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Dear Reader,

The biopharmaceutical sector has been evolving rapidly over the past years from traditional extraction-based pharmaceuticals, such as plasma proteins, to recombinantly expressed therapeutic monoclonal antibodies (mAbs). More recently, this sector has embraced advances in the form of mRNA vaccines against COVID-19 and viral vector-based therapies.

To ensure the quality of the biotherapeutics in-process and for product release, various high-quality reference materials are needed for both characterization and quantification to ensure safety and precision of dosing. Extensively studied and certified “performance“ or “system suitability” reference materials like the NISTmAb and the USP (United States Pharmacopeia) IgG standards are getting more popular to be used as reference points alongside the active biopharmaceutical for the analytical methods as well as for method development. In addition to these, the SILu™MAb product line provides stable isotope labelled mAbs as well as non-labelled calibrants, adding efficient options for improved accuracy and precision in clinical studies relying on mass spectrometric detection.

Many methods based on different modes of liquid chromatography (LC) and mass spectrometry (MS) are gaining even more importance when present in guidelines from regulatory agencies and pharmacopeias. These techniques are fundamental for advancing biopharmaceutical research and production processes. The methods are typically aimed to provide fast, accurate and reliable characterization and to assess and monitor critical quality attributes (CQA) of the biotherapeutic. These assays enable manufacturers to deliver safe, more consistent, and effective products that improve patient treatment outcomes.

In this issue of Analytix Reporter, we discuss methods for the effective protein fingerprinting of the viral vector AAV5 (Adeno Associated Virus, Serotype 5) by LC-MS and we present a fast titer determination of mAbs using a protein A modified monolithic wide pore silica column (page 10). If you are interested in learning more about the chromatography of biopharmaceutical compounds for research and quality control, visit us at **SigmaAldrich.com/PharmaQC**.

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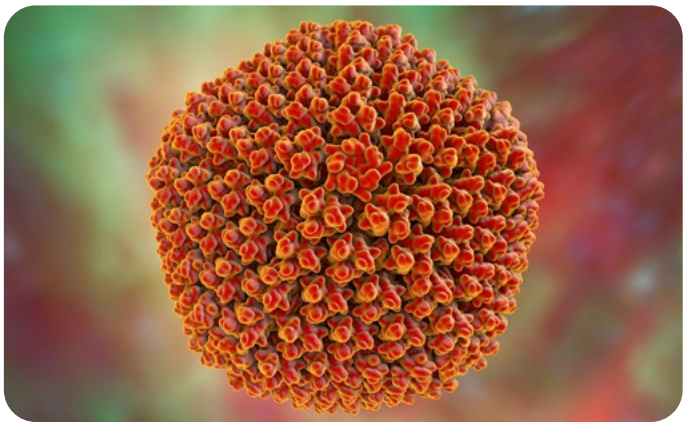
PHARMA & BIOPHARMA

Protein Fingerprinting of a Viral Vector, AAV5

Geoffrey Rule, Kevin Ray, Uma Sreenivasan, Cory Muraco, Pei Liu, Analytix@merckgroup.com

Introduction



The culture and use of adeno-associated virus (AAV) as a gene delivery device has seen much interest in recent years as a strategy for delivering targeted gene therapies towards muscle, nerve, liver, and eye disorders. This includes different AAV serotypes that target specific tissues as well as genetically engineered hybrid types with altered tissue specificity. In-depth characterization of the viral capsid proteins, as well as the genomic content, is essential to verify critical quality attributes of these particles. Both the amino acid sequence as well as post-translational modifications (PTMs) that are found on the viral capsid proteins can have an impact on the tissue tropism, efficacy, and immunogenicity of AAV.[1] PTMs that have been identified on AAV include phosphorylation, SUMOylation, ubiquitination, acetylation, methylation, and glycosylation.[2]

The USP released draft guidelines[3] for the analytical characterization of viral vectors in 2022 to provide method starting points for determination of critical quality attributes. A variety of tests are described to characterize the identity, purity, concentration, and potency of these viral vectors, among other traits. For determination of capsid identity, starting methods are provided that use Western blotting, reversed-phase HPLC with UV detection, and HPLC with MS detection. The latter approach describes the use of both intact mass analysis of the capsid proteins, as well as amino acid sequence analysis using peptide mapping. Here we describe our work to develop methods for protein fingerprinting of AAV serotype 5 using both intact mass analysis and peptide mapping. Several post-translational modifications of the viral proteins VP1, VP2 and VP3 were identified.

Experimental Methods

A system suitability mix, **MSRT1**, was prepared according to the instructions on the data sheet but with a final acetonitrile concentration of 1.6% (v/v). The injection volume was 10 µL. This solution is a mix of 14 isotopically labelled peptides injected prior to injection of samples to verify instrument performance.

AAV Production

AAV5 subtype (Q9YIJ1) was produced in HEK293 non-T VirusExpress® 293 AAV Production Cells using EX-CELL® CD HEK293 Viral Vector Medium. Three transfection plasmids carrying: 1. Replication and capsid genes; 2. certain adenovirus genes on a helper plasmid and 3. a plasmid with the gene of interest, in this case green fluorescence protein (GFP), were used. After three days of transfection, full AAV capsids, as well as product related impurities such as empty capsids and capsids filled with host DNA fragments or plasmid backbones, were produced. The HEK cells were then lysed with detergent, treated with benzonase/magnesium, to digest all unused plasmid DNA and host nucleic acids, and finally treated with 0.5 M sodium chloride. The clarified lysate was affinity purified on a fast protein liquid chromatography (FPLC) system (ÄKTA) using commercially available resin, from host cell proteins and digested nucleic acids. This process resulted in viral particle (vp) concentrations between 4E+12 and 2E+13 vp/mL as determined by droplet digital polymerase chain reaction (ddPCR).

Intact Mass Analysis

AAV particles were treated with 10% acetic acid for 15 minutes to dissociate AAV capsid assembly before centrifugation at 12,000 rpm for five minutes. Approximately 0.6 µg of total AAV capsid protein was injected for each run.

Chromatography was performed on BIOshell™ A400 Protein C4, 3.4 µm particle, and BIOshell™ IgG 1000 Å C4, 2.7 µm particle columns, along with a competitor column, mentioned in the draft guideline, for comparison. All had dimensions of 100 x 2.1 mm. Mass spectrometry was conducted on a Waters™ Xevo™ G2-S QTof in positive ion mode. The chromatographic conditions and instrument parameters used for the applied LC-UV-MS method are shown in **Tables 1** and **2** below.

Table 1. HPLC conditions for intact mass analysis

LC Conditions	
Instrument:	ACQUITY™ Premier UPLC
Columns:	BIOshell™ A400 Protein C4, 100 x 2.1 mm I.D., 3.4 µm (**66825-U**); BIOshell™ IgG 1000 Å C4, 100 x 2.1 mm I.D., 2.7 µm (**63288-U**); Competitor C4 column, 300 Å, 1.7 µm FPP, 100 x 2.1 mm I.D.
Mobile phase:	[A] 95:5 Water (0.1% (v/v) TFA: acetonitrile (0.1% (v/v) TFA) [B] Acetonitrile (0.1% (v/v) TFA)
Gradient:	

Time (min)	A%	B%
0.0	80	20
1.0	68	32
16.0	64	36
20.0	20	80
21.5	20	80
22.0	80	20
30.0	80	20

Flow rate:	0.2 mL/min
Column temp.:	80 °C
Detector:	UV @ 280 nm and mass spectrometry (**Table 2**)
Injection:	5 µL
Sample:	As described in text

Table 2. MS Conditions used for intact mass analysis

MS Conditions	
Instrument:	Waters™ Xevo™ G2-S QTof
Polarity:	Positive ion
Capillary (kV)	3.0 kV
Sampling Cone	120 V
Source Offset	120 V
Source Temperatures	125 °C
m/z range:	500-4000

Peptide Mapping

Digestion of purified AAV particles was conducted with both trypsin and, separately, chymotrypsin, using a Low-Artifact Digestion Buffer and a filter-assisted, sample preparation protocol. For details on this protocol, please see the online application note.[4]

BIOshell™ A160 Peptide C18, 2.7 µm particle column and an identical BIOshell™ column with 2 µm particles were used with gradient conditions outlined in the USP draft guidelines.[3] Both columns were 150 x 2.1 mm in dimension. For comparison, a competitor C18 column, mentioned in the draft guidelines, was evaluated using the same conditions (**Table 3**) and column dimensions.

Mass spectrometry was conducted on a Thermo QE Plus in positive ion mode using a scan range of 200 to 2000 m/z and data dependent MS2 of the top ten ions (**Table 4**).

Table 3. HPLC conditions used for peptide mapping

LC Conditions	
Instrument:	ACQUITY™ UPLC
Columns:	BIOshell™ A160 Peptide C18, 2.7 µm (**66905-U**); BIOshell™ A160 Peptide C18, 2 µm (**67243-U**); Competitor C18, 130 Å, 2.5 µm FPP column All 150 x 2.1 mm
Mobile phase:	[A] 0.1% Formic acid in water (v/v) [B] 0.1% Formic acid in acetonitrile (v/v)
Gradient:	

Time (min)	A%	B%
0.0	97	3
0.5	97	3
50.0	45	55
50.1	10	90
55.0	10	90
55.1	97	3
75.0	97	3

Flow rate:	0.25 mL/min
Column temp.:	40 °C
Pressure:	2890 psi/198 bar (2.7 µm column); 5725 psi/392 bar (2 µm column); 3555 psi/244 bar (2.5 µm column) at start of run.
Detector:	MS (**Table 4**)
Injection:	5 µL
Sample(s):	As described in text

Table 4. Mass spectrometer conditions used for peptide mapping

MS Conditions	
Instrument:	Thermo QE Plus
Polarity:	Positive ion
Spray Voltage:	3.5 kV
Capillary Temperature:	320 °C
Sheath Gas:	10
Aux gas:	5
S-Lens	50 V
m/z range:	200-2000
ddMS2:	Top 10

Results

Protein Fingerprinting

The draft USP guidelines provided a starting point for MS characterization of viral vectors by both intact capsid fingerprinting and peptide mapping. The draft guideline also describes an additional method, using UV detection and a 2-hour chromatographic run, for determination of capsid stoichiometry. While we did not replicate this method, we did use UV detection in conjunction with mass spectrometry fingerprinting of the intact viral capsids over a shorter 30-minute run. Integration of the UV detected peaks was then used to evaluate capsid stoichiometry. The combined LC-UV-MS analysis provided a convenient, one-method assessment of fingerprint and stoichiometry in a shorter run time.

Intact Mass Analysis of VP1, VP2, and VP3

The comparison of columns for separation of the intact capsid proteins is shown in **Figure 1**. Both the BIOshell™ A400 C4 and BIOshell™ IgG 1000 Å C4 columns provided good retention of the proteins using the gradient described in the draft guideline. Separation of VP1 from VP2 and VP3 was also achieved. The BIOshell™ A400 C4 column also provided partial separation of a VP3-clip from VP3. The competitor column gave faster elution with the same conditions but did not show a distinct peak for VP1. Deconvolution of the peaks observed on the BIOshell™ A400 C4 column resulted in the mass determinations as shown in **Figure 2**.

Both VP1 and VP3 were observed to be highly acetylated while VP2 showed a degree of phosphorylation. The presence of 0.1% TFA in the mobile phases was found to result in a substantial amount of TFA adduction on each of the proteins. Interestingly, other AAV serotypes (AAV2, AAV8) we have examined have not shown this same degree of TFA adduction. Replacing the TFA with 0.1% formic acid resulted in a loss of chromatographic separation of the three capsid proteins although the TFA adduction was eliminated (data not shown). The use of difluoroacetic acid (DFA) could be evaluated as a compromise between chromatographic separation and MS sensitivity.

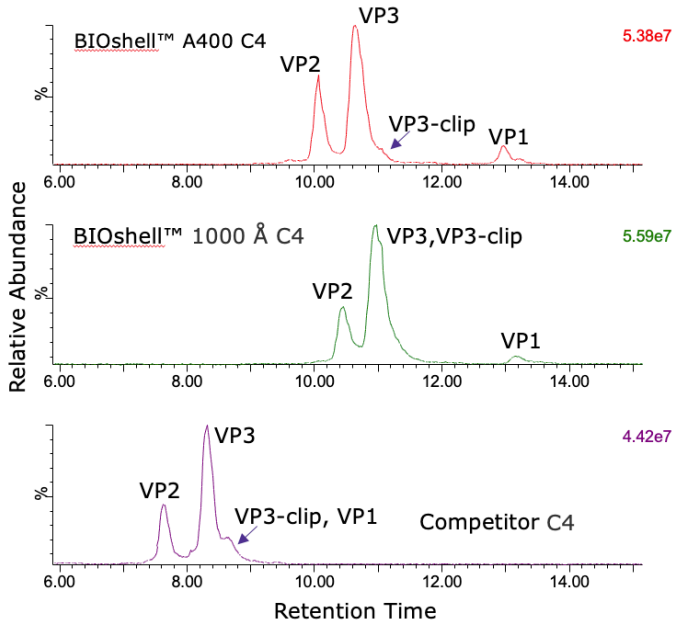


Figure 1. Total ion current (TIC) profile comparison of AAV5 capsid protein retention and separation on three columns evaluated.

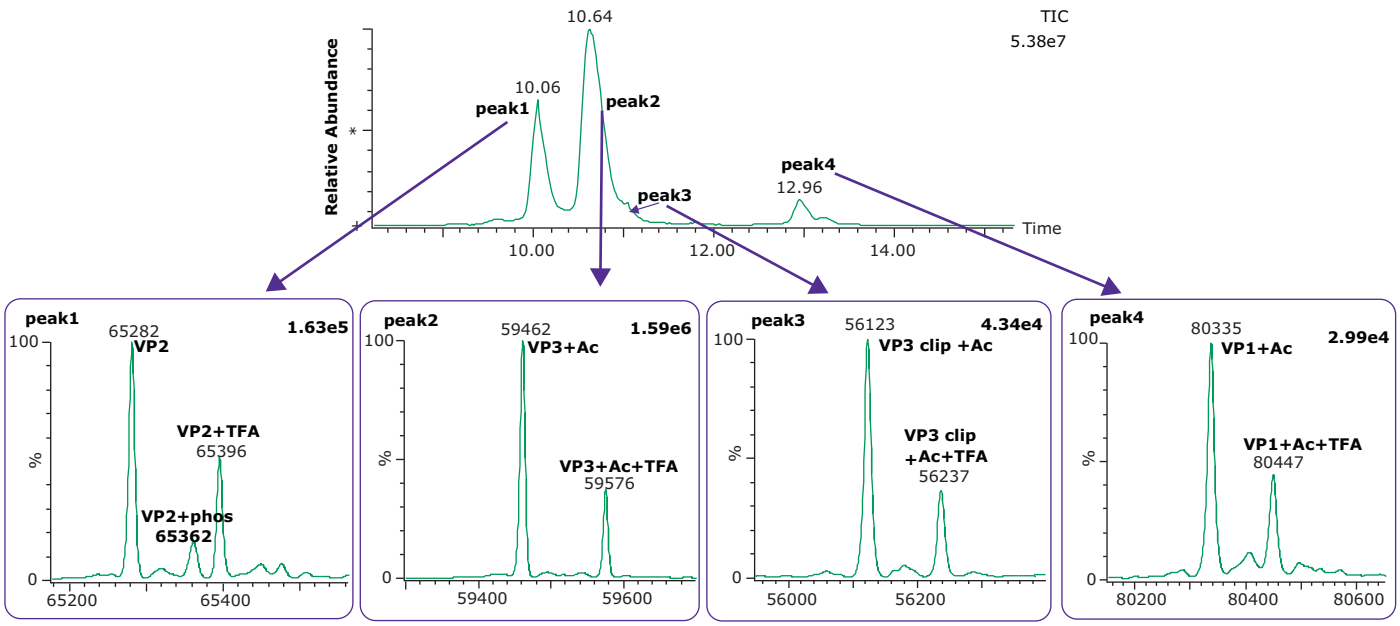


Figure 2. Deconvoluted spectra from each of four dominant peaks observed on the BIOshell™ A400 Protein C4 column.

A clipped form of VP3 was also observed, corresponding to the cleavage of the aspartic acid (695) - proline (696). This bond is known to be particularly labile to hydrolysis under acidic conditions and elevated temperature, and similar clip proteoforms have been reported by others.[5,6] **Table 5** shows the close agreement of the observed masses and the theoretical masses for the capsid proteins.

Table 5. Observed masses and the theoretical masses for the capsid proteins

Viral Protein	AA seq	Modification	Theoretical mass (Da)	Observed mass (Da)	Mass error
VP1	2-724	N-term Acetylation	80336	80335	0.0012%
VP2	138-724		65283	65282	0.0015%
VP3	194-724	N-term Acetylation	59463	59462	0.0017%
VP3-clip	194-695	N-term Acetylation	56125	56123	0.0036%

Using the same conditions described above, but with UV detection, a measurement of the relative abundance of the four main peaks was made, as shown in **Figure 3**.

