Chiral Separation and Detection Enhancement of Propranolol Using Automated Pre-column Derivatization

LC

Varian Application Note
Number 3

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Introduction

Drugs with chiral centers exist naturally in racemic mixtures, that is, a mixture of nearly equal proportions of the enantiomers such that the mixture is optically inactive. Since enantiomers of drugs can have different therapeutic characteristics and effectiveness, the determination of enantiomeric purity has become increasingly important in the pharmaceutical industry.

Currently there are three different LC methods for chiral separations:

1. Converting enantiomers to diastereomers by pre-column derivatization with optically active reagents.
2. Using chiral mobile phase.
3. Using chiral stationary phase.

Of the three methods, the first one offers multiple advantages:

1. No special column or mobile phase required.
2. Micro-scale derivatization keeps cost low.
3. Additional benefit of detection enhancement.

The derivatization process is easily automated using the sample preparation features on the Star 9100 AutoSampler.

Methodology

In this application, Propranolol, existing in racemic form, is analyzed by pre-column derivatization with (+)-1-(9-Fluorenyl)ethyl chloroformate (FLEC), a pure enantiomer, which is fluorescent (Figure 1).

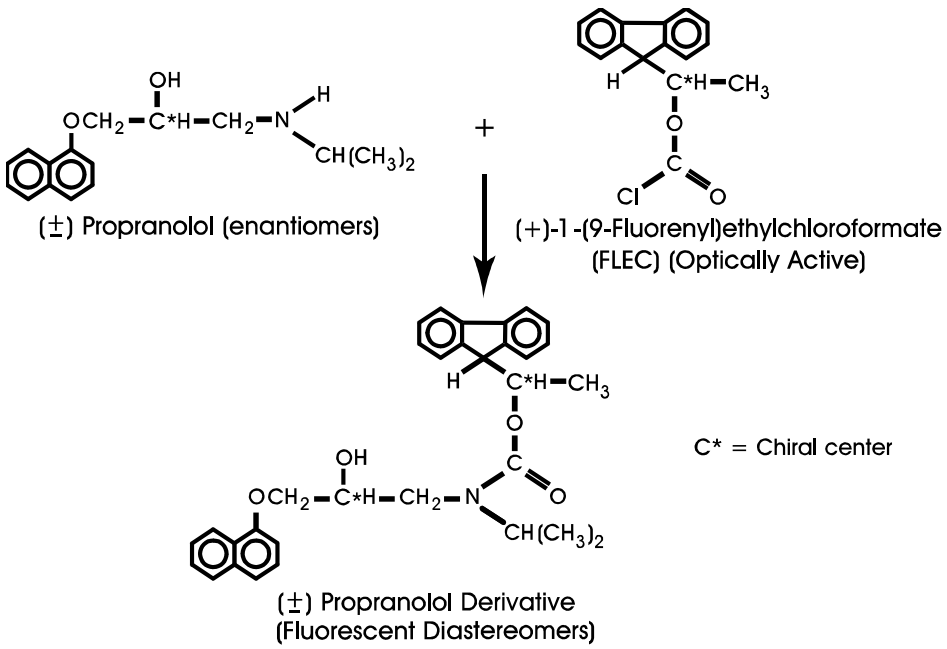


Figure 1. Analysis of (±) Propranolol Using Pre-column Derivatization (FLEC)

Three areas of accomplishment are:

1. Chiral separation.

2. Enhancement of detection sensitivity.
3. Enhancement of detection selectivity.

9100 AutoSampler Procedure

1. Transfer and mix FLEC with Propranolol sample.
2. Wait for programmed reaction time (2-5 minutes).
3. Inject.

HPLC Conditions

Column:	MicroPak SP C8 4 mm x 15 cm
Mobile Phase:	30:70::Acetate:Acetonitrile, 2 mL/min.
Detection:	Fluorescence, Ex. 265 nm, Em. 345 nm

Results

1. The enantiomers are well separated with no interference from the blank, as shown in Figure 2.
2. Using fluorescence, the response is at least 10 times that of UV absorbance. See Figure 3.
3. Fluorescence detection eliminates the interference detected by UV absorbance. See Figure 3.

