Extraction of Buprenorphine and Norbuprenorphine from Whole Blood Using ISOLUTE® SLE+ Supported Liquid Extraction Prior to LC-MS/MS Analysis

Victor E. Vandell, Ph.D, Dan Menasco, Ph.D

Biotage, 10430 Harris Oaks Blvd., Suite C, Charlotte, NC USA 28269

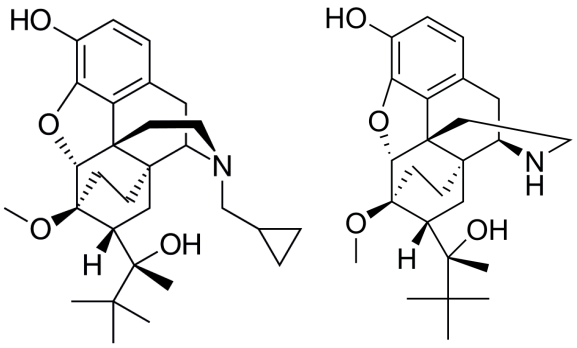

Figure 1. Structure of Buprenorphine and Norbuprenorphine

Introduction

This application note describes the extraction of buprenorphine and norbuprenorphine from whole blood using ISOLUTE® SLE+ Supported Liquid Extraction prior to LC-MS/MS analysis.

ISOLUTE® SLE+ Supported Liquid Extraction products 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.

This application note describes a fast extraction protocol for the qualitative and quantitative analysis of buprenorphine and norbuprenorphine extracted from whole blood matrix.

Analytes

Buprenorphine and Norbuprenorphine

Sample Preparation Procedure

Format:

ISOLUTE® SLE+ 400 µL Supported Liquid Extraction plate, part number 820-0400-P01

Sample Pre-treatment

To 100 µL of sample (calibrator, QC or patient) add 250 µL of 0.1% (v/v) aqueous ammonium hydroxide. Add an appropriate amount of internal standard separately or mix internal standard into the ammonium hydroxide pre-treatment solution prior to adding to sample. Mix thoroughly.

Sample Loading

Pipette 350 µL (total volume) of the pre-treated sample into each well of the ISOLUTE® SLE+ plate. Using a Biotage® PRESSURE+96 Positive Pressure Manifold , apply 2–5 psi of pressure to load samples onto the sorbent. Wait 5 minutes for the sample to equilibrate on the sorbent.

Analyte Extraction

Apply 700 µL of an ethyl acetate: acetonitrile: conc. ammonium hydroxide (95:4:1, v/v) solution to each well. Elute slowly at a rate of 1 mL/min (10–12 drops/min). Add a second 700 µL aliquot of extraction solvent and repeat elution protocol. Apply pressure (5–10 seconds) to elute any remaining extraction solvent.

Post Extraction

Dry the extract in a stream of air or nitrogen using a Biotage® SPE Dry 96 (40 °C at 60 L/min) or TurboVap® 96 (40 °C at 1.0 bar).

Reconstitution

Reconstitute with 100 µL of methanol:water (60:40, v/v) with 0.1% formic acid to each well and let sample equilibrate for 15 minutes.

HPLC Conditions

Instrument
Agilent 1260 Liquid Handling System (Agilent, Santa Clara, CA.)

Column
Restek Raptor Biphenyl (50 mm x 2.1 mm, 3 µm)

Mobile Phase
A: Water with 0.1% formic acid

B: Methanol with 0.1% formic acid

Flow Rate
0.3 mL/min

Injection
10 µL

Column Temperature
35 °C

MS Conditions
A Sciex 4000 Q-Trap triple quadrupole mass spectrometer (Sciex, Foster City, CA.) was used equipped with a Turbo Ionspray® interface for mass analysis. Positive ions were acquired in the multiple reaction monitoring (MRM) mode with the ion source temperature at 500 °C.

Table 1. HPLC Gradient Conditions.

Step	Time (min)	Flow Rate (µL/min)	% A	% B
1	0.0	300	40	60
2	0.20	300	40	60
3	0.6	300	15	85
4	1.0	300	15	85
5	1.0	300	40	60
6	5.0	300	40	60

Table 2. MS conditions for target analytes.

Analyte	MRM Transition	Declustering Potential (v)	Collisopn Energy (CE)
Buprenorphine	468 > 396.2	40	70
Norbuprenorphine	414 > 83	40	70
Buprenorphine-D_4	472.1 > 58.9	40	80
Norbuprenorphine-D_3	417.1 > 83.0	40	80

Results/Discussion

Chromatography
Buprenorphine and norbuprenorphine (analyte and internal standard) were chromatographically separated on a biphenyl column using a linear gradient of methanol and water, with 0.1% formic acid. The extracted ion chromatogram (see **Figure 2**) shows baseline separation of the two analytes and their corresponding deuterated internal standards.

