AUTOMATION OF BIOANALYTICAL SAMPLE EXTRACTION TECHNIQUES USING ANDREW+™ PIPETTING ROBOT WITH THE EXTRACTION+ CONNECTED DEVICE

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INTRODUCTION

Bioanalytical sample preparation methods range from simple techniques such as, protein precipitation to more targeted and specialized techniques like solid phase extraction (SPE) or immunoaffinity purification (IP). Generally, the simpler techniques have wider applicability and require minimal method development with a trade-off of limited cleanliness, specificity, and sensitivity. One of the major challenges of bioanalytical sample preparation in modern laboratories is to maximize lab efficiency supporting many bioanalytical sample preparation techniques, with little to no method develop, whilst achieving consistent results from day-to-day, user-to user, and lab-to-lab. Automating the various sample preparation workflows can minimize this variability while freeing up scientists for other tasks.

In this work, the Andrew+ Pipetting Robot configured with the Extraction+ Connected Device was used to fully automate the sample preparation and extraction of the therapeutic drug, apixaban from plasma. The easy to use, web-based OneLab™ Software enabled rapid sample extraction method creation and implementation for the most common bioanalytical sample preparation techniques including: protein precipitation (PPT), PPT with phospholipid (PL) removal, solid-supported liquid extraction (SSLE), reversed-phase (RP) SPE, RP-SPE with PL removal, and mixed-mode SPE. For each extraction method, all pipetting steps, sample extraction vacuum settings, waste disposal, and final collection was fully automated on Andrew+ configured with with Extraction+. Apixaban extraction recovery and matrix effects were used to compared extraction performance and cleanliness, across the extraction techniques, while standard curve and QC analytical performance were used to assess overall method performance.

EXPERIMENTAL METHODS

Automation
The Andrew+ Pipetting Robot configured with the Extraction+ Connected Device, controlled by the OneLab Software, was used to develop and execute all sample extraction methods. (Figures 1 & 2) A representative OneLab protocol visualization, system component designation, and deck layout for sample extraction are shown in Figure 3.

Solution Preparation
The analyte, apixaban was obtained from Cerilliant, while isotopically labeled apixaban (13C-d3), sourced from Cayman Chemicals, was used as internal standard. Concentrated stock solutions of apixaban and its internal standard were prepared in methanol. These solutions were then used to prepare standard curve and quality control (QC) samples in plasma.

Sample Extraction Techniques
The sample extraction techniques, protocols, and extraction product with ordering information are shown in Figure 4. Manufacturers extraction protocol guidelines for each extraction technique/product were followed, including sample diluents, suggested wash and elution solutions, and suggested volumes for the wash and elution steps.

Analytical Detection & Quantitation
LC-MS/MS detection and quantification of apixaban extracted samples was performed using a Waters Xevo™ TQ-XS tandem quadrupole mass spectrometer (ESI+). Chromatographic separation was achieved using an ACQUITY™ I-Class PLUS UPLC™ system and an ACQUITY UPLC BEH™ C_{18}, 1.7 μm, 2.1 x 50 mm column. Mobile phases A and B consisted of 0.1% formic acid in water and acetonitrile, respectively. A linear gradient from 5-100% B over 4.0 minutes was used at a flow rate of 0.5 ml/min.

