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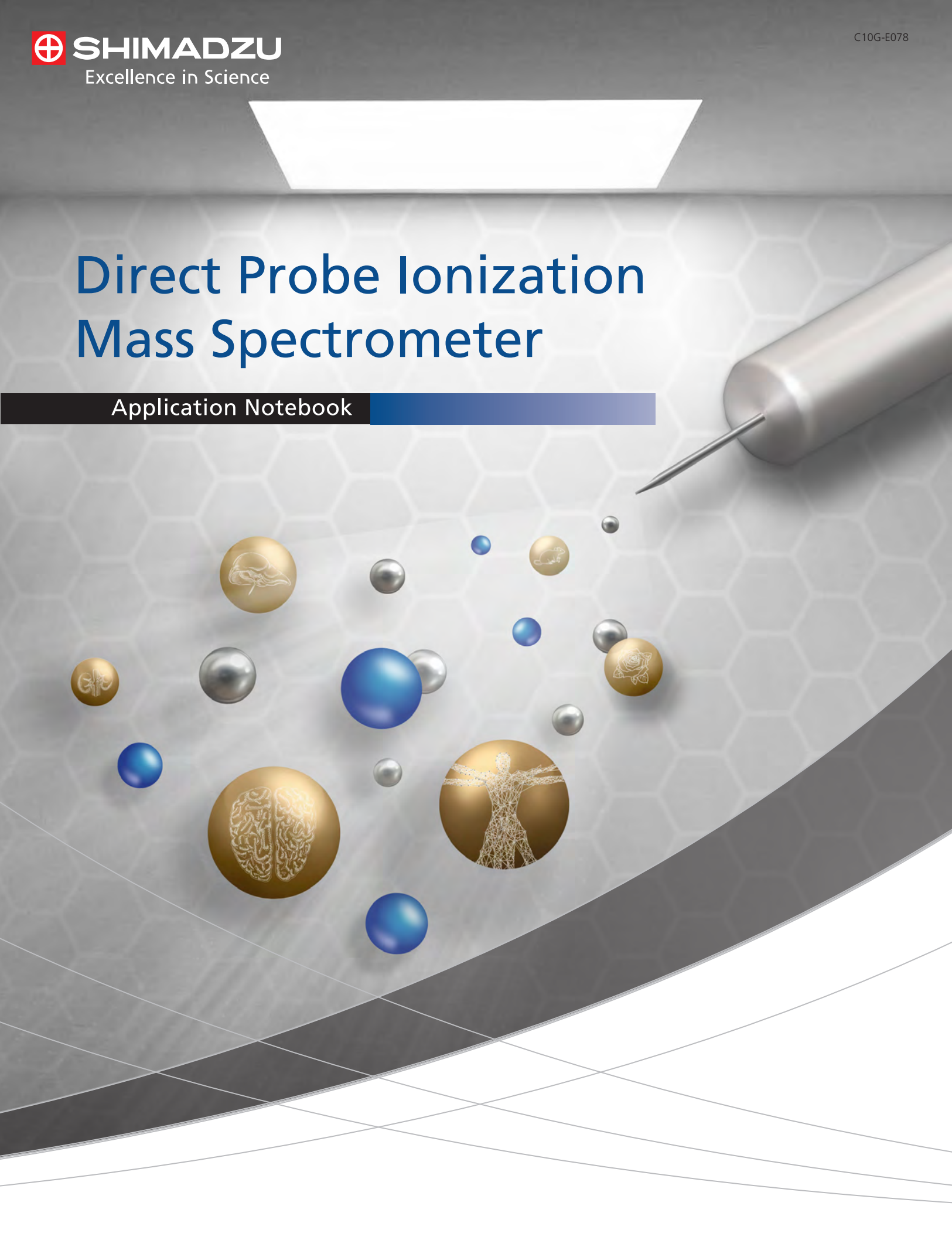
C10G-E078

Direct Probe Ionization Mass Spectrometer

Application Notebook



Index

DPiMS: Direct Probe Ionization Mass Spectrometer

Introduction

Invention of electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) technique were undoubtedly the milestones in the history of mass spectrometry (MS), fully deserving the Nobel Prize awarded in year 2002. ESI enabled high-efficiency ionization directly from a liquid phase, which made liquid-chromatography mass spectrometry (LC-MS) the most sensitive and quantitative method for a wide variety of applications. On the other hand, MALDI enabled direct ionization of biomolecules and significantly reduced the sample preparation burden for analyses requiring high throughput, such as bacterial identification. Whilst evolution of mass spectrometry hardware has continuously improved the quality of data, it was the new ionization method that drastically changed the landscape of MS applications.

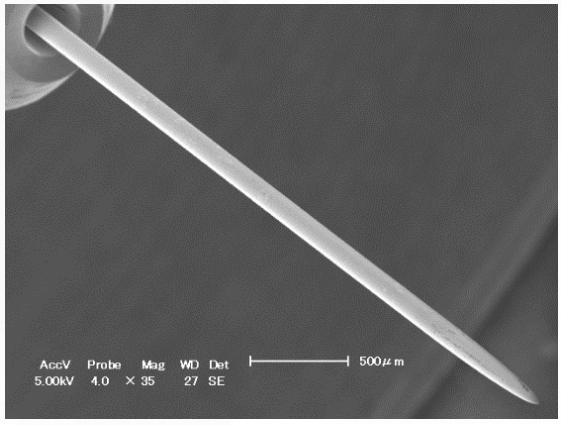


Fig.1 Scanning Electron Microscopy image of a PESI probe used in DPiMS.

In this regard, Direct Probe Ionization Mass Spectrometer (DPiMS) has the potential to pioneer new types of applications with the new ionization interface, which was initially introduced by Hiraoka *et al.*[1]. The technique successfully incorporated the best of both worlds; high ionization efficiency of ESI and simplicity of MALDI. This was achieved by using an ultrafine needle (Fig. 1) to sample an extremely small volume of sample on its surface and subsequently applying high voltage to the needle to imitate an ESI probe. Hiraoka explained that the applied voltage allows electron transfer from the solvent and causes migration of solute and solvent towards the needle-tip by electrostatic repulsion, which eventually form a jet of nano-droplets and a Taylor cone like observed in a normal ESI process. The new ionization technique was named Probe-ESI (PESI).

DPiMS: Direct Probe Ionization Mass Spectrometer

DPiMS, with the PESI technology implemented, is characterized by the microscopic sampling volume and high sensitivity for the amount sampled. In a typical application, compounds at a low ng/mL concentration range can be readily detected by DPiMS, in which case the actual abundance of compound subjected to MS can be as small as a few attograms. For this, DPiMS can be a robust solution for the routine analysis of high-complexity matrices, such as plasma or food extract, as contamination of MS hardware can be perfectly mitigated. Moreover, direct sampling from solid samples causing minimal destruction might enable new applications such as real-time analysis of live tissues or cells.

This application book features eight cutting-edge applications of DPiMS, aimed at exemplifying the potential of this novel instrument in addressing the unmet analytical needs in food safety, forensics, clinical toxicology and biological research.

Reference:

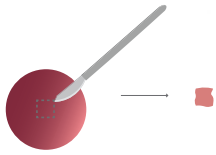
[1] K. Hiraoka, K. Nishidate, K. Mori, D. Asakawa, S. Suzuki, Development of probe electrospray using a solid needle, *Rapid Commun. Mass Spectrom.* **21** (18) (2007) 3139e3144.

Click **here** to inquire more on the DPiMS-8060

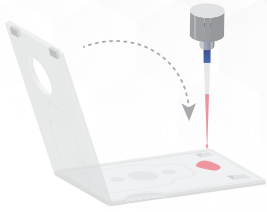
DPiMS: Mechanism of Action

1. DPiMS works with 'wet' samples

1) For Solid Samples

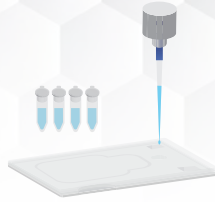


i) Cut approximately 2-3 mm square of sample.



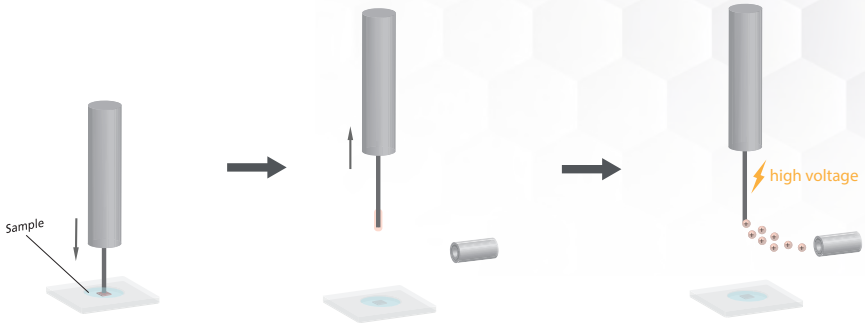
ii) Place solid samples in a biological sample plate. Snap close the plate, then add a small volume of wetting agent on top.

1) For Liquid Samples



Liquid samples can be crude but ionization occurs more efficiently after appropriate dilution. Spot samples directly into a disposable liquid sample plate.

2. Single data acquisition completes within seconds

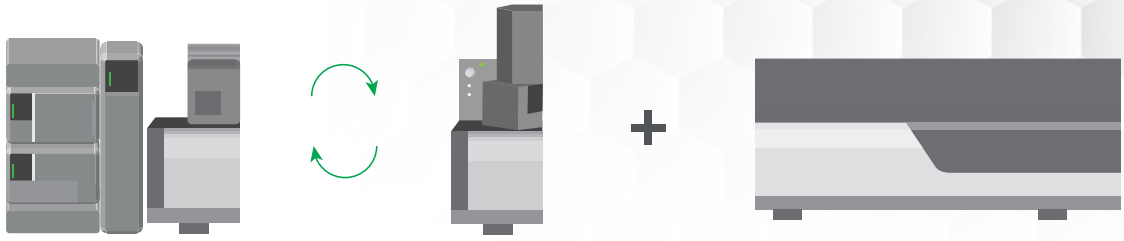


i) The probe is first lowered into the sample.

ii) The probe is raised to the top position. As the probe raises, samples are evenly coated on the surface.

iii) Voltage applied to the probe needle causes migration and subsequent electrospray from the tip-end. Ions generated travel towards the orifice and into the mass spectrometer.

3. DPiMS can be used interchangeably with LC/MS



The ionization unit of DPiMS can be detached and replaced with the heated ESI unit to enable LC/MS analysis using the same MS hardware and operating software. Switching takes only a few minutes without the need for special tools or service engineers.

Addressing unmet analytical needs of food safety

Challenge Polar compounds are challenging to analyze in complex food matrices

Solution

Quick and Sensitive Screening for Tetrodotoxin Contamination

Tetrodotoxin (TTX) is the lethal poison contained in pufferfish *(fugu)* in its inner organs, especially liver and ovaries. Upon rare incidents of suspected poisoning, detection of TTX from leftover food is needed to identify the cause. However, LC/MS analysis of TTX requires extensive cleanup and special chromatography due to high polarity and hydrophilicity. DPiMS offers much quicker and simpler analysis thanks to its unique ionization mode that enhances detection of polar compounds.

Samples:

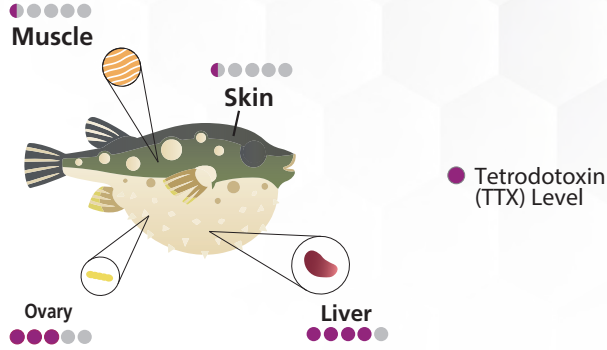
Muscle, Skin, Liver and Ovary

Target Compound:

Tetrodotoxin

MS Acquisition:

Product ion scan (confirmation)
MRM (quantitation)



Takifugu Poecilonotus

Challenge Some pesticides are not amenable to chromatographic separation

Solution

Direct Analysis of Pesticides in Agricultural Products

Due to the vast chemical diversity of pesticides, there is no chromatographic method that can cover all suspected pesticides in a single run. Hence, analytical laboratories are required to combine various techniques for full pesticide screening. DPiMS has the potential to fill the gap between LC and GC techniques, particularly for polar compounds that are not amenable to conventional chromatography.

