Determination of lumiracoxib in tablets

Development and validation of HPLC and UV spectrophotometric methods for the determination of lumiracoxib in tablets

Moreira, T.S.; Pierre, M.B.R.; Fraga, C.A.M.; Sousa, V.P.*

1Departamento de Medicamentos, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, UFRJ, Rio de Janeiro, RJ, Brasil.
2Departamento de Fármacos, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, UFRJ, Rio de Janeiro, RJ, Brasil.

*Autor correspondente: Valeria Pereira de Sousa - Departamento de Medicamentos - Faculdade de Farmácia - Universidade Federal do Rio de Janeiro, UFRJ - Av. Carlos Chagas Filho, 373, Bloco B sub-solo, sala 15 - CEP: 21.941-902 - Rio de Janeiro - RJ, Brasil - Telefone/ Fax: (21) 2564-7380 - e-mail: valeria@pharma.ufrj.br

ISSN 1808-4532

ABSTRACT

In this study, two methods, based on high-performance liquid chromatography (HPLC) and UV spectrophotometry, were developed and validated for the quantitative determination of lumiracoxib in tablets. The HPLC was carried out on a column of propylsulfonic acid bonded to silica gel (250 x 4.6 mm; 5 μm), with a mobile phase of phosphate buffer (pH 7.4; 10 mM)-water-acetonitrile (10:40:50, v/v/v) flowing at 1.0 mL/min and detection of the drug at 278 nm. The UV method was based on absorbance at 275 nm, with ethanol as solvent. Both assays were linear over the concentration range of 2–30 μg/mL (R≈0.999), as well as accurate and precise, with recoveries between 98 and 100% and relative standard deviation (%RSD) < 2.0%. The proposed methods are highly sensitive, precise and accurate and were successfully applied to the quantitation of lumiracoxib in the commercial formulation. The spectrophotometric method is a simple, cheap and less time-consuming method. However, the chromatographic method is selective for the determination of the degradation products of lumiracoxib.

Keywords: lumiracoxib; tablets; HPLC; UV spectrophotometry; pharmaceutical analyses.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used as the agents of choice to treat the chronic pain of osteoarthritis (Gorsline & Kaeding, 2005; Moskowitz et al., 2007). However, this class of drug prevents prostaglandin synthesis by the nonselective inhibition of both isoforms of cyclooxygenase (COX) (Hungin & Kean, 2001; Brandt, 2003) and this profile accounts for their common side effects, including gastric irritation, renal impairment and inhibition of platelet aggregation (Rainsford, 1999; Green, 2001; Laine, 2003; Tomisato et al., 2004). The use of NSAIDs is associated with an increased risk of gastrointestinal ulcers and ulcer complications such as bleeding and perforation (Rainsford, 1999). COX-2 selective inhibitors have demonstrated analgesic and anti-inflammatory effectiveness comparable to that of traditional NSAIDs in patients with arthritis, combined with an improved safety profile (Buttgereit et al., 2001; Laine et al., 2003; Radi & Khan, 2006; Rostom et al., 2007).

Lumiracoxib: {2-[(2-fluoro-6-chlorophenyl)amino]-5-methyl-benzenecacetic acid} (Figure 1) is a novel COX2-selective with a quite different structure from others in the class, which are typically sulfonamides (Farkouh et al., 2004; Niederberger et al., 2006), and possesses a carboxylic acid group, which confers weakly acidic properties (pKa 4.7). It has a high lipophilicity, high selectivity and a fairly short plasma half-life (3–6 h) compared to other COX2-selective inhibitors (Schnitzer et al., 2004; Niederberger et al., 2006; Mysler et al., 2006). These properties lead to a lower systemic drug exposure. The pharmacokinetics of lumiracoxib is characterized by good oral bioavailability (Kalbag et al., 2004), dose proportionality with no accumulation, and no significant influence of age, sex or body weight on apparent plasma clearance (Esser et al., 2005). In addition, lumiracoxib has demonstrated sustained higher concentrations in the synovial fluid than in the plasma, in patients with rheumatoid arthritis (Hawkey et al., 2006).

Figure 1. The chemical structure of lumiracoxib.
Until now, no method for the direct analysis of lumiracoxib in pharmaceutical formulations has been published. In the present study, rapid, specific, precise and validated HPLC and UV spectrophotometric methods for the direct quantitative estimation of lumiracoxib in tablets are reported.

