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Determination of Centanafadine and Lactam Metabolite in a Comparison of Dried Plasma Spot vs. Exact-Volume Plasma Fractionation in a Microfluidic Device

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OVERVIEW

PURPOSE

To investigate the application of Dried Plasma Spot (DPS) vs. Microfluidic "all-in-one" capillary microsampling for the determination of centanafadine and its lactam metabolite.

METHOD

Human blood was sampled onto either Novilytic™ Duo Plasma Prep cards or collected by capillary microsampling using the Shimadzu MSW^2 microfluidic device. Collection discs from the plasma prep cards were allowed to dry 24 hr in the presence of desiccant prior to homogenization in optimized extraction solvent containing SLIS (d_6-centanafadine and lactam). MSW^2 capillary devices were centrifuged and fractionated plasma carefully removed at the scored zones representing a volumetric collection of 5.6 µL. Plasma was washed out of the device in extraction solvent containing SLIS. Analysis of extracts was performed by LC-MS/MS in multiple reaction monitoring mode under +ESI conditions.

RESULTS

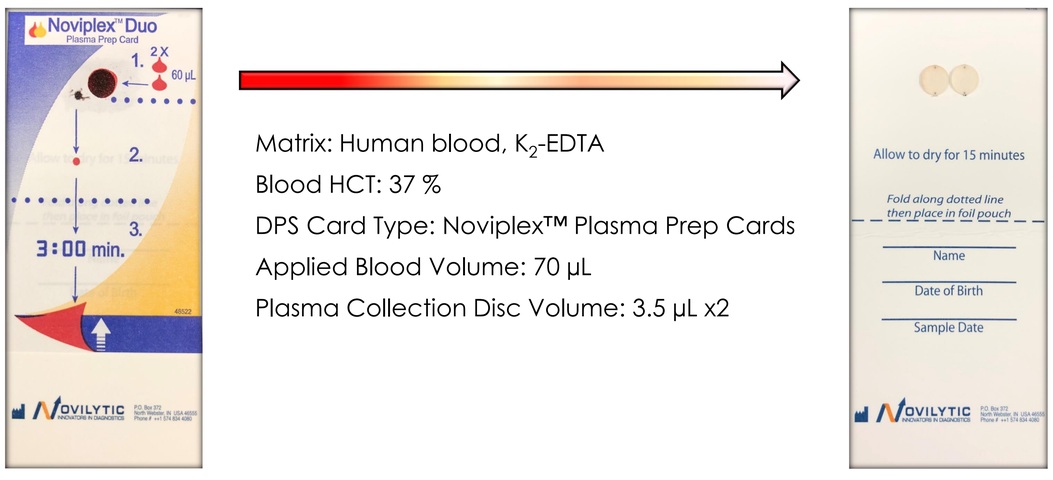
Recoveries > 90% from DPS facilitated an LOQ of 5.0 ng/mL (140 fg on-column, S/N > 15:1) for each of centanafadine and its lactam metabolite, with all acceptance criteria being met for within-run precision and accuracy supporting a calibration range three-orders of magnitude (**Tables 1 and 2**). Similar sensitivity and assay performance could be achieved from MSW^2 microsamples when extracting 5.6 µL of centrifugal plasma (**Figure 4**).

INTRODUCTION

Recent advances in microsampling techniques for the generation of plasma include blood hematocrit (HCT) removal membranes and microfluidic technology, which supports both blood collection and exact-volume plasma harvesting within the same device. The latter approach obviates the requirement for transfer of centrifugal plasma to exact-volume capillaries, a difficult and arduous sample processing task prone to procedural error. In the current research, centanafadine and its lactam metabolite were selected to evaluate each approach to plasma generation, as represented by Noviplex™ Plasma Prep cards for blood HCT removal and Shimadzu MicroSampling Wings (MSW^2) for microfluidic blood collection and plasma processing.

METHODS

DRIED PLASMA SPOT CARD PREPARATION



Matrix: Human blood, K_2-EDTA
Blood HCT: 37 %
DPS Card Type: Noviplex™ Plasma Prep Cards
Applied Blood Volume: 70 µL
Plasma Collection Disc Volume: 3.5 µL x2

Figure 1. Dried plasma spot card preparation. Following a 24 hour drying period in the presence of desiccant, both plasma collection discs from a single blood aliquot were carefully removed and placed in a 96w plate for extraction.

