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Measurement of Orotic Acid in Urine by Supercritical Fluid Chromatography

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Abstract: This work presents a simple, rapid and reliable supercritical fluid chromatography (SFC) method for a sensitive measurement of orotic acid in human urine. The samples were diluted with deionized water and analyzed directly without any pretreatment.

Keywords: SFC, orotic acid, human urine.

Orotic acid is an intermediate in the pathway of pyrimidine synthesis. It is an important substance but difficult to analyze in biological samples because it is present in trace amount in normal human urine, but greatly increased in inherited metabolic diseases and drug treatment. The methods used to determine orotic acid in urine were mainly high performance liquid chromatography (HPLC)[1] and capillary zone electrophoresis (CZE)[2]. To date, the analysis of orotic acid in urine with SFC has not been reported. The aim of this work is to set up a method to determine orotic acid in urine with SFC.

The packed column SFC was a Gilson Model SF_3 system (Anachem, UK). It consists of a 308 master pump and a 306 slave pump. The separation was achieved in a 250×4.6 mm i.d. column with a cyano stationary phase (5 μm particle size). The temperature was 50°C and the pressure was 2×10^4 kPa. Detection was performed with a Jasco 875-CE UV detector (Japan), fitted with a high pressure cell, the wavelength was 280 nm. The samples were injected *via* an injector valve with a 10 μL injection loop.

To elute components, supercritical carbon dioxide was used as mobile phase. As orotic acid could not be eluted without a modifier, methanol with 0.2% trifluoroacetic acid (TFAA) was used as a modifier. When the flow rate of the mobile phase was 2.5 mL/min, the concentration of the modifier increased from 10% to 25%, the retention times of orotic acid decreased from 3.986 minutes to 1.591 minutes, and the peak shapes were improved. When 20% modifier was used, and the flow rate of the mobile phase increased from 1.5 mL/min to 3.0 mL/min, the retention times decreased from 3.267 minutes to 1.519 minutes. The concentration of the TFAA had a little effect to the shape and the retention times.

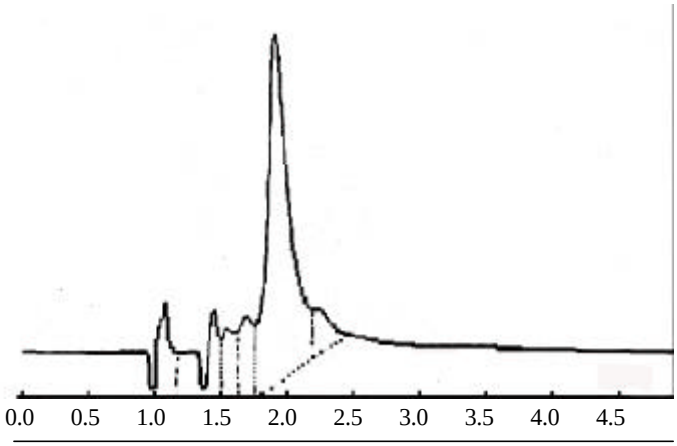
Precision was determined by measuring repeatability of the standard orotic acid. The values of the retention time and the peak area were determined by injecting ten times. The relative standard deviation (RSD) values for retention time and peak area were 0.47% and 2.33%, respectively. Calibration curve was constructed at six concentration

levels in the range from 30 to 500 ng injected in column. Three independent determinations were performed at each concentration. The calibration equation was Y=163.55X+4300.9 and the correlation coefficient (R^2) was 0.9991, indicating a good linearity of response over the concentration range considered.

Human urine samples were diluted with deionized water (1:10 v/v) and analyzed directly without any pretreatment. **Figure 1** showed that the analysis could be completed within 3 minutes. The content of the orotic acid in the human urine was 2.19 ±0.058 μmol/mL (average ± SD).

It showed that SFC could be used to determine orotic acid in urine. It does not need time-consuming sample preparation, and it is suitable for the rapid measurement of orotic acid in urine.

Figure 1 SFC-UV Chromatogram of a urine sample



Mobile phase: CO_2-20%CH_3OH (containing 0.2%TFAA), pressure: 20 MPa, flow rate: 2.5 mL/min, retention time of orotic acid: 1.887 min.

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