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RESEARCH ARTICLE

Effect of modulation of alimentary and formulation pH on the pharmacokinetics of BCS-Class I, II, III, and IV compounds in rats following cassette dosing

Satish Kumar[a,b], Lakshmi Prasanna Tattala[a], Ravi Akkireddy[a], Satinder Singh[a], Sudhir Kumar Tiwari[a], Pratima Srivastava[a], Sumeet Gupta[b] and Anroop Nair[c]

[a]Aragen Life Sciences Limited, IDA Nacharam, Hyderabad, India; [b]MM College of Pharmacy, M. M. (Deemed to be University), Ambala, India; [c]Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Ahsa, Saudi Arabia

ABSTRACT

Objective: This study was performed to appraise the effect of modulation of formulation pH and abdominal pH on the pharmacokinetics (PK) of the selected compounds from different drugs of the Biopharmaceutics Classification System (BCS) when administered via cassette dosing in rats.

Methods: Pharmacokinetics of four different BCS class drugs viz. propranolol HCl, diclofenac sodium, atenolol, and acetazolamide (BCS-I, II, III, and IV, respectively) in different formulation pH conditions and different abdominal pH conditions were evaluated. The animal groups were dosed orally.

Results: Propranolol HCl exhibited a significant increase in area under the curve (AUC_{0-last}) when the alimentary canal pH was modulated to acidic (195%), and a significant increase in maximum concentration at a given time point (C_{max}) (102%) for formulation with basic pH in healthy animals. Diclofenac exhibited a significant increase in AUC_{0-last} when the alimentary canal pH was modulated to acidic (95%), and a significant decrease in C_{max} (55%) for formulation with basic pH in healthy animals. Atenolol displayed a substantial increase in AUC_{0-last} in basic pH conditions in the alimentary canal (38%), and a significant decrease in AUC_{0-last} (49%) for basic pH formulation in healthy animals. Acetazolamide demonstrated a considerable increase in AUC_{0-last} when alimentary canal pH was modulated to basic (55%), and a substantial enhancement in AUC_{0-last} (39%) for acidic pH formulation in healthy animals.

Conclusion: The results suggest that both alimentary canal conditions with acidic and basic pH and formulation with acidic and basic pH have a considerable influence on the PKs of different compounds not only based on their acid dissociation constant (pKa) but also independently on the pH in the area of absorption in the alimentary canal and salt form of the compound.

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Cassette administration; acidic formulation; basic formulation; pharmacokinetics; pH modulation

Background

In early drug discovery, out of many molecules, after *in vitro* few selected for *in vivo* studies based on their *in vitro* data, but even after very good *in vitro* data, many of them are rejected in pre-clinical studies. This could be because of poor absorption, degradation at stomach pH or not reaching proper site of absorption or site of absorption is not suitable for same drug. For example, if drug is weak acid and absorption site is small intestine or weak base with absorption site stomach. In that case, we may end up killing a good compound may be because of low exposure. According to literature, around 90% of drugs fail in clinical trials and it increases if we count pre-clinical failures. *In vivo* pharmacokinetics (PKs), such as bioavailability (*F*), drug exposure (AUC, area under the curve), maximum concentration (C_{max}), half-life ($t_{1/2}$), clearance (CL), and volume distribution (Vd), as well as solubility, permeability, protein binding, metabolic stability, and other strict selection criteria for drug-like properties during drug optimization, contribute to this improvement. The best lead compounds have been chosen based on a set of cutoff values for certain drug-like characteristics [1]. The odds of a molecule found in its early stages reaching the market are one in 10,000, or about 0.01%. For a variety of reasons, including lack of efficacy, toxicity,

CONTACT Sumeet Gupta sumeetgupta25@gmail.com MM College of Pharmacy, M. M. (Deemed to be University), Mullana, Ambala, Haryana 133207, India

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poor absorption, distribution, metabolism, and elimination (ADME) qualities, economic interest, and market rivalry, the compounds are discarded at the preclinical stage and removed from further research during clinical studies. Literature suggests poor PK profile contributed majorly (39.4%) to the drop of the clinical candidates [2].

While drug candidates with low plasma exposure are frequently discarded without further research, those with higher plasma exposure are frequently chosen to move on to clinical trials. Choosing the right compound based on Drug Metabolism and Pharmacokinetics (DMPK) of a compound, we need to mimic human like PKs. We take care of food/fasting, formulation strategy, dose escalation, and few more type of studies for lead compounds, but what happens if we kill compound because of intrinsic properties like abdominal pH. The first organ where a medicine taken orally comes into close touch with gastrointestinal (GI) fluids is typically the stomach. Drug absorption is limited by the stomach's short transit time and thick mucous layer, despite its comparatively wide epithelial surface. These GI characteristics can affect how drugs are made and behave. Gastric emptying is frequently the rate-limiting stage since the majority of absorption takes place in the small intestine. Food slows gastric emptying, which explains why taking some medications on an empty stomach accelerates absorption. This is especially true for fatty foods. The pace at which other pharmaceuticals are absorbed is impacted by medications that alter stomach emptying. Food may also have effect (e.g. omeprazole), decrease the amount of absorption for medications that are broken down in the stomach (e.g. penicillin), or increase it for medications that are poorly soluble (e.g. griseofulvin) [3]. If food is playing such a big role now question arises, is it just the transit time or does pH also plays a major role. Because of this, we made some efforts here where we validated one PKs method by modulating the pH of both formulation and alimentary canal and 3–5 compounds can be screened in one go.

For novel drug development, various guidelines suggest generating the key parametric data in the initial stage, for example, Log*P* (logarithm of the octanol–water partition coefficient), log*D* (measurement of a molecule's lipophilicity), pKa (acid dissociation constant) value, etc. as the traits have an impact on physicochemical characteristics like aqueous solubility, which in turn regulates the medication manufacturing strategies. Their impact on physiological properties such as absorption, diffusion, metabolism, and elimination is very significant [4], which can give an idea about the selection of better formulation, according to the site of absorption in the alimentary canal during the *in vivo* studies. Published studies have unequivocally demonstrated that individuals with high gastric pH may have diminished systemic exposure to weak basic medications, which have poor solubilization at higher pH, leading to decreased and varying bioavailability [5]. In order to ward off any threat of drug exposure loss in clinical settings, understanding how the substance responds to changes in stomach pH is crucial which can lead to a significant loss of compound efficacy. The pH of the stomach and intestine can change in a patient due to several reasons, i.e. food, age, acidity medications, disease conditions, etc.

To overcome the high gastric acidic conditions clinically, proton pump inhibitors (PPIs) are the main drugs given as supportive treatments. PPIs (such as pantoprazole and omeprazole) decrease the formation of gastric fluid by inhibiting the adenosine triphosphatase enzyme system, often termed the gastric proton pump, in the stomach parietal cells [6]. A substituted benzimidazole derivative known as omeprazole significantly reduces both baselines and stimulates stomach acid production [7]. The first PPI to enter the market was omeprazole. PPIs are mainly metabolized by CYP2C19 and CYP3A4 [8].

The chronic use of stomach acid-suppressing drugs has increased as a result of the high incidence of gastroesophageal reflux disorder in people of all ages, the increase in the newest approval of PPIs for long-term use, and the availability of over-the-counter anti-secretory agents in many Western countries. Antacids are another available option for reducing the acid already present in the gut. In their formulas, antacids are a blend of different substances that may include crystals of magnesium, aluminum, calcium, sodium, carbon, or bismuth. The antacids function by bringing the stomach's pH back to normal and blocking the action of the protease enzyme pepsin [9]. By altering the ionization state of weak bases and acids, which are affected by urine pH, antacids can modify excretion [10].

Increased acidic content may lead to increased oral bioavailability of weak acidic drugs. Histamine was dosed to increase the acid secretion in stomach. Basically, three major pathways (the histamine–gastrin pathway, the somatostatin–CCK pathway, and the neural pathway) normalize the acid discharge in the alimentary canal [11]. In general, acetylcholine, gastrin, and histamine are three local substances that control the release of gastric acid [12]. The parietal cells are stimulated by histamine to release HCl. Studies showed

that a 50 mg/kg/SC dose of histamine leads to an increase in acid secretion [13,14]. The same procedure is used to evaluate the effect of increased acid secretion on the PKs of different compounds.

