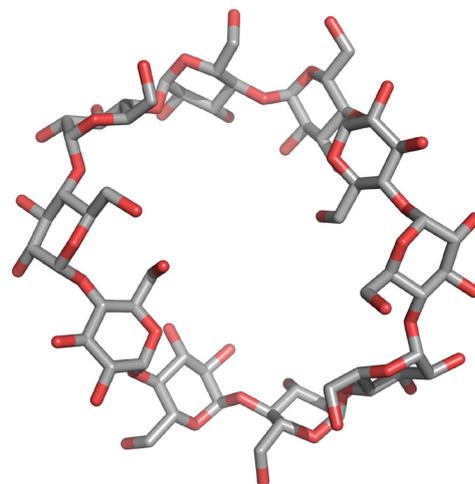


Cyclodextrin purification (Part 2): Method transfer and purification

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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 sub-

units (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 cyclodextrin purification were developed and optimized prior in analytical scale (see application note [VPH0066](#)). The mass and volume overload studies revealed that 100 μ L injections of 100 mg/mL cyclodextrin mixture would still allow highly pure purification of at least four cyclodextrin ([VPH0066](#)). A linear scale-up from analytical to preparative scale was performed. The column length and particle size remained the same (250 mm; 5 μ m), 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 μ RIU 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](#)).

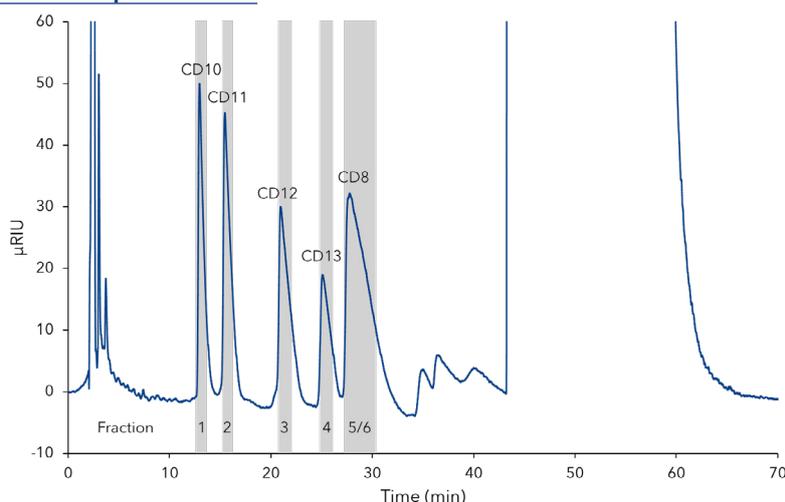


Fig.1 Separation of CDmix on C18H 250x20mm column, 2 mL injection, 100 mg/mL CD mix; indication of collected fractions

