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Research Article

Simultaneous Estimation of Olmesartan medoxomil and Hydrochlorothiazide by RP-HPLC Method in Combined Tablet Dosage Forms and its Invitro Dissolution Assessment.

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ABSTRACT
A simple, sensitive, rapid and reproducible reversed- phase HPLC method has been developed and validated for estimation of Olmesartan medoxomil and Hydrochlorothiazide simultaneously and also the comparative study of invitro data in tablet formulation. The assay involved an isocratic elution of these two component on Inertsil-phenyl column (25cm X 4.6mm, 5µm) using a mobile phase composition of Buffer: Acetonitirile (480:520) and pH adjusted to 3.0 with dilute orthophosphoric acid. The flow rate was 1.0 mL/min and the analytes monitored at 257nm.Calibration curves were linear with coefficient correlation between 0.99 to 1.0 over a concentration range of 24 to 74 µg/mL of Olmesartan medoxomil and 15 to 47 µg/mL for Hydrochlorothiazide respectively. The invitro release of various test units was compared for their similarity using the f_2 test which limits were found with in the acceptance criteria. All the validation parameters were with in the acceptance range according to ICH norms. The described method was successfully employed for quality control assay of both the component simultaneously and dissolution data helpful in generating the further information regarding invivo absorption rate in tablet dosage form.

Keywords: Olmesartan medoxomil; Hydrochlorothiazide; Invitro dissolution study; Reversed-phase HPLC; Antihypertensive agent.

INTRODUCTION

Olmy-H fixed dose combination tablet contains Olmesartan medoxomil and Hydrochlorothiazide as Antihypertensive agent[1]. Olmesartan medoxomil is a prodrug, which, after ingestion, liberates the only active metabolite, Olmesartan. It is a competitive and selective AII type 1 receptor antagonist that is used alone or with other Antihypertensive agents to treat hypertension[2,3]. The hydrolysis of olmesartan medoxomil occurs readily by the action of esterases which are present abundantly in the gastrointestinal tract, liver and plasma. Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle[4,5]. Hydrochlorothiazide is a thiazide diuretic. Thiazides affect the renal tubular mechanisms of electrolyte reabsorption, directly increasing excretion of sodium and chloride. The renin-aldosterone link is mediated by angiotensin II, so co-administration of an angiotensin II receptor antagonist tends to reverse the potassium loss associated with these diuretics. Fixed dose combination of both these component in one tablet is 20 milligrams of olmesartan/12.5 milligrams of hydrochlorothiazide once daily[6].

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Several methods have been described in the literature for the determination of olmesartan medoxomil by ultraviolet[7], by Capillary zone electrophoresis[8], in urine by LC/MS[9] and hydrochlorothiazide individually in plasma by LC/MS[10] , antihypertensive efficiency in combination of both drugs[11], hydrochlorothiazide in combination with other drugs were estimated by derivative spectroscopy, HPLC and LCMS[12-16] , HPLC Analysis of olmesartan medoxomil and hydrochlorothiazide in *vitro* Dissolution Studies and in Combined Tablets[17, 18] and HPTLC Analysis of olmesartan medoxomil and hydrochlorothiazide in Combined Tablets[19].

In the proposed work, a successful attempt has been made to develop analytical method and generation of invitro data with due consideration of accuracy, sensitivity, rapidity, economy and simplicity. Also prior to the human clinical studies dissolution data must usually be generated which provide useful recommendation for their evaluation[20, 21].

MATERIAL AND METHODS

Chemicals and Materials:

MSN and Ipca Laboratories supplied Olmesartan medoxomil and Hydrochlorothiazide respectively. Acetonitrile (HPLC grade) and Sodium dihydrogen

orthophosphate were purchased from Spectrochem and E-Merck Limited respectively. In-house purified water (USPgrade) was used throughout the study.

