HICHROM

Chromatography Columns and Supplies

LC COLUMNS
SIELC
Primesep

Catalogue 9

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SIELC TECHNOLOGIES

- Novel silica-based mixed-mode phases
- Suitable for RP, NP, ion-exchange and ion-exclusion chromatography
- Unique adjustable selectivity
- Stable in 100% aqueous eluents
- LC-MS and preparative chromatography applications

Primesep® mixed-mode stationary phases have been developed by SIELC Technologies for separating a wide range of polar and non-polar compounds by different separation modes, based only on eluent selection. Ionizable compounds interact with the stationary phase by reversed-phase, ion-exchange or ion-exclusion mechanisms. In addition, Promix™ phases are available for biomolecule analysis. Obelisc™ mixed-mode phases are described on pages 220-221 and the newer SHARC™ columns on page 222.

Primesep® Phases

Primesep Phase	Particle Size (µm)	Pore Size (Å)	Main Separation Modes	Typical Applications
A	5, 10	100	RP + cation-exchange + ion-exclusion	Neutral and weak basic compounds
100	5, 10	100	RP + cation-exchange + ion-exclusion	Neutral and basic compounds
200	5, 10	100	RP + cation-exchange + polar interaction	Neutral and strong basic compounds
500	5, 10	100	RP + cation-exchange + ion-exclusion	Neutral and basic compounds
C	5, 10	100	RP + cation-exchange + complex formation	Amines, sulphonium, phosphonium and metal ions
P	5, 10	100	RP + strong cation-exchange + π-π interaction	Neutral and basic compounds Structural isomers of aromatic compounds
AB	5, 10	100	RP + cation-exchange + anion-exchange	Neutral, acidic and basic compounds
B	5, 10	100	RP + anion-exchange + ion-exclusion	Neutral and acidic compounds
B2	5, 10	100	RP + anion-exchange + ion-exclusion	Neutral and acidic compounds
SB	5, 10	100	RP + anion-exchange + ion-exclusion	Neutral and acidic compounds
D	5, 10	100	RP + anion-exchange	Small hydrophobic and acidic compounds Low MW plasma components and biofluids

Table 1 summarises the retention of polar compounds on Primesep phases compared with typical C18 phases at various eluent pHs.

Table 1. Retention of Polar Compounds

Polar Compounds	Primesep	Typical C18	
		Acidic pH	Basic pH
Basic	Good	Poor	Good
Acidic	Good	Good	Poor
Zwitterionic	Good	Poor	Poor

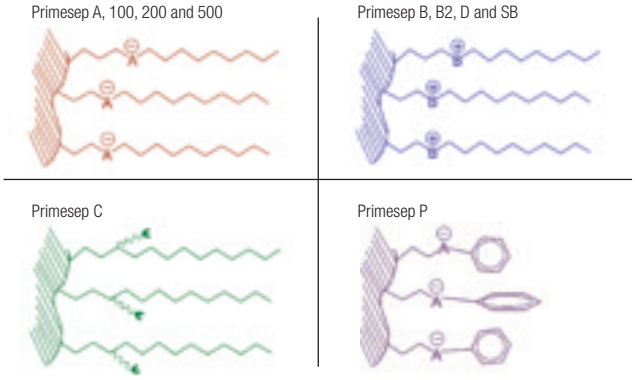


Figure 1. Primesep phases

Each Primesep column has a dual chemistry stationary phase containing a hydrophobic long alkyl chain and an ionisable cationic or anionic embedded group. Reversed-phase and ion-exchange interactions can be controlled independently; reversed-phase by organic concentration and ion-exchange by eluent ionic strength and pH. All columns offer the same hydrophobic retention properties but differ in their ion-exchange and other properties.

Primesep columns are suitable for analytical and preparative scale separations in isocratic and gradient modes, and are compatible with all common detection techniques.

These columns are resistant to dewetting in 100% aqueous eluent and are stable in pure organic and highly acidic conditions down to pH 1.5. They can efficiently separate organic and inorganic ions on the same column at the same time. This enables an organic pharmaceutical to be quantified simultaneously with its inorganic counter ion. Also, inorganic cations and anions can be run together without a specialised ion chromatography system.

The choice of buffer for use with Primesep columns depends on the detection technique. For UV detection, TFA, sulphuric acid, phosphoric acid and their salts are recommended. For LC-MS or ELSD detection, the best choices are TFA, ammonium formate, ammonium acetate, formic and acetic acids.

