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Application News

No. C143

Liquid Chromatograph Mass Spectrometry

Simultaneous Analysis of Tyrosine Kinase Inhibitors in Human Blood Plasma with LC/MS/MS

Cancer treatment in recent years employs drugs known as molecular targeted drugs that were developed to target molecules related to the growth, invasion, and metastasis of tumor cells in order to inhibit tumor cell growth.

Lung cancer treatment employs tyrosine kinase inhibitors (TKIs), which target the epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK), and new molecular targeted drugs, referred to as second and third generation drugs, continue to be developed.

This article introduces an example of simultaneous analysis of EGFR-TKIs, ALK-TKIs, and metabolites in human blood plasma for the purpose of research into pharmacokinetics using the triple quadrupole high performance liquid chromatograph mass spectrometer LCMS-8050.

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Simultaneous Analysis of Four EGFR-TKIs and Three ALK-TKIs

Samples with the standard EGFR-TKIs and ALK-TKIs listed in Table 1 added to a control human blood plasma were deproteinized according to the process in Fig. 1 and the resulting supernatants were submitted for analysis. MRM measurement with LC/MS/MS can selectively detect target drugs according to their molecular mass and structure (Fig. 2 and Fig. 3).

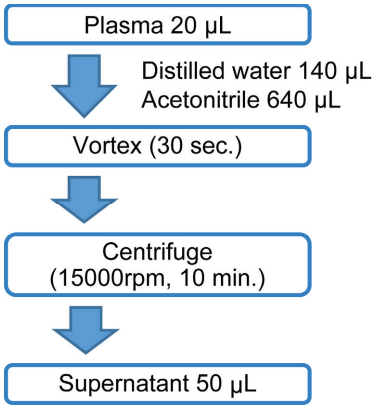

Fig. 1 Pretreatment Workflow of Blood Plasma Samples

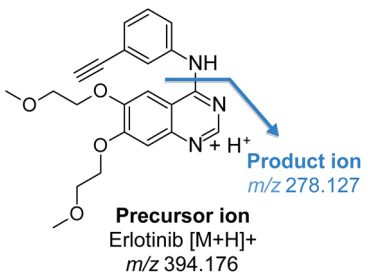

Fig. 2 Precursor Ion and Product Ion of Erlotinib

Table 1 EGFR-TKIs, ALK-TKIs, and Metabolites

	Compound	Molecular formula	Monoisotopic mass [u]	MRM transition *m/z*
EGFR-TKIs	Afatinib	$C_{24}H_{25}ClFN_5O_3$	485.163	486.2 > 371.1
	Erlotinib	$C_{22}H_{23}N_3O_4$	393.169	394.2 > 278.1
	OSI-420 *1	$C_{21}H_{21}N_3O_4$	379.153	380.2 > 278.1
	Gefitinib	$C_{22}H_{24}ClFN_4O_3$	446.152	447.2 > 128.1
	Osimertinib	$C_{28}H_{33}N_7O_2$	499.270	500.3 > 72.1
	AZ5104 *2	$C_{27}H_{31}N_7O_2$	485.254	486.3 > 72.2
ALK-TKIs	Alectinib	$C_{30}H_{34}N_4O_2$	482.268	483.3 > 396.3
	Crizotinib	$C_{21}H_{22}Cl_2FN_5O$	449.119	450.1 > 260.2
	Ceritinib	$C_{28}H_{36}ClN_5O_3S$	557.223	558.2 > 433.1

*1 Erlotinib metabolite, *2 Osimertinib metabolite

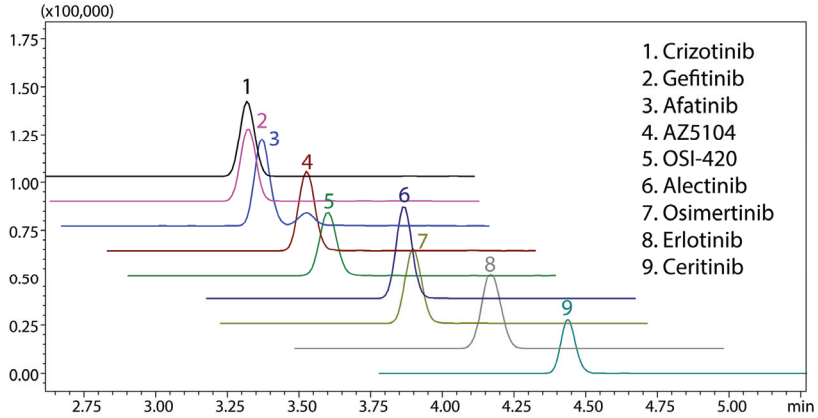

Fig. 3 Mass Chromatograms of Human Blood Plasma Samples with Standard Additives

A calibration curve was created from the control blood plasma with standards added and the integrity of accuracy and precision were evaluated. Good linearity was obtained in the set concentration range for all TKIs and accuracy in the entire range, including the quantitative lower limit, was within 100±15 %. In the same manner, precision (%RSD) was within 15 % and good repeatability was obtained (Table 2).

