

# APPLICATION

## Quantitative Analysis of Cocaine Metabolites and Adulterants in Oral Fluid

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**Sean Orłowicz**

*When not in the lab, Sean enjoys just about anything involving the outdoors: hiking, climbing, surfing, etc. He is especially at home in the mountains, being an avid skier and motorcyclist.*

### Introduction

Cocaine is frequently diluted throughout the creation process with numerous cutting agents and adulterants. It has been found that the majority of cocaine positive samples detected levamisole (88 %) and lidocaine (14.7 %), which are responsible for potential toxicity and numerous public health concerns.<sup>1,2</sup> In addition, procaine, benzocaine, caffeine, and other adulterants that are commonly found in these samples, can be important markers in benzoylecgonine-positive test samples in forensic toxicology labs.<sup>3</sup> Since adulterants are correlated with cocaine positive samples, potential cocaine use can be uncovered when samples are benzoylecgonine-negative. Oral fluid, being a non-intrusive form of biological sample collection, becomes an obvious choice for drug testing cases especially where drivers are under the influence (DUID). This sample matrix is time sensitive and demands on-site (roadside or a traffic stop) specimen collection for an accurate drug quantification.

A polymeric strong cation-exchange Solid Phase Extraction (SPE) sorbent, Strata<sup>®</sup>-X-C, in a 96-Well Plate format is utilized for sample extraction and clean-up of cocaine-positive samples. A Kinetex<sup>®</sup> 2.6 μm XB-C18 LC column in conjunction with a SCIEX 5000 Triple Quad mass spec was utilized for LC-MS/MS analysis of cocaine metabolites (benzoylecgonine, cocaethylene, norcocaine) along with its most common adulterants. A mobile phase consisting of 0.1 % Formic acid and Acetonitrile was utilized in a 3 minute gradient for an analytical run.

### Materials and Methods

#### Reagents and Chemicals

Analytical reference standards, internal standards and human saliva were purchased from Cerilliant<sup>®</sup> Corporation (Round Rock, TX, USA) and Bioreclamation/VT<sup>®</sup> (Chastertown, MD, USA), respectively. The Intercept i2<sup>®</sup> oral fluid collection device was obtained from Orasure Technologies, Inc. (Bethlehem, PA). All other chemicals, were obtained from Sigma-Aldrich<sup>®</sup> (St. Louis, MO). Ultrapure D.I. water was obtained from Sartorius<sup>®</sup> arium<sup>®</sup> comfort II, courtesy of Sartorius Corporation (Bohemia, NY).

### Experimental Conditions

#### Sample Pre-treatment

1 mL human oral fluid was collected on the cellulose pad of the applicator tip provided by the Intercept i2 oral fluid collection device. Saturated pad was placed into transport tube containing the Intercept i2 buffer solution to sit overnight. The sample was then centrifuged at 600 g for 15 minutes to collect supernatant.

#### Solid Phase Extraction Protocol

- 96-Well Plate:** Strata-X-C, 30 mg/well  
**Part No.:** 8E-S029-TGB  
**Condition:** 1 mL Methanol  
**Equilibrate:** 1 mL DI Water  
**Load:** Combine 0.5 mL of pre-treated sample spiked with internal standards and 1 mL 1 % Formic acid in Water. Mix/vortex for 10-15 seconds and load onto Strata-X-C plate.  
**Wash 1:** 1 mL DI Water  
**Wash 2:** 1 mL Acetone/Water (50:50)  
**Dry Down:** 5 minutes at maximum vacuum (15" Hg or higher)  
**Elute:** 2x 500 μL Methanol/Acetonitrile/30 % Ammonium Hydroxide (5:5:2)  
**Dry Down:** Evaporate to dryness under gentle Nitrogen at 45-50 °C  
**Reconstitute:** 200 μL of initial mobile phase

#### HPLC Conditions

- Column:** Kinetex 2.6 μm XB-C18  
**Dimensions:** 50 x 4.6 mm  
**Part No.:** 00B-4496-E0  
**Mobile Phase:** A: 0.1 % Formic acid in Water  
 B: 0.1 % Formic acid in Acetonitrile  

