Chromatographic Evaluation of a New Organic/Inorganic Hybrid Reversed-Phase HPLC Packing

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Summary:

An organic/inorganic hybrid porous particle has been synthesized for use as an HPLC packing. This new chromatographic hybrid particle has been additionally surface bonded to attach multiple C_8 and C_{18} groups. These surface bonded hybrid particles not only exhibit extended pH stability, but show improved performance for basic compounds. On silica-based reversed-phase columns, broad and tailing peaks for many basic analytes have been attributed to surface silanols. We attribute the improved performance of the surface bonded hybrid particles to their reduced surface silanol concentration. Peak shape for a variety of basic, neutral, and acidic analytes was studied on reversed-phase bonded hybrid packings. Data is presented illustrating peak shape trends as a function of injected mass, mobile phase pH, and organic modifier.

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Poster Outline

1-4 Introduction

5 Nortriptyline example
- –XTerra™ RP_{18} shows the best peak shape.

6 The effects of mass load and retention factor on tailing shown in detail for nortriptyline
- –Tailing on C_{18}-Silica columns increases with increasing retention, a trend not observed on the Hybrid columns.
- –Increasing mass loads gave increasing tailing factors on C_{18}-Silica. The increases were less pronounced on Hybrid.

8-9 Tailing factors are compared for 8 analytes at various pH's and organic mobile phase modifiers at two mass loads
- –The best tailing factors across all mobile phases were observed for XTerra™ RP_{18}.

10-12 The effect of mass load on tailing for our test analytes
- –XTerra™ RP_{18} provided the lowest average tailing factors and the best overall peak shape.

13-15 Experimental conditions and references

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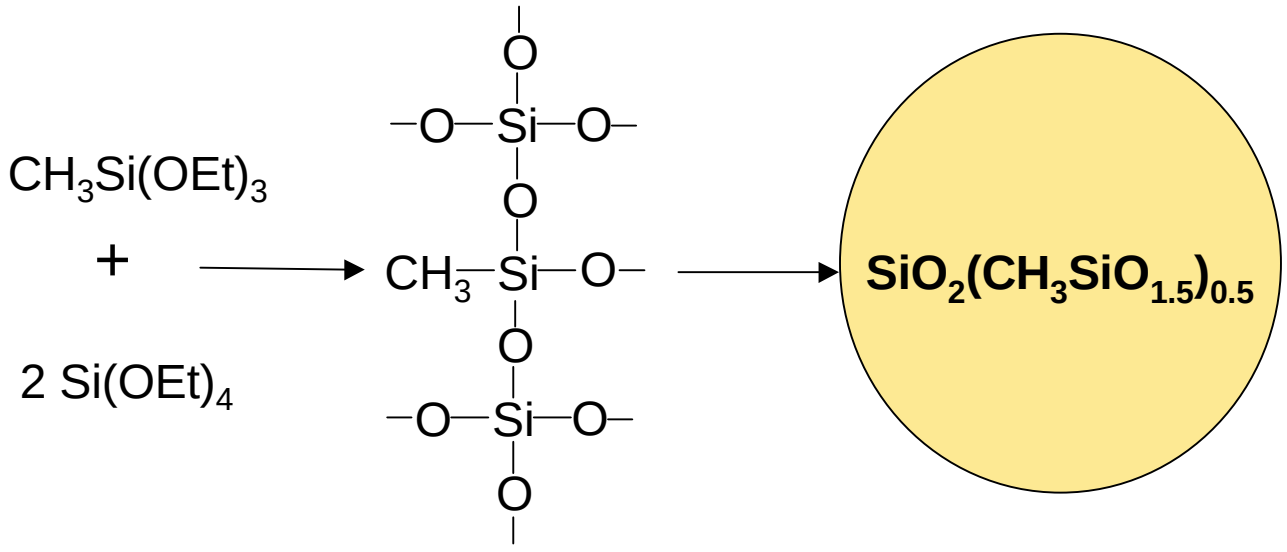
Hybrid Technology Particle

- Hybrid Organic/Inorganic materials contain both organic and inorganic components

- The hybrid particles described here were synthesized from $Si(OEt)_4$ and $CH_3Si(OEt)_3$:



- Hybrid particles combine:
  - efficiency and mechanical strength of silica
  - extended pH range and absence of base tailing of organic polymers

