SIMULTANEOUS DETERMINATION OF METOPROLOL, PROPRANOLOL AND PHENOL RED IN SAMPLES FROM RAT IN SITU INTESTINAL PERFUSION STUDIES

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ABSTRACT

Single-pass intestinal perfusion technique (SPIP) is the most used classic technique employed in the study of intestinal absorption of compounds in which a non-absorbable marker such as phenol red is used to correct the water flux. A simple and rapid reversed-phase high performance liquid chromatographic method with UV detection at 227 nm was developed for simultaneous quantitation of propranolol and metoprolol along with phenol red for in-situ permeability studies. The mobile phase was a mixture of 55% methanol, 45% of 0.05 M KH_2PO_4 aqueous solution (adjusted to pH 6) and 0.2 % (v/v) triethylamine. Analysis was run at a flow rate of 1 ml/min with a 9 min run time. The calibration curves were linear for all three compounds ($r > 0.999$) across the concentration range of 7.5-125 µg/ml with a limit of detection of 4.24, 2.18 and 8.57 ng/ml and limit of quantification of 14, 7.2 and 28.3 ng/ml for metoprolol, propranolol and phenol red respectively. The coefficient of variation for intra-assay and inter-assay precision was less than 8% and the accuracy was between 93.6-107%. Using the SPIP technique and the suggested HPLC method for sample analysis, the mean values of 0.49 e^{-4} (±0.19) cm/sec and 0.32 e^{-4} (± 0.09) cm/sec were obtained for propranolol and metoprolol intestinal permeability coefficients respectively.

Keywords: Propranolol; Metoprolol; Phenol red; liquid chromatography; Permeability

INTRODUCTION

Propranolol hydrochloride and metoprolol tartrate are clinically important beta blockers which are used orally in the treatment of disorders such as hypertension, arrhythmia and angina pectoris (1). Oral administration is the most convenient and useful route for drug delivery which involves gastrointestinal absorption of drugs. For the oral route the amount of drug reaching the general circulation depends on many different factors. Among these, the ability of a molecule to cross the biological membranes (permeability) is a very important biopharmaceutical parameter. In fact the prediction of drug absorption is very important for the design of an oral preparation. Since human in vivo studies are not usually possible in the early phases of drug development, therefore, some experimental methods such as animal *in vivo* and *ex vivo* models have so far been evolved to estimate gastrointestinal absorption of drugs (2-7). One of the most used classic technique in the study of intestinal absorption of compounds has been the single-pass intestinal perfusion (SPIP) model (8, 9), which provides experimental conditions closer to what is faced following oral administration. This technique has lower sensitivity to pH variations because of a preserved microclimate above the epithelial cells and it maintains an intact blood supply to the intestine (10, 11). Because water absorption and secretion during the perfusion may cause errors in the calculated effective permeability (P_{eff}) values, a non-absorbable marker to correct water flux through the intestinal wall is needed (8). For this purpose phenol red as a non-absorbable marker which was introduce in 192(12).is co-perfused with drug compounds in each experiment.

Nowadays interest has grown for using *in vitro* and *in situ* methods to predict, as early as possible, *in vivo* absorption potential of a drug. For this purpose, each laboratory should carefully establish its own calibration curve relating human intestinal permeability to observed *in vitro/in situ* permeability. To establish a correlation between human and rat intestinal permeability in our own laboratory, we used a series of drugs with known

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human permeability values were used. Actually there are a limited number of drugs which their human intestinal permeability values are available. Among drugs that were used, propranolol and metoprolol are the two beta blockers which their in vivo absorption potential in human have been studied. On the other hand, since the membrane permeability for passively absorbed compounds such as metaprolol and propranolol(13) is a function of their partitions coefficient and pk_a's (2), they do not interfere in intestinal permeability of each other and the combination of propranolol and metoprolol together with phenol red as a marker, which is not absorbed across the intestinal membrane, can be perfused through the intestinal segment in an experiment to determine intestinal permeabilities at the same time. Therefore for the purpose of time saving and using fewer animals, perfusion of metoprolol together with propranolol (in the presence of phenol red) in an experiment and simultaneous determination of these two drugs and phenol red is meaningful. Various HPLC methods for determination beta of blockers using different mobile phases and wavelengths for each beta blocker (14-16) and optimization of a mobile phase to separate beta blockers by HPLC simultaneously (1) have been reported. These currently available methods failed to resolve phenol red from the interferences and there is still no method available for simultaneous determination of a beta blocker and phenol red in biologic and non-biologic samples.

