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pH-dependent functional activity of P-glycoprotein in limiting intestinal absorption of protic drugs 1. Simultaneous determination of quinidine and permeability markers in rat in situ perfusion samples

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Abstract

A simple, specific and sensitive reversed-phase high performance liquid chromatographic (RP-HPLC) method with UV absorbance detection was developed and validated for simultaneous determination of quinidine, verapamil and passive permeability markers, in samples obtained from rat intestinal in situ single-pass perfusion studies. Chromatography was carried out on C18 column with mobile phase comprising of acetate buffer (pH 5.0) and methanol in the ratio of 40:60 (v/v) pumped at a flow rate of 0.6 ml/min and UV detection was employed at 230 and 275 nm. The average retention times for hydrochlorthiazide, frusemide, quinidine, propranolol, and verapamil were 4.9, 5.8, 6.9, 8.9 and 11.3 min, respectively. The calibration curves were linear ($R^2 > 0.9995$) in the selected range for each analyte. The method is specific and sensitive with limit of quantification as 25 ng/ml for quinidine and verapamil. The intra- and inter-day accuracy and precision were found to be good for all the five analytes. The method was found to be reliable in permeability determination and to estimate pH-dependent P-glycoprotein (P-gp)-mediated efflux transport of quinidine. Weak bases quinidine, propranolol and verapamil showed pH-dependent permeability, where quinidine permeability increased by 3.6-fold when the luminal pH was changed from pH 4.5–7.4. Inhibition of P-gp by verapamil (200 μM) indicated that about 68% and only 35% of passive transport of quinidine was attenuated by P-gp-mediated efflux at pH 4.5 and 7.4, respectively. In conclusion, low passive transport rates of weakly basic P-gp substrates at lower pH, may lead to more accessibility of these molecules to P-gp within enterocytes thus resulting in pH-dependent functional activity of P-gp as protic drugs moves along the gastrointestinal tract.

Keywords: P-glycoprotein; Quinidine; pH-dependent; Rat in situ permeability; RP-HPLC

1. Introduction

Intestinal permeability is a fundamental parameter describing the rate and extent of intestinal drug absorption and thus correlates well to the in vivo performance of the drug or the dosage form. Efflux transport mediated by P-glycoprotein (P-gp) is widely accepted as a reason for low and variable oral absorption for a number of drugs. P-gp, a 170 kDa transmembrane protein, is an ATP-dependent efflux membrane transporter apically expressed throughout the GIT and acting as major biochemical barrier to oral bioavailability [1]. P-gp also plays a critical role in the distribution, metabolism and elimination of many clinically important therapeutic substrates [2]. P-gp has broad substrate specificity and a number of potential drugs including HIV protease inhibitors like indinavir, saquinavir, ritonavir and anticancer drugs like paclitaxel, vinblastine, doxorubicin have been reported to be substrates for P-gp [3]. An understanding of the physiologic regulation of P-gp is a key to design strategies for the improvement of therapeutic efficacy of these drugs. Quinidine

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being a substrate to P-gp, has been used for (i) elucidating the quantitative contribution of P-gp-mediated efflux to its intestinal absorption [4,5], (ii) regional variability of P-gp expression, (iii) pharmacokinetic interactions [6,7] and (iv) P-gp kinetic profiling [8]. Further, quinidine is being increasingly used for understanding mechanistic aspects of intestinal P-gp [9].

According to pH-partition theory, the membrane permeability of the uncharged species of a protic or ampholytic molecule is much higher than that of the charged species. P-gp-mediated efflux activity becomes insignificant for highly permeable compounds, thus the change in passive permeability as a function of luminal pH may influence the functional activity of P-gp for the same drug as it travels along the Gl tract. Based on this hypothesis, quinidine was selected for this study as it is weak base and a strong P-gp substrate. A suitable analytical method for determining quinidine in the transport buffer in presence of P-gp modulator is necessary for this purpose and further to study the role of P-gp on overall permeability, kinetic profiling of P-gp-mediated efflux transport and for exploring the use of P-gp modulators for improving absorption. P-gp modulation studies are mostly done with radio-chemical analysis [4,5], which is not always possible because of problems related to the storage, handling and waste disposal of radioactive materials. The FDA guidelines suggest internal standards and markers to characterize the permeability of drug substances for classifying them into biopharmaceutical classification system (BCS) [10–12]. Further these markers are useful in monitoring the integrity and functional status of the intestinal membrane and thus can be routinely used for accurate permeability characterization [13].

