Influence of bioinert (U)HPLC hardware on the analysis of four different biomolecules

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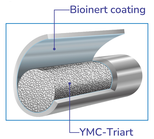
Introduction

It is well known that certain biomolecules can adhere to stainless-steel column hardware. When adherence to the stainless-steel column hardware occurs, reduced recovery and carry-over become significant issues. In this study, the benefits of using bioinert hardware for the analysis of biomolecules are demonstrated. Four distinct biomolecule types are used to demonstrate the benefits of bioinert hardware. One type of biomolecule used in this study is phosphopeptides. YMC-Accura Triart columns with bioinert surface coating were used to separate phosphopeptides, and the results were compared to standard stainless-steel hardware using the same chromatographic conditions. The second type of biomolecule is an Adeno-associated virus (AAV) capsid protein. A YMC-Accura Triart Bio C4 column is used for the AAV analysis and compared to standard column hardware. A YMC-Accura Triart Diol-HILIC column is used in hydrophilic interaction liquid chromatography (HILIC) mode for the third type of biomolecule analysis, deoxythymidine and RNA oligonucleotides. Phospholipids, LPC (16:0) and PC (34:2), are the fourth and final type of biomolecule with reversed phase chromatography coupled to mass spectrometry (MS) being used for their analysis with YMC-Accura Triart C18.

Model Case Study

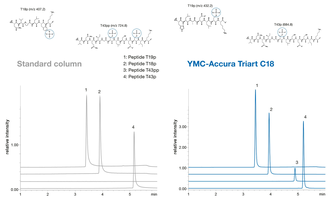


- Comparison of bioinert YMC-Accura (U)HPLC columns to standard stainless steel columns
- YMC-Accura Triart columns are equipped with a bioinert surface coating of the column body and frits
- Analysis of phosphopeptides, phospholipids, AAV capsid proteins and oligonucleotides using bioinert hardware
- Use of IP-RP LC, RP LC/MS and HILIC modes

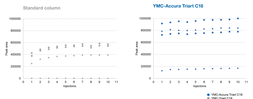
Acknowledgment

- Bioinert column hardware leads to higher recovery, better peak shapes, greater reproducibility
- Little to no conditioning is necessary when using bioinert hardware
- Use of bioinert columns prevent non-specific absorption of the analytes by the hardware
- The bioinert YMC-Accura Triart columns are the best choice for the analysis of metal-sensitive analytes such as biomolecules containing phosphate groups
- Bioinert columns are ideally combined with a bioinert system

Operation of phosphopeptides



The use of the bioinert YMC-Accura Triart C18 column led to higher intensities and peak areas for all peaks. Due to the high recovery rates of YMC-Accura Triart C18 the challenging phosphopeptide T43 pp, which contains two phosphate residues, can be detected. This is not achieved using the standard stainless steel column.

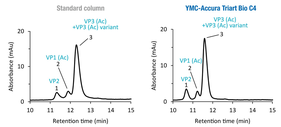


Analysis of phosphorylated peptides with the bioinert YMC-Accura Triart C18 column gives very stable peak areas. About 6-9 % deviation is seen for T18p, T19p and T43p within the first 10 injections. In comparison, the standard stainless steel column shows deviations of up to 56 %.

Application data courtesy of Shimadzu Europa.

Column:	YMC-Accura Triart C18 (1.9 µm, 12 nm) 100 x 2.1 mm ID (bioinert hardware)
	YMC-Triart C18 (1.9 µm, 12 nm) 100 x 2.1 mm ID (standard hardware)
Eluent:	A) water + 0.1 % formic acid
	B) acetonitrile + 0.1 % formic acid
Gradient:	0.7 % - 25 %B (0-5 min), 25 %B (5 - 6.6 min),
	0.7 %B (6.6 - 8 min)
Flow rate:	0.6 mL/min
Temperature:	60 °C
Detection:	ESI-MS
Injection:	2 µL (10 pmol/µL)
Sample:	Massprep phosphopeptide enolase standard (Waters)
System:	Shimadzu Nexera XS inert
	Shimadzu LCMS-2020

Adeno-associated virus (AAV) capsid protein analysis



Both, the YMC-Accura Triart Bio C4 and the standard YMC-Triart Bio C4 column enable the separation of the AAV capsid proteins VP1 and VP2. The bioinert YMC-Accura Triart Bio C4 additionally prevents adsorption of analytes, which leads to improved peak shape.

Application data courtesy of Prof. S. Uchiyama, Osaka University.

