ScienceAsia 31 (2005): **13-21**

Spectrophotometric and High Performance Liquid Chromatographic Determination of Amlodipine Besylate in Pharmaceuticals

Kanakapura Basavaiah,* Umakanthappa Chandrashekar and Paregowda Nagegowda

Department of Chemistry, University of Mysore, Manasagangothri, Mysore – 570 006, India.

* Corresponding author, E-mail: basavaiahk@yahoo.co.in

Received 1 Jun 2004
Accepted 1 Dec 2004

ABSTRACT: Two rapid assay procedures based on visible spectrophotometry and high performance liquid chromatography (HPLC) have been developed for the determination of amlodipine besylate (ADB) in pharmaceutical formulations. Spectrophotometric method is based on the bromination of ADB with a known excess of bromate-bromide mixture in acid medium followed by the determination of surplus bromine by reacting with Metanil Yellow and measuring the absorbance at 530 nm. The HPLC determination was carried out on a reversed phase C_{18} column using 0.1% orthophosphoric acid (pH 3): acetonitrile (20:80) at a flow rate of 1.0 ml min^{-1} with UV-detection at 238 nm. In the spectrophotometric method, the absorbance is found to increase linearly with increasing concentration of ADB, which is corroborated by the calculated correlation coefficient of 0.9975. The system obeys Beer's law for 1.25-7.50 μg ml^{-1} ADB with a molar absorptivity of 2.51 x 10^4 l $mol^{-1}cm^{-1}$ and a Sandell sensitivity of 16.37 ng cm^{-2}. The limits of detection and quantification are calculated to be 0.17 and 0.56 μg ml^{-1}, respectively. In the HPLC method, a rectilinear relationship was observed between 7.55 – 241.6 μg ml^{-1} ADB with a detection limit of 1.51 μg ml^{-1} and a quantification limit of 3.02 μg ml^{-1}. The statistical evaluation of the methods was examined by determining intra-day and inter-day precision. The methods, when applied to the determination of ADB in tablets, gave satisfactory results. Accuracy and reliability of the proposed methods were further ascertained by parallel determination by the reference method and by recovery studies.

KEYWORDS: amlodipine besylate, determination, spectrophotometry, HPLC, pharmaceuticals.

INTRODUCTION

Amlodipine besylate (ADB) is an important calcium channel blocker belonging to the dihydropyridine family. It is more selective for arterial vascular smooth muscle than for cardiac tissue and is approved for the treatment of hypertension and for variant and stable angina[1-3]. It is chemically known as (4 R, S)-3-ethyl-5-methyl 2-(2-amino-ethoxy-methyl)-4-(2-chloro phenyl)-1,4-dihydroxy-6-methyl pyridine-3,5-dicarboxylate monobenzene sulphonate[4] and its structure is given in Fig. 1. The main effects of this drug are confined with peripheral and coronary vasodilator properties. Therefore, the analysis of its dosage forms is very important.

The assay procedure listed in European Pharmacopoeia describes a reversed phase high performance liquid chromatographic (HPLC) method[5] for the determination of drug in bulk and pharmaceutical formulations. Most methods developed for ADB in pharmaceuticals are applicable to combined dosage forms and a limited number of them have been reported for the quantification of drug in single dosage forms. Visible spectrophotometry is widely used for the assay of ADB in dosage forms. Procedures based on ion-pair complex formation followed by extraction[6-13], charge-transfer complex formation[14-17],

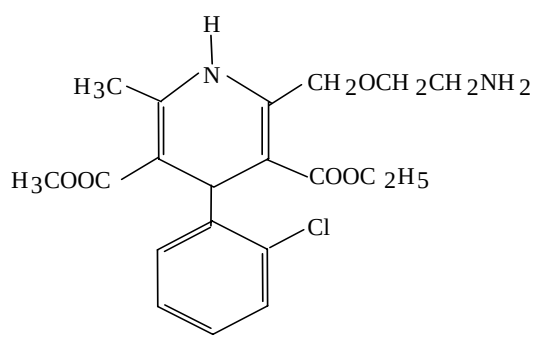

Fig 1. Structure of amlodipine.

derivatization[18,19], redox[20], oxidative coupling[6] and complex formation[17,21,22] reactions have been proposed by several workers. However, these procedures suffer from such disadvantages as poor sensitivity and selectivity, heating or extraction step and narrow range of linear response (Table 1).

Methods based on several chromatographic techniques like liquid chromatography tandem-mass spectrometry[23], high performance thin layer chromatography[24-27], unicellar electrokinetic chromatography[28], packed column supercritical fluid chromatography[29] and HPLC[30-36] with photometric[30-35] and electrochemical[36] detection systems have been used for the quantitative determination of amlodipine in pharmaceuticals. However, majority of the HPLC procedures[32-36] are applicable to combined dosage forms, and even those procedures [31,32] used for assay in single dosage forms have either narrow range of applicability (0.5-16 μg ml^{-1})[30] or poor sensitivity (2-10 mg ml^{-1})[31]. Other methods reported for the determination of ADB in formulations include UV-spectrophotometry[37], difference UV-spectrophoto metry[38], fluorimetry[39] and anodic stripping voltametry[40].

