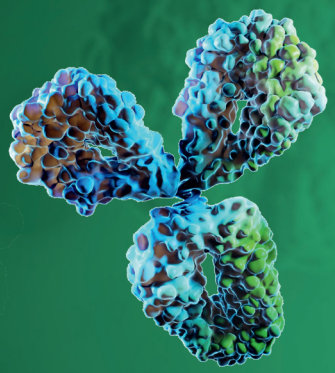
YMC EUROPE GMBH
The Selectivity Company

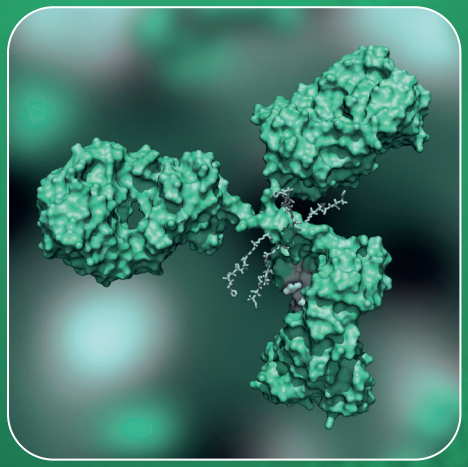
YMC Application Collection

Antibodies & ADCs



IEX
SEC
HIC
RP

Intact mAbs
Digested mAbs
DAR determination
Native LC-MS



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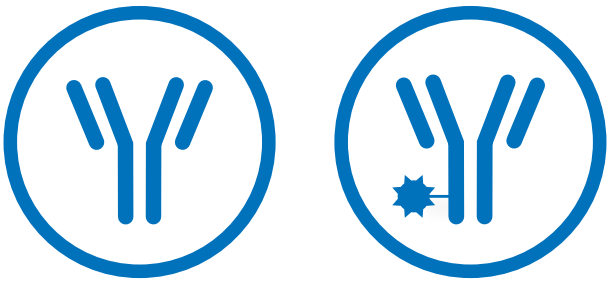
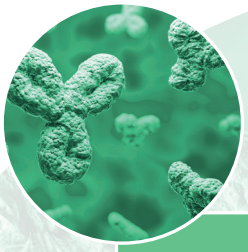




(Monoclonal) Antibodies

IEX

- BioPro IEX QA
- BioPro IEX QF
- BioPro IEX SP
- BioPro IEX SF

SEC

- YMC-Pack Diol-200
- YMC-Pack Diol-300
- YMC-SEC MAB

HIC

- BioPro HIC HT
- BioPro HIC BF

RP

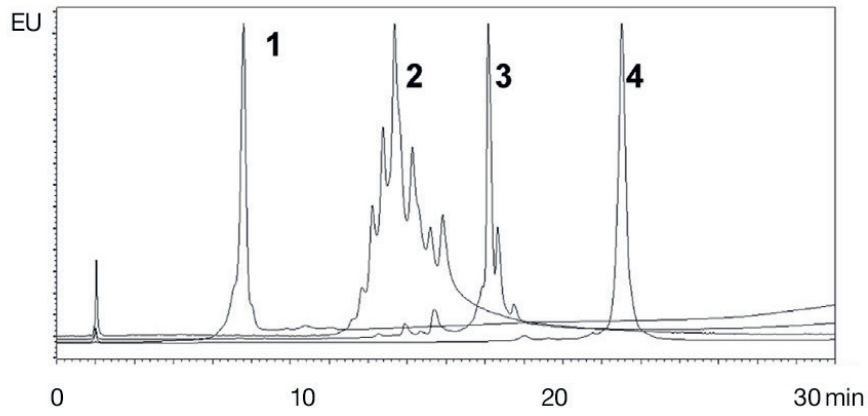
- YMC-Triart Bio C4

IEX

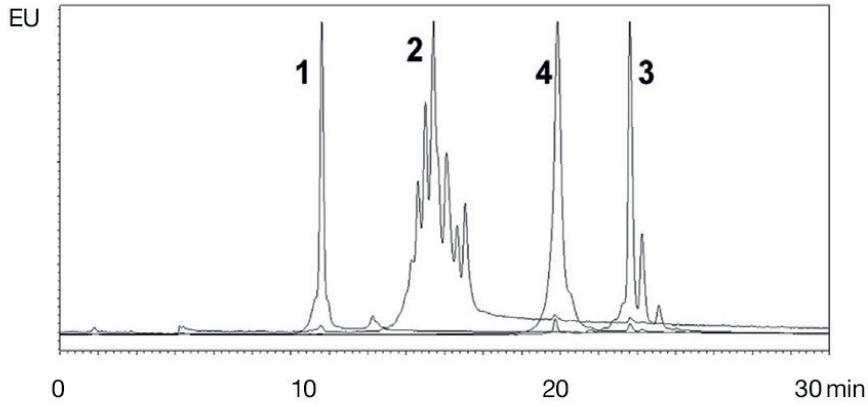
Ion exchange chromatography (IEX) is one of the standard methods for the characterisation of charge-sensitive biomolecules such as monoclonal antibodies (mAbs). Acidic and basic variants caused by chemical or enzymatic modifications can be separated from the main isoform of the mAb. These antibody variants have to be critically evaluated as differences in impurities and/or degradation products could lead to severe undesirable side effects.

CEX analysis via pH or salt gradient

pH gradient



salt gradient



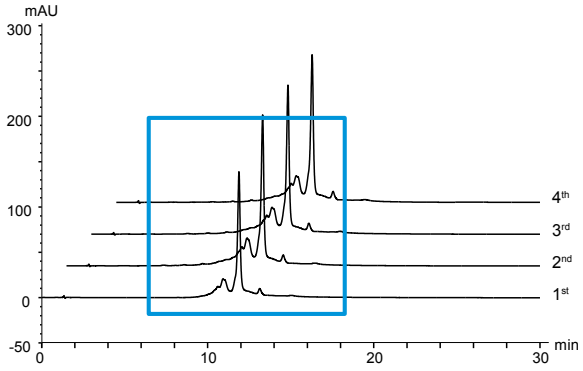
Column:	BioPro IEX SF (5 µm) 100 x 4.6 mm ID
Part No:	SF00S05-1046WP
Eluents:	pH gardient: A) CX-1 pH Gradient Buffer A* (pH 5.6) B) CX-1 pH Gradient Buffer B* (pH 10.2)
	Salt gradient: A) 10 mM MES**-NaOH (pH 5.7) B) 10 mM MES**-NaOH (pH 5.7) containing 1 M NaCl
Gradient:	pH: 0–100%B (0–20 min) salt: 0–20%B (0–20 min)
Flow rate:	0.6 mL/min
Temperature:	30 °C
Detection:	Fluorescence: ex 280 nm, em 350 nm
Injection:	2 µL (100 µg/mL each)
Sample:	Natalizumab (Tysabri®) Cetuximab (Erbitux®) Adalimumab (Humira®) Denosumab (Prolia®; XGEVA®)

*commercially available from Thermo Fisher Scientific; 10 times diluted; **MES: (N-morpholino)ethanesulfonic acid

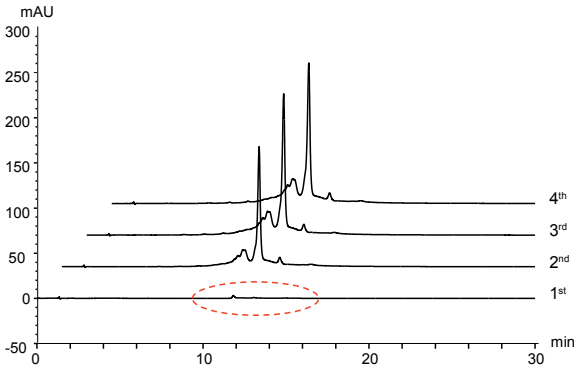
Reference: S. Fekete, A. Beck, D. Guillarme, Characterisation of cation exchanger stationary phases applied for the separations of therapeutic monoclonal antibodies, J. Pharm. Biomed. Anal., 2015, 111, 169–176.

No preconditioning required for reliable results

YMC Accura BioPro IEX SF

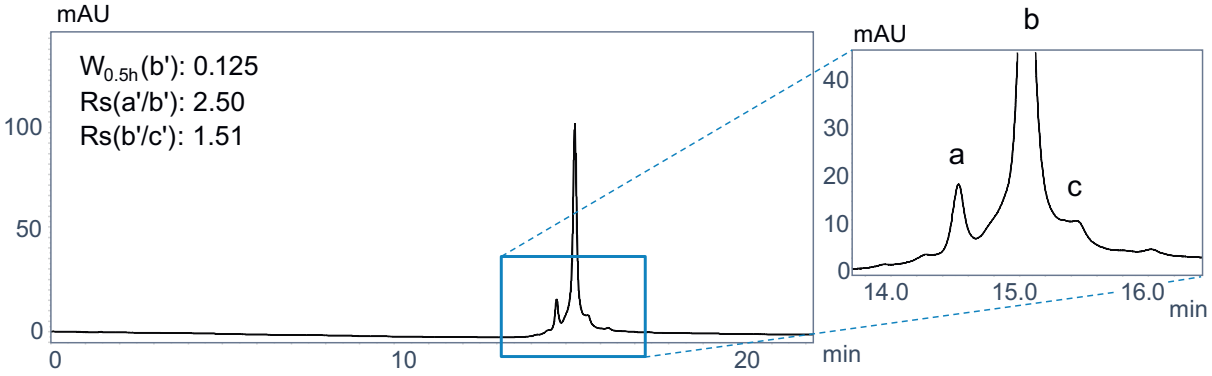


Standard PEEK column



Columns:	YMC Accura BioPro IEX SF (5 µm) 100 x 4.6 mm ID (bioinert coated hardware) BioPro IEX SF (5 µm) 100 x 4.6 mm ID (standard hardware)
Part Nos.:	SF00S05-1046PTC SF00S05-1046WP
Eluent:	A) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8) B) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8) containing 0.2 M NaCl
Gradient:	0–50%B (0–30 min), 0%B (30–45 min)
Flowrate:	0.5 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Injection:	5 µL
Sample:	Bevacizumab (5 mg/mL)
System:	bioinert UHPLC

