Extraction of Illicit Drugs from Hemolyzed Whole Blood using ISOLUTE® SLE+ Columns Prior to LC-MS/MS Analysis

This application note describes the extraction of different drugs in hemolyzed whole blood, which are typically screened for forensic toxicology panels, using ISOLUTE® SLE+ supported liquid extraction columns in the 1 mL and 2 mL sample capacity formats.

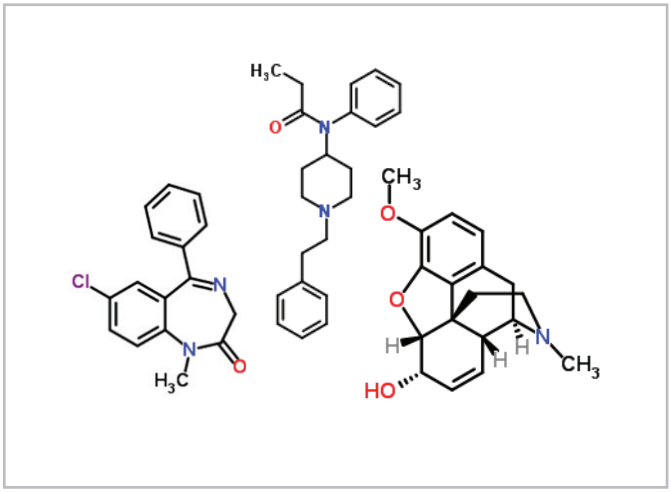

Figure 1. Structures of Diazapam, Fentanyl, and Codeine

Introduction

Toxicology screening for various illicit drugs can be conducted using different biological matrices collected from a subject. Of the matrices that can be tested, whole blood is considered one of the most difficult. Post mortem whole blood has been found to be even more difficult to analyze. The characteristics of hemolyzed blood (increased viscosity and cellular content) are similar to that of post-mortem blood, and hemolyzed blood is used in this application note to demonstrate the use of ISOLUTE SLE+ columns in its preparation for LC-MS/MS analysis.

ISOLUTE® SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation time.

Analytes

Alprazolam, Clonazepam, Diazepam, Flunitrazepam, Oxazepam, Temazepam, Nitrazepam, Normeperidine, Naltrexone, Morphine, Codeine, Oxymorphone, Hydromorphone, Oxycodone, Hydrocodone, 6-Acetyl Codeine, 6-Acetyl Morphine, Fentanyl, Buprenorphine, EDDP

Sample Preparation Procedure

Format:	ISOLUTE SLE+ 1 mL sample volume column, part number 820-0140-C or ISOLUTE SLE+ 2 mL sample volume column, part number 820-0290-D
Whole Blood:	The negative whole blood matrix was completely hemolyzed to simulate consistency of post-mortem blood samples.
Sample Pre-treatment:	Fortify 500 µL of negative blank whole blood with test analytes as needed to prepare calibration standards and quality control standards. Add 400 µL of ammonium hydroxide (2%, v/v) to calibration standards, QC standards and patient samples then gently vortex the solutions. Add up to 100 µL of internal standard solution to each sample. **NOTE**: Total internal standard solution volume should not exceed 10 % of the recommended loading capacity.
Sample Loading	Load pre-treated samples onto columns. Apply a short pulse of vacuum (VacMaster-10 Sample Processing Manifold) or positive pressure (PRESSURE+48 Positive Pressure Manifold) to initiate flow and then allow sample to absorb on column for 5 minutes.
Analyte Elution:	Apply 2 x 3 mL of ethyl acetate (1 mL ISOLUTE SLE+ cartridge) or 2 x 4 mL (2 mL ISOLUTE SLE+ cartridge) to each cartridge and allow solvent to gravity flow. Apply positive pressure or pull slight vacuum as needed during collection process to facilitate a flow rate of 1 mL per minute
Post Extraction:	Evaporate sample to dryness and reconstitute in water:methanol (90:10, v/v, 500 µL).
Additional Information:	A white film was observed post dry down in each sample tube, believed to be residual components from the original samples. To obtain a clear extract and prevent solubilizing this white material, we recommend using an aqueous/methanol mix as the reconstitution solvent.

