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Titrimetric and Modified Spectrophotometric Methods for the Determination of Amlodipine Besylate Using Bromate-Bromide Mixture and Two Dyes

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ABSTRACT: One direct titrimetric method and two indirect spectrophotometric methods for the determination of amlodipine besylate (ADB) are described. The methods use bromate-bromide mixture and two-dyes, namely Methyl Orange and Indigo Carmine, as reagents. In titrimetry (Method A), an acidified solution of ADB is titrated directly with bromate-bromide mixture using methyl orange as an indicator. Spectrophotometry involves the addition of known excess of bromate-bromide mixture to an acidified solution of ADB and the determination of the residual bromine based on its ability to bleach the dyes Methyl Orange (Method B) or Indigo Carmine (Method C) quantitatively. Titrimetry allows the determination of 1-10 mg of ADB, whereas spectrophotometry is applicable over the concentration ranges of 0.5-3.0 μg ml^{-1} and 1.25-12.50 μg ml^{-1} for method B, and method C, respectively. Method B with a calculated molar absorptivity of 6.56×10^4 l mol^{-1} cm^{-1}, is the most sensitive method ever developed for ADB. The limits of detection and quantification are reported for both the spectrophotometric methods. The methods described could usefully be applied to routine quality control of tablets containing ADB. No interference was observed from common pharmaceutical adjuvants. Statistical comparison of the results with the reference method shows an excellent agreement, and indicates no significant difference in accuracy and precision. The reliability of the methods has been ascertained by recovery studies.

KEYWORDS: Amlodipine besylate, determination, bromate-bromide, methyl orange, indigo carmine, pharmaceuticals.

INTRODUCTION

Amlodipine besylate (ADB) is 2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl), 1,4-dihydroxy-6-methyl-3,5- pyridine dicarboxylic acid, 3-ethyl-5-methyl ester[1] (Fig. 1). ADB is a calcium channel blocker with a vasodilatory activity similar to that of nifedipine and is mainly used for its antianginal, antihypertensive and antiarrhythmic activities[2-4]. ADB in biological fluids has been determined mostly by chromatographic techniques such as gas chromatography,[5] liquid chromatography-tandem mass spectrometry (LC-TMS)[6-9], high performance liquid chromatography (HPLC)[10-13] and high performance thin layer chromatography (HPTLC)[14]. A limited number of methods have been reported for the quantitation of this drug when present alone in dosage forms. Methods based on several techniques such as LC-TMS[15], HPLC[16-18], HPTLC[19-21], packed column super critical fluid chromatography[22], micellar electrokinetic chromatography[23], UV spectrophotometry[19,24], differential spectrophotometry[25] fluorimetry[26] and voltammetry,[27] are found in the literature for the determination of ADB in pure form and in tablets. Despite being one of the most widely used antihypertensive, antiangial and coronary vasodilator drugs, no titrimetric method has been developed for ADB and even a few visible spectrophotometric methods reported suffer from one or the other disadvantage.

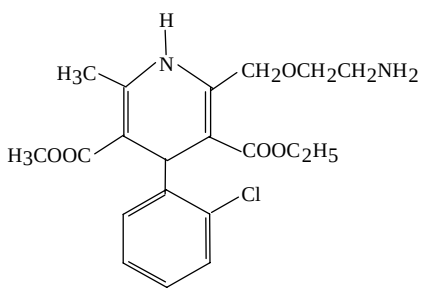


Fig 1. Structure of ADB.

Spectrophotometric methods based on ion-pair formation and using Bromothymol Blue[28], Bromocresol

Green, Bromophenol Blue and Methylene Blue[29], Rhodizonic acid[30], Fast Green FCF[31] and trinitrobenzene sulphonic acid[32] as ion-pair agents, have been reported by several authors, but the procedures involve extraction step and are less sensitive (ε = ~10^3 l mol^{-1} cm^{-1}). Procedures based on charge-transfer complex formation using chloranilic acid[33] and tetrachloro quinone[34] as reagents are also less sensitive, besides requiring a heating step[34] for a quantitative reaction. Iskender and Sagirli[35] have developed a method based on the derivatization reaction involving the amino group of the drug molecule and 1,2-napthaquinone-4-sulphonic acid. However, their procedure involves heating at 50° C for 20 minutes and is also poorly sensitive with the concentration range of applicability being 10-80 μgml^{-1}. Redox reaction using Folin-Cioealteu reagent[36] and oxidative coupling reaction[28] using 3-methyl-2-benzothiazolinone hydrazone hydrochloride in the presence of cerium (IV) have also been the basis of spectro photometric methods for the assay of ADB in pharmaceuticals. The yellow product obtained by treating ADB with NaOH in dimethyl formamide medium was used by Meyyanathan et al.[37] for the determination of the drug in the range of 20-100 μg ml^{-1}. Rahman and Hoda[38] have proposed two methods based on the use of dichlorodicyano benzoquinone (DDBQ) and ascorbic acid in dimethyl formamide medium. The methods are less sensitive and the coloured species (DDBQ radical anion) is less stable. In addition, the procedure using ascorbic acid involves a boiling step at 100° C for 25 min. The same group of authors[39] have recently reported three procedures based on redox and complexation reactions, and using iron(III) and ammonium molybdate as oxidants and orthophenanthroline and bipyridyl as chelating agents. The procedures are sensitive enough (ε = 10^4 l mol^{-1} cm^{-1}) but involve a heating step to force the reaction to completion. Very recently[40], bromate-bromide mixture and metanil yellow have been employed for the sensitive spectrophotometric determination of ADB.

