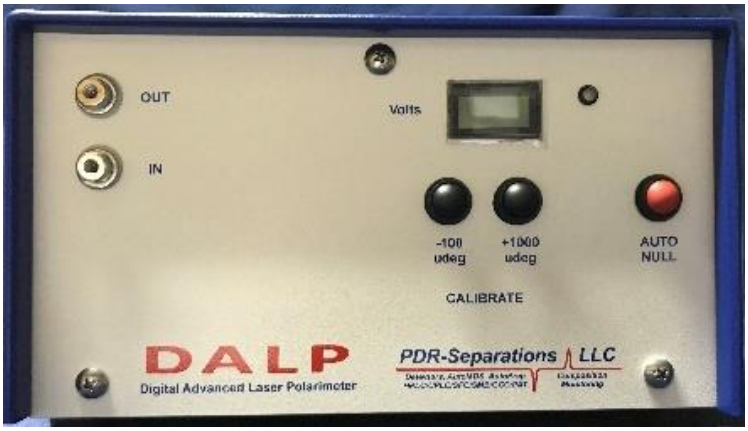
DALP (Digital Advanced Laser Polarimeter) Optical Activity Detector

DALP is a new digital implementation of our traditional ALP (Advanced Laser Polarimeter) that has been in production for more than 20 years. DALP uses digital signal processing (DSP) with our proprietary algorithms to successfully ignore changes in laser power and absorbance to consistently delivery improved sensitivity, drift, and accuracy. PDR's companion **DALP App** sets time constant and supports on-site optimization, calibration, and software upgrades. Existing ALPs can be upgraded to be DALP with all-new electronics and improved capabilities. for about $10K.



100 - 240 VAC 50/60 Hz
20 watts
10 x 6 x 16 inches
20 lbs
CE, UL, CSA, TUV
Flow Cells: HPLC, UHPLC, SFC, Prep, CCC/CPC, SMB/MCC, PAT, etc.

DALP responds to optical activity with a unique positive or negative deflection. Amplitude is proportional to concentration and specific rotation while +/- sign indicates clockwise or counterclockwise rotation: regardless of elution order, eluent, or separation method. Operating at 635 nm, DALPs only respond to optical activity and ignores absorbance – all peaks are optically active, gradients do not affect the baseline, and no chromophore is required. Detection is based on polarization (optical phase) changes not absorbance (amplitude) changes. In method development applications DALPs show elution order of enantiomers and confirms that peaks are optically active. In preparative purification applications DALPs control peak collection based on peak +/- polarity and amplitude, rather than just amplitude. DALPs do not suffer from the overload (if too much light is absorbed, there is nothing left to detect) and non-linearity problems of absorbance-based detectors.

DALP Advantages:

- Universal Optical Activity Detector
- HPLC, UHPLC, SFC, CCC/CPC, SMB/MCC, PAT, etc.
- Pharmaceuticals, Antibiotics, Pesticides, Proteins, Foods, Flavors, Fragrances, etc.
- No Chromophore Required, no Absorbance effects
- Sensitive, Large Linear Dynamic Range (> 100,000)
- Robust (10+ year MTBF at 24/7/365)
- Analog and Digital Output, can be connected to any data system
- DALP can be optimized and calibrated onsite using DALP App and a Calibration Shim
- Fully Automatic, No User Adjustments or Stop Flow Scans required
- Consistent Assignment (+/-) for Enantiomers
- Real Time Data – Multiple Scans Not Required
- Two "real" Calibration Peaks that rotate plane of polarization same as Analytes
- Flow Cells available for Any Application
- Confirm Enantiomeric Separation in Method Development
- Measure Specific Rotation from Chromatogram Peak
- Control Fraction/Peak Collection in Preparative Purification

Theory of Operation:

DALP measures the rotation of a highly-polarized 635 nanometer laser-beam passing through a flow cell with microdegree sensitivity in microliter volume – without absorbance anomalies or interferences, and no chromophore is required. See Figure DALP-01 below of typical chromatogram. Calibrates are real rotations exactly same as analytes. DALP's rotational calibration is based on geometry using a mechanical shim, not chemistry. DALPs are very accurate and specific rotation can be calculated directly from a chromatogram peak.

