



Original Article

***In vitro*–*in vivo* studies of the quantitative effect of calcium, multivitamins and milk on single dose ciprofloxacin bioavailability** [☆]Baishakhi Dey ^a, Prakash Katakam ^b, Fathi H. Assaleh ^b, Babu Rao Chandu ^b, Shanta Kumari Adiki ^c, Analava Mitra ^{a,*}^a School of Medical Science and Technology, IIT Kharagpur, Kharagpur 721302, India^b Faculty of Pharmacy, University of Zawia, Az Zawiyah, Libya^c Department of Pharmaceutical Analysis, Nirmala College of Pharmacy, Guntur, India

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ABSTRACT

Ciprofloxacin, commonly used in India as an anti-microbial for prolonged use in chronic and non-specific indications, may affect the bioavailability of the drug. The drug prescribed is commonly taken with multivitamins, calcium and milk. A simple and reliable analytical methodology obtaining a correlation with *in vivo* urinary excretion studies using UV and HPLC and *in vitro* dissolution studies (IVIVC) has shown a significant increase in elimination rate of ciprofloxacin co-administered with multivitamins, calcium and milk. Appreciable IVIVC results proved that dissolution studies could serve as an alternative to *in vivo* bioavailability and also support bio-waivers.

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1. Introduction

Development of antimicrobial resistance is a challenging problem in world health scenario, despite the increasing demand for antimicrobials in global pharmaceutical market worths a current value of about \$66.5 billions [1]. However, antibiotics are one of the most widely used and frequently misused drugs. Due to wide predominance of infectious diseases and emergence of resistant strains, there is an increased inclination among clinicians to prefer newer-generation antibiotics as one of the first-line therapeutic regimens. However, prolonged antibiotic therapy may give rise to some side effects like anemia, dyspepsia, hyperacidity, gastritis, hepatotoxicity and nephrotoxicity, which necessitate co-mediations. Such co-mediations include common antacids or multivitamins (MVs) which are either prescribed by physicians or self-advised and that may adversely affect the bioavailability of the antibiotic prescribed. Thus, the anti-microbial effect of the drug is compromised, leading to prolonged consumption of the antibiotic and development of antimicrobial resistance against that particular antibiotic [2–4].

Fluoroquinolones administered orally have been a preferred choice for clinicians in treatment of grave infections for decades. Ciprofloxacin (CPFX) is a fluoroquinolone carboxylic acid derivative and broad-spectrum antibiotic active against Gram-positive and Gram-negative organisms. Pharmacokinetically, CPFX undergoes renal and hepatic bio-degradative pathways. After 24 h of administration, approximately 50% of oral dosage or 80% of intravenous dosage appears in the urine either as parent compounds or as metabolites. Renal excretion of CPFX is nearly thrice the glomerular filtration rate, highlighting the role of tubular secretion and anionic transport mechanism in the elimination of this carboxyfluoroquinolone. As a popular oral antibiotic with rapid onset of action, it is used in treatment of numerous infectious diseases afflicting the skin, bones, gastrointestinal tract (GI-tract), genitourinary tract, and meninges. Mechanistically, CPFX acts by effective inhibition of DNA gyrase preventing DNA replication in susceptible bacteria [5]. Patients under prolonged CPFX therapy are reported to suffer from hyperacidity, nausea, vomiting, gastritis, pain in the abdomen, and anemia and hence they consume antacids often without medical supervision. Calcium or magnesium containing antacids is more popular among the consumers than the H₂ antagonists or proton pump inhibitors. Calcium carbonate tablets or supplements are widely consumed by patients with osteoporosis or bone-related disorders for therapeutic purpose. Similar is the case with intake of calcium-rich foods like milk

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or yogurt providing dietary supplementation of osteo-calcium. Anemia is another side effect with the sustained use of CPFV and the preventive measure goes to prescribing MVs. Sometimes, secondary disease conditions also necessitate the rational use of MVs along with CPFV. MVs affect the bioavailability of the drug, leading to further research to make it evidence based [2–8]. Indians are mainly lacto-vegetarian and use milk as a culturally acceptable measure for various conditions of GI tract disorders and anemia.

