

# Combining MALDI Imaging and Liquid Surface Extraction for Spatial Metabolomics



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## Introduction:

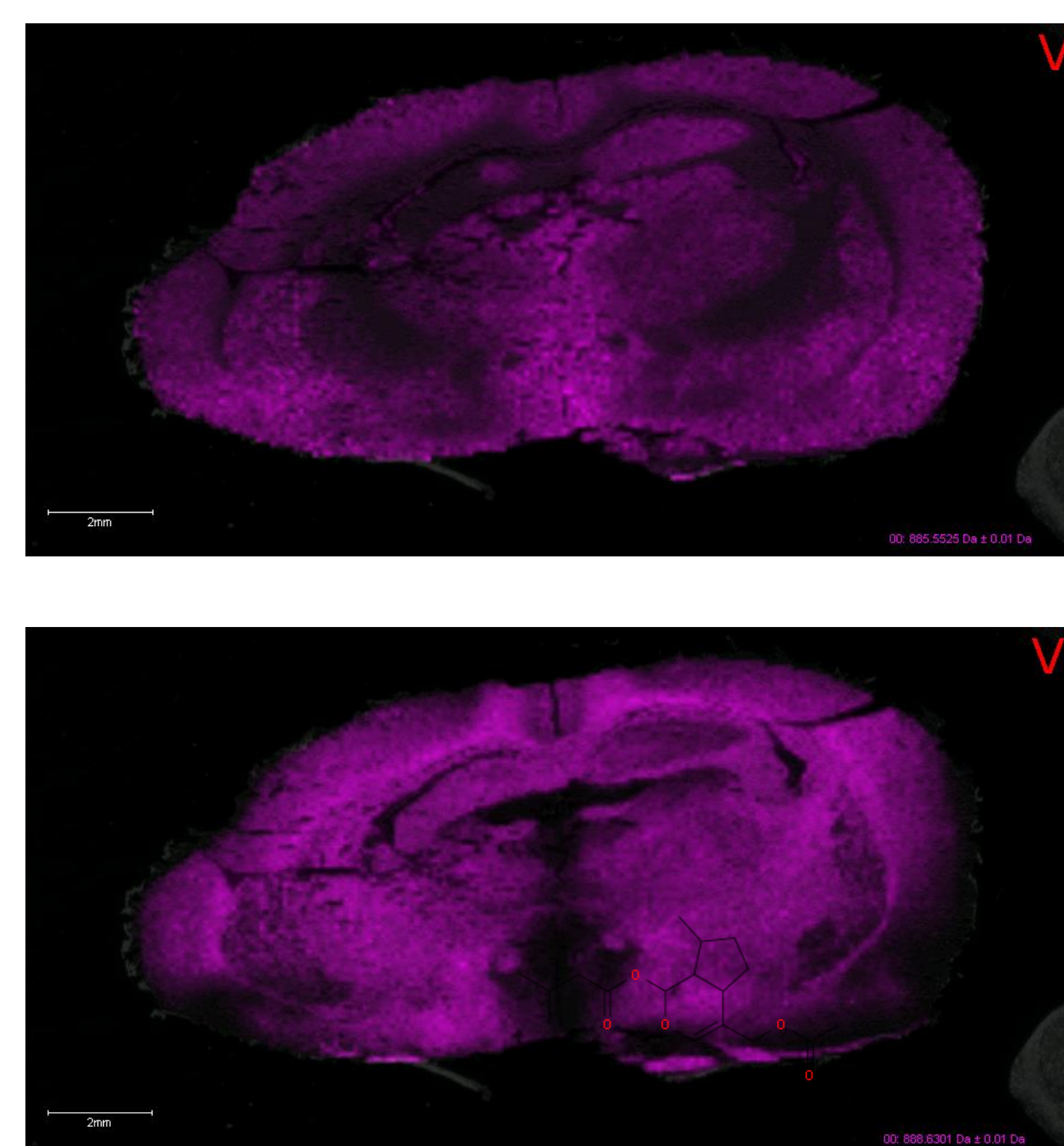
- While MALDI Imaging does an excellent job of providing localization of metabolites, lipids, and proteins, it can be challenging to ID and quantify these molecules.
- There is great interest in combining MALDI Imaging with Metabolomics to produce Spatial Metabolomics, where metabolites of interest can 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.