Samples:

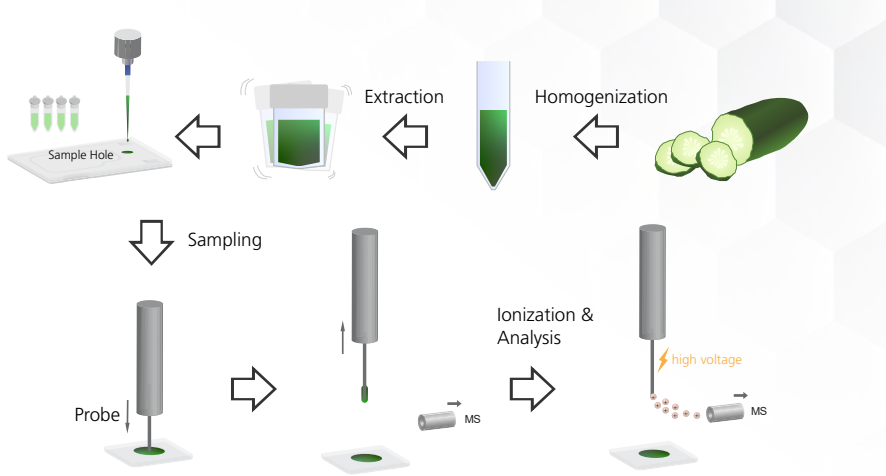
Cucumber

Target Compound:

Pesticides

MS Acquisition:

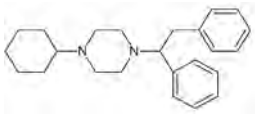
MRM (quantitation)



Alleviating the difficulties of forensic analysis

Challenge Sample states and types are wide-ranging particularly in post-mortem examination

Solution Rapid and Quantitative Analysis of Drugs in Forensic Specimens



Structural Formula of MT-45

Samples:

Organ Samples (Brain, Lung, Heart, Liver, Kidney)

Target Compound:

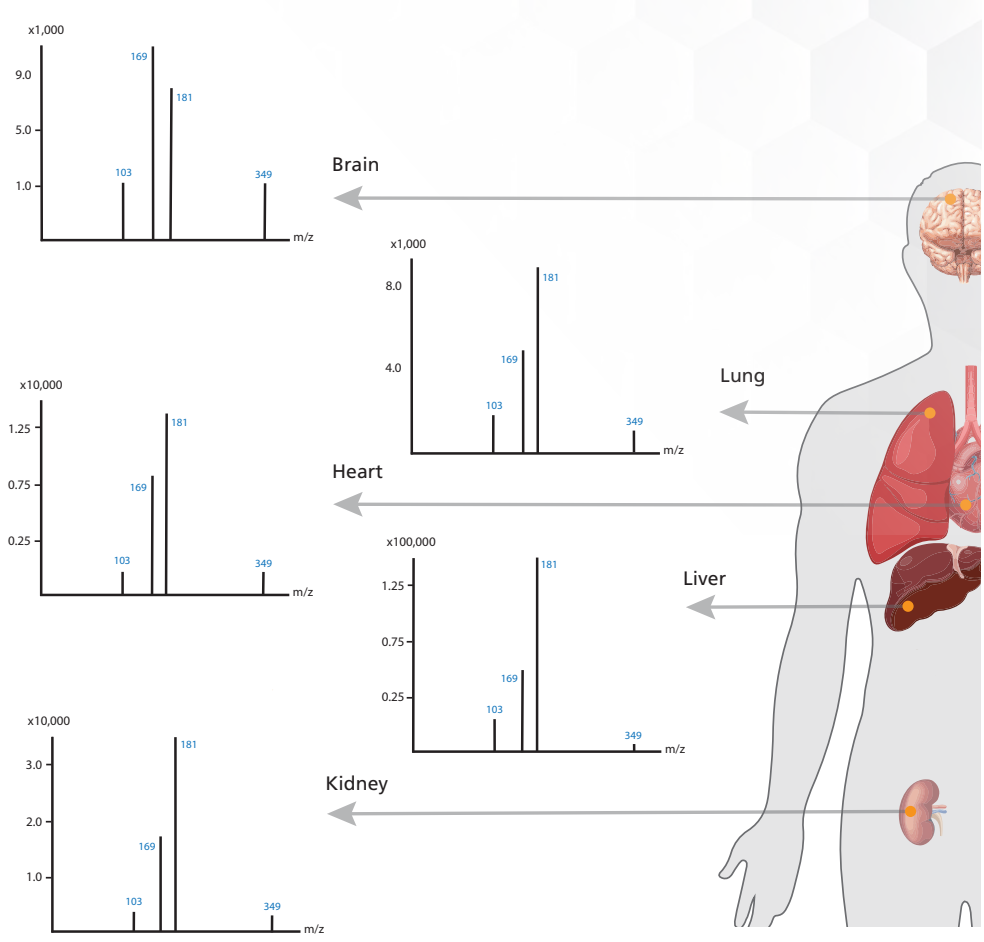
MT-45

MS Acquisition:

MRM (quantitation)

Product ion scan (confirmation)

Comparison with LC-MS/MS



Product Ion Scan Results MT-45 in each organ using DPiMS-8060

Sample pretreatment protocol for LC/MS is largely established for body fluids such as blood. In contrast, due to large variation in composition and mechanical properties, organ tissues require tailored protocols or experiences to ensure effective pretreatment, hence making body fluid the first choice sample for forensic analysis. However, there are cases when organs must be analyzed, for example for postmortem examinations, where DPiMS can provide a method that works provisionally for a variety of tissues.

An archetype of future point-of-care tests

Challenge Initial screening needs to be simple, quick and low-cost

Solution

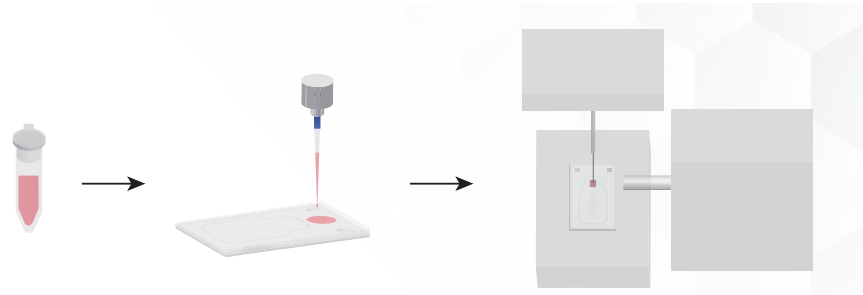
A 3-Step Screening Method for 64 Drugs in blood

Screening for drugs of abuse is perhaps the most frequently performed LC-MS/MS analysis in a forensic/clinical laboratory. Tests are intended to give a quick indication of presence or absence of a panel of drugs, and in-depth confirmatory analyses are subsequently conducted for those that were screened positive. Thus, there are enormous economic benefits in making the screening test quicker and low-cost.

Samples:
Whole Blood

Target Compound:
Panel of 64 drugs of abuse

MS Acquisition:
MRM (quantitation)



Step 1. Dilution and Centrifugation

Step 2. Sample Loading

Step 3. Sample Analysis

Challenge Low sample volume and high-throughput have always been the trade-off

Solution

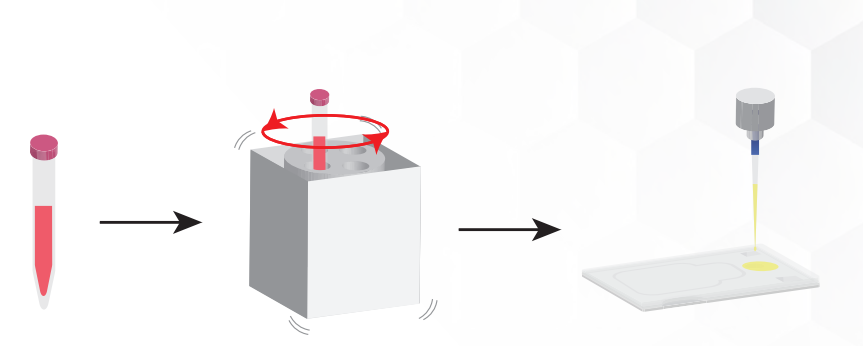
Quantitation of Everolimus and Abiraterone without Chromatography

The future of clinical testing is such that a microvolume of sample is analyzed instantly at site, enabling the clinicians to make timely decisions for better treatment. While LC/MS can deliver reliable results quickly, it needs to be operated and managed in a laboratory environment because of consumables such as columns and solvents. DPiMS, with its uniquely simple configuration, has the potential to deliver point-of-care testing after years of validation researches and developments.

Samples:
Plasma

Target Compound:
Everolimus and Abiraterone

MS Acquisition:
MRM (quantitation)
Product ion scan (confirmation)



Dilution

Centrifugation

Sample Loading

DPiMS™-8060 is for Research Use Only.
Not for use in diagnostic procedures.

Advancing biological researches with ‘fresh’ data

Challenge Sample preparation for mass spectrometry is destructive and limits the experimental design

Solution ## Proof-of-Concept of *ex vivo* Brain Metabolomics

Samples:

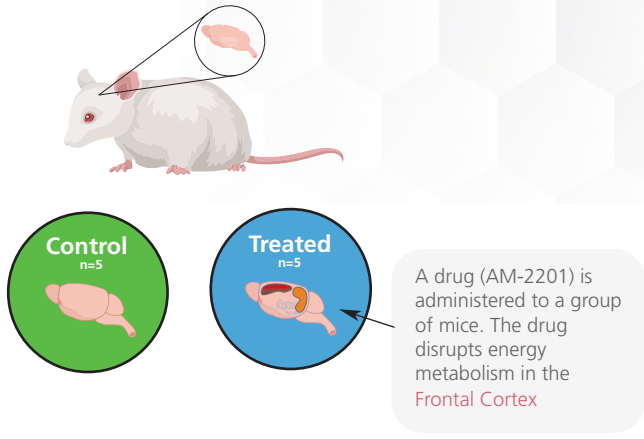
Brain Frontal Cortax and Hippocampus

Target Compound:

26 Metabolites
(incl. amino acids, organic acids, and sugars)

MS Acquisition:

MRM (quantitation)



A drug (AM-2201) is administered to a group of mice. The drug disrupts energy metabolism in the Frontal Cortex

Solution ## Proof-of-Concept of *ex vivo* Liver Metabolomics

Samples:

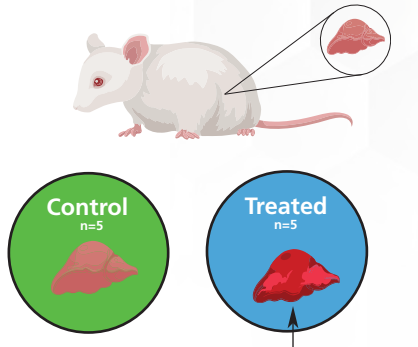
Liver

Target Compound:

26 Metabolites
(incl. amino acids, organic acids, and sugars)

MS Acquisition:

MRM (quantitation)



CCl_4^- is used to induce liver failure

In principle, mass spectrometry (MS) cannot be used for *in vivo* analysis as MS requires the actual sample to be ionized and measured, unlike photometric technologies that allows for non-invasive analysis of live specimens. Moreover, the sampling procedure for MS requires certain time, during which unwanted changes might occur to the sample. DPiMS provides the unique means to sample from intact tissues in real time without sample destruction (*ex vivo* analysis).

Aquiring data directly from live samples

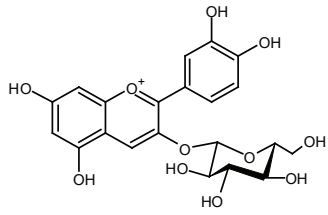
Challenge Mass spectrometry of live samples have not been possible

Solution ## Direct Analysis of Anthocyanin Pigment in Petals

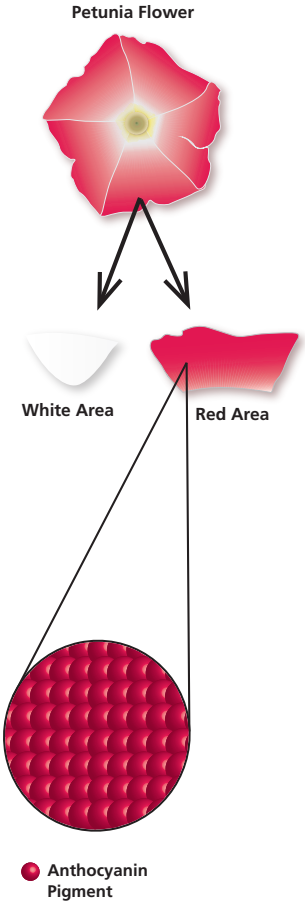
Samples:
Petunia Flower

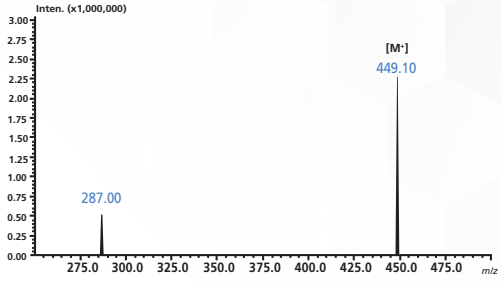
Target Compound:
Cyanidin-3-glucoside (Anthocyanin)

MS Acquisition:
Product ion scan (confirmation)

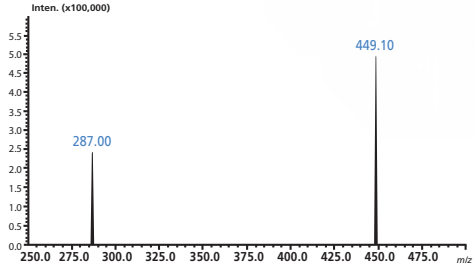


Structural Formula of Anthocyanin

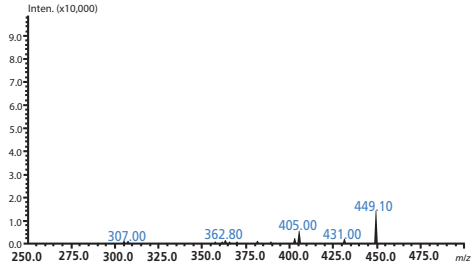




Product ion scan result of the Anthocyanin



Product ion scan result of the Petunia Flower (Red Area)



Product ion scan result of the Petunia Flower (White Area)

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Application News

No. B102

Probe Electro Spray Ionization Mass Spectrometer

Direct Rapid Analysis of Tetrodotoxin Contained in Fugu Using DPiMS™-8060

Fugu (pufferfish) has long been a familiar to the Japanese as high-quality food fish, and is now consumed in other countries as well. Even though it is widely known that certain parts and species of fugu contain the deadly poison tetrodotoxin (TTX, also called fugu poison), incidents of fugu poisoning occur frequently due to careless control.

Establishment of a quick and simple detection method for TTX originating from fugu has been strongly desired, not only for sites involved in food hygiene and quality control, but also for general consumers, who have a heightened awareness of food safety.

This article introduces a quick TTX analysis method using the new Shimadzu DPiMS-8060 mass spectrometer (Fig. 1), which combines the new ionization method called probe electro spray ionization (PESI) and a tandem-type mass spectrometer. A direct rapid analysis method for TTX contained in fugu which does not require pretreatment is also introduced. This method is applicable not only to the liver and ovaries of poisonous fugu, which are widely known to contain TTX, but also to the skin and muscles, which may contain TTX depending on the species.

T. Murata

Measurement of Tetrodotoxin Standard Sample

As a standard sample, Tetrodotoxin, from fugu (FUJIFILM Wako Pure Chemical Corporation) was prepared in a 50 % ethanol solution, and 10 µL of the sample solution was injected in the dedicated liquid sample plate of the DPiMS-8060 and measured.

