Analysis of aldosterone by LC-MS/MS: a performance comparison between a conventional electrospray ionization source and a new atmospheric pressure ionization source

Elisa Danese[1], Mairi Pucci[1], Gian Luca Salvagno[1], Francisco Ferron[2], Natalia Oxana Lupsac[2] , Giulia Sartori[3], Francesca Pizzolo[3], Giuseppe Lippi[1]

(1) Clinical Biochemistry section, Department of Neurological, Biomedical and Movement Sciences, University of Verona, Italy
(2) Waters S.p.a. Sesto San Giovanni, Milano, Italy
(3) Internal Medicine Section, Department of Medicine, University of Verona

Background

Aldosterone, a mineralcorticoid steroid hormone, plays a central role in regulation of blood pressure. The usual serum or plasma concentration is in the range of pg/mL, which makes the analysis rather challenging. Liquid chromatography and tandem mass spectrometry (LC-MS/MS) offer several advantages over conventional radioimmunoassay (RIA)-based assays in terms of both higher sensitivity and better specificity.

Purpose

In this work we report early results on performance comparison between a conventional electrospray ionization (ESI) source and a new atmospheric pressure ionization source, UniSpray (US) (Waters Corp.) for LC-MS/MS analysis of aldosterone.

Methods

Sample extraction was performed on Oasis MAX µElution Plate (Waters) according to the application note of the producer. Chromatographic separation was performed on ACQUITY UPLC I-Class System, using CORTECS UPLC C18 column (Waters) followed by detection on TQS-Micro Tandem Quadrupole Mass Spectrometer. Standard solutions of aldosterone and its labeled internal standard were purchased by Cayman Chemical. The calibration curves were prepared in surrogate matrix of stripped human serum purchased by Chromsystems (range: 15-2000 pg/mL). Sixty five serum samples were analyzed in duplicated with ESI and US sources, and results were then compared with those obtained by the RIA method currently used in the local laboratory.

Unispray Ionization source

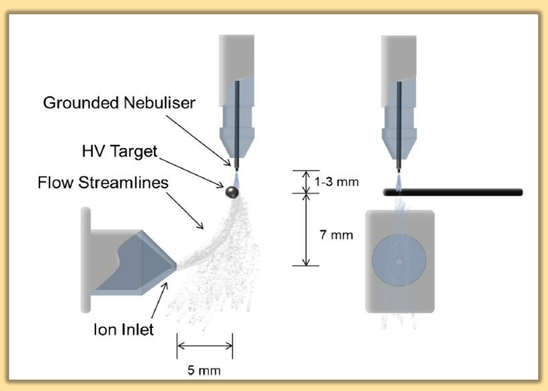

Figure 1. Internal view of the UniSpray

UniSpray shares some common features with high flow rate ESI sources in that the liquid flow is nebulised by a high velocity, concentric nitrogen gas flow. However, in comparison to ESI, the high voltage is applied to a cylindrical target rod that is positioned in close proximity to the grounded nebulizer such that the near-supersonic jet impinges on the target surface.

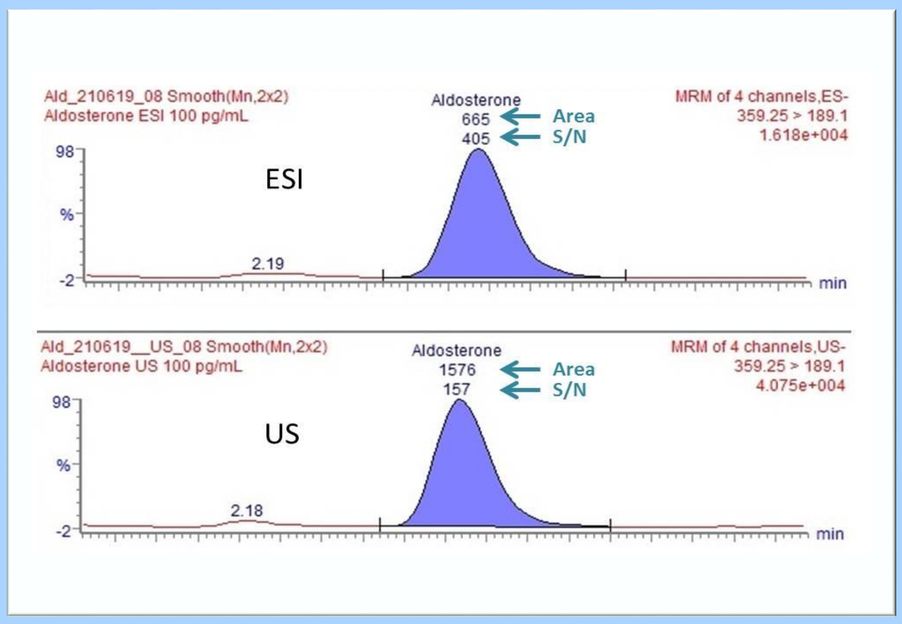

Figure 2

Representative chromatograms showing the peaks comparison between the fourth point of the calibration curve (at aldosterone concentration of 100 pg/mL) quantified with the ESI source and the UniSpray source. Differences in signal intensity and signal to noise ratio are highlighted.

Sample extraction

Sample pre-treated with zinc sulphate in methanol (50%) and phosphoric acid (0,05%) were mixed, centrifugated, loaded on the Oasis MAX µElution Plate and slowly pulled throw at low vacuum. After consecutive washes with phosphoric acid (0,05%) and ammonia (0,1%) in methanol and water, aldosterone was eluted using methanol (70%) and then water.

