Automated extraction of basic peptides and small molecules from human urine using EVOLUTE® EXPRESS WCX prior to UHPLC-HRMS analysis

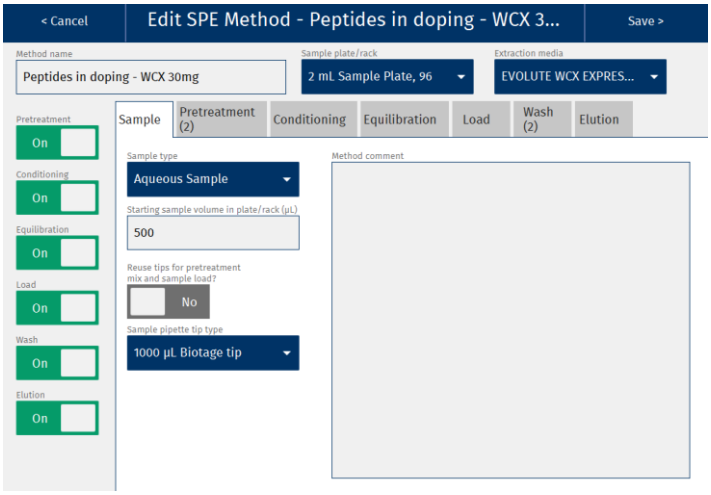
Appendix

Biotage® Extrahera™ Classic method

The method outlined in this application note was automated on the Biotage® Extrahera™ Classic, using Biotage® EVOLUTE® EXPRESS WCX 30mg plates. This appendix provides the necessary software settings to configure the Extrahera™ system for this method.

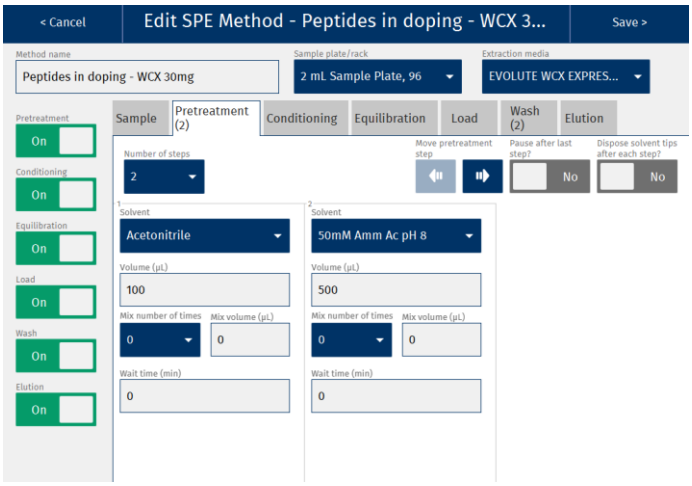
Total extraction time for 96 samples is approximately 50 minutes for the full protocol, including conditioning and equilibration. For an optimized load-wash-elute, total extraction time is approximately 40 minutes.

Sample tab



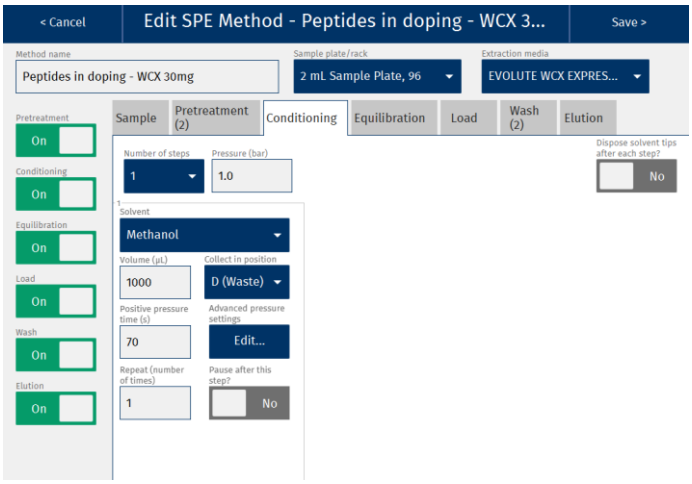
Settings	
Sample Type	Aqueous Sample
Starting sample vol.	500
Reuse tips	No
Sample pipette tip type	1000 µL Biotage tip
Method comment	

