

HPLC 2010

Newsletter

BOSTON

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June 2010

More information about DPX



For more information about DPX system, please visit www.gerstel.com (Applications/Technology/Extraction Techniques):

AppNote-2009-07
Analysis of Drugs of Abuse using Automated Disposable Pipette Extraction and LC/MS/MS

AppNote-2009-06
Comprehensive Analysis of Drugs of Abuse in Blood and Urine with Automated Disposable Pipette Extraction and HPLC/MS/MS

AppNote-2009-01
Automated Multi-Residue Pesticide Analysis in Fruits and Vegetables by Disposable Pipette Extraction (DPX) and Gas Chromatography/Mass Spectrometry

AppNote-2008-11
Analysis of Drugs and Metabolites in Blood and Urine using Disposable Pipette Extraction

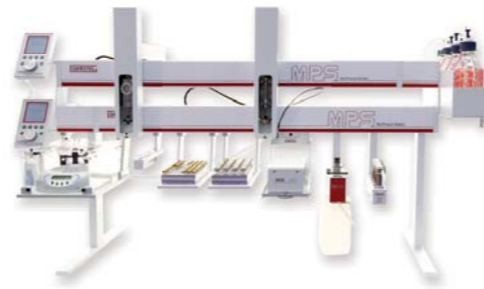
AppNote-2008-09
Automated Extraction, Derivatization and GC/MS Analysis of Tetrahydrocannabinol and Metabolites in Whole Blood Using Disposable Pipette Extraction

AppNote-2008-01
Automated Disposable Pipette Extraction of Pesticides from Fruits and Vegetables

Automated Vortex / Centrifuge for MPS

GERSTEL is presenting the novel Vortex and Centrifuge option for the GERSTEL MultiPurpose Sampler (MPS) at HPLC 2010.

Sample preparation steps such as liquid-liquid extraction can now be performed faster and more efficiently with the benefit of improved phase separation by using vortexing and medium level centrifugation, for example when performing QuEChERS clean-up. Replaceable rotors for 2 mL or 10 mL vials are available. For more information, please visit the GERSTEL booth.

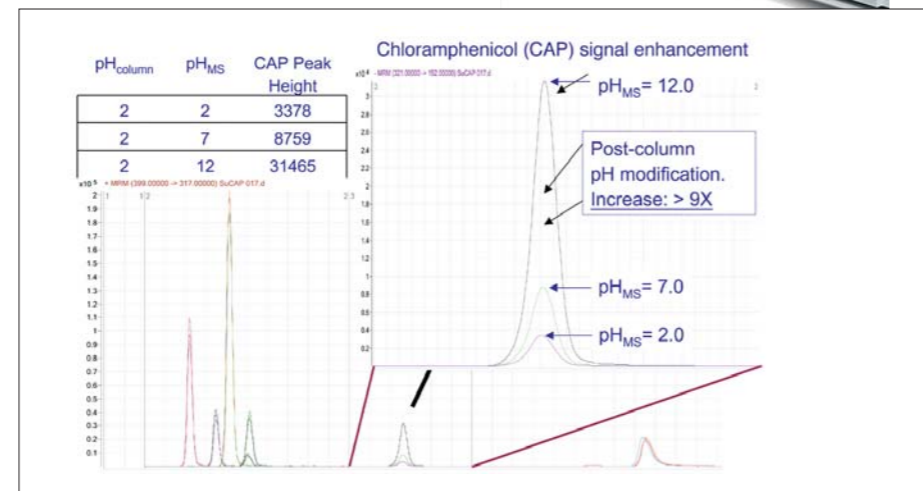


GERSTEL LC/MS Effluent Optimizer (LEO)

In LC/MS, we work hard to reach the goal of achieving the perfect LC separation and combining it with the most efficient ionization and lowest achievable MS detection limits for our analytes.

