Comprehensive Screen of Acidic/Neutral/Basic Drugs from Blood and Urine Featuring a Direct Elution to Analysis Technique via LC-MS/MS

UCT Part Numbers

SSHLD063
Styre Screen® HLD
60 mg, 3 mL Column

SPHACE5001-5
Select PH Buffer Pouches
100 mM Acetate Buffer pH 5.0

UASBETA-GLUC--50
50mL Abalonase™ Ultra
3X Concentrated Purified
β - glucuronidase Solution

SLPFPP100ID21-3UM
Selectra® PFPP HPLC Column
100 X 2.1 mm, 3 µm

SLPFPPGDC20-3UM
Selectra® PFPP Guard Column
10 X 2.0 mm, 3 µm

SLGRDHLDR
Guard Column Holder

CLINICAL FORENSICS

Summary:

Comprehensive screening is often referred to as general unknown or systematic toxicological analysis. It is in most cases the first line of defense when determining the presence of drugs of abuse. Because of this, it is imperative that the method of choice is robust, sensitive, and targeted to allow for the isolation of analytes of interest while simultaneously removing any interfering compounds. By combining a streamlined solid-phase extraction approach, featuring direct elution to analysis, with the use of LC-MS/MS and full screen QTrap methodology, both illicit and prescribed drugs can be accurately detected.

This featured application note exploits the highly retentive, universal nature of UCT's polymeric Styre Screen® HLD sorbent for the analysis of acidic, neutral, and basic compounds in blood and urine. The generalized protocol not only minimizes unnecessary sample preparation steps, but also it allows for the LC-MS friendly elution solvent to go directly into an autosampler vial for further analysis. HPLC separation was carried out using UCT's Selectra® PFPP column prior to detection by LC-MS/MS. The pentafluorophenylpropyl phase can undergo dipole–dipole, and pi–pi interactions, imparting unique selectivity and retention mechanisms to the column that distinguish it from a traditional biphenyl phase. Excellent absolute recoveries (71 - 118%) and relative standard deviations (RSD% ≤ 24.33%) were obtained.

Sample Pretreatment:

1. A) To 1 mL blood sample, add 100 mM Acetate buffer (pH 5.0) and appropriate amount of internal standard.
   B) To 1 mL of urine sample, add 1 mL of Abalonase™ Ultra Purified β-Glucuronidase working solution, vortex for 30 sec and heat at 65°C for 30 minutes. Allow sample to cool.
2. Vortex Samples for 30 seconds to mix. Sample pH should be 5.0 ± 0.5.
3. Centrifuge for 10 minutes at 3000 rpm

SPE Procedure:

1. CONDITION STYRE SCREEN®HLD EXTRACTION COLUMN:

   a) 1 mL CH_3OH

   b) 1 mL D.I. H_2O

2. APPLY SAMPLE: Load at 1 to 2 mL/minute

3. WASH COLUMN:

   a) 2 mL D.I. H_2O

   b) 2 mL 5% CH_3OH

   c) Dry column (5 minutes at full vacuum or pressure)

4. ELUTE:

   a) 1 mL of 0.1% Formic Acid in CH_3OH directly in autosampler vials

   b) Cap Vial and Vortex mix before analysis on LC-MS/MS

LC-MS/MS Parameters:

System: Shimadzu LC30AD w/ MS-8050
Column: Selectra® PFPP HPLC Column 100 X 2.1 mm, 3 µm
Guard Column: Selectra® PFPP Guard Column 10 X 2.0 mm, 3 µm
Column Temperature: 40 °C
Column Flow Rate: 0.3 mL/min
Injection Volume: 5 µL
Auto-sampler temperature: 10 °C

Gradient Program:		
Time (min)	% Mobile Phase A 5 mM Ammonium Formate with 0.1% Formic Acid in H_2O	% Mobile Phase B 5 mM Ammonium Formate with 0.1% Formic Acid in CH_3OH
0	100	0
8.0	0	100
10.0	0	100
10.1	100	0
15.0	100	0

