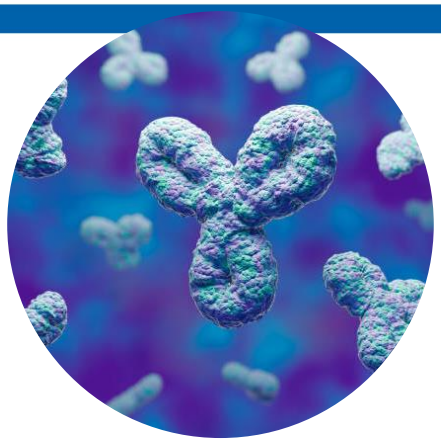


A Simple Quantitative Method for Monoclonal Antibody Coformulations

Ivan Lededev, PhD, Brian Rivera, and Bryan Tackett, PhD
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Overview

Monoclonal antibodies (mAbs) are well-established therapeutics for a wide range of medical applications. Recent trends have investigated the administration of multiple mAbs with the goal of improving efficacy without sacrificing safety. Although coformulation approaches offer significant advantages, the complexity of drug product development, characterization, and quality control increase as well.

One such example is the requirement to quantify each mAb in coformulation. Similarities in physicochemical properties, as well as marked differences in concentrations between two mAbs, can present unique challenges for analysis. Many mAbs chosen for coformulation are standard format (i.e., IgG1 humanized or chimeric) and expressed from Chinese Hamster Ovary (CHO) cell lines. Consequently, coformulated mAbs will have very similar molecular weight, glycosylation, and isoelectric point, limiting chromatographic method options.

These challenges demand that the analytical method offer sufficient selectivity to separate these highly similar biomolecules. Differences in the hydrophobicities of each mAb, and thus their relative affinities for the stationary phase, make reverse phase chromatography a suitable too for analysis. In this application note, we present a simple, quantitative method for mAb coformulations by intact protein reversed phase LC using a Biozen 2.6 µm WidePore C4 column.

The use of a superficially porous, widepore particle morphology offers both higher efficiency separations and improved mass transfer kinetics for large biomolecules. This, combined with the use of a shallow gradient, ensures maximum resolution is achieved between chemically similar proteins. The initial scouting method used was 30-40 % mobile phase B in 5 minutes (~0.6 % B per column volume). Resulting chromatogram overlays for two mAbs, NIST and Trastuzumab, are shown in **Figure 1**. The retention time difference indicate that sufficient resolution can be achieved.

As seen in **Figure 2**, utilizing the same method conditions while injection a 1 mg/mL coformulation solution of both mAbs shows baseline separation (USP resolution of 3.2). Further optimization of the method by modifying the gradient to 40-50 % mobile phase B using the same gradient slope (**Figure 3**) yields a marginal improvement in resolution (USP resolution of 4).

Finally, temperature was investigated to improve peak shape. As observed in Figure 4, increasing temperature to 80 °C yields further improvement in resolution (USP resolution of 4.4).

In summary, by utilizing Biozen™ WidePore C4 chemistry, sufficient retentivity, efficiency, and resolution can be achieved to implement a simple reversed phase method to quantify coformulated mAbs with similar physicochemical properties. Potential limitations to this proof-of-concept method may arise as discrepancies in concentration broaden between mAbs. Further optimization may be required to ensure applicable linearity for the method is achieved.

LC Conditions

Columns: Biozen 2.6 µm WidePore C4

Dimensions: 150 x 2.1 mm

Part No.: 00F-4786-AN

Mobile Phase: A: 0.1 % Trifluoroacetic Acid in Water
B: 0.1 % Trifluoroacetic Acid in Acetonitrile

Gradient:

	Figures 1 and **2**		**Figures 3** and **4**	
	Time (min)	**%B**	**Time (min)**	**%B**
	0	30	0	40
	5	40	5	50
	6	80	6	80
	8	80	8	80
	8.1	30	8.1	30
	12.1	30	12.1	30

Flow Rate: 0.8 mL/min

Injection Volume: 1 µL

Temperature: 60 °C (**Figures 1**-**3**)
80 °C (**Figure 4**)

Detector: UV-Vis @ 280 nm

LC System: Waters® ACQUITY® UPLC I-Class

Sample: Trastuzumab (1 mg/mL)
NIST mAb (1 mg/mL)

Figure 1. Overlay of Trastuzumab and NIST mAb on a Biozen™ 2.6 µm WidePore C4 Column.

