

## Fully Automated Solid Phase Extraction Sample Preparation, using the Andrew+™ Pipetting Robot configured with the Extraction+ Connected Device

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### Abstract

The following work demonstrates the new capabilities and flexibility of the Andrew+ Pipetting Robot in combination with the Extraction+ Connected Device, controlled by OneLab™ Software, for fully automated Solid Phase Extraction (SPE) sample preparation in cartridge and plate SPE formats.

### Benefits

- Fully automated SPE sample extraction using the Andrew+ Pipetting Robot and the Extraction+ Connected Device, eliminating the need for user intervention
  - Automation compatibility with SPE plates and cartridge formats
  - Liquid transfer flexibility into collection in plates, tubes, and bottles
  - Fully programmable vacuum pressure profiles extraction performance variability
  - Automated sample preparation and extraction increase efficiency, providing walk-away time for the analyst
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- Transferrable methods on the easy-to-use OneLab Software allow for easy implementation of the same preparation procedure across users and labs

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## Introduction

Liquid chromatography-mass spectrometry (LC-MS) bioanalytical SPE sample preparation is time-consuming and complex, often perceived as the “bottle-neck” in the LC-MS bioanalytical workflow, due to the many protocol steps required to accurately and reliably isolate or purify the target analyte(s) from various biological matrices. Implementing fully automated SPE sample preparation greatly simplifies the analytical workflow, 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.

The work presented herein demonstrates fully automated sample preparation and SPE extraction solution using the Andrew+ Pipetting Robot (Andrew+) and newly introduced Extraction+ Connected Device (Extraction+), controlled via OneLab Software (Figure 1), to ensure accurate, reliable, and robust SPE sample preparation.

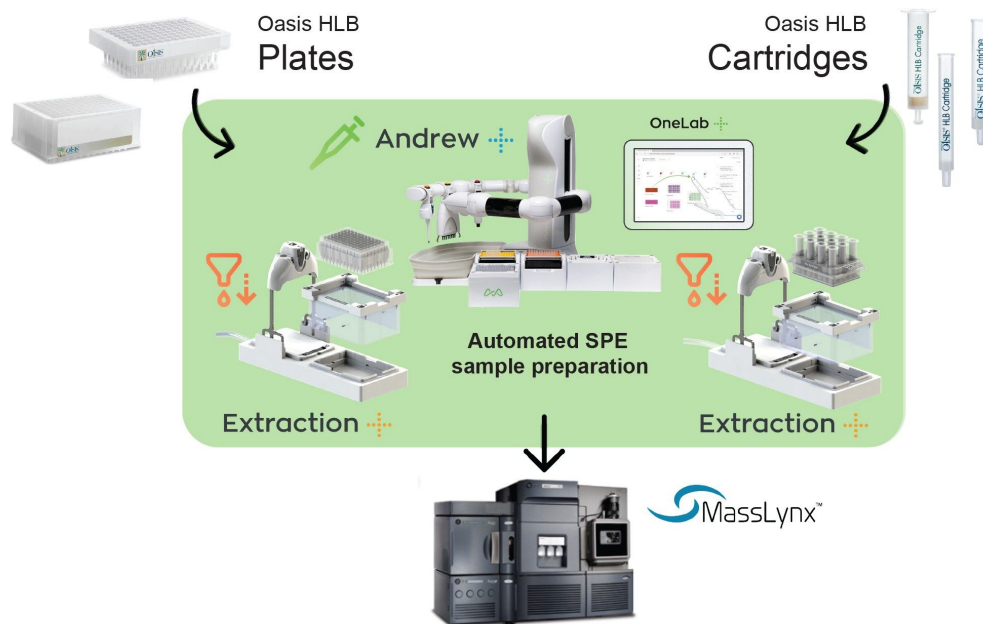


Figure 1. Graphical illustration of the automated SPE sample extraction and LC-MS analysis workflow using Oasis HLB SPE plates and cartridges and the Andrew+ Pipetting Robot configured with the Extraction+ connected device.

## Experimental

### LC-MS Conditions

LC system:	ACQUITY UPLC I-Class, FL with Column Manager (CMA)
MPA:	0.1% FA in Water
MPB:	0.1% FA in Acetonitrile

Column/Sorbents:	HSS PFP 1.8 $\mu$ m, 2.1 mm x 50 mm Column (p/n: 186005965)
Column temp.:	35 °C
Sample temp.:	10 °C
Injection volume:	5 $\mu$ L
WNW:	9:10 water:acetonitrile
SNW:	25:25:25:25 water:methanol:acetonitrile:water
MS system:	Xevo® TQ-XS
Capillary (Kv):	3.0
Cone voltage:	30 V
Desolvation temp.:	500 °C
Desolvation flow:	1100 L/Hr
Cone gas flow:	150 L/Hr

## LC Gradient

Time (min)	Flow (mL/min)	% MPA	% MPB
0.0	0.5	100.0	0.0
5.0	0.5	5.0	95.0
6.0	0.5	5.0	95.0
6.1	0.5	100.0	0.0
7.0	0.5	100.0	0.0

## MS Settings

Ionization mode:	ESI+
Acquisition range:	MRM
Capillary voltage:	2.00 kV
Cone voltage:	60 V
Desolvation temperature:	500 °C
Desolvation flow:	1000 L/Hr
Cone gas flow:	150 L/Hr
Collision gas flow:	0.2 mL/min
Nebulizer gas flow:	7 Bar

## Data Management

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Instrument control software: MassLynx™ (v4.2)

