A Novel Drug Screening Protocol for Acidic, Basic, and Neutral Drugs in Hydrolyzed Urine using Supported Liquid Extraction prior to LC-MS/MS Analysis

Victor Vandell±, Dan Menasco±, Elena Gairloch±, Lee Williams¥, Paul Roberts¥

±Biotage, 10430 Harris Oaks Blvd., Charlotte, North Carolina, 28269, USA

¥Biotage GB Limited, Dyffryn Business Park, Cardiff, CF82 7TS, UK.

INTRODUCTION

The ability to extract a broad range of different drugs from a biological matrix allows for the expedited analysis of a patient sample using LC-MS-MS. Typically small molecules are extracted from matrices like urine based on their polarities. A fast and reliable sample preparation method that could be implemented to extract drugs of different polarities from urine could be used as a screening tool to quickly identify the presence of illicit drugs in patient samples using LC-MS-MS.

This poster will demonstrate the utility of supported liquid extraction for the extraction of over 30 different acidic, basic and neutral drugs in urine prior to LC-MS-MS.

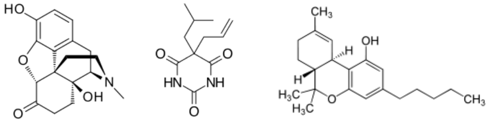

Figure 1. *From left-to-right: structures of Oxymorphone, Butalbital and Tetrahydrocannabinol*

SAMPLE PREPARATION TECHNOLOGY

ISOLUTE® Supported Liquid Extraction

Supported Liquid Extraction (ISOLUTE® SLE+) is a modified diatomaceous sorbent that has a high adsorption affinity for aqueous solutions and the analytes solubilized in the aqueous solution. The sample preparation methodology of Supported Liquid Extraction (SLE) works on the same chemical premises as a liquid-liquid extraction experiment, but is carried out on a solid phase. The development of a sample preparation method using SLE will enable the user to load the aqueous matrix, containing the target analytes and interferences onto a pre-packed SLE column or 96 well plate. Any water-immiscible organic solvent (e.g. dichloromethane, ethyl acetate, diethyl ether, etc.) can then be gravity fed through the column or well to extract and collect target analytes with endogenous interferences retained on the sorbent. Our recommended sample preparation technology allows for the expeditious recovery and isolation of the desired analyte from endogenous interferences such as proteins, lipids, phospholipids, salts and other unwanted components that attenuate chromatographic separation and subsequent MS detection of the desired analytes.

EXPERIMENTAL PROTOCOL

Reagents

HPLC grade Water, Methanol, Ethyl Acetate, Acetone, Acetonitrile, Isopropanol, Ammonium Hydroxide and Formic Acid were purchased from Sigma-Aldrich Co. (Atlanta, GA.). The negative urine was collected from drug free donors.

ISOLUTE SLE+ Sample Prep: Hydrolyzed Urine

Negative urine was spiked with drug standards at concentration ranges from 1.25 ng/mL to 100 ng/mL. An aliquot of 100 µL of spiked urine was enzymatically hydrolyzed using B-glucoronidise. The urine was pretreated with 200 µL of water. The total sample was loaded onto ISOLUTE® SLE+ 400 well plate. The sample was allowed to equilibrate on the sorbent for 5 minutes. The analytes were extracted with 1400 µL of dichloromethane/isopropanol (90:10). The extracted sample was dried down on SPE-Dry 96 at 40 C and reconstitute in mobile phase.

Liquid Chromatography

Instrument: Agilent 1260 HPLC (Santa Clara, CA.)
Column: Restek Raptor Biphenyl column (3.0 µm, 50 x 2.1 mm)
Mobile Phase: A: 0.1% FA (aq) **B:** 0.1% FA in MeOH.
Flow Rate: 0.5 mL/min
Injection Volume: 15-20 µL
Column Temperature: 50 ˚C

Table 1. *Gradient parameters for positive and negative analytes.*

Time (min)	% Mobile Phase B Positive ions	% Mobile Phase B Negative ions
0	60	60
0.2	60	60
1.5	90	100
2.5	90	100
2.6	60	60
6.0	60	60

Mass Spectrometry

Instrument: Sciex 4000 Q-Trap (Foster City, CA.) equipped with a Turbo Ionspray® interface for analysis.
Ion Source Temperature: 500 °C

Table 2. *SCIEX 4000 Q-Trap parameters and analyte retention times.*

Analyte	MRM Transition	Declustering Potential (V)	Collision Energy (CE)	Retention Time (mins)
Codeine	300.2>215	40	25	1.11
Hydrocodone	300>199	40	25	1.37
Oxycodone	316>241	40	30	1.28
Norcodeine	286.1>225	40	25	0.94
Oxymorphone	302>227	40	30	0.59
6-acetyl codeine	342.4>215	40	30	2.99
Morphine	286.1>165	40	30	0.90
Alprazolam	308.8>280.5	40	30	3.96
Clonazepam	315.8>269.8	40	30	3.66
Diazepam	284.9>154	40	30	4.05
Flunitrazepam	313.9>267.9	40	30	3.87
Nitrazepam	282.1>180	40	30	3.67
Oxazepam	288>242	40	30	3.64
Temazepam	300.9>255	40	30	3.88
Dextromethorphan	272>215	40	30	3.32
Buprenorphine	468.2>396.2	55	55	3.23
Norbuprenorphine	414.1>83.1	55	55	3.09
Fentanyl	337>188	40	30	3.23
EDDP	278>234	40	30	3.39
benzoylecgonine	290>168	40	30	2.87
THC	315.2>193	40	28	4.17
Naltrexone	342>323.8	40	30	1.28
hydromorphone	286.2>185.1	40	30	0.58
Propoxyphene	340.3>266.3	40	30	3.29
Pentazocine	286.3>69.1	40	30	3.08
Amphetamine	136>91	40	30	1.88
Norfentanyl	233.1>84	40	35	1.02
MDEA	208.1>105.1	40	45	2.33
Butalbital	223.1>180.1	-50	-22	3.47
Pentobarbital	225.1>182	-50	-18	3.63
Phenobarbital	231.1>188	-50	-18	3.36
Secobarbital	237.1>193.9	-50	-23	3.72
THC-COOH	343.2>299	-50	-35	4.6
d_3-11-OH THC	332.2>314	-50	-30	4.42

