Sample Preparation Method for Determination of Axitinib in Plasma Using ISOLUTE® SLE+

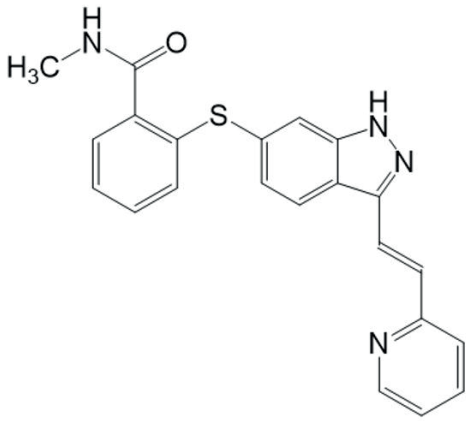

Figure 1. Structural formula of Axitinib.

Introduction

Axitinib (Figure 1) is a type of molecularly targeted drug used in renal cell carcinoma. To avoid serious side effects and achieve maximal therapeutic efficacy, it is crucial to determine the correlation between side effects on blood levels and treatment efficacy. Therapeutic Drug Monitoring (TDM) is one of the measures to achieve this.

In this application note, ISOLUTE® SLE+ was used as a sample preparation column for Axitinib determination in plasma using the principles of supported liquid extraction (SLE).

ISOLUTE® SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation time.

Analytes

Axitinib (Axitinib, CAS:319460-85-0)

Internal Standards

Osimertinib (Osimertinib, CAS: 1421373-65-0)

Sample Preparation Procedure

Format

ISOLUTE® SLE+ 400 µL Sample Volume columns, (part number: 820-0055-B).

An equivalent 96-well plate format, (part number 820-0400-P01) is also available.

Sample Pre-Treatment

To plasma (200 µL), add 5.0 ng/mL of Osimertinib as an internal standard. Add water (HPLC grade or higher) (200 µL) and vortex mix for 30 seconds.

Sample Loading

Load 400 µL of the sample solution onto the ISOLUTE® SLE+ column and apply light pressure (3 psi) or vacuum (-0.2 bar) to initiate flow. Allow the sample to absorb for at least 5 minutes and wait for samples to stabilize. Ensure that all sample solution is absorbed onto the diatomaceous earth.

Sample Elution

Add 900 µL of methyl tert-butyl ether (MTBE) and allow to flow under gravity for 5 minutes. Then, add another 900 µL of the solvent, and allow to stand for 5 minutes or more. If required, the extraction can be completed with application of gentle pressure (3 psi) or vacuum (-0.2 bar) (10 to 30 seconds).

Evaporation and Reconstitution

Evaporate the extract with a nitrogen gas evaporator, and reconstitute the extract with water:methanol (1:1, v/v, 2 mL).

Biotage®

UHPLC Conditions

Instrument
Nexera LC-30AD (Shimadzu)

Column
ACQUITY UPLC® BEH C18 1.7 µm (2.1 mm × 50 mm column; Waters)

Mobile Phase
A: 10 mmol/L ammonium acetate aqueous solution

B: acetonitrile

Flow Rate
0.4 mL/min

Gradient Conditions

Time (mins.)	% Mobile Phase B
0	5
3	95
4	95
4.1	5
6.5	5

Column Temperature
40 °C

Injection Volume
1 µL

Mass Spectrometry Conditions

Instrument
LCMS-8060 (Shimadzu)

Ionization Mode
ESI positive

Nebulizer Gas Flow Rate
3.00 L/min

Drying Gas Flow Rate
10.00 L/min

Heating Gas Flow Rate
10.00 L/min

Interface Temperature
350 °C

DL Temperature
200 °C

Heat Block Temperature
350 °C

CID Gas
270 kPa

SRM Transitions
Axitinib: m/z 387.40 › 356.05, Rt 2. 14 min, Collision Energy -20

Osimertinib (IS): m/z 500.30 › 72.15, Rt 2. 43 min, Collision Energy -27

Results

SRM (Selected Reaction Monitoring) chromatograms of Axitinib and Osimertinib (IS) are shown in Figure 2. Under the LC conditions used in this application note, two peaks were detected in the Axitinib chromatogram. This was attributed to the separation of the isomers. In this study, we determined the peak area value for the peak at elution time of 2.14 minutes and used this to create the calibration curve*. The calibration curve generated is shown in Figure 3.

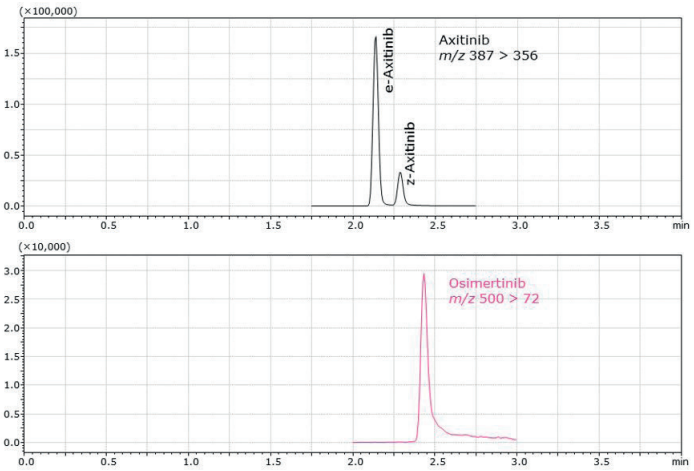

Figure 2. SRM chromatograms of Axitinib (top) and Osimertinib (IS) (bottom).

