Application Note

Biotherapeutics and Biosimilars

Agilent

Trusted Answers

Imaging Lipids in a Brain Injury Model with AP-MALDI on the Agilent 6495 Triple Quadrupole and 6560 Ion Mobility Mass Spectrometers

Authors

Xi Qiu, Daniel Cuthbertson, and John Sausen
Agilent Technologies, Inc.

Ludovic Muller, William T. Andrews, Anh Tran, Maureen A. Kane, and Jace W. Jones
University of Maryland, School of Pharmacy, Baltimore, MD, U.S.

Eugene Moskovets, Nivedita Bhattacharya, and Venkateswarlu Panchagnula
MassTech Inc, Columbia, MD, U.S.

Abstract

This application note demonstrates the interface of an atmospheric pressure matrix-assisted laser desorption/ionization (AP-MALDI) source to an Agilent 6495 triple quadrupole (TQ) mass spectrometer and an Agilent 6560 ion mobility Q-TOF mass spectrometer. The ability to image endogenous lipids across mouse brain tissue using selective precursor to product ion transitions on the TQ mass spectrometer is demonstrated. It is further demonstrated that the same AP-MALDI source can be used to generate mass spectrometry images of endogenous lipids using an ion mobility-enabled Q-TOF mass spectrometer. As a result, these data highlight the compatibility of using AP-MALDI on Agilent mass spectrometers to selectively detect and image endogenous mouse brain lipids.

Introduction

Traumatic brain injury (TBI) is a neurological condition that can lead to cognitive decline and physical and psychological impairments. The use of a controlled cortical impact (CCI) mouse model can effectively replicate TBI and the localized nature of the injury provides an ideal platform for visualizing *in situ* molecules affected by the injury, e.g., lipids within brain tissue. AP-MALDI mass spectrometry imaging (MSI) allows the study of localized, *in situ* molecules that have resulted from brain injury. This study highlights AP-MALDI imaging with two complimentary mass spectrometry systems: a high sensitivity 6495 triple quadrupole (TQ) as shown in Figure 1 and a high selectivity 6560 ion mobility quadrupole time of flight (IM-Q-TOF) as shown in Figure 2. The ability to image endogenous lipids across mouse brain tissue using selective precursor to product ion transitions on a TQ mass spectrometer is demonstrated, and the same AP-MALDI source can be used to generate mass spectrometry images of endogenous lipids using an ion mobility-enabled Q-TOF mass spectrometer.

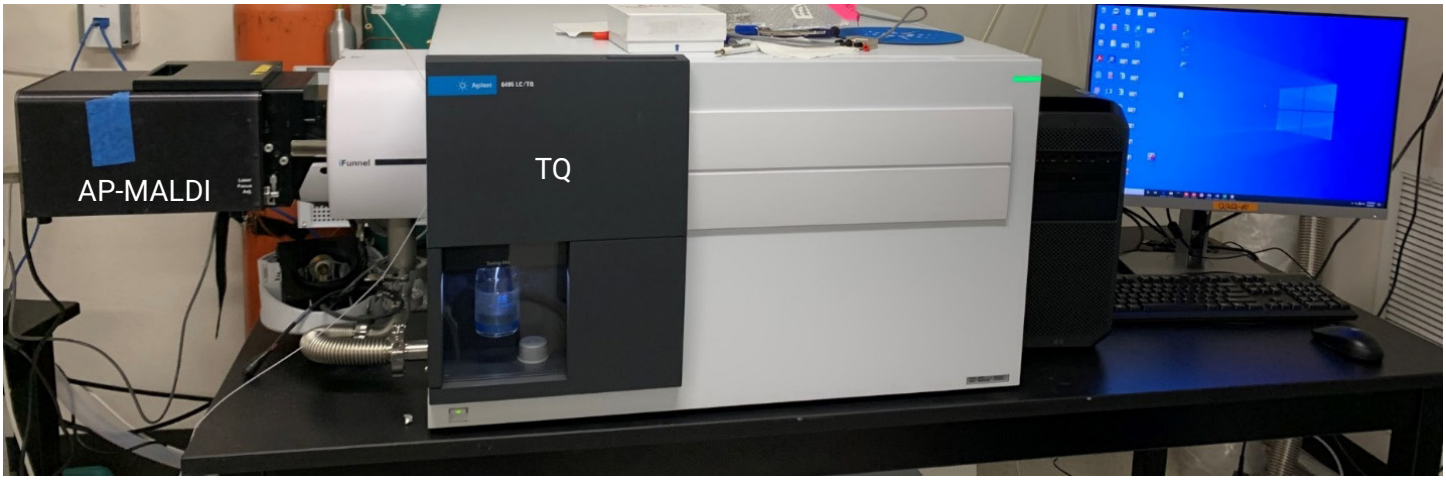

Figure 1. MassTech AP-MALDI ion source and Agilent 6495 triple quadrupole systems.

