Comparison of the Chromatography of Octadecyl Silane Bonded Silica and Polybutadiene Coated Zirconia Phases Based on a Diverse Set of Cationic Drugs

Xiqin Yang, Jun Dai, Peter W. Carr, University of Minnesota,
Department of Chemistry, 207 Pleasant Street SE, Minneapolis, MN 55455 USA

Robert T. Moody, MAC-MOD Analytical, Inc.,
103 Commons Court, Chadds Ford, PA 19317 USA

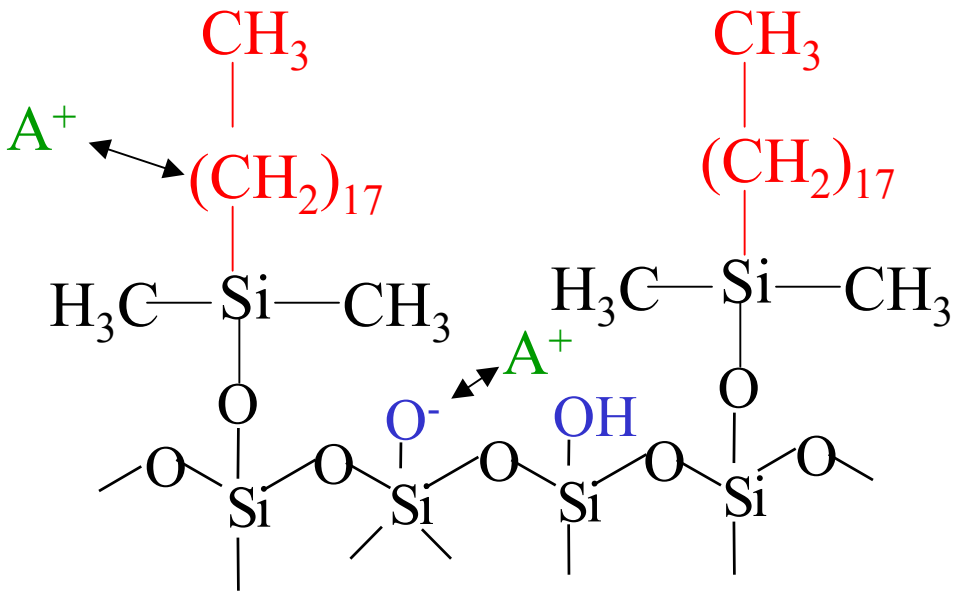
Outline

- Mixed-mode retention on silica and zirconia based stationary phases
  - Reversed-phase vs. ion-exchange interactions

- Comparison of the chromatography on ODS and PBD-ZrO_2 based on a diverse set of cationic drugs
  - Retention of basic drugs at pH 6.0
  - Retention of basic drugs at pH 3.0
  - Comparison of columns based on K-K plot
  - Comparison of columns based on plate count and symmetry factor

- Conclusions
  - Very different selectivity of PBD-ZrO_2 vs. ODS
  - Column performance depends on both condition and solute

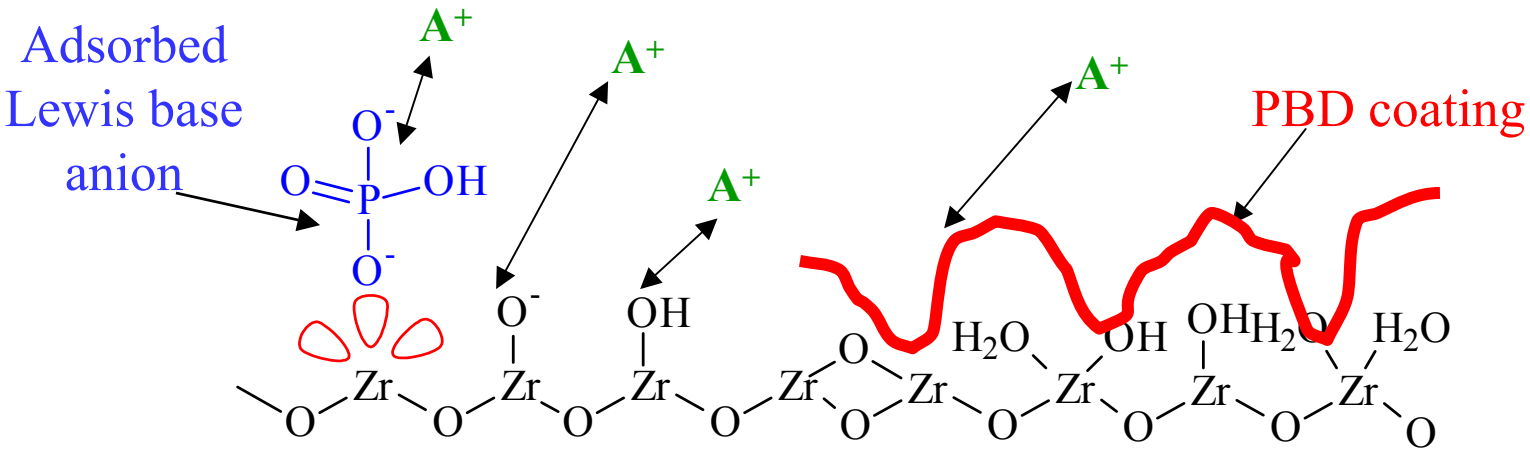
Mixed-Mode Separation on ODS Phases



- Bonded C_{18} Chains — Reversed-Phase (RP) Interactions
- Ionized Silanol Groups — Ion-Exchange (IEX) Interactions

✓ Mixed-mode retention mechanism!

