Stability of pain-related medications, metabolites, and illicit substances in urine

Elizabeth Gonzales [a,*], Gary Ng [b], Amadeo Pesce [a], Cameron West [a], Robert West [a], Charles Mikel [a], Sergey Latyshev [a], Perla Almazan [a]

[a] Millennium Research Institute, 16980 Via Tazon, San Diego, CA 92127, USA
[b] Millennium Laboratories, 16981 Via Tazon, San Diego, CA 92127, USA

ABSTRACT

Background: Effective urine drug testing requires an understanding of the stability of medications, metabolites and other substances excreted in the urine matrix. When the testing results do not fit the clinical picture, physicians frequently request repeat testing of the original specimen in order to corroborate the results. We determined the stability in urine of various medications, metabolites, and illicit substances commonly requested for testing by physicians treating patients with pain and pain-related disorders.
Methods: Quantitative analyses of urine specimens were performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Two replicates at a high and low concentration were analyzed at time 0, and after 2, 3 and 6 months following storage at $+4$ °C and -20 °C. At each time interval, the percent difference from time 0 for each analyte was calculated and averaged for each storage condition.
Results: For the majority of medications, the percent differences were within 20% of the original measurement for all 3 storage conditions. All were within 30% of the original measurement after 2, 3 and 6 months in all storage conditions, except for 7-amino-clonazepam, and carboxy-tetrahydrocannabinol.
Conclusions: The findings from the current study confirm that the majority of medications, metabolites, and illicit substances commonly requested for testing by physicians treating patients with pain and pain-related disorders are stable within 20% of the original concentration when stored refrigerated or frozen for up to 6 months. Thus, delayed testing, repeat testing, and add-on testing of urine specimens can yield reliable results for up to 6 months following the urine collection date.

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1. Introduction

Physicians treating patients with chronic pain and pain-related disorders often utilize urine drug testing as part of their comprehensive treatment and monitoring plan [1,2]. Urine drug testing is commonly carried out at reference laboratories, requiring that specimens be transported to the testing facility. The time between collection and analysis can be several days. Because of this time interval, to assure that results are accurate, it is necessary to demonstrate that specimens are stable during this time frame.

In addition, situations may arise when the time between collection and analysis is more than several days. These situations include repeat testing for analytical purposes, repeat testing when the test results do not fit the clinical picture, repeat testing to add additional drugs to the test panel, and repeat testing when a specimen produces an unexpected result [3,4]. In the event that the analyte in question is not stable in the urine specimen, degradation can occur in the time between the initial

test and the re-test. In these instances, the results of the re-test may vary from the original test result.

Previous studies have been conducted to determine the stability of select drugs in urine under different storage temperatures. However, there has not been a comprehensive study conducted to determine the stability of medications, metabolites, and illicit substances commonly requested for testing by physicians treating patients with pain and pain-related disorders. See Table 1 for a summary of the extant literature [5–12].

The majority of the published studies on drug stability in urine have addressed mostly illicit substances such as heroin, methamphetamine, THC, and cocaine, and only few select opioid medications, such as morphine and codeine. These studies have shown that specimens frozen at -20 °C and those stored at $+4$ °C are stable over a period of months [5–9,11]. Most often, specimens sent to reference laboratories are stored at $+4$ °C because freezing and then thawing large numbers of urine specimens is impractical. However, those specimens that need to be set aside for legal purposes may be stored frozen. There is a dearth of information on the stability in urine over time of the types of medications requested for testing by physicians treating patients with pain and pain-related disorders.

* Corresponding author. Tel.: +1 888 756 1627; fax: +1 858 217 1756.
E-mail address: egonzales@millenniumlabs.com (E. Gonzales).

Table 1
Stability of medications in urine: Summary of the extant literature.

Compound(s)	Temperature	Length of storage	Outcomes	Study
Morphine, codeine, methadone, and amphetamine	+24 °C, +37 °C, and −20 °C	14 days	No detectable loss	Frings and Queen [5]
Benzoylecgonine, cTHC, phencyclidine, codeine, morphine, amphetamine, methamphetamine, and lysergic acid diethylamide	−16 to −18 °C	45 days	No significant loss except for cTHC (average cTHC loss: 11%, range: 0 to 34%)	Paul et al. [6]
Amphetamine, benzoylecgonine, codeine, methamphetamine, morphine, cTHC, phencyclidine	−20 °C	12 months	Changes within ± 25% of initial concentration	Dugan et al. [7]
Morphine, codeine	+24 to +30 °C, +4 to +8 °C, and < −15 °C	11 months	Refrigerator and freezer: 10-40% decline; room: results highly variable	Lin et al. [8]
Morphine	−20 °C	12 months	Average percent change: -4% (range −41.4 to +13.9%)	Chang et al. [9]
MDMA	+20 °C, +4 °C, and −20 °C	21 weeks	No significant losses	Clauwaert et al. [10]
Amphetamine and methamphetamine	+25 °C, +4 °C	150 days	No noticeable changes	Zaitsu et al. [11]
6-Acetylmorphine	+25 °C, +4 °C	150 days	+25 °C: significant decreases; +4 °C: no significant losses	
cTHC	−20 °C	1 year	Decline (27.6–81.8%)	Parlar et al. [12]

2. Methods

2.1. Test method

Quantitative analysis of urine specimens was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) at Millennium Laboratories in San Diego, CA. An Agilent® 1200 series binary pump system, well-plate sampler, thermostatted column compartment, paired with an Agilent® triple Quadrupole mass spectrometer and Agilent® MassHunter software were used for analysis.

