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Enhancement of specificity of aldosterone measurement in human serum and plasma using 2D-LC–MS/MS and comparison with commercial immunoassays

Julie A. Ray[a,*], Mark M. Kushnir[a], John Palmer[b], Seyed Sadjadi[c], Alan L. Rockwood[a,d], A. Wayne Meikle[a,d,e]

[a] ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT 84108, United States
[b] Agilent Technologies Inc., Santa Clara, CA 95051, United States
[c] Phenomenex, Torrance, CA 90501, United States
[d] Department of Pathology, University of Utah, Salt Lake City, UT 84108, United States
[e] Department of Medicine, University of Utah, Salt Lake City, UT 84108, United States

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ABSTRACT

Background: Accurate measurement of aldosterone is important for the standardized testing of primary hyperaldosteronism. Commercial immunoassays show substantial between-method variations resulting in significant clinical consequences. We developed a specific two dimensional (2D)-LC–MS/MS method for measuring aldosterone in human serum and plasma and compared it with three commercial immunoassays and an LC–MS/MS method.
Methods: 250 μL samples, controls and calibrators spiked with d4-aldosterone were subjected to liquid–liquid extraction. The samples were analyzed using negative mode electrospray and 2D-LC followed by MS detection using an ABSciex 5500 mass spectrometer and compared with immunoassays of Siemens (Coat-A-Count), DiaSorin (CLIA-LIAISON), and IBL (ELISA). Data was acquired using multiple reaction-monitoring mode.
Results: LOQ and LOD of the method were 0.04 and 0.02 nmol/L respectively. The assay was linear up to 166 nmol/L. Inter and intra-assay imprecision at 0.13, 1.38 and 8.30 nmol/L were <10%. Interferences were absent and no differences were observed between serum and plasma matrices. Method recovery ranged from 95% to 113%. Ion suppression was not observed. Evaluated immunoassays showed positive biases ranging between 22% and 37% when compared with the developed method.
Conclusions: We developed and validated an accurate method for measurement of aldosterone in human serum and plasma using 2D-LC–MS/MS which is suitable for clinical purposes. The method is faster than previously published LC–MS/MS methods, uses less sample, has adequate sensitivity while being able to preserve high specificity in a cost effective manner. Linearity of the assay makes it promising for urine and adrenal venous samples. Comparison with three commercial immunoassays demonstrates the advantages of the developed method.

1. Introduction

Aldosterone produced in the adrenal cortex of the adrenal gland is the principal regulator of salt and water balance in the body. Primary hyperaldosteronism caused by an aldosterone-producing adenoma is amongst the most recurrent cause of secondary hypertension [1–4], damage or disease of the kidney being the most common cause. Accurate measurement of aldosterone is clinically important in the diagnosis of hypertension. Currently used radioimmunoassay (RIA) as well as chemiluminescence-based methods (CLIA) have poor specificity and show wide variations in

Abbreviations: 2D-LC–MS/MS, two dimensional liquid chromatography tandem mass spectrometry; LOQ, limit of quantitation; LOD, limit of detection; MRM, multiple reaction monitoring; LLE, liquid–liquid extraction; IA, immunoassay; RIA, radioimmunoassay; CLIA, chemiluminescent immunoassay; SPE, solid phase extraction; SLE, supported liquid extraction.

* Corresponding author at: 500 Chipeta Way, Salt Lake City, UT 84108, United States. Tel.: +1 801 583 2787; fax: +1 801 587 1568.
E-mail address: julie.ray@aruplab.com (J.A. Ray).

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results [5–9]. These antibody-based kit methods sometimes require an initial cleanup step or are direct methods [10,11]. But high discrepancies in the measurement of the analyte between such platforms have impressed the need for improvement of aldosterone measurement using more reliable methods such as LC–MS/MS [12–19].

LC–MS/MS is a reliable technique that is progressively being used to measure analytes at low levels with high precision as well as high specificity [20–24]. Sample cleanup prior to LC–MS/MS, is important in order to improve background of chromatograms leading to enhanced life time of the assay as well as the mass spectrometer. In the past several sample pretreatment methods such as SPE (solid phase extraction) and SLE (supported liquid extraction) have been suggested for improving the LC–MS/MS signal to noise ratio of aldosterone [14,25–28]. We report here LLE (liquid–liquid extraction) using lower sample volume than most published methods and achieve the separation of aldosterone from its interferences using 2 dimensional-LC–MS/MS (2D-LC–MS/MS) in half the run time. The method has been compared with commercial immunoassay methods as well as LC–MS/MS methods of other reference laboratories.

