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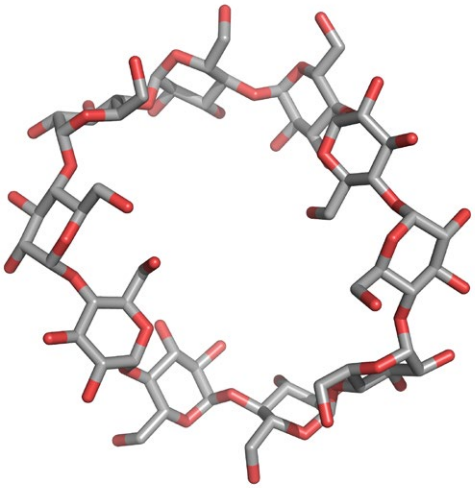
# Cyclodextrin purification (Part 2):
## Method transfer and purification

Yannick Krauke*, Sebastian Thürmann*, Christian Sonnendecker**, Kate Monks* – applications@knauer.net

*KNAUER Wissenschaftliche Geräte GmbH, Hegauer Weg 38, 14163 Berlin – www.knauer.net

** Fakultät für Lebenswissenschaften / Institut für Biochemie AG Mikrobiologie und Bioverfahrenstechnik, Johannisallee 21-23, 04103 Leipzig



## SUMMARY

Cyclodextrins (CD) are macrocyclic compounds composed of five or more glycopyranosides. These ring structures can function as micro-capsules. Hence, CDs often find interesting applications in drug delivery. In this application, five CDs were purified in high purity from a CD mixture derived from a biocatalytic synthesis.

## INTRODUCTION

Cyclodextrins (CD) are oligosaccharides of glucopyranose that are bound in a cyclic form of five to up to 12 units. The ring structure can function as a micro-capsule for other molecules. Due to their unique chemical structure they find different applications i. e. as drug carriers, in cosmetics, or in food industry. High amounts of CDs with more than 10 subunits (CD>10) are of special interest since larger molecules can be inserted into their rings [1]. Therefore, new approaches for synthesis and purification especially of CD >10 are conducted increasingly. A purification method previously developed in analytical scale was here transferred to preparative scale (KNAUER application note VPH0066).

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## RESULTS

The method parameters for cyclodextrine purification were developed and optimized prior in analytical scale (see application note VPH0066). The mass and volume overload studies revealed that 100 µL injections of 100 mg/mL cyclodextrine mixture would still allow highly pure purification of at least four cyclodextrine (VPH0066). A linear scale-up from analytical to preparative scale was performed. The column length and particle size remained the same (250 mm; 5 µm), the inner diameter was increased from 4 mm to 20 mm. The KNAUER scale up converter was used for fast determination of preparative method parameters, the flow rate was increased to 20 mL/min and sample injection volume to 2 mL. The chromatogram of the preparative CD mix run showed baseline separation of five CD peaks **(Fig. 1)**. These five peaks were collected using the threshold function of PurityChrom software exceeding a certain µRIU value. Chromatograms from samples of each fraction were compared to chromatogram of the whole CD mixture. The overlay clearly showed that all fractions were 100 % pure and no contamination from neighbor peaks **(Fig. 2)**.

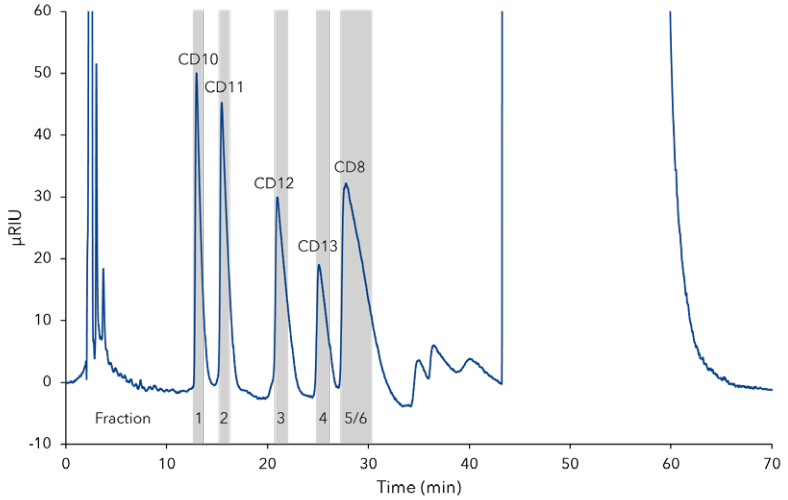



**Fig. 1** Separation of CDmix on C18H 250x20mm column, 2 mL injection, 100 mg/mL CD mix; indication of collected fractions