Scan to be directed to the Andrew Alliance section of Waters.com

AUTOMATED SAMPLE PREPARATION AND EXTRACTION

ANDREW+ PIPETTING ROBOT CONFIGURED WITH THE EXTRACTION+ CONNECTED DEVICE

1. Andrew+ Pipetting Robot
2. Microplate Gripper
3. Extraction+ Vacuum Pump
4. Waste Container
5. SPE Cartridges in a Rack
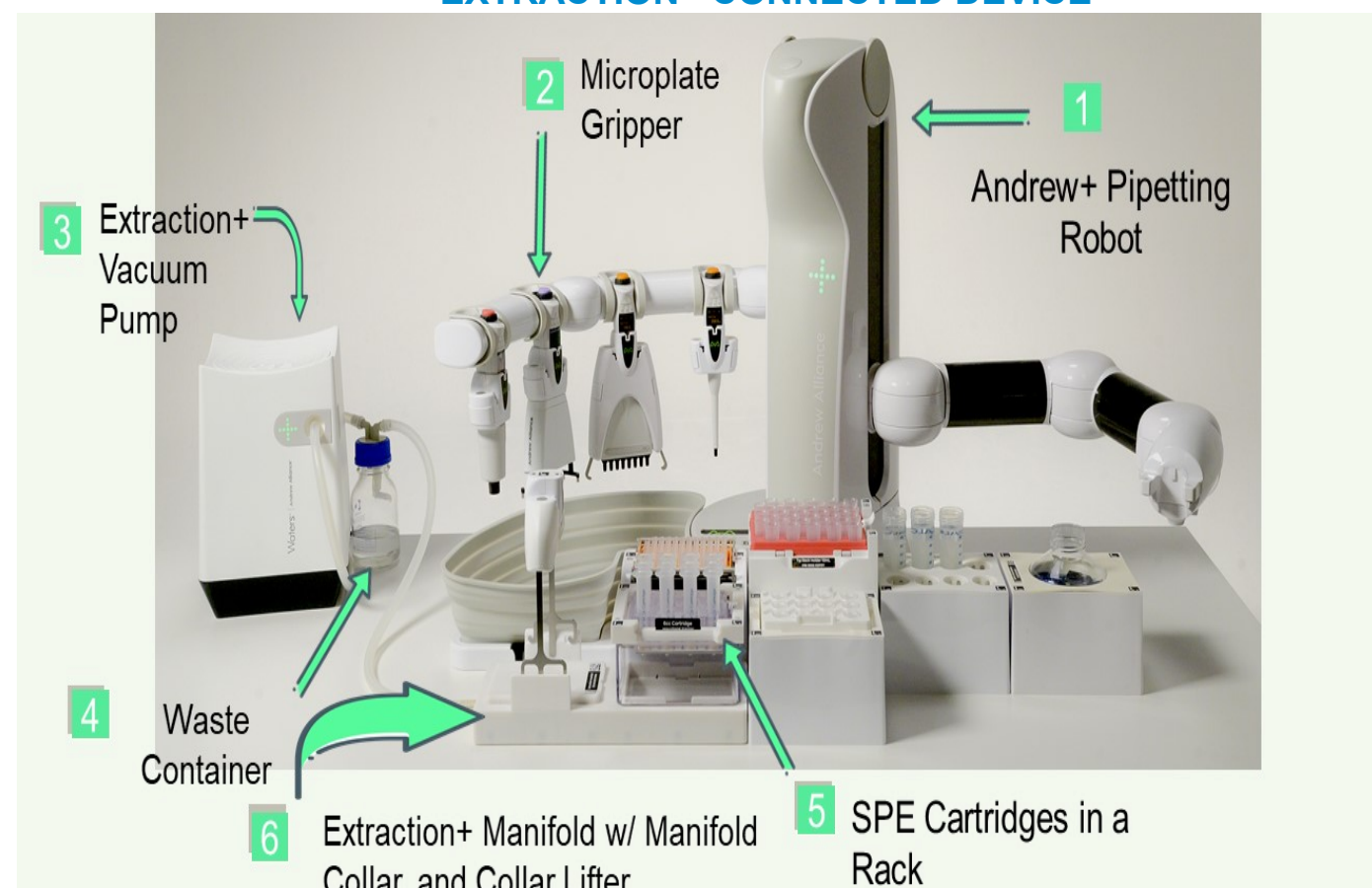6. Extraction+ Manifold w/ Manifold Collar, and Collar Lifter

Figure 1. The Andrew+ Pipetting Robot configured with Extraction+ Connected Device used for sample preparation and extraction.