Ideally suited for native IEX-MS

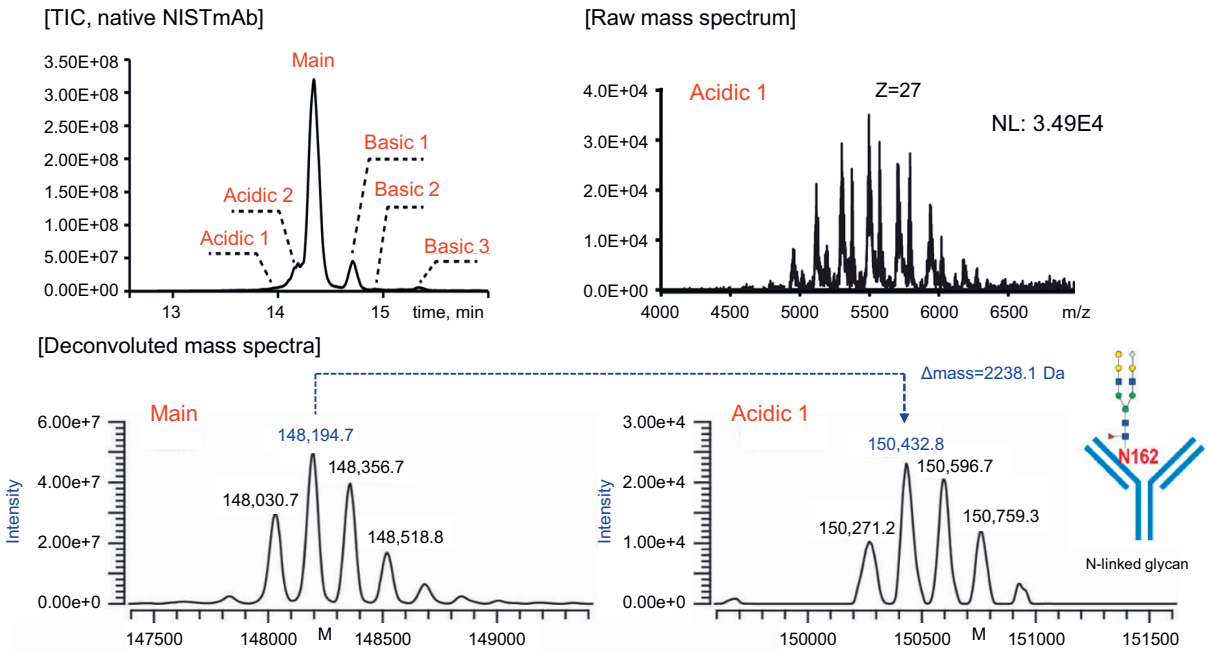


Column:	YMC Accura BioPro IEX SF (3 µm) 100 x 2.1 mm ID
Part No.:	SF00S03-10Q1PTC
Eluent[1]:	A) 20 mM CH_3COONH_4-CH_3COOH (pH 5.6) B) 140 mM CH_3COONH_4-10 mM NH_4HCO_3 (pH 7.4)
Gradient:	20%B (0–2 min), 20–100%B (2–18 min), 100%B (18–22 min)
Flow rate:	0.1 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Injection:	2 µL
Sample:	Trastuzumab (1 mg/mL)
System:	bioinert UHPLC

Reference: Y. Yan, A. P. Liu, S. Wang, T. J. Daly und N. Li, Ultrasensitive Characterization of Charge Heterogeneity of Therapeutic Monoclonal Antibodies, Anal. Chem., 2018, 90, 13013-20.

Smaller column IDs that allow lower flow rates and volatile mobile phases are necessities for coupling to mass detection. Such high sensitivity analyses are ideally performed using YMC Accura BioPro IEX columns. Another positive effect is the decreased solvent consumption and lower amount of sample required.

Native online CEX-MS analysis of monoclonal antibodies (IgG1 type)



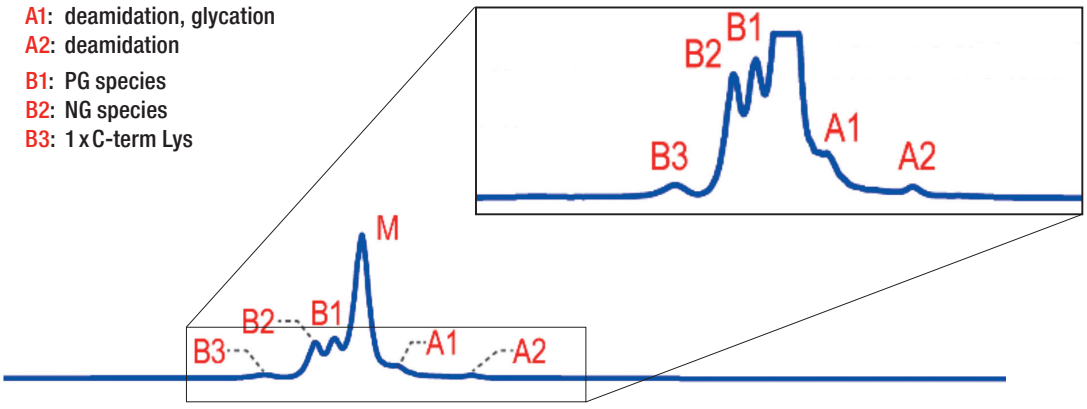
Column:	BioPro IEX SF (5 µm) 100 x 4.6 mm ID	Temperature:	45 °C
Part No.:	SF00S05-1046WP	Detection:	nanospray ionisation-mass spectrometry (NSI-MS)
Eluent:	A) 20 mM CH_3COONH_4-CH_3COOH (pH 5.6) B) 140 mM CH_3COONH_4-10 mM NH_4HCO_3 (pH 7.4)	Load:	50 µg
		System:	LC) ACQUITY UPLC I-Class system (Waters) MS) ExactiveTM Plus EMR mass spectrometer (Thermo Fisher Scientific)
Gradient:	0%B (0–2 min), 0–100%B (2–18 min), 100%B (18–22 min)		
Flow rate:	0.4 mL/min (To enable online simultaneous UV and MS detection, a post-column analytical splitter (~400:1 ratio) was connected)		

By courtesy of S. Wang, Regeneron Pharmaceuticals Inc.

Reference: Y. Yan, A. P. Liu, S. Wang, T. J. Daly und N. Li, Ultrasensitive Characterization of Charge Heterogeneity of Therapeutic Monoclonal Antibodies, Anal. Chem., 2018, 90, 13013-20.

Native online AEX-MS of IgG4 type mAbs

A1: deamidation, glycation
A2: deamidation
B1: PG species
B2: NG species
B3: 1 x C-term Lys

