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Validation of a LC method for the analysis of zafirlukast in a pharmaceutical formulation☆

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Abstract

A reversed-phase high-performance liquid chromatographic (HPLC) method was developed and validated for estimation of zafirlukast in a pharmaceutical formulation. Assay samples were extracted utilizing acetonitrile. Drug and internal standard were chromatographed on reversed-phase C_{18} columns, using mixtures of acetonitrile/water and the eluents were monitored at different wavelengths. The method was validated statistically for its linearity, accuracy, robustness and precision. Experimental design was used during validation to evaluate method robustness and for the determination of intermediate precision. Factors examined for statistical approaches include laboratory, day, analyst, instrument, different percentage of organic modifier, temperature, wavelength and flow-rate. Due to its simplicity and accuracy, the method may be used for routine quality control analysis. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Zafirlukast; Reversed phase high performance liquid chromatography; Validation; Intermediate precision; Robustness testing; Experimental design

1. Introduction

Zafirlukast (4-(5-cyclopentyloxy-carbonylamino-1-methyl-indol-3-ylmethyl)-3-methoxy-*n*-*o*-tolylsulfonylbenzamide) (Fig. 1) is a synthetic, selective peptide leukotriene receptor antagonist indicated for the prophylaxis and treatment of chronic asthma. The rationale for the development of leukotriene antagonists was based on in vitro and in vivo data demonstrating the extensive role of the cysteinyl leukotrienes C_4 (LTC_4), D_4 (LTD_4) and E_4 (LTE_4) in the pathogenesis of asthma.

Initial data have demonstrated an improvement in pulmonary function and symptom control and a reduction in the use of short-acting inhaled

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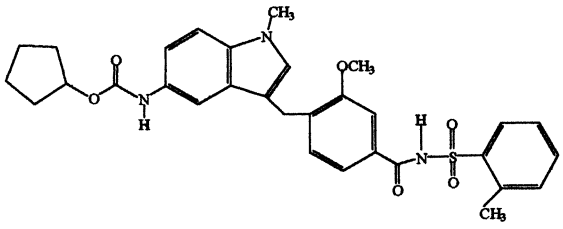

Fig. 1. Chemical structure of zafirlukast.

β_2-adrenoceptor agonist therapy in patients with mild to moderate asthma treated with oral zafirlukast at the recommended dosage of 20 mg twice daily. Available data also suggest that zafirlukast may significantly reduce the incidence of asthma exacerbations [1].

Because zafirlukast is administered orally, it may be particularly beneficial in patients poorly compliant with asthma therapy as a result of poor inhaler technique.

Some HPLC methods [2,3] have been described for the determination of zafirlukast in biological fluids, based on two different detection and separation techniques. The first published method uses normal phase chromatography with fluorescence detection; the second, unpublished, uses reversed phase HPLC with ultraviolet detection.

This paper reports a rapid and sensitive HPLC method with UV detection for routine control of zafirlukast in a pharmaceutical formulation (Accoleit®). The method was validated by linearity, accuracy, precision and robustness. Experimental design was used during validation to evaluate method robustness and for the determination of intermediate precision.

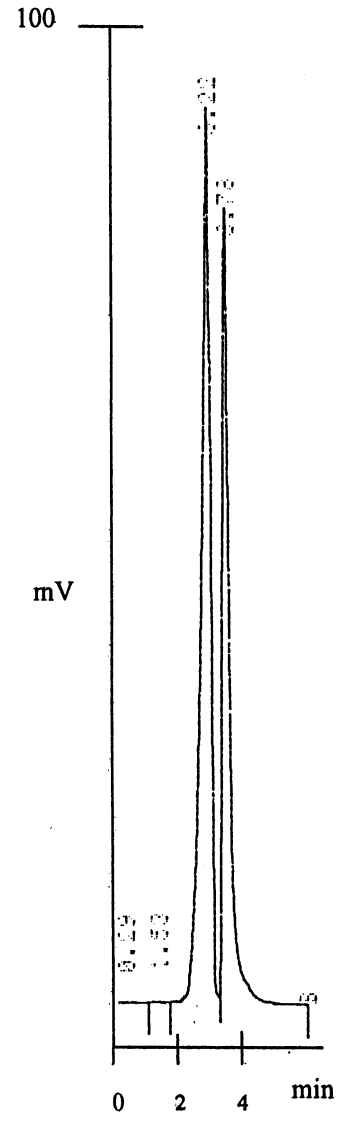

Fig. 2. Chromatogram of a solution containing zafirlukast (tr 3.22) at a concentration of 1.2 mg/ml and internal standard (tr 3.78) at a concentration of 0.12 mg/ml at the described chromatographic conditions.

2. Experimental

2.1. Apparatus

Different HPLC systems were used at the two laboratories involved in the studies. The specifics are provided below.

LAB. A: the HPLC 1 apparatus was a Perkin Elmer chromatographic system (series 410 liquid chromatograph) equipped with a septumless injector (Rheodyne 7125-075) and a column heater (Perkin Elmer TC 931). A variable wavelength diode array detector (Perkin Elmer LC 235) was used. Peak area integration were performed using a chromatographic data system (Perkin Elmer LCI 100 laboratory computing integrator). A Vydac reversed-phase C_{18} column (25 cm × 4.6 mm i.d., particle size 10 μm), thermostated at 24, 26 and 28°C, was used as the stationary phase.

