

DPX Manual Processing Instruction Sheet – Determination of Acidic Drugs in Urine**GERSTEL AUTOMATION**

The above application has been shown to be readily automated using a GERSTEL MPS autosampler under MAESTRO control. The 12 × 75mm test tubes containing the acetonitrile/HCl/urine samples are simply placed onto the sample tray of the MPS autosampler. All the required sample preparation steps listed in the Manual DPX Extraction section of this method are subsequently performed automatically by the MPS. Automation of the DPX extraction process ensures that sample extractions are performed in a highly reproducible manner while

eliminating tedious and labor intensive manual steps and reducing exposure to potentially harmful solvents. Recoveries of non-polar compounds approach 100 % when the elution solvent is added to the top of the DPX tip, which is readily performed with the GERSTEL MPS autosampler. Analyte concentration factors of 10 times can be realized by injecting 20µL of the eluent into a GERSTEL CIS inlet combined with solvent venting. DPX automation for the GERSTEL MultiPurpose Sampler is expected to be available for customer shipment in 2009

DPX Manual Processing Instruction Sheet**Determination of Acidic Drugs in Urine****Introduction**

The analysis of urine for the presence of drugs by chromatographic methods incorporates sample preparation. The required sample preparation is typically time-consuming and is generally considered to be the main “bottle neck” for laboratory analysis throughput.

Solid phase extraction (SPE) is a widely used, proven method for sample preparation and sample clean-up in the field of forensic analysis. A number of SPE products are available that offer various sample preparation functions and cover a wide range of procedures. Most SPE products require relatively large volumes of solvents leading to increased cost of analysis per sample and higher limits of detection. Disposable Pipette Extraction (DPX) was developed as an alternative to traditional SPE, combining efficient and rapid extraction with significantly reduced solvent consumption. In addition to urine, sample matrices such as plasma, serum, oral fluid (saliva) and sweat can also be extracted using DPX.

The following application uses a sample volume of 0.2 mL and has been shown to be readily automated using the GERSTEL MultiPurpose Sampler (MPS) and MAESTRO software control.

This instruction sheet is provided for the GERSTEL DPX Evaluation Kit to enable the user to evaluate the ease, speed, extraction recovery, and reproducibility obtained when performing manual DPX sample preparation prior to GC-MS determination of acidic drugs from a urine sample.

Please note: This instruction sheet is intended for qualified staff with chemical/technical training and appropriate expertise, particularly in the safe handling of chemicals and laboratory instrumentation and in health and safety protection at the workplace.

Experimental

The 0.4 ppm acidic drug sample in urine was prepared by combining a commercially available SPE drug mix stock (Alltech P/N: 01479) with “drug-free” urine. For the acidic drugs that were analyzed, d5-pentobarbital (Cerilliant, catalog # P-009) was used as an internal standard.

Neat SPE drug mix control preparation:

1. Prepare a 4 ppm working stock solution of the SPE drug mix by combining 80 µL of the 50 ppm SPE drug mix (Alltech P/N: 01479) with 920 µL acetonitrile.
2. Prepare a 4 ppm working stock solution of the internal standard by adding 25 µL of the 100 µg/mL stock solution to 475 µL acetonitrile.
3. Prepare a 0.4 ppm SPE drug mix control standard and internal standard by combining 50 µL of the 4 ppm intermediate SPE drug mix stock and 50 µL of the working stock solution of internal standard to 400 µL acetonitrile in a 2 mL glass autosampler vial.

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Sample Pre-treatment:

1. Prepare 1 mL of a 1 ppm SPE drug mix in urine (or deionized water) by combining 20 µL of a 50 ppm SPE drug mix (Alltech P/N: 01479) with 480 µL of urine (or deionized water). Vortex mix.
2. Pipette 0.2 mL of the 1 ppm sample into a 12 x 75mm borosilicate glass test tube.
3. Add 10 µL of the 100 µg/mL internal standard stock solution to the sample and vortex mix.
4. Add 50 µL 0.1 M HCl and 50 µL acetonitrile to the sample and vortex mix.

Manual DPX extraction:

The DPX tips used for this application (DPX-RP-1-mL, DPX Labs, LLC) contain styrene divinyl benzene (SDVB) resin with reverse phase characteristics. The DPX procedure is as follows:

1. Attach the 5 mL syringe with adaptor to the DPX-RP-1mL tip.
2. Aspirate 500µL of conditioning solvent (30% acetonitrile in water) into the DPX tip and mix by slowly aspirating air (app. 1mL). Small air bubbles will create a perturbation of the solution, providing highly efficient mixing of sorbent and solution.
3. Dispense into the corresponding test tube or waste container.
4. Slowly aspirate the entire sample solution into the DPX tip followed by slowly aspirating 2 mL of air to mix sorbent with solution. The formation of a Gel indicates that the analyte has been thoroughly mixed with the sorbent.
5. Wait for 20 seconds for the analyte to adsorb and partition into the sorbent.
6. Slowly dispense the solution and air, back into the original test tube or into a waste receptacle.
7. Aspirate 500µL of wash solution (20% acetonitrile in deionized water) into the DPX tip and mix with air (1mL). Wait 10 seconds, then dispense back into the corresponding test tube or waste container.
8. Aspirate 500 µL acetonitrile elution solvent into the DPX tip and then aspirate 1 mL air to mix. Wait 10 seconds, then dispense into the corresponding GC vial.