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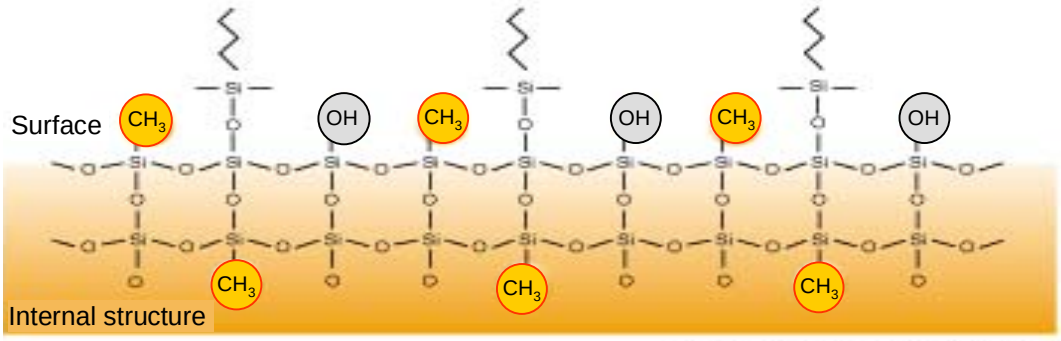
XTerra™ Bonded Phases

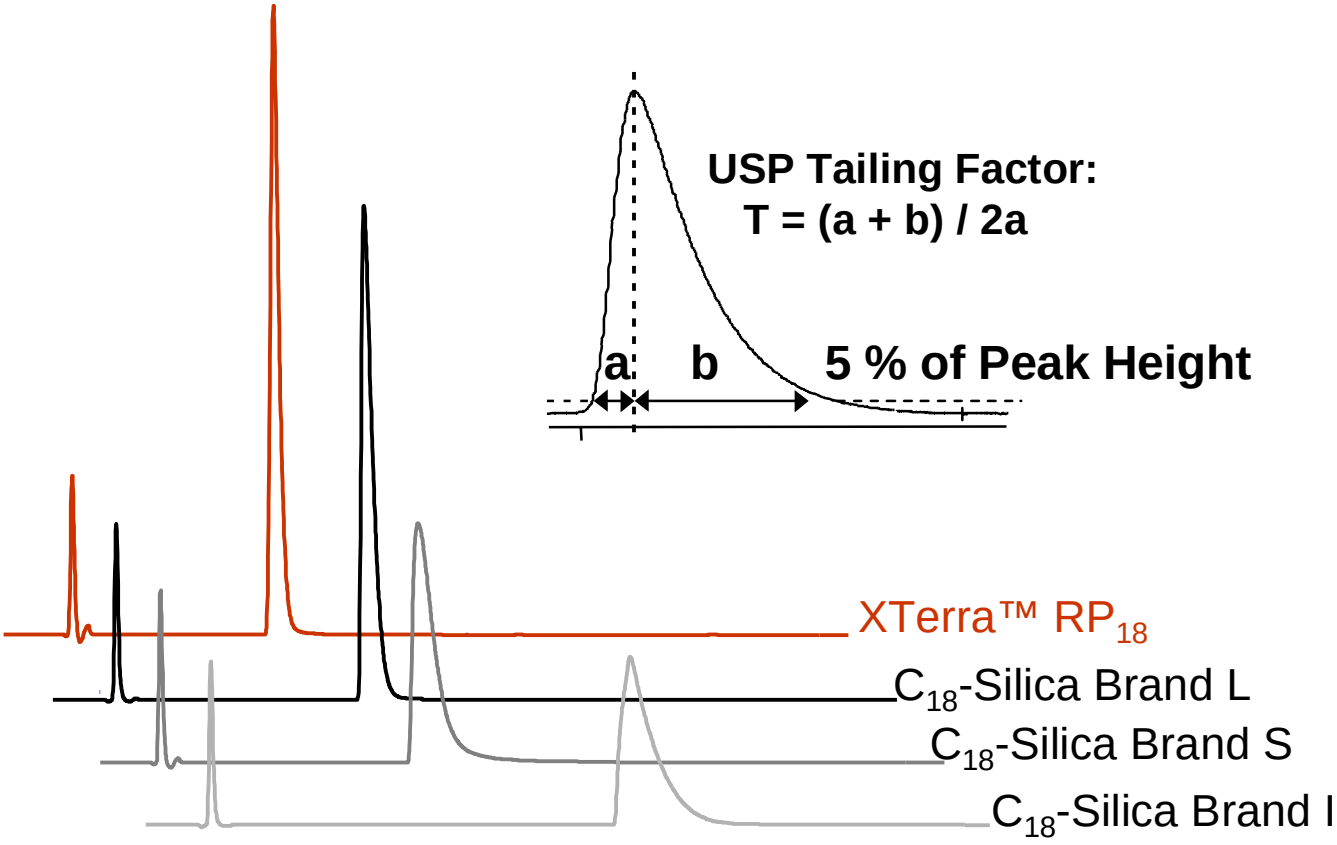


Bonded XTerra™ Particle

- XTerra™ bonded phases are based on the hybrid particles shown in the previous slide
- XTerra™ RP_{18}
  - Embedded carbamate C_{18} phase with a trimethylsilyl endcap
  - 14.95% C
  - 2.32 µmol/m²
- XTerra™ MS C_{18}
  - Trifunctional C_{18} phase with a trimethylsilyl endcap
  - 15.45% C
  - 2.22 µmol/m²

