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DEVELOPING *IN VITRO-IN VIVO* CORRELATION (IVIVC) FOR TRIMETAZIDINE, INDAPAMIDE AND CIPROFLOXACIN EXTENDED-RELEASE SOLID ORAL DOSAGE FORMS

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ABSTRACT

The current paper describes *in vitro-in vivo* correlation (IVIVC) development for extended-release solid oral dosage forms containing BCS Class I, II and III APIs: trimetazidine, indapamide and ciprofloxacin. Dissolution kinetics (in 3 media: 0.1 M or 0.01 M hydrochloric acid, acetate buffer pH 4.3, phosphate buffer pH 6.8) and pharmacokinetic properties of evaluated drugs were studied. Level B IVIVC was established for each of evaluated solid oral dosage forms, correlation coefficients (r) were 0.97, 0.94 and 0.96 for trimetazidine, indapamide and ciprofloxacin drugs, respectively.

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KEYWORDS

In vitro-in vivo correlation (IVIVC), dissolution, trimetazidine, indapamide, ciprofloxacin.

INTRODUCTION

Over the last three decades, *in vitro-in vivo* correlation (IVIVC) became a powerful tool for drug manufacturers and regulatory authorities for understanding of the in vivo and in vitro performance of dosage forms. A good IVIVC can allow the use of in vitro dissolution studies for prediction of product *in vivo* performance and, therefore, to waive of bioequivalence studies on healthy volunteers to establish interchangeability of multisource drugs[1,2].

U.S. Food and Drug Administration (FDA) defines IVIVC as "a predictive mathematical model describing the relationship between an in vitro property of an extended release dosage form (usually the rate or extent of drug dissolution or release) and a relevant in vivo response, e.g., plasma drug concentration or amount of drug absorbed)"[3]. One more definition is stated in monograph 1088 "*In Vitro* and *In Vivo* Evaluation of Dosage Forms" of U.S. Pharmacopoeia (USP 30-NF 25): "the establishment of a (quantitative) relationship between a biological property or a parameter derived from a biological property produced by a dosage form, and a physicochemical property or characteristic of the same dosage form"[4].

There are three main levels of IVIVC, based on different parameters of relationship: A, B and C. Level A correlation is the highest level of correlation, and represents a point-to-point relationship between *in vitro* dissolution profile and *in vivo* absorption curve. Level A IVIVC relates the entire *in vitro* dissolution curve to the *in vivo* concentration profile, so the *in vitro* dissolution can become a surrogate tool for establishment of the drug vivo performance. Such correlation is considered to be the most informative and is recommended for regulatory purposes. Level B IVIVC uses the principles of statistical moment analysis to compare a summary parameter of the mean *in vitro* dissolution rate (e.g., mean dissolution time, $MDT_{in\ vitro}$) and a mean *in vivo* summary parameter (e.g., mean residence time, $MRT_{in\ vivo}$). Although level B correlation does not reflect the full *in vivo* concentration-time profile, it is also useful for regulatory purposes. A Level C correlation establishes a single point correlation between an *in vitro* dissolution parameter (e.g., the time to release 50 % or 90 % of the API ($T_{50\%}$, $T_{90\%}$) and corresponding in vivo parameter (e.g., C_{max}, T_{max} or AUC). Level C IVIVC does not reflect the complete shape of the plasma concentration-time curve, and hence is not very useful for regulatory support[1-4].

Successful development of IVIVC has high expectance, if drug release from dosage form and its dissolution is the rate-limiting step in absorption process into the systemic circulation. Therefore, it is easier to develop IVIVC for extended-release solid oral dosage forms than it is for immediate-release products. Also IVIVC depends on biopharmaceutical properties of API based on a biopharmaceutical classification system, which classifies drugs on their aqueous solubility and intestinal permeability[5].

Thus, IVIVC development for extended-release solid oral dosage forms is an important aim of regulatory science. In our study we have chosen 3 extended-release preparations containing APIs included in Russian List of Essential Medicines[6]. This study may be also important for Russian regulatory authorities to accept our first IVIVC guidance, which is now in preparation.

MATERIALS AND METHODS

Chemicals

Analytical grade concentrated hydrochloric acid, glacial acetic acid, sodium acetate, potassium dihydrophosphate, disodium hydrophosphate dodecahydrate, diethyl ether, chloroform, dichloromethane, citric acid, sodium hydroxide, HPLC grade acetonitrile, methanol and water for chromatography were used.

Evaluated drugs

Trimetazidine: Preductal MR, modified-release tablets, 35 mg, Servier, France (innovator drug). It is an anti-ischemic (anti-anginal) metabolic agent, which improves myocardial glucose utilization through inhibition of fatty acid metabolism[7].

