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Presented at HPLC, Nice France, 15th-19th June 2003

OVERVIEW

Six 3 μ m state-of-the-art C18 silica packings were chromatographically evaluated using two different mobile phase conditions:

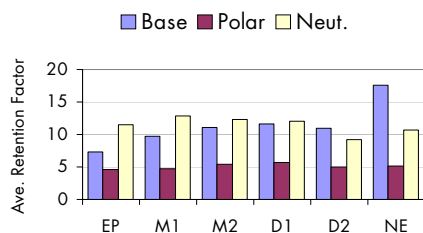
- 20mM K₂HPO₄, pH 7.0/methanol, 35:65 (v,v)
- 20mM formic acid (~0.1% FA), pH 2.7/acetonitrile, 90:10 or 72:28 (v,v)
(An extremely popular MS compatible mobile phase)

These mobile phases were selected from recent publications^{1,2}. The trends between efficiency and tailing factors were examined under the two test conditions. In the pH 7 test, tailing factors did not correlate with efficiency because, in addition to other not clearly understood factors, the efficiencies were significantly affected by the higher than optimum flow rates used in the test. Recently defined selectivity parameters were calculated for each of the packings¹. The peak shape data was examined for correlations between efficiencies or tailing factors and the selectivity parameters. No correlations were found.

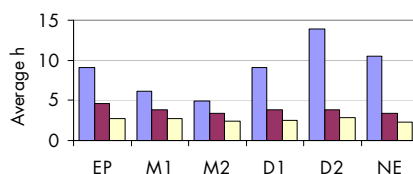
Although the optimum flow rate was selected for the pH 2.7 test, tailing factors still did not correlate with efficiency. Tailing factors continued to increase at flow rates below the optimum on the M1 packing. On the D1 packing efficiency and tailing remained constant across the entire flow rate range (0.1-0.7mL/min). The observed differences are attributed to differences in loading capacity.

DATA SUMMARY

pH 7 Retention and h

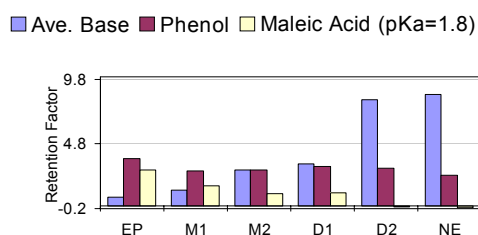


Similar retention for all solutes except for the bases on the non-encapped (NE) and EP materials.

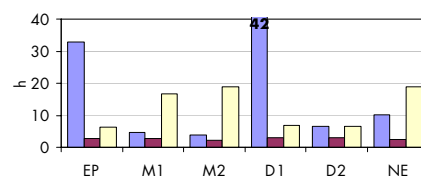


Basic and polar solutes showed elevated reduced plate height (h) due to flow rate selection.

pH 2.7 Retention and h



Similar k' for phenol but very different k' for bases. High k' for bases trended with low k' for MA to the point of exclusion.

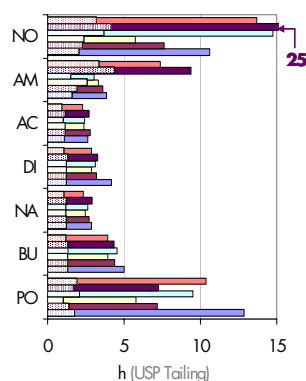


Similar h for phenol but very different h for bases. The D2 packing had good k' for bases with good h.

[1] U. Neue, K. Tran, P. Iraneta, B. Alden, *J. Sep. Sci.* 2003, 26, 174-186
 [2] D.V. McCalley, *J. Chrom. A* 2003, 987, 17-28
 [3] D.V. McCalley, *J. Chrom. A* 1998, 793, 31-46

