Chromatography

GC
Chiral Gas Chromatography

Chiral phases overview poster included

Stop scrying – start planning

LIPODEX® and HYDRODEX cyclodextrin phases for high enantiomeric recognition

MACHEREY-NAGEL

www.mn-net.com

AZ Chrom s.r.o. azetchrom@hplc.sk www.azetchrom.sk

MN Since 1911

MACHEREY-NAGEL – A pioneer in chiral Gas Chromatography

Chiral chromatography has a growing share in modern analytical chemistry. While pharmaceuticals are mostly separated by chiral HPLC, flavors and other volatile compounds are analyzed by enantiomeric gas chromatography. The reason for this is simple. Pharmaceuticals can be easily dilutet in typical HPLC-solvents, while the flavors are mainly olfactory substances.

Brief history of chiral chromatography

In the 1960s, it was Prof. E. Gil-Av, who succeeded first in separating L- and D-amino acid esters with chiral stationary phases.

In 1977 H. Frank, G. Nicholson and E. Bayer developed Chirasil-VAL, the first polysiloxane bonded chiral stationary phase. This breakthrough regarding temperature stability (> 200 °C), as well as enantiomeric selectivity made the first enantiomeric separations in gas chromatography possible. Until then, mostly amino acids could be separated, because stationary phases in chiral columns only showed a thermal stability of less than 110 °C.

Another landmark was the introduction of cyclodextrin-based phases, like those developed at the end of the 1980s by Prof. W. A. König in cooperation with MACHEREY-NAGEL. Modern cyclodextrins, used as chiral stationary phases, enabled the separation of a wide array of optically active compounds for the first time.

MACHEREY-NAGEL, with more than 50 years of experience in chromatography, launched the first capillary columns coated with cyclodextrins in 1987.

Decades-long expertise and a broad variety of twelve different chiral GC phases qualify MACHEREY-NAGEL to provide solutions for your enantiomeric separation tasks and ensures the best possible support and service.

MN Cyclodextrin columns – Hints and remarks on chiral Gas Chromatography

How to approach a separation task in enantiomeric GC? Sometimes, it can prove to be rather complicated to successfully separate enantiomers in GC, as it is very difficult to predict how a molecule will interact with a specific cyclodextrin. In the majority of cases, it will be inevitable to screen pre-selected columns on suitability for the particular sample, although some useful hints on successful separations may be found in the literature or in online databases like ***www.mn-net.com/apps***. A basic classification of CD based columns can be done by distinguishing the different ring sizes of the cyclodextrin.

Cyclodextrins are cyclic oligosaccharides, consisting of six (α-cyclodextrin), seven (β-cyclodextrin) or eight (γ-cyclodextrin) glucose units bonded through α-1,4-linkages. MN Cyclodextrins are either lipophilic phases (LIPODEX®) with long non-polar ligants or hydrophilic phases (HYDRODEX) with short polar ligants. The resulting FS capillary column is a chemically non-bonded phase. Hence, water is strictly forbidden for all LIPODEX® and HYDRODEX GC columns.

