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High Sensitivity Analysis of Steroid Hormones with modified ESI to improve desolvation efficiency

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1. Overview

Development of a high-sensitivity method to assay a steroid panel in serum samples. Thanks to LC-MS/MS with the newly developed ion source IonFocus Unit, the sensitivity of steroid hormone was improved.

2. Introduction

Steroid hormones play a major role in the control of metabolism, neurotransmission, intracellular signaling, gene expression, reproduction and cardiovascular. Therefore, steroid hormones are very important in elucidating the mechanisms of various diseases. Furthermore, not only do steroids play roles in sedation and seizure prevention, they are known to be effective in cancer treatment and regenerative medicine. Therefore, highly sensitive analytical technologies to steroid hormones quantitation in biological samples are required in clinical research. Here we investigated higher sensitive analytical methods to steroid hormones by improving desolvation efficiency in LC-MS/MS ion source.

3. Materials and Methods

3-1. Improvement of Desolvation Efficiency

The newly developed ion source IonFocus Unit for the LCMS-8060NX is equipped with an improved heat-assisted ESI probe. The desolvation efficiency has been improved through increasing the heat transfer efficiency of the ESI heater and the maximum flow rate of the heating gas (Figure 1). This means that challenging molecules like steroid hormones can be analyzed with optimum ionization conditions and high sensitivity. As for the detail of the heater, some mesh parts are newly introduced. The mesh parts increase the surface area of the heater to improve the heat transfer efficiency.

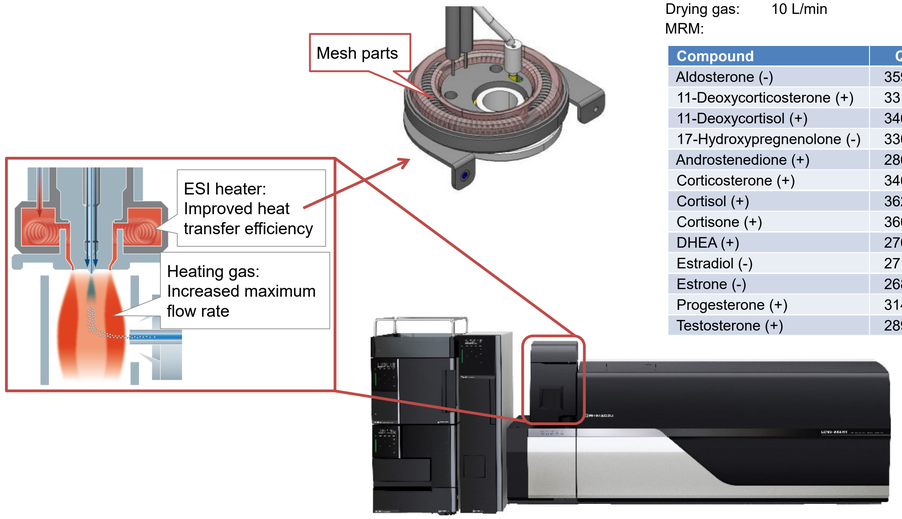

Figure1 LCMS-8060NX with IonFocus Unit

3-2. Regents

Standard of aldosterone, androstenedione, corticosterone, cortisol, cortisone, dehydroepiandrosterone (DHEA), 11-deoxycorticosterone, 11-deoxycortisol, estradiol, estrone, 17α-hydroxypregnenolone, progesterone, and testosterone were obtained from Sigma-Aldrich (St Louis, USA). Human serum sample was a pool of healthy anonymous donor and obtained from BioWest (Nuaillé, France).

3-3. Sample Preparation.

800 µL of methanol was added to 200 µL serum sample for precipitation of proteins. After 1 minute of mixing by vortex mixer, sample was centrifuged at 14 000 g for 15 minutes. 800 µL of supernatant was transferred in a new microtube and evaporated to dryness with a vacuum concentrator. After evaporation to dryness, sample was reconstituted with 400 µL of 50% methanol and transferred to a vial with glass-integrated insert prior to injection in the system.

3-4. Analytical Conditions

UHPLC (Nexera X2^{TM} system)

Column: Shim-pack Velox Biphenyl (50 mmL.×3.0 mmI.D., 2.7 µm)
Mobile phase A: 0.2 mmol/L Ammonium fluoride/water
B: 0.2 mmol/L Ammonium fluoride/acetonitrile
Flow rate: 0.8 mL/min
Injection vol.: 30 µL
Column temp.: 30°C

MS (LCMS-8060NX)

Ionization: IonFocus (ESI, Positive/Negative)
DL temp.: 150°C Heating gas: 10-25 L/min
Interface temp.: 200-400°C HB temp.: 500°C
Drying gas: 10 L/min Nebulizing gas: 3.0 L/min
MRM:

Compound	Quant. (m/z)	Qual. (m/z)
Aldosterone (-)	359.40 > 189.30	359.40 > 331.35
11-Deoxycorticosterone (+)	331.20 > 109.20	331.20 > 97.20
11-Deoxycortisol (+)	346.70 > 109.20	346.70 > 97.20
17-Hydroxypregnenolone (-)	330.70 > 109.20	330.70 > 97.10
Androstenedione (+)	286.70 > 97.20	286.70 > 109.15
Corticosterone (+)	346.70 > 329.10	346.70 > 121.20
Cortisol (+)	362.70 > 327.10	362.70 > 121.20
Cortisone (+)	360.90 > 163.25	360.90 > 121.15
DHEA (+)	270.90 > 213.10	270.90 > 253.25
Estradiol (-)	271.40 > 145.25	271.40 > 169.30
Estrone (-)	268.80 > 143.10	268.80 > 145.05
Progesterone (+)	314.80 > 97.15	314.80 > 109.20
Testosterone (+)	289.10 > 96.90	289.10 > 109.00

