



Application Note: Automated DPX of Basic Drugs in Blood (updated: 3/22/09)

Product: DPX-CX (1 mL, TA)

INTRODUCTION

The analysis of drugs in whole blood is challenging due to the complex sample matrix. In addition, low detection limits are required for many drugs.

Most sample preparation methods in the literature focus on either multiple liquid-liquid extractions or solid-phase extraction (SPE). For basic drugs, which represent the vast majority of drugs of interest in both forensic and clinical toxicology, high recoveries can be achieved using SPE with cation exchange mechanisms. Unfortunately, conventional SPE methods still require multiple steps and use relatively large volumes of solvent.

In contrast, disposable pipette extraction (DPX) uses minimal solvent, is readily automated, and can be performed in a few short minutes. In this application note, a completely automated method is presented, where the extraction of one sample of blood is completed while the previous sample is being chromatographically separated and analyzed by GC/MS. This method utilizes a large volume injection system (GERSTEL CIS-4) that concentrates the analyte in the inlet without requiring a separate solvent evaporation step. A dual rail MPS-2 (GERSTEL) processes the samples by using one syringe (2.5 mL) to perform the DPX extractions and the other syringe (100 μ L) to perform injections into the GC/MS inlet. This completely automated method represents the highest throughput reported for basic drugs analyzed by GC/MS.

EXPERIMENTAL

Materials:

The drug mix (0.5 mg/mL, Quik-check drug solution) was purchased from Alltech (State College, PA).

Initial Sample Preparation:

Spike 0.25 mL whole blood sample with internal standard (d_5 -PCP)

Precipitate proteins with 0.50 mL acetonitrile

Centrifuge and transfer to clean labeled tube

Add 0.1 mL 0.1 M HCl

Place on sample tray rack of MPS

Instrumentation:

Agilent Technologies 6890 GC with 5972A MSD (inert XL), equipped with a dual rail MPS-2 (GERSTEL) for automated extractions and injections.

column--30 m DB-1701 (J&W Scientific), 30 m, 0.25 mm ID, $d_f = 0.25\mu$ m

carrier gas--He at constant flow of 1 mL/min

oven--80 $^{\circ}$ C for 1 min, ramp at 20 $^{\circ}$ C/min to 300 $^{\circ}$ C, hold 7 min for 19 minute run



Inlet: CIS-4, GERSTEL. Injection of 50 μ L was performed slowly at 1.32 μ L/s into a deactivated quartz wool inlet liner. Injection was performed using stopped flow at an initial T at 20 $^{\circ}$ C, split vent open for 1 min, and the inlet T was ramped at 12 $^{\circ}$ C/min to 300 $^{\circ}$ C with the split vent closed.

MSD: scan 40-550 amu

RESULTS AND DISCUSSION

A schematic of the automated extraction method is shown in Figure 1, and a picture of the system is shown in Figure 2. The DPX tip is processed using an attached transport adaptor, which makes an air-tight seal with the syringe needle of the MPS-2. The syringe aspirates sample solution into the tip, and the sorbent is mixed with the solution by aspirating air. The solution is then dispensed to waste, or back into its original container. Wash solution is subsequently delivered through the top of the DPX tip to waste. In the last step of the extraction, the basic elution solvent is added to elute the basic drugs into the GC/MS vial. It is important to note that this syringe never comes in contact with the sample solution. The total time for extraction is approximately 6 minutes, which is less than the chromatographic analysis.

The second syringe of the dual rail MPS-2 is used to perform the injection of the extract into the GC/MS inlet. By using a CIS-4, large volumes are injected, which provides a concentration step without requiring separate solvent evaporation off-line like conventional SPE methods.

A concern for large volume injections of whole blood samples is that the inlet will become very dirty and cause deleterious effects in the analysis. However, multiple injections were performed with little loss of peak intensities, as shown in Figures 3 and 4. Most importantly, all of the compounds were readily identified in full scan at concentrations of 0.5 ppm (0.5 μ g/mL) and 0.1 ppm (0.1 μ g/mL) with just 0.25 mL of whole blood. To achieve similar results using traditional SPE and GC/MS methods, at least 1 mL of whole blood would have been required to be extracted in addition to an approximately 10-15 minute concentration and solvent evaporation step.

Table 1 shows the results from this automated DPX method for basic drugs. The extraction recoveries were approximately 70% or greater for most of the drugs, and the % C.V. (% RSD) was less than 10% for almost all of the drugs in spite of using d_5 -PCP as internal standard. The % C.V. would be expected to be less than 5% for all of the drugs when using deuterated analogues of each compound of interest.

By using selected ion monitoring with this automated method, detection limits less than 10 ng/mL are readily achievable for most of these basic drugs of abuse.

CONCLUSION

Basic drugs were readily analyzed with little sample preparation by using automated DPX with the GERSTEL dual rail MPS-2. This method demonstrates the fastest analysis and highest throughput possible for these compounds by using GC/MS analysis.

