Efficient and sensitive peptide mapping approach by µPAC columns with ultralow sample loading

Yuan Lin¹, Xuefei Sun¹, Jeff Op de Beeck², Shanhua Lin¹, ¹Thermo Fisher Scientific Sunnyvale, CA, ²Thermo Fisher Scientific, Ghent, Belgium

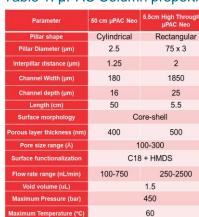
ABSTRACT

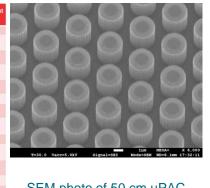
Peptide mapping is an important approach to analyze monoclonal antibodies for the identification of sequences, post-translational modifications and mutations. Traditional packed columns are usually used for peptide mapping at analytical flow rate with large sample loading. For improved separation and sensitivity, low flow chromatography has become the preferred LC method. Microfabricated pillar array columns (µPAC[™]) were introduced as an innovative technology for low flow. Here, peptide mapping is conducted using 50 cm µPAC Neo and 5.5cm High Throughput µPAC Neo columns. With only 20ng NISTmAb tryptic digest, 96.4% and 98.6% are achieved for heavy chain (HC) and light chain (LC) in 15 mins elution time using 50cm µPAC Neo column. The same sequence coverages are achieved in a 5 mins elution time using 5.5cm High Throughput µPAC Neo column.

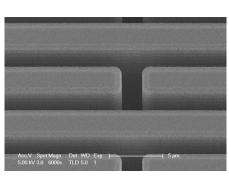
INTRODUCTION

Compared with packed bed (and monolithic) column technology microfabricated pillar array columns (µPAC[™]) are an innovative technology that enables high peak capacity separations at moderate LC pump pressures. Through the implementation of lithographic pattern transfer and deep reactive ion etching (DRIE) into silicon wafers, separation channels can be manufactured that contain micrometer sized silicon features that are perfectly positioned according to a pre-defined design. The introduction of perfectly-ordered separation beds eliminates any Eddy dispersion originating from heterogenous flow paths through the column and increases column permeability. It also provides high peak capacity separations at low flow rate with enhanced ionization sensitivity.

Table 1. µPAC Column properties







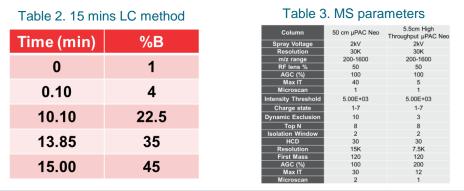
SEM photo of 50 cm µPAC SEM photo of 5.5 cm High Neo column

Throughput µPAC Neo column

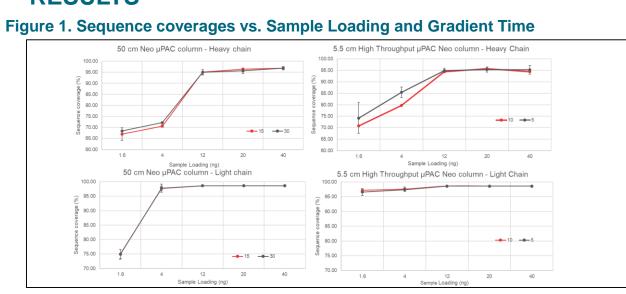
MATERIALS AND METHODS

Standard NISTmAb tryptic digest was dissolved in water with 0.1% formic acid. Ultra-low sample amounts of respectively 1.6, 4, 10, 20 and 40 ng were loaded on 50cm µPAC Neo and 5.5cm µPAC Neo High Throughput columns. In search of optimal performance, different flow rate and gradient time were investigated. Digested peptides mixtures were separated by the Thermo Fisher[™] Vanguish[™] Neo UHPLC system then directly analyzed by MS/MS on Thermo Scientific[™] Orbitrap Exploris[™] 480 Mass Spectrometers. Raw data were analyzed by Thermo Scientific[™] BioPharma Finder[™] 5.1 with automatic parameter values. Peptides with $|ppm error| \le 10ppm$, identified only by MS2, MS Area $\ge 1E5$, and Miss cleavage ≤ 2 are selected to calculated sequence coverage. Glycopeptides were manually confirmed by MS2 spectrum.

Solvent A is water with 0.1% formic acid (FA), and solvent B is 80% acetonitrile with 0.1% FA. 15 mins method and mass spectrometry parameters are listed as an example.

