

# ION PAIRING REAGENTS AND BUFFERS



# 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.

## 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 limitations 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).

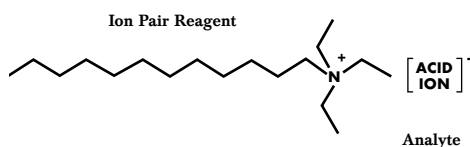


Figure 1. Quaternary Amine (Q-Series) Ion Pair Reagent.

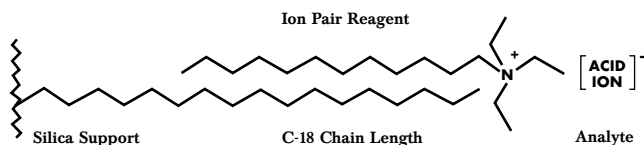


Figure 2. Quaternary Amine (Q-Series) Ion Pair Reagent interacting with C-18 Support.

## ULTRAPURE ION PAIRING REAGENTS AND BUFFERS

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.)

S-Series	Optical Absorbance (AUFS)		Q-Series	Optical Absorbance (AUFS)	
	200 nm	210 nm		200 nm	210 nm
S5	0.006	0.002	Q5	0.060	0.001
S6	0.048	0.018	Q6	0.059	0.006
S7	0.008	0.001	Q7	0.022	0.009
S8	0.001	0.003	Q8	0.082	0.003
S12	0.002	0.003	Q12	0.102	0.013
CH3CN	0.076	0.013			
CH3OH	0.940	0.510			

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

### 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 and Rivier Buffers to complement the separation capabilities of the Sulfonate S-Series and Quaternary Amine Q-Series.

# ULTRAPURE ION PAIRING REAGENTS AND BUFFERS

## 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).

Q-Series	Retention Times (min)		Retention Ratio Acid/Alcohol
	Benzoic Acid	Benzyl 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.

## Effect of Q-Reagent Chain Length on Retention

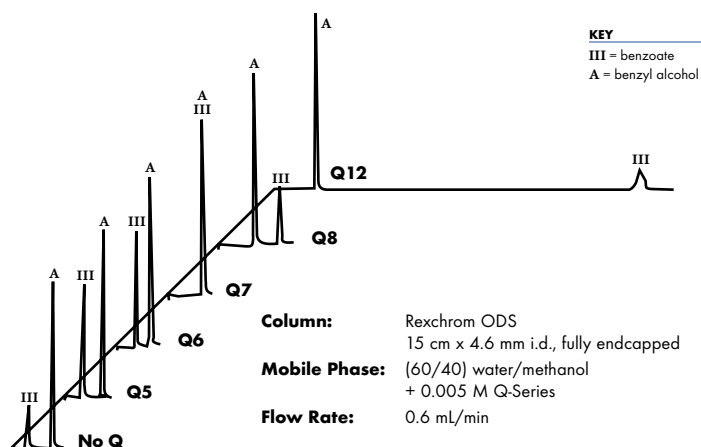


Figure 3. Retention increases with Q-Reagent chain length.

## Choosing Appropriate Ion Pair Reagents

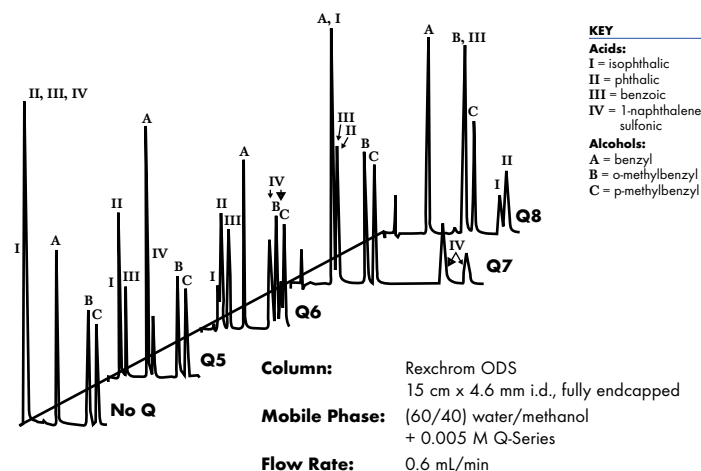


Figure 4. In a mixture of ionic and nonionic compounds, first separate the nonionic compounds from each other (See above). Then choose the ion pair reagent that retains the ionic compounds as desired. Here, Q6 seems to be the reagent of choice since all peaks are visibly separated.