Apart from intrinsic HCl excretion and pH, formulation pH is also a major factor that can influence the drug PKs; for example, weak acid drugs are well absorbed in the stomach with high acid content. However, if the formulation has excipients having basic pH (i.e. magnesium trisilicate, sodium carbonate, etc.), this can lead to precipitation of ionized drug in the stomach and eventually lead to lower absorption. In this case, the change of formulation pH determines the PKs of the drug. Our aim herein was to develop the *in vivo* model to assess the effect of the alimentary canal and formulation pH on compound PKs. Literature suggests that there are only a few variables that affect a drug's degree of ionization in its solution: the pH of the solution and the drug's pKa. Of these two, pKa is an inherent attribute. However, changes in pH can be managed externally. In the current study, compounds were dosed as cassettes for three reasons: first, the dosing of the cassettes helps us reduce the number of animals. With the individual animal approach, 120 animals were required for this study. With the cassette approach, we completed the same procedure with 30 animals only. Second, the pH was uniform in both formulations and animals for all four medications. This helped us minimize profile changes due to drug and alimentary canal pH variations. Lastly, the data was generated quickly due to the fourfold decrease in sample size [15].

The selection of test drugs was done based on two main factors. First, two of the selected drugs are weak acids (propranolol HCl and acetazolamide) and the rest two are weak base (atenolol and diclofenac sodium) category. Second, all four drugs are commonly used drugs but from different BCS classes. This helps to avoid interaction at solubility and permeability level. Although the chance of interaction is still there in case of active absorption and metabolism level, comparative data are provided in Table 1.

The GI pH of rats is substantially different from that of humans (e.g. the pH of a fasting rat's stomach is approximately 3.5–3.9, whereas that of a human is approximately 1.2–2). To more closely resemble human 'fasted' or 'fed' states, pH-modifying drugs in rodents, such as pentagastrin or histamine to drop pH and omeprazole or pantoprazole for increasing pH can be used. The basic principle of drug absorption suggests that weak acid gets better absorption at acidic pH but in actuality many of the cases, we even get good exposure for weak basic drugs also and less bioavailability for weak acids also. This mainly depends upon the area of absorption and degree of ionization. Here, we tried to develop the *in vivo* method which can help to test the compound real pH based absorption before going to investigational new drug (IND) candidate selection. Characterizing how changes in physiological pH impact a drug's absorption, distribution, and stability is the main justification for carrying out a pH-based PK study in rats. This is crucial for extrapolating preclinical results to people. This novel screening method facilitates the evaluation of new chemical entities (NCEs) from the preclinical to clinical stages of drug development. It enables the assessment of NCE PKs across all four Biopharmaceutics Classification System (BCS) classes by simulating conditions in both acidic and basic regions of the alimentary canal, a process guided by the compound's pKa. To our knowledge, this is the first reported

Table 1. A comparison of properties of different drugs studied.

Drug properties	Propranolol	Diclofenac	Atenolol	Acetazolamide
Absorption site	Whole intestine [16].	Stomach and duodenum, however, mainly depend on salt form. The potassium salt gets absorbed maximally in the stomach and sodium salt in the intestine [17].	Primarily intestine [18].	Stomach and upper part of the intestine [19].
Drug type	Weak base	Weak acid	Weak base	Weak acid
pKa	9.42	4.15	9.6	7.2
Metabolism and excretion	CYP1A2 and inhibits CYP2D6. Due to considerable first-pass liver metabolism (aromatic hydroxylation, N-de-alkylation followed by further side-chain oxidation, and direct glucuronidation), only about 25% of propranolol generally enters the systemic circulation. Urine metabolites are visible.	CYP2B6, CYP2C9, and slightly by CYP3A4 Very little diclofenac is removed intact; instead, it is biotransformed and excreted in urine by glycoconjugate and sulfate metabolites that are. Conjugate excretion and renal function may be connected [20].	Eliminated through urine only 5% metabolized using the liver as hydroxylated metabolite [18].	Most of the drug excreted unchanged through the kidney very small part in bile

model to rapidly screen the PKs of NCEs under these varied conditions within a single rodent study, offering significant efficiency over traditional methods.

Materials and methods

Materials

Atenolol, acetazolamide, diclofenac sodium, propranolol HCl, telmisartan, and methylcellulose were purchased from a local supplier and were manufactured by Sigma-Aldrich (St. Louis, MO). Lithium-heparin microtainers were purchased from PreQ Company (Mumbai, India). Merck Pvt Ltd (Mumbai, India) provided the HPLC grade methanol, formic acid, and acetonitrile.

Animals

Animals were purchased from Charles River's partner, Hylasco Bio-Technology Pvt Ltd (Hyderabad, India). The research protocol for animal investigation was approved by the Institutional Animal Ethics Committee (IAEC) according to the protocol mentioned in B-011. Animals used were Sprague Dawley (SD) rats which have an average age of 2 months and were quarantined for three days in an OLAW and AAALAC certified vivarium. The animal house was maintained at relative humidity (40–70%), temperature (22 ± 2 °C), and light/dark cycle (12–12 h). According to the laboratory animal veterinarian's consent, normal, healthy animals were chosen and gradually acclimated for at least three days before the initiation of the experiment. Throughout, the experimental duration, animals were observed for their health as per the facility's standard operating procedures (SOPs). Animals have free access to reverse osmosis water and common rat feed pellets (Samitek Instruments, Safe Diet, New Delhi, India). Five groups of animals (n = 6) were randomly assigned. Prior to oral therapy, animals were allowed access to water with a 12 h food fasting condition.

Cannulation of jugular vein

The animals were catheterized with a silicone tubing cannula having exterior width of 1.25 mm and interior width of 0.75 mm. During the cannulation procedure, rats were given 5% isoflurane through inhalation, and 2% isoflurane as maintenance dosage using E-Z Anesthesia equipment (Palmer, PA). Cannulation was performed on seven animals to guarantee protection in the event blood extraction patency is affected. Once the right jugular vein became visible, a slightly loose ligation was used to ligate the vein's cranial end. The catheter was introduced through a tiny incision made between the ligatures. The tube was kept in position by a loose ligature that was further wrapped. Through a scapular incision, the catheter was exteriorized and subcutaneously tunneled. Sterile suturing material was used to stitch the animal. The surgery was carried out on a surgical pad with temperature control. The sutured area and the region around the scapula on the rat were cleaned with an antiseptic solution. The catheter was then sealed with a stainless-steel stopper after being checked for patency and injected with a locking solution (heparinized saline).

Pharmacokinetics

Animals were dosed and bled in accordance with SOPs. Table 2 summarizes the detailed PKs study plan. Briefly, there were five groups of rats (n = 6)

Table 2. Pharmacokinetics study plan.

Study variables	Group 1	Group 2	Group 3	Group 4	Group 5
Animal species	Rat/Sprague-Dawley				
Test item	Propranolol, diclofenac, atenolol, and acetazolamide				
Sex	Male				
Body weight	200–230 g				
Gastric pH	Normal	Acidic (histamine 50 mg/kg/SC)	Basic (pantoprazole 10 mg/kg/PO, and digene – 1 mL/kg)	Normal	Normal
Formulation pH	Neutral	Neutral	Neutral	Acidic (using HCl – 2.5 pH)	Basic (using NaOH 9 pH)
Number of animals	6	6	6	6	6
Saline infusion	0.05 mL	0.05 mL	0.05 mL	0.05 mL	0.05 mL
Route of administration	PO				
Feeding condition	Fasting overnight, food was provided 4 h post-dosing				
Dose (mg/kg)	0/5	0/5	10/5	0/5	0/5
Dose volume (mL/kg)	2/5	2/5	2/5	2/5	2/5
Concentration (mg/mL)	0/1	0/1	5/1	0/1	0/1
Formulation vehicle	Tween 80 (1% v/v) + methylcellulose (0.5% w/v)				
Sample/collection – type	Blood/serial, jugular vein				
Anti-coagulant	Lithium-heparin				
Time points	0.25, 0.5, 1, 2, 4, 8, and 24 h				

who were dosed (5 mg/kg; 5 mL/kg) orally with the drug dispersed in 0.5% *w/v* methylcellulose + Tween 80 (1% *v/v*). Feed was provided 4 h post dosing. Group 1 was administered suspension formulation in normal conditions. Group 2 was given cassette suspension 30 min after administering the histamine (50 mg/kg/SC (subcutaneous) to increase the acid secretion). Group 3 was dosed with cassette suspension for 60 min after administering pantoprazole 10 mg/kg (to stop the acid secretion), and subsequently administered antacid after 20 min (2 mL/kg, digene to neutralize the existing acid). Group 4 normal animals were administered cassette suspension after adjusting the formulation pH to 2.5. Group 5 normal animals were administered cassette suspension after adjusting the formulation pH to 9. Before collecting blood, the initial few drops of samples were excluded to guarantee that there was no more extra heparinized saline in the tube. The tube containing blood (0.12–0.20 mL) was attached to the external catheter. Heparinized saline was placed within the cannula and then sealed. Before centrifugation, the collected blood samples were kept on ice. At 4 °C and 4000 rpm, centrifugation was carried out for 10 min. The samples were further taken in labeled centrifuge vials and immediately stored at −80 °C.