Pretreatment tab



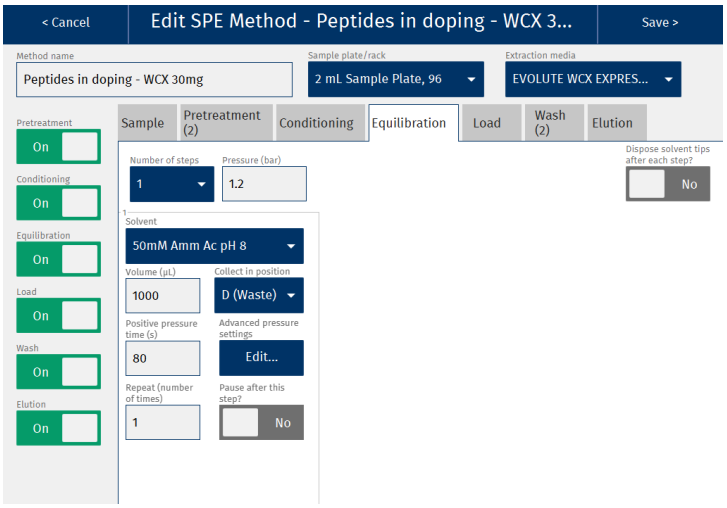
Number of steps	2
Pause after last step?	No
Dispose solvent tips?	No
Pretreatment step 1	
Solvent	Acetonitrile
Volume (µL)	100
Mix number of times	0
Wait time (mins)	0
Pretreatment step 2	
Solvent	50mM Ammonium Acetate (pH8)
Volume (µL)	500
Mix number of times	0
Wait time (mins)	0

Conditioning tab



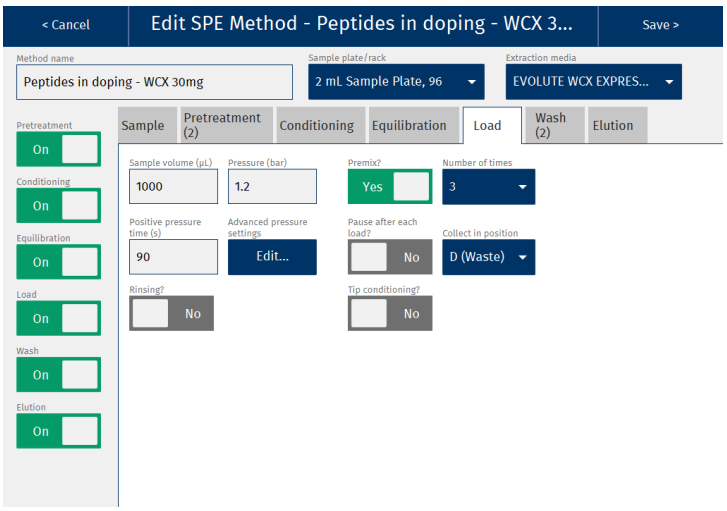
Number of steps	1
Pressure (bar)	1.0
Dispose solvent tips?	No
Conditioning step 1	
Solvent	Methanol
Volume (µL)	1000
Collect in position	D (Waste)
Positive pressure time (s)	70
Advanced pressures	No
Repeat	1
Pause after this step	No

Equilibration tab



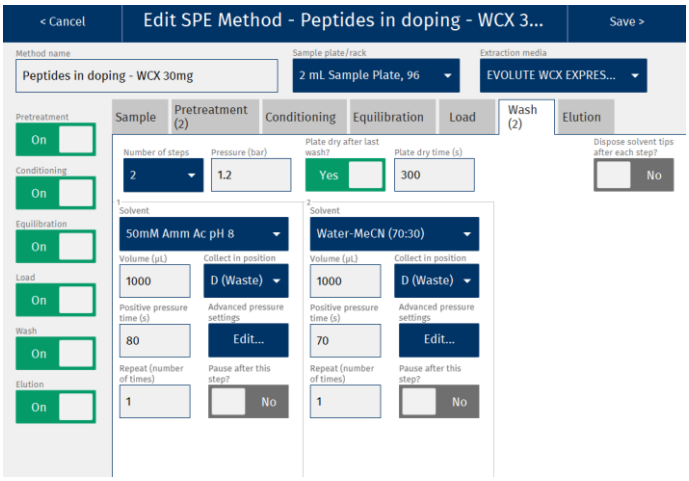
Number of steps	1
Pressure (bar)	1.2
Dispose solvent tips?	No
Equilibration step 1	
Solvent	50mM Ammonium Acetate (aq) (pH8)
Volume (µL)	1000
Collect in position	D (Waste)
Positive pressure time (s)	80
Advanced pressures	No
Repeat	1
Pause after this step	No

Load tab



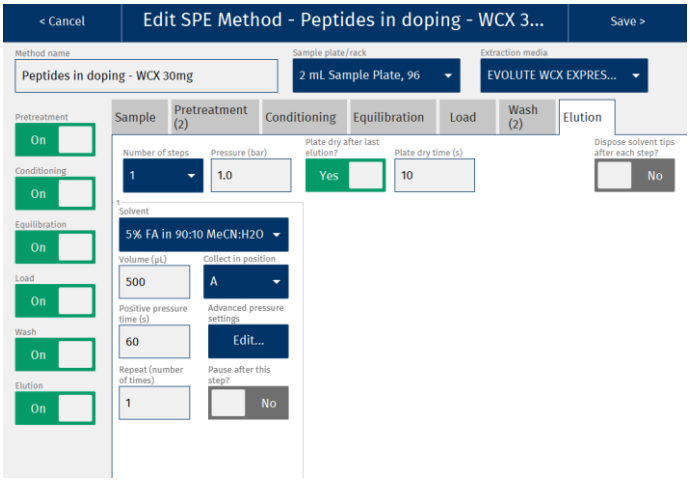
Sample volume (µL)	1000
Pressure (bar)	1.2
Premix?	Yes
Number of times	3
Positive pressure time (s)	90
Advanced pressures	No
Pause after each load?	No
Collect in position	D (Waste)
Rinsing	No
Tip conditioning	No

