PROJECT REVIEW

"Development of detection method of hydrolysed rapid-acting insulin analogues in human urine by ion trap mass spectrometry"

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Insulin issued from the recombinant technology present the same amino acid sequence as the human endogenous hormone and a molecular weight of 5807 Da. Different analogues have been commercialized with slight amino acid modifications affecting their molecular weight, except for Humalog (Eli Lily) that presents only two interchanged amino acids resulting in a polypeptide chain of same molecular weight than human insulin. A LC-MS/MS detection method for rapid-acting analogues in plasma and urine was recently published (Thevis et al., 2005; Thevis et al., 2006). The urine method proposes an immunoaffinity purification from a 25 mL urine aliquot and a top-down sequencing based mass spectrometric approach on a triple quadrupole instrument. The study of the lowest molecular weight ions after fragmentation in the collision cell allows differentiation of the analogues, even if their precursor mass is identical. This approach is innovative but can not be adapted on regular 3D ion trap as fragmentation of large molecule is difficult. As a result, no low molecular weight ions can be observed. Preliminary works have shown that a bottom-up approach using peptide hydrolysis can release smaller fragments that can easily fragment to the desired diagnostic ions.

It is proposed that hydrolysis can be performed prior to analysis on an ion trap to monitor the smaller peptide containing the susceptible amino acid interchange, and then fragmentation pattern can qualify the analogues. The goal of this project is to develop a detection method for hydrolysis products of insulin and rapid-acting analogues using an ion trap mass spectrometer. Complementarily, a simpler purification method using single-use immunoprecipitation will be tentatively developed and compared with the 3-steps approach.

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Results and Conclusions

Insulin testing is not widespread; as a matter of fact, in 2012 it is available in only a handful of laboratories. This method consuming a quite important volume of the urine sample and requiring cumbersome sample preparation must be applied aside from those routinely employed, the testing organizations have been reluctant as well to request insulin testing considering the costs involved. Whilst research is still ongoing, this relatively pragmatic approach was put in place for the analysis of the 2010 Olympic Games samples. The simplified purification with immunoprecipitation allowed the analysis of a greater number of specimens. As a result, for the first time during Olympic Games, over 200 samples, i.e. 10% of samples could be analyzed.

With current advancements in LC-MS/MS technologies (nanoflow, nanospray ionization, selexION, Qtrap 6500 etc), this method could be further improved as extraction volumes could be reduced to a more practical volume in the low mL range and as well, lower injection volumes would reduce ion suppression.