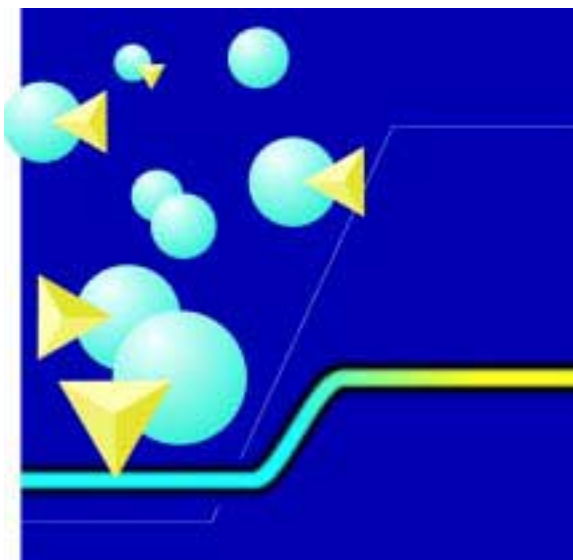


## Ion Pair Chromatography – The Short Track



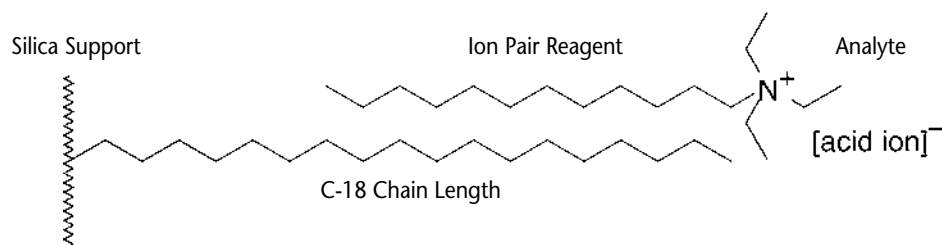
One of the most important techniques in chromatography is reversed-phase HPLC, although it is restricted to the separations of non-ionic and non-polar compounds. Regarding the fact that many biological substances of interest are either polar or ionic, HPLC analysts achieved in the past fair results with the method of ion suppression. This method is based on pH adjustment of the mobile phase to result in a non-ionized analyte. Ion suppression is suitable for weak acids and weak bases, but not for samples containing more than one ionizable component. In addition, extensive method development is required to determine the optimum pH of the mobile phase. A further limitation is given by the instability of the silica support in bonded-phase columns, requiring a pH within the range of 2-8. Below pH 2 hydrolysis of the bounded functional groups occurs and above pH 8, silica dissociates and the silica support starts to dissolve.

Strong acids and strong bases can be analyzed on reversed-phase columns using the technique of ion-pair chromatography (IPC) that was developed in 1973 by Dr. Gordon Schill. To promote the formation of ion pairs, commonly a pH of 7.5 is suitable for analysis of acids, pH 3.5 for bases, respectively. Retention is then modified by including a bulky organic molecule, oppositely charged to the analyte, into the mobile phase. The mechanism of IPC is described in at least three models: the ion-pair model, the dynamic ion-exchange model, and the ion-interaction model. In brief, retention is most likely affected by both, electrostatic and solvophobic effects. For a detailed description of these theories we refer to the original literature. Separation of charged molecules can also be obtained by using ion exchange chromatography. In contrast to this method, ion pair chromatography has the following advantages:

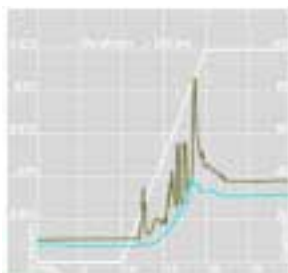
- Reduced separation time
- Highly reproducible results
- Sharper peak shapes
- Simultaneous separation of ionized and non-ionized analytes in one run
- Wide choice of additives to improve separation.

