An Evaluation and Comparison of the Evidence MultiSTAT to GC/MS for the Detection of Morphine, Codeine, and Thebaine in Urine and Oral Fluid

Amanda L. Pacana[1], MSFS; Stacie Terry, MS; Madeleine J. Swortwood[1,2], PhD; Britni N. Skillman[1], PhD

[1]*Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340*

[2]*Robson Forensic, Denver, CO 80246*

INTRODUCTION

With the ongoing opioid epidemic in the United States, the "poppy seed defense" has become a tactic for explaining away opiate positive samples. One proposed method to differentiate poppy seed vs opiate or heroin use is to use thebaine as a biomarker as thebaine is prevalent in the opium poppy but unlikely to be seen after heroin use[1]. Research has shown that poppy seed consumption can produce positives for presumptive and confirmatory tests, prompting the SAMHSA cutoff to increase from 300 ng/mL to 2000 ng/mL for opiates in urine[2].

The Randox Evidence MultiSTAT is a platform with available opiate screening assays that exhibit limited cross-reactivity with thebaine[3,4]. This study used the Evidence MultiSTAT to analyze authentic human urine (UR) and oral fluid (OF) specimens after poppy seed ingestion. Samples were also analyzed using a developed and validated (ASB 036) GC/MS method with SPE. By comparing these two platforms using authentic samples, laboratories can make an informed decision on screening methods. This research also provides insight into the utility of thebaine as a poppy seed biomarker in two different matrices.

CONCLUSIONS

- The Randox **Evidence MultiSTAT is capable of detecting opiates** after the consumption of poppy seeds
- **Thebaine poses analytical challenges** in terms of stability and performance in assays combined with other opiates
- **Alternative methods should be considered**, such as:
  - A thebaine-only confirmatory method
  - An LC-MS/MS confirmatory method

ACKNOWLEDGEMENTS

Sam Houston State University and the Forensic Sciences Foundation Lucas Grant for funding this project, and Randox Toxicology for providing the Evidence MultiSTAT for immunoassay testing.

RESULTS & DISCUSSION

Table 1: Validation Results

Validation Results		Morphine	Codeine	Thebaine
Monitored ions (M^+)		*m/z* 429*, 236, 287	*m/z* 178*, 371, 196, 234	*m/z* 311*, 296
Limit of Detection (ng/mL)	Oral Fluid	7.5	7.5	7.5
Cutoff		15	15	15
Carryover (40 ng/mL)		None	None	None
Stability (7.5 ng/mL)		≥ 48 h	≥ 48 h	≤ 24 h
Stability (30 ng/mL)		≥ 48 h	≥ 48 h	≤ 48 h
Limit of Detection (ng/mL)	Urine	100	100	100
Cutoff		200	200	200
Carryover (2000 ng/mL)		< LOD	None	< LOD
Stability (100 ng/mL)		≥ 48 h	≥ 48 h	≤ 24 h
Stability (400 ng/mL)		≥ 48 h	≥ 48 h	≥ 48 h
No interferences observed for any analyte in either matrix				
*Indicates quantification ion				

