ENHANCED ION MOBILITY SEPARATION OF DERIVATIZED ISOBARIC STEROIDS BY DESI AND MALDI TOF MASS SPECTROMETRY

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INTRODUCTION

Steroids play an important biological role, often functioning as signalling molecules. This important class of molecule is poorly ionised by both DESI and MALDI so derivatization is required to enhance the ionisation.

An additional analytical challenge is faced as several biologically important steroids have an inactive isomeric form, like dehydroepiandrosterone (DHEA), which is highly abundant but seemingly inert, versus testosterone which has a wide range of biological effects but is present at a lower level. Isomeric steroids can be separated by liquid chromatography or with limited efficiency by ion mobility separation (IMS).

In this work we present the use of several reactive agents to derivatise isobaric steroids, which show enhanced IMS separation for direct sample analysis techniques like DESI-MS.

METHODS

Derivatisation of the steroids

Standard steroids were selected because of their biological activity (active vs. inert) and/or being chemically isobaric. The steroid 17a-Estradiol is biologically inert, whereas its isobaric steroid 17b-Estradiol is active. Androsterone and trans-androsterone are not known to have any biological activity but are isobaric. Finally testosterone is highly biologically active, however epitestosterone and DHEA are known to be inactive (figure 1).
Three derivatization reagents were tested following a literature review: *p*-tolenesulfonyl (PTSI)[1], (+)-O,O'-Diacetyl-L-tartaric anhydride[2] (DATAN) (see figure 2) and mono-(dimethylaminoethyl) succinyl (MDMAES) ester[3].

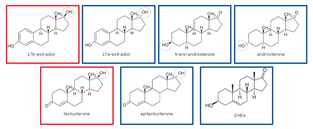

Figure 1. Chemical formulae of the seven steroids: 17a-estradiol, 17b-estradiol, androsterone, trans-androsterone, epitestosterone, testosterone (blue box: inert and red box: active biologically).

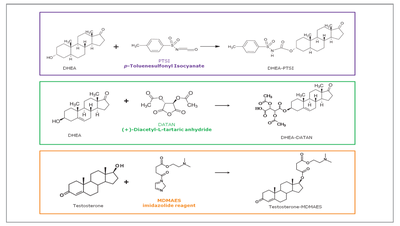

Figure 2. Derivatization reactions used in this study.

Tissue sections

Tissue sections analyzed were consecutive sections from a mouse liver. The tissue sample was snap frozen in liquid nitrogen and stored at -80°C, prior to cryo-sectioning at 10 µm thickness.

Mass spectrometry

All data was acquired using a DESI SYNAPT G2-*Si* ion mobility enabled mass spectrometer.
For DESI analysis, derivatized steroids were spotted individually and mixed onto Prosolia Teflon well glass slides and liver tissue sections.
When the 2D DESI stage (Prosolia, US) was mounted, the MALDI source was uncoupled and the electrospray inlet block was installed along with an inlet capillary. DESI spray conditions were set at 1.5 µL/min, 98:2 MeOH:water with nebulising gas pressure of 5 bar.

Instrument settings

Polarity:	Negative and Positive ion
Mass range:	100 -1,200 *m/z*
IMS pressure:	3.1 mbar
Wave Height:	40 V
Wave Velocity:	1000 to 300 m/s

Data management

Data acquired from the derivatized steroids spotted onto glass slides and MALDI targets were mined using MassLynx and DriftScope software packages.
Data acquired from the derivatized steroids spotted onto liver tissue sections were processed and visualized using High Definition Imaging 1.4(HDI) software for detailed image analysis.

RESULTS

Ion mobility separation of the steroids derivatized by PTSI in DESI negative mode.

17a-estradiol-PTSI (α-E_2-PTSI), 17b-estradiol-PTSI ((b-E_2-PTSI) , androsterone-PTSI (α-A-PTSI), trans-androsterone-PTSI (b-A-PTSI) were first spotted individually to assess their separation in the gas phase by IMS (drift time).