## Methods:

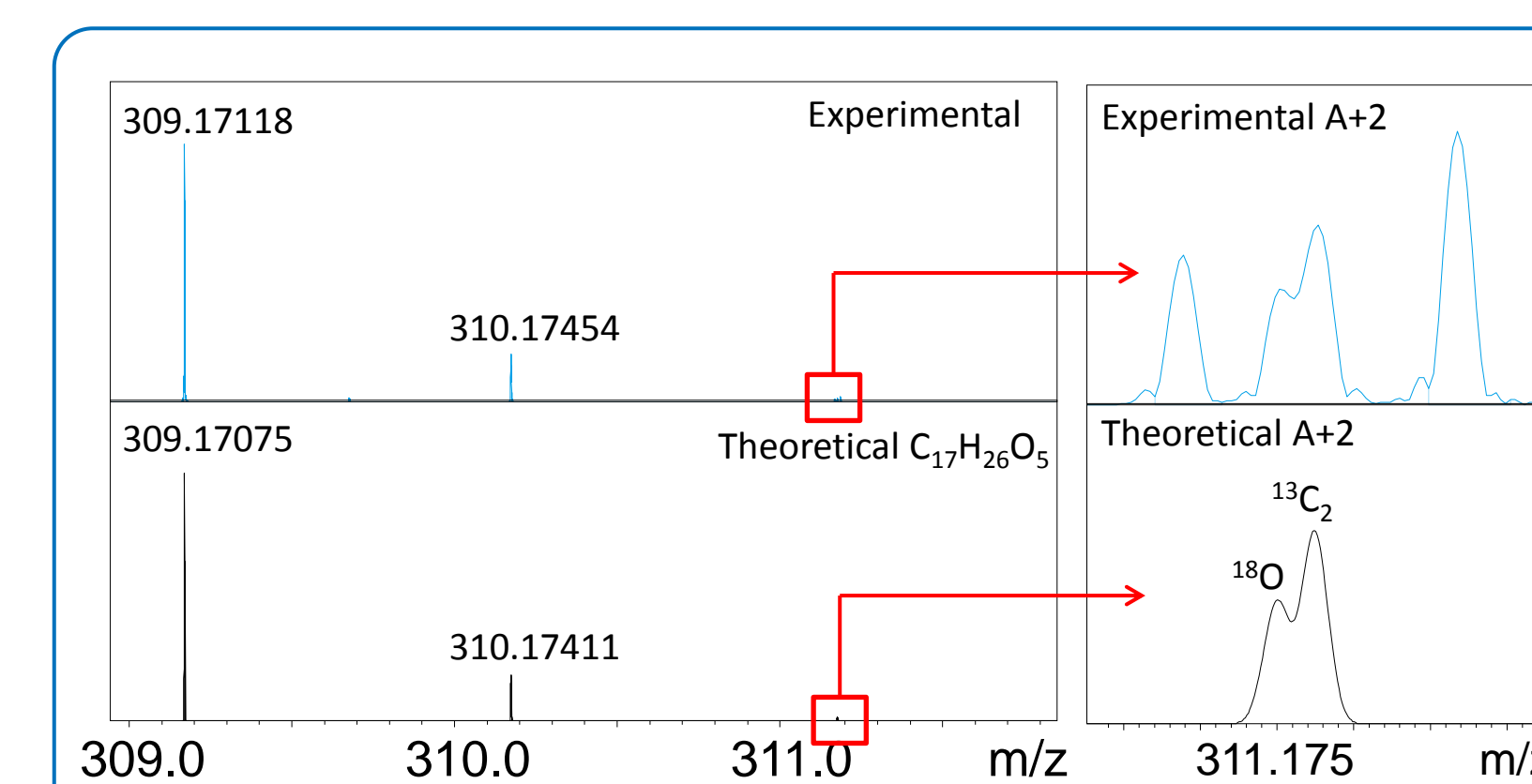
- Fresh frozen rat brain was sagittally sectioned at 10  $\mu\text{m}$  and mounted on standard glass slides for SepQuant analysis, or ITO-coated glass slides for MALDI Imaging analysis.
- For SepQuant analysis, room-temperature tissue sections were extracted with three, 0.5 – 1  $\mu\text{L}$  extractions of 50:50  $\text{H}_2\text{O}$ :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  $\mu\text{L}/\text{min}$ , 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  $\mu\text{m}$  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.