The objective of this study was to suggest a simple and rapid RP-HPLC method for simultaneous quantification of metoprolol and propranolol in the presence of phenol red.

MATERIALS and METHODS

Chemicals

Propranolol was provided from ICI-Pharma (Madrid, Spain) and metoprolol was from Ciba-Geigy (Barcelona, Spain). Phenol red was purchased from Sigma chemical company (St. Louis, MO, USA). Acetonitrile and methanol were HPLC grade and obtained from Merck (Darmstadt, Germany). KH_2PO_4, NaH_2PO_4, Na_2HPO_4, Orthophosphoric acid, NaOH, NaCl and triethylamine were purchased from Merck (Darmstadt, Germany). Double distilled water was used during the entire HPLC procedure.

Apparatus

A liquid chromatographic system (Beckman, USA) comprising of 126 gold solvent delivery module equipped with a Rheodyne (Cotati, CA) injector and a variable wavelength ultraviolet spectrophotometric detector (166 gold, Beckman, USA) set at 227 nm was used in this study. Analytical column which were used for chromatographic separations was Shimpack VP-ODS 5 µm 4.6 x 250 mm (Shimadzu, Kyoto, Japan) with a Shimpack VP-ODS 5 µm 4.6 x 50 mm guard column (Shimadzu, Kyoto, Japan). System Gold software was used for data acquisition and System Gold nouveau software was used for data reporting and analysis.

Chromatographic conditions

The mobile phase was a mixture of 55% methanol and 45% of 0.05 M KH_2PO_4 aqueous solution adjusted to pH 6, to which was added 0.2 % (v/v) triethylamine. The mobile phase was filtered through sintered glass filter P5 (1-1.6 micron) (Winteg, Germany) and degassed in sonicator (Liarre, Italy) under vacuum. The mobile phase was pumped in isocratic mode at a flow rate of 1 ml/min at ambient temperature. The UV detection was accomplished at 227 nm and samples of 20 µl were injected using Hamilton injector syringe (Hamilton, Bonaduz, Switzerland) onto the column.

Validation procedure

A full validation of the assay consisting of linearity, lower limit of detection and quantitation (LOD and LOQ), intraday and interday accuracy and precision of the method was performed. To access linearity, known concentrations of compounds in PBS buffer were prepared. The concentrations were in the range of 7.8-125 µg/ml. The accuracy and precision data were obtained by analyzing four aliquots of samples at different concentrations. Intraday reproducibility was determined using four aliquots of samples and interday reproducibility was determined over a 4-day period (n=4). Finally to determine the LOD, the signal to noise ratio was used by comparing test results from samples with known concentrations to blank samples. The LOQ is also defined as the lowest concentration that can be quantitate with acceptable precision and accuracy under the stated experimental condition.

Composition of perfusion solution

The perfusion buffer was prepared by dissolving 5.77 g Na_2HPO_4 (anhydrous), 4.085 g $NaH_2PO_4.2H_2O$ and 7g NaCl in one liter of distilled water. The pH of prepared buffer was 7.2. Preliminary experiments showed that there were no considerable adsorption of the compounds on the tubing and syringe. Samples from perfusion study were filtered and directly injected onto HPLC column and required no sample preparation prior to analysis.

Preparation of standard solutions

Primary stock solution of all three compounds was prepared in phosphate buffered saline (PBS) to obtain a concentration of 2 mg/ml of each compound. Then it was diluted to 250 µg/ml to make a working solution and standards for calibration curves and quality control samples were prepared using serial dilution of working solution in PBS. The concentration range for working standard solutions was 7.8-125 µg/ml. This range was selected based on the concentration that were going to used in animal studies. Preliminary studies showed that there is no chemical interactions and stability problem in the solution for all components.

