# Combining MALDI Imaging and Liquid Surface Extraction for Spatial Metabolomics

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## Introduction:

- While MALDI Imaging does an excellent job of providing localization of metabolites, lipids, and proteins, it can be challenging to ID and quantify these molecules.
- There is great interest in combining MALDI Imaging with Metabolomics to produce Spatial Metabolomics, where metabolites of interest can be both localized, identified, and accurately quantified.
- Current spatial metabolomics methods rely on excision of small regions of tissue, followed by homogenization/extraction and analysis by HPLC-MS.
- While this method can be used to accurately identify and quantify metabolites, it is not high throughput, cannot be easily automated, and has poor spatial resolution.
- Here, we combine liquid surface extraction of tissue slices using an HTX SepQuant with MALDI Imaging.

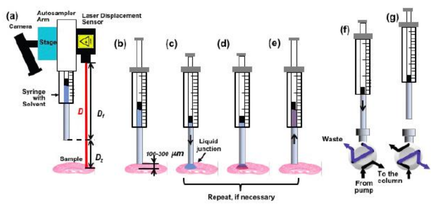


**Figure 1.** HTX SepQuant DropletProbe Workflow. **(A)** Drawing of surface sampling device using a PAL autosampler with a 10 µL syringe. **(B)** Syringe is positioned 100 – 200 µm above the tissue surface. **(C)** 0.5 µL of extraction liquid is dispensed, making contact with the surface. **(D)** Extraction liquid is held on surface for ~2 seconds. **(E)** Extraction liquid is drawn back into syringe. Steps C – D are repeated 2 more times to optimize extraction. **(F)** Extraction liquid is injected into sample loop (LOAD) on a switching valve. **(G)** Switching valve switches (INJECT) and sample is separated on HPLC column.

## Methods:

- Fresh frozen rat brain was sagittally sectioned at 10 µm and mounted on standard glass slides for SepQuant analysis, or ITO-coated glass slides for MALDI Imaging analysis.
- For SepQuant analysis, room-temperature tissue sections were extracted with three, 0.5 – 1 µL extractions of 50:50 $H_2O$:ACN, and injected onto a 2.1 x 100 mm C18 column.
- The SepQuant workflow is shown in **Figure 1**. A zoom of the SepQuant performing liquid surface extraction is shown in **Figure 2**.
- A Bruker Elute pump performed a 5 minute separation gradient (10 – 90% B) at 500 µL/min, and the eluted species were analyzed on the Bruker timsTOF and solariX XR instruments.
- For MALDI Imaging, tissue sections were coated with 9-AA using an HTX TM Sprayer. The coated tissue was analyzed on a Bruker solariX XR with 90 µm pixel resolution.
- For MALDI Imaging, data was analyzed using FlexImaging and SCiLS. For liquid surface extraction, data was analyzed using DataAnalysis.
- Metabolites were identified by accurate mass, isotopic fine structure, MSMS fragmentation, and HMDB.

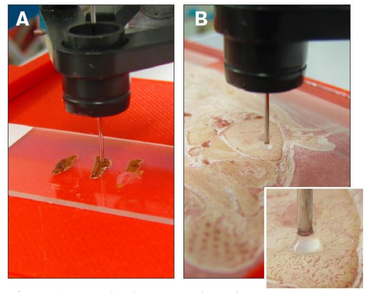


**Figure 2.** HTX SepQuant DropletProbe extracting sample. SepQuant probe positioned above **(A)** liver and **(B)** whole rat body section. A zoom of the liquid extraction interface is shown in the inset.

## Results:

- MALDI Imaging of the rat brain produced typical negative ion results, as shown in **Figure 3**.

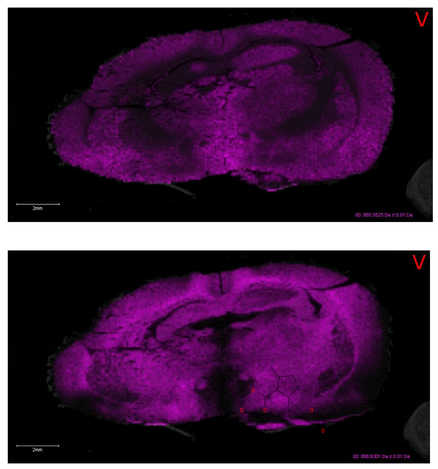


**Figure 3.** Negative ion MALDI Imaging results of rat brain, showing unique localization of lipids. **(A)** Localization of the lipid at m/z 885.55 and **(B)** localization of the lipid at m/z 888.630.

