ELIMINATION AND EXCHANGE OF TRIFLUOACETATE COUNTER-ION IN A CATIONIC PEPTIDE: IMPROVEMENTS IN THE PROCESS

Ever Pérez Hernández, Hilda Elisa Garay Pérez, Ania Cabrales Rico, Reinier Acosta Martin, Juan Hernández Piñero, Yordanka Masforrol Gonzales, Mercedes Arencibia Leyva, Yamilet Pérez Chabas, Cecilia Sagardoy Prieto, José Manuel Perryman Figueroa, Cristian Estrada, Antonio Antequera, David Diago Abreu and Osvaldo Reyes Acosta.
e-mail:ever.perez@cigb.edu.cu. Peptides Synthetic Department. CIGB, Cuba.

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Abstrac

Most synthetic peptides are nowadays produced using solid-phase procedures. Due to cleavage and purification process, they are mainly obtained in the presence of trifluoroacetic acid (TFA) and, for cationic peptides, as trifluoroacetate (TF-acetate) salts. The present work describes improvements introduced in the process of replacement of the counter-ion in a cationic peptide, intended to lower the acidity of the purified peptide by using ion exchange to substitute the trifluoroacetate counter-ion from RP-HPLC by acetate, had been implemented as a non-scalable batch chromatography operation, and needed therefore to be replaced by an alternative amenable to industrial scale-up. In order to achieve this objective, a column chromatography step was implemented using the ion exchange resin Dowex 1x8, determining optimum flow and sample concentration in order to substitute one anion for the other.

Methods

All solvents were from Merck (Germany) and the peptide used for this study is produced and supplied by the Department of synthesis of CIGB. The anion resin (DOWEX 1X8, Chloride form) was from Fluka. For the industrial scale-up we use a chromatography column XK 26 and peristaltic pump from LKB.

Determining the load of synthetic peptide for Dowex 1x8 matrix

A chromatographic column PD-10 loaded with 0.416 g of resin, corresponding to 1 ml of gel was used. The experiment is performed to a workflow by gravity. It starts with 100 mg peptide and is increased by the same amount in each experiment up to 400 mg / mL, 3 replicates for each concentration to be studied is performed.

Resin-ion Exchange to laboratory scale.

Weigh 0.416 g of Dowex 1x8 corresponding to an equal volume of 1 ml and added to a chromatography column HR-5, washing with 6 volumes of purified water, 3 volumes of NaOH 1 mol/L, 8 volumes of purified water, 4 volumes of 50% acetic, 2 volumes of 0.6% acetic acid. The entire process is performed in an analytical HPLC system Merck-Hitachi. Suspend the synthetic peptide CIGB 300 0.6% acetic acid and pass through the column at once, it becomes finally lyophilized for 72 hours. We studied three linear velocity (180, 270 and 360cm/h) and three concentrations (65, 100 and 135 mg/ml of matrix) of injection to column. Analyses were performed by TOF mass spectrometry (Figure 1).

Industrial scale-up.

To perform the scale-up of the process of exchange the counter-ion was taken into account parameters keeping constant load, packed bed height (h) and linear flow (Fl). We use the equations of area (A), Volume (V) and volumetric flow (Fv).

$A = \Pi \bullet D2/4$; $V = A \bullet h$; $Fv = A \bullet Fl$

Conclusions

- In this study, we have shown that the use of chromatography process is an efficient method to exchange a peptide-TF-acetate salt for an acetate salt, keeping the same purity.

References

- Gaussier H. y col. Replacement of trifluoroacetic acid with HCl in the hydrophobic purification steps of pediocin PA-1: a structural effect. Appl. Environ. Microbiol. 2002; 68: 4803-4008.
- Roux S. et al. Elimination and exchange of trifluoroacetate counter-ion from cationic peptides: a critical evaluation of different approaches. J Pept Sci. 2008; 14(3):354-9.

Results

The exchange capacity of the 1x8 Dowex matrix was known through the pH measurement of the peptide eluted during the exchange process. Figure 1 shows the pH values obtained from each eluate, according to the applied peptide concentration. pH corresponding to concentrations among 100 and 300 mg/mL was 4.25 as an average; value greater in 1.25 units to the one obtained for the 400 mg/mL concentration (pH=3). This demonstrates that the entire trifluoroacetate ion applied to the matrix in the loading experiments among 100 and 300 mg/mL was exchanged with the acetate ion. However, the chromatographic matrix was not able to interchange amounts of peptide greater than 300 mg/mL.

