AUTOMATED SAMPLE PREPARATION PLATFORM FOR BIOANALYTICAL SPE METHOD DEVELOPMENT & OPTIMIZATION USING ANDREW+™ PIPETTING ROBOT

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INTRODUCTION

Bioanalysis, or the analysis of drugs and their metabolites extracted from biological fluids, are key assays used in support of drug discovery, development, clinical and forensic toxicology research. Bioanalytical method development focuses on three major aspects: 1- detection, 2- separation and 3- extraction of analytes from various biomatrices. Common sample preparation techniques for bioanalysis include: dilution, protein precipitation (PPT), liquid-liquid extraction (LLE), and solid phase extraction (SPE). The choice of sample preparation technique is driven by throughput, sensitivity, selectivity, and robustness requirements of the analyte target.

Regardless of the extraction technique, development and optimization of the sample extraction step is often time consuming and complex, with the need to optimize all steps of the protocol. Implementing fully-automated sample preparation, greatly simplifies sample extraction method development, reduces human error, and improves analytical method reproducibility (day-to-day, user-to-user, and lab-to-lab), while freeing up the analyst to do other tasks, thus streamlining the sample preparation process.

This work aims to demonstrate a fully automated SPE method development strategy using a novel sequential elution strategy [1], optimizing pH solvent polarity, and various aqueous/organic compositions to determine optimum analyte elution profiles. This automation strategy incorporates the use of a compact robotic platform, and automated liquid handling device, which simplifies and streamlines the bioanalysis sample preparation SPE workflow, (Figure 1) maximizing productivity, reducing risk of errors, while ensuring analytical method performance.

METHODS

LC-MS/MS conditions: The chromatographic separation was performed using a Waters ACQUITY™ I-Class UPLC™ and ACQUITY UPLC HSS T3 C18 Column (100Å 2.1 x 50 mm, 1.7µm) and gradient elution using water and acetonitrile mobile phases containing 0.5 % formic acid. Detection was performed with a Waters Xevo™ TQ-S Mass Spectrometer (ESI +). And Multi Reaction Monitoring (MRM) of individual analytes. The MRM transitions used for carbamazepine used for quantification were 237.1 → 179.1 and 237.1 → 194.1.

Automation Platform: The Andrew Alliance Andrew+ Pipetting Robot, an automated liquid handling device, controlled by its cloud-based, OneLab™ Software (Figure 2) was used design and execute the sample preparation and extraction protocols.

SPE Extraction: Reversed-phase (RP) SPE extraction was performed using an Oasis™ HLB 96-well plate (30 mg sorbent per well).

Elution Profile RP- SPE Scouting Method: The Andrew+ Pipetting Robot deck layout (Figure 3.) used for elution profile assessment with sequential elution profiling. The equipped with 4-50 mL centrifuge tube dominos and one 250 mL glass bottle domino. Neat, aqueous solutions spike with 10 ng/ml of each analyte was used for this evaluation. The representative OneLab Software graphical protocol used for the 3x3 elution profile scouting method is shown in Figure 4. The associated SPE 96-well plate map is shown in Figure 5.

Final RP-SPE Method Evaluation: The top 3 elution profile conditions resulting from the scouting method were used for final RP-SPE method development. Performance assessment (recovery, selectivity/specificity, and matrix effects) was performed with an aqueous standard solution and bioomatrices (Rat plasma from Rockland) spiked with 10 ng/mL of carbamazepine. The Andrew+ deck layout is illustrated in Figure 7, while the associate SPE 96-well plate map is shown in Figure 8.

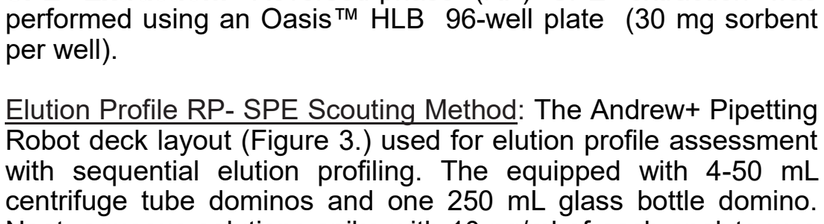

Figure 1. Sample preparation & LC-MS/MS workflow used for RP-SPE method development.

