ENHANCED MOLECULAR COVERAGE, RESOLUTION AND SPEED FOR IN-SITU PHARMACEUTICAL TABLET MSI ANALYSIS BY COMBINING DESI AND MALDI USING MULTI-REFLECTING Q-TOF

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INTRODUCTION

Characterization of pharmaceutical tablets is important in the drug development process to ensure their quality and effectiveness. The traditional tablet dissolution method requires the use of LC-MS. However, this approach doesn't indicate the localization of different components of the tablet formulation, such as the active pharmaceutical ingredient (API) or excipients. Therefore, a method to directly analyze the tablet in-situ is required. Mass spectrometry imaging (MSI) allows the analysis of samples without the need for dissolution.

Matrix-assisted laser desorption/ionization (MALDI) and desorption electrospray ionization (DESI) have proven to be powerful ionization techniques used for MSI applications. Their complementarity has been published in the recent years, especially for lipids analyzed from moue brain tissue samples[1].

In this study, DESI and MALDI ion sources mounted on an ultra-high MS resolution Q-Tof spectrometer (up to 200,000 FWHM with a scan speed of up to 10 Hz) were used to evaluate the molecular coverage of the individual ionization techniques for the analysis of homemade and commercially available tablets.

METHODS

Sample information

Experiments were performed analyzing homemade and commercially available tablets called "Lysopaine (20 mm diameter) without sugar 36 lozenges to suck".

The in-house tablets were generated by heterogeneously depositing aspartame powder and topping with 200mg of lactose on a tablet press coated with magnesium stearate. All was compressed to a standard force of 1000 kg. The tablets were easily removed from the mold with care thanks to the lubrication property of magnesium stearate. Tablets were relatively flat and had a thickness of between 2 and 3 mm.

Mass spectrometry

MSI experiments were carried out on a MALDI/DESI™ XS SELECT SERIES™ MRT (a quadrupole Multi-Reflecting Time-of Flight) mass spectrometer (figure 1) with a mass resolution >200,000 FWHM across the lipid mass range and a mass accuracy <500 ppb (figure 1).

For MALDI experiments, samples were prepared using a HTX M5 (HTX Technologies) nebulizing spray device. A 10 mg/mL solution in acetonitrile/water/TFA (70/30/0.1 *v/v/v*) of α-Cyano-4-hydroxycinnamic acid (CHCA) MALDI matrix was evenly applied on the tablets (eight passes).

DESI imaging experiments require no sample preparation as desorption and ionization are initiated by charged droplets impacting directly on the surface. The DESI solvent composition was 95% MeOH, 5% water with 50 pg/µL Leu-enkephalin, with a flow rate of 2 µL/min using a µBSM nanoflow pump and a N_2 nebulizing gas pressure of 10 psi. The DESI High-Performance sprayer (HPS) was used for improved sensitivity, DESI spray focus, robustness and ease-of-use.

All MSI experiments were performed at 10 Hz with a pixel size of 50 µm.

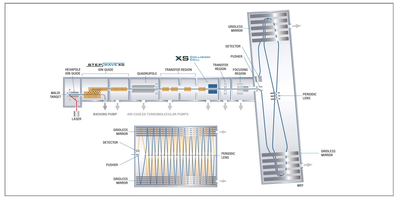

Figure 1. Schematic of SELECT SERIES MRT showing multi reflecting ToF analyzer.

Data management

MALDI and DESI imaging datasets were mined using MassLynx™ as well as processed and visualized using High Definition Imaging Software (HDI™) v1.7 (Waters).

Datasets were also subjected to a Uniform Manifold Approximation and Projection (UMAP) algorithm using in-house software to automatically explore and perform unsupervised segmentation of pixels into clusters of similar spectral characteristics.

RESULTS

1) Analyses of the homemade tablet by MALDI and DESI MSI

Initial analysis was performed using the 2 mm thick tablet by MALDI in positive mode where sodiated/potassiated lactose and protonated/ sodiated aspartame were detected (figure 2). The heterogenous distribution of the aspartame on the tablet press gave a distribution in packets on the surface of the tablet, whereas lactose was used to hold the tablet together.

In DESI positive mode, the spectra were more rich, with many more peaks detected. In figure 3A), it can be seen that protonated/sodiated/ potassiated monomer and dimer were detected. Furthermore , a series of doubly charged ions with a mass difference corresponding to lactose as mass unit of 342 Da were detected. Further work is underway to confirm identification.

Singly charged protonated monomer, dimer and trimer of aspartame ions were detected in the part of the tablet with the highest peak intensity.

In DESI negative mode, singly charged monomer and dimer of lactose and aspartame were detected. Interestingly, a polymeric series of peaks that were doubly and triply charged were detected with a mass difference of m/z 171 (doubly charged) and m/z 114 (triply charged) which is consistent with lactose.

