Low Cost Entry for Automation in Laboratory Settings

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Introduction

In the forensic world, many cases are decided based on the laboratory test results. Yet, while forensic testing is critically important for legal demands, many labs remain constrained by tight budgets and limited resources. Purchasing, replacing, or expanding capacity with expensive liquid handlers or other laboratory automation systems are economically impossible for many labs. By considering options that allow a more modular approach, with "Islands of Automation", labs can afford to automate their processes and achieve higher throughput. This option is affordable, reliable and applicable to any matrix, including urine and whole blood.

Methods

Reagents and Materials

Standards

Blank whole blood was purchased from UTAK laboratories (Valencia, CA). All standards were purchased from Cerilliant (Round Rock, TX). HPLC grade water and methanol (MeOH) were purchased from Sigma Aldrich (St. Louis, MO) in addition to reagent grade dichloromethane (DCM), formic acid, phosphoric acid (H_3PO_4), isopropanol, and ammonium hydroxide (NH_4OH). EVOLUTE® EXPRESS CX (30 mg bed) extraction plate (601-0030-PX01), and Collection Plate, 2 mL Square (121-5203) Biotage®.

Instruments

Biotage® Extrahera™ (Figure 1) is an automated Sample Preparation Platform equipped with an 8 channel pipetting head, with a built in positive pressure processing functionality. The system is interconvertible between 4 and 8 channel pipetting into 24 (6 x 4 arrangement) columns or 96-well plates, respectively. Biotage® PRESSURE+ 96 Positive Pressure Manifold (PPM-96) (Figure 2). Biotage® SPE Dry 96 (SD-9300-DHS-NA) (Figure 3), and Biotage® ACT Plate Adapter (Figure 4) (414355SP) to prevent cross contamination from evaporation were supplied by Biotage. Analysis was conducted using a Nexera X2 HPLC system (Shimadzu, Columbia, MD) coupled to a 5500 mass spectrometer (Sciex, Framingham MA).

Figure 1. Biotage® Extrahera™ More Processing – Less Programming.

Figure 2. *Biotage® PRESSURE+ 96*

Figure 3. *Biotage® SPE Dry 96*

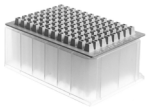

Figure 4. *Biotage® ACT Plate Adapter*

Sample Preparation, pretreatment and Extraction

Whole Blood Sample Preparation

25 mL of whole blood was spiked with 20 ng/mL of drugs of abuse compounds. These DOA compounds included drugs from multiple drug classes: anticonvulsants, benzodiazepines, opioids, antidepressants, stimulants, hallucinogens, and antipsychotics. 100 µL of DOA spiked whole blood was added initially to each well of the 96 deep well sample plate. Then 200 µL of 4% phosphoric acid were added to each well.

Extraction Procedures

Extractions was performed both manually and automated using the Biotage® PRESSURE+ 96, Biotage® Extrahera™, respectively. the Biotage® SPE Dry 96 was used for evaporation. 300 µL of the pretreated sample was used for the solid phase extraction method using EVOLUTE® EXPRESS CX 30 mg. The extraction procedure is shown in table 1 for the automated extraction & table 2 for the manual extraction.

Automated Extraction

Step	Volume (µL)	Solvent	Time (min)	Pressure (bar)
Sample Pretreatment	200	4% H_3PO_4	4	N/A
Conditioning	1000	Methanol	5	2
Equilibration	1000	4% H_3PO_4	5	2
Sample Load	300	Sample	7	0.5
Wash #1	1000	4% H_3PO_4	5	0.8
Wash #2	1000	50% Methanol	5	0.8
Plate Dry	N/A	N/A	2	5
Elution	2 x 600	DCM/IPA/NH_4OH [78:20:2]	10	0.5
Plate Dry	N/A	N/A	20	5
Recon step	100	Mobile phase (5:95)	2	
Total Time			65	

Table 1. Extrahera™ Automated Steps Processing Parameters .

Manual Extraction

Step	Volume (µL)	Solvent	Time (min)	Pressure (psi)
Sample Pretreatment	200	4% H_3PO_4	10	N/A
Conditioning	1000	Methanol	7	2-4
Equilibration	1000	4% H_3PO_4	7	2-4
Sample Load	300	Sample	10	2-4
Wash #1	1000	4% H_3PO_4	7	2-4
Wash #2	1000	50% Methanol	7	2-4
Plate Dry	N/A	N/A	2	20
Elution	2 x 600	DCM/IPA/NH_4OH [78:20:2]	10	2-4
Plate Dry	N/A	N/A	20	20
Recon step		Mobile phase (5:95)	4	
Total Time			85	

Table 2. *Manual Extraction processing steps Parameters.*

Dry Down and Sample Reconstitution:

Elution solvent was collected into a collection plate. All samples were evaporated to dryness at 40°C with 20 L/min of nitrogen using a Biotage® SPE Dry 96. Extracts were then reconstituted with 100 µL of 95:5 mobile phase A/mobile phase B and analyzed via LC-MS/MS.