The LC separation may require a certain pH and polarity range of the eluent, while analyte ionization in the LC/MS ionization source requires yet another pH, a different buffer – or even derivatization of the analyte for best possible efficiency or optimized spectral information. How to optimize both? The logical answer is to take the effluent from the perfect LC separation and optimize it for MS analysis. This task is easily possible when you add the GERSTEL LC/MS Effluent Optimizer (LEO) module to your LC/MS/MS system. Application examples show sensitivity gains of up to a factor of 40 by simply adding a salt solution to the LC effluent and/or changing its pH. The LEO module is quickly and easily installed in your LC/MS system. A solvent mixture, buffer solution or reagent is then easily added to the effluent ensuring that the LC separation can be performed under optimal conditions while also enabling maximum yield in the MS ionization process, helping to reduce or eliminate Ion Suppression. Whether you are

looking to perform pH adjustment or post-column derivatization, for method development or routine analysis, when you use LEO and the GERSTEL MAESTRO software you can easily and efficiently control all parameters as part of the overall method. Just one sequence table controls the entire system from sample preparation through LC separation and effluent optimization to MS analysis. It is all done with the click of a mouse.



Enhancing signal in LC/MS through post-column pH modification. Example: Chloramphenicol (CAP)



Automated Sample Preparation

Doing Drugs? II

Comprehensive Analysis of Drugs of Abuse in Urine with Automated Disposable Pipette Extraction (DPX) and HPLC/MS/MS with dynamic MRM.

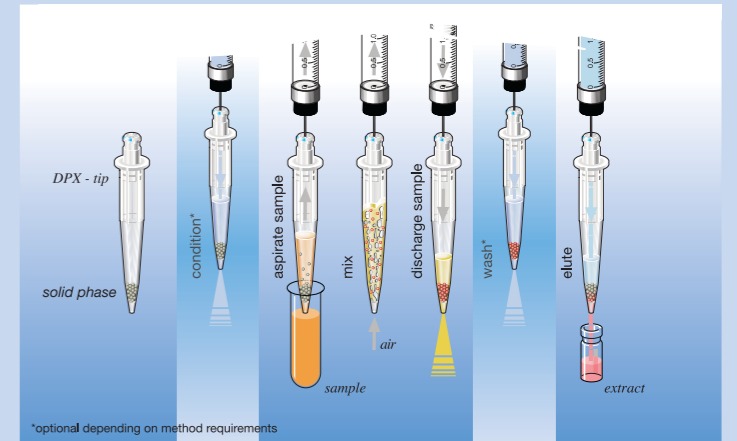
In order to analyze biological specimens for drugs such as benzodiazepines, it is necessary to perform sample preparation to eliminate matrix interference and ion suppression. Solid-phase extraction is generally the preferred sample preparation technique, in this study Disposable Pipette Extraction (DPX) was utilized. DPX is a fast 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 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), cation exchange (DPX-CX) or immunoaffinity sorbent material. For determination of target drugs, GC/MS or HPLC/MS/MS are generally the preferred techniques. The advantage of LC/MS/MS is that chemical derivatiza →

Automated DPX process

All steps are performed automatically by the MPS. If needed, the sorbent is conditioned with solvent prior to the extraction process.

- 1 Sample is drawn into the pipette tip for direct contact with the solid phase sorbent. There is no contact between the sample and the syringe used to aspirate the sample and therefore no risk of cross contamination.
- 2 Air is drawn into the pipette tip from below through the frit. Turbulent air bubble mixing creates a suspension of sorbent in the sample, ensuring optimal contact, highly efficient extraction, and high recovery.
- 3 The extracted sample is discharged, typically after 30 seconds.

If needed, the sorbent can be washed to remove unwanted residue.



- 4 Extracted analytes are eluted using a suitable solvent, which is added from above for most efficient elution. The eluate is collected in a vial for subsequent sample introduction to LC/MS or GC/MS.

The total time required for extraction in the examples shown in this article was always less than 6.5 minutes. Sample preparation and GC/MS or LC/MS determination can be performed in parallel for best possible throughput and system utilization.

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Eberhard-Gerstel-Platz 1
45473 Mülheim an der Ruhr
Germany

Editorial Director
Guido Deußing
ScienceCommunication
Neuss, Germany
guido.deussing@t-online.de