Quantification software: TargetLynx™

## Sample Preparation and SPE Extraction

A stock solution containing a mixture of small molecule pharmaceuticals (10 µg/mL) was prepared in methanol from individual 1.0 mg/mL concentrated analyte stock solutions. This solution was then used to prepare a 100 ng/mL working analyte stock solution in water, which was then used to assess automated and manual reversed-phase (RP) SPE analytical performance. Neat recovery experiments were conducted using the 100 ng/mL analyte mix working stock solution in 1, 3, and 6 cc Oasis HLB SPE cartridges, as well as in the µElution and macro 96-well Oasis HLB SPE plates (N=4 per SPE format), using a simple 3-step SPE protocol (load, wash, elute). Information regarding the basic RP SPE procedure can be found in the Waters SPE Wall Chart (p/n 720001981 < <https://www.waters.com/webassets/cms/library/docs/720001981en.pdf> > ). Briefly, an aqueous 4% phosphoric acid solution was directly loaded to the SPE plate or cartridge followed by the addition of the spiked 100 ng/mL analyte solution and mixed using a six-time pipette mixing step. The 4% aqueous phosphoric solution is often used to ensure sufficient disruption of the target analytes from the biological matrix proteins. This pretreated sample was loaded on the SPE cartridge or plates. Following sample loading, the SPE device was washed with a 5% methanol solution, and then eluted into appropriate collection vessels using a methanol solution. Samples were then diluted with water to ensure compatibility with the initial LC conditions. Respective volumes used for the sample pretreatment, SPE extraction, and sample dilution are provided in Table 1. Following SPE extraction, resulting samples were covered with screw caps or a 96-well cap mat and vortexed. Subsequent detection and analysis of the extracted samples was performed by LC-MS/MS using an ACQUITY UPLC I-Class System coupled to the Xevo TQ-XS Mass Spectrometer. Full LC-MS conditions can be found in the Experimental Section and using the MS MRM parameters listed in Table 2 for detection and analysis of the pharmaceuticals.

Step/SPE device	1 cc cartridge (30 mg)	3 cc cartridge (60 mg)	6 cc cartridge (150 mg)	$\mu$ Elution 96-well plate (2 mg)	Macro elution 96-well plate (30 mg)
OneLab protocol	1	2	3	4	5
Solution	SPE pipetting volume ( $\mu$ L)				
Sample	500	1000	2000	250	500
Pretreatment	500	1000	2000	250	500
Wash	1000	1500	4000	300	700
Elution	2 x 125	2 x 250	2 x 1000	2x50	2x125
Dilution	250	500	2000	100	250
Collection vessel	Waters 2 mL LC/GC vial	Waters 2 mL LC/GC vial	5 mL VWR centrifuge tube	700 $\mu$ L 96-round well collection plate	700 $\mu$ L 96-round well collection plate

Table 1. Respective volumes used for each of the reversed-phase Oasis HLB SPE steps in 1, 3, and 6 cc cartridges, as well as the 96-well  $\mu$ Elution and Macro Elution plates.

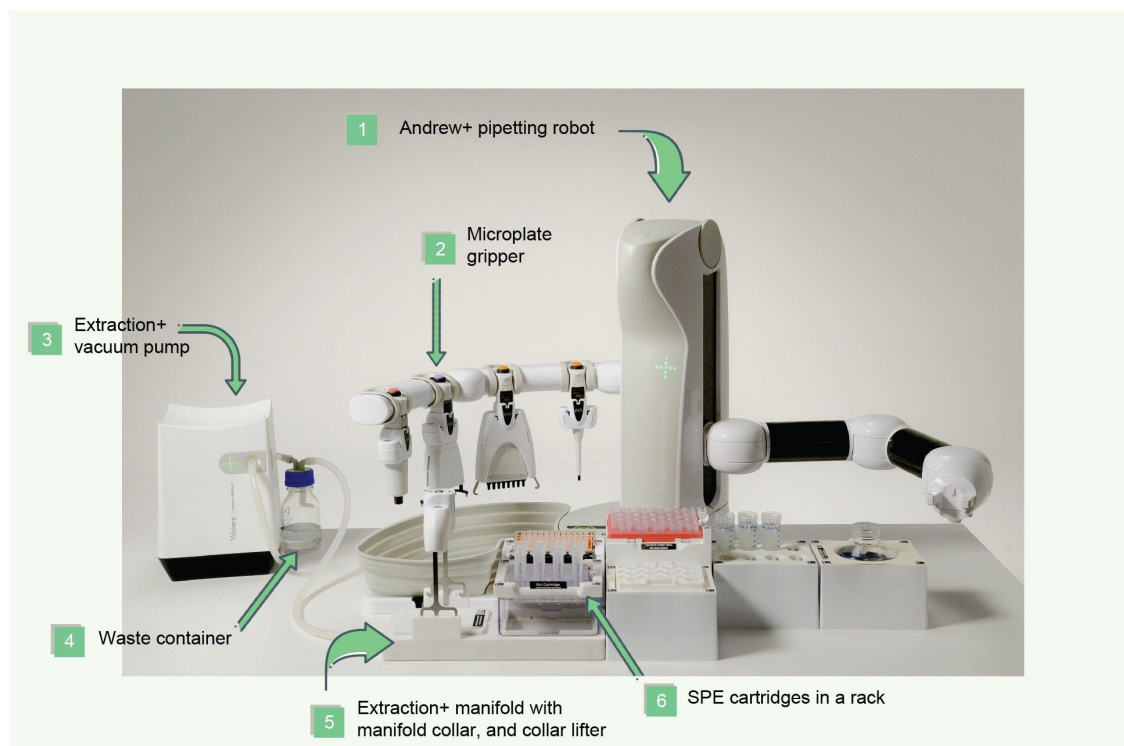
Compound	M+H <sup>+</sup>	Fragment ion (primary)	Collision energy (primary)	Fragment ion (confirmatory)	Collision energy (confirmatory)
Acetazolamide	221.1	79.0	20	83.0	16
Acetaminophen	152.1	65.0	24	110.1	14
Prednisone	359.2	313.1	12	147.1	28
o-toluamide	136.2	77.1	18	44.1	16
Cortisone	361.2	163.0	22	121.1	26
Metoprolol	268.2	116.1	18	98.1	18
Amitriptyline	278.2	105.0	22	91.0	28
Apixaban	460.3	199.1	35	443.2	30

Table 2. Small molecule pharmaceuticals MS parameter settings used for analytical SPE performance evaluation of the Andrew+ configured with the Extraction+.

## SPE Automated Extraction

The Andrew+ configured with the Extraction+, controlled by the OneLab Software, was used to carry out all automated SPE evaluations and is shown in Figure 2. A negative vacuum using a pressure step ramp controlled via OneLab Software was applied to the SPE steps. An illustration of a typical pressure profile defined in the OneLab protocol is shown in Figure 3. Visual representation of the OneLab protocols used for the SPE cartridge

and plate evaluation of Extraction+ performance, and available for download from [Andrew Alliance OneLab library <https://onelab.andrewalliance.com/library>](https://onelab.andrewalliance.com/library) , are shown in Figures 4–8. These protocols were created using the SPE conditions in Table 1 for the various SPE cartridge and plate formats. Contained in these figures, are the OneLab protocols with protocol visualization and drag-and-drop method creation, key information such as system components (e.g., dominos, connected devices, pipettes, pipette tips, bottles, tubes, etc.), lab consumables, and the Andrew+ deck layout for easy automated method implementation and execution with the Extraction+.



