GERSTEL Solutions

Completely Automated Hydrolysis, Extraction and Analysis of Opioids in Urine using a New Robotic Autosampler and LC/MS/MS Platform

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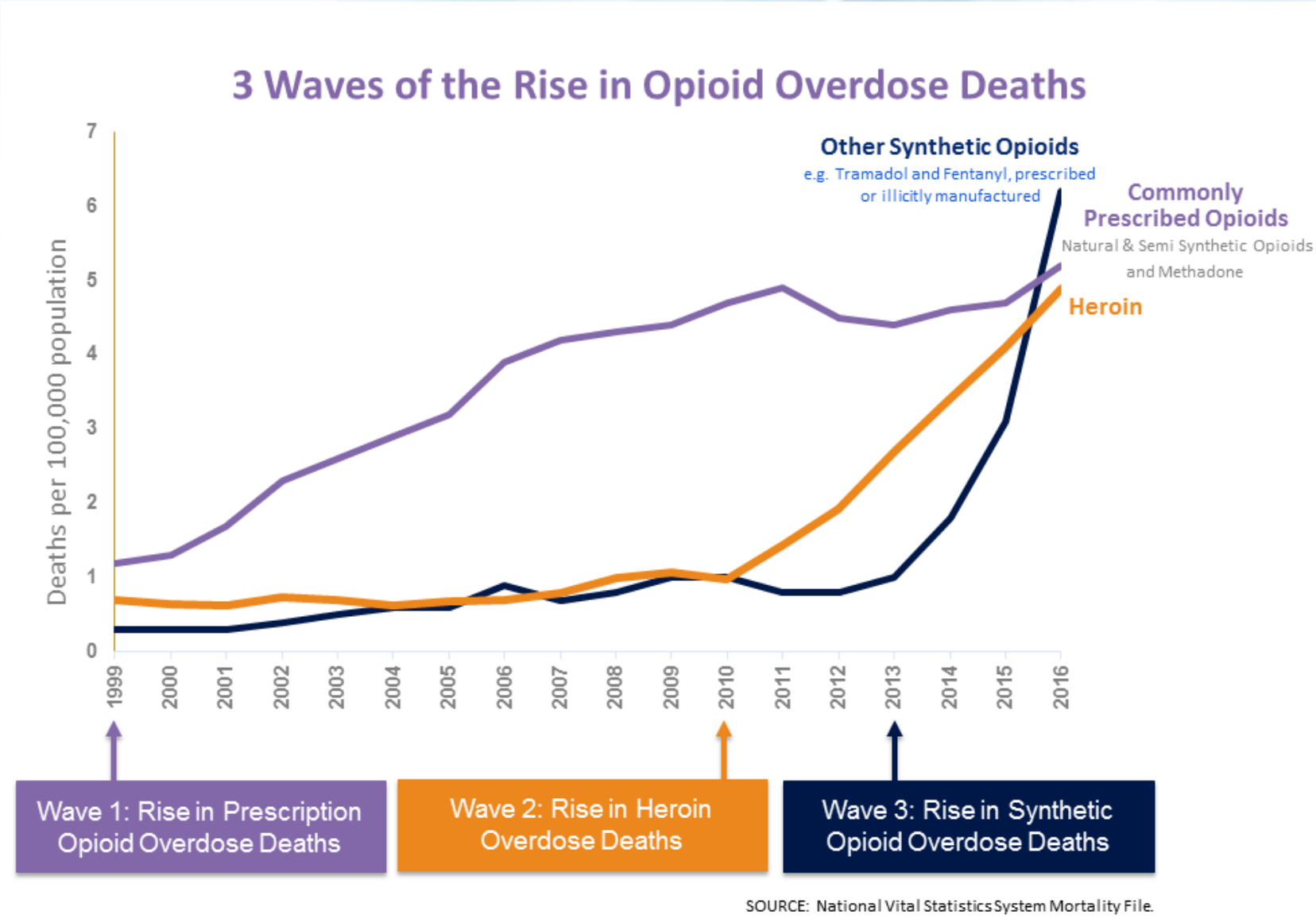
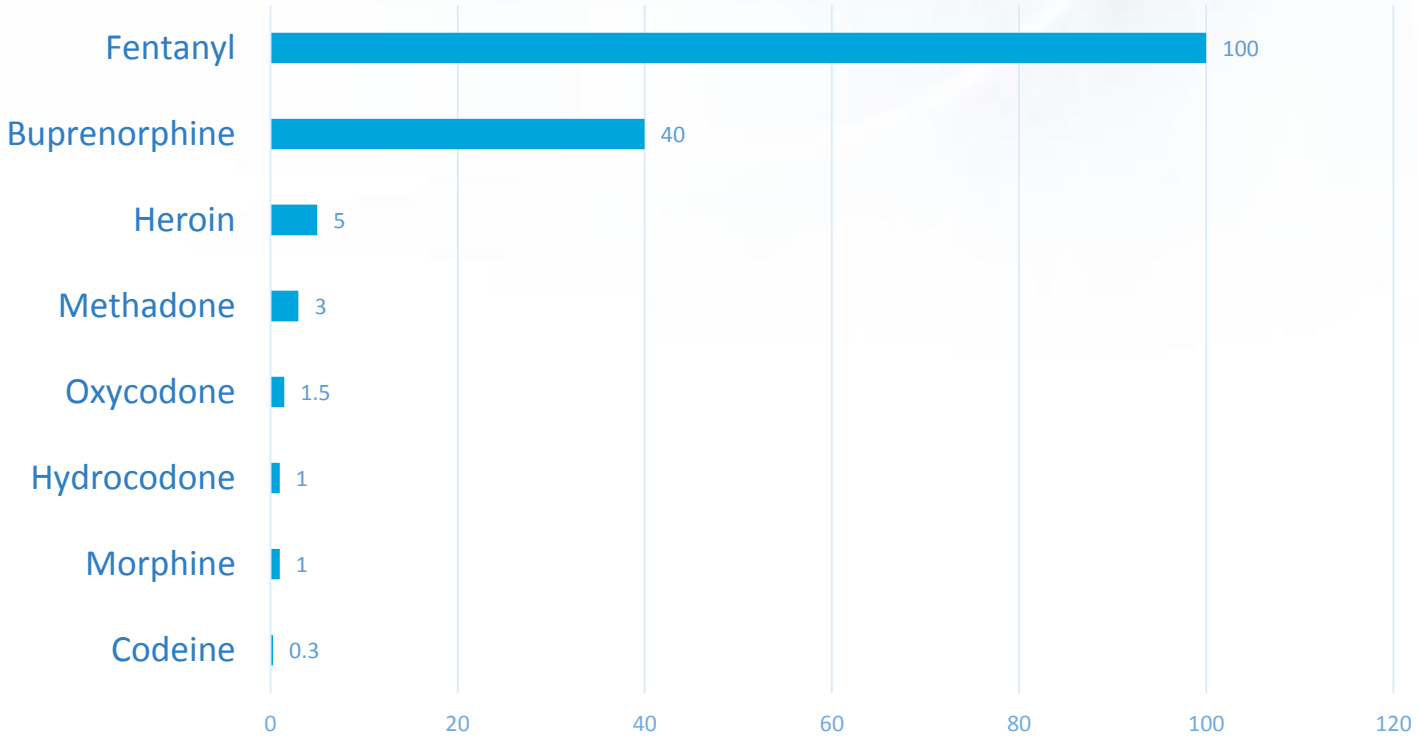


