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Bioequivalence Study of Easyrect 100 Mg Tablets Versus Spedra® 100 mg Tablets after a Single Oral Dose Administration of Each to Healthy Volunteers Male Adults under Fasting Conditions

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Abstract

This study was a comparative single-dose, open-label, randomized, two-treatment, two-sequence, four-period, full-replicate crossover, in-vivo study to determine the bioequivalence of Easyrect 100 mg Tablets (Avanafil100 mg) manufactured by Egyptian Group for Pharmaceutical Industries for Zeta Pharm for Pharmaceutical Industries versus Spedra® 100 mg Tablets(Avanafil100 mg) manufactured by Menarini International Operations Luxembourg S.A. after a single dose administration given to healthy adult volunteers under fasting conditions. The subjects who conform to the study entry criteria were dosed according to a randomization schedule. The study was designed and completed according to the good clinical and laboratory practices.

Keywords: Bioequivalence parameters (C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$); Healthy volunteers; Avanafil ; MRC

Introduction

To investigate single-dose bioequivalence of Easyrect 100 mg Tablets manufactured by Egyptian Group for Pharmaceutical Industries for Zeta Pharm for Pharmaceutical Industries (Avanafil100 mg) and Spedra® 100 mg Tablets manufactured by Menarini International Operations Luxembourg S.A. (Avanafil100 mg) given to healthy adult males under fasting conditions [1]. For the ln-transformed ratio (test product/reference product) for the bioequivalence parameters (Cmax, AUC0-t, and AUC0-∞) while other pharmacokinetic parameters of ke, t1/2, Tmax, and (AUCt/AUC∞)% were reported [2]. The influence of sequence, product, and period effect were tested by ANOVA [3,4].

The study was carried out by the Makin Research Center (MRC), Nasr city, Cairo, Egypt. This is a submission to obtain registration for a new chemical entity avanafil tablets (Spedra) with proposed indications for the treatment of erectile dysfunction in adult men [5].

Dosage Forms and Strengths

The submission proposes registration of the following dosage forms and strengths:

- Spedra (avanafil) 50 mg tablets blister package
- Spedra (avanafil) 100 mg tablets blister package
- Spedra (avanafil) 200 mg tablets blister package

Methods and Procedures

Study Drug Administration: On study day 1 of each study period, the study drugs were administered according to a randomization plan. The administration of the study drugs was documented in the drug administration form.

- Treatment A: One Easyrect 100 mg Tablets (Avanafil100 mg) taken with 240 mL of water (measured with a 100-mL cylinder) at room temperature.
- Treatment B: One Spedra® 100 mg Tablets (Avanafil100 mg) taken with 240 mL of water (measured with a 100-mL cylinder) at room temperature.
- Prior and Concurrent Medication: According to the study's protocol, no prescription medication or nonprescription medication was to be taken starting one week before the first study's drugs administration until the end of the study (collection of the last sample of period II) [6].
- Special Precautions to be taken: Co-administration of avanafil with any form of organic nitrate is contraindicated due to the potentiation of hypotension. Nitrates should not be administered to subjects for at least 12 hours after the last dose of avanafil and should be administered under close medical supervision with appropriate hemodynamic monitoring [7].

Dietary Restrictions, Standardized Diet and Fluid Intake

No consumption of alcohol was permitted for the subjects 48 hours prior to the study's drugs administration until the collection of the last sample of the respective study period. No consumption of any beverages or foods containing methylxanthines, e.g., caffeine (coffee, tea, cola, cocoa, chocolate, etc.) was permitted for the subjects 48 hours prior to the study's drugs administration until the collection of last blood sample of the respective study period [8].

In addition, the consumption of any beverages or foods containing grapefruit was prohibited one week before the first study's drugs administration and throughout the entire study.

Food and fluid intake were identical in both study periods, starting from the dinner served 10 hours before study's drugs administration on study day 1until the end of confinement. Meals were standardized in composition and amount in both periods. The subjects were not allowed to consume any additional beverages or foodstuffs other than those provided throughout the period of confinement [9].

No excessive fluid intake (>150 mL of water per hour) was allowed from 1to10 hours prior to dosing. From one hour before study's drugs administration to two hours after, no fluid intake was allowed apart from the 240 mL of water used for the administration. Following the four hours, subjects were allowed to drink water but not exceeding 150 mL per hour.

Collection and Handling of Blood Samples for Analysis

In the morning of study day 1of each study period and before study's drugs administration, a cannula was inserted into the subject's forearm vein and it remained there until the last blood sample was collected [10].

The volume of blood taken for the determination of Avanafil in plasma was 5 mL per sample. The following blood samples for the analysis of Avanafil in plasma were collected: at the following intervals: 0, 5 min, 10 min, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24 & 48 hours after dosing. The number of blood collections for drug analysis was 17 samples in each study period [11].

