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Short communication

Liquid chromatographic determination of lumiracoxib in pharmaceutical formulations

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ABSTRACT

A stability-indicating reversed-phase liquid chromatography (LC) method was developed and validated for the determination of lumiracoxib in pharmaceutical formulations. The LC method was carried out on a Synergi fusion C_{18} column (150 mm × 4.6 mm), maintained at 30 °C. The mobile phase was composed of phosphoric acid (25 mM; pH 3.0)/acetonitrile (40:60, v/v), run at a flow rate of 1.0 mL/min, and detection at 272 nm. The chromatographic separation was obtained within 10 min and it was linear in the concentration range of 10–100 μg/mL (r^2 = 0.9999). Validation parameters such as the specificity, linearity, precision, accuracy, and robustness were evaluated, giving results within the acceptable range. Stress studies were carried out and no interference of the degradation products was detected. Moreover, the proposed method was successfully applied for the assay of lumiracoxib in pharmaceutical formulations.

1. Introduction

Lumiracoxib (LMC, Fig. 1), is a selective cyclo-oxygenase (COX)-2 inhibitor developed for the management of acute pain and chronic pain associated with osteoarthritis and rheumatoid arthritis, with lower gastrointestinal complications than non-selective non-steroidal anti-inflammatory drugs [1,2]. LMC, 2-[(2-fluoro-6-chlorophenyl) amino]-5-methyl-benzeneacetic acid (MW, 294 Da), is chemically distinct from the other COX-2 inhibitors in that it lacks a sulfur-containing moiety and possesses a carboxylic group that confers weakly acidic properties (pK_a 4.7) [3,4]. It was recently withdrawn from the market in some countries [5], however it could be available in others.

Bioanalytical methods have been published for the study of the pharmacokinetic and metabolism of LMC in healthy male subjects [4], pharmacokinetic in plasma and synovial fluid [6] and for determination of LCM in human plasma [7]. At the moment, as also described by the literature in an overview of the analytical methodologies for determination of COX-2 inhibitors in bulk drugs, pharmaceuticals and biological matrices [8], there are no published methods validated for the quantitative analysis of lumiracoxib in pharmaceutical formulations, therefore the aim of the present work was to develop and validate a stability-indicating method for the determination of lumiracoxib in pharmaceutical formulations.

2. Experimental

2.1. Chemical and reagents

LMC was extracted from commercial tablets (Prexige® 400 mg) with tert-butyl methyl ether, filtered and then submitted to a SpeedVac concentrator (Model SPD 1010, Thermo Electron Corporation, Milford, MA, USA) until dryness. The obtained powder was analyzed for purity by the proposed method and by LC–MS. To confirm the identity, additional techniques such as diffuse reflectance infrared Fourier transform spectroscopy (DRIFT) and nuclear magnetic resonance (NMR) were carried out (data not shown). A total of six batches of Prexige® (Novartis, Stein, Switzerland) tablets containing 100 or 400 mg of LMC were obtained from commercial sources. All chemicals used were of pharmaceutical or special analytical grade. For all the analyses, ultrapure water was used (Milli-Q Gradient System, Millipore, Bedford, MA, USA).

2.2. Methods

2.2.1. Liquid chromatography (LC)

A Shimadzu LC 10A vp system (Shimadzu, Kyoto, Japan) was used. The detector was set at 272 nm and peak areas were integrated automatically by computer using a Shimadzu Class VP® V

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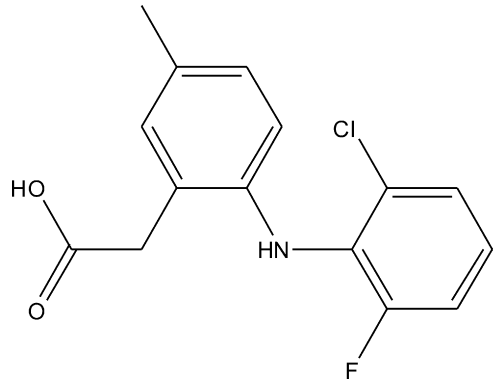

Fig. 1. Chemical structure of lumiracoxib (LMC).