In situ permeation studies

In situ permeation studies were performed using established methods adapted from the literature (17, 18). Briefly, male Wistar rats (250-300 g) were maintained on 12 h light- dark cycle and fasted 12-18 h before experiment. However drinking water was readily accessible. The rats were anaesthetized using an intraperitoneal injection of pentobarbital (60 mg/kg) and placed on a heated pad to keep normal body temperature. By making a midline abdominal incision, a 10 cm section of the proximal rat jejunum was located gently with plastic tubing (0.04 in. i.d.,0.085 in. o.d.) rinsed with saline (37^{o}C) and attached to the perfusion assembly which consisted of a syringe pump (Palmer, UK) and a 60 ml syringe connected to it. Care was taken to handle the small intestine gently and to minimize the surgery in order to maintain an intact blood supply. The entire surgical area was then covered with Parafilm to reduce evaporation. Blank perfusion buffer was infused for 10 min by a syringe pump followed by perfusion of compounds (0.13 mM and 0.07 mM for propranolol and metoprolol respectively) at a flow rate of 0.2 ml/min for 90 min. The concentrations were selected based on the oral dose of drug products. Outlet samples were collected every 10 min in microtubes. The volume of sample for each time interval was 2.1 ml. When the experiment was completed, the length of segment was measured and the animal was euthanitized with a cardiac injection of saturated solution of KCl. Samples were stored at -20^{o}C until analysis. In all animal studies "Guide to the care and use of experimental animals" by Canadian Council on Animal Care, was followed (19).

RESULTS AND DISCUSSION

Chromatography and specificity

In fig. 1 the representative chromatogram of a collected sample containing propranolol, metoprolol and phenol red is presented. The retention times were 4.1, 4.9 and 7.4 min for metoprolol, phenol red and propranolol respectively. The chromatographic run time of 9 min which was sufficient for sample analysis allows analyzing a large number of samples in a short period of time. Injection of blank PBS buffer collected from outlet tubing (before perfusion of the drug solution) onto HPLC column showed that no interfering peak could be observed on chromatogram. Moreover System suitability test parameters were checked to ensure that the system is working correctly during the analysis (20). Parameters which are typically used in suitability evaluations are reported as follows: The important parameter t_0 which is marked in most cases by the center of the first band or baseline disturbances following sample injection is 2.56 min in the present analysis. This is the time at which PBS buffer peak appears. Capacity factor (k') values were 0.60, 0.88 and 1.87 for metoprolol, phenol red and propranolol respectively. Selectivity factor (**α**) was found to be 1.54 for separation of metoprolol and phenol red. The value of 2.13 was obtained for separation of phenol red and propranolol as well. The respected resolution parameters were 4.32 and 9.27 respectively. The calculated Tailing factors of 1.14, 1 and 1.07 were obtained for metoprolol, phenol red and propranolol peaks respectively.

Linearity

The five-point calibration curves for all three compounds were prepared in the range of 7.8-125 µg/ml. This concentration range was selected based on the drug concentrations which was used in permeability studies. Since in each experiment there was already an inlet solution with defined concentration of compounds (0.13 mM and 0.07 mM for propranolol and metoprolol respectively), the external standard method was employed in this investigation. The concentration-peak area relationships were described by a simple regression analysis. The minimum correlation coefficient of the calibration curves for three substances was 0.9998. The standard curves were prepared on 4 consecutive days and regression parameters are listed in table 1.

Accuracy and Precision

Four quality control samples with concentrations within calibration range were used in triplicates (n=3) to determine the accuracy and precision of the method. The samples were prepared in PBS which is a well-known, accepted medium for permeability studies. The mean of the observed concentrations for all three compounds from the prepared samples were calculated. The repeatability (intra-assay precision) and the intermediate (between-assay) precision were calculated from data obtained during 4 day validation. Results are shown in table 2 and table 3 respectively.

Table 1. Analytical parameters of calibration curves of propranolol, metoprolol and phenol red

Regression equation‡	Propranolol	Metoprolol	Phenol Red
range	7.8-125.0 µg/ml	7.8-125.0 µg/ml	7.8-125.0 µg/ml
Slope (b ± SD)♣	129648 (± 323.1)	24318 (± 338.7)	22533 (± 2863)
Intercept (a ±SD)♣	418079 (±162744)	67599 (± 38312)	59464 (± 26807)
r (n) ♠	0.9999 (4)	0.9998(4)	0.9999 (4)

‡ Linear regression analysis with a regression equation of y = a + bx, in which x is the concentration in µg/ml and y is the peak area, ♣ S.D. is the standard deviation of intercept and slope, ♠ r is the correlation coefficient and n is the number of points in calibration curves. Each point is the mean of four measurements.

