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Pharmacokinetic evaluation of an azithromycin controlled release dosage form in healthy human volunteers: a single dose study

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Abstract

Azithromycin (AZI) follows a two-compartment model pharmacokinetically. The purpose of this study was to evaluate the in vivo performance of a controlled release (CR) formulation of AZI, which would eliminate the risk of high peak plasma concentrations obtained within 2–3 h after *peroral* administration of immediate release (IR) products. The study was conducted in twelve healthy male human volunteers to compare an experimental NIPER product (CR tablets) with Vicon® (IR tablets) at the same dose level as a single-dose, randomized, one-period, two-treatment, and parallel-study. Concentrations of AZI in serum samples were assessed using the validated HPLC method. From the serum concentration–time profiles various pharmacokinetic parameters ($AUC_{0–96}$, $AUC_{0–inf}$, C_{max} and T_{max}) were calculated for both products. Results showed that the high peak concentration obtained by administration of a conventional IR formulation were eliminated with the CR product. A mean dosage form index (DI) of 1.17 with fluctuations of 7.57% was obtained with the CR product at steady state level, indicating reduced fluctuations at the steady state serum concentrations. Elimination of the pronounced peak as well as fluctuations reduced or minimized AZI adverse effects associated with the IR product.

Keywords: Azithromycin; Controlled release; Pharmacokinetics

1. Introduction

Azithromycin (AZI) is a semi-synthetic, acid stable erythromycin derivative with an expanded spectrum of activity and improved tissue pharmacokinetic characteristics relative to erythromycin (Christopher and Barradel, 1996; Hardman and Limbird, 1996). It is very effective against upper and lower respiratory tract infections, sexually transmitted diseases, skin and soft tissue infections. A three to five days *peroral* regimen of once-daily 500 mg AZI has been shown to be effective in patients with acute bronchitis, pneumonia, sinusitis, pharyngitis, tonsillitis and otitis media. On *peroral* administration, AZI absorbs rapidly and follows a two-compartment model. It has a narrow therapeutic range (0.1–0.4 μg/ml) and a long elimination half-life (≈60 h) (Ritschel and Kearns, 1999). AZI treatment is often associated with various adverse effects related to the GIT (cramping, diarrhea, nausea and vomiting) and central nervous system (headache and dizziness). The incidence or severity of these adverse effects is mainly because of high peak plasma concentrations of AZI that are seen within 2–3 h after *peroral* administration of immediate release (IR) dosage forms (Foulds et al., 1990; Christopher and Barradel, 1996).

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In controlled release drug delivery systems (CRDDS), drug release is slower than the intrinsic absorption rate (i.e. the rate of absorption from a conventional dosage form); therefore drug release from the system becomes the apparent rate of absorption. If absorption is slower than distribution, a two-compartment model collapses into a one-compartment model eliminating the pronounced peak obtained with two-compartment model (Ritschel, 1989). A drug with a long half-life is inherently sustained and usually considered as a poor candidate for CRDDS. However, a CRDDS of AZI with an appropriate apparent rate of absorption (less than the distribution rate) will eliminate high peak plasma concentration and minimize fluctuations.

The serum concentration–time profile of AZI, reported for the IR product of AZI was used in calculation of dose and release rate of AZI from the CR formulation (Foulds et al., 1990). The two-compartment model of AZI shown by IR products was collapsed into a one compartment model using Ritschel's method (Ritschel, 1989; Ritschel and Sathyan, 1990). Based on theoretical collapsing of compartment model and pharmacokinetic parameters of AZI, calculated dose and release rate (first-order kinetics) for CRDDS were found to be 506.4 mg (approximately 500) and $0.164\,h^{-1}$, respectively. In order to achieve target release profile matrix based controlled release (CR) formulation of AZI (NIPER tablets) was developed which showed first order release rate that was very close to theoretically calculated release rate (unpublished data). This paper deals with pharmacokinetic study of the developed NIPER experimental product in comparison to IR marketed tablets (Vicon® Pfizer Ltd., India) in healthy male human volunteers to evaluate the in vivo performance of developed CR formulation.

