



# Sepax Monomix H2P SAX Tween Quantification

**Monomix H2P SAX, 2.1 x 20 mm SS**

Part Number: 282640990-2102

**Monomix H2P SAX, 2.1 x 50 mm SS**

Part Number: 282640990-2105



# Summary

- Monomix H2P-SAX columns are designed to effectively quantify neutral amphiphilic surfactants, such as Tween 80, Tween 20, and Poloxamers, which are commonly present in formulated biological samples, such as Monoclonal Antibodies.
- In this study, Erbitux and Tween 20 and Tween 80 mixtures were analyzed as an example to demonstrate the effectiveness of tween quantification by Monomix H2P-SAX column. The calibration range and capacity of Tween 20/80 were also analyzed on Monomix H2P-SAX column, respectively:
  - Tween 20: Calibration range 0.0016%- 0.0250%; Calibration range up to 0.0125 µg.
  - Tween 80: Calibration range 0.0010%- 0.0156%; Calibration range up to 0.0078 µg.
  - As you go above the CMC of Tween, micelles will form in solution. Dilution of sample is suggested for high concentration Tween quantification.  
*(Larger column sizes are available if higher capacity is needed)*
- Monomix H2P-SAX columns are more hydrophilic than other surfactant quantification column brands on the market. 10% IPA which is commonly used in the binding condition to reduce hydrophobic interaction, is not needed in the binding condition of Monomix H2P-SAX.
- With a narrow size distribution ( $D_{90}/D_{10} < 1.3$ ), Monomix H2P-SAX column offers higher separation and better peak shape than other vendors.



# Technical Specification

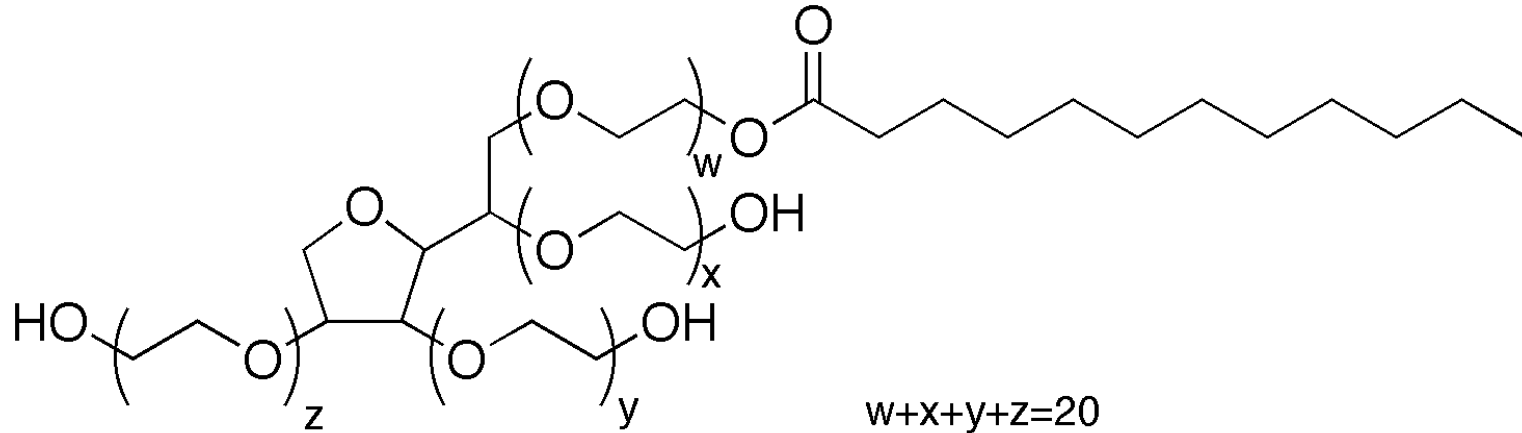
Application:	Surfactant quantification
Brand:	Monomix H2P-SAX
Part Number:	282640991-2102
Chemistry:	Mixed-Mode
Ion exchange capacity:	0.5 meq/gram
Mass Spec. Compatibility:	Yes
Particle Size:	40 $\mu\text{m}$
pH Stability:	1-13
Operating Temperature Limit:	80 $^{\circ}\text{C}$
Operating Pressure Limit:	3,000 psi
Flow Rate:	0.1-1 mL/min
Mobile Phase Compatibility:	Aqueous solvents, organic solvents, or their mixtures

## Experimental Method

Mobile Phase	A: 2% formic acid in water B: 2% formic acid in IPA
Instrument:	HPLC
Detector:	ELSD
Column Temperature	23 $^{\circ}\text{C}$
Flow Rate:	1.00 mL/min
Injection Volume:	50 $\mu\text{L}$
Column Pressure Range:	40-125 bar



