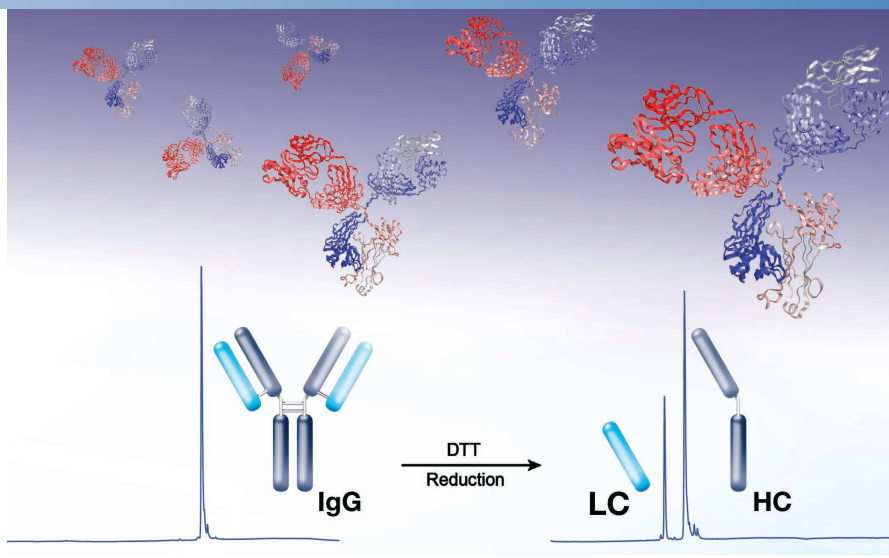
# MAbPac RP Column

## High-performance reverse phase chromatography column for monoclonal antibody analysis

The Thermo Scientific™ MAbPac™ RP is a reverse phase (RP) liquid chromatography column designed for mAb characterization, including the separation of mAb and variants, light chain (LC) and heavy chain (HC), Fc and Fab fragments, scFc and F(ab')$_2$ fragments, using LC/UV or LC/MS. The unique column chemistry provides excellent performance under a broad range of pH, temperature, and mobile phase composition.

Product Highlights

- Superior resolution power for monoclonal antibodies and related substances
- High efficiency with low carry-over
- Excellent MS compatibility
- Wide operating pH range: 0–14
- High temperature stability: up to 110 °C
- High throughput




### Introduction

The monoclonal antibody (mAb) therapeutics market is growing at a rapid rate owing to increasing demand for targeted treatments. Therapeutic mAbs are mostly produced from mammalian cells. These biological products are heterogeneous due to post-translational modifications. Additional modifications such as oxidation can be introduced during the manufacturing process. A comprehensive characterization of mAb purity, aggregates, and variants is required for the final biopharmaceutical product approval and subsequent manufacturing processes. There is a growing trend to obtain intact mass information as well as the glycan profile in the QC of mAbs using reverse phase chromatography coupled with high resolution mass spectrometry detection. LC/MS analysis of mAb fragments such as light chain (LC), heavy chain (HC), Fc, Fab, scFc and F(ab')$_2$, can accurately reveal the location, as well as nature, of the modification. Moreover, in most QC environments, LC/UV analysis of mAb fragments has been established as a high throughput assay.

### Column Technology

MAbPac RP is a reverse phase (RP) column specifically designed for separation of intact monoclonal antibodies (mAbs) and mAb fragments. The stationary phase is fully compatible with mass spectrometry friendly organic solvent such as acetonitrile and isopropanol, as well as low pH eluents containing trifluoroacetic acid or formic acid. The MAbPac RP is based on wide-pore 4 µm polymer particles that are stable at extreme pH (0–14) and high temperature (up to 110 °C). The wide-pore size of polymeric particles enables efficient separation of protein molecules with low carry-over.

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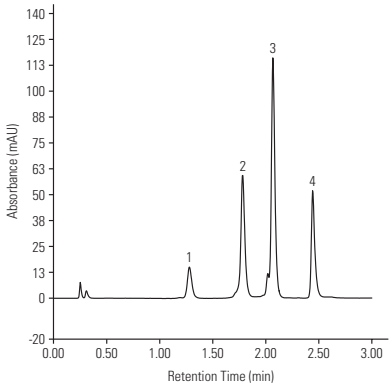
## Applications

### Fast Separation of Intact Proteins/mAbs

The MAbPac RP column is designed for high resolution and fast separation of proteins. High throughput and high-resolution can be achieved by adjusting both gradient slope and flow rate. The wide-pore nature of polymer particles combined with 4 µm particle size provides excellent resolution for intact proteins/mAbs with high-throughput and low carry-over. Figure 1 shows the baseline separation of four proteins: ribonuclease A, cytochrome C, lysozyme, and a mAb, on a 2.1 × 50 mm MAbPac RP column within 3 min.



