Selecting an Ion Pairing Reagent for Anion Separation

Tips for Developing a Successful Method Using Ion Pairing Reagents

- An endcapped ODS (octadecylsilyl) column is the most common column for ion pair separations and should be tried first.
- Use only HPLC-grade water and chromatography solvents in mobile phase preparation.
- If non-ionic components are present in the sample, optimize the resolution of those components prior to attempting ionic separations.
- Select the appropriate ion pair reagent to provide the necessary counter ion. Use the Q-series for acidic compounds and the sulfonates for basic compounds.
- Through experimentation, find the alkyl chain length which results in the best separation [figure 2].
- Once the reagent has been selected, adjust the pH of the mobile phase to maximize resolution. Because slight modifications of pH can profoundly affect retention and selectivity, make all adjustments in small increments and monitor carefully. [table 2].
- Ideally, the ion pair reagent concentration in the mobile phase should be 0.005 M. However, small adjustments in reagent concentration may increase retention slightly and optimize the separation.

When choosing the proper reagent, alkyl chain lengths must be taken into consideration. The chain lengths enable selective separation of the analyte. The longer the chain, the more hydrophobic the counter ion, and therefore, greater the retention. The retention may increase by a factor of 12 when going from hexyl (Q6) to dodecyl (Q12), as illustrated in figure 1 and table 1. Both figure 1 and table 1 demonstrate that the Q-reagent chain length governs benzoic acid retention times but does not affect the benzyl alcohol retention times.

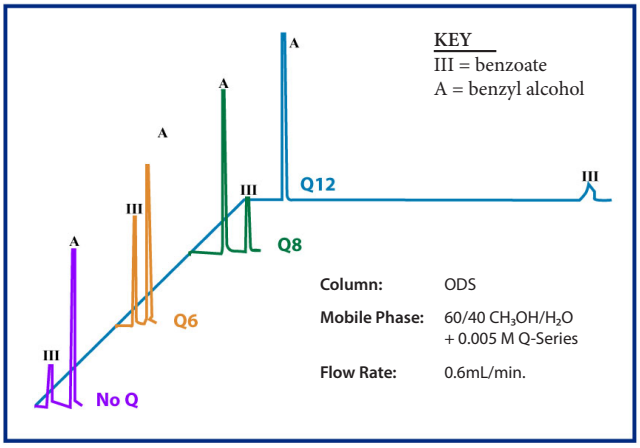

Figure 1: Effect of Q-reagent chain length on retention

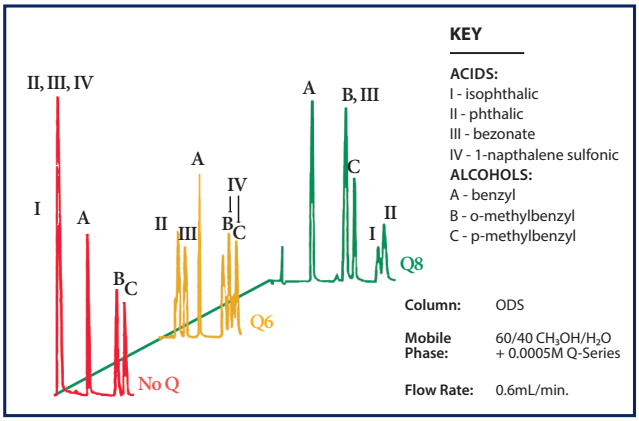

Figure 2: Choosing appropriate ion pairing reagents

Retention Times (min)		Retention Ratio	
Q-Series	Benzoic Acid	Benzyl Alcohol	Acid / Alcohol
Q6	6.50	8.60	0.76
Q8	12.36	8.94	1.38
Q12	79.53	8.52	9.33

Table 1: Retention vs. chain length

Benzoic acid / benzyl alcohol in (60/40) CH_3OH/H_2O			
Q6		Q8	
pH	R	pH	R
7.50	0.59	7.51	1.06
6.50	0.70	6.54	1.29
5.50	0.96	5.50	1.59

Table 2: Retention ratio R as a function of pH

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Ordering Information

PRODUCT	SIZE	CATALOG #
Q-SERIES ION PAIR CONCENTRATES FOR ANIONS		
Ion Pair Concentrate Q6	100 mL	1-404036-200
Ion Pair Concentrate Q8	100 mL	1-404038-200
Ion Pair Concentrate Q12	100 mL	1-404031-200
ION PAIR REAGENTS FOR ANIONS		
TBAP (Tetrabutylammonium Phosphate)	50 mL	1-680505-200
TBAP (Tetrabutylammonium Phosphate)	500 mL	1-680503-200
TBAP (Tetrabutylammonium Phosphate)	1 L	1-680504-200
ION PAIR REAGENTS FOR CATIONS		
1-Pentanesulfonate	25 g	1-403025-200
1-Pentanesulfonate	100 g	1-403125-200
1-Pentanesulfonate	1000 g	1-403325-200
1-Hexanesulfonate	25 g	1-403026-200
1-Hexanesulfonate	100 g	1-403126-200
1-Hexanesulfonate	500 g	1-403226-200
1-Hexanesulfonate	1000 g	1-403326-200
1-Heptanesulfonate	25 g	1-403027-200
1-Heptanesulfonate	100 g	1-403127-200
1-Heptanesulfonate	1000 g	1-403327-200
1-Octanesulfonate	25 g	1-403028-200
1-Octanesulfonate	100 g	1-403128-200
1-Octanesulfonate	1000 g	1-403228-200
HFIP USED IN CONJUNCTION WITH TEA FOR OLIGONUCLEOTIDE SEPARATION		
HFIP (Hexafluoro-2-Propanol)	10 g	1-270701-200
HFIP (Hexafluoro-2-Propanol)	25 g	1-270702-200
HFIP (Hexafluoro-2-Propanol)	100 g	1-270704-200

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