



Advances of Optical Rotation Detection in Chiral HPLC

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Abstract

In chiral chromatography, the technology of detecting chiral molecules using optical devices has lagged behind until now. The stability and sensitivity of this type of instrument is an important issue in chiral chromatography. However, with the availability of the single wavelength LED, increased sensitivity and increased economics with simplified instrument design was possible. In addition, a newly designed glass coated steel flow cell has produced a superior, third generation optical rotation detector. Aided with the Faraday compensation technique, this instrument gives high optical rotation power with excellent signal to noise ratio.

This instrument is an excellent supplemental detector to standard UV detection. For chiral separations, it permits the identification of elution order which is important when quantifying to establish minimum levels of detection. It allows for the identification of enantiomeric pairs when dealing with multiple chiral centers. During the method development process, this detector detects marginal selectivity when UV detection fails. The important features of this instrument will be described. Also, examples of absolute sensitivity, identification of enantiomer pairs and reversed elution order will be included.

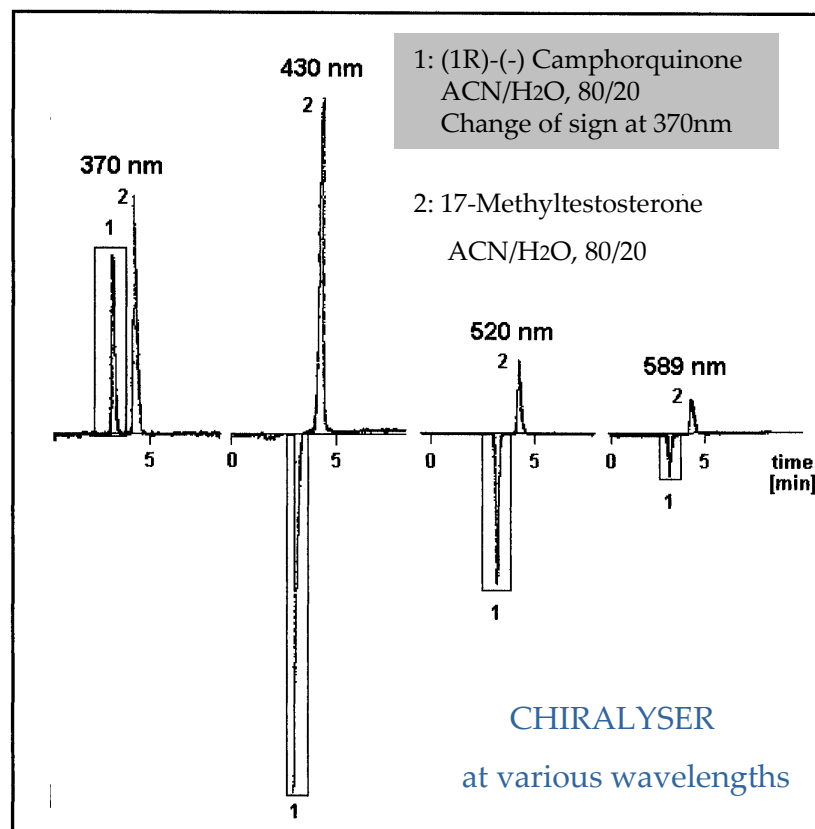


Polarimetry and Chiral Detection

- Identifies the chirality of a molecule with a positive or negative sign of rotation.
- Detects only optically active compounds.
- Can detect optically active compounds with no or low UV absorbance.
- Works with gradients.
- Simple determination of enantiomeric excess (ee) and optical purity.

Optical Rotation as a Function of Wavelength

At 430nm from a high power blue LED, the maximum optical rotation dispersion is obtained with minimum absorption effects. Although intensity is lower than laser sources the distribution is constant with no interference effects (low noise) commonly seen with laser sources.