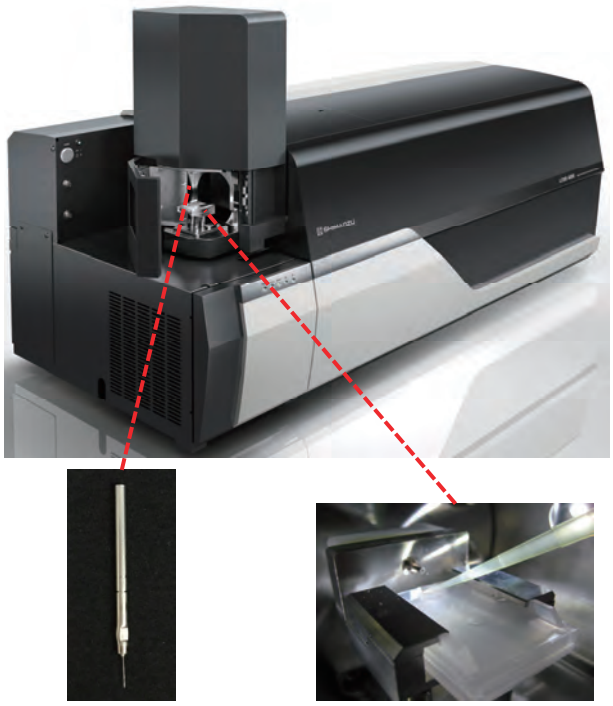
A product ion scan was carried out, conditions which enable confirmation of the characteristic fragment ion *m/z* 162.1 of TTX (Fig. 2) were studied, and the conditions shown in Table 1 were set. The results obtained by the product ion scan are shown in Fig. 3.

Next, 1, 5, 25, 50, 100, and 300 ng/mL of the TTX standard sample were prepared. The samples were measured under the MRM (Multiple Reaction Monitoring) condition, and a calibration curve was prepared.

Based on the results, the detection limit and the quantitative lower limit of TTX by DPiMS-8060 analysis were calculated. The calibration curves and the values of these limits are shown in Fig. 4.

Table 1 TTX Analysis Conditions for DPiMS-8060

Collision Energy	: -30 V
MRM Transition	: *m/z* : 320.2 > 162.1 (Monitoring conducted using proton adduct as precursor ion.)
Survey Event : Product Ion Scan MS Range	: *m/z* : 100-370
Scan Speed	: 5,000 u/sec
Event Time	: 0.06 sec
Desolvation Line	: 250 °C
Heat Block	50 °C
Polarity	: Positive
Acquisition time	: 0.5 min



Probe (Tip diameter 700 nm) Sample plate for liquids

Fig. 1 DPiMS™-8060

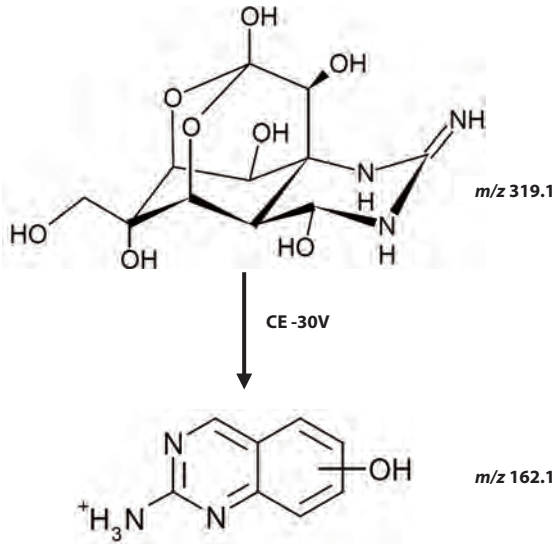


Fig. 2 TTX and Fragment Ion

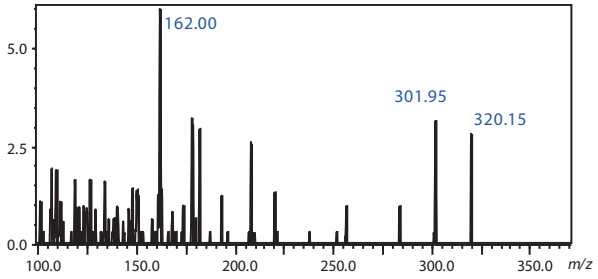


Fig. 3 Product Ion Scan of TTX Standard Sample

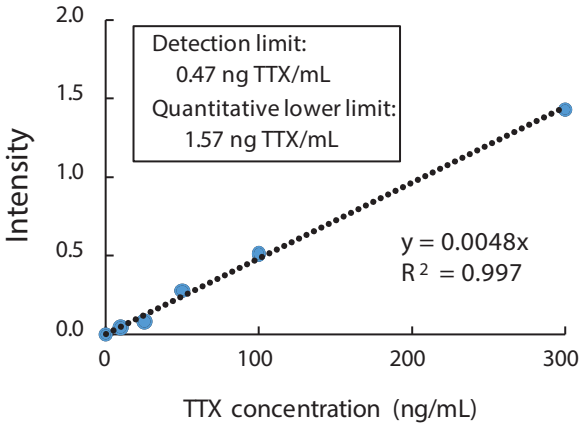


Fig. 4 Calibration Curve of TTX Standard Sample

*Values shown here are reference values and are not guaranteed values.

Detection of TTX Contained in Fugu

Real samples approximately 3 mm square were taken from the muscle, skin, liver, and ovary of the finepatterned puffer (*Takifugu poecilonotus*, Fig. 5), which is one species of poisonous fugu. The samples were inserted in the dedicated biological sample plate of the DPiMS-8060, 35 µL of the 50 % ethanol solution was dripped on the top part as an ionization accelerator, and a product ion scan was conducted. The results are shown in Fig. 6. Fragment ions of TTX were detected from all of the tissues. Furthermore, the fact that differences in detection sensitivity could also be seen, depending on the part, suggested that the magnitude of the TTX concentration contained in the respective parts of poisonous fugu can be measured simply without pretreatment by using the DPiMS-8060.

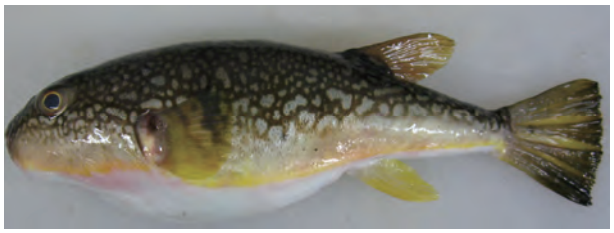


Fig. 5 Finepatterned Puffer

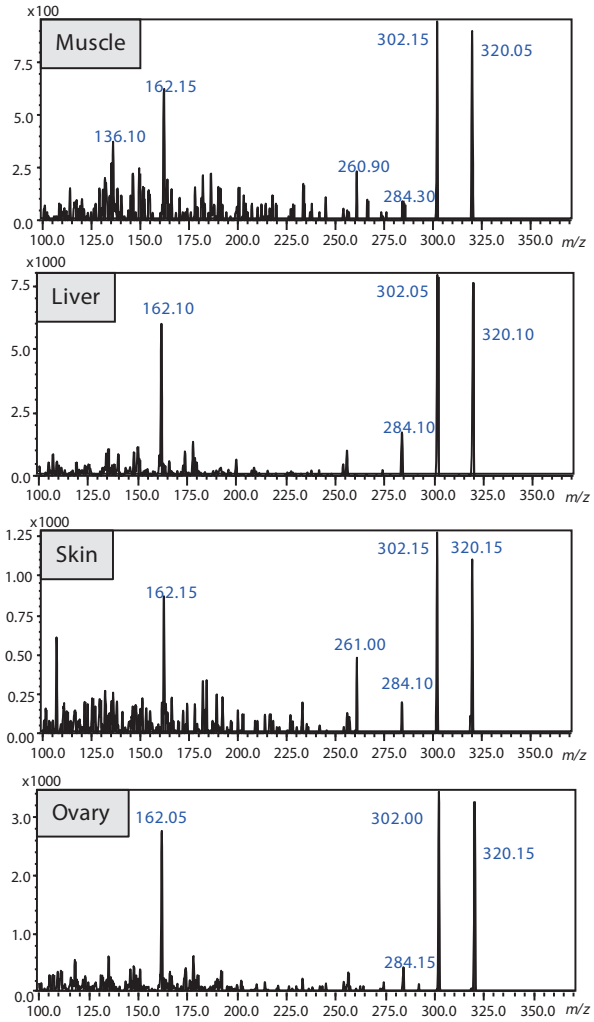


Fig. 6 Product Ion Scans of Various Parts of Poisonous Fugu (Finepatterned Puffer)

Conclusion

As a result of an analysis of a standard sample of tetrodotoxin (TTX), which is the deadly poisonous component contained in the tissue of fugu fish, it was shown that simple and high sensitivity mass spectrometry in analysis of TTX is possible by using the Shimadzu DPiMS-8060, even though analysis of this high polarity component by the conventional LCMS method tends to be complicated, for example, requiring sample pretreatment.

Moreover, quick and simple detection of TTX in fugu tissues was possible without pretreatment. This suggested the possibility that the DPiMS-8060 may become an effective analytical method in the field of inspections for protection of food safety.

<Acknowledgments>
The samples used here were provided by Prof. Yuji Nagashima of Niigata Agro-Food University. We wish to express our deep appreciation for this cooperation.

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Fast screening of pesticides in foods and agricultural products with probe electrospray ionization (PESI) tandem mass spectrometry

ASMS 2019 ThP-043

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Beijing,100020.

Fast screening of pesticides in foods and agricultural products with probe electrospray ionization (PESI) tandem mass spectrometry

Introduction

Pesticides screening in food and agricultural products have been intensively emphasized due to the increasing food safety requirement. Recently, high resolution mass has been thought to a promising screening method, but it is very costly. Pesticides usually have broad polar range and both GCMS and LCMS methods should be used for their determination. Probe electrospray ionization (PESI) source, as an ambient ionization technique, which could be used for the determination of both polar and semi-polar compounds. Meanwhile, as an in situ ionization, PESI source usually needs less sample pretreatments. In this study, 80 pesticides are selected as research targets, which are commonly monitored in vegetables every year in China. A PESI-MS/MS approach is developed aiming at both high throughput and quantification using MRM acquisition.

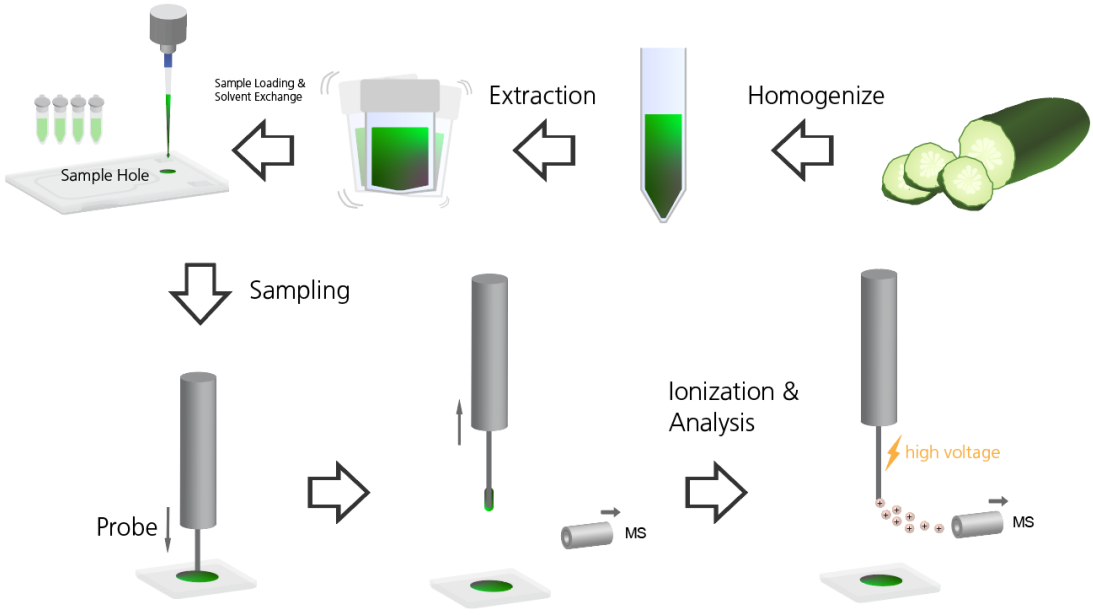

Figure 1 Sample pretreatment flow and schematic diagram of PESI

Material and Methods

Commercially available pesticides were used for this experiment. Standards of pesticides were diluted with water / isopropanol = 1/1 to an appropriate concentration and then analysed by DPiMS-8060. The water / isopropanol (1/1) solution consisted of 2 mM ammonium acetate buffer and 0.2% formic acid. The commercially available pesticides were used for the confirmation of MS spectrum and the optimization of MRM conditions.
Automated MRM parameter optimization with both APCI and ESI source were carried out by flow injection analysis of authentic standards with a function of the LabSolutions LCMS control software. The DPiMS-8060 consisted of PESI source (Shimadzu) and a triple quadrupole mass spectrometer (LCMS-8060, Shimadzu).

For sample pretreatment, 1.5 kg of fresh cucumber was pulverized with a fruit and vegetable processing machine (AUX-20B, Aux) for 3 min, and stored in 50 ml polyethylene centrifuge tube for use. 10 (±0.01) g sample were put in a 50 ml centrifuge tube, 5ml ultrapure water and 10 ml of acetonitrile (5% acetic acid) were added and then vortexed for 3min. Afterwards, 4g Mg_2SO_4 and 1g NaCl were added and vortexed for another 3min. Finally, the sample was centrifuged at 4200 rpm for 5 min and supernatant was stored for use.

For sample analysis, 10 µl of the supernatant was loaded in the sample plate and after evaporation at room temperature (ca.10mins), 10 µl of the water / isopropanol was added in the same sample plate.

