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Technical Report

Ultra-Fast Analysis of Drugs in Biological Fluids with the SIL-40 Autosampler

- Analytical Intelligence Part 5 -

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Abstract:

There is increasing demand in modern clinical/pharmaceutical laboratories for high-throughput liquid chromatography-tandem mass spectrometry analysis for drug quantitation in biological fluids. The analysis of drugs and metabolites using LC-MS/MS typically requires analysis times of a few minutes per sample. In this report, we describe the use of the SIL-40 series autosampler (SIL-40) for the precise and ultra-fast quantitation of drugs in plasma samples within less than 20 sec, thanks to the extremely low injection cycle time, introducing real examples of increased productivity in the lab.

Keywords: Ultra-fast injection, High-throughput analysis, SIL-40 series

1. Importance of the Injection Cycle Time in High-Throughput Analysis

There is growing need for increased throughput of liquid chromatography-tandem mass spectrometry analysis in modern laboratories. For example, in clinical/pharmaceutical laboratories, the maximization of analytical throughput and associated higher efficiency facilitates more rapid reporting of results, increasing the effectiveness of all corrective actions for treatments such as dose adjustments.

Development of bioanalytical systems with higher throughput is also a prime concern for drug metabolism and pharmacokinetic (DMPK) evaluation, a crucial step in early drug discovery which deals with a large number of samples.

The analysis of drugs and metabolites using LC-MS/MS typically requires analysis times of a few minutes per sample. Recent advances in LC technology, such as improved LC column quality and higher pressure tolerance, allow the shortening of analytical cycle time to the range of several tens of seconds. Under these analytical conditions, autosampler injection cycle time plays a critical role in reducing overall analysis time and thereby achieving higher throughput.

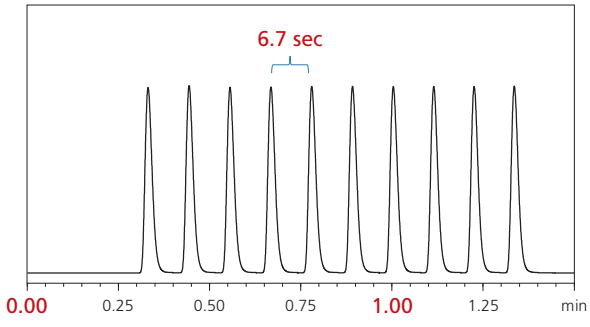

Fig. 1 UV chromatogram of caffeine obtained with the SIL-40, showing the injection cycle time

Table 1 Analytical conditions

Mobile Phase:	Water / MeOH = 4 / 6 (v/v)
Column:	Shim-pack™ XR-ODS II (3.0 mm I.D.× 75 mmL., 2.2 µm)
Flow rate:	1.4 mL/min
Injection volume:	0.5 µL
Detection:	UV-VIS 273 nm

2. System Configuration

In this report, we describe the use of the SIL-40 for the precise and ultra-fast quantitation of drugs in plasma samples, with the goal of maximizing the throughput of a clinical laboratory.

Fig. 1 shows the ultra-fast injection of a caffeine standard solution using the SIL-40. An injection cycle under 7 seconds was achieved.

In order to evaluate the effectiveness of sample analysis with this ultra-fast injection performance, we carried out an ultra-fast analysis of drugs in blood plasma using the triple quadrupole mass spectrometer LCMS-8050 from Shimadzu. Fig. 2 shows the test compound and the internal standard. A 5 mmL guard column was used to reduce matrix effects in the short time window. Analysis conditions are shown in Tables 2 and 3. Analysis was performed with the system volume as low as possible (through direct connection of the column to the ESI source) to prevent post-column dilution. Even with extremely narrow columns (<5 sec), it was possible to collect enough data points with Shimadzu UFMS technology.

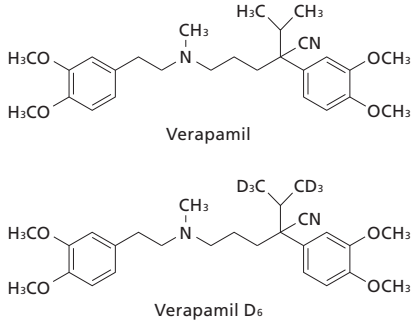

Fig. 2 Verapamil and IS chemical formulae.