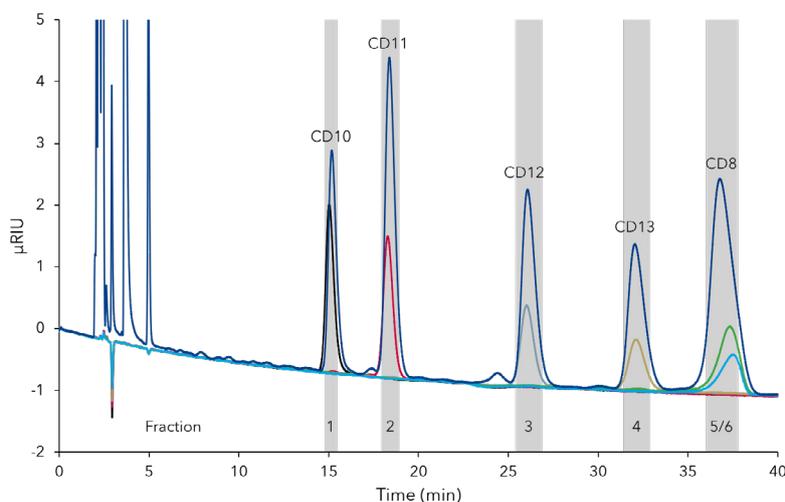


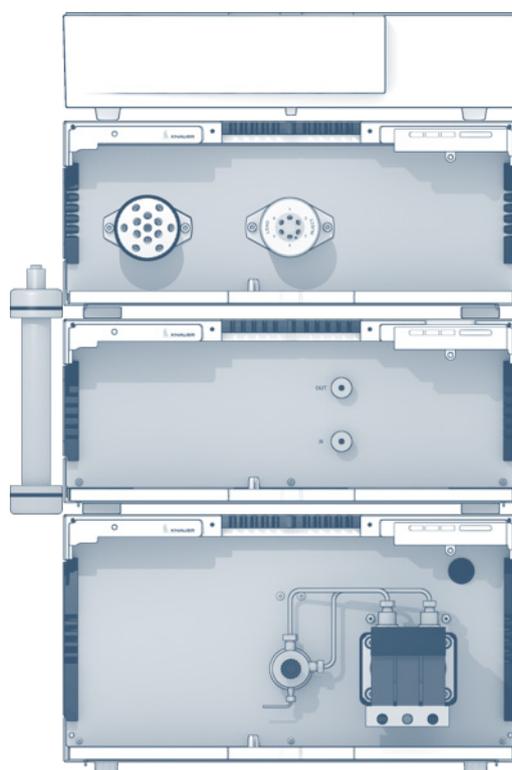
Fig.2 Overlay analytical chromatograms of CDmix and the fractions collected from purification step ([Fig. 1](#)) black Frc1; red Frc2, light blue Frc3, yellow Frc4, green and blue Frc5/Frc6

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 fractionated with threshold function over μ RIU 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

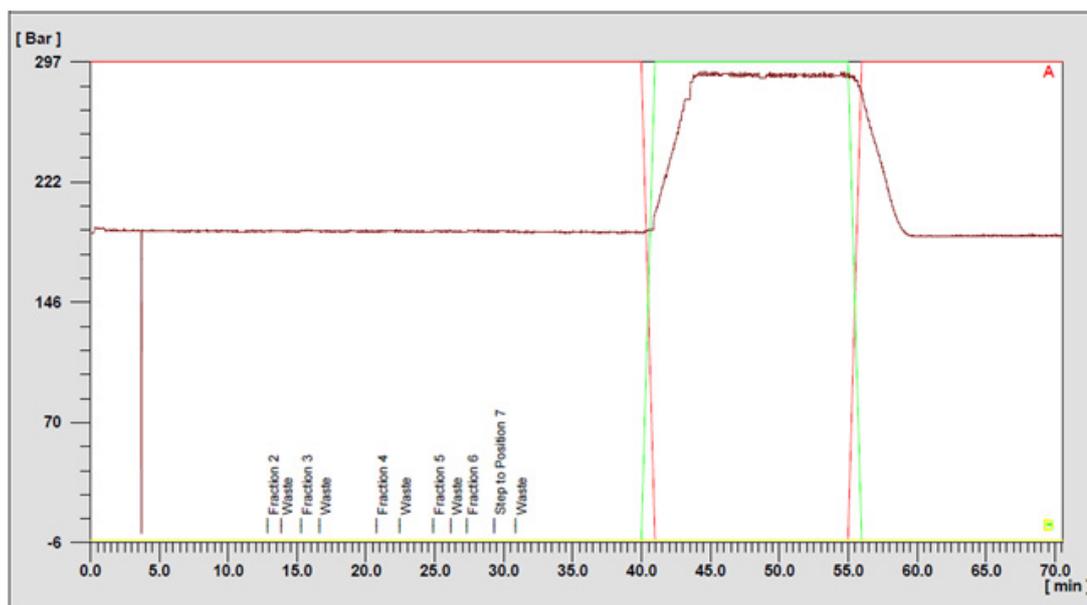


Fig.A1 Pressure fractionation, prep run

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 Middle:6Port2Pos,1/16",sst Right:P4.1S, 50ml,sst	AYFAEABR
Column	Eurospher II 100-5 C18H 250x20mm	25PE185E2J
	Eurospher II 100-5 C18H 30x20mm	03PE185E2J
	Eurospher II 100-5 C18H 250x4mm	25WE185E2J
Software	PurityChrom basic	A2650
	KNAUER Scale up converter	A1696

RELATED KNAUER APPLICATIONS

[VPH0066](#) - Cyclodextrin purification (Part 1): Method screening and overload studies