The current research work aims at detecting the quantitative effect of calcium (Ca), MVs and milk on the elimination of single-dose CPFV in the urine of human volunteers by UV and HPLC. Further research will be extended to see CPFV interactions with Ca, milk and MVs by *in vitro* dissolution studies and establish *in vitro*–*in vivo* correlation (IVIVC) which is a predictive mathematical treatment describing the relationship between an *in vitro* property of a dosage form (e.g. extent of drug release) and a relevant *in vivo* response (e.g. amount of drug absorbed) [9–11]. In ciprofloxacin assessment, urinary samples were preferred to blood samples considering volunteer's compliance, convenient methodology, concentration of drugs in the urine being higher than in the blood, detection that can be made by simple analytical methods of UV, lack of protein in the urine of a healthy volunteer obviating the need for denaturation step, and no extra sample pretreatment (the only caution is to look after urine dilutions) [12–16]. A novel applicational aspect of the current research was to establish IVIVC using dissolution test as a surrogate for *in vivo* CPFV interactions and bioavailability studies, which can waive the need for expensive human trials and hence is a suitable substitute for bioequivalence studies [9–11,15,16].

2. Materials and methods

2.1. Chemicals and reagents

All solvents used were of HPLC grade and reagents were of analytical grade. HPLC grade water was used. Reagents as specified like phosphoric acid, triethylamine and acetonitrile were purchased from Merck and Sigma (Mumbai, India). CPFV was a gift sample from M/s East India Pharmaceuticals Ltd., Kolkata and Lomefloxacin (internal standard, IS) from Macleods (Mumbai, India). CPFV film coated tablets (500 mg), calcium tablets (500 mg) and multivitamin syrup (Polybion[®]) were purchased from the local market. Packed fresh cow milk was obtained from local dairy. Each 5 mL of Polybion syrup contained vitamin B₁, 2 mg; vitamin B₂, 2.5 mg; vitamin B₆, 0.75 mg; nicotinamide, 15 mg; pantothenyl alcohol, 3 mg; and vitamin-B₁₂, 2 mg. The syrup specification of MV content was 16.03 for 100 mL, 34.28 for 250 mL, and 52.27 for 400 mL.

2.2. Instrumentation

UV spectrophotometric determinations were done in Thermo Scientific (Mumbai, India). HPLC analyses were carried out in Shimadzu LC 10 ADVp (Japan) with UV detector, and dissolution studies were performed using USP Type II dissolution test apparatus (Labindia DS 8000, Mumbai, India).

2.3. Volunteer selection and urine collection

The study protocol followed the ethical principles for biomedical research involving human volunteers (ICMR 2009). Healthy adult human volunteers (four males and females each) of 25–35 years were selected (lottery) from a pool of 25 volunteers residing locally with the body weight of 65–78 kg. Study time was

winter season. Volunteers were clinically assessed by physicians of School of Medical Science and Technology, IIT Kharagpur. Anthropometric status, blood biochemistry and hematology values were within the normal limits in all these subjects. Smokers, alcoholics, those with enzyme deficiencies, particularly renal and hepatic insufficiencies, children, pregnant and lactating women, non-cooperative and inconsistent individuals were excluded from the study. Written consents of eight volunteers were obtained after explaining them the research protocol in vernacular. The volunteers were asked to maintain standard dietary conditions (avoiding high caloric and junk food), definite water (1 L) intake, normal physical activities, and avoidance of strenuous exercises and work overloads in the ensuing days of study.

The volunteers were given one CPFV tablet (500 mg) on the first day; one CPFV tablet (500 mg) and vitamin B-complex (Polybion, 15 mL) on the third day (out of four days of trial, a gap of one day was kept between each day of trial so as to ensure complete renal clearance of CPFV); one CPFV tablet (500 mg) and calcium tablet (Calbon 500, Saga Lab; calcium carbonate 1.25 g) on the fifth day; and one CPFV tablet (500 mg) and 250 mL of cow-milk immediately after tablet intake on the seventh day and no other food or drink was ingested for the next 6 h on the seventh day. Midstream urine samples of the eight volunteers were collected in sterilized vials properly labeled on four days of drug intake maintaining the same time intervals (2, 4, 8, 12, and 16 h), stored in refrigerator and protected from light.