Here we report a simple, selective, sensitive, reproducible and reliable RP-HPLC method with short run time of 14 min for the routine quantitative analysis of quinidine and verapamil in perfusion samples in presence of permeability markers, hydrochlorthiazide (less permeable), frusemide (less permeable) and propranolol (highly permeable), routinely used in permeability studies. This method utilizes isocratic-mode with UV detection, which is more feasible and less time-consuming than any other method of detection. Although a number of methods are available for quantification of quinidine in various biological matrices [14–16], the uniqueness of the present method is its ability to simultaneously quantify passive integrity markers. The proposed method was further used to elucidate the pH-dependent functional activity of P-gp in limiting intestinal absorption of quinidine.

2. Materials and methods

2.1. Chemicals

Frusemide was obtained from Dr. Reddy's Lab. (Hyderabad, India). Hydrochlorthiazide was received from Aristo Pharmaceutical Ltd. (Daman, India). Propranolol HCl was from Sun Pharmaceutical Industries Ltd. (Mumbai, India). Verapamil and quinidine were purchased from Sigma–Aldrich Co. (MO, USA). All the solvents used were of HPLC grade (J.T. Baker, Mexico) and reagents and chemicals were of analytical grade.

2.2. Instruments

For analysis, Waters HPLC system (Milford, MA, USA) consisting of 600 E multisolvent delivery system, 717 plus autosampler and 2487 dual λ absorbance detector was used. MILLENIUM^{32} software (version 3.05.01) was used for data acquisition and processing. Other instruments include Elgastat (Elga Ltd., Bucks, UK), electronic balance AG245 (Griefensee, Switzerland), Branson 3210 sonicator (The Hague, The Netherlands), Millipore syringe filtration assembly (Bangalore, India) and Brand autopippetes from E. Merck (Mumbai, India).

2.3. Methodology

2.3.1. Chromatography

The column used for chromatographic separations was 4.6 mm i.d., 250 mm length and 5 μm particle size C18 (Symmetry®, Waters, USA) attached to a guard column of C18 (Waters, USA). The chromatographic separations were accomplished using mobile phase consisting of acetate buffer (10 mM, adjusted to pH 5.0 with glacial acetic acid) and methanol (40:60, v/v), filtered through 0.45 μm filter (Millipore) and deaerated in ultrasonic bath (Branson 3210). Mobile phase was pumped in isocratic mode at a flow rate of 0.6 ml/min at ambient temperature. Samples of 20 μl were automatically injected after filtration through 0.45 μm filter (Millipore). Elution of quinidine, propranolol and verapamil was monitored at 230 nm and frusemide and hydrochlorthiazide was monitored at 275 nm, using a dual wavelength detector.

2.3.2. Preparation of standard solutions

Primary stock solutions of 2 mg/ml of all the five analytes in ultra pure water were prepared, individually, and were diluted to obtain a common secondary stock solution of 200 μg/ml of each analyte. Aliquots of secondary stock solution of all the analytes were then diluted in blank in situ perfusion samples to obtain calibration standards in the concentration range of 0.2–2.2 μg/ml.

2.4. Method validation

The chromatographic method was validated on three different days to determine various parameters viz. linearity, sensitivity, precision and accuracy for each analyte. The calibration curves and validation studies were performed with blank perfusion solution collected from in situ single-pass perfusion (see Section 2.5). Interference in the presence of

any endogenous constituents of perfusion solution was assessed by analysis of blank perfusion samples [17]. A six-point calibration curve was constructed by spiking blank perfusion solution with appropriate volumes to obtain working standards over the concentration range of 0.2–2.2 μg/ml.