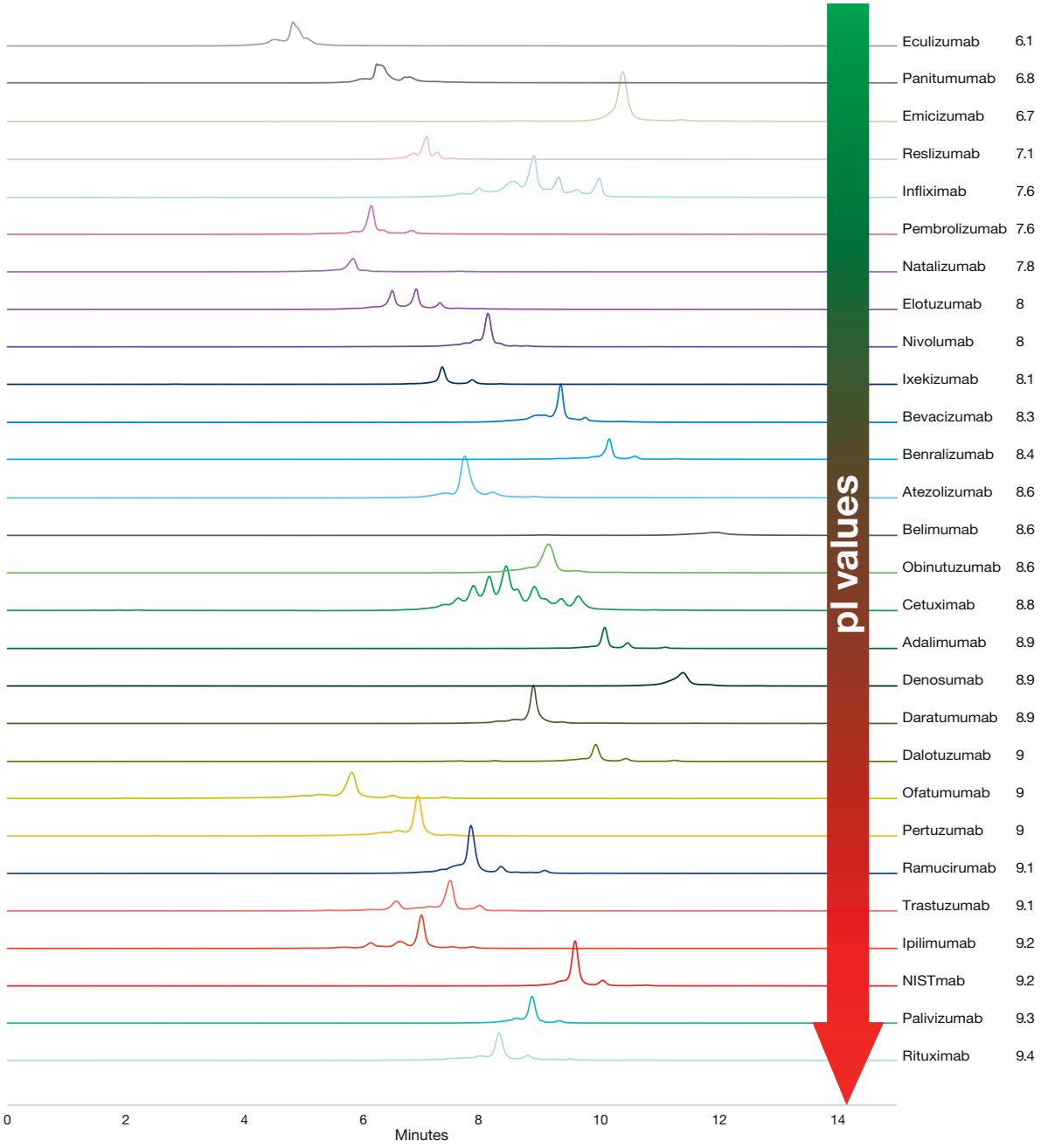


Column:	BioPro IEX QF (5µm) 100 x 4.6 mm ID	Detection:	NSI-MS (nanoelectrospray ionisation) UV
Part No.:	QF00S05-1046WP	Sample:	Inhouse IgG4-based mAb, pI=6.6 (Regeneron)
Eluent:	A) 10 mM ammonium acetate, pH 6.7 B) 300 mM ammonium acetate, pH 6.8	Setup:	Post column stainless-steel tee to direct the majority to the UV detector Remaining sub-microlitre per minute flow directed to the NSI-MS
Gradient:	0%B (0–2 min), 0–100%B (2–18 min), 100%B (18–22 min)		
Flow rate:	0.4 mL/min		
Temperature:	45 °C intact mAb 25 °C subunit analysis		
Injection:	5 or 10 µg mAb sample		

By courtesy of S. Wang, Regeneron Pharmaceuticals Inc.

Reference: A. Liu, Y. Yan, S. Wang, N. Li, Coupling Anion Exchange Chromatography with Native Mass Spectrometry for Charge Heterogeneity Characterization of Monoclonal Antibodies, Anal. Chem. 2022, 94, 6355–6362.

MS compatible charge variant analysis



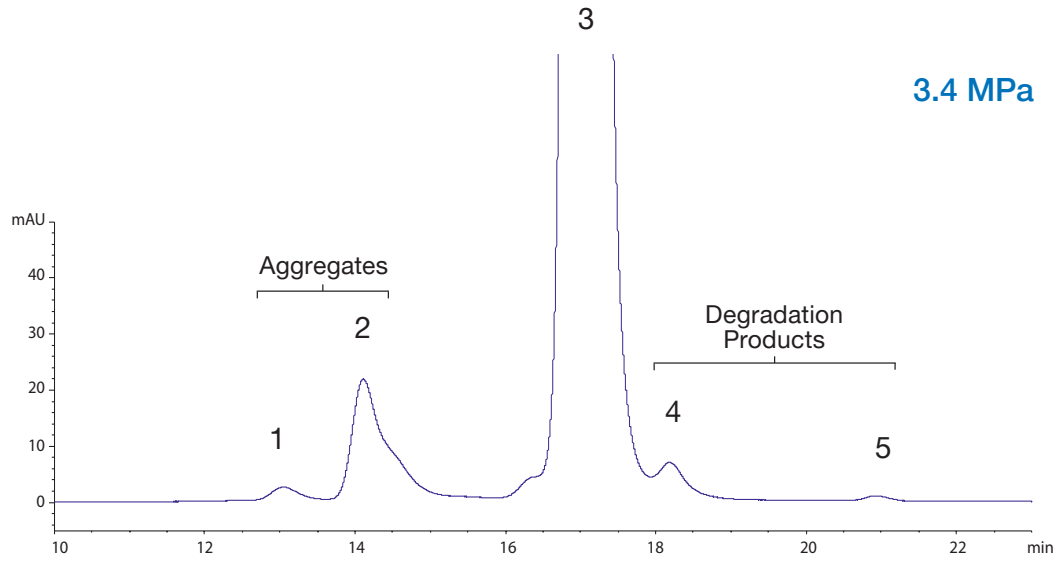
Column:	BioPro IEX SF (5 µm) 100 x 4.6 mm ID
Part No:	SF00S05-1046WP
Eluent:	A) 20 mM CH_3COONH_4-CH_3COOH (pH 5.6) B) 140 mM CH_3COONH_4-10 mM NH_4HCO_3 (pH 7.4)
Gradients:	Depending on the pI of MAb starting with 20–30 %B Inital %B (0–2 min), inital–100%B (2–18 min), 100%B (18–22 min)
Flow rate:	0.4 mL/min
Temperature:	ambient
Detection:	Fluorescence: ex 280 nm, em 360 nm

By courtesy of D. Guillarme. Institute of Pharmaceutical Sciences of Western Switzerland (University of Geneva), Geneva, Switzerland

SEC

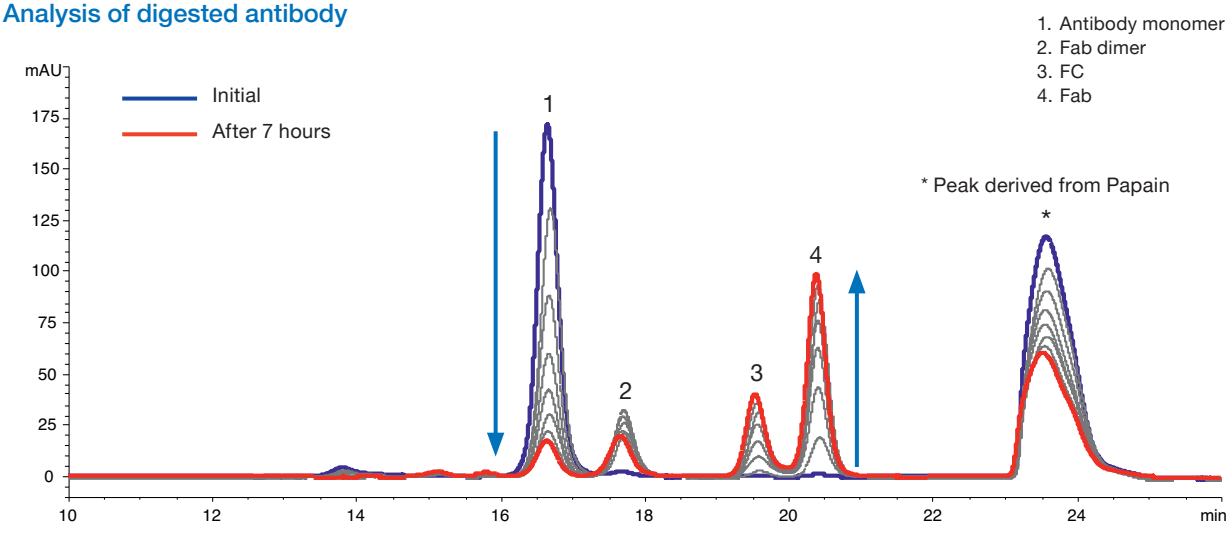
Due to the sizes of monoclonal antibodies (mAbs, about 150 kDa), size exclusion chromatography (SEC) is a standard technique for analysing mAbs such as bevacizumab (Avastin®). It is also a standard separation mode used in quality control to obtain information about aggregation and/or fragmentation of the mAbs.

Bevacizumab and its fragments and aggregates

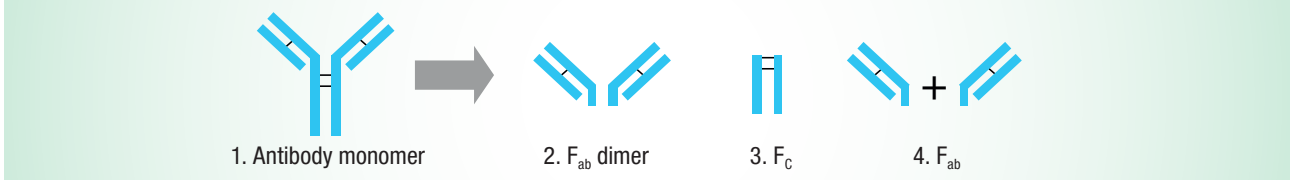


Column:	YMC-SEC MAB (3 µm, 25 nm) 300 x 4.6 mm ID	Detection:	UV at 280 nm
Part No.:	DLM25S03-3046WT	Cell path:	10 mm
Eluent:	0.1 M phosphate buffer (pH = 7) containing 0.2 M NaCl	Injection:	10 µL (5 mg/mL)
Flow rate:	0.165 mL/min	Sample:	Bevacizumab (Avastin®)
Temperature:	25°C		

