Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 46 (2008) 583–586
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Simultaneous determination of metoprolol succinate and amlodipine besylate in pharmaceutical dosage form by HPLC

Vaijanath G. Dongre [a,*], Sweta B. Shah [a,b], Pravin P. Karmuse [a,b], Manisha Phadke [b], Vivek K. Jadhav [b]

[a] *Department of Chemistry, University of Mumbai, Vidyanagari, Kalina, Santacruz (East), Mumbai 400098, India*
[b] *Analytical Development Laboratory, Ipca Laboratories Ltd., Kandivli Industrial Estate, Kandivli (West), Mumbai 400067, India*

Received 18 June 2007; received in revised form 26 October 2007; accepted 9 November 2007
Available online 17 November 2007

Abstract

A simple, precise, specific and accurate reverse phase HPLC method has been developed for the simultaneous determination of metoprolol succinate (MS) and amlodipine besylate (AB) in tablet dosage form. The chromatographic separation was achieved on Hypersil BDS cyano (250 mm × 4.6 mm, 5 μm) column using PDA detector. The mobile phase consisting of buffer (aqueous triethylamine pH 3) and acetonitrile in the ratio of 85:15 (v/v) at a flow rate of 1.0 mL/min was used. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness.

Keywords: Metoprolol; Amlodipine; HPLC; Simultaneous determination; Validation

1. Introduction

High blood pressure can be treated with number of drugs depending upon the causes which are responsible for it. It is increasingly appreciated that the elusive goal of a 'normal' blood pressure is achieved only if multi-drug therapy is employed [1].

Amlodipine besylate (AB), 2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl) 1,4-dihydro-6-methyl-3,5-pyridine-dicarboxylic acid-3 ethyl-5 methyl ester, is a calcium channel blocker (Fig. 1a). It is used in the treatment of hypertension and angina [2].

Metoprolol succinate (MS), 1-[4-(2-methoxyethyl)-phenoxy]-3-[(1-methylethylamino]-2-propanol (Fig. 1b) is a beta-adrenergic blocking agent, which reduces chest pain and lowers high blood pressure [2].

AB is not official with USP as well as with EP. USP 2006 describes an HPLC method for determination of MS as extended release tablets [3]. Few HPLC methods are reported in the literature for the simultaneous determination of some antihypertensive drugs from pharmaceutical dosage forms [4–11]. So far, to our present knowledge, no HPLC method is reported for the simultaneous determination of metoprolol succinate and amlodipine besylate in dosage forms.

This paper describes the development and validation of a screening method to simultaneously quantify AB (immediate release type) and MS (sustained release type) by liquid chromatography.

2. Experimental

2.1. Instrumentation

HPLC system (Waters 2695, Milford, USA) consisting of quaternary gradient pump, autosampler, column oven and photodiode array detector (PDA, Waters 2996) was employed for analysis. Chromatographic data was acquired using Empower software.

2.2. Reference substances, sample, reagents and chemicals

Active pharmaceutical ingredient (API) working standards of amlodipine besylate (AB), metoprolol succinate (MS) and

* Corresponding author. Tel.: +91 22 26526091x564; fax: +91 22 26528547.
E-mail addresses: dongrevg@yahoo.com (V.G. Dongre), vivek@ipca.co.in (V.K. Jadhav).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.11.006

