



ORIGINAL ARTICLE

Utility of oxidation–reduction reaction for the spectrophotometric determination of amlodipine besylate

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Abstract A simple, rapid, accurate, precise and sensitive spectrophotometric method for the determination of amlodipine besylate (ADB) in bulk sample and in dosage forms is described. The method is based on oxidation of the drug by potassium permanganate in acidic medium and determine the unreacted oxidant by measuring the decrease in absorbance for five different dyes; methylene blue (MB), acid blue 74 (AB), acid red 73 (AR), amaranth dye (AM) and acid orange 7 (AO) at a suitable λ_{\max} 663, 609, 511, 520, and 484 nm, respectively. Regression analysis of Beer's law plots showed good correlation in the concentration ranges 1.0–24, 0.9–22, 1.2–26, 0.9–12.8 and 1.0–14 $\mu\text{g ml}^{-1}$, respectively. The apparent molar absorptivity, Sandell sensitivity, detection and quantitation limits were calculated. For more accurate results, Ringbom optimum concentration ranges were 1.2–22.4, 1.1–20, 1.4–24.5, 1.0–12.3 and 1.3–13.2 $\mu\text{g ml}^{-1}$, respectively. Statistical treatment of the results reflects that the proposed procedures are precise, accurate and easily applicable for the determination of amlodipine besylate in pure form and in pharmaceutical preparations.

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1. Introduction

Amlodipine besylate is 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridindicarboxylate-3-ethyl-5-methyl ester mono-benzene sulphonate. It is a new calcium

channel-blocking agent with vasodilator activity similar to that of nifedipine (Reynolds, 1996). It is mainly used for its antian-ginal, antihypertensive and antiarrhythmic activity. The drug in pure form and its formulations are not official in USP pharmacopoeia, and therefore require much more investigation. Different analytical methods that have been reported for its determination including, high-performance liquid chromatography (Baranda et al., 2004, 2005; Sudhakar et al., 2006; Zarghia et al., 2005; Josefsson et al., 1995; Naidu et al., 2005; Avadhanul et al., 1996; Sankar et al., 1997), liquid chromatography coupled with tandem mass spectrometry (Yasuda et al., 1996), gas liquid chromatography (Bersford et al., 1987), gas chromatography coupled with mass spectrometry (Feng et al., 1998), high-performance thin layer liquid chromatography (Chandrashekhara et al., 1994; Pandya et al., 1995; Ilango et al., 1997) high-performance capillary electrophoresis (Jiang et al., 2003) and

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fluorimetry (Mohamed et al., 1998). Visible spectrophotometric methods are commonly used in industrial laboratories because of their simplicity, selectivity and sensitivity. The amlodipine besylate in pharmaceutical preparations was determined by the spectrophotometric method (Sridhar et al., 1997; Rahman et al., 2004) involving oxidation of the drug, voltammetrically (Gazy, 2004). A number of other extractive spectrophotometric methods (Lokesh et al., 1996; Reddy et al., 1997; Singhvi and Chaturvedi, 1998, 1999; Rahman and Azmi, 2000, 2001; Prabhakaran and Giridhar, 2002) have been also reported. However, some of these methods are somewhat tedious and time consuming. Therefore, the need for a fast, low cost, accurate, precise and sensitive method is obvious, especially for a routine quality control analysis of pharmaceutical products containing ADB.

All five dyes, methylene blue (MB), basic blue 9 [122965-43-9]; acid blue 74 (AB 74), indigocarmine, indigo-5,5'-disulfonic acid disodium salt [860-22-0]; acid red 73 (AR 73), brilliant crocein MOO C.I. 27290; amaranth (AM), acid red 27, azorubin S, [915-67-3] and acid orange 7, (AO), orange II sodium salt [633-96-5] are well known for their high absorptivity and have been utilized for estimation of excess oxidant. The work aims to demonstrate a simple, rapid, accurate, precise and sensitive spectrophotometric method suitable and convenient for the determination of amlodipine besylate in pure and in dosage forms.

