

Using the DPiMS-8060 Mass Spectrometer to Analyze Drugs in Plasma (1) -A Quantitative Analysis of Everolimus-

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User Benefits

- ◆ The concentration dependence of drugs in plasma is easily analyzed with this method.
- ◆ The analytical results obtained are unaffected by column condition or degradation.

Introduction

Measuring the concentration of drugs in blood or plasma is a type of analysis frequently performed in the context of research and clinical sampling. Accordingly, techniques are needed for obtaining results quickly and easily. Currently, LC/MS systems are typically used to measure drug concentrations in samples. However, because of the columns used in LC/MS systems, matrix components originating from metabolic products, such as proteins in blood and plasma, must be carefully removed. If the samples are not pretreated in this way, they can cause degradation or changes to the condition of columns, which can affect the analytical results. Furthermore, inadequate pretreatment of samples can lead to instrument contamination, increasing the frequency of maintenance required.

This article introduces an analysis method using the LCMS-8045 system equipped with the DPiMS-8060 probe electrospray ionization (PESI) unit. The concentration of everolimus in commercial plasma is then measured with only simple deproteinization pretreatment.

Principles behind the PESI method and Applicable samples

When an LC/MS system is used to analyze drugs in plasma, the deproteinization process must be performed very carefully to avoid degradation of flow lines including injector and the column. Therefore, the process from pretreatment to analysis takes approximately 30 minutes per sample, although the time can vary depending on the mobile phase and analytical method used. The PESI method used here is a direct ionization technique that does not involve the use of a column. This eliminates any impact on the analytical results due to column degradation. Furthermore, only a few dozen pL of solution are analyzed per analysis, so this method minimizes instrument contamination and the impact of matrix effects that could interfere with ionization. Given its advantages, the PESI method is ideal for simple measurements such as measuring drug concentrations in samples.

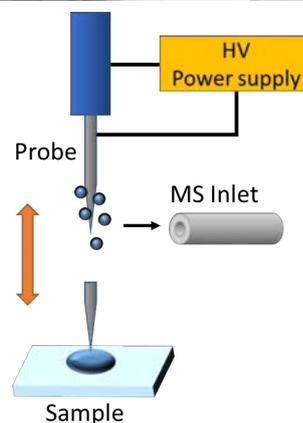


Fig. 1 DPiMS™-8060 System and the Principles behind the PESI Method

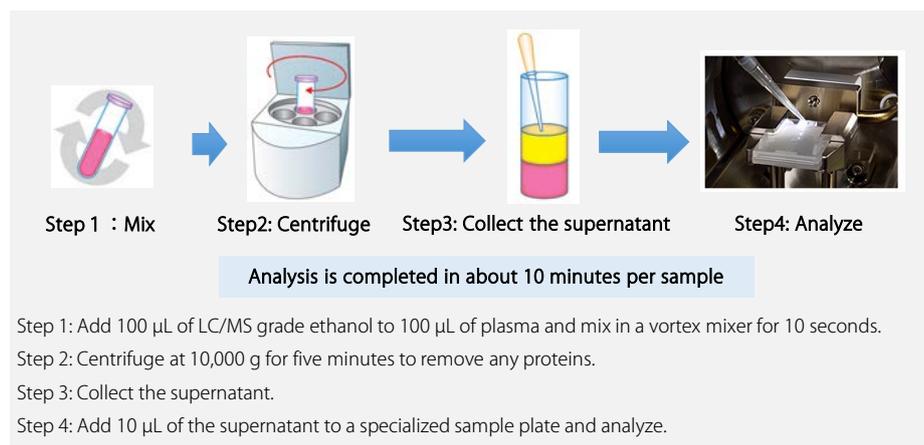


Fig. 2 Sample Preparation Process

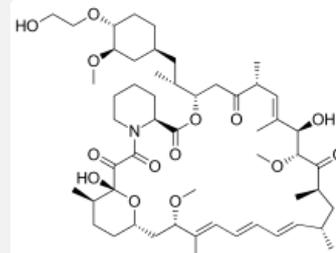


Fig. 3 Everolimus Structure

Measuring the Everolimus Standard Sample

One of the molecular target drug everolimus was used in this experiment (Fig. 3). Although plasma contains a large number of matrix components that interfere with ionization, it was confirmed that the concentration in plasma could be quantified using the PESI method under these conditions.

Standard samples of everolimus dissolved in a 50 % ethanol-water solution (v/v) were analyzed in Q3 Scan mode with the instrument parameter settings in Tables 1 and 2. The results showed that everolimus was detected primarily as three types of adduct ions (Fig. 4 A). A product ion scan was then performed. The Na adduct ion (m/z 980.8), which had the highest intensity of the three types of ions detected by Q3, was set as the precursor ion. The analysis results showed that specific fragment ions were generated when a CE value of -51.0 was specified (Fig. 4 B). Of these, the fragment ion with the highest signal intensity (m/z 389.3) was used for measuring everolimus in plasma.