Bromate-bromide mixture along with dyes such as Methyl Orange and Indigo Carmine have been used as reagents for the titrimetric and spectrophotometric determination of several pharmaceuticals[41-46]. In this paper, simple and sensitive titrimetric and spectrophotometric methods are described for the determination of ADB in pure drug and in tablets using this combination of reagents.

Materials and Methods

Apparatus

All absorbance measurements were made using a Systronics model 106 digital spectrophotometer provided with matched 1-cm quartz cells.

Reagents and Solutions

All chemicals used were of analytical reagent grade and solutions were made in double distilled water.

Bromate-bromide mixture (2.5 mM $KBrO_3$-25 mM KBr): The bromate-bromide mixture was prepared by dissolving 0.4175 g of $KBrO_3$ (Sarabhai M. Chemicals, Baroda, India) and 3 g of KBr (Qualigens Fine Chem, Mumbai, India) in water and diluting to one liter in a calibrated flask, and used for the titrimetric work. The solution was diluted appropriately to obtain 10 μg ml^{-1} and 30 μg ml^{-1} solutions with respect to $KBrO_3$ for use in the spectrophotometric methods B and C, respectively.

Methyl Orange indicator (0.5%): 50 mg of dye (S.d. Fine Chem., India) was dissolved in 10 ml of water.

Methyl Orange solution (50 μg ml^{-1}): A 500 μg ml^{-1} stock solution was first prepared by dissolving 52.4 mg of dye (S.d. Fine Chem., Mumbai, India; dye content 85 %) in water and diluting to 100 ml in a calibrated flask, and filtered. The filtrate was appropriately diluted to get a 50 μg ml^{-1} dye solution for use in the spectrophotometric method B.

Indigo Carmine solution (100 μg ml^{-1}): A 1000 μg ml^{-1} dye solution was prepared by dissolving 112 mg of dye (S.d. Fine Chem. Mubai, India; 90 % dye content) in water and diluting to 100 ml with water in a calibrated flask, and filtered. The filtrate was diluted 10-fold with water to get a 100 μg ml^{-1} solution for use in the spectrophotometric method C.

Hydrochloric acid (5 M): 111 ml of concentrated hydrochloric acid (Qualigens Fine Chem. Mumbai, India, Sp. gr. 1.18) was diluted to 250 ml with water, and used in spectrophotometric studies. This was further diluted to get 2 M acid for use in the titrimetric investigations.

Standard Drug Solution

Pharmaceutical grade ADB was kindly provided by Cipla India Ltd., Mumbai, India and was used as received. A stock standard solution containing 1 mg ml^{-1} ADB was prepared by dissolving 250 mg of pure drug in 15 ml of glacial acetic acid and diluting to the mark in a 250 ml calibrated flask with water, and was used in the titrimetric assays. The stock solution (1000 μg ml^{-1} ADB) was diluted stepwise to obtain working concentrations of 10 μg ml^{-1} and 25 μg ml^{-1} for use in the spectrophotometric determination by methods B and C, respectively.

Sample Solution

Twenty tablets were weighed and ground into a fine powder. An amount of powder equivalent to 100 mg of ADB was transferred into a 100 ml calibrated flask, 20 ml of glacial acetic acid added and shaken for 20 min. Then, the volume was diluted to the mark with water, mixed well and filtered using a Whatman No 42 filter paper. The filtrate equivalent to 1000 μg ml^{-1} ADB was diluted appropriately with water for assay by the spectrophotometric methods.

General Procedures

Titrimetry (Method A): A 10 ml aliquot of pure drug solution containing 1-10 mg of ADB was accurately measured into a 100 ml titration flask. Five ml of 2 M HCl was added, and titrated with 2.5 mM bromate-bromide mixture using 2 drops of Methyl Orange an indicator until the indicator colour disappeared. A blank titration was run and the volume of bromate was subtracted from the volume required for sample titration. The amount of ADB in the measured aliquot was calculated from:

$$\text{Amount (mg)} = VM_wR$$

where

V =volume of bromate-bromide solution consumed, ml

M_W = relative molecular mass of drug, and

R = molarity of bromate-bromide mixture with respect to $KBrO_3$.