In vitro dialysis dissolution study

An *in vitro* dialysis-based dissolution study was conducted to evaluate the release profile of cassette formulation in Tween 80 (0.5%) + 0.5%MC (99.5%) at a concentration of 2 mg/mL/each of propranolol, diclofenac, atenolol, and acetazolamide across physiologically relevant pH conditions using a beaker setup with orbital shaking. Dialysis was carried out using 3.5 kDa MWCO cellulose membranes (Bioneau, Hyderabad, India). The following dissolution media were prepared fresh: pH 3.0 citrate buffer, pH 9.0 borate buffer, fasted-state simulated intestinal fluid (FaSSIF) at pH 3.0, and FaSSIF at pH 9.0 (10 mL per beaker). A 1.0 mL aliquot of sample (2 mg/mL cassette formulation) was loaded into pre-hydrated dialysis bags. Dialysis bags filled with samples were immersed in 10 mL preheated medium (37 °C), with stirring initiated at $t = 0$. Samples (0.3 mL) were withdrawn from the receiver compartment at 0.5, 4, 8, and 24 h, immediately replaced with an equal volume of fresh preheated medium, filtered (0.45 µm PTFE), and analyzed by liquid chromatography with tandem mass spectrometry (LC–MS/MS) and then percentage release was calculated.

Estimation of the drug in plasma

Calibration curve and quality control sample preparation

Atenolol, acetazolamide, diclofenac, propranolol HCl, and internal standard telmisartan stock solutions were made in dimethyl sulfoxide (DMSO) at a concentration of 1 mg/mL. Working standard solutions were made utilizing serial dilution from the master stock in a proportion of 40:20:40 (v/v/v), water:DMSO:methanol. The concentrations of the working standard solutions were 25-fold higher than those of the final extracted blood calibration curve (CC) and quality control (QC) sample concentrations. All of the above standards were created independently, a total of nine CC and three QC samples. CC standards for atenolol, acetazolamide, diclofenac, and propranolol HCl had lower limits of quantification (LLOQ) of 1.00, 5.00, 5.00, and 1.00 ng/mL, and upper limits of quantification (ULOQ) of 1000, 5000, 5000, and 1000 ng/mL. The QC samples (laboratory QC; 4.58, 22.40, 22.40, and 4.58 ng/mL, mid QC; 528.00, 2400.00, 2400.00, and 528.00 ng/mL, high quality control (HQC); 880.00, 4000.00, 4000.00 and 880.00 ng/mL) for all compounds were set in plasma by adding individually (1 µL in 24 µL of drug free plasma) from the main working standard. Dilution of a portion of the main stock with solvent produced the working internal standard solution (200 ng/mL).

Sample preparation

A 96-well polypropylene plate was used to hold 25 µL aliquots of plasma (PK study samples), which were then allowed to precipitate using acetonitrile (200 µL) that contained telmisartan (200 ng/mL). Following a 5 min vortex at 1000 rpm, the solution was centrifuged at 4 °C for 5 min at a speed of 4000 rpm. In order to conduct the analysis, the supernatant (110 µL) was taken off and put on a different plate. The supernatant was diluted with 110 µL of a mixture containing an equal proportion of methanol:water and a volume of 5 µL was used for estimation.

Estimation by LC–MS/MS

The mass spectrometric estimation was carried out using the Turbo VTM ionization source interface on the QTRAP 6500+ instrument (SCIEX, Marlborough, MA). Data collected were processed and quantified with an Analyst program *version 1.6.3* (SCIEX, Marlborough, MA). The mobile phase constitutes a mixture of methanol:acetonitrile:water in the ratio of 60:20:20 while the flow rate was initially fixed at 10 µL/min. The flow

dependent source properties were optimized using flow injection analysis and a mobile phase flow rate of 0.6 mL/min without a column. The Turbo V source with electrospray ionization probe was adjusted to the following settings: Positive polarity, ion spray voltage of 5500 V, source temperature of 550 °C, curtain gas pressure of 40 psi, nebulizer pressure of 55 psi, and heater pressure of 65 psi. The device was operated in MRM mode that identifies the parent and fragment ions efficiently. The parent and fragment ions of atenolol, acetazolamide, diclofenac, and propranolol HCl were measured at *m/z* values of 267–145, 223.1–181.0, 296.1–215, and 260.0–116.2, with optimal declustering potentials of 55, 70, 70, and 55 V and collision energies of 47, 19, 25, and 31 V, respectively. Similarly, for the internal standard (telmisartan), the *m/z* values noticed were 515.2 and 276.2, while the collision energy and declustering potential were 64 and 90 V, respectively.

A column consisting of Kinetex EVO (Phenomenex, Torrance, CA), C18 with particle sizes of 5 µm and diameters of 30 × 3 mm was used. The solvent mixture of ammonium acetate (10 mM) and formic acid in water (0.1%) and 100% acetonitrile was allowed to flow at 0.6 mL/min to separate the actives. The gradient flow was allowed for 3.7 min utilizing a program (time (min)/% *B* = 0.00/10, 0.3/10, 2.0/90, 2.20/90, 3.70/10). The representative chromatogram images of all samples are shown in supplementary figure file. The validation of analytical procedures for all the drugs in plasma was performed according to the earlier study [21]. Method was validated by performing the different studies (specificity, selectivity, linearity, precision, accuracy, stability, and matrix effect) as per Kumar et al. [21].

Pharmacokinetic parameter analysis

The PK parameters for each rat plasma sample were calculated using Phoenix software version 8.1. The parameters included oral (time for maximum concentration) T_{max} (h), (maximum concentration in profile) C_{max} (ng/mL), and others, for example (terminal phase volume of distribution) $V_{z\_}$ obs_F (L/kg), AUC_{0-inf} (ng·h/mL), (clast based clearance) Cl_obs_F (mL/min/kg), MRT_{0-last} (maximum residence time, h), and bioavailability estimated as ($F = \{(AUC_{PO}/AUC_{IV}) \times (Dose_{IV}/Dose_{PO})\} \times 100$). In other instances, the plasma concentration was only measured for short time intervals since there was no detectable drug level found after that point or it was below the quantification limits. As a result, the AUC in these situations was determined using the most recent time point indicated. IV data from our most recent published study were used to calculate bioavailability [21].

Statistical analysis

The average number of animals for all the data is 6 (mean ± S.D.). Dunnett's multiple range test and one-way ANOVA were used to analyze group differences; a, b, and c indicate significant differences compared to the healthy control group and/or the arthritic control group, respectively ($^{a}p < 0.0001$, $^{b}p < 0.001$, $^{c}p < 0.05$).

Results

The plasma concentration versus time was plotted for atenolol, acetazolamide, diclofenac, and propranolol HCl following oral cassette delivery to male SD rats at a dose of 5 mg/kg and was presented in Figures 1–4, respectively.

Propranolol HCl – (BCS Class-I)

A substantial enhancement in AUC_{0-last} (195%; $n = 6$), AUC_{0-inf} (184%; $n = 6$), and F (195%) was noticed in group 2 (alimentary canal with acidic pH conditions) when compared to group 1 (normal animals) after suspension formulation administration. A significant increase in C_{max} (102%; $n = 6$) was noticed in group 5 (normal animals with basic formulations) when compared to (group 1) control animals after suspension formulation administration (Table 3; Figure 1).

Diclofenac sodium – (BCS Class-II)

A considerable enhancement in AUC_{0-last} (95%; $n = 6$), AUC_{0-inf} (86%; $n = 6$), and F (95%; $n = 6$) was noticed in group 2 (alimentary canal with acidic conditions) when compared to group 1 (normal animals) after suspension formulation administration. A significant decrease in C_{max} (55%; $n = 6$), AUC_{0-last} (55%; $n = 6$), AUC_{0-inf} (56%; $n = 6$), and F (55%; $n = 6$) was noticed in group 5 (normal animal with basic formulation) when compared to group 1 (control group animals) after suspension formulation administration (Table 4, Figure 2).

