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Analytical Products

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Ascentis® Express 120 Å C18 pH+ U/HPLC Columns

Enhancing HPLC Performance in High pH and High-Temperature Environments

The Ascentis® Express 120 Å C18 pH+ U/HPLC column is built on the reliable Fused-Core® particle technology, enhancing speed and efficiency. It features surface-modified organo-silane technology that provides alkaline resistance, ensuring excellent stability in high pH environments. With a broad operational range of pH 2-12, Ascentis® Express 120 Å C18 pH+ facilitates robust method development and improved separations for basic compounds that often face challenges like poor peak shapes, insufficient retention, or limited load tolerance at low pH. It is particularly well-suited for use with high pH mobile phases.

Features & Benefits

- Exceptional stability for high pH and high-temperature applications
- Versatile performance across a wide range of operating conditions for selective separation of acids, bases, neutrals, and zwitterions
- Established Fused-Core® technology for enhanced separation speed and maximum chromatographic efficiency
- Excellent suitability for LC-MS applications

Ascentis® Express 120 Å C18 pH+	
Silica:	Type B (High purity silica)
Particle Platform:	Superficially porous particles (SPP)
Phase Chemistry:	Dimethyloctadecylsilane - surface modified
Endcapped:	Yes
USP:	L1
Particle Size:	2.7 µm
Pore Size:	120 Å
Carbon Load:	5.6%
Surface Area:	75 m^2/g
pH-Range:	2-12
Max. Temperature:	60 °C
Pressure Stability:	600 bar

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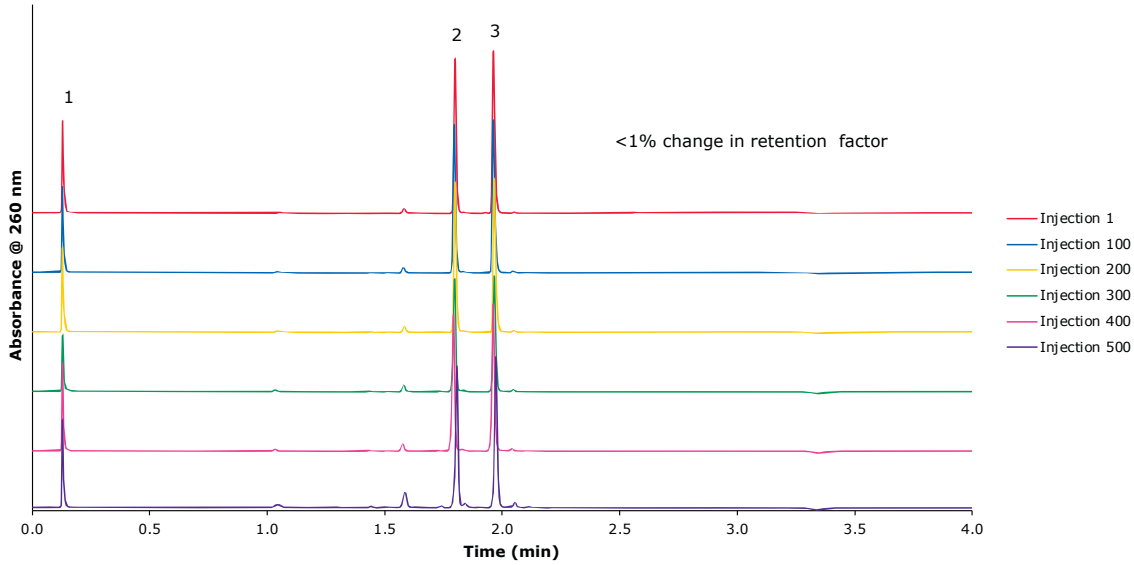
Designed for Exceptional Stability

The stability of the Ascentis® Express 120 Å C18 pH+ column was evaluated using 10 mM ammonium bicarbonate at pH 10 and 60 °C. Remarkably, there was less than a 1% change in retention over 20,000 column volumes. Additionally, the column exhibited symmetrical peak shapes with no increase in back pressure throughout the 500-injection stability test.