Table 2. Intra-assay precision obtained from 4 calibration curves with 4 levels of QC samples

Compound	Added concentration (µg/ml)	Mean measured concentration (µg/ml)	SD (µg/ml)	CV (%)	Accuracy (%)
Metoprolol	125.0	124.2	0.41	0.33	99
	62.5	64.1	0.07	0.12	100
	31.2	29.4	2.09	7.10	98
	15.6	16.2	0.32	2.00	102
Propranolol	125.0	124.2	0.03	0.02	99
	62.5	64.0	0.01	0.02	102
	31.2	31.7	0.48	1.51	100
	15.6	15.3	1.07	6.98	93
Phenol Red	125.0	125.0	0.53	0.42	100
	62.5	64.7	1.32	2.04	102
	31.2	29.9	2.09	6.98	100
	15.6	16.4	0.36	2.29	107

Table 3. Inter-assay precision obtained from 4 calibration curves with 4 levels of QC samples

Compound	Added concentration (µg/ml)	Mean measured concentration (µg/ml)	SD (µg/ml)	CV (%)	Accuracy (%)
Metoprolol	125.0	123.7	0.75	0.60	99
	62.5	65.0	1.50	2.30	10
	31.2	31.6	1.95	6.17	101
	15.6	16.0	0.23	1.43	102
Propranolol	125.0	125.3	1.85	1.47	100
	62.5	65.3	2.28	3.49	104
	31.2	32.4	1.80	5.52	103
	15.6	15.3	1.23	7.99	97
Phenol Red	125.0	124	1.80	1.44	99
	62.5	64.0	1.02	1.60	102
	31.2	32.2	2.00	6.30	103
	15.6	16.7	0.26	1.56	107

Table 4. Concentrations used for compounds tested in SPIP method and Intestinal permeability coefficients determined in rats

Compound	C_{in} (mM)	Rat no.	P_{eff} ($\times 10^4$) cm/sec	Mean P_{eff} ($\times 10^4$) cm/sec	MeanNWF (µl/h/cm)
Propranolol	0.13	Rat 1	0.45	0.49 (±0.19)	28 (±12)
		Rat 2	0.29		
		Rat 3	0.75		
		Rat 4	0.5		
Metoprolol	0.07	Rat 1	0.35	0.32 (± 0.09)	-8 (± 2)
		Rat 2	0.4		
		Rat 3	0.19		
		Rat 4	0.37		

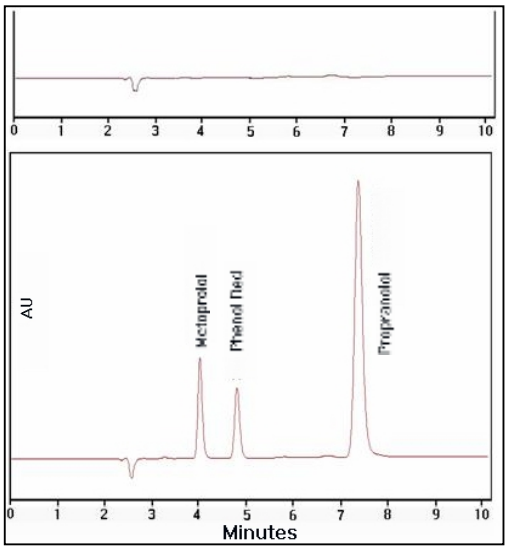

Figure 1. Representative chromatogram of a blank perfused PBS (up) and sample from intestinal perfusion containing metoprolol, propranolol and phenol red (down).

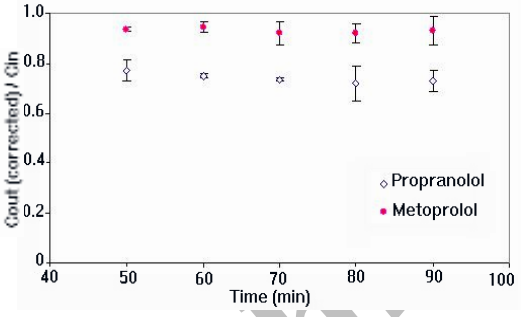

Figure 2. Plot of concentration ratio of the inlet and outlet tubing (Cin/Cout) vs. time. (n=4, error bars represent S.D)

Limits of detection (LOD) and quantitation (LOQ)

The LOD and LOQ decide about the sensitivity of the method. The LOD is the lowest concentration of the analyte detected by the method while the LOQ is the minimum quantifiable concentration. The signal-to-noise ratio of 3:1 and 10:1 were taken as LOD and LOQ, respectively, which were calculated using Gold nouveau software, and then confirmed by taking dilutions from the secondary stock solution. In the present study the detection limits for metoprolol, propranolol and phenol red were 4.24, 2.18 and 8.57 ng/ml respectively. The LOQ values were 14, 7.2 and 28.3 ng/ml respectively. The obtained values are good compared to other reported HPLC methods. For comparison, the LOQ's of 195 and 98 ng/ml has been reported for metoprolol and propranolol respectively. The reported values for LOD's were 10 ng/ ml for both compounds (1).

