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Separation of racemic drugs on chiral resorcinarene-bonded HPLC-columns

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Chiral separations are becoming more important for drug quality control. Because existing methods are often not sufficient to separate the enantiomers, the development of new selective stationary phases for HPLC-separations is of continued interest.

Calixarenes are macrocycles made up of phenolic units coupled to aldehydes [1]. Among them, resorcinarenes are known as host molecules for various compounds [2–5]. Relatively few papers have dealt with calix[4]arenes as immobilized [6] or solved [7–8] chiral selectors in CE. Healy et al. [9] described the use of silica-bonded calix[4]arenes modified at the lower rim with ephedrin units for chiral separations in HPLC.

To our knowledge, this is the first time that chiral resorcinarenes have been employed in HPLC. We tested eight stationary phases with different bound resorcinarenes. Chiral compounds were chosen that have broad variety in the structures around the chiral centers. Among them, basic, acidic and neutral compounds were tested due to the potential ability of complex formation of resorcinarenes with neutral as well as charged analytes [2–5]. Methanol (MeOH) or acetonitrile (MeCN)/20 mM NaH_2PO_4 (pH = 3.5) mixtures were used in the reversed-phase mode. Normal-phase separations were achieved with eluents containing hexane/isopropanol (Hex/IPA) and addition of 0.1% triethylamine (TEA) or 0.1% acetic acid (AA), respectively.

Two of the resorcinarene phases containing L-phenylalanine ethyl ester (RES-Phe) and S-1-(2-naphthyl)-ethylamine (RES-Naph) were most appropriate to separate a variety of compounds of pharmaceutical interest (Fig. 1).

Separations on the RES-Naph-phase were achieved in normal-phase systems, indicating that the main retention mechanism is due to interactions between polar structures of analytes and the stationary phase. Beside π-basic naphthyl substituents, resorcinarene possesses secondary amino and phenolic groups. Obviously, these substituents are involved mainly in a three point chiral recognition with the

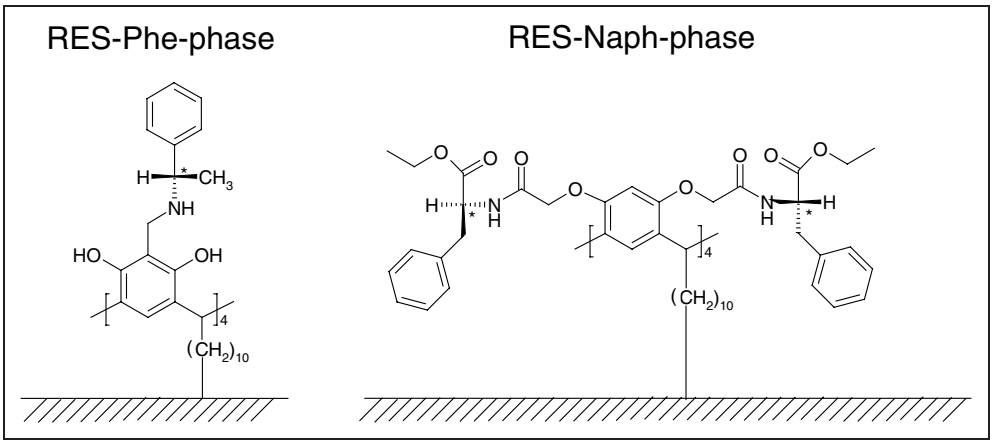

Fig. 1: Structures of investigated chiral resorcinarenephases
RES-Phe: L-phenylalanin ethyl ester linked with resorc[4]arene
RES-Naph: S-1-(2-naphthyl)-ethylamine linked with resorc[4]arene

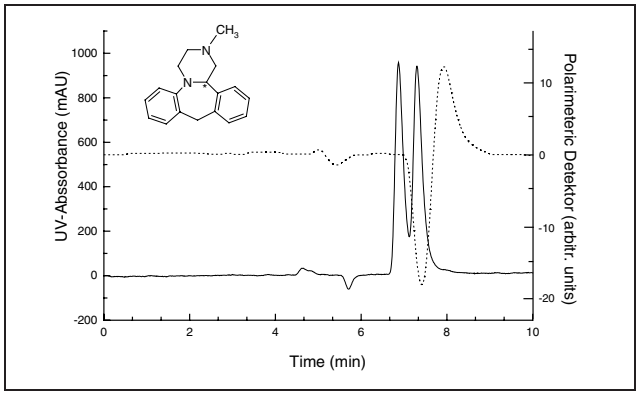

Fig. 2: Separation of the enantiomers of mianserin on the RES-Naph-phase conditions: Hex/IPA 8 : 2 (v/v) + 0,1% TEA; 0,5 ml/min; 260 nm; 30 °C; 250 × 4 mm

analytes. Interestingly, the free phenolic groups are quite distant from the chiral center of the S-1-(2-naphthyl)-ethylamine substituent. Thus, an inclusion of the analytes into the cavities of resorcinarenes is likely occuring.