### Properties of IPC Reagents

IPC reagents from Fluka are of highest purity and have only little extinction in the low UV. For example, Tetrabutylammonium hydrogensulfate shows excellent transparency down to 200 nm, at even high concentrations. In addition, they are tested for the absence of insoluble matter and non-absorbing impurities like redox traces, which may interfere with the sample. The final check is performed with an application test, using a very rough gradient (figure 1).



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Fluka IPC quality  
commercially available high purity reagent

Figure 1: Gradient test

column: Nucleosil,  
100-7 C18  
dimensions: 120 mm x 4 mm  
detection: UV 205 nm  
flow rate: 2.0 ml/min.  
eluent: CH<sub>3</sub>CN:TBAHSO<sub>4</sub>  
0.005 M  
gradient: 0': 0% CH<sub>3</sub>CN  
10': 0% CH<sub>3</sub>CN  
20': 100% CH<sub>3</sub>CN  
30': 100% CH<sub>3</sub>CN

## Hints for using IPC reagents

In general, IPC reagents can be used with all stationary phases. To avoid precipitation, the eluent should contain at least 10 % water. Solubility problems may occur, especially when acetonitrile is used as the organic compound. For the preparation of the mobile phases, always use HPLC grade solvents.

Longer equilibration (10 column volumes) may be necessary using low concentrated additives in the mobile phase.

Special attention should be given using long-chained IPC reagents, since irreversible adsorption can occur on the stationary phase.

Therefore, the column should be reserved for this special purpose, otherwise it may lead to changes in separation behavior.

## Selection guide

Commercially available end-capped octadecylsilyl (C18) columns are the most commonly used for IPC.

If you have a mixture of ionic and non-ionic analytes, we recommend, first, to optimize the method for the separation of the non-ionic components. Afterwards, select the appropriate ion pair reagent to provide the necessary counter ion. Alkyl sulfonates are a good first choice for basic solutes, whereas quaternary amines are useful for acidic solutes. Notice that halogenated IPC reagents are only suitable for isocratic applications and should not be used running a gradient.

The evaluation of the right alkyl chain length to obtain best resolution is not easy. So far, no concrete rules or tools are existing to support analysts in their choice. However, some approaches are described in the literature (see below). A good approach is to start with Heptanesulfonic acid sodium salt for cations, Tetrabutylammo-

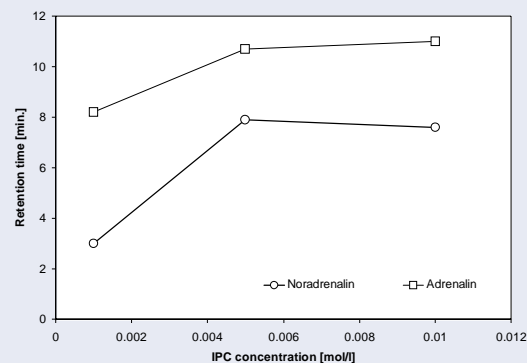


Figure 2: Relation between the concentration of the ion pair reagent and the retention time.

Nucleosil C18 column, using heptane sulfonic acid as IPC reagent at pH 3.0. At a concentration of about 0.005 M we get saturation and an increase of the concentration does not result in a better separation.

nium hydrogen sulfate or dihydrogen phosphate for anions, respectively.

After you elected the IPC reagent that gives the best separation, you can optimize your method by adjusting concentration and pH.

Normally, a concentration of 0.005 M of a short or middle chain ion pairing reagent in the mobile phase is suitable for most separations. Long chain reagents have their optimum between 0.0005 and 0.002 M. Figure 2 shows the relation between the retention time and the concentration of the ion pair reagent. However, slight variations of the concentration can increase the resolution by affecting the retention times.

Application table: pH values of the ready-to-use eluents, adjusted by the indicated combinations of ion-pairing reagent concentrates and phosphate concentrates; the reagent concentrations refer to the prepared eluents.

