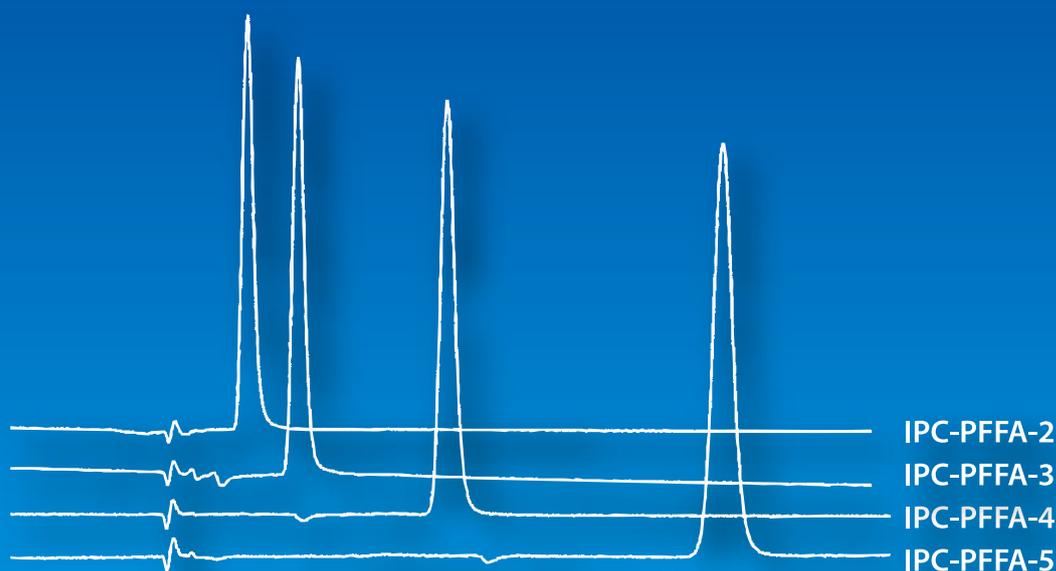


Ion-Pair Reagents for HPLC



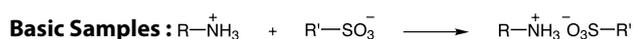
Ion-Pair Chromatography for Acidic Samples

Ion-Pair Chromatography for Basic Samples



Ion-Pair Reagents for HPLC

Ion-exchange chromatography systems have previously been utilized in HPLC analysis of ionic samples. Recently, reversed phase partition chromatography using ion-pair reagents has been developed and utilized. The ionic samples form an ion-pair with ion-pair reagents in the mobile phase to become electrically neutral. The increase in hydrophobic character of the ion-pair results in a greater affinity for the reverse stationary phase and leads to sample resolution.



UV and fluorescence detectors are widely used. Therefore ion-pair reagents must lack UV absorption and fluorescence

themselves to obtain highly sensitive detection of samples. The UV absorption of sodium alkanesulfonates and quaternary ammonium salts is minimal so that these reagents can be used for reliable HPLC analysis. On the other hand, when a sample lacks sufficient UV absorption or fluorescence, the use of sodium 9,10-dimethoxyanthracene-2-sulfonate allows for high-sensitivity detection as a fluorimetric ion-pair reagent.

Recently, use of LC-MS in which mass spectrometry is incorporated in HPLC as a detector has become widespread. Sodium alkanesulfonates, a general ion-pair reagents, being non-volatile crystals pose a problem in that they contaminate the interface. The IPC-PFFA series is made of highly volatile ion-pair reagents allowing for continuous LC-MS analysis without contaminating the interfaces.

Ion-Pair Chromatography for Acidic Samples

- Analysis is performed with pH adjusted to 7.5 with the addition of quaternary ammonium salts to the mobile phase.
- Acidic samples form an electrically neutral ion-pair with the quaternary ammonium salt and are retained in the reverse phase systems.
- The ion-pair reagents for acidic samples for LC-MS are supplied as 0.5 M aqueous solutions and were adjusted to pH 7.5. The solution can be used as a neutral mobile phase after dilution with the LC solvents (acetonitrile/water or methanol/water) to 5 mM. Since the acidic substances are ionized under the neutral conditions, they are facilitated to form an ion-pair.

[Examples]

1. When 0.5 mol/L Tetrabutylammonium Phosphate is used:

The reagent (10 mL) is diluted to 1 L with an aqueous solvent such as methanol - water.
(pH adjustment is not required because the reagent is already buffered.)

2. When Tetrabutylammonium Hydroxide is used:

- 1) The reagent (12.5 mL) is diluted to 1 L with an aqueous solvent such as methanol - water.
- 2) The pH is adjusted to 7.5 by the addition of an aqueous phosphoric acid (10%).

