

Celecoxib Identification Methods

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SUMMARY. Celecoxib is a nonsteroidal anti-inflammatory drug used in the treatment of pain and inflammation, associated with rheumatoid arthritis, and several other inflammatory disorders. The objective of this study was the identification of celecoxib through several analytical methods. Celecoxib was identified by its melting range, ultraviolet and infrared spectrophotometry, thin layer chromatography, and nuclear magnetic resonance, all of them performed in accordance with the methodologies of the official codes. The methods used have been found to be fast, efficient, reproducible and are suitable for the identification of celecoxib.

RESUMEN. "Métodos para la Identificación del Celecoxib". Celecoxib es un antiinflamatorio no esterooidal utilizado en el tratamiento del dolor y la inflamación, asociado con la artritis y otras enfermedades. El presente trabajo tiene como objetivo la identificación de celecoxib utilizándose distintos métodos. Celecoxib fue identificado por su punto de fusión, espectrofotometría en el ultravioleta e infrarrojo, cromatografía en capa delgada y resonancia magnética nuclear, todos de acuerdo con las especificaciones internacionales. Los métodos propuestos fueran adecuados, eficientes y reproducibles y pueden ser utilizados en la correcta identificación de celecoxib.

INTRODUCTION

Celecoxib (Fig. 1) is a nonsteroidal anti-inflammatory drug (NSAIDS). This drug is still among the most widely used drugs in the world. It is effective in the treatment of pain and inflammation ^{1,2}. Celecoxib is a 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide, with the empirical formula C₁₇H₁₄F₃N₇O₂S, and molecular weight of 381,37.

Despite the worldwide investigation of circulatory side effects associated with this class of drugs, celecoxib have been recently approved in many countries, therefore only little information about quality control techniques for this medication has been published and it is still not included in any official code.

This new agent may be as effective as traditional NSAIDS, showing anti-inflammatory, analgesic and antipyretic activities, and it is comparable to known traditional NSAIDS that inhibits the enzyme cyclooxygenase (COX) which is involved in the synthesis of prostaglandin's ^{3,4}.

Two distinct COX isoforms (COX-1 and COX-2) have been identified ⁵. Traditional

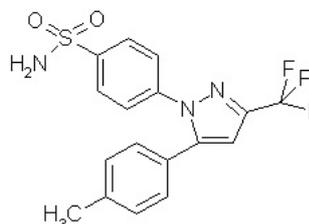


Figure 1.
The chemical structure of celecoxib.

NSAIDS are not selective and inhibit both COX-1 and COX-2, thus reducing the inflammation, but causing adverse effects. These side effects may be different according to the degree of inhibition of each COX isoform ^{5,6}.

Celecoxib is a COX-2 specific inhibitor, promoting a reduction of the inflammatory process and maintaining normal physiological levels of prostanoids in stomach and kidneys. It appears to have a gastrointestinal safety profile superior to the traditional NSAIDS ⁷.

Although available in the world market in the last few years, not many quality trials have been published to date regarding celecoxib (CELEBRATM). And in addition it is not listed in any official code.

KEY WORDS: Celecoxib, chromatography, identification, nuclear magnetic resonance, spectrophotometry

PALABRAS CLAVE: Celecoxib, cromatografía, espectrofotometría, identificación, resonancia magnética nuclear.

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For instance, further studies are required to determine ultraviolet and infrared spectra. So it felt necessary to propose procedures which would serve as a rapid and reliable method for the identification of celecoxib in pharmaceutical formulations, including some spectral data.

MATERIAL AND METHODS

All methods were validated according to ICH recommendations^{8, 9}.

Materials

Samples of celecoxib and its process impurities were kindly provided by Dr. Reddy's Research Foundation, Hyderabad, India. Solvents used were all analytical grade. Capsules of Celebra™, produced by Pfizer, USA, were purchased in the local market. Capsules containing 100 or 200 mg of celecoxib and excipients such as lactose, sodium lauryl sulfate, povidone, croscarmellose sodium, magnesium stearate, and titanium dioxide were used to evaluate if the methods were suitable to this dosage form.

Sample preparation

The proposed methods were tested in capsules after extraction, according to the extraction procedure developed by Guirguis *et al.*¹⁰ and described below.

Celecoxib extraction

Celecoxib was extracted from Celebra™ 100 mg and 200 mg capsules. Ten capsules were crushed to fine powder, using agate mortar and pestle, transferred to a 50 ml volumetric flask and diluted to volume with methanol. The solution was shaken for 5 min and filtered. The residue was recrystallized from acetonitrile after evaporation of the solvent in a water bath at 50 °C, under a stream of nitrogen.

