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® (Molinar-Institute Berlin, Germany) and MODDE® (MODDE® 12 User Guide, Sweden).

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

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 UltraMate™ 3600 (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.

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

Results and discussion

On HPLC column LiChrospher 100 RP18 250 x 0.4mm (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® Plackett-Burman design matrix with 11 experiments, different batches of HPLC columns as one of the variables, was proven as a critical factor (Maskovic, M. 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 0.4mm (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 to and 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 chromatographic condition:

<table>
<thead>
<tr>
<th>Chromatographic condition</th>
<th>HPLC columns</th>
<th>Mobile phase</th>
<th>Flow rate</th>
<th>Column temperature</th>
<th>Injection volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zorbax 5 SB C18 250 x 0.4mm, 5µm</td>
<td>Zorbax 5 SB C18 250 x 0.4mm, 5µm</td>
<td>gradient grade elution with water R as mobile phase A and methanol as mobile phase B</td>
<td>2.0 mL/min</td>
<td>25°C</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

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.

References


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Molinar-Institute for applied chromatography, Berlin, Germany
DryLab (molinar-institute.com)

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