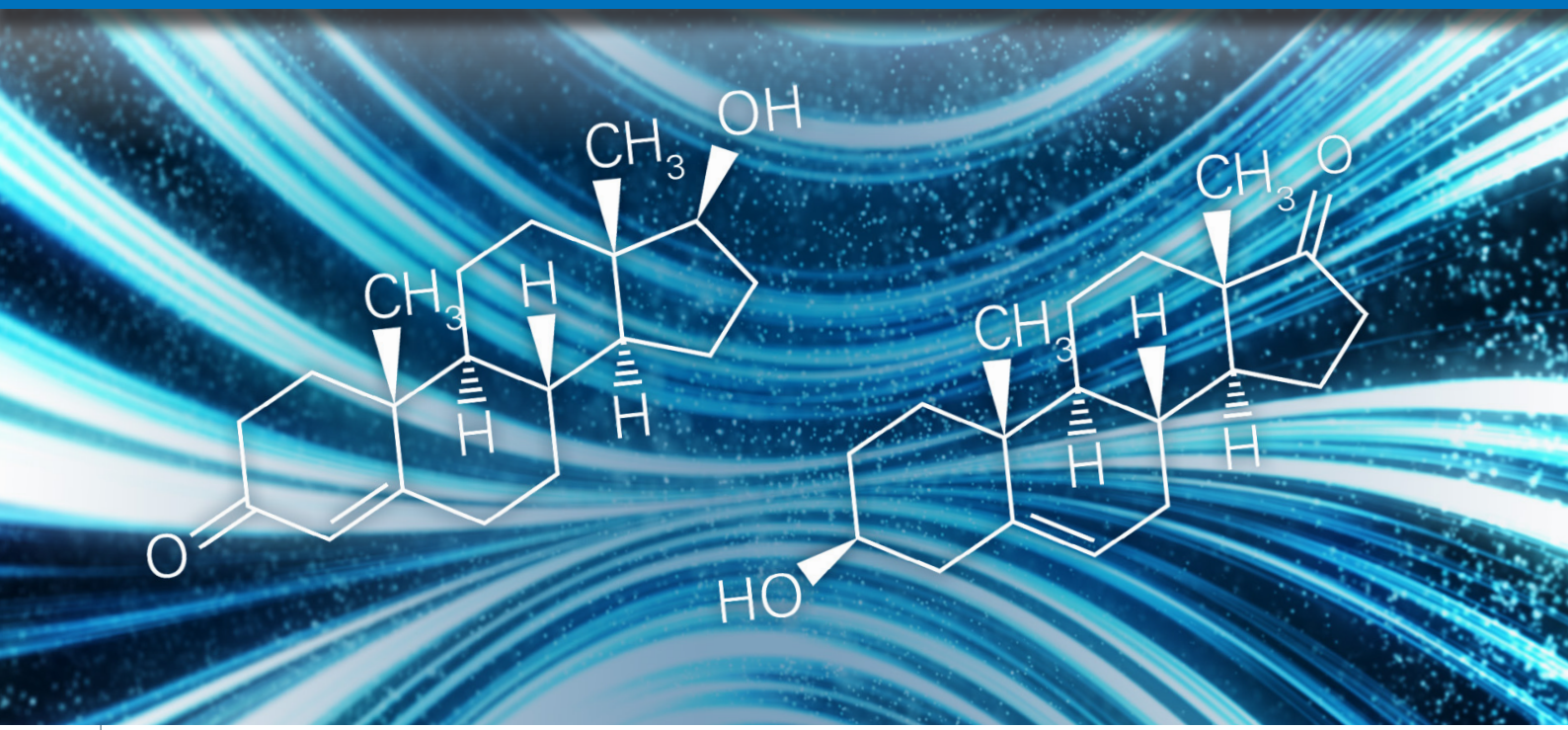

Separation of isobaric androgens in target tissues using chemical derivatization and MALDI-2 TIMS Imaging

Decreasing the levels of circulatory androgens is a common technique to block tumor cell growth for the treatment of prostate cancer [1].