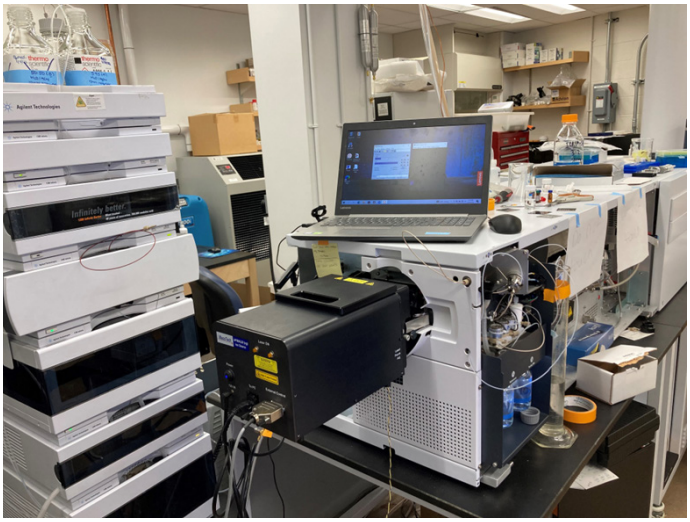

Figure 2. MassTech AP-MALDI ion source and Agilent 6560 ion mobility TOF systems.

Experimental

Materials

2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (CHCA), and 9-aminoacridine (9-AA) were purchased from Sigma-Aldrich (St. Louis, MO).

Instrumentation

- MassTech AP-MALDI (UHR) ion source
- Agilent 6495 triple quadrupole system
- Agilent 6560 ion mobility time-of-flight system

Sample preparation

Mouse brain tissue sections were deposited on standard stainless steel MALDI plates. The slides were coated with a matrix using a TM-sprayer (HTX Imaging). DHB and CHCA matrices were used for positive ion mode and 9-aminoacridine (9-AA) was used for negative ion mode. The AP-MALDI source (MassTech Inc.) was interfaced either to the 6495C TQ or the 6560 IM-Q-TOF mass spectrometers. The number of TQ transitions were optimized as a function of dwell time, lipid abundance, and laser raster speed. IM-Q-TOF data were acquired over an *m/z* range of 150 to 1,700. The data were collected using multiplexing mode (4-bit) and postprocessing using the Agilent HRdm software.

MS analysis and data processing

Images for both instruments were processed using SpectroSwiss Mozaic software.

Results and discussion

Method optimization for metabolites

The MassTech AP-MALDI source was interfaced to the Agilent 6495C TQ and tissue samples were acquired at 60-micron resolution. Dwell times for each transition were set to 50 ms, and 1.2 amu resolution was used on the quad except where mentioned. The raster speed of the AP-MALDI source was synced with the cycle time of the TQ for each experiment so that one pixel equals one instrument cycle. Table 1 and Table 2 show TQ source parameters and lipid MRM transitions.

Table 1. Agilent 6495C TQ source parameters.

Agilent 6495 TQ Parameters	
Gas Temperature (°C)	280
Gas Flow (L/min)	11
Capillary (V)	2,000
Positive High Pressure RF (V)	200
Positive Low Pressure RF (V)	110

Table 2. Targeted lipid MRM transitions.