## Results:

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

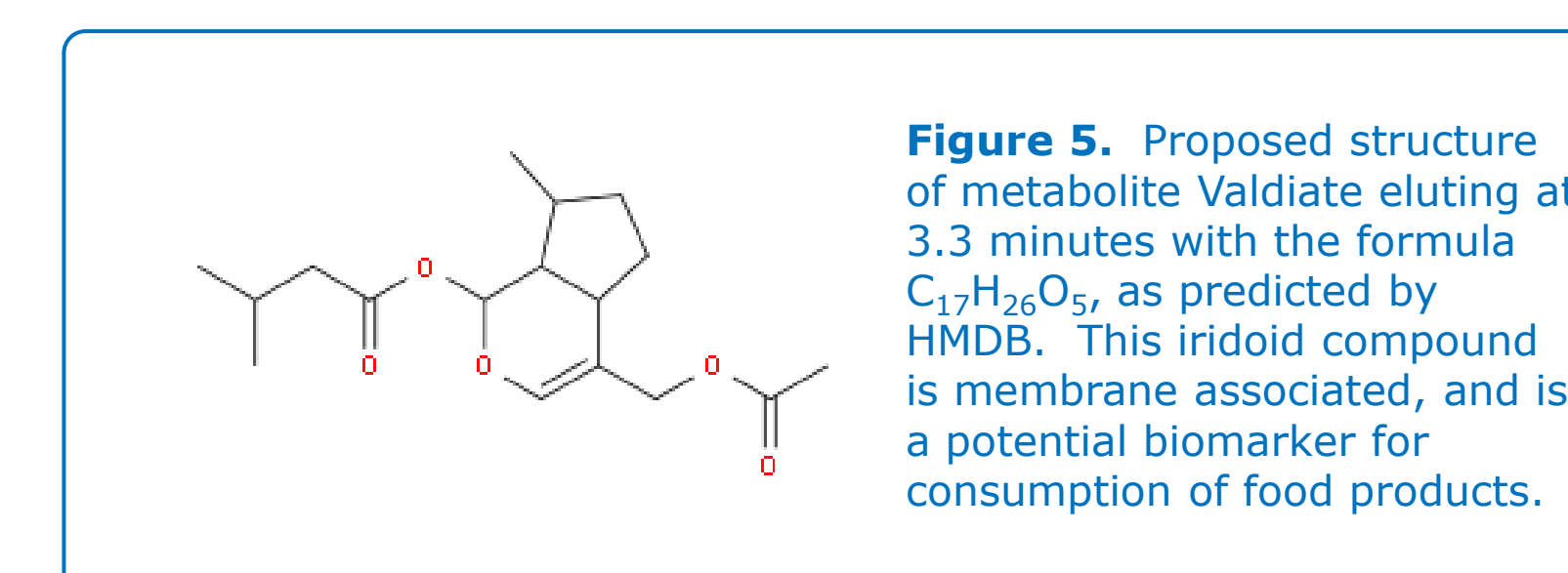


**Figure 3.** Negative ion MALDI Imaging results of rat brain, showing unique localization of lipids. (A) Localization of the lipid at  $m/z$  885.55 and (B) localization of the lipid at  $m/z$  888.630.