Wash tab



Number of steps	2
Pressure (bar)	1.2
Plate dry after last wash?	Yes
Plate dry time (s)	300
Dispose of solvent tips?	No
Wash step 1	
Solvent	50mM Ammonium Acetate (aq) (pH8)
Volume (µL)	1000
Collect in position	D (Waste)
Positive pressure time (s)	80
Advanced pressures	No
Repeat	1
Pause after step?	No
Wash step 2	
Solvent	Water:Acetonitrile (70:30)
Volume (µL)	1000
Collect in position	D (Waste)
Positive pressure time (s)	70
Advanced pressures	No
Repeat	1
Pause after step?	No

Elution tab



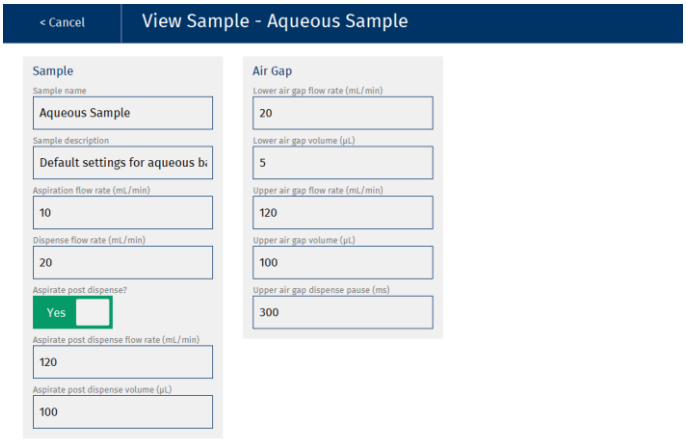
Number of steps	1
Pressure (bar)	1.0
Plate dry after last elution?	Yes
Plate dry time (s)	10
Dispose solvent tips?	No
Elution step 1	
Solvent	5% formic acid in 90:10 MeCN:H_2O
Volume (µL)	500
Collect in position	A
Positive pressure time (s)	60
Advanced pressures	No
Repeat	1
Pause after this step	No

Solvent Properties

Solvent description	
1	Acetonitrile
2	50mM Ammonium Acetate in Water (pH8)
3	Methanol
4	70:30 Water:Acetonitrile
5	5% Formic Acid in 90:10 Acetonitrile:Water

Solvent	**1**	**2**	**3**	**4**	**5**
Reservoir	Refillable				
Aspiration flow rate (mL/min)	10	10	10	10	10
Dispense flow rate (mL/min)	20	20	20	20	20
Aspirate post dispense?	Yes	Yes	Yes	Yes	Yes
Aspirate post dispense flow rate (mL/min)	120	20	120	120	120
Aspirate post dispense volume (µL)	100	100	100	100	100
Serial dispensing?	No	No	No	No	No
Chlorinated?	No	No	No	No	No
Highly volatile?	No	No	No	No	No
Lower air gap flow rate (mL/min)	20	20	20	20	20
Lower air gap volume (µL)	5	5	5	5	5
Upper air gap flow rate (mL/min)	120	20	120	120	120
Upper air gap volume (µL)	100	100	100	100	100
Upper air gap dispense pause (ms)	200	300	300	300	200
Tip conditioning?	Yes	Yes	Yes	Yes	Yes
Conditioning frequency	First aspiration	First aspiration	First aspiration	First aspiration	First aspiration
Number of times	3	3	3	2	3
Conditioning flow rate (mL/min)	20	10	10	20	20
Conditioning volume (% tip capacity)	100	100	100	100	100

Sample tab



Sample name	Aqueous sample
Aspiration flow rate (mL/min)	10
Dispense flow rate (mL/min)	20
Aspirate post dispense?	Yes
Aspirate post dispense flow rate (mL/min)	120
Aspirate post dispense volume (µL)	100
Lower air gap flow rate (mL/min)	20
Lower air gap volume (µL)	5
Upper air gap flow rate (mL/min)	120
Upper air gap volume (µL)	100
Upper air gap dispense pause (ms)	300

Extraction media tab



Name	EVOLUTE EXPRESS WCX 30mg 96-well plate
Manufacturer	Biotage
Part number	602-0030-PX01
Capacity volume (µL)	1000
Format	96 Positions, Plate/Columns
Comment	
Solvent dispensation height (mm)	-125.0
Sample dispensation height (mm)	-135.0
Aspiration height (mm)	-135.0

Sample plate tab



Name	2 mL Sample Plate, 96
Capacity volume (µL)	1800
Plate/rack height (mm)	-114.5
Format	96 Positions, Plate/Columns
Pipetting height	
Aspiration height (mm)	-162.0
Pretreatment dispensation height (mm)	-128.0