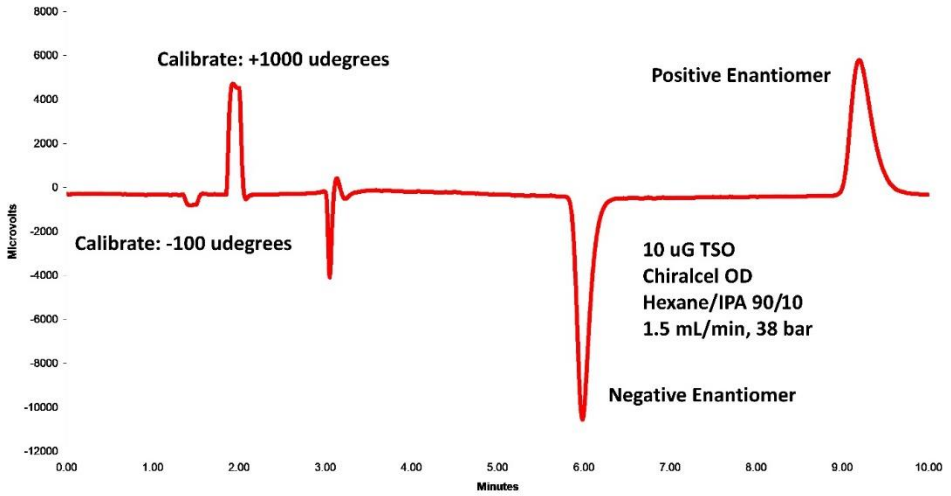


Figure DALP-01: Typical DALP Chromatogram of Separated Racemate

Measured optical activity for a particular analyte depends on a variety of parameters including wavelength. At wavelengths between 200 and 400 nm optical activity values typically changes quickly with wavelength and zero crossings (sign inversions) are not unusual. Variations in measured optical activity and zero crossings are not common above 500 nm. Traditional polarimeters use the sodium D line emission at 590 nm and DALP uses laser diodes at 635 nm. Measurements at these wavelengths (590 and 635 nm) have proven to be extremely stable and reproducible with no absorbance-related interferences while shorter wavelengths prove problematic in many cases. Measured optical activity is affected by sample matrix (including the diluent) and to a lesser extent by temperature.

Light can be described as having properties similar to that of a traveling wave, see Figure DALP-02A below. Wave motion results from vibrations (oscillations) that produce crests and troughs. The distance between successive crests or troughs, called wavelength, corresponds to light's color. The magnitude of vibration, see Figure DALP-02B, corresponds to its intensity or amplitude. Most light sources emit waves of light vibrating in many different planes, see Figure DALP-02C.

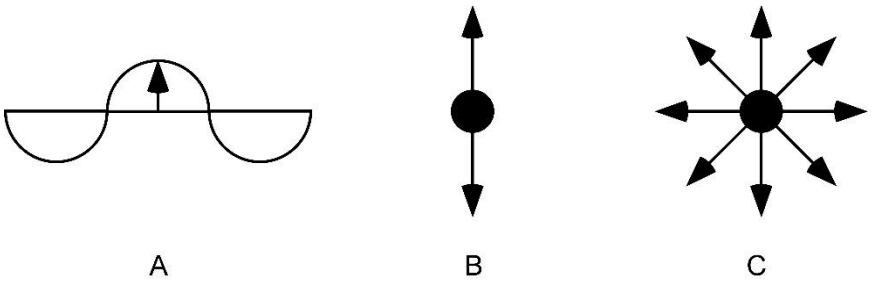


Figure DALP-02: Wave-Motion of Light

If passed through a polarizing filter the emerging light will be limited primarily to waves vibrating in a single plane, see Figure DALP-03 below. The resulting light is called plane-polarized because the light is primarily polarized in a single optical plane.

