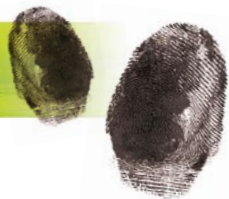




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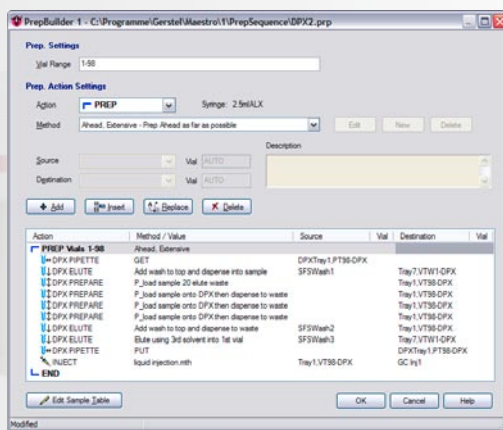
Automated Disposable Pipette Extraction





DPX



**Revolutionary SPE technique
Fast extraction and high recovery
Low limits of detection
Fully automated**





-  **ADD**
Add solvent, internal standard or reagent
-  **DPX PIPETTE**
Get a new DPX-pipette
-  **DPX EXTRACT**
Perform the DPX extraction
-  **INJECT**
Introduce an aliquot of the DPX-eluate to the GC or LC system

MAESTRO PrepBuilder page for the DPX method. All sample preparation steps are conveniently and easily selected from a drop down menu and added to the method.

Disposable Pipette Extraction DPX

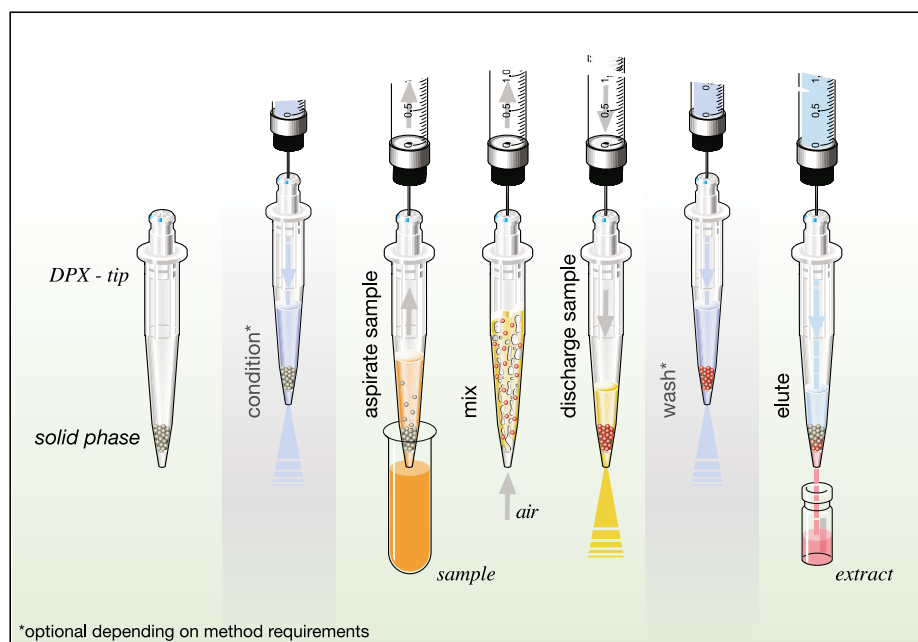
Disposable Pipette Extraction (DPX) is a fast and efficient SPE technique used for a wide range of applications such as drugs of abuse, therapeutic drug monitoring, comprehensive screening, pharmacology studies, as well as pesticides in fruit and vegetables. DPX is based on unique and patented SPE devices: Pipette tips that incorporate loosely contained sorbent material, which is mixed with the sample solution. Turbulent air bubble mixing creates a suspension of sorbent in the

sample ensuring optimal contact and highly efficient extraction. DPX is performed much faster than traditional SPE techniques because time-consuming conditioning steps are not required. Elution requires only a small amount of solvent, which means that DPX effectively provides a concentration step. For many applications, such as pesticides in fruit and vegetables, solvent evaporation is not required. DPX methods are readily automated using the GERSTEL MPS, which can

introduce the extract into a GC/MS or LC/MS system. Additional sample preparation steps can be performed, including derivatization or adding an internal standard. The analyst only needs to place the samples in the MPS autosampler and activate the sequence table from the MAESTRO software. Everything else is handled automatically including GC/MS and LC/MS analysis.



