Albumin (I-80.5 KDa) is the most abundant protein in blood. Its presence in urine is often the first sign of kidney damage. Thus, it has become an important biomarker for renal disease. As a result, accurate measurement in urine is of high interest in drug discovery and clinical research.

Under normal kidney function, urinary albumin levels are quite low (< 30 mg/L). However, albuminuria is associated with chronic kidney disease, and the higher the level of albuminuria, the greater the risk of future renal dysfunction. The presence of albumin in the urine is an early indicator of renal disease. However, this workflow can be complex and time consuming, often taking 24 hours to achieve analytically sensitive and accurate quantification.

This work describes the accurate quantification of urinary albumin over 3.5 orders of magnitude (0.1–500 µg/mL), which is completed in <4 hours using commercially available digestion and peptide purification kits and genetic protocols from only 15 µL of sample. This first standardized analytical method is shorter (3–4 h) and more sensitive (0.1 µg/mL) than published methods.1

METHODS

Sample Preparation
Human serum albumin (HSA) was isolated from pooled human serum. Urine samples (15 µL) were prepared for LC-MS analysis using the ProteinWorks aXpress Digest Kits and Protocol.2 After digestion, peptides were clean-up using the ProteinWorks µElution SPE Clean-up Kit and Protocol.

LC-MS Conditions
LC-MS/MS quantification of signature peptides was performed using a Waters Xevo TQ-XS triple quadrupole MS (ESI). Chromatographic separation was achieved using an ACQUITY UPLC system with an ACQUITY UPLC Peptide BEH C18 1.7 µm, 2.1 mm x 150 mm column and 0.1% formic acid in water and acetonitrile mobile phases. A total of 11 HSA tryptic peptides (highlighted in blue, Figure 1), and 2 MRM transitions per peptide, were monitored for quantification. MS conditions are summarized in Table 1.

RESULTS

UPLC chromatographic separation of albumin tryptic peptides, digested in human urine (30 µg/mL).

Table 1. Final MS conditions for HSA tryptic peptides, including precursor and fragment ions. Primary tryptic peptides used for quantification are highlighted in blue.

Table 2. Linear dynamic range and standard curve statistics for the 4 primary albumin tryptic peptides: YLYEIAFR, FQNALLVR, LVNEVTEFAK, and FQNALLVR/PLVEPONLK. Calculated endogenous urinary albumin concentrations in each lot of human urine using the YLYEIAFR, FQNALLVR, LVNEVTEFAK, and FDNQPLVEPONLK tryptic peptides of HSA.

Table 3. Summary of the QC sample statistics (precision and accuracy) in Urine Lots #1-3 for the 4 primary tryptic peptides used for quantification. Urine samples were digested and extracted protein quantification digestion and peptide purification kit.

Table 4. Calculated endogenous urinary albumin concentrations in 3 lots of human urine using the YLYEIAFR, FQNALLVR, LVNEVTEFAK, and FDNQPLVEPONLK tryptic peptides of HSA.

INTRODUCTION

Alkaline phosphatase (ALP) is an enzyme that is found in the plasma of humans and is used to measure the activity of ALP. It is also used to measure the activity of other enzymes such as amyloglucosidase, which is involved in the breakdown of starch.

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DISCUSSION

Using a generic kit-based approach (with simple step-wise protocols and standardized, pre-measured reagents) for digestion and subsequent peptide purification, an accurate and reproducible quantification of albumin concentrations was achieved. The analytical sensitivity (8.1 µg/mL), broad linear dynamic range, and selectivity of this LC-MS method reliably quantifies both low endogenous and elevated concentrations of albumin in urine.

REFERENCES