GC/MS Application Note

Number 72
April 2003

Quantitation of Orotic Acid in Urine Samples by Stable Isotope Dilution uSIS Mass Spectrometry: Diagnostic and Exploration of OCT Deficiency

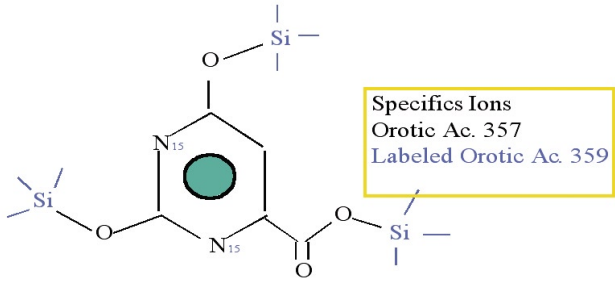
G. Briand[1,2], M. Fontaine[1], F. Hottevart[1], D. Dillies[1], D. Dobbelaere[3], L. Vallee[4], N. Porchet[1] and M. Lesieur[5]

[1]Laboratoire de Biochimie et Biologie Moléculaire, Hôpital C. Huriez, CHRU, Lille, France, [2]Service Commun de Spectrométrie de Masse de Lille, Faculté de Médecine, Lille, France, [3]Unité des Maladies Métaboliques, Clinique de Pédiatrie, Hôpital Jeanne de Flandres, CHRU, Lille, France, [4]Service des Maladies Infectieuses et de Neurologie Infantile, Hôpital R. Salengro, CHRU, Lille, France, [5]Varian Analytical Instruments, France

Introduction

Medical biology uses mass spectrometric techniques ever more frequently, particularly for biochemical investigations of metabolism. We will show here, a characteristic example, the quantitation of the orotic acid, which is an intermediate metabolite in the synthesis of pyrimidic nucleotides. Orotic acid is only present in trace amounts in normal human urine but its excretion can increase significantly in some pathologies such as the ornithine carbamyltransferase (OCT) deficiency, a urea cycle anomaly. The urea cycle is the body's main system for removing waste nitrogen produced by the metabolism of proteins. Only the nitrogen atoms derived from ammonia and aspartate are destined for urea and thus identified as waste nitrogen atoms. The manifestations of a reduction or failure in urea synthesis are: hyperammonaemia, urea cycle substrate accumulation, and urinary orotate excretion. A sensitive method for detecting even mild increases orotic acid is clinically important.

Tri-TMS Acid Orodic Structure



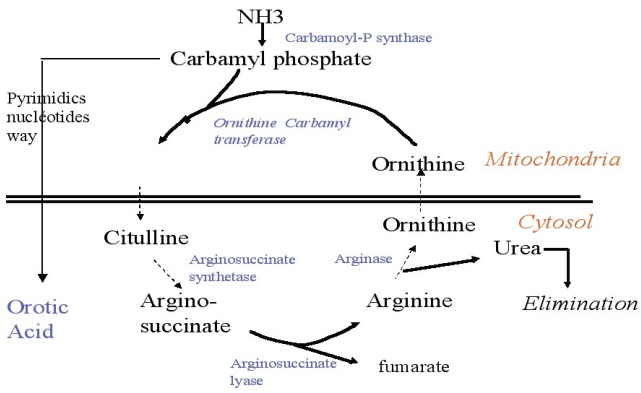
Molecular Weight 372 (N^{14}), 374 (N^{15})

Experimental

The method employs stable isotope dilution with 1-3 [^{15}N] orotic acid and analysis by gas chromatography – mass spectrometry. A Varian 3800 GC is coupled to a Saturn 2200 in electronic impact ionization mode; the autosampler is a CP-8400. This technique replaces a colorimetric method that may give false positive results.

1-3 [^{15}N] orotic acid (7 µg) is added to the urine (1 mL) as the internal standard. The organic acids are extracted three times with 2 mL ethyl acetate. After drying, derivatization to form trimethylsilyl (TMS) derivatives of the organic acids is accomplished with 100 mL of BSTFA/TMCS in chloroform for 45 min at 80 °C.