Blood samples were collected into tubes containing heparin as an anticoagulant slightly shaken, and centrifuged at approximately 3500 rpm for 10 minutes [12]. After centrifugation, plasma samples were transferred directly into a 5-mL plastic tube. These samples were immediately stored at the study site in an ultradeep freezer at a nominal temperature of -80 ^{o}C.

The label of the collecting tubes had the study's code number, subject number, study period, and the

designated sample number. It did not contain information that would allow identifying the given treatment. This assured that the analysts at MRC analyzed the samples blindly. The total amount of blood loss during the whole study (including blood for laboratory tests) did not exceed 350 mL in a period of one month. All procedures involving handling of blood samples will be documented [13].

Bio-analytical Drug Determination Methodology

A high performance liquid chromatographic (Shimadzu Prominence with rack changer) method coupled with mass spectrometric detection (LC-MS/MS) was developed, optimized and validated at MRC laboratories for the determination of Avanafil in human plasma. The method was fully validated according to the "FDA Bioanalytical Method Validation Guidelines 2003". Linearity of the assay method was verified within the concentration range of 65 - 7500 ng/mL. All results were within the acceptance criteria as stated in the recommended guidelines. The mean recovery of Avanafil was 102.82% at 65 ng/mL, and 91.91% at 7500 ng/ml. The described method is proved to be sensitive, accurate and reproducible with lower limit of quantification of 65 ng/mL for Avanafil [14].

Data Quality Assurance

The MRC's quality assurance procedures were implemented to assure the built-in quality system. All data entry was done by the trained staff of MRC and checked by the QAU personnel. All procedures were performed according to the internal MRC-approved SOPs with the results being documented and reported. Deliberately, all in-use manuals were archived by the QAU.

All sheets used to document results were issued and approved by the QAU serially, and ultimately reserved in the QAU.

Logbooks were audited internally by the MRC QAU personnel during the internal audit of both the clinical part and the analytical part of the study. All laboratory (clinical and analytical) results were checked and their source documents retained by the QAU [15]. Source document verification was done by the QAU after each data entry. Instrumental outputs after calculations were checked by the QAU personnel. Necessary actions were taken and corrective and/or preventive measures were recommended. A report after each audit period was delivered to the MRC management. Report of audits were followed up and reserved by the QAU. The QAU implements an internal quality system to keep all essential records related to the study guaranteeing the appropriate authorized direct access and traceability of data with utmost confidentiality [16].

All audit trails were enabled within the operated software. After the study report preparation, the QAU audited the report and released its quality assurance statement, which evidenced each audit task [17].

Pharmacokinetic Calculations

The pharmacokinetic parameters of Avanafil were estimated using standard non-compartmental methods. The maximal plasma concentration was taken directly from the measured data. The area under the plasma concentration–time curve (AUCt) was calculated from measured datapoints from the time of administration to the time of last quantifiable concentration (Clast) by the linear trapezoidal rule.

The area under the plasma concentration–time curve extrapolated to infinity (AUC∞) was calculated according to the following formula:

AUC0-∞ = AUC0-t + Clast / [ln (2)/t½], where Clast is the last quantifiable concentration. The ratio AUC0-t/AUC0/-∞ as a percent was determined as an indicator for the adequacy of sampling time.

The elimination half-life t½ was calculated as t½ = ln (2)/(-b) where b was obtained as the slope of the linear regression of the ln-transformed plasma concentrations versus time in the terminal period of the plasma curve.

Statistical Analysis

Statistical analysis was performed using a Kinetica version 5.1 (Thermo Scientific, USA).

Reagents, Chemicals & Standards

- Avanafil working standard
- Ezogabine working standard
- Water for chromatography (Sharlau, Spain)
- Acetonitrile, HPLC grade (Sigma Aldrich Chemie GmbH, Steinheim-Germany)
- Ammonium Formate, Dichloromethane, (Sigma Aldrich Chemie GmbH, Steinheim-Germany)
- Blank plasma obtained from the Holding Company for Biological Products & Vaccines (VACSERA), Giza, Egypt.

Statistical Results

Plasma Concentration-Time Profiles for Each Volunteer

Time (h.)	Test 1st time		Test 2nd time		Reference 1st time		Reference 2nd time	
	Dosage Form		Dosage Form		Dosage form		Dosage form	
	Mean ± SD		*Mean ± SD*		*Mean ± SD*		*Mean ± SD*	
0	-	-	-	-	-	-	-	-
0.08333	121.08	18.55	312.38	306.11	254.24	310.16	181.86	78.4104
0.16667	569.59	733.38	1020.3	861.78	745.98	805.23	943.441	740.035
0.25	727.19	811.81	847.89	898.53	1071.22	738.78	1405.95	859.106
0.5	1190.91	951.12	1119.71	778.2	1660.35	683.43	1544.44	787.087
0.75	1062.53	800.5	1390.97	658.9	1463.24	491.45	1604.9	677.476
1	1416.05	511.97	1263.14	850.72	1188.29	550.87	1224.1	494.923
1.5	927.33	559.95	723.85	388.32	800.36	463.7	788.952	422.62
2	867.95	426.71	606.4	210.65	560.55	308.5	576.79	316.955
3	500.91	242.32	338.05	158.02	344.6	188	348.737	192.337
4	267.85	126.63	213.61	119.26	204.48	106.38	196.863	97.4729
6	209.02	211.91	195.11	186.34	120.99	53.49	167.15	172.089
8	116.85	61.01	106.92	39.45	88.23	23.87	90.7	14.9401
10	117.58	80.38	74.15	0.21	85.1	12.16	89.76	6.58696
12	115.93	80.92	85	25.46	88.17	24.9	78.1	16.8291
24	102	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	-

Table 1: Plasma concentration Average ± SD (ng/mL) of Avanafil following oral administration of Treatment (A) test product Easyrect 100 mg Tablets and Treatment (B) reference product Spedra® 100 mg Tablets to 21 volunteers.

Pharmacokinetic Parameter	Treatment (Mean)	
C_{max} (ng/ml)	1871.62	1986.02
T_{max} (*h*) median	0.88	0.50
AUC_{0-t} (*ng.h/ml*)	3237.21	3001.36
AUC_{0-inf} (*ng.h/ml*)	3572.64	3204.00
$t_{1/2}$	1.94	1.64
$K_{elimination}$	0.49	0.60

Table 2: Pharmacokinetic Parameter.

	Point Estimate	Lower Confidence Limit	Upper Confidence Limit
C_{max} (ng/mL)	93.72%	83.89%	104.69%
AUC_t (ng.hr/mL)	108.11%	95.88%	121.90%
$AUC_{0-\infty}$ (ng.hr/mL)	110.74%	98.93%	123.96%

Table 3: 90% Confidence Interval & Point Estimate for Cmax, AUC0-t & AUC0-∞.

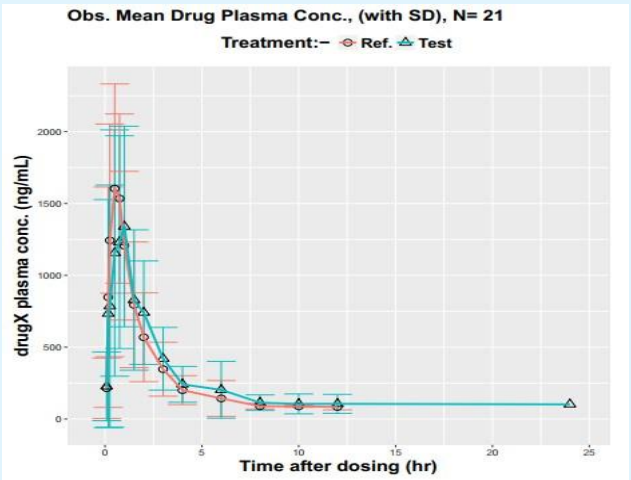

Figure 1: Mean plasma concentration vs time profile for avanafil after administration of an oral single-dose of 100 mg avanafil of the test product (Easyrect 100 mg tablets) and the reference product Spedra® 100 mg tablets).

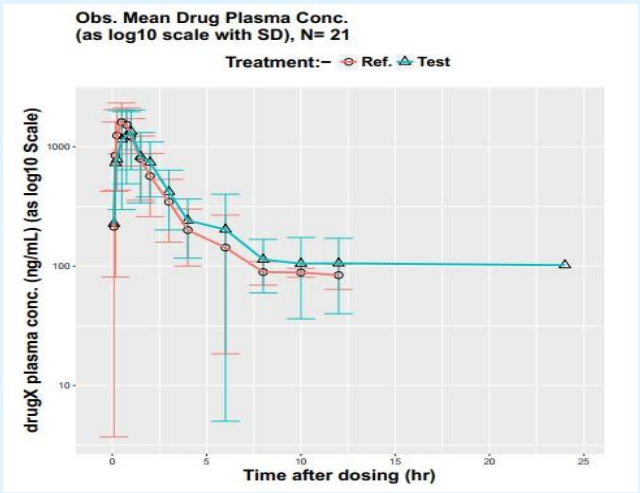

Figure 2: Logarithmic mean plasma concentration vs time profile for avanafil after administration of an oral single-dose of 100 mg avanafil of the test product (Easyrect 100 mg tablets) and the reference product (Spedra® 100 mg tablets).

Statistical Program

- ANOVA Tables for the Pharmacokinetic Parameters

Effect	DF	Sum of Squares	Mean Square	F Value	Pr > F	Significance	CV Within-Treatment
Formula	1.00	0.09	0.09	0.96	0.33	Non-Significant	.
Period	3.00	0.02	0.01	0.07	0.97	Non-Significant	.
Subject(Sequence)	19.00	1.97	0.10	1.13	0.35	Non-Significant	.
(Within) Mean Square Error	59.00	5.43	0.09	.	.	--	31.04%
Sequence	1.00	0.01	0.01	0.07	0.79	Non-Significant	.

Table 4: ANOVA table with Confidence Interval for Ln C_{max}.

Effect	DF	Sum of Squares	Mean Square	F Value	Pr > F	Significance	CV Within-Treatment
Formula	1.00	0.13	0.13	1.18	0.28	Non-Significant	.
Period	3.00	0.73	0.24	2.24	0.09	Non-Significant	.
Subject(Sequence)	19.00	7.80	0.41	3.80	<.0001	Significant	.
(Within) Mean Square Error	59.00	6.38	0.11	.	.	--	33.78%
Sequence	1.00	0.58	0.58	1.42	0.25	Non-Significant	.

Table 5: ANOVA table with Confidence Interval for Ln AUC_t.

Effect	DF	Sum of Squares	Mean Square	F Value	Pr > F	Significance	CV Within-Treatment
Formula	1.00	0.22	0.22	2.29	0.14	Non-Significant	.
Period	3.00	0.67	0.22	2.36	0.08	Non-Significant	.
Subject(Sequence)	19.00	8.26	0.43	4.56	<.0001	Significant	.
(Within) Mean Square Error	59.00	5.63	0.10	.	.	--	31.65%
Sequence	1.00	0.49	0.49	1.12	0.30	Non-Significant	.

Table 6: ANOVA table with Confidence Interval for Ln AUC_{inf}.

Effect	DF	Sum of Squares	Mean Square	F Value	Pr > F
Formula	1	0.334821	0.334821	3.73362	0.06838
Period	1	0.43006	0.43006	4.79563	0.04121
Subject(Sequence)	19	2.01563	0.106086	1.18297	0.359 NS
(Within) Mean Square Error	19	1.70387	0.089677	.	.
Sequence	1	0.040923	0.040923	0.456332	0.5075

Table 7: ANOVA table with Confidence Interval for T_{max}.

Conclusions

Bioequivalence could be demonstrated for Avanafil within the prescribed 90% confidence interval of 80.00% to 125.00% for AUC_{0-t} and $AUC_{0-\infty}$ and for C_{max} to be within 80.00% to 125.00% with respect to the parametric method on ln-transformed data. The test product, Easyrect 100 mg Tablets by Egyptian Group for Pharmaceutical Industries for Zeta Pharm for Pharmaceutical Industries, investigated in this study was shown to be bioequivalent with the reference product; Spedra® 100 mg Tablets by Menarini International Operations Luxembourg S.A.. Plasma levels may be used as surrogate parameters for therapeutic response. Therefore, the data obtained in this study prove, by appropriate statistical methods, the essential similarity of plasma levels of Avanafil from the test product Easyrect 100 mg Tablets and from the reference product Spedra® 100 mg Tablets suggesting equal clinical efficacy of these two products. The product, Easyrect 100 mg Tablets by Egyptian Group for Pharmaceutical Industries for Zeta Pharm for Pharmaceutical Industries, may be used interchangeably with the reference product Spedra® 100 mg Tablets by Menarini International Operations Luxembourg S.A.. That was shown the tested product has an acceptable therapeutic efficacy.

What Is Already Known About This Subject?

It is known about this subject comparing the activity of the active ingredient of the drugs to ensure the effectiveness of same active ingredient.

What This Study Adds?

The study ensures the same level of drug alternatives for the safety of the patient.

This study is comparing two different drugs but the same active ingredient, and to ensure the same level of alternatives to medicine.

For Example: Augmentin (amoxicillin and clavulanic acid) and Megamox (amoxicillin and clavulanic acid), differ in trade name but same active ingredient.

The Test product, Easyrect 100 mg Tablets manufactured by Egyptian Group for Pharmaceutical Industries for Zeta Pharm for Pharmaceutical Industries is bioequivalent to the reference drug, Spedra® 100 mg The Test product, Easyrect 100 mg Tablets manufactured by Egyptian Group for Pharmaceutical Industries for Zeta Pharm for Pharmaceutical Industries is bioequivalent to the reference drug, Spedra® 100 mg Tablets manufactured by Menarini International Operations Luxembourg S.A.

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