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Extraction of Mycophenolic Acid (MPA) and Mycophenolic Acid Glucuronide (MPAG) from Serum Using ISOLUTE® SLE+ prior to LC-MS/MS

This application note describes the extraction of the immunosuppressant drug mycophenolic acid and its metabolite mycophenolic acid glucuronide from fortified serum using ISOLUTE SLE+ in a 96-well plate format.

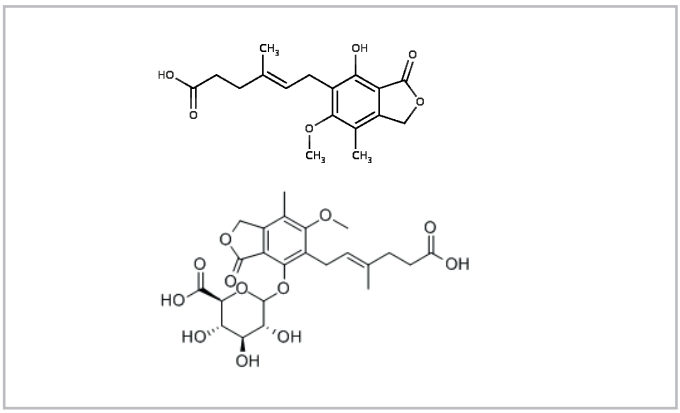

Figure 1. Structure of Mycophenolic Acid (MPA) and Mycophenolic Acid Glucuronide (MPAG)

Introduction

Immunosuppressant drugs are instrumental in preventing organ and tissue rejection in patients undergoing transplant surgery. Mycophenolic Acid is a common immunosuppressant drug used in patient transplant therapy. The ability to monitor the trough levels in patients to evaluate dosing is important for the administration of the drug. The free drug and its glucuronidated metabolite can be found in patient serum. The ability to quantitate the amount of free drug and metabolite in patient serum is supported by using a fast and efficient supported liquid extraction sample preparation method using ISOLUTE® SLE+plates. The free drug and metabolite can be recovered from serum using ISOLUTE SLE+ with high enough efficiency to allow for quantitation at target trough levels.

Analytes

Mycophenolic Acid (MPA) and Mycophenolic Acid Glucuronide (MPAG)

Sample Preparation Procedure

Format:	ISOLUTE SLE+ 200 µL Supported Liquid Extraction Plate, part number 820-0200-P01
Matrix:	Pooled human serum
Sample Pre-treatment:	Fortify 100 µL of negative serum with target analytes as needed to prepare target concentrations ranging from 0.1 µg/mL to 10 µg/mL (up to 10 µL of working standard). Add 90 µL of 20% aqueous formic acid to the samples then gently vortex the solutions. **NOTE:** Total sample load volume should not exceed the recommended load capacity (200 µL) for each well. For larger sample volumes, the method can be scaled up for use with higher capacity ISOLUTE SLE+ plates or columns.
Sample Loading:	Load pre-treated samples onto wells. Apply a short pulse of vacuum (VacMaster-96 Sample Processing Manifold) or positive pressure (PRESSURE+ 96 Positive Pressure Manifold) to initiate flow and then allow sample to absorb on column for 5 minutes.
Analyte Elution:	Apply ethyl acetate (2 x 500 µL) to each well and allow solvent to gravity flow. Apply positive pressure or pull slight vacuum as needed during collection process to facilitate a flow rate of 1 mL per minute.
Post Extraction:	Evaporate sample and reconstitute in water:acetonitrile (50:50, v/v, 500 µL).
Additional Information:	Working standards were prepared in 100% acetonitrile

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HPLC Conditions

Instrument: Agilent 1200 Liquid Handling System (Agilent Technologies, Berkshire, UK)

Column: Restek Allure Organic Acids, 5µm analytical column (150 x 4.6 mm id) (Restek, Bellefonte, PA).

Mobile Phase: A: 0.1% Formic Acid
B: Methanol containing 0.1% Formic Acid

Gradient:

Step	Time (min)	Flow Rate (µL/min)	%A	%B
1	0.0	1000	90	10
2	0.30	1000	90	10
3	0.80	1000	2.0	98
4	1.5	1000	2.0	98
5	2.0	1000	90	10
6	5.0	1000	90	10

Mass Spectrometry Conditions

Applied Biosystems/MDS Sciex 4000 Q-Trap triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA.) equipped with a Turbo Ionspray® interface for mass analysis.