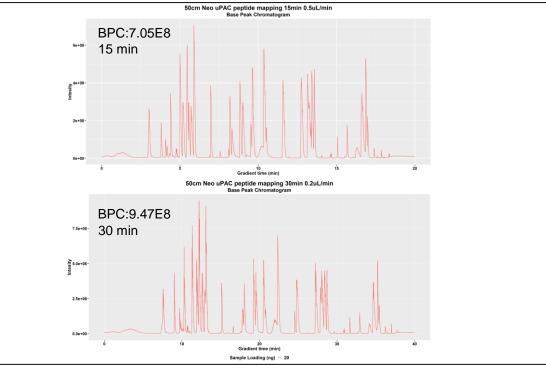


RESULTS



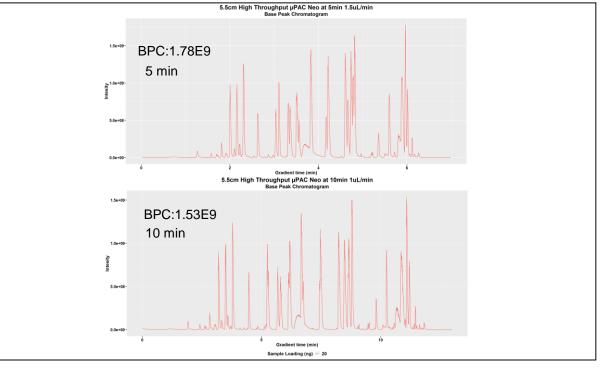
For both columns, sample loading increasing from 12 ng to 40 ng doesn't impact the sequence coverage and reaches the plateau with 20ng sample loading. The impact of gradient time is also investigated, for 50 cm µPAC Neo, 15min and 30min gradient gave the same results, which significant increase the analysis efficiency. For 5.5cm High Throughput µPAC Neo column, short gradient is better for low sample loading, like 1.6 ng of sample

Figure 2. Base peak chromatography of 50cm µPAC Neo column (15min vs 30min)



Base peak chromatography of 50cm µPAC Neo column shows that both 15 mins and 30 mins method can provide good separation for the NIST mAb digests.

Figure 3. Chromatography of 5.5cm High Throughput µPAC Neo column (5min vs 10min)

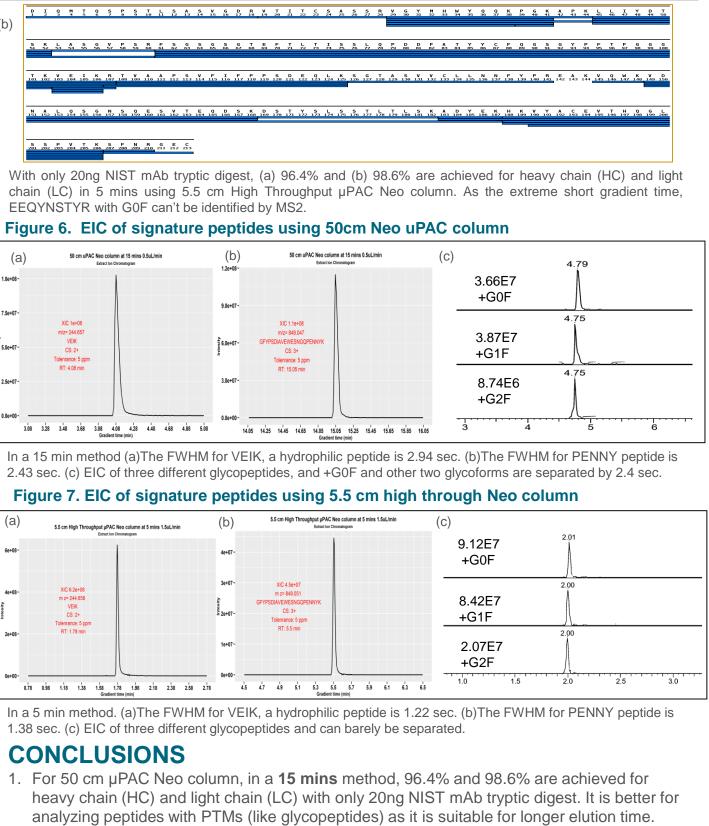


Base peak chromatography of 5.5cm High Throughput µPAC Neo column shows that both 5 mins and 10 mins method can provide good separation for the NIST mAb digests.

