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Spectrophotometric method for the determination of amlodipine besylate with ninhydrin in drug formulations

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Abstract

A spectrophotometric method has been developed for the determination of amlodipine besylate in pure form and in pharmaceutical preparations. The method is based on the reaction of the primary amino group of the drug with ninhydrin in *N*,*N*′-dimethylformamide (DMF) medium producing a coloured complex which absorbs maximally at 595 nm. Beer's law is obeyed in the concentration range of 10–60 μg ml^{-1} with RSD of 0.66% and molar absorptivity of 6.52×10^3 l mol^{-1} cm^{-1}. All variables were studied in order to optimize the reaction conditions. The proposed method has been applied successfully to the analysis of the bulk drug and its dosage forms. No interference was observed from common pharmaceutical adjuvants. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision.

Keywords: Amlodipine besylate; Ninhydrin; Pharmaceutical preparations; Spectrophotometry

1. Introduction

Amlodipine besylate 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. It is chemically known as (4*R*,*S*)-3-ethyl 5-methyl 2-(2-amino-ethoxy-methyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl pyridine-3,5-dicarboxylate monobenzene sulphonate and is official in Martindale The Extra Pharmacopoeia [1]. The main effects of this drug are confined with peripheral and coronory vasodilator properties. Therefore, the analysis of its dosage forms is very important.

The assay procedure listed in European Pharmacopoeia for amlodipine besylate describes the reversed phase high performance liquid chromatographic method [2] for determination of the drug in bulk and pharmaceutical formulations. Other methods based on high performance liquid chromatography [3–8], reversed phase high performance liquid chromatography [9–11], high performance thin layer chromatography [12–15], gas chromatography [16], gas chromatography coupled with mass spectrometry [17], liquid chromatography coupled with tandem mass spectrometry [18] and fluorimetry [19] have been described in the literature. Spectrophotometry as a quantitative analytical method still belongs to the most frequently used analytical techniques in pharmaceutical analysis. It provides practical and significant economic advantages over other methods. Prasad and coworkers [20,21] have developed two derivative spectrophotometric methods in combined tablet preparations. The drug content in pharmaceutical preparations has been determined by two spectrophotometric methods [22]. The first method is based on the formation of an ion–pair complex of the drug with bromothymol blue, which was extracted in chloroform and measured at 405 nm. The other method involved the formation of an oxidative coupling product of the drug with 3-methyl-2-benzothiazolinone hydrazone–HCl in the presence of ceric ammonium sulfate. The charge-transfer reaction of amlodipine with π-acceptors such as *p*-chloranilic acid [23] and chloranil

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[24] has been used for its assay in bulk and pharmaceutical formulations. Recently, Jain and Agrawal [25] have proposed spectrophotometric methods for simultaneous determination of amlodipine besylate and lisinopril in tablet formulations based on multicomponent mode of analysis over the range 300–190 nm using four sampling points at 300, 271, 242 and 213 nm. The other extractive spectrophotometric methods [26–30] are also utilized for its assay in dosage forms. However, many of these methods are limited in their applications or rather much tedious and time consuming. There is, therefore, a need for a simple spectrophotometric method for the assay of amlodipine besylate.

The present paper describes a rapid, simple and sensitive visible spectrophotometric method for the determination of amlodipine besylate. The determination is based on the reaction of the primary amino group of the amlodipine besylate with ninhydrin in N,N′-dimethylformamide medium.

2. Experimental

2.1. Apparatus

A Milton Roy Spectronic 20D$^+$ spectrophotometer (Milton Roy Co., USA) was used for all absorbance measurements. A water bath shaker (NSW 133, India) was used to control the heating temperature for colour development.

