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Simultaneous determination of insulin and its analogues in pharmaceutical formulations by micellar electrokinetic chromatography☆

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ABSTRACT

A simple and efficient MEKC method was developed to simultaneously determine human insulin, its five analogues, the main degradation products and the excipients usually present in injection formulations. A very fast method with a total analysis time of 3 min was then successfully validated for the analysis of human insulin and the quality control of commercial formulations was carried out.

1. Introduction

Insulin is an important hormone secreted by pancreatic β-cells regulating principally glucose metabolism. Currently synthesised by recombinant DNA technology, this hormone is commonly administered by subcutaneous injection for the treatment of insulin-dependent diabetes mellitus. Human insulin consists of two peptide chains – A and B – containing 21 and 30 amino acid residues, respectively, and connected via two disulphide bridges (Fig. 1). As can be seen in this figure, the different insulin variants are quite similar since they differ only by one to three amino acids. Lispro, aspart and glulisin are rapid-acting analogues that mimic postprandial insulin secretion. Glargin and detemir are long-acting analogues that mimic basal insulin secretion. Protamine is sometimes associated with human insulin, lispro or aspart to provide an intermediate action profile [1]. The excipients of pharmaceutical injections are mainly meta-cresol and phenol.

Diabetes is one of the most common metabolic diseases in the world and the prevalence is increasing every year, especially for type 2 diabetes. A lot of insulin formulations are then produced; they are expensive and require a prescription. Therefore they are an important target for counterfeiting. Even if the proportion of counterfeit drugs is superior in developing countries, it is worth noting that it is affecting the whole world and more particularly the e-commerce in more economically developed countries. Counterfeited insulins contribute to therapeutic failures and in some cases can also lead to death. Therefore, it is essential for public health to strengthen the control of pharmaceutical products such as insulin formulations by developing efficient and easily applicable quality control methods.

The determination of insulin has been already described in the literature but most papers report immunochemical methods for the monitoring of biological samples [2–11]. Some instrumental analytical methods based on LC or CE were also developed for the analysis of pharmaceutical formulations but only for human insulin quantification in formulations without protamine [12–15]. Recently, CE and MEKC were applied to the separation of various

Abbreviations: ACN, acetonitrile; BGE, background electrolyte; CM-β-CyD, carboxymethyl-β-CyD; DNA, deoxyribonucleic acid; EOF, electroosmotic flow; HDAS-β-CyD, heptakis (2,3-di-*O*-acetyl-6-*O*-sulfo)-β-CyD; HDMS-β-CyD, heptakis (2,3-di-*O*-methyl-6-*O*-sulfo)-β-CyD; IS, internal standard; N, number of theoretical plates, separation efficiency; OS-γ-CyD, octakis (2,3-dihydroxy-6-*O*-sulfo)-γ-CyD; pI, isoelectric point; SBE-β-CyD, sulfobutylether-β-CyD; TM-β-CyD, heptakis (2,3,6-tri-*O*-methyl)-β-CyD.

