The Use of Circular Dichroism Detection in UHPLC to Determine Enantiomeric Ratios without Peak Resolution

by D.J. Tognarelli, Jasco Inc, 28600 Mary's Court, Easton, MD, 21601 USA

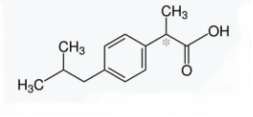
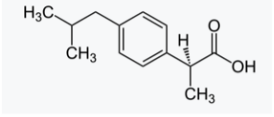
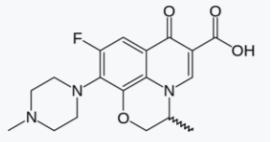
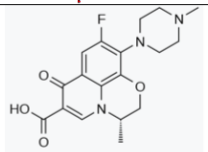
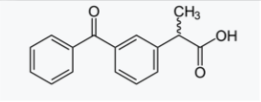
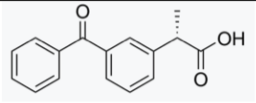
The determination of enantiomeric ratios is imperative in characterising and studying the pharmacological effects of each enantiomer. The FDA requires individual testing of chiral drug molecules as significant differences in behaviour between enantiomers in the body can occur as history has shown. Some enantiomers just differ in potency, but others can be inhibitive to each other and even some detrimental. In the 1950s one enantiomer of thalidomide was used against morning sickness, however, the other enantiomer caused birth defects [1]. Tuberculosis is treated with a variety of medications including the ethambutol (S,S)-(+) enantiomer, but the (R,R)-(-) enantiomer causes blindness [2].

Currently, the time-consuming task of achieving baseline resolution of enantiomers is required for determining the enantiomeric purity. This typically requires SFC screening employing many columns and solvent combinations under a variety separation conditions. The resulting conditions are applicable to the separation of those specific enantiomers, while other racemic mixtures require a new solvent-column screen.

The circular dichroism detector (CD) measures the differential absorption of right and left circularly polarised light. Although enantiomers will absorb UV light equally, they will absorb right and left circularly polarised light differently. The ability to measure this differential absorption between the enantiomers will allow the determination of the enantiomeric ratio without resolving the peaks, thereby eliminating the time-consuming process of achieving enantiomeric separation.

Advil® is a very common anti-inflammatory with the active ingredient ibuprofen in the racemic form, while Seractil® is the enantiomerically pure form. Floxin® is an antibiotic used for treating a variety of bacterial infections with the active ingredient ofloxacin which is racemic. Levaquin® is the enantiomerically pure form of that drug. Actron is another anti-inflammatory drug with the racemic active ingredient ketoprofen. Keral is the enantiomerically pure form. The structures of these drugs are shown in Table 1. The determination of the enantiomeric purity of these drugs is imperative since they are among many commercially available drugs that are offered as completely different drugs in the racemic and enantiomeric pure forms. To meet the FDA requirements for chiral drug production it is necessary to confirm the correct form of the chiral drug is being produced and the enantiomeric purity must be analysed.

Table. 1. Structures of the drug compounds.

Racemic mixture	Single Enantiomer
 Ibuprofen	 Dexibuprofen
 Ofloxacin	 Levofloxacin
 Ketoprofen	 Dexketoprofen

Experimental

Test compounds Camphorsulfonic Acid D, Camphorsulfonic Acid L, Camphorsulfonic Acid DL, Levofloxacin, Ofloxacin, (S)-(+)-Ibuprofen, Ibuprofen, (S)-(+)-Ketoprofen and Ketoprofen were all purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade water and HPLC grade acetonitrile was purchase from Sigma-Aldrich (St. Louis, MO, USA). The C18 (2.1 x 50mm, 1.9um) column was purchased from Restek (Bellefonte, PA, USA).

The Jasco UHPLC consisted of a quaternary pump, autosampler and circular dichroism

detector controlled under ChromNAV 2 software. The concentration of each sample was 1.0mg/mL with the injection volume of 1uL and flow rate of 0.5mL/min. The CD wavelength varied for each compound and is listed for each in the results and discussion section.

Results and Discussion

Figure 1 shows the overlaid CD spectra of camphorsulfonic acid D, camphorsulfonic acid L and camphorsulfonic acid DL. Camphorsulfonic acid D and camphorsulfonic acid L show equal and opposite differential absorption of the circularly polarised light from the CD detector with a maximum around 287nm. The racemic camphorsulfonic acid DL shows no signal in the spectral scan and chromatogram as the equal amounts of D and L enantiomers present cancel their respective absorption. The overlaid chromatograms at 287 nm is shown in figure 2. The UV signal of the 3 are almost identical as expected, but the CD chromatograms illustrate the ability to determine the enantiomeric excess without resolving the enantiomers.

Camphorsulfonic acid is an ideal compound for demonstrating the principles and operation of circular dichroism, however to show the applicability to enantiomeric determinations without resolution for active drug molecules, the active ingredients in Advil®, Seractil®, Actron, Keral, Floxin® and Levaquin® were analysed. Figures 3 shows the chromatograms at 225nm of Ketoprofen comparing the racemic mixture to its enantiomerically pure counterpart. Ofloxacin and levofloxacin at 295 nm and ibuprofen at 260 nm produced similar results (not shown). In all three examples, the racemic mixture shows no CD absorption while the enantiomerically pure compounds (S)-(+)-ketoprofen and levofloxacin show negative CD absorption and (S)-(+)-ibuprofen shows positive CD absorption. The lack of CD absorption proves the mixtures are truly racemic as the equal and opposite CD absorption of the enantiomers cancel each other out.