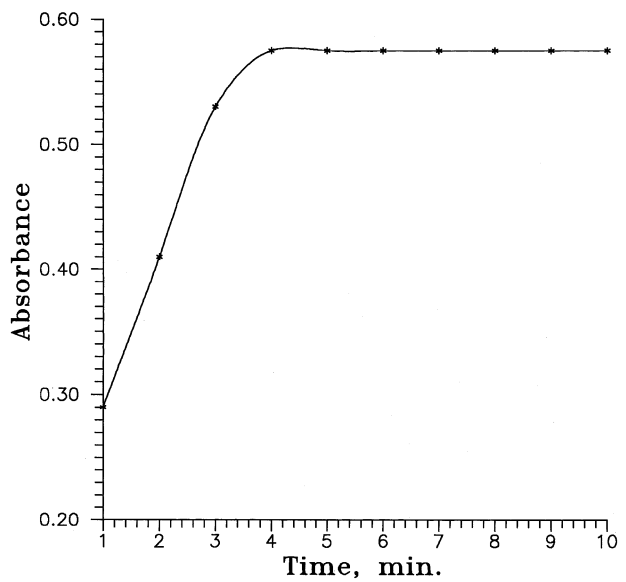

Fig. 1. Effect of heating time on the formation of coloured product (50 μg ml^{-1} drug + 9.0 × 10^{-3} M ninhydrin).

2.2. Reagents and standards

All the reagents used were of analytical grade. A 0.1% amlodipine besylate (Wockhardt Ltd., India) solution was prepared in DMF. A 0.06 M ninhydrin (E. Merck) solution was also prepared in DMF. Freshly prepared ninhydrin solution was always used.

2.3. Procedure for the assay of amlodipine besylate

Aliquots of 0.10–0.60 ml amlodipine besylate standard solution (1 mg ml^{-1}) were pipetted into a series of boiling test tubes. To each test tube 1.5 ml of ninhydrin solution was added, mixed well and heated on a water bath at 100 ± 1°C for 5 min. After heating, the solutions were cooled at room temperature and transferred to a 10 ml volumetric flask and diluted to volume with DMF which corresponds to 10–60 μg ml^{-1} concentration in the final reaction solution. The absorbance was measured at 595 nm against a reagent blank treated similarly. The concentration of amlodipine besylate was calculated from calibration graph.

2.4. Procedure for the assay of amlodipine besylate in pharmaceutical preparations

Twenty tablets were accurately weighed and powdered. A portion equivalent to 50 mg amlodipine besylate was stirred with 20 ml of DMF, let stand for 10 min, the residue was filtered on a Whatman no. 42 filter paper and washed with DMF. The filtrate and washing were diluted to volume in a 50 ml volumetric flask and subjected to the procedure for determination.

3. Results and discussion

The ninhydrin has been known as a reagent for the detection of amino acids and amines since 1910 and therefore, a number of theories have been put forward to explain the mechanism of its reaction. It was suggested that the reactions of ninhydrin with amine, amino acids and imino acids all proceed by the same mechanism [31] to give diketohydrindylidene–diketohydrindamine or the Ruhemann's purple. This compound would further react with amino group to give the product which absorbed maximally at 595 nm.

The optimum conditions for determination of amlodipine besylate were established via a number of preliminary experiments.

3.1. Effect of heating time

A 0.5 ml of 0.1% amlodipine besylate was mixed with 1.5 ml of 0.06 M ninhydrin solution. The reaction mixture was heated on a water bath at 100 ± 1°C. A

coloured product was obtained and the intensity of the colour was reached to maximum after four min of heating and remained constant up to 10 min. The content of the boiling tube was transferred to 10 ml standard volumetric flask and diluted to volume with DMF. Hence, the absorbance was measured after 5 min of heating. The results are shown in Fig. 1.

3.2. Effect of ninhydrin concentration

To 0.5 ml of 0.1% amlodipine besylate, different volumes (0.2–1.5 ml) of 0.06 M ninhydrin were added. The reaction mixtures were heated for 5 min on a water bath at 100 ± 1°C. The coloured product was diluted to 10 ml with DMF and the absorbance was measured against a reagent blank at 595 nm. The results showed that the highest absorbance was obtained with 1.3 ml which remained unaffected with higher amounts of ninhydrin (Fig. 2). A 1.5 ml of the reagent, therefore, was added for the determination.

