Accurate Mass Analysis of Components Eluting from Dried Blood Spots

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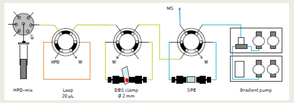
1 Introduction

Dried blood spots have drawn increasing attention as a versatile means of sample collection to support pharmacokinetic studies, with many distinctive advantages. The mass spectrometric analysis of compounds eluted from dried blood spots (DBS) is a well accepted technique, with the majority of analyses using multiple reaction monitoring on triple quadrupole instruments. Under these conditions, only selected molecules of interest are detected, while other components from the dried blood spots are not analyzed. However, identification of these components may benefit the development of new clinical assays, or lead to improvements in the performance of established assays.



Schematic diagram of the Spark Holland dried blood spot and solid phase extraction device (above). PerkinElmer AxIon 2 TOF mass spectrometer (right).

2 Materials and Methods

A prototype dried blood spot (DBS) clamping device (Spark Holland) was used to investigate components extracted from DBS. The device clamps a circular (2 mm) region of the DBS providing a leak-tight region for flow-through desorption. Solid phase extraction was performed with an ACE™ automatic cartridge exchanger and a HPD™ high pressure dispenser (Spark Holland). Internal standard was added by loop injection from the online DBS device.

Accurate mass electrospray time-of-flight (TOF) MS analysis was performed with a PerkinElmer AxIon 2 TOF mass spectrometer, enabling high resolution (>12,000 FWHM) and mass accuracy up to 2 ppm. Gradient elution was provided by a PerkinElmer Flexar UHPLC analytical pump.

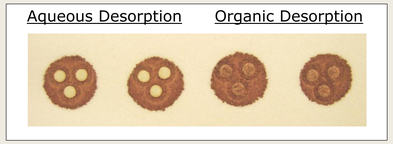
HILIC separations were performed on a Brownlee SPP HILIC 2.1x5 mm (2.7 µm) guard cartridge (amino acids, lipids, electrolytes) or the same guard cartridge inline with a Brownlee SPP HILIC 2.1x50 mm column (acylcarnitines). Mobile phase A was water with 0.1% formic acid (FA) and 10 mM ammonium formate, and B was acetonitrile with 0.1% FA. DBS cards were desorbed at 0.7 ml/min for 0.75 min directly to the column, which was then switched out of line from the DBS card and a gradient of 95-75% B over 4 min was run at the same flow rate. PerkinElmer NeoBase™ high level control cards were used for HILIC DBS samples.

2 Materials and Methods (cont.)

Reversed phase separations were performed with a 10x2 mm HySphere 7 µm C18 HD SPE cartridges (Spark Holland) and a Brownlee SPP 2.1x50 mm C18 (2.7 µm) column (PerkinElmer) fitted with a 2.1x5 mm guard column. Mobile phase A was water + 0.1% FA, and B was methanol + 0.1% FA. The SPE cartridge was switched in-line with the column and eluted with a gradient of 5-100% B over 9.5 min. DBS cards were prepared from whole blood spiked with 80 ng/ml nortriptyline. Nortriptyline d3 internal standard (10 µl of 20 ng/ml stock in 5% methanol + 0.1% FA) was added by loop injection from the online DBS device during DBS desorption.

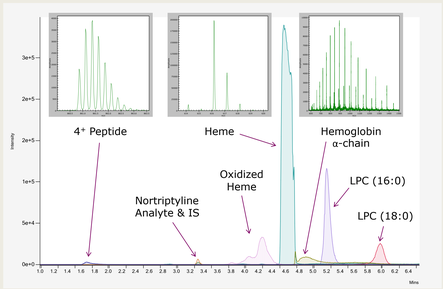
3 Dried Blood Spot Desorption

Dried blood spots were desorbed with either high aqueous solvent (5% methanol : 95% water + 0.1% FA) for reversed phase separations or high organic solvent (95% acetonitrile : 5% water + 0.1% FA) for HILIC separations. The figure below shows DBS that were desorbed with either high aqueous or high organic solvents.



