International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:4, No:3, 2010

Metoprolol Tartrate-Ethylcellulose Tabletted Microparticles: Development of a Validated In-vitro In-vivo Correlation

Fatima Rasool, Mahmood Ahmad, Ghulam Murtaza*, Haji M. S. Khan,
Shujaat A. Khan, Sonia Khiljee and Muhammad Qamar-Uz-Zaman

***Abstract*—**This study describes the methodology for the development of a validated in-vitro in-vivo correlation (IVIVC) for metoprolol tartrate modified release dosage forms with distinctive release rate characteristics. Modified release dosage forms were formulated by microencapsulation of metoprolol tartrate into different amounts of ethylcellulose by non-solvent addition technique. Then in-vitro and in-vivo studies were conducted to develop and validate level A IVIVC for metoprolol tartrate. The values of regression co-efficient (R^2-values) for IVIVC of T2 and T3 formulations were not significantly ($p<0.05$) different from 1 while the values of R^2 for IVIVC of T1 and Mepressor® were significantly ($p<0.05$) different from 1. Internal prediction errors of IVIVC, calculated from observed Area under Curve (AUC) and predicted AUC, were less than 10%. This study successfully presents a valid level A IVIVC for metoprolol tartrate modified dosage forms.

***Keywords*—**Metoprolol tartrate, Dissolution, Bioavailability, Validated in-vitro in-vivo correlation.

I. Introduction

CURRENTLY, an increasing role of validated in-vitro in-vivo correlation (IVIVC) in the development of modified release dosage forms is observed. In-vivo performance of an alternative formulation of predefined specifications can be predicted by using particular dissolution specifications and IVIVC function [1]. Thus, the development of a good correlation needs a discriminating dissolution test which can act as an alternative of gastrointestinal conditions. Food and Drug Administration (FDA) propose very descriptive guidelines for the development and validation of IVIVC. According to FDA, three different release rates of a drug are needed for the development of an IVIVC. FDA also suggests the internal or external validation of IVIVC [2, 3].

Fatima Rasool is with Department of Pharmacy, Faculty of Pharmacy & Alternative Medicine, the Islamia University of Bahawalpur, Bahawalpur, Pakistan (phone: 0092-62-9255243; fax: 0092-62-9255565; e-mail: fatimashoaib21@yahoo.com).
Mahmood Ahmad, ma786_786@yahoo.com
Ghulam Murtaza, gmdogar356@gmail.com
Haji Muhammad Shoaib Khan, fatimashoiab21@hotmail.com
Shujaat Ali Khan, shujaat786_786@yahoo.com
Sonia Khilji, allah_pharmacist@yahoo.com
Muhammad Qamar-Uz-Zaman, qamarpharmacist@hotmail.com
Department of Pharmacy, Faculty of Pharmacy & Alternative Medicine, the Islamia University of Bahawalpur, Bahawalpur, Pakistan (phone: 0092-62-9255243; fax: 0092-62-9255565).

Matrix tablets and microparticles are important classes of most commonly used oral sustained release (SR) dosage forms. The later class i.e. microparticulate dosage form development has currently attracted much attention of researchers. It involves the coating of release retardant material (polymer) around drug molecules [4]. Ethylcellulose, a hydrophobic plastic polymer, is one of the most commonly used release retardant materials. Hydrophobic plastic polymeric dosage forms, composed of ethylcellulose for instance, do not erode and swell. Thus, diffusion as a result of liquid penetration is the mode of drug release from ethylcellulose microparticles. Many researchers have excellently described ethylcellulose, its properties and functions. Ethylcellulose is available in various viscosity grades on the basis of ethoxyl group substitution. The viscosity of ethylcellulose depends upon the ethoxy substitution [5].

Metoprolol tartrate is classified as BCS (Biopharmaceutics Classification System) class I drug as it is highly water soluble and permeable drug [6]. The dissolution is the rate limiting step in the absorption of metoprolol tartrate, therefore level A IVIVC is expected for its modified release dosage forms. FDA recommends level A IVIVC as the highest correlation for the submission of New Drug Application (NDA) and Abbreviated New Drug Application (ANDA) [7]. The literature survey shows many studies regarding the development of matrix tablets of metoprolol tartrate [4, 6]. Moreover, some studies have also been conducted for the development of IVIVC for matrix SR tablets metoprolol tartrate [6, 8]. Literature shows no study regarding IVIVC development of metoprolol tartrate tabletted microparticles.