While the stoichiometry of AAV capsids is sometimes suggested to have a specific ratio of capsid proteins, such as 1:1:10, Wörner et al.[7] nicely describe the process of capsid formation as being a stochastic sampling of capsid proteins available in the cell pool. AAV capsids are then made up of a highly heterogenous composition of three capsid proteins so that measured stoichiometry represents only an average composition. Combining both the UV and MS detection in the same run is an added convenience in the evaluation of samples.

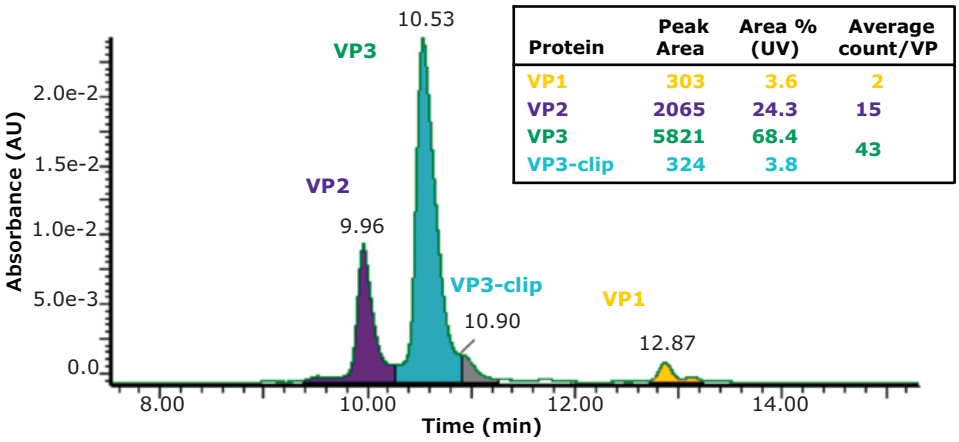
Peptide Mapping

For the comparison here, we used the column and conditions suggested in the draft USP guidelines; a competitor column and gradient conditions shown in **Tables 3** and **4**, above. This gradient is delivered over a 50-minute period. On both the competitor and BIOshell™ columns, good sequence coverage was obtained using tryptic digestion but with slightly better sequence coverage on the BIOshell™ columns due to retention of several small, early eluting peptides including VVTK, ADEVAR, GEPVNR, SLRVK, RIDDHFPKR (**Figure 4**). Importantly, the N-terminal sequence of VP2, (APTGK) was also identified on these columns, in addition to the N-terminal sequences of VP1 and VP3 as shown in **Figure 5**.

One large section of 60 amino acids was not covered by tryptic digestion alone, and so a separate digestion was performed with chymotrypsin to increase the overall sequence coverage. Similar multi-enzyme digestion of AAV capsids has been shown useful by others.[8] Using the two enzymes, separately, increased the coverage to 100% on the BIOshell™ columns.

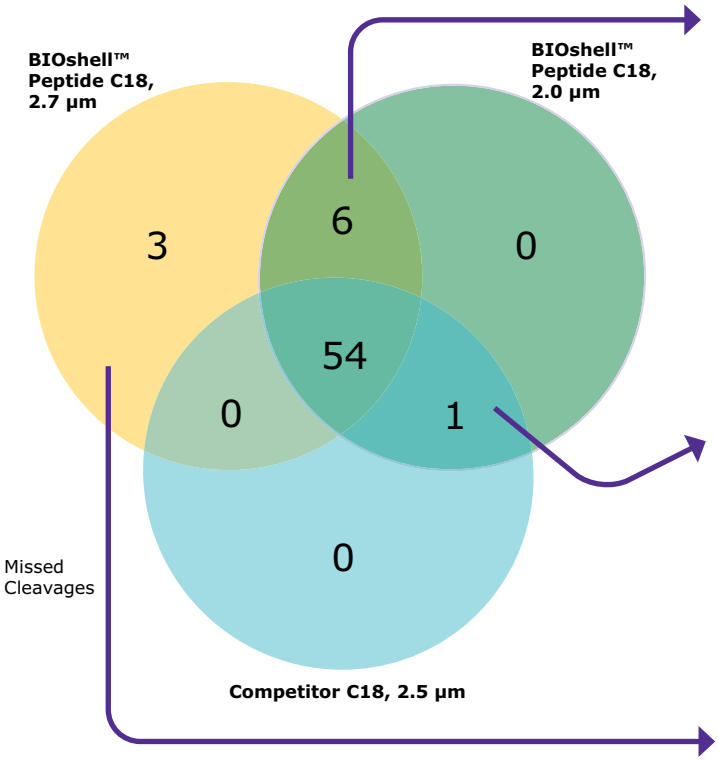
A summary of the PTMs identified, along with their percent abundance on each of the three columns, is shown in **Table 6**.

Both VP1 and VP3 were found to be 100% acetylated while at least four sites of phosphorylation were identified. Acetylation of the N-terminus of AAV capsids appears to be highly conserved across serotypes.[2] In addition to the deamination site, four sites of oxidation were observed with all being less than 1% abundant than the unoxidized forms. Overall, the agreement in the abundances of the PTMs between the columns was very good.



Protein	Peak Area	Area % (UV)	Average count/VP
VP1	303	3.6	2
VP2	2065	24.3	15
VP3	5821	68.4	43
VP3-clip	324	3.8	

Figure 3. Stoichiometry determination using UV (280 nm) detection on the BIOshell™ A400 Protein C4 column.



	Sequence	Δppm	RT	MS Area	Protein	start	end
Polar/Early eluters	VVTK	-1.84	1.89	4.24E+06	VP1,2,3	229	232
	ADEVAR	-0.65	3.05	3.35E+05	VP1	67	72
	GEPVNR	-0.45	3.22	2.44E+05	VP1	61	66
	SLRVK	-1.07	4.11	1.88E+05	VP1,2,3	301	305
	RIDDHFPKR	-0.77	7.98	1.99E+05	VP1,2	143	151
VP2 N-terminus	APTGK	1.18	1.58	8.11E+04	VP2	138	142

Sequence	Δppm	RT	MS Area	Protein	start	end
SGSVDGS NANAYFG YSTPWGY FDFNRFH SHWSPR	0	28	4.46E+06	VP1,2,3	252	285

Sequence	Δppm	RT	MS Area	Protein	start	end
STRTWVLPSY NNHQYREIK	0.36	15.3	1.02E+05	VP1,2,3	233	251
STRTWVLPS YNNHQYR	0.39	15.5	1.03E+05	VP1,2,3	233	248
DVYLQGPIW AKIPETGAHF HPSPAMGG FGLK	0.94	24.9	6.95E+04	VP1,2,3	600	630

Figure 4. Venn diagram showing the number of peptides identified using each of the three columns evaluated.

Table 6. Post-translational modifications and their abundance determined

Protein	Residue #	Modification	Peptide Sequence	% Abundance Competitor C18	% Abundance BIOshell™ 2 µm	% Abundance BIOshell™ 2.7 µm
VP1	1	S1+Acetylation	SFVDHPPDWLEEVGEGLR	100.00	100.00	100.00
VP3	1	S1+Acetylation	SAGGGGPLGDNNQGADGV GNASGDWHCDSTWMGDR	100.00	100.00	100.00
VP1	55	N55+Deamidation	GLVLPGYNYLGPGNGLDR	28.2	30.2	25.20
VP1,2,3	468	M468+Oxidation	NWFPGPMGR	0.53	0.56	0.67
VP1,2,3	474	W474+Oxidation	TQGWNLGSGVNR	0.21	0.21	0.20
VP1,2,3	568	M568+Oxidation	VAYNVGGQMATNNQSSTTAP ATGTYNLQEIVPGSVWMER	0.73	0.76	0.59
VP1,2,3	623	M623+Oxidation	IPETGAHFHPSPAMGGFGLKHPPPMMLIK; IPETGAHFHPSPAMGGFGLK	0.28	1.24	0.34
VP1,2,3	530	S530+Phosphorylation	IFNSQPANPGTTATY	5.70	5.80	5.90
VP1,2,3	~648	~S648+Phosphorylation	MLIKNTPVPGNITSF; IKNTPVPGNITSF	2.12	2.70	2.54
VP2	1-41	Phosphorylation	APTGKRIDDHFPKRKKARTEEDS KPSTSSDAEAGPSGSQQL	9.55	5.50	9.51
VP1,2	155-227	Phosphorylation	TEEDSKPSTSSDAEAGPSGSQQLQIPAQ PASSLGADTMSAGGGGPLGDNNQGADGV GNASGDWHCDSTWMGDR	7.68	11.59	9.10

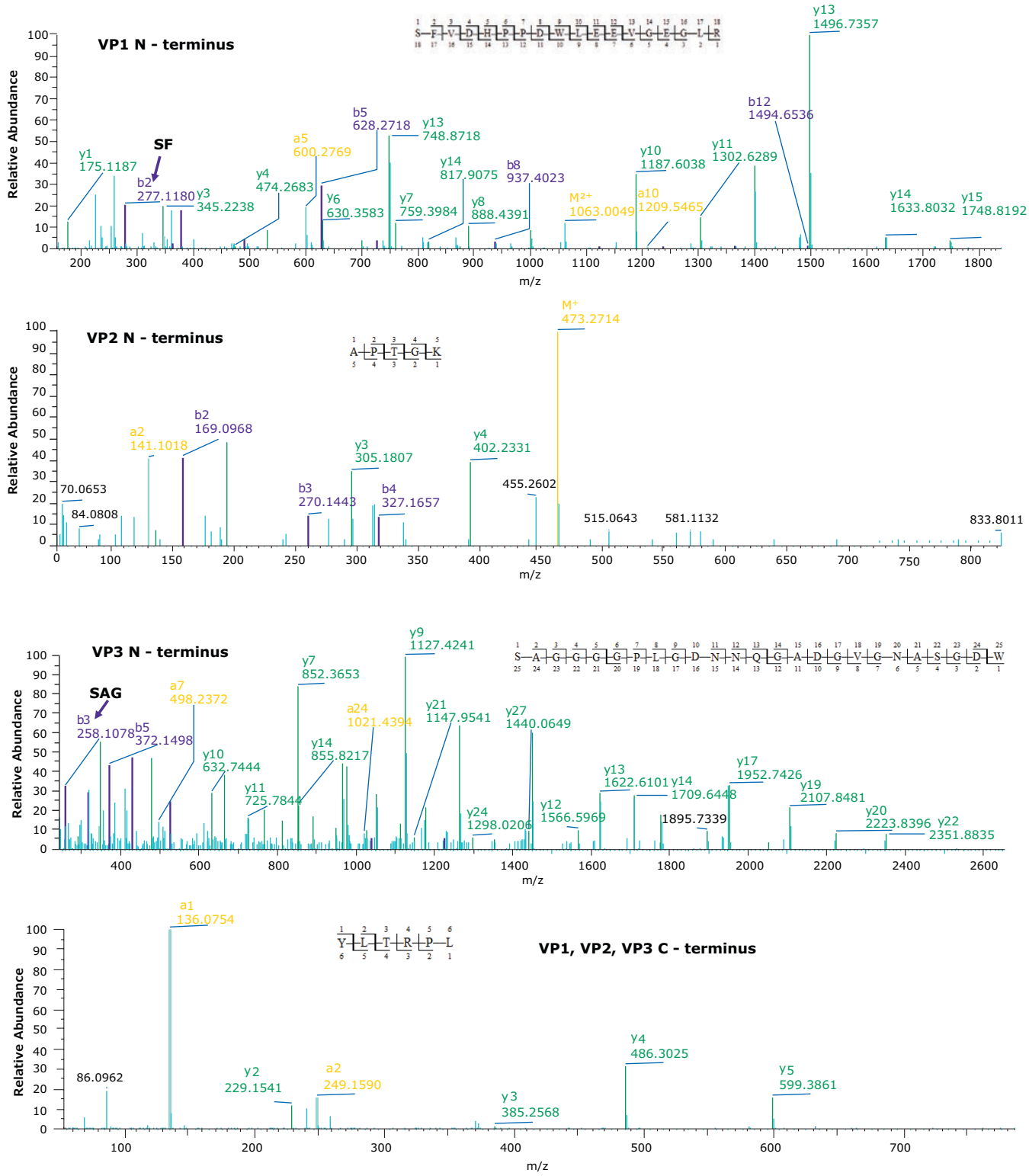


Figure 5. Product ion spectra providing sequence coverage of VP1, VP2, and VP3 N-termini as well as C-terminus of each.

Conclusions

Several column comparisons were shown to demonstrate uses of the BIOshell™ line of columns for characterizing viral vectors, in this case AAV serotype 5. Conditions outlined in the USP draft guideline were used for both intact mass fingerprinting of viral capsids and for peptide mapping experiments, but we suggest that further improvements in chromatography might be made with additional gradient optimization.

The BIOshell™ columns, particularly the A400 Protein C4 column, have proven to be effective in separating the three capsid proteins of AAV5 for intact mass analysis and stoichiometry evaluation. In addition, the BIOshell™ A400 Protein C4 column provides partial separation of the VP3-clip from VP3. The competitor column also shows partial separation of VP3-clip from VP3 but with coelution of VP1.

The BIOshell™ A160 Peptide C18 columns, in both the 2.7 and 2.0 µm particle sizes, proved to be useful in retaining short, polar peptides to provide slightly improved sequence coverage over the competitor column. Retention of the N-terminus of VP2 was only provided by the BIOshell™ columns.

The USP draft conditions for the mobile phase and gradient conditions were used in both approaches for characterizing capsids, but we suggest these conditions might benefit from further optimization with other AAV serotypes or specific PTMs.

Acknowledgements

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Featured Products

Description	Cat. No.
HPLC	
BIOshell™ A160 Peptide C18, 15 cm x 2.1 mm I.D., 2.7 µm	**66905-U**
BIOshell™ A160 Peptide C18, 15 cm x 2.1 mm I.D., 2.0 µm	**67243-U**
BIOshell™ A400 Protein C4, 10 cm x 2.1 mm I.D., 3.4 µm	**66825-U**
BIOshell™ IgG 1000 Å C4, 10 cm x 2.1 mm I.D., 2.7 µm	**63288-U**
Acetonitrile, LiChrosolv® hypergrade for LC-MS	**1.00029**
Formic acid, for LC-MS LiChropur™	**533002**
Trifluoroacetic acid, eluent additive for LC-MS, LiChropur™	**80457**
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VirusExpress® 293 AAV Production Cells	**VP002**
EX-CELL® CD HEK293 Viral Vector Medium, Chemically defined, animal component-free, without L-glutamine	**14385C**
Sample Preparation	
Acetic acid, for LC-MS LiChropur™	**5.33001**
Chymotrypsin Sequencing Grade, 4x25 µg	**11418467001**
Iodoacetamide (IAM), Single use vial of 56 mg	**A3221**
Low Artifact Digestion Buffer	**EMS0011**
SOLu-Trypsin, recombinant, expressed in Pichia pastoris, Proteomics Grade, liquid	**EMS0004**
Tris(2-carboxyethyl)phosphine (TCEP), 0.5 M, pH 7.0	**646547**
Microcon-30kDa Centrifugal Filter Unit with Ultracel-30 membrane	**MRCF0R030**

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Description	Cat. No.
Sample Preparation	
α-Chymotrypsin from bovine pancreas, suitable for protein sequencing, salt-free, lyophilized powder	**C6423**

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PHARMA & BIOPHARMA

Monoclonal Antibody Titer Determination Using a Chromolith® WP 300 Protein A Wide Pore Monolithic Silica HPLC Column

Analyzing Cetuximab, Trastuzumab, and Universal Antibody Standard by HPLC-UV

Gisela Jung, R&D Scientist, Benjamin Peters R&D Lab Head, Cory Muraco, Biomolecule Workflows Product Manager,
Analytix@merckgroup.com

Introduction

Monoclonal antibodies (mAbs) are often produced by fermentation processes where the amount and quality of the expressed product are essential and are monitored via affinity chromatography. This antibody titer determination can be carried out by using a Chromolith® WP 300 Protein A HPLC column. In this work, bind and elute experiments are shown for three different monoclonal antibodies: cetuximab, trastuzumab, and the universal antibody standard, human, on a Chromolith® WP 300 Protein A column (**Figure 1**).

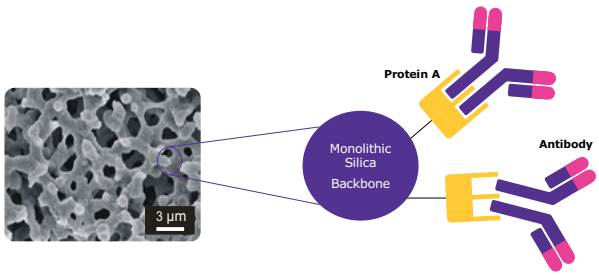

Figure 1. Schematic of the antibody interaction in the bimodal pore structure of the Chromolith® WP 300 Protein A column.

All three immunoglobulin variants were analyzed with the addition of a matrix-standard to demonstrate the ability of this column to handle high matrix load.

Experimental

Three mAbs in standard solutions and three simulated matrix samples were prepared using SILu™ Lite SigmaMAb standards and the matrix standard, SigMatrix Serum diluent, a 6% recombinant HSA (Human Serum Albumin) in phosphate-buffered saline (PBS) solution, pH 7.4, that can be used as a blank or matrix/diluent for proteinaceous analytes in LC-MS/MS calibrators, controls, or samples. The standard solutions and matrix samples were analyzed on a 25 x 2 mm I.D. Chromolith® WP 300 Protein A column under the conditions shown in **Table 1**.

