VertiSep™ pHendure HPLC Columns

Practical Extreme pH in RP-HPLC Using Hybrid Particle Based Packings

Introduction

Spherical hybrid organic–inorganic particles combine the high efficiency and excellent mechanical strength of silica with the wide pH stability range and reduced silanol effects of polymers. Reversed-phase HPLC packing based on hybrid particles exhibits excellent efficiency, selectivity and long lifetimes at elevated temperatures.

The problem of extreme pH in silica column

Silica-based materials are the most widely used packings for RP-HPLC due to the high efficiency and excellent mechanical strength. However, there are some limitations on these conventional silica-base materials.

- **Short lifetimes below pH 2 (hydrolysis of bonded phase)**
- **Short lifetimes above pH 8 (dissolution of silica)**
- **Broad, tailing peaks for basic compounds**
- **Short life time at high temperatures**

Why operate outside pH 2 – 8?

The mobile phase pH is an important factor in retention, selectivity and efficiency for polar compounds. pH also influences analyte ionisation, which is necessary for LC-MS. Finally, the stability of the analyte may be improved at high or low pH. A good column for low and high pH should offers acceptable lifetime, no bleed, stable retention and efficiency.

What is Hybrid particle based packings?

The Hybrid particle contains both silica and organosiloxane and shares the advantages of both. The Hybrid particle is prepared by sol-gel synthesis using organosilanes. This particle is as hard as silica and does not swell or shrink in the presence of organic solvents. The pH stability of bonded phases based on this Hybrid particle exceeds that of silica-based bonded phases.

Specifications	C18	Phenyl-Hexyl
Particle sizes (µm)	2.5, 5	2.5, 5
Pore Sizes (Å)	100	100
Surface area (m^2/g)	350	350
Carbon load (%C)	14	12
Endcapping	propriety	propriety
pH Range	1-12	2-12
USP	L1	L11

What is VertiSep™ pHendure

The new VertiSep™ pHendure from Vertical Chromatography is a Hybrid organic-inorganic particle based packings providing extended pH Stability from 1-12, a novel solution to the problems exhibited by conventional silica-based and polymer-based packing materials.

What are the features?

- **long lifetimes below pH 2 (Low pH stability)**
- **long lifetimes above pH 8 (high pH stability)**
- **Long life time at high temperatures(stability of hybrid particle)**
- **Low bleed, ideal for both LC-MS and routine HPLC method development (stability of hybrid particle)**

VertiSep™ pHendure HPLC columns are packed with hybrid silica-organosilane particle based packings providing pH stability pH 1-12 and high resolution, a novel solution to the problems on acidic or basic conditions exhibited by conventional silica-based columns.

Hydrolysis of bonded phase will occur and causes loss of efficiency under acidic conditons with conventional silica-based column. VertiSep™ pHendure with the hybrid particle based packings solves this problem and provides excellent stability under acidic conditions offerring long lifetime.

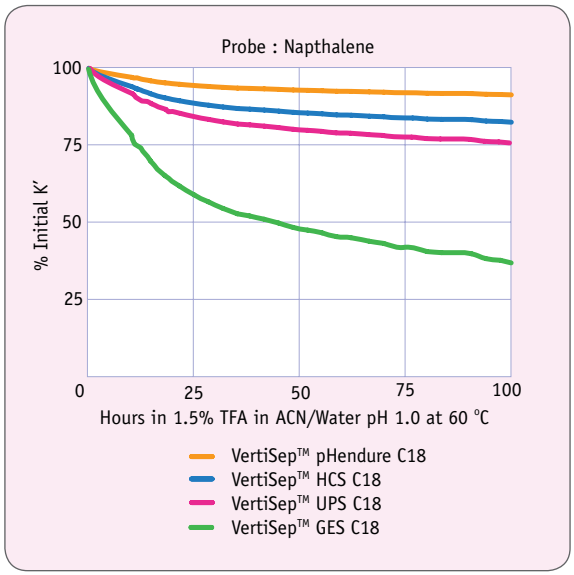
Dissolution of silica will occurs and causes tailing peaks under basic conditions with conventional silica-based column. VertiSep™ pHendure with the hybrid particle based packings solves this problem and provides excellent stability under basic conditions offering symmetrical peaks.

VertiSep™ pHendure is low bleed, ideal for both LC-MS and routine HPLC method development.

VertiSep™ pHendure C18 is 14% carbon loading offering high capacity and great resolution for all HPLC applications including USP L1.

VertiSep™ pHendure Phenyl-Hexyl is 12% carbon loading offering an alternative selectivity to C18 for analytes containing aromatic rings or USP L11.