Dissolution parameters:

Medium	Phosphate buffer pH 6.8, 0.1 N HCL buffer pH-1.2 and Water.
Volume	900 mL
Apparatus	Paddle
RPM	100
Temperature	37 ± 0.5^0C
Time	45 minutes

HPLC Condition:

Detector	257 nm for assay
Injection volume	20 µL
Flow rate	1.0 mL/min
Temperature	30˚ C
Mobile phase	Buffer: Acetonitrile: Methanol (480:520)
Diluent	Acetonitrile: Methanol (1:1)

Instrumentation:

The chromatographic separations were performed using Shimadzu LC 2010C integrated system equipped with quaternary gradient pump, 2010C UV-VIS detector, 2010C Column Oven and 2010C programmable auto sampler controlled by CLASS-VP software. The Inertsil-Phenyl (250X4.6 mm), 5µm was used as a stationary phase. The system suitability results displayed in Table 1 were evaluated throughout the study. Electrolab TDT-08L autosampler dissolution apparatus were used for comparative dissolution study.

Buffer preparation:

Dissolve 2.9g of sodium dihydrogen orthophosphate in to 1000 mL of Milli Q water and adjust pH 3.0 with orthophosphoric acid. Filtered it through 0.45 µ HVLP nylon filter.

For Dissolution:

Standard preparation:

Standard stock solutions were prepared in Diluent and dilute it further for second dilution with dissolution media and then dilute 5.0 mL of this to 10.0mL with buffer solution pH 1.2 to make final concentration Olmesartan medoxomil 11 µg and Hydrochlorothiazide 7 µg respectively.

Sample preparation:

Place 1 tablet each in six different vessels and operate the instrument as mentioned above. Withdraw about 10 mL of the sample solution, filter and dilute 5.0 mL of this to make the final concentration.

The samples withdrawn above were analyzed on HPLC.

Applied method to compare dissolution profiles:

The description of the in vitro dissolution profiles was calculated by using model-independent method[22-25]. In this study, as model-independent approaches, two fit factors were applied to the dissolution data that compare the dissolution profiles of a pair of drug product. These fit factors directly compare the difference between the percent drug dissolved per unit time for a test and reference product. The fit factors are f_1 (difference factor) and f_2 (similarity factor).

For Assay:

Standard preparation:

Standard stock solutions were prepared in diluent and further for second dilution, dilute it with mobile phase to make final concentration Olmesartan medoxomil 50 µg and Hydrochlorothiazide 31.5 µg respectively.

Sample preparation:

Weigh accurately tablets powdered equivalent to about 100 mg of Olmesartan medoxomil and 62.5 mg of Hydrochlorothiazide in to 200-mL volumetric flask. Add about 100-mL diluent and sonicate it for 30 minute to dissolve. Filtered it through 0.45 µ HVLP nylon filter and made further dilution 5.0 mL to 50.0 mL with mobile phase.

RESULTS

The detection wavelength of 257 nm was chosen in order to achieve a good sensitivity for quantitative determination of Olmesartan medoxomil and Hydrochlorothiazide in tablet dosage. The mobile phase consisting of Buffer: Acetonitirile (480:520) (pH 3.0) with orthophosphoric acid helped to produce well resolved chromatogram at ambient temperature using a flow rate of 1.0 mL/min and a runtime of 10 min, Hydrochlorothiazide elutes at first and then Olmesartan

Table 1.System Suitability and System Precision

Compound	Retention time(Mean ± SEM)	n	k'	R	T	α
HCTZ	4.078 ± 0.0020	4448.75	0.4561	-	1.32	-
Olmesartan Medoxomil	7.290 ± 0.0024	3812.24	0.7882	9.18	1.35	1.728

HCTZ:Hydrochlorothiazide, n: Theoretical plates, k': Capacity Factor, R: Resolution,T: Asymetry,α = Selectivity

