Introduction

Purifying synthetic peptides is necessary since impurities can potentially affect therapeutic safety. Here, we show a mass-directed peptide isolation method that significantly improves the purity of the targeted peptide. It is also necessary to analyze the purified peptide for process related impurities and post-purification degradation products. Selecting optimal column chemistry is an important step in developing a synthetic peptide impurity analysis. The use of LC/MS as an effective tool for analytical RP column screening will be demonstrated. By specifically interrogating a RP chromatographic separation of synthetic peptides for process impurities such as deamidation, misincorporation, and fragmentation, a high degree of confidence can be achieved in the analytical method even when performed with only a UV absorbance detector.

Methods

I. Synthetic Peptide Purification

Eledoisin belongs to the tachykinin family of neuropeptide, which is believed to exhibit a wide spectrum of pharmacological and physiological activities such as vasodilation, hypothermia, and stimulation of extracellular smooth muscle. Eledoisin (Glu-Pro-Ser-Lys-Pro-His-Leu-Gly-Leu-Met-His) is an 11 amino acid peptide, with a molar mass of 1188.4 Da. Formic acid mobile phases, with a pH of 10 mobile phases gave the best separation, so it was used for the fraction collection in large scale.

Table 1. Impurities of bivalirudin, identified by HRMS.

Table 2. Impurities of ceruletide, identified by HRMS.

Results

Figure 1. For purification, separation of eledoisin was optimized on a 4.6 x 100 mm SelectPeptide CSH C18 Peptide column, using various mobile phases with focused gradients (0.36% B change/column volume). The pH 10 mobile phases gave the best separation, so it was used for the fraction collection in large scale.

Figure 2. Crude eledoisin was purified on a XSelect Peptide CSH C18 OBD Prep Column, 150 x 3.0 mm, 100 μm. The retention characteristics of the two columns were compared using Ammonium Bicarbonate pH 10 mobile phases.

Figure 3. A complementary reversed phase method was used to estimate the purity of the fractions (55 and 56) of eledoisin: a peptide BEH C18 130 Å, 1.7 μm, 2.1 x 150 mm column with TFA mobile phases. The purity of eledoisin increased significantly from 45% ( crude ) to 99% ( purified fraction).

II. Column Selection for Synthetic Peptide Impurity Analysis

A. Bivalirudin

Structure (Phe-Pro-Aig-Glu-Gly-Gly-Gly-Aig-Glu-Aig-Asp-Glu-Glu-Ile-Leu)

B. Ceruletide

Structure (Pro-Glu-Asp-Tyr-SO\textsubscript{3}-H-Tyr-Gly-Tyr-Met-Asp-Phe-NH\textsubscript{2})

Conclusion

Peptides are gaining more and more attention as potential biotherapeutics. Currently, more than 100 peptides are marketed worldwide. The synthetic peptide approach has the advantage of being generational in a quick and well-controlled way using solid phase peptide synthesis (SPPS). However, impurities do exist inevitably. They are originated from raw material, manufacturing process and storage conditions.

In this study, optimization of purification conditions of the synthetic peptide was first carried out using a small-scale sample. After the optimization, a large amount of sample (>35 mg) was purified on a Prep column. Mass-directed purification was used to identify the purity of the sample in a single step purification.

Further, different columns under different mobile phase conditions. Column screening revealed unique characteristic of different column chemistry. Below are some of the observations:

1. Selectivity

Selectivity changes not only among columns, but also under different mobile phase conditions.

2. Retention

For most of the synthetic peptides, CSH columns have the least retention, while the HSS T3 column has the most retention.

For peptides that have extremely low pKa ( e.g. Ceruletide ), CSH columns have the most retentivity, likely because the net charge on the peptide is negative even with acidic mobile phases and it interacts with the positive surface of the CSH particles, resulting in longer retention.

3. Lability

CSH type columns generally have the highest peak capacity. Therefore, more sample can be loaded onto these columns without sacrificing the resolution.

Based on the chromatographic results, it is clear that there is no single column that is the most effective for all of the synthetic peptide impurity analyses attempted. As a result, screening columns for this application can be beneficial. As a starting point, below are some of the characteristics of the peptide columns that Waters offer:

- Peptide BEH C18 columns (130 Å and 300 Å)
- Peptide CSH C18 130 Å column
- Peptide HSS T3 100 Å column
- Peptide CSH C18 130 Å column

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