https://www.cdc.gov/drugoverdose/images/epidemic

Potency Compared to Morphine

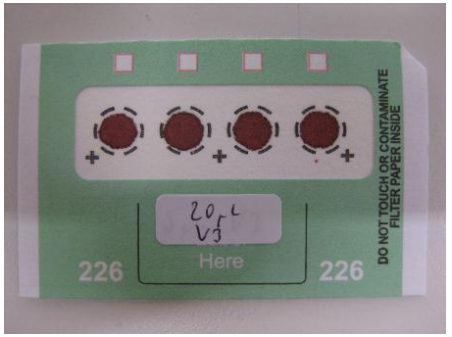


https://www.washingtonpost.com/graphics/2017/health/opioids-scale

Opioid Testing

Matrix

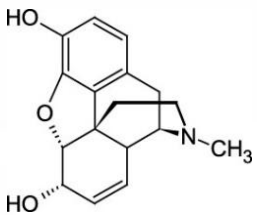
- Blood
- Plasma
- Serum
- Urine
- Dried Blood Spots
- Saliva



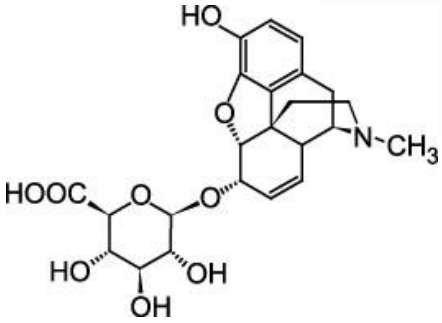
Analytical Technique

ELISA, GC/MS, LC/MS/MS

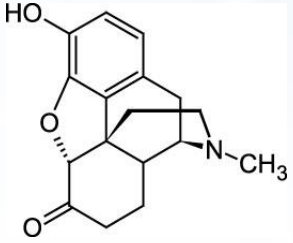
Morphine



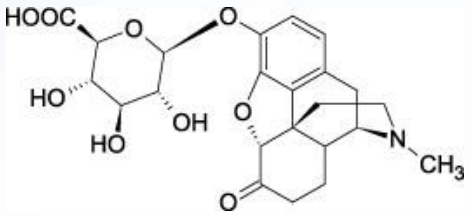
Morphine-6-B-D-glucuronide



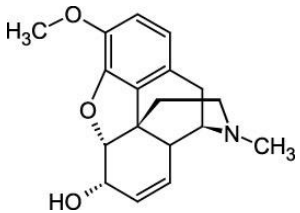
Hydromorphone



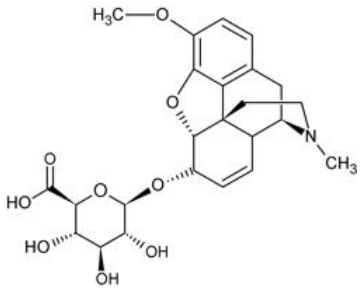
Hydromorphone-3-B-D-glucuronide



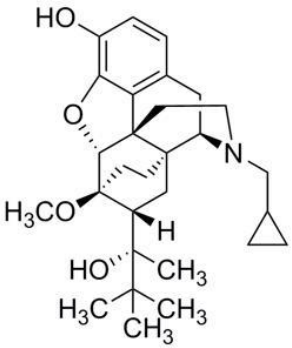
Codeine



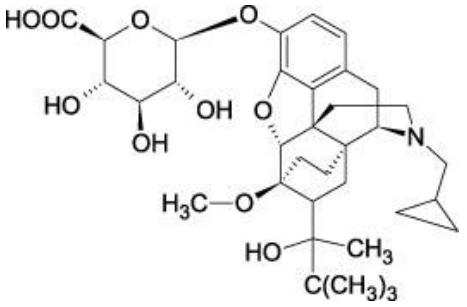
Codeine-6B-glucuronide



Buprenorphine



Buprenorphine-3B-glucuronide



Hydrolysis – acid hydrolysis or enzymatic hydrolysis

"Degradation of Opioids and Opiates During Acid Hydrolysis Leads to Reduced Recovery Compared to Enzymatic Hydrolysis"

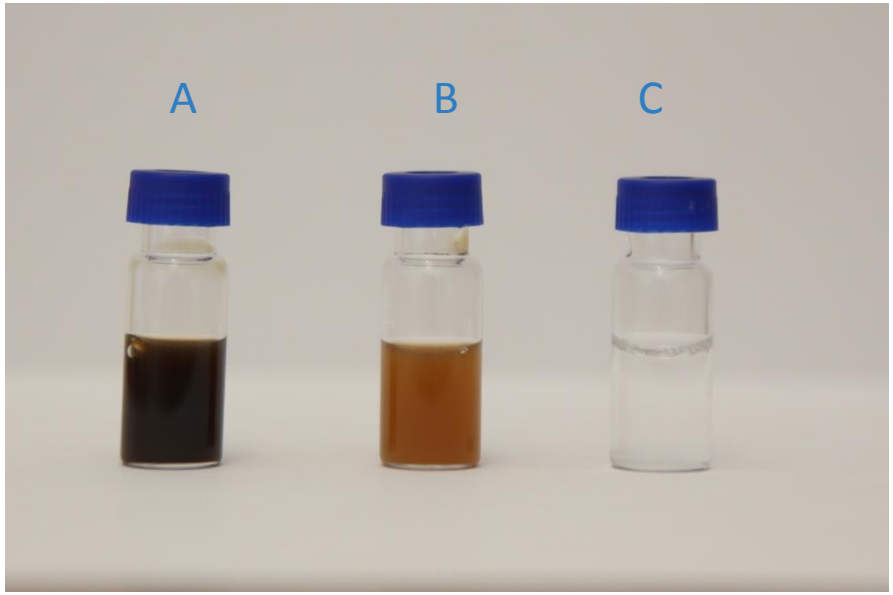
- P. Sitasuwan, et al, Journal of Analytical Toxicology, Volume 40, Issue 8, 1 October 2016, Pages 601–607

Variety of B-glucuronidase enzymes:

A) Helix-Pomatia
B) Abalone
C) Recombinant Protein

https://imcstips.com/IMCSzyme

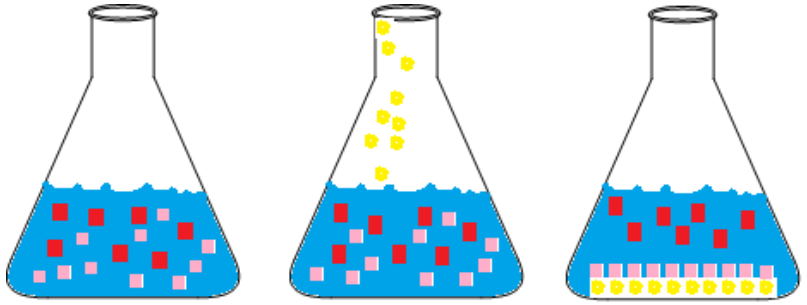
Urine Samples treated with different β-Glucuronidase enzymes.

