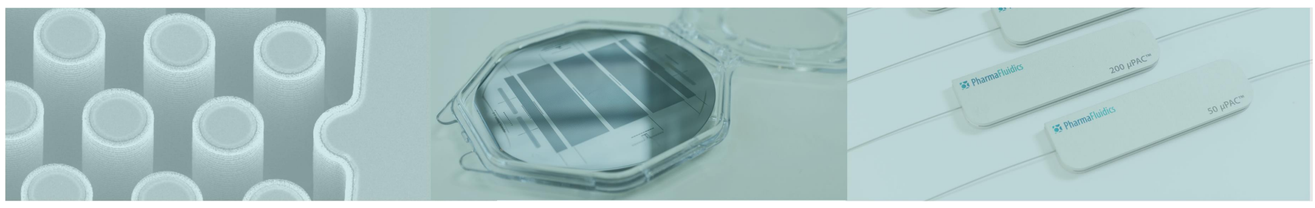

Micro Pillar Array Column Technology Coupled to PASEF: A Sensitive Approach to Analyze Host Cell Proteins

Packed bed technology

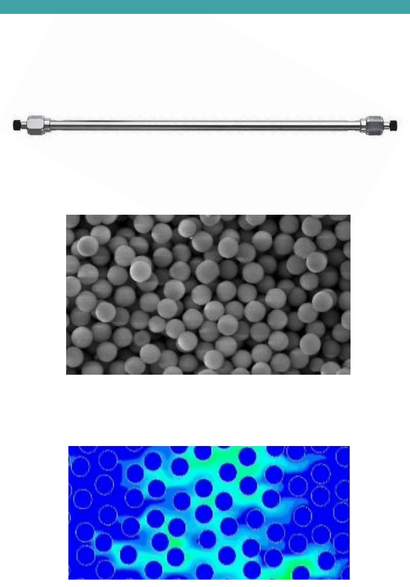

Fig. 1 Top: Packed bed column, Middle: SEM image showing porous silica particles, Bottom: Screenshot of CFD simulation of the flow in a heterogenous separation bed.

Pillar array technology

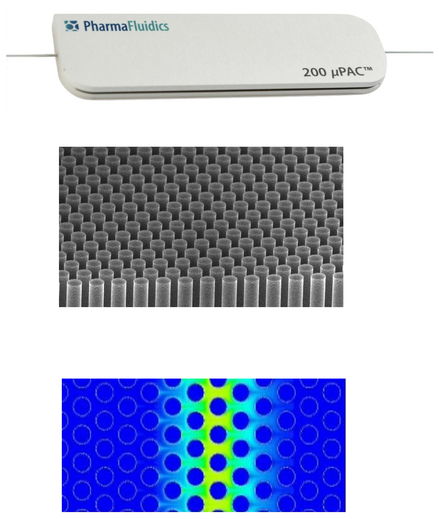

Fig. 2 Top: Micro pillar array column (µPAC™), Middle: SEM image showing the pillar array bed, Bottom: Screenshot of CFD simulation of the flow in a homogenous separation bed.