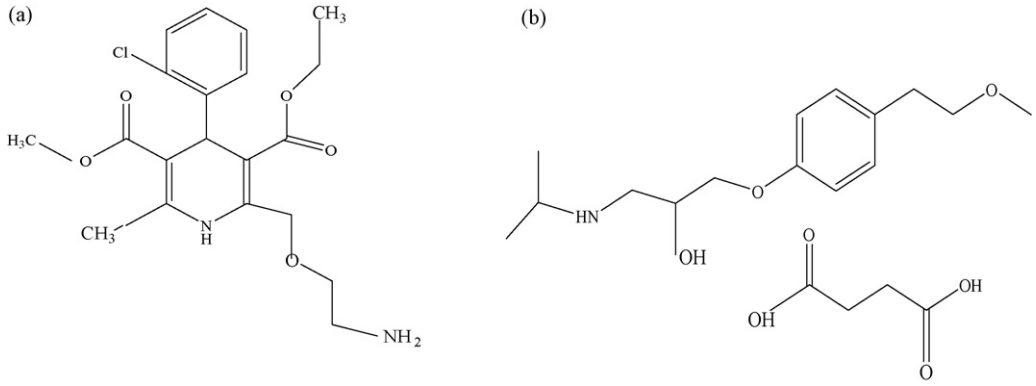

Fig. 1. Structures of (a) amlodipine besylate and (b) metoprolol succinate.

test samples (bilayered tablets with composition MS-50 mg and AB, equivalent to amlodipine-5 mg) were obtained from Ipca Laboratories Limited, Mumbai, India. HPLC grade acetonitrile, methanol and orthophosphoric acid were obtained from Merck, Mumbai, India Limited. Triethylamine was from Spectrochem Mumbai, India. High purity deionised water was obtained from Millipore, Milli-Q (Bedford, MA, USA) purification system.

2.3. Chromatographic conditions

Hypersil BDS Cyano (250 mm × 4.6 mm, 5 μm) (Bellefonte, PA, USA) column was used as a stationary phase. The isocratic mobile phase consisting of a mixture of buffer (0.7% aqueous triethylamine, pH 3.0 adjusted with orthophosphoric acid) and acetonitrile in the ratio of 85:15 (v/v) was used throughout the analysis. The flow rate of the mobile phase was 1.0 mL/min. Detector signal was monitored at a wavelength of 254 nm. The column temperature was kept ambient and injection volume was 20 μL.

2.4. Solution preparation

2.4.1. AB stock solution

AB standard stock solution was prepared by transferring 35.0 mg of amlodipine besylate working standard into a 100 mL volumetric flask. A 20 mL portion of methanol was added, sonicated and cooled to room temperature. The solution was diluted to the mark with mobile phase.

2.4.2. Standard solution

Portion of 50.0 mg of metoprolol succinate working standard was transferred into 100 mL volumetric flask and dissolved by adding 30 mL of mobile phase. Further, a 20 mL of AB stock solution was added, volume was adjusted with mobile phase to obtain a required solution containing 500 μg/mL of metoprolol succinate and 70 μg/mL of amlodipine besylate, respectively.

2.5. Sample solution

Sample solution was prepared by transferring five tablets in 500 mL volumetric flask. One hundred millilitres methanol was added to disintegrate tablets completely by using ultrasonicator for 20 min. To this, 100 mL of mobile phase was added, sonicated and shaken for 15 min each. The volume was diluted to the mark with mobile phase and mixed thoroughly. This solution was filtered through 0.45 μm membrane filter. The standard and sample solutions were found to be stable for at least 24 h.

2.6. Validation procedure

The specificity of the method was determined by injecting the sample solution containing excipients without drug having concentration same as that of the sample. Linearity solutions were prepared at 10 concentration levels from 10% to 200% of analyte concentration.

The accuracy of the method was carried out by adding known amount of each drug corresponding to three concentration levels 80%, 100% and 120% of the label claim along with the excipients in triplicate The samples were given the same treatment as described in Section 2.5.

Precision of the method was checked by carrying out six independent assays of MS and AB test samples against qualified working standard. Intermediate precision was performed by analyzing the samples by two different analysts using different instruments on different days.

Robustness was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 mL/min to 0.8 mL/min and 1.2 mL/min. The organic strength was varied by ±2%, while pH was varied by ±0.2 units. Standard solution was injected six times in replicate for each change.

Respective peak areas, dilution factors, sample and standard weights were taken into account to quantitate the amounts of AB and MS in mg per tablet.