An eluent with Hex/IPA 8 : 2 (v/v) and addition of 0.1% TEA gave the best results for the separation of the antidepressant mianserin ($\alpha = 1.23$) (Fig. 2), the anaesthetic ketamine ($\alpha = 1.18$) and for 2-(*n*-propylthio)-3-naphthyl-4(3*H*)-chinazolinone ($\alpha = 1.29$). Possible interactions with the resorcinarenes could occur through the amino, keto or thio groups. Furthermore, aromatics of the compounds could interact with aromatic moieties of the basic structure of the resorcinarene or with the naphthyl substituents, respectively.

Interestingly, no separations were observed in systems with acid aqueous eluents. This could be due to the comparative polar cavities with free phenolic units, which are obviously poor capable for stronger hydrophobic interactions with apolar parts of analytes.

In contrast, the RES-Phe-phase shows separations in normal-phase as well as in reversed-phase mode. Mianserin was separated on this column as on RES-Naph within 13 min ($\alpha = 1.16$); however, MeCN/20 mM NaH_2PO_4 (pH = 3.5) 6 : 4 (v/v) was used as eluent. The greater hydrophobicity of the cavity of RES-Phe, due to the substituted phenolic units, may be responsible for stronger lipophilic interactions with the host-molecule. This could be the reason for successful separations in reversed-phase mode in contrast to the RES-Naph-phase.

With hexane containing eluents, no discrimination of the enantiomers of mianserin was observed whereas other analytes were separated under such conditions. A separation of the diuretic chlorthalidone was achieved with Hex/IPA 8 : 2 (v/v) and addition of 0,1% AA ($\alpha = 1.04$). Furthermore, the enantiomers of the analgetic ketoprofen were separated with Hex/IPA 9 : 1 (v/v) and addition of 0,1% TEA ($\alpha = 1.10$). As shown, the choice of a suitable mobile phase system had a significant effect on the selectivity of the column. Although the phenols of the resorcinarene are substituted by phenylalanin ethyl ester by a spacer, the polarity of this selector is high enough to interact in normal-phase mode. Possibly, the eight chiral centers per resorcinarene compared to four chiral centers of the RES-Naph allow for more favorable interactions. Furthermore, the higher degree of substitution of this resorcinarene leads to a more rigid host. Thus, adjacent phenylalanine ethyl ester substituents could contribute to a chiral recognition of the analytes and lead to a three point interaction.

Experimental

1. Chemicals

Mianserin was obtained from Salutas Pharma GmbH (Magdeburg, Germany). Ketamine was supplied from Arzneimittelwerk Dresden (Dresden, Germany). 2-(*n*-Propylthio)-3-naphthyl-4(3*H*)-chinazolinone was synthesized at the Institute of Pharmacy, University of Greifswald. Chlorthalidone was obtained from Novartis Pharma GmbH (Nürnberg, Germany) and ketoprofen was supplied from Rhône-Poulenc Rorer GmbH (Köln, Germany). NaH_2PO_4, triethylamine (TEA) and acetic acid (AA) were obtained from Merck KgaA (Darmstadt, Germany).

HPLC grade methanol, acetonitrile, isopropanol and hexane were purchased from Applichem GmbH (Darmstadt, Germany). Water was bidestilled.

2. Equipment

Chromatographic experiments were performed on a Shimadzu system with two LC-10AD high pressure pumps, a SIL-10A autosampler and SPD-M10A diode array detector. A Chiralyser from IBZ Meßtechnik (Hannover, Germany) was used as polarimetric detector.

3. Columns

Resorcinarene phases were obtained from Synaptec GmbH (Greifswald, Germany). They were prepared by immobilizing resorcinarenes via an undecenyl spacer onto Kromasil® silica (Kromasil Si 100, 5 µm, specific surface area/BET: 311 m^2/g, pore volume: 0.9 ml/g, manufacturer: EKA Chemicals AB (Bohus, Sweden)) by a patented procedure [DE 19602393, EP 0786661 A2 and Wo 97/27479]. All phases have dimensions of 250 × 4 mm I.D.

4. Chromatography

Chromatographic runs were performed isocratically throughout. The eluents were degassed by ultrasonication prior to use. The column temperature was set to 30 °C and injection volumes were 10 µl. The flow rate varied between 0.5 and 1.0 ml/min.

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