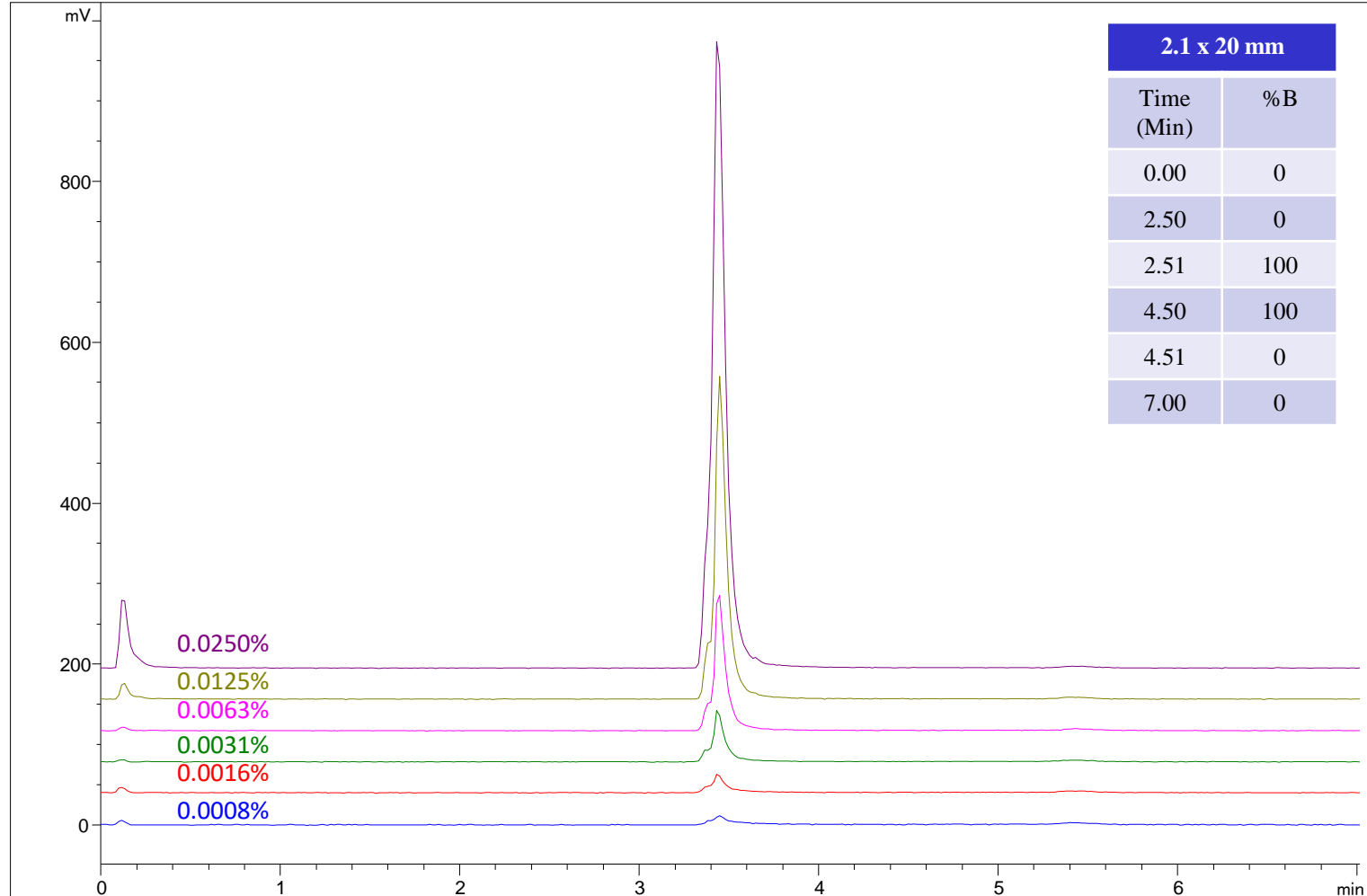
# Tween 20 Structure



Tween 20 is a mixed population sample.



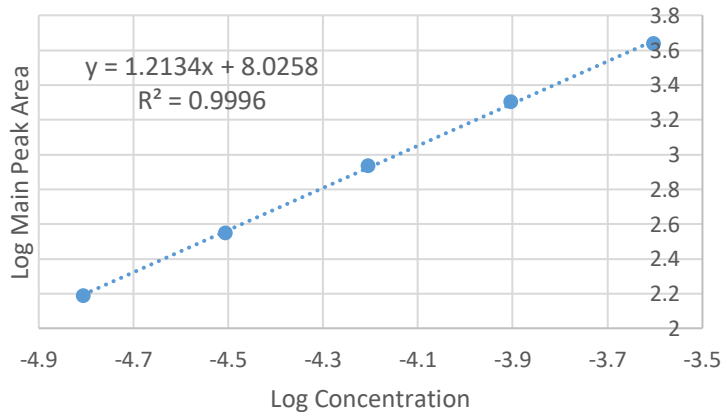
# Tween 20 Quantification Test on Monomix H2P-SAX Column