Spectrophotometric method B: Varying aliquots (0.5-3.0 ml) of 10 μg ml^{-1} ADB solution were accurately measured into different 10 ml calibrated flasks and the total volume was adjusted to 3 ml by adding water. To each flask were added 1 ml each of the bromate-bromide mixture (10 μg ml^{-1} with respect to $KBrO_3$) and 5 M HCl. The content was mixed well after stoppering the flasks and let stand for 15 minutes with occasional shaking. Then, 1 ml of 50 μg ml^{-1} Methyl Orange solution was added to each flask and diluted to the mark with water. The absorbance of solution was measured at 520 nm against a reagent blank after 5 minutes.

Spectrophotometric method C: Different aliquots (0.5-5.0 ml) of standard 25 μg ml^{-1} ADB solution were taken in a series of 10 ml calibrated flasks, water was added to bring the total volume to 5 ml. To each flask was then added 1 ml of 5 M HCl followed by 1.5 ml of the bromate-bromide mixture (30 μg ml^{-1} with respect to $KBrO_3$). After stoppering the flasks, the content was mixed well and let stand for 15 minutes with occasional shaking. Finally, 2 ml of 100 μg ml^{-1} Indigo Carmine solution was added to each flask and the volume was diluted to the mark with water and mixed well. The absorbance of each solution was measured at 610 nm against a reagent blank after 5 minutes.

In methods B and C, the measured absorbance was plotted against the concentration of ADB to obtain the calibration graphs. The concentration of the unknown was read from the respective calibration graph or computed from the respective regression equation, derived from the Beer's law data.

Convenient aliquots of tablet extract were taken for analysis by titrimetry (1 mg ml^{-1}) and spectrophotometric method B (10 μg ml^{-1}) and method C (25 μg ml^{-1}) applying the procedures described above.

Results and Discussion

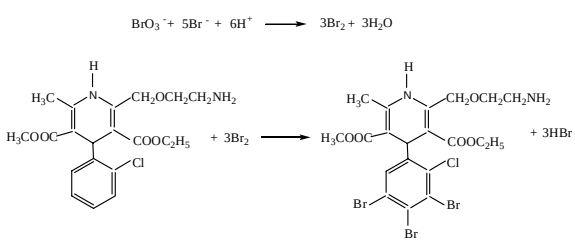
Preliminary experiments revealed that ADB is prone to substitution reaction by bromine-generated *in situ* by the action of acid on bromate-bromide mixture. In titrimetry, the reaction is followed by direct titration with the bromate-bromide mixture using Methyl Orange as an indicator. In spectrophotometry, the reaction is followed by measuring the increase in absorbance of either Methyl Orange at 520 nm or Indigo Carmine at 610 nm, as the absorbance change is caused by bleaching action of bromine on the dyes.

Method Development

Optimization of reaction conditions

Experimental variables, such as the choice of acid medium, acid concentration, and standing time, were optimized.

Titrimetry: The quantitative nature of the reaction between ADB and bromate was checked by titrating 1-10 mg of drug in acid medium with the bromate-bromide mixture (2.5 mM with respect to $KBrO_3$). The



stoichiometry of ADB : $KBrO_3$ was calculated to be 1:1. The reaction between ADB and *in situ* generated bromine could be represented by the above reaction scheme:

The titration was carried out in hydrochloric acid medium, producing a very sharp end point with Methyl Orange. The reaction stoichiometry was found to be unaffected when 4-6 ml of 2 M HCl was used in a total volume of 20-25 ml. Using 2.5 mM bromate solution

and in the presence of a large excess of bromide, 1-10 mg of ADB could be determined with a fair degree of accuracy and precision. The linearity between the drug amount and end point is apparent from the correlation coefficient of 0.9984 obtained by the line of best fit via least squares treatment.

Spectrophotometry: Many dyes are prone to oxidation to form colourless products in acid medium,[47] thus offering a suitable analytical approach for the indirect assay of oxidisable pharmaceuticals by using various oxidizing agents[48-54] including *in situ* generated bromine[41-46]. Gyory[55] used Methyl Orange as an indicator in the direct titration with the bromate-bromide mixture, as the dye was irreversibly bleached at the end point. Quantitative decolouration of azo dyes by bromine is widely used for its spectrophotometric determination[56]. In the present investigation, the reaction between bromine and Methyl Orange or Indigo Carmine was used for the indirect spectrophotometric determination of ADB. In the proposed methods, different amounts of ADB were reacted with a fixed and excess amount of the bromate-bromide mixture in HCl medium, and the unreacted bromine was determined by treating with a fixed amount of Methyl Orange or Indigo Carmine and measuring the absorbance either at 520 nm or at 610 nm, respectively. The absorbance was found to be linearly dependent on the ADB concentration.

