Boosting Bioanalysis

As our biological knowledge expands and therapies become increasingly complex, analytical methods must evolve to ensure safe and effective treatment for patients. Novel column technologies from YMC make use of alternative analytical conditions to enable faster and more sensitive analysis of biological materials.

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Monoclonal Antibody Analysis Using HIC with an Isopropanol Modifier

Achieve narrow peak width while reducing antibody denaturation and improving recovery by using the nonporous BioPro HIC BF column

Due to poor solubility and low bioavailability, understanding the complex structures of antibodies is more important than ever. Monoclonal antibodies provide immune responses by binding to cells and proteins bearing a specific antigen. These molecules can be modified to stimulate specific immune attacks in applications such as the treatment of cancer and autoimmune diseases.

Hydrophobic interaction chromatography (HIC) is one method of analysis for antibodies, owing to the non-denaturing conditions used and the high selectivity of changes in the antibody structure to the hydrophobic surface. The protic solvent isopropanol (15 percent) can be added to the eluent to limit denaturation and improve antibody recovery – as demonstrated with the non-porous BioPro HIC BF column from YMC.

These conditions provide high resolution and narrow peak widths. In this application note, developed by the Department of Analytical Pharmaceutical Chemistry at the University of Geneva, we demonstrate a robust analytical system for separation of the monoclonal antibodies adalimumab (Humira), brentuximab (commercially available as the antibody-drug conjugate brentuximab vedotin; Adcetris), pembrolizumab (Keytruda) and reslizumab (Cinqaero). Analysis is complete in 12 minutes at a column temperature of 20 °C, using a gradient with decreasing ammonium sulphate and increasing isopropanol content in the sodium phosphate buffer.

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Generic Monoclonal Antibody Analysis by RP-(U)HPLC

Rapid and robust analysis of generic antibodies using YMC-Triart Bio C4

Monoclonal antibodies have emerged as useful tools in the fight against cancer and infectious diseases, due to their ability to bind to specific antigens. The large size of these molecules (often about 150 kDa) means that their analysis is often carried out using ion exchange, size exclusion or hydrophobic interaction chromatography. Reversed-phase columns are also useful in combination with MS, but issues with sensitivity and resolution have traditionally limited their use.

The solution: to use a wide-pore (300 A, 30 nm), temperature-stable stationary phase such as YMC-Triart Bio C4 to analyze monoclonal antibodies in reversed-phase mode. The enhanced stability allows the use of temperatures up to 90 °C. This application note describes a robust method for the analysis of commercially available antibodies trastuzumab (Herceptin), rituximab (MabThera), adalimumab (Humira), bevacizumab (Avastin) and NISTmAb. The analysis is completed in 7 minutes using 1.9 µm UHPLC particles and a column temperature of 80 °C with water, acetonitrile and TFA as eluents. The greater sensitivity and sharper peaks for all molecules analyzed demonstrates the potential of the method for any UHPLC monoclonal antibody analysis.

