NOVEL TARGETED DESI MRM TQ SYSTEM PROVIDING INCREASED SENSITIVITY AND ACQUISITION SPEED FOR MASS SPECTROMETRY IMAGING

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INTRODUCTION

Mass spectrometry imaging (MSI) has been successfully used for spatial biomarker discovery, drug biodistribution and for clinical research applications. Most of the studies are conducted using a high resolution TOF mass spectrometers, equipped with MALDI and more recently DESI ionisation techniques. However, once the targeted molecules have been identified (drug or biomarker), there is an increasing need for methods that enhance specificity for the detection of low-level analytes considering tissue complexity and the decreasing amounts of analytes present. Tandem quadrupole (TQ) MS are renowned for their sensitivity and specificity in targeted applications using MRM modes of acquisition.

Here a DESI source with a TQ MS was used to determine limits of detection (LOD's) for several pharmaceutical compounds spotted on control tissue section and distributions of drug and metabolites in dosed tissue sections.

METHODS

Tissue sample preparation

Several dilution series of pharmaceutical compounds (chloroquine, propranolol, olanzapine, erlotinib, moxifloxacin, terfenadine and irinotecan) were spotted on a control porcine liver tissue sections in a 10 fold dilution series.

Gefitinib, a drug belonging to a class of tyrosine kinase inhibitors (TKIs), was administrated IV at 10 mg/kg via the tail vein of male C57Bl6 mice. Livers were collected at 0.5, 1, 3, 8 and 24 hours post dosed. Livers were stored at -80°C until sectioning at 18 µm using a cryostat.

Mass spectrometry

MSI experiments were carried out using a DESI XS source mounted on a TQ MS in positive ionization mode. The DESI XS source was used on both mass spectrometers with the High-Performance sprayer (HPS) for improved sensitivity, spray focus, robustness and ease-of-use.

All experiments were performed in MRM mode. DESI HPS conditions were set at 2 µL/min, 95:5 MeOH: water, with a N_2 nebulising gas pressure set at 10-15 psi and capillary voltage set between 0.55 to 0.8 kV forming a focused spray. The samples were imaged at different pixel sizes from 15 up to 75 µm and a range of acquisition speeds from 5 Hz up to 50 Hz.

Data management

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

Regions of Interest (ROIs) ,defined in HDI, and associated intensities were averaged in the form of a .csv file which was loaded directly into MetaboAnalyst[1] (https://www.metaboanalyst.ca/MetaboAnalyst/faces/home.xhtml) to generate the intensity box plot.

RESULTS

1) LOD of pharmaceutical compounds spotted on tissue and analyzed using the targeted MRM DESI MSI

Chloroquine was spotted on a control porcine liver tissue section in different concentrations from 0.003 µM up to 30 µM in a 10 fold dilution series. One series was imaged at a speed of 10 Hz with two MRM transitions for chloroquine (320.2 > 142.17 and 320.2 > 274.1) and MRM transition for protonated PC (34:6) (782.55 > 184) which gave a dwell time of 21 ms per MRM transition per pixel. A second series was acquired with only one of the MRM transitions for chloroquine and one for the lipid at 50 Hz, leading to a dwell time of 6 ms. For both acquisition speeds, chloroquine was detected from the 0.3 µM spot with a S/N=14.

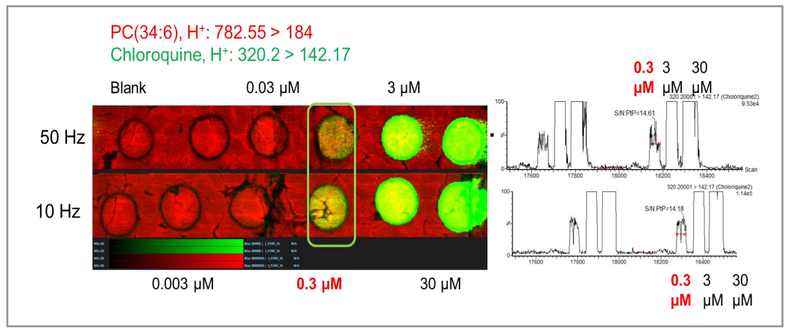

Figure 1. Chloroquine dilution series spotted on porcine liver tissue (0.003 to 30 µM). RGB overlay ion image with protonated lipid PC(36:4) (in red) and Chloroquine (in green).