In order to get reproducible urinary excretion studies, water loading was done by ingesting 400 mL of water to the volunteers after overnight fasting prior to the trial to promote diuresis and enable collection of sufficient urine samples [12]. Before drug administration, the volunteers were instructed to ensure complete urination; 1 h after water loading, the urine sample collected at this time was taken as the blank sample. The volunteers were instructed to take CPFV with 200 mL of water, followed by another 200 mL given at hour intervals for the next 4 h. The same procedures were used for the four days of drug intake of trials and the volunteers were instructed for complete emptying of the bladders to collect sufficient urine samples [12,15].

2.4. UV spectrophotometric analysis of urine samples

The UV analysis of the urine samples collected from the volunteers at different time intervals was carried out in the following manner. Urine samples (0.4 mL) collected at different time intervals were diluted with 20 mL of methanol: water (80:20, v/v). The samples were scanned using the same diluents (methanol: water, 80:20, v/v) as blank and the wavelength range was fixed between 200 and 400 nm [16–21].

For the quantitative estimation of CPFV release under different study conditions, 0.4 mL of urine was diluted to 20 mL. The volume of urine collected in each case was 50 mL. UV absorbance values of 0.4 mL of diluted urine samples at different time intervals (2, 4, 8, 12, and 16 h) were plotted in the standard calibration curve of CPFV and intrapolated to get the corresponding concentration of CPFV. Thus, the amount of CPFV present in 50 mL of urine samples was calculated by unitary method.

2.5. HPLC analysis of the urine samples

HPLC analysis was carried out using inertsil ODS C₁₈ column, (150mm × 4.6mm, 5 μm) maintaining a column temperature of 20 °C, injection volume of 10 μL with a flow rate of 1.2 mL/min, and a run time of 10 min. The UV detector was set at a wavelength of 278 nm.

A 7 mL of triethylamine was diluted in 1000 mL of HPLC grade water and then adjusted to a pH of 3.0 with phosphoric acid, well

mixed and degassed. The mobile phase consisted of a filtered and degassed mixture of 0.025 M H₃PO₄ (adjusted to a pH of 3.0 with triethylamine) and acetonitrile in the ratio of 87:13 (v/v).

In HPLC studies, quantitative estimation of CPF_X release under different study conditions was determined according to the following equation:

$$\% \text{ assay} = \frac{A_t \times W_s \times D_t \times P \times A_w}{A_s \times W_t \times D_s} \times 100$$

where A_t is the average area of major peak in test chromatogram, A_s is the average area of major peak in standard CPF_X chromatogram, W_t is the wt of test taken in mg (to get from HPLC calibration curve), W_s is the wt of std CPF_X taken in mg (11/30/60 mg), P is the purity of working standard (99.5%), D_t is the dilution of test samples (50 times diluted), D_s is the dilution of standard solution, and A_w is the average wt of tablet (500 mg).

2.6. Dissolution studies

Absorption and hence bioavailability of the drug greatly depend on the dissolved state of the drug. Therefore, dissolution rate is a critical step in the performance of a drug and its dosage forms. This step of a drug is found to be affected by co-administered drugs and other food ingredients [9,10,14,22–26].

In vitro CPF_X interaction with calcium, MVs and milk was studied in USP Type II dissolution test apparatus with certain modifications of the reported literature [24,27–31]. Briefly, dissolution study of CPF_X tablets was carried out in 500 mL of dissolution media under simulated gastric and intestinal pH conditions (pH 1.2 and 6.8, respectively), thermostated at 37 ± 5 °C and operated at 100 rpm. 5 mL of aliquots of dissolution media were withdrawn at predetermined time intervals (5, 15, 30, 45, 60, 90 and 120 min) and their absorbance was measured at 276 nm in UV spectrophotometer.

