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Rapid analysis of drugs in plasma using probe electrospray ionization mass spectrometry

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Overview

A system combining a probe electrospray ionization (PESI) method and a tandem mass spectrometer enabled rapid and simple quantitative analysis of Everolimus and Abiraterone in commercial plasma components by simple pretreatment with deproteinization only.

Introduction

Drugs with low effective blood concentration such as immunosuppressant have large pharmacokinetic variability and therapeutic drug monitoring is required to minimize toxic side effects. Therefore, measuring drug concentration quickly and accurately is important for clinicians to carry out timely and appropriate treatment for patients.

However, complicated pretreatment is required for measurement of drugs as complex metabolite components in the blood influence on measurement. Therefore, it is difficult to obtain the analysis result quickly.

In this study, PESI/MS/MS system is enabled rapid and simple quantitative analysis of Everolimus and Abiraterone in commercial plasma components by simple pretreatment with deproteinization only.

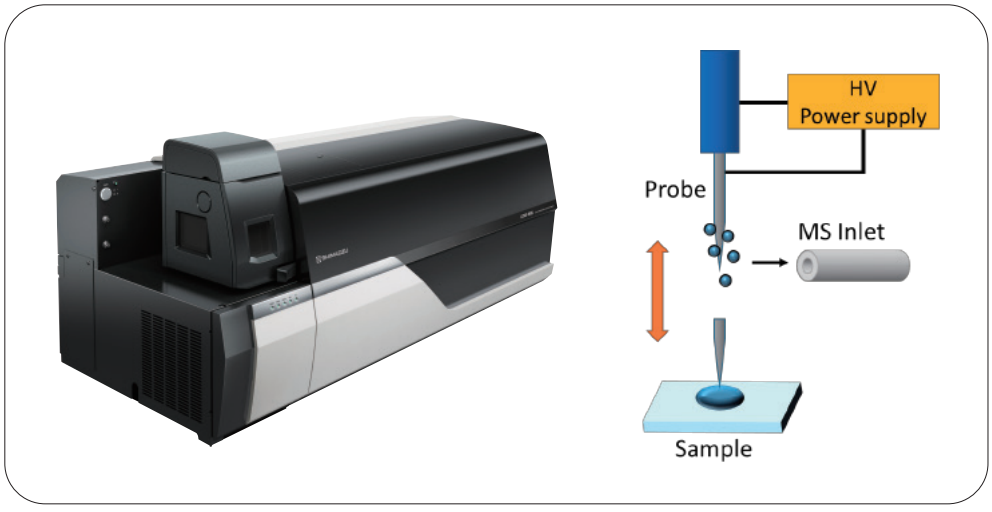
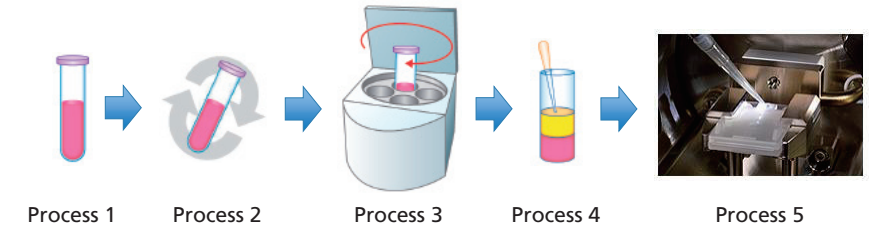


Figure 1 DPiMS-8060 and LCMS-8060 triple quadrupole mass spectrometer

Methods

A commercially available standard plasma was used for this experiment. 100 µL of ethanol for LCMS was added to 100 µL of standard plasma to which predetermined amounts of Everolimus and Abirateron were added, and mixed by vortexing. Ten µL of the supernatant after centrifugation at 10,000 g for 5 minutes was used for DPiMS measurement. The MS / MS system was used with a triple quadrupole mass spectrometer (LCMS 8060, Shimadzu, Kyoto, Japan) connected with a PESI(Probe Electro Spray Ionization)) unit (DPiMS-8060, Shimadzu, Kyoto, Japan) as an ion source.



Total measurement time (including sample pretreatment) about 10 minutes

Process 1 : Add 100 µL of ethanol for LCMS to 100 µL of reagent added standard plasma (Adjust the ethanol concentration to 50%)

Process 2 : Mix by vortexing (about 10 sec)

Process 3 : Centrifugation (10,000 g x 5 min)

Process 4 : Separate the supernatant

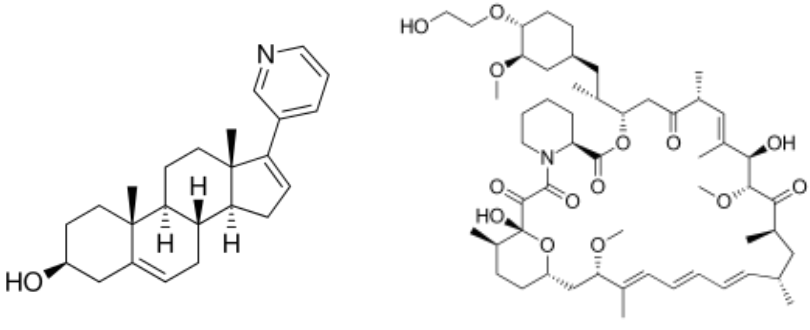
Process 5 : Place 10 µL of supernatant on the measurement plate and start measurement

