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Application News

Imaging Mass Microscope iMScope™ QT

Confirmation of Synthesis of Sparingly Soluble Compounds by Accurate MALDI-TOF Mass Spectrometry

Ei-ichi Matsuo[1], Andreas Baumeister[2]
1 Shimadzu Corporation, 2 Shimadzu Europa GmbH

User Benefits

- iMScope QT enables MALDI mass spectrometry of poorly soluble compounds that are difficult to analyze with LC/MS.
- Using LCMS-9030, mass calibration with external standards enables accuracy to be maintained over a long period of time.
- It is possible to confirm the accurate mass of poorly soluble synthetic products.

■ Introduction

The MALDI method is tolerant to the characteristics of the sample and thus, for example, can be used to perform mass spectrometry of poorly soluble compounds that are difficult to measure with LC-MS. For the analysis of less polar compounds, an ionizing agent is sometimes added to the sample and matrix to promote ionization. They must be mixed and dissolved, and then spotted and dried on a sample plate to form cocrystals. Therefore, it is necessary to be able to dissolve all components in the same solvent. On the other hand, for highly accurate mass measurement in MALDI-TOF MS analysis, it is desirable to perform mass calibration using internal standards. However, it takes time and effort to find calibrants with similar physical properties and similar molecular weight to the sample compound.

The Q-TOF type mass spectrometer LCMS-9030 provides stable and accurate mass spectrometry over a long period of time with mass calibration using external standards. By attaching the iMScope QT (Fig. 1) with a MALDI ion source to the LCMS-9030 in place of the ESI, accurate mass measurement of poorly soluble compounds can be achieved.

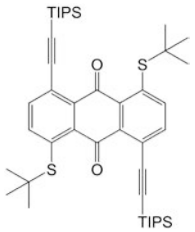
Fig. 1 iMScope™ QT - LCMS-9030

Table 1 Theoretical monoisotopic masses of poorly soluble compounds

	SIE1	SIE3	SIE4
$[M]^+$	744.38808	545.98246	845.42185
$[M+H]^+$	745.39590	546.99028	846.42967
$[M+Na]^+$	767.37785	568.97223	868.41162
$[M+K]^+$	783.35178	584.94616	884.38556
$[M+NH_4]^+$	762.42245	564.01683	863.45622

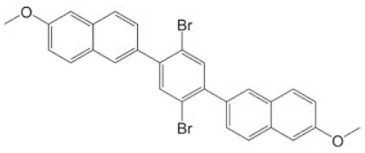
TIPS = Tri-Isopropylsilane

SIE1



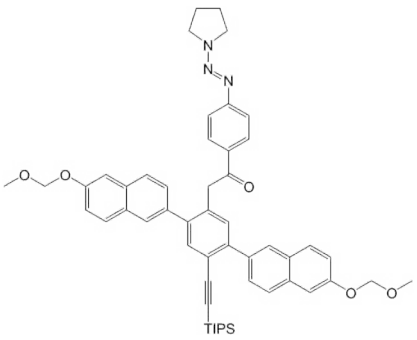
Chemical Formula: $C_{44}H_{64}O_2S_2Si_2$
Exact Mass: 744.3886

SIE3



Chemical Formula: $C_{28}H_{20}Br_2O_2$
Exact Mass: 545.9830

SIE4



Chemical Formula: $C_{53}H_{59}N_3O_5Si$
Exact Mass: 845.4224

Fig. 2 Structures of poorly soluble compounds

■ Mass calibration using an external standard

A matrix solution was prepared and used as the external standard. Specifically, DHB (2, 5-dihydroxybenzoic acid) was dissolved in 70% aqueous acetonitrile solution containing 0.1% trifluoroacetic acid (TFA) to give a final concentration of 50 mg/mL. Mass calibration was performed using DHB cluster ions (3M to 7M) as the standard peak for calibration.

