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Research Article

Quantitative Determination of Amlodipine Besylate without Derivatized in Pure Form and Tablet Dosage Forms with UV Spectrophotometric Method

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ABSTRACT:
The present work describes the development and validation of UV Spectrophotometric method for direct determination of underivatized amlodipine besylate (ADB) in pure and tablet dosage forms. The validation parameters of linearity, precision, accuracy, recovery, specificity, limit of detection and limit of quantification were studied. The range of quantification for proposed method was 2-17 µg/mL. The precision of method was calculated as the relative standard deviation (RSD) and less than 2 %, and accuracy (relative error) was better than 6 % (n = 6). The developed method was successfully applied for the assay of pharmaceutical dosage forms which do not require any preliminary separation or treatment of the samples. The RSD values for Norlopin® tablet (5 mg) and Norvasc® tablet (5 mg) was found to be less than 2 %. The results obtained from this method were compared with two reference method reported in literature and no significant difference was found statistically ($p>0.05$).

Keywords : Amlodipine besylate, tablets, UV spectrophotometric method

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1. INTRODUCTION

Amlodipine besylate (ADB), (4R,S)-3-ethyl 5-methyl 2-(2-amino-ethoxy-methyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methylpyridine-3,5-dicarboxylatemonobenzene sulphonate (its empirical formula is: $C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$, Fig.1), is a dihydropyridine type long acting channel blocker with slow onset of vasodilatory action [1-3].

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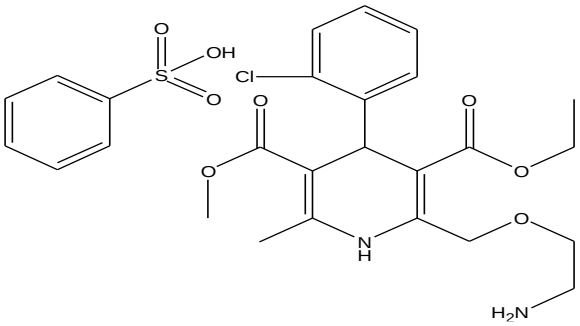

Figure 1. Chemical structure of amlodipine besylate (ADB)

It may also be used for dilated cardiomyopathy and exhibits ameliorating effects on plasma and myocardial catecholamines with a significant reduction of calcium deposition [4,5]. 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 [3-5]. Several analytical procedures are available in the literature for the analysis of ADB in pharmaceutical dosage, such as High performance liquid chromatography [6-12], high performance thin layer chromatography [13-15], electrochemical analysis [16,17] and titrimetric and spectrophotometric method using bromate-bromide mixture [18]. As far as sensitivity and economical methods of assay are concerned, the determination of ADB by spectrophotometric methods have been proposed based on the extractable ion-pair complex formation followed by extraction and charge-transfer complexes formation [19-26], complex reaction with 2,3-dichloro 5,6-dicyano 1,4-benzoquinone and ascorbic acid in N,N-dimethylformamide medium [27], oxidative coupling with 3-methyl 2-benzothiazoli- none hydrazone hydrochloride [28], complex formation with sodium hydroxide and ninhydrine [29,30], derivative spectroscopy [31-32], the reduction of iron (III) in acid medium [33] and application of oxidants [34]. However, many of these methods are limited in their applications because ADB was derivatizated with different substances. These procedures suffer from such disadvantages as poor selectivity, heating or extraction step and are expensive and time consuming. Therefore there is a need for a simple spectrophotometric method for the assay of ADB without derivatization in pharmaceutical preparations.

Therefore, in this study we are aim at developing simple and sensitive method, which would overcome the difficulties encountered in most spectrophotometric method. The UV Spectrophotometric method was developed for determination without derivatizated of ADB in tablet dosage forms and completely validated (by using linearity, precision, accuracy and sensitivity parameters). We also indicated that determination of ADB without derivatization in tablet dosage forms was possible. The proposed method is recommended for the routine analysis since it is rapid, simple, accurate and also sensitive and specific by no

derivatized. The results obtained this developed and validated method was statistically compared with two reference method [12,18].