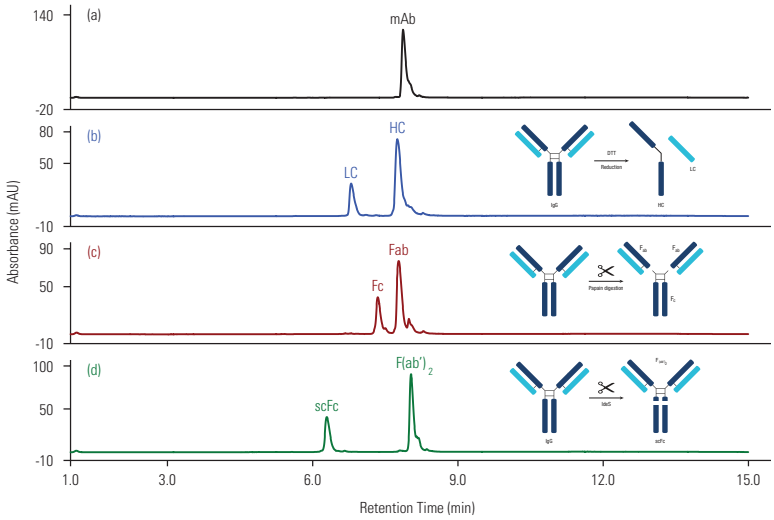

| | |
|---|---|
| Column: | **MAbPac RP**, 4 µm |
| Format: | 2.1 × 50 mm |
| Mobile phase A: | $H_2O$/TFA (99.9 : 0.1 v/v) |
| Mobile phase B: | MeCN/ $H_2O$/TFA (90: 9.9 :0.1 v/v/v) |
| Gradient: | Time (min) %A %B |
| | 0.0 80 20 |
| | 2.5 50 50 |
| | 2.7 50 50 |
| | 2.8 80 20 |
| | 4.0 80 20 |
| Flow Rate: | 0.6 mL/min |
| Inj. Volume: | 1 µL |
| Temp.: | 80 °C |
| Detection: | UV (280 nm) |
| Sample: | 1. Ribonuclease A (0.5 mg/mL)<br>2. Cytochrome C (0.5 mg/mL)<br>3. Lysozyme (0.5 mg/mL)<br>4. mAb (1 mg/mL) |

Figure 1: Fast separation of intact proteins/mAb

### Separation of mAb Fragments

Monoclonal antibodies are heterogeneous. Comprehensive analysis of mAb post-translational modifications, such as deamidation, C-terminal lysine truncation, N-terminal pyroglutamation, methionine (Met) oxidation, and glycosylation, requires complete digestion of mAbs and sequencing of all the peptides. However, "peptide mapping" can be time consuming.

A simpler and more direct way to analyze mAb variants and locate the modifications is to measure mAb fragments, which can either be generated by chemical reduction or by enzymatic digestion. Figure 2 shows the analysis of trastuzumab intact molecule and fragments. LC and HC (Figure 3b), Fc and Fab (Figure 3c), scFc and $F(ab')_2$ (Figure 3d) are baseline separated on a 3.0 × 50 mm MAbPac RP column using a 10 min gradient. Light chain (LC) and heavy chain (HC) are generated by the reduction of mAb with DTT, Fc and Fab fragments are generated by papain digestion, and single-chain Fc (scFc) and $F(ab')_2$ fragments are generated by IdeS digestion.




| | |
|---|---|
| Column: | **MAbPac RP**, 4 µm |
| Format: | 3.0 × 50 mm |
| Mobile phase A: | $H_2O$/FA/TFA (99.88 : 0.1:0.02 v/v/v) |
| Mobile phase B: | MeCN/ $H_2O$/FA/TFA (90: 9.88 :0.1:0.02 v/v/v/v) |
| Gradient: | Time (min) %A %B |
| | 0.0 80 20 |
| | 1.0 80 20 |
| | 11.0 55 45 |
| | 12.0 55 45 |
| | 14.0 80 20 |
| | 15.0 80 20 |
| Flow Rate: | 0.5 mL/min |
| Inj. Volume: | 5 µL |
| Temp.: | 80 °C |
| Detection: | UV (280 nm) |
| Sample: | (a) Trastuzumab (5 mg/mL)<br>(b) Trastuzumab + DTT (4 mg/mL)<br>(c) Trastuzumab + Papain (2 mg/mL)<br>(d) Trastuzumab + IdeS (2 mg/mL) |