Table1 Instruments Parameters for DPiMS-8060

PESI	LCMS-8060
Ionization Outage time: 200 msec	Desolvent line: 200 ℃
Take sample time: 50 msec	Heat block: 30 ℃
Discharge voltage: 2.3 kV(+)/-3.0 kV(-)	Sample amount: 10 µL
Cleaning time: 0.05 min(+)/0.05 min(-)	MRM method: 0.075 min/5 pesticides
Cycle time of probe: 2.78 Hz	Switch time: 1 msec
Ionization time: 250 msec	Residence time: 2 msec

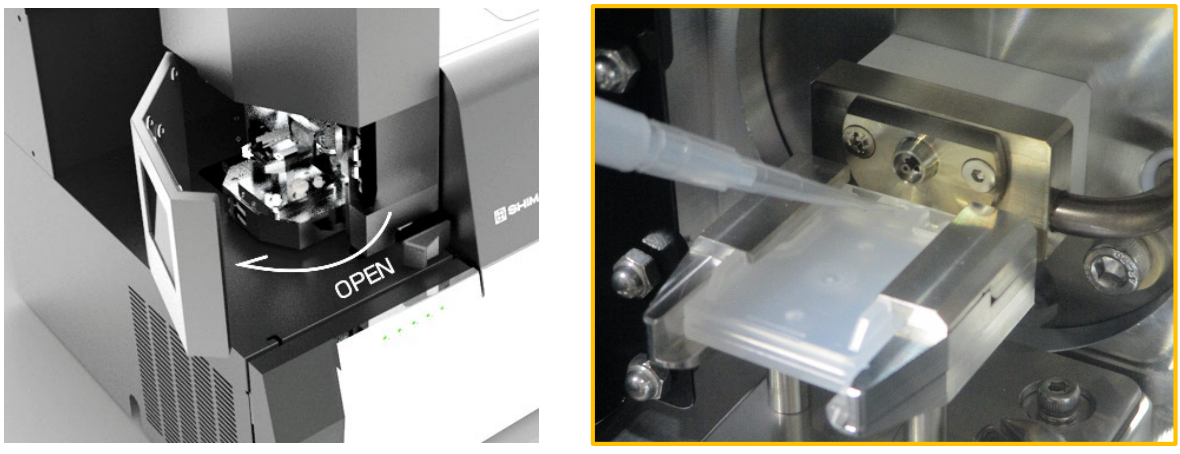

Figure 2 Injection mode for DPiMS-8060

Results

Ionization confirmation and sensitivity check

Automated MRM parameter optimization with ESI or APCI source were carried out by flow injection analysis with an embedded function of Labsolutions. Afterwards, ionization confirmation of 80 pesticides was applied using DPiMS-8060. Fortunately, among the 80 pesticides, 74 compounds can be confirmed by DPiMS-8060 in water/isopropanol (1/1) solution. The unconfirmed compounds include dicofol, fugiclor and hexacyclohexane (4 isomers), which are usually detected with GCMS.

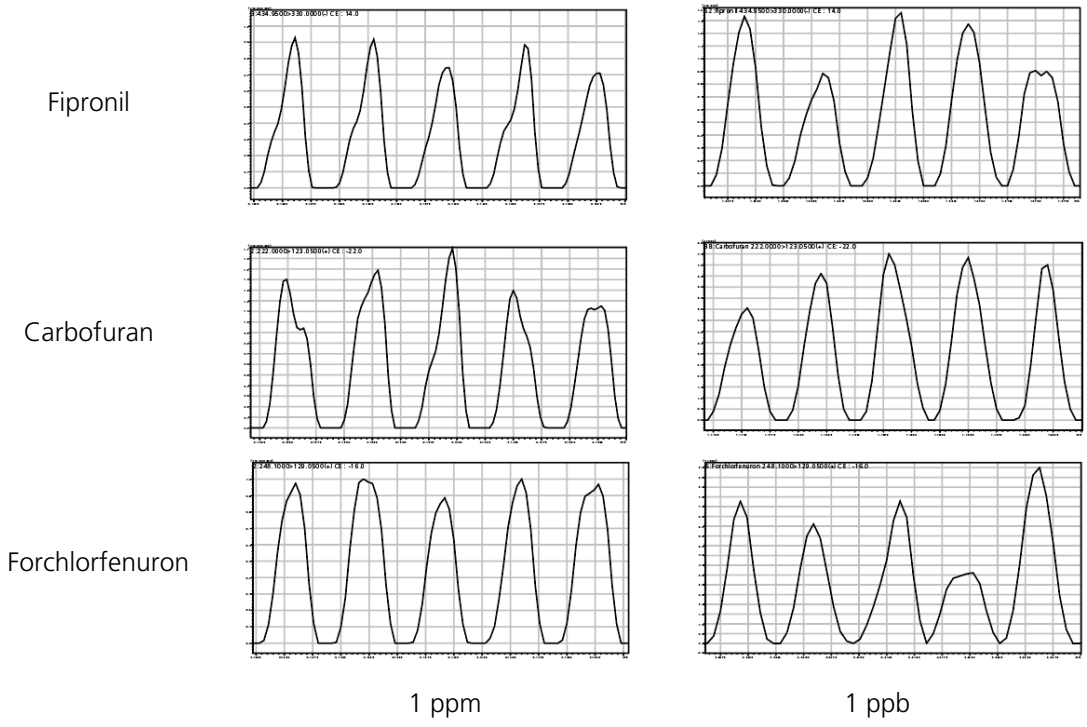

Figure 3 Typical mass chromatograms of isocarbophos, carbofuran, and forchlorfenuron
(1ppm for ionization confirmation and 1ppb for sensitivity check)

Single pesticide standard solution was prepared in 1, 10, and 100ppb for sensitivity check on DPiMS-8060. Among the 74 compounds, 27 compounds are traditionally detected with GCMS method and 47 compounds are with LCMS methods.

Generally, over 90% pesticides can be detected at lower than 10ppb, which demonstrated the high sensitivity of DPiMS-8060 (Table 2).1ppb responses of typical pesticides are shown in Figure 3.

Table 2 Linearity of typical pesticides

Traditional Method	Total	1 ppb	10 ppb	100 ppb	>100 ppb
GCMS	27	12	8	3	4
LCMS	47	45	2	0	0

Quantitative Analysis of real samples

Blank matrix was obtained the same way as normal samples. 1ppm stock solution was diluted to 5, 10, 20, 50, and 100ppb with water / isopropanol. The calibration curves were treated the same way as samples but standard solutions were added. Among the 74 compounds, the linear correlation coefficient of 36 compounds are over 0.98 (Table 3). The mass chromatogram of cucumber blank matrix with 100ppb standard are show in Figure 4. DPiMS-8060 can test 74 pesticides within 1.5 min, which is suitable for fast screening of pesticides in vegetables.For most compounds traditionally detected with GCMS, they show poor or no linearity under PESI detection. This is probably due to the matrix component strongly affect the ionization of these compounds. Especially for pyrethroids, their precursors are usually ammonium adducts. Under current pretreatment procedure, none of them could be detected.

Table 3 Linearity of typical pesticides

No.	Name	Equations	R^2
1	Methamidophos	y=10271x-3973	0.997
2	Acephate	y=242.1x+121.7	0.988
3	Omethoate	y=1324x-1757	0.995
5	Sulfone aldicarb	y=597.4x+1027.3	0.997
7	Thiamethoxam	y=436.4x+1740.4	0.998
8	Imidacloprid	y=347.2x-57.9	0.998
9	Carbofuran-3-hydroxy	y=1371x+1863	0.998
10	Dimethoate	y=503.7x+2466	0.996
11	Acetamiprid	y=335.8x+282.2	0.998
13	Aldicarb	y=271.5x+17.6	0.992
14	Carbofuran	y=545.7x+3104	0.984
15	Carbaryl	y=78.14x-327.39	0.999
16	Phosemet	y=63.11x+218.85	0.995
17	Azoxystrobin	y=858.1x+6042.2	0.999
18	Malathion	y=99.25x+10389.78	0.99
19	Dimethomorph	y=787.7x+5140.4	0.992
20	Triadimefon	y=58.36x+2903.33	0.989
21	Triazophos	y=794.9x-1005.5	0.997
24	Diazinon	y=12809x+3795	0.999
26	Phoxim	y=72.14x+3293.62	0.992
29	Difenoconazole	y=214.5x+3198.5	0.998
30	Profenofox	Y=146.2x+1198.6	0.99

Fast screening of pesticides in foods and agricultural products with probe electrospray ionization (PESI) tandem mass spectrometry

31	Tridemorph	y=911.2x+4730.8	0.998
32	Chlorpyrifos	y=47.29x+2052.55	0.998
35	Pyridaben	y=487.3x+14122.1	0.982
37	Cyromazine	y=830.1x+100260.9	0.999
40	Paclobutrazol	y=188.1x+587.0	0.997
43	Chlorantraniliprole	y=94.29x+306.3	0.997
45	Phorate	y=43.58x+343.69	0.993
46	Isofenphos-methyl	y=164.7x+868.7	0.992
60	Pyrimethanil	y=1535x+7138	0.999
64	Etofenprox	y=133.8x+6467.5	0.998
68	Fipronil	y=708.3x+13232.6	0.986
69	Fipronil Desulfinyl	y=369.8x+21422.8	0.987
70	Fipronil sulfide	y=1083x+7228	0.993
71	Fipronil Sulfone	y=1562x+13836 0.996	0.996

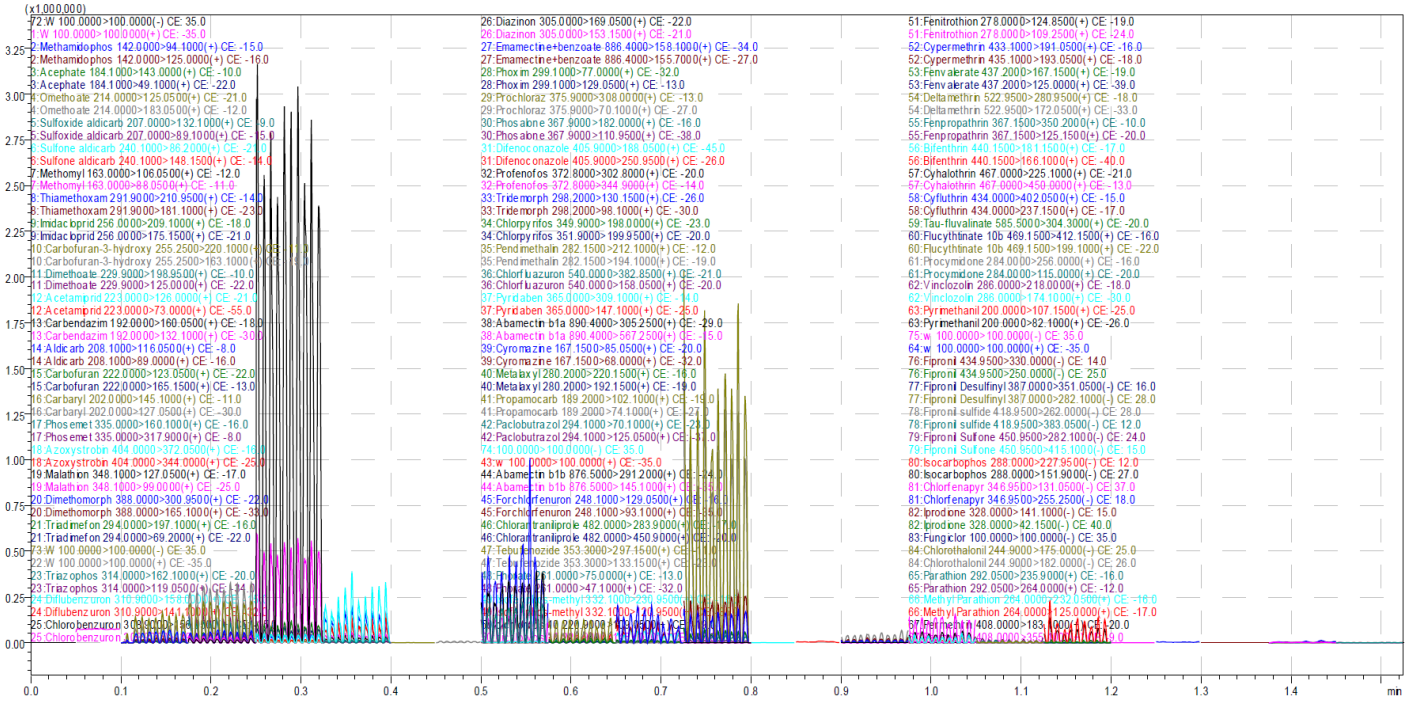

Figure 4 Typical mass chromatograms for 74 pesticides (100ppb)

10 µg/kg and 100 µg/kg spike recovery experiments were performed in cucumber. Table 4 shows the spike recovery rate for some pesticides, which demonstrate the DPiMS-8060 can be used for the fast screening analysis. For the other pesticides, the recovery rate is not within the range of 60-120%.
Another easy and simple extraction method should be developed for them.

Table 4 Pesticides spike recovery in cucumber

No.	Name	10(µg/kg) spike	100(µg/kg) spike	MRL(µg/kg)*
1	Methamidophos	122%	66.6%	50
2	Acephate	85.4%	74.5%	300
3	Omethoate	99.5%	67.4%	20
5	Sulfone aldicarb	85.6%	85.0%	20
7	Thiamethoxam	85.0%	113%	500
8	Imidacloprid	70.6%	111%	200
9	Carbofuran-3-hydroxy	73.0%	93.0%	20
10	Dimethoate	67.1%	116%	200
14	Carbofuran	89.9%	124%	20
15	Carbaryl	93.7%	102%	1000
25	Emamectine	66.0%	86.8%	20
29	Difenoconazole	79.8%	130%	200
40	Paclobutrazol	63.9%	123%	50
43	Chlorantraniliprole	95.4%	147%	20
60	Pyrimethanil	69.1%	127%	2000
64	Etofenprox	124%	81.7%	500

Conclusion

- On DPiMS-8060, the analytical work flow is presented as high-throughput and robust measurement for pesticides in agriculture products.
- MRM methods of 74 pesticides have been confirmed on DPiMS-8060, which enables fast screening analysis in short time.
- Matrix calibration curves are necessary to obtain satisfactory recovery.

