MULTI-STEP WORKFLOW FOR VISUALIZATION OF DRUG/METABOLITES AND METABOLISM WITH DESI TQ AND DESI Q-TOF MASS SPECTROMETERS

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INTRODUCTION

Typically DMPK studies are performed using LC/MS with ESI of biofluids and tissues that have been homogenized and therefore the molecular localization is lost.

DESI mass spectrometry imaging (MSI) allows the determination of small molecule distributions directly from tissue, such as pharmaceutical compounds and their metabolites as well as endogenous molecules. TOF based mass spectrometers have been demonstrated for untargeted analysis but there is a need for MSI methods that enhance sensitivity for the detection of low level analytes in tissues that are present in relatively low concentrations.

Tandem quadrupole (TQ) MS instruments are renowned for their sensitivity and specificity in targeted applications using multiple reaction monitoring (MRM) modes of acquisition.

Gefitinib is a TKI inhibitor, developed for the treatment of non-small cell lung cancer. Metabolism and pharmacokinetics have been recently reported[1,2,3].

Here a DESI source mounted on either a high-resolution Q-Tof or a TQ MS were used in a multi-step workflow allowing the localization of Gefitinib and its metabolites in liver, with timed collection, to monitor DMPK and the metabolism over time, directly from tissue section.

METHODS

Tissue sample preparation

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

Two types of experiments were performed for this study:

1) Full scan MS using a SELECT SERIES™ MRT spectrometer (a multi -reflecting Q-ToF) with a mass resolution >200,000 FWHM across the lipid mass range and a mass accuracy <500 ppb
2) MRM using a tandem quadrupole mass spectrometer for high sensitivity and specificity

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.

Table 1 summarizes the DESI parameters used for the full scan experiment on the MRT and the MRM experiment on the TQ.

Table 2 lists the MRM transitions for Gefinitib and16 of its known metabolites and potassiated PC [34:1].

	Full scan MS MRT	MRM TQ
Ionisation mode	(+)	(+)
Flow rate	2 µl/min	2 µl/min
DESI solvent composition	95% MeOH, 5% water with 100pg/µL Leu-enkephalin	95% MeOH, 5% water
Capillary voltage	1.05 kV	1 kV
Nebulising gas	15 psi	15 psi
Acquisition speed	10 Hz	5 Hz
Pixel size (lateral)	100 µm	50 µm

Table 1. DESI parameters used for the full scan experiment on the MRT and the MRM experiment on the TQ.

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

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 and TIC normalized in the form of a .csv file which was loaded directly into MetaboAnalyst [4] (https://www.metaboanalyst.ca/MetaboAnalyst/faces/home.xhtml) for extensive statistical analyses.

Putative lipid identification were performed using suitable databases (LIPID MAPS® Lipidomics Gateway website).

RESULTS

1) Untargeted discovery high mass resolution MSI

The first step of the workflow was to analyze the 5 post dosed liver tissue sections in full scan MS mode to be able to image gefitinib, potentially its metabolites and endogenous molecules.

In figure 1 and 2 are displayed the ion images of putatively identified lipids and gefitinib are diplayed, demonstrating the ultra mass accuracy of the MRT, providing extra confidence in the compound identification.

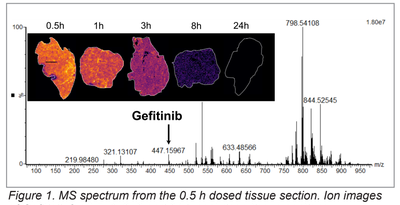

Figure 1. MS spectrum from the 0.5 h dosed tissue section. Ion images of Gefitinib for 0.5, 1, 3, 8 and 24 h post dose tissue sections.

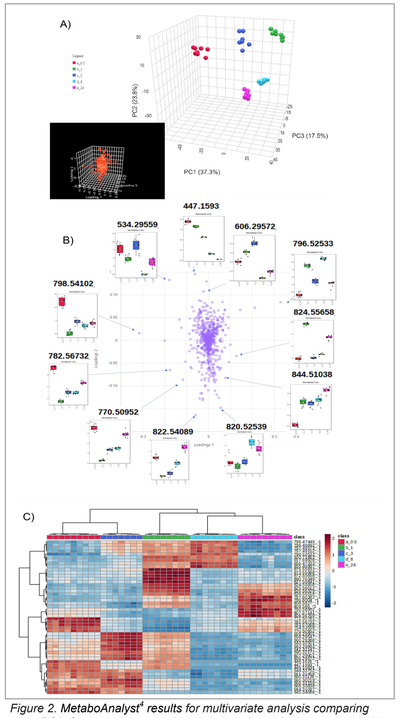

Figure 2. MetaboAnalyst[4] results for multivariate analysis comparing eight ROIs for each tissue section (0.5, 1, 3, 8 and 24 h post-dose). A) PCA score plot, B) PCA loading plot with examples of box plots) and C) heatmap

Gefinitib was detected in all tissue sections expect the 24h post dose (Figure 1), displaying a signal decrease with thereafter. None of Gefitinib's metabolites were detected in full scan MS mode.