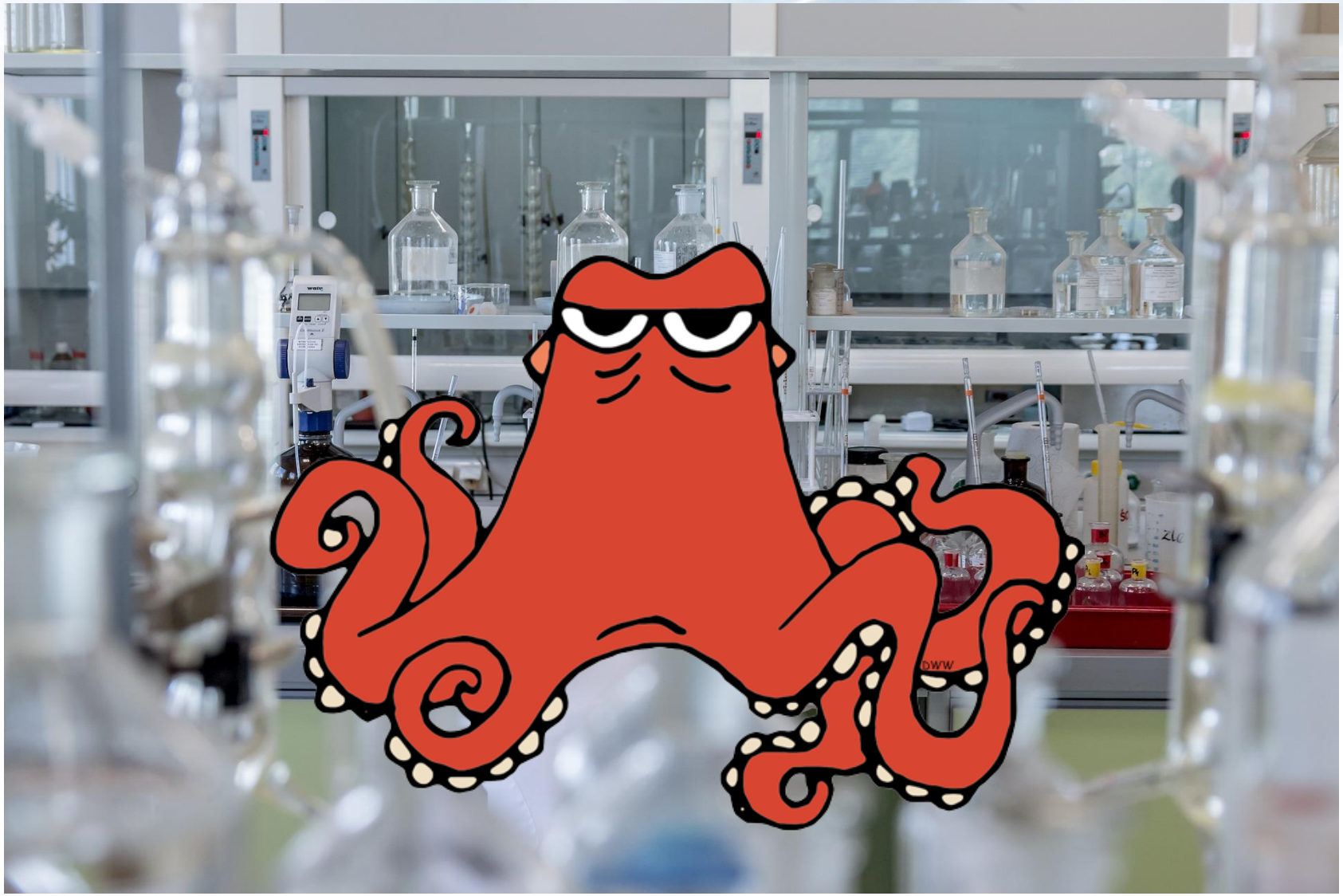


Extraction

- Dilute and shoot
- Precipitation (“solvent crash”)
- Liquid-Liquid extraction
- Solid Phase Extraction

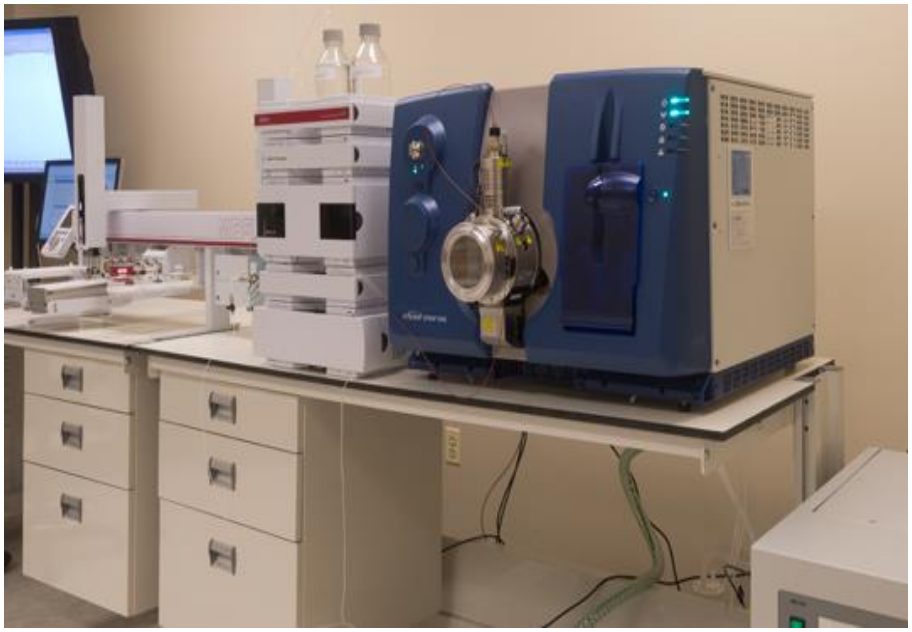
Solid Phase Extraction





MultiPurpose Sampler (MPS)

Integrated with LC/MS/MS



MultiPurpose Sampler (MPS)

