Short Communication

Pak. j. sci. ind. res., vol. 34, no. 9, September 1991

A Direct Method for the Determination of Phenylalanine, Tyrosine, Leucine and Valine in Wheat by Reverse Phase HPLC

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(Received February 17, 1990; revised November 14, 1991)

The availability of reverse phase HPLC has led to the development of newer and more efficient methods for the analysis of amino acids. Schuster *et al*. [1] resolved some free amino acids without derivatization procedures and gradient mobile phase system. Recent methods include pre and post-column derivatization with a wide range of multi step solvent gradient systems and fluorescent or electrochemical detection to analyse complex mixture of amino acids in various matrices [2-9].

A simple, inexpensive and direct method is described here for the determination of some free amino acids in protein hydrolysates of wheat.

Apparatus. A Hitachi Model 635-A liquid chromatographic system consisting of pressure constant flow pump with a multi wavelength UV monitor was used. Analytical columns 250 x 4 mm i.d. containing Lichrosorb and also Unisil RP-18, 5 µm as stationary phase were used.

Chromatographic procedure. The separation was performed using ortho phosphoric acid pH 2.2 as the mobile phase. Before initial use the column was conditioned with methanol for one hr. at ambient temperature. Detection was performed with a UV detector at a wavelength 210 nm.

Mobile phase containing orthophosphoric acid pH 2.2 was found to be the most suitable at a wavelength of 210 nm and an octadecylsilica as the stationary phase. The elution profile of a standard mixture of amino acids in reverse phase HPLC separation mode is shown in Fig. 1. This separation method was applied for the analysis of amino acids from protein hydrolysates of some new varieties of wheat. Fig. 2 is a typical chromatogram from one such analysis. Regression analysis of peak height vs. concentration in the range of 0.01 to 5.0 µg. 10.0 µl showed excellent linearity (0.999) for each amino acid. Recovery experiments were also carried on the real samples by adding known amounts of pure amino acids to the protein hydrolyzates of wheat samples. Mean recovery for these amino acids was found to be consistently above 96%. The reproducibility of the method is of the same

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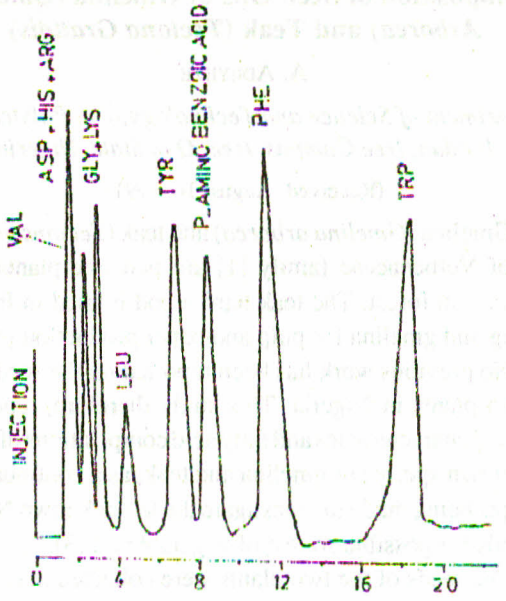

Fig. 1. Reverse phase HPLC chromatogram of amino acids using O-phosphoric acid pH 2.2 as mobile phase and Lichrosorb RP-18 as a stationary.

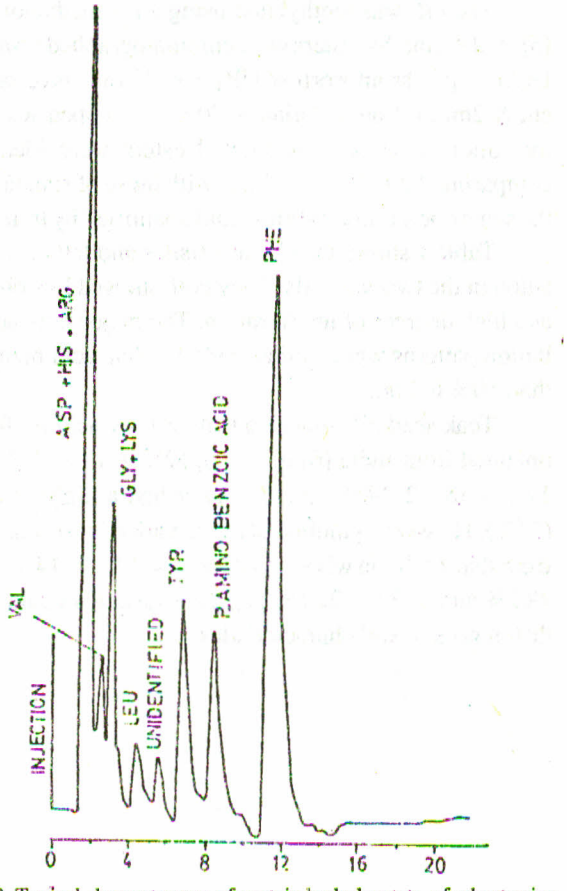

Fig. 2. Typical chromatogram of protein hydrolysatets of wheat using O-phosphoric acid pH 2.2 and Lichrosorb RP-18 as stationary phase.

order as that of established standard procedures. Thus, the proposed method is very simple, inexpensive and can be done on a simple liquid chromatograph with single pump and a UV detector. Nevertheless, solvent programming and sample derivatizing has extensively been used by several previous workers [3,9] when the composition of the mixture was not known or when the solute had wide ranging hydrophobicities.

Work is in progress in our laboratory to apply this method for the separation of some more complex mixtures of amino acids and also to estimate quantitatively amino acids in different biological samples.

Key words: Phenyl-alanine, Wheat, Tyrocine.

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