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UHPLC Columns



Columns for ultra high performance liquid chromatography

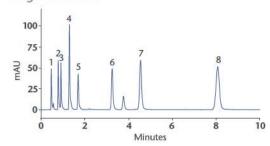
BlueOrchid sub-2 μ m columns represent a new level of performance in HPLC, offering faster separations with improved resolution.

Compared to conventional HPLC applications using 3–5 µm particle sizes, ultra fast separations with superior efficiency are made possible through the use of sub-2 µm particles on UHPLC systems, with significant improvements in sensitivity as well as in resolution. BlueOrchid columns packed with 1.8 µm particles feature a very narrow particle size distribution to minimize column back pressures to values in reach of conventional HPLC systems. BlueOrchid also offers outstanding peak symmetry, even for basic compounds.

Advantages

- faster analysis (up to 10 times)
- improved resolution
- enhanced sensitivity
- low back pressure
- excellent peak shape

Engelhardt test



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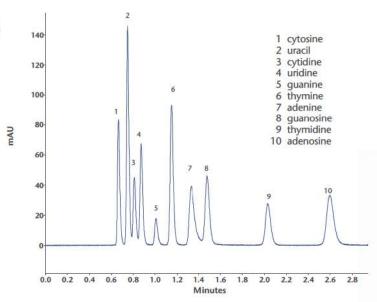


Application areas Ultra high performance liquid chromatography (UHPLC) columns with sub-2 µm particles can be used to full extent with HPLC systems which feature low system dead volume, fast data aquisition, sensitive detection, and the capability to operate at pressures up to 1000 bar. Compared with other sub-2 µm columns, BlueOrchid UHPLC columns induce relatively low back pressure, and thus show excellent results also in HPLC systems designed for fast LC separations. By decreasing the particle size of the packing material, the analyst can maximize the number of theoretical plates, making shorter column lengths possible. At the same time, the optimum range of mobile phase linear velocities is greatly expanded. As a result, higher flow rates can be used without loss of separation performance. Both factors result in faster analysis times and increased sample throughput. Cost per sample is also significantly reduced due to less mobile phase consumption and waste.

BlueOrchid UHPLC columns have a wide range of applications.

| Modification | Application areas | Separation mechanism |
|-----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|
| C18 | offers an extended pH range for analysis of acidic, basic and neutral analytes in reversed phase mode (sulphonamides, anabolic steroids, anti-psychotics, beta blockers, Sudan dyes, phenols, preservatives etc.) | hydrophobic interaction |
| C18 A | polar endcapped C18 phase for alternative selectivity; designed for use with 100% aqueous eluents for analysis of very polar compounds, basic pharmaceutical ingredients, water soluble vitamins, catecholamines as well as organic acids | hydrophobic and polar interaction |
| C8 | similar selectivity to C18 phase but less retention due to the lower hydrophobicity, useful for analysis of water soluble vitamins, steroids, catecholamines, sedatives etc. | reduced hydrophobic interaction compared to C18 phase |
| PFP | alternative selectivity to C18 phase, especially developed for analysis of fluorinated and other halogenated polar compounds (halogenated phenols, taxane samples, polar compounds with hydroxyl, carboxyl, nitro or other polar groups) | hydrophobic and polar interaction |
| C4 | low hydrophobicity with short alkyl chains, ideal for reversed phase separations of proteins and peptides | weak hydrophobic and hydrophilic interactions |
| Phenyl | alternative selectivity for aromatic and moderately polar analytes or mixtures with varying polarity and aromaticity | pi-pi interaction with aromatics |
| CN | for a wide range of applications in normal phase mode as well as reversed phase mode (steroids, carbohydrates, polar compounds) | hydrophobic and hydro- philic interactions |
| SAX | strong anion exchanger, ideally suited for use with smaller organic molecules, including nucleotides and organic acids | ion exchange inter- actions |
| HILIC | hydrophilic interaction chromatography phase which retains highly polar and hydrophilic com- pounds | hydrophilic interactions |
| PAL-HILIC | the polymer amine ligands are ideal for separating proteins, peptides, other anionic species and polar molecules; suited as anion exchange column and for HILIC separations | hydrophilic and ionic interaction |
| NH ₂ | suited to the analysis of sugars and carbohydrates, applicable in three modes: weak anion exchange, reversed phase and normal phase | hydrophobic and hydrophilic interactions |
| Si | suited for non-polar and moderately polar organic compounds using normal phase LC | hydrophilic interactions |

