Measurement of Albumin in Human Urine by Liquid Chromatography-Isotope Dilution Tandem Mass Spectrometry

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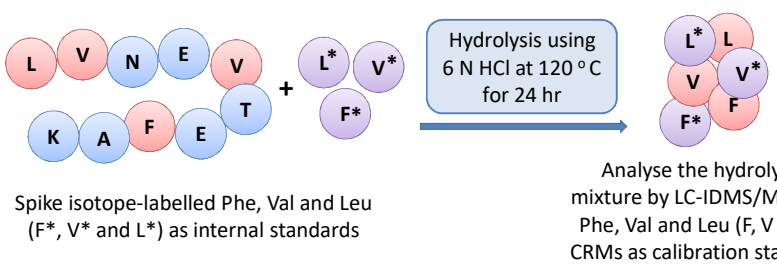
Introduction

Urine albumin is an important biomarker for assessing the health status of the kidneys. Urine albumin-to-creatinine ratio (ACR) helps to identify early-stage kidney disease. As diabetes and hypertension are the leading causes for kidney disease, it is imperative to routinely measure the urine albumin of patients with these chronic diseases in order to provide timely treatment and prevent the onset of kidney failure. The IFCC has formed a Working Group for standardisation of albumin in urine with the objectives of developing reference measurement procedures and commutable CRMs, as well as harmonising routine measurement procedures with reference measurement procedures.

Method 1 (Peptide Calibrator)

In method 1, the signature peptide, LVNEVTEFAK (L-K) was quantified after digestion of the urine samples by trypsin. The calibration standard used was custom-synthesised L-K which has been purity assessed using the peptide impurity corrected amino acid analysis (PICAA) approach. An isotope-labelled analogue of L-K, [Leu(^{13}C6, ^{15}N)]LVNEVTEFAK (L*-K), was used as the internal standard. To validate the method, a recovery check was conducted after spiking the urine samples with an albumin CRM solution.

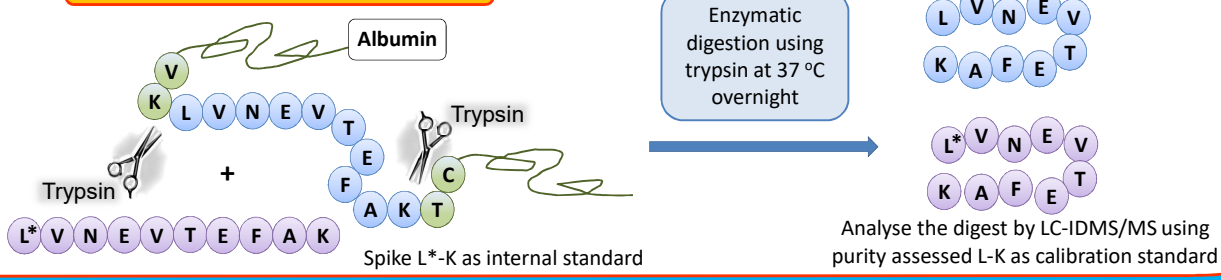
Quantification of L-K Calibration Standard by Amino Acid CRMs



Spike isotope-labelled Phe, Val and Leu (F*, V* and L*) as internal standards

Analyse the hydrolysed mixture by LC-IDMS/MS using Phe, Val and Leu (F, V and L) CRMs as calibration standards

Quantification of Albumin by L-K



Spike L*-K as internal standard

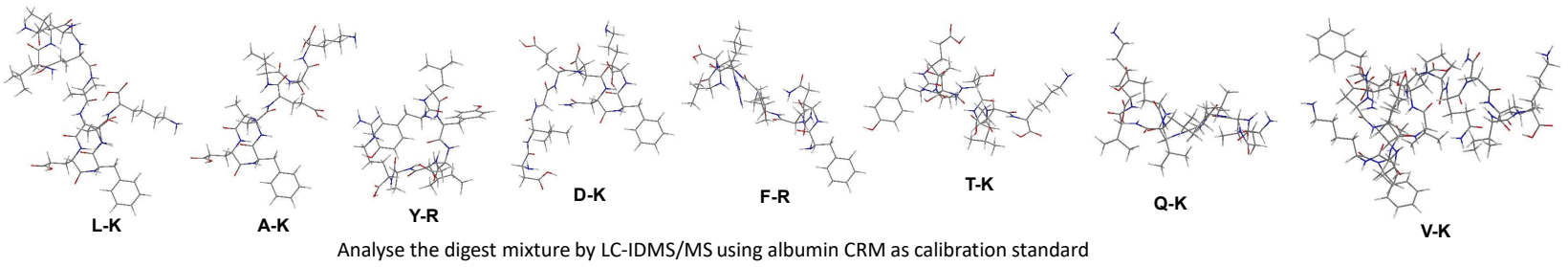
Analyse the digest by LC-IDMS/MS using purity assessed L-K as calibration standard

Method 2 (Protein Calibrator)

In method 2, an albumin CRM was used as a calibrator and ^{15}N-labelled albumin was used as the internal standard. Urine samples were digested using trypsin and eight resulting peptides, L-K, AEFAEVSK (A-K), YLYEIAR (Y-R), DLGEENFK (D-K), FQNALLVR (F-R), TYETTLEK (T-K), QTALVELVK (Q-K), and VFDEFKPLVEEPQNLIK (V-K) were quantified by LC-IDMS/MS simultaneously.