Primesep® Embedded Acidic Phases

Primesep A, 100, 200 and 500

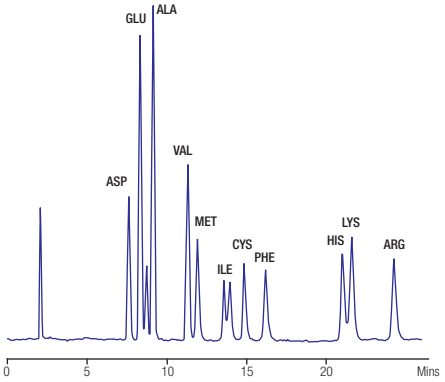
Primesep® A, 100, 200 and 500 are reversed-phase columns with different strengths of embedded acidic (anionic) ion-pairing groups. Primesep A is the strongest acidic column, while Primesep 500 is the weakest acidic column. Differences in functional group acidity allow selection of the most appropriate column for a particular set of basic compounds that differ in their pka value.

The embedded acidic functional group can be in an ionised form, or in a non-ionised form, depending on the pH of the eluent. When these two forms (see below) are in equilibrium, the phase is half ionised and half non-ionised. In order to achieve component retention by ion-exchange on these Primesep columns, eluent pH should be close to or above the transition value, as shown below.

Primesep Phase	Transition pH
A	Ionized at all working pH
100	pH 1
200	pH 2
500	pH 5
C	pH 3.5

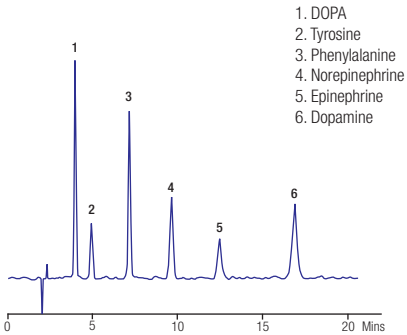
Ionised and non-ionised forms of Primesep embedded acidic phases

Primesep 100 and 200 are versatile columns for separation of a broad range of compounds. Figure 2 shows the separation of underivatized amino acids on Primesep 100. Figure 3 illustrates the application of Primesep 200 for catecholamines.



Column: Primesep 100 (5µm, 150 x 4.6mm)
Eluent: CH_3CN - H_2O (30:70)
Gradient of 0.05 to 0.3% TFA in 25mins
Flow rate: 1ml/min
Detection: ELSD

Figure 2. Analysis of amino acids on Primesep 100



Column: Primesep 200 (5µm, 150 x 4.6mm)
Eluent: CH_3CN - 10mM ammonium formate, pH 2.9 (90:10)
Flow rate: 1ml/min
Detection: UV, 210nm

Figure 3. Analysis of catecholamines on Primesep 200

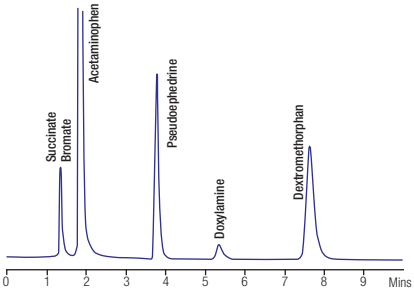
Primesep C

Primesep C also contains an embedded anionic group (carboxylic acid) but with additional complex-formation properties. The latter facilitates retention of amines, sulphonium, phosphonium and metal ions. The degree of ion-exchange and complex formation can be effectively adjusted by the alteration of eluent pH, within the range 3 – 7. The unique complex forming properties of Primesep C columns lead to a reversal of elution order compared to ion-exchange, eg. t_R $Li^+ > Na^+ > K^+$. Similarly primary amines are retained longer than secondary and tertiary amines on Primesep C columns.

Figure 4 shows the analysis of the active ingredients in a cough and cold medicine on Primesep C.

Primesep P

The Primesep P phase provides three interactions with analytes – reversed-phase, π-π interaction and strong cation-exchange. It contains embedded acidic ion-pairing groups combined with an aromatic moiety and is useful for the separation of structural isomers of aromatic compounds. Enhanced π-π interaction can be achieved by adding THF to CH_3CN-H_2O eluents.



