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High throughput quantitation for therapeutic drug monitoring with Open access LC/MS/MS system

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Introduction

In this paper, we introduce four research use only LC/MS/MS methods for therapeutic drug monitoring (TDM), mycophenolic acid, sunitinib and axitinib, voriconazole, itraconazole. TDM is indispensable for managing drug dosage based on the drug concentration in blood in order to conduct a rational and efficient drug therapy. Liquid chromatography coupled with tandem quadrupole mass spectrometry (LC/MS/MS) is increasingly used in TDM because it can perform selective and sensitive analysis by simple sample pretreatment. In the field of TDM, it is necessary to measure the specimen such as plasma or serum quickly after suitable pretreatment and report the precise result. LC/MS/MS system with a simple and user-friendly interface can provide a streamlined workflow and reduce the load of analysts. We developed high-throughput LC/MS/MS methods for TDM with a new data acquisition and processing software.

Method

Instruments and LC/MS/MS analytical conditions

For LC/MS/MS analysis, a LCMS-8050 triple quadrupole mass spectrometer coupled to a Nexera X2 UHPLC system with mobile phase switching unit (Shimadzu corporation, Japan) was used. In all the methods, the compounds were separated by a reversed phase mode using a common column, Shim-pack GIS (Shimadzu corporation, Japan). All data acquisition and processing were performed by Open Solution QuantAnalytics (Shimadzu corporation, Japan), a software package for acquiring and reviewing quantitative LC/MS/MS data with ease.

High throughput quantitation for therapeutic drug monitoring with Open access LC/MS/MS system

Table 1 LC/MS/MS consitions

	Method 1	Method 2
Target	Mycophenolic acid (Immuno-suppressant)	Voriconazole (Triazole antifungal agent)
Column	Shim-pack GIS (2.1 mmI.D. x 75mmL., 3um)	
Column Oven	40°C	
Flow rate	0.3 mL/min	0.4 mL/min
Mobile phase A	1% Acetic acid-water	10 mM Ammonium acetate-water
Mobile phase B	Acetonitrile	Methanol
Gradient	A/B = 1/1, isocratic	Bconc. 30% (0 - 0.50min) → 100% (1.50 - 3.00min) → 30% (3.01 - 5.00min)
Injection volum	5 µL	1 µL
Ionization	ESI-positive	
MRM transition	321.40 > 207.30	350.20 > 281.20
Run time	4 min.	5 min.
	Method 3	**Method 4**
Target	Sunitinib and Axitinib (Anti-cancer drug)	Itraconazole (Triazole antifungal agent)
Column	Shim-pack GIS (2.1 mmI.D. x 75mmL., 3um)	
Column Oven	40°C	
Flow rate	0.3 mL/min	0.4 mL/min
Mobile phase A	10 mM Ammonium acetate-water	10 mM Ammonium acetate-water
Mobile phase B	Acetonitrile	Acetonitrile
Gradient	Bconc. 10% (0 - 0.25min) → 80% (2.00 - 3.00min) → 10% (3.01 - 5.00min)	Bconc. 65% (0 - 1.00 min) → 95% (1.50 - 2.50min) → 65% (2.51 - 4.50min)
Injection volum	5 µL	3 µL
Ionization	ESI-positive	
MRM transition	Sunitinib 399.40 > 283.30 Axitinib 387.40 > 356.30 SU12662 (Active metabolite of Sunitinib) 371.40 > 283.30	Itraconazole 705.40 > 392.40 Hydroxy Itraconazole (Active metabolite of Itraconazole) 721.40 > 408.40
Run time	5 min.	4.5 min.

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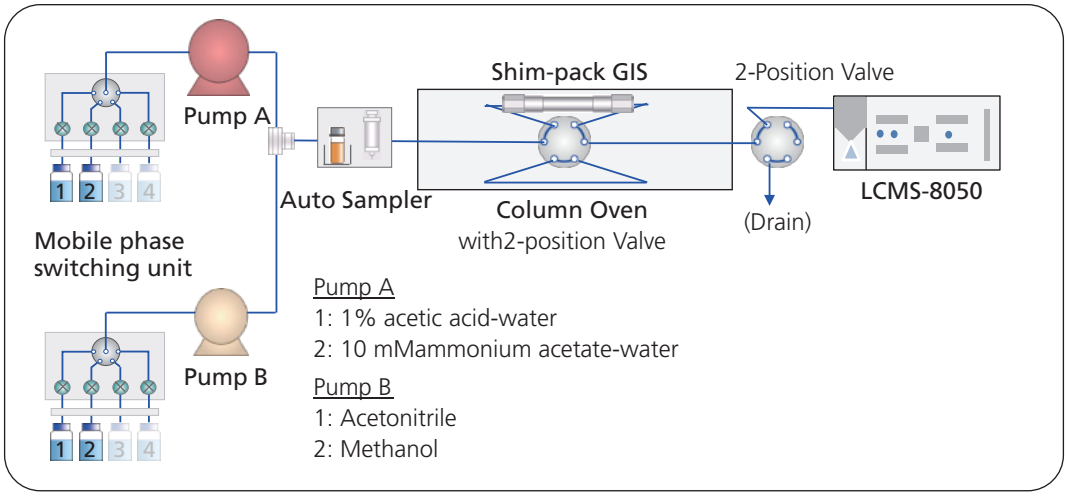