Extraction Recoveries and Matrix Suppression
A working stock solution of a 0.1 µg/mL of buprenorphine and norbuprenorphine was prepared in methanol. The working stock was spiked into whole blood at a concentration level of 5.0 ng/mL and allowed to equilibrate for a minimum of 30 minutes. The spiked whole blood samples were then extracted using the ISOLUTE® SLE+ 96-well plate following the protocol described. The extracted solutions were clear in color. The samples were then dried down and reconstituted as described. All of the reconstituted samples were clear in appearance. The samples were analysed using the LC-MS/MS method outlined above.

Analyte recoveries were determined using fortified blanks containing the same amount of extracted matrix. The average recoveries (n=8) for buprenorphine and norbuprenorphine were subsequently calculated as 69.9 and 101, respectively, and are shown in **Figure 3** for both analytes at 5.0 ng/mL spiked concentration. The samples were extracted in replicates of 8 with RSDs less than 10%.

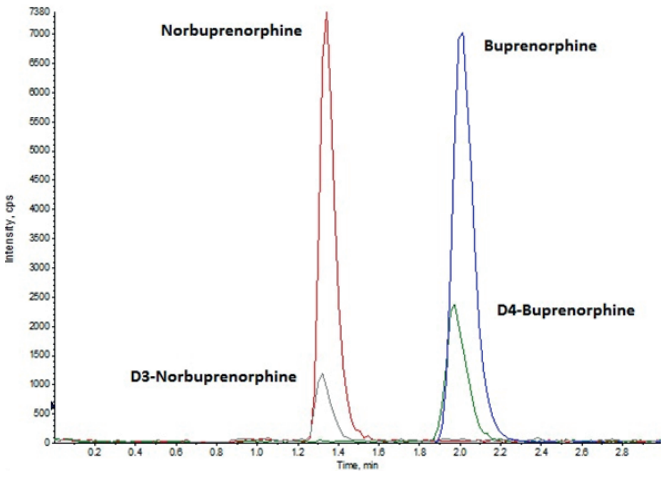

Figure 2. Extracted Ion Chromatogram for Buprenorphine and Norbuprenorphine with Internal Standards.

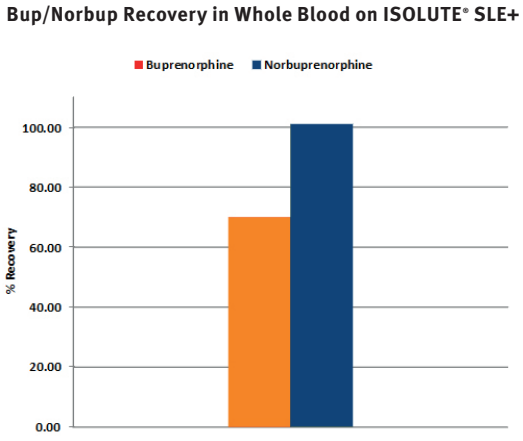

Figure 3. Averaged percent recovery (n=8) for Buprenorphine and Norbuprenorphine spiked into whole blood at 5.0 ng/mL.

Matrix effects were determined at 5.0 ng/mL for the extracted samples. Figure 4 shows the observed percent of suppression of 28% and 16% for buprenorphine and norbuprenorphine, respectively. The matrix effect data coupled with the recovery data indicate that the extraction protocol would be viable for qualitative and quantitative analysis of buprenorphine and norbuprenorphine in whole blood.

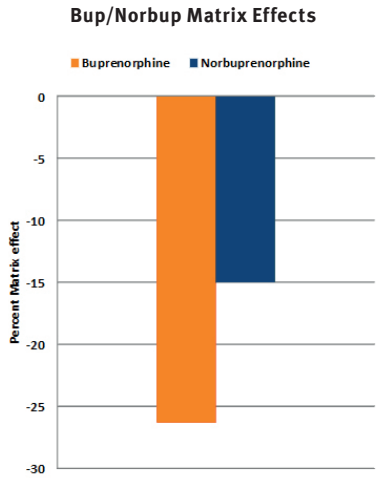

Figure 4. Plot of measured matrix effect for buprenorphine and norbuprenorphine spiked at 5.0 ng/mL into whole blood and extracted using ISOLUTE® SLE+.

Ordering Information

Part Number	Description	Quantity
820-0400-P01	ISOLUTE® SLE+ 400 Supported Liquid Extraction Plate	1
PPM-96	Biotage® PRESSURE+ 96 Positive Pressure Manifold	1
SD-9600-DHS-EU	Biotage® SPE Dry Sample Concentrator System 220/240 V	1
SD-9600-DHS-NA	Biotage® SPE Dry Sample Concentrator System 100/120 V	1
C103263	TurboVap®96, Evaporator 100/120V	1
C103264	TurboVap® 96, Evaporator 220/240V	1

EUROPE
Main Office: +46 18 565900
Toll Free: +800 18 565710
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com
Support Tel: +46 18 56 59 11
Support Fax: + 46 18 56 57 11
eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
Outside US: +1 704 654 4900
us-1-pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
jp-1-pointsupport@biotage.com

CHINA
Tel: +86 21 2898 6655
Fax: +86 21 2898 6153
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