

Efficient Quality Control of Peptide Pools by UHPLC and Simultaneous UV and HRMS Detection

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Perpendicular drop method:

Alternative definition of peak groups based on resolution.

Table S1. Reproducibility (relative standard deviation, RSD) of peak areas for all peptides in the CEF advanced peptide pool using the perpendicular drop method. High relative standard deviations in peak areas of some peptides with low peak-to-valley ratio (p/v) indicate the need for peak groups. Peaks are grouped until nearly baseline separation ($R_s \geq 1.5$) is achieved to ensure robustness against resolution (R_s) changes. Peptides 8+14, 12+11, 21+9, 13+23, and 19+18+20+15 were combined into five peak groups.

Peptide number	Sequence	R _s ^{1,2}	Peak area ³ [μAU×min]	RSD peak area [%]
8*	KTGGPIYKR	/	/	/
14*	AVFDRKSDAK	/	/	/
10	ILRGSVAHK	1.8	0.939	0.2
12*	RLRAEAQVK	1.4	0.514	0.1
11*	RVRAYTYSK	10.3	1.224	0.1
32	TPRV TGGGAM	3.3	0.685	0.2
28	YPLHEQHGM	8.5	1.358	0.8
16	ATIGTAMYK	2.2	0.960	0.8
25	SRYWAIRTR	2.0	1.922	0.1
1	VSDGGPNLY	2.9	1.000	0.2
21*	RAKFKQLL	1.0	0.721	0.3
9*	RVLSFIKGTK	2.3	0.897	0.2
29	IPSINVHHY	4.3	2.021	0.1
22	FLRGRAYGL	/	1.208	0.4
13*	SIIPSGPLK	/	1.181	2.7
23*	QAKWRLQTL	/	1.749	0.3
19*	RPPIFIRRL	/	0.691	2.0
18*	LPFDKTTVM	/	1.461	0.8
20*	ELRSRYWAI	1.4	2.270	0.2
15*	IVTDFSVIK	1.9	1.162	0.2
17	DYCNVLNKEF	1.7	1.102	0.2
26	ASCMGLIY	1.5	1.343	0.2
27	RRIYDLIEL	1.8	0.818	0.4
24	SDEEEAIVAYTL	1.5	1.374	0.3
7	NLVPMVATV	9.5	0.989	0.2
4	FMYSDFHFI	3.3	2.184	0.1
31	EFFWDANDIY	4.3	2.924	0.2
30	EENLLDFVRF	6.1	0.867	0.1
6	GLCTLVAML	4.8	0.552	0.4
3	GILGFVFTL	2.0	0.895	1.0
2	CTELKLSDY	/	/	/
5	CLGGLTMV	/	/	/
8+14		2.1	1.891	0.2
12+11		10.3	1.726	0.1
21+9		2.3	1.618	0.1
13+23		2.6	2.947	0.2
19+18+20+15		3.2	5.574	0.1

¹ Calculated for peaks resulting from injection #10 using perpendicular drop method.

² Determined according to the formula of the European Pharmacopoeia, related to the next main peak.

³ Peak areas were normalized using peptide 1 as an internal standard and averaged over seven injections (injections #4-10).

* Evaluation as part of a peak group; / Values are not available mainly due to poorly resolved peaks.
grey: Values below 1.5, which suggest the formation of a peak group.