*Figure 2. Andrew+ configured with the required Dominos, the Andrew Alliance Bluetooth electronic pipettes and microplate gripper on the tool stand and 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 in the corresponding adaptor.*



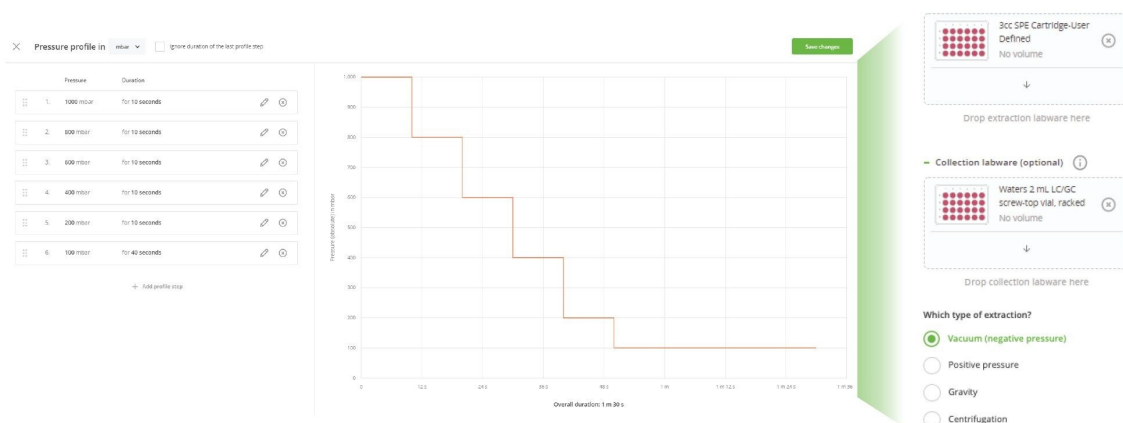
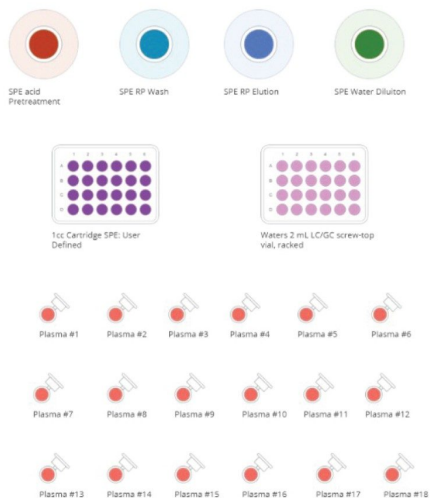


Figure 3. Illustration of the OneLab full vacuum pressure control, for evacuation of the liquid from SPE cartridges and collection into 2 mL LC/GC vials during the SPE method execution.

# OneLab Protocol 1 | Oasis RP-HLB 1 cc Cartridge SPE

## OneLab protocol visualization



## Andrew+ system components *Dominos, electronic pipettes and tips*

← Material list  
Check you've got all the required materials

**Devices <sup>2</sup>**

- Extraction+ 518.7004.175EH  
518.7004.175EH
- Andrew+ 718.8141.175R  
718.8141.175R

**Tools <sup>3</sup>**

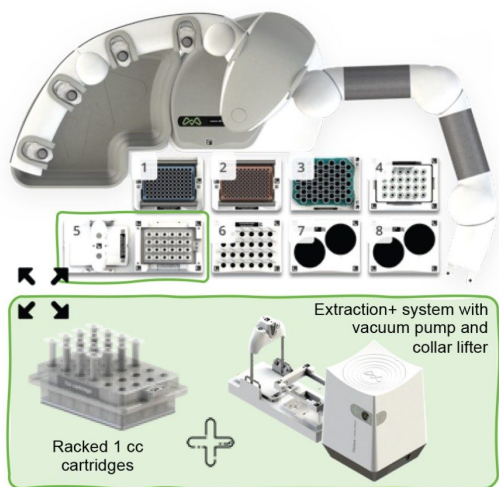
- 1-ch 300 Andrew Alliance Picus Pipette - 98300844
- 1-ch 1000 Andrew Alliance Picus Pipette - 19044258
- 1-ch 5000 Andrew Alliance 1 98388973
- Microplate Gripper Microplate Gripper - 478.4523.1456

**Tips <sup>3</sup>**

- 10-300 µL Optifit tips AT 16811 2 505
- 50-1000 µL Optifit tips AT 16801 26 036
- 100-5000 µL Optifit tips AT 16811 1 50

**Domino blocks <sup>8</sup>**

- Extraction+ 518.7000
- Microtube 218.2152
- 2 x 250mL Duran bottle 218.3701
- Collection Labware Rack 218.4611
- 2 x Tip Insertion System 218.1101
- Tip Rack Holder 5mL 218.1252



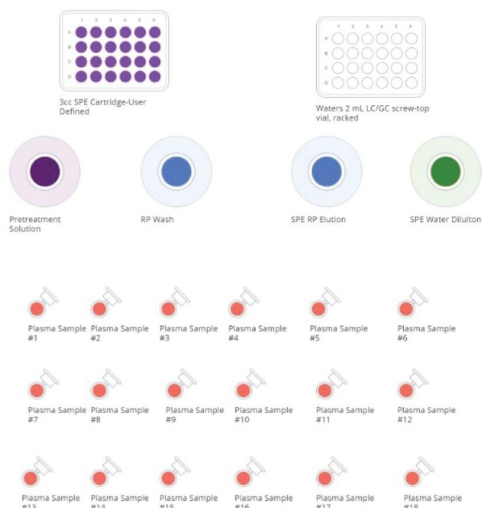
## Andrew+ deck layout with Extraction+ connected device — 1 cc Oasis HLB SPE cartridge —

Position	Dominos and connected devices with Labware
1, 2	Tip insertion system domino
3	5 mL tip rack holder domino
4	Collection labware rack domino with 2 mL LC/GC screw-top vials
5	Extraction+ connected device with HLB Oasis 1 cc vac cartridges
6	Microtube domino with 2 mL snap-cap centrifuge tubes
7, 8	250 mL Duran Bottle Domino

Figure 4. Andrew+ configured with Extraction+ with protocol visualization, system components identification, and Andrew+ deck layout.

