Evaluation and Limitations of Dried Plasma Spot (DPS) Testing for Opioids

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INTRODUCTION

Opioid overdoses in the United States have been on the rise for several decades. This has caused many forensic laboratories to become overwhelmed with the number of opioid cases to process. In order to quicken the analysis process, some laboratories have looked to alternative specimen collection. Alternative matrices like urine and oral fluid have been being used for many years in clinical and forensic toxicology testing. Dried plasma spots (DPS) have begun to be investigated for clinical testing but with little research investigating their applicability in forensic drug testing. DPS collection may be a quick and easy collection method that does not require a trained collector like a traditional blood draw. This study focused on the extraction and detection of 9 opioids in dried plasma spots using liquid-chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) and Telimmune™ dried plasma spot cards.

MATERIALS AND METHODS

Extraction protocol:

- 250 µL of blood + 25 µL of standard
- 50 µL applied to card and dried for 3 mins
- Top layer removed and disc dried for 10 mins
- +1 mL of extraction solvent and 10 µL of ISTD
- Vortex, sonication, vortex, and transfer to clean tube
- Dry down and reconstitute in 25 µL of mobile phase

Extraction efficiencies calculated for analytes:

- 8 extraction solvents evaluated for all compounds
- 3 lots of blood assessed (to troubleshoot morphine):
  - Commercial (unopened and opened tubes)
  - Freshly collected

Fresh whole blood collection:

- 28 Gauge needle with lancing device
- Max depth on device (level 6) with side finger puncture
- Blood allowed to flow freely into a microcentrifuge tube then fortified for immediate analysis

LC Conditions	
Mobile Phase	A: 0.01% FA with 5mM AF in DI water B: 0.01% FA in ACN
Column	Agilent Poroshell 120 EC-C18 (100 mm x 2.1 mm x 2.7 µm)
Flow Rate	0.4 mL/min
MS Conditions	
Acquisition	Targeted MS/MS Positive ESI mode
Drying Gas	300°C 13 L/min
Sheath Gas	300°C 12 L/min

RESULTS AND DISCUSSION

Table 1: Extraction efficiencies for each extraction solvent for all analytes.

	1% FA in 50:50 MeOH:ACN	1% FA in ACN	1% FA in MeOH	ACN Only	MeOH Only	50:50 MeOH:ACN	1% NH_4OH in 50:50 MeOH:ACN	EtOAc
Drug	**% Efficiency**	**% Efficiency**	**% Efficiency**	**% Efficiency**	**% Efficiency**	**% Efficiency**	**% Efficiency**	**% Efficiency**
HYM	2.3%	ND	1.9%	ND	2.4%	ND	ND	0.6%
MOR	ND	ND	ND	ND	ND	ND	ND	0.4%
HYC	3.2%	1.0%	1.9%	1.3%	2.9%	1.6%	ND	1.0%
COD	5.2%	1.1%	2.9%	1.6%	3.6%	1.5%	ND	1.7%
OXM	2.7%	ND	2.7%	1.4%	3.0%	ND	ND	0.9%
MET	20.6%	2.1%	3.8%	2.3%	2.3%	2.3%	1.4%	3.8%
OXC	5.3%	1.5%	3.4%	2.1%	4.1%	1.8%	ND	2.0%
6AM	1.8%	ND	1.0%	ND	1.6%	ND	ND	0.6%
BUP	1.3%	ND	1.2%	0.8%	1.3%	ND	ND	0.7%

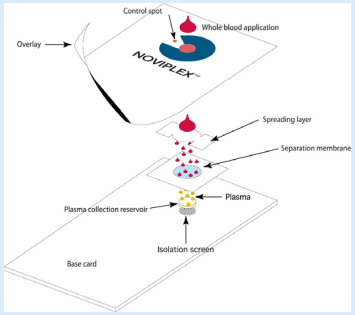

Figure 1: Layout of a Telimmune™ DPS card.

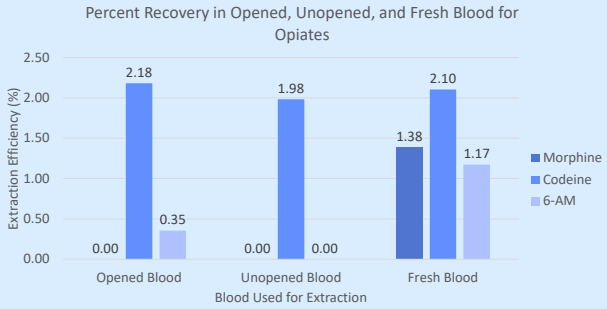

Figure 2: Percent (%) recoveries of opiates using different bloods.

Key results per experiment:

- Extraction solvents for all analytes:
  - Every analyte, except morphine, extracted with at least 4 out of the 8 solvent systems tested.
  - EtOAc was the only solvent able to extract morphine from the DPS disc.
- Commercial blood (opened and unopened) vs fresh blood for opiates:
  - As commercial blood aged, morphine could no longer be extracted.
  - The use of fresh blood increased the recovery of morphine from 0.4% to 1.3%
  - The hemolysis that may occur with aged blood may affect the removal of the red blood cells via the separation membrane and spreading layer. Morphine is affected by this and may get captured in the one of the top layers.
  - Collection of fresh blood may mitigate the hemolysis issue and allow for more accurate detection.

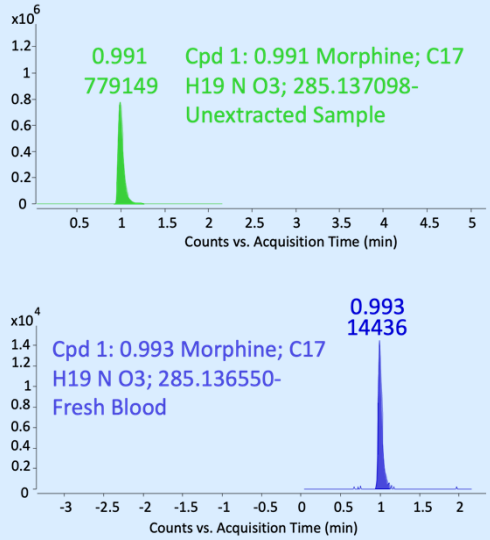

Figure 3: Extracted ion chromatograms for morphine unextracted (left) as compared to morphine extracted with EtOAc in fresh blood (right).

CONCLUSIONS

- DPS may be used as a quick and easy collection method, but using DPS for forensically relevant drugs may demonstrate challenges.
- Commercial blood may hemolyze, causing challenges for the DPS filter to properly remove red blood cells.
- Freshly collected whole blood may mitigate hemolysis but could be difficult to obtain for use in validation studies.
- These results indicate that DPS would not be suitable for postmortem casework.
- More research is required to gauge the utility of DPS in driving under the influence of drug (DUID) cases.

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