A simplified, rapid LC-MS/MS assay for serum and salivary creatinine

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Introduction

Creatinine in serum is produced from conversion of muscle creatine, of which between 1 and 2% is converted to creatinine each day. The amount produced is proportional to an individual’s muscle mass and there are therefore variations with age and sex. Renal excretion of creatinine usually mirrors endogenous production and consequently it can be used as a marker of glomerular filtration rate (GFR). Although the reference ranges for serum creatinine are wide due to inter-individual variation, formulae to calculate an estimated GFR (eGFR) from the serum creatinine concentration (for example taking into account age and sex) are now widely employed and this is more accurate than considering the concentration alone (Levey et al., 2009).

In routine clinical laboratories serum creatinine is usually measured by colourimetric or enzymatic assays using automated analysers, due to high workload and requirements for rapid turn-around-time. In the UK, the majority of laboratories utilise the alkaline picrate (Jaffé) method, whereby creatinine reacts with picrate under alkaline conditions to form a coloured complex which is then detected spectrophotometrically. However this method is prone to interferences from compounds such as bilirubin, ketones, glucose and protein (Watkins et al., 1976). Although modifications such as kinetic Jaffé assays and compensation factors have improved performance, interference remains an issue. Enzymatic methods rely on the use of enzymes such as creatinine iminohydrolase or creatininase with the eventual production of a chromophore or ammonia. These methods also suffer from interferences, for example from haemoglobin and bilirubin, however they are thought to have improved specificity compared to the Jaffé assay (Cobbaert et al., 2009).

Methods

We have developed an improved liquid chromatography tandem mass spectrometry assay for serum creatinine using a 4 mm x 3 mm strong cation exchange SecurityGuard column. To prepare the samples, 500 µL methanol was added to 10 µL of sample and 10 µL of internal
standard (1 mg/L creatinine-d3) and the samples were mixed and centrifuged. 2 µL of supernatant was injected onto the column and eluted with 100% mobile phase A as initial conditions (2 mmol/L ammonium acetate, 0.1% formic acid in water) stepping up to 100% mobile phase B (50 mmol/L ammonium acetate, 0.1% formic acid and 10% methanol in water) at 0.4 minutes, holding for 0.2 minutes before returning to initial conditions for 0.5 minutes. Total run time was 1.1 minutes. A Waters Xevo TQD tandem mass-spectrometer was used and the specific transitions monitored were m/z 114>44.5 (creatinine) and 117>47.5 (creatinine-d3).

**Results**

The intra-batch imprecision of the assay was found to be 1.4% at 50 µmol/L and 1.1% at 600 µmol/L creatinine, and the assay was linear to at least 1000 µmol/L. Ion suppression was found to be minimal when comparing serum samples and methanol. A comparison of patient serum samples using an Abbott Architect C16000 analyser with an Abbott Architect kinetic alkaline picrate assay revealed a generally good correlation, which would be expected unless the samples contained interferants. Initial testing of a small number of external quality assessment samples showed an average bias of +6.88% compared to the all-method mean for the scheme.

Creatinine can also be measured in saliva (Lloyd et al., 1996) and we have tested 42 paired serum and saliva samples from patients with renal impairment using this assay. On average salivary creatinine concentrations were 15.4% of serum levels although large variations between sample pairs were seen, ranging from 0.036% to 77.7%. This variation was not concentration dependent.

**Conclusions**

This assay represents a simple and extremely rapid means of determining creatinine in serum and saliva using a small sample volume. With improved specificity compared to colourimetric and enzymatic assays, this method may offer benefits for patients where interferences are likely such as jaundiced patients, or where small samples are obtained.

**References**

