HPLC Derivatization Reagents: Dabsyl Chloride

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Dabsyl chloride is a common amine-derivatization reagent for detecting proteins and other biomolecules by HPLC. Dabsyl-derivatives can be detected at visible wavelengths and at picomole concentrations.

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Dabsyl chloride (4-N,N-dimethylaminoazobenzene-4'-sulfonyl chloride) is the reagent of choice for preparing primary and secondary amines, amino acids, thiols, imidazoles, phenols and aliphatic hydroxyl groups for analysis by HPLC. The reagent forms colored derivatives at room temperature that are easily detectable between 420-460 nm. This higher UV range eliminates interferences from most other biological compounds. The intense color permits detection of amines at nanomole levels.

**Figure 1** ......... Dabsyl Chloride

**Application**

**Fast, Simple Separation of Dabsylated Amino Acids** Precolumn derivatization of amino acids with DABS-Cl takes only 10 minutes at 70 °C and permits complete reaction of primary and secondary amino acids. Dimethylaminoazobenzene sulfonyl amino acids (DABS-AA) can be detected at visible light wavelengths. This permits HPLC analysis of amino acids at picomole concentrations and eliminates baseline noise that can occur when using UV wavelengths. The HPLC method discussed here takes only 25 minutes for a room temperature separation of approximately 35 DABS-AAs. It was suggested that the best analysis is delivered using a Sigma-Aldrich’s dabsyl chloride reagent, produced by our Supelco brand, is tested for both purity and reactivity. This ensures high yield of derivative, and prevents loss of sample due to unwanted side reactions. Complete instructions and a Certificate of Analysis are supplied with each purchase. If you need assistance our knowledgeable staff of Technical Service chemists can help you with reagent selection, derivatization procedures and troubleshooting. Please e-mail us at techservice@sial.com

15 cm x 4.6 mm ID, 3 μm particles SUPELCOSIL LC-DABS column, a 2 cm x 4.6 mm ID, 5 μm particles Supelguard™ LC-18-T guard column, and a two-eluent mobile phase – consisting of 25 mM potassium dihydrogen phosphate (pH 6.8) as solvent A, and acetonitrile:2-propanol (75:25) as solvent B (Figure 2). This method also resolves DABS derivatives of taurine, D-hydroxylysine, norleucine, cysteic acid, cystine, S-carboxymethylcysteine, and S-sulfocysteine. The separation of DABS-norleucine adds dimension to the method because this derivative can serve as an internal standard.

For more detailed information, use the enclosed reply card to request Supelco Application Note, T397124 (BHL). This publication can also be requested by phone or downloaded from our website: www.sigma-aldrich.com/supelco-library
Figure 2: DABS Amino Acids by HPLC

Column: SUPELCOSIL LC-DABS, 15 cm x 4.6 mm ID, 3 µm particles (Cat. No. 59137)
Sample: 5 µL DABS-derivatized amino acids
Mobile Phase: A = 25 mM potassium dihydrogen phosphate (pH 6.8), B = acetonitrile:2-propanol, 75:25
Gradient Program (Time: %B):
0-1 min.: 20%; 1-4 min.: 20-23%; 4-9 min.: 23%; 9-10 min.: 23-27%; 10-14 min.: 27%; 14-19 min.: 27-35%; 19-25 min.: 35-60%; 25-26 min.: 60-70%; 26-29 min.: 70%; 29-29.1 min.: 70-20%;
29.1-35.1 min.: 20
Flow Rate: 2 mL/min.
Det.: 436 nm UV

DABS-Cl derivatives are prepared by

1. Add 50 mL of 1.5 M NaHCO₃ (pH 9.0), followed by 100 mL of 2 mg/mL dabsyl chloride in acetone, to each.
2. 110 mL aliquot of amino acid standard
3. Vortex the mix
4. Heat to 70 °C for 10 minutes (or hold at room temperature for 30 minutes)
5. Dry under vacuum
6. Resuspend the mix on 200 mL of 70% ethanol and centrifuge for 2 minutes @ 14,000 x g, and transfer to a vial
7. Derivatives are very stable between temperatures of –20 °C to room temperature

NOTE: the pH of NaHCO₃ is very important

Materials

References

3. Supelco Application Note 124, T397124 (BHL)