Ultrapure Ion Pairing Reagents and Buffers

Ion Pair Chromatography is a method for improving the separation of charged analytes. In the resolution of organic ions with conventional HPLC methods, use of ion pair reagents can enhance peak shape and retention time when common remedies such as modifying eluent ratios or changing stationary phase fail.

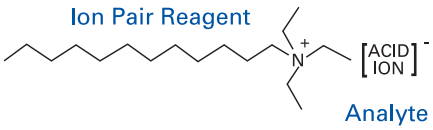

Figure 1. Quaternary Amine (Q-Series) Ion Pair Reagent.

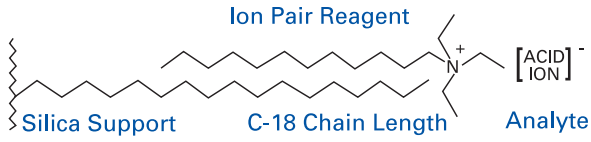

Figure 2. Quaternary Amine (Q-Series) Ion Pair Reagent interacting with C-18 Support.

The Advantages of Ion Pair Chromatography

In the past, chromatographic separation of charged analytes has been achieved by ion suppression (the careful adjustment of the mobile phase pH to result in a nonionized analyte). Determining the optimum mobile phase pH in ion suppression, however, often requires extensive method development. Samples containing more than one ionizable component were often unusable. The imitations of ion suppression led to the development of a new, more generally applicable approach to separation of ionized components: *ion pair chromatography*.

Developed by Dr. Gordon Schill in 1973, ion pair chromatography relies upon the addition of ionic compounds to the mobile phase to promote the formation of ion pairs with charged analytes. These reagents are comprised of an alkyl chain with an ionizable terminus (figure 1). When used with common hydrophobic HPLC phases in the reversed-phase mode, ion pair reagents can be used to selectively increase the retention of charged analytes (figure 2).

Although ion exchange chromatography has become a popular mode of separation, it is not useful in all situations. The advantages of ion pair chromatography over ion exchange chromatography are:

- Simple preparation of buffers
- Wide choice of carbon chain lengths for improved retention and separation
- Significantly reduced separation time
- Simultaneous separation of both ionized and nonionized solutes
- Highly reproducible results
- Improved peak shape

Regis Provides a Choice of Reagents

Regis manufactures both ultrapure anionic Sulfonate (S-Series) and cationic Quaternary Amine (Q-Series) ion pair concentrates in the following alkyl chain lengths: pentyl, hexyl, heptyl, octyl, and dodecyl. Alkyl chains are represented by cardinal numbers in the naming of our products, i.e., 5, 6, 7, 8, and 12. (See product descriptions on the following pages.)

Ultrapure Ion Pairing Reagents and Buffers

Optical Absorbance (AUFS) S-Series	200 nm	210 nm
S5	0.006	0.002
S6	0.048	0.018
S7	0.008	0.001
S8	0.001	0.003
S12	0.002	0.003
CH_3CN	0.076	0.013
CH_3OH	0.940	0.510

Optical Absorbance (AUFS) Q-Series	200 nm	210 nm
Q5	0.060	0.001
Q6	0.059	0.006
Q7	0.022	0.009
Q8	0.082	0.003
Q12	0.102	0.013

Table 1. Typical optical absorbances (AUFS) at 0.005 M.

Q-Series	Retention Times (min) Benzoic Acid	Benzyl Alcohol	Retention Ratio Acid/ Alcohol
Q5	4.53	9.17	0.49
Q6	6.50	8.60	0.76
Q7	8.24	9.13	0.90
Q8	12.36	8.94	1.38
Q12	79.53	8.52	9.33

Table 2. Retention vs. chain length.

[benzoic acid/benzyl alcohol in (60/40) water/ methanol]					
Q6		Q7		Q8	
pH	R	pH	R	pH	R
7.50	0.59	7.50	0.88	7.51	1.06
6.50	0.70	6.51	1.00	6.54	1.29
5.50	0.96	5.52	1.23	5.50	1.59

Table 3. Retention ratio R as a function of pH.

Purity is a Key Ingredient

Purity is of key importance in the manufacture of our Ion Pair Reagents. Regis S- and Q-Series products are synthesized in accordance with the industry's highest quality standards, resulting in exceptional purity and integrity. This is demonstrated in table 1: UV transparency as low as 200 nm can be achieved for both the S- and Q-Series reagents. In most cases, these absorbances are lower than those for HPLC grade acetonitrile and methanol. Although the S- and Q-Series ion pair reagents can be used at wavelengths less than 210 nm, the crucial factors in determining what wavelength to use are the integrity of the detector optics and the purity of the organic modifiers.

Regis also supplies bulk Sulfonate and several additional bulk Ion Pair Reagents to complement the separation capabilities of the Sulfonate S-Series and Quaternary Amine Q-Series.