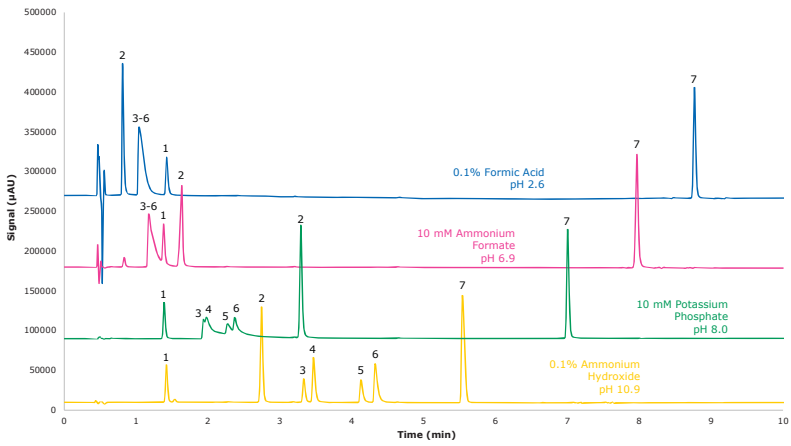
Sample compounds:

1. Uracil
2. Acenaphthene
3. Amitriptyline

Utilizing pH for Enhanced Separation Optimization

Adjusting pH is an effective approach for improving selectivity in method optimization. In this case study involving common drugs of abuse, the broad pH versatility of the Ascentis® Express 120 Å C18 pH+ column was employed for separation, accommodating various pKa values to create an optimized method that focuses on the key compounds of interest, particularly the amphetamines. A mixture of drugs of abuse and their metabolites was analyzed at different pH levels to identify the optimal separation conditions. Given that amphetamines have high pKa values, a high pH mobile phase is most suitable. This application illustrates how using low pH for high pKa compounds can lead to poor chromatography, while increasing the pH enhances chromatographic performance. The acidic compound (peak 7) and multiple charged species (peak 1) are less affected by the rising pH.

Chromatographic conditions		
Column:	Ascentis® Express 120 Å C18 pH+ (2.7 µm) 100 x 2.1 mm I.D. (**70016-U**)	
Mobile phase:	[A] aqueous as indicated: • 0.1% Formic acid (pH 2.6) • 10 mM Ammonium formate (pH 6.9) • 10 mM Potassium Phosphate buffer (pH 8.0) • 0.1% Ammonium hydroxide (pH 10.9) [B] Methanol	
Gradient:	Time	%B
	0.00	25.00
	3.00	50.00
	5.00	60.00
	10.00	95.00
	10.10	25.00
	15.00	25.00
Flow rate:	0.4 mL/min	
Pressure:	353 bar	
Column temp.:	40 °C	
Detector:	UV/PDA, 214 nm	
Injection:	5.0 µL	
Sample:	in 93:7 Water:methanol	

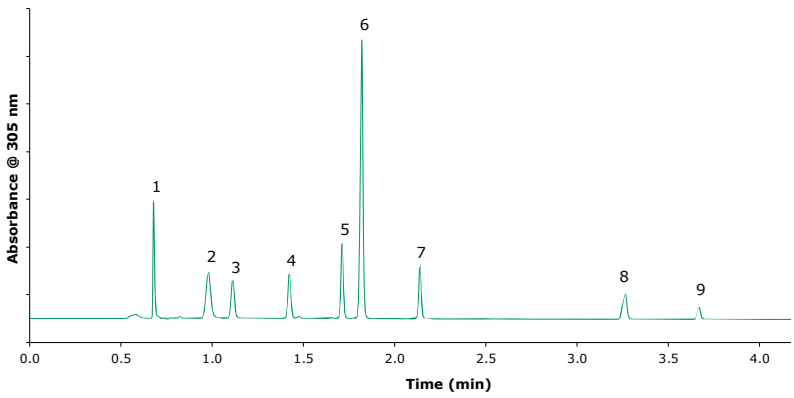


Sample compounds:

