The Pharma Innovation

CODEN : PIHNBQ

ISSN 2277-7695

Vol. 1 No. 4 2012

Online Available at www.thepharmajournal.com

THE PHARMA INNOVATION

In vitro – in vivo correlation of marketed formulations of cetirizine dihydrochloride

Kuldeep Malodia

Guru Jambheshwar University, Hisar, Haryana, India

IVIVC can impart *In vivo* meaning to the *in vitro* dissolution test and can be useful as surrogate for bioequivalence. Further, IVIVC can also allow setting of more meaningful dissolution specifications. Ultimately conclusion is that IVIVC cannot be done for immediate release formulation. It can be done only in conventional products, where adsorption of drug is dissolution limited but in the case of mouth dissolving tablets of Cetirizine dihydrochloride tablets the ratio of dissolution was font and the absorption was dependent on the rate of permeation and therefore in case of permeation rate limited absorption the IVIVC correlation is very poor and mostly impossible. Cetirizine dihydrochloride is histamine H_1-receptor antagonist. It is an anti allergic drug with selective inhibitory effect on peripheral H_1 receptors.

Experimental work on the cetirizine dihydrochloride 10 mg tablet shows the result of t-test for formulation A (2.35), formulation B (2.23), formulation C (2.35) respectively. It shows there is no correlation between plasma data v/s dissolution data. Another test also performed to confirm IVIVC to calculate the correlation coefficient (r) and found the results for different formulation between plasma data and dissolution data. From the results of all experimental works we can conclude that IVIVC are not possible in Cetirizine dihydrochloride tablets because the test don't show the calculated value within the limit of tabulated value (at 5% level ± 2.228 on 10 degree of freedom). For cetirizine mouth dissolving tablets, Ethical Committee doesn't give the permission to us because no anyone market formulation available. Similarly for SR tables which are not available in market the permission was not granted.

Keyword: IVIVC, cetirizine dihydrochloride, bioequivalence etc.

INTRODUCTION: IVIVC is one of the most significant tools for formulation development and optimization which involves varying excipients levels, processing methods, identifying discriminating dissolution methods and subsequent scale up of the final product because quantitative and qualitative changes in a formulation may alter drug release and *in vivo* performance. Dissolution test is the only *in vitro* quality control test available till date, which can provide an insight to predict *in vivo* behavior of the drug product. It serves as a tool to distinguish between "acceptable and unacceptable" (bioequivalent or bio-inequivalent) drug products. The value of the dissolution test as a quality control tool is significantly enhanced if an IVIVC is established.

Corresponding Author's Contact information:
Kuldeep Malodia
Guru Jambheshwar University, Hisar, Haryana, India
E-mail: drkuldeepmalodia@gmail.com

In order to established IVIVC we selected cetirizine dihydrochloride plain 10 mg (three tablets of every formulation) for accurate use of data from both the fasted and fed state IVIVC. The dissolution grade was determined by using marketed tablets for which permission was granted by ethical committee of the university. The absorption rate was determined by giving the marketed tablets to healthy human volunteer and the samples were analysed by HPLC for amount of drug in blood at different time. The blood concentration was plotted against time and from this we determined the rate of absorption, AUC and rate of elimination of drug.

Cetirizine dihydrochloride is histamine H_1-receptor antagonist. It is an anti allergic drug with selective inhibitory effect on peripheral H_1 receptors. The molecular formula of the drug is C_{21} H_{27} Cl_3 B_2 O_3. The molecular weight of the drug is 461.8. It is a white or almost white powder, freely soluble in water, practically insoluble in acetone and in methylene chloride. Cetirizine dihydrochloride contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of (RS)-2-[2-[4-[(4- chlorophenyl) phenylmethyl] piperazin- 1-yl] ethoxy] acetic acid dihydrochloride, calculated with reference to the dried substance. The drug is store in a well closed container which is protected from light.

Materials and Methods

Cetirizine dihydrochloride and its internal standard tolbutamide for HPLC analysis were supplied by Vardhman Healthcare, Mullana. Triethylamine AR grade, chloroform, dichloromethane, sodium dihydrogen phosphate and HPLC grade acetonitrile, methanol, isooctane and isopropanol were purchased from Qualigens fine chemicals, Mumbai. The commercial tablet of cetirizine dihydrochloride 10 mg were provided by Cope 10 mg (Mankind Pvt. Ltd.), Pancet 10 mg (Panjon Pharma Ltd.) and Setride 10 mg (Wockhardt Ltd.).

