were calculated for each animal using the formula, percent decrease in activity= $(1-W_a/W_b)\times100$ , where  $W_a$  and  $W_b$  are average activity scores before and after drug administration, respectively and the average decrease in activity was calculated for all groups. The results are presented in Table 3. The data were analysed using student's 't' test (paired) where readings of the animals before drug administration served as control and the level of significance was set at P<0.001.

In the tail flick method<sup>2-4</sup>, it was found that all the extracts showed significant narcotic analgesic activity. The activity was found to be maximum for petroleum ether extract and minimum for chloroform extract. The activities were about 30-60% of that of morphine sulphate. Similar results were obtained from acetic acid-induced writhing test also, showing significant activity for all extracts. The activity was maximum for petroleum ether extract and minimum for chloroform extract and the activity was comparable to that produced by standard aspirin.

In activity evaluation study, it was found that all three extracts significantly depressed the locomotor activity, which was found to be slightly lower than that produced by the

standard, chlorpromazine. Here also, the activity was found to be maximum for petroleum ether extract and minimum for chloroform extract. Thus, it can be concluded that, on preliminary screening of crude extracts of *Cleome rutidosperma*, it was found that petroleum ether extract exhibited analgesic and locomotor depressant activities followed by methanol and chloroform extracts, which possessed these activities to a lesser extent.

### REFERENCES

- 1. Kritikar, K.R., and Basu, B.D., In; Indian Medicinal Plants, Vol. VI, Lalit Mohan Basu, Deharadun, India, 1991, 181.
- Kulkarni, S.K., In; Handbook of Experimental Pharmacology, 2nd Edn, Vallabh Prakashan, Delhi, 1993, 43.
- Turner, R.A., In; Screening Methods In Pharmacology, Academic Press, New York and London, 1965, 112.
- Varma, S., Handard, M.E. and Dandiya, P.C., Indian J. Pharmacol., 1989, 21, 46.
- Hazare, S.W., Chandra, S., Tandan, S.K., Sharma, J., Lal, J. and Telang, A.G., Indian J. Pharmacol., 2000, 32, 357.
- 6. Singh, T.J. and Garg, B.D., Indian J. Pharmacol., 1987, 19, 32.
- 7. Dews, P.B., Brit. J. Pharmacol., 1953, 1, 46.
- Ghosh, M.N., In; Fundamentals of Experimental Pharmacology,
   2nd Edn, Scientific Book Agency, Calcutta, 1984, 179.

## Spectrophotometric Method for the Determination of Lacidipine in Tablets

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A simple and sensitive spectrophotometric method has been developed for the determination of lacidipine in bulk and tablets. The method is based on the reaction of lacidipine with ferric chloride, potassium ferricyanide and hydrochloric acid to form a bluish green colored chromogen with an absorption maximum of 740 nm. Beer's law was obeyed in the range of 0-10  $\mu$ g/ml. The proposed method has been successfully applied to the analysis of the bulk drug and its dosage forms. Statistical comparison of the result with that obtained with reported method showed good agreement and indicated no significant difference in precision. This method does not require any extraction or heating.

\*For correspondence E-mail: phravi75@rediffmail.com Lacidipine is chemically 4-{2-[3-(1,1-dimethylethoxy)-3-oxo-1-propenyl phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridine dicarboxylic acid}diethyl ester¹. Reported methods of

analysis include HPLC<sup>2-5</sup> and spectrophotometry<sup>6</sup>. The aim of the present investigation was to develop an improved spectrophotometric method with greater precision, accuracy and sensitivity for the determination of lacidipine in bulk and tablets.

Pure lacidipine was obtained from GlaxoSmithKline Pharmaceuticals Ltd., Mumbai. An Elico UV/Vis spectrophotometer model SL 150 with 1 cm matched quartz cells was used for all absorbance measurements. All other chemicals

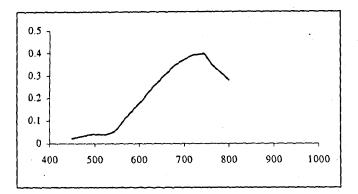


Fig. 1: Absorption spectrum of colored chromogen of lacidipine

## Concentration of lacidipine is 6 $\mu$ g/ml.

used were of Analar grade. Aqueous solution of hydrochloric acid (1N), ferric chloride (0.3% w/v) and potassium ferricyanide (0.1% w/v) was prepared.

Stock solution of pure lacidipine was prepared by dissolving 100 mg of the drug in 100 ml of methanol. The final concentration of lacidipine was brought to 100  $\mu$ g/ml with distilled water. In to a series of 10 ml volumetric flasks, 0.2 to 1.0 ml of lacidipine solution (100  $\mu$ g/ml) was pipetted separately and to each flask 1 ml of 0.3% w/v ferric chloride solution was added and shaken for 5 min. Then 0.5 ml of 0.1%

w/v potassium ferricyanide was added and shaken for 5 min. To this solution, 1 ml of 1N hydrochloric acid was added and shaken for 5 min. Then final volume was made up to 10 ml with distilled water. The absorbance of the bluish green color developed was measured at 740 nm against the reagent blank.

