Robustness Evaluation of ekspert™ microLC 200 system in the Quantitation of Drugs in Hydrolyzed Urine

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Introduction

The interest in microflow UHPLC for quantitation is increasing due to its ability to improve the throughput and sensitivity while reducing costs and instrument maintenance intervals.

Historically, microflow UHPLC has been overlooked in the development of LC/MS/MS methods which involve challenging sample matrices because of robustness issues. The ekspert microLC 200 is specifically designed to provide reliable and robust performance typically associated with 2.1 mm ID column based analytical separations.

This application note presents the results of a long term robustness study using the ekspert microLC 200 and AB SCIEX QTRAP® 5500 for the quantitation of drugs in hydrolyzed urine

Key benefits of microflow UHPLC in drugs quantitation

- Improvements to sensitivity and assay throughput.
- Reduction of matrix effect and sample consumption.
- Reduction in operation costs and frequency of scheduled maintenance

Challenges in the quantification of drugs using microflow UHPLC

- Robust quantitative analysis of drugs in biological matrices.
- Reduced column life often associated with analysis of hydrolyzed human urine sample.
- Achieve rugged conventional HPLC robustness with microLC 200 µUHPLC system.

Unique features of AB SCIEX ekspert microLC 200

- Dedicated high performance microflow pumping system with Microfluidic Flow Control™ (MFC).
- Seamless MS Integration within a dynamic flow range of 5-200 µL/min using the AB SCIEX microflow ESI source.
- Robust UHPLC performance capable of operating at pressures up to 10,000 psi.
- ekspert microLC 200 robustness upgrade kit and guard column promote rugged system performance.

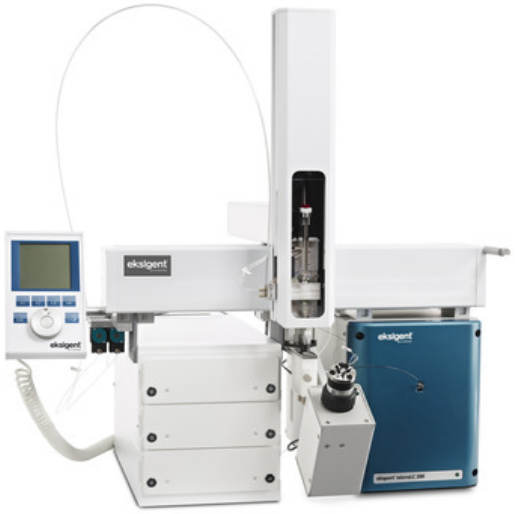


Table 1. HPLC gradient for the analysis of triazenes mix experiments

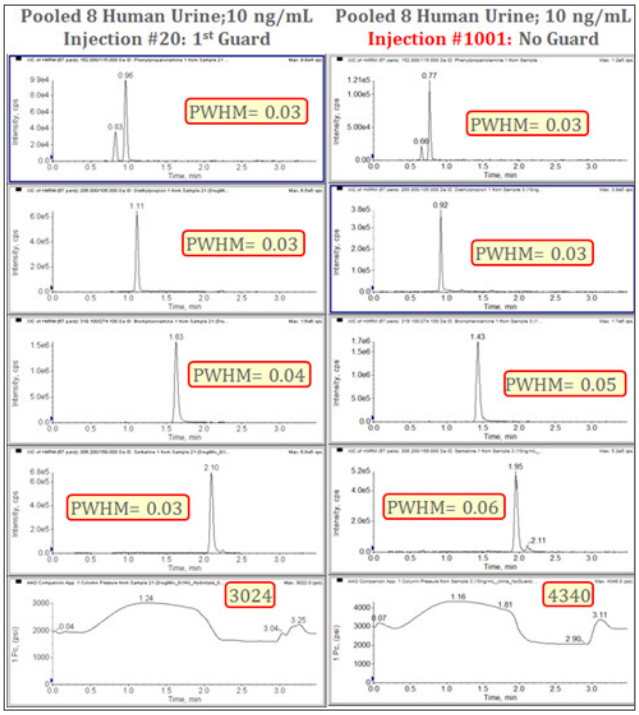


Figure 1: Chromatography and column pressure comparison between 20th and 1000th run with guard replaced every 250 runs.

Materials and Methods

Sample Preparation

Sample preparation is based upon a simple “dilute and shoot” methodology. Eight human urine samples were pooled in order to normalize matrix effects; enzymatic hydrolysis was then performed using industry standard procedures. The dilution factor of the hydrolyzed urine injected was 4x.