Results

- The limit of detection (LOD) and limit of quantification (LOQ) defined as the lowest concentration generating a signal to noise ratio (S/N) >3 and >10 were 5 and 10 pg/mL respectively for both sources. Within-run coefficients of variations were <6% over a broad range of values (4 sample pools).
- The matrix effect, evaluated as peak area of extracted post-spiked aldosterone serum samples taken as percentage of extraction solvent samples spiked to equivalent concentrations displayed a RSD of 0.5%. The recovery percentage, expressed as ratio of [(Peak Area of Pre-Spike) / (Average Peak Area of n Post-Spikes)] x 100 was 64%.
- A mean increase in signal intensity of 51.5% and a mean decrease in S/N ratio of 48% was observed for US compared to ESI (Figure 2). Linear regression analysis between RIA and LC-MS/MS on the 65 routine samples yielded correlation coefficient of 0.84 for both ESI and US (Figure 3).
- The LC-MS/MS assay with US and ESI sources displayed a mean negative proportional bias of -42% and -67% respectively compared to RIA (Figure 4). Data obtained in US and ESI were perfectly correlated (r=0.998).

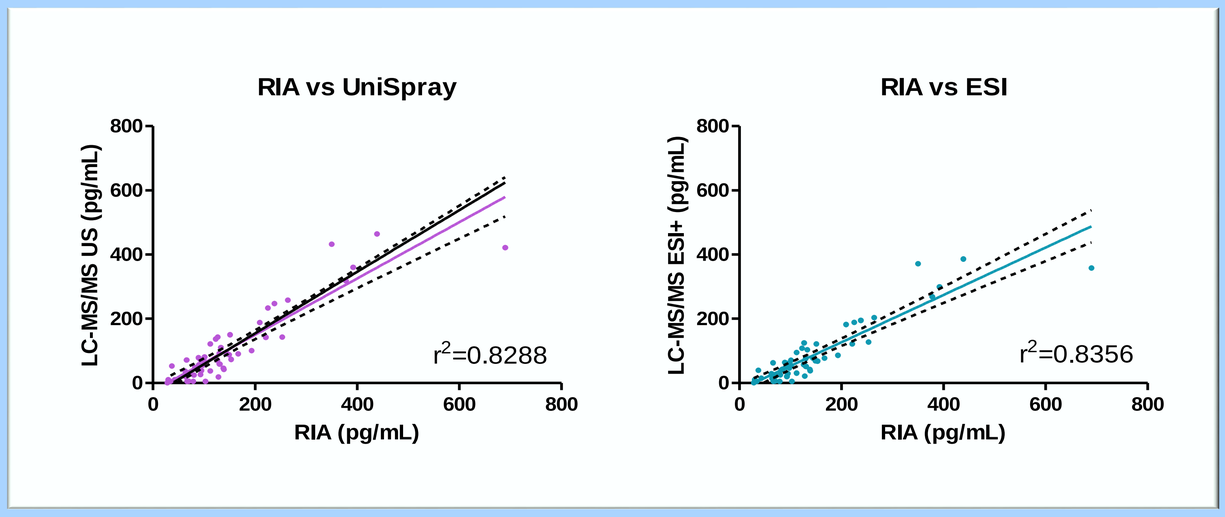

Figure 3 Linear regression analysis between RIA and LC-MS/MS

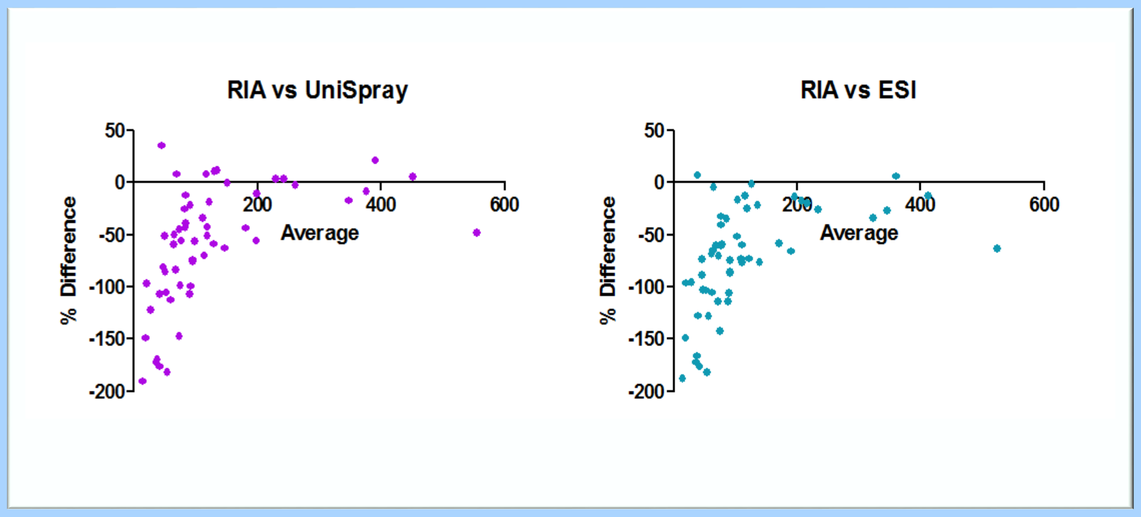

Figure 4 Bland-Altman analysis showing the presence of a significant proportional bias between the quantification of aldosterone by RIA and by LC-MS/MS method with both UniSpray and ESI sources

Conclusion

For the measurement of compounds such as aldosterone, where sensitivity is critical, the present LC–MS/MS method displayed optimal analytical performance, showing advantages of using US over ESI source in term of signal intensity. Further adjustments would be needed for improving extraction protocol efficiency.

Reference

Pizzolo F, et al. Fully automated chemiluminescence vs RIA aldosterone assay in primary aldosteronism work-up. J Hum Hypertens. 2017;31:826-30.