In vivo Studies

Pharmacokinetic Studies Using Healthy Human Volunteers

Study Protocol (COPE 10 mg)

A study protocol was prepared for conducting the pharmacokinetic studies using healthy human volunteers. After approval of the study protocol by the Institutional Ethics Committee (IEC), it was adopted for the pharmacokinetic studies. Prior to the pharmacokinetic studies the volunteers were checked for any sort of hypersensitivity reactions to the drug. 10 mg of Cetirizine dihydrochloride was given to all the volunteers at the same time. During 24 hours they were observed for any hypersensitivity reactions. No hypersensitivity was observed in any of the volunteers. The study was performed as a single-dose, randomized, crossover design in 8 healthy volunteers. Informed written consent was obtained from all the volunteers before the study.

Table 1: Pharmacokinetic Studies Protocol on Healthy Volunteers

Drug	**Cetirizine dihydrochloride**
Dose	10 mg daily, od
Dosage form	Tablet
Brand name	Cope 10 mg (Mankind Pvt. Ltd.)
Treatment	Dose administered at 8:30 am
Collection of Blood samples	Blood samples collected at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24 hrs after dosing
Number of volunteers	8 healthy males, age group 20-24 years
Food	Food provided at 10.00, 14.00, and 22.00 hrs
Washout period	7 days
Analysis of the Blood	Analysis done by Reverse Phase High
Samples	High Performance Liquid Chromatography (HPLC)
Statistical analysis	Comparison of the various pharmacokinetic parameters *viz* C_{max}, t_{max}, AUC, K_e, $t_{1/2}$ done by paired t-test.

Drug Analysis

The samples were taken under medical supervision. Volunteers remained under clinical observation until collection of the last blood sample. During study, participants were provided with standardized meals. The consumption of alcohol or caffeine or any other drug was not allowed during this time. Preparations were administered with approximately 200 ml of water. 3 ml of venous blood was collected at 0 (per dose) 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h after dosing. Immediately after the collection of blood samples, the samples were centrifuged at 4000 rpm and clear plasma was separated, transferred into sealable tubes and was stored at -20°C. Plasma concentration was measured by reverse phase ion pair HPLC with UV absorbance detection using the sample preparation method described below.

Calibration Curve of Cetirizine Dihydrochloride in Human Plasma

Plasma samples were prepared in the range of 0.2 µg/ml to 2.0 µg/ml (0.2, 0.4, 0.6, 0.8, 1.5, 2.0 µg/ml) of Cetrizine dihydrochloride by taking 0.5 ml aliquots of plasma. In each sample, 0.5 ml of standard solution containing different concentrations of Cetirizine dihydrochloride and 10 µg/ml Tolbutamide was added. Tolbutamide was used as the internal standard. The drug was extracted using the above said extraction procedure and was analyzed by HPLC. The procedure for preparation of standard solutions of Cetirizine dihydrochloride in human plasma is shown in Table 2.

Table 2: Preparation of Standard Solutions of Cetirizine Dihydrochloride in Human Plasma

Volume of Plasma (ml)	Volume of Stock Solution (ml)	Volume of Organic solvent (ml)	Reconstituted in methanol (ml)	Final Concentration (nh/ml)	Retention time (min)
0.5	0.5	2	0.5	50	6.110
0.5	0.5	2	0.5	100	6.019
0.5	0.5	2	0.5	150	6.026
0.5	0.5	2	0.5	200	6.020
0.5	0.5	2	0.5	250	6.017
0.5	0.5	2	0.5	300	6.024
0.5	0.5	2	0.5	350	6.124

*Stock solutions of Cetirizine dihydrochloride were prepared in the range of 0.2, 0.4, 0.6, 0.8, 1.5 and 2.0 µg/ml using methanol as the solvent. For each 0.5 ml aliquot of plasma, 0.5 ml stock solution of Cetirizine dihydrochloride was used from 0.2 µg/ml to 2.0 µg/ml to achieve a final concentration ranging from 0.2 µg/ml to 2.0 µg/ml of Cetirizine dihydrochloride in plasma.