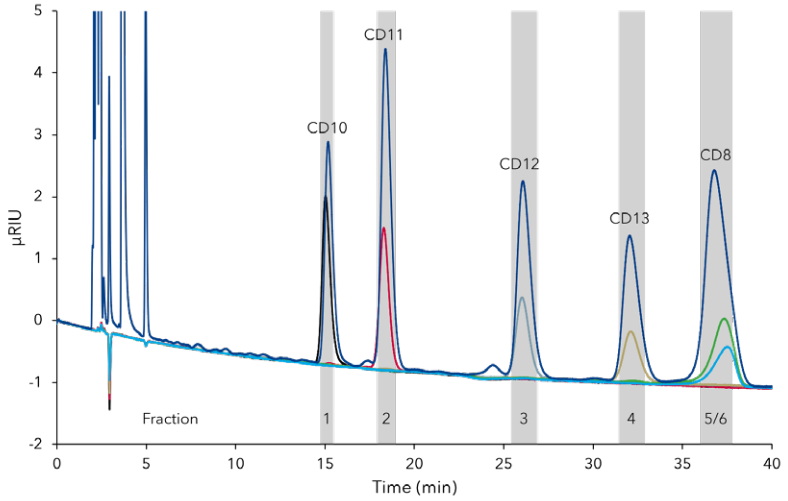
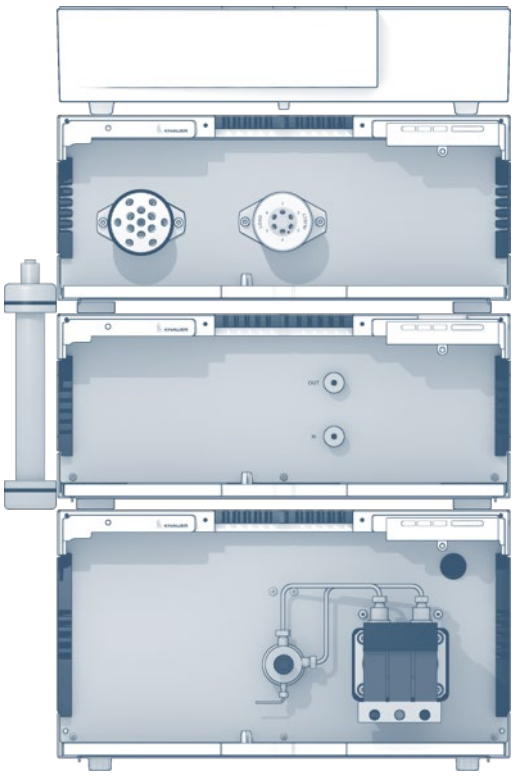



**Fig. 2** Overlay analytical chromatograms of CDmix and the fractions collected from purification step (Fig. 1) black Frc1; red Frc2, light blue Frc3, yellow Frc4, green and blue Frc5/Frc6

## MATERIALS AND METHOD

The AZURA Preparative HPLC system consisted of AZURA P2.1L 100 mL sst pump with ternary LPG module, AZURA RID 2.1L high flow detector and AZURA assistant module with 12 port multi position 1/8" sst valve (fractionation), 6 port 2 position 1/16" sst injection valve and P4.1S 50 ml sst feed pump. Final purification method was as follow: 20 mL/min, RT, 40 min 3% methanol, 14 min 30% methanol, 20 min 3 % methanol. Peaks were fractioned with threshold function over µRIU signal. Fraction analysis was performed with AZURA analytical RI system as described in application note VPH0066.



## CONCLUSION

A previously in analytical scale developed purification method for cyclodextrine was transferred to semi-preparative scale by linear scale-up. Five cyclodextrines were purified in nearly 100 % purity and the results showed that higher loading would be possible without to much loss in purity. The preparative refractive index detector AZURA RID 2.1L HighFlow allowed detection at flow rates of 20 mL/min without using a flow splitter thus facilitating the purification process. All together a new approach for cyclodxtrine purification was developed.