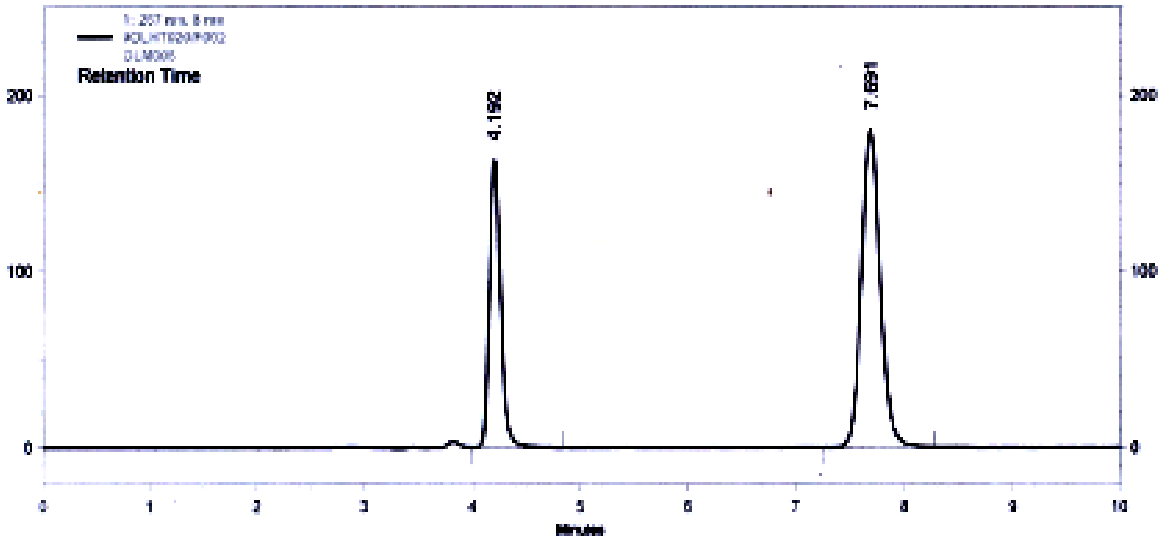
Table 2. Characteristics of the Analytical Method Derived from the Standard Calibration Curve

Compound	LOD	LOQ µg/mL	Linearity µg/mL range n=(5)	Correlation co-efficient µg/mL	Residual std. regression σ	Slope of regression (S)
HCTZ	0.007	0.021	15 to 47	0.99993	7815.145	34502.838
Olmesartan Medoxomil	0.012	0.041	24 to 74	0.99997	10653.250	46087.139