Disposable Pipette Extraction Process



The DPX process

If needed, the sorbent is conditioned with solvent prior to the extraction

- 1) Sample is drawn into the pipette tip for direct contact with the solid phase sorbent
- 2) Turbulent air bubble mixing creates a suspension of sorbent in the sample ensuring optimal contact and highly efficient extraction
- 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 into a vial for subsequent sample introduction to LC/MS or GC/MS

The DPX advantage

1 Rapid sample extraction

- Equilibration and elution occur in seconds
- Conditioning steps generally not required
- DPX clean-up is faster than the GC or LC run and is easily performed in parallel to the analysis. The next sample is always ready for introduction when the GC or LC has finished its run for optimized productivity and throughput

2 Low detection limits

- Less than 250 µL of blood or urine required to reach very low detection limits, when combined with large volume injection
- Minimal elution volume, effectively providing a concentration step
- Multiple Large Volume Injections (LVI) can be performed without contaminating the inlet liner and GC/MS with residue
- LVI replaces solvent evaporation step, which is normally performed in order to reach the required limits of determination

3 Reliable and Reproducible

- Close to 100% recovery on all basic compounds
- Excellent reproducibility and accuracy
- Extremely clean extracts ensure low signal background, low detection limits and good GC/MS and LC/MS system stability
- Just in time sample preparation, every sample is prepared at the exact same point in time prior to sample introduction to the GC/MS or LC/MS for best possible reproducibility and accuracy

4 Complete Automation

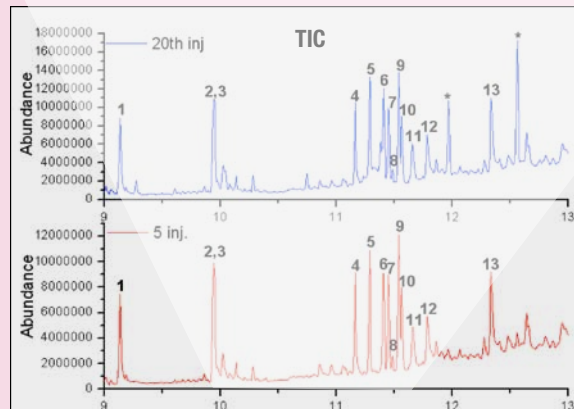
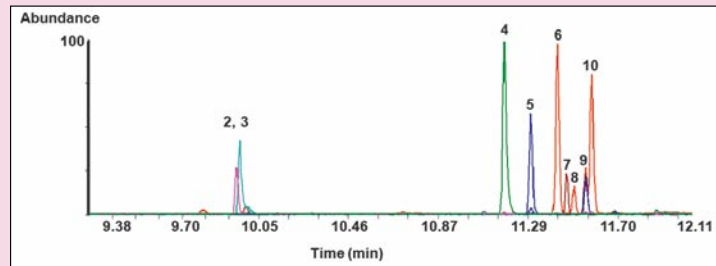
- Automation based on proven MPS 2 PrepStation technology
- No manual intervention required
- Complete extraction controlled by GERSTEL MAESTRO software
- DPX method integrated with ChemStation method and sequence table
- Automated derivatization can be performed by the MPS autosampler either in the vial or in the CIS inlet eliminating tedious manual procedures

GC/MS Applications



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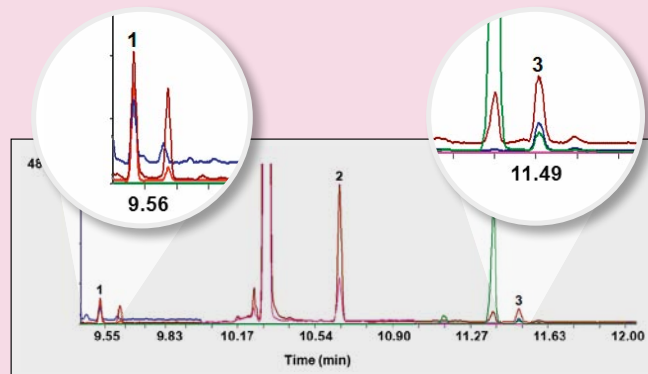
Drugs of abuse



Drug	Average RSD
Amphetamine	13.9 %
Methamphetamine	14.4 %
Meperidine	5.8 %
PCP	2.2 %
Methadone	3.3 %
Methaqualone	3.6 %
Amtriptyline	3.1 %
Cocaine	3.8 %
Cis Doxepin	2.8 %
Imipramine	3.3 %
Trans Doxepin	3.2 %
Pentazocine	5.3 %
Codeine	4.2 %
Desipramine	6.4 %

