

The role of high performance liquid chromatography in a study to extend the shelf life of injected solutions of the recombinant monoclonal antibody bevacizumab (Avastin).

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Monoclonal antibody therapies currently approved by the Food and Drug Administration.

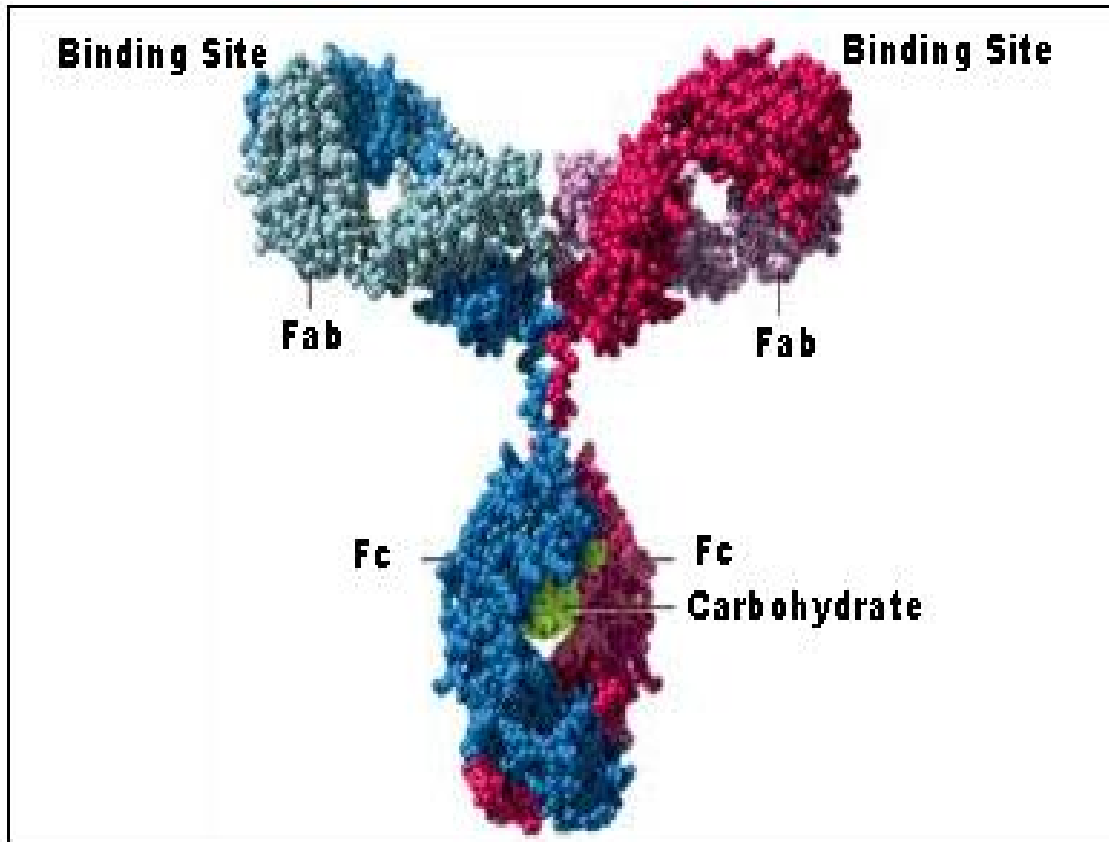
- Abciximab
- Adalimumab
- Alemtuzumab
- Baliliximab
- Bevacizumab
- Cetuximab
- Daclizumab
- Eculizumab
- Efalizumab
- Ibritumomab tiuxetan
- Infliximab
- Muromonab DC3
- Natalizumab
- Omalizumab
- Palivizumab
- Panitumumab
- Ranibizumab
- Gemtuzumab ozogamicin
- Rituximab
- Tositumomab
- Trastuzumab

International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use:

ICH harmonised tripartite guideline Q5C;

Quality Testing of Biotechnological Products: Stability Testing of Biotechnological/Biological Products.