Atenolol – (BCS Class-III)

A substantial enhancement in C_{max} (29%; $n = 6$), AUC_{0-last} (38%; $n = 6$), and F (38%; $n = 6$) was noticed in group 3 (alimentary canal with basic pH conditions) when compared to group 1 (control group animals) after suspension formulation administration. A significant decrease in AUC_{0-last} (49%; $n = 6$), AUC_{0-inf} (60%; $n = 6$), and F (49%; $n = 6$) was noticed in group 5

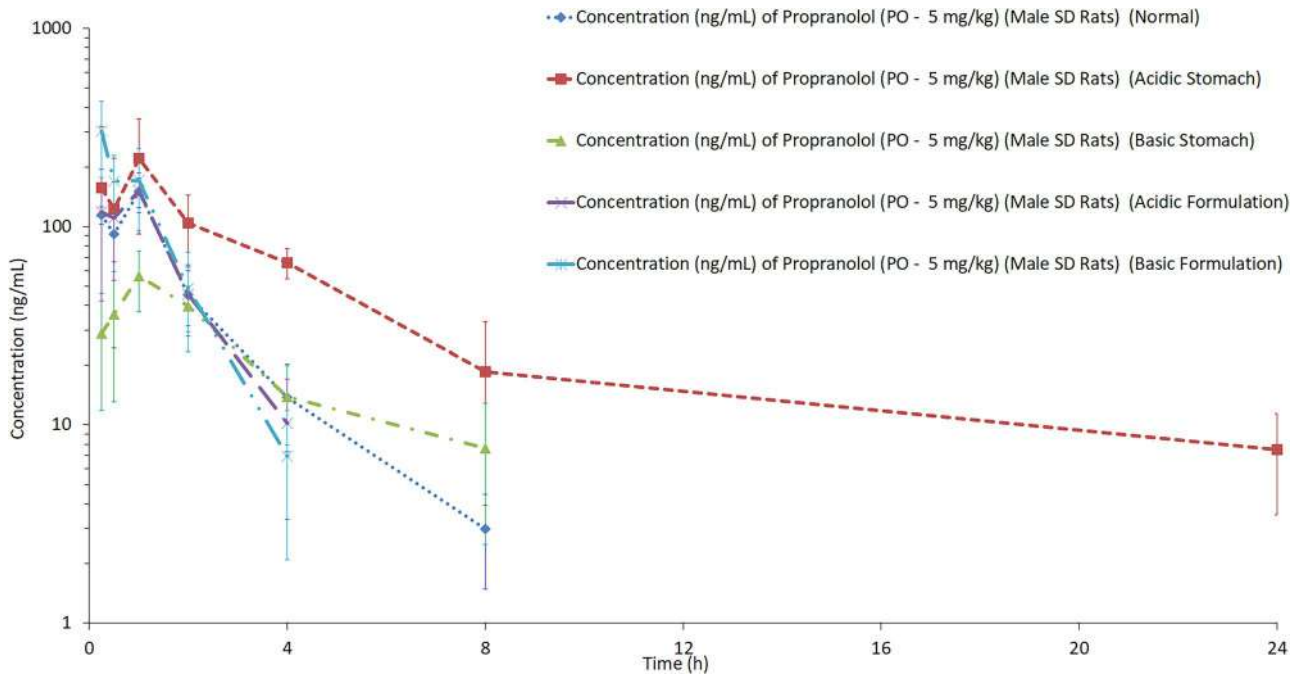

Figure 1. Concentration–time profiles of propranolol HCl after per oral cassette administration to male Sprague Dawley rats with different formulations at 5 mg/kg. The data presented are mean with S.D. error bars (n = 6) and pharmacokinetic parameters were analyzed by one-way ANOVA followed by Dunnett's multiple range test.

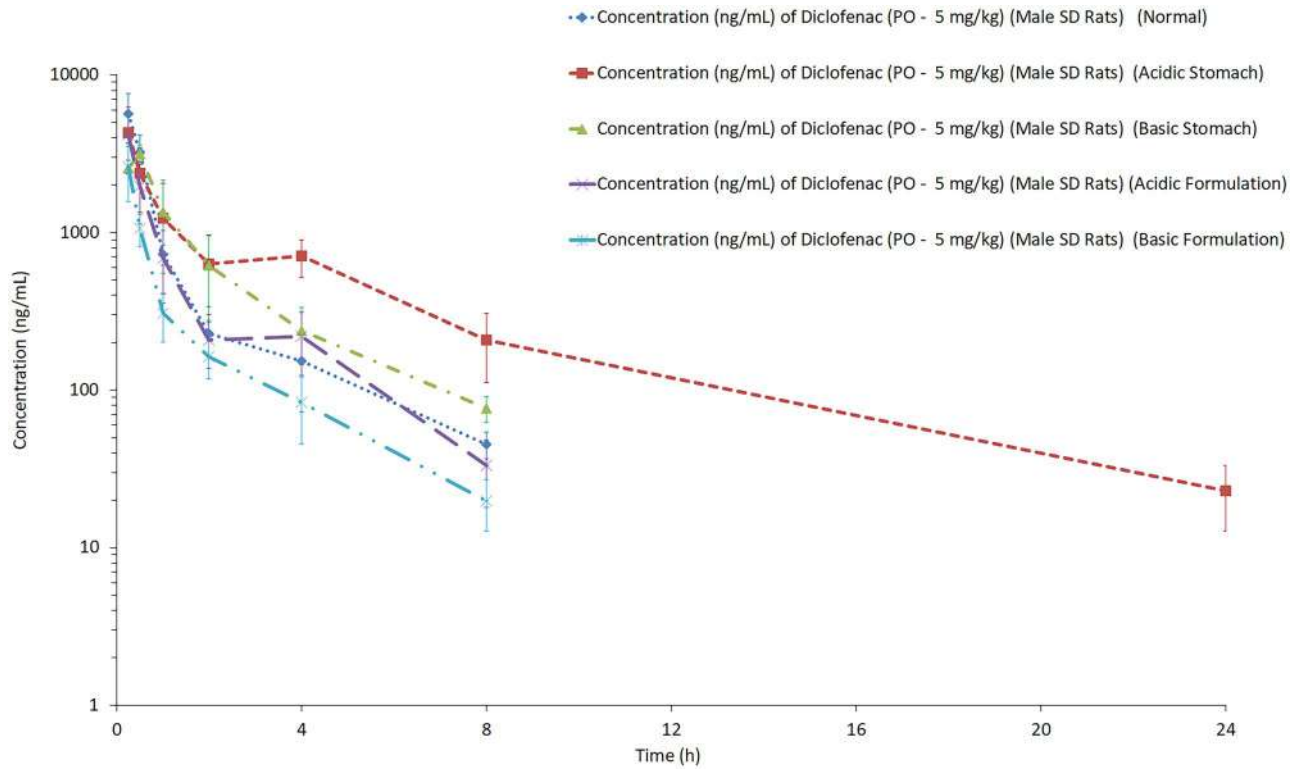

Figure 2. Concentration–time profiles of diclofenac after per oral cassette administration to male Sprague Dawley rats with different formulations at 5 mg/kg. The data presented are mean with S.D. error bars (n = 6) and pharmacokinetic parameters were analyzed by one-way ANOVA followed by Dunnett's multiple range test.

