



Application Note: Analysis of Basic, Acidic and Neutral Drugs in Urine (updated: 7/06/08)

Product: DPX-CX (1 mL)

INTRODUCTION

Analysis of urine for the presence of drugs and metabolites by chromatographic methods incorporates sample preparation. This procedure is typically very time-consuming and is generally the “bottle neck” for laboratory analysis and throughput.

Solid-phase extraction (SPE) methods have been demonstrated to be very useful in sample preparation. Numerous types of SPE products are available with various procedures and equipment for their use. However, most SPE products require relatively large volumes of solvents and numerous steps, such as conditioning steps. Also, most SPE products are not readily automated, and the automated SPE methods generally take too long to perform for practical use.

In this research, the goal is to develop an efficient and rapid extraction method using Disposable Pipette Extraction (DPX). Another goal is to develop a single DPX method that can efficiently extract basic, acidic and neutral drugs and metabolites. Finally, the method is developed to be sensitive for low volume applications (0.2 mL) so that this method can be readily automated for future applications and can be readily adapted for the analysis of alternative matrices such as oral fluid (saliva) and sweat samples. The analysis of alternative matrices requires a comprehensive screening and analysis due to their low volumes.

EXPERIMENTAL

Sample pretreatment:

Urine sample was obtained from a “drug-free” volunteer (with the exception of caffeine). This sample was used throughout this study. Just 0.2 mL of urine was used, and it was spiked at the appropriate concentration of analyte in a small test tube. Addition of 50 uL of 0.1 M HCl and 50 uL of acetonitrile was made to each sample and vortex mixed. For the general drugs that were analyzed, an internal standard was not utilized, but SKF was added to the final solutions as an external standard. For targeted drugs such as benzoylecgonine (BE), morphine, codeine, 6-monoacetyl morphine, amphetamine, and methamphetamine, deuterated internal standards of each analyte were used in the studies. The use of internal standards drastically improves the quality of the data in terms of reproducibility, but this use was cost prohibitive to use for all of the analytes incorporated in this study.



DPX extraction:

The DPX tips used in this study were DPX-CX-1mL (DPX Labs, LLC), which contain cation exchange resin which has some reverse phase characteristics. To more readily process multiple samples, wash and elution solvents were pre-dispensed into disposable labeled tubes located in corresponding adjacent tube rack positions. The wash solvent consisted of 500 uL of DI water, the first elution solvent (acidic/neutral drugs) consisted of 500 uL of acetonitrile and the second elution solvent (basic drugs) consisted of 500 uL of 78/20/2 of CH₂Cl₂/isopropanol/ammonium hydroxide. Using multiple syringe devices, several samples could be processed almost simultaneously to improve laboratory throughput. The DPX procedure consisted of the steps:

- 1) slowly draw in aqueous sample solution into DPX tip and draw in air to mix sorbent with solution, being careful not to draw in too fast and making sure the sorbent is thoroughly mixed;
- 2) wait approximately 30 seconds for the analyte to adsorb and partition into the sorbent;
- 3) dispense solution back into to test tube or waste container;
- 4) draw in wash solution and air to mix and wash sorbent (this should be done carefully to prevent the sorbent from just floating on the top).
- 5) wait 10 seconds and dispense solution to test tube or waste container;
- 6) draw in elution solvent 1 and air to mix sorbent with solvent;
- 7) wait approximately 30 seconds and dispense solution into GC vial;
- 8) draw in elution solvent 2 and air to mix sorbent with solvent;
- 9) wait approximately 30 seconds and dispense solution into another GC vial.

Concentration and chemical derivatization:

For the general drugs that do not require derivatization, the eluents from the DPX extract were evaporated using nitrogen gas and low heat (40 °C). This procedure was carefully monitored for the basic eluent (from the second elution step) to reduce the evaporation of the volatile amphetamine compounds, and this step took only approximately 3 minutes to perform (due to the low solvent volume) for both eluents. These samples were subsequently reconstituted in 50 uL of 80/20 ethyl acetate/methanol.

For the analysis of the more polar compounds, such as benzoylecgonine, morphine, and 6-monoacetyl morphine, the extracts were evaporated in the GC vial to complete dryness using nitrogen and heat. They were subsequently derivatized by adding 50 uL BSTFA (with 1% TMS), capped under low nitrogen gas flow, and vortex mixed. They were placed in a heat block at approximately 80 °C for 20 minutes. The derivatives were cooled to room temperature, then the cap was removed, 50 uL of hexanes was added, and the solution was transferred to a vial insert. The insert was then replaced in the same corresponding vial and capped and placed on the autosampler (GERSTEL MPS-2) for analysis.



