Srinivasa Rao, Y., **Acta Ciencia Indica.**, 2002, 28, 41.
11. Seshagiri Rao, J.V.L.N., Srinivasa Rao, Y., Murthy, T.K. and Sankar, D.G., **Asian. J. Chem.**, 2002, 14, 647.
12. Rao, Y.S., Murthy, T.K., Chowdary, K.P.R. and Rao, J.V.L.N.S., **Indian Drugs**, 2002, 39, 348.
13. Srinivasa Rao, Y., Rajanikumar, V. and Seshagiri Rao, J.V.L.N., **Asian. J. Chem.**, 2002, 14, 1791.

Spectrophotometric Method for Estimation of Some COX-2 Inhibitors in Pure form and in Pharmaceutical Formulations

NEELAM SEEDHER*, A. GARG AND SONU BHATIA
Department of Chemistry, Panjab University, Chandigarh-160014.

Accepted 29 July 2003
Revised 19 May 2002
Received 27 January 2003

Ultraviolet absorption spectrophotometric method for the estimation of celecoxib, rofecoxib, meloxicam and nimesulide in pure form and in pharmaceutical formulations has been developed. The solvents employed were 10% (v/v) aqueous dimethylsulfoxide for rofecoxib, 10% (v/v) aqueous dimethylformamide for meloxicam, 20% (v/v) aqueous acetonitrile for celecoxib and nimesulide. 0.1 N sodium hydroxide was also used as solvent for all the drugs except rofecoxib. All the solvents were found to give accurate, sensitive and reproducible results for the estimation of drugs in pure form. These solvent systems could also be used for the estimation of drugs from solid formulations. For the estimation of drugs in pure form, method involving the use of 0.1 N sodium hydroxide was found to be relatively more precise, economical and safer than the one involving the use of organic solvents. For the estimation of celecoxib, rofecoxib and meloxicam from the formulations, on the other hand, the use of organic solvents gave better results and should be preferred. For the estimation of nimesulide from formulations, however, again 0.1 N sodium hydroxide was found to be a better solvent.

Nonsteroidal antiinflammatory drugs (NSAIDs), are the most widely used medications in the world. The adverse effects associated with traditional NSAIDs are primarily the result of the inhibition of prostaglandin synthesis by blocking the enzyme cyclooxygenase (cox) activity throughout the body. The new category of NSAIDs , the selective cox-2 inhibitors, has potential advantages over the traditional NSAIDs[1]. Four cox-2 inhibitors, celecoxib, rofecoxib, meloxicam and nimesulide have been selected for the present investigations. Spectrophotometric estimation of nimesulide has been reported by a number of workers using different solvents[2,3]. However, very few spectrophotometric[4-7] and other methods[8,9] are available for the estimation of rofecoxib, celecoxib and meloxicam. Moreover, none of the methods have reported the use of organic solvent-water mixtures with low concentrations of organic solvents. In the present paper, an attempt has been made to develop a quick, sensitive, economical and safer spectrophotometric method for the estimation of these drugs.

Rofecoxib and celecoxib were obtained as gift samples from M/s. Ranbaxy Research Laboratories, Gurgaon. Meloxicam and nimesulide were also gift samples from Ms. Sun Pharmaceutical Industries Ltd., Mumbai and Panacea Biotec Ltd., Lalru, respectively. All solvents were of analytical grade. They were first dried by keeping in contact with Linde type 4A molecular sieves overnight.

***For correspondence**
E-mail: nseedher@yahoo.com

Dimethylsulphoxide (DMSO) and dimethylformamide (DMF) were further purified by distillation under reduced pressure. Acetonitrile (ACN) was refluxed with 1 % (w/v) phosphorus pentoxide for half an hour and then distilled. Water used was double distilled in all glass apparatus.