# OneLab Protocol 2 | Oasis RP-HLB 3 cc Cartridge SPE

## OneLab protocol visualization



## Andrew+ system components Dominos, electronic pipettes and tips

### Material list

Check you've got all the required materials

#### Devices <sup>1</sup>

Extraction+ 518.7004.175EH  
Extraction+  
518.7004.175EH

Andrew+ 718.8141.175DR  
Andrew+  
718.8141.175DR

#### Tools <sup>3</sup>

1-ch 300  
Andrew+ Intouch Plus Pipette +  
38385644

1-ch 1000  
Andrew+ Intouch Plus Pipette +  
18544236

1-ch 5000  
Andrew+ Intouch Plus +  
38388973

Microplate Gripper  
Microplate Gripper +  
418.4522.1486

#### Tips <sup>3</sup>

10-300 µL Opette tips  
AT 1602 2 Tips  
791000

50-1000 µL Opette tips  
AT 1602 20 Tips  
791000

100-5000 µL Opette tips  
AT 1602 2 Tips  
792004

#### Domino blocks <sup>3</sup>

Extraction+  
518.7000

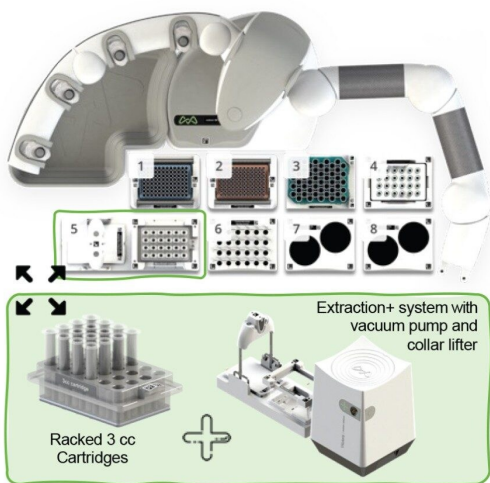
Microtube  
218.2152

2 x 250mL Duran bottle  
218.3701

Collection Labware Rack  
318.4911

2 x Tip Insertion System  
318.1101

Tip Rack Holder 5mL  
318.1202



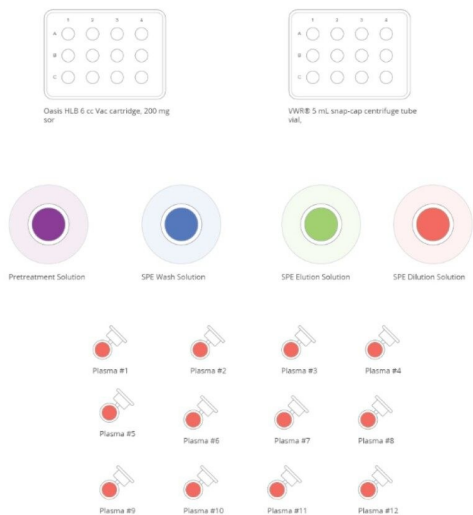
## Andrew+ deck layout with extraction+ connected device — 3 cc Oasis HLB SPE cartridge —

Position	Dominos and connected devices with labware
1, 2	Tip insertion system domino
3	5 mL tip rack holder domino
4	Collection labware rack domino with 2 mL LC/GC screw-top vials
5	Extraction+ Connected Device with HLB Oasis 3 cc vac cartridges
6	Microtube domino with 2 mL snap-cap centrifuge tubes
7, 8	250 mL Duran bottle domino

Figure 5. Andrew+ configured with Extraction+ with protocol visualization, system components identification, and Andrew+ deck layout.

# OneLab Protocol 3 | Oasis RP-HLB 6 cc Cartridge SPE

## OneLab protocol visualization



## Andrew+ system components

*Dominos, electronic pipettes and tips*

### Material list

Check you've got all the required materials

#### Devices <sup>2</sup>

**Extraction+ 518.7004.175EH**  
Extraction+  
518.7004.175EH

Ready

**Andrew+ 718.8141.1715R**  
Andrew+  
718.8141.1715R

Ready

#### Tools <sup>2</sup>

**1-ch 1000**  
Andrew Alliance Picos Pipette -  
19044258

100%

**1-ch 5000**  
Andrew Alliance Picos Pipe  
98389973

100%

**Microplate Gripper**  
Microplate Gripper -  
418.4522.149C

100%

#### Tips <sup>2</sup>

**50-1000 µL Opsetfit tips**  
All Nest 28 tips  
791000

**100-5000 µL Opsetfit tips**  
All Nest 28 tips  
780304

#### Domino blocks <sup>7</sup>

**Extraction+**  
518.7000

**Microtube**  
218.2152

**2 x 250mL Duran bottle**  
218.3701

**Collection Labware Rack**  
218.8011

**Tip Insertion System**  
218.1101

**Tip Rack Holder 5mL**  
218.1252



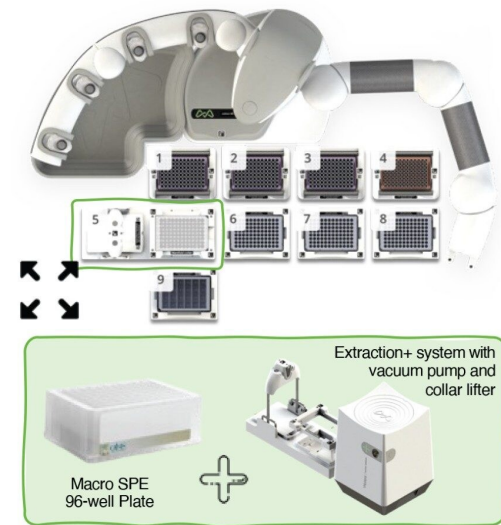
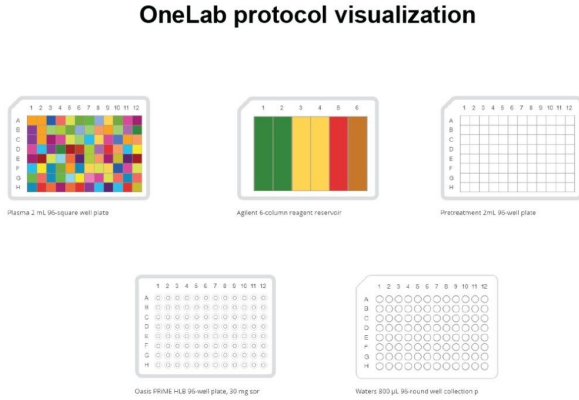
### Andrew+ deck layout with Extraction+ connected device