Nucleosides and bases: BlueOrchid PFP



Column: Mobile phase:

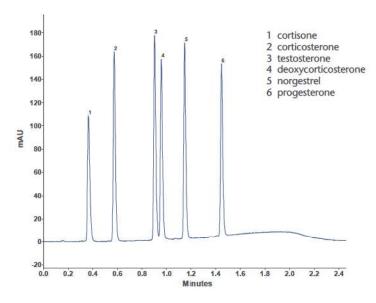
BlueOrchid PFP, 1.8 μ m 100 x 2 mm A: 20 mM NH₄ac pH 3.5 B: A/MeOH 90:10 (v/v) 90% A / 10% B

Isocratic: 0.5 ml/min Flow rate: Inj. volume:

1 μl 210 nm (100 Hz, 0.001 s), 10 mm, 2 μl flow cell 30 °C Detection:

Temp:

Anabolic steroids: BlueOrchid C18



BlueOrchid C18, 1.8 µm 50 x 2 mm A: water (+0.1 % formic acid) Column: Mobile phase: B: ACN (+0.1 % formic acid)

0–1.5 min 35%–75% B 1.5–2 min 75% B Gradient:

Flow rate: 1 ml/min Inj. volume: 0.5 µl

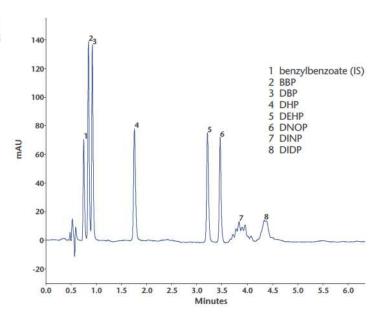
Detection: 254 nm (100 Hz, 0.005 s), 10 mm, 2 µl flow cell

Temp:





Phthalates: BlueOrchid C18





BlueOrchid C18, 1.8 μm, 100 x 2 mm A: H₂O / ACN 15 : 85 Column:

Mobile phase: B: ACN

Gradient:

0% B 1.2 min hold 0%-100% B 1.2-3.2 min 100% B 1.8 min hold

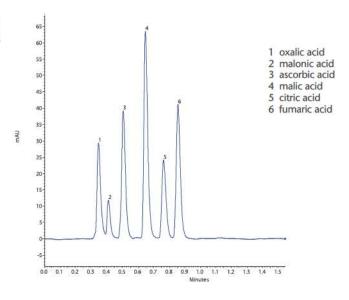
Flow rate: 0.5 ml/min

Inj. volume: 2 µl

225 nm (50 Hz, 0.05 s), 10 mm, 2 µl flow cell Detection:

Temp:

Organic acids: BlueOrchid C18 A



Column: BlueOrchid C18 A, 1.8 µm 50 x 2 mm $0.2 \text{ g NaH}_2\text{PO}_4$ (pH 2.5); isocratic 0.7 ml/min Mobile phase: Flow rate:

Inj. volume:

210 nm (100 Hz, 0.001 s), 10 mm, 2 µl flow cell Detection:

Temp:





Back pressure Due to its narrow particle size distribution, the column back pressure of BlueOrchid columns is lower than other high speed column materials on the market.

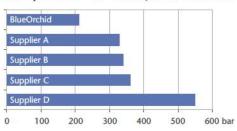
> Pressures up to 1000 bar (15000 psi) are possible. To preserve the column's lifetime, we recommend that BlueOrchid columns generally be used at pressures below 800 bar (10000 psi). This is an optimal balance between speed, resolution and column lifetime.