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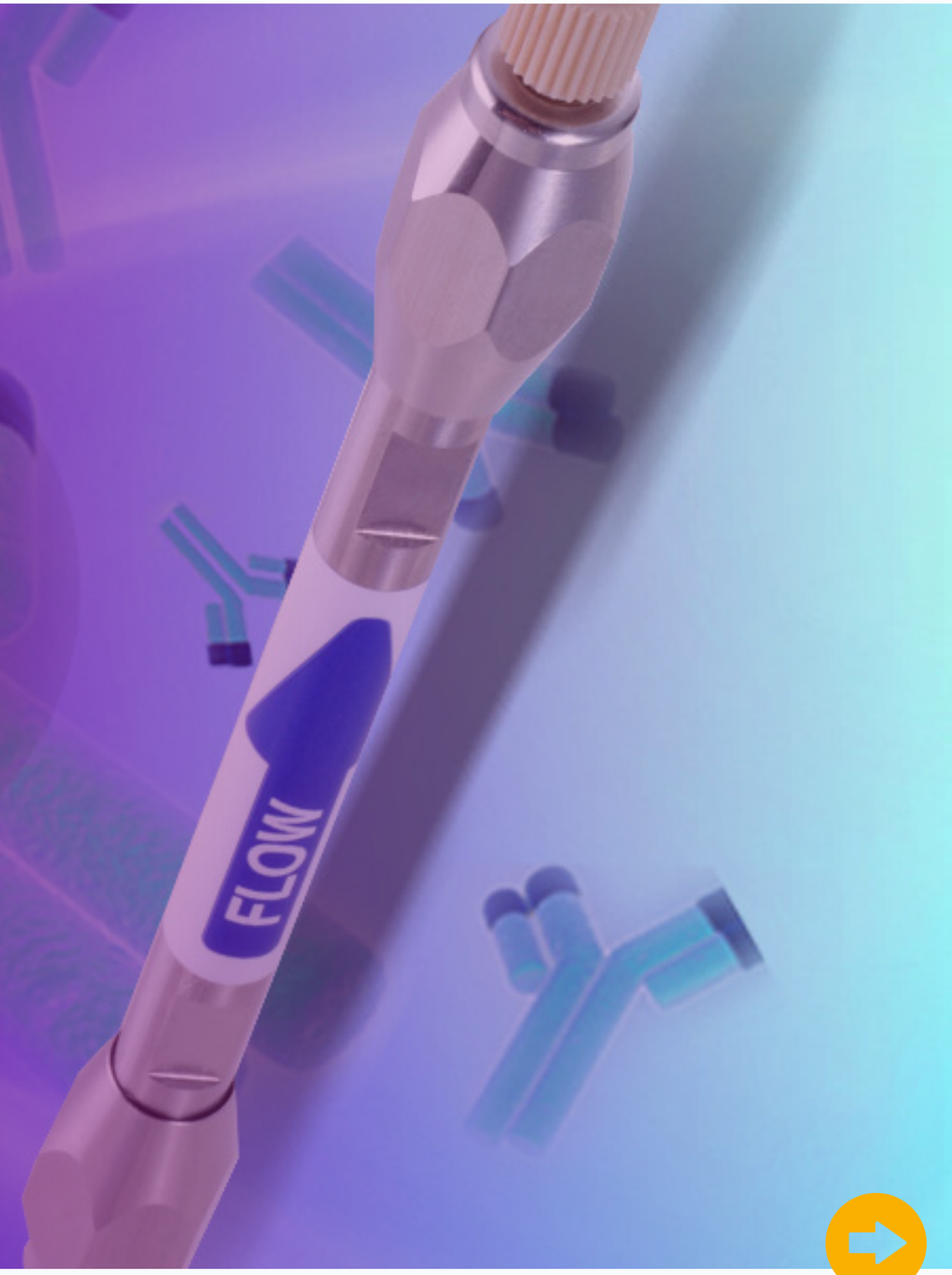

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Simultaneous Analysis of Amyloid β Proteins by HPLC

Rapid analysis of four amyloid β proteins using YMC-Triart Bio C4 assists research into Alzheimer's disease

Amyloid β (Aβ) peptides consist of 36-43 amino acids produced by the cleavage of amyloid precursor proteins by secretase enzymes. Aβ molecules can aggregate to form flexible soluble oligomers that are implicated in Alzheimer's disease, but their biological functioning remains unexplained. Despite suggestions of Aβ neurotoxicity, further research is needed to give reliable analysis into the structures of these peptides.

We present a method for the simultaneous determination of four Aβ peptides. This overcomes the problem of their high hydrophobicity, which can often complicate analysis by leading to the formation of aggregates.

Given the need for a stationary phase with lower hydrophobicity and larger pores, YMC-Triart Bio C4 was chosen to develop optimized rapid methods for analyzing complex mixtures of peptides and proteins across a wide temperature and pH range. Full details can be found in our application note.

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A Non-Porous Anion Exchanger for Oligonucleotide Separation

BioPro IEX QF offers optimized nucleotide separations with reduced carryover and improved peak shape

Nucleic acid therapeutics such as antisense, siRNA and aptamers are expected to be the next generation of pharmaceuticals. However, the very similar structures of oligonucleotides following synthesis means that chromatographic methods for their analysis and purification must be highly specific.

Our application note presents useful tips and guidelines for the optimization of ion exchange chromatography (IEX) for separating nucleotides. Specifically, we describe the use of BioPro IEX QF to reduce carryover and improve peak shape by optimizing both the buffer type (changing from Tris-HCl to NaOH) and the counter ion used (changing from NaCl to $NaClO_4$).

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Sphingolipid Analysis by LC-MS/MS Using Metal-Free Columns

High-sensitivity sphingolipid analysis can be achieved using the YMC-Triart C18 metal-free column

Sphingolipids are central components of biological membranes, and also serve a crucial role in cell signalling processes. These molecules are known to impact the pathology of many human diseases including obesity, Alzheimer's disease and diabetes. LC analysis of sphingolipids such as sphingosine-1-phosphate (S1P) and ceramide-1-phosphate (C1P) is complicated, however, due to the presence of phosphate groups, which are known to induce significant peak tailing.

We present an improved method of analysis using the YMC-Triart C18 metal-free column, which was recently reported by Dr Gowda et al (1). Metallic surfaces are known to adsorb phosphate groups but this column uses hardware consisting of PEEK-lined stainless steel and PEEK frits. This eliminates phosphate adsorption and so improves peak shapes, which leads to improved sensitivity of these analyses, namely LC-MS.

