



Automated Sample Preparation

Doing Drugs ?

Comprehensive Analysis of Drugs of Abuse in Blood and Urine with Automated Disposable Pipette Extraction and HPLC/MS/MS

In order to analyze biological specimens for drugs and their metabolites, it is necessary to perform sample preparation to eliminate matrix interference. Solid-phase extraction is generally the preferred sample preparation technique, in this study Disposable Pipette Extraction (DPX) was utilized. DPX is a novel dispersive solid-phase extraction technique that uses loosely contained sorbent in a disposable pipette tip. The sample is aspirated into the tip where it is actively mixed with the sorbent and forms a suspension. The main advantages of the DPX technology are that the extraction is very

rapid, minimal solvent waste is generated, and the entire process can be fully automated including introduction of the extract to the chromatographic system. The GERSTEL MPS autosampler performs DPX extractions in approximately 5 minutes using reversed phase (DPX-RP) or cation exchange (DPX-CX) sorbent material. For chemical analysis of target drugs, GC/MS or HPLC/MS/MS are generally the preferred techniques. The advantage of LC/MS/MS is that chemical derivatization of the analytes is not required, making sample preparation simpler and less time consuming. In addition,

highly efficient ionization, in combination with tandem mass spectrometry results in high sensitivity and selectivity. This study focused on performing automated extraction of reduced sample volumes coupled with LC/MS/MS to provide high throughput analysis “one sample at a time”. The sample preparation time was decreased sufficiently to allow the extraction of a sample during the chromatographic analysis of the previous sample in the sequence.

Experimental

Instrumentation. Sample extraction and introduction in the LC/MS/MS system was automated using a GERSTEL MPS dual rail PrepStation with DPX option.

Analysis conditions LC

Mobile Phase: A - 4.5 mM

Ammonium acetate

B - Methanol

Gradient:	Initial	90 % A / 10 % B
	2 min	85 % A / 15 % B
	3 min	65 % A / 35 % B
	4 min	50 % A / 50 % B
	6 min	35 % A / 65 % B
	8 min	90 % A / 10 % B

(4 min)

Flowrate: 350 µL/min

Column: 2.1 mm x 30 mm, 3.5 µm, Eclipse XDB C18 (Agilent)

Inj. volume: 10 µL

Analysis conditions MS

Positive ion mode, Single reaction monitoring

Run time: 10 min

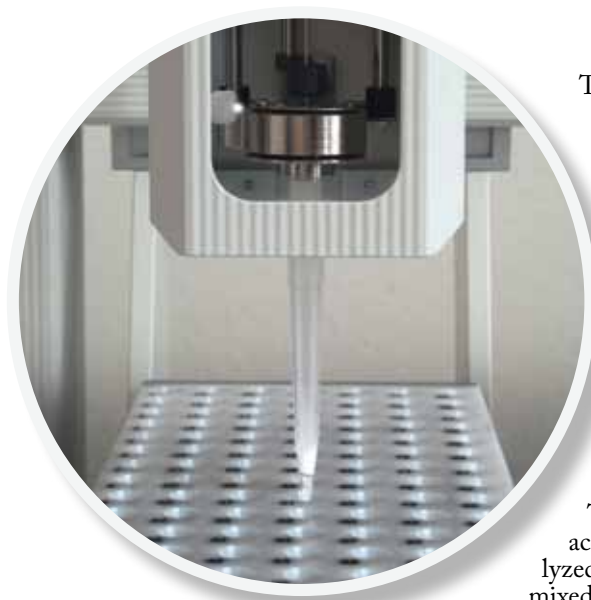
Capillary: 3 kV

Extractor: 2.81 V

Source Temp.: 130 °C

Desolvation Temp.: 391 °C

Compound	M + H [m/z]	Dwell Time [ms]	Cone Voltage [V]
d ₃ -Oxymorphone	305	50	30
Oxymorphone	302	50	30
Morphine	286	50	30
Hydromorphone	286	50	30
d ₃ -Oxycodone	319	50	30
Oxycodone	316	50	30
6-MAM	328	50	30
Codeine	300	50	30
Hydrocodone	300	50	30



The supernatant was decanted into a clean labeled sample tube. 100 μL of 0.1 M HCl was added to the solution, and the sample tube was placed on the MPS 2 sample tray for automated DPX extraction.

Urine sample preparation.

