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Extractive spectrophotometric methods for the determination of nifedipine in pharmaceutical formulations using bromocresol green, bromophenol blue, bromothymol blue and eriochrome black T

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Abstract

Four simple, sensitive and accurate spectrophotometric methods have been developed for the determination of nifedipine in pharmaceutical formulations. These methods are based on the formation of ion-pair complexes of amino derivative of the nifedipine with bromocresol green (BCG), bromophenol blue (BPB), bromothymol blue (BTB) and eriochrome black T (EBT) in acidic medium. The coloured products are extracted with chloroform and measured spectrophotometrically at 415 nm (BCG, BPB and BTB) and 520 nm (EBT). Beer's law was obeyed in the concentration range of 5.0–32.5, 4.0–37.5, 6.5–33.0 and 4.5–22.5 μg ml^{-1} with molar absorptivity of 6.41×10^3, 4.85×10^3, 5.26×10^3 and 7.69×10^3 l mol^{-1} cm^{-1} and relative standard deviation of 0.82%, 0.72%, 0.66% and 0.68% for BCG, BPB, BTB and EBT methods, respectively. These methods have been successfully applied for the assay of drug in pharmaceutical formulations. No interference was observed from common pharmaceutical adjuvants. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision.

Keywords: Nifedipine; Extractive spectrophotometry; Ion-pair formations; Pharmaceutical formulations

1. Introduction

Nifedipine, chemically dimethyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl) pyridine-3,5-dicarboxylate is an important calcium channel blocker with peripheral and coronary vasodilator activity [1–5]. The drug is frequently used as antihypertensive and potent arterial vasodilator in the treatment of angina pectoris and various other cardiovascular disorders [6,7]. The drug and its formulations are official in USP [8] and BP [9], which recommended HPLC and non-aqueous titration for its assay, respectively.

The drug has been determined by a variety of analytical techniques, such as HPLC [10–31], reversed-phase HPLC [32–34], HPTLC [35], gas chromatography [36–53], micellar electrokinetic chromatrography [54], electroanalytical methods [55–60], flow-injection analysis [61], mass spectrometry [62] and UV spectrophotometry [63–65].

The estimation of nifedipine alone was carried out using second-order derivative spectra [66] of the compound in 0.1 M HCl, whereas first-order derivative spectra were utilized for its assay in combined dosage forms [67,68]. The methanolic solution of the drug reacts with 4-dimethylaminobenzaldehyde in H_3PO_4 resulting in the formation of yellow-coloured product, which forms a basis for its determination at 380 nm [69]. Two spectrophotometric methods have been recommended, one is based on the formation of blue-coloured complex with Folin-Ciocalteau reagent [70] and the second method involves the charge transfer complex formation with chloranil [71]. A kinetic spectrophotometric method has also been described based on oxidation of drug with $KMnO_4$ at neutral pH [72]. The other two spectrophotometric methods were developed which involved the reduction of nifedipine with Zn/NH_4Cl and Zn/HCl to hydoxylamino derivative and primary aromatic amino derivative, respectively [73,74]. The hydoxylamino derivative was reacted with 4-(methylamino) phenol and potassium dichromate to give a chromophore, which absorbed maximally at 525 nm. The aromatic amino derivative formed Schiff's base with 3,4,5-trimethoxybenzaldehyde and subsequently determined at 365 nm. So far, no extractive spectrophotometric

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method for the assay of nifedipine in pharmaceutical formulations was reported.

In this communication, four new extractive spectrophotometric methods for the determination of nifedipine have been discussed. The methods are based on the reduction of nitro group of nifedipine by Zn/HCl into primary amino derivative which forms chloroform-extractable ion-pair complexes with bromocresol green (BCG), bromophenol blue (BPB), bromothymol blue (BTB) and eriochrome black T (EBT).

2. Experimental

2.1. Apparatus

A Spectronic 20D$^+$ spectrophotometer (Milton Roy, USA) was used to measure the absorbance.

An Elico model Li-10 pH meter was used for pH measurements.

