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High Performance Liquid Chromatograph LC-2050C 3D/ RF-20Axs

Application News

Analysis of Methionine Sulfone and Cysteic Acid Using Automated Pretreatment Functions

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User Benefits

- This method can provide significantly short analysis time compared to the post-column derivatization method generally used for amino acid analysis.
- This method without manual pretreatment is easy to use because complicated derivatization process is well automated.
- Twenty proteinogenic amino acids[1)] can be analyzed as well using the same column and the derivatization reagents as this method.

■ Introduction

Hydrochloric acid hydrolysis is commonly used as a pretreatment when analyzing amino acids. However, sulfur-containing amino acids such as methionine, cysteine, and cystine are oxidized in the process. For accurate determination of these amino acids, methionine and cysteine/cysteine should be oxidized preliminarily to methionine sulfone and cysteic acid respectively by performic acid treatment, followed by hydrolysis procedures to determine methionine sulfone and cysteic acid.

The post-column derivatization method is often used for the analysis of methionine sulfone and cysteic acid. However, this method is not suitable for high-speed analysis due to cation exchange column. On the other hand, the pre-column derivatization method for amino acids provides very easy analytical operation because of automatic derivatization of the complicated process.

This article introduces the analysis of methionine sulfone and cysteic acid using the automatic pretreatment function of LC-2050C integrated high-performance liquid chromatograph.

■ Automatic pre-column derivatization

LC-2050C is equipped with the automatic pretreatment function as standard, which can be set to execute desired operation such as sample dilution and reagent addition. Table 1 shows how to prepare derivatization reagents for this derivatization procedure. After adding the reagents listed in Table 2 into vials and locating them to the designated position numbers, necessary settings were input as Fig. 1 to mix the sample and derivatization reagents within the needle of the autosampler. The derivatization reaction can be performed automatically within the needle by the above-mentioned settings.

Table 1 How to prepare derivatization reagents

● 0.1 mol/L Borate buffer Add 0.62 g of boric acid and 0.20 g of sodium hydroxide into 100 mL of ultrapure water.
●Mercaptopropionic acid Reagent(MPA Reagent) Add 10 µL of 3-mercaptopropionic acid into 10 mL of 0.1 mol/L borate buffer.
●OPA Reagent Add 0.3 mL of ethanol into 10 mg of *o*-phthalaldehyde and dissolve completely. Then add 0.7 mL of 0.1 mol/L borate buffer and 4 mL of ultrapure water.
●MPA / OPA Solution Mix 300 µL of MPA Reagent and 600 µL OPA Reagent.
●Phosphoric acid aqueous solution Add 0.5 mL of phosphoric acid into 100 mL of ultrapure water.

Table 2 Allocations and addition volumes of derivatization reagents

Vial	Reagent	Addition volume (µL)
52	MPA/OPA Solution	15
53	Acetonitrile	0.5
54	Phosphoric acid solution	5

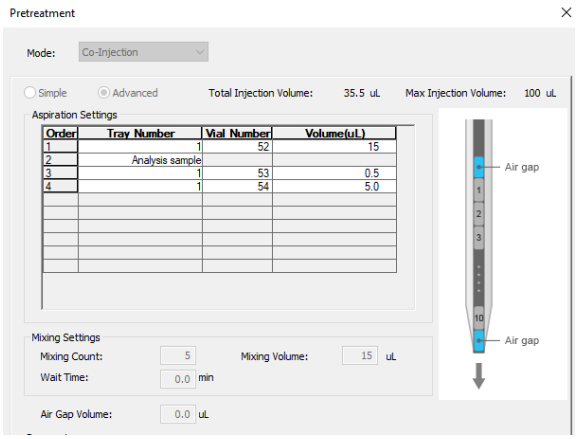

Fig. 1 Pretreatment setting screen

■ Analysis of standard solution

Fig. 2 shows a chromatogram of standard solutions of methionine sulfone and cysteic acid. The HPLC analysis of methionine sulfone and cysteic acid can be done in 12 min including the automatic pre-treatment procedure. Analytical conditions are shown in Table 3.

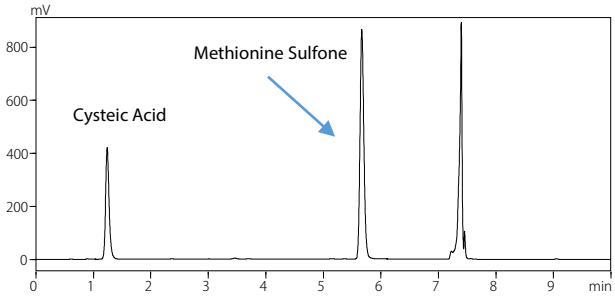

Fig. 2 Chromatogram of standard solution
(25 µmol/L for each compound)

