Effective Extraction Strategies for Buprenorphine and Norbuprenorphine in Urine, Oral Fluid and Whole Blood using Cation Exchange Solid Phase Extraction and Supported Liquid Extraction prior to HPLC-MS/MS Analysis

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INTRODUCTION

Buprenorphine and Norbuprenorphine are typically problematic for analysis due to analyte stability issues during sample preparation. A fast, reliable and robust sample preparation method that could be implemented to extract these drugs from complex biological matrices with good analyte recovery and minimum matrix effects would be ideal for toxicology labs.

This poster will demonstrate two fast and robust methods for the extraction of buprenorphine and norbuprenorphine in urine, oral fluid and whole blood.

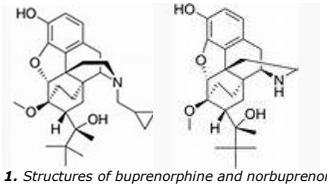

Figure 1. *Structures of buprenorphine and norbuprenorphine.*

EXPERIMENTAL PROTOCOL:

Reagents

HPLC grade Water, Methanol, Ethyl Acetate, Acetone, Acetonitrile, Isopropanol, Ammonium Hydroxide and Formic Acid were purchased from Sigma-Aldrich Co. (Atlanta, GA.). The negative urine and oral fluid was collected from drug free donors.

Sample Preparation/Extraction Technologies

EVOLUTE EXPRESS Cation Exchange Resin

EVOLUTE® EXPRESS CX is functionalized hydroxylated polymer-based SPE sorbent that contains both non-polar and strong cation exchange functionality to give a mix-mode retention characteristic. The EVOLUTE® EXPRESS CX format promotes optimized flow efficiencies, often eliminating the need for conditioning and/or equilibration of individual wells or columns. The sorbent can extract a wide range of basic and neutral analytes from biological fluids (e.g. oral fluid, urine) using various buffers and simple wash steps for delivering cleaner extracts.

Supported Liquid Extraction Procedure

Supported Liquid Extraction (ISOLUTE® SLE+) is a modified diatomaceous sorbent that has a high adsorption affinity for aqueous solutions and the analytes solubilized in the aqueous solution. The sample preparation methodology of Supported Liquid Extraction (SLE) works on the same chemical premises as a liquid-liquid extraction experiment, but is carried out on a solid phase. The development of a sample preparation method using SLE will enable the user to load the aqueous matrix, containing the target analytes and interferences onto a pre-packed SLE column or 96 well plate. Any water-immiscible organic solvent (e.g. dichloromethane, ethyl acetate, diethyl ether, etc.) can then be gravity fed through the column or well to extract and collect the target analytes. The water-soluble endogenous interferences (i.e. proteins, lysophospholipids, phospholipids and salts) are retained on the sorbent, which can subsequently be discarded. The desired target analytes will be solubilized in various biological matrices. Endogenous interferences like proteins, lipids, phospholipids, salts and other unwanted components that will cause problems with the chromatographic separation and subsequent detection of the desired analytes will be cleaned up using the recommended sample preparation technology.

EVOLUTE EXPRESS CX Methodology: Urine

100 µL of urine was pretreated with 0.1% aqueous formic acid at a 1:9 dilution. Add the internal standard to the samples. Load total sample onto EVOLUTE® EXPRESS CX cartridge or well plate. Wash sorbent with 50% aqueous acetone solution (2 x 1000 µL). Wash the sorbent with HPLC grade water (1 x 1000 µL). Dry the sorbent bed under positive or negative pressure. Elute target analytes with 600 µL of a ethyl acetate: acetonitrile: conc. ammonium hydroxide (70:27:3). Dry eluent down and reconstitute in mobile phase.

EVOLUTE EXPRESS CX Methodology: Oral Fluid

100 µL of oral fluid was pretreated with 0.1% aqueous formic acid at a 1:9 dilution. Add the internal standard to the samples. Load total sample onto EVOLUTE® EXPRESS CX cartridge or well plate. Wash sorbent with 50% aqueous acetone solution (2 x 1000 µL). Wash the sorbent with HPLC grade water (1 x 1000 µL). Elute target analytes with 600 µL of isopropanol: acetonitrile: conc. ammonium hydroxide (45:55:3). Dry eluent down and reconstitute in mobile phase.

ISOLUTE SLE+ Methodology: Whole blood

100 µL of whole blood was pretreated with 0.1% aqueous ammonium hydroxide solution containing the internal standard at a 1:2.5 dilution. Load total sample onto ISOLUTE® SLE+ 400 cartridge or well plate. Let sample sit for 5 minutes. Elute target analytes with 1400 µL of ethyl acetate: acetonitrile: conc. ammonium hydroxide (95:4:1). Dry eluent down and reconstitute in mobile phase.

