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Short communication

DryLab® software application for development of HPLC gradient method suitable for determination of three components in drug product

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Introduction

Quality by design (QbD) approach by including a set of experiments accelerates the process of achieving good chromatography using modern development and optimization software as DryLab® (Molnar-Institute Berlin, Germany) and MODDE® (MODDE® 12 User Guide, Sweden)

The DryLab® software package is used for experimental modeling and involves forecasting which is simulation of chromatographic behavior of a stationary phase based on a small number of pre-defined experimental parameters, for e.g., elution mode - isocratic or gradient, cell phase composition, flow, column temperature, cell phase pH, then column filling characteristics, column length and diameter, as well as type of stationary phase, particle dimensions. Using the DryLab® software package enables time saving, reduction of resources, expensive laboratory equipment, instruments, columns, solvents etc.

The MODDE® software package is software based on the "Design of Experiment" (DoE) concept (Das and Maity, 2017). The basic idea is with the assistance of the most famous experiments, their systematic and planned performance and processing of the obtained results, to take the maximum amount of information with the smallest possible number of experiments, preferably. The final goal of using the DoE approach is obtaining stability indicating, accurate and robust analytical methods with relevant and effective experiments performed for a short time.

Optimisation of the method for determination of assay on three components in multicomponent drug product was required because the present method doesn't provide selectivity between placebo peak and the main peak of Component 1 and also, between unknown impurity and the main peak of Component 2. Overlapping between placebo peaks with the Component 3 also was noticed.

This manuscript briefly describes optimization of a High Pressure Liquid Chromatography (HPLC) gradient method for assay of three components in multicomponent drug product. The final method was achieved through best responses generated by QbD software and the analytical knowledge gained from the optimization phase.

Materials and methods

All reagents used during the study were with HPLC grade purity. Reference standards of three components separately, were used for preparation of standard solution with analytical concentration of 0.014 mg/mL, 0.006 mg/mL and 0.02 mg/mL, respectively. The sample solution was prepared from the present formulation of the drug product.

In order to obtain a proper separation between the peaks of interest (active substance and two preservatives) and between the main peaks and peaks that originate from the matrix, the Gradient Mode 2 runs model from the software package DryLab® was preferred.

Experiments were made on Dionex UltiMate™ 3000 (Thermo Scientific) HPLC system, using water as mobile phase A and methanol as mobile phase B in gradient mode, starting with 10% mobile phase B and ending with 80% mobile phase B. Two gradient times were proposed in DryLab®, 60 and 180 minutes. Flow rate was 2.0 mL/min. Injection volume was 20µL. UV detection was performed at 254nm.

One of the advantages of the DryLab® software package is the ability to predict the optimal gradient, which is given in a visually quite acceptable solution. DryLab®

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provides peak resolution information and generates millions of unique, virtual chromatograms. Gradient changes associated with a virtual chromatogram, which are allowed by Gradient Editor tool, correspond to a predicted gradient. Proposed optimal gradient from DryLab®, was transmitted in real time and generated data was very similar to the software prediction.

Results and discussion

The optimal proposed gradient of the software DryLab® was evaluated in real time on several different octadecyl HPLC columns.

On HPLC column LiChrospher 100 RP18 250 x 4.0mm (5µm) using chromatography with gradient time of 60 minutes, all peaks were separated and all parameters for system suitability were acceptable. During performing the robustness with MODDE® Placket-Burman design matrix with 11 experiments, different batches of HPLC columns as one of the variables, was proven as a critical factor (Maskovic et al., 2010).

Of the other tested HPLC columns, using chromatography with gradient time of 60 minutes all parameters for system suitability were acceptable, but an overlapping of placebo peaks with the Component 3 was obtained.

Then, The Gradient Mode 2 runs model from the software package DryLab® on Zorbax 5 SB C18 250 x 4.0mm (5µm), was chosen as most adequate HPLC column. Peaks were integrated and chromatogram was put back in the software DryLab® in order to predict the movement of the peaks when gradient mode is changed and to separate peaks from placebo and the main peak of Component 3.

With the new optimal proposed gradient, chromatography of 30 minutes is achieved, where there is a complete separation of all nine peaks with suitable system suitability criteria. Five peaks were originating from placebo, three peaks from the main components and additionally one peak from an unknown impurity.

Using the QbD approach, method for assay of three components in multicomponent drug product was developed and optimized as following: HPLC column: Zorbax 5 SB C18 250 mm x 4.0 mm; 5µm, gradient grade elution with water R as mobile phase A and methanol as mobile phase B, flow rate of 2 mL/min, run time 30 minutes, column temperature 25°C and injection volume 20 µL. Gradient elution steps: Elute isocratically for 10 minutes with 30% of mobile phase B and 70% of mobile phase A. Use linear gradient elution increasing the concentration of mobile phase B to 60% after 2 minutes. Carry out a linear gradient elution for 3 minute to 75% of mobile phase B, elute isocratically for a further 10 minutes with a mixture of 75% of mobile phase B and 25% of mobile phase A. Degrease the concentration of mobile phase B of 30% for 0.1 minute, and elute isocratically for 10 minutes more.

Robustness of the final method was performed with MODDE® Placket-Burman design matrix, with 11 experiments. Adequate results were accomplished.

After method optimization for determination of assay of Component 1, Component 2 and Component 3 in combination with both MODDE® and DryLab® Software specificity, linearity, accuracy and precision were proved with analytical method validation.

Conclusion

This study highlights significant efficacy of the DoE in optimization of analytical method for determination of assay of three components in multicomponent drug product. Using the one factor at time (OAFT) approach, method optimization could be long lasting process with deficiency in method performance regarding to robustness and reproducibility.

By the implementation of software MODDE® and DryLab®, method was developed and chromatographic conditions were optimized to achieve adequate chromatography with suitable peak selectivity. When theoretical obtained response for best separation was included in testing analysis the obtained result matched with the software prediction. It can be concluded that software assisted method optimization for assay of multicomponent drug product could effectively replace the trial and error based OAFT approach.

Method was validated for specificity, linearity, accuracy, precision, robustness, stability of the solutions and filter study and met the predetermined acceptance criteria. Hence, this method could be introduced into the routine use for the determination of assay.

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