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Direct Surfactant Analysis for Antibody Drug and DAR Analysis for ADC Using Polymer-Based Reverse Phase Column

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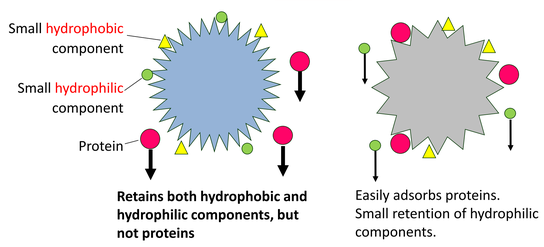
Introduction

Various evaluation items are requested in the quality control of antibody drugs. Analysis of surfactants in antibody drugs and DAR (drug antibody ratio) in ADCs (antibody drug conjugates) are two of them. Shodex™ ODP2 HP series columns, packed with polyhydroxymethacrylate-base spherical gel, capable of providing these two analyses.

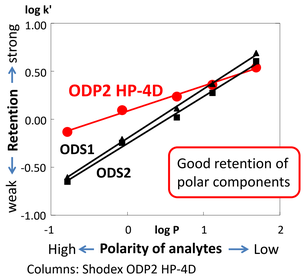
Because of very low UV absorption of surfactants and the presence of other matrices (e.g., protein and salt) in the drugs makes the quantification of surfactants in antibody drugs difficult. We developed a rapid HPLC method using ODP2 HP series columns and an ELSD for the quantifications of surfactants in commercial antibody drugs without the necessity of sample pretreatment. Moreover, by using the column's unique future of providing hydrophobic interaction under controlled eluent conditions, a method was also developed for the analysis of DAR in ADCs.

Comparison between the ODP2 HP and an ODS columns

	ODP2 HP	ODS
Base Material	Polyhydroxy-Methacrylate (Polymer Base)	Silica
Functional Group	None	Octadecyl (C18)
Average Pore Size	40 Å	100 – 120 Å
Workable pH Range	3 – 12 (Alkaline Durable)	2 - 8

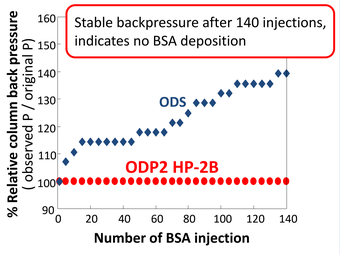


Retention of Polar Components



Columns: Shodex ODP2 HP-4D
ODSs from two other manufacturers
4.6 mm I.D. x 150 mm each
Eluent: H_2O / CH_3CN = 75 / 25
Flow rate: 1.0 mL/min
Column temp.: 40 °C

Protein Deposition Study



Columns: Shodex ODP2 HP-2B
ODS from other manufacturer
2.0 mm I.D. x 50 mm each
Eluent: 1 mM CH_3COONH_4 aq. / CH_3CN = 90 / 10
Flow rate: 0.2 mL/min
Column temp.: 30 °C
Sample: Repeated injections of
7 mg/mL BSA (in H_2O), 5 µL

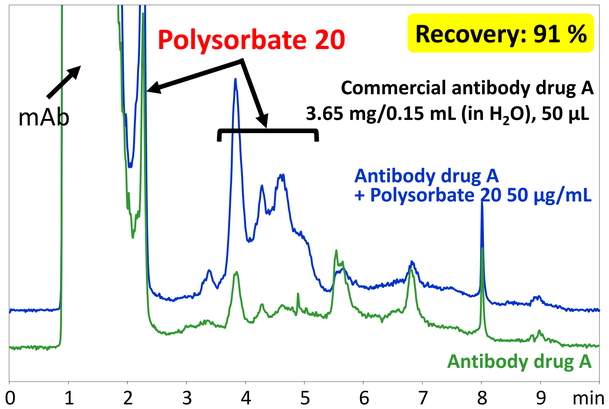
ODP2 HP Specifications

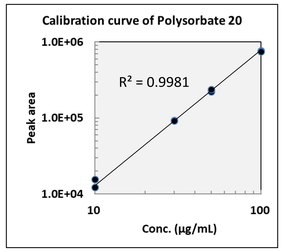
Product name	Plate Number (TP/Column)	Particle Size (µm)	Column Size (mm) I.D. × Length
ODP2 HP-4B	≥ 3,500	5	4.6 × 50
ODP2 HP-4D	≥ 13,000	5	4.6 × 150
ODP2 HP-4E	≥ 17,000	5	4.6 × 250
ODP2 HPG-4A	(guard column)	5	4.6 × 10
ODP2 HP-2B	≥ 3,000	5	2.0 × 50
ODP2 HP-2D	≥ 7,000	5	2.0 × 150
ODP2 HPG-2A	(guard column)	5	2.0 × 10

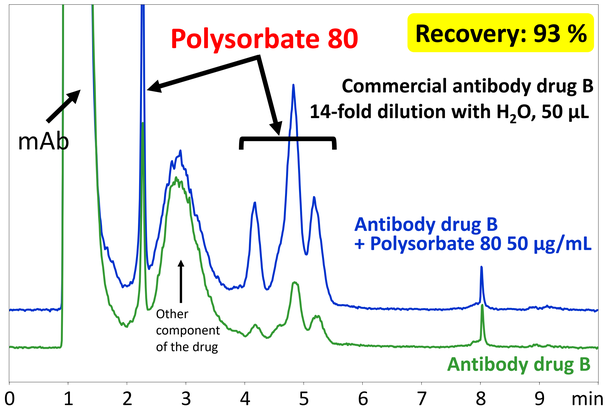
Housing: SUS
Workable Temp. Range: 20 - 60 °C
Usable Solvents: H_2O, CH_3CN, CH_3OH