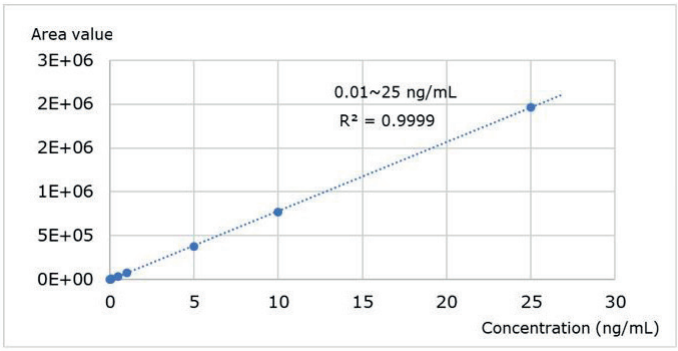

Figure 3. Standard curve for Axitinib.

Axitinib has a wide dynamic range of 0.01 to 25 ng/mL covering the range of blood concentrations required for TDM (0.1 to 10 ng/mL) and has a good linearity with a multiple correlation coefficient (r^2) of 0.9999 or more.

(* Reference: Biomedical Chromatography. 2018;32:e4147)

Confirmation of Analyte Recovery and Matrix Factors

Sample pre-treatment is very important in samples of biological origin. ISOLUTE® SLE+ supported liquid extraction columns were used to eliminate the effects of matrix components such as proteins, phospholipids, and salts and to allow improved quantitative analyses. Figure 4 shows the SRM chromatograms obtained by SLE pre-treatment of the control plasma spiked with 50.0 ng/mL axitinib. No interference from contaminants in the plasma was observed.

Recovery rates and matrix factors after SLE pre-treatment for 3 axitinib plasma concentrations (0.5, 5.0, and 20.0 µg/mL) are shown in Table 1. Recovery was calculated by comparing the peak area of samples spiked with axitinib pre-extraction (A) with that of blank samples spiked after extraction (B).

Matrix factors were calculated by comparing the area values of (B) and the standard solution (S). As a result, a recovery rate of more than 95% was obtained at each concentration, and the values of matrix factors were sufficiently low. It was quantitatively confirmed that ISOLUTE SLE+ pre-treatment effectively eliminates matrix effects. In this application note, we recommend the use of osimertinib as IS for axitinib, but use of stable isotope dilution as an internal standardization approach would also be appropriate.

Table 3. Recovery and Matrix Factors in Tests with plasma (n=3).

Blood Concentrations (ng/mL)	Recovery rate* (%)	Matrix Factor* (%)
0.5	111.3	-1.1
5.0	105.9	13.3
50.0	96.5	1.8

* Recovery rate = [A]/[B] × 100; Matrix Factor = 1-[B]/[S] × 100

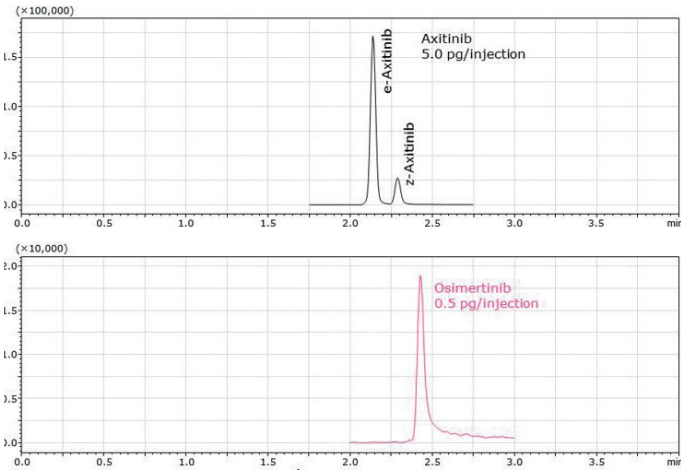

Figure 4. SRM chromatograms of 50.0 ng/mL Axitinib in plasma after ISOLUTE® SLE+ extraction.

Ordering Information

Part Number	Description	Quantity
820-0055-B	ISOLUTE® SLE+ 400 µL Sample Volume	50
820-0400-P01	ISOLUTE® SLE+ 400 µL 96-Well Plate	1
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold	1
121-2016	Biotage® VacMaster™ 20 Sample Processing Station (with 16 mm Rack)	1

This application note was prepared in collaboration with the Pharmaceutical Department of Gunma University Hospital, Japan.

EUROPE
Main Office: +46 18 565900
Toll Free: +800 18 565710
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com
Support Tel: +46 18 56 59 11
Support Fax: + 46 18 56 57 11
eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
Outside US: +1 704 654 4900
us-1-pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
jp-1-pointsupport@biotage.com

CHINA
Tel: +86 21 68162810
Fax: +86 21 68162829
cn_order@biotage.com
cn-1-pointsupport@biotage.com

KOREA
Tel: + 82 31 706 8500
Fax:+ 82 31 706 8510
korea_info@biotage.com
kr-1-pointsupport@biotage.com

INDIA
Tel: +91 22 4005 3712
india@biotage.com

Distributors in other regions are listed on www.biotage.com

Literature Number: AN937

Biotage®