ADB, when added in increasing amounts to a fixed amount of *in situ* generated bromine, consumes the latter and there will be a concomitant decrease in the latter's concentration. When a fixed amount of either dye is added to decreasing amounts of bromine, a concomitant increase in the dye concentration results. This is observed as a proportional increase in absorbance at the respective wavelengths of maximum absorption with increasing concentration of ADB (Fig. 2 & 3).

Preliminary experiments were performed to fix the upper limits of the two dyes that could be spectrophotometrically determined in acid medium, and these were found to be 5 μg ml^{-1} and 20 μg ml^{-1} for Methyl Orange and Indigo Carmine, respectively. A bromate concentration of 1 μg ml^{-1} in the presence of a large excess of bromide was found to bleach the red colour of 5 μg ml^{-1} Methyl Orange, and the blue colour due to 20 μg ml^{-1} Indigo Carmine was completely destroyed by 4.5 μg ml^{-1} bromate in the presence of bromide. Hence, different amounts of ADB were reacted with 1 ml of 10 μg ml^{-1} bromate in method B (Methyl Orange) and 1.5 ml of 30 μg ml^{-1} bromate in method C (Indigo Carmine) in acid medium and in the presence of a large excess of bromide, followed by determination of residual bromine as described under procedures B and C.

Hydrochloric acid was found to be the convenient medium for bromination of drug by *in situ* generated bromine, and the determination of the latter using the two dyes. The absorbance of the dyes was not affected in 0.125-1.25 M HCl. However, since 1.0 M acid concentration was found to be optimal for the bromination reaction in a reasonable time of 5 and 10 min in methods B and C, respectively, the same concentration was maintained for the determination of the unreacted bromine with the dyes, and even this contact time is not critical. Any delay up to 30 minutes had no effect on the absorbance. A 5 min standing time was found to be necessary for the complete bleaching of the dye colour by the residual bromine. Though the colour of either dye in acid medium is indefinitely stable, it was found to be stable for several days in the presence of the reaction product.

Quantitative parameters of spectrophotometric methods

A linear correlation was found between absorbance at λ_{max} and the concentration ranges given in Table 1. The correlation coefficients, intercepts and slopes for the calibration plots are also presented in Table 1. The graphs showed negligible intercept as described by the regression equation:

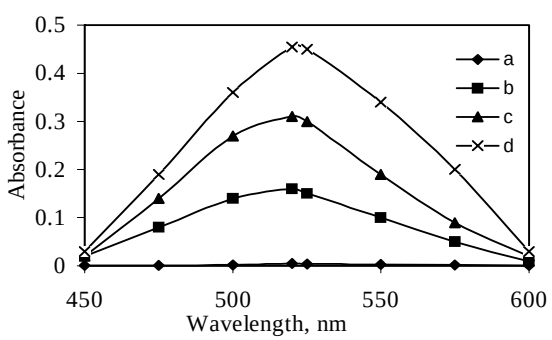


Fig 2. Absorption spectra of Method B: a. reagent blank, b, c and d are after addition of 1.0, 2.0 and 3.0 μg ml^{-1} ADB.

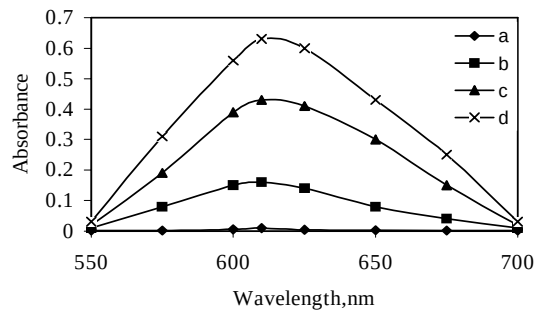


Fig 3. Absorption spectra of Method C: a. reagent blank, b, c and d are after addition of 2.5, 7.5 and 12.5 μg ml^{-1} ADB.

Table 1. Quantitative parameters of spectrophotometric methods.

Parameter	Method B	Method C
λ_{max}, nm	520	610
Beer's law limits, μg ml^{-1}	0.5 – 3.0	1.25 – 12.5
Molar absorptivity, l mol^{-1} cm^{-1}	6.56 x 10^4	1.86 x 10^4
Sandell sensitivity, ng cm^{-2}	6.23	22.0
Limit of detection, μg ml^{-1}	0.06	0.18
Limit of quantification, μg ml^{-1}	0.19	0.63
Regression equation (A*)		
Slope (b)	0.15	0.052
Intercept (a)	0.013	-0.037
Correlation coefficient (r)	0.9995	0.9934

* A = a + bC, where A is the absorbance and C concentration in μg ml^{-1}.

$$A = a + b\,C$$

(where A is the absorbance; a, y-intercept; b, slope and C, concentration in μg ml^{-1}), which was obtained by the method of least squares. Other sensitivity parameters, such as molar absorptivity, Sandell sensitivity, detection as well as quantification limits, are also compiled in Table 1 and are indicative of the high sensitivity of the methods.

