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Short communication

Validated spectrophotometric methods for the determination of amlodipine besylate in drug formulations using 2,3-dichloro 5,6-dicyano 1,4-benzoquinone and ascorbic acid

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Abstract

Two simple and sensitive spectrophotometric methods have been proposed for the determination of amlodipine besylate either in pure form or in pharmaceutical formulations. The first method is based on the charge transfer complexation reaction of the drug with 2,3-dichloro 5,6-dicyano 1,4-benzoquinone (DDQ) to give coloured product having maximum absorbance at 580 nm. The second procedure depends on the measurement of purple red colour produced by the interaction of drug with ascorbic acid in *N*,*N*-dimethylformamide medium (DMF) which absorbed maximally at 530 nm. Under the optimized experimental conditions, Beer's law was obeyed in the concentration ranges of 1–125 and 10–140 μg ml^{-1} with DDQ and ascorbic acid, respectively. Both the methods were applied successfully for the analysis of amlodipine besylate in dosage forms. Results of analyses were validated statistically and through recovery studies.

Keywords: Amlodipine besylate; 2,3-Dichloro 5,6-dicyano 1,4-benzoquinone; Ascorbic acid; Spectrophotometry

1. Introduction

Amlodipine besylate is listed in Martindale, The Extra Pharmacopoeia and European Pharmacopoeia [1,2] which is chemically (4*R*,*S*)-3-ethyl 5-methyl 2-(2-amino-ethoxy-methyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl pyridine-3,5-dicarboxylate monobenzene sulphonate, approved for the treatment of variant and stable angina and hypertension. It is relatively a new long acting calcium channel blocker with slow onset of vasodilatory action [3,4]. It may also be used for dilated cardiomyopathy and exhibits ameliorating effects on plasma and myocardial catecholamines with a significant reduction of calcium deposition [5,6]. In addition to calcium channel blocking ability, amlodipine also inhibits vascular smooth muscle cell growth through interactions with targets other than L-type calcium channels [7]. Amlodipine is more selective for arterial vascular smooth muscle than cardiac tissues. Due to these important pharmacological responses, develop-

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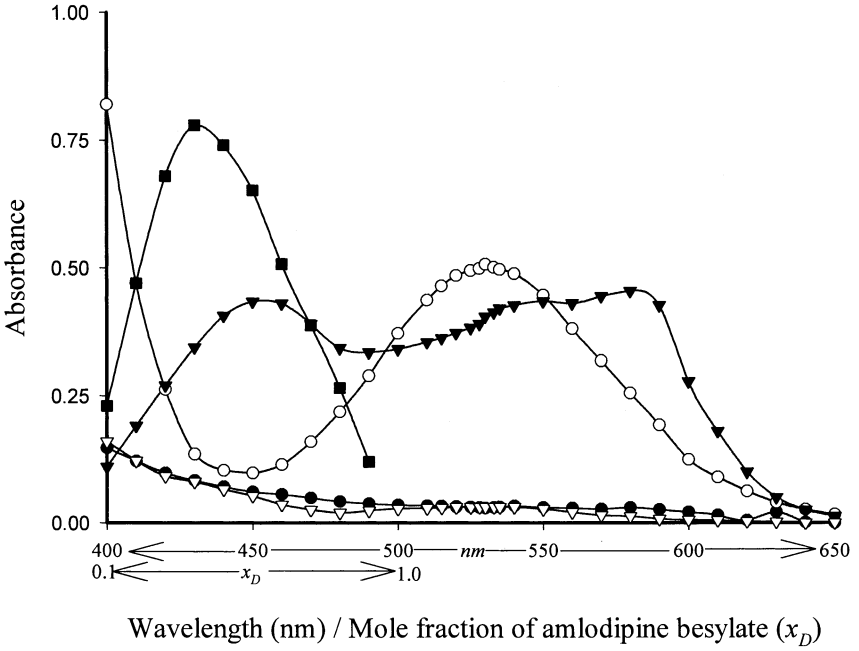