“...The evaluation for stability may necessitate complex analytical methodologies. Assays for biological activity, where applicable, should be part of the pivotal stability studies. Appropriate physicochemical, biochemical and immunochemical methods for the analysis of the molecular entity and the quantitative detection of degradation products should also be part of the stability program whenever purity and molecular characteristics of the product permit the use of these methodologies...”



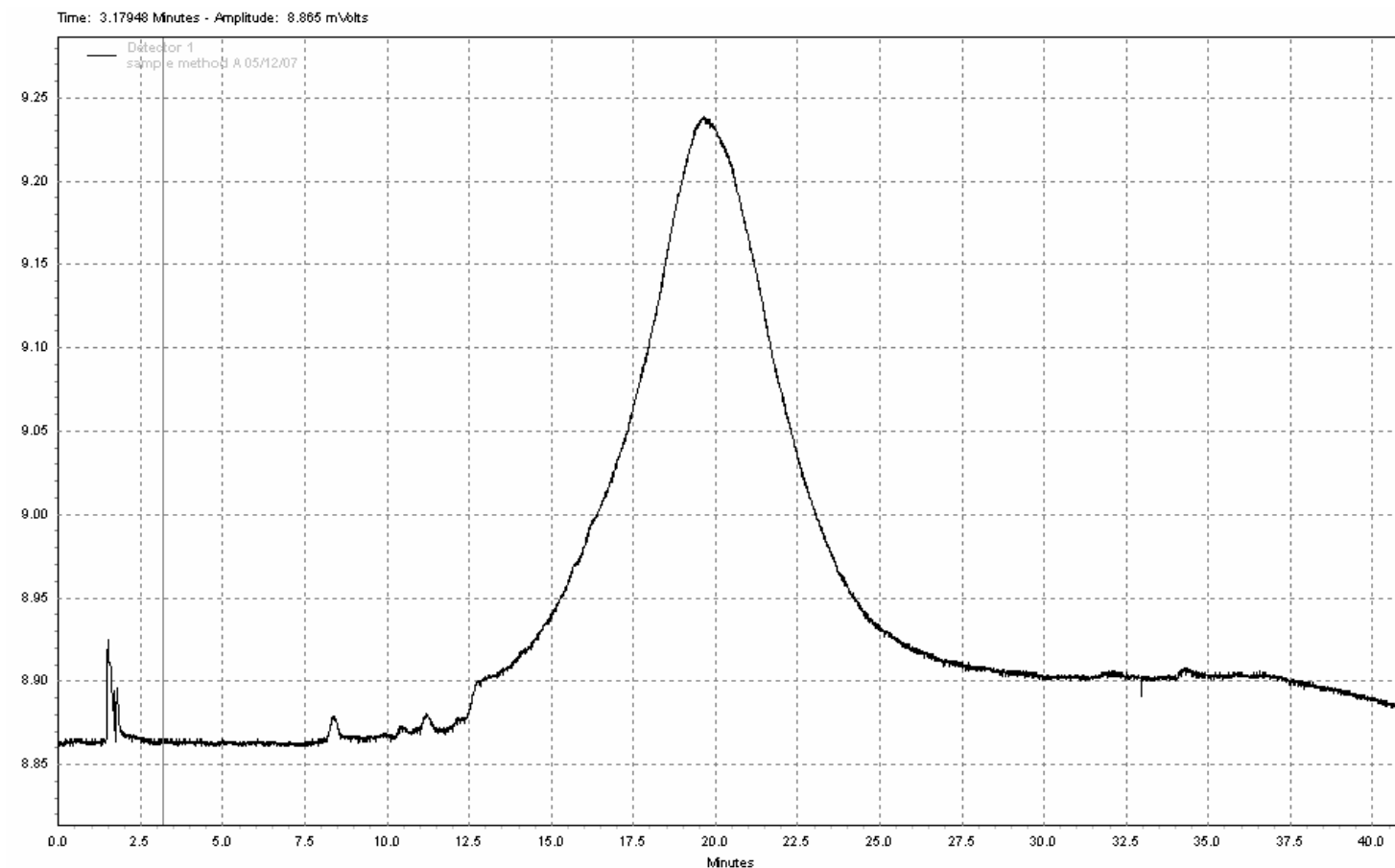
Fab – Fragment antibody binding.
Fc – Fragment constant region.

