IMPROVED SELECTIVITY AND ACQUISITION SPEED USING DESI QUADRUPOLE BASED MASS SPECTROMETER

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INTRODUCTION

Mass spectrometry imaging (MSI) is a well-established technique for the mapping of molecules directly from tissue sections. It has been applied in pharmacokinetic and toxicological studies for drug discovery and development in the pharmaceutical industry.
Desorption electrospray ionization (DESI™) is a technique that is gaining momentum, mainly due to its ease of use with minimal sample preparation, its ambient nature, and the possibility to re-use the tissue sample multiple times. When analyzing tissue sections directly, the biological complexity means that high specificity is required in the method of analysis. This can be provided by Single Ion Recording (SIR) or Multiple Reaction Monitoring (MRM) modes of acquisition, which are used as standard practice with LC-MS (ESI) experiments in the pharmaceutical industry. Recently, work showing the advantages of combining DESI and MRM experiments have been reported[1]. The main advantages are the high specificity, speed of acquisition, simplicity and cost of the equipment.
Here we present the results of a study where four cassette drug dosed brain, kidney and liver tissues were analysed using the DESI XS source mounted on a Xevo™ TQ-XS tandem quadrupole mass spectrometer using the High-Performance DESI sprayer. DESI imaging data of the four drugs and their main metabolites are presented.

METHODS

Sample preparation

A first series of experiments was performed using a mixed solution of Propanolol, Olanzapine, Erlotinib, Moxifloxacin, Terfenadine and Irinotecan that were dissolved and ten-fold diluted in MeOH/H_2O 1:1 v/v solution.
1 µL of each solution were spotted on 16 µm porcine liver tissue sections covering the range 1 pg to 10,000 pg.

A second series of experiment were performed using mouse animals that were orally dosed with four drug compounds: Olanzapine (10 mg/kg), Erlotinib (10 mg/kg), Moxifloxacin (25 mg/kg) and Terfenadine (25 mg/kg). Brain, kidney and liver tissue sections were obtained from a control, 2 and 6 hours post dosed animals.

DESI XS Conditions

Solvent delivery:	2 µL/min 98:2 MeOH: H_2O with 0.1% formic acid and 200 pg/µL of leucine-enkephalin
Voltage:	0.8 kV
Nebulizing gas:	Nitrogen at 10 psi (0.7 bar)
Pixel size:	50 µm
Sprayer	New High-Performance Sprayer

MS Conditions

MS Instrument:	**Xevo TQ-XS**
Ionization mode:	DESI positive ion mode
MS experiment	MRM experiment
Cone Voltage	20 V
Source temperature:	100°C
Speed of acquisition:	10 pixels per second

The implementation of the DESI XS on Xevo TQ-XS mass spectrometer has been developed using High Definition Imaging (HDI™) 1.6 software via a plugin, where the captured optical image is co-registered, MRM method is defined (MRM transitions, polarity, pixel size, MS scan rate) and area to be imaged is drawn (see figure 1).
The acquisition can either be started directly from the plugin or from the sample list in MassLynx™ SCN 1021. After acquisition, the data can be automatically processed and viewed in HDI.

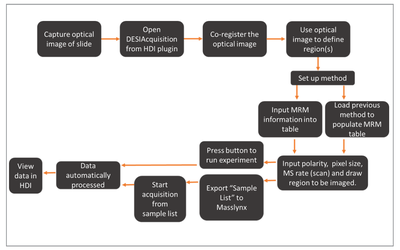

Figure 1. Workflow of DESI imaging using Xevo TQ-XS: from capturing an optical image, to the co-registration, set-up of the method and starting the experiment from the same software, automated processing and visualisation in HDI.

	Name	Precursor ion (m/z)		Product ion (m/z)	Dwell time (ms)	Collision energy (V)
1	Propranolol_01	260.15	>	73.9	6	15
2	Propranolol_02	260.2	>	116	6	15
3	Propranolol_03	260.25	>	71.9	6	15
4	Olanzapine_01	313.15	>	256.2	6	23
5	Olanzapine_02	313.2	>	213	6	30
6	Olanzapine_03	313.25	>	282	6	23
7	Erlotinib_01	394.15	>	278.2	6	32
8	Erlotinib_02	394.2	>	336.2	6	22
9	Erlotinib_03	394.25	>	304	6	22
10	Moxifloxacin_01	402.15	>	384.2	6	20
11	Moxifloxacin_02	402.2	>	358.2	6	20
12	Moxifloxacin_03	402.25	>	261	6	20
13	Terfenadine_01	472.25	>	436.3	6	26
14	Terfenadine_02	472.3	>	454.2	6	20
15	Terfenadine_03	472.35	>	128.9	6	20
16	leucine-enkephalin	556.5	>	397.2	6	22
17	Irinotecan_01	587.25	>	195	6	30
18	Irinotecan_02	587.3	>	124	6	38
19	Irinotecan_03	587.35	>	167	6	30
20	Lipid	798.5	>	163	6	35

Table 1. MRM transitions for the Moxifloxacin, Irinotecan, Propranolol, Olanzapine, Erlotinib and Terfenadine ten-fold dilution series DESI imaging experiment using Xevo TQ-XS.

