Extraction of Loperamide and N-Desmethyl Loperamide from Blood Followed by LC-MS/MS Analysis

UCT Part Numbers

CSXCE106
Clean Screen® XCEL I
130mg / 6mL SPE Cartridge

SLC-18100ID21-18UM
Selectra® C18 HPLC
100 X 2.1 mm, 1.8 µm

SLC-18GDC20-18UM
Selectra® C18 Guard Column
10 X 2.1 mm, 1.8 µm

SLGRDHLDR
Guard Column Holder

Summary:

Loperamide is an over the counter antidiarrheal drug that has been considered to be safe when used as directed. When taken properly, loperamide acts to slow gastrointestinal activity in order to help control diarrhea. However, as the opioid epidemic continues to ravage our nation there has been an increasing number of reported cases of loperamide overdose. Loperamide activates the same receptors as opioids, but when taken as instructed at normal doses, very little of it is absorbed into the bloodstream. As a result, it is unable to cross the blood-brain barrier. Therefore, it wouldn't cause people to feel high as with other opioids, nor would it provide pain relief. However, when Loperamide is taken at extremely high doses, it can produce the same euphoric effects as traditional opioids, which has led to an upward trend of abuse and misuse of the drug.

This application note outlines a simple 3 step method for the extraction of Loperamide and its main metabolite, N-desmethyl Loperamide, from blood. UCT's Clean Screen® XCEL I column provides users with the same level of sample clean up as traditional SPE while allowing the elimination of timely conditioning and wash steps. Sample extracts are then analyzed via UHPLC-MS/MS utilizing UCT's Selectra® C18 Column.

CLINICAL FORENSICS

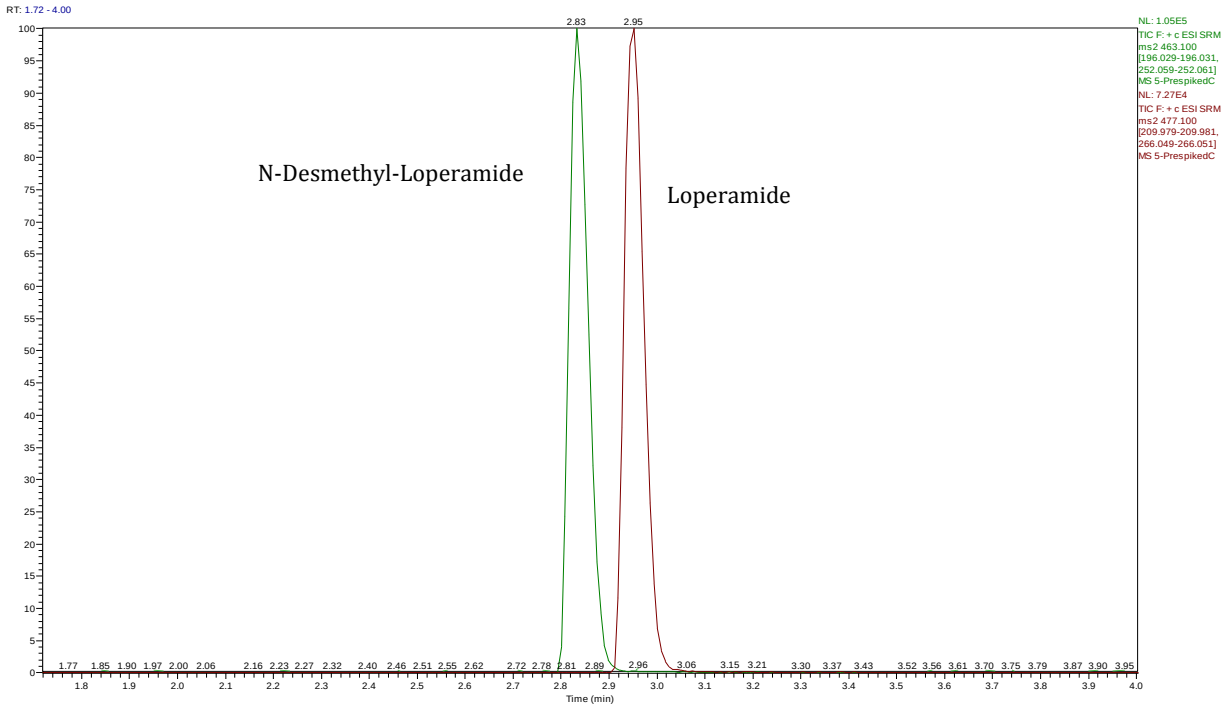
Sample Pretreatment:

1. To 1 mL blood sample add 3 mL Acetate Buffer pH 5 and appropriate amount of internal standard
2. Vortex Samples for 30 seconds to mix

