Integration of mycophenolate and its metabolite analysis in plasma using LC-MS/MS with full-automated sample preparation

Toshikazu Minohata [1,2], Stéphane Moreau[3], Fanny Dayot[1], Jean-François Hoeffler[1]
[1] ALSACHIM SAS, Illkirch, France, [2] Shimadzu Corporation, Kyoto, Japan, [3] Shimadzu Europe GmbH, Duisburg, Germany

1. Introduction

Currently sample preparation for the detection of drugs in biological samples by liquid chromatography-mass spectrometry (LC-MS/MS) involves complex offline extraction methods such as solid phase extraction or liquid/liquid extraction, all of which require additional sample concentration and reconstitution in an appropriate solvent. These sample preparation methods are time-consuming, often taking 1 hour or more per sample, and are more vulnerable to variability due to errors in manual preparation. Our approach to offering a high sensitivity drug detection method and timely, automated analysis of multiple samples is to use the automated sample preparation system coupled to the detection capabilities of a high-sensitivity triple stage quadrupole mass spectrometer.

2. Method

Mycophenolic acid (MPA) and its glucuronide (MPA-G) in plasma were verified using DOSIMYCO™ (Alsachim, France). Plasma sample was loaded directly into the automated sample preparation system (CLAM-2000 Shimadzu, Japan). The CLAM-2000 was programmed to perform protein precipitation using methanol followed by filtration and sample collection. The sample is then transported using an arm from the CLAM-2000 to the HPLC without human intervention for LC-MS/MS analysis.
The treated samples were trapped using a DOSIMYCO™ C8 column and then separated by DOSIMYCO™ C18 column at 65 ℃ in 1.5 min.

MRM transition :

	ion	polarity	target ion	reference ion
MPA	+NH4	pos	338.10>207.10	338.10>159.10
MPA-G	+NH4	pos	514.10>207.10	514.10>159.10
	ion	**polarity**	**target ion**	**reference ion**
[13C, 2H3] MPA	+NH4	pos	342.10>211.10	342.10>159.10
[13C, 2H3] MPA-G	+NH4	pos	518.10>211.10	518.10>159.10

Samples preparation for manual handling

1. Put 25 µL of samples/calibrators in 1.5 mL microtube
2. Add 25 µL of Internal Standard
3. Add 450 µL of Extraction buffer
4. Shake for 1 min
5. Centrifuge at 15,000 g for 7 min
6. Transfer 200 µL of supernatant to vial

Samples preparation for CLAM-2000

1. Take 20 µL of IPA/H_2O(75/25) to sample cup
2. Add 10 µL of samples/calibrators
3. Add 180 µL of Extraction buffer
4. Add 10 µL of Internal Standard
5. Shake for 1 min at 1,900 rpm
6. Filtrate for 1min

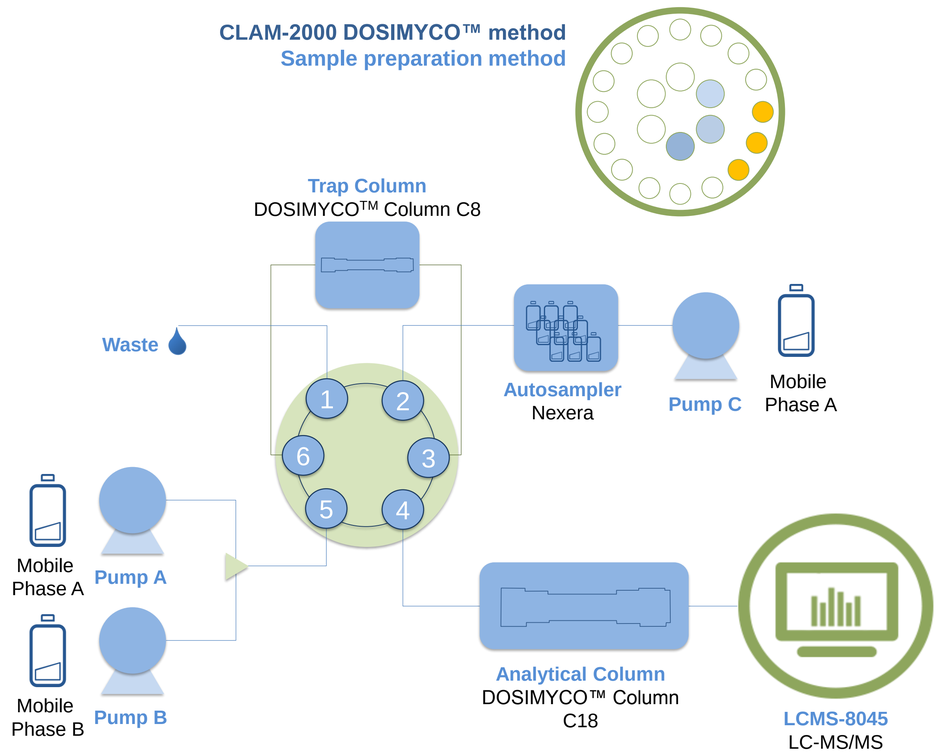


Figure 1. Schematic representation of the CLAM-2000 LC-MS/MS method for DOSIMYCO™