This study is focused on the development of sustained release tabletted microparticles of metoprolol tartrate of different release characteristics by varying ethylcellulose concentration. Then in-vitro and in-vivo studies were conducted to develop validateed IVIVC for metoprolol tartrate.

II. Materials and Methods

A. Materials

Pure metoprolol tartrate and ethyl cellulose (22 cp) were obtained from Novartis Pharma-Pakistan and Sigma-USA, respectively. Metoprolol tartrate tablets (Mepressor®, Novartis Pharma-Pakistan) was purchased from market. The chemicals

such as dichloromethane, liquid paraffin and n-hexane were of analytical grade supplied by Merck (Germany).

B. Formulations

Three tabletted microparticulate formulations (T1- fast, T2- moderate and T3- slow release) of metoprolol tartrate with different release rates were prepared by its microencapsulation (Non-solvent addition coacervation) into different amounts of ethyl cellulose i.e. 1:1, 1:2 and 1:3 drug : polymer ratios, respectively [9]. Each formulation contained 200 mg metoprolol tartrate. Fourth formulation was Mepressor® 200 mg, Novartis Pharma-Pakistan.

C. In-vitro dissolution data and its analysis

In-vitro dissolution tests of all formulations were conducted in phosphate buffer pH 6.8 stirred at 50 rpm and 37±0.5 ºC using USP Apparatus II to get drug dissolved (%) versus time (h) profiles (Figure 1). The samples (5 ml) were collected by automatic sampler at pre-defined time points for 12 hours and then analyzed at 275 nm using UV-Vis spectrophotometer-Shimadzu 1601, Japan. To compare the obtained dissolution profiles of all formulations by similarity factor (f_2), following equation was used [5].

$$f_{2=} 50 \log \{[1+ (1/P) \sum_{i=1}^{P} (R_t-T_t)^2]^{-1/2} *100\} \quad (1)$$

Where "Rt" and "Tt" are the cumulative percentage dissolved at time point "t" for reference and test formulations, respectively, and "n" is the number of sample points. The two dissolution profiles are considered similar if average difference between all compared dissolution samples is less than 15% and f_2 value is greater than 50%.

D. Statistical analysis

One way analysis of variance for various statistical calculations was conducted using SPSS, version 12.0. The level of significance was set at $p<0.05$.

E. Bioavailability data and its analysis

Drug Plasma concentration versus time profiles of all formulations were obtained by an in-vivo study involving 20 non-smoking healthy young male human subjects (confirmed by physical and biochemical examination) approved by the Board of Advance Studies and Research, the Islamia University of Bahawalpur, Pakistan. Four periods, four treatments, single dose and randomized cross over study design with a wash out period of seven days was adopted. Blood samples (5 ml) were collected at pre-defined time points for 24 hours, centrifuged to separate plasma for analysis using High Performance Liquid Chromatography (HPLC)-UV, Perkin-Elmer, Japan [11]. The study subjects were continually monitored for blood pressure and pulse rate. The Software, Kinetica 4.0 was used to calculate pharmacokinetic parameters from the obtained data following one-compartment model. Percent drug absorbed at all time point were calculated by Wagner-Nelson equation as given below [3].

$$\text{Percent Absorbed} = \{(C(t) / Ke + AUC(0\text{-}t) / AUC(0\text{-}\infty)) \times 100 \quad (2)$$

Where C (t) = plasma drug concentration at time t, K = elimination rate constant. Its value used in this equation was obtained from Mepressor. AUC (0-t) = area under the concentration time curve from time "0" to time "t", AUC (0-∞) = area under the concentration time curve from time "0" to infinity.

F. Development of IVIVC and its internal validation

The data obtained from in-vitro dissolution tests and bioavailability studies was used to develop IVIVC. A graph was plotted between percent drug absorbed and dissolved for all formulation and regression analysis was performed (Figure 2). IVIVC was considered good if the value of regression co-efficient was not different from 1. The predictability of developed IVIVC was assessed by its internal prediction error (%) of Cmax (maximum plasma drug concentration) or AUC, calculated by following formula [3].

$$\text{Percent Prediction Error} = [(AUC_{observed} - AUC_{predicted}) / AUC_{observed}] \times 100 \quad (3)$$

According to FDA guidelines, an IVIVC is predictive if the internal prediction error for a formulation is not more than 15% for AUC and Cmax and the internal prediction error across formulations is not more than 10% for AUC and Cmax.