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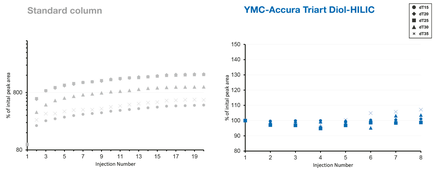
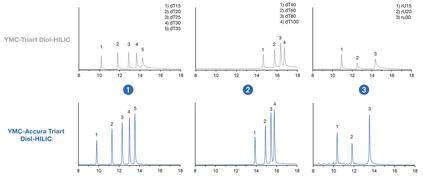
Column:	YMC-Accura Triart Bio C4 (1.9 µm, 30 nm)
	150 x 2.1 mm ID (bioinert hardware)
	YMC-Triart Bio C4 (1.9 µm, 30 nm)
	150 x 2.1 mm ID (standard hardware)
Eluent:	A) water + 0.1% difluoroacetic acid
	B) acetonitrile + 0.1% difluoroacetic acid
Gradient:	20-32%B (0-1 min), 32-36%B (1-16 min),
	36-80%B (16-20 min)
Flow rate:	0.2 mL/min
Temperature:	80 °C
Detection:	UV at 280 nm
	ESI-MS (positive ion mode)
Injection:	50 µL
Sample:	Denatured AAV2

HILIC analysis of oligonucleotides

Column:	YMC-Accura Triart Diol-HILIC (1.9 µm, 12 nm) 150 x 2.1 mm ID (bioinert hardware)
	YMC-Triart Diol-HILIC (1.9 µm, 12 nm) 150 x 2.1 mm ID (standard hardware)
Eluent:	A) 50 mM ammonium acetate (pH 6.9)
	B) acetonitrile
Gradient:	75-45 %B (0 - 30 min)
Flow rate:	0.3 mL/min
Temperature:	40 °C
Detection:	UV at 260 nm
Injection:	2 µL
Sample:	deoxythymidine oligonucleotides: dT15-35 (2 µM) and dT40-100 (2 µM)
	RNA oligonucleotides: rU15-30 (2 µM)
System:	bioinert system



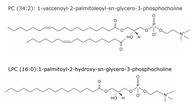
The bioinert YMC-Accura Triart Diol-HILIC needs only 8 injections for conditioning, assessed by the difference between the initial and 8th peak area being less than 10 % [1]. In contrast, with the stainless steel column, 20 injections are required until full conditioning is achieved [2].

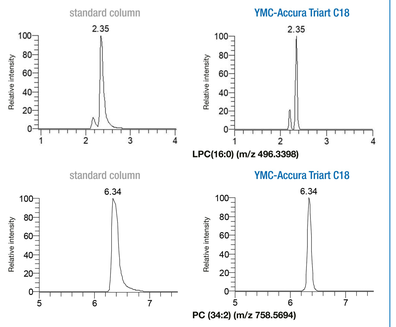


Use of the bioinert YMC-Accura Triart Diol-HILIC column results in higher sensitivities, peak areas and less tailing for all sizes and types of nucleotides tested [1]. Non-specific adsorption did not vary according to nucleotide length, even though the adsorption is usually higher for longer oligonucleotides in IP-RP. On the contrary, the relative peak areas varied strongly for the stainless steel column, which indicates that non-specific absorption is still significant [2].

[1] H. Lardeux, A. Goyon, K. Zhang, J.M. Nguyen, M.A. Lauber, D. Guillarme, V. D'Atri, The impact of low adsorption surfaces for the analysis of DNA and RNA oligonucleotides, J. Chromatogr. A 1677 (2022) 463324.
[2] Application data courtesy of Honorine Lardeux, Institute of Pharmaceutical Sciences of Western Switzerland (University of Geneva), Geneva, Switzerland.

Phospholipid separation





Column:	YMC-Accura Triart C18 (1.9 µm, 12 nm) 100 x 2.1 mm ID
Eluent:	A) 10 mM ammonium formate/acetonitrile + 0.1 % formic acid (40/60)
	B) 10 mM ammonium formate in 2-propanol/acetonitrile + 0.1 % formic acid (40/60)
Gradient:	20 - 55 %B (0 - 3.5 min), 55 - 95 %B (3.5 - 15 min),
	95 %B (15 - 17 min)
Flow rate:	0.4 mL/min
Detection:	ESI positive mode, Orbitrap QExactive Focus
	(FS 70k at mz200, AGC 1e6 charges, IT 50ms; DDA top4 175.k at mz200, IT 50ms)
Temperature:	50 °C
Injection:	2 µL
Sample:	100 µL pooled human plasma precipitated with 2-propanol
	(Sample-to-solvent ratio 1:6; reconstituted with 100 µL of 2-propanol)

By using bioinert YMC-Accura Triart C18 column, excellent peak shapes are achieved. When using the standard stainless steel column, massive peak tailing is obtained. Despite the challenging matrix of precipitated human plasma, YMC-Triart columns show high matrix tolerance due to their large surface area of 360 m2/g and their robustness.

Application data courtesy of Sergey Girel, Institute of Pharmaceutical Sciences of Western Switzerland (University of Geneva), Geneva, Switzerland.

Conclusions

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