Figure 1: Authentic Samples (OF) MultiSTAT Results

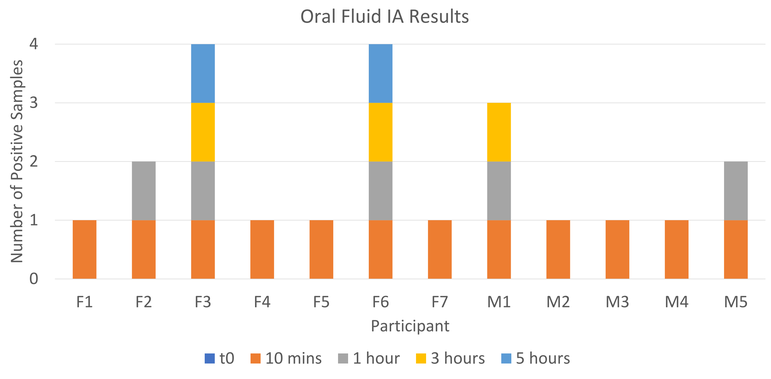

Figure 2: Authentic Samples (UR) MultiSTAT Results

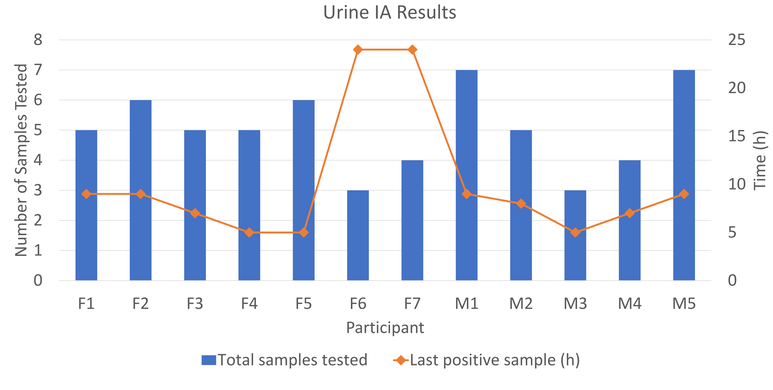

Table 2: Positive GC/MS Authentic Samples

Participant	Samples > LOD	Samples > Cutoff
F1	-	-
F2	-	-
F3	10 mins (OF) 3 h (OF)	10 mins (OF)
F4	-	-
F5	-	-
F6	-	-
F7	-	-
M1	10 mins (OF)	-
M2	10 mins (OF) 8 h (UR)	8 h (UR)
M3	-	-
M4	-	-
M5	10 mins (OF)	-

Study Limitations:

- Authentic samples not screened and confirmed in tandem
- Instability of analytes in the delay between screening and confirmation
- Refrigeration issues during sample storage between screening and confirmation
- Limited resources for both screening and GC/MS analysis leading to an inability to measure every authentic sample collected

MATERIALS & METHODS

Authentic Samples (n=12):

- Participant consumption of two poppy seed muffins (Betty Crocker™)
- UR collected at every void until 24 h
- OF collected: 10 min, then 1, 3, 5, 8, & 24 h

Sample Preparation:

- Fortify 0.5 mL sample or QC with ISTD (morphine-D3)
- Add 3 mL 100 mM phosphate buffer (pH 6)
- Centrifuge (4200 rpm, 15 mins)

SPE (Cerex Clin II SPE columns):

- Load sample and wash:
  - 3 mL DI water, 1 mL HCl, 3 mL MeOH
- Dry columns 5 min, then elute:
  - 3 mL of 2% NH_4OH in 80:20 DCM:IPA
- Dry at 40˚C and reconstitute:
  - 25 µL ethyl acetate
- Derivatize:
  - 25 µL BSTFA (1% TMS), 70 ˚C for 20 min

- *Presumptive Testing:*
  - Biochip array cartridge prepared and loaded according to manufacturer guidelines
- *GC/MS Instrumentation:*
  - Agilent 7890A GC with Agilent 7683B MS

Injection Port Parameters	2 µL injection
	280 ˚C inlet
	Split (10:1) mode
GC Parameters	DB-5ms column (30 m x 25 µm x 0.25 µm)
	Helium; 1.2 mL/min
	150 ˚C to 300 ˚C over 8 mins
MS Parameters	EI+ and SIM mode
	Source temp 230 ˚C

REFERENCES

1. Cassella G, Wu AHB, Shaw BR, Hill DW. The Analysis of Thebaine in Urine for the Detection of Poppy Seed Consumption. Journal of Analytical Toxicology 1997;21(5):376–83. https://doi.org/10.1093/jat/21.5.376.
2. Rohrig TP, Moore C. The Determination of Morphine in Urine and Oral Fluid Following Ingestion of Poppy Seeds. Journal of Analytical Toxicology 2003;27(7):449–52. https://doi.org/10.1093/jat/27.7.449.
3. DoA MultiSTAT-Oral Fluid II. Randox Technology. https://www.randoxtoxicology.com/doa-ms-of/
4. DoA MultiSTAT-Urine I. Randox Technology. https://www.randoxtoxicology.com/doa-ms-urine-i/