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

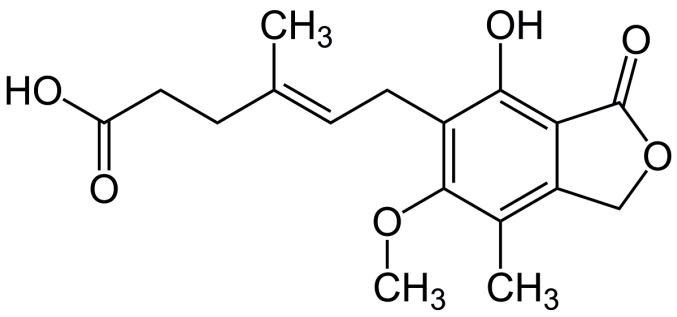

Figure 1. Structural formula of mycophenolic acid.

Introduction

Mycophenolic acid (Figure 1) is an active metabolite produced by the hydrolysis of the immunosuppressive agent Mycophenolate Mofetil in the body. It is considered important to control pharmacokinetic parameters such as AUC (area under the blood concentration-time curve) and trough concentration in order to prevent acute rejection after organ transplantation.

In this application note, ISOLUTE® SLE+ was used as a sample pretreatment column for mycophenolic acid determination in plasma using the principles of supported liquid extraction (SLE). UV based methods are commonly used to measure mycophenolic acid. In this application note, we have developed a method that can be used for both UV measurement and LC/MS/MS measurement. LC/MS/MS assays are highly selective and less susceptible to the effects of other drugs used in combination during treatment.

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.

Analytes

Mycophenolic acid (Mycophenolic acid, CAS: 137234-62-9)

Internal Standards

Indomethacin (Indomethacin, CAS: 53-86-1)

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 200 µL of plasma, add 5.0 µg/mL of indomethacin (internal standard) followed by 200 µL of 2% formic acid aqueous solution. Vortex mix for 30 seconds.

Sample Loading

Load 400 µL of the sample solution onto the column and apply gentle 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 bed.

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 extractions can be completed with application of gentle pressure (3 psi) or vacuum (-0.2 bar) (10–30 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).

In the case of UV measurement, the reconstituted solution should be measured as it is, or the reconstituted solution can be concentrated to a volume of less than 2 mL, depending on the sensitivity of UV measurement.

Dilution for LC/MS/MS

Additional dilution of the reconstituted assay solutions should be performed depending on the range of calibrated concentrations in the LC/MS/MS used*.

*In this application note, a 10-fold dilution was performed using water : methanol (1:1, (v/v)).

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: 0.1% (v/v) Formic acid aqueous solution

B: Acetonitrile

Flow Rate
0.4 mL min

Gradient Condition
Time/% of B: 0/5 →3/95 →4/95→ 4.1/5 →6.5/5

Column Temperature
40 °C

Injection Volume
1 µL

Mass Spectrometry Conditions

Equipment
LCMS-8060 (Shimadzu)

Ionization Mode
ESI positive

Nebulizer Gas Flow Rate
3 L/min

Flow Rate of Drying Gas
10 L/min

Heating Gas Flow
10 L/min

Interface Temperature
350 °C

DL Temperature
200 °C

Heat Block Temperature
350 °C

CID Gas
270 kPa

SRM Transition
Mycophenolic acid: m/z 321. 00 › 207.15, Rt 2.26 min, Collision Energy;-22

Indomethacin (IS): m/z 358. 00 › 139.05, Rt 2.66 min, Collision Energy;-22

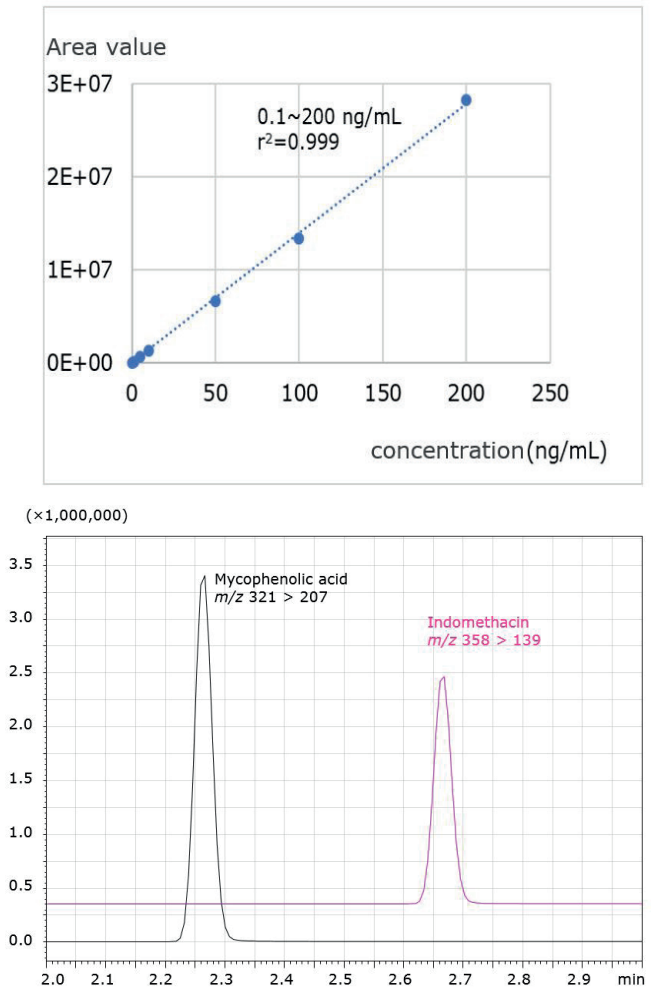

Figure 2. Calibration Curves and SRM Chromatograms of Mycophenolic Acids.