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Application News

No. B90

Probe Electrospray Ionization Mass Spectrometer

Rapid Analysis of Drugs in Forensic Specimens Using the DPiMS™-8060

The analysis of drugs and toxic substances in forensics generally uses body fluid samples such as blood and urine due to their ease of handling and consideration for data collection. However, there are cases where the sampling of body fluid is difficult, such as from decomposed or charred bodies or hemorrhagic deaths. In such situations, organs may be used as samples instead. Since the instrumental analysis of organs requires complex pretreatment due to the complexity of the components within the organs, it takes time until the analysis results can be obtained.

Researches until now have focused on speeding up and simplifying the pretreatment of organs using the QuEChERS method. In order to further shorten the time needed for organ analysis, this article introduces a direct analysis method of drug content in organs with minimal pretreatment and analysis time using the newly developed DPiMS-8060 mass spectrometer (Fig. 1) which combines probe electrospray ionization (PESI), a novel ionization method, with tandem mass spectrometry.

T. Murata

Fig. 1 **DPiMS™-8060**

Qualitative Analysis

Analysis using the DPiMS-8060 involves repeatedly piercing the sample organ with a probe. At the same time, a voltage is applied to the probe tip to ionize the sample that adheres to the probe surface and introduce it directly into the mass spectrometer.

On June 26, 2016, 1-cyclohexyl-4-(1,2-diphenylethyl)-piperazine (hereinafter, MT-45) was designated as a narcotic in Japan. We used forensic samples of organs (liver, brain, kidney, heart, lung) with MT-45 intake and performed qualitative analysis of the MT-45 in those organs by product ion scanning. The results are shown in Fig. 2. We also analyzed the expected metabolites of MT-45 by product ion scanning, of which the results are shown in Fig. 3.

Preparation of samples is simple with no complex pretreatment. The analysis samples for this research were prepared by cutting a 3 mm square section from each organ and setting each section in a sample plate. Then 35 µL of a 1:1 solution of 10 mmol/L ammonium formate aqueous solution and ethanol was dripped onto each sample. With this step the samples are ready for analysis.

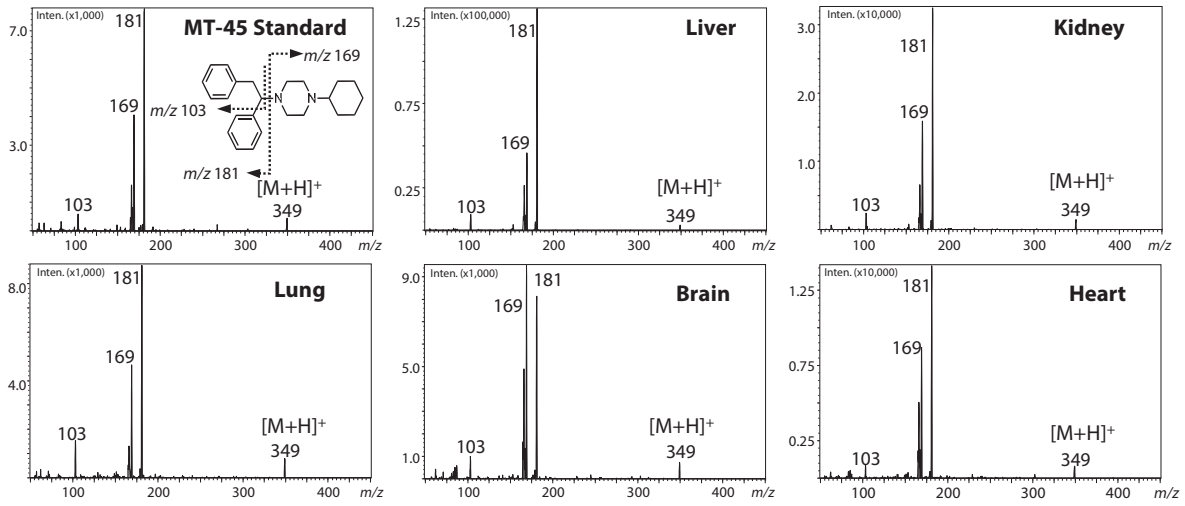

Fig. 2 **Structural Formula of MT-45 and Product Ion Scan Results of MT-45 in Each Organ**

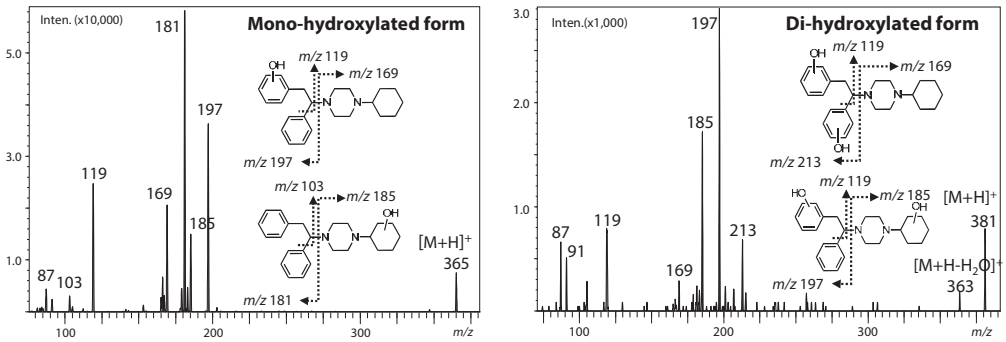

Fig. 3 **Structural Formulae of MT-45 Metabolites and Product Ion Scan Results of Expected MT-45 Metabolites in Liver**

Quantitative Analysis

Organ samples were prepared by the QuEChERS method. Using the obtained sample solution and the standard addition method, quantitation was performed using the DPiMS-8060 and an LC-MS/MS to compare quantitation values.

A calibration curve (n=6) was created by analyses using the conditions listed in Table 1. The linearity (r) of the calibration curve was higher than 0.996 and the accuracy (%RE) was within -5.0 to 9.4, demonstrating favorable analysis precision. Fig. 4 shows the calibration curve.

The drug concentration in each organ was quantitated using this calibration curve and the results are shown in Table 2.

The results show that the values obtained using the DPiMS-8060 and those with an LC-MS/MS are overall of the same level. Furthermore, since the DPiMS-8060 does not require the time for elution that an LC does, the time required for a single analysis is only 0.5 min. This means that by using the DPiMS-8060, the measurement time can be reduced by 97.5 %.

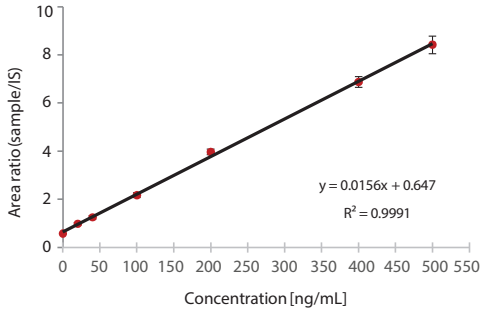

Fig. 4 **MT-45 Calibration Curve by Standard Addition**

Table 1 **Analytical Conditions Using DPiMS-8060**

Collision Energy	25 V
Mass Range	*m/z* 50-450
Scan Speed	405 u/sec
Event Time	1 sec
Desolvation Line	250 °C
Heat Block	50 °C
Polarity	Positive
Acquisition time	0.5 min/event

Table 2 **Comparison of DPiMS-8060 and LC-MS/MS Quantitation Results**

Section	DPiMS-8060 (µg/mL)	LC-MS/MS (µg/mL)
Liver	4.1	3.9
Brain	1.6	1.5
Heart	1.8	2.0
Lung	8.7	10.9
Kidney	1.7	1.5
Measurement time	**0.5 min**	20 min

Conclusion

The DPiMS-8060 enabled rapid detection of a narcotic in organs without any pretreatment.

In addition, application of the DPiMS-8060 to quantitative analysis is also possible with minimal pretreatment.

These results suggest that the use of the DPiMS-8060 is viable as a simple and rapid method for analyzing the drug and toxic substance content in organs in the field of forensics.

Acknowledgments
The data in this article was obtained through collaborative research with Kiyotaka Usui, lecturer at the Tohoku University Graduate School of Medicine. We would like to thank him for his generous support and collaboration.

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Rapid and quantifiable screening method for 64 drugs in human blood by direct probe ionization/ tandem mass spectrometry (DPiMS)

ASMS 2019 MP 216

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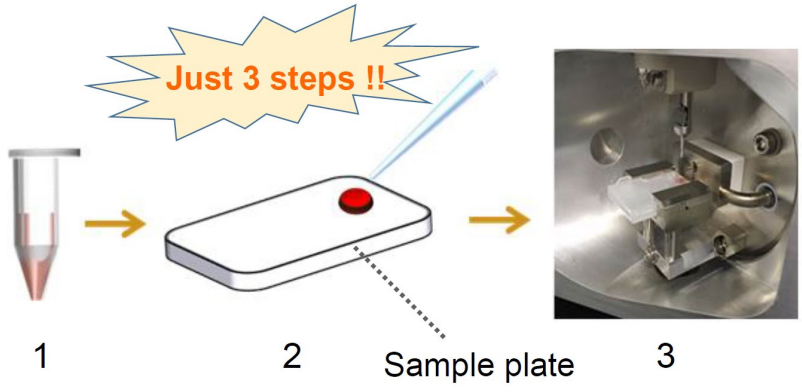
Rapid and quantifiable screening method for 64 drugs in human blood by direct probe ionization/tandem mass spectrometry (DPiMS)

Introduction

LC-MS/MS-based drug screening analysis is a gold standard in forensic toxicology because LC-MS/MS allows for the simultaneous determination of drugs in a single run. LC-MS/MS has gradually achieved high-throughput analysis, though more rapid and higher user-friendly method are preferable for drug screening. In particular, sample preparation such as extraction is mandatory for instrumental analysis, though it is a time-consuming process for analysts. Therefore, innovative analytical method without tedious sample preparation step is strongly required for next-generation drug screening analysis. Recently, ambient ionization techniques (AITs) have been improved and they are able to directly analyze target compounds in biological specimens. Probe electrospray ionization (PESI) is an ambient ionization technique invented by Prof. Kenzo Hiraoka in 20071, and it enables us to analyze drugs directly in biological specimens including tissue samples. We have first combined PESI with tandem mass spectrometry and have succeeded in analyzing intact endogenous metabolites not only in mouse liver but also in brain2-4. Here, we present a novel ultra-rapid drug screening analysis by direct probe ionization-tandem mass spectrometry (DPiMSTM-8060) and demonstrate its usability.

Material and Methods

Sample Preparation



1. Whole blood (10 µl) is diluted 10-fold with an IS* (50 ng/ml) aq.
   Then the diluted solution is further diluted 2-fold with ethanol.
2. 10 µl of the final diluted sample is pipetted onto a sample plate.
3. Start direct analysis!

*50ng/mL Diazepam-d5 aq.

⇒ Total 5 min

Fig.1 Schematic of analytical protocol

Analytical Condition

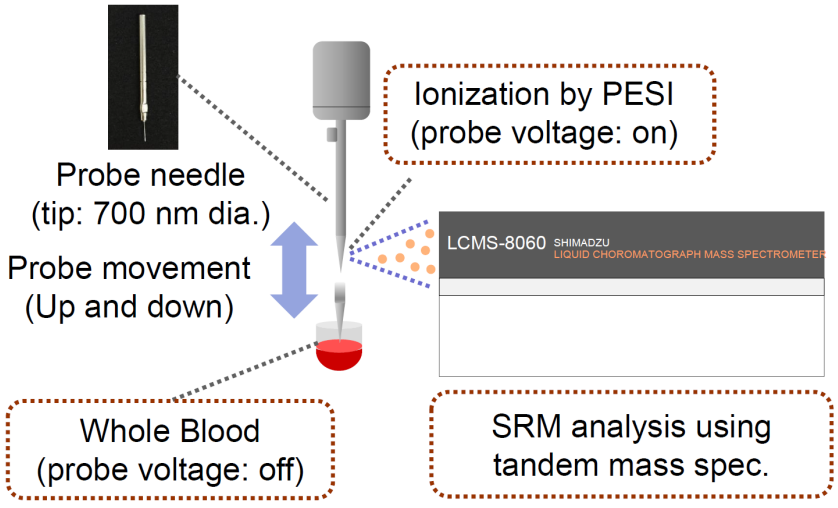


Fig. 2 Schematic of DPiMS-8060 system

Instruments: LCMS-8060 tandem mass spec. with DPiMS-8060 (Shimadzu Corporation)
Total number of the target analytes: 64 compounds (Table 2)
Data acquisition mode: SRM mode for each compound with scheduled SRM (Fig. 3).
Ionization mode: PESI positive/negative
Dwell time: 1 msec for each transition
Applied voltage: ±3.0 kV

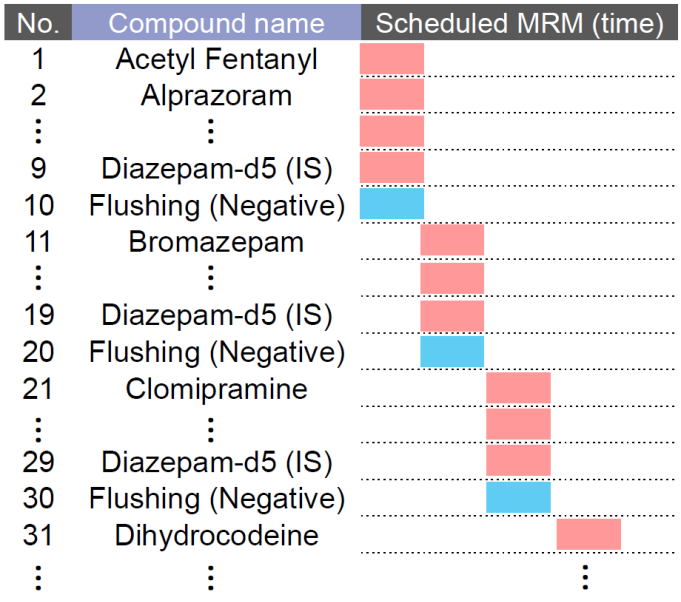


Fig. 3 Schematic of scheduled SRM method

Table 1 Comparison of data acquisition time between DPiMS-8060 and LC-MS/MS

	DPiMS/MS	LC-MS/MS
Data Acquisition time (64 compounds screening)	**3.2** mins ⇒ ultra-rapid!!	> 30 mins

Results

The method was optimized to contain 64 MRM transitions for drugs compounds to be monitored simultaneously, and their quantitative performance was evaluated in control blood samples (Table 2). The limits of quantitation were found to be 1 ng/mL for 25 compounds (Figure 3), demonstrating more than sufficient sensitivity for the screening purpose. Further validation experiment is in progress, evaluating intra-day and inter-day accuracy and precision at spike levels of 40 ng/mL and 80 ng/mL (data not shown).