Method Validation

Accuracy and precision of methods: The accuracy of the proposed methods was established by analyzing the pure drug at three different levels, and the precision was evaluated by calculating the relative standard deviation (RSD) of seven replicate determinations using the same solution containing pure drug at three different levels. The relative error (%) and percent RSD values summarized in Table 2 reveal the high accuracy and precision of the methods. For a better picture of reproducibility on a day-to-day basis, a series was run, in which the standard drug solution at three levels was analyzed each for five days. The day-to-day RSD values were in the range of 1.34-2.75%, and represented the best approval of the methods in routine use.

Determination of ADB in tablets: Thirty-six brands of tablets in 2.5 mg, 5.0 mg and 10 mg doses are commercially available in the local market. The proposed methods were applied to assay ADB in some representative tablets, and the results are compiled in Table 3. The drug content of the same batch tablets was checked by the reported method according to Sane *et al*[19]. It is clear from Table 3 that there is a close agreement

Table 2. Accuracy and precision of methods.

Method A				Method B				Method C			
ADB taken, mg	ADB found*, mg	RE, %	RSD %	ADB taken, μg	ADB found*, μg	RE, %	RSD %	ADB taken, μg	ADB found*, μg	RE, %	RSD %
3.0	3.07	2.33	0.85	10.0	10.12	1.20	0.62	30.0	29.46	1.80	1.26
6.0	5.92	1.33	0.33	20.0	20.16	0.80	0.46	60.0	60.14	0.23	0.74
9.0	8.85	1.88	1.28	30.0	30.42	1.40	1.35	90.0	89.04	1.06	0.94

RE – Relative error.
RSD – Relative Standard Deviation.
* Mean value of seven determinations.

Table 3. Results of array of tablets containing ADB by the proposed methods.

Tablet brand name*	Nominal amount mg per tablet	Found** (% of nominal amount ± SD)			
		Reference method	Method A	Method B	Method C
Amlopres[a]	2.5	102.65+1.04	101.92+0.84 t = 1.22,F = 1.53	100.36+156 t = 2.78,F = 2.25	101.26+0.96 t = 2.20,F = 1.17
	5.0	99.26+0.64	101.03+1.26 t = 3.68,F = 3.87	100.96+1.06 t = 3.16,F = 2.74	101.04+1.36 t = 2.81,F = 4.51
	10.0	101.38+0.96	102.66+1.56 t = 1.61,F = 2.64	100.68+1.66 t = 0.84,F = 2.99	102.06+1.04 t = 2.11,F = 1.17
Amlocor[b]	2.5	98.74+0.48	99.26+0.64 t = 1.47,F = 1.78	100.04+0.78 t = 3.26,F = 2.64	99.66+1.11 t = 2.42,F = 5.35
	5.0	99.33+0.72	100.14+0.43 t = 2.21,F = 2.80	98.76+1.16 t = 0.96,F = 2.59	100.68+1.34 t = 2.07,F = 3.46
	10.0	101.62+0.85	102.16+0.34 t = 1.42,F = 6.25	101.74+1.45 t = 0.29,F = 2.91	102.55+1.26 t = 1.40,F = 2.20

* Marketed by: [a] Cipla India Ltd., [b] Torrent Drugs and Chemicals Pvt. Ltd.,
** Mean value of five determinat.ions
Tabulated t- value at 95% confidence level is 2.77
Tabulated F- value at 95% confidence level is 6.39 ions.

between the results obtained by the proposed methods and the established method.

The results were also compared statistically by a Student's t-test for accuracy and a variance ratio F-test for precision with those of the reference method at 95 % confidence level. The calculated t and F-values (Table 3) did not exceed the tabulated values (t = 2.77, F=6.39) for four degrees of freedom suggesting that there was no significant difference between the proposed methods and the reference method in terms of accuracy and precision.

Recovery studies: The accuracy and validity of the proposed methods were further ascertained by performing recovery studies via standard addition technique. Pre analyzed tablet powder was spiked with pure ADB at three different levels and the total amount of drug was determined by the proposed methods. Each determination was repeated three times. The recoveries of the pure ADB added were quantitative (Table 4), and revealed that the common additives, such as talc, starch, gum acacia, lactose, sodium alginate, magnesium stearate, calcium gluconate and calcium dihydrogen orthophosphate, and experiments did not interfere in the determination.