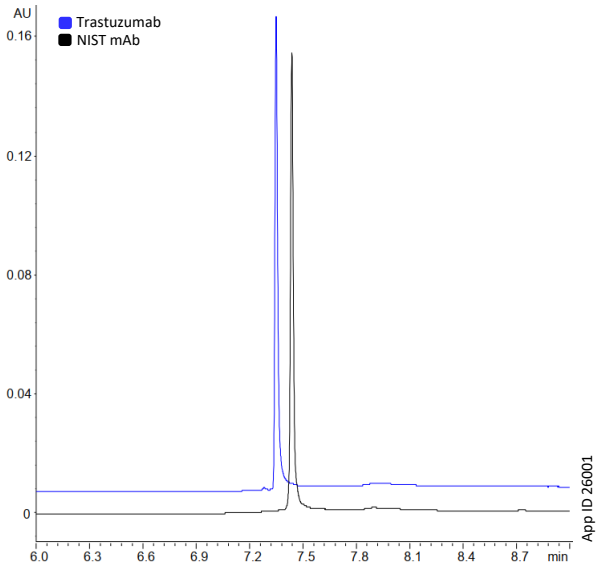


Figure 2. Trastuzumab and NIST mAb on a Biozen 2.6 µm WidePore C4 Column, 30-40 % Mobile Phase B, 60 °C.

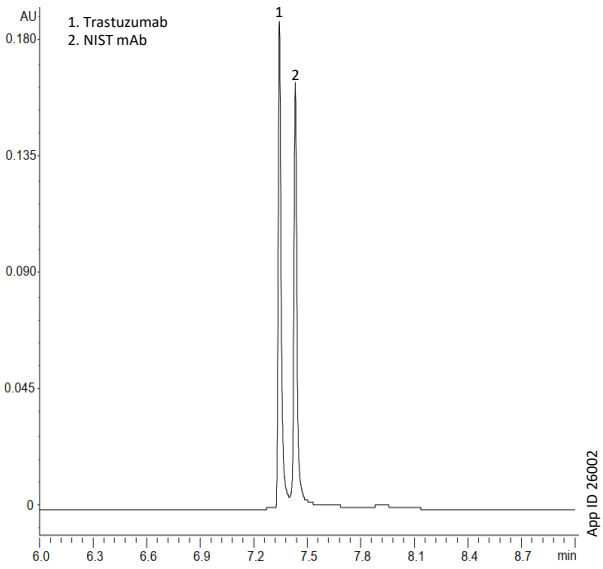


Figure 3. Trastuzumab and NIST mAb on a Biozen 2.6 µm WidePore C4 Column, 40-50 % Mobile Phase B, 60 °C.

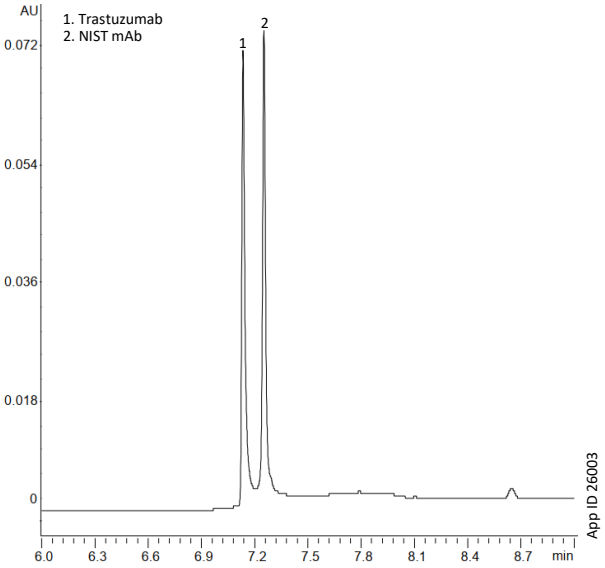
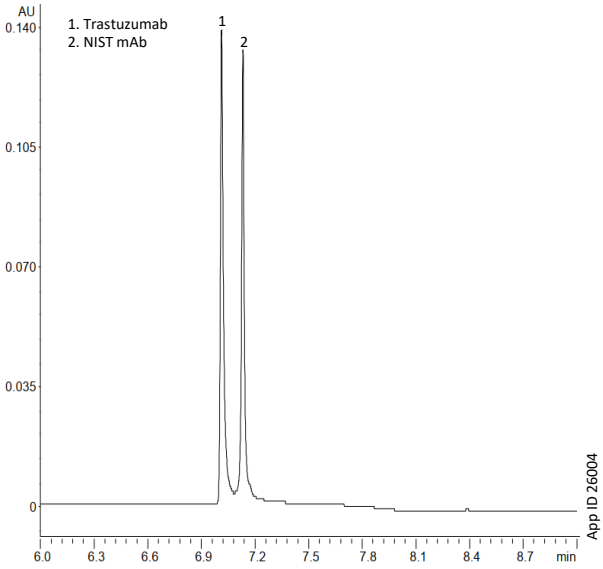


Figure 4. Trastuzumab and NIST mAb on a Biozen 2.6 µm WidePore C4 Column, 40-50 % Mobile Phase B, 80 °C.



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t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic
t: +420 272 017 077
cz-info@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong
t: +852 6012 8162
hkinfo@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia
t: +62 21 5019 9707
indoinfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Japan
t: +81 (0) 120-149-262
jpinfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Poland
t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 6559 4364
sginfo@phenomenex.com

Slovakia
t: +420 272 017 077
sk-info@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand
t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
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