Introduction

It is key to understand the tissue-specific steroids metabolic pathway, to be able to develop novel therapeutic treatments. Mass Spectrometry Imaging (MSI) is a powerful tool to visualize the distribution of such molecules involved in the metabolism in tissue. However, the detection of androgens by mass spectrometry is a challenge, due to their low abundance and poor ionization efficiency [2]. In the past, on-tissue chemical derivatization was found to be a valuable tool to successfully increase sensitivity for these compounds in MSI [3]. But still, isomeric/isobaric interferences of key androgens derivatives such as testosterone (T) and dehydroepiandrosterone (DHEA) is an issue preventing researchers to study the intracrinology in several diseases.

Keywords:
MALDI-2, TIMS Imaging, On Tissue Derivatization

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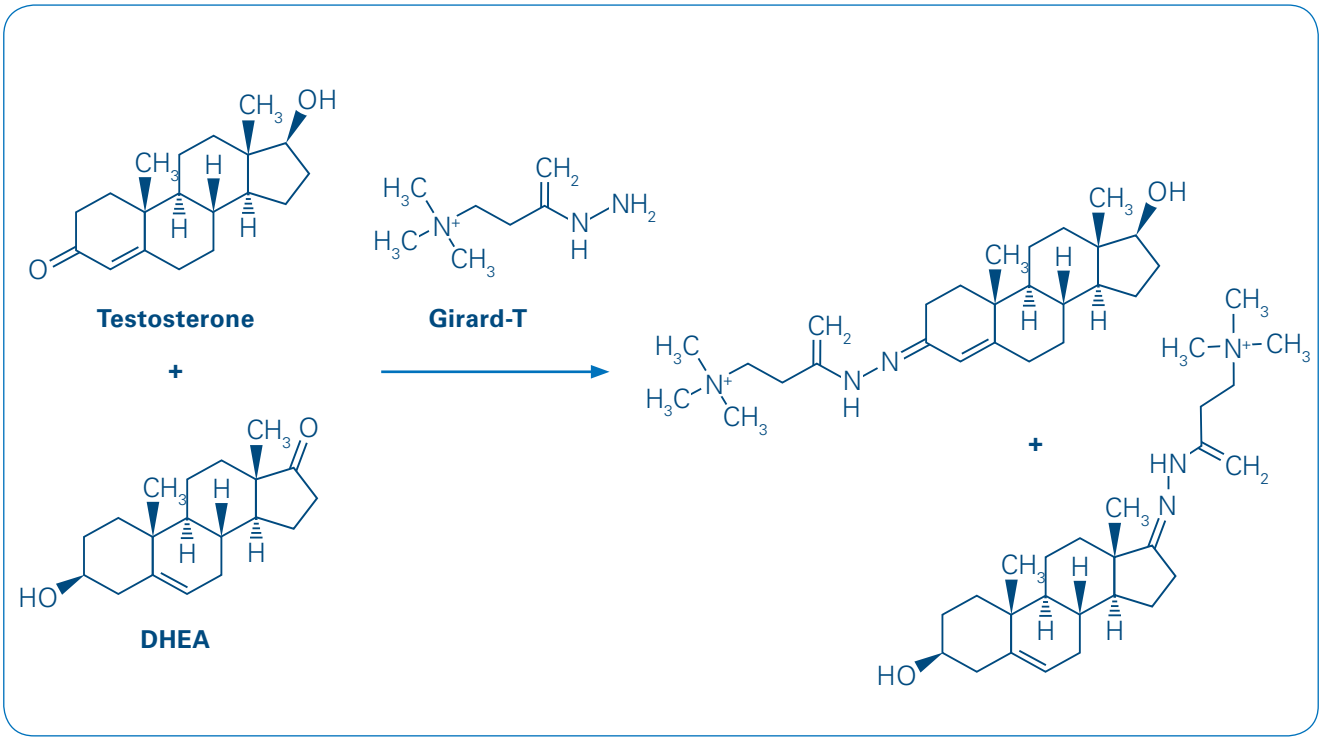