Linearity was measured from calibration curve prepared form standard solutions ($n = 5$) containing a mixture of five analytes in the selected range (0.2–2.2 μg/ml for each analyte). The interpolation of areas obtained for each analyte in the sample matrix in the calibration curve permitted quantification. Precision (%R.S.D.) of the method was determined by analyzing quality control (QC) samples of three different concentrations within the calibration range. Accuracy of the assay was shown as percentage relative error (%RE) and calculated based on the difference between the calculated concentration and the spiked concentration. QC samples were prepared in blank perfusion solution spiked with all analytes at different concentrations (0.5, 1.5 and 2.0 μm/ml) following the same procedure as for calibration standards, using a different primary stock. To assess intra- and inter-day variation, calibration standards and QC samples were prepared on the same day ($n = 5$) and on three consequent days ($n = 11$) and analyzed. Intra- and inter-day precision was depicted as %R.S.D. while intra-day accuracy was depicted with %RE. Limit of quantification (LOQ) was determined by serial dilution of working standards, while the LOD was taken as one-third of LOQ [18,19].

2.5. In situ single-pass intestinal permeability and P-gp inhibition studies

2.5.1. Animals and legal prerequisites

Sprague–Dawley rats (270–350 g) were used to perform in situ single-pass perfusion. Anesthesia, surgical and perfusion procedures were justified in detail and were approved by the Institutional Animal Ethics Committee (IAEC, NIPER). The study complied with local and federal requirements for animal studies.

2.5.2. Methodology

The surgical procedure and the in situ single-pass perfusion experiments were performed according to the methods described previously [20]. Rats were fasted for 16–18 h (tap water ad libidum) prior to study. Anaesthesia was induced with an intraperitoneal injection of thiopental sodium (50 mg/kg). To maintain normal body temperature rats were placed on a heating pad (37 °C). The abdomen was opened with a midline incision and a 10–12 cm jejunum segment was isolated and cannulated at both ends with glass tubing. The segment was rinsed with phosphate buffer saline (10 ml) and the perfusion solution maintained at 37 °C was pumped at a flow rate of 0.1 ml/min using syringe pump (Harvard Apparatus PHD 2000 pump, MA, USA). The perfusion solution (pH 4.5 and 7.4) consisted of NaCl 48 mM, KCl 5.4 mM, Na_2HPO_4 2.8 mM, NaH_2PO_4 4 mM and D-glucose 1 g/l; and contained drugs with or without verapamil (200 μM) as P-gp inhibitor. Final concentrations used in the studies were 50 μM, hydrochlorthiazide; 100 μM, propranolol; 50 μM, frusemide and 10 μM, quinidine.

A two-step perfusion procedure was followed to determine the permeability of compounds with and without P-gp inhibitor [20]. This included sampling every 5 min for a 30 min perfusion period with perfusion solution containing test compounds (quinidine, hydrochlorthiazide, frusemide and propranolol) only, after 20 min equilibration, and then switched over to perfusion solution containing both test compounds and verapamil, and similarly sampling every 5 min for 30 min perfusion period after 20 min equilibriation. Permeability was determined in four individual rats ($n = 4$). Equilibrium of 20 min prior to sampling was found to be sufficient to reach an initial steady-state. Water flux was quantified by volume measurements [20,21]. Perfusion samples were filtered through 0.45 μm filters and were automatically injected (20 μl) without involving any sample preparation procedures.

2.5.3. Data treatment and statistics

Permeabilities (without and with P-gp inhibitor) are calculated after correcting the outlet concentration for water flux on the basis of the ratio of volume of perfusion solution collected and infused for each sampling point (5 min).

$$P_{\text{eff, control}} \quad \text{(or)} \quad P_{\text{eff, inh}} = \frac{Q[(C_{\text{in}}/C_{\text{out}}) - 1]}{2\Pi rl}$$

where Q is the flow rate, C_{in} and C_{out} the respective inlet and outlet concentrations, r the radius of intestine (0.21 cm) and l is the length of intestine measured after completion of perfusion [22,23].

Values were indicated as mean ± S.D. for permeability in four independent rats. Statistical difference between the permeabilities of the drugs without and with P-gp inhibitor was evaluated by one way ANOVA (SigmaStat version 2.03, SPSS Inc., IL, USA) at $P < 0.05$ and <0.01.