The present work is aimed at developing two simple and sensitive methods, which would overcome the difficulties encountered in most visible spectrophotometric and HPLC methods. The spectrophotometric method involves treating a fixed amount of bromate-bromide solution in acid medium with ADB solution and determining the unreacted bromine by treating with a fixed amount of Metanil Yellow dye solution and measuring the absorbance at 530 nm. The HPLC analysis was carried out by injecting the drug solution on to an Accurasil ODS C_{18} column with the elution being effected by a mobile phase consisting of 0.1% orthophosphoric acid (pH 3)-acetonitrite (20:80) and UV-detection at 238 nm. The methods are fairly rapid and sensitive. In fact, the spectrophotometric method is the most sensitive method ever reported for ADB (Table 1). Both methods are characterized by a fairly high degree of accuracy and precision. The methods were proved to be successful in determining ADB in tablet formulations.

Table 1. Comparision of the existing spectrophotometric methods with the proposed method.

S l No.	Reagent/s	λ_{max} (nm)	Linear range (μgml^{-1})	ε(l mol^{-1} cm^{-1})	Remarks	Ref
1.	Bromothymol blue	405	5-40	-	Involves extraction	6
2.	Bromocresol green bromophenol blue or methylene blue	410	0-80	-	Involves extraction	7
3.	Rhodizinic acid	450	0.1×10^3 - 1.5×10^3	-	Involves extraction/ requires rigid pH control, least sensitive	8
4.	Fast green FCF or	625	2.5-20	$2.2x10^4$	Involves extraction,	9
	Orange II	485	2.5-20	$1.9x10^4$	requires rigid pH control	
5.	Trinitrobenzene sulfonic acid	337	6.0-30.0	-	Involves extraction	10
6.	Tetrachloroquinone	346	5-25	-	Involves incubation at 55° C for 10 min and requires strict pH control	15
7.	DDBQ*	580	1-125	$6x10^3$	Uses non-aq. medium, coloured species less stable; less sensitive	17
8.	NQS**	462	10-80	-	Involves incubation at 50° C for 20 min, requires strict pH control	18
9.	NQS**	477	1-80	$4.4x10^2$	Requires strict pH control, least sensitive	19
10.	Chloranil	362	1-70	$1.3x10^3$	Requires rigid pH control, less sensitive	19
11.	Ascorbic acid	530	10-140	$3.2x10^3$	Requires boiling at 100±1°C for 25 min, less sensitive	17
12.	NaOH	456	20-100	-	Requires non-aq. medium, less sensitive	21
13.	Ninhydrin	595	10-60	$6.5x10^3$	Requires non-aq. medium	22
14.	BrO_3^- Br^-/Metanil Yellow	530	1.25-7.50	$2.5x10^4$	Non stringent experimental conditions, highly sensitive	Present method

* 2, 3-dichloro-5, 6 dicyano-1, 4-benzoquinone
** 1,2-naphthaquinone-4-sulfonic acid

Materials and Methods

Apparatus: A Systronics model 106 digital spectrophotometer with 1 cm matched quartz cells was used for absorbance measurements. The chromatographic system consisted of an Agilent 1100 series chromatograph equipped with an in built solvent degasser, quaternary pump, photodiode array detector with variable injector and auto sampler, and a reversed phase 5 μm Accurasil ODS C_{18} column (250 x 4.6 mm).

Reagents and Solutions

A bromate-bromide mixture equivalent to 1500 μg ml^{-1} $KBrO_3$ and 20000 μg ml^{-1} KBr was prepared by dissolving 0.150 g of $KBrO_3$ (Sarabhai M. Chemicals, Baroda, India) and 2.0 g of KBr (Indian Drugs and Pharmaceuticals Ltd, Hyderabad, India) in water and diluting to 100 ml in a calibrated flask. This was diluted stepwise to get a working concentration of 30 μg ml^{-1} with respect to $KBrO_3$.

Metanil Yellow (400 μg ml^{-1}) was first prepared by dissolving 56.3 mg of the dye (S.d. Fine Chem. Ltd., Mumbai, India, Dye content 71 %) in water and diluting to 100 ml in a calibrated flask. This was diluted 10-fold to get a working concentration of 40 μg ml^{-1} dye.

Hydrochloric acid (5 M) was prepared by diluting 111.0 ml of concentrated acid, (S.d. Fine Chem. Mumbai, India) specific gravity 1.18 to 250 ml with water.

HPLC grade acetonitrile (Rankem, India), AR grade orthophosphoric acid (Qualigens Fine Chemicals, India) and triethylamine (Loba Chemie, India) and distilled water filtered through a 0.45 μm filter (Millipore) were used. A 0.1% orthophosphoric acid solution was prepared by adding 1 ml of the acid to 1 litre of water, mixed well, pH adjusted to 3.0 by using triethylamine and filtered through a 0.45 μm filter. The dilutent solution was prepared by mixing acetonitrile and water at a ratio of 60:40. The mobile phase used consisted of 0.1% orthophosphoric acid (pH 3) and acetonitrile (20:80).

