

3,3'-Diaminobenzidine (DAB) by LC-MS
LC-MS method with excellent retention and peak shape

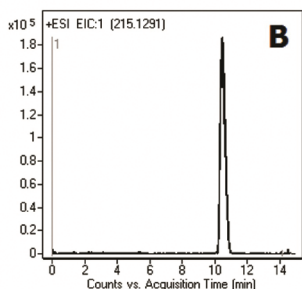
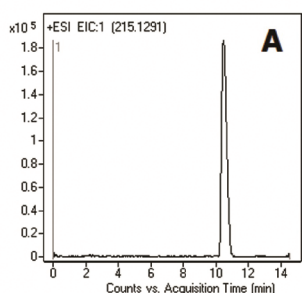
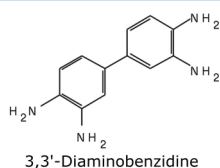


Figure A: single injection
Figure B: 5-run overlay, 3 injections done one day, 2 injections done the next

Note: DAB reacts with hemoglobin (an oxidation reaction catalyzed by the heme groups) in the presence of hydrogen peroxide producing a dark brown color. This reaction is used to stain cells that were prepared with hydrogen peroxidase enzyme. DAB tablets are used in immunohistology for the detection of peroxidase activity. Diaminobenzidine is a known mutagen (a compound that can induce changes in the genetic information of an organism).

Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100A
Catalog No.: 70000-15P-2
Dimensions: 2.1 x 150 mm
Solvents: A: 50% DI H₂O/ 50% MeOH/ 0.1% formic acid
 B: Acetonitrile/ 0.1% formic acid
Gradient:

time (min.)	%B	time (min.)	%B
0	80	9	30
4	30	10	80

Post Time: 5 min
Injection Vol.: 1 microL
Flow Rate: 0.4 mL/min
Detection: ESI – POS - Agilent 6210 MSD TOF mass spectrometer
Sample: Stock Solution: 1 mg/mL in DI water diluent. The solution was filtered through a 0.45 µm nylon syringe filter (MicroSolv Tech Corp). Working Solution: Stock solution was diluted 1:100 with 50/50 solvent A/solvent B mixture.
Peak: 3,3'-Diaminobenzidine 215.1291 m/z (M +H)⁺
t₀: 0.9 min

Discussion

DAB (3,3'-Diaminobenzidine) is a very challenging compound for analysis by HPLC. It is highly polar and hence difficult to retain when RP-HPLC columns are used. Moreover, when there are a significant number of silanol groups present on the surface of the column packing material, the peak for DAB becomes very broad (5 – 10 min peak width). As can be seen from the accompanying chromatograms, a Cogent Diamond Hydride™ column was an excellent choice for the analysis of DAB. The peak shape is symmetrical with high efficiency. The repeatability of the analysis is also remarkable as can be seen in Figure B.

For more information visit www.MTC-USA.com

Cat. No.	Description
70000-15P-2	Diamond Hydride™ HPLC Column, 100A, 4µm, 2.1 x 150 mm