Figure 1: Androgen Girard-T derivatization reaction scheme

To improve the ion yields for androgens in a MALDI-MS imaging experiment, MALDI-2, a recently introduced laser post-ionization technique, can be employed. It has been shown that MALDI-2 allows for the sensitive analysis of lipids, steroids, liposoluble vitamins, glycans and many other different species [4]. Due to reduced ion suppression, up to 2-3 orders of magnitude higher ion signals were obtained upon use of the laser post-ionization module compared to a traditional MALDI experiment.

For the first time, we present here a proof-of-concept study showing data on the use of laser post-ionization (MALDI-2) with trapped ion mobility separation (TIMS) in the detection and distribution of isobaric androgens derivatives in murine tumor xenograph and mouse prostate control tissue.

Experimental

Full experimental details including all mass spectra and detailed supporting information have been described recently [5]. Only the most relevant experimental aspects are summarized here.

Off-tissue derivatization reaction screening using standard substances

Derivatization agent Girard-T (0.1 mg/mL in 9:1 MeOH:H_2O + 0.01% TFA) was mixed with standard solutions of androgens, testosterone and DHEA (10 ng/mL in MeOH), at a ratio of 2:1 (derivatization agent:androgen) independently. The reaction was left for 1 h at 40°C. Afterwards the reaction solution was mixed with HCCA matrix (5 mg/mL 6:4 ACN:H_2O + 0.1% FA) at a ratio of 1:1 and spotted on a MALDI target plate using the dried droplet method. An equimolar mixture of both androgens was prepared using the same protocol.

Tissue preparation

LNCaP human prostate cancer cells were implanted subcutaneously onto the flank of male nude CD-1 mice. When the tumor volume reached approximately 100-150 mm^3, mice were subjected to DHEA treatment (0.5 μg/kg). After tumor excisement, cryosectioning was performed to slices of 12 μm tissue thickness. Sections were mounted on conductive glass slides and vacuum dried. Application of Girard-T reagent (1 mg/ slide) was realized using an ImagePrep device (total reaction time 1 h) followed by HCCA matrix application using a modified 3D printer.