Standard Drug Solution

Pharmaceutical grade ADB certified to be 99.7% pure was received as a gift from Cipla India Ltd., Mumbai, India and was used without further purification. A stock standard solution containing 250 μg ml^{-1} ADB was prepared by dissolving 25 mg of ADB in 10 ml of glacial acetic acid and diluting to the mark in a 100 ml calibrated flask with water. This was diluted to get 25 μg ml^{-1} working concentration for the spectrophotometric study. For the chromatographic work, a stock standard solution equivalent to 302 μg ml^{-1} ADB was prepared by dissolving 30.2 mg of pure drug in the dilutent solution and diluting to the mark in a 100 ml calibrated flask.

Dosage Forms

Thirty-six brands of tablets are currently marketed in India. Only two brands, Amlopres (2.5 mg, 5.0 mg and 10.0 mg dosage forms) marketed by Cipla India Ltd and Amlocor (2.5 mg, 5.0 mg and 10.0 mg dosage forms) marketed by Torrent Drugs and Chemicals Ltd., India were purchased from local commercial sources for investigation.

General Procedures

Spectrophotometry: In a series of 10 ml calibrated flasks were placed 0.5-3.0 ml aliquots of 25 μg ml^{-1} ADB solution and the total volume was adjusted to 4 ml by adding requisite volume of water. To each flask was then added 2 ml of 5 M HCl followed by 1 ml of 30 μg ml^{-1} bromate solutions. The flasks were stoppered, contents mixed well and let stand for 10 min with occasional shaking. Then, 1 ml of 40 μg ml^{-1} Metanil Yellow solution was added to each flask, the volume adjusted to the mark with water, mixed well, and absorbance of each solution measured at 530 nm against a reagent blank after 5 min. The calibration graph was prepared by plotting the absorbance versus concentration of drug solution. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the Beer's law data.

HPLC Method

Chromatographic Conditions. Chromatographic separation was achieved at ambient temperature on a reversed phase Accurasil ODS 5 μm C_{18} column (250x4.6 mm) using a mobile phase consisting of 0.1% orthophosphoric acid (pH 3) – acetonitrile (20:80) at a flow rate of 1.0 ml min^{-1}. The detector wavelength was set at 238 nm with a sensitivity of 0.2 a.u.f.s.

Calibration Graph: Working standard solutions equivalent to 7.55-241.6 μg ml^{-1} ADB were prepared by appropriate dilution of stock standard solution with the diluent solution. Twenty μl aliquot of each solution was injected automatically on to the column in duplicate and the chromatograms were recorded. Calibration graph was constructed by plotting the mean peak area versus concentration of ADB. The concentration of the unknown was read from the calibration graph or calculated from the regression equation derived from the concentration and peak area data.

Procedure for Tablets: Twenty tablets were weighed and ground into a fine powder. An amount of powder equivalent to 25 mg of ADB was accurately weighed into a 100 ml calibrated flask, 10 ml glacial acetic acid and 40 ml of water added, and shaken for about 20 min. The volume was diluted to the mark with water, mixed well and filtered using a Whatman No.42 filter paper.

The filtrate (250 μg ml^{-1}) was diluted 10-fold and a suitable aliquot was analyzed by spectrophotometry.

For assay by the HPLC method, an amount of powdered tablet equivalent to 30 mg of ADB was accurately weighed into a 100 ml calibrated flask, 60 ml of diluent solution added and shaken for about 20 minutes, the volume was diluted to the mark and mixed well. A small portion of this solution (≈ 10 ml) was withdrawn and filtered through a 0.2 μm filter to ensure the absence of particulate matter. The filtered solution was appropriately diluted with the diluent solution for analysis.

Results and Discussion

Method Development

Spectrophotometry: Many dyes are prone to oxidation to form colourless products in acid medium, thus offering a suitable analytical approach for the indirect spectrophotometric determination of different pharmaceutical substances[41-46] using oxidizing agents including *in situ* generated bromine. In the proposed spectrophotometric method, the susceptibility of ADB to undergo bromination by *in situ* generated bromine and the latter's ability to quantitatively decolorise Metanil Yellow have been used for the determination of ADB. The possible reaction scheme is given in Fig. 2