Figure 1 Nexera X2 UHPLC system with mobile phase switching unit

Calibration standards and QC samples

For each compound, more than five calibration standards and three QC samples were prepared. Samples were precipitated in a simple way of deproteination using organic solvent such as methanol or acetonitrile. The resulting supernatant was diluted and injected into LC/MS/MS without filtration.

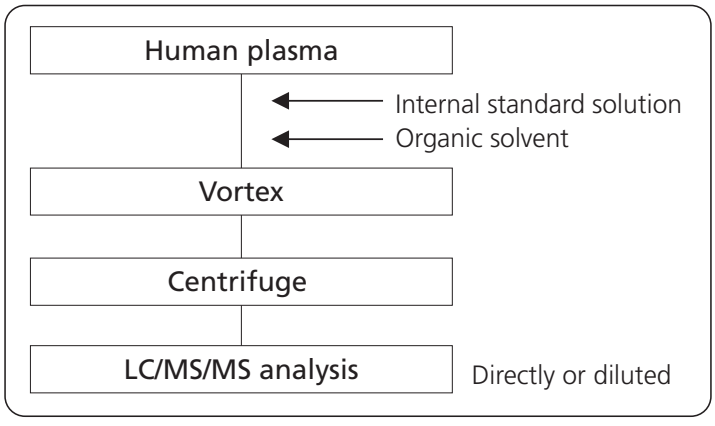

Figure 2 Work flow of the pretreatment

Result and discussion

Precision, accuracy and linearity

Table 2 illustrates linearity of all compounds and Table 3 illustrates accuracy and precision of the QC samples. Determination coefficient (r2) of all calibration curves was larger than 0.995, the precision (n=6) was within 15% RSD, and the accuracy (n=6) was within 80-120%.

Excellent linearity, accuracy and precision were obtained within a specific concentration range. Furthermore, All the methods took less than 5 minutes per one LC/MS/MS analysis, including column rinsing.

Table 2 Linear dynamic range for each compound

	Compound	Linearity (µg/mL)			r2
Method 1	Mycophenolic acid	0.2	~	20	0.999
Method 2	Voriconazole	0.1	~	10	0.999
Method 3	Sunitinib	3	~	300	0.999
	Axitinib	0.3	~	30	0.999
	SU12662	3	~	300	0.999
Method 4	Itoraconazole	10	~	1000	0.999
	Hydoroxy Itorazonazole	10	~	1000	0.999

Table 3 Precision and accuracy for analysis of QCs

	Compound	Concentrations of QC samples (µg/mL)			Precision (%)			Accuracy (%)		
		Low	Middle	High	Low	Middle	High	Low	Middle	High
Method 1	Mycophenolic acid	0.5	5	15	1.6	3.66	2.55	108.5	104.9	103.5
Method 2	Voriconazole	0.2	4	8	1.74	1.56	1.7	98.6	103.6	99
Method 3	Sunitinib	5	50	250	4.05	1.36	2.24	100.7	95.5	96
	Axitinib	0.5	5	25	8.26	4.56	4.43	85.3	88.5	91.2
	SU12662	5	50	250	4.07	1.26	3.17	97.2	96.5	97.8
Method 4	Itoraconazole	25	250	750	0.65	0.82	0.38	98.7	98.8	103.3
	Hydoroxy Itorazonazole				3.27	4.36	3.1	81.3	90.4	86.9