2.2. Reagents and standards

2.2.1. Preparation of amino derivative of the nifedipine

A 0.05% solution of reduced nifedipine was prepared by treating a mixture of 12.5 mg of pure nifedipine (J.B. Chemicals and Pharmaceuticals Ltd., Mumbai, India), dissolved in 3 ml ethanol, 1.8 ml of 5 M HCl for BTB (2.5 ml for BCG and BPB; 3.0 ml for EBT required for ion-pair formation) and 3.0 g of Zn dust. The content was allowed to stand for 20 min at 25 ± 1 °C. The solution was then filtered through a Whatmann No. 42 filter paper; the residue was washed with two 5-ml portions of doubly distilled water. The filtrate and washings were diluted to volume in a 25-ml standard volumetric flask with doubly distilled water.

The 0.025% solutions of BCG (S.D. Fine-Chem Limited, India), BPB (S.D. Fine-Chem Limited, India), BTB (S.D. Fine-Chem Limited, India) and EBT (Fluka Chemie AG, Germany) were prepared by dissolving 25 mg of each dyestuff in 100-ml standard volumetric flask and diluting to volume with doubly distilled water.

2.3. Procedure of calibration curve

Into a series of 50-ml separating funnel 7 ml of BCG (BPB, BTB or ETB) followed by an appropriate volume of 0.05% reduced nifedipine (0.10–0.65 ml for BCG; 0.08–0.75 ml for BPB; 0.13–0.66 ml for BTB and 0.09–0.45 ml for EBT) were placed and mixed well. Then 10 ml of chloroform was added to each funnel. The contents were shaken for 2 min and allowed to separate the two layers. The absorbance of the organic layer was measured at 415 nm for BCG, BPB and BTB ion-pair complexes and 520 nm for EBT ion-pair complex against a reagent blank prepared similarly in each case. The calibration curve was constructed in each case, by considering the absorbance measured at seven concentration levels of nifedipine. The amount of the drug was computed either from calibration curve or from regression equation.

The colour of the complexes was stable for at least 2 h.

2.4. Procedure for the assay of drug in dosage forms

An amount of the tablet or capsule equivalent to 12.5 mg of nifedipine was weighed accurately, and extracted into 25 ml chloroform with shaking. Filtration through a Whatmann No. 42 filter paper was performed. The filtrate was evaporated to dryness under vacuum and the residue was dissolved in 3 ml ethanol and converted into amino derivative following the procedure given under the head "preparation of amino derivative of the nifedipine" and then subjected to the recommended procedure for the determination.

3. Results and discussion

Nifedipine contains $-NO_2$ group, which is reduced to amino derivative by zinc dust and HCl. In the present study, the reduced nifedipine possessing primary aromatic amino group is protonated in acidic medium, which forms ion-pair complexes with each of the acid dyes such as BCG, BPB, BTB and EBT. The ion-associated complexes are quantitatively extracted with chloroform. The absorption spectra are shown in Fig. 1, which revealed that the ion-pair complexes with BCG (BTB or BPB) and EBT absorbed maximally at 415 and 520 nm, respectively. The reagent blanks prepared under similar conditions showed no absorption.

3.1. Composition and formation constant of ion-pair complexes

The stoichiometry of ion-pair complexes of the reduced drug with each of the dyestuffs was established following the method of continuous variations [75]. The results (Fig. 2) indicated that the molar ratio of the drug to dyestuff in each compound is 1:1. The formation constants [76,77] were also calculated and found to be 2.91×10^6, 3.32×10^6, 5.82×10^5 and 5.54×10^6 for complexes with BCG, BPB, BTB and EBT, respectively.

3.2. Optimization of the reaction conditions

The optimum conditions for quantitative estimation of the drug were established via a number of preliminary experiments.

3.2.1. Effect of concentration of hydrochloric acid

The influence of the concentration of HCl on the reduction of the nifedipine and subsequent ion-pair formation of amino derivative of the drug with various dyestuffs has been studied. The results are shown in Fig. 3. It is apparent from the figure that the absorbance of ion-pair complexes with BCG (or BPB), BTB and EBT was found to be constant when the reduction was done in the range of 0.47–0.53, 0.34–0.40 and 0.56–0.64 M, respectively. For the most efficient extraction of ion-pair with chloroform the optimum value was fixed at 0.50, 0.36 and 0.60 M for ion-pair formation with BCG (or BPB), BTB and EBT, respectively.

