Drug imaging and uptake kinetics in *Fasciola hepatica* parasite samples using AP-SMALDI MSI

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**Biological background**
Fascioliasis is a food-borne infection that is counted among the neglected tropical diseases (NTDs).¹ Caused by *Fasciola* parasites, fascioliasis affects various types of mammals, including humans. Prevalence is estimated to be 2.4 – 17 million worldwide.²,³ The only medically approved drug for human treatment is triclabendazole (TCBZ). However, the mode of action of TCBZ is not fully understood. Emerging resistance indicates the necessity for developing new drugs.

**MALDI-MSI background**
- Mass Spectrometric Imaging (MS Imaging) supported by Thermo Scientific™ Orbitrap™ technology results in High Resolution in Mass and Space.
- High Resolution in Mass and Space is achieved by Atmospheric-Pressure Scanning microprobe Matrix-Assisted Laser Desorption/Ionization (AP-SMALDI) coupled with Orbitrap MS.
- AP-SMALDI⁵ AF ion source is a product of TransMIT GmbH, Giessen, Germany.

**Objectives**
- The drug uptake of two compounds is investigated by means of MS Imaging.
- *F. hepatica* tissue sections were analyzed to uncover routes of uptake, uptake kinetics, and distributions of drug triclabendazole (TCBZ).
- Also, a new drug candidate – imatinib – is investigated with AP-SMALDI MSI.

**Keywords**
Orbitrap technology, tissue samples, high resolution accurate mass, high spatial resolution, SMALDI, Imaging, 3D-surface topography, lipids, metabolites, drug compounds
Methods and workflow

Figure 1. Spiking experiment of *F. hepatica* with 100 μM drug solution – here displayed in the entire workflow from tissue to slicing, matrix spraying, MS analysis by means of MS Imaging, data processing and comparison to the adjacent tissue slice (H&E stained). Preparation: *In vitro* exposure of adult worms to 100 μM drug solution. Washing (with PBS and H2O). The specific parts of the figure (A-F) are described in detail here:

A. Embedding in aqueous gelatin solution and freezing
B. Cryosectioning (20 μm thick, transversal) at −23 °C using a Cryostat HM525
C. Matrix application with pneumatic sprayer system (SMALDI®Prep, TransMIT GmbH)
D. AP-SMALDI® AF ion source coupled to Thermo Scientific™ Q Exactive™ HF MS [4]. The scanning mode is illustrated to the left. Pixel sizes applied for the data shown here are 10 μm. AP-SMALDI® AF setup is detailed in [7].
E. Data evaluation by means of MS Imaging is performed in Mirion software, TransMIT GmbH [5].
F. Hematoxylin & Eosin (H&E) staining of analyzed section — for organ assignment and visualization by means of optical microscopy
Figure 2 illustrates the details of the laser path with the laser beam (1) passing through a focusing lens (2) and two mirrors directing the light through an objective lens (3) close to the sample. The objective lens focuses the pulsed laser light to the tissue sample (base plate, 4). An off-axis camera (bottom, 5) allows for sample inspection while the autofocusing (top, 6) laser ensures the analyzing laser is always in focus.

Technical details of the AP-SMALDI\textsuperscript{5} AF setup coupled to Orbitrap mass spectrometry for MS Imaging application are found in [7].

Also refer to the Technical Note TN000659 for instrumental setup with Orbitrap Exploris MS and AP-SMALDI\textsuperscript{5} AF ion source.
Results for imatinib

![Chemical structure of imatinib](image)

$m/z$ 812.6186  $m/z$ 494.2665  $m/z$ 810.5982

PC 38:3; [M+H]$^+$  imatinib; [M+H]$^+$  PC 36:1; [M+Na]$^+$

AP-SMALDI MS images  

<table>
<thead>
<tr>
<th>Time</th>
<th>Corresponding Optical Images</th>
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<tbody>
<tr>
<td>20 min</td>
<td>B2, C2, D2</td>
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<td>4 h</td>
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<td>12 h</td>
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Figure 3. Drug uptake and kinetics for imatinib, $m/z$ 494.2665 in *F. hepatica* with time points at 20 min, 4 h, and 12 h after exposure.[8]

A. Chemical structure of the drug imatinib

B1, C1, D1. AP-SMALDI MS images for the tissue sections harvested after different times of drug uptake

B1. 20 min, with imatinib in red color

C1. 4 h, with PC 38:3 (green) and PC 36:1 (blue) to display the tissue by some endogenous lipids – and with imatinib in red color

D1. 2 h, with PC 38:3 (green) and PC 36:1 (blue) to display the tissue by some endogenous lipids – and with imatinib in red color.

