Quantitative Urine Pain Management Drug Screening with HPLC-MS/MS

Background Information

Pain management drugs are among the most prescribed medications, and they are also the most abused class of prescription drugs.[1-2] Therefore, many states including Ohio have enacted laws governing the prescription of pain management drugs. Patients enrolled in pain management programs need to be monitored for compliance, which means appropriate use of prescribed drugs and abstinence from non-prescribed drugs.

Urine is the most commonly used specimen in monitoring pain management and illicit drug use. A single urine specimen can be tested for a number of drugs. Immunoassay (IA) can be used as a screening method, but it lacks specificity and sensitivity. For example, most immunoassay tests cannot distinguish various opiate drugs and cannot detect oxymorphone at all.[3-4] In addition, immunoassay is only semi-quantitative and clinicians in some cases need quantitative assessment of urine drug concentrations.[3] For example, the morphine-to-codeine ratio can help identify heroine abusers.[5]

High performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) offers high resolving power, high selectivity and wide dynamic range, all of which enable simultaneous quantification of a broad spectrum of drugs present in biological matrices.[3] In clinical laboratories, HPLC-MS/MS can be used as a confirmation method in conjunction with a prior immunoassay-based screening method, or it can be used as a stand-alone screening method offering quantitative results with high confidence.

Some drugs/metabolites (e.g. morphine, codeine and THCA) are present in urine as both free and glucuronide conjugates. The conjugation rate varies significantly. In order to have consistent results, conjugated drugs are usually converted to free drugs prior to HPLC-MS/MS analysis. Chemical hydrolysis is very efficient but may result in unwanted reactions. For example, acid hydrolysis will convert heroin and its metabolite, 6-monoacetylmorphine (6-MAM), to morphine.[6] In contrast, enzymatic hydrolysis is much milder, but needs longer incubation time to achieve sufficient (>80%) hydrolysis efficiency.[7]

Cleveland Clinic Laboratories offer an HPLC-MS/MS method for quantitative screening of 11 pain management drugs and five other commonly abused drugs in urine.[8] In this method, total drug concentration is measured after enzymatic hydrolysis. This assay also includes an adulteration test using urine test strips to ensure specimen quality. In addition to pain management, this test can also be used for monitoring patient compliance in rehabilitation programs.

The high sensitivity of this method is capable of detecting drug manufacturing impurities. As an example, codeine is a known manufacturing impurity in morphine (~0.04%) and can be detected by this method.[9]

Clinical Information

This test detects the following 11 pain management drugs in urine:

- Morphine
- Codeine
- Dihydrocodeine
- Oxycodone
- Oxymorphone
- Hydrocodone
- Hydromorphone
- Methadone and metabolite
- Fentanyl and metabolite
- Tramadol and metabolite
- Buprenorphine and metabolite

Also included are five commonly abused drugs that are not prescribed for pain management:

- Amphetamine
- Methamphetamine
- Cocaine metabolite
- Heroin metabolite
- Marijuana metabolite

In most cases, both parent drug and metabolite are quantified. Tetrahydrocannabinol (THC, the main active component of marijuana), cocaine and heroin have rather short half-lives and therefore only the stable metabolites are analyzed. As a result, a total of 20 analytes are measured in this test (Table 1).

This test can assist clinicians to determine patient compliance. Presence of prescribed drugs/metabolites and absence of non-prescribed drugs/metabolites indicate patient compliance. Test results are for medical purposes only.

Interpretation

Table 1 lists the lower limit of quantification (LLOQ) and higher limit of quantification (HLOQ) for each analyte in the Pain Panel. A result greater than the LLOQ for a parent drug and/or metabolite indicates use of the drug.

Table 1. Analytical Measurement Ranges

Analyte	LLOQ (ng/ml)	HLOQ (ng/ml)
Morphine	5	5365
Codeine	11	5484
Dihydrocodeine	5	5184
Oxycodone	5	4719
Oxymorphone	5	4581
Hydrocodone	8	5270
Hydromorphone	5	4980
Methadone	16	4897
EDDP	6	4178
Fentanyl	6	5971
Norfentanyl	6	5514
Tramadol	25	5208
O-desmethyl Tramadol	20	5000
Buprenorphine	20	5000
Norbuprenorphine	20	4300
Amphetamine	5	5365
Methamphetamine	8	5339
Benzoylecgonine	24	5410
6-Acetylmorphine	5	4800
THCA	21	5008

The level of drugs/metabolites in urine is affected by dose, metabolic rate, sample collection time relative to the drug use and hydration status.

Detailed data interpretations for each analyte are listed below:

Morphine: Morphine may arise from morphine-containing drugs, poppy seeds or by metabolism of codeine and heroin. Morphine is metabolized to hydromorphone.

Codeine: Codeine is not a recognized metabolite of other opiates and its presence indicates use of a codeine-containing drug. Codeine is metabolized to morphine. Other minor metabolites include hydrocodone, hydromorphone and dihydrocodeine. Use of heroin is indicated when 1) both codeine and morphine are detected in urine; 2) morphine concentration is >10,000 ng/mL; and 3) morphine-to-codeine ratio is above 2.

Dihydrocodeine: The presence of dihydrocodeine may arise from dihydrocodeine-containing drugs or from the metabolism of hydrocodone. Dihydrocodeine is also metabolized to hydrocodone.

Oxycodone: Oxycodone is not a recognized metabolite of other opiates and its presence indicates use of an oxycodone-containing drug. Oxycodone is metabolized to oxymorphone.

Oxymorphone: Oxymorphone may arise from oxymorphone-containing drugs or by metabolism of oxycodone.