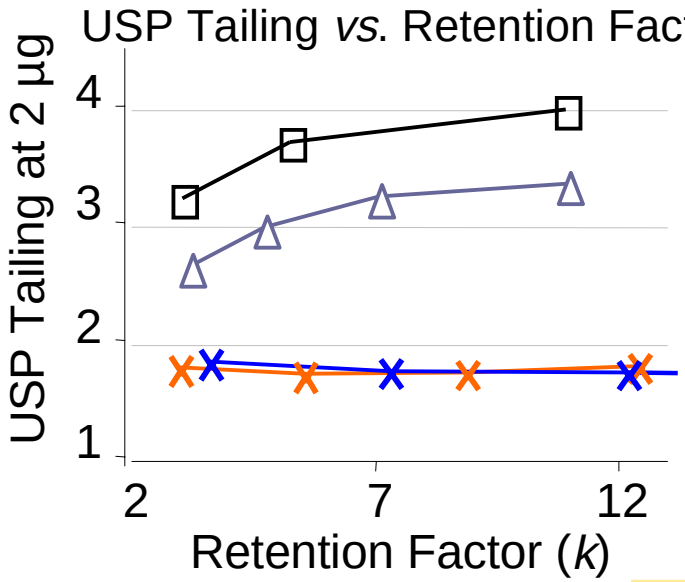
Nortriptyline Example:
pH 7/MeCN Mobile Phases

2 µg Load (88/12) 20 mM K_2HPO_4 pH 7/Acetonitrile



C_{18} Packings	*k* Retention Factor	N 5 sigma	USP Tailing
XTerra™ RP_{18}	3.151	3759	1.8
C_{18}-Silica Brand L	3.955	3051	2.9
C_{18}-Silica Brand S	4.336	684	3.4
C_{18}-Silica Brand I	6.507	759	2.9

Nortriptyline: pH 7 / MeCN
Tailing *vs.* Retention Factor and Mass Load



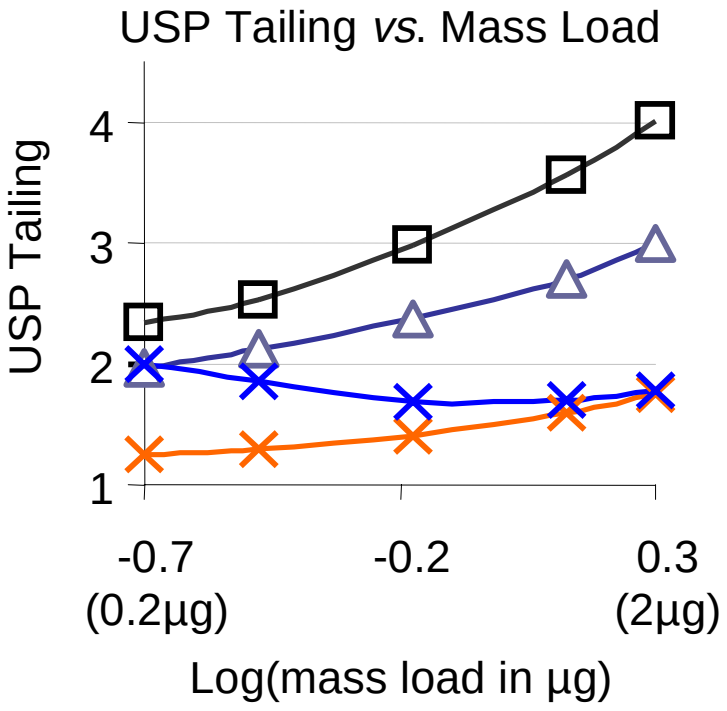
C_{18}-Silica Brand L

C_{18}-Silica Brand I

XTerra™ RP_{18}

XTerra™ MS C_{18}

XTerra™ packings showed less increase in tailing with increasing retention and mass



C_{18}-Silica Brand L, 30% MeCN (k'=11.2)

C_{18}-Silica Brand I, 36% MeCN (k'=4.8)

