Tandem Quadrupole DESI Imaging To Visualize Spatial Distribution of Gefitinib and Related Metabolites in Rat Liver

Anthony J. Midey,[1] Robert Plumb,[1] Nikunj Tanna,[1] and Ian D. Wilson[2]

[1]Waters Corp., Milford, MA, USA; [2]Imperial College London, London, UK

Introduction

Identification and quantification of drugs and metabolites in discovery and development studies is critical, usually supported by LC-MS. The distribution-disposition of drugs and metabolites typically require a radiolabeled version of the drug not normally available at the drug discovery stage. Desorption Electrospray Ionization (DESI) is a label-free, ambient ionization method with minimal sample preparation for mapping this spatial localization, precluding the need for antibody tagging to detect these compounds.

Here, DESI-MS/MS imaging on a tandem quadrupole MS analyzed liver tissue after single subcutaneous (SC) dosing of gefitinib to male Wistar rats at therapeutic dose. Targeted DESI MRM imaging detected gefitinib and six metabolites in the post-dose liver samples. The majority were most intense at 1 h post-dose and were still above detection limits at 24 h without using high, lethal doses. The DESI MS imaging signal intensity of the drug and metabolites correlated with the pharmacokinetics of the drug and metabolite from LC-MS/MS.

Samples

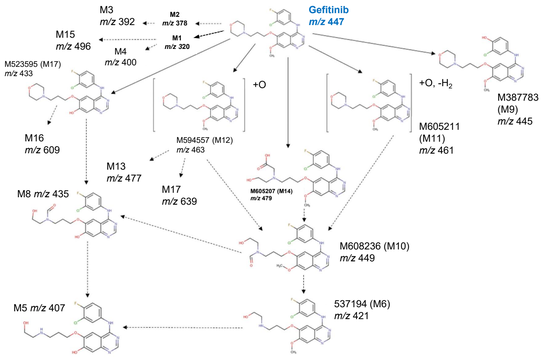
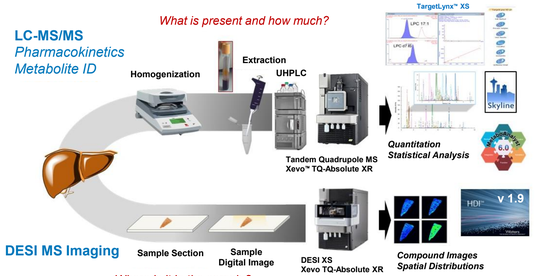


***Figure 1**. Gefitinib drug metabolism pathway[1,2]*

Study Design

- Single SC administration of gefitinib to male rats at 10 mg/kg
- Rat plasma, urine samples collected periodically over 24 h post-dose for pharmacokinetics (PK), metabolite ID (Met ID)
- Fresh frozen liver samples taken at 0.5, 3, 6, 8, and 24 h post-dose from 4 rats for DESI MS imaging
- Animal dosing and sampling was performed at Evotec (France) SAS (Toulouse, France) after management review and in accordance with National and EU guidelines.

Gefitinib Metabolism – LC-MS/MS and MS Imaging



Plasma Pharmacokinetics	Urine Elimination	Plasma Metabolite ID	Urine Metabolite ID
• SPE (10 µL, Waters OASIS HLB™)	• 5 µL, Protein Precipitation 4:1 Acetonitrile:Methanol (v/v)	• 5 µL, Protein Precipitation of each 4:1 Acetonitrile:Methanol (v/v)	
• Gefitinib-d6 internal std.	• Gefitinib-d6 internal std.	• Gefitinib-d6 internal std.	

ACQUITY Premier UPLC™ system ACQUITY Premier BEH C18 column (2.1 x 100 mm, 1.7 µm particle)	**A:** Water, 0.1% Formic Acid, 10 mM Ammonium Acetate **B:** Acetonitrile. 0.1% Formic Acid, 10 mM Ammonium Acetate Gradient: 3 min. method of Molloy, BJ, et al. (Ref. 2)

