HICHROM

Chromatography Columns and Supplies

LC COLUMN SELECTION HILIC Phases

Catalogue 9

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HYDROPHILIC INTERACTION CHROMATOGRAPHY (HILIC) PHASES

Introduction

Hydrophilic Interaction Chromatography (HILIC) is a variant of normal-phase chromatography which is performed using polar stationary phases with partially aqueous eluents. The technique combines the characteristics of 3 major liquid chromatography techniques – reversed-phase, normal-phase and ion chromatography. HILIC is an alternative approach to reversed-phase for the effective separation of polar compounds. Solutes elute in the order of increasing hydrophilicity (polarity), the opposite of reversed-phase, thus providing an orthogonal selectivity.

Mode of Operation

Retention in HILIC is proportional to the amount of organic solvent in the eluent. Typical HILIC eluents contain 65-90% acetonitrile or methanol. The low proportion of water in the eluent generates a water-rich layer on the surface of the polar stationary phase. This enables solutes to partition between the eluent and this water-rich layer (Figure 1). In addition, weak electrostatic interactions between solute and stationary phase contribute to overall selectivity. Gradient elution may be performed either with a decreasing organic or increasing salt gradient. Salt is not required for uncharged solutes such as carbohydrates, but typically 10mM salt is necessary with charged solutes such as peptides. Ammonium formate and acetate are suitable volatile buffers for LC-MS.

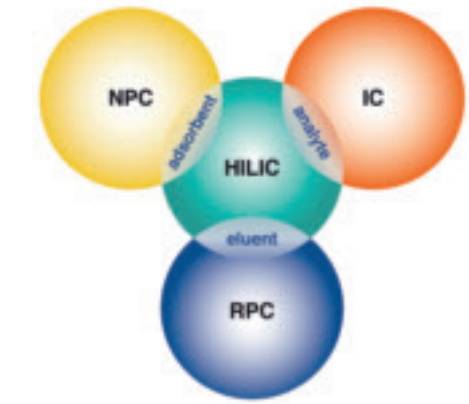
Several types of HILIC phases have been developed, including unbonded silica, neutral bonded ligands (eg. amide, diol), charged ligands (eg. amino), zwitterionic phases and mixed reversed-phase/HILIC phases. A wide selection of HILIC phases is summarised in the table below.

ERLIC, also referred to as eHILIC, is a subset of HILIC separations which employs charged interactions and their subsequent orientation effects (see PolyLC section for further details).

Aqueous normal-phase (ANP) is a further technique related to HILIC (see pages 186-188 for further details).

Applications

HILIC phases are particularly useful for compounds that are weakly retained by reversed-phase columns. Typical application areas include carbohydrates, oligonucleotides, peptides and proteins, amino acids and natural products.



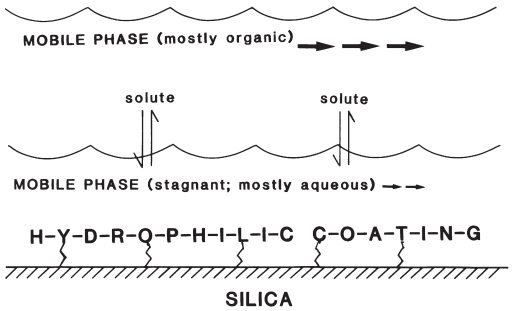


Figure 1. Hypothetical partition mechanism of hydrophilic interaction chromatography (HILIC)

HILIC Phases

Phase	Manufacturer	Functional Group	Particle Size (µm)	Pore Size (Å)	Page
Acclaim HILIC-10	Thermo Scientific	Proprietary	3	120	235, 238
Accucore HILIC[1]		Proprietary	2.6	80	223, 225, 227
Accucore Urea-HILIC[1]		Urea	2.6	80	223, 225, 227
Accucore 150-Amide-HILIC[1]		Amide	2.6	80	223, 226, 227
BioBasic AX		Polyethyleneimine	5	300	239, 242
Brownlee SPP HILIC[1]	Perkin Elmer	Unbonded silica	2.7	90	195
COSMOSIL HILIC	Nacalai Tesque	Triazole	2.5, 5	120	91, 93, 94
Epic HILIC-HC	ES Industries	Polyhydroxylated polymer	1.8, 3, 5, 10	120	100
HALO and HALO-5 HILIC[1]	Advanced Materials Technology	Unbonded silica	2.7, 5	90	6, 125, 127-129
HALO and HALO-5 Penta-HILIC[1]		Penta-hydroxy	2.7, 5	90	6, 125, 128
Hypersil GOLD HILIC	Thermo Scientific	Polyethyleneimine	1.9, 3, 5	175	228, 230
Inertsil HILIC	GL Sciences	Propyl alcohol	3, 5	100	108, 111, 112-114
Kromasil HILIC-D	Akzo Nobel	Diol	5	60	147
NUCLEODUR HILIC	Macherey-Nagel	Zwitterionic ammonium sulphonic acid	1.8, 3, 5	110	157, 158, 160
NUCLEOSHELL HILIC[1]			2.7	90	162
Obelisc N	SIELC	Proprietary	5	100	220, 221
PolyGLYCOPLEX	PolyLC	-	5, 12	-	196, 197, 199
Syncronis HILIC	Thermo Scientific	Zwitterionic	1.7, 3, 5	100	234
TSKgel Amide-80	Tosoh Bioscience	Carbamoyl	3, 5	100	250
TSKgel NH2-100		Ethylamino	3	100	250
VisionHT HILIC	Grace	-	1.5, 3, 5, 10	120	123
YMC-Triart Diol-HILIC	YMC	Diol	1.9, 3, 5	120	266, 267
ZIC-HILIC	Merck	Zwitterionic sulphobetaine	3.5, 5	100, 200	173-175, 177
ZIC-*p*HILIC		Zwitterionic sulphobetaine	5	-	173, 175, 177
ZIC-cHILIC		Zwitterionic phosphorylcholine	3	100	176, 177

[1] Superficially porous phase