XTerra™ RP_{18}, 30% MeCN (k'=5.6)

XTerra™ MS C_{18}, 30% MeCN (k'=7.5)

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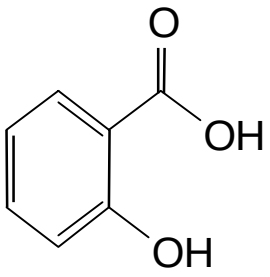
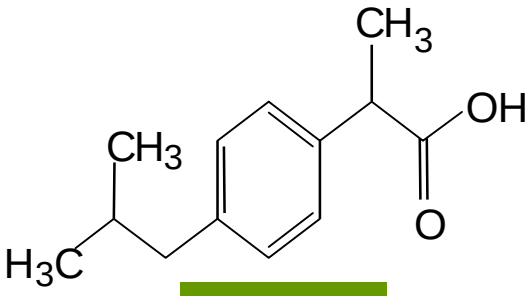
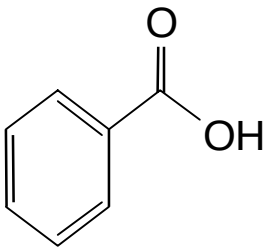
Analyte Structures



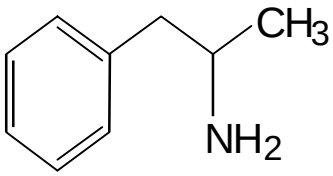
Salicylic Acid
pK_a 3.0



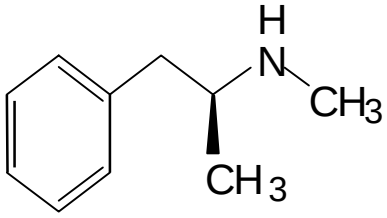
Ibuprofen
pK_a 4.4



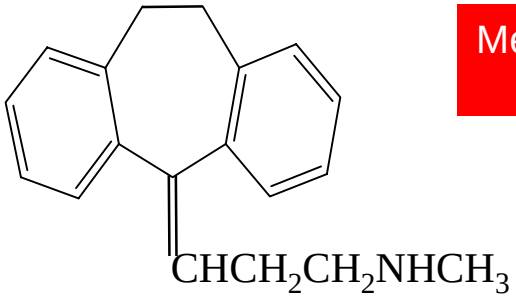
