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Research Article

Bromothymol blue and Bromocresol purple as efficient reagents for the development and validation of visible spectrophotometric assay methods for Felodipine

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Abstract

Bromothymol blue (BTB) and Bromocresol purple (BCP) are introduced as reagents that can help in developing and validating two simple and low cost visible spectrophotometric methods for the determination of Felodipine drug in pure form as well as in formulations. The new methods are based on attraction between positive charge on the nitrogen of drug molecule and the negative charge of BTB as well as BCP to produce coloured complexes. The coloured complexes exhibited absorption maxima at 420 nm and 415 nm respectively for BTB and BCP. It was found that the results obtained by the proposed methods and the labeled amounts are in good agreement. The relative standard deviations of the proposed methods are found to be 1.557% and 1.807%. Based on all the results, it is concluded that the proposed methods offer low cost, simple, sensitive and rapid process of determining and validating the selected drug using visible spectrophotometry.

Keywords: Spectrophotometric analysis, Felodipine, economical methods, validation.

Introduction

Felodipine is a long-acting 1,4-dihydropyridine calcium channel blocker derivative with chemical name, ethylmethyl-1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridin-dicarboxylate (Fig. 1). It is used for the management of hypertension and angina pectoris [1,2]. Felodipine is a slightly yellowish, crystalline powder with the molecular formula of $C_{18}H_{19}Cl_2NO_4$. Felodipine binds to a number of calcium-binding proteins, exhibits competitive antagonism of the mineral corticoid receptor, and blocks calcium influx through voltage-gated T-type calcium channels. Felodipine decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through voltage-gated L- type calcium channels. Felodipine may be used to treat mild to moderate essential hypertension. It is available in local pharmacy as Plendil tablets containing 2.5 mg, 5 mg, or 10 mg of Felodipine for oral administration.

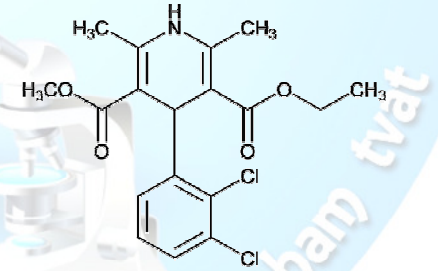

Fig. 1 Chemical structure of Felodipine

Various literature reports [3-19] are available based on determination of Felodipine in pharmaceutical dosage form or in biological fluids. But they suffer from drawbacks like large sample volumes and longer retention times. Hence, simple, cost effective and stability indicating analytical methods are developed by the authors for the assay of Felodipine in pure and in pharmaceutical dosage forms by employing spectrophotometric technique. Moreover, keeping the current regulatory requirements in mind, the developed me-

thods were extensively validated. The paper describes in detail a simple, sensitive, rapid and economical visible spectrophotometric methods that are studied for the assay of Felodipine in pure and in dosage forms. The two reagents selected for the purpose are bromothymol blue and bromocresol purple.

2. Materials and methods

2.1 Preparation of reagents

Pure sample of Felodipine was procured as gifted sample by Dr. Reddy's Laboratories, Limited, Hyderabad, India. Commercially available tablets of Plendil containing 5.0 mg of Felodipine were purchased from the local pharmacy. All the chemicals and reagents used were of analytical grade and solutions were prepared using doubled distilled water. A 0.1 % solution of bromothymol blue (BTB) was prepared by dissolving the 100 mg dye obtained from Rankem, India, (95% dye content) in water and filtered to remove the insoluble residue. Phthalate buffer (pH = 3.0) was prepared by dissolving 10.21 g of potassium hydrogen phthalate and 223 mL of 0.1 M HCl. 0.1% solution of bromocresol purple (BCP) was prepared by dissolving the 100 mg BCP dye received from Rankem, India, in 100 mL double distilled water and filtering it to remove the insoluble residue.

2.2 Preparation of stock and working standard solution

The stock solution (1 mg/mL) of Felodipine (FLP) was prepared by dissolving 100 mg of the drug in 10.0 mL of methanol and made up to 100 mL with distilled water to get a clear solution. In order to get the working standard solutions of concentrations 200 µg/mL and 160 µg/mL respectively for BTB and BCP, appropriate volumes of stock solution were taken and diluted accordingly.

