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Application News

Liquid Chromatograph Mass Spectrometer LCMS-2050

Quantitative Analysis of Mycophenolic Acid and Metabolites in Serum Using a Single Quadrupole Mass Spectrometer

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User Benefits

- Mycophenolic acid and glucuronic acid conjugate can be analyzed in 2 minutes per sample using a single Q-MS.
- The LCMS-2050 is compact, thus allowing it to be used in the same installation space as an HPLC instrument.

■ Introduction

Immunoassay is commonly used for measuring drug concentrations in biological samples. However, non-specific reactions and lot-to-lot differences in antibody reagents have been noted. Liquid chromatography is a way to overcome these problems. Diode array detectors and fluorescence detectors are the mainstays of LC. Still, they can require long analysis times due to separation from matrices, and sensitivity may also be insufficient.

A mass spectrometer (MS) is highly selective because it is capable of separation according to *m/z* and is superior in terms of throughput and sensitivity. Therefore, LC/MS is now replacing immunoassay as the mainstream analysis method.

This article presents an example of the analysis of mycophenolic acid (MPA) and glucuronic acid conjugate (MPA-G) in plasma using a single quadrupole mass spectrometer. Single quadrupole mass spectrometers are less expensive than triple quadrupole mass spectrometers and have simple condition settings, making it easy even for those new to mass spectrometry to begin an analysis.

■ Sample Preparation

In this report, a reagent kit (DOSIMYCO™) was used with MPA and MPA-G standards added to plasma. Here, DOSIMYCO calibration samples and control samples were used. The respective isotope-labeled compounds [^{13}C,$^{2}H_3$]-MPA and [^{13}C,$^{2}H_3$]-MPA-G were used as internal standards.

The pretreatment of plasma samples with MPA and MPA-G is shown in Fig. 1. Here, 25 mL of the internal standard and 350 mL of DOSIMYCO extract were added to 50 mL of plasma sample, stirred, centrifuged, and the supernatant was dispensed into vials for LC/MS analysis.

1. Calibrators/Controls 50 µL
2. Add 25 µL of internal standard ([^{13}C,$^{2}H_3$]-MPA, [^{13}C,$^{2}H_3$]-MPA-G)
3. Add 350 µL of DOSIMYCO extract
4. Vortex (60 sec)
5. Centrifuge (15,000 xg, 7 min)
6. Dispense 200 µL of supernatant to LC vial

Fig. 1 Protocol

■ Conditions

A Nexera™ X3 ultra-high performance liquid chromatograph and an LCMS-2050 single quadrupole mass spectrometer were used (Fig. 2). The LCMS-2050 is a compact, easy-to-use, high-performance single quadrupole mass spectrometer with heated DUIS™ ionization, which has the advantages of both ESI and APCI methods, and a mass range from *m/z* 2-2000.

Fig. 2 Nexera™ and LCMS -2050

Table 1 shows the analytical conditions for HPLC and MS, and Table 2 shows the conditions for SIM.

Table 1 Analytical Conditions

HPLC conditions	
System:	Nexera X3
Column:	DOSIMYCO trapping column
	DOSIMYCO analytical column
Mobile Phases:	DOSIMYCO mobile phases A, B
Flowrate:	A/B 0.8 mL/min (for analysis)
	C 2 mL/min (for trap)
Column Temp.:	65 °C
Injection Volume:	10 µL
MS Conditions	
Instrument:	LCMS-2050
Ionization:	ESI
Mode:	SIM (Selected Ion Monitoring)
Nebulizing Gas Flow:	3.0 L/min
Drying Gas Flow:	5.0 L/min
Heating Gas Flow:	7.0 L/min
Desolvation Line Temp.:	355 °C
DL Temp.:	100 °C

Table 2 SIM Conditions

Compound	Polarity	*m/z*
MPA	+	338.0
[^{13}C,$^{2}H_3$]-MPA	+	342.0
MPA-G	+	514.0
[^{13}C,$^{2}H_3$]-MPA-G	+	518.0

Fig. 3 shows the flow path configuration. The injected sample is trapped by the trap column, separated by the analytical column, and introduced into the mass spectrometer.