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Absolute Recovery - Blood				
Analyte	25 ng/mL (n=3)	Rel. Std Dev (%)	80 ng/mL (n=3)	Rel. Std Dev (%)
3,4-Methylenedioxypyrovalerone	94%	3.94	96%	2.94
6-MAM	101%	3.45	94%	2.14
Alphahydroxy-Alprazolam	88%	4.45	92%	1.54
Alprazolam	94%	7.00	99%	3.46
Amitriptyline	107%	7.48	115%	5.78
Amphetamine	103%	3.91	96%	3.32
Atenolol	104%	3.55	100%	1.43
Baclofen	118%	8.69	100%	4.81
Benzoylecgonine (BE)	97%	4.87	101%	2.37
Bupivacaine	91%	2.70	87%	2.67
Buprenorphine	96%	3.25	89%	2.72
Cannabidiol (CBD)	86%	4.69	88%	2.79
Cannabinol (CBN)	95%	2.49	97%	2.96
Clonazepam	96%	1.11	98%	1.32
Cocaine	93%	4.13	94%	3.43
Codeine	97%	3.82	91%	3.18
Dextrorphan	97%	2.72	95%	2.31
Diazepam	95%	1.58	103%	2.82
EDDP	100%	2.97	108%	4.85
Fentanyl	96%	3.51	96%	2.57
Hydrocodone	100%	3.86	97%	1.95
Hydromorphone	97%	3.59	93%	1.50
Imipramine	99%	2.14	96%	3.71
Ketamine	98%	3.19	100%	3.06
Levorphanol	99%	2.39	96%	1.33
Lorazepam	101%	4.40	103%	3.46
MDEA	98%	2.84	102%	1.83
MDMA	99%	4.45	102%	2.86
Meperidine	97%	4.83	98%	3.31
Meprobamate	107%	3.75	103%	3.07
Methamphetamine	95%	3.95	96%	2.94
Methylphenidate	99%	3.39	102%	2.42
Midazolam	93%	4.91	97%	3.90
Morphine	99%	3.77	90%	1.73
Naltrexone	101%	4.78	96%	3.98
Nicotine	93%	3.43	93%	1.81
Norbuprenorphine	97%	4.99	96%	2.55
Norcodeine	95%	7.39	98%	3.25
Nordiazepam	93%	1.20	95%	2.91
Norketamine	98%	5.03	100%	1.83
Nortriptyline	107%	7.11	108%	7.13
Oxazepam	101%	7.47	100%	3.88
Oxycodone	95%	3.26	93%	1.85
Oxymorphone	99%	1.78	92%	2.10
PCP	100%	2.01	104%	1.54
Phentermine	98%	3.34	93%	2.85
Ritalinic Acid	103%	8.81	96%	7.28
Temazepam	95%	2.85	95%	1.59
THC	101%	10.83	110%	6.94
THC-COOH	107%	12.71	108%	9.01
THC-OH	99%	6.24	105%	7.37
Tramadol	97%	3.40	99%	1.30
Zolpidem	94%	3.10	90%	4.00

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Absolute Recovery - Urine				
Analyte	25 ng/mL (n=3)	Rel. Std Dev (%)	80 ng/mL (n=3)	Rel. Std Dev (%)
3,4-Methylenedioxypyrovalerone	92%	1.69	97%	1.44
6-MAM	98%	1.62	99%	1.51
Alphahydroxy-Alprazolam	92%	3.3	97%	3.37
Alprazolam	87%	2.4	92%	6.16
Amitriptyline	96%	2.71	100%	1.50
Amphetamine	96%	1.63	99%	1.59
Atenolol	99%	3.38	98%	2.33
Baclofen	99%	3.21	95%	1.65
Benzoylecgonine (BE)	91%	1.00	97%	2.04
Bupivacaine	96%	0.80	97%	0.44
Buprenorphine	101%	1.95	98%	2.05
Cannabidiol (CBD)	84%	8.80	76%	24.33
Cannabinol (CBN)	69%	6.02	71%	23.61
Clonazepam	95%	2.51	97%	2.50
Cocaine	93%	2.59	96%	2.19
Codeine	99%	0.47	97%	1.16
Dextrorphan	95%	2.02	95%	1.49
Diazepam	94%	2.73	103%	0.77
EDDP	94%	1.99	101%	2.52
Fentanyl	97%	1.30	101%	1.37
Hydrocodone	92%	1.21	93%	1.42
Hydromorphone	100%	0.50	100%	1.05
Imipramine	94%	6.00	94%	0.93
Ketamine	91%	2.34	97%	1.72
Levorphanol	97%	0.36	95%	1.70
Lorazepam	94%	3.16	96%	2.85
MDEA	98%	3.06	97%	3.21
MDMA	93%	2.23	97%	0.64
Meperidine	93%	2.39	96%	1.85
Meprobamate	93%	8.29	96%	4.02
Methamphetamine	93%	2.07	99%	1.80
Methylphenidate	93%	2.51	95%	2.40
Midazolam	94%	4.81	100%	2.07
Morphine	101%	1.71	99%	1.42
Naltrexone	90%	0.62	94%	1.24
Nicotine	93%	1.32	95%	1.40
Norbuprenorphine	98%	2.06	97%	1.19
Norcodeine	98%	1.48	95%	3.21
Nordiazepam	91%	1.68	94%	2.73
Norketamine	92%	1.94	95%	2.81
Nortriptyline	97%	2.56	96%	2.00
Oxazepam	98%	3.05	98%	1.67
Oxycodone	84%	0.96	85%	1.05
Oxymorphone	102%	1.96	100%	0.99
PCP	92%	4.60	96%	2.11
Phentermine	95%	1.93	97%	1.72
Ritalinic Acid	104%	2.85	104%	2.38
Temazepam	91%	3.45	97%	0.51
THC	89%	6.07	98%	7.34
THC-COOH	80%	2.82	93%	11.39
THC-OH	90%	5.69	97%	3.80
Tramadol	97%	0.92	99%	0.84
Zolpidem	91%	2.95	94%	1.60