- <http://www.chennaionline.com/science/BiotechCorner/10Bio06.asp#>

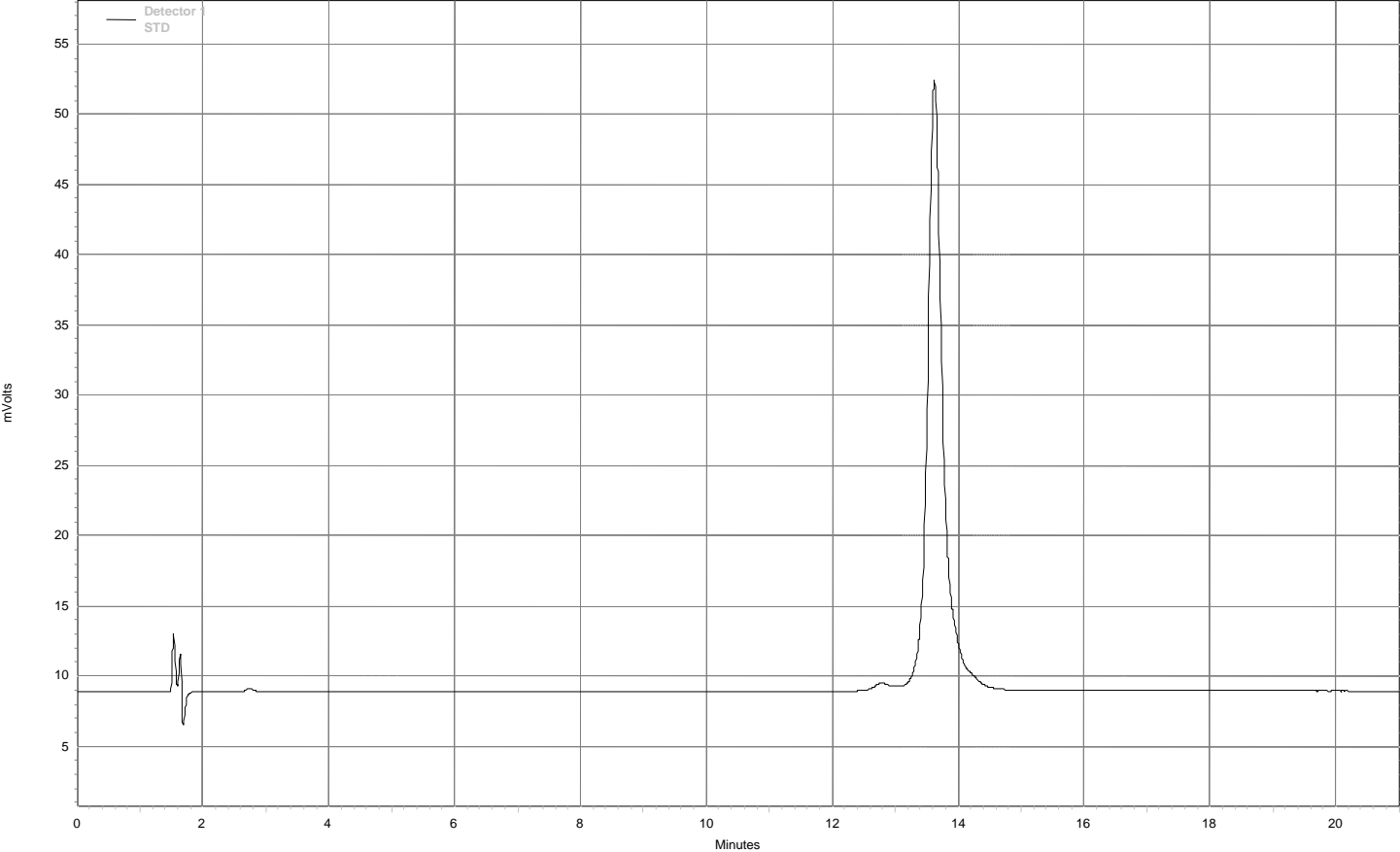
Reverse Phase Chromatography.

