

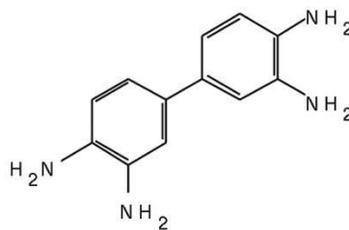
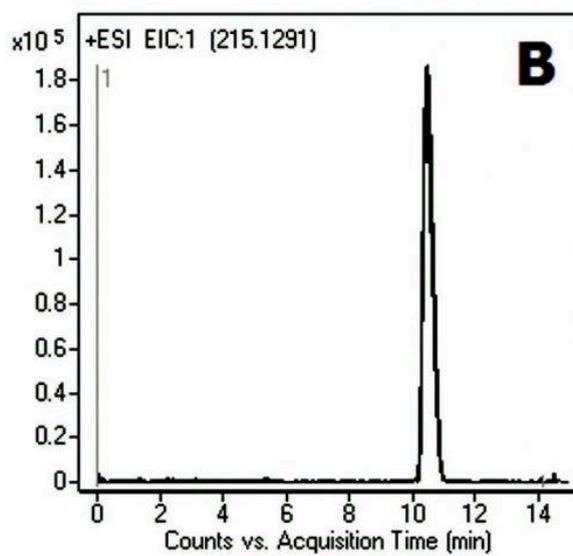
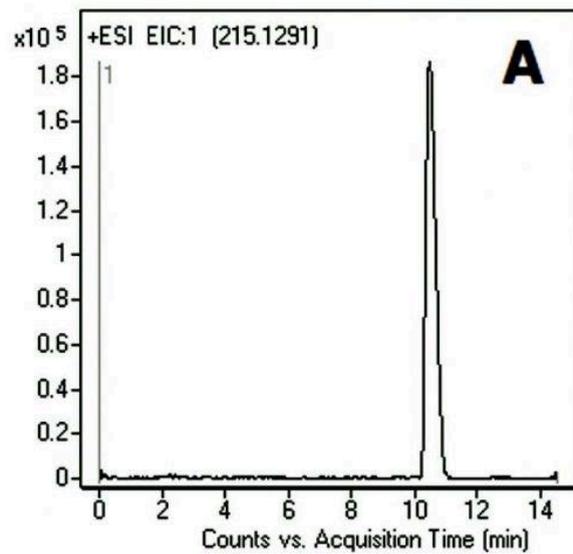
Diaminobenzidine Analyzed with LCMS - AppNote

Retention and Separation is Easy with this Method

3,3'-Diaminobenzidine (*DAB*) is a very challenging compound for analysis using HPLC with a UV detector. It is highly polar and hence difficult to retain when Reversed Phase HPLC Columns are used. Moreover, when there are a significant number of Silanol Groups present on the surface of the Column Stationary Phase, the Peak for *DAB* becomes very broad (*5 – 10 minute Peak Width*).

As can be seen from the Chromatograms below, this method is an excellent choice for the analysis of *DAB* and resolves the issues of band broadening. The Peak shape is Symmetrical with high Efficiency.

The Repeatability of the Analysis is also remarkable as can be seen in a duplicate run shown in *Figure B*.



Peak:

3,3'-Diaminobenzidine 215.1291 m/z (M+H)⁺

Method Conditions:

Column: Cogent Diamond Hydride™, 4 μm , 100Å

Catalog No.: [70000-15P-2](#)

Dimensions: 2.1 x 150 mm

Mobile Phase:

A: 50:50 DI Water / Methanol with 0.1% Formic Acid

B: Acetonitrile with 0.1% Formic Acid

Gradient:

Time (minutes)	%B
0	80
4	30
9	30
10	80

Post Time: 5 minutes

Injection vol.: 1 µL

Flow rate: 0.4 mL / minute

Detection: ESI – POS – Agilent 6210 MSD TOF Mass Spectrometer

Sample preparation:

Working Solution: Stock solution was diluted 1:100 with 50:50 Solvent A / Solvent B mixture.

Stock Solution: 1mg / mL in DI Water Diluent. The Solution was filtered through a 0.45µm Nylon Syringe Filter (MICROSOLV Tech Corp.).

t_o : 0.9 minutes

Notes: 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 immuno-histology for the detection of peroxidase activity. Diaminobenzidine is a known mutagen (a compound that can induce changes in the genetic information of an organism).



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