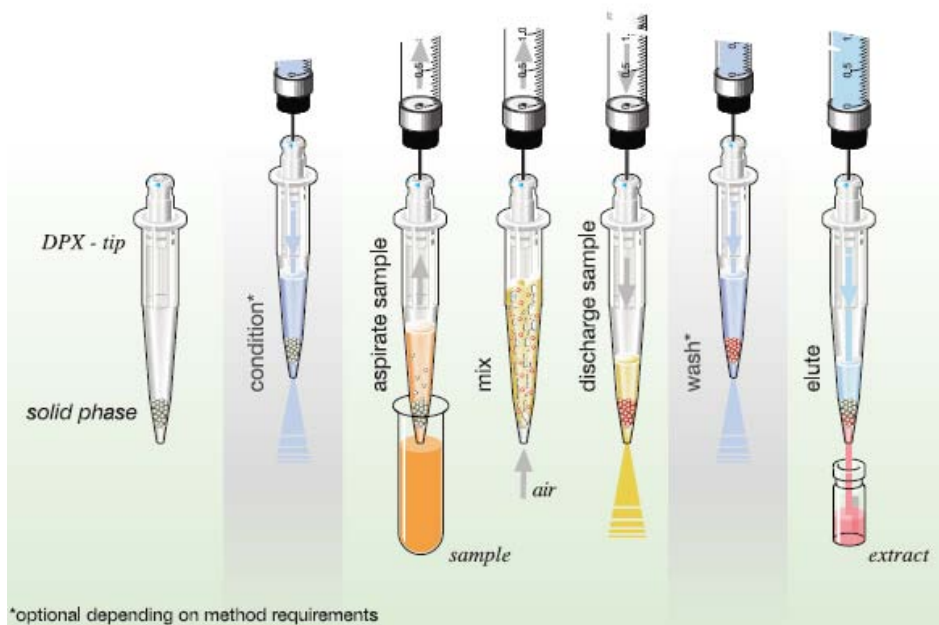


Figure 1. Schematic of the steps used in the automated DPX extraction method. The DPX tip is fitted with a transport adaptor (left), which permits a syringe and holder to process the extraction steps.





Figure 2. A picture of the dual rail MPS-2 system, which stands on top of the GC/MS system. The left syringe performs large volume injections, and the right syringe performs automated DPX extractions.

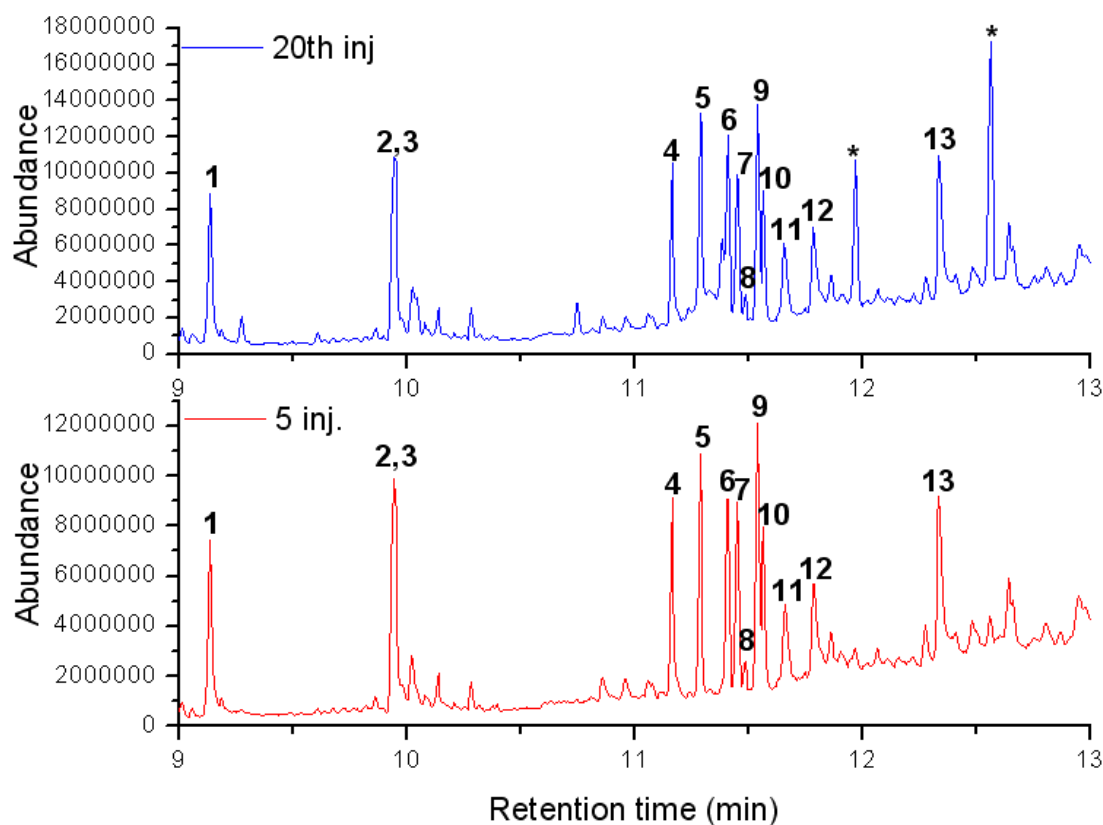


Figure 3. GC/MS total ion chromatogram (full scan) of the 5th (bottom) and 20th injection of 0.5ppm drugs extracted from 0.25 mL of whole blood. Compounds are: 1) Meperidine, 2) PCP-*d*₅ (ion 205), 3) PCP, 4) Methadone, 5) Methaqualone, 6) Amitriptyline, 7) Cocaine, 8) *cis*-Doxepin, 9) Imipramine, 10) *trans*-Doxepin, 11) Desipramine, 12) Pentazocine, 13) Codeine. *-Denotes septum bleed from the GC vial cap due to repeated injections for this study (which would not show up under normal analyses).