Table 2 Data acquisition and analysis conditions for mass spectrometry

MS Data Acquisition Parameters	
Instrument	iMScope QT
Pitch (Spatial resolution)	10 [μm]
Polarity	Positive
Mass range	300 – 1000 (SIE1 & SIE4) 400 – 600 (SIE3)
Data point (X)	32 [points]
Data point (Y)	32 [points]
Data point	1,024 [points]
Sample voltage	3.70 [kV]
Detector voltage	2.20 [kV]
Number of laser shots	50 [shots]
Laser repetition rate	10000 [Hz]
Laser diameter setting	1
Laser intensity	55 / 65 (SIE1 & SIE4 / SIE3)
Data Analysis	
Mass spectra	IMAGEREVEAL MS

■ Sample preparation

A trace amount of each sample was dissolved in chloroform (approx. 50 to 100 μL), and used directly for analysis. DCTB (trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene] malononitrile) was used as a matrix and dissolved in chloroform at a concentration of 10 mg/mL. Sodium trifluoroacetate dissolved in tetrahydrofuran (THF) at a concentration of 2 mg/mL was added as a cation donator only for SIE3. The sample solution, the matrix solution, and the cation donator solution (in the case of SIE 3) were mixed in equal amounts, and a small amount (about 0.5 to 1.0 μL) was spotted on the SUS target, dried, and used for measurement.

■ Accurate mass confirmation for synthetic compounds

Each poorly soluble compound (SIE1, SIE3, SIE4; Fig. 2 and Table 1) was measured with the iMScope QT - LCMS-9030 under the analytical conditions shown in Table 2, with or without the sample compound, and peaks derived from the sample were confirmed (Fig. 3 (a, b), Fig. 4 (a, b), and Fig. 5 (a, b)). As a result, SIE1 and SIE4 were observed as $[M+H]^+$ ions, and SIE3 was observed as $[M]^+$ ions (Table 1). Then, the accurate mass was measured three times (Fig. 3 (c-e), Fig. 4 (c-e), and Fig. 5 (c-e)), and the average value was calculated (Table 3). It was found that the accurate mass of each compound could be measured with an accuracy within 1 ppm of the theoretical value. In this case, the exact mass was confirmed by accurate mass measurement, but this approach is also useful for formula prediction and structure analysis of unknown compounds.