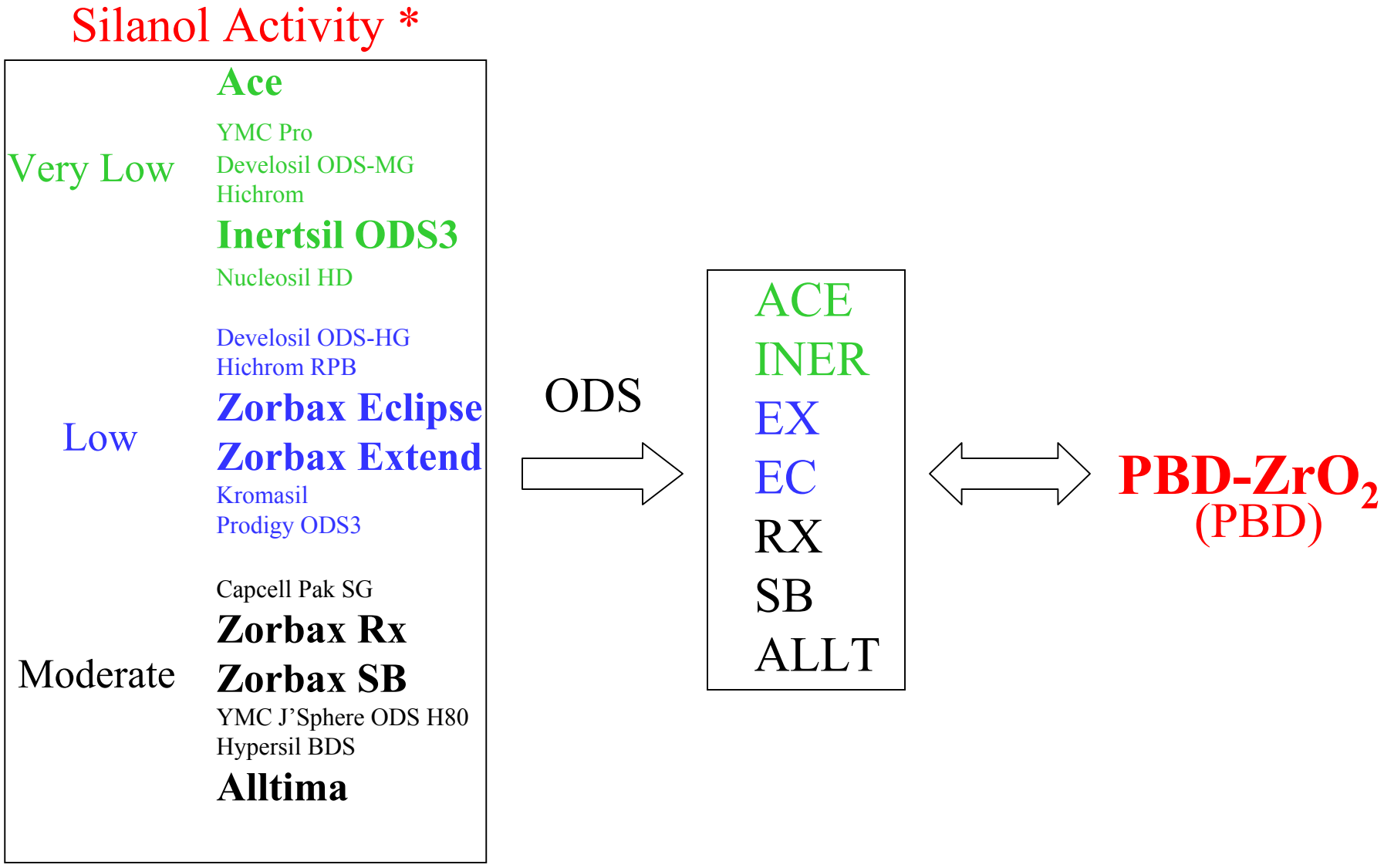
Mixed-Mode Separation on PBD-ZrO_2



- PBD Coating — Reversed-Phase (RP) Moieties
- Lewis Base Anions — Ion-Exchange (IEX) Sites

✓ Mixed-mode retention mechanism!

Silanol Activity Ranking of Stationary Phases



* Adapted from Mac-Mod Analytical, Inc. "*Column Comparison Guide*", based on plate count of amitriptyline

Characteristics of the Stationary Phases

Column[a]	Designation	Surface Area (m^2/g)	Pore Size (Å)	Carbon Content (% w/w)	Endcapped
ACE	ACE	300	100	15.5	Yes
Zorbax Eclipse	EC	186	80	10	Yes
Inertsil ODS-3	INER	436	95	14.7	Yes
Zorbax Extend	EX	179	80	10	Yes
Zorbax SB	SB	180	80	10	No
Alltima	ALLT	350	100	16	Yes
Zorbax RX	RX	172	80	10	No
PBD-ZrO_2	PBD	11.2 [b]	500	2.5	No

[a] Data provided by the manufacturers unless noted otherwise.
[b] Data obtained by BET.

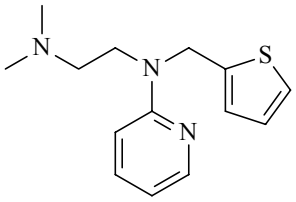
Comparison of Different RPLC Columns for Ion-Exchange and Reversed Phase Interactions

Column	k'_{am}	k'_{ac}	N_{am}	Rank 1	As_{am}	Rank 2	k'_{am}/k'_{ac}	Rank 3
PBD	5.16	0.88	52,600	1	0.9	3	5.89	8
ACE	1.9	3.76	50,700	2	0.99	1	0.51	2
EC	2.32	4.21	49,600	3	0.81	4	0.55	3
INER	3.02	5.46	37,500	4	0.95	2	0.55	4
EX	2.07	4.38	35,600	5	0.67	5	0.47	1
SB	3.2	3.25	34,300	6	0.6	6	0.99	7
ALLT	4.51	5.26	17,800	7	0.34	7	0.86	6
RX	2.97	4.09	11,000	8	0.31	8	0.73	5

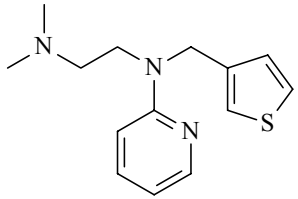
Conditions: 80/20 MeOH/25mM ammonium phosphate buffer, pH 6, temperature ambient (about 28 °C)
am = amitriptyline ac = acenapthalene

✓**Different parameter gives different column ranking.**

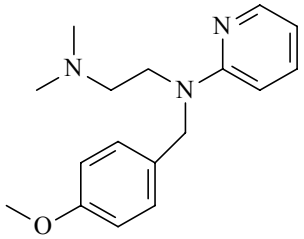
Antihistamines



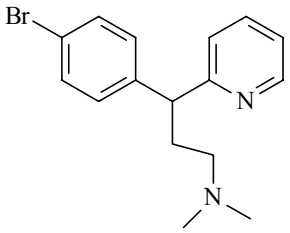
Methapyrilene
(3.7, 8.9)



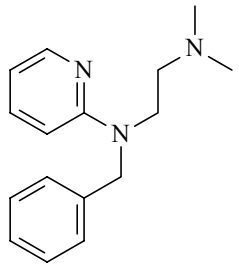
Thenyldiamine
(3.9, 8.9)



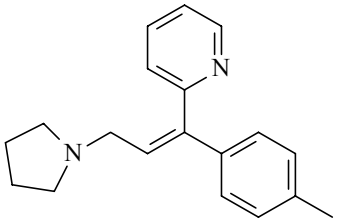
Pyrilamine
(4.0, 8.9)



Brompheniramine
(3.6, 9.8)

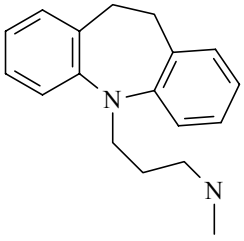


Tripelennamine
(4.2, 8.7)

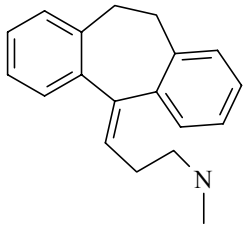


Triprolidine
(6.6)

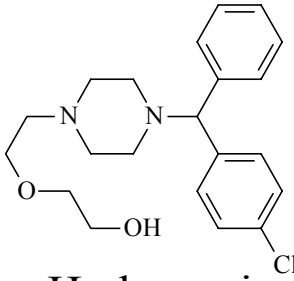
Antidepressants



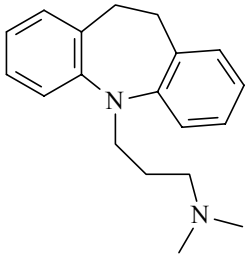
Desipramine
(10.4)



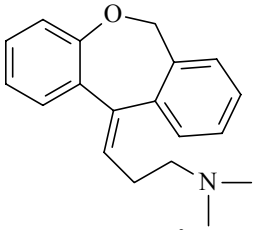
Nortriptyline
(9.7)



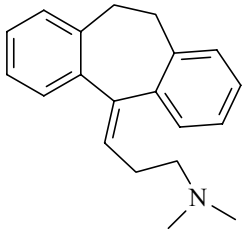
Hydroxyzine
(2.0, 7.1)



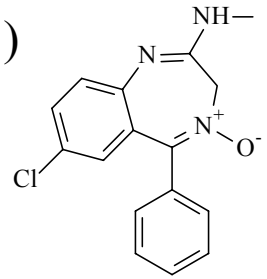
Impramine
(9.5)