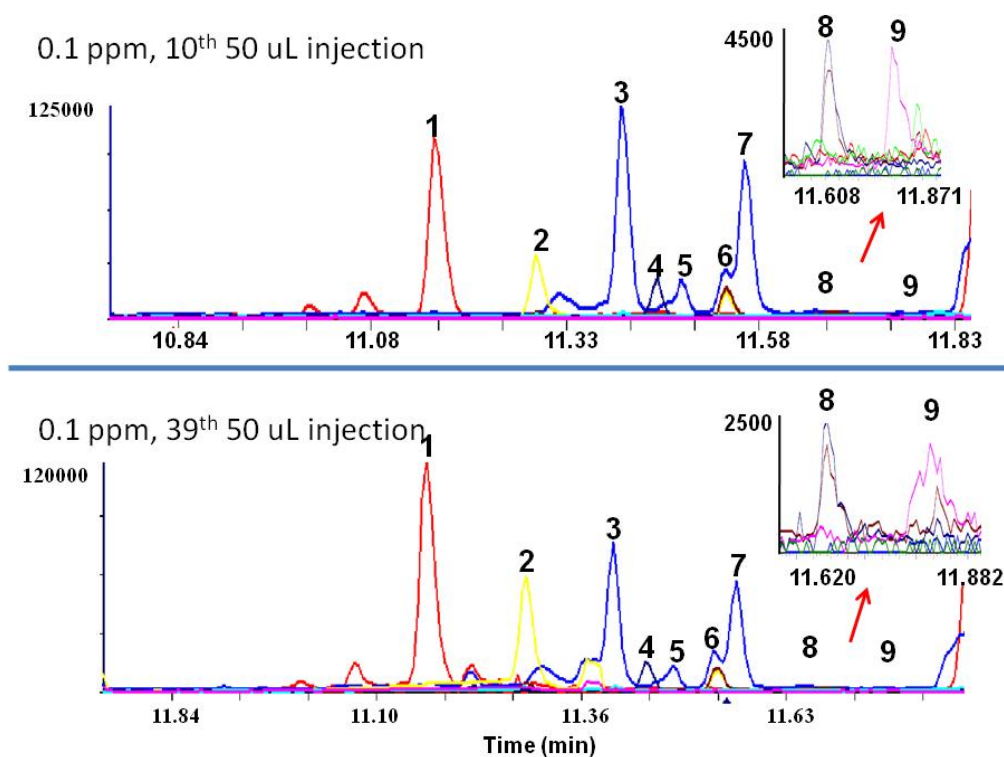


Figure 4. The GC/MS (recorded in full scan) extracted ion chromatograms of basic drugs at 0.1 ppm extracted and injected with 10 (top) and 39 (bottom) large volume injections of 50 uL. Little losses of intensities were observed, even for compounds that are typically not analyzed without chemical derivatization. Compounds are: 1) Methadone, 2) Methaqualone, 3) Amitriptyline, 4) Cocaine, 5) *cis*-Doxepin, 6) Imipramine, 7) *trans*-Doxepin, 8) Desipramine, 9) Pentazocine



Table 1. Extraction efficiencies and reproducibility results for basic drugs extracted by automated DPX using d_5 -PCP as internal standard.

Compound	m/z	Internal Std.	% Recovery*	% C.V. *
Meperidine	247	PCP- d_5	94.1	9.2
PCP	200	PCP- d_5	72.5	2.2
Methadone	72	PCP- d_5	69.7	6.3
Methaqualone	235	PCP- d_5	78.9	7.7
Amitriptyline	58	PCP- d_5	70.3	7.2
Cocaine	182	PCP- d_5	67.9	7.7
<i>cis</i> -Doxepin	58	PCP- d_5	78.2	8.8
Imipramine	234	PCP- d_5	64.3	6.3
<i>trans</i> -Doxepin	58	PCP- d_5	110	3.2
Desipramine	234	PCP- d_5	52.0	14.1
Pentazocine	217	PCP- d_5	86.9	16.8
Codeine	299	PCP- d_5	67.0	9.6

REFERENCES

W. E. Brewer, S. T. Ellison, S. L. Morgan, J. R. Stuff, and F. D. Foster, "Completely Automated GC/MS Analysis of Drugs and Metabolites Using Disposable Pipette Extraction with Cooled Injection System", Society of Forensic Toxicologists 2008 Annual Meeting, Phoenix, AZ, Oct. 2008.

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