2. Materials and methods

2.1. Materials

AZI and roxithromycin were gift samples from Alembic Chemical Ltd., India and Panacea Biotec Ltd., India, respectively. Vicon®, AZI immediate release tablets (Pfizer India Ltd., India) containing 250 mg of AZI were purchased from local market. All other reagents were either of HPLC or AR grade procured from Loba Chemie, India and Mallinckrodt, USA. Ultra pure water prepared by reverse osmosis was filtered through 0.45 μm membrane filter and used in all experiments.

2.2. Instruments

The analysis of AZI was performed in Waters HPLC system (Milford, USA) configured with two 515 pumps, 717 plus auto-sampler and 464-pulsed electrochemical detector. Millennium software (version 3.05.01) was used for data acquisition and processing. Other instruments used in this study include an Electrolab tablet dissolution tester (USP XXIII) (Mumbai, India), Elgastat (ELGA Ltd. UK), electronic balance AG 245 (Greifensee, Switzerland), Branson 3210 sonicator (The Hague, The Netherlands), Maxi dry lyo from Heto (Allerod, Denmark), Biofuge primo from Heraeus (Hanau, Germany), Brand autopipettes from E. Merck (Mumbai, India) and microlitre syringes from Hamilton (Bonaduz, Switzerland).

2.3. In vitro quality control tests

In order to judge the quality of formulations and for better prediction of in vivo performance, drug release studies were carried out for both the NIPER product and Vicon® using USP apparatus II (Electrolab, India) in 900 ml of sodium phosphate buffer (pH 6.0) at $37 \pm 0.5\,^{\circ}C$ and 50 rpm. Moreover, drug content of both products were also determined. For the determination of AZI content, accurately weighed quantities of powder equivalent to 250 mg of AZI was extracted with methanol and analyzed using the validated HPLC method developed in our laboratory (Gandhi et al., 2000).

2.4. Pharmacokinetic study

2.4.1. Experimental design

Study was designed as a single-dose, randomized, one-period, two-treatment, parallel-study in healthy male human volunteers, and conducted at NIPER bioavailability center after the approval of Drug Controller General of India and NIPER Ethical Committee. The ethical committee is equivalent to Institutional Review Board (IRB) of western developed countries.

2.4.2. Inclusion criteria

A group of 12 male volunteers, ranging in age 23–30 years, weight 50–85 kg were screened by performing physical examination, liver function tests, haemogram, and routine urine analysis. The scope of the study was explained to all the subjects and an informed consent form was obtained before the start of study.

2.4.3. Dosing schedule

On the day of study, formulations were administered with a glass of water (approximately 200 ml) on an empty stomach after overnight fast. A light breakfast and lunch was provided after 2nd and 6th hours sample, respectively. A group of 12 healthy subjects received either two tablets of the NIPER product (the test formulation containing 250 mg of AZI) or two Vicon® tablets (IR marketed tablets containing 250 mg of AZI). Half of the volunteers were randomly allocated to ingest the NIPER product while the other half was given Vicon® tablets.

2.4.4. Collection of blood samples

Venous blood samples (3 ml) were collected into vacutainers at 0 h (shortly before the ingestion of drugs) 1, 2, 3, 4, 6, 9, 12, 24, 36, 48, 72 and 96 h after the ingestion of drug doses. After collection of blood samples, serum was separated and stored at −25 °C till analysis.

2.4.5. Monitoring of adverse effects

During the study, the incidence and severity of reported side effects were checked by filling "visual analogue scales" in which the subject was required to rate, on the scale of 1–10. The subjects were instructed that "0" indicates no effect and "10" indicates the worst possible effect. After the study, for a particular side effect (e.g. abdominal pain) in a particular subject, all visual-analogue-scale scores (over the 96 h post-dose period) were summed to give a "cumulative score" over the entire time period of evaluation. "Cumulative scores" for all members of a treatment group were summed, and divided by the number of subjects in the group, to give a mean cumulative score. The scale of this mean cumulative score does not correspond to the original 0–10 scale, since it reflects the summation of all non-zero scores over the entire evaluation period. The incidence and severity of particular side effects were compared on the basis of mean cumulative scores.

2.5. Determination of pharmacokinetic parameters

2.5.1. Chromatographic analysis

A reported method (Kees et al., 1998) was modified and validated to quantify AZI in serum samples. Chromatographic separation was performed on Nucleosil CN column (250 mm × 3.9 mm, 5 μm) using mobile phase consisting of sodium phosphate buffer (50 mM), acetonitrile and methanol (60:33:7 v/v, pH 7.0) at the flow rate of 1.0 ml/min. The drug was quantified using an amperometric electrochemical detector with dual glassy carbon electrodes operated in the oxidative screen mode with electrode 1 set at 650 mV and electrode 2 set at 850 mV.