Column:	Zorbax 300A C18 SB Column														
Mobile Phase A:	0.15% Trifluoroacetic acid in water														
Mobile Phase B:	0.15% Trifluoroacetic acid in; 70% isopropanol, 20% Acetonitrile and 10% water.														
Gradient:	<table><thead><tr><th>Time</th><th>% Mobile Phase A</th></tr></thead><tbody><tr><td>0min</td><td>70%</td></tr><tr><td>5min</td><td>70%</td></tr><tr><td>13min</td><td>64%</td></tr><tr><td>20min</td><td>64%</td></tr><tr><td>21min</td><td>70%</td></tr><tr><td>26min</td><td>70%</td></tr></tbody></table>	Time	% Mobile Phase A	0min	70%	5min	70%	13min	64%	20min	64%	21min	70%	26min	70%
Time	% Mobile Phase A														
0min	70%														
5min	70%														
13min	64%														
20min	64%														
21min	70%														
26min	70%														
Flow Rate:	1ml/min														
Detection:	280nm														
Oven Temperature:	80°C														
Standard:	Avastin (bevacizumab) 400mg in 16ml vial.														
Standard Preparation:	Dilute 0.1ml of standard stock solution to 5.0ml with 0.01M KH_2PO_4 (pH6.0)														
Sample Preparation:	Dilute 0.1ml of sample solution to 5.0ml with 0.01M KH_2PO_4 (pH6.0)														

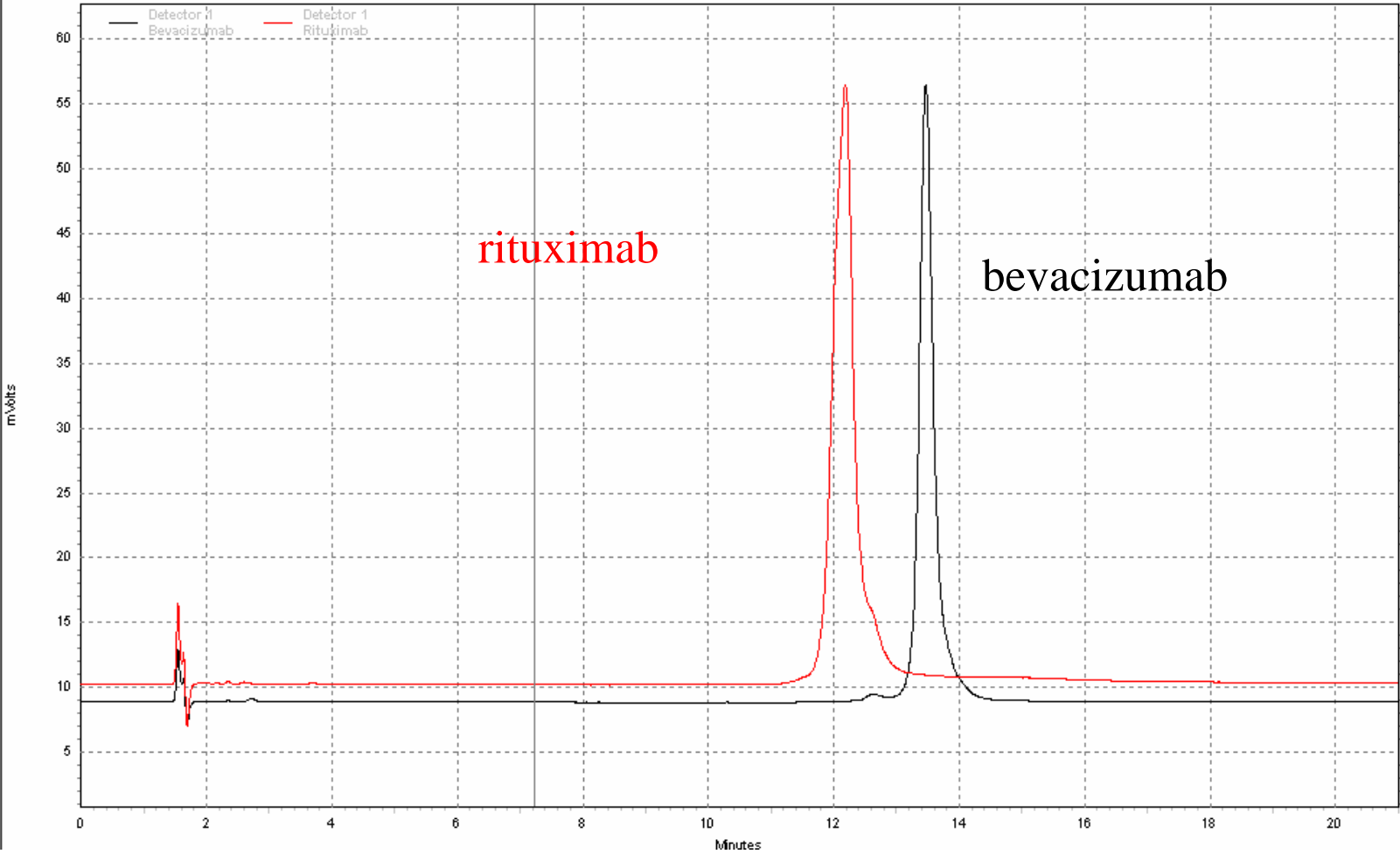
Reverse phase chromatogram of bevacizumab with an oven temperature of 50°C.