## REFERENCES

[1] E.M.Martin Del Valle, Cyclodextrins and their uses: a review, Process Biochemistry, Volume 39, Issue 9, 2004, Pages 1033-1046,ISSN 1359-5113

[2] Sonnendecker, C. , Thürmann, S. , Przybylski, C. , Zitzmann, F. D., Heinke, N. , Krauke, Y. , Monks, K. , Robitzki, A. A., Belder, D. and Zimmermann, W. (2019), Large-Ring Cyclodextrins as Chiral Selectors for Enantiomeric Pharmaceuticals. Angew. Chem. Int. Ed.. doi:10.1002/anie.201900911

# ADDITIONAL RESULTS

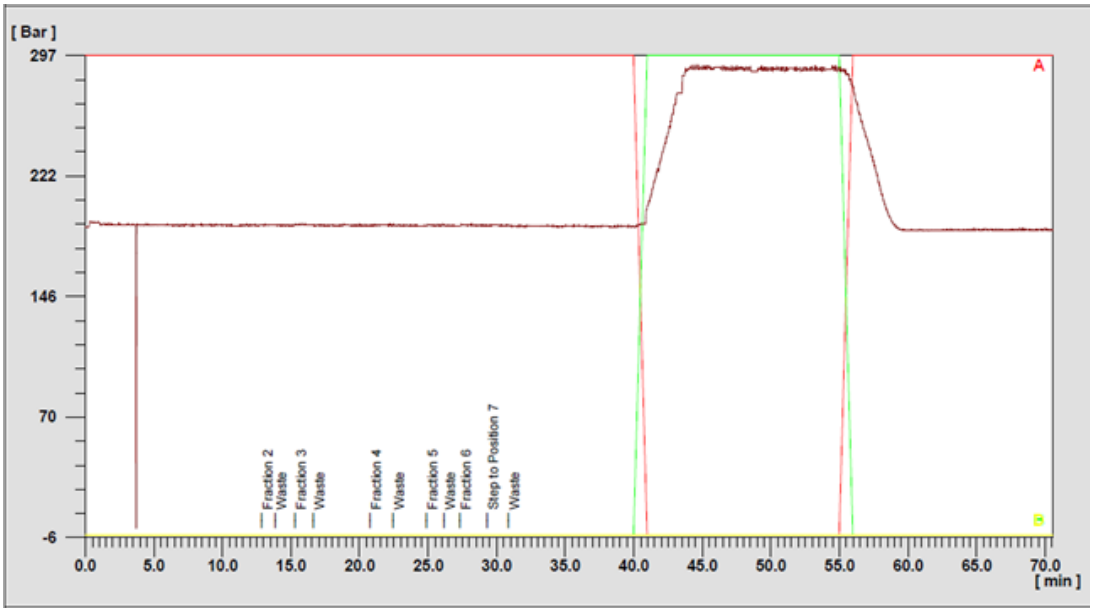



**Fig. A1** Pressure fractionation, prep run

# ADDITIONAL MATERIALS AND METHODS

**Tab. A1** Method parameters

| | | | |
|---|---|---|---|
| **Column temperature** | RT | **Detection wavelength** | RI |
| **Injection volume** | 2 mL | **Data rate** | 20 Hz |
| **Injection mode** | Full loop | **Time constant** | 0.05 s |

**Tab. A2** Pump parameters

| | | | |
|---|---|---|---|
| Eluent A | 3 % methanol | | |
| Eluent B | 30 % methanol | | |
| Flow rate | 20 mL/min | | |
| Pump program | Time [min] | % A | % B |
| | 0-40 | 100 | 0 |
| | 40-55 | 0 | 100 |
| | 55-75 | 100 | 0 |

**Tab. A3** System configuration

| Instrument | Description | Article No. |
|---|---|---|
| Pump | AZURA P2.1L, 100 mL, SST | APE20KA |
| | AZURA LPG ternary module for Pump P 2.1L | AZZ00AB |
| Detector | AZURA RID 2.1L High flow | ADD38 |
| Assistant | Left: 12Mpos,1/8"",sst<br>Middle:6Port2Pos,1/16",sst<br>Right:P4.1S, 50ml,sst | AYFAEABR |
| Column | Eurospher II 100-5 C18H 250x20mm<br>Eurospher II 100-5 C18H 30x20mm<br>Eurospher II 100-5 C18H 250x4mm | 25PE185E2J<br>03PE185E2J<br>25WE185E2J |
| Software | PurityChrom basic | A2650 |
| | KNAUER Scale up converter | A1696 |

# RELATED KNAUER APPLICATIONS

**VPH0066** - Cyclodextrin purification (Part 1): Method screening and overload studies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