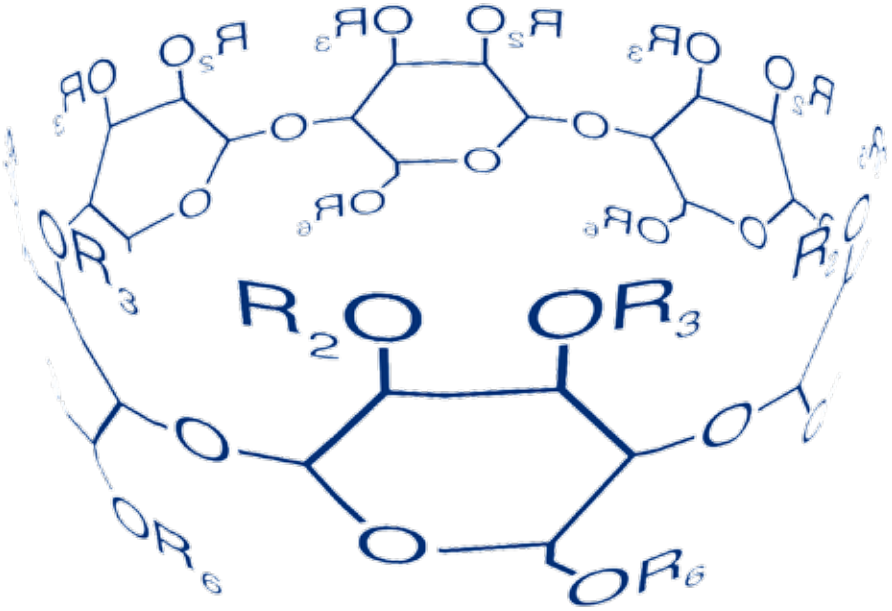


Fig. 1: Schematic α-cyclodextrin

Apart from the number of glucose units and the resulting ring sizes, all cyclodextrins have, as shown in the chart below, covalently bonded ligands at position 2, 3 and 6 of each molecule. Their functionality and polarity have an impact on the separation capabilities of the chiral stationary phase as well.

Phase	CD	Ligand		
		R_2	R_3	R_6
LIPODEX® A	alpha	Pentyl	Pentyl	Pentyl
LIPODEX® B	alpha	Pentyl	Acetyl	Pentyl
LIPODEX® C	beta	Pentyl	Pentyl	Pentyl
LIPODEX® D	beta	Pentyl	Acetyl	Pentyl
LIPODEX® E	gamma	Pentyl	Butyryl	Pentyl
LIPODEX® G	gamma	Pentyl	Pentyl	Methyl
HYDRODEX β-PM	beta	Methyl	Methyl	Methyl
HYDRODEX β-3P	beta	Methyl	Pentyl	Methyl
HYDRODEX β-6TBDM	beta	Methyl	Methyl	TBDMS
HYDRODEX β-TBDAc	beta	Acetyl	Acetyl	TBDMS
HYDRODEX γ-TBDAc	gamma	Acetyl	Acetyl	TBDMS
HYDRODEX γ-DiMOM	gamma	Methoxymethyl	Methoxymethyl	TBDMS

Table 1: Overview of MN cyclodextrin phases

Interactions

The following interactions between an analyte and the cyclodextrin have an influence on the selectivity of the column.

- Inclusion (size of the molecule)
- Dipole/dipole interactions (functional groups)
- Hydrophobic interactions (carbon content)
- Hydrogen bonds (functional groups)
- Steric interactions