— 6 cc Oasis HLB SPE cartridge —

Position	Dominos and connected devices with Labware
1	Tip insertion system domino
2	5 mL tip rack holder domino
3	Collection labware rack domino with 5 mL snap-cap centrifuge tubes
4	Microtube domino with 2 mL snap-cap centrifuge tubes
5	Extraction+ Connected Device with HLB Oasis 6 cc vac cartridges
6, 7	250 mL Duran bottle domino

Figure 6. Andrew+ configured with Extraction+ with protocol visualization, system components identification, and Andrew+ deck layout.

# OneLab Protocol 4 | Oasis RP-HLB 96-well Macro Plate SPE



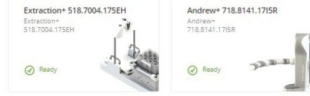
## Andrew+ system components

*Dominoes, electronic pipettes and tips*

### Material list

Check you've got all the required materials

#### Devices <sup>2</sup>



#### Tools <sup>3</sup>



#### Tips <sup>2</sup>



#### Domino blocks <sup>3</sup>



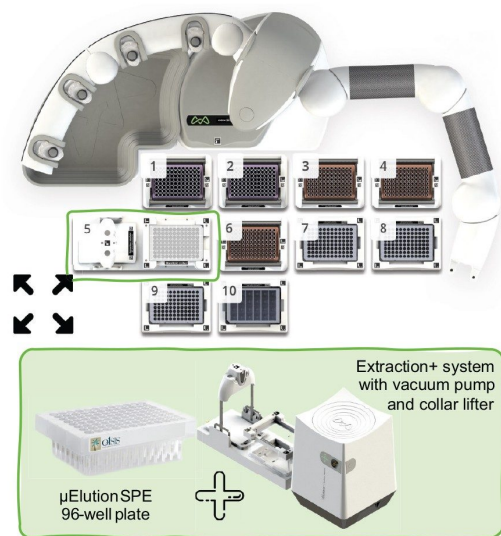
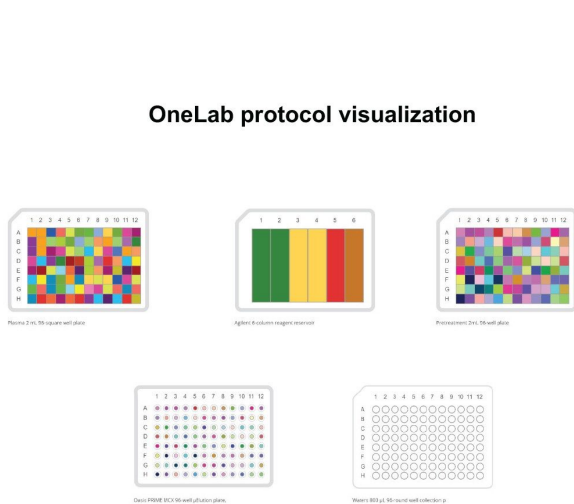
### Andrew+ deck layout with Extraction+ connected device

— Oasis HLB SPE Macro 96-well plate —

Position	Dominoes and connected devices with Labware
1 to 4	Tip insertion system domino
5	Extraction+ connected device with Oasis HLB 96-well macro plate
6 to 8	Storage plate domino
9	Deepwell microplate domino with 6-column reagent reservoir

Figure 7. Andrew+ configured with Extraction+ with protocol visualization, system components identification, and Andrew+ deck layout.

# OneLab Protocol 5 | Oasis RP-HLB 96-well $\mu$ Elution Plate SPE



## Andrew+ system components

*Dominos, electronic pipettes and tips*

### Material list

Check you've got all the required materials

#### Devices <sup>2</sup>

**Extraction+** 518.7004.175EH

Extraction+  
518.7004.175EH

Ready

**Andrew+** 718.8141.175R

Andrew+  
718.8141.175R

Ready

#### Tools <sup>3</sup>

**8-ch 300**

Andrew Alliance Pippa Pipette +  
38564294

100%

**8-ch 1200**

Andrew Alliance Pippa Pipette +  
38389639

100%

**Microplate Gripper**

Microplate Gripper +  
418.4022.1495

100%

#### Tips <sup>2</sup>

**3 x 10-300  $\mu$ l Opifit tips**

At least 2 boxes = 6 tips  
790380

**2 x 50-1200  $\mu$ l Opifit tips**

At least 1 box = 24 tips  
791210

#### Domino blocks <sup>10</sup>

**Extraction+**

518.7000

**Deepwell Microplate**

218.2001

**3 x Storage plate**

218.2001

**5 x Tip Insertion System**

218.1101

## Andrew+ deck layout with Extraction+ connected device

— Oasis HLB SPE  $\mu$ Elution 96-well plate —

Position	Dominos and connected devices with labware
1 to 4 and 6	Tip insertion system domino
5	Extraction+ connected device with Oasis HLB 96-well $\mu$ Elution plate
7 to 9	Storage plate domino
10	Deepwell microplate domino with 6-column reagent reservoir

Figure 8. Andrew+ configured with Extraction+ with protocol visualization, system components identification, and Andrew+ deck layout.