3. Results and discussion

3.1. Chromatography and specificity

Representative chromatograms of blank and in situ perfusion samples containing the five drugs are shown in Fig. 1. Under the chromatographic conditions used for analysis the retention times of frusemide, hydrochlorthiazide, propranolol, quinidine and verapamil were 5.8, 4.9, 8.9, 6.9 and 11.3 min, respectively. No other interfering peaks were observed in the blank perfusion solution and the method was specific to all the analytes at the corresponding wavelength (230 or 275 nm). Although the interference from the blank perfusate is negligible for hydrochlorthiazide and frusemide at 230 nm, chromatogram of these two drugs was monitored at 275 nm to provide high specificity (Fig. 1). The chromatographic run time was 14 min, which permitted the analysis of

Table 1
Regression analysis of calibration curves for all drugs in the specified range of 0.2–2.2 μg/ml[a]

	Retention time (min)	Slope	Intercept	Correlation coefficient
Hct	4.9	289087 (±11519)	−2474 (±21204)	0.9996 (±0.00003)
Fru	5.8	237993 (±2280)	1248 (±8620)	0.9996 (±0.0005)
Qui	6.9	415531 (±7169)	−6558 (±7543)	0.9998 (±0.0001)
Ppn	8.9	562335 (±7524)	−7408 (±8782)	0.9998 (±0.0001)
Ver	11.3	169128 (±5925)	−7684 (±4235)	0.9995 (±0.0005)

[a] Six concentrations across the range were evenly distributed. Values represent mean (±S.D.) of five calibration curves.

a large number of samples in a short time. Thus the method is simple and highly selective.

3.2. Validation

The chromatographic method was validated on three different days to determine the sensitivity, accuracy, and precision of the present HPLC method.

3.2.1. Range and linearity

The six point calibration curves that were constructed for each assay batch were linear ranging between 0.2 and 2.2 μg/ml with correlation coefficient (r) over 0.999 for all standard curves (Table 1).

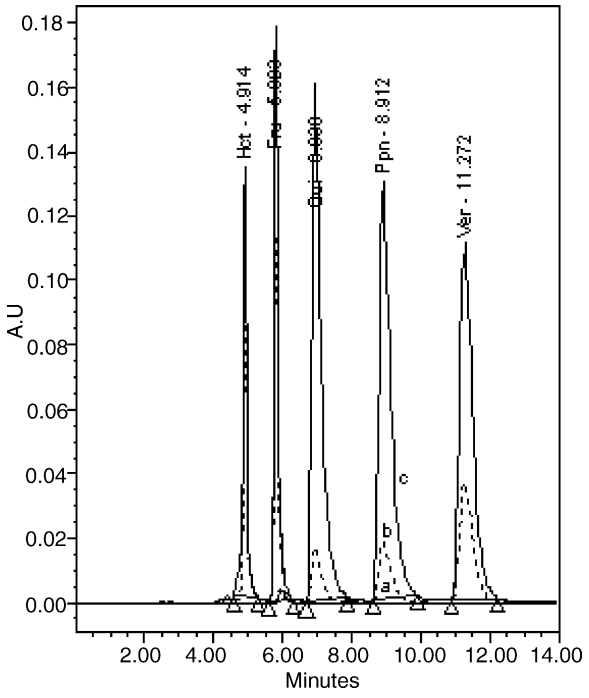

Fig. 1. Chromatograms of (a) blank in situ perfusion sample, (b) perfusion sample containing all five analytes, that included hydrochlorthiazide (Hct), frusemide (Fru), quinidine (Qui), propranolol (Ppn) and verapamil (Ver), monitored at 275 nm and (c) perfusion samples containing all five analytes monitored at 230 nm. The peaks are annotated with their respective names and retention times. Chromatograms were obtained from the blank sample and the sample containing Hct 50 μM, Fru 50 μM, Qui 100 μM, Ppn 100 μM and Ver 200 μM, collected after in situ perfusion through rat intestine.

3.2.2. Accuracy and precision

Analysis of calibration and QC standards were used to assess the accuracy (%RE) and precision (%R.S.D.). The data is summarized in Tables 2 and 3. Both intra- (n = 5) and inter-day (n = 11) R.S.D. were less than 3% for QC standards over the selected range for all drugs, except for hydrochlorthiazide, which is still within the acceptable limits (<4%). Further calibration standards also showed acceptable accuracy and precision (Tables 2 and 3). %RE for quinidine QC samples ranged from −2.45 to −0.92% and for verapamil it was −1.36 to 2.18% while for rest of the drugs accuracy ranged from −2.26 to 1.61%. Overall, data showed good accuracy and precision of the method for each analyte.

