Arabian Journal of Chemistry (2009) **2**, 59–63

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

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

Sayed A. Shama, Alaa S. Amin *, El Sayed M. Mabrouk, Hany A. Omara

Chemistry Department, Faculty of Science, Benha University, Benha, Egypt

Received 10 July 2009; accepted 10 July 2009
Available online 6 August 2009

KEYWORDS

Amlodipine besylate;
Spectrophotometry;
Redox reaction;
Potassium permanganate;
Pharmaceutical analysis

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 μg 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 μg 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.

1. Introduction

Amlodipine besylate is 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridindicarboxy-late-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 antianginal, antihypertensive and antiarrhythic 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 (Chandrashekhar et al., 1994; Pandya et al., 1995; Ilango et al., 1997) high-performance capillary electrophoresis (Jiang et al., 2003) and

* Corresponding author.
E-mail address: asamin2002@hotmail.com (A.S. Amin).

1878-5352 © 2009 King Saud University. All rights reserved. Peer-review under responsibility of King Saud University.
doi:10.1016/j.arabjc.2009.07.002

Production and hosting by Elsevier

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; Prabhakan 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′-disulfononic 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 μg 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 (5.0×10^{-4} 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 0.1–2.6 ml ADB (100 μg ml^{-1}) to 1.0 ml of 5.0×10^{-4} M $KMnO_4$ and 1.0 ml of 0.2 M H_2SO_4. The solution was heated in a water bath at 50 ± 1 °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 μg 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 ± 1 °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 5.0×10^{-5} M; higher concentration of $KMnO_4$ caused the color to 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 ± 1 °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.

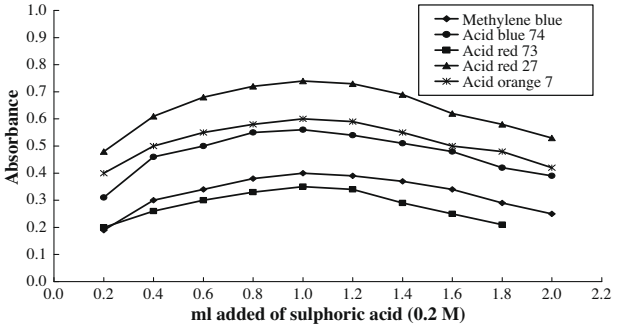

Figure 1 Effect of ml added of sulphuric acid (0.2 M) on absorbance of 10 μg ml^{-1} of amlodipine besylate with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M).

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 (μg ml^{-1})	1.0–24	0.9–22	1.2–26	0.9–12.8	1.0–14
Ringbom limits (μg ml^{-1})	1.2–22.4	1.1–20	1.4–24.5	1.0–12.3	1.3–13.2
Molar absorptivity (L mol^{-1} 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 (μg ml^{-1})	0.277	0.249	0.329	0.239	0.272
Quantitation limits (μg 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 μg 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 (μg 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 (%)	*t*-value*	*F*-ratio*	Recovery (%)	*t*-value*	*F*-ratio*	Recovery (%)	*t*-value*	*F*-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 (%)	*t*-value*	*F*-ratio*	Recovery (%)	*t*-value*	*F*-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 μg 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 μm 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 laboratorie.

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 μg 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.

References

Avadhanul, A.B., Srinivas, J.S., Anjaneyul, Y., 1996. Indian Drugs 33, 3.
Baranda, A.B., Jiménez, R.M., Alonso, R.M., 2004. J. Chromatogr. 1031, 913.
Baranda, A.B., Etxebarria, N., Jiménez, R.M., Alonso, R.M., 2005. Talanta 67, 933.
Basset, J., Jeffery, G.H., Mendham, J., 1978. Vogels Text Book of Quantitative Inorganic Analysis I, 308.
Basset, J., Denney, R.C., Jeffery, G.H., Mendham, J., 1986. Vogels Text Book of Quantitative Inorganic Analysis, fourth ed. Prentice Hall, p. 350.

Bersford, A.P., V. Macrae, P., Stopher, D.A., Wood, B.A., 1987. J. Chromatogr. 420, 178.
The British Pharmacopoeia (BP), 2005. Her Majesty's Stationary Office, London, I, p. 125.
Chandrashekhar, T.G., Rao, P.S.B., Smrita, K., Vyas, S.K., Dutt, C., 1994. J. Planar Chromatogr. Mod. TLC 7, 458.
Feng, Y., Gou, X., Yand, D., Ne, Y., 1998. Guangdong Yaoxueyuan Xuebao 14, 111.
Gazy, A.A., 2004. Talanta 62, 575.
Ilango, K., Kumar, P.B., Prasad, V.R.V., 1997. Indian J. Pharm. Sci. 59, 336.
IUPAC, 1978. Spectrochim. Acta B 33, p. 242.
Jiang, T.F., liang, B., Li, B.J., Li, C., Ou, Q.Y., 2003. Fenxi Kexue Xuebao 19, 33.
Josefsson, M., Zackrisson, A.-L., Norlander, B., 1995. J. Chromatogr. B: Biomed. Appl. 672, 310.
Lokesh, B.V.S., Reddy, M.N., Sanker, D.G., Sreedhar, K., 1996. East. Pharm. 39, 125.
Miller, J.C., Miller, J.N., 1993. Statistics in Analytical Chemical, third ed. Ellis Horwood, Chichester, UK.
Mohamed, Y.E., Naglaa, M.E.K., Bahia, A.M., Nashwa, G.M., 1998. Bull. Fac. Pharm. 36, 1.
Naidu, K.R., Kale, U.N., Shingare, M.S., 2005. J. Pharm. Biomed. Anal. 39, 147.
Pandya, K.K., Satia, M., Gandhi, T.P., Modi, I.A., Modi, R.I., Chakravarthy, B.K., 1995. J. Chromatogr. B: Biomed. Appl. 667, 315.
Prabhakan, A.H., Giridhar, R., 2002. Indian Drugs 39, 204.
Rahman, N., Azmi, S.N.H., 2000. Anal. Sci. 16, 1353.
Rahman, N., Azmi, S.N.N., 2001. IL Farmaco 56, 731.
Rahman, N., Singh, M., Hoda, M.N., 2004. IL Farmaco 59, 913.
Reddy, M.N., Rani, G.T., Rao, K.V.S.P., Sankar, D.G., Sreedhar, K., 1997. Indian J. Pharm. Sci. 59, 188.
Reynolds, J.E.F. (Ed.), 1993. Martindale The Extra Pharmacopoeia, 31st ed. Royal Pharmaceutical Society, London, p. 819.
Sankar, S.R., Nanjan, M.J., Vasudevan, M., Shaat, N., Suresh, B., 1997. Indian J. Pharm. Sci. 59, 171.
Singhvi, I., Chaturvedi, S.C., 1998. Indian J. Pharm. Sci. 60, 309.
Singhvi, I., Chaturvidi, S.C., 1999. Indian J. Pharm. Sci. 61, 190.
Sridhar, K., Sastry, C.S.P., Reddy, M.N., Sankar, D.G., Srinivas, K.R., 1997. Anal. Lett. 30, 121.
Sudhakar, P., Nirmala, M., Babu, J.M., Vyas, K., Reddy, G.M., Bhaskar, B.V., Reddy, P.P., Mukkanti, K., 2006. J. Pharm. Biomed. Anal. 24, 605.
Yasuda, T., Tanaka, M., Iba, K., 1996. J. Mass Spectrom. 31, 879.
Zarghia, A., Foroutanb, S.M., Shafaatia, A., Khoddam, A., 2005. IL Farmaco 60, 789.