LOD= Limit of detection, LOQ= Limit of quantification

Table 3. Method Precision

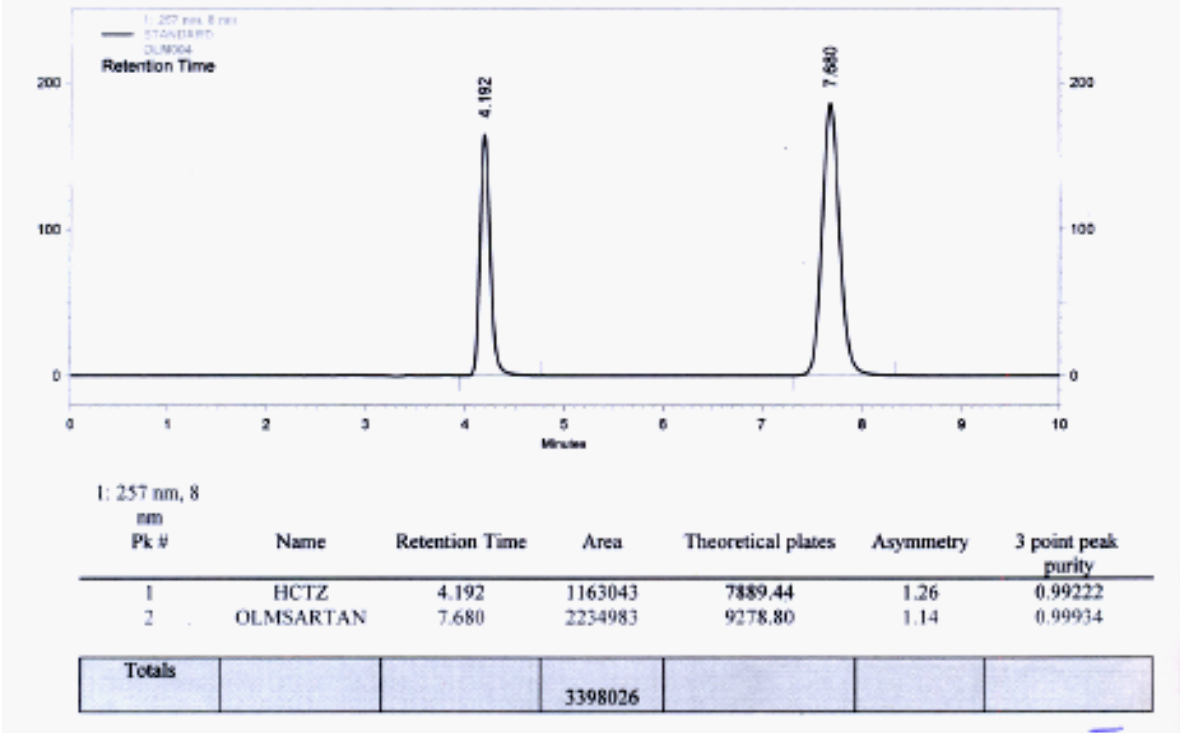
Compound	Concentration (µg/mL) (n=6)	Retention time Mean ± SEM (n=6)	% Assay Mean ± SEM (n=6)	% RSD of Assay
HCTZ	31.5	4.10 ± 0.0000	99.66 ± 0.272	0.7
Olmesartan Medoxomil	50	7.20 ± 0.0000	96.01 ± 0.195	0.5



1: 257 nm, 8 nm

Pk #	Name	Retention Time	Area	Theoretical plates	Asymmetry	3 point peak purity
1	HCTZ	4.192	1165326	7367.35	1.31	0.99892
2	OLMSARTAN	7.691	2176572	9352.95	1.09	0.99930
Totals			3342098			

Fig. 1: - Chromatogram for Test solution



1: 257 nm, 8 nm

Pk #	Name	Retention Time	Area	Theoretical plates	Asymmetry	3 point peak purity
1	HCTZ	4.192	1163043	7889.44	1.26	0.99222
2	OLMSARTAN	7.680	2234983	9278.80	1.14	0.99934
Totals			3398026			

Fig. 2: - Chromatogram for Standard solution.

medoxomil shown in the chromatogram, Fig. 1 and 2 which illustrate the separation of both active ingredients. The isocratic program throughout HPLC method was adopted to analyze both components in a short single run

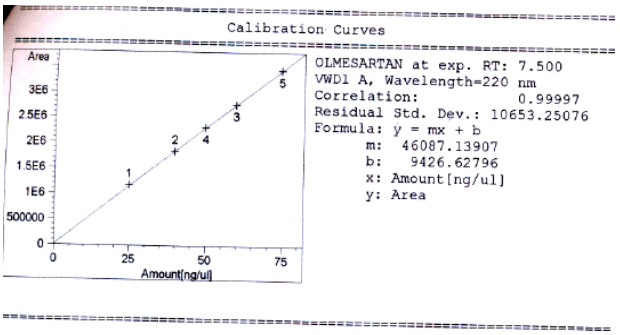


Fig. 3: - Linear calibration curve for Olmesartan medoxomil.

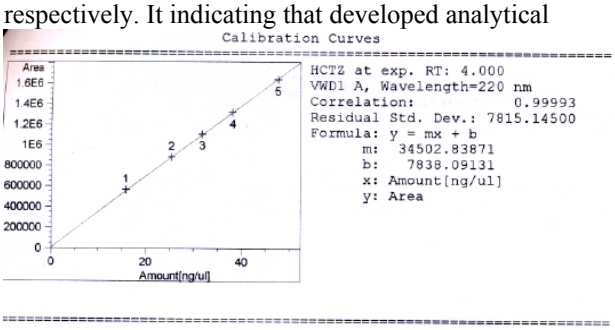


Fig. 4 - Linear calibration curve for Hydrochlorothiazide.

time. The proposed method is simple and economic, which don't require extraction or separation of the analyte.

