

# Comparison of Drug Distribution Images from Thin Tissue Sections Obtained Using Desorption Electrospray Ionization Tandem Mass Spectrometry and Whole-Body Autoradiography

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## OVERVIEW

### Goals:

- Compare desorption electrospray ionization tandem mass spectrometry (DESI-MS/MS) and whole-body autoradiography (WBA) methodologies for examining the distribution of intravenously dosed propranolol in whole-body mouse thin tissue sections
- Evaluate the possibility to acquire useful DESI-MS/MS images of whole-body mouse thin tissue sections in 1 hr

### Methods:

- Comparison of DESI-MS/MS and WBA:
  - Mice dosed with same level of drug evaluated in parallel
  - Spatial distribution investigated
  - Relative quantitation attempted
- Rapid imaging
  - Signal level and image quality dependence on speed investigated

### Results:

- Comparison of the DESI-MS/MS signal for propranolol and the radioactivity attributed to propranolol from WBA sections indicated nominal agreement between the two techniques for the relative amount of propranolol in the brain, lung, and liver. Data from the kidney showed an unexplained disparity between the two techniques.
- Attempts to detect and image the distribution of the known propranolol metabolites were unsuccessful using DESI-MS/MS.
- Although signal decreased with increasing scan rate, useful whole-body images for propranolol were obtained even at 7 mm/s, which required just 79 min of analysis time.

## INTRODUCTION

- This research is focused on comparing desorption electrospray ionization mass spectrometry (DESI-MS) [1-3] and whole-body autoradiography (WBA) [4] methodologies for examining the distribution of intravenously dosed propranolol in whole-body mouse thin tissue sections.
- To date, DESI-MS has not been used to image whole-body thin tissue sections from small animals dosed with a drug. Recently published data by Wiseman et al. [5] demonstrate DESI-MS imaging of a sagittal brain section from a rat dosed with 250 mg clozapine via an intracerebral ventricular injection.
- In this work we report on
  - comparable qualitative distributions of parent drug levels with DESI-MS/MS and WBA for most, but not all, organs examined
  - obtaining useful chemical images of the parent drug in whole-body mouse tissue sections in less than 1.5 hours (7 mm/s scan speed) with DESI-MS/MS

## EXPERIMENTAL

**Chemicals.** HPLC grade methanol and water were purchased from Burdick & Jackson (Muskegon, MI). D,L-propranolol hydrochloride and D,L-propranolol-[4-<sup>3</sup>H] hydrochloride (in ethanol; 27 Ci/mmol) were purchased from Sigma-Aldrich (St. Louis, MO). A 0.1 nM propranolol test solution was prepared in 50/50 (v/v) methanol/water.

**Tissue Preparation for MS Tissue Imaging.** Mice (Male CD-1; Charles River Laboratories) were administered propranolol intravenously via the tail vein at 7.5 mg/kg as an aqueous solution in 0.9% NaCl. At 20 or 60 min post-dose, mice were euthanized, frozen, and whole-body sagittal sections were prepared. For more details on tissue preparation see ref. 6.

**Tissue Preparation for Quantitative Whole-Body Autoradiography (QWBA):** Total Radiolabeled Drug-Related Material. The radiolabeled dosing solution was prepared by evaporating ethanol from a portion of D,L-propranolol-[4-<sup>3</sup>H] hydrochloride (in ethanol; 27 Ci/mmol) under a stream of nitrogen followed by the addition of non-radiolabeled propranolol and 0.9% NaCl. The specific activity of the resulting solution was 35.27 nCi/nmol. Mice were administered the [<sup>3</sup>H] propranolol solution intravenously via the tail vein at 7.5 mg/kg (1 mCi/kg radioactive dose) as an aqueous solution in 0.9% NaCl. At 20 or 60 min post-dose, mice were euthanized, frozen, and prepared for WBA. For more details on tissue preparation see ref. 6.

**Radioprofiling of Drug-Related Material and Metabolite Identification from Brain, Kidney, Liver, and Lung Tissue Samples.** Brain, kidney, liver, and lung were excised and rinsed in saline from a mouse dosed intravenously via the tail vein at 7.5 mg/kg [<sup>3</sup>H] propranolol in 0.9% NaCl (1 mCi/kg radioactive dose) then sacrificed 60 minutes post-dose. The specific activity of the resulting solution was 40.21 nCi/nmol. Organs were homogenized in three portions (w/v) water using a small tissue homogenizer. Samples were extracted in two portions (v/v) 50/50 acetonitrile:water (v/v). Samples were dried down under N<sub>2</sub> and reconstituted in 10 mM ammonium acetate.

