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The bioequivalence study of Folifer-Z®: a new formulation of sustained-release iron and zinc

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Abstract

The bioequivalence of Folifer-Z® tablets, a new sustained-release iron and zinc formulation was evaluated and compared to that of Fefol-Z® capsules in 30 healthy male subjects. Each subject received a single oral dose of either product according to a randomized two-way crossover design. A washout period of 1 week was allowed after each treatment. Blood samples were obtained over a 24-h period, and iron and zinc concentrations were measured. The pharmacokinetic parameters of Folifer-Z® were C_{max} (103 ± 46.2 μg/dl), T_{max} (5.93 ± 2.94 h) and $AUC_{0-24\ h}$ (1937 ± 706 μg/dl per h), whereas the corresponding Fefol-Z® values were C_{max} (109 ± 41.5 μg/dl), T_{max} (6.64 ± 2.54) and $AUC_{0-24\ h}$ (1865 ± 699 μg/h per dl). Analysis of variance on log-transformed data for C_{max} and $AUC_{0-24\ h}$ revealed lack of significant differences among the two formulations. The mean relative bioavailability of $AUC_{test}/AUC_{reference}$ was 1.07 (90% confidence interval range: 99–115%) and for $C_{max\ test}/C_{max\ reference}$ was 0.96 (90% confidence interval range: 88–105%). Regarding the zinc results, the pharmacokinetic parameters of Folifer-Z® values were C_{max} (101 ± 20.7 μg/dl), T_{max} (4.86 ± 1.53 h) