TP123

High-Sensitivity Analysis of Aldosterone in Low-Volume Serum Samples using Micro-Flow LC-MS/MS for Clinical Research

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1. Overview

Development of a high-sensitivity method to assay aldosterone in serum samples.
Thanks to micro-flow LC/MS/MS , serum sample volume can be greatly reduced.

2. Introduction

Accurate and sensitive measurement of aldosterone in plasma or serum is a key parameter for assessment of primary aldosteronism (PA) or other adrenal diseases. Immunoassays suffer from cross-reactivity with metabolites while GC-MS methods require extensive sample preparation and derivatization. High-sensitivity analysis using LC-MS/MS, while being usually more specific and simple, is also challenging because of low ionization yield and may require high sample volume.
Micro-flow electrospray is a good compromise between ionization efficiency improvement and ease-of-use, compared to nano-electrospray. Here we present a method to quantify aldosterone in serum samples by micro-flow LC/MS/MS to reach low concentrations using a limited volume of sample.

3. Methods

3-1. Reagents

Certified aldosterone stock solution at 100µg/mL in acetonitrile was purchased from Sigma-Aldrich (St Louis, USA). Stock solution of d4-Aldosterone at 100µg/mL was purchased from IsoSciences (Ambler, USA). All solvents were of LCMS grade from Wako Chemicals (Osaka, Japan).
Double charcoal-stripped serum (pool of healthy donors) from BioIVT (Westbury, USA) was used to prepare calibration curves and quality controls. 6 individual serum samples from anonymous donor (BioIVT) were assayed as unknowns to evaluate matrix effect and assay precision.
The calibration curve was ranging from 1 to 2 500 pg/mL with levels regularly dispatched within the range. QC were prepared at 3, 1 000 and 2 250 pg/mL. Organic solvent addition to plasma was limited to 0.5% (v/v).

3-2. Sample Preparation

10 µL of serum sample were spiked with 5 µL of d4-aldosterone solution at 500 pg/mL in water/methanol 1/1. Then, 30 µL of acetonitrile were added for precipitation of proteins. After centrifugation at 10 000g, supernatant was transferred to a vial with glass-integrated insert prior to injection in the system.

3-3. Analytical Conditions

Nexera™ Mikros LC-MS/MS system with trap-and-elute configuration was used for analysis (Shimadzu Corp., Japan). The micro-LC system composed of a binary gradient microflow pump (LC-Mikros), a binary gradient with two analytical pumps for trapping process (LC-30AD), an autosampler (SIL-30AC), a 6-ports/2-positions switching valve (FCV-32AH) and a column oven (CTO-Mikros).

This system was coupled to high-sensitivity triple quad mass spectrometer (LCMS-8060) with micro-ESI ionization source (Figure 1).
Other analytical parameters are described in Tables 1 and 2.

Figure 1 Mikros LC/MS/MS system in trap-and-elute configuration

Table 1: LC Conditions

Trapping Column	: CERI C8 5µm 5*0.3mm
Trapping Flow Rate	: 100 µL/min
Trapping Mobile Phases	: A: Water/Methanol 95/5 v/v B: Acetonitrile
Trapping Gradient	: 100% A (1.5min) → 100 %B (1.5-5min) → 100 %A (5-10min)
Injection Volume	: 10 µL
Microbore Column	: Shimadzu PLONAS C18 2.7µm 100*0.2mm
Temperature	: 50ºC
Flow Rate	: 4 µL/min
Gradient	: 30% B (0-2 min) → 30 to 95%B (2-4.5 min) → 95%B (4.5-5.5 min) → 30%B (5.5-10 min)

Table 2: MS/MS conditions

System	: LCMS™-8060		
Ionization	: Micro-ESI		
Probe Voltage	: -2.4 kV (negative ionization)		
Temperature	: Interface: 125 ° C Desolvation Line: 250 ° C Heater Block: 300 ° C		
Gas Flow	: Nebulizing Gas: 3 L/min Heating Gas: 3 L/Min Drying Gas: Off		
Dwell Time	: 115 ms		
Pause time	: 3 ms		
MRM	: Compound	MRM Quant	MRM Qual
	Aldosterone	359.20 > 189.30	359.2 > 331.35
	d4-aldosterone	363.00 > 190.20	

4. Results

4-1. Calibration

Calibration curve was calculated by linear regression with $1/x^2$ weighting using internal standardization. Acceptance criteria for calibration levels was an accuracy comprised between 85-115 (80-120% at LOQ).
A typical calibration curve is shown in Figure 2.

Standard	Concentration (pg/mL)	Accuracy (%)
Cal 1	1.000	100
Cal 2	4.000	99.6
Cal 3	500.0	100
Cal 4	1000	98.4
Cal 5	1500	98.4
Cal 6	2000	102
Cal 7	2500	101

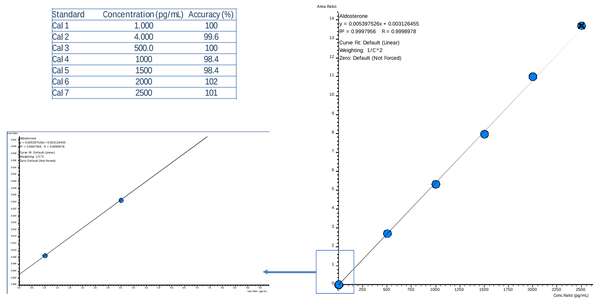

Figure 2 - Aldosterone typical calibration curve

4-2. Lower Limit of Quantitation

The lower limit of quantitation (LOQ) was established was injecting 3 independent replicates of a Quality Control prepared at 1 pg/mL within 3 independent series. Acceptance criteria were an accuracy comprised between 80-120% and a %RSD< 20%.
The Figure 3 shows an overlay of the replicate LOQ QC and Table 3 the results obtained.

Table 3: LOQ Results

Sample	Concentration (pg/mL)	Mean Accuracy (n=9)	Min Accuracy	Max Accuracy	Intra-day Precision	Inter-day Precision
QC LOQ	1	103 %	94.4 %	114 %	5.2 %	4.9 %

Table 4: Intra and Inter-day Precision

Sample	Aldosterone Mean Concentration (pg/mL, n=15)	Intra-day Precision	Inter-day Precision
Male 1	58.98	3.7%	3.4%
Male 2	44.99	3.4%	3.5%
Male 3	1498	1.5%	1.7%
Female 1	1398	1.7%	1.8%
Female 2	8.054	7.5%	6.9%
Female 3	1102	2.4%	2.2%

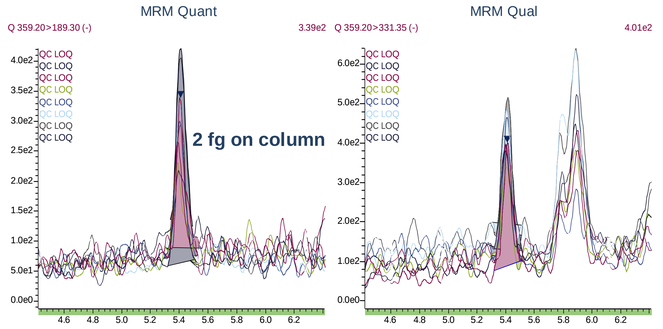

Figure 3 – Overlaid chromatograms of LOQ Replicates

4-3. Assay Performance on Real Samples

6 individual samples from anonymous donors (3 males, 3 females) were assayed within 3 days, each with 5 replicates. The results of intra and inter-day precision are presented in Table 4. For all samples, %RSD was inferior to 8%, making this assay suitable for routine analysis.
Due to the lack of certified sample, accuracy could not be evaluated. Some comparison with reference methods will be conducted in future work.
Due to the stripping process, serum used to prepare calibration standards and QC could be altered in a way that modify the matrix effect. To evaluate this parameter, we compared the peak area of the internal standard in neat solution, QC and real samples. Results are presented in Table 5. It is shown that there was no significant difference between these samples, demonstrating that matrix effect was controlled within acceptable values for such bioanalytical assay.

Table 5: Matrix Effect Evaluation

d4-Aldosterone	QC (n=12)	Samples (n=30)	Solution (n=3)
Mean area	125212	130671	126899
%RSD	18%	16%	2%
Recovery	99%	103%	---

5. Conclusions

A high-sensitivity assay was developed for aldosterone in human serum samples to support clinical research and reached LOQ as low as 1 pg/mL, but also with high dynamic range.
As a merit of higher sensitivity obtained with microflow LC/MS/MS, such limit of quantification can be obtained with low volume samples of 10 µL, enabling its application to pediatric or small animal samples.
The method also proved to be rugged and stable enough to be applied in routine.