A second experiment was performed analyzing a mixture of pharmaceutical compounds (propranolol, olanzapine, erlotinib, moxifloxacin, terfenadine and irinotecan). Ion images and summary table is shown in figure 2.

Overall targeted MRM DESI imaging achieved very good LOD as all compounds were detected sub 1 µM, with terfenadine detected with the lowest LOD of 0.022 µM.

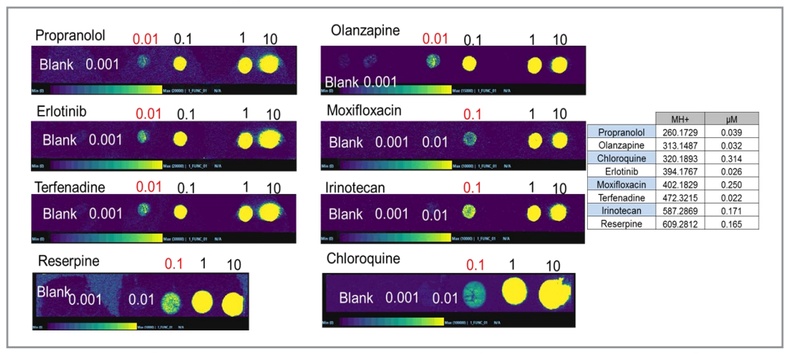

Figure 2. DESI MRM dilution series images for each drug and ROIs summed intensities plotted in Excel for propranolol, olanzapine, erlotinib, moxifloxacin, terfenadine, irinotecan.

2) Drug and its metabolites dosed tissue study - Gefitinib drug in mouse livers

MSI experiments were performed in targeted MRM mode using the DESI XS source mounted on a TQ MS. MRM transitions which were obtained from previous UHPLC MS/MS studies[2,3,4] that were transferred onto the DESI TQ MS that was optimized with Gefitinib and M11 metabolite standards, running confirmatory transition experiments.

Table 1 lists the MRM transitions for Gefitinib and 16 of its known metabolites and potassiated PC [34:1].

Compound name	Precursor m/z		Product m/z	Cone voltage (V)	Collision energy (V)	Dwell time (msec)
M1	320	>	304	15	18	7
M2	378	>	305	15	18	7
M3	*392*	>	*318*	*15*	*18*	*7*
M4	*400*	>	*320*	*15*	*18*	*7*
M5	*407*	>	*306*	*15*	*18*	*7*
M6 (M537194)	422	>	320	15	18	7
M7 (M523595, Odesmethyl Gefitnib)	433	>	128	15	18	7
M9 (M387783)	445	>	128	15	20	7
Gefitnib	**447**	**>**	**128**	**15**	**20**	**7**
M10	449	>	130	15	20	7
M11 (M605211)	461	>	142	15	20	7
M12 (M594557)	463	>	128	15	20	7
M13	477	>	158	15	20	7
M14 (M605207)	479	>	160	20	23	7
M15	*496*	>	*320*	*15*	*20*	*7*
M16	*609*	>	*433*	*20*	*25*	*7*
M17	*639*	>	*463*	*25*	*25*	*7*
Lipid PC [34:1],K+	798.5	>	163	80	35	7

Table 1: MRM transitions for Gefitinib, the 16 recently reported metabolites and potassiated PC [34:1].

Gefitinib was detected with a S/N ~ 2,500 and was at least 15-20 times more intense than the most concentrated metabolite (M11 (m605211)). Out of 16 metabolites, ten were detected in livers from dosed mice (figure 4). The order of intensity was gefitinib > M11 > M10 > M13 > M7 > M12 > M6 > M9 > M2 > M14 > M1.