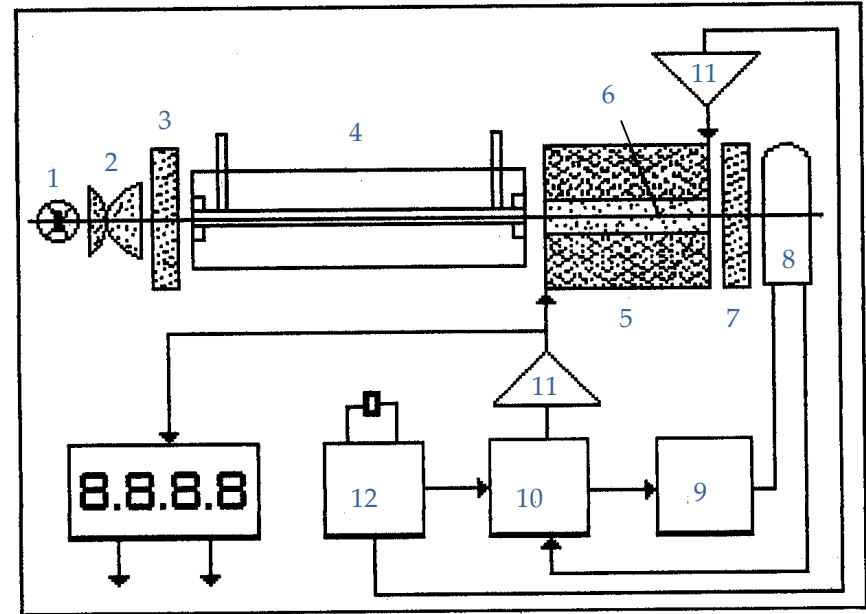


Faraday Effect

The Faraday effect describes the rotation of the plane of polarized light produced when plane polarized light is passed through a substance in a magnetic field, the light traveling in a direction parallel to the direction of propagation. For a given substance, the angle of rotation is proportional to the length of the column which the light traverses and the strength of the magnetic field.

Since a compensating current can be made proportional to the optical rotation of the sample, the analysis is in real time simpler and substantially faster than the mechanical manipulation of previous devices.

The Faraday modulation measures the rotation angle independent of the light source intensity or absorption of the sample. This fast electrical compensation is proportional in real time to the amount of optically active substance inside the cell which is easily transformed to a calibrated readout.



- | | |
|---------------|--------------------------------|
| 1. LED, 430nm | 7. Analyser |
| 2. Lenses | 8. Photomultiplier |
| 3. Polarizer | 9. HV-Generator |
| 4. Cell | 10. Phase Sensitive Integrator |
| 5. Coil | 11. Amplifier |
| 6. Rod | 12. Time Base |

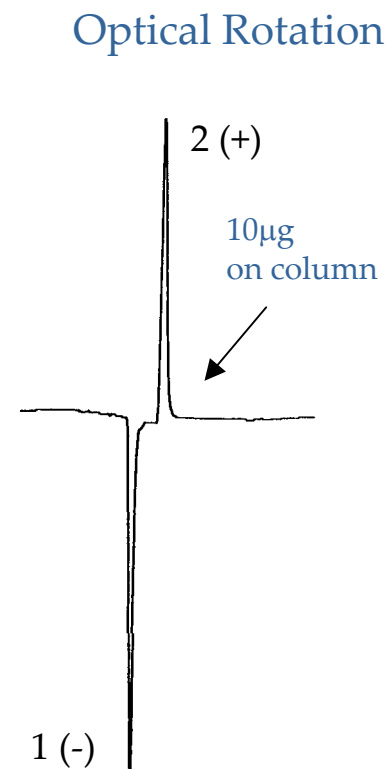
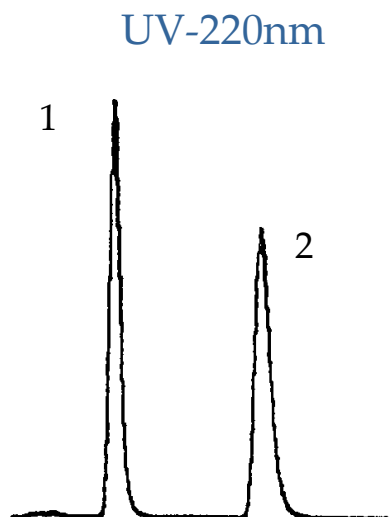
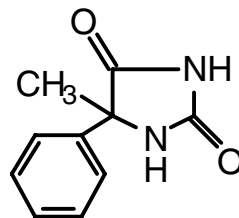
UV vs Optical Rotation

Analyte: 5-Methyl-5-phenylhydantoin

Column: CHIROBIOTIC T, 250x4.6mm

Mobile Phase: 100% Methanol

Flow Rate: 1.0 mL/min.



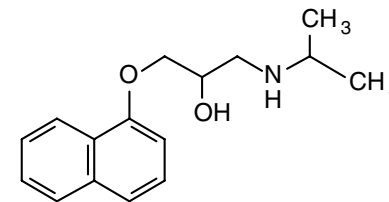
Identifying Reversal of Elution Order

Analyte: Propranolol

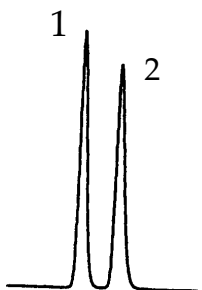
Column: CHIROBIOTIC T and CHIROBIOTIC TAG, 250x4.6mm

Mobile Phase: 100/0.1%wt.:MeOH/ATFA

Flow Rate: 1.0 mL/min.