Data analysis

Effective permeability coefficient (P_{eff}) was calculated from the steady-state concentrations of compounds in the collected perfusate collected which is considered to be attainable when the concentration level of phenol red is stable. It was reached about 40 min after the beginning of the perfusion which is confirmed by plotting the ratio of the outlet to inlet concentrations (corrected for water transport) versus time. Representative results are plotted in Fig.2. P_{eff} was calculated using following equation according to the parallel tube model (21, 22):

$$P_{eff} = -Q \ln[C_{out\ corrected} / C_{in}] \ / 2\pi r l$$

In which C_{in} is the inlet concentration and $C_{outCorrected}$ is the outlet concentration of compound which is corrected for volume change in the segment using phenol red concentration in inlet and outlet tubing. Q is the flow rate (0.2 ml/min), r is the rat intestinal radius (0.18 cm) (19) and l is the length of the segment. It has been demonstrated that in humans at a Q_{in} of 2-3 ml/min, P_{eff} is membrane-controlled. In the rat model the Q_{in} is scaled to 0.2 ml/min, since the radius of the rat intestine is about 10 times less than that of human. The intestinal net water flux (NWF, µl/h/cm) was calculated from the following equation:

$$NWF = \frac{(1 - [Ph.red_{(out)} / Ph.red_{(in)}] * Q_{in}}{l}$$

where [Ph.red $_{(in)}$] and [Ph.red $_{(out)}$] are the inlet and outlet concentrations of the non-absorbable, water flux marker phenol red. A negative net water flux indicates loss of fluid from the mucosal side (lumen) to the serosal side (blood) while a positive net water flux indicates secretion of fluid into the segment (22). The determined P_{eff} and NWF values and concentrations used for compounds tested in the single pass intestinal perfusion are listed in table 4. As it is seen, the overall mean for NWF is low for both series of experiments. The reported value for intestinal net water flux according to Fagerholm et al was -56 (± 59) µl/h/cm (22). The stable water fluxes and P_{eff} values, with time, for tested compounds indicated that intestinal barrier function was maintained in all experiments. Any un-physiological leakage across the jejunal mucosa makes changes in the intestinal barrier function which is probably one of the main reasons for several of the contradictory results obtained by the in situ and in vitro models. The mean P_{eff} values obtained are in agreement with previously reported values for the effective intestinal permeability coefficients of $0.59e^{-4}$ (±0.13) cm/sec and 0.66 e^{-4} (±0.29) cm/sec for

metoprolol and propranolol respectively (6). The respected value for metoprolol according to Fagerholm et al was 0.33 e^{-4} (±0.20) cm/sec (22). Both of these compounds belong to Class I of Biopharmaceutics Classification System. That means both of them are highly soluble and highly permeable. The fractions (%) of dose absorbed in human as reported in literature are 95% and 90% for metoprolol and propranolol respectively (22).

Determination of P_{eff} values using SPIP method in the rat intestine for large number of compounds with different physicochemical properties, which is in process in our lab, could provide a prediction of oral dose absorbed in human. Since human in vivo studies are difficult and time consuming, this prediction is of considerable interest at an early stage of drug development.

CONCLUSION

The results indicated that this analytical method has acceptable precision, accuracy and linearity. Isocratic elution with UV detector and short run time, make the method suitable to be used in intestinal permeability studies.