GC/MS analysis:

GC parameters:

Agilent Technologies 6890N GC.
Agilent Technologies HP 5-MS Column: 30m, 0.25mm I.D., 0.25 μ m film.
Flow rate: 1.0 mL/min @ constant flow.
Injector: 280 °C
Transfer line temperature: 300 °C

Oven Program: Initial temp. 80 °C for 1 minute.

<u>Rate (°C/min)</u>	<u>Final Temp.</u>	<u>Final Time</u>
40.0	280	10.00

Total Run Time = 22.0 minutes

MS parameters:

Agilent Technologies 5975 MSD
General screen: scan 40-550 amu, scan rate of 14
SIM analysis (dwell times of 20 ms) of the targeted analytes (TMS derivatives):

BE = *82, 240, 361
BE-D₃ = *85
morphine = *429, 236
morphine-D₃ = *432
6MAM = *399, 340, 287
6MAM-D₃ = *402
codeine = *371, 196
codeine-D₃ = *374
oxycodone = *387, 372
oxycodone-D₃ = *390

PCP = *200, 242, 91
PCP-D₅ = *205

RESULTS AND DISCUSSION

The statistical results are shown in tables 1-3. The recoveries and limit of detection (LOD) results for all of the drugs are exceptional. The relative standard deviation (%RSD) is better for the drugs that utilize deuterated internal standards as expected. Surprisingly, the general screens (Table 1) using full scan GC/MS provided very low LODs. The LOD was calculated using the equation $3.3*s/m$, where s is the standard deviation (calculated at 0.1 ppm) and m is the slope. This is based on the area of an extracted ion (from table 1), so the LOD will actually be slightly greater when incorporating qualifier ions.

In Figure 1, a total ion chromatogram of the acidic fraction is shown. The extracts are very “clean” and free from interferences, even when analyzed in full scan mode. In Figure 2, the extracted ion chromatogram of the acidic fraction is shown. The ions utilized are listed in Table 1.



In Figure 3, a total ion chromatogram of the basic fraction is shown. The extracts are very “clean” with some interference from creatinine when analyzed in full scan mode. It has been found that the creatinine can be removed by washing with pH 6 buffer solution. In Figure 4, the extracted ion chromatogram of the basic fraction is shown. The ions utilized are listed in Table 1.

Table 1. Statistical results for acidic, basic and neutral drugs recorded in full scan using extracted ion chromatograms. The %RSD was calculated using 6 replicates at 0.5 ppm.

Compound	Ion	Internal Standard	Recovery	% RSD	R ²	LOD* (ug/mL)
Butabarbital	156	Pentobarbital- <i>d</i> ₅	96.33%	2.63%	0.9988	0.0120
Amobarbital	156	Pentobarbital- <i>d</i> ₅	99.59%	2.57%	0.9988	0.0106
Pentobarbital	156	Pentobarbital- <i>d</i> ₅	98.56%	1.58%	0.9985	0.0152
Secobarbital	168	Pentobarbital- <i>d</i> ₅	97.55%	1.41%	0.9995	0.0261
Phenobarbital	204	Pentobarbital- <i>d</i> ₅	95.27%	2.54%	0.9988	0.0129
Glutethimide	189	Pentobarbital- <i>d</i> ₅	99.40%	2.74%	0.998	0.0216
Meperidine	247	PCP- <i>d</i> ₅	93.16%	1.43%	0.9931	0.0099
PCP	200	PCP- <i>d</i> ₅	96.78%	1.04%	0.9992	0.0003
Methadone	72	PCP- <i>d</i> ₅	96.38%	2.65%	0.9992	0.0051
Methaqualone	235	PCP- <i>d</i> ₅	99.39%	1.85%	0.9902	0.0134
Amitriptyline	58	PCP- <i>d</i> ₅	95.60%	1.99%	0.999	0.0038
Cocaine	182	PCP- <i>d</i> ₅	102.50%	1.31%	0.9968	0.0160
<i>cis</i> -Doxepin	58	PCP- <i>d</i> ₅	98.53%	1.18%	0.9988	0.0200
Imipramine	234	PCP- <i>d</i> ₅	96.03%	3.72%	0.9984	0.0099
<i>trans</i> -Doxepin	58	PCP- <i>d</i> ₅	98.50%	2.30%	0.9987	0.0146
Pentazocine	217	PCP- <i>d</i> ₅	95.70%	4.37%	0.9946	0.0676
Codeine	299	PCP- <i>d</i> ₅	105.03%	6.85%	0.9864	0.0520
Oxycodone	315	PCP- <i>d</i> ₅	111.11%	8.80%	0.9761	0.0638
Desipramine	234	PCP- <i>d</i> ₅	91.49%	9.60%	0.9522	0.1027

Table 2. Statistical results for TMS derivatized polar drugs (based on deuterated internal standards for each drug and SIM analysis).

