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Applikationsbericht

High-Performance DESI-MSI using a DESI XS Xevo™ TQ Absolute XR system for Sensitive and Specific Targeted Imaging of Small Molecules in Tissue Sections

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Abstract

Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI-MSI) has emerged as a powerful, minimal-preparation method to ionize and image small molecules—especially phospholipids—directly from tissue sections. High spatial resolution MSI comes at the cost of relatively long acquisition times, so system stability and robustness are paramount.

The system solution described in this study combines an ACQUITY™ µBSM binary pump for ultra-steady solvent flow, the DESI High-Performance Sprayer (HPS) for spray stability, and a DESI XS source on a Xevo TQ Absolute XR for highly sensitive, selective MRM-based imaging of lipids and small endogenous metabolites in rodent brain tissue sections.

Benefits

- The sensitive and specific mapping of endogenous small metabolites and lipids in rodent brain using targeted MS imaging workflows
- Robustness of targeted DESI MSI using an ACQUITY µBSM binary pump DESI solvent delivery combined with the DESI XS source on a Xevo TQ Absolute XR mass spectrometer
- Superior specificity and sensitivity of targeted MS imaging DESI workflows for detecting and visualizing low abundance molecules directly from surfaces
- Illustrating the application of targeted MS imaging workflows with MRM acquisition to visualize isobaric and isomeric species through the use of diagnostic product ions

Introduction

Over the past decade, DESI has become a growing technique for directly ionizing and imaging small molecules from tissue sections with virtually no sample preparation. Phospholipids draw intense interest across biology and medicine because they underpin membrane structure and fluidity, and govern key processes such as signalling and molecular transport. Also, shifts in phospholipid profiles can flag disease states, metabolic dysregulation or inflammatory responses. In particular, studying brain, where they are especially abundant—they inform studies of development, aging and neurodegeneration (e.g. Alzheimer's, Parkinson's diseases).

Furthermore, by mapping endogenous small molecules in situ, DESI-MSI exposes regional metabolic and signalling changes that conventional bulk assays simply can't resolve. Generating higher spatially resolved DESI imaging in the low tens of microns is becoming a necessity to better understand tissue margins, microenvironmental heterogeneity or metabolic region-specific shift. This can induce longer acquisition times and therefore system stability and robustness are key factors.

In our workflow, an ACQUITY µBSM binary pump is harnessed to deliver steady DESI solvent flow rate combined with the DESI HPS for a stable spray and a DESI XS source on a Xevo TQ Absolute XR Mass Spectrometer for the sensitivity and specificity of the targeted MRM-MS imaging mode.

Experimental

Sample Preparation

Consecutives rodent brain tissue sections were generated from snap-frozen tissue that were stored at -80 °C, using a cryostat (Leica) at 18 µm thickness and thaw-mounted onto standard microscope slides (1 x 3 inches). Tissue sections were kept at -80 °C until analysis.

Mass Spectrometry

Experiments were performed using a DESI XS source mounted on a Xevo TQ Absolute XR Triple Quadrupole Mass Spectrometer in MRM mode of acquisition. The DESI spray conditions were set at 2 µL/min, 98:2 MeOH/H_2O using an ACQUITY µBSM binary pump and a N_2 nebulizing gas pressure of 15 psi.

A DESI HPS was utilized for improved sensitivity, DESI spray focus, robustness and ease-of-use. Furthermore, a heated transfer line (HTL) was mounted directly onto the ion block of the TQ Absolute XR. It was set at room temperatures for the small molecules experiments and heated to 450 °C for the negative mode isomeric phospholipid experiment. This enhanced the desorption and transfer of the charged droplets into the mass spectrometer resulting in increased sensitivity.

