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 ± 0.5^{0}.

The optical characters such as Beer's law limits 0-10 μg/ml, molar absorptivity 6.6 (l/mol/cm)$\times10^{2}$, Sandell's sensitivity 0.0151 $\mu g/cm^2/0.001$ 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.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Nalla G. Palaniswamy, Chairman and Dr. Thavamani Palaniswamy, Trustee, K. M. C. H. College of Pharmacy, Coimbatore for providing the necessary facilities.

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Visible Spectrophotometric Determination of Levofloxacin in Tablet Dosage Forms

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Accepted 16 October 2004

Revised 10 June 2004

Received 12 November 2003

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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and the statistical analysis for all the methods were satisfactory. Interference studies were carried out using common excipients and the percentage recovery of levofloxacin using method A, B and C was found to be 99.9, 101.6, 100.5 %, respectively.

Levofloxacin (LFN), (-)-(S)-9-fluro-2, 3 dihydro-3-methyl-10 (4-methyl-1-piperazinyl)-7-oxo-7H pyrido (1,2,3-de)-1,4-benzocaine-6-carboxylic acid hemihydrate, is indicated for various bacterial infections[1]. The literature on the extractive spectrophotometric methods for the assay of the drug is scanty, although these methods are well suited. Few HPLC methods[2-6] for the determination of the drug in biological samples, which are tedious and time consuming, have been reported.

In the present work, three visible spectrophotometric methods have been developed, of which methods A and B are based on the formation of ion–association complexes of drug with Eriochrome black–T (EBT) and bromocresol green (BCG). Method C is based on the formation of a blue coloured chromogen with Folin-Ciocalteau (FC) reagent. The colour formation by FC reagent with LFN may be explained based on the analogy with the reports of earlier workers[7]. The mixed acids in the FC reagent preparation are the final chromogen and involve the following chemical species; $3H_2O.\ P_2O_5.\ 13WO_3.\ 5MoO_3.\ 10H_2O$ and $3H_2O.\ P_2O_5.\ 14WO_3.\ 4MoO_3.\ 10H_2O$. The proposed methods have been extended to the assay of the drug in tablet formulations.

Absorbance measurements were made on a Shimadzu UV/Vis Spectrophotometer – 1601 with 1 cm matched quartz cells. All chemicals used were of analytical or pharmaceutical grade (procured from CDH (P) Ltd., Mumbai) and quartz–processed high purity water was used throughout.

Standard solution of the drug was prepared by dissolving 100 mg of pure sample in methanol and diluted up to the mark in a 100 ml volumetric flask. A 0.5 % of EBT and 0.04 % of BCG solutions were prepared by dissolving in a few drops of methanol and then diluted to the mark with distilled water in 100 ml calibrated flasks separately. Acetate buffer of pH 4.0 and 10% sodium carbonate solution were prepared as per Indian Pharmacopoeia.

For method A, aliquots of standard LFN solution ranging from 1 to 8 ml (25 μg/ml) were transferred in to a series of 125 ml separating funnels. To this, 2 ml of EBT solution was added and the volume was made up to 10 ml with distilled water. To the resulting solution 10 ml of chloroform was added to each funnel and mixed well. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulfate. The absorbance of the colored species was measured at 490 nm, against the corresponding reagent blank.

For method B, aliquots of standard drug solutions ranging from 0.5 to 3 ml (100 μg/ml) were transferred in to a series of 125 ml separating funnels. To this 2 ml of acetate buffer of pH 4.0, 2 ml of BCG were added and the solutions were diluted to 10 ml with distilled water. About 10 ml of chloroform was added to each funnel, the contents were shaken well and the absorption of the separated chloroform layer was measured at 420 nm against the corresponding reagent blank.

For method C, to a series of 10 ml volumetric flasks, aliquots of standard drug solutions ranging from 1 to 6 ml of stock solution (250 μg/ml), were added. This was followed by the addition of 0.5 ml of FC reagent and 1ml of 10 % sodium carbonate and the volume was made upto 10 ml with distilled water. After shaking for 2 min, the volumetric flasks were allowed to stand for 10 min. The absorbance of blue coloured chromogen was measured at 720 nm against reagent blank. Calibration graphs were plotted for all the methods.

Twenty tablets (Alevo-500 mg, Alkem and Levobact-00 mg, Microlabs) were weighed separately, finely powdered, powder equivalent to 100 mg of the drug was dissolved in methanol and filtered. Aliquots of LFN solutions ranging from 1 to 6 ml (25 μg/ml) for method A, 0.5 to 3 ml (100 μg/ml) for method B, 1 to 6 ml (250 μg/ml) for method C and 1 to 6 ml (100 μg/ml) for UV method were used and analyzed using the procedure described.

The optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity values, regression equation and correlation coefficient for all the methods are given in Table 1. The precision[8] of the proposed methods were found to be excellent, as indicated by the relative standard deviation (less than 1.0) calculated from six replicate analyses of the drug.

The effects of common excipients and other substances were tested for possible interferences in the assay. It was observed that talc, starch, lactose, dextrose and magnesium stearate did not interfere with the determination at the lev-

TABLE 1: OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY DATA

Parameter	Method A	Method B	Method C
λmax (nm)	490	420	720
Beer's law limits (μg/ml)	2.5 – 20	5 – 30	25 – 150
Molar absorptivity (l/mol.cm)	$1.08x10^4$	$0.416x10^4$	$1.01x10^3$
Sandell's Sensitivity (ng/cm²)	34.29	89.01	36.60
Correlation Coefficient (r)	0.986	1.001	0.991
Regression Equation (Y)*			
Slope, b	0.0296	0.0093	0.0017
Intercept, a	0.002	0.044	0.135
Relative Standard deviation** (%)	0.713	0.625	0.49
Range of error**			
(at 95% confidence limit)	0.570	0.502	0.392

*Y=a+bx, where x is the concentration in μg /ml. **For six replicate analysis within Beer's law limits.

els found in dosage forms. The applicability of the methods was also checked by analyzing synthetic mixtures of the drug containing the following amount of excipients in mg, LFN (300), sucrose (50), starch (50), lactose (50) and dextrose (50). A suitable amount of the synthetic mixture was analyzed using methods A, B and C. Percent recovery of LFN using methods A, B and C was found to be 99.9, 101.6 and 100.5, respectively, with RSD values less than 1.0 for six replicates.

The accuracy[8] of the proposed methods was examined by performing recovery experiments using drug solutions of known strengths. The results were found to be satisfactory. The performance of the proposed methods was judged by calculating the student's "t" and F values. At the 95 % confidence level, the calculated t and f values did not exceed the theoretical values as shown in Table 2, indicating that there is no significant difference between the values obtained with the proposed visible spectrophotometric methods and the UV method.

TABLE 2: DETERMINATION OF LEVOFLOXACIN IN TABLET FORMULATIONS

Dosage form	Label claim mg/tab	UV Method (%)	Proposed methods*		
			Method A	Method B	Method C
Tablet 1	500	100.1±1.4	99.6±1.12 (F=1.005; t=0.268)	99.8±0.88 (F=1.12; t=1.96)	101.14 (F=1.43; t=1.36)
Tablet 2	500	99.8±0.81	99.3±1.5 (F=1.012; t=0.347)	99.1±1.09 (F=1.02; t=1.57)	100.4 (F=1.24; t=1.65)

*Figures are expressed in recovery ±SD (n=6); Tablet 1 is Alevo, Alkem Laboratories., Mumbai and Tablet 2 is Levobact, Microlabs., Bangalore.

These methods could provide successful accuracy, while utilizing common laboratory reagents in a simple, easy to follow procedure. The methods can be successfully applied to routine analysis of formulations containing levofloxacin.

ACKNOWLEDGEMENT

The authors are indebted to Madras Pharmaceuticals India Ltd., for the gift sample of LFN.

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Variability of Serum Phenytoin Concentrations in Indian Epileptic Patient Population

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Accepted 18 October 2004

Revised 14 June 2004

Received 9 January 2004

Due to varied pharmacokinetic behavior and inter individual variation of phenytoin, serum level monitoring is necessary to optimize the drug therapy. The present study was designed to study the relationship between serum levels of phenytoin and different patient related factors like the dose administered, effects of age, sex, control of seizures and the influence of co-medication on the phenytoin concentration. Blood samples were collected from the selected patient group (126 patients) and analyzed using a sensitive HPLC method. Of the 111 patients receiving phenytoin as mono therapy, complete seizure control could be achieved in 80 patients. Seventy four percent of these patients had seizures completely controlled with serum phenytoin level below therapeutic range. The following are the conclusions: In the epileptic patients on monotherapy, the seizure control with phenytoin was observed in the concentration range of 3.9 to 29 μg/ml with a mean (± SD) of 11.56±13 μg/ml. Patients showing therapeutic benefit ranged with serum drug level from sub therapeutic level to toxic level indicating the reference therapeutic range (10 to 20 μg/ml) has to redefined for Indian epileptic patient population.

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Serum level monitoring of antiepileptic drugs is important for optimizing drug therapy since relationships between serum levels and therapeutic and toxic effects have been clearly established for several drugs. Routine monitoring of