***In-vitro* Studies**

Dissolution study was carried out with 10 mg Cetirizine dihydrochloride tablet (Cope). The study was carried out using dissolution medium alkaline borate buffer, 900 ml, dissolution apparatus type II (Paddle type) at 50 rpm with 37 ± 0.5 °C temperature and samples were analyzed by double beam UV- Spectrophotometer at 230 nm (lambda max).

Standard Curve of Citirizine Dihydrochloride in Distilled Water

Weighed quantity of Citirizine dihydrochloride 10 mg pure API was dissolved in 100 ml of distilled water to make a stock solution (100 µg/ml). The absorbance of solution was measured at 230 nm. From the stock Solution 2.5, 5, 7.5, 10, 12.5, 15 ml were pipetted out and diluted to 50 ml with distilled water to get 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml and 30 µg/ml Solutions.

Table 3: Concentration versus Absorbance Reading of Citirizine Dihydrochloride at λ_{max} 230 nm

S. No.	Concentration (µg/ml)	Absorbance
1	5	0.159
2	10	0.335
3	15	0.508
4	20	0.683
5	25	0.856
6	30	0.981

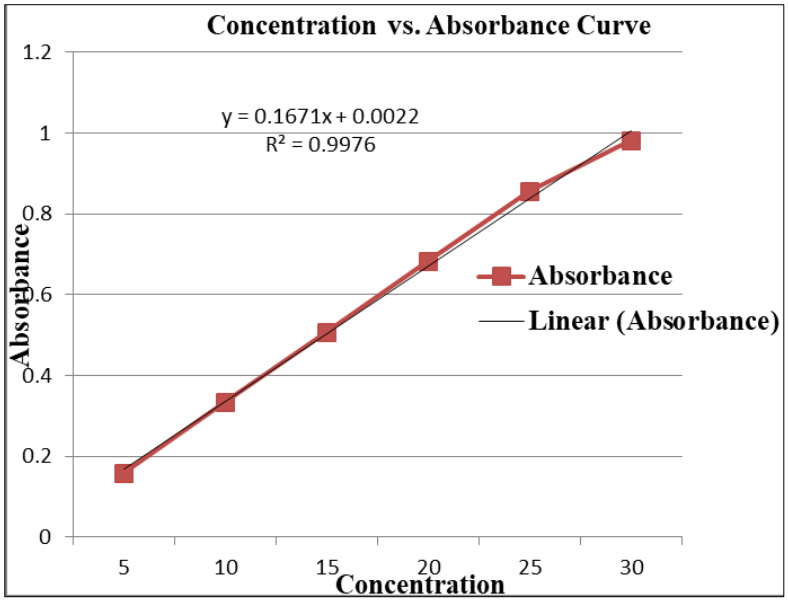

Fig 1: Standard Curve of Citirizine Dihydrochloride at λ_{max} 230 nm

Comparison between Three Formulations of Same Drug by Dissolution Data

Table 4: Mean Dissolution Data (Setride 10 mg)

Time (Min)	Absorbance	Conc. (µg/ml)	Drug Release (mg)	% Release
0	0	0	0	0
5	0.142	4.36	3.92	39.2
10	0.197	6. 02	5.48	54.8
20	0.260	7.96	7.16	71.6
30	0.277	8.49	7.64	76.4
40	0.299	9.17	8.25	82.5
50	0.321	9.84	8.86	88.6
60	0.329	10.11	9.01	90.1
80	0.327	10.03	9.03	90.3
N = 3				

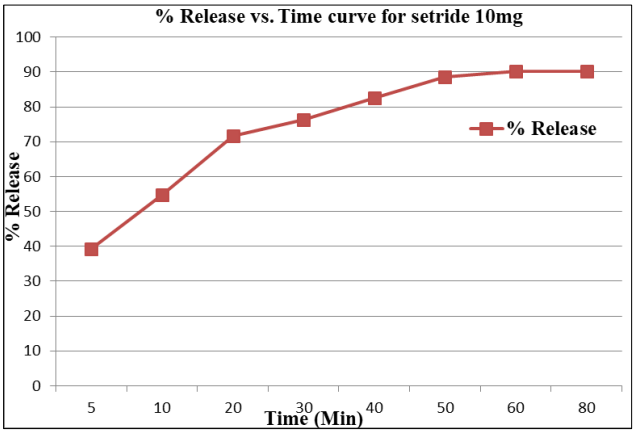

Fig 2: % release vs. time curve for setride 10 mg

Table 5: Mean Dissolution Data (Pancet 10 mg)