Figure 1. Propranolol Percentage from Radioprofile Data

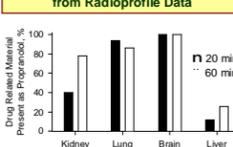


Figure 2. DESI-MS with a PDI Interface

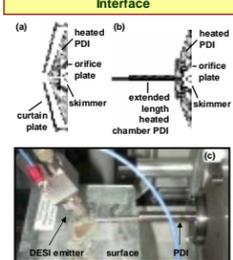
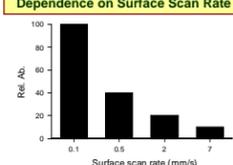


Figure 3. DESI-MS/MS Signal Dependence on Surface Scan Rate



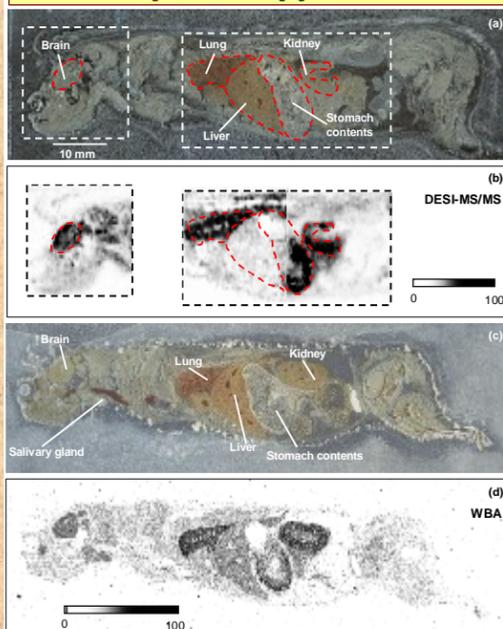
The tissue extract samples were injected into the Agilent HPLC. The flow from the HPLC was split 4:1 between a radiochemical detector (INUS Beta-RAM) and a Finnigan TSQ Quantum Ultra MS (ESI /positive ion mode). Radioprofile of the brain sample indicated the presence of only the parent drug. In the lung, liver and kidney the major [<sup>3</sup>H] metabolites were identified as two different hydroxypropranolol glucuronides (m/z 452).

DESI-MS System. All DESI-MS experiments were performed on a 4000 QTRAP mass spectrometer equipped with a particle discriminator interface (PDI, Figures 2a and 2b). Evaluation of PDIs with 2.0, 4.0, 7.0 and 12 cm long heated chambers was accomplished by measuring the continuous infusion ES-MS/MS signal (m/z 260 @ 116) from a 0.1 nM propranolol solution with each of the modified configurations. The ES-MS/MS signal decreased with increasing chamber length. For the imaging discussed here, we used the 7.0 cm heated chamber as this extension was the shortest chamber that allowed us to image a whole-body mouse thin tissue section in our current instrument configuration (Figure 2c). For more details on instrumental setup and evaluation of PDIs see ref. 6.

Figure 3 shows relative DESI-MS/MS signal levels of propranolol as a function of surface scan rates for the same lung tissue. The observed trend was in agreement with our previous study, where signal level was shown to decrease with increasing surface scan rate for analytes deposited on retaining/porous thin-layer chromatography (TLC) plates. The lower signal levels with higher surface scan rate were most likely due to decreased effectiveness of the solid-liquid extraction step inherent to DESI.