Figure 2 Scheme of sample pretreatment for PESI analysis

Results

Method development for Everolimus and Abiraterone

The analytical conditions of PESI-MSMS were investigated using the standard reagents of Everolimus and abiraterone dissolved in 50% EtOH solvent.



Abiraterone Everolimus

Figure 3 Structure of Everolimus and Abiraterone

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The mass spectrometer was operating in the positive ion mode and configured in multiple reaction monitoring (MRM) mode for quantification of abiraterone (m/z 350 → 156).

To screen for metabolites of abiraterone, the Mass Spectrometer was operated in Product IonScan Mode.

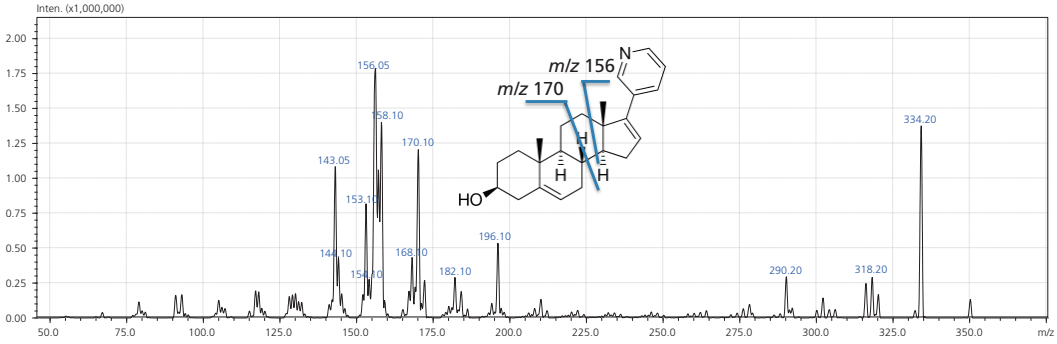


Figure 4. Mass chromatogram of product ion scan for abiraterone (compound concentration: 5 ppm)

Abiraterone has product ions at *m/z* 170 and 156 at an ionization voltage of 2.45 kV, CE-50.0 V. Product ion of *m/z* 156 was used for quantitative analysis and m/z 170 was used as a confirmation ion.

In Everolimus, multiple adduct ions were observed in a standard Q3 scan. In the product ion scan, a Na adduct (*m/z* 980.8) with high ion intensity among the adduct ions was set as a precursor ion.

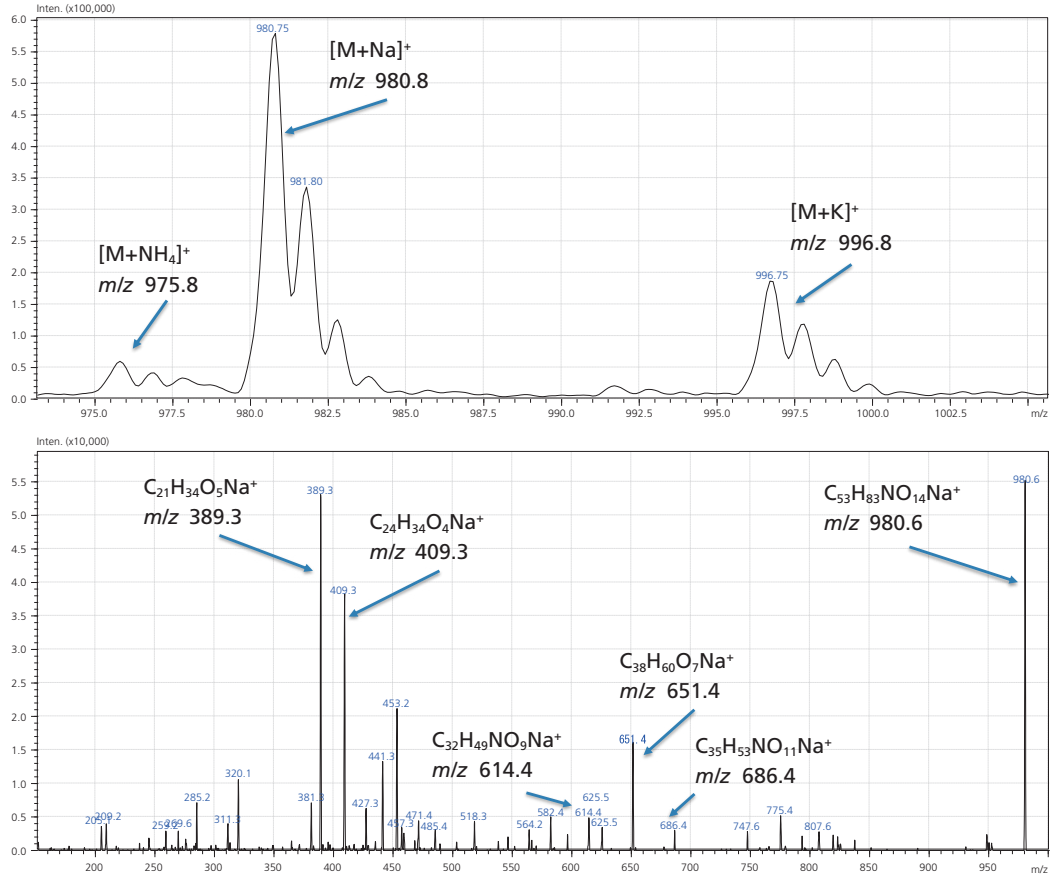
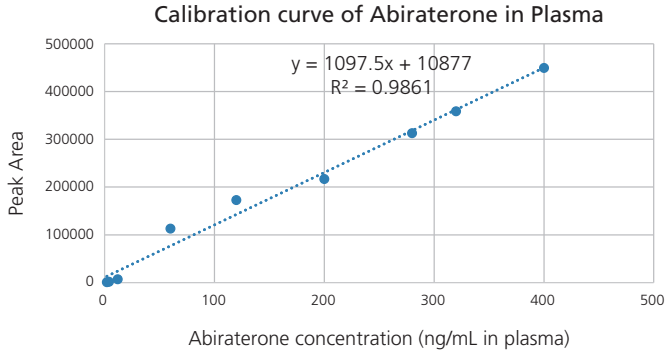