Figure 2: Separation of mAb and mAb fragments

## LC/MS analysis of Intact mAb and mAb fragments

High resolution mass spectrometers such as the Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer provide accurate mass information of large biologic molecules such as mAbs. The MAbPac RP column can be directly coupled to the mass spectrometer for MS detection of mAb and mAb fragments. While trifluoroacetic acid (TFA) as ion-pairing reagent provides excellent separation results, TFA can suppress ionization in the LC/MS interface, causing a drop in signal. This can be mitigated by reducing the TFA concentration to 0.02% in the presence of 0.1% formic acid (FA). Figure 3 shows the intact mass detection of trastuzumab. The top trace shows the total ion-current chromatogram. The middle trace shows the MS spectrum of trastuzumab in the mass range of 2,000 to 4,000 *m/z*. The bottom trace shows a zoom-in spectrum of trastuzumab with 50+ charges. The cluster shows the glycosylation profile of trastuzumab.

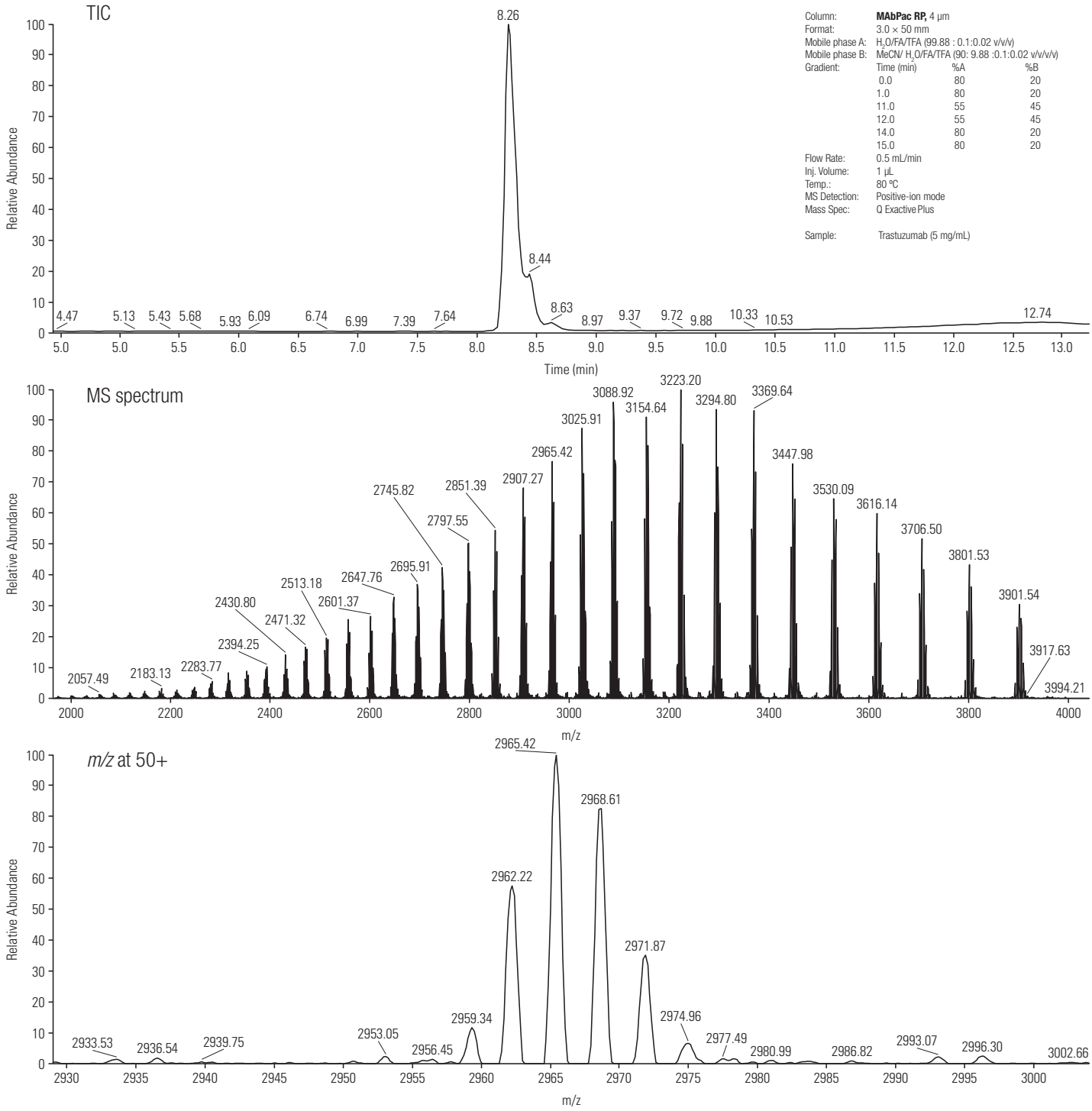



Figure 3: Intact mAb and glycosylation profile by LC/MS