Benchtop Workstation

Integrated with GC/MS

MPS

Evaporative Concentration mVAP

Extraction
Derivatization
Addition of Standards

Solid Phase Extraction SPE & Filtration

Cold storage of samples

DisposablePipette-Extraction DPX

MPS for LC & LC/MS

CF200 Centrifugation

Dried Blood Spot Autosampler DBS-A

Online-SPE SPExos

Sample Preparation

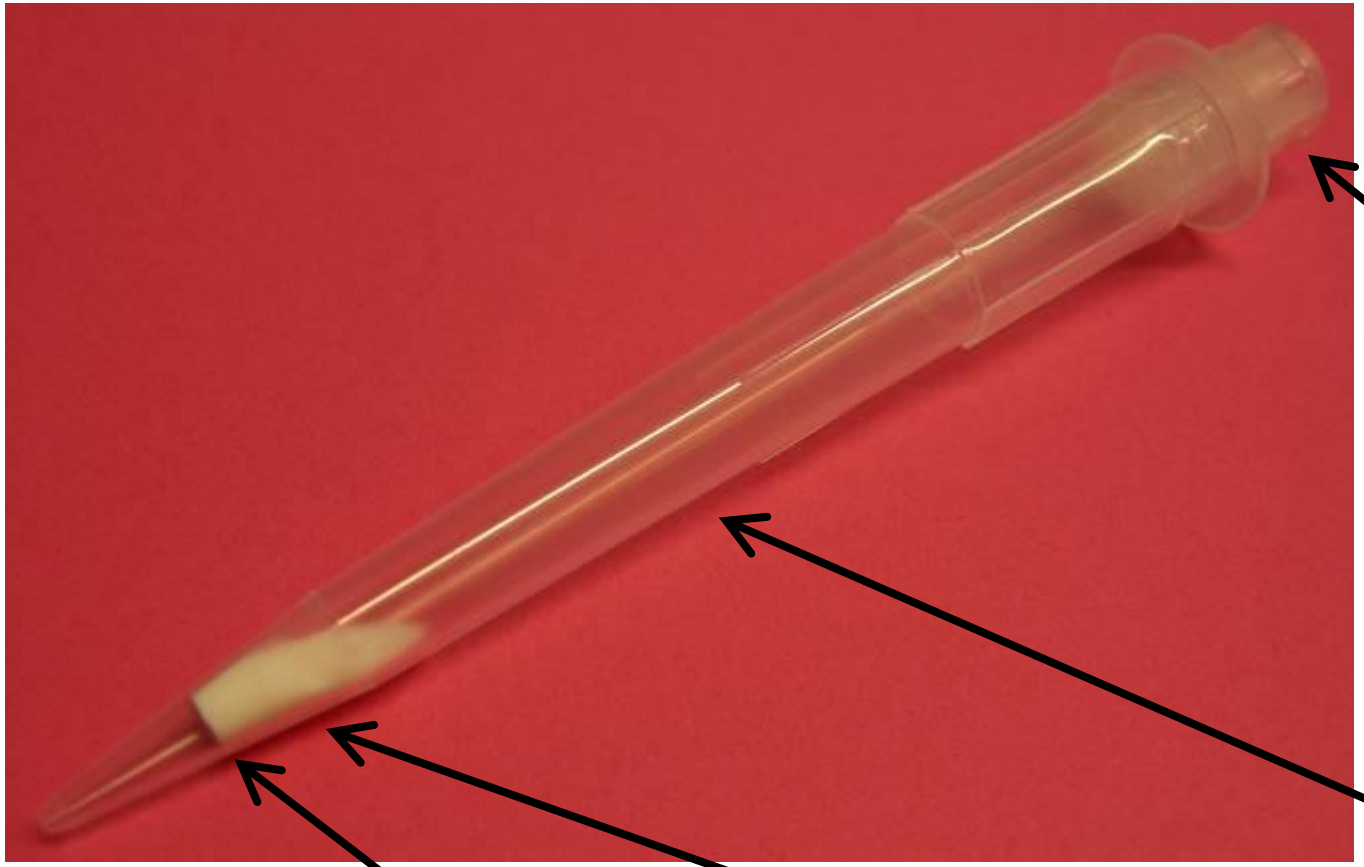
Typical Manual Sample Preparation Techniques

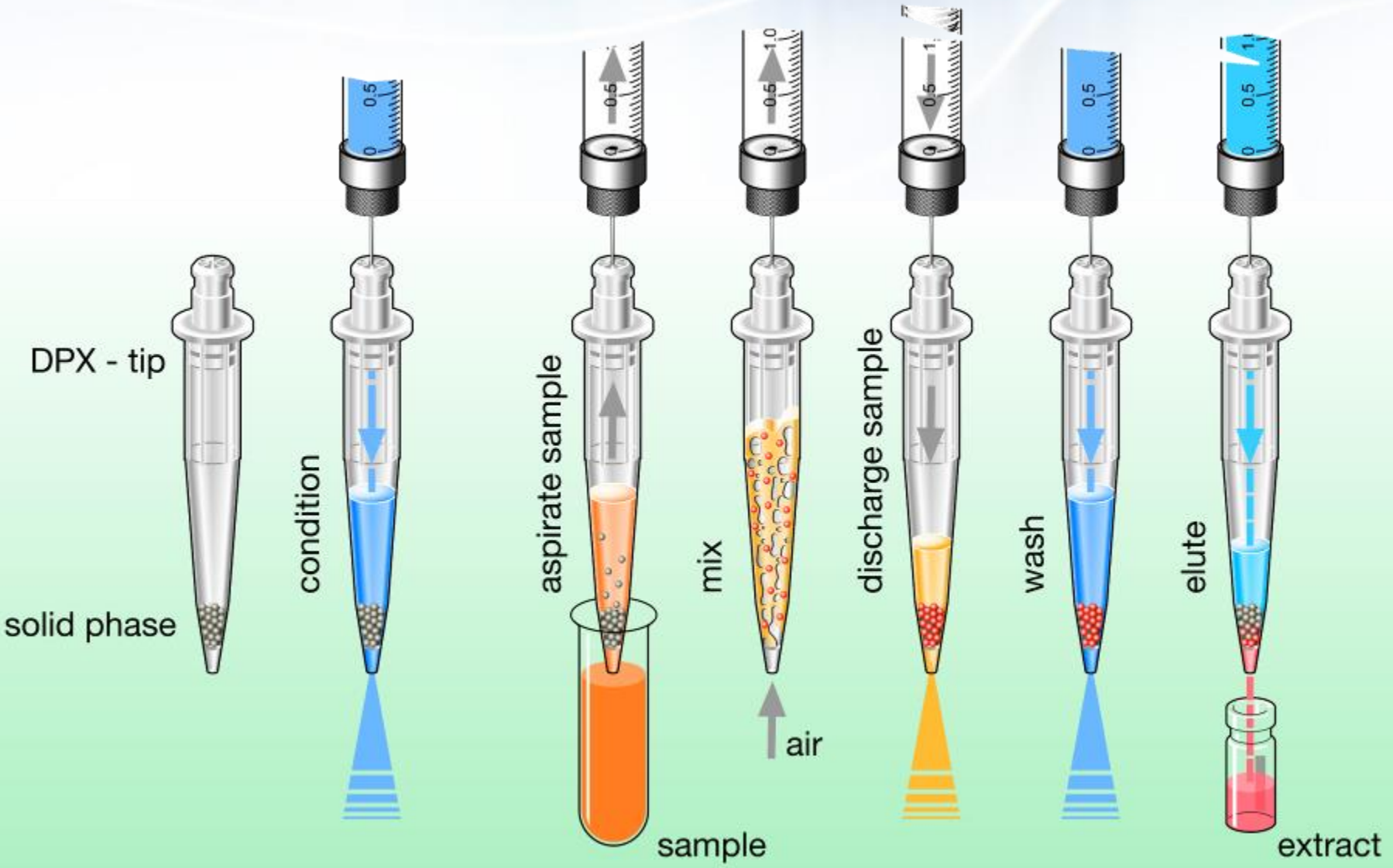
- Pipetting
- Dilution
- Vortexing/Mixing
- Heating
- Sonication
- Evaporation
- Calibration Standard Preparation
- Liquid-Liquid Extraction
- Solid Phase Extraction

