Quantitative analysis of cortisol and cortisone in human urine using solid-phase extraction (SPE)

Pierre Negri[1], Aymeric Morla[2], Arie-Jan de Graaf[3], and Fabian Reijn[3],

[1] SCIEX, USA, [2]SCIEX, France, [3]Diagnotix, The Netherlands

This technical note demonstrates the accurate quantitation of cortisol and cortisone in human urine using a solid-phase extraction (SPE) procedure and analysis by the SCIEX QTRAP 4500 system. Low- to mid-nmol/L level sensitivity was achieved at the lowest calibrator with signal-to-noise ratios (S/N) of 1330:1 for cortisol at 34.0 nmol/L and 1688:1 for cortisone at 41.0 nmol/L. In addition, excellent linearity was observed across clinically relevant concentrations, with r^2 values above 0.99 across the calibration range (34.0-601 nmol/L for cortisol and 41.0-693 nmol/L for cortisone).

Key benefits of cortisol and cortisone analysis from human urine using the QTRAP 4500 system

- **Efficient sample preparation.** Cortisol and cortisone were efficiently extracted from human urine samples using solid-phase extraction (SPE), starting with 100 µL of human urine
- **Excellent linearity.** Calibration curves for cortisol and cortisone showed r^2 values above 0.99 across the calibration range (34.0-601 nmol/L for cortisol and 41.0-693 nmol/L for cortisone)
- **Low-nmol/L level sensitivity and excellent quantitative performance.** Sensitive quantitation for cortisol and cortisone was achieved with excellent precision (2.9% for cortisol and 0.4% for cortisone) and accuracy (101.7% for cortisol and 95.9% for cortisone) at the lowest calibrator levels (34.0 nmol/L for cortisol and 41.0 nmol/L for cortisone)

