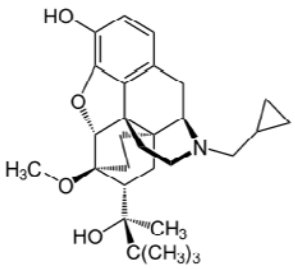
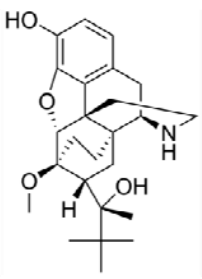
# Buprenorphine in Urine

## - Maintenance medication for opioid addiction

Buprenorphine is a synthetic opioid used for pain treatment and maintenance medication for opioid addiction. Buprenorphine is metabolized to nor-buprenorphine involving enzyme CYP3A4 in the liver. Both the parent compound and its metabolite conjugate to glucuronoides. Maximum plasma concentrations are reached within 30 minutes to three hours after administration. On the following pages, we show a method for analysis of buprenorphine and norbuprenorphine using reversed phase LC-MS/MS aided by isotope internal standards. Patient samples (urine) were analysed along with standards and control samples.




Buprenorphine




Norbuprenorphine

# Buprenorphine in Urine

## Purospher® STAR RP-18 endcapped

Recommended column:
Purospher® STAR RP-18 endcapped (2 µm) Hibar® HR 50-2.1 mm (1.50646.0001)

Recommended solvents and reagents:

Acetonitrile: hypergrade for LC-MS LiChrosolv® (1.00029)

Water: Water for chromatography LiChrosolv® (1.15333)
or freshly purified water from Milli-Q® water purification system

Formic acid 98-100% for analysis EMSURE® ACS, Reag. Ph Eur (1.00264)

Mobile phase: A: 0.1% formic acid in Milli-Q water
B: 0.1% formic acid in acetonitrile

| Time (min) | A (%) | B (%) |
|---|---|---|
| 0.00 | 90 | 10 |
| 0.25 | 90 | 10 |
| 2.00 | 10 | 90 |
| 2.10 | 90 | 10 |
| 3.00 | 90 | 10 |

**Sample preparation:**
Take 1 ml of urine sample to 10 mL glass tube, add 50 µL internal standard followed by 1 ml 0.1 M acetate buffer pH. Thereafter mix samples thoroughly and place in centrifuge (4000 rpm) for 5 minutes. Take a mixed-mode solid phase extraction cartridge (6 mL) and condition with 2 ml methanol followed by 2 ml of 0.1 M acetate buffer, pH 4. Apply samples, standards and internal controls on solid phase extraction (SPE) cartridges and let the samples slowly run through the cartridges by gravity. Wash the cartridge with 2 mL of 0.05 M HCl followed by 2 mL of a solution containing methanol/water (50:50 v/v), and allow the fluids to flow through the cartridges. Dry the cartridge with full vacuum for 4 minutes. Elute SPE tubes with 2 ml solution of dichloromethane/isopropanol/2% ammonia. Take the eluate and from each sample and evaporate all solvent. Add 200 µL of mobile phase and make sure all surface is wetted to re-constitute the sample completely. Transfer sample to autosampler vials and run analysis on LC-MS/MS system.

**Quantitation of buprenorphine and norbuprenorphine in urine**
Linear range: 1 – 100 ng/ml for buprenorphine.
Linear range: 5 – 1000 ng/ml for norbuprenorphine

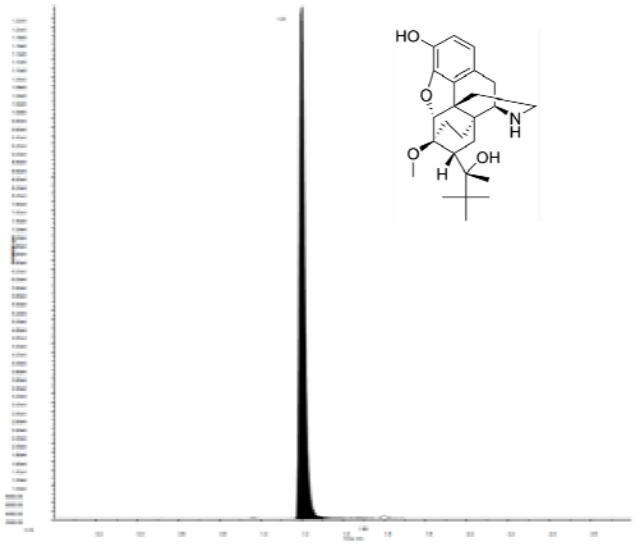
# Buprenorphine in Urine

## Purospher® STAR RP-18endcapped

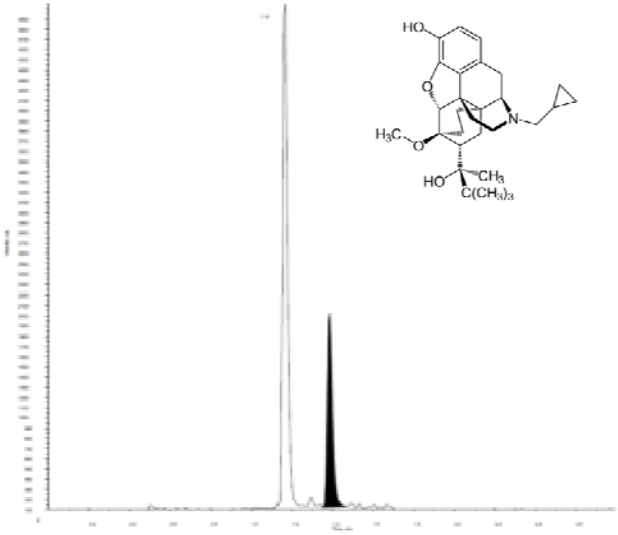
### Chromatographic Conditions

| | |
|---|---|
| Column: | Purospher® STAR RP-18 endcapped (2 µm) Hibar® HR 50-2.1 mm (1.50646.0001) |
| Injection: | 10 µL |
| Detection: | LC-MS/MS ESI; MRM transitions: 468.6/55.2 (Bup) and m/z 414.6/83.2 (Norbuprenorphine) |
| Flow Rate: | 0.7 mL/min |
| Gradient | See table |
| Mobile Phase: | A: 0.1% formic acid in Milli-Q water<br>B: 0.1% formic acid in acetonitrile |
| Temperature: | 40 °C |
| Sample: | Urine samples treated according to sample preparation protocol. |
| Backpressure: | 230 bar (3312 psi) at start of gradient |

| Time (min) | A (%) | B (%) |
|---|---|---|
| 0.00 | 90 | 10 |
| 0.25 | 90 | 10 |
| 2.00 | 10 | 90 |
| 2.10 | 90 | 10 |
| 3.00 | 90 | 10 |




Norbuprenorphine concentration: 642 ng/ml




Buprenorphine concentration: 2.16 ng/ml

### Chromatographic Data

| No. | Compound | Retention Time (min) | Precursor ion (m/z) | Product ions (m/z) |
|---|---|---|---|---|
| 1 | Void volume | 0.2 | - | |
| 2 | Norbuprenorphine | 1.2 | 414.6 | 83.2 |
| 3 | Buprenorphine | 1.4 | 468.6 | 55.2 |

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
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