PASEF

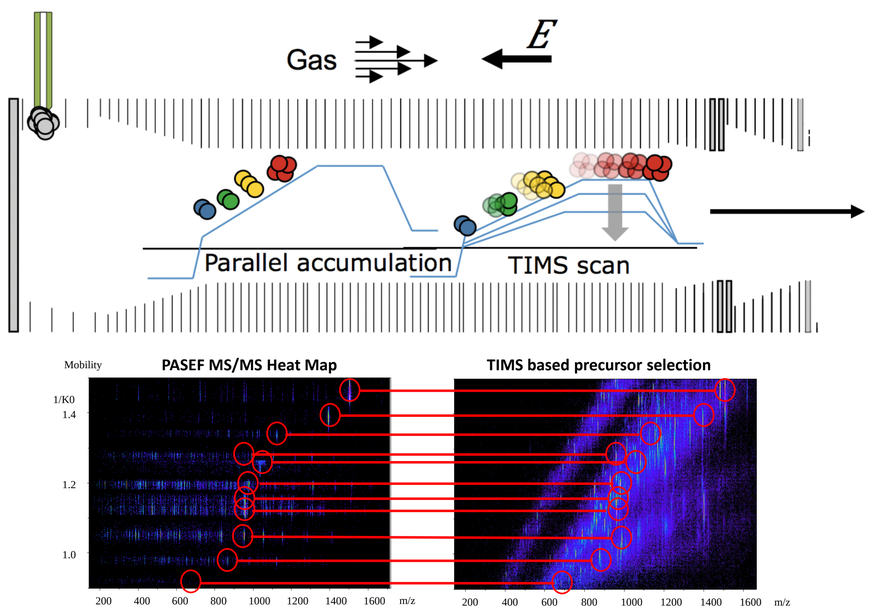

Fig. 3 Top: Accumulation and focusing of precursor ions in the TIMS cell. Bottom: PASEF scan allows 2 dimensional precursor isolation. (Courtesy of Bruker Daltonics)

INTRODUCTION

In biotherapeutic drug development and production, it is required to monitor the presence of host cell proteins (HCPs) down to the ppm level during manufacture and prior to product release. Whereas immunospecific assays such as ELISA are currently the gold standard to detect and quantify these proteins, this method has the disadvantage that potential protein impurities can be 'missed' during analysis (1). By using aspecific methods such as LC-MS/MS, these limitations can be overcome and previously unreported HCPs can be detected. In this workflow, we show how the combination of micro-chip based pillar array chromatography columns (µPAC™) (Fig. 2) (3) and parallel accumulation and serial fragmentation scans (PASEF) on the Bruker timsTOF PRO QTOF (Fig. 3) can be implemented to improve detection of HCPs in antibody preparations (4).

METHODS

The NISTmAb Reference Material 8671 was reduced using DTT and alkylated with iodacetamide prior to partial tryptic digestion according to (2). Peptides were separated on a PharmaFluidics 200 cm µPAC™ C18 column and a 25 cm long packed bed alternative using a nanoELUTE HPLC coupled to a timsTOF Pro ion mobility QTOF mass spectrometer (Bruker Daltonics). Using a direct sample injection method (without preconcentration onto a trap column or SPE cartridge), 1.5 µg of partially digested NISTmAb Reference Material 8671 was injected in triplicate on all columns. A 180 min gradient was used in a total run time of 210 min, and this at a flow rate of 500 nl/min. PASEF scans were recorded and searched against the mouse SwissProt database using Mascot at 1% FDR.

RESULTS

The fundamental benefit of nano LC-MS/MS towards analysis at higher flow rates (microflow LC and analytical scale LC) lies in the increased detection sensitivity that can be obtained. This increase in sensitivity is mainly due to an increase in electrospray ionization efficiency, but the quality of the LC separation definitely also plays an important role. The uniform separation bed of PharmaFluidics µPAC™ columns ensure that peptides elute sharper, and therefore they will enter the mass spectrometer at higher concentrations, hereby having a positive effect on detection sensitivity.

When comparing the µPAC™ column to a leading manufacturer's best packed bed alternative and previously reported data (2), a substantial increase in the number of HCPs that could be identified was observed (Fig. 4). With a total of 316 HCPs, this is the most comprehensive list of NISTmAb HCPs ever reported.

The high quality of the data is reflected by the increase in proteins that have been identified with high confidence (≥ 5 peptide matches). For these high confidence proteins, an increase up to 40% could be achieved when comparing to the packed bed alternative. Similar conclusions can be drawn from Fig. 5 where 14 documented HCPs have been compared in terms of peptide matches. The description and sequence coverage that was obtained for all HCPs identified with at least 3 peptides is listed in Table 1.