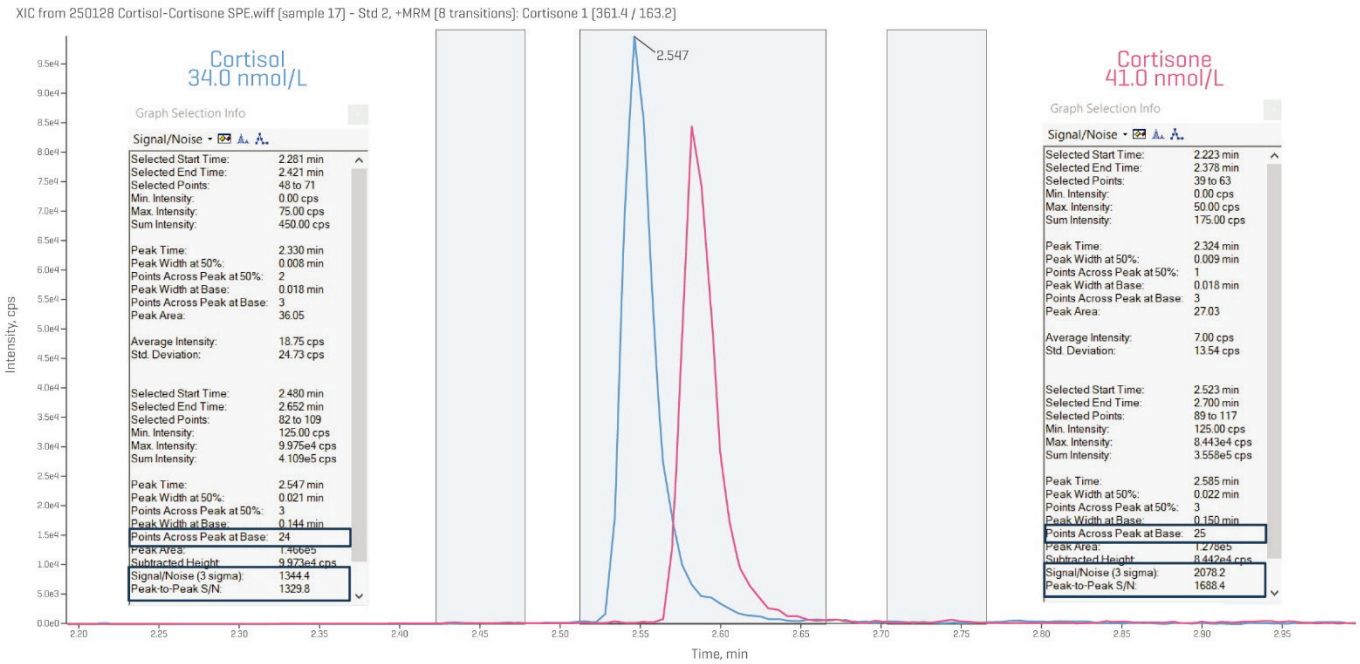

Figure 1. Extracted ion chromatogram (XIC) of cortisol (blue) and cortisone (pink) extracted from urine matrix using SPE. Overlaid XIC is shown for calibration standards in matrix for cortisol at 34.0 nmol/L and cortisone at 41.0 nmol/L. Signal-to-noise ratios (S/N) of 1330:1 for cortisol and 1688:1 for cortisone, respectively, were achieved based on a peak-to-peak algorithm.

Introduction

Cortisol and cortisone play pivotal roles in governing the body's stress response, metabolic pathways, and immune regulation. Their concentrations vary widely and can shift in response to both normal physiology and underlying disease, making reliable detection in human urine especially important. Robust quantification of these glucocorticoids offers valuable information about adrenal function and hormonal homeostasis, supporting a broad range of clinical assessments and research applications.

Methods

Sample preparation: Sample preparation was performed using Diagnotix's cortisol & cortisone reagents set [https://www.diagnotix.com/en/products/cortisol-cortisone-urine-lc-ms-kit-2] according to the manufacturer's specifications. 100 µL calibrators in human urine were used for the procedure. This reagent set is only available in certain EU countries.

Liquid chromatography conditions: Chromatographic separation was achieved using a Phenomenex Kinetex Luna Omega Polar C18 column (100 x 2.1 mm, 2.1 µm, 00D-4760-AN). Mobile phases A and B from the reagents set were used. The total run time was 5 minutes at a flow rate of 550 µL/min. The injection volume was 5 µL. The LC gradient program is presented in **Table 1**.

Table 1: Chromatographic gradient for the analysis of cortisol and cortisone in human urine.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	85	15
4.2	40	60
4.21	0	100
5.2	0	100
5.21	85	15
7.5	85	15

Mass spectrometry conditions: Mass spectrometry analysis was performed using the QTRAP 4500 system operating in positive electrospray ionization mode. Source and gas conditions are presented in **Table 2**. Compound-dependent parameters were optimized by infusion.

Table 2: Source and gas parameters for the analysis of cortisol and cortisone in human urine.

Parameter	Value
Polarity	Positive
Ion source gas 1	50 psi
Ion source gas 2	60 psi
Curtain gas	30 psi
Source temperature	400 °C
Ion spray voltage	5500 V
CAD gas	9

Data processing: Data processing was performed using SCIEX OS software (version 3.1.6). Peak integration was achieved using the MQ4 algorithm. Quantitative analysis was conducted in the Analytics module of SCIEX OS, where calibration curves, concentration calculations, assay precision, and accuracy statistics were automatically generated.

Results and discussion

Figure 1 shows the chromatographic separation of cortisol and cortisone in a control human urine sample at a final concentration of 34.0 nmol/L for cortisol and 41.0 nmol/L for cortisone, respectively. The extracted ion chromatograms showed a S/N of 1330:1 for cortisol and 1688:1 for cortisone, at the lowest matrix calibrator measured (34.0 nmol/L for cortisol and 41.0 nmol/L for cortisone), calculated using a peak-to-peak algorithm.

Figure 2 shows the representative extracted ion chromatograms (XICs) for A) cortisol and B) cortisone across their respective concentration ranges (34.0-601 nmol/L for cortisol and 41.0-693 nmol/L for cortisone). The peaks shown for the lowest matrix calibrators measured (34.0 nmol/L for cortisol and 41.0 nmol/L for cortisone) are well above the blank for both analytes.

The quantitative performance of the method was investigated by injecting a series of calibrator samples spiked at concentrations ranging from 34.0-601 nmol/L for cortisol and 41.0-693 nmol/L for cortisone, respectively. Linearity, accuracy and precision were assessed across the calibration ranges for each of the two analytes. **Figure 3** shows the calibration curves for cortisol (top) and cortisone (bottom) over the analytes' respective calibration ranges. The plots show excellent linear responses across the calibration series, with r^2 values greater than 0.99 for both analytes.