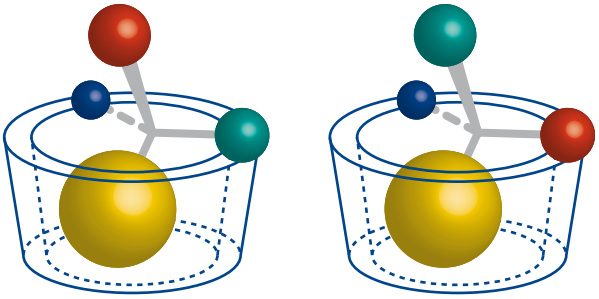
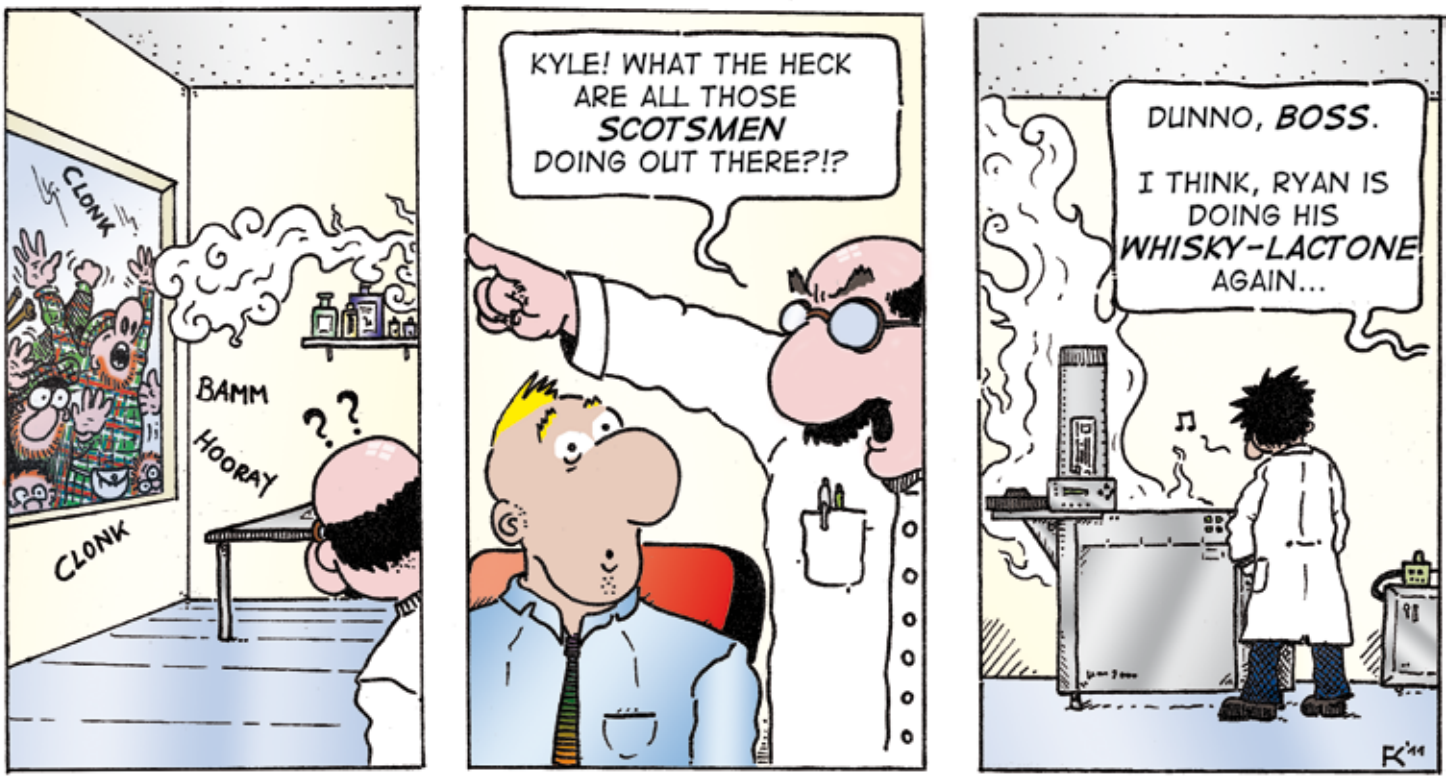


Fig 2: Schematic enantiomer separation

To achieve a successful separation, it is inevitable to have more than one of these interactions involved, preferably with comparable strenght. Needless to say, molecules containing functional groups with strong influence on these interactions, like alcohols or amines, have a tendency to separate less well than others.

To avoid interference from active polar groups, derivatization of the analyte can be the key. Derivatization offers a variety of methods, like alkylation, acylation or silylation that may actually help in separating enantiomers by diminishing or eliminating interactions of e.g. alcohols and amines. Another advantage is a reduced eluting temperature, resulting in an increased resolution. Please find more information about derivatization reagents in our database ***www.mn-net.com/apps*** or order a free sample directly under ***www.mn-net.com/Derivatization***.



Even if it is nearly impossible to predict the actual outcome of an enantiomer separation a priori, there are some parameters that have a considerable influence on the quality of separation.

Molecular weight

A long series of empiric tests has shown that there seems to be an overall tendency that, with increasing weight, larger cyclodextrins yield better results.