		IPC reagents	Fluka 86847 TBAHSO <sub>4</sub> 0,005M	Fluka 86846 TBABr 0,005M	Fluka 51834 Heptane sulfonic Na salt 0,005M	Fluka 86899 TBAH <sub>2</sub> PO <sub>4</sub> 0,005M	Fluka 86851 TBAOH 0,005M
<b>Phosphate salts</b>							
			~ 2.7			~ 4.9	
H <sub>3</sub> PO <sub>4</sub> 0,01M	Fluka 79626	~ 2.2	~ 2.1	~ 2.2	~ 2.3	~ 2.4	~ 2.3
H <sub>3</sub> PO <sub>4</sub> / KH <sub>2</sub> PO <sub>4</sub> 0,005M	Fluka 79628	~ 2.7	~ 2.4	~ 2.6	~ 2.6	~ 2.8	~ 4.3
KH <sub>2</sub> PO <sub>4</sub> 0,01M	Fluka 60232	~ 4.7	~ 2.8	~ 4.8	~ 4.8	~ 4.9	~ 7.0
H <sub>3</sub> PO <sub>4</sub> / Na <sub>2</sub> HPO <sub>4</sub> 0,005M	Fluka 79629	~ 5.0	~ 2.9	~ 4.9	~ 5.0	~ 5.0	~ 7.1
Na <sub>2</sub> HPO <sub>4</sub> 0,005M	Fluka 71648	~ 9.1	~ 6.8	~ 9.1	~ 9.2	~ 7.1	
Na <sub>2</sub> HPO <sub>4</sub> / KH <sub>2</sub> PO <sub>4</sub> 0,005M	Fluka 71653	~ 7.0	~ 6.4	~ 7.1	~ 7.0	~ 6.9	~ 8.5
Na <sub>2</sub> HPO <sub>4</sub> 0,01M	Fluka 71651	~ 9.3	~ 7.2	~ 9.3	~ 9.3	~ 7.4	

## Literature

Effects of eluent additives on separation:

Zou, H.F., Zhang, Y.K., Hong, M.F., Lu, P.; Effects of organic modifier and ion-pair-reagent in liquid chromatography; *Chromatographia*, 35 No. 7/8 (1993).

Zou, H.F., Hang, Y., Hong, M., Lu, P.; Retention values of sulfonic acids as a function of the nature and concentration of inorganic salt in RP IPC; *J. Liq. Chromatogr.*, 17 (3), 707-719 (1994).

Zou, H.F., Zhang, Y.K., Hong, M.F., Lou, P.C.; Effect of Hammett's constant on the quantitative correlation of benzoic acid retention in RP IPC with octanol/water partition coefficients; *J. Liq. Chromatogr.*, 16 (6), 3433-3443 (1993).

The three IPC models:

Horvath, C., Melander, W., and Molnar, I., *J. Chromatogr.* 125, 129-156 (1976).

Kissinger, P.T., *Anal. Chem.* 49, 883 (1977).

Bidlingmeyer, B.A., *J. Chromatogr.* 141, 289-312 (1977).

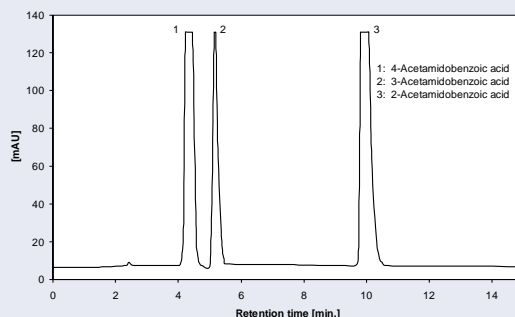
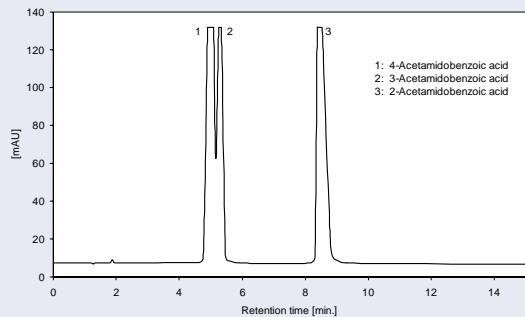