HPLC Conditions

Analytical column	: DOSIMYCO™ Column C18 2,1x50 mm, 5 µm
Trap column	: DOSIMYCO™ Column C8 4,6x30 mm, 5 µm
Pump A	: DOSIMYCO™ Mobile Phase A
Pump B	: DOSIMYCO™ Mobile Phase B
Pump C	: DOSIMYCO™ Mobile Phase A
Rinse solution (Internal & External)	: (R0) DOSIMYCO™ System Cleaning Phase (R1) DOSIMYCO™ Mobile Phase B
Isocratic flow rate	: 2 mL/min (for trap) 0.8 mL/min (for analysis)
Oven temperature	: 65 °C

MS Conditions LCMS-8045

Ionization	: ESI Positive
DL temp.	: 100 °C
Heat Block temp.	: 200 °C
Interface temp.	: 200 °C
Nebulizer gas flow	: 3 L/min
Drying gas flow	: 10 L/min
Heating gas flow	: 10 L/min

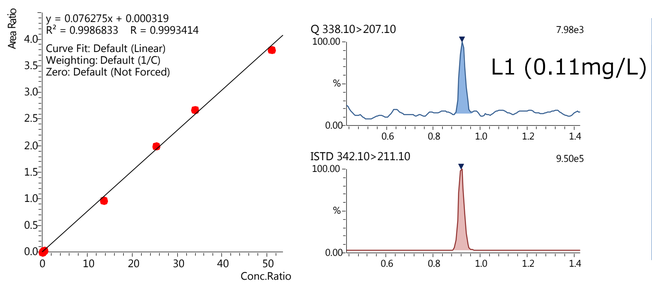
Time program :

Time (min)	event	
0.25	Pump C Flow	2 mL/min
0.26	Pump C Flow	0.02 mL/min
0.25	Valve Position	1
1	Pump B conc.	60
1.01	Pump B conc.	100
1.5	Pump B conc.	100
1.51	Pump B conc.	60
1.51	Valve Position	0

3. Result and discussion

We evaluated this system using calibrator and control plasma spiked with mycophenolic acid and its glucuronide in DOSIMYCO™ and carried out concurrent analysis over a range of concentrations in 0.1 to 50 mg/L for mycophenolic acid and 1 to 250 mg/L for its glucuronide. The calibration curves that were generated had linear regression values of r^2 >0.99 for each curve. The reproducibility (N=3) at 3 concentrations for control was excellent.

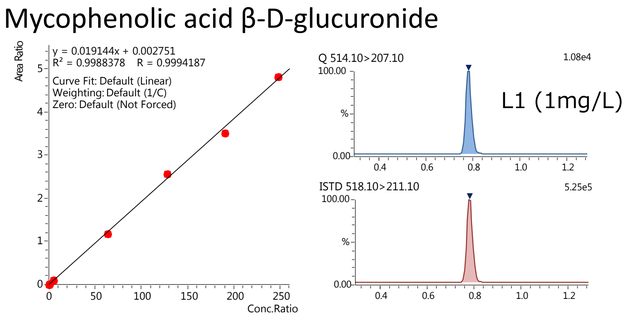
Mycophenolic acid



Molecule	MPA		
Level	C1	C2	C3
Target conc. (mg/L)	0.3	20.9	35.7
Actual conc.(mg/L)	0.28	17.95	35.69
	0.27	19.81	35.03
	0.31	20.21	30.59
Average (mg/L)	0.29	19.32	33.77
CV (%)	5.7	6.2	8.2
Deviation (%)	-4.0	-7.6	-5.4

N=3

Mycophenolic acid β-D-glucuronide



Molecule	MPA-G		
Level	C1	C2	C3
Target conc. (mg/L)	3.2	102.9	208.4
Actual conc.(mg/L)	2.95	101.31	188.67
	2.98	87.42	172.70
	2.76	102.83	208.56
Average (mg/L)	2.90	97.19	190.0
CV (%)	4.1	8.7	9.5
Deviation (%)	-9.4	-5.5	-8.8

N=3

Figure 2. Calibration curves, MRM chromatograms and summary of Mycophenolic acid and its glucuronide

Sample preparation and LC-MS/MS analysis can be performed in parallel to accelerate throughput using CLAM-2000.

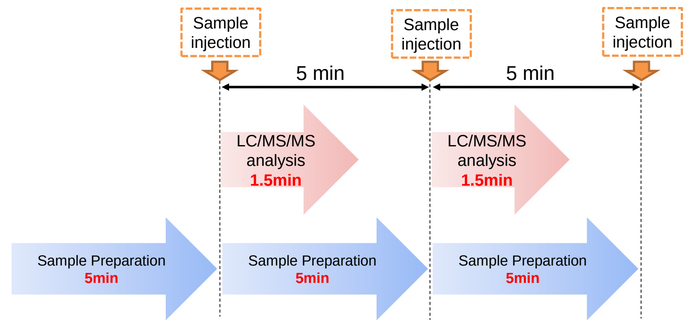


Figure 3. Analytical flow with parallel processing

4. Conclusion

We completed mycophenolic acid analysis using the automated sample preparation system coupled to LC-MS/MS. The results show the capability of the system for large sample set analyses with improved accuracy and precision by eliminating human error associated with manual sample handling.