Benzoic Acid
pK_a 4.2



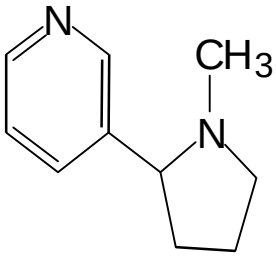
Amphetamine
pK_a 9.8



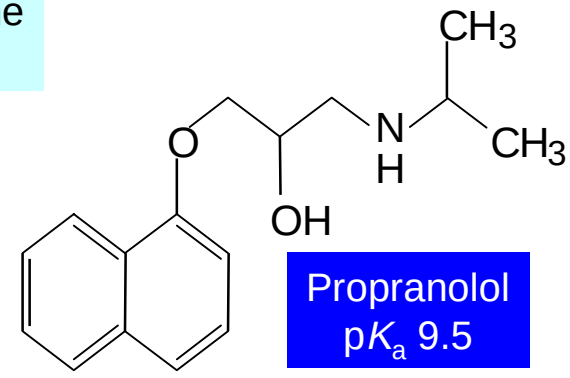
Methamphetamine
pK_a 9.5



Nortriptyline
pK_a 9.7



Nicotine
pK_{a1} 6.2, pK_{a2} 11



Propranolol
pK_a 9.5

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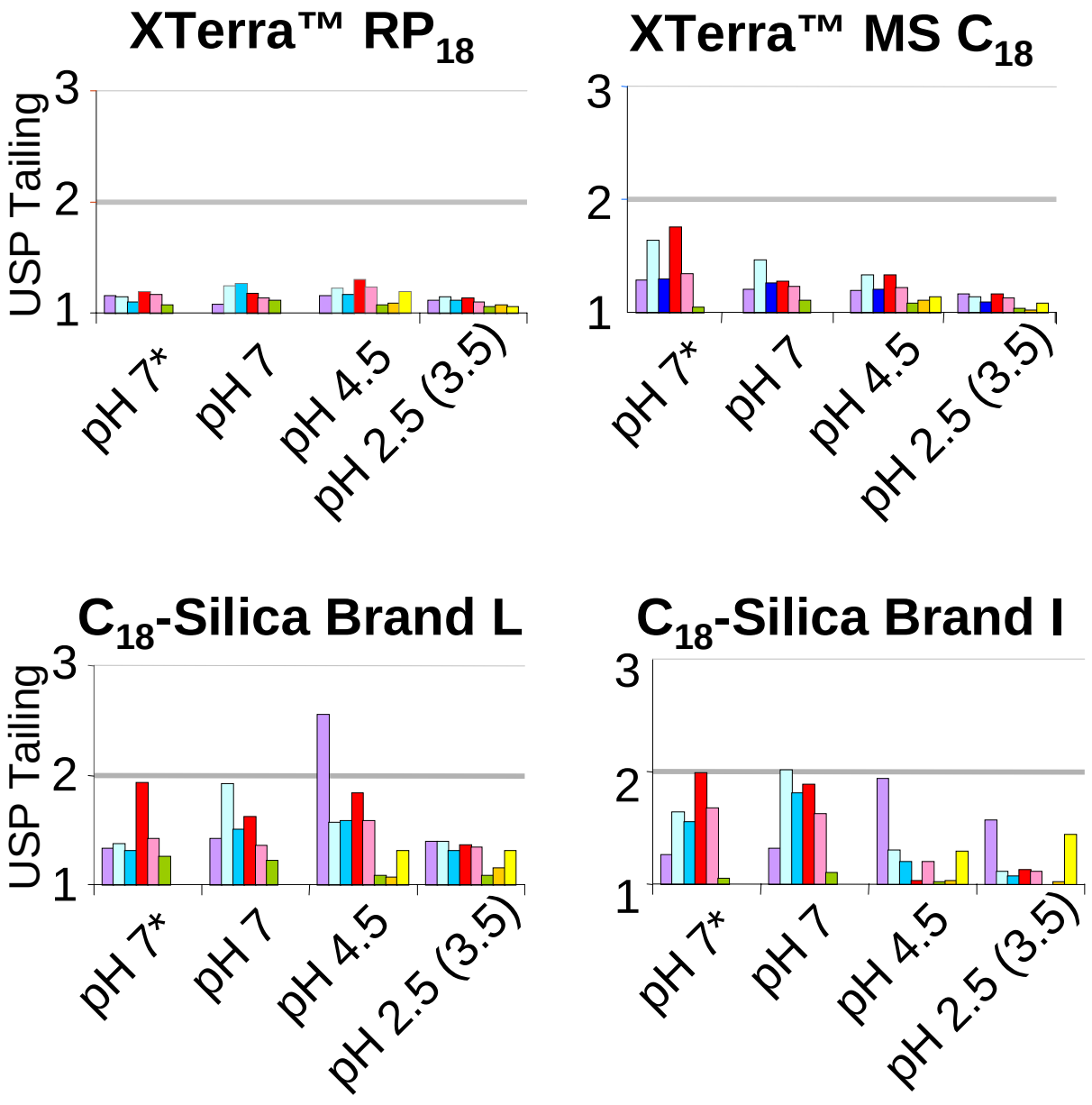
Comparison at 0.2µg Load
Effect of Mobile Phase pH on Tailing



MeCN mobile phase modifier except for pH 7* (MeOH)