2	3 4	5	E 5 6 7	8	P A 9 10	L 11	12	K 13 J	P T 14 15	Q 5 16	T 17	L 18	T 19	L 20	T 21	C 22 2	T F 23 2	S S	G 5 26	F 27	S 28	L 29	S 30 3	Γ Α 11 3:	G 2 33	M 34	S 35	V 36 :	G W 37 38	I 3 39	R 40	Q 41 4	P P 12 43	G G 44	K 45	46	47 47	E W 48 49
D 52	I W 53 54	W 55 :	D D 56 57	K 58	K H	Y 61	N 62	P 63 (S L 64 65	K 5 66	D 67	R 68	L 69	T 70	I 71	S 72 7	K [73 7	D T 14 75	5 76	K 77	N 78	Q 79	V 1 80 8	/ L 11 82	. K 2 83	V 84	T 85	N 86 i	M D 37 88	P 8 89	A 90	D 91 9	T A 92 93	5 94	Υ 95	Y 96	C 97	A R 98 99
I 102	F N 103 104	F 105 1	Y F 06 107	D 108 1	V W 09 110	G 111	Q 112 1	G 113 1	T T 14 11	V 5 110	T 5 117	V 118	S 119	S 120 1	A 121 1	S 122 1	T 23 12	(G 24 12	Р 5 126	S 127	V 128	F 129	P 130 1	- A 31 13	P 12 13	S 3 134	S 135	K 136_1	S T 37 13	S 8 139	G 140	G 141 1	T A 42 14	A 3 14	L 1 145	G 146	C 147 1	L V 48 14
¥ 152 :	F P 153 154	E 155 1	PV 56157	T 158 1	V S 59 160	W 161	N 162 J	S 163 1	G A 64 16	L 5 16	T 5 167	S 168	G 169	V 170 1	H 171 1	T 172	F F 73 17	74 17	V 5 176	L 177	Q 178	S 179	S 180 1	G L 81 18	Y 2 18	S 3 184	L 185	S 186 1	S V 87 18	V 8 189	T 190	V 191 1	P S 92 19	5 3 19	S 4 195	L 196	G 197 1	T Q 98 19
I 202	C N 203 204	V 205 2	N H 96 207	K 208 2	P S 09 210	N 211	T 212 2	K 213_2	V D 114 21	K 5 210	R 217	V 218	E 219	P 220 2	K 221 2	S 222 2	C [23 22	D K 24 22	5 226	H 227	T 228	C 229	P 230 2	р С 31 23	P 2 233	A 3 234	P 235	E 236 2	L L 37 23	G 8 239	G 240	P : 241 2	S V 42 24	F 3 24	L 1 245	F 246	P 247 2	P K 248 249
D 252	T L 253 254	M 255 2	I S 56 257	R 258 2	T P 59 260	E 261	V 262 2	T 263 2	C V 164 26	V 5 260	V 267	D 268	V 269	S 270 2	H 271 2	E 272 2	D F 73 2	ЭЕ 7427	5 27€	K 277	F 278	N 279	W ' 280 2	Y V 81 28	D 12 283	G 3 284	V 285	E 286 2	V H 87 28	N 8 289	A 290	K 291 2	T K 92 29	Р 329	R 1 295	E 296	E 297 2	Q Y 198 291
T 302 :	Y R 303 304	V 305 3	V S 06 307	V 308 3	L T 09 310	V 311	L 312 3	H 313 3	Q D 114 31	W 5 31 (L 3 317	N 318	G 319	K 320 3	E 321 3	Y 1 322 3	K (23 32	C K 24 32	₩ 5 326	S 327	N 328	K 329	A 330 3	- P 31 33	A 2 333	P 3 334	I 335	E 336 3	K T 37_33	I 8 339	S 340	K 3 341 3	A K 142 34	G 3 34	0 1 345	P 346	R 347 3	E P 148 341
Y 352	T L 353 354	P 355 3	P S 56 357	R 358 3	E E 59 360	M 361	T 362 3	K 363 3	N Q 164 36	V 5 360	S 367	L 368	T 369	C 370 3	L 371 3	V 1 372 3	K (73_3;	S F 74 37	Υ 5 376	P 377	S 378	D 379	I 1 380 3	4 V 81 38	E 2 383	W 3 384	E 385	S 386 3	N G 87 38	Q 8 389	P 390	E 391 3	N N 92 39	Ι Υ 13 39	K 1 395	T 396	T 397 3	P P 198 391
D 402	S D 403 404	G 105 1	S F 06 407	F 408 4	L Y 09 410	S 411	K 412 4	L 113 4	T V 114 41	D 5 410	K 5 417	S 418	R 419	W 420 4	Q 121 4	Q (122 4)	G 1 23 42	N V 24 42	F 5 426	S 427	C 428	S 429	V 130 1	M H 31 43	E 12 433	A 3 434	L 435	H 436 4	N H 37 43	Y 8 439	T 440	Q 441 4	K S	L 3 44	S 1 445	L 446	S 447 4	P G
I 2	Q M 3 4	Ţ) 5 5 7	P S	5 T 10	L 11	S 1 12 1	A \$	5 V 4 15	G 16	D 17	R 18	V 19	T 20 2	I . 21 2	T C	5 S 3 24	A 1 25	S 26	S 27	R 28	V 29	G Y 30 3	M 1 32	H 33	W 34	Y 35	Q (36 3) K 7 38	P 39	G 40	K A	P 2 43	K 44	L 45	L 46	I \ 47 4	/ D 8 49
K 52	L A 53 54	S (55 5	5 V 6 57	P 9 58 5	R 60	F 61	S 62 6	G 9 03 0	5 G 14 65	S 66	G 67	T 68	E 69	F - 70 7	т I Л 7	L T 72 7:	· I 3 7	5 1 75	S 76	L 77	Q 78	P 79	D C 30 8	F 82	A 83	T 84	¥ 85	Y (86 8	F 7 88	Q 89	G 90	S G 91 9	2 93	P 94	F 95	Т 96	F (97 9	5 G 18 99
K 102 1	V E 03 104	I 105 10	(R 96 107	T V 108 10	A 19 110	A	P : 112 1	S V 13 13	(F	I 116	F 117	P 118	P 119 1	S 1 20 1	D I 21 1	E Q 22 12) L 3 12	K 4 125	5 126	G 127	T 128 J	A .29 1	S V 30 13	V 1 132	2 133	L 134	L 135 J	N N .36 13	4 F 37 136	Y 139	P 140 J	R E	í A 12 143	K 3 144	V 145	Q 146	W 1 147 1	K V 18 149