Translation and editing
Kaj Petersen
kaj_petersen@gerstel.de

Scientific advisory board
Eike Kleine-Benne, Ph.D.
eike_kleine-benne@gerstel.de

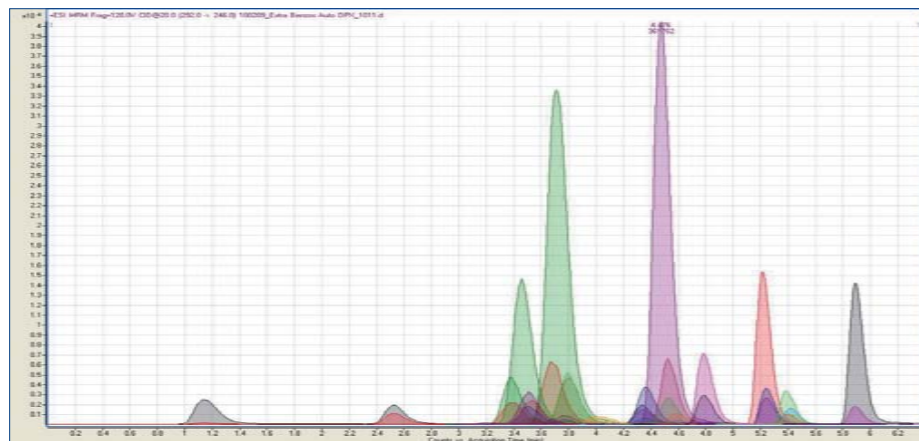
Oliver Lerch, Ph.D.
oliver_lerch@gerstel.de

Malte Reimold, Ph.D.
malte_reimold@gerstel.de

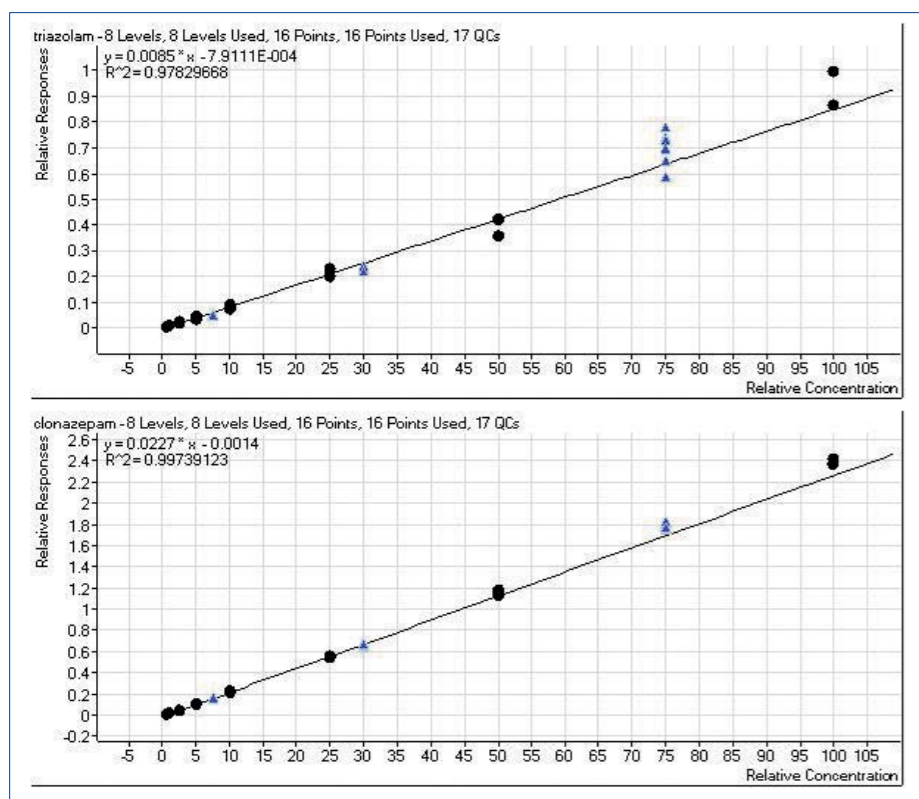
Contact
gerstel@gerstel.com

Design
Paura Design, Hagen, Germany
www.paura.com

www.gerstel.com



Extracted Ion Chromatogram of DPX extracts of urine samples spiked at 7.5 ng/mL.



Representative Calibration Curve of Triazolam and Clonazepam.