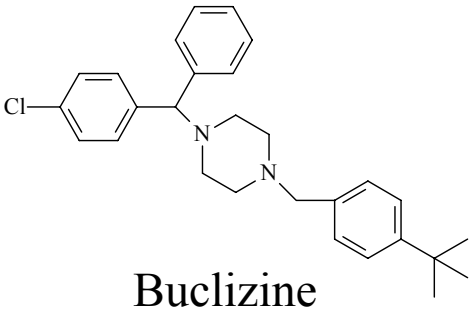
Doxepin
(9.0)



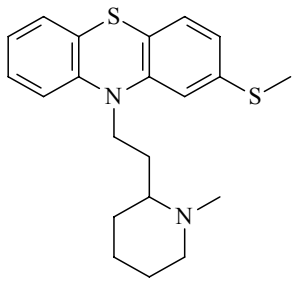
Amitriptyline
(9.4)



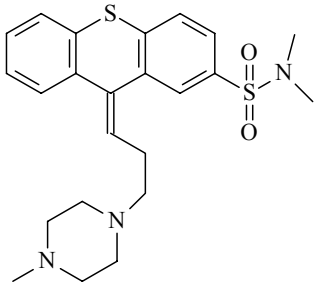
Chlordiazepoxide
(4.8)



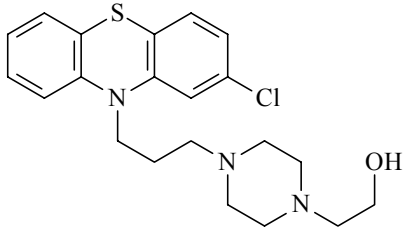
Buclizine
(3.1, 6.2)



Thioridazine
(9.5)

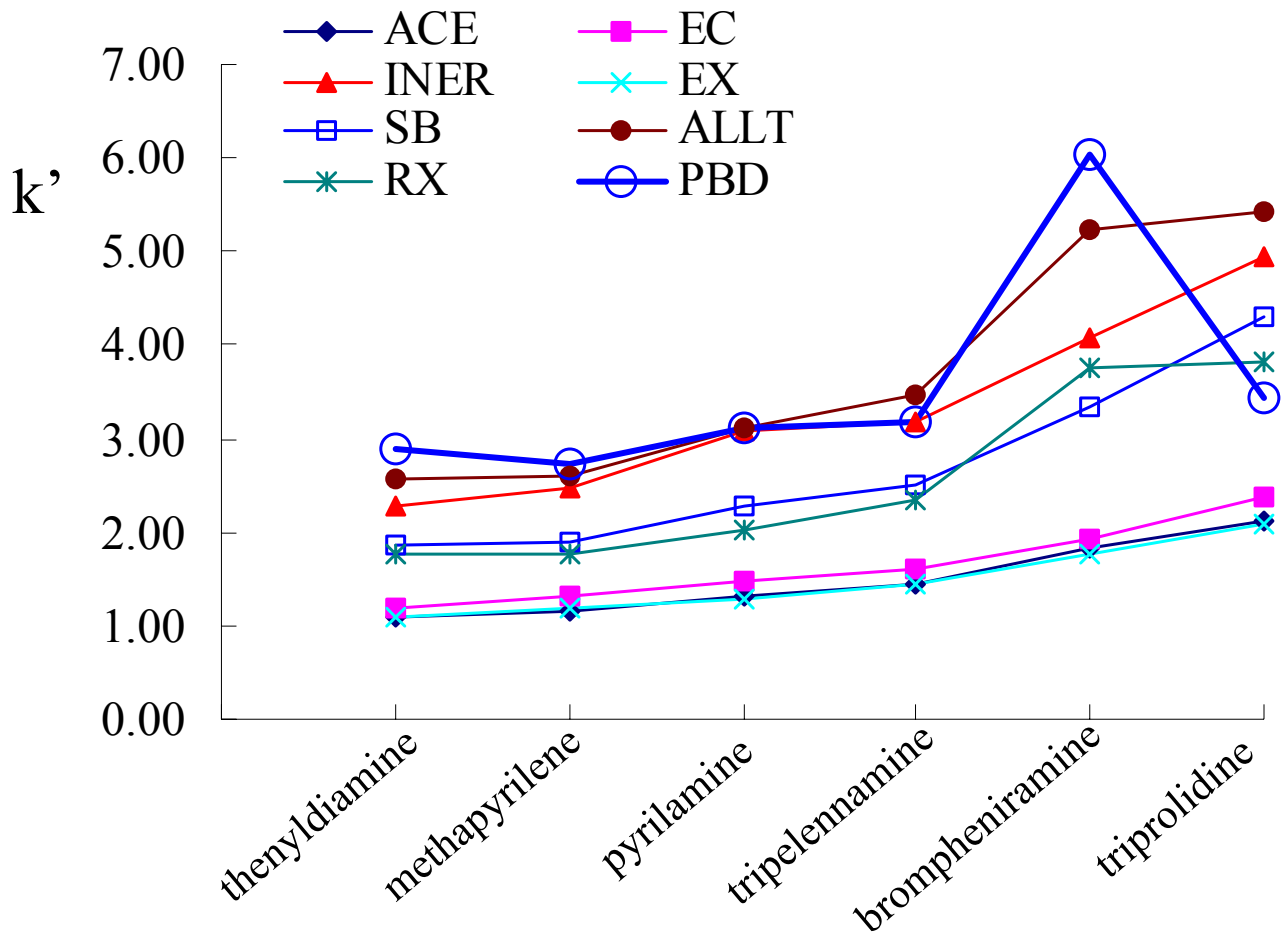


Thiothixene
(7.7, 7.9)



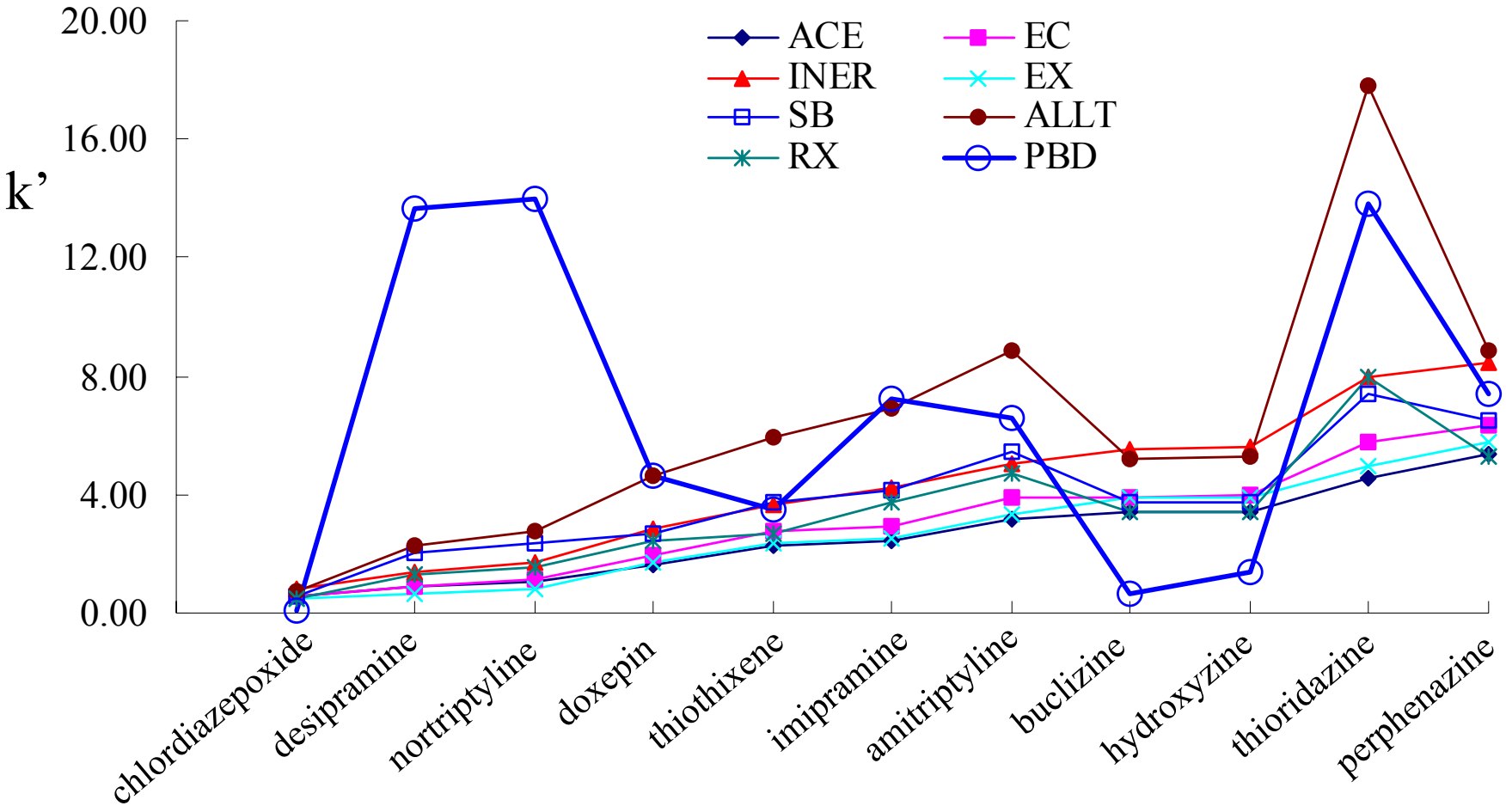
Perphenazine
(3.7, 7.8)