Table 1. Conditions for titer determination of mAbs by HPLC-UV

LC Conditions		
Column:	Chromolith® WP 300 Protein A 25 x 2 mm I.D. (**1.52358**)	
Mobile phase:	[A] 100 mM Disodium hydrogen phosphate pH 7.4 [B] 100 mM Disodium hydrogen phosphate pH 2.5	
Gradient:	**Time (min)**	**A%** / **B%**
	0	100 / 0
	0.50	100 / 0
	0.55	0 / 100
	2.60	0 / 100
	2.65	100 / 0
	5.00	100 / 0
Flow rate:	0.38 mL/min	
Pressure:	As indicated	
Temperatures:	Column: 25 °C	
	Autosampler: 10 °C	
Detector:	UV @ 280 nm (analytical flow cell; 10 µL)	
Injection:	1.0 µL or as indicated for linearity determination	
Samples		
Universal Antibody Standard, Human	Sample without matrix (5 mg/mL): 1 mg SILu™ Lite SigmaMAb Universal Antibody Standard, human, in 200 µL water	
	Sample with matrix (1 mg/mL): 1 mg SILu™ Lite SigmaMAb Universal Antibody Standard, human, in 200 µL water + 800 µL 6% SigMatrix Serum diluent	
Cetuximab	Sample without matrix (5 mg/mL): 0.5 mg SILu™Lite SigmaMAb Cetuximab Monoclonal Antibody in 100 µL water	
	Sample with matrix (1 mg/mL): 0.5 mg SILu™Lite SigmaMAb Cetuximab Monoclonal Antibody in 100 µL water + 400 µL 6% SigMatrix Serum diluent	
Trastuzumab	Sample without matrix (5 mg/mL): 0.5 mg SILu™Lite SigmaMAb Trastuzumab Monoclonal Antibody in 100 µL water	
	Sample with matrix (1 mg/mL): 0.5 mg SILu™Lite SigmaMAb Trastuzumab Monoclonal Antibody in 100 µL water + 400 µL 6% SigMatrix Serum diluent	

Results

Analysis of Universal Antibody Standard, Human

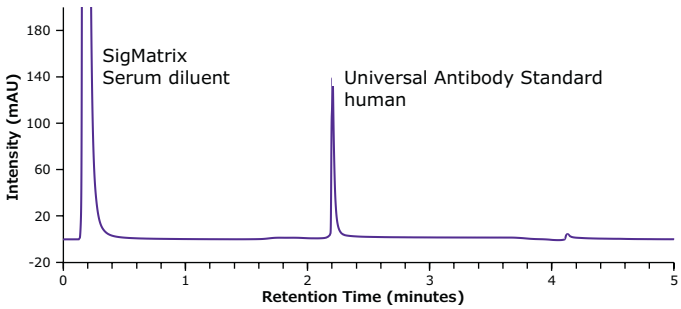

Figure 2. Analysis of SILu™Lite SigmaMAb Universal Antibody Standard, human, in matrix on a Chromolith® WP 300 Protein A (25 x 2 mm).

Table 1. Chromatographic data for analysis of SigmaMAb Universal Antibody Standard, human, in matrix

Peaks	Compound	Retention Time (min)	Peak Width at 50% (min)	Peak area (mAU* min)	Tailing factor	Column back-pressure (bar)
1	SigMatrix Serum diluent	0.22	-	-	-	
2	SILu™ Lite SigmaMAb Universal Antibody Standard, human	2.20	0.023	4.33	2.24	13

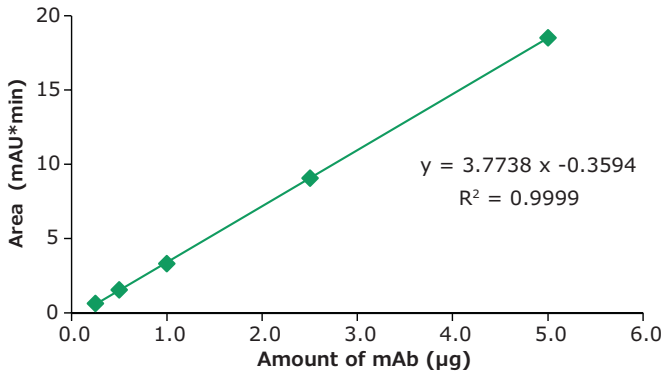

Figure 3. Linearity, Universal Antibody Standard, human, peak area vs injected amount of mAb.

Table 2. Linearity data for Universal Antibody Standard, human, using different injection volumes

Concentration (µg/µL)	Injection volume (µL)	Amount (µg)	Mean Area (mAU*min)
5.00	0.05	0.25	0.66
5.00	0.10	0.50	1.55
5.00	0.20	1.00	3.31
5.00	0.50	2.50	9.06
5.00	1.00	5.00	18.53

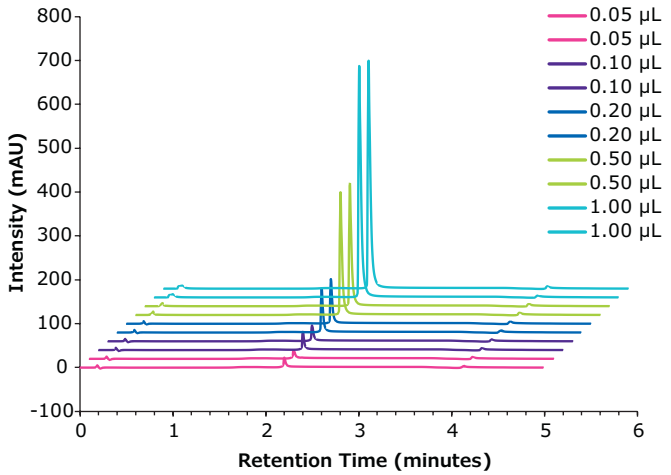

Figure 4. Linearity, Universal Antibody Standard, human, peak intensity with different injection volumes vs retention time.

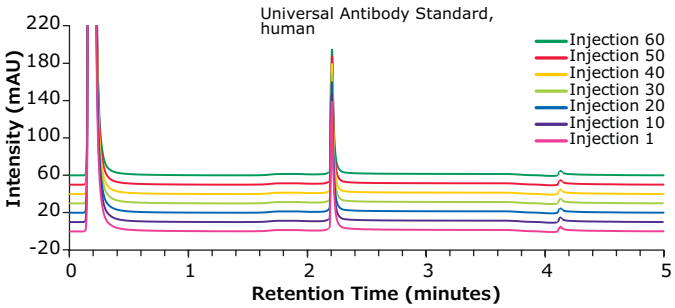

Figure 5. Analysis of Universal Antibody Standard, human, in matrix on a Chromolith® WP 300 Protein A (25 x 2 mm) – multiple injections.

Table 3. Chromatographic data for multiple injections of Universal Antibody Standard, human, in matrix solution

Injection #	Retention Time (min)	Peak Width at 50% (min)	Peak area (mAU* min)	Recovery (%)	Column back-pressure (bar)
1	2.20	0.023	4.28	99	13
10	2.20	0.024	4.16	96	14
20	2.20	0.023	4.34	100	16
30	2.21	0.024	4.22	97	18
40	2.21	0.024	4.25	98	19
50	2.21	0.024	4.29	99	21
60	2.21	0.025	4.23	98	22

Did you know...

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Analysis of Cetuximab

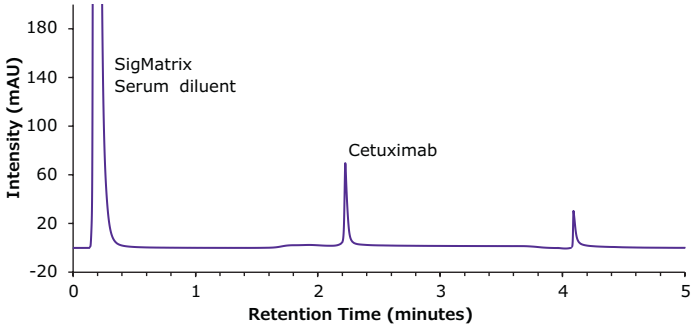

Figure 6. Analysis of SILu™Lite Cetuximab in matrix on Chromolith® WP 300 Protein A (25 x 2 mm).

Table 4. Chromatographic data for analysis of cetuximab in matrix

Peaks	Compound	Retention Time (min)	Peak Width at 50% (min)	Peak area (mAU* min)	Tailing factor	Column back-pressure (bar)
1	SigMatrix Serum diluent	0.21	-	-	-	
2	SILu™ Lite SigmaMAb Cetuximab	2.22	0.023	2.16	1.68	15

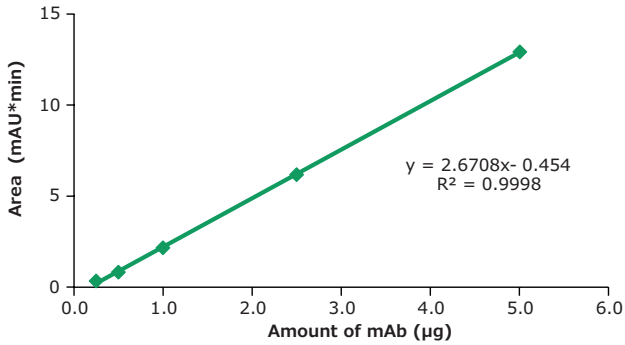

Figure 7. Linearity cetuximab peak area vs injected amount of mAb.

Table 5. Linearity data for cetuximab using different injection volumes

Concentration (µg/µL)	Injection volume (µL)	Amount (µg)	Mean Area (mAU*min)
5.00	0.10	0.50	0.77
5.00	0.20	1.00	2.34
5.00	0.50	2.50	7.31
5.00	1.00	5.00	16.04

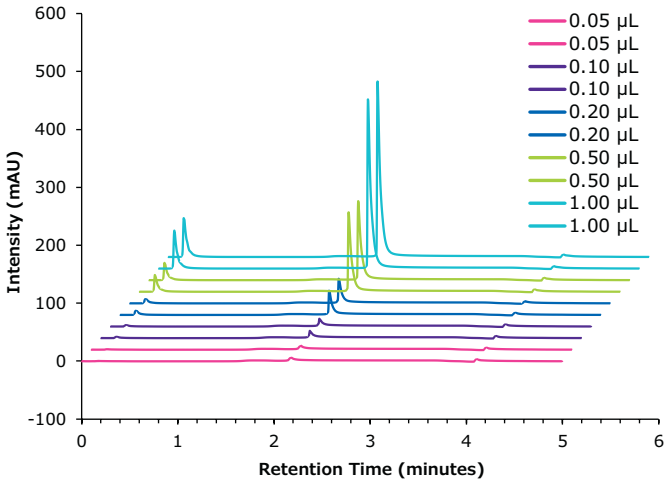

Figure 8. Linearity cetuximab peak intensity with different injection volumes vs retention time.

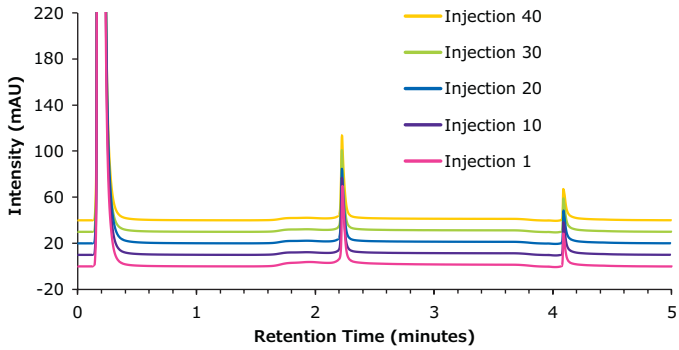

Figure 9. Analysis of cetuximab in matrix on a Chromolith® WP 300 Protein A (25 x 2 mm) - multiple injections.

Table 6. Chromatographic data for multiple injections of cetuximab in matrix solution

Injection #	Retention Time (min)	Peak Width at 50% (min)	Peak area (mAU* min)	Recovery (%)	Column back-pressure (bar)
1	2.23	0.024	2.17	99	15
10	2.22	0.024	2.14	98	15
20	2.22	0.024	2.11	96	16
30	2.22	0.024	2.21	101	19
40	2.22	0.024	2.39	109	20

Analysis of Trastuzumab

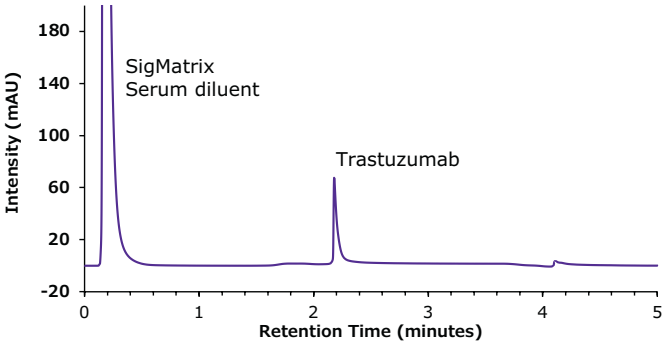

Figure 10. Analysis of SILu™ Lite Trastuzumab in Matrix on Chromolith® WP 300 Protein A (25 x 2 mm).

Table 7. Chromatographic data of analysis of trastuzumab in matrix

Peaks	Compound	Retention Time (min)	Peak Width at 50% (min)	Peak area (mAU* min)	Tailing factor	Column back-pressure (bar)
1	SigMatrix Serum diluent	0.22	-	-	-	
2	SILu™Lite SigmaMAb Trastuzmab	2.18	0.028	2.96	4.47	16

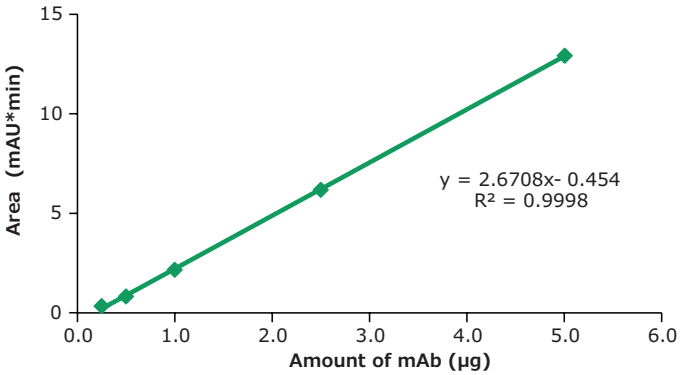

Figure 11. Linearity trastuzumab peak area vs injected amount of mAb.

Table 8. Linearity data for trastuzumab using different injection volumes

Concentration (µg/µL)	Injection volume (µL)	Amount (µg)	Mean Area (mAU*min)
5.00	0.05	0.25	0.33
5.00	0.10	0.50	0.82
5.00	0.20	1.00	2.17
5.00	0.50	2.50	6.18
5.00	1.00	5.00	12.93

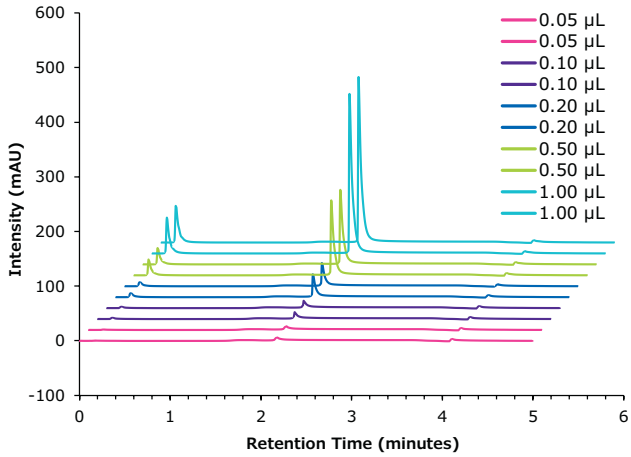

Figure 12. Linearity trastuzumab peak intensity with different injection volumes vs retention time.

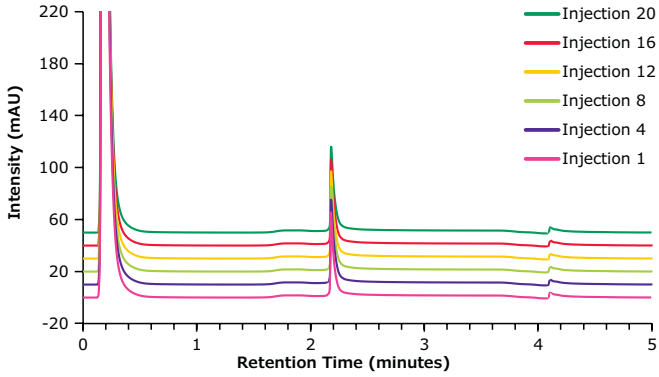

Figure 13. Analysis of trastuzumab in matrix on a Chromolith® WP 300 Protein A (25 x 2 mm) - multiple injections.