50 μL of 0.6 M sodium acetate buffer (pH = 4) and 10 μL of β -glucuronidase was added to a 200 μL sample of urine. The solution was thermostated at 70°C for 2 hours, and then cooled to room temperature. To precipitate proteins, 250 μL of acetonitrile was added to the hydrolyzed urine and the sample was vortex mixed and centrifuged. The supernatant was decanted into a clean labeled sample tube. 200 μL of 0.1 M HCl was added to the sample solution, and the sample tube was placed on the MPS sample tray for automated DPX extraction.

Sample preparation

All opiate standards were obtained from Cerilliant (Round Rock, TX). A 10 ppm stock solution was prepared in methanol for all sample fortifications. Two internal standards were used, d_3 -Oxymorphone for quantitation of Oxymorphone and d_3 -Oxycodone for all other opiates. All solvents used were of HPLC grade or higher.

Blood sample preparation.

A 250 μL blood sample was spiked at the specified concentration with the stock opiates mix. To precipitate proteins, 500 μL of acetonitrile was added and the solution was vortex mixed and centrifuged.

Extraction

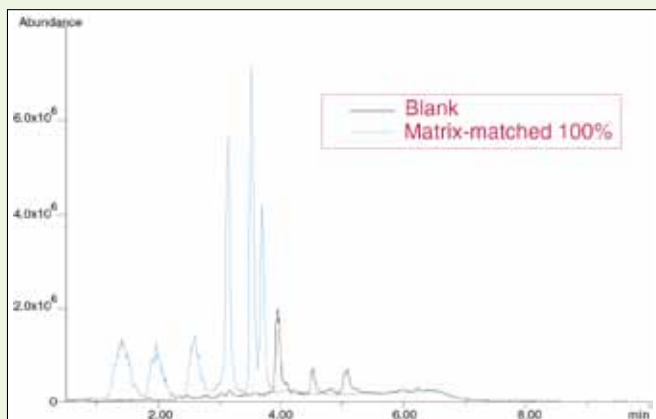
A GERSTEL MPS dual rail PrepStation was set up with 1 mL DPX-CX tips (DPXLabs, LLC, Columbia, SC) for extraction of drugs from blood and hydrolyzed urine. The following automation method was used: 250 μL of 30% acetonitrile/water was slowly added through the top of the DPX tip at a rate of 50 $\mu\text{L}/\text{s}$ to wet the sorbent. The sample was then aspirated into the DPX tip at a rate of 90 $\mu\text{L}/\text{s}$ and mixed with the sorbent by drawing in an additional 2 mL of air. After

a 30 s equilibration time to allow analyte binding, the resulting solution was dispensed to waste. To wash off excess matrix, a 500 μL wash of 10% acetonitrile/water was added to the sorbent material through the top of the DPX tip and dispensed to waste followed by an additional wash using 500 μL of acetone. For elution of the analytes, 700 μL of 78/20/2 (v/v) of methylene chloride/isopropanol/ammonium hydroxide was added to the sorbent material through the top of the DPX tip and dispensed directly into a clean HPLC vial. All eluents were dried and reconstituted with 100 μL of methanol and 400 μL of 4.5mM ammonium acetate before injection.

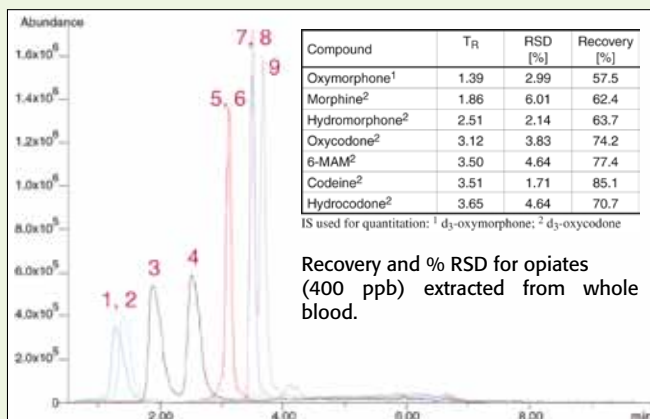
Results and Discussion

The DPX-CX extractions were readily performed using the GERSTEL MPS dual rail PrepStation. These DPX tips are ideal for basic drugs due to their mixed-mode cation exchange and reversed phase characteristics. The entire extraction process took approximately 5 minutes per sample. Because a basic eluent is used with the cation exchange sorbent, the eluents had to be solvent exchanged into the HPLC mobile phase. The extract was dried in about 4 minutes using low heat and nitrogen gas flow. All HPLC/MS spectra were collected using single reaction monitoring (SRM) because under the HPLC conditions used we were unable to generate quality daughter ions for the opiate drugs using multiple reaction monitoring (MRM). Although SRM/MS analysis may not provide the best sensitivity