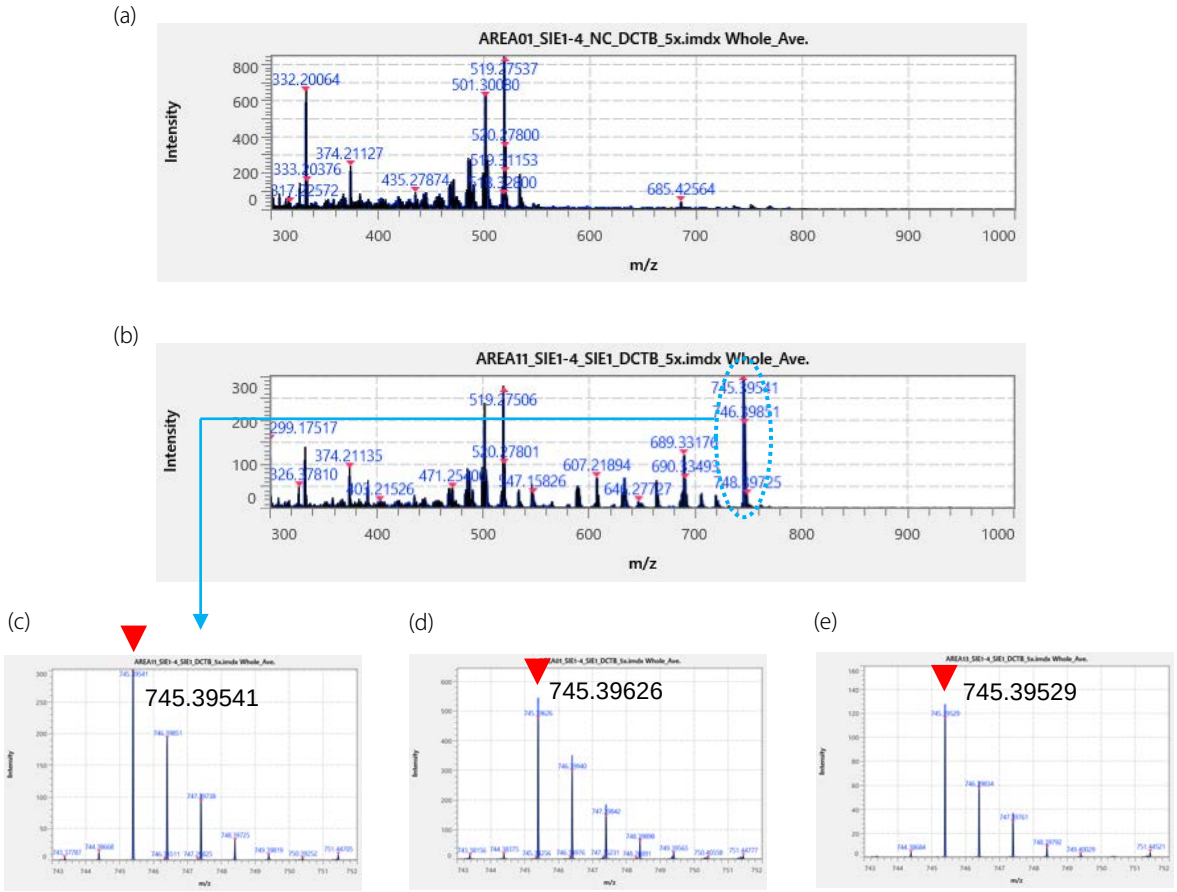


Fig. 3 Measurement results for SIE1

Mass spectra of negative control (no sample) (a) and SIE1 (b) and enlarged mass spectra of repeated measurements of SIE1 (n = 3) (c-e)