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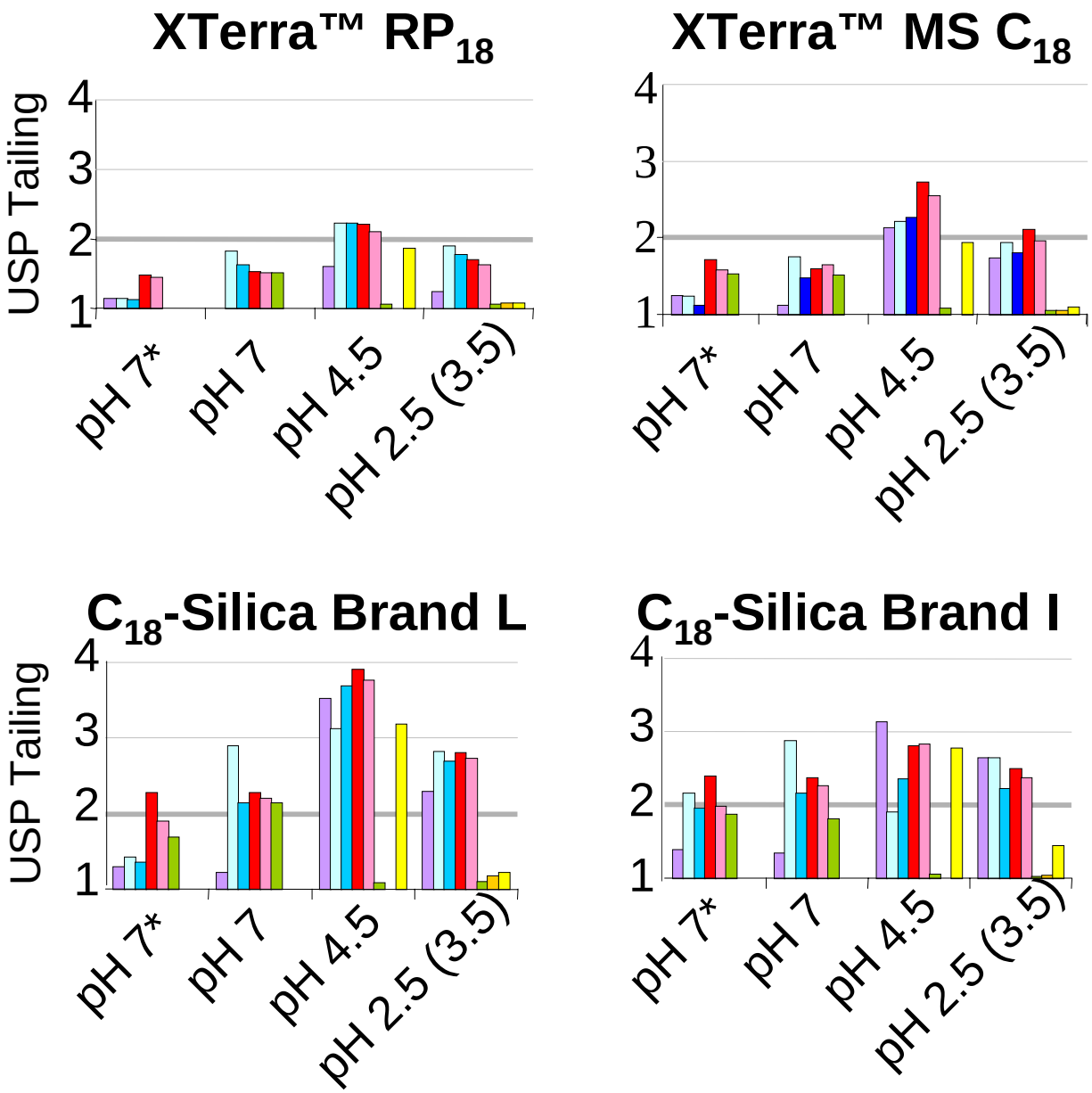
Comparison at 2µg Load
Effect of Mobile Phase pH on Tailing



MeCN mobile phase modifier except for pH 7* (MeOH)

