Application Note 124

Fast, Simple Separation of Dabsylated Amino Acids

Thirty-five DABS-AA can be separated in 25 minutes at room temperature on a 15cm x 4.6mm ID SUPELCOSIL LC-DABS column under HPLC conditions. DABS derivatization takes only 10 minutes at 70°C, permitting complete reaction of primary and secondary amino acids. These derivatives can be detected at visible wavelengths, at picomole concentrations. They also are stable at temperatures from -20°C to room temperature.

Key Words:
- DABS-AA • DABTH-AA • reversed phase HPLC
- derivatization

SUPELCOSIL™ LC-DABS and SUPELCOSIL LC-18-DB columns (15cm x 4.6mm ID, 3µm particles) will separate dimethylaminoazobenzene sulfonyl amino acids (DABS-AA) and dimethylaminoazobenzene thiohydantoin amino acids (DABTH-AA), respectively. Using reversed phase HPLC, these separations are fast, simple, and reproducible.

Precolumn derivatization of amino acids with DABS-chloride (Cl) takes only 10 minutes at 70°C and permits complete reaction of primary and secondary amino acids. DABS derivatives are also very stable at temperatures ranging from -20°C to room temperature.

DABS-AAs 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.

To develop this HPLC method for analyzing DABS-AAs, various reversed phase columns and several potassium dihydrogen phosphate and sodium acetate buffers were tested. Different pHs, flow rates, gradients, and organic solvents and modifiers also were evaluated. A 15cm x 4.6mm ID, 3µm particles SUPELCOSIL LC-DABS column, a 2cm x 4.6mm ID, 5µm particles Supelguard™ LC-18-T guard column, and a two-eluent mobile phase — consisting of 25mM potassium dihydrogen phosphate (pH 6.8) as solvent A, and acetonitrile:2-propanol (75:25) as solvent B — delivered the best analysis (Figure A1).

Along with the separation of 35 DABS-AAs, 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 checking different steps of the sample handling procedure. Resolution of cysteic acid, cystine, S-carboxy methylcysteine and S-sulfocysteine indicates that the HPLC method will continue to work, even when different experimental conditions are used to treat a protein before hydrolysis.

Changing solvent B to contain acetonitrile and methanol in a 70:30 ratio delivers a chromatographic profile (Figure A2) similar to Figure A. This second analysis adds hydroxyproline, methionine sulfone, and methionine sulfoxide to the profile.

The resolution of hydroxyleucine, under conditions of the second HPLC procedure, makes this method useful for studying different aspects of metabolism in connective tissue. The method is also useful for evaluating distribution of hydroxyleucine in biological fluids. Resolution of methionine sulfone and methionine sulfoxide makes it possible to follow methionine oxidation of a protein *in vivo* under different biological conditions, or when treating it with performic acid.

In any amino acid analysis, tryptophan poses a special problem. Tryptophan is unstable under hydrochloric acid (HCl) hydrolysis conditions. Hydrolysis with methanesulfonic acid and derivatization with DABS-Cl prepare tryptophan for HPLC analysis, without adversely affecting the separation of other amino acids. But this procedure loses its effectiveness for samples with a sugar concentration of more than 20%. For analyzing tryptophan in protein samples with sugar concentrations greater than 20%, sodium hydroxide hydrolysis followed by DABS-Cl derivatization and HPLC analysis is a more effective procedure.

Resolution of DABTH-AAs on a 15cm x 4.6mm ID, 3µm particles SUPELCOSIL LC-18-DB column is fast and sensitive, permitting analyses of very small amounts of material. Figure B shows the separation of DABTH-AAs, including DABTH-norleucine, in only 18 minutes, by two HPLC methods. The gradient conditions remove excess reagent from the column during the analysis. The column is protected with a 2cm x 4.6mm ID, 5µm particles Supelguard LC-18-DB guard column. Although many analyses of DABTH derivatized amino acids can be performed on the same analytical column, you should change the guard column every 200-250 runs.

The 3µm particles in the SUPELCOSIL LC-18-DB column deliver chromatographic peaks nearly twice as high as those obtained from a column packed with 5µm particles. The columns also display excellent column-to-column reproducibility, indicating very reliable methodology. The consistently reproduced retention times for the DABTH-AAs confirm that these HPLC methods are excellent for routine analysis and identification of amino acids.

These methods offer exceptional sensitivity for detailed biological and structure-function studies of even trace concentrations of proteins and peptides.