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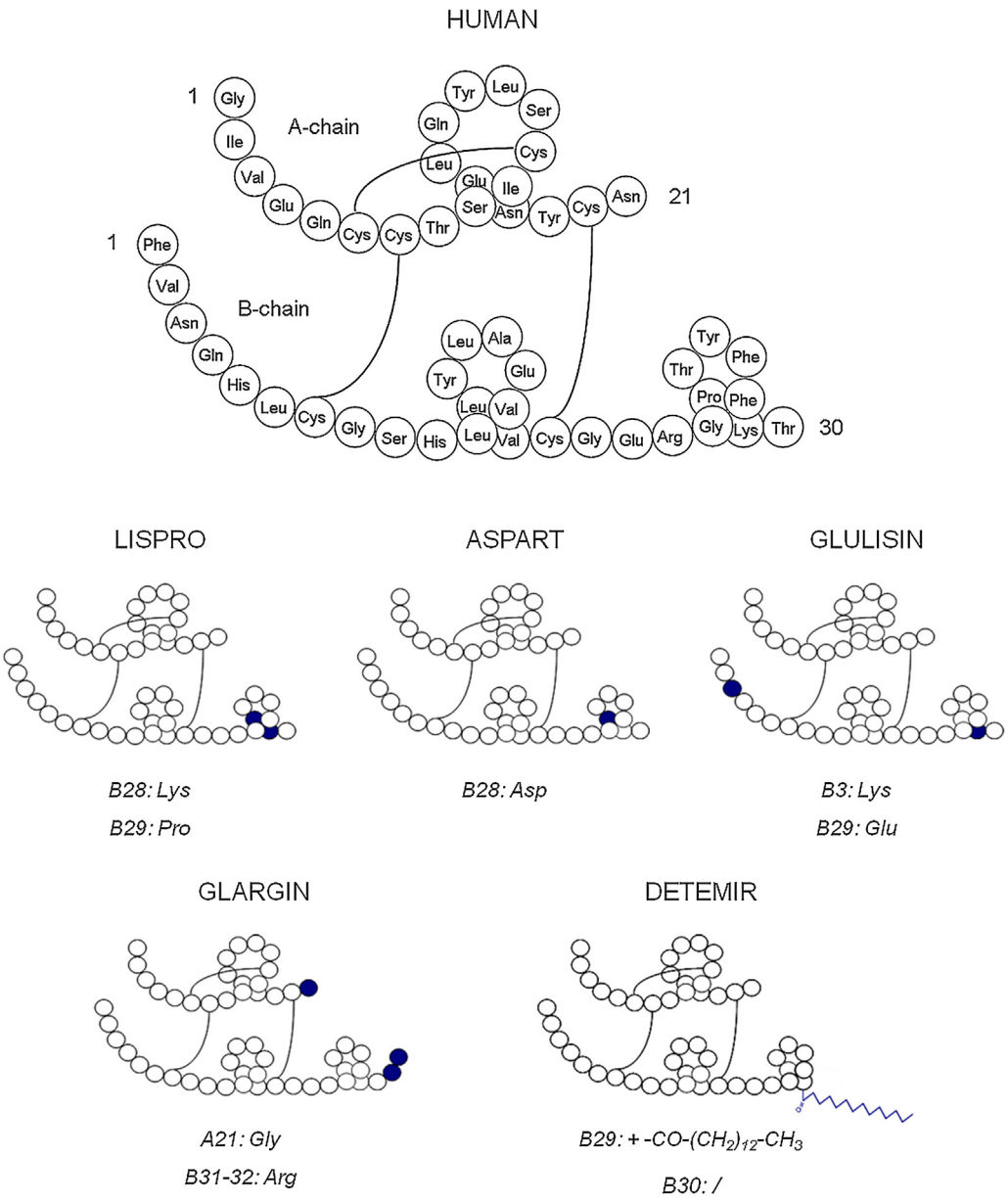

Fig. 1. Structure of human insulin and its analogues.

insulin analogues [16–18]. Indeed, CE is an attractive technique with its well-known advantages such as simplicity, high separation efficiency, short analysis time and low sample and solvent consumption. It is considered as a powerful alternative to HPLC and is frequently used for the separation of large biomolecules [19,20].

The aim of this work was to develop an easy and fast MEKC method for the simultaneous determination of human insulin, its five analogues and the excipients usually present in commercial formulations. A stability study was also performed and degradation products could be separated with the same method. The method was shortened, adjusted and was then fully validated for human insulin analysis. It was also applied to the quality control of pharmaceutical formulations, including those containing protamine.

2. Materials and methods

2.1. Chemicals and reagents

Acetic acid, ammonia, HCl (37%), ACN, SDS and $ZnCl_2$ were purchased from Merck (Darmstadt, Germany). Protamine, sodium benzoate, glycerol, phenol and meta-cresol were obtained from Sigma-Aldrich (St. Louis, MO, USA). γ-CyD was from Sigma-Aldrich. OS-γ-CyD was kindly provided by Professor G. Vigh (Texas A&M University, TX, USA). CM-β-CyD, TM-β-CyD and HDMS-β-CyD were purchased from Cyclolab (Budapest, Hungary). SBE-β-CyD was from CyDex Pharmaceuticals (Lenexa, KS, USA) and HDAS-β-CyD was obtained from Antek Instruments (Houston, TX, USA).

Human insulin standard was obtained from Sigma-Aldrich. Different pharmaceutical formulations were also used: Humuline NPH® (human insulin) and Humalog® (insulin lispro) were obtained from Eli-Lilly (Indianapolis, IN, USA). Novomix 30® (insulin aspart) and Levemir® (insulin detemir) were purchased from Novo Nordisk (Bagsvaerd, Denmark). Insuman Rapid® (human insulin), Apidra® (insulin glulisin) and Lantus® (insulin glargin) were obtained from Sanofi Aventis (Bridgewater, NJ, USA). Actrapid® and Mixtard® (human insulins) were collected on the internet.

Ultra-pure water was supplied by a Milli-Q equipment (Millipore, Bedford, MA, USA) and Chromafil® syringe filters (0.20 μm) were purchased from Macherey-Nagel (Düren, Germany).