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

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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.

Experimental

The method employs stable isotope dilution with 1-3 [¹⁵N] 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 [¹⁵N] 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 µL of BSTFA/TMCS in chloroform for 45 min at 80 °C.
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


These data represent typical results.
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