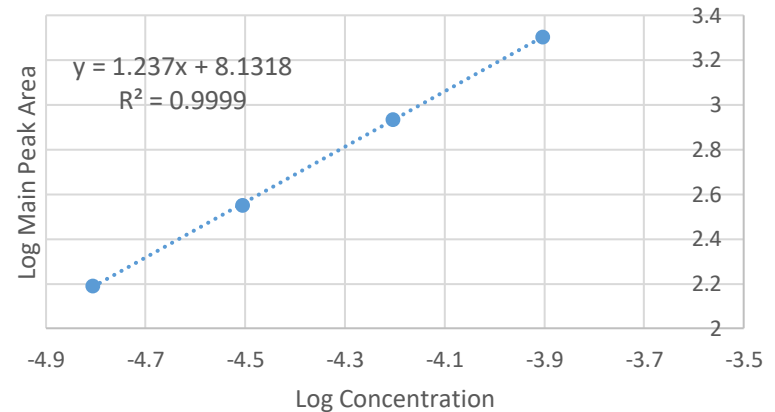


# Tween 20 Quantification Test on Monomix H2P-SAX Column Calibration Curve

**Tween 20**  
**(Include 0.025% data point)**



**Tween 20**  
**(Not include 0.025% data point)**



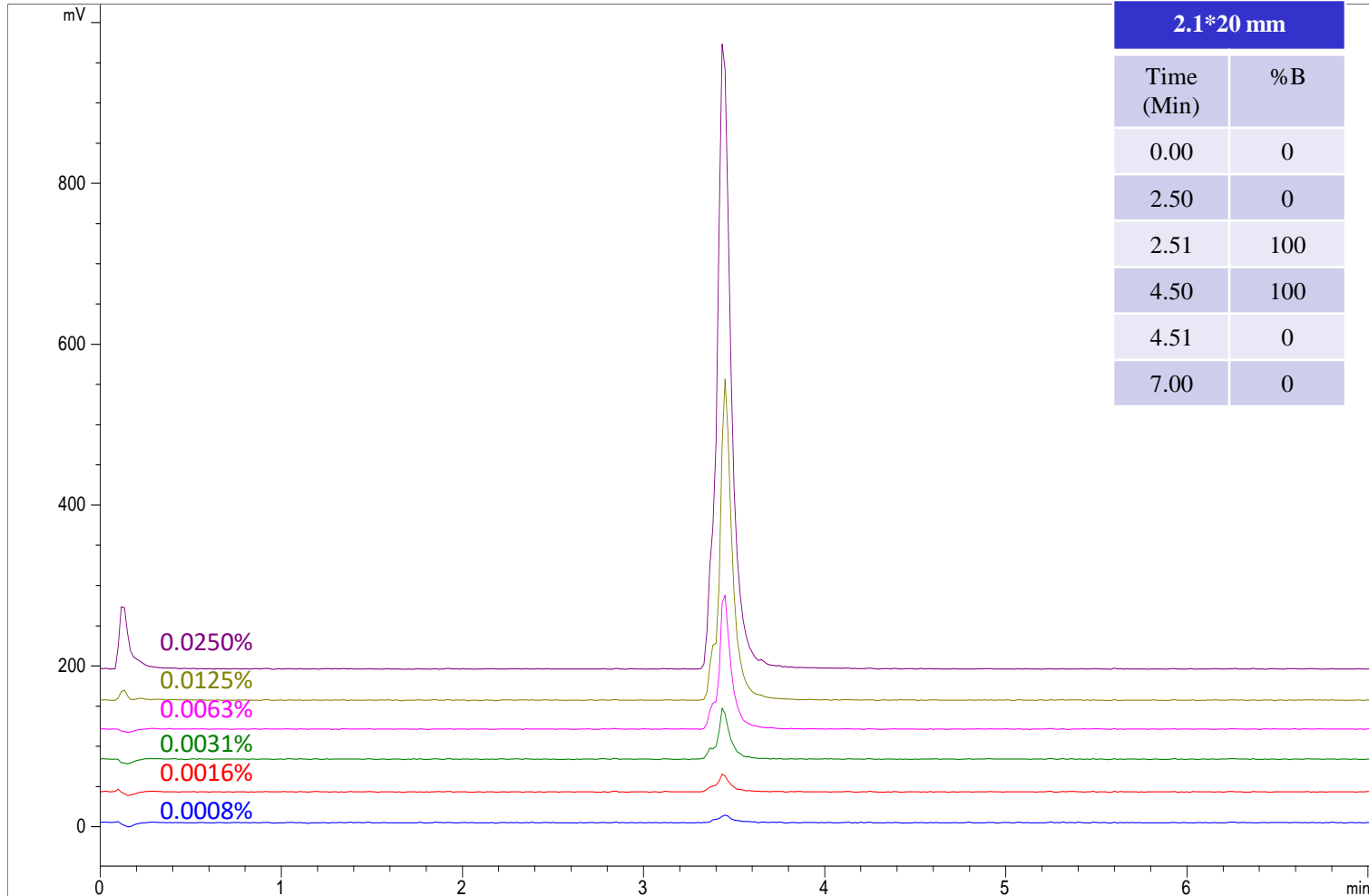
Calibration range up to 0.0125  $\mu\text{g}$

Concentration w/v	Flowthrough Area	Main Peak Area	Log (Concentration)	Log (Flowthrough Area)	Log (Main Peak Area)	Flowthrough Peak Area Percentage
0.0008%	14.9	85.5	-5.10721	1.173186	1.931966	
0.0016%	22	155	-4.80618	1.342423	2.190332	12.4%
0.0031%	9.8	356.3	-4.50515	0.991226	2.551816	2.7%
0.0063%	18	862.2	-4.20412	1.255273	2.935608	2.0%
0.0125%	75	2011.9	-3.90309	1.875061	3.303606	3.6%
0.0250%	349.8	4373.4	-3.60206	2.54382	3.640819	7.4%

The calibration range selection is based on the ELSD detector.

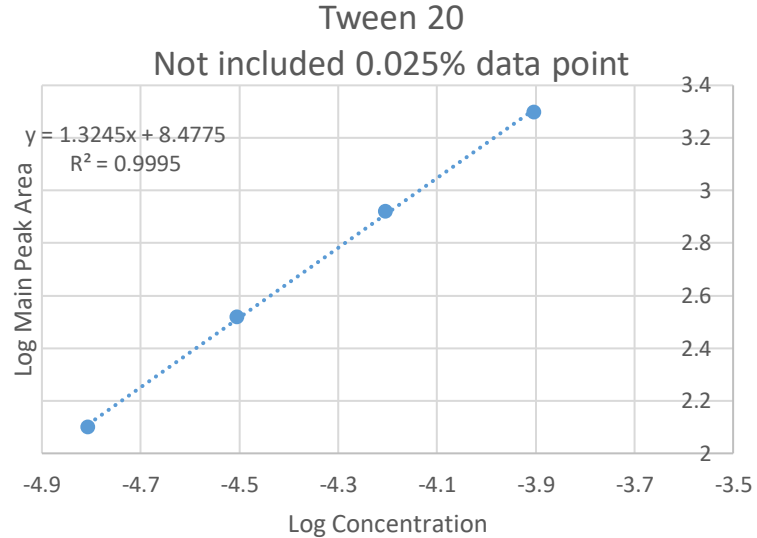
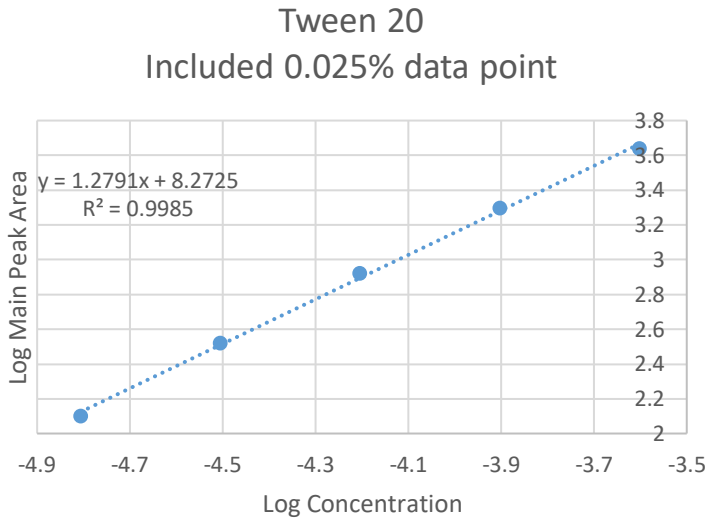


