TISSUE IDENTIFICATION BY RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY (REIMS) BASED ON FRAGMENTATION PROFILE OF GLYCEROPHOSPHOLIPIDS

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OVERVIEW

- **Rapid characterization of tissues is an important problem in the surgical diagnostic area.**
- **Near real-time tissue identification using the recently developed REIMS technique is based on the profile of the different lipids, mainly phospholipids and triglycerides, present in cell membrane.**
- **Due to extensive isomerism, one molecular formula represented by one MS peak usually covers multiple different chemical species.**
- **The isomer composition of MS peaks might carry additional information about the actual phenotype of the tissue.**
- **The objective of the present study was the investigation of this hypothesis.**

METHODS

- Samples: liver tissue from different animals (pig, chicken, rabbit)
- Workflow and instrumentation can be seen on Figure 1 below.
- Negative ionization mode, Xevo G2-XS Q-ToF
- m/z range 600-900 for ToF mode
- m/z range 50 – 600 for MS/MS mode
- Collision energy 25 eV

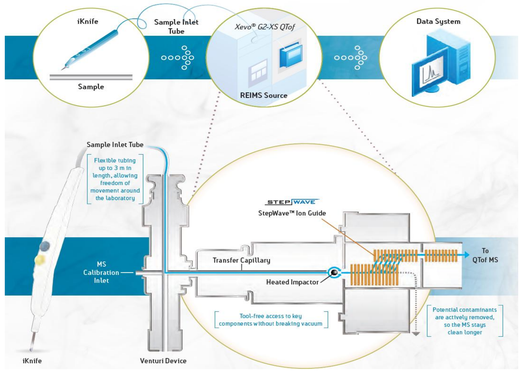


Figure 1. Vapor was transfered to the mass spectrometer for analysis. MSMS spectra were acquired from previously selected phospholipids and subjected to multivariate classification algorithms. The models were then used to identify the origin of animal liver samples online.

RESULTS

In order to identify the most important compounds involved in the separation of the different tissue types, a classification model based on the full profile of different animal livers was built (Figure 2).

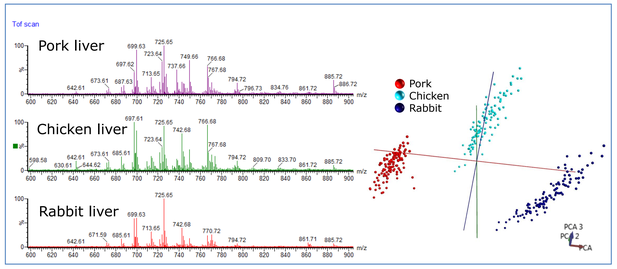


Figure 2. Typical mass spectra of membrane phospholipides of animal liver (left) and 3D PCA model (right)

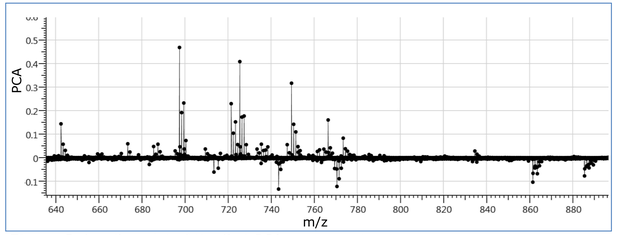
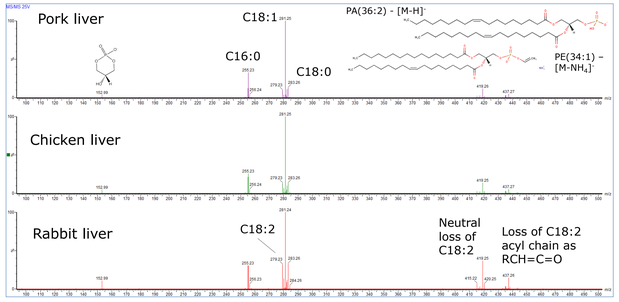


Figure 3. Loading plot of the first PCA component

According to the mass spectra and the loading plot the following m/z values were selected for MS/MS analysis:
m/z 699.497 m/z 725.513 m/z 749.513 m/z 766.539

The acquired MSMS spectra feature more than one phospholipid species contributing to the individual peaks. Using this observation, the previously selected precursor ions were fragmented during REIMS sampling of all different livers. Figure 3 below shows an example for the MS/MS spectrum of the same precursor ion in different liver tissue.



According to MS/MS spectra the parent ion m/z 699.497 presumably represents the following phospholipids:

PA(36:2) - [M-H]-
PA(18:1/18:1)
PA(18:0/18:2)
PA(18:2/18:0)

PE(34:1) - [M-NH4]-
PE(16:0/18:1)
PE(18:1/16:0)

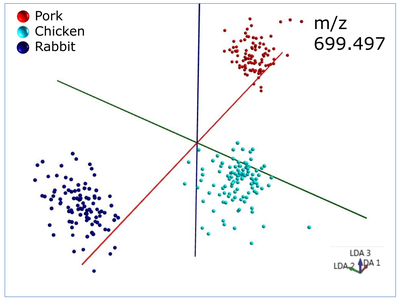
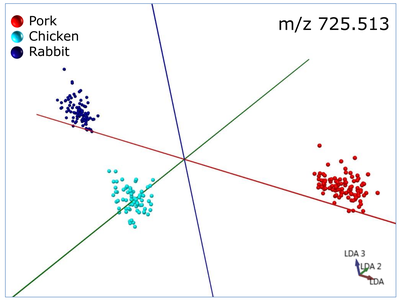
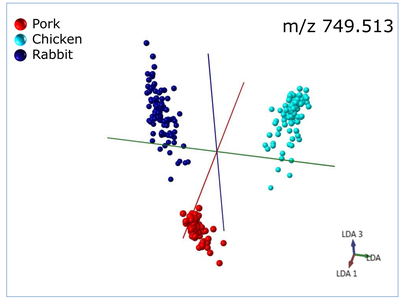


Figure 4. Example for MS/MS spectra of m/z 699.497 obtained from animal liver





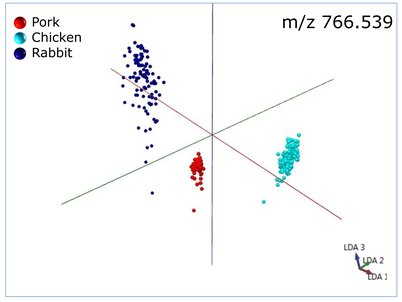


Figure 5. 3D LDA plot of the three liver sample based on different fragment ion spectra. The leave 20% out cross-validation resulted 100% correct classification rate. LDA plots indicate that tissue can be distinguished based on only one MS peak.

CONCLUSION

- **The presented data clearly demonstrates that REIMS has the potential of distinguishing different tissues based on the MS/MS spectra of only one precursor ion.**
- **Our findings suggest that the MS peaks contain several different phospholipid species, possibly from different classes.**
- **Since the relative contribution of isomeric phospholipids to a specific exact m/z value peak varies between different tissues, we have an additional information for the identification of different tissue types.**

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