How to Select a Regis Ion Pair Reagent For Method Development

To choose 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 counterion, and therefore, greater the retention. Retention may increase by a factor of almost 20 when going from pentyl (Q5) to dodecyl (Q12), as illustrated in table 2 and figure 3. Both table 2 and figure 3 demonstrate that the Q-reagent chain length governs benzoic acid retention times, but does not affect the benzyl alcohol retention times. Similar behavior can also be achieved with the S-Series.

The following are guidelines to developing a successful method using Regis' ion pair reagents:

- Select a column — endcapped ODS (octadecylsilyl) is most common.
- Use only HPLC-grade water and chromatography grade reagents in mobile phase preparation.
- Choose the mobile phase components and concentrations that give the best separation.
- If nonionic components are present in the sample, optimize the resolution prior to attempting ionic separations.
- Select the appropriate ion pair series to provide the necessary counterion. Use the Q-series for acidic compounds and the S-series for basic compounds.
- Through a process of elimination, choose the alkyl chain length which results in the best separation (figure 4).
- Once the reagent has been selected, adjust the pH of the mobile phase to maximize resolution. Because slight modification of pH can profoundly effect retention and selectivity, make all adjustments in small increments and monitor carefully (table 3).
- 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 (figure 5).

Ultrapure Ion Pairing Reagents and Buffers

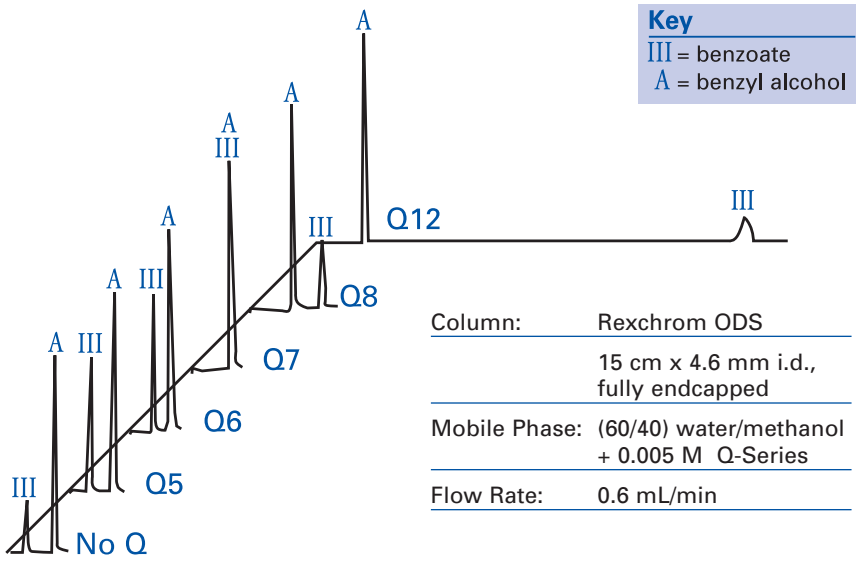

Figure 3. Retention increases with Q-Reagent chain length.

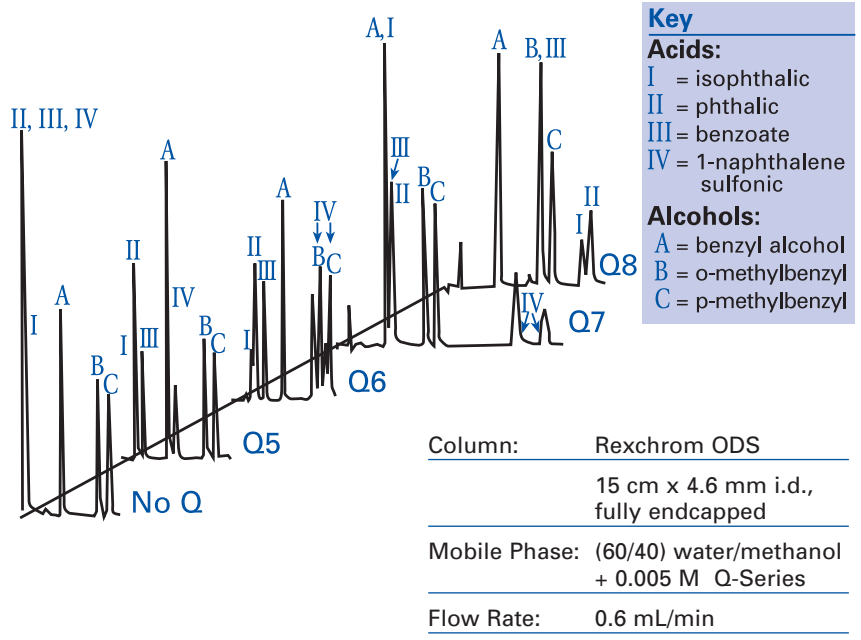

Figure 4. In a mixture of ionic and nonionic compounds, first separate the nonionic compounds from each other (See above). Then choose the ion pair reagent that retains the ionic compounds as desired. Here, Q6 seems to be the reagent of choice since all peaks are visibly separated.