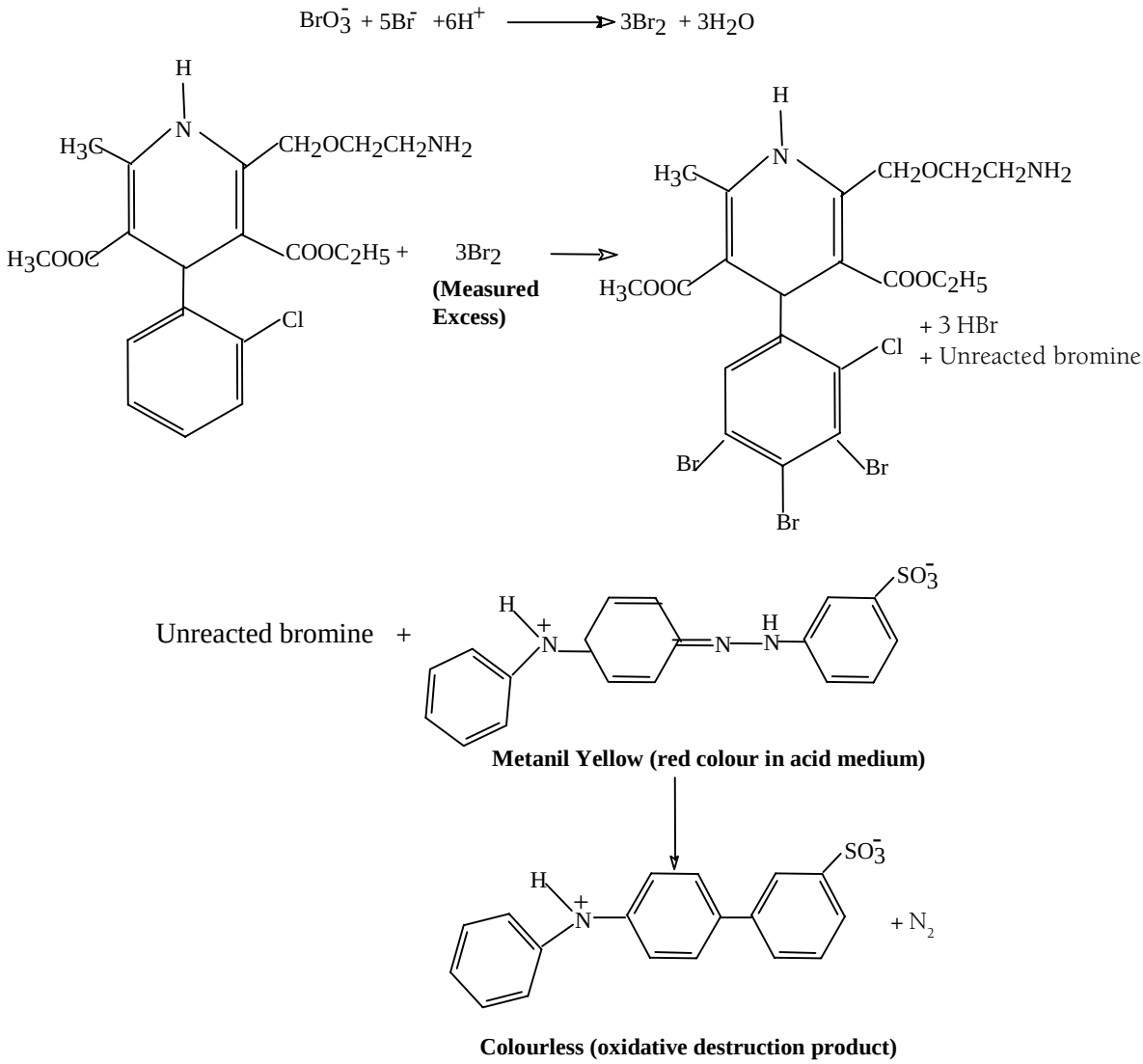

Fig 2. Reaction scheme.

In this method, different amounts of ADB were added to a fixed and known excess of *in situ* generated bromine, and after the bromination reaction was judged to be complete, the residual bromine was determined by reacting it with a fixed amount of Metanil Yellow dye and measuring the change in absorbance at 530 nm. The absorbance was found to increase linearly with the concentration of ADB. ADB, when added in increasing amounts to a fixed and known excess amount of *in situ* generated bromine, consumes the latter, and there will be a concomitant decrease in the amount of bromine. When a fixed amount of Metanil Yellow is added to decreasing amounts of bromine, a proportional increase in the dye concentration results which is reflected in the proportional increase in absorbance at 530 nm (Fig. 3).

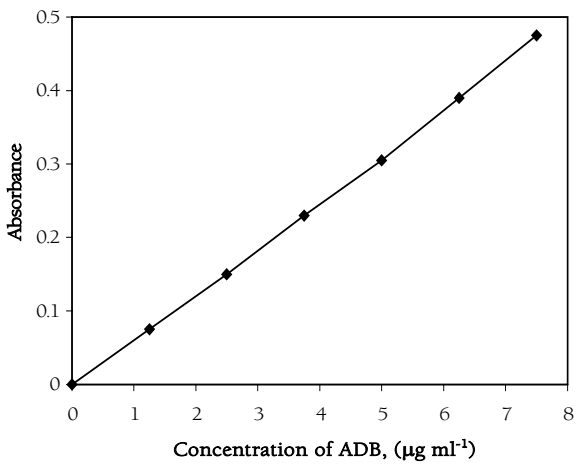

Fig 3. Beer's law curve.

By performing a preliminary experiment, it was found that 4 μg ml^{-1} Metanil Yellow could be determined spectrophotometrically at 530 nm in acid medium. The red colour due to 4 μg ml^{-1} dye was quantitatively bleached by 3 μg ml^{-1} bromate solution in the presence of a large excess of bromide. Hence, different amounts of ADB were reacted with 30 μg of $KBrO_3$ in the presence of bromide and in acidic conditions, and the unreacted bromine was determined by treating with 40 μg of Metanil Yellow. This was done to establish the concentration range over which the method is applicable for the determination of ADB.