3.3. Analytical data

Beer's law limit, molar absorptivity, detection limit, regression equation and correlation coefficient were obtained by least square treatment of results. The linear relationship was found between absorbance at λ_{max} and concentration of drug in the range 10–60 μg ml^{-1}. Regression analysis of Beer's law plot at 595 nm yielded the regression equation, $A = 0.001 + 1.149 \times 10^{-2}C$ (where C = concentration in μg ml^{-1}). High values of correlation coefficient ($r = 0.9999$) and small value of intercept validated the linearity of calibration curve and

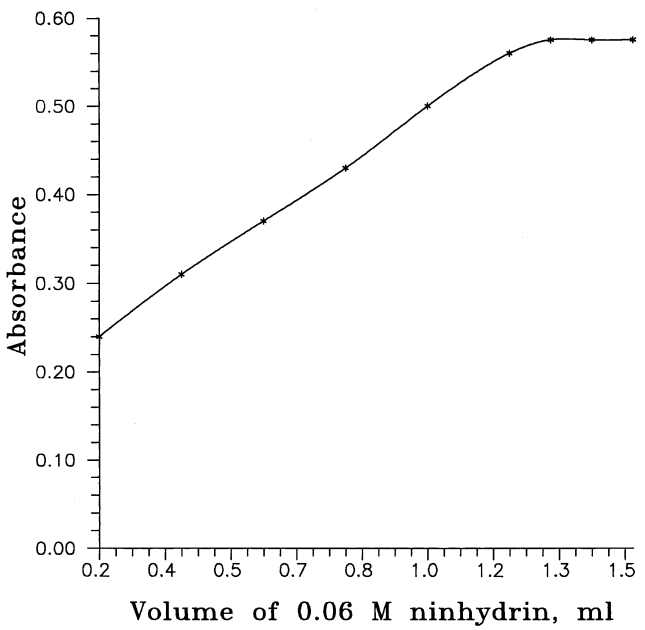

Fig. 2. Effect of ninhydrin concentration on the absorbance at λ_{max} of the coloured product (drug = 50 μg ml^{-1}).

obedience to Beer's law. The confidence limit for intercept value at 95% confidence level was computed using the relation $a \pm tS_a$ [32] and found to be $0.001 \pm 5.065 \times 10^{-4}$. This indicated the high reproducibility of the proposed method. The molar absorptivity and the detection limit were found to be 6.52×10^3 l mol^{-1} cm^{-1} and 0.22 μg ml^{-1}, respectively. The detection limit was calculated using the equation [33,34]:

$$\text{Detection limit} = \sqrt{S_o^2 \frac{n-2}{n-1}} \frac{t}{b}$$

where n = number of samples; b = slope of line of regression; t = student's t-value at 95% confidence level and S_o^2 = variance. The detection limit and the slope of the calibration graph indicated the good sensitivity of the method and the small value of variance, 9.96×10^{-7}, confirmed the small degree of scattering of experimental data points around the line of regression.

The reproducibility of the proposed method was checked with ten replicate determinations of 0.4 mg of amlodipine besylate, which gave average recovery of 100.09% with relative standard deviation of 0.66%.

Beer's law limit and percent relative standard deviations of the proposed method are comparable with that of other spectrophotometric procedures described in the literature (Table 1). It is worth noticing that Beer's law limit of the proposed method is quite comparable with high precision.

In order to investigate the applicability of this spectrophotometric method to the determination of amlodipine besylate alone or in pharmaceutical preparations, the effect of the presence of some common excipients such as starch, talc, lactose and magnesium stearate was studied. It was found that the common excipients did not interfere in the determination.