Table 1
Calibration curve

mg/ml	$A/A_{I.S.}$	Slope	RSD slope	Intercept	RSD intercept	r^2
0.12	1.4679	12.2442	0.0362	-2.38×10^{-4}	0.0028	0.9999
0.10	1.2278					
0.07	0.8536					
0.04	0.4891					
0.02	0.2459					

LAB. B: the HPLC 2 apparatus was a Merck Hitachi chromatographic system pump (LaChrom L-7100) equipped with a septumless injector (Rheodyne 7125-075) and a column oven (LaChrom L-7300). An UV detector (LaChrom L-7400) was used. Peak area integration were performed using a D-7000 HPLC system manager program. A Vydac reversed-phase C_{18} column Sil X-10 (25 cm × 4.6 mm i.d., particle size 10 μm), thermostated at 24°C, was used as the stationary phase.

The experimental design was produced, and statistical analysis of the data was performed, by Nemrod software [4] (LPRAI, Universitè de Marseille III, France).

2.2. Reagents

Lichrosolv® acetonitrile was purchased from Merck (Darmstadt, Germany). Water used in the mobile phase was deionized, distilled and filtered through a 0.22 μm Millipore (Bedford, USA) before use.

The determination of zafirlukast in commercial formulation was carried out on Accoleit® tablets (ZENECA, Milano, Italy). The composition of one tablet was: zafirlukast mg 40, and inactive ingredients croscarmellose sodium, lactose, magnesium stearate, microcrystalline cellulose, povidone, hydroxypropylmethylcellulose and titanium oxide.

2.3. Extraction procedure

Due to the lack of zafirlukast reference standard, a composite of ten tablets of Accoleit® was prepared by reducing the tablets to a 'fine', uniform particle size powder and extracted with 50 ml of acetonitrile (three times for 30 min) under magnetic stirring at 30°C. The extractive solutions were collected, filtered and evaporated under vacuum. The residue was treated with methanol and a white precipitate was obtained. Purity control was carried out by melting point, TLC and HPLC.

2.4. Preparation of standard solutions

Two working stock solutions were prepared in volumetric flasks at a concentration of 0.2 mg/ml of zafirlukast (solution A) and 0.8 mg/ml of internal standard flavone (solution B), using acetonitrile.

Table 2
Accuracy/recovery for zafirlukast

Concentration (mg/ml)	n	Recovery (%)	RSD (%)
0.04	4	100.25	1.79
0.07	4	99.02	0.77
0.12	4	99.79	0.37
Mean		99.68	0.62

Table 3
Method settings and range investigated during robustness testing

Variable	Optimized value	Range investigated
Mobile phase (CH_3CN/H_2O)	80/20	75/25–85/15
Temperature (°C)	24	24–28
λ	245	242–248
Flow (ml/min)	1	0.8–1.2

Table 4
Experimental matrix for robustness testing

Exp. no.	Run order	U_1	U_2	U_3	U_4
1	5	1	1	1	−1
2	6	−1	1	1	1
3	8	−1	−1	1	1
4	12	1	−1	−1	1
5	1	−1	1	−1	−1
6	3	1	−1	1	−1
7	11	1	1	−1	1
8	9	−1	−1	−1	−1
9	4	0	0	0	0
10	2	0	0	0	0
11	7	0	0	0	0
12	10	0	0	0	0

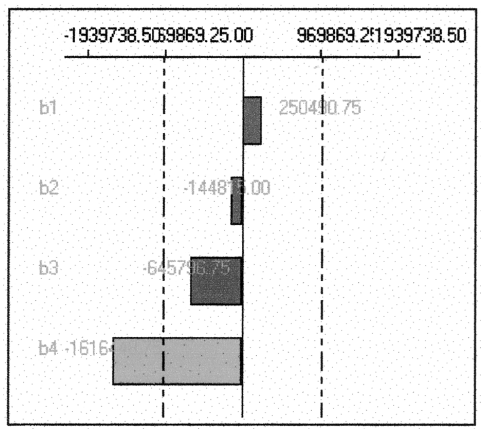

Fig. 3. Graphic analysis of effects for the response peak area during robustness test.

2.5. Calibration procedure

Aliquots of sol. A equal to 0.12, 0.10, 0.07, 0.04 and 0.01 mg/ml were accurately withdrawn and added with 1 ml (0.8 mg) of sol. B. Both solutions were utilized for the response linearity study of zafirlukast. Before injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. Quantitation was accomplished using an internal standard method. Five determinations were carried out for each solution. Peak areas were recorded for all the solutions. The correlation graph was constructed by plotting the peak areas obtained at the optimum wavelength of detection versus the injected amounts.

2.6. Chromatographic conditions

The mobile phase was a mixture of acetonitrile/water (80/20, v/v).

The flow rate was 1 ml/min. The UV detector wavelength was set at 245 nm and was used an attenuation of 0.05 a.u.f.s. The temperature was set at 24°C.