# Tween 20 Quantification Test on Monomix H2P-SAX Column- Background Deducted





# Tween 20 Quantification Test on Monomix H2P-SAX Column- Background Deducted



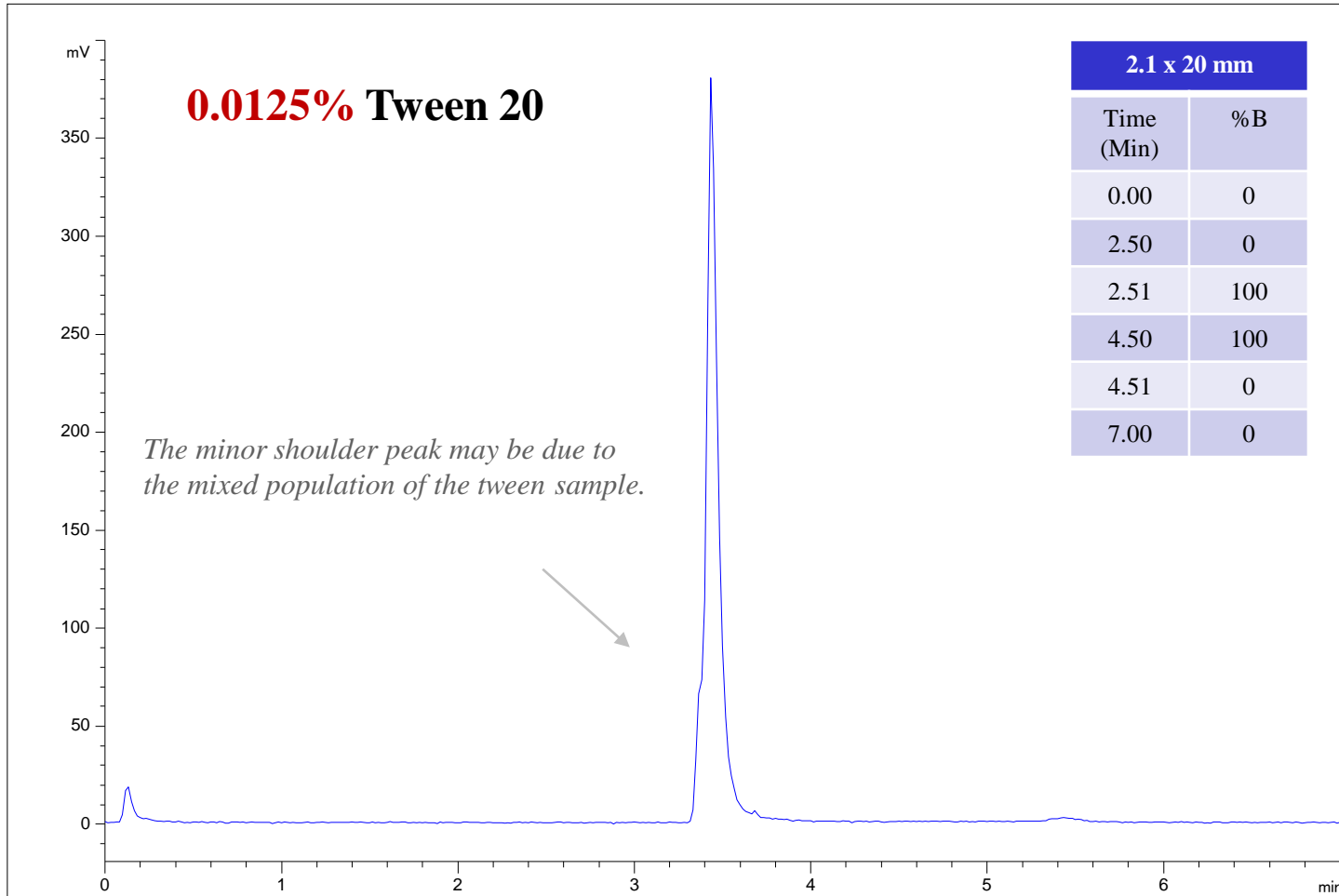
sample #	concentration w/v	Flowthrough Area	Main Peak Area	Log (concentration)	Log (Flowthrough Area)	Log (main Peak Area)	Flowthrough Peak Area Percentage
4	0.0008%	0	68.7	-5.10721	#NUM!	1.836957	0
5	0.0016%	0	126.4	-4.80618	#NUM!	2.101747	0
6	0.0031%	0	330.9	-4.50515	#NUM!	2.519697	0
7	0.0063%	0	830.1	-4.20412	#NUM!	2.91913	0
8	0.0125%	39.1	1984.7	-3.90309	1.592177	3.297695	1.9%
9	0.0250%	315.2	4344.3	-3.60206	2.498586	3.63792	6.8%