Comparison of Antihistamine Separation on Different RPLC Phases



MeOH / 25 mM ammonium phosphate buffer (60/40, v/v, pH = **6.0**), T = 35 °C

Comparison of Antidepressant Separation on Different RPLC Phases



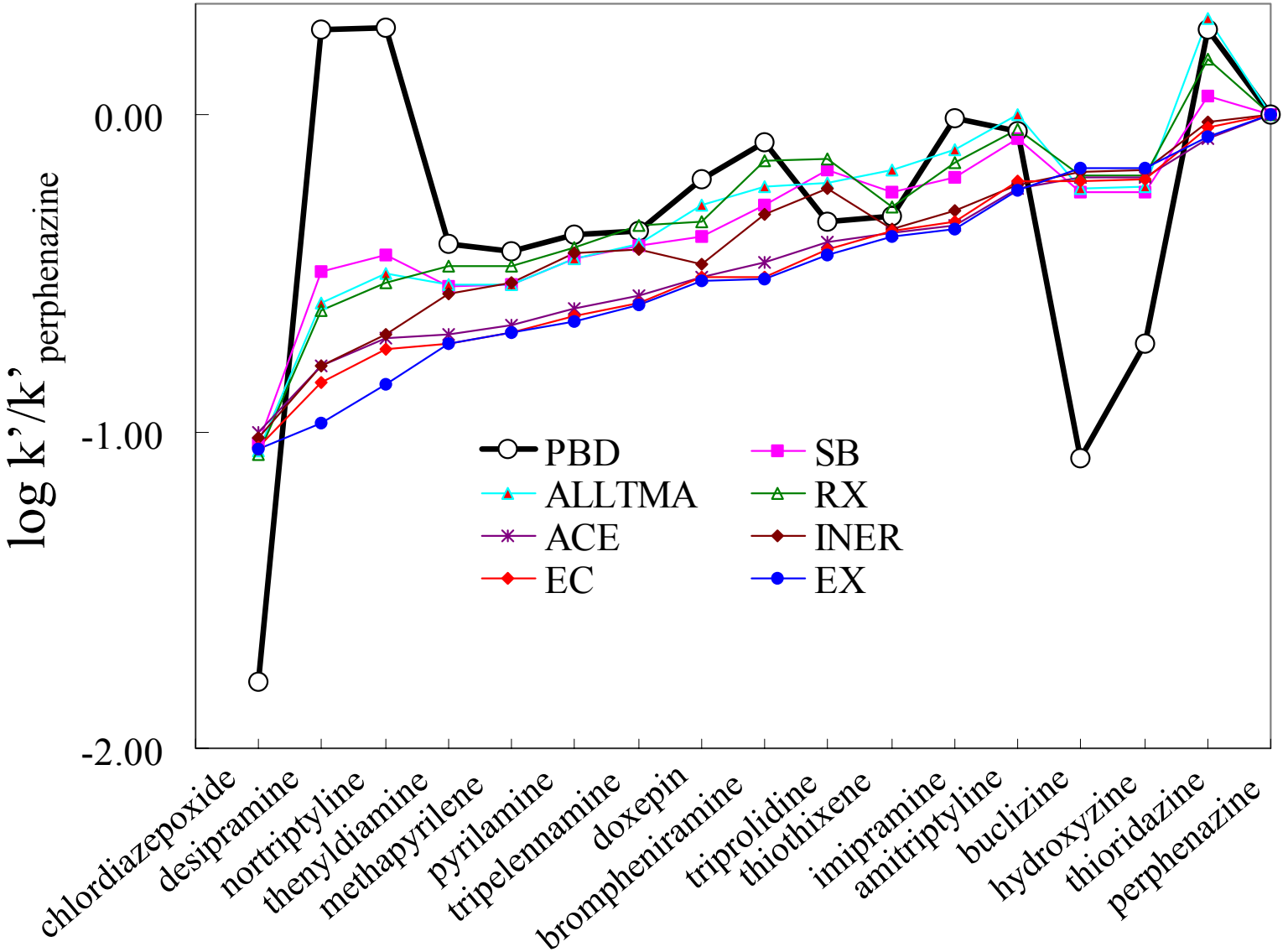
MeOH / 25 mM ammonium phosphate buffer (72/28, v/v, pH = **6.0**), T = 35 °C

Selectivity Comparison of Antidepressant on Different RPLC Phases

Column	ACE	EC	INER	EX	SB	ALLT	RX	PBD
$\alpha_{2/1}$	1.64	1.60	1.70	1.20	3.49	2.94	2.84	114
$\alpha_{3/2}$	**1.22**	**1.27**	**1.26**	**1.33**	**1.13**	**1.24**	**1.22**	**1.02**
$\alpha_{4/3}$	1.56	1.69	1.66	2.14	1.15	1.64	1.56	0.33
$\alpha_{5/4}$	1.38	1.39	1.30	1.38	1.39	1.30	1.12	0.76
$\alpha_{6/5}$	1.05	1.07	1.14	1.05	1.11	1.16	1.38	2.05
$\alpha_{7/6}$	1.31	1.33	1.20	1.33	1.31	1.29	1.26	0.91
$\alpha_{8/7}$	1.08	1.00	1.10	1.17	0.68	0.58	0.72	0.09
$\alpha_{9/8}$	**1.00**	**1.01**	**1.01**	**1.01**	**1.00**	**1.01**	**1.00**	**2.30**
$\alpha_{10/9}$	1.34	1.47	1.42	1.25	2.01	3.38	2.34	9.86
$\alpha_{11/10}$	1.19	1.10	1.06	1.17	0.87	0.50	0.66	0.54
Median	1.27	1.30	1.23	1.22	1.23	1.47	1.39	2.17
Maximum	1.64	1.69	1.70	2.14	3.49	3.38	2.84	114
Minmum	1.00	1.00	1.01	1.01	1.00	1.01	1.00	1.02