Chromatographic separation was performed using an acetonitrile formic acid water gradient running at 0.4 mL per minute and a 2.1 × 50mm, 1.8μ Zorbax SB-C18 column. HPLC grade H2O, acetonitrile, methanol, and formic acid were from VWR (West Chester, PA). Mobile phase A = 0.1% formic acid in water, B = 0.1% formic acid in acetonitrile, and column temperature was 50 °C.

Specimens were hydrolyzed prior to analysis using standard enzymatic hydrolysis methods using β-Glucuronidase Type L-II from Patella vulgate (keyhole limpet) (Sigma Aldrich) in 0.4 mol/l pH 4.5 acetate buffer at elevated temperatures. Deuterated internal standards were added prior to hydrolysis. Analyte and deuterium-labeled internal standards are purchased from Cerilliant® Corporation, Round Rock, TX, USA. The deuterated internal standards were diluted to 1000 ng/mL by adding them to synthetic urine (Microgenics®, Fremont, CA).

All spectra were collected using positive electrospray ionization. Optimal instrument parameters were set for each analyte of interest. Typical parameters include: gas temperature, 350 °C; drying gas, 12 L per minute; nebulizer gas (nitrogen), 35 psi (24,100 Pa); capillary voltage, 3000 V; fragmentor voltage, 110 V. Multiple reaction monitoring mode was used for quantitation and product ion scan mode was used to acquire full scan tandem mass spectra (MSMS) spectra. In product ion scan mode, MS-MS spectra were collected with collision energy set to 5 V, 10 V, 20 V, and 30 V.

Product ion spectra were collected in the range of m/z 50 to 350. Scan times were set to 500 ms. In multiple reaction monitoring mode, 2 transitions were used to identify and quantitate a single compound. A quantitative transition was used to calculate concentration based on the quantifier ion, and a qualitative transition was used to ensure accurate identification of the target compound based on the ratio of the qualifier ion to the quantifier ion. The quantitative transitions used are shown in Table 2.

Quantitative analysis was performed using Agilent® MassHunter Quantitative Analysis software. Accepted accuracy for calibrators was ±20% of the target value and the coefficient of determination (R^2) was required to be ≥0.99 as verification of linearity and goodness-of-fit. Ion suppression was accounted for by addition of deuterated internal standards and the quantitation of each analyte based on the relative response of the analyte to its deuterated analogue.

Lower limits of quantitation were defined as the lowest point of the calibration curve and fulfilled the requirement of the lower limit of quantitation (LLOQ) signal-to-noise ratio of 10:1. The measured upper limit of linearity (ULOL) for the assays were determined using solutions spiked at the ULOL concentrations from pure standards. Replicates of the spiked solutions were quantitated with the same calibration curve used for patient specimen calibration, and the spiked solutions were calculated to be within 20% of their theoretical values (coefficient of variation [CV], 10%); and R^2 was ≥0.99 when the spiked ULOL specimen was included in the calibration curve.

The accuracy and precision of the assays were assessed by the determination of within-run and between-run variation. Quality control (QC) materials were obtained from UTAK® laboratories (Valencia, CA). For within-run variation, twenty-five replicates of QC sample at 2 concentrations (low and high) were run in the same batch. Twenty replicates of 2 concentrations of cTHC QC were run separately. The average concentration and %CV were calculated for both concentrations for each analyte. The %CV was <15 for all analytes (Table 3). For between-run variation, the average concentration and %CV were calculated for both the high and low QC concentrations for each analyte processed in a single month (N > 100,000 specimens). The %CV was <15 for all analytes (Table 4).

2.2. Specimen storage conditions

Urine was collected from a healthy control subject and confirmed negative for any medications using LC-MS/MS testing. Thirty-three medications, metabolites, and illicit substances commonly requested for testing by physicians treating patients with pain and pain-related disorders were individually added to aliquots of this negative urine. Specimens were prepared at 2 concentrations (levels 1 and 2) which correspond to the LC-MS/MS low and high calibrators. Two replicates at each concentration were analyzed at time 0, and after 2, 3 and 6 months following storage at +4 °C and −20 °C. Specimens stored at −20 °C were aliquotted and tested both with and without a freeze thaw cycle at each time interval, resulting in a total of 396 specimens (replicates of 2 concentrations for 3 storage conditions for 33 analytes). The analytes investigated are shown in Table 5. At each time interval, the percent difference from time 0 for each analyte was calculated and averaged for the four specimens (2 each at levels 1 and 2) at each storage condition. There were no exclusion criteria; every specimen concentration result was included in the percent difference calculations.

3. Results

The stability results after 2, 3, and 6 months are shown in Table 5. For the majority of medications, metabolites, and illicit substances, the