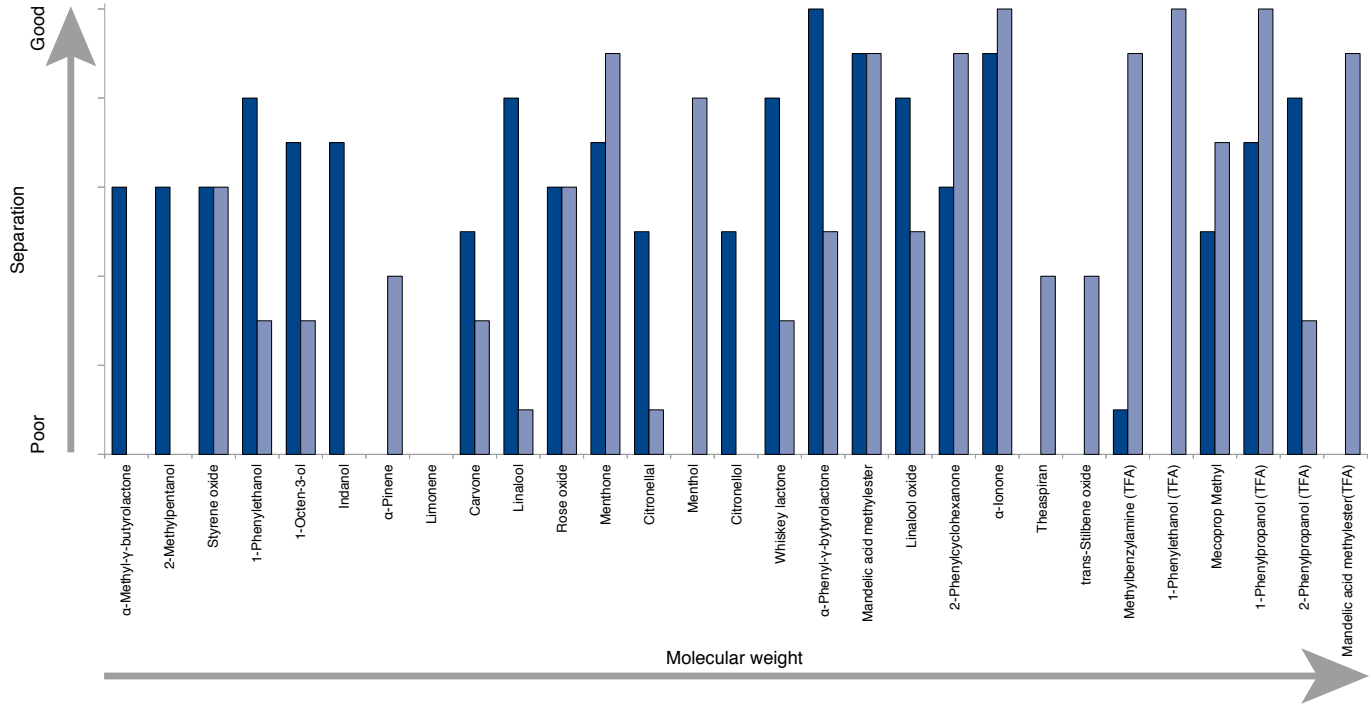


Fig. 3: Comparison of chiral recognition (■ β-TBDAc, ■ γ-TBDAc)

It also seems that HYDRODEX cyclodextrins, seem to show a broad selectivity with seven (β) glucose units in the ring, while LIPODEX® cyclodextrins get a broad selectivity with eight (γ) glucose units. A reason could be, that the LIPODEX® cyclodextrins actually appear smaller to the analyte, due to their rather bulky pentyl ligands.