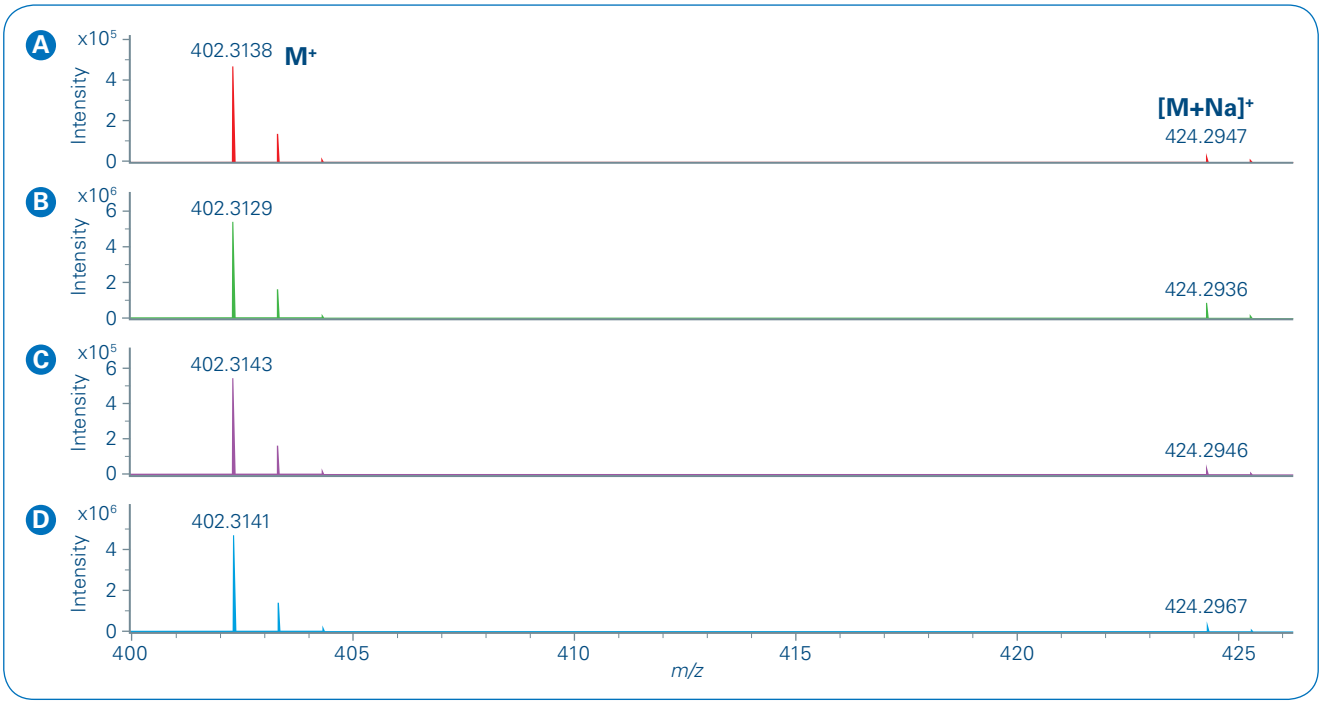

Figure 2: Mass spectra of Girard-T (GT) derivatives from standard substances of (A) Testosterone with MALDI-2 disabled (B) Testosterone with MALDI-2 enabled (C) DHEA with MALDI-2 disabled (D) DHEA with MALDI-2 enabled.

MALDI-2 TIMS Imaging

Data was acquired on a timsTOF fleX MALDI-2 instrument in the mass range of *m/z* 300 to 800. A "single" laser spot with active beam scan was used to ablate a resulting pixel size of 50 µm. The instrument was operated in positive ion mode, and ions were accumulated from 769 laser shots per pixel. For TIMS analysis, a ramp time of 769 ms was chosen including a range for $1/K_0$ from 1.1 to 1.75 Vs/cm^2. For imaging, a raster width of 50 µm was used, respectively. Laser power was optimized for each experiment to give the best resulting data. Experiments were also conducted in MALDI-2 mode using a trigger delay of 10 µs and a laser frequency of 1 kHz. Data analysis was performed with DataAnalysis 5.3, TIMS data viewer 1.0 and SCiLS™ Lab 2022a.

Results

Two different derivatization agents, namely Girard-T (GT) and Dansyl Hydrazone (DS), were tested for the detection of testosterone and DHEA. In this application note only results from GT derivatization are shown for molecular mapping of these androgens, because this achieved the best sensitivity and well-resolved mobilograms. In Figure 1 the reaction scheme of derivatization agent and androgen is shown.

Off-tissue derivatization reaction screening using standard substances

During the derivatization reaction a charged molecule is formed, so that the M^+ ion is detected. As shown in Figure 2, endogenous T and DHEA were detected as GT derivatives at *m/z* 402.31 (M^+ species) and *m/z* 424.29, corresponding to the $[M+Na]^+$ species.

Signal enhancement upon laser-induced post-ionization was evaluated using standard substances as described in the method section. It is clearly shown, that using standard substances at high concentrations, GT-derivatives exhibit just a low signal enhancement upon MALDI-2. Since GT-derivatives are already charged species, they apparently do not benefit from the MALDI-2 process. Detection of derivatized androgens from tissue showed a different effect. There the MALDI-2 effect was stronger, most likely because of ion suppression, that is happening during traditional MALDI. On the contrary, signal intensity of DS-derivatives was enhanced upon MALDI-2 by about

20 times for DS-DHEA and 40 times for DS-T derivatives (data not shown).