MS Conditions

MS systems:	Xevo TQ Absolute XR Mass Spectrometer
Source type:	DESI XS
Polarity:	Positive and Negative
Source temperature (°C):	150
MS1 resolution:	Unit (0.7 Da)

MS2 resolution:	Unit (0,7 Da)
Dwell time:	6 ms
Experiment type:	MRM

DESI Setup

Capillary voltage (kV):	0.70 (negative) and 0.75 (positive)
Nitrogen flow (psi):	15
Solvent delivery:	ACQUITY μBSM binary pump
Solvent:	98% Methanol, 2% Water
Solvent flow rate:	2 μL/min
Pixel size:	25 and 35 μm

Data Mangement

MS software:	MassLynx™ v4.2 Software (SCN 1050) (For Xevo TQ Absolute XR)
Informatics:	High Definition™ Imaging (HDI™) 1.8

Results and Discussion

A) Phospholipids

Two analyses of the same rodent brain tissue section have been performed visualizing phospholipids in positive and negative ionisation mode by combining the ACQUITY µBSM binary pump and DESI XS mounted onto the Xevo TQ Absolute XR. The acquisition speed was 5 scans/seconds, and the total time of acquisition were approximately 11-12 hours.

As seen in figures 1 and 2, the non-normalised ion images show the great signal stability and robustness of the whole system from the steady solvent delivery using the ACQUITY µBSM, the DESI HP sprayer and Xevo TQ Absolute XR Mass Spectrometer. Furthermore, the signal to noise (S/N) for the targeted lipids are reported in table 1 and 2 alongside the MRM transition information, where some of them were isobaric and/or isomeric such as lipids *m/z* 772.55 in positive ionisation mode and lipids *m/z* 788.55 in negative ionisation mode.

Name		*m/z* Precursor		*m/z* Product Ion	Cone Voltage (V)	Col. Energy (eV)	S/N
PC (32:0), Na^+	Phospholipids	756.55	>	147	40	35	1:690
PC (34:2), H^+	Phospholipids	758.55	>	184	40	35	1:444
PC (34:1), H^+	Phospholipids	760.55	>	184	40	35	1:620
PC (32:0), K^+	Phospholipids	772.55	>	163	40	30	1:3159
PC (35:2), H^+	Phospholipids	772.55	>	184	40	35	1:474
PC (34:2), Na^+	Phospholipids	780.55	>	147	40	40	1:246
PC (34:1), Na^+	Phospholipids	782.55	>	147	40	35	1:1723
PC (36:4), H^+	Phospholipids	782.55	>	184	40	30	1:1770
PC (36:2), H^+	Phospholipids	786.55	>	184	40	35	1:355
PC (34:2), K^+	Phospholipids	796.6	>	163	40	35	1:448
PC (34:1), K^+	Phospholipids	798.6	>	163	40	35	1:3092
PC (36:4), Na	Phospholipids	804.55	>	147	40	40	1:957
PC (36:3), Na	Phospholipids	808.6	>	147	40	40	1:663
PC (38:6), H^+	Phospholipids	808.6	>	184	40	40	1:318
PC (36:2), K^+	Phospholipids	820.6	>	163	40	40	1:905
PC (36:4), K^+	Phospholipids	824.6	>	163	40	40	1:206
PC (38:4), Na	Phospholipids	832.6	>	147	40	40	1:603
PC (38:5), K^+	Phospholipids	846.6	>	163	40	40	1:332
PC (38:4), K^+	Phospholipids	848.55	>	163	40	40	1:1350

Table 1. MRM transition information of the targeted putatively identified phospholipids acquired in positive ionization mode with the signal to noise (S/N) acquired from a rodent brain tissue section.