Melting Range

The samples and the working standard were previously dried, to determine the melting range. This determination was obtained using a capillary melting point apparatus (Mettler Toledo) model FP 90 and FP 81 HT.

Ultraviolet spectrophotometry

For the identification trials a spectrophotometer UV-VIS SHIMADZU, model UV 2201, and 1 cm quartz cells were used, with automatic wavelength accuracy of 0.1nm. To identify the samples, a stock solution (10 µg.ml⁻¹) of cele-

coxib was prepared and the ultraviolet spectrum recorded.

Nuclear Magnetic Resonance (NMR)

Proton nuclear magnetic resonance spectrum of celecoxib were carried out using a Bruker DRX 500 AVANCE spectrometer operating at 500.13 MHz and equipped with a dedicated 5-mm proton probe. The spectrums were recorded using DMSO as the solvent, with all the chemical shifts in the range 0-10 ppm.

Infrared spectrophotometry

The infrared absorption spectra were obtained in a potassium bromide disk. The spectra were recorded on a SHIMADZU model FTIR 8101 infrared spectrophotometer.

Thin layer Chromatography (TLC)

In this procedure it was used silica gel 60 F₂₅₄ plates (20 x 20 cm) with a thickness of 0.25 mm. All plates used were commercially prepared by MERCK (lot # 040422153). The mobile phase used to develop the system consisted of chloroform-ethyl acetate-ether (10:5:1, v/v/v).

Just in order to verify the selectivity of the proposed system, another COX-2 inhibitor (rofecoxib) with similar properties was used. 20 µl of celecoxib reference substance and extracted from tablets and rofecoxib solutions (50 µg.ml⁻¹) were spotted on the TLC plates, and transferred to a developing tank containing the mobile phase. The plate was then examined under UV light (254 and 365 nm).

RESULTS AND DISCUSSION

Melting range

The information given by the determination of the physical constants such as the melting range can be applied in the characterization of a compound, and it is an important index of purity, when compared to standards. Just a small amount of impurity can cause a depression (and broadening) of the observed melting range. Thus this procedure of determining the melting range becomes strongly recommended in qualitative tests.

A modest amount of the working standard and the drug sample were transferred to a 6-cm-long melting point tubes of capillary size (1 mm width). The capillary tubes were then held vertically into the melting point apparatus and its melting range was determined. The obtained melting range of celecoxib was 161.3 °C - 162.2

°C. Presenting a medium value of 161.7 °C, and a standard deviation of 0,93 and a RSD of 0.58%. These values are the average of 9 determinations. The melting range values obtained for the working standard and the drug sample indicate quite similar results.

According to the data obtained, this procedure has been found to be precise, accurate and suitable for the analysis of celecoxib. It should be noted that the method gave similar and favorable results with respect to the low RSD values. The melting point determined in a capillary tube is one of the most accurate and more meaningful criterions of purity.

Ultraviolet spectroscopy

In the development of this method, several solvents were used, like methanol, acetonitrile,

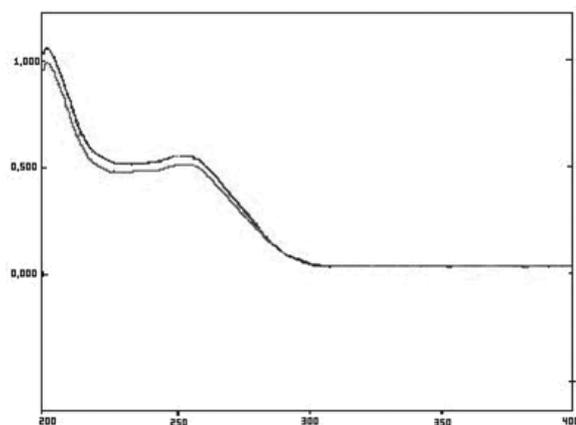


Figure 2. The ultraviolet spectra of methanol solutions ($10 \text{ g } \mu\text{l}^{-1}$) of celecoxib working standard (upper line) and the sample (lower line).

NaOH (0.1 M) and HCl (0.1 M). The above solvents were also used in combinations. The decision for using a 100% methanol was based on its sensitivity, stability and preparation time.

A stock solution of celecoxib was prepared by dissolving 10 mg of the sample and the reference material, in 200 ml of methanol, to achieve a final concentration of $50 \text{ } \mu\text{g ml}^{-1}$. From this solution was prepared a solution containing $10 \text{ } \mu\text{g ml}^{-1}$, and the absorbance was measured. The solvent used in the solubilization was also used as a blank.