2.3 Preparation of dosage forms

Twenty tablets of Plendil of strength 5.0 mg of Felodipin were weighed and ground into a fine powder. An amount of powder equivalent to 100 mg of Felodipine was accurately weighed into a 100 mL calibrated flask, 50 mL of methanol was added and the contents were shaken for 15–20 minutes. Then, the volume was finally made up to the mark with distilled water, mixed well and filtered using a Whatman No.42 filter paper. A suitable volume of the filtrate was accurately diluted with distilled water and this solution was used for the determination of Felodipine as per the recommended procedures.

2.4 Instrumentation

A UV-Visible Spectrophotometer Elico (SL-160 Model) with 1.0 cm matched quartz cuvettes was used for all spectral and absorbance measurements. An electronic balance (Model Shimadzu AUW-220D) with 0.001g Readability, 200g Capacity, 0.001g Repeatability, was used to weigh the required amount of the drug and the reagents. A Systronics digital pH meter (Model-362) was used for pH measurements.

3. Results and Discussion

3.1 Optimization studies

Optimization studies involve the study of the influence of various factors such as reagent concentration, order of addition, time, temperature and choice of solvent for maximum color development. Aliquots of working standard solutions of Felodipine (0.5-2.5 mL, 80 ◎g/mL) were taken in different 125 mL separating funnels. To each of the separating funnels BTB dye solution (5.0 ml), buffer solution (5.0 ml, pH-3.0) and chloroform (10 ml) were added and the contents were mixed for 2 minutes. The layers were allowed to separate. The separated layers were collected in dry test tubes and the absorbance of each organic layer was measured in 1.0 cm cell at 420 nm against blank. The concentration of the unknown was read from the calibration graph or computed from the regression equation. In a similar way, control experiments were performed in case of BCP by varying one and fixing the other parameters such as type and volume of acid, concentration of dye, organic solvent used for extraction, ratio of organic phase to aqueous phase during extraction, shaking time and temperature in order to establish the optimum conditions in each proposed methods. The optimum conditions established for both the methods BTB and BCP are presented in Table 1 and Table 2 respectively.

Table 1 Optimum conditions established for the method involving BTB

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	400 – 420	420	-
Effect of buffer on colour development	pH = 3.0-4.0	pH = 3.0	Variation of pH 3.0 buffer beyond the upper and lower limits resulted in low absorbance values
Effect of volume of BTB	1.0 – 5.0 mL	5.0 mL	5.0 mL dye solution was necessary for covering broad range of Beer's law limits
Choice of organic solvent for extraction of the coloured complex	$CHCl_3$	$CHCl_3$	Several water immiscible solvents were tested for the extraction of the coloured complex into organic phase. Chloroform was preferred for its selective extraction of the coloured drug-dye complex from the aqueous phase.
Effect of shaking time on extraction	1 – 5 minutes	2 minutes	Constant absorbance values were obtained for shaking periods between 1 to 5 minutes
Stability of the coloured species in organic solvent	12 hours	-	-

3.2 Recommended procedures

After a systematic and detailed study of the various parameters involved, as described above in the optimum conditions, the following procedures are proposed for the assay of Felodipine in bulk samples. Accurately measured portions (0.5-2.5 mL, 200 ◎g/mL) of standard solutions of Felodipine were taken into a series of 125 mL separating funnels and the volume to 5.0 mL with phthalate buffer solution (pH-3.0). To each of the separating funnels, BTB dye solution (5.0 mL) and chloroform (10.0 mL) were added and the separating funnels were shaken for 2 minutes. The layers were allowed to separate. The separated layers were collected in dry test tubes containing anhydrous sodium sulphate. The absorbance of each organic layer was measured in 1.0 cm cell at 410 nm against blank. The concentration of the unknown was read from the calibration graph. Similar procedure described above was followed using BCP also. BCP dye solution (5.0 mL) and buffer solution (5.0 mL, pH=3.0) and chloroform (10.0 mL) was used for aliquots (0.5-2.5 mL) of standard Felodipine (160 ◎g/mL) solution. The absorbance of organic layer was measured at 415 nm against blank. The unknown concentration of the Felodipine was obtained from calibration graph.