Table 2 Integrity Evaluation Results for Simultaneous Analysis of EGFR-TKIs, ALK-TKIs, and Metabolites

Compounds	Range (ng/mL)	Accuracy (%)				Precision (%RSD, n=5)			
		LLOQ *1	Low *2	Medium *3	High *4	LLOQ *1	Low *2	Medium *3	High *4
Afatinib	5-2000	98.5	103.2	106.3	95.7	12.4	13.9	7.6	7.4
Erlotinib	5-2000	95.1	103.2	102.7	92.3	4.7	5.4	0.8	2.0
OSI-420	5-2000	95.1	104.3	103.4	91.9	9.0	2.7	2.7	3.2
Gefitinib	5-2000	93.0	110.2	103.9	93.5	12.1	6.4	3.4	1.3
Osimertinib	5-2000	94.5	107.0	103.5	93.1	4.6	3.7	2.4	1.9
AZ5104	5-2000	94.7	106.5	102.7	92.5	9.8	6.0	2.7	3.1
Alectinib	5-2000	96.9	101.4	104.0	93.1	13.4	6.3	1.8	1.9
Crizotinib	5-2000	95.4	106.1	106.6	95.3	10.9	11.1	4.1	2.5
Ceritinib	10-2000	94.4	105.2	103.6	91.6	8.0	6.7	1.7	2.8

*1: 5 ng/mL (10 ng/mL for Ceritinib), *2: 10 ng/mL (25 ng/mL for Ceritinib), *3: 100 ng/mL, *4: 1000 ng/mL

Analysis of Blood Plasma Specimens

Fig. 4 and Fig. 5 show examples of blood plasma specimen analysis. Erlotinib and the erlotinib metabolite OSI-420 were detected in specimen A and alectinib was detected in specimen B. Like the control blood plasma used to create the calibration curve, no significant interference by impurities in the blood plasma was observed for either specimen.

This analysis method that uses LC/MS/MS is expected to be utilized as an analysis technique for TKIs in blood plasma specimens.

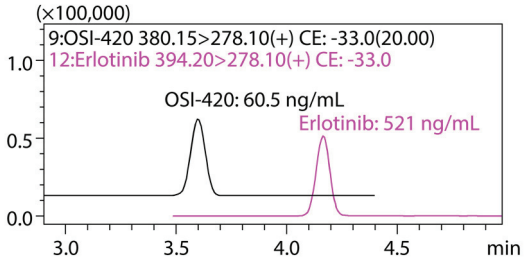

Fig. 4 Analysis Result of Blood Plasma Specimen A

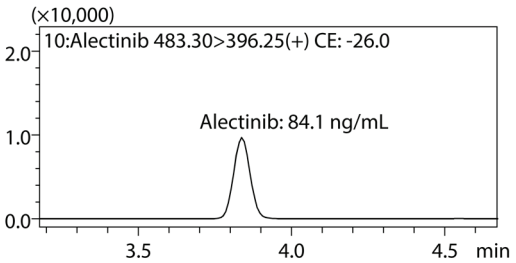

Fig. 5 Analysis Result of Blood Plasma Specimen B

Table 3 Analysis Conditions

Column	:	Shimadzu GLC Mastro C18 (100 mm L. × 2.1 mm I.D., 3 µm)			
Mobile Phase	:	A 10 mmol/L Ammonium formate buffer - water, B Acetonitrile			
Flow Rate	:	0.3 mL/min			
Time program	:	B Conc. 10 % (0 min) – 100 % (5 – 7 min) – 10 % (7.01 – 10 min)			
Column Temp.		50 °C	Injection Volume	:	3 µL
Probe Voltage	:	1.0 kV (ESI-positive mode)			
Interface Temp.	:	300 °C	DL Temp.	:	250 °C
Block Heater Temp.	:	400 °C	Nebulizing Gas Flow	:	3 L/min
Heating Gas Flow	:	10 L/min	Drying Gas Flow	:	10 L/min

<Acknowledgments>

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References
- Guidance for Industry : Bioanalytical Method Validation (2001, US FDA)
- Guideline on Bioanalytical Method Validation in Pharmaceutical Development (2013, Ministry of Health, Labour and Welfare, Japan)

Notes
- The product described in this document has not been approved or certified as a medical device under the Pharmaceutical and Medical Device Act of Japan.
- It cannot be used for the purpose of medical examination, treatment or related procedures.
- The specimens described in this document were all sampled and measured at the National Cancer Center Hospital. Permission was obtained in accordance with proper procedures regarding the publication of measurement data.

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