2. MATERIALS AND METHODS

2.1. Equipments

A Thermospectronic (HEλIOSβ) double-beam UV-Visible spectrophotometer with a fixed slid width 2 nm and its data processing system was used. The curves of the UV-Visible spectra (N=6. Δλ=18.0 nm) of standard and tablet solutions were recorded in a 1 cm quartz cells between in wavelength ranges of 250-450 nm at scan speed of 600 nm/min.

2.2. Reagents and Solutions

ADB standard was kindly supplied by Novartis Pharmaceutical Industry (Ankara-Turkey). Purity of this substance checked by standard methods (melting point, NMR and IR spectra) and no impurities were found. Ethanol, acetonitrile and other analytical chemicals were purchased from Merck (Germany). Pharmaceutical dosage forms (Norlopin®, Norvasc®) containing ADB were obtained commercially in pharmacy and were claimed to contain 5 mg of the drug and other substance as excipien.

The stock solution of ADB was prepared in ethanol-acetonitrile (30/70, v/v) solvent mixture to a final concentration 100 μg/mL. For calibration, standard solutions containing 2, 4.5, 7, 9.5, 12 and 17 μg/mL were daily prepared by diluting the stock solution to a constant volume with ethanol-acetonitrile mixture. The quality control samples (QC) were prepared at 5.75, 10.75 and 14.50 μg/mL concentrations from stock solution of ADB. These samples were used in analysis of standard samples as quality controls for the purpose of checking recovery of analyte in the daily analyses of standard samples.

2.3. Tablets

Twenty tablets each of Norvasc and Norlopin tablet were carefully weighed and ground to finely divided powders. Accurate weights equivalent to 5 mg ADB was dissolved in 100 mL ethanol-acetonitrile (30/70, v/v) solvent mixture, mixed well. Solution was stand for about 10-15 min and filtered up using 12 mm filter paper and then the solution volume was made up to the 250 mL with same solvent mixture**.** The final concentration of these solutions was 20 μg/mL.

2.4. Method Validation

The validation of method was carried out by establishing specifity, linearity, recovery values, limits of detection (LOD), limit of quantification, within- and between-day precision and accuracy according to International Conference on Harmonization guidelines (ICH) [35] for validation of analytical procedures.

3. RESULTS AND DISCUSSION

3.1. Method Development

To develop a sensitive the UV spectrophotometric method, various solvent systems were tried, such as water, methanol, ethanol and acetonitrile individually or in combinations of different proportions. The final decision of

using ethanol-acetonitrile (30/70, v/v) mixture was based on sensitivity, interference, easy of preparation, suitability for drugs, content estimation and cost in that order. Methanol-asetonitrile mixture can be used. But, methanol was abandoned because of its toxicity.

The standard solutions were scanned wavelength range of 250-450 nm in the 2 nm band width against a similarly prepared blank in spectrophotometer. The λ_{max} was found to be 360 nm. This wavelength was used for all measurements. The UV absorption spectrums of ADB were monitored: the one broad shouldered peak with maximum wavelengths at 360 nm in ethanol-acetonitrile (30/70, v/v) solvent medium was observed as shown in Fig. 2 and 3. However, these shouldered peaks were not appeared when the bandwidth was chosen as 5 nm and it was monitored a single well-defined maximum peak in UV spectrums. These maximum wavelengths were broader at low concentrations so that analysis couldn't be performed; at higher concentrations the peaks were sharper but analysis couldn't be carried out because of the shoulder.

3.2. Linearity

Linearity of the assay was demonstrated over concentration range of 2 to 17 µg/mL ADB in six replicate at separate concentrations. The linearity was evaluated by linear regression analysis, which was calculated by least square regression method. Linear range was determined by plotting the absorbance at its λ_{max} versus sample concentration. The regression equation of calibration curve and regression coefficient (r) for standard samples were found as A=0.0122x+0.0298 and 0.999, respectively (Fig.2.).