Targeted DESI MS/MS Imaging

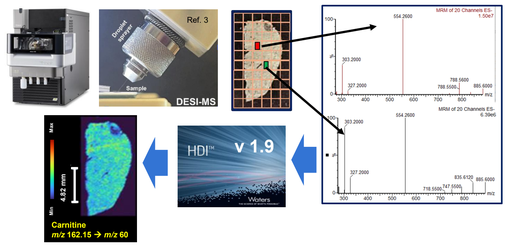


***Figure 2**. Workflow: Targeted DESI MS/MS Imaging by MRM on TQ-Absolute XR MS*

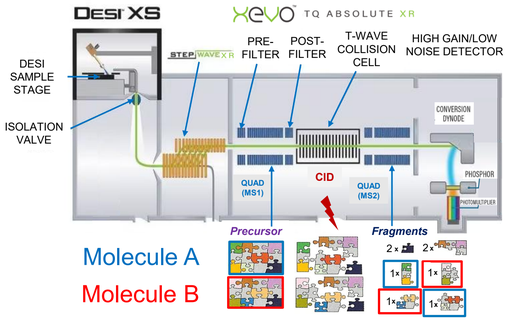


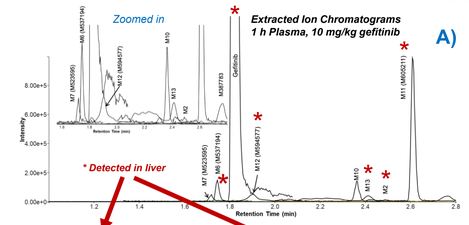
***Figure 3**. MS Imaging by MRM on TQ: fingerprint fragment of precursor for selectivity, provides ID by MS/MS.*

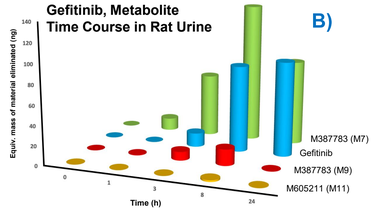
DESI MRM Imaging Experiment

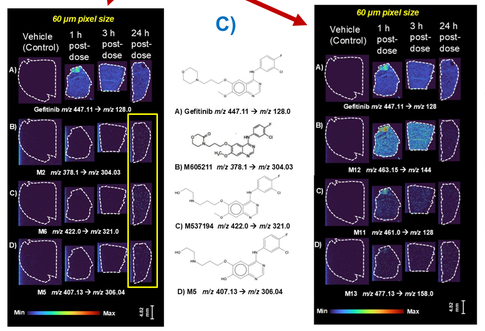
- Fresh frozen rat liver cryosectioned to 10 µm thickness, thaw mounted on std. glass specimen slides
- Samples stored @ -80ºC; thawed for 15 min. in vacuum desiccator, then analyzed

DESI emitter V	0.65 kV; + mode
DESI Solvent	98:2 methanol-water w/ 0.01% (v/v) formic acid
DESI Solvent Flow	2 µL/min
Cone Voltage	13 to 22 V
Sprayer Pressure	0.9 bar
Source Temperature	120˚C
MS1, MS2 Resolution	0.75 Da FWHM

Results







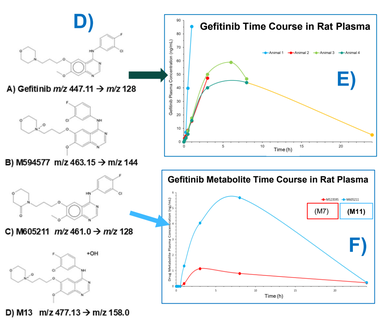


***Figure 4**. A) LC-MS/MS plasma extr. ion chromatogram, gefitinib/metabolites, 1 h post-dose; B) Urine LC-MS/MS gefitinib, metabolites elim. profile, 24 h period; C) DESI targeted MS images: gefitinib, M2, M5, M6 metabolites (top to bot.) in liver at ctrl., 1, 3, and 24 h post-dose; D) DESI targeted MS images: gefitinib, M12, M11, M13 metabolites (top to bot.) in liver at ctrl., 1, 3, and 24 h post-dose; E) LC-MS/MS gefitinib plasma conc. (ng/mL) in 4 dosed rats, 24 h period; F) LC-MS/MS M7, M11 plasma conc. (ng/mL), 24 h period.*

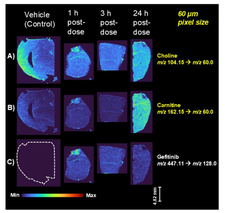


***Fig.5**. DESI targeted MS images: A) carnitine; B) carnitine; C) gefitinib in liver for ctrl., 1, 3, 24 h post-dose.*

Conclusions

- Spatial metabolomics of gefitinib, its metabolites over 24 h post-dose in rat liver by targeted DESI MRM imaging on a tandem quadrupole MS augmented plasma, urine LC-MS/MS studies on the same instrument.
- Enhanced sensitivity of DESI imaging on a tandem quad MS without any labeling required mapped gefitinib, 6 drug metabolites in liver at therapeutic dose, better representing the drug metabolism in typical use vs. higher lethal doses often needed for detection.
- Drug and M11 metabolite liver intensities matched the elimination profiles.

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The authors declare no competing financial interest.

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