## RESULTS AND DISCUSSIONS

Figure 4. DESI-MS Imaging at 100 mm/s



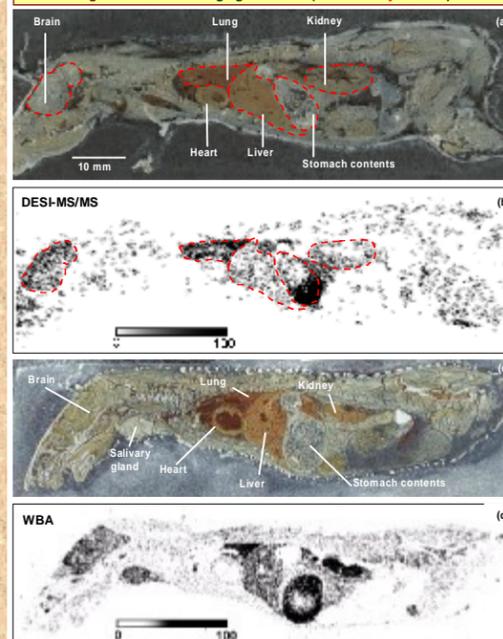
(a) Scanned optical image of a whole-body tissue section of a mouse dosed intravenously with 7.5 mg/kg propranolol and euthanized 20 min after dose. (b) Distribution of propranolol in 20 mm x 20 mm and 38 mm x 20 mm areas measured by DESI-MS/MS (SRM: m/z 260 @ 116) using 80/20 (v/v) methanol/water as DESI solvent at a flow rate of 5 µl/min. Surface scan rate was 100 µm/sec, dwell time was 100 ms and the images were created from 41 lanes with 500 µm spacing. Pixel size was 84 µm (h) x 500 µm (v) and experiment times were 150 and 285 min for the 20 mm x 20 mm and 38 mm x 20 mm areas, respectively. (c) Scanned optical image of a 40 mm thick sagittal whole-body tissue section of a mouse dosed intravenously with 7.5 mg/kg [<sup>3</sup>H] propranolol and euthanized 20 min after dose. (d) Autoradioluminograph of [<sup>3</sup>H] propranolol related material in the tissue section presented in (c).

Total analysis time was 7.5 hours. Based on the predicted metabolic pathway of propranolol, eight transitions were monitored during imaging: m/z 260 @ 116 (propranolol), m/z 260 @ 183 (propranolol), m/z 276 @ 116 (hydroxypropranolol), m/z 292 @ 116 (dihydroxypropranolol), m/z 436 @ 116 (propranolol glucuronide), m/z 452 @ 116 (hydroxypropranolol glucuronide), m/z 468 @ 116 (dihydroxypropranolol glucuronide) and m/z 482 @ 116 (propranolol glucuronic acid). An additional transition of hydroxypropranolol glucuronide (m/z 452 @ 276) was tested after imaging.

Only the transitions for propranolol provided signal above background levels. The inability to detect hydroxypropranolol glucuronide was unexpected, because this was identified as the major metabolite in lung, kidney and liver extracts using LC-MS/MS (see Experimental section).

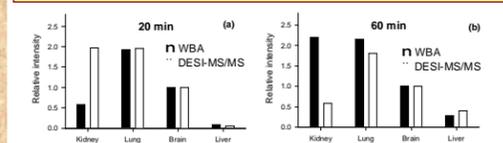
Furthermore, another sampling approach used in our laboratory, namely, the liquid micro-junction surface sampling probe/electrospray ionization mass spectrometry system (LMJ-SSP), clearly detected the parent drug (m/z 260 @ 116) and the hydroxypropranolol glucuronide metabolite (m/z 452 @ 116) from these organs under the same instrumental conditions (see Figure 7).

Figure 5. DESI-MS Imaging at 7 mm/s (79 min Analysis Time)



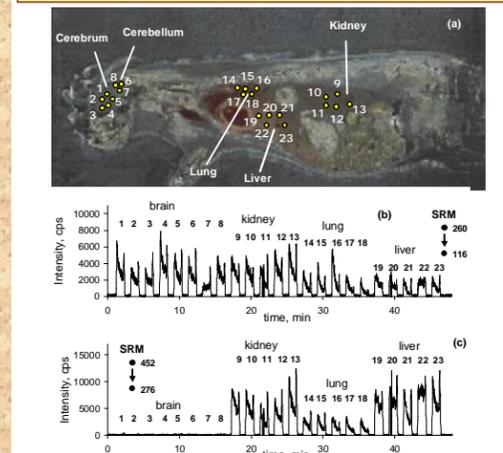
(a) Scanned optical image of a whole-body tissue section of a mouse dosed intravenously with 7.5 mg/kg propranolol and euthanized 60 min after dose. (b) Distribution of propranolol in the 94 mm x 30 mm tissue section presented in (a) measured by DESI-MS/MS (SRM: m/z 260 @ 116) using 80/20 (v/v) methanol/water as DESI solvent at a flow rate of 5 µl/min. Surface scan rate was 7 mm/sec, dwell time was 20 ms and the image was created from 151 lanes with 200 µm spacing. Pixel size was 140 µm (h) x 200 µm (v) and total experiment time was 79 min. (c) Scanned optical image of a 40 mm thick sagittal whole-body tissue section of a mouse dosed intravenously with 7.5 mg/kg [<sup>3</sup>H] propranolol and euthanized 60 min after dose. (d) Autoradioluminograph of [<sup>3</sup>H] propranolol related material in the tissue section presented in (c).