Gradient	Time (min)	% B
	0	10
	3	40
	3.5	40
	3.51	10
	6	10

**Flow Rate:** 1 mL/min  
**Injection:** 5 μL  
**Temperature:** Ambient  
**LC Sytem:** Agilent<sup>®</sup> 1260  
**Detection:** MS/MS (SCIEX 5000 Triple Quad) ESI+



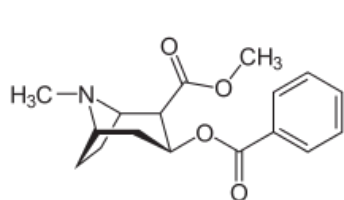
### HPLC Conditions (Q1 Scan)

**Column:** Kinetex<sup>®</sup> 2.6 μm C18  
**Dimensions:** 50 x 3.0 mm  
**Part No.:** 00B-4462-Y0  
**Mobile Phase:** A: 0.1 % Formic acid in Water  
 B: 0.1 % Formic acid in Methanol  
**Gradient:**

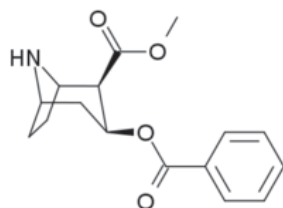
Time (min)	% B
0	10
4	95
5.5	95
5.51	10
7	10

  
**Flow Rate:** 0.5 mL/min  
**Injection:** 10 μL  
**Temperature:** Ambient  
**LC System:** Shimadzu<sup>®</sup> Nexera<sup>®</sup>  
**Detection:** MS/MS (SCIEX 4000 QTRAP<sup>®</sup>) ESI<sup>+</sup>

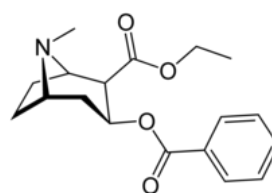
### Chemical Structures of Analytes from Test Panel