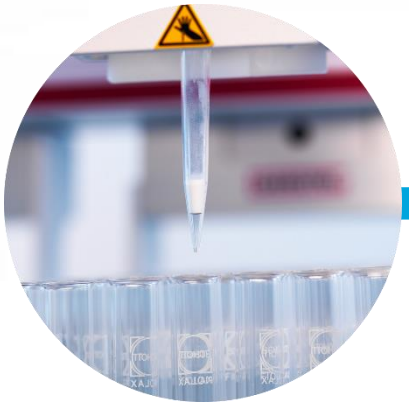
Automated
Disposable Pipette Extraction DPX

DPX

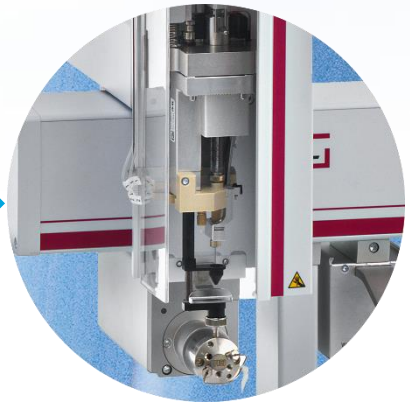
GERSTEL DPX automation

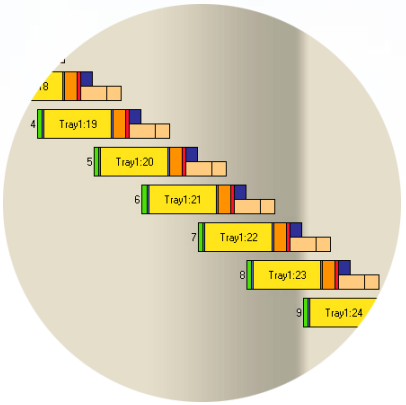



DPX - tip
solid phase
condition
aspirate sample
sample
mix
air
discharge sample
wash
elute
extract




mVAP
GERSTEL




Tray1:19
Tray1:20
Tray1:21
Tray1:22
Tray1:23
Tray1:24

LC Method Parameters

Mobile Phase: A - 5mM ammonium formate with 0.05% formic acid
B –0.05% formic acid in methanol

LC Gradient:

Time (min)	Flow (mL/min)	Pressure (bar)	% B
0	0.5	800	5
0.5	0.5	800	5
1.5	0.5	800	30
3.5	0.5	800	70
4.5	0.5	800	95
6.49	0.5	800	95
6.5	0.5	800	5

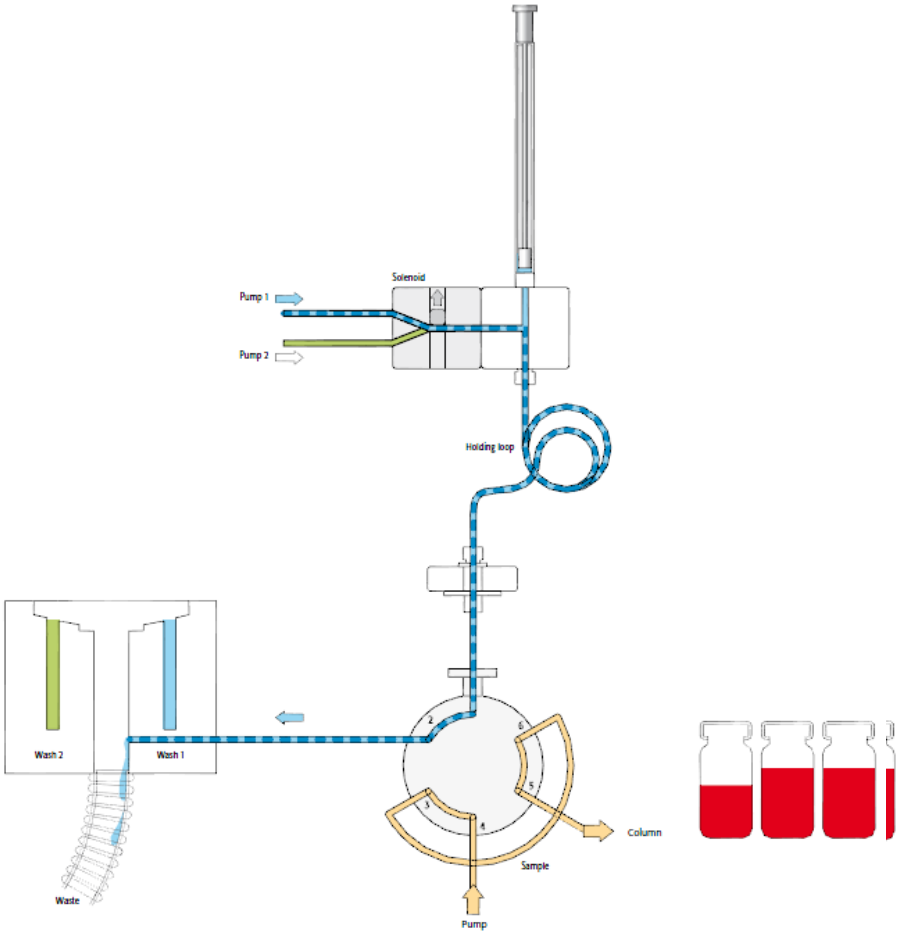
Run time: 8 minutes.
Injection volume: 2.0uL (loop over-fill technique)
Analytical Column: Agilent Poroshell 120 EC-C18 column, (3.0 x 50mm, 2.7um)
Column Temperature: 55 ^{o}C

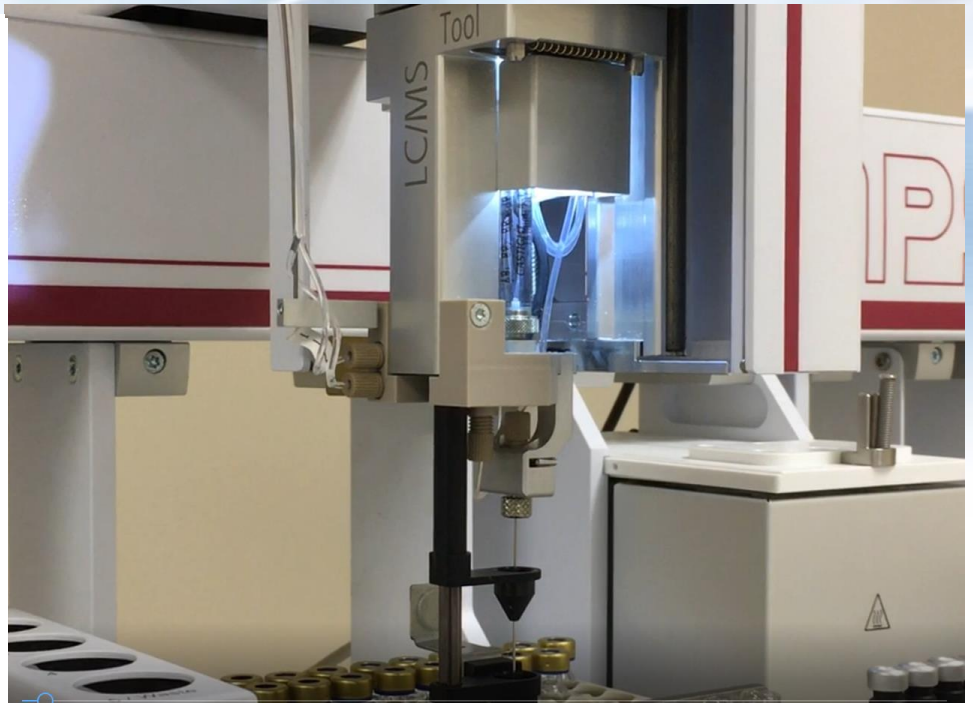
Mass Spectrometer Parameters:

