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Short Communication

Quantitation of aldosterone in human plasma by ultra high performance liquid chromatography tandem mass spectrometry

Edward Hinchliffe[a,*], Stephanie Carter[a], Laura J. Owen[b], Brian G. Keevil[b]

[a] Department of Clinical Biochemistry, University Hospital of South Manchester, Wythenshawe, Manchester M23 9LT, United Kingdom
[b] Manchester Academic Health Science Centre, The University of Manchester, University Hospital of South Manchester, Manchester M23 9LT, United Kingdom

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ABSTRACT

Aldosterone is a mineralocorticoid steroid hormone whose measurement in the clinical laboratory is principally performed for the investigation of primary hyperaldosteronism. Traditionally measurement of aldosterone has been performed by radioimmunoassay, however these assays lack specificity as they are prone to interference from structurally related steroid hormones. Herein, we have developed a novel, sensitive and specific method utilising solid phase extraction and quantitation of aldosterone from human plasma by UPLC–MS/MS. Standards, quality controls and samples (250 μL) were extracted using Oasis® HLB 96-well plates. Extract (30 μL) was injected onto a Krudcatcher UPLC In-Line Filter, 0.5 μm guard column, coupled to a Kinetex PFP, 100 mm × 2.1 mm, 2.6 μm column with methanolic mobile phase gradient elution. Eluant was connected to a Waters® Xevo TQS tandem mass spectrometer operating in electrospray negative mode. We detected multiple reaction monitoring (MRM) transitions of m/z 359.0 > 189.1 for aldosterone and 366.0 > 194.1 for d7-aldosterone respectively, which co-eluted at 2.65 min. Ion suppression was negligible. Mean recovery was 89.6%, limit of detection and lower limit of quantitation were 26 pmol/L and 30 pmol/L respectively. The assay was linear up to 3200 pmol/L (r^2 = 0.9999). Mean intra- and inter-assay imprecision and bias were all <10%. Comparison of the UPLC–MS/MS method with an immunoassay in routine clinical use in the UK yielded the equation UPLC–MS/MS = 0.789(RIA)–41.7, linear regression r^2 = 0.88, n = 54. We have developed a sensitive and specific method for the extraction and measurement of aldosterone from human plasma. The method features a simple 96-well plate solid phase extraction procedure, highly selective column chemistry and short chromatographic run times.

1. Introduction

Aldosterone is a mineralocorticoid steroid hormone produced by the zona glomerulosa of the adrenal cortex. The principle action of aldosterone is to regulate sodium reabsorption and potassium excretion in the renal collecting ducts and distal convoluted tubule. Control of synthesis and secretion of aldosterone is primarily exerted by the renin–angiotensin system (RAS), which is itself stimulated by a fall in systemic blood pressure and sodium depletion.

Clinically, measurement of aldosterone is important to identify patients with primary hyperaldosteronism. This condition is most commonly caused by autonomous aldosterone production by an adrenal adenoma (classical Conn's syndrome), or hyperplasia of one or both adrenal glands. Hyper-secretion of aldosterone typically presents clinically with drug resistant hypertension and, in up to 37% of cases, hypokalaemia [1]. The prevalence of primary hyperaldosteronism in patients with essential hypertension may be up to 10% [2], but due to non specific signs and symptoms the condition is frequently overlooked. Where clinical suspicion arises, screening is indicated as effective medical and surgical management options exist to control or cure the condition. The recommended initial screening test for primary hyperaldosteronism is assay of the plasma aldosterone to renin ratio (ARR) [2].

Measurement of aldosterone in human plasma in clinical laboratories has most frequently been performed using radio immunoassay (RIA) or enzyme labelled immunoassay [3]. The specificity of immunoassays is often poor due to antibody cross reactivity to other structurally related steroid hormones, which may result in falsely elevated concentrations. To overcome these limitations, in recent years there has been growing interest in the use of liquid chromatography tandem mass spectrometry (LC–MS/MS) for steroid hormone analysis in clinical laboratories. Recently, a small number of reports have been published detailing methods for the quantitation of aldosterone from human plasma by LC–MS/MS. Such methods utilise liquid–liquid extraction (LLE) with relatively long chromatographic run times [4–6], or require highly specialised automated extraction platforms [5] which may not be suitable for many clinical laboratories. Herein, we report the development of a highly sensitive and specific method for the

* Corresponding author.
E-mail addresses: ed.hinchliffe@uhsm.nhs.uk, ed_hinchliffe@hotmail.com (E. Hinchliffe).

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measurement of aldosterone from human plasma by UPLC–MS/MS. The method utilises a simple 96-well plate solid phase extraction (SPE) technique, highly selective column chemistry and short chromatographic run times. The method provides a useful option for laboratories wishing to analyse aldosterone in routine clinical practice with relatively high throughput.

2. Methods

2.1. Materials

Oasis® HLB 96-well extraction plates were purchased from Waters (Hertfordshire, UK). LC–MS grade methanol was purchased from Greyhound (Birkenhead, UK). LC–MS grade acetonitrile, aldosterone, bilirubin and haemoglobin were purchased from Sigma (Dorset, UK). d7-aldosterone was purchased from Isosciences (Pennsylvania, USA). Polypropylene 2 mL 96-deep-well plates were purchased from Porvair Sciences (Surrey, UK), and 'Easy Pierce' heat sealing foil sheets from Thermo Scientific (Surrey, UK). Intralipid was purchased from Kabi Pharmacia (Uppsala, Sweden).