3.2.3. Sensitivity

LOD and LOQ decide the sensitivity of the method. LOD is defined as the lowest concentration of the analyte that the analytical process can reliably differentiate from background levels [18,24]. LOQ is defined as lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability. LOQ was assessed by serial dilution to obtain the mean chromatographic response (peak area) 10 times more than the SD of six independent determinations and LOD was taken as one-third of LOQ.

Table 2
Intra- and inter-day precision and accuracy of hydrochlorthiazide and frusemide estimations of the method monitored at 275 nm

Concentration (μg/ml)	Hct			Fru		
	Intra-day[a]		lnter-day[b]	Intra-day[a]		Inter-day[b]
	%R.S.D.	%RE	%R.S.D.	%R.S.D.	%RE	%R.S.D.
Calibration standards						
0.2	5.21	4.27	4.57	0.51	−6.28	2.96
0.6	2.73	3.05	3.75	2.53	0.25	2.27
1.0	2.94	1.51	3.28	1.99	1.40	2.18
1.4	3.03	−0.74	2.64	2.33	1.97	2.47
1.8	2.86	−0.60	2.19	0.99	1.89	2.11
2.2	2.99	0.45	2.78	2.88	−0.94	2.14
Quality control samples						
0.5	1.44	1.61	2.19	2.11	1.19	1.69
1.5	1.76	−1.99	2.67	2.16	0.89	1.82
2.0	2.56	−0.26	3.64	0.42	0.95	0.84

[a] Intra-day accuracy and precision was determined with five replicates for each concentration.

[b] Inter-day precision was determined with eleven replicates (day 1, n = 5; day 2, n = 3; day 3, n = 3) for each concentration.

Table 3
Intra- and inter-day precision and accuracy of quinidine, propranolol and verapamil estimations of the method monitored at 230 nm

Cone (μg/ml)	Qui			Ppn			Ver		
	Intra-day[a]		lnter-day[b]	Intra-day[a]		lnter-day[b]	Intra-day[a]		lnter-day[b]
	%R.S.D.	%RE	%R.S.D.	%R.S.D.	%RE	%R.S.D.	%R.S.D.	%RE	%R.S.D.
Calibration standards									
0.2	1.08	−3.17	4.62	2.08	−2.42	4.73	4.25	−4.45	6.81
0.6	1.48	0.76	2.96	1.30	1.78	2.02	3.12	0.00	3.25
1.0	2.21	−0.52	1.83	3.44	−0.14	2.58	2.38	2.50	3.78
1.4	2.81	1.38	2.08	1.71	0.54	1.96	2.78	−1.67	3.43
1.8	2.27	−0.53	1.62	0.79	−0.61	1.92	0.63	0.91	1.06
2.2	3.11	−0.13	2.60	0.84	0.12	1.37	2.58	−0.41	1.76
Quality control samples									
0.5	1.20	−1.51	2.49	2.31	−1.36	2.15	2.70	−1.36	2.65
1.5	0.36	−2.45	2.84	1.34	−2.66	1.48	1.29	−0.21	2.15
2.0	0.87	−0.92	2.69	2.55	−0.37	2.09	0.74	2.18	1.66

[a] Intra-day accuracy and precision was determined with five replicates for each concentration.
[b] Inter-day precision was determined with eleven replicates (day 1, $n = 5$; day 2, $n = 3$; day 3, $n = 3$) for each concentration.

The LOD and LOQ for quinidine, verapamil and hydrochlorthiazide were 8.3 and 25 ng/ml, respectively, while for frusemide and propranolol LOD and LOQ are 16.6 and 50 ng/ml, respectively. Thus, the method shows high sensitivity with the added advantage of no complex sample preparation procedures.

