• METABOLOMICS • CLINICAL APPLICATIONS

Overcoming Clinical Metabolomics Barriers with Remote Sampling and Microchip CE-MS

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Introduction

Remote sampling technologies (such as capillary blood collection onto paper matrices) have the potential to overcome key barriers in clinical metabolomics by enabling non-clinical sample collection. Capillary electrophoresis-mass spectrometry (CE-MS) provides advantages over traditional liquid chromatography-mass spectrometry (LC-MS), by operating at ultra-low flow rates, minimizing matrix effects, shortening run-to-run times, and standardizing electrophoretic mobility for improved laboratory-to-laboratory harmonization. We evaluated the metabolomics analysis of specimens from OneDraw capillary blood sampling with both LC-MS and microchip CE (ZipChip, 908 Devices) for high-throughput metabolomics analysis.

Highlights

- CE-MS provides excellent resolution and high reproducibility detection of polar and ionic metabolites. It requires minimal sample preparation and is well suited to studies involving limited sample volumes, such as with DBS and clinical remnant material.
- Dextromethorphan and its metabolites, dextorphan and dextrorphan O-glucuronide, were measured in CE-MS samples. In contrast, only dextorphan O-glucuronide was detectable via LC-MS.
- Neutral and nonpolar metabolites are a particular weakness of CE-MS. For example, caffeine was not detectable via CE-MS. A 3 hours kinetic analysis of caffeine is presented from LC-MS results.
- A proof-of-concept derivatization sample pre-treatment is explored as a potential technique to expand CE-MS metabolite coverage.

Methods

Plasma, serum, calibrators, and quality control samples were extracted with methanol containing stable-isotope internal standards and ammonium acetate. OneDraw strips containing 75 µL of dried blood "spots" on paper matrices (DBS) were cut into sub-strips, which were extracted in parallel for CE-MS and LC-MS analysis.

CE-MS: Blood strips were extracted with either: (1) the addition of methanol first, or (2) with hydration in water followed by methanol. Compounds were separated using the ZipChip microchip CE system (908 Devices), operating under standardized conditions. MS conditions used 60k MS1 and analyzer-optimized DDA in positive ion mode on a Thermo Scientific Exploris 240 mass spectrometer. The resulting files were processed with the ZipChip metabolomics software package (908 Devices), Skyline, MS-DIAL and custom software.

LC-MS: Blood strips were weighed and extracted with 20x volume prechilled 80:20 (v:v) methanol:water fortified with CIL QC1. Centrifuged extracts were dried and reconstituted in 1:1 ACN:H2O + 0.1% FA fortified with CIL QC2. Reconstitution volume was normalized to strip weight to account for blood sampling differences. LC-MS analysis was carried out with HILIC and C18 methods on an Agilent Infinity II LC system coupled to a Bruker timsTOF 2 operating in AutoMS/MS scanning mode for both positive and negative ionization. Bruker Metaboscape, MS-DIAL, and Skyline Daily were used for feature extraction and statistical analysis.

Results

In this pilot study, a set of pooled male plasma, pooled male serum, and DBS samples from an individual in a metabolomic kinetic study were used to evaluate OneDraw based sampling and microchip CE-MS (ZipChip metabolomics platform) for coverage and reproducibility and to compare/contrast datasets derived from CE-MS and LC-MS. NIST SRM-1950 was also utilized to evaluate quantification accuracy of amino acids results. Within a study pool quality control (SPQC), validated metabolites showed <10% CV and produced PCA with tight grouping in the center of all analyzed samples.

Comparing extraction techniques for the blood samples, we observed a slight positive bias towards improved extraction when adding methanol first, compared to pre-hydration.

Dextromethorphan (DXM) and caffeine drug kinetics were measured over a 3-hour timecourse. DXM and its metabolites, dextorphan (DOR) and dextorphan O-glucuronide (DORGlu), were detectable on CE-MS, while only DORGlu and additionally caffeine were detected from LC-MS.

Despite good performance across a variety of analyte classes, limitations of the current ZipChip metabolomics platform methodologies include the inability to measure molecules like caffeine (neutral at pH 2.3), and negatively charged metabolites. We provide analysis showing the overlap of compounds from LC and CE-MS to demonstrate how these methods complement each other in metabolomic profiling. Finally, we show a proof-of-concept derivatization method for expanding compound coverage of CE-MS.

Acknowledgements

This study was approved by the WCG IRB, approval number WCG IRB Protocol #20240699, approval data 2024-02-28

Conflict of Interest

The authors declare no competing financial interests.

Study Workflow

Analyzing capillary blood collection (DBS) using both capillary electrophoresis (CE) and liquid chromatography (LC)

Dry blood spot/strip (DBS) technology is an exciting tool, providing access to self administered, remote sampling collection of biological samples, with excellent capacity for long term storage at ambient conditions.