$R_4-N^+ X^-$	10363	IPC-TEA-OH (Tetraethylammonium Hydroxide) (10% in Water)	25mL
	10364	IPC-TBA-OH (Tetrabutylammonium Hydroxide) (10% in Water)	25mL 100mL
	10365	IPC-TBA-Br (Tetrabutylammonium Bromide)	25g 100g 500g
	10366	IPC-TBA-Cl (Tetrabutylammonium Chloride)	5g 25g
	10367	IPC-TBA-P (Tetrabutylammonium Phosphate) (0.5mol/L in Water)	10mL 100mL
	10368	IPC-TBA-HS (Tetrabutylammonium Hydrogen Sulfate)	25g 100g
	10453	IPC-DTMA-Cl (Dodecyltrimethylammonium Chloride)	25g 500g

for LC-MS

$R_2-NH_2^+ X^-$	A5703	IPC-DPAA (Dipropylammonium Acetate) (ca. 0.5mol/L in Water)	10mL
	A5702	IPC-DBAA (Dibutylammonium Acetate) (ca. 0.5mol/L in Water)	10mL 100mL
	A5704	IPC-DAAA (Diamylammonium Acetate) (ca. 0.5mol/L in Water)	10mL 100mL
	A5705	IPC-DHAA (Dihexylammonium Acetate) (ca. 0.5mol/L in Water)	10mL 100mL

Using of IPC-DRAA

Column : Kaseisorb LC ODS 2000
2.0 mm I.D. X 150 mm

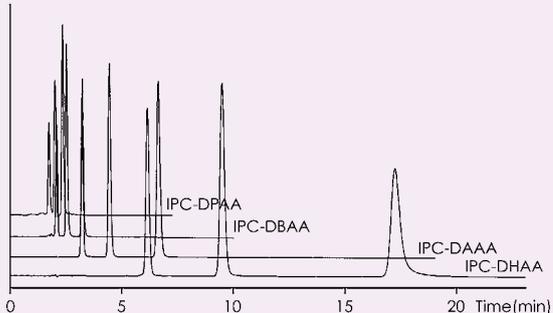
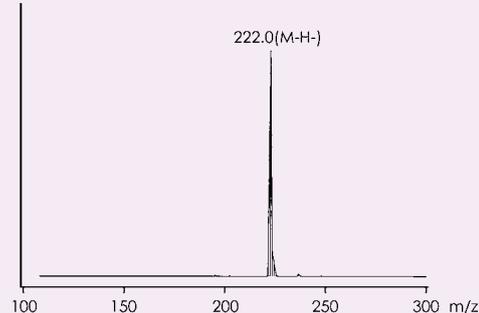
Mobile phase : CH₃CN / H₂O = 30 / 70
containing 5mM IPC Reagent
(Dialkylammonium Acetate)

Flow rate : 0.2 mL/min
Temperature: 25°C

Aminonaphthalenesulfonic Acid

Detection : UV 254 nm
MS : 1100 MSD (Agilent)
ESI (Negative)

Sample : 1. 4-Amino-1-naphthalenesulfonic Acid
2. 1-Amino-8-naphthalenesulfonic Acid
3. 8-Amino-2-naphthalenesulfonic Acid

Ion-Pair Chromatography for Basic Samples

- Analysis is performed by the addition of sodium alkanesulfonate to the mobile phase.
- The basic samples form an electrically neutral ion-pair with sodium alkanesulfonate and are retained in the reverse phase system.
- In the case of sodium alkanesulfonate, the greater the number of carbons in the alkyl group, the greater the partition ratio.
- The solubility of the products such as sodium 1-decanesulfonate (IPC-ALKS-10) may decrease depending upon the composition of the mobile phase solvents; especially after the addition of the buffer for pH adjustment. Resulted turbidity of the mobile phase and crystal formation may interfere with the analysis. To avoid the trouble, modification of the solvent system composition should be considered.
- The ion-pair reagents for basic samples in LC-MS analysis are supplied as 0.5 M aqueous solutions. The solution can be used as an acidic mobile phase after dilution with the LC solvents (acetonitrile/water or methanol/water) to 5 mM. Since the basic substances are ionized under the acidic conditions, they are facilitated to form an ion-pair.
- We launched the high-quality products of PFFA-6, 7 and 8 (A5722, A5721, A5720) for high-sensitive detections.

[Examples]

- 1) Sodium 1-Heptanesulfonate 1.011 g (0.005 mol) is weighed out.
- 2) The reagent is dissolved in 1 L of an aqueous solvent such as methanol - water.
- 3) The pH is adjusted to 3.5 by the addition of aqueous phosphoric acid (50%).