RESULTS

OneLab+ Software

Design & Execute Protocols

Pipettes

Dominoes & Connected Devices

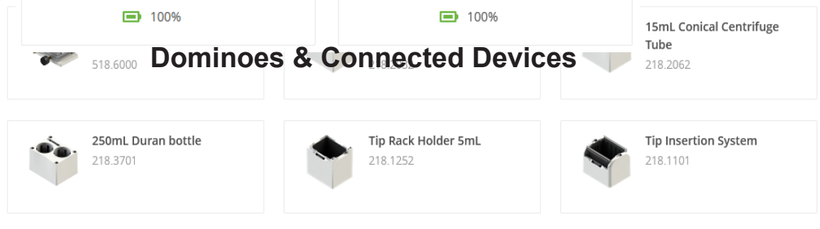

Figure 2: Visual representation of Andrew+ Pipetting robot with a compact and versatile working deck controlled with its cloud-native and easy-to-use OneLab Software. The easy -to-assemble dominoes and connected devices provides fast and easy operation, accommodating a diversity of simple to complex workflows.

SPE Elution Profile Scouting Method

Andrew+ Pipetting Robot Deck Layout

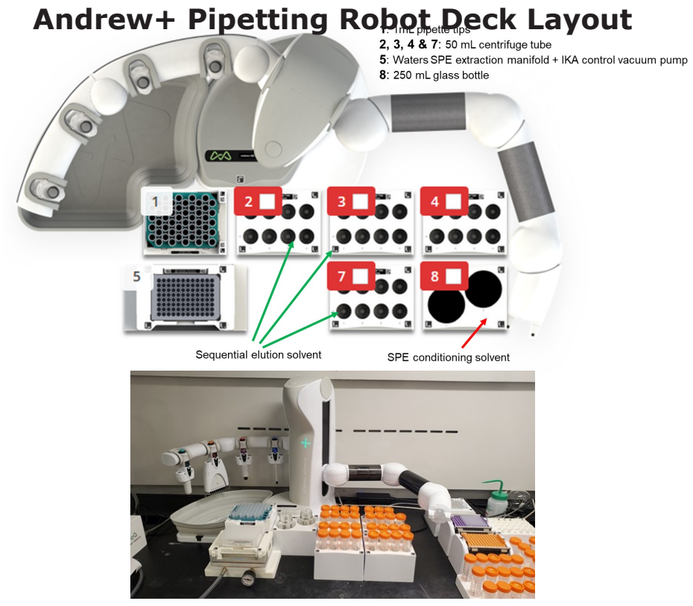

Figure 3. Andrew+ Pipetting Robot deck layout with connected devices and dominoes used for RP-SPE sequential elution profile scouting, evaluating pH, organic solvent, and organic composition.

OneLab Software Graphical Protocol

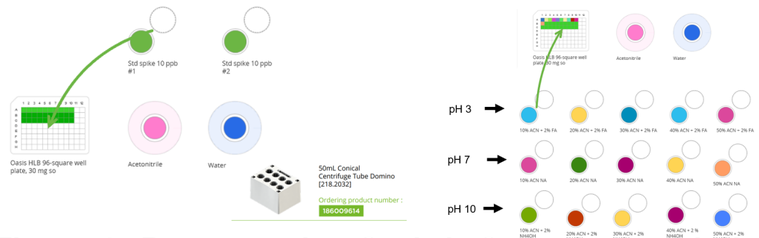

Figure 4. Representative OneLab Software graphical protocol creation for the SPE 3x3 Elution Profile Scouting Method.

3x3 Grid Elution Profile SPE 96-Well Plate Map

Row	Column	Organic Composition	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%	X	X
A		Methanol											10%	90%
B		Methanol											20%	100%
C		Methanol											30%	
D		Acetonitrile											40%	
E		Acetonitrile											50%	
F		Acetonitrile											60%	
G		Acetone											70%	
H		Acetone											80%	

Figure 5. 96-well RP-SPE plate map for the Elution Profile Scouting Method used with Andrew+ Pipetting Robot and using deck layout in Figure 3.

Carbamazepine SPE Elution Profile Map

		10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
MeOH	pH3	0	19	27	33	21	0	0	0	0	0
	pH7	0	0	0	59	24	18	0	0	0	0
	pH10	0	17	42	21	20	0	0	0	0	0
ACN	pH3	0	12	67	21	0	0	0	0	0	0
	pH7	0	0	31	19	49	0	0	0	0	0
	pH10	0	17	63	20	0	0	0	0	0	0
Acetone	pH3	0	89	8	3	0	0	0	0	0	0
	pH7	11	86	3	0	0	0	0	0	0	0
	pH10	26	64	10	0	0	0	0	0	0	0

Figure 6. Summarized sequential elution profile results for the carbamazepine demonstrating a wide distribution profile in methanol and acetonitrile at all pH's and high solubility and recovery in 30% acetone.