For testing the action of calcium on the dissolution behavior of CPF_X, calcium tablet was added to the dissolution medium with an appropriate concentration of CPF_X at the start of the experiment while other conditions remained the same. Aliquots of the dissolution medium were withdrawn and CPF_X concentrations were determined spectrophotometrically at 276 nm.

In vitro milk–CPF_X interaction studies were conducted in 500 mL of dissolution media, and other conditions remained the same as above. One FDA glass (250 mL) of low fat milk was added to the dissolution media with an appropriate concentration of CPF_X at the start of the experiment; CPF_X concentrations were determined spectrophotometrically at 276 nm.

In vitro MVs–CPF_X interaction studies were conducted in 500 mL of dissolution media, and other conditions remained as before. A 55 mL of Polybion syrup was added to the dissolution media with an appropriate concentration of CPF_X at the start of the experiment; CPF_X concentrations were determined spectrophotometrically at 276 nm. In all the above *in vitro* dissolution studies, the aliquots withdrawn were not replenished with fresh equivalent amount of dissolution media.

Cumulative percentage of CPF_X release profiles was characterized by the area under the dissolution curves (AUC) up to a time 't' in simulated gastric and intestinal fluids (SGF and SIF) under different study conditions. The AUC for each dissolution profile was calculated by the trapezoidal rule [32–34].

2.7. Comparison of dissolution data

The dissolution profiles were further analyzed by difference factor (f_1) and similarity factor (f_2). Difference factor (f_1) is the percentage difference between two curves at each point and is a

measurement of the relative error between the two curves. The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent dissolution between the two curves [35–37]. The following equations were used to calculate f_1 and f_2 values:

$$f_1 = \left\{ \frac{[\sum_{t=1}^n |R_t - T_t|]}{[\sum_{t=1}^n R_t]} \right\} \times 100$$

$$f_2 = 50X \log \{ [1 + (1/n) \sum_{t=1}^n (R_t - T_t)^2]^{-0.5} \times 100 \}$$

where n is the number of points, R_t is the dissolution value of the reference product at time t , and T_t is the dissolution value for the test product at time t . For dissolution curves to be considered similar, f_1 should be close to zero and f_2 should be close to 100. Generally, f_1 value ranges up to 15 (0–15) and f_2 values are greater than 50 (50–100), which ensures equivalence between the two curves.

In this research, CPF_X was used as the standard substance and CPF_X+Ca, CPF_X+MV_s and CPF_X+milk were used as the reference substances. Comparison was made for both *in vitro* SIF and SGF conditions using f_2 values. The more the deviation from the standard value, the greater the accuracy of the experimental methodology.

2.8. IVIVC studies

IVIVC is a predictive mathematical treatment describing the relationship between *in vitro* properties of a dosage form (e.g. extent of drug release) and a relevant *in vivo* response (plasma drug concentration or amount of drug absorbed). IVIVC has drawn the attention and is worthy of consideration by pharmaceutical industries, academics and regulatory sectors. If *in vitro* tests can suitably reflect bioavailability data, it is helpful to waive bioequivalence studies on healthy volunteers. Among the three main levels (A, B and C) of IVIVC, level A (the highest grade) relates the entire *in vitro* dissolution curve to *in vivo* concentration profile (*in vivo* absorption curve) so that *in vitro* dissolution can serve as a surrogate marker for *in vivo* drug actions study; level B of IVIVC utilizes Statistical Moments Analysis to compare the mean of any parameter of *in vitro* dissolution (e.g. mean dissolution time, $MDT_{in vitro}$) with the mean of any *in vivo* parameter (e.g. mean residence time, $MRT_{in vivo}$); level C of IVIVC establishes a single point correlation between an *in vitro* dissolution parameter (e.g. time to release 50% or 90% of the active drug, $T_{50\%}$ or $T_{90\%}$) and corresponding *in vivo* parameter (e.g. C_{max} , T_{max} or AUC) [9–11,32,38–41].