MATERIAL AND METHODS

Chemicals and reagents

Prexige® 100 mg tablets (Novartis, Switzerland) were purchased from a commercial outlet. Standard Lumiracoxib (98% pure) was extracted from tablets by refluxing with ethanol in a Soxhlet extractor and purified by acid-base partition. The purity was confirmed by acid-base titration, IR spectroscopy and NMR. HPLC-grade acetonitrile was obtained from TEDIA (Rio de Janeiro, Brazil). Deionized water was prepared by the Milli-Q system (Millipore, MA, USA) and used to prepare all solutions for the HPLC method. Distilled water was used to prepare all solutions for the UV method. All solutions were prepared daily. All other chemicals used were of analytical grade.

Instrumentation and analytical conditions

A Shimadzu (Kyoto, Japan) HPLC system was used, equipped with a LC-10AD pump, SPD-M10A PDA detector and SIL-10AD auto-injector with a 50 μL loop. Data was acquired with Class-VP 6.1 software. The chromatographic column (250 x 4.6 mm; 5 μm), from J. T. Baker (Phillipsburg, Canada), was packed with propylsulfonic acid-bonded silica gel. The mobile phase consisted of phosphate buffer (pH 7.4; 10 mM)-water-acetonitrile (10:40:50; v/v/v), the pH being adjusted with 0.1M sodium hydroxide. The mobile phase was filtered through a 0.45 μm membrane filter and degassed before use. Samples and standards (25 μL) were injected, eluted at room temperature at a flow rate of 1.0 mL/min and fractions were analysed by absorbance at 278 nm.

The UV analysis was performed on a Shimadzu UV-Vis recording spectrophotometer (UV PC 2401) at 275 nm, using 1.0 cm quartz cuvettes. The absorbance measurements were handled by Shimadzu UVPC software.

Preparation of standard solutions

Accurately weighed 25 mg of lumiracoxib standard was transferred to a 100 mL volumetric flask and dissolved in acetonitrile, for HPLC determination, and in ethanol, for UV determination. The mixture was sonicated for 10 min or until the standard dissolved completely. This solution was further diluted with the mobile phase and ethanol, respectively, to obtain five working standards at concentrations of 2, 5, 10, 15 and 30 μg/mL. In the chromatographic method, the solutions were passed through a 0.45 μm membrane filter prior to injection.

Preparation of sample solution

Twenty tablets, each containing 100 mg lumiracoxib, were accurately weighed and finely powdered. A quantity of powder equivalent to 50 mg lumiracoxib was weighed and transferred to a 100 mL volumetric flask and dissolved in acetonitrile, for HPLC determination, and in ethanol, for UV determination. Aliquots of this solution were diluted in mobile phase and ethanol, respectively, at a concentration of 10 μg/mL. The solution was passed through a 0.45-μm membrane filter before injection into the HPLC system.

Method validation

The methods were validated in accordance with Brazilian guidelines and the International Conference on Harmonization guidelines for validation of analytical procedures (Brasil, 2003; ICH, 2005). The following validation characteristics were assessed: linearity, precision, accuracy, specificity, robustness and limits of detection and quantitation.

Linearity

Calibration curves were constructed with five concentrations of the standard solution (2-30 μg/mL), for the HPLC and UV methods. The solutions were prepared in triplicate. The linearity of these curves was calculated by linear least-squares regression analysis.

Precision

The precision of the methods was determined at two levels, repeatability (intra-day) and intermediate precision (inter-day). Repeatability of an analytical procedure is tested within one laboratory over a short period of time, by assaying samples on the same day. Triplicate samples were tested at 80%, 100% and 120% of the analytical concentration. The intermediate precision was studied by comparing the assays on two different days.

The system precision for the HPLC method was assessed by six replicate analyses of the drug at five concentrations from 2 to 30 μg/mL. The acceptance criterion was ±2% for the percent relative standard deviation (%RSD) for the peak area and retention time of the drug.

Accuracy

Accuracy was determined as the percent recovery of a known amount of standard lumiracoxib added to diluted sample solutions. From the powdered tablets, an accurately weighed amount equivalent to 50 mg of lumiracoxib was transferred to a 100 mL volumetric flask and dissolved in
Determination of lumiracoxib in tablets

acetonitrile, for the HPLC determination, and in absolute ethanol, for the UV determination (final concentration of 0.5 mg/mL). Aliquots of 1.0 mL of this solution were transferred to 50 mL volumetric flasks containing 1.0, 2.0 and 3.0 mL of lumiracoxib standard solution (0.25 mg/mL). The volume was made up with mobile phase, for the HPLC, and ethanol, for the UV determination, to give a final concentration of 15, 20 and 25 μg/mL. For both methods, all samples were prepared in triplicate. The percentage recovery was calculated by comparing the known added amount of lumiracoxib standard with the measured amount, after subtracting the unspiked samples.