Table 9. Chromatographic data for multiple injections of trastuzumab in matrix solution

Injection #	Retention Time (min)	Peak Width at 50% (min)	Peak area (mAU* min)	Recovery (%)	Column back-pressure (bar)
1	2.18	0.027	2.83	94	16
4	2.18	0.029	3.06	102	16
8	2.18	0.029	2.99	99	17
12	2.18	0.028	3.01	100	18
16	2.18	0.029	3.04	101	19
20	2.18	0.029	3.01	100	20

Conclusion

It could be shown that all three antibodies (universal antibody standard, human, cetuximab, trastuzumab) can be analyzed reproducibly using the Chromolith® WP 300 Protein A column.

A fast separation, within about 2 min, can be achieved, with high linearity values, for a broad range of injected sample amounts.

Furthermore, it was demonstrated that the column can handle a high matrix load for multiple injections. A standardized matrix was used for this study (SigMatrix Serum diluent).

Featured Products

Description	Cat. No.
Chromolith® WP 300 Protein A 25 x 2 mm I.D.	**1.52358**
SILu™Lite SigmaMAb Universal Antibody Standard human, 1 mg	**MSQC4**
SILu™Lite SigmaMAb Cetuximab Monoclonal Antibody, recombinant, expressed in CHO cells, 0.5mg	**MSQC18**
SILu™Lite SigmaMAb Trastuzumab Monoclonal Antibody, recombinant, expressed in CHO cells, 0.5 mg	**MSQC22**
SigMatrix Serum diluent 6%	**D5322**
Water for chromatography (LC-MS grade) LiChrosolv® or tap fresh from an appropriate Milli-Q® system	**1.15333**
Disodium hydrogen phosphate anhydrous EMPROVE®	**1.06585**

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CANNABIS

Natural Cannabinoid and Cannflavin Profiling by HPLC-PDA

Caleb King, Director of Process Development; Reginald Gaudino, PhD, Chief Science Officer - Front Range Biosciences; Dominika Gruszecka, Shimadzu Scientific; Katherine Stenerson, Product Manager; Analytix@merckgroup.com

Introduction

Cannabinoids are a diverse group of diterpenoid compounds primarily observed in Cannabis and Rhododendron species. To date, over 120 phytocannabinoids have been identified and quantified in Cannabis extracts using analytical techniques such as High Performance Liquid Chromatography (HPLC). With the federal legalization of hemp, a type of Cannabis, and state-supported legalization measures for high-THC Cannabis, HPLC testing of dried plant material for psychotropic potency and therapeutic dosing has become part of nearly every piece of legislation. While numerous chromatographic methods have been developed for the detection and quantification of THCA, CBDA, CBGA, CBNA, and their decarboxylated forms, many do not account for the possibility of coelutions with other secondary metabolites in plant samples such as cannabinoids, flavonoids, and terpenes. To complicate analyses further, the metabolomes of different Cannabis varieties can vary greatly, resulting in chromatographic coelutions that are present in some extracts but not in others.

The method presented in this application note attempts to resolve most of the significant coelutions common to different types of Cannabis and was designed for laboratories interested in the quantification of minor cannabinoid and cannflavin constituents. Using this method, a total of 34 unique Cannabis analytes were quantified in less than 32 minutes. (**Table 1**). The method described has been successfully applied to not only leaf and flower Cannabis tissue, but cannabis/hemp products such as concentrates, oils, and cosmetic products.

Table 1. Abbreviations used for cannabinoids included in method

Compound	Abbreviation
Cannabidiorcin	CBDO
Cannabidivarinic acid	CBDVA
Cannabidivarin	CBDV
Cannabigerivarin	CBGV
Cannabigerovarinic acid	CBGVA
Cannabielsoin	CBE
Cannabidibutol	CBDB
Cannabichromeorcin	CBCO
Cannabidiolic acid	CBDA
Cannabigerolic acid	CBGA
Cannabigerol	CBG
Cannabidiol	CBD
Δ^9-Tetrahydrocannabivarin	Δ^9-THCV
Δ^9-Tetrahydrocannabivarinic acid	Δ^9-THCVA
Cannabichromevarin	CBCV
Cannabidiphorolic acid	CBDPA
Cannabichromevarinic acid	CBCVA
Cannabinol	CBN
Cannabinolic acid	CBNA
Cannabidiphorol	CBDP
Δ^9-Tetrahydrocannabinol	Δ^9-THC
Δ^8-Tetrahydrocannabinol	Δ^8-THC
Cannabicyclol	CBL
Δ^9-Tetrahydrocannabinolic acid	Δ^9-THCA
Cannabichromene	CBC
Cannabichromenic acid	CBCA
Cannabicyclolic acid	CBLA
Cannabidiol monomethyl ether	CBDM
Cannabigerol monoethyl ether	CBGM
Δ^9-Tetrahydrocannabiphorol	Δ^9-THCP
Cannabicitran	CBT
Δ^9-Tetrahydrocannabiphorolic acid	Δ^9-THCPA

Experimental

Sample Preparation

Air dried samples were milled to a powder using stainless steel ball-bearings with stems and seeds mechanically removed after pulverization. Between 0.2 and 0.5 grams of powder aliquots were solvent extracted in 10 mL of HPLC-grade acetone using ultrasonication for a total of 30 minutes, at a water temperature no greater than 35 °C. Sample extracts were syringe-filtered with 0.22 µm PTFE filters, followed by either a 2-fold dilution for leaf extracts or a 5-fold dilution for floral extracts.

Method

A Shimadzu Prominence-i LC-2030C Plus system, equipped with an Ascentis® Express C18 column and a photodiode array detector (PDA) was utilized to quantitate cannabinoid and cannflavin analytes in dried hemp tissues (**Table 2**).

Table 2. Instrument and mobile phase conditions

LC Conditions	
Column:	Ascentis® Express C18, 2.7 µm x 150 x 3 mm (**53816-U**)
Mobile Phase:	[A] Water, 8% (v/v) Methanol, 0.035% (v/v) Formic Acid, 1.8 mM Ammonium Formate; [B] Acetonitrile
Gradient:	See **Table 3**
Flow rate:	0.45 mL/min
Column temp.:	24 °C
Autosampler temp.:	15 °C
Detector:	PDA (various wavelengths, see **Table 4**)
Injection:	2 µL
Samples:	leaf extracts and floral extracts

Table 3. Gradient Conditions

Time (min)	% A	% B
0	59	41
1	58	42
10	37	63
16	32	68
26	19	81
28	13	87
29.5	0	100
30.5	0	100
31	59	41

Recommended Equilibration Time: 4 minutes

Table 4. Photodiode array detector conditions

Analyte	Quantitative Wavelength	Analyte	Quantitative Wavelength	Analyte	Quantitative Wavelength
Cannflavin B	340 nm	CBG	230 nm	CBL	230 nm
CBDO	230 nm	CBD	230 nm	Δ⁹-THCA	270 nm
CBDVA	270 nm	THCV	230 nm	CBC	280 nm
CBDV	230 nm	Δ⁹-THCVA	270 nm	CBCA	258 nm
CBGV	230 nm	CBCV	280 nm	CBLA	270 nm
CBGVA	270 nm	CBDPA	270 nm	CBDM	230 nm
CBE	230 nm	CBCVA	258 nm	CBGM	230 nm
CBDB	230 nm	CBN	280 nm	Δ⁹-THCP	230 nm
CBCO	280 nm	CBNA	258 nm	CBT	230 nm
CBDA	270 nm	CBDP	230 nm	Δ⁹-THCPA	270 nm
Cannflavin A	340 nm	Δ⁹-THC	230 nm	PDA Conditions Lamp: D2 Cell Temperature: 40 °C Polarity: + Slit Width: 8nm	
CBGA	270 nm	Δ⁸-THC	230 nm		

Calibrations

Calibration standards were prepared gravimetrically from certified reference materials (CRMs) or research grade isolates for 34 unique cannabinoids and cannflavins at concentrations ranging from 0.1 to 800 µg/mL. The linearity for all compounds was $R^2 \geq 0.99$ using linear correlations and a best-fit weighting of 1/concentration. The UV spectra of each analyte was recorded in a spectral library to assist in positive identification of cannabinoids and cannflavins in plant tissue extracts.

Results and Discussion

A cannabinoids/cannflavin standard and hemp flower extract are presented in **Figures 2A** and **B**, showing the signal at 230 nm. By monitoring the multiple wavelengths described in **Table 4**, sufficient resolution was obtained for all peaks to allow for adequate identification and consistent integration. A solvent containing no analytes was applied to all standards and samples for consistent baseline identification.

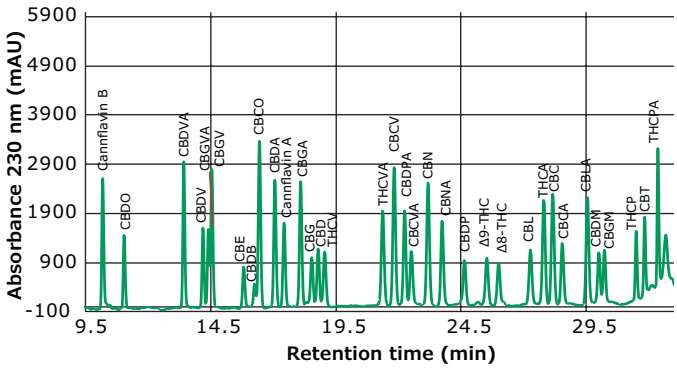

Figure 2A. Chromatogram of 34 cannabinoids/cannflavins at approximately 1 µg/mL. (Trace at 230 nm only shown.)

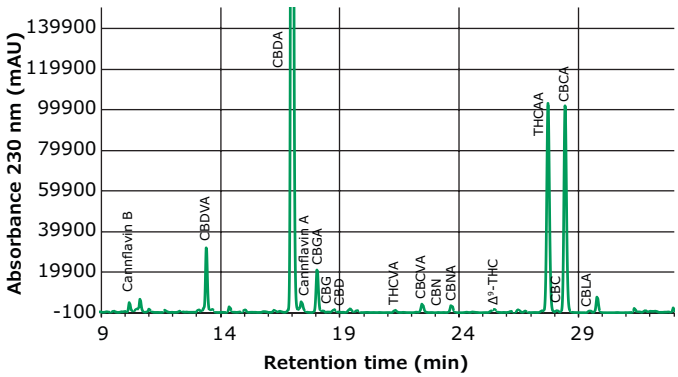

Figure 2B. Chromatogram of mix of hemp flower acetone extracts at a total dilution of 50X. (Trace at 230 nm only shown.)

Accuracy and Precision

Accuracy and precision were evaluated by spiking all 34 analytes onto homogenized low-cannabinoid producing Cannabis plant material (**Table 5**). The concentration of cannabinoids and cannaflavins present in non-spiked Cannabis plant material was subtracted from the observed concentrations in the spiked samples. To further evaluate the method's accuracy and precision, performance test (PT) samples provided by Merck were diluted by 5X and analyzed (**Table 6**).

Table 5. Average percent recoveries and percent relative standard deviations at approximately 5 µg/mL on-column or approximately 0.05 Weight %. (N=3 replicates).

Analyte	Avg. Recovery (%)	RSD (%)
Cannflavin B	101.0	3.5
CBDO	120.0	7.5
CBDVA	97.6	2.5
CBDV	112.0	2.8
CBGV	108.0	2.0
CBGVA	108.0	3.2
CBE	111.0	2.9
CBDB	99.5	1.8
CBCO	101.0	2.8
CBDA	103.0	2.3
Cannflavin A	111.0	7.5
CBGA	100.0	1.5
CBG	89.7	3.7
CBD	103.0	3.7
Δ⁹-THCV	94.2	3.3
Δ⁹-THCVA	91.5	0.6
CBCV	102.0	2.0
CBDPA	102.0	2.4
CBCVA	97.4	2.3
CBN	95.3	2.8
CBNA	97.0	2.4
CBDP	114.0	3.3
Δ⁹-THC	99.8	1.9
Δ⁸-THC	103.0	1.3
CBL	105.0	5.0
Δ⁹-THCA	99.7	2.1
CBC	85.6	4.9
CBCA	98.4	2.4
CBLA	98.7	2.6
CBDM	101.0	1.4
CBGM	98.9	2.1
Δ⁹-THCP	103.0	2.6
CBT	101.0	2.1
Δ⁹-THCPA	96.0	0.7

Table 6. Average percent recoveries and percent relative standard deviations of PT test samples. N=3 replicates per performance test.

Analyte	Avg. Recovery (%)	RSD (%)
CBDVA	106.0	2.0
CBDV	103.0	1.3
CBDA	123.0	1.8
CBGA	113.0	2.0
CBG	113.0	1.1
CBD	102.0	1.5
Δ⁹-THCV	104.0	1.4
Δ⁹-THCVA	98.8	1.9
CBN	101.0	1.3
Δ⁹-THC	111.0	1.2
Δ⁸-THC	107.0	1.4
Δ⁹-THCA	108.0	1.9
CBC	106.0	1.3
CBCA	105.0	2.1

Limits of Detection

Determined Limits of Detection (LOD) as S/N > 3 by weight percent are shown in **Table 7**.

Table 7. Calculated method Limits of Detection (LOD*) at 50X total dilution factor and 0.2 g of sample.

Analyte	LOD* (Wt%)
Cannflavin B	0.002
CBDO	0.003
CBDVA	0.006
CBDV	0.003
CBGV	0.003
CBGVA	0.006
CBE	0.003
CBDB	0.001
CBCO	0.003
CBDA	0.005
Cannflavin A	0.003
CBGA	0.005
CBG	0.003
CBD	0.003
Δ⁹-THCV	0.003
Δ⁹-THCVA	0.006
CBCV	0.003
CBDPA	0.006
CBCVA	0.006
CBN	0.003
CBNA	0.006
CBDP	0.003
Δ⁹-THC	0.003
Δ⁸-THC	0.003
CBL	0.003

Table 7. (Continued) Calculated method Limits of Detection (LOD*) at 50X total dilution factor and 0.2 g of sample

Analyte	LOD* (Wt%)
Δ⁹-THCA	0.005
CBC	0.003
CBCA	0.006
CBLA	0.006
CBDM	0.006
CBGM	0.006
Δ⁹-THCP	0.003
CBT	0.003
Δ⁹-THCPA	0.006

*LOD as S/N > 3:1

Conclusions

A gradient HPLC method was developed for the quantification of 34 unique compounds in Cannabis within a single injection. Solvent consumption per injection was less than 16 mL with an injection-to-injection runtime of 35 minutes. The method described allows for the quantitation of major and minor phytocannabinoids in Cannabis with minimal coelutions from flavonoids or terpenes; thus, reducing limits of detection while maintaining accuracy at ≤ ±20% and precision at ≤ ±10%.

Featured & Related Products

Description	Cat. No.
Ascentis® Express C18 column, 15 cm x 3 mm, 2.7 µm	**53816-U**
Millex® syringe filters, hydrophilic PTFE 0.20 µm, 33 mm, non-sterile	**SLLG033**
Water, 4L, HPLC grade	**WX0008***
Methanol, OmniSolv®, HPLC gradient grade	**MX0488****
Acetonitrile, HPLC gradient grade, OmniSolv®	**AX0142#**
Acetone, OmniSolv®	**AX0116'**
Formic Acid, 98-100%, for HPLC, LiChropur™	**543804**
Ammonium Formate for LC-MS, LiChropur™	**70221**
Cannflavin A, phyproof ® reference substance	**PHL85954**
Cerilliant® Cannabinoid Certified Reference Materials	
Cannabinoid Mixture (Acids) – 6 component solution, 500 µg/mL each analyte	**C-218**
Cannabinoid Mixture (Neutrals) – 8 component solution, 500 µg/mL each analyte	**C-219**
Δ⁹-tetrahydrocannabinol (Δ⁹-THC), 1 mg/mL in methanol	**T-005**
Δ⁸-tetrahydrocannabinol (Δ⁸-THC), 1 mg/mL in methanol	**T-032**
Δ⁹-tetrahydrocannabinolic acid (THCA), 1 mg/mL in acetonitrile	**T-093**
Tetrahydrocannabivarin (THCV), 1 mg/mL in methanol	**T-094**
Tetrahydrocannabivarinic acid (THCVA), 1 mg/mL in acetonitrile	**T-111**
Δ⁹-tetrahydrocannabiphorol (Δ⁹-THCP) - 1 mg/mL in methanol	**T-168**
Cannabidiol (CBD), 1 mg/mL in methanol	**C-045**
Cannabidiolic acid (CBDA), 1 mg/mL in acetonitrile	**C-144**
Cannabigerol (CBG), 1 mg/mL in methanol	**C-141**
Cannabigerolic acid (CBGA), 1 mg/mL in acetonitrile	**C-142**
Cannabichromene (CBC), 1 mg/mL in methanol	**C-143**
Cannabichromenic acid (CBCA), 1 mg/mL in acetonitrile	**C-150**
Cannabidivarin (CBDV), 1 mg/mL in methanol	**C-140**
Cannabidivarinic acid (CBDVA), 1 mg/mL in acetonitrile	**C-152**
Cannabinol (CBN), 1 mg/mL in methanol	**C-046**
Cannabinolic acid (CBNA), 1 mg/mL in acetonitrile	**C-153**
(±)- Cannabicyclol (CBL), 1 mg/mL in acetonitrile	**C-154**
Cannabicyclolic acid (CBLA), 0.5 mg/mL, in acetonitrile	**C-171**
Cannabigerivarin (CBGV), 1 mg/mL in methanol	**C-227**
Cannabigerovarinic acid (CBGVA), 1 mg/mL in acetonitrile	**C-226**
Cannabichromevarin (CBCV), 1 mg/mL in degassed methanol	**C-242**
Cannabichromevarinic acid (CBCVA), 1 mg/mL in 1% DIPEA/.05% ascorbic acid in acetonitrile	**C-256**
Cannabielsoin (CBE), 1 mg/mL in degassed methanol	**C-246**
Cannabichromeorcin (CBCO), 1 mg/mL in degassed methanol	**C-251**
Cannabidiphorol (CBDP), 1 mg/mL in 1% DIPEA/.05% ascorbic acid in acetonitrile	**C-240**
Cannabicitran (CBT), 1 mg/mL in degassed acetonitrile	**C-241**

*Available in North America only, alternative 270733 or **34877-M** or ultra high purity from suitable Milli-Q® system

** Available in North America only, alternative **1.06007**

Available in North America only, alternative **1.00030**

' Available in North America only, alternative **1.00020**

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CANNABIS

A New Thermal Desorption Tube for Air Sampling of Cannabis Terpenes

Jamie Brown, Senior R&D Scientist, Analytix@merckgroup.com

Introduction

There is a growing interest in sampling terpenes from the air.[1,2,3] Since gas chromatography is the most common technique used for terpene analysis, a new thermal desorption (TD) tube specific for sampling terpenes relevant to cannabis and hemp was developed. This provides a means to monitor/determine terpene concentrations in the cultivation environment.

