# Fast Extraction of Basic Drugs from Urine with No Dry Down Step Using EVOLUTE® EXPRESS CX Mixed-Mode Cation Exchange SPE prior to LC-MS/MS

This application note describes the solid phase extraction of a variety of basic drugs in urine which are typically screened for forensic toxicology panels using EVOLUTE® EXPRESS CX in a 10 mg 96-well plate format.

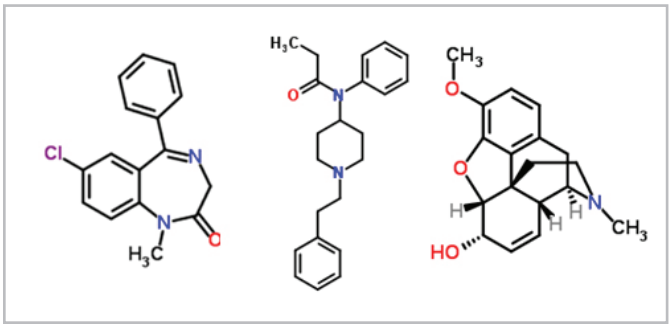


**Figure 1.** Structure of Diazepam, Fentanyl, and Codeine.

## Introduction

The screening of patient urine samples for basic drugs is typically performed following a lengthy sample preparation methodology that requires a time consuming dry down step. A novel elution protocol coupled to the EVOLUTE EXPRESS advanced plate technology affords a fast sample preparation solution that follows a LOAD-WASH-ELUTE-ANALYZE strategy. Basic drugs are fortified into urine and extracted in 3 easy steps using EVOLUTE EXPRESS CX solid phase extraction in a 96-well plate format.

EVOLUTE EXPRESS CX mixed-mode cation exchange polymer-based SPE sorbent extracts a wide range of basic analytes from biological fluids and other aqueous matrices using new plate design technology that reduces processing time through the elimination of the traditional conditioning and equilibration steps. EVOLUTE EXPRESS CX removes matrix components such as proteins, salts, non-ionizable interferences and phospholipids, delivering cleaner extracts with reproducible recoveries for accurate quantitation.

## Analytes

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

## Sample Preparation Workflow: Load » Wash » Elute » Analyze

| | |
|---|---|
| **Format:** | EVOLUTE® EXPRESS CX 10 mg Fixed Well Plate, part number 601-0010-PX01 |
| **Sample Pre-treatment** | Pipette patient urine (200 µL) into a container and add appropriate amount of internal standard. Pipette urine calibrators and QC samples (200 µL) and add appropriate amount of internal standard. Pre-treat each of the samples with HCl (0.01N, 200 µL) and gently vortex the solutions. |
| **Sample Loading:** | Load pre-treated samples onto wells. Allow to flow through sorbent via gravity. |
| **Sample Washing:** | 1) Add water (1000 µL) to each well and allow to flow by gravity.<br>2) Add 0.01N HCl/isopropanol (80:20, v/v, 1000 µL) to each well and allow to flow by gravity. Collect into waste container. Apply vacuum or positive pressure as needed between each step. |
| **Analyte Elution:** | Add a solution of tetrahydrofuran:methanol:acetonitrile:2% aqueous ammonium hydroxide (60:20:19:1, v/v/v/v, 600 µL) and allow solvent to gravity flow into a sample collection plate. Apply positive pressure or pull slight vacuum as needed during collection process to facilitate a flow rate of 1 mL per minute. |
| **Post Extraction** | Add water (600 µL) to the eluent in each well. Load the collection plate onto the HPLC autosampler. |
| **Additional Information:** | Prepare elution solution at a 50 mL total volume by mixing THF (30 mL): MeOH (10 mL): Acetonitrile (9.5 mL): 2% aq $NH_4OH$ (0.5 mL). Mix thoroughly before use. |



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)

**Mobile Phase:** Solvent A: 5mM Ammonium Formate with 0.01% (v/v) Formic Acid
Solvent B: Acetonitrile with 0.01% (v/v) 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