DRIED PLASMA SPOT EXTRACTION

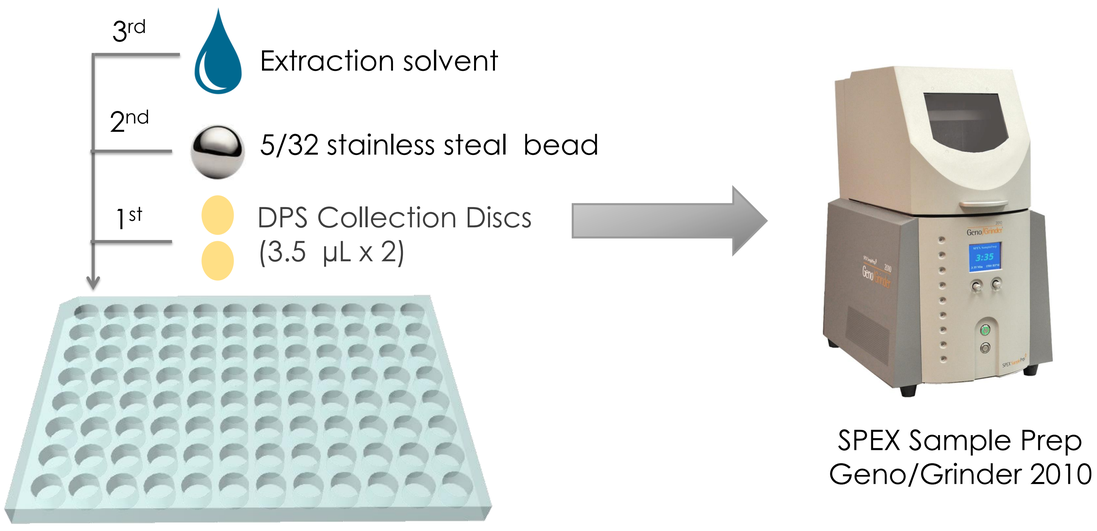


Figure 2. Order of addition and apparatus required for the homogenization of DPS collection discs. Optimal extraction solvent was MeOH:H_2O (4:1; 250 µL) for homogenization at 1750 rpm for 10 minutes. Prior to homogenization, DPS collection discs were pre-soaked in extraction solvent for 20 minutes at 25°C, 500 rpm. Following homogenization, 96w plates were centrifuged and a 50 µL aliquot of supernatant diluted with 200 µL of water.

MICROSAMPLING WING PREPARATION AND EXTRACTION

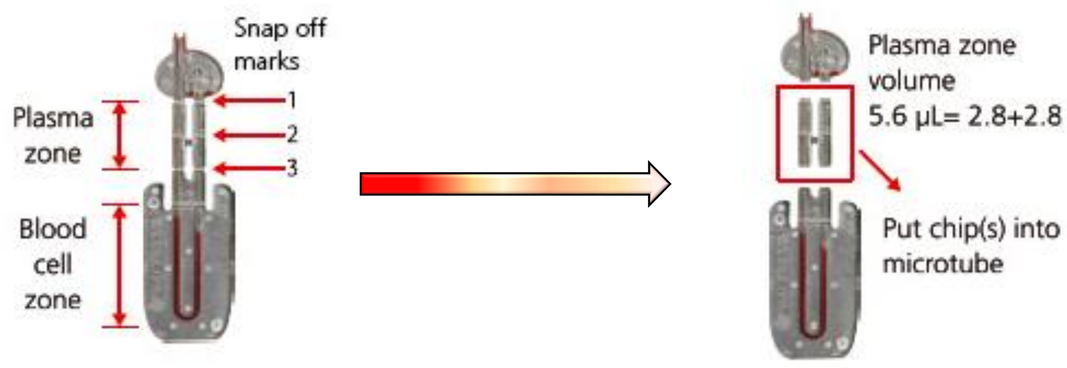


Figure 3. Shimadzu Microsampling Wing (MSW^2) microfluidic device constructed of an olefin polymer whose capillary is treated with K_2-EDTA. Whole blood (23 µL) was collected and the device centrifuged (*2000g*, 10 min) with 5.6 µL of plasma removed between volumetric zones 1 and 3. Harvested plasma was placed into a 96w plate and washout solution containing SLIS added (MeOH:H_2O, 4:1; 500 µL). The well plate was mixed for 60 sec at 1800 rpm using the SPEX Geno/Grinder 2010. Following centrifugation, an aliquot of extract supernatant (100 µL) was diluted with H_2O (200 µL).

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RESULTS

TABLE 1: CENTANAFADINE WITHIN-RUN PRECISION AND ACCURACY
6 replicates/QC level; blood HCT 37%; 5.00 – 1500.0 ng/mL

Statistics	LOQ QC (5.00 ng/mL)		QC1 (15.0 ng/mL)		QC2 (750.0 ng/mL)		QC3 (1125.0 ng/mL)	
	DPS	*MSW^2*	*DPS*	*MSW^2*	*DPS*	*MSW^2*	*DPS*	*MSW^2*
% C.V.	9.0	3.3	4.2	6.1	4.8	3.4	4.4	4.1
% Nominal	100.8	110.1	95.4	94.7	106.3	105.4	103.3	90.9

TABLE 2: CENTANAFADINE LACTAM WITHIN-RUN PRECISION AND ACCURACY
6 replicates/QC level; blood HCT 37%; 5.00 – 1500.0 ng/mL range

Statistics	LOQ QC (5.00 ng/mL)		QC1 (15.0 ng/mL)		QC2 (750.0 ng/mL)		QC3 (1125.0 ng/mL)	
	DPS	*MSW^2*	*DPS*	*MSW^2*	*DPS*	*MSW^2*	*DPS*	*MSW^2*
% C.V.	9.4	5.9	9.4	8.0	7.3	2.9	3.9	4.2
% Nominal	96.1	105.3	96.1	97.4	103.5	106.1	101.3	94.1

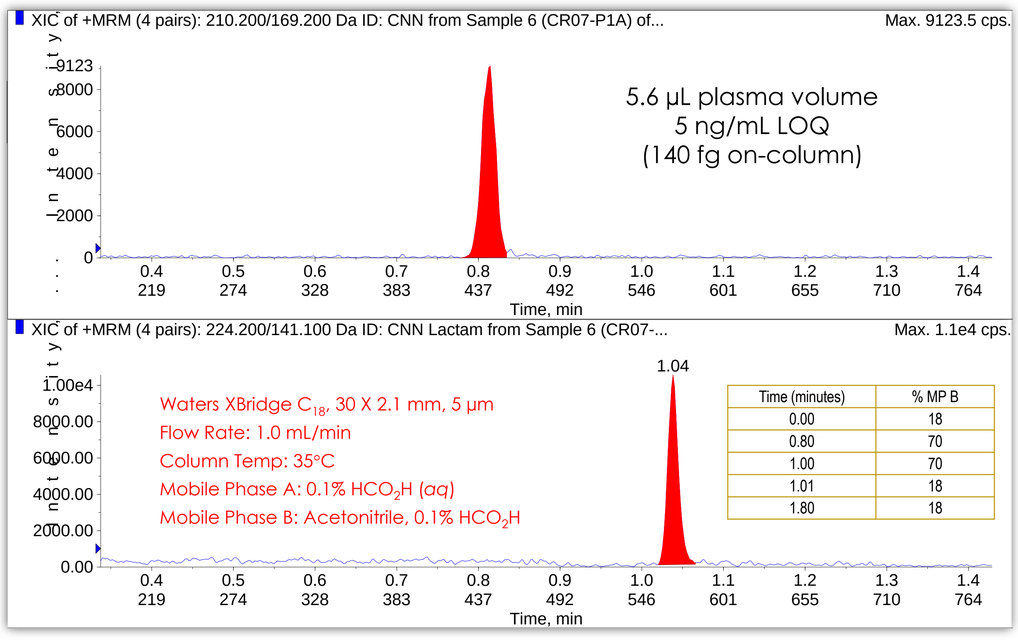

FIGURE 4: EXTRACTED LOQ RESPONSE FROM MSW2

DRIED PLASMA SPOTS

- Requires high blood volume (60 µL minimum)
- Collection discs amenable to homogenization with > 90% recovery for centanafadine and lactam metabolite
- Occasional blood breakthrough noted
- LC-MS/MS optimization allows LOQ detection from only 7 µL of DPS matrix; 3.5 µl feasible with larger injection volume
- Recommended bridging study vs. centrifugal plasma to demonstrate HCT filtration does not introduce quantitation bias
- Future experiments include:
  - Impact of blood HCT on recovery
  - Applicability of DPS when filtered plasma is hemolyzed
  - Correlation of DPS with centrifugal plasma
  - Confirmation of equivalent sampling volume between collection discs

CAPILLARY MSW^2

- Exceptionally easy to implement for sample collection; only 23 µL blood required
- Processing somewhat more laborious than DPS at collection site due to centrifugation requirement, however collected samples can be frozen and the plasma zone removed at the bioanalytical lab
- Rapid sample preparation consisting of washout in extraction solvent containing SLIS with subsequent dilution to mobile phase conditions; > 90% recovery
- No HCT bias on retrieved plasma zone volume, but analyte concentration in plasma increases with increasing HCT as expected. Between 30 – 50% blood HCT, quantitation bias is within acceptance criteria
- LC-MS/MS optimization allows LOQ detection from only 5.6 µL of plasma; assay sensitivity will support 2.8 µL of plasma allowing for sample replicates
- Centrifugal plasma microsamples more likely to correlate with established data

CONCLUSIONS

Both DPS cards and MSW^2 capillary microsampling devices offer volumetric collection of plasma, albeit with the MSW^2 device requiring ca. 1/3 the blood volume of DPS. For each assay, within-run precision and accuracy data met all acceptance criteria with LOQ S/N's > 15:1. In comparison to traditional capillary microsampling requiring exact volume transfer to a second capillary tube, the blood sampling, fractionation and volumetric collection of plasma was enormously simplified using the "all-in-one" MSW^2 microfluidic device.

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