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Effect of food supplement constituents Quercetin, Silibinin and Luteolin on oral uptake of protease inhibitor saquinavir: synthetic meets natural

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ABSTRACT

Saquinavir is the BCS class IV drug, it is a first protease inhibitor for HIV infection treatment, having poor permeability. The main purpose of this study was to study the effect of natural compounds on its bioavailability. In this work, binary systems of SQU with the natural bioenhancers were prepared using physical mixing method. The effect of these compounds were studied using different sophisticated experimental protocols. Firstly the compatability was tested for the three used bioenhancers quercetin (QU), silibinin (Sil), Luteolin (LT). Oral uptake was studied by analyzing the transport of SQU across the human colorectal adenocarcinoma cell line (Caco-2) cell lines. Permeation through the goat intestine tissue was also studied. Pharmacokinetic analysis was also performed in rabbits by administered SQU with different bioenhancers in the form of suspension, and the whole analytical studies for the estimation of SQU in different studies were conducted using LC-MS. In the compatibility studies, bioenhancers found to be showing no or minimal interaction with the SQU. Permeation in the intestinal tissue of goat was significantly increased as compared to the plain drug. The transport of SQU across the Caco-2 cell lines also found to be improved than the plain drug. Pharmacokinetic study showed there was increase in the Cmax by approx. 3 folds using the different bioenhancers. AUC was also found to be increase by more than 2 folds with the each bioenahncer. The maximum oral uptake enhancement was found with the QU following by the Sil and then LT.

Keywords: saquinavir; protease inhibitor; quercetin; silibinin; luteolin; Caco-2 cell monolayer;

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Introduction

Oral route is the most convenient and the preferred route for drug delivery. It has been reported that more than 40% of drug products available in the market are administered via oral route.[1] Many of these drug candidates having a major drawback of lower oral bioavailability due to low permeability which is a foremost obstacle in achieving adequate drug therapy. Most of the drug candidates used in anti-viral therapy having the issue of low bioavailability due to poor permeation. It has been reported that a huge amount of drug candidates in development could fail because of permeation problems. Thus, the development of safe and effective formulations that improve the oral absorption of poorly bioavailable drugs may increase the success rate of drugs in development and it can consequently increase the rate of success of therapy.[2, 3]

Natural bioenhancers typically acts as drug facilitators, these compounds does not show any activity at a given dose but whenever they are used in combination with the drug candidates they facilitates their permeation or bioavailability by several activities like P-gp inhibition and CYP inhibition.[4] Quercetin (QU) is a plant-derived flavonoid found in fruits, vegetables, leaves and grains. It exhibited activities including antioxidant, radical scavenging, anti-inflammatory, antiathero sclerotic, anticancer, and antiviral effects [5]. It is a potent inhibitor of CYP3A4 and a modulator of P-glycoprotein [5-7]. Silibinin is the one of the chief and mostly abundant component approximately 70% of silymarin [8]. It has been reported that silibinin could inhibit human CYP1A2, CYP2D6 and 3A4 enzymes responsible for the metabolism [9, 10]. Silibinin has also been reported as P-gp inhibitor [11]. Luteolin (LT) is one of the main constituents of P. barbatus herbal tea [12]. However, there is a very little known about the way that these compounds affect the bioavailability. Nevertheless, it is known that flavonoids interact with transport systems in intestinal cells, such as the ABC transporters P-glycoprotein (Pgp) and multidrug resistance proteins (MRP), which actively inhibit them [13]. The major reason to choice these bioenhancers is their P-gp inhibition activity and to explore new ways to make more effective oral therapies for HIV infection as the major issue with the SQU is the P-gp efflux pump.

Saquinavir (SQU) was the first drug candidate which gets approved as protease inhibitior for the HIV infection treatment. In the last decade, SQU has become a chief component of highly active antiretroviral therapies [14, 15]. SQU having molecular weight 670.8 with partition coefficient (LogP) 4.1. It is class IV drug in the Biopharmaceutical Classification System (BCS) having low permeability and low solubility [16]. It is usually administered in the salt form mesylate, the solubility of the SQU is pH-dependent [17]. Apart from having the not very much favorable physicochemical properties for permeability, it is also reported that efflux protein (P-gp) also hampers the transport of SQU from the gut wall [18, 19]. Inspite of this SQU is also metabolized by both human hepatic and small intestinal enzymes, which also further results in low oral bioavailability (4%) and displays wide inter-individual variability [20, 21].

The aim of the present study was to study the effect of QU, Sil and LT in the enhancement of the oral absorption of SQU. A physical mixture of SQU and bioenhacers (different concentrations) was prepared by blending method. The compatibility of the prepared SQU-QU, SQU-Sil and SQU-LT binary systems were characterized by Fourier transform infrared spectroscopy (FTIR) and Differential scanning calorimetry (DSC). The absorption of the physical mixture (PM) at different concentration levels were studied in Caco-2 cells and an in vitro franz diffusion studies. In addition, the oral bioavailability of the PM was evaluated in rabbits. Therefore, in the present study, we focused on the exploration and inclusion of natural compounds in an oral formulation with the focused aim of improving the oral bioavailability of SQU.

Materials and Methods

Materials

Saquinavir (SQU) mesylate was obtained as a gift sample from Aurbindo Pvt Ltd, Mumbai. Quercetin, Silibinin and Luteolin was purchased from Sigma-Aldrich. Caco-2 cell lines were obtained from NCCS Pune. All other chemicals and reagents used in the study were of analytical grade and were commercially obtained.

