Routine Drug Screening of Human Urine via GC-TOFMS following Automated Solid Phase Extraction (SPE) Cleanup

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Key Words

drug screening, Pharmacovigilance (PV), Gas Chromatograph Time Of Flight Mass Spectrometer (GC-TOFMS) Solid Phase Extraction (SPE), SmartPrep, Urine Drug Testing (UDT), drug metabolites

Introduction

Routine research at the LVR-Hospital Viersen (Clinic for Psychiatry and Psychotherapy of the National Rhineland Association) includes screening of drugs in human urine. Successful treatment of pain disorders rely on sustainable and objective patient data. Routine Urine Drug Testing (UDT) is fast becoming a common method for obtaining regular results that can be used to effectively manage a patient’s pain therapy.

In 1961, the World Health Organization (WHO) established a program dedicated to international drug monitoring, coining the term pharmacovigilance (PV), to focus on testing and monitoring patients for their care and the safe use of medicines. PV is directly applied to the development of new medicines where a series of phase 1-3 clinical trials on humans is used to determine drug efficacy and viability for human treatment or therapy of a disease or condition.

Drug monitoring looks for both the parent drug compounds as well as their metabolites to provide insight on when and how the drugs were taken. The screening process may expect certain drugs and their metabolites to be present. In certain cases, patient monitoring is searching for a pattern of drug presence from the screening process.

In therapeutic monitoring, it is expected that certain levels of drugs will be present. Typical screening looks for drugs such as methadone, opiates, amphetamines, and others. The results from screening are used to develop a therapeutic patient treatment. The reliability of data obtained is essential and often calls for an objective means for sample cleanup prior to final analysis. A common method for urine cleanup is the use of SPE, and the ability for automation to maintain 24/7 consistency in any laboratory and without technician bias increases data confidence. This application note shows the simple transfer of a manual method to automation, even for challenging biological matrices such as small volumes of urine.
Experimental

Prior to SPE, human urine samples were subjected to enzymatic splitting. The SPE process was performed both manually and using the SmartPrep Extractor. Manual SPE was performed with a vacuum.

Instrumentation

Materials

- Sample - human urine
- SPE Cartridges - HyperSep™ Verify-CX SPE cartridge, 200mg, 3mL, Thermo Scientific
- Solvents and standards – laboratory or reagent grade
  - Acetate buffer
  - Methanol
  - Formamide (FA)
  - Ammonia
  - Glucuronidase/arylsulfatase
  - Creatinine
  - N-Acetyltyramine

Reliability of data from an automated system often involves testing a clean sample matrix to confirm the biological sample can be run routinely without causing systemic issues. The SmartPrep® Extractor automated SPE cartridge system was used for running a clean sample of human urine. The automated SPE test showed no leaks or blockages in the valves or the Teflon® tubing, giving confidence the SmartPrep Extractor could perform regular testing using human urine.

Enzymatic splitting of glucuronide or sulfated medicinal and narcotic drugs is generally done in the sample preparation step to free the analytes for better measurement. To accomplish this, the human urine is mixed with beta-glucuronidase/arylsulfatase prior to preparation. Glucuronidase consists of 629 amino acids and is an enzyme that cleaves beta-glucuronide. The split allows for better separation and measurement of the free analytes compared to the glucuronide substances.

The manual sample preparation steps for enzymatic splitting prior to SPE are indicated here:

1. 1.5 mL of urine
2. 100 µL 2M acetate buffer pH 4.8
3. 15 µL glucuronidase/arylsulfatase
4. Intensive mixing
5. Heat for 30 min at 55°C

SmartPrep Extractor for Automated SPE
To test the capability of the instrument several tests have been performed. The SmartPrep System showed no leaks or blockages in the valves or the Teflon® tubing with the urine matrix. To exclude the influence of carryover on blank values, several test runs were made measuring highly concentrated standards. Each contact point (valves, connectors, syringe, etc.) was rinsed with methanol and examined for residues. These tests did not show any cross contamination. Figure 1 shows a chromatogram of a blank with only normal contamination levels. This ensures that if a sample with a high concentration of analytes is run before a low sample that both measurements will be accurate.

