Throughput and Sample Preparation Improvements for Endocrine Research by Micro-flow LC-MS/MS

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ABSTRACT

Simple and rapid analysis of endocrine samples is necessary in a clinical research laboratory. Additionally, as financial and environmental concerns become more prevalent, heavy use of solvents for high flow LC-MS/MS methods, particularly those involving online extraction, can be an issue. Microflow LC offers a solution to these concerns, whilst greatly enhancing on column sensitivity and chromatography performance, reducing sample consumption and improving system robustness and uptime. As the sample injection volume is minimized, robustness and reproducibility of the entire method is dramatically increased as matrix effects are significantly reduced.

INTRODUCTION

The measurement of steroids in serum with LC/MS/MS presents several challenges . The low level intrinsic sensitivity, coupled in some cases with a very low concentration in plasma, creates difficulties in obtaining adequate functional sensitivity and levels of quantification. Microflow LC, enabling high mass sensitivity and the use of small amounts of sample, is an attractive option for quantitation. Here, we investigate the ability of micro-flow liquid chromatography to allow sensitive and rapid analysis of steroids in serum, enabling simple sample extraction methodologies and high sample throughput.

Micro-flow liquid chromatography has become a compelling alternative to conventional HPLC for many analyses given its benefits in solvent saving, high throughput, and low sample consumption. Lower solvent consumption resulting from the use of micro-flow rates (5-50 µl/min), as compared to a typical flow rate of 500 µl/min using conventional HPLC, significantly reduces solvent and waste disposal costs. In addition, high on-column linear velocities and low mixing and delay volumes allow for fast chromatography and thus higher sample throughput.

These benefits are realized while sensitivity is maintained or enhanced as compared to conventional HPLC, making micro- flow LC an excellent fit for the analysis of steroids in serum given the sensitivity and throughput requirements of this assay.

MATERIALS AND METHODS

Sample Preparation:
20µL serum calibrators & QCc samples were pipetted into a standard 1.5ml eppendorf tube. 80µL precipitation reagent (a solution of methanolic zinc sulphate) was added, and the sample vortex mixed. Following centrifugation, 50µL of supernatant was added to 50µL LC-MS grade water, the sample vortex mixed again, and was injected onto the micro-flow LC-MS/MS System.

HPLC Conditions:
µHPLC separation was provided by a Sciex M3 MicroLC micro-flow liquid chromatography system equipped with a YMC C8 50x0.5mm µHPLC column, maintained at 45ºC. A gradient of water and methanol (both containing 0.1% formic acid) was used at a flow rate of 15µL/min. The injection volume was set to 3µL. The total run time for all compounds, including column reequilibration time, was 3 minutes.

MS/MS Conditions:
A Sciex TripleQuad™ 4500 LC/MS/MS system with a Turbo V source and a 50µm electrospray capillary was used. Two MRM transitions per compound were analysed. MRM transitions were optimised using individual standards and direct infusion. Source and gas conditions were optimised by flow injection of the lowest sensitivity compound (Testosterone).

SCIEX M3 MicroLC

SCIEX TripleQuad® 4500 LC/MS/MS system

RESULTS

Comparison of performance vs conventional HPLC
Commercial control serum was obtained at low levels (0.1nmol/L for Androstenedione, Testosterone and 17-OHP) and analysed using the proposed approach, having been previously analysed using conventional HPLC under standard conditions for the analysis of steroids. Figure 1 shows sensitivity data on a commercial serum calibrator at the expected limit of detection analysed by the proposed method.

Figure 1. Sensitivity data on a commercial serum calibrator at approximately the proposed LOD

Micro-flow chomatography to reduce background and increase robustness
The scalability of micro-flow chromatography allows for significantly reduced sample requirements and injection volumes. As a consequence, background is significantly reduced and sensitivity with respect to amount on column is greatly enhanced. Figure 2 shows the comparison of an extract of a stripped serum spike of Testosterone at 0.1nmol/L, ran by conventional and micro-flow LC. As can be seen, particularly in the internal standard channel, interference from additional peaks is greatly reduced.

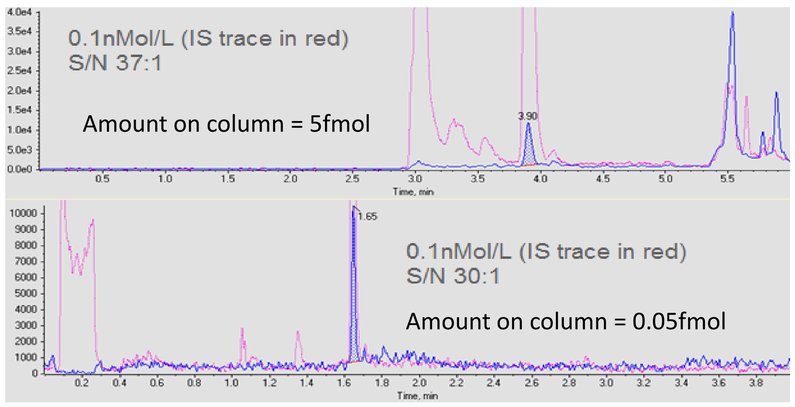

Figure 2. Comparison of conventional (top) and micro-flow (bottom) LC for a low level testosterone extract.