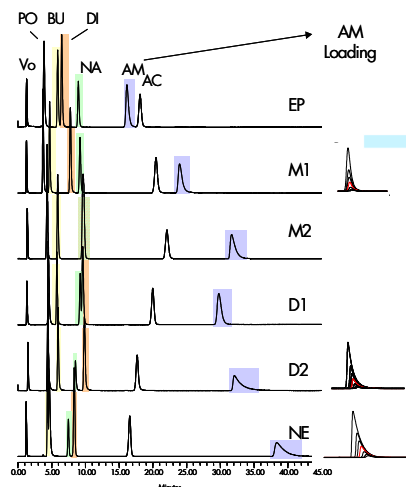
pH 7.0: 20mM K₂HPO₄/MeOH EVALUATION DATA

pH 7 Reduced Plate Height



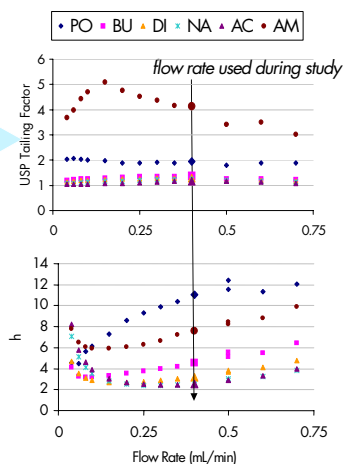
EP M1 M2 D1 D2 NE

These experimental conditions were selected for historical purposes. Ideally the test should be performed at ~0.1 mL/min with much lower on-column mass loading.



Can peak shape for ionized solutes be predicted based on the selectivity differences?

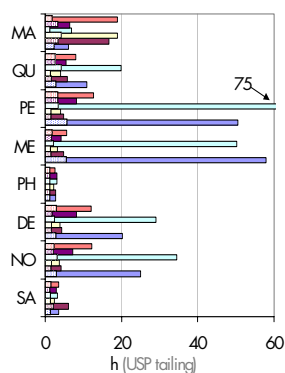
Effect of Flow Rate on Tailing and h



The flow rate was selected based on the minimum h for AC. However, the optimum flow rate for the bases and polar solutes was ~0.1 mL/min.

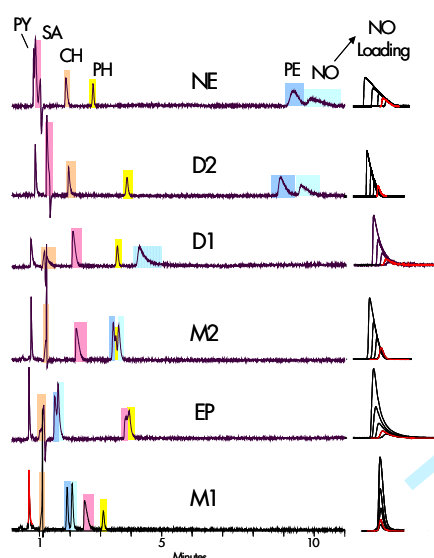
pH 2.7: 20mM FORMIC ACID/MeCN EVALUATION DATA

pH 2.7 Reduced Plate Height



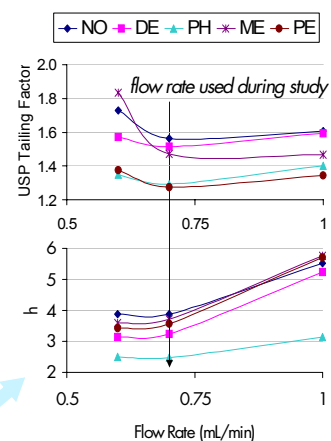
EP M1 M2 D1 D2 NE

Solutes (PE and ME) containing a piperazine ring gave particularly high h on 2 out of the 6 packings. Although only 54ng (except 540ng for ME) was injected on-column, mass overload occurred on 4 out of the 6 packings.



Mass overload was the major cause of poor peak shape.

Effect of Flow Rate on Tailing and h



The optimum flow of 0.7 mL/min was selected for this pH using the M1 packing. However, this was one of the few packings that did not suffer from mass overload.

MATERIALS KEY

Code	Solutes	Chemical Features	mobile phase (pH)
PO	propranolol	Ether, OH, 2°amine	65%MeOH(7)
BU	butylparaben	Ester, phenol	65%MeOH (7)
NA	naphthalene	Neutral	65%MeOH (7)
DI	dipropylphthalate	Di-ester	65%MeOH (7)
AC	acenaphthene	Neutral	65%MeOH (7)
AM	amitriptyline	3°amine	65%MeOH (7)
NO	nortriptyline	2°amine	65%MeOH (7)
SA	salicylic acid	Phenol, acid	28%MeCN (2.7)
NO	nortriptyline	2°amine	28%MeCN (2.7)
DE	desipramine	2° and weak 3°amine	28%MeCN (2.7)
PH	phenol	Phenol	28%MeCN (2.7)
ME	meclizine	Piperazine	28%MeCN (2.7)
PE	perphenazine	Piperazine, phenothiazine	28%MeCN (2.7)
MA	maleic acid	Di-acid	28%MeCN (2.7)
QU	quinine	Ether,OH, 3°amine, quinoline	10%MeCN (2.7)