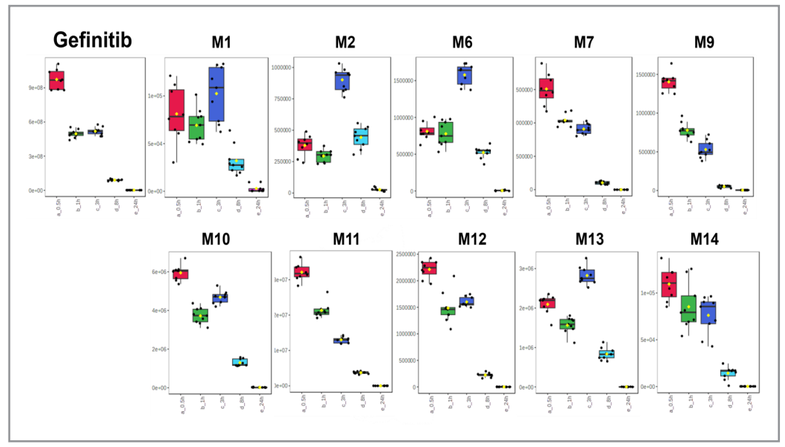

Figure 3. MetaboAnalyst box plots of the intensities averaged from 0.5, 1, 3, 8 and 24h post-dose tissue section ROIs for Gefitinib and 16 of the known metabolites (M1, M2, M6, M7, M9, M10, M11, M12, M13 and M14).

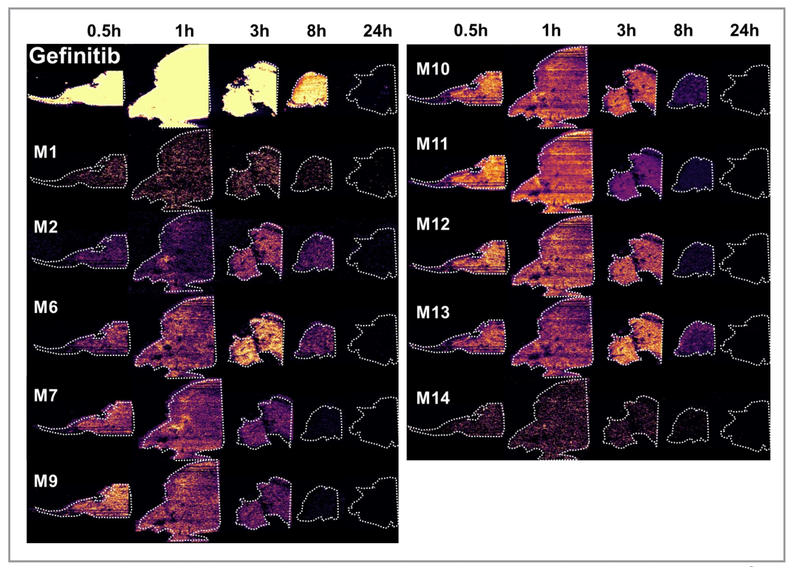

Figure 4. 0.5, 1, 3, 8 and 24h post-dose tissue sections ion images of Gefitinib and 16 of the known metabolites (M1, M2, M6, M7, M9, M10, M11, M12, M13 and M14).

CONCLUSION

- **Efficient ionization of a wide range of compounds by DESI, without the need for further sample preparation.**
- **All compounds were detected with a concentration between 0.03 - 0.5 µM spotted on tissue section, at 10 Hz acquisition speed.**
- **Acquisition at high speed, up to 50 Hz, had minimal decrease in signal intensity, opening the possibility to analyze samples at higher throughput than current methods.**
- **Gefitinib was detected using DESI combined with full scan MS and MRM acquisition modes.**
- **Results from traditional DMPK studies were successfully transferred to the DESI MRM imaging experiment where ten metabolites were detected and imaged.**

References

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