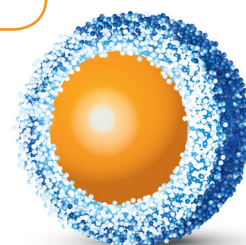
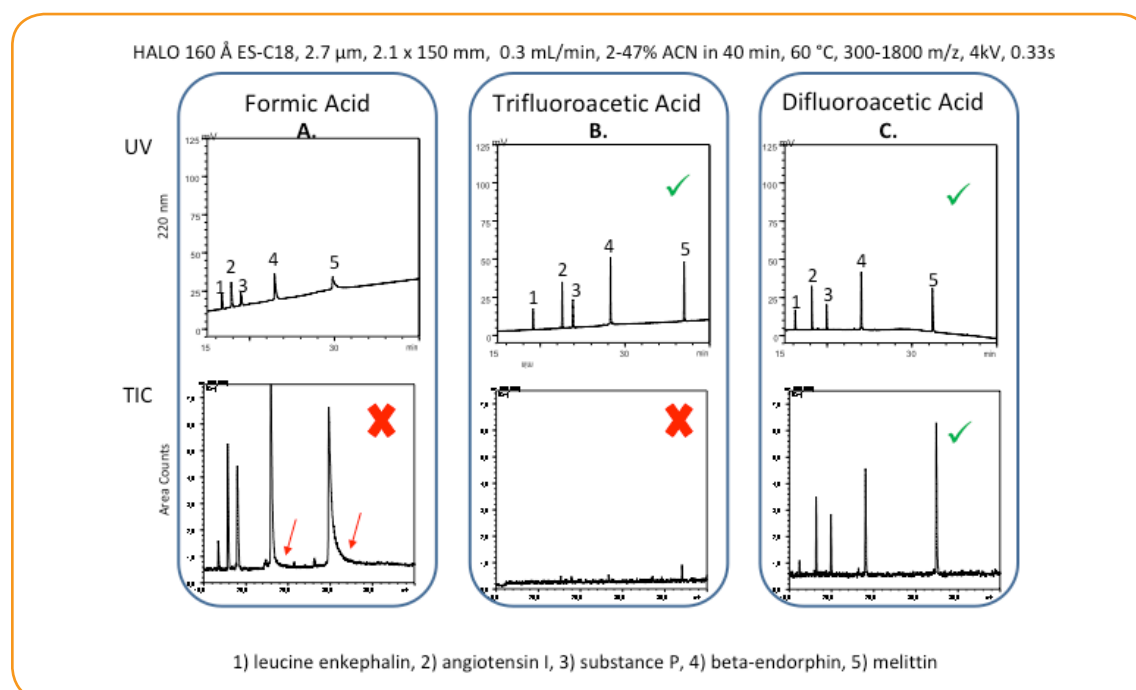