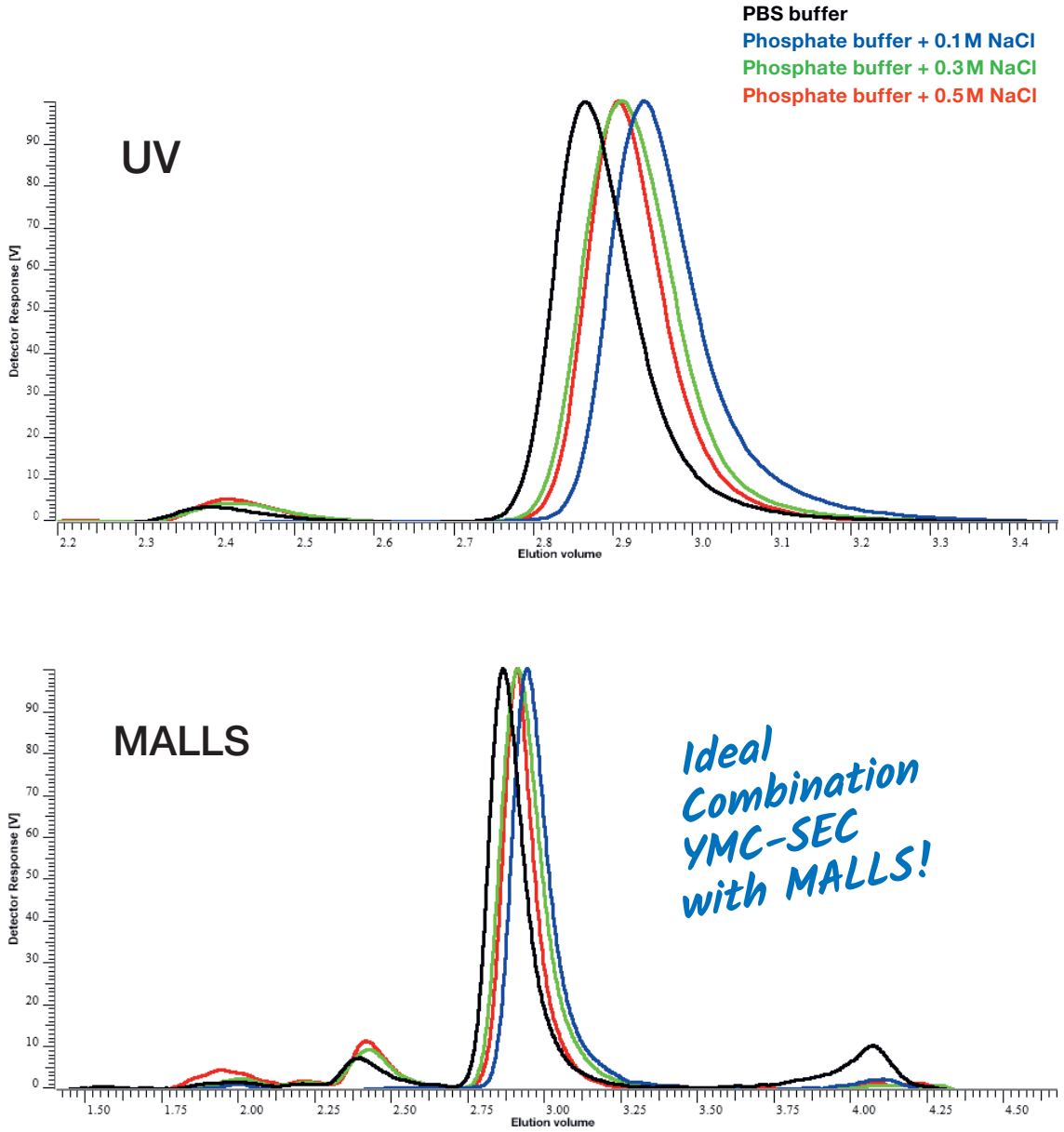
Analysis of digested antibody



Column:	YMC-SEC MAB (3 µm, 25 nm) 300 x 4.6 mm ID	Temperature:	25 °C
Part No.:	DLM25S03-3046WT	Detection:	UV at 280 nm
Eluent:	0.1 M phosphate buffer (pH = 7) containing 0.2 M NaCl	Injection:	2 µL (3 mg/mL)
Flow rate:	0.165 mL/min	Sample:	Humanised monoclonal IgG1 + Papain



Detection of higher molar mass species by MALLS



Column:	YMC-SEC MAB (3 µm, 25 nm) 300 x 4.6 mm ID
Part No.:	DLM25S03-3046WT
Eluent:	Phosphate buffer pH 6.6 containing 0.3 M NaCl
Flow rate:	0.33 mL/min
Temperature:	25 °C
Detection:	MALLS at 90° angle (PSS SLD7100), UV at 280 nm
Injection volume:	10 µL
Sample:	Bevacizumab (Avastin®) dosage form (10 mg/mL, diluted to 1 mg/mL)
System:	PSS-SECcurity GPC systems, 1260 Infinity II
Software:	WinGPC Unichrom

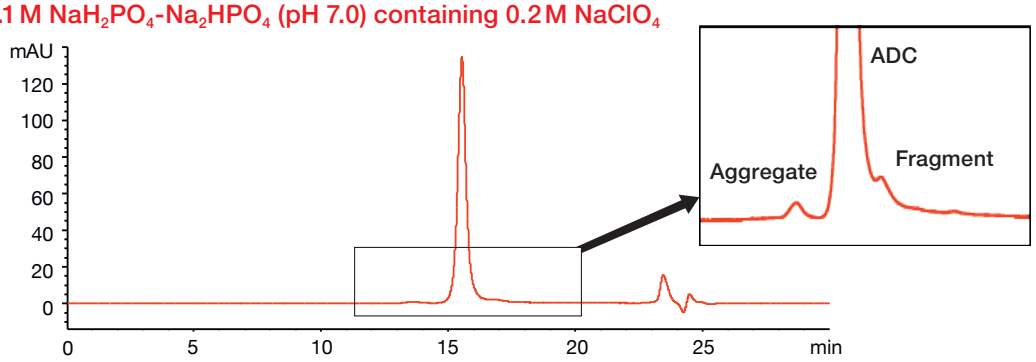
By courtesy of T. Hofe, PSS Polymer Standards Service GmbH, Mainz, Germany.

Four different buffers, a phosphate buffered saline (PBS) pH 7.4 and phosphate buffers pH 6.6 with varying concentrations of NaCl, were used to develop a suitable MALLS detection method for mAbs.
A defined minimum ionic strength is necessary to achieve a robust method with good resolution. The phosphate buffer with 0.3 M NaCl appeared to be the most suitable eluent.
Compared to UV detection, the MALLS signal shows 2 higher molar mass species, aggregates of bevacizumab, at about 2.0 mL and 2.3 mL elution volume.

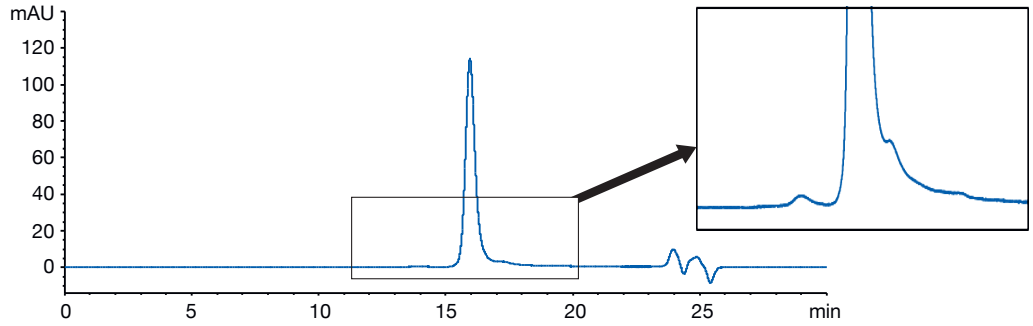
Analysis of Antibody-Drug-Conjugates (ADCs)

Brentuximab vedotin and its fragments and aggregates

0.1 M NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 0.2 M $NaClO_4$



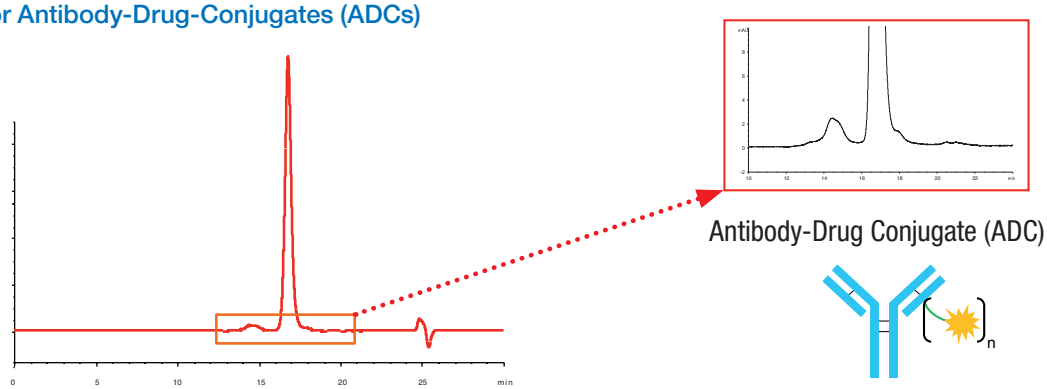
0.1 M NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 0.2 M NaCl/2-propanol (85/15)



Column: YMC-SEC MAB (3 µm, 25 nm) 300 x 4.6 mm ID
Part No.: DLM25S03-3046WT
Eluent: 0.1 M phosphate buffer (pH=7) cont. 0.2 M $NaClO_4$
0.1 M phosphate buffer (pH=7) cont. 0.2 M NaCl/2-propanol (85/15)
Flow rate: 0.165 mL/min
Temperature: 25 °C
Detection: UV at 280 nm
Injection: 4 µL (2.5 mg/mL)
Sample: Brentuximab vedotin (Adcetris®) for injection

By courtesy of Prof. S. Manabe, Hoshi University, Tokyo/Tohoku University, Sendai Japan.