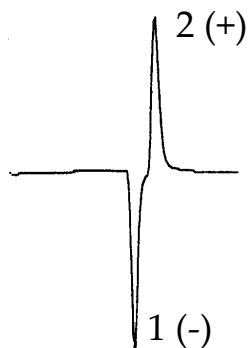


UV-254nm

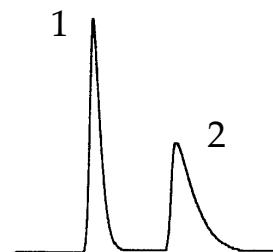


CHIROBIOTIC T

Optical Rotation

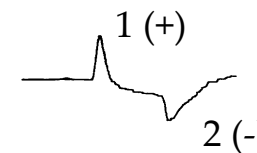


UV-254nm



CHIROBIOTIC TAG

Optical Rotation



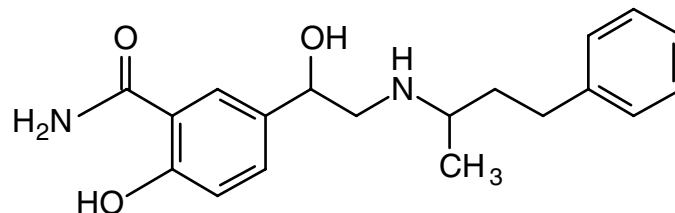
Identification of Enantiomeric Pairs

Analyte: Labetalol

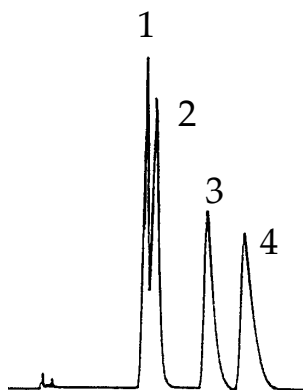
Column: CHIROBIOTIC V, 250x4.6mm

Mobile Phase: 100/0.1%wt.:MeOH/AFTA

Flow Rate:1.0 mL/min.



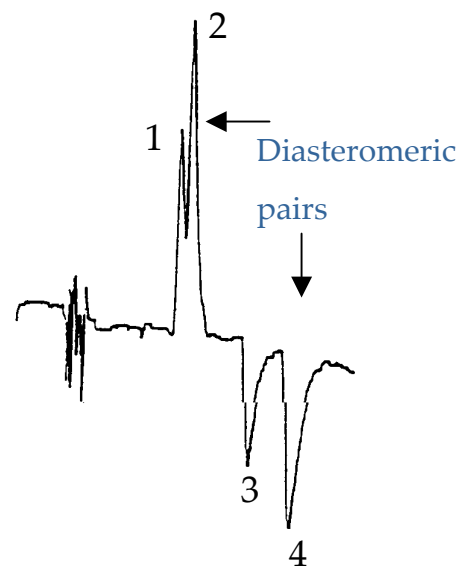
UV-254nm



Peak 1 (+);Peak 3 (-) Enantiomers

Peak 2 (+);Peak 4 (-) Enantiomers

Optical Rotation



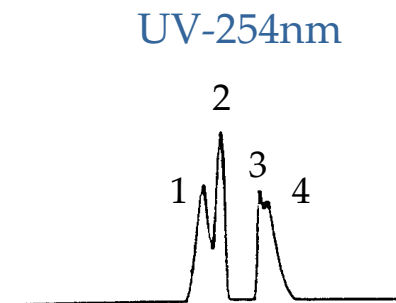
Identification of Enantiomeric Pairs

Analyte: Unknown Sample (2 chiral centers)

Column: CHIROBIOTIC V

Mobile Phase: 100/0.15/0.05; MeOH/HOAc/TEA

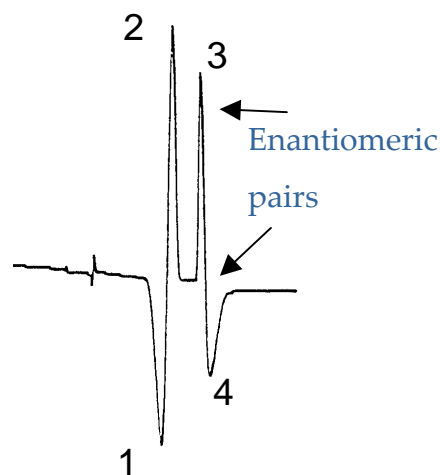
Flow Rate: 1.0 mL/min.