3.3. Application of the method to determine pH-dependent P-gp-mediated efflux

Intestinal permeability was determined in rat jejunum using in situ single-pass perfusion technique and the samples were analyzed by the proposed method. The permeability ($P_{eff,control}$) of quinidine was found to be 0.23 (± 0.03) $\times 10^{-4}$ cm/s, at pH 7.4 (Fig. 2), while hydrochlorthiazide, frusemide and propranolol showed permeabilities 0.023 (± 0.004) $\times 10^{-4}$ cm/s, 0.069 (± 0.054) $\times 10^{-4}$ cm/s and 0.48 (± 0.035) $\times 10^{-4}$ cm/s, respectively. Based on permeability data, quinidine and propranolol can be classified into high permeability class drug as per BCS [25,26]. Inhibition of P-gp by verapamil (200 μM) significantly increased the permeability ($P_{eff,inh}$) of quinidine ($P < 0.05$) while no change in the permeability was found for hydrochlorothiazide, frusemide and propranolol, indicating P-gp-mediated efflux of quinidine. In the present case, hydrochlorothiazide, propranolol and frusemide were used as permeability markers so as to check the integrity of the intestinal membrane on different occasions and to obtain accurate permeability data for quinidine and verapamil under different conditions (viz. presence and absence of P-gp-mediated efflux and to study the effect of quinidine permeability in the presence of other drugs or P-gp modulators).

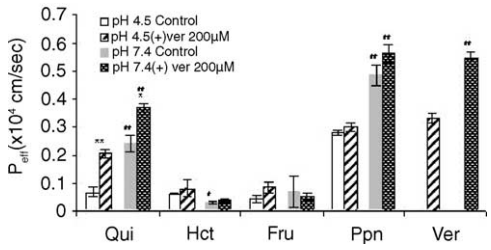

Fig. 2. Intestinal permeability of hydrodrochlorthiazide (Hct), frusemide (Fru), quinidine (Qui) and propranolol (Ppn) in the absence and presence of P-gp modulator, verapamil (Ver). The method allowed the estimation of Ver permeability in the same set of experiments. Values are the mean ± S.D. of four independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$; statistically significant difference from permeability in the presence P-gp-mediated efflux (control) at same pH (one-way ANOVA). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; statistically significant difference from permeability at pH 4.5 (one-way ANOVA).

The $P_{eff,control}$ of weak bases, quinidine and propranolol, increased by 3.61- and 1.72-folds when the pH of perfusion solution was changed from pH 4.5 to 7.4 (Fig. 2). However, no difference in permeability of hydrochlorthiazide and frusemide (weak acids) was observed with change in luminal pH. Inhibition of P-gp-mediated efflux by verapamil (200 μM) increased the permeability ($P_{eff,inh}$) of quinidine at both pH, however, the difference between $P_{eff,control}$ and $P_{eff,inh}$ was found to be more at pH 4.5. The proposed HPLC method also was able to quantify verapamil, which showed pH-dependent permeability (Fig. 2). However, the data represents verapamil permeability in the presence of quinidine (100 μM), where P-gp-mediated efflux is partially or completely inhibited [27].

To evaluate the quantitative functional role of P-gp, the intestinal efflux inhibition ratio (EIR), i.e. the ratio of permeability due to P-gp-mediated efflux activity ($P_{P\text{-}gp}$) and the passive permeability (P_{PD}), was calculated. $P_{P\text{-}gp}$ was obtained by subtracting $P_{eff,control}$ from $P_{eff,inh}$ while P_{PD} is equal to $P_{eff,inh}$ [28]. Assuming complete P-gp inhibition by verapamil (200 μM), EIR for quinidine was found to be 0.68 and 0.35, indicating that about 68% of passive transport of quinidine is attenuated by P-gp-mediated efflux, at pH 4.5, while only 35 of passive transport is attenuated by P-gp at pH 7.4.

The pH-dependent permeability of quinidine, propranolol and verapamil support the pH partition theory [29]. This theory states that the fraction of unionized species of a basic drug, which drives the passive transmembrane transport, increases from pH 4.5 to 7.4. The pH-dependent passive permeability of weak bases has also been observed in the in situ perfusion rat intestine [30]. Different permeability patterns of protic drugs at different luminal pH may be observed because of the difference in lipophilicity of ionized and unionized species. The composition of enterocvtic membrane favors the uptake and transit of drug molecules with appropriate lipophilicity [31]. However there are a number of exceptions for the general belief that only uncharged species can cross membranes. A formal electrical charge in ionization state can be highly delocalized and therefore be less of an impediment to membrane partitioning, especially when log *D* is sufficiently high [32].