Specificity

Specificity of the HPLC method was determined with Class VP software, which gives the peak pureness in two different ways: peak similarity and ratiograms. The peak similarity is an analysis of spectra recorded at the beginning, middle and end of the peak, comparing the sample and standard UV absorption spectra at each of these retention times. In the ratiograms, the absorbance ratio for two wavelengths is plotted against elution time. To indicate high purity in a peak, the graph must exhibit a rectangular shape, showing the peak purity index to be constant and near unity.

Robustness

The robustness of the HPLC method was evaluated by analyzing the samples in differing conditions, produced by small changes in the pH (6.8–7.4) and temperature (25–30 °C) and changing the HPLC equipment to a Waters system, with Waters 510 pump, Waters UV-Vis 486 detector, Rheodyne 7725i injection valve with a 20 μL loop and a Waters 746 integrator. The effects on retention time and peak parameters were studied.

Limit of detection (LOD) and limit of quantitation (LOQ)

The parameters LOD and LOQ were determined on the basis of the response and slope of the regression equation. LOD was calculated as three times the noise level on the calibration curve and LOQ as ten times.

Table 1 - Results of regression analysis of data for quantitation of lumiracoxib by HPLC and UV methods

<table>
<thead>
<tr>
<th>Features</th>
<th>UV method</th>
<th>HPLC method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range (μg/mL)</td>
<td>2-30</td>
<td>2-30</td>
</tr>
<tr>
<td>Regression equation(^a)</td>
<td>(y = 0.048x + 0.0053)</td>
<td>(y = 69589x + 26134)</td>
</tr>
<tr>
<td>Correlation coefficient ((r^2))</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>Standard error of intercept</td>
<td>(1.0 \times 10^{-2})</td>
<td>15789.1</td>
</tr>
<tr>
<td>Standard error of slope</td>
<td>(6.0 \times 10^{-4})</td>
<td>908.8</td>
</tr>
</tbody>
</table>

\(^a\)\(y = bx + a\), where \(x\) is the concentration in μg/mL, \(y\) is the absorbance, for the UV method, and the peak area, for the HPLC method, \(a\) is the intercept and \(b\) is the slope.

Table 2 - Precision of determination of lumiracoxib in tablets by the HPLC method

<table>
<thead>
<tr>
<th>Sample Concentration (μg/mL)</th>
<th>Day</th>
<th>Purity (%)</th>
<th>Intra-day %RSD</th>
<th>Inter-day %RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.0</td>
<td>1</td>
<td>93.50</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>92.56</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>1</td>
<td>94.22</td>
<td>0.90</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>94.06</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>12.0</td>
<td>1</td>
<td>94.76</td>
<td>0.23</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>94.84</td>
<td>1.55</td>
<td></td>
</tr>
</tbody>
</table>

269
Determination of lumiracoxib in tablets

Stress testing

For degradation studies, 50 mg of lumiracoxib was accurately weighed and transferred to a 100 mL volumetric flask. From this solution, 1 mL aliquots were transferred to 50 mL volumetric flasks, each with 1 mL of 1N NaOH (alkaline degradation) or 1 mL of 1N HCl (acid degradation) or 1 mL of 3% H2O2 (oxidative degradation) and kept at room temperature for 12 h before the analysis. A solution at the same dilution was kept at 100°C for 1 h and a control solution was kept for 12 h in a refrigerator. The volume was made up with acetonitrile, for HPLC determination, and absolute ethanol, for the UV determination.

RESULTS

HPLC method

A high-performance liquid chromatography-based method was proposed for the estimation of lumiracoxib in pharmaceutical dosage form. The chromatographic conditions were adjusted to improve the performance of the assay. Initially, reversed-phase columns, such as C8 and C18, were tested, but they yielded asymmetric peaks. The propylsulfonic acid bonded silica gel column (250 x 4.6 mm; 5 μm) produced symmetric peaks and an adequate retention time. The mobile phases investigated were phosphate buffer (pH 7.4; 10 mM)-water-acetonitrile in the ratios 13:47:40 (v/v/v), 11:39:50 (v/v/v) and 10:40:50 (v/v/v). Changing the percentage of acetonitrile (40-50%) resulted in a significant change in peak symmetry, but none in retention time. Mobile phase selection was based on peak parameters (symmetry, tailing), run time, easy of preparation and cost. Figure 2A shows a typical chromatogram obtained in the analysis of lumiracoxib with the selected mobile phase, phosphate buffer (pH 7.4; 10 mM)-water-acetonitrile 10:40:50 (v/v/v).