Regis Sulfonates (S-Series) For Basic Compounds

S-Series Ion Pair Concentrates (For Cations)

The sulfonates are sodium salts that act as an anionic counterion for the separation and resolution of positively charged analytes. The sulfonates are available as: Ion pair concentrates—premixed 0.5 M solutions of alkyl sulfonates. When diluted to 1 L with HPLC-grade water, a 10 mL bottle forms a 0.005 M solution.

Larger quantities are available upon request. Please call Regis for pricing.

Product	Size	Catalog #
S5 (1-pentylsodiumsulfonate)	(5) 10 mL bottles 100 mL bottle	405025 405035
S6 (1-hexylsodiumsulfonate)	(5) 10 mL bottles 100 mL bottle	405026 405036
S7 (1-heptylsodiumsulfonate)	(5) 10 mL bottles 100 mL bottle	405027 405037
S8 (1-octylsodiumsulfonate)	(5) 10 mL bottles 100 mL bottle	405028 405038
S12 (1-dodecylsodiumsulfonate)	(5) 10 mL bottles 100 mL bottle	405021 405031
0.5 M solutions of Alkyl Sulfonates *(Each 10 mL bottle, diluted to 1 L, produces a 0.005 M solution)*		
S-Series Method Development Kit *Each kit contains a 10 mL bottle of each of the following:*		
S5, S6, S7, S8, S12		405020

Bulk Ion Pair Reagents (For Cations)

Bulk powder—fine, purified crystals, for use as a buffer in large-scale mobile phase preparation.

Larger quantities are available upon request. Please call Regis for pricing.

Product	Size	Catalog #
1-Pentanesulfonate, Sodium Salt	25 gm 100 gm	403025 403125
1-Hexanesulfonate, Sodium Salt	25 gm 100 gm	403026 403126
1-Heptanesulfonate, Sodium Salt	25 gm 100 gm	403027 403127
1-Octanesulfonate, Sodium Salt	25 gm 100 gm	403028 403128

Regis Quaternary Amines (Q-Series) For Acidic Compounds

Q-Series Ion Pair Concentrates (For Anions)

The Q-series is comprised of quaternary alkyltriethylamines that can be used for the resolution of negatively charged species. This unique set of cationic reagents was developed to complement the Sulfonate Series (S-Series) and is exclusively manufactured by Regis. The Quaternary Alkyltriethylamines are available as:

Ion pair concentrates —premixed 0.5 M solutions of alkylamines. When diluted to 1 L with HPLC-grade water, a 10 mL bottle forms a 0.005 M buffered solution.

Product	Size	Catalog #
Q5 (1-pentyltriethyl-ammonium phosphate)	(5) 10 mL bottles 100 mL bottle	404025 404035
Q6 (1-hexyltriethyl-ammonium phosphate)	(5) 10 mL bottles 100 mL bottle	404026 404036
Q7 (1-heptyltriethyl-ammonium phosphate)	(5) 10 mL bottles 100 mL bottle	404027 404037
Q8 (1-octyltriethyl-ammonium phosphate)	(5) 10 mL bottles 100 mL bottle	404028 404038
Q12 (1-dodecyltriethyl-ammonium phosphate)	(5) 10 mL bottles 100 mL bottle	404021 404031
0.5 M solutions of Quaternary Alkyltriethylamines *(Each 10 mL bottle, diluted to 1 L, produces a 0.005 M solution)*		
Q-Series Method Development Kit *Each kit contains a 10 mL bottle of each of the following:*		
Q5, Q6, Q7, Q8, Q12		404020

Other Regis Bulk Ion Pair Reagents (For Anions)

Other bulk Ion Pair reagents such as Tetrabutylammonium phosphate, Trihexylamine and Triheptylamine are complementary reagents used for the resolution of negatively charged analytes.

Product	Size	Catalog #
Tetrabutylammonium phosphate 0.5 M, pH 7.5	10 mL	680502
Tetrabutylammonium phosphate 0.5 M, pH 7.5	500 mL	680503

Ion Pair References

1. Perry, J. A.; Glunz, L. G.; Szczerba, T. J.; Hocson, V. S.; Reagents For Ion Pair Reversed-Phase HPLC; American Laboratory 1984, 16(10), 114—119.
2. Eksborg, S.; Lagerstrom, P.; Modin, R.; Schill, G.; Ion Pair Chromatography of Organic Compounds J. Chrom. 1973, 83, 99.
3. Eksborg, S.; Schill, G.; Ion Pair Partition Chromatography of Organic Ammonium Compounds Anal. Chem. 1973, 45, 2092.

For additional information on Ion Pair Reagents, contact us by phone,

800.323.8144 ext. 662
847.967.6000 ext. 662
or e-mail,
sales@registech.com