6.12 software program. The experiments were carried out on a reversed-phase Phenomenex (Torrance, CA, USA) Synergi fusion C_{18} column (150 mm × 4.6 mm I.D., 4 μm). The LC system was operated isocratically at 30 °C using a mobile phase of phosphoric acid (pH 3.0; 25 mM)/acetonitrile (40:60, v/v). This was filtered through a 0.45 μm membrane filter and run at a flow rate of 1.0 mL/min. The injection volume was 20 μL for both standard and samples.

2.2.2. Mass spectrometry (MS)

The MS experiments were performed on a triple quadrupole mass spectrometer (Micromass, Manchester, UK), model Quattro LC, equipped with an electrospray ionization (ESI) source in positive mode, set up in scan mode, using a Masslynx (v 3.5) software program. The mass spectrometer conditions were optimized with the direct injection (syringe pump, KD Scientific, Holliston, MA, USA) of the LMC reference solution (1500 ng/mL) into the system. The best response was obtained with an electrospray capillary potential of 2.5 kV, cone voltage of 27 V, RF lens voltage of 0.3 V, source temperature of 120 °C, and ESI probe temperature of 400 °C, respectively. Before the analysis, the samples were diluted to 1:10 in acetonitrile:water (50:50, v/v), and introduced into the mass spectrometer by direct infusion at 10 μL/min. The mass spectrometry data were acquired in the *m*/*z* range between 100 and 500 amu.

2.3. Procedure

2.3.1. Preparation of reference solutions

The stock solutions of LMC were prepared by weighing 50 mg, transferred to individual 50 mL volumetric flasks and diluted to volume with acetonitrile, obtaining a concentration of 1 mg/mL. The stock solutions were stored at 2–8 °C protected from light. Working standard solutions were prepared daily by diluting the stock solutions to an appropriate concentration in mobile phase.

2.3.2. Preparation of sample solutions

To prepare the sample stock solution, tablets containing 100 or 400 mg of LMC were accurately weighed and crushed to a fine powder. An appropriated amount was transferred into an individual 50 mL volumetric flask, diluted to volume with acetonitrile, and sonicated for 15 min, obtaining the final concentration of 1 mg/mL of the active pharmaceutical ingredient. This solution was stored at 2–8 °C protected from light. Working sample solutions were prepared daily by diluting the stock solutions to an appropriate concentration in mobile phase.

2.3.3. Validation of the method

Analytical method development and validation play a major role in the discovery, development, and manufacture of pharmaceuticals [9]. The International Conference on Harmonization (ICH) [10] requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance.

2.3.4. Specificity and forced degradation studies

A stability-indicating method is the one that accurately quantifies the active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities [11,12]. This greatly contributes to the possibility of improving drug safety [13]. The stability-indicating capability of the method was determined by subjecting a reference sample solution (200 μg/mL) to accelerated degradation by acidic, basic, neutral, oxidative, and photolytic conditions. The acidic degradation was induced by storing the samples solutions in hydrochloric acid (0.05 M) under reflux at 80 °C for 1.5 h, and the solution was cooled and neutralized with base. A sample solution prepared in sodium hydroxide (2 M) was used for base hydrolysis evaluation and the solution was refluxed at 80 °C for 4 h, cooled and neutralized with acid. For the study under neutral thermal degradation, the drug was dissolved in water and heated at 80 °C for 8 h. Oxidative degradation was induced by storing the sample solutions in 5% hydrogen peroxide, at ambient temperature for 20 h, protected from light. Photodegradation was induced by exposing the samples in a photostability chamber to 200 W h/m^2 of near ultraviolet light for 3 h. After the procedures, the samples were diluted in mobile phase to a final concentration of 50 μg/mL. The interference of the excipients of the pharmaceutical formulation was determined by the injection of a sample containing only placebo (in-house mixture of all the tablet excipients) and a sample containing placebo added with LMC at a concentration of 50 μg/mL. Then, the stability-indicating capability of the method was established by determining the peak purity of LMC in the degraded samples using a PDA detector. Additionally, the peak of LMC and the principal degraded form were collected and analyzed by MS.

2.3.5. Linearity

Linearity was determined by constructing three independent calibration curves. For the construction of each calibration curve five standard concentrations of LMC in the range of 10–100 μg/mL were prepared in mobile phase and three replicates of 20 μL injections were performed.