Effect of Acid Modifier on mAb Peak Shape in Reversed-Phase HPLC

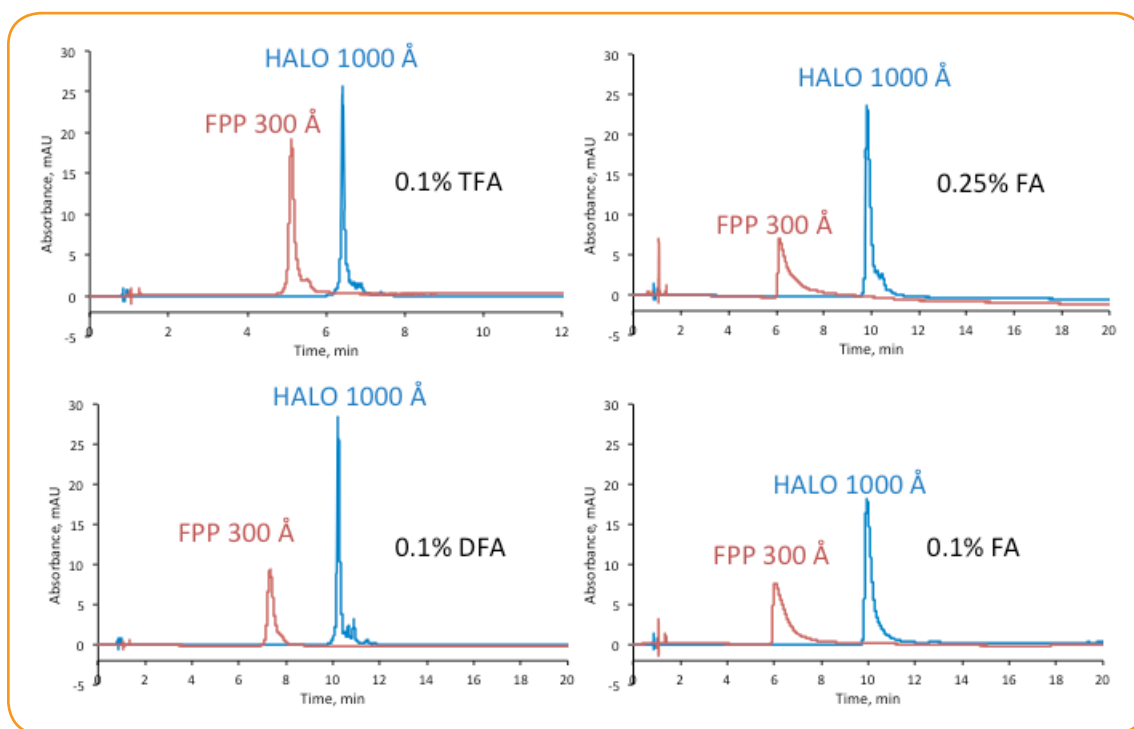
While there are several factors that contribute to peak shape, the choice of acid modifier is one of the most significant. The example in Fig. 1 shows the effect of three different acid modifiers on the peak shape and ionization efficiency of five different peptides. Formic acid (FA) does not have the capability to ion-pair, but provides great ionization efficiency so it is widely used in MS analysis (Fig. 1A). Trifluoroacetic acid (TFA) is known for its low pH and its ability to ion-pair. TFA is ideal for UV detection, but less preferred for MS detection since it greatly inhibits ionization efficiency (Fig. 1B). Difluoroacetic acid (DFA) is also able to ion-pair resulting in excellent peak shapes with increased ionization efficiency over TFA for MS detection (Fig. 1C). For a compromise of sharp peak shape and good ionization efficiency, one should choose DFA.

Figure 1. Example of acid modifier effects on peptide peak shape in both UV and MS conditions.



The chromatograms in Fig. 2 show the effect of acid modifier on intact trastuzumab peak shape run on both a HALO 1000 Å C4 Superficially Porous Particle (SPP) column and a Fully Porous Particle (FPP) 300 Å C4 column.

Figure 2. Effect of acid modifier on trastuzumab peak shape using HALO 1000 Å and FPP 300 Å columns.

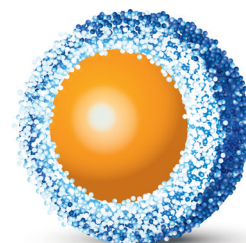


Conditions: 2.1 x 150 mm, A = water/acid, B = ACN/acid, 0.4 mL/min, 32-38% B in 12 min (TFA), 27-37% B in 20 min (DFA) 25-35% B in 20 min (FA), 80 °C, 280 nm

In Table 1, the same trend is observed for both columns with 0.1% TFA giving the sharpest peak shape, followed by 0.1% DFA, then 0.25% FA, and finally 0.1% FA giving the broadest peak shape. Note the improved performance on the HALO 1000 Å column demonstrating much narrower and taller peak widths compared to the FPP 300 Å column.