Final RP-SPE Method Evaluation

Andrew+ Pipetting Robot Deck Layout

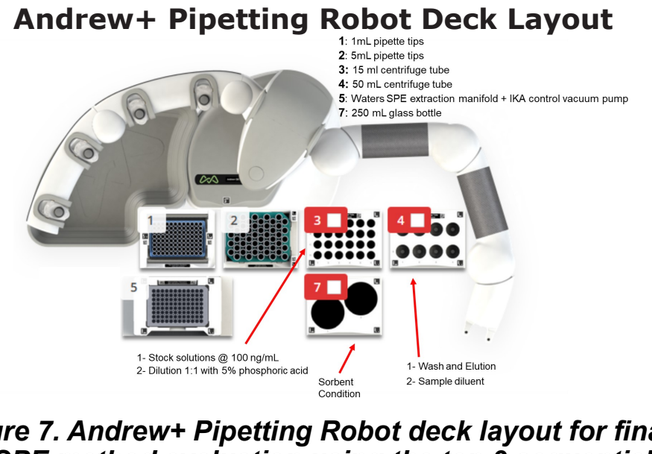

Figure 7. Andrew+ Pipetting Robot deck layout for final RP-SPE method evaluation using the top 3 sequential elution methods. Samples were extracted from neat, non-matrix samples and plasma to determine analyte recovery and matrix effects.

Final Method SPE 96-Well Plate Map

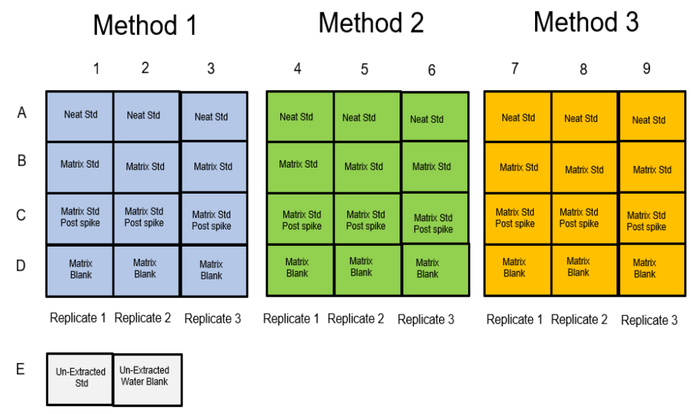

Figure 8. 96-well plate map for Final RP-SPE Method Protocol used with Andrew+ Pipetting Robot with deck layout in Figure 7.

Table 1. Final method comparison for carbamazepine, illustrating that Method 3, with a 30% MeOH pH 7 wash and 30% Acetone pH10 elution solution, provided the best recovery (84%) with the lowest matrix effects (3%) in extracted rat plasma.

	Method 1	Method 2	Method 3
Wash	100% Water	10% Acetone pH 3	30% MeOH pH 7
Elution	30% Acetone pH 10	30% Acetone pH 10	30% Acetone pH 10
	Mean % Recovery (Mean Matrix Effects)	Mean % Recovery (Mean Matrix Effects)	Mean % Recovery (Mean Matrix Effects)
Neat (non-matrix) SPE Extracted Standard	67	87	93
Rat Plasma (matrix) SPE Extracted standard	63(11)	69(11)	84(3)

STUDY HIGHLIGHTS

- An automated RP-SPE method development protocol was created using the Andrew+ Pipetting Robot platform, an automated liquid handling device, which simplifies and streamlines the sample preparation and SPE workflow, maximizing productivity, reducing risk of errors, while ensuring analytical method performance.
- The intuitive OneLab Software, incorporates "user actions" with image and video guidance, along with graphical protocol creation, for fast protocol development.
- Using an SPE scouting method, with a novel sequential elution strategy, optimizing pH, organic solvent, and organic composition, was created to determine optimum analyte elution profiles.
- Final RP-SPE method evaluation was performed using the top 3 SPE elution profiles determined from the scouting method, assessing SPE performance (Recovery and Matrix Effects) in neat and extracted plasma.
- In 2 experiments, optimal wash and elution conditions were determined for carbamazepine (Table 1) which yielded 84 % recovery and only 3% matrix effects in extracted plasma.

CONCLUSION

The Andrew+ Pipetting Robot and its intuitive cloud-based OneLab Software greatly simplify protocol creation and execution for simple to complex sample preparation and extraction protocols, thus providing a fast, standardized, and fully automated approach to SPE method development.

References
1.Novel Extraction Techniques with ACQUITY UPLC and 2D Technology: Part I Pesticide Screening in Drinking Water (720006588EN)
https://www.waters.com/webassets/cms/library/docs/720006588EN

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