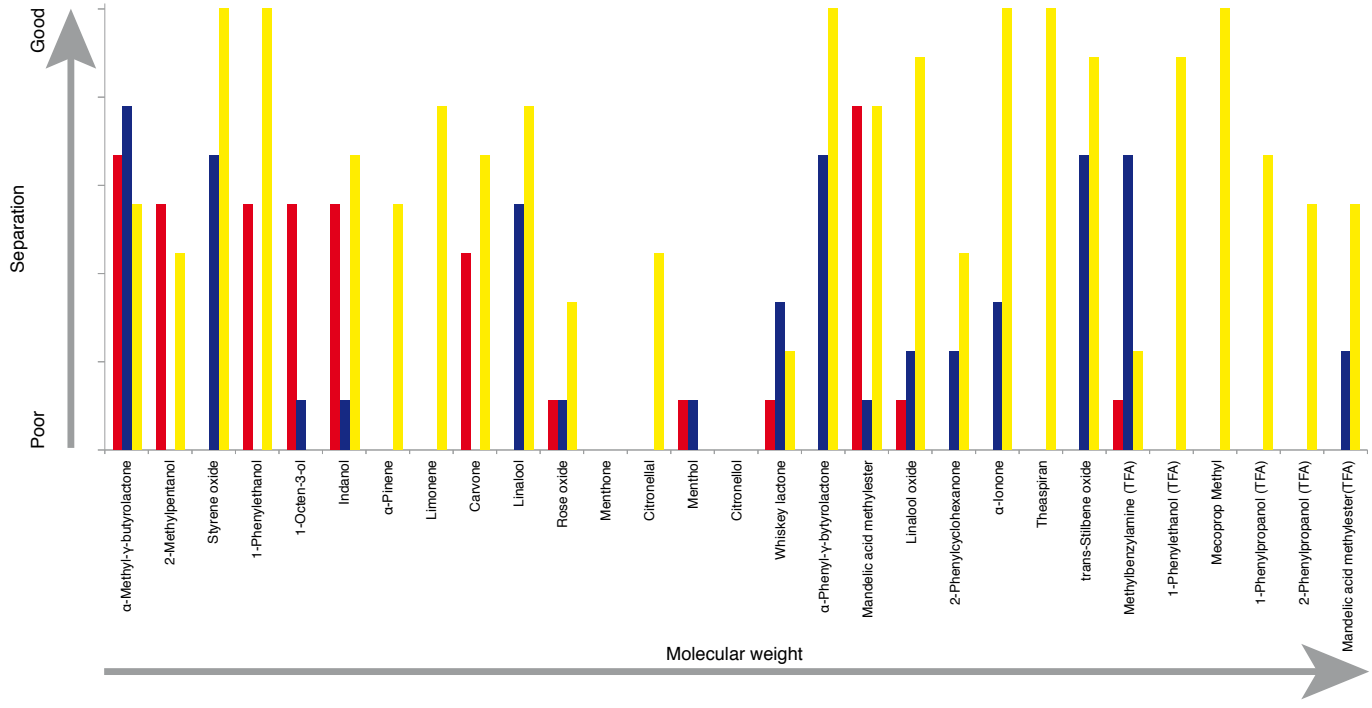
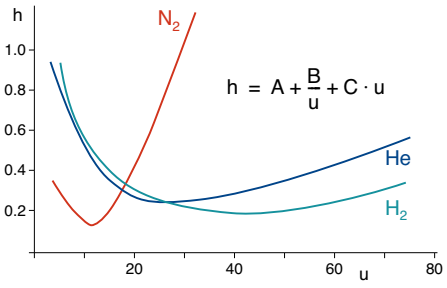


Fig 4: Comparison of chiral recognition (■ LIPODEX® A, ■ LIPODEX® C, ■ LIPODEX® E)

Carrier gas

There are three types of carrier gases in use in GC, hydrogen, helium and nitrogen. They all have their advantages and setbacks, be it price, volatility or operability. In chiral Gas Chromatography it is important that the carrier gas has a high linear velocity in the column, because the height of a theoretical plate directly corresponds with the speed of the transporting media. Therefore, even if hydrogen is the most expensive (and not always easy to handle in terms of safety) carrier gas, its advantages over helium and nitrogen regarding separation efficiency are significant. Therefore hydrogen remains the best choice for all enantiomer separations.