The spectrum of celecoxib in methanol is shown in Figure 2. A maximum absorption at 252 was found.

The spectra obtained for the sample and reference material was compared and analyzed, suggesting that the extraction procedure was adequate for the dosage form tested (Celebra[®]) and the ultraviolet spectrum can be used for identification of both bulk substance and tablets.

Nuclear Magnetic Resonance (NMR)

The NMR spectroscopy is a powerful technique for the characterization of the exact structure of a compound, providing much more information about molecular structure than any other technique. The proton NMR is the most commonly used form of NMR because its sensitivity and the large amount of structure information it yields. The combination of infrared and NMR spectroscopy data is often sufficient to determine the structure of an unknown molecule.

The spectrum shown in Figure 3 possesses two characteristic sharp singlet peaks at 2.3 and

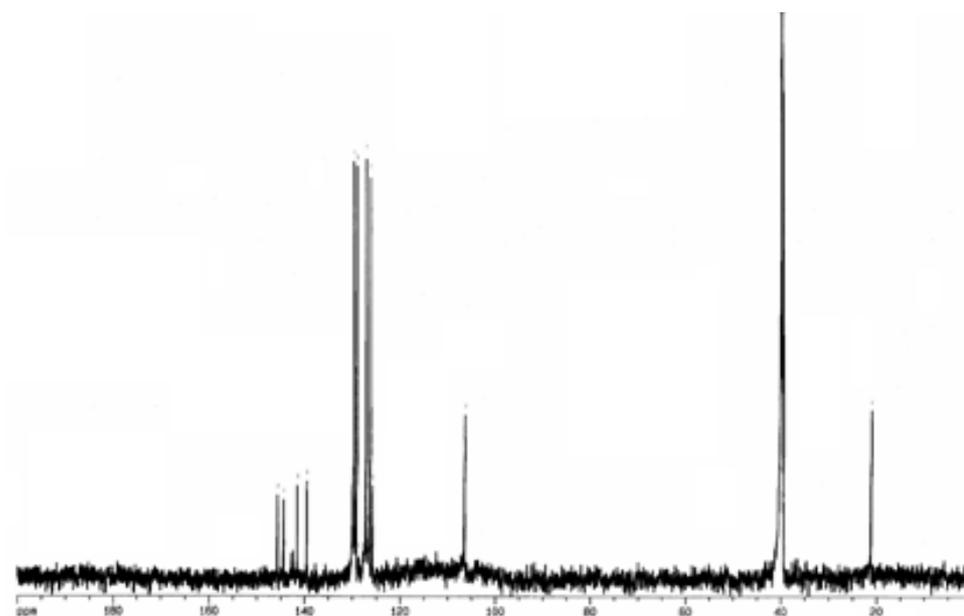


Figure 3. NMR spectrum of celecoxib in DMSO.

3.3 ppm that belong to the methyl and sulfonamide protons of celecoxib. The spectrum also reveals peaks from 7.3 to 7.9, which is due to the protons of aromatic groups. The characteristic peaks from the working standard agree well with those observed in the samples, considering the fact that a constant shift is observed in all peaks ^{11, 12}. The characteristic peaks in the range of 2.0-4.0 ppm can be used for identification of extracted sample and working standard.

Infrared Spectroscopy

The infrared spectroscopy is one of the most commonly used technique for the identification of compounds, this fact is true because its spectra is almost insensitive by the presence of impurities in low concentrations.

The infrared spectrum method can be used for molecules much as a fingerprint. If the infrared spectra of two substances are compared and match peak for peak, it is possible to say that they are the same compound ¹². This methodology is also important to determine structural information about the molecule.

The infrared absorption spectrum were obtained by mixing 1.5 mg of the finely ground extracted sample or the working standard, with 150 mg of powered potassium bromide (previously dried), and then pressed this mixture under high pressure, in order to obtain a KBr disk. This KBr disk was then inserted into a holder in the spectrometer. Triturate 1.5 mg of the substance to be examined with 150 mg of finely powdered and dried potassium bromide. These quantities were sufficient to give a KBr disc of 13-mm diameter and a spectrum of suitable intensity. Carefully the substance and the potassium bromide were grinded. The mixture was then, spread it uniformly in a suitable die, and submit it in vacuum to a pressure of about 800 MPa.

The working standard and the extracted sample were examined by the same procedure and record the spectra between 4000 cm⁻¹ and

Band Frequency (cm ⁻¹)	Assignment
1150 - 1350	S = O stretching (sulfonamide group)
1550 - 1600	N - H stretching
3300 - 3500	NH ₂ stretching

Table 1. Infrared frequency assignments of celecoxib.

