TECHNICAL REPORT N° 27 **November 2000**

HPLC CHIRAL DETECTION

Interest toward chirality of drugs and need of reliable analytical method to detect and quantify the enantiomeric purity has been addressed in many papers and conferences (see for example proceedings of recent ISCD12 in Chamonix).
Since HPLC has been in the last 20 years the basic analytical technique in pharmaceutical industries, the role of chiral HPLC detectors is expected to grow in the future.
Optical activity detectors may be based either on polarimetry or on CD.
A few papers compared the two techniques for the task[1] [2]; we want to stress here that different approaches, within the same category, are furthermore possible.

Polarimetric detectors:
Dedicated commercial units are based either on single wavelength (nowadays typically a diode laser source) in a range shifted in the red to be outside from absorption phenomena, or, viceversa, on the *white* spectra from a halogen or a Hg/Xe source.
Monochromatic approach is by definition more general purpose, while the use of white light may increase sensitivity, but with potential problems if Cotton effects are present in the eluted sample.
Retrofit of standard polarimeters with flow cell and laser source is the other way to go, mainly if forecasted use is not heavy.
Sensitivity and baseline drift are the main concern of these detectors, polarimetric detectors are indeed very similar to the refractive index (RI) ones most chromatographers are familiar with.

CD detectors:
These can be based on a conventional CD spectrometer with flow cell or on dedicated units (Jasco CD-1595 is the only commercial example so far).
The advantage of CD detection is evident: you increase selectivity and sensitivity selecting the analytical wavelength for the highest response or, better, for the best g factor ($g = \Delta A/A$)[3] [4].
The second main advantage of the CD approach is that from the same detector you get simultaneously both CD (chiral information) and Absorption (mass information). Indeed it's also possible to get g factor directly or as a post run calculation from the same unit.
On the fly or off line spectral acquisition is the third main advantage of these units, able indeed to provide much more information.
These are however only theoretical arguments since:
-sensitivity is still often a main concern (apart from preparative applications)
-precision is strictly related to actual g factor of the sample
-normal HPLC users have typically little experience about polarimetry and none about CD, so a CD detector may be more difficult to optimize.

We must pass from the stage in which chiral HPLC detection was a special techniques in the hands of a restricted group of specialists (sometime looking for the sample to *prove* their ideas and not to a general purpose analytical approach), to the one in which the technique is applied in a routine way by *normal* chromatographers.
The step is not trivial, it'll take time and a deeper education to see the technique really expanding.
In this respect we would recommend to read the HPLC-CD chapter[5] of the recent CD book printed this year, which includes also an ample bibliography.

---

[1] Kudo K., Ajima K., Sakamoto M., Saito M., Morris S., Castiglioni E. *Chromatography* 20, 1999, 59
[2] Castiglioni E. *Chim.Ind.* 81, 1999, 217
[3] Drake A., Gould J.M., Mason S.F. *J.Chromatogr.* 202, 1980, 239
[4] Salvadori P., Bertucci C., Rosini C. *Chirality* 3, 1991, 376
[5] Salvadori P., Di Bari L., Pescitelli G.in *Circular Dichroism: Principles and Applications,Second Edition* edited by Berova N., Nakanishi K., Woody R., 2000, John Wiley & Sons, 797

E.C.S. European Chirality Services srl via Verdi 6A 23870 Cernusco Lombardone (Lc) Italy
tel/fax (0039)-039-9284368 mob 0349-6907288 e-mail ecssrl@libero.it