Hydrochloric acid was found to be an ideal medium for bromination as well as the determination of residual bromine. Two ml of 5 M HCl in a total volume of 4 ml was found to be adequate for the bromination reaction, which was complete in 10 min, and the same concentration was maintained for the determination of the residual bromine by its bleaching action on Metanil Yellow. Contact time of 10 min is not critical and any delay up to 30 min in the determination of the residual bromine had no effect on the absorbance. A 5 min standing time was found necessary for the quantitative bleaching of the dye colour by the residual bromine. The absorbance of the dye colour was stable for 60 min in the presence of the bromination product.

Analytical Data

In the spectrophotometric method, Beer's law was obeyed over the concentration range 1.25-7.50 μg ml^{-1}. The apparent molar absorptivity and Sandell sensitivity values were 2.51x10^4 l mol^{-1} cm^{-1} and 16.37 ng cm^{-2}, respectively. The linear plot gave the regression

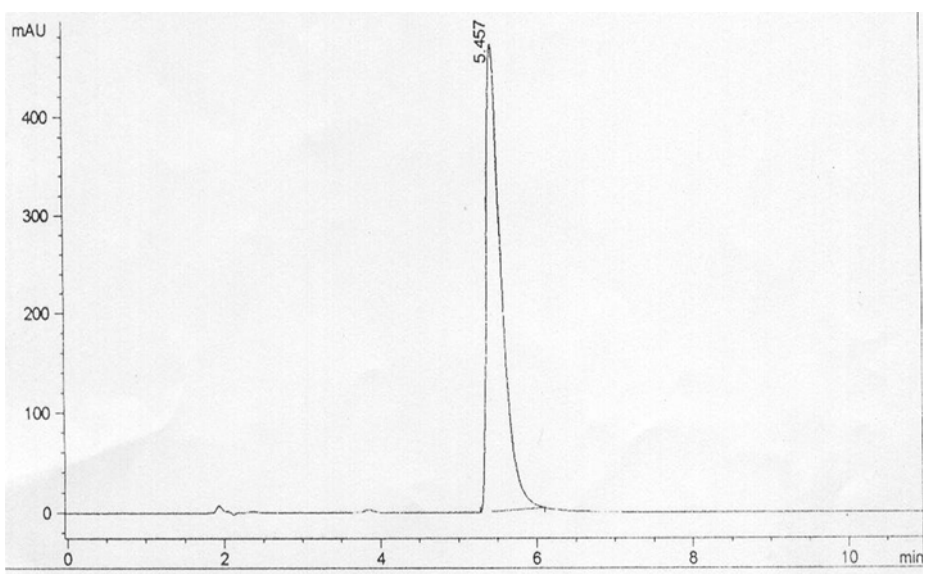

Fig 4. Typical chromatogram (ADB, 181.1 μg ml^{-1}).

Table 2. Evaluation of Accuracy and Precision.

Spectrophotometric method					HPLC Method						
ADB taken μgml^{-1}	ADB found* μgml^{-1}	RE %	RSD %	Range of error,%	ADB taken μgml^{-1}	ADB found* μgml^{-1}	RE %	RSD@ %	RSD# %	ROE@ %	ROE# %
2.0	1.98	1.0	0.86	±0.83	50.0	48.56	2.68	0.56	1.04	±0.54	±1.00
4.0	4.03	0.75	0.72	±0.69	100.0	97.32	2.68	0.32	0.85	±0.31	±0.82
6.0	6.05	0.83	1.26	±1.21	150.0	148.42	1.01	0.75	1.18	±0.72	±1.14

* Mean value of seven determinations
@ Based on retention time
# Based on peak area
RE = Relative error
RSD = Relative standard deviation
ROE = Range of error at 95 % confidence level

equation:

$$A = 2.3x10^{-3} + 0.063\ C\ (r = 0.9975, n=6)$$

where A is absorbance and C is concentration in μg ml^{-1}. The detection and quantification limits were calculated from the standard deviation of absorbance measurements from a series of 7 blank solutions. The limits of detection and quantification were established according ICH guidelines [47] and were calculated to be 0.17 μg ml^{-1} and 0.56 μg ml^{-1} respectively.

Table 3. Results of analysis of tablets and statistical evaluation.

.

Brand name of tablet	Nominal amount, mg/ tablet	Found** (% of nominal amount ± SD) Reference method	Spectrophotometric method	HPLC method
Amlopres	2.5	102.65 ±1.04	100.88 ±0.83 t = 2.98 F = 1.57	101.32 ±0.62 t = 2.53 F = 2.81
	5.0	99.26 ±0.64	100.36 ±1.26 t = 1.83 F = 3.88	97.65 ±1.44 t = 2.42 F' = 5.06
	10.0	101.38 ±0.96	99.85 ±1.56 t = 3.18 F' = 2.64	98.83 ±1.85 t = 2.85 F = 3.71
Amlocor	2.5	98.74 ±0.48	99.94 ±0.94 t = 2.67 F = 3.84	97.84 ±0.75 t = 2.29 F = 2.44
	5.0	99.33 ±0.72	101.26 ±1.76 t = 2.46 F = 5.98	100.66 ±0.63 t = 3.62 F = 2.80
	10.0	101.62 ±0.85	99.38 ±1.86 t = 2.62 F = 4.79	99.04 ±1.58 t = 3.39 F = 3.45