Targeted Lipid Transitions				
No.	Lipid	Ion	Precursor	Product Ion
1	Cer d42:2	$[M+H-H_2O]+$	630.6	264.2
2	SM(d34:1)	[M+H]+	703.6	184.1
3	HexCer(d42:2)	$[M+H-H_2O]+$	792.7	264.2
4	LPE 18:0	[M+H]+	482.3	341.3
5	LPC 16:0	[M+H]+	496.3	184.1
6	LPC 18:0	[M+H]+	524.4	184.1
7	PC 32:0	[M+H]+	734.6	184.1
8	PC 34:1	[M+H]+	760.6	184.1
9	PC 36:1	[M+H]+	788.6	184.1
10	PC 38:4	[M+H]+	810.6	184.1
11	PE 36:1	[M+H]+	746.6	605.6
12	PE 38:2	[M+H]+	772.6	631.6
13	PE 40:6	[M+H]+	792.6	651.6
14	PE P-38:6	[M+H]+	748.5	364.3
15	PE P-38:4	[M+H]+	752.6	361.3
16	PE P-40:6	[M+H]+	776.6	385.3

Selected tissue lipid images demonstrating that various lipid classes can effectively be targeted and imaged using a combination of the MassTech AP-MALDI source and the Agilent 6595C TQ are shown in Figure 3.

The quadrupole isolation window was also evaluated to assess signal and specificity for targeted lipid imaging at two specific isolation thresholds: enhanced unit resolution at 0.7 amu and a wide isolation of 1.2 amu (Figure 4).

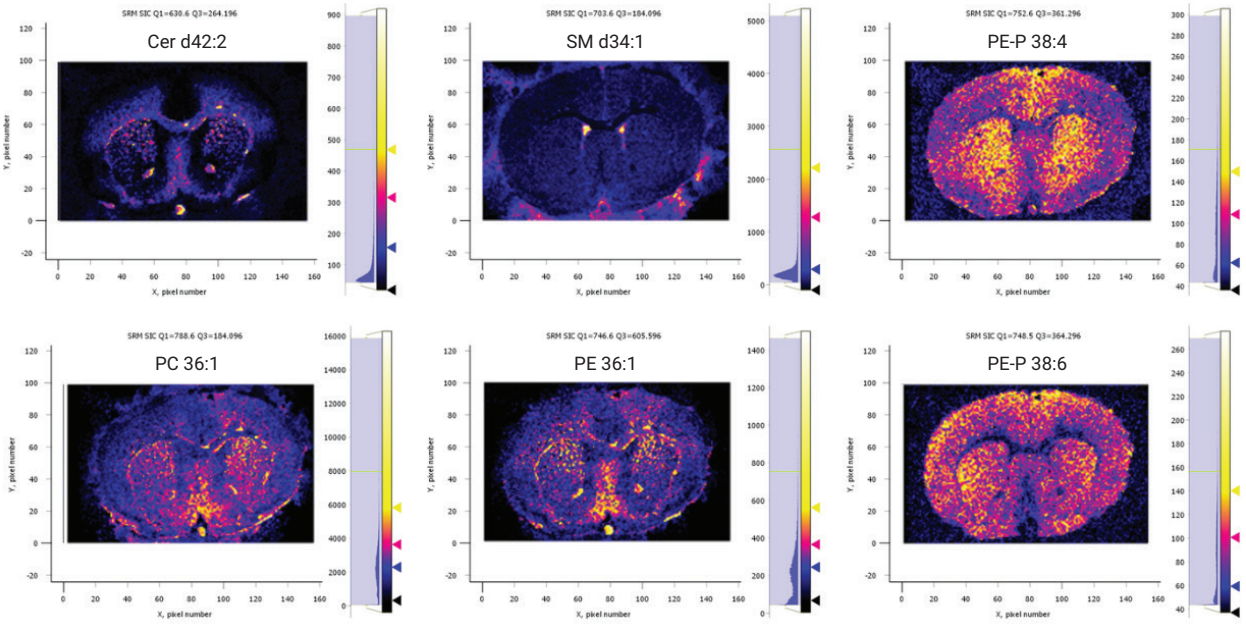

Figure 3. AP-MALDI triple quadrupole images of selected lipids.