Analytical performance
Figures 3 and 4 show that limits of detection and linearities are within acceptable limits. Enhanced performance achieved by the micro-flow LC technology does not compromise analytical performance.

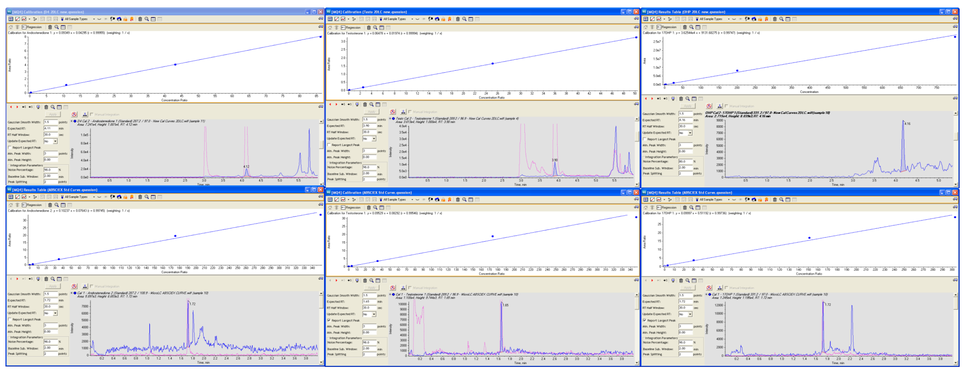

Figure 3. Standard curves and low level QC chromatograms, Androstenedione, Testosterone and 17-OHP

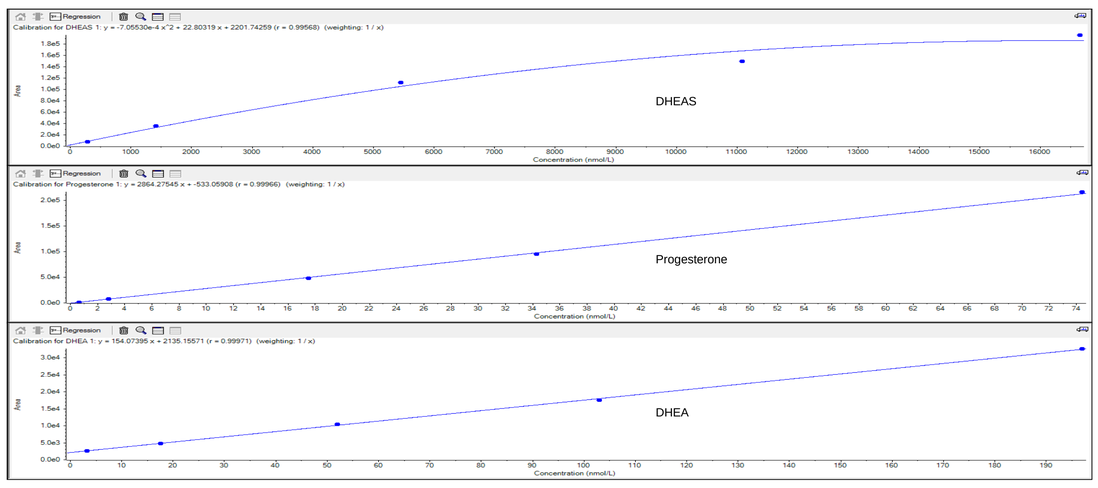

Figure 4. Additional Standard Curves in serum for DHEAS, Progesterone and DHEA. Note no deuterated internal standard was obtainable for DHEAS, therefore a quadratic fit was necessary.

Reproducibility
A series of commercial serum QC samples were obtained at low, varied concentrations and analysed by the proposed method. Statistics were generated on the results and are shown in figure 5.

Analyte	Concentration in QC (nmol/L)	S/N (1SD)	%CV (n = 6)
Testosterone	0.1	30	6.2
Androsteinedione	0.1	16.4	8.2
Progesterone	0.65	9.6	9.5
DHEAS	282	51.3	4.4
DHEA	3.28	20	9.4
17-hydroxyprogesterone	0.1	42	8.6

Figure 7. Statistical results generated from the analysis of commercial QC samples

CONCLUSIONS

- We present here a proof-of-concept analysis for the use of micro-flow liquid chromatography for the analysis of steroids in serum.
- The proposed method offers advantages over conventional HPLC in terms of sample requirements, throughput and solvent consumption/disposal.
- In addition, reduction in injection volume and therefore amount of extracted matrix introduced to the system allows improvements in assay robustness and instrument uptime.
- Microflow LC shows good analytical performance across relevant concentration ranges.
- Further research to expand the compounds analysed is ongoing.

TRADEMARKS/LICENSING

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