Suitable for Antibody-Drug-Conjugates (ADCs)



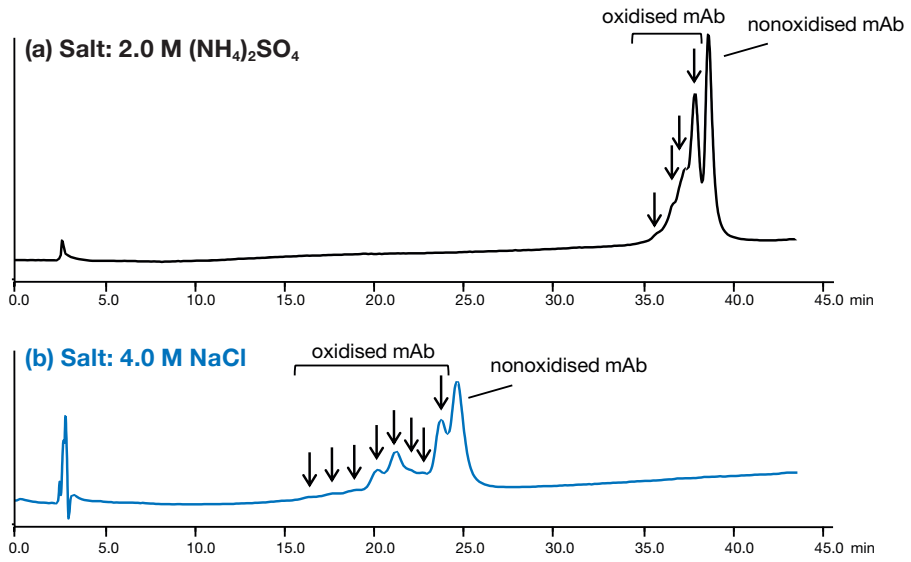
Column: YMC-SEC MAB (3 µm, 25 nm) 300 x 4.6 mm ID
Part No.: DLM25S03-3046WT
Eluent: 0.1 M phosphate buffer (pH = 7) containing 0.2 M NaCl / 2-propanol (85 / 15)
Flow rate: 0.165 mL/min
Temperature: 25 °C
Detection: UV at 280 nm
Injection: 4 µL (2.5 mg/mL)
Sample: SigmaMAb Antibody Drug Conjugate Mimic

YMC-SEC MAB is also suitable for the analysis of Antibody-Drug Conjugates (ADCs). The addition of an organic solvent to the mobile phase can improve the results obtained for ADC analysis.

HIC

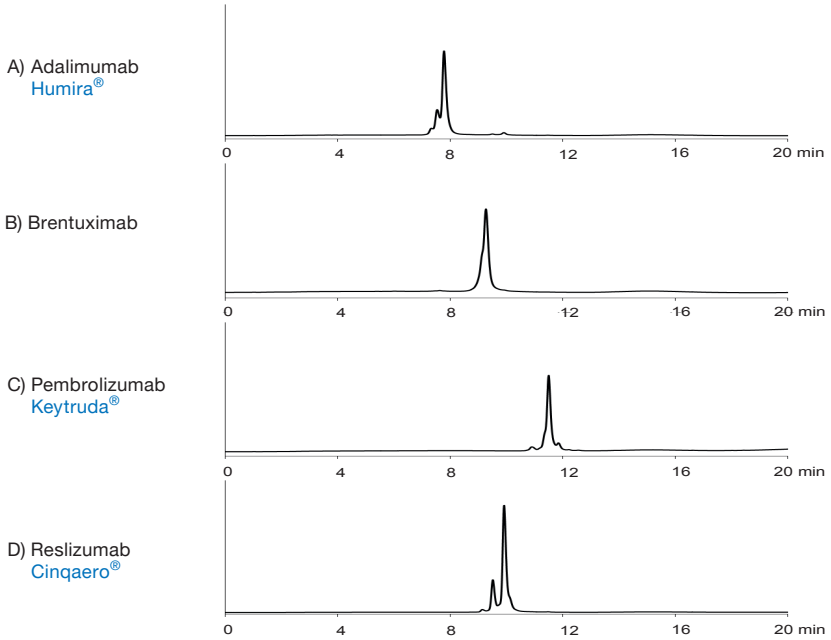
Monoclonal antibodies (mAbs) are often analysed using hydrophobic interaction chromatography (HIC) because of their high hydrophobicity. Furthermore, the determination of drug-to-antibody ratios (DAR) of antibody-drug-conjugates (ADCs) such as brentuximab vedotin is important for their therapeutic efficacy and pharmacokinetics. Therefore, control of DAR is a key factor for ADC quality control.

Analysis of oxidised monoclonal antibodies



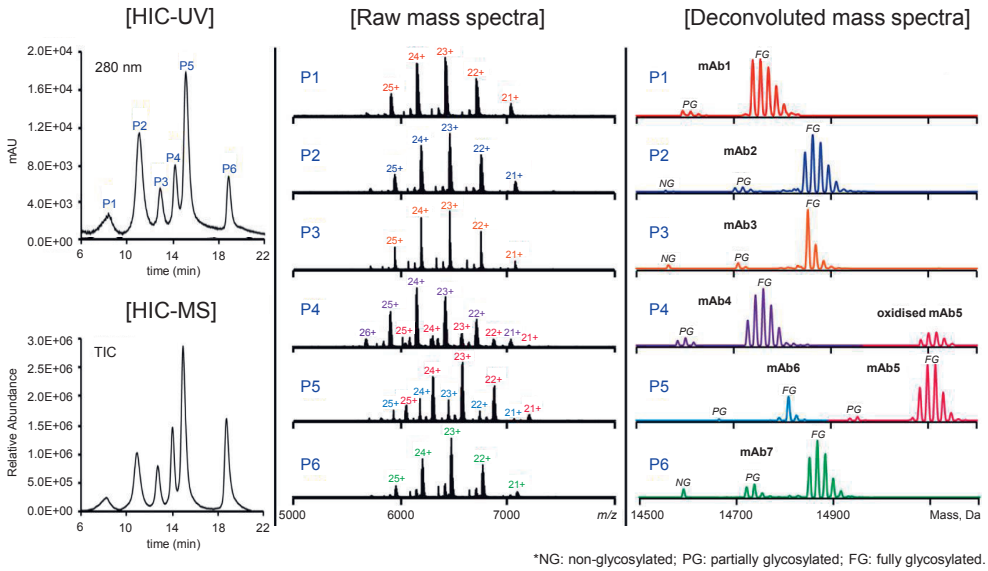
Column:	BioPro HIC BF (4 µm) 100 x 4.6 mm ID	Flow rate:	0.3 mL/min
Part No.:	BHB00S04-1046WT	Temperature:	25 °C
Eluent:	A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing salt B) 100mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)	Detection: Injection:	UV at 280 nm 5 µL (1.0 mg/mL)
Gradient:	40–80%B (0–40 min), 80%B (40–45 min)	Sample:	oxidised NISTmAb

HIC analysis of different monoclonal antibodies using isopropanol as modifier



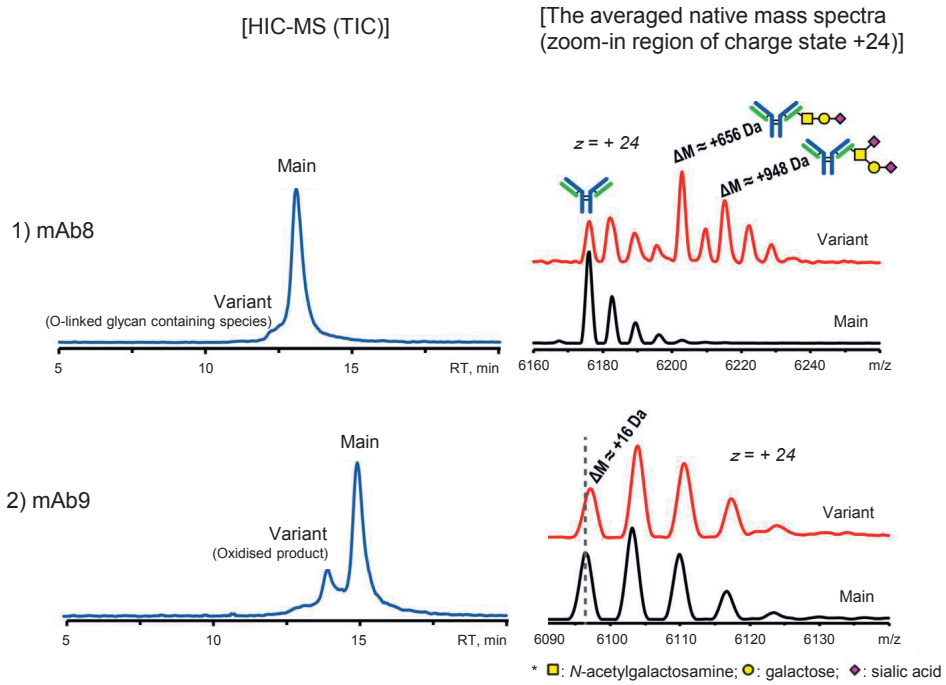
Column:	BioPro HIC BF (4 µm) 100 x 4.6 mm ID	Temperature:	20 °C
Part No.:	BHB00S04-1046WT	Detection:	Fluorescence: ex 280 nm, em 360 nm
Eluent:	A) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 7.4) containing 1.5 M $(NH_4)_2SO_4$ B) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 7.4) / 2-propanol (85/15)	Injection:	3 µL (2 mg/mL)
Gradient:	0–100%B (0–20 min)		By courtesy of D. Guillarme. Institute of Pharmaceutical Sciences of Western Switzerland (University of Geneva), Geneva, Switzerland
Flow rate:	1.0 mL/min		

Native online HIC-MS analysis of seven different mAbs



*NG: non-glycosylated; PG: partially glycosylated; FG: fully glycosylated.