The specification of dissolution method is set by considering the solubility, permeability, dissolution and pharmacokinetics of the drug substance. A model-independent method was used for the comparison of in vitro dissolution profiles. In this study f_1 (difference factor) and f_2 (similarity factor) was calculated. The use of these factors was also recommended for dissolution profile comparison in the FDA's guides for industry.

Table 4. Method Accuracy For HCTZ For Olmesartan Medoxomil

Drug	Level	Drug Added	Drug recovered	% Assay(Mean ± SEM)	% Assay(n=3)
HCTZ	50%	32.00	32.38	101.4 ± 0.057	0.1
	100%	62.83	63.50	101.3 ± 0.120	0.2
	150%	94.00	95.09	101.4 ± 0.152	0.3
Olmessartan Medoxomil	50%	50.43	50.46	100.5 ± 0.378	0.7
	100%	100.36	100.55	100.6 ± 0.404	0.7
	150%	150.06	150.82	100.9 ± 0.264	0.5

Table 5: Method Ruggedness

Day	Compound	% Assay Mean ± SEM (n=6)	% RSD of Assay(n=6)
Day 1	HCTZ	99.66 ± 0.272	0.7
	Olmesartan Medoxomil	96.01 ± 0.195	0.5
Day 2	HCTZ	102.23 ± 0.261	0.6
	Olmesartan Medoxomil	95.98 ± 0.214	0.6

Linearity:

The plot of peak area responses against concentration is shown in fig 3 and 4. It can be seen that plot is linear over the concentration range of 24 to 74 µg/mL and 15 to 47 µg/mL for Olmesartan medoxomil and Hydrochlorothiazide respectively with a correlation coefficient (r^2) 0.9999. The results of linearity, limit of detection and limit of quantification were presented in Table 2.

Specificity:

There was no interference from sample placebo and peak purity of Olmesartan medoxomil and Hydrochlorothiazide were 0.99930 and 0.99892 respectively. It indicating that developed analytical method was specific for its intended purpose.

Standard and sample solution stability:

Standard and sample solution stability was evaluated at room temperature for 24 h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 24 h at room temperature.

Method precision:

The relative standard deviation for six replicate injections was less than 1.0 %, which met the acceptance criteria established for the method. The results obtained were presented in Table III.

Accuracy/recovery:

The data presented in Table IV show excellent recoveries at all levels. The average recoveries for triplicate determinations at 50,100, and 150% levels were with in the acceptable criteria. Excellent recovery and low relative standard deviation value showed that the method is suitably accurate for potency assay of Olmesartan Medoxomil and Hydrochlorothiazide simultaneously in the drug substances.

Method robustness:

The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance. The content of the drug was not adversely affected by various changes.

Method Ruggedness:

Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results of ruggedness were presented in Table V.

Comparative Dissolution Data:

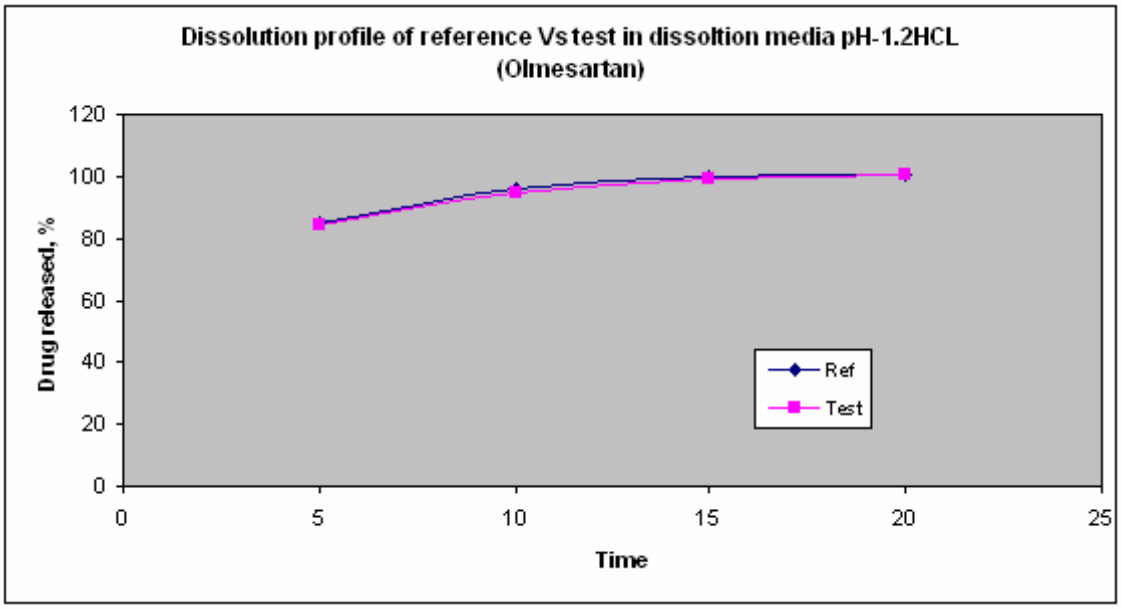


Fig. 5 Dissolution profile of reference vs test in dissolution media pH-1.2 HCl (Olmesartan)

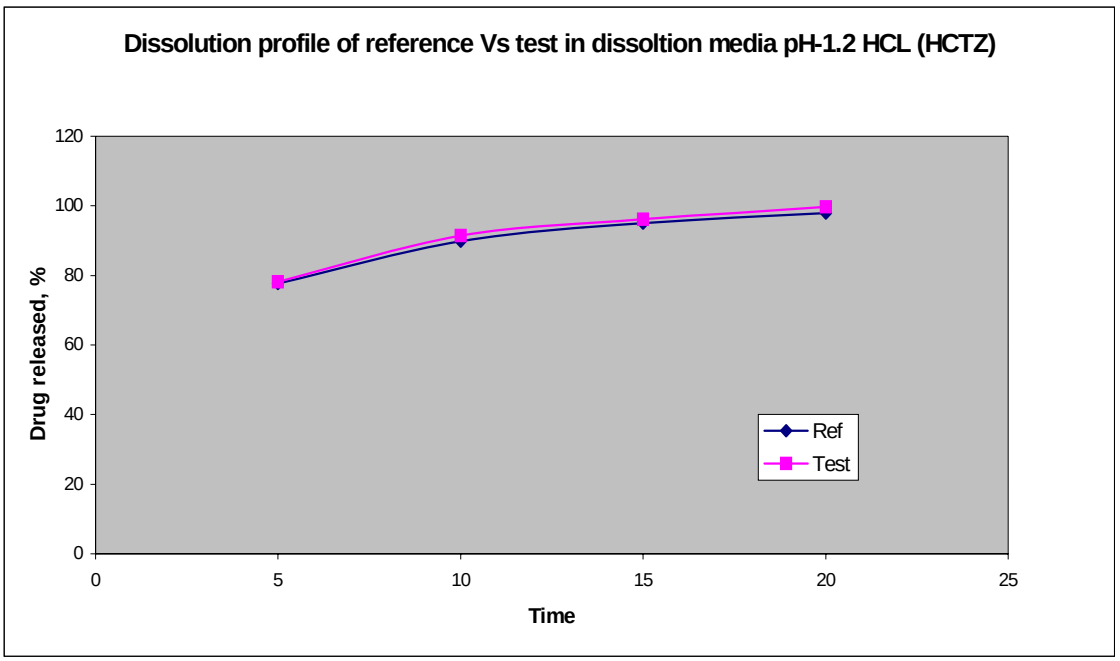


Fig. 6 Dissolution profile of reference vs test in dissolution media pH-1.2 HCl (HCTZ)

The values of factor f_1 and f_2 were calculated for the dissolution in three different media up to 45 minutes. As can be seen in Table VI and VII the data obtained for f_1 and f_2 were found to be with in the acceptable criteria. In three different media pH 6.8 phosphate buffer shows the better result and water was not found to be suitable as dissolution media.