Conclusions

The assay results demonstrate that it is possible to use bromate-bromide mixture as a reagent for the direct titrimetric and indirect spectrophotometric determination of amlodipine in authentic samples. The titrimetric method is applicable over a micro scale (1-10 mg), and as small a concentration as 0.5 μg ml^{-1} drug can be determined by spectrophotometric method with a fair degree of accuracy and precision. The methods developed can be rapidly carried out, are simple to perform, and do not require specific sample treatments. The spectrophotometric methods use an inexpensive instrument compared to many reported procedures,[15-23,26] and do not require any expensive or toxic reagents or organic solvents. The methods are rapid, and do not entail any stringent experimental conditions, which affect the sensitivity and reliability of the methods. A significant advantage of the spectrophotometric methods presented here is the high sensitivity, which surpasses that of all the spectrophotometric methods reported previously[28-39]. In terms of the linear range of applicability, the spectrophotometric methods are even more sensitive than the chromatographic[16-18] as well as fluorimetric[26] methods. Thus, using an inexpensive instrument, it is possible to achieve a better sensitivity than is possible with costly experimental set up. An additional advantage is that the absorbance measurement is made at 520 or 610 nm, where the interference from the associated inactive ingredients is usually far less at longer wavelength than at shorter wavelength (350-450 nm) used in most of the reported methods. The proposed methods are advantageous

Table 4. Results of recovery study.

Tablet studied	Method A				Method B				Method C			
	Amount of drug in tablet, mg	Amount of pure drug added, mg	Total found, mg	Recovery* of pure drug added, %	Amount of drug in tablet, μg	Pure drug added, μg	Total found, μg	Recovery* of pure drug, %	Amount of drug in tablet, μg	Pure drug added, μg	Total found, μg	Recovery* of pure drug added, %
Amlopres (2.5 mg)	2.55	3.0	5.59	101.36	5.02	10.0	15.25	102.28	25.32	30.0	54.50	97.26
	2.55	4.0	6.56	100.28	5.02	15.0	20.57	103.67	25.32	40.0	65.19	99.68
	2.55	5.0	7.68	102.66	5.02	20.0	24.98	99.83	25.32	50.0	77.19	103.75
Amlopres (5 mg)	2.53	3.0	5.48	98.32	5.05	10.0	14.78	97.28	25.26	30.0	56.21	103.16
	2.53	4.0	6.60	101.67	5.05	95.0	20.21	101.07	25.26	40.0	65.77	101.28
	2.53	5.0	7.49	99.24	5.05	20.0	24.99	99.68	25.26	50.0	75.13	99.74
Amlopres (10 mg)	2.57	3.0	5.65	102.66	5.03	10.00	15.20	101.73	25.52	30.0	55.60	100.26
	2.57	4.0	6.63	101.38	5.03	15.00	20.04	100.04	25.52	40.0	67.15	104.08
	2.57	5.0	7.58	100.26	5.03	20.00	24.68	98.24	25.52	50.0	76.39	101.74

*Mean value of three trials.

than many of the reported spectrophotometric methods for the determination of amlodipine in pharmaceutical dosage forms.