**Mean value of five determinations
Tabulated t-value at 95% confidence level is 2.77
Tabulated F-value at 95% confidence level is 6.39

HPLC

ADB was also determined by HPLC. A solution of ADB was injected in duplicate on to the column and was monitored by UV-detection at 238 nm. The composition and pH of the mobile phase were varied to optimize the chromatographic conditions. A mobile phase consisting of 0.1% orthophosphoric acid (pH 3): acetonitrile (20:80) was used after several preliminary runs. Acetonitrile and phosphoric acid increase the solubility of ADB and prevent its adherence to the packing material in the column. At a flow rate of 1.0 ml min^{-1}, the retention time for ADB was 5.4 min (Fig. 4). Under the described experimental conditions, the analyte peaks were well defined and free from tailing. ADB was determined by measuring the peak area. A plot of peak area against concentration (Fig. 5) gave a linear relationship (r=0.9999) over the concentration range 7.55-241.6 μg ml^{-1}. Using regression analysis, the linear equation, Y = 2.79 + 34X was obtained where Y is the mean peak area and X is concentration in μg ml^{-1}. The limits of detection and quantifications calculated according to ICH guidelines[47] were 1.51 μg ml^{-1} and 3.02 μg ml^{-1}, respectively.

Method Validation

Accuracy and Precision: To determine the accuracy and precision of the methods, pure ADB solutions containing three different concentrations were analysed in seven replicates. The results obtained from this investigation are summarised in Table 2. The per cent relative error which is an index of accuracy is <3% and is indicative of good accuracy. The relative standard deviation (≤ 1.5%) and the range of error (≤ 1.5%) at the 95% confidence level can be considered to be satisfactory. The inter-day precision was established by performing analyses over a five-day period on solutions prepared freshly each day. The RSD values were no more than 2.3% for the spectrophotometric method. The peak-area based and rention time based RSD values were 3.2% and 1.8% respectively.

Application

The proposed methods were successfully applied to the assay of ADB in two brands of tablets with 2.5,

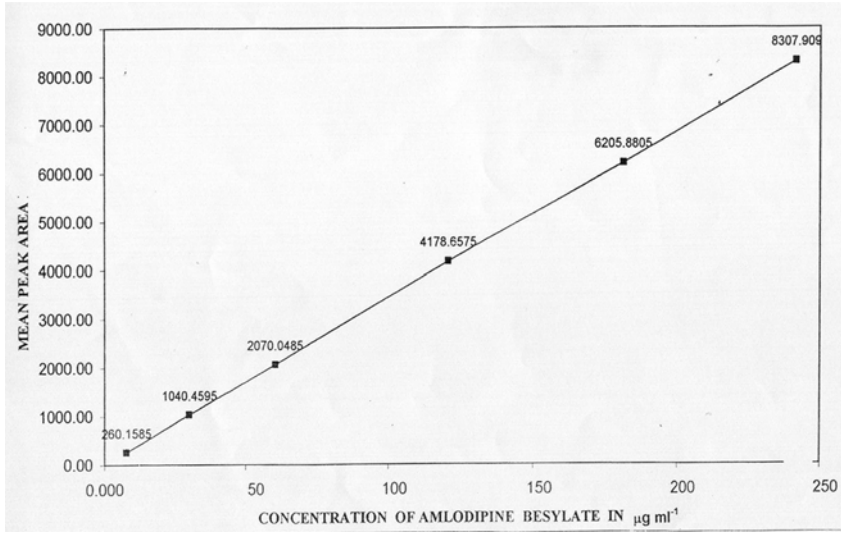

Fig 5. Linearity graph.

5.0 and 10.0 mg dosage forms. The results obtained are presented in Table 3 and compare well with the label claim. The drug content of the same batch tablets was determined by the reported method according to Lu et al[37]. It is clear from Table 3, that there is close agreement between the results obtained by the proposed methods and the reference method[37].

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 and 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.

To further ascertain the accuracy and validity of the proposed methods, recovery experiments were performed. Pre-analyzed tablet powder was spiked with pure drug at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The recoveries of pure ADB were quantitative (Table 4) and revealed that common additives and excipients such as talc, starch, gumacacia, lactose, calcium gluconate, calcium dihydrogenorthophosphate, magnesium stearate and sodium alginate did not interfere in the determination.

Conclusions

Amlodipine besylate was determined in pharmaceuticals by two different techniques. The methods are simple, rapid and convenient since they do not require any special working conditions. The striking feature of the spectrophotometric method is that it is free from heating or extraction step unlike

Table 4. Results of recovery experiment.