In this study, IVIVC was determined by plotting the percentage of CPF_X released (CPF_{X,rel}) *in vitro* (from dissolution studies) against the cumulative percentage of CPF_X excreted in urine for a sufficient period of time which is directly related to the total amount of CPF_X absorbed.

3. Results

The mean (±SD) values of various parameters of the eight volunteers were determined as follows: age, 27.3 ± 4.16 years; weight, 69.5 ± 8.01 kg; height, 170.3 ± 6.45 cm; body surface area (BSA), 1.7 ± 6.95 m²; systolic blood pressure, 123 ± 0.82 mmHg; and diastolic blood pressure, 80 ± 0.91 mmHg. Linearity details of the standard calibration curves of CPF_X for UV, HPLC and dissolution studies are presented in Table 1. The quantitative elimination of CPF_X at hourly intervals (2, 4, 8, 12, and 16 h) was determined from the standard calibration curve of UV and HPLC studies and is shown in Figs. 1–3. The peak areas (±SD) values of

CPFX eliminated at hourly intervals in the urine of eight volunteers as detected in HPLC analysis are provided in Table 2. The pharmacokinetic parameters determined from the urinary excretion data obtained from UV and HPLC studies are summarized in

Table 1
Data of calibration curve of CPFX for UV, HPLC and dissolution studies.

Parameters	UV studies	HPLC studies	Dissolution studies
95% confidence intervals			
Slope	0.105–0.108	12.82–13.78	0.141–0.163
Y-intercept	–0.0013–0.0109	–11.84–22.54	–0.0406–0.0468
X-intercept	–0.103–0.0125	–1.748–0.864	–0.330–0.250
Goodness of fit			
R ²	0.999	0.999	0.997
P value	< 0.0001	< 0.0001	< 0.0001
Equation	Y=0.1072X+0.004	Y=13.30X+5.348	Y=0.1522X+0.003
Best fit values			
Slope (± SD)	0.1072 ± 0.0004	13.30 ± 0.18	0.1522 ± 0.004
Y-intercept (± SD)	0.004785 ± 0.002	5.348 ± 6.68	0.003133 ± 0.015
X-intercept	–0.0446	–0.402	–0.0205

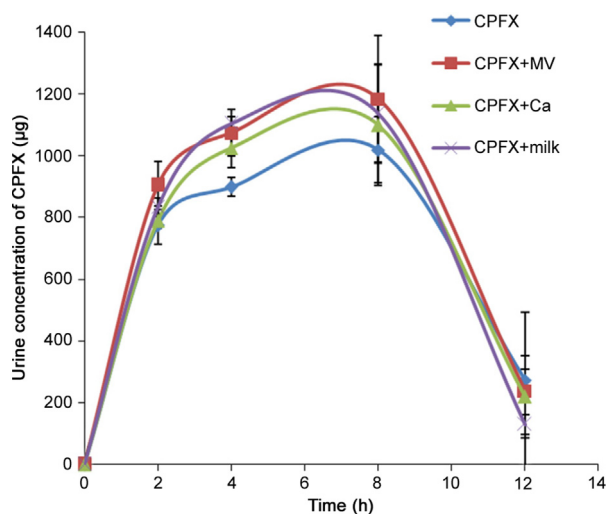


Fig. 1. Quantitative elimination of CPFX at hourly intervals under different study conditions (UV studies).

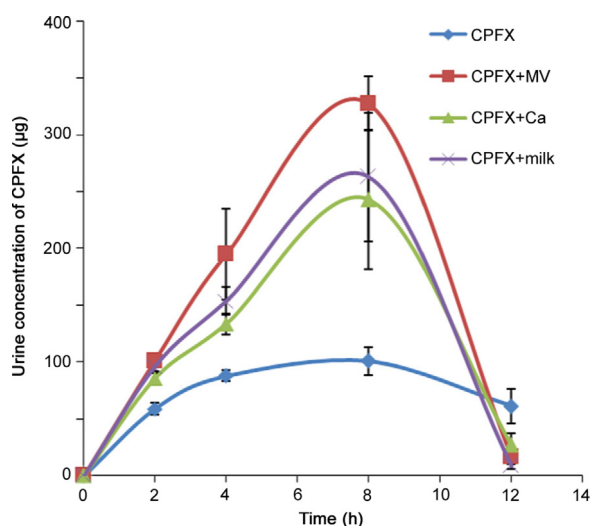


Fig. 2. Quantitative elimination of CPFX at hourly intervals under different study conditions (HPLC studies).