III. Results and Discussion

The purpose of this study was to develop formulations with different release rates and to evaluate for in-vitro and in-vivo studies to establish an IVIVC. The formulations with different release rates were prepared by using different amounts of polymer for the microencapsulation of metoprolol tartrate followed by direct compression in to tablets. The drug release versus time data for all prepared tabletted microparticles is plotted in Figure 1. Increase in polymer concentration as 1:1, 1:2 and 1:3 (drug: polymer ratios) drastically reduced the release rate of metoprolol tartrate. As the release of drug from ethyl cellulose matrix takes place by diffusion mechanism [5], thus with the increase in ethyl cellulose concentration, the surface pores of formulations may reduce in number resulting in the retardation of drug release.

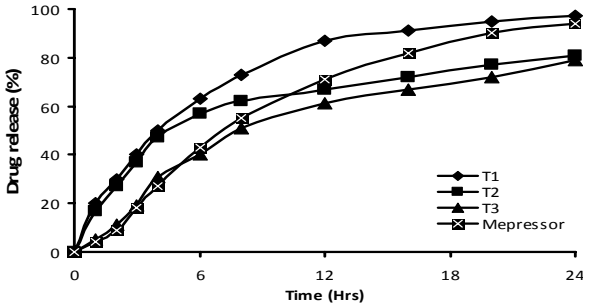

Fig. 1 Percentage of drug release versus time profiles of metoprolol tartrate tabletted microparticulate formulations

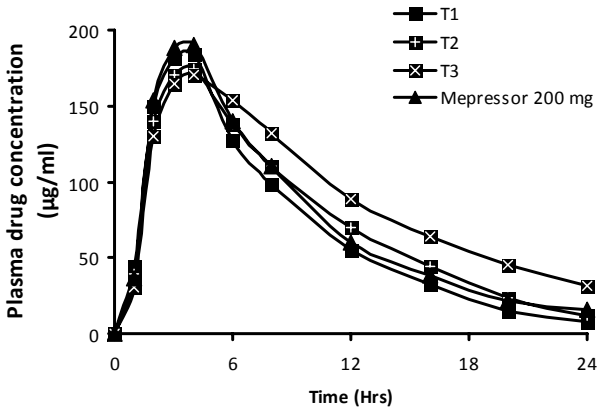

Fig. 2 Plasma drug concentration versus time profile of metoprolol tartrate tabletted microparticulate formulations

Figure 1 illustrates cumulative dissolution profiles from T1, T2 and T3 formulations in phosphate buffer pH 6.8 stirred at 50 rpm using USP Apparatus II. Table 1 shows f_2-values for T1 versus T2, T2 versus T3 and T1 versus T3 comparison and elaborated that the two dissolution profiles in above mentioned pairs are dissimilar to each other. The f2-values of each dissolution profile comparison were below 50.

TABLE I
F2-VALUES OBTAINED FROM THE COMPARISON OF METOPROLOL TARTRATE TABLETTED MICROPARTICULATE FORMULATIONS

	f2-values
T1 versus T2	46.31
T2 versus T3	44.02
T1 versus T3	31.77

Table 2 illustrates the average values of various pharmacokinetic parameters for tabletted microparticles of metoprolol tartrate.

TABLE II
AVERAGE VALUES OF VARIOUS PHARMACOKINETIC PARAMETERS METOPROLOL TARTRATE TABLETTED MICROPARTICULATE FORMULATIONS

	T1	T2	T3	Mepressor®
AUC (µg.hr/ml)	1658.74	1846.66	2497.42	1891.02
Cmax (µg/ml)	184	174	170	190
Tmax (Hrs)	4	4	4	4

Figure 2 shows percent drug dissolved from T1, T2 and T3 formulations versus time. While Figure 3 illustrates a plot between percent drug dissolved and percent drug absorbed.

TABLE III
REGRESSION CO-EFFICIENT OF IVIVC FOR METOPROLOL TARTRATE TABLETTED MICROPARTICULATE FORMULATIONS

	T1	T2	T3	Mepressor®
R^2-values	0.8727	0.9669	0.9553	0.7628
Prediction error	9.1%	7.3%	7.7%	8.9%

Table 3 shows the values of regression co-efficient of IVIVC (percent drug dissolved versus percent drug absorbed) for metoprolol tartrate formulations and internal prediction error. The R2-values for IVIVC of T2 and T3 formulations were not significantly ($p<0.05$) different from 1 while the values of R^2 for IVIVC of T1 and Mepressor® were significantly ($p<0.05$) different from 1.