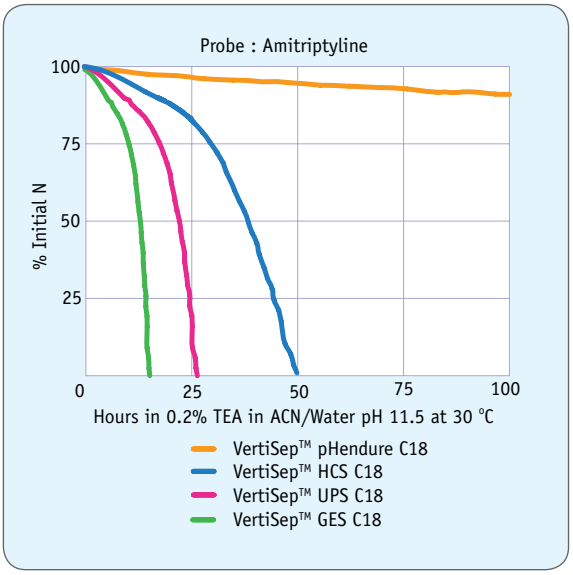
Stability under acidic and high temperature conditions

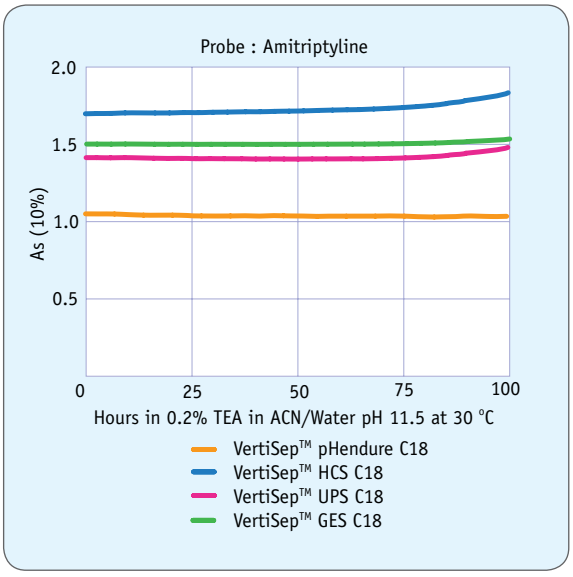


VertiSep™ pHendure C18

Hybrid Particle columns were exposed to 1.5% Trifluoroacetic acid in ACN/Water at pH 1.0 at 60 °C for 100 hours at a flow rate of 1mL/min, then tested the columns with a standardized test condition and Napthalene as test probe. VertiSep™ pHendure hybrid particle exhibits excellent stability under acidic conditions better than conventional silica-based packings.

Stability under basic conditions



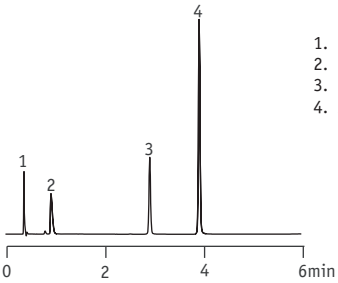


VertiSep™ pHendure C18

Hybrid Particle columns were exposed to 0.2% Triethylamine in ACN/Water at pH 11.5 at 30 °C for 100 hours at a flow rate of 1mL/min, then tested the columns with a standardized test condition and amitriptyline as test probe. VertiSep™ pHendure hybrid particle exhibits excellent stability and provides symmetrical peak for basic compounds under basic conditions better than conventional silica-based packings.

VertiSep™ pHendure

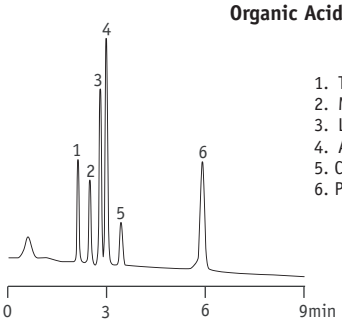
Test Mixed



1. Uracil
2. Procaine
3. Fenuron
4. 3-Nitrobenzoic acid

Column:	VertiSep™ pHendure C18 5µm 4.6x150mm
Mobile Phase:	ACN:20 mM Sodium phosphate (A:B), pH 2.1
Gradient:	0 min 10%ACN, 5.5 min 50%ACN
Flow Rate:	1.5mL/min
Detection:	UV254nm

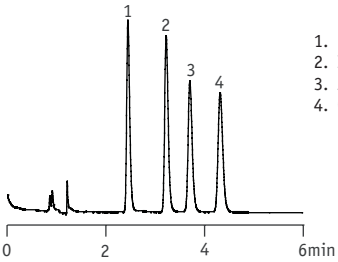
Organic Acid



1. Tartaric acid
2. Malic acid
3. Lactic acid
4. Acetic acid
5. Citric acid
6. Propionic acid

Column:	VertiSep™ pHendure Phenyl-Hexyl 5µm 4.6x150mm
Mobile Phase:	20 mM Phosphate buffer, pH 2.5:Methanol (97:3)
Flow Rate:	1.0mL/min
Detection:	UV220nm