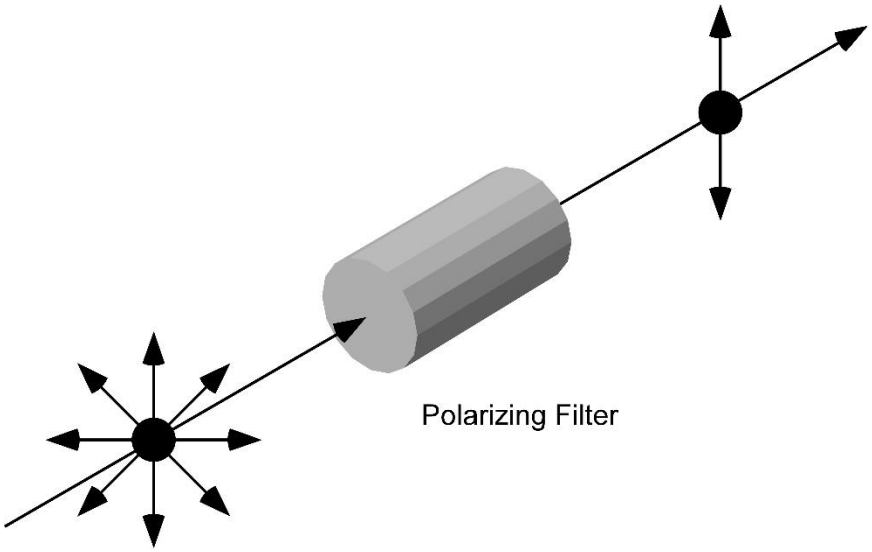


Figure DALP-03: Producing Plane Polarized Light

So, what is optical activity? To answer that question, we shift from physics to chemistry. Some organic compounds have a molecular geometry that affects plane polarized light. These compounds contain a carbon atom to which is attached four different functional groups (see Phenylalanine example in Figure DALP-04 below). Because a single bonded carbon atom has a tetrahedral geometry, the functional groups can be attached in either of two configurations with one molecule being a mirror image of the other (similar to your right and left hands). Otherwise the molecules are structurally identical. This geometry rotates (changes) the angle of the plane of polarized light passing through, is called optical activity, and is unique. The "rotation" of the plane of polarization is actually an optical phase phenomenon that locally affects the light beam inside the flow cell. Optical activity is defined as the ability of a compound to rotate plane polarized light in one direction or another (positive versus negative rotation).

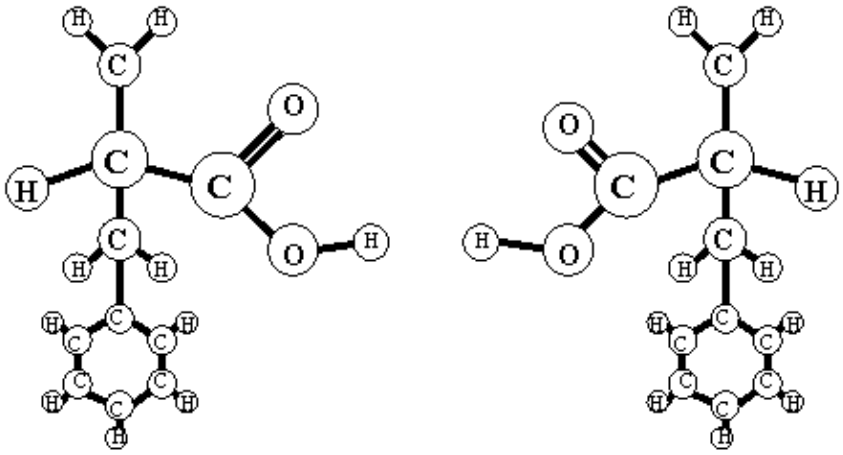


Figure DALP-04: Phenylalanine Chiral Geometry

Mirror image, non-superimposable isomers are called enantiomers. Enantiomers are chemically identical but contain a difference in 3D molecular geometry that produces an equal but opposite rotation of plane polarized light. Using a polarimeter we can measure the optical rotation produced by a liquid or gas phase sample, see Figure DALP-05 below.

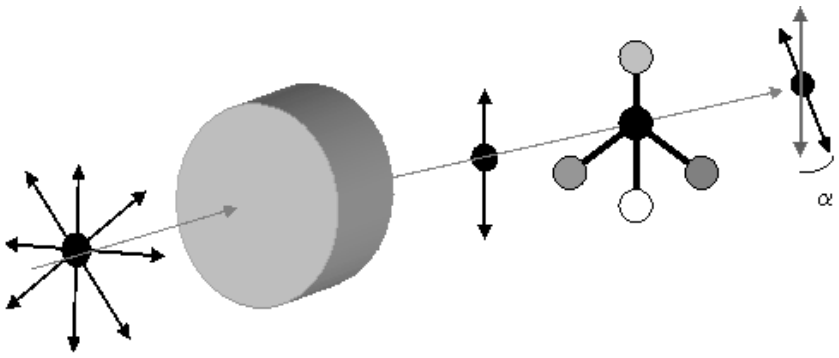


Figure DALP-05: Rotation of Plane-Polarized Light by Analyte

DALP optical system is illustrated in Figure DALP-06 below and consists of a laser diode, polarizing prism, Faraday rotator, flow cell, analyzing prism, and photodiode.