Tables 3 and 4. The *in vitro* cumulative CPFX elimination in SGF and SIF under different study conditions is shown in Tables 5 and 6 and Suppl. Figs. 1 and 2. Comparisons of dissolution profiles under different study conditions both in SGF and SIF were made based on

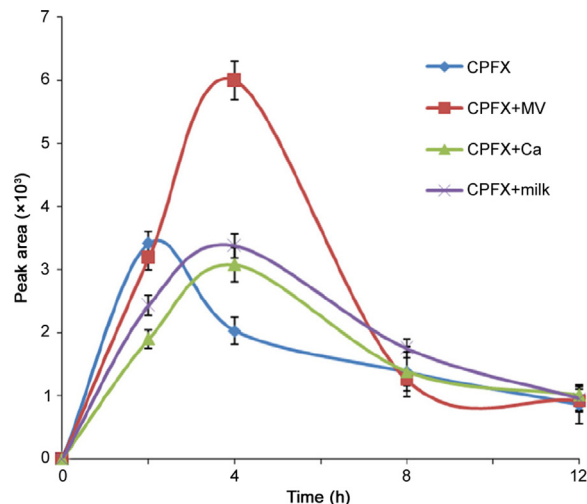


Fig. 3. Cumulative elimination of CPFX under different study conditions (HPLC studies).

Table 2
Peak area values of CPFX eliminated in the urine of the 8 volunteers in HPLC studies under different conditions.

Time (h)	CPFX alone	CPFX + MVs	CPFX + calcium	CPFX + milk
2	3411 ± 185	3184 ± 187	1893 ± 153	2430 ± 157
4	2027 ± 210	5990 ± 300	3073 ± 262	3372 ± 190
8	1374 ± 391	1254 ± 95	1382 ± 310	1746 ± 146
12	857 ± 299	923 ± 177	1009 ± 154	954 ± 187

CPFX, ciprofloxacin; MVs, multivitamins.

Table 3
Pharmacokinetic parameters of urinary excretion data by UV.

Pharmacokinetic parameters	CPFX (alone)	CPFX + MVs	CPFX + Ca	CPFX + milk
AUC _{0-t} (µg/mL h)	8903.92	10,238.64	9490.73	9742.78
AUC _{0-∞} (µg/mL h)	11,702.83	13,121.11	12,237.17	12,196.97
t _{1/2} (h)	3.203	3.101	3.118	3.067
k _a (1/h)	0.2440	0.2568	0.2529	0.2768
Cl/F (mg)/((µg/mL)/h)	0.04272	0.03810	0.04085	0.04099
C _{max} (µg/mL)	988.21	1154.62	1065.87	1118.50
T _{max} (h)	4.348	4.170	4.214	3.991
AUMC (µg/mL h ²)	102,029.75	109,795.28	103,441.46	98,026.88
MRT (h)	8.718	8.367	8.453	8.037

Table 4
Pharmacokinetic parameters of urinary excretion data by HPLC.

Pharmacokinetic values	CPFX (alone)	CPFX + MVs	CPFX + Ca	CPFX + milk
AUC _{0-t} (µg/mL h)	924.87	1897.50	1527.87	1515.68
AUC _{0-∞} (µg/mL h)	1531.31	7204.61	2342.38	1515.68
t _{1/2} (h)	4.212	26.584	4.245	0.327
k _a (1/h)	0.175	3.632	0.214	4.235
Cl/F (mg)/((µg/mL)/h)	0.3265	0.0694	0.2134	0.3298
C _{max} (µg/mL)	95.64	181.26	160.12	1603.94
T _{max} (h)	5.887	1.368	5.332	0.327
AUMC (µg/mL h ²)	18,042.98	278,306.31	25,287.54	1074.26
MRT (h)	11.782	38.628	10.795	0.709

Table 5
In vitro data of CPFX release in SGF (dissolution studies).