SAMPLE EXTRACTION PROTOCOLS

PPT: Waters Sirocco Plate (p/n 186003873)

1	PRECIPITATION	• Add 300 μL ACN to well of Sirocco Plate • Add 100 μL plasma to ACN
2	VORTEX	• Vortex for 30 seconds (Off deck)
3	ELUTE	• Elute with 5 psi vacuum for 5 minutes.

PPT Phospholipid Removal: Waters Ostro™ Plate (p/n 186005518)

1	SAMPLE	• Add 200 uL plasma to Ostro well
2	PRECIPITATION	• Add 600 μL ACN with 1% FA to plasma • Aspirate 6x to mix
3	ELUTION	• Elute under vacuum for 3 minutes at 5 psi

SSLE: Analytical Sales & Services Diatomaceous Earth Plate (p/n 96260-1)

1	DILUTION	• Dilute 200 μL sample 1:1 with water
2	LOAD	• Load 200 μL of diluted sample to SSLE plate • Apply low vacuum for 3 sec. and wait 5 mins.
3	ELUTE	• 2 x 500 μL of MTBE – Wait for 5 minutes • Apply high vacuum for 30 sec
4	EVAPORATE	• Evaporate to dryness (Off deck)
5	RECON	• Recon. with 200 μL of 97:2:1 Water:ACN:FA

Reversed-Phase SPE: Waters Oasis™ HLB Plate (p/n WAT058951)

1	DILUTION	• Dilute with 600 μL plasma 1:1 with 4% H_3PO_4
2	LOAD	• Load 1000 μL ptx. sample on Oasis HLB plate
3	WASH	• 1 mL of 95:5 Water:MeOH
4	ELUTE	• 2 x 250 μL MeOH
5	DILUTION	• Dilute with 500 μL water

Reversed-Phase SPE PL Removal : Waters Oasis HLB PRiME Plate (p/n 186008054)

1	SAMPLE DILUTION	• Add 600 μL plasma to well of collection plate • Dilute with 600 μL of 4% H_3PO_4
2	LOAD	• Load 1000 μL pretreated sample onto Oasis PRiME HLB plate
3	WASH	• 1 mL of 95:5 Water:MeOH
4	ELUTE	• 2 x 250 μL 90:10 ACN:MeOH
5	DILUTION	• Dilute with 500 μL water

Mixed-Mode SPE: Waters Oasis MCX Plate (p/n 186002482)

1	SAMPLE DILUTION	• Add 600 μL plasma to collection plate • Dilute with 600 μL of 4% H_3PO_4
2	LOAD	• Load 1000 μL ptx sample onto MCX plate
3	WASH	• 1 mL of 2% formic acid in water
4	ELUTE	• 2 x 250 μL MeOH
5	DILUTION	• Dilute with 500 μL water

Figure 4. Sample extraction protocols used for apixaban plasma extraction.

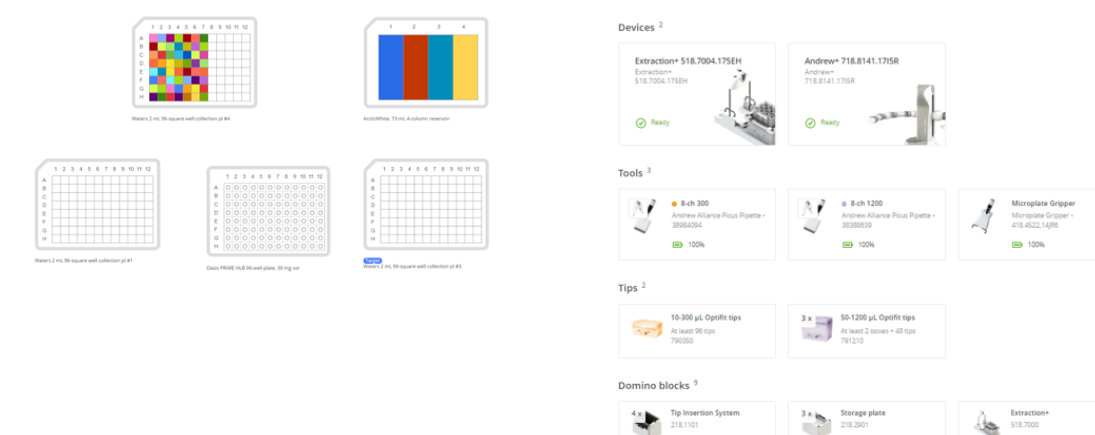### EXTRACTION+ CONNECTED DEVICE SOLUTION