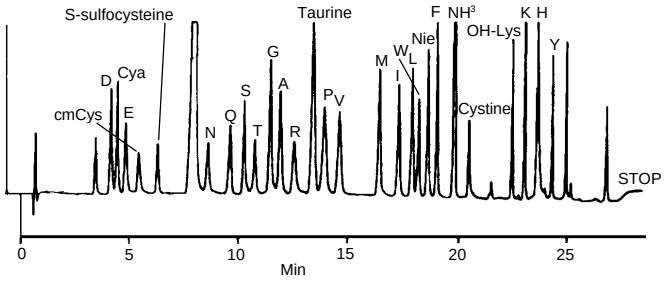
Figure A. DABS Amino Acids by HPLC

Column: **SUPELCOSIL LC-DABS, 15cm x 4.6mm ID, 3µm particles**
Cat. No.: **59137**
Mobile Phase: A = 25mM potassium dihydrogen phosphate (pH 6.8), B = acetonitrile:2-propanol, 75:25 (A1) or acetonitrile:methanol, 70:30 (A2)
Flow Rate: 2mL/min
Det.: 436nm UV (A1) or variable wavelength UV (A2)
Sample: 5µL DABS-derivatized amino acids

A1-System I

Gradient Program

Time (min.)	%B
0-1	20
1-4	20-23
4-9	23
9-10	23-27
10-14	27
14-19	27-35
19-25	35-60
25-26	60-70
26-29	70
29-29.1	70-20
29.1-35.1	20



A2 — System II

Gradient Program

Time (min.)	%B
0-1	25
1-5	25-28
5-12	28
12-13	28-30
13-19	30
19-30	30-40
30-39	40-65
39-40	65-75
40-44	75
44-44.1	75-25
44.1-53.1	25

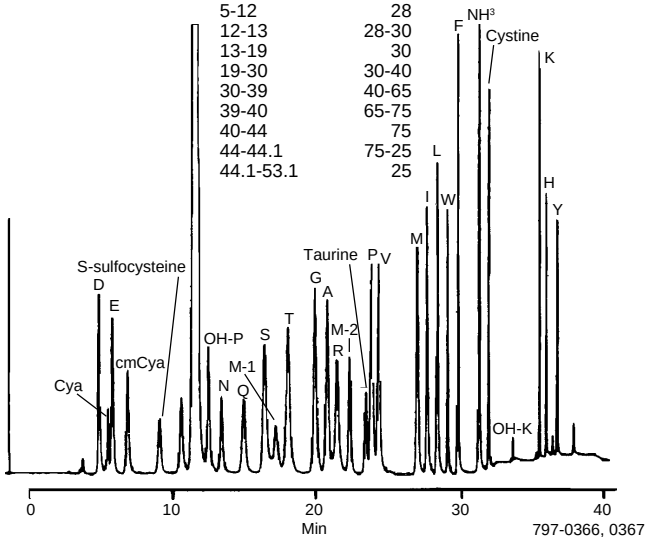


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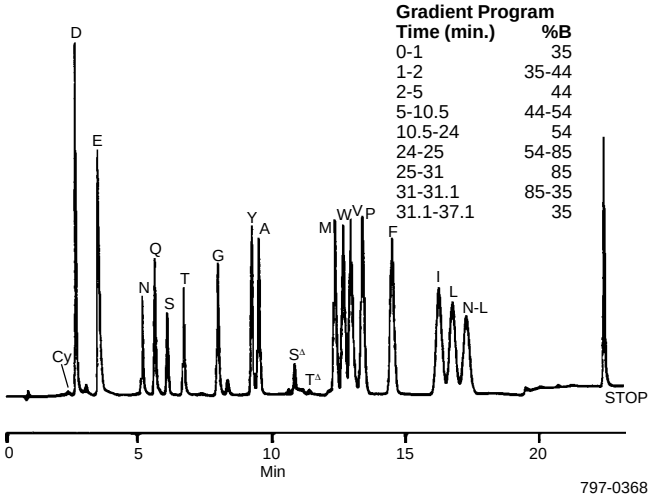
Figure B. DABTH-Amino Acids by HPLC

Column: **SUPELCOSIL LC-18-DB, 15cm x 4.6mm ID, 3µm particles**
Cat. No.: **58993**
Mobile Phase: gradient analysis, A = 35mM sodium acetate (pH 5.1), B = acetonitrile
Flow Rate: 2mL/min
Det.: 436nm UV
Sample: 5µL DABTH-derivatized amino acid standards, 5-15 picomoles (A) or 0.5-1.5 picomoles (B)

Gradient Program

Time (min.)	%B
0-1	35
1-2	35-44
2-5	44
5-10.5	44-54
10.5-24	54
24-25	54-85
25-31	85
31-31.1	85-35
31.1-37.1	35



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Ordering Information:

Description	Cat. No.
SUPELCOSIL LC-DABS Column	
15cm x 4.6mm ID x 3µm particles	**59137**
SUPELCOSIL LC-18-DB Column	
15cm x 4.6mm ID x 3µm particles	**58993**
Supelguard Guard Column Kits includes guard column (2cm x 4.6mm ID x 5µm particles), holder, and connecting hardware	
Supelguard LC-18-T (for LC-DABS column)	**59620**
Supelguard LC-18-DB	**59555**
Replacement Supelguard Guard Columns, pk. 2	
Supelguard LC-18-T (for LC-DABS column)	**59621**
Supelguard LC-18-DB	**59565**

Acknowledgment

We gratefully acknowledge the contributions of Vilberto Stocchi, Instituto di Chimica Biologica, Universita degli Studi di Urbino, Urbino, Italy.

Reference

1. Stocchi, V., *et al.,* Anal. Biochem., **179,** 107-117 (1989).

Reference not available from Supelco.

Contact our Technical Service Department (phone 800-359-3041 or 814-359-3041, FAX 800-359-3044 or 814-359-5468) for expert answers to your questions.

Note 124

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