Thermal desorption is less wasteful and more environmentally friendly than single-use devices since the TD tubes can be reused and no (toxic) solvents are required for the sample preparation workflow. TD is capable of sampling a wide range of terpene concentrations by either adjusting the sample volume (how much air pulled through the tube during sampling) or by adjusting the split flow ratio of the thermal desorber prior to the analysis to transfer more or less of the sample to the GC instrument and detector.

Air sampling near live plants or dried plant materials provides a simple way to determine the concentration of the different terpenes released by the plant at a given time and under specific growing or drying conditions. With thermal desorption, only the volatile compounds like the terpenes are collected by the TD tubes. Less volatile compounds, such as tetrahydrocannabinol (THC), and cannabinoids, are not released into the air by the plant due to their much higher boiling points. So, for analysis only the TD tubes are sent to the laboratory, not any plant material. The technique is non-invasive/less destructive.

Knowing the terpene concentration emitted from the dried plant material could provide valuable information to growers and dispensaries, since the terpene odor can influence customer purchasing decisions. Below are examples of where Thermal Desorption tubes could be used for the determination/quantification of terpenes:

What is Thermal Desorption?

Thermal Desorption (TD) is a sample preparation technique used in the gas chromatographic analysis of volatile and semi-volatile organic compounds (VOCs and SVOCs).

Typically, an instrument called a Thermal Desorber is connected to a GC instrument and in most cases, it replaces the common GC inlet/injector port.

There are a variety of ways to collect samples onto TD tubes (active & passive). The most common way is to pull air through the TD tube using an air sampling pump. As the air travels through the tube the VOCs and SVOCs of interest are retained by one or more adsorbents packed into the tube while the main components of air (nitrogen, oxygen, argon etc.) passe through the adsorbents un-retained. Essentially the more volume of air that is pulled through the tube, the more VOCs and SVOCs are retained on the adsorbents in the TD tube (if no breakthrough occurs).

After sampling, the TD tube is sealed and sent to a laboratory where it is placed on a thermal desorber to be analyzed. During analysis the tube is rapidly heated to 250 – 330 °C while an inert carrier gas sweeps the VOC /SVOCs (thermally desorbed from the TD tubes adsorbent) onto the GC column, where they are separated and transferred to a detector such as flame ionization detector (FID) or a mass spectrometer (MS).

- Workplace Exposure – Industrial Hygiene
  - to measure greenhouse worker's exposure to terpenes.
- Emission & Odor Testing – Regulatory
  - to quantify terpene emissions from a cultivation area (greenhouses or outdoor farms).
- Terpene Profiling – Growers & Testing Labs
  - To characterize different strains
  - Does a certain terpene ratio provide answers to questions like:
    - When is the right time to harvest?
    - When are the plants in distress?
- Testing terpene profiles can also be useful for other sources such as hops, fruits, and essential oils.

Experimental

To quantify the terpene concentration, a thermal desorption tube must be able to retain the terpenes during sampling, but it also must efficiently release them during the desorption step. During our research, we discovered no single adsorbent could provide good recoveries for all the terpenes relevant to cannabis and hemp samples. Therefore we set out to develop a new multi-bed TD tube we named the Carbotrap® T420. It contains two different graphitized carbon black adsorbents that offer different surface areas and retentivity (weak and medium), resulting in excellent recoveries of the target terpenes (see **Figure 1** for a schematic of the new tube). To demonstrate the performance of this new Carbotrap® T420 tube, we tested it alongside a TD tube packed with Tenax® TA, which is a commonly used adsorbent in the field of thermal desorption.

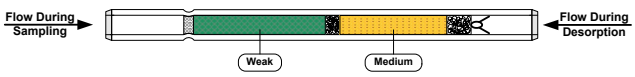

Figure 1. Schematic of the glass Carbotrap® T420 thermal desorption tube.

Terpene Test Standard

We combined two multi-component terpene test mixes and then added neat β-myrcene to the final mix to create a comprehensive terpene test mix for calibration and to challenge the TD tubes. See **Table 1** for the specific details.

Table 1. Overview of the composition of the terpene test mixes used for challenging the TD tubes.

Compound Name	Mix A	Mix B	Neat	Final Conc.
α-Pinene	✓			400 µg/mL
Camphene	✓			400 µg/mL
β-Pinene*	✓	✓		800 µg/mL
β-Myrcene			✓	400 µg/mL
3-Carene*	✓	✓		800 µg/mL
α-Terpinene	✓			400 µg/mL
p-Cymene		✓		400 µg/mL
Limonene*	✓	✓		800 µg/mL
γ-Terpinene	✓			400 µg/mL
Terpinolene		✓		400 µg/mL
L-Fenchone	✓			400 µg/mL
Linalool		✓		400 µg/mL
Fenchol	✓			400 µg/mL
Camphor*	✓	✓		800 µg/mL
Isoborneol	✓			400 µg/mL
Borneol		✓		400 µg/mL
DL-Menthol	✓			400 µg/mL
α-Terpineol		✓		400 µg/mL
Citronellol	✓			400 µg/mL
Pulegone	✓			400 µg/mL
Geraniol		✓		400 µg/mL
Geranyl Acetate	✓			400 µg/mL
β-Caryophyllene		✓		400 µg/mL
α-Cedrene	✓			400 µg/mL
α-Humulene	✓			400 µg/mL
Nerolidol I*	✓	✓		800 µg/mL
Nerolidol II		✓		400 µg/mL
Cedrol	✓			400 µg/mL
β-Eudesmol	✓			400 µg/mL
α-Bisabolol	✓			400 µg/mL

* The concentrations of these terpenes are doubled when the A&B mixes are combined.

A final concentration of 400 µg/mL and 800 µg/mL was made by adding the following mixes to a 5 mL glass volumetric flask:

- 1 mL Terpene Mix A 2000 µg/mL (**CRM40755**)
- 1 mL Terpene Mix B 2000 µg/mL (**CRM40937**)
- 2.53 µL Myrcene neat (**64643**)*
- Fill flask to the 5 mL mark with methanol

* Volume calculated by using the density of myrcene 0.791 g/cm^3 at 25°C

Analytical Conditions

For the analysis of the collected samples on the TD tubes the conditions and instrument parameters outlined in **Table 2** & **3** were used.

Table 2. Thermal desorber parameters

Thermal Desorber	
Instrument:	PerkinElmer® TurboMatrix 150
Carrier gas:	Helium
Temperatures	
Primary tube desorption:	300 °C
Focusing trap low temperature:	0 °C
Focusing trap desorption temperature:	300 °C
Heating rate of focusing trap:	99 °C/s
Valve & transfer line temp:	175 °C
Timings	
Pre-purge prior to desorption:	2.0 min
Primary tube desorption time:	5.0 min
Focusing trap desorption time:	8.0 min
Pneumatics	
Column pressure:	13.8 psi (analyzed at constant pressure, the calculated flow rate is 1.7 mL/min at 45 °C)
Desorb flow:	10 to 25 mL/min*
Inlet split (between primary tube and the focusing trap):	50 to 75 mL/min*
Outlet split (between focusing trap and GC column):	10 to 50 mL/min*
Dry-purge:	Not Used

*The desorption flow and split flow varied based on the application on how much sample was collected on the TD tubes, and the anticipated concentration expected from the samples.

Table 3. Gas chromatograph parameters

GC Conditions	
Instrument:	Agilent 7890B GC / 5977A (Single Quadrupole)
Column:	SLB®-5ms 30 m x 0.25 mm ID x 0.25 µm (**28471-U**)
Oven:	45 °C for 2 min, 10 °C/min to 120 °C hold for 3 min, 30 °C/min to 300 °C hold for 2 min
Column flow:	Column Pressure was controlled by the thermal desorber (**Table 2**). The transfer line of thermal desorber was connected directly to the GC Column, thus by-passing the inlet of the GC
Detector:	MS
Mass Spec Parameters	
Tune EMV:	1912
Transfer line:	230 °C
MS Source:	230 °C
MS Quad:	150 °C
Scan range:	40-265 amu
Solvent delay:	0 min

The chromatogram in **Figure 2** shows 0.5 µL of the terpene test standard (as described above) spiked onto a glass Carbotrap® T420 tube and then desorbed.

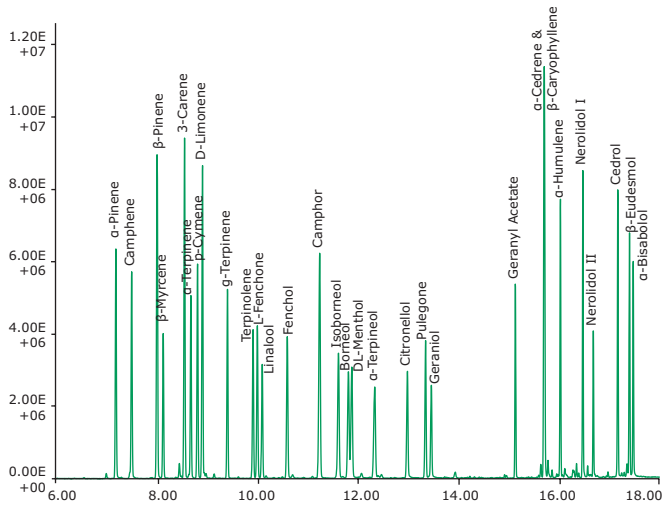

Figure 2. Desorption of the terpene test mix from the Carbotrap® T420 TD tube.

Results & Discussion

Recovery Experiments

To test the recovery, we spiked each of the TD tubes with 0.5 µL of the terpene test mix described above and challenged them with 2 and 10 liters of nitrogen gas. To spike the TD tube we used the Adsorbent Tube Injector System (ATIS). It is a sample preparation device designed to transfer calibration standards or test mixes onto a TD tube. ATIS employs the technique of flash vaporization to vaporize the sample in a continuous flow of inert gas, which carries the analytes to the tube. For this experiment, the ATIS flow controller was adjusted to deliver a constant flow of 0.1 L/min. (**Figure 3** illustrates how the TD tubes were spiked). The temperature of the ATIS glassware was set to 100 °C. Using a Hamilton® 7000 series syringe, a 0.5 µL volume of the terpene test mix was injected into the ATIS glassware. The hot glassware vaporized the terpenes, which were then carried to the TD tubes by the nitrogen gas. The tubes remained attached until a total of 2 liters passed through the tubes. These steps were repeated with another TD tube, but the tube remained attached until a total of 10 liters had passed through the tube. After the tubes were challenged, they were analyzed to determine the recovery of each terpene desorbed from the TD tubes. To determine the recovery, a four-point calibration curve was created by spiking four Carbotrap® T420 tubes with 0.25, 0.35, 0.5, and 0.6 µL of the terpene test mix. For the calibration curve, the amount of nitrogen gas was reduced to a very small “challenge volume” of only 0.2 liters. This is enough to transfer the terpenes to the adsorbents packed in the tube but doesn’t present any sort of real challenge for the adsorbent since the volume is very low. **Table 4** lists the results of this challenge experiment and compares the recoveries of those terpenes relevant to cannabis and hemp from a single-bed TD tube packed with Tenax® TA vs the new Carbotrap® T420 tube. Recoveries greater than 92% were achieved with the Carbotrap® T420 tubes at both

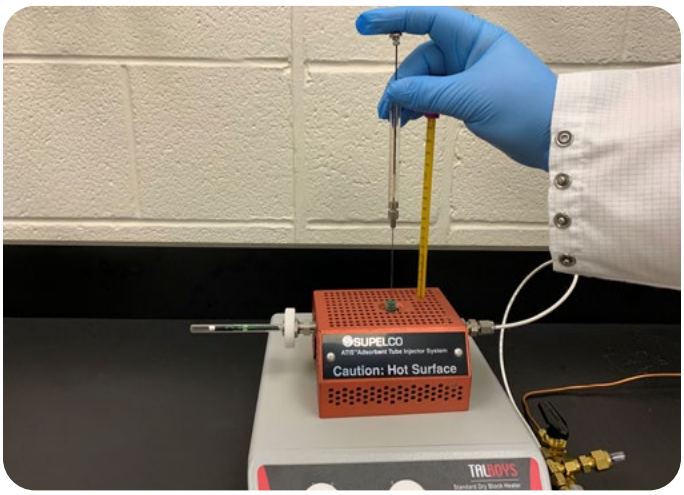

Figure 3. Spiking the Carbotrap® T420 tube with the terpene test mix using Supelco® Adsorbent Tube Injector System (ATIS).

2 and 10 liters. Whereas the recovery on the Tenax® tube for α-pinene and camphene was less than 80% only after the 2-liter challenge and dropped to less than 25% together with β-pinene to 42% after being challenged with 10-liters.

Table 4. Comparison of the terpene recoveries from Tenax and Carbotrap® T420 TD tubes at 2 challenge volumes

Compound/ Challenge Volume	Carbotrap® T420		Tenax® TA	
	2 L N_2	10 L N_2	2 L N_2	10 L N_2
α-Pinene	97%	97%	78%	24%
Camphene	99%	104%	74%	16%
β-Pinene	93%	92%	94%	42%
β-Myrcene	109%	107%	95%	103%
3-Carene	97%	94%	101%	101%
α-Terpinene	96%	94%	88%	98%
p-Cymene	98%	97%	101%	103%
Limonene	99%	97%	100%	101%
γ-Terpinene	97%	94%	99%	100%
Terpinolene	97%	94%	90%	96%
α-Terpineol	97%	96%	100%	99%
β-Caryophyllene	101%	97%	96%	94%
α-Humulene	99%	97%	100%	98%

To compare the performance of the Carbotrap® T420 tubes under actual field conditions, we collected air samples from inside the trim room of a cannabis greenhouse using both the Carbotrap® T420 and Tenax® TA TD tubes. The air sample collection took place with both the TD tubes positioned side by side to at a flow rate of 0.1 L/min for a total sample volume of 10 Liters. **Table 5** shows that the concentration obtained from the Tenax tubes was significantly lower for α-pinene, camphene, and β-pinene. Typically, a difference of +/- 25% suggests that breakthrough (insufficient retention) could be occurring. This large difference suggests that with Tenax® TA an underestimation of the concentration for these more volatile terpenes can occur. This issue was not detected with the Carbotrap® T420. The two employed Graphitized Carbon Black (GCB) adsorbents in the Carbotrap® T420 with increasing adsorption strengths, make the tube more efficient and suitable for sampling a wider range of terpenes compared to tubes packed with just Tenax® TA

Table 5. Terpene concentrations determined from sampling air inside a cannabis-growing facility.

	Carbotrap® T420	Tenax®-TA	Carbo trap T420 vs. Tenax®-TA
	(µg/m³)	(µg/m³)	% Diff
α-Pinene	297.9	165.9	57%
Camphene	20.1	8.8	78%
β-Pinene	229.9	162.9	34%
β-Myrcene	1473.2	1531.7	-4%
3-Carene	8.6	7.8	10%
α-Terpinene	3.8	3.9	-2%
p-Cymene	6.7	5.9	14%
Limonene	499.0	522.0	-4%
γ-Terpinene	2.9	2.9	-2%
Linalool	42.1	46.8	-11%
β-Caryophyllene	78.5	86.8	-10%
α-Humulene	18.2	19.5	-7%

The lower recoveries of α-pinene, camphene, and β-pinene obtained from the Tenax® TA tubes from both the lab challenge and actual air samples taken in the cannabis-growing facility shows the importance of choosing a thermal desorption tube like the Carbotrap® T420 to produce accurate terpene air concentration values when collecting samples.