Preliminary experiments were carried out to estimate the minimum concentration of organic solvent required to prepare stable drug solutions of sufficient concentration. Following solvents were selected; 10% (v/v) aqueous DMSO for rofecoxib, 10% (v/v) aqueous DMF for meloxicam and 20% (v/v) aqueous ACN for celecoxib and nimesulide. For preparation of stock drug solutions, drug dissolved in pure solvent was diluted by slow addition of water while stirring to obtain a solution of appropriate solvent and drug concentration. Alternatively, 0.1N aqueous sodium hydroxide solution could also be used as solvent for all the drugs except rofecoxib. Five dilutions of the stock solution were prepared in each case and ultraviolet absorption spectra were determined against solvent blank. Absorbance at wavelength corresponding to absorption maxima (λmax) versus drug concentration plots were used to calculate extinction coefficients and the Beer's law limits for the analysis of pure drugs. The stability of drug solutions was checked by measuring absorbance of the solutions after different time intervals.

For estimation of the drugs in drug formulations, three different solid formulations, each of celecoxib, rofecoxib, meloxicam and nimesulide were used. Celecoxib formulations were in capsule form while all other formulations were tablets. Twenty tablets of each formulation were crushed and appropriate weight of the homogeneous mixture was taken for preparation of stock solution. The contents of the capsules were used in the same way but without crushing. The formulation was thoroughly shaken with the solvent on a vortex mixer for sufficient time. Preliminary experiments showed that for maximum drug extraction, shaking for a period of five minutes was enough in the case of all the drugs using organic solvents and in the case of meloxicam and nimesulide using 0.1N sodium hydroxide. Dissolution of celecoxib from formulations by 0.1 N NaOH, however, required about 20 min of shaking. Drug stock solution was filtered through Whatman filter paper number one and five dilutions were prepared with the appropriate solvents. Ultraviolet absorption spectra of the diluted solutions were determined and the concentration of drug was obtained from the measured absorbance and the extinction coefficients of pure drugs at (max, determined previously. Concentrations, so obtained were multiplied by the appropriate dilution factor to obtain the amount of estimated drug in the formulation. Percentage recovery was calculated by dividing this amount by the labeled amount and multiplying with 100. All the data reported for pure drugs and drug formulations are the average of five replicates. Ultraviolet absorption spectra were determined on a Hitachi UV-VIS double beam spectrophotometer with the respective solvent as blank. Statistical parameters were calculated using statistical software, SPSS for windows® (SPSS Inc., Chicago, IL)

Various optical parameters; λmax, Beer's law limits, extinction coefficient at λmax(ε) and Statistical parameters; correlation coefficient (r), coefficient of determination (r^2) and standard error of estimation (SE) were obtained for various drugs in different solvents. Sensitivity (=M/ε), defined as the smallest weight of substance that can be detected in a column of solution having a unit cross section, was also determined in each case[4,5,10]. Sensitivity values were expressed in $\mu g/cm^2/0.001$ absorbance units. Lower the value of sensitivity, the more efficient the method of estimation. Various optical and statistical parameters for various drugs suggested that the use of aqueous solutions of various organic solvents as well as 0.1N NaOH can be employed as solvents for development of sensitive and precise methods for the estimation of drugs in pure form. All the drug solutions except rofecoxib, in various solvents studied at concentrations within the Beer's law limits were stable for at least 24 h (the maximum time studied). Rofecoxib in 10 % DMSO was, however, stable only for 2 h.

On comparing the data for various solvents, it was observed that in 0.1 N sodium hydroxide, the extinction coefficients and Beer's law limits were relatively higher and sensitivity values were lower. This was especially true for nimesulide, where the extinction coefficient was almost double and sensitivity was almost half in sodium hydroxide as compared to aqueous solutions of organic solvents. Thus 0.1 N sodium hydroxide should be the preferred solvent for the estimation of pure drugs. It is also economical and safer since the organic solvents are more toxic and more expensive than the alkali. However, due to the very low solubility of rofecoxib in 0.1 N NaOH, it could not be used as a solvent for the estimation of rofecoxib.