2. Experimental

2.1. Apparatus

All the absorption spectral measurement were made using JASCO V-530 (UV-VIS) spectrophotometer (Japan), with scanning speed 400 nm min^{-1} and band width 2.0 nm , equipped with 10 mm matched quartz cells.

2.2. Reagents

All chemicals used were of analytical or pharmacopoeial grade purity and bidistilled water was used. Standard amlodipine besylate was obtained from Egyptian International Pharmaceutical Industries Co. (EIPICO) 10th of Ramadan City, Egypt. Stock amlodipine besylate solution ($100 \mu\text{g ml}^{-1}$) was prepared by dissolving 0.01 g in bidistilled water and adjusted to 100 ml with bidistilled water in 100 ml measuring flask. Working solutions of lower concentration were prepared by serial dilutions.

Aqueous solutions of 10^{-3} M AB (Merck), and AO, AM and AR (Aldrich), or 10^{-4} M for MB (Merck) were prepared by dissolving an appropriate weight in 100 ml bidistilled water. A stock ($5.0 \times 10^{-4} \text{ M}$) solution of KMnO_4 (Aldrich), was freshly prepared by dissolving an accurate weight in bidistilled water, and standardized as recommended (Basset et al., 1986).

A solution of 0.2 M H_2SO_4 , was prepared by adding exact volume from stock (98%) concentrated acid to bidistilled water, cooled to room temperature, transferred to 500 ml measuring flask, diluted to the mark and standardized as recorded (Basset et al., 1978).

2.3. General procedure

The method depends on oxidation of amlodipine besylate by addition of $0.1\text{--}2.6 \text{ ml}$ ADB ($100 \mu\text{g ml}^{-1}$) to 1.0 ml of

$5.0 \times 10^{-4} \text{ M}$ KMnO_4 and 1.0 ml of 0.2 M H_2SO_4 . The solution was heated in a water bath at $50 \pm 1 \text{ }^\circ\text{C}$ for 10 min , the mixture was cooled and 2.0 ml (10^{-4} M) of MB, 0.8 , 0.35 , 0.8 and 0.7 ml (10^{-3} M) of AB, AR, AM and AO, respectively, was added. The volume was completed to 10 ml with bidistilled water. The decrease in color intensity of dyes were measured spectrophotometrically against a blank solution containing the same constituent except drug treated similarly, at their corresponding λ_{max} 663 , 609 , 511 , 520 or 484 nm , respectively. The concentration range was determined in each case by plotting the concentration of amlodipine besylate against absorbance at the corresponding maximum wavelengths.

2.4. Procedure for determination of dosage forms

At least ten tablets of ADB were weighed into a small dish, powdered and mixed well. A portion equivalent to 10 mg was weighed and dissolved in 100 ml bidistilled water, mixed well for 15 min using a magnetic stirrer and filtered through a sintered glass crucible G4. A 1.0 ml aliquot of the test solution ($100 \mu\text{g ml}^{-1}$ of ADB) was treated as described above in the general procedure.

3. Results and discussion

An analytical procedure based on the specific reactivity of an amino group was investigated. The method involves two steps namely:

- (1) Oxidation of amlodipine besylate with KMnO_4 in acidic medium by heating in water bath of $50 \pm 1 \text{ }^\circ\text{C}$.
- (2) Determination of unreacted oxidant by measuring the decrease in absorbance of dyes at a suitable λ_{max} .

4. Optimization

The influence of each of the following variables on the reaction was tested.

4.1. Effect of permanganate concentration

The influence of KMnO_4 concentration was studied in the range from 10^{-5} to 10^{-4} M , as final concentration. The optimum results were obtained with $5.0 \times 10^{-5} \text{ M}$; higher concentration of KMnO_4 caused the color to be disturbed.

