PEPTIDE CONTENT AND AMINO ACID COMPOSITION DETERMINATIONS BY THE EZ:FAAST AMINO ACID ANALYSIS PROCEDURE

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Introduction

Methods used with the most frequency to determine protein's solutions concentration or the peptide content in a sample are: aminoacids analysis [1], total nitrogen determination by Kjeldahl, the dry weight method, the Edelhoch method and the colorimetric methods such as the Bradford techniques or the BCA. From all of them, the aminoacid analysis technique is the most accurate; one of the advantages of this method is its independence from the abundance of aromatic residues in the protein or the peptide analyzed; also it is precise at low concentrations. The Phenomenex EZ:faast™ Reagents Kit (Phenomenex, USA)[2] is use to derivatize the aminoacids released during a peptide hydrolysis. The volatile derivatives are separated using gas chromatography (GC) with Flame Ionization Detectors (FID).
Here we shows the determination of the peptide content for different peptides, using the Phenomenex EZ:faast™ Reagents Kit. This will allow knowing the exact peptide content in the sample, which will allow solutions preparation at exact sample concentrations.

Methods

Peptide sample hydrolysis, derivatization and analysis: acid hydrolysis in vapor phase, with 6M HCl, was used to hydrolyze the samples. The derivatization was performed by using the EZ:faast amino acid analysis procedure (Phenomenex ®). Derivatized samples are quickly analyzed by GC-FID. Composition and quantity of hydrolyzed amino acids where determined quickly and accurately . Samples theoretical concentration were prepared by precise weight and dilution in water (aa free).
Gas Chromatographic Analysis: GC-FID Agilent Technologies 7890A. Injection Split 1:15 @ 250 C, 2.0µLCarrier Gas Helium, 1.5mL/min); Oven Program 32 C/min from 110 to 320 C Detector 320 C.

Results

Experimental design

Table 1. Amount and volume of each peptide used for the preparation of each sample.

Sample name	Theoretical concentration (mg/mL)	Amount of peptide to hydrolyze (nmoles)	Volume of the solution (mL)	Amount of aa nLeu added as internal standar of the hydrolysis
BSA-1/BSA-2	2.00	0.500	16.6	25 (nmoles) = 2.8 mL
Sample A-1A Sample B-1B	1.00	16	34.2	32 (nmoles) = 3.6 mL
Sample A-1A, Sample B-2B	1.00	16	34.2	16 (nmoles) = 1.8 mL

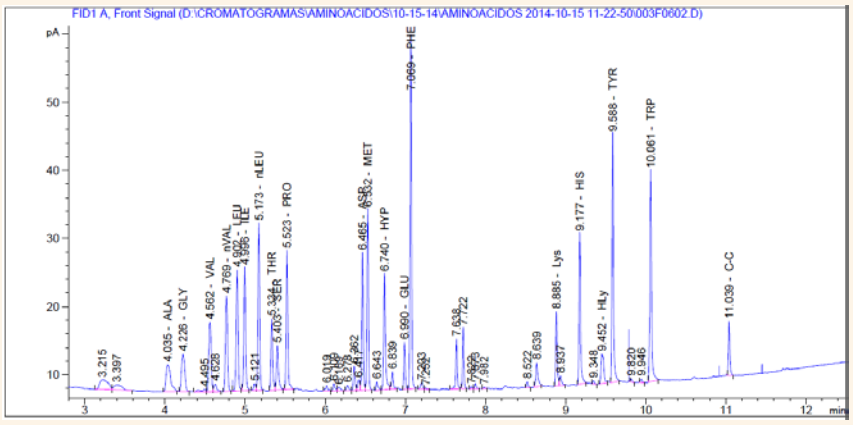

Figure 1. Chromatogram showing the 3rd level of the **standard calibration sample** (20 nmol of each aminoacid). Aminoacids **nLeu** and **nVal** are used as internal standards for hydrolysis and derivatization respectively. Above each peak appear the retention time and aminoacid identification in the 3 letters code.