2. Materials and methods

2.1. Reagents and samples

Aldosterone was purchased from Cerilliant (Sigma; St Louis, MO) and d_4-aldosterone from Iso Sciences (King of Prussia, PA). Stock standards were prepared in methanol at concentrations of 1 g/L. Working calibration standards of aldosterone and d_4-aldosterone were prepared at 13.84 and 17.30 nmol/L respectively in methanol:water (ratio 1:1). Calibration standards were prepared in 0.05% BSA (bovine serum albumin) at 0.13, 0.27, 0.69, 2.76, 5.53 and 8.30 nmol/L. Water, methanol and MTBE (methyl t-butyl ether) were purchased from VWR (Radnor, PA). Ammonium acetate was purchased from Sigma Aldrich (St Louis, MO). Serum and plasma samples used for the method evaluation were deidentified discard samples submitted to ARUP Laboratories for routine analysis. All studies with human serum and plasma samples were approved by IRB of the University of Utah.

2.2. Sample treatment

250 μL aliquots of patient samples, calibrators and controls were transferred to 2 mL polypropylene microcentrifuge tubes. 20 μL of working internal standard and 1.5 mL MTBE were added to each tube, shaken, centrifuged and frozen. The organic layer was transferred to a 96-well plate and evaporated under nitrogen at 50 °C. The residues were reconstituted using 100 μL of methanol:water (ratio 1:4). The plate was shaken, centrifuged for 5 min at 4000 × *g* and 30 μL was injected for 2D-LC–MS/MS analysis.

2.3. LC–MS/MS conditions

The HPLC system consisted of two binary 1260 series Agilent HPLC pumps, HTC PAL autosampler (LEAP Technologies, NC) and a fast wash station. Chromatographic separation was performed by 2D-LC–MS/MS in a gradient LC elution program. The HPLC columns used in the method were Zorbax Eclipse XDB CN 50 mm × 2.1 mm × 5 μm (Agilent Technologies) fitted with in-line filter as the loading column and a Poroshell 120 EC C18 50 mm × 2.2 mm × 2.7 μm (Agilent Technologies) as the analytical column fitted with a guard cartridge (Poroshell EC C18 2.1 mm × 5 mm × 2.7 μm). Column temperature was maintained at 50 °C and mobile phases used in both dimensions of separation were 2 mM ammonium acetate in water (A) and methanol (B). The gradients and 6-port valve configuration are shown in Table 1 and Supplementary Fig. 1, respectively. Peak of interest eluting from the loading column between 1.2 and 2.7 min was heart-cut and focused in the front of the analytical column. Total analysis time was 4.4 min.

Table 1
2D Gradient elution program.

Time (min)	Flow rate (μL/min)	% A	% B
Zorbax XDB CN (Loading)			
0.0	500	80	20
0.10	500	80	20
2.80	500	15	85
2.90	500	5	95
3.70	500	5	95
3.80	500	80	20
4.40	500	80	20
Poroshell EC C18 (Eluting)			
0.0	450	60	40
0.10	450	60	40
3.0	450	30	70
3.10	450	30	70
3.11	450	5	95
3.51	450	5	95
3.52	450	60	40
4.40	450	60	40

Table 2
Mass transitions and corresponding optimized voltages used in the method.

Mass transitions	Q1 (Da)	Q3 (Da)	CE (V)	CXP (V)
Aldosterone-1	359.1	189.0	−25.5	−12.0
Aldosterone-2	359.1	331.1	−22.0	−16.0
d4-aldosterone-1	363.2	190.2	−26.4	−30.8
d4-aldosterone-2	363.2	335.2	−21.4	−16.9

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Quadrupoles Q1 and Q3 were tuned to unit resolution and the MS parameters optimized for maximum signal intensity for each mass transition. The instrument was operated with electrospray ionization (ESI) in negative mode; ion-spray voltage was -3500 V, gases 1, 2 and curtain gas were 45, 60 and 25, respectively; entrance potential (EP) and declustering potential (DP) were -10 V and -50 V respectively while ion source temperature was maintained at 700 °C. Collision energies (CE) and exit potentials (CXP) of the two monitored MRMs (multiple reaction monitoring) were *m/z* 359.1/189.0 and 359.1/331.1 for aldosterone and 363.2/190.2 and 363.2/335.2 for the internal standard (Table 2). The ratio of primary (1) to secondary mass transition (2) was monitored to evaluate specificity of the analysis [29]. Quantitative calibration was performed with each batch of samples, the curve being forced through zero; data acquisition and processing was performed with Analyst™ 1.5.2.

3. Method validation

3.1. Assay performance characteristics

Performance of the assay was established based on results obtained for limit of quantitation (LOQ), limit of detection (LOD), upper limit of linearity (ULOL), imprecision, method comparison, carryover and interference studies, extraction recovery and ion suppression experiments.

LOQ was determined by preparing a pool of serum containing high concentration of aldosterone. An aldosterone depleted serum sample purchased from Golden West Biologicals (MSG 3000) was