2.3.6. Precision and accuracy

The precision of the method was determined by repeatability and intermediate precision. Repeatability was examined by six evaluations of the same concentration sample, on the same day, under the same experimental conditions. The intermediate precision was assessed by carrying out the analysis on three different days (inter-days) and also by other analysts performing the analysis in the same laboratory (between-analysts). The accuracy was evaluated by the recovery of known amounts of the reference substance added to a sample solution (containing 25 μg/mL of LMC and tablet excipients) to obtain solutions with final concentrations of 40, 50, and 60 μg/mL, corresponding to 80, 100, and 120% of the nominal analytical concentration, respectively. The accuracy was calculated as the percentage of the drug recovered from the formulation matrix.

2.3.7. Limits of quantitation (LOQ) and detection (LOD)

The LOQ was taken as the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy, and the LOD was taken as the lowest absolute concentration of analyte in a sample that can be detected but not necessarily quantified. The LOD and LOQ were calculated from the slope and the standard deviation of the intercept of the mean of three calibration curves, determined by a linear regression model, as defined by ICH.

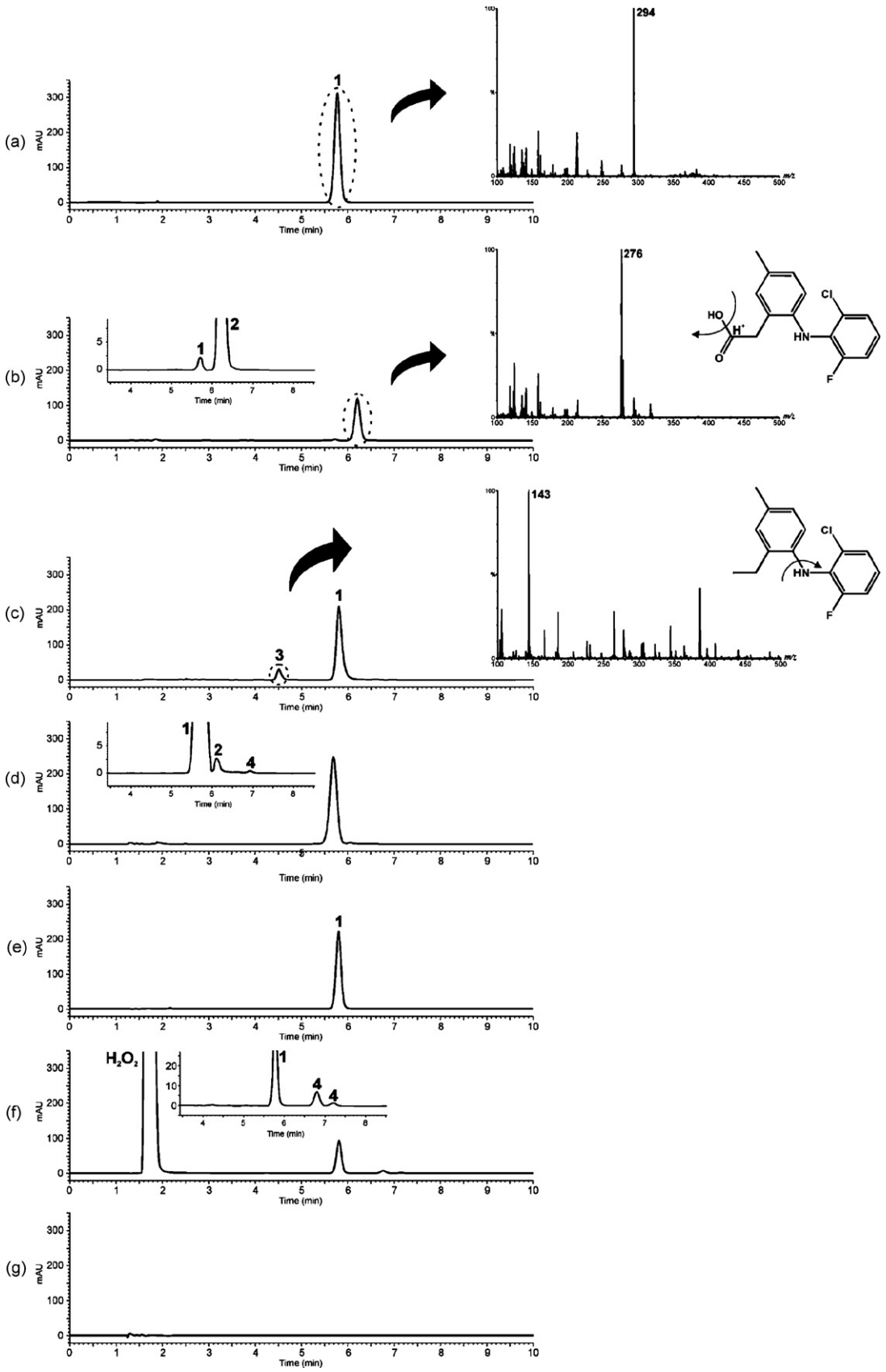

Fig. 2. Chromatograms of LMC (50 μg/mL). (a) LMC reference substance solution with mass spectrum of the peak, and after; (b) acidic hydrolysis with the mass spectrum and proposed structure of late eluting peak; (c) photodegraded condition and proposed structure of degradation peak; (d) base hydrolysis; (e) thermal degradation; (f) oxidative condition; (g) blank sample containing the excipients. Peak 1: lumiracoxib; 2: dehydro-lumiracoxib; 3: 2-[(2-fluoro-6-chlorophenyl) amino]-lumiracoxib; 4: unidentified degradation product. Chromatographic conditions: Phenomenex Synergi fusion C18 column (150 mm × 4.6 mm), 30°C; mobile phase: phosphoric acid (25 mM; pH 3.0)/acetonitrile (40:60, v/v); flow rate: 1.0 mL/min; detection: 272 nm.

2.3.8. Robustness

The robustness was determined by analyzing the same samples under a variety of conditions of the method parameters, such as flow rate, column temperature, changing the mobile phase composition and pH. The response surface method design was applied to evaluate the relationships between one or more measured responses. An experimental design is always recommended because the effect of a given factor is calculated at several level combinations of the other factors, and it represents more globally what is happening around the nominal situation [14,15]. Moreover, the D-optimal criteria was used to select design points to minimize the variance associated to the estimates of specified model coefficients, with a low number of experiments.