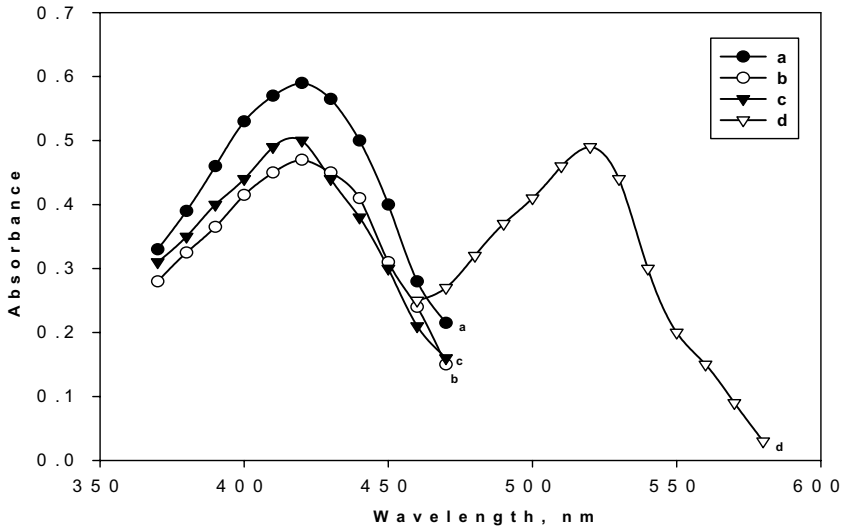

Fig. 1. Absorption spectra of nifedipine–dye complex extracted in chloroform: (a) amino derivative of nifedipine–BCG complex, (b) amino derivative of nifedipine–BPB complex, (c) amino derivative of nifedipine–BTB complex and (d) amino derivative of nifedipine–EBT complex.

