Easy and Robust Automated Sample Preparation and Extraction for LC-MS/MS Bioanalytical Workflows

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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 (SLE), 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+ Robot configured with the Extraction+ Connected Device. 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.

EXPERIMENTAL METHODS

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™ UPLC™ I-Class PLUS 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.

ANDREW+ PIPETTING ROBOT CONFIGURED WITH THE EXTRACTION+ CONNECTED DEVICE

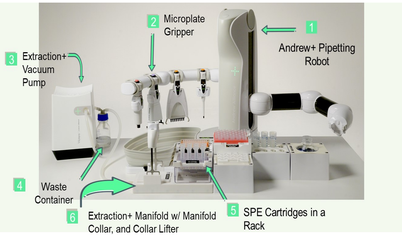
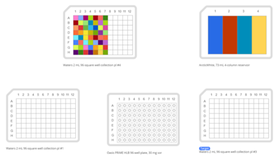


Figure 1. The Andrew+ Pipetting Robot configured with Extraction+ Connected Device used for sample preparation and extraction.

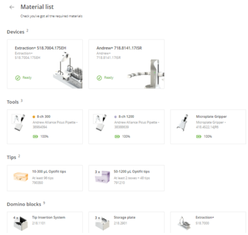
Figure 2. The Extraction+ system including the connected vacuum pump, flow-through waste container, Extraction+ manifold with the manifold collar, the integrated collar lifter, and SPE cartridges (1, 3 and 6 cc) with the corresponding adaptors.

Figure 3. Andrew+ Pipetting Robot OneLab Method

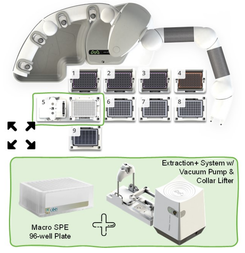
OneLab Protocol Visualization



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

SAMPLE EXTRACTION PROTOCOLS

Figure 4. Extraction protocols for all sample preparation products.

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 µL 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 mins at 5 psi

SLE: 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 SLE 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 1 mL 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

RESULTS

Figure 5. Recoveries and matrix effects for Apixaban using the automated sample preparation methods shown in Figure 4.

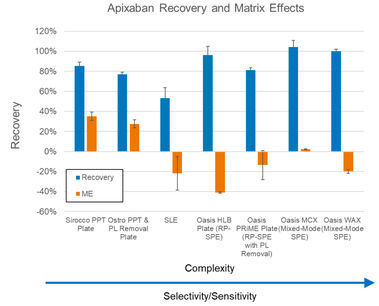


Table 1. Apixaban sample preparation and LC-MS quantitative performance for all sample extraction techniques automated on Andrew+ Robot with Extraction+ Connected Device using OneLab Software. All techniques achieved excellent linearity (>0.99), accuracy (± 15 %) and precision (± 15 %).

Apixiban Quantitative Performance Extracted from Plasma							
	Calibration Curve Performance					Sample Extraction 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)

CONCLUSIONS

- **Greatly simplified and streamlined sample extraction, with no method development required**
- **Library Methods minimize protocol development work**
- **Easy qualitative comparison of multiple sample preparation techniques**
- **Accurate and precise quantitative results easily meet bioanalytical regulatory requirements**
- **Lab productivity is maximized , reducing errors, and ensuring overall analytical method performance.**

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