Operation: Electrospray positive mode (Jet Stream)
Gas Temperature: 350^{o}C
Gas Flow (N_2): 5 L/min
Nebulizer pressure: 35 psi
Sheath Gas Flow (N_2): 11 L/min
Sheath Gas Temperature: 250 ^{o}C
Capillary voltage: 4000 V
Nozzle voltage: 500 V
Delta EMV: 0 V

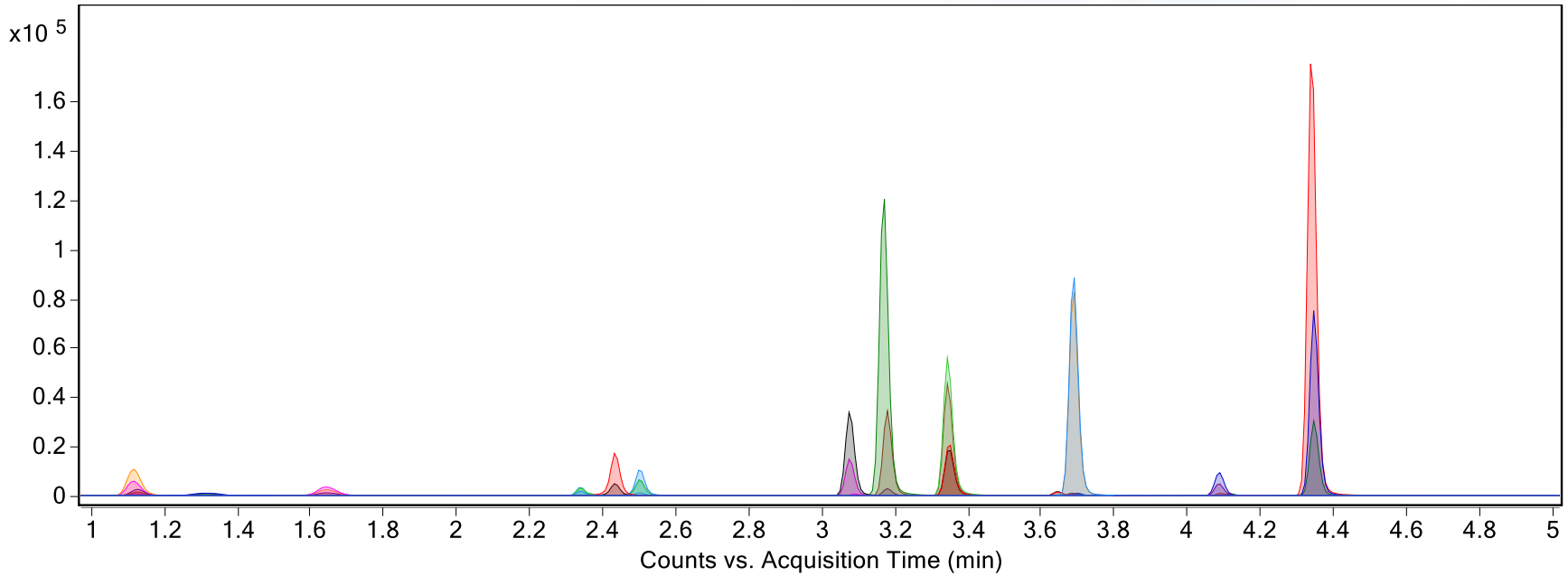
Compound Name	Precursor Ion (m/Z)	Product Ion (m/z)		Frag (V)		CE (V)		Ret Time (min.)	High Std Conc. (ng/mL)	MRL (ng/mL)	Limit of Quantitation (ng/mL)
Buprenorphine[4]	468.3	396.2	55.1	200	200	39	58	4.08	200	10.0	5.00
Codeine[3]	300.2	165.1	128	158	158	45	60	2.33	1000	50.0	25.0
D3-morphine[1]	289.0	165.1	152	153	153	38	66	1.09	-	-	-
D3-Norbuprenorphine[2]	417.3	152	55.1	190	190	124	76	3.62	-	-	-
D3-Tramadol[3]	268.2	58.1	-	102	-	14	-	3.16	-	-	-
D4-Buprenorphine[4]	472.3	400.2	59.1	210	210	40	56	4.06	-	-	-
D4-Meperidine[5]	252.2	224.2	178.2	140	140	15	15	3.33	-	-	-
D5-Fentanyl[6]	342.3	188.1	105.1	92	92	20	40	3.67	-	-	-
D5-Norfentanyl[7]	238.2	84.1	55.1	125	125	20	50	3.06	-	-	-
D9-Methadone[8]	319.3	268.1	-	118	-	8	-	4.31	-	-	-
Fentanyl[6]	337.2	188.1	105.1	143	143	17	37	3.68	20.0	1.00	0.500
Furanyl Fentanyl[6]	375.2	188.1	105	150	150	15	25	3.67	20.0	n/a	0.500
Hydrocodone[3]	300.2	199	128	159	159	27	63	2.70	1000	50.0	25.0
Hydromorphone[1]	286.2	185	157	159	159	27	43	2.20	1000	50.0	25.0
Meperidine[5]	248.2	220.1	174.1	128	128	17	13	3.33	1000	50.0	25.0
Methadone[8]	310.2	265.1	105	112	112	7	27	4.32	1000	50.0	25.0
Morphine[1]	286.2	165.1	152	158	158	45	64	1.10	1000	50.0	25.0
Norbuprenorphine[2]	414.3	187.1	83.1	205	205	37	53	3.63	200	10.0	5.00
Norfentanyl[7]	233.2	84.1	55.1	112	112	12	36	3.08	20.0	1.00	0.500
Oxycodone[3]	316.2	298.1	241.1	143	143	13	25	2.42	1000	50.0	25.0
Oxymorphone[1]	302.1	227.1	198.1	133	133	24	44	1.29	1000	50.0	25.0
Sufentenil[8]	387.2	238.1	110.9	145	145	10	20	4.07	20.0	n/a	0.500
Tramadol[3]	264.2	58.1	42.1	107	107	14	106	3.16	500	25.0	12.5