A second experiment was carried out where the isobaric PTSI steroids were mixed 1:1 and analysed by DESI.

Figure 3 displays the mobilograms of the individual PTSI steroids as well as the mixed samples.

The isobaric steroids 17a-estradiol-PTSI and 17b-estradiol-PTSI showed clear separation with a drift time difference of 0.38ms with a IMS cycle of 10.8ms. When the two isobaric compounds were mixed 1:1, there was a clear separation with a R_s=1.

Androsterone-PTSI and trans-androsterone-PTSI demonstrated a greater drift time difference of 0.55ms. When mixed 1:1, the separation was almost baseline with a R_s=1.2.

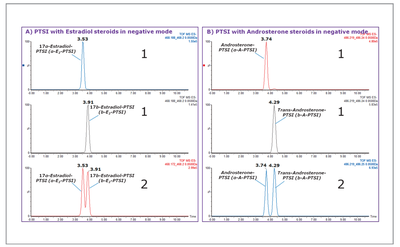

Figure 3. Mobilograms for the individual (1) and mixed (2) steroids A) 17a-estradiol-PTSI and 17b-estradiol-PTSI and B) Androsterone-PTSI and trans-androsterone-PTSI.

IMS comparison of epitestosterone, testosterone and DHEA using different derivatization agents (DESI +/-).

The remaining three isobaric steroids (epitestosterone (α-T), testosterone (b-T) and DHEA) proved to be more challenging. The three isobaric steroids were initially derivatized using the PTSI reagent. As seen in figure 4A, epitestosterone-PTSI is baseline separated by IMS from testosterone-PTSI, whereas DHEA-PTSI is partially separated by IMS from testosterone-PTSI.

Derivatization with (+)-O,O'-Diacetyl-L-tartaric anhydride (DATAN), was used and can be analyzed in negative mode (figure 4B) but also in positive mode (figure 4D). In negative mode, the IMS separation resolution was similar to the ones obtained with PTSI steroids. In positive ion mode, α-T and b-T were baseline resolved. DHEA-DATAN was not detected which could emphasize the detection of the low abundant biologically active testosterone when analyzed directly from tissue.

Steroids derivatized with MDMAES were analyzed in positive mode (figure 4C). However, this resulted in little separation of the isobaric steroids by IMS, especially for b-T-MDMAES and DHEA-MDMAES.

Table 1 summarizes the separation resolution (Rs) from all the experiments carried out with α-T, b-T and DHEA.

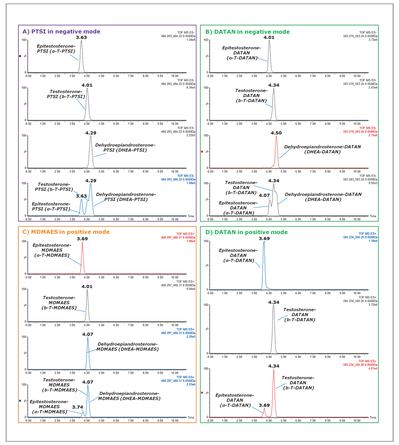

Figure 4. Mobilograms for the individual and mixed epitestosterone, testosterone and DHEA, using derivatization reagents A) PTSI (negative ionisation mode), B) DATAN (negative ionisation mode), C) MDMAES (positive ionisation mode) and D) DATAN (positive ionisation mode).

	α-T & b-T	b-T & DHEA	α-T & DHEA
PTSI negative mode	0.9	0.6	1.4
DATAN negative mode	0.9	0.4	1.3
DATAN positive mode	1.7		
MDMAES positive mode	1	0.2	1.1

Table 1. Summary of the obtained Resolution (Rs) for the IMS separation of different derivatized forms of epitestosterone, testosterone and DHEA

DESI imaging: PTSI-Standards spotted onto liver tissue sections

DESI imaging was performed by spotting the PTSI derivatized isobaric steroids standards on liver tissue sections, to evaluate if endogenous molecules from the tissue could effect the IMS of the isobaric species, and if other isobaric species would have similar drift times to the steroids.