Figure 6. Comparison of WBA and DESI-MS/MS Data - Propranolol



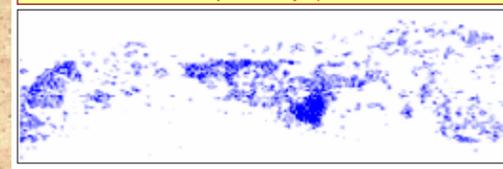
Nominal agreement for the relative distributions of propranolol in the brain, lung, and liver between the DESI-MS/MS signal for propranolol and the radioactivity attributed to propranolol from WBA sections. In the case of kidney, the data exhibited an unexplained disparity between DESI-MS/MS and WBA. Signal levels for all organs were normalized to that of the brain tissue.

Figure 7. Detection of Metabolite using a Liquid Microjunction Surface Sampler Probe



(a) Photograph of a 40 mm thick sagittal whole-body tissue section of a mouse dosed intravenously with 7.5 mg/kg propranolol and euthanized 60 min after dose. Signal levels for (b) propranolol (m/z 260 @ 116), and (c) hydroxypropranolol glucuronide (m/z 452 @ 276), were recorded during the sampling at each point using an LMJ-SSP/ESI-MS/MS system. Dwell time was 100 ms for each position monitored. Eluting solvent was 80/20 (v/v) methanol/water, 0.1% formic acid at a flow rate of 5 µl/min.

Figure 8. Prognosis - Simulated DESI-MS Image with 400 µm Lane Spacing (32 min Analysis)



Using faster reverse speed (14 mm/s) and higher lane spacing (400 µm) targets the total analysis time, but does not change the (forward) surface scan rate (i.e. net time spent on actual analysis of a lane defined only by the surface scan rate and the length of the lane) and DESI signal levels.

Our studies of signal dependence on lane spacing showed, that increasing the lane spacing above 200 µm did not result in increased DESI signal level. This means that an image acquired with 14 mm/s reverse speed (which does not affect the resulting image) and with 400 µm lane spacing can be easily simulated by using Figure 5b (by using data from every second lane).

- Calculation:
  - Area: 94 mm x 30 mm, lane spacing: 400 µm → 76 lanes
  - Surface scan rate: 7 mm/s → 13.5 s/lane
  - Lowering the stage at the end of the lane: 1 s
  - Reverse speed: 14 mm/s → 7 s/lane (time also used to position above next lane)
  - Lifting the stage at the end of the lane: 1 s
  - Additional -2 s/lane for position sensing, etc. → 25 s/lane analysis time
  - 76 lanes x 25 s/lane → 32 min

## CONCLUSIONS

- Nominal agreement for the relative distributions of propranolol in the brain, lung, and liver was shown between the DESI-MS/MS signal for propranolol and the radioactivity attributed to propranolol from WBA sections.
- In the case of kidney, the data exhibited an unexplained disparity between DESI-MS/MS and WBA.
- Image quality was reduced as surface scan rate increased, due to reduced signal levels and larger pixel sizes.
- Chemically informative DESI-MS/MS images of the parent drug were obtained at surface scan speeds up to 7 mm/s, the top speed for the stage used.
- Imaging time of just 79 minutes for a whole-body section of a mouse.
  - Simple changes in the surface scan scheme or use of a stage with faster movement capabilities should result in images of the same quality obtained in as little as approximately 32 minutes.
- Current performance characteristics of this DESI-MS imaging system were not sufficient to detect hydroxypropranolol glucuronide, the major metabolite known to be present in kidney and liver tissues at levels close to that of the parent drug.
- These discrepancies, low signal levels for the parent drug, and the inability to detect known metabolites present in the tissue will need to be addressed and overcome to make this tissue section imaging by DESI-MS a practical drug discovery tool.

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