Plate height and gas velocity



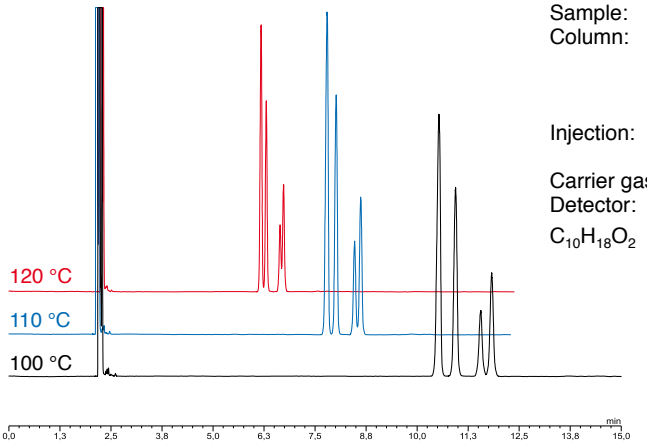
The Van Deemter equation shows how the plate height h depends on the flow velocity u for 3 different GC gases:

A Eddy diffusion; for WCOT capillary columns A = 0
B molecular axial diffusion; B is a function of the diffusion coefficient of the component in the respective carrier gas
C resistance to mass transfer

In practice often higher velocities than $u_{opt.}$ are chosen, if separation efficiency is sufficient, since higher carrier velocities mean shorter retention times.

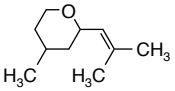
Temperature

The temperature can have, as mentioned above, a beneficial influence on the resolution. Rose oxide below is a very good example for temperature optimization



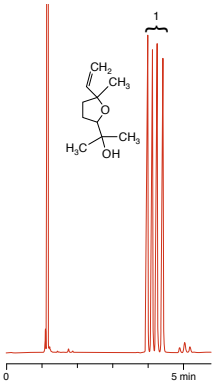
Sample: Rose oxide
Column: FS-HYDRODEX γ-TBDAc, 50 m x 0.25 mm ID, REF 723387.50, max. temperature 220/240 °C
Injection: 1.0 µL (1 % in C_6H_{14}) split 50 mL/min
Carrier gas: 120 kPa H_2 (1.7 mL/min)
Detector: FID 250 °C

$C_{10}H_{18}O_2$



Applications

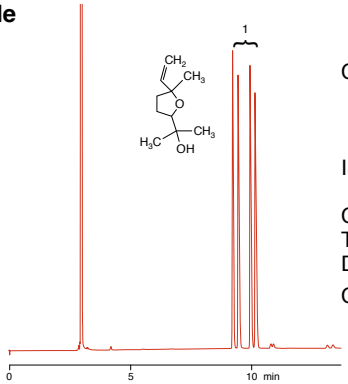
An interesting application is linalool oxide on a HYDRODEX β-3P compared with HYDRODEX β-PM. Even though both phases are β-cyclodextrins and β-3P differs from β-PM only by a pentyl instead of a methyl ligand in Pos. 2, the β-3P, in this case, shows superior chiral separation power. This allows a baseline separation in less than 5 minutes on a 25 m column.



Enantiomer analysis of Linalool Oxide
MN Appl. No. 201950

Column: HYDRODEX β-3P, 25 m x 0.25 mm ID, REF 723358.25, max. temperature 230/250 °C
Injection: 0.1 µL (1 % in CH_2Cl_2) split 130 mL/min
Carrier gas: 60 kPa H_2 (1.9 mL/min)
Temperature: 124 °C
Detector: FID 250 °C

$C_{10}H_{18}O_2$



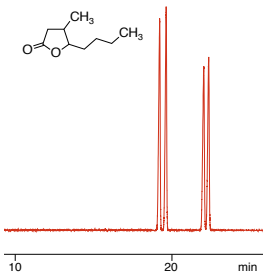
Enantiomer analysis of Linalool Oxide
MN Appl. No. 201940