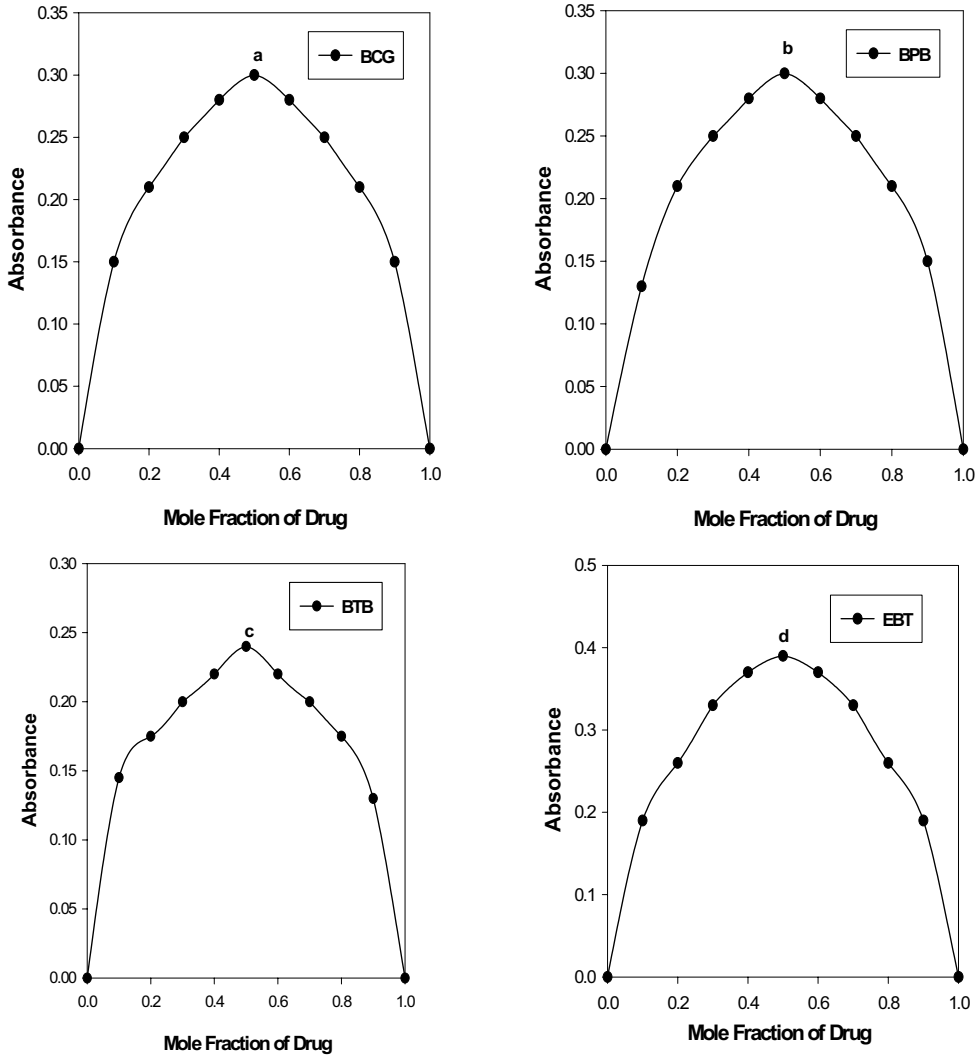

Fig. 2. Job's method of continuous variation of drug–dye (1.443×10^{-4} M) systems: (a) BCG, (b) BPB, (c) BTB and (d) EBT.

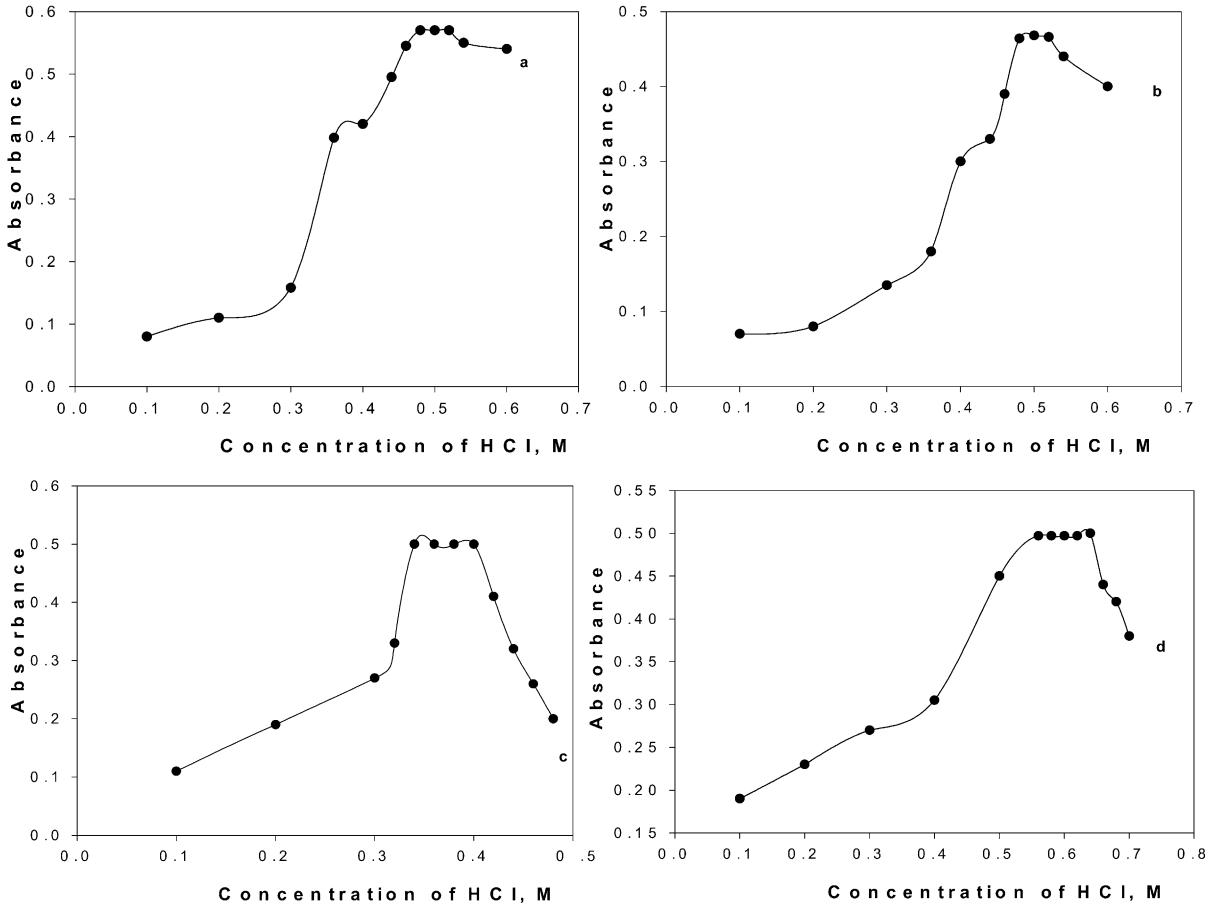

Fig. 3. Effect of the concentration of HCl: (a) 31 μg ml^{-1} drug + 7 ml of 0.025% BCG, (b) 33 μg ml^{-1} drug + 7 ml of 0.025% BPB, (c) 33 μg ml^{-1} drug + 7 ml of 0.025% BTB and (d) 22.5 μg ml^{-1} drug + 7 ml of 0.025% EBT.