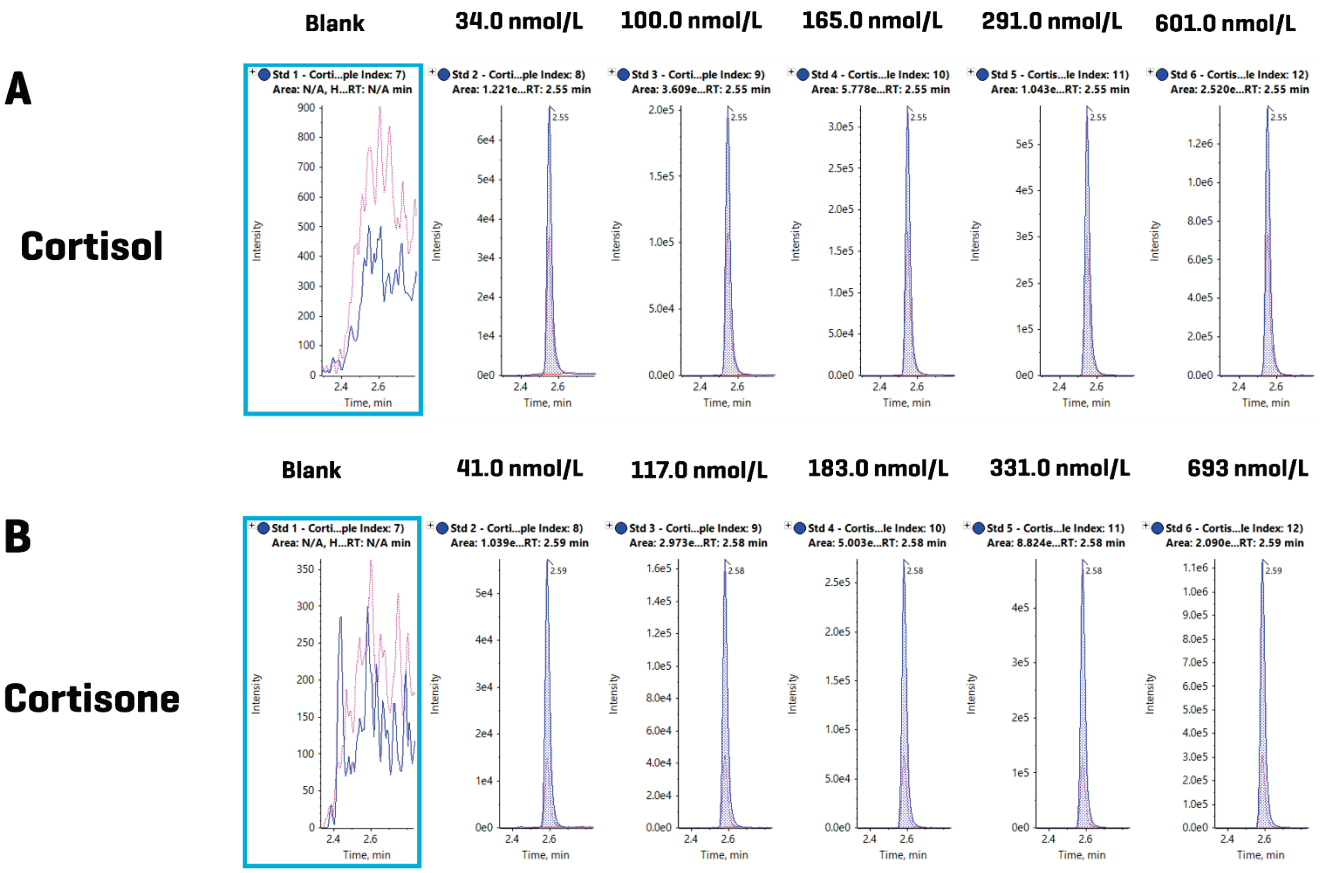

Figure 2. Extracted ion chromatograms (XICs) for cortisol and cortisone in an extracted urine matrix using the QTRAP 4500 system. The XICs show the signal for A) cortisol and B) cortisone across their respective concentration ranges (34.0-601 nmol/L for cortisol and 41.0-693 nmol/L for cortisone).