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References

1. *The Merck Index* (1994) 12th Ed., Merck & Company, INC, White House Station, New Jersey, p. 942.
2. *Indian Drug Review* (1998) Mediworld Publications Group, New Delhi 4, 153.
3. Current Index of Medical Specialties (1999) BIO-GARD Pvt. Ltd., Bangalore 22, 102.
4. Martindale, *The Extra Pharmacopoeia* (1989) 26th ed., The Royal Pharmaceutical Society, London, p. 1492.
5. Beresford A P, Macrae P V, Stopher D A and Wood B A (1987) Analysis of amlodipine in human plasma by gas chromatography. *J Chromatogr Biomed Appl* **64,** 178-83.
6. Yasuda T, Tanaka M and Iba K (1996) Quantitative determination of amlodipine in serum by liquid chromatography with atmospheric pressure chemical ionization-tandem spectrometry. *J Mass Spectrom* **31,** 879-84.
7. Feng Y, Zhang L, Shen Z, Pan F and Zhang Z (2002) Analysis of amlodipine in human plasma by liquid chromatography-mass spectrometry. *J Chromatogr Sci* **40,** 49-53.
8. Zhang D, Chen X, Gu J and Li X (2001) Applications of liquid chromatography-tandem spectrometry in drug and biomedical analyses. *J Guo Clin Chim Acta* **313,** 147-50.
9. Marza A, Dal Bo L, Mazzucchelli P, Nunzia C, Crivelli F, Ismail S, Uhr M R and La P (2000), Amlodipine bioequivqlence achieved with a very sensitive liquid chromatography tandem mass spectrometric bioassay. *Arzeneim-Forsch* **50**, 688-94.
10. Josefsson M and Norlander B (1996) Coupled column chromatography on a chiral AGP phase for determination of amlodipine besylate enantiomers in human plasma: an HPLC assay with electrochemical detection. *J Pharm Biomed Anal* **15,** 267 -77.
11. Josefsson M, Zackrisson A L and Norlander B (1995) Sensitive high performance liquid chromatographic analysis of amlodipine in human plasma with amperometric detection and a single step solid phase sample preparation. *J Chromatogr Biomed Anal* **672,** 310-13.
12. Shimooka K, Sawada Y and Tatematsu H (1989) Analysis of amlodipine in serum by a sensitive high performance liquid chromatographic method with amperometric detection. *J Pharm Biomed Anal* **7,** 1267-72.
13. Tatar S and Atmaca S (2001) Determination of amlodipine in human plasma by high performance liquid chromatography with fluorescence detection. *J Chromatogr Biomed Sci Appl* **758,** 305-10.
14. Pandya K K, Satia M, Gandhi T P, Modi I A, Modi R T and Chakravarty B K (1995) Detection and determination of total amlodipine by high performance thin layer chromatogarphy: a useful technique for pharamcokinetic studies. *J Chrmatogr Biomed Appl* **667,** 315-20.
15. Carvalho M, Oliveira C H, Mendes G D, Sucupira M, Moraes M E A and De Nucci G (2001) Amlodipine bioequivalence study: quantification by liquid chromatography coupled to tandem mass spectrometry. *Biopharmaceutics and Drug Disposition* **22,** 383-90.
16. Patki R V, Tamhankar C P and Tipnis H P (1994) Simple and rapid high performance liquid chromatographic estimation of amlodipine from pharmaceutical dosages. *Indian Drugs* **31,** 560-1.
17. Avadhanulu A B, Srinivas K S, Anjaneyulu Y (1996) Reverse phase HPLC determination of amlodipine besylate in drug and pharmaceutical dosage forms. *Indian Drugs* **33,** 36-40.
18. Patel Y P, Patil S, Bhoir I C and Sundaresan M (1998) Isocratic, simultaneous reversed phase high performance liquid chromatographic determination of six drugs for combined hypertension therapy. *J Chromatogr A* **828,** 283-6.
19. Sane R T, Padke M, Hijji P S, Shah M and Patel P H (1998) UV-spectrophotometric and high performance thin layer chromatographic determination of benidipine HCl from its bulk drug. *Indian Drugs* **35,** 79-85.
20. Chandrashekar T G, Rao P S N, Smrita K, Vyas S K and Dutt C (1994) Analysis of amlodipine besylate by HPTLC with fluorimetric detection: a sensitive method for assay of tablets. *J Planar Chromatogr Mod TLC* **7,** 458-60.
21. Ilango K, Kumar P B and Prasad V R V (1997) Simple and rapid high performance thin layer chromatographic determination of amlodipine in pharmaceutical dosage forms. *Indian J Pharm Sci* **59,** 336-7.
22. Bhoir I C, Raman B, Sundaresan M and Bhagawat A M (1998) Separation and estimation of seven vasodilators using packed column supercritical fluid chromatography. *J Pharm Biomed Anal* **17,** 539-46.
23. Bretnali A E and Clarke G S (1995) Investigation and optimization of the use of micellar electrokinetic chromatography for the analysis of six cardiovascular drugs. *J Chromatogr A* **700,** 173-8.
24. Lu P R, Bi X L and Xie YZ (1995) Improved determination of the homogeneity of the content of luohuoxi tablets. *Yaowu Fenxi Zaghi* **15,** 42-3
25. Khopade S A and Jain N K (2000) Differecne spectrophotometric estimation of amlodipine besylate. *Indian Drugs* **37,** 351-3.
26. Mohamed Y E, Naglaa M K, Bahia A M and Nashwa G B (1998) Fluorimetric determination of amiodarone, amlodipine and propafenone. *Bull Fac Pharm (Cairo Univ)* **36,** 1- 9.
27. Gazy A A K (2004) Determination of amlodipine besylate by adsorptive anodic stripping voltammetry on glassy carbon electrode in tablets and biological fluids. *Talanta* **62,** 575–82.
28. Sridhar K, Sastry C S P, Reddy M N, Sankar D G and Ramasrinivas K (1997) Spectrophotometric determination of amlodipine besylate in pure form and tablets. *Anal Lett* **30,** 121-33.
29. Singhvi I and Chaturvedi S C (1998) Visible spectrophotometric methods for estimation of amlodipine besylate in tablets. *Indian J Pharm Sci* **60,** 309-10.
30. Singhvi I and Chaturvedi S C (1999) Spectrophotometric method for determination of amlodipine besylate and binidipine hydrochloride from tablets. *Indian J Pharm Sci* **61,** 190-1.
31. Murthy T K, Reddy M N, Reddy M D and Sankar D G (2001) Extractive spectrophotometric methods for the determination of amlodipine besylate. *Asian J Chem* **13,** 771-3.
32. Yucesoy C and Goelcue A Y (2001) Spectrophotometric