During characterization, mAbs are often reduced to LC and HC. Mass spectrometry analysis of these fragments can quickly reveal and localize the modifications. Figure 4 shows the separation of trastuzumab LC and HC on a 3.0 × 50 mm MAbPac RP column. Total ion chromatogram (TIC) and UV spectrum show identical retention time of mAb fragments. The mass spectrum of LC shows multiple charge states of a single polypeptide chain while the mass spectrum of the HC shows multiple glycosylation forms of the heavy chain.

| | | | |
|---|---|---|---|
| Column: | **MAbPac RP,** 4 µm | | |
| Format: | 3.0 × 50 mm | | |
| Mobile Phase A: | $H_2O$/FA/TFA (99.88 : 0.1:0.02 v/v/v) | | |
| Mobile Phase B: | MeCN/ $H_2O$/FA/TFA (90: 9.88 :0.1:0.02 v/v/v/v) | | |
| Gradient: | Time (min) | %A | %B |
| | 0.0 | 80 | 20 |
| | 1.0 | 80 | 20 |
| | 11.0 | 55 | 45 |
| | 12.0 | 55 | 45 |
| | 14.0 | 80 | 20 |
| | 15.0 | 80 | 20 |
| Flow Rate: | 0.5 mL/min | | |
| Inj. Volume: | 1 µL | | |
| Temp.: | 80 °C | | |
| UV Detection: | 280 nm | | |
| MS Detection: | Positive-ion mode | | |
| Mass Spec: | Q Exactive Plus | | |
| Sample: | Reduced trastuzumab (4 mg/mL) | | |