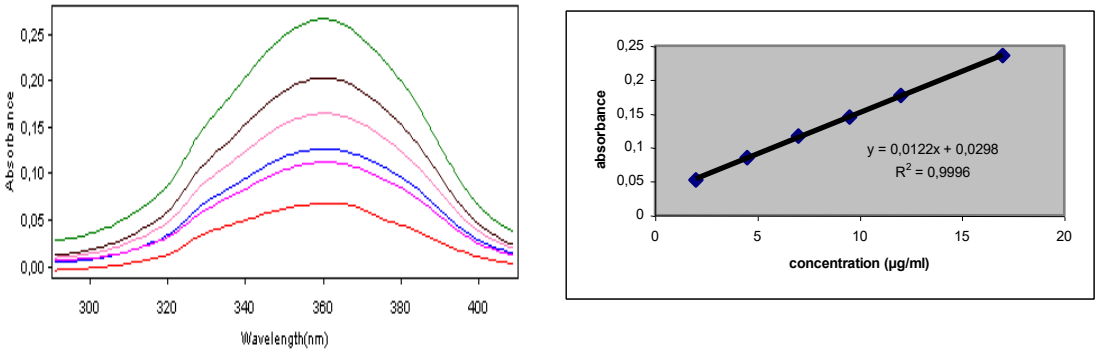

Figure 2. UV Spectrums and calibration curve of standard solutions of ADB

3.3. Limit of Detection and Quantification

Spectrophotometrically, LOD and LOQ were determined by an empirical method that consisted of analyzing a series of standard solutions containing decreasing amounts of ADB. This method, although not applicable for complex matrices, is useful for simple samples. The LOQ was defined as the lowest concentration on the calibration curve that presented a RSD that did not exceed 10% and the LOD was defined as the lowest concentration that presented a RSD that did not exceed 20%. LOQ and LOD values for standard samples were approximately deemed to be 2 µg/mL and 1.5 µg/mL, respectively.

3.4. Precision and Accuracy

Repeatability was evaluated by assaying samples, at same concentration and during the same day. Assay precision and accuracy were assessed by assaying three quality control samples (5.75, 10.75 and 14.50 µg/mL) in six replicate on one day for within-day precision and once daily for six days for between-day precision. Concentrations of ADB in quality control samples were determined by application of the appropriate standard curve obtained on that occasion. The within-day and between-day relative standard deviation (RSD) were <2% for standard samples (n=6). Precision studies of UV spectrophotometric method showed acceptable RSD values. The accuracy of this analytic method for assay determination was checked for quality control samples and the relative errors for accuracy were < 6 % (Table 1).

Table 1. Precision and accuracy of the method for determination of ADB (n=6)

Added (µg/mL)	Within-day			Between-day		
	Found±SD[a] (µg/mL)	Precision RSD %	Accuracy[c] RE%	Found±SD[a] (µg/mL)	Precision RSD%	Accuracy RE%
5.75	5.91±0.15	1.96	2.85	6.08±0.14	1.82	5.65
10.75	10.68±0.13	1.04	-0.65	10.80±0.20	1.57	0.45
14.50	14.17±0.21	1.30	-2.24	14.41±0.14	0.88	-0.63

SD[a]: Standard deviation of six replicate determinations, RSD: % Relative standard deviation
Accuracy[c]: %Relative Error

3.5. Recovery

The accuracy was determined by recovery of known amounts of ADB reference standard added the tablet samples at the beginning of the process. For recovery study, the tablet solutions according to the procedure described at Section *2.4.* was prepared. The tablet solutions to 2.5, 5.0 and 7.5 µg/mL concentrations were transferred in ADB standard solution to 7.5 µg/mL concentration. The final concentrations of these solutions were 10, 12.5 and 15 µg/mL. The absorbance of solutions prepared was measured with spectrophotometer. The percentage recovery of added ADB standard was calculated by comparing the found and added concentrations (C_{found}/C_{added}x100) in each case. The mean recoveries and the RSD values for these recovery values were found ranged 99.0- 110.0% and 1.0-2.0%, respectively. No interference from the common excipients was observed. The results showed in Table 2.

