LC-MS/MS DETERMINATION OF LOW-MOLECULAR-WEIGHT ANALYTES IN HUMAN URINE AND SERUM AFTER AUTOMATED SAMPLE PREPARATION

Orsolya Geda[1], Mónika Vezse[2], Éva Csöndör[1], Róbert Farkas[1], Gellért Balázs Karvaly[1]

[1]Laboratory of Mass Spectrometry and Separation Technology, Department of Laboratory Medicine, Faculty of Medicine, Semmelweis University, 4 Nagyvárad Square, 1089 Budapest, Hungary

[2]Biotage Sweden AB, Uppsala, Sweden

Introduction

Automated sample preparation offers significant advantages for diagnostic laboratories in the execution of assays involving a large sample load and labor-intensive manual preparation steps. In our laboratory, the measurement of cortisol in urine and symmetric dimethylarginine (SDMA) in serum is routinely performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Multi-step sample preparation is carried out with 96-well deep-well plates. For cortisol analysis, urine samples are processed using supported liquid extraction (SLE), while serum is prepared for SDMA analysis using protein precipitation (PPT). We present the feasibility of automating these workflows using the novel and versatile Biotage® Extrahera™ LV-200 sample preparation platform.

Objective

Our aim was to evaluate the efficiency of sample preparation using the Biotage® Extrahera™ LV-200 automated sample preparation system.

Methods

The efficiency of method transfer was assessed by preparing serum and urine samples collected for routine diagnostic purposes, by using both the manual and automated procedures (Figure 1). A total of 84 human urine and 36 serum samples were processed for the analysis of cortisol and SDMA, respectively.

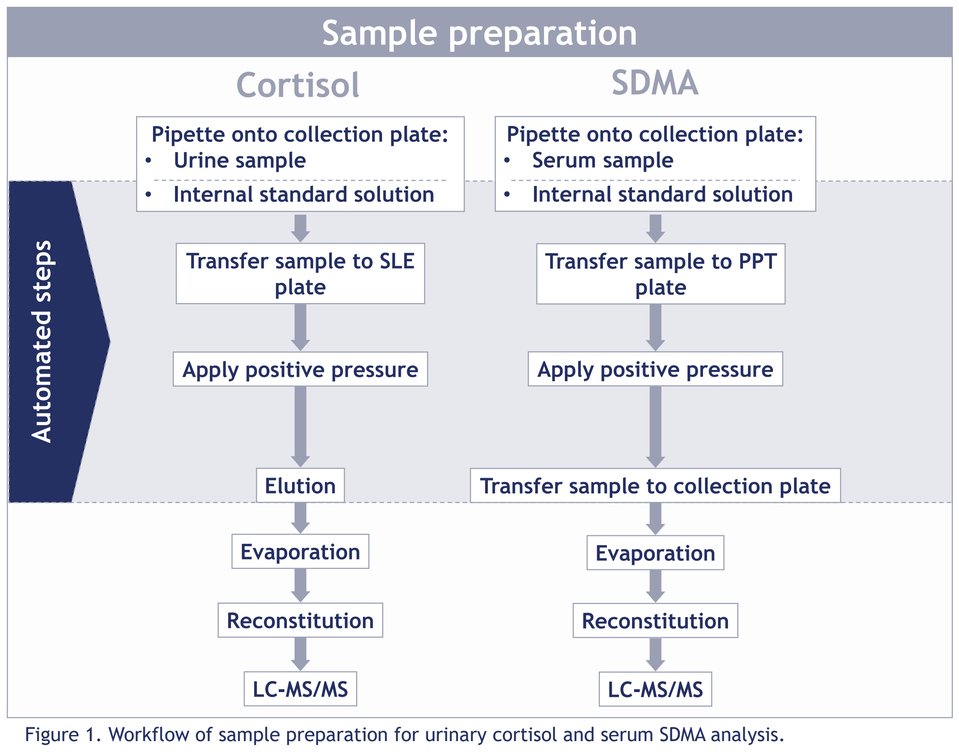

Figure 1. Workflow of sample preparation for urinary cortisol and serum SDMA analysis.

The measurement of cortisol and SDMA was carried out using LC-MS/MS. Mass spectrometric detection was performed in multiple reaction monitoring (MRM) mode. The following ion transitions were applied: cortisol, 363.3 → 121.1; $^2H_6$-cortisol internal standard, 369.3 → 142.1; SDMA at 338.1 → 172.3, and $^2H_6$-SDMA internal standard, 344.1 → 175.2. Further analytical details are provided in Table 1.

Table 1. General analytical settings.