Total ion chromatogram of DPX extract of 250 µL of whole blood spiked at 0.5 ppm of drugs of abuse with d₅-PCP as internal standard. The inserted chromatogram above shows the extracted ion chromatogram (EIC). The sample was protein precipitated with 0.5 mL of acetonitrile, and the supernatant was transferred to clean tubes with 0.1 mL of 0.1 M HCl added, and then automated DPX was performed.
 1) Meperidine, 2) PCP-d₅ (ion 205), 3) PCP, 4) Methadone, 5) Methaqualone, 6) Amtriptyline, 7) Cocaine, 8) cis-Doxepin, 9) Imipramine, 10) trans-Doxepin, 11) Desipramine, 12) Pentazocine, 13) Codeine
 *-Denotes septum bleed from vial cap after repeat injections.

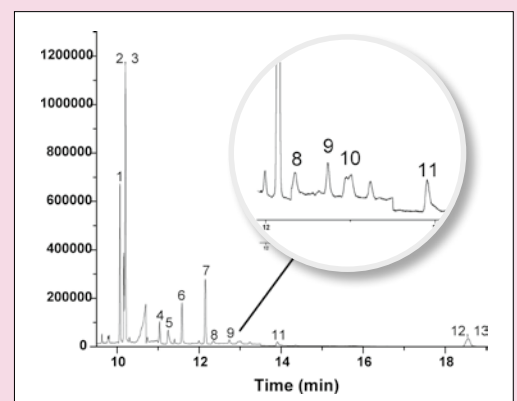
THC and metabolites



Total ion chromatogram of 10 ng/mL THC and metabolites extracted from 0.5 mL whole blood following protein precipitation, centrifugation and DPX-RP. Derivatization was performed in the CIS inlet by injecting 50 µL of DPX eluent together with 20 µL of 50/50 BSTFA/acetonitrile. No additional solvent evaporation or derivatization step was performed.
 Analytes:
 1) THC-TMS, 2) OH-THC-TMS, 3) COOH-THC-2TMS

Benzodiazepines

Total ion chromatogram of 0.2 ppm benzodiazepines in 0.2 mL of urine following enzymatic hydrolysis and DPX. Derivatization was performed in the CIS inlet by injecting 50 µL of DPX eluent together with 20 µL of 50/50 MTBSTFA/acetonitrile. No separate solvent evaporation step was performed. Increasing the sample volume to 0.5 mL using multiple DPX extractions would increase the sensitivity.
 1) Diazepam, 2) Nordiazepam-d₅-TBDMS, 3) Nordiazepam-TBDMS, 4) Flunitrazepam, 5) 7-aminoflunitrazepam, 6) Oxazepam-2TBDMS, 7) Temazepam-TBDMS, 8) Nitrazepam, 9) Lorazepam-2TBDMS, 10) Clonazepam-TBDMS, 11) Alprazolam, 12) α-OH-Alprazolam-d₅-TBDMS, 13) α-OH-Alprazolam-TBDMS



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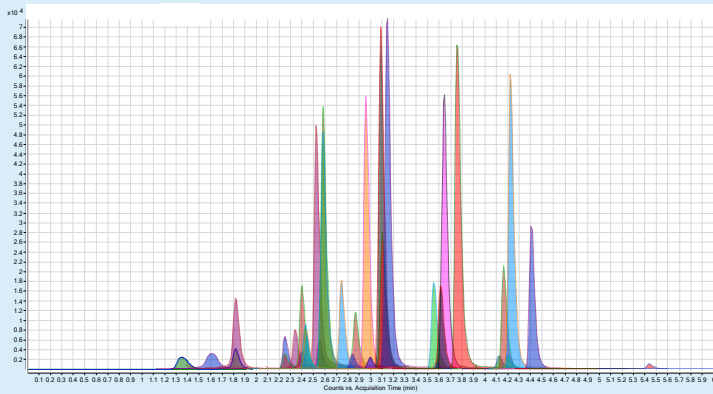
DPX-LC/MS/MS Drug Screening

Automated extraction and determination of 40 drugs in urine using automated DPX-LC/MS/MS. Samples of 276 μL hydrolyzed urine spiked with drugs were diluted with 500 μL acetonitrile, vortexed and transferred to a DPX-SC tip. After automated DPX the sample was evaporated and reconstituted using 100 μL of methanol / 0.05% formic acid (1:1 v/v).

