

was done for 24 h at 37°. The assessment of antibacterial activity was based on the measurement of inhibition zone diameter formed around the disc. Four independent determinations were conducted for each extract is given in the following Table 1.

These results suggest the presence of an active principle (s) with good antibacterial potency of high concentration of a moderately active principle in the extract. The aqueous and methanolic extracts gave positive test for polyphenolics. Work is in progress on separation and structure elucidation of the compounds responsible for antibac-

terial action. This antibacterial activity would support the therapy of infections and traditional therapeutics of this plant.

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Estimation of Rofecoxib by Difference Spectroscopy in Pharmaceutical Formulations

S. J. RAJPUT* AND M. G. SANKALIA

Pharmacy Department, Faculty of Technology and Engineering,
The M. S. University of Baroda, Kalabhavan, Vadodara-390001.

Accepted 24 March 2003

Revised 30 January 2003

Received 30 March 2002

Difference spectrophotometric method was developed for the estimation of rofecoxib in bulk drug and in pharmaceutical formulations. Rofecoxib exists in two different forms in acidic and basic medium which differs in their UV spectra. Difference spectrum, obtained by keeping rofecoxib in 0.1 N NaOH in reference cell and rofecoxib in 0.1 N HNO₃ in sample cell, showed two characteristic peaks at 219.2 nm and 260.8 nm with negative and positive absorbance respectively. Difference of absorbance between these two maxima was calculated to find out the amplitude, which was plotted against concentration. The method was found to be linear in the ranges of 2-16 µg/ml.

Rofecoxib¹ is described chemically as 4-[4-(methylsulfonyl) phenyl]-3-phenyl-2(5H)-furanone. Rofecoxib is comparatively a new non-steroidal antiinflammatory drug², which is active at a low dose³. Rofecoxib is not official in any of the pharmacopeia. Various methods for estimation of rofecoxib reported in literature are HPLC with post-column photochemical derivatization⁴⁻⁵, reverse-phase HPLC⁶, HPLC⁷⁻⁹, HPLC with tandem mass spectrometry¹⁰⁻¹¹, LC¹², LC-MS¹³⁻¹⁵, monolithic silica LC¹⁶, fluorescence detection¹⁷ and UV/vis spectrophotometry¹⁸⁻¹⁹. Not a single difference

spectrophotometric method is reported in literature till date. So the objective of this study was to develop accurate, precise, sensitive, selective, reproducible and quick difference spectrophotometric methods for estimation of rofecoxib in pharmaceutical formulations. Difference method is more sensitive than simple UV method because the absorbances of the same concentration solutions at different maxima are going to be added to each other in the difference method. So amplitude of difference method is more as compared to absorbance of simple method for the same concentration of chromogen and makes method more sensitive. Rofecoxib exists in two different forms in acidic and basic medium which differs in their UV spectra. Difference spectrum, obtained by

*For correspondence

E-mail: srajput@rediffmail.com

keeping rofecoxib in 0.1 N NaOH in reference cell and rofecoxib in 0.1 N HNO₃ in sample cell, showed two characteristic peaks at 219.2 nm and 260.8 nm with negative and positive absorbance, respectively.

Double distilled water was used throughout the study. Methanol, nitric acid and sodium hydroxide used in the study were of analytical grade. 0.1 N HNO₃ and NaOH were prepared and standardized as per IP-1996^{20,21}. Hitachi U-2000 UV/Vis spectrophotometer with 1-cm matched quartz cells was used for all the spectral and absorbance measurements. The commercially available tablet and suspension were procured from the local market (Torox tablets of 12.5 mg strength and Torox suspension 12.5 mg/5 ml of Torrent pharmaceuticals).

Stock solution of 100 µg/ml in methanol was prepared from standard drug. Suitable samples were taken in 10 ml volumetric flasks, and volumes were made up with 0.1 N HNO₃ and 0.1 N NaOH to prepare a series of standard solutions. Difference spectra were obtained by keeping basic form (i.e. rofecoxib in 0.1 N NaOH) in reference cell and acidic form (i.e. rofecoxib in 0.1 N HNO₃) in sample cell. Difference of absorbance between 219.2 nm and 260.8 nm was calculated to find out the amplitude.

