The Determination of Citric Acid in Human Urine by LC/MS/MS

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Introduction
Citrate is indicated for the management of renal tubular acidosis with calcium stones. The quantitation of citric acid is challenging due to the high endogenous level of citric acid in human urine. Here we describe a very simple, sensitive and cost-effective LC/MS/MS method for the determination of citric acid in human urine. The urine used to make calibration standards and quality control samples was citric acid depleted using the enzymatic conversion in dialysis bags. Citric acid and iso-citric acid was chromatographically separated. This method offered good precision and accuracy and was successfully applied for the pharmacokinetic and bioequivalence studies of 10 mg Extended-Release Tablets.

Calibration standards and quality control samples were prepared by spiking known amount of citric acid to citric acid depleted human urine. Citric acid and internal standard, citric acid-d4, were diluted and then chromatographed using reversed phase HPLC. The analytical column was a Prevail Organic Acid 3 μm (150 x 4.6 mm). An AP3000 or AA4000 with ESI source was operated in negative ion mode using transition m/z 191.0→111.0 for citric acid and m/z 195.0→115.0 for citric acid-d4. No matrix interference and matrix effects were observed. Acceptable intra-day and inter-day assay precision and accuracy were observed over a linear range of 5.00 to 1000 mcg/mL (low curve) and 50.0 to 2000 mcg/mL (high curve).

Method
Since citric acid is an endogenous compound in human urine, the urine used to make standards and quality control samples was citric acid depleted by means of dialysis. Dialysis is a method for removing small molecules from a solution through a semi-permeable membrane. Large molecules are retained within the dialysis bag while small molecules and ions pass through. The enzymatic conversion of citrate to oxaloacetate and acetate accomplished was accomplished by citrate lyase, a bacterial enzyme from Aerobacter aerogenes. The enzyme used for conversion also contains oxaloacetate decarboxylase. This decarboxylates part of the oxaloacetate formed from citrate with citrate lyase to pyruvate. The reaction is shown as follows:

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\text{Citric acid} \rightarrow \text{Oxaloacetate} + \text{Acetate}
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The enzymes were loaded in the dialysis bag. Citric acid is a small molecule and easily diffuses into the dialysis bag. Then the enzymes digested the citric acid molecules inside the bag. The reaction time was set according to concentration gradient and continuous mixing resulting in a urine matrix depleted of citrate.

The citric acid depleted urine was screened by LC/MS/MS to confirm the absence of interference at the retention time of the citric acid. This urine was then used to prepare an amount of citric acid to prepare calibration standards and quality control samples.

Results
The method was fully validated over a range of 5.00 to 1000 mcg/mL and 50.0 to 2000 mcg/mL with weighted linear regression (1/x^2). The correlation coefficients for first three validation batches were 0.997 or better (Figure 3). Figures 4 through 7 show typical chromatograms of extracted lower and upper limits of quantitation (LOD 5.00 mcg/mL, ULQ 1000 mcg/mL), an extracted blank matrix, and an extracted patient sample. No carryover or interference was observed in the chromatogram of the blank matrix.

Conclusion
Validation results obtained in this study indicate that a robust and reliable method has been established for the measurement of citric acid in human urine by LC/MS/MS. This validated method is suitable for determination of citric acid in the human urine samples.