Ion	Qualifier ions	Internal Standard	Recovery	% RSD	R ²	LOD* (ng/mL)
82	240, 361	Benzoylcegonine- <i>d</i> ₃ -TMS	72.88%	5.47%	0.9942	4.57*
371	178, 196	Codeine- <i>d</i> ₃ -TMS	87.41%	1.84%	0.9981	1.04
429	236	Morphine- <i>d</i> ₃ -2TMS	84.25%	1.45%	0.9956	3.08
399	287, 340	6-monoacetylmorphine- <i>d</i> ₃ -TMS	87.74%	3.75%	0.9970	1.21
387	372	Oxycodone- <i>d</i> ₃ -TMS	86.14%	0.89%	0.9935*	21.3



Table 3. Statistical results for COOH-THC (TMS-derivatized) extracted using DPX-CX. Surprisingly, the CX sorbent has ample reverse phase characteristics to recover this analyte.

Ion	Qualifier ions	Internal Standard	Recovery	% RSD	R ²	LOD (ng/mL)
371	473, 488	COOH-THC- <i>d</i> ₃ -2TMS	57.17%	0.73%	0.9987	3.38*

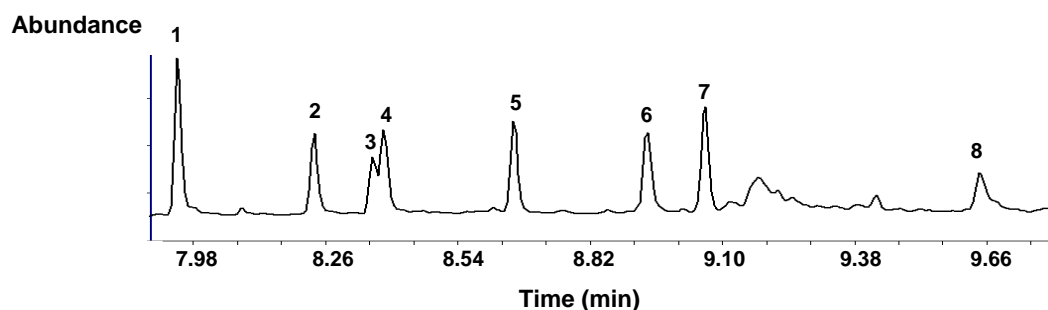


Figure 1. GC/MS total ion chromatogram of acidic drugs in full scan mode at 0.5 ppm. The peaks are: (1) Butabarbital, (2) Amobarbital, (3) Pentobarbital-D5 (ion 161), (4) Pentobarbital, (5) Secobarbital, (6) Caffeine (ion 194), (7) Glutethimide, and (8) Phenobarbital.

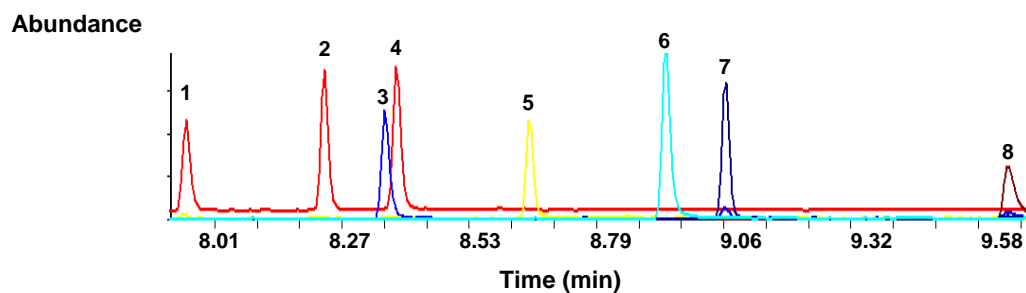


Figure 2. GC/MS extracted ion chromatograms of acidic drugs at 0.5 ppm. The peaks are: (1) Butabarbital, (2) Amobarbital, (3) Pentobarbital-D5 (ion 161), (4) Pentobarbital, (5) Secobarbital, (6) Caffeine (ion 194), (7) Glutethimide, and (8) Phenobarbital.