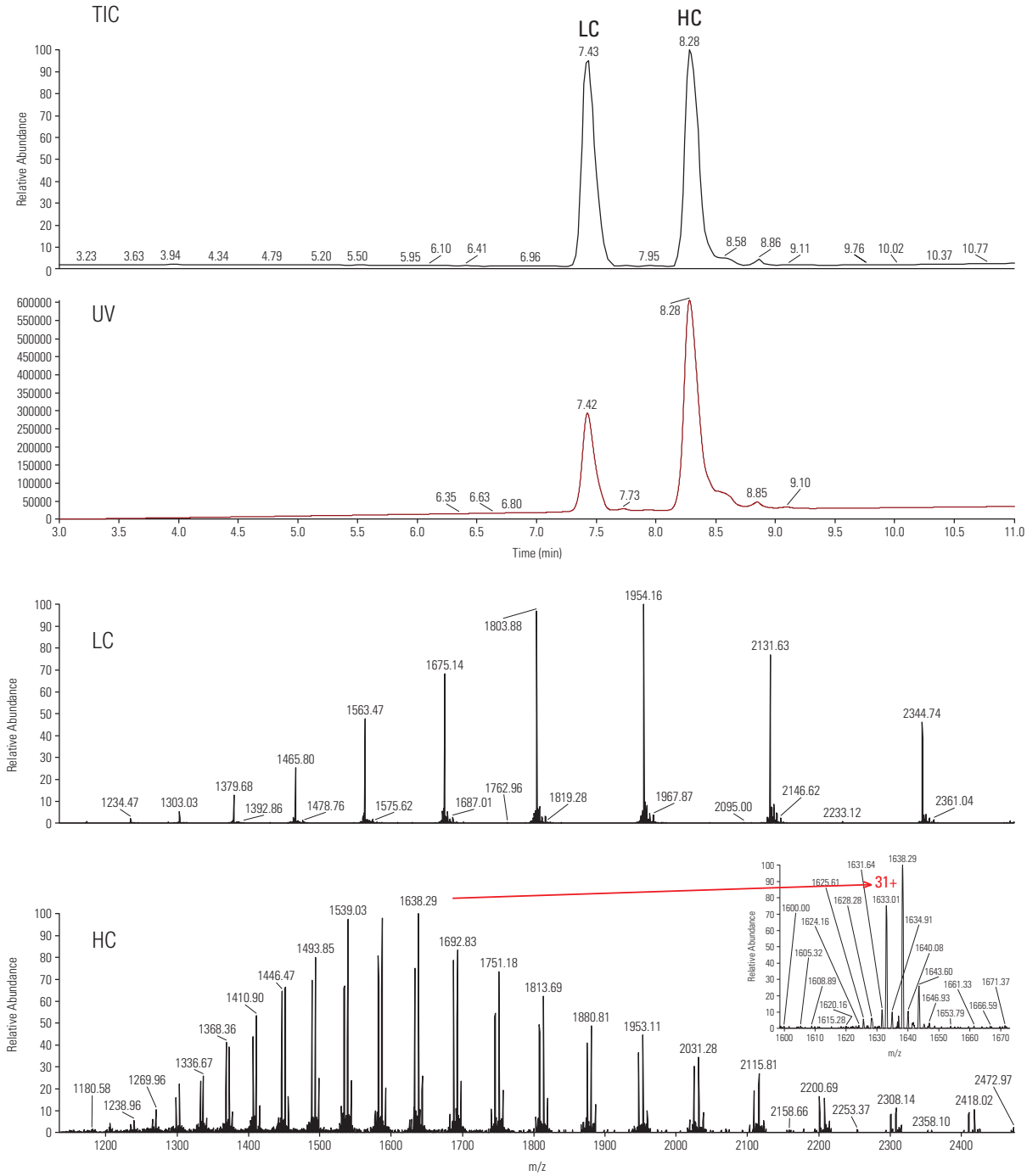
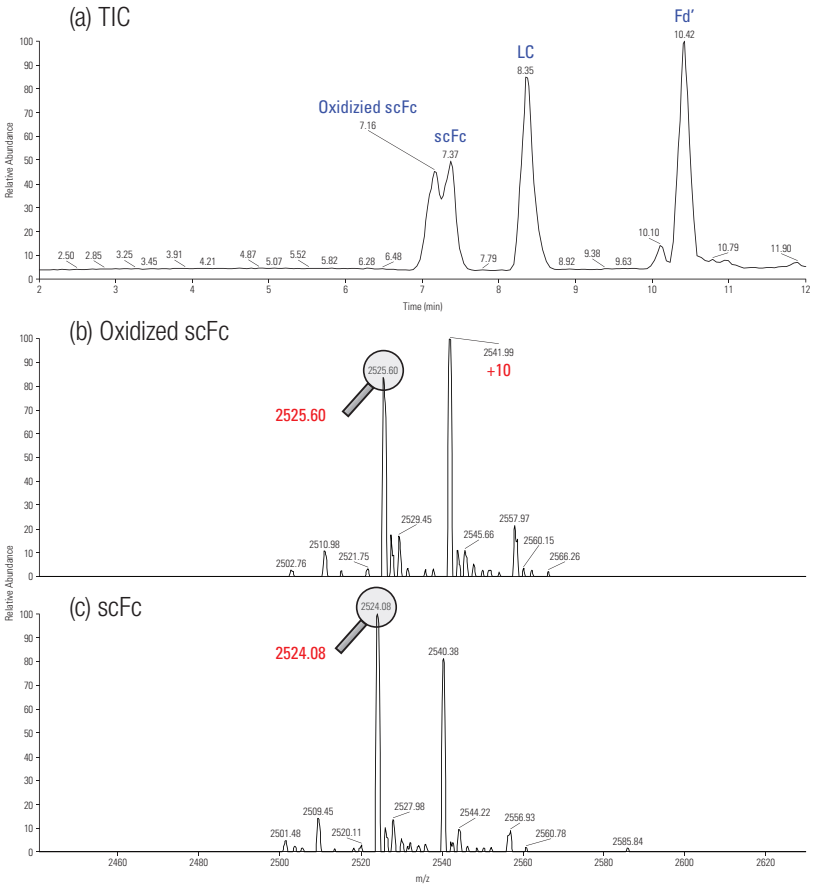