- Liquid Surface Extraction was used to probe various areas of a serial slice of rat brain. Extraction volumes of 0.5 - 1 µL were used, which probed an area of 1 – 3 mm.
- Many small molecules were measured by both instruments and both ionization techniques. We observed a mix of ions that were detected by both ionization techniques, and were unique to each technique.
- One of the small molecules measured by the SepQuant with LCMS is the peak at *m/z* 309.1711, which eluted at ~3.35 minutes as shown in **Figure 4**.
- Using the high resolving power of the **solariX XR** to measure isotopic fine structure on an LC timescale, the formula for this peak was identified as $C_{17}H_{26}O_5$, as shown in **Figure 4**.

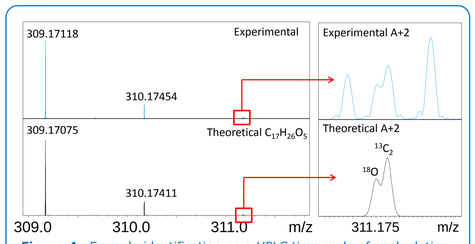


**Figure 4.** Formula identification on a UPLC time scale of peak eluting at ~3.3 minutes from SepQuant using isotopic fine structure. Using SmartForula XR, the formula $C_{17}H_{26}O_5$ was generated from the peak at *m/z* 309.17118.

- Searching HMDB for either the accurate *m/z* or the formula generates the iridoid compound HMDB0040980 with the common name Valdiate. The structure of this compound is shown in **Figure 5**.

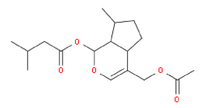

**Figure 5.** Proposed structure of metabolite Valdiate eluting at 3.3 minutes with the formula $C_{17}H_{26}O_5$, as predicted by HMDB. This iridoid compound is membrane associated, and is a potential biomarker for consumption of food products.

- Searching the MALDI imaging data for Valdiate at *m/z* 309.1696 produces the image shown in **Figure 6.** The compound is not strongly localized and is dispersed throughout the brain.
- The non-localization may be due to Valdiate associated with the membranes of the rat brain.

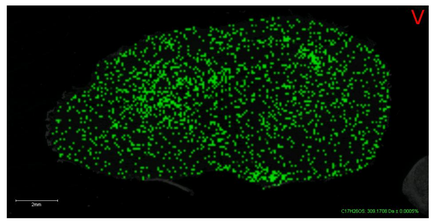


**Figure 6.** Formula identification on a UPLC time scale of peak eluting at ~3.3 minutes from SepQuant using isotopic fine structure. Using SmartForula XR, the formula $C_{17}H_{26}O_5$ was generated from the peak at *m/z* 309.17118.

- A similar extraction and LC analysis was performed on the **timsTOF Pro**, to generate ion mobility and MSMS data to aide in molecule identification.
- The peak at *m/z* 309.17 eluted at the same time with SepQuant LCMS on the timsTOF compared to SepQuant LCMS on the solariX XR, as show Figure 7.

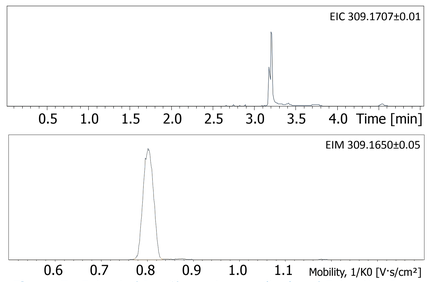


**Figure 7.** Extracted Ion Chromatogram (top) and Extraction Ion Mobiligram (bottom) for the peak at *m/z* 309.17 using the timsTOF Pro. The peak at m/z 309 eluted at the same time on the timsTOF Pro and produced a reduced mobility of 0.8 and a CCS of 167.8Å².

- Using TIMS, the peak at *m/z* 309.17 produced a single mobility as shown in the Extracted Ion Mobilogram in **Figure 7**. This peak has a reduced mobility of 0.8 and a measured CCS of 167.8 Å².

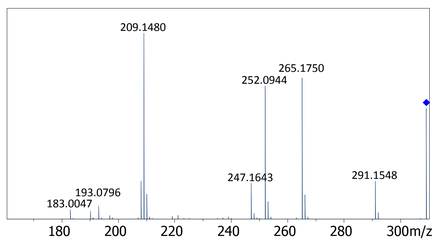


**Figure 8.** QCAD MSMS spectrum of *m/z* 309.17 generated by the timsTOF Pro. This MSMS spectrum does not match the theoretical spectrum for Valdiate from HMDB.

- Using the SepQuant and the timsTOF Pro, LCMSMS data for the peak at *m/z* 309.17 was measured. The MSMS spectrum of the peak at *m/z* 309.17 is shown in **Figure 8**.
- This MSMS spectrum did not match the theoretical MSMS spectrum for Valdiate from HMDB; further work is needed to confirm this MSMS data with Valdiate.

## Conclusions:

MALDI Imaging coupled with liquid surface extraction using the SepQuant provides a powerful tool to couple molecule identification and localization using isotopic fine structure, CCS measurements, and MSMS.

MALDI Imaging