Uptake of Moisture During Air Sampling

Generally, it is important to minimize the amount of water vapor transferred to the GC during a thermal desorption process. As a rule of thumb, it is advisable to make sure that the amount of water retained on the TD tubes is always <1 mg prior to desorption. This can be achieved by choosing hydrophobic adsorbents (like used in the Carbotrap® T420) to reduce water pick up during sampling, and/or dry purging the tubes prior to the thermal desorption step. Since humidity inside a greenhouse can easily exceed 50%RH, it is important to keep this in mind when collecting air samples in this environment. Too much water vapor in the desorbed sample can affect split flow ratios as water vapor expands differently than dry helium (used carrier gas) since the water vapor is quickly released from the TD tube during desorption. Water vapor can also cause separation problems on the GC and quench some detectors. A commonly successful way to address this issue is to choose thermal desorption tubes that contain adsorbents that do not retain moisture during sampling. For this study, it was tested how much water was retained by the new Carbotrap® T420 TD tube. For comparison, we also included TD tubes packed with Tenax® TA and silica gel. Tenax® TA was selected because it is known to be very hydrophobic. Silica gel was also tested. Due to its hydrophilic nature, it can

retain up to ~40% of its own weight in water vapor before reaching saturation. Therefore, it would be indicative of the amount of water vapors being pulled through the other TD tubes during this experiment. For the experiment, we thermally conditioned the silica gel tubes at 120 °C and both the Carbotrap® T420 and Tenax® TA at 320 °C for one hour to assure they were completely dry before we obtained the tare weight of each tube using a laboratory balance. Then the tubes were connected to the exposure chamber where a dynamic atmosphere of 70% humidity was generated. We used an air sampling pump to pull the humidified air through the tubes at a flow rate of 0.1 L/min. After every 1-liter of humidified air pulled through the tubes, they were removed and weighed on a laboratory balance. This was repeated until a total of 10-liters of the humidified air had passed through the tubes. **Table 6** shows the water retained by each type of tube. The results showed no significant amount of water vapor retained on the Carbotrap® T420 or the Tenax® TA tubes. The tare weight of the tubes fluctuated between +/- 1 mg which indicated that the water vapors passing through these tubes were not retained. As expected, the silica gel tube did retain a significant amount of water vapor and continued to increase in weight as more humid air was pulled through the tube. Only very little water vapor was retained by the Carbotrap® T420 TD tubes even after 120 mg of water vapor (10 L air sample) had passed through them, demonstrating it is on par with the Tenax® TA tube in terms of moisture retention. This shows that the Carbotrap® T420 TD tube can be used for effective sampling in high humidity environments. This simplifies the desorption, as no dry-purge step is required prior to analyzing the tubes.

Table 6. Amount of water vapor concentrated in TD tube while sampling in high humidity (70%RH).

Volume of Humidified Air pulled through the tubes	Silica Gel	Carbotrap® T420	Tenax® TA
0-Liter	0 mg	≤ 1mg	≤ 1mg
1-Liter	18 mg	≤ 1mg	≤ 1mg
2-Liter	35 mg	≤ 1mg	≤ 1mg
3-Liter	50 mg	≤ 1mg	≤ 1mg
4-Liter	64 mg	≤ 1mg	≤ 1mg
5-Liter	76 mg	≤ 1mg	≤ 1mg
6-Liter	87 mg	≤ 1mg	≤ 1mg
7-liter	97 mg	≤ 1mg	≤ 1mg
8-Liter	106 mg	≤ 1mg	≤ 1mg
9-Liter	113 mg	≤ 1mg	≤ 1mg
10-Liter	120 mg	≤ 1mg	≤ 1mg

Conclusion

The Carbotrap® T420 thermal desorption tube has been specifically designed for sampling terpenes in air. It exhibits excellent recoveries of terpenes relevant to the cannabis and hemp industry for sample volumes of up to 10 liters of air. This new adsorbent tube is also suitable for sampling in humid atmospheres like greenhouses as it does not retain water.

Thermal desorption can be considered as an eco-friendly sample collection and analysis technique since the used tubes, like the Carbotrap® T420, can be re-conditioned and reused multiple times. Furthermore no (hazardous) solvents are required in the desorption process, that would need to be sourced and later disposed after the analysis.

The Carbotrap® T420 is available as both glass and stainless-steel tubes. Glass tubes do have the advantage of allowing a visual observation of the integrity of the adsorbent packing with repeated use and are generally considered to be more inert. Stainless-steel tubes are more durable and will not break when sampling under harsher conditions in the field. Each TD tube contains a unique barcode for easy sample identification and tracking. The tubes are designed to function with any thermal desorption instrument that accepts ¼ in. O.D. x 3.5 in. long tubes (6.35 mm O.D. x 89 mm length).

Featured Products

Description	Cat. No
Thermal Desorption Sampling Tubes	
Carbotrap® T420 89 mm Glass-Fritted TD Tube, 1/4 in. (6.35 mm) O.D., preconditioned, Pk.10	**28689-U**
Carbotrap® T420 89 mm SS TD Tube, 1/4 in. (6.35 mm) O.D., preconditioned, Pk.10	**28687-U**
GC Column	
SLB®-5ms Capillary GC Column, L × I.D. 30 m × 0.25 mm, df 0.25 µm	**28471-U**
Reference Materials & Standards	
Terpene Mix A, certified reference material, 2000 µg/mL each component in methanol	**CRM40755**
Terpene Mix B, certified reference material, 2000 µg/mL each component in methanol, ampule of 1 mL	**CRM40937**
Myrcene, analytical standard, neat	**64643**
Accessories	
Adsorbent Tube Injector System (ATIS) 110 VAC	**28520-U**
Adsorbent Tube Injector System (ATIS) 230 VAC	**28521-U**
ATIS Replacement standard injection glassware	**28526-U**
Hamilton® syringe 7101, volume 1 µL, needle size 22s ga (bevel tip)	**20979**

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FOOD & BEVERAGE

LC-MS/MS Analysis of 16 Per- and Polyfluoroalkyl Substances (PFAS) in Milk using QuEChERS based on FDA method C-010.02

Lara Rosenberger, Yannick Hövelmann, LC-MS Experts, Olga Shimelis, R&D Manager, Analytix@merckgroup.com

Introduction

Per- and polyfluorinated alkyl substances (PFAS) are a class of compounds that have been widely used in commercial product applications over the past decades due to their versatile physical and chemical properties (e.g., water repellent, firefighting foams, cookware, food packaging). Owing to their chemical stability, these compounds are also widely present in our environment and have the potential to bioaccumulate in humans over time. Regulatory agencies such as the EPA and FDA have introduced limit values for certain substances and the development of analytical methods to avoid possible human health risks (such as low infant birth weights, cancer, and effects on the immune system).[1-4]

The U.S. Food and Drug Administration (FDA) has issued a methodology (C-010.02) for PFAS extraction from food samples applying a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction technique and further clean-up step using dispersive solid phase extraction (dSPE), followed by LC-MS/MS analysis.[4]

For FDA method C-010.02, an extraction salt mixture containing 6.0 g $MgSO_4$ as well as 1.5 g NaCl, and a dSPE clean-up mix containing 900 mg $MgSO_4$, 300 mg PSA and 150 mg graphitized carbon, are specified. The Supel™ QuE non-buffered extraction salt mix and the specifically designed Supel™ QuE PSA/ENVI-Carb™ Tube 3 for clean-up have been used to meet the method requirements.

This application note describes the analysis of 16 PFAS compounds in milk and was performed in accordance with FDA method C-010.02.

Experimental

Solutions and Standards Preparation

Native and isotopically labeled PFAS standards were used as methanolic 50 µg/mL stock solutions. These standards were then diluted following the dilution scheme of the method C-010.02 to obtain calibration standards in the required concentrations (external calibration: 0.01, 0.05, 0.10, 0.50, 1.0, 5.0, 10, and 25 ng/mL).

Sample Preparation

Evaluation of Background Contamination

In accordance with U.S. FDA method C-010.02, analysis was performed for water and milk samples. UHPLC-MS grade water was used to test PFAS background contamination and found to be free of the 16 analytes covered by the FDA method. The water samples (5 mL) were fortified with the isotopically labeled internal standards and were further mixed with 5 mL water, 150 µL formic acid, and 10 mL acetonitrile. After addition of the Supel™ QuE extraction salt package (Cat. No. **55295-U**), the mixture was placed on a shaker (1500 rpm for 10 minutes) and the PFAS analytes were extracted from the water phase into the organic phase. For further clean-up of complex samples like food matrices, dSPE is required. The organic layer was therefore transferred into a second tube, containing Supel™ QuE PSA/ENVI-Carb (Cat. No. **55479-U**) and shaken for 10 minutes at 1500 rpm. After centrifugation (4000 g for 10 minutes), the sample was filtered (Millex® filters, Cat. No. **SLGNX13**) and used for LC-MS/MS analysis.

Method Performance Assessment

Following the background assessment of the method using the Supel™ QuE materials, method performance was investigated using milk as an exemplary sample matrix for quantitation of PFAS in processed foods. For that purpose, 5 mL of UHT, reduced-fat (1.5%) milk were spiked at 0.5 or 2.0 ng/mL with 16 native PFAS and 8 isotopically labeled surrogate standards. The samples were analyzed using the same methodology for the presence of PFAS analytes. Extraction and purification were performed as described in FDA method C-010.02.

LC-MS/MS analysis

An Agilent 1290 Infinity II instrument coupled to an Agilent 6495C triple quadrupole mass spectrometer was used for the LC-MS/MS analysis. Analyte separation was achieved using Ascentis® Express PFAS 90 Å (15 cm x 2.1 mm, 2.7 µm, Cat. No. **53560-U**) as analytical column. In addition, a delay column (Ascentis® Express 90 Å PFAS Delay Column, 5 cm x 3.0 mm, 2.7 µm,

Cat. No. **53572-U**), was installed after the mixing valve and before the autosampler to offset potential PFAS contamination potentially originating from the LC system (e.g., pump, tubings, fittings, filters). Polypropylene snap cap vials were used instead of standard glass vials to avoid possible PFAS adherence to the glass surface. The LC conditions used are shown in **Table 1**.

Table 1. LC Conditions used for analysis of 16 PFAS compounds

LC Conditions	
Instrument:	Agilent 1290 Infinity II instrument coupled to an Agilent 6495C triple quadrupole mass spectrometer
Columns:	Ascentis® Express 90 Å PFAS HPLC Column, 2.7 µm, 15 cm x 2.1 mm (**53560-U**) Delay column: Ascentis® Express 90 Å PFAS Delay Column, 2.7 µm, 5 cm x 3.0 mm (**53572-U**)
Mobile phase:	[A] 5 mM Ammonium acetate*; [B] methanol

LC Conditions (continued)			
Gradient:	**Time (min)**	**A%**	**B%**
	Initial	90.0	10.0
	3.0	90.0	10.0
	3.1	60.0	40.0
	26.0	10.0	90.0
	26.1	90.0	10.0
	28.0	90.0	10.0
Flow rate:	0.30 mL/min		
Pressure:	320 bar		
Column temp.:	40 °C		
Detector:	MS/MS, ESI (-), MRM (see **Table 2** for details)		
Injection:	10 µL		
Sample(s):	See text		

* mobile phase A was modified compared to FDA method C-010.02 and used without addition of 1-methyl piperidine

Table 2. MRM, chromatographic and linearity (R^2) data for 16 PFAS analytes

Peak	Compound	Acronym	MRM	Collision Energy (eV)	RT (min)	R^2
1	PFBA	Perfluorobutanoic acid	213.0->169.0	4	6.3	0.9965
2	PFPeA	Perfluoropentanoic acid	263.0->219.0	4	8.8	0.9967
3	PFBS	Perfluorobutanesulfonic acid	298.9->80.0	40	9.3	0.9975
4	PFHxA	Perfluorohexanoic acid	313.0->269.0	4	11.7	0.9964
5	PFPeS	Perfluoropentanesulfonic acid	348.9->99.0	37	12.1	0.9951
6	HFPO-DA	Hexafluoropropylene oxide dimer acid	285.0->169.0	4	12.5	0.9965
7	PFHpA	Perfluoroheptanoic acid	363.0->319.0	4	14.4	0.9960
8	PFHxS	Perfluorohexanesulfonic acid	398.9->99.0	41	14.6	0.9949
9	NaDONA	Sodium dodecafluoro-3H-4,8-dioxanonanoate	377.0->251.0	8	14.7	0.9956
10	PFOA	Perfluorooctanoic acid	413.0->369.0	8	16.7	0.9964
11	PFHpS	Perfluoroheptanesulfonic acid	448.9->99.0	45	16.8	0.9961
12	PFNA	Perfluorononanoic acid	463.0->419.0	8	18.7	0.9974
13	PFOS	Perfluorooctanesulfonic acid	498.9->80.0	76	18.7	0.9976
14	9Cl-PF3ONS	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	530.9->351.0	28	19.7	0.9961
15	PFDA	Perfluorodecanoic acid	513.0->469.0	8	20.4	0.9961
16	11Cl-PF3OUdS	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	630.9->451.0	32	22.4	0.9953

Results and Discussion

A chromatogram of a solvent calibration standard containing the 16 native compounds is shown in **Figure 1**. All 16 compounds demonstrated a lower limit of quantitation (LLOQ) of 0.01 ng/mL for the HPLC method and an LLOQ of 0.02 ng/mL in the context of the milk sample. Linear calibration curves (0.01-25 ng/mL) with R^2 ≥0.99 were obtained for all PFAS analytes (**Table 2**).

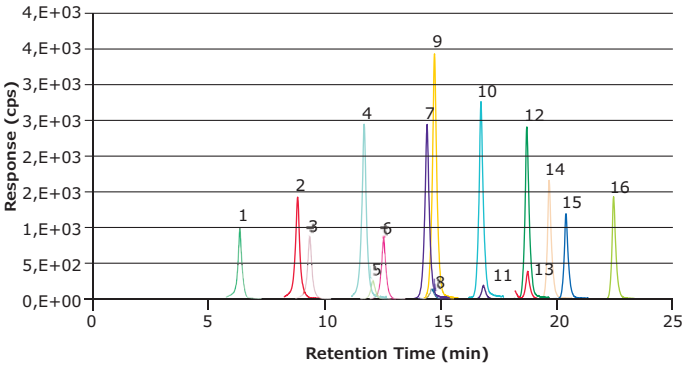

Figure 1. 16 PFAS compounds at 1 ng/mL in methanol (Peak IDs see **Table 2**)

The background evaluation of the FDA method C-010.02 using the recommended salt package and dSPE material showed negligible background levels for all the studied PFAS compounds (**Table 3**), as shown by values below the respective lower limits of quantification (LLOQ) of the LC-MS/MS method of 0.01 ng/mL (0.02 ng/mL in relation to the milk sample). Furthermore, an upfront screening of PFAS compounds in the UHPLC-MS solvents revealed concentrations below 0.01 ng/mL.

Table 3. Results of method background testing for the evaluation (LLOQ of 0.01 ng/mL)

Analyte	Method Background using UHPLC-MS water as sample
PFBA	Below LLOQ
PFPeA	Below LLOQ
PFBS	Below LLOQ
PFHxA	Below LLOQ
PFPeS	Below LLOQ
HFPO-DA	Below LLOQ
PFHpA	Below LLOQ
PFHxS	Below LLOQ
NaDONA	Below LLOQ
PFOA	Below LLOQ
PFHpS	Below LLOQ
PFNA	Below LLOQ
PFOS	Below LLOQ
9Cl-PF3ONS	Below LLOQ
PFDA	Below LLOQ
11Cl-PF3OUdS	Below LLOQ

The acceptable recovery range for the investigated PFAS analytes based on the FDA guidelines for the validation of chemical methods is 40-120% (including RSD ≤ 22%) for concentrations at lower levels (i.e. 1 ng/mL). **Table 4** displays the recoveries and %RSD from the experimental study where 16 compounds were spiked in quintuplicate in milk samples. All recoveries and %RSD met the requirements of the FDA method and were thus in the recommended range.

Table 4. Precision and recovery (n = 5) of PFAS in milk samples at 2 fortification/spike levels (0.5 ng/mL and 2.0 ng/mL)

Analyte	Fortified conc. (ng/mL)	Mean recovery (%)	% RSD	Fortified conc. (ng/mL)	Mean recovery (%)	% RSD
PFBA	0.5	94	9.3	2.0	86	4.7
PFPeA		91	5.0		84	3.7
PFBS		86	4.1		84	3.2
PFHxA		85	2.3		84	3.2
PFPeS		80	7.1		84	3.7
HFPO-DA		87	8.2		86	1.9
PFHpA		94	10.9		96	4.3
PFHxS		88	5.1		83	3.0
NaDONA		81	2.6		82	3.3
PFOA		101	3.6		89	2.3
PFHpS		89	5.8		81	5.7
PFNA		100	4.5		90	3.0
PFOS		81	1.8		83	2.7
9Cl-PF3ONS		95	4.8		84	2.9
PFDA		95	5.0		82	4.7
11Cl-PF3OUdS		95	6.1		85	2.8

Conclusions

In this application note, the workflow for FDA method C-010.02 to analyze 16 PFAS in processed food using the QuEChERS method was investigated for milk samples. The background values of all used consumables and the LC-MS system resulted in levels below the LLOQs given in the method, thus ensuring an appropriate analysis of low levels of PFAS analytes. At both 0.5 ng/mL and 2.0 ng/mL fortified concentration levels, recoveries for all 16 compounds were well within the FDA method acceptable range of 40—120%. The calculated %RSDs were less than 11%, indicating satisfactory precision. Hence, the Supel™ QuE PSA/ENVI-Carb clean-up mix 3, Supel™ QuE extraction salt mix (non-buffered), Ascentis® Express PFAS columns, and Millex® syringe filters proved to be suitable tools for this PFAS analysis in milk samples.