Compound	R ²	LOQ [ng/mL]
temazepam ^C	0.9917	0,5
alprazolam ^C	0.9901	0,5
oxazepam ^C	0.9950	0,5
lorazepam ^C	0.9908	0,5
estazolam ^D	0.9955	0,5
α-OH-alprazolam ^A	0.9958	0,5
flurazepam ^B	0.9937	0,5
midazolam ^B	0.9919	0,5
flunitrazepam ^E	0.9959	0,5
diazepam ^B	0.9969	0,5
clobazam ^C	0.9974	0,5
bromazepam ^E	0.9973	0,5
triazolam ^C	0.9783	0,5
nordiazepam ^B	0.9941	0,5
nitrazepam ^C	0.9910	0,5

Calibration Curve Results Calculated using d₅-α-Hydroxyalprazolam (A), d₅-Nordiazepam (B), d₅-Oxazepam (C), d₅-Estazolam (D) and d₄-Clonazepam (E).

Recovery for 16 benzodiazepines using our DPX-LC/MC/MS-Method. The low recovery of clobazam is based on its low extraction efficiency. Unlike the other benzodiazepines it does not contain a tertiary amine in its chemical structure and therefore binds less tightly to the DPX-CX cation exchange material. This result supports the specificity of the DPX-CX sorbent for tertiary amines.

tion 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: All Analyses were performed using an Agilent 1200 HPLC with an Agilent 6410 Triple Quadrupole Mass Spectrometer with ESI source. Sample Preparation and Sample Introduction was performed using a GERSTEL MultiPurpose Sampler (MPS XL).

Materials

Benzodiazepine Multi-Component Mixture 8, containing Clonazepam, Temazepam, Nitrazepam, Alprazolam, Diazepam, Flunitrazepam, Lorazepam, and Oxazepam at 250 µg/mL each in acetonitrile, Nordiazepam, Clobazam, Bromazepam, Estazolam, Flurazepam, Midazolam, and Triazolam, at 1.0 mg/mL each in methanol, and α-hydroxyalprazolam, at 100 µg/mL in methanol, were purchased from Cerilliant. Intermediate stock solutions of the sixteen benzodiazepines were prepared in water from appropriate dilutions of these stocks.

Deuterated analogues d₅-nordiazepam, d₅-α-hydroxyalprazolam, d₅-oxazepam, d₄-clonazepam, and d₅-estazolam, at 100 µg/mL each in methanol, were purchased from Cerilliant. These stocks were combined and diluted with water to be used as internal standards during analysis.

Compound	Recovery [%]
Alprazolam	65
α-OH alprazolam	100
Clonazepam	91
Diazepam	80
Flunitrazepam	86
Lorazepam	49
Nitrazepam	93
Nordiazepam	91
Oxazepam	91
Temazepam	84
Estazolam	90
Clobazam	6
Triazolam	54
Flurazepam	82
Midazolam	57
Bromazepam	43

Urine Sample Pretreatment

- Pipette 260µL of hydrolyzed urine sample into a clean 12 x 75mm culture tube.
- Pipette 250µL of 1.0M HCl into the tube and vortex mix for a few seconds.
- Pipette 250µL of acetonitrile into the tube and vortex mix for a few seconds.
- Filter the sample using an Agilent 2 in 1 syringe filter and collect the filtrate into a clean 12 x 75mm culture tube.
- Place the filtered urine sample onto the GERSTEL MPS 2XL multi-purpose sampler with DPX Option.

Whole Blood Sample Pretreatment

- Pipette 200µL of whole blood sample into a clean 12 x 75mm culture tube.
- Pipette 800µL of acetonitrile into the tube and vortex mix for a few seconds.
- After centrifugation for 10 minutes to pellet the precipitated proteins, transfer the supernatant into a clean 12 x 75mm culture tube.
- Place the sample onto the GERSTEL MPS 2XL multi-purpose sampler with DPX Option.

Extraction

A GERSTEL MultiPurpose Sampler (MPS) was set up with 1 mL DPX-CX tips (DPX Labs, LLC, Columbia, SC) for extraction of hydrolyzed urine samples. 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 µL/s to wet the sorbent. The