Measuring Everolimus in Plasma

Samples were prepared as per the sequence in Fig. 2. Plasma samples with various final concentrations of everolimus were prepared. Next, equal amounts of ethanol were added, and the solutions were mixed in a vortex mixer. The mixed solutions were centrifuged, and then collected supernatant for use as analytical samples. In terms of the analytical conditions, the values configured are indicated in Tables 1 and 2. The calibration curve prepared from the MRM mode sample analysis results is shown in Fig. 5.

The calibration curve for everolimus in plasma was good linearity over the concentration ranges from 3 to 50 ng/mL, with $R^2 = 0.9972$ coefficient of determination.

Table 1 PESI Actuation Conditions

Ionization Position	: -37 mm
Ionization Stop Time	: 200 msec
Sampling Position	: -45.0 mm
Sampling Stop Time	: 50 msec
Probe Speed	: 250 mm/s
Probe Acceleration	: 0.63 G

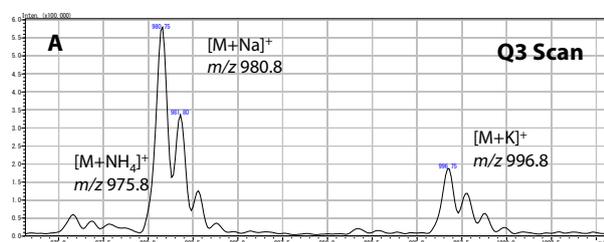


Fig. 4 Everolimus Standard Sample Measurement Results (5 ppm)

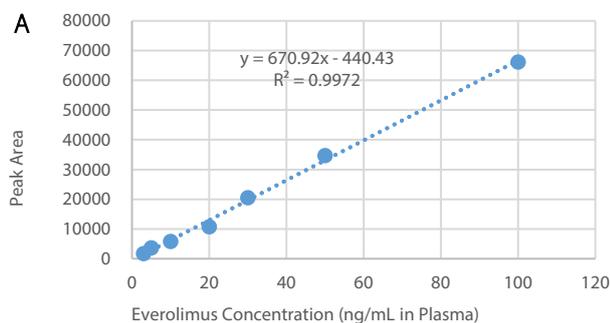


Fig. 5 Results for the Measurement of Everolimus in Plasma

A: Calibration Curve for Everolimus Concentration in Plasma (n = 3);

B: %RSD Value Calculated from Mean Area and Standard Deviation (SD) Values for Each Concentration

In terms of variability, the %RSD value was approximately 20 % or less over the linear range, which is more than adequate for semi-quantitative analysis (n = 3). In this method, the analysis time was approximately 10 minutes per sample including pretreatment process. These results suggest that drug measurements can be performed quickly and easily in this way.

Conclusion

This article gives an example that the DPiMS-8060 system has a good capability of performing concentration-dependent measurements of everolimus added to plasma. The fact that the %RSD value was at most approximately 20 % suggests that the system can even be used for quantitative analysis (Fig. 5). The time required for analysis, including pretreatment, was about 10 minutes per sample, which means that the time spent on sample measurements can be significantly shortened. Also, because this analysis method does not involve liquid chromatography, the analytical results cannot be affected by factors such as column or flow line contamination.

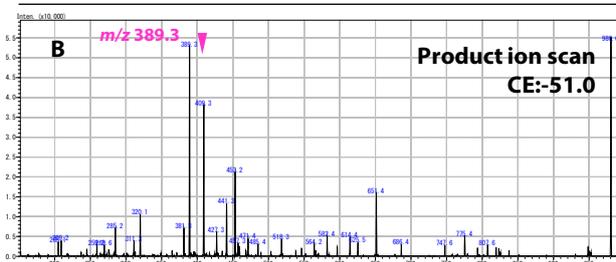
This article also demonstrates that the DPiMS system can be used with the techniques described to measure target substances in solvents that contain a variety of matrix components. Subsequent usage for analyzing clinical samples is thus anticipated.

Acknowledgements

This article was prepared in collaboration with Takehiro Inoue at Kyoto University. His assistance is deeply appreciated.

Table 2 Mass Spectrometer Analytical Conditions

DL Temperature	: 250 °C
Heat Block Temperature	: 50 °C
Interface Voltage	: + 2.45 kV (ESI-positive mode)
Sample Acquisition Stop time	: 50 msec
Scan Range	: m/z 10 – 1200 (Q3 Scan)
	: m/z 10 – 500 (Product ion scan)
Scan Speed	: 5000 u/sec



Everolimus Concentration (ng/mL)	Mean Peak Area	SD	%RSD
3	1712	162.3	9.5
5	3635	467.2	12.8
10	5857	943.2	16.1
20	10756	1158.6	10.8
30	20487	2898.4	14.1
50	34640	4256.4	12.3
100	66089	13650.1	20.6

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