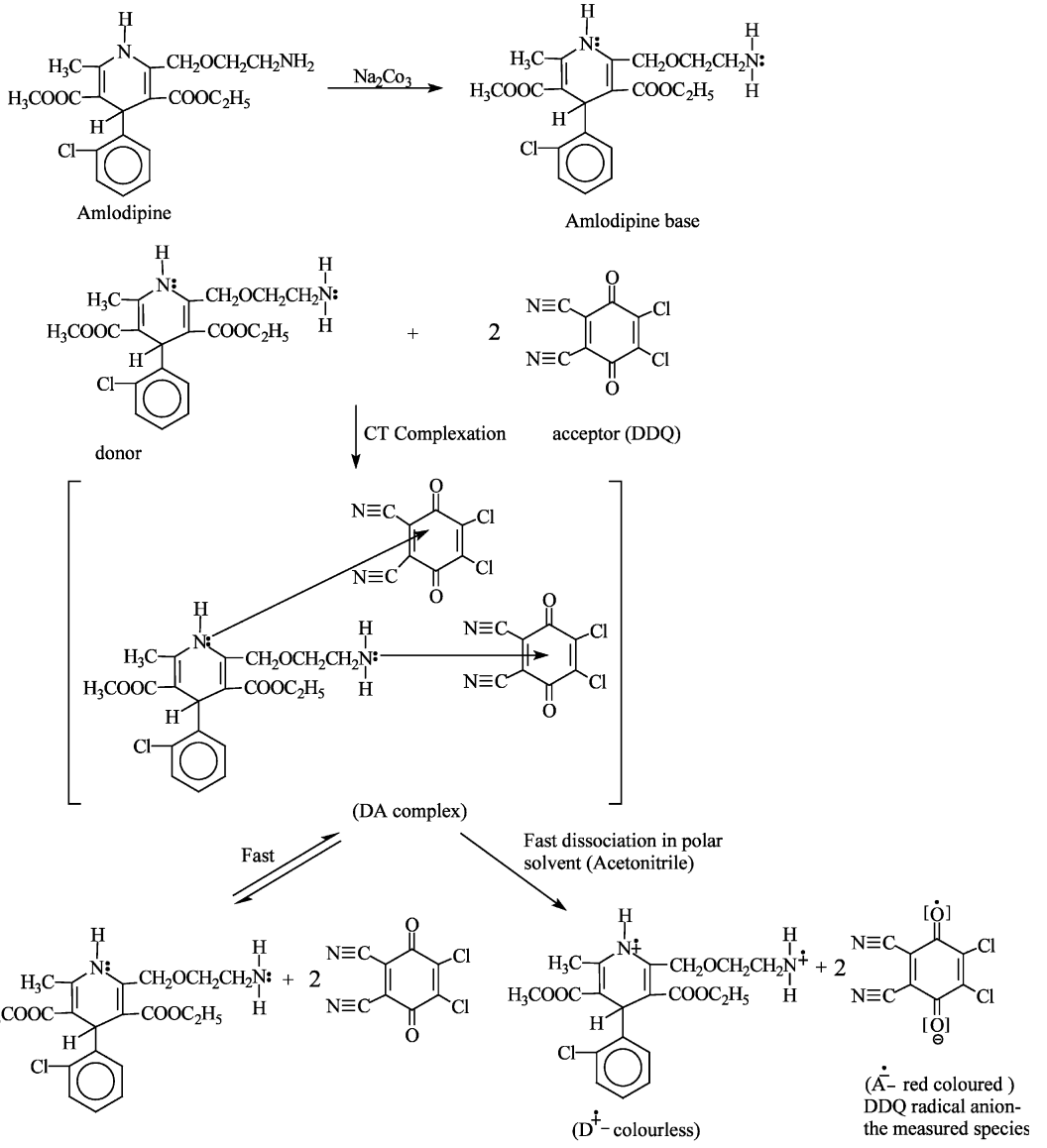
Fig. 1. Absorption spectra of the reaction products of amlodipine besylate with DDQ (▼) and ascorbic acid (○) and their respective reagent blanks. Job's plot for amlodipine–DDQ complex (■).

ment of sensitive and accurate methods for the determination of amlodipine besylate is desired.

Different methods for the quantification of amlodipine besylate have been reported which include high performance liquid chromatography [8–13], reversed phase high performance liquid chromatography [2,14–16], high performance thin layer chromatography [17–20], gas chromatography [21], gas chromatography–mass spectrometry [22], liquid chromatography with tandem mass spectrometry [23] and fluorimetry [24]. Though these methods are sensitive enough, they are expensive and not easily manageable. On the other hand, spectrophotometry is still the technique of choice since it is sensitive, economical, rapid and more easily manageable for third world countries.

Few spectrophotometric methods have been reported for the assay of amlodipine besylate based on extractable ion-pair complexes [25–29], oxidative coupling with 3-methyl 2-benzothiazolinone hydrazone hydrochloride [30], with sodium hydroxide [31], derivative spectroscopy [32,33], simultaneous multicomponent mode of analysis [34] and charge transfer complexation with *p*-chloranilic acid [35] and chloranil [36]. It has also been determined based on the reaction of $-NH_2$ group with ninhydrin in drug formulations [37].