Corresponding optical images (B2, C2, D2) of the previously analyzed tissue slices – after matrix removal by washing and subsequent H&E staining of the tissue slices, as above, after different times of drug uptake

B2. 20 min

C2. 4 h – here with various exemplary organ annotations provided in the figure

D2. 12 h
Results for TCBZ

\[
\text{Cl} \quad \begin{array}{c}
\text{Cl} \\
\text{O} \\
\text{N=S} \\
\end{array} \\
\text{H}
\]

\[m/z \ 828.5961 \quad m/z \ 358.9571 \quad m/z \ 751.5039\]

HexCer 38:0;O4; [M+K]\[m/z \ 358.9571\]

TCBZ; [M+H]\[m/z \ 751.5039\]

PA O-38:3, [M+K]\[m/z \ 828.5961\]

AP-SMALDI MS images

corresponding optical images

Figure 4. Drug uptake and kinetics for TCBZ, \textit{m/z} 358.957099 in \textit{F. hepatica} with time points at 20 min, 4 h, and 12 h after exposure.

A: chemical structure of the drug triclabendazole TCBZ

B1, C1, D1: AP-SMALDI MS images for the tissue sections harvested after different times of drug uptake in red color and endogenous compounds from the tissue, here illustrated with images for PA O-38:3 (blue) and HexCer 38:0; O4 (green)

B1: 20 min, TCBZ in tegument and subtegumental area — tegumental uptake with TCBZ in red to the top left and bottom part of tissue

C1: 4 h, accumulation in gastrodermis and ovary (see arrows single-channel image) with PA O-38:3 (blue) and HexCer 38:0; O4 (green) to display the tissue by some endogenous lipids

D1: 12 h, almost uniform distribution in tissue after 12 h of incubation with PA O-38:3 (blue) and HexCer 38:0; O4 (green)

C1*: 4h, compare to C1 here single channel image for the drug compound TCBZ – its accumulation in gastrodermis and ovary is observed

The raw data were investigated for sulfoxide and sulfone metabolites as well – they were not detected in TCBZ-treated worms. It can be speculated that the worm is not able to produce the metabolites by itself in significant amounts.

Corresponding optical images (B2, C2, D2) of the previously analyzed tissue slices — after matrix removal by washing and subsequent H&E staining of the tissue slices, as above, after different times of drug uptake

B2: 20 min

C2: 4 h — here with various exemplary organ annotations provided in the figure

D2: 12 h
Conclusion

- AP-SMALDI MS Imaging enabled by Orbitrap technology allows to successfully track anti-Fasciola compounds during their penetration into parasitic tissues upon time course studies.
- Spatial resolution of AP-SMALDI MS imaging allows to display the worm organs and shows perfect correspondence with H&E stained tissue slices.
- In parallel, localization of lipid marker signals and localization of the drug compound are achieved.
- Subsequent H&E staining of previously measured sections is applicable and allows to localize the drug compound and metabolites in its identical section environment.
- Differences between the investigated drug compounds regarding route of uptake, kinetics, and regions of drug accumulation are clearly observed.
- AP-SMALDI MS Imaging enabled by Orbitrap technology is perfectly suited to gain information on drug uptake, kinetics, and distributions of drug compounds and its metabolites.
- Lipid markers found (by MS imaging data processing) and subsequent histological staining of the identical tissue slices (optical microscopy) are crucial for assigning detected drugs to organs in the tissue.

References


Results shown in this Technical Note have been published in a larger scope and with more background and details — including AP-SMALDI images for a third compound. Refer to the paper from C.M. Morawietz et al. https://doi.org/10.1007/s00436-021-07388-1 for more details.

Ethics approval

Animal experiments were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS No 123; revised Appendix A) and the German Animal Welfare act. The experiments were approved by the Regional Council (Regierungspraesidium) Giessen (V54-19c20 15 h 02 Gl 18/10 Nr. A16/2018).

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