4.2. Effect of acid concentration

Different types of acid were examined (HCl , H_2SO_4 , H_3PO_4 , CH_3COOH and HNO_3). The most suitable acid to achieve maximum yield of redox reaction was found to be sulphuric acid. Moreover, various volumes of H_2SO_4 were tested and found to be 1.0 ml of 0.2 M as shown in Fig. 1.

4.3. Effect of temperature and time

The oxidation process of amlodipine besylate is catalyzed by heating in water bath of $50 \pm 1 \text{ }^\circ\text{C}$. The time required to complete the reaction is 10 min . After oxidation process, the solution must be cooled at least for 3.0 min before addition of dye.

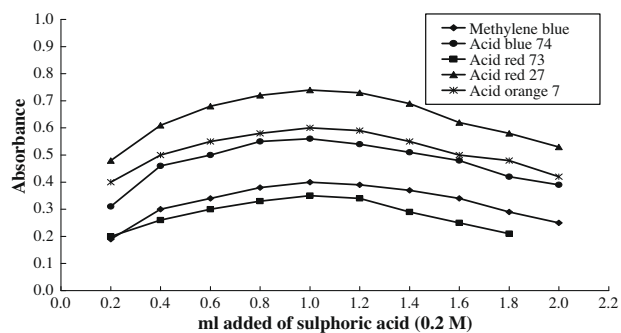


Figure 1 Effect of ml added of sulphuric acid (0.2 M) on absorbance of $10 \mu\text{g ml}^{-1}$ of amlodipine besylate with KMnO_4 (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M).

4.4. Effect of dye concentration

The optimum volume of dye used for production of maximum color intensity is 2.0 ml of 10^{-4} M MB, or 0.8, 0.35, 0.8 and 0.7 ml of 10^{-3} M AB, AR, AM and AO, respectively. The effect of time after the addition of dye indicated that shaking for 1.0 min is sufficient to give reliable results for all dyes. The color remains constant for at least 48 h.

4.5. Analytical data

Beer's law limits, molar absorptivities, Sandell sensitivities, regression equations and correlation coefficients were calculated and recorded in Table 1. The limits of detection ($K = 3$) and quantitation ($K = 10$) were established according to IUPAC definitions (IUPAC, 1978) and recorded in Table 1. In order to determine the accuracy and precision of the

Table 1 Optical and regression characteristics of amlodipine besylate with five different dyes.

| Parameters | MB | AB | AR | AM | AO |
|---|----------------------|----------------------|-----------------------|-----------------------|-----------------------|
| λ_{max} (nm) | 663 | 609 | 511 | 520 | 484 |
| Beer's law limits ($\mu\text{g ml}^{-1}$) | 1.0–24 | 0.9–22 | 1.2–26 | 0.9–12.8 | 1.0–14 |
| Ringbom limits ($\mu\text{g ml}^{-1}$) | 1.2–22.4 | 1.1–20 | 1.4–24.5 | 1.0–12.3 | 1.3–13.2 |
| Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$) | 2.25×10^4 | 3.12×10^4 | 2.01×10^4 | 22×10^4 | 3.42×10^4 |
| Sandell sensitivity (ng cm^{-2}) | 25.19 | 18.15 | 28.17 | 13.44 | 16.58 |
| Detection limits ($\mu\text{g ml}^{-1}$) | 0.277 | 0.249 | 0.329 | 0.239 | 0.272 |
| Quantitation limits ($\mu\text{g ml}^{-1}$) | 0.923 | 0.831 | 1.096 | 0.798 | 0.907 |
| Regression equation*: slope (b) | 0.0397 | 0.0551 | 0.0355 | 0.0744 | 0.0603 |
| Intercept (a) | 5.3×10^{-3} | 8.5×10^{-3} | -9.9×10^{-3} | -4.6×10^{-3} | -3.1×10^{-3} |
| Correlation coefficient (r) | 0.9998 | 0.9999 | 0.9998 | 0.9996 | 0.9999 |
| RSD**% | 0.66 | 1.01 | 0.82 | 0.51 | 0.73 |

* With respect to $A = a + bC$ where C is concentration of drug in $\mu\text{g ml}^{-1}$ and A is absorbance.