Since enantiomeric excess determinations are most often performed to identify the enantiomeric purity typically of 95% or higher, Figure 4 shows the overlay of 95%, 96%, 97%, 98% and 99% (S)-(+)-ketoprofen. Figure 5 shows the correlation coefficient of 0.99 for 95-100% (S)-(+)-ketoprofen.

Conclusion

CD detection has shown the ability to differentiate between enantiomers when other detection methods such as UV do not. This ability when combined with the rapid elution and separation of a UHPLC can provide enantiomeric excess determinations without resolving the enantiomers. This application could also be applied to racemisation studies offering very short time intervals for those chiral molecules that rapidly racemise.

1. C. Blakemore and S. Jennett. The Oxford Companion to the Body. Oxford University Press. 2001.

2. N. Chhabra, M. Aseri and D. Padmanabhan. A review of drug isomerism and its significance. Int. J. Appl. Basic Med. Res. 2013. 3 (1). 16-18.

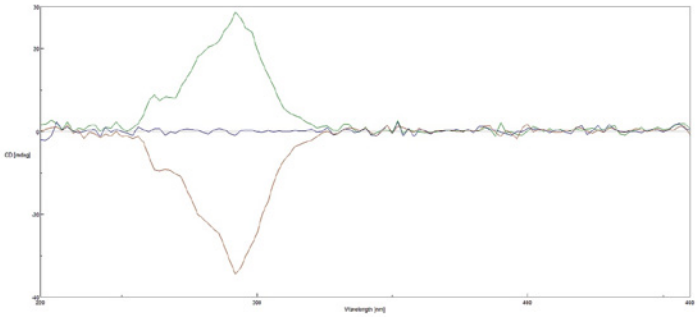


Figure 1. CD Spectra of Camphorsulfonic Acid, D, L and Racemic.
This overlay shows the CD scan from 220nm to 460nm of camphorsulfonic acid D (green), camphorsulfonic acid DL (blue) and camphorsulfonic acid L (orange).

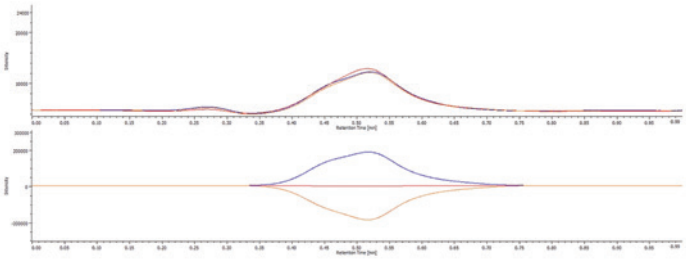


Figure 2. Camphorsulfonic Acid, D, L and Racemic
This is the UV (Top) and CD (Bottom) chromatogram overlay at 287nm of camphorsulfonic acid D (blue), camphorsulfonic acid DL (red) and camphorsulfonic acid L (orange).

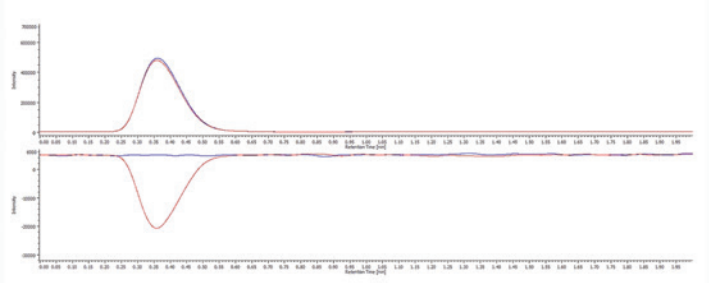


Figure 3. Ketoprofen and (S)-(+)-Ketoprofen
This is the UV (Top) and CD (Bottom) chromatogram overlay at 225nm of ketoprofen (blue) and (S)-(+)-ketoprofen (red).

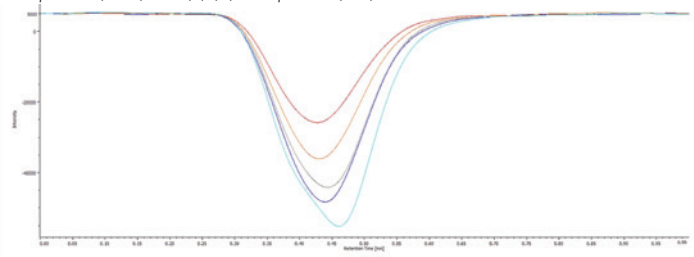


Figure 4. Overlay of 95-99% (S)-(+)-Ketoprofen
These are CD chromatogram overlays at 225nm of 95% to 99% (S)-(+)-ketoprofen.

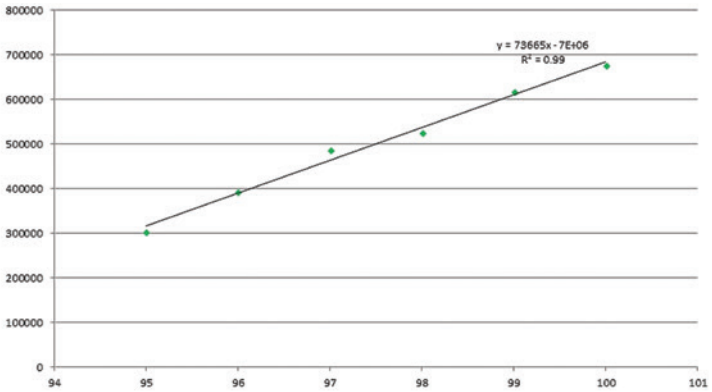


Figure 5. Calibration Curve of (S)-(+)-Ketoprofen
The calibration curve of 95% to 100% (S)-(+)-ketoprofen.
The correlation coefficient is 0.99.