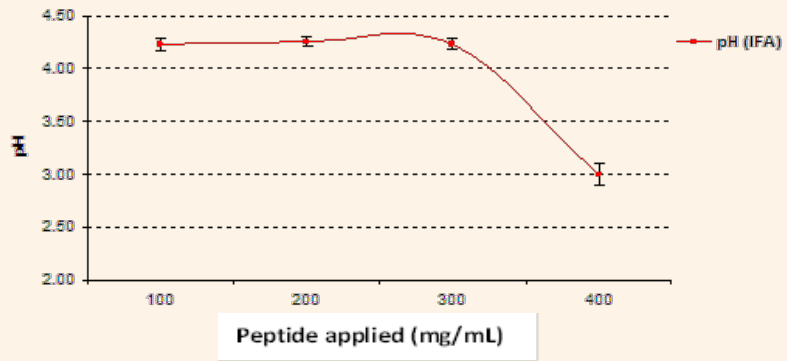

Figure 1.Behavior of the pH for the CIGB-300 peptide eluted from the ion Exchange experiments in order to establish the load of the 1x8 Dowex resin.

The best results in the study of the Exchange process at laboratory scale were reached in those corresponding to a linear velocity of 270 cm/h and at a concentration of the applied peptide to the chromatographic matrix of 100 mg/mL. Those conditions were used at industrial scaling and Figure 3 shows a comparison of four lots produced; the exchange in lots 1201 and 1202 was carried out using a chromatographic column, observing that the concentration of the trifluoroacetate counter-ion is cero, while for lots 1203 and 1301, process carried out in batch, the exchange is incomplete.

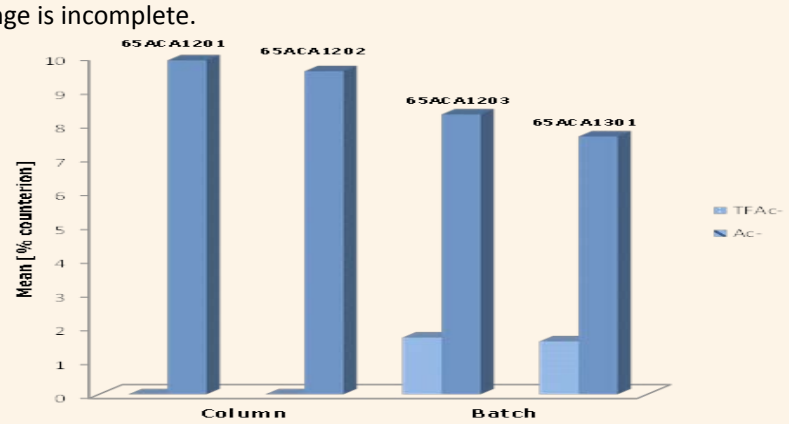

Figure 2.Comparison of four lots produced, two were carried out using a chromatographic column and others twos in batch.

Figure 3 shows chromatographic profiles corresponding to the peptide in trifluoroacetate salt form and then as acetate salt. We can observe that the purity levels obtained remain above 95%, which corresponding to the established specification.

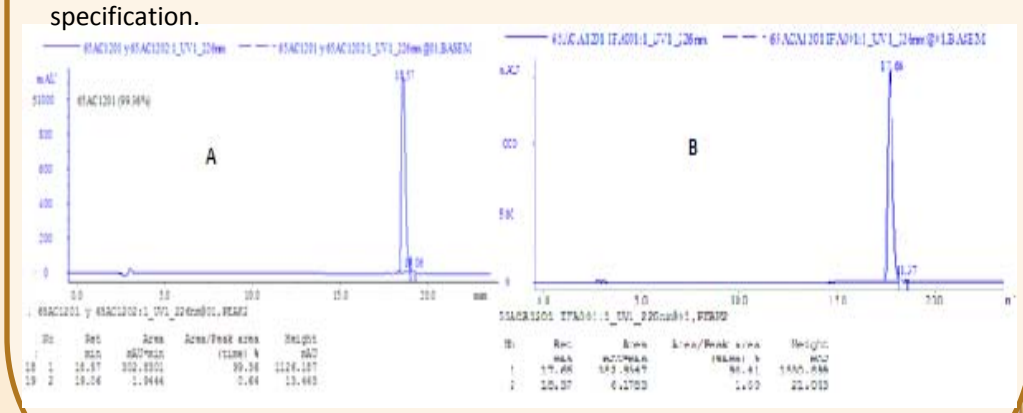

Figure 3. Comparison of chromatographic profiles in RP-HPLC, (A) peptide in trifluoroacetate salt and (B) in acetate salt.