LCMS Tool

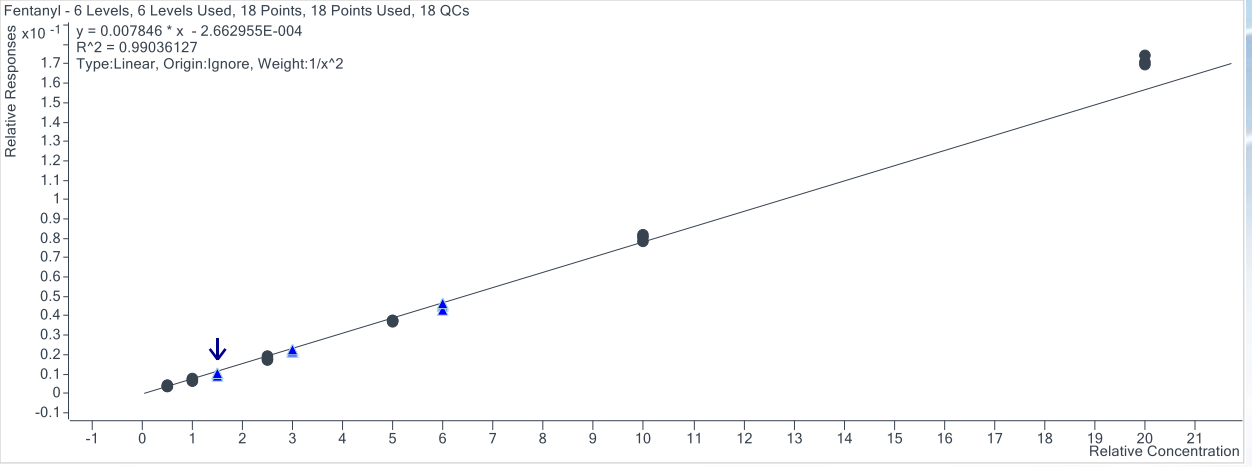


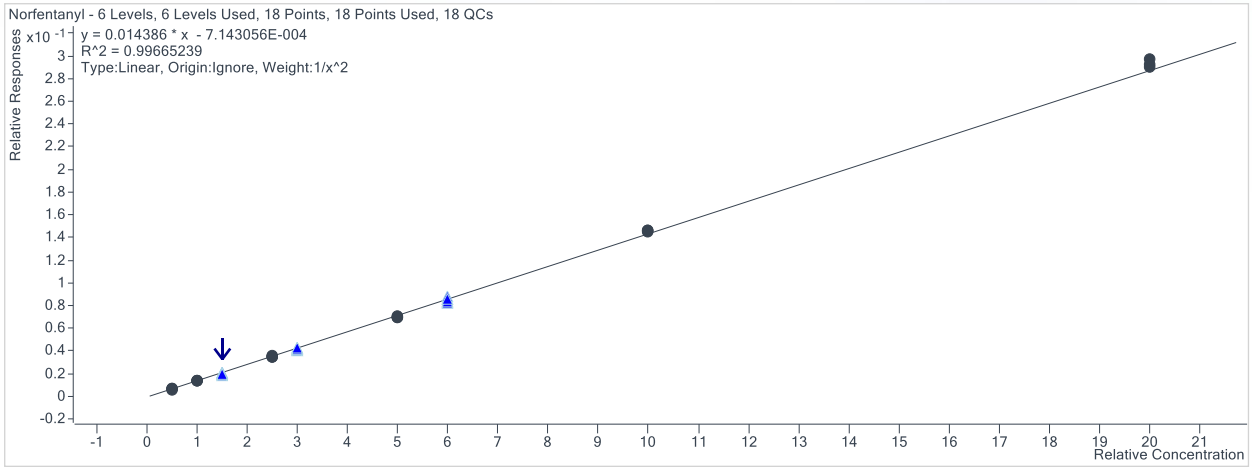


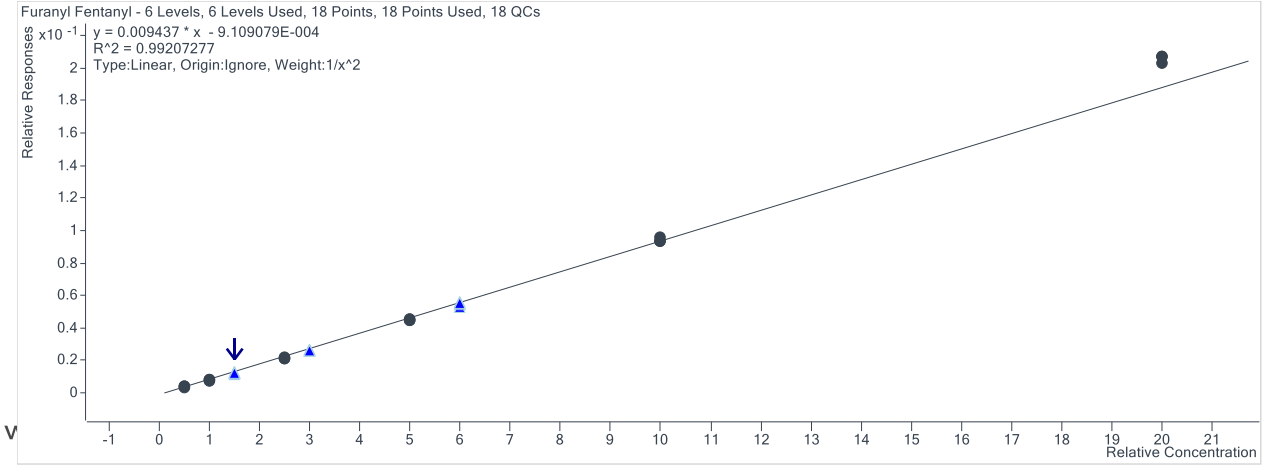
- Near-Zero carryover
- Low detection limits
- Fast
- Reproducible
- Integrated MAESTRO software control


x10 5
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
1.2
1.4
1.6
1.8
2
2.2
2.4
2.6
2.8
3
3.2
3.4
3.6
3.8
4
4.2
4.4
4.6
4.8
5
Counts vs. Acquisition Time (min)


12000
10000
8000
6000
4000
2000
0
-2000
Morphine Response
0 min
15 min
30 min
1 hour
Incubation Time


Fentanyl - 6 Levels, 6 Levels Used, 18 Points, 18 Points Used, 18 QCs
y = 0.007846 * x - 2.662955E-004
R^2 = 0.99036127
Type:Linear, Origin:Ignore, Weight:1/x^2
Relative Responses
x10 -1
Relative Concentration


Norfentanyl - 6 Levels, 6 Levels Used, 18 Points, 18 Points Used, 18 QCs
y = 0.014386 * x - 7.143056E-004
R^2 = 0.99665239
Type:Linear, Origin:Ignore, Weight:1/x^2
Relative Responses
x10 -1
Relative Concentration


Furanyl Fentanyl - 6 Levels, 6 Levels Used, 18 Points, 18 Points Used, 18 QCs
y = 0.009437 * x - 9.109079E-004
R^2 = 0.99207277
Type:Linear, Origin:Ignore, Weight:1/x^2
Relative Responses
x10 -1
Relative Concentration

Resulting QC Accuracy and Precision

Additional Application Notes

GERSTEL Application Note No. **192**, 2017

Drugs of Abuse in Oral Fluids: Automated SPE Extraction and LC/MS/MS Determination using a Robotic Autosampler

Fred D. Foster[1], John R. Stuff[1], Jacqueline A. Whitecavage[1], Edward A. Pfannkoch[1], Mark Hayward[2]

1. GERSTEL, Inc., 701 Digital Drive, Suite J, Linthicum, MD, 21090, USA
2. ITSP Solutions, Inc., 10 South Carolina St, Hartwell, GA 30643, USA

Keywords
Forensics, Sample Preparation, LC/MS/MS, High Throughput Lab Automation, SPE

Abstract
Solid phase extraction (SPE) is one of the sample preparation methods most widely used by chromatographers, as can be seen from the large number of SPE methods found in the literature. Typically, a liquid sample is passed across an adsorbent bed to retain and concentrate target analytes and eliminate interference from the sample matrix. Alternatively, the adsorbent can be used to retain interferences while allowing the target analytes to pass through. Manual SPE cartridge formats can vary from disks to individual cartridges with various volumes of sorbent to 96-well plates. However, solid phase extraction can be tedious and time consuming when performed manually and there is increasing demand for automation of SPE methods.