3. Results and discussion

3.1. Optimization of chromatographic conditions

In order to achieve simultaneous elution of the two components, different chromatographic conditions were attempted. Stationary phases like C_8, C_{18} and cyano were used. Metoprolol eluted in all the stationary phases, while amlodipine was retained with C_8 and C_{18} using different mobile phase compositions of

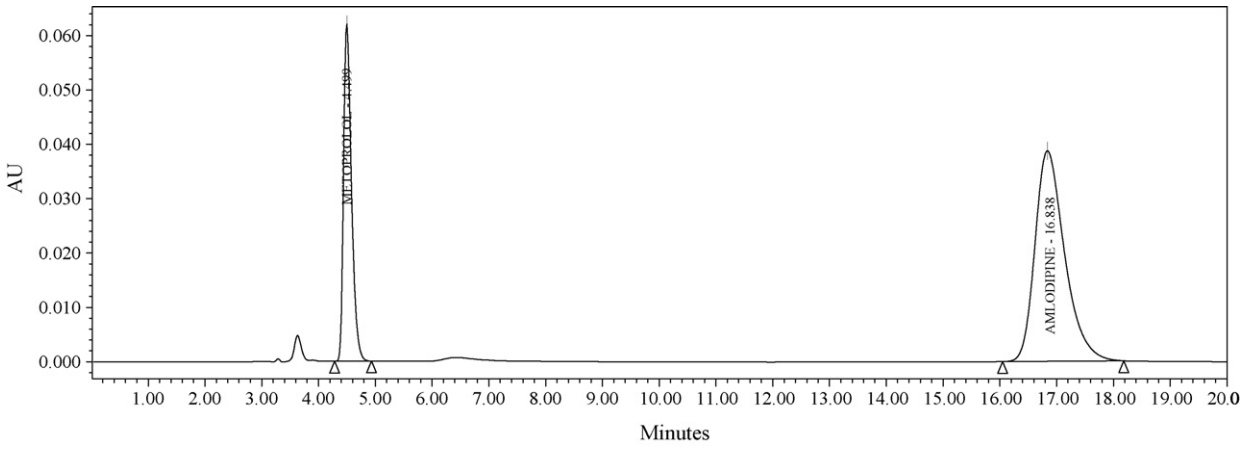

Fig. 2. Chromatogram of tablet extract, showing well separated peaks of amlodipine (besylate peak at RT 3.9) and metoprolol.

Table 1
System suitability parameters

Component ($n = 6$)	Area	Peak symmetry[a]	Theoretical plates[a]	Capacity factor[a]	USP resolution[a]
MS	619,697	1.77	4916	224.45	–
AB	1,063,488	1.68	4514	842.13	19.09

[a] USP–NF 29 section 621, pp. 2135.

water (pH 3.0 adjusted with *o*-phosphoric acid) and acetonitrile (65:35, 70:30 and 85:15 (v/v)). Both the components were eluted with cyano column. To avoid merging of besylate peak ($t_r = 3.9$ min) with metoprolol peak ($t_r = 4.6$ min) and to reduce runtime, the mobile phase composition was selected as water (pH 3)–acetonitrile, in the ratio of 85:15 (v/v). Although substantial separation was achieved with this mobile phase, a tailing (>2) was observed for amlodipine peak. To minimize the peak tailing, triethyl amine (0.7%) was added as an organic modifier. Peak tailing for amlodipine was found to be well within the limit of 2 at pH 3. To reduce the analysis time the gradient system was also employed but the peak area reproducibility for amlodipine was found to be poor.

Under these optimized conditions, the analyte peaks were well resolved and free from tailing. The tailing factors were <1.5 for both the peaks. The elution order was MS ($t_r = 4.6$ min) and AB ($t_r = 17.3$ min), at a flow rate of 1.0 mL/min. The chromatogram was recorded at 254 nm as the overlaid PDA spectrum of amlodipine and metoprolol showed maximum response at this wavelength. A chromatogram of tablet extract was recorded and shown in Fig. 2.