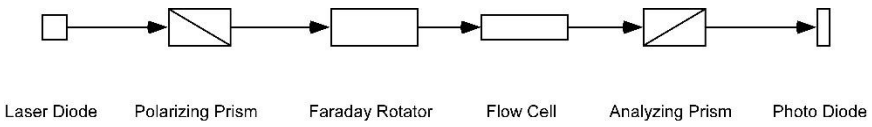


Figure DALP-06: DALP Optical System

Polarized light from a laser diode is further filtered with a polarizing prism and this highly polarized light passes through a Faraday rotator and then a flow cell. The Faraday rotator imparts an oscillating rotation to the plane of polarization. A sample exhibiting net optical activity flowing through the flow cell will offset the average oscillating rotation. Advanced electronics and mathematical algorithms extract the magnitude and sign of net optical activity from the photodiode signal. Faraday oscillation is above 300 Hz, so integration of results is not required and real time values are available continuously. Faraday oscillation is part of our phase detection and noise rejection scheme.

DALP in Method Development:

DALPs are useful in method development to differentiate enantiomer peaks from achiral peaks, track enantiomer elution order, identify peaks in multiple chiral center compounds, detect optically active compounds, etc.

DALPs confirm enantiomeric separation by showing the characteristic positive and negative enantiomeric peaks. UV cannot differentiate enantiomeric peaks from unseparated racemate and/or achiral peaks. DALPs only reacts to optical activity, not absorbance. Compounds with multiple chiral centers exhibit noticeable differences in specific rotation between enantiomer pairs, see Figure DALP-07 below. This characteristic makes it easy to properly identify and quantitate peaks in diastereoisomer (and more complex) separations. Using a DALP along with UV/DAD in method development and analysis provides instant verification of enantiomers, showing amplitude and unique +/- polarity that is not dependent on elution order or eluent composition. DALPs ignore peaks and gradients with no optical activity.

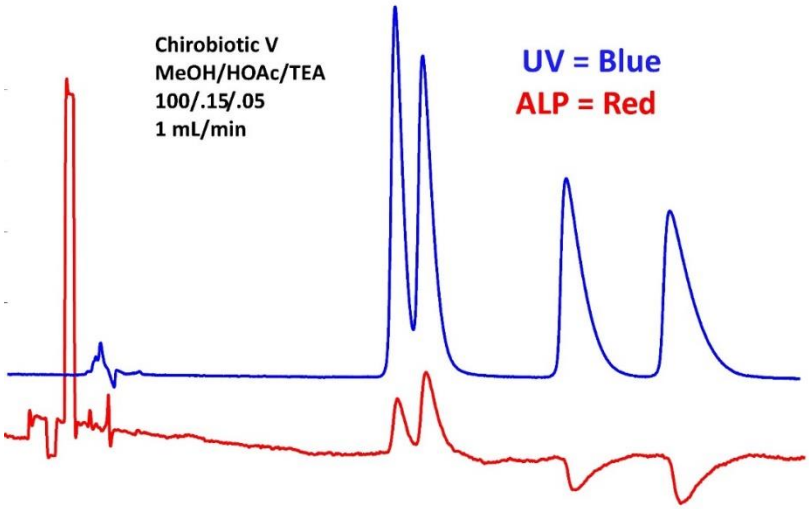


Figure DALP-07: Labetalol Diastereomer

See Figure DALP-08 below where an ALP was used to purify cypermethrin enantiomers using SFC. Cypermethrin has three chiral centers and eight enantiomers. There are no commercially available cypermethrin single enantiomer standards. The ability of ALP to identify enantiomers by peak polarity and area was a big help in interpretation of chromatograms and peak identification for preparative purification separations. The last peak in the UV chromatogram is an impurity, not a cypermethrin enantiomer. This was easy to see on the ALP trace.

