

## Improving Separation in Reversed Phase HPLC

Separation in reversed phase liquid chromatography depends on non-polar analytes being retained by a hydrophobic stationary phase such as a C18 column. If the sample contains ionic (polar) components, they may not be sufficiently retained by the stationary phase for adequate separation. The primary methods of improving retention are via ion pairing or pH modification.

### Improving separation via ion pair chromatography

Ion pairing is a technique that can aid in separation by pairing an alkyl salt with the ionic components in the sample. The carbon chain will interact with the stationary phase, and the salt with the ion, thus allowing improved separation of ionic species. Typically, the longer the alkyl chain, the greater the retention by the column. Basic species react best with sodium alkyl sulfonates, and acidic with quaternary ammonium salts or alkyl amines.

**Table 1. Ion pair chromatography chart**

Product code	Use	Product description	Alkyl chain length	Molecular weight	Suggested molarity (mM*)
T/0931	Acidic Species	Tetramethylammonium Chloride, for HPLC	1	109.5	5
T/0935	Acidic Species	Tetramethylammonium Hydrogen Sulfate, for HPLC	1	171.2	5
T/3203	Acidic Species	Triethylamine, for HPLC	2	101.2	5
T/0621	Acidic Species	Tetraethylammonium Bromide, for HPLC	2	210.2	5
T/3258	Acidic Species	Trifluoroacetic acid, for HPLC	2	114	0.05% v/v
T/0481	Acidic Species	Tetra-N-butylammonium hydroxide solution 0.5M Aq., for HPLC	4	259.5	5
T/0486	Acidic Species	Tetra-N-butylammonium hydroxide solution 0.1M phosphate buffered, for HPLC	4	259.5	5
T/0611	Acidic Species	Tetradecyltrimethylammonium Bromide, for HPLC	10	336.4	0.5
D/5311	Acidic Species	Dodecyltrimethylammonium Bromide, for Ion pair chromatography	12	308.3	0.5
C/3961	Acidic Species	Cetyltrimethylammonium Bromide, for Ion pair chromatography	16	364.4	0.5
M/3876	Basic Species	Methane Sulfonic Acid sodium salt, for Ion pair chromatography	1	118.1	5
P/1038	Basic Species	1-Pentane Sulfonic Acid sodium salt, for Ion pair chromatography	5	174.2	5
H/0438	Basic Species	1-Hexane Sulfonic Acid sodium salt, for Ion pair chromatography	6	188.2	5
H/0168	Basic Species	1-Heptane Sulfonic Acid sodium salt, for Ion pair chromatography	7	202.4	5
H/0166	Basic Species	1-Heptane Sulfonic Acid sodium salt solution 0.1M, for Ion pair chromatography	7	202.4	5
O/0028	Basic Species	1-Octane Sulfonic Acid sodium salt, for Ion pair chromatography	8	216.7	5
O/0026	Basic Species	1-Octane Sulfonic Acid sodium salt solution 0.1M, for Ion pair chromatography	8	216.7	5
D/0030	Basic Species	1-Decane Sulfonic Acid sodium salt, for Ion pair chromatography	10	244.3	0.5
D/5296	Basic Species	1-Dodecane Sulfonic Acid sodium salt, for Ion pair chromatography	12	272.4	0.5

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\*Except where noted

All mobile phases using salts must contain at least 10% water in order to avoid precipitation. Using a dedicated column for ion pair chromatography, particularly with longer alkyl chain lengths, is recommended for silica based stationary phases, as non-reversible adsorption may occur.

### Using pH modification and additives to improve separation

Improved separation for non-polar compounds can also be attained via pH adjustment with buffers or mobile phases. Retention for acidic compounds can be enhanced by separation under low pH conditions. Similarly, organic bases can benefit from separation at a pH higher than the pK<sub>b</sub> of the base, although care must be taken in column selection since silica may degrade above pH 8.

**Table 2. Additives and blends for pH modification**

Product code	Product description	Grade - brand
A/0406	Acetic Acid	HPLC
A113	Acetic Acid	LCMS
FE/0200	Acetonitrile with 0.1% Trifluoroacetic Acid	HPLC gradient grade
FE/1100	Acetonitrile with 0.1% Formic Acid	
J/0100	Ammonia Solution 0.25M	
J/0200	Ammonium Acetate Solution 0.25M	
T006024000	Ammonium Bicarbonate 10mM in Water with 5% Methanol (pH 10)	Mobile phase buffer solution for HPLC
A117	Formic Acid	LCMS
J/4200	Formic Acid Solution 0.25M,	HPLC gradient grade
J/7600	Sodium Dihydrogen Orthophosphate 0.25M Solution	
J/7650	Di-Sodium Hydrogen Orthophosphate 0.25M Solution	
S/4940	Sodium Hydroxide 46-51% Solution	HPLC for electrochemical detection
O/0515	Orthophosphoric Acid 85%,	
P/1290	Perchloric Acid 60%	HPLC
FE/1200	Water with 0.1% Formic Acid	HPLC gradient grade
FE/0100	Water with 0.1% Trifluoroacetic Acid	

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### Solvents for reversed phase HPLC

We offer several grades of solvents for HPLC, covering many individual applications. Those selected in table 3 are suitable for gradient analysis. Thermo Scientific™ Chromplete™ is the recommended grade for labs that are using more than one technique such as spectrophotometry or GC or need products that meet the analytical requirements of either USP or ACS.

**Table 3. Reversed phase HPLC solvents**

Product code	Product description	Grade - brand
A/0627	Acetonitrile	HPLC gradient grade - Fisher Chemical
T00101	Acetonitrile	Chromplete for HPLC, GC, ACS & spectrophotometry - Thermo Scientific
E/0665DF	Ethanol, Denatured	HPLC - Fisher Chemical
M/4058	Methanol	
T00102	Methanol	Chromplete for HPLC, GC, ACS & spectrophotometry - Thermo Scientific
P/7508	2-Propanol	HPLC - Fisher Chemical
T00104	2-Propanol	Chromplete for HPLC, GC, ACS & spectrophotometry - Thermo Scientific
W/0106	Water	HPLC - Fisher Chemical
T00103	Water	Chromplete for HPLC, GC, ACS & spectrophotometry - Thermo Scientific

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