Reverse phase chromatogram of bevacizumab with an oven temperature of 80°C.



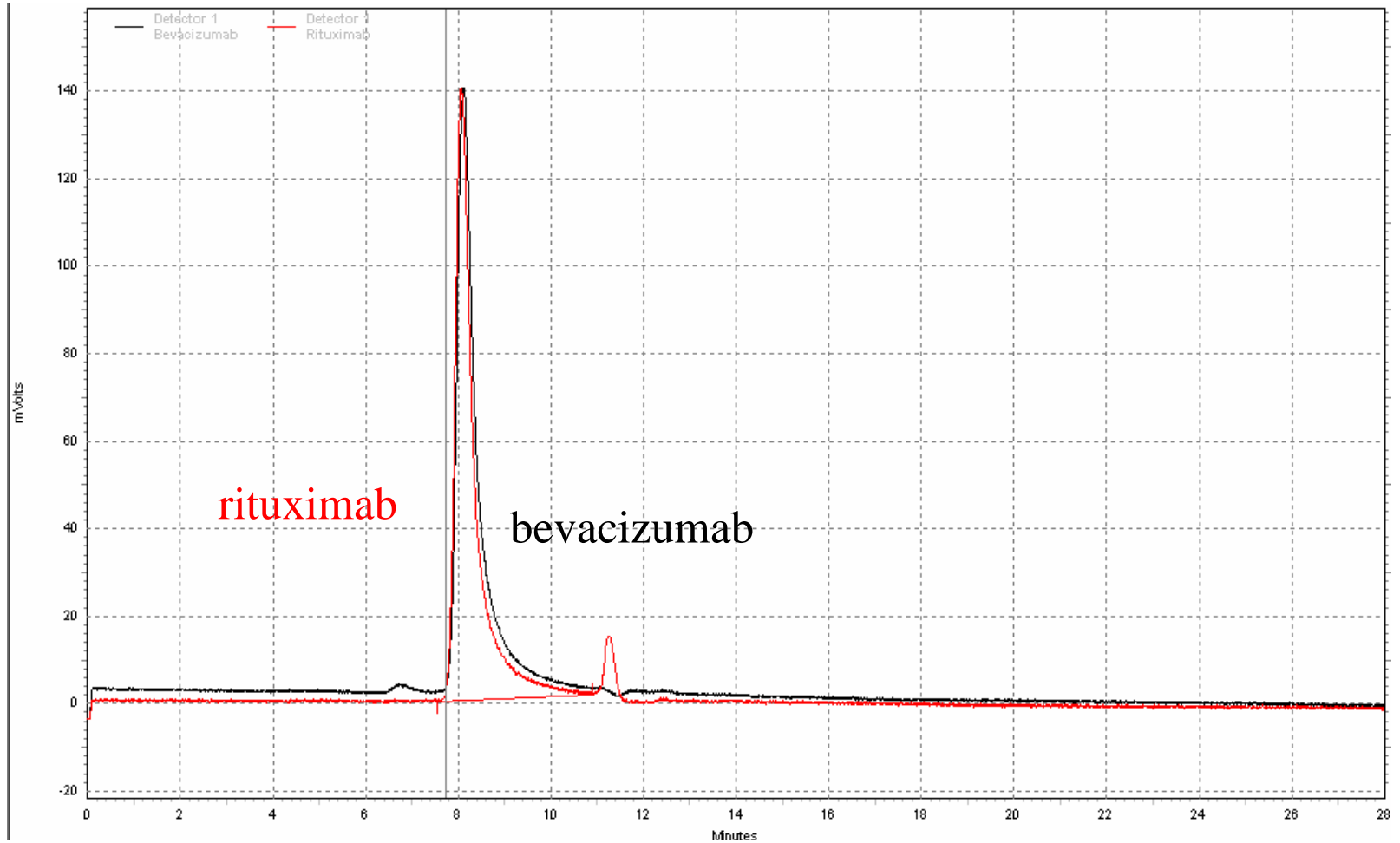
Overlay of reverse phase chromatograms of rituxumab and bevacizumab.