4 Reversed Phase Separation

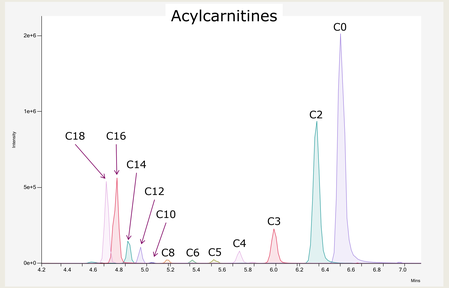
Desorption of DBS with high aqueous solvent followed by reversed phase separation yielded abundant heme B ($C_{34}H_{32}N_4O_4Fe$). Also identified were iron-containing heme degradants such as oxidized heme, hemoglobin α and β subunits, peptides, lipids, and nortriptyline at a therapeutic level (80 ng/ml).

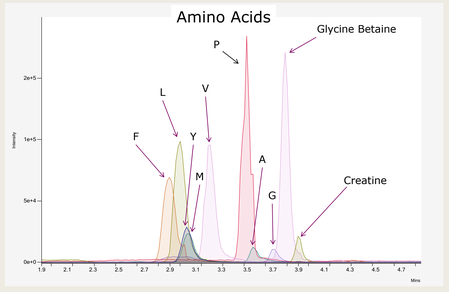


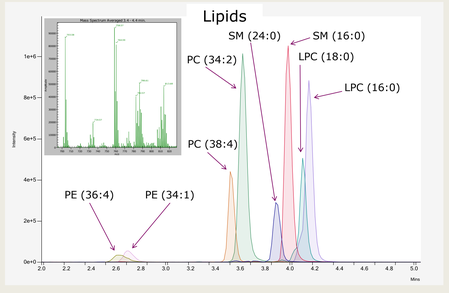
Base peak chromatograms of selected components desorbed from DBS with high aqueous solvent and analyzed by reversed phase LC-TOF-MS.

5 HILIC Separation

Desorption of dry blood spots with high organic solvent combined with HILIC separation (below) showing detection of components with widely varied polarities: acylcarnitines (top panel), amino acids (middle panel), lipids (bottom panel). The ratios of amino acid and acylcarnitine peaks are in agreement with the ratios of the spiked levels, showing that all components are desorbing from the paper.



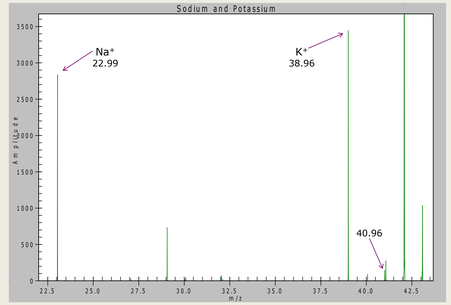




HILIC separation of acylcarnitines, amino acids, and lipids. PC, phosphatidyl choline; LPC, lysophosphatidyl choline, PE, phosphatidyl ethanolamine. C0, free carnitine; C8, octanoylcarnitine; C18, octadecanoylcarnitine.

5 HILIC Separation (cont.)

Dried blood spot components were separated by HILIC chromatography and the low mass region of the TOF MS spectrum was monitored for the presence of metal ions.



Mass spectra averaged from 3.1 to 3.3 min during LC-MS analysis.

5 Summary

- The online DBS system has the advantage of avoiding paper punching and offline extraction. Addition of internal standards is readily incorporated.
- High resolution and high mass accuracy TOF MS analysis allows for simultaneous detection of a large number of analytes without loss of sensitivity. Examples include: metal ions, metabolites, therapeutics drugs, peptides, and proteins.
- TOF MS analysis, in contrast to MRMs, allows for retrospective analysis of data to identify novel or unexpected compounds which would be of benefit, for example, in determining interferences, troubleshooting matrix effects, or identification of drugs of abuse.
- Using a combination of reversed phase and HILIC separation allows for detection of compounds with widely varied molecular weights and polarities, using a single flexible analytical system.

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