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Short communication

Software assisted method optimization for related and degradation products of angiotensin converting enzyme inhibitor in drug product containing two active ingredients

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Introduction

Angiotensin converting enzyme inhibitor (Component 1) in combination with long-acting calcium antagonist (Component 2) is widely used as therapy in patients with mild to moderate essential hypertension. To avoid therapy with multiple formulations, combination from the two active ingredients in one tablet is the ideal solution for patients (Gawade et al., 2019; Springall et al., 2019). From analytical aspect, method development for related and degradation products for the two active ingredients is a challenge, hence impurity profile study of both active substances should be performed.

According to the literature review there are several high performance liquid chromatography (HPLC) methods for formulations with only one of the active components, but only a few for a formulation containing both of these components.

Method development for related and degradation products of Component 1 was time consuming process in which impurities were not well separated from each other or from the impurities of the second active compound.

Using the One factor at time (OAFT) approach for method optimization of the gradient and the temperature, robust method was not developed.

The aim of this study is implementation of software which uses Design of experiments (DoE) plans as an efficient and fast tool for method development. By using statistical software tools in method optimization, the effect of each factor on the separation can be calculated and the data can be used to find the optimum separation (Barnett et all, 2013).

Materials and methods

All reagents in the study were gradient grade. For the active substance Component 1, CRS standard was provided by the EDQM (Strasbourg, France). Also system suitability standard, provided by EDQM, was used for identification of specified impurities of the active substance. The experiment was performed on a sample with concentration of 1 mg/mL, spiked with system suitability standard (mix from three known impurities). Using spike sample, improved visualization of the two critical separations was achieved, between specified impurity of Component 1 and unknown impurity of Component 2 and between placebo peak and main peak of Component 1.

The development study was carried out on an Agilent 1290 Infinity Automated Method Development system equipped with a column and solvent selection valves that allow automated exploration and screening of a wide range of conditions that are common critical parameters in HPLC (different columns, pH ranges and organic modifiers).

The screening experiment was conducted on Fusion AE for establishing basic method variables (pH, column type, temperature, wavelength, type of solvent and flow) (Garber et al., 2011). Optimization of the method was needed because of the unsatisfying separation between placebo peak and the main peak of Component 1 and also between unknown impurity from Component 2 and known and specified impurity from Component 1.

Software assisted method optimization for chromatography DryLab 4.0 software package (Molnar-Institute) was used. Based on a small number of

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experiments, these software applications can predict the movement of peaks when parameters such as eluent composition or pH, flow rate, column temperature, column dimensions, and particle size are changed.

Experiments were made on two Dionex UltiMate™ 3000 (Thermo Scientific) systems, on two different columns ACE 5 C-18 250-4.6 mm and ACE 5 C-8 250-4.0 mm (5µm), on two temperatures 30 °C and 55 °C using mobile phase A (phosphate buffer pH 3.8 : ACN = 97:3) and mobile phase B (phosphate buffer pH 3.8 : ACN = 70:30) in gradient mode, starting with 100% mobile phase A and ending with 100% mobile phase B. Two gradient times were proposed in DryLab 4.0, 50 minutes and 80 minutes. Flow rate was 1.5 mL/min. Injection volume was 20µL. UV detection was performed at 210 nm.

Results and discussion

Results on column ACE 5 C-8 250 : 4.0 mm (5µm), were not satisfying because the peak of placebo was not separated from the peak of Component 1, nor the unknown impurity of Component 2 from the known and specified impurity from Component 1.

With the second column, ACE 5 C-18 250-4.6 mm, using chromatography with gradient time of 80 minutes and temperature of 55 °C all peaks were separated. Peaks were integrated and chromatography was evaluated by the software DryLab 4.0 so he could predict the movement of the peaks when gradient mode is changed.

Knowing this information from the experiment, best gradient mode was found and samples were analyzed by the proposed method. After achieving satisfying chromatography, all specified impurities from Component 2 were analyzed with the proposed method to assure that there is no interaction between them and the specified impurities from Component 1. Because of the late elution of the specified impurities from Component 2, rum time was adjusted to 110 minutes.

After establishing optimized method for related and degradation products of Component 1, in combination with Fusion AE and DryLab 4.0, specificity, linearity, accuracy, robustness and precision were verified with method validation (Little, 2014).

Conclusion

This study highlights significant utility of DoE in optimization of method development especially for related and degradation products of active components. Using the OAFT approach for method optimization, non-robust method can be developed in long period of time.

With the implementation of softwares Fusion AE and DryLab 4.0, method was developed and conditions were optimized to obtain good chromatography with optimum peak separation. When estimations for best separation was put in practice the obtained result was successful and matched the software prediction. This suggests that software assisted method optimization for related and degradation products can replace the trial and error OAFT approach used to achieve good chromatography.

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