	I0341	IPC-ALKS-3	(Sodium 1-Propanesulfonate)	5g	25g
	I0342	IPC-ALKS-4	(Sodium 1-Butanesulfonate).....	5g	25g
	I0343	IPC-ALKS-5	(Sodium 1-Pentanesulfonate)	5g	25g 100g
	I0344	IPC-ALKS-6	(Sodium 1-Hexanesulfonate)	5g	25g 100g
	I0345	IPC-ALKS-7	(Sodium 1-Heptanesulfonate)	5g	25g 100g
R-SO ₃ ⁻ Na ⁺	I0346	IPC-ALKS-8	(Sodium 1-Octanesulfonate).....	5g	25g 100g
	I0347	IPC-ALKS-9	(Sodium 1-Nonanesulfonate)	5g	25g
	I0348	IPC-ALKS-10	(Sodium 1-Decanesulfonate)	5g	25g
	I0349	IPC-ALKS-11	(Sodium 1-Undecanesulfonate)	5g	25g
	I0350	IPC-ALKS-12	(Sodium 1-Dodecanesulfonate)	5g	25g
	I0351	IPC-ALKS-13	(Sodium 1-Tridecanesulfonate)	5g	25g
	I0352	IPC-SDS	(Sodium Dodecyl Sulfate).....	5g	100g 500g

for LC-MS

	A5711	IPC-PFFA-2	(Trifluoroacetic Acid) (ca. 0.5mol/L in Water)	10mL	
	A5712	IPC-PFFA-3	(Pentafluoropropionic Acid) (ca. 0.5mol/L in Water)	10mL	
Rf-COOH	A5713	IPC-PFFA-4	(Heptafluorobutyric Acid) (ca. 0.5mol/L in Water)	10mL	100mL
	A5714	IPC-PFFA-5	(Nonafluorovaleric Acid) (ca. 0.5mol/L in Water)	10mL	
	A5715	IPC-PFFA-6	(Undecafluorohexanoic Acid) (ca. 5mmol).....	1sample	

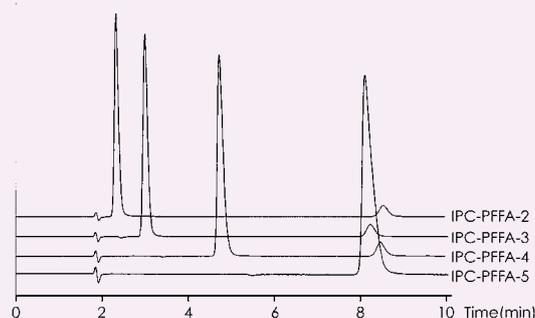
Ion-Pair Reagents for HPLC

A5716	IPC-PFFA-7	(Tridecafluoroheptanoic Acid) (ca. 5mmol)	1sample
A5717	IPC-PFFA-8	(Pentadecafluorooctanoic Acid) (ca. 5mmol)	1sample
A5722	IPC-PFFA-6 HG	(Undecafluorohexanoic Acid High Grade)	1g 5g
A5721	IPC-PFFA-7 HG	(Tridecafluoroheptanoic Acid High Grade)	1g 5g
A5720	IPC-PFFA-8 HG	(Pentadecafluorooctanoic Acid High Grade)	1g 5g

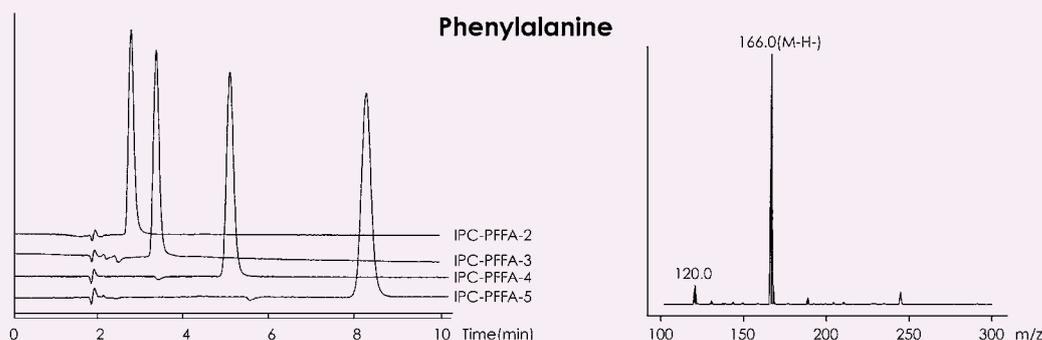
Using of IPC-PFFA

Column	: Kaseisorb LC ODS 2000 2.0 mm I.D. X 150 mm	Temperature:	25°C
Mobile phase	: CH ₃ OH / H ₂ O = 40 / 60 containing 5 mM IPC Reagent	Detection	: UV 254 nm
Flow rate	: 0.2 mL/min	MS	: 1100 MSD (Agilent) ESI (Positive)

Benzylamine



Phenylalanine



Fluorimetric Ion-Pair Reagent

A5701	Sodium 9,10-Dimethoxyanthracene-2-sulfonate	1g
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