

Waters

Lab Highlights

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NEW APPLICATIONS FOR THE DETERMINATION OF AMINO ACIDS USING PRECOLUMN DERIVATIZATION WITH PHENYL ISOTHIOCYANATE

THOMAS L. TARVIN, STEVEN A. COHEN, and BRIAN A. BIDLINGMEYER,
Applications Development Group, Waters Chromatography Division, Millipore Corp.
34 Maple Street, Milford, MA 01757

The Waters PICO-TAGTM method for amino acid analysis using phenyl isothiocyanate (PITC) precolumn derivatization has proven to be a powerful tool for the determination of amino acids in samples such as purified proteins and peptides, intravenous solutions, and cell culture broths. The benefits of the method are 1) extremely rapid analysis (under 12 minutes) with excellent resolution throughout; 2) 1 picomole detection limit, which allows analysis of as little as 100ng of protein; and 3) low operating costs.

This paper will report on the application of the PITC method to more complex samples, including modified protein amino acids, hydrolyzed feeds and foods, free amino acids in foods and plant extracts, and amino acids derived from biological sources such as serum, urine, and tissue. We shall discuss strategies for optimizing separations for specific samples, including modification of mobile phase composition and pH, temperature variations, and altering gradient profiles. Also discussed will be sample preparation techniques for dealing with complex matrices, including removal of proteins, fats and vitamins, drugs, and other UV-absorbing compounds.

As an example, 20mg samples of several types of feed hydrolyzates were hydrolyzed by weighing into a 16 X 125mm screwtop PyrexTM brand vial, adding 2 ml of 6N HCl, thoroughly flushing with nitrogen, and quickly capping. The vials were placed in an oven at 110 C for 24 hours. After cooling, 8 ml of a solution containing the internal standard, alpha-aminobutyric acid (0.25nM/ μ l), was added. One ml aliquots were filtered through Millex^R HV filters, and 5 μ l of the filtrate from each was placed into a 6 X 50mm sample tube for derivatization. Derivatization was performed as previously reported¹, except that methanol was substituted for ethanol. All of the amino acids are well resolved in these samples, and there are no major unknown peaks. For all of the feeds examined, results from the PICO-TAGTM method agree well with analysis by conventional ion exchange methods. The reproducibility of the method is also comparable to conventional AAA; relative standard deviations for sixteen samples of a soy flour hydrolyzate, hydrolyzed and derivatized individually, ranged from 3 to 10 percent.

1. B. A. Bidlingmeyer, S. A. Cohen and T. L. Tarvin, J. Chromatogr., 336 (1984), 93-104.