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Table 2 Analytical Conditions

System:	Nexera XR
Column:	Shim-pack Velow EXP guard column cartridge (2.1 mm I.D., 5 mmL, 2.7 µm)
Column temperature:	Room temp.
Mobile phases:	A: water + 0.1% formic acid B: acetonitrile + 0.1% formic acid A / B = 27 / 13 (v/v)
Flow rate:	750 µL/min
Average cycle time:	18 sec
Injection volume:	0.5 µL

Table 3 MS/MS Acquisition Parameters

	MRM
Verapamil:	455.1>165.1, 105.3, 303.3
Verapamil D_6:	461.9>165.2, 150.2, 309.3

Plasma samples were spiked with an appropriate concentration of Verapamil from 0.39 to 100 µg/L and subsequently underwent protein precipitation in a plasma to precipitant solution ratio of 1:3. After vortex (1 min) and short incubation at room temperature (5 min), samples were centrifugated and 200 µL of supernatant was collected and transferred into micro-vials.

During analysis the needle was actively rinsed after sample aspiration using the SIL-40 rinsing pump (1 sec). As a result, the carryover was kept extremely low while achieving an overall analysis cycle time of under 18 sec.

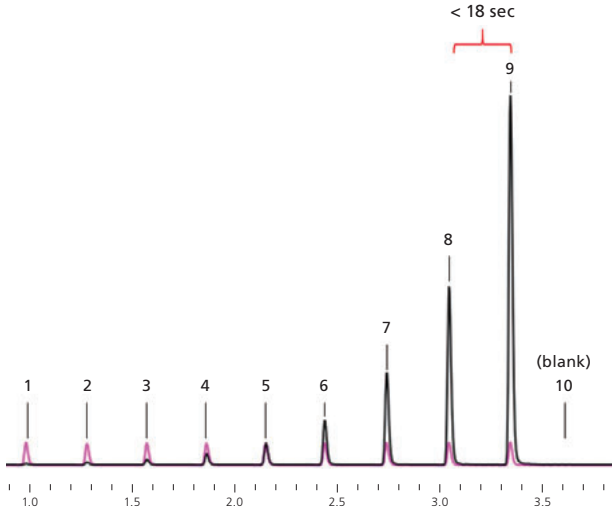

Fig. 3 Linearity over the bioanalytically relevant conc. range. Black: Verapamil chromatogram. Pink: Verapamil D_6 chromatogram. 1: Calib 1 (0.39 µg/L). 2: Calib 2 (0.78 µg/L). 3: Calib 3 (1.56 µg/L). 4: Calib 4 (3.12 µg/L). 5: Calib 5 (6.25 µg/L). 6: Calib 6 (12.5 µg/L). 7: Calib 7 (25 µg/L). 8: Calib 8 (50 µg/L). 9: Calb 9 (100 µg/L). 10: Blank.

The 9 peaks shown in Fig. 3 (peaks 1-9) represent the standard samples added to the blood plasma. The calibration curves obtained from these peaks show good linearity (R2=0.9998). In addition, after injection of the most concentrated sample, a blank solution was injected (peak 10). The blank solution did not produce a peak above the average noise level, demonstrating that carryover was negligible.

In addition, even after 300 injections, the analytical stability remained high (Fig. 4). The RSD% of the internal standard (shown in blue) was 2.4%, stable even under ultra-fast injection conditions with a cycle under 18 sec. This confirmed the ability to measure a large number of samples with ultra-fast injection.

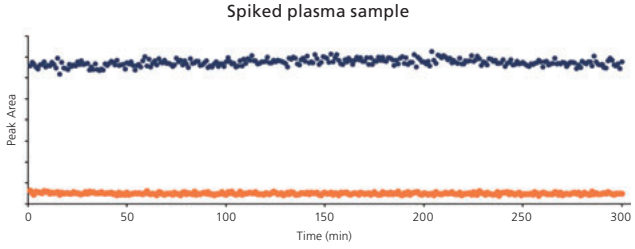

Fig. 4 Spiked plasma sample (0.39 µg/L), 300 consecutive injections. RSD% peak area without any smoothing: 2.4% for IS (blue), 9.3% for Verapamil (orange).

3. Conclusions

- The use of the SIL-40 in combination with the UFMS LCMS-8050 allows accurate drug quantitation in plasma samples.
- With a 5 mm column and active rinsing of the needle using a rinsing pump, it was possible to prevent carryover as well as matrix effects from non-target compounds in the samples, in addition to reducing the overall analysis cycle time to under 18 seconds.

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