The observed significant difference in permeability of weak bases propranolol (pK_a 9.5), verapamil (pK_a 6.5) and quinidine (pK_a 4.2, 7.9) at pH 4.5 and 7.4 is thus attributed to the degree of ionization of these drugs at the luminal pH. Converse to basic drugs, acidic drug hydrochlorthiazide showed decrease in permeability when the luminal pH was changed from pH 4.5 to 7.4. The low passive transport rate of quinidine at pH 4.5 is attributable for more passive transport attenuation by P-gp efflux [9]. Passive transport and P-gp-mediated efflux act in mutually opposite directions at the apical membrane contributing to the overall permeability of P-gp substrates. Drugs with slow transit across the enterocytes are more accessible to P-gp within the enterocytes, may be at sub-saturation concentrations and thus their permeability is more influenced by P-gp-mediated efflux [33,34]. In a similar way, because of the high degree of ionization at a low pH, passive transport of quinidine is significantly reduced leading to high P-gp-meditated efflux. Overall, the data indicated that passive pH-dependent permeability of quinidine influences the P-gp-mediated efflux rates and thus may influence the drug absorption as it moves down the gastrointestinal tract.

A basic drug tends to have high solubility and less passive permeability in the upper intestine, while less solubility with high passive permeability in the lower Gl tract (Gl pH above drug pK_a). pH of the lumen determines the fraction of unionized species of protic molecules available for passive diffusion. Thus, as basic drug moves down the Gl tract, absorption may switch from permeability control to solubility control. In contrast, an acidic drug shows solubility control in acidic environment and permeability control in basic environment. The findings from the present study indicates that when the drug absorption is permeability limiting, the functional activity of P-gp in limiting intestinal absorption of various protic or ampholytic drugs is more significant. Considering these factors in compartmental absorption transit models may improve their predictability and also may explains various drug interactions associated with protic or ampholytic drugs.

4. Conclusion

The proposed RP-HPLC method is simple, sensitive, reliable, and provides satisfactory accuracy, precision with lower quantification limit and short retention times for the determination of quinidine and verapamil from in situ intestinal permeability samples with an additional advantage of simultaneous quantification of US FDA suggested permeability markers. This technique eliminated any endogenous interference from biological matrix, and permitted analysis involving no sample preparation procedures. Hence this method can be easily and conveniently adopted for routine quantification in permeability studies. Based on the sensitivity results (LOQ), it may be concluded that this method will be useful for permeability estimations using in vitro everted sac, intestinal sections (Ussing chambers) and cell cultured monolayer models. The advantage of the developed method lies in the simultaneous determination of passive permeability markers (hydrochlorthiazide, frusemide and propranolol) routinely employed in permeability studies. Overall, this methodology provides a simple quantification and can be useful for understanding functional role of P-gp-mediated efflux in the intestinal drug absorption.

Degree of ionization of protic drugs changes as a function of luminal pH which ultimately influences the passive permeability. Data indicated that pH-dependent passive permeability of quinidine determines the functional activity of P-gp where at low pH, because of slow transit across the enterocytes, drug molecules are more accessible to P-gp and thus passive permeability is more attenuated by P-gp at lower pH for basic drugs. Results add new dimensions to the development of intestinal drug absorption prediction models incorporating the functional role of P-gp in limiting permeability of various protic drugs.

References

[1] S.V. Ambudkar, S. Dey, C.A. Hrycyna, M. Ramchandra, I. Pastan, M.M. Gottesman, Annu. Rev. Pharmacol. Toxicol. 39 (1999) 361.
[2] M.V.S. Varma, Y. Ashokraj, C.S. Dey, R. Panchagnula, Pharmacol. Res. 48 (2003) 347.
[3] W.C. Williams, P.J. Sinko, Adv. Drug Del. Rev. 39 (1999) 211.
[4] Y. Adachi, H. Suzuki, Y. Sugiyama, Pharm. Res. 18 (2001) 1660.
[5] Y. Adachi, H. Suzuki, Y. Sugiyama, Pharm. Res. 20 (2003) 1163.
[6] E.D. Kharasch, C. Hoffer, D. Whittington, Br. J. Clin. Pharmacol. 57 (2004) 600.
[7] E.D. Kharasch, C. Hoffer, T.G. Altuntas, D. Whittington, J. Clin. Pharmacol. 44 (2004) 224.
[8] T.T. Tran, A. Mittal, T. Gales, B. Maleeff, T. Aldinger, J.W. Polli, A. Ayrton, H. Ellens, J. Bentz, J. Pharm. Sci. 93 (2004) 2108.
[9] S. Neuhoff, A.L. Ungell, I. Zamora, P. Artursson, Pharm. Res. 20 (2003) 1141.
[10] G.L. Amidon, H. Lennernas, V.P. Shah, J.R. Crison, Pharm. Res. 12 (1995) 413.
[11] R. Lobenberg, G.L. Amidon, Eur. J. Pharm. Biopharm. 50 (2000) 3.
[12] Guidance for Industry, Waivers of in vivo bioavailability and bioequivalence studies for immediate release solid oral dosage forms based on a biopharmaceutics classification system, August 2000, CDER/FDA.