A literature survey of charge transfer complexation reactions of polyhalo/polycyanoquinones with basic nitrogenous centres reveals that 2,3-dichloro 5,6-dicyano 1,4-benzoquionone (DDQ) is one of the sensitive reagents among them which acts as an electron acceptor and yields more sensitive results in comparison to other polyhaloquinones [38–41]. In 1964, Jaroslav Bartos introduced ascorbic acid as a sensitive and economical reagent for the detection and determination of primary amino group in *N*,*N*-dimethyformamide medium [42]. Since then the mechanism of this reaction has not been exactly elucidated yet, so not much attention has been paid to the use of ascorbic acid as an economical spectrophotometric reagent for the determination of amino group specially in pharmaceutical analysis.



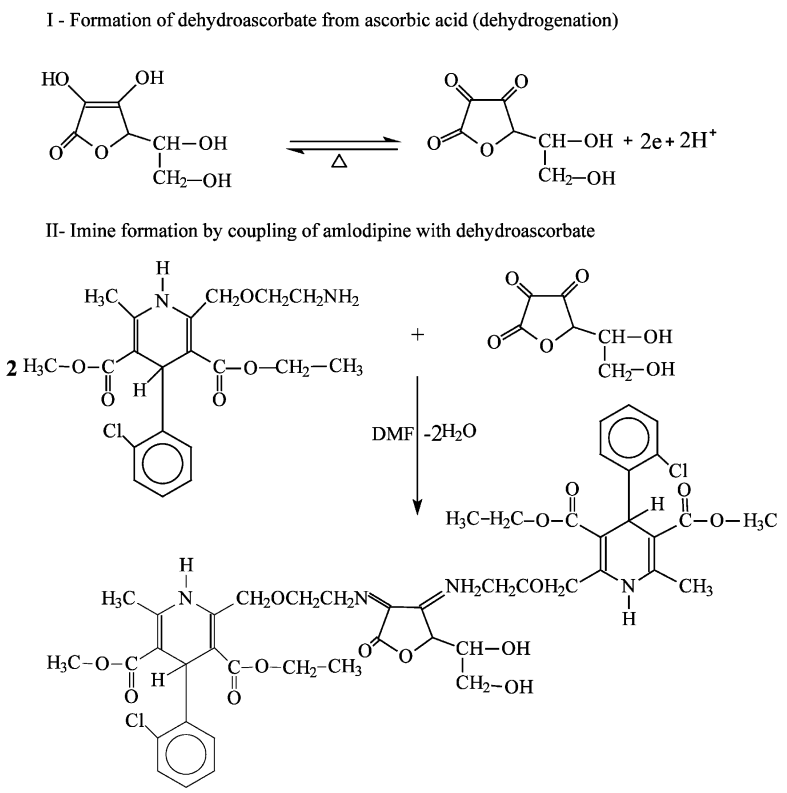
Scheme 1.

This paper describes two sensitive, fast, simple and economical methods for the determination of amlodipine besylate in pure and dosage forms. The first method is based on the charge transfer complexation reaction of amlodipine with DDQ. The second procedure utilizes the reaction of primary $-NH_2$ group of the drug with ascorbic acid in *N*,*N*-dimethylformamide medium. The proposed methods are validated statistically.

2. Experimental

2.1. Reagents and materials

All chemicals used were of AR-grade. Water was doubly distilled. A 0.1% solution of amlodipine besylate (Wockhardt Ltd., India) was prepared in chloroform. 0.1% solution of amlodipine besylate was also prepared in *N*,*N*-dimethylfor-



Scheme 2.

mamide (S.D. Fine Chem. Ltd., India). As reagents 0.05% DDQ (Fluka, Switzerland) solution in acetonitrile and 0.5 M aqueous sodium carbonate (E. Merck, India) solution were prepared for DDQ method. For the second method, 0.2% ascorbic acid was prepared by dissolving 100 mg of ascorbic acid (S.D. Fine) in 0.5 ml of water in a 50 ml standard flask and diluting to the volume with DMF.

2.2. Recommended procedure

2.2.1. DDQ method

2.2.1.1. Preparation of amlodipine base solution. In a 150 ml separatory funnel, 50 ml of 0.1% amlodipine besylate solution in chloroform was transferred followed by 75 ml of 0.5 M aqueous sodium carbonate solution. The content was mixed well and shaken for a few minutes. The organic layer was separated and dried over anhydrous sodium sulphate. A 25 ml portion of organic layer containing amlodipine base was evaporated to dryness and the residue was taken up with acetonitrile and transferred to 50 ml standard volumetric flask, diluting to volume. This corresponds to 0.05% amlodipine base solution.