In the imaging experiment a raster pattern was defined using HDI 1.4 over the region of interest around the tissue sections (figure 5A).

The scan speed and line spacing were selected for the target pixel dimensions. For 100 µm resolution images, the stage was scanned at 0.3 mm per second on the x-axis and stepped 0.10mm in the y-axis between each DESI line scan. The MS scan time was 0.33 second

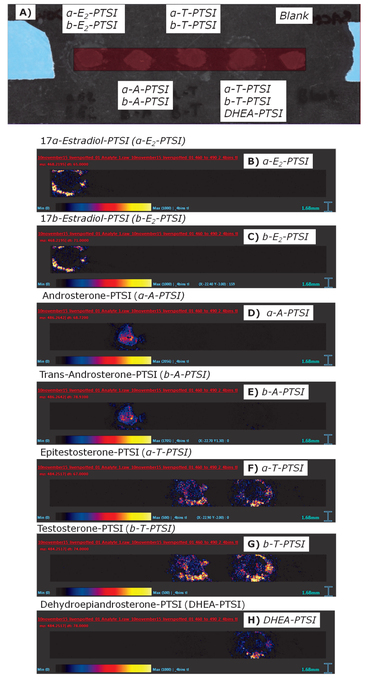

Figure 5. Ion images of the PTSI derivatized steroids spotted onto liver tissue sections. A) photographic image of the samples, B) ion image of α-E_2-PTSI, C) ion image of b-E_2-PTSI, D) ion image of α-A-PTSI, E) ion image of b-A-PTSI, F) ion image of α-T-PTSI, G) ion image of b-T-PTSI, H) ion image of DHEA-PTSI.

The drift time for each PTSI derivatized steroid obtained from the DESI imaging data corresponded closely to those drift times obtained from the experiments performed on standards.

Figure 5 displays the ion images obtained for each derivatized steroid. Even in the case of testosterone–PTSI and DHEA-PTSI, that have the closest drift time, the specificity during the DESI imaging experiment is sufficient to clearly observe only DHEA-PTSI on the tissue liver where it is spiked (figure 5H).

Figure 6 displays the processed DESI imaging of a Red-Green-Blue ion image overlay of m/z 219 (background species), 885.6 (PI(38:4)) and 187.1 with HDI 1.4.

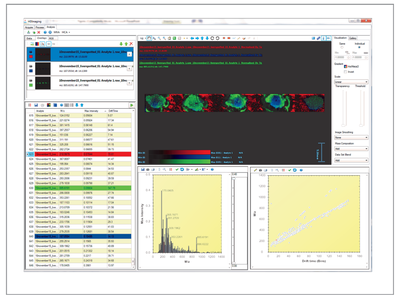

Figure 6. HDI 1.4 display showing a RGB overlay of m/z 219 (background species), 885.6 (PI(38:4)) and 187.1.

CONCLUSION

- **Ion mobility allows the separation of isobaric derivatized steroids.**
- **PTSI and DATAN derivatization of steroids gave good ionisation efficiency of the steroids in negative mode with effective IMS separation.**
- **DATAN derivatization, in positive ion mode, provided the biggest drift time difference between α-T and b-T. However DHEA was not detected.**
- **MDMAES derivatization, in positive ion mode, allowed the detection of α-T, b-T and DHEA. However poor IMS separation between b-T and DHEA was seen.**
- **DESI imaging of the PTSI derivatized steroids was consistent with the standard data when spotted onto the liver tissue sections.**

References

1. Kostiainen et al. Journal of Chromatography A, 1310 (2013) 133-137
2. Li et al. Talanta79 (2009) 1204-1208
3. Johnson et al. Journal of Lipid Research, Vol 42, 2001, 1699-1705.

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