[13] J.W. Polli, S.A. Wring, J.E. Humphreys, L. Huang, J.B. Morgan, L.O. Webster, C.S. Serabjit-Singh, J. Pharmacol. Exp. Ther. 299 (2001) 620.
[14] G. Carignan, K. Carrier, S. Laganiere, M. Lessard, J. Chromatogr. B 672 (1995) 261.
[15] F. Nielsen, K.K. Nielsen, K. Brosen, J. Chromatogr. B 660 (1994) 103.
[16] G.L. Hoyer, D.C. Clawson, L.A. Brookshier, P.E. Nolan, F.I. Marcus, J. Chromatogr. 572 (1991) 159.
[17] J.E. Visich, P.R. Byron, J. Pharm. Biomed. Anal. 15 (1996) 105.
[18] R. Panchagnula, T. Bansal, M.V.S. Varma, C.L. Kaul, J. Chromatogr. B 806 (2004) 277.
[19] M. Page-Sharp, T.W. Hale, L.P. Hackett, J.H. Kristensen, K.F. Ilett, J. Chromatogr. B. 796 (2003) 173.
[20] A. Hanafy, P. Langguth, H. Spahn-Langguth, Eur. J. Pharm. Sci. 12 (2001) 405.
[21] M. Bermejo, A. Avdeef, A. Ruiz, R. Nalda, J.A. Ruell, O. Tsinman, I. Gonzalez, C. Fernandez, G. Sanchez, T.M. Garrigues, V. Merino, Eur. J. Pharm. Sci. 21 (2004) 429.
[22] P.J. Sinko, G.D. Leesman, G.L. Amidon, Pharm. Res. 8 (1991) 979.
[23] Y.Y. Chiu, K. Higaki, B.L. Neudeck, J.L. Barnett, L.S. Welage, G.L. Amidon, Pharm. Res. 20 (2003) 749.
[24] P. Sharma, H. Chawla, R. Panchagnula, J. Chromatogr. B 768 (2002) 349.
[25] U. Fagerholm, M. Johansson, H. Lennernas, Pharm. Res. 13 (1996) 1336.
[26] E. Rinaki, G. Valsami, P. Macheras, Pharm. Res. 20 (2003) 1917.
[27] R. Sandstrom, A. Karlsson, L. Knutson, H. Lennernas, Pharm. Res. 15 (1998) 856.
[28] M.D. Troutman, D.R. Thakker, Pharm. Res. 20 (2003) 1210.
[29] P.A. Shore, B.B. Bordie, J. Pharmacol. Exp. Ther. 119 (1957) 361.
[30] D.C. Taylor, R. Pownall, W. Burke, J. Pharm. Pharmacol. 37 (1985) 280.
[31] M.V.S. Varma, S. Khandavilli, Y. Ashokraj, A. Jain, A. Dhanikula, A. Sood, N.S. Thomas, O.P. Pillai, P. Sharma, R. Gandhi, S. Agrawal, V. Nair, R. Panchagnula, Curr. Drug Metab. 5 (2004) 375.
[32] K. Palm, K. Luthman, J. Ros, J. Grasjo, P. Artursson, J. Pharmacol. Exp. Ther. 291 (1999) 435.
[33] K. Ito, H. Kusuhara, Y. Sugiyama, Pharm. Res. 16 (1999) 225.
[34] M.V.S. Varma, S. Khandavilli, R. Panchagnula, Mol. Pharm., in press.