Table 2. Target drugs in the panel and their calibration ranges after optimization

Compound Name	Calibration Range (ng/mL)	Linearity (R^2)	Compound Name	Calibration Range (ng/mL)	Linearity (R^2)
Acetyl Fentanyl	1 – 100	0.995	Cloxazolam	5 – 100	0.980
Alprazolam	10 – 100	0.965	Clozapine	1 – 100	0.996
Amitriptyline	5 – 100	0.993	Cocaine	1 – 100	0.992
Amoxapine	5 – 100	0.998	Colchicine	5 – 100	0.936
Atropine	1 – 100	0.999	Desipramine	1 – 100	0.991
Blonanserin	1 – 100	0.999	Diazepam	5 – 100	0.973
Bromazepam	10 – 100	0.973	Dihydrocodeine	1 – 100	0.993
Brotizolam	5 – 100	0.976	Diphenhydramine	5 – 100	0.989
Bupivacaine	1 – 100	0.998	Diphenidine	10 – 100	0.933
Carbamazepine	1 – 100	0.991	Dosulepin	5 – 100	0.981
Carpipramine	1 – 100	0.972	Duloxetine	5 – 100	0.979
Chlorpromazine	5 – 100	0.960	Escitalopram	1 – 100	0.991
Clobazam	10 – 100	0.969	Estazolam	5 – 100	0.969
Clocapramine	5 – 100	0.958	Etizolam	5 – 100	0.976
Clomipramine	25 – 100	0.983	Fludiazepam	10 – 100	0.97
Clotiazepam	1 – 100	0.988	Flunitrazepam	50 – 100	0.99

Table 2. (continued)

Compound Name	Calibration Range (ng/mL)	Linearity (R^2)	Compound Name	Calibration Range (ng/mL)	Linearity (R^2)
Flurazepam	1 – 100	0.999	Perphenazine	25 – 100	0.998
Fluvoxamine	5 – 100	0.986	Pimozide	10 – 100	0.964
Levomepromazine	5 – 100	0.985	Prazepam	5 – 100	0.995
Lidocaine	1 – 100	0.993	Promethazine	5 – 100	0.996
Maprotiline	1 – 100	0.996	Propericiazine	1 – 100	0.997
MDA	5 – 100	0.995	Quetiapine	1 – 100	0.993
MDMA	5 – 100	0.999	Quazepam	5 – 100	0.980
Medazepam	25 – 100	0.975	Risperidone	1 – 100	0.988
Methamphetamine	5 – 100	0.984	Sildenafil	25 – 100	0.968
Mianserin	5 – 100	0.972	Sulpiride	1 – 100	0.989
Midazolam	1 – 100	0.985	Tandospirone	1 – 100	0.999
Mirtazapine	1 – 100	0.992	Tofisopam	1 – 100	0.999
Morphine	25 – 100	0.940	Trazodone	1 – 100	0.995
Nortriptyline	5 – 100	0.986	Triazolam	5 – 100	0.991
Nitrazepam	25 – 100	0.970	Zolpidem	1 – 100	0.992
Pemoline	50 – 100	0.986	Zotepine	5 – 100	0.986

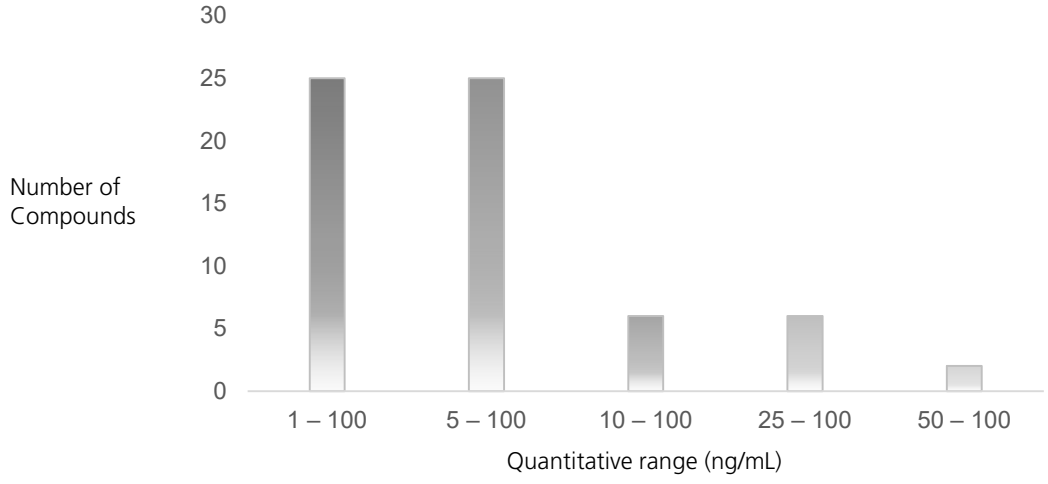


Figure 3. Quantitative ranges of 64 drugs in blood measured by DPiMS

Rapid and quantifiable screening method for 64 drugs in human blood by direct probe ionization/tandem mass spectrometry (DPiMS)

Comparison of screening results for samples between DPiMS-8060 and LC-MS/MS

Table 3 Comparison of screening results

Sample No. (Real postmortem whole bloods)	DPiMS-8060	LC-MS/MS With in-house library
	Detected Compounds	
1	Alprazolam, Amitriptyline, Flunitrazepam, Nortriptyline, Fluvoxamine	Alprazolam, Amitriptyline, Flunitrazepam, Nortriptyline
2	Atropine, Lidocaine, Clomipramine, Fluvoxamine	Atropine, Lidocaine
3	Estazolam, Risperidone, Trazodone, Diphenhydramine, Diazepam, Fludiazepam, Flunitrazepam	Estazolam, Risperidone, Trazodone
4	Methamphetamine, Amitriptyline	Methamphetamine
5	Methamphetamine	Methamphetamine

Screening result by DPiMS-8060 correlated with the results by the established LC-MS/MS, demonstrating its applicability to real postmortem whole bloods.

Conclusion

Ultra-rapid and highly user friendly drug screening without cumbersome sample preparation was achieved by DPiMS-8060, and quantitative performance of the method was fully validated for 64 various drugs, demonstrating the practicality of the method.

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

ASMS 2019 WP 030

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

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 in˜uence on measurement. Therefore, it is difÿcult 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.

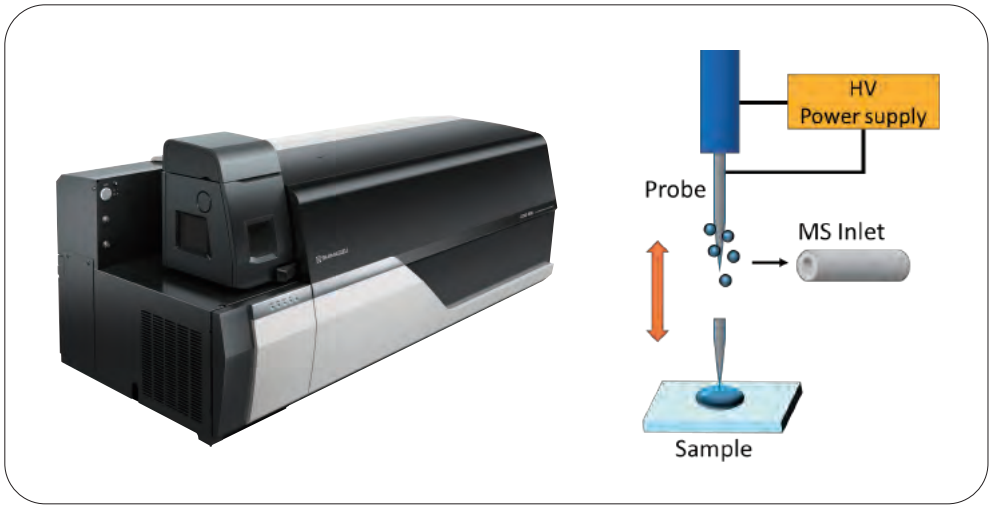
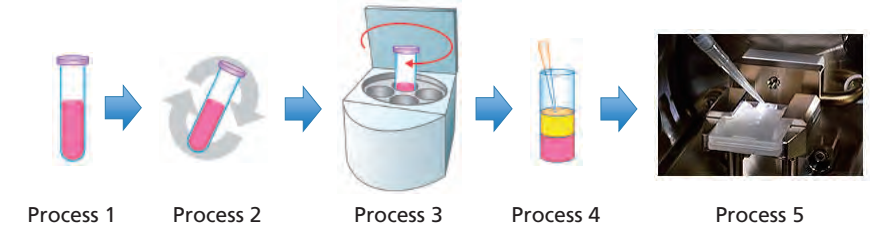


Figure 1 DPiMS-8060 and LCMS-8060 triple quadrupole mass spectrometer

Methods

A commercially available standard plasma was used for this experiment. 100 ¿L of ethanol for LCMS was added to 100 ¿L of standard plasma to which predetermined amounts of Everolimus and Abirateron were added, and mixed by vortexing. Ten ¿L 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.

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



Total measurement time (including sample pretreatment) about 10 minutes

Process 1 : Add 100 ˜L of ethanol for LCMS to 100 ˜L 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

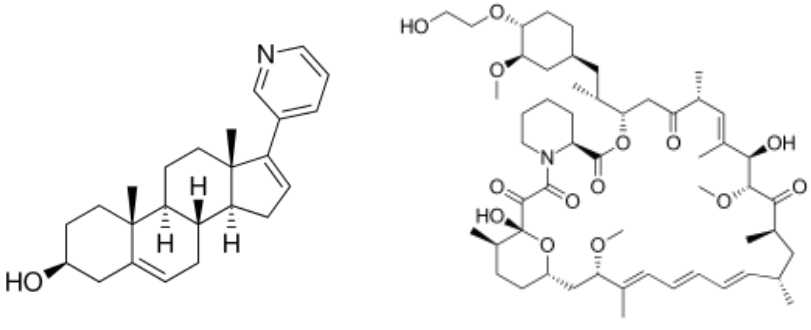
Process 5 : Place 10 ˜L of supernatant on the measurement plate and start measurement

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

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

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

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

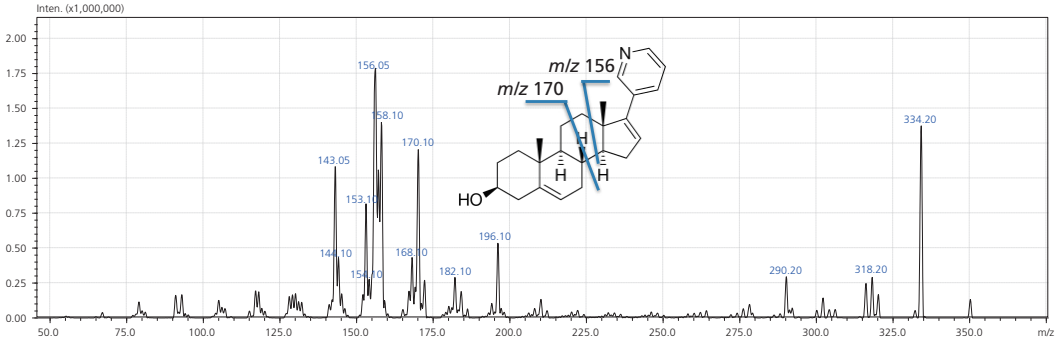


Figure 4. Mass chromatogram of product ion scan for abiraterone (compound concentration: 5 ppm)

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 conÿrmation 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.