Code	Bonding	End-cap	%C	SSA m ² /g	Dp μ m
NE	mC18	no	10	180	3.50
D2	Low coverage dC18	yes	12.1	323	3.00
D1	High coverage dC18	yes	17.3	402	2.99
M2	High coverage mC18	yes	15.9	338	3.50
M1	High coverage mC18	yes	20.2	344	3.49
EP	Embedded polar group (EPG) C18	yes	17.7	339	3.36 _{pH7} 3.57 _{pH3}

METHOD

- 20mM K₂HPO₄/KH₂PO₄, pH 7/MeOH, 35:65(v,v)
 - Instruments:
 - Waters 2690XE, Waters 2487 DUV with high pressure flow cell, NesLab RTE-111 Circulating Bath
 - Chromatographic conditions:
 - Flow Rate: 0.4mL/min, Temp: 23.4°C, Detection: 254nm, 0 Filter TC, 10pps, 6 μ L injection volume, on-column mass load was 0.6 μ g (AM), 1.2 μ g (AC), 2.1 μ g (DI), 0.36 μ g (NA), 0.12 μ g (BU), 2.4 μ g (PO)
 - Columns:
 - 3x100mm, nominal 3 μ m packings, efficiencies reported as tangent at 61% inflection
- 20mM formic acid, pH2.7/MeCN, 72:28 or 90:10 (v,v)
 - Instruments:
 - Waters 2690XE, Waters 996 PDA with a μ bore flow cell, Waters 2690XE column heater
 - Chromatographic conditions:
 - Flow Rate: 0.7mL/min, Temp: 30.0°C, Detection: 213-254nm, 0 Filter TC, 10pps, 0.9 μ L injection, on-column mass load was 54ng for all solutes except meclizine for which 9 μ L (540ng) was used
 - Columns:
 - 3x150mm, nominal 3 μ m packings, efficiencies reported at 1/2ht
 - Loading studies
 - 0.030, 0.54, 0.108, 0.216, 0.432 μ g on column
- Data acquisition and processing
 - Millennium³² V3.2
 - All retention times and efficiencies were corrected for extra-column volume and dispersion
 - $t_{Rcorr} = t_{Rmeas} - (\text{extra-column volume})/(\text{flow rate})$
 - $N_{1/2ht\ corr} = 5.54(t_{Rcorr})^2/(W_{0.5}^2 - W_{0.5sys}^2)$
 - $N_{tang\ corr} = 16(t_{Rcorr})^2/(W_{tang}^2 - W_{tangsys}^2)$

Reduced Plate Height

$$h = \frac{\text{column length (cm)} * 10,000}{\text{Efficiency} * \text{particle diameter } (\mu\text{m})}$$

CONCLUSION

1. In this study, the causes for poor peak shape were found to be flow rate selection, silanol activity and/or mass overloading. With these C18 packings, differences in mass loading capacity was a significant contributor to poor peak shape.
2. Strong retention for ionizable solutes was often associated with poor loading capacity and poor peak shape. The ideal packing would have high k' and h .
3. The impact of flow rate on the peak shape for solutes could help differentiate between the possible sources of poor peak shape. Additional studies are needed.
4. At pH 2.7, the retention behavior of acids and bases indicate that the particle surface is ionized and that the distribution of surface charge ranges from predominantly negative for the materials with high retention for the bases (and exclusion of maleic acid) to predominantly positive on the packings with low retention for the bases, but significant retention for maleic acid.
5. The poor peak shape observed for the pH 2.7 FA mobile phase is not typical of different pH 2.7 buffer systems². The importance of avoiding mass overload in peak shape evaluations has previously been noted³. Overloading occurred at unexpectedly low mass loads and for some of the packings no measurable mass load could be found that did not overload the packings even when k' was reduced to the point of exclusion.

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