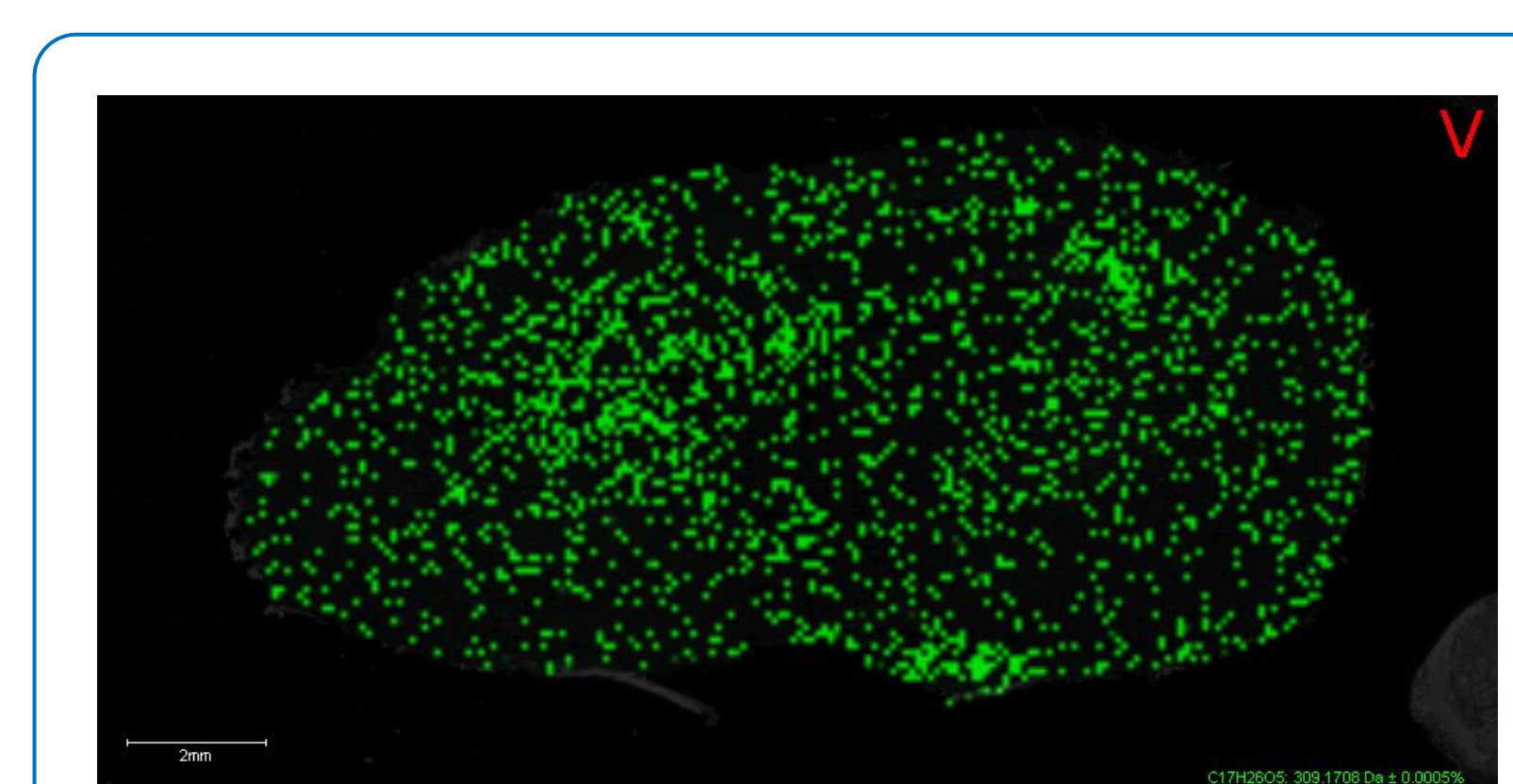
**Figure 4.** Formula identification on a UPLC time scale of peak eluting at  $\sim 3.3$  minutes from SepQuant using isotopic fine structure. Using SmartFormula XR, the formula  $\text{C}_{17}\text{H}_{26}\text{O}_5$  was generated from the peak at  $m/z$  309.17118.

