

3,3'-Diaminobenzidine (DAB) by LC-MS

LC-MS method with excellent retention and peak shape

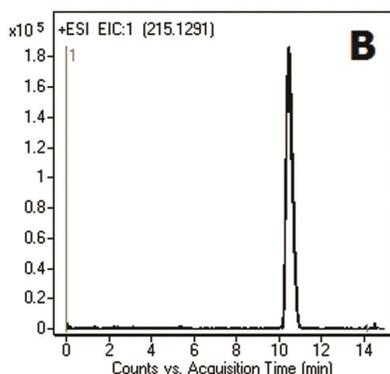
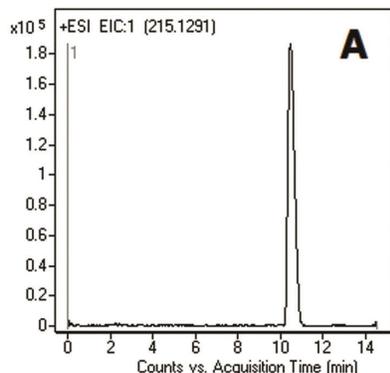
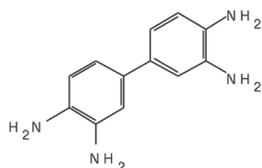


Figure A: single injection
Figure B: 5-run overlay, 3 injections done one day, 2 injections done the next



3,3'-Diaminobenzidine

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, 100Å

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
	0	80
	4	30
	9	30
	10	80

Post Time: 5 min

Injection vol.: 1µL

Flow rate: 0.4 mL/min

Detection: ESI - POS - Agilent 6210 MSD TOF mass spectrometer

Sample: Stock Solution: 1mg/mL in DI H₂O diluent. The solution was filtered through a 0.45µm 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.