A Broad Application of Migration Time Indexing and Metabolite Libraries to Evaluate Quantitative Coverage in Microchip CE-MS Metabolomics

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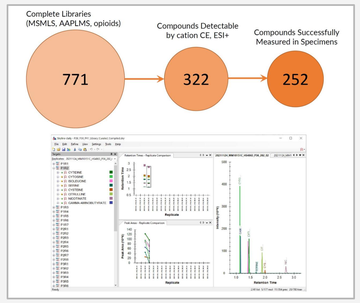
Abstract

In the field of metabolomics, microchip capillary electrophoresis (mCE) has a number of potential advantages over traditional liquid chromatography (LC), including higher throughput due to decreased separation times, increased separation efficiency, and lower required sample volumes. A drawback of microchip CE is the comparative (to LC) variability in migration time. Previous work done on the microchip-CE platform PATsmart™ ZipChip® System (Repligen) demonstrated improved accuracy for metabolite identification using indexed migration times (iMT). This work expands on the use of migration time indexing to include several metabolite libraries and evaluate the number of compounds addressable in common matrices of interest in metabolomics including human and mouse plasma, human serum, various mouse tissues, and immortalized human cell lines. Out of 322 potential "detectable" library compounds, we measured 252 metabolites in a 4-minute method under standardized conditions, in one or more matrix tested.

Library Technologies

IROA Technologies MSMLS (603) and AAPMLS (176) libraries, along with a library of 14 opioids were purchased and combined according to the manufacturer's protocol into groups of 12 non-isobaric molecules. These groups were diluted into 0.1 M ammonium acetate with 50/50 Methanol/water containing 5 uM NSK-A, for a final concentration of approximately 5uM for each library compound. These were analyzed 2x each using a ZipChip HS chip and Peptides BGE on an Exploris 240. Initial data analysis was performed in MLSDiscovery (IROA) to determine precursor adducts and MS/MS fragment ions present for each library compound. Using the most abundant precursor adducts, raw data was imported to Skyline for library and iMT annotation.

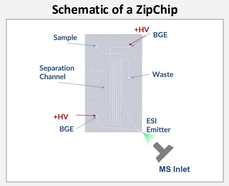


To the left is a screenshot of library curation in Skyline, with the compounds organized by plate and row (left), duplicate runs of each compound set (middle), and a representative electropherogram from MSMLS Plate 1 Row 2 compound set (right). Library compounds which are neutral or acidic at pH 2.5 would not be expected to be observed with this cation CE method.

Methods and Materials

Sample Prep: Biological specimens for evaluation of metabolite coverage were purchased from BioIVT. Sample protocols were customized to each sample type; biofluids were precipitated/extracted directly, while tissues and cells were homogenized with probe sonication. Briefly, samples were protein precipitated with MeOH containing internal standards, followed by addition of aqueous ammonium acetate to a final concentration of 0.1 M. **Data Collection:** Separations were performed on a ZipChip microchip CE platform using Peptides BGE and an HS chip (Repligen) with a field strength of 750 V/cm. Data dependent analysis (DDA) and quantitative analysis was run on an Exploris 240 Orbitrap mass spectrometer (Thermo Fisher Scientific). Data was analyzed in Skyline –daily v21.2.1.1485.