Data for the estimation of drugs in formulations has been reported as the average of five determinations and are expressed as mean±SEM (standard error of mean). The average of the percentage recovery for three formulations of each drug was also calculated. It is seen that in the case of celecoxib, rofecoxib and meloxicam, the recovery was almost 100%, using aqueous solutions of organic solvents

TABLE 1. ANALYSIS OF VARIOUS DRUGS IN FORMULATIONS USING THE PROPOSED METHOD.

Formulation*	Labelled amount	(mg)Amount found	(mg)**% Recovery	Average% Recovery
C I	100	103.5± 0.48	103.5	100.80
C II	100	100.0±0.89	100.0	
C III	100	98.92±0.60	98.92	
R I	12.5	12.50±0.10	100.0	100.05
R II	12.5	12.48±0.12	99.81	
R III	12.5	12.54±0.10	100.4	
M I	7.50	7.54±0.18	100.6	99.66
M II	7.50	7.43±0.13	99.01	
M III	7.50	7.46±0.05	99.43	
N I	100	99.54±0.30	99.54	100.32
N II	100	100.8±0.44	100.8	
N III	100	100.6±0.31	100.6	

*C=celecoxib, R=rofecoxib, M=meloxicam, N=nimesulide. All formulations were tablets except celecoxib. Celecoxib formulations were capsules. Solvents used were 10% (v/v) aqueous DMSO for rofecoxib, 10% (v/v) aqueous DMF for meloxicam, 20% (v/v) aqueous ACN for celecoxib and 0.1N NaOH for nimesulide. **Values reported are the average of five determinations and are expressed as mean±SEM (Standard Error of Mean).

whereas in the case of nimesulide it was about 100% using 0.1 N NaOH as solvent. NaOH (0.1N) gave lower average percentage recovery for all drugs except nimesulide. Also the extraction of celecoxib from formulations required a longer time using 0.1 N NaOH as solvent. Thus although for pure drugs 0.1N NaOH was a better solvent, for the estimation of celecoxib, rofecoxib and meloxicam from formulations, aqueous solutions of organic solvents were found to be better and should be preferred. For nimesulide, however, sodium hydroxide was found to be better solvent even for the estimation from formulations. Data for the estimation of drug from formulations using the preferred solvents is shown in Table 1.

The suggested method is quick, simple, sensitive and precise for the determination of cox-2 inhibitors in pure form and in formulations. The excipients present in formulations do not interfere with the drug estimations. The use of organic solvent-water mixtures with low concentrations of organic solvents offer a number of advantages such as low cost, lesser pollution and smaller error due to evaporation of the solvent.

For the estimation of celecoxib and rofecoxib, spectrophotometric methods reported in the literature[5,7] involve the use of 0.1 N sodium hydroxide and pure methanol, respectively as solvents. For celecoxib, the results presented here using 0.1 N NaOH agree with the reported method. However, it has been shown here that for the analysis of celecoxib in formulations, solvent consisting of 20% ACN as solvent, should be preferred. For rofecoxib, 10% DMSO has shown to be an equally good solvent. A visible spectrophotometric method involving the use of Folin-Ciocalteu reagent[4] and an ultraviolet absorption spectrophotometric method involving the use of pure DMF as solvent[6] have been reported in the literature for the estimation of meloxicam. However, the methods proposed in this paper are simpler than the visible spectrophotometric method and economical and more precise than the ultraviolet absorption spectrophotometric method reported in the literature. For the estimation of nimesulide, various methods are available[2,3]. The most convenient and accurate method[2] involves the use of 50% and 100% ACN as the solvent. It has been shown in this paper that equally good results can be obtained using 20% ACN and 0.1 N NaOH as solvents, which makes the proposed methods more economical and safer.