The linearity of the proposed method was assessed by determining the peak area of lumiracoxib in the analytical range of 2-30 μg/mL. The linear regression equation and correlation coefficient values are summarized in Table 1. The limits of detection (LOD) and quantitation (LOQ) were calculated and found to be 0.69 and 2.0 μg/mL, respectively.

The precision was determined by repeatability (intra-day) and intermediate precision (inter-day) and was expressed as %RSD of a series of three experiments at different concentrations. The experimental results are shown in Table 2. Intra-day variability was calculated for assays on two distinct days and showed RSD < 2.0%. The accuracy results for lumiracoxib in the tablets were determined at three concentrations by the technique of standard addition. The recovery ranged from 98.8 to 99.9% (Table 4), comparing the known amount of standard added and the concentration detected in the spiked sample. The system precision was estimated from the %RSD of six peak areas obtained by consecutive injections of each of five standard solutions in the range of concentration studied (2-30 μg/mL). The measured values of RSD for the peak area ranged between 0.14 and 1.34%. The mean retention time was 2.52 min and its %RSD ranged between 4.47 x 10^-3 and 0.0110%.

The HPLC method described here was specific, as seen in Figure 3, where the spectrum obtained at 2.5 min does not show any significant differences from those obtained at 2.4 and 2.6 min, proving the absence of interfering substances. Figure 4 exhibits a rectangular ratiogram with a peak purity index of 1.000, showing that the ratio was constant, but bigger than the threshold.

Figure 2. Elution profile of lumiracoxib: standard (A) and after exposure to oxidative (B), acidic (C), basic (D) and high temperature (E) stress conditions.
In order to study the robustness of the proposed method, deliberate modifications were made in the pH value of the mobile phase, temperature and the HPLC system brand. The method was considered robust because no appreciable changes in the chromatographic performance were observed.

Stability studies indicated that the samples were stable when subjected to acidic conditions and after one hour in the 100°C bath. In alkaline and oxidative degradation conditions, new peaks appeared with retention times of 2.5 and 4.3 min, respectively, both showing absorption at 200-230 nm (Figure 2).

UV method

The absorption spectrum of lumiracoxib is shown in Figure 5. The $\lambda_{\text{max}}$ was found to be 275 nm. This wavelength was used for all measurements. Calibration curves were constructed by plotting absorbance versus concentration in the range 2-30 µg/mL and the results of the regression analysis are given in Table 1. The correlation coefficient obtained was 0.999 (Table 1). The limits of detection (LOD) and quantitation (LOQ) were calculated and found to be 0.44 and 1.5 µg/mL, respectively.

Table 3 shows the precision obtained in the determination of lumiracoxib in samples at three final concentrations. Intra-day variability was calculated from assays on two days and shows RSD < 2.0%. The method achieved good accuracy, with a mean recovery of 99.3% (Table 4).

The reference standard and sample prepared at the same concentration gave identical absorption spectra. The stability studies of the lumiracoxib solutions showed that in alkaline and oxidative conditions the spectrum profile changed (Figure 5).

### Table 3 - Results of determination of lumiracoxib in tablets by the UV method

<table>
<thead>
<tr>
<th>Sample concentration (µg/mL)</th>
<th>Day</th>
<th>Purity (%)</th>
<th>Intra-day %RSD</th>
<th>Inter-day %RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.0</td>
<td>1</td>
<td>96.36</td>
<td>97.52</td>
<td>97.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>95.81</td>
<td>95.64</td>
<td>95.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.13</td>
</tr>
<tr>
<td>10.0</td>
<td>1</td>
<td>96.45</td>
<td>97.35</td>
<td>97.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>96.23</td>
<td>97.85</td>
<td>96.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>96.71</td>
<td>97.14</td>
<td>96.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.32</td>
</tr>
<tr>
<td>12.0</td>
<td>1</td>
<td>96.54</td>
<td>97.23</td>
<td>97.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>97.64</td>
<td>98.11</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 - Percent recovery of lumiracoxib standard used to spike sample solution of tablets of lumiracoxib (10 µg/mL) for HPLC and UV methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Concentration of standard (µg/mL)</th>
<th>Recovery mean (%) ± %RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added</td>
<td>Found</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.98</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.89</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>14.88</td>
</tr>
<tr>
<td>UV</td>
<td>5</td>
<td>4.94</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.95</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>14.99</td>
</tr>
<tr>
<td>HPLC</td>
<td>5</td>
<td>4.94</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.95</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>14.99</td>
</tr>
</tbody>
</table>
Determination of lumiracoxib in tablets

Figure 3. Chromatographic purity obtained by similarity.