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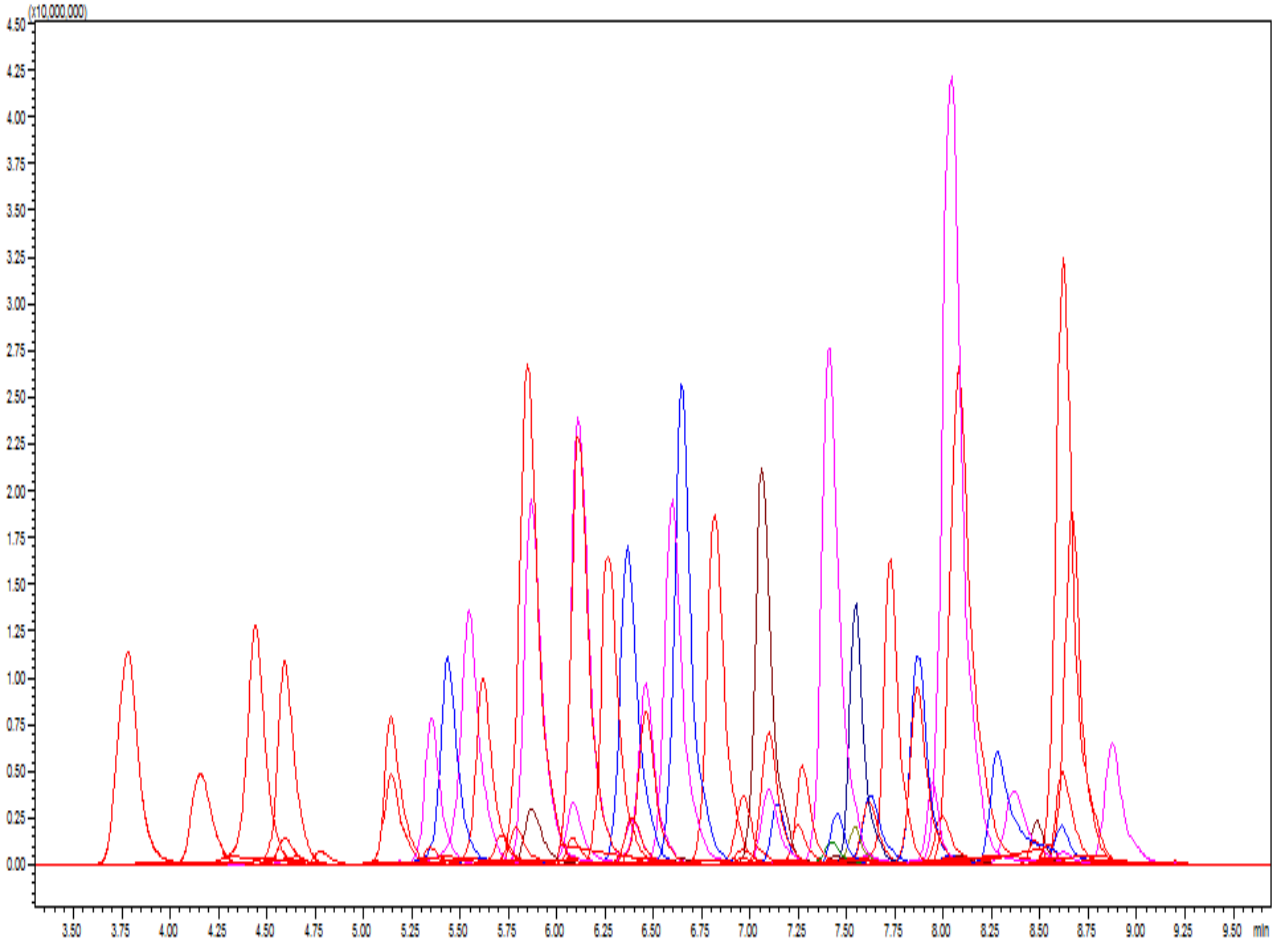

Figure 1: Chromatogram of 100 ng/mL Extracted Standard

Conclusion:

UCT's new universal screening method is designed for the efficient extraction of Acidic/Neutral/Basic Drugs from both blood and urine. The use of the highly-crosslinked, polymeric Styre Screen® HLD sorbent chemistry allows for increased analyte sensitivity, enhanced specificity for selected functional groups, low organic solvent consumption, ease of automation, and optimizied chromatographic resolution. It features a direct elution to analysis approach where the LC-MS friendly elution solvent collects directly into an autosampler vial for further analysis. By eliminating several traditional steps within the featured protocol, this minimizes overall sample preparation time and cost and reduces the risk for error and subsequent sample rework.

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