Development of an LC-MS/MS method to quantify urinary 18-hydroxycortisol,18-oxocortisol and Tetrahydroaldosterone

Silvia Bérgamo & Brian Keevil .

Dept. Clinical Biochemistry, University Hospital of South Manchester NHS Foundation Trust, Manchester Academic Health Science Centre , UK

INTRODUCTION

Primary hyperaldosteronism (PA) is the most common form of secondary hypertension. It is important to distinguish between surgically treatable unilateral aldosterone producing adenomas (APA) and bilateral adrenal hyperplasias (BAH) best treated by medication. The most reliable method to identify the principal subtypes of PA is adrenal vein sampling. Unfortunately this technique is technically demanding and available only in highly specialised centres. Evidence suggests that PA subtype classification through specific urinary steroid quantification may help guide treatment (1) including 18 oxo cortisol (18 oxoF), 18 hydroxycortisol (18OHF) and terahydro aldosterone (THAldo).

METHODS

An aliquot of urine (150 µl) , standard or QC was placed in a 96 deep well block and was mixed with 150 µl of phosphate buffer ,10 µl of IS solution and 10 µl of β-glucuronidase from E-Coli (KURA Biotec, Chile) . After vortex mixing, the mixture was incubated for one hour at 45 degrees. The reaction was stopped by rapid cooling and samples were transferred to a supported liquid extraction plate (Biotage, UK) and the analytes were eluted with 1000 µl of MTBE. After evaporation to dryness, the residue was dissolved in 100 µL of 30% aqueous methanol solution . Chromatography was performed using a ACQQUITY UPLC BEH phenyl 1.7 µm 2.1x 100mm. The mobile phases used were A (water containing 11mg/L ammonium fluoride) and B (methanol). The gradient consisted of 50 % B initially, a step up to 80% B for 3 min, another step up 95% B for 1 sec, followed by a step down to 50% B for 50 sec. Analytes were measured using a Xevo TQ-S MicroTM tandem mass spectrometer (Waters, UK). 18 oxo F and 18OHF were run in pos ion mode whereas THAldo was run in neg ion mode. Calibrators included all 3 compounds to make extraction easier but two separate batched runs in either pos or neg mode were used .

RESULTS

Quantifier and qualifier transitions used are shown in table 1

Compound	Quantifier m/z	Qualifier m/z
18-oxocortisol	377.2>313.2	379.3>121
18-hydroxycortisol	379.3>267.2	379.3>109
tetrahydroxyaldosteron a	363.2>193.1	363.2>335.1
d4- 18 hydroxycortisol	383.3 > 121	
d6- ThAldo	369.3 > 196.1	

Table 1. Quantifiers and qualifiers transitions

Chromatographic separation was achieved between THAldo, 18oxo F and 18OHF (figure 1). The run time was 4.5 minutes per sample. Negligible ion suppression was observed in the region of the chromatogram where 18 oxoF, 18 OHF and THAldo elute.

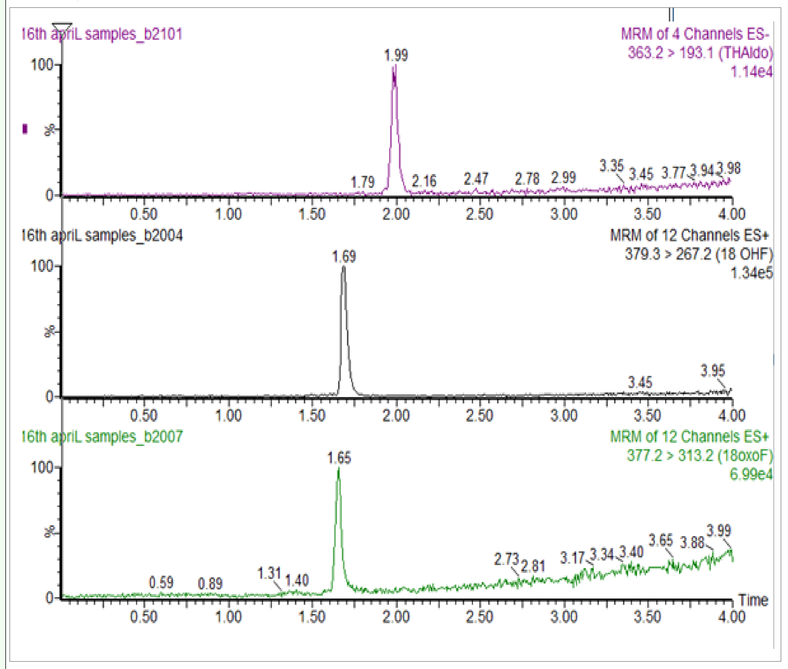

Figure 1: Example chromatogram for a urine sample

RESULTS

The standard curve was linear to 1000 µg/L, 25 µg/L and 500 µg/L for, 18OHF, 18 oxoF and THaldo respectively and reproducible between batches. The curves showed good correlation with the assigned standard values with an R2 value of 0.99

The lower limit of quantifications for 18-oxoF, 18-OHF, THAldo was,0.3 µg/L, 0.9 µg/L and 0.25 µg/L respectively. At these concentrations, the % CV and bias of 10 replicates were less than 10 %.(table 2) None of the steroids tested to check for interference, endogenous or exogenous, gave a response above the LLOQ at the retention time for either compound or their internal standards.

Intra- and inter-assay imprecision was within the admissible range from 3.2 – 10.9% over 100-600 µg/L (18-OHF), 2.5-15.0 µg/L (18-oxoF) and 50-300 µg/L (ThAldo).

	18OHF	18 oxo	ThAldo
LLOQ	0.9	0.3	0.25
CV%	6.76	10.06	5.03
Bias	5.6	14.29	1.04

Table 2. LLOQ of the three compounds.

DISCUSSION

We have developed an assay for 18-OHF, 18-oxoF, and THAldo that has a simple sample preparation. This LC-MS/MS method offers precise and accurate quantitative measurement of these steroids, and will be used in the evaluation of hyperaldosteronism.

REFERENCES

Lang K. Urine steroid metabolomics as a diagnostic tool in primary aldosteronism. *Endocrine Abstracts* (2015) 38 OC1.6