Twenty tablets of lacidipine (Sinopil, 4 and 2 mg, GlaxoSmithKline Pharmaceutical Ltd, Lacivas, 4 mg, Aztec-Sun division) were accurately weighed and powdered. Tablet powder equivalent to 10 mg of lacidipine was dissolved in 10 ml of methanol and filtered. Two different aliquots of this solution giving analyte concentrations of 4 and 6  $\mu$ g/ml were obtained by sequential dilution. Each aliquot of this solution was treated as described above.

Mixture containing lacidipine and common excipients such as lactose, talc, methyl cellulose, magnesium stearate and starch were prepared. A portion of the mixture equivalent to 100 mg of lacidipine was weighed accurately and dissolved in 100 ml of methanol and the solution was treated as described above. The effect of different concentrations and volumes of reagents (hydrochloric acid, ferric chloride and potassium ferricyanide) were studied at 740 nm for a fixed concentration of lacidipine (6 μg/ml).

To ensure the accuracy and reproducibility of the results obtained, 2 mg of lacidipine reference was added to previously analyzed tablet powder extract and the solution so obtained was treated as described above. Absorbance of the colored solution (6  $\mu$ g/ml) was measured at 740 nm at intervals of 5 min up to 90 min to investigate the stability of the color.

Ferric chloride oxidizes the 1,4-dihydro pyridine molecule in lacidipine and get reduced itself into ferrous ion. This ferrous fon reacts with potassium ferricyanide and gives bluish green colored chromogen. The absorption spectrum

TABLE 1: ESTIMATION OF LACIDIPINE IN TABLETS

| Sample   | Labeled<br>amount<br>(mg/tablet) | Amount found by*           |                            | Recovery Studies*       |                         |               |
|----------|----------------------------------|----------------------------|----------------------------|-------------------------|-------------------------|---------------|
|          |                                  | Proposed<br>method<br>(mg) | Reported<br>method<br>(mg) | Amount<br>added<br>(mg) | Amount<br>found<br>(mg) | %<br>Recovery |
| Tablet 1 | 4                                | 4.06                       | 4.08                       | 2                       | 2.02                    | 101.00        |
| Tablet 2 | 4                                | 4.13                       | 4.18                       | 2                       | 1.98                    | 99.00         |
| Tablet 3 | 2                                | 2.02                       | 1.97                       | 2                       | 2.01                    | 100.50        |

<sup>\*</sup>Average of 5 determinations

of the colored solution showed maximum absorption at 740 nm. The reagent blank has no absorbance at this wavelength. The bluish green color has been found to be stable for 60 min after which the intensity of color decreases gradually.

Temperature of the reaction, quantity, concentration and addition of various reagents were optimized after several experiments. The optimum quantity and concentration of hydrochloric acid, ferric chloride and potassium ferricyanide were found to be 1 ml, 1 ml, 0.5 ml and 1N, 0.3% w/v, 0.1% w/v, respectively. Replacement of hydrochloric acid with sodium hydroxide, sulphuric acid and phosphoric acid was also tried, but it was found that with these reagents there was no colour development. The optimum temperature was found to be  $37\pm0.5^{\circ}$ .

The optical characters such as Beer's law limits 0-10  $\mu$ g/ml, molar absorptivity 6.6 (l/mol/cm)×10², Sandell's sensitivity 0.0151  $\mu$ g/cm²/0.001 absorbance unit were found. Precision was determined by analyzing five replicate samples containing a known amount of lacidipine and the results obtained showed a correlation coefficient of 0.9974, % relative standard deviation of 1.08 and % error of ±0.71.

The validity of the method for the assay of tablets was determined. Results obtained using the developed method

and a reported method were found to be in good agreement (Table 1). Percent recovery experiments revealed good accuracy of the data. It was found that it is unnecessary to separate soluble excipients present in various marketed tablets before analysis since the results of analysis were always reproducible and equivalent to the labeled contents of the preparations.

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#### REFERENCES

- 1. Reynolds, J.E.F., In; Martindale, The Extra Pharmacopoeia, 31st Edn., The Pharmaceutical Press, London, 1996, 897.
- Pellegatti, M., Braggio, S., Sartori, S., Frances Chatti, F and Bolelli, G.F., J. Chromatograph. Biomed. Appl., 1992, 3, 105.
- 3. Hamdan, M., Scandola, M., Franchi, D., Gaviragi, G and Teriza, G., Org. Mass Spectrum, 1992, 27, 240.
- 4. Tiller, P.R., J. Chromatogra., 1993, 647, 101.
- Rossato, P., Scandola, M and Grossi, P., J. Chromatogr., 1993, 647, 155.
- Meyyanathan, S.N., Tonio, M.T., Rama Sarma, G.V.S. and Suresh, B., Eastern Pharmacist, 1999, 42, 117.

# Visible Spectrophotometric Determination of Levofloxacin in Tablet Dosage Forms

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The present paper describes three simple, accurate, rapid and precise visible spectrophotometric methods for the estimation of levofloxacin in tablet formulations. Methods A and B were based on the formation of ion-association complex of the drug with Eriochrome black T and bromocresol green. The absorbance of chloroform extracted complexes was measured at 490 and 420 nm, respectively. Method C was based on the formation of blue coloured chromogen with Folin-

Ciocalteau reagent which showed maximum absorbance at 720 nm. The optical characteristics

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