3.2.2. Effect of dye concentration

The effect of dye concentration on the intensity of the colour developed at the selected wavelength and constant nifedipine concentration was critically examined using different millilitres of the reagent (0.025%). The results indicated (Fig. 4.) that the maximum absorbance, in each case, was found with 6.0 ml of the reagent, beyond which absorbance become constant. Therefore, 7.0 ml of each dyestuff was used for ion-pair formation throughout the experiment.

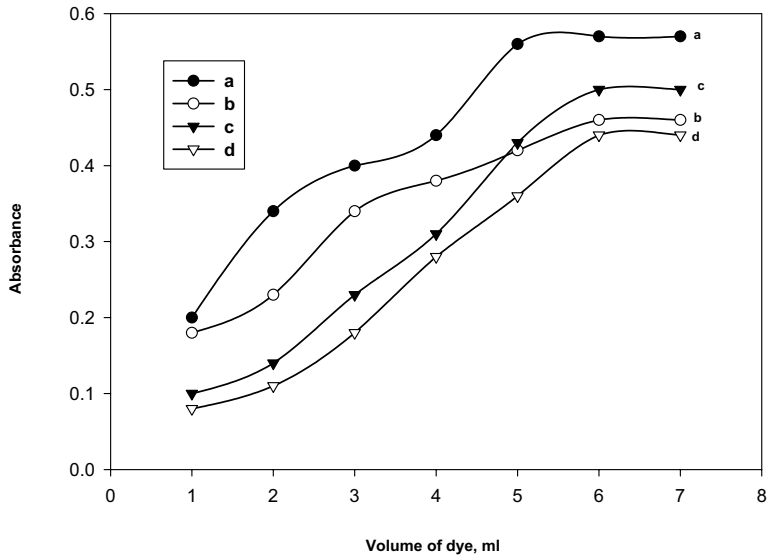

Fig. 4. Influence of the volume of 0.025% dye: (a) BCG, (b) BPB, (c) BTB and (d) EBT.

Table 1
Analytical characteristics of the proposed methods

Parameter(s)	Extraction methods with			
	BCG	BPB	BTB	EBT
λ_{max} (nm)	415	415	415	520
Beer's law limit (μg ml^{-1})	5.0–32.5	4.0–37.5	6.5–33.0	4.5–22.5
Molar absorptivity (l mol^{-1} cm^{-1})	6.41×10^3	4.85×10^3	5.26×10^3	7.69×10^3
Linear regression equation [a]	$A = 4.10 \times 10^{-4} + 1.849 \times 10^{-2}C$	$A = 3.20 \times 10^{-4} + 1.400 \times 10^{-2}C$	$A = 1.070 \times 10^{-3} + 1.515 \times 10^{-2}C$	$A = 2.200 \times 10^{-4} + 2.211 \times 10^{-2}C$
Intercept (a)	4.100×10^{-4}	3.200×10^{-4}	1.070×10^{-3}	2.200×10^{-4}
S_a	8.300×10^{-4}	3.800×10^{-4}	5.700×10^{-4}	1.480×10^{-3}
tS_a [b]	2.113×10^{-3}	9.770×10^{-4}	1.465×10^{-3}	3.805×10^{-3}
Slope (b)	1.849×10^{-2}	1.400×10^{-2}	1.515×10^{-2}	2.211×10^{-2}
S_b	4.00×10^{-5}	2.00×10^{-5}	3.00×10^{-5}	3.00×10^{-5}
tS_b [c]	1.028×10^{-4}	5.142×10^{-5}	7.713×10^{-5}	2.828×10^{-4}
Correlation coefficient (r)	0.9999	1.0000	0.9999	0.9999
Variance (S_0^2)	7.00×10^{-5}	2.84×10^{-7}	5.19×10^{-7}	3.58×10^{-6}
Detection limit (μg ml^{-1})	1.06	0.09	0.11	0.20
Relation standard deviation (%) [d]	0.82	0.72	0.66	0.68
Recovery (%)	100.08	99.80	100.86	100.24

[a] With respect to $A = a + bC$, where C is the concentration (μg ml^{-1}) and A is absorbance.
[b] Confidence intervals of the intercept at 95% confidence limit.
[c] Confidence intervals of the slope at 95% confidence limit.
[d] Ten replicate samples.

