

Thebaine determination in urine by liquid chromatography tandem mass spectrometry after poppy seed consumption

Charlotte Walter¹, Kenneth Goldschneider¹, Blaise Ostertag², Junfang Zhao², Kenneth Setchell¹.

¹Department of Anesthesia, ²Department of Pediatrics, Division of Pathology and Laboratory Medicine, Cincinnati Children's Hospital Medical Center

Background

- Urine drug testing (UDT) is common with potential severe consequences.^{1,2}
- Poppy seeds can cause positive UDT for opiates secondary to harvesting contamination.^{3,4}
- Thebaine is present in poppy seeds but is not present in commonly used street or prescription drugs.^{2,5}
- Thebaine is a potential biomarker present in urine after poppy seed ingestion to distinguish poppy seed ingestion from opioid use.^{2,5}

Aims and objectives:

Aim: Develop and validate a high-sensitivity stable-labeled isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to detect and quantify thebaine in the urine and study its pharmacokinetics after poppy seed ingestion

Hypothesis: Thebaine will reliably be found in the urine after poppy seed ingestion for at least 8 hours.

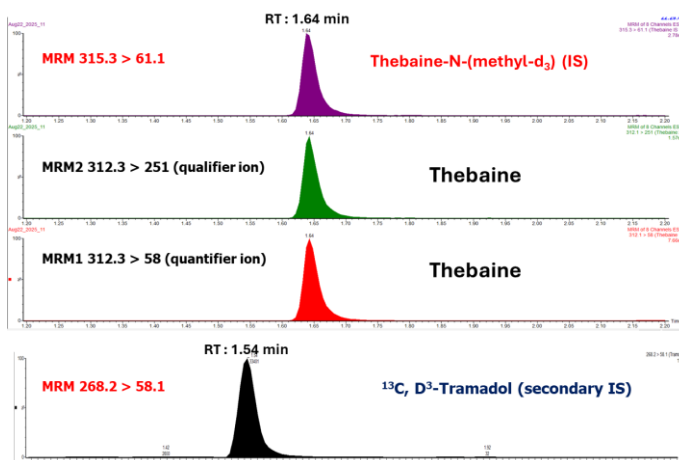


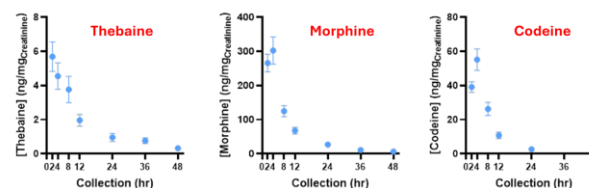
Figure 1: Thebaine elutes similarly to 2 internal standards (tramadol metabolite and labeled thebaine) as well as the qualifier ion

Methods

- After IRB approval, enrolled 20 healthy adults with no recent opioid use.
- Each consumed a commercially available poppy-seed muffin.
- Collected 8 total spot urine specimens (baseline then 2, 4, 8, 12, 24, 36, and 48 h post ingestion).
- Thebaine, codeine and morphine in urine were all extracted by solid-phase extraction after enzymic hydrolysis with β -glucuronidase and analyzed by LC-MS/MS.
- Method validation followed by CAP/CLIA guidelines.
- 6 poppy seed muffins were analyzed for thebaine, morphine and codeine. (See Figure 4)

Results

- Assay specificity was confirmed by co-elution of the quantifier and qualifier transition for the thebaine and the internal standard (See Figure 1). The assay was linear over the dynamic range 0-50 ng/mL with detection limit of 0.2 ng/mL.
- Precision and accuracy at varying concentrations were 7.9-12.8% CV with thebaine being stable in the urine at -20°C for 45 days.
- At baseline, thebaine, codeine, and morphine were not detected in all urines.
- After poppy seed ingestion, thebaine, morphine and codeine all rapidly appeared in 20 subjects (See Figure 2)
- After 24 hours, 3 subjects had clinically significant levels of morphine (>25 ng/mg Cr) but no detectable thebaine. (See Figure 3).



Creatinine Corrected Levels						
Collection	Thebaine		Morphine		Codeine	
	[Average] (ng/mg creatinine)	N	[Average] (ng/mg creatinine)	N	[Average] (ng/mg creatinine)	N
Baseline (0 h)	Not detected	20	Not detected	20	Not detected	20
Post-2 h	5.69 ± 0.86	20	265.79 ± 25.63	20	39.09 ± 3.08	20
Post-4 h	4.55 ± 0.78	20	302.37 ± 40.20	20	55.16 ± 6.31	20
Post-8 h	3.76 ± 0.77	20	124.87 ± 16.09	20	26.21 ± 3.87	17
Post-12 h	1.97 ± 0.34	19	67.75 ± 9.84	20	10.77 ± 1.89	17
Post-24 h	0.96 ± 0.23	12	26.84 ± 3.00	19	2.66 ± 1.57	2
Post-36 h	0.76 ± 0.16	10	10.95 ± 1.38	18	Not detected	20
Post-48 h	0.32 ± 0.08	7	6.46 ± 0.89	15	Not detected	20

*Average ± SEM

Figure 2

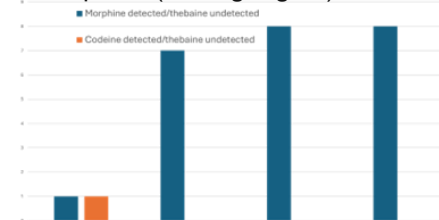


Figure 3: Limits of thebaine testing

Analyte	Average (mcg/muffin, N=6)
Thebaine	41.8 ± 4.6
Codeine	39.1 ± 4.9
Morphine	6.5 ± 2.8
Hydrocodone	< 1
Hydromorphone	< 1

Figure 4: Opiate and thebaine levels in a whole lemon poppy seed muffin.

Discussion

- Thebaine was consistently detected in urine for 8 h post-ingestion.
- It is useful to reliably support poppy-seed ingestion as the cause of a positive UDT.
- Morphine persisted in the urine longer – a limitation on relying on thebaine alone to assess for poppy-seed ingestion.

Conclusion

Thebaine in urine indicates recent poppy seed ingestion, though it cannot exclude concurrent drug use. Absence of thebaine does not necessarily rule out poppy seeds as the source of a positive UDT.

References

- Price HR et al Frontiers in Pharmacology. 2018;9(AUG)961.
- Walter S. She ate a poppy seed salad just before giving birth. Then they took her baby away. USA Today. 9/9/24.
- Knutsen HK, et al. Efsa j. May 2018;16(5):e05243.
- Shetge SA et al J Agric Food Chem. May 6 2020;68(18):5241-5248.
- Cassella G et al. J Anal Toxicol. Sep 1997;21(5):376-83.