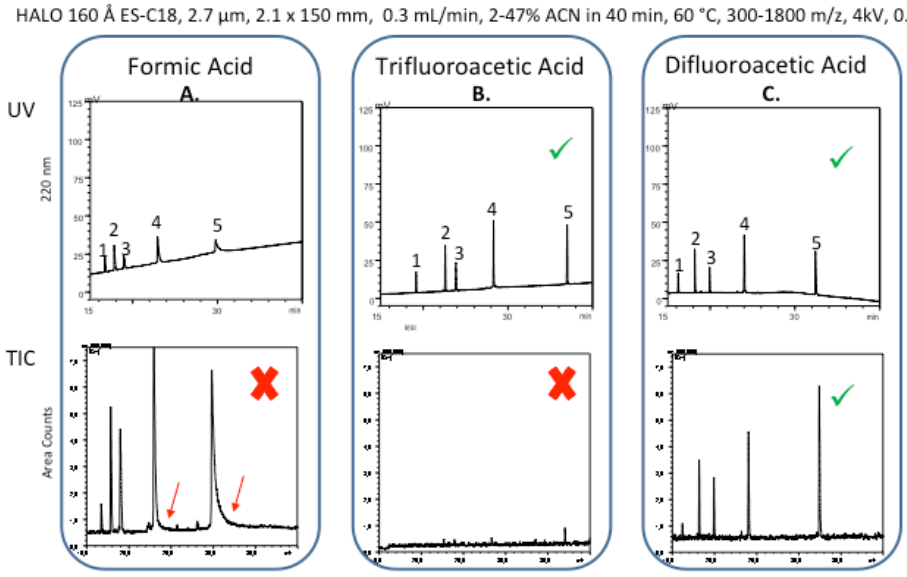
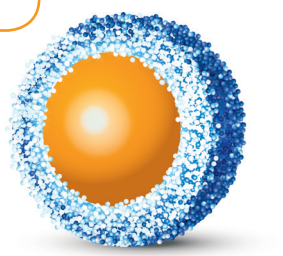
Effect of Acid Modifier on mAb Peak Shape in Reversed-Phase HPLC

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). Trifluoracetic 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.





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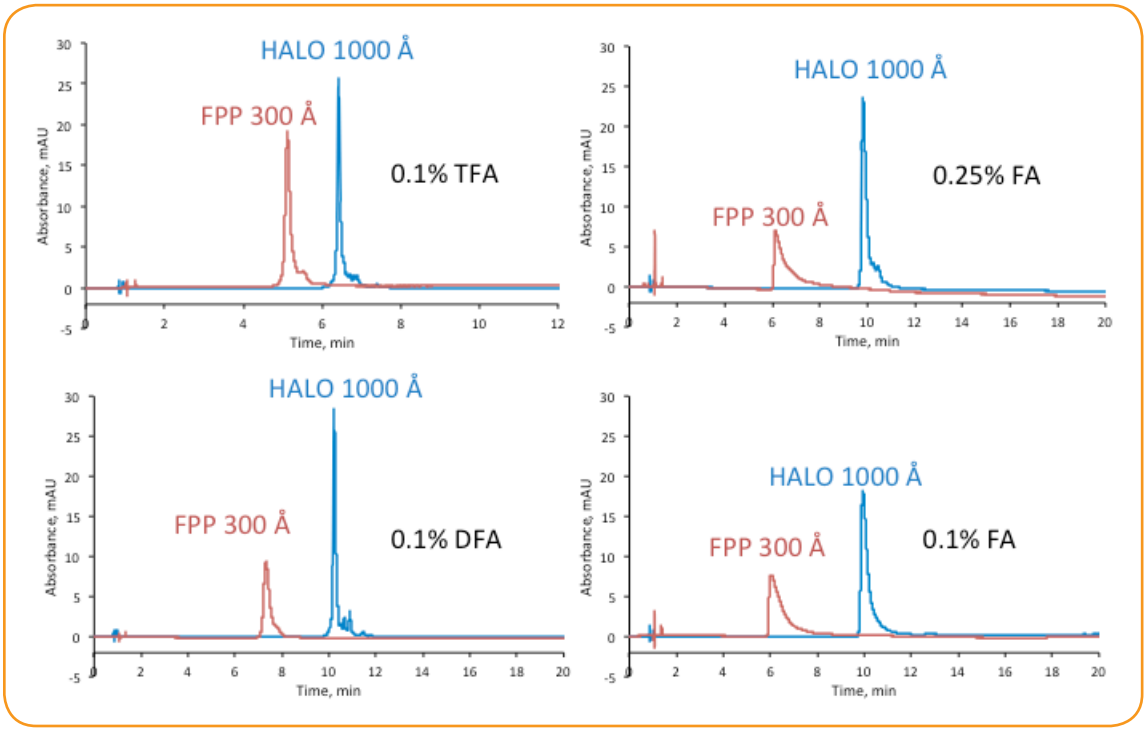
The chromatograms in Fig. 2 show the effect of acid modifier on intact trastuzumab peak shape run on both a HALO 1000 Å C4 Superficially Porous Particle (SPP) column and a Fully Porous Particle (FPP) 300 Å C4 column.