LC-MS method for estimation of SQU in different studies

The samples were analyzed using ekspertTM ultraLC with ekspertTM ultraLC 100 pump system (eksigent-AB Sciex, USA) coupled with 3200 QTRAP mass spectrometer (AB Sciex, USA). 20 µL of each sample was injected. The autosampler system (ekspertTM ultraLC 100 XL, eksigent-AB Sciex, USA) was tempered to 4°C equipped with column oven (ekspertTM ultraLC 100, eksigentAB Sciex, USA) fixed at 40°C. Chromatographic elution of analyte was achieved using a Phenomenax C18 5µm (250*4.6) mm column at a flow rate of 0.5 mL min^{-1} for having run time 8 mins. The composition of mobile phase was eluent a (20 mM ammonium formate buffer) and eluent b (acetonitrile) was in 55:45 % v/v. The SQU was quantified in the multiple reaction monitoring (MRM) mode at ion transitions m/z 671.00 432.80. LC-MS Conditions for analysis were in the positive ion mode with a potential of 5.5 kV applied on the electro spray ionization needle. The ionization source temperature was 600 °C. The curtain gas (CUR) was at 25.0 psi, the nebulizer source gas 1 at 50.0 psi and the turbo ion source gas 2 at 50.0 psi was utilized. The optimized declustering potential and entrance potential were 60.0 V and 5.6 V respectively. SQU fragmentation was achieved by collision activated dissociation (CAD) with nitrogen gas. The collision gas pressure was fixed at 2.0 psi for MRM quantitation. The collision energy 22.0 V and collision cell exit potential 3.0 V were optimized. Dwell time 200 ms was used. Chromatographic responses were found to be linear over an analytical range of 25–2000 ng/ml and found to be quite satisfactory and reproducible. Extraction efficiency was greater than 88 %. Accuracy data in the present study ranged from 98.42 to 100.07 % indicates that there was no interference from endogenous plasma or other components. Inter-day as well as intra-day replicates of SQU, gave R.S.D. below 5.0 (should be less than 15 according to CDER guidance for Bio-analytical Method Validation), revealed that proposed method is highly precise and accurate.

Ex-vivo permeation studies (Franz diffusion cell)

Diffusion cell with an area of 3.80 cm^2 having donor and acceptor compartment were used. The intestinal tissue of goat was collected from local slaughter house and was stored in normal saline. Tissue was cleaned and intestinal content was removed by a slow infusion of normal saline and air before setting up for the experimentation.

Caco-2 Cell line culture

Caco-2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with high glucose, fetal bovine serum, penicillin, and streptomycin at 37 °C and 5% CO_2.

Animal preparation for *In-vivo* studies

In vivo pharmacokinetic study performed in New Zealand white rabbits (2-3 Kg) provided by the animal house at Pharmacy Department, The Maharaja Sayajirao university of Baroda. All the experiments were performed under guidelines approved by the Institutional Animal Ethics Committee (IAEC Registration number 404/01/a/CPCSEA). The rabbits were given free access to food and water. The rabbits were fasted for 12 h prior to the experiments with free access to water.

Preparation of Physical Mixture of saquinavir and bioenhancers

SQU–QU, SQU–Sil, SQU–LT binary system were prepared at the five different weight ratio levels 5:0.5, 5:1, 5:1.5, 5:2, 5:2.5, 5:3 w/w. Physical mixture method was adapted for the

binary system, in this the required amounts were accurately weighed and were sealed in a polythene bag and were blended for 30 minutes.

Compatibility studies of binary system

SQU–QU, SQU–Sil, SQU–LT binary system were characterized for their compatibility in different ratios. In this FTIR and DSC were used. The FTIR spectrum of the SQU and binary mixtures were recorded and were interpreted for any physical interaction. Similarly the DSC chromatogram for the SQU and binary mixtures were recorded and were interpreted for physical interaction.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR transmission spectra of a pure SQU, QU, Sil, LT, SQU–QU, SQU–Sil and SQU–LT and binary systems of PM method were obtained using Avatar™ 360 E.S.P™ FTIR spectrometer, Thermo Nicolet Corp., Madison, WI, USA. Samples were mixed with dry KBr and converted to a fine powder before compressing into KBr disc. Each sample was scanned for 16 times over a wave number region of 500–4000 cm^{-1}. The characteristic peaks were recorded.

Differential Scanning Calorimetry (DSC)

DSC thermograms were recorded for SQU, QU, Sil and LT and their respective binary systems using Mettler-Toledo, Schwerzenbach, Switzerland. The temperature range was 0–350^{o}C. An empty aluminium pan was used as the reference for all measurements.

***Ex-vivo* permeation studies (Franz diffusion cell)**

The prepared binary systems using the physical mixture method was assessed for the permeation using the intestinal tissue of the goat which is very much morphological similar to human intestine tissue. In this experimentation the tissue was mounted in between the donor and acceptor chamber in the diffusion cell. The donor compartment was filled with the sample (SQU and binary systems) having concentration (5 mg/ml) and acceptor cell was filled up with the simulated intestinal fluid (SIF) with aid to continuous stirring and then samples of the solution (1 mL each) were withdrawn at different time intervals (0,15,30,60,120,180,240,360,480 min) and passed through the syringe filters. The SQU amount was quantified using validated LC-MS method for the SQU. The amount of the drug permeated through the tissue was determined. The amount permeated was plotted against the different time points. The permeability coefficients (Peff) and permeation enhancement ratios (from Peff values) were calculated as per equation 1 and 2 respectively.

$$Peff\,(cm/\sec) = \frac{dQ/dt}{A*Cd} \qquad \text{Eq. 1}$$

Where, A = the surface area, dQ/dt = amount of drug permeated per unit time at steady state, Cd = donor drug concentration.

$$R = \frac{Papp(sample)}{Papp(control)} \qquad \text{Eq. 2}$$

The results of experiments performed (n = 3).

