Mass Spectrometry Imaging Characterization of Ethiodized Oil, a Radiopaque Hydrophobic Multipurpose Liquid Used in Cancer Therapy

Shubhneet Warar[1], Danielle Stolley[2], A. Colleen Crouch[2], Erik Cressman[2]

1: Baylor University, Waco, TX; 2: University of Texas MD Anderson Cancer Center, Houston, TX

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Making Cancer History®

Introduction

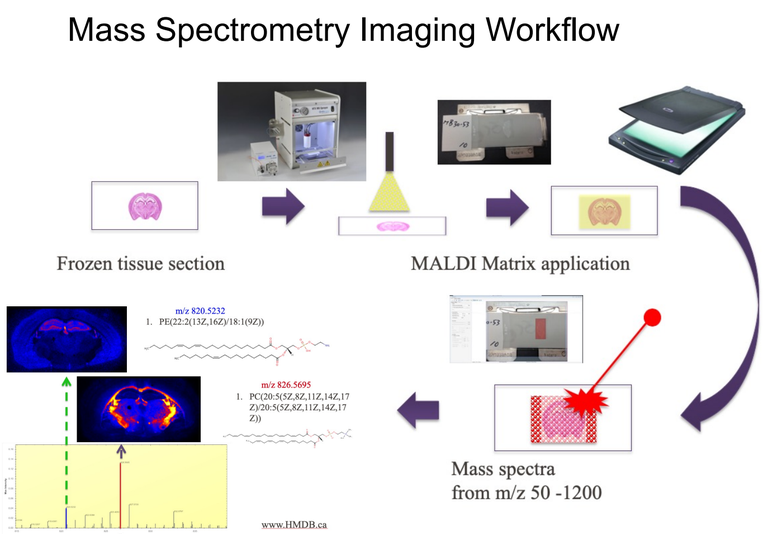
The incidence and mortality of liver cancer are both increasing and there is no curative medical treatment [1]. Embolization is a promising treatment option in which an embolic agent, ethiodized oil (iodinated ethyl esters from poppy seed oil), is often utilized in combination with drugs to disrupt tumor arterial supply [2]. The oil is an ideal radiopaque contrast agent for imaging using computed tomography (CT) due to the presence of iodine [3]; however, characterization of ethiodized oil with mass spectrometry imagining (MSI) was unknown. Herein, we characterize the mixture of compounds comprising ethiodized oil using matrix-assisted laser desorption ionization (MALDI)-MSI.

Methods

MALDI Matrices: Three matrices were tested on ethiodized oil (Guerbet LLC) by spotting a 1:1 ratio of solution to matrix on a standard MALDI well plate as follows: for positive ion mode, 2,5-dihydroxybenzoic acid (DHB) and alpha-cyano-4-hydroxycinnamic acid (CHCA); for negative ion mode, 9-aminoacridine hydrochloride (9AA).

Tissue Preparation: Ethiodized oil was spotted on sectioned tissue and matrix was applied uniformly across by a HTX M5 robotic sprayer.

Analysis: MALDI time-of-flight (TOF) mass spectrometry was performed on a Waters Synapt G2-Si. MS/MS was done with optimized transfer collision energies for selected peaks to further characterize features. Data processing and images were reconstructed using HDImaging software.



Scheme 1:
Typical MSI workflow. Cryosectioned tissue is frozen-mounted on a regular glass slide. After drying under vacuum for 10 minutes, MALDI matrix is applied using a robotic sprayer. Data was acquired from m/z 50-1200 and processed using HDImaging software.

Results

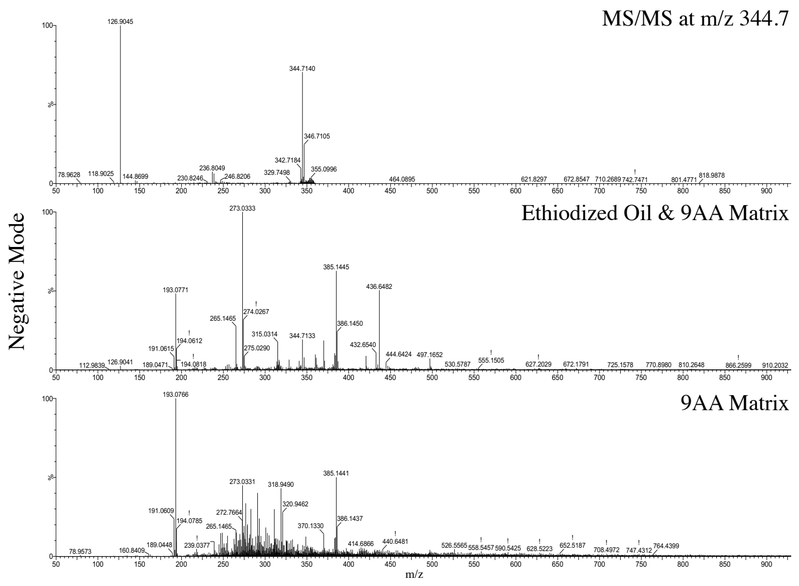


Figure 1:
Mass spectra of ethiodized oil and 9AA matrix spotted on standard MALDI plate in negative mode. Spectra labels are as follows: bottom spectrum shows only matrix, middle spectrum shows matrix and ethiodized oil, top spectrum shows MS/MS fragments at m/z 344.7. Distinct peaks were seen at m/z 344.71, 436.68, and 508.58. For both precursor ions m/z 344.71 and 436.68, only a fragment peak at 126.90 was seen and assigned to iodide as ethiodized oil is an iodinated mixture. For the precursor ion m/z 508.58, fragment peaks were seen at 126.90, 169.46, 210.83, and 316.74. Oil spotted on tissue again showed an iodine peak at m/z 126.90.