Figure 4: LC/MS analysis of mAb fragments




| | | | |
|---|---|---|---|
| Column: | **MAbPac RP**, 4 µm | | |
| Format: | 3.0 × 50 mm | | |
| Mobile Phase A: | $H_2O$/FA/TFA (99.88 : 0.1:0.02 v/v/v) | | |
| Mobile Phase B: | MeCN/ $H_2O$/FA/TFA (90: 9.88 :0.1:0.02 v/v/v/v) | | |
| Gradient: | Time (min) | %A | %B |
| | 0.0 | 75 | 25 |
| | 1.0 | 75 | 25 |
| | 11.0 | 63 | 37 |
| | 12.0 | 63 | 37 |
| | 14.0 | 75 | 25 |
| | 15.0 | 75 | 25 |

| | |
|---|---|
| Flow Rate: | 0.5 mL/min |
| Inj. Volume: | 2 µL |
| Temp.: | 80 °C |
| MS Detection: | positive-ion mode |
| Mass spec: | Q Exactive Plus |
| Sample: | Oxidized trastuzumab, reduced by DTT and digested by IdeS (1 mg/mL) |

Figure 5: LC/MS analysis of oxidized scFc

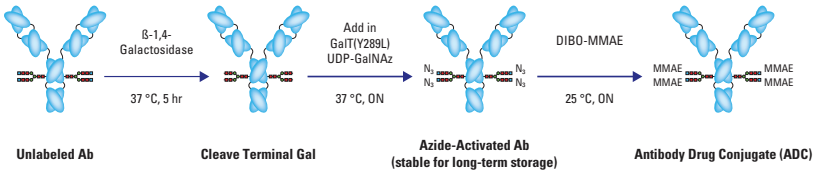
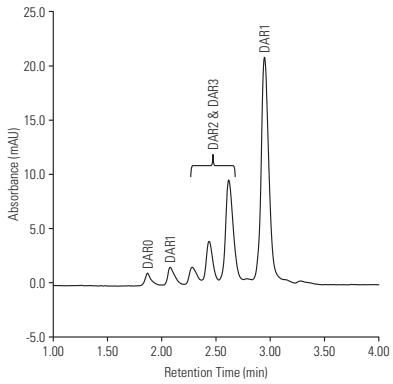



Figure 6: Site-selective antibody-drug conjugates (ADCs)




| | | | |
|---|---|---|---|
| Column: | **MAbPac RP**, 4 µm | | |
| Format: | 2.1 × 50 mm | | |
| Mobile Phase A: | $H_2O$/TFA (99.9 : 0.1 v/v) | | |
| Mobile Phase B: | MeCN/ $H_2O$/TFA (90: 9.9 :0.1 v/v/v) | | |
| Gradient: | Time (min) | %A | %B |
| | 0.0 | 65 | 35 |
| | 0.5 | 65 | 35 |
| | 4.5 | 45 | 55 |
| | 5.0 | 45 | 55 |
| | 5.5 | 65 | 35 |
| | 6.0 | 65 | 35 |

| | |
|---|---|
| Flow Rate: | 0.6 mL/min |
| Inj. Volume: | 2 µL |
| Temp.: | 80 °C |
| Detection: | UV (280 nm) |
| Sample: | Trastuzumab-MMAE |

Figure 7: MMAE modified trastuzumab ADC

## LC/MS analysis of mAb fragments containing oxidation variants

Development of high-throughput release and characterization assays is critical for rapidly growing biologics pipeline for biotherapeutics. Oxidation of mAbs is commonly monitored during process optimization, formulation development, and stability studies. The mAb can be broken down into scFc, LC, and Fd' fragments using DTT reduction followed by IdeS enzyme. Figure 5a shows the baseline separation of the scFc, LC, and Fd' fragments of trastuzumab. This sample was previously treated with $H_2O_2$, resulting in oxidation of a methione in the Fc region (confirmed by peptide mapping, data not shown). The oxidized scFc eluted before the unmodified scFc. Figure 5b shows the +10 charge state of scFc at *m/z* 2525.60 and Figure 5c shows the +10 charge state of unmodified scFc at *m/z* 2524.08. The delta mass between oxidized peak and unmodified peak corresponds to one oxygen mass. This example demonstrates that oxidation occurring in the scFc, LC, and Fd', regions can be simultaneously monitored by this assay, without going through complete digestion of mAb and peptide mapping. This simple reduction/digestion assay coupled with fast separation of the mAb fragments using MAbPac RP and high resolution accurate mass detection by Q Exactive Plus Instrument makes it an ideal method for high throughput screening of mAb oxidation.