determination of amlodipine besylate in tablets with trinitrobenzenesulfonic acid. *Ankara Universitesi Eczacilik Fakultesi Dergisi* **30,** 1-8.
33. Rahman N and Azmi S N K (2000) Spectrophotometric determination of amlodipine besylate by charge-transfer complex formation with p-chloranilic acid. *Anal Sci* **16,** 1353-6.
34. Golcu A, Yucesoy C and Serin S (2000) The use of charge-transfer complexation in the spectrophotometric determination of amlodipine besylate. *Sci Pharm* **68,** 235-46.
35. Iskender G and Sagirli A O (2000) Spectrophotometric determination of amlodipine besylate and aspartime in tablets. *Acta Pharm Turc* **42,** 1-5.
36. Lokesh B S and Narayana R M (1999) Colorimetric determination of amlodipine. *Ethiop Pharm J* **17,** 56-8.
37. Meyyanathan S N, Joel J, Scaria S, Sowmya S and Suresh B (1998) Simple spectrophotometric analysis of amlodipine besylate. *Indian Drugs* **95,** 296-7.
38. Rahman N and Hoda M N (2003) Validated spectrophotometric methods for the determination of amlodipine besylate in drug formulations using 2,3-dichloro-5,6-dicyano-benzoquinone and ascorbic acid. *J Pharm Biomed Anal* **31,** 352-81.
39. Rahman N, Singh M and Hoda M N (2004) Application of oxidants to the spectrophotometric determination of amlodipine besylate in pharmaceutical formulations. *IL Farmaco* **59,** 913-19.
40. Basavaiah K, Chandrashekar U and Nagegowda P (2005) Spectrophotometric and high performance liquid chromatographic determination of amlodipine besylate in pharmaceuticals, *Science Asia* 31, 13-21.
41. Basavaiah K and Nagegowda P (2003) Determination of captopril in pharmaceutical preparations using chloramine-T. *Bulg Chem Commun* **35,** 48-53.
42. Basavaiah K, Chandrashekar U and Nagegowda P (2005) Rapid titrimetric and spectrophotometric determination of frusemide (furosemide) in tablets using bromate-bromide mixture and methyl orange. *Indian J Chem Tachnol* **12,** 149-55.
43. Basavaiah K and Prameela H C (2003) Three useful bromimetric methods for the determination of salbutamol sulphate. *Anal Bioanal Chem* **376,** 879-83.
44. Basavaiah K, Chandrashekar U, Prameela H C and Nagegowda P (2003) Quantitative determination propranolol hydrochloride. *Acta Ciencia Indica Chem* **29,** 25-9.
45. Basavaiah K and Prameela H C (2003) Three new bromimetric methods for the estimation of famotidine. *Science Asia* **29,** 147-53.
46. Basavaiah K and Nagegowda P (2004) Determination of ranitidine hydrochloride in pharmaceutical preparations by titrimetry and visible spectrophotometry using bromate and acid dyes. *IL Farmaco* **59,** 147-53.
47. Kolthoff I M, Belcher R, Stanger VA and Matsuyama (1957) G Volumetric Analysis, Vol. III, Interscience Publishers, INC, New York.
48. Sastry C S P, Rao S G, Rao J S V M L and Naidu P Y (1997) Application of azine dyes for the determination of ranitidine hydrochloride in pharmaceutical formulations. *Anal Lett* **30,** 2377-90.
49. Sharma C S N, Kamala Sastry C and Sastry C S P(2002) Determination of stavudine and lamivudine by visible spectrophotometry. *Acta Ciencia India Chem*. **28** 221-5.
50. Sastry C S P and Rao J S V M L (1996) Indirect spectrophotometric determination of methotrexate by oxidimetry. *East Pharm* **39,** 117.
51. Sastry C S P, Sarma V A N, Prasad V V and Lakshmi C S R (1997) N-bromosuccinimide as an analytical reagent for the spectrophotometric determination of benzimidazole derivatives. *Indian J Pharm Sci* **59,** 161-4.
52. Basavaiah K and Prameela H C (2003) Use of oxidation reaction for the quantitative determination of albendazole with chloramine-T and acid dyes. *Anal Sci* **19,** 779-84.
53. Basavaiah K and Manjunathaswamy J (2001) A facile and highly sensitive spectrophotometric method for the determination of some phenothiazine antipsychotics using chloramine-T and indigo carmine. *Anal Sci* **17,** 963-7.
54. Amin A S, Ahmed I S, Dessouki H A and Gouda E A (2003) Utility of oxidation-reduction reaction for the determination of ranitidine hydrochloride in pure form, dosage forms and in the presence of its oxidative degradates. *Spectrochim Acta Part A* **59,** 695-703.
55. Gyory S (1893) *Z anal Chem* **32,** 415.
56. Laitinen H A and Boyer K W (1972) Simultaneous determination of bromine and chlorine with methyl orange. *Anal Chem* **44,** 920-6.