Column: HYDRODEX β-PM, 50 m x 0.25 mm ID, REF 723370.50, max. temperature 230/250 °C
Injection: 0.1 µL (1 % in CH_2Cl_2) split 150 mL/min
Carrier gas: 120 kPa H_2 (1.7 mL/min)
Temperature: 120 °C
Detector: FID 250 °C

$C_{10}H_{18}O_2$

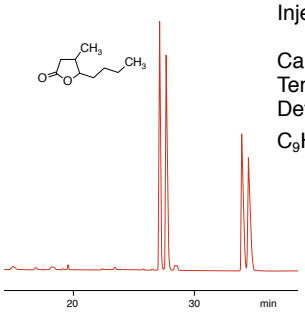
Applications (cont.)

It isn't imperative that enantiomers are separated on only one specific type of cyclodextrin column. It cannot be claimed that a specific racemic mixture will only separate on an α-,β- or γ-cyclodextrin, as it is not clear, which of the aforementioned interactions is predominant. The chromatograms below e.g. show the successful separation of 4-Butyl-3-methylbutyrolactone (Whisky-Lactone) on three columns with different ring sizes.



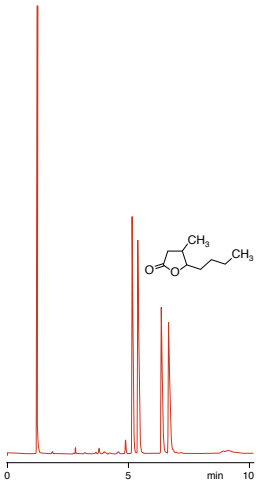
Enantiomer analysis of 4-butyl-3-methylbutyrolactone (Whisky lactone)
MN Appl. No. 202852

Column: FS-LIPODEX® A, 25 m x 0.25 mm ID, REF 723360.25, max. temperature 200/220 °C
Injection: 1.0 µL (1 % in CH_2Cl_2) split 180 mL / min
Carrier gas: 60 kPa H_2 (1.8 mL / min)
Temperature: 80 °C $\xrightarrow{1\ °C/min}$ 120 °C
Detector: FID 250 °C
$C_9H_{16}O_2$



Enantiomer analysis of 4-butyl-3-methylbutyrolactone (Whisky lactone)
MN Appl. No. 202882

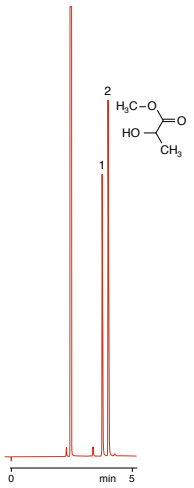
Column: HYDRODEX β-PM, 50 m x 0.25 mm ID, REF 723370.50, max. temperature 230/250 °C
Injection: 1.0 µL (1 % in CH_2Cl_2) split 150 mL / min
Carrier gas: 120 kPa H_2 (1.7 mL / min)
Temperature: 130 °C
Detector: FID 250 °C
$C_9H_{16}O_2$



Enantiomer analysis of 4-butyl-3-methylbutyrolactone (Whisky lactone)
MN Appl. No. 202862

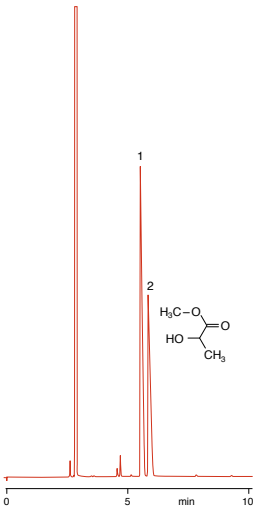
Column: FS-LIPODEX® E, 25 m x 0.25 mm ID, REF 723368.25, max. temperature 200/220 °C
Injection: 0.1 µL (1 % in CH_2Cl_2) split 95 ml/min
Carrier gas: 60 kPa H_2 (1.7 mL / min)
Temperature: 145 °C
Detector: FID 250 °C
$C_9H_{16}O_2$

Although the majority of the separations shows that the size of the cyclodextrin seems to have a large impact on the separation capability, in the sense of the bigger the better, it is not the ultimate parameter to indicate if a chiral compound will be separated on a column. Overall, the chiral separation power is a result from the sum of all aforementioned interactions. This may lead to a totally different separation on two columns. A good example is methyl lactate on the HYDRODEX β-PM compared with LIPODEX® A. The result is a reversed order of peaks, implying that the separation mechanism is different, however in both cases with baseline separated enantiomers.



