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 inhouse 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.

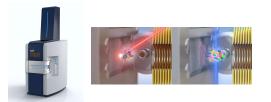


Figure 1. MALDI-2 timsTOF fleX instrument

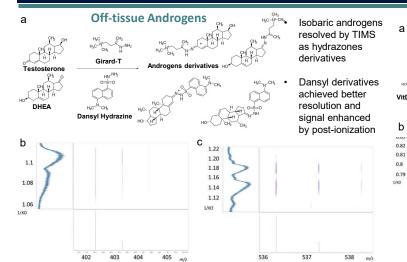


Figure 2. a) Androgens (T and DHEA) derivatization reaction scheme using Hydrazine-type reagents. Off-tissue Mobilogram of equimolar T/DHEA mix standard as b) Girard derivatives, c) Dansyl derivatives

Androgens imaging on prostate tissue section

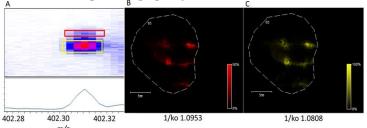
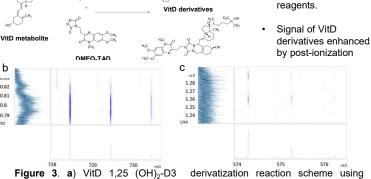


Figure 4. On-tissue a) Mobilogram of T and DHEA as Girard derivatives. Spatial distribution of **b**)Testosterone and c) DHEA as Girard derivatives.

Off-tissue Vitamin D metabolite • PTAD VitD metaboli

RESULTS



Cookson-type reagents. Off-tissue Mobilogram of equimolar 1,25 (OH)2-D3/C3epimer 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 azodicarbonvls 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.

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Westfälische

VitD C3-epimers

resolved by TIMS

upon derivatization

with Cookson-type







