INTRODUCTION

The European Pharmacopoeia 6.7 describes a liquid chromatography (LC) method for the quantification of sulindac, using a quaternary mobile phase including chloroform and with a rather long run time. In the present study, a new method using a short sub-2µm column, which can be used on a classical HPLC system, was developed. The new LC conditions (without chloroform) were optimized by means of a new methodology based on design of experiments in order to obtain an optimal separation.

MATERIALS AND METHODS

Analyses were performed on an Agilent technologies HPLC 1100 series.

Chromatographic conditions: reference method
- Analytical column: Alltima Silica column (250 x 4.6 mm i.d., 10 µm particle size)
- Mobile phase: Acetic acid/ethanol/ethylacetate/chloroform (1:4:100:400 (v/v/v/v))
- Flow-rate: 2.0mL/min
- Temperature: 20° C
- Detection: UV at 280 nm
- Injection volume: 20µL

RESULTS

Apparatus
- Chromatographic conditions: Optimised method
- Analytical column: Platinum C18 Rocket column (53 x 7 mm i.d., 1.5µm particle size)
- Mobile phase: ACN/buffer pH2 (see experimental design section)
- Flow-rate: 3.0mL/min
- Temperature: 35° C
- Detection: UV at 340 nm
- Injection volume: 100µL

Experimental design
Four HPLC factors were investigated using DoE methodology through a design matrix. All of the factors were quantitative (see table 1). The objective of this study was to determine the optimal chromatographic conditions allowing us to obtain a separation criterion of at least 0 minutes (i.e. baseline-resolved peaks) with a probability of at least 90%.

A summary of the optimal values for each factor allowing the achievement of the higher probability ensuring a separation of at least 0 minutes with baseline-resolved peaks is shown in Table 2.

CONCLUSIONS

The developed HPLC method for the quantification of sulindac and its related impurities divided the run time of analyses by three compared to the reference method. Figure 4a and 4b show the optimal predicted and experimental chromatograms. As can be seen, the predicted retention times were found to be very close to the experimental values and an acceptable separation was obtained within an analysis time of 6 minutes.

ACKNOWLEDGEMENTS

The authors acknowledge the Walloon Region of Belgium and Arlenda®.

Table 2: Summary of the optimal values for each factor of the experimental design

<table>
<thead>
<tr>
<th>Optimal values</th>
<th>P(separation&gt; 0)&gt;0.9</th>
<th>Plateinit (min)</th>
<th>ACNlower (%)</th>
<th>ACNupper (%)</th>
<th>Gradient time (min)</th>
</tr>
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<tr>
<td>Experimental</td>
<td>1</td>
<td>0.5</td>
<td>40</td>
<td>55</td>
<td>3.6</td>
</tr>
<tr>
<td>Optimised</td>
<td></td>
<td></td>
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</table>

Figure 1: Chromatogram of the reference method.

Figure 2: Experimental retention times versus predicted ones. Residuals are depicted at the bottom right corner.

Figure 3: Surface of probability to reach S>0. The design space is surrounded by black lines for an expected probability to have well-separated peaks is 0.9. Factors optimal values are placed between parentheses.

Figure 4: (a) Experimental chromatogram recorded at optimal condition. (b) Predicted chromatogram at optimal condition.

Figure 5: Accuracy profiles of (a) sulindac, (b) E-sulindac, (c) sulphide and (d) sulphone.

Figure 6: Accuracy profiles of (a) sulindac, (b) E-sulindac and (c) sulphide.

Figure 7: Predicted chromatogram at optimal condition. (1: sulindac, 2: sulphide, 3:sulphone, 4: E-sulindac)