Chromatography Parameters

The LC gradient started with 95% aqueous and gradually decreased to 5% aqueous over an 8.5-minute total run time. This allowed for full separation of all compounds in the panel. Figure 5 show the extracted chromatograms for the 55 compound panel.

UPLC	Parameter
Column	Restek Raptor Biphenyl 2.7 µm, 100 x 3.0 mm
MPA	0.1% Formic Acid (aq)
MPB	0.1% Formic Acid in MeOH
Flow Rate	0.45 mL/min
Column Temp.	40 °C
Sample Temp.	20 °C
Injection Volume	10 µL

Table 3. Shimadzu Nexera X2 UPLC.

Mass Spectrometry Parameters

Instrument: A SCIEX 5500 triple quadrupole Mass Spectrometer with Turbo Ionspray® Ion interface (Foster City, CA) was used. Optimized source parameters are shown in table 3. Detection window for sMRM was set at 48 seconds with target scan time at 3.0 seconds.

Ionization Spray Voltage	+1500(V)	CAD	Medium
Source Temp	600 °C	GS1	55
Curtain	30 (V)	GS2	65

Table 4. SCIEX 5500 Triple Quadrupole ESI (+/-) Turbo Ionspray® Source Parameters.

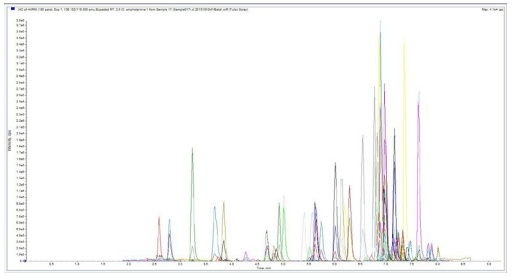

Figure 5. *Extracted Ion Chromatogram for analytes in the panel.*

Results

Extraction Timing

From Tables 1&2, the observed total time for the automated extraction was 43 min, in addition to 22 min for evaporation and reconstitution. The manual extraction took 65 min, in addition to the 24 min evaporation and reconstitution.

Extraction Consistency

Figure 6 shows area counts of two analytes, ketamine and amitriptyline, for 96 injections at a concentration of 20 ng/mL. The manual extraction showed some variability and inconsistency. On the other hand, the benchtop automated system, Biotage® Extrahera™, provided a more reliable and reproducible extraction in comparison to the manual process, exhibiting good repeatability and consistency between samples.

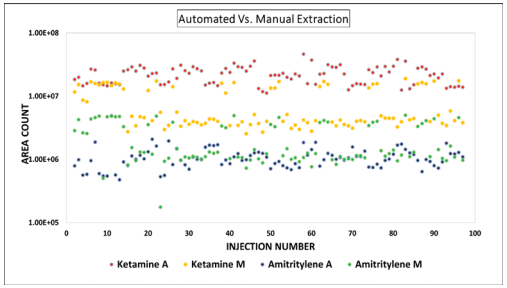

Figure 6. *Variations in area count seen for compounds manually extracted Vs. automation.*

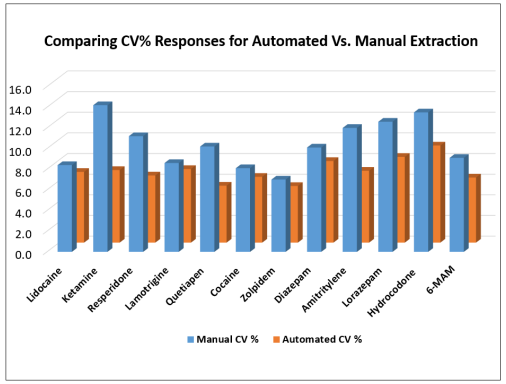

Figure 7. *Variations in responses for 12 analytes at 20 ng/mL from different classes using both manual and automated extraction techniques.*

Conclusions

- **The Biotage® Extrahera™ offers a reliable and rugged option for pretreatment, mixing, pipetting and processing sample extractions.**
- **By implementing the Extrahera™ in our laboratory workflow, we decreased extraction time by almost 25% and minimized the exposure to unhealthy chemicals.**
- **Automation of the SPE method was found to produce more reliable and consistent results for the spiked samples vs. those from the existing manual procedure.**

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