Reference

1. *SG Gowda et al., "Facile determination of sphingolipids under alkali condition using metal-free column by LC-MS/MS", Anal Bioanal Chem, 410, 4793–4803 (2018). DOI: 10.1007/s00216-018-1116-5*

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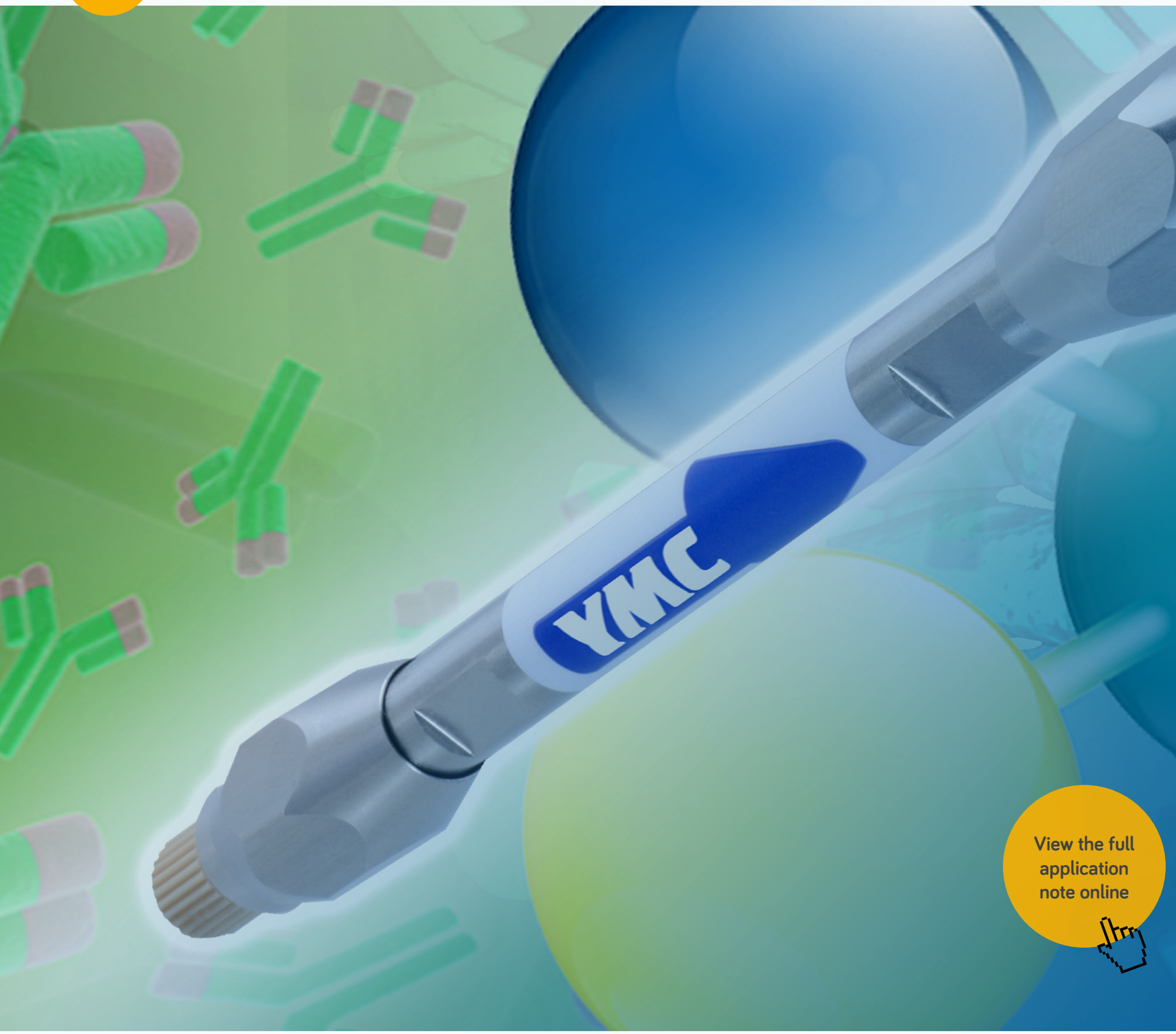

LC-MS-Compatible Mobile Phases for ADC Analysis

LC-MS analysis of reduced antibody-drug conjugates using YMC-Triart Bio C4

LC-MS methods rely on volatile buffers and additives in the mobile phase due to the vaporization of solvents in the ionization process. Formic acid is often used for the measurement of small molecules at low pH, despite it producing broader peaks, since it is more MS-compatible than TFA and prevents non-volatile additives contaminating the detector. TFA cannot be used in negative ion MS and remains in the detector.

This application uses YMC-Triart Bio C4 to analyze a reduced monoclonal antibody and a reduced antibody-drug conjugate-mimic with formic acid and TFA as additives. Using YMC-Triart Bio C4 and the addition of 0.1 percent TFA, excellent resolution and peak shapes are obtained. Despite slightly broader peaks and shorter retention times being observed when using 0.1 percent formic acid, the separation is also suitable for structural analysis using LC-MS.

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