Liquid Chromatography

Instrument: Agilent 1260 HPLC (Santa Clara, CA.)
Column: Restek Raptor Biphenyl column (3.0 µ, 50 x 2.1)
Mobile Phase: A: 0.1% FA (aq) B: 0.1% FA in MeOH.
Flow Rate: 0.3 mL/min
Injection Volume: 10 µL
Column Temperature: 30 °C

Table 1. *Gradient parameters.*

Time (min)	% Mobile Phase B
0	60
0.2	60
0.6	85
1.0	85
1.1	60
5.0	60

Mass Spectrometry

Instrument: Sciex 4000 Q-Trap (Foster City, CA.) equipped with a Turbo Ionspray® interface for analysis.
Ion Source Temperature: 500 °C

Table 2. *SCIEX 4000 Q-Trap parameters.*

Analyte	MRM Transition	Declustering Potential (V)	Collision Energy (CE)
Buprenorphine	468 > 396.2	40	70
Norbuprenorphine	414 > 83	40	70
Buprenorphine-D4	472.1 > 58.9	40	80
Norbuprenorphine-D3	417.1 > 83.0	40	80

Results

The recoveries of both buprenorphine and norbuprenorphine from spiked urine, oral fluid and whole blood at concentration ranging from 5.0 ng/mL to 500 ng/mL were determined and are shown below (Figure 2).

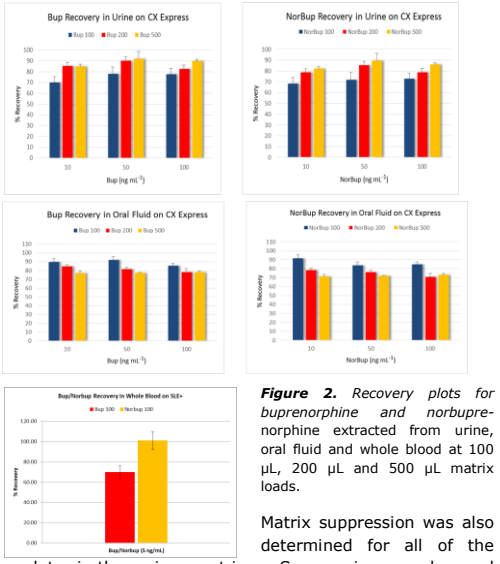

Figure 2. *Recovery plots for buprenorphine and norbupre-norphine extracted from urine, oral fluid and whole blood at 100 µL, 200 µL and 500 µL matrix loads.*

Matrix suppression was also determined for all of the analytes in the various matrices. Suppression was observed for urine and whole blood while a small amount of enhancement was observed for oral fluid. Figure 3 shows a plot for buprenorphine and norbuprenorphine.

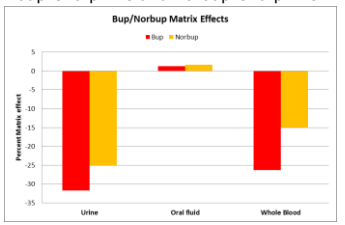

Figure 3*. Matrix effects plot for buprenorphine and norbuprenorphine in urine, oral fluid and whole blood.*

Calibrators for buprenorphine and norbuprenorphine were prepared ranging from 0.5 ng/mL to 100 ng/mL. Linearity was determined for samples spiked in urine, oral fluid and blood across the target dynamic range. Table 3 shows typical data observed for analytes extracted from oral fluid. Similar results were observed for urine and whole blood (data not shown).

	Buprenorphine		Norbuprenorphine	
Target Conc	**Calculated Conc (ng/mL)**	**Accuracy (%)**	**Calculated Conc (ng/mL)**	**Accuracy (%)**
0.50	0.44	88.57	0.48	95.05
1.00	0.95	95.60	1.10	109.90
5.00	5.25	104.93	4.81	96.10
10.00	9.67	96.73	10.42	104.17
25.00	23.9	95.75	23.30	93.21
50.00	50.1	101.59	50.17	100.34
100.0	100.4	100.43	101.2	101.23

Table 3. *Accuracy for extracted calibrators in oral fluid.*

Conclusion

- Buprenorphine and Norbuprenorphine were successfully extracted from three complex matrices using two robust extraction methods.
- The optimized extraction protocols were evaluated for recovery and matrix effects and found to be acceptable for achieving linearity across the dynamic range of 0.5 - 100 ng/mL.
- All of the extraction protocols were performed manually but could be automated using the Biotage Extrahera® Sample Preparation Workstation for high throughput production capacity.

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