Tween 20 CMC: 0.006% w/v



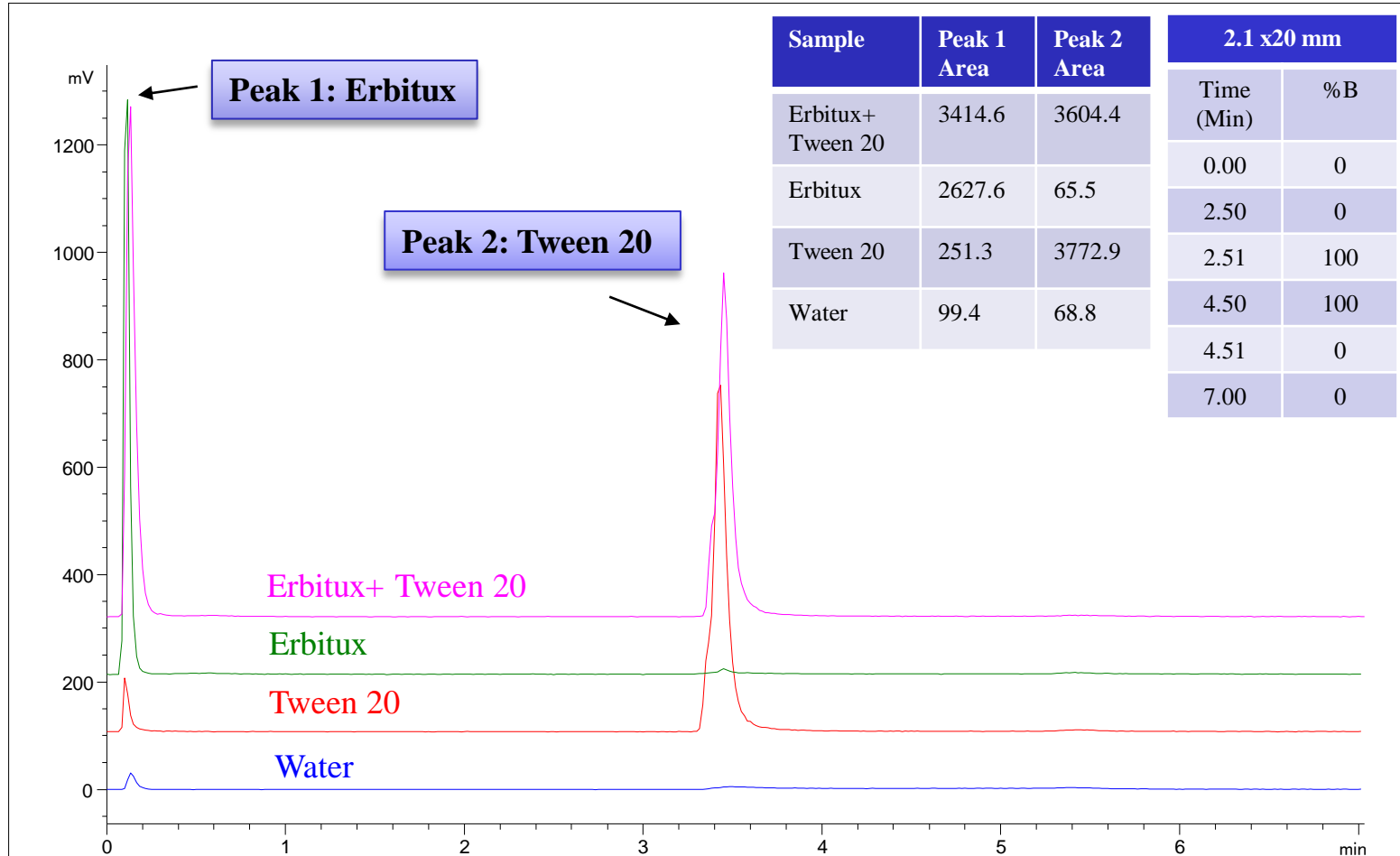


# Tween 20 Test on Monomix H2P-SAX





# Tween 20 and Erbitux Test on Monomix H2P-SAX

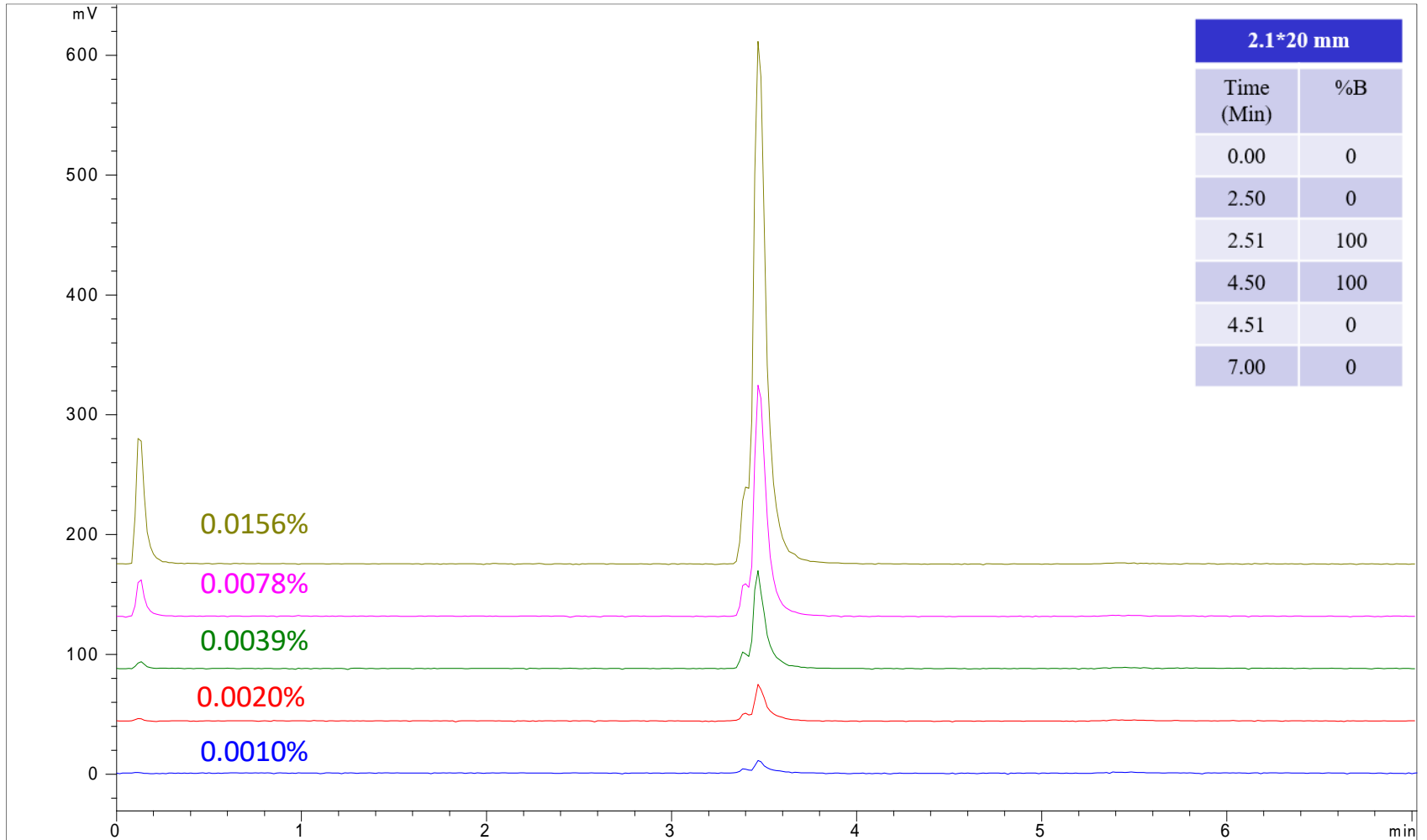


Sample: 0.04 mg/mL Erbitux+ 0.02% Tween 20, 0.04 mg/mL Erbitux, 0.02% Tween 20





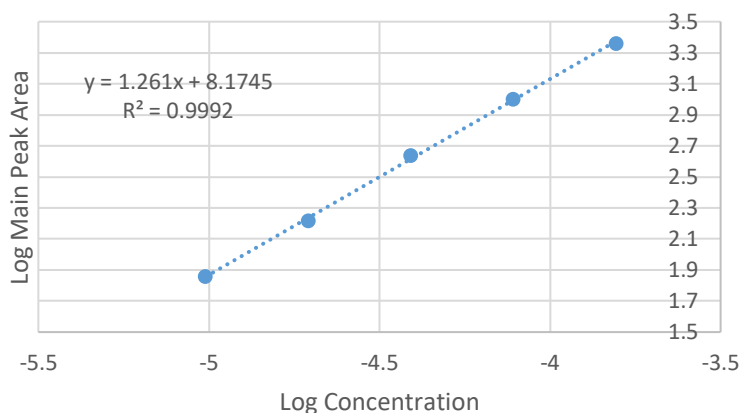
# Tween 80 Quantification Test on Monomix H2P SAX