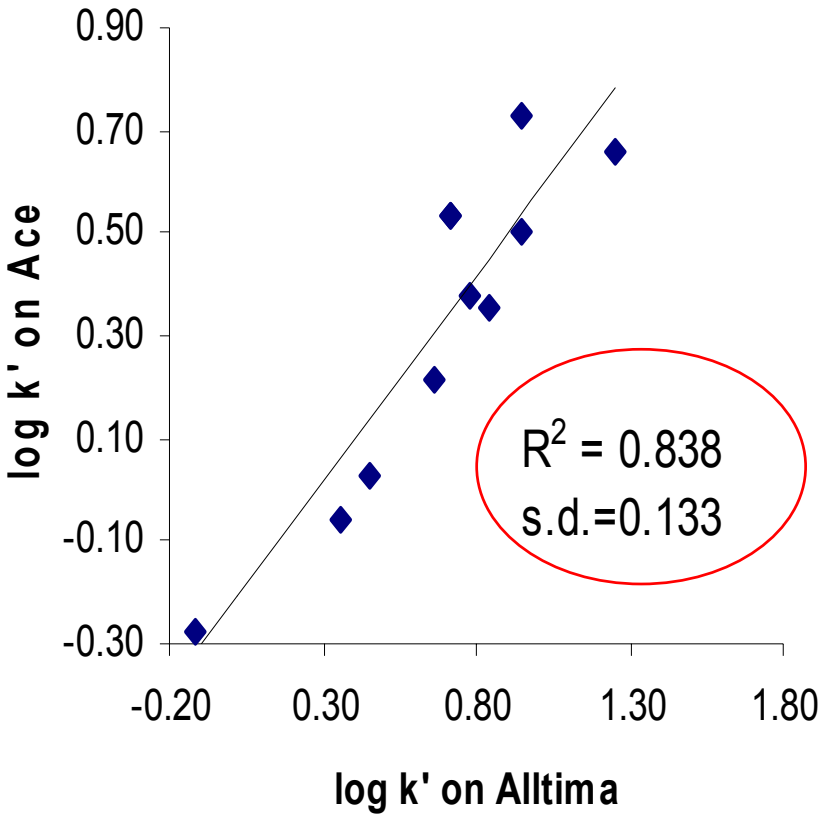
MeOH / 25 mM ammonium phosphate buffer (72/28, v/v, pH = **6.0**), T = 35 °C
11 antidepressant are ordered according to the k' on ACE column

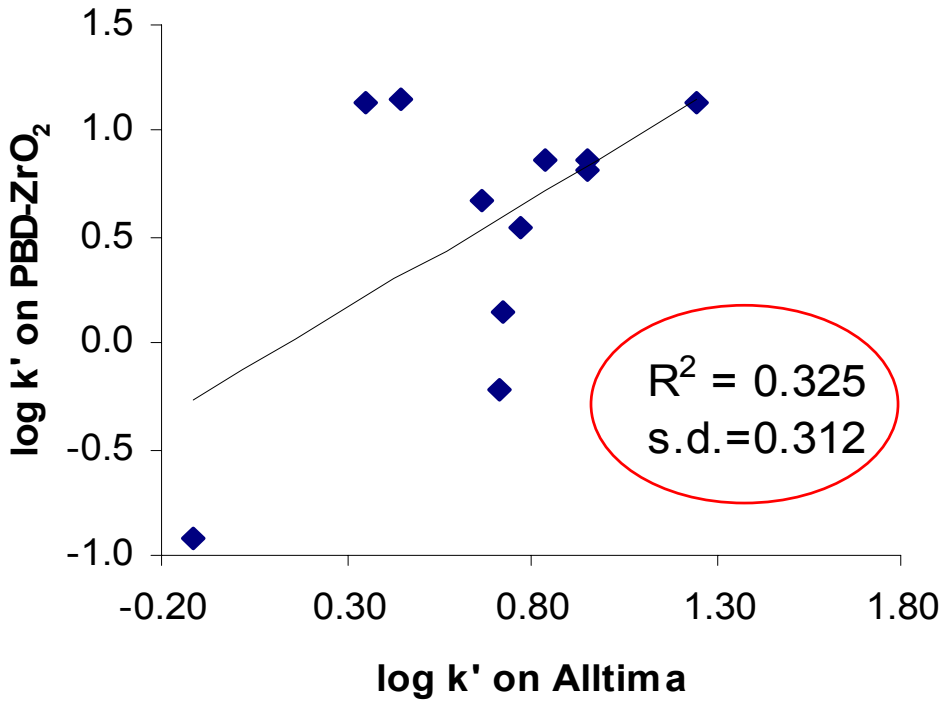
Comparison of Selectivity via Relative Retention



✓**PBD-ZrO_2 is very different.**

K-K Plot (log k' vs. log k') on Different Columns



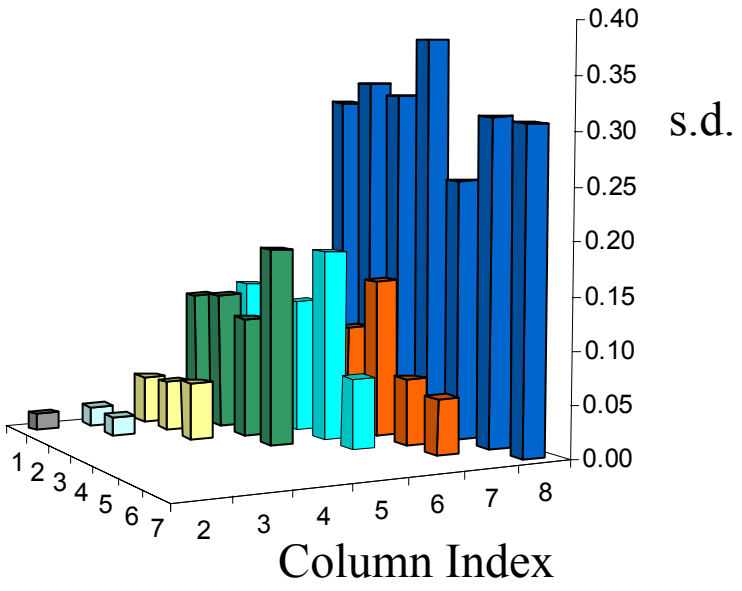


MeOH / 25 mM ammonium phosphate buffer (72/28, v/v, pH = **6.0**), T = 35 °C
11 antidepressant.

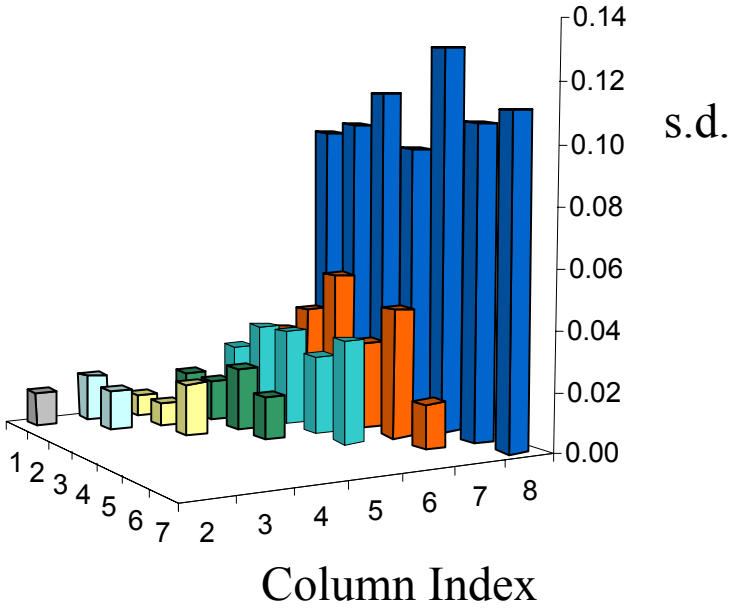
- ✓ **Poor correlations in the κ–κ plot indicate changes in selectivity.**
- ✓ **PBD-ZrO_2 is really different.**

Comparison of the Standard Error of K-K Plots

Antidepressant



Antihistamines



MeOH / 25 mM ammonium phosphate buffer (72/28, v/v, pH = **6.0**),T = 35 °C, 11 antidepressants.

