Increase Productivity and Accuracy in Chiral HPLC/SFC/CCC by using an Advanced Laser Polarimeter (ALP)

ALP Applications:
- Confirm Enantiomeric Separation
- Identify Elution Order of Enantiomers
- Measure “ee” and Specific Rotation from Chromatograms
- Control Fraction Cuts in Purification
- Detect Enantiomers without Chromophores
- Monitor changes in Optical Activity during Chemical Reactions
- Monitor Protein/Peptide Conformation

Advanced Laser Polarimeters (ALPs) are used in HPLC, SFC, SMB, CCC, and Process applications covering analyte sizes ranging from micrograms to tons. The productivity and accuracy of these applications depend on ALPs unique characteristics. ALPs are very easy to integrate into new or existing systems and operation is fully automatic.

Small Molecule Pharmaceuticals

The majority (80+ %) of small molecule pharmaceutical candidates are chiral compounds containing one or more chiral centers. Most have specific rotation values between 10 and 40 degrees. ALPs are very beneficial for development, scale up, and production of optically active compounds.

See the chromatogram below of the antihypertensive Labetalol. The two enantiomeric pairs are separated into 4 separate components. UV is blue and ALP is red. With UV alone, it is not possible to determine which peaks are from which pair of enantiomers. However, with the ALP in series, additional information is obtained demonstrating that the first and third peaks are from one enantiomeric pair, and the second and fourth peaks comprise the other pair. There is one positive and one negative peak pair with the same area and another positive and negative peak pair with a different area in the ALP plot. This is the result of different specific rotation values for each +/- pair and the same absorbance for each +/- pair.

Based on this example, it is easy to extrapolate to the usefulness of ALP for compounds that with more than two stereogenic centers.

Advanced Laser Polarimeter (ALP) Benefits:
- Sensitive only to Optical Activity
- Not affected by Absorbance
- Very large Linear Dynamic Range (1 million)
- Long Lifetime (10 year MTBF) – no lamp to replace
- Wide variety of Flow Cells for HPLC, SFC, & Process
- Ideal for Automated Applications
  - No user adjustments
  - No stop flow scans
  - Data is real time

Foods, Flavors, and Fragrances

Because taste and smell are chiral effects, foods, flavors, and fragrances can be successfully analyzed by ALPs.

Fertilizers and Pesticides

Many new and developing fertilizers and pesticides are chiral for the same reasons that most small molecule pharmaceuticals are chiral -- improved specificity and efficacy with reduced toxicity. ALPs are very useful with these compounds.

Theory

Advanced Laser Polarimeters (ALPs) measure the net optical activity of the contents of their flow cells in real time. The measurement is usually made dynamically as in a flowing HPLC or SFC system but can be made statically with sample stationary in a flow cell. ALPs measure optical activity directly and exhibit no interferences and no non-linearities across a large dynamic range (one million). These selective and robust characteristics allow exactly the same optical bench technology to be applied from analytical to prep to production level applications with excellent correlation between units operating at drastically different analyte scales from micrograms to tons.

Measures optical activity for a particular analyte depends on a variety of parameters including the interrogation wavelength. Between 200 and 400 nm amplitude variations due to wavelength are large and zero crossings (sign inversions) are typical.

Wavelength variations and zero crossings are seldom observed beyond 500 nm. Traditional polarimeters use sodium D line emission at 590 nm and ALPs use laser diode emitters at 635 nm. Measurements at these wavelengths (590 and 635 nm) have proven to be extremely stable and accurate.
reproducible with essentially no absorbance-related interferences while shorter wavelengths prove problematic in many cases. Measured optical activity is also affected by solvents and to a lesser extent by temperature.

The large linear dynamic range of ALPs and their ease of operation make them ideal detectors for method development, prep, and process applications.

Comparison to UV and CD

UV (ultra violet absorption) and CD (circular dichroism) are both absorbance based detection schemes and share some characteristics. They require a chromophore in the analyte and excitation with a wavelength in the chromophore’s absorbance band. Their linear dynamic range is naturally limited (more so for CD) because as absorbance increases the photon flux at the photodetector is approaching zero and noises increase. In addition to a chromophore, CD requires a chiral center coupled to that chromophore for detection of optical activity. Both UV and CD are often affected by solvent gradients and CD suffers from other absorbance related interferences.

ALPs do not require chromophores or wavelength tuning to match an absorbance band because they are not absorbance based. ALPs detect optical activity by measuring the angle of rotation of a highly polarized laser beam -- a phase measurement not an amplitude measurement. Large angular rotation only changes the phase angle and does not reduce photon flux at the photodetector.

To compare sensitivity is complicated because of variations in analyte characteristics and chromatographic conditions as well as variations in instruments. For most pharmaceutical compounds, UV is more sensitive than CD or ALP. Exceptions include compounds with no chromophore (sugars, antibiotics, etc.) where only ALPs are sensitive. If CD is first used with an appropriate analyte captive in the flow cell, a stopped flow wavelength scan is performed, and the wavelength of maximum signal is selected then subsequent analysis of that particular analyte by CD will be competitive with ALPs. In automated analytical screening and prep purification applications ALPs almost always perform better because no adjustments or stop flow scans are required and the linear dynamic range is much larger.

HPLC/SFC Method Development

In method development an ALP will confirm enantiomeric separation by showing the characteristic positive and negative enantiomeric peaks. UV detectors are usually more sensitive than ALPs but UV detectors cannot uniquely identify enantiomeric peaks as compared to peaks from unseparated racemate and achiral compounds. Compounds with multiple chiral centers exhibit noticeable differences in specific rotation between enantiomer pairs. This characteristic makes it easier to properly identify and quantitate peaks in diastereoisomer (and more complex) separations.

See the ALP chromatogram below (TSO, OD, Hexane/IPA). The 2 small square peaks eluting before the void volume spike are internal calibrates showing -100 and +1000 microdegrees of rotation. The first eluting enantiomer is negative and the second enantiomer is positive - clearly.

HPLC/SFC Prep

In prep purification applications the tendency of UV and other absorbance detectors to overload makes ALP the preferred detector. Chiral prep performance is best when an ALP is used to detect and collect enantiomeric peaks and a UV detector is used to track impurities that are to be avoided in collected fractions. ALPs are robust with long lifetimes and they operate without adjustment or attention. Flow cell are available to accommodate any application at any scale.

The chromatogram below shows prep collection triggered by ALP derivative value and sign. This robust collection mode always puts the (+) enantiomer in the (+) bottle regardless of elution order and is dynamically adaptive to variations in loading and retention time. Notice that the smaller injections on the right are collected correctly.

Summary

Unique patented features, robust construction, and excellent support result in improved productivity.