Ionization Source Temperature: 300 °C

Scan Function	Analyte	MRM Transition	Declustering Potential (DP)	Collision Energy (CE)	Cell Exit Potential (CXP)
1	MPA	321.0→207.1	40	30	16
2	MPAG-1	321.1→207.1	40	30	16
3	MPAG-2	514.2→207.1	40	30	16
4	MPAG-3	497.1→207.1	40	30	16

Table 1. MRM transitions for MPA and MPAG in positive mode ESI-MS/MS

Results

The MRM transitions were identified for the MPA and MPAG (**see table 1**). It became apparent immediately that the MPAG was fragmenting in the ionization source and losing the glucuronide moiety. The in-source fragmentation of MPAG to give mass transition MPAG-1 (MRM 321.1›207.1) yielded a significantly better response than the MPAG2 and MPAG-3 mass transitions. The MPAG-1 mass transition with in-source loss of glucuronide, makes the MPAG-1 transition a pseudo isobar of the parent compound (MPA). To address the isobar issue of using the same transition for MPA and MPAG, chromatographic separation was achieved at baseline resolution for the metabolite and free drug (**Figure 2**).

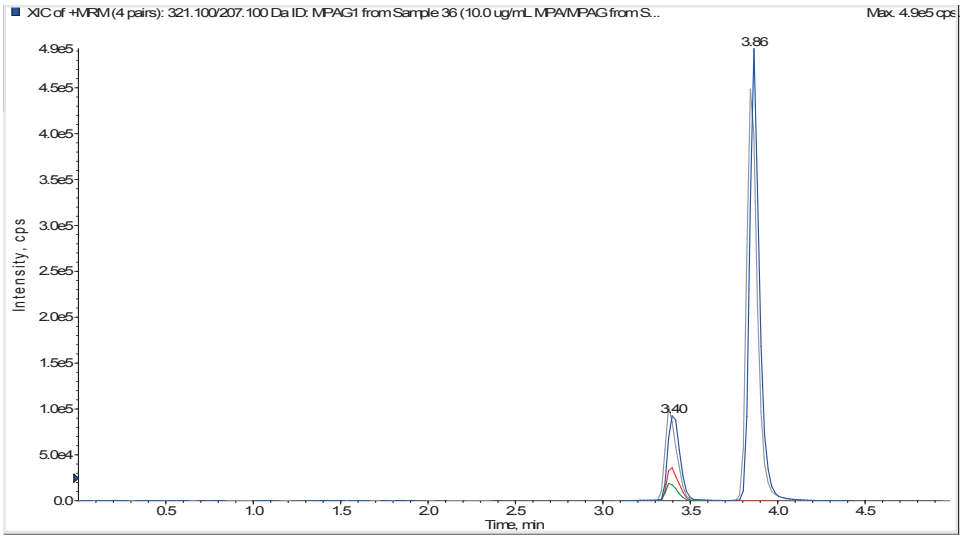

Figure 2. Extracted Ion chromatogram for 10 µg/mL MPA and MPAG extracted from serum