Impurity: Not Detected
Peak purity index: 1.000000
Single point threshold: 0.999931
Minimum peak purity index: 69

Figure 4. Peak purity obtained by chromatographic ratio.

Impurity: Not Detected
Peak purity index: 1.000000
Single point threshold: 0.999931
Minimum peak purity index: 69
DISCUSSION

The development of HPLC methods for the determination of drugs has received considerable attention in recent years because of their importance in routine quality control analysis. The choice of the mobile and stationary phases was based on good performance of the system.

The HPLC method showed excellent linear proportionality between the analyte peak area and concentration of lumiracoxib, in the analytical range of 2-30 μg/mL. The analytical concentration used in this method is much higher than the LOD and LOQ. The method showed an adequate recovery of lumiracoxib in the tablet solutions spiked with standard. The repeatability, intermediate precision and system precision all showed RSD < 2.0%, indicating acceptable precision. The similarity of the absorption spectra of fractions at different retention times and the rectangular form of the ratiogram showed the specificity of the proposed method. The method was considered robust in that no notable changes were seen in the chromatographic performance when the system was altered slightly to simulate operational variation.

Stability studies indicated that the samples were stable when subjected to acidic conditions and after exposure to high temperatures. In alkaline and oxidative conditions, new peaks appeared, but in no case did the degradation products overlap the elution peak of the drug, showing the specificity of the analytical procedure with respect to these likely contaminants.

The proposed UV method to determine lumiracoxib allows a shorter time of analysis and an economical and simple procedure for sample preparation. Moreover, spectrophotometric methods involve simpler instrumentation than other instrumentation techniques such as HPLC. The correlation coefficient indicated good linearity and the LOD and LOQ were adequate. Intra-day and inter-day variability showed RSD < 2.0%, indicating satisfactory precision. The accuracy of the method was good, with a mean recovery of 99.3%.

The assay value obtained with the HPLC method was slightly lower than that obtained with the UV method, probably because of the higher sensitivity of the chromatographic technique. Nonetheless, all results were statistically the same (p-value > 0.05) according to the Student t-test.

Both the HPLC and UV methods developed for the determination of lumiracoxib in tablets were found to be simple, rapid, precise, accurate and sensitive. In summary, the proposed methods can be used for the analysis of this drug form in routine quality control.

ACKNOWLEDGEMENTS

This research received financial support from the Brazilian agency FAPERJ.

RESUMO

Desenvolvimento e validação de método por HPLC e espectrofotometria de UV para a determinação de lumiracoxibe em comprimidos
Determination of lumiracoxib in tablets

No presente estudo foram desenvolvidas e validadas metodologias por cromatografia líquida de alta eficiência (CLAE) e espectrofotometria de absorção na região do UV para a determinação quantitativa de lumiracoxibe em comprimidos. A análise cromatográfica foi realizada em coluna propilsulfônica ligada a sílica gel (250 x 4,6 mm; 5 μm) usando tampão fosfato (pH 7,4; 10 mM)-água-acetonitrila (10:40:50, v/v/v), com fluxo de 1,0 ml/min e detecção a 278 nm. O método por UV foi realizado a 275 nm usando etanol como solvente. A determinação foi linear para ambos os métodos na faixa de concentração de 2–30 μg/ml (R²=0,999). Os métodos por CLAE e UV foram precisos e exatos, com recuperação na faixa de 98 e 100% e desvio padrão relativo (DPR) < 2,0%. Os métodos propostos são sensíveis, precisos e exatos e podem ser aplicados com segurança na quantificação de lumiracoxibe em formulações comerciais. O método espectrofotométrico é um método simples, econômico e rápido. Porém, o método cromatográfico é seletivo para a quantificação dos produtos de degradação do lumiracoxibe.

Palavras-chave: lumiracoxibe; comprimidos; CLAE; espectrofotometria de UV; análise farmacêutica.

REFERENCES


