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Application News

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Liquid Chromatography Mass Spectrometry

High-Speed Analysis of Sunitinib and Axitinib in Plasma Using Triple Quadrupole LC/MS/MS (LCMS-8050)

This article introduces an example measurement of the molecularly targeted drugs sunitinib and axitinib using the LCMS-8050 high-sensitivity triple quadrupole mass spectrometer. Although a simple method of sample pre-treatment was used that involves only deproteinization, the quantitative results obtained were excellent in terms of accuracy and precision.

Sample Pretreatment

The pretreatment workflow is shown in Fig. 1.
Standard (STD) samples of each drug in plasma were prepared to create a calibration curve, and QC samples of each drug in plasma were prepared to ensure validity of results. Standard samples and QC samples were created at the plasma concentrations shown in Table 3. An internal standard solution and acetonitrile were added to these standard samples and QC samples, and the deproteinized supernatant was then diluted in 10 mmol/L of aqueous ammonium formate solution and used for analysis. The stable isotope-labeled compound of imatinib (imatinib-d8) was used as the internal standard solution.
The pretreatment method used is simple and requires no labor-intensive steps such as solid phase extraction, which allows pretreatment to be performed at low cost and in a short period of time.

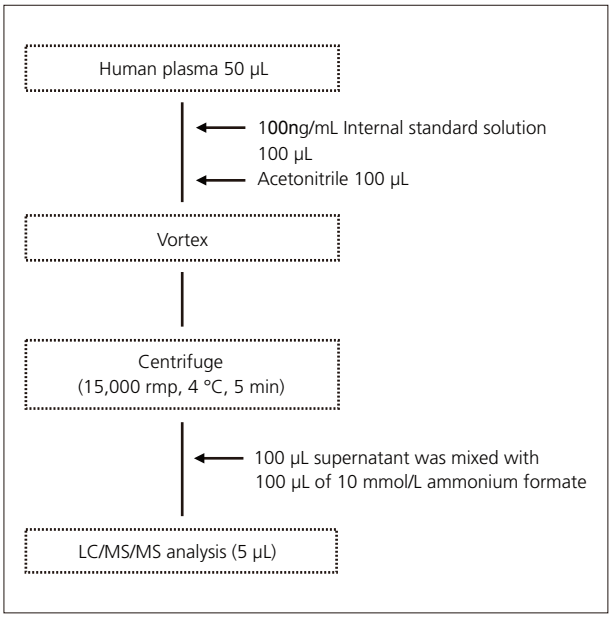

Fig. 1 Pretreatment Workflow

LC/MS/MS Analytical Conditions

LC/MS/MS analytical conditions are shown in Table 1, and MRM transitions are shown in Table 2. Sunitinib, axitinib, and the sunitinib metabolite SU12662 were measured.
A Shim-pack GIS column was used, and separation was performed in reverse-phase mode. Electrospray ionization (ESI) was used as the method of ionization, and MRM measurements were performed in positive ion mode.

Table 2 MRM Parameters

Compound Name	Polarity	MRM Transition
Sunitinib	+	399.40 > 283.30
Axitinib	+	387.40 > 356.30
SU12662	+	371.40 > 283.30
Imatinib-d8	+	502.50 > 394.40

Table 1 Analytical Conditions

Column	: Shim-pack GIS (75 mm L. × 2.1 mm I.D., 3 µm)
Mobile Phase	: A) 10 mmol/L Ammonium formate in water B) Methanol
Time Program	: B.Conc. 10 % (0.25 min) → 80 % (2.00 - 3.00 min) → 10 % (3.01 - 5.00 min)
Flowrate	: 0.3 mL/min
Column Temperature	: 40 °C
Injection Volume	: 5 µL
Probe Voltage	: +4.0 kV (ESI-positive mode)
DL Temperature	: 150 °C
Block Heater Temperature	: 200 °C
Interface Temperature	: 300 °C
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 5 L/min
Heating Gas Flow	: 15 L/min

Analysis Results

The calibration curves for each compound in plasma are shown in Fig. 2, and representative chromatograms are shown in Fig. 3.

The chromatograms for sunitinib, the sunitinib metabolite SU12662, and axitinib show two peaks representing different isomers of these compounds. Calibration curves were created by grouping the peaks representing different isomers of same compound and summing the area of those peaks. Good linearity with a correlation coefficient of 0.999 or higher was obtained for all calibration curves.