** Relative standard deviation for six determinations.

Table 2 Evaluation of the accuracy and precision of the proposed procedure of amlodipine besylate.

| Dye | Taken ($\mu\text{g ml}^{-1}$) | Recovery (%) | RSD ^a (%) | RE ^b (%) | Confidence limits ^c |
|-----|---------------------------------|--------------|----------------------|---------------------|--------------------------------|
| MB | 8.0 | 100.1 | 0.86 | 0.90 | 8.01 ± 0.0724 |
| | 10 | 100.2 | 0.89 | 0.93 | 10.02 ± 0.0933 |
| | 12 | 99.9 | 0.53 | 0.55 | 11.99 ± 0.0661 |
| AB | 8.0 | 99.8 | 0.74 | 0.78 | 7.98 ± 0.0619 |
| | 10 | 100.5 | 0.46 | 0.48 | 10.05 ± 0.0483 |
| | 12 | 99.7 | 0.38 | 0.39 | 11.96 ± 0.0472 |
| AR | 8.0 | 100.3 | 0.65 | 0.68 | 8.02 ± 0.0546 |
| | 10 | 99.6 | 0.71 | 0.95 | 9.96 ± 0.0745 |
| | 12 | 99.8 | 0.40 | 0.42 | 11.98 ± 0.0504 |
| AM | 8.0 | 99.5 | 0.70 | 0.74 | 7.96 ± 0.0588 |
| | 10 | 99.9 | 0.88 | 0.92 | 9.99 ± 0.0923 |
| | 12 | 100.3 | 0.67 | 0.71 | 12.04 ± 0.0850 |
| AO | 8.0 | 100.4 | 0.80 | 0.84 | 8.03 ± 0.0672 |
| | 10 | 100.6 | 0.77 | 0.80 | 10.06 ± 0.0808 |
| | 12 | 100.6 | 0.33 | 0.34 | 12.07 ± 0.0409 |

^a Relative standard deviation for six determinations.

^b Relative error.

^c 95% confidence limits and five degrees of freedom.

Table 3 Determination of ADB in pharmaceutical formulations using the proposed and official methods.

| Pharmaceutical formulations | Proposed methods | | | | | | | | |
|-----------------------------|------------------|------------------|------------------|--------------|------------------|------------------|--------------|------------------|------------------|
| | MB | | | AB | | | AR | | |
| | Recovery (%) | <i>t</i> -value* | <i>F</i> -ratio* | Recovery (%) | <i>t</i> -value* | <i>F</i> -ratio* | Recovery (%) | <i>t</i> -value* | <i>F</i> -ratio* |
| Norvasc 5 mg ^a | 99.2 | 0.27 | 1.36 | 99.8 | 0.58 | 2.11 | 100.1 | 0.82 | 2.85 |
| Amilo 5 mg ^b | 100.2 | 1.02 | 3.12 | 99.6 | 0.80 | 2.69 | 100.4 | 0.62 | 1.84 |
| Alkapress 5 mg ^c | 99.8 | 0.58 | 1.45 | 100.1 | 0.62 | 2.11 | 99.6 | 0.36 | 1.14 |
| Amlodipin 5 mg ^d | 100.5 | 0.19 | 1.21 | 99.6 | 0.37 | 1.27 | 99.7 | 0.34 | 1.64 |
| Myodura 5 mg ^e | 100.3 | 0.92 | 1.58 | 99.8 | 0.36 | 1.89 | 99.6 | 0.64 | 1.91 |
| | Official | | | | | | | | |
| | AM | | | AO | | | Recovery (%) | Recovery (%) | |
| | Recovery (%) | <i>t</i> -value* | <i>F</i> -ratio* | Recovery (%) | <i>t</i> -value* | <i>F</i> -ratio* | | | |
| Norvasc 5 mg ^a | 99.5 | 0.90 | 2.58 | 99.7 | 0.57 | 1.89 | 100.1 | 98.9 | |
| Amilo 5 mg ^b | 99.9 | 0.15 | 1.27 | 99.7 | 1.08 | 2.93 | 100.4 | 99.4 | |
| Alkapress 5 mg ^c | 100.4 | 0.48 | 2.12 | 99.7 | 0.54 | 1.58 | 99.6 | 99.5 | |
| Amlodipin 5 mg ^d | 99.8 | 0.39 | 1.77 | 100.2 | 0.95 | 1.89 | 99.7 | 99.4 | |
| Myodura 5 mg ^e | 99.5 | 0.54 | 1.85 | 100.1 | 0.34 | 2.11 | 99.6 | 99.4 | |