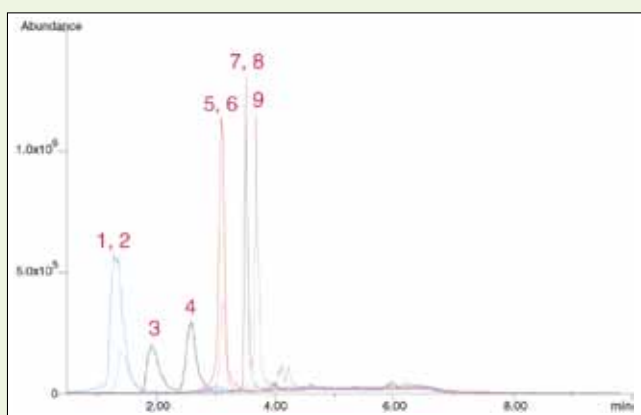




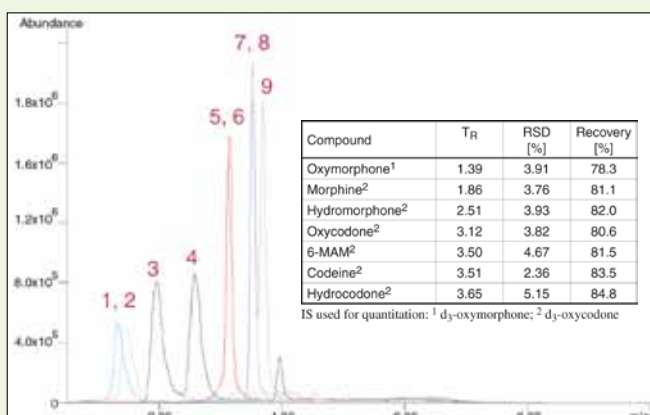
Overlay total ion LC/MS chromatograms of DPX extracts of a blank blood sample and of a matrix matched sample, both spiked at 0.5 ppm. The overlay shows that interferences are negligible



Recovery and % RSD for opiates (400 ppb) extracted from whole blood.



Extracted ion chromatogram of a DPX extract of whole blood spiked with 100 ppb of the opiate mix and with 400 ppb of the internal standards (d₃-oxymorphone and d₃-oxycodone) in whole blood. Even when performing SRM MS analysis, the sensitivity is more than sufficient, demonstrating the high ionization efficiency of the electrospray system. Most importantly, no matrix effect or ion suppression was observed, showing that sample extraction and cleanup with DPX is well suited for the analysis. (1) d₃-oxymorphone, (2) oxymorphone, (3) morphine, (4) hydromorphone, (5) d₃-oxycodone, (6) oxycodone, (7) 6-MAM, (8) codeine, and (9) hydrocodone.



Analysis of urine spiked with 500 ppb of opiates. Again, extracts were free from interferences. No matrix effect or ion suppression for opiates was seen. (1) d₃-oxymorphone, (2) oxymorphone, (3) morphine, (4) hydromorphone, (5) d₃-oxycodone, (6) oxycodone, (7) 6-MAM, (8) codeine, and (9) hydrocodone.

velocity for the analysis of these drugs at low concentrations, this study focused on the automated DPX extraction and the utility of this automated sample preparation for HPLC/MS analysis of opiates. A rapid resolution HPLC column was chosen to generate chromatographic data in less than 10 minutes.

Conclusion

Automated DPX extraction of opiates from biological specimens can be performed successfully using the GERSTEL MPS dual rail PrepStation. In the work presented here, the total extraction time was 5 minutes, additionally 4 minutes were required for evaporation and solvent exchange. The total sample preparation

time was less than the chromatographic run time, which means that the next sample can be prepared while separation of the current sample is in progress. Whenever the LC/MS/MS system has finished a run, the next sample is ready to be introduced ensuring the highest possible throughput. Additionally, "just in time" sample preparation helps to ensure that the prepared sample is not kept in the autosampler for a long time prior to being analyzed, reducing the risk of analyte degradation and helping to maintain sample integrity. The DPX-CX tips work very well for extraction of opiates, recoveries were in the range from 60 to 85 % with RSD's below 6 %. Future work will focus on determining the lower limits of detection and quantitation using tandem

mass spectrometry with multiple reaction monitoring. Also, automated DPX combined with HPLC/MS/MS will be optimized for other drugs and metabolites.

Further information:
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