

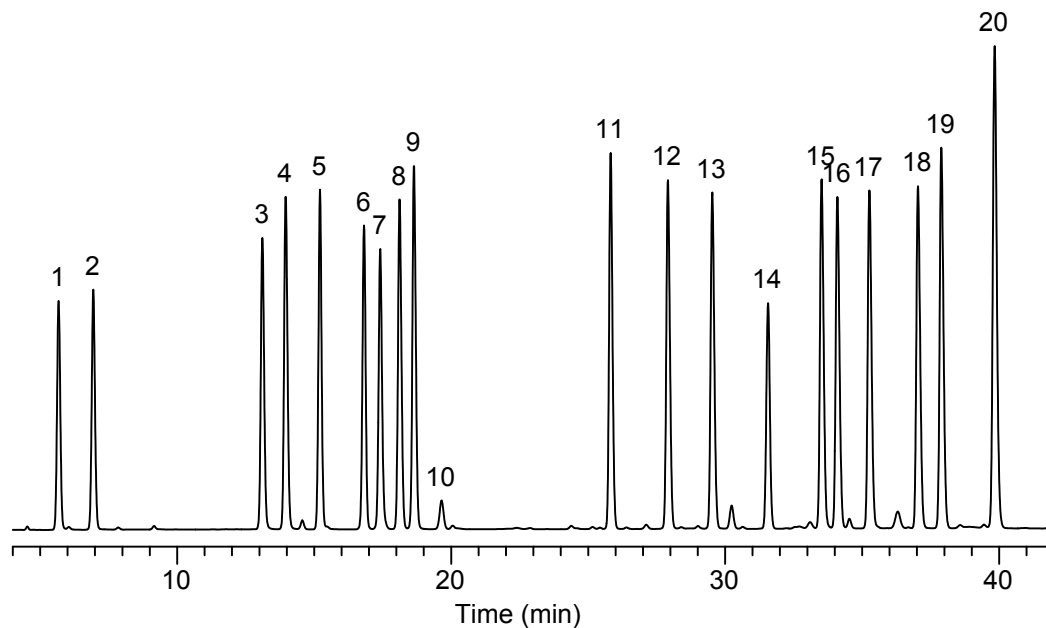
**Instructions of 18 Natural Amino Acids Analysis with HPLC  
by Phenylisothiocyanate (PITC) Pre-column Derivatization Method**

Column:	Diamonsil AAA 250×4.6 mm , 5 μm ( Cat.#: 99751 )
Mobile Phase A:	0.05 mol/L sodium acetate (adjusted to pH=6.50±0.05 with glacial acetic acid)
Mobile Phase B:	Methanol : Acetonitrile : H <sub>2</sub> O=20 : 60 : 20(v/v/v)
Flow Rate:	1.0 mL/min
Detector:	UV 254 nm
Column Temp:	45 °C
Injection Volume:	10 μL
Sample Solution:	Pipetted 200 μL mixed solution(19 Natural Amino Acids +NH <sub>3</sub> ; concentration of Amino Acids is 2.0 mmol/L, excepted concentration of Cystine is 1.0 mmol/L) and derivatization solution(100 μL 1 mol/L triethylamine-ACN solution+100 μL 0.2 mol/L PITC-ACN solution) into a 1.5 mL centrifuge tube, mixed thoroughly and reacted for 1 h at ambient temperature. Then added 400 μL n-hexane and shaken gently for 5-10 s, rested for a while, pipetted 200 μL lower solution and mixed with 800 μL H <sub>2</sub> O, filtered with 0.22 μm syringe filter.

Gradient Procedures

Time/min	0	39	40	45	46	60
A/%	95	52	0	0	95	95
B/%	5	48	100	100	5	5

## HPLC Chromatogram for 18 Natural Amino Acids, Norleucine and other compounds



1 Aspartate	2 Glutamate	3 Serine	4 Glycine
5 Histidine	6 Arginine	7 Threonine	8 Alanine
9 Proline	10 NH <sub>3</sub>	11 Tyrosine	12 Valine
13 Methionine	14 Cystine	15 Isoleucine Ile	16 Leucine
17 Norleucine	18 Phenylalanine	19 Tryptophan	20 Lysine

### Notes

#### 1 Pretreatment

Firstly, successively using 60 mL 15% methanol aqueous and methanol to wash the HPLC system with flow rate 0.5-1.0 mL/min.

Secondly, connecting the Diamonsil AAA column with HPLC system, then, respectively using 30 mL methanol and 15 mL 15% methanol aqueous to wash the whole system with flow rate 0.5-1.0 mL/min.

Thirdly, balancing the whole system with mobile phase until the baseline is stable.

2 After working, respectively using 30 mL 15% methanol aqueous and methanol to wash the whole system with flow rate 0.5-1.0 mL/min.

3 Must keep room temperature at 25 °C.