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## Results and Discussion

Extraction+ is the new, fully automatable SPE system solution used in combination with Andrew+, which eliminates the need for user intervention during SPE sample preparation and extraction. The Extraction+ device is comprised of two modules, an SPE manifold and a vacuum pump, both controlled via the OneLab Software. An image of the Extraction+ configured with the Andrew+ is shown in Figure 2. Key features of the Extraction+ include:

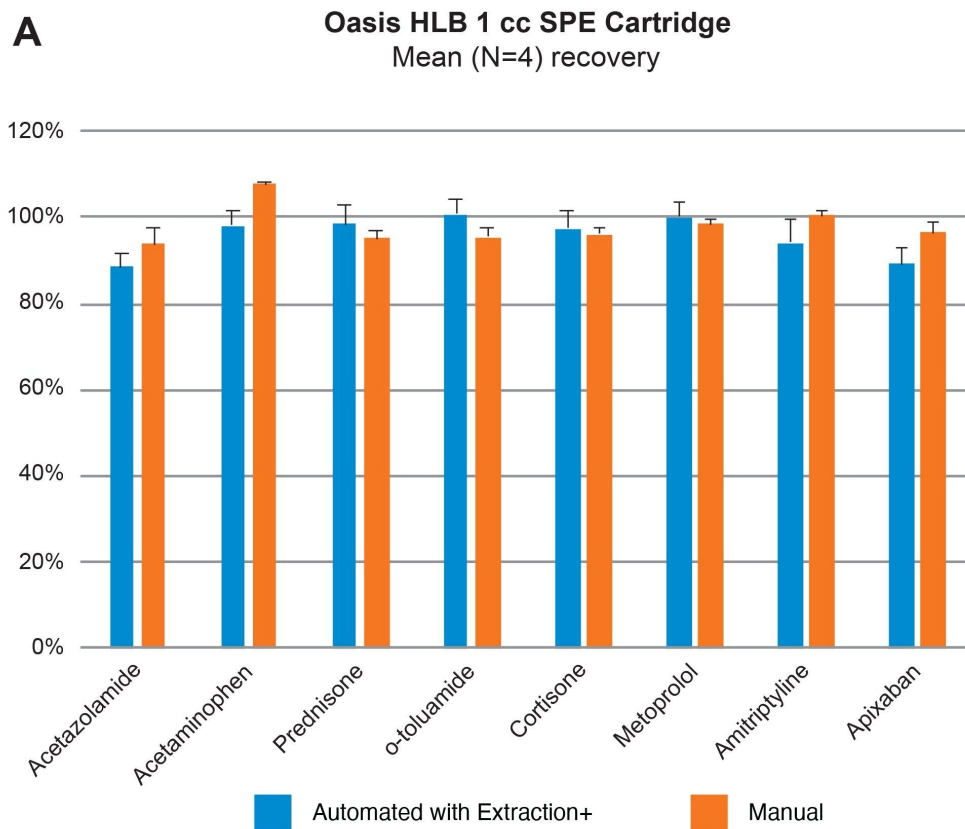
1. Compatibility to perform SPE in both cartridge and plate formats,
2. Liquid dispensing, collection, and evacuation into plates, tubes, or bottles,
3. Flow-through waste collection,
4. Full vacuum pressure control, defined within the OneLab protocol, for evacuation of the liquid during the SPE method execution (Figure 3).

The labware gripper installed on Andrew+ facilitates the movement of waste and sample collection vessels to and from the Extraction+ vacuum manifold, while the collar lifter on the Extraction+ manifold, moves the extraction manifold collar with the SPE device into a 'parking spot' (idle position) during the insertion of the collection vessels onto the vacuum manifold with the labware gripper.

Both Andrew+ and Extraction+ are controlled through the user-friendly, cloud-based OneLab Software. The Extraction+ when used with Andrew+, can carry out the liquid handling and the sample extraction fully automated (no manual intervention), providing complete walk-away sample preparation, whilst ensuring robust and reliable sample preparation from lab-to-lab, day-to-day, and user-to-user.

Comparable SPE extraction performance using the Andrew+ configured with the Extraction+ using 1, 3, 6 cc RP Oasis HLB cartridges and 96-well Macro plate as compared to manual SPE is shown in Figure 9, panels A–D. Furthermore, robust and repeatable, automated SPE performance in 1 cc Oasis HLB cartridges is illustrated in Tables 3 and 4, highlighting single digit intra/inter-assay performance (mean recovery and RSD). Figure 10 highlights comparable Oasis HLB automated SPE extraction performance for both cartridge and 96-well plate formats, using the same sorbent bed size (30 mg). Collectively these results demonstrate highly flexible and reproducible Oasis HLB SPE sample preparation in both cartridges and plates, with the developed SPE protocols created in the OneLab Software and executed with the Andrew+ Pipetting Robot configured with the

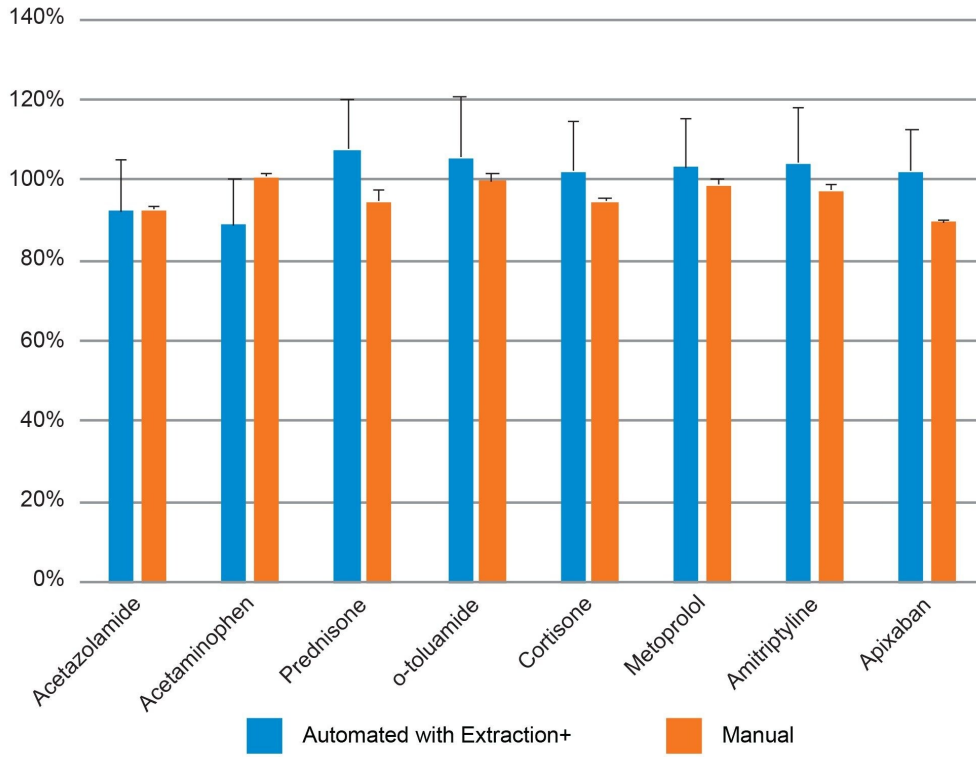
Extraction+ connected device.