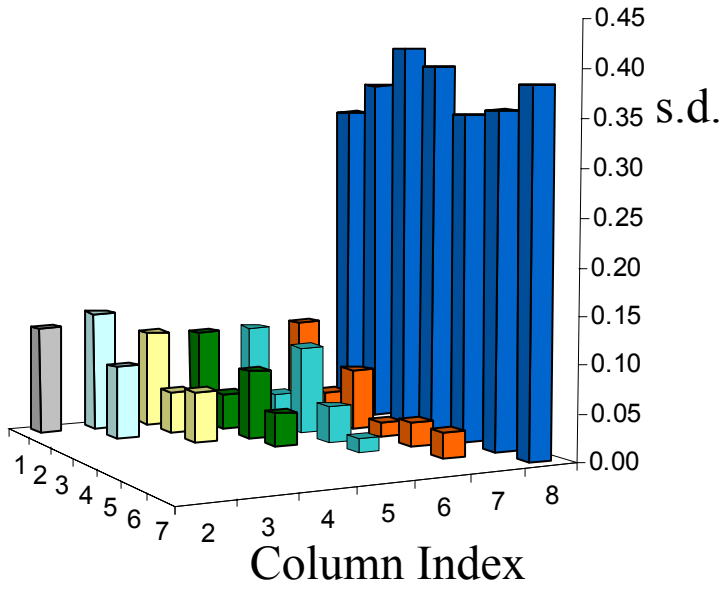
MeOH / 25 mM ammonium phosphate buffer (60/40, v/v, pH = **6.0**),T = 35 °C 6 antihistamines.

Column index, 1 = ACE, 2 = EC, 3 = INER, 4 = EX, 5 = SB, 6 = ALLT, 7 = RX, and 8 = PBD.

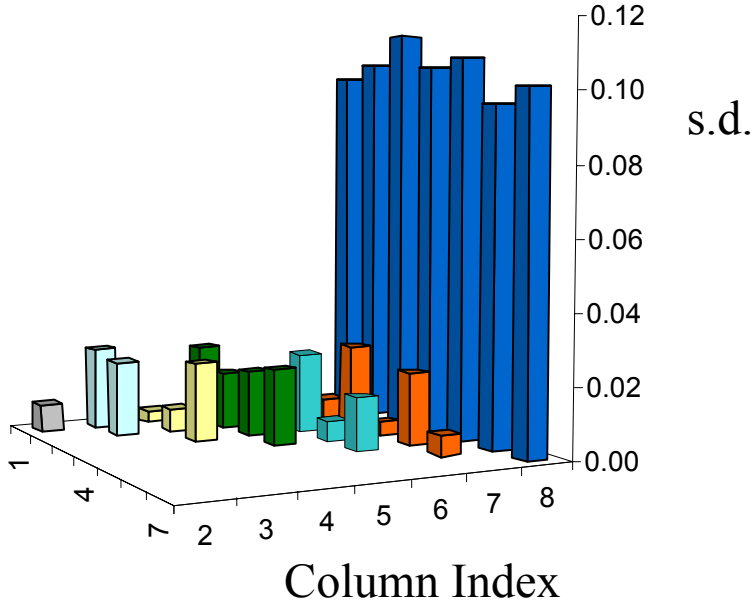
✓**The very large s.d. for PBD-ZrO_2 vs. all other phases indicates a dramatic difference in selectivity from ODS.**

Comparison of the Standard Error of K-K Plots

Antidepressant



Antihistamines

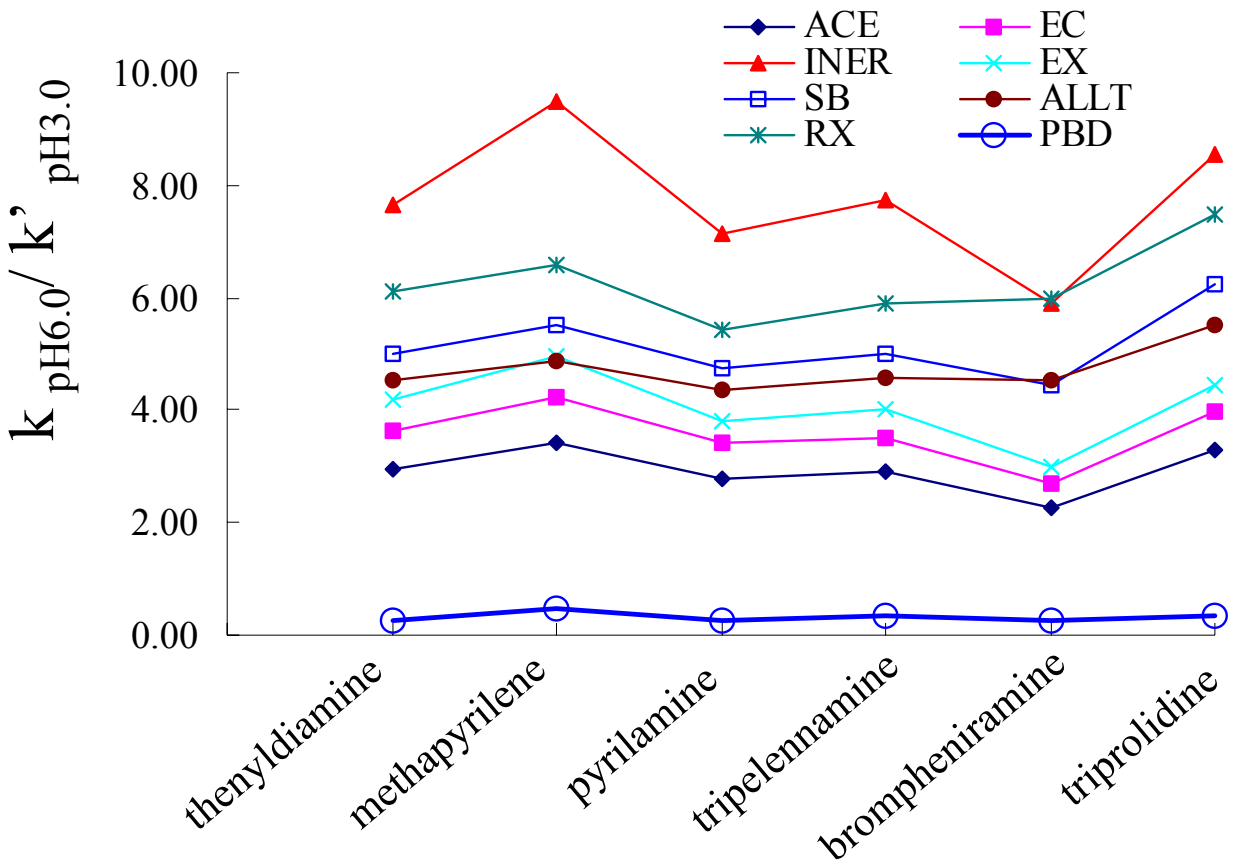


MeOH / 25 mM ammonium phosphate buffer (72/28, v/v, pH = **3.0**),T = 35 °C, 11 antidepressants.

MeOH / 25 mM ammonium phosphate buffer (60/40, v/v, pH = **3.0**),T = 35 °C 6 antihistamines.