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



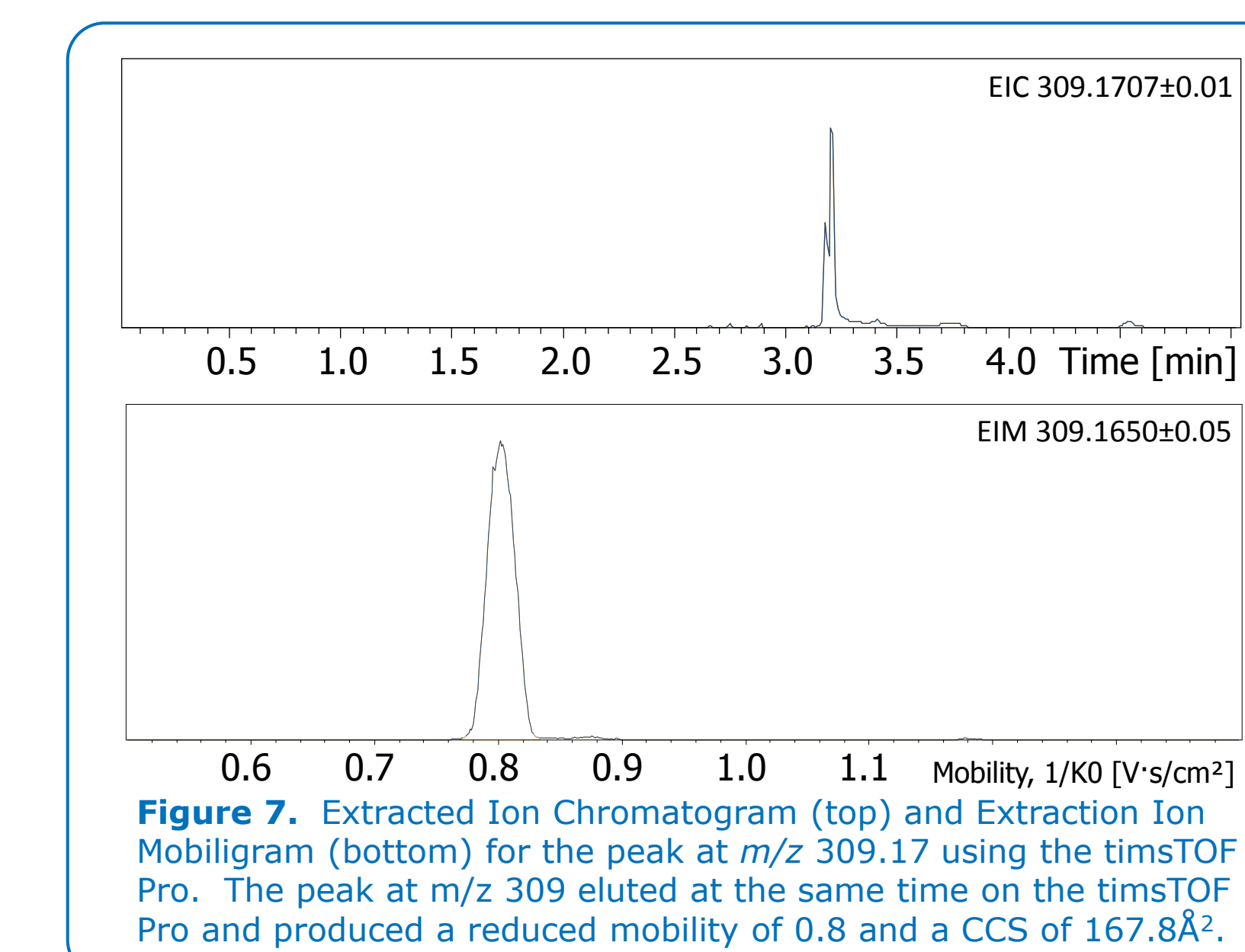
**Figure 5.** Proposed structure of metabolite Valdiolate eluting at 3.3 minutes with the formula  $\text{C}_{17}\text{H}_{26}\text{O}_5$ , as predicted by HMDB. This iridoid compound is membrane associated, and is a potential biomarker for consumption of food products.

- Searching the MALDI imaging data for Valdiolate 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 Valdiolate associated with the membranes of the rat brain.



