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HPLC CD DETECTORS

In the previous TN 27 we outlined status of current HPLC chiral detectors. With the present note we would like to compare in more details application of conventional CD units equipped with HPLC flow cell versus the dedicated HPLC-CD detector available commercially only from Jasco[1] [2].
So far a real comparison is missing, but we would like to stress here a few points coming from direct experience.
CD is a challenging technique in respect to sensitivity.
Assuming to deal with a sample with anisotropy factor (g) of 10^{-3} if our eluted component shows an absorption of 0.010 absorbance the expected ΔA will be of 0.00001 absorbance (or 0.33 millideg since most CD units are using for historical reason this sort of scale).
This is a rather difficult signal to detect (and quantify), considering also that you operate in flow mode with a small volume cell.
And g factor of 10-3 is often quite high compared to what you get from *normal* samples.
So a dedicated CD detector is built to improve sensitivity of conventional unit using a simpler optics, with wider spectral bandpass and long path cell (25 mm).
But you get nothing free of charge: aberrations in CD measurement will be higher using simpler optics, while the large bandpass may decrease selectivity and degrade the accuracy; at last the long path cell may be a limiting factor due to mobile phase absorption.
Finally the wavelength range of dedicated current CD detector (Jasco CD-1595) is more limited, particularly in the UV region, than in a normal unit.

So generally speaking we think that the optimal use of an HPLC-CD detector needs some preliminary steps, to be carried on also with the help of a bench top conventional CD apparatus.
Real CD and absorption spectra of the pure enantiomers (if not easy available the fraction may be collected by semiprep chiral chromatography …) are indeed the basic prerequisite to quantify the expected g factor and select the proper analytical wavelength. These must be measured dissolving sample in same mobile phase and also absorption spectra of mobile phase itself must be carefully considered, not to exceed linearity range of the HPLC detector.
Same operation can be carried on using CD and absorption spectral capabilities of the CD detector, but comparison with the same data from a conventional spectrometer may help a lot to see where limits are.
For example the relative shape of the spectra will indicate potential effects of the wide bandpass used in the HPLC detector.
From all these data you can evaluate the potential limits of confidence in your chromatographic analysis using the dedicated CD detector. Limits to be further verified running standard samples in chromatographic way.
For example on line calculation of the g factor is an appealing feature, but this too will be strictly limited if expected (and actual) drift of the CD and absorbance zero will be significative. In these cases only way to get a reliable g factor data is by post run data processing

In several cases we do have the feeling that a conventional CD spectrometer equipped with a proper HPLC cell and focussing optics is still a superior way to approach the problem, despite a somehow lower sensitivity and much larger size and cost, but this should not be a surprise for the people knowing difficulties and limitations in CD measurements.

Conclusion:
The small size, the (relatively) limited cost and the nice features (CD + absorbance + g factor simultaneous outputs, on line spectral capabilities , ….) of the CD-1595 detector are very appealing, but miracles are not possible.
In any HPLC detector sensitivity, accuracy, precision and linearity are the parameters to rely on, these are not strictly correlated eachother and are strongly depending on the sample to be analyzed. So the first task to achieve is to understand where limits are for the specific task.
An ideal HPLC-CD detector based on current state of the art technology would be probably larger and more expensive than a conventional *dicrograph*!

[1] Brandl F., Pustet N., Mannschreck A. *Internat.Lab.* 29, 1999, 10C
[2] Bertucci C., Andrisano V., Cavrini V., Castiglioni E. *Chirality* 12, 2000, 84

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