4. Result

4-1. Effect of Heating Gas on Sensitivity

The relationship between interface temperature (IFT, temperature of heating gas) and sensitivity of steroid hormones was investigated. Figure 2 shows the relationship for 12 of steroid hormones when the flow rate of heating gas (HG) is 10, 20, or 25 L/min. 11 of steroid hormones except for aldosterone had higher peak intensity at higher interface temperature and flow of heating gas. In particular, estradiol was strongly influenced by these parameters.

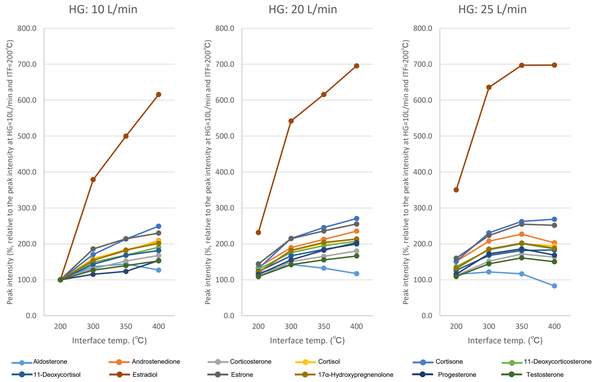

Figure 2 Relationship between interface temperature and sensitivity of 12 steroid hormones

Figure 3 shows the relationship between interface temperature and sensitivity of DHEA when the flow of HG is 10, 20, or 25 L/min. The peak intensities of DHEA was more affected by these parameters than other steroid hormones and improved dramatically.

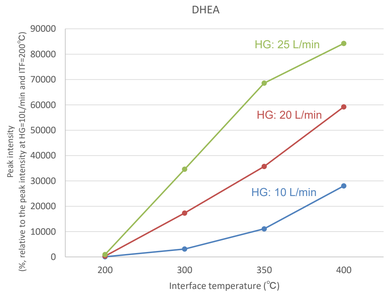

Figure 3 Relationship between interface temperature and sensitivity of DHEA

From the above results, it was found that desolvation greatly affects the sensitivity of DHEA. Therefore, we evaluated the sensitivity improvement of DHEA by the newly developed ion source IonFocus Unit in the analytical condition where more desolvation is required (flow rate of LC pump: 1.5 mL/min.)

When the flow rate of LC pump was 1.5 mL/min, the peak intensity of DHEA was improved by 3.0 times by IonFocus Unit. The intensity was improved more than when the flow rate of LC pump was 0.8 mL/min (Figure 4). It was found that the newly developed ion source IonFocus Unit can efficiently desolvate even when the flow rate of LC pump is high.

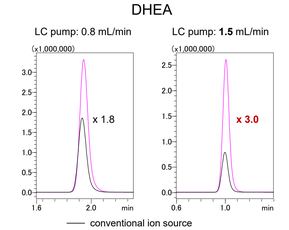

Figure 4 Sensitivity improvement of DHEA at high flow rate of LC pump

4-2. Analysis of Steroid Hormones in Human Serum

As a result of the analysis of the human serum using the newly developed ion source IonFocus Unit, 9 of steroid hormones (androstenedione, cortisol, cortisone, DHEA, 11-deoxycorticosterone, estrone, 17-hydroxypregnenolone, progesterone, testosterone) were detected (Figure 5). By improving the desolvation efficiency, low-concentration DHEA could be detected with sufficient peak intensity without complicated pretreatment such as solid phase extraction.

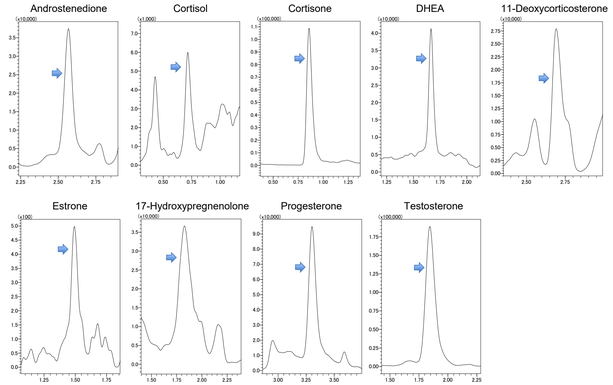

Figure 5 MRM chromatograms of steroid hormones in human serum

5. Conclusions

- It was found that increasing the desolvation efficiency improves the sensitivity of steroid hormones (especially DHEA). The sensitivity of steroid hormone was improved by the newly developed ion source IonFocus Unit.
- In pediatric or post-menopausal samples, circulating levels of reproductive hormones are very low and therefore challenging to measure. In addition, sample volume availability can be limited adding difficulties for high-sensitivity analysis. Therefore, LC-MS/MS with newly developed ion source IonFocus Unit will support these clinical researches.