Drug free serum (300 μl) was taken in micro-centrifuge tube and spiked with different volumes of working stock (10 μg/ml AZI in acetonitrile) to give calibration standards of 10, 20, 50, 100, 300, 500 ng/ml and quality control samples of 30, 200, 400 ng/ml within calibration range. Then 10 μl of working stock of internal standard roxithromycin (1 mg/ml in acetonitrile) was added to each tube and serum proteins were precipitated with 1.0 ml of acetonitrile followed by centrifugation. The supernatant was evaporated to dryness at 50 °C in a vaccum concentrator (Maxi dry lyo, Denmark). Dried residue thus obtained was reconstituted in 150 μl of acetonitrile:water mixture (1:1) and 90 μl of supernatant was injected into HPLC system.

The peak area ratio of AZI to roxithromycin (ROX) was used for constructing calibration curves and analysis of AZI in quality control as well as bio-study samples. The modified method was validated in terms of linearity, range, precision and accuracy. In addition, various system suitability parameters such as plate count, tailing factor, resolution and capacity factor were also determined.

2.5.2. Data analysis

The serum concentration–time profiles of AZI were used to calculate various pharmacokinetic parameters by compartment independent model. $AUC_{0–96}$ (area under curve from time 0–96 h) and $AUC_{0–inf}$ (area under curve from time 0 to ∞) were calculated by linear trapezoidal method. The peak serum concentration

(C_{max}) and the time to reach peak (T_{max}) were directly read from the individual serum concentration–time plots. The pharmacokinetic parameters were evaluated statistically using a paired *t*-test.

2.5.3. Comparison of in vitro and in vivo data

Drug release parameters (first order release rate (k_r) and time of delivery (t_{del})) obtained from in vitro data of NIPER tablets and drug's pharmacokinetic properties were used for predicting blood AZI concentration–time profiles (Ritschel, 1989). Values of C_{max}, t_{max} and $AUC_{0–96}$ thus obtained were compared to the values calculated from theoretically developed controlled drug release profile and actual AZI serum concentration–time profile of NIPER tablets.

2.5.4. Prediction of in vivo profiles at steady state

The single dose AZI serum concentration–time profile was used to generate steady state concentration levels on multiple dosing by using superposition method (Ritschel, 1989). Obtained values of maximum steady state concentration (C_{ssmax}) and minimum steady state concentration (C_{ssmin}) were compared to the desired values calculated from theoretically developed controlled drug release profile. The goodness of CRDDS was evaluated by percentage fluctuations at steady state and dosage form index (DI) values (Gibaldi and Perrier, 1982).

3. Results and discussions

3.1. In vitro quality control tests

From the dissolution studies, the AZI release of NIPER tablets was found to be controlled and at a rate close to theoretical AZI release rate (Table 1). In case of Vicon® tablets more than 90% AZI was dissolved in medium within specified USP limit of 45 min for IR products (USP24/NF19, 2000). On the other hand AZI content in Vicon® and NIPER tablets was found to be 98.99 ± 2.95% and 104.32 ± 0.52%, respectively of labeled content. Hence, these formulations were used for pharmacokinetic studies.

Table 1
Comparison of drug release parameters of NIPER and Vicon® tablets

Parameters	First order kinetics		
	Theoretical	NIPER product	Vicon® IR product
k_r (h^{-1})	0.164	0.171	4.189
r	0.999	0.983	0.989

k_r: first order release rate constant; *r* correlation coefficient.

Table 2
Standard curve parameters of AZI in serum samples ($n = 3$)

Day	Concentration range (ng/ml)	Correlation coefficient	Equation of line
Day 1	10–500	0.994	$Y = 0.00031X + 0.03287$
Day 2	10–500	0.990	$Y = 0.00190X + 0.07869$
Day 3	10–500	0.989	$Y = 0.00049X + 0.03204$

Concentration range was selected on the basis of expected concentration of AZI in human serum samples. Analysis of AZI was performed on Nucleosil CN column.