2.2.1.2. Procedure for the determination. Aliquots of 0.05% amlodipine base corresponding to 1–125 μg ml^{-1} were transferred into a series of 5 ml volumetric flasks. 1.0 ml of 0.05% DDQ solution was added in each flask and diluted to volume with acetonitrile. The coloured product formed immediately and remained stable from 3 to 25 min. Therefore, the absorbances were measured within the stability period at 580 nm against the reagent blank prepared simultaneously.

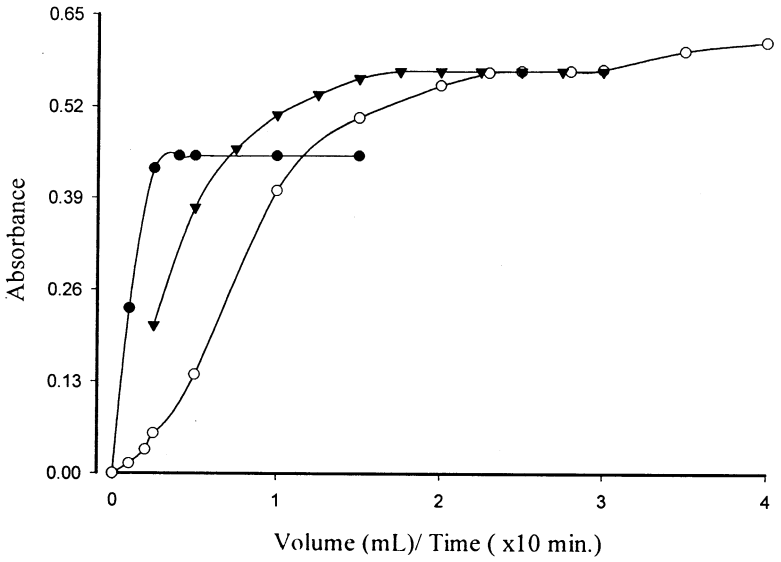


Fig. 2. Effect of the volume of 0.05% DDQ (●) and 0.2% ascorbic acid (▼) and the heating time (○).

2.2.2. Ascorbic acid method

Into a series of boiling tubes, aliquots of 0.1% amlodipine besylate solution in DMF (10–140 μg ml^{-1}) were pipetted. To each tube, 2.5 ml of 0.2% ascorbic acid solution was added. The total volume in each tube was maintained to 5 ml by adding DMF. The contents were mixed well and placed on a water bath maintained at 100±1 °C for 25 min. The solutions were cooled to room temperature. The reaction mixture and their corresponding washings were transferred and collected in a series of 10 ml volumetric flasks. They were diluted to volume with DMF. The absorbances were measured within the stability period of 4 h at 530 nm against the reagent blank treated similarly.

2.2.2.1. Analysis of pharmaceutical formulations. Ten tablets (claiming for 10 mg of amlodipine besylate per tablet) were finely powdered and extracted into sufficient volume of chloroform with shaking. The residue was filtered on whatmann filter paper no. 42 and the filtrate was diluted to 100 ml with chloroform. The solution of amlodipine base was prepared as discussed above and the drug was analyzed following the recommended procedure using DDQ as the reagent.

An accurately weighed portion of powdered tablets equivalent to 100 mg of amlodipine besylate was stirred with sufficient volume of DMF and left standing for 10 min. The residue was filtered on whatmann filter paper no. 42 and washed well with DMF. The filtrate and washings were diluted to volume in a 100 ml volumetric flask. The assay was completed following the recommended procedure using ascorbic acid as reagent.

3. Results and discussion

3.1. Reaction mechanism and IR studies

The molecular interactions between electron donors and acceptors are generally associated with the formation of intensely coloured charge transfer complexes or radical anions depending on the polarity of the solvent used. DDQ is a π-acceptor which readily forms charge transfer complexes with basic nitrogenous compounds as n-donors [38–41]. Some salts of amines do not react with σ- or π-acceptors because of non-