With only 20ng NIST mAb tryptic digest, (a) 96.4% and (b) 98.6% are achieved for heavy chain (HC) and light chain (LC) in 15 mins using 50cm µPAC Neo column.

Figure 5. Sequencies of the sequence of the se	uence coverage for HC and LC for 5.5 cm High Throughput µPAC Neo
QVTLRESGF	PALVKPTQTLTLTCTFSGFSLSTAGNSVGWIRQPPGKALEWL
123456789	9 10 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50
A D I W W D D K K	K H Y N P S L K D R L T I S K D T S K N Q V V L K V T N M D P A D T A T Y Y C A R D
1 52 53 54 55 56 57 58 5	99 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100
I F N F Y F D V	W W G Q G T T V T V S S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K
11 102 103 104 105 106 107 108 10	99 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 154
) Y F P E P V T V	VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
11 152 153 154 155 156 157 158 15	9 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 296
Y I C N V N H K F	PSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
01 202 203 204 205 206 207 208 20	09 210 211 212 213 214 215 216 217 218 219 229 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 24
(D T L M I S R T	T P E V T C V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N
51 252 253 254 255 256 257 258 25	59 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 298 299 298 294 295 295 296 297 288 299 386
S T Y R V V S V L 01 302 303 304 305 306 307 308 30	LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ 09 310 311 312 313 314 315 316 317 318 310 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 35
V Y T L P P S R E	E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V
551 352 353 354 355 356 357 358 35	59 360 361 362 363 364 365 366 367 388 389 390 391 392 393 394 395 396 397 398 399 400
L D S D G S F F L	LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
181 402 403 404 405 406 407 408 40	09 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449



- up the total analysis.

REFERENCES

1. Kavan, D. and Man, P. "MSTools - Web based application for visualization and presentation of HXMS data" Int. J. Mass Spectrom. 2011, 302: 53-58. http://dx.doi.org/10.1016/j.ijms.2010.07.030.

TRADEMARKS/LICENSING

© 2023 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others.

PO2023-70EN

2. For 5.5 cm High Throughput µPAC Neo column, in a **5 mins** method, 96.4% and 98.6% are achieved for heavy chain (HC) and light chain (LC) with only 20ng NIST mAb tryptic digest. It is better for high throughput screening to get the sequence coverage in 5 mins.

3. Small tryptic peptides such as TKPR and VSNK in heavy chain are not covered because their MS1 signals are too low to be detected in a 20ng sample loading.

4. Compared with traditional microflow columns which usually need 5 up of sample loading for peptide mapping, this ultralow sample loading of 20 ng using µPAC Neo columns will speed