3.2. Method validation

The newly developed method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, and robustness [12]. System suitability was established by injecting standard solution and results are shown in Table 1.

3.2.1. Specificity

The chromatograms were checked for the appearance of any extra peaks. No chromatographic interference from the tablet excipients was found. Peak purity was verified by confirming homogeneous spectral data for metoprolol and amlodipine.

3.2.2. Linearity

AB and MS showed linearity in the range of 42–98 μg/mL and 300–700 μg/mL, respectively. Linear regression equations and correlation coefficient (R^2) are: $Y_{AB} = 21529x - 8719$ ($R^2 = 1.00$) and $Y_{MS} = 1199x + 18782$ ($R^2 = 1.00$).

3.2.3. Accuracy

The accuracy was expressed as the percentage of analytes recovered by the assay method. It was confirmed from results that the method is highly accurate (Table 2).

3.2.4. Precision

The relative standard deviations (R.S.Ds.) were 0.10% for AB and 0.04% for MS, which are well within the acceptable limit

Table 2
Accuracy data (analyte recovery)

Theoretical (% of target level)	Amount added (mg)	Amount recovered (mg)	Recovery (%)
Metoprolol			
80	200.60	199.36	98.9
100	250.00	247.49	98.5
120	300.20	295.82	98.0
Amlodipine			
80	20.70	20.32	98.3
100	25.50	25.22	99.0
120	30.50	30.37	99.7

$n = 3$ determinations.

of 2.0%. The R.S.D's. for intermediate precision were found to be 0.31% for MS and 0.68% for AB.

3.2.5. Robustness

In all deliberately varied conditions, the RSD of peak areas of metoprolol and amlodipine were found to be well within the acceptable limit of 2%. The tailing factor for both the peaks was found to be <1.5.

4. Conclusion

Proposed HPLC method is specific, accurate and precise for the simultaneous determination of amlodipine besylate (immediate release type) and metoprolol succinate (sustained release type) from pharmaceutical dosage form.

The described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination.

Acknowledgements

The authors wish to thank the Head, Department of Chemistry, University of Mumbai and the management of Ipca Laboratories for providing necessary facilities.

References

[1] A. Domenic, Sica Drugs 62 (2002) 443–462.
[2] The Merck Index, 14th ed., 2006, p. 493 and 6149.
[3] The United States Pharmacopoeia, NF 29, US Pharmacopoeial Convention, Rockville, MD, 2006, p. 1419.
[4] M.M. Baing, V.V. Vaidya, R.T. Sane, S.N. Menon, K. Dalvi, Chromatographia 64 (2006) 293–296.
[5] K.R. Naidu, U.N. Kale, M.S. Shingare, J. Pharm. Biomed. Anal. 39 (2005) 147–155.
[6] K.R. Rajeshwari, G.G. Sankar, A.L. Rao, M.S. Shingare, J. Pharm. Sci. 68 (2006) 275–277.
[7] E. Caudron, S. Laurent, E.M. Billaud, P. Prognon, J. Chromatogr. B 801 (2004) 339–345.
[8] D.A. Shah, K.K. Bhatt, M.B. Shankar, R.S. Mehta, T.R. Gandhi, S.L. Baldana, Ind. J. Pharm. Sci 68 (2006) 796–799.
[9] K.H. Kim, J.H. Lee, M.Y. Ko, K.S. Shin, J.S. Kang, W.C. Mar, J.R. Youm, Chromatographia 55 (2002) 1–2.
[10] S.I. SA'SA', I.M. Jalal, H.S. Khalil, J. Liq. Chromatogr. 11 (1988) 1673–1696.
[11] F. Belal, I.A. AI-zaagi, E.A. Gadkariem, M.A. Abounassif, J. Pharm. Biomed. Anal. 24 (2001) 335–342.
[12] ICH, Q2B, Harmonized tripartite guideline, validation of analytical procedure: methodology, IFPMA, in: Proceedings of the International Conference on Harmonization, March 1996.