Table 3 Analytical conditions

System	: LC-2050C*1
Column	: Shim-pack ™ XR-ODSII*2 100 mm × 3.0 mm I.D., 2.2 µm
Mode	: Low pressure gradient
Mobile phase	: A) 20 mmol/L (Sodium) citrate buffer (pH 4.6) B) Water/Acetonitrile = 1:9
Flow rate	: 1.0 mL/min
Time program	: B.Conc. 15% (0 min) → 22% (5~6 min) → 100% (6.01~8 min) → 15% (8.01~10 min)
Column temp.	: 40 ℃
Injection volume	: 1 µL
Sample cooler	: 4 ℃
Detection	: Fluorescence detector (Cell temp. : 25 ℃) : Ex. 350 nm, Em. 450 nm
Vial	: SHIMADZU LabTotal™ for LC 1.5 mL, Glass*3

*1 : Applicable for LC-2050C 3D
*2 : P/N 228-41624-92
*3 : P/N 227-34001-01

■ Linearity

The linearities (coefficients of determination, r2) over the concentration ranges of 2.5, 10, 25, 50, and 100 µmol/L for methionine sulfone and cysteic acid were both more than 0.999. Related results are shown in Table 4 and Fig. 3.

Table 4 Coefficients of determination

Compound	Linearity (r^2)
Cysteic Acid	0.9999
Methionine Sulfone	1.0000

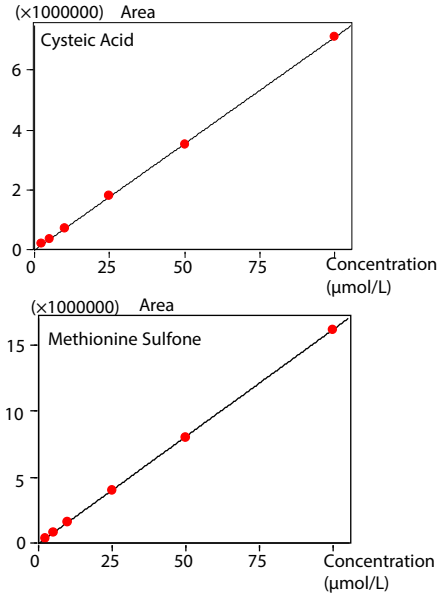

Fig. 3 Calibration curves
(Upper : Cysteic Acid Lower : Methionine Sulfone)

■ Real sample analysis

A livestock feed was subjected to HPLC analysis after performic acid treatment and hydrochloric acid hydrolysis as shown in Fig. 4. The chromatogram of the livestock feed is shown in Fig. 5, and the quantitative results are shown in Table 5. Nine consecutive analyses were performed for evaluating peak area standard deviations as repeatability test. Obtained results are shown in Table 6.

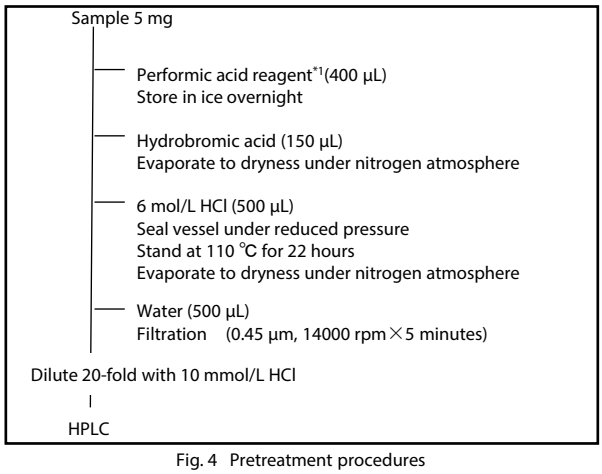

Fig. 4 Pretreatment procedures

*1: Mix 9 mL of formic acid and 1 mL of 30% hydrogen peroxide. Stand at room temperature for 1 hour.

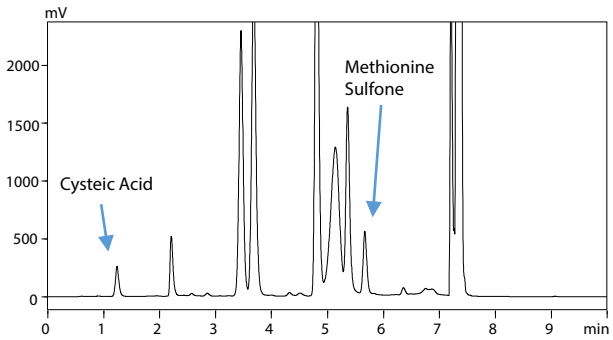

Fig. 5 Chromatogram of livestock feed

Table 5 Determination results

	Concentrations in sample solution (µmol/L)	Content rates (µmol/g)
Cysteic Acid	15.9	30.6
Methionine Sulfone	15.4	29.6

Table 6 Peak area repeatabilities (%RSD, n=9)

Compound	Livestock Feed
Cysteic Acid	1.08
Methionine Sulfone	0.61

■ Conclusion

This article presents the analysis of methionine sulfone and cysteic acid using the automated pretreatment function. Manual derivatization is not required, and one analysis can be performed in only 12 min using this pretreatment function. LC-2050C used for this analysis can also be applied for the analysis of the twenty proteinogenic amino acids[1]. Consequently, improved instrument availability and a wide range of sample applicability can be expected.

<Reference>
1) Analysis of Amino Acids in Foods Using Automatic Pretreatment Function of Integrated HPLC Application News No.01-00028-EN

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