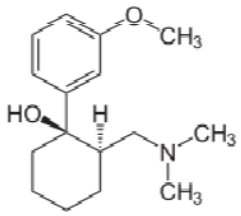
Tramadol in Urine

- Analgesics

Tramadol is a weak μ-opioid receptor agonist and inhibitor of serotonin/noradrenalin re-uptake used for pain treatment and other indications such as treatment for restless leg syndrome and fibromyalgia. Tramadol has a structural similarity to the natural opiate codeine. Tramadol is reported to have a lower risk of developing dependence than codeine. Tramadol is a racemate, where the (+) form inhibit the reuptake of serotonin and the (-) form inhibit norepinephrine. In addition, tramadol is extensively metabolized, and where a total of 24 metabolites have been identified. The active metabolite 0-desmethyl tramadol has a higher affinity for opioid receptors than the parent compound.

Urine samples from patients were analysed in both methods along with standards and control samples.



Tramadol

Tramadol in Urine

Purospher® STAR RP-18 endcapped

Recommended column:
Purospher® STAR RP-18endcapped (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 (%)	Flow Rate (mL/min)
0.00	95	5	0.40
0.20	95	5	0.40
2.00	50	50	0.40
2.50	10	90	0.40
2.80	10	90	0.40
3.00	95	5	0.50
4.50	95	5	0.50

Sample preparation:
Take 0.2 ml of each sample into a 5 mL glass tube with screw cap. Add 25 µL internal standard followed by 1.0 ml 0.1 M acetate buffer pH 4. Use vortex mixer for a few seconds to homogenize the samples. Centrifuge, if necessary, at 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 5 minutes. Elute SPE tubes with 2 ml solution of dichloromethane/isopropanol/2% ammonia. Take the eluate from each sample and evaporate all solvent to dryness. Add 4.0 mL of mobile phase and make sure all surface is wetted to re-constitute the sample completely. Transfer 2.0 mL sample to autosampler vials and run analysis on LC-MS/MS system.

Quantitation of Tramadol in urine

Linear range: 25 – 1500 ng/mL

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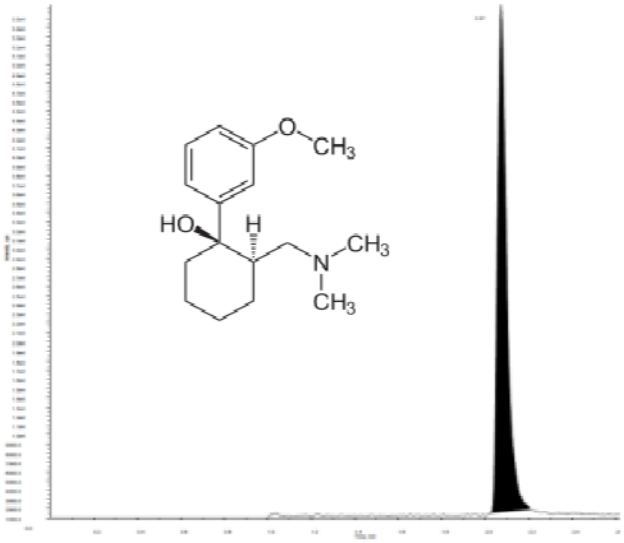
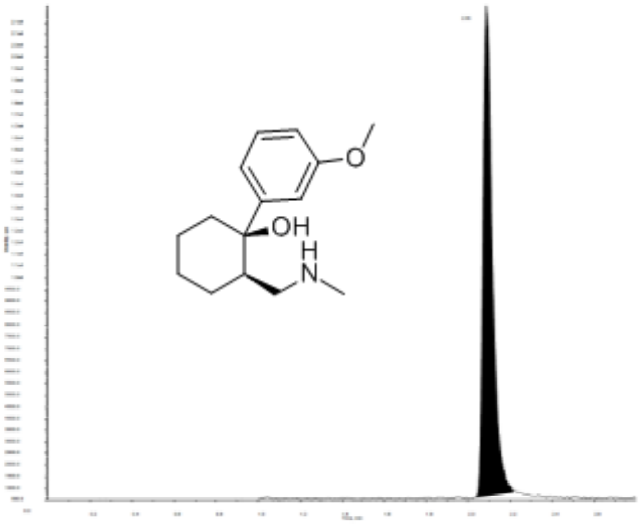
Purospher® STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (2 µm) Hibar® HR 50-2.1 mm (1.50646.0001)
Injection:	5 µL
Detection:	LC-MS/MS ESI; MRM transitions: m/z 264/58 (Tramadol) and m/z 250/44
Flow Rate:	0.4 mL/min
Gradient	See table
Mobile Phase:	A: 0.1% formic acid in Milli-Q water B: 0.1% formic acid in acetonitrile
Temperature:	40 °C
Sample:	Urine samples treated according to sample preparation protocol.
Backpressure:	170 bar (2448 psi) at start of gradient



Tramadol concentration: 23.2 ng/mL



N-desmethyl-tramadol concentration: 21.2 ng/mL

Chromatographic Data

No.	Compound	Retention Time (min)	Precursor ion (m/z)	Product ions (m/z)
1	Void volume	0.2	-	
2	Tramadol	2.07	264.0	58.0
3	N-desmethyl-tramadol	2.09	254.0	44.0