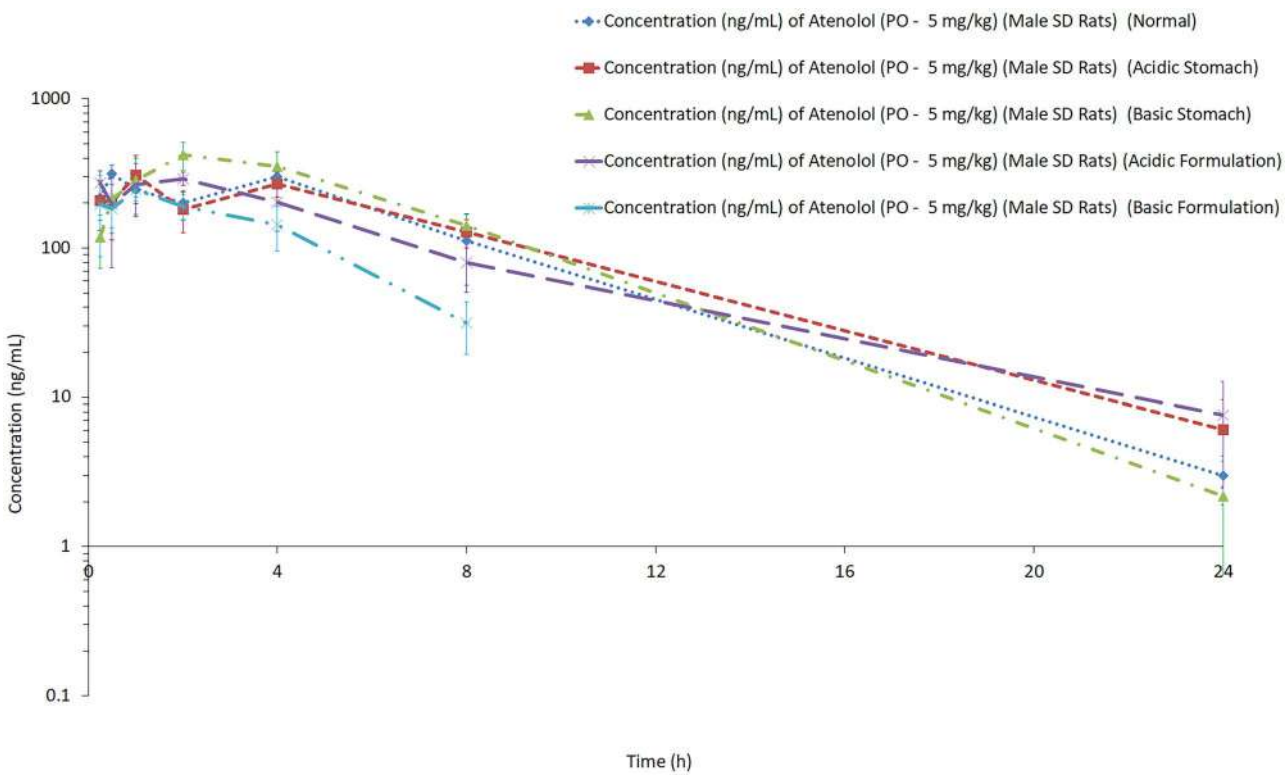

Figure 3. Concentration–time profiles of atenolol after per oral cassette administration to male Sprague Dawley rats with different formulations at 5 mg/kg. The data presented are mean with S.D. error bars (n = 6) and pharmacokinetic parameters were analyzed by one-way ANOVA followed by Dunnett's multiple range test.

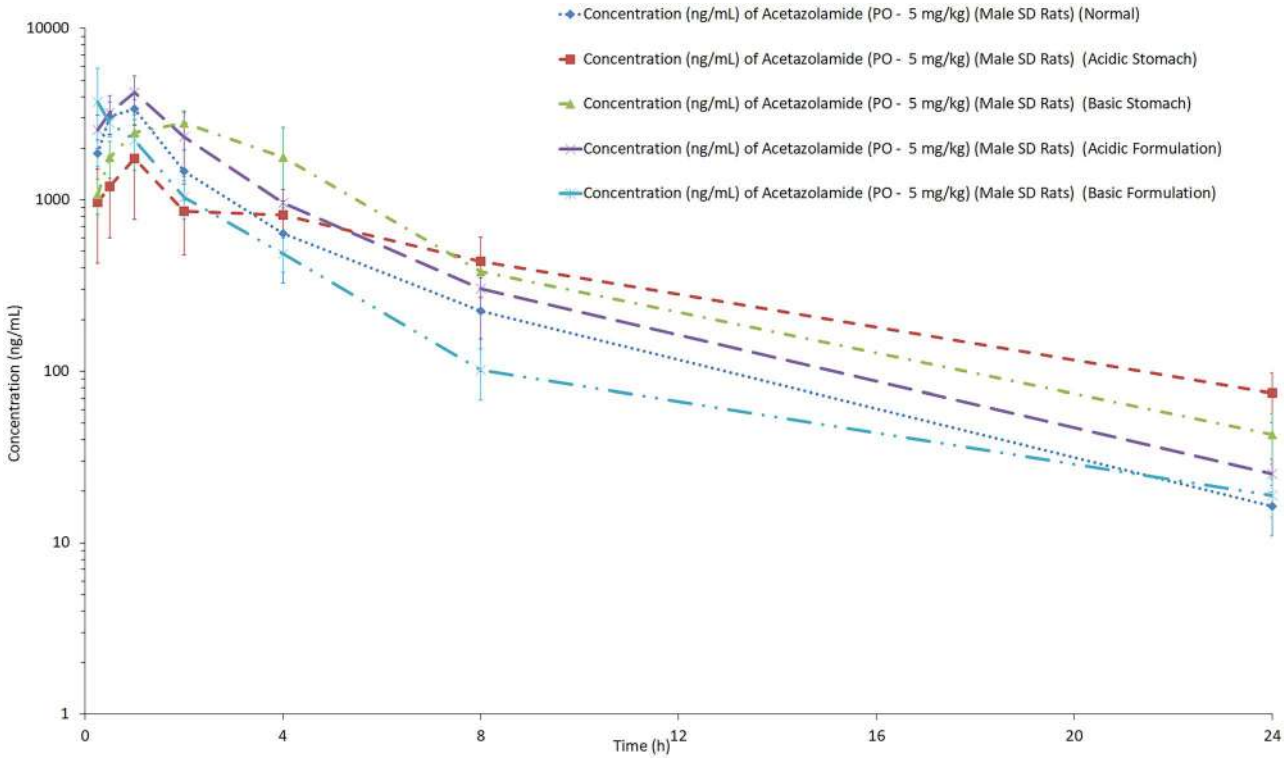

Figure 4. Concentration–time profiles of acetazolamide after peroral cassette administration to male Sprague Dawley rats with different formulations at 5 mg/kg. The data presented are mean with S.D. error bars (n = 6) and pharmacokinetic parameters were analyzed by one-way ANOVA followed by Dunnett's multiple range test.

Table 3. Pharmacokinetic parameters of propranolol after cassette oral administration of propranolol, diclofenac, atenolol, and acetazolamide in different formulations to male Sprague Dawley rats (n = 6) at 5 mg/kg b.wt.

PK parameters	Group 1	Group 2	Group 3	Group 4	Group 5		
	Mean ± S.D.	Mean ± S.D. (% change)	Mean ± S.D. (% change)	Mean ± S.D. (% change)	Mean ± S.D. (% change)	*F* Value	*p* Value
Dose (mg/kg)	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	–	–
C_{max} (ng/mL)	150.41 ± 25.42	224.70 ± 137.02 (49.39)	63.48 ± 14.02 (−57.80)	164.99 ± 53.06 (9.69)	**304.07 ± 127.52[c] (102.15)**	**6.214**	**0.0013**
T_{max} (h)	1.00 ± 0.00	0.88 ± 0.31	1.08 ± 0.49	0.79 ± 0.33	0.25 ± 0.00	–	–
$t_{1/2}$ (h)	1.40 ± 0.36	4.53 ± 2.38	2.87 ± 1.70	1.01 ± 0.41	0.62 ± 0.13	–	–
Vd/*F* (L/kg)	37.22 ± 12.12	40.23 ± 18.34	96.72 ± 48.95	29.18 ± 14.23	15.84 ± 7.27	–	–
Cl/*F* (mL/min/kg)	302.39 ± 34.37	110.55 ± 26.58	426.56 ± 111.65	334.04 ± 89.72	298.95 ± 129.40	–	–
AUC_{0-last} (ng·h/mL)	249.74 ± 49.31	**736.53 ± 174.81[a] (194.91)**	177.56 ± 35.57 (−28.90)	239.84 ± 76.42 (−3.96)	312.58 ± 133.57 (25.16)	**26.21**	**<0.0001**
AUC_{0-inf} (ng·h/mL)	278.42 ± 31.33	**792.97 ± 198.06[a] (184.81)**	207.04 ± 54.46 (−25.64)	267.03 ± 84.43 (−4.09)	320.93 ± 133.83 (15.27)	**24.94**	**<0.0001**
AUCExtra (%)	4.27 ± 4.01	6.82 ± 3.65	12.47 ± 11.17	5.92 ± 3.82	3.19 ± 3.32	–	–
MRT_{0-last} (h)	1.57 ± 0.36	4.71 ± 2.42	3.08 ± 1.43	1.33 ± 0.28	1.00 ± 0.15	–	–
Rsq	0.9496 ± 0.07	0.9267 ± 0.08	0.8776 ± 0.23	0.9616 ± 0.05	0.9488 ± 0.08	–	–
%*F*	24.88 ± 4.91	**73.37 ± 17.41[a] (194.91)**	17.69 ± 3.54 (−28.90)	23.89 ± 7.61 (−3.96)	31.14 ± 13.31 (25.16)	**26.22**	**<0.0001**

C_{max}: maximum concentration; T_{max}: time at which maximum concentration was observed; AUC_{0-last}: area under the curve for all plasma time points; AUC_{0-inf}: area under the curve from time zero extrapolated to infinite time; MRT: mean residence time; Vd_F obs: volume of distribution for free fraction based on the terminal phase; Cl_F_obs: clearance based on free fraction on the terminal phase; %*F*: bioavailability.
All the values are mentioned as mean ± S.D. and analyzed by one-way ANOVA followed by Dunnett's multiple range test.
[a]$p < 0.0001$, [c]$p < 0.05$ compared with negative control group. Bold values denote the values significantly changed in comparison to control group.