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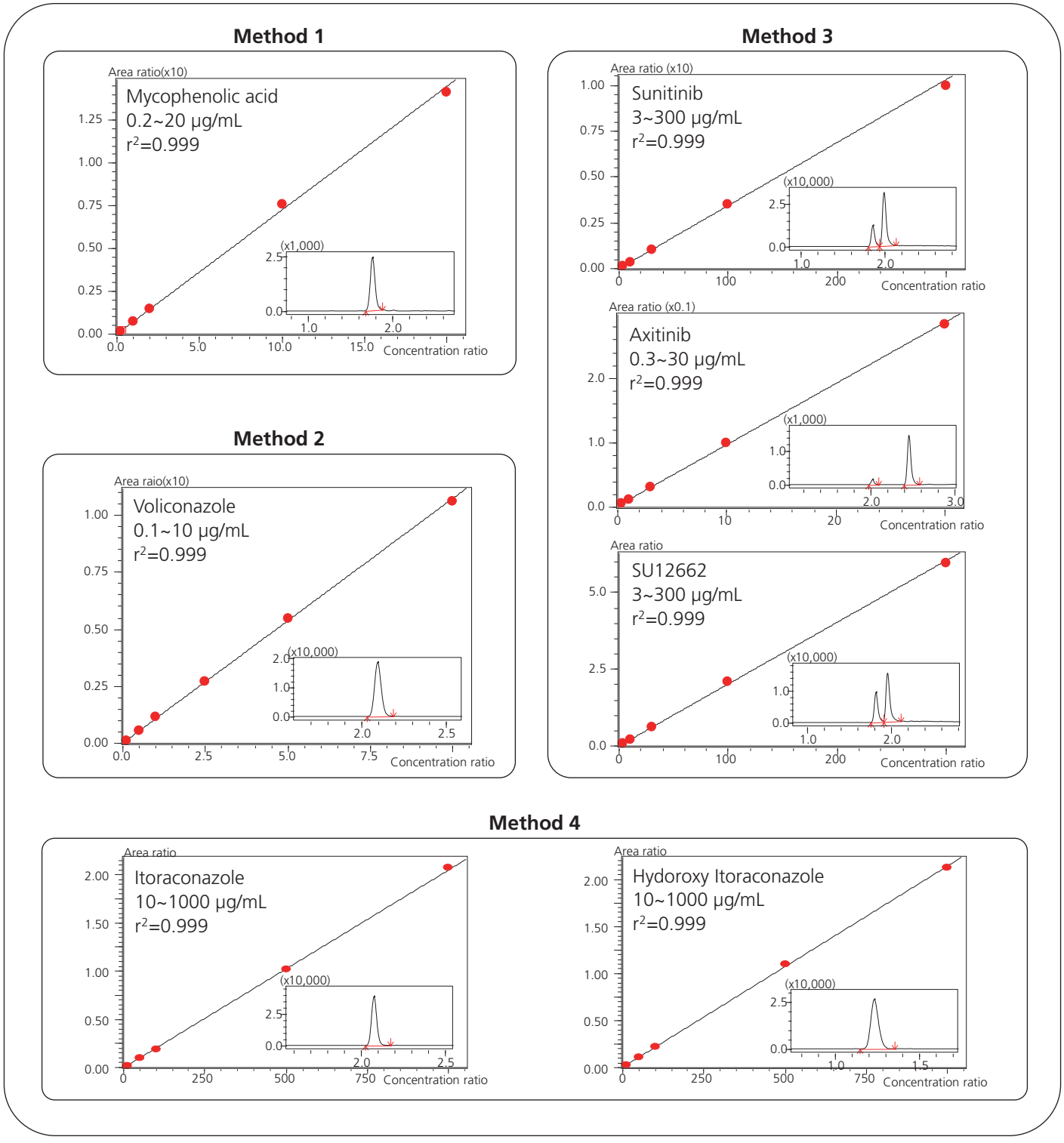

Figure 3 Calibration curves for each compound

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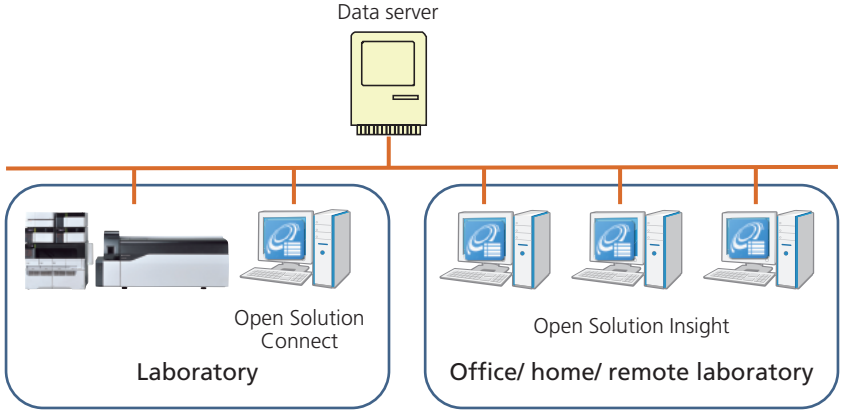
Easy data acquisition with Open access software

