

Supplementary Data 11: LC Settings for LC-MS.

ACE_0353_MB04

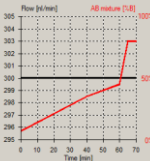
MS device	Thermo Orbitrap Elite																				
LC device	Thermo Easy-nLC 1000																				
ion source	Thermo Nanospray Flex																				
Analytical column	Self-packed fused silica capillary with integrated pico frit emitter; New Objectives PF360-75-15-N-5																				
column diameter	Length (L _c) = 22 cm; ID = 75µm; OD = 360 µm; emitter 15 µm																				
stationary phase	Reposil-Pur 120 C18-AQ, Dr. Maisch GmbH																				
particle diameter (d _p)	1.9 µm																				
Pore size	120 Å																				
Column ID	AC59																				
Column oven	Sonation column oven PRSO-V1																				
Column oven temp.	45°C																				
solvents	A: 0.1% FA in UPLC water B: 0.1% FA in UPLC ACN																				
gradient	Gradient <table><thead><tr><th>Time</th><th>Duration</th><th>Flow [nl/min]</th><th>%B</th></tr></thead><tbody><tr><td>00:00</td><td>N/A</td><td>300</td><td>7</td></tr><tr><td>50:00</td><td>50:00</td><td>300</td><td>45</td></tr><tr><td>60:00</td><td>10:00</td><td>300</td><td>80</td></tr><tr><td>70:00</td><td>10:00</td><td>300</td><td>80</td></tr></tbody></table> Add Del Up Down Flow [nl/min] AB mixture [%B] Solvents A: water B: acetonitrile	Time	Duration	Flow [nl/min]	%B	00:00	N/A	300	7	50:00	50:00	300	45	60:00	10:00	300	80	70:00	10:00	300	80
Time	Duration	Flow [nl/min]	%B																		
00:00	N/A	300	7																		
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60:00	10:00	300	80																		
70:00	10:00	300	80																		

MS device	Thermo Orbitrap Elite																				
LC device	Thermo Easy-nLC 1000																				
ion source	Thermo Nanospray Flex																				
Analytical column	Self-packed fused silica capillary with integrated pico frit emitter; New Objectives PF360-75-15-N-5																				
column diameter	Length (L _c) = 35 cm; ID = 75µm; OD = 360 µm; emitter 15 µm																				
stationary phase	Reprosil-Pur 120 C18-AQ, Dr. Maisch GmbH																				
particle diameter (d _p)	1.9 µm																				
Pore size	120 Å																				
Column ID	AC60																				
Column oven	Sonation column oven PRSO-V1																				
Column oven temp.	45°C																				
solvents	A: 0.1% FA in UPLC water B: 0.1% FA in UPLC ACN																				
gradient	Gradient <table><thead><tr><th>Time</th><th>Duration</th><th>Flow [nl/min]</th><th>%B</th></tr></thead><tbody><tr><td>00:00</td><td>N/A</td><td>300</td><td>7</td></tr><tr><td>50:00</td><td>50:00</td><td>300</td><td>45</td></tr><tr><td>60:00</td><td>10:00</td><td>300</td><td>80</td></tr><tr><td>70:00</td><td>10:00</td><td>300</td><td>80</td></tr></tbody></table> Add Del Up Down Flow [nl/min] AB mixture [%B] Solvents A: water B: acetonitrile	Time	Duration	Flow [nl/min]	%B	00:00	N/A	300	7	50:00	50:00	300	45	60:00	10:00	300	80	70:00	10:00	300	80
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00:00	N/A	300	7																		
50:00	50:00	300	45																		
60:00	10:00	300	80																		
70:00	10:00	300	80																		

