

MALDI-2-MS, chemical derivatization and Ion Mobility on the timsTOF-flex-MS for enhanced MSI to assess Vit-D metabolism and androgen intracrinology

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INTRODUCTION

- Growth of many prostate cancer tumors are dependent on active androgens such as testosterone (T) and dehydroepiandrosterone (DHEA).
- Vitamin D's active metabolite (1,25-(OH)₂-D₃), modulates the androgen intracrine pathway

Aims of the study

- To evaluate the application of ion mobility and MALDI-2 on a timsTOF flex for determining the spatial distribution of derivatized androgens and vitamin D metabolites to improve sensitivity, and separate biological active isobaric species.

MATERIALS AND METHODS

- Instrument was a MALDI-2-IM-MSI using a timsTOF-flex in-house modified with a 1 kHz, frequency-quadrupled Nd:YAG post-ionization laser (266 nm).
- Several derivatization reagents for both androgens (Girard-T and Dansyl Hydrazine) and VitD metabolite (PTAD and DMEQ-TAD) were screened to assess ionization enhancement and mobility separation on standards and tissue sections
- On-tissue chemical derivatization (OTCD) was performed by the Bruker ImagePrep using prostate tumour (10µm) tissue and matrix was applied using a modified 3D printer
- The source and TIMS pressures, as well as the CCS were calibrated using a low-molecular weight tune-mix.

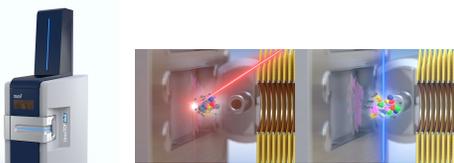


Figure 1. MALDI-2 timsTOF flex instrument

RESULTS

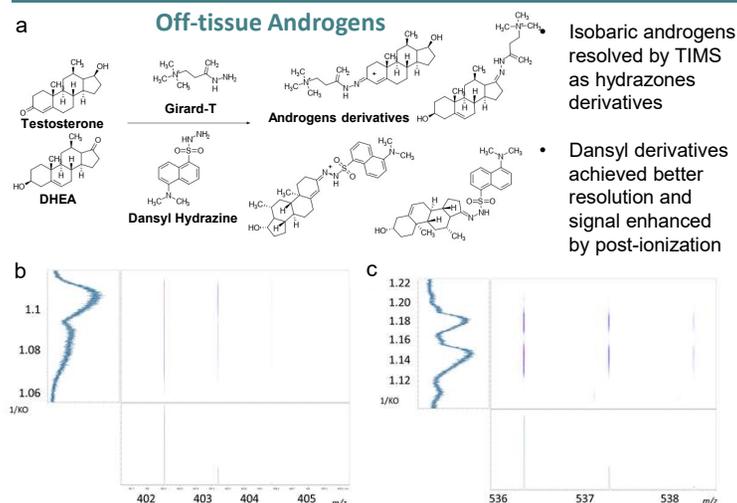


Figure 2. a) Androgens (T and DHEA) derivatization reaction scheme using Hydrazine-type reagents. Off-tissue Mobilogram of equimolar T/DHEA mix standard as b) Girard derivatives, c) Dansyl derivatives

Androgens imaging on prostate tissue section

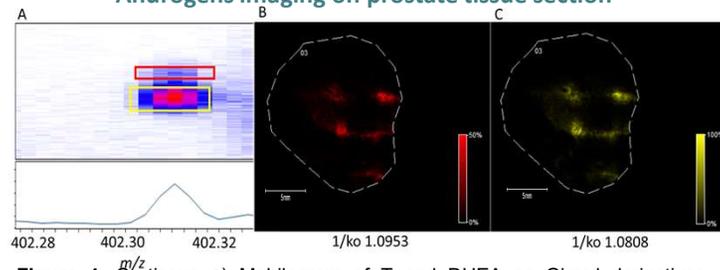


Figure 4. On-tissue a) Mobilogram of T and DHEA as Girard derivatives. Spatial distribution of b) Testosterone and c) DHEA as Girard derivatives.

Off-tissue Vitamin D metabolite

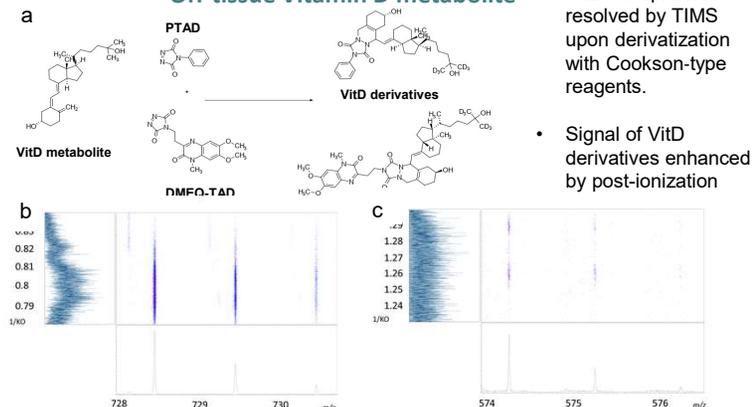


Figure 3. a) VitD 1,25 (OH)₂-D₃ derivatization reaction scheme using Cookson-type reagents. Off-tissue Mobilogram of equimolar 1,25 (OH)₂-D₃/C₃-epimer standard mix as b) DMEQ-TAD derivatives c) PTAD derivatives

CONCLUSIONS

- Increase in ionization efficiency and isobaric separation of Dansyl DHEA/T derivatives achieved using MALDI-2-TIMS
- MALDI-2-MS shown an increase in sensitivity for azodicarbonyls VitD derivatives and C₃-epimers successfully resolved using TIMS.
- First time spatial distribution of isobaric androgens achieved at tissue level by OTCD-MALDI-2-IM-MSI.

REFERENCES

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