DISCUSSION

Considering the efficiency of HPLC, attempt has been made to develop simple, accurate, precise, rapid and economic method for simultaneous estimation of Olmesartan medoxomil and Hydrochlorothiazide in a tablet dosage form. Thus method described enables to the quantification of Olmesartan medoxomil and Hydrochlorothiazide. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. It has been found that this method is also applicable for Inertsil $C_{8\ and}$ C_{18} column (250X4.6 mm), 5µm. The contribution of another important factor is its LOD. Dissolution testing is very important invitro test to evaluate drug product. This data form the part of the pharmaceutical development report, but can also be included in the bioequivalance study report. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility. Hence, this HPLC method can be used for analysis of commercial formulation and dissolution data provides useful information for invivo studies.

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Table 6. Comparative Dissolution Profile for Hydrochlorothiazide Content

	Reference (Benicar-H Tab 20:12.5mg)	Test (Olmesartan +HCTZ Tab)
Manufactured by	Sankyo Pharma	Zydus Cadila Limited
Apparatus	USP Type II, RPM- 50	
Method of Analysis	HPLC	
Dissolution medium(I)	900 ml, 0.1 N Hydrochloric acid	
% of Drug release for Hydrochlorothiazide		
Time in minutes	Reference	Test
5	77.7	78.2
10	89.8	91.5
15	95.0	96.2
20	97.9	99.7
F_1 (Similarity factor)	1.443	
F_2(Dissimilarity factor)	88.236	
Dissolution medium(II)	900 ml, Water	
Time in minutes	Reference	Test
5	83.3	83.9
10	89.4	87.7
15	92.2	92.4
20	94.8	96.2
F_1 (Similarity factor)	1.084	
F_2(Dissimilarity factor)	90.898	
Dissolution medium(I)	900 ml, pH-6.8 Phosphate buffer	
Time in minutes	Reference	Test
5	89.7	87.1
10	96.8	95.0
15	98.7	98.9
20	100.4	98.6
F_1 (Similarity factor)	1.660	
F_2(Dissimilarity factor)	84.113	

Table 7. Comparative Dissolution Profile for Olmesatan Medoxomil Content

	Reference (Benicar-H Tab 20:12.5mg)	Test (Olmesartan +HCTZ Tab)
Manufactured by	Sankyo Pharma	Zydus Cadila Limited
Apparatus	USP Type II, RPM- 50	
Method of Analysis	HPLC	
Dissolution medium(I)	900 ml, 0.1 N Hydrochloric acid	
% of Drug release for Olmesartan Medoxomil		
Time in minutes	Reference	Test
5	85.3	84.6
10	96.0	94.8
15	99.8	99.2
20	100.7	100.8
F_1 (Similarity factor)	0.681	
F_2(Dissimilarity factor)	95.068	
Dissolution medium(II)	900 ml, Water	
Time in minutes	Reference	Test
5	25.8	25.1
10	27.7	27.5
15	30.3	31.4
20	32.4	34.1
F_1 (Similarity factor)	3.184	
F_2(Dissimilarity factor)	91.651	
Dissolution medium(I)	900 ml, pH-6.8 Phosphate buffer	
Time in minutes	Reference	Test
5	93.8	94.0
10	97.3	96.5
15	99.0	98.5
20	98.7	99.9
F_1 (Similarity factor)	0.694	
F_2(Dissimilarity factor)	94.948	

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