1. Benzoylecgonine, pKa 3.35/pKb 10.82
2. 6-Acetylmorphine, pKa 9.08
3. MDA, pKa 9.67
4. d-Amphetamine, pKa 9.9
5. MDMA, pKa 9.9
6. Methamphetamine, pKa 9.87
7. 11-nor-Δ^9-carboxy-THC (THC-COOH), pKa 4.8

Separation of Omeprazole and Related Compounds at High pH

HPLC separation of Omeprazole requires high pH stability due to its sensitivity to pH changes, which affect its ionization and stability. With a pKa around 4.0, Omeprazole is mainly protonated at lower pH and deprotonated at higher pH, impacting its interaction with the stationary phase and retention times. Its instability in acidic conditions makes stable pH essential for accurate quantification. Efficient separation relies on consistent retention behavior, which stable pH enhances, leading to better resolution and peak shape. Reproducibility is crucial, as pH variations can alter retention times and peak areas. Therefore, high pH stability is vital for maintaining Omeprazole's ionization state and achieving reliable results.



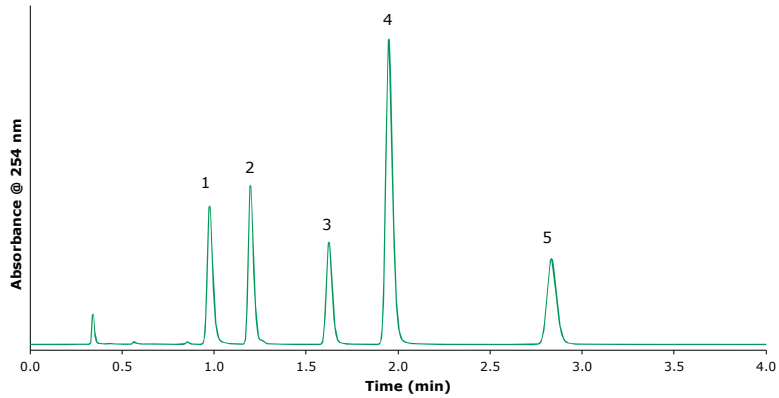
Sample compounds:

1. Related Compound F & G
2. Related Compound B
3. Related Compound E
4. Related Compound A
5. Impurity B
6. Omeprazole
7. Impurity H
8. N'-Methyl Omeprazole
9. Impurity C

Chromatographic conditions	
Column:	Ascentis® Express 120 Å C18 pH+ (2.7 µm) 150 x 2.1 mm I.D. (**70017-U**)
Mobile phase:	[A] 0.1% Ammonium hydroxide pH 10.6) in water [B] Acetonitrile
Gradient:	Time %B
	0.00 13.00
	3.30 53.00
	3.80 53.00
	3.90 13.00
	9.00 13.00
Flow rate:	0.4 mL/min
Column temp.:	60 ºC
Detector:	PDA, 305 nm
Injection:	1.0 µL
Sample:	Omeprazole and related compound in USP diluent

Fast Separation of Antidepressants

Tricyclic antidepressants (TCAs) are a class of medications mainly utilized for treating depression. The separation of these antidepressants is performed under high pH conditions using an Ascentis® Express 120 Å C18 pH+ column. This approach results in excellent peak shape and resolution in under 3 minutes, showcasing the benefits of Fused-Core® particle technology.



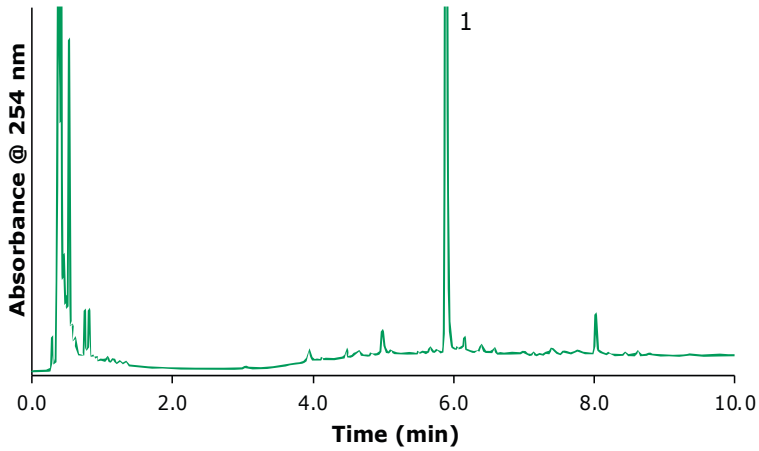
Chromatographic conditions	
Column:	Ascentis® Express 120 Å C18 pH+ (2.7 µm) 100 x 2.1 mm I.D. (**70016-U**)
Mobile phase:	[A] 10mM Ammonium bicrbonate (pH 10) [B] Acetonitrile
Isocratic:	60% B
Flow rate:	0.5 mL/min
Column temp.:	35 ºC
Detector:	UV/PDA, 254 nm
Injection:	0.2 µL