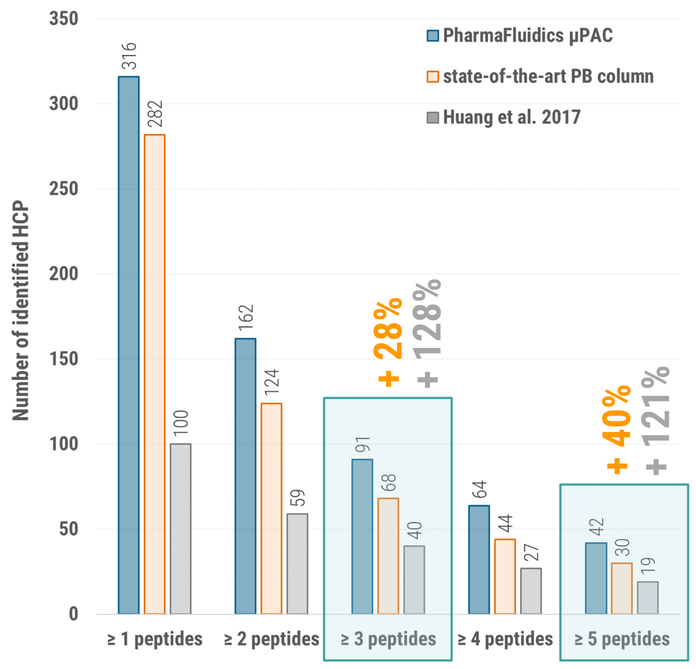

Fig. 4 HCPs identified in NISTmAb after partial digestion and LC-MS/MS analysis

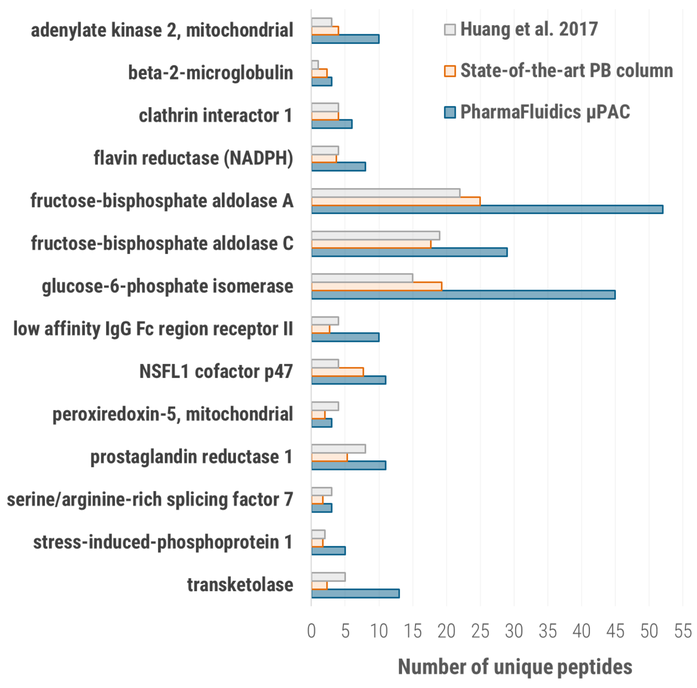

Fig. 5 Number of unique peptides identified for a selection of HCPs present in the NISTmAb standard.

CONCLUSIONS

- Comprehensive analysis of HCPs can be obtained by using µPAC™ nano LC columns in combination with PASEF technology
- 316 HCPs were identified in the NISTmAB reference material 8671, 91 of which with more than 3 peptide matches

