

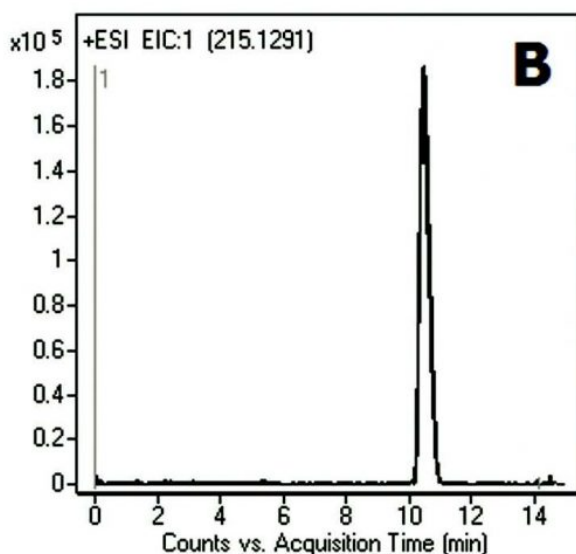
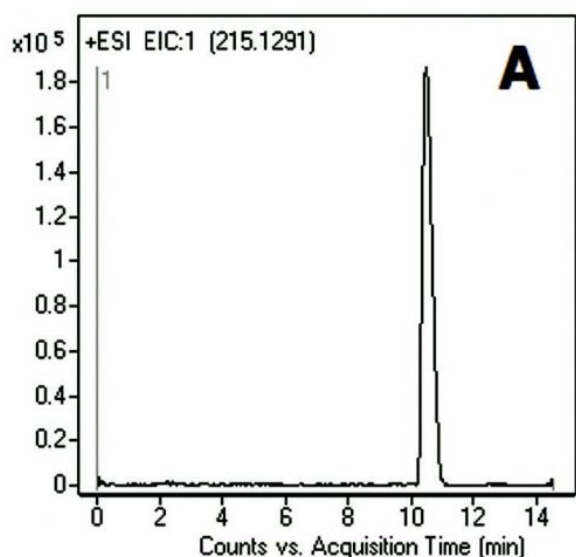
## 3,3'-Diaminobenzidine Analyzed with LCMS – AppNote

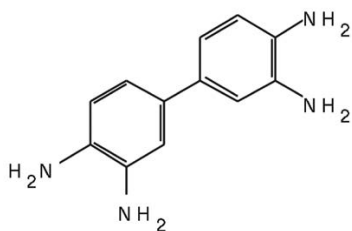
### Retention and Separation is Easy with this Method

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™, 4µm, 100Å

**Catalog No.:** 70000-15P-2

**Dimensions:** 2.1 x 150mm

**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.:** 1µL

**Flow rate:** 0.4mL / 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<sub>0</sub>:** 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).



**Attachment**

**No 151 Diaminobenzidine Analyzed with LCMS pdf** 0.4 Mb [Download File](#)

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