Transport of SQU across the Caco-2 cell monolayers

The Caco-2 cells were seeded at the density of approximately 1×10^5 cells per well in to a 12-well transwell polycarbonate cell culture inserts having 12-mm diameter and 3-µm pore size purchased from Costar®, (Corning Costar Co, Cambridge, MA, USA). The cells were cultured and used after 25 days so that full maturation and confluence must obtained. It also includes P-glycoprotein (P-gp) expression and the formation of tight junctions in the cell monolayer. For the very first week of the culturing the medium was replaced every other day. After 7 days the medium was changed daily. The basolateral (BL) and apical (AP) compartments contained 1.5 and 0.5 mL of culture medium, respectively.

The trans-epithelial electric resistance (TEER) values were measured using Millicell®-ERS (Millipore, Bedford, MA, USA) before and immediately after the transport studies to evaluate the integrity of the Caco-2 cell monolayers. The transport medium (HBSS) resistance was subtracted from the TEER value

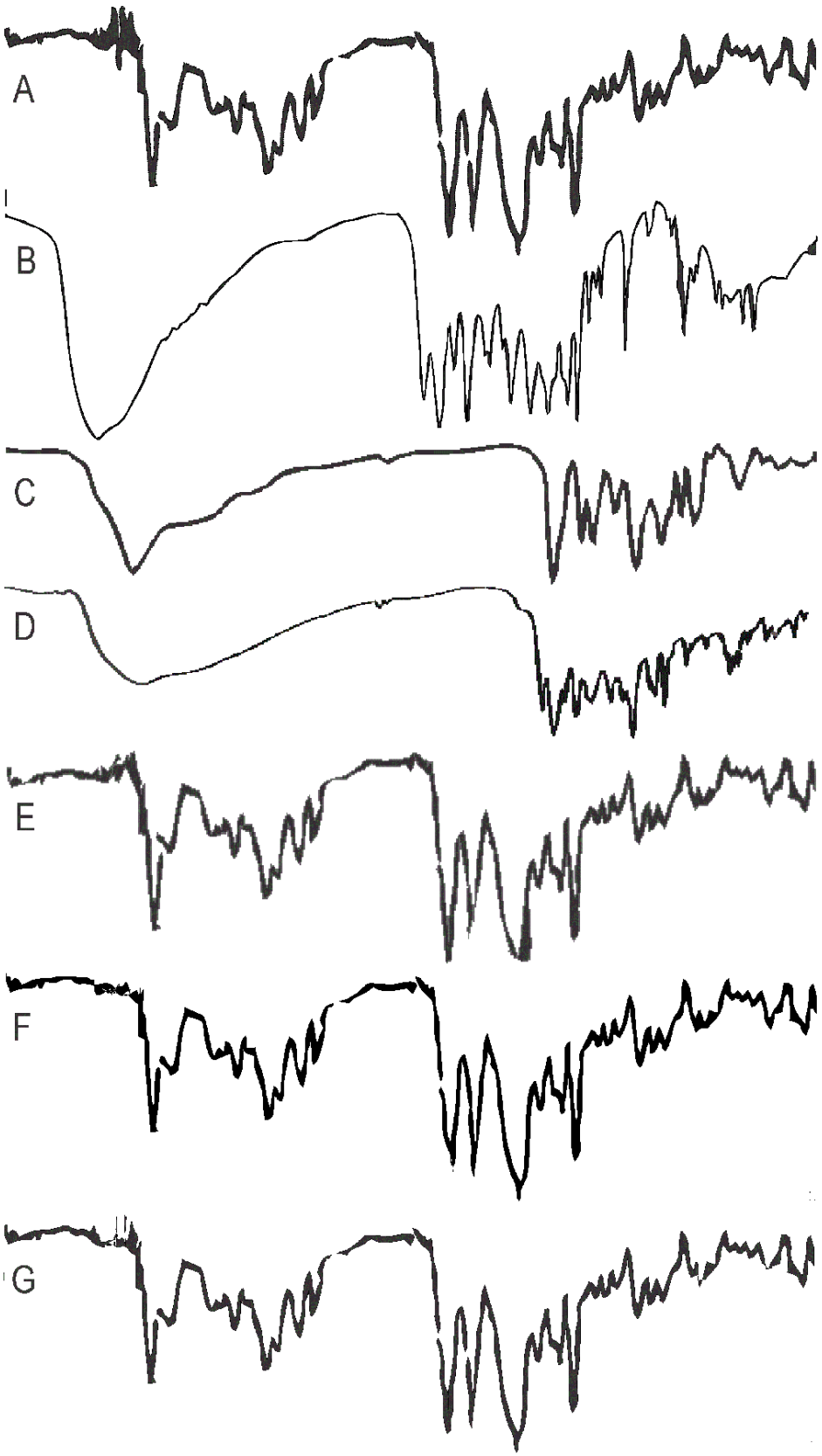

Figure 1 FTIR spectrum of (A) SQU (B) QU (C) Sil (D) LT (E) SQU:QU (F) SQU:Sil (G) SQU:LT for compatibility studies.

considering it as the background resistance. The cell monolayers having TEER values below 300 $\Omega \cdot cm^2$ were excluded from the study design.

The cell monolayer was equilibrated at 37 °C with warm HBSS (37 °C) for 30 min before starting the experimentation (transport studies). After 30 mins, the HBSS was removed and the sample solutions (SQU, SQU-QU, SQU-Sil, and SQU-LT containing 10 µM/mL SQU in HBSS) were added to the AP compartments. Sample (100 µL) were withdrawn from the receiver chamber at different time points (30, 60, 90, 120, 240, 480 mins) the equal volume (100 µL) of fresh HBSS was added to the chamber so as to maintain a constant volume.

The drug concentration in the samples was determined by validated LC-MS method as described above. The experiments were performed in triplicate. The apparent permeability coefficient (Papp, cm/s) was calculated using the following equation:[22]

$$Papp(cm/\sec) = \frac{dQ/dt}{A*C0}$$

Where, dQ/dt is the transport rate, C0 is the initial drug concentration on the apical side, and A is the surface area of the membrane filter (1.12 cm^2).

