Reversed-Phase Antibody Separations with Jupiter™ 300 C4

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Introduction

The number of antibody therapeutics has increased dramatically over the last ten years. This is partly because of the ability of antibodies to target specific biomolecules in vivo thereby either blocking or activating specific biological activities. While antibodies have proved incredibly useful as therapeutic agents, there have been many challenges in analysing and purifying such proteins.

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Immunoglobulin G (Ig-G), which is the traditional recombinant antibody used, is a 150 KDa glycoprotein consisting of two heavy chains (~50 KDa each) and two light chains (~25 KDa each) that are disulfide linked forming a “Y”-like structure. Because of its large size and moderate hydrophobicity, as well as its non-globular structure, antibodies have often proved difficult to analyse by reversed-phase chromatography.

Antibody protein peaks are often broad and poorly defined with poor recoveries. Such poor chromatography can make separating minor components in an antibody sample difficult. Efforts were undertaken to improve reversed-phase chromatography of Ig-G by investigating different mobile phase conditions as well as flow-rate and temperature.

Materials and Methods

Analyses were performed using an HP 1100 LC system (Agilent Technologies, Palo Alto, California, USA) equipped with a binary pump and UV detector and using HP Chemstation software (Version A.08.03) for data analysis. The HPLC columns used for the analyses were Jupiter 300 C4, C5 and C18 150 × 2.0 mm (Phenomenex, Torrance, California, USA). Recombinant dog immunoglobulin G (Ig-G), trifluoroacetic acid (TFA) and dithiothreitol were purchased from Sigma Chemicals (St. Louis, Missouri, USA). Solvents were purchased from Fisher Scientific (Fairlawn, New Jersey, USA).

For all separations an aliquot of 4 µg of dog Ig-G was injected on selected columns running at a flow-rate of 0.25 mL/min. Mobile phase A was 5% acetonitrile/0.1% trifluoroacetic acid in water for all runs. Mobile phase B was either 95% acetonitrile/0.85% trifluoroacetic acid in water or 75% acetonitrile/20% isopropanol/0.85% trifluoroacetic acid in water. A gradient from 20% B to 95% B over 20 minutes was used for all separations.

For HPLC analysis of antibody heavy and light chain mixtures, dog Ig-G was reduced with 2 mM DTT in 8 M urea at 45º C for 30 minutes.

Results

Different organic mobile phase conditions as well as Jupiter phases were evaluated to improve overall peak shape for antibody separations. Figure 1 demonstrates the utility of using low amounts of isopropanol in the organic mobile phase to improve peak shape of hydrophobic proteins. Figure 1(a) shows the elution of Ig-G on a Jupiter 300 C4 column where Acetonitrile is used as the organic mobile phase. Figure 1(b) shows the same Ig-G run on the same column using an organic mobile phase that contains 20% isopropanol. Note the dramatic improvement in both recovery and peak shape for the Ig-G peak. The addition of isopropanol to the organic mobile phase improves the efficiency as well as the peak shape for large hydrophobic proteins mainly because of the increased hydrophobicity of the organic mobile phase upon addition of isopropanol.

Different Jupiter 300 phases were evaluated to determine which phase provided the best peak shape and efficiency. The dog Ig-G sample was run on the Jupiter 300 C4, C5 and C18 phases and results are shown in Figure 2. Surprisingly, the Jupiter 300 C5 and Jupiter 300 C18 do demonstrate reasonable peak shape and recovery. However, as expected, the Jupiter 300 C4 phase demonstrated the least retention as well as the best peak shape and recovery of the three phases. This clearly demonstrates that Jupiter 300 C4 is the best column for the chromatographic separation of large hydrophobic proteins.

An additional area of application interest for many working with antibodies is in the separation of heavy and light chains of Ig-G. For this example dog Ig-G was reduced with dithiothreitol and injected on a Jupiter 300 C4 column. Results are shown in Figure 3; one can see that the heavy and light chains are baseline resolved using the conditions previously described. These results demonstrate the ability of the Jupiter 300 C4 to resolve differences...
Phenomenex has produced a brochure highlighting how its Zebron™ ZB-5ms column can be used to optimize the analysis of semi-volatile organic compounds to a 14-min run time. Typically this method must be extended for >20 min in order to achieve passing resolution for isomeric pairs, such as benzo[b] and benzo[k]fluoranthene. The company's Arylene Matrix Technology™, a proprietary bonding process, makes this separation possible as columns are produced with enhanced selectivity for multi-ring aromatics. The various applications of this column can be seen in the brochure.

Phenomenex Inc., Torrance, California, USA.

Applications for GC column

If you would like more information on these columns or any of the applications listed, please contact Phenomenex or a local distributor. Also, if you are new to protein and peptide HPLC or are doing method development work call today to reserve your FREE copy of our 75-page A User's Guide — Introduction to Peptide and Protein HPLC.

Ordering Information

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Extraction of basic drugs from biological matrices

Application note TN-008 from Phenomenex reportedly provides a simple solid-phase extraction (SPE) method for the extraction of basic drugs from biological samples. Analysis of basic compounds in urine or blood is hampered by the presence of endogenous compounds in the sample. These interferences are present in higher concentrations than the target analytes and thus may mask their presence. The extraction method uses the company's strata™ X-C — a patent pending 33 µm mixed mode polymeric resin.

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