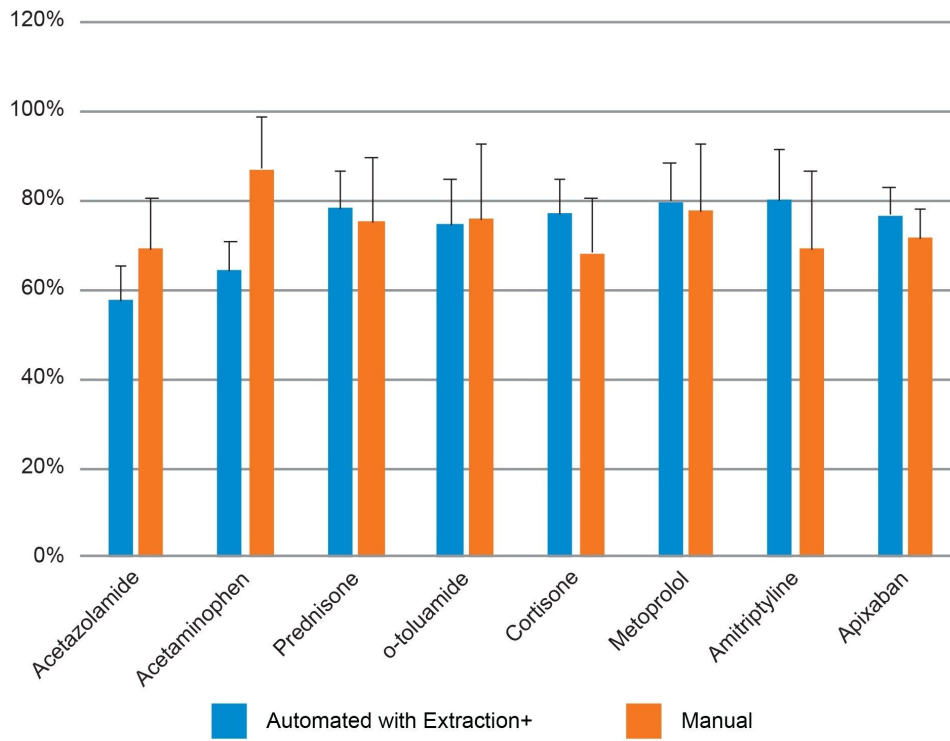
B

Oasis HLB 3 cc SPE Cartridge  
Mean (N=4) recovery



C

Oasis HLB 6 cc SPE Cartridge  
Mean (N=4) recovery



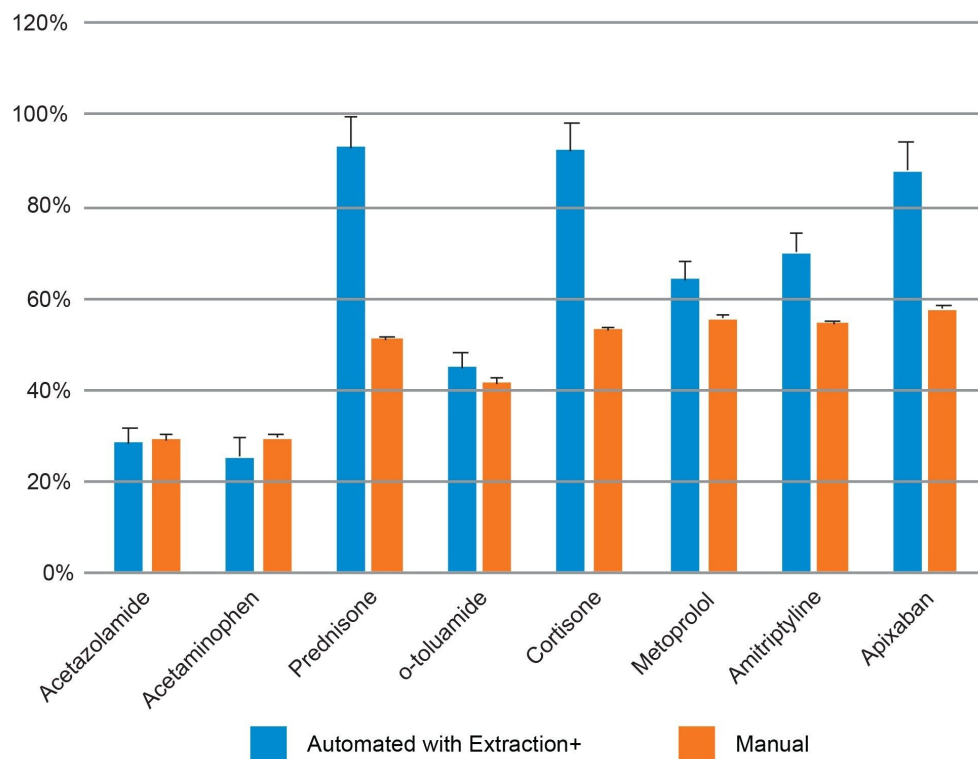
**D****Oasis HLB 96-well Macro Plate**  
Mean (N=4) recovery

Figure 9. Comparison of automated SPE extraction performance using Andrew+ configured with the Extraction+ device as compared to manual SPE extraction using (A) 1 cc (B) 3 cc (C) 6 cc RP Oasis HLB cartridges and the (D) 96-well macro plate.