Indapamide: Arifon retard, controlled-release tablets, 1.5 mg, Servier, France (innovator drug). It is indicated for the treatment of hypertension, alone or in combination with other antihypertensive drugs[8].

Ciprofloxacin: Cifran OD, extended-release tablets, 500 mg, Ranbaxy, India. It is indicated for the treatment of the infections caused by ciprofloxacin sensitive bacteria[9].

All preparations have current Marketing Authorization in Russian Federation.

In vitro dissolution studies

All dissolution studies were performed using USP Apparatus 2 (Sotax AT7 smart, Allschwil, Switzerland) at 50 rpm. Dissolution media were Ph. Eur. 6.0-6.8 0.1 M (or 0.01 M) hydrochloric acid solution, acetate buffer solution pH 4.3 and phosphate buffer solution pH 6.8 at 37 ± 0.5 °C. Twelve tablets of each preparation were studied to obtain statistically significant results. In all experiments, 20-mL sample aliquots were withdrawn at 1, 2, 3, 4, and 6 h using hollow shaft™ and immediately replaced with equal volumes of fresh medium at the same temperature to maintain constant total volume during the test. All samples were filtered through 0.45-μm membrane filters (Millipore, Billerica, MA, USA), the first portion of filtrate was discarded. Drug release was assayed spectrophotometrically using a UV-vis spectrophotometer (Lambda 25 Perkin Elmer, Waltham, MA, USA) using corresponding dissolution media as compensation liquid. Statistical treatment was carried out using Microsoft Excel software. To confirm the validity of results, relative standard deviation (RSD, %) was calculated for each time point. Dissolution specifications for all evaluated drugs are shown in Table 1.

Table 1

Dissolution specifications for trimetazidine, indapamide and ciprofloxacin drugs.

Evaluated drug	Dissolution media	Media volume, mL	Assay, λ_{max}, nm	Reference solution
Preductal MR	0.01 M hydrochloric acid solution, acetate buffer solution pH 4.3, phosphate buffer solution pH 6.8	500	270 nm for phosphate buffer solution, 268 nm for other media	0,007 % trimetazidine CRS solution in corresponding medium
Arifon retard	0.1 M hydrochloric acid solution, acetate buffer solution pH 4.3,	500	240 nm	0,0003 % indapamide CRS solution in corresponding medium

	phosphate buffer solution pH 6.8			
Cifran OD	0.1 M hydrochloric acid solution, acetate buffer solution pH 4.3, phosphate buffer solution pH 6.8	900	323 nm for phosphate buffer solution, 328 nm for other media	0,003 % ciprofloxacin CRS solution in corresponding medium

In vivo pharmacokinetics studies

In vivo pharmacokinetics studies for each of evaluated drugs were carried out during bioequivalence studies (were Preductal MR, Arifon retard and Cifran OD were used as reference preparations) in State Hospital № 23 "Medsantrud" according to Russian Minzdrav Guidance "Bioequivalence evaluation of dosage forms"[10]. All studies were approved by local ethic committee and accepted by Federal Service on Surveillance in Healthcare and Social Development. Study design was randomized, two-way, crossover, with 14 days washout period, in fasted state. It was conducted on 18 healthy male and female volunteers. Sampling times were 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h for trimetazidine; 2, 3, 5, 8, 10, 11 and 12 h for indapamide and 0,5, 1, 1.5, 2, 2,5, 3, 4, 5, 6, and 8 h for ciprofloxacin after oral administration. Pharmacokinetic parameters AUC_{0-t}, C_{max}, T_{max}, $MRT_{in\ vivo}$, and C_{max}/AUC_{0-t} were calculated using Kinetika™ (Thermo Scientific, Germany) software. To confirm the validity of results, coefficient of variation (CV, %) was calculated for each time point. HPLC procedures (Agilent 1100, Santa Clara, CA, USA) for determination of APIs in plasma are shown in Table 2.

Table 2
HPLC procedures for determination of APIs in plasma.