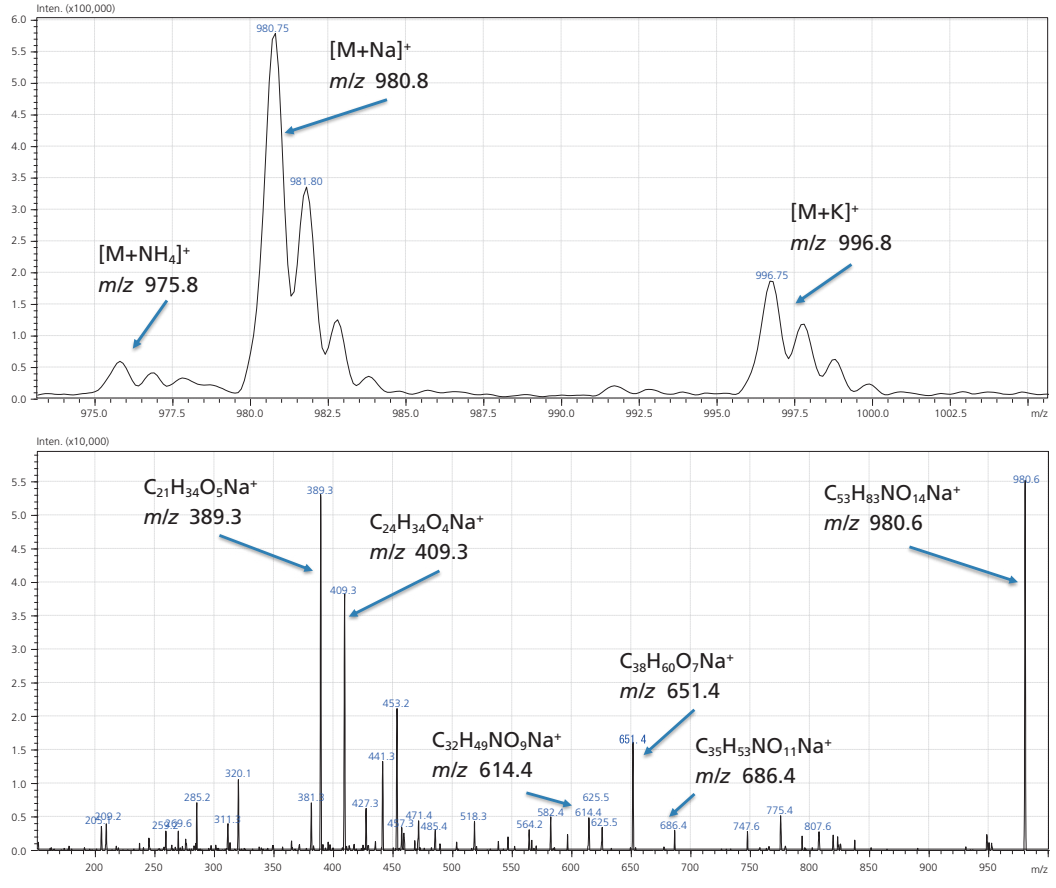


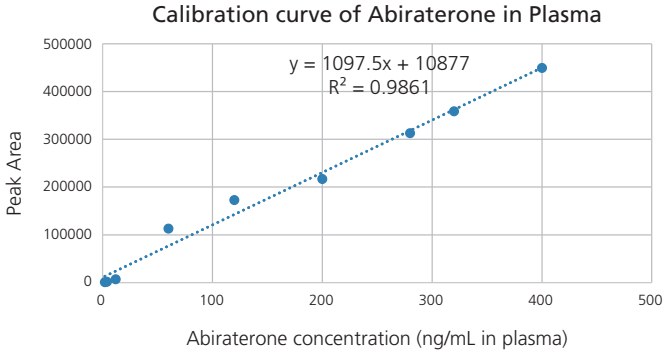
Figure 5. Mass chromatogram of Q3 Scan and product ion scan for Everolimus (compound concentration: 5 ppm)

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

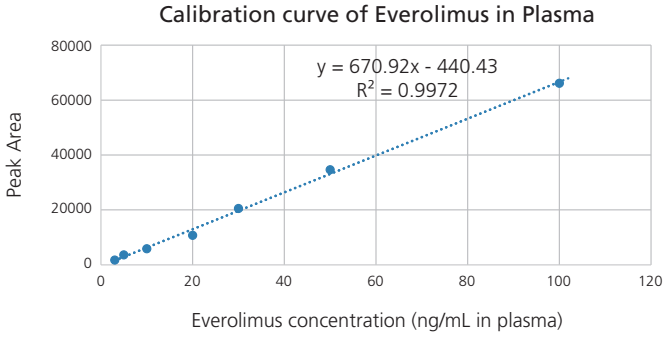
Although Evelolimus produces very many product ions, m / z 389.3 with high ionic intensity was used for quantitative analysis.

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 signiÿcantly the measurement time including pretreatment. These results demonstrated that the PESI/MS/MS system can contribute to the quantiÿcation 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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Rapid analysis of drugs in plasma using probe electrospray ionization mass spectrometry

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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Application News

No. B87

Probe Electrospray Ionization Mass Spectrometer

Establishment of a Method for Direct Analysis of the Mouse Brain Metabolome Using the DPiMS™-8060

Metabolome analysis is a method for comprehensively analyzing endogenous metabolites such as amino acids, organic acids, fatty acids, and sugars, and in recent years has come to be widely used in the field of life science. Probe electrospray ionization (PESI) is a direct ionization method in which an ultrafine and minimally invasive probe is used for sampling. Acquired samples are ionized by applying a high voltage to the probe tip and therefore components can be analyzed without a chromatograph. By using the DPiMS-8060 probe electrospray ionization tandem mass spectrometer (Fig. 1), which combines PESI with tandem mass spectrometry, direct analysis of the metabolome of a biospecimen is possible.

Generally, pretreatment is indispensable for the metabolome analysis of a brain sample since there are many lipids in the brain which cause interference in analysis. However, by using the DPiMS-8060, we succeeded in a direct and rapid analysis of metabolites in a brain sample without any pretreatment.

This article introduces a method for analyzing brain metabolites using a PESI tandem mass spectrometer.

T. Murata

Fig. 1 **DPiMS™-8060**

Analysis of the Mouse Brain Metabolome

This research attempted direct analysis of brain metabolites without any complex pretreatment by using the newly developed DPiMS-8060 mass spectrometer which combines probe electrospray ionization (PESI), a novel ionization method that uses an ultrafine probe, with tandem mass spectrometry.

Standard metabolite samples including amino acids, organic acids, and sugars (25 metabolites) were prepared by diluting them with 50 % ethanol solution and dripping 10 µL of each sample into sample plates for liquid samples. We then selected the MRM transitions for each compound and optimized the mass spectrometer conditions such as collision energy (CE). The resulting values are listed in Table 1.

Table 1 **MRM Transitions of 25 Metabolites**

Name	Polarity	Transition (*m/z*)	Collision Energy (V)
3-hydroxybutyrate	(–)	103.1>59.0	35
Citric acid/isocitric acid	(–)	191.3>111.2	20
D-glucose	(–)	179.0>89.1	20
Glucose-6-phosphate	(–)	259.1>96.9	20
Glutaric acid	(–)	131.0>87.3	20
Glycine	(–)	74.2>74.2	20
L-asparatic acid	(–)	131.9>88.1	20
L-glutamic acid	(–)	146.0>102.1	20
L-lactic acid	(–)	89.0>43.2	20
L-malic acid	(–)	133.0>114.9	20
L-serine	(–)	103.9>74.2	20
N-acetyl-L-asparate	(–)	174.0>88.2	20
Pyruvic acid	(–)	87.1>43.1	20
Succinic acid	(–)	117.1>73.0	20
Uracil	(–)	111.0>41.8	20
α-ketoglutaric acid	(–)	144.6>100.8	20
L-histidine	(–)	154.0>93.1	20
L-methionine	(–)	147.9>46.9	20
L-phenylalanine	(–)	164.2>147.0	20
L-threonine	(–)	118.1>74.1	20
L-tryptophan	(–)	203.3>116.0	20
2-aminobutyric acid	(+)	104.1>58.1	35
GABA	(+)	104.2>45.0	20
L-glutamine	(+)	147.1>84.2	20
L-leucine/L-isoleucine	(+)	132.1>86.2	20

Next, we rapidly analyzed brain metabolites (0.2 min per analysis) without any pretreatment. The metabolites in the frontal cortex of a model group of mice administered a drug to disrupt energy metabolism in the brain (AM-2201) and a control group of mice were analyzed by DPiMS/MS. Multivariate analysis (PLS-DA) results are shown in Fig. 2. Since the metabolome of the model group and the control group is clearly separated as shown in Fig. 2 (a), we can see that the metabolome profile differs between the groups. Using the loading plot shown in Fig. 2 (b), it is possible to identify the metabolites that differ greatly between the two groups. In this case, we can see that metabolites such as glutamic acid and succinic acid differ greatly. These results match well with the metabolome analysis results using a GC/MS/MS system introduced previously, indicating that the DPiMS-8060 is capable of obtaining metabolome analysis results of the same level as with existing methods even without any pretreatment.

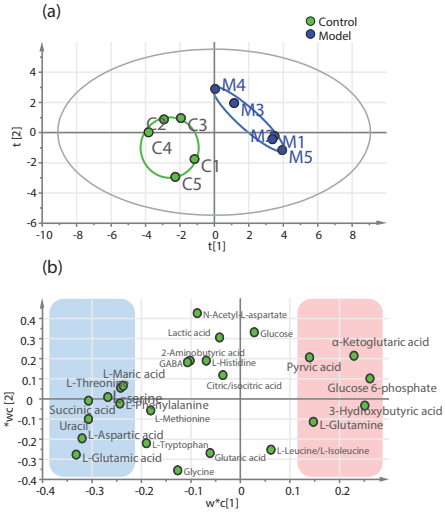

Fig. 2 **Results of Multivariate Analysis of Frontal Cortex Metabolites of the Control Group and the Model Group**

Since the probe used for sampling and ionization is ultrafine with a tip diameter of about 700 nm, the DPiMS-8060 may be applicable to distribution analysis of extremely minute areas. We next therefore analyzed the metabolites in the frontal cortex and the hippocampus using the DPiMS-8060. The results are shown in Fig. 3. As indicated by the results, direct analysis of the difference in the local distribution of frontal cortex and hippocampus metabolites was successful without pretreatment. Upon evaluating matrix effects regarding the frontal cortex and hippocampus using glutamic acid labeled with a stable isotope, no significant difference was observed between the two (Fig. 4).

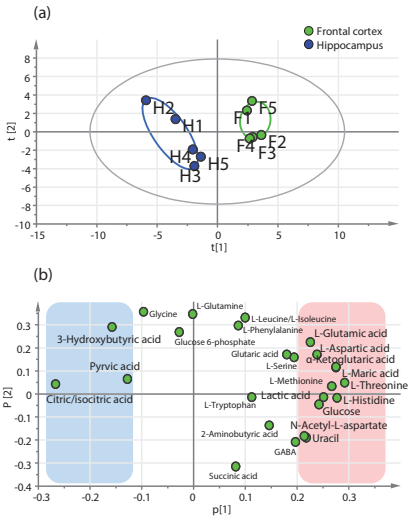

Fig. 3 **Results of Multivariate Analysis of Metabolites in the Frontal Cortex and Hippocampus**

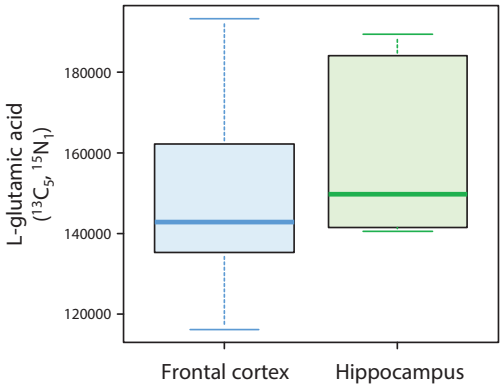

Fig. 4 **A Comparison of Matrix Effects Regarding the Frontal Cortex and Hippocampus**

Conclusion

We confirmed that brain metabolites can be detected very easily and rapidly without any complex pretreatment by using the new DPiMS-8060 mass spectrometer, even for brain samples with high lipid content.

In addition, by taking advantage of the fact that an ultrafine probe is used, local analysis of brain metabolites is also possible.

Acknowledgments

The data in this article was obtained through collaborative research with Associate Professor Kei Zaitsu and Lecturer Yumi Hayashi at the Nagoya University Graduate School of Medicine. We would like to thank them for their generous support and collaboration.

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Hayashi, Y.; Zaitsu, K.; Murata, T., et al. *Anal. Chim. Acta* **2017**, *983*, 160-165.

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Application News

No. B88

Probe Electrospray Ionization Mass Spectrometer

Establishment of a Method for Direct Analysis of the Mouse Liver Metabolome Using the DPiMS™-8060

In the analysis of endogenous metabolites (metabolome analysis), it is difficult to perfectly remove biases caused by pretreatment and sampling. Therefore, in order to accurately grasp the changes in the metabolome of a biospecimen, the establishment of a method for direct analysis of the metabolome is indispensable. Probe electrospray ionization (PESI) is a new direct ionization method in which an ultrafine and minimally invasive probe is used for sampling. Acquired samples are ionized by applying a high voltage to the probe tip and therefore components can be analyzed without using a chromatograph.

By using the DPiMS-8060 probe electrospray ionization tandem mass spectrometer (Fig. 1), which combines PESI with tandem mass spectrometry, direct analysis of the metabolome of a biospecimen is possible.

This article introduces a method established to directly analyze the metabolome of a tissue sample (intact metabolome analysis) using a PESI tandem mass spectrometer together with the application of the method to the metabolome analysis of CCl_4-induced acute liver failure model mice.

T. Murata

Fig. 1 **DPiMS™-8060**

Sample Preparation and Analytical Conditions

Standard metabolite samples including amino acids, organic acids, and sugars (26 metabolites) were prepared by diluting them with 50 % ethanol solution and dripping 10 µL of each sample into dedicated sample plates for liquid samples (Shimadzu Corporation). We then selected the MRM transitions for each compound and optimized the mass spectrometer conditions such as collision energy (CE). The information on the optimized MRM transitions of the 26 metabolites is listed in Table 1.

Next, liver samples were collected from mice by dissection. Square sections about 3 mm in size were taken from a healthy mouse and liver failure model mouse (liver failure induced by administering carbon tetrachloride) each. The sections were placed in dedicated sample plates for solid samples and then set on the instrument. Since solid samples can be analyzed by the DPiMS-8060 by simply placing them in a sample plate, there is no need for complex pretreatment.