## Fine-Tuning a Separation

**Column:** Rexchrom ODS  
15 cm x 4.6 mm i.d., fully endcapped  
**Mobile Phase:** (60/40) water/methanol  
+ Q-Series  
**Flow Rate:** 0.6 mL/min

Increasing Q7 concentration from 0.005 M (left) to 0.0055 M (right) moves the benzoate peak off the benzyl alcohol peak.



Figure 5. Increasing the Q-Reagent concentration may increase retention slightly and optimize the separation.

## 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.

### 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	Product #	U.S. Price
S5 (1-pentylsodiumsulfonate)	(5) 10 mL bottles	405025	
	100 mL bottle	405035	
S6 (1-hexylsodiumsulfonate)	(5) 10 mL bottles	405026	
	100 mL bottle	405036	
S7 (1-heptylsodiumsulfonate)	(5) 10 mL bottles	405027	
	100 mL bottle	405037	
S8 (1-octylsodiumsulfonate)	(5) 10 mL bottles	405028	
	100 mL bottle	405038	
S12 (1-dodecylsodiumsulfonate)	(5) 10 mL bottles	405021	
	100 mL bottle	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:

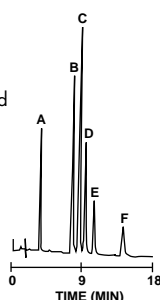
S5, S6, S7, S8, S12	405020
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Product	Size	Product #	U.S. Price
1-Pentanesulfonate, Sodium Salt	25 gm	403025	
	100 gm	403125	
1-Hexanesulfonate, Sodium Salt	25 gm	403026	
	100 gm	403126	
1-Heptanesulfonate, Sodium Salt	25 gm	403027	
	100 gm	403127	
1-Octanesulfonate, Sodium Salt	25 gm	403028	
	100 gm	403128	
1-Dodecanesulfonate, Sodium Salt	5 gm	403021	
	25 gm	403022	

## ION PAIR APPLICATIONS

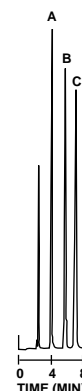
### Separation of Catecholamines using Ion Pair Reagents

<b>Column:</b>	REXCHROM ODS, 5 $\mu$ m, 100 $\text{\AA}$ 15 cm x 4.6 mm i.d.
<b>Mobile Phase:</b>	(89/11) 0.005 M S8 ion pair concentrate/ acetonitrile, pH 2.5
<b>Flow Rate:</b>	1.0 mL/min
<b>Load:</b>	10 $\mu$ L
<b>Detection:</b>	UV 280 nm
<b>Peak Identities:</b>	A. 3,4-Dihydroxyphenylacetic acid B. Norepinephrine C. 3,4-Dihydroxyphenylalanine D. Epinephrine E. 3,4-Dihydroxybenzylamine F. Dopamine



### Separation of Artificial Sweeteners

<b>Column:</b>	REXCHROM Little Champ II (ODS), 3 $\mu$ m, 100 $\text{\AA}$ 5 cm x 4.6 mm i.d.
<b>Mobile Phase:</b>	(86/14) 0.005 M Q7 ion pair concentrate/ acetonitrile, pH 6.2
<b>Flow Rate:</b>	1.0 mL/min
<b>Load:</b>	10 $\mu$ L
<b>Detection:</b>	UV 210 nm
<b>Peak Identities:</b>	A. Aspartame B. Acesulfame Potassium (Acesulfame-K) C. Saccharin



## 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 alkyl amines. When diluted to 1 L with HPLC-grade water, a 10 mL bottle forms a 0.005 M buffered solution.

Product	Size	Product #	U.S. Price
Q5 (1-pentyltriethylammonium phosphate)	(5) 10 mL bottles 100 mL bottle	404025 404035	
Q6 (1-hexyltriethylammonium phosphate)	(5) 10 mL bottles 100 mL bottle	404026 404036	
Q7 (1-heptyltriethylammonium phosphate)	(5) 10 mL bottles 100 mL bottle	404027 404037	
Q8 (1-octyltriethylammonium phosphate)	(5) 10 mL bottles 100 mL bottle	404028 404038	
Q12 (1-dodecyltriethylammonium 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
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### 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	Product #	U.S. Price
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, check our Web site at [www.registech.com/ionpair/](http://www.registech.com/ionpair/) or contact Regis directly at:

(800) 323-8144 ext. 649

(847) 967-6000 ext. 649

e-mail us at: [sales@registech.com](mailto:sales@registech.com).