Evaluated drug	Extraction*	Procedure
Preductal MR	Mix 1 mL of plasma with 1 mL of phosphate buffer pH 6.8 and inject into solid phase extraction cartridge, previously washed with mixture of 1 mL of methanol and 1 mL of phosphate buffer pH 6.8. Elute with 1 mL of mixture methanol–phosphate buffer pH 6.8 (20:80) and evaporate eluate to dryness *in vacuo* at 37 °C. Reconstitute the residue with 150 µL of eluent.	*Stationary phase*: Hypersil C18, 250*4,6 mm, 5 µm *Temperature*: 30 °C *Mobile phase*: methanol–buffer solution pH 3.0 (1:2) *Flow rate*: 1,2 mL/min *Injection*: 100 µL *Detection*: spectrophotometer at 270 nm
Arifon retard	To 2 mL of plasma, add 5 mL mixture of ether–chloroform (2:1) and 1 mL of phosphate buffer pH 6.8, shake for 10 min, and centrifuge at 4500 *g* for 5 min.	*Stationary phase*: Hypersil C18, 250*4,6 mm, 5 µm *Temperature*: room temperature *Mobile phase*: acetonitrile–0.05

	Discard aqueous layer and evaporate to dryness *in vacuo* at 37 °C. Reconstitute the residue with 200 µL of mobile phase.	M potassium dihydrophosphate solution (35:65) *Flow rate*: 1 mL/min *Injection*: 100 µL *Detection*: spectrophotometer at 240 nm
Cifran OD	To 0.5 mL of plasma, add 3.5 mL dichloromethane, shake for 10 min, and centrifuge at 4500 *g* for 5 min. Discard aqueous layer and evaporate to dryness *in vacuo* at 37 °C. Reconstitute the residue with 250 µL of mobile phase.	*Stationary phase*: Nova-Pak C18, 300*3,9 mm,4 µm *Temperature*: room temperature *Mobile phase*: acetonitrile–0.02 M potassium dihydrophosphate solution (3:4) *Flow rate*: 0.8 mL/min *Injection*: 30 µL *Detection*: fluorimeter at λ_{ex} 270 nm and λ_{em} 440 nm

**** - all buffer solutions were Ph. Eur. 6.0-6.8 buffers.***

RESULTS AND DISCUSSIONS

Dissolution profiles of each preparation are shown in Figures 1-3. All profiles were linearizsed, and summary dissolution parameter, mean dissolution time, was calculated by following equation: $MDT_{in\ vitro} = 1/k$, where k is 1st order dissolution constant.

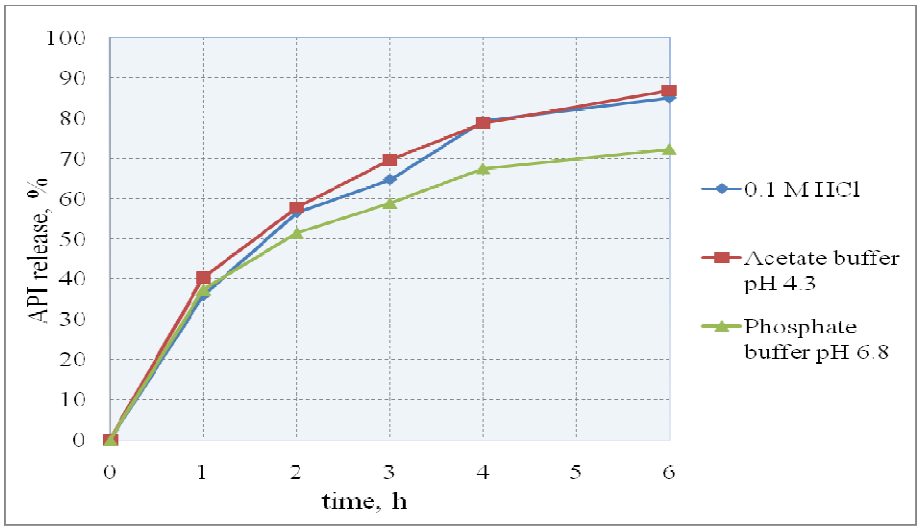

Figure 1.
Dissolution profiles of Preductal MR in three different media.

