AccuTOF™ LC/MS

Determination of Phenylalanine Isotope Ratio Enrichment by LC/Time-of-Flight Mass Spectrometry

Zhanpin Wu[1], Xiao-Jun Zhang[2], Robert B. Cody[1], and Robert R. Wolfe[2]
[1]Department of Analytical Instruments, JEOL USA, Inc.
[2]Metabolism Unit, Shriners Burns Institute, University of Texas Medical Branch at Galveston

Introduction

Time-of-flight mass spectrometry has the advantages of high resolution, high sensitivity, and high mass accuracy but with relatively narrow dynamic range if a TDC (Time-to-Digital Converter) is used as a data acquisition system. This disadvantage of narrow dynamic range hinders the applications for isotope ratio enrichment measurement. Recently, a new LC/TOF-MS system was introduced that achieved a wide dynamic range by using an ADC (Analog-to-Digital Converter) instead of a conventional TDC. We used this system to evaluate the measurement of phenylalanine (Phe) isotope ratio enrichment. The accuracy, reproducibility, and sensitivity for the method were determined. The method is simple, rapid, and accurate and presents an attractive alternative to traditional GC/MS applications.

Experimental

The system included an Agilent 1100 HPLC and a JEOL AccuTOF™ time-of-flight mass spectrometry system. Complete system control and data evaluation were carried out using a JEOL MassCenter™ workstation.

All solvents used were of HPLC grade. The standard solutions were prepared by mixing unlabelled Phe and $^{13}C_6$-Phe in 0.1 % formic acid solution to make solutions with isotope ratio enrichments ranging from 0.02 % to 9.2 % and a constant total concentration of 100 nmol/mL. The isotope ratio enrichment was expressed as tracer/tracee ratio (TTR).

Chromatographic Conditions		**MS Conditions**	
Column:	Luna C_{18} (2), 5µm, 2.0 x 150 – mm	Source:	ESI
		Ionization mode:	positive
Mobile phase:	A = 0.1 formic acid in water	Resolving power:	5,000 (FWHM) at m/z 166
	B = acetonitrile	Needle voltage:	2200 V
Gradient:	Start with 100% A to 12% B in 12 minutes.	Orifice 1 voltage:	35 V
		Orifice 1 temp.	80 °C
Flow rate:	0.2 mL/min	MCP voltage:	2700V
Injection volume:	10 µL	Desolvating gas:	2.5 L/min
		Desolvating chamber:	200 °C
		Nebulizing gas:	1.0 L/min

Results and Discussion

The system was tuned by using Phe standard solution in order to achieve high sensitivity and high resolution. Figure 1 shows the mass chromatograms with different resolving powers for a low level (0.07%) deuterium-enriched sample of Phe extracted from rabbit muscle protein. The S/N increased from 22 for the low-resolution mass chromatogram to 36.9 for the high-resolution mass chromatogram.

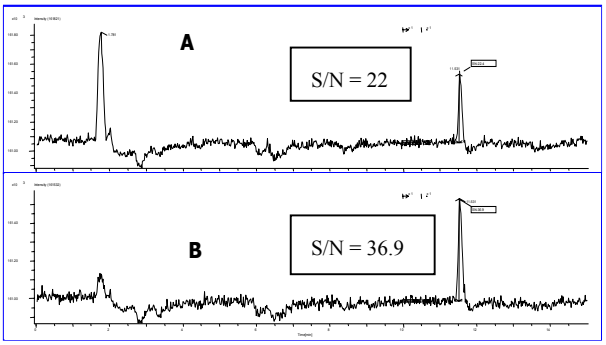

Figure 1. Mass chromatograms for a low level (0.07%) d_5-Phe extracted from rabbit muscle protein. (A) low resolution for m/z 171+/- 0.5; (B) high resolution for m/z 117.118 +/- 0.03

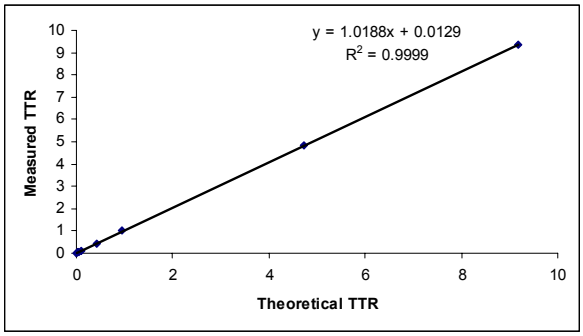

Figure 2. Relation between the theoretical ratios and the measured ratios.

The accuracy and precision of the isotope ratio measurement was determined by running standard $^{13}C_6$-Phe solution with the concentration of 100 nmol/mL and the isotope ratios ranging from 0.02% to 9.2%. Figure 2 shows the relation between the theoretical ratios and the measured ratios. The linear correlation R^2 of 0.9989, the slope of 1.0861, and the y-intercept of 0.0918 indicate excellent precision and accuracy. The enrichment detection limit is 0.05%.

The reproducibility was determined by running the standard $^{13}C_6$-Phe solution with 0.94% enrichment 5 times. The coefficient of variation was 1.9%.

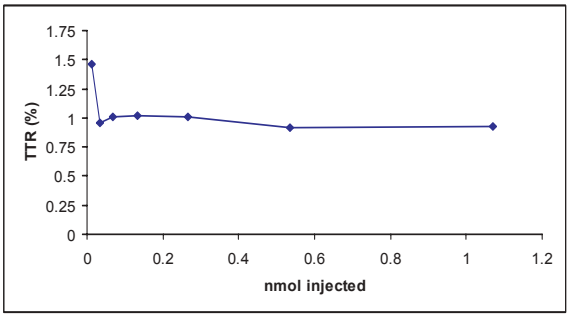

Figure 3. Relation between the injected amount and isotope ratio measurement

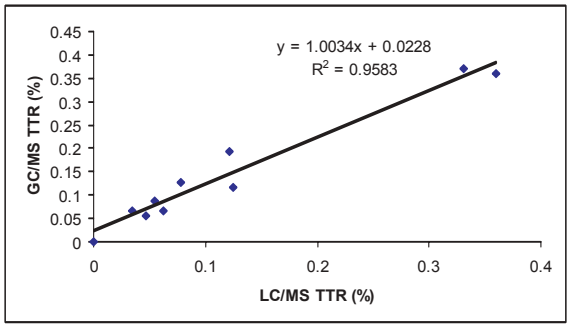

Figure 4. Correlation between measurements of Phe enrichment by LC/MS and GC/MS.

The dose dependency was determined by running standard $^{13}C_6$-Phe solutions with 0.94% enrichment and the concentration ranging from 1.1 nmol/mL to 106.0 nmol/mL. In Figure 3, the relation between the injected amount and the isotopic ratio is shown. The lowest concentration required to get an accurate enrichment measurement is 3 nmol/mL.

A comparison of Phe enrichment obtained by this method with the values obtained by traditional GC/MS is shown in Figure 4 for rabbit muscle protein after an isotope tracer infusion experiment. There was excellent agreement between the two methods.

Conclusion

An LC/TOF-MS system with an ADC as the data acquisition system can be used to measure isotope ratio enrichment. The method is simple, rapid, and accurate and presents an attractive alternative to traditional GC/MS applications.