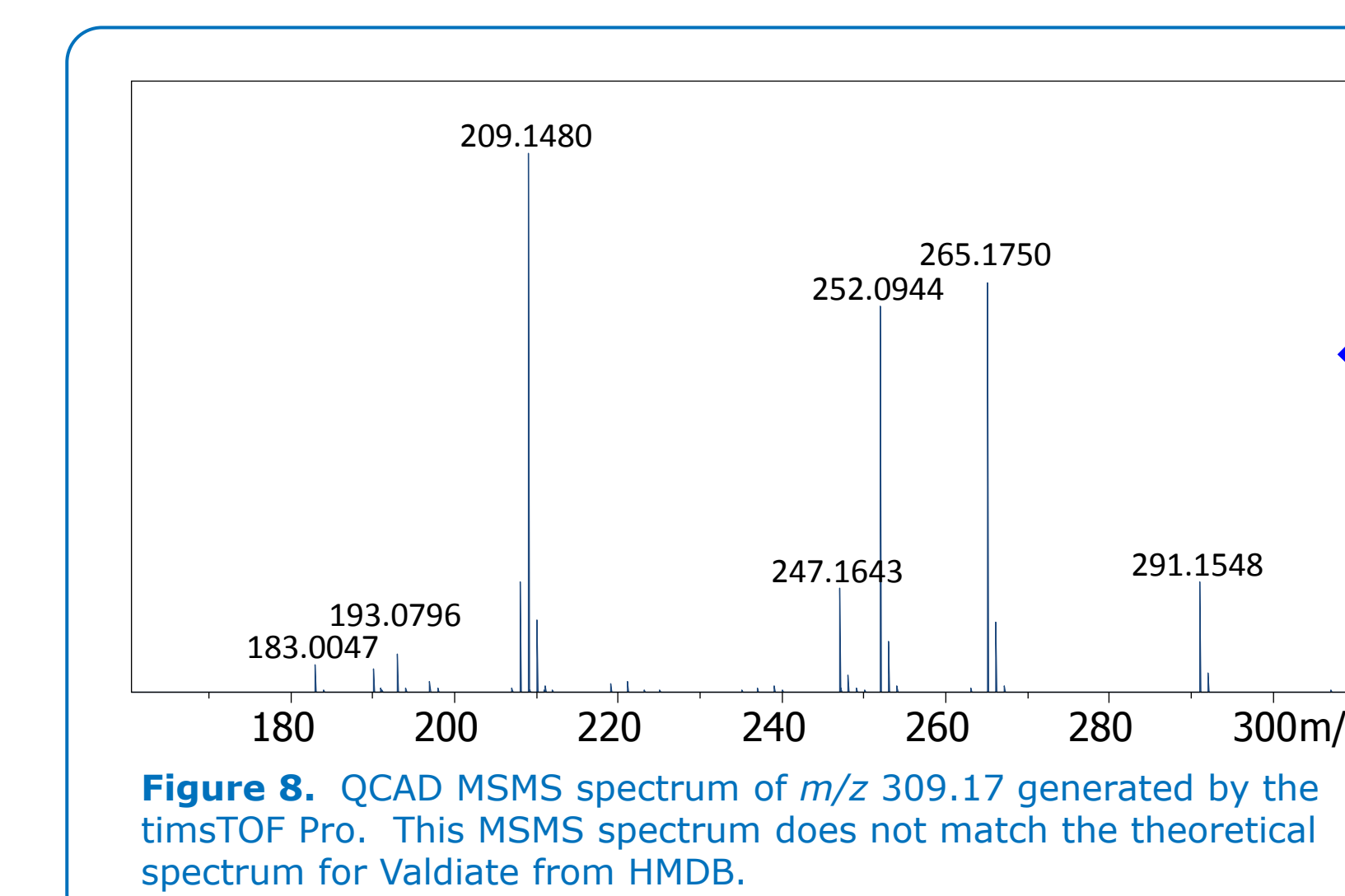
**Figure 6.** Formula identification on a UPLC time scale of peak eluting at  $\sim 3.3$  minutes from SepQuant using isotopic fine structure. Using SmartFormula XR, the formula  $\text{C}_{17}\text{H}_{26}\text{O}_5$  was generated from the peak at  $m/z$  309.17118.

- A similar extraction and LC analysis was performed on the **timsTOF Pro**, to generate ion mobility and MSMS data to aid 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  $167.8 \text{ \AA}^2$ .

- 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  $167.8 \text{ \AA}^2$ .