3.2.3. Choice of organic solvent

A number of organic solvents such as chloroform, carbon tetrachloride, dichloromethane, benzene and toluene were examined for extraction of the ion-pair complex in order to provide an applicable extraction procedure. Chloroform was preferred for its selective extraction of ion-pair complex from the aqueous solution. Shaking time of 0.5–4.0 min. provided a constant absorbance and hence, 2.0 min was used as an optimum shaking time throughout the experiment. The ion-pair complexes were quantitatively recovered in one extraction only and were also stable for at least 2 h.

3.2.4. Effect of excipients

A systematic study of the effect of excipients was performed, following the proposed procedures for a 10-ml sample system, by adding a known amount of excipients to the fixed nifedipine concentration (22.5 μg ml^{-1}). The results revealed the fact that no significant interference was observed from the excipients, such as glucose, fructose, sucrose, lactose and starch commonly present in pharmaceutical formulations. However, the drug content from the powdered tablets or capsules was extracted into chloroform, which completely eliminates the common excipients found in drug formulations.

3.3. Analytical data

Calibration graphs were constructed, by measuring the absorbance at seven concentration levels, which showed linear response of absorbance in relation to concentration of nifedipine over the range of 5.0–32.5, 4.0–37.5, 6.5–33.0 and 4.5–22.5 μg ml^{-1} for BCG, BPB, BTB and EBT methods, respectively. Regression analysis of calibration graphs indicated linear relationship with negligible intercepts. Table 1 summarizes the analytical parameters, molar absorptivity and the results of statistical analysis of the experimental data: regression equations computed from calibration graphs along with standard deviation of slope (S_b) and intercept (S_a), confidence interval of slope (tS_b) and intercept (tS_a) on the ordinate. The detection limits [78,79] were found to be 1.06, 0.09, 0.11 and 0.20 μg ml^{-1} for BCG, BPB, BTB and EBT methods, respectively. The small value of variance, further, suggested least scatter of experimental data points around the line of regression.

The repeatability of the proposed procedures was checked by performing 10 replicate determinations of nifedipine at concentration levels of 20 and 30 μg ml^{-1} with BCG (or EBT) and BPB (or BTB), respectively. The percent relative standard deviations (% RSDs) and recoveries were found to vary over the range of 0.66–0.82% and 99.8–100.7%, respectively.

The accuracy of proposed methods was demonstrated by recovery experiments, which were carried out by adding a fixed amount of pure drug to the pre-analysed dosage forms. The analytical results obtained are summarized in Table 2. The percentage of RSDs (0.12–0.62%) can be considered to be very satisfactory.

The performance of the proposed methods was compared with that of other existing UV–visible spectrophotometric methods (Table 3). It is evident from the table that the proposed methods are more sensitive than the other reported methods due to their higher molar absorptivities and present better accuracy with narrow linear dynamic range. The methods are found to be simple and can compete with other existing spectrophotometric methods in determining drug in lower concentrations. The order of performance of the proposed methods is EBT > BCG > BTB > BPB.

Table 2
Determination of nifedipine in pharmaceutical formulations by standard addition method