Typical back pressure¹⁾ **Dimensions**

| 320 bar (4800 psi) | 50 x 2 mm | |
|----------------------|------------|--|
| 550 bar (8 250 psi) | 100 x 2 mm | |
| 780 bar (11 700 psi) | 150 x 2 mm | |

1) measured with BlueOrchid C18 columns at ambient temperature at 500 µl/min, acetonitrile/water, 50:50

Back pressure²⁾ for sub-2 µm RP columns



2) measured with BlueOrchid C18, 50 x 2 mm at ambient temperature, flow rate: 500 µl/min, acetonitrile/water, 65:35

Column types

Eleven bonded phases and one plain silica material are available for reversed phase and normal phase chromatography. Every BlueOrchid column type features:

- exceptional peak symmetry and resolution
- 1.8 µm narrow particle size distribution for low back pressure
- next-generation ultra pure spherical silica
- outstanding pH stability

Modification Carbon load (%) rec. pH range Endcapping USP code Order no.

| C18 | 10 | 1-11 | yes | L1 | XXBI181B0E |
|-----------------|-----|------|-------------|-----|------------|
| C18 A | 12 | 2-9 | yes (polar) | L1 | XXBI184B0E |
| C8 | 8 | 2-9 | yes | L7 | XXBI081B0E |
| PFP | 8 | 2-8 | no | L43 | XXBI057B0E |
| C4 | 5 | 2-8 | no | L26 | XXBI040B0E |
| Phenyl | 8 | 2-8 | no | L11 | XXBI050B0E |
| CN | 4 | 2-8 | no | L10 | xxBI200B0E |
| SAX | 2.5 | 2-8 | no | L14 | XXBI150B0E |
| HILIC | 6 | 2-8 | no | | XXBI120B0E |
| PAL-HILIC | 6 | 2-8 | no | | XXBI130B0E |
| NH ₂ | 2 | 2-8 | no | L8 | XXBI190B0E |
| Si | _ | 2-8 | no | L3 | XXBT000B0E |

UHPLC column hardware The BlueOrchid stationary phase is packed in our new Vertex plus UHP hardware, specially design ned for ultra high-performance applications which demand pressure resistance up to 1000 bar (15 000 psi) and extremely low dead volume. Instead of traditional sieve-filter sandwiches, thin frits retain the packing material in the column for low dispersion. Various dimensions are available, providing more flexibility to adapt resolution and analysis speed.

Available column dimensions

| 30 x 2 mm | 50 x 2 mm | 100 x 2 mm | 150 x 2 mm |
|-----------|-----------|------------|------------|
| | | | |

Reproducibility

To obtain the most accurate results when performing quantitative analyses, it is important that retention time and peak area remain consistent. BlueOrchid columns are manufactured in ISO 9001:2008 accredited laboratories employing strict manufacturing procedures and 100% quality control testing. BlueOrchid columns show excellent reproducibility, column after column.

BlueOrchid columns are your first choice for new method development. Experience a new level of performance with BlueOrchid columns from KNAUER.