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

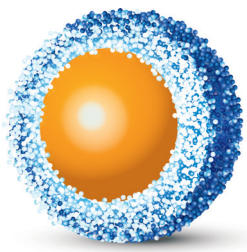


Conditions: 2.1 x 150 mm, A = water/acid, B = ACN/acid, 0.4 mL/min, 32-38% B in 12 min (TFA), 27-37% B in 20 min (DFA) 25-35% B in 20 min (FA), 80 °C, 280 nm

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.

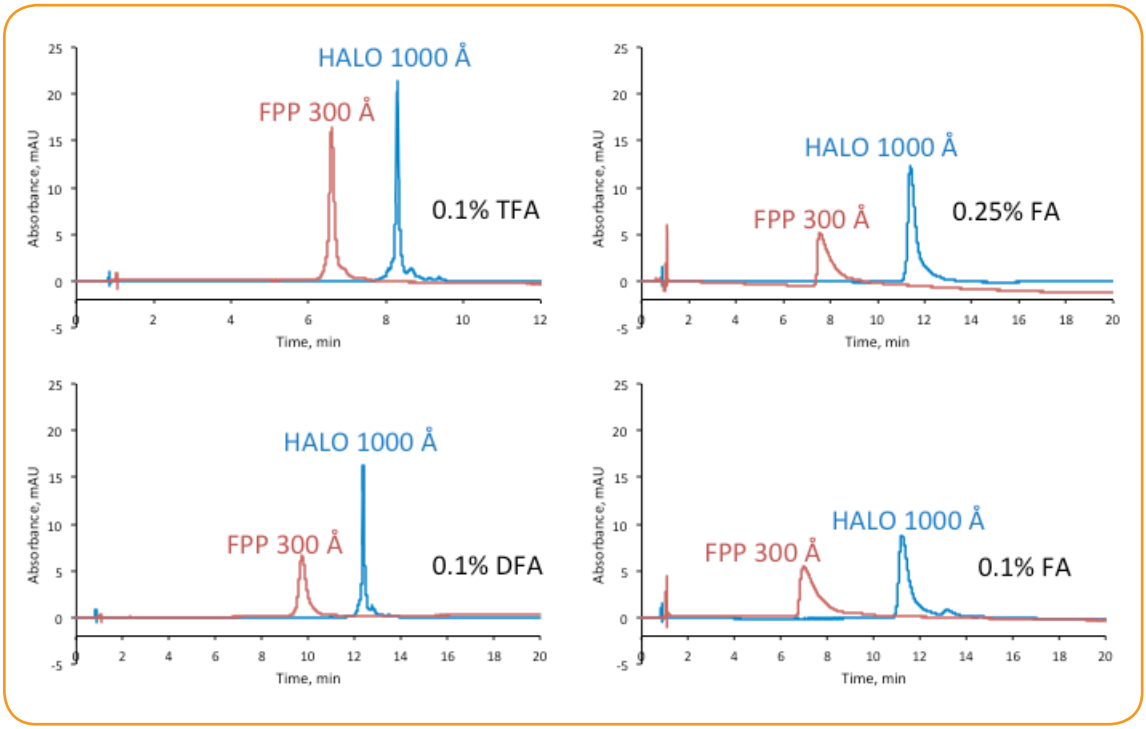
Table 1

	TRASTUZUMAB PEAK WIDTH	
% Acid	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

	SIGMAMAB PEAK WIDTH	
% Acid	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.