**Ion Source Temperature:** Ambient

## Mass Spectrometry Conditions

**Instrument:** 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

**Table 1.** MRM transitions for drugs in positive mode Turbo Ionspray.

| 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 | 40 | 40 | 16 |
| 8 | Normeperidine | 234>160 | 30 | 30 | 16 |
| 9 | Naltrexone | 342.2>323.8 | 30 | 40 | 16 |
| 10 | Morphine | 286>165 | 45 | 40 | 16 |
| 11 | Codeine | 300>199 | 30 | 40 | 16 |
| 12 | Oxymorphone | 302>227 | 30 | 30 | 16 |
| 13 | Oxycodone | 316>241 | 45 | 40 | 16 |
| 14 | Hydrocodone | 300>199 | 25 | 25 | 16 |
| 15 | 6-Acetyl Codeine | 342.4>255 | 30 | 40 | 16 |
| 16 | 6-Acetyl Morphine | 328>165.5 | 30 | 45 | 16 |
| 17 | Fentanyl | 337>188 | 30 | 30 | 16 |
| 18 | Buprenorphine | 468.2>396.2 | 30 | 60 | 16 |
| 19 | EDDP | 278>234 | 30 | 40 | 16 |

## Discussion

The advanced plate design technology utilized in the EVOLUTE® EXPRESS formats affords a **LOAD-WASH-ELUTE** strategy that yields a relatively abbreviated solid phase extraction methodology. Blank urine samples were fortified with basic drug standards to yield final sample concentration of 10 ng/mL. The fortified samples were diluted 1:1 with 0.01N HCL solution to pH adjust the urine samples and ionize the basic drugs in solution. These samples were then loaded onto the sorbent without the need for conditioning or equilibration steps. The sorbent was subsequently washed with water and then aqueous acidified isopropanol to remove urea, creatinine, salts, and other interferences.

The novel elution strategy utilizes tetrahydrofuran (THF) which is a water miscible organic solvent as the major polar elution solvent instead of ethyl acetate or other water immiscible organic solvents typically used to elute basic drugs. The THF can be mixed with other HPLC compatible solvents to yield an effective elution solvent that can be further diluted with water post elution and directly injected onto an HPLC column. The novel solution of THF:MeOH:ACN:$NH_4OH$ (2%aq.) prepared as reported above, yields recoveries for the basic drug analytes ranging from 52–110% with typical averaged recoveries for most analytes greater than 75%. (**Figure 2**) The elution sample is collected as a clear solution prior to dilution and injection onto LC-MS/MS system. The percent relative standard deviation for each series was typically ‹ 10%.

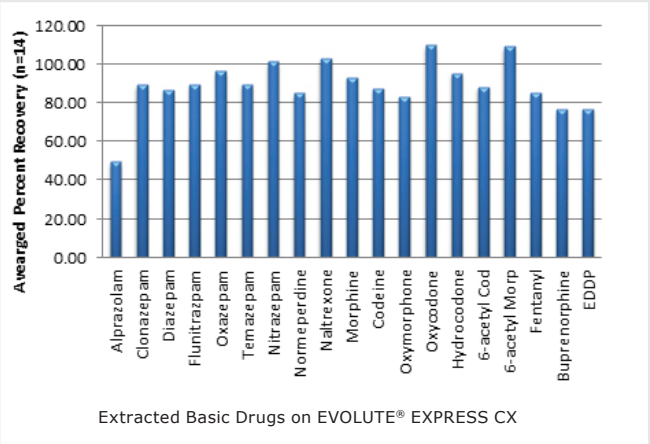


**Figure 2.** Plot of averaged recoveries for basic drugs extracted from fortified urine using EVOLUTE EXPRESS CX plates. The drug standards were fortified at 10 ng/mL.

## Ordering Information

| Part Number | Description | Quantity |
|---|---|---|
| 601-0010-PX01 | EVOLUTE® EXPRESS CX 10 mg Fixed Well Plate | 1 |
| PPM-96 | Biotage® PRESSURE+ 96 Positive Pressure Manifold 96 well | 1 |

For the latest application notes visit www.biotage.com

**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

**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

**JAPAN**
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com

**CHINA**
Tel: +86 21 2898 6655
Fax: +86 21 2898 6153
cn_order@biotage.com

To locate a distributor, please visit our website at www.biotage.com


**Part Number: AN806**