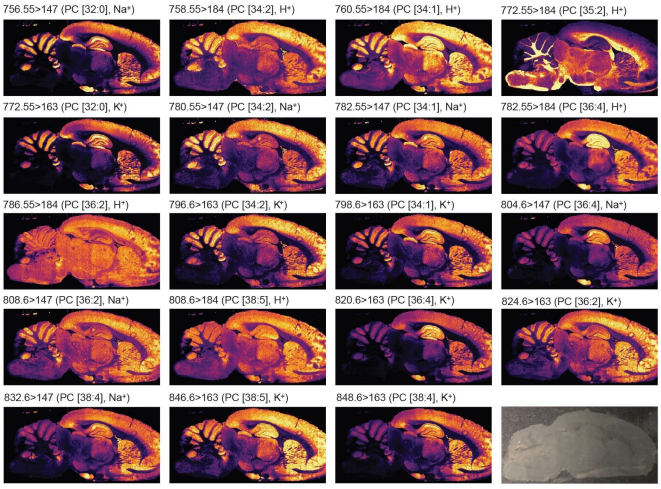

Figure 1. MRM ions images of rodent brain putatively identified phospholipids acquired in positive ionization mode at 35 x 35 µm pixel size.

Name		*m/z* Precursor		*m/z* Product Ion	Cone Voltage (V)	Col. Energy (eV)	S/N
PE [16:0_18:1],H^-	Phospholipids	716.5	>	281.03	30	20	1:455
PE [P-16:0_20:4], H^-	Phospholipids	722.55	>	303.22	30	20	1:426
PE [17:2_18:1], H^-	Phospholipids	726.55	>	281.22	30	35	1:4314
PE [16:0_20:4], H^-	Phospholipids	738.55	>	303.22	30	35	1:169
PE [18:1/18:1], H^-	Phospholipids	742.55	>	281.22	30	35	1:2000
PE [P-16:0_22:6], H^-	Phospholipids	746.55	>	327.22	30	35	1:16
PE (P-16:0_22:4)	Phospholipids	750.5	>	331.3	30	35	1:893
PE (P-18:0_20:4)	Phospholipids	750.5	>	303.3	30	35	1:1250
PE [16:1_22:6], H^- or PS [16:0_18:0], H^-	Phospholipids	762.55	>	283.22	30	35	1:215
PE [16:0_22:6], H^-	Phospholipids	762.55	>	327.22	30	35	1:17
PE (16:0_22:4)	Phospholipids	766.55	>	331.3	30	35	1:222
PE (18:0_20:4)	Phospholipids	766.55	>	303.3	30	35	1:173
PE [P-18:0_22:6], H^- or PE [17:1_22:6], H^-	Phospholipids	774.55	>	327.22	30	35	1:53
PS [18:0_18:1], H^-	Phospholipids	788.55	>	419.25	30	35	1:606
PE [18:1_22:6], H^-	Phospholipids	788.55	>	327.2	30	35	1:50
PE [18:0_22:4], H^-	Phospholipids	794.55	>	331.22	30	20	1:242
PS [18:0_20:4], H^-	Phospholipids	810.55	>	283.22	30	20	1:618
PS (18:0_22:6)	Phospholipids	834.55	>	283.25	30	35	1:16100
PI (16:0_20:4)	Phospholipids	857.55	>	303.2	30	45	1:124
PI (18:0_20:4)	Phospholipids	885.55	>	303.2	30	45	1:916

Table 2. MRM transition information of the targeted putatively identified phospholipids acquired in negative ionization mode with the signal to noise (S/N) acquired from a rodent brain tissue section.

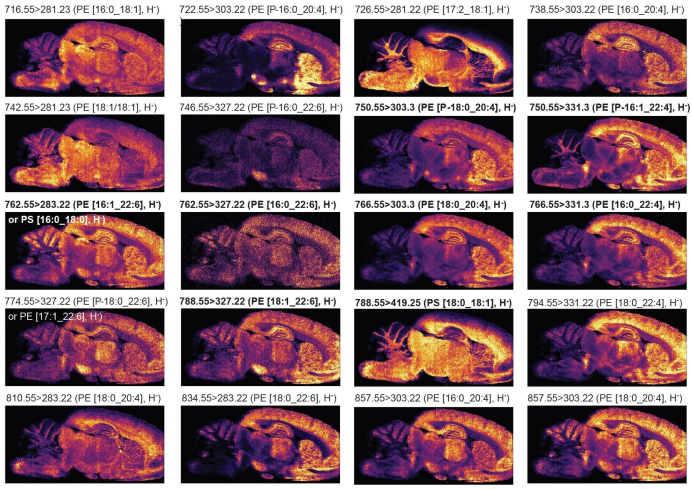

Figure 2. MRM ions images of rodent brain putatively identified phospholipids acquired in negative ionization mode at 35 µm pixel size.