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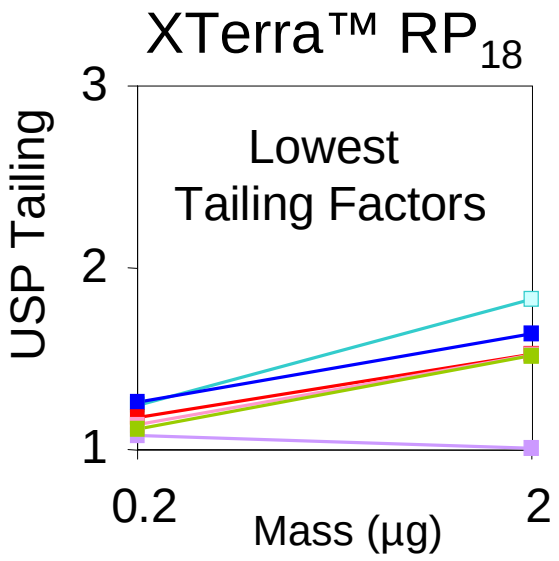
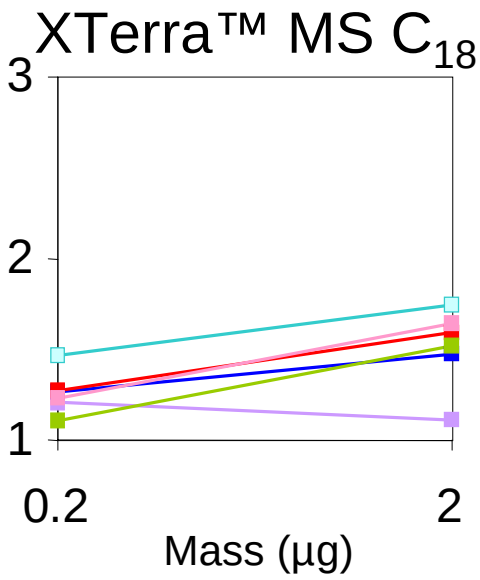
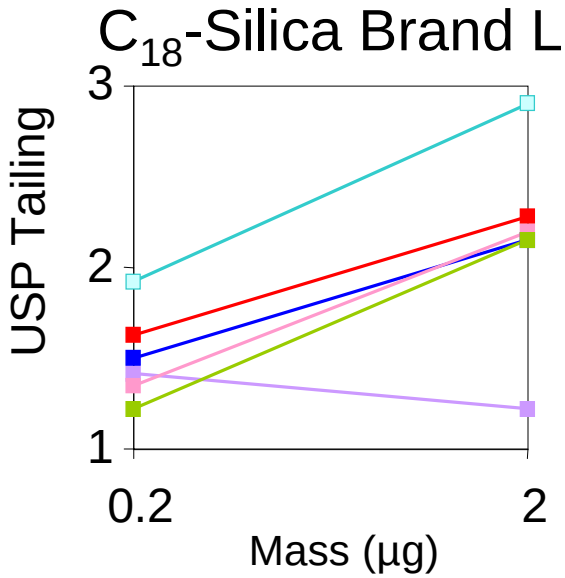
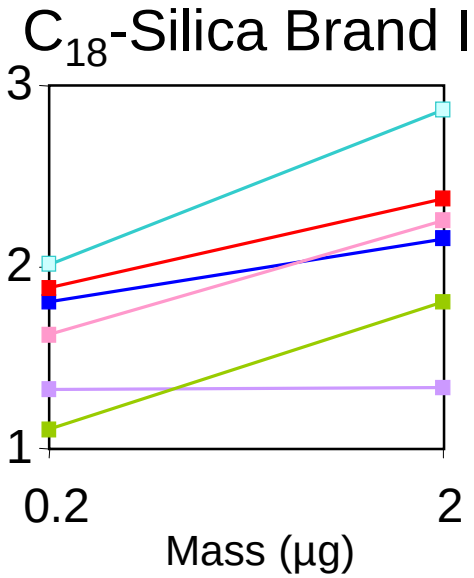
Effect of Mass Load on Tailing
pH 7/ MeCN Mobile Phases