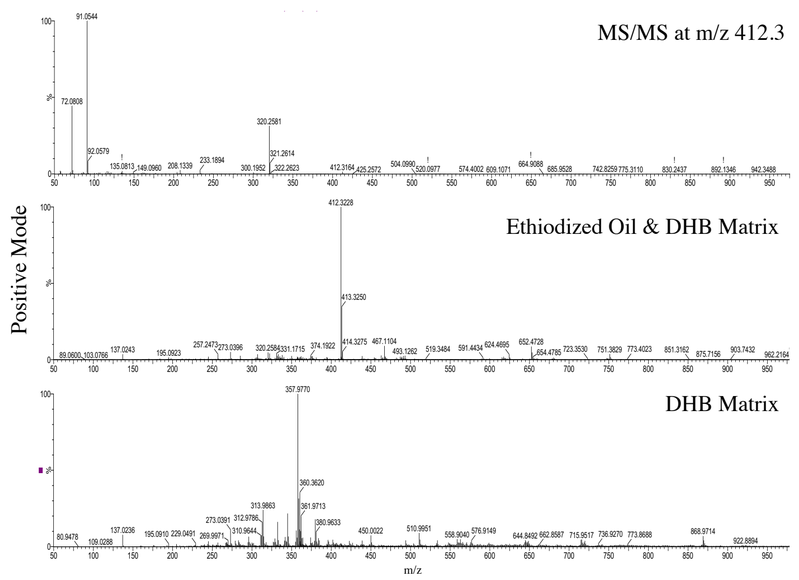


Figure 2:
Mass spectra of ethiodized oil and DHB matrix spotted on standard MALDI plate in positive mode. Spectra labels are as follows: bottom spectrum shows only matrix, middle spectrum shows matrix and ethiodized oil, top spectrum shows MS/MS fragments at m/z 412.3. Using either DHB or CHCA, distinct MS peaks were seen at m/z 320.25 and 412.32. For the precursor feature at m/z 320.25, MS/MS analysis showed fragment peaks at 72.08 and 208.13. For the precursor ion m/z 412.32, fragment peaks were seen at 72.08, 91.05, 208.13, and 320.26. Mass spectra of spotted tissue in both positive and negative mode showed similar precursor peaks but with weaker intensity as endogenous lipids were of higher intensity.

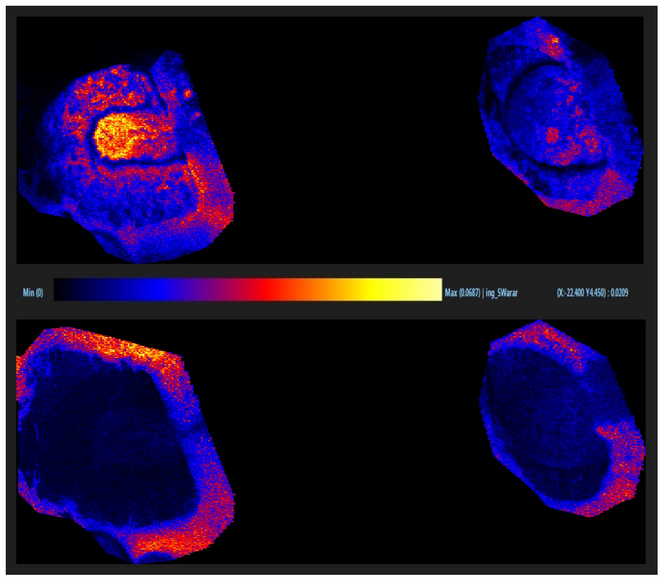


Figure 3:
HDImaging software image reconstruction of ethiodized oil spotted on sectioned tissue in negative mode at m/z 292.7711 (top image) and m/z 370.1321 (bottom image). Left tissues show ethiodized oil spotted in the middle. Right tissues show a mixture of ethiodized oil, dichloroacetic acid, caffeine, and benzethonium chloride spotted in the middle. Intensity of compounds is depicted by the shown color scale. Yellow circular spot on the left tissue in the top image likely shows ethiodized oil. The bottom image shows that matrix was possibly not adhering to the ethiodized oil, a slippery and difficult to dry substance, and thus not ionizing.

Conclusions

Ethiodized oil is a common clinical contrast agent, but exact structures of the mixture are not reported. To determine chemical composition, MS peaks can be analyzed and compared to endogenous peaks in tissue. The mass spectra were consistent with the oil being a mixture of compounds with iodinated long-chain fatty acid esters. Compounds were determined to have between 1-3 iodines per structure. By characterizing MS peaks, researchers can now track distribution of ethiodized oil in tissues. By detecting the oil in tissue, other drugs dissolved in the oil could be more easily traced.

References

[1] Cressman, E. N.; Guo, C. *CardioVascular and Interventional Radiology* **2018**, *41* (10), 1611–1617.

[2] Uchida, H.; Ohishi, H.; Matsuo, N.; Nishimine, K.; Ohue, S.; Nishimura, Y.; Maeda, M.; Yoshioka, T. *Cardiovascular and Interventional Radiology* **1990**, *13* (3), 140–145.

[3] Lipiodol (Ethiodized Oil) Injection; Guerbet US: Princeton, NJ, USA, 2018.

Acknowledgements

Thank you to MD Anderson Cancer Center and the Baylor University Honors College for sponsoring the University Outreach Summer Program's Undergraduate Cancer Research Internship. This work was also supported by a training fellowship from The University of Texas Health Science Center at Houston Center for Clinical and Translational Sciences TL1 Program (NIH Grant TL1 TR003169).