Size exclusion chromatography.

Column:	Tosoh TSKgel G3000SWXL Column
Mobile Phase:	0.1M sodium chloride in (0.05M sodium dihydrogen orthophosphate and 0.05M disodium hydrogen phosphate, at pH 6.0)
Flow Rate:	1ml/min
Detection:	280nm
Standard:	Avastin (bevacizumab) 400mg in 16ml vial.
Standard Preparation:	Dilute 0.1ml of standard stock solution to 5.0ml with 0.01M KH_2PO_4 (pH6.0)
Sample Preparation:	Dilute 0.1ml of sample solution to 5.0ml with 0.01M KH_2PO_4 (pH6.0)

Overlay of Size exclusion chromatograms of rituximab and bevacizumab



Validation

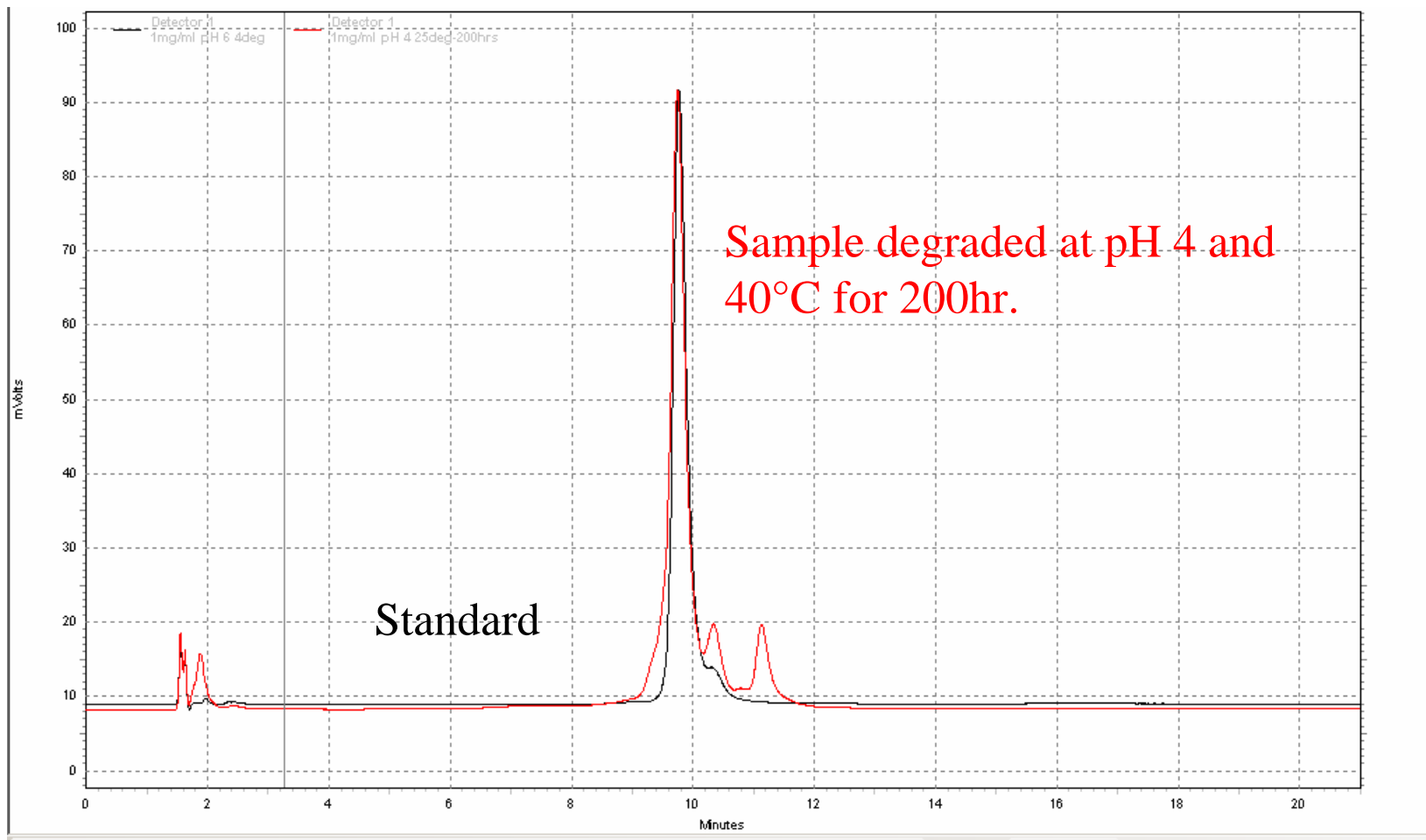
Reverse Phase Chromatography:

- Precision:
 - Repeatability RSD = 0.124%
(n=6)
- Accuracy:
 - Linearity = 0.99998

