Chromai Pre-column Automatic Derivatization Amino Acid Analysis Application

As the basic unit of proteins, amino acids are essential substances for human physiological functions. The detection of amino acids is of great significance in pharmaceuticals, food, and environmental industries. In laboratory liquid-phase analysis of amino acids, since most amino acids are highly polar and lack ultraviolet absorption, derivatization is usually required prior to analysis to improve detection sensitivity and separation selectivity.

The *Chinese Pharmacopoeia* (2020 Edition) First Supplement newly includes the "9120 Amino Acid Analysis Guiding Principle", which incorporates four pre-column derivatization methods. Among them, PITC, AQC, and DNFB derivatization require manual operation with cumbersome procedures. The OPA and FMOC amino acid derivatization assay can realize automatic derivatization in an autosampler, where OPA derivatizes primary amino acids and FMOC derivatizes secondary amino acids. This method adopts dual-wavelength detection: the derivative products of primary amino acids are detected at 338 nm, and the derivative product of the secondary amino acid (proline) is detected at 262 nm.

This method uses OPA and FMOC derivatization reagents combined with the online derivatization function of the Chromai autosampler, enabling automated online derivatization of amino acids and greatly improving the efficiency of amino acid analysis and detection.

1. Instrument System

Chromai Leaps system (quaternary pump P40, autosampler A10CV, column oven C10, UV detector D10)

2. Standard Solution

Amino acid mixed standard solution (Aspartic acid, Glutamic acid, Serine, Histidine, Glycine, Threonine, Arginine, Alanine, Valine, Methionine, Phenylalanine, Isoleucine, Leucine, Lysine, Proline)

3. Derivatization Reagents

- **OPA**: Weigh 40 mg of o-phthalaldehyde, add 3.5 mL of borate buffer, 0.5 mL of acetonitrile, and 64 μL of 3-mercaptopropionic acid, dissolve and mix well.
- **FMOC**: Weigh 40 mg of FMOC, add 8 mL of acetonitrile, dissolve and mix well.

4. Chromatographic Conditions

- **Mobile Phase A**: 0.05 mol/L sodium acetate solution - triethylamine - tetrahydrofuran (950:0.14:5, adjusted to pH 6.8 with 2% acetic acid)
- **Mobile Phase B**: 0.16 mol/L sodium acetate solution (adjusted to pH 6.8 with 2% acetic acid) - methanol - acetonitrile (200:400:400)
- **Elution Gradient**: 0–27 min: 92%–40% A; 27–31 min: 40%–0% A; 31–38 min: 0% A; 38.01–41 min: 92% A
- **Column**: Chromai C18 (4.6 × 250 mm, 5 μm)
- **Flow Rate**: 1 mL/min
- **Column Temperature**: 40 ℃
- **Injection Volume**: 20 μL
- **Detection Wavelength**: 338 nm (0–27 min); 262 nm (27–41 min)

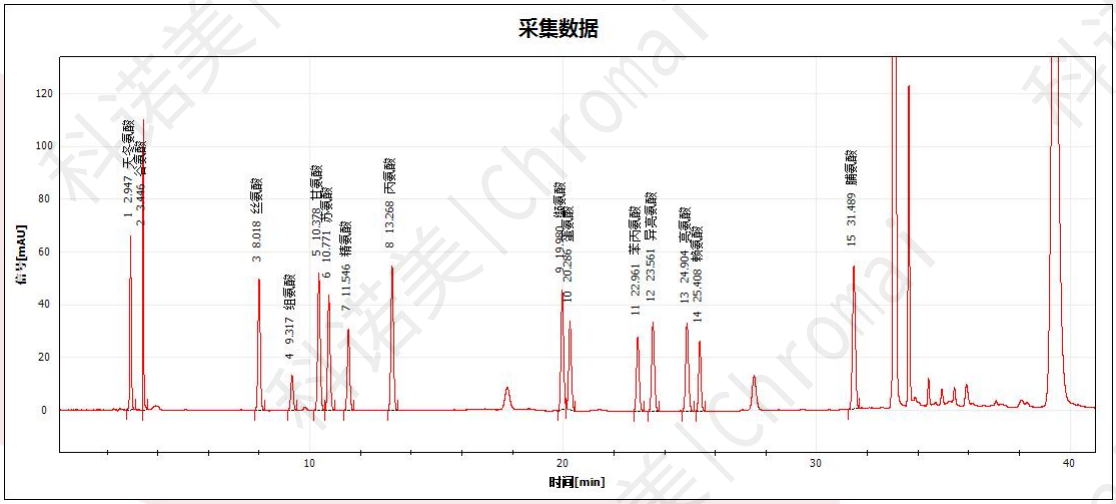
Autosampler Pre-column Derivatization Program