Linearity

Range: 0 - 400 pmoles

Correlation coefficient for Derivative 1 = 0.99960

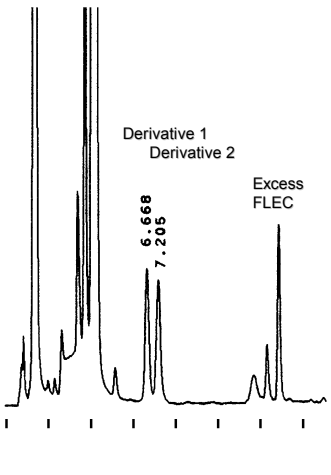
Correlation Coefficient for Derivative 2 = 0.99998

Reproducibility of 6 Runs at 200 picomoles

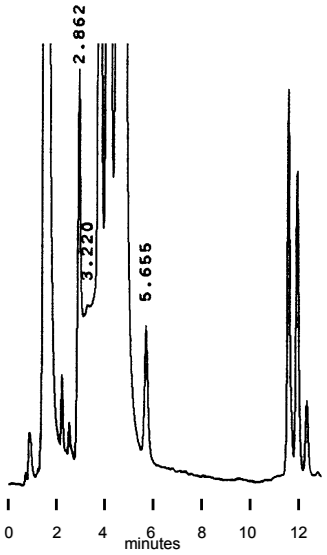
	Derivative 1	Derivative 2
Average	189.2 pmoles	191.2 pmoles
Std. Deviation	1.9 pmoles	2.3 pmoles
Rel. Std. Deviation	1.1%	1.8%

References

1. FLEC APPLICATIONS: *Resolution of Beta-blockers*, Eka Nobel AB, S-44501 Surte, Sweden. Telefax +4631-981954
2. S. Einarsson, B. Josefsson, P. Moeller, D. Sanchez, *Separation of amino acid enantiomers and chiral amines using precolumn derivatization with (+)-1-(9-Fluorenyl) ethyl chloroformate and reversed-phase liquid chromatography*, Anal. Chem., 1987, Vol. 59, No. 8, P.1191-5.
3. D. Sanchez, P. Moeller, S. Einarsson, B. Josefsson, *FLEC, a new development in chiral separation and the determination of amino acids, amines and alcohols*, Janssen Chimica Acta, 1988, Vol. 6, No. 1, P. 10-14.
4. Souter, R.W., *Chromatographic separation of stereoisomers*, CRC Press, 1985.



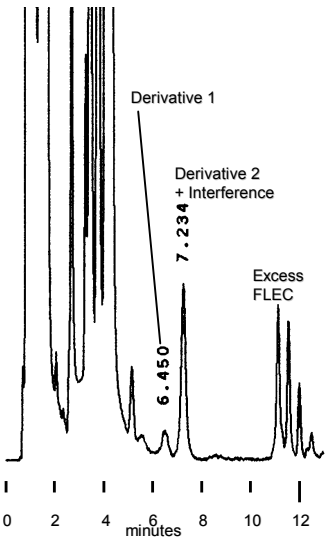
Chromatogram of FLEC - (±) Propranolol, 200 pmoles



Chromatogram of FLEC-Buffer (Blank)

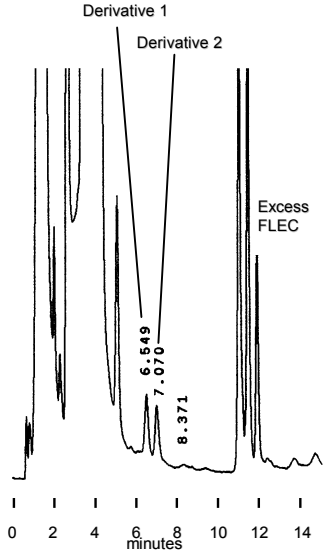
Detection = Fluorescence: Ex. 265 nm, Em. 345 nm
Mobile Phase: 30:70::Acetate:Acetonitrile

Figure 2. FLEC-Derivatization of (±) Propranolol



FLEC-Derivatized (±) Propranolol (100 pmoles) Using UV at 254 nm

Det. Limit = 10 pmoles



FLEC-Derivatized (±) Propranolol (100 pmoles) Using Fluorescence Ex. 265 nm, Em. 345 nm

Det. Limit = 1 pmole

Figure 3. Detection Enhancement Using Fluorescence vs. UV

These data represent typical results.
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