Tablet studied	Spectrophotometric method				HPLC method			
	Amount of ADB in tablet, µg	Amount of pure ADB added, µg	Total found, µg	Recovery of pure* drug added,%	Amount of ADB in tablet, µg	Amount of pure ADB added, µg	Total found, µg	Recovery of pure* drug added, %
Amlopres	20.18	30.0	49.09	96.38	506.6	600.0	1144.70	106.35
(2.5 mg)	20.18	40.0	59.96	99.44	506.6	900.0	1394.36	98.64
	20.18	50.0	71.47	102.58	506.6	1500.0	2062.55	103.73
Amlopres	20.07	30.0	51.17	103.66	488.3	600.0	1067.78	96.58
(5 mg)	20.07	40.0	59.97	99.75	488.3	900.0	1364.54	97.36
	20.07	50.0	68.89	97.64	488.3	1500.0	1984.70	99.76
Amlopres	19.97	30.0	50.38	101.38	494.3	600.0	1121.66	104.56
(10 mg)	19.97	40.0	59.47	98.74	494.3	900.0	1401.05	100.75
	19.97	50.0	71.63	103.32	494.3	1500.0	1973.60	98.62

*Mean value of three determinations

most methods reported earlier. It involves the least number of experimental variables, which is reflected in high degree of accuracy and precision. The most significant advantage of the method is its sensitivity which is 10-20 fold higher than that achieved by the previously reported methods. The HPLC method is characterized by a shorter retention time and a long dynamic range of concentration over which the method is applicable. In both methods, there was no interference from matrix sources.

Acknowledgements

The authors gratefully acknowledge the receipt of pure sample of drug as a gift from M/s. Cipla India Ltd., Mumbai, India. Two of the authors (UC and PN) thank the authorities of the University of Mysore, Mysore, for facilities.

References

1. *Indian Drug Review* (1998) Mediworld Publication Group, New Delhi, 4, 153.
2. Current Index of Medical Specialities (1999) BIO-GARD Pvt. Ltd., Bangalore, India, 22, 102.
3. Martindale, *The Extra Pharmacopoeia* (1989) 26th ed., The Royal Pharmaceutical Society, London, pp 1492.
4. *The Merck Index* (1994) 12th ed., Merck & Co., INC, White House Station, New Jersey, pp 942.
5. *The European Pharmacopoeia* (2001) 3rd ed., Supplement, Council of Europe, Strarbourg, pp 819.
6. Sridhar K, Sastry C S P, Reddy M N, Shankar D G and Rama Srinivas K (1997) Spectrophotometric determination of amlodipine besylate in pure form and tablets. *Anal Lett* **30,** 121-33.
7. Singhvi I and Chaturvedi S C (1998) Visible spectrophotometric methods for estimation of amlodipine besylate in tablets. *Indian J Pharm Sci* **60,** 309-10.
8. Singhvi I and Chaturvedi S C (1999) Spectrophotometric method for determination of amlodipine besylate and benidipine hydrochloride from tablets, *Indian J Pharm Sci* **61**, 190-1.
9. Murthy T K, Reddy M N, Reddy M D and Shankar D G (2001) Extractive spectrophotometric methods for the determination of amlodipine besylate, *Asian J Chem* **13**, 771-3.
10. Yucessoy C and Goelcue A Y (2001) Spectrophotometric determination of amlodipine besylate in tablets with trinitrobenzene sulphonic acid. Ankara Universities Eczacilick Fakultesi Dergisi **30**, 1-8.
11. Cetin G and Sungur A (1995) A spectrophotometric method for the determination of amlodipine in pharmaceutical formulations. *Sci Pharm* **63,** 93-8.
12. Lokesh B V S, Reddy M N, Shankar D G and Sreedhar K (1996) Extractive spectrophotometric determination of amlodipine. *East Pharm* **39,** 125-6.
13. Reddy M N, Rani G T, Rao K V S P, Sankar D G and Sreedhar K (1997) Extractive spectrophotometric determination of amlodipine using eriochrome black T and indigocarmine. *Indian J Pharm Sci* **59,** 188-9.
14. Rahman N and Azmi S N (2000) Spectrophotometric determination of amlodipine besylate by charge transfer complex formation with p-chloranilic acid. *Anal Sci* **16**, 1353-6.
15. Golcu A, Yuccesoy C and Serin S (2000) The use of charge-transfer complexation in the spectrophotometric determination of amlodipine besylate. *Sci Pharm* **68,** 225-46.
16. Ebeid M Y, El-Kousy N M, Mousa B A and Mohammed N G (1998) Contribution to the methods of determination of some cardiovascular drugs. *Egypt J Pharm Sci* **39,** 31-43.
17. 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-1, 4-dibenzoquinone and ascorbic acid. *J Pharm Biomed Anal* **31,** 381-92.
18. Iskender G and Sagirli A (2000) Spectrophotometric determination of amlodipine besylate and aspartame in tablets. *Acta Pharm Turc* **42,** 1-50.
19. Anu P, Suvarna G K and Sathyanarayana D (2000) Simple spectrophotometric methods for the determination of amlodipine besylate in solid dosage formulations. *East Pharm* **43,** 111-2.
20. Lokesh B S and Narayana R M (1999) Colorimetric determination of amlodipine. *Ethiop Pharm J* **17,** 56-8.
21. Meyyanathan S N, Joel J, Scaria S, Sowmya S and Suresh B (1998) Simple spectrophotometric analysis of amlodipine besylate. *Indian Drugs* **35,** 296-7.
22. Rahman N and Azmi S N H (2001), Spectrophotometric method for the determination of amlodipine besylate with ninhydrin in drug formulations. *IL Farmaco* **56,** 731-5.
23. Carvalho M, Oliveira C H, Mendes G D, Sucupira M, Moraes M E A and De Nucci G (2001) Amlodipine biequivalence study: quantification by liquid chromatography coupled to tandem mass spectrometry. *Biopharmaceutics Drug Disp* **22,** 385-90.
24. Chandrashekar T G, Rao P S N, Simritha K, Vyas K and Dutt C (1994) Analysis of amlodipine besylate by HPTLC with fluorimetric detection: a sensitive method for the assay of tablets. *J Pharm Chromatogr Mod* TLC **7,** 458-60.
25. Ilango K, Kumar P B and Prasad V R V (1997) Simple and rapid HPTLC determination of amlodipine in pharmaceutical dosage forms. *Indian J Pharm Sci* **59,** 336-7.
26. Pandya K K, Salia M, Gandhi T P, Modi I A, Modi R I and Chakravarthy B K (1995) Detection and determination of total amlodipine by high performance thin layer chromatography: a useful technique for pharmacokinetic studies. *J Chromatogr Biomed Appl* **667,** 315-20.
27. Agrekar A P and Pawar S G (2000) Simultaneous determination of atenolol and amlodipine in tablets by high performance thin layer chromatography. *J Pharm Biomed Anal* **21,** 1137-42.
28. Bretnall 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.
29. 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.
30. Avadhanulu A B, Srinivas K S and Anjaneyulu Y (1996) Reverse phase HPLC determination of amlodipine besylate in drug and pharmaceutical dosage forms. *Indian Drugs* **33,** 36-40.
31. Patki R V, Tamhankar C P and Tipnis H P (1994) Simple and rapid HPLC estimation of amlodipine from pharmaceutical dosages. *Indian Drugs* **31,** 560-561.

