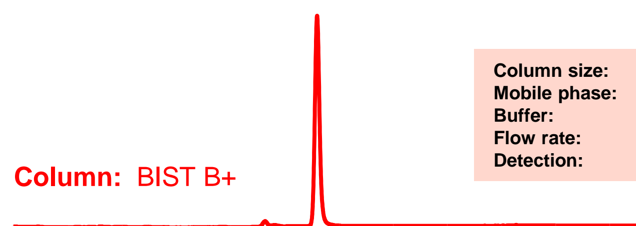
CoAp # 2023-2

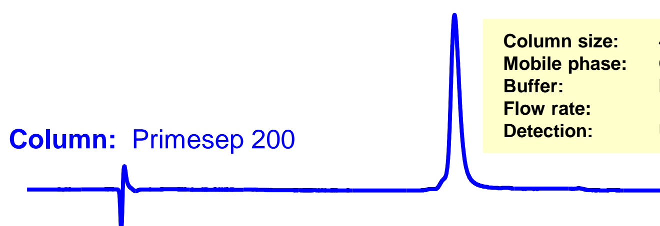
Cool Applications

“Making Tough LC Applications Look Cool”

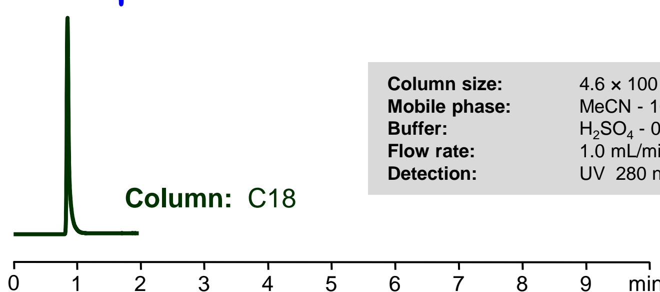
HPLC Methods for Analysis of DAB (3,3’- Diaminobenzidine)



Column size:	4.6 × 100 mm, 5 µm
Mobile phase:	Gradient MeCN 80-30%, 10 min
Buffer:	H_2SO_4 -0.2%
Flow rate:	1.0 mL/min
Detection:	UV 280 nm

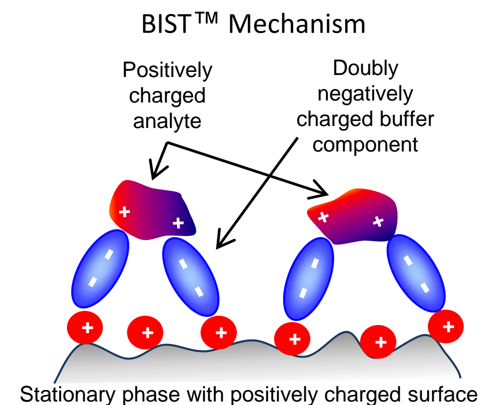


Column size:	4.6 × 100 mm, 5 µm
Mobile phase:	Gradient MeCN 10-40%, 10 min
Buffer:	H_2SO_4 -0.05%
Flow rate:	1.0 mL/min
Detection:	UV 280 nm

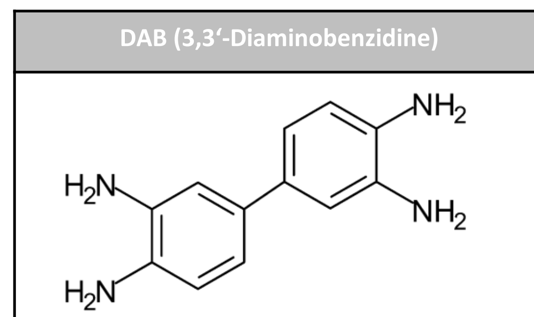


Column size:	4.6 × 100 mm, 5 µm
Mobile phase:	MeCN - 10%
Buffer:	H_2SO_4 - 0.1%
Flow rate:	1.0 mL/min
Detection:	UV 280 nm

BIST™ Mechanism



DAB (3,3‘-Diaminobenzidine)



Application Comments

3,3'-Diaminobenzidine (DAB) is most commonly used in biological and medical research as a chromogen for staining procedures. It is employed in various laboratory techniques such as immunohistochemistry (IHC), immunoblotting (commonly known as Western blotting), and enzyme-linked immunosorbent assays (ELISA).

DAB is a suspected carcinogen, meaning that it may have the potential to cause cancer. This is due to the mutagenic effect when DAB is metabolized in the body with some of the products being mutagenic.

DAB cannot be retained using a standard reverse-phase column. Here, we provide two different method for its retention. The first method uses our new Bridge Ion Separation Technique (BIST), while the second method uses a mixed-mode Primesep 200 column. On a Primesep 200, DAB is retained primarily through the ion exchange mechanism (though hydrophobicity does play a role as without the gradient, the retention is slightly higher and peak is wider), and to affect its retention/elution, buffer concentration can be adjusted. While in BIST, DAB is retained on the stationary phase until the water concentration is increased sufficiently that the ion solvation breaks the bridge formed between the stationary phase and the analyte. This bridge is formed by the double-charged ions of the MP.

Visit www.sielc.com to learn more about Primesep 200 and BIST B+ columns.