The distribution of the two main ingredients (lactose and aspartame) were similar between MALDI positive, DESI positive and DESI negative mode.

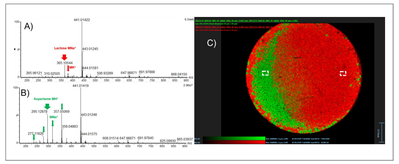

*Figure 2. **MALDI positive mode** results of the homemade tablet A) MS spectrum where lactose is abundant, B) MS spectrum where aspartame is abundant, C) overlayed ion images of sodiated lactose (red) and protonated aspartame (green).*

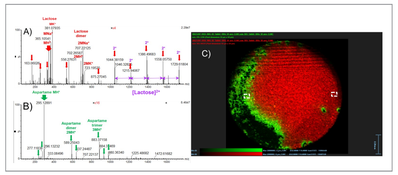

*Figure 3. **DESI positive mode** results of the homemade tablet A) MS spectrum where lactose is abundant, B) MS spectrum where aspartame is abundant, C) overlayed ion images of sodiated lactose (red) and protonated aspartame (green).*

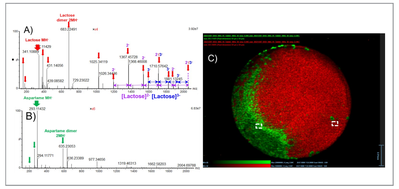

*Figure 4. **DESI negative mode** results of the homemade tablet A) MS spectrum where lactose is abundant, B) MS spectrum where aspartame is abundant, C) overlayed ion images of MH^- lactose (red) and MH^- aspartame (green).*

To obtain high mass accuracy, internal lockmass was used to correct the m/z. For MALDI in positive mode, a CHCA matrix peak was used: $[2CHCA+H]^+$ *m/z* 379.09246. For DESI, leucine enkephalin was spiked into the DESI solvent. In positive mode, *m/z* 556.27658 and negative mode *m/z* 554.26202 were used.

Mass accuracies were calculated for the different detected ion types in the three experiments: MALDI (+), DESI (+) and DESI (-) and are reported in table 1.

Mode of acquisition	Compounds	Ion type	Calculated ion mass (m/z)	Observed ion mass (m/z)	Mass delta (ppm)
MALDI Positive mode	Aspartame	MH^+	295.12885	295.12878	**-0.24**
	Aspartame	MNa^+	317.11079	317.11075	**-0.13**
	Lactose	MNa^+	365.10543	365.10544	**+0.03**
DESI Positive mode	Aspartame	MH^+	295.12885	295.12891	**+0.20**
	Aspartame	$2MH^+$	589.25042	589.25043	**+0.02**
	Aspartame	$3MH^+$	883.37199	883.37158	**-0.46**
	Lactose	MNa^+	365.10543	365.10541	**-0.5**
	Lactose	MK^+	381.07937	381.07935	**-0.05**
	Lactose	$2MNa^+$	707.22164	707.22125	**-0.55**
DESI Negative mode	Aspartame	MH^-	293.11430	293.11432	**+0.07**
	Lactose	MH^-	341.10894	341.10889	**-0.15**
	Lactose	$2MH^-$	683.22515	683.22491	**-0.35**

Table 1. Putative identification with sub 0.5 ppm mass accuracy of different ion types of lactose and aspartame from the MALDI positive mode, DESI positive mode and DESI negative mode experiments

2) Analyses of the commercially available Lysopain tablet by MALDI and DESI MSI

Experiments were performed analyzing commercially available tablets called Lysopain (20 mm diameter). This tablet was chosen because of the flatness of the tablet and its compatibility with MSI analysis. The active ingredients are a mixture of small molecules, an API named cetylpyridinium chloride ($C_{21}H_{38}ClN$) and a protein-based molecule lysozyme hydrochloride.

In MALDI positive ion mode, the most intense signal was *m/z* 304.29990 which corresponds to cetylpyridinium choloride $[M-HCl+H]^+$ and displayed a patch distribution in small clusters across the tablet (figure 5). Additional signals, unique to MALDI, that were ubiquitously distributed across the tablet were detected and remain to be identified.

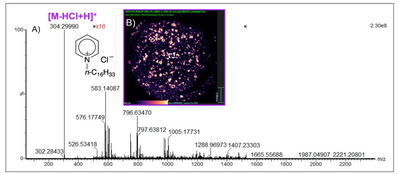

*Figure 5. **MALDI positive mode** A) MS spectrum of Lysopain tablet demonstrating the presence of cetylpyridinium chloride multimers, B) ion image of cetylpyridinium chloride [M-HCl+H]+ m/z 304.29990.*

In DESI positive ion mode, similarly to MALDI, the strongest signal detected from the tablet surface was *m/z* 304.30002 (cetylpyridinim chloride $[M-HCl+H]^+$). The distribution of the small molecule API was also patchy with clusters of the API smaller than 600 x 600 µm on the surface of the tablet. Further signals with a similar distribution as $[M-HCl+H]^+$ were identified in the ppb mass accuracy to be multimers of the small API such as $[M_2-HCl+H]^+$, $[M_3-HCl+H]^+$, $[M_4-HCl+H]^+$.

