Distribution analysis of galanthamine, a plant alkaloid, by MS imaging

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1. Introduction

Plant alkaloids are used in various pharmaceuticals, such as anticancer drugs and analgesics. Among these plant alkaloids, galanthamine is a Amaryllidaceae-type alkaloid with acetylcholinesterase inhibitors used in the treatment of neurological diseases such as Alzheimer's disease. Although the chemical synthesis of galanthamine has been successfully achieved, *Narcissus* is the main source of its production. Research indicates that galanthamine content varies not only with the type of *Narcissus*, but also with the developmental stage and the part of the plant[1)]. Pharmaceutical companies are pursuing plant species with higher galanthamine content to increase pharmaceutical productivity.

In this study we were able to quickly confirm the presence of galanthamine in our *Narcissus* sample using the DPiMS^{TM} QT probe electrospray ionization (PESI) kit and the LCMS quadrupole time-of-flight (Q-TOF) mass spectrometer (MS). Subsequently, we analyzed the distribution of galanthamine by MS imaging (MSI) using the iMScope QT and a LCMS Q-TOF MS.

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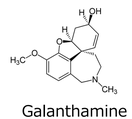
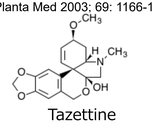
Narcissus tazetta



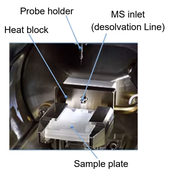
Galanthamine
$C_{17}H_{21}NO_3$

Lycorine
$C_{16}H_{17}NO_4$



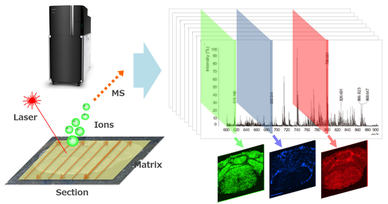
Tazettine
$C_{18}H_{21}NO_5$

Fig. 1 Structural formula of alkaloids in *Narcissus tazetta*



In the DPiMS QT, the sample adhering to the probe surface is ionized by applying a voltage to the probe tip and then introduced directly into the mass spectrometer.

Fig. 2 Principles of PESI



In MS imaging, the section to be analyzed is divided into small areas (pixels) of several um each, and MALDI mass spectrometry is performed on each pixel. The distribution of the molecule of interest can be confirmed visually by shading each pixel according to the ionic strength of the molecule of interest in the obtained spectrum.

Fig. 3 Principles of MSI

2. Sample Preparation and Analysis Conditions

For our sample, we used *Narcissus tazetta* with leaves growing to about 15 cm in length. Galanthamine was extracted with 50% EtOH aq. from freeze-dissolved leaves. The solution was analyzed via the DPiMS QT and the LCMS-9030 (Shimadzu Corporation). Data analysis was performed using the LabSolutions Insight Explore^{TM} software (Shimadzu).

Frozen leaves and bulbs were sliced to a thickness of 20 μm using a microtome and mounted on indium tin oxide (ITO) coated glass slides (Matsunami). These were coated with α-cyano-4-hydroxycinnamic acid (CHCA) via vapor deposition by using the matrix sublimation apparatus iMLayer^{TM} (Shimadzu) at a thickness of 0.7 μm. MSI analysis was performed using the iMScope QT atmospheric MALDI equipped with an optical microscope and the LCMS-9030 (Shimadzu). Data analysis was performed using the IMAGEREVEAL^{TM} MS (Shimadzu).

Table1. Analytical settings of PESI

Mass spectrometer	
System	: DPiMS QT+LCMS-9030
Polarity	: Positive
DL temp	: 250 ˚C
Heat block temp	: 50 ˚C
Interface Voltage	: 3.5 kV
MS Range	: MS / MS/MS *m/z* 50-2,000
Measurement Time	: 0.5 min

Easy switching

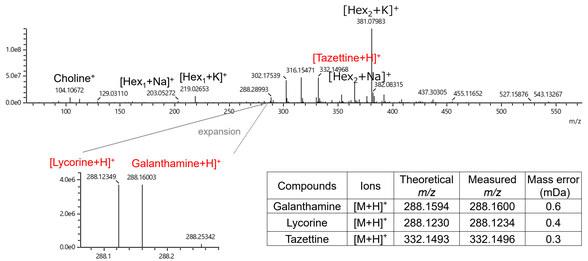
Table2. Analytical settings of MSI

Mass Spectrometer	
System	: iMScope QT+LCMS-9030
Polarity	: Positive
DL temp	: 290 ℃
Heat block temp	: 450 ℃
MS Range	: *m/z* 280-335
Spatial Resolution (Pitch)	: 10 / 25 mm
Laser Diameter Setting	: 1 / 2
Laser Intensity	: 50 / 60
Laser Repetition Frequency	: 20 kHz
Matrix Coating	
System	: iMLayer^{TM}
Matrix Used	: a-Cyano-4-hydroxycinnamic Acid (CHCA)
Coating Method	: Deposition with 0.7 mm Thickness

3. Results

Rapid Detection of Galanthamine in *Narcissus tazetta* by PESI

Galanthamine was rapidly detected in the leaf extract solution using the DPiMS QT and the LCMS-9030 in only 0.5 min. Accurate mass analysis confirmed the presence of Galanthamine in *Narcissus tazetta*. Other plant alkaloids, Lycorine and Galanthamine, metabolite Choline, and sugars composed of Hexose were also detected.