Peak 1(-); Peak 2 (+) Enantiomers

Peak 3 (+); Peak 4 (-) Enantiomers

Optical Rotation



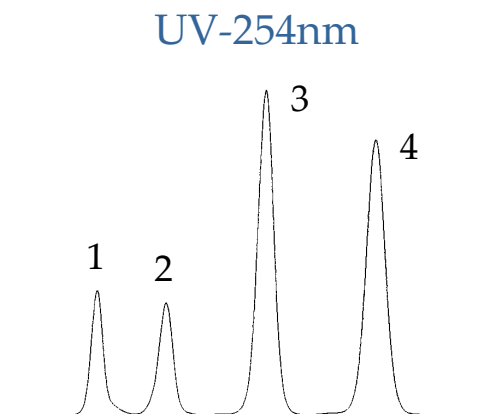


Determination cis/trans Enantiomers

Column: CYCLOBOND I 2000 RSP, 250x4.6mm

Mobile Phase: 40/60: ACN/5mM NH₄OAc, pH 3.8

Flow Rate: 0.8 mL/min.



Optical Rotation



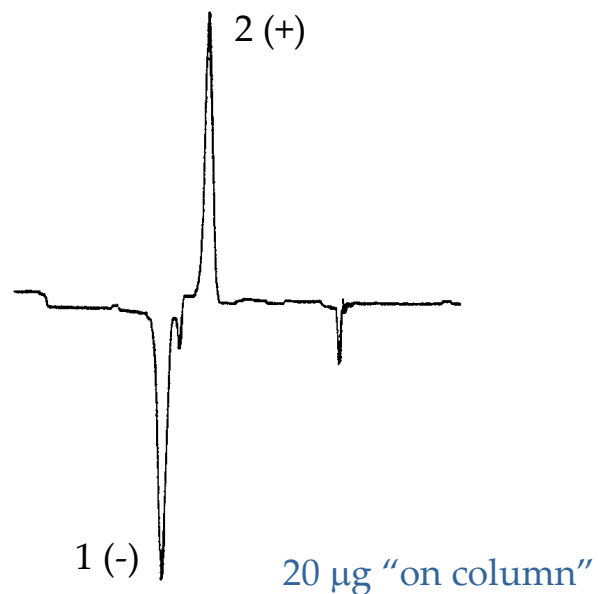
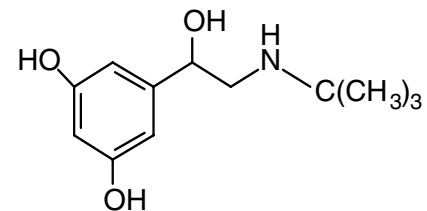
Optical Rotation of Amino Alcohol

Analyte: Terbutaline

Column: CHIROBIOTIC T, 250x4.6mm

Mobile Phase: 100/0.1%w: MeOH/ATFA

Flow Rate: 1.0 mL/min.



Determining Rotation of Analyte with No UV

Analyte: Carnitine

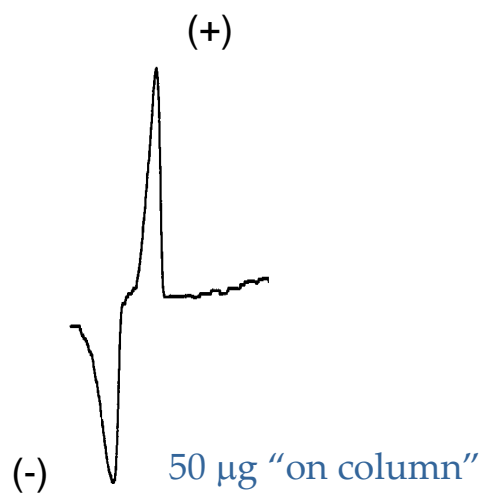


Column: CHIROBIOTIC TAG, 250x4.6mm

Mobile Phase: 85/15: MeOH/20mM NH₄OAC, pH 6.0

Flow Rate: 1.0 mL/min.

Polarimetry, unlike circular dichroism does not require that the analyte have a detectable UV wavelength. This makes it a very useful tool for detecting non-aromatic amino acids and other similar substances.





Conclusions

A stable, sensitive, economical and simple polarimetry device has been described with a 430nm 'blue' LED light source for optimal rotation determinations.

This device is a useful aide to chiral chromatography work by providing:

- The sign of rotation of chiral molecules with or without UV chromaphores.
- Information on any potential for a reversal of elution order.
- Information on the presence of enantiomeric pairs or diastereomers.
- Optical purity/enantiomeric excess.