Table 2. Recovery values of ADB in pharmaceutical preparations (n=6)

Formulation name	Taken (μg/mL)	Added (μg/mL)	Found±SD (μg/mL)	Recovery±RSD (%)
Norlopin-5 mg	2.50	7.5	9.93±0.10	99.3±1.00
	5.00	7.5	12.54±0.15	100.3±1.19
	7.50	7.5	16.27±0.28	108.5±1.72
Norvasc-5 mg	2.50	7.5	9.97±0.18	99.7±1.80
	5.00	7.5	12.61±0.14	100.9±1.11
	7.50	7.5	15.04±0.18	100.3±1.19

SD: Standard deviation of six replicate determinations, RSD: Relative standard

3.6. Stability

To determine of the stability of ADB standard solutions at ambient and refrigeration temperature, ADB solutions to 4.5, 12 and 16 μg/mL concentrations and stock solution were stored at ambient, 4°C and deepfreeze for 24 h and 1 week. Then the stability measurements were carried out. The results were evaluated comparing these measurements with those of standards and expressed as percentage deviation. The results indicated that the stock solution and solutions to 4.5, 12 and 16 μg/mL concentrations were found to be stable after one week with no significant change in concentration.

3.7. Interferences study

The effects of common excipients and additives were tested for their possible interferences in the assay of ADB. In addition to the active ingredient, amlodipine besylate, each tablet contains the following inactive ingredients: microcristaline cellulose, dibasic calcium phosphate anhydrous, sodium starch glycolate and magnesium stearate. It has been determined any interference of these substances at the levels found in dosage forms.

3.8. Comparison of Proposed Method with Two Reference Methods

The suggested UV spectrophotometric method was applied to the quantitative determination of ADB in tablet dosage forms. Also the developed and validated method was statistically compared with two reference methods in literature [12, 18]. One of reference methods is HPLC method (I reference method) and other is indirect spectrophotometric method (II reference method). In the indirect spectrophotometric method, ADB had been reacted with bromate-bromide mixture in HCl medium and then the unreacted bromine was measured absorbance with spectrophotometer.

The proposed method was linear over the concentration range 2-17 μg/mL. The I and II reference method had been found as linear over the concentration range 7.55-241.6 μg/mL and 1.25-12.5 μg/mL, respectively. The average recovery value for ADB in 5 mg tablet composites ranged to 98.0- 100.0% and the RSD (Table 3) values obtained within- and between-day assay of quality control samples ranged from 0.5 to 2.0% for proposed method, which indicated high accuracy and precision.

Table 3: Statistical comparison (Student *t*-test and F-test) of two reference methods and proposed method

	Method	n	X	RSD (%)	Std. Error Mean	*t*-values	P-Values
First comparision	Proposed method	10	101.36	2.2498	1.2989	-2.149	0.098
	HPLC	10	97.90	1.6573	0.9568		
Second comparision	Proposed method	10	101.37	2.2498	1.2989	0.016	0.988
	Metod C	10	101.39	1.7128	0.9888		

n: Number of determination, [a]X: mean, RSD: Relative Standard Deviation, t_c: Calculated t values
H_o: Hypothesis: no statically significant difference exists between two methods $t_t > t_c$; H_o hypothesis in accepted (α=0.05)

In I and II reference method**,** the average recovery value had been ranged to 97.58-99.76% and 100.0-101.0% for ADB in 5 mg tablet composites, respectively, and also the RSD values had been ranged from 0.85 to 1.04% and from 0.5 to 1.26%, respectively. The results obtained were compared statistically by Student's *t*-test (for accuracy) and variance *F*-test (for precision) with reference methods [12, 18] at 95 % confidence level with five degrees of freedom**.** The results showed that the P-values were higher than P=0.05 indicating that there was no significant difference between the proposed and reference methods (Table 4).

Table 4: Comparision of the proposed method with existing spectrophotometric methods for the determination of ADB in pharmaceutical formulations

Reagent	λ_{max} nm	Range μg mL^{-1}	RSD %	Reference
Bromothymol Blue[a]	405.0	5-40	0.99	28
MBTH[b]	630.0	5-40	0.87	28
Eriochrome Black T[a]	495.0	5-50	0.88	22
Indigocamine[a]	590.0	25-150	0.87	22
Rhodizonic acid[a]	450.0	100-1500	-	20
p-Chloranilic acid	540.0	100-600	0.52	26
Sodium hydroxide	465.0	20-100	1.90	29
Underivatized ADB	360.0	2-17	0.5-2.0	This work

[a]Extractive method, [b] 3-Methyl-2-benzothiazolinone hydrazone hydrochloride

Also, the newly proposed method for the determination of ADB in pharmaceutical preparations compared favorably with other spectrophotometric methods used derivatized (Table 4). It was found that method has advantages of high performance (RSD <2 % for pure samples and dosage forms) and fast response. Therefore can be used applied as an alternative to the existing methods.