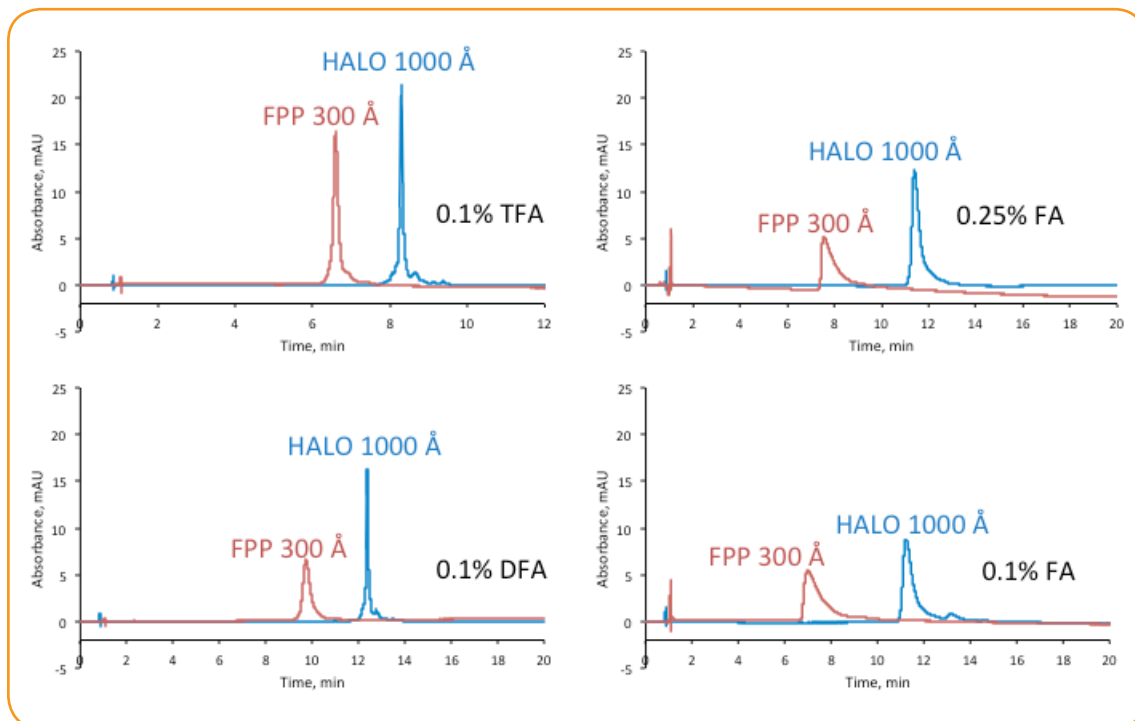
Table 1

% Acid	TRASTUZUMAB PEAK WIDTH	
	HALO 1000 Å	FPP 300 Å
0.1% TFA	0.0756	0.1318
0.1% DFA	0.1113	0.2723
0.25% FA	0.2104	0.4307
0.1% FA	0.2893	0.5489



Another monoclonal antibody, SILu™ Lite SigmaMAb, shows the same trend as was observed with trastuzumab. Both TFA and DFA provide similar narrow peak widths. Broader peak widths are observed with 0.25% FA and 0.1% FA. Resolution of the minor components is reduced when formic acid is used. Table 2 lists the peak widths measured at 50% height for each condition.

Figure 3. Effect of acid modifier on SigmaMAb peak shape using HALO 1000 Å and FPP 300 Å columns.



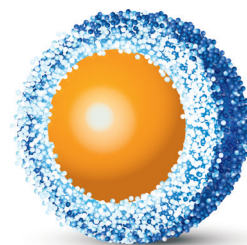
Conditions: same as listed in Fig. 2.

Table 2

% Acid	SIGMAMAB PEAK WIDTH	
	HALO 1000 Å	FPP 300 Å
0.1% TFA	0.0773	0.1318
0.1% DFA	0.0930	0.3412
0.25% FA	0.3303	0.5777
0.1% FA	0.4449	0.7309

