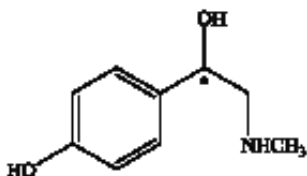


CHIRAL-CBH - an excellent choice for the separation of synephrine enantiomers. Comparison of three chiral columns

Pellati et al. has compared three commercially available chiral columns, CHIRAL-CBH, LiChroCart Chiradex and Sumichiral OA-6000 for the analysis of the enantiomers of synephrine in *C. aurantium* L. var. *amara* fruits. The article is published in *J. Pharm. Biomed. Anal.* 37 (2005) 839-849. CHIRAL-CBH demonstrated outstanding chromatographic performance and resolution.

Synephrine is a chiral drug and is used clinically as a racemic mixture despite the fact that the enantiomers have different pharmacological activity. The R-synephrine is more active than the S-form. The structure of synephrine is shown below.



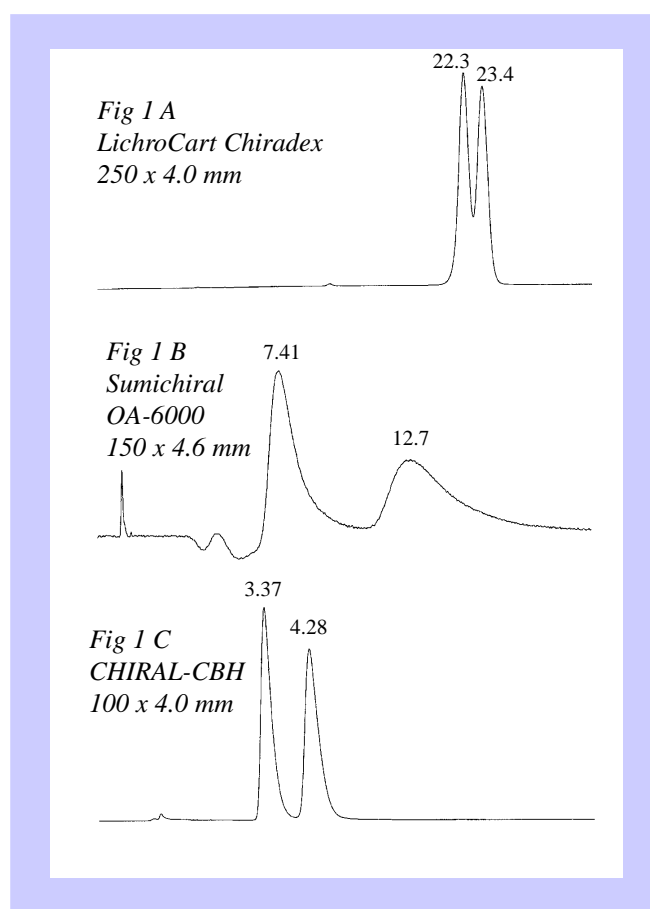
Considering the increasing use of *C. aurantium* herbal medicines in recent years, it is very important to analyse these preparations due to the pharmacological activity and the possible toxicity of the plant extracts containing synephrine. It is also very important to be able to separate and analyse the individual enantiomers in plant extracts due to the different pharmacological effects of the enantiomers.

Three different columns were tested for the separation of synephrine enantiomers; CHIRAL-CBH (100 x 4.0 mm, 5 µm), LiChroCart Chiradex (250 x 4.0 mm, 5 µm) and Sumichiral OA-6000 (150 x 4.6 mm, 5 µm).

LiChroCart Chiradex is a cyclodextrine based chiral column and the optimum mobile phase conditions for the separation of synephrine was methanol-NaH₂PO₄ (pH3.5; 25 mM) and 10 mM tetrabutylammonium hydrogen sulfate. The flow-rate was 0.4 ml/min. The column temperature was 2°C. A chromatogram is demonstrated in Fig 1 A. As can be seen only partial separation was obtained despite the 250 mm long column. The retention is very high on this column. The last enantiomer is eluted after 25 min.

The chiral stationary phase on the Sumichiral OA-6000 column consists of a coordination compound of copper(II) ion and the chiral ligand (R,R)- tartaric acid mono-R-1-(α -naphthyl)-ethylamide. The optimum mobile phase composition was; 1 mM copper (II) acetate and 10 mM ammonium acetate in aqueous solution pH 6.4. The flow-rate was 1.7 ml/min. and the temperature 26°C. As can be seen in Fig 1 B only partial resolution with very broad peaks and high retention were observed. The last peak was eluted after about 19 min

The CHIRAL-CBH column is the shortest of the three tested columns but despite that fact it demonstrated outstanding resolution and very good chromatographic performance. In addition the base-line resolved enantiomers of synephrine were eluted after less than 5 min. The mobile phase used for the separation demonstrated in Fig.1 C was; 5% (w/w) 2-propanol in 10 mM sodium phosphate buffer pH and 50 µm disodium EDTA. The flow-rate was 0.8 ml/min and the column temperature 20°C.



Due to the outstanding resolution, short separation time and the very good chromatographic performance the authors used the CHIRAL-CBH column for the quantification of the enantiomers of synephrine in plant extracts. The method was validated in accordance with the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (1996) and US Pharmacopeia 24 (2000). Excellent linearity, accuracy, precision and sensitivity was obtained.