4. CONCLUSIONS

Today, HPLC method with different detection and spectrophotometric methods are widely used as analytical techniques for determination of ADP in tablet dosage forms. However, the determination of ADP reacted with different

substances in the most of these methods has been made. For this, the UV spectrophotometric method were developed and completely validated for quantitative determination of underivatized ADB in tablet dosage forms in this study and also the results show good recoveries of ADB from the spiked tablet samples, without any interference from the excipients. The proposed method are rapid, simple, accurate, reproducibility and convenient since it do not require any special working conditions. For this reasons, it can be used for determination from pharmaceutical preparations of ADB in routine quality control measurement, where economy and time are essential.

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Conflict of Interest

Author has no personal financial or non-financial interests.

REFERENCES

1. Reynolds EF, In:Martindale-The Extra Pharmacopoeia (31.st ed.) The Royal Pharmaceutical Society, London, 1996, pp. 819-820.

2. European Pharmacopoeia, third ed., Council of Europe, Strasbourg, 2001 supplement, pp. 431-432.

3. Civantos B, Aleixandre A, Blood pressure and α-vascular reactivity in hypertensive rats treated with amlodipine and dietary Ca, Europ. J Pharmacol. 2004; 489: 101-110.

4. Khattar RS, Senior R, Swales JD, Lahiri A, Value of ambulatory intra-arterial blood pressure monitoring in the long-term prediction of left ventricular hypertrophy and carotid atherosclerosis in essential hypertension, J. Hum.Hypertens. 1999; 3: 111-116.

5. Resnick LM, Laragh JH, Renin, calcium and the pathophysiologic basis of antihypertensive theraphy, Am. J. Cardiol. 1985; 56: 68-74.

6. Patki RV, Tamhanker CP, Tipnis HP, Simple and rapid high performance liquid chromatographic estimation of amlodipine in pharmaceutical dosage forms, Indian Drugs 1994; 3: 560-565.

7. Sankar SR, Nanjan MJ, Vasudevan M, Shaat N, Suresh B, Simultaneous estimation of atenolol and amlodipine in formulations by reversed phase-HPLC. Indian J. Pharm. Sci. 1997; 59: 171-175.

8. Dhorda VJ, Shetkar NB, Reversed phase liquid chromatographic determination of ramipril and amlodipine in tablets. Indian Drugs 1999; 36: 638-642.

9. Avadhanulu AB, Srinivas JS, Anjaneyulu Y, Reverse phase HPLC determination of Amlodipine besylate in drug and its pharmaceutical dosage forms. Indian Drugs 1996; 33:36-40.

10. Baranda AB, Jimenez RM, Alonso RM, Simultaneous determination of Five 1,4-dihydropyridines in pharmaceutical formulations by high-performance