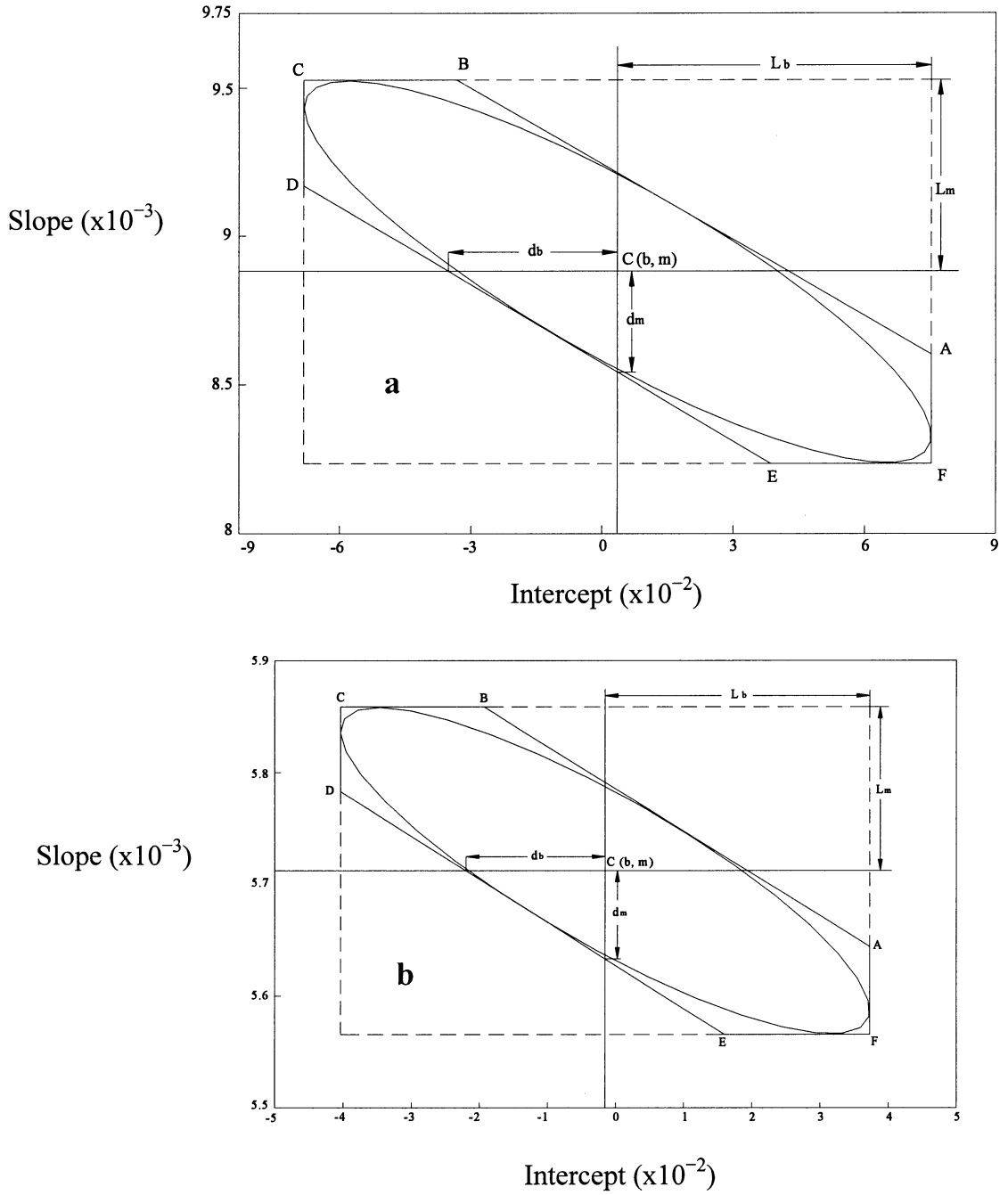


Fig. 3. Joint confidence region ($P = 0.05$) for the slope and intercept of regression line of DDQ method (a). Joint confidence region ($P = 0.05$) for the slope and intercept of regression line of ascorbic acid method (b).

availability of lone pair of electrons. In a similar manner, amlodipine besylate does not react with DDQ. In order to determine amlodipine besylate, the drug was dissolved in chloroform and shaken with a 0.5 M aqueous sodium carbonate solution. This treatment yielded amlodipine base in chloroform layer and evaporated to dryness. The residue was taken up in acetonitrile, a more polar solvent. The amlodipine base acts as an n-donor to form reddish violet coloured charge transfer complex with DDQ showing absorption maxima at 435, 550 and 580 nm (Fig. 1). These bands may be attributed to the formation of DDQ radical anions, which probably resulted through the dissociation of the donor–acceptor complex in a highly polar solvent like acetonitrile. In order to avoid the maximum interference from the reagent blank, the absorption band at λ_{max} 580 nm was chosen for analytical studies. The Job's plot (Fig. 1) suggested a donor to acceptor ratio of 1:2, confirming the presence of two n-donating centres in the amlodipine base molecule [43]. On the basis of the literature background and our experimental observations, a reaction mechanism is proposed and given in Scheme 1.