Featured & Related Products

Description	Cat. No.
Sample Preparation	
Supel™ QuE PSA/ENVI-Carb™ Tube 3, volume 15 mL, Pk. 50	**55479-U**
Supel™ QuE Non-Buffered Tube 2, pk. 50	**55295-U**
Brand® PP graduated centrifuge tube, screw cap volume 50 mL, without base, non-sterile, Pk. 300	**BR114820**
Millex® Syringe Filter, Nylon, Non-sterile, 0.20 µm pore size, 13 mm diameter	**SLGNX13**
HPLC Analysis	
Ascentis® Express 90 Å PFAS HPLC Column, 2.7 µm, 15 cm x 2.1 mm	**53560-U**
Ascentis® Express 90 Å PFAS Delay Column, 2.7 µm, 5 cm x 3.0 mm	**53572-U**
Water for UHPLC-MS LiChrosolv®	**1.03728**
Methanol hypergrade for LC-MS LiChrosolv®	**1.06035**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**1.00029**
Ammonium acetate LiChropur™, eluent additive for LC-MS	**73594**
Formic acid for analysis EMSURE® ACS,Reag. Ph Eur	**1.00264**
Standardization and Calibration	
Perfluorobutanoic acid, analytical standard, 25 mg	**68808**
Perfluoropentanoic acid, analytical standard, 25 mg	**68542**
Perfluorohexanoic acid, analytical standard, 25 mg	**43809**
Perfluoroheptanoic acid, analytical standard, 25 mg	**43996**
Perfluorooctanoic acid, analytical standard, 100 mg	**33824**
Perfluorodecanoic acid, analytical standard, 25 mg	**43929**
Pentadecafluorooctanoic acid, 100 µg/mL in methanol, analytical standard, 1 mL	**33603**
Heptadecafluorooctanesulfonic acid, 100 µg/mL in methanol, analytical standard, 1 mL	**33607**

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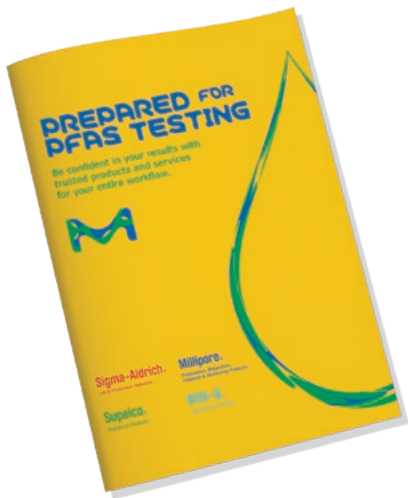

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Comparison of Syringe Filters in Retaining Particulates and Maintaining Optimal UHPLC Back Pressure

Lindsay D. Lozeau, Senior Scientist; Maricar Dube, Global Technical Marketing Manager, Analytix@merckgroup.com

Abstract

It is well known that proper sample preparation techniques are critical to achieving high-quality data. Filtration is sometimes omitted as a sample preparation step prior to (U)HPLC and LC-MS analyses, but this omission can have unwanted consequences since the presence of particulates in samples and mobile phases can compromise column and instrument performance. The first part of this paper investigates the ability of different syringe filters to retain particles. The particle retention efficiency (or percent retention) was then correlated to a column lifetime study in the second part of this paper. Both filtered and unfiltered samples were injected into a UHPLC system and the back pressure was monitored up to 500 injections or until a set pressure cutoff was exceeded. The data obtained demonstrated the importance of filtration in optimizing HPLC column lifetime.

Introduction

High-performance, ultra-high performance liquid chromatography (HPLC, UHPLC), and liquid chromatography-mass spectrometry (LC-MS) have become common analytical methods across many sectors, from the pharmaceutical, food and beverage, and cannabis industries, to academic and government institutions, as well as clinical and environmental labs. Although the technological developments in modern instruments offer increased throughput and better sensitivity, these advantages can only be realized with adequate sample preparation.[1]

There are many sample preparation techniques used for HPLC, with the goal(s) of clearing particulates, increasing analyte concentration, solvent or matrix switching, and/or chemical derivatization of the analyte of interest to improve its detection and peak shape.[2] One of the simplest and least expensive sample preparation techniques is filtration, which removes particulates from the sample.[3] Undissolved particulates in a sample, even at low concentrations, could potentially clog the HPLC column by accumulating on the column inlet frit, leading to poor quality data, high instrument back pressure and reduction of column lifetime.[4,5] Thus, the removal of these particulates from samples via filtration could avoid these challenges while providing better chromatographic data.[6]

Filtration is also important for mobile phase preparation in (U)HPLC and LC-MS.[7,8,9] Commercially available HPLC, UHPLC, LC-MS, and MS grade solvents come pre-filtered, and there is no need for additional filtration when using these solvents directly out of the bottle as a mobile phase. However, there are many methods that require buffers and/or mixtures for mobile phases, and these require the addition of salts such as phosphates and acetates.[9] In such cases, it is recommended that the buffer be filtered prior to use[10] and always used fresh.[11]

This study has two parts: (1) testing the retention efficiency of various syringe filters and (2) assessment and comparison of column lifetime when samples are filtered and when they are not. Factors that influence the retention by syringe filter devices, as well as the mobile phase, filtration will also be discussed in more detail.

Experimental

Part 1: Filter Retention Study

Studies were performed on syringe filters (0.2 µm and 0.45 µm pore sizes) from four manufacturers. The syringe filters were made of either hydrophilic polytetrafluoroethylene (PTFE) or regenerated cellulose (RC). 0.05% (v/v) polystyrene bead solutions in water were made using 0.5 µm and 0.24 µm diameter polystyrene beads for testing 0.45 µm and 0.2 µm pore size syringe filters, respectively. Bead solution was passed through n=4 syringe filters per lot, and in most cases, multiple lots were tested. Filtrate was collected after 3 mL of bead solution was filtered, and then characterized fluorescently or spectrophotometrically compared to a six-point standard curve.

Part 2: Column Lifetime Study

Column lifetime was assessed with repeated 10 µL injections of either filtered or unfiltered 0.05% (v/v) bead solutions. Filtration was carried out into 30 HPLC-certified vials using n=30 devices from the 0.45 µm syringe filters tested in Part 1. In most cases, multiple

lots were tested. After each injection, the change in back pressure was monitored, up to 500 column injections or a set pressure cutoff of 8000 psi/548 bar. This cutoff was set to ensure that the system did not reach an unsafe pressure level. The HPLC conditions are provided in **Table 1**.

Table 1. HPLC Conditions

Column:	Ascentis® Express C18, 2.7 µm, 50 x 2.1 mm I.D. (**53822-U**)
Mobile phase:	Acetonitrile:water 35:65 v/v, filtered through 0.2 µm hydrophilic PTFE membrane (**JGWP04700**) with Millicup™-FLEX (**MCFLX4702**)
Flow rate:	0.75 mL/min
Column temp:	35 °C
Injection:	10 µL
Back pressure cutoff:	8000 psi/548 bar

A new column was used for each test with each 0.45 µm syringe filter type from Part 1. Tubing, injector, seals, and the whole system were all extensively cleaned between each test, both with and without a column installed, to ensure all particulates from the prior run were cleared from the system. When each new column was installed, the system was flushed with 70:30 acetonitrile:water (1 mL/min) for 10 minutes, and then equilibrated with mobile phase (**Table 1**) until a stable back pressure baseline was established, approximately 10-15 minutes.

Results and Discussion

Sample Filtration using syringe filters

Part 1. Filter Retention Study

Percent retention or retention is an indicator of the percentage of particulates that will be successfully removed from a sample during filtration or allowed to pass through the filter and into the instrument. Retention of 0.5 µm diameter beads by 0.45 µm syringe filters, and 0.24 µm diameter beads by 0.2 µm syringe filters were tested after filtration of 0.05% (v/v) bead solutions.

Filtration using 0.45 µm syringe filters is commonly used to remove particulates prior to analytical methods. This procedure is done to avoid interferences that could affect the accuracy of data and to protect the instrument. **Table 2** shows the retention efficiency of the four 0.45 µm syringe filters that were tested. Retention efficiency varied from one membrane filter material to the other, with RC showing the lowest retention of 48.2±4.3% versus the PTFE demonstrating approximately 98-100% retention of the polystyrene beads.

Table 2. Percent retention of 0.5 µm diameter beads (0.05% solution) by 0.45 µm pore size syringe filters.

			% Retention	
Manufacturer (MFR)	**Material**	**Lot Numbers**	**Individual (n=4 devices)**	**Average (all lots)**
Millipore®	PTFE (hydrophilic)	1a	96.6±0.10	98.3±1.8
		1b	100±0.10	
MFR-2	PTFE (hydrophilic)	2a	100±0.10	100±0.10
		2b	100±0.10	
MFR-3	PTFE (hydrophilic)	3a	100±0.10	100±0.10
MFR-4	Regenerated cellulose (RC)	4a	52.6±3.6	48.2±4.3
		4b	45.1±2.8	
		4c	46.8±1.8	

In HPLC, filtration through a 0.45 µm filter membrane is sufficient unless the column is packed with small particle sizes (e.g., sub-2 µm particles), in which case a 0.2 µm filter is needed.[7,12] UHPLC columns are more prone to clogging due to smaller porosity of inlet frits, interstitial spaces between silica particles and tubing diameter. Thus, the retention of 0.24 µm diameter beads by 0.2 µm syringe filters was also evaluated (**Table 3**). The retention efficiency of the regenerated cellulose filter is less than 20%, indicating that greater than 80% of the particulates to be filtered passed through the membrane. The three hydrophilic PTFE syringe filters showed varying retention efficiencies, with MFR-2 giving the lowest value overall while also demonstrating inconsistency from lot to lot.

Table 3. Percent retention of 0.24 µm diameter beads (0.05% solution) by 0.2 µm pore size syringe filters.

			% Retention	
Manufacturer (MFR)	**Material**	**Lot Numbers**	**Individual (n=4 devices)**	**Average (all lots)**
Millipore®	PTFE (hydrophilic)	1a	96.7±0.70	96.0±1.6
		1b	95.4±1.9	
MFR-2	PTFE (hydrophilic)	2a	78.8±17	49.8±32
		2b	20.9±0.90	
MFR-3	PTFE (hydrophilic)	3a	98.5±1.5	98.5±1.5
MFR-4	Regenerated cellulose (RC)	4a	14.4±1.1	15.8±2.2
		4b	14.2±0.70	
		4c	18.7±0.60	

The retention efficiency data suggest that not all syringe filters of the same pore size rating will perform equally in sample filtration.

Part 2. Column Lifetime Study

Filtering samples prior to (U)HPLC analyses is considered a good practice.[5] This process prevents premature clogging of the column and increased back pressure of the instrument, which avoids unnecessary system shutdown

and loss of lab productivity while also maintaining data quality. The results of the retention efficiency studies in the previous section imply that these syringe filters may vary in the way that they protect columns when they are used to filter particulate-containing samples prior to (U)HPLC injection.

The 0.45 µm syringe filters in Part 1 were used to filter 0.05% (v/v) solutions of 0.5 µm diameter polystyrene beads into HPLC-certified vials. The filtrates were then injected into the UHPLC instrument. Repeated injections of only 10 µL were made until the back pressure cut off (8000 psi/ 548 bar) was reached or exceeded. Unfiltered samples were also directly injected. The results are shown in **Figure 1**.

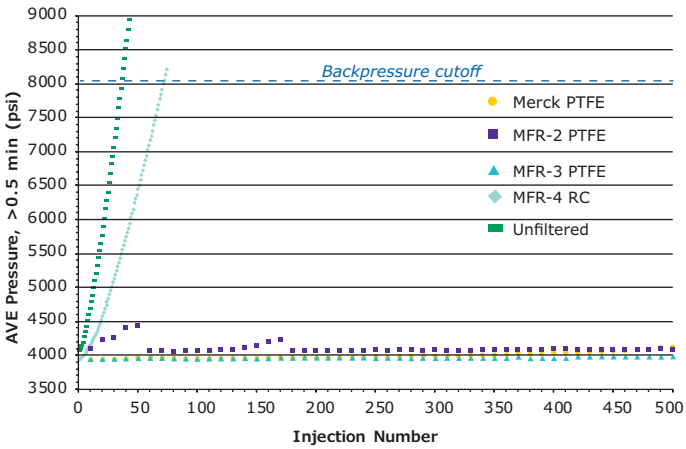

Figure 1. Effect of sample filtration on UHPLC system back pressure. The average back pressure (psi) is plotted against injection number of filtered and unfiltered 0.05% (v/v) solutions of 0.5 µm diameter beads. Filtered samples were from three 0.45 µm hydrophilic PTFE syringe filters and one RC syringe filter, each from a different manufacturer (MFR). Unfiltered solutions were also injected.

Only 36 injections of the unfiltered sample were made before the back pressure exceeded the cutoff value of 8000 psi. The cutoff was exceeded after 71 injections for the RC syringe filter filtrates. This result indicated that the column was quickly clogged from particles, and that even an injection of 10 µL significantly reduced column lifetime. This observation correlates with the retention efficiency data where the regenerated cellulose syringe filter retained only 48% of the particles in a solution, meaning that approximately 50% of the particulates to be filtered were instead injected into the UHPLC system. The samples filtered through PTFE all could be injected over 500 times without showing appreciable changes in column back pressure. This finding was due to nearly 100% of the particles were retained by these syringe filters, as described in the previous section.

Filtration of mobile phase using membrane filters

The importance of filtration in (U)HPLC and LC-MS applications is not limited to sample filtration. The presence of particulates in the mobile phase can also cause serious problems in (U)HPLC. A previous study by Joshi, et. al. demonstrated that incomplete removal of particles from mobile phase also leads to an increase in UHPLC column back pressure (**Figure 2**).[7,13] In that study, mobile phase composed of 50:50 acetonitrile:water was filtered through polypropylene (PP) and hydrophilic PTFE membrane filters (0.2 µm and 0.45 µm pore sizes). The mobile phase was allowed to continuously flow to an UHPLC column at a flow rate of 0.25 mL/min, and the back pressure was monitored over 600 minutes.

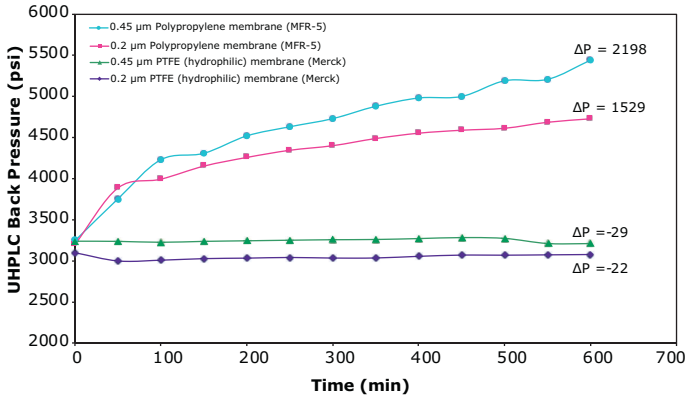

Figure 2. Change in UHPLC system back pressure (psi) vs. time (min) as mobile phase flowed at 0.25 mL/min for 600 minutes. The mobile phase was filtered through 0.2 µm and 0.45 µm polypropylene (PP) and PTFE membranes. (Reprinted with permission from reference 13.)

Of the four membrane filters tested, mobile phase filtered through the 0.45 µm and 0.2 µm polypropylene membrane showed the biggest increase in back pressure. Aside from causing the same column issues that have already been discussed, particulates in the mobile phase can cause premature breakdown of instrument components such as pump check valves, pistons, and seals.[14] Particulates can also affect the mixing efficiency of high or low pressure mixers and skew the results of gradient LC separations as mobile phase is not mixed properly. It is important to note that compared to a sample injection, a significantly higher volume of mobile phase contacts the column during an experiment, which if any particulates are present, could accelerate and exacerbate the column clogging effects observed in Part 2.

Factors that influence membrane filter retention

In this paper, the filtration of samples and mobile phases was performed using membrane filters, serving as microporous barriers. The pores in the membranes are responsible for the physical size exclusion of particulates from a bulk matrix. However, certain situations may arise where particulates larger than the expected or claimed pore size of a membrane may pass through it, or particulates smaller may not, leading to varied results of retention.

The different features of the pores of a microfilter, such as shape, size, frequency, distribution, and symmetry, define how the filter will retain certain sizes of particles. In the filter retention study, different PTFE filters of the same pore size, especially the 0.2 µm pores, from different manufacturers showed varying retention

of the same particles. This observation may be due to pore size distribution and porosity being broader or narrower from manufacturer to manufacturer. In other words, the range of individual pore sizes from smallest to largest may be vastly different. Due to the varied nature of casting membrane filters, pore shape can vary widely from material to material, and even from lot to lot, if the process is not carefully controlled. Further, manufacturers have different ways of measuring porosity and pore size, which can be based on tests such as air permeability, bubble point, pure water permeability, BET analysis, porometry or retention.