Column: Primesep C (5µm, 150 x 4.6mm)
Eluent: CH_3CN - 50mM TEA phosphate, pH 3.0 (60:40)
Flow rate: 1ml/min
Detection: UV, 205nm

Figure 4. Analysis of active ingredients in cough and cold drugs on Primesep C

Primesep® Embedded Basic Phases

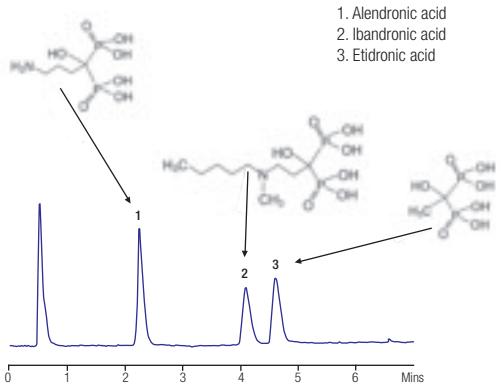
Primesep B, B2 and SB

Primesep® B, B2 and SB contain embedded basic ion-pairing groups. In addition to improving the retention of acidic compounds by anion-exchange, the phases separate bases by an ion exclusion mechanism. Primesep B and SB are strong basic columns for operation in the pH range 1.5 to 4.5 and 1.5 to 5 respectively, created by the addition of TFA, phosphoric or perchloric acids to the eluent. Primesep B2 is a weak basic column that offers an extended pH range from 1.5 to 7, suitable for use with appropriate buffered solutions. For development of new methods, Primesep B2, SB or D are recommended, due to the extended pH stability range. Primesep B, B2, SB and D are fully ionised at all working pH values.

Figure 5 shows the simultaneous separation of dronic acids on Primesep SB.

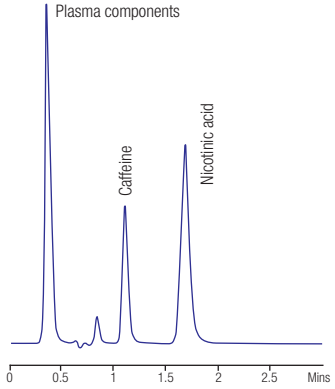
Primesep D

Primesep D comprises an anion-exchange group embedded in a long alkyl chain. It is a weaker basic column than Primesep B2. In addition, Primesep D allows direct injection of plasma and other biofluids, enabling a broad range of small molecules to be analysed via a single column without any sample preparation. At pH 3.0, most proteins become positively charged and are excluded from the stationary phase, whilst small hydrophobic molecules are retained. Figure 6 illustrates a direct plasma injection on Primesep D.



Column: Primesep SB (5µm, 50 x 4.6mm)
Eluent: CH_3CN - TFA (10:90)
TFA gradient 0.03 to 0.3% in 7mins
Flow rate: 1.0ml/min
Detection: ELSD

Figure 5. Analysis of dronic acids on Primesep SB



Column: Primesep D (5µm, 50 x 4.6mm)
Eluent: CH_3CN - H_2O - HCO_2H (33:67:0.2)
Flow rate: 1ml/min
Detection: UV, 250 nm

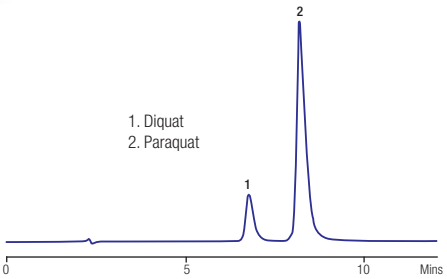
Figure 6. Direct plasma analysis on Primesep D

Embedded Acidic and Basic Phase

Primesep AB

Primesep AB is a zwitterionic reversed-phase column with embedded cation-exchange and anion-exchange functionalities, combining the properties of both in a single column. With basic analytes, Primesep AB behaves as if it has a negatively charged surface. Conversely, with strong acids Primesep AB behaves as if it has a positively charged surface. Anions and cations can be separated at the same time as neutrals. This is useful for complex mixtures which include polar ionisable compounds both of acidic and basic nature. The column is also capable of separating both the anion and cation of the same salt. This is important in analysis of pharmaceutical formulations, drug substances and other organic and inorganic salts.

Figure 7 shows the separation of a mixture of the quaternary compounds diquat and paraquat on Primesep AB.