96-Well Plate or Cartridge Sample Extraction

Figure 2. The Extraction+ system including the Extraction+ connected vacuum pump, flow-through waste container, Extraction+ manifold with the manifold collar, the integrated collar lifter, and SPE cartridges (1, 3 and 6 cc) with the corresponding adaptors.

ANDREW+ PIPETTING ROBOT ONELAB METHOD

OneLab Protocol Visualization

Andrew+ System Components: Dominos, Electronic Pipettes & Tips

Andrew+ Deck Layout with the Extraction+ Connected Device for 96-well Extraction Plate Formats

Andrew+ Deck Layout with Extraction+ Connected Device
— Oasis HLB SPE Macro 96-well Plate —

Position	Dominos and connected devices w/ Labware
1 to 4	Tip Insertion System Domino
5	Extraction+ Connected Device w/ Oasis HLB 96-well macro plate
6 to 8	Storage Plate Domino
9	Deepwell Microplate Domino w/ 6-column reagent reservoir

Figure 3. Representative Andrew+ Pipetting Robot with Extraction+ Connected Device and OneLab Software method with protocol visualization, system components identification, and representative Andrew+ deck layout.

RESULTS

Table 1. Apixaban sample preparation and LC-MS quantitative performance for all sample extraction techniques automated on Andrew+ with Extraction + using OneLab Software. All techniques achieved excellent linearity (>0.99), accuracy (± 15 %) and precision (± 15 %).

Apixaban Quantitative Performance Extracted from Plasma							Sample Extraction Performance	
	Calibration Curve Performance							
Extraction Technique	Dynamic Range (ng/mL)	Linear Fit (R^2)	Weighting	% Accuracy Range (N=3)	%RSD Range (N=3)	Low,Mid, High QC % Accuracy Range	% Recovery	% Matrix Effects
PPT (Sirocco)	2-500	0.993	$1/x^2$	92.4-105.5	0.2-3.7	92.6-97.9	85.4 (4.6)	35.1 (4.1)
PPT with PL removal (Ostro)		0.997		97.6-103.4	0.2-14.9	94.6-99.9	77.0 (2.5)	27.5 (4.0)
SSLE		0.993		86.0-109.1	0.4-6.1	90.8-97.0	53.2 (19.9)	-21.8 (16.7)
RP SPE (HLB)		0.996		93.4-109.9	0.7-2.4	89.9-100.9	96.2 (9.0)	-41.0 (0.8)
RP SPE with PL removal (Oasis PRiME HLB)		0.986		87.9-110.8	0.3-3.8	93.3-103.0	81.1 (3.2)	-13.6 (14.6)
Mixed Mode SPE (Oasis MCX)		0.996		94.2-109.7	0.2-3.7	96.1-105.7	104.3 (6.2)	2.4 (0.4)
Mixed Mode (Oasis WAX)	NA						100.1 (2.2)	-19.8 (1.8)

CONCLUSION

This work highlights successful automated sample preparation and extraction for various bioanalytical LC-MS extraction workflows. Use of the Andrew+ Pipetting Robot configured with the Extraction+ Connected Device greatly simplified and streamlined sample extraction, with no required method development. Automation of these bioanalytical sample preparation and extraction techniques, maximizes lab productivity, reduces errors, and ensures overall analytical method performance.