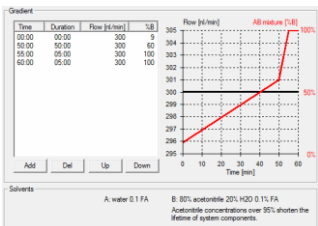
ACE_0372_MB16/18

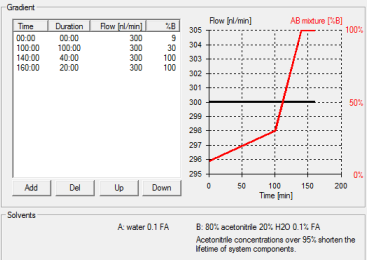
MS device	Thermo Orbitrap Elite
LC device	Thermo Easy-nLC 1000
ion source	Thermo Nanospray Flex
Analytical column	Self-packed fused silica capillary with integrated pico frit emitter; New Objectives PF360-75-15-N-5
column diameter	Length (L _C) = 40 cm; ID = 75µm; OD = 360 µm; emitter 15 µm
stationary phase	Reposil-Pur 120 C18-AQ, Dr. Maisch GmbH
particle diameter (d _p)	1.9 µm
Pore size	120 Å
Column ID	AC64
Column oven	Sonation column oven PRSO-V1
Column oven temp.	50°C
solvents	A: 0.1% FA in UPLC water B: 0.1% FA in UPLC ACN
gradient	

ACE_0393_MB01/02

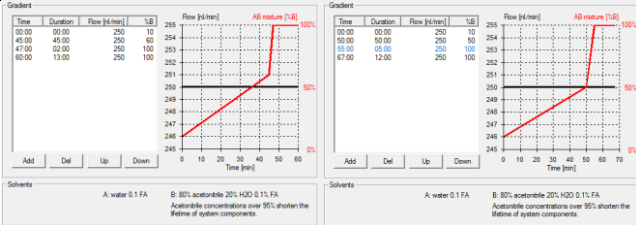
MS device	Thermo Orbitrap Elite																								
LC device	Thermo Easy-nLC 1000																								
ion source	Thermo Nanospray Flex																								
Analytical column	Self-packed fused silica capillary with integrated pico frit emitter; New Objectives PF360-75-15-N-5																								
column diameter	Length (L _c) = 40 cm; ID = 75µm; OD = 360 µm; emitter 15 µm																								
stationary phase	Reposil-Pur 120 C18-AQ, Dr. Maisch GmbH																								
particle diameter (d _p)	1.9 µm																								
Pore size	120 Å																								
Column ID	AC64																								
Column oven	Sonation column oven PRSO-V1																								
Column oven temp.	45°C																								
solvents	A: 0.1% FA in UPLC water B: 0.1% FA in UPLC ACN																								
gradient	Gradient <table><thead><tr><th>Time</th><th>Duration</th><th>Flow (µl/min)</th><th>%B</th></tr></thead><tbody><tr><td>00:00</td><td>00:00</td><td>300</td><td>0</td></tr><tr><td>40:00</td><td>40:00</td><td>300</td><td>35</td></tr><tr><td>40:00</td><td>00:00</td><td>300</td><td>45</td></tr><tr><td>45:00</td><td>05:00</td><td>300</td><td>80</td></tr><tr><td>50:00</td><td>05:00</td><td>300</td><td>80</td></tr></tbody></table> Flow (µl/min) AB mixture (%B)  Time [min]	Time	Duration	Flow (µl/min)	%B	00:00	00:00	300	0	40:00	40:00	300	35	40:00	00:00	300	45	45:00	05:00	300	80	50:00	05:00	300	80
Time	Duration	Flow (µl/min)	%B																						
00:00	00:00	300	0																						
40:00	40:00	300	35																						
40:00	00:00	300	45																						
45:00	05:00	300	80																						
50:00	05:00	300	80																						

MS device	Thermo Orbitrap Fusion LUMOS																				
LC device	Thermo Easy-nLC 1000																				
ion source	Thermo Nanospray Flex																				
Analytical column	Self-packed fused silica capillary with integrated pico frit emitter; New Objectives PF360-75-15-N-5																				
column diameter	Length (Lc) = 50 cm; ID = 75µm; OD = 360 µm; emitter 15 µm																				
stationary phase	Reprosil-Pur 120 C18-AQ, Dr. Maisch GmbH																				
particle diameter (dp)	1.9 µm																				
Pore size	120 Å																				
Column ID	AC73																				
Column oven	Sonation column oven PRSO-V2																				
Column oven temp.	50°C																				
solvents	A: 0.1% FA in UPLC water B: 0.1% FA, 20% H2O in UPLC ACN																				
gradient	Gradient <table><thead><tr><th>Time</th><th>Duration</th><th>Flow [µl/min]</th><th>S:B</th></tr></thead><tbody><tr><td>00:00</td><td>00:00</td><td>300</td><td>9</td></tr><tr><td>50:00</td><td>50:00</td><td>300</td><td>60</td></tr><tr><td>55:00</td><td>05:00</td><td>300</td><td>100</td></tr><tr><td>60:00</td><td>05:00</td><td>300</td><td>100</td></tr></tbody></table> Add Del Up Down Flow [µl/min] AB: solvent (%) 100% 60% 0% Solvents A: water 0.1% FA B: 80% acetonitrile 20% H2O 0.1% FA Acetonitrile concentrations over 95% shorten the lifetime of system components.	Time	Duration	Flow [µl/min]	S:B	00:00	00:00	300	9	50:00	50:00	300	60	55:00	05:00	300	100	60:00	05:00	300	100
Time	Duration	Flow [µl/min]	S:B																		
00:00	00:00	300	9																		
50:00	50:00	300	60																		
55:00	05:00	300	100																		
60:00	05:00	300	100																		