Biotage®

HPLC Conditions

Instrument: Agilent 1200 Liquid Handling System (Agilent Technologies, Berkshire, UK)

Column: Phenomenex Gemini C18, 150 mm x 4.6 mm (5 µm) (catalog# 00F-4435-E0)

Mobile Phase: A: 5mM Ammonium Formate with 0.01% Formic Acid
B: Acetonitrile with 0.01% Formic Acid

Gradient:

Step	Time (min)	Flow Rate (µL/min)	% A	% B
1	0.0	1000	90	10
2	0.50	1000	90	10
3	2.5	1000	10	90
4	3.5	1000	10	90
5	4.0	1000	90	10
6	7.0	1000	90	10

Injection Volume: 5 µL

Temperature: Ambient

MS Conditions

Applied Biosystems/MDS Sciex 4000 Q-Trap triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA.) equipped with a Turbo Ionspray® interface for mass analysis.

Ion Source Temperature: 500 °C

Scan Function	Analyte	MRM Transition	Declustering Potential (DP)	Collision Energy (CE)	Cell Exit Potential (CXP)
1	Alprozolam	308.8>280.5	30	35	16
2	Clonazepam	315.8>269.8	30	30	16
3	Diazepam	284.9>154	30	30	16
4	Flunitrazepam	313.9>267.9	30	30	16
5	Oxazepam	288>242	30	40	16
6	Temazepam	300.9>255	30	30	16
7	Nitrazepam	282.1>180	30	30	16
8	Normeperdine	234>160	30	40	16
9	Naltrexone	342.2>323.8	45	40	16
10	Morphine	286>165	30	40	16
11	Codeine	300>199	30	30	16
12	Oxymorphone	302>227	45	40	16
13	Oxycodone	316>241	25	25	16
14	Hydrocodone	300>199	30	40	16
15	6-Acetyl Codeine	342.4>255	30	45	16
16	6-Acetyl Morphine	328>165.5	30	30	16
17	Fentanyl	337>188	30	60	16
18	Buprenorphine	468.2>396.2	30	40	16
19	EDDP	278>234	30	40	16

Table 1. MRM transitions in positive mode Turbo Ionspray.

Results

The whole blood matrix was completely hemolyzed yielding a relatively thick consistency as compared to un-hemolyzed blood. The purpose of using hemolyzed whole blood was to replicate post-mortem blood consistency and demonstrate the utility of the 1 mL and 2 mL ISOLUTE SLE+ column formats.

The whole blood was spiked at a final concentration of 20 ng/mL. The spiked samples were pretreated with 2% ammonium hydroxide and loaded onto ISOLUTE SLE+ columns. Figure 2 shows photos highlighting the processing for whole blood extraction. As can be seen in Figure 2, the whole blood sits on top of sorbent during initial loading. After the application of the vacuum, the whole blood migrates down the column and absorbs to the sorbent. The elution solvent is collected as a clear solution prior to dry down and reconstitution into a suitable mobile phase for LC-MS/MS analysis.

The recoveries for the drug analytes spiked into the whole blood are shown in Figure 3. The averaged recoveries (n=7) for drugs spiked into whole blood ranged from 45%-92% and 63%-104% for the 1 mL and 2 mL column formats, respectively. The percent relative standard deviation for each series was typically ‹ 10%.