## Analysis of Antibody-Drug Conjugate (ADC)

Antibody-drug-conjugates are proven to be a highly effective cancer therapy. Due to the heterogeneous nature of the ADC, it is critical to characterize its multiple drug-to-antibody ratio (DAR) forms. The MAbPac RP column can be utilized in the separation of mAb and its conjugates. In Figure 6, ADCs were prepared by enzymatically activating mAb Fc domain glycans with azides using the mutated beta-galactosyltransferase enzyme. The azide-activated antibodies were then conjugated with dibenzocyclooctyne (DIBO)-activated Val-Cit-PAB-Monomethyl Auristatin E (MMAE) toxin in a copperless click reaction, resulting in a mixture of drug-loaded antibody species with 0 to 4 MMAE molecules. The unmodified mAb and ADCs with DAR values ranging from 0 to 4 are well resolved on the MAbPac RP column (Figure 7).

## Analysis of PEGylated Protein

Protein PEGylation technology has been used successfully to increase the circulating half life and decrease antigenicity of protein drugs. PEGylated protein contains multiple PEGylated forms. Figures 8a and 8b illustrate the separation of a PEGylated protein and its de-PEGylated form on a 2.1 × 50 mm MAbPac RP column. At least eight PEGylated forms are resolved based on the degree of PEGylation.

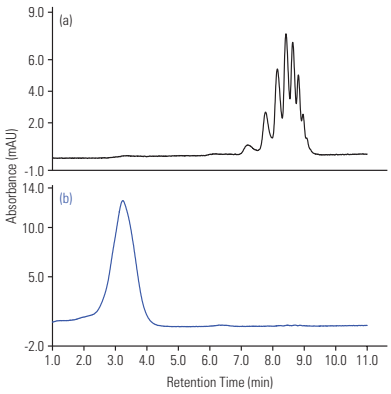



Figure 8: PEGylated protein

| | | | |
|---|---|---|---|
| Column: | **MAbPac RP,** 4 µm | | |
| Format: | 3.0 × 50 mm | | |
| Mobile Phase A: | $H_2O$/TFA (99.9 : 0.1 v/v) | | |
| Mobile Phase B: | MeCN/ $H_2O$/TFA (90: 9.9 :0.1 v/v/v) | | |
| Gradient: | Time (min) | %A | %B |
| | 0.0 | 55 | 45 |
| | 1.0 | 55 | 45 |
| | 11.0 | 25 | 75 |
| | 12.0 | 25 | 75 |
| | 14.0 | 55 | 45 |
| | 15.0 | 55 | 45 |
| Flow Rate: | 0.5 mL/min | | |
| Inj. Volume: | 10 µL | | |
| Temp.: | 80 °C | | |
| Detection: | UV (280 nm) | | |
| Sample: | (a) PEGylated protein (11 mg/mL)<br>(b) de-PEGylated protein (1.24 mg/mL) | | |

## Excellent Reproducibility and Chemical Stability

Column ruggedness is a critical characteristic for accurate and reproducible results, as well as good column lifetime. MAbPac RP columns are packed using a carefully developed packing protocol to ensure excellent packed bed stability, column efficiency and peak asymmetry. Figure 9 demonstrates that the excellent performance of the MAbPac RP is maintained throughout 1,000 runs at 80 °C providing consistent retention time, peak shape, and peak efficiency. The RSDs of retention time from four protein peaks are tabulated in Figure 9.

MAbPac RP chemistry also offers excellent chemical stability in a wide pH range. Figure 10 shows the MAbPac RP column maintains its performance after 6 hours of wash with 0.8 M NaOH at 80 °C. Such chemical stability, especially under alkaline condition provides a great advantage over RP columns based on pure silica or organo-silica hybrid particles.