Furthermore, multiply charged signals were detected with high charge states (+8 to +12) and were putatively identified as the domain C-type lysozyme protein. The distribution of the high charge states was also blotchy with clusters smaller than 150 x150 µm, localized differently to the small molecule API.

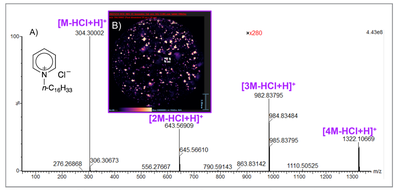

*Figure 6. **DESI positive mode** A) MS spectrum of Lysopain tablet demonstrating the presence of cetylpyridinium chloride multimers, B) ion image of cetylpyridinium chloride [M-HCl+H]+ m/z 304.30002.*

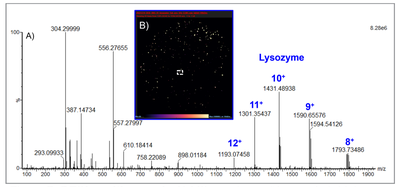

*Figure 7. **DESI positive mode** A) MS spectrum of Lysopain tablet demonstrating the presence of lysozyme protein, B) composite ion image of 9+, 10+ and 11+ lysozyme protein envelop.*

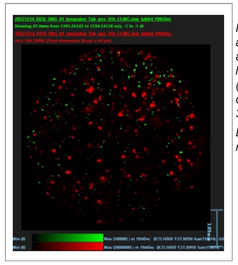

Figure 8. Overlay ion image of composite ion image of 9+, 10+ and 11+ lysozyme protein envelop (red) and cetylpyridinium chloride [M-HCl+H]+ m/z 304.3 (green).

DESI positive mode experiment of Lysopain tablet.

Mass accuracies were calculated for the different ion types that were detected in the three experiments: MALDI (+), DESI (+) and are reported in table 2.

Mode of acquisition	Compounds	Ion type	Calculated ion mass (m/z)	Observed ion mass (m/z)	Mass delta (ppm)
MALDI Positive mode	Cetylpyridinium Chloride	$[M-HCl+H]^+$	304.29988	304.29990	**+0.07**
DESI Positive mode	Cetylpyridinium Chloride	$[M-HCl+H]^+$	304.29988	304.30002	**+0.46**
	Cetylpyridinium Chloride	$[2M-HCl+H]^+$	643.56915	643.56909	**-0.09**
	Cetylpyridinium Chloride	$[3M-HCl+H]^+$	982.83843	982.83795	**-0.49**

Table 2. Putative identification with sub 0.5 ppm mass accuracy of different ion types of cetylpyridinium chloride from the MALDI positive mode, DESI positive mode experiments.

The DESI positive mode dataset was subjected to an automated and unsupervised segmentation in-house microapp software called MSI Segmentation. In this case Uniform Manifold Approximation and Projection (UMAP) algorithm was used to automatically explore and perform unsupervised segmentation of pixels into clusters of similar spectral characteristics . Within the parameters used six clusters (+ the cluster for the pixels outside of the tablets) were identified and mapped on the image (figure 9A). Furthermore the pixel clustering results were visualized in the projected UMAP space to assess clustering quality (figure 9B) .

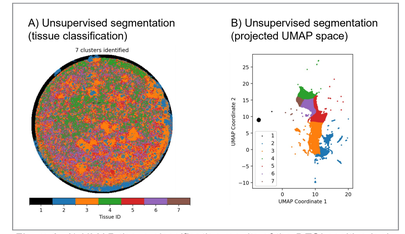

Figure 9. A) UMAP tissue classification results of the DESI positive ionization mode Lysopain tablet analysis. B) Projected UMAP space

CONCLUSION

- **Homemade and commercially available tablets were successfully imaged by MALDI and DESI MRT at 10 Hz acquisition speed.**
- **The main ingredients of the tablets were detected by both ionization techniques apart from the protein based ingredient which was only detected by DESI in positive mode as a multiply charged species.**
- **DESI MS spectra were more rich with the presence of adduct and multimer ion species.**
- **Distributions of the main ingredients between the different ionization techniques were similar.**
- **In-house tissue segmentation software efficiently clustered pixels to simplify the complex and large amount of data.**

References

1: K. Škrášková, E. Claude, E.A. Jones, M. Towers, S.R. Ellis, R.M.A. Heeren; Methods. 2016 Jul; 104:69-78

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