3.3 Method validation

The developed visible spectrophotometric methods were validated for linearity, limit of quantification, limit of detection, precision and accuracy in accordance with pharmacopeial norms.

3.3.1 Spectral characteristics

The maximum absorption (λ_{max}) of the coloured species formed in the above proposed methods was ascertained by following the above procedures with specified amounts of Felodipine. The absorption spectra for each proposed method was scanned on a spectrophotometer in the wavelength region of 340 to 900 nm against similar reagent blank or distilled water and were recorded graphically that are presented in Figs. 2 and 3.

3.3.2 Optical characteristics

In case of the coloured species formed in the above methods, Beer's law plots were recorded with the absorbance vs. wavelength for a set of solutions containing varying amounts of Felodipine and specified amounts of reagents, against the corresponding reagent blanks and the Beer's law plots of these systems are shown in Figs. 4 and 5. The Beer's law limits, molar absorptivity, Sandell's sensitivity and optimum photometric range for Felodipine in both the proposed me-

thods were calculated, and the least square regression analysis was carried out for each proposed method, and all these values are presented in Table 3.

Table 2 Optimum conditions established for the method involving BCP

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	410 – 420	415	-
Effect of buffer on colour development	pH = 3.0-4.0	pH = 3.0	Variation of pH 3.0 buffer beyond the upper and lower limits resulted in low absorbance values
Effect of volume of BCP	1.0–5.0 mL	5.0 mL	5.0 mL dye solution was necessary for covering broad range of Beer's law limits
Choice of organic solvent for extraction of the coloured complex	$CHCl_3$	$CHCl_3$	Several water immiscible solvents were tested for the extraction of the coloured complex into organic phase. Chloroform was preferred for its selective extraction of the coloured drug-dye complex from the aqueous phase.
Effect of shaking time on extraction	1 – 5 minutes	2 minutes	Constant absorbance values were obtained for shaking periods between 1 to 5 minutes
Stability of the coloured species in organic solvent	24 hours	-	-