^a Pfizer S.A.E. Cairo, Egypt under authority of Inc., USA.

^b Alpha Chem Advanced of Pharmaceutical Industries Company (ACAPI), Bader Industrial City, Cairo, Egypt.

^c Alkan Pharmaceutical Company, Cairo, Egypt.

^d Pharaonia Pharmaceutical, Pharo-pharma Company, Cairo, Egypt.

^e Global Napi Pharmaceuticals Company (GNP) under license from Merck & Co. Inc., USA, Egypt.

* Theoretical value for *t*- and *F*-values for five degrees of freedom and 95% confidence limits are 2.57 and 5.05, respectively.

methods, solution containing three different concentrations of ADB were prepared and analyzed in six replicates. The analytical results obtained from this investigation were summarized in Table 2.

5. Interference

A systematic quantitative study was undertaken by measuring the absorbance of solutions containing $10 \mu\text{g ml}^{-1}$ of ADB with varying concentration of the additives and excipients such as calcium hydrogen phosphate, magnesium stearate and starch. Under the experimental conditions, the effect of excipients frequently found in formulations was evaluated using the proposed method. The additives and excipients in all tablets are not interfere.

6. Analytical applications

The proposed method was successfully applied to determine ADB in its dosage forms. The results obtained were compared statistically by Student's *t*-test (for accuracy), and variance ratio *F*-test (for precision) (Miller and Miller, 1993), with the official method (British Pharmacopoeia (BP), 2005) at 95% confidence level as recorded in Table 3. The results showed that the *t*- and *F*-values were lower than the critical values indicating that there was no significant difference between the proposed and official methods. The proposed method was more accurate with high recoveries compared to the official method (depended on liquid chromatography using stationary phase, octadecylsilyl silica gel $5.0 \mu\text{m}$ and mobile phase, mix 15 volumes of acetonitrile, 35 volumes of methanol and 50 volumes of a solution prepared as follows: dissolve 7.0 ml of triethylamine in 1000 ml bidistilled water and adjust

to pH 3.0 ± 0.1 with phosphoric acid), so the proposed method can be recommended for routine analysis of ADB in pure and dosage forms in the majority of drug quality control laboratories.

7. Conclusion

The proposed method was advantageous over other reported visible spectrophotometric and colorimetric methods, related to their high reproducibility, high sensitivity, less time consuming and using simple and inexpensive reagents. Moreover, this method allowed the determination of ADB up to $0.9 \mu\text{g ml}^{-1}$, in addition to simplicity, rapidity, precision and stability of colored species for more than 48 h. The proposed method may be applied for routine analysis and in quality control laboratories for the quantitative determination of the ADB in raw materials and in pharmaceutical formulations. The stability constant was determined and the free energy change was calculated potentiometrically. The positive value of ΔG reveals that the dissociation of this drug is not spontaneous.

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