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Chromatographic and Mass Spectrometric Analysis of Antidiabetic Biopharmaceuticals

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ABSTRACT

In the present communication various chromatography and chromatography coupled mass spectrometric methods used for the routine analysis of antidiabetic biopharmaceuticals such as insulin and its analogs were reviewed. The study revealed the fact that High Performance Liquid Chromatography (HPLC) was extensively used for quality control analysis of insulin and its analogs and mass spectrometric technique was widely used for the identification of impurities.

Key words: Antidiabetic, Biopharmaceuticals, Chromatography, Mass spectrometry, Impurities

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INTRODUCTION

Biopharmaceuticals are preparations that exhibits structural similarities and pharmacological activities of compounds found within the body. They are protein in nature and are produced using recombinant DNA technology. They have been employed in treating dreadful disorders like cancer, diabetes, hepatitis and many other. Antidiabetic biopharmaceuticals are those biological preparations that are designed to fight against diabetes which includes insulin and its analogs that are derived from recombinant DNA technology and are at high risk of being contaminated by impurities or related substances. Impurities are undesirable substance's that can occur in final drug substances or drug products found either due to product related substances, process related substances or host cell related substances. Contamination of insulin and its analogs by impurities can induce severe adverse immune reactions to patient and can slaughter a promising drug candidate from the market. Hence it becomes necessary to develop analytical methods that could be used for routine analysis of insulin and its analogs and also for the identification of impurities associated with them. Many analytical methods have been developed for the analysis of biologics. Chromatography is a separation technique that is widely employed for routine analysis of insulin and its analogs. Among chromatography, high performance liquid chromatography play a prominent role. Mass spectrometry is also a versatile analytical tool for the characterization of biopharmaceuticals. However, Chromatography coupled with mass spectrometry play a vital role in analysis of impurities in insulin and its analogs. Observing these facts, in the present study chromatography and chromatography coupled mass spectrometric methods adopted for the routine analysis of insulin and its analogs were reviewed.

MJ Ansari *et al.* have developed and validated a simple and rapid RP-HPLC method for routine analysis of human insulin in dosage form by using RP-C18 (150×4.6mm, 5µm) column. The mobile phase used consists of 55 volume of 1mmol sodium sulphate in HPLC water pH 3.2 adjusted by phosphoric acid and 45 volume of acetonitrile. The flow rate was 1mL/min and the retention time of insulin was 4.3min at a detector wavelength of 214nm [1].

BA Mousa*et al.* have developed and validated RP-HPLC method for the determination of recombinant human insulin in bulk and pharmaceutical dosage form, with reduced retention time. They assessed the effects of the column temperature, pH of mobile phase and the presence of vial additives or impurities on the accuracy of the

assay. They used C-18 column and mobile phase was composed of Solution A (aqueous solution of anhydrous Na_2SO_4) and solution B (anhydrous Na_2SO_4 in 50:50 mixture of water and acetonitrile) [2].

Y Bilal *et al.*have developed and validated a simple and reliable HPLC method with diode array detector for determining the human insulin in pharmaceutical preparations. They used reversed-phase column. The mobile phase consists of acetonitrile and 0.2M sodium sulfate buffer (pH 2.4) solution (25:75, v/v). This method was validated for specificity, linearity, precision, accuracy, and limit of quantitation. This method was applied for the quality control of commercial Actrapid formulation [3].

DS Rajan *et al.* have developed a simple and sensitive method for the analysis of human insulin injection by using reverse-phase high performance liquid chromatography technique. C-18 Column was used as stationary phase, and mobile phase consist of 60 volume of 1mmol sodium sulphate and 0.2% trimethylamine in water, pH 3.2 adjusted by phosphoric acid and 40 volume of acetonitrile. Flow rate was adjusted to 1mL/min. Sample size of 20µL was used and eluent was monitored with a UV detector set at 214nm at room temperature [4].

O Alexis *et al.* have developed two high performance liquid chromatography techniques like RP and SEC-HPLC for the separation of insulin and its monodesamido derivatives and aggregation products in pharmaceutical preparations. The developed method was validated and the results of validation shows that the method used were specific, precise, reproducible, and robust in nature. Further characterization of insulin and its derivatives were done by static and dynamic light scattering technique [5].

S Joanna *et al.* isolated and characterized the acetylated derivative of recombinant Insulin Lispro produced in *Escherichia coli*. Ion-exchange chromatography was used to collect the related substance in Insulin Lispro and it was subjected to MALDI analysis which showed a +42Da mass increment. Glu-c enzyme cleavage enabled indication of modified peptide, tandem mass spectrometry allowed to explore the location and type of modification. In addition peptide was isolated and sequenced by Edman degradation [6].

H Jia-Chuan *et al.* identified recombinant human insulin and its analogs such as insulin lispro, insulin aspart, insulin detemir, insulin glargine, and insulin glulisine by peptide mapping technique. They used *S. aureus* V8 endoproteinase for enzymatic digestion. The digestion was carried out for 6 hours at 25°C and then the digested samples were analysed using RP-HPLC with diode array detector. V8 endoproteinase digestion results in formation of fragments which were detected by UV-absorbance at 214nm and compared to the reference standard. Finally they concluded that the peptide mapping technique was of great importance in identification and evaluation of protein variants [7].

Y Hongmei *et Al.* developed a simple, fast, effective, robust method to identify disulfide bonds in proteins/peptides by using a specific modification of sulfhydryl groups of proteins with α-, β-unsaturated carboxyl group containing matrices in on-target MALDI-MS analysis. The developed method gave a platform for the identification of disulfide in peptide without resorting to the alkylation of disulfide bonds and HPLC separation. The formation of peptide-matrix adducts in solution phase instead of gas phase was provided. The nucleophilic addition mechanism and the factors affecting generation of the matrix adducts discussed and they reported that the pH of the solution is the most important factor to control the formation of adducts. The developed method was successful in examining all disulphide bonds in human insulin and lysozyme [8].

G Sofia *et al.* structurally characterized three forms of glycatedbovine insulin when glycated under reducing conditions. Simultaneously characterization of glycated insulin produced under pseudo physiological conditions was also done. HPLC purification of mono-, di-, and triglycated insulin forms, followed by enzymatic digestion and mass spectrometry (MALDI-TOF/TOF) for the unambiguous assignment of the glycation sites was their approach [9].

V Kannan *et al.* isolated monoglycosylated insulin glargine and tri-glycosylated insulin glargine, two Glycoforms of glargine expressed in *Pichia pastoris* by high-performance liquid Chromatography. These isolated fractions were subjected to ESI and MALDI mass spectrometry to identify the glycosylation site, its nature and structure of glycoform. Then they concluded that glycosylations were O-linked and the first glycosylation happens at T8 and consecutive glycosylation occurs at T30 in the B-chain. Dimannosylated glargine covalently attached to threonine at position 30 was confirmed by tandem mass spectrometry techniques. The mannose nature of glycan was confirmed by using α-mannosidase [10].

CONCLUSION

Chromatographic methods and mass spectrometric based methods were reviewed and found that the methods adopted can be used for the routine analysis of antidiabetic biopharmaceuticals in bulk and pharmaceutical dosage forms.

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