A purple red coloured product is obtained on heating amlodipine besylate with ascorbic acid in DMF, which absorbed maximally at 530 nm (Fig. 1). On heating in a water-bath, the oxidation of ascorbic acid occurs mainly due to the formation of dehydroascorbic acid [44]. The carbonyl group further reacts with $-NH_2$ group of amlodipine to form a purple red coloured imine. The IR spectrum of amlodipine besylate displayed a band in the region 3120–2950 cm^{-1} attributed to N^+–H stretching mode. A sharp band at 1697 cm^{-1} may be due to C=O stretching vibration. The aromatic nature of the drug is characterized by the bands appearing in the region 1610–1450 cm^{-1}. The band at 1303 cm^{-1} and other bands in the region 1200–1000 cm^{-1} may be assigned to C–N (ring) and C–N (aliphatic amine) stretching vibrations [45]. The IR spectrum of the reaction product exhibits a broad band in the region 3600–3300 cm^{-1} which may be attributed to –OH and –CH stretching vibrations whereas N^+–H stretching mode disappeared. Another sharp and strong band at 1670 cm^{-1} suggested the formation of

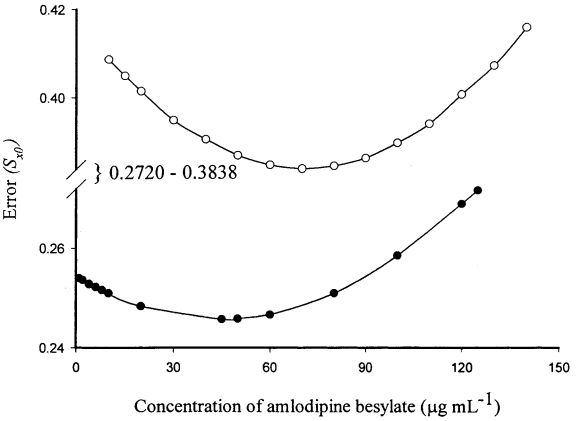


Fig. 4. Plot of error in the determination of the concentration of amlodipine besylate by DDQ method (●) and ascorbic acid method (○).

C=N group. The C=O stretching vibration is also shifted to a lower value and may appear in the C=N group region. The IR spectrum of the product has also indicated the OH deformation vibrations and the presence of C–O–C stretching appearing at 1256 and 1102 cm^{-1}, respectively. Thus the comparative study of the IR spectra of amlodipine besylate and the reaction product suggested an imine formation. The reaction mechanism is proposed in Scheme 2.

3.2. Optimization of variables

In DDQ method, the only effective variable is the concentration of DDQ since the reaction gets stabilized within 3 min and remains unaffected for a further 20 min. To study the effect of the concentration of DDQ, varying volumes of 0.05% reagent was mixed with 0.5 ml of drug in a 5 ml standard flask and diluted to volume with acetonitrile. The absorbance was measured after 3 min of mixing at 580 nm against the reagent blank. It was found that 0.5 ml of the reagent gave the highest absorbance (Fig. 2); above this volume the absorbance remains constant. A volume of 1.0 ml was, therefore, used in all further measurements.

To optimize heating time for ascorbic acid method, 1.0 ml of 0.1% amlodipine besylate was mixed with 2.5 ml of 0.2% ascorbic acid and heated at 100 ± 1 °C. The absorbance was mea-

Table 1
Evaluation of the accuracy and precision of the two proposed procedures

Proposed methods	Amount taken (mg ml^{-1})	Amount found (mg ml^{-1}) ± S.D.[a]	RSD (%)[a]	SAE[b]	Confidence limit[c]
DDQ	10	10.01 ± 0.06	0.64	0.03	0.061
	60	60.00 ± 0.31	0.51	0.14	0.297
	100	100.03 ± 0.54	0.54	0.24	0.516
Ascorbic acid	20	20.00 ± 0.23	1.14	0.10	0.216
	80	80.03 ± 0.46	0.57	0.20	0.434
	100	100.05 ± 0.51	0.51	0.23	0.490

[a] Mean ± S.D. for five determinations.
[b] SAE, standard analytical error.
[c] Confidence limit at 95% confidence level and four degrees of freedom ($t = 2.132$) [51].

sured at 530 nm against the reagent blank as a function of heating time. The results (Fig. 2) show that the absorbance remains constant between 22 and 32 min of heating. There is an abrupt change in the absorbance above 32 min of heating and therefore, 25 min of heating time was used throughout the experiment.