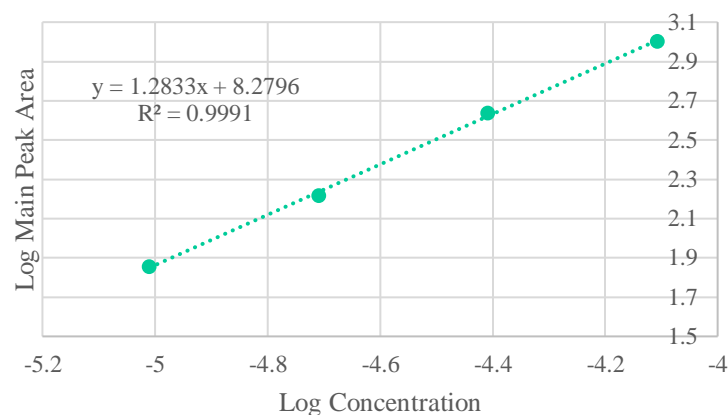


# Tween 80 Quantification Test on Monomix H2P SAX Calibration Curve

Tween 80  
(Included 0.0156 % data point)



Tween 80  
(Not included 0.0156 % data point)



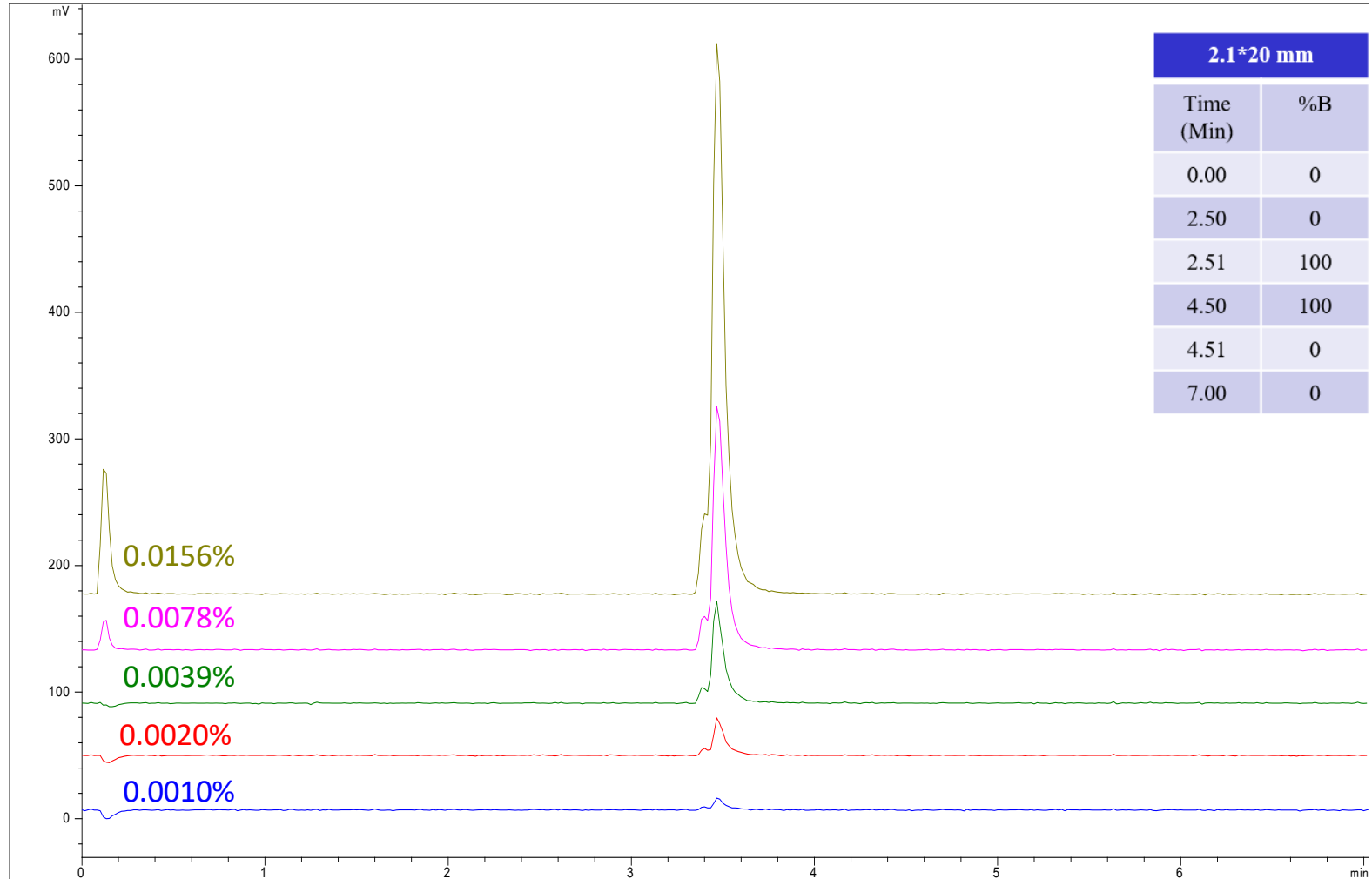
Calibration range up to 0.0078  $\mu\text{g}$

Sample	Concentration w/v	Flowthrough Area	Main Peak Area	Log (Concentration)	Log (Flowthrough Area)	Log (Main Peak Area)	Flowthrough Peak Area Percentage
1	0.0010%	1.9	71.8	-5.0103	0.278754	1.856124	2.6%
2	0.0020%	7.5	165.4	-4.70927	0.875061	2.218536	4.4%
3	0.0039%	17.6	435.3	-4.40824	1.245513	2.638789	3.9%
4	0.0078%	104	1008.7	-4.10721	2.017033	3.003762	9.4%
5	0.0156%	369.7	2298.8	-3.80618	2.567849	3.361501	13.9%

The calibration range selection is based on the ELSD detector.