3.2. Validation of HPLC method

During serum sample analysis, resolution of AZI and ROX from biological artifacts was difficult. A method described by Kees et al. (1998) was modified in order to overcome this problem and modified method involves the use of acetonitrile to precipitate serum proteins. Linearity of response of analytical method was studied by running the standard curve of AZI in the concentration range of 10–500 ng/ml. Concentration–area under peak responses were linear over the range selected. The calibration range was selected on the basis of expected concentration of AZI in human serum samples. Table 2 lists the calibration curve parameters for the AZI in serum samples at three different days. Correlation coefficients with six calibration points were always greater than 0.989. Performing replicate analysis of quality control samples of serum assessed precision and accuracy. Three different concentrations (three replicates per concentration over 1–3 days) within calibration range were prepared in serum and analyzed. Precision and accuracy results shown in Table 3 demonstrate good precision and accuracy over the concentration range selected. Percent recoveries were in the range of 90–100% for three different quality control samples and coefficient of variation was found to be less than 10% in all cases. All system suitability parameters such as plate count, tailing factor, resolution and capacity factor were found to be acceptable (Swartz and Krull, 1998).

Table 3
Precision and accuracy of AZI in serum samples (mean (S.D.) $n = 3$)

Actual concentration (μg/ml)	Concentration calculated (μg/ml)	R.S.D. (%)	Recovery (%)
30	28.14 (00.61)	2.16	93.81
200	196.73 (15.80)	8.03	98.36
400	390.30 (11.07)	2.83	97.57

Percent RSD: percent relative standard deviation.

3.3. Pharmacokinetic results

Mean AZI serum concentration–time profiles after administration of 500 mg AZI from NIPER tablet (CR formulation) and Vicon® (IR) tablets are shown in Fig. 1. All calculated pharmacokinetic parameters such as $AUC_{0–96}$, $AUC_{0–inf}$, C_{max} and T_{max} are listed in Table 4. As can be seen from Fig. 1 that two-compartment model was successfully collapsed into one compartment and initial pronounced peak obtained with IR product (Vicon®) was eliminated in CR product. As a result of collapsing of compartment model CR product gave a flattened drug concentration–time profile whereas IR product showed distinct peak and trough concentrations. On the other hand, with Vicon® tablets C_{max} of 0.494 μg/ml was achieved in 3 h. However, in case of the NIPER product there was no distinct C_{max} peak as the concentration–time curve was flattened and concentration attained in initial hours were maintained till 24 h. These results clearly demonstrate the controlled release of AZI and elimination of pronounced peak after administration of NIPER tablets. In addition, calculated $AUC_{0–96}$ and $AUC_{0–inf}$ values for the NIPER product were found to be higher than Vicon® tablets. AZI is better absorbed from intestine than stomach and therefore, higher values were not unexpected as AZI absorption was improved from CR formulation due to slower release throughout GIT (Hardman and Limbird, 1996). Further, serum concentrations of AZI were detectable in reasonable amounts even at 96 h after administration of both products supporting long half-life of AZI (Christopher and Barradel, 1996).

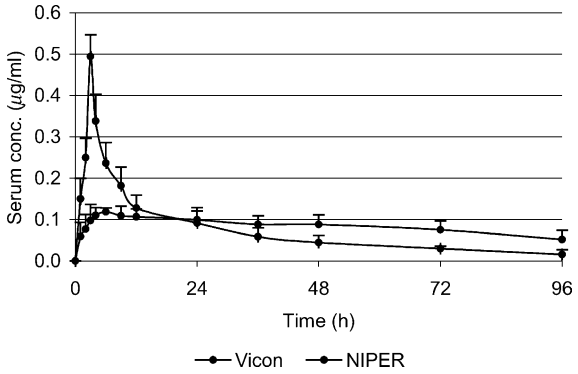

Fig. 1. Mean serum AZI concentration–time profiles for NIPER and Vicon® tablets after *peroral* administration to healthy human volunteers (mean ± S.D.; $n = 6$).