	Cortisol	SDMA
Instrument	Shimadzu Nexera XR HPLC system coupled to AB Sciex QTrap 5500 mass spectrometer	Shimadzu Nexera X2 HPLC system coupled to LCMS 8050 CL mass spectrometer
Stationary phase	Kinetex Biphenyl	Poroshell C18
Mobile phase	A: water containing 0.1% formic acid (v/v) B: methanol containing 0.1% formic acid (v/v)	A: water containing 0.1% formic acid (v/v) B: methanol containing 0.1% formic acid (v/v) C: acetonitrile containing 0.1% formic acid (v/v)
Flow rate	0.3 mL/min	0.3 mL/min
Injection volume	2 µL	1 µL

The calibrated concentration ranges were 2–400 ng/mL for cortisol, and 25–500 ng/mL for SDMA. The results obtained using manual and automated sample preparation were compared by using Bland-Altman analysis, Passing-Bablok regression and Lin's concordance correlation coefficient (CCC). The strength of concordance was evaluated according to McBride's criteria[1].

Results of Cortisol Sample Preparation (urine)

The evaluation of the cortisol measurements are summarized in Table 2. In the SLE-based sample preparation, Bland-Altman analysis indicated a systematic bias of 1.77% between the manual and automated methods (Figure 2). Passing-Bablok regression revealed no significant proportional error, and the constant error was also negligible (Figure 3). Excellent agreement was observed between the manual and automated procedures (CCC = 0.984).

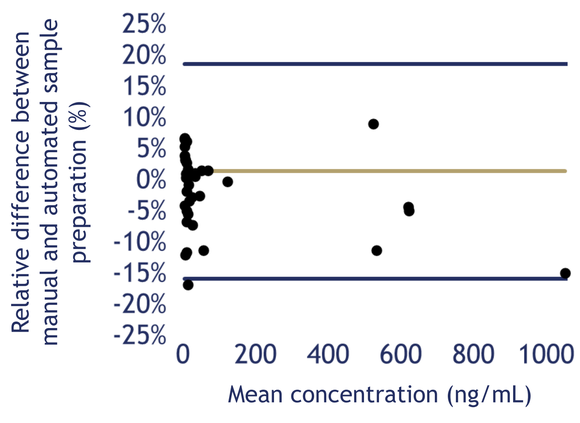

Figure 2. Bland–Altman comparison of manual and automated sample preparation for cortisol determination.

Table 2. Analytical comparison of cortisol concentrations obtained with manual and automated preparation.

Bland-Altman mean difference, c_{manual}–$c_{automated}$ (95% CI)		1.77% (-17.4—20.9%)
Passing-Bablok regression	Slope (95% CI)	1.01 (0.98—1.05)
	Intercept (95% CI)	-0.28 (-0.55—-0.39)
	τ	0.943
Lin's concordance correlation coefficient		0.984 (substantial agreement)

τ: Kendall correlation coefficient.

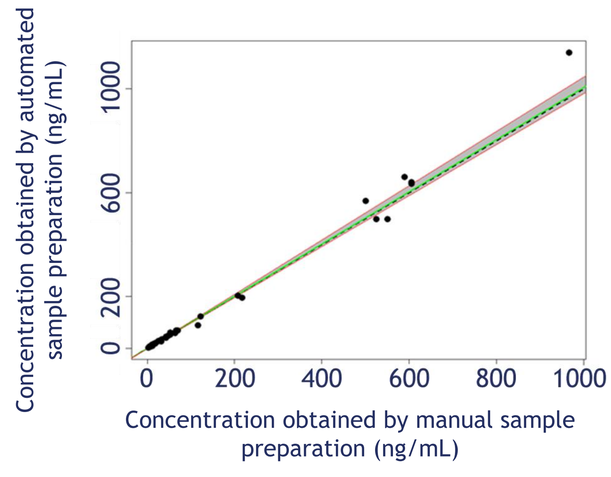

Figure 3. Passing–Bablok comparison of manual and automated sample preparation for cortisol determination.

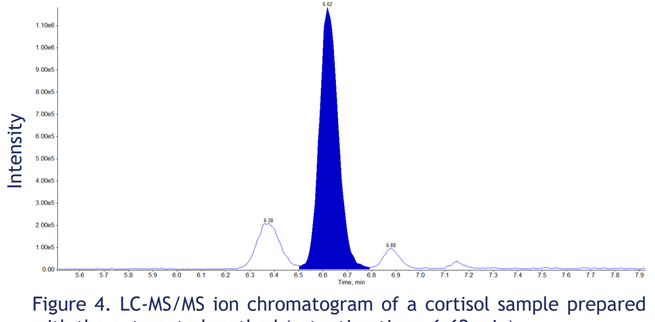

Figure 4. LC-MS/MS ion chromatogram of a cortisol sample prepared with the automated method (retention time: 6.62 min).