#	Identified Proteins (91)	Unique pept	Coverage
1	Fructose-bisphosphate aldolase A	52	70%
2	Glucose-6-phosphate isomerase	45	56%
3	Fructose-bisphosphate aldolase C	29	65%
4	Protein ABHD11	21	38%
5	Polypeptide N-acetylgalactosaminyltransferase 2	15	24%
6	Polypeptide N-acetylgalactosaminyltransferase 6	15	18%
7	Heat shock cognate 71 kDa protein	13	22%
8	Polyadenylate-binding protein 1	13	20%
9	Semaphorin-4B	13	16%
10	Transketolase	13	15%
11	Heterogeneous nuclear ribonucleoprotein A1	12	23%
12	Apoptosis-inducing factor 1, mitochondrial	11	20%
13	NSFL1 cofactor p47	11	35%
14	Prostaglandin reductase 1	11	30%
15	Protein disulfide-isomerase A6	11	14%
16	Semaphorin-7A	11	18%
17	Adenylate kinase 2, mitochondrial	10	32%
18	Dystroglycan	10	7%
19	Low affinity immunoglobulin gamma Fc region receptor II	10	17%
20	Glycoprotein endo-alpha-1,2-mannosidase	9	13%
21	Eukaryotic translation initiation factor 4B	8	9%
22	Flavin reductase (NADPH)	8	38%
23	Protein NipSnap homolog 3B	8	34%
24	Bcl-2-associated transcription factor 1	7	4%
25	Elongation factor 1-alpha 1	7	13%
26	Fumarate hydratase, mitochondrial	7	16%
27	Syntaxin-12	7	21%
28	THO complex subunit 4	7	20%
29	Thyroid hormone receptor-associated protein 3	7	5%
30	Clathrin interactor 1	6	10%
31	Focal adhesion kinase 1	6	6%
32	Heterogeneous nuclear ribonucleoprotein A/B	6	20%
33	Heterogeneous nuclear ribonucleoproteins A2/B1	6	18%
34	Interferon regulatory factor 4	6	15%
35	Malate dehydrogenase, mitochondrial	6	19%
36	Methionine--tRNA ligase, cytoplasmic	6	6%
37	Non-specific lipid-transfer protein	6	8%
38	Programmed cell death protein 5	6	47%
39	Splicing factor 3A subunit 1	6	9%
40	Transgelin-2	6	19%
41	Adenylyl cyclase-associated protein 1	5	11%
42	Anionic trypsin-2	5	12%
43	Ataxin-2	5	5%
44	Cytokine receptor common subunit gamma	5	13%
45	E3 ubiquitin-protein ligase DTX3L	5	7%
46	Keratin, type I cytoskeletal 10	5	8%
47	Nipped-B-like protein	5	2%
48	Pancreatic lipase-related protein 2	5	12%
49	Splicing factor, proline- and glutamine-rich	5	6%
50	Stress-induced-phosphoprotein 1	5	9%
51	Transaldolase	5	17%
52	Ubiquitin-conjugating enzyme E2 variant 2	5	31%
53	UMP-CMP kinase	5	28%
54	ARF GTPase-activating protein GIT1	4	7%
55	B-cell linker protein	4	8%
56	Beta-hexosaminidase subunit beta	4	8%
57	Drebrin-like protein	4	11%
58	ELAV-like protein 1	4	14%
59	Exostosin-like 2	4	14%
60	Glutathione S-transferase P 1	4	24%
61	Golgi SNAP receptor complex member 2	4	15%
62	L-lactate dehydrogenase A chain	4	15%
63	Myelin expression factor 2	4	8%
64	Nucleoside diphosphate kinase B	4	28%
65	Papilin	4	4%
66	Peptidyl-prolyl cis-trans isomerase FKBP2	4	18%
67	Protein enabled homolog	4	7%
68	Protein PRRC2C	4	1%
69	Ribosome-binding protein 1	4	2%
70	Sulfhydryl oxidase 1	4	7%
71	Ubiquitin carboxyl-terminal hydrolase 8	4	4%
72	Beta-2-microglobulin	3	24%
73	Bifunctional glutamate/proline--tRNA ligase	3	3%
74	Cathepsin D	3	9%
75	CD2-associated protein	3	6%
76	Cleavage and polyadenylation specificity factor subunit 5	3	17%
77	Eukaryotic translation initiation factor 3 subunit G	3	12%
78	Heterogeneous nuclear ribonucleoproteins C1/C2	3	13%
79	High mobility group protein B1	3	13%
80	Keratin, type II cytoskeletal 1	3	4%
81	Keratin, type II cytoskeletal 5	3	5%
82	La-related protein 4B	3	6%
83	Mortality factor 4-like protein 2	3	13%
84	Peptidyl-prolyl cis-trans isomerase D	3	9%
85	Peroxiredoxin-5, mitochondrial	3	14%
86	pre-mRNA 3' end processing protein WDR33	3	4%
87	Protein LYRIC	3	7%
88	Ras GTPase-activating protein-binding protein 1	3	7%
89	RNA polymerase II-associated protein 3	3	5%
90	Serine/arginine repetitive matrix protein 1	3	4%
91	Treacle protein	3	3%

Table 1 List of HCPs detected in NISTmAb with at least 3 peptides.

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2. Huang et al.; Anal. Chem. 2017, 89, 5436-5444
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