The developed platform was used one common column for all methods and mobile phase changeover could be automatically done by just selecting method file. Open Solution QuantAnalytics software enables users to submit sample queue and set the LC/MS/MS condition easily and quickly. Users can intuitively start LC/MS/MS measurement by just selecting the predefined method and placing sample vials in the specified autosampler plate positions guided by software. The resulting data can be reviewed in office as soon as it becomes available on the designated data server. This system enables easy and quick data acquisition without tedious manual operation such as replacement of a column and mobile phases.



(1) Import sample list and select methods.

(2) Confirm sample list then start analysis.

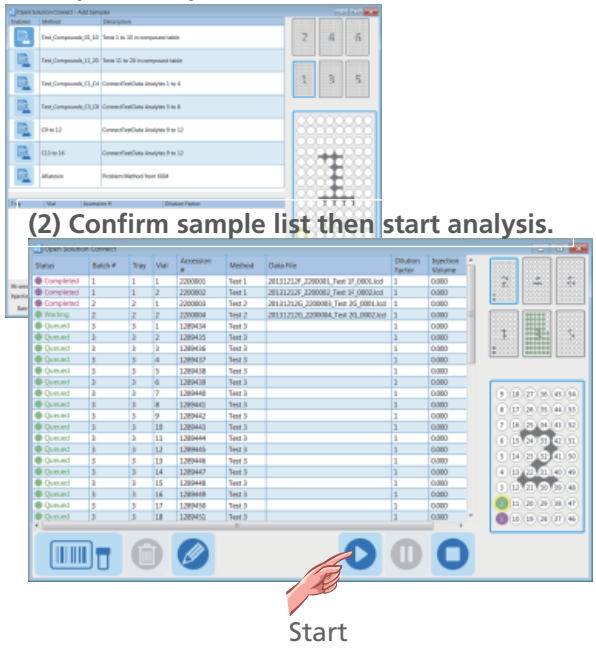


Figure 4 User interface for sample queue submission

(1) Select batch.

(2) Expert checks data, decides next step.

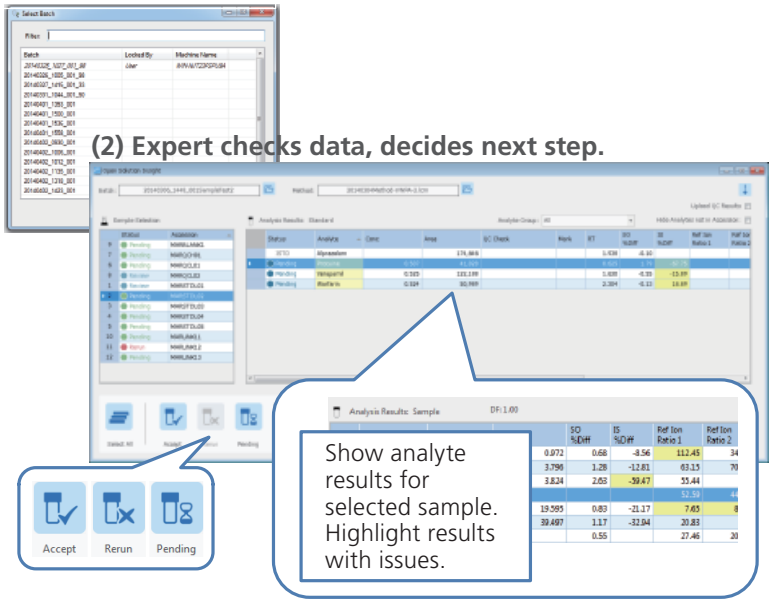


Figure 5 User interface for checking the result

High throughput quantitation for therapeutic drug monitoring with Open access LC/MS/MS system

Conclusions

- The combination of the Open Solution QuantAnalytics software and LC/MS/MS system with mobile phase switching unit enables easy sample submission and efficient data acquisition. The user only describes the vial position of samples, chooses a predefined method and the resulting data can be reviewed in office as soon as it becomes available on the designated data server.
- Saving time and effort for changing system conditions among each target compounds was achieved with mobile phase switching system and high through-put methods using a common column.

Disclaimer: Shimadzu LCMS-8050 CL and certain Nexera X2 UHPLC components are registered in the U.S. as a Class I device and is not specifically cleared for TDM. Other UHPLC components, Shim-pack GIS, and OpenSolution QuantAnalytics are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

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