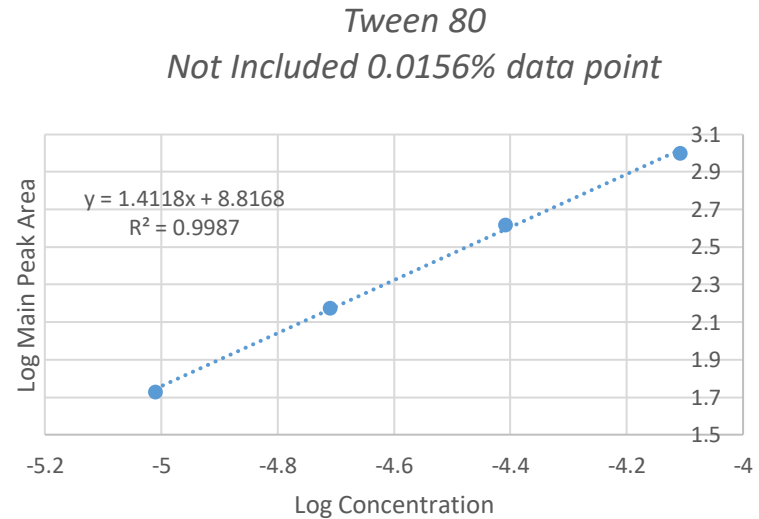
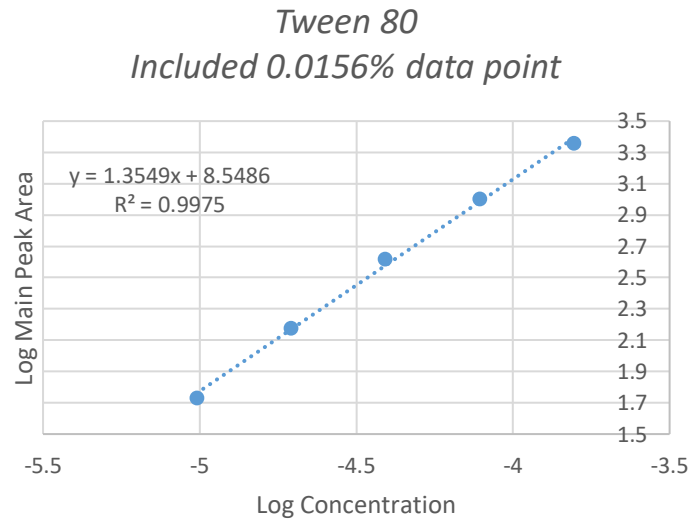
# Tween 80 Quantification Test on Monomix H2P SAX Background Deducted



2.1*20 mm	
Time (Min)	%B
0.00	0
2.50	0
2.51	100
4.50	100
4.51	0
7.00	0



# Tween 80 Quantification Test on Monomix H2P SAX Calibration Curve (Background Deducted)



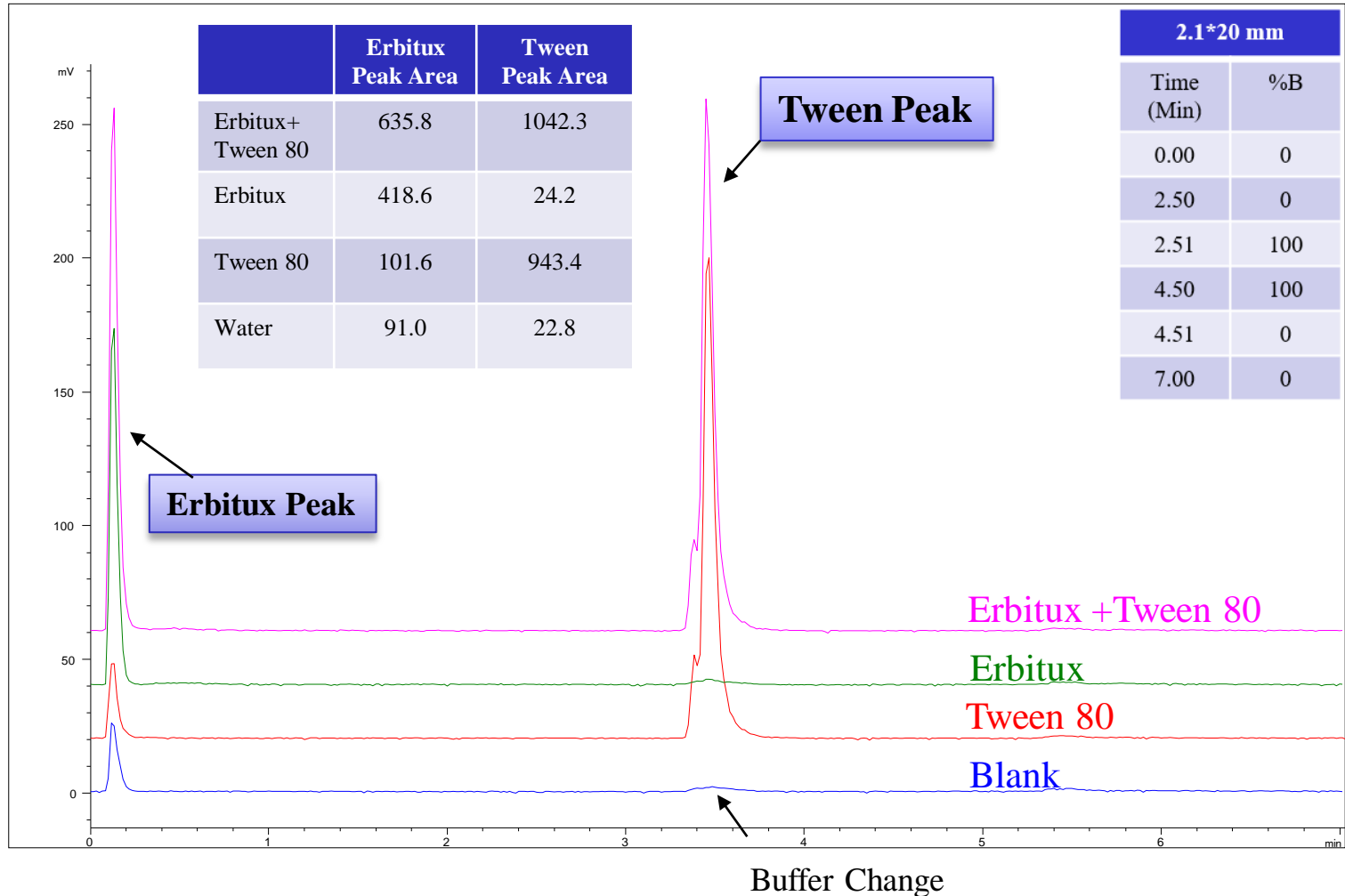
Calibration range up to 0.0078  $\mu\text{g}$