32. Patel Y P, Patil S, Bhoir I C and Sundaresan M (1998) Isocratic simultaneous reversed phase HPLC determination of six drugs for combined hypertension therapy. *J Chromatogr A*, **828,** 283-6.
33. Zarapkar S S, Kolte S S and Rane S H (1997) High performance liquid chromatographic determination of amlodipine and atenolol simultaneously from pharmaceutical preparations. *Indian Drugs* **34,** 350-3.
34. Dhorda V J and Shetkar N B (1999) Reversed phase high performance liquid chromatographic determination of ramipril and amlodipine in tablets. *Indian Drugs* **36,** 638-41.
35. Halkar UP, Bhandari NP and Rane SH (1998) High performance liquid chromatographic determination of amlodipine and enalapril maleate from pharmaceutical preparations. *Indian Drugs*, **35,** 168-9.
36. Baranda AB, Jimenez RM and Alonso RM (2004) Simultaneous determination of five 1, 4-dihydropyridines in pharmaceutical formulations by high performance liquid chromatography – amperometric detection. *J Chromatogr* A **1031,** 275-80.
37. Lu P R, Bi H L and Xie Y Z (1995) Improved determination of the homogeneity of the content of luohuoxi tablets. *Yaowu Fenxi Zazhi* **15,** 42-3.
38. Khopade SA and Jain NK (2000). Difference spectrophotometric estimation of amlodipine besylate. *Indian Drugs* **37,** 351-3.
39. Gazy A A K (2004) Determination of amlodipine besylate by adsorptive square wave anodic stripping voltammetry on glassy carbon electrode in tablets and biological fluids. *Talanta* **62,** 575-82.
40. Mohammed Y E, Naglaa M E, Bahia A M and Nashwa G M (1998) Fluorimetric determination of amiodarone, amlodipine and propafenone. *Bull Fac Pharm* (Cairo University) **36,** 1-9.
41. Basavaiah K and Prameela H C (2003) Three new bromimetric methods for the estimation of famotidine, *Science Asia* 29, 147-3.
42. Basavaiah K, Chandrashekar U, Prameela HC and Nagegowda P (2003) Quantitative determination of propranolol with bromate and methyl orange. *Acta Ciencia Indica Chem* **29,** 25-30.
43. 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,** 47-153.
44. Basavaiah K and Nagegowda P (2003) Determination of captopil in pharmaceutical preparations using chloramine-T. *Bulg Chem Commun* **35,** 48-53.
45. Basavaiah K and Prameela H C (2003) Use of an oxidation reaction for quantitative determination of albendazole using Chloramine T and acid dyes, *Anal Sci* **19,** 779-84.
46. Basavaiah K and Manjunathaswamy J (2001) A facile and highly sensitive spectrophotometric method for the determination of some phenothiazine psychotropics using chloramine-T and indigo carmine. *Anal Sci* **17,** 963-7.
47. ICH Expert Working Group (1994) Text on validation of analytical procedures, pp1-8.