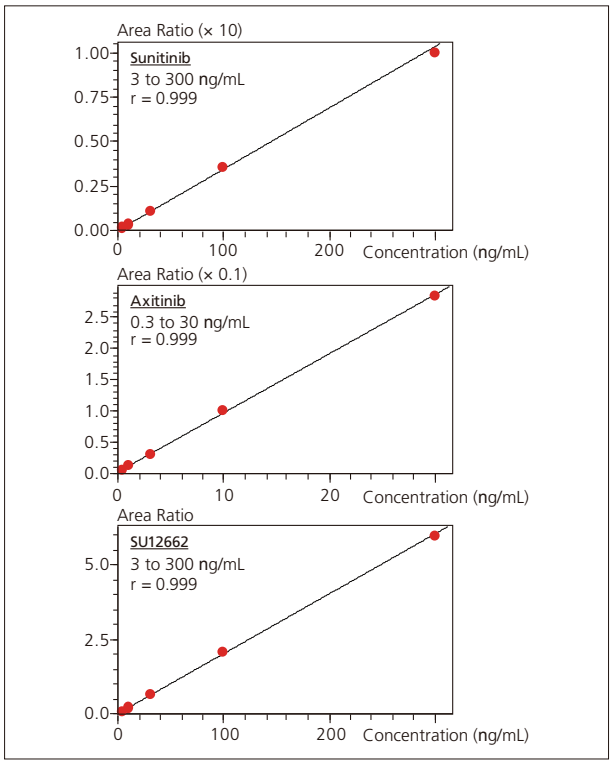

Fig. 2 Calibration Curves for Each Compound

The accuracy and repeatability of each point on the calibration curves for each compound concentration in plasma and each QC sample concentration is shown in Table 3.

Good results were obtained for all points on calibration curves and all QC sample concentrations, with repeatability of 10 % or below and accuracy within 100 ± 15 %.

Table 3 Measurement Results of Each Compound in Plasma and QC Samples

		Concentration in Plasma (ng/mL)	Accuracy (%)	Concentration Repeatability (%)
Sunitinib	STD1	3	100.1	5.73
	STD2	10	98.9	2.19
	STD3	30	102.0	2.58
	STD4	100	102.3	2.34
	STD5	300	96.7	2.74
	QC1	6	100.7	4.05
	QC2	50	95.5	1.36
	QC3	250	96.0	2.24
Axitinib	STD1	0.3	102.5	8.71
	STD2	1	94.2	6.36
	STD3	3	101.2	3.67
	STD4	10	103.1	2.97
	STD5	30	99.0	3.06
	QC1	0.5	85.3	8.26
	QC2	5	88.5	4.56
	QC3	25	91.2	4.43
SU12662	STD1	3	97.1	4.70
	STD2	10	98.7	2.73
	STD3	30	102.1	1.66
	STD4	100	103.5	1.92
	STD5	300	98.7	2.67
	QC1	5	97.2	4.07
	QC2	50	96.5	1.26
	QC3	250	97.8	3.17

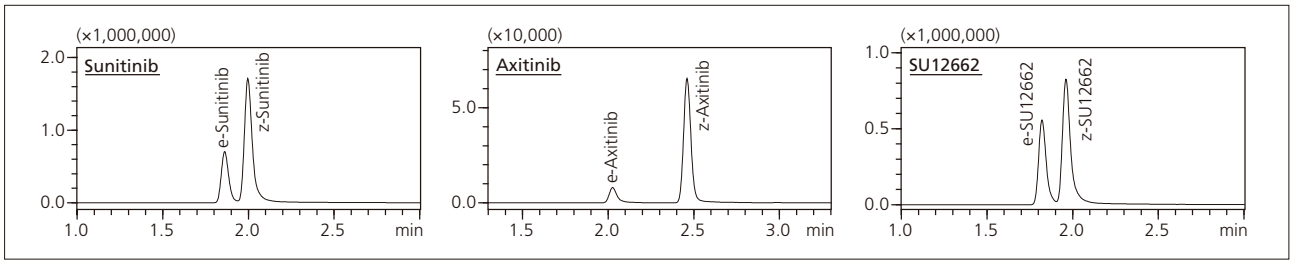

Fig. 3 Representative Chromatograms for Each Compound

This Application News was created with the cooperation of the Pharmaceutical Sciences Department of Tohoku University Hospital.

Notes: · The products mentioned in this article have not received approval for use as medical devices based on the Pharmaceutical and Medical Device Act.
· The analytical methods mentioned in this article cannot be used for diagnostic purposes.

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