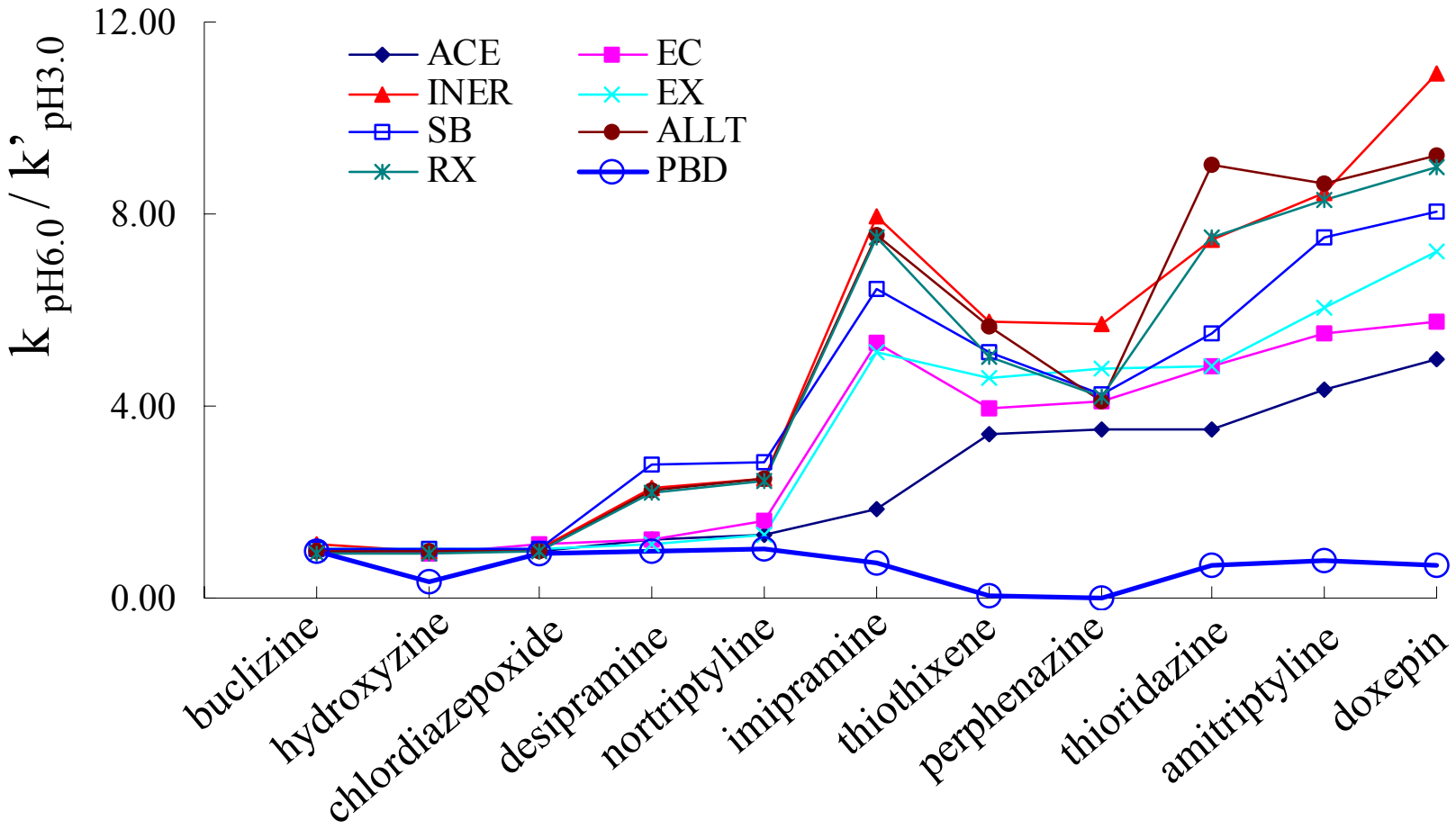
Column index, 1 = ACE, 2 = EC, 3 = INER, 4 = EX, 5 = SB, 6 = ALLT, 7 = RX, and 8 = PBD.