The validity of the proposed method for the determination of drug in pharmaceutical preparations was tested by applying the standard addition technique. The results obtained were reproducible with low relative standard deviations (0.21–0.45%) and the mean recoveries were in the range of 99.96–100.12%. The results are reported in Table 2.

Commercial formulations containing amlodipine besylate were assayed successfully by the proposed method. The results were compared to those obtained by the reference method [35], based on reaction with sodium hydroxide, which are listed in Table 3. The performance of the proposed method was judged by calculating t- and F-values [36]. At 95% confidence judged level, the calculated t- and F-values do not exceed the theoretical values indicating no significant difference between the proposed method and the reference method.

Amlodipine besylate, as all dihydropyridines, is sensitive to day and UV light and undergoes decomposition

Table 1
Comparison of Beer's law limit and precision of proposed method with those from other spectrophotometric methods for the determination of amlodipine besylate

Reagent	λ_{max} (nm)	Beer's law limit (μg ml^{-1})	RSD (%)	References
Bromocresol green [a]	409.0	0–80		[29]
Bromophenol blue [a]	409.0	0–80		[29]
Methylene blue [a]	668.2	0–80		[29]
Sodium hydroxide	456.0	20–100	1.90	[35]
Bromothymol blue [a]	405.0	5–40	0.99	[22]
MBTH [b]	630.0	5–40	0.87	[22]
Ninhydrin	595.0	10–60	0.66	present method

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

Table 2
Spectrophotometric determination of amlodipine besylate in pharmaceutical preparations by standard addition method

Preparations [a]	Amount taken (μg ml^{-1})	Amount added (μg ml^{-1})	Total amount found (μg ml^{-1})[b]	Recovery (%)	RSD (%) [b]
Myodura-10	10.00	20.00	29.99	99.96	0.28
	20.00	20.00	39.99	99.97	0.21
Amlong-10	10.00	20.00	30.02	100.06	0.44
	20.00	20.00	40.05	100.12	0.45
Amlogard-10	10.00	20.00	30.02	100.06	0.44
	20.00	20.00	40.02	100.05	0.33
Amlosun-10	10.00	20.00	30.02	100.06	0.44
	20.00	20.00	40.02	100.05	0.45
Amloz-10	10.00	20.00	30.02	100.06	0.44
	20.00	20.00	40.05	100.12	0.45
Amtas-10	10.00	20.00	29.99	99.96	0.28
	20.00	20.00	39.99	99.97	0.21

[a] Drug samples are in tablet form.
[b] Five independent analyses.

Table 3
Spectrophotometric determination of amlodipine besylate in pharmaceutical preparations by the proposed method and reference method

Pharmaceutical preparations	Labelled amount (mg)	Proposed method		Reference method [35]		t_{calc} [b]	F_{calc} [c]
		Recovery (%)	RSD (%) [a]	Recovery (%)	RSD (%) [a]		
Myodura	10	100.02	0.42	100.02	0.27	0.023	2.33
Amlong	10	99.97	0.34	100.02	0.27	0.128	1.55
Amlogard	10	100.10	0.50	100.02	0.35	0.047	2.08
Amlosun	10	100.10	0.50	100.02	0.35	0.139	2.08
Amloz	10	100.15	0.42	100.05	0.24	0.238	2.94
Amtas	10	100.02	0.42	100.15	0.40	0.239	1.06

[a] Five independent analyses.
[b] Theoretical value for t at 95% confidence level is 2.306.
[c] Theoretical value for F at 95% confidence level is 6.39.

to give pyridine derivative. The drug and its photoproducts contain amino group. Therefore, the proposed method is unable to distinguish the drug from its photoproducts. However, the determination was done under a condition where contact with light was completely avoided.

It is concluded that the proposed method is sensitive, simple, rapid and selective for determination of amlodipine besylate in bulk and commercial formulations with good accuracy and precision. The short analysis time and low cost are the main advantages of the developed method for routine analysis.

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