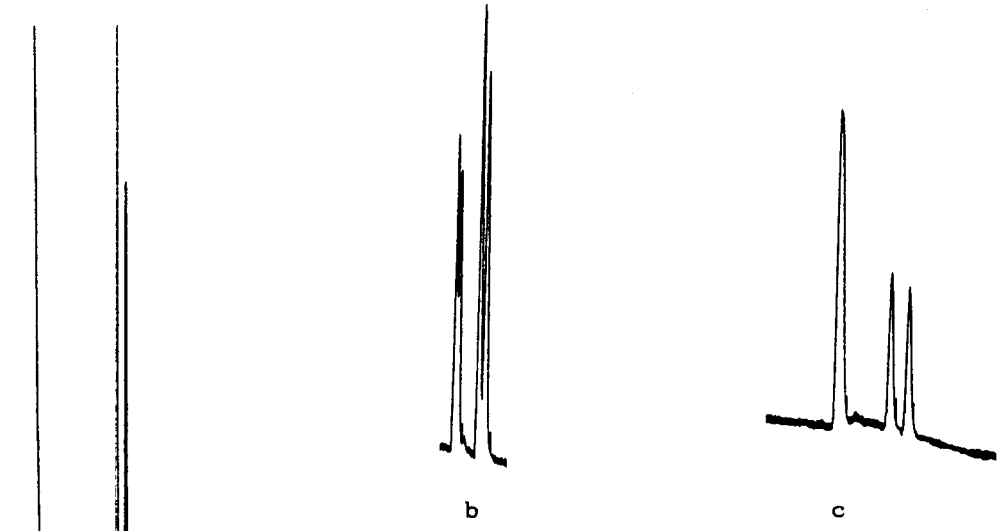


Fig. 1.
a. 1st peak: Trans-2-methylcyclohexanol, k'= 3,18
   2nd peak: Cis-2-methylcyclohexanol, k'= 3,52
b. Trans-2-methylcycloheptanol, k'= 7,48/7,59
   Cis-2-methylcycloheptanol, k'= 8,28/8,48
   Temperature: 105°C (a and b)
c. Cis-2-methylcyclooctanol, k'= 12,21
   Trans-2-methylcyclooctanol, k'= 13,80/14,34
   Temperature: 110°C
Column: CP CYCLODEX β 236 M, 25m x 0.25mm I.D., carrier: Hydrogen

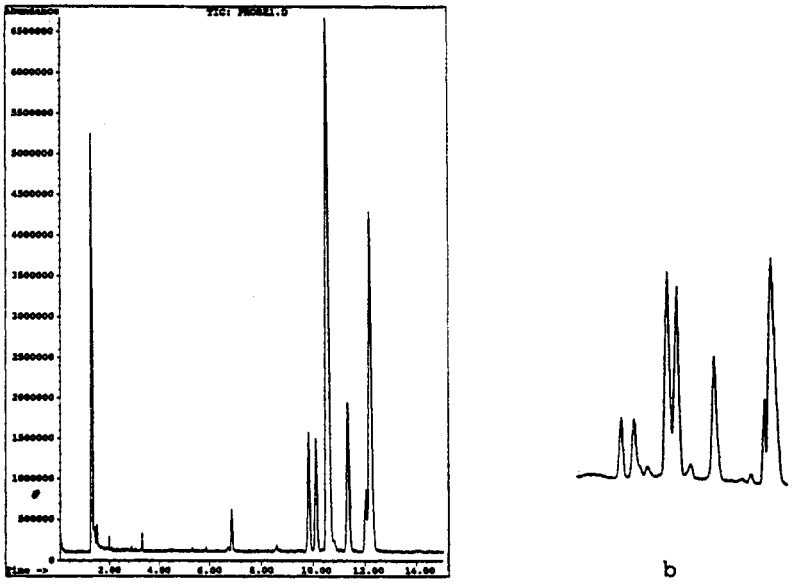
again exhibits a more rigid conformation (ref. 3). According to our experience in the field of hydrocarbon analysis, the retention differences of isomeric cycloheptane derivatives are smaller than those for the corresponding cyclohexane derivatives (ref. 4). Surprisingly, the separation of all 4 enantiomers was the best in the case of 7 membered ring. In the case of 8 membered ring only one isomer (trans) can be separated into the corresponding enantiomers.

USING MULTIDIMENSIONAL GAS CHROMATOGRAPHY

Multidimensional gas chromatography is an efficient method of analysis specially used for very difficult separation problems. The improved resolution can be reached by changing the polarity and/or the temperature (ref. 5).

Multidimensional chromatography was widely used for the enantio-specific analysis of natural product mixtures (ref. 6-8). The first column was intended for the preseparation of the complex mixture, whereas the main chiral column was used for the enantio-specific separation of the selected cuts. However, the coupling of achiral high-efficiency separation columns with chiral main columns is not only a useful means for the analysis of complex mixtures, but can also be used for solving difficult separation problems (ref. 9/10). An example is given in Fig. 2.

The isomeric cyclotrideca-1,5,9-trienes are used as model compounds for studying conformative effects. The compounds were available as synthesis mixture (ref. 11/12). As it shown in a comparison between the two chromatograms, a complete separation of the isomers is only possible by means of multidimensional GC.



a b

Fig.2 Chromatogram of a mixture of isomeric cyclotrideca-1,5,9-trienes
a. Column: CP CYCLODEX β 236 M, 25m x 0.25mm I.D., 130°C, carrier: helium, Inj./MSD 250°C/280°C, total time 12 minutes
b. Column 1: PB-1, 40m x 0.32mm I.D., 145°C, carrier: hydrogen, Inj./FID 300°C,
Column 2: CP CYCLODEX β 236 M, 25m x 0.25mm I.D., 120°C, carrier: hydrogen, Inj./FID 300°C, total time 35 minutes, Sichromat-2

The outstanding suitability of the cyclodextrin phase for diasteromere and enantiomere analysis makes it possible to separate all compounds included in the mixture and in this way it also enables an exact identification and quantification of the individual components.

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