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REFERENCES

1. Basci N.E., Temizer A., Bozkurt A., Isimer A., Optimization of mobile phase in the separation of β-blockers by HPLC, J. Pharm. Biomed. Anal., 1998, 18: 745-750.
2. Amidon G.L., Sinko P.J., Fleisher D., Estimating human oral fraction dose absorbed: a correlation using rat intestinal membrane permeability for passive and carrier-mediated compounds, Pharm Res., 1988, 5: 651-654.
3. Artursson P., Karlsson J., Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial cells. Biochem. Biophys. Res. Commun., 1991,175: 880-885.
4. Hillgren K.M., Kato A., Borchardt R.T., In vitro systems for studying intestinal drug absorption. Med. Res. Rev., 1995, 15: 83-109.
5. Rubas W, Jezyk N., Grass G.M., Comparison of the permeability characteristics of a human colonic epithelial (Caco-2) cell line to colon of rabbit, monkey, and dog intestine and human drug absorption. Pharm. Res., 1993, 10: 113-118.
6. Salphati L., Childers K., Pan L., Tsutsui K., Takahashi L., Evaluation of a single-pass intestinal-perfusion method in rat for the prediction of absorption in man., J. Pharm. Pharmacol., 2001, 53: 1007-1013.
7. Cook T.J., Shenoy S.S., Intestinal permeability of chlorpyrifos using the single-pass intestinal perfusion method in the rat., Toxicology, 2003, 184: 125-133.
8. Crouthamel W., Sarapu A. (Editors)., Animal models for oral drug delivery in man, Amer. Pharm. Assoc., Washington DC, 1983, pp. 27-106.
9. Sutton S.C., Rinaldi M.T.S., Vukovinsky K.E., Comparison of the gravimetric, Phenol red and 14C-PEG-3350 methods to determine water absorption in the rat single-pass intestinal perfusion model. AAPS Pharm. Sci., 2001, 3(3): article 25.
10. Hogerle M.L., Winne D., Drug absorption by the rat jejunum perfused *in situ*. Dissociation from the pH-partition theory and role of microclimate-pH and unstirred layer., Naunyn-Schmiedeberg's Arch Pharmacol., 1983, 322: 249-255.
11. Shiau Y.F., Frenandez P., Jackson M.J., McMonagle S., Mechanisms maintaining a low-pH microclimate in the intestine. Ant. J. Physiol., 1985, 248: G608.
12. Gorham F. D., The factor of dilution in gastric analysis, J. Amer. Med. Ass., 1923, 81: 1735-1742.
13. Toropainen E., Ranta V.P., Vellonen K.S., Palmgren J., Talvitie A., Laavola M., Suhonen P., Hamalainen K. M., Auriola S., Urtti A.; Paracellular and passive transcellular permeability in immortalized human corneal epithelial cell culture model, Eur. J Pharm. Sci., 2003, 20: 99-106.
14. Modamio P., Lastra C.F., Marino E.L., Error structure for the HPLC analysis for atenolol, metoprolol and propranolol: a useful weighting method in parameter estimation, J. Pharm. Biomed. Anal., 1998, 17: 507-513.
15. Augustijns P., Mols R., HPLC with programmed wavelength florescence detection for the simultaneous determination of marker compounds of integrity and P-gp functionality in the Caco-2 intestinal absorption model, J. Pharm. Biomed. Anal., 2004, 34: 971-978.

16. Panchagnula R., Bansal T., Varma M.V.S., Kaul C.L., Rversed-phase liquid chromatography with ultraviolet detection for simultaneous quantitation of indinavir and propranolol from ex-vivo rat intestinal permeability studies, J. Chrom. B., 2004, 806: 277-282.
17. Sinko P.J., Hu P., Waclawski A.P., Patel N.R.., Oral absorption of anti-AIDS nucleoside analogues. 1. Intestinal transport of didanosine in rat and rabbit preparations, J. Pharm. Sci., 1995, 84: 959-965.
18. Svensson U.S., Sandstorm R., Carlberg O., Lennernas H., Ashton M., High *in situ* rat intestinal permeability of artemisinin unaffected by multiple dosing and with no evidence of P-gp involvement, Drug Metab. Dispos., 1999, 27: 227-232.
19. Ernest D., Alfert ED., Brenda M., Cross B.M., McWilliam A.A. (Editors), CCAC, Guide to the care and use of experimental animals, Canadian Council on Animal Care, Volume 1, Second edition, Bradda Printing Services Inc, Ottava, Ontario, Canada,1993, p. 15-52.
20. S. Lindsay, Analytical chemistry by open learning; High Performance Liquid Chromatography, John Wiley & Sons, Chichester, England, 1995, p.17-23.
21. Levitt M.D., Kneip J. M., Levitt D.G., Use of laminar flow and unstirred layer models to predict intestinal absorption in the rat, J. Clin. Invest., 1988, 81: 1365-1369.
22. Fagerholm U., Johansson M., Lennernas H., Comparison between permeability coefficients in rat and human jejunum, Pharm. Res., 1996, 13(9): 1336-1342.