The analysis was performed using an Agilent 1200 HPLC with a Zorbax Eclipse Plus column DB-C18, 2.1x100 mm, 1.8 μm and an Agilent 6410 TripleQuad Mass Spectrometer (ESI, dynamic MRM, positive mode).

LC-Conditions

Injection Volume: 2.5 μL
 Mobile Phase: **A:** 5 mM ammonium formate with 0.05% formic acid
B: Methanol with 0.05% formic acid
 Gradient: initial 95 % A / 5 % B
 0.5 min 95 % A / 5 % B
 1.5 min 70 % A / 30 % B
 3.5 min 30 % A / 70 % B
 4.5 min 5 % A / 95 % B
 6.5 min 5 % A / 95 % B
 7.5 min 95 % A / 5 % B
 Flow Rate: 500 μL / min



Extracted Ion Chromatograms of 40 drugs and metabolites in urine.

Compound	RT [min]	Precursor Ion (m/z)	Product Ion (m/z)	Recovery [%]
Codeine	2,297	300,2	152,0	95
Hydrocodone	2,446	300,2	199,0	113
Hydromorphone	1,881	286,2	185,0	74
6-MAM	2,476	328,2	165,0	89
Morphine	1,426	286,2	152,1	72
d3-morphine	1,414	289,2	152,1	100
Oxycodone	2,392	316,2	241,0	91
Oxymorphone	1,620	302,2	227,1	73
Buprenorphine	4,131	468,4	414,0	59
EDDP	3,807	278,2	234,1	81
Fentanyl	3,691	337,2	188,2	59
d5-Fentanyl	3,680	342,3	188,1	100
Methodone	4,256	310,2	265,1	75
Norbuprenorphine	3,631	414,3	187,0	68
Norfentanyl	3,046	233,1	150,1	79
Alprazolam	4,483	309,0	281,0	117
a-OH-alprazolam	4,369	325,0	297,0	128
Clonazepam	4,228	316,0	270,0	121
Diazepam	4,811	285,0	257,0	78
Flunitrazepam	4,283	314,0	268,0	123
Lorazepam	4,447	321,0	275,0	150
Nitrazepam	4,243	282,0	236,0	101
Nordiazepam	4,691	271,0	165,0	79
d5-nordiazepam	4,674	276,0	213,0	100
Oxazepam	4,444	287,0	269,0	142
Temazepam	4,565	301,0	255,0	96
o-desmethyltramadol	3,283	250,1	189,1	65
Norpropoxyphene	4,251	326,2	252,1	70
Propoxyphene	4,220	340,3	58,0	78
d5-propoxyphene	4,210	345,3	271,2	100
Tramadol	3,164	264,2	58,0	70
Carisoprodol	4,500	261,2	176,1	72
d7-Carisoprodol	4,490	268,2	183,1	100
Gabapentin	2,504	172,1	154,0	61
Meprobamate	3,709	219,1	158,0	73
PCP	3,620	244,3	91,2	71
d5-PCP	3,609	249,3	164,3	100
COOH-THC	5,512	345,0	193,0	47
Amphetamine	2,583	136,2	91,1	50
d5-Amphetamine	2,573	141,1	93,0	100
Benzoyllecgonine	3,041	290,3	168,3	89
Cocaine	3,151	304,2	182,1	145
MDA	2,580	180,0	163,3	79
MDEA	2,806	208,0	135,0	120
MDMA	2,643	194,0	163,0	85
Methamphetamine	2,642	150,2	91,1	65
Methylphenidate	3,206	234,2	84,1	103

GERSTEL MAESTRO Software

Next generation software for automated sample preparation and sample introduction. MAESTRO optimizes performance and throughput of GERSTEL modules and systems.

- Stand-Alone operation or fully integrated in the Agilent ChemStation Software
- One sequence table operates the entire system including LC/MS or GC/MS
- Sample Prep by Mouse-Click using the PrepBuilder functions
- Scheduler for easy planning
- PrepAhead: Automated overlapping of sample prep and analysis for optimum productivity and throughput
- Priority samples can be added to the system at any point in the analysis sequence
- LOG file and Service LOG file functions ensure traceability
- Automated E-mail notification if the sequence is stopped
- Control of up to 4 systems from one PC
- Real-time monitoring of all modules and parameters
- Remote support tool included



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