Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 4, 2013

Research Article

SIMULTANOUS ESTIMINATION OF AMLODIPINE BESYLATE AND CLOPIDOGREL BISULFATE IN TABLET DOSAGE FORM BY RP-HPLC

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Received: 06 Aug 2013, Revised and Accepted: 25 Sep 2013

ABSTRACT

Objective: To develop & validate a simple, precise, specific and accurate reverse phase high pressure liquid chromatographic method for the simultaneous determination of Amlodipine besylate (AB) and Clopidogrel bisulphate in tablet dosage form. Method: Chromatographic separation was achieved on reverse phase (Microsorb-MV 100-5 C-18 (250x4.6mm, 5 µm) column using UV detector. The sample was analyzed using acetonitrile & phosphate buffer (3.4g of potassium dihydrogen phosphate in 1000 ml of double distilled water, pH adjusted to 3 with ortho phosphoric acid) in the ratio of 60:40 (v/v) as mobile phase at a flow rate of 1.0 mL/min. and detection at 250 nm. Result: The retention time for Amlodipine besylate & clopidogrel bisulfate was found to be 4.2 & 9.1 min. respectively. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. The method was found to be specific for simultaneous estimation of both the drugs in tablet dosage form & for all parameters, the S.D. & R.S.D. values were found to be well within the acceptable limit of 2.0%. Conclusion: Proposed HPLC method is specific, accurate and precise for the simultaneous determination of Amlodipine besylate and Clopidogrel bisulphate 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.

Keywords: Amlodipine besylate, Clopidogrel bisulfate, HPLC, Simultaneous determination, Validation.

INTRODUCTION

Amlodipine besylate (AB) is the besylate salt of Amlodipine, a long acting, calcium channel blocker, AB is a calcium antagonist that inhibits the trans-membrane influx of calcium ions into vascular smooth muscles and cardiac muscles, which in turn affects their contractile process and results in reduced blood pressure. It is used in the treatment of hypertension and angina. Amlodipine besylate (AB), chemically, 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridinedicarboxylic acid3-ethyl, 5-methyl ester (±)-monobenzenesulfonate [1-5].

Clopidogrel is an analogue of Ticlopidine, it is an anti platelet agent which selectively inhibits the binding of adenosine diphosphate (ADP) to its platelet receptor and blocks the subsequent ADP-mediated activation of the glycoprotein GPIIb/IIIa complex, thereby inhibiting platelet aggregation. It is used worldwide for the long-term prevention of atherothrombotic events (myocardial infarction, stroke, peripheral arterial disease, acute coronary syndrome, cardio-vascular death) [7-10]. Chemically, Clopidogrel bisulphate (CPS) is methyl-2-chlorophenyl-(4, 5, 6, 7-tertrahydrothieno [3, 2-c] pyridine-5yl) acetate bisulphate used in the treatment of cardiovascular diseases [2-3]. AB is official in USP, BP, EP & IP whereas CPS is official in USP [1-5]. Chemical structure of AB & CPS are shown in Fig. 1.Combination drug products of AB and CPS are widely marketed and used for the treatment of hypertension and cardiac disorders.

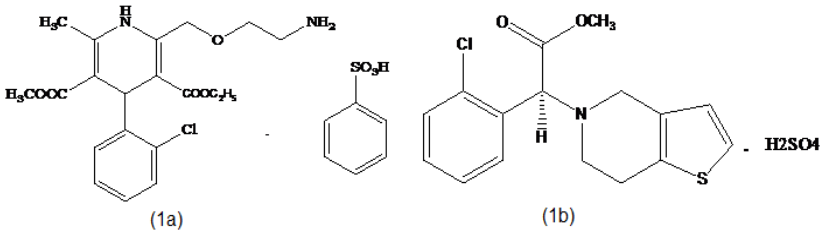

Fig. 1: Chemical structures of the analytes (1a) AB & (1b) CPS.

This paper describes the development and validation of an analytical method for simultaneous estimation of AB and CPS by liquid chromatography.

MATERIALS AND METHODS

Instrumentation

HPLC system (Cyber lab LC 100) consisting of binary gradient pump, Microsorb-MV 100-5 C-18 column (250x4.6mm, 5 µm), UV detector was employed for analysis. Chromatographic data was acquired using WS-100 Workstation software.