**Oasis HLB 1 cc Cartridge (30 mg) vs. 96-Well Macro Plate (30 mg)**  
Mean (N=4) recovery

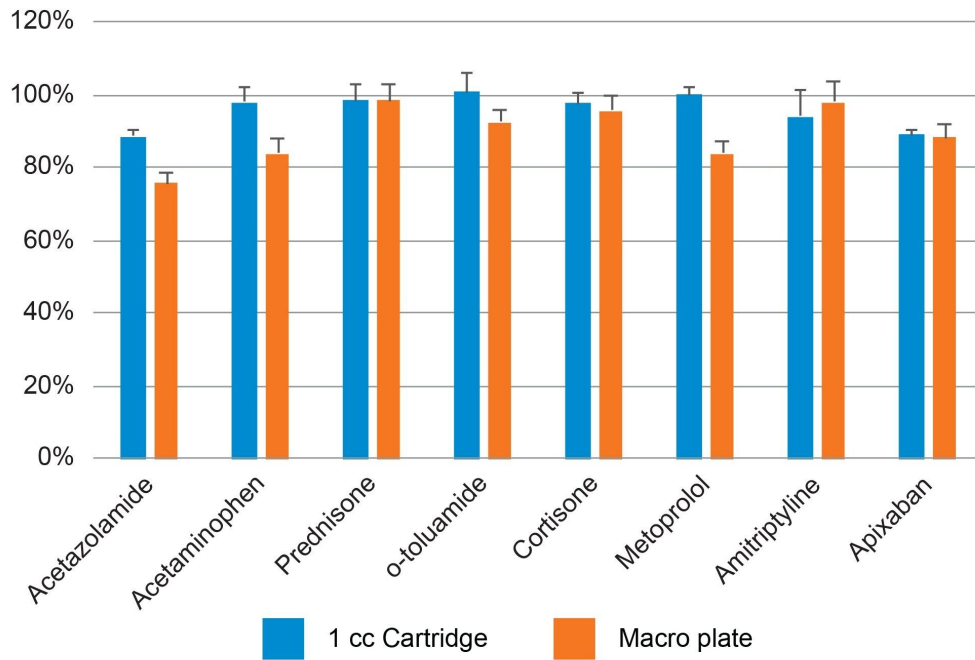


Figure 10. Comparison of automated SPE extraction performance using Andrew+ configured with the Extraction+ device using 1 cc Oasis HLB cartridge (30 mg) and the Oasis HLB Macro 96-well plate (30 mg).

Target analyte	User 1			User 2			User 3		
	% Mean (N=4) recovery	S.D.	%RSD	% Mean (N=4) recovery	S.D.	%RSD	% Mean (N=4) recovery	S.D.	%RSD
Acetazolamide	91.2	1.4%	1.5	88.7	3.2%	3.6	102.2	4.0%	3.9
Acetaminophen	99.3	4.0%	4.1	98.1	3.8%	3.9	110.6	4.4%	4.0
Prednisone	104.5	4.5%	4.3	98.6	4.4%	4.4	107.9	5.5%	5.1
o-toluamide	105.6	5.2%	5.0	100.9	3.6%	3.6	114.7	6.1%	5.3
Cortisone	92.3	2.8%	3.0	97.5	4.2%	4.3	106.6	5.4%	5.1
Metoprolol	102.6	2.3%	2.3	100.2	3.3%	3.3	113.1	4.7%	4.1
Amitriptyline	92.7	7.6%	8.2	94.1	5.4%	5.8	107.9	5.7%	5.3
Apixaban	75.3	1.7%	2.3	89.1	4.0%	4.5	84.0	4.2%	4.9

Table 3. Intra-batch automated SPE extraction performance using 1 cc RP Oasis HLB cartridges with Andrew+ configured with the Extraction+ device, demonstrating comparable percent analyte recoveries with single digit %RSDs across the three SPE experiments conducted by three different analysts.

Target analyte	User 1 % mean (N=4) recovery	User 2 % mean (N=4) recovery	User 3 % mean (N=4) Recovery	Users 1-3 % mean recovery	S.D.	Users 1-3 mean % RSD
Acetazolamide	91.2	88.7	102.2	<b>94.0</b>	7.2%	7.7
Acetaminophen	99.3	98.1	110.6	<b>102.6</b>	6.9%	6.7
Prednisone	104.5	98.6	107.9	<b>103.7</b>	4.7%	4.5
o-toluamide	105.6	100.9	114.7	<b>107.1</b>	7.0%	6.5
Cortisone	92.3	97.5	106.6	<b>98.8</b>	7.3%	7.3
Metoprolol	102.6	100.2	113.1	<b>105.3</b>	6.8%	6.5
Amitriptyline	92.7	94.1	107.9	<b>98.2</b>	8.4%	8.5
Apixaban	75.3	89.	84.0	<b>82.8</b>	7.0%	8.4

Table 4. Inter-batch automated SPE extraction performance using 1cc Oasis HLB SPE cartridges with Andrew+ configured with the Extraction+ device. Results demonstrate comparable percent mean analyte recoveries (83–107%) with single digit RSDs  $\leq 8.5\%$  across the three SPE experiments conducted by three different analysts.

## Conclusion

This application highlights fully automated SPE sample preparation in cartridge and plate formats, leveraging the capabilities of the newly introduced connected device, Extraction+ configured on Andrew+. In combination with its fully software-controlled pipetting, vacuum pump control, and liquid transfer in collection plates, tubes, and bottle, the use of the static and core grippers with Extraction+ facilitates movement to and from the extraction manifold, eliminating the need for user intervention during the SPE sample preparation.

The OneLab protocols for the described SPE cartridges and plate formats are downloadable from the OneLab Library providing ease of use and transferability across Andrew+ systems, which allows for easy implementation of the same preparation procedure across users in the same lab or between connected labs. Andrew+ with Extraction+ simplified and streamlined sample preparation and extraction procedures, maximizing productivity, reducing errors, and ensuring the overall analytical performance of a typical bioanalytical LC-MS workflow.

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[robot/](https://www.andrewalliance.com/pipetting-robot/)

[Andrew+ Pipetting Robot](https://www.andrewalliance.com/pipetting-robot/)

[OneLab Software Automation Workflow Kits and Scripts](https://onelab.andrewalliance.com/automation-workflow-kits-and-scripts.html)

[ACQUITY UPLC I-Class PLUS System](https://www.waters.com/waters/nav.htm?cid=134889751)

[Xevo TQ-XS Triple Quadrupole Mass Spectrometry](https://www.waters.com/waters/nav.htm?cid=134889751)

[Oasis Sample Extraction Products](https://www.waters.com/waters/nav.htm?cid=134889751)

720007711, September 2022



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