670 cm⁻¹ under the same operational conditions ¹³.

The transmission minimum (absorption maximum) in the spectrum obtained with the substance to be examined corresponds in position and relative size to those in the spectrum obtained with the reference substance.

Using the positions of the bands of the working standard as reference, the positions of the significant bands in the spectrum of the sample to be examined and the reference spectrum should correspond to the wave-number scale. The relative sizes of the bands are concordant in both spectra. The two spectra were compared and the major observed bands and their assignments for celecoxib are found in Table 1.

The working standard and the samples were analyzed and compared peak for peak, through their characteristics spectral bands. The spectrum itself is shown in Figure 4.

Thin-layer chromatography

One of the most effective screening methods is the thin-layer chromatography (TLC), which is the simplest of all the widely used chromatographic methods to perform. In the determination of this method, different chromatography systems were tested and analyzed according to the classification proposed by Moffat ¹³, which divide the drugs in three categories: acid, basic and neutrals based on their polarity and acid characteristics. This procedure is important in

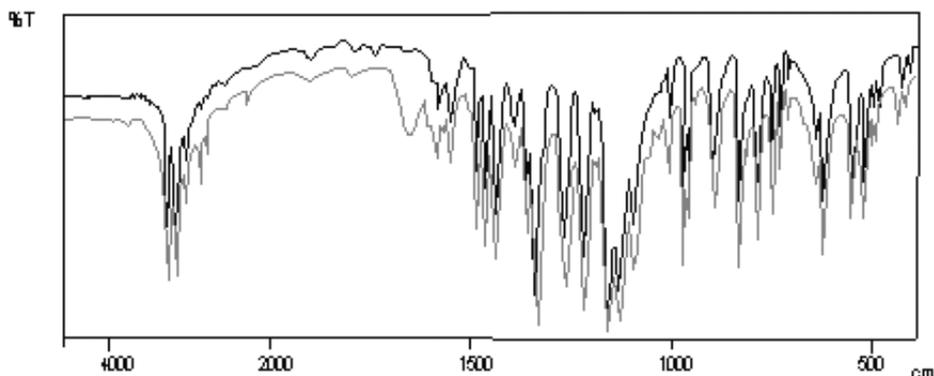


Figure 4. The infrared spectrum of the working standard (upper line) and the sample (lower line).

order to increase the information obtained only by changing the mobile phase, and thus resulting in a significant change in selectivity.

These chromatographic systems consisted of mixtures of the following solvents: chloroform-acetonitrile, ethyl acetate, acetone and methanol, in different concentrations. Nevertheless, all the mobile phases tested were not adequate for the proper identification of the drug. With the objective to develop more reliable chromatographic method, a modification in the system tested was proposed, in order to improve the chromatographic resolution. The mobile phase used consisted of chloroform-ethyl acetate-ether (10:5:1 v/v/v). The system was chosen due to its sensitivity, simplicity and efficacy.

The preference to use commercially prepared silica gel plates was due to its durability and homogeneity of the absorbent layer.

A sample solution of the working standard and the drug sample ($50 \mu\text{g ml}^{-1}$) were spotted onto a silica gel plate with a micropipette and the chromatogram was developed by placing the plate in a tank containing the mobile phase. Following development the individual solute spots were identified under UV lamp (254 and 365 nm). The spots in the drug sample and the working standard presented similar Rf values. The Rf value obtained for drug sample and the working standard were 0.45 and for rofecoxib 0.32. The picture of the TLC plate is shown in Figure 5.

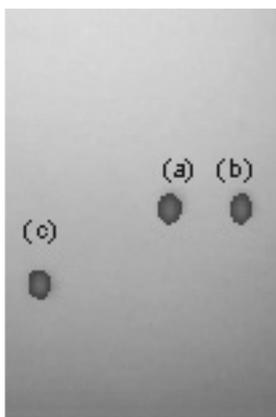


Figure 5. Graphical representation of the spots of the drug sample (a), the working standard (b) and rofecoxib (c).

Based on the results, this developed TLC method has shown to be specific, simple and fast, for the identification of celecoxib. It has been successfully employed for the quality evaluation of celecoxib.

CONCLUSION

The objective of this research was to apply analytical methodologies for the correct identi-

cation of celecoxib. Being a relatively new drug, there are not many publications concerning its analysis, and so far it is not included in any official code.

Some of the methodologies are simple, and have been found to be accurate, precise and suitable, giving, by this way, reliable results.

Hence, the methods evaluated in this study are applicable for the identification of celecoxib, in routine quality control laboratories, for the identification of this drug.

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