Table 4. Pharmacokinetic parameters of diclofenac after cassette oral administration of propranolol, diclofenac, atenolol, and acetazolamide in different formulations to male Sprague Dawley rats (n = 6) at 5 mg/kg b.wt.

	Group 1	Group 2	Group 3	Group 4	Group 5		
PK parameters	Mean ± S.D.	Mean ± S.D. (% change)	Mean ± S.D. (% change)	Mean ± S.D. (% change)	Mean ± S.D. (% change)	*F* Value	*p* Value
Dose (mg/kg)	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	–	–
C_{max} (ng/mL)	5770.76 ± 1836.97	4312.75 ± 1953.91 (−25.27)	**3339.59 ± 273.33[c] (−42.13)**	4187.67 ± 1305.98 (−27.43)	**2624.13 ± 1053.64[c] (−54.53)**	**4.156**	**0.0102**
T_{max} (h)	0.29 ± 0.10	0.25 ± 0.00	0.50 ± 0.27	0.25 ± 0.00	0.25 ± 0.00	–	–
$t_{1/2}$ (h)	3.48 ± 3.49	4.09 ± 0.50	2.71 ± 1.23	1.92 ± 0.44	1.94 ± 0.44	–	–
Vd/*F* (L/kg)	6.39 ± 6.76	4.26 ± 1.40	4.38 ± 2.33	4.63 ± 1.84	8.27 ± 2.62	–	–
Cl/*F* (mL/min/kg)	21.19 ± 3.02	12.01 ± 3.71	18.25 ± 2.10	27.29 ± 5.14	48.93 ± 8.13	–	–
AUC_{0-last} (ng·h/mL)	3751.35 ± 633.22	**7308.33 ± 2059.81[a] (94.82)**	4382.02 ± 547.08 (16.81)	3051.03 ± 646.52 (−18.67)	**1689.13 ± 317.19[c] (−54.97)**	**23.88**	**<0.0001**
AUC_{0-inf} (ng·h/mL)	3999.83 ± 566.06	**7445.28 ± 2096.73[a] (86.14)**	4620.34 ± 553.06 (15.51)	3146.90 ± 628.73 (−21.32)	**1747.80 ± 326.42[c] (−56.30)**	**24.26**	**<0.0001**
AUCExtra (%)	6.30 ± 7.42	1.84 ± 0.79	5.13 ± 3.94	3.21 ± 2.12	3.38 ± 1.76	–	–
MRT_{0-last} (h)	1.25 ± 0.22	4.57 ± 0.81	1.98 ± 0.37	1.58 ± 0.38	1.43 ± 0.24	–	–
Rsq	0.9045 ± 0.13	0.9382 ± 0.07	0.9712 ± 0.03	0.8739 ± 0.12	0.9499 ± 0.05	–	–
%*F*	56.94 ± 9.61	**110.93 ± 31.27[a] (94.82)**	66.51 ± 8.30 (16.81)	46.31 ± 9.81 (−18.67)	**25.64 ± 4.81[c] (−54.97)**	**23.87**	**<0.0001**

C_{max}: maximum concentration; T_{max}: time at which maximum concentration was observed; AUC_{0-last}: area under the curve for all plasma time points; AUC_{0-inf}: area under the curve from time zero extrapolated to infinite time; MRT: mean residence time; Vd_F obs: volume of distribution for free fraction based on the terminal phase; Cl_F_obs: clearance based on free fraction on the terminal phase; %*F*: bioavailability.
All the values are mentioned as mean ± S.D. and analyzed by one-way ANOVA followed by Dunnett's multiple range test.
[a]$p < 0.0001$, [c]$p < 0.05$ compared with negative control group. Bold values denote the values significantly changed in comparison to control group.

(normal animal with basic pH formulation) when compared to group 1 (control group animals) after suspension formulation administration (Table 5, Figure 3).

Acetazolamide – (BCS Class-IV)

A considerable improvement in AUC_{0-last} (55%; n = 6), AUC_{0-inf} (57%; n = 6), and %*F* (55%; n = 6) was noticed in group 3 (alimentary canal with basic conditions) when compared to group 1 (control group animals) after suspension formulation administration. A significant increase in AUC_{0-last} (39%; n = 6), AUC_{0-inf} (39%; n = 6), and %*F* (39%; n = 6) was noticed in group 4 (normal animal with acidic formulation) when compared to group 1 (control group animals) after suspension formulation administration (Table 6, Figure 4).

Table 5. Pharmacokinetic parameters of atenolol after cassette oral administration of propranolol, diclofenac, atenolol, and acetazolamide in different formulations to male Sprague Dawley rats (n = 6) at 5 mg/kg b.wt.

PK parameters	Group 1	Group 2	Group 3	Group 4	Group 5		
	Mean ± S.D.	Mean ± S.D. (% change)	Mean ± S.D. (% change)	Mean ± S.D. (% change)	Mean ± S.D. (% change)	*F* Value	*p* Value
Dose (mg/kg)	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	–	–
C_{max} (ng/mL)	336.69 ± 25.28	338.33 ± 88.08 (0.49)	**435.02 ± 65.72[c] (29.21)**	343.83 ± 56.19 (2.12)	266.67 ± 58.84 (−20.79)	**5.566**	**0.0024**
T_{max} (h)	1.90 ± 1.92	2.10 ± 1.75	2.33 ± 0.82	1.15 ± 0.82	0.75 ± 0.39	–	–
$t_{1/2}$ (h)	5.06 ± 4.04	3.65 ± 0.74	2.71 ± 0.53	4.17 ± 0.91	2.33 ± 0.80	–	–
Vd/*F* (L/kg)	11.92 ± 3.44	11.62 ± 2.90	7.34 ± 2.31	15.07 ± 2.58	15.15 ± 5.70	–	–
Cl/*F* (mL/min/kg)	33.91 ± 12.69	36.64 ± 4.63	30.82 ± 4.09	42.97 ± 10.70	75.53 ± 12.08	–	–
AUC_{0-last} (ng·h/mL)	1989.84 ± 402.92	2269.16 ± 303.73 (14.04)	**2736.73 ± 391.13[c] (37.53)**	1971.57 ± 398.95 (−0.92)	**1010.59 ± 180.25[b] (−49.21)**	**19.96**	**<0.0001**
AUC_{0-inf} (ng·h/mL)	2827.91 ± 1367.10	2304.16 ± 299.28 (−18.52)	2746.25 ± 385.87 (−2.89)	2022.58 ± 423.75 (−28.48)	**1126.24 ± 173.18[b] (−60.17)**	**6.111**	**0.0014**
AUCExtra (%)	15.12 ± 29.04	1.55 ± 1.26	0.38 ± 0.43	2.35 ± 1.92	10.28 ± 7.57	–	–
MRT_{0-last} (h)	4.75 ± 1.03	6.15 ± 0.28	5.23 ± 0.37	5.55 ± 1.05	2.95 ± 0.19	–	–
Rsq	0.9135 ± 0.16	0.9947 ± 0.01	0.9976 ± 0.00	0.9712 ± 0.04	0.9550 ± 0.06	–	–
%*F*	46.33 ± 9.38	52.84 ± 7.07 (14.04)	**63.72 ± 9.11[c] (37.53)**	45.91 ± 9.29 (−0.92)	**23.53 ± 4.20[b] (−49.21)**	**19.96**	**<0.0001**

C_{max}: maximum concentration; T_{max}: time at which maximum concentration was observed; AUC_{0-last}: area under the curve for all plasma time points; AUC_{0-inf}: area under the curve from time zero extrapolated to infinite time; MRT: mean residence time; Vd_F obs: volume of distribution for free fraction based on the terminal phase; Cl_F_obs: clearance based on free fraction on the terminal phase; %*F*: bioavailability.
All the values are mentioned as mean ± S.D. and analyzed by one-way ANOVA followed by Dunnett's multiple range test.
[b]$p < 0.001$, [c]$p < 0.05$ compared with negative control group. Bold values denote the values significantly changed in comparison to control group.