In vivo pharmacokinetic study in rabbits

Dosing

In pharmacokinetics study rabbits were divided in to five groups (3 per group). The SQU (oral), SQU (IV), SQU-QU, SQU-Sil, SQU-LT were orally administered as suspension, at a dose of 50 mg/kg. The rabbits had free access to water during the entire experiment. Blood samples of 600 µL were collected at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hr after dosing via the orbital venous plexus using isoflurane as anesthesia. The whole blood was collected in heparinized tubes, and the plasma from the sample was separated by centrifugation at 9000 rpm for 8 min and stored at -20 °C prior to analysis by LC-MS.

Analysis of plasma samples

The concentration of SQU in the plasma samples were estimated by LC-MS. The extraction for the analysis of sample was carried out using protein precipitation method. The extraction procedure was identical for the all samples (Standard validation samples, cell line samples, in-vivo samples). In 100 µL of plasma samples 400 µL of acetonitrile was added and then vortexed for 5 mins. Following the vortexing, the samples were centrifuged at 9000 rpm for 12 mins. The supernatant was then transferred in to fresh micro-centrifuged tubes and adequately diluted with the mobile phase prior injecting in to LC-MS. The drug concentrations in the samples were calculated as described above.

Pharmacokinetic and Statistical analysis

The drug concentration obtained from the LC-MS analysis was used to derive pharmacokinetic parameters. WinNonlin 6.3 was used to determined parameters maximum plasma concentration (Cmax), time for maximum plasma concentration (Tmax), Area under the curve (AUC) etc. All other mathematical calculations were done using the Microsoft Excel 2013.

Results and Discussion

Compatibility studies of binary systems prepared

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectrums of SQU, QU, Sil, LT and their binary systems were recorded. The recorded spectrums were interpreted in terms of change in any characteristic peaks of the SQU. The characteristics peaks in the FTIR studies of SQU exhibited C=O stretching at 1623 cm^{-1}, OH stretching at 3528 cm^{-1} and NH_2 stretching at 3102 cm^{-1} which confirms the structure of SQU. All these characteristic peaks remains regular in the prepared different binary systems. Although, there was a slight (not significant) change observed in the intensities of the peaks of SQU. These primary results of the FTIR spectra

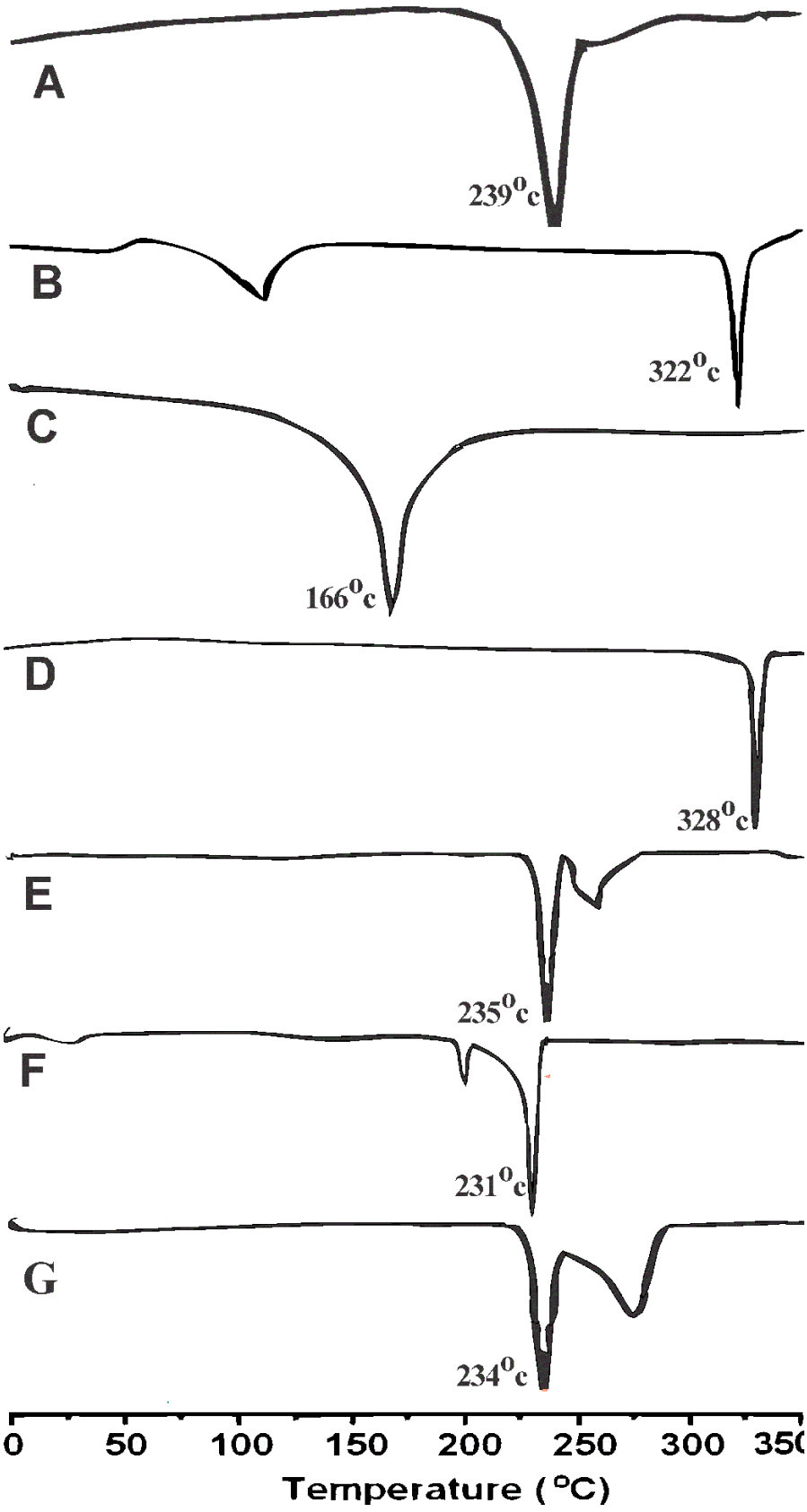

Figure 2 DSC thermogram of (A) SQU (B) QU (C) Sil (D) LT (E) SQU:QU (F) SQU:Sil (G) SQU:LT for compatibility studies.