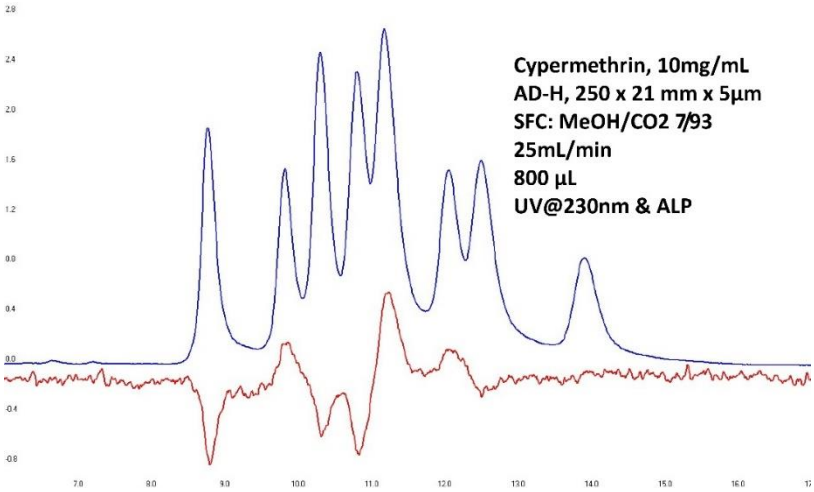


Figure DALP-08: Cypermethrin Separation, 4 chiral centers, ALP+UV, SFC

See Figure DALP-09 below where an ALP was used to purify Gentamicin analogs using HPLC. UV detection is challenging because these compounds lack a UV absorbing chromophore. All analogs (C1A, C2, C2A, C2B, C2A, and C1) were separated along with impurities and sisomicin.

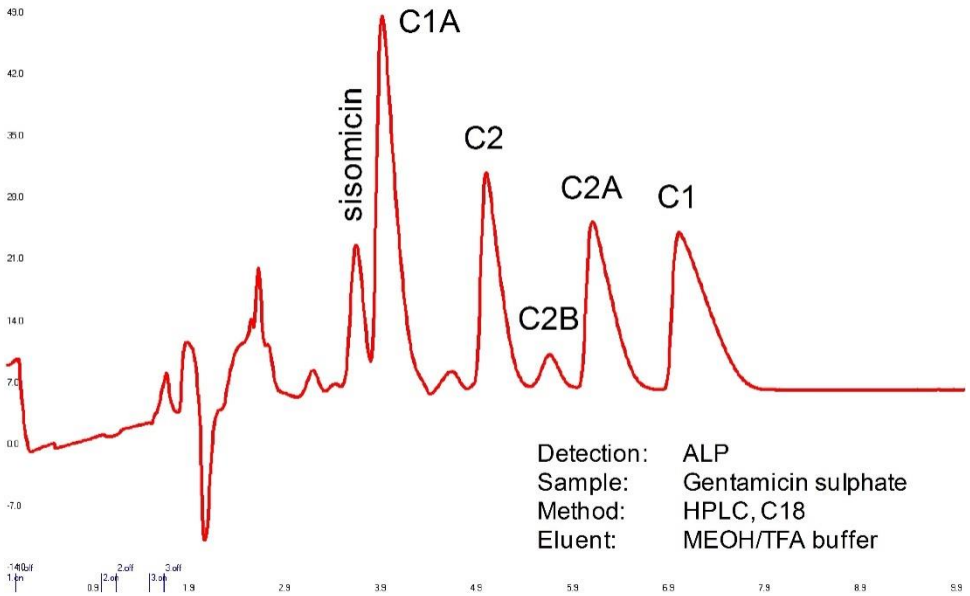


Figure DALP-09: Gentamicin Preparative Purification Separation with ALP Detection

See Figure DALP-10 below where an ALP was used to separate 4 Sugars.