Along with detecting the drug and potentially its metabolites, metabolic phenotyping changes can be studied in full scan MS mode to examine the effect of Gefinitb. Untargeted and unbiased multivariate statistical analysis (MVA) was performed and results are displayed in figure 2.

Endogenous metabolic profiles can be clearly grouped according to the post-dose timing tissue from the principal component analysis (PCA) score plot (2,A).

Putative identifications were performed with great confidence thanks to the sub 500 ppb mass accuracy achieved with the MRT mass spectrometer.

Sodiated and potassiated PC (34:1) lipid was more intense in the 0.5 h liver then signal dropped in the 1h tissue with increased signal with a longer post-dose time.

Figure 3. Ion images of endogenous lipids for 0.5, 1, 3, 8 and 24h post-dose tissue sections.

2) Targeted MSI for increase specificity and sensitivity

Further MSI experiments were performed in targeted MRM mode using the DESI mounted on a TQ MS. MRM transitions which were obtained from previous UPLC MS/MS studies that were transferred onto the DESI TQ MS that was optimized with Gefitinib and M11 metabolite standards, running confirmatory transition experiments.

Gefitinib, 16 metabolites's MRM transitions were set and imaged, as well as potassiated lipid PC (34:1).

Out of 16 metabolites, ten were detected in livers from dosed mice but not in the control tissue (figure 5).

From the ion images and also average intensities for multiple ROIs for each datasets, it can be seen in figure 4 that Gefitinib, M7, M9, M10, M11, M12 and M14 concentrations were maximal at 0.5h, declining thereafter, whereas metabolites M1, M2, M6 and M13 reached their maximum concentrations at 3h post dosing.

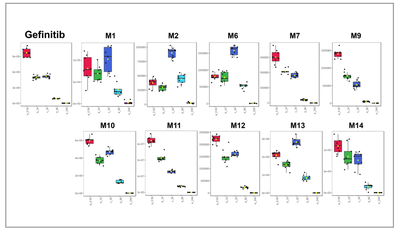

Figure 4. MetaboAnalyst box plots of the intensities averaged from 0.5, 1, 3, 8 and 24h post-dose tissue sections ROIs for Gefitinib and16 of the known metabolites (M1, M2, M6, M7, M9, M10, M11, M12, M13 and M14).

CONCLUSION

- **A multi-step approach, combining HRMS discovery untargeted and MRM targeted successfully imaged Gefitinib, its metabolites and the effects of the drug on lipid metabolism.**
- **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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[2] R.S.Plumb; *J. Proteome Res.* **2022**, 21, 3, 691–701
[3]B.Molloy; XENOBIOTICA, **2021**, VOL. 51, NO. 4, 434–446
[4]Chong, J.; Nucl. Acids Res., **2018**, 46, W486-494.

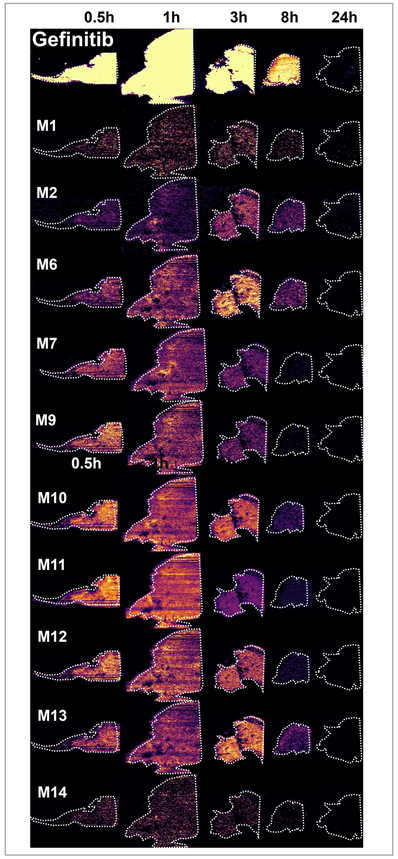

Figure 5. 0.5, 1, 3, 8 and 24h post-dose tissue sections ion images of Gefitinib and 16 of the known metabolites (M1, M2, M6, M7, M9, M10, M11, M12, M13 and M14),

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