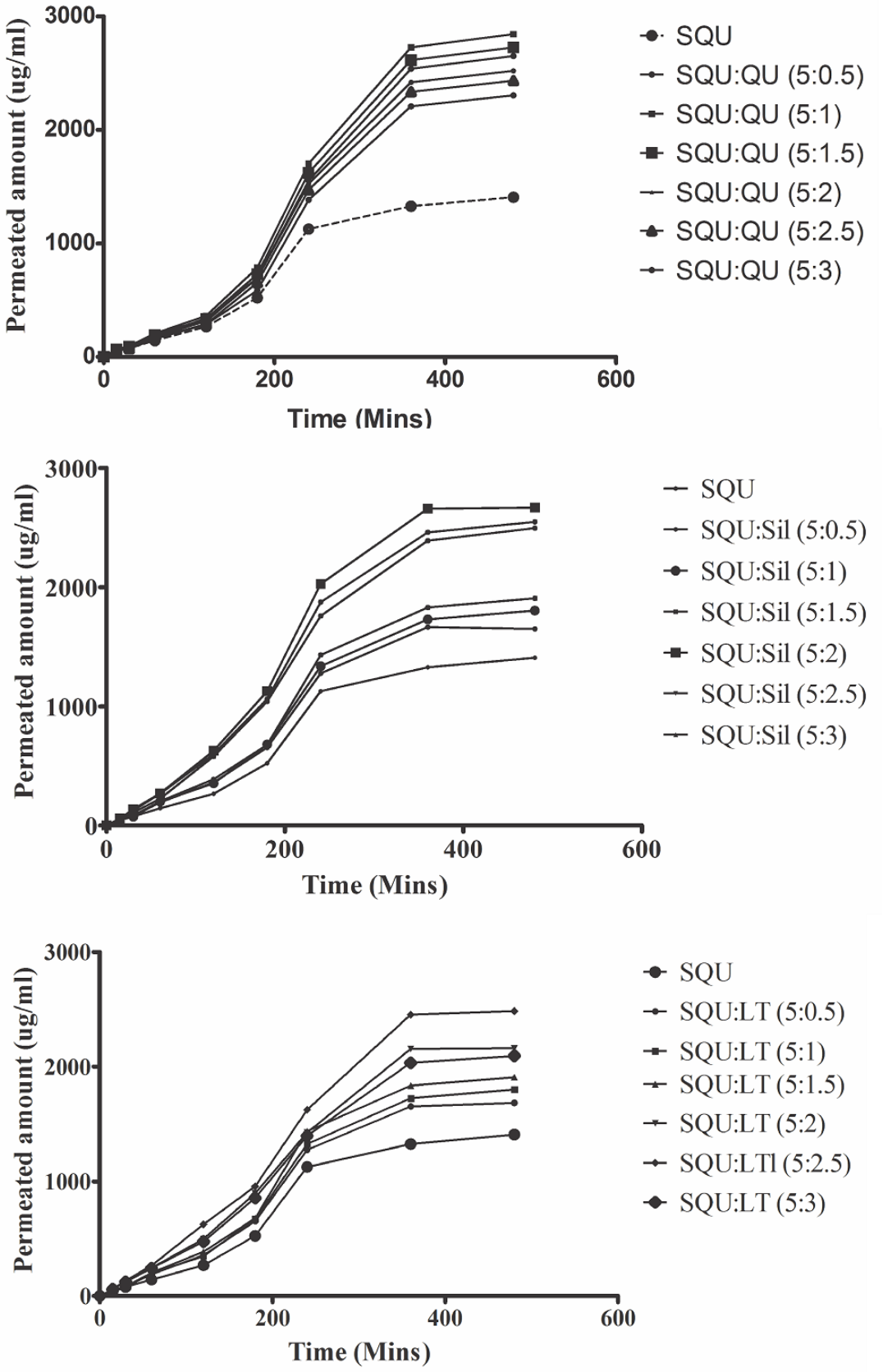

Figure 3 Time profile for permeation of the SQU and binary system from the intestinal tissue.

revealed that there was no interaction between SQU, QU, Sil and LT in binary system. It also confirms that there is no interaction at the molecular level in the SQU and their binary system. The FTIR spectrum of SQU and different binary system has been shown in Figure 1.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) thermograms of the SQU, QU, Sil and LT has been recorded. These recorded thermograms were interpretated to know the interactions. Interpretations of the thermograms revealed that there is no physical interaction between the SQU, QU, Sil and LT in their respective binary systems. The DSC thermogram of SQU showed an endothermic peak at 239°C which was corresponding to its melting point. While the thermogram of QU shows an endothermic peak at 322°C. Sil shows a broad endothermic peak in the range of 166-173°C. The luteolin thermogram showed endothermic peak on 328.16°C, which again attributed to its melting point. DSC thermogram of SQU-QU physical mixture shows an endothermic peak at 235°C which is very near to the pure SQU, which reveals that there is no interaction in the QU and SQU. The DSC thermogram of all the pure compounds and binary mixtures has been illustrated in Figure 2. DSC thermogram of SQU-Sil physical mixture shows an endothermic peak at 231°C which is slightly lower than the pure SQU, although it is not significant, the endothermic peak of binary mixture suggest minimal or no physical interaction between the QU and Sil. DSC thermogram of SQU-LT physical mixture shows an endothermic peak at 234°C which is at the lower side of the pure SQU, the endothermic peak of SQU-LT binary mixture suggests no physical interaction between the SQU and LT. The DSC data of all the samples encourages and clears the way for the researchers to move further for the permeation studies.

