Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2012, 4(4):1512-1516
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Development and validation of RP-HPLC method for simultaneous determination of metoprolol and aspirin in fixed dose combinations

Boyka G. Tsvetkova*, Ivanka P. Pencheva, Plamen T. Peikov

Medical University – Sofia, Faculty of Pharmacy, Department of Pharmaceutical chemistry,

---

ABSTRACT

In this study, reverse phase high performance liquid chromatographic method have been developed and validated for the simultaneous determination of metoprolol and aspirin in combined pharmaceutical formulation. The chromatographic separation was achieved in a Phenomenex-Luna, C18 (250 mm x 4.6 mm i.d., 5 µm) as a stationary phase and phosphate buffer (pH adjudted to 4.6 with ortho-phosphoric acid):methanol (20:80 v/v) as eluent, at a flow rate of 0.8 ml/min. UV detection was performed at 230 nm. The retention time of metoprolol and aspirin was found to be 3.06 and 6.97 min, respectively. The results of analysis were validated statistically and by recovery studies. Linearity, accuracy and precision were acceptable in the ranges (20-100 µg/ml) for aspirin and (10-50 µg/ml) for metoprolol. The calibration curves were linear (R^2 > 0.9999) in the range of each analyte. The % recovery for metoprolol and aspirin is 99.17 and 99.75, respectively. No chromatographic interference from the tablet excipients was found. The results of the studies showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which can be used for the routine determination of metoprolol and aspirin in bulk and its pharmaceutical dosage forms.

Key words: Liquid chromatography, validation, metoprlol, aspirin, quality control

---

INTRODUCTION

Monotherapy with various antihypertensive agents is not always sufficient to control the blood pressure, and concomitant use of two or more drugs is necessary in 50% of the hypertensive patients [1-4]. The primary goal of any antihypertensive therapy is therefore achievement of normotension without addition of intolerable side effects, which can be accomplished by combination of drugs with different mechanism of action.

Beta-blockers are clinically important drugs used in the treatment of disorders such as hypertension, angina pectoris and arrhythmia [5]. Metoprolol (1-[4-(2-Methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-2-propanol) has been used extensively for more than 25 years to treat such cardiovascular disorders as hypertension, arrhythmia and heart failure [6]. Several methods have been reported for determination of metoprolol including gas chromatography-mass spectrometry (GC-MS) [7-9], high performance liquid chromatography [10, 11-13], LC-MS [14, 15], LC-MS-MS [16], high performance thin layer chromatography [17] and spectrophotometry [18].

Aspirin is 2-acetyloxybenzoic acid, often used as an analgesic, antipyretic, anti-inflammatory and an antiplatelet agent [19]. It suppresses the production of prostaglandins and thromboxanes due to inactivation of the cyclooxygenase enzyme [20]. There are various analytical methods for estimation of aspirin in pharmaceutical dosage forms and biological samples [21-27].

A combination of metoprolol and aspirin is used in the treatment and prevention of cardiovascular disease such as stroke and to regulate blood pressure. Literature survey reveals that there is no RP-HPLC method available for the

determination of these analytes in combination; therefore the aim of this paper is to develop a specific, precise and accurate chromatographic method that could be applied in quality control for the simultaneous determination of metoprolol and aspirin in pharmaceutical preparations. The proposed method was validated as per the International Conference on Harmonization (ICH) method validation guidelines [28].

MATERIALS AND METHODS

Chemicals and Reagents

Working standards of aspirin RS (Purity 100.09) and metoprolol succinate RS (purity 99.81) were provided by (Sigma-Aldrich). The pharmaceutical preparation of combination of aspirin (100 mg) and metoprolol succinate (50 mg) was obtained commercially. LC-grade methanol and ortho-phosphoric acid were procured from Merck (Germany). All other chemical reagents were of analytical grade.

Instrumentation and chromatographic conditions

Chromatographic separation was performed on modular HPLC system LC-10A Shimadzu (Japan) arranged with a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector, column oven CTO-10A, SPD-M10A diode array detector and communication bus module CBM-10A. Separation was achieved isocratically with a LiChrosorb C18, 250 mm x 4.6 mm, 5 µm column eluted with a mixture of phosphate buffer (pH adjudted to 4.6 with ortho-phosphoric acid) and methanol (20:80 *v/v*) as the mobile phase at flow rate of 0.8 ml/min. Detection was carried out by absorbance at 230 nm. The analysis was carried out at an ambient temperature and injection volume was 20 µl.

Preparation of standard solutions

For aspirin: 20 mg of aspirin was accurately weighed and transferred to a 20 ml volumetric flask and volume was made up to 20 ml with methanol (Stock solution A-1000 µg/ml). From Stock solution A, 5.0 ml was taken into a 50 ml volumetric flask and volume was made up to 50 ml with methanol (Stock solution B-100 µg/ml).

