# Optimization of Matrix Assisted Rapid Evaporative Ionisation Mass Spectrometry (MA-REIMS) System.

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## INTRODUCTION

- Rapid Evaporative Ionisation Mass Spectrometry (REIMS) is a powerful tool for fast classification and identification of complex biological samples based on their mass spectral fingerprints.
- The Matrix Assisted REIMS (MA-REIMS) includes the introduction of a matrix, for example isopropanol (IPA), mixed into the sample aerosol, resulting in increased sensitivity and enhancement of the signal to noise ratio.
- **The purpose of this study was to investigate the effect of the addition of different doping agents to the matrix on the following parameters:**
  - **Spectral signal intensity and signal to noise ratio.**
  - **Tissue classification accuracy.**

## METHODS

The MA-REIMS method is shown schematically in Fig 1 and 2.

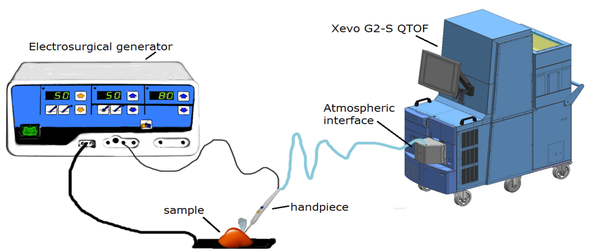


***Figure 1.:*** *Schematic of the MA-REIMS system: the aerosol generated by an electrosurgical cautery device is sampled and transferred to the distant mass spectrometer via a venturi air jet pump.*

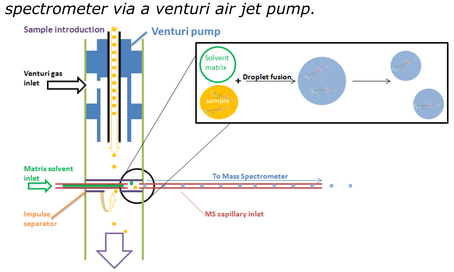


***Figure 2.:*** *The details of the matrix introduction; an external syringe pump is used to introduce the matrix component into an impulse separator in front of the inlet capillary. It is thought that the matrix droplets fuse with the aerosol entering the capillary.*

The experimental settings were as follows:

- Waters Xevo G2-S Mass spectrometer, Negative Ion mode, 600 – 900 m/z range
- Porcine liver and muscle tissue as sample
- Isopropanol as matrix.

The investigated matrix doping additives are shown in Fig 3.

| Alkalic doping additives: | | Acidic doping additives: | |
|---|---|---|---|
| Ammonia |  | Formic acid |  |
| Diethylamine (DEA) |  | | |
| Triethylamine (TEA) |  | Trifluoroacetic acid (TFA) |  |
| Piperazine |  | | |

***Figure 3.:*** *Doping additives examined in this study.*

## RESULTS

### Effect of different doping additives

- Porcine liver was used as a sample source to test the effects of different doping additives.
- 10 sec combined spectra with 1 sec scan time were used for comparison of different doping additives (Fig 4).
- The spectra featured mainly phospholipids with a negative charge.

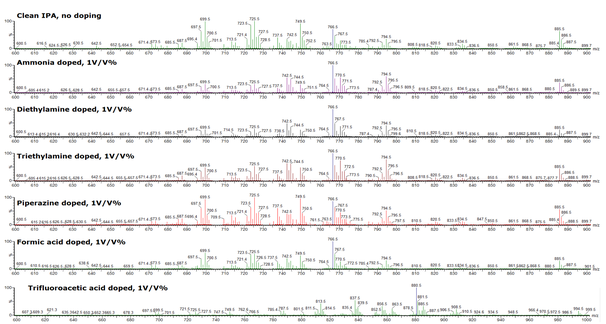


***Figure 4.:*** *Spectra obtained using the different doping additives. Most doping additives have a significant effect on the total ion intensities and the different phospholipids ratios. TFA, however, acts as an adduct to the investigated phospholipids without any beneficial effect towards ion formation.*