Time (min)	CPF ^a	CPF ^a +Ca ^b	CPF ^a +milk	CPF ^a +MV ^c
0	0	0	0	0
5	26.80	8.14	5.7	8.1
15	38.20	36.54	12.8	10.9
30	50.45	40.91	15.4	16.7
45	66.50	42.15	16.9	22.9
60	70.23	42.91	25.3	29.4
90	82.45	45.01	28.1	32.1
120	98.01	47.24	30.9	35.5

^a CPF^a, ciprofloxacin.

^b Ca, calcium.

^c MVs, multivitamins.

Table 6
In vitro data of CPFX release in SIF (dissolution studies).

Time (min)	CPF ^a	CPF ^a +Ca ^b	CPF ^a + milk	CPF ^a +MV ^c
0	0	0	0	0
5	27.1	10.6	6.2	5.9
15	43.5	25.3	11.1	10.7
30	57.8	32.4	12.7	18.1
45	73.5	39.0	16.7	24.2
60	80.1	45.2	22.8	28.0
90	92.7	47.4	31.5	34.5
120	99.0	49.5	36.2	38.9

^a CPF^a, ciprofloxacin.

^b Ca, calcium.

^c MVs, multivitamins.

Table 7
Correlation coefficient (R^2) values obtained from IVIVC data of CPFX release under different conditions.

Media	CPF ^a	CPF ^a +MV ^b	CPF ^a +Ca ^c	CPF ^a +milk
UV method				
SGF–urine	0.999	0.918	0.664	0.908
SIF–urine	0.997	0.953	0.786	0.974
HPLC method				
SGF–urine	0.984	0.860	0.651	0.899
SIF–urine	0.995	0.924	0.801	0.951

^a CPF^a, ciprofloxacin.

^b MVs, multivitamins.

^c Ca, calcium.

f_2 values. f_2 values in SGF were 35.81 (CPF^a+Ca), 29.77 (CPF^a+milk) and 31.31 (CPF^a+MV^c), and in SIF they were found to be 36.36 (CPF^a+Ca), 31.2 (CPF^a+milk) and 32.16 (CPF^a+MV^c). For IVIVC studies, the correlation coefficient of CPF^a release both *in vitro* (dissolution studies) and *in vivo* (urinary excretion studies) are presented in Table 7 and Suppl. Figs. 3–6.

4. Discussion

Our research data showed that elimination of CPF^a (either cumulative release or at hourly intervals) increased significantly when co-administered with MV^c, Ca-tablets and milk as evident by *in vivo* UV and HPLC urinary excretion studies and *in vitro* dissolution studies. Elimination of CPF^a was markedly increased in the order of MV^c>milk>calcium in contrast to CPF^a release when administered alone.

Analyses of pharmacokinetic parameters showed that an increase in k_a values increased the C_{max} and *vice versa*. In contrast, T_{max} values were found longer with smaller k_a values and shorter with larger k_a values. However, k_a values did not show any influence on the AUC.

The similarity factor, f_2 values, ranged from 29.77 to 36.36, suggesting the accuracy of our *in vitro* experimental methodology [35–37]. From the point of IVIVC studies, our research data showed R^2 values ranging from 0.651 to 0.999 under different study conditions, thus exhibiting a good correlation. Estimation of CPF^a elimination under different conditions and determination of the IVIVC based on urinary excretion data were found to be simple, reliable and cost-effective. The simultaneous dissolution studies could serve as a bio-waiver for expensive human trials and hence this method could be a suitable substitute for bioequivalence studies. Dissolution studies can serve as a surrogate for *in vivo* bioavailability and support bio-waivers.

