

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

D, L-Amino Acid Analysis System

Testing of optical purity is important in pharmaceutical industry, biotechnology-related industry, food industry, clinical chemistry industry, and many other fields. With respect to amino acid, inasmuch as physiological effect of synthesized peptides and the derivatives are much affected by the optical purity particularly, optical separation of the amino acids forming the peptides and the derivatives is important.

Separation of optical isomers by HPLC is possible by three methods, chiral solid phase method, chiral mobile phase method, and chiral derivatization method; here in this article, separation by the chiral derivatization method is introduced.

For chiral derivatization method, OPA *N*-acetylcysteine reagent⁽¹⁾ is used. It is a high-sensitivity analytical method⁽²⁾ that permits automatic derivatization and analysis only by setting the sample solution in the SIL-10A auto sampler. Shown in Fig.1 is the process of automatic derivatization.

Shown in Fig.2 is a chromatogram of D, L-amino acid standard solution by the above method.

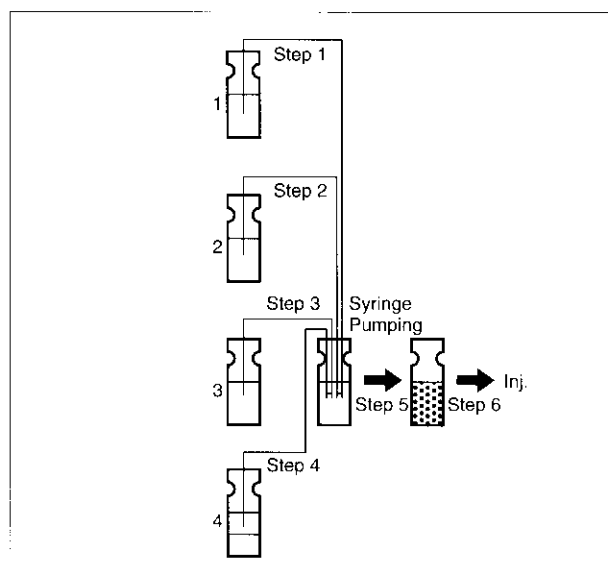