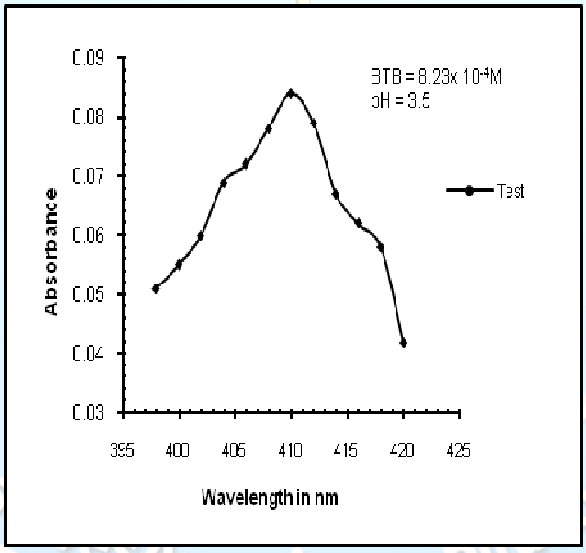

Fig. 2 Absorption spectrum of Felodipine with BTB-$CHCl_3$

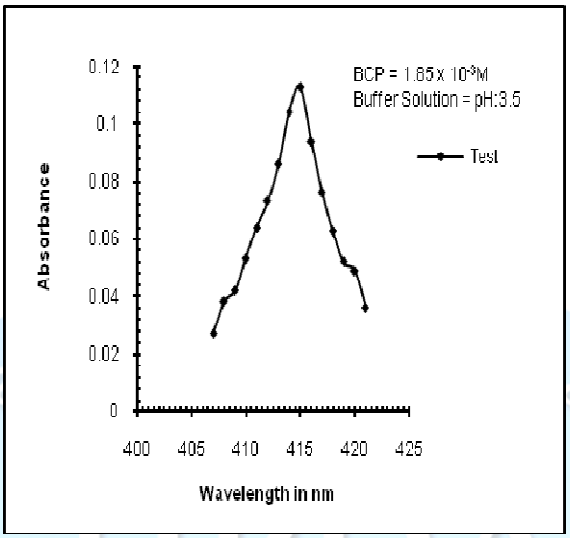

Fig. 3 Absorption spectrum of Felodipine with BCP-$CHCl_3$

3.3.3 Precision and accuracy

The precision of both the proposed methods was obtained by actual determination of six replicates of a fixed amount of Felodipine in total solution. The percentage relative standard deviation and percentage range of error at 0.05 and 0.01 confidence limits were calculated and presented in Table 3. In order to obtain the accuracy of the proposed methods, different amounts of bulk samples of Felodipine within the Beer's law limits were taken and analyzed by the proposed methods. The results (percentage error) are shown in Table 3.

3.3.4 Analysis of formulations

Commercial formulations (tablets) of Felodipine (Plendil-5.0 mg) were analyzed by the proposed methods. The values obtained by the proposed methods and the reference method [6] for formulations were compared statistically with F-test and t-test and found that they are not significantly different. Percentage recoveries were determined by adding standard drug to initially analyzed formulations. The results of percentage recoveries by the proposed methods were summarized in Table 4.

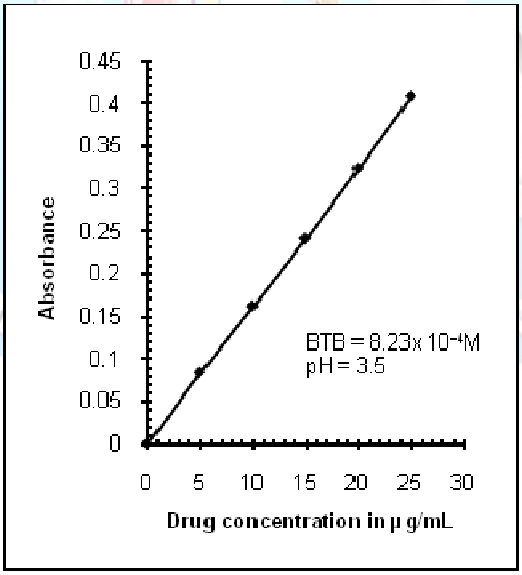

Fig. 4 Beer's law plot of Felodipine with BTB-$CHCl_3$

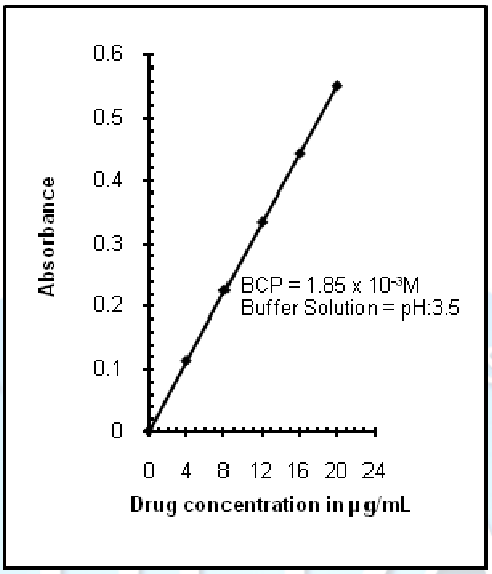

Fig. 5 Beer's law plot of Felodipine with BCP-$CHCl_3$

The secondary nitrogen group of Felodipine involves in the formation of ion association complex with acid dyes, BTB and BCP. These complexes are extractable into chloroform from the aqueous phase. The protonated nitrogen moiety (positive charge) of Felodipine is expected to attract the oppositely charged (negative charge) of dye, that behaves as a single unit being held together by electrostatic attraction. Based on analogy, the structures of ion association complexes are shown in Fig. 6.