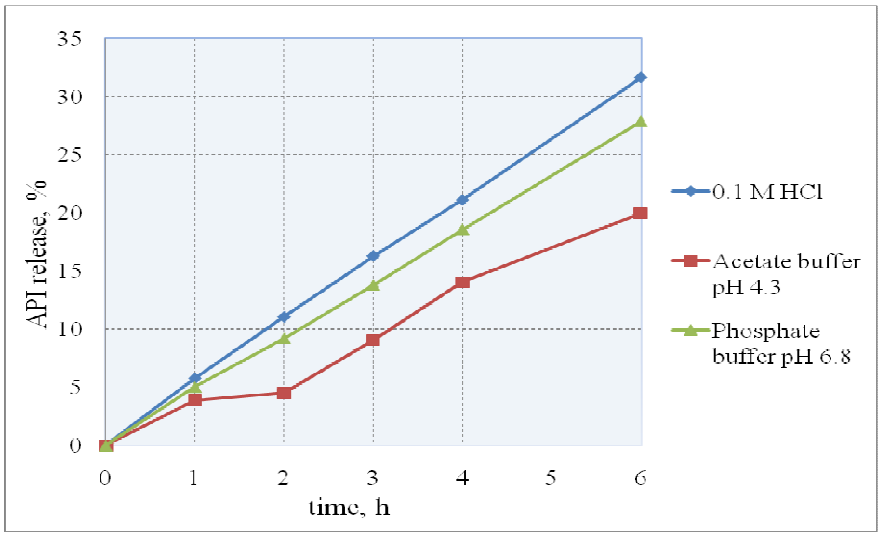

Figure 2.
Dissolution profiles of Arifon retard in three different media.

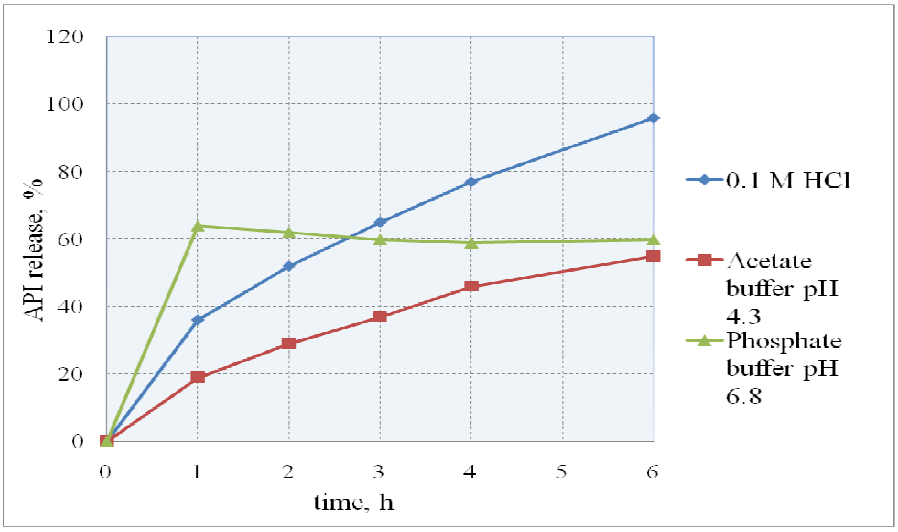

Figure 3
Dissolution profiles of Cifran OD in three different media.

Main pharmacokinetic parameters of studied drug products, together with mean dissolution time, are shown in Table 3. Level B IVIVC was evaluated between two summary parameters: mean dissolution time, $MDT_{in\ vitro}$, and mean residence time, $MRT_{in\ vivo}$ using linear regression analysis

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Table 3
Pharmacokinetics parameters and mean dissolution time ($MDT_{in\ vitro}$), mean values.

Evaluated drug product	C_{max}, ng/ mL	T_{max}, h	AUC_{0-t}, ng*h/mL	C_{max}/AUC_{0-t}, h^{-1}	$MRT_{in\ vivo}$, h	$MDT_{in\ vitro}$, h
Preductal MR	64.2	2.9	743.7	0.087	10.198	6.721
Arifon retard	25.5	8.6	531.1	0.047	6.856	43.391
Cifran OD	1306.5	3.4	7704.5	0.174	4.090	5.287

Linear regression equation parameters for all three IVIVCs are shown in Table 4.

Table 4
Linear regression equation parameters.

Evaluated drug product	Equation parameter				
	a	b	r^2	n	p
Preductal MR	1,089	0,766	0,967	18	< 0,0001
Arifon retard	40,97	7,17	0,941	18	< 0,0001
Cifran OD	0,982	1,07	0,957	18	< 0,0001

where linear regression equation is y = a + bx;
r^2 – correlation coefficient;
n – number of points;
p - the p-value.

All calculated correlation coefficient (r^2) values for studied drug products exceeded 0.9, what indicates good level B IVIVC. Therefore, a quantitative relationship between pharmacokinetic property and *in vitro* dissolution property is established.

CONCLUSION

Level B IVIVC was developed for all evaluated drug products. Although level B correlation do not reflect the full *in vivo* concentration-time profile, the results of the current study indicate, that *in vivo* behavior of trimetazidine, indapamide, and ciprofloxacin extended-release solid oral dosage forms may be evaluated by *in vitro* dissolution kinetic studies. This may allow waiving long and expensive *in vivo* bioequivalence studies for generic drug products to establish their interchangeability with reference preparation.

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