A Rapid iMethod™ Test for the Analysis of Drugs of Abuse in Urine

**iMethod™ Test for NIDA 5 Drugs Version 1.0 for Cliquid® Software**

The most commonly used screening category for drugs of abuse is called the NIDA-5 (National Institute on Drug Abuse), which include Cannabinoids, Opiates, Amphetamines, Cocaine and Phencyclidine (PCP). In urine, the target drugs and/or metabolites are amphetamine, methamphetamine, PCP, codeine, morphine, benzoylecggonine, 6-monoacetylmorphine (6-MAM) and, (±)-11-nor-9-Carboxy-Δ9-THC (THC-COOH).

Laboratory-based drug testing is usually performed in a two step fashion using two different detection methods. The first step is a screening test and is usually done by an immunoassay technique (ELISA, EMIT, or RIA). Suspected positive samples are confirmed using a mass spectrometry technique (commonly GC/MS). Recently, LC/MS/MS has emerged as a reliable method to perform both screening and confirmation of drugs in urine without the setup requirements of immunoassays or the time-consuming sample preparation and derivatization required for GC/MS confirmation. Under SAMHSA (Substance Abuse & Mental Health Services Administration) NIDA's current guidelines for federally-mandated testing, urine is the only specimen included for testing government employees and private sector employees covered under the US Department of Transportation or other agency guidelines. The described sample extraction technique provides an effective means to detect and quantify drugs of abuse in urine at or below the typical GC/MS cutoff values.

The following description outlines the instrument requirements and expected results obtainable from AB SCIEX iMethod™ test for the analysis of NIDA-5 drugs in urine when using an AB SCIEX 3200 QTRAP® or API 3200™ LC/MS/MS system in conjunction with a Shimadzu Prominence or Agilent 1200SL LC System. This method includes all the method documentation, MS and LC method files, processing method files and report templates, to deploy this new iMethod™ test in Cliquid® software. It is suggested that all documentation be reviewed completely prior to using the enclosed test.

This method included is for the routine screening (via MRM ratios) and quantification of Cannabinoids, Opiates, Amphetamines, Cocaine and Phencyclidine (PCP) and their related metabolites. This method document contains two sample extraction methodologies, one utilizing CHAPS and one without CHAPS. The recommended method utilizes CHAPS and was found to improve recovery for THC-COOH due to disruption of non-specific binding. Calibration was performed using stock solutions of known concentrations from 2 to 20 000 ng/mL. Control samples of spiked urine at relevant low and high concentrations served to establish the target analyte range. The method uses a deuterated internal standard as an internal standard for each analyte.

The separation was based upon using a Restek DB Biphenyl HPLC Column with a mobile phase gradient of water and acetonitrile with formic acid with a total run time of 5.5 minutes.
This method was evaluated using a nine point calibration curve for each analyte with control sample responses obtained at the relevant low and high concentration ranges of the linear detection range from 20 to 20 000 ng/mL for morphine, codeine, amphetamine, benzoylecgonine and 6-Acetylmorphine and from 2 to 2 000 ng/mL for methamphetamine and PCP. Detection limits were estimated using a signal to noise greater than 10:1 based upon the concentration of the lowest level calibrator. The estimated detection limits for each analyte are more than sufficient to allow the analytical method to be used for either screening or for confirmation.

![Chromatogram](attachment:chromatogram.png)

**Figure 1. Chromatogram of a spiked urine sample at 100 ng/mL for Methamphetamine and PCP and 1000 ng/mL for the other compounds shown.**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Limit of quantitation (ng/mL)</th>
<th>%CV at LOQ</th>
<th>Accuracy at LOQ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCP</td>
<td>2</td>
<td>12.3</td>
<td>98.9</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>20</td>
<td>5.08</td>
<td>100</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>20</td>
<td>5.10</td>
<td>86.4</td>
</tr>
<tr>
<td>Morphine</td>
<td>20</td>
<td>12.2</td>
<td>100</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>2</td>
<td>4.17</td>
<td>99.0</td>
</tr>
<tr>
<td>Codeine</td>
<td>20</td>
<td>9.70</td>
<td>95.7</td>
</tr>
<tr>
<td>6-Acetylmorphine</td>
<td>20</td>
<td>7.44</td>
<td>109</td>
</tr>
<tr>
<td>THC-COOH</td>
<td>20</td>
<td>2.00</td>
<td>101</td>
</tr>
</tbody>
</table>
Calibration

The following example calibration curves illustrate the linearity and dynamic range expected for the analytes.

![Calibration Curves](image)

Figure 2: The following are representative calibration curves for four of the compounds included in the method using CHAPS.

System Requirements

In order to run this method as outlined above, the following equipment and reagents are required:

- An AB SCIEX 3200 series (3200 QTRAP® or API 3200™) LC/MS/MS system
- A Shimadzu Prominence 20A LC system with reservoir tray and bottles, system controller CBM-20A, 100 µl mixer, 2 isocratic LC-20AD, 3 channel degasser, SIL-20AC autosampler, and CTO-20AC column oven or Agilent 1200SL LC system with binary pump (without static mixer), well plate autosampler and thermostated column oven.
- Standard and deuterated drug compounds (www.cerilliant.com)
- Reagent grade formic acid, acetic acid (glacial), sodium acetate (anhydrous), CHAPS, and zinc sulfate heptahydrate
- LC/MS grade water and acetonitrile
- A Restek Analytical Column, 5 µm, DB Biphenyl Column, 50 x 2.1 mm
• A Restek Guard Cartridge, 5 µm, DB Biphenyl Column, 10 x 2.1 mm
• Restek Guard Cartridge Holder, 1 cm
• Pipettes and standard laboratory glassware

Please note that a column is provided with this iMethod™ Test Kit. Also, that this method can be run on other HPLC systems, given that they are supported for use by Cliquid® Software and the retention times are updated in the method according to the HPLC configuration used.

**Ordering Information**

<table>
<thead>
<tr>
<th>Product name</th>
<th>Part number</th>
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</thead>
<tbody>
<tr>
<td>iMethod™ Test for NIDA-5 Drugs V 1.0</td>
<td>1034375</td>
</tr>
</tbody>
</table>

**Important Note**

The purchase and use of certain of the chemicals listed above may require the end user to possess any necessary licenses, permits or approvals, if such are required in accordance with local laws and regulations. It is the responsibility of the end user to purchase these chemicals from a licensed supplier, if required in accordance with local laws and regulations. The suppliers and part numbers listed above are for illustrative purposes only and may or may not meet the aforementioned local requirements. AB SCIEX is not responsible for user’s compliance with any statute or regulation, or for any permit or approval required for user to implement any iMethod™ Test procedure.

The iMethod™ Test described above has been designed by AB SCIEX to provide the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. This method is provided for information purposes only. The performance of this method is not guaranteed due to many different potential variations, including instrument performance, tuning, and maintenance, chemical variability and procedures used, technical experience, sample matrices, and environmental conditions. It is up to the end user to make adjustments to this method to account for slight differences in equipment and/or materials from lab to lab as well as to determine and validate the performance of this method for a given instrument and sample type. Please note that a working knowledge of Analyst® Software may be required to do so.

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