Table 3 Optical and regression characteristics, precision and accuracy of the proposed methods for Felodipine

Parameter	Method involving BTB	Method involving BCP
λ_{max} (nm)	410	415
Beer's law limits (µg/mL)	5.0-25.0	4.0-20.0
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	5.738 x 10^3	4.952 x 10^3
Sandell's sensitivity (µg.cm^{-1}/0.001 absorbance unit)	0.0417	0.0498
Optimum photometric range (µg/mL)	7.0-20.0	5.0-17.7
Regression equation (Y=a+bc); slope (b)	0.0249	0.0262
Intercept (a)	0.00033	-0.0022
Correlation coefficient (r)	0.9999	0.9993
Relative standard deviation (%) (Average of six determinations)	1.557	1.807
Range of error (%) (confidence limits)	1.793 (0.05 level) 2.802 (0.01 level)	2.076 (0.05 level) 3.254 (0.01 level)

3.3.5 Nature of the coloured species

An attempt has been made to indicate the nature of coloured species in each of the proposed methods for Felodipine based on analogy of reactive functional moiety (secondary amine group) in the drug with appropriate reagents. The method is described in Fig-6.

Table 4 Results of assay and recovery of the drug Felodipine in formulation, namely Plendil

Method	Labeled amount (mg)	Amount found (mg)	t-test value	F-test value	Found by reference [6] method	% Recovery by proposed method
BTB-based	5.0	4.91	0.26	3.53	4.98	98.2
BCP-based	5.0	4.97	0.27	2.53		99.4

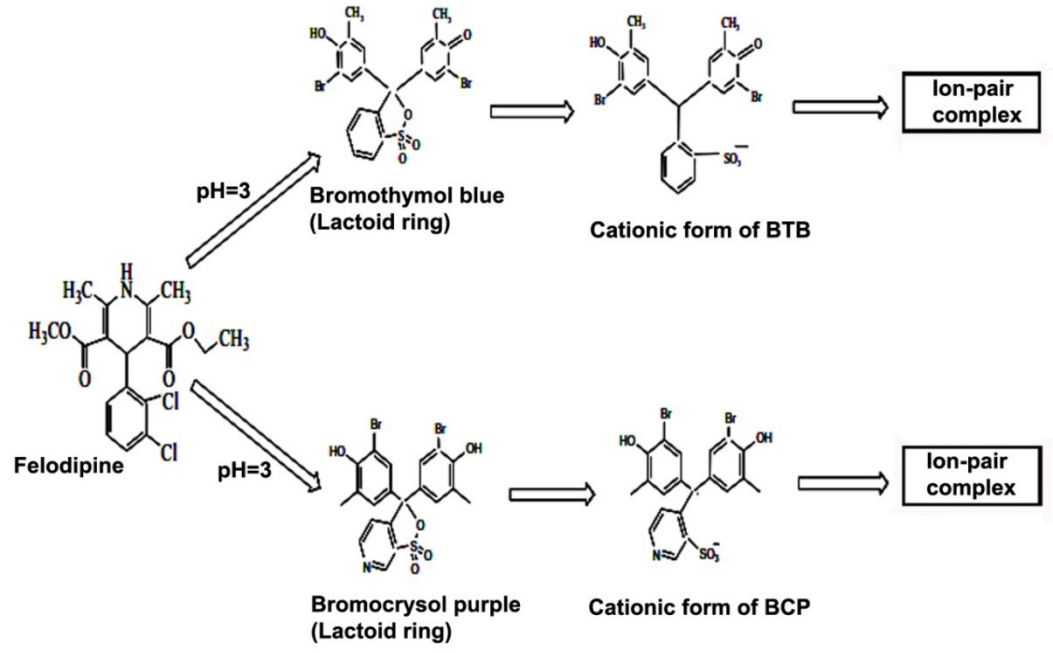

Fig. 6 Reaction scheme of Felodipine with BTB and BCP

4. Conclusions

The visible spectrophotometric methods developed in the present study for the determination of Felodipine are simple, accurate, precise and economical. The data of statistical analysis reveal that the proposed methods are in good agreement with those of the reported methods. The analytical performance of the developed spectrophotometric methods was achieved by comparing the validation results with the data obtained from various scientific works reported. It was observed that the proposed methods of determining the Felodipine with the organic reagents, namely bromothymol blue and bromocresol purple perform well from the analytical point of view on par with the reported work in the literature, with an increased range of linearity and have the ability to detect Felodipine even at a lower concentration level. Moreover, the proposed methods do not require any pretreatment of the drug and tedious extraction procedure. Thus, the proposed methods can be used for routine analysis of Felodipine in pharmaceutical industries and research laboratories.

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