3. Results and discussion

A chromatogram of zafirlukast and internal standard is shown in Fig. 2. The substances are well-resolved with retention times of 3.22 and 3.78, respectively, by using the selected chromatographic conditions.

The method was validated statistically for its linearity, accuracy, robustness and precision.

Table 5
Experimental plan to study intermediate precision and obtained responses

Analyst	Day	Instrument	Response (%)
1	1	HPLC 1	101.43
1	1	HPLC 1	101.83
1	1	HPLC 1	99.28
2	1	HPLC 1	99.59
2	1	HPLC 1	98.8
2	1	HPLC 1	101.43
1	2	HPLC 1	99.18
1	2	HPLC 1	100.27
1	2	HPLC 1	99.59
2	2	HPLC 1	98.59
2	2	HPLC 1	100.26
2	2	HPLC 1	100.4
1	1	HPLC 2	101.26
1	1	HPLC 2	99.86
1	1	HPLC 2	100.28
2	1	HPLC 2	100.7
2	1	HPLC 2	99.7
2	1	HPLC 2	99.72
1	2	HPLC 2	100.14
1	2	HPLC 2	100.4
1	2	HPLC 2	100.4
2	2	HPLC 2	99.85
2	2	HPLC 2	99.87
2	2	HPLC 2	101.43

3.1. Linearity

The linearity of peak area responses versus concentrations were studied from 0.02 to 0.12 mg/ml for zafirlukast. The peak area ratio of analyte was divided by the peak area of the internal standard. A linear response was observed over the examined concentration range. Table 1 summarizes the correlation coefficient, slope and intercept.

3.2. Accuracy/recovery

Accuracy was studied using simulated preparations at three different concentrations, corresponding to 0.04, 0.07 and 0.12 mg/ml. Recovery data obtained were within the range 99.02–100.25% and RSD was 0.62% (Table 2), satisfying the acceptance criteria for the study.

3.3. Repeatability

The system repeatability was assessed from ten replicate injections of a sample solutions of zafirlukast at the analytical concentration of $\sim$ 0.07 mg/ml. The RSD for the active principle was found to be 0.78%.

3.4. Robustness testing

Robustness testing was performed in order to obtain information about those critical parameters affecting the response (peak area) [5]. The robustness of a method can be tested using experimental design in order to study the simultaneous variation of the factors. As a result of the data analysis, one is able to indicate which of the tested factors are not robust for the considered response. When factors that are not robust are detected one can decide to change the method or to control the factor in question more strictly [6–10].

To carry out robustness testing with experimental design, it is necessary to select the factors and the levels at which to test them, followed by the selection of the suitable design which depends on the postulated relationship. In general linear models are sufficient and advisable because of the small experimental domain and for the reduction in the number of experiments. For each controlled factor it is necessary to know its optimized value in order to define the interval within it can be controlled.

In the assessment of HPLC method for zafirlukast all the studied factors during the optimization process (organic modifier percentage, U_1; temperature, U_2; λ, U_3; flow rate, U_4) were considered. The experimental domain of selected variables is reported in Table 3. The ranges examined were small deviations from the method settings and the considered response was the peak area.

A linear relationship (Eq. (1)) with four variables was postulated and an eight-run Plackett–Burman design was chosen for the coefficients evaluation [11].

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 \quad (1)$$

To test the model linearity, four experiments with the optimized conditions, corresponding to the center of experimental domain, were carried out. The experimental matrix is reported in Table 4.

The regression model assumed was found not significant by means of analysis of variance but the graphic analysis of effects (Fig. 3) pointed out that the factor flow rate caused a statistically significant variation of the response. Graphic analysis of effects is a tool of experimental design in which the numerical values of the effects are displayed. This analysis requires the construction of a bar graph in which the length of each bar is proportional to the absolute effect value. The effects that exceed the reference lines, corresponding to the 95% confidence interval, are those significant for the response [12,13]. In this case the variation in flow rate was significant and then exerts a critical effect on the response. Concluding the method can be considered robust but a precautionary statement about flow rates have to be included in the procedure.

3.5. Intermediate precision

The intermediate precision is a measure of precision between repeatability and reproducibility. It is obtained when the assay is performed by

multiple analysts, using multiple instruments, on multiple days, in one laboratory [5]. Because these parameters influence the response together, it is advisable to study these effects simultaneously. In this case the factors considered were the analyst (analyst 1 and analyst 2), the instrument (HPLC 1 and HPLC 2) and the day (day 1 and day 2). A liner model ($y = b_0 + b_1x_1 + b_2x_2 + b_3x_3$) was assumed and a full factorial design 2^3 was employed to estimate the model coefficients [13]. The considered response was the zafirlukast found amount. Each experiment was repeated three times in order to evaluate the experimental error. The analyses were carried out in a randomized order according to the experimental plan reported in Table 5. The level of zafirlukast was ~ 0.07 mg/ml. The regression model was found not significant, thus indicating that no factor considered influence the response. Besides the RSD found in this conditions (0.85%, $n = 24$) was acceptable with respect to the RSD (0.78%, $n = 10$) found in the repeatability study.

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