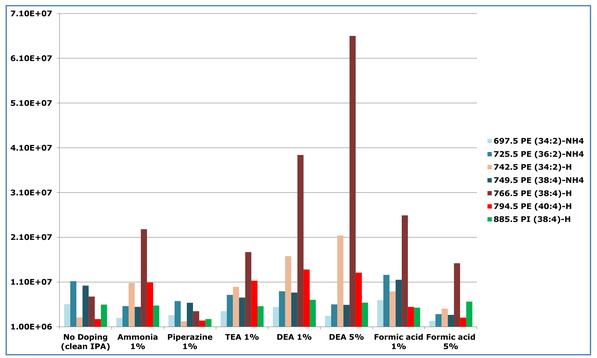


***Figure 5.:*** *The intensities of different phospholipids as a function of the doping additive.*
*Alkaline doping additives, ammonia, DEA and TEA enhance ionisation via increased deprotonation of phosphatidyletanolamines (PE), whilst decreasing the degree of $NH_4^-$ loss. Other lipids e.g. phosphatidylinositols (PI) are not effected. Whilst piperazine is also an alkaline substance, it seems to act as an ion suppressor.*
*Acidic additives such as formic acid were expected to suppress the negative ion signal, however at the correct concentration signal enhancement is observed.*

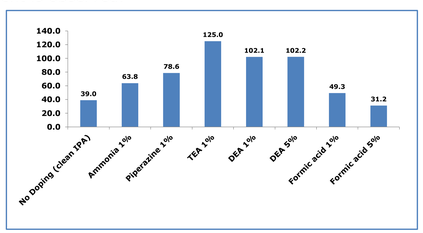


***Figure 6.:*** *Signal to noise ratio as a function of doping additive. The ratio was calculated by dividing the average intensity of significant peaks by the average noise level. The enhancement of signal quality is significant with alkaline additives.*

### Effect of doping on classification

Principal Component Analysis (PCA) was used to test the separation of spectra gained from tissues with different methods. A double binary model (DEA doped vs no doping; Porcine liver vs porcine muscle) was created to study the effect of doping on the classification accuracy.

Each group contains 30 spectra. The model consists of 120 spectra. Leave 20%-out cross-validation resulted in 100% correct classification rate.

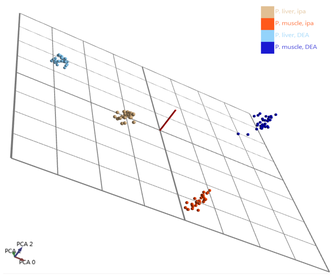


***Figure 7.:*** *PCA model of porcine liver and muscle spectra containing both DEA doped and pure IPA. There is a clear separation between all groups, however the DEA groups are more compact due to the better spectral quality.*

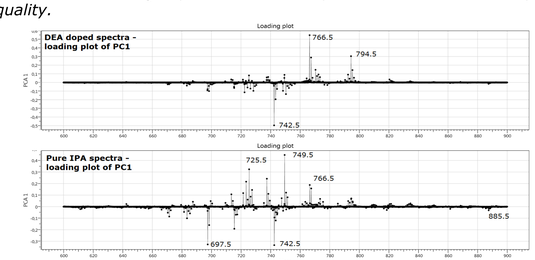


***Figure 8.:*** *PC1 loading plots of different classification systems. There is a shift in the significant species responsible for the separation between porcine liver and muscle tissues.*

## CONCLUSIONS

- **The tested doping additives for Matrix Assisted REIMS had a significant impact on the quality of acquired spectra from biological tissues.**
- **Alkaline additives (Ammonia, TEA, DEA) have enhanced the ionisation of glycerophospholipids (especially PEs), while other compounds (formic acid, piperazine) mostly had an ion suppression effect.**
- **Our future plans include the test of matrix additives in positive ion mode.**
- **The study showed that doping and a refined MA-REIMS method could be a powerful tool in improving the sensitivity and performance of REIMS technique.**

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