Preparations	Amount taken (μg ml^{-1})	Amount added (μg ml^{-1})	Recovery (%)				RSD (%) [a]			
			BCG	BPB	BTB	EBT	BCG	BPB	BTB	EBT
Tablet										
Nicardia retard-10	10	10	100.04	100.09	99.91	100.04	0.31	0.32	0.43	0.26
	5	10	100.01	99.94	100.02	100.09	0.30	0.62	0.37	0.45
Calciguard-10	10	10	100.10	100.09	99.91	100.04	0.41	0.41	0.43	0.26
	5	10	100.01	99.94	99.94	100.03	0.30	0.62	0.37	0.37
Adalat retard-10	10	10	99.99	100.027	100.04	100.04	0.52	0.19	0.28	0.26
	5	10	100.01	99.94	100.03	100.03	0.30	0.62	0.50	0.37
Capsule										
Nicardia-10	10	10	99.94	99.95	100.04	100.04	0.43	0.30	0.28	0.12
	5	10	100.22	100.13	100.03	100.03	0.41	0.54	0.50	0.37
Calciguard-10	10	10	100.04	100.09	100.041	99.99	0.31	0.32	0.28	0.12
	5	10	100.01	100.13	100.029	100.15	0.47	0.54	0.39	0.17

[a] Five independent analyses.

Table 3
Comparison of the proposed methods with existing spectrophotometric methods for the assay of nifedipine in pharmaceutical formulations

Reagents	λ_{max} (nm)	Beer's law limit (μg ml^{-1})	Molar absorptivity (l mol^{-1} cm^{-1})	Recovery (%)	RSD (%)	References
Potassium permanganate	530	18.0–44	–	99.5–101.3	1.50	71
3,4,5-Trimethoxybenzaldehyde	365	10.0–70	–	100.2–102.4	1.50	73
4-Dimethylaminobenzaldehyde	380	5.0–60	–	97.8–98.5	–	69
Ethanol and phosphate buffer saline	340	–	–	99.7–99.9	–	63
Derivative UV	400	4.0–12	–	98.5–101.3	1.40	68
4-Methylaminophenol and dichromate	525	5.0–175	1.90×10^3	99.7–100.5	0.60	72
BCG	415	5.0–32.5	6.41×10^3	99.9–100.1	0.82	This work
BPB	15	4.0–37.5	4.85×10^3	99.9–100.1	0.72	This work
BTB	415	6.5–33.0	5.26×10^3	99.8–100.9	0.66	This work
EBT	520	4.5–22.5	7.69×10^3	100.0–100.2	0.68	This work

Table 4
Determination of nifedipine in dosage forms by the proposed methods and reference method [9]

Preparations	Proposed methods												Reference method			F [c]			
	Recovery (%)				RSD (%) [b]				t [a]				Recovery (%)	RSD (%) [b]	t [a]				
	BCG	BPB	BTB	EBT	BCG	BPB	BTB	EBT	BCG	BPB	BTB	EBT				BCG	BPB	BTB	EBT
Tablet																			
Nicardia retard-10	99.94	100.02	99.84	100.03	0.43	0.41	0.38	0.26	0.32	0.15	0.93	0.34	100.02	0.09	0.49	3.40	3.13	2.67	1.26
Calciguard-10	99.99	99.95	100.04	100.17	0.52	0.30	0.28	0.19	0.23	0.34	0.33	2.08	100.10	0.14	1.58	2.15	1.40	1.64	3.54
Adalat retard-10	99.88	100.09	100.04	100.12	0.35	0.32	0.49	0.36	0.72	0.69	0.19	0.81	100.02	0.09	0.49	2.33	1.92	4.51	2.40
Capsule																			
Nicardia-10	99.80	99.88	99.90	100.17	0.55	0.36	0.43	0.19	0.87	0.73	0.47	2.08	100.02	0.09	0.49	5.76	2.41	3.48	1.50
Calciguard-10	100.04	100.09	100.04	100.03	0.31	0.41	0.28	0.26	0.35	0.54	0.33	0.34	100.10	0.14	1.58	1.32	1.24	1.64	1.88

[a] t-value at 95% confidence level is 2.776.
[b] Five independent analyses.
[c] F-value at 95% confidence level is 6.39.

The proposed methods have been successfully applied to the determination of nifedipine in commercial tablets and capsules purchased locally. The results (Table 4) obtained by the proposed methods were compared by BP method [9]. The calculated Student's t-values and F-values did not exceed the theoretical ones at 95% confidence level. Therefore, there is no significant difference between the proposed methods and BP method.

4. Conclusion

The proposed methods are advantageous in comparison to other existing spectrophotometric methods because the de-

tection limits are as low as 0.09 μg ml^{-1}. The molar absorptivities are also comparable with low RSDs. No interference from common excipients was encountered. Thus the proposed methods are simple, sensitive, accurate, precise, economical and suitable for routine analysis of nifedipine in drug formulations.

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