CE-MS is well suited for clinical and pharmacokinetic metabolomics, offering minimal sample preparation, small sample volume, and robust quantification. We evaluated the metabolomics analysis of OneDraw DBS in parallel using both CE-MS and traditional LC-MS analyses (**Fig. 1**).

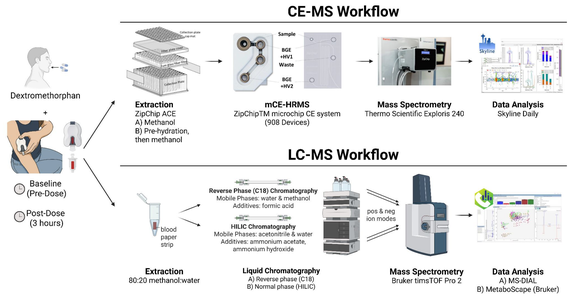

Figure 1: Study workflow with CE-MS (top) and LC-MS (bottom) analyses. Blood was collected onto paper strips using the DrawBridge OneDraw device from a single donor. After drying, paper strips were collected and divided into three portions for analysis with either capillary electrophoresis (CE) or liquid chromatography (LC). Data analysis was performed through Skyline (CE), MetaboScape (LC), and MS-DIAL (both) for untargeted and targeted analyses of QCs and metabolism of exogenous compounds.

CE-MS Quality Assessment

Analyses of internal standards and sample-to-sample variation metrics display high resolution & reproducibility

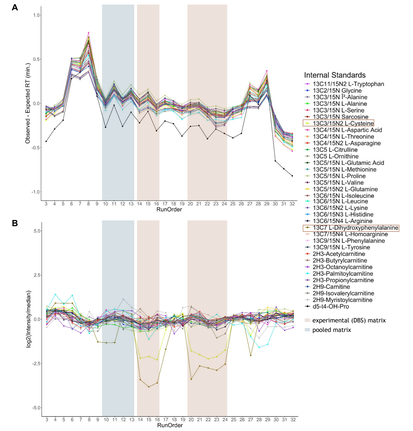

Figure 2: Expected RT drift (A) and intensity variation (B) of CE-MS internal standards across 30 injection batch run. Highlighted areas differentiate the background matrix of experimental samples versus QCs. The two matrix sensitive compounds (B) are boxed in the figure legend.

Pooled male plasma, pooled male serum, and DBS samples from an individual in a metabolomic kinetic study were used to evaluate OneDraw based sampling and microchip CE-MS (ZipChip) for coverage and reproducibility. The calibration standards provided in the kit, plotted in sample run order, show overall consistent retention time and intensity variations (**Fig. 2**). Aligning with expected matrix effects, RT shift variations can be seen between samples and QCs (**Fig 2A**). For signal intensity variation, a two internal standards (dihydroxyphenylalanine and cysteine) notably appear to be uniquely impacted by sample type (**Fig 2B**); we hypothesize that this may be a result of interaction with the OneDraw paper absorbent material.

A set of validated metabolites (155 compounds) were assessed in the study pool quality control (SPQC) with an average of <10% CV. PCA of validated metabolites (**Fig. 3**) showed tight grouping of the SPQC in the center of all samples, as expected. Using the calibration standards provided in the kit, the value assignment for amino acids from NIST (n=10) on average showed 10.6% error for the NIST SRM-1950 (data not shown).

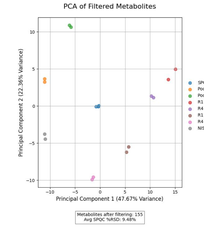

Figure 3: Principal Component Analysis (PCA) grouped by sample type shows SPQC in the center of all samples.

Clinical Metabolomics Kinetics

Dextromethorphan, its metabolites dextrorphan & dextrorphan O-glucuronide, and caffeine

OneDraw-derived DBS were assessed for drug metabolism from a single donor pre- and 3-hours post-consumption of dextromethorphan (DXM) alongside a standard energy drink beverage. DXM and its metabolites, dextorphan (DOR) and dextrorphan O-glucuronide (DORGlu) were measurable in post-dose samples via CE-MS (**Fig. 4**).

Comparing extraction techniques (shown as different colored data points in **Fig. 4**), one-step extraction with methanol improved the extraction efficiency over pre-hydration, resulting in overall larger metabolite signals. This trend is the opposite for some dipeptides, potentially due to unquenched protease activity (data not shown).

We wished to expand the pre-/post-dose drug kinetics with additional sampling times that were available for LC-MS analysis. Of the DXM metabolites, DORGlu was detectable via LC-MS, but the parent DXM and intermediate DOR were not. A potential reason for absence of DXM and DOR from LC-MS results may be due to a lower instrument sensitivity in the LC-MS workflow, leading to sample signals falling below the LOD. DORGlu kinetics show increasing signals both at 60 min and 3 hour timepoints, indicating a multi-hour kinetic profile (**Fig. 5A**).