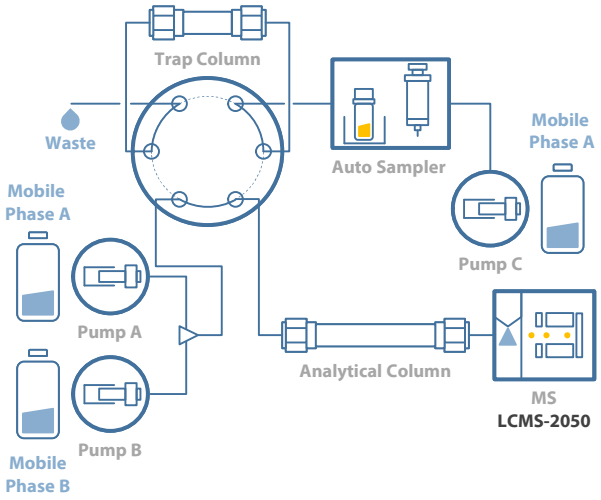

Fig. 3 Flow Path Configuration of the Pretreatment and LC-MS System

■ Results for Calibration Samples

Six calibrators were analyzed in triplicate for each point. The obtained calibration curves and chromatograms of the lowest point of the calibration curves are shown in Fig. 4. Good linearity with an R^2 value of 0.999 or higher was obtained in the concentration range (MPA: 0.1-50 mg/L, MPA-G: 1-250 mg/L). The accuracy of each point was also good, within 100 ± 10 % for each compound.

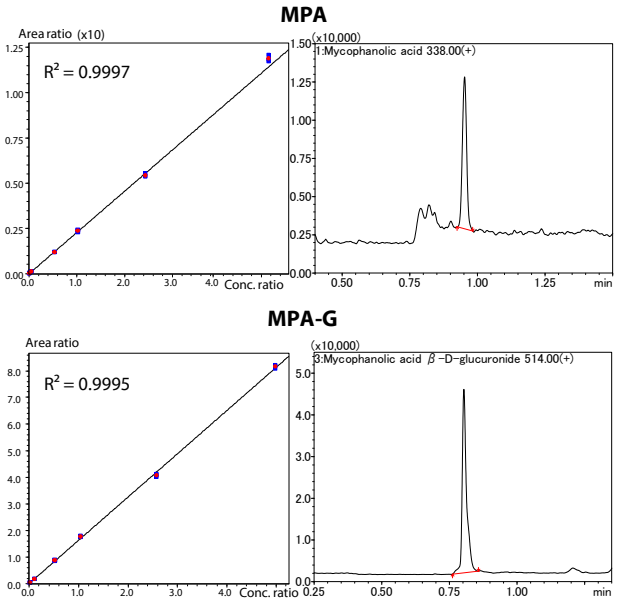

Fig. 4 Calibration Curves and Chromatograms of the Lowest Point of the Curve

■ Results for Controls

Three control samples were analyzed in triplicate for each point and the results obtained are shown in Table 3. Good results were obtained for all compounds, with accuracy within 100 ± 10 % and concentration %RSD within 5 %.

Table 3 Results for Controls

MPA			
Sample	Conc. (mg/L)	Accuracy % (average, n=3)	%RSD (n=3)
Control 1	1.02	90.4	1.03
Control 2	8.13	92.3	0.19
Control 3	31.6	104.8	1.54

MPA-G			
Sample	Conc. (mg/L)	Accuracy % (average, n=3)	%RSD (n=3)
Control 1	10.1	98.6	0.64
Control 2	43.1	97.8	0.33
Control 3	168.3	103.6	4.03

■ Conclusion

Mycophenolic acid (MPA) and glucuronic acid conjugate (MPA-G) in plasma were analyzed using a single quadrupole mass spectrometer. Both components obtained good linearity and accuracy in a set concentration range in a short analysis time of 2 min. It was shown that the single quadrupole mass spectrometer could be used to analyze drugs in biological samples with high sensitivity and high throughput.

The single quadrupole mass spectrometer is a relatively low-cost instrument that is easy to handle, even for those without mass spectrometer experience. A wide range of drug applications is expected to lead to the development of mass spectrometry in this field.

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