	Name	Precursor ion (m/z)		Product ion (m/z)	Dwell time (ms)	Collision energy (V)
1	Desmethylolanzapine	299.2	>	256.2	6	40
2	Olanzapine	313.15	>	256.2	6	23
3	Hydroxyolanzapine	329.2	>	272	6	23
4	Didesmethylerlotinib	366.2	>	278.2	6	32
5	Desmethylerlotinib	380.2	>	278.2	6	30
6	Erlotinib	394.15	>	278.2	6	32
7	Moxifloxacin	402.2	>	358.2	6	20
8	Terfenadine	472.25	>	436.3	6	26
9	Carboxyterfenadine	502.3	>	466.25	6	30
10	Lipid	798.5	>	163	6	35

Table 2. MRM transitions for the four drug dosed tissue with metabolites DESI imaging experiment using Xevo TQ-XS.

RESULTS

In the first series of experiment, all six pharmaceutical compounds were detected when spotted on liver tissue by DESI TQ-XS .

For all tested compounds the level of detection was between 10 pg and 100 pg spotted onto liver tissue section as seen in figure 2.

Moreover, by drawing Regions of Interest (ROIs) on each spot on the ion images in HDI, intensities for each compound were exported and plotted in Excel to generate calibration curves, resulting in R^2 over 0.9997 for the six dilution series, demonstrating the excellent linearity of the method.

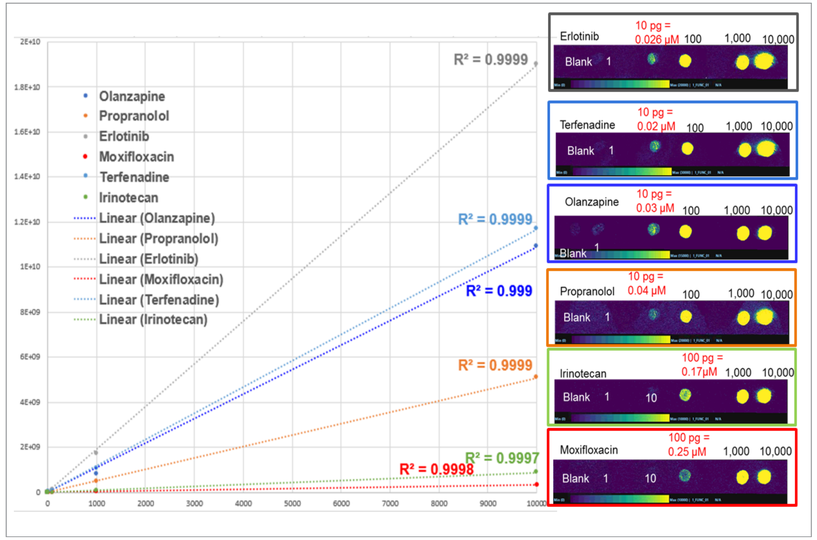

Figure 2. Calibration curve with DESI ion images of the ten-fold dilution series of Moxifloxacin, Irinotecan, Propranolol, Olanzapine, Erlotinib and Terfenadine on DESI XS Xevo TQ-XS mass spectrometer, in positive ionisation mode with the High-Performance DESI sprayer.

In the second series of experiment, four drugs and their metabolites were targeted in the DESI TQ-XS imaging analysis.

In figure 3 is shown the localisation of the four drugs that were detected in all dosed tissues and not in the control tissues as well as the metabolites hydroxyolanzapine, desmethylerlotinib and carboxyterfenadine.

Whereas desmethylolanzapine and didesmthylerlotinib were detected in the kidney and liver tissue sections.

CONCLUSION

- **A simplified workflow and software solution have been implemented for DESI imaging applications on a Xevo TQ-XS mass spectrometer.**
- **All pharmaceutical compounds were detected at 10-100 pg on tissue with excellent linearity.**
- **Four drugs and five metabolites have been successfully imaged by MRM imaging using DESI XS with a Xevo TQ-XS tandem mass spectrometer.**
- **MRM imaging has enhanced level of detection due to the specificity of the method**

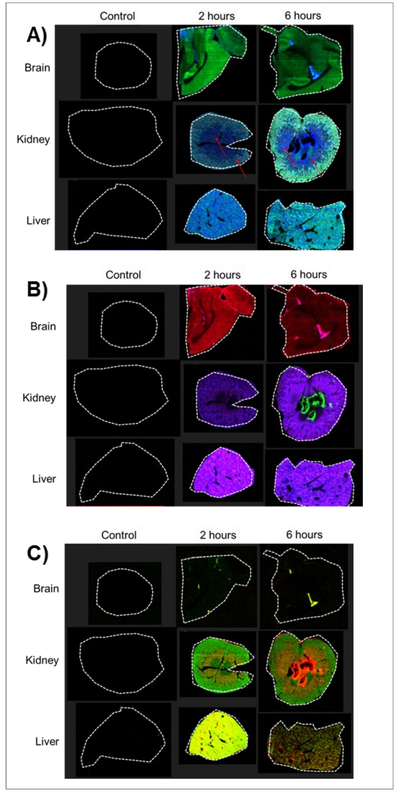

Figure 3. A) RGB overlay ion images of desmethylolanzapine (red, highlighted by red arrows). olanzapine (green), hydroxyolanzapine (blue). B) RGB overlay ion images of erlotinib (red), Didesmethylerlotinib (green), desmethylerlotinib (blue) and C) RBG overlay ion images of carboxyterfenadine (red), terfenadine (green).

References
1. L.Lamont, D.Hadavi, B.Viehmann, B.Flinders, R.M.A.Heeren, R.Vreeken, T.Porta Siegel; Quantitative mass spectrometry imaging of drugs and metabolites: a multiplatform comparison; Analytical and Bioanalytical Chemistry; 413, 2779-2791 (2021)

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