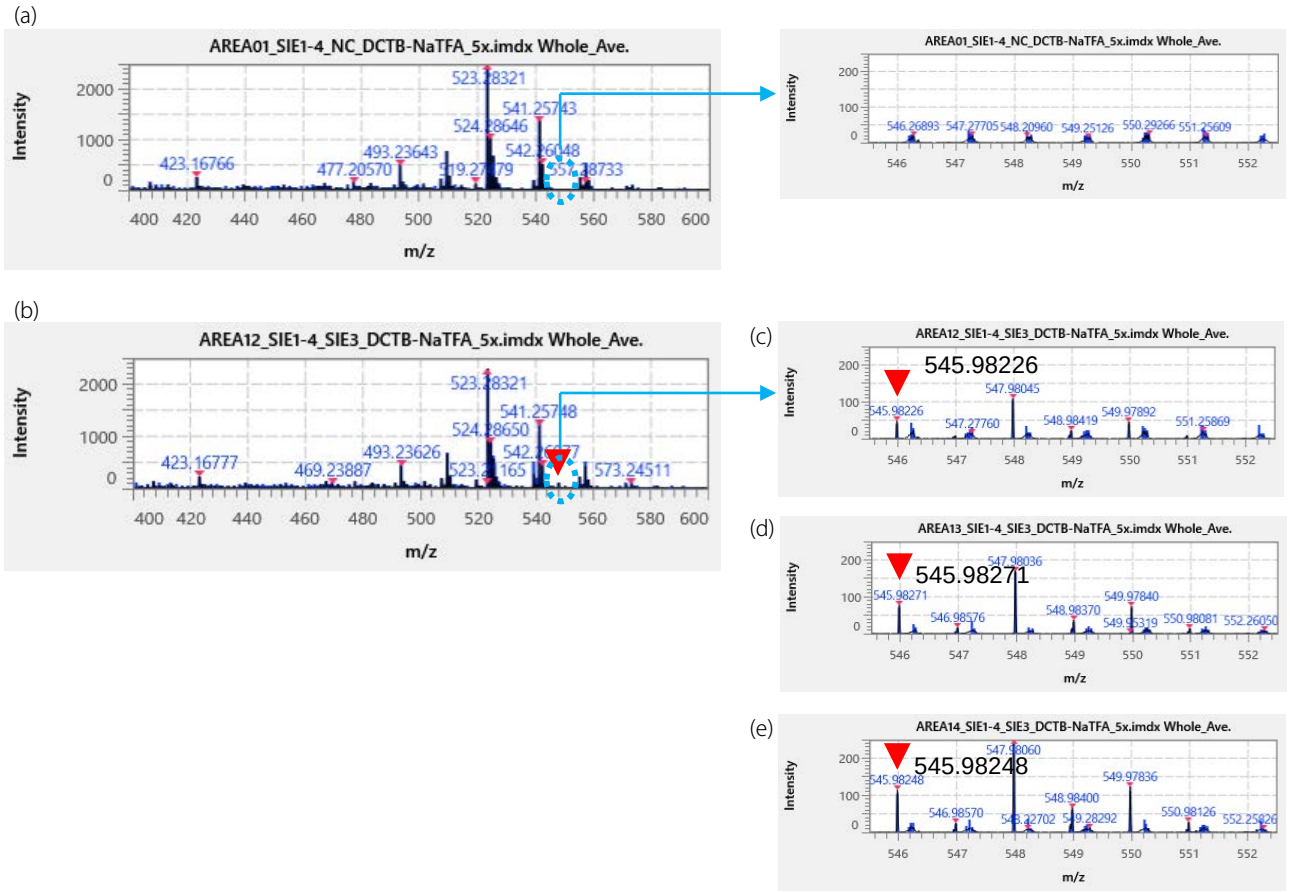


Fig. 4 Measurement results for SIE3

Mass spectra of negative control (no sample) (a) and SIE3 (b) and enlarged mass spectra of repeated measurements of SIE3 (n = 3) (c-e)

