

## **LC/MS/MS method for the detection of narcotic analgesics and non-steroidal anti-inflammatory drugs in human urine**

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### ***Introduction***

The use and misuse of drugs and medications by competitors in sport has been recognized as an important problem. Athletes might use substances to eliminate any obstacle they might encounter during their training. One of the obstacles they might encounter is pain felt from injury or excessive training. The narcotic analgesics and the non-steroidal anti-inflammatory drugs (NSAIDs) are some of the drugs abused by competitors to overcome the effects of pain. Although the World Anti-Doping Agency (WADA) does not prohibit the use of NSAIDs, there is evidence that it is being used in endurance sport. The increased sensitivity accomplished by the introduction of liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) makes it the preferred choice for the detection of these substances. The aim of this study was to develop a screening method for the simultaneous detection of an extended list of narcotic analgesics and NSAIDs in human urine using LC/MS/MS

### ***Material and methods***

#### ***Chemicals and reagents***

Reference standards of narcotic analgesics and NSAIDs were obtained from the Department of Pharmacology at the University of the Free State or from Pharmaceutical companies.  $\beta$ -glucuronidase/arylsulphatase was obtained from Roche Diagnostics, sodium hydrogen phosphate, potassium dihydrogen phosphate and formic acid were obtained from Merck. The diethyl ether was obtained from Burdick and Jackson and the acetonitrile from Sigma-Aldrich. The buffer and mobile phase were prepared in MilliQ water from our own system.

### *Sample preparation*

A new sample preparation method was developed for this study. This included the hydrolysis of two (2) ml of urine samples at pH 5.2 with  $\beta$ -glucuronidase/arylsulphatase for 2 hours at 50 °C. One ml of phosphate buffer at pH 7.0 was added and extracted with 5 ml of diethyl ether. After centrifugation for 5 minutes the organic phase was evaporated to dryness under a stream of nitrogen-gas. The residue was dissolved in 100  $\mu$ l of mobile phase and 10  $\mu$ l injected onto the LC-MS/MS system

### *Instrument Parameters*

Instrument: AB Sciex API 2000 MS/MS System with an Agilent 1100

Series HPLC system

LC column: Zorbax Eclipse XDB-C18 (50 mm x 2.1 mm) 1.8  $\mu$

Mobile Phase A: 0.1 % Formic Acid

Mobile Phase B: Acetonitril

Gradient: 0 - 2 minutes A: 90% B: 10%

2 – 8 minutes A: 10% B 90%

8 – 9 minutes A: 90% B 10%

9 – 15 minutes A: 90% B 10% (equilibrate)

Method runtime: 15 minutes runtime per sample

### *Validation*

Validation was performed for qualitative purposes for non-threshold substances according to the International Standards for Laboratories (ISL).

Specificity: Ten different blank urine samples were analyzed and compared with 10 corresponding control sample spiked with various narcotic analgesics and NSAIDs.

Robustness: Five aliquots from the same control sample at low, medium and high concentration were analyzed over 5 days (5 aliquots each day). Coefficient of variation (CV %) were calculated

Limit of detection: Sequentially diluted urine were analyzed and the signal to noise (S:N) calculated. The LOD is where S:N > 3.

## Results and discussion

Table 1: Characteristic parameters for the compounds

Compound name	RT (min)	Precursor ion	Product ion	LOD (ng/ml)	Repeatability CV %
Apomorphine (ISTD)	4.45	268	237	-	2.0
Buprenorphine	5.43	468	187	0.6	3.0
Codeine	1.73	300	128	0.7	7.0
Dextromoramide	5.62	393	306	0.8	1.0
Dextropropoxyphene	5.58	340	266	0.5	9.0
Diclofenac	6.79	262	234	0.5	4.0
Ethoheptazine	5.32	337	188	0.6	3.0
Fenoprofen	5.54	300	199	0.5	7.0
Fentanyl	5.45	286	185	0.9	4.0
Hydrocodone	1.65	310	265	2.7	6.0
Hydromorphone	1.08	286	115	1.7	7.0
Ibuprofen	6.90	358	340	0.5	6.0
Indomethacin	6.80	316	298	0.6	7.0
Ketoprofen	6.18	302	284	0.7	6.0
Ketorolac	5.97	287	218	1.7	7.0
Mefenamic acid	7.25	248	220	0.9	5.0
Meloxicam	6.39	274	155	0.9	9.0
Methadone	5.56	264	58	0.4	1.0
Morphine	1.07	268	237	0.9	10.0
Nabumetone	6.78	468	187	0.8	9.0
Nalbuphine	5.73	300	128	0.5	5.0
Naproxen	6.35	393	306	3.0	10.0
Oxycodone	2.39	340	266	1.9	2.0
Oxymorphone	0.98	262	234	0.8	9.0
Paracetamol	1.69	152	110	18.0	4.0
Pentazocine	5.32	300	199	0.5	5.0
Pethidine	5.28	286	185	1.3	5.0
Phenylbutazone	6.99	310	265	0.5	15.0
Piroxicam	6.04	286	115	34.0	1.0
Salicylic acid	2.38	358	340	0.8	9.0
Sulindac	5.90	316	298	0.8	6.0
Tenoxicam	5.62	302	284	2.0	7.0
Tiaprofenic acid	6.20	287	218	2.0	8.0
Tilidine	5.36	248	220	0.5	1.0

## **Conclusion**

There is evidence that NSAIDs is being abused by endurance athletes. This abuse is also observed in veterinary drug analysis, especially in equine sports. Although the use of NSAIDs is not prohibited by WADA, a fast and reliable LC/MS/MS method for the simultaneous detection of narcotic analgesics and NSAIDs was developed for future studies to determine the extend of this abuse by athletes.

## **References**

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