Amino acid analysis by HPLC with FLD detector

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Abstract

Quantitative and qualitative amino acid analysis was performed by HPLC with pre-column derivatisation.

Procedure

For amino acid analysis, purified protein was hydrolyzed with 6N HCl for 24 h at 120 oC in the oil bath. The resultant mixture was analyzed by an Agilent 1260 HPLC system (Agilent, USA) with a fluorescent detector (FLD) after derivatisation with OPA (O-phthalaldehyde) (SRL, India). For proline and hydroxyproline identification, 9-fluorenylmethoxycarbonyl (FMOC-Cl) (SRL, India) was applied to derivatise the sample. The derivatized sample was loaded (50 µl) onto an HPLC column (ZORBAX-SB-C-18 column (250× 4.6m, 5 micron particle size) using sample injector (Agilent 1260). For the gradient elution method, the column was eluted using 0.01M Na_2HPO_4 buffer and acetonitrile (100%) as a mobile phase solvent system. The flow rate was maintained at 1 ml/min. Data from the system was collected and evaluated using Agilent open LAB control panel software. Amino acid from sample and standard was quantified via comparison to the retention time and absorbance. The amino acid content was expressed as the number of residues/1000 residues.

Gradient System:

A-Buffer (0.01M Na_2HPO_4); B- Acetonitrile

Excitation:230, Emission: 460

Time	A%	B%	Flow rate (ml/min)	Max. pressure
0.00	91.0	9.0	1.000	400.00
2.00	80.0	20.0	1.000	400.00
35.00	68.0	32.0	1.000	400.00
55.00	20.0	80.0	1.000	400.00
57.00	91.0	09.0	1.000	400.00

OPA preparation:

OPA: 5 mg

Methanol: 50 microlit

Sodium Borate Buffer: 450 microlit

Beta-Mercaptoethanol: 25 microlit

An equal volume of the hydrolysed sample and OPA solution was mixed and incubated for two minutes prior to HPLC injection.

FMOC-Cl Preparation:

For proline and hydroxyproline identification in HPLC, FMOC-Cl (SRL, India) was used to derivatize the COLII sample. Necessary modifications have been made in the manuscript. Briefly, hydrolyzed collagen sample (20 µL of 1mg/ml)) was mixed with 80 µL borate (20 µmol/mL, pH 10.5) buffer and 100 µL of FMOC-Cl solution in acetone (10 µmol/mL) was added in the mixture. The resultant mixture was incubated at room temperature for 30 m followed by addition of 300 µL pentane. Excess FMOC-Cl, hydrolysed product of FMOC-OH and acetone would be extracted by pentene from the mixture. Finally, the derivatized COLII sample was ready to inject into HPLC column for detection of proline and hydroxyproline.

References

Jeevithan, E. et al. Type II Collagen and Gelatin from Silvertip Shark (Carcharhinus albimarginatus) Cartilage: Isolation, Purification, Physicochemical and Antioxidant Properties. Mar. Drugs 12, 3852–3873 (2014)