Table 1 **MRM Transitions of 26 Metabolites**

Name	Polarity	Transition (*m/z*)	Collision Energy (V)
3-hydroxybutyrate	(–)	103.1>59.0	35
Citric acid/isocitric acid	(–)	191.0>111.1	20
D-glucose	(–)	179.1>59.2	20
Glucose-6-phosphate	(–)	259.1>96.9	20
Glutaric acid	(–)	131.0>87.3	20
Glycine	(–)	74.2>74.2	20
L-asparagine	(–)	131.0>113.3	20
L-asparatic acid	(–)	131.9>88.1	20
L-glutamic acid	(–)	146.0>102.1	20
L-lactic acid	(–)	89.0>43.2	20
L-malic acid	(–)	133.0>114.9	20
L-serine	(–)	103.9>74.2	20
Pyruvic acid	(–)	87.1>43.1	20
Succinic acid	(–)	117.1>73.0	20
Taurine	(–)	124.0>80.0	20
2-aminobutyric acid	(+)	104.1>58.1	20
L-glutamine	(+)	147.1>84.2	20
L-histidine	(+)	156.1>110.3	20
L-leucine/L-isoleucine	(+)	132.1>86.2	20
L-methionine	(+)	150.3>104.1	20
L-ornithine	(+)	132.9>70.0	20
L-phenylalanine	(+)	166.2>120.2	20
L-proline	(+)	116.2>70.0	20
L-threonine	(+)	120.1>74.0	20
L-tryptophan	(+)	205.2>146.1	20
L-tyrosine	(+)	182.1>136.1	20

Intact Metabolome Analysis of a Healthy Mouse and a Liver Failure Mouse

Carbon tetrachloride (CCl_4) is known to induce acute liver failure. Taking a CCl_4-induced acute liver failure model mouse group (hereinafter, model group) and a control group, we performed intact metabolome analysis by DPiMS/MS. Principal component analysis (PCA) of the obtained results revealed that the two groups are well separated along the first principal component axis as shown in the PCA score plot in Fig. 2 (a). Since the PCA loading plot in Fig. 2 (b) suggests that taurine is greatly influencing the separation, we created a box-whisker plot for taurine as shown in Fig. 2 (c) and performed a significance test. We found that there is a significant difference between the model group and the control group ($p<0.001$, Welch's t-test).

The amount of released enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are used as an index for the diagnosis of acute liver failure. Looking at these two enzymes, there is a significant increase in the model group and a significant negative correlation with the taurine level of the model group (Pearson correlation coefficient r=–0.975 (ALT) and –0.785 (AST)). The liver of a mouse that is administered CCl_4 produces trichloromethyl radicals from the CCl_4 by function of the metabolism enzyme CYP2E1 and it is regarded that these trichloromethyl radicals induce acute liver failure. On the other hand, it is understood that taurine acts as a scavenger of radicals in the liver. Therefore, the taurine concentration in the model group liver is likely to have decreased due to the trichloromethyl radicals that were produced from CCl_4.

Conclusion

The mouse liver metabolome was successfully analyzed without any complex pretreatment by using the DPiMS-8060.

Based on the fact that we successfully observed the changes in metabolites caused by liver failure in CCl_4-induced liver failure model mice, we were able to confirm the applicability of this method to practical use.

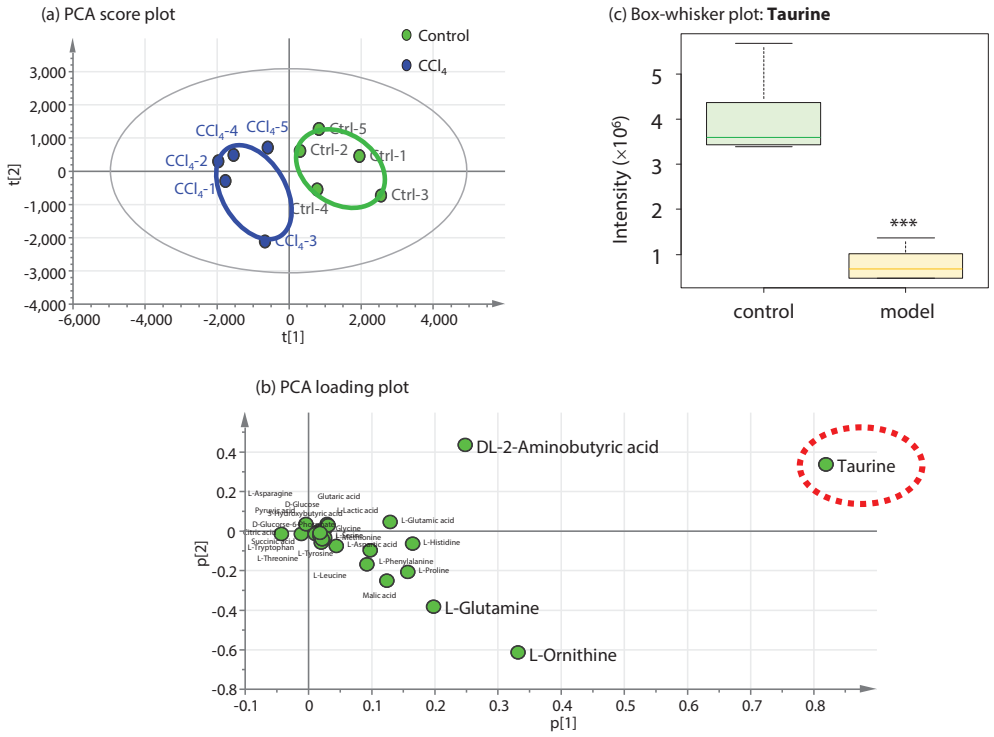

Fig. 2 **Results of Intact Metabolome Analysis of a Healthy Mouse and a Liver Failure Model Mouse**

Acknowledgments

The data in this article was obtained through collaborative research with Associate Professor Kei Zaitsu and Lecturer Yumi Hayashi at the Nagoya University Graduate School of Medicine. We would like to thank them for their generous support and collaboration.

References

Zaitsu, K.; Hayashi, Y.; Murata, T., et al. *Anal. Chem.* **2016**, *88*, 3556-3561.

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Application News

No. B89

Probe Electrospray Ionization Mass Spectrometer

Direct and Rapid Analysis of Anthocyanin Pigment in Petunia Flower Petals Using DPiMS™-8060

In recent years, foods with function claims and functional plants are gaining great attention in the fields of food and agriculture in terms of preventing illnesses and improving health. It is expected that they will grow into a large industry.

As technological advances are being made daily to add high functions and high added values to plants, needs are growing for the establishment of technologies for measuring the substances that contribute to the functions and added values of developed and produced functional plants from the viewpoint of controlling the quality of such products.

With the intent to develop a rapid and simple method for measuring the substances which cause the high functions and values that are added to functional plants, this article introduces a pretreatment-free analysis method of anthocyanin pigment that exists locally in petunia flower petals. The method uses the newly developed DPiMS-8060 mass spectrometer (Fig. 1) which combines probe electrospray ionization (PESI), a novel ionization method, with tandem mass spectrometry.

T. Murata

Qualitative Analysis of Anthocyanin Pigment in Petunia Flower Petals

Square sections a few mm in size were cut from petunia flower petals and placed in sample plates for solid samples. Preparation for analysis is finished by simply dripping 35 µL of 50 % aqueous ethanol on the sample to promote ionization with no need for pretreatments such as extraction.

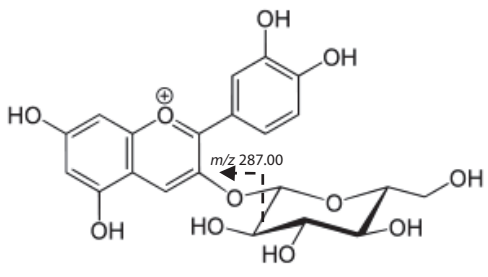
Substances of the flower petal placed in the sample plate are attached to the DPiMS-8060 probe tip by repeatedly piercing the petal. At the same time, a voltage is applied to the probe tip to ionize the sample that adheres to the probe surface and introduce it directly into the mass spectrometer. The probe used for sampling is ultrafine with a tip diameter of about 700 nm, allowing the measurement of analysis target substances in specific areas of the petal. This is not possible if employing pretreatment such as extraction.

Product ion scanning was used for the qualitative analysis of anthocyanin pigment in petunia flower petals. Table 1 lists the analytical conditions and Fig. 2 shows the result of a product ion scan.

Fig. 1 **DPiMS™-8060**

Table 1 **Analytical Conditions Using DPiMS-8060**

Collision Energy	10 V
Mass Range	*m/z* 50-464
Scan Speed	3000 u/sec
Event Time	0.15 sec
Desolvation Line	300 °C
Heat Block	50 °C
Polarity	Positive
Acquisition time	0.2 min



cyanidin-3-glucoside (MW=449.4)

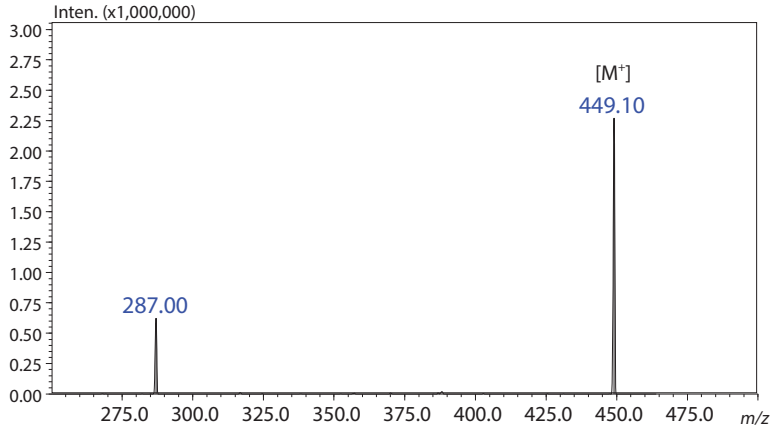


Fig. 2 **Structural Formula of Anthocyanin Pigment (Cyanidin-3-glucoside) and Product Ion Scan Result of a Standard Sample**

Local Distribution of Anthocyanin Pigment

Both the red area and white area of a petunia petal were sampled and analyzed by product ion scanning. The obtained qualitative results are compared as follows.

As shown in Fig. 3, anthocyanin pigment was found in the red area of a petunia flower petal, but not in the white area. This demonstrates that the correlation between anthocyanin pigment distribution and the color of a flower petal can be verified by mass spectrometry.

Since there is no need for pretreatment such as extraction or complicated operations using an analysis instrument, one sample can be analyzed in merely 0.2 min, indicating that the DPiMS-8060 can be used for rapid and simple measurements of substances contained or locally present in plants.

Conclusion

The anthocyanin pigment in petunia flower petals was rapidly detected by using the DPiMS-8060 without any pretreatment.

In addition, the local distribution of the anthocyanin pigment within petunia flower petals was measured easily.

These results suggest that for the quality control of foods with function claims and functional plants, the DPiMS-8060 may be used for the simple and rapid analysis of the substances which cause high functions and high added values.

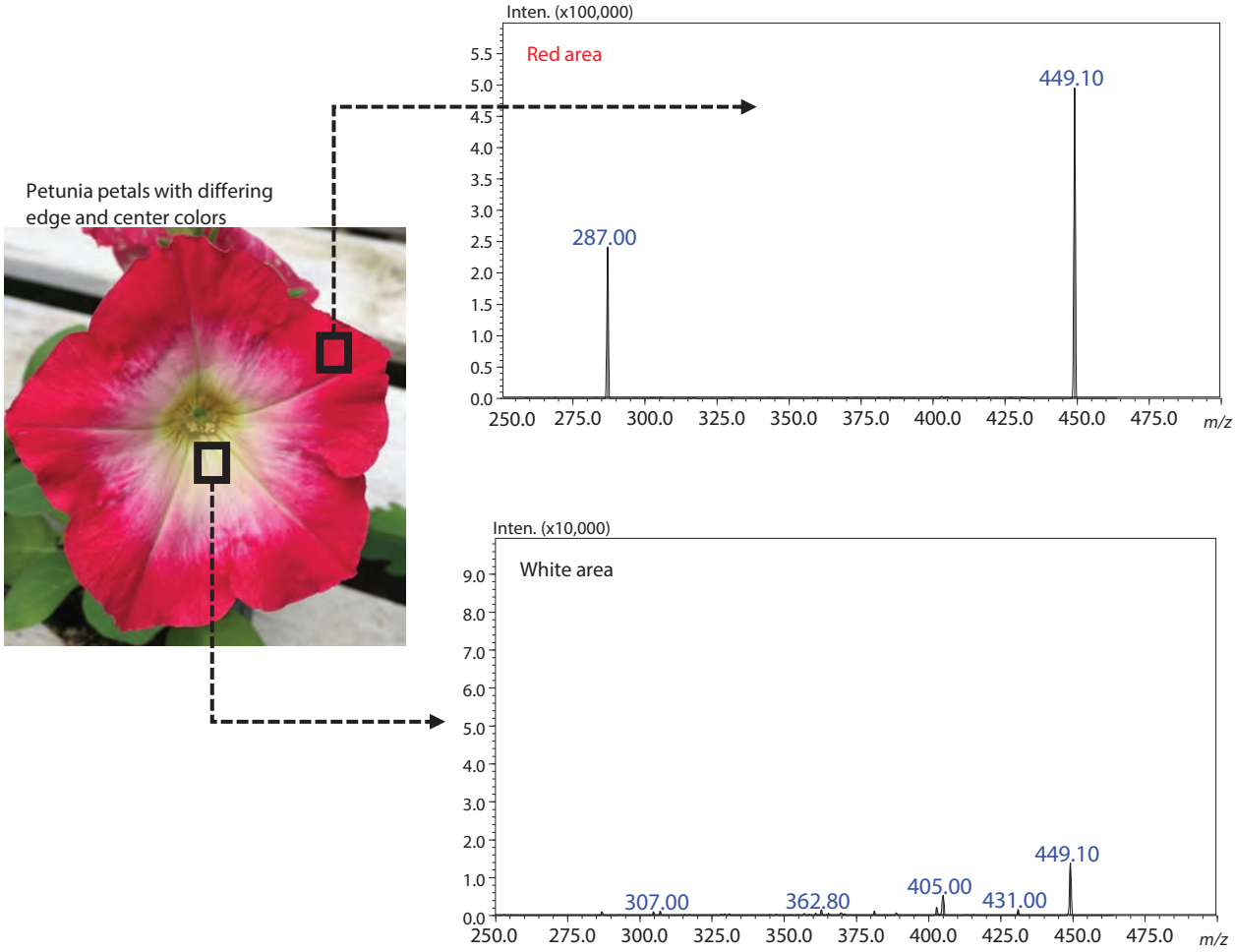


Fig. 3 **Product Ion Scan Results of Petunia Flower Petals**

Acknowledgments
The data in this article was provided by Associate Professor Katsuhiro Shiratake at the Graduate School of Bioagricultural Sciences and Associate Professor Kei Zaitsu at the Graduate School of Medicine, both of Nagoya University.
We would like to thank them for their generous support and collaboration.

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