Column: Primesep AB (5µm, 150 x 4.6mm)
Eluent: CH_3CN - 0.3% TFA (10:90)
Flow rate: 1ml/min
Detection: UV, 250 nm

Figure 7. Separation of mixture of diquat and paraquat on Primesep AB

Selectivity of Primesep® Phases

Figure 8 shows a comparison of the relative selectivities of each of the Primesep® phases for a standard mixture of benzonitrile, dopamine and 3,5-dihydroxybenzoic acid. The neutral benzonitrile is retained similarly on all columns, due to a reversed-phase mechanism. Under these analytical conditions, the cation-exchange Primesep phases A, 100, 200 and P show greater retention for the basic dopamine (peak 2), with Primesep A showing the strongest interaction. Conversely, the anion-exchange Primesep phases B, D and B2 (and SB) show the greatest retention for the acidic 3,5-dihydroxybenzoic acid (peak 3).

Table 2 highlights the relative cationic and anionic strengths of the various Primesep phases.

Table 2. Relative Strengths of Primesep Phases

Cation-Exchange		Anion-Exchange
Primesep A	Strong ↓	Primesep SB
Primesep 100, P		Primesep B
Primesep 200		Primesep D
Primesep C	Weak	Primesep B2

Method Development Kits

As an aid to method development, kits containing 3, 4 or 5 columns of the same dimensions can be supplied. Custom kits allow customer selection of the most appropriate phases for a given application.

eg. Most popular kit: Primesep 100, B2 and C
Most diverse kit: Primesep 100, B2, C and 200
Most possible applications: Primesep 100, B2, C, D and 200

Please contact Hichrom for ordering information for method development kits.

Screening service

SIELC offer a free and confidential screening service on their Primesep and Obelisc columns, to assist in column selection. Please contact Hichrom for details of this service.

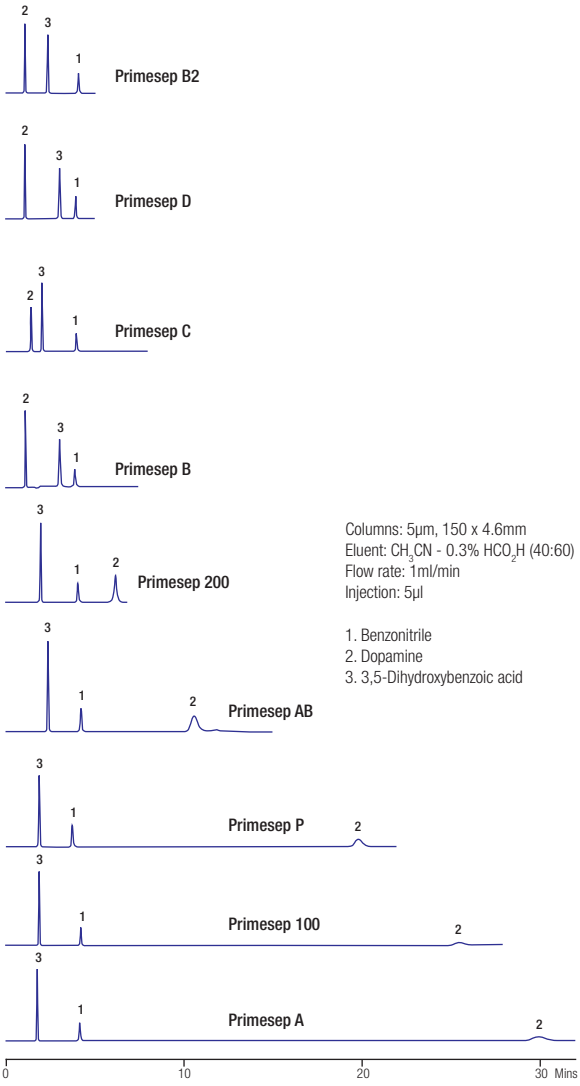


Figure 8. Selectivity comparison of Primesep phases

Please contact Hichrom to request a copy of the latest Primesep product brochures.