MS device	Thermo Orbitrap Fusion LUMOS
LC device	Thermo Easy-nLC 1200
ion source	Thermo Nanospray Flex
Analytical column	Self-packed fused silica capillary with integrated pico frit emitter; New Objectives PF360-75-15-N-5
column diameter	Length (L _c) = 50 cm; ID = 75µm; OD = 360 µm; emitter 15 µm
stationary phase	Reprosil-Pur 120 C18-AQ, Dr. Maisch GmbH
particle diameter (d _p)	1.9 µm
Pore size	120 Å
Column ID	AC73
Column oven	Sonation column oven PRSO-V2
Column oven temp.	50°C
solvents	A: 0.1% FA in UPLC water B: 0.1% FA, 20% H ₂ O in UPLC ACN
gradient	

MS device	Orbitrap Fusion Lumos
LC device	Thermo Easy-nLC 1200
ion source	Thermo Nanospray Flex
Analytical column	Self-packed fused silica capillary with integrated emitter (no frit); ESI Source Solutions PTC3-75-50-SP
column diameter	Length (L _c) = 44 cm; ID = 75 µm; OD = 360 µm; emitter 5 µm
stationary phase	Reprosil-Pur 120 C18-AQ, Dr. Maisch GmbH
particle diameter (d _p)	1.9 µm
Pore size	120 Å
Column ID	AC98
Column oven	Sonation column oven PRSO-V2
Column oven temp.	50°C
solvents	A: 0.1% FA in UPLC water B: 0.1% FA, 20% H ₂ O in UPLC ACN
gradient	

MS device	Orbitrap Fusion Lumos																																																								
LC device	Thermo Easy-nLC 1200																																																								
ion source	Thermo Nanospray Flex																																																								
Analytical column	Self-packed fused silica capillary with an integrated sintered frit; CoAnn Technologies ICT36007515F-50-5																																																								
column diameter	Length (LC) = 41 cm; ID = 75µm; OD = 360 µm; emitter 15 µm																																																								
stationary phase	Phenomenex Kinetex C18-XB core shell																																																								
particle diameter (dp)	1.7 µm (core shell)																																																								
Pore size	100 Å																																																								
Column ID	AC127																																																								
Column oven	Sonation column oven PRSO-V2																																																								
Column oven temp.	50°C																																																								
solvents	A: 0.2% FA in UPLC water B: 0.2% FA, 20% H2O, 80% ACN																																																								
gradient	Gradient <table><thead><tr><th>Time</th><th>Duration</th><th>Flow [nl/min]</th><th>%B</th></tr></thead><tbody><tr><td>00:00</td><td>00:00</td><td>350</td><td>2</td></tr><tr><td>05:00</td><td>05:00</td><td>350</td><td>6</td></tr><tr><td>47:00</td><td>42:00</td><td>350</td><td>25</td></tr><tr><td>62:00</td><td>15:00</td><td>350</td><td>40</td></tr><tr><td>63:00</td><td>01:00</td><td>350</td><td>98</td></tr><tr><td>70:00</td><td>07:00</td><td>350</td><td>98</td></tr></tbody></table> AddDelUpDown Flow [nl/min] AB mixture [%B] 0%50%100% Solvents A: water 0.1 FA B: 80% acetonitrile 20% H2O 0.1% FA Acetonitrile concentrations over 95% shorten the lifetime of system components. Gradient <table><thead><tr><th>Time</th><th>Duration</th><th>Flow [nl/min]</th><th>%B</th></tr></thead><tbody><tr><td>00:00</td><td>00:00</td><td>300</td><td>4</td></tr><tr><td>10:00</td><td>10:00</td><td>300</td><td>9</td></tr><tr><td>141:00</td><td>131:00</td><td>300</td><td>30</td></tr><tr><td>186:00</td><td>45:00</td><td>300</td><td>55</td></tr><tr><td>196:00</td><td>10:00</td><td>300</td><td>98</td></tr><tr><td>210:00</td><td>14:00</td><td>300</td><td>98</td></tr></tbody></table> AddDelUpDown Flow [nl/min] AB mixture [%B] 0%50%100% Solvents A: 98% H2O, 2% ACN, 0.2 FA B: 20% H2O, 80% ACN, 0.2% FA Acetonitrile concentrations over 95% shorten the lifetime of system components.	Time	Duration	Flow [nl/min]	%B	00:00	00:00	350	2	05:00	05:00	350	6	47:00	42:00	350	25	62:00	15:00	350	40	63:00	01:00	350	98	70:00	07:00	350	98	Time	Duration	Flow [nl/min]	%B	00:00	00:00	300	4	10:00	10:00	300	9	141:00	131:00	300	30	186:00	45:00	300	55	196:00	10:00	300	98	210:00	14:00	300	98
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210:00	14:00	300	98																																																						