Tablet powder or suspension equivalent to 2.5 mg rofecoxib was taken into beaker and 15 ml methanol was added followed by stirring on magnetic stirrer for about 30 min at 40°. Then it was filtered through Whatman filter paper No. 42 into the 25 ml of volumetric flask. Filter paper was washed thrice with 2 ml of methanol and volume was made up to the mark with methanol to prepare working stock solution of 100 µg/ml. Suitable samples were taken in 10 ml volumetric flasks, and volumes were made up with 0.1 N HNO₃ and 0.1 N NaOH.

Calibration curve was repeated for five times and RSD at each concentration level was found to be less than 1%, which indicates that method can be used for analysis of bulk drug samples. The calibration curve was found to follow Beer-Lambert's Law in the concentration range of 2-16 µg/ml. The linearity equation was $y=0.0813x+0.1288$ with coefficient of determination (r^2) = 0.9994. The values for molar absorptivity and Sandell's sensitivity were found to be 2.5557×10^4 l/mol·cm and 1.23×10^{-2} µg/cm²/0.001 absorbance unit, respectively. Recovery study was carried out by adding known amount of standard rofecoxib to previously analyzed samples at three levels. Recovery was found to be 99.8% and 100.4% for tablet and suspension respectively. Satisfactory recovery

indicated no interference of excipients on extraction efficiency.

Developed method was subjected to analytical validation and analytical parameters such as accuracy, precision, linearity, limit of detection (LoD), limit of quantitation (LoQ) and robustness were studied. For marketed formulations, the accuracy of the methods was greater than 98% and RSD did not exceed 2%. Thus proposed method is accurate and precise. LoD and LoQ were found to be 0.1059 µg/ml and 0.3529 µg/ml respectively. Large variation in the amplitude with change in normality of NaOH and HNO₃ for the same concentration of rofecoxib indicates that method is not robust enough to withstand small changes in normality of HNO₃ and NaOH.

Thus proposed method is simple, accurate, precise, sensitive, reproducible, repeatable, quick and inexpensive. In absence of any reported method for estimation of rofecoxib by difference spectrophotometry, proposed methods can be used for the estimation of rofecoxib from bulk drug and its marketed formulations.

ACKNOWLEDGEMENTS

We are grateful to Torrent Research Center, Gandhinagar for providing rofecoxib as a gift sample. We also thank Dr. S. H. Mishra, Head of Pharmacy Department, for providing necessary facilities in the department.

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Design and Evaluation of Mucoadhesive Buccal Patches of Diclofenac Sodium

J. S. PATIL* AND K. P. RAO¹

B. V. V. S's H. S. K. College of Pharmacy, Bagalkot-587 101.

¹H. K. E's College of Pharmacy, Gulbarga-585 105.

Accepted 25 March 2003

Revised 6 February 2003

Received 4 December 2001

The goal of the present investigation was to design and evaluate mucoadhesive buccal patches of diclofenac sodium, which is used as analgesic and antiinflammatory agent. Patches were fabricated by casting technique with different polymer combinations and were evaluated for *in vitro* release, bio-adhesion strength, duration of bioadhesion, folding endurance, surface pH and percentage of elongation. The release profile and test for adhesion were found to be the function of the type of polymer used. The formulation containing hydroxy propyl cellulose and carbapol 934 P was found to give the better results.

The usage of most of the NSAIDs by oral route associated with potential disadvantages such as peptic ulceration and gastric bleeding¹. Diclofenac sodium is a new generation NSAID, which is widely used in the long-term treatment of rheumatoid arthritis. Short biological half-life of 1-2 h necessitates multiple dosing for maintaining therapeutic effect throughout the day. Diclofenac sodium suffers from several drawbacks like irritation, peptic ulceration and gastric bleeding, this may eventually cause wall perforation. Such effects usually are associated with chronic high dose treatment^{2,3}. There is also a substantial first pass effect, only about 50 %

of drug is available systemically⁴. These severe drawbacks create a potential need for development of mucoadhesive patches, which are capable of avoiding the first pass effect and gastrointestinal side effects with delayed release system. The development of technology for release of drug at a controlled rate in to systemic circulation using buccal cavity as port of entry has become popular. In the present study an attempt was made to design the mucoadhesive buccal patches of diclofenac sodium with various polymers. The patches were characterized by keeping uniformity, flexibility, clarity and homogeneity as the tools.

*For correspondence

BLDEA's College of Pharmacy, Ashram Road,
Bijapur-586 103.

Diclofenac sodium IP and polymers such as carbapol-934P, hydroxypropylmethyl cellulose (6 cps), hydroxypropyl cellulose (HPC), sodium carboxymethyl cellulose and ethyl