Sample	Concentration w/v	Flowthrough Area	Main Peak Area	Log (Concentration)	Log (Flowthrough Area)	Log (Main Peak Area)	Flowthrough Peak Area Percentage
1	0.0010%	0	53.7	-5.0103	#NUM!	1.729974	0
2	0.0020%	0	149.8	-4.70927	#NUM!	2.175512	0
3	0.0039%	0	414.9	-4.40824	#NUM!	2.617943	0
4	0.0078%	73.8	998.1	-4.10721	1.868056	2.999174	6.9%
5	0.0156%	337.9	2277.3	-3.80618	2.528788	3.35742	12.9%

Tween 80 CMC: 0.00157% w/v



# Quantification of Tween 80 in Erbitux by Monomix H2P SAX



Sample: 0.01 mg/mL Erbitux, 0.01 mg/mL Erbitux + 0.008% Tween 80



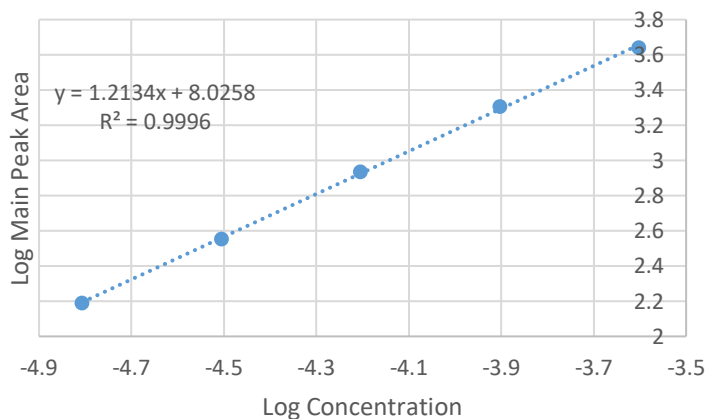


# Tween 20 vs Tween 80 Capacity

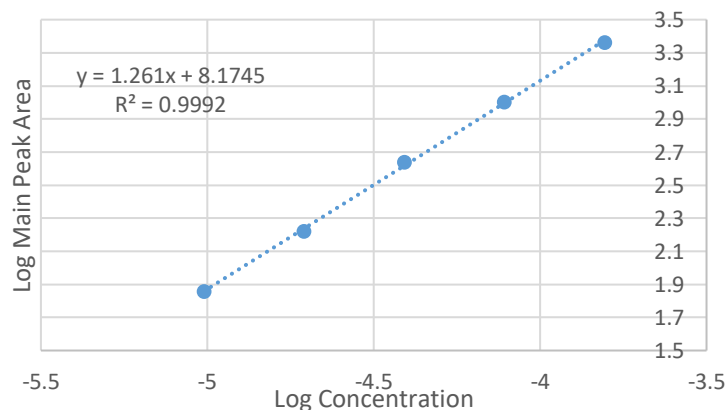
Tween 80 capacity lower than Tween 20 capacity on Monomix H2P-SAX may be due to:

1. Tween 80 forms micelle at a lower concentration than Tween 20.
2. Tween 80 micelle is larger than Tween 20 micelle, and the Monomix H2P-SAX resin is porous.

Tween 20



Tween 80



Tween 20 Calibration range up to 0.0125  $\mu\text{g}$

Tween 20 Sample	Concentration w/v	Flowthrough Area	Main Peak Area
2	0.0016%	22	155
3	0.0031%	9.8	356.3
4	0.0063%	18	862.2
5	0.0125%	75	2011.9
6	0.0250%	349.8	4373.4

Tween 80 Calibration range up to 0.0078  $\mu\text{g}$

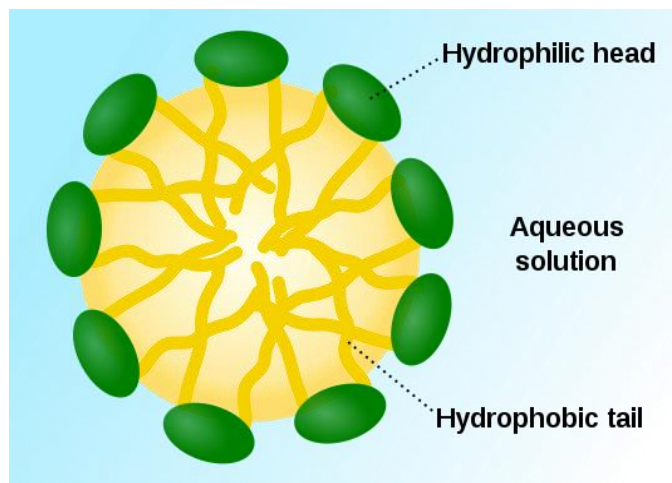
Tween 80 Sample	Concentration w/v	Flowthrough Area	Main Peak Area
1	0.0010%	1.9	71.8
2	0.0020%	7.5	165.4
3	0.0039%	17.6	435.3
4	0.0078%	104	1008.7
5	0.0156%	369.7	2298.8



# Understanding Tween Micelles

As you go above the CMC of Tween, micelles will form in solution. As the hydrophobic functional groups are encapsulated inside of the micelle, possibly micelles will not bind to the Monomix H2P-SAX resin with the same affinity as free Tween. Dilution of sample is suggested for high concentration Tween 20/80 quantification on Monomix H2P-SAX column.

- ❖ Tween 80 form micelle at 0.00157% w/v in water.<sup>1</sup>
- ❖ Tween 20 form micelle at 0.006% w/v in water.<sup>2,3</sup>



**Figure 1.** Typical micelle structure.<sup>4</sup>

- Ref:
1. Chou DK, Krishnamurthy R, Randolph TW, Carpenter JF, Manning MC (June 2005). "Effects of Tween 20 and Tween 80 on the stability of Albutropin during agitation". *J Pharm Sci.* 94 (6): 1368–81. doi:10.1002/jps.20365. PMID 15858848
  2. Helenius, A., et al., Properties of Detergents. *Methods in Enzymology*, 56, 734-749 (1979).
  3. <https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/217/731/p5927pis.pdf>
  4. [https://openwetware.org/wiki/Critical\\_micelle\\_concentration\\_\(CMC\)](https://openwetware.org/wiki/Critical_micelle_concentration_(CMC))



# Order Information

Column	Part Number
Monomix H2P SAX, 2.1 x 20 mm SS	282640990-2102
Monomix H2P SAX, 2.1 x 50 mm SS	282640990-2105

## CONTACT US

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