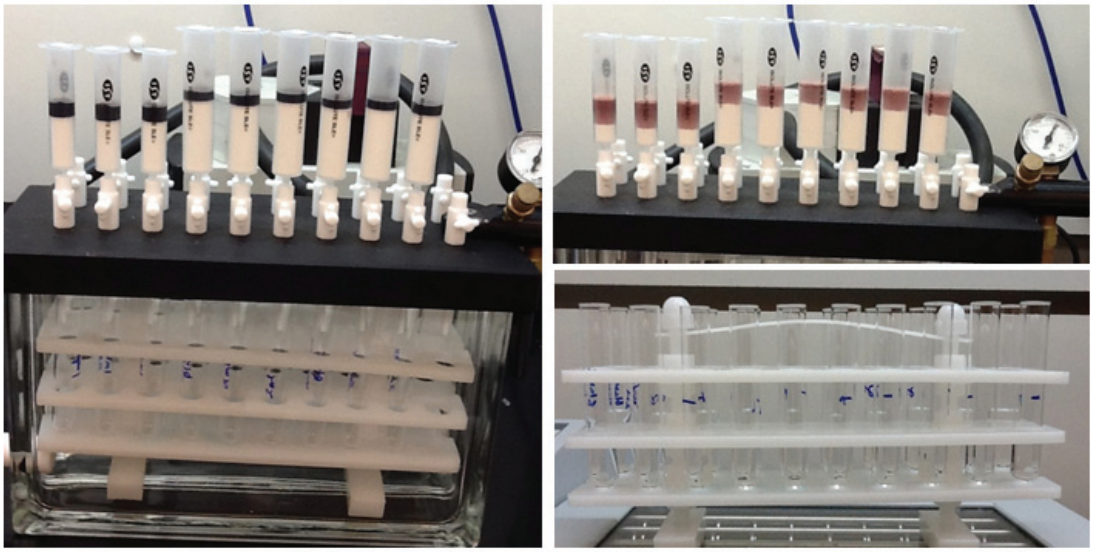

Figure 2: Photographs of whole blood sample processing on ISOLUTE SLE+ 1 mL and 2 mL columns depicting loading of sample onto sorbent bed, absorption of whole blood sample onto column packing and subsequent collection of clear extraction solvent.

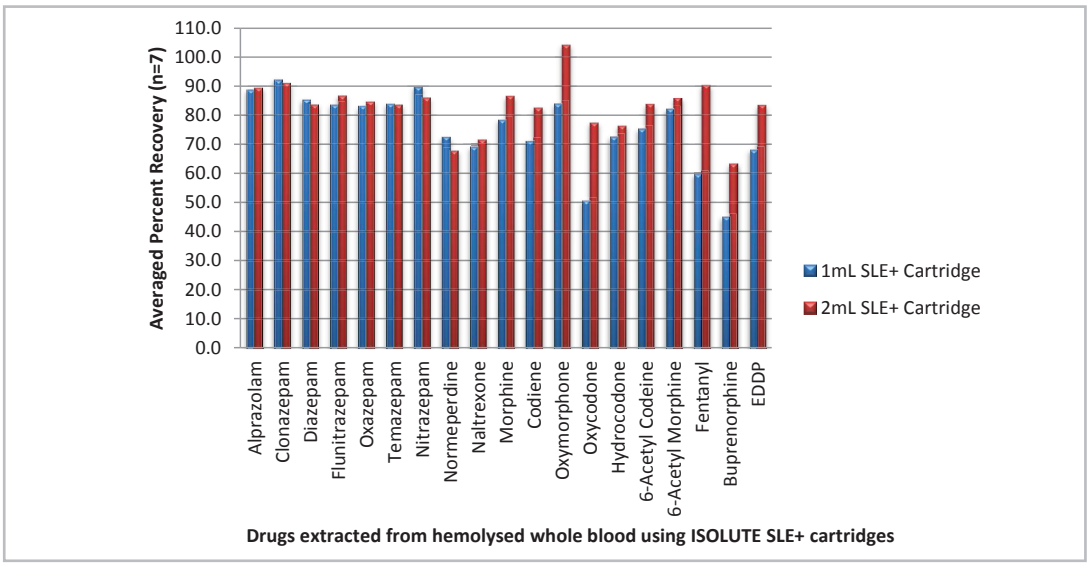

Figure 2: Plot of averaged (n=7) recovery of spiked drugs of abuse (10 ng/mL) from hemolyzed whole blood using ISOLUTE SLE+ columns

Ordering Information

Part Number	Description	Quantity
820-0140-C	ISOLUTE SLE+ 1 mL Sample Volume Columns	30
820-0290-D	ISOLUTE SLE+ 2 mL Sample Volume Columns	20
PPM-48	Biotage® PRESSURE+ 96 Positive Pressure Manifold	1
121-1016	Biotage® VacMaster™-10 Sample Processing Manifold	1
C103198	TurboVap® LV, 120V	1
C103199	TurboVap® LV, 230V	1

For the latest application notes and more information about ISOLUTE® SLE+ visit www.biotage.com

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