MS device	Thermo Orbitrap Fusion Lumos
LC device	Thermo Easy-nLC 1200
ion source	Thermo Nanospray Flex
Analytical column	Self-packed fused silica capillary with integrated pico frit emitter; CoAnn Tech. ICT36007515F-50-5
column diameter	Length (L _c) = 42 cm (AC107) and 46 cm (AC106); ID = 75µm; OD = 360 µm; emitter 15 µm
stationary phase	AC107: Reprosil-Pur 120 C18-AQ, Dr. Maisch GmbH AC106 : Reprosil Fluosil 120 PFP, Dr. Maisch GmbH
particle diameter (d _p)	1.9 µm (AC107); 2.2 (AC106)
Pore size	120 Å
Column ID	AC107; AC106
Column oven	Sonation column oven PRSO-V2
Column oven temp.	50°C
solvents	A: 0.1% FA in UPLC water B: 0.1% FA in UPLC ACN
gradient	 Solvents A: water 0.1 FA B: 80% acetonitrile 20% H₂O 0.1% FA Automatic concentration over 95% shortens the lifetime of system components.

MS device	Thermo Orbitrap Fusion Lumos																				
LC device	Thermo Easy-nLC 1200																				
ion source	Thermo Nanospray Flex																				
Analytical column	Self-packed fused silica capillary with integrated pico frit emitter; CoAnn Tech. ICT36007515F-50-5																				
column diameter	Length (L _c) = 42 cm; ID = 75µm; OD = 360 µm; emitter 15 µm																				
stationary phase	Reposil-Pur 120 C18-AQ, Dr. Maisch GmbH																				
particle diameter (d _p)	1.9 µm																				
Pore size	120 Å																				
Column ID	AC107																				
Column oven	Sonation column oven PRSO-V2																				
Column oven temp.	50°C																				
solvents	A: 0.1% FA in UPLC water B: 0.1% FA in UPLC ACN																				
gradient	Gradient <table><thead><tr><th>Time</th><th>Duration</th><th>Flow [nl/min]</th><th>%B</th></tr></thead><tbody><tr><td>00:00</td><td>00:00</td><td>250</td><td>10</td></tr><tr><td>50:00</td><td>50:00</td><td>250</td><td>50</td></tr><tr><td>55:00</td><td>05:00</td><td>250</td><td>100</td></tr><tr><td>67:00</td><td>12:00</td><td>250</td><td>100</td></tr></tbody></table> AddDelUpDown Flow [nl/min] AB mixture [%B] Solvents A: water 0.1% FA B: 80% acetonitrile 20% H2O 0.1% FA Acetonitrile concentrations over 95% shorten the lifetime of system components.	Time	Duration	Flow [nl/min]	%B	00:00	00:00	250	10	50:00	50:00	250	50	55:00	05:00	250	100	67:00	12:00	250	100
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