For metoprolol: 20 mg of metoprolol was accurately weighed and transferred to a 20 ml volumetric flask and volume was made up to 20 ml with methanol (Stock solution C-1000 µg/ml). From Stock solution C|, 5.0 ml was taken into a 50 ml volumetric flask and volume was made up to 50 ml with methanol (Stock solution D-100 µg/ml).
For mixed standard: From the stock solutions B and D dilutions of different concentration were made in the ratio 2:1 for aspirin and metoprolol as mentioned in the Table 1.

Table 1. Preparation of mixed standards

Stock solution → Volume taken (ml)		Total volume (ml)	Concentration in µg/ml	
B	D		aspirin	metoprolol
2	1	10	20	10
4	2	10	40	20
6	3	10	60	30
8	4	10	80	40
10	5	10	100	50

Analysis of pharmaceutical preparation

The pharmaceutical preparation containing aspirin 100 mg and metoprolol 50 mg was analyzed using this method. The content of 20 capsules was taken and powdered. The powder equivalent to 25 mg of aspirin and 12.5 mg of metoprolol was accurately weighed and transferred into a 25 ml volumetric flask, 10 ml of methanol was added and the content was ultrasonicated for 20 min. The volume was then diluted to the mark and mixed well. A small portion was withdrawn and filtered through a 0.22 µm filter to ensure the absence of particulate matter. The filtered solution was appropriately diluted with methanol as already described. The amount of drug present in the sample solution was determined using the calibration curves of standard drugs.

RESULTS AND DISCUSSION

The proposed method was validated as per ICH guidelines with respect to specificity, linearity, precision and accuracy.

Specificity

The specificity of the method was determined by checking the interference with the components from placebo. No interference was observed for any of the components like excipients of both drugs. The Fig. 1 showed typical chromatogram obtained from analysis of standard solution using the proposed method. The retention time observed –3.06 min for aspirin and 6.97 min for metoprolol permits a rapid assay, which is important for routine analysis.

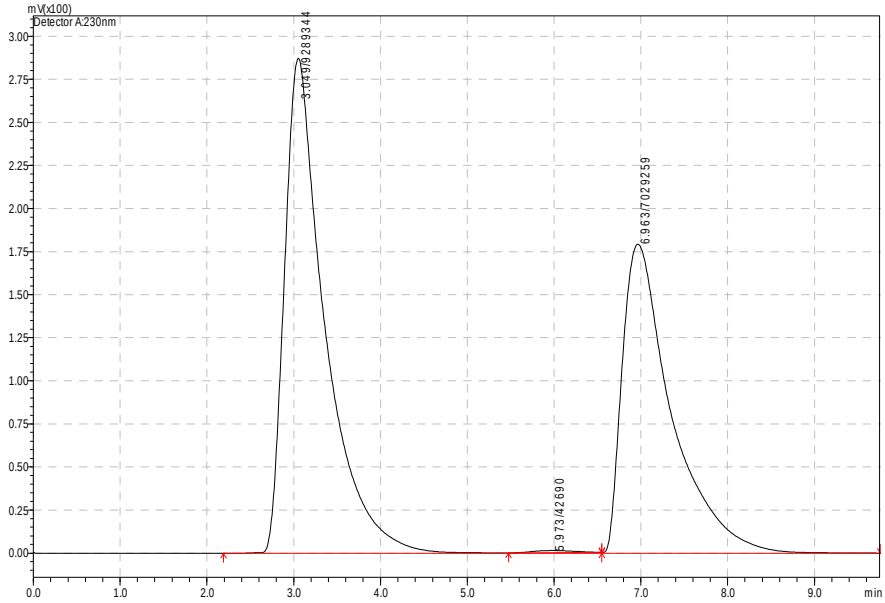

Fig.1. Chromatogram obtained from analysis of sample solution

The system suitability studies were carried out to determine theoretical plates, resolution and tailing factors. The results were given in Table 2. The values obtained demonstrated the suitability of the system for the analysis of investigated drug combination, system suitability parameters may fall within ±3% standard deviation range during routine performance of the method.

Table 2. System suitability test parameters for aspirin and metoprolol

Parameter	aspirin	metoprolol
Retention time (min)	3.06	6.97
Resolution	2.68	-
Tailing factor	0.82	0.78
Theoretical plates	4875	5412
LOQ (ng)	10	50
LOD (ng)	5	10

Linearity and calibration curves

To establish the linearity of analytical method, a series of dilution ranginng from 20-100 µg/ml for aspirin and 10-50 µg/ml for metoprolol was prepared in the same manner described in the Table 1. All the solutions were filtered through 0.22 µm membrane filter prior to use and injected in chrommatograph. A calibration curves were plotted between the mean peak areas vs. respective concentrations. The corresponding linear regression equation was *y=10547.x-1320.2* with square of correlation coefficient R^2 of 0.9999 for aspirin and *y=17120.x-1202.4* with square of correlation coefficient R^2 of 0.9999 for metoprolol, respectively.

Table 3. Precision of the method

aspirin		metoprolol	
Amount claimed (mg/tablet)	Amount found (mg/tablet)	Amount claimed (mg/tablet)	Amount found (mg/tablet)
100.0	99.85	50.00	50.41
	99.47		49.54
	100.5		49.26
	99.85		49.87
	100.4		50.62
	100.1		50.45
Mean	100.0	Mean	50.03
S_d	0.385	S_d	0.553
%RSD	0.39	%RSD	1.11

Precision

The precision of the method was evaluated by performing six independent determinations of the test sample preparation and calculating RSD (%). The RSD values measured during assessment of precision was <2.0% for both analytes, confirming the method is precise (Table 3).

