Nanoscale liquid chromatography was performed on an humidity not controlled). Each stress test sample was stored at humidity accelerated stability sample) both for 6 months.

The glucagon stress test sample (powder) was stored at 25 °C and stored for 3 hours at 75 °C. This sample was used as a system suitability test (SST). Approximately 0.7 fmol of each products can be identified and quantified using nano-LC-coupled to a nano-LC system.

Both peptides were stored at accelerated conditions including high temperature and high humidity. Characterization of the degradation products is important in understanding how the quality of the peptide varies due to storage conditions, and to validate the analytical procedures used.

Degradation products can occur as a modification of an amino acid in the sequence or as a hydrolys of the peptide backbone. Both events can be monitored using MS.

As shown in Figure 1, calcitonin contains a well-characterized N-terminal acetylation site. It also contains a possible O-Acetylation site on the Ser13. Acetylation of an amino acid results in a mass shift of +42.011 (Thermofisher Scientific, Reinach, Switzerland)

Calcitonin is a 32-amino acid polypeptide hormone that regulates the blood sugar level in the body. Glucagon is a 29-amino acid polypeptide hormone that regulates the blood sugar level in the body. Both peptides were stored at accelerated conditions including high temperature and high humidity. Both peptides were stored as a powder we observed mostly N-terminal and C-terminal peptide hydrolysis products. When stored in solution, internal peptide hydrolysis products were detected.

Although degradation products were identified in all samples most of them were below the limit of quantitation (signal to noise ratio). Deamidation product could only be quantified in the sample stored in solution (SST) for 3 h at 75 °C. About 45% of the total peak area was deamidated.

The identity of the peptide backbone hydrolysis products were confirmed using PEAKS Studio 5.3 de novo sequencing software. As shown in Figure 9, the amount of hydrolysis products increased if the peptide was stored at high temperature and high humidity. If glucagon was stored as a powder we observed mostly N-terminal and C-terminal peptide hydrolysis products. When stored in solution, internal peptide hydrolysis products were detected.

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In order to identify both expected and unexpected peptide degradation products, a database search algorithm must be used in conjunction with a de novo sequencing approach.

References