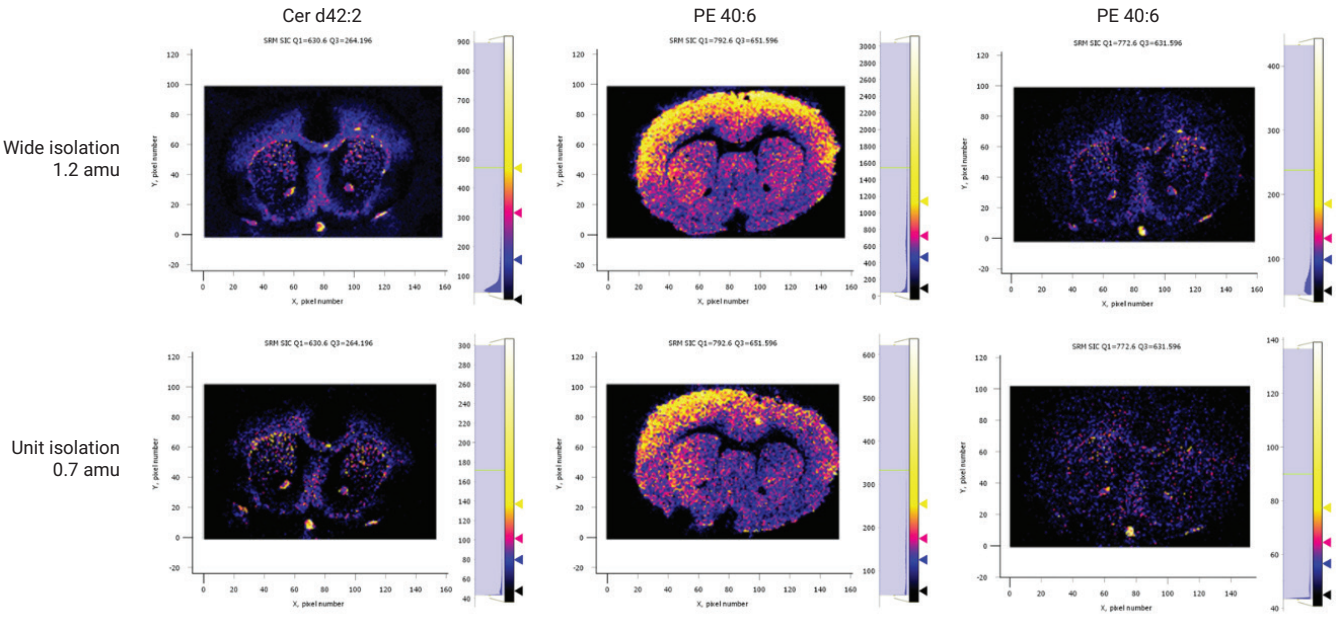

Figure 4. Tissue image comparison between unit resolution (0.7 amu) and wide isolation (1.2 amu).

Analysis of brain total lipid extract highlighting the complementary nature of high-resolution mass spectrometry and high-resolution ion mobility is shown in Figure 5. Spatial localization of endogenous lipids across mouse brain tissue (preliminary ID based on accurate mass) is shown in Figure 6. Drift spectra across tissue detailing differential spatial localization of isomeric species is shown in Figure 7.

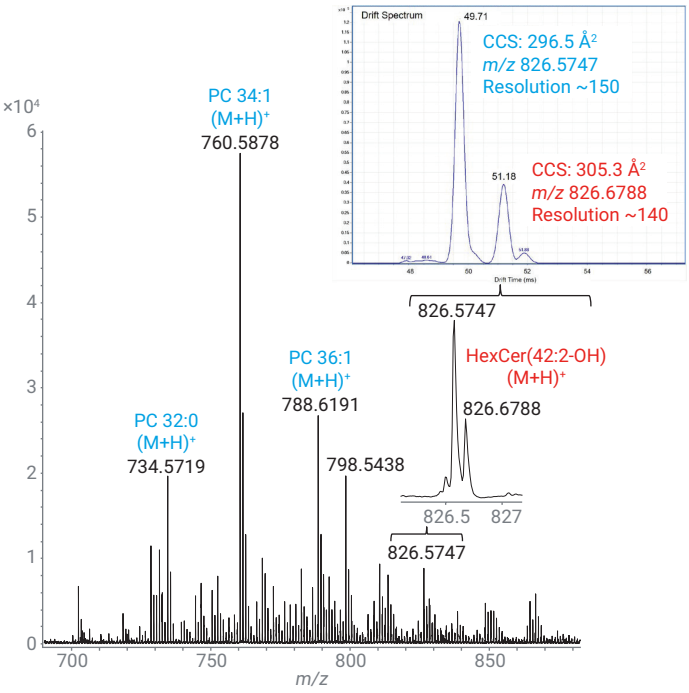

Figure 5. Spot analysis example from the AP-MALDI coupled to the Agilent 6560 IM-Q-TOF mass spectrometer.