Hydrocodone: Hydrocodone may arise from hydrocodone-containing drugs or by metabolism of dihydrocodeine. Hydrocodone is metabolized to hydromorphone and dihydrocodeine.

Hydromorphone: Hydromorphone may arise from hydromorphone-containing drugs or by metabolism of morphine and hydrocodone.

Amphetamine: Amphetamine may arise from amphetamine-containing drugs (eg. Adderall and Benzedrine) or by metabolism of methamphetamine. Clobenzorex, famprofazone, fenethylline, fenproporex and mefenorex contain amphetamine pro-drugs that can be metabolized to amphetamine. Selegiline is metabolized to both amphetamine and methamphetamine.

Methamphetamine: Methamphetamine may arise from methamphetamine-containing drugs or metabolism of selegiline, which is metabolized to both methamphetamine and amphetamine. Over-the-counter inhalers for nasal decongestion may cause positive methamphetamine results. Methamphetamine is metabolized to amphetamine.

Benzoylecgonine: Benzoylecgonine is a metabolite of cocaine. Presence of benzoylecgonine indicates use of cocaine.

Methadone: Presence of methadone indicates use of a methadone-containing drug. Methadone is metabolized to EDDP (2-ethylidene-1,5-dimethyl1-3,3-diphenylpyrrolidine). Urine methadone levels vary widely depending on metabolism and urine pH. However, urine EDDP levels are less affected by pH and are preferable for assessing compliance with methadone therapy. The absence of EDDP and presence of methadone in a urine specimen very likely indicate adulteration by direct addition of methadone to the urine specimen.

EDDP: EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine) is a metabolite of methadone, and its presence indicates use of a methadone-containing drug.

Fentanyl: Presence of fentanyl indicates use of a fentanyl-containing drug. Fentanyl is metabolized to norfentanyl.

Norfentanyl: Norfentanyl is a metabolite of fentanyl, and its presence indicates use of a fentanyl-containing drug.

Tramadol: Presence of tramadol indicates use of a tramadol-containing drug (Ultram). Tramadol is metabolized to O-Desmethyltramadol.

O-Desmethyltramadol: O-Desmethyltramadol is an active metabolite of Tramadol and its presence indicates use of a tramadol-containing drug (Ultram).

THCA: Tetrahydrocannabinol carboxylic acid (THCA) is a metabolite of delta-9-tetrahydrocannabinol, which is the main active component of marijuana. Presence of THCA indicates use of marijuana.

6-MAM: 6-MAM (6-monoacetylmorphine, also known as 6-acetylmorphine) is a unique metabolite of heroin. Presence of 6-MAM indicates use of heroin. 6-MAM is further metabolized to morphine and absence of 6-MAM does not rule out the use of heroin.

Buprenorphine: Presence of buprenorphine indicates use of buprenorphine-containing drugs (e.g. Suboxone and Buprenex). Buprenorphine is metabolized to norbuprenorphine.

Norbuprenorphine: Norbuprenorphine is the primary active metabolite of buprenorphine. Presence of norbuprenorphine indicates use of buprenorphine-containing drugs (e.g. Suboxone and Buprenex).

Table 2. Adulteration test results

Urine Test Strip Results	Clinical Interpretation
Creatinine 10 - 20 mg/dL and specific gravity < 1.003	Dilution
Creatinine <10 mg/dL and specific gravity < 1.003 or >1.020	Substitution
Presence of oxidant and/or pH<3 or pH>10	Adulteration

Methodology

1) Isotope-labeled internal standards are added to each urine sample.
2) All samples are subjected to enzymatic hydrolysis (16-24 hours) to convert glucuronide conjugates to free drugs. Hydrolysis efficiency is >90% for all drugs.
3) After hydrolysis, samples are analyzed by turbulent flow online extraction and HPLC-MS/MS in the multiple reaction monitoring mode.
4) Analyte concentration is calculated based on analyte to internal standard peak area ratio with the use of a calibration curve.
5) A specimen quality test is included with this testing that measures creatinine concentration, specific gravity, pH and presence of oxidant.

References

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Test Overview

Test Name	Quantitative Pain Panel, Urine
Reference Ranges	Morphine <5 ng/ml; Oxymorphone <5 ng/ml; Hydromorphone <5 ng/ml; Dihydrocodeine <5 ng/ml; Codeine <11 ng/ml;Benzoylecgonine <24; ng/ml; Amphetamine <5 ng/ml; O-desmethyl Tramodol <20 ng/ml; Oxycodone <5 ng/ml; Methamphetamine <8 ng/ml; Hydrocodone <8 ng/ml; Norfentanyl <6 ng/ml; Tramodol <25 ng/ml; Fentanyl <6 ng/ml; Methadone <16 ng/ml; EDDP <6 ng/ml; Buprenorphine <20 ng/ml; Norbuprenorphine <20 ng/ml; 6-Acetylmorphine <5 ng/ml; THC <21 ng/ml Quantitative Pain panel, Urine: Creatinine: >19 mg/dL Quantitative Pain panel, Urine: Creatinine: >19 mg/dL Quantitative Pain panel, Urine: pH: 4-10 Quantitative Pain panel, Urine: Specific Gravity: 1.005-1.020 Quantitative Pain panel, Urine: Oxidants: Negative
Specimen Requirements	Testing Volume/Size: 10 mL; Type: Urine, random; Tube/Container: Clean container; Transport Temperature: Refrigerated; Note: No preservative. Minimum Specimen Requirements: Testing Volume/Size: 2.5 mL; Note: No preservative.
Ordering Mnemonic	UQNTPP
Disclaimers or Notations	For medical purposes only; not intended for forensic use.
CPT Codes	80299; 82145(x2); 82520; 82646; 83840(x2); 83925(x13)
Billing Code	82347

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