However, the challenge is still to be able to distinguish between the two isobaric androgens. Using trapped ion mobility, it was possible to separate the androgen derivatives by their different collisional cross section.

In Figure 3 the respective mobilograms of derivatized Testosterone (a) and DHEA (b) at its sodiated cluster are shown. At m/z 424.29 testosterone is detected at $1/K_0$ of 1.259, whereas DHEA shows the mobility signal at $1/K_0$ 1.236. The mobilogram of an equimolar solution of both androgens exhibits a clear separation in the mobility dimension.

On-tissue derivatization reaction screening

The observed signal enhancement upon laser-induced post-ionization was evaluated on tumor xenograph tissue (treated and control), in which the levels of endogenous tumor T and DHEA are high (around 20 ng/g as determined by LC/MS, data not shown). Tissue sections were derivatized using GT reagent and covered with matrix as described in the methods section.

Isobaric T and DHEA were successfully detected and resolved.

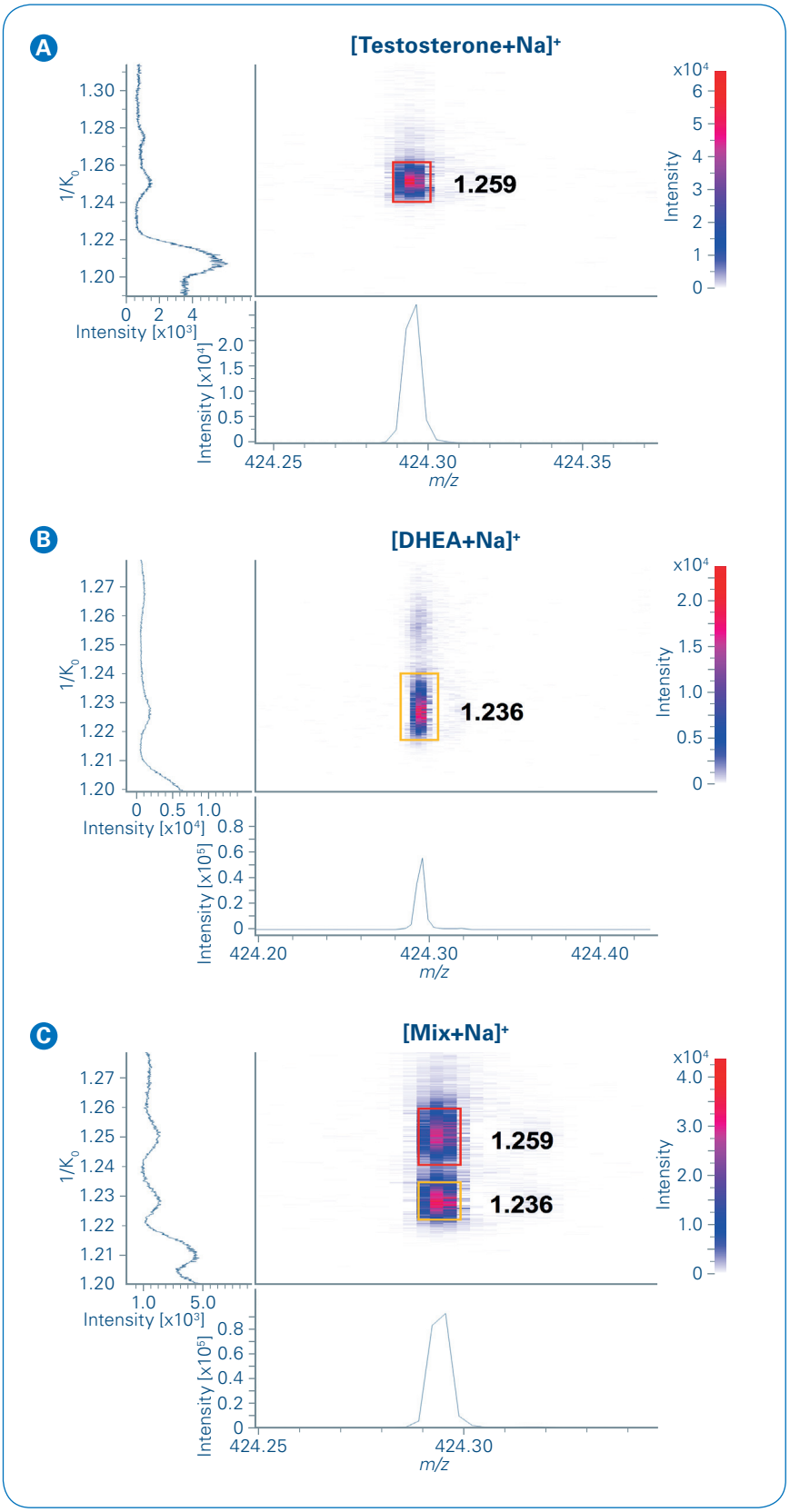

Figure 3: Off-tissue mobility assessment of Girard-T (GT) derivatized androgen standards by MALDI-2-TIMS. Mobilograms of derivatized (A) Testosterone at its sodiated cluster at m/z 424.2947 showing one mobility signal at $1/K_0$ 1.259 (B) DHEA at its sodiated cluster at m/z 424.2946 showing one mobility signal at $1/K_0$ 1.236 and (C) equimolar solution of T and DHEA at their isobaric sodiated cluster at m/z 424.2950 showing respective mobility signals at $1/K_0$ 1.259 (T) and $1/K_0$ 1.236 (DHEA).

