3.2 Chromatography and Electrophoresis (P. Schulze)

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This department provides central analytical services for in-house scientists, including qualitative and quantitative analysis, ee determinations, and preparative separations of chemical mixtures using modern chromatographic and electrophoretic methods as well as hyphenated techniques. Part of the group is involved in the development of detection technology.

Gas chromatography

The GC team applies a variety of modern capillary gas chromatographic techniques for routine analysis such as high temperature GC, GC x GC, SPME or KAS-TDU. Most analytes are detected via flame ionization or thermal conductivity detection. Unknown substances are identified using mass spectrometric detection (GC-MS). The team also develops analytical methods, e.g. the quantification of analytes in samples with complex or aqueous matrices.

Liquid chromatography and electrophoresis

The liquid chromatography and electrophoresis laboratory applies liquid phase separations e.g. high pressure liquid chromatography and capillary electrophoresis. In 2014-2016 the HPLC-group focused on increasing the separation efficiency and selectivity. For this reason several new achiral sub 2 µm and chiral 3 µm stationary phases were tested and applied. Likewise, the application and the development of 2D-HPLC were extended.

Two HPLC-systems for column switching and backflush techniques have been installed for analysis and simultaneous determination of saccharides and their reduction products. These systems are also in use for the analysis of oxidation products of glucose, glycerin and 5-(hydroxymethyl)furfural.

In January 2015 a 2D-system for comprehensive and heart cutting HPLC has been installed. It was the first commercial system for multiple heart cutting techniques and allows separations of complex mixtures at up to 120 MPa operation pressure.

With a combination of 2 µm achiral and 3 µm chiral reversed phase columns, the separation of diastereomers or enantiomers were performed for more than 50 samples.

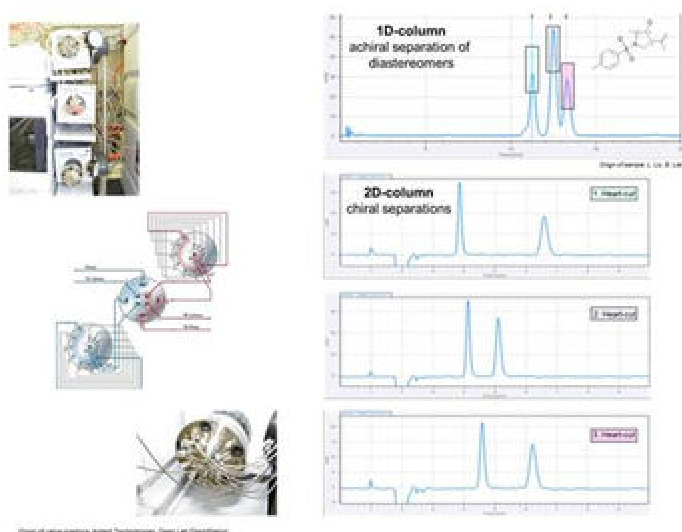

Fig. 1 **Chiral separation of three diastereomers in a single run. Achiral separation of the diastereomers in the 1st dimension with the multiple heart cuts and corresponding chiral separations in the 2nd dimension.**

Additionally, the LC group was involved in the following projects:

- Development of 372 new chiral HPLC methods; installation of method scouting systems for chiral separations.
- Micro preparative separation of a huge number of reaction products for NMR and MS.
- Separation and identification of peptides by multi charge HPLC/MS analysis.
- Separation and identification of steroids by LC/MS/MS.
- Separation and HPLC/MS identification of 65 different catalysts of the IDPi-project with molecular weights of up to 2800 on special 300 Å C3- and C8-stationary phases.
- Trace analysis of methanol and 2-propanol by ion chromatography.
- Achiral and chiral separations of several helicene derivatives using 2D-HPLC.

Preparative liquid chromatography

In the preparative HPLC, mainly reaction batches of up to 20 grams total amount are separated using upscaled HPLC methods. Different stationary phases such as NP-, RP- or chiral stationary phases are utilized in separation columns with inner diameters

between 10 and 50 mm. Most samples are detected *via* UV absorbance. Analytes lacking UV absorbing double bonds are detected by differences in refractive index.
To increase the separation efficiency of i.e. constitutional aromatic isomers, peak recycling is applied by fractionating the pure parts of each peak and redirecting the remaining peaks to the beginning of the column (Figure 2).

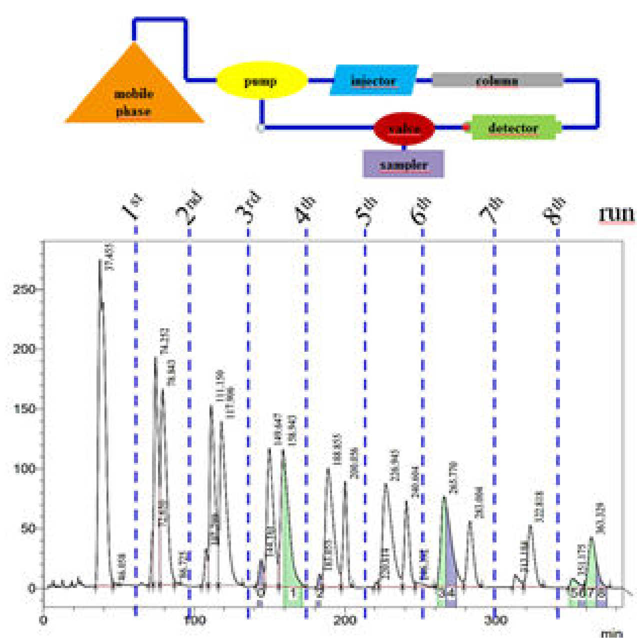

Fig. 2 Peak recycling diagram and chromatogram of a natural product.

Column switching in preparative HPLC increases the separation speed of lately eluting substances.

Pre-purification of the sample is conducted on a short column and the elution area of interest is transferred on the second dimension applying a longer column which is used to purify the target analytes.

Independent research projects

A low fluidic volume fluorescence detection cell was developed and built based on UV LED excitation and an alternative optical design. Its configuration facilitates high signal-to-noise detection and allows for up to three excitation sources to be used either simultaneously or in sequence resulting in potentially higher selectivity.

In another project, a manufacturing process for capillary-based liquid core waveguides (LCW) based upon a low refractive index polymer coating was developed. Due to its large path length, low volume and small emitting cross-section, this design is advantageous for spectrally resolved detection setups in HPLC i.e. analyte characterization via Raman.

Publications resulting from this research area:

(1) Gitlin, L.; Schulze, P.; Ohla, S.; Bongard, H.-J.; Belder, D.; *Electrophoresis* **2015**, *36*, 449-456.

(2) Gliemann B. D.; Petrovic A.G.; Zolnhofer E. M.; Dral P. O.; Hampel F.; Breitenbruch G.; Schulze P.; Raghavan V.; Meyer K.; Polavarapu P. L.; Berova N.; Kivala M.; *Chem. Asian J.* **2016**, DOI: 10.1002/asia.201601452R1.

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