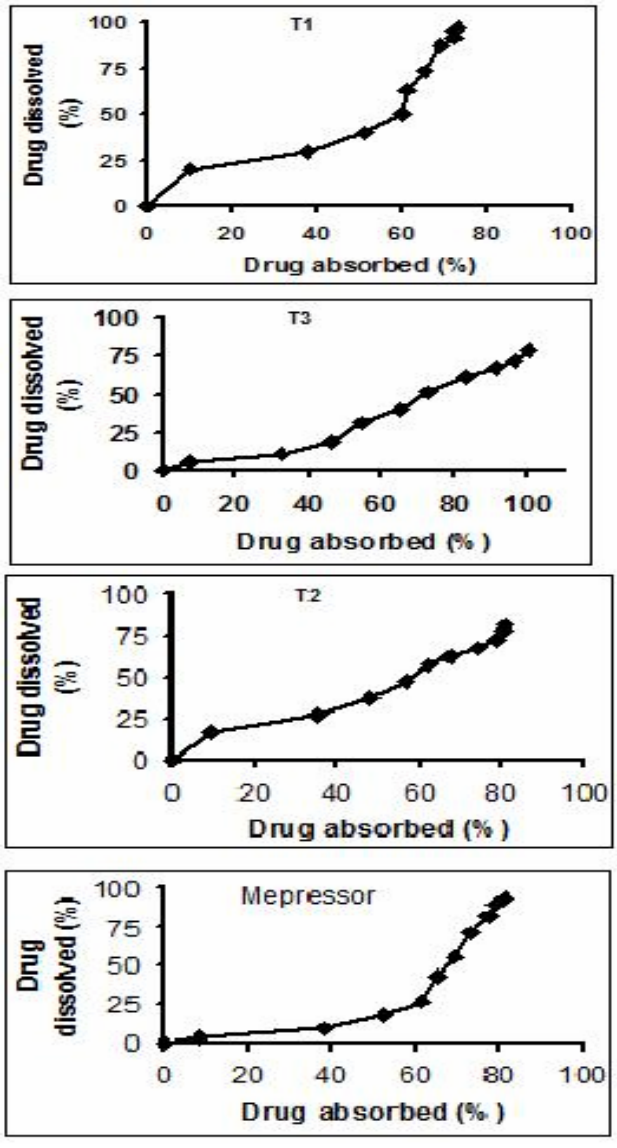

Fig. 3 Plots between percent absorbed and dissolved of metoprolol tartrate from its tabletted microparticulate formulations

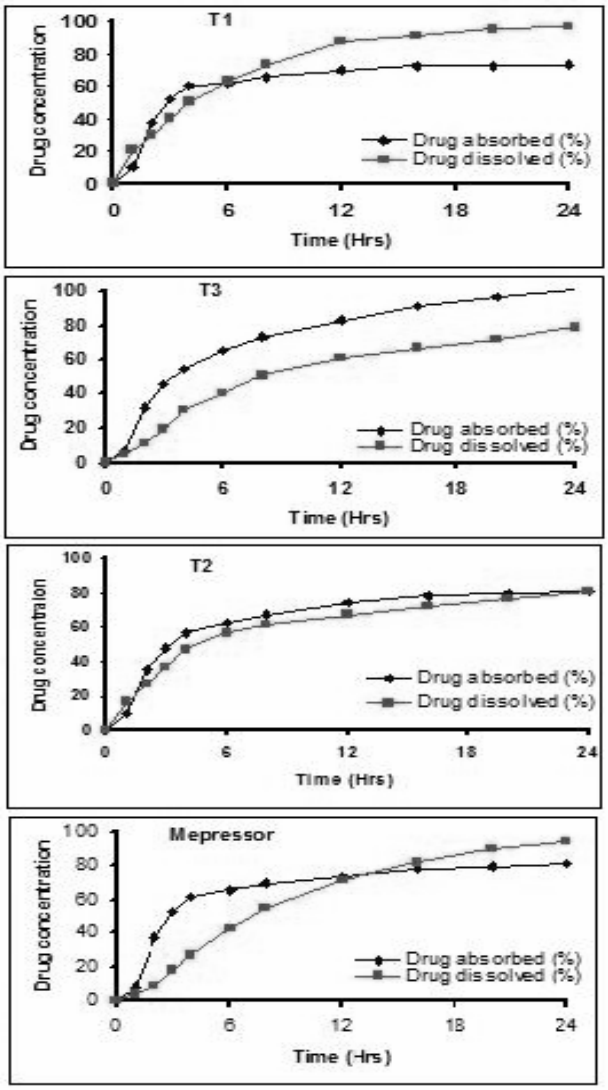

Fig. 4 Percent drug dissolved and absorbed from metoprolol tartrate tabletted microparticulate formulations versus time

A valid IVIVC is helpful for the use of in-vitro dissolution data instead of bioavailability studies, formulation optimization and post-approval changes [7]. Thus the present study was also designed to develop to develop a high quality IVIVC and its internal validation by assessing percent prediction error of Cmax or AUC. Therefore, present study is a predictable analysis for T2 and T3 type formulations because regression co-efficient of IVIVC was not significantly ($p<0.05$) different from 1.