REFERENCES

1. Silas, S. and Clegg, D.O., **Bull. Rheum. Dis.**, 1999, 40, 1.
2. Chandran, S., Saggar, S., Priya, K.P. and Saha, R.N., **Drug Develop. Ind. Pharm.**, 2000, 26, 229.

3. Altinoz, S. and Dursun, O.O., **J. Pharm. Biomed. Anal.**, 2000, 22, 175.
4. Kuchekar, B.S., Late, S.G., Singavi, A.A. and Shinde, D.B., **Indian J. Pharm. Sci.**, 2001, 63, 321.
5. Murthy, T.K., Reddy, M.N. and Sankar, D.G., **Indian J. Pharm. Sci.**, 2001, 63, 521.
6. You, W.W., Liv, Y. and Wang, Z.B., **Fenxi Huaxue**, 1999, 27, 841.
7. Reddy, M.N., Murthy, T.K. and Shanta Kumar, S.M., **Indian Drugs**, 2002, 39, 39.
8. Joseph, C. and Bertucat, M., **Anal. Lett.**, 1999, 32, 2051.
9. Depre, M., Ehrich, E., Van Hecken, A., De Lepeleire, I., Dallop, A., Wong, P., Porras, A., Gertz, B.J. and De Schepper, P.J., **Eur. J. Clin. Pharmacol.**, 2000, 56, 167.
10. Sandel, E.B., In; Colorimetric Estimation of Traces of Metals, Interscience Publishers Inc., New York 1944.

Antibacterial activity of *Saraca asoca* Bark

Y. N. SEETHARAM*, H. SUJEETH, G. JYOTHISHWARAN, A. BARAD
G. SHARANABASAPPA AND SHABANA PARVEEN
Biosystematics and Medicinal Plants Laboratory, Department of Botany,
Gulbarga Univeristy, Gulbarga-585 106.

Accepted 6 August 2003
Revised 26 May 2003
Received 26 March 2002

Bark extracts of *Saraca asoca* (Roxb.) de Willde were investigated for *in vitro* antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus aureus* and *Klebsiella pneumoniae* at 4 mg/ml using agar well diffusion method. The ethanol and distilled water extracts showed significant broad spectrum antibacterial activity.

Saraca asoca (Roxb.) de Willde is a medium sized endangered evergreen tree (distributed throughout India), mostly cultivated in gardens[1]. The bark of this plant is used as astringent to the bowels, anthelminitic, for curing diseases of the blood, in fever, dyspepsia, dysentery, burning sensation and leucorrhoea[2]. Flavonoids and sterols have been isolated from this plant[3,4]. In the light of above information the present investigation was undertaken which deals with the antibacterial activity of petroleum ether, butanol, ethanol and distilled water extracts of bark of *Saraca asoca* (Roxb.) de Willde against various Gram positive and Gram negative bacteria. The results of which are being reported in the present communication.

The bark of *Saraca asoca* (Roxb.) de Willde was collected from Lal Bagh, Bangalore, Karnataka in December 1999. The identity of the bark has been confirmed using all official monographic specifications[5]. The shade dried bark was pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered bark (500 g) was extracted with petroleum ether (PE, 40-60°), successively butanol (BT), ethanol (EE), and distilled water (DW) using Soxhlet extractor method. The extracts were then distilled separately and condensed to yield solid mass completely free from solvents. (PE-3.22%, BT-8.67%, EE-12.43% and DW-27.43%). The solid mass were redissolved in dimethylformamide (DMF) to evaluate antibacterial efficiency.

Bacterial cultures used for testing included *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus aureus* and *Klebsiella pneumoniae*. These bacterial cultures were obtained from Department of Microbiology, Gulbarga University, Gulbarga, India. The stock cultures were maintained on nutrient agar medium at 37°.

Antibacterial activity of the above mentioned extracts tested separately using agar well diffusion method[6]. Four

***For correspondence**
E-mail: Suzz@rediffmail.com