Step	Operation
1	Aspirate 40 μL of borate buffer
2	Aspirate 10 μL of sample solution
3	Aspirate 10 μL of OPA derivatization solution
4	Mix 5 times in the sample vial
5	Aspirate 10 μL of FMOC derivatization solution
6	Mix 5 times in the sample vial
7	Aspirate 120 μL of diluent
8	Mix 10 times in the sample vial

5. Results and Discussion

As shown in Figure 1, after derivatization with OPA and FMOC, all 15 amino acids are well separated.

Figure 1 Chromatogram of amino acid standard solution by pre-column automatic derivatization



Standard Curve and Repeatability

All 15 amino acids show good linearity in the concentration range of 5 - 100 μg/mL, with linear correlation coefficients all greater than 0.999.

At a concentration of 100 μg/mL, six replicate injections were performed. The repeatability of peak area and retention time is shown in Table 1 and Table 2.

Table 1 Linearity results of amino acid standard curves

Component	Linear Equation	r^2
Aspartic acid	y = 2.6486x - 1.5526	0.9999
Glutamic acid	y = 2.1444x - 4.2082	0.9996
Serine	y = 3.0669x - 0.9696	0.9999
Histidine	y = 0.8647x - 0.3588	0.9999
Glycine	y = 3.5035x - 4.1195	0.9998
Threonine	y = 2.7598x - 0.8775	1.0000

Component	Linear Equation	r^2
Arginine	y = 1.9488x - 0.8450	0.9999
Alanine	y = 3.8840x - 1.5092	0.9999
Valine	y = 2.9139x - 0.6303	1.0000
Methionine	y = 2.2942x - 0.3111	0.9999
Phenylalanine	y = 2.0334x - 0.0421	1.0000
Isoleucine	y = 2.5605x - 1.2149	0.9999
Leucine	y = 2.6490x - 0.3133	0.9999
Lysine	y = 1.8708x - 4.7725	0.9996
Proline	y = 3.9281x + 15.2049	0.9997

Table 2 Repeatability results of retention time and peak area of amino acids (100 μg/mL)

Component	Retention Time RSD (%)	Peak Area (mAU·s) RSD (%)
Aspartic acid	0.06	1.36
Glutamic acid	0.08	1.12
Serine	0.03	1.26
Histidine	0.03	1.43
Glycine	0.03	0.96
Threonine	0.02	1.24
Arginine	0.04	1.27
Alanine	0.03	1.18
Valine	0.01	1.23
Methionine	0.01	1.23
Phenylalanine	0.01	1.39
Isoleucine	0.01	1.08
Leucine	0.01	1.20
Lysine	0.01	1.98
Proline	0.04	4.14

6. Conclusion

Using the pre-column derivatization function equipped with the autosampler of the Chromai Leaps system and selecting OPA and FMOC derivatization reagents, automated online derivatization of primary and secondary amino acids can be achieved. In this experiment, all 15 amino acids are completely separated, with excellent linearity and repeatability, significantly improving the accuracy and detection efficiency of amino acid analysis.