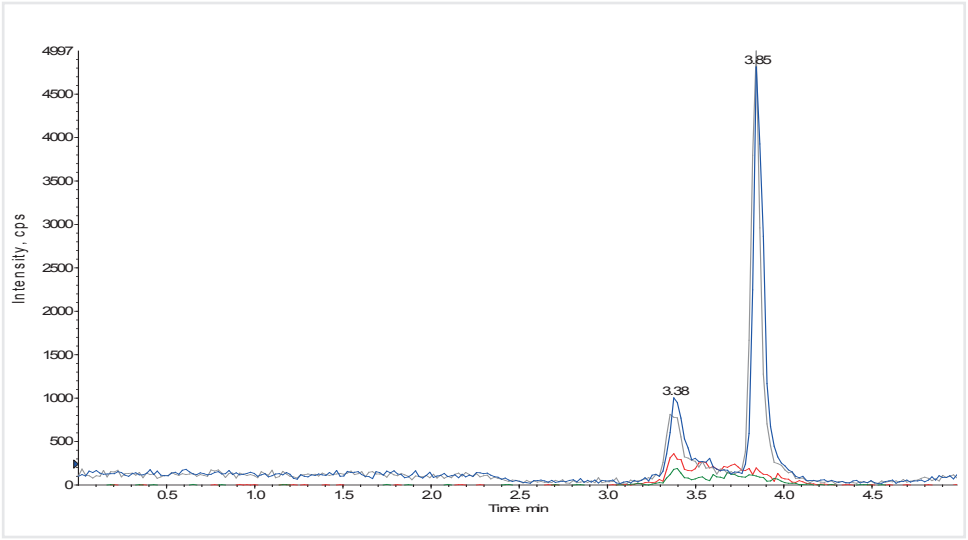

Figure 3. Extracted Ion chromatogram for 0.1 µg/mL MPA and MPAG extracted from serum

At concentrations >3 µg/mL any of the MPAG mass transitions can be used to qualitatively and quantitatively analyze the target analyte. At concentrations ‹ 3 µg/mL the MPAG2 and MPAG3 mass transitions become less prevalent. Figure 3 shows a typical extracted ion chromatogram for the target analytes at LOD trough level of 0.1 µg/mL. The MPAG1 mass transition is the only transition with enough signal response to yield a peak.

The free drug and metabolite were fortified into serum across a dynamic range of trough levels to demonstrate the recovery of each analyte using supported liquid extraction. Figure 4 shows the averaged recoveries (n=7) for MPA and MPAG in serum from 0.1 µg/mL to 10 µg/mL. The %RSD for each concentration level was ‹ 10%. The recoveries are sufficient to facilitate quantitation of each analyte across the concentration range.

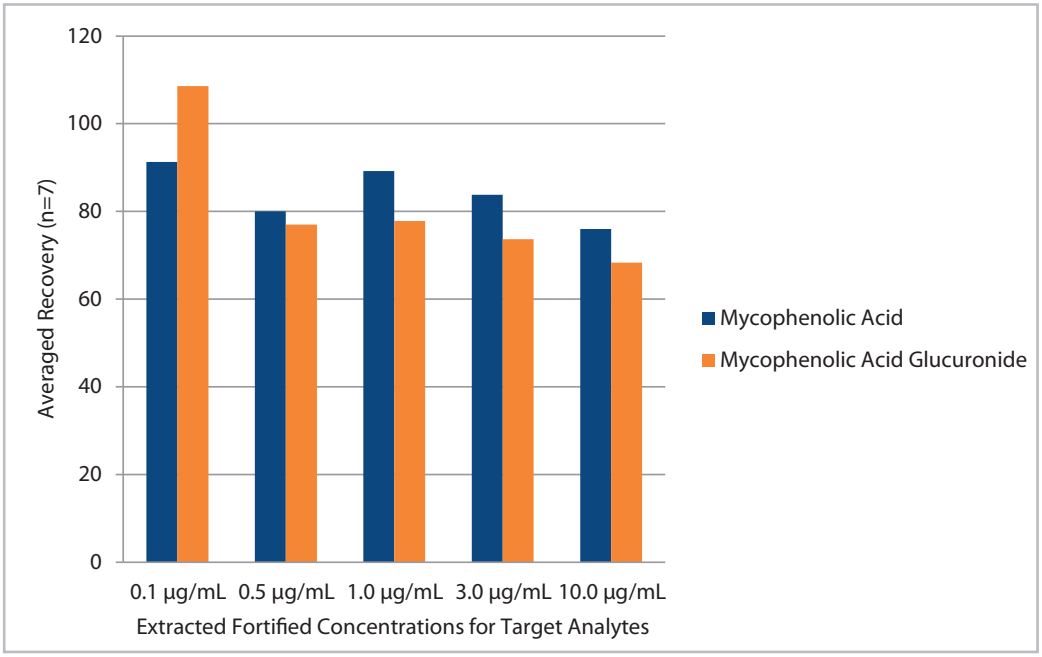

Figure 4. Plot of averaged recoveries across a concentration range for MPA and MPAG fortified and extracted from serum.

Ordering Information

Part Number	Description	Quantity
820-0200-P01	ISOLUTE® SLE+ 200 µL Supported Liquid Extraction Plate	1
SD-9600-DHS-EU	Biotage® SPE Dry Sample Concentrator System 220/240 V	1
SD-9600-DHS-NA	Biotage® SPE Dry Sample Concentrator System 100/120 V	1
PPM-96	Biotage® Positive Pressure Manifold 96 position	1

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