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Short communication

Application of experimental design for determination of insulin analogs

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Introduction

Determination of human insulin and its analogs and their related compounds is a challenge as a consequence of very similar chemical structure of these compounds. The most common methods for determination of insulin and its related compounds in pharmaceutical formulations are: radioimmunoassay, enzyme immunoassay, luminescent immunoassay, high performance liquid chromatography and capillary electrophoresis (Arcelloni et al., 1998; Dezier et al,. 1987). Capillary electrophoresis (CE) is a popular method permitting separation of analytes from small molecules to large biomolecules. Its advantages include simplicity, high separation efficiency, short analysis time and low sample and solvent consumption, making this a powerful alternative method to High performance liquid chromatography (HPLC). Consequently, there were several methods of CE developed for pharmaceutical analysis of insulin formulations (Arcelloni et al., 1998; Lamalle et al., 2015 Ortner et al., 2009).

The International Chemometrics Society (ICS) defines chemometrics as the science of relating measurements made on a chemical system or process to the state of the system via application of mathematical or statistical methods (Gemperline, 2006). Design of experiments (DoE) is a statistical method to develop optimization that is used across a variety of industries. It possesses a numerous advantages over the traditionally employed "one variable at a time" (OVAT) approach, such as increased experimental efficiency, as well as an ability to resolve factor interactions and provide detailed maps of a process's behavior (Bowden et al., 2019). To improve the separation process, CE coupled with chemometric optimization could provide a complete profile of separation, offering the useful information of factors influencing the separation as well as their interactions. The principle of DoE is arrangement of the experiments at changed combination of factors with the purpose of gaining the maximum information with minimal runs. This process is arranged by changing all the applicable factors simultaneously, according to a planned set of experiments which determine the effect of each factor and their significance (Aboul-Harnisch et al., 2018; Enein and Abdel-Megied, 2019; Orlandini et al., 2014).

Therefore, in this study, we demonstrate research of the main factors influencing migration behavior of insulin and its analogues. For this purpose, application of Response Surface Methodology (RSM) enabled the optimization of a CE method with the objective to improve the separation of insulin analogs.

Materials and methods

Chemical and reagents

Insulin and analogues were used from pharmaceutical formulations including Apidra® SoloStar® (Sanofi), Humalog® (Lilly), Humulin®R (Lilly), Lantus® SoloStar® (Sanofi), Levemir® FlexPen® (Novo Nordisk), NovoRapid® Penfill® (Novo Nordisk). The analytical reagents: sodium hydroxide, ammonium acetate, ammonia, Acetic acid and hydrochloric acid were purchased from Sigma-Aldrich.

Instrumentation

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The instrumentation used was the CE 7100 system (Agilent, Waldbronn, Germany). For separation the fussed silica capillaries (Polymicro Technology, Phoenix, AZ, USA) with an internal diameter of 50 μm and a total length of 65 cm in positive mode using constant voltage were used. Samples were introduced with a pressure of -50 mbar for 4s at the cathodic end of capillary. Aiming to prevent the adsorption of proteins in the capillary wall, a strong preconditioning was used. This procedure included conditioning of the capillary between each injection for 18 min with 0.1M NaOH, 6 min with acetonitrile and 24 min with the running buffer. The statistical software MODDE® was used for the DoE.

Experimental design

In this study the factors with higher influence in the migration behavior of each insulin analogue were selected and analyzed using Experimental design. After a preliminary study these factors were chosen to be the pH and buffer concentration of the background electrolyte and the voltage applied. The impact of the above-mentioned factors separately and their interaction (combination of the factors) were analyzed by RSM design called CCF. The levels of factors selected for optimization were the pH value (8, 9, 10) and the concentration (40, 50, 60) of the background electrolyte (BGE) and voltage (10, 20, 30). The number of total experiments calculated for these 3 factors was 15.

Results and discussion

Six insulin analogues were determined using this Experimental design. After finishing the required CE runs in different conditions, for each insulin analog was calculated the migration time and the influence of these factors on the migration behavior was studied. The most influencing factor for migration time of each insulin analog resulted to be the voltage. Its influence was more significant than any other factor, or the combination of the factors (pH and BGE concentration, pH and voltage or BGE concentration and voltage). Other influencing factor is pH value of the BGE, which also has an important effect on the separation time. These discoveries suggest that the pH variation appears to be the most effective method of controlling a CE separation and accordingly analysis time.

Conclusion

Applying the RSM design, was able to find the optimal range of the most crucial factors including buffer pH and concentration, in relation of analysis time reduction. In consideration of the fact that when determining proteins and peptides with CE, a long preconditioning time is necessary to remove the adsorbed molecules in capillary wall, the appropriate analysis time for this determination would be 8-10 min. After several analysis for each insulin analogue, the optimal scope of specified factors to obtain acceptable analysis time were: applied voltage 20-25 kV, the buffer pH: 8.5-9.5 and the buffer concentration 40 mM ammonium acetate.

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