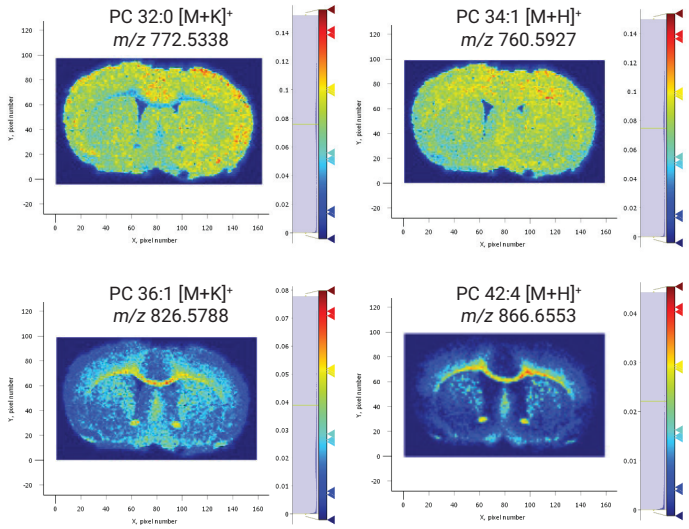

Figure 6. Spatial localization of endogenous lipids across mouse brain tissue (preliminary ID based on accurate mass).

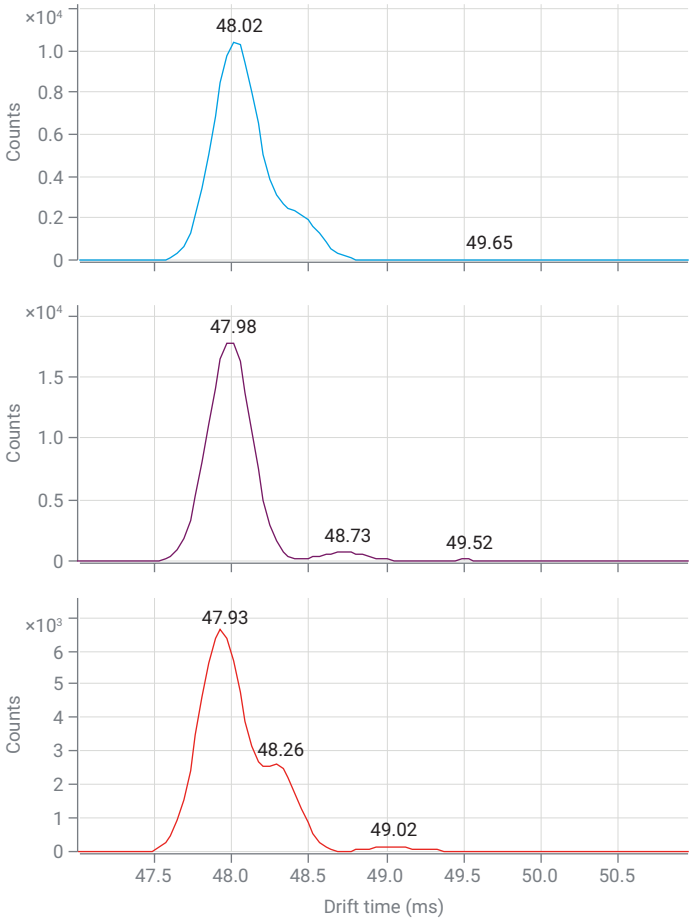

Figure 7. Drift spectra across tissue detailing differential spatial localization of isomeric species (*m/z* 760.5927), ion mobility resolution >125.

Conclusion

An AP-MALDI source was interfaced to both an Agilent 6495 triple quadrupole mass spectrometer and an Agilent 6560 ion mobility Q-TOF mass spectrometer for mass spectrometry imaging. The TQ platform demonstrated that the targeted approach had sufficient selectivity and sensitivity to spatially localize lipids across brain tissue. The IM-Q-TOF platform enabled the use of high-resolution ion mobility to separate endogenous, isobaric, and potentially isomeric lipid species on-tissue. AP-MALDI allows interfacing a MALDI source to a mass spectrometer with desired data acquisitions features such as selective, targeted analysis (e.g., TQ), or highly selective separation of endogenous on-tissue analytes (e.g., IM-Q-TOF).

www.agilent.com

For Research Use Only. Not for use in diagnostic procedures.

RA45064.6208333333

This information is subject to change without notice.

Printed in the USA, June 22, 2023
5994-6247EN

Agilent
Trusted Answers