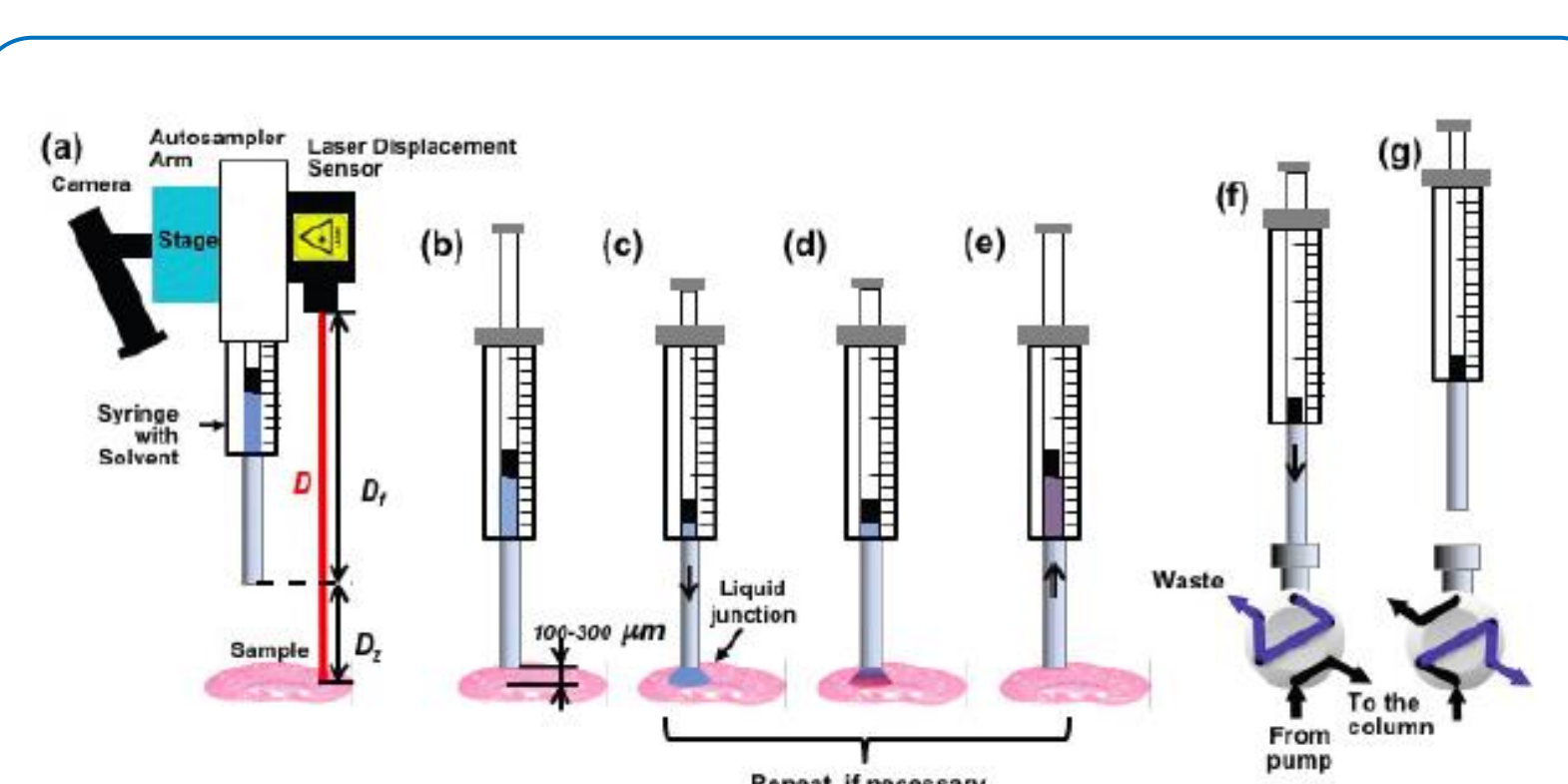
**Figure 8.** QCAD MSMS spectrum of  $m/z$  309.17 generated by the timsTOF Pro. This MSMS spectrum does not match the theoretical spectrum for Valdiolate from HMDB.

- 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 Valdiolate from HMDB; further work is needed to confirm this MSMS data with Valdiolate.