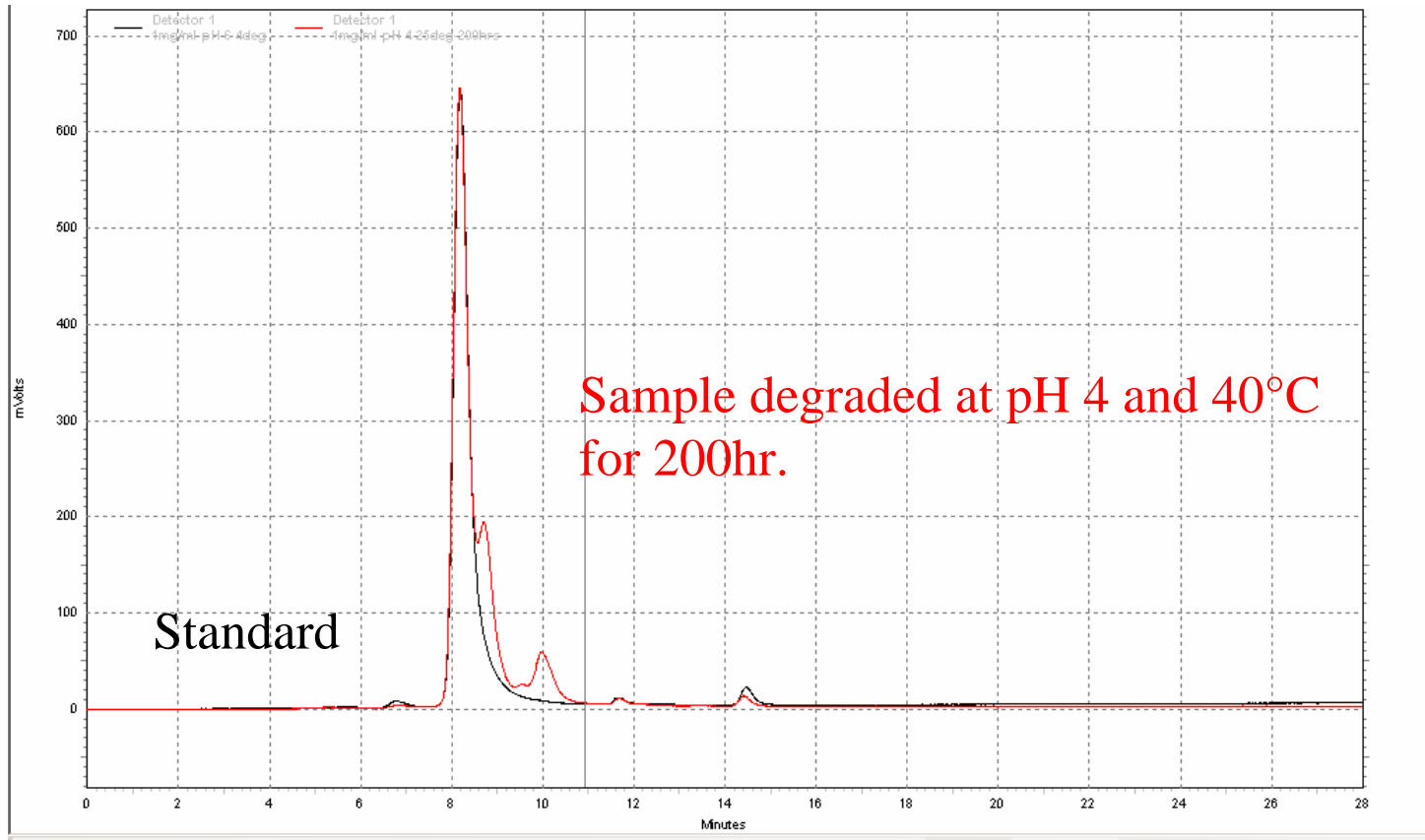
Size Exclusion Chromatography:

- Precision:
 - Repeatability RSD = 0.644%
(n=6)
- Accuracy:
 - Linearity = 0.9999

Overlay of reverse phase chromatograms of standard and sample degraded at pH 4 and 40°C for 200hours.



Overlay of size exclusion chromatograms of standard and sample degraded at pH 4 and 40°C for 200hours.



Internally normalised data from reverse phase chromatography after 1 months refrigerated storage in syringes.

	RT = 2.6 min	RT = 12.4 min	bevacizumab
Vial contents	0.31	1.48	98.20
1 week syringe	0.30	1.45	98.24
Vial contents	0.34	1.41	98.21
2 week syringe	0.37	1.41	98.22
Vial contents	0.33	1.42	98.22
4 weeks syringe	0.33	1.34	98.32

Internally normalised data from size exclusion chromatography after 1 months refrigerated storage in syringes.

	RT = 4.5 min	RT = 6.7 min	bevacizumab
	trimer	dimer	monomer
Vial contents	Not quantifiable	1.29	98.56
1 week syringe	Not quantifiable	1.39	98.53
Vial contents	1.21	1.51	96.56
2 week syringe	1.33	1.44	98.23
Vial contents	Not quantifiable	1.38	97.93
4 weeks syringe	Not quantifiable	1.44	98.57

ICH guideline Q5C:

“... The use of relevant physicochemical, biochemical and immunochemical analytical methodologies should permit a comprehensive characterisation of the drug substance and or drug product (eg molecular size, charge and hydrophobicity) and the accurate detection of degradation changes that may result from deamidation, oxidation, sulphoxidation, aggregation or fragmentation during storage...”