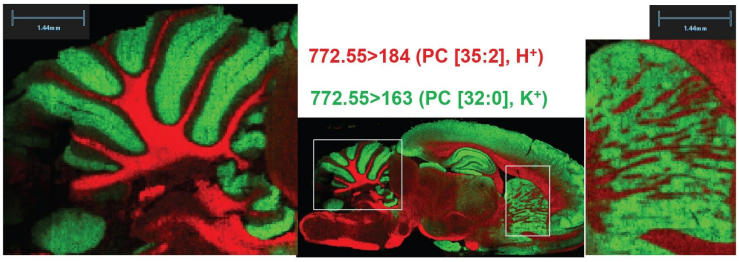

Figure 3. Positive ionization mode rodent brain RGB overlay ion images of isobaric putatively identified PC lipids acquired at 35 µm pixel size m/z 772.55 (protonated PC [35:2] 782.55 > 184 and potassiated PC [32:0] 772.55 > 163 demonstrating the image quality.

B) Small molecule metabolites

Further experiments were conducted analysing consecutive rodent brain tissue sections, targeting endogenous small molecules in negative and positive ionisation mode.

Firstly, in negative mode, three experiments were performed targeting some amino acids, TCA cycle metabolites, arachidonic acid metabolites (which are also called oxylipins or lipid mediators) and fatty acids. Each experiment lasted 11-13 hours. In table 3, 4 and 5 the MRM transitions and S/N are reported. In table 4, some of the HETEs S/N using the Xevo TQ Absolute XR are similar to those ones reported when coronal rodent tissue sections were analysed using a previous iteration of the DESI MSI System (a Xevo TQ Absolute Mass Spectrometer)[1].

Name		*m/z* Precursor		*m/z* Product Ion	Cone Voltage (V)	Col. Energy (eV)	S/N
Serine	amino acid	104	>	74	15	10	X
Creatinine	amino acid derivative	112	>	42	15	20	1:7
Succinate	TCA cycle	117	>	73	20	10	X
Taurine	sulfonic acid	124	>	79.9	20	20	1:33
Creatine	amino acid derivative	130	>	88	15	10	1:70
Aspartic_acid	amino acid	132.1	>	87.9	20	11	1:3
Malic_acid	organic acid	133	>	115	20	15	1:74
L-glutamic acid	amino acid	146	>	102	20	14	1:33
Uric acid		167	>	124	25	15	1:6
N-acetylaspartate (NAA)		174.1	>	88	10	10	1:263
Ascorbic acid (Vit C)		175	>	89	20	15	1:23
Citric acid	organic acid	191	>	97	15	20	1:24
Hexose+Cl	Sugar	215		35	10	20	1:7
Hexose Phosphate	Glycolis pathway	259.1	>	97	30	20	1:40
Inosine	purine nucleoside	267	>	135	25	25	1:72
Adenosine monophosphate (AMP)	Nucleotide	346	>	79	25	35	1:15
prostaglandin E2 (PGE2)	Lipid mediator	351.2	>	169.1	17	19	1:6
Thromboxane B2 (TXB2)		369.2	>	169.1	26	16	1:3
Flavin mononucleotide (FMN)	Co-enzyme	455	>	79	20	35	1:8

Table 3. MRM transition information of the targeted putatively identified small molecules metabolites such as amino acids, TCA cycle acquired in negative ionization mode with the signal to noise (S/N) acquired from a rodent brain tissue section.