Prolonged antibiotic therapy causes anemia, gastric irritation or acidity; thus, antacids and multi-vitamin formulations are the commonly co-administered drugs with antibiotics [1,5,8]. Calcium either in the form of carbonate or chloride is a constituent of many marketed antacid formulations [2,3,42,43]. Multivitamin formulations, especially with mineral contents, are found to reduce the bioavailability of fluoroquinolones [4,5,8]. It is a common practice of many patients to intake drugs with drinks like milk, yogurt or juices other than water. Moreover, calcium-enriched foods and dairy products are often included in the breakfast menu of patients undergoing treatment [6,7,24]. Our research findings showed that the bioavailability of CPF^a was significantly reduced by co-administration of MV^c followed by that of milk and calcium. Calcium or its salts chelates or binds with CPF^a, preventing its absorption and thereby reducing its bioavailability. However, milk was found to inhibit absorption of CPF^a more than calcium alone mostly because of CPF^a interactions with the milk matrix which decreased CPF^a absorption by complexation with metal ions present in milk and by adsorption at the surface of milk protein molecules mostly casein (forms ~84% of total milk protein), thus providing additional inhibitory effect and preventing absorption of CPF^a [24]. Structurally, each CPF^a molecule has one R–COOH group which readily interacts with Ca²⁺ ions (also other poly cations) [41]. The R–COOH group of CPF^a becomes negatively charged, releasing H⁺ ion, determined by its pK_a value. The pK_a for carboxyl groups ranges between 1.8 and 2.4 [12,13]. Thus, a pH above this pK_a would result in a greater percentage of the carboxyl groups on CPF^a being ionized. Depending on other mechanisms like intake of antacids containing metals like calcium, H₂ antagonists, proton pump inhibitors; the type of food ingested by the patient; and the pH of the stomach which mostly ranges from 1 to 6, it is likely that the pK_a of the carboxyl group will be exceeded [13,41]. However, if the patient takes only CaCO₃ containing formulations with CPF^a on an empty stomach, CaCO₃ can interact with gastric HCl to form CaCl₂, CO₂ and H₂O, thus increasing the gastric pH value. Therefore, the magnitude of change in gastric pH, whether it is from CaCO₃ itself or from combination of CaCO₃ and other medications, can influence the amount of ionized carboxyl groups found on CPF^a. Owing to such ionization, the exposed and negatively charged groups on CPF^a bind with the positively charged Ca²⁺ ions that are present in the medication or supplement administered, referred to as chelation. The degree or significance of this interaction is, however, dependent on the time of exposure to one another and the pH of the environment at that time [2,13,24,26,41–44]. A 2 h separation of the calcium containing products and the CPF^a can avoid this interaction. The same logic can be applied for MV^c necessitating a gap of about 4 h between CPF^a and intake of MV. If the co-administration of other drugs or food ingested decreases absorption or bioavailability of CPF^a, it

potentially increases the risk of infection treatment failure and a drug producing sub-therapeutic actions for a long time leads to development of antimicrobial resistance against that particular antibiotic. Thus, the potency of a renowned antibiotic becomes greatly hindered due to drug–drug and drug–food interactions.

5. Conclusions

Drug–food and drug–drug interactions are matters of concern since the therapeutic efficacy and bioavailability of renowned drugs get greatly suppressed. Moreover, severe adverse drug reactions may also be precipitated. Our current research showed a significant reduction in the bioavailability of popular antibiotic CFX on interactions with MVs, calcium tablets and milk. Thus, to prevent sub-therapeutic effects and protect against development of anti-microbial resistance, patients should consider taking CFX and other Ca-containing medicaments at a lag period of 2 h and above. The same solution can be applicable to other dairy and milk products. For MVs, a lag period of at least 4 h is recommended.

The UV-HPLC analytical methodology followed the estimation of CFX elimination under different study conditions was found to be simple, reliable and cost-effective. Determination of IVIVC based on urinary excretion data showed that dissolution studies can serve as a bio-waiver for expensive human trials and hence the developed methods can be suitable substitutes for bioequivalence studies.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jpha.2015.02.003>.

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