Separation of two mAbs from their molecular variants



* □: *N*-acetylgalactosamine; ○: galactose; ◆: sialic acid

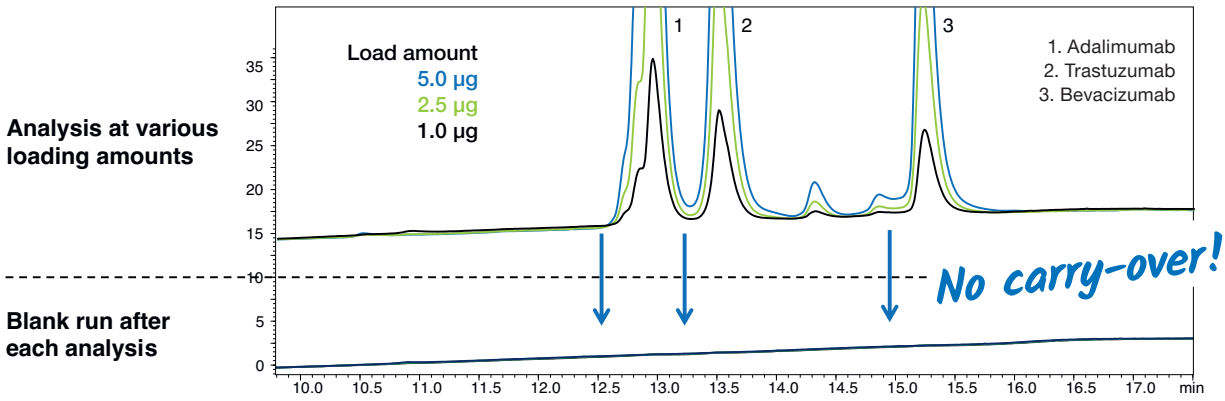
Column:	BioPro HIC BF (4 µm) 100 x 4.6 mm ID
Part No.:	BHB00S04-1046WT
Eluent:	A) 3 M ammonium acetate in water B) 100 % water
Gradient:	0 %B (0–2 min), 0–90 %B (2–18 min), 90 %B (18–22 min)
Flow rate:	0.3 mL/min
Temperature:	ambient
Detection:	UV at 280 nm, NSI-MS
Injection:	mAb mixture: 3 µL (3–6 µg) mAb 8 and mAb 9: 10 µg each
Sample:	Mixture of 7 in-house mAbs at 1–2 mg/mL each 2 in-house mAbs with molecular variants
Setup:	Post-column makeup flow: 100 % water at 1.5 mL/min (reducing salt conc. 6-fold) Splitter to reduce the flow rate to 1–5 µL/min

By courtesy of S. Wang, Regeneron Pharmaceuticals Inc.

To enable simultaneous UV and MS detection a post-column makeup flow and a splitter were used. The makeup flow decreases the salt concentration while the splitter reduces the flow rate to enable the coupling to MS. A nanospray ionisation (NSI) was chosen because of its high sensitivity and salt tolerance.

Reference: Y. Yan, T. Xing, S. Wang, T. J. Daly, N. Li, Online coupling of analytical hydrophobic interaction chromatography with native mass spectrometry fo the characterization of monoclonal antibodies and related products, J. Pharm. Biomed. Anal. 186 (2020) 113313.

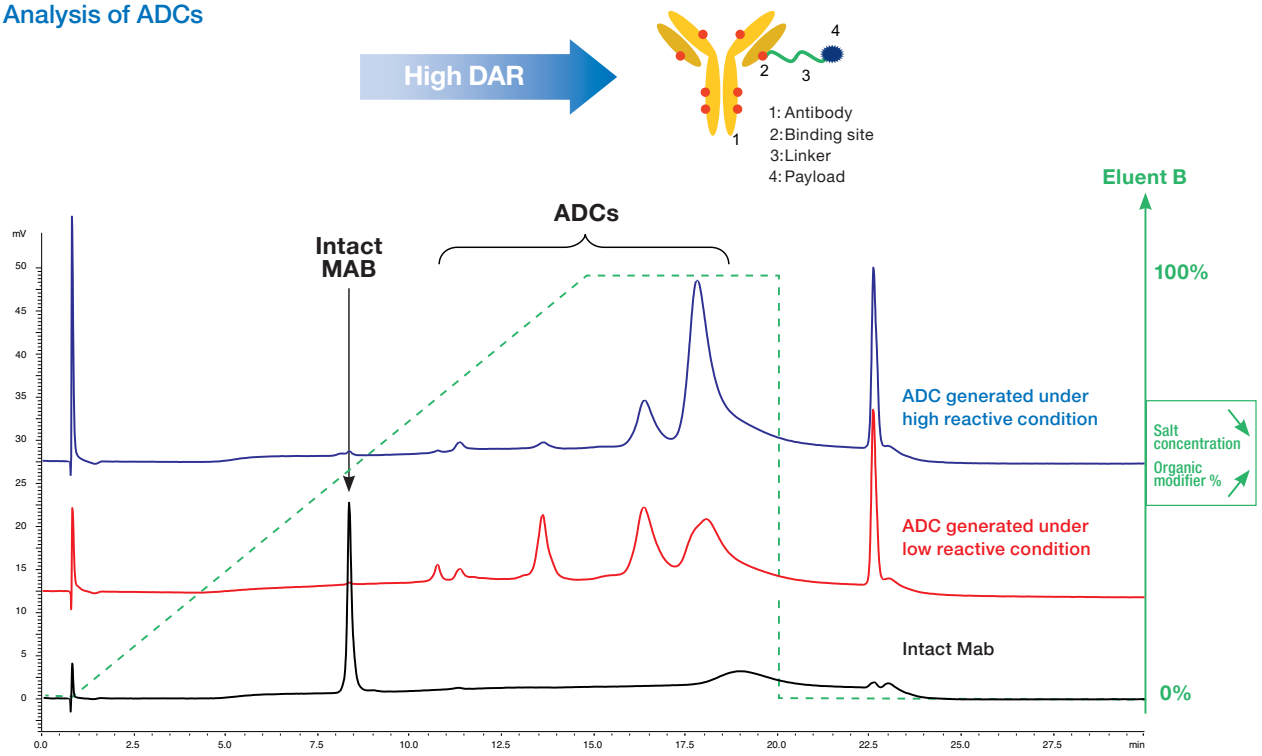
Highly accurate quantification of ADCs and antibodies



Column: BioPro HIC HT (2.3 µm) 100 x 4.6 mm ID
Part No.: BHH00SQ3-1046PTH
Eluent: A) 100 mM NaH_2PO_4-Na_2HPO_4 containing 2.0 M $(NH_4)_2SO_4$ (pH 7.0)
B) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)
Gradient: 0%B (0–1 min), 0–100%B (1–11 min), 100%B (11–15 min)
Flow rate: 0.5 mL/min
Temperature: 25 °C
Detection: UV at 280 nm

BioPro HIC HT offers higher linearity over wide loading and virtually no carry-over. This contributes to highly accurate quantitation of ADCs and antibodies.

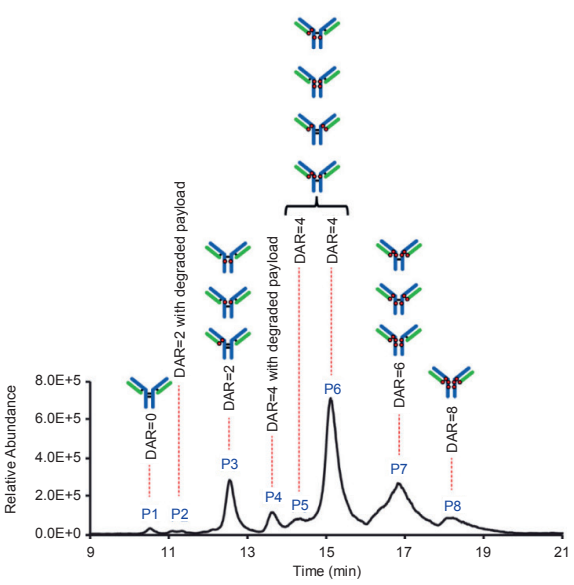
Analysis of ADCs



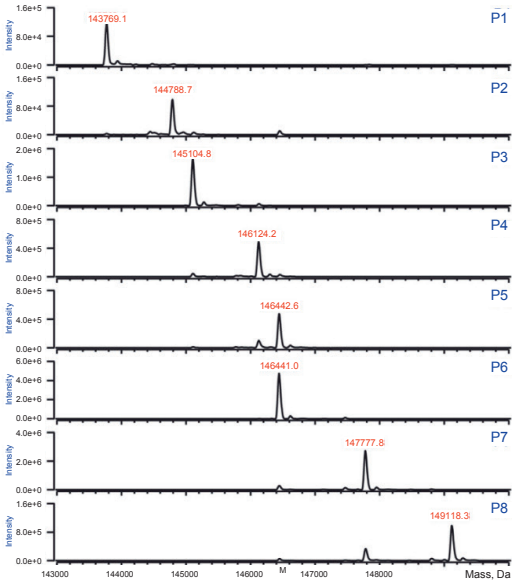
Column: BioPro HIC BF (4 µm) 100 x 4.6 mm ID
Eluent: A) 50 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 1.5 M $(NH_4)_2SO_4$/2-propanol (95/5)
B) 50 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)/2-propanol (80/20)
Gradient: 0%B (0–1 min), 0–100%B (1–15 min), 100%B (15–20 min), 0%B (20–30 min)
Flow rate: 1.0 mL/min
Temperature: 25 °C
Detection: UV at 280 nm
Sample: Antibody-drug-conjugate (courtesy of RIKEN, Japan)

Native online HIC-MS analysis of cys-linked ADCs

[HIC-MS (BPC: base peak chromatogram)]



[Deconvoluted mass spectra]



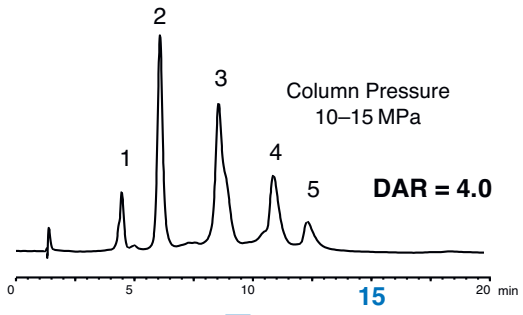
Column:	BioPro HIC BF (4 µm) 100 x 4.6 mm ID	Injection:	10 µg
Part number:	BHB00S04-1046WT	Sample:	SigmaMAb ADC-mimic
Eluent:	A) 3 M ammonium acetate in water B) 2-propanol/water (30/70)	Setup:	Post-column makeup flow: 100% water at 1.5 mL/min (reducing salt conc. 6-fold) Splitter to reduce the flow rate to 1–5 µL/min
Gradient:	10%B (0–2 min), 10–97%B (2–18 min), 97%B (18–22 min)		
Flow rate:	0.3 mL/min		
Temperature:	ambient		
Detection:	UV at 280 nm, NSI-MS		

By courtesy of S. Wang, Regeneron Pharmaceuticals Inc.