Calibration Curve

Figure 2 shows the calibration curves for mycophenolic acid and the SRM (Selected Reaction Monitoring) chromatograms.

SRM mode allows measurements that are more selective than UV-detection. The quantitation range was 0.1 to 200 ng/mL, which covers the required blood concentration range (0.5 to 20 µg/mL) for TDM (Therapeutic Drug Monitoring) of mycophenolic acid, and the multiple correlation coefficient (r^2) was 0.999 or more, showing good linearity.

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 3 shows the SRM chromatograms obtained by pretreatment of the control plasma spiked with 5.0 µg/mL mycophenolic acid. No interference from contaminants in the plasma was observed.

Recovery rates and matrix factors after SLE pretreatment for 3 mycophenolic acid 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 mycophenolic acid 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 90% was obtained at each concentration, and the values of matrix factors were sufficiently low. It was quantitatively confirmed that ISOLUTE SLE+ pretreatment effectively eliminates matrix effects.

Table 1. Recovery and Matrix Factors in Recovery Tests with plasma (n=3).

Blood Concentration (µg/mL)	Recovery Rate* (%)	Matrix Factors* (%)
0.5	98.4	19.7
5.0	99.4	20.8
20.0	101.0	16.3

*Recovery rate = [A]/[B] × 100 Matrix factor = 1-[B]/[S] × 100.

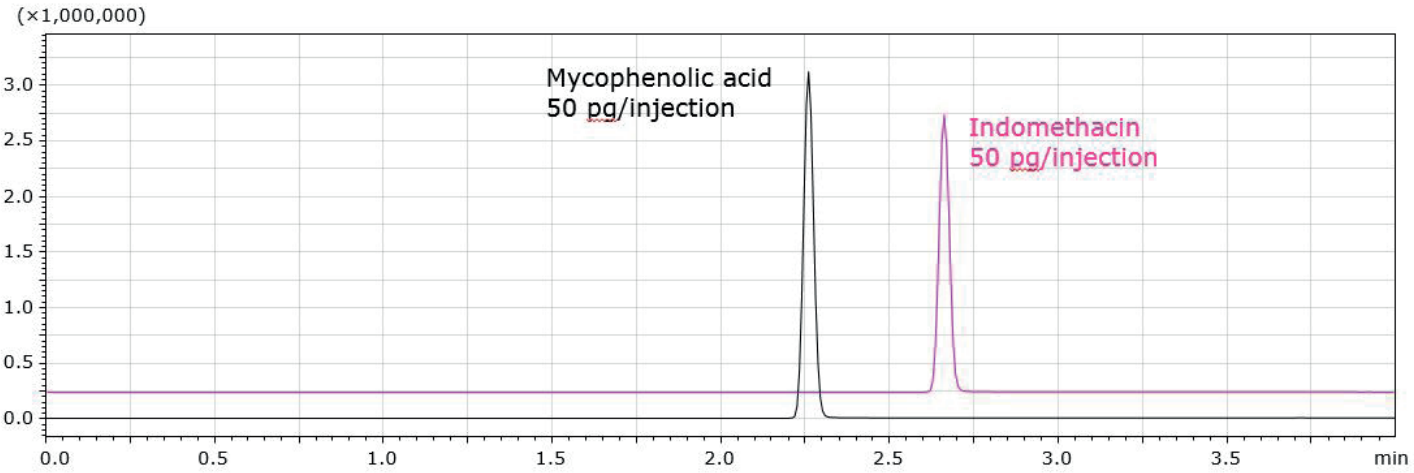

Figure 3. SRM chromatograms after ISOLUTE® SLE+ treatment with 5.0 µg/mL of mycophenolic acid in plasma.

Tips and Tricks for Sample Pretreatment with ISOLUTE® SLE+

In supported liquid extraction, the charge (ionization) of the solute (analyte) should be suppressed as much as possible to facilitate the distribution of the target compound into the organic solvent. This is especially important for highly polar compounds. Changing the pH of the sample solution improves the efficiency of extracting the sample solution using supported liquid extraction. For acidic compounds such as mycophenolic acid, acidification of the sample solution to inhibit analyte ionization can improve the transfer into organic solvents and increase extraction efficiency. Table 2 shows the changes in recovery rates due to pH differences in the sample solutions and extraction solvents.

In addition, unlike solid-phase extractions, in which the applied samples passes through the column, ISOLUTE® SLE+ absorbs all the added samples into diatomaceous earth. Therefore, it is crucial that samples be processed in volumes appropriate for each column size. If the volume of sample is too small to ensure adequate application, appropriate sample dilution is required to ensure an adequate volume is applied. Table 3 shows the change in the recovery rate due to the difference in the volume of sample applied.

Table 2. Changes in recovery rates due to differences in sample solutions and extraction solvents.

Dilution Medium	pH of the Sample	Extraction Solvent	Recovery Rate %
Water	Neutral	MTBE	2.7
Water	Neutral	Ethyl acetate	19.5
Water	Neutral	Diethyl ether	2.7
2% formic acid aqueous solution	Acidic	MTBE	101.0

Table 3. Changes in the recovery rate due to the difference in the amount of sample application.

Amount of Application	Extraction Solvent	Recovery Rate (%)
200 µL	MTBE	98.8
100 µL	MTBE	89.9
50 µL	MTBE	81.5
10 µL	MTBE	54.9
10 µL→200 µL*	MTBE	86.3

*10 µL of the samples were diluted with 2% formic acid aqueous solution to 200 µL and applied to ISOLUTE® SLE+.

Ordering Information

Part Number	Description	Quantity
820-0055-B	ISOLUTE® SLE+ 400 µL Sample Volume Columns	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 Manifold	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: AN926