ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Department od Analytical Chemistry

Candidate: Bc. Dominika Krestová

Supervisor: prof. PharmDr. Lucie Nováková, Ph.D.

Consultant: Mgr. Pavel Šištík, Ph.D.

Title of Diploma Thesis: Analysis of insulin and its selected analogues in biological samples by liquid chromatography with mass spectrometry detection

The aim of this thesis was to develop a rapid, simple, specific and sensitive method for the determination of insulin and its selected analogues aspart, lispro, glulisine, glargine, detemir, and degludec in biological matrix, namely plasma. Insulin is a hormonally active peptide involved in maintaining a stable blood glucose concentration. Insulin, together with its structural analogues, is used for the treatment of diabetes mellitus. A limiting factor in the analysis of these substances is their instability and very low concentration in blood samples, which is in the order of pg/ml.

Method development included optimization of mass spectrometry (MS) parameters for the detection of individual analytes and optimization of ultra-high performance liquid chromatography (UHPLC) to ensure separation of the analytes.

Two systems, namely 1D (one dimensional) and 2D (two dimensional) UHPLC, were tested in coupling with tandem mass spectrometry using triple quadrupole (QqQ). Several UHPLC columns, different compositions of mobile phase and additives and gradient elution settings were tested during method development. The aim was to separate the individual insulins while identifying selected reaction monitoring (SRM) transitions for quantification. Mainly focus was put on human insulin and insulin lispro, which have the same molecular weight and their only difference is the arrangement of two amino acids in the structure of these compounds.

The method of plasma sample preparation was adopted from AN (application notes) Waters **Chyba! Nenalezen zdroj odkazů.** and involved protein precipitation (PPT) followed by solid-phase microextraction (µSPE) using a multi-modal Oasis Max µSPE sorbent.

The final 2D-UHPLC-MS/MS method for the analysis of insulin and its structural analogues was performed on a Xevo TQ - XS instrument, using an XBridge C18 2.1 x 20 mm trap column with a particle size of 3.5 µm, a Cortecs UPLC C18+ 2.1 x 100 mm analytical column with a particle size of 1.6 µm with a total chromatographic analysis time of 11 minutes. The mobile phase contained aqueous and acetonitrile components with the addition of formic acid.

The newly developed method will be used for identification and quantification of insulin and its selected analogues in unexplained deaths for the needs of the Department of Forensic Medicine, Faculty of Medicine, Ostrava University Hospital.

Keywords: insulin and its structural analogues, 2D-UHPLC-MS/MS, method development, optimization

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