Surrogate addition:

The addition of a surrogate to both the DPX extract and the neat SPE drug mix control standard is used to compensate for any variations that may occur when samples are injected and to ensure accurate calculation of extraction efficiencies.

1. Prepare a 10ppm ketamine surrogate solution by adding 10 µL of a 1 mg/mL ketamine HCl stock (Cerilliant P/N: K-002) to 990 µL of methanol.
2. Add 10 µL of the 10 ppm ketamine surrogate sample to both the DPX eluent and the neat SPE drug mix control standard in their corresponding GC vials. Cap each vial and vortex mix prior to injection into the GC-MS system.

GC/MS analysis:

GC parameters:

- Agilent Technologies 6890N GC.
- Agilent Technologies HP 5-MS Column: 30m, 0.25mm I.D., 0.25µm film.
- Carrier Gas Flow rate: 1.0 mL/min @ constant flow.

CIS4:

- Sample mode: Splitless mode
- Purge flow: 20.0 mL/min.
- Purge time: 1.20 min.
- Initial temperature: 40 °C; 12 °C/second
- End temperature: 280 °C
- Hold time: 3.00 min.
- Injection volume: 1 µL

GC Oven Program:

- Initial temperature: 80 °C
- Initial time: 1 minute
- Rate: 20.0 °C/min
- Final Temperature: 280 °C
- Final Time: 9.00 minutes
- Total Run Time: 20.0 minutes

MS parameters:

- Agilent Technologies 5975 MSD

SIM analysis:

- Dwell times: 20 ms
- Masses monitored: 141, 156, 168, 204, 189

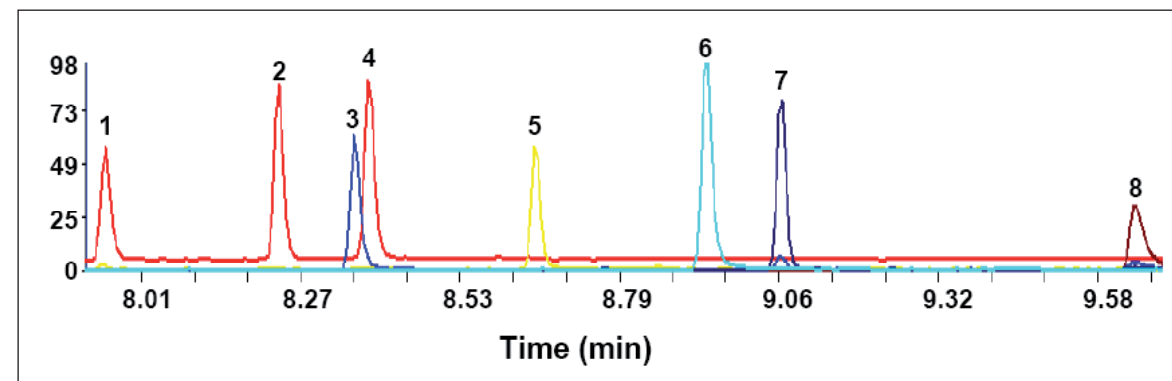
RESULTS AND DISCUSSION

The statistical results for the extraction of acidic drugs from urine are shown in Table 1. The recovery is calculated by taking the area of the peak for each drug and dividing by the area of the surrogate, and then dividing this value by the ratio of the drug to surrogate of the neat standard (multiplied by 100%). The RSD is determined by performing this study for 4 samples.

Table 1. Statistical Results for the Extraction of Acidic Drugs using DPX-RP.

Compound	Ion	% Recovery	% RSD
Butabarbital	156	60	3.46
Amobarbital	156	80	0.91
Pentobarbital	156	78	2.87
Secobarbital	168	88	2.68
Phenobarbital	204	66	8.08
Glutethimide	189	97	3.02

Figure 1. Example GC/MS Chromatogram of acidic drugs extracted from urine using DPX-RP.



(1) Butabarbital, (2) Amobarbital, (3) Pentobarbital-D5, (4) Pentobarbital, (5) Secobarbital, (6) Caffeine, (7) Phenobarbital, (8) Glutethimide