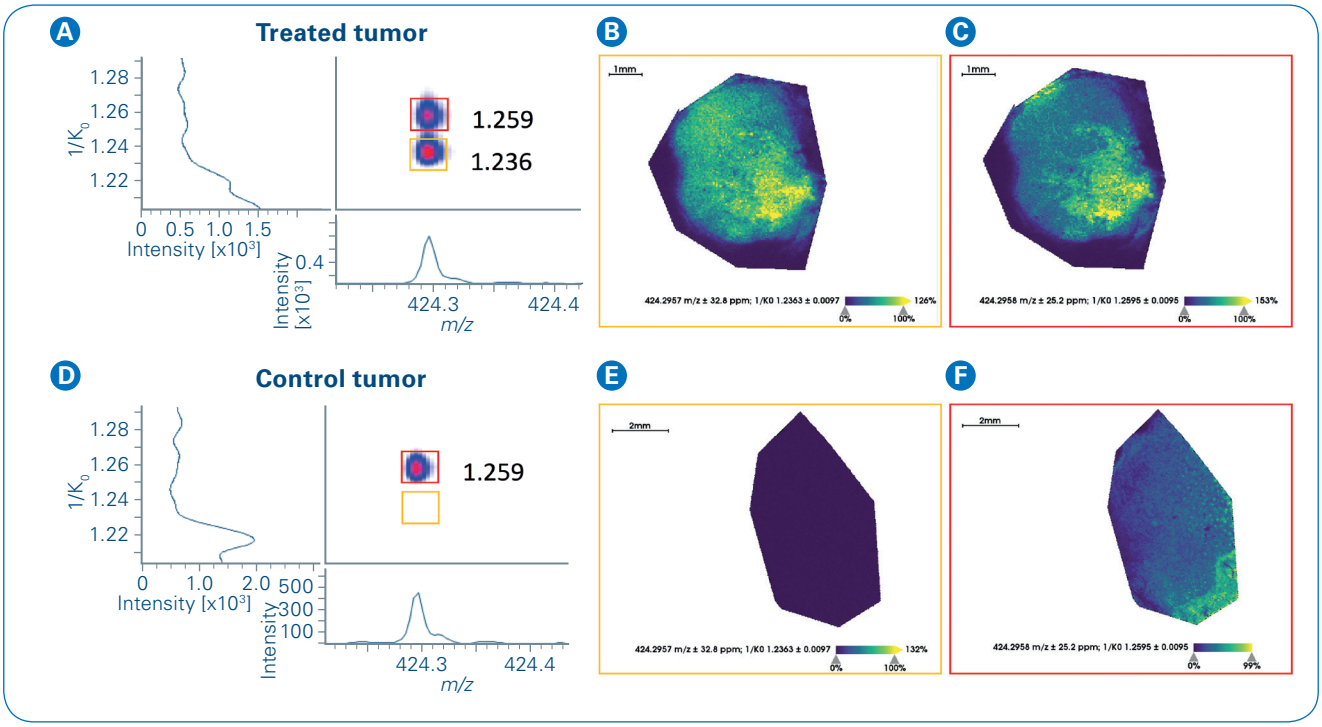

Figure 4: Molecular distribution of endogenous isobaric androgens detected as GT derivatives in a LNCaP tumor tissue from a xenograft mouse and mouse prostate control tissue. (A) + (D) Average mobilograms showing isobaric androgens as GT-derivatives at their sodiated cluster m/z 424.2940 at $1/K_0$ 1.259 for Testosterone and $1/K_0$ 1.236 for DHEA. Ion images from tissue of (B) + (E) DHEA-GT derivative and (C) + (F) Testosterone-GT derivative.

Figure 4 illustrates the average mobilograms of the separated isobaric androgens as GT-derivatives for the treated tumor section (Figure 4a) and the control tumor section (Figure 4d). It is obvious that in the treated tumor both androgens, testosterone, and DHEA are detectable whereas in the control tumor only testosterone is visible. This makes sense in a biological context as rodents do not produce this steroid. The information from the average mobilograms were extracted and molecular distributions in tissue for the different androgens are shown here. For the treated tumor tissue section, ion images for derivatized testosterone (Figure 4c) and derivatized DHEA (Figure 4b) reveal the presence of both androgens. Looking at the ion images for the control tumor section (Figure 4e and Figure 4f), only a distribution for testosterone can be observed.

Conclusion

- In this proof of principle study, it has been shown that imaging of derivatized isobaric androgens within tissues is possible using OTCD-MALDI-2-TIMS technology. To our knowledge, this is the first method capable of spatially resolved T and DHEA, two biological relevant androgens, at tissue level. On-tissue