Name		*m/z* Precursor		*m/z* Product Ion	Cone Voltage (V)	Col. Energy (eV)	S/N
6 or 12- HTTrE	AA metabolite	279.2	>	179.1	20	13	1:5
16-HOTrE	AA metabolite	293.2	>	235.2	25	13	1:24
8 or 13-HOTrE	AA metabolite	293.2	>	195.2	25	15	1:13
10 or 12 or 13-DiHODE)	AA metabolite	313.2	>	183.1	25	20	1:6
5-HETE	AA metabolite	319.2	>	115	30	15	1:18
8-HETE	AA metabolite	319.2	>	155	30	15	1:8
9-HETE	AA metabolite	319.2	>	151	30	15	1:11
11-HETE or 9-HETE	AA metabolite	319.2	>	167	30	20	1:37
12-HETE	AA metabolite	319.2	>	179	30	15	1:139
12-HETE	AA metabolite	319.2	>	135	30	15	1:20
15-HETE	AA metabolite	319.2	>	175	30	15	1:22
15-HETE	AA metabolite	319.2	>	219	30	15	1:7
16-HETE	AA metabolite	319.2	>	233	30	15	X
16-HETE	AA metabolite	319.2	>	189	30	15	1:3
17-HETE	AA metabolite	319.2	>	247	30	15	X
19-HETE	AA metabolite	319.2	>	231	30	15	X
20-HETE	AA metabolite	319.2	>	289.2	30	15	X

Table 4. MRM transition information of arachidonic acid metabolites acquired in negative ionization mode with the signal to noise (S/N) acquired from a rodent brain tissue section.

Name		*m/z* Precursor		*m/z* Product Ion	Cone Voltage (V)	Col. Energy (eV)	S/N
FA (18:1)	Fatty acid	281.2	>	281.2	20	10	1:19
FA (20:4)	Fatty acid	303.2	>	259.2	20	15	1:540
FA (22:6)	Fatty acid	327.2	>	283.3	20	15	1:300
FA (22:5)	Fatty acid	329.2	>	285.2	20	15	1:9
FA (22:4)	Fatty acid	331.2	>	287.2	20	15	1:16

Table 5. MRM transition information of the targeted fatty acids acquired in negative ionization mode with the signal to noise (S/N) acquired from a rodent brain tissue section.

Figure 4 displayed some examples of the distributions of a panel of small endogenous molecules in rodent brain tissue sections.

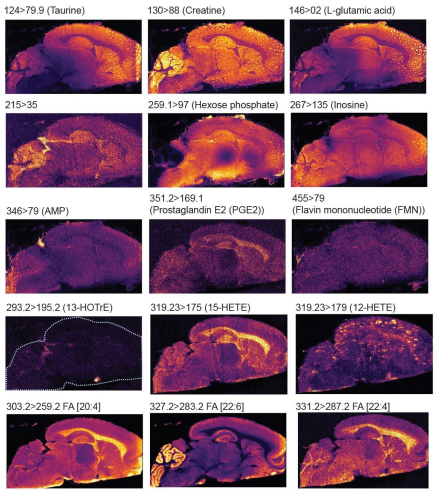

Figure 4. MRM ions images of rodent brain putatively identified small endogenous molecules such as amino acids, TCA cycle metabolites, arachidonic acid metabolites and fatty acids acquired in negative ionization mode at either 25 or 35 µm pixel sizes.

A further two experiments were carried out in positive ionisation focusing more on small metabolites that ionise preferentially in positive ionisation mode, such as certain amino acids, neurotransmitters, carnitine. A selection of their distributions are displayed in figure 5.