3.4. Comparison of in vitro and in vivo data

AZI serum concentration–time profile for the NIPER product obtained from single dose study in

Table 4
Pharmacokinetic parameters of AZI determined from serum concentration–time profiles obtained after *peroral* administration of NIPER and Vicon® tablets to 12 healthy human volunteers (six each) following parallel-study design

Volunteer code	NIPER formulation				Volunteer code	Vicon® IR product			
	C_{max} (μg/ml)	T_{max} (h)	$AUC_{0–96}$ (μg h/ml)	$AUC_{0–inf}$ (μg h/ml)		C_{max} (μg/ml)	T_{max} (h)	$AUC_{0–96}$ (μg h/ml)	$AUC_{0–inf}$ (μg h/ml)
2	0.142	4	6.664	8.502	1	0.534	3	6.532	10.040
4	0.106	4	5.969	10.308	3	0.526	3	5.034	7.220
6	0.131	3	9.003	15.030	5	0.459	3	7.658	8.633
9	0.130	6	9.246	14.236	7	0.555	3	6.445	6.896
10	0.112	6	7.707	11.862	8	0.466	3	8.622	9.969
12	0.121	9	9.870	18.609	11	0.422	3	6.810	7.597
Mean	0.124	5.333	8.076	13.091	Mean	0.494	3	6.850	8.393
S.D.	0.013	2.160	1.550	3.629	S.D.	0.052	0.000	1.213	1.379

C_{max}, peak serum concentration; T_{max}, time to reach peak serum concentration; AUC, area under the curve for 0–96 h and 0–∞.

Table 5
Calculated pharmacokinetic parameters from AZI concentration–time profile of human study and profiles generated from theoretical calculated release rate as well as in vitro drug release data of NIPER tablets

Profile	$AUC_{0–96}$ (μg h/ml)	$AUC_{0–inf}$ (μg h/ml)	C_{max} (μg/ml)	T_{max} (h)
Predicted from theoretical calculated release rate	8.00	12.43	0.124	16.5
Predicted from in vitro data of NIPER tablets	8.04	12.48	0.125	16.1
In vivo data of NIPER tablets	8.07	13.09	0.119	5.3

C_{max}, peak serum concentration; T_{max}, time to reach peak serum concentration; AUC, area under the curve for 0–96 h and 0–∞.

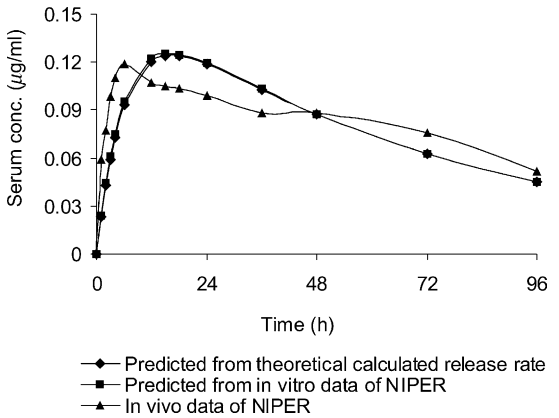

Fig. 2. Comparison of AZI concentration–time profile for NIPER tablets obtained from single dose study in human volunteers with profiles generated from theoretical calculated release rate as well as in vitro drug release data of NIPER.

human subjects were compared to in vivo profiles generated from theoretical calculated release rate as well as in vitro release data of NIPER tablets (Fig. 2). All calculated pharmacokinetic parameters results are summarized in Table 5. Since in vitro AZI release rate ($0.171\,h^{-1}$) was similar to theoretical desired rate ($0.164\,h^{-1}$) predicted AZI concentration–time profiles were also similar. As clearly shown in Fig. 2, both predicted release profiles were similar and there was no statistical difference between calculated AUC values (paired *t*-test, $P < 0.05$). Similarly predicted AZI concentration–time profile from theoretical calculated release rate was also found to be comparable with serum concentration–time profiles of human study as shown by $AUC_{0–96}$, $AUC_{0–inf}$ and C_{max} values. However, differences were observed in T_{max} values due to variable pharmacokinetics properties (e.g., variability in drug absorption and half-life) (Foulds et al., 1990; Hardman and Limbird, 1996).

3.5. Monitoring of adverse effects

During study, incidence and severity of various side effects (i.e., nausea, vomiting, diarrhea, abdominal pain, headache and dizziness) associated with AZI therapy were monitored. The mean cumulative score results of each side effect are shown in Table 6. In case of NIPER formulation, none of the volunteer reported the incidence of nausea and abdominal cramping as was reported in case of Vicon® tablets. It is clear from mean cumulative score that in case of CR product, the incidence/severity of reported side effects was very less as compared to marketed IR formulations. Thus by avoiding the peak serum concentration with CR product having lower release rate of AZI as compared to IR product it was possible to avoid most of the side effects associated with conventional therapy of AZI.

