PHARMACEUTICAL *Application Briefs*

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Brief Number 1001

Chiral Analysis by Automated Online Precolumn Derivatization Using the Waters MilliLab Workstation/Autosampler

Highlights

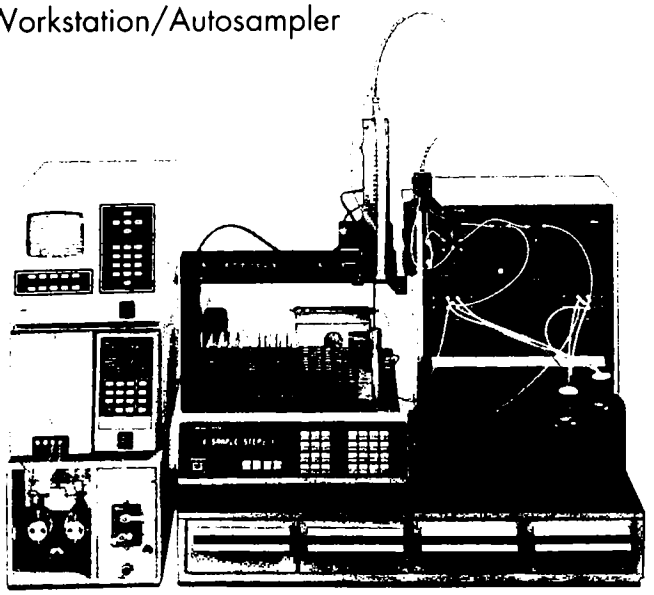
The determination of the enantiomeric purity of drug products is becoming an increasingly important analysis in the pharmaceutical laboratory. Guidelines are in the process of being established by the FDA, and in the European Economic Community, for the approval of racemates and pure stereoisomers. As an integral part of these guidelines, chiral liquid chromatographic (HPLC) methodologies will play a major role.

The separation of enantiomers poses a significant challenge because the physical and chemical properties of the two optical isomers are identical. Diastereomers, since they are not mirror images, have different physical properties and are therefore more easily separated.

There are currently three different modes of LC available for chiral analyses:

- Derivatization with optically active (homochiral) reagents
- Chiral mobile phase additives
- Chiral stationary phases

Precolumn derivatization has become a popular approach to the LC analysis of chiral compounds because it offers total flexibility in the selection of LC parameters, allowing one to exploit all of the advantages of conventional LC methods. Derivatization with optically active or homochiral reagents converts enantiomers into the corresponding diastereomers with different physical properties, allowing them to be separated using achiral chromatographic columns and mobile phases. This approach has several advantages in addition to the use of conventional columns and mobile phases, including reduced expense, improved detection properties of the analyte (such as sensitivity), and the potential for automation.



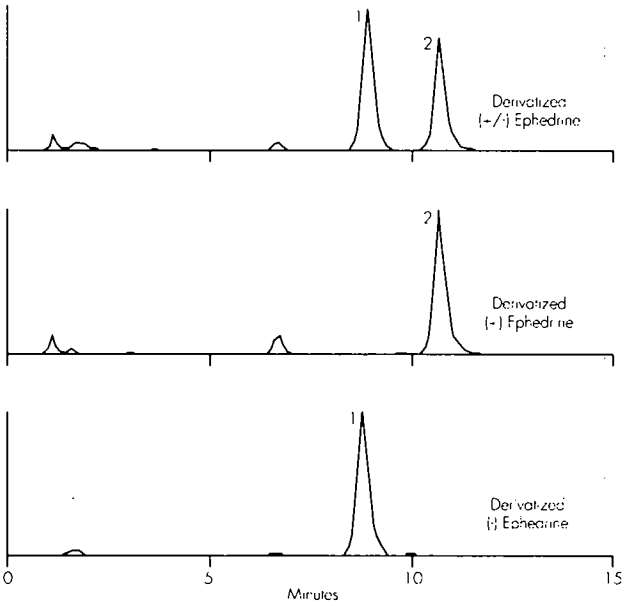
The Waters MilliLab Workstation/Autosampler (right) configured as part of an LC system (left).

In spite of these advantages, derivatization approaches are all too often avoided, due to the additional sample and reagent handling involved, as well as the potential for errors in accuracy and precision. In order to address some of these concerns, this application brief describes the use of Waters MilliLab™ Workstation as an automated, online, precolumn derivatizer/autosampler for the analysis of chiral compounds.

