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Acquisition of SRM Chromatographic Traces of Human SRMAtlas peptides using an Agilent 6460 Triple Quadrupole LC-MS system

This document provides the LC gradient applied for the targeted analysis of peptides in the Human SRMAtlas (www.srmatlas.org) and the elution time of 21 peptides as well as 11 iRT peptides acquired with this gradient to support dynamic SRM acquisition and transfer of elution / retention times.
The human SRMAtlas was developed with an Agilent G6530A Q-TOF LC-MS system to generate high-resolution, high-mass accuracy reference fragment ion spectra that were used to develop SRM assays on several G6460A QQQ LC-MS systems. The peptides described below were used to ensure strict retention time standardization across multiple MS instruments. Selecting 'RT_catalog Chipcube' under 'Elution Time Type' on the SRMAtlas query page provides retention time values that refer to the gradient below.

Equipment

- G6460A QQQ LC-MS system equipped with a HPLC Chip Cube interface and a 1260 Infinity HPLC (Agilent Technologies Inc., Santa Clara, CA)
- ProtID-Chip-150 (II), C18, 150 mm, 300 Å, 40 nL enrichment column (Agilent Technologies Inc., Santa Clara, CA)

Reagents

- Loading solvent: 0.1% formic acid in 3% acetonitrile / 97% water (CapPump)
- Solvent A: 0.1% formic acid in water (NanoPump)
- Solvent B: 0.1% formic acid in acetonitrile (NanoPump)

Peptide Separation

Peptides were loaded by the capillary pump delivering 0.1% formic acid in 3% acetonitrile / 97% water at a flow rate of 3 µL/min. Peptide separation was performed with a ProtID-Chip-150 (II) using 0.1% formic acid in water (A), 0.1% formic acid in acetonitrile (B) and a gradient from 3% to 43% B in 80 min and 43%-63% B from 80-85 min at a flow rate of 300 nL/min delivered by the nanoflow pump. The timetable is provided below.

	Time	B%	Flow	Max. Press.
1	0.00	3.0	0.3 µl/min	150 bar
2	80.00	43.0	0.3 µl/min	
3	85.00	63.0	0.3 µl/min	
4	85.10	80.0	0.3 µl/min	
5	90.00	80.0	0.3 µl/min	
6	90.10	3.0	0.3 µl/min	
7	98.00	3.0	0.3 µl/min	

Observed elution times (RT) in minutes of 21 peptides acquired with the gradient described above to ensure retention time standardization across multiple instruments.

Sequence	RT [min]	SSR	MW
LGGGGGGDGSR	3.1	3.6	888.4049
TSTSPPPEK	5.9	4.1	942.4658
SVSQQASQER	6.6	4.5	1118.5316
APEETIQSK	10.3	6.6	1001.5029
LGGGGGGDFR	14.0	12.9	891.4198
EGNSTVDCLK	14.5	13.9	1121.5023
VLDVNDNAPK	17.5	14.8	1083.5560
LGDPACPEIK	21.1	18.3	1098.5379
LLGGGGDFR	23.1	21.5	890.4610
HWYITTGPVREK	26.6	25.7	1485.7728
LLLGGDFR	34.3	29.4	889.5021
ADSYYEYLLK	37.8	30.9	1263.6023
VAQVLEGFITR	40.1	35.7	1231.6924
ILAIELENLK	41.4	34.5	1154.6910
LLLLDFR	46.4	36.5	888.5433
SVVLCLLEVAR	49.1	46.5	1257.7115
ILAPLLILDK	52.1	37.4	1107.7267
WNVEDVYEFIR	52.6	44.2	1468.6987
LLLLLDFR	54.7	41.7	1001.6273
DILLLPLQLPR	56.1	41.7	1289.8071
WPGFYILQWK	56.7	43.3	1336.6968

Observed elution times of iRT (Biognosys) peptides acquired with the gradient described above to support transfer of retention times.

iRT peptide sequence	RT [min]
LGGNEQVTR	11.2
GAGSSEPVTGLDAK	19.1
VEATFGVDESNAK	22.6
YILAGVENSK	25.1
TPVISGGPYEYR	27.8
TPVITGAPYEYR	29.5
DGLDAASYYAPVR	31.5
ADVTPADFSEWSK	35.4
GTFIIDPGGVIR	41.7
GTFIIDPAAVIR	46.9
LFLQFGAQGSPFLK	50.6