Table 6
Mean cumulative scores for various side effects calculated from "visual analogue scales" during in vivo study

Treatment	Volunteer[a]	Mean cumulative score[b]					
		Headache	Dizziness	Abdominal cramping	Abdominal pain	Nausea	Vomiting
Vicon®	6	1.5	0.5	0.5	0	1.66	0
NIPER	6	1.6	0	0	0	0	0

[a] Number of volunteers received same treatment.
[b] Cumulative scores for all members of a treatment group were summed and divided by the number of subjects in the group, to give a mean cumulative score.

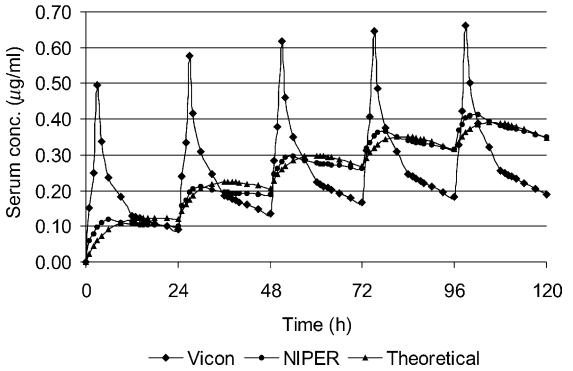

Fig. 3. Predicted steady state AZI concentration–time profiles for NIPER product and Vicon® tablets in comparison to desired theoretical profile calculated from mean serum concentration–time profile of single dose (500 mg) of AZI.

3.6. Prediction of in vivo profile at steady state

Steady state AZI serum concentration–time profiles predicted from in vivo data for both products and also a comparison with AZI steady state desired concentration–time profile predicted from theoretical calculated AZI release rate are presented in Fig. 3. All calculated in vivo parameters from serum concentration–time profiles are summarized in Table 7. Results demonstrated that NIPER product behaved similarly in vivo as the theoretically designed formulation, which was indicated by their predicted maximum steady state concentration values of 0.41 and 0.395 μg/ml for NIPER product and theoretical desired formulation, respectively. Moreover, no significant difference was found in values at the level of significance tested (paired *t*-test, $P < 0.05$).

Goodness of CRDDS was evaluated by percentage fluctuations at steady state levels and DI values (Gibaldi and Perrier, 1982). DI for CRDDS should be less than that obtained with the conventional dosage forms. All results are depicted in Table 7. In case of NIPER formulation % fluctuations were found to be lower (7.57) as compared to Vicon® tablets (22.75). In addition, percentage fluctuations after administration of NIPER product were very close to theoretical desired product (5.80). On the other hand, mean DI of 1.17 was predicted from NIPER product at steady state, which was comparable with theoretical desired product (1.13) and significantly lower than the value predicted for Vicon® tablets (3.47).

Table 7
Predicted AZI concentration levels, dosage form index (DI) and percentage fluctuations in steady state concentration levels for AZI from NIPER product and Vicon® tablets

Product	k_r (h^{-1})	C_{sspred} (μg/ml)	DI	Percentage fluctuations
Theoretical	0.164	0.346–0.390	1.13	5.80
NIPER	0.174	0.350–0.412	1.17	7.57
Vicon®	4.189	0.190–0.660	3.47	22.75

C_{sspred}: $C_{sspredmin} - C_{sspredmax}$.

4. Conclusions

Human study results showed that high serum peak obtained with IR products was eliminated in CR products by successful pharmacokinetic collapsing of compartment model. Moreover, in case of CR product, none of the volunteers reported any incidence of GIT related side effects. From the present investigation CR delivery of a long biological half-life drug is found to be more advantageous over conventional immediate release systems. Hence, this study serves as platform to develop CR systems for similar long biological half-life drugs. Although of, pharmacokinetically such drugs are not considered as potential candidates for CR delivery, the advantages in terms of avoiding toxic manifestations (dose dependant, release dependant) provide a strong drive to formulate them as CR systems.

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