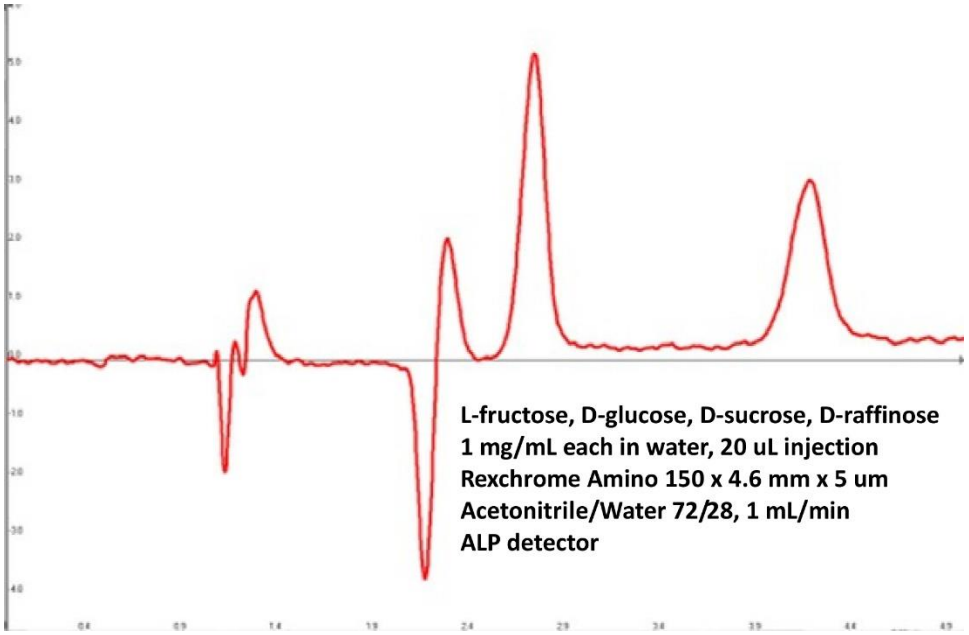


Figure DALP-10: Separation of 4 Sugars

DALP in Preparative Purification:

In preparative purification applications the tendency of UV and other absorbance-based detectors to overload makes a DALP the preferred detector (DALPs have huge linear dynamic range). Chiral preparative purification performance is best when a DALP 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. Flow cells are available to accommodate any application at any scale.

Figure DALP-11 below shows the end of an SFC stacked injection preparative purification run where sample bottle was being sucked-dry resulting in smaller injections. UV (Blue) and ALP (Red) traces shown with peak collection by ALP derivative. This robust AutoPrep + ALP collection mode always puts the (+) enantiomer in the (+) bottle regardless of elution order and dynamically adapts to variations in loading and retention time. This purification mode does not use time, except to define time between injections. Notice that the smaller injections on the right are collected correctly without manual intervention.

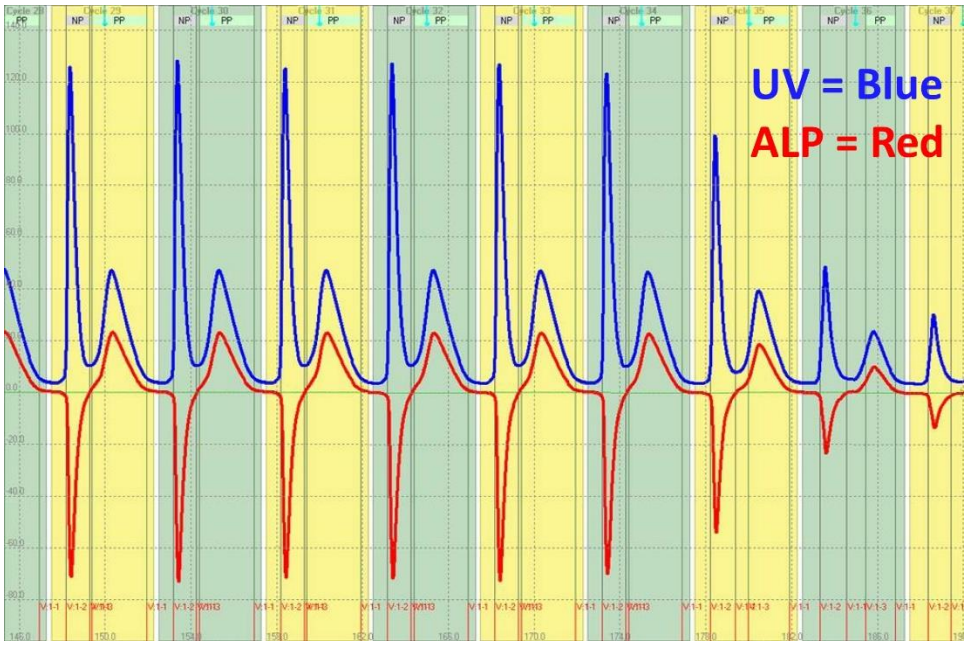


Figure DALP-11: Preparative Purification Stacked Injections, SFC job at PDR 2006.11.06

Using AutoPrep + DALP to purify enantiomers means a new job can usually be started by specifying only: injection volume, number of injections, time between injections, eluent composition, and flow rate (assuming column and solvents are proper). Elution order and collection via time windows are not required because AutoPrep + DALP will send positive peaks to the positive-designated collection port and negative peaks to the negative-designated collection port consistently based on real-time amplitude and +/- sign of DALP signal derivative. This mode of collecting enantiomers is very easy to use and very efficient. In most cases concentration and purity of collected fractions will be better than other techniques because each collection is actively controlled by conditions inside the DALP flow cell. Collections never start or stop too soon or too late, but just right consistently.