Time (Min)	Absorbance	Conc. (µg/ml)	Drug Release (mg)	% Release
0	0.00	0.00	0.00	0.00
5	0.134	4.10	3.69	36.9
10	0.218	6. 69	5.28	52.8
20	0.251	7.67	6.90	69.0
30	0.280	8.58	7.72	77.2
40	0.296	9.04	8.14	81.4
50	0.316	9.68	8.72	87.2
60	0.323	9.93	8.94	89.4
80	0.333	10.23	9.21	92.1
N = 3				

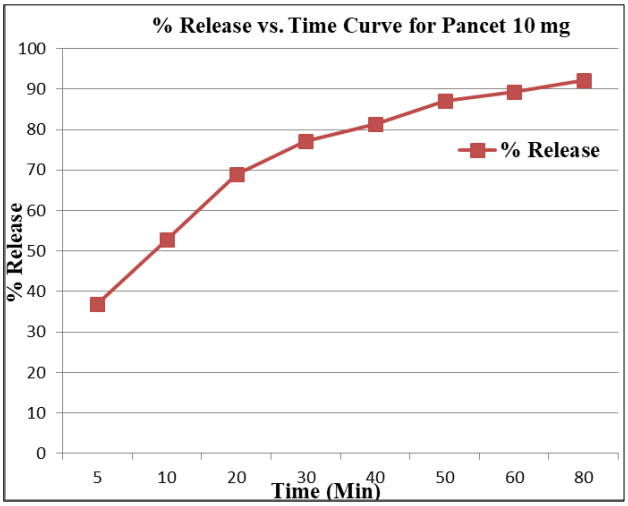

Fig 3: % release vs. time curve for Pancet 10 mg

Table 6: Mean Dissolution Data (Cope 10 mg)

Time (Min)	Absorbance	Conc. (µg/ml)	Drug Release (mg)	% Release
0	0.00	0.00	0.00	0.00
5	0.127	3.90	3.51	35.1
10	0.205	6.28	5.65	56.5
20	0.262	8.03	7.23	72.3
30	0.279	8.54	7.69	76.9
40	0.301	9.22	8.30	83.0
50	0.319	9.77	8.79	87.9
60	0.330	10.15	9.14	91.4
80	0.335	10.28	9.26	92.6
N = 3				