![Figure 1: Automated SmartPrep Extractor SPE Process]

<table>
<thead>
<tr>
<th>Condition 3 mL</th>
<th>10 mM FA in methanol @ 10 mL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load Small Volume Sample 1.5 mL</td>
<td>human urine @ 5 mL/min</td>
</tr>
<tr>
<td>Clean sample line 1.5 mL</td>
<td>0.1% FA in water @ 20 mL/min</td>
</tr>
<tr>
<td>Load Small Volume Sample 1.5 mL</td>
<td>human urine @ 5 mL/min</td>
</tr>
<tr>
<td>Wash 1 mL</td>
<td>0.1% FA in water @ 10 mL/min</td>
</tr>
<tr>
<td>Wash 1 mL</td>
<td>10 mM FA in methanol @ 10 mL/min</td>
</tr>
<tr>
<td>Wash 3 mL</td>
<td>vent @ 10 mL/min</td>
</tr>
<tr>
<td>Nitrogen purge cartridge for 5 min</td>
<td></td>
</tr>
<tr>
<td>Clean plunger 3 mL</td>
<td>1% ammonia in methanol @ 20 mL/min</td>
</tr>
<tr>
<td>Elute 1.5 mL</td>
<td>1% ammonia in methanol @ 5 mL/min</td>
</tr>
<tr>
<td>Elute 2 mL</td>
<td>vent @ 5 mL/min</td>
</tr>
<tr>
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<td>Clean plunger 3 mL</td>
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</tr>
</tbody>
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Results

The eluted fractions from manual extraction and from the automated SmartPrep Extractor were both measured on a Pegasus® GC-TOFMS. To exclude the influence of carryover on blank values, several test runs were made measuring highly concentrated standards containing drug analytes. Each contact point (valves, connectors, syringe, etc.) was rinsed with methanol and examined for residues. These tests did not show any cross contamination. Figure 2 shows a chromatogram of a blank with only normal contamination levels. This ensures that if a sample with a high concentration of drug analytes is run before a low sample that both measurements will be accurate.

Figure 2: Sample blank chromatogram run on SmartPrep Extractor showing no residual carryover

Figure 3: Manual SPE of a urine sample, arrow indicating creatinine matrix
Spectra results from automated SPE and manual SPE procedures were overlayed to better show the levels of creatinine present in each sample preparation. Figure 4 shows that much less creatinine is retained on the cartridge and eluted when the flow is more controlled using an automated system.

Due to the dominant sample matrix effect, the ability to see the presence of N-Acetyltyramine is increased when the chromatogram is overlayed on the spectra, as shown in Figures 5 and 6. The sample matrix is greatly minimized in the results overlayed from the SmartPrep Extractor used for sample preparation versus the manual SPE sample preparation. Note that the time differences in the chromatogram between Figures 5 and 6 are due to differences in chromatographic column length.
Conclusion

In clinical laboratories, time and quality are crucial factors for patient monitoring of drugs. To generate reliable data without bias, automation is a key tool. The SmartPrep Extractor is a simple sequential system for automated SPE sample preparation. It is this sequential process that allows laboratory personnel to be available for more important tasks due to its ability to operate the SPE system for multiple samples without any manual intervention.

Qualitative results confirmed the positive presence of N-Acetyltietyramine using manual solid-phase extraction and SmartPrep automated SPE. Transfer of the manual SPE method was effectively performed for automation with the SmartPrep Extractor, proven by the similar qualitative presence of drugs (Figures 5 and 6). Because of controlled flow rates, automated SPE sample preparation shows less sample matrix background effect than manual SPE, increasing confidence in long term data generation.

References