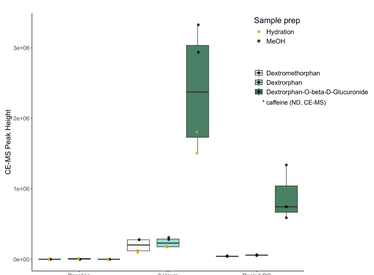

Figure 4: Dextromethorphan metabolism. DXM, DOR, and DORGlu were identified using CE-MS. Peak height is shown from baseline (pre-dose) and 3-hours post dosing alongside SPQC. Caffeine was not detected on CE-MS.

Results in **Fig. 5** show LC-MS signals normalized to tryptophan internal standard as ($Trp_{sample}/Trp_{baseline}$) to preserve the relative C18/HILIC intensities. Additionally, LC-MS allowed measurement of caffeine kinetics. For caffeine, we see a faster metabolism profile than DORGlu (**Fig. 5B**), capturing the concentration peak at 60 min and beginning decline at the 3 hour time point. These results align with the reported 3-6 hour half-life for both DXM and caffeine. As DORGlu is the product of DXM, its concentration peak would be delayed from the parents products, as we see here.

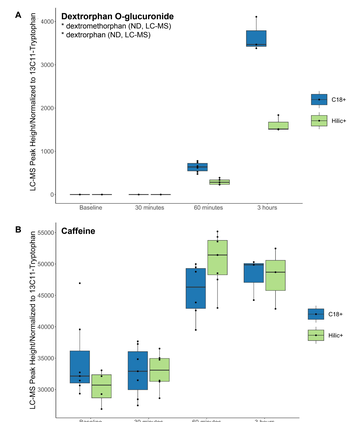

Figure 5: Expanded kinetics timepoints of dextrorphan O-glucuronide (A) and caffeine (B) via LC-MS. Both C18 and HILIC chromatography results are included side-by-side for each compound.

Limitations

Metabolite coverage of LC and CE varies, Specific considerations for neutral and anionic species

CE-MS (electrophoretic mobility) and LC-MS (hydrodynamic forces) have notable analytical differences, resulting in unique chemical compatibilities. To investigate chemical coverage overlap from the starting perspective of an LC-MS metabolomics lab, we generated compound lists for each chromatography channel (C18+ & HILIC+) from DBS samples through high-confidence MS/MS library matching in MetaboScape. Combining lists produced 239 unique compounds, which were targeted in Skyline to verify presence/absence of peaks. **Fig. 6** summarizes the overlap for these LC-biased compounds in each method. The compound overlap is quite modest (~½), highlighting the complementary nature between LC and CE. The limitation of this approach is that the CE unique compounds are not captured, and an inverse approach would likely yield similar CE-biased results.

Most commercial CE-MS systems are optimized for cationic analyte separation. For ZipChip, neutrals and anions divert to waste prior to the MS. Adaptation for MS negative mode through background electrolyte and interface polarity modification requires significant re-optimization that may compromise metabolite coverage. While techniques like pressure loading allow for broader sample introduction, detection remains biased due to system chemistry favoring cation migration. As a potential solution, we tested a proof-of-concept derivatization workflow using 4-hydrazinyl-n,n-dimethylaniline (4-HDMA) for the detection of short-chain fatty acids and TCA cycle intermediates (**Fig. 7**). This technique produced ions with mass M+119.0968 for 9 SCFA and TCA cycle metabolites that were otherwise not detectable via CE-MS. Of these compounds, some were not readily detectable via LC-MS, further supporting the strengths of this CE-MS approach.

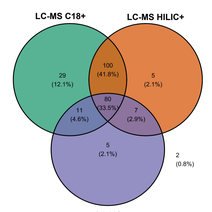

Figure 6: Compound overlap between LC and CE methods from an LC-biased target list.

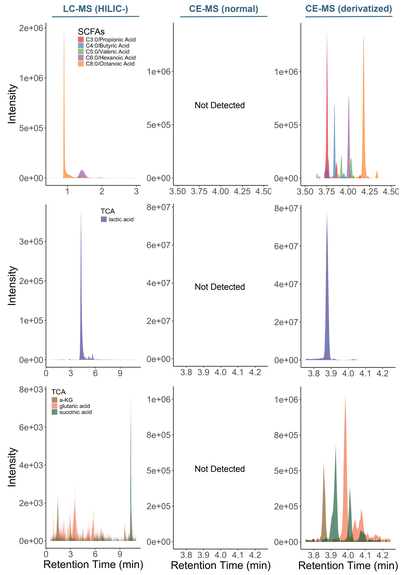

Figure 7: LC-MS (left) and CE-MS (middle) of SCFAs and TCA cycle intermediates alongside CE-MS from a derivatized DBS sample (right).

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