Table 6. Pharmacokinetic parameters of acetazolamide after cassette oral administration of propranolol, diclofenac, atenolol, and acetazolamide in different formulations to male Sprague Dawley rats (n = 6) at 5 mg/kg b.wt.

PK parameters	Group 1	Group 2	Group 3	Group 4	Group 5		
	Mean ± S.D.	Mean ± S.D. (% change)	Mean ± S.D. (% change)	Mean ± S.D. (% change)	Mean ± S.D. (% change)	*F* Value	*p* Value
Dose (mg/kg)	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	–	–
C_{max} (ng/mL)	3595.73 ± 450.31	**1820.90 ± 843.43[c] (−49.36)**	3197.86 ± 480.51 (−11.07)	4311.78 ± 994.99 (19.91)	4064.35 ± 1881.67 (13.03)	**5.081**	**0.0039**
T_{max} (h)	0.70 ± 0.27	1.60 ± 1.34	2.00 ± 1.10	0.83 ± 0.26	0.42 ± 0.30	–	–
$t_{1/2}$ (h)	3.60 ± 0.71	5.90 ± 1.11	4.20 ± 1.04	3.68 ± 0.25	4.10 ± 0.72	–	–
Vd/*F* (L/kg)	2.78 ± 0.86	4.23 ± 1.10	2.14 ± 0.91	2.03 ± 0.28	4.15 ± 1.31	–	–
Cl/*F* (mL/min/kg)	8.82 ± 0.91	8.21 ± 1.12	5.74 ± 1.02	6.40 ± 0.88	11.51 ± 1.67	–	–
AUC_{0-last} (ng·h/mL)	9439.27 ± 951.47	9631.21 ± 1524.92 (2.03)	**14646.08 ± 2795.39[b] (55.16)**	**13093.04 ± 1863.49[c] (38.71)**	7244.68 ± 1007.56 (−23.25)	**17.34**	**<0.0001**
AUC_{0-inf} (ng·h/mL)	9526.88 ± 928.86	10294.53 ± 1384.53 (8.06)	**14921.49 ± 2704.06[b] (56.63)**	**13227.45 ± 1880.00[c] (38.84)**	7358.79 ± 986.87 (−22.76)	**18.62**	**<0.0001**
AUCExtra (%)	0.95 ± 0.57	6.64 ± 3.42	2.00 ± 1.66	1.02 ± 0.22	1.61 ± 0.72	–	–
MRT_{0-last} (h)	3.70 ± 0.48	6.88 ± 1.26	4.83 ± 0.35	3.86 ± 0.62	3.32 ± 0.50	–	–
Rsq	0.9283 ± 0.03	0.9565 ± 0.05	0.9531 ± 0.02	0.9442 ± 0.06	0.8938 ± 0.08	–	–
%*F*	100.76 ± 10.16	102.81 ± 16.28 (2.03)	**156.34 ± 29.84[b] (55.16)**	**139.76 ± 19.89[c] (38.71)**	77.33 ± 10.76 (−23.25)	**17.34**	**<0.0001**

C_{max}: maximum concentration; T_{max}: time at which maximum concentration was observed; AUC_{0-last}: area under the curve for all plasma time points; AUC_{0-inf}: area under the curve from time zero extrapolated to infinite time; MRT: mean residence time; Vd_F obs: volume of distribution for free fraction based on the terminal phase; Cl_F_obs: clearance based on free fraction on the terminal phase; %*F*: bioavailability.
All the values are mentioned as mean ± S.D. and analyzed by one-way ANOVA followed by Dunnett's multiple range test.
[b]$p < 0.001$, [c]$p < 0.05$ compared with negative control group. Bold values denote the values significantly changed in comparison to control group.

In vitro drug release results

A significantly high release of propranolol AUC_{0-last} (ng·h/mL) (acidic conditions = 732692.94; basic conditions 543784.65, 1.35-fold higher in acidic condition) was observed in acidic pH 3. Whereas diclofenac AUC_{0-last} (ng·h/mL) was more in basic condition (acidic conditions = 323321.7; basic conditions 1034753.79, 3.2-fold higher in basic condition). There was no change in AUC_{0-last} (ng·h/mL) observed for atenolol (acidic conditions = 1471885.8; basic conditions 1464959.67, 1.0 fold) under the *in vitro* conditions between acidic and basic conditions. Again, a significantly high release of acetazolamide AUC_{0-last} (ng·h/mL) (acidic conditions = 2132119.34; basic conditions 1759536.56, 1.35-fold higher in acidic conditions) was observed at acidic pH 3 (Table 7).

Table 7. A comparison table of drug release data under *in vitro* conditions.

Drug	Propranolol		Diclofenac		Atenolol		Acetazolamide	
Condition	Acidic	Basic	Acidic	Basic	Acidic	Basic	Acidic	Basic
AUC_{0-last} (ng·h/mL)	732692.94	543784.65	323321.70	1034753.79	1471885.80	1464959.67	2132119.35	1759536.56

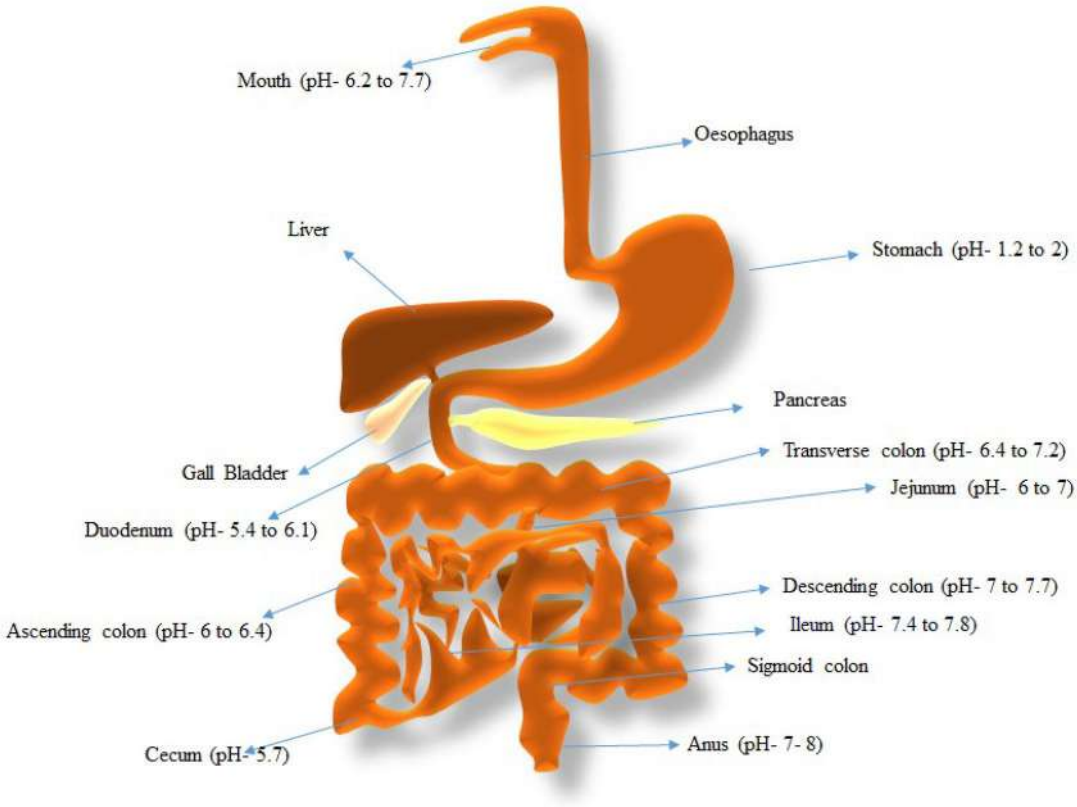

Figure 5. Pictorial depiction of the alimentary canal and pH in different regions.