Ordering Information – Primesep® (5µm, 100Å phases)[1]

When ordering please replace '**X**' with phase type ie. A, 100, 200, 500, C, B, B2, D, P, AB, SB

Column i.d.[2] (mm)	Column Length[2] (mm)				Guards[3] (2/pk)
	50	100	150	250	
2.1	**X**-21.050.0510	**X**-21.100.0510	**X**-21.150.0510	**X**-21.250.0510	**X**-21.G.0510
3.2	**X**-32.050.0510	**X**-32.100.0510	**X**-32.150.0510	**X**-32.250.0510	**X**-32.G.0510
4.6	**X**-46.050.0510	**X**-46.100.0510	**X**-46.150.0510	**X**-46.250.0510	**X**-46.G.0510
10	**X**-100.050.0510	**X**-100.100.0510	**X**-100.150.0510	**X**-100.250.0510	-
22	**X**-220.050.0510	**X**-220.100.0510	**X**-220.150.0510	**X**-220.250.0510	-
22 (10µm)	**X**-220.050.1010	**X**-220.100.1010	**X**-220.150.1010	**X**-220.250.1010	-

[1] Other pore sizes and particle sizes available [2] Other dimensions available [3] Direct connect - no holder required

Primesep direct connect guard column

Preparative Separations

Primesep® columns offer high capacity ion-exchange mechanisms for the retention of polar compounds. This enables these columns to be successfully used for scale-up separations. Conditions can be developed to be efficient and economical for preparative separations. For example, for ionisable compounds, conditions can be chosen where a high concentration of organic modifier is present, reducing the cost of solvent removal. In addition, the ability to reverse the elution order of differently charged components in the mixture, makes Primesep particularly effective for isolating specific components. Figure 9 demonstrates the high loading capacity of a Primesep 100 analytical column, which infers a high capacity ion-exchange mechanism on scaling-up to preparative dimensions.

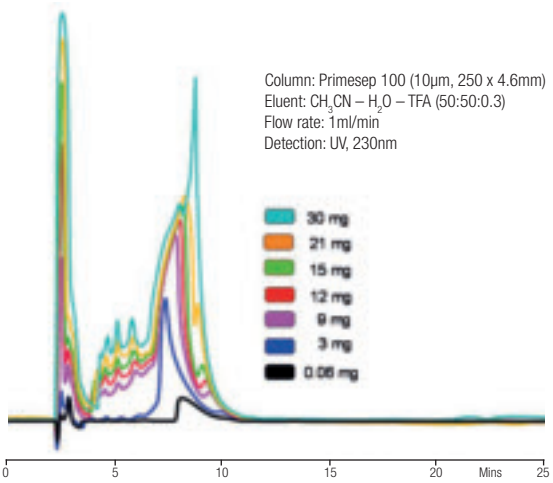


Figure 9. Loading study of HCl salt of polar compound

PROMIX™

- Peptide and protein separations
- 2D HPLC with single column
- Alternative selectivity to reversed-phase 300Å columns
- Scalable from capillary to preparative

Promix Phase	Particle Size (µm)	Pore Size (Å)
SP	5, 10	100
AP	5, 10	100, 300
MP	5, 10	300, 800
LP	5, 10	800

The Promix™ range of columns, manufactured by SIELC, is designed for biomolecule analysis, in particular the analysis and purification of peptides and proteins, and for proteomics applications. Promix columns have the benefit of performing 2D HPLC with a single column, based on a combination of ionic and reversed-phase interactions.

Independent control of acid/buffer concentration and organic modifier offers almost unlimited control of retention and selectivity. Promix columns show enhanced selectivity compared to dedicated reversed-phase or ion-exchange columns for closely related peptides and proteins, being able to separate compounds differing in sequence by a single amino acid pair only. Methods on these columns are completely scalable from capillary to preparative. Figure 10 shows the separation of insulin analogues differing in sequence by a single amino acid pair.

Please contact Hichrom for ordering information for Promix columns.

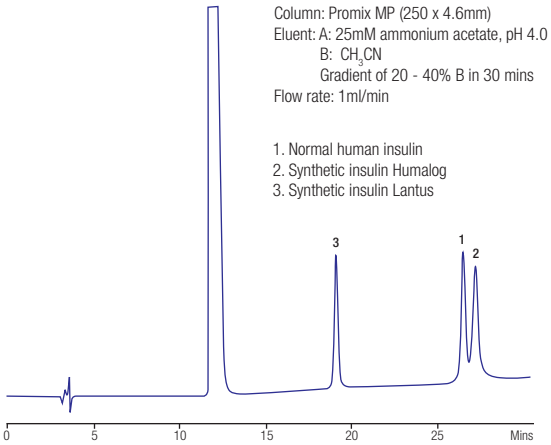


Figure 10. Separation of human and synthetic insulins on Promix