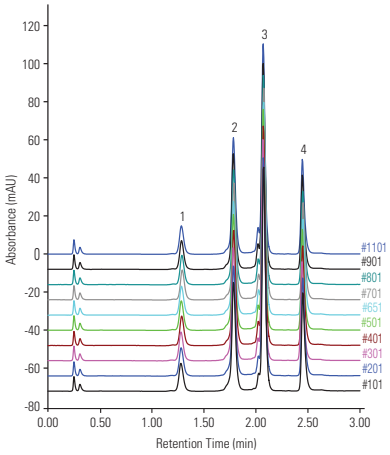



Figure 9: Excellent reproducibility

| | | | |
|---|---|---|---|
| Column: | **MAbPac RP**, 4 µm | | |
| Format: | 2.1 × 50 mm | | |
| Mobile Phase A: | $H_2O$/TFA (99.9 : 0.1 v/v) | | |
| Mobile Phase B: | MeCN/ $H_2O$/TFA (90: 9.9 :0.1 v/v/v) | | |
| Gradient: | Time (min) | %A | %B |
| | 0.0 | 80 | 20 |
| | 2.5 | 50 | 50 |
| | 2.7 | 50 | 50 |
| | 2.8 | 80 | 20 |
| | 4.0 | 80 | 20 |
| Flow Rate: | 0.6 mL/min | | |
| Inj. Volume: | 1 µL | | |
| Temp.: | 80 °C | | |
| Detection: | UV (280 nm) | | |
| Sample: | 1. Ribonuclease A (0.5 mg/mL)<br>2. Cytochrome C (0.5 mg/mL)<br>3. Lysozyme (0.5 mg/mL)<br>4. mAb (1 mg/mL) | | |

| Peaks | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| RSD of Retention Time | 0.535 | 0.262 | 0.225 | 0.123 |

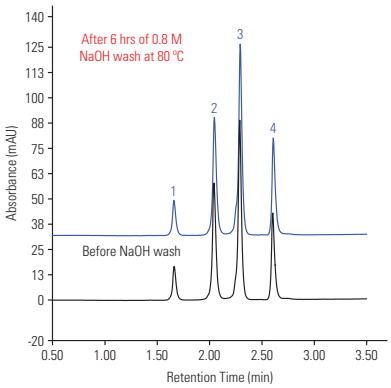



Figure 10: Superior chemical stability

| | | | |
|---|---|---|---|
| Column: | **MAbPac RP**, 4 µm | | |
| Format: | 2.1 × 50 mm | | |
| Mobile Phase A: | $H_2O$/TFA (99.9 : 0.1 v/v) | | |
| Mobile Phase B: | MeCN/ $H_2O$/TFA (90: 9.9 :0.1 v/v/v) | | |
| Gradient: | Time (min) | %A | %B |
| | 0.0 | 85 | 15 |
| | 2.5 | 50 | 50 |
| | 2.7 | 50 | 50 |
| | 2.8 | 85 | 15 |
| | 5.0 | 85 | 15 |
| Flow Rate: | 0.6 mL/min | | |
| Inj. Volume: | 1 µL | | |
| Temp.: | 80 °C | | |
| Detection: | UV (280 nm) | | |
| Sample: | 1. Ribonuclease A (0.5 mg/mL)<br>2. Cytochrome C (0.5 mg/mL)<br>3. Lysozyme (0.5 mg/mL)<br>4. mAb (1 mg/mL) | | |

## Consistent Manufacturing

Each MAbPac RP column is manufactured according to stringent specifications to ensure column-to-column reproducibility. Each column is shipped with a test chromatogram.

## Physical Data

| | |
|---|---|
| Chemistry | Phenyl |
| Substrate | Spherical polymer particles |
| Particle size | 4 µm |
| Pore size | 1,500 Å |
| Column housing | Stainless steel |

## Operational Specifications

| Column | Column ID (mm) | Flow Rate (mL/min) | Pressure Limit (psi) | Temperature (°C) | pH Range |
|---|---|---|---|---|---|
| MAbPac RP | 3.0 | 0.40–1.00 | 4,000 | < 110 | 0–14 |
| MAbPac RP | 2.1 | 0.20–0.60 | 4,000 | < 110 | 0–14 |

## Ordering Information

| Description | Particle Size | Part Number |
|---|---|---|
| MAbPac RP, Analytical, 3.0 × 100 mm | 4 µm | 088644 |
| MAbPac RP, Analytical, 3.0 × 50 mm | 4 µm | 088645 |
| MAbPac RP, Guard, 3.0 × 10 mm | 4 µm | 088646 |
| MAbPac RP, Analytical, 2.1 × 100 mm | 4 µm | 088647 |
| MAbPac RP, Analytical, 2.1 × 50 mm | 4 µm | 088648 |
| MAbPac RP, Guard, 2.1 × 10 mm | 4 µm | 088649 |

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+91 27 1766 2352
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