Discussion

The study was executed to appraise the alteration of PKs parameters of propranolol HCl, diclofenac sodium, atenolol, and acetazolamide under different formulation pH and GI pH conditions. As it may have a sizable impact on drug dissolution and solubility, drug release, drug stability, and intestinal permeability, GI pH is a critical component that can significantly alter oral drug absorption and bioavailability. Sometimes pH of GIT could change even because of formulation excipients. To avoid the change in GI because of formulation, the most chemically inert and safe suspension formulation was used which is easy to use clinically. Again, the same formulation is used for control group to avoid group to group variation. GIT exhibits region-specific pH (pH 6.2–7.7 – buccal cavity) [22], pH 1.2–2 – stomach [23], pH 5.7 – starts with the duodenum, and increases to pH 7.4 in the small intestine and again decreases to 5.7 in cecum [24] (Figure 5). The pancreatic juice and bicarbonates neutralize the stomach acid in the duodenum, raising the pH in the small intestine [25]. The properties of all four drugs (propranolol, diclofenac sodium, atenolol, and acetazolamide) studied were presented in Table 1.

Propranolol HCl

AUC_{0-last} increases with acidic pH conditions in the alimentary canal and C_{max} significantly increases with formulation with basic pH. This observation could be due to the change in the drug form from free base to HCl salt and the increase in the stomach pH augmented the plasma exposure for propranolol HCl. Propranolol is such a weak base whereas the propranolol HCl is weak acid, which leads to increased exposure in acidic alimentary canal conditions. A drug's lipid solubility, degree of ionization, and molecule size and shape all have a significant impact on passive diffusion absorption [20]. The change in degree of ionization because of change in drug form from free base to salt and change in alimentary pH is the main reason for drug exposure in case of propranolol HCl [16].

Diclofenac sodium

AUC_{0-last} increases with acidic pH in the alimentary canal. The C_{max} decreases with the basic formulation. Increased acid secretion in the alimentary canal led to increased exposure to diclofenac sodium. %*F* is twice as compared to normal control group animals. Also, an increase in $t_{1/2}$ was observed. Exposure decreased by twofold in basic formulation, which could be due to the precipitation of the drug at the absorption site. The effect on the PKs of diclofenac has been reported for pantoprazole, wherein there is no or very little cytochrome P450 (CYP) interaction. However, omeprazole has considerable CYP interaction with diclofenac. As the reported absorption site for diclofenac sodium is small intestine, we are getting higher exposure after 2 h timepoint when drug crossed stomach and reached small intestine in slightly basic environment [17,18].

Atenolol

AUC_{0-last} of atenolol increases with basic pH in the alimentary canal and decreases if the formulation pH is basic. Atenolol is a weak base and weak basic drugs show better absorption in basic pH, which is there in the intestine. Yet more increase in pH toward basic, increased the plasma exposure by 40%. An increase of the formulation pH toward basic pH could have precipitated the drug in stomach pH before reaching the intestine, which decreased the plasma exposure by 60% [19].

Acetazolamide

Basic pH in the alimentary canal increases the AUC_{0-last} of acetazolamide and AUC_{0-last} significantly increases ($p < 0.05$) with a formulation having acidic pH. pKa for acetazolamide is between weak acid and weak base. pH of the compound has been changed externally by making salt with HCl. Literature suggests that the drug is a weak acid but increased exposure to the basic alimentary canal indicates that the compound is weakly basic. Acidic formulation helped the drug to sustain the stomach pH and as an absorption site in the stomach and small intestine, the drug got relatively better absorbed in the intestine. Although acetazolamide is class 4 compound, it is reported to have 60–100% bioavailability already by Alex Yartsev (https://derangedphysiology.com/main/cicm-primary-exam/renal-system/Chapter-023/acetazolamide) [26,27].

The PKs of a drug can vary depending on its pKa because various parts of the body ionize and deionize drugs differently. Drugs that are not ionized pass through membranes more readily than charged species. Weakly acidic pharmaceuticals are unionized at the acidic pH of the stomach and are thus preferentially absorbed from the gastric area because drug ionization relies on the pH of the location. To cross a membrane barrier, the drug substance needed to be soluble in the lipid composition of the membrane for entry and soluble in the aqueous phase for departure that depends most of the time on the pH of the formulation and pH of the alimentary canal. Some of the compounds are acidic and basic pH sensitive and get precipitated above solubilizing pH which in turn leads to compromised PKs. Propranolol HCl can be absorbed along the whole intestine till the colon [16] and is a substrate for Pgp [28]. Diclofenac is reported to undergo enterohepatic circulation [29] and also causes interaction at the Pgp level [30,31]. All the CYP based and Pgp based interactions can be considered to be normalized as the four drugs were administered in all the groups. A single formulation was prepared for all five groups so accuracy based changes also stand nullified.

While a pH above the pKa increases absorption for bases, a pH below the pKa increases absorption for acids. The biopharmaceutical categorization system demonstrated that the two main parameters that control drug absorption after oral administration are the drug's solubility/dissolution and its intestinal permeability. The pKa suggests that both basic and acidic drugs may become more soluble as the luminal pH rises in more distal areas of the small intestine. Drug solubility in the GI environment may vary in various intestine segments, for instance, as a result of pH variations, in a relatively predictable manner. While basic drugs are better absorbed in the intestine, where the pH is greater, acidic drugs will be more readily absorbed in the extremely acidic stomach. The four BCS classes draw attention to the constraints on the absorption procedure. Apart from the acid release by histamine, it also plays a role in other physiological changes like vasodilatation and bronchoconstriction. Lowering of blood pressure because of vasodilation could be another reason for extended exposure to a few drugs [32]. All parts of the body, including blood vessels, smooth muscle cells in the airways, and neurons, have H1 receptors. H2 receptors are mostly located in the heart, smooth muscle cells, and parietal cells of the stomach mucosa. H2 receptor activation promotes flushing, headache, tachycardia, bronchoconstriction, vascular permeability, hypotension, and gastric acid secretion. Given their limited quantifiable impact on airway functions, the clinical significance of H2 receptors is not well established. Increased adenylate cyclase system activity can result from H2 receptor activation, raising intracellular cyclic AMP. Duodenal ulcers can be treated with H2 antagonists, which also stop them from coming back. Histaminergic neurons, which regulate the release

of histamine, dopamine, serotonin, noradrenaline, and acetylcholine in the central nervous system, are mostly home to H3 receptors. Both peripheral hematopoietic cells and bone marrow include H4 receptors. Histamine can affect PGP expression and its capacity to efflux medications across cell membranes via its receptors (H1, H2, H3, and H4) [33,34].

Our *in vitro* and *in vivo* data demonstrate that *in vitro* release profiles are not necessarily sufficient for establishing a robust *in vitro–in vivo* correlation (IVIVC). This highlights the critical need for a reliable *in vivo* PK method capable of evaluating compound properties directly within varying *in vivo* acidic and basic conditions, thereby overcoming the limitations of *in vitro* dissolution assays and improving the predictability of preclinical models. Specifically, while *in vitro* studies showed that diclofenac had a 3.2-fold higher AUC_{0-last} in basic conditions, the *in vivo* study revealed a significantly higher systemic exposure under acidic conditions. A similar reversal was observed for acetazolamide: the *in vitro* assay indicated a 1.23-fold higher AUC_{0-last} in acidic conditions, but the *in vivo* data showed greater exposure in basic conditions. Furthermore, atenolol exhibited no change in AUC_{0-last} *in vitro*, yet an increase in AUC_{0-last} was observed during the *in vivo* study at basic pH.

Conclusion

A systematic investigation was carried out to assess the PKs of four different BCS class drugs viz. propranolol HCl, diclofenac, atenolol, and acetazolamide (BCS-I, II, III, and IV, respectively) in different formulation pH conditions and abdominal pH conditions via cassette dosing in male SD rats. The data observed in this investigation imply that the PKs of the four different BCS class chemicals investigated are significantly influenced by both the pH conditions of the alimentary canal and the formulations. The observed effect generally depends on the pKa, salt form and area of absorption in the alimentary canal for the drug. Further investigation in humans is recommended to support this claim.

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Ethical approval

This article does not contain any studies with human subjects. Animal studies are performed under the protocol approved by IAEC of Aragen Life Sciences Limited, protocol number B-011.

Author contributions

CRediT: **Satish Kumar**: Conceptualization, Data curation, Investigation, Writing – original draft; **Lakshmi Prasanna Tattala**: Investigation; **Ravi Akkireddy**: Investigation; **Satinder Singh**: Supervision, Writing – review & editing; **Sudhir Kumar Tiwari**: Software, Writing – review & editing; **Pratima Srivastava**: Conceptualization, Supervision, Validation, Writing – review & editing; **Sumeet Gupta**: Conceptualization, Writing – review & editing; **Anroop Nair**: Software, Validation, Writing – review & editing.

Data availability statement

Data will be available on the basis of request.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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