Figure 3: Relation between pH and retention:

In this example we separated three isomers of acetamino benzoic acid on a Nucleosil C18 column. The mobile phase was supplemented with 0.005 M Tetrabutylammonium hydrogen sulfate and 0.005 M di-sodium hydrogen phosphate. pH was adjusted to 2.4 (left) and 4.5 (right). Whereas at pH 2.4 two of the isomers were poorly separated, we could observe a base line separation at pH 4.5.

More significant is the adjustment of the pH. A pH of about 7.5 is a good starting point for acids, 3.5 for bases, respectively. Since selectivity and retention are affected by the pH, you can optimize the separation by changing the pH in small increments. Use the application table on page 2 to easily prepare the mobile phase at a defined pH containing the appropriate IPC reagent. Figure 3 (above) gives an example of the dependence between pH and resolution.

## Summary: IPC – The Short Track

Ion pairing reagents are used to separate ionizable analytes by reversed phase HPLC. Adjusting the pH to suppress ions is often not possible and requires extensive method development. With Fluka IPC reagents you can work at an optimized pH and achieve fast separations of ionized and non-ionized analytes in one run.



In reversed phase HPLC, purity of the eluent additives means better reproducibility, reliable methods and more accurate results. Eluent additives such as buffers or ion pairing reagents, can be the reason for impurity peaks if the quality is not sufficient for your application. Often these impurities vary from batch to batch. Gradient elution and detection in the low UV range require high purity reagents. Fluka offers the ion pairing reagents and buffers with the highest quality for faster method development and more reproducible results.

## Get started with IPC

To start with IPC we recommend the following reagents and buffers:

### For [+] charged compounds

Fluka 51832	1-Heptanesulfonic acid sodium salt Monohydrate
Fluka 74882	1-Octanesulfonic acid sodium salt Monohydrate
Fluka 30631	1-Decanesulfonic acid sodium salt
Fluka 71726	Sodium dodecyl sulfate

### For [-] charged compounds:

Fluka 87724	Tetramethylammonium hydrogen sulfate
Fluka 86626	Tetraethylammonium hydrogen sulfate
Fluka 86853	Tetrabutylammonium hydrogen sulfate
Fluka 87299	Tetrahexylammonium hydrogen sulfate

### Buffers

Fluka 79607	Ortho phosphoric acid 50 %
Fluka 60221	Potassium dihydrogen phosphate
Fluka 71636	di-Sodium hydrogen phosphate Dihydrate
Fluka 71686	Sodium hydroxide solution 50 %

The reagents mentioned above are available in several package sizes and even in very convenient ampoules

Go to our web site to find detailed descriptions of more than 75 IPC reagents and buffer additives

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Search on the keyword: 'IPC'

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Fluka 09749	Acetic acid:Triethylamine 2M:1M Concentrate
Fluka 09748	Acetic acid:Triethylamine 2M:2M Concentrate
Fluka 09751	Formic acid:Triethylamine 2M:1M Concentrate
Fluka 09752	Formic acid:Triethylamine 2M:2M Concentrate
Fluka 03387	Phosphoric acid:Triethylamine 2M:1M Concentrate
Fluka 03388	Phosphoric acid:Triethylamine 2M:2M Concentrate
Fluka 09745	Sulfuric acid:Triethylamine 2M:1M Concentrate
Fluka 09744	Sulfuric acid:Triethylamine 2M:2M Concentrate
Fluka 09746	Trifluoroacetic acid:Triethylamine 2M:1M Concentrate
Fluka 00747	Trifluoroacetic acid:Triethylamine 2M:2M Concentrate

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