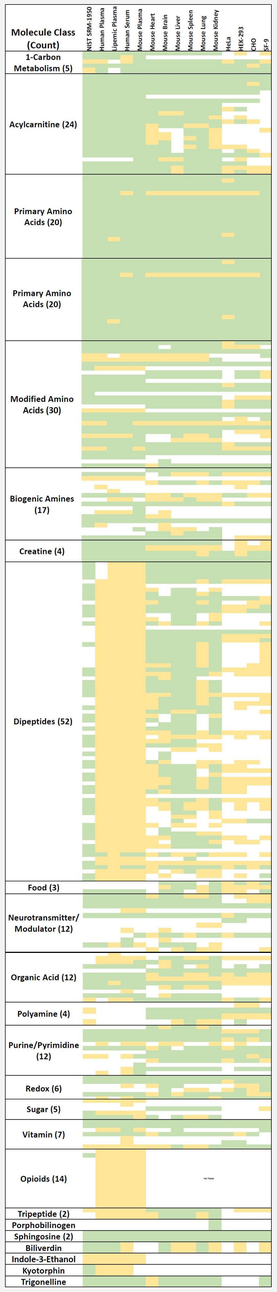
Schematic of a ZipChip



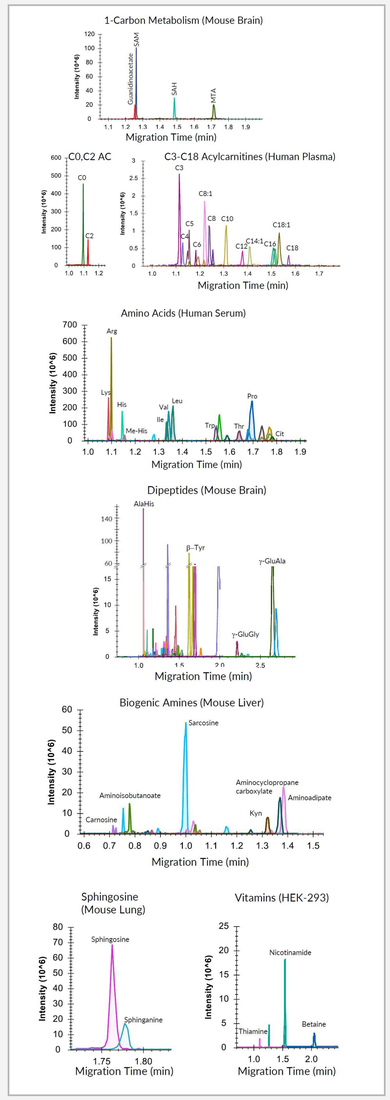
ZipChip mCE cartridge

ZipChip Interface on Exploris 240

Metabolite Coverage



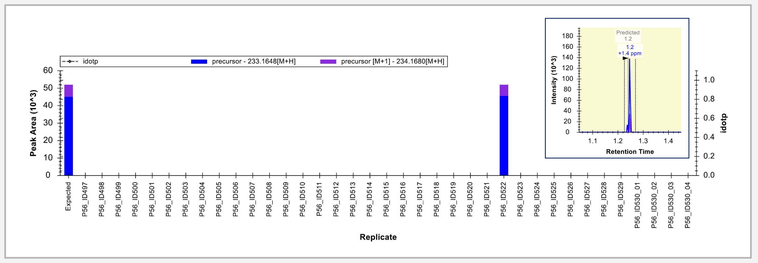
The microchip CE based workflow allowed the targeted measurement of greater than 250 metabolites (left) with a single extraction, in a single method, with a 4-minute analysis time. In the table, **green** indicates the compound was reproducibly measured in all samples of that type; **yellow** indicates detection in only some specimens, and white/blank indicates not observed. Example electropherograms are grouped by molecule class for clarity. Representative matrices were chosen to exemplify the separation.



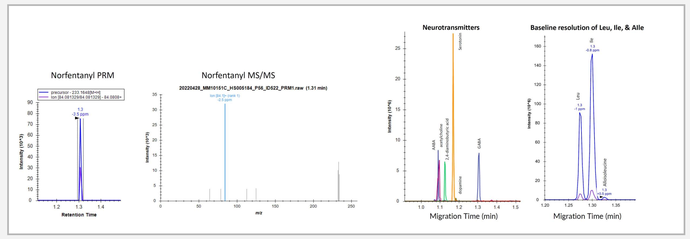
Unique Capabilities

Opioid Detection in Screened Plasma: Prescription pharmaceuticals and illicit drugs are major contributors to metabolite variability in clinical specimens destined for metabolomics analysis, leading to wasted resources in metabolomics data collection or statistical analysis, or incorrect conclusions (L St. John-Williams et al. Sci Data. 2017 Oct 17;4:170140). We included a variety of opioids in our iMT library as a proof-of-concept for drug detection in clinical specimens. During our analysis of 35 randomly selected human plasma and serum specimens obtained from BioIVT, a single sample (ID522) screened as preliminarily positive for norfentanyl (a metabolite of fentanyl) and tramadol.

Below: Peak area for norfentanyl as a function of serum or plasma specimen. Inset shows the norfentanyl peak in specimen ID522 along with mass error and predicted migration time.



The observation of norfentanyl and tramadol in the human serum specimen was corroborated using a targeted MS/MS analysis (ZipChip-PRM) on the Exploris 240. Extracted Ion Electropherogram from the clinical specimen and the MS/MS spectrum is shown below. This suggests that a ZipChip CE-MS metabolomics platform could be used to improve or validate clinical specimen annotation for illicit, prescription, or OTC pharmaceuticals.



Uncommonly-Combined Measurements: As compared to other broad-spectrum, single-shot metabolomics techniques, ZipChip CE-MS offers a unique combination of measurements that can be made simultaneously from the same specimen. Typically, very polar (i.e. amino acids), nonpolar (i.e. sphingosine/sphinganine), and higher molecular weight (peptide) species might require two or more extraction and analysis methods. Because of the separation mechanism of CE-MS, we demonstrate below and at left some of the combined analyses which would be difficult-to-impossible with any single chromatography method.

Conclusions and Next Steps

- Using an indexed Migration Time and accurate mass library of over 300 compounds amenable to analysis by ZipChip CE-HRMS, we detected 252 different small molecules and metabolites in a set of specimens including human serum/plasma, mouse tissues, and 4 cultured cell lines.
- Comparable assays using liquid chromatography would likely require multiple extractions and multiple separation methods; mCE-MS could represent a threefold or more throughput improvement.
- Future automation of quantitative metabolomics data analysis will enable turn-key CE-MS metabolomics pipelines.

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