liquid chromatography-amperometric detection. J. Chromatogr. A 2004; 1031: 275-280.
11. Malesuik MD, Cardoso SG, Bajrski L, Lanzanova FA, Determination of Amlodipine in Pharmaceutical Dosage Forms by liquid Chromatography and Ultraviolet Spectrophotometry. J AOAC Inter. 2006; 89: 359-364.
12. Basavaiah K, Chandrashekar U, Nagegowda P, Spectrophotometric and High performance liquid chromatographic determination of amlodipine besylate in pharmaceuticals. Science Asia. 2005; 31: 13-21.
13. Chandrashekhar TG, Rao PSN, Smrita K, Vyas SK, Dutt C, Analysis of amlodipine besylate by HPTLC with fluorimetric detection: a sensitive method for assay of tablets. J Planar Chromatogr Mod. TLC 1994; 7: 458-462.
14. Argekar AP, Powar SG, Simultaneous determination of atenol and amlodipine in tablets by high-performance thin-layer chromatography. J Pharm Biomed Anal. 2000; 21: 1137-42.
15. Gowri N, Vaidhyalingam V, Shantha A, HPTLC method for the simultaneous estimation of amlodipine besylate and Benazepril hydrochloride in tablets. Indian Drugs 2003; 40: 645-648.
16. Altiokka G, Dogrukol-Ak D, Tunçel M, Aboul-Enein HY, Determination of amlodipine in pharmaceutical formulations by differential-pulse voltammetry with a glassy carbon electrode. Archiv der Pharmazie 2002; 335: 104-8.
17. Gazy AAK, Determination of Amlodipine besylate by adsoptive square-wave anodic stripping voltammetry on glassy carbon electrode in tablets and biological fluids. Talanta 2004; 62: 575-82.
18. Basavaiah K, Chandrashekar U, Nagegowda P, Titrimetric and modified spectrophotometric methods for the determination of amlodipine besylate using bromate-bromide mixture and two dyes. Science Asia 2006; 32: 271-278.
19. Prabhakar AH, Giridhar R, A spectrophotometric method for the determination of amlodipine besylate in pure form and in tablets. Indian Drugs. 2002; 39: 204-208.
20. Singhvi I, Chaturvedi SC, Visible spectrophotometric methods for estimation of amlodipine besylate in tablets, Indian J Pharm Sci. 1998; 60: 309-310.
21. Singhvi I, Chaturvedi SC, Spectrophotometric method for determination of amlodipine besylate and benidipine hydrochloride from tablets. Indian J Pharm Sci. 1999; 61: 190- 191.
22. Reddy MN, Rani GT, Rao KVSP, Sankar DG, Sreedhar K, Extractive spectrophotometric determination of amlodipine using erichrome black T and indigocarmine. Indian J Pharm Sci. 1997; 59: 188-9.
23. Çetin G, Sungur S, A spectrophotometric method for the determination of amlodipine in pharmaceutical formulations. Sci Pharm. 1995; 63: 93-8.
24. Lokesh BVS, Reddy MN, Sankar DG, Sreedhar K, Extractive spectrophotometric determination of amlodipine. East Pharm. 1996; 39: 125-6.

25. Golcu A, Yuccesoy C, Serin S, The use of charge transfer complexation in the spectrophotometric determination of amlodipine besylate. Sci Pharm. 2000; 68: 225-46.
26. Rahman N, Azmi SN, Spectrophotometric determination of amlodipine besylate by charge transfer complex formation with p-chloranilic acid. Anal Sci. 2000; 16: 1353-56.
27. Rahman N, Hoda MN, 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. J Pharm Biomed Anal. 2003; 31: 381-92.
28. Sridhar K, Sastry CSP, Reddy MN, Shankar DG, Srinivas KR, Spectrophotometric determination of amlodipine besylate in pure form and tablets. Anal Lett. 1997; 30: 121-133.
29. Meyyanathan SN, Joel J, Scaria S, Sowmya S, Suresh B, Simple spectrophotometric analysis of amlodipine besylate. Indian Drugs 1998; 35: 296-7.
30. Rahman N, Azmi SNH, Spectrophotometric method for the determination of amlodipine besylate with ninhydrin in drug formulations. Il Farmaco. 2001; 56: 731-5.
31. Prasad CVN, Parihar C, Chowdhary TR, Purohit S, Parimoo P, Simultaneous determination of atenolol-amlodipine and haloperidol-trihexaphenidyl in combined tablet preparation by derivative spectroscopy. Pharm Pharmacol Commun. 1998; 4: 325-30.
32. Prasad CVN, Saha RN, Parimoo P, Simultaneous determination of amlodipine-enalapril maleate and amlodipine-lisinopril in combined tablet preparations by derivative spectrophotometry. Pharm Pharmacol Commun. 1999; 5: 383-8.
33. Basavaiah K, Chandrashekar U, Prameela HC, Sensitive spectrophotometric determination of amlodipine and felodipine using iron (III) and ferriciyanide. Il Farmaco. 2003; 58: 141-8.
34. Rahman N, Singh M, Hoda MN, Application of oxidants to the spectrophotometric determination of amlodipine besylate in pharmaceutical formulations. IL Farmaco. 2004; 59: 913-9.
35. Validation of Analytical Procedures, Proceedings of the International Conference on Harmonisation (ICH). Commision of the European Communities, 1996.