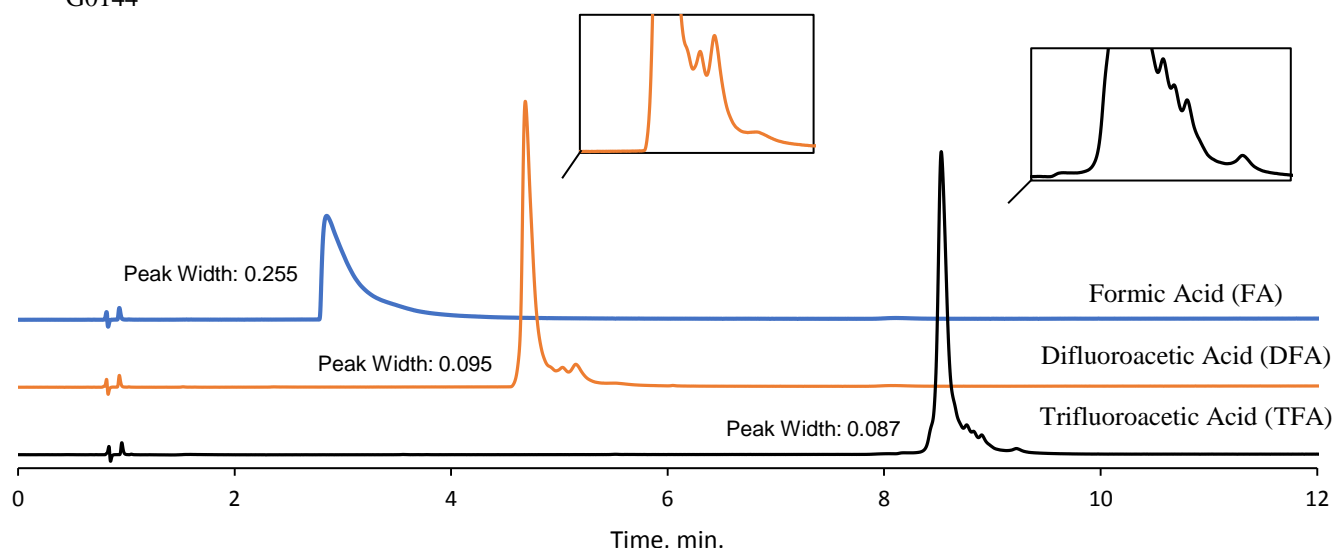
Conclusions

Depending on the requirements of the separation, one may select an ion-pairing agent (TFA or DFA) for improved peak shape and resolution of minor mAb components or formic acid to maximize ionization efficiency for MS analysis when using HALO 1000 Å columns. The larger pore size of HALO 1000 Å columns provides sharper peaks and better resolution of minor mAb components than fully porous 300 Å columns under all run conditions tested.



Effect of Acid Modifiers on Intact mAb Peak Shape

G0144



TEST CONDITIONS:

Column: HALO 1000Å C4, 2.7µm, 2.1 x 150 mm
Part Number: 92712-714

Mobile Phase:

A= Water, with 0.1% FA, DFA, or TFA as noted

B= 80/20 ACN/Water, with 0.1% FA, DFA, or TFA as noted

Gradient:

Time (min.)	%B
0.00	35.0
12.00	47.5

Flow Rate: 0.4 mL/min

Initial Pressure: 218 bar

Temperature: 80 °C

Injection Volume: 2 µL

Sample Solvent: 30/70 ACN/Water

Detection: UV 280 nm, PDA

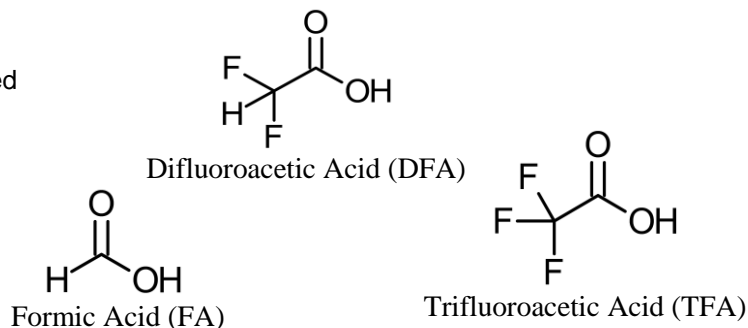
Data Rate: 12.5 Hz

Response Time: 0.05 sec

Flow Cell: 1 µL

LC System: Shimadzu Nexera X2

STRUCTURES:



Trastuzumab (~148 kDa) is a monoclonal antibody (mAb) used to treat breast cancer. TFA and DFA can be used as mobile phase additives instead of formic acid to provide much narrower and more symmetrical peaks, and to allow adjustments to retention and resolution among minor variants.