2.2. Calibration standards and quality control material

Stock solutions of aldosterone and d7-aldosterone were prepared by weighing pure aldosterone powder and solubilising in methanol. Calibration standards and quality controls (QCs) were prepared by spiking stock aldosterone solution into phosphate buffered saline (PBS) containing 0.1% (w/v) bovine serum albumin (BSA). Final calibrator concentrations ranged from 0 to 3200 pmol/L. Three QC samples were prepared to achieve final concentrations of 150, 500 and 1500 pmol/L, representing clinically relevant aldosterone concentrations.

2.3. Sample preparation

Patient plasma samples were obtained using K^+EDTA or lithium heparin collection tubes. Deuterated d7-aldosterone was prepared to a final concentration of 60 μg/L in methanol, and 10 μL added to 250 μL standards, QC's and patient plasma samples. To all samples, 125 μL 0.1 mol/L zinc sulphate was added, and samples vortexed for 20 s. Following this, 250 μL methanol was added to each sample and the samples vortexed for 1 min, followed by centrifugation for 5 min at 8000 × *g*. Individual wells of an Oasis® HLB 96-well extraction plate were washed consecutively with 1 mL acetonitrile, 1 mL methanol, then 0.5 mL H_2O. The total volume of supernatants obtained following zinc sulphate and methanol precipitation were applied to individual wells of the extraction plate and washed with 0.5 mL H_2O then 0.5 mL 40% methanol. Bound material was eluted from the SPE adsorbent using 1 mL acetonitrile and collected into 2 mL 96-deep-well polypropylene plate. Acetonitrile was evaporated to dryness and wells re-constituted in 80 μL of 30:70 methanol:water (v/v). Finally, the plate was sealed with 'Easy Pierce' heat sealing foil, vortexed for 2 min and centrifuged for 5 min at 8000 × *g*.

2.4. Liquid chromatography

Chromatography was performed on a Waters® Acquity™ UPLC system. Extracted sample (30 μL) was injected directly from the 96-deep well plate onto a Krudcatcher UPLC In-Line Filter, 0.5 μm × 0.004″ (Phenomenex, Macclesfield, UK) guard column, coupled to a Kinetex PFP, 100 mm × 2.1 mm, 2.6 μm column (Phenomenex, Macclesfield, UK).

Mobile phase A contained laboratory grade de-ionised H_2O, and mobile phase B contained methanol. Initial conditions were 70:30 (v/v) mobile phase A:B. Following sample injection, elution was performed by means of a gradient from 30% to 65% mobile phase over 3 min, followed by 95% mobile phase B held for 30 s. Following this, the columns were re-equilibrated back to initial conditions and held for a further 1 min prior to the next sample injection. Total chromatographic run time was 4.5 min and total injection-to-injection run time was 5 min. The mobile phase flow rate was maintained at 0.45 mL/min, and the column maintained at 45 °C.

2.5. Tandem mass spectrometry

Eluate from the analytical column was injected directly into a Waters® Xevo TQS tandem mass spectrometer operating in the negative electrospray ionisation mode (Waters, Hertfordshire, UK). The instrument conditions were as follows: electrospray capillary voltage 2.8 kV, collision energy 20 eV, sample cone voltage 30 V and source offset 40 V. Desolvation gas flow and temperature were maintained at 500 L/h and 600 °C respectively, cone gas flow was maintained at 150 L/h, and the source temperature was 150 °C. Aldosterone and d7-aldosterone were detected in multiple reaction monitoring (MRM) mode with a dwell time of 0.125 s per channel. MRM transitions were m/z 359.0 > 189.1 (quantifier) and 359.0 > 297.0 (qualifier) for aldosterone, and 366.0 > 194.1 for d7-aldosterone. Collision gas flow was maintained at 0.15 mL/min. Resolution was 2.7 (low mass) and 14.7 (high mass) for both MS1 and MS2.

2.6. LC–MS/MS method validation

2.6.1. Ion suppression

Ion suppression/enhancement was assessed by continuous post-column infusion of d7-aldosterone (1 mg/L in 50:50 mobile phase *A*:*B*) directly into the mass spectrometer at a flow rate of 10 μL/min. Ten patient plasma samples were extracted and injected, as described in Sections 2.3 and 2.4, along with a methanol and H_2O blank. Ion suppression or enhancement was interpreted as a fall or increase in baseline count greater than 10% at the retention time of aldosterone.

2.6.2. Linearity

Linearity of the assay was assessed by repeat (n = 6) analysis of calibrators, with concentrations ranging from 0 to 3200 pmol/L. LC–MS/MS response was plotted against nominal concentration values using TargetLynx™ software (Waters, Hertfordshire, UK). In addition, patient plasma samples were assayed neat, diluted with PBS/BSA and assayed over a range of dilutions. Measured aldosterone concentrations were compared to expected concentrations. Linearity of the assay was confirmed by weighted linear regression with a correlation coefficient r^2 value > 0.99.

2.6.3. Accuracy and recovery

To confirm the utility of PBS/0.1% BSA matrix for preparation of standards and QCs, the following accuracy and recovery experiments were performed.

The method of standard additions was used with plasma and PBS/BSA-based spiked calibrators. Samples were spiked with a range of aldosterone concentrations from 50 to 2000 pmol/L. The PBS/BSA-based calibrators were deemed acceptable for use if calculated aldosterone concentrations were within 10% of plasma matrix following standard additions.

To calculate recovery, pure aldosterone stock solution was used to spike into six different patient plasma samples. These samples had endogenous aldosterone concentrations ranging from 104 to 222 pmol/L. The concentrations of aldosterone added to aliquots of each of these six plasma samples were 64, 353 and 1221 pmol/L. Each sample was assayed in triplicate and % recovery calculated from measured compared to expected concentrations. According