Name		*m/z* Precursor		*m/z* Product Ion	Cone Voltage (V)	Col. Energy (eV)	S/N
2-Aminobutyric or Dimethylglycine	non-proteinogenic amino acid or choline metabolite	104	>	58	15	15	1:16
Choline		104.1	>	60	15	22	1:41
GABBA	Neurotransmitter	104.1	>	87.1	14	10	1:77
Serine	amino acid	106.1	>	60	20	10	X
Niacinamide		123	>	80	15	23	1:6
Taurine	sulfonic acid	126	>	108	20	12	1:3
Leucine	amino acid	132.1	>	43	20	24	1:15
4-Hydroxyproline		132.1	>	86	20	15	X
Creatine	amino acid derivative	132.1	>	90	15	15	1:175
Hypoxanthine	purine nucleoside	137	>	110	15	20	1:11
Acetylcholine	Neurotransmitter	146.1	>	60	10	10	1:10
Glutamine	amino acid	147.1	>	84.1	20	13	1:2
Dopamine	Neurotransmitter	154.1	>	137.1	20	10	V
Histidine	amino acid	156.1	>	110	20	13	1:2
Phenylalanine	amino acid	166.1	>	120.1	20	10	1:2
Norepinephrine	Neurotransmitter	170	>	152	20	10	1:24
Arginine	amino acid	175.1	>	70	20	22	1:7
Serotonin	Neurotransmitter	177.1	>	160.2	15	10	X
Cystathionine	modified amino acid	223	>	134	20	10	1:12
Cytidine	pyrimidine nucleoside	244.1	>	112	20	13	1:6
Adenosine	purine nucleoside	268.1	>	136.1	15	19	1:5
Inosine	purine nucleoside	269.1	>	137	20	12	1:16
Guanosine	purine nucleoside	284	>	152	20	15	1:4
Car C16	Carnitine	400.3	>	85	50	26	1:32
Car C18:1	Carnitine	426.3	>	85	50	28	1:74

Table 6. MRM transition information of the targeted putatively identified small molecules metabolites such as amino acids, neurotransmitters, carnitine acquired in positive ionization mode with the signal to noise (S/N) acquired from a rodent brain tissue section.

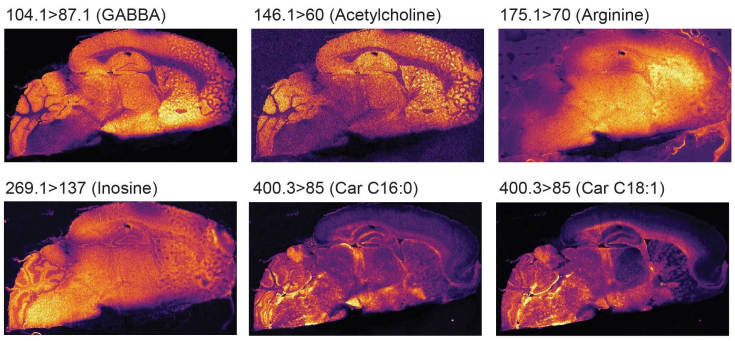

Figure 5. MRM ions images of rodent brain putatively identified small endogenous molecules such as amino acids, neurotransmitters, carnitines acquired in positive ionization mode at 35 µm pixel size.

Conclusion

The targeted MS imaging workflow combining a ACQUITY µBSM binary pump, DESI HPS, and a DESI XS source on a Xevo TQ Absolute XR Mass Spectrometer was demonstrated to deliver many hours of signal stability for the consistent generation of high-quality ion images over long periods of time.

Low abundant small molecules were detected directly from brain tissue section thanks to the sensitivity of the Xevo TQ Absolute XR Mass Spectrometer. Moreover, the specificity of the system allowed the differentiation of isobaric and isomeric molecules such as phospholipids, oxylipins, and small metabolites.

References

1. Using Targeted MRM MS Imaging With DESI to Visually Localize Isobaric And/or Low Abundance

Lipids, Waters application note. 720008692.

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DESI XS <https://www.waters.com/nextgen/global/products/mass-spectrometry/mass-spectrometry-ion-sources/desi-xs.html>

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High Definition Imaging (HDI) Software <https://www.waters.com/nextgen/global/products/informatics-and-software/mass-spectrometry-software/high-definition-imaging--hdi--software.html>

MassLynx Mass Spectrometry Software <https://www.waters.com/nextgen/global/products/informatics-and-software/mass-spectrometry-software/masslynx-mass-spectrometry-software.html>

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