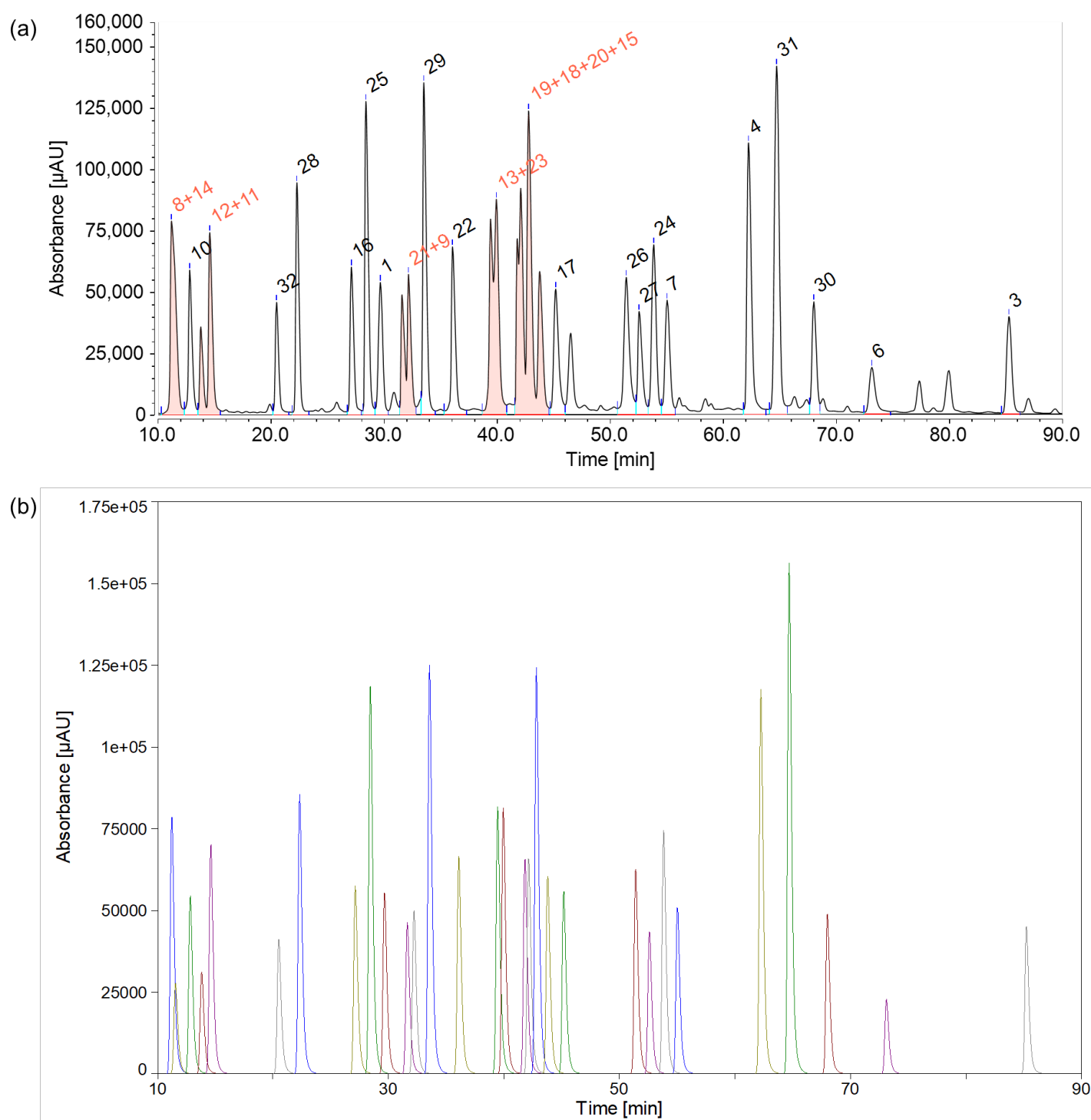


Figure S1: Examination of the CEF advanced peptide pool consisting of 32 peptides, listed in Table S1. **a)** Integration by a routine software based on a perpendicular drop algorithm. Some peaks (peptides 8+14, 12+11, 21+9, 13+23 and 19+18+20+15) have been combined due to a lack of separation ($R_s < 1.5$) indicated by a grey background in Table S1. **b)** Integration by peak fitting software PeakFit Vers. 4.12. The good reproducibility of peak areas eliminated the need for peak groups. Colors are assigned to the peaks for better visibility.