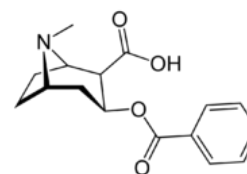
Cocaine



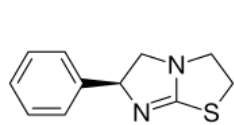
Norcocaine



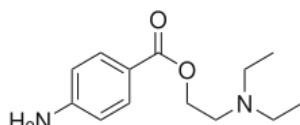
Cocaethylene



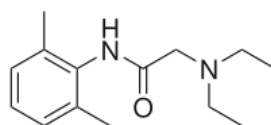
Benzoecgonine



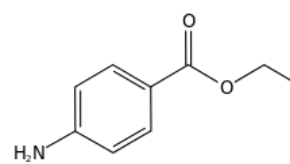
Levamsole



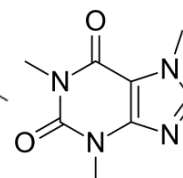
Procaine



Lidocaine

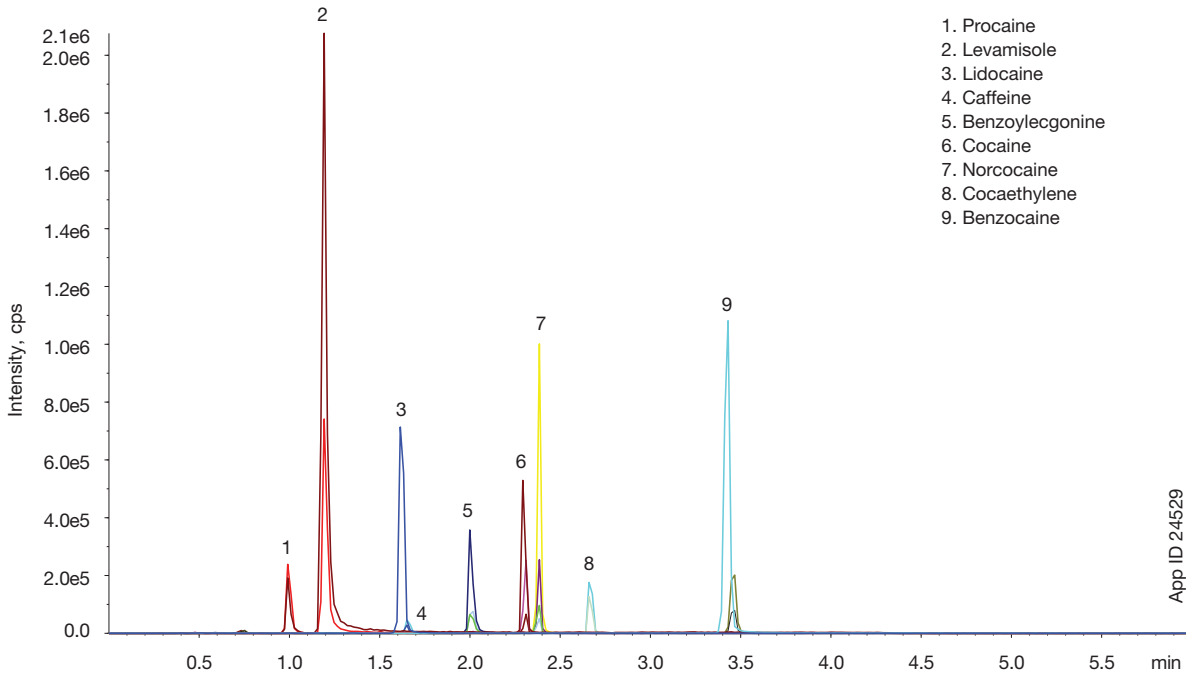


Benzocaine

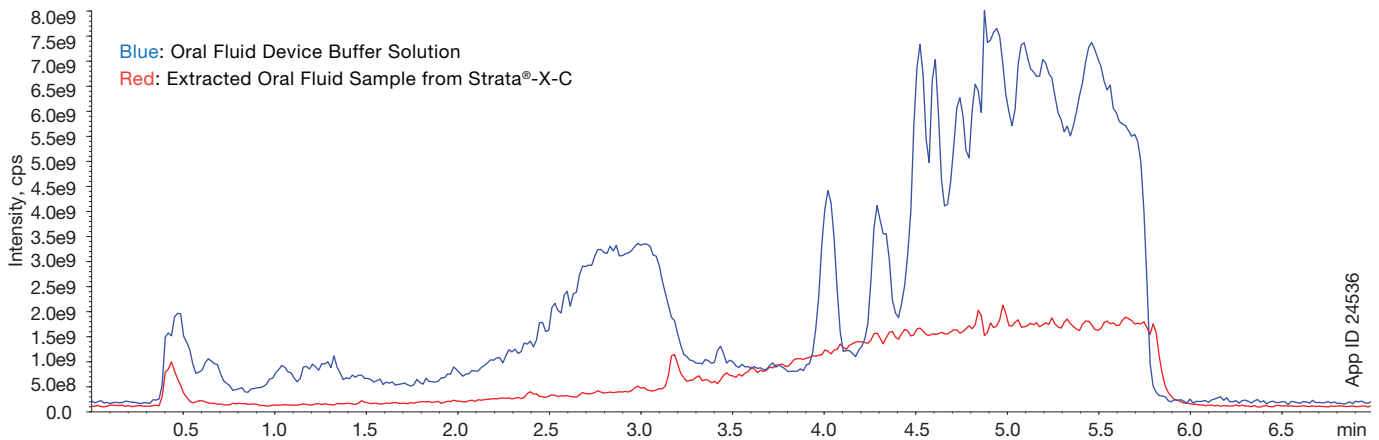


Caffeine

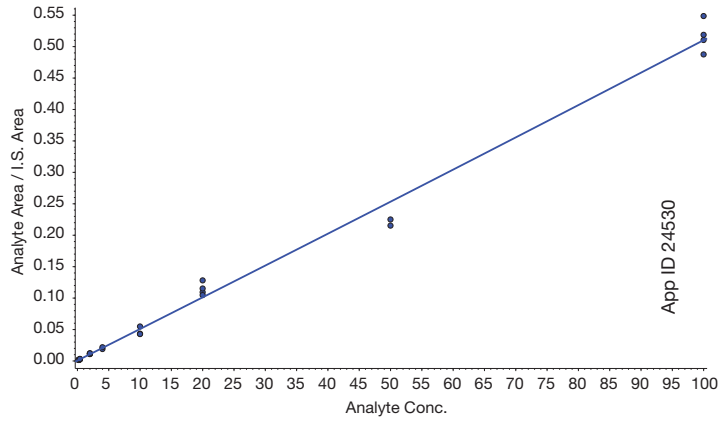
**Figure 1.**  
Representative TIC Chromatogram of Cocaine Metabolites and Adulterants