Figure 5. Mass chromatogram of Q3 Scan and product ion scan for Everolimus (compound concentration: 5 ppm)

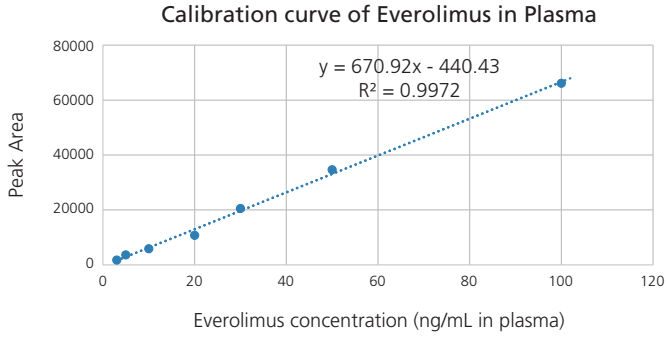
Although Evelolimus produces very many product ions, m / z 389.3 with high ionic intensity was used for quantitative analysis.

Quantitative Analysis of Evelolimus and Abiraterone in Plasma

The quantitative analysis of Everolimus and Abiraterone in Plasma was achieved using this method. Standard reagent-added plasma was mixed with an equal volume of ethanol, proteins were removed using centrifugation, and The supernatant was analyzed directly by PESI / MS / MS using MRM method created using standard reagents. A linear calibration curve was obtained with concentration ranges of each 3-100 ng/mL, 2-400 ng/mL with Everolimus and Abiraterone. The % RSD within the quantitative range of each drug was also about 20%, and it was possible to obtain enough quantitativeness. Furthermore, the analysis time including the pretreatment was also possible within 10 minutes per sample at a very short time. Ionization by the PESI technique is not susceptible to ionization inhibition due to matrix effect. Therefore, as in this case, when the same m/z component as in the target is not present in the solution, it is unnecessary to separate matrix components causing ionization inhibition by liquid chromatography or complicated pretreatment. When analyzing drugs in plasma by LC-MS/MS, careful deproteinization process is required because it will damage the column. Therefore, preprocessing time is required. However, by using the PESI/MS/MS system using the PESI technique which is one of the direct ionization techniques, it was possible to shorten significantly the measurement time including pretreatment. These results demonstrated that the PESI/MS/MS system can contribute to the quantification of drug component in complex matrices.



Abiraterone conc ng/mL	Peak Area Average	SD	%RSD
2	690	212.3	30.8
4	1439	194.3	13.5
12	6651	304.9	4.6
60	112840	17144.2	15.2
120	172832	5437.3	3.1
200	216994	6537.4	3.0
280	312745	21145.7	6.8
320	358782	16537.9	4.6
400	449258	9537.8	2.1



Everolimus conc ng/mL	Peak Area Average	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

Figure 6 Quantitative analysis results of abiraterone and everolimus

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Conclusions

- Quantitative analysis of drug in Plasma was achieved within 10 minutes including pretreatment time.
- PESI/MS/MS system can contribute to quantitative analysis of drug components in complicated matrix components with short time measurement.

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