Comparison of Antihistamine Retention Under Different pH Conditions



MeOH / 25 mM ammonium phosphate buffer (60/40, v/v), T = 35 °C

Comparison of Antidepressant Retention Under Different pH conditions



MeOH / 25 mM ammonium phosphate buffer (72/28, v/v), T = 35 °C

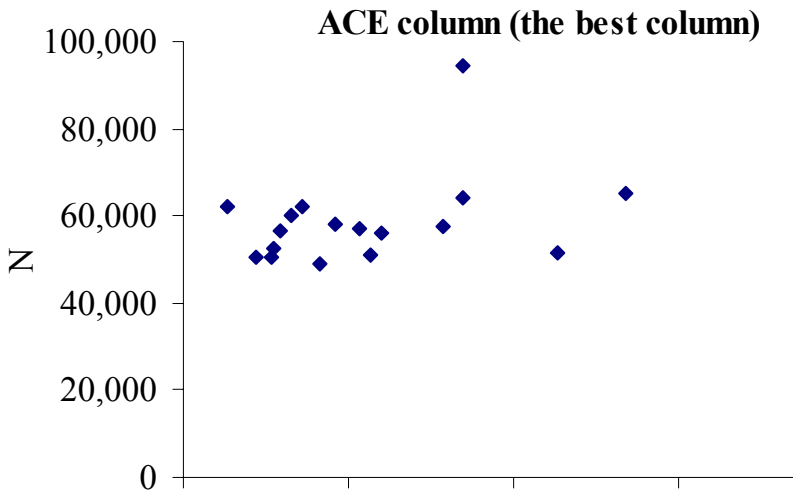
Comparison of Normalized Plate Count

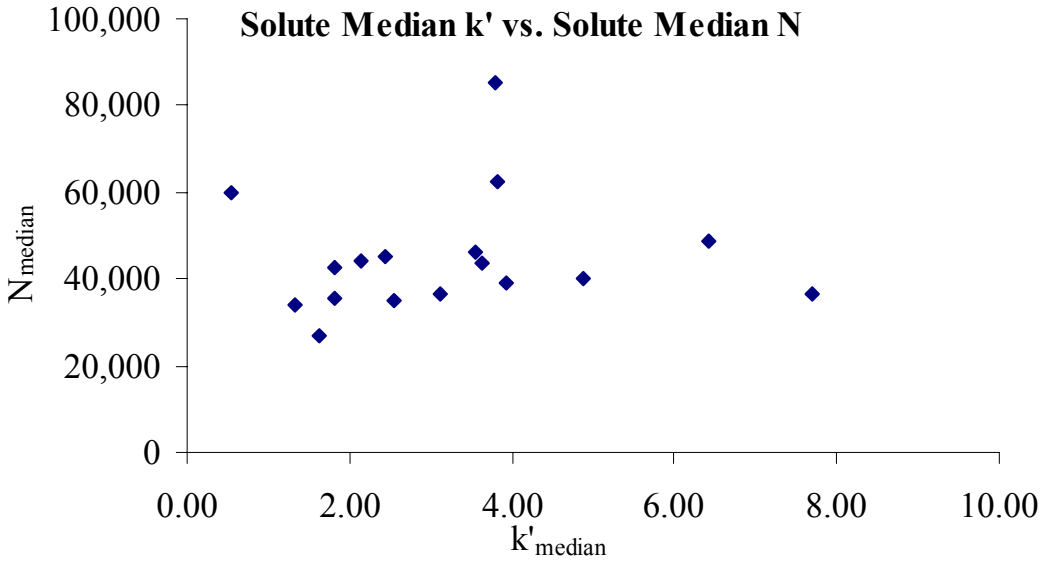
Solute	Median per meter	Normalized Plate Count, $N/N_{solutemedian}$							
		ACE	EC	INER	EX	SB	ALLT	RX	PBD
$N_{solutemedian}$ / $N_{columnmedian}$		57,300	56,000	42,400	40,550	33,300	18,900	9,900	51,300
nortriptyline	26,700	1.90	1.67	0.88	0.99	1.01	0.39	0.50	2.12
desipramine	34,050	1.49	1.34	0.72	0.86	1.14	0.38	0.30	1.60
doxepin	34,800	1.41	1.40	1.05	0.95	0.96	0.56	0.40	1.04
thenyldiamine	35,650	1.48	1.45	1.25	0.75	0.59	0.53	0.13	1.58
thiothixene	36,400	1.40	1.40	0.98	1.11	0.93	0.65	0.19	1.02
thioridazine	36,600	1.41	1.49	1.05	0.93	0.95	0.19	0.13	1.29
imipramine	39,000	1.44	1.42	1.01	0.92	0.99	0.46	0.30	1.32
amitriptyline	39,900	1.44	1.39	1.04	0.96	0.70	0.33	0.25	1.29
methapyrilene	42,450	1.33	1.33	1.04	0.77	0.68	0.96	0.08	1.35
triprolidine	43,500	1.32	1.33	0.97	1.03	0.19	0.25	0.03	1.13
pyrilamine	44,350	1.35	1.34	0.97	1.03	0.48	0.40	0.09	1.18
tripelennamine	45,400	1.37	1.35	1.01	0.99	0.43	0.34	0.10	1.24
brompheniramine	46,100	1.26	1.21	0.89	0.94	0.13	1.15	0.02	1.06
perphenazine	48,750	1.33	1.29	0.95	1.05	1.07	0.81	0.77	0.31
chlordiazepoxide	59,750	1.04	1.14	0.81	0.95	1.07	0.96	1.04	0.66
hydroxyzine	62,450	1.03	0.97	1.12	0.82	1.38	1.36	0.84	0.70
buclizine	85,400	1.11	1.10	0.85	1.01	1.04	0.93	0.99	0.67

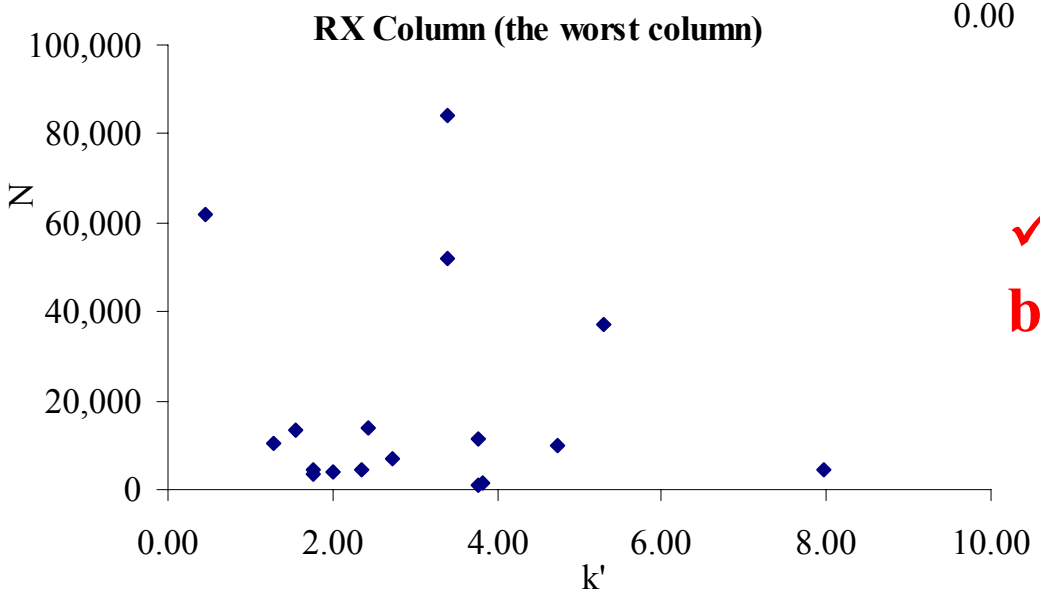
Red: N below $N_{solutemedian}$ (median for that solute on 8 columns); blue: best N of that solute.

✓No universal trend in plate count among the columns involved in the present study is observed. Column performance is solute dependent.

Plot of Retention Factor vs. Plate count







✓**There is no clear relationship between k' and N!**

Comparison of Asymmetry Factor

Solute	Median	Absolute Value of (1-As)							
		EC	ACE	INER	EX	SB	RX	ALLT	PBD
$(1\text{-As})_{median}$		**0.17**	**0.19**	**0.21**	**0.31**	**0.47**	**0.56**	**0.61**	**0.05**
buclizine	**0.06**	0.02	**0.08**	**0.09**	**0.08**	**0.00**	0.02	0.03	**0.21**
hydroxyzine	**0.06**	**0.01**	0.04	**0.09**	**0.07**	0.02	0.02	**0.08**	**0.09**
perphenazine	**0.13**	**0.02**	0.13	0.04	0.10	0.12	**0.23**	**0.37**	**0.66**
chlordiazepoxide	**0.15**	0.09	**0.19**	**0.07**	**0.18**	0.11	0.14	0.15	**0.52**
pyrilamine	**0.23**	**0.01**	**0.01**	**0.24**	0.22	**0.54**	**0.53**	**0.62**	0.07
thiothixene	**0.24**	0.17	**0.27**	0.16	0.20	**0.44**	**0.63**	**0.40**	**0.02**
tripelennamine	**0.28**	0.03	**0.00**	0.24	**0.31**	**0.55**	**0.56**	**0.68**	0.01
doxepin	**0.28**	0.19	**0.17**	0.25	**0.31**	**0.43**	**0.66**	**0.42**	0.25
amitriptyline	**0.30**	0.28	0.23	0.16	**0.31**	**0.54**	**0.71**	**0.70**	**0.06**
triprolidine	**0.32**	0.14	0.14	**0.35**	0.28	**0.76**	**0.65**	**0.72**	**0.09**
thioridazine	**0.39**	0.36	0.32	0.05	**0.41**	**0.41**	**0.70**	**0.63**	**0.05**
methapyrilene	**0.39**	0.30	0.25	0.21	**0.47**	**0.49**	**0.63**	**0.70**	**0.01**
brompheniramine	**0.41**	**0.04**	0.11	**0.47**	0.34	**0.69**	**0.57**	**0.69**	0.05
thenyldiamine	**0.44**	0.36	0.32	**0.03**	**0.51**	**0.61**	**0.67**	**0.73**	0.05
desipramine	**0.44**	**0.46**	0.25	0.41	**0.47**	0.36	**0.52**	**0.58**	**0.03**
nortriptyline	**0.45**	0.41	0.27	0.44	**0.46**	**0.67**	**0.48**	**0.61**	**0.02**
imipramine	**0.46**	0.46	0.35	0.30	**0.48**	**0.47**	**0.54**	0.46	**0.02**

Red: As below As $_{solutemedian}$ (median for that solute on 8 columns); blue: best As of that solute.

Conclusions

1. Very different selectivity of PBD-ZrO_2 and ODS phases towards basic solutes
2. Column ranking is very condition and solute dependent
3. pH has very dramatic effect on the retention basic compounds
4. PBD-ZrO2 is a good alternative to ODS phase
5. Column ranking based on one or two solutes is not reliable

Acknowledgments

National Institute of Health

University of Minnesota

Alltech Associates, Inc

Agilent Technologies

Mac-Mod Analytical

ZirChrom Separations, Inc