SPE Procedure:

1. Apply samples to SPE tubes without any preconditioning
    a. Allow samples to flow through the column at a rate of 1-2 mL/minute
2. Wash samples with 2 mL of D.I H_2O
3. Wash SPE column with 2 mL of 98:2 MeOH:Glacial Acetic Acid
4. Dry column for 5 minutes at full vacuum or pressure
5. Wash samples with 2 mL of Hexane
6. Dry column for 10 minutes at full vacuum or pressure
7. Elute compounds with 2 mL of 78:20:2 DCM:IPA:NH4OH
    a. Collect eluate at a rate of 1- 2 mL/min
8. Evaporate to dryness at < 50°C
9. Reconstitute sample in mobile phase

LC-MS/MS Parameters:



MRM transitions (ESI⁺, 50 ms dwell time)					
Compound		Rt (min)	Q1 ion	Q3 ion 1	Q3 ion 2
1	N-Desmethyl-Loperamide	2.83	463.1	252.06	196.03
2	Loperamide	2.95	477.1	266.05	209.98

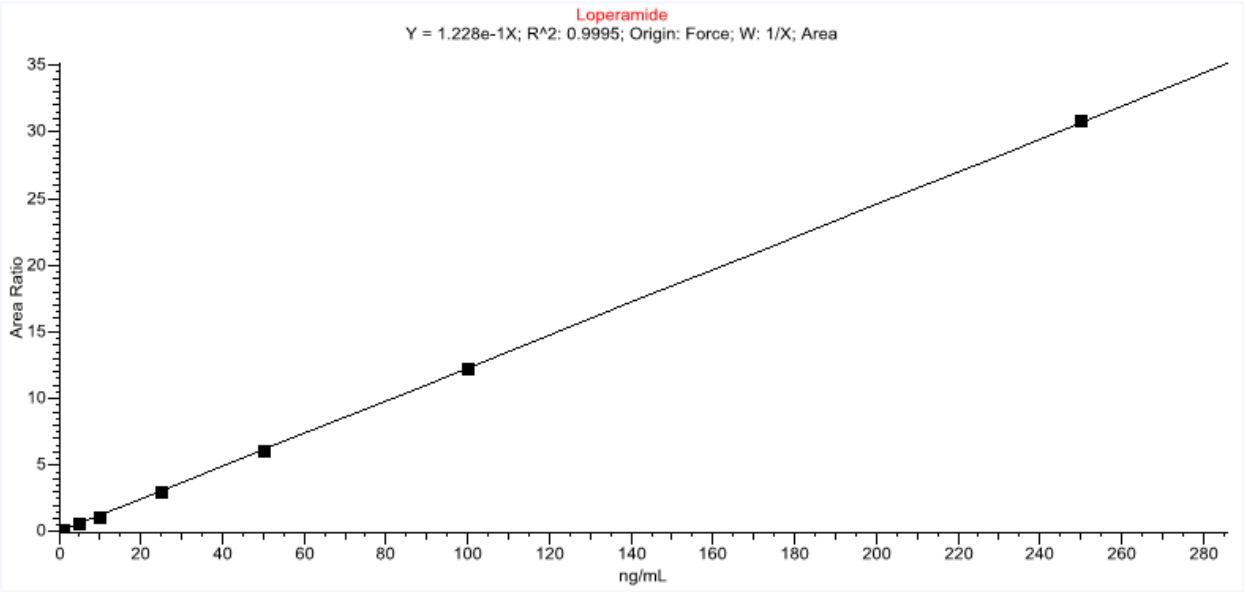
CLINICAL FORENSICS

System: Thermo Scientific™ Dionex™ Ultimate™ 3000 (LC) Thermo Scientific™ TSQ Vantage™ (MS/MS)		
Column: UCT Selectra® C18, 100 x 2.1 mm, 1.8 µm		
Guard Column: UCT Selectra® C18, 10 x 2.1 mm, 1.8 µm		
Column Temperature: 50 °C		
Column Flow Rate: 0.3 mL/min		
Injection Volume: 1 µL		
Gradient Program:		
Time (min)	**% Mobile Phase A** (0.1% Formic Acid in Water)	**% Mobile Phase B** (0.1% Formic Acid in ACN)
0	90	10
1	35	65
4	35	65
4.1	90	10
6	90	10

Results:

Recovery (%) from Blood (n=3)			
Concentration	**5 ng/mL**	**10 ng/mL**	**50 ng/mL**
Loperamide	102	95	93
N-Desmethyl-Loperamide	106	97	96
Average Recovery	104	96	94.5

Matrix-Matched Calibration Curve of Loperamide (R^2=0.9995) Range 0.5-250 ng/mL



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