chemical derivatization in combination with laser post-ionization is a powerful tool, which enables detecting endogenous concentrations of the androgens within murine xenograft tissue, a key tissue model for prostate cancer intracrinology. The sensitivity of these compounds in tissue is increased using MALDI-2. Additionally, trapped ion mobility separation efficiently unravelled the complex isobaric features.
- The possibility to differentiate between isobaric androgens, like testosterone and DHEA, offers the opportunity to study tissue intracrinology within target tissues to gain novel insights into tissue-specific androgens biology. Understanding the distribution of these androgens in the prostate tumor is strategic at aiming to establish how potential effects of a tumor environment may have on the intracrine pathway of steroidogenesis. It is also key to design therapeutic treatment that may cause desirable effects to reduce the formation of cancer cells. Importantly, the use of this platform facilitates the analysis of previously inaccessible biologically relevant isobaric steroids through adaptation of existing chemical derivatization methods.

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References

[1] Chang KH, et al. (2011). Proceedings of The National Academy of Sciences, **108**, 13728-13733.

[2] Higashi T, et al. (2005). Journal of Chromatography B, **825**, 214-222.

[3] Cobice DF, et al. (2018). Biochemical Pharmacology, **148**, 88-99.

[4] Soltwisch J, et al. (2020). Analytical Chemistry, **92**, 13, 8697–8703.

[5] Cobice DF, et al. RSC Advances, in press.

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