In order to study the effect of volume of reagent on the absorbance, varying volume of 0.2% ascorbic acid was mixed with 1.0 ml of 0.1% amlodipine besylate in different boiling tubes and the contents were heated on the water bath at 100 ± 1 °C for 25 min. The highest absorbance was obtained with 1.75 ml of the reagent (Fig. 2); above which the absorbance remains unaffected. 2.5 ml of the reagent was taken as optimum value.

3.3. Analytical data

Under the optimum experimental conditions, linear calibration graphs were obtained over the concentration ranges 1–125 and 10–140 μg ml^{-1} of amlodipine besylate with molar absorptivities of 0.60×10^4 and 0.32×10^4 l mol^{-1} cm^{-1} using DDQ and ascorbic acid, respectively. The calibration data were fitted by least square treatment and the regression equations obtained for DDQ and ascorbic acid methods were $A = 3.54 \times 10^{-3} + 8.88 \times 10^{-3}C$ and $A = -1.55 \times 10^{-3} + 5.71 \times 10^{-3}C$, respectively. In each case, the correlation coefficient was found to be 0.9999, indicating the good linearity of both the calibration graphs and the intercepts are all close to zero. The confidence intervals of intercepts at 95% confidence level were calculated (1.45×10^{-3} and 1.90×10^{-3} for DDQ and ascorbic acid methods, respectively) which confirmed that these are not different from zero. Thus the present methods are free from constant errors independent of the concentration of amlodipine besylate. There is also strong correlation existing between the slope and intercept. In order to judge the reliability of strong correlation of these parameters, more rigorous treatment of calibration data was made to draw a joint confidence region (Fig. 3a and b) following the method of Mandel and Linnig [46]. The joint confidence region for slope and intercept is a tiltless ellipse having the point of best fit as its centre. It is evident from Fig. 3a and b that the points for which intercept is zero fall well within the ellipse.

The variance was calculated using the equation $S_o^2 = \Sigma(A_{exp} - A_{calc.})^2/n - 2$ [47] and found to be 4.44×10^{-6} and 4.50×10^{-5} for DDQ and ascorbic acid methods, respectively. The small values of variance obtained for both the methods indicated negligible scattering of the experimental data points from the line of best fit. The values of correlation coefficients were not sufficient enough to evaluate the linearity of the calibration graphs. The linearity was evaluated by the percent relative standard deviation of the slope ($S_{b\ rel}$%) [48]. The values were found to be 0.11 and 0.18 for DDQ and ascorbic acid methods, respectively, which indicated better linearity of the former method.

The statistical analysis of the calibration data also allows the calculation of error (S_{xo}) in the determination of a given concentration of amlodipine besylate and may be helpful to establish the

Table 2
Standard addition method for the determination of amlodipine besylate in dosage forms

Formulation name	DDQ method						Ascorbic acid method					
	Amount (μg ml^{-1})			Recovery (%) ± RSD (%)[a]	SAE[b]	Confidence limit[c]	Amount (μg ml^{-1})			Recovery (%) ± RSD (%)[a]	SAE[b]	Confidence limit[c]
	Taken	Added	Found ± S.D.[a]				Taken	Added	Found ± S.D.[a]			
Amdepin-10	25	25	50.05 ± 0.26	100.10 ± 0.53	0.12	0.251	15	15	30.03 ± 0.29	100.17 ± 0.98	0.13	0.280
	35	35	70.01 ± 0.32	100.01 ± 0.45	0.14	0.303	45	45	89.83 ± 0.49	99.81 ± 0.55	0.22	0.468
Amlogard-10	25	25	50.06 ± 0.25	100.12 ± 0.49	0.11	0.234	15	15	30.10 ± 0.29	100.33 ± 0.95	0.13	0.274
	35	35	70.01 ± 0.38	100.03 ± 0.54	0.17	0.359	45	45	90.08 ± 0.51	100.09 ± 0.56	0.23	0.486
Amlong-10	25	25	49.75 ± 0.30	99.50 ± 0.60	0.13	0.284	15	15	29.96 ± 0.31	99.87 ± 1.05	0.14	0.299
	35	35	69.54 ± 0.34	99.34 ± 0.49	0.15	0.328	45	45	89.86 ± 0.47	99.84 ± 0.52	0.21	0.446
Amlopin-10	25	25	50.03 ± 0.29	100.06 ± 0.59	0.13	0.281	15	15	30.00 ± 0.31	100.01 ± 1.02	0.14	0.293
	35	35	69.85 ± 0.36	99.79 ± 0.52	0.16	0.346	45	45	89.97 ± 0.45	99.97 ± 0.49	0.20	0.424
Amlopres-10	25	25	49.93 ± 0.31	99.86 ± 0.62	0.14	0.294	15	15	29.98 ± 0.27	99.93 ± 0.91	0.12	0.260
	35	35	69.97 ± 0.37	99.96 ± 1.53	0.17	0.351	45	45	89.91 ± 0.48	99.90 ± 0.53	0.21	0.453
Myodura	25	25	50.08 ± 0.29	100.16 ± 0.58	0.13	0.275	15	15	30.01 ± 0.28	100.03 ± 0.95	0.13	0.271
	35	35	70.14 ± 0.33	100.20 ± 0.46	0.15	0.312	45	45	90.05 ± 0.44	100.05 ± 0.49	0.20	0.419