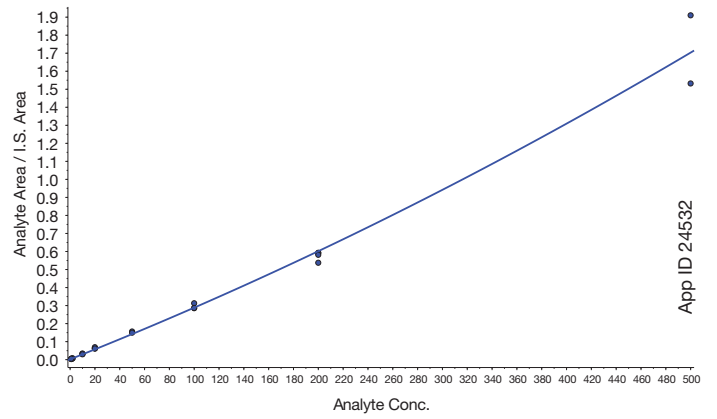
**Figure 2.**  
Representative Q1 Scan of Samples (over 100-2000 m/z range) showing Sample Cleanliness by SPE



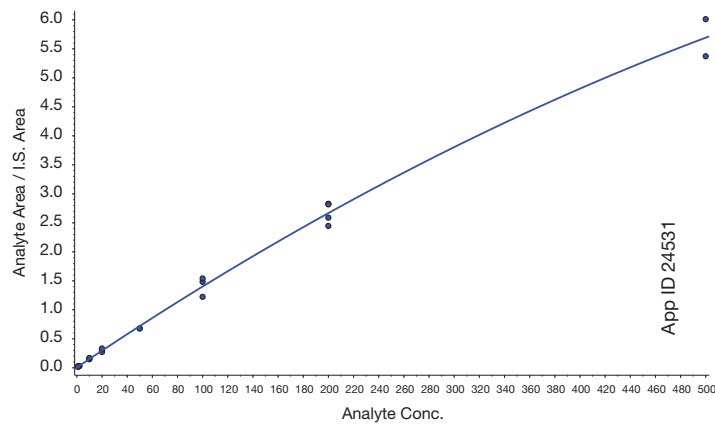
**Figure 3.**  
Calibration Curve of Cocaine  
(0.2-100ng/mL); R=0.9957



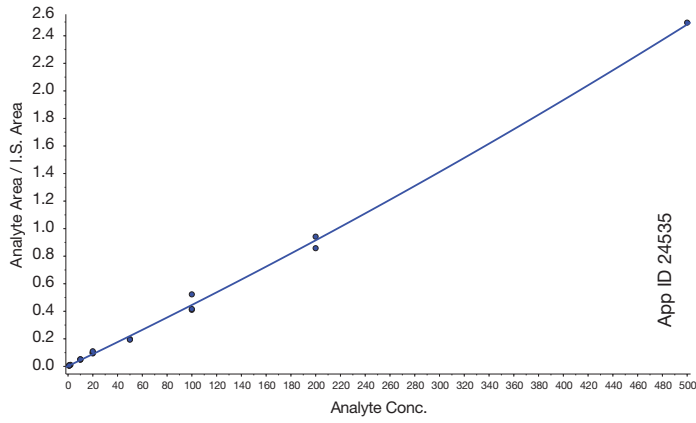
**Figure 5.**  
Calibration Curve of Benzoylecgonine  
(1-500ng/mL); R=0.9956