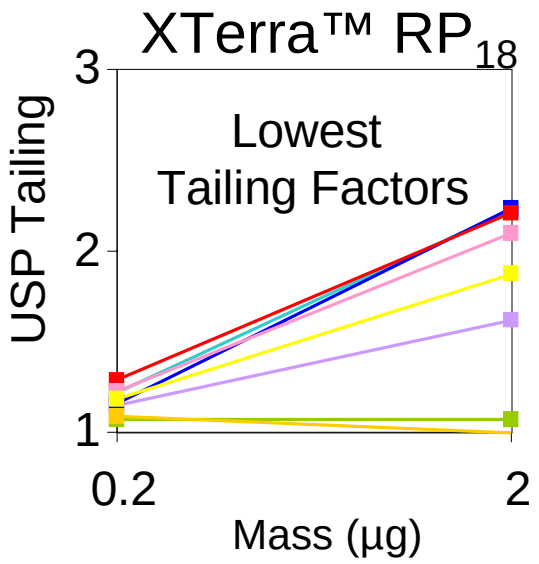


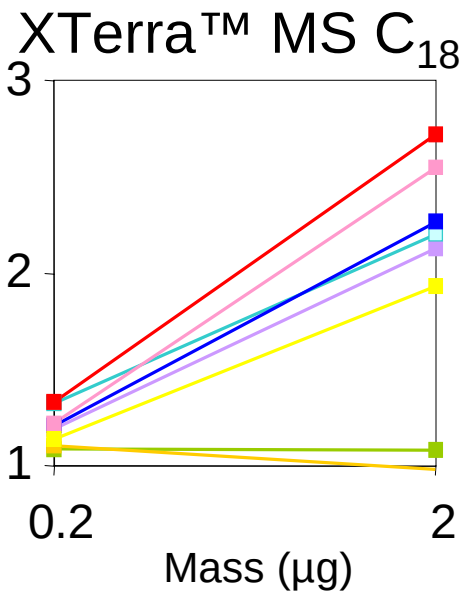


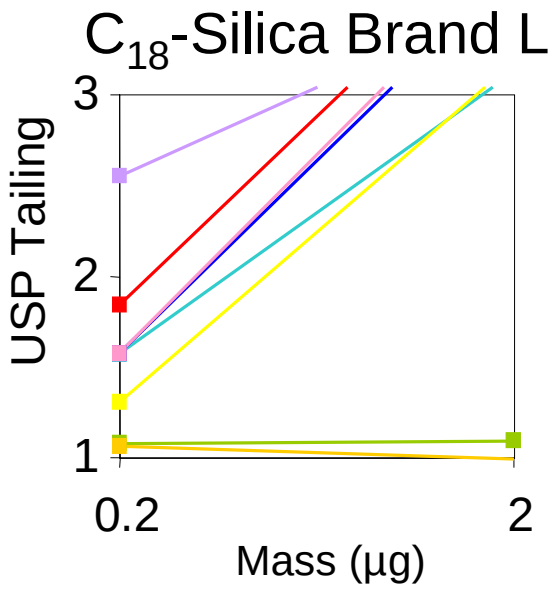


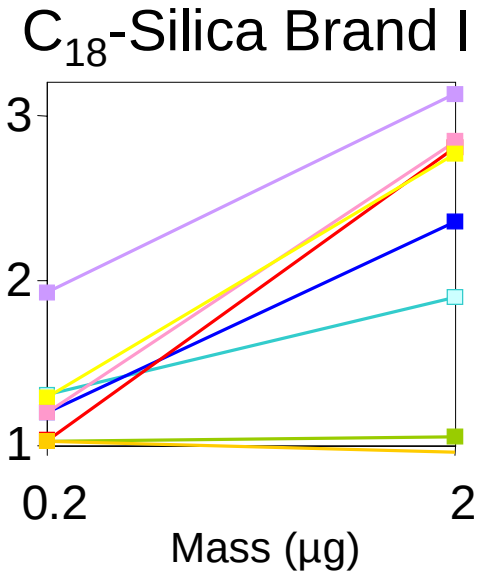


Effect of Mass Load on Tailing
pH 4.5/ MeCN Mobile Phases









Conclusions

The lowest average tailing factors were obtained:

- on XTerra™ RP_{18} across all mobile phase pH's
- at pH 2.5 (3.5) with 0.2 µg loads
- at pH 7 with methanol with 2 µg loads

Average USP Tailing Factors at 0.2µg Mass Load

C_{18} Packing	pH 7 MeOH	pH 2.5 (3.5) MeCN	pH 4.5 MeCN	pH 7 MeCN
XTerra™ RP_{18}	1.13	1.10	1.17	1.15
XTerra™ MS C_{18}	1.35	1.10	1.19	1.23
C_{18}-Silica Brand L	1.39	1.28	1.54	1.44
C_{18}-Silica Brand I	1.47	1.16	1.24	1.55

Average USP Tailing Factors at 2µg Mass Load

C_{18} Packing	pH 7 MeOH	pH 2.5 (3.5) MeCN	pH 4.5 MeCN	pH 7 MeCN
XTerra™ RP_{18}	1.06	1.42	1.76	1.44
XTerra™ MS C_{18}	1.35	1.57	1.95	1.44
C_{18}-Silica Brand L	1.59	2.08	2.86	1.99
C_{18}-Silica Brand I	1.85	1.92	2.18	1.99

Experimental Conditions: HPLC Mobile Phases

Organic:	%MeOH	%MeCN			
Buffer pH:	pH 7	pH 10	pH 7	pH 4.5	pH 2.5 or 3.5[1]
Nicotine[1]	32	23	7	0	0
Nortriptyline	68	60	35	35	30
Propranolol	50	43	27	20	20
Meth-amphetamine	14	-	12	7	7
Amphetamine	14	-	7	7	7
Ibuprofen	50	23	27	55	50
Benzoic Acid	-	0	-	20	20
Salicylic Acid	-	0	-	7	30

Experimental Conditions: HPLC Conditions

- Column Configuration: 4.6 x150 mm
- C_{18} Stationary Phases: 5 µm, high purity, low silanol activity, endcapped phases. Brands I and L are leading non-Waters brands.
- Flow Rate: 1.4 mL/min
- Temperature: 30 °C
- Injection Volume: 3 µL (0.2 µg), 30 µL (2 µg)
- Sample: 0.070 mg/mL analyte in 80/20 H_2O/MeOH (v/v)
- Void Volume: 0.016 mg/mL uracil determined in the mobile phase with the highest % organic
- Detection: 214nm, 220nm, or 254nm

Experimental Conditions: Buffers

- pH 10: Borate
  - 20 mM H_3BO_3
- pH 7: Phosphate
  - 20 mM K_2HPO_4
- pH 4.5: Acetate
  - 10 mM CH_3COONH_4
- pH 2.5 or 3.5: Phosphate
  - 20 mM KH_2PO_4

References:
David Victor McCalley, “Influence of sample mass on the performance of reversed-phase columns in the analysis of strongly basic compounds by high-performance liquid chromatography,” *Journal of Chromatography A*, 793 (1998) 31-46