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To the preanalysed sample solution, a definite concentration of standard drug was added and then its recovery was analysed. The percent recovery for aspirin was found to be 99.75 % and for metoprolol it was 99.17 % (Table 4).

Table 4. Statistical data for accuracy

Statistical data	aspirin	metoprolol
% Recovery	99.75	99.17
SD	1.179	1.73
% RSD	1.18	1.75

Analysis of pharmaceutical preparation

The results from analysis of pharmaceutical formulation are shown in Table 5.

Table 5 Results for assay of aspirin and metoprolol in pharmaceutical preparation

Labeled amount (mg)		Observed amount ± SD		%RSD	
aspirin	metoprolol	aspirin	metoprolol	aspirin	metoprolol
100	50	99.80±0.41	99.78±0.0.55	0.40	0.55

CONCLUSION

The newly developed LC method is specific, precise, accurate and rapid. The analytical procedure is suitable for quality control of pharmaceutical preparation containing aspirin and metoprolol.

REFERENCES

[1] I.Os, T. Hotens, J. Dollerup, C.E. Mogensen. *Amer. J. Hypertens*., **1991**, 10, 899.
[2] E. Schmieder. *Expert Opin. Pharmacother*., **2004**, 5, 2303.
[3] R. Collins, R. Peto, S. MacMohan; *Lancet*, **1990**, 335, 827.
[4] S. Suharmi, B. Santoso. *Eur. J. Pharmacol*., **1990**, 183, 2375.
[5] J.V. Okopski. *J. Clin. Pharm. Ther*., **1987**, 12, 369.
[6] J. Fang, H.A. Semple, J. Song. *J. Chromatogr. B*, **2004**, 809, 9.
[7] K.Kim, J. Lee, K. Shin, J. Kang, W. Mar. *Chromatographia*, **2002**, 55, 81.
[8] M. Angier, R. Lewis, A. Chaturvedi, D. Canfield. *J. Anal. Toxicol.*, **2005**, 29, 517.
[9] B. Yilmaz, S. Arslan. V. Akba, *Talanta*, **2009**, 80, 346.
[10] S. Albers, J. Elshoff, C. Volker, A. Richter, S. Laer. *Biomed. Chromatogr.*,**2005**, 19, 202.
[11] A. Braza, P. Modamio, C. Lastra, E. Marino. *Biomed. Chromatogr*., **2002**, 16, 517.
[12] V. Boralli, E. Coelho, P. Cerqueira, V. Lanchote. *J. Chromatogr. B.*, **2005**, 823, 195.
[13] R. Johnson, R. Lewis. *Forensic Sci. Int.*, **2006**, 156, 106.
[14] B. Jensen, C. Sharp, S. Gardiner, E. Begg. *J. Chromatogr. B.*, **2008**, 865, 48.
[15] C. Dupuis, J. Gaulier, A. Pellisier-Alicot, P. Marquet, G. Lashatre. *J. Anal. Toxicol.*, **2004**, 28, 674.
[16] K. Gowda, U. Mandal, P. Selvan, W. Solomon, A. Ghosh, A. Sarkar, S. Agarwal, T. Rao, T. Pal. *J. Chromatogr. B.*, **2007**, 858, 13..
[17] B. Sagar, R. Nitin, S. Sohan. *Der Pharmacia Lettre*, **2011**, 3, 1.
[18] G. Alpdogan, S. Sungur. *Spectrochim. Acta Part A.*, **1999**, 55, 2705.
[19] P.S. Hervey, K.L. Goa. *Drugs*, **1999**, 58, 469.
[20] H. Tohgi, S. Konno, K. Tamura, B. Kimura, K. Kawano. *Stroke*, **1992**, 23, 1400.
[21] M. Ahmed, H. Biswas, G. Sadic. *J. Med. Sci.*, **2001**, 1, 61.
[22] P. Umapathi, P. Parimoo, S. Thomas, V. Agarwal. *J. Pharm. Biomed. Anal.*, **1997**, 15, 1703.
[23] M. Gandhimathi, T. Ravi, N. Snukla. *Indian Drugs*, **2006**, 43, 397.
[24] W. Morden, I. Wilson. *Rapid Commun. Mass Spectrom.*, **1999**, 10, 1951.
[25] M. Sawyer, V. Kumar. *J. Chromatogr. Sci.*, **2003**, 41, 393.
[26] W. Chao, T. Vieckers, C. Mann. *J. Pharm. Biomed. Anal.*, **1997**, 16, 87.
[27] T. Yamane, M. Nakashima, M. Wada, K. Nakashima. *Chromatography*, **2006**, 27, 71.

[28] ICH, Q2B, Validation of Analytical Procedures: Methodology, International Conference on Harmonization, Geneva, November, **1996.**