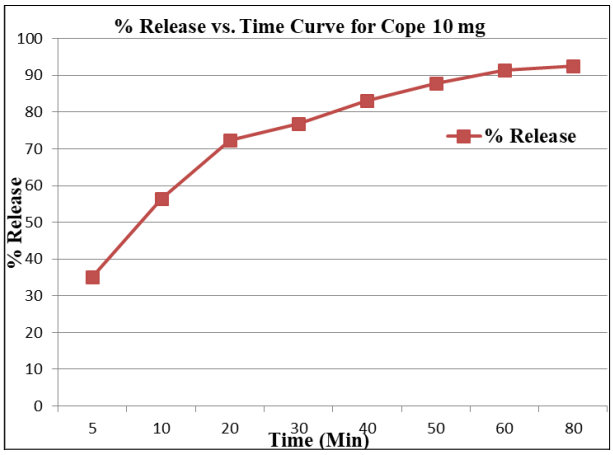

Fig 4: % release vs. time curve for Cope 10 mg

Comparison between Three Formulations

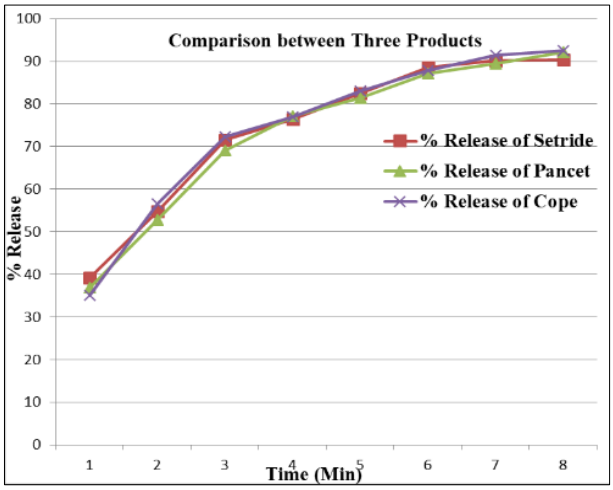

Fig 5: Comparison between Three Formulations

Table 7: Plasma Concentration Profile of Cetirizine Dihydrochloride

Time (Hr)	Conc.(ng/ml)	AUC	Cumulative AUC	Conc. × time	AUMC
0	0	0	0	0	0
0.5	225.3	56.33	56.33	112.65	28.16
1	222.5	111.95	168.28	222.50	83.79
2	197.5	210.00	378.28	395.00	308.75
3	202.2	200.00	578.28	607.50	501.25
4	190.2	196.35	774.63	760.80	684.15
6	155.0	345.20	1119.83	930.00	1690.80
8	132.5	287.50	1407.33	1060.00	1990.00
12	90.2	222.70	1603.03	1082.40	2142.40
24	52.5	856.20	2486.23	1260.00	14054.40

For IVIVC we perform t-test for two individual data (plasma data vs. dissolution data) for cetirizine dihydrochloride after completing the experimental work as showing in following tables.

Table 8: t-test for IVIVC (Setride 10 mg)

In vitro (X_1) µg/ml)	*In vivo* (X_2) µg/ml)	$(X_1)^2$	$(X_2)^2$
4.36	225.30	19.01	50760.09
6.02	222.50	36.24	49506.25
7.96	197.50	63.36	39006.25
8.49	202.50	72.08	41006.25
9.17	190.20	84.09	36176.04
9.84	155.00	96.82	24025.00

Table 9: t-test for IVIVC (Pancet 10 mg)

In vitro (X_1) µg/ml)	*In vivo* (X_2) µg/ml)	$(X_1)^2$	$(X_2)^2$
4.10	225.30	16.81	50760.09
6.69	222.50	44.75	49506.25
7.67	197.50	58.82	39006.25
8.58	202.50	73.61	41006.25
9.04	190.20	81.72	36176.04
9.68	155.00	93.70	24025.00

Table 10: t-test for IVIVC (Cope 10 mg)

In vitro (X_1) µg/ml)	*In vivo* (X_2) µg/ml)	$(X_1)^2$	$(X_2)^2$
3.90	225.30	15.21	50760.09
6.28	222.50	39.44	49506.25
8.03	197.50	64.48	39006.25
8.54	202.50	72.93	41006.25
9.22	190.20	85.01	36176.04
9.77	155.00	95.45	24025.00

Table 11: Cetirizine Dihydrochloride 10 Mg – Mean Time Parameters Using Statistical Moments

Factor	Cetirizine dihydrochloride tablet			IVIVC correlation coefficient (r)	
	A	B	C	A	0.813
MRT (Plasma data)	15.17	15.17	15.17	B	0.805
MRT (disso. Data)	0.589	0.744	0.752	C	0.860

Table 12: t-test Values for IVIVC (Cetirizine Dihydrochloride 10 mg) in Different Tablets

Formulation + plasma data (pd)	t-test	Values ($p< 0.05 \pm 2.228$, D.F. 10)
A + PD	T1	2.35
B + PD	T2	2.23
C + PD	T3	2.35

Note: A – Seteride (10 mg); B – Pancet (10 mg); C – Cope (10 mg)

Summary

Experimental work on the cetirizine dihydrochloride 10 mg tablet shows the result of t-test for formulation A (2.35), formulation B (2.23), formulation C (2.35) respectively. It shows there is no correlation between plasma data v/s dissolution data. Another test also performed to confirm IVIVC to calculate the correlation coefficient (r) and found the results for different formulation between plasma data and dissolution data. From the results of all experimental works we can conclude that IVIVC are not possible in Cetirizine dihydrochloride tablets because the test don't show the calculated value within the limit of tabulated value (at 5% level ± 2.228 on 10 degree of freedom). For cetirizine mouth dissolving tablets, Ethical Committee doesn't give the permission to us because no anyone market formulation available. Similarly for SR tables which are not available in market the permission was not granted.