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Ordering information

| Ordering info | rmation | | |
|---------------|-----------------------|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Order No. | BlueOrchid C18 | Order No. | BlueOrchid HILIC |
| 03BI181B0E | 1.8 µm, 30 x 2 mm ID | 03BI120B0E | 1.8 μm, 30 x 2 mm ID |
| 05BI181B0E | 1.8 µm, 50 x 2 mm ID | 05BI120B0E | 1.8 μm, 50 x 2 mm ID |
| 10BI181B0E | 1.8 µm, 100 x 2 mm ID | | 1.8 µm, 100 x 2 mm ID |
| 15BI181B0E | | | 1.8 μm, 150 x 2 mm ID |
| Order No. | BlueOrchid C18 A | Order No. | BlueOrchid PAL-HILIC (weak) |
| 03BI184B0E | 1.8 µm, 30 x 2 mm ID | 03BI130B0E | 1.8 μm, 30 x 2 mm ID |
| 05BI184B0E | 1.8 μm, 50 x 2 mm ID | 05BI130B0E | 1.8 μm, 50 x 2 mm ID |
| 10BI184B0E | 1.8 μm, 100 x 2 mm ID | 10BI130B0E | 1.8 μm, 100 x 2 mm ID |
| 15BI184B0E | 1.8 μm, 150 x 2 mm ID | 15BI130B0E | 1.8 μm, 150 x 2 mm ID |
| Order No. | BlueOrchid C8 | Order No. | BlueOrchid NH ₂ |
| | 1.8 μm, 30 x 2 mm ID | 03BI190B0E | 1.8 μm, 30 x 2 mm ID |
| 05BI081B0E | | 05BI190B0E | |
| 10BI081B0E | 1.8 µm, 100 x 2 mm ID | 10BI190B0E | 1.8 μm, 100 x 2 mm ID |
| 15BI081B0E | 1.8 μm, 150 x 2 mm ID | 15BI190B0E | 1.8 μm, 150 x 2 mm ID |
| Order No. | BlueOrchid PFP | Order No. | BlueOrchid Si |
| 03BI057B0E | 1.8 μm, 30 x 2 mm ID | 03BI000B0E | 1.8 μm, 30 x 2 mm ID |
| 05BI057B0E | 1.8 μm, 50 x 2 mm ID | 05BI000B0E | 1.8 μm, 50 x 2 mm ID |
| 10BI057B0E | 1.8 μm, 100 x 2 mm ID | 10BI000B0E | 1.8 μm, 100 x 2 mm ID |
| 15BI057B0E | 1.8 μm, 150 x 2 mm ID | 15BI000B0E | 1.8 μm, 150 x 2 mm ID |
| Order No. | BlueOrchid C4 | Order No. | BlueOrchid RP Method Development Kit |
| 03BI040B0E | 1.8 µm, 30 x 2 mm ID | A66050 | C18 1.8 µm, 50 x 2 mm ID |
| 05BI040B0E | 1.8 μm, 50 x 2 mm ID | | C18A 1.8 µm, 50 x 2 mm ID |
| 10BI040B0E | 1.8 µm, 100 x 2 mm ID | | C8 1.8 μm, 50 x 2 mm ID |
| 15BI040B0E | 1.8 μm, 150 x 2 mm ID | A66100 | C18 1.8 µm, 100 x 2 mm ID C18A 1.8 µm, 100 x 2 mm ID |
| Order No. | BlueOrchid Phenyl | | C8 1.8 µm, 100 x 2 mm ID |
| 03BI050B0E | 1.8 µm, 30 x 2 mm ID | | The Company of the C |
| 05BI050B0E | 1.8 µm, 50 x 2 mm ID | | |
| 10BI050B0E | 1.8 µm, 100 x 2 mm ID | | |
| 15BI050B0E | 1.8 μm, 150 x 2 mm ID | | MANAGE |
| Order No. | BlueOrchid CN | | |
| 03BI200B0E | 1.8 µm, 30 x 2 mm ID | | Marie Control of the |
| 05BI200B0E | 1.8 µm, 50 x 2 mm ID | | |
| 4 ADTT AAADAE | 4.0 4.00 0 10 | | |





Technical data are subject to change without notice.

BlueOrchid SAX

10BI200B0E 1.8 µm, 100 x 2 mm ID 15BI200B0E 1.8 µm, 150 x 2 mm ID

03BI150B0E 1.8 μm, 30 x 2 mm ID 05BI150B0E 1.8 μm, 50 x 2 mm ID 10BI150B0E 1.8 μm, 100 x 2 mm ID 15BI150B0E 1.8 μm, 150 x 2 mm ID

Visit www.knauer.net for details on complete HPLC systems, HPLC columns, and osmometers.

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