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Critical appraisal for achiral HPLC with polarimetric detection for the determination of drug enantiomers

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Despite the clear potential of achiral HPLC with polarimetric detection for the determination of drug enantiomers (Lloyd *et al*, 1989), interest in the early 90's soon faded when limitations of commercial instrumentation for polarimetric detection, particularly with respect to limits of detection (LOD's), became apparent. However, especially in the light of data reported and sentiments expressed in a recent report by Sims *et al* (1997) on solvent effects on optical rotation (OR) detection, it was felt that there was hope that the benefits of polarimetric detection for HPLC might yet be more fully exploited if there was more study of the factors which affect the performance of this class of detector.

For such a detector, the Chiramonitor^R, it was found that baseline noise was dependent on pump performance and the length of time the laser light source had been switched on. Therefore absolute values of limits of detection varied significantly from day to day and accordingly, only qualitative observations were made based on measurements made at a similar time. For example, it was confirmed for HPLC of N-acetyl-L-phenylalanine ethyl ester using both straight phase and reversed phase solvent systems that UV-OR detection in series gave lower OR LOD's than OR-UV. While solvent effects were observed, the range of LOD's found (1.7-7.2 μg on-column, when all normalised to a 200 μl peak volume) using five different solvent systems did not suggest that manipulation of solvent systems might become a practical means of achieving order of magnitude dimunitions in LOD, especially in view of the amount of compound-specific method development that would be involved. The use of microbore HPLC with on-column sample focussing in order to reduce the concentration LOD was unsuccessful for N-acetyl-L-phenylalanine ethyl ester. This was because it was not sufficiently well retained on a Spherisorb-S5-ODS2 phase to allow a combination of sample solvent and mobile phase that would give effective focussing. However this would almost certainly be a viable approach for more hydrophobic compounds.

As these studies progressed it became apparent that artefacts affecting peak shapes and areas presented every bit as much of a problem as high LOD's. For example these had an adverse effect on studies of solvent effect on detection of (-)-2-phenylbutyric acid and of UV/OR signal for phenylalanine. It was therefore decided to also use the Jasco OR-990, a newer design of instrument.

Surprisingly, LOD's for a range of compounds such as S-naproxen, gossypol, l-propranolol, S-nicotine, quinine and N-acetyl-L-phenylalanine ethyl ester were still only in the low μg range. Importantly though symmetrical, artefact-free peaks were obtained thus making quantitative measurement much easier. It was therefore decided to investigate the application of the OR-990 to the determination of trace enantiomeric impurity in a single enantiomer drug by achiral HPLC, using the ratioing of UV and OR detector signals. By using 5 $mg.ml^{-1}$ rather than 1 $mg.ml^{-1}$ sample solutions to deal with the OR sensitivity issue and working off λ_{max} to maintain UV linearity, it was found that use of the OR-990 in series with a UV detector could be used to comfortably distinguish between naproxen samples differing in enantiomeric purity by 1%. Since this work may be carried out simultaneously with the determination of other structurally-related impurities, it is clear that, with the OR-990, polarimetric detection may now become a much more effective tool for chiral drug analysis.

References

Lloyd, D.K., Goodall, D.M., Scrivener, H. (1989) Diode-laser-based optical rotation detector for high-performance liquid chromatography and on-line polarimetric analysis. Anal.Chem. 61: 1238-1243.

Sims, J.L., Carriera, J.A. Bragg, R.A. (1997) Optical rotation detection for reversed phase HPLC: investigation of solvent effects. Chirality 9: 122-125.