2.3.9. Analysis of LMC in pharmaceutical formulations

For the quantitation of LMC in the tablet formulations, the respective stock solutions were diluted to appropriate concentration with mobile phase, filtered, injected in triplicate and the percentage recoveries of the drug calculated against the reference substance.

3. Results and discussion

To obtain the best chromatographic conditions, the mobile phase was optimized to provide adequate peak symmetry and sensitivity. Potassium phosphate, sodium phosphate, sodium acetate, formic acid, and phosphoric acid buffers were tested. The use of phosphoric acid (25 mM; pH 3.0) in combination with acetonitrile (40:60, v/v), at 30 °C, resulted in a relatively short retention time of 5.7 min, better peak symmetry (1.04), and a simple mobile phase (without salt buffer addition). For the selection of the best wavelength detection a PDA detector was used.

Forced degradations studies were performed to provide indications of the stability-indicating properties of the analytical method, particularly when there is no information available about the potential degradation products. The chromatograms of forced degradation studies and mass spectra are shown in Fig. 2. The acid hydrolysis exhibited a significant decrease of area and only one eluting peak was observed. This eluting peak was collected and analyzed by mass spectrometry as the dehydro-LMC degradant (m/z 276). Additionally, the evaluation of the main peak from the LC method showed only an m/z of 294 attributable to LMC, and no other mass determinations were observed. The photolytic condition exhibited a decrease of area with one additional peak detected, which was collected and analyzed by mass spectrometry (m/z 143) suggesting the 2-[(2-fluoro-6-chlorophenyl) amino] part of the LMC structure, after cleavage in the amino group of the molecule. The basic condition also exhibited a decrease of area with two additional peaks detected. Based on the retention time, the first degradation peak was supposed to be the dehydro-LMC, and the second peak was not identified. The neutral thermal degradation resulted in decrease of the area and did not produce any detectable eluting degradation product. Under the oxidative condition a significant decrease of the area was observed with two additional peaks detected. Some degradation products found in the forced degradation studies could not be collected from LC column for mass spectrometry analysis due to their short time appearance and low yield. Besides, no interference from formulation excipients was also demonstrated (Fig. 2g), showing that the peak was free from any coeluting peak, with values of peak purity index higher than 0.9999. These results indicated that the proposed method is specific and stability-indicating for the analysis of LMC under the conditions of this study.