Results of SDMA Sample Preparation (serum)

The outcomes of the statistical comparison of SDMA results are summarized in Table 3. The systematic bias between manual and automated methods was -3.3% (Figure 5). Statistically, Passing–Bablok regression revealed neither proportional nor constant bias (Figure 6). A substantial agreement was found between the manual and automated procedures (CCC = 0.981).

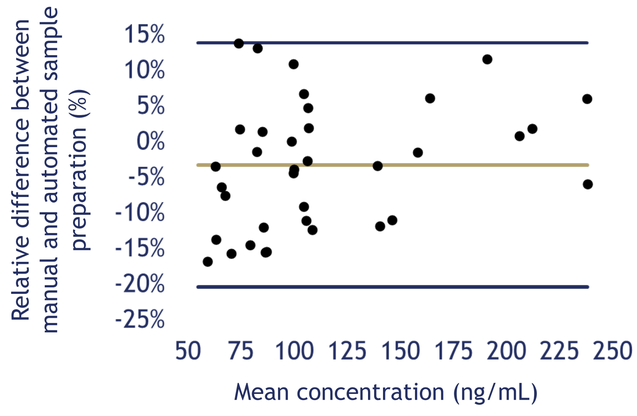

Figure 5. Bland–Altman comparison of manual and automated sample preparation for SDMA determination.

Table 3. Analytical comparison of SDMA concentrations obtained with manual and automated sample preparation.

Bland-Altman mean difference, c_{manual}–$c_{automated}$ (95% CI)		-3.3% (-20.4—13.8%)
Passing-Bablok regression	Slope (95% CI)	1.03 (0.94—1.12)
	Intercept (95% CI)	-6.51 (-16.3—-2.64)
	τ	0.828
Lin's concordance correlation coefficient		0.981 (substantial agreement)

τ: Kendall correlation coefficient.

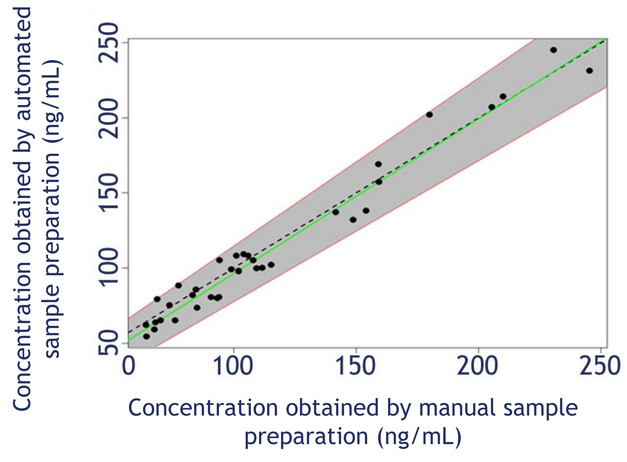

Figure 6. Passing–Bablok comparison of manual and automated sample preparation for SDMA determination.

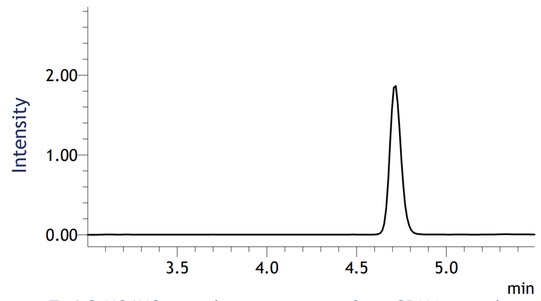

Figure 7. LC-MS/MS ion chromatogram of an SDMA sample prepared with the automated method (retention time: 4.68 min).

Conclusions

The manual and the Biotage® Extrahera™ LV-200–based automated sample preparation procedures proved to be analytically equivalent across both clinical laboratory methods investigated. The automation of conventionally manual sample preparation steps demonstrated sufficient effectiveness. For urinary cortisol, the automated workflow included internal standard addition, transfer of the sample onto the SLE plate, and elution. In the case of SDMA, all steps (internal standard addition, protein precipitation and derivatization) could be accomplished using Biotage® Extrahera™ LV-200.

Reference: 1. McBride, G., A proposal for strength-of-agreement criteria for Lin's concordance correlation coefficient. NIWA client report: HAM2005-062, 2005. 45: p. 307-310.

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