Enantiomer separation of methyl lactate
MN Appl. No. 202762

Column: FS-LIPODEX® A, 50 m x 0.25 mm ID, REF 723360.50, max. temperature 200 / 220 °C
Injection: 0.1 µL (1 % in CH_2Cl_2) split 320 mL / min
Carrier gas: 120 kPa H_2 (2.2 mL / min)
Temperature: 80 °C
Detector: FID 250 °C
Peaks: ($C_4H_8O_3$)
1. S-(–)
2. R-(+)



Enantiomer separation of methyl lactate
MN Appl. No. 202772

Column: FS-HYDRODEX β-PM, 50 m x 0.25 mm ID, REF 723370.50, max. temperature 230/250 °C
Injection: 0.1 µL (1 % in CH_2Cl_2) split 150 mL / min
Carrier gas: 120 kPa H_2 (1.7 mL / min)
Temperature: 90 °C
Detector: FID 250 °C
Peaks: ($C_4H_8O_3$)
1. R-(+)
2. S-(–)

MACHEREY-NAGEL

Your competent partner in analytical chemistry

Tradition and Modernity – 100 years of experience

· Worldwide operating German company (founded 1911)
· Subsidiaries in France, Switzerland and USA
· Distributors in over 150 countries
· Longtime tradition in filter papers
· Development, production and sales of special products for water, environmental and food analysis, for biotechnology, chemical and pharmaceutical industry and medical diagnostics

Quality and Diversity – 5 product ranges with over 25 000 products "Made in Germany"

Filtration	· Filter Papers · Filter Membranes · Extraction Thimbles
Rapid Tests	· Test Papers and Test Strips · Urine Test Strips
Water Analysis	· Colorimetric and Titrimetric Test Kits · Photometric Water Analysis · Microbiology
Chromatography	· High Performance Liquid Chromatography (HPLC) · Gas Chromatography (GC) · Thin Layer Chromatographie (TLC) · Sample Preparation (SPE)
Bioanalysis	· Kits for Purification of Nucleic Acids · Kits for Purification of Proteins · Transfer Membranes

Ordering information

Length	10 m	25 m	50 m
all columns 0.4 mm OD	**0.10 mm ID**	**0.25 mm ID**	**0.25 mm ID**
FS-LIPODEX® A		723360.25	723360.50
FS-LIPODEX® B		723362.25	723362.50
FS-LIPODEX® C		723364.25	723364.50
FS-LIPODEX® D		723366.25	723366.50
FS-LIPODEX® E	723382.10	723368.25	723368.50
FS-LIPODEX® G		723379.25	723379.50

Length	10 m	25 m	50 m
all columns 0.4 mm OD	**0.10 mm ID**	**0.25 mm ID**	**0.25 mm ID**
FS-HYDRODEX β-PM		723370.25	723370.50
FS-HYDRODEX β-3P		723358.25	723358.50
FS-HYDRODEX β-6TBDM	723383.10	723381.25	723381.50
FS-HYDRODEX β-TBDAc		723384.25	723384.50
FS-HYDRODEX γ-TBDAc		723387.25	723387.50
FS-HYDRODEX γ-DiMOM		723388.25	723388.50

www.mn-net.com

MACHEREY-NAGEL

AZ Chrom s.r.o., Robotnícka 10, 831 03 Bratislava
Tel. 0907 244526, Fax. 02-20715811, azetchrom@hplc.sk, www.azetchrom.sk

MN Since 1911