Optimisation and validation of a fast HPLC method for the quantification of sulindac and its related impurities

Université de Liège

F. Krier[1], M. Brion[1], B. Debrus[2], P. Lebrun[2], A. Driesen[1], E. Ziemons[2], B. Evrard[1] and Ph. Hubert[2]

[1] Laboratory of Pharmaceutical Technology, CIRM, University of Liège, Belgium
[2] Laboratory of Analytical Chemistry, CIRM, University of Liège, Belgium
E-mail: Fabrice.Krier@ulg.ac.be
URL: http://hdl.handle.net/2268/62350

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

Apparatus

Analyses were performed on an Agilent technologies HPLC 1100 series.

Chromatographic conditions: reference method (NPLC)

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

Chromatographic conditions: Optimised method (RPLC)

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

Experimental design

Four HPLC factors were investigated using DoE methodology through a full factorial design. 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%.

Table 1: Description of the levels of four factors involved in the experimental design

	Plateinit (min)	ACN lower (%)	ACNupper (%)	Gradient time (min)
Levels	0-1	15-30-55	55-60-65	1-3-5
Central point	0-0.5-1	30	60	3
Maximum effect	linear	quadratic	quadratic	quadratic

RESULTS

Reference method

As can be seen in Figure 1, the reference method enabled the separation of all the compounds within 18 minutes and was completed within 25 minutes.

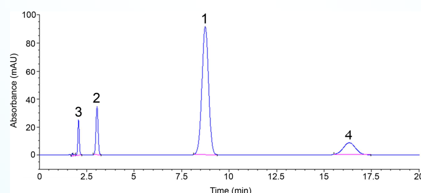

Figure 1 : Chromatogram of the reference method.

Optimised method

Figure 2 illustrates the quality of the fit of the observed retention times versus the predicted retention times. Most of the residuals were mainly located within the interval [-0.2 min, 0.2 min]. Figure 3 shows the probability surfaces in different directions of the space around the optimal solution (for each graph, two factors were fixed at their optimal values). As we can see, the best probability surface was obtained when the duration of the initial isocratic plate was around 1 minute.

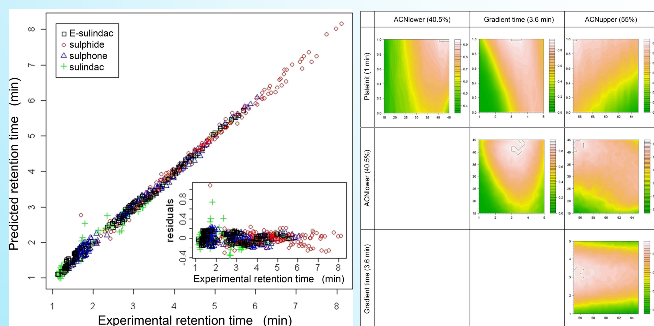

Figure 2 : Experimental retention times versus predicted ones. Residuals are depicted at the bottom right corner.

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.

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

Table 2: Optimal factor setting maximising the separation of the compounds

	Plateinit (min)	ACNlower (%)	ACNupper (%)	Gradient time (min)
Optimal values P(separation> 0)>0.9	1	40.5	55	3.6

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.

Figure 4 : (a) Experimental chromatogram recorded at optimal solution. (b) Predicted chromatogram at optimal condition. (1: sulindac, 2: sulphide, 3: sulphone, 4: E-sulindac)

Validation method

The calibration and validation standards were prepared by mixing and diluting the stock solutions with phosphate buffer solution (pH 7.4; 50 mM) to reach the concentration levels: 100/10; 100/5; 100/1; 100/0.5; 50/0.25; 25/0.125; 1/0.005; 0.5/0.0025 µg/ml (sulindac concentration/ concentrations of related impurities, respectively.

An original approach using accuracy profiles based on tolerance intervals was applied to evaluate the reliability of the results. The tolerance interval used was a "β-expectation tolerance interval" defining an interval in which it is expected that each future result will fall with a defined probability (β). It is therefore a predictive methodology. This tolerance interval is computed for each validation standard concentration level, using their estimated intermediate precision standard deviation and bias. By joining together the upper tolerance limits on the one hand and the lower tolerance limits on the other hand, the method defines an accuracy profile (fig.5)

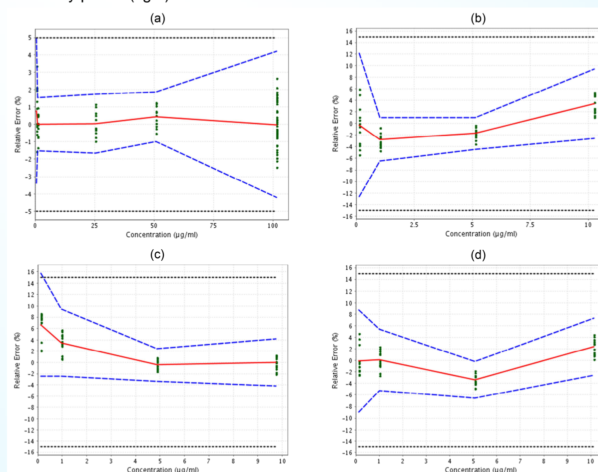

Figure 5 : Accuracy profiles of (a) sulindac, (b) E-sulindac, (c) sulphide and (d) sulphone. Relative bias (—), ± 5% acceptance limits (- - -), 95% (sulindac) or 85% (related impurities) β-expectation tolerance limits (– – –), and relative back-calculated concentrations (●).

CONCLUSIONS

An analytical method for the quantification of sulindac and its related impurities was developed using a short column with sub-2 µm particles on a classical HPLC system. This method was optimised using DoE methodology and the DS concept. Under optimised conditions, the analysis time was considerably reduced (by about 3-fold). Furthermore, we did not use chloroform unlike in the Eur. Ph. reference method. Finally, this particular method was validated successfully using accuracy profiles approach for sulindac and its related substances.

ACKNOWLEDGEMENTS :The authors acknowledge the Walloon Region of Belgium and Arlenda®