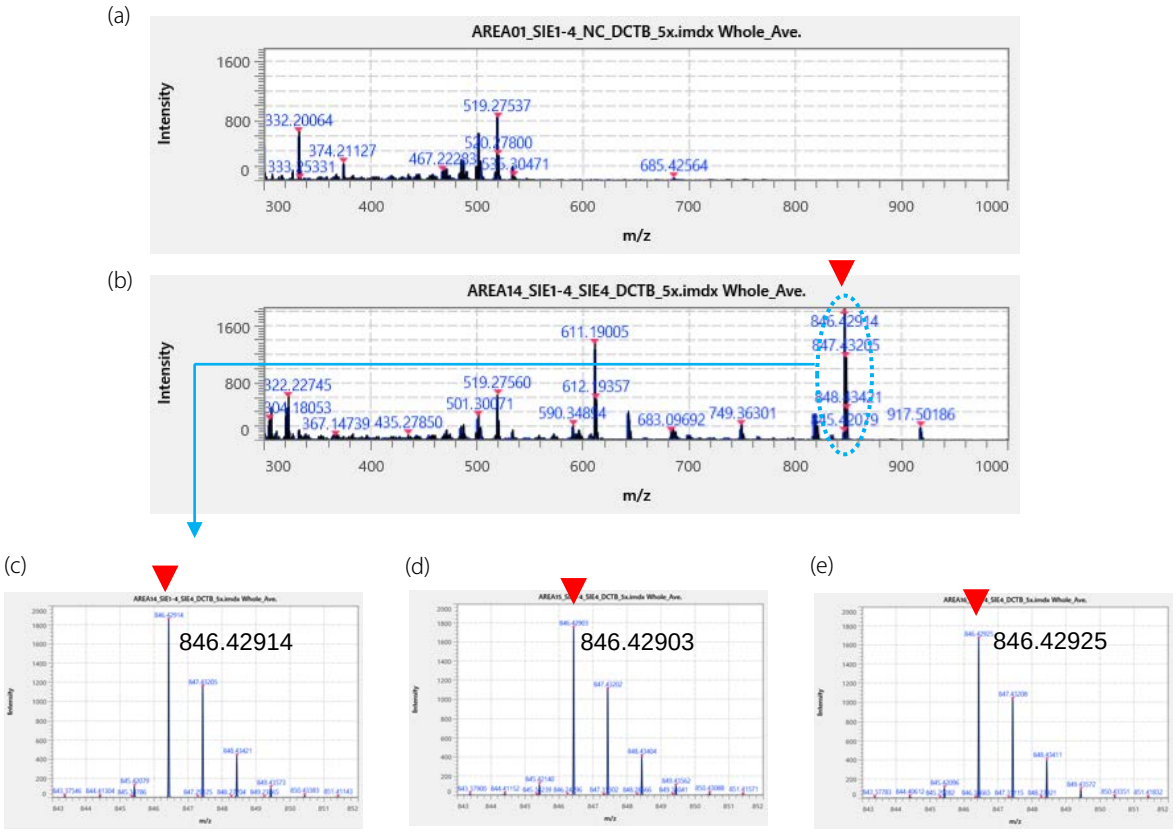


Fig. 5 Measurement results for SIE4

Mass spectra of negative control (no sample) (a) and SIE4 (b) and enlarged mass spectra of repeated measurements of SIE4 (n = 3) (c-e)

Table 3 Summary of accurate mass spectrometry results

SIE1	*m/z*	Difference	
		mDa	**ppm**
Theoretical $[M+H]^+$	745.39590		
Measured 1	745.39541	-0.49	**-0.66**
Measured 2	745.39626	0.36	**0.48**
Measured 3	745.39529	-0.61	**-0.82**
Average (of absolute value)	745.39611	0.49	**0.65**

SIE3	*m/z*	Difference	
		mDa	**ppm**
Theoretical $[M]^+$	545.98246		
Measured 1	545.98226	-0.20	**-0.37**
Measured 2	545.98271	0.25	**0.46**
Measured 3	545.98248	0.02	**0.04**
Average (of absolute value)	745.39611	0.16	**0.29**

SIE4	*m/z*	Difference	
		mDa	**ppm**
Theoretical $[M+H]^+$	846.42967		
Measured 1	846.42914	-0.53	**-0.63**
Measured 2	846.42903	-0.64	**-0.76**
Measured 3	846.42925	-0.42	**-0.50**
Average (of absolute value)	846.43041	0.53	**0.63**

■ Conclusion

Examples of poorly soluble compounds include liquid crystal materials in liquid crystal displays, poorly soluble resin materials used in industrial products, and synthetic polymers, all of which are indispensable in our daily lives. It is very useful to be able to confirm the synthesis of these compounds with accurate mass spectrometry. With the iMScope QT – LCMS-9030, accurate mass measurement is achievable even with external calibration methods. As a result, even in the case of poorly soluble compounds, analysis can be performed easily without the need to find suitable internal calibrants or perform solvent studies. When combined with LC/MS, the iMScope QT provides accurate mass spectrometry for compounds with a variety of physical properties.

The original purpose of iMScope QT is to perform high spatial resolution MS imaging integrated with microscope images. It has been reported that liquid crystal materials can be analyzed by laser desorption ionization (LDI), and we believe that MS imaging of (organic) liquid crystal displays can be used for impurity analyses. In addition to MS imaging, we propose this attractive and practical new MALDI application for poorly soluble compounds.

■ Acknowledgements

We would like to thank the Laboratory of Synthetic Organic Chemistry (Eric Sidler from the Mayor group), Department of Chemistry, University of Basel for kindly providing us with the phenylene-ethynylene derivatives.

01-00384-EN First Edition: June 2022

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