IV. Conclusion

This validated IVIVC allows the use of associated dissolution data for biowaiver study. This study can also be used as a guideline for the development of valid IVIVC for other BCS class I drugs.

References

[1] H. Rettig, J. Mysicka. (2008, February). IVIVC: Methods and Applications in Modified-Release Product Development. *Dissol. Technol.* [Online]. 15. pp. 6-9. Available: www.dissolutiontech.com/DTresour/.../DT200802_A01.pdf

[2] U.S. Department of Health and Human Services. (1997, September). Guidance for industrial. Extended release oral dosage forms: Development, evaluation and application of In Vitro/In Vivo correlations. Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Rockville, MD. Available: www.fda.gov/.../Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070239.pdf

[3] G. Murtaza, M. Ahmad, N. Akhtar. (2009, August). Biowaiver study of oral tabletted ethylcellulose microcapsules of a BCS class I drug. *Bull. Chem. Soc. Ethiop*. [Online]. 23 (2). pp. 1-16. Available: http://ajol.info/index.php/bcse/article/view/44959/0

[4] R. V. Nellore, G. S. Rekhi, A. S. Hussain, G. L. Tillman, L. L. Augsburger. (1998, January). Development of metoprolol tartrate extended-release matrix tablet formulations for regulatory policy consideration. J. Control. Release [Online]. 50 (2). pp. 247-256. Available: http://www.ncbi.nlm.nih.gov/pubmed/9685891

[5] G. Murtaza, M. Ahamd, N. Akhtar, F. Rasool. (2009, July). A comparative study of various microencapsulation techniques: Effect of polymer viscosity on microcapsule characteristics. *Pak. J. Pharm. Sci*. [Online]. 22 (3). pp. 291-300. Available: www.pjps.pk/CD-PJPS-22-3-09/Paper-10.pdf

[6] N. D. Eddington. In Vitro In Vivo Correlation with Metoprolol Extended Release Tablets Using Two Different Releasing Formulations: An Internal Validation Evaluation. Published at http://www.locumusa.com. *Int. J. Generic Drugs* [Online]. pp. 417-429. Available: http://www.locumusa.com

[7] U.S. Department of Health and Human Services. (1995, November). Guidance for industry: Modified release solid oral dosage forms scale-up and postapproval changes: chemistry, manufacturing, and controls, in vitro dissolution testing, and in vivo bioequivalence documentation. Center for drug Evaluation and Research, Food and Drug Administration. Rockville, MD. Available:www.fda.gov/.../Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070636.pdf

[8] N. D. Eddington, P. Marroum, R. Uppoor, A. Hussain, L. Augsburger. (1998, March). Development of an In Vitro-In Vivo Correlation for a hydrophilic Metoprolol tartaret extended release tablet formulation. *Pharm. Res*. [Online]. 15(3). pp. 466-473. Available: www.locumusa.com/pdf/members/ivivc-03.pdf

[9] F. Rasool, M. Ahmad, G. Murtaza, S. A. Khan, M. N. Aamir. [2010, 21 January]. Hydrophilic plastic polymeric tabletted microparticles loaded with metoprolol tartrate: Formulation and in-vitro evaluation. Submitted for publication in *Latin Am. J. Pham*. [Online]. Available: http://www.latamjpharm.org/

[10] S. Klein, J. B. Dressman. [2007, February]. Comparison of drug release from metoprolol modified release dosage forms in single buffer versus a pH-gradient dissolution test. *Dissol. Technol*. [Online]. 13: 6-12. Available: www.dissolutiontech.com/DTresour /.../DT200602_A01.pdf

[11] M. Aqil, A. Ali, A. Ahad, Y. Sultana, A. K. Najmi, N. Saha. [2007, December]. A validated HPLC method for estimation of metoprolol in human plasma. *Acta Chromatogr*. [Online]. 19. pp. 130-140. Available:livewww.us.edu.pl/uniwersytet/jednostki/wydzialy/chemia/.../11_AC19.pdf