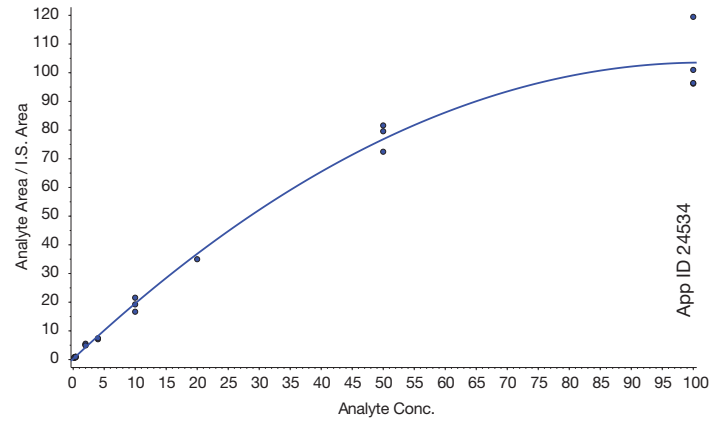
**Figure 4.**  
Calibration Curve of Norcocaine  
(1-500ng/mL); R=0.9975



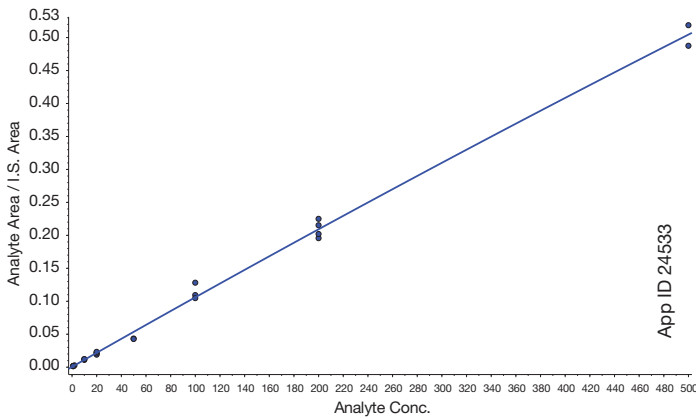
**Figure 6.**  
Calibration Curve of Cocaethylene  
(1-500ng/mL); R=0.9971



**Figure 8.**  
Calibration Curve of Lidocaine  
(0.2-100ng/mL); R=0.9955



**Figure 7.**  
Calibration Curve of Levamisole  
(1-500ng/mL); R=0.9971



**Table 1.**  
Precision, Accuracy, Linearity and % CV

Analyte	R2 (Linearity curve)	% Accuracy Low QC	% CV	% Accuracy Mid QC	% CV	% Accuracy High QC	% CV
Cocaine	0.9971	106.5	14.1	106.7	5.3	84.5	9.8
Norcocaine	0.9975	108.4	12.9	99.4	5.4	87	3
Benzoyllecgonine	0.9956	115.2	7.3	110.4	7.7	94.6	1.5
Cocaethylene	0.9971	115.5	10.4	118.3	3.2	87.8	14.1
Levamisole	0.9971	111.2	7	94.2	6.5	86.1	2.7
Lidocaine	0.995	115	10.2	102.5	15	86.8	14.4
Procaine	0.9952	119.1	10.8	106.6	15.7	97.6	10.3
Benzocaine	0.9978	101.9	11.25	103.7	6.4	95.6	6.8

Note: Caffeine data not shown due to significant loss in recovery

Low QC = 4 ng/mL for all analytes (0.8 ng/mL for cocaine, procaine and lidocaine)

Mid QC = 40 ng/mL (8 ng/mL for cocaine, procaine and lidocaine)

High QC = 150 ng/mL (30 ng/mL for cocaine, procaine and lidocaine)

## Results and Discussion

**Figure 1** shows good resolution of all analytes of interest. Parallel Q1 scan (100-2000 m/z range) for qualitative measurement demonstrates sufficient clean-up (**Figure 2**) of the majority of the excipients that are present in the oral fluid device's buffer solution used to secure sample stability during transport. Calibration curves drawn for analytes in extracted samples, covering the range from 0.2 ng/mL to as high as 500 ng/mL (**Figures 3-8**). A quadratic calibration with 1/x weighting, applied, encountered saturation around or after 500 ng/mL concentration level for most of the analytes from the panel. The linearity curve demonstrate regression value, R, larger than 0.995 (**Figures 3-8**) for all analytes showing robustness of the assay. Several analytes, norcocaine, benzoyllecgonine, lidocaine, (**Figures 4, 5, and 8**) displayed non-linear curve. The very polar, neutral analyte caffeine was not well retained during the aggressive organic wash comprising of 50 % acetone (data not presented). The developed extraction method shows good precision and accuracy for compounds of interest. Precision (2-14 %) and accuracy data (85-119 %) for all three levels of QC samples are comparable and within acceptable industry standard (**Table 1**).