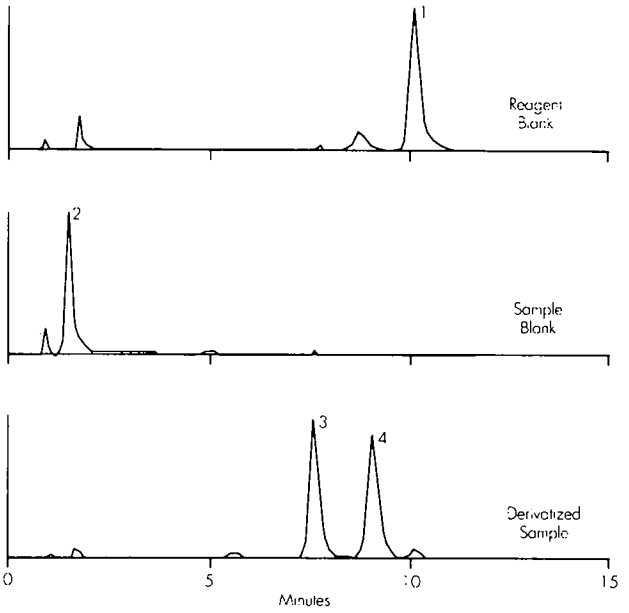
The Waters MilliLab Workstation automates precolumn derivatization to yield reproducible results at a lower cost than either manual methods or more complex robotic systems can provide. When equipped with the LC autoinjector option, the Workstation becomes a fully automated online precolumn derivatizer that also functions as an autosampler. In addition, the Workstation can also perform a broad range of automated sample preparation tasks including filtration, solid phase extraction, liquid/liquid extraction, evaporation, single and serial dilution, mixing, and gas purging to accommodate the wide range of demanding sample matrices often encountered in pharmaceutical chiral analyses.

Operating Conditions

Sample: (+/-) Ephedrine, 5 mg/mL in acetonitrile
Reagent: 2,3,4,6-tetra-O-acetyl-b-D-glucopyransylisothiocyanate (GITC), 60 mg/mL in acetonitrile
Column: Nova Pak C18 (3.9 x 150 mm), ambient temperature
Mobile Phase: 65/35 20mM monobasic ammonium phosphate/acetonitrile, 1 mL/min.
Detection: UV at 254 nm
Derivatization Procedure Reference: J. Gal, J. of Chrom., 307, 220 (1987) The reagent is transferred, mixed, and a delay time is programmed to allow the reaction to take place. Diluent is then transferred, mixed, and the resulting product mixture is injected. All transfers, mixing, etc. for both sample and blanks were accomplished using the Workstation in an unattended online, automated method.
Injection: MilliLab Workstation, 20 µL



These three chromatograms were used to assign the elution order of the derivatized enantiomers in the racemic mixture. From top to bottom, a separation of a derivatized racemic mixture of (+/-) Ephedrine, the (+) isomer derivative, and the (-) isomer derivative. Peak identities are: 1) derivatized (-) Ephedrine, and 2) derivatized (+) Ephedrine.



The above three chromatograms illustrate, from top to bottom, separation of a reagent blank, a sample blank, and a derivatized (+/-) Ephedrine sample. The sample blank and reagent blank samples were generated under the same conditions as those used to carry out the derivatization. Peak identities are: 1) GITC reagent, 2) underivatized (+/-) Ephedrine, 3) derivatized (-) Ephedrine, and 4) derivatized (+) Ephedrine.

Results

The series of chromatograms were obtained using the MilliLab Workstation in the automated online mode both as an auto-derivatizer, and as a sample injector. These results correlate exactly with those reported in the literature (see reference). Reproducibility of each of the derivatives formed for six separate derivatizations was evaluated and found to be less than 1%, yielding equivalent results obtained by off-line manual methods. Automation of the method results in higher sample throughput, less opportunity for error, and reduced cost due to reagent and solvent conservation, as well as unattended operation. Although this brief outlines the adaptation of an existing manual method, a key feature of the Workstation is the ability to perform methods development by linking different methods together. This allows the chemist to optimize such reaction parameters as volume, time and temperature.