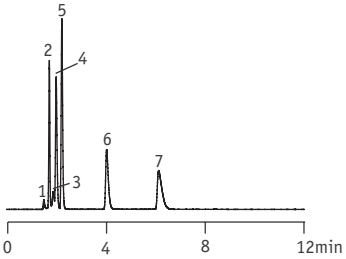
Tricyclic Antidepressants



1. Nortriptyline
2. Imipramine
3. Amitriptyline
4. Clomipramine

Column:	VertiSep™ pHendure C18 5µm 4.6x150mm
Mobile Phase:	10 mM Ammonium bicarbonate buffer pH 10.5:ACN (25:75)
Flow Rate:	1.5mL/min
Detection:	UV254nm

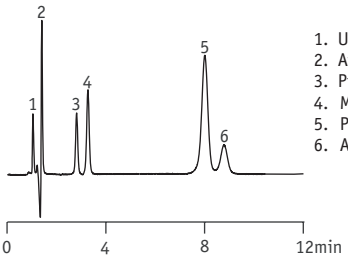
Cold Remedy Mix



1. Chloride
2. Phenylephrine
3. Bromide
4. Maleic acid
5. Acetaminophen
6. Chlorpheneramine Maleate
7. Dextromethorphan Hydrobromide

Column:	VertiSep™ pHendure C18 5µm 4.6x150mm
Mobile Phase:	Phosphate buffer, pH 2.5:Methanol:ACN (60:24:16)
Flow Rate:	1.5mL/min
Detection:	UV215nm

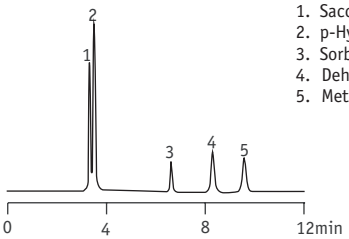
Mix of ß-Blocker



1. Uracil
2. Atenolol
3. Pindolol
4. Metoprolol
5. Propanolol
6. Alprenolol

Column:	VertiSep™ pHendure C18 5µm 4.6x150mm
Mobile Phase:	ACN:50 mM Triethylamine lamine acetate (60:40), pH 11
Flow Rate:	0.43mL/min
Temp:	20 °C
Detection:	UV230nm

Food Additives



1. Saccharin
2. p-Hydroxybenzoic Acid
3. Sorbic Acid
4. Dehydroacetic Acid
5. Methylparaben

Column:	VertiSep™ pHendure Phenyl-Hexyl 5µm 4.6x150mm
Mobile Phase:	20mM Potassium Phosphate pH 2.5:ACN/Water (25/75)
Flow Rate:	1.0mL/min
Detection:	UV220nm

Ordering Information

Phase	Particle Size (µm)	I.D. Length (mm)	QTY	Part No.
VertiSep™ pHendure				
C18	2.5	2.1 x 50	1	033A-B291
	2.5	2.1 x 100	1	033A-B391
	2.5	4.6 x 50	1	033A-E291
	2.5	4.6 x 100	1	033A-E391
	5	4.6 x 50	1	033A-E221
	5	4.6 x 100	1	033A-E321
	5	4.6 x 150	1	033A-E421
	5	4.6 x 250	1	033A-E521
	5	10 x 150	1	033A-H421
	5	10 x 250	1	033A-H521
	5	21.2 x 150	1	033A-I421
	5	21.2 x 250	1	033A-I521
	5	30 x 150	1	033A-J421
	5	30 x 250	1	033A-J521
Phenyl-Hexyl	2.5	2.1 x 50	1	0334-B291
	2.5	2.1 x 100	1	0334-B391
	2.5	4.6 x 50	1	0334-E291
	2.5	4.6 x 100	1	0334-E391
	5	4.6 x 50	1	0334-E221
	5	4.6 x 100	1	0334-E321
	5	4.6 x 150	1	0334-E421
	5	4.6 x 250	1	0334-E521
	5	10 x 150	1	0334-H421
	5	10 x 250	1	0334-H521
	5	21.2 x 150	1	0334-I421
	5	21.2 x 250	1	0334-I521
	5	30 x 150	1	0334-J421
	5	30 x 250	1	0334-J521

Ordering Information

Phase	Particle Size (µm)	I.D. Length (mm)	QTY	Part No.
VertiSep™ pHendure Guard Cartridges*				
C18	2.5	2.1 x 10	2	033A-B193
	5	4.6 x 10	2	033A-E123
	5	10 x 10	2	033A-H123
	5	21.2 x 10	2	033A-I123
Phenyl-Hexyl	2.5	2.1 x 10	2	0334-B193
	5	4.6 x 10	2	0334-E123
	5	10 x 10	2	0334-H123
	5	21.2 x 10	2	0334-I123

*Guard holder required

0300-0001

0300-0002

0300-0003

Ordering Information

Description	QTY	Part No.
Guard Holder with Coupler		
For column I.D. 2.1-7.8 mm	1	0300-0001
For column I.D. 10 mm	1	0300-0002
For column I.D. 21.2 mm	1	0300-0003