ChromTech Application Bulletin No.7 (2001)

Strategies for bioanalytical work on the CHIRAL-AGP column

The CHIRAL-AGP column has been used for the determination of many different kinds of enantiomers present at low concentration in biological material, i.e. plasma and urine. The updated reference list for CHIRAL-AGP contains more than 200 publications. Around 50% of these publications discuss bioanalytical methods.

In analysis of drugs from biological material there are at least two main sources for interferences in the chromatogram:
1. endogenous compounds
2. metabolites

Although the CHIRAL-AGP column shows high enantioselectivity for a wide variety of compounds, the selectivity for structure-related compounds, i.e. parent compound-metabolite, may be lower.
There are several methods that can be used to overcome these interference problems.

Different ways to separate enantiomers in complex samples (plasma, urine)

The examples given for each way are taken from the CHIRAL-AGP reference list. The latest version of the reference list can be found in the new "Chiral Application Handbook" and on www.chromtech.se.

1. CHIRAL-AGP column alone

Retention and enantioselectivity are regulated with the mobile phase composition, i.e. pH, organic modifier nature and concentration, buffer type and concentration.
MS detection is preferable due to the high detection selectivity, however also UV and fluorescense detectors are used.
Examples: Bupivacaine, Mepivacaine, Ibuprofen, Naproxen, Metoprolol, Verapamil, Alfuzosin, Oxamniquine, Methadone, Ketoprofen, Labetalol, Vamicamide, Mefloquine, Ifosfamide, Ketorolac, Ketamine

2. CHIRAL-AGP column + preparation of enantiomeric derivatives

Can affect both chiral selectivity and selectivity between endogenous compounds and the drug. When preparing an enantiomeric derivative, the derivatization reagent used is nonchiral, as for example acetylation of amines by acetic acid anhydride or an acid chloride. Acids can be transformed to enantiomeric ester derivatives.
Examples: Atenolol, Verapamil

3. Non-chiral column coupled in series with the CHIRAL-AGP column

Used to increase the resolution between endogenous compounds and the drug and the metabolites
Examples: Disopyramide, Methadone,

4. Non-chiral reversed-phase column in off-line combination with the CHIRAL-AGP column

Collect the eluate from the reversed-phase column and inject it onto the CHIRAL-AGP column after concentration. Suitable if the mobile phase on the reversed phase column is not suitable for obtaining selectivity on the CHIRAL-AGP column.
Examples: Verapamil, Hydroxychloroquine, Terfenadine, Mosapride, Albendazole sulphoxide

5. Non-chiral normal phase column in off-line combination with the CHIRAL-AGP column

Collect the eluate, containing the drug and the metabolite separately, from the normal phase column, evaporate the solvent, dissolve in the mobile phase used for the chiral chromatography and inject on the chiral column. The method is used for very complicated samples
Examples: Disopyramide, Chloroquine, Trimipramine, Mianserin

6. Column Switching

Cut a band from a non-chiral reversed-phase column and switch it automatically over to the CHIRAL-AGP column using a valve. The method is used for very complicated samples.
Examples: Metoprolol, Verapamil, Bupivacaine, Warfarin, Terbutaline, Amlodipine

ChromTech Ltd., Congleton, U.K. Tel: +44 1260 270153 Fax: +44 1260 274394 E-mail: ct@chromtech.co.uk