**Title: Liquid chromatography tandem mass spectrometry: challenges in introducing published methods into the clinical laboratory**

Milad Khedr,1 Anna M Milan,1 Andrew S Davison1,\*

Department of Clinical Biochemistry and Metabolic Medicine, Liverpool Clinical Laboratories, Royal Liverpool University and Broadgreen University Hospitals Trust, Liverpool L7 8XP, UK1

**\*Corresponding Author:** Andrew S Davison

**Corresponding Author Address:** Department of Clinical Biochemistry and Metabolic Medicine, Liverpool Clinical Laboratories, Royal Liverpool University Hospitals Trust, Liverpool, L7 8XP, UK; Telephone: 0151 706 4011; Fax: 0151 706 4250

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We read with interest the recent article by Marshall *et al* 1 on combined quantification of urinary citrate and oxalate using liquid chromatography tandem mass spectrometry (LC-MS/MS). We have attempted to develop a method for the simultaneous measurement of urinary citrate and oxalate using a ‘dilute and shoot’ approach on an Acquity® liquid chromatography module coupled to a Xevo TQS tandem mass spectrometer (Waters, Wilmslow, UK) with limited success.

Oxalate tuning experiments were performed using an oxalate solution (9mg/mL, Sigma Aldrich, Dorset, UK), which was diluted in deionised water to produce a solution of 1mg/mL (pH 2). Equipment was operated in electrospray negative ionisation mode (capillary voltage 2.7kV, collision energy 9.0eV, source temperature 150°C, desolvation temperature 200°C and desolvation gas flow 500L/hr). Mobile phase A was deionised water and mobile phase B methanol. Both mobiles phases contained 2mmol/L ammonium acetate (Sigma-Aldrich, Dorset, UK) and 0.1% formic acid (Biosolve BV, Netherlands). The composition of the mobile phase was manipulated to oxalic acid’s highest pKa of 4.2. An oxalate precursor ion was generated (m/z 88.9), but it proved challenging to generate an optimal product ion (m/z 60.8) even at micromolar concentrations (collision energies of 5-50eV evaluated). Consequently further assay development for oxalate was not performed. The exact reason for the poor product ion signal is unknown, but it may result from poor ionisation efficiency due to the small size of the oxalate molecule,2 and fragility in the stepwave and/ or collision cell of the mass spectrometer used in our study.

In contrast, citrate ionised more efficiently to generate both precursor and product ions (191.1>111.0). An Atlantis C18 column (3x100 mm, 3.0µ, Waters, Wilmslow, UK) was used for chromatographic isolation of citrate (run time 5 minutes). The analytical measuring range for citrate was linear up to 10 000 μmol/L, the lower limit of the measuring interval was 50 μmol/L. Inter-assay precision was less than 15%, between 200–10 000 μmol/L. Method comparison with a citrate lyase enzymatic assay (ILab Aries, Cheshire, UK) showed a mean bias of 31.4% (95% CI 24.0-38.8%). Post column infusion of citrate (1mg/mL) did not show ion suppression at the area of citrate elution (retention time 2.29 min). However, matrix factor experiments revealed ion enhancement at 1 mmol/L and ion suppression at 9 mmol/L. The percentage coefficient of variation for the internal standard normalised matrix factor was 19.4 and 13% at 1 and 9 mmol/L, respectively. This was likely due the use of phosphate buffered saline (PBS) based calibrators. In contrast, Marshall *et al*1 reported a matrix factor of <10% for citrate using PBS calibrators. This is likely due to the choice of column (0.8μm HSS T3 Vanguard column coupled to an HSS T3 2.1 x 50mm 1.8μm, Waters, UK) as well as the solid phase extraction step. Our findings make an excellent case for undertaking both qualitative (i.e. post column infusion) and quantitative assessments of the matrix effects when developing LC-MS/MS methods, as per recommendations of the CLSI C62A guidance for LC-MS/MS based methods in the clinical laboratory.4

LC-MS/MS has increasingly become a popular technique in the clinical laboratory. Nonetheless, it is still not mainstream for measurement of citrate and oxalate. A review of the urinary citrate and oxalate methods used by the WEQAS scheme participants shows that they are dominated by enzymatic methods (personal communication, WEQAS 2017). This is not surprising as these are robust and avoid the need for technical expertise.

Our experience highlights some of the difficulties in introducing published LC-MS/MS methods in the clinical laboratory. With harmonisation gaining momentum in the clinical laboratories accreditation and with the introduction of LC-MS/MS specific guidance4, it is hoped that the assay development process for newly published methods should become more robust and less daunting.

**References**

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