The accuracy and precision values were calculated by 3 replicates in matrix at the lowest matrix calibrators measured (34.0 nmol/L for cortisol and 41.0 nmol/L for cortisone). The accuracy was 101.7% for cortisol and 95.9% for cortisone, respectively. The precision (%CV) was 2.9% for cortisol and 0.4% for cortisone.

Conclusions

A fast and accurate LC-MS/MS method for the quantitation of cortisol and cortisone extracted from human urine samples was developed using the SCIEX QTRAP 4500 system. The method demonstrated:

- Sample preparation using solid-phase extraction (SPE), starting with 100 µL of human urine
- Excellent sensitivity at the lowest calibrator level, resulting in S/N of 1330:1 for cortisol at 34.0 nmol/L and 1688:1 for cortisone at 41.0 nmol/L.
- Excellent linear responses across the calibration series consisting of 5 calibrators, with r^2 values greater than 0.99 for both analytes
- High quantitative performance of the method, resulting in excellent precision (2.9% for cortisol and 0.4% for cortisone) and accuracy (101.7% for cortisol and 95.9% for cortisone) at the lowest calibrator levels (34.0 nmol/L for cortisol and 41.0 nmol/L for cortisone)

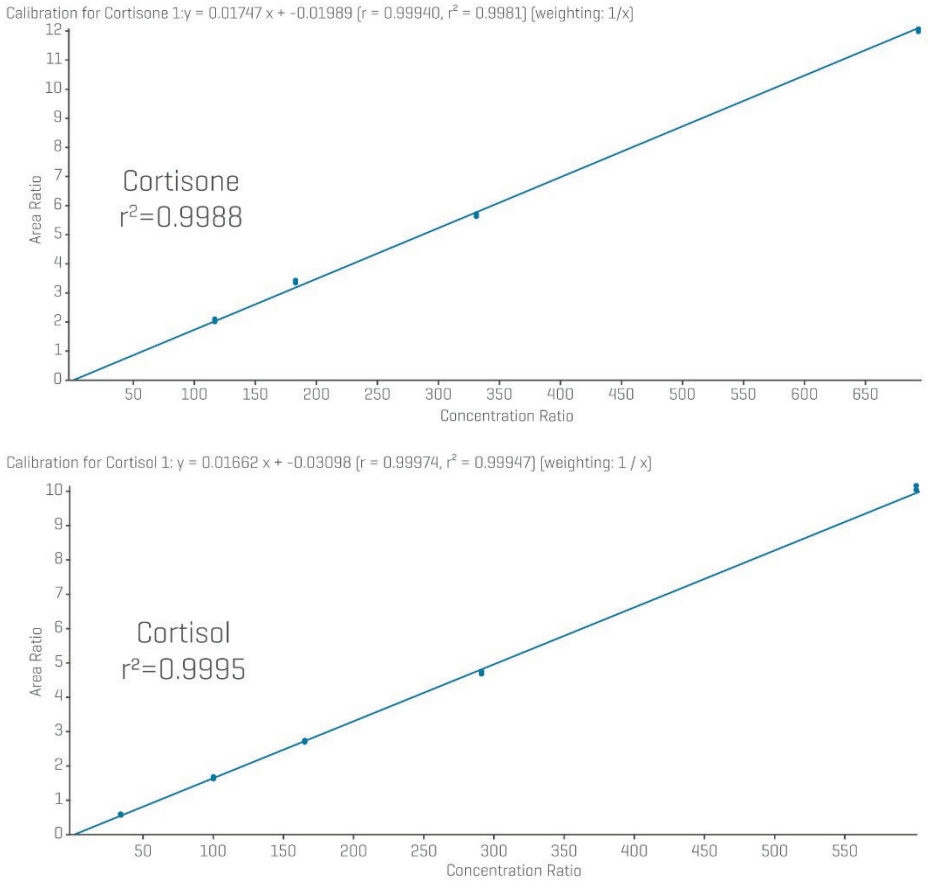

Figure 3. Linear calibration curves for cortisol (top) and cortisone (bottom) in urine matrix using SPE. The calibration curves were run across the concentration ranges (34.0-601 nmol/L for cortisol and 41.0-693 nmol/L for cortisone, respectively). The curves were generated using linear regression and 1/x weighting for cortisol and cortisone in urine, resulting in r^2 values of 0.9995 and 0.9988, respectively.

Headquarters
250 Forest Street, Marlborough,
MA 01752 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices