An Improved Platform for the Recovery and Analysis of Cannabinoids from Dried Blood Samples

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Introduction

The use of dried blood spots (DBS) from a finger stick to measure blood levels of cannabinoids could be a simple and cost-effective means to determine exposure levels from cannabis consumption. Two recent papers (1,2) reported analysis of delta-9-tetrahydrocannabinol (THC) and major metabolites from dried blood spots collected on 903™ Cards. We undertook a comparison of the performance of two cotton based absorbents; HemaSpot-HF™ and the 903™ Card as well as two glass fiber based membranes; Agilent's DMS™ Card and the HemaSpot-SE™. Our effort involved recovery and analysis of the three major constituents of *Cannibis*; delta-9-tetrahydrocannabinol (THC), cannabinol (CBD and cannabindiol (CBD).

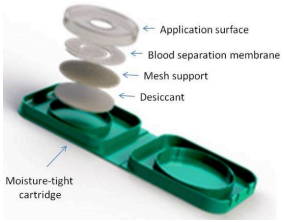

Figure 1. Exploded View of the HemaSpot-SE™

Figure 2. Spiral membrane showing location of plasma punches.

The HemaSpot-SE™ device (Figure 1) contains a glass fiber membrane cut into a spiral shape (Figure 2) that separates cellular components of whole blood (WB) from plasma.

Methods

Sample Preparation for recovery trials: Fresh blood (495 µL) from volunteers was spiked with 5 µL of a methanolic solution of each of the analytes to give three QC levels of 1000, 125 and 15.625 ng/mL.

- Blood samples gently mixed 15 minutes. 40 µL of each QC level applied to absorbent.
- Dry 4 hours, collect whole blood spot, extract with 500 µL mixture of ACN/H2O/FA (90/10/0.1%) containing all three internal standards (IS) at 5 ng/mL. Sonicate 30 minutes, vortex overnight at ambient temperature.
- Decant and evaporate to dryness at 30 ºC under a stream of N2.
- Add 50 µL extraction mixture without IS, vortex 15 minutes, centrifuge, analyze.

Sample Preparation for correlation trials: Fresh blood was spiked with the three analytes to give 9 standard concentrations ranging from 3.9 ng/mL to 2000 ng/mL.

- Standard solutions applied to the HemaSpot-SE (150 µL) device, allowed to wick out briefly then sealed and dried over one or two nights.
- Two 6-mm punches removed from the plasma portion of the SeraForm for extraction using 500 µL of the above IS extraction solution.
- Controls of 40 mL each of the QC WB samples were treated in the same manner.

HPLC Analysis: Chromatographic system; Shimadzu SIL-HT autosampler, LC-AT10 pumps. Phenomenex Kinetex C8 Column (50 X 2.1 mm, 2.6 um), mobile phase A (MPA): 0.5% formic acid in water and (MPB): 0.5% formic acid in acetonitrile, gradient elution from 20% MPB, 1 minute, ramping to 90% over 7 minutes followed by a 1 minute hold before returning to 20% MPB and re-equilibrating.

Mass spectrometry: Waters (Milford, MA, USA) Micromass Quattro Ultima triple quad mass spectrometer. Acquisitions carried out in multiple reaction monitoring scan mode using positive electrospray ionization (ESI+). Instrument parameters; 3.0 kV capillary voltage; 120 ºC source temperature; 220 ºC desolvation temperature; 500 L/hr desolvation gas flow; 40 V cone voltage. Analyte specific values are given in Table 1.

Analyte	Q1 Mass (m/z)	Q3 Mass (m/z)	CE (eV)
CBD	315.5	193.1	20
CBD-*d*3	318.6	196.0	20
CBN	311.5	222.5	25
CBN-*d*3	314.6	223.5	25
THC	315.5	193.1	20
THC-*d*3	318.6	196.0	20

Table 1. MS/MS parameters used for cannabinoids and their deuterated standards.

Results

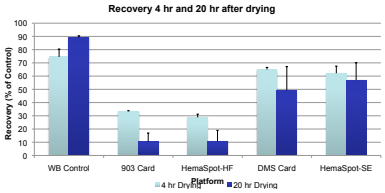

Figure 3. Improved recovery from glass based membranes

[a]Whatman 903™ Protein Saver Cards, [b]HemaSpot-HF™ Device, [c]Agilent DMS Cards, [d]HemaSpot-SE Device

Figure 3 shows how the average recovery of the three cannabinoids at the three QC levels from the two cotton based matrices was only 30% of the amount applied after only 4 h drying. This recovery dropped further to only 10% after 20 h drying.

Recovery from the two glass based membranes remained better than 50% over the two time periods.

Figure 4 shows the correlation of the HemaSpot-SE punches from two separate donors, tested after one day drying and again after 6 week storage and compared to their wet plasma controls. Recovery of the three analytes from multiple 6 mm punches of the dried plasma was found to correlate well (>99%) with the wet plasma standard curve when corrected for plasma volume.

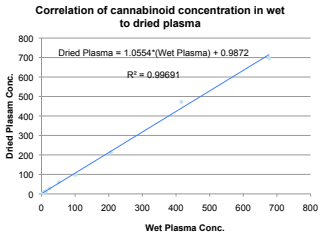

Figure 4. Dried plasma from HemaSpot-SE show a high correlation to wet plasma.

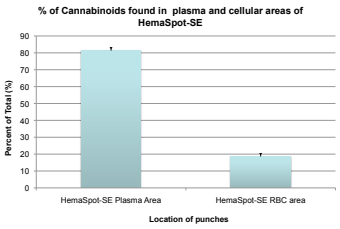

Figure 5. Cannabinoids are present mainly in plasma and not in the cells.

Figure 5 shows distribution for three cannabinoids between the plasma and the cellular portions of the separated WB spot. Spiked whole blood samples (150 uL) collected on HemaSpot-SE devices had 6 mm punches pulled from both areas of the forms. The amount of cannabinoids found in the plasma portion of the form was 4 times that collected from the cellular portion.

Conclusions and Next Steps

- Cannabinoids show good recovery from glass based membranes when compared to cotton-based filter paper matrices
- Dried plasma separated on HemaSpot-SE devices shows high correlation with wet plasma for cannabinoid quantitation
- Recovery of the three cannabinoids remained high after several weeks when recovered from the plasma portion of HemaSpot-SE
- Samples (ca. 100) collected from volunteers will be analyzed and compared to results from plasma assay.

References

1. L. Mercolini, R. Mandrioli, V. Sorella, L. Somaini, D. Giocondi, G. Serpelloni, M.A. Raggi, "Dried blood spots: Liquid chromatography-mass spectrometry analysis of delta-9-tetrahydrocannabinol and its main metabolites", Journal of Chromatography A, 1271 (2013) 33-40
2. A. Thomas, H. Geyer, W. Schanzer, C. Crone, M. Kellmann, T. Moehring, M. Thevis, "Sensitive determination of prohibited drugs in dried blood spots (DBS) for doping controls by means of a benchtop quadrupole/Orbitrap mass spectrometer", Anal Bioanal Chem (2012) 403: 1279-1289.