***Ex-vivo* permeation studies**

The permeability of binary systems of SQU-QU, SQU-Sil and SQU-LT in goat intestinal tissue shows a significant rise as compared to the plain SQU. Permeation coefficient (P_{eff}) was calculated for SQU and SQU-QU, SQU-Sil and SQU-LT binary systems. The permeation coefficients calculated for the all weight ratios has been summed up in Table 1**.** The permeation coefficient for plain SQU was (2.135±0.387) × 10^{-6} cm/s. The QU shows an increase in the amount permeated having permeation coefficient (4.395±0.15) × 10^{-6} cm/s at weight ratios (5:1), while Sil shows maximum permeation coefficient (4.283±0.18) × 10^{-6} cm/s at weight ratios (5:2) and LT shows maximum enhancement at weight ratio (5:2.5) with permeation coefficient (3.956±0.458) × 10^{-6} cm/s. Release profile of the SQU and binary systems at different time points has been shown in Figure 3.

Transport of SQU across the Caco-2 cell monolayers

To study the effect of the bioenhancers on SQU transport through cell monolayers, drug transport across Caco-2 cell monolayers from the AP side to the BL side were studied. TEER was determined in all the experiments, which clearly shows there was no cellular damage. Effect of presence of different concentration of QU, Sil and LT has been studied. Figure 4A shows effect of different concentration of QU, Figure 4B and 4C shows effect of different concentration of Sil and LT respectively. Figure 5A, 5B and 5C shows the time profiles of SQU permeation through the Caco-2 cell monolayers in the presence of QU, Sil and LT respectively. It is clearly seen that the amount of drug at the BL side increased with time for all the samples. Although the concentration in the samples having bioenhancers was higher than the plain SQU sample.

The *P*app (AP to BL side) of SQU and its binary systems were calculated for 24 hrs. As shown in Figure 4A, 4B, 4C maximum *P*app was observed with the QU in the ratio of 5:1 while in the Sil and LT maximum was observed in the ration of 5:2

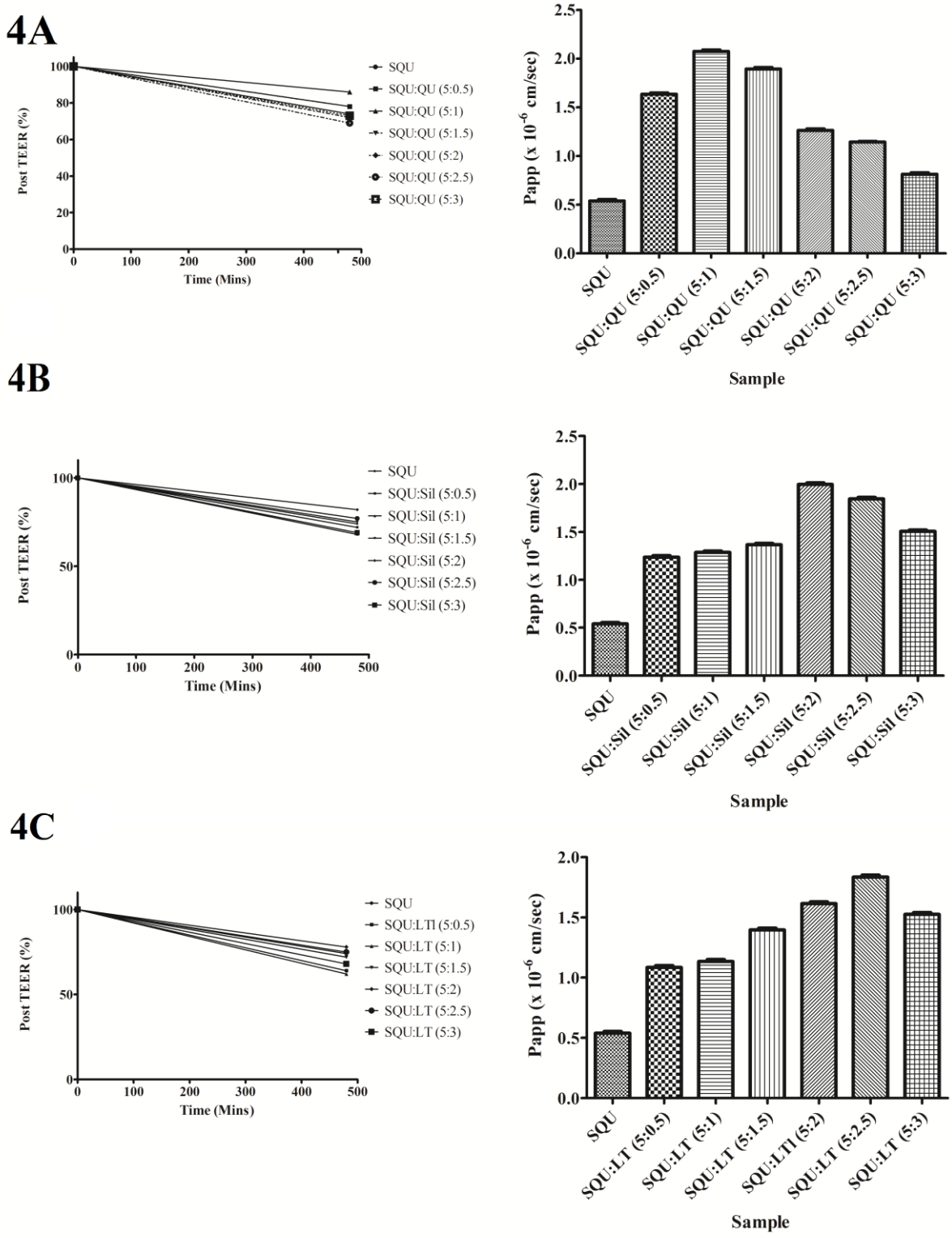

Figure 4 Papp values and deviation in post experimental TEER values in Caco-2 cell line studies at different weight ratios (**4A**) SQU:QU binary system (**4B**) SQU:Sil binary system (**4C**) SQU:LT binary system