Conclusion

IVIVC can impart *In vivo* meaning to the *in vitro* dissolution test and can be useful as surrogate for bioequivalence. Further, IVIVC can also allow setting of more meaningful dissolution specifications. Ultimately conclusion is that IVIVC cannot be done for immediate release formulation. It can be done only in conventional products, where adsorption of drug is dissolution limited but in the case of mouth dissolving tablets of Cetirizine dihydrochloride tablets the ratio of dissolution was font and the absorption was dependent on the rate of permeation and therefore in case of permeation rate limited absorption the IVIVC correlation is very poor and mostly impossible.

References

1. Al-Behaisi S, Antal I, Morovjan G, Szunyog J, Drabant S, Marton S. Klebovich *In vitro* simulation of food effect on dissolution of deramciclane film-coated tablets and correlation with *in vivo* data in healthy volunteers. Eur J Pharm Sci. 2002 Mar;15(2):157-62.
2. Amarjit S, Paramjit S, Kapoor VK. Analytical profile of drug substances and excipients 2001;28:197-249.
3. Amidon GL, Lennernas H, Shah VP, Crisson JRA. Theoretical basis for a Biopharmaceutic Classification System: The correlation of invitro drug product dissolution & *in vivo* bioavailability Pharm. Res. 1995;(12):413-420.
4. Anil Singla K, Chawla M, Singh A. Nimesulide: Some pharmaceutical and pharmacological aspects --- An update. J Pharm. Pharmacol. 2000;(52):467-486.
5. Balan G, Timmins P, Greene DS, Marathe PH. IVIVC model for glibenclamide after administration of metformin/glibenclamide tablets to healthy human volunteers. J Pharmacy and pharmacology. 2000;52(7):831-38.
6. Bernareggi A. Clinical pharmacokinetics of nimesulide Clin. Pharmacokinet. 1998;(35):247-274.
7. Bernareggi A. The pharmacokinetic profile of nimesulide in healthy volunteers Drugs. 1993;46(1):64-72.
8. Chandra Rao GS, Nagoji KEV, Rao MEB. Formulation and evaluation of nimesulide

dispersible tablet, Ind. J Pharm. Sci. 2002 Nov-Dec, 598-99.
9. Cheung RY, Kuba R, Rauth AM, Wu XY. A new approach to the *in vivo* and *in vitro* investigation of drug release from locoregionally delivered microspheres. J Control Release. 2004 Nov 5;100(1):121-33.
10. Chowdary KPR, Buchi NN. Effect of PH on the solubility & dissolution rate of nimesulide. The Eastern Pharmacist. 1999 Dec, 125-126.
11. FDA Guidance for Industry: SUPAC-MR: Modified release solid oral dosage forms: scale-up and post-approval changes: chemistry, manufacturing and controls, *in vitro* dissolution testing, and *in vivo* bioequivalence documentation. 1997.
12. FDA, Draft Guidance for industry: Waiver of *in vitro* bioavailability & bio chemical studies for immediate release solid oral dosage form containing active ingredient based on Biopharmaceutic Classification System. 1999.
13. Gillespie WR. Convolution-based approaches for *in vitro-in vivo* correlation modeling. In: Young D, DeVane J, Butler J, eds. *In vitro-In vivo* Correlations. New York, NY: Plenum Press. 1997;423:53-65.
14. Huang X, Brazel CS. The importance and mechanisms of burst release in matrix-controlled drug delivery systems. J Control Rel. 2001;73:121-136.
15. Humbert H, Cabiac MD, Bosshardt H. *In vitro-in vivo* correlation of a modified-release oral form of ketotifen: *in vitro* dissolution rate specification. J Pharm Sci. 1994;83:131-136.
16. Hwang SS, Gorsline JJ, Louie J, Dye D, Guinta D, Hamel L. *In vitro* and *in vivo* evaluation of a once-daily controlled release pseudoephedrine product. J Clin Pharmacol. 1995;35:259-267.