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.

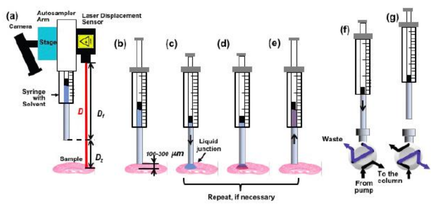

Figure 1. HTX SepQuant DropletProbe Workflow. **(A)** Drawing of surface sampling device using a PAL autosampler with a 10 µL syringe. **(B)** Syringe is positioned 100 – 200 µm above the tissue surface. **(C)** 0.5 µL of extraction liquid is dispensed, making contact with the surface. **(D)** Extraction 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.

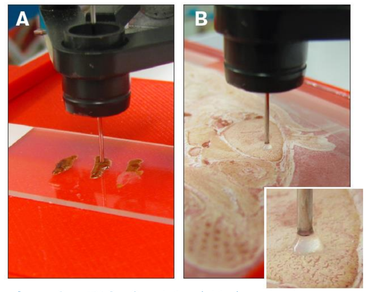

Figure 2. HTX SepQuant DropletProbe extracting sample. SepQuant probe positioned above **(A)** liver and **(B)** whole rat body section. A zoom of the liquid extraction interface is shown in the inset.

Results:

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

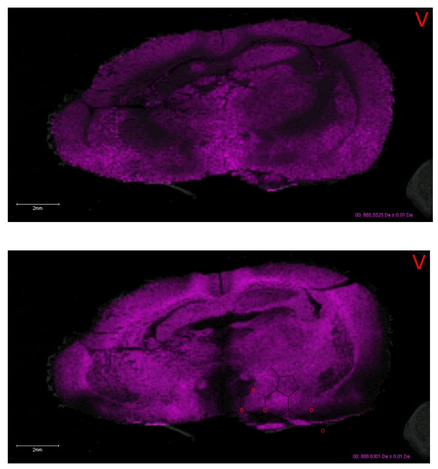

Figure 3. Negative ion MALDI Imaging results of rat brain, showing unique localization of lipids. **(A)** Localization of the lipid at m/z 885.55 and **(B)** localization of the lipid at m/z 888.630.

- 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**.

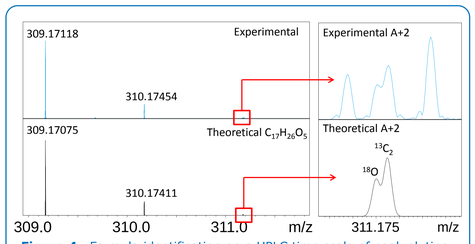

Figure 4. Formula identification on a UPLC time scale of peak eluting at ~3.3 minutes from SepQuant using isotopic fine structure. Using SmartForula XR, the formula $C_{17}H_{26}O_5$ was generated from the peak at *m/z* 309.17118.

- 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**.

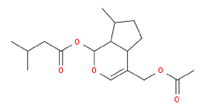

Figure 5. Proposed structure of metabolite Valdiate eluting at 3.3 minutes with the formula $C_{17}H_{26}O_5$, as predicted by HMDB. This iridoid compound is membrane associated, and is a potential biomarker for consumption of food products.

- 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.

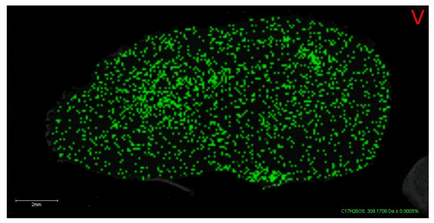

Figure 6. Formula identification on a UPLC time scale of peak eluting at ~3.3 minutes from SepQuant using isotopic fine structure. Using SmartForula XR, the formula $C_{17}H_{26}O_5$ was generated from the peak at *m/z* 309.17118.

- 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.

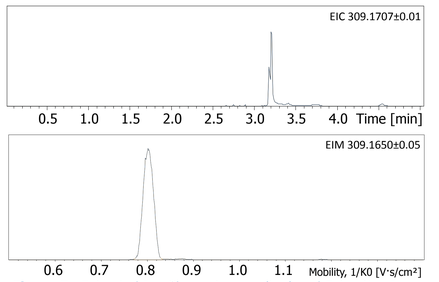

Figure 7. Extracted Ion Chromatogram (top) and Extraction Ion Mobiligram (bottom) for the peak at *m/z* 309.17 using the timsTOF Pro. The peak at m/z 309 eluted at the same time on the timsTOF Pro and produced a reduced mobility of 0.8 and a CCS of 167.8Å².

- 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.

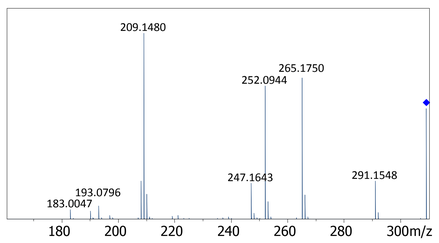

Figure 8. QCAD MSMS spectrum of *m/z* 309.17 generated by the timsTOF Pro. This MSMS spectrum does not match the theoretical spectrum for Valdiate from HMDB.

- 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