## Conclusion

The developed assay significantly eliminated the amount of excipients present in the oral fluid device transport buffer, which can potentially reduce mass spec instrument sensitivity and increase downtime. The precision and accuracy data are in good agreement for bioanalytical method validation.

## References

1. Lynch, K; Dominy, S. ; Graf, J. ; "Detection of Levamisole Exposure in Cocaine Users by LC-Tandem Mass Spectrometry"; J. Journal of Analytical Toxicology, Vol. 35, page 176-178.
2. Lee, K; Ladizinski, B.; Federman, D. "Complications Associated With Use of Levamisole-Contaminated Cocaine"; Mayo Clinic Proceedings; 2012 Jun; 87(6); page 581-586.
3. Fucci, N. ; De Giovanni, N; "Adulterants Encountered in the Illicit Cocaine Market"; Forensic Sci Int. 1988 Aug 12; 95(3): page. 247-252

**Ordering Information**





**Kinetex® Core-Shell LC Columns**

2.6 µm Columns (mm)				SecurityGuard ULTRA Cartridges <sup>†</sup> (mm)			SecurityGuard ULTRA Cartridges <sup>†</sup> (mm)			SecurityGuard ULTRA Cartridges <sup>†</sup> (mm)	
Phases	50 x 2.1	150 x 2.1	3/pk	50 x 3.0	100 x 3.0	3/pk	50 x 4.6	100 x 4.6	150 x 4.6	3/pk	
C18	00B-4462-AN	00F-4462-AN	AJO-8782 for 2.1 mm ID	00B-4462-YO	00D-4462-YO	AJO-8775 for 3.0 mm ID	00B-4462-E0	00D-4462-E0	00F-4462-E0	AJO-8768 for 4.6 mm ID	

2.6 µm Analytical Columns (mm)				SecurityGuard ULTRA Cartridges <sup>†</sup>
Phases	50 x 4.6	100 x 4.6	150 x 4.6	3/pk
XB-C18	00B-4496-E0	00D-4496-E0	00F-4496-E0	AJO-8768 for 4.6 mm ID

<sup>†</sup>SecurityGuard ULTRA Cartridges require holder, Part No.: AJO-9000

**Strata®-X-C Solid Phase Extraction**

Format	Sorbent Mass	Part Number	Unit
<b>Tube</b>			
	30 mg	8B-S029-TAK**	1 mL (100/box)
	30 mg	8B-S029-TBJ	3 mL (50/box)
	60 mg	8B-S029-UBJ**	3 mL (50/box)
	100 mg	8B-S029-EBJ	3 mL (50/box)
	100 mg	8B-S029-ECH	6 mL (30/box)
	200 mg	8B-S029-FBJ	3 mL (50/box)
	200 mg	8B-S029-FCH	6 mL (30/box)
	500 mg	8B-S029-HBJ	3 mL (50/box)
	500 mg	8B-S029-HCH	6 mL (30/box)
<b>Giga™ Tube</b>			
	500 mg	8B-S029-HDG	12 mL (20/box)
	1 g	8B-S029-JDG	12 mL (20/box)
	1 g	8B-S029-JEG	20 mL (20/box)
	2 g	8B-S029-KEG	20 mL (20/box)
	5 g	8B-S029-LFF	60 mL (16/box)
<b>96-Well Plate</b>			
	10 mg	8E-S029-AGB	2 Plates/Box
	30 mg	8E-S029-TGB	2 Plates/Box
	60 mg	8E-S029-UGB	2 Plates/Box
<b>96-Well Microelution Plate</b>			
	2 mg	8M-S029-4GA	ea

\*\*Tab-less tubes available. Contact Phenomenex for details.



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AH0-9347	Presston 100 Positive Pressure Manifold, 3 mL Tube Complete Assembly
AH0-9343	Presston 100 Positive Pressure Manifold, 6 mL Tube Complete Assembly

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Part No.	Description
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