LC Conditions

LC System	AB SCIEX ekspert microLC 200
Analytical Column	AB SCIEX ChromXP™ C18-CL 0.5 x 50mm; 3 µm (PN: 805-10013)
Guard Column	C18, 1×5 mm, 5 µm; Guard columns replaced every 250 injections
Flow Rate	40 µL/ min
Injection Volume	1 µL full loop (3 µL sample consumption)
Column Temp	40°C
Mobile Phase A	Water (0.1% Formic Acid, 5 mM ammonium fomate)
Mobile Phase B	Methanol (0.1% Formic Acid, 5 mM ammonium fomate)

MS Conditions

Q Trap 5500 system

Turbo VTM ion source equipped with 65um stainless steel microflow electrode (PN: 800-00420)

TEM: 500.00

CUR: 20.00

TEM: 500.00

CUR: 20.00

CAD: Medium

GAS1: 20.00

GAS2: 60.00

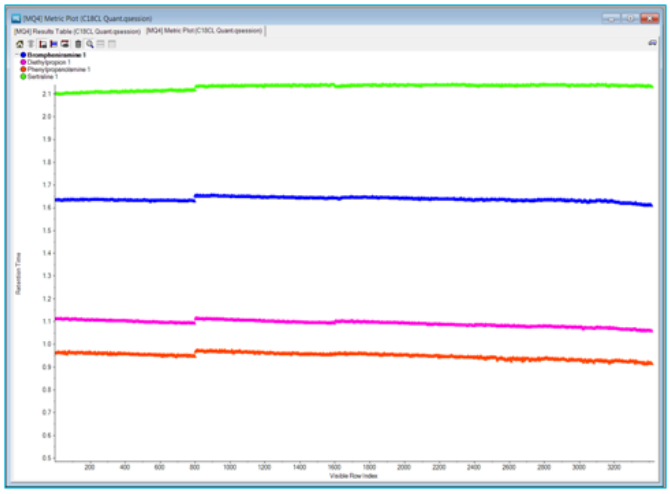
Gradient Conditions

Time (min)	Mobile phase A%	Mobile phase B%
0.0	*98*	*2*
0.2	*98*	*2*
0.8	*60*	*40*
1.8	*30*	*70*
1.9	*5*	*95*
2.9	*5*	*95*
3.0	*98*	*2*
3.5	*98*	*2*

Software

Data Acquisition	*Analyst® 1.6.2software*
Quantitation	MultiQuant™2.1software

Figure 2. Retention time plot of QC samples over 1000 injections.



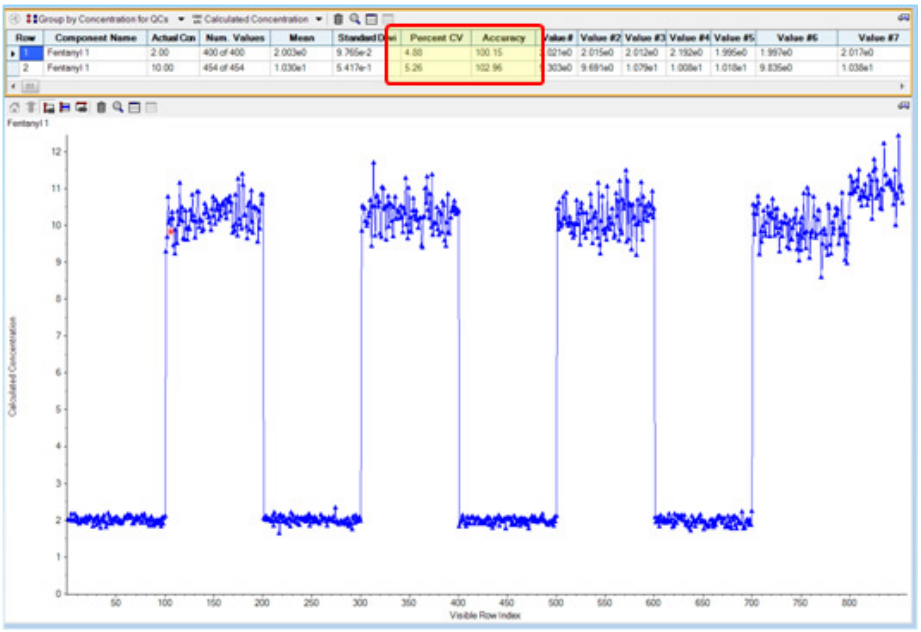


Figure 3. Long term accuracy and precision of QC samples (2,10ng/mL in hydrolyzed urine); %CV < 6%; 100%< Accuracy < 103%.

Conclusions

1. The ekspert microLC 200 system can provide rugged, reliable chromatographic performance over the course of 1000 injections of hydrolyzed urine.
2. Microflow UHPLC is a viable analytical strategy in the quantification of common pain panel compounds.
3. Advantages common to microflow UHPLC can be realized without compromising the robustness of the LC/MS/MS system.\
4. The utilization of guard columns and robustness kit improves the long term system performance in the presence of complex matrices.

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