Sample compounds:

1. Nortriptyline
2. Doxepin
3. Imipramine
4. Amitriptyline
5. Trimipramine

Analyzing Nicotine from Cigarette Sample

High pH mobile phases are optimal for analyzing nicotine, as they enhance retention and improve peak shape compared to low pH conditions. A cigarette sample is analyzed using an Ascentis® Express 120 Å C18 pH+ column with a mobile phase consisting of ammonium hydroxide and acetonitrile at pH 10.15. Given that nicotine has a pKa of 8, it is typically analyzed at an elevated pH of 10. In this analysis of a commercial cigarette, the active stimulant, nicotine, is easily identified using the Ascentis® Express 120 Å C18 pH+ column.



Sample compounds:

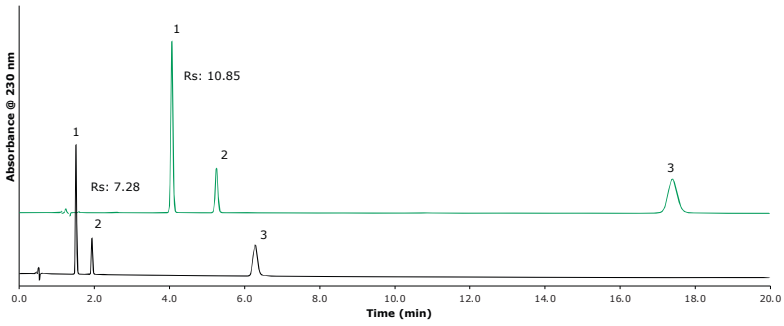
1. Nicotine

Chromatographic conditions		
Column:	Ascentis® Express 120 Å C18 pH+ (2.7 µm) 100 x 2.1 mm I.D. (**70016-U**)	
Mobile phase:	[A] Ammonium hydroxide (pH 10.15) in water [B] Acetonitrile	
Gradient:	Time	%B
	0.0	5.0
	2.0	5.0
	10.0	45.0
	11.0	45.0
	12.0	5.0
Flow rate:	0.4 mL/min	
Column temp.:	30 ºC	
Detector:	UV/PDA, 254 nm	
Injection:	0.5 µL	
Sample solvent:	95/5 Water/MeCN	

Modernization of the USP Method for Separating Lidocaine and Related Impurities

Lidocaine is widely used as a local anesthetic in pharmaceutical formulations. The USP organic impurities test method utilizes a C18 (L1) column for the HPLC separation. Under equal conditions the Ascentis® Express 120 Å C18 pH+ column delivers excellent performance with a high pH mobile phase (green trace).

By reducing the column ID and length the method runtime reduces by over 50% and mobile phase consumption significantly decreases, all while still achieving the impurity resolution requirement of at least 1.5 (black trace).



Sample compounds:

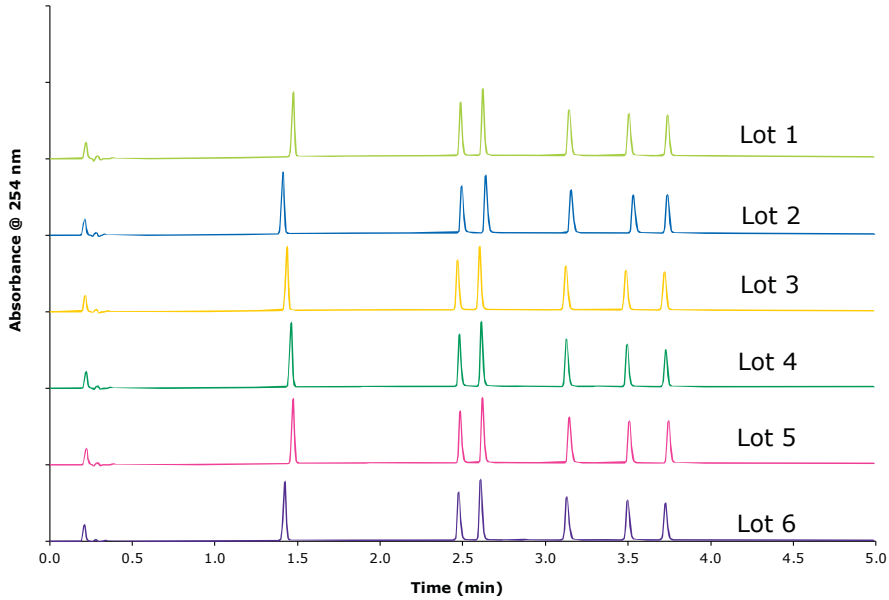
1. 2-Cl-N(2 - 6-dimethylphenyl) Acetamine (Imp. H)
2. 2 - 6-Dimethylaniline Hydrochloride (Imp. A)
3. Lidocaine

Chromatographic conditions	
Column:	Ascentis® Express 120 Å C18 pH+ (2.7 µm) 150 x 4.6 mm I.D. (**70023-U**) Ascentis® Express 120 Å C18 pH+ (2.7 µm) 100 x 2.1 mm I.D. (**70016-U**)
Mobile phase:	[A] Potassium phosphate buffer (pH 8) [B] Acetonitrile
Isocratic:	30% B
Flow rate:	1.0, 0.4 mL/min
Column temp.:	30 ºC
Detector:	UV/PDA, 230 nm
Injection:	20.0, 3.4 µL
Sample solvent:	Mobile phase

Excellent Reproducibility for Reliable Results

Lot-to-lot reproducibility is essential for method validation. Six distinct lots of Ascentis® Express 120 Å C18 pH+ columns were evaluated using a mixture of one neutral compound and five basic compounds. The separation was performed using a gradient with a Mobile Phase A: 0.1% NH_4OH, pH:10.7 and Mobile Phase B: Acetonitrile at 40 °C and a flow rate of 0.4 mL/min.

The average % RSD for the retention time across all compounds was less than 0.6%.



Sample compounds in order of elution:

1. Butyl Paraben
2. Doxylamine
3. Chlorpheniramine
4. Doxepin
5. Amitriptyline
6. Trimipramine

Ordering Information

Length (mm)		I.D. (mm)	Part Number	Description
50	x	1.5	**70012-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, 5 cm x 1.5 mm I.D.
100	x	1.5	**70013-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, 10 cm x 1.5 mm I.D.
150	x	1.5	**70014-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, 15 cm x 1.5 mm I.D.
50	x	2.1	**70015-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, 5 cm x 2.1 mm I.D.
100	x	2.1	**70016-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, 10 cm x 2.1 mm I.D.
150	x	2.1	**70017-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, 15 cm x 2.1 mm I.D.
50	x	3.0	**70018-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, 5 cm x 3.0 mm I.D.
100	x	3.0	**70019-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, 10 cm x 3.0 mm I.D.
150	x	3.0	**70020-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, 15 cm x 3.0 mm I.D.
50	x	4.6	**70021-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, 5 cm x 4.6 mm I.D.
100	x	4.6	**70022-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, 10 cm x 4.6 mm I.D.
150	x	4.6	**70023-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, 15 cm x 4.6 mm I.D.
250	x	4.6	**70024-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, 25 cm x 4.6 mm I.D.
5	x	2.1	**70025-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, Guard column 5 mm x 2.1 mm I.D.
5	x	3.0	**70026-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, Guard column 5 mm x 3.0 mm I.D.
5	x	4.6	**70027-U**	Ascentis® Express 120 Å C18 pH+, 2.7 µm, Guard column 5 mm x 4.6 mm I.D.

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