RESULTS AND DISCUSSION

A total of 34 drug analytes with varied polarities were successfully extracted from a small volume of hydrolyzed urine using Supported Liquid Extraction (SLE+). The analytes are classified as positive, negative and neutral drugs which are typically extracted separately using both cation and anion exchange solid phase extraction. A list of the extracted analytes along with the mass spectrometry optimized parameters and the chromatographic conditions are shown in **Table 1 and Table 2,** respectively. The analytes were detected in both positive and negative mode as a function of analyte ionization potentials. The extracted samples were injected twice to analyze for both positive and negative ions. Sample extracted ion chromatograms for both positive and negative mode are shown in **Figure 2.**

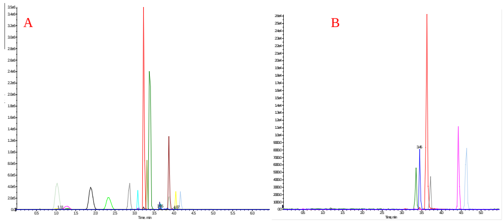

Figure 2. *Extracted ion chromatograms for positive (A) and negative (B) analytes.*

Analyte Recovery Determinations

A 1000 ng/mL drug analyte stock solution was prepared from Cerilliant standards in methanol. A target volume of 100 µL of negative urine was then spiked at the three different concentration levels of 25, 50 and 100 ng/mL. The spiked urine was then pretreated and extracted as per protocols mentioned above. **Figure 3** shows typical recovery data observed for analytes extracted from urine across the dynamic concentration range.

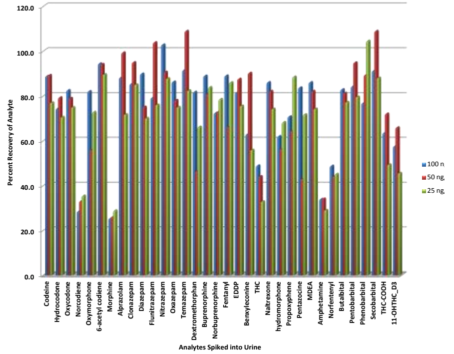

Figure 3. *Percent recoveries for drugs at different concentration levels.*

The screening protocol on the SLE+ yields > 65% recoveries for most of the analytes. Further optimization could be employed by using either an acidic or basic pretreatment step (instead of water) which would boost recoveries for the acidic or basic drugs, respectively.

Matrix Effects/Limit of Detection

Suppression and enhancement was observed for the analytes in urine. **Figure 4** shows a plot of the calculated percent suppression and enhancement for all of the analytes. Matrix suppression/enhancement was < 30% for most of the analytes.

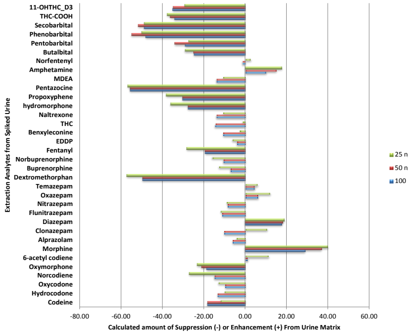

Figure 4. *Plot of observed matrix suppression and enhancement across a dynamic concentration range from 25, 50 and 100 ng/mL*

The measured recoveries and the matrix effects did not prevent the analytes from being detected at the lower concentration levels ranging from 1.25 to 5.0 ng/mL. An acceptable limit of detection was defined as a signal to noise ratio of 3:1 or better. The screening method was found to be effective supported by the observed limit of detection (LOD). Figure 5 shows a tally of the LODs observed for each of the analytes.

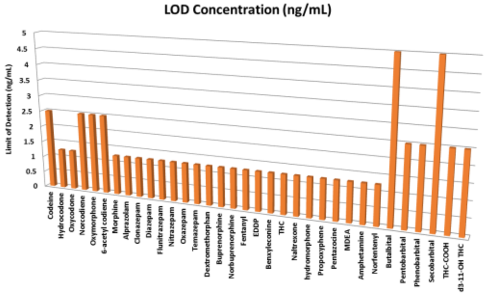

Figure 5. *Plot of observed limits of detection for each drug*

CONCLUSION

- A viable screening method for positive, negative and neutral analytes can be facilitated using the Supported Liquid Extraction (SLE) sorbent.
- While the recoveries are varied on the sorbent, sufficient detection limits can be achieved for analytes amenable to being extraction on the SLE+ sorbent.
- The same extraction screening protocol could be used to subsequently confirm the analyte concentration via a separate injection or possibly from the same injection to maximize work flow.

www.biotage.com

MSACL, 2016 Palm Springs
Part Number: P144