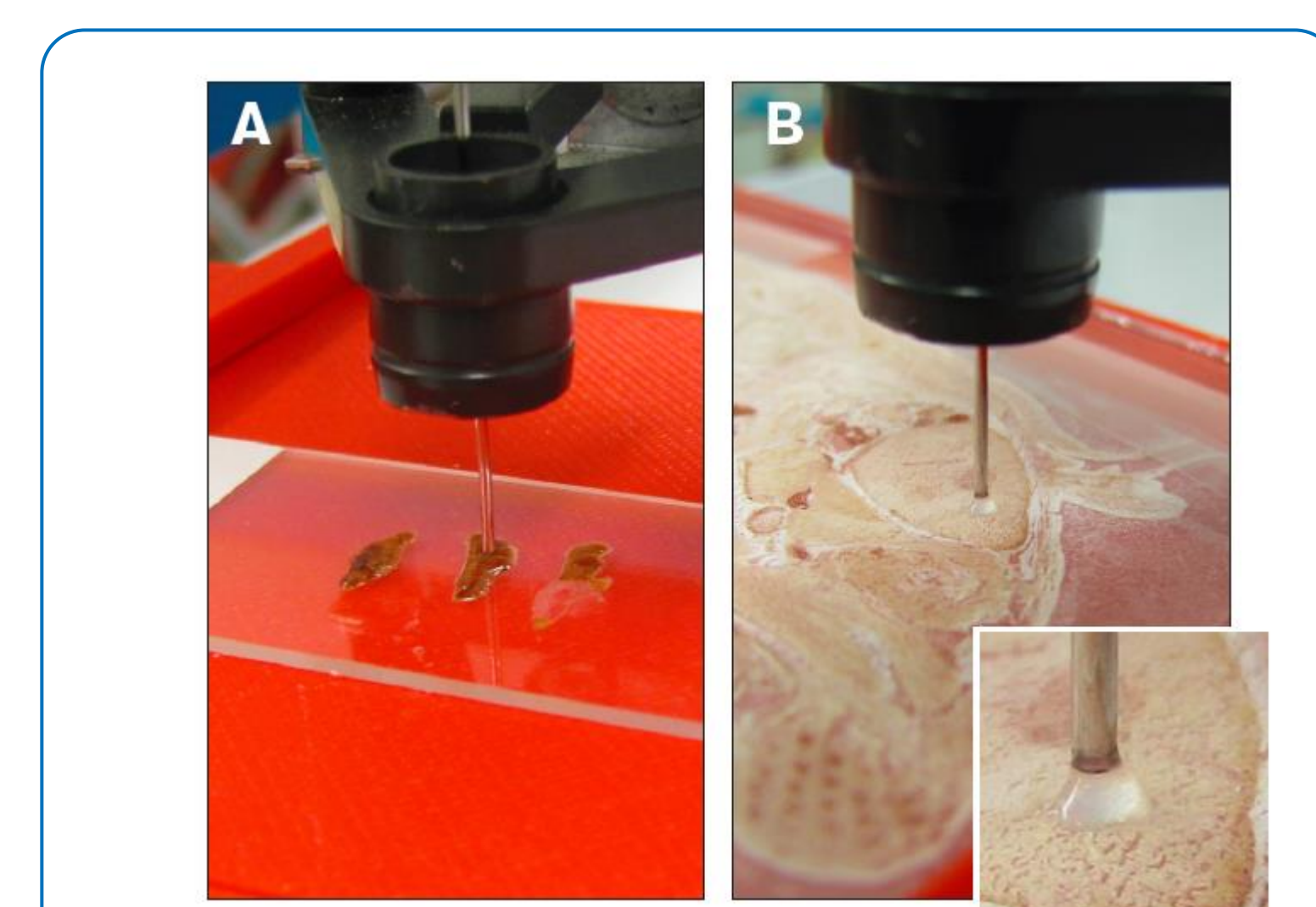
## Conclusions:

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



**Figure 1.** HTX SepQuant DropletProbe Workflow. (A) Drawing of surface sampling device using a PAL autosampler with a 10  $\mu\text{L}$  syringe. (B) Syringe is positioned 100 – 200  $\mu\text{m}$  above the tissue surface. (C) 0.5  $\mu\text{L}$  of extraction liquid is dispensed, making contact with the surface. (D) Extraction liquid is held on surface for  $\sim 2$  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.



**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.