Tri-TMS Acid Orodic Structure



Gas Chromatograph

Column	Varian CP-SIL8 CB Low Bleed/MS 30m x 0.25 mm x 0.25 µm (Varian Part No. CP5860)
Flow Rate	1 mL/min
Oven Program	40 °C no hold, 30 °C/min to 70 °C, hold 6 min, 5 °C/min to 280 °C
Injection Temperature	250 °C
Injection Mode	Splitless
Injection Volume	0.5 µL
Injection Speed	2.5 µL/sec
Transfer Line	280 °C
Ion Trap	200 °C

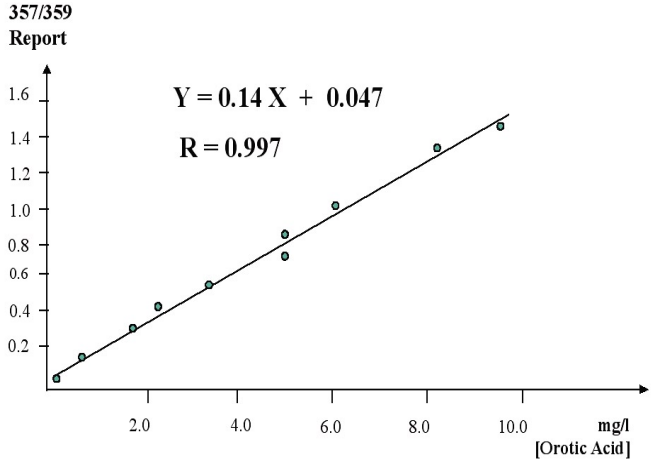
Mass Spectrometer

Ionization Mode	EI
Ion Preparation Technique	uSIS
Target	5000
Prescan Ionization Time	1500µs
Mass Range	350-365 m/z
Isolated Ions	357.1 m/z and 359.1m/z
Isolation Window	1
Ionization Storage Level	156u and 157u
Isolation Time	5 ms
Ejection Amplitude	20V
Scan Time	0.6 sec
Segment Start Time	20 min
End Time	54 min
Filament	80 µA
Multiplier	+300V
Threshold	0
Mass Defect	0
Background Mass	300
RF Dump Value	375

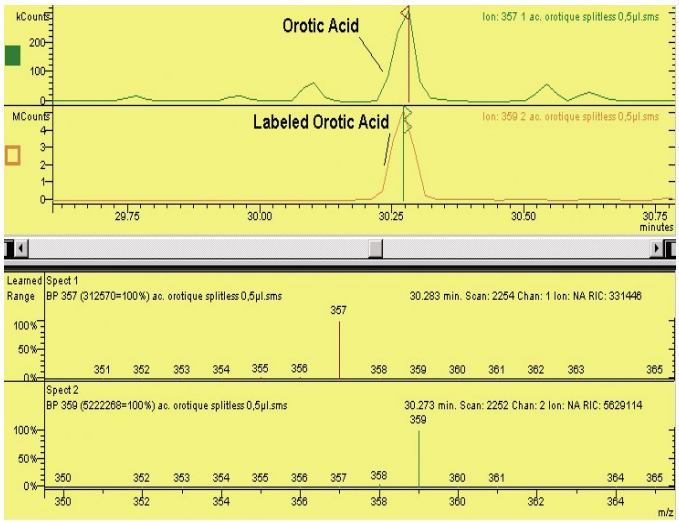
Results and Discussion

The following ions are monitored by uSIS (unit resolution Selected Ion Storage): m/z 254 and 357 for the native molecule and m/z 256 and 359 for the ^{15}N-labeled orotic acid. Quantitation is performed with standard curves of ratios of the more stable ions m/z 357/359. The calibration curve is linear up to 10 mg/L. The equation for the response curve is: y = 0.14x + 0.047 (correlation coefficient: 0.997). The limit of detection is 0.05 mg/L. To avoid excess isotopic contribution on the internal standard value, the more concentrated samples are diluted. The orotic acid concentrations in random urines from 30 healthy children ranged from 0.48 to 4.8 µg/mg of creatinine. The RSD for the analysis of sample injected twice is 4%.

Calibration Curve



Chromatograms and Ions



Conclusion

The uSIS technique uses the same algorithm employed to isolate the parent ion with the MS/MS option. Compared to SIS, uSIS is well suited to isolate ions with a window of 1 m/z over the entire mass range. Thus uSIS is comparable to the SIM technique with a quadrupole mass filter. The only precaution is to be sure that the ions being isolated are stable enough. The stable isotope dilution GC/MS analysis is a very powerful method to quantitate analytes in a biological fluid.

Reference

McCann M.T., Thompson M.M., Gueron I.C. and Tuchman M., Clin. Chem, 1995, 41(5), 739-743.

These data represent typical results.
For further information, contact your local Varian Sales Office.