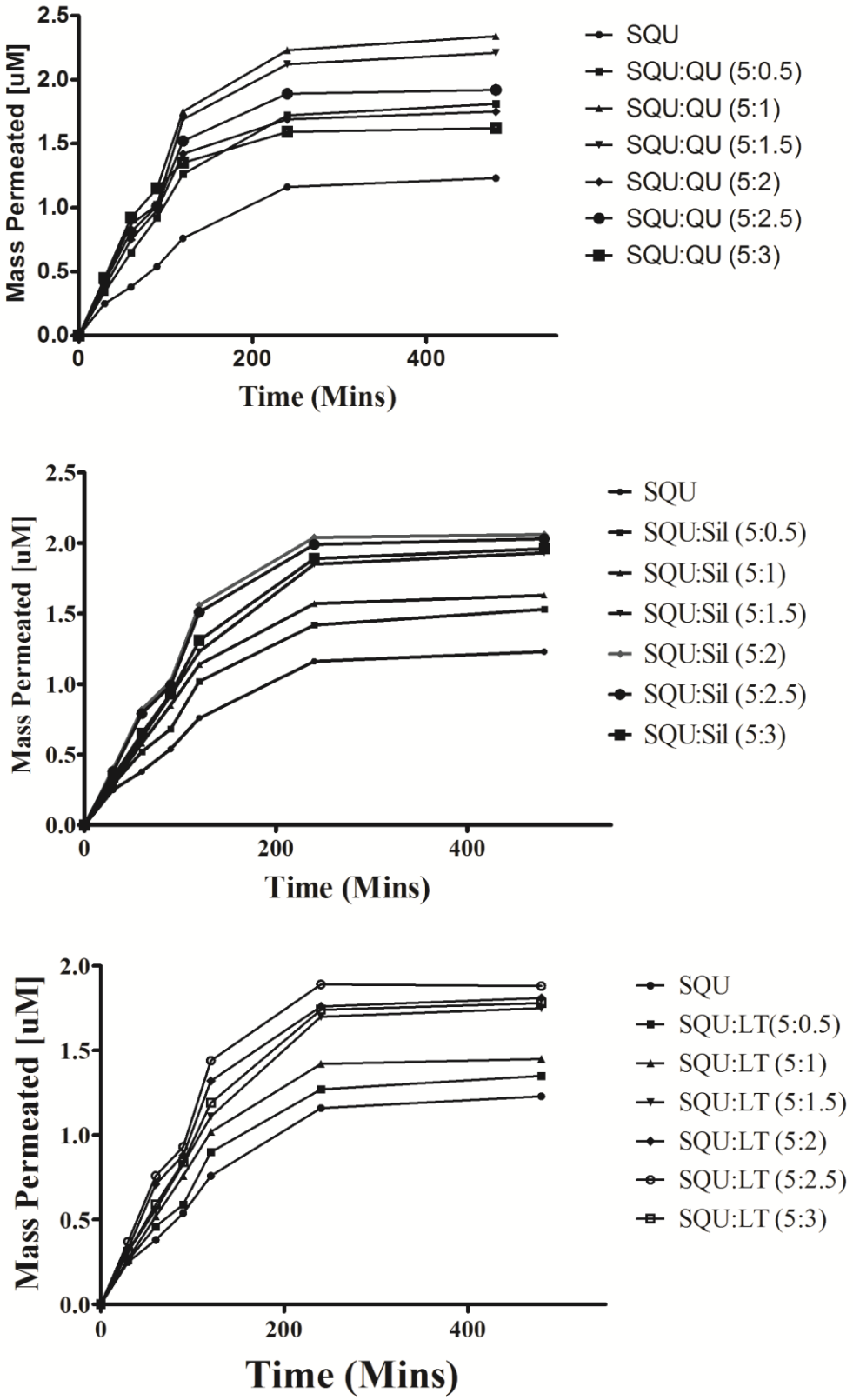

Figure 5 Time profile for mass permeation of the SQU and binary system in Caco-2 monolayer.

and 5:2.5 respectively. Although it was also observed that the ratio of Sil 5:2 and 5:2.5 was very close, but 5:2 concentration also shows maximum enhancement in the ex-vivo studies so this was chosen as optimum concentration for pharmacokinetic studies.

Pharmacokinetic studies of SQU in rabbits

The pharmacokinetics of SQU were studied in rabbits to evaluate the enhancement in the absorption efficiency of the SQU in combination with QU, Sil and LT. Plasma drug concentration versus time profile for SQU and SQU-QU, SQU-Sil and SQU-LT were plotted as shown in Figure 6. After the oral administration of the SQU and binary systems there was significant difference was observed in the pharmacokinetic profile. AUC increase by 2.51 folds in the case of the QU. While, 2.34 and 2.09 folds increase was observed with the Sil and LT respectively. The pharmacokinetic parameters for all the three bioenhancers are shown in Table 2.