[a] Mean ± S.D. for five determinations.
[b] SAE, standard analytical error
[c] Confidence limit at 95% confidence level and four degrees of freedom ($t = 2.132$) [51].

Table 3
Comparison of the two proposed methods with the reference method [30]

Pharmaceutical preparations	Labelled amount (mg)	DDQ method				Ascorbic acid method				Reference method	
		Recovery (%)[a]	RSD (%)[a]	*t*-value[b]	*F*-value[b]	Recovery (%)[a]	RSD (%)[a]	*t*-value[b]	*F*-value[b]	Recovery (%)[a]	RSD (%)[a]
Amdepin	10	100.13	0.73	0.0404	4.51	99.90	0.59	0.7370	2.93	100.11	0.34
Amlogard	10	100.20	0.67	0.2952	4.20	99.98	0.57	0.3664	2.98	100.09	0.33
Amlong	10	99.95	0.32	0.0614	1.85	99.98	0.36	0.0351	2.41	99.98	0.24
Amlopin	10	99.96	0.38	0.0434	1.11	99.72	0.84	0.6133	4.46	99.97	0.40
Amlopres	10	99.98	0.51	0.5369	1.22	99.75	0.95	0.8667	2.81	100.14	0.55
Myodura	10	100.19	0.68	0.4451	2.72	99.80	0.67	0.6463	2.60	100.03	0.41

[a] Average of five independent analyses.
[b] Theoretical *t*-value (DOF = 8) and *F*-value (DOF = 4,4) at 95% confidence level are 1.860 and 6.39, respectively [51].

confidence limits at the selected levels of confidence in the determination of unknown concentrations. These parameters were evaluated by using the following formula [49].

$$S_{xo} = \frac{S_{y/x}}{b}\left[1 + \frac{1}{n} + \frac{(y_0 - \bar{y})^2}{b^2 \sum_i (x_i - \bar{x})^2}\right]^{1/2} \quad (1)$$

where $\bar{y}$ and $\bar{x}$ are the average absorbance and concentration values, respectively for n standard specimens. Fig. 4 shows the graphs of S_{xo} versus concentration of amlodipine besylate. The error is minimum when the actual absorbance is equal to the average absorbance, which corresponds to about 45 and 70 μg ml^{-1} for DDQ and ascorbic acid methods, respectively. The accuracy and precision of the proposed methods were evaluated by the repeated analyses at three different concentration levels. The results are summarized in Table 1. The standard deviations, relative standard deviations and standard analytical errors [50] can be considered to be very satisfactory.

As an additional demonstration of accuracy, recovery experiments were performed by adding a known amount of amlodipine besylate to the preanalyzed dosage forms. The results showed (Table 2) that the mean recoveries were in the range of 99.34–100.20%. No interference from the common excipients was observed.

The methods were successfully applied to the determination of amlodipine besylate in pharmaceutical formulations. The results of the proposed method (DDQ or ascorbic acid) were compared with those of the reference method [30]. Table 3 shows that the calculated *t*- and *F*-values are less than the theoretical ones, confirming accuracy and precision at 95% confidence level.

Under the experimental conditions described, the linearity and sensitivity were the best with the charge transfer complex formation procedure. Both the proposed spectrophotometric methods are simple, sensitive and reproducible. Moreover, these procedures are likely to be very suitable for the routine analysis of amlodipine besylate in dosage forms.

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