When syringe filters are used for (U)HPLC and LC-MS sample preparation, particle retention is not the only characteristic that must be considered. Other aspects of the membrane such as its chemistry and chemical compatibility, filter diameter, thickness and housing, as well as chemical characteristics of both the solution and analyte being filtered can also have significant impact on the overall membrane filter performance.[15]

Conclusions

Syringe filters with the same pore size rating have different particle retention efficiency. It is important to select a filter that efficiently retains particles in order to protect the (U)HPLC column from premature clogging. Mobile phases also need to be filtered because particulates not only cause column clogging, but they can also cause instrument components to fail over time. Thus, to protect HPLC column lifetime and to produce consistent, high-quality data, filtration of both the sample and mobile phase should always be considered.

Featured Products

Description	Cat. No.
Syringe Filters	
Millex® Syringe Filter, PTFE 0.2 µm	**SLLG033NS**
Millex® Syringe Filter, PTFE 0.45 µm	**SLCR033NS**
Mobile Phase Filtration	
47mm Omnipore™ hydrophilic PTFE 0.2 µm	**JGWP04700**
47mm Omnipore™ hydrophilic PTFE 0.45 µm	**JHWP04700**
47mm LCR hydrophilic PTFE 0.45 µm	**FHLC04700**
Millicup™-FLEX Vacuum Filter	**MCFLX4710**
Classic Glass Filter Holder - Kit 47 mm	**XX1014700**
LC-MS/MS	
Ascentis® Express C18, 50 x 2.1 mm I.D., 2.7 µm	**53822-U**
Acetonitrile, for UHPLC-MS LiChrosolv®	**1.03725**
Water, for UHPLC-MS LiChrosolv®	**1.03728**

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Supelco® IQ/OQ/PQ Kit enables an efficient and reliable Instrument Qualification

Dr. Holger Keller, Sr. Product Manager Service, Metrohm AG, Matthias Nold, Product Manager, Reference Materials, Merck KGaA, Analytix@merckgroup.com

Reliability and consistency of analytical results requires a proper instrument qualification at the time of installation as well as periodic performance checks. This article outlines the tests that should be part of the instrument qualification process for ion chromatography instruments.

Ion (-exchange) chromatography (IC) is a well-established analytical technology that separates ions and polar molecules based on their respective charged groups. Ion chromatography systems consist of the following main components (**Figure 1**): A pump that moves the mobile phase (eluent; e.g. water), an injector that introduces the sample containing the ions into the mobile phase, a separation column as a stationary phase that holds the ion exchanger material, and finally a detector that registers the ion load after release from the column.

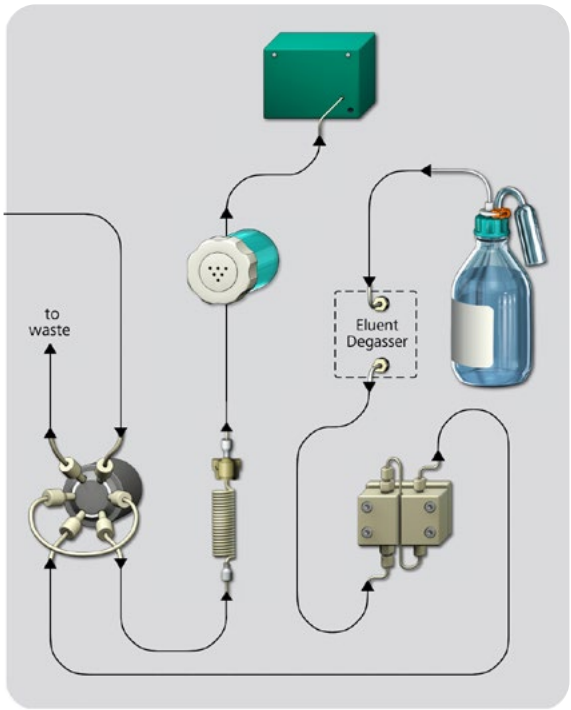


Figure 1. Functional principle IC (example)

IC can handle a wide range of varying analyte concentrations, from parts per trillion to the percent range. IC is commonly used in the pharma, food, and environmental sectors to analyze samples for specific components and to verify compliance with norms and standards.

Today's Ion Chromatographs are sophisticated analytical instruments. To guarantee reliable, reproducible, and traceable results from day one, professional installation and system qualification are key to their success. To keep the instrument in its peak performance over time, regular maintenance and periodic requalification are strongly advised.

During initial qualification or periodic requalification, the system undergoes a range of standardized tests to evaluate performance characteristics of individual and critical system components. Next to mechanical and electronical quality parameters, performance is also tested and evaluated in a series of measurements using traceable Certified Reference Materials (CRMs).

Steps of Instrument Qualification (quick introduction)

Especially in the pharmaceutical industry, instrument qualification and documentation of the findings is a must to fulfill the requirements of the various regulatory bodies. USP <1058> for instance defines the Analytical Instrument Qualification (AIQ) as “the collection of documented evidence that an instrument performs suitably for its intended purpose”. To support customers in this task, the Metrohm Compliance Service has developed standardized test procedures and extensive documentation to comply with these regulatory demands.

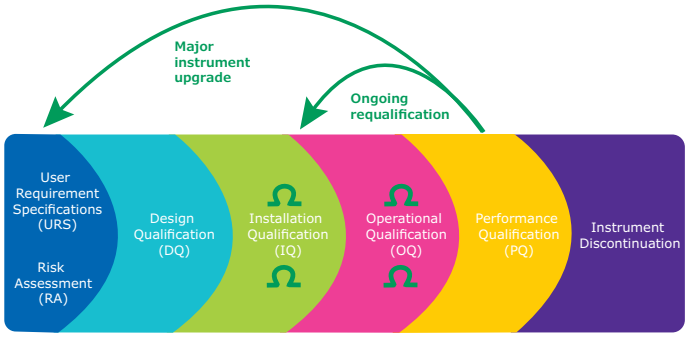


Figure 2. Analytical Instrument Qualification

This procedure focuses on two key elements of the overall qualification process - Installation Qualification (IQ) and Operational Qualification (OQ).

IQ documents the installation process of the system and ensures that the instrument, software, and accessories have been delivered and are set up correctly at a suitable workplace environment.

OQ encompasses a mechanical/electrical component test, including the calibration of all relevant hardware components, functional specification testing of the installed software, and a holistic system test where the main components are used to execute predefined test sequences with traceable CRM solutions.

To focus as much as possible on the performance evaluation of the system itself, it is essential to exclude or at least minimize as much as possible, sources of error that are not directly linked to the tested components.

One source of error that can easily be controlled, are the standard solutions used in the holistic system tests. Relying on prepared CRM solutions is the safest way to exclude critical sources of error related to standards preparation from raw materials or stock solutions. Such sources of error could be originating from pipetting, weighing, and dilution steps, in addition to other risk factors like possible contamination of glassware and/or tools, and operator performance.

Specific Tests involving Standard Solutions

The holistic test procedures used during IQ/OQ of Ion Chromatography systems can also be applied to periodic system checks to satisfy recommendations and requirements based on GLP (Good Laboratory Practice).

Performance assessment of key components and statistical evaluation of achieved results (e.g., reproducibility and accuracy) should be carried out using a PEEK capillary (0.18 mm ID, 10 m) instead of a column, and pre-prepared *Trace***CERT**® CRM standard solutions.

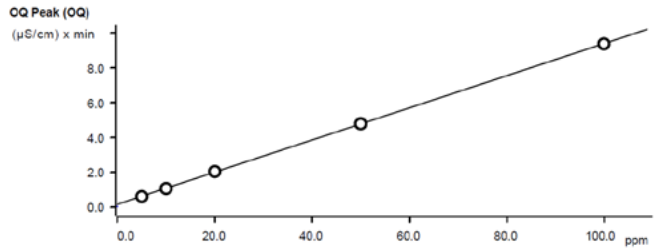
Test procedures for the main system components include (**Table 1**):

- Linearity test of detector modules
- Injector tests such as precision, retention time stability, and cross contamination
- Injection volume linearity (e.g. for Metrohm Inline Sample Preparation (MiPT) technique)
- Reproducibility of suppressor chambers

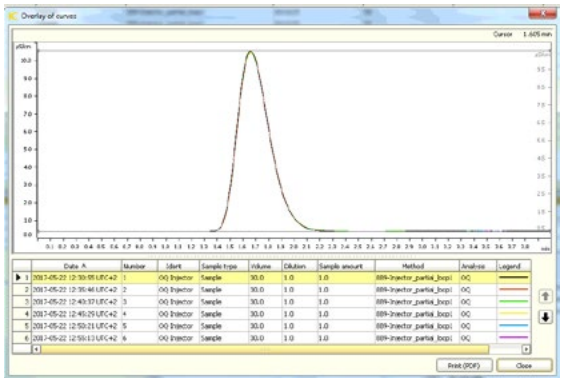
Table 1. Overview on tests and the procedures

Test	Description	Acceptance*
Detector test	Injection of 20 µL standard solution each: • 5 mg/L bromide • 10 mg/L bromide • 20 mg/L bromide • 50 mg/L bromide • 100 mg/L bromide	Linearity: Correlation coefficient, % RSD (≥ 0.999, ≤ 3.0%)
Injector test: Repeatability	Injection of 6 times 20 µL standard solution 50 mg/L bromide	• Precision: % RSD (≤ 0.5%) • Retention time stability: SD [abs] (≤ 0.02 min)
Injector test: cross-contamination	Injection of 20 µL ultrapure water, followed by 20 µL standard solution 1000 mg/L bromide, followed by 20 µL ultrapure water.	Amount of carried over sample (|x| ≤ 0.1 %)
Injector test: Linearity (MiPT technique)	Six injections of 5 mg/L standard solution with the following volumes: • 4 µL • 8 µL • 16 µL • 40 µL • 80 µL • 200 µL	Linearity: Correlation coefficient, % RSD (≥ 0.999, ≤ 3.0%)
Suppressor test: Repeatability of suppression in suppressor chambers	Injection of 20 µL; standard solution 20 mg/L bromide Full loop (20 µL)	Precision: % RSD (MSM/MSM-LC ≤ 0.5 % MSM-HC ≤ 2.0 %)

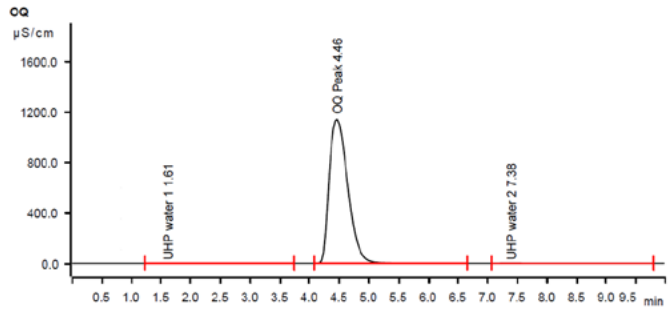
* Acceptance values as applied by Metrohm shown in parentheses



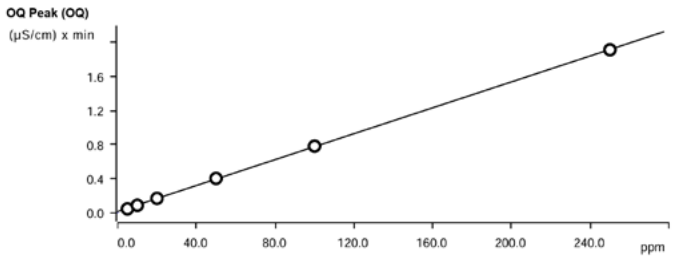
Example 1: Detector test - linearity



Example 3: Injector test - cross contamination



Example 2: Injector test - repeatability



Example 4: Injector test - linearity of injection volumes

*Trace*CERT® IQ/OQ/PQ Kit (Cat. No. 12674)

This ready to use IQ/OQ/PQ kit for Ion Chromatography contains six certified bromide standard solutions with nominal concentrations between 5 and 1000 mg/kg as required for the IC instrument qualification tests as described above. The standards are manufactured under ISO 17034 accreditation (accreditation for CRM manufacturers) using high purity sodium bromide and high-purity water (18.2 MΩcm, 0.22 µm filtered) as starting materials.

The product features include:

- Values traceable to NIST SRM
- Expanded Uncertainty provided considering all contribution factors (**Figure 5**)
- Stability and Homogeneity tested
- The kit includes 6 sodium bromide solutions (See concentrations on next page):

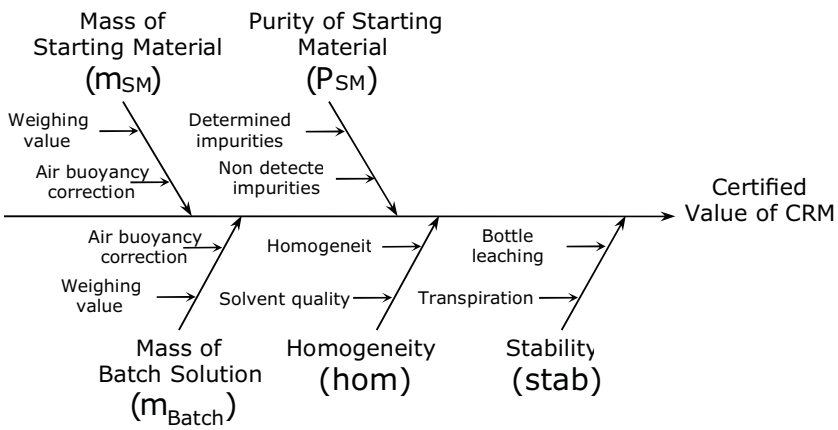


Figure 5. Contribution factors of uncertainty

Typical relative contributions are:

$u(m_{SM})$	< 0.01 %
$u(m_{Batch})$	< 0.01 %
$u(P_{SM})$	< 0.10 %
u_{hom}	< 0.03 %
u_{stab}	< 0.18 %

- Bromide Standard 1: 5.00 mg/kg
- Bromide Standard 2: 10.00 mg/kg
- Bromide Standard 3: 20.00 mg/kg
- Bromide Standard 4: 50.00 mg/kg
- Bromide Standard 5: 100.00 mg/kg
- Bromide Standard 6: 1000.0 mg/kg

To guarantee top reliability of the values for these *Trace***CERT**® certified reference materials, three independent procedures were followed:

After the purity of the starting material is confirmed and if contamination and loss of material are strictly prevented, the solutions are produced gravimetrically. This approach allows the highest accuracy and small uncertainties. The certified value of *Trace***CERT**® reference materials is based on this approach and is directly traceable to the SI unit of kilogram.

The starting material (sodium bromide) is measured against the NIST Standard Reference Material 999 (using argentometric titration), followed by gravimetric preparation using balances calibrated with SI-traceable weights. Consequently, the value calculated by this unbroken chain of comparisons is traceable to the reference to which the starting material is compared.

To underpin the certified gravimetric value, the bottled *Trace***CERT**® solution is compared to a second reference material (NaCl, certified by BAM, Cat No. 71387) by argentometric titration.

Featured Products

Description	Cat No
IQ/OQ/PQ Kit for Ion Chromatography, 1 Kit (6x100 mL)	**12674**
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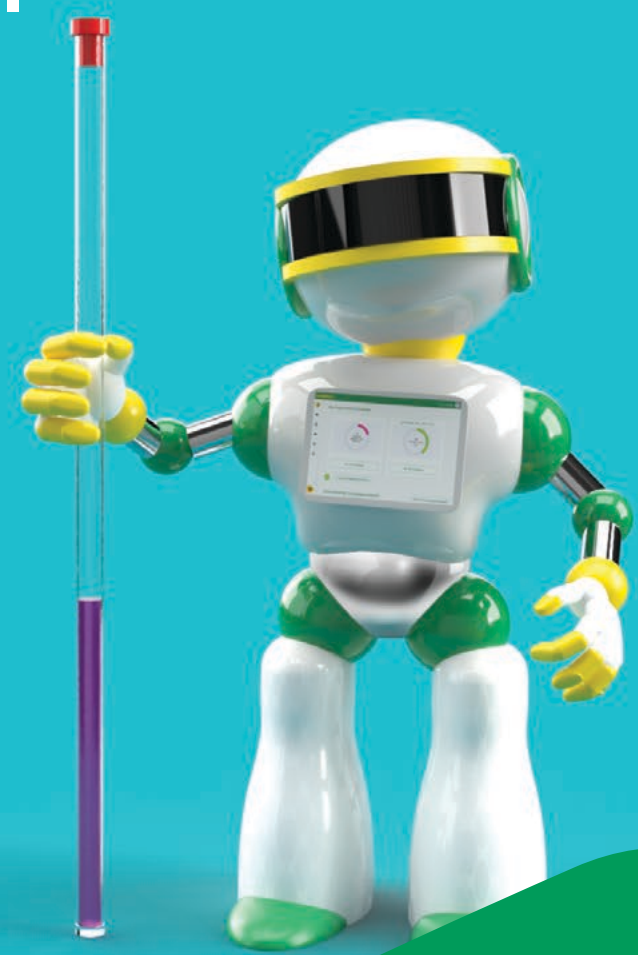

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