Reference substances, sample, reagents and chemicals

Active pharmaceutical ingredient (API) working standards of Amlodipine besylate (AB), Clopidogrel bisulphate (CPS) were obtained as gift sample from Lupin Laboratories limited Pune, India and test samples (tablets with composition CPS-75 mg and AB, equivalent to amlodipine-5 mg) were procured from the local market, Pune, India. HPLC grade acetonitrile, methanol and ortho-phosphoric acid obtained from Merck, Mumbai, India Limited. HPLC grade water obtained from MOLYCHEM, Thane, India.

Chromatographic conditions

Microsorb MV 100-5 C-18 column (250mm×4.6 mm, 5µm) was used as a stationary phase. The isocratic mobile phase consisting of a mixture of phosphate buffer (pH 3.0 adjusted with ortho-phosphoric acid) and acetonitrile in the ratio of 60:40 (v/v) was used throughout the analysis. The flow rate of the mobile phase was 1.0 mL/min., ambient column temperature and 20µL injection volume. Elution of analytes was monitored at 250 nm using a LC-100 UV absorbance detector (190– 600 nm). Detector signal at a wavelength of 250 nm was monitored.

Solution preparation

Phosphate buffer solution

Dissolve 3.4g of potassium dihydrogen phosphate in 900mL HPLC grade water. Adjust the pH 3.0 with o-phosphoric acid and make up the volume up to 1000 ml with same solution [9].

AB stock solution

AB standard stock solution was prepared by transferring 100mg of Amlodipine besylate working standard into a 100mL volumetric flask. A 20mL portion of acetonitrile was added, sonicated and cooled to room temperature. The solution was diluted up to the mark with mobile phase to give a solution of strength 1000µg/mL.

CPS stock solution

CPS standard stock solution was prepared by transferring 100 mg of Clopidogrel bisulphate working standard into a 100mL volumetric flask. A 20mL portion of acetonitrile was added, sonicated and cooled to room temperature. The solution was diluted up to the mark with mobile phase to give a solution of strength 1000µg/mL.

Sample solution

Twenty tablets, labeled as containing 5 mg of AB, and 75 mg of CPS together with excipients, were accurately weighed, transferred to a clean and dry mortar and ground into a fine powder.

A weight of the powder equivalent to one tablet content (390 mg) was accurately weighed, then transferred to a clean 50 ml volumetric flask, 20 ml of acetonitrile was added, and the flask was attached to a rotary shaker for 10 min to disperse the material completely. The mixture was then sonicated for 10 min. and diluted up to the mark with mobile phase to give a solution containing 1000 µg/ml. This solution was filtered through a 0.45µm pore size Nylon 66 membrane filter.

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 [5].

Linearity solutions were prepared at 10 concentration levels from 10% to 400% 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 [11].

Precision of the method was checked by carrying out six independent assays of AB and CPS test samples against qualified working standard [5].

Intermediate precision was performed by analyzing the samples by two different analysts on different days [5].

Robustness was performed by deliberately changing the chromatographic conditions [5].

The flow rate of the mobile phase was changed from 1.0 mL/min to 1.1 mL/min. The organic strength was varied by ±5%, while pH was varied by ±0.5 units. Standard solution was injected six times in replicate for each change [13].

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

RESULTS & DISCUSSIONS

Optimization of chromatographic conditions

In order to achieve simultaneous elution of the two components the different chromatographic conditions were attempted. The stationary phase C18 was used in different mobile phase compositions. Optimization of the mobile phase was performed based on resolution, asymmetric factor and peak area obtained for both Amlodipine and Clopidogrel. The mobile phase combination of phosphate buffer (pH 3.0 adjusted with o-phosphoric acid) and acetonitrile (65:35, 70:30 and 85:15 (v/v)) were tried. Acetonitrile: phosphate Buffer (60:40) pH 3.0 (adjusted with Ortho-phosphoric acid) at a flow rate of 1.0 mL/min found to be satisfactory and gave two symmetric and well-resolved peaks for Amlodipine and Clopidogrel. The chromatogram recorded at 250 nm, as spectrum of Amlodipine and Clopidogrel showed maximum response at this wavelength. The retention time for Amlodipine and Clopidogrel were 4.2 and 9.1, respectively (Fig. 2). A chromatogram of tablet extract was recorded and shown in Fig. 2.