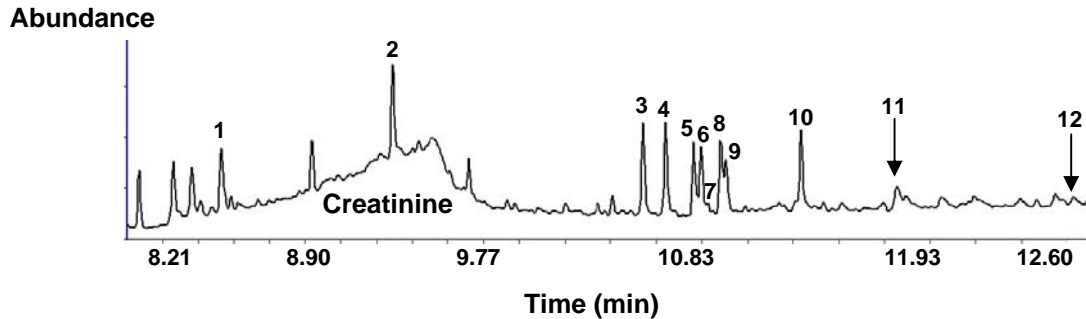


Figure 3. GC/MS total ion chromatogram of basic drugs in full scan mode at 0.5 ppm. The peaks are: (1) Meperidine, (2) PCP, (3) Methadone, (4) Methaqualone, (5) Amitriptyline, (6) Cocaine, (7) *cis*-Doxepin, (8) Imipramine, (9) *trans*-Doxepin, (10) SKF 525A (ext. std.), (11) Codeine, and (12) Oxycodone.

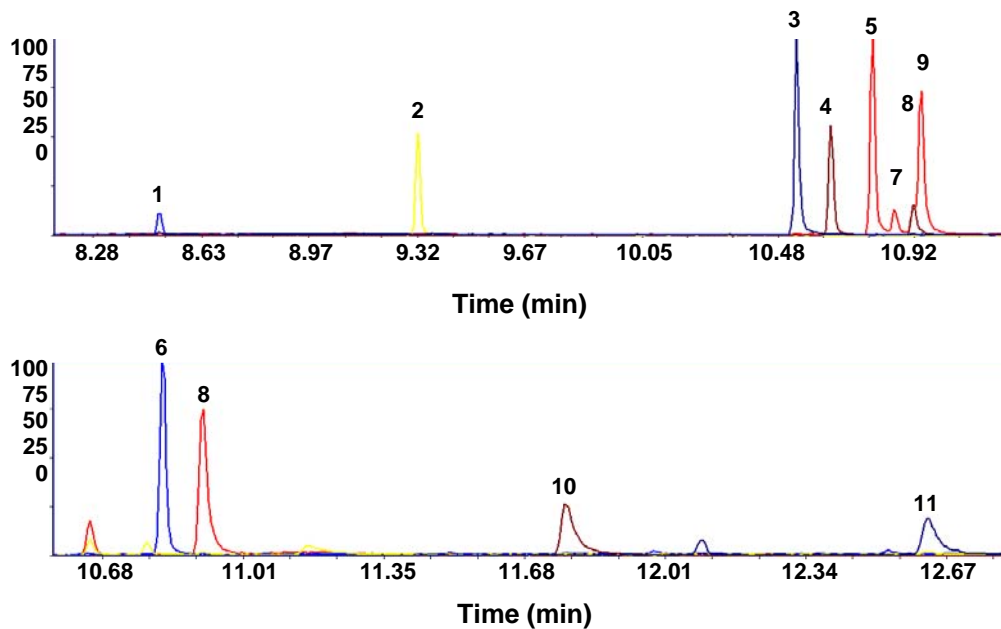


Figure 4. GC/MS extracted ion chromatograms of basic drugs at 0.5 ppm. The peaks are: (1) Meperidine, (2) PCP, (3) Methadone, (4) Methaqualone, (5) Amitriptyline, (6) Cocaine, (7) *cis*-Doxepin, (8) Imipramine, (9) *trans*-Doxepin, (10) Codeine, (11) Oxycodone.

CONCLUSION

This method proves to be very reproducible and efficient for analyzing numerous drugs of various drug classes. This study demonstrates a rapid, convenient and efficient method for comprehensive drug screening. The sensitivity for the analysis is very good, though the LODs may be lowered by using a larger sample volume utilizing DPX-CX 5 mL tips.



REFERENCES

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