The MPS robotic[pro] is a highly efficient LC or GC autosampler with extended robotic functionality. The MPS robotic[pro] provides reliable processing of complex tasks including automation of SPE procedures. Syringe holders and syringes are integrated in special syringe modules, which can be exchanged automatically within a running sequence for maximum flexibility. The system is controlled in a simple and efficient manner using the proven GERSTEL MAESTRO software. In this study, we show that an existing SPE method using ITSP cartridges [1] can be automated using the MPS robotic[pro] autosampler under MAESTRO control. An example of a solid phase extraction method illustrating the determination of drugs of abuse in oral fluid is shown.

Introduction
Drug testing based on the analysis of oral fluid samples is increasingly used due to sample collection advantages over other biological matrices and due to the early detection window it offers compared with other approaches. The collection of oral fluid is noninvasive and can be performed under directly observed conditions. The simplicity of collecting an oral fluid sample also reduces the likelihood of test sample adulteration or sample swapping helping to ensure the validity of the results. Some collection devices, including the Quantisal devices [2] used within this study, have an indicator to ensure an exact volume of oral fluid is collected. Following the collection step, the devices are subsequently placed in storage tubes containing stabilization buffer, ensuring that the sample can be shipped or stored for subsequent analysis without analyte degradation.

While the buffer helps to stabilize analytes present in the oral fluid sample, it may also cause analyte suppression effects in the LC/MS/MS analysis leading

GLOBAL ANALYTICAL SOLUTIONS

GERSTEL

…mating Liquid-Liquid Extractions
…a Bench-top Workstation

…ter, Oscar Cabrices, Jackie Whitecavage,
…ff, Edward Pfannkoch
…*c., 701 Digital Dr. Suite J,*
…MD 21090, USA

…DS
…reparation, Lab Automation, LC/MS/MS

…T
…id extractions have long been performed manually
…sed to extract and concentrate analytes from
…atrices. Inclusion of liquid-liquid extraction in
…al methods attests to the wide acceptance of the
Following solvent extraction it is also common
…n evaporation and reconstitution step to improve
…nits or exchange solvents for compatibility with
…hromatographic separations. Modern analytical
…ing to automation to help reduce solvent usage
… sample throughput while ensuring the high
… resulting data.
…Z coordinate autosampler, the GERSTEL
… Sampler (MPS), commonly used for sample
…n GC or HPLC can be used to perform a wide
…mple preparation techniques using a single
…d controlling software. The sampler can be
…art of a GC or LC system or can be configured
…workstation and can also include a six position
…tion.
…t, the automation of liquid-liquid extractions
… autosampler is discussed. Examination of a
… vortexing option that allows samples to be
…ctively mixed using speeds of up to 3000 rpm
…omated liquid-liquid extractions methods for
…lytes from different matrices are examined
…cision and accuracy data are provided.

GERSTEL Application Note No. **199**, *2018*

N A L Y T I C A L S O L U T I O N S

GERSTEL

Automated Desorption, SPE Extraction, and LC/MS/MS Analysis of Dried Blood Spots

Fred D. Foster, John R. Stuff, Edward A. Pfannkoch
GERSTEL, Inc., 701 Digital Dr. Suite J,
Linthicum, MD 21090, USA

Keywords
Forensics ,Veterinary, Sample Preparation, LC/MS/MS, High Throughput Lab Automation

Abstract
The extraction of dried blood spots (DBS) typically involves manual intervention. First, a small disc is punched out of the center of a dried blood spot placed on a DBS card. Following solvent extraction of the sample, it is also common to include further cleanup steps, using solid phase extraction (SPE) to improve detection limits or exchanging solvents for compatibility with subsequent chromatographic separations. Modern analytical labs are looking to automate the process to help reduce solvent usage and to increase sample throughput while ensuring the high quality of the resulting data.

In this report, the complete automation of dried blood spot analysis is demonstrated and the results evaluated. A novel, automated DBS Autosampler (DBS A) automatically inserts DBS cards into a Flow Through Desorption (FTD™) cell in which individual blood spots are rapidly and effectively desorbed. The DBS A is integrated into a complete clean-up and analysis system using online SPE with replaceable cartridges combined with automated injection to an LC/MS/MS system. Automated DBS extraction methods for a variety of analytes from different matrices are examined along with the use of different SPE cartridge sorbents. The resulting precision and accuracy data are provided.

GERSTEL AppNote

…nd Determination of
…botic Autosampler and

…ns were then introduced into the new Agilent
…C-MS/MS instrument.

…UCTION
…y of sample handling steps are required prior to
…ysis of urine samples to accurately determine
… concentrations. These steps typically begin
…e enzymatic hydrolysis of analytes from their
…ated forms to the native drug using enzymes
…beta-glucuronidase. The genetically modified,
…eta-glucuronidase *IMCSzyme* can hydrolyze
…le drug classes within 30 minutes with high
…ncy [2]. To ensure that the hydrolysis process
…plete and reproducible, the pH, temperature
…ration must be controlled and optimized for the
…e used.
…achieve the very low limits of detection necessary
…ug compounds and their metabolites, it is often
…sary to remove interfering matrix. Interferences
…e produced as a result of the hydrolysis procedure
…cur naturally from the biological nature of the
… samples themselves. Solid phase extraction (SPE)
…idely used, proven method for sample preparation
…ample clean-up of hydrolyzed urine samples in the
… of forensic analysis. Disposable Pipette Extraction
…X) was developed as an alternative to traditional
…, combining efficient and rapid extraction with
…ificantly reduced solvent consumption. DPX relies

S O L U T I O N S

GERSTEL

…The Completely
…w for the Hydrolysis
…e Samples by

…T I O N S

…RSTEL

…, 29201, USA

…omated, 96 well plate
…w including enzymatic
…described. A GERSTEL
…pled to an AB SCIEX
…a was used for a fast
…minutes), dilution and
…edure was applied to
…(e.g., opiates, opioids,
…llucinogens) in urine.
…loyed an ultra-pure
…olysis efficiencies of
…r the analytes tested.
…d the reproducible
…mples on the same
… %. Moreover, the
…ysis, injection and
…e than 200 samples

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…d using the
…zed such as
a reduction of the sample volume used from 1 to 0.5 mL serum and the use of smaller 1 mL format SPE cartridges. The analysis method has been validated according to GTFCh guidelines (Society of Toxicological and Forensic Chemistry). Limits of quantification below 1 ng/mL for THC and THC-OH, extraction efficiencies between 70 and 93 % and relative standard deviations between 3.3 and 10 % were achieved. The SPE system performs sample preparation in

Thank you for your attention!

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