Fig.1 Automatic Derivatization

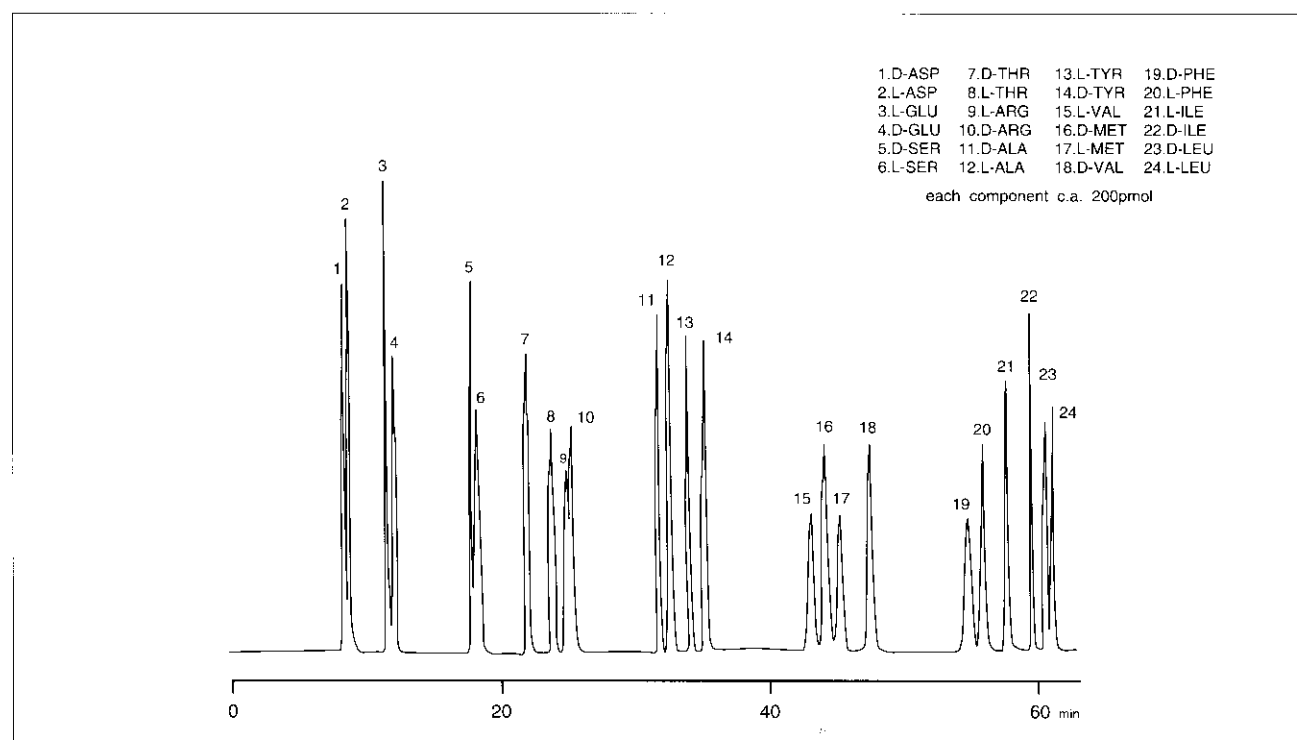


Fig.2 Chromatogram of Standard Solution

Shown in Fig.3 is the chiral derivatization, and shown in Fig.4 is the procedure for derivatization.

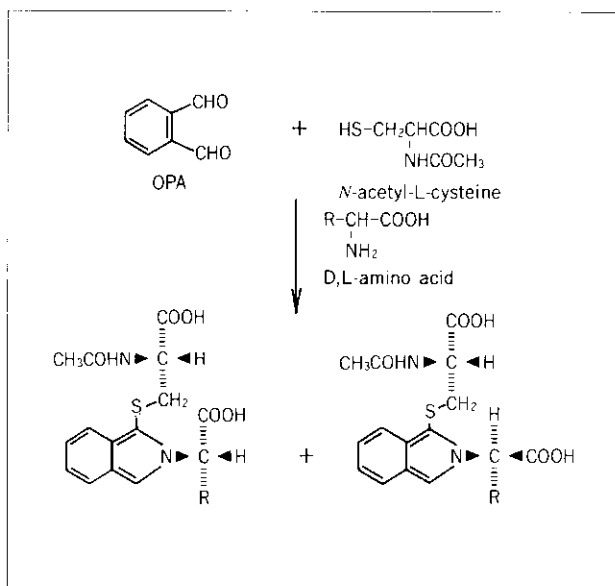


Fig.3 Chiral Derivatization

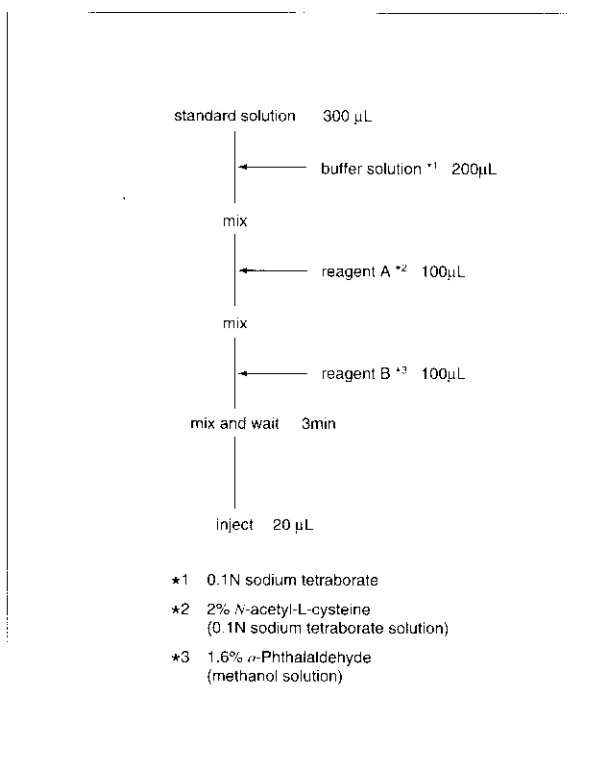


Fig.4 Derivatization Procedure

The analytical conditions are shown in Table 1. Setting of gradient program was carried out by using the software for optimizing the conditions of separation, DryLab/Windows, available from Shimadzu. By using this program, good analytical conditions can be found out speedily.

Table 1 Analytical Conditions

Instrument	: Shimadzu LC-10A system		
Column	: Develosil ODS-UG-5 (6mm I.D. X 200mm L.) with guard column (6mm I.D. X 35mm L.)		
Precolumn	: Shim-pack GRD-ODS (4mm I.D. X 250mm L.)		
Column temp.	: 35°C		
Mobile Phase	: A ; 50mm Sodium Acetate B ; Methanol Gradient elution Initial condition B 0%		
Flow rate	: 1.2mL/min		
Detection	: RF-550 Ex=350nm Em=450nm		
Gradient program	TIME	FUNCTION	VALUE
	16	B CONC	24
	24	B CONC	24
	29	B CONC	40
	50	B CONC	40
	59	B CONC	67
	59.01	B CONC	80
	64	B CONC	80
	64.01	B CONC	0
	65	STOP	