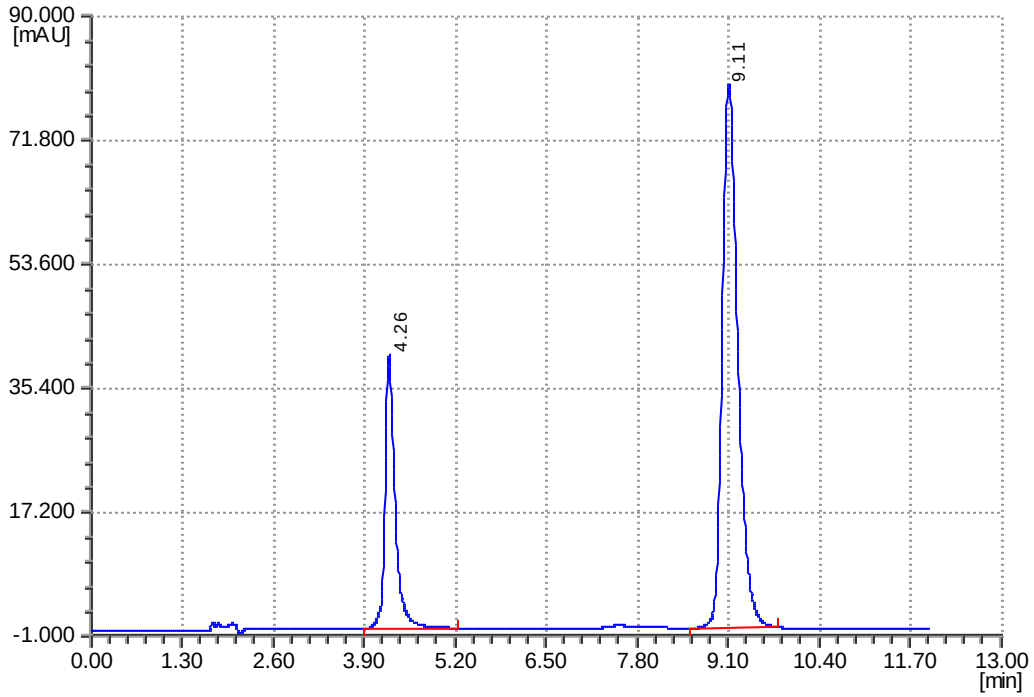

Fig. 2: Chromatogram of Amlodipine besylate (peak at RT 4.2) and Clopidogrel bisulphate (peak at RT 9.1)

Table 1: System suitability parameters for AB & CPS

Component (n = 6)	Area Peak	Symmetry	Theoretical plates[a]	Capacity factor[a]	USP resolution[a]
Amlodipine	40248.85	1.632	3990.4	1.66	2.57
Clopidogrel	124300.117	1.375	6821.45	4.69	13.94

(a - USP–NF 29 section 621, p. 2135)

Method validation

The newly developed method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, and robustness [5]. System suitability was established by injecting standard solution and results are shown in Table 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 AB and CPS.

Linearity

AB and CPS showed linearity in the range of 10-50 µg/mL and 100–400µg/mL, respectively. Linear regression equations and correlation coefficient (R^2) are: Y_{AB} = 1850x−6010 (R^2 = 0.999) and Y_{CPS} = 688.1x + 1297 (R^2 = 0.997).

Precision

The relative standard deviations (R.S.Ds.) were 1.81% for AB and 1.50% for CPS, which are well within the acceptable limit of 2.0%. The R.S.D's. for intermediate precision were found to be 0.67% for AB and 1.71% for CPS.

Accuracy

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

Robustness

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

Table 2: Accuracy study of AB & CPS

Theoretical (% of target level)	Amount added (µg/ml)	Amount recovered (µg/ml)	Recovery (%)
Amlodipine besylate			
80	18	18.44	102.6
100	20	20.14	100.7
120	22	22.14	100.6
Clopidogrel bisulfate			
80	270	273.4	101.2
100	300	306.9	102.3
120	330	328.2	99.4

n = 3 determinations

CONCLUSION

Proposed HPLC method is specific, accurate and precise for the simultaneous determination of Amlodipine besylate and Clopidogrel bisulphate 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.

ACKNOWLEDGEMENT

The authors thank to Hon. Principal Marathwada Mitra Mandal's College of Pharmacy and Lupin Pharmaceuticals Ltd. for providing Gift sample.

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