Spike ^{15}N-labelled albumin as internal standard

Enzymatic digestion using trypsin at 37 °C overnight



Analyse the digest mixture by LC-IDMS/MS using albumin CRM as calibration standard

Results

Recovery of Method 1:

	Mid Level (~ 40 mg/kg)	High Level (~ 220 mg/kg)
Replicates, n	8	6
% Recovery	96.4	98.8
% CV	1.04	2.33

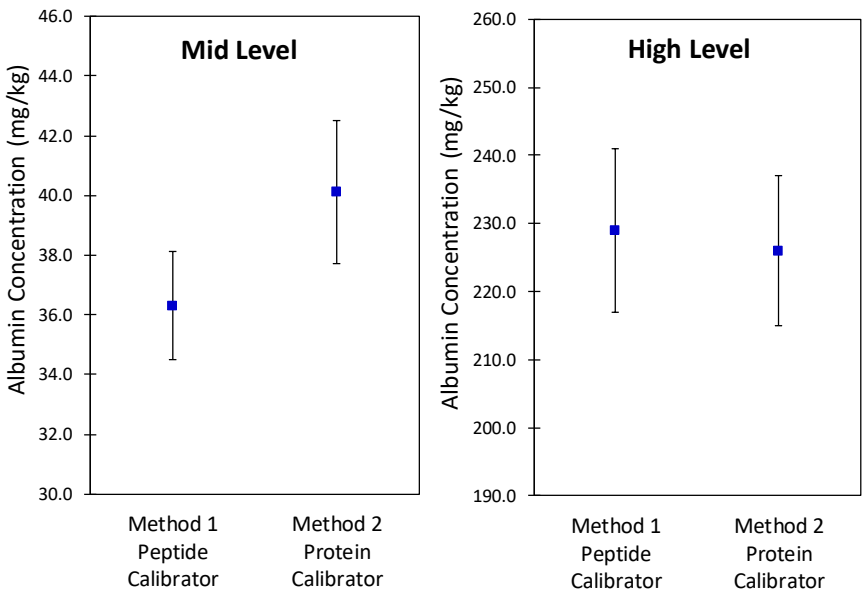
Recovery of Method 2:

	Low Level (~ 7 mg/kg)	Mid Level (~ 40 mg/kg)
No. of Peptides	8	8
% Recovery	101.9	103.2
% CV	1.85	1.60

Recovery by Using Different Peptides in Method 2:

Peptides	% Recovery Low Level, n = 6	% CV	% Recovery Mid Level, n = 7	% CV
L-K	100.2	2.99	103.1	4.42
A-K	100.5	3.38	100.2	3.55
Y-R	104.1	2.73	103.3	3.34
D-K	104.9	4.52	104.4	2.49
F-R	100.1	2.25	104.2	3.24
T-K	101.1	2.36	105.6	5.63
Q-K	101.4	1.57	101.8	3.19
V-K	103.2	4.19	102.9	3.30

Comparison of Two Methods



Conclusion

The newly developed LC-IDMS/MS methods have been shown to be accurate and precise. Method 2 (protein calibration) was found to be suitable for urine samples with a wide albumin concentration range, while Method 1 (peptide calibration) was suitable for urine samples with albumin concentrations close to or higher than 40 mg/kg. Methods 1 and 2 were successfully applied in the value assignments of albumin in urine samples in the 2017 & 2018 HSA EQA Programmes, respectively. The EQA Programme was participated by over 30 clinical laboratories across Singapore. Majority of the participating laboratories achieved satisfactory z-scores. These findings further support the good reproducibility and precision of the developed methods.

With additional work on stability, HSA has successfully launched a new CRM for albumin in human urine. This CRM will be useful to clinical laboratories for validating their methods or use as QC materials.

Certified Reference Material (HRM-3004A)

Albumin and Creatinine in Human Urine

Certified Values of Albumin (mg/L)*	
STY-0018-053	STY-0018-054
40.1 ± 2.4	226 ± 11

*Converted from mg/kg using urine density

References

1. Beasley-Green A, Burris NM, Bunk DM and Phinney KW. Multiplexed LC–MS/MS Assay for Urine Albumin. *Journal of proteome research* 2014; 13: 3930-3939.
2. Seegmiller JC, Barnidge DR, Burns BE, Larson TS, Lieske JC and Kumar R. Quantification of urinary albumin by using protein cleavage and LC-MS/MS. *Clinical chemistry* 2009; 55:1100-1107.