The calibration curves constructed for LMC were found to be linear in the 10–100 μg/mL range. The value of the determination coefficient calculated ($r^2 = 0.9999$, $y = 63392x \pm 694x - 2247 \pm 5064$, where, x is concentration and y is the peak absolute area) indicated the linearity of the calibration

Table 1
Inter-day and between-analysts precision data of the method.

Sample	Inter-day			Between-analysts		
	Day	Recovery[a] (%)	RSD[b] (%)	Analysts	Recovery[a] (%)	RSD[b] (%)
1	1	100.03	0.51	A	100.45	0.32
	2	99.47		B	100.08	
	3	100.18		C	99.81	
2	1	98.54	0.60	A	98.57	0.79
	2	99.52		B	97.41	
	3	99.60		C	98.92	

[a] Mean of three replicates.
[b] Relative standard deviation.

Table 2
Chromatographic conditions and range investigated during robustness testing.

Experimental	Factors					Responses[a]		
	H_3PO_4 (mM)	Buffer (%)	Flow (mL/min)	pH	Temp (°C)	RSD (%)[b]	Assay (%)	Peak symmetry
1	25	40	1.00	3.0	30	0.38	99.95	1.02
2	28	42	1.10	3.0	27	1.06	100.25	1.06
3	28	42	1.10	3.0	33	1.47	99.41	1.06
4	22	38	0.90	3.0	27	1.24	99.63	1.12
5	25	38	0.90	2.8	27	0.96	99.72	1.10
6	25	38	1.10	2.8	27	1.36	99.50	1.10
7	25	38	1.10	2.8	33	0.34	99.98	1.08
8	22	38	0.90	2.8	30	0.14	99.99	1.07
9	22	42	1.10	2.8	30	0.28	100.03	1.04
10	22	40	0.90	2.8	27	1.07	99.95	1.07
11	22	40	0.90	2.8	33	0.55	100.16	1.04
12	28	42	0.90	2.8	30	0.20	99.94	1.01
13	28	38	0.90	2.8	30	0.77	99.87	1.07
14	22	42	0.90	3.2	30	0.35	99.85	1.05
15	22	38	0.90	3.2	30	0.50	99.78	1.10
16	22	40	1.10	3.2	27	0.27	100.08	1.09
17	22	40	1.10	3.2	33	0.45	100.02	1.05
18	28	38	1.10	3.2	33	0.95	99.93	1.06
19	28	38	1.00	3.2	27	0.22	100.09	1.09
20	28	42	0.90	3.2	30	0.15	100.05	1.03

[a] Mean of three replicates.
[b] Relative standard deviation.

curve for the method. The validity of the assay was verified by means of ANOVA, which demonstrated significant linear regression and non-significant linearity deviation ($P < 0.01$).

The precision evaluated as the repeatability resulted in a RSD value of 0.18% ($n = 6$). Intermediate precision was assessed by analyzing two samples of the pharmaceutical formulation on three different days (inter-day); the RSD values obtained were 0.51 and 0.60%. Between-analysts precision was determined by calculating the RSD for the analysis of two samples of the pharmaceutical formulation by three analysts; the values were found to be 0.32 and 0.79% (Table 1).

The accuracy was assessed from three different added standard solutions containing 15, 25 and 35 μg/mL of LMC. The results obtained of 14.81, 24.87 and 35.39 μg/mL, with RSDs lower than 0.07%, corresponding to an accuracy of 98.72, 99.46 and 101.11%, respectively, demonstrated that the method was accurate within the desired range. The values calculated for LOD and LOQ were 0.24 and 0.80 μg/mL, respectively, and were also confirmed experimentally.

The experimental ranges of the selected variables evaluated in the robustness testing are given in Table 2. The analysis of variance ANOVA was performed and the model terms (variables) were not significant ($P < 0.05$), thus demonstrating that the method was robust. Moreover, the stability of the analytical solution was analyzed and it was found to be stable up to 48 h (99.43%, assay).

The LC method validated in this paper was applied for the determination of LMC in tablet dosage forms, without prior separation of the excipients. The values obtained ranged from 99.78 to 101.24%, with RDS values lower than 0.30%

4. Conclusion

The results of the validation studies showed that the LC method is specific, accurate and possesses significant linearity and precision characteristics without any interference from the formulation excipients and degradation products. Moreover, the proposed method was successfully applied for the quantitative analysis of lumiracoxib in pharmaceutical formulations.

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