Zebrafish larvae as toxicity model for drug development using imaging mass spectrometry

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Introduction

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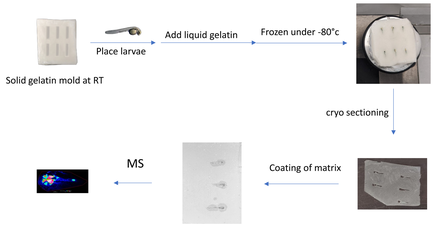
Organ specific distribution can impact efficacy, toxicity, and drug level kinetics. Using the zebrafish model to predict tissue distribution of compounds could allow early assessment of this characteristic and allow comparisons between compounds early in compound selection to optimize for ideal distribution at lower cost and a quicker turnover. Mass spectrometry imaging provides a way to determine drug level through virtual dissection of these microscopic specimens.
This poster presents the method development of zebrafish larvae embedding/ sectioning, and imaging result of toxicity model compounds dosed zebrafish larvae.

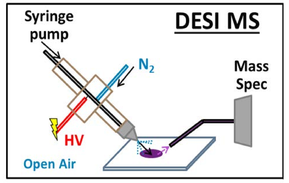
Method

Zebrafish of 7 days-post-fertilization were dosed separately with four compounds (clozapine, diltiazem, buspirone and loperamide), and placed on the fish shaped surface of solid gelatin block (20%) made from 3D printed mold, the site of larvae was covered by liquid gelatin (20%). Sections of 10 µm thickness obtained using a HM550 Cryostat at -16°C were coated with DHB on an HTX TM sprayer or sublimation. MALDI Imaging was carried out on a Bruker 7T Solarix FT-ICR or a timsTOF Flex MALDI 2 mass spectrometer. Following imaging acquisition, H&E optical images of the same section was obtained on a Meiji microscope MT4300LV or a Motic slide scanner EasyscanPro1. In parallel, DESI mass spectrometry imaging was applied to the whole fish to obtain the DESI ion images.

MALDI imaging of zebrafish larvae workflow



DESI-MS imaging of zebrafish larvae workflow



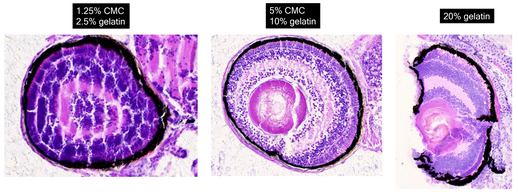
- Charged Solvent performs extraction/desorption of analyte
- Common charge and nebulizing gas remove solvent
- Simple thaw mount of tissues and analysis in ambient conditions

Control of fish placement

Better reproducibility reached with gelatin block that has fish shape surface made from 3D printed mold

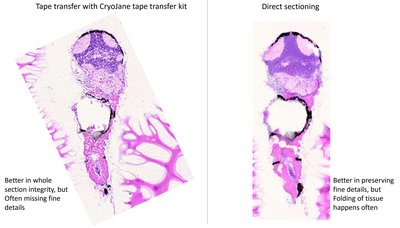
Optimization of embedding media

tested media: CMC/gelatin[1], gelatin[1], HPMC/PVP[2], pHPMA[3]
prefered media are: room temperature gelatin block (20%gelatin made at 50°C) combines liquid gelatin (20% made at 85°C)



Challenges of sectioning zebrafish larvae

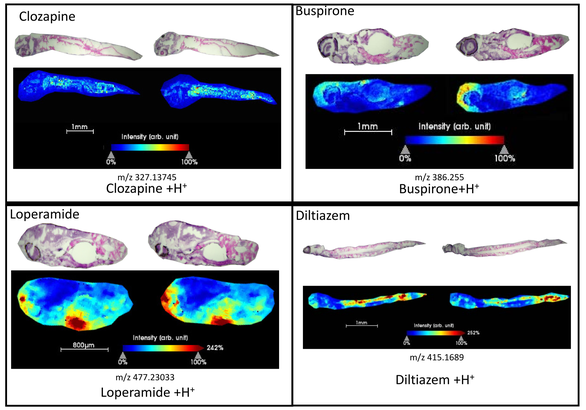
with or without tape transfer



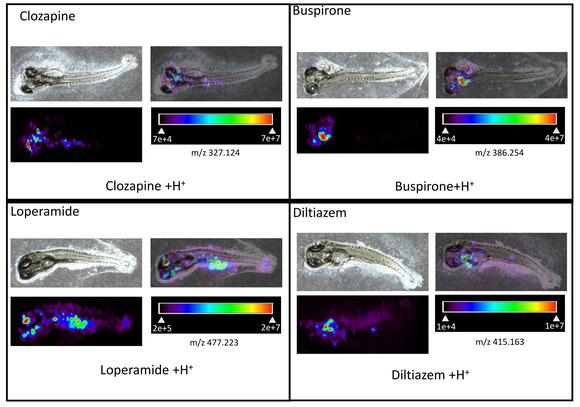
Drug distribution in dosed zebrafish larvae

- Mass spectrometry imaging allows virtual dissection of the zebrafish larva to compare tissue specific drug distribution between molecules
- Images demonstrate greater abundance of buspirone and clozapine in the CNS relative to loperamide in accord with mammalian tissue distribution

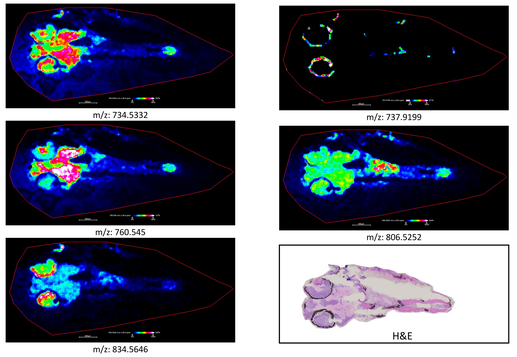
MALDI imaging results



DESI imaging results



Zebrafish organ ion marker by MALDI



Summary

- Zebrafish larvae embedding media and method were optimized.
- Gelatin made at 50°C stays solid at RT and gelatin made at 85°C stays liquid at RT. Ambient temperature placement of fish larvae makes the operation feasible to control the fish body orientation for obtaining of quality sections.
- Zebrafish larvae as toxicity model was demonstrated. The distribution of model compounds correlate the organ ion marker well, indicating the usefullness of using zebrafish larvae as funneling tool in early drug developement.
- Further imaging of zebrafish larvae to determine more organ ion marker is under process to facilitate the correlation between drug distribution and organs.
- Comparing MALDI to DESI, MALDI imaging provides higher spatial resolution, DESI has minmal sample preparation.

References:

1) J Biomol Tech. 2013, Sep,24(3):119-27
2) Anal. Chem. 2020, 92, 16, 11080-11088
3) Anal. Chem. 2011, 83, 5458-5462

Disclosures:

- All authors are employees of AbbVie and may own AbbVie stock.
- AbbVie sponsored and funded the study; contributed to the design; participated in the collection, analysis, and interpretation of data, and in writing, reviewing, and approval of the final presentation.