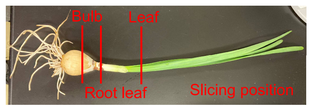


Compounds	Ions	Theoretical *m/z*	Measured *m/z*	Mass error (mDa)
Galanthamine	$[M+H]^+$	288.1594	288.1600	0.6
Lycorine	$[M+H]^+$	288.1230	288.1234	0.4
Tazettine	$[M+H]^+$	332.1493	332.1496	0.3

Fig. 4 Mass Spectra of *Narcissus* Leaves and Mass Accuracy of Detected Alkaloids

Distribution analysis of galanthamine in *Narcissus tazetta* by MSI

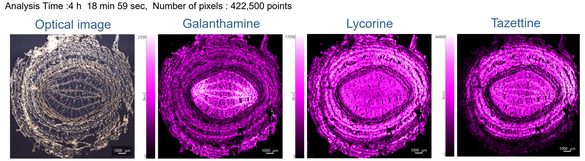
The distribution of galanthamine, lycorine, and tazettine in sections prepared from the bulb and leaves (two locations) of the *Narcissus tazetta* shown in Fig. 5 was analyzed by MSI. The MS imaging results of the entire section at 25 μm spatial resolution and the area observed with an optical microscope at 5x objective lens at 10 μm spatial resolution are shown in Fig. 6. The results showed good MS images of galantamine, lycorine, and tazettine from all samples. The MS images of the bulb showed that galanthamine was distributed mostly in the section of the bulb that later grows and becomes the leaves, whereas MS images of lycorine and tazettine did not show such distribution, suggesting that the distribution area of plant alkaloids in the bulb differs from species to species, but no such differences in distribution were observed in the leaf cross sections.



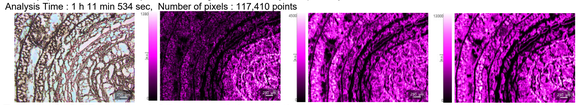
Narcissus tazetta were grown in soil culture with leaves growing to about 15 cm. Three types of sections were prepared: bulb, root leaf, and leaf cross section.

Fig5. Location of *Narcissus tazetta* section

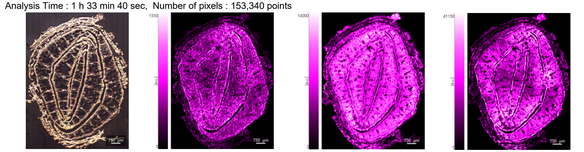
a. Bulb (CCD camera) 25 μm spatial resolution
Analysis Time :4 h 18 min 59 sec, Number of pixels : 422,500 points



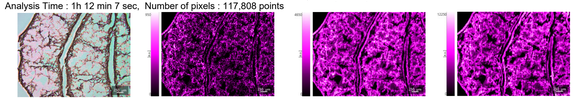
d. Bulb (optical microscope at 5x objective lens) 10 μm spatial resolution
Analysis Time : 1 h 11 min 534 sec, Number of pixels : 117,410 points



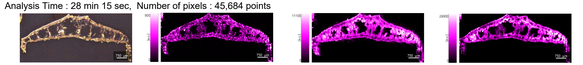
c. Root leaf (CCD camera) 25 μm spatial resolution
Analysis Time : 1 h 33 min 40 sec, Number of pixels : 153,340 points



d. Root leaf (optical microscope at 5x objective lens) 10 μm spatial resolution
Analysis Time : 1h 12 min 7 sec, Number of pixels : 117,808 points



e. Leaf (CCD camera) 25 μm spatial resolution
Analysis Time : 28 min 15 sec, Number of pixels : 45,684 points



f. Leaf (optical microscope at 5x objective lens) 10 μm spatial resolution
Analysis Time : 43 min 20 sec, Number of pixels : 70,983 points

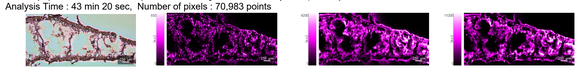


Fig. 6 Mass images of alkaloids in *Narcissus*

4. Conclusion

The combination of PESI and Q-TOF was able to detect galanthamine in narcissus with a high mass accuracy of within 1 mDa. The time required for analysis was significantly reduced compared to that by LC or LC/MS with a measurement time of 0.5 min, suggesting that rapid screening is possible. The specific distribution of galantamine in narcissus bulbs was confirmed by MS imaging analysis using a combination of iMScope QT and Q-TOF, with the ionization unit replaced from PESI to MALDI. Such distribution analysis enables identification of regions with high galanthamine content, facilitates efficient isolation and purification in the pharmaceutical manufacturing process, and is expected to contribute to the reduction of extraction costs.