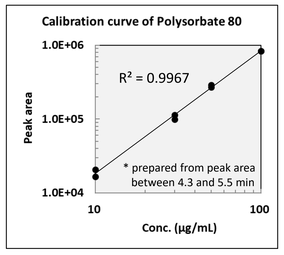
Direct Analysis of Surfactants in Antibody Drugs

Polysorbates in Commercial Antibody Drugs



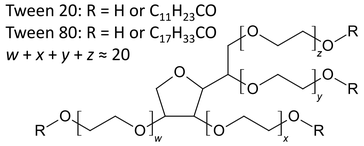




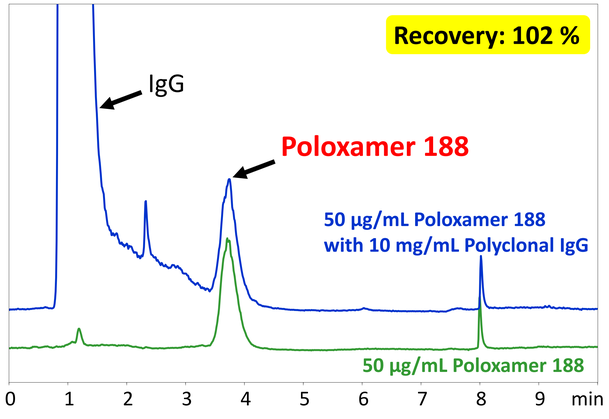


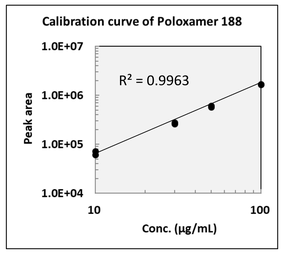
Polysorbate 20 and 80

Tween 20: R = H or $C_{11}H_{23}CO$
Tween 80: R = H or $C_{17}H_{33}CO$
$w + x + y + z \approx 20$



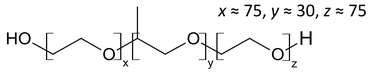
Poloxamer 188 and IgG





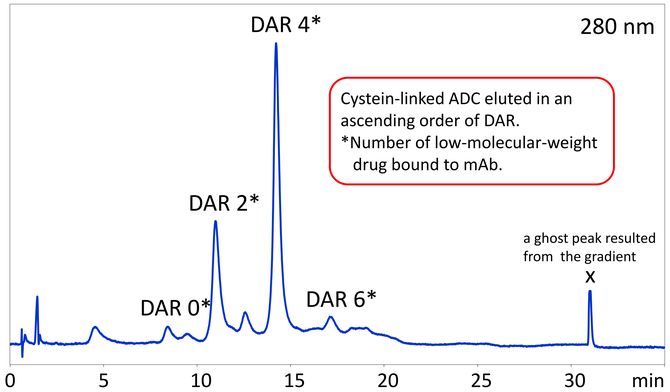
Poloxamer 188

$x \approx 75$, $y \approx 30$, $z \approx 75$

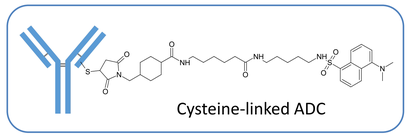


Instrument: Shimadzu Prominence-i
Column: Shodex ODP2 HP-4D (4.6 mm I.D. x 150 mm)
Eluent: (A) 0.1 % NH_3 aq. /(B) CH_3CN
Low pressure linear gradient; B % = 20 % (0 min) → 80 % (5-6 min) → 20 % (6.01-10 min)
Flow rate: 1.0 mL/min
Detector: ELS (Shimadzu ELSD-LTII)
Column temp.: 40 °C

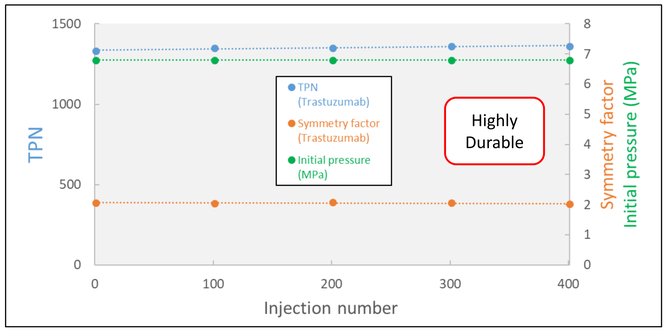
DAR Analysis of ADC



Sample: SigmaMAb ADC Mimic 1 mg/mL (in H_2O), 5 µL
Instrument: Shimadzu Nexera X2 (conventional spec.)
Column: Shodex ODP2 HP-4B (4.6 mm I.D. x 50 mm)
Eluent: (A) 1.5 M $(NH_4)_2SO_4$ in 50 mM Sodium phosphate buffer (pH 7.0)
(B) 50 mM Sodium phosphate buffer (pH 7.0)/IPA=75/25
High pressure linear gradient;
B % = 0 % (0 min) → 100 % (20-30 min) → 0 % (30.01-35 min)
Flow rate: 0.8 mL/min
Detector: PDA (190-350 nm)
Column temp.: 30 °C



Repeated Injection of ADC



Conclusions

Shodex™ ODP2 HP series columns when used with alkaline eluent and ELSD enable direct and rapid surfactant analysis in antibody drug formulations without the necessity of deproteinization nor desalting sample pretreatment steps.

It is also applicable for the DAR analysis of ADC (cysteine-linked type) using a gradient method with decreasing ammonium sulfate concentration.

Shodex™ ODP2 HP series are powerful tools for the quality control of antibody drugs.

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