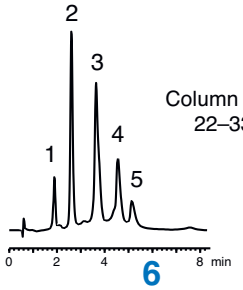
Reference: Y. Yan, T. Xing, S. Wang, T. J. Daly, N. Li, Online coupling of analytical hydrophobic interaction chromatography with native mass spectrometry for the characterization of monoclonal antibodies and related products, J. Pharm. Biomed. Anal. 186 (2020) 113313.

High throughput DAR determination by shortening analysis time

1. DAR 0
2. DAR 2
3. DAR 4
4. DAR 6
5. DAR 8



Flow rate
0.5 mL/min



2.5 x faster

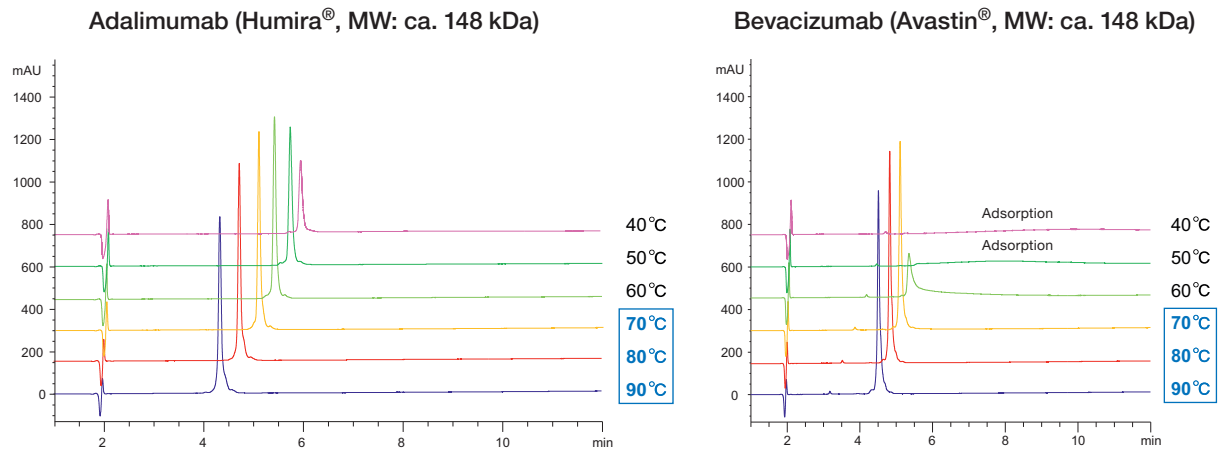
Flow rate
1.2 mL/min

Column:	BioPro HIC HT (2.3 µm) 100 x 4.6 mm ID	Temperature:	25°C
Part No.:	BHH00SQ3-1046PTH	Detection:	UV at 280 nm
Eluent:	A) 20 mM NaH_2PO_4-Na_2HPO_4 containing 1.0 M $(NH_4)_2SO_4$ (pH 7.0) B) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)/2-propanol (85/15)	Injection:	10 µL
Gradient:	0–100%B (0–15 min), 100%B (15–20 min) 0–100%B (0–6.25 min), 100%B (6.25–8.3 min)	Sample:	Brentuximab vedotin (Adcetris®) (2.5 mg/mL)

RP

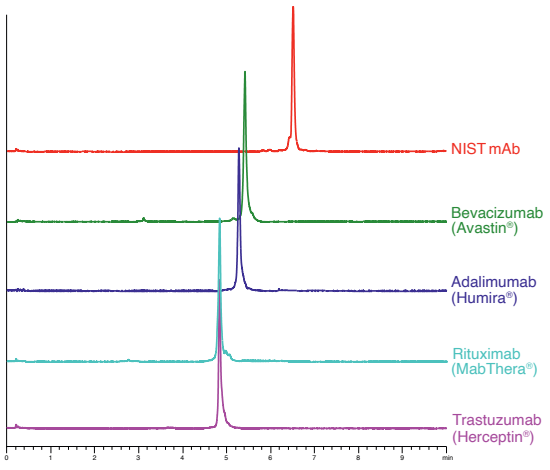
Due to their molecular weight of about 150 kDa, intact antibodies are usually analysed by IEX, SEC or HIC. In addition, RP methods have become an easy tool which is compatible with mass spectrometry (MS). However, lack of sensitivity and resolution has been a hurdle in the past. With modern RP phases addressing the requirements of these analytes, it is now easier to find a suitable method. Successful analysis in RP mode for mAbs is enhanced by employing a widepore, temperature-stable stationary phase, such as YMC-Triart Bio C4.

RP analysis of intact monoclonal antibodies



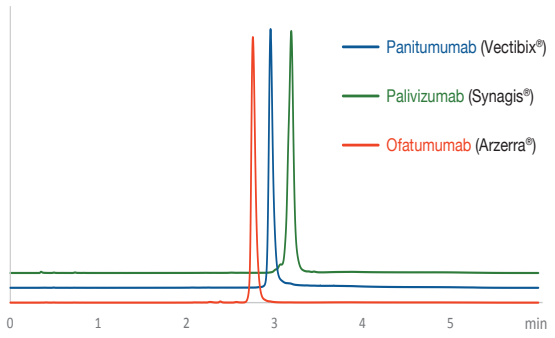
Column: YMC-Triart Bio C4 (3 µm, 30 nm) 150 x 3.0 mm ID
Part No.: TB30S03-1503PTH
Eluent: A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
Gradient: 30–60%B (0–15 min), 90%B (15–30min)
Flow rate: 0.4 mL/min
Detection: UV at 220 nm
Injection: 4 µL
Sample: Adalimumab (0.5 mg/mL) or Bevacizumab (0.5 mg/mL)

Analysis of different monoclonal antibodies



Column: YMC-Triart Bio C4 (1.9 µm, 30 nm) 50 x 2.1 mm ID
Part No.: TB30SP9-05Q1PT
Eluent: A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
Gradient: 25–45%B (0–10 min)
Flow rate: 0.4 mL/min
Temperature: 80 °C
Detection: UV at 280 nm (0.13s, 40Hz)
Injection: 2 µL (0.5 mg/mL)

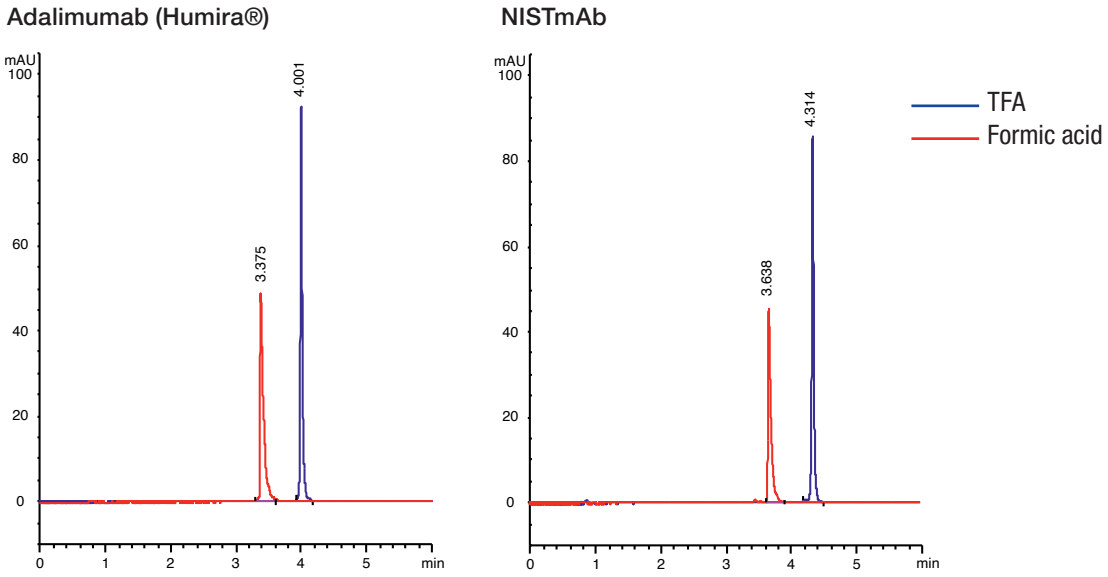
Analysis of challenging monoclonal antibodies



Column: YMC-Triart Bio C4 (1.9 µm, 30 nm) 50 x 2.1 mm ID
Part No.: TB30SP9-05Q1PT
A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
Gradient: 25–50 %B (0–4 min)
Flow rate: 0.4 mL/min
Temperature: 90 °C
Detection: Fluorescence: ex 280 nm, em 350 nm
Injection: 0.5 µL

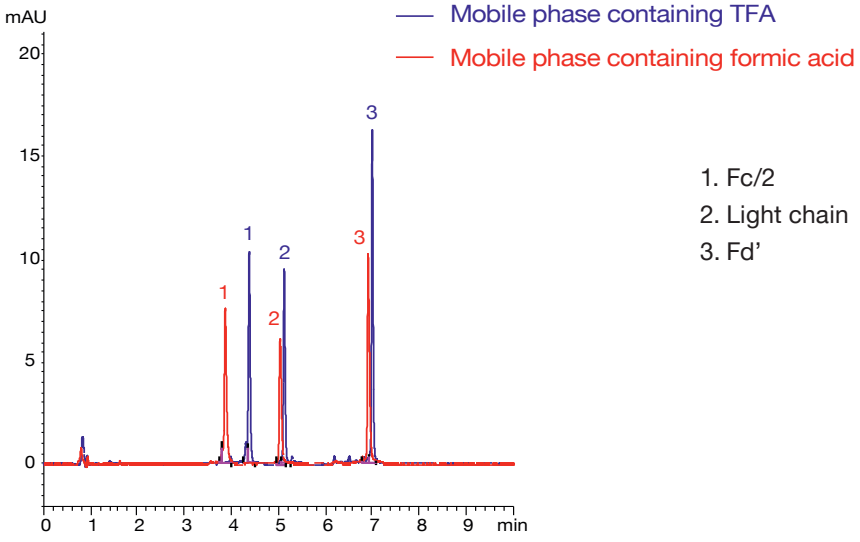
By courtesy of D. Guillarme. Institute of Pharmaceutical Sciences of Western Switzerland (University of Geneva), Geneva, Switzerland

Use of MS compatible conditions for antibody analysis by RP



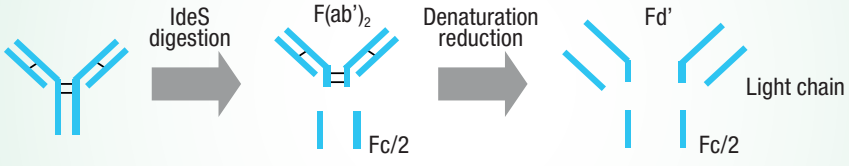
Column:	YMC-Triart Bio C4 (1.9 µm, 30 nm) 150 x 2.1 mm ID	Flow rate:	0.4 mL/min
Part No.:	TB30SP9-15Q1PT	Temperature:	80 °C
Eluent:	A) water/TFA or formic acid (100/0.1)	Detection:	UV at 280 nm (0.13 s, 40 Hz)
	B) acetonitrile/TFA or formic acid (100/0.1)	Injection:	2 µL (0.5 mg/mL)
Gradient:	10–95%B (0–10 min)		

LC/MS compatible analysis of monoclonal antibody fragments



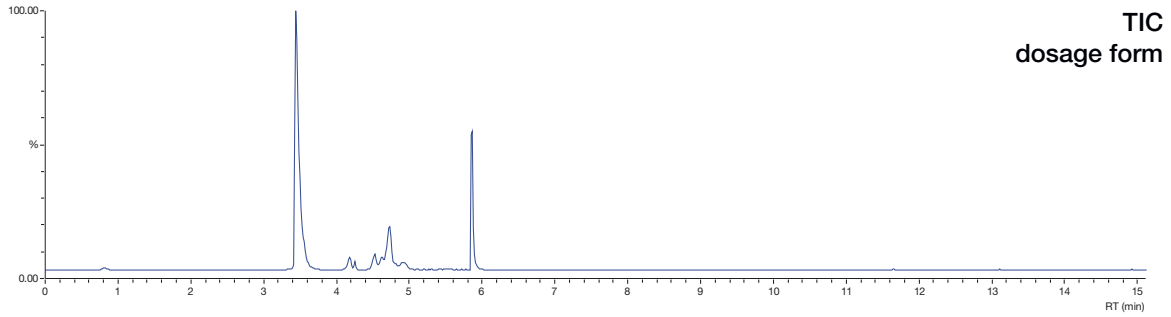
1. Fc/2
2. Light chain
3. Fd’

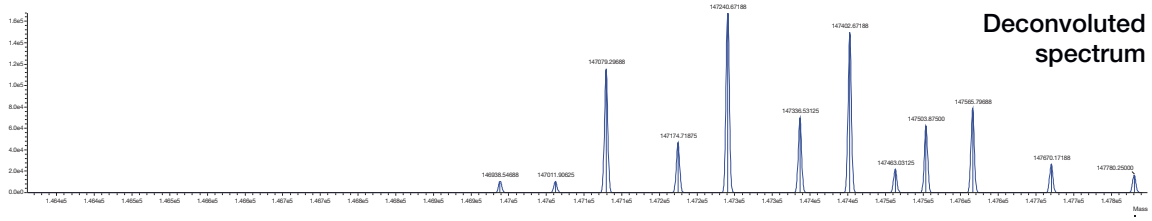
Column:	YMC-Triart Bio C4 (1.9 µm, 30 nm) 150 x 2.1 mm ID	Gradient [formic acid]:	20–45%B (0–10 min), 90%B (10–12.5 min)
Part No.:	TB30SP9-15Q1PT	Flow rate:	0.4 mL/min
Eluent [TFA]:	A) water/TFA (100/0.1)	Temperature:	80°C
	B) acetonitrile/TFA (100/0.1)	Injection:	4 µL (0.25 mg/mL)
Gradient [TFA]:	25–50%B (0–10 min), 90%B (10–12.5 min)	Detection:	UV at 280 nm
Eluent [formic acid]:	A) water/formic acid (100/0.1)	Sample:	mAb Subunit Standard (Waters Corp.)
	B) acetonitrile/formic acid (100/0.1)		



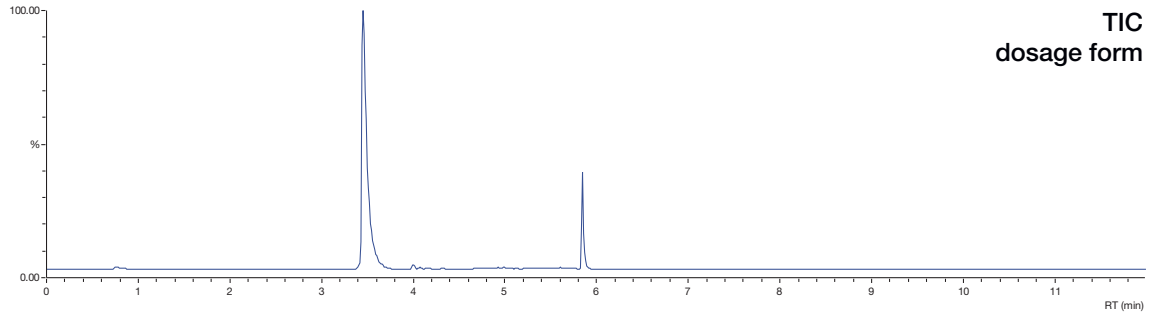
Commercial monoclonal antibodies by MicroLC-MS

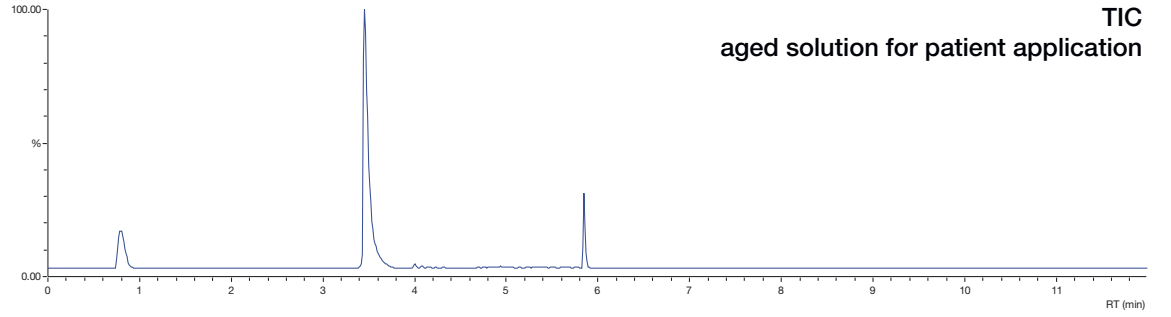
Rituximab (MabThera®)

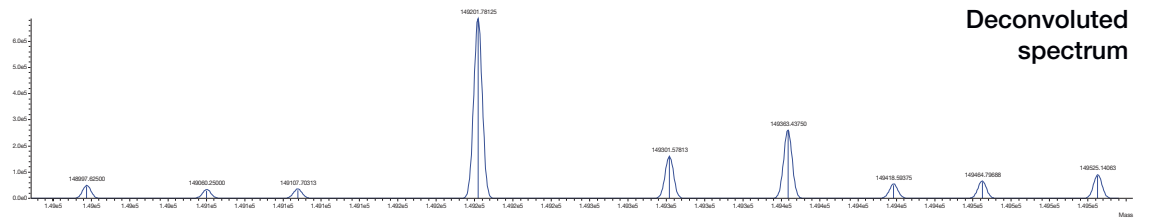




Bevacizumab (Avastin®)







Column:	YMC-Triart Bio C4 (30 nm, 3 µm) 100 x 0.3 mm ID, 1/16" end fittings	Detection:	Shimadzu LCMS-9030 QTOF
Part No.:	TB30S03-10H0AU	Injection:	0.1 µL
Eluent:	A) H_2O + 0.1 % formic acid B) acetonitrile + 0.1% formic acid	Sample:	Rituximab dosage form (10 mg/mL, diluted to 0.1 µg/mL) Bevacizumab dosage form (10 mg/mL, diluted to 0.1 µg/mL) Bevacizumab solution for patient application (3 mg/mL, diluted to 0.03 µg/mL)
Gradient:	20%B (0–2.5 min), 20%–100 %B (2.5–4 min), 100 %B (4–5 min), 20%B (5–7 min)	LC system:	Shimadzu Nexera Mikros
Flow rate:	15 µL/min		
Temperature:	75 °C, 4 °C Autosampler		

In collaboration with Shimadzu Europa GmbH