Discussion

Low bioavailability of SQU presents a considerable challenge for the researcher from the very beginning. As, it is a very important and potent drug in anti-HIV therapy. In our proposed work, binary system of the SQU was prepared with three different bioenhancers using physical mixing method. This method has very crucial step of the blending or mixing as if mixing is not proper it can alters and effects result very much. So, the blending must be proper as to achieve uniformity of content. Analytical method for the estimation of SQU was an important step for the whole research process, so for this purpose the most sophisticated and highly reliable instrument LC-MS was used. The results of LC-MS was very much reproducible and the method was properly validated before using for the samples.

Ex-vivo studies were primary studies and were of important as they confirms the hypothesis behind the research work and encourages the team to go for further sophisticated and reliable technologies such as cell lines and *In-vivo* studies. The results of Ex-vivo studies revealed that the bioenhancers can plays a crucial role in the permeation enhancement of the SQU. The transport studies in caco-2 cell lines suggested that there is increase in the cellular uptake of SQU in the presence of the bioenhancers. The effective transport of the SQU in the presence of the bioenhancers most likely due to their P-gp inhibitory effect. The caco-2 cell lines also helps in optimization of the best ratios that can be used for the pharmacokinetic studies.

Pharmacokinetic studies were conducted in rabbits, these studies shows a significant increase in the plasma concentration of the SQU in the presence of the bioenhancers. It is well known fact that SQU is the substrate of the P-gp so it limits its oral uptake. As the bioenhancers used in the research work are P-gp inhibitor so the oral absorption has increased in the case of binary systems. In addition, to this it has also been reported that the hepatic and intestinal first pass metabolism also effects on the bioavailability of the SQU. This east meets west technique, in combination with P-gp inhibition also helps in the metabolism as they also shown some effect of the CYP enzymes. So these bioenhancers may further advantage the bioavailability of SQU. The study of hepatic metabolism will be our future perspective.

Conclusion

In this research work, SQU binary systems were prepared with the bioenhancrs to study their effect on the oral absorption. The studies carried out during this research work are compatability studies, ex-vivo permeation studies, and transport across Caco-2 cell monolayers and pharmacokinetic studies. The results were promising showing significant changes in the permeation and transport across cell lines. Oral absorption also increases in the rabbits. The effect of the three bioenhacrers when compared QU shows the maximum enhancement following by the Sil and then by the LT. These promising results encourages us to focus on hepatic first

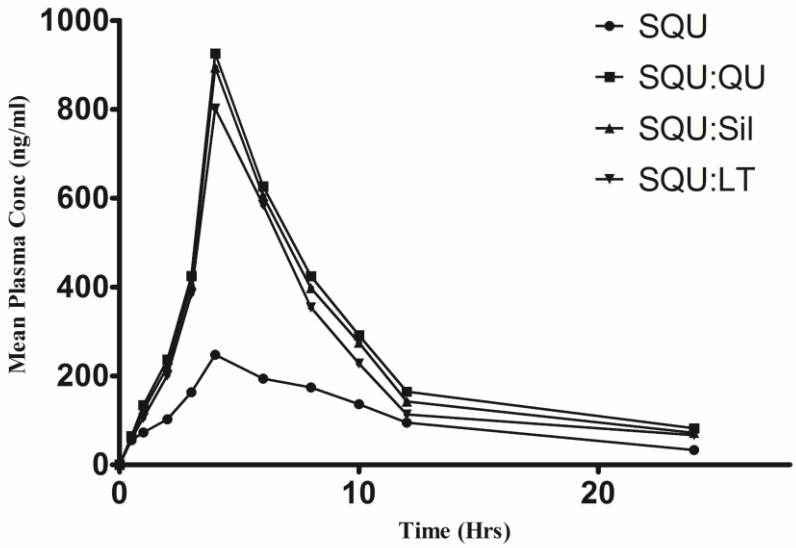

Figure 6 Time profile for mean plasma concentration for SQU and SQU:QU, SQU:Sil and SQU:LT.

Table 1 Permeation coefficient for SQU and SQU-QU, SQU-Sil, SQU-LT at different weight ratios

Permeation coefficient and enhancement ratio of SQU and its different binary systems (x 10^{-6} cm/s)												
SQU	2.135 ± 0.38											
Weight Ratio	**5:0.5**		**5:1**		**5:1.5**		**5:2**		**5:2.5**		**5:3**	
	Peff	**ER**	**Peff**	**ER**	**Peff**	**ER**	**Peff**	**ER**	**Peff**	**ER**	**Peff**	**ER**
SQU-QU	4.093 ±0.18	1.92	**4.395 ±0.15**	**2.06**	4.217 ±0.22	1.98	3.898 ±0.25	1.83	3.764 ±0.32	1.76	3.561 ±0.25	1.67
SQU-Sil	2.664 ±0.26	1.25	2.778 ±0.28	1.30	2.939 ±0.15	1.38	**4.283 ±0.18**	**2.01**	3.961 ±0.34	1.86	3.846 ±0.28	1.80
SQU-LT	2.345 ±0.38	1.10	2.458 ±0.32	1.15	3.009 ±0.26	1.41	3.473 ±0.15	1.63	**3.956 ±0.28**	**1.85**	3.278 ±0.32	1.54

Table 2 Pharmacokinetic parameters for SQU, SQU: QU, SQU: Sil and SQU: LT in rabbits

Parameter	**Cmax**	**Tmax**	**AUC (0-24)**	**Relative Bioavailability**
SQU	247.66	4	2593.583	1
SQU:QU	925.67	4	6514.5	2.51
SQU:Sil	894.33	4	6072.333	2.34
SQU:LT	802	4	5424.25	2.09

Cmax, maximum blood concentration of drug; Tmax, the time taken to reach the Cmax; AUC, the area under the blood drug concentration-time curve;

pass metabolism bypass for further increase the absorption, so these factors will be further needed to explore.

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