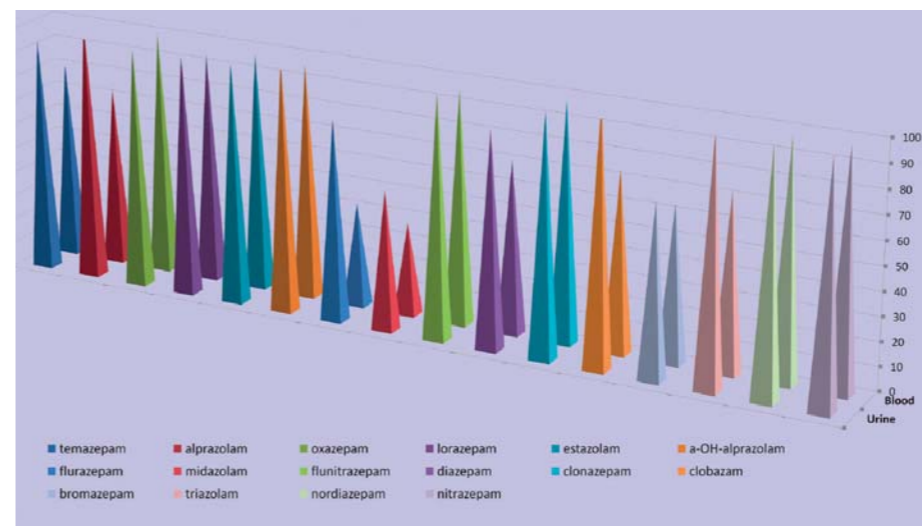
Analysis Conditions HPLC

Column:	Zorbax Eclipse, XDB C18 RRHT (2.1 mm x 50 mm, 1.8 µm)
Mobile Phase:	A – 20 mM ammonium-formate, pH 8.6 B – 10 % isopropyl alcohol in methanol
Gradient:	
Time (min)	Flow (mL/min) Pressure (bar) % B
0	0.2 600 45
2	0.2 600 45
5	0.2 600 95
9	0.2 600 95
9.5	0.2 600 45
Column temperature:	35 °C
Injection volume:	2,5 µL
Run time:	10 min
MS Parameters	
Ionisation:	ESI positive ion mode
Gas Temperature:	350 °C
Gas Flow (N2):	12 L/min
Nebulizer pressure:	35 psi
Capillary:	4,5 kV

sample was then aspirated into the DPX tip and mixed with the sorbent by drawing in an additional 1.3 mL of air. After a 20 s equilibration time to allow analyte binding, the resulting solution was dispensed to waste. To wash off excess matrix, 500 µL of a 10% acetonitrile/water wash solution 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 100% acetonitrile. 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 the eluate dispensed directly into a clean 2 mL autosampler vials. All eluents were dried and reconstituted with 50 µL of water before injection.

Results and Discussion

The DPX-CX extractions were readily performed using the GERSTEL MultiPur-



Matrix effects on the ionization of benzodiazepines in hydrolyzed urine and whole blood compared to the neat standard solutions in pure water (100%).



MPS 2XL MultiPurpose Sampler with the GERSTEL DPX Option.

pose Sampler. The entire extraction process took approximately 6.5 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. In light of the world-wide acetonitrile shortage, it was found that the use of 10% isopropyl alcohol in methanol as the organic modifier of the mobile phase provided an alternative to using acetonitrile. All HPLC/MS spectra were collected using dynamic multiple reaction monitoring (MRM) providing good sensitivity for the analysis of these drugs at low concentrations. A rapid resolution HPLC column was chosen in order to perform the chromatographic data in approximately 10 minutes.

Conclusion

Automated DPX extraction of benzodiazepines from urine can be performed successfully using the GERSTEL MPS. In the work presented here, the total extraction time was 6.5 minutes. 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.

Using this DPX-LC/MS/MS method for determination of benzodiazepines in urine high recoveries for all benzodiazepines were achieved. The calibration curves showed good linearity (R² ≥ 0.98) with limits of quantitation of 0.5 ng/mL. The method proved to be accurate and precise. Accuracy data averaged 102% (range: 84.9% - 136%) and precision data averaged 5.48%RSD (range: 1.01% -26.4%) for all benzodiazepines analyzed.

AUTHORS: Fredrick D. Foster, John R. Stuff, Edward A. Pfannkoch; GERSTEL, Inc., 701 Digital Dr. Suite J, Linthicum, MD 21090, USA. Sparkle T. Ellison, William E. Brewer, Stephen L. Morgan; Department of Chemistry and Biochemistry, University of South Carolina, 631 Sumter Street, Columbia, SC 29208, USA. Tom Gluodenis; Agilent Technologies, 2850 Centerville Rd., Wilmington, DE 19808, USA.