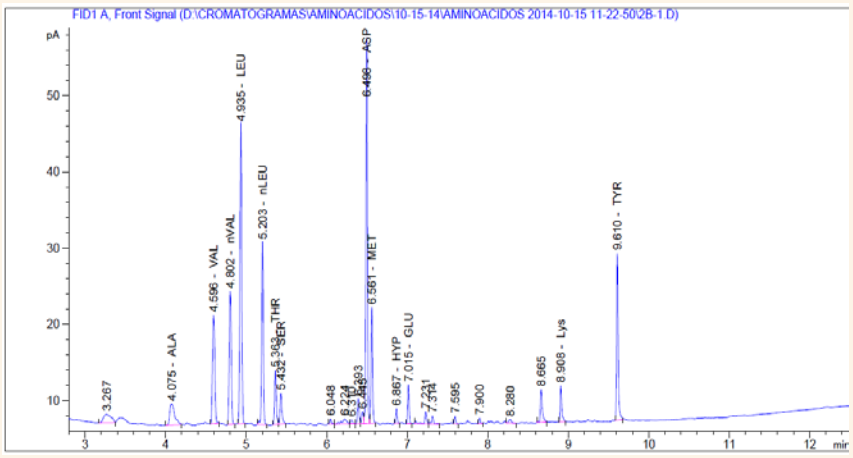

Figure 2. Chromatogram showing aminoacids of one of **the peptides under study (Sample A).** Aminoacids **nLeu** and **nVal** are used as internal standards for hydrolysis and derivatization respectively. Above each peak appear the retention time and aminoacid identification in the 3 letters code.

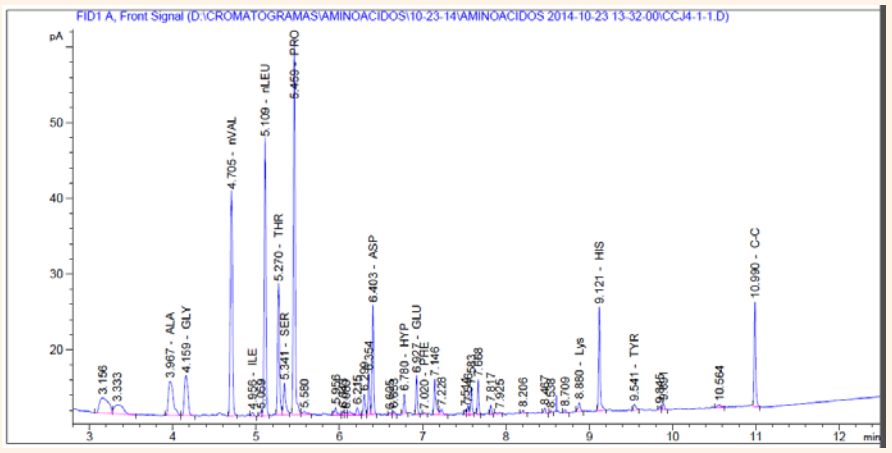

Figure 3. Chromatogram showing aminoacids of one of the **peptides under study (Sample B).** Aminoacids **nLeu** and **nVal** are used as internal standards for hydrolysis and derivatization respectively. Above each peak appear the retention time and aminoacid identification in the 3 letters code.

Table 1. Values obtained from the experimental concentration for three injections of the BSA sample used as standard of concentration (theoretical value 2 mg/mL). Also the Mean, Standard Deviation (STDEV), Variation Coefficient (CV) and % Error, are showed.

Sample	Experimental concent. (mg/mL)	Mean	STDEV	CV	% Error (|Vexp. – Vteo.|/Vteo.)
BSA 1-1	1.933	1.95	0.02	0.9%	2.6%
BSA 1-2	1.943				
BSA 1-3	1.968				

Table 2. Table 4. Values obtained from the experimental concentration of two replicas of the peptide CCJ4 and the injections (in triple) for each one of them, including the mean, the STDEV and the CV.

	Conc. (mg/mL)	Media	STDEV	CV	Media	STDEV	CV
Sample A-1-1	0.622	0.63	0.01	1.2%	0.66	0.04	5.3%
Sample A-1-2	0.624						
Sample A-1-3	0.635						
	Conc. (mg/mL)	Media	STDEV	CV			
Sample A-2-1	0.685	0.69	0.01	0.7%			
Sample A-2-2	0.691						
Sample A-2-3	0.695						

As can be observed in the table , there is a low variability between the values obtained from the different injections in the GC, which is in the agreement with an automatic injection ; the variation coefficient are between 0.7 % and 1.2%.
Including both preparation of the same sample, the variation coefficient was 5.3% which is good taking in account the amount of manual steps during the whole procedure.
This analysis was applied to Sample B in the same way.

Conclusions

1. The peptide content of sample A was 66% according to the experimental concentration obtained (0.66±0.04 mg/mL) in comparison to he theoretical (1.00 mg/mL).
2. This procedure allows a precise method to calculate the experimental concentration of a peptide solution, the peptide content and composition analysis of the sample.

References

1. Anders, J.C; Parten, B.F.; Petrie, G.E.; Marlowe, R.L. y McEntire, J.E. (2003) Using Amino Acid Analysis to Determine Absorptivity Constants. A validation case study using Bovine Serum Albumin. BioPharma 17, 30-37.
2. EZ:faast. Amino Acid Analysis of Protein Hydrolysates by GC-FID or GC-NPD. User´s Manual. Phenomenex.