Overview

- Paper spray MS provides rapid biological sample screening with minimal sample preparation
- A self-contained paper spray cartridge with built-in plasma fractionation capability would aid in rapid analysis
- This cartridge would require a membrane capable of obtaining plasma from whole blood without causing cell lysis or changing the concentration of drug.

Introduction

- Paper spray mass spectrometry
  - The spray substrate is a wedge of paper with a macroscopic point
  - Solvent is applied to the paper and an applied voltage produces a cone of charged solvent droplets similar to ESI
- Analysis of dried blood spots
  - As the solvent wicks through the wedge it elutes analytes of interest that can be detected in via spray ionization (figure 1)
  - MS/MS signal is integrated for the duration of the spray (figure 2)
- Paper spray MS was conducted from a plastic cartridge by adding solvent to top of cartridge and applying a voltage

Results

- Extracted plasma was evaluated for red blood cell lysis
  - Hemoglobin was measured by analyzing the fractionated plasma by nano-drop UV/VIS
  - A calibration curve was created by spiking centrifuged plasma with whole blood
  - Samples were extracted and their absorbance was measured around 413 nm
- Drug levels were measured by paper spray MS in both centrifuged plasma and membrane fractionated plasma
  - A measurable drop in the ratio between the analyte and internal standard indicated drug binding
- The Cytosep membrane was selected for further testing
  - To improve separation the membrane was treated with both alum and human fibrinogen
  - The set of analytes was expanded to evaluate what properties lead to increased drug binding
  - A quantitative experiment was performed on subset of analytes shown to be unaffected by extraction

Methods

- Evaluating different membranes
  - Vivid GR polysulphone membrane, Noviplex plasma prep card and grade 1660 Cytosep membrane were evaluated
  - Plasma extraction was carried out by spotting drugged blood on a membrane with a 3 mm paper collection punch beneath
  - An isotopically labeled solution was added to the dried plasma spot
  - Concentrations were determined by paper spray MS
  - Paper spray MS was conducted from a plastic cartridge by adding solvent to top of cartridge and applying a voltage

- Evaluating red blood cell separation efficiency
  - Vivid GR and Noviplex card showed negligible amounts of red blood cell lysis
  - Cytosep membrane showed around 7.6% red blood cell concentration

- Evaluating drug binding
  - Significant decreases in drug concentration were observed for the Noviplex and Vivid GR membranes
  - Cytosep membrane was selected for further testing
  - The relatively high red blood cell concentration of the Cytosep membrane extracted plasma needed to be addressed
Evaluating effects of agglutination agents

- The Cytosep membrane was treated with two agglutination agents to improve red blood cell retention
- Alum and human fibrinogen were evaluated at various concentrations
- Alum improved retention but reproducibility of plasma amount was poor
- Fibrinogen reduced red blood cell penetration to negligible levels but induced drug binding

Conclusions

- While Noviplex cards and Vivid GR membranes can extract plasma from whole blood with minimal red blood cell lysis, the analyte concentrations in the plasma are significantly different than plasma obtained by centrifuge
- Agglutination agents can be added to Cytosep membranes to reduce lysis, but, it is difficult to find a concentration that significantly reduces lysis while not inhibiting extraction or causing drug binding
- The untreated Cytosep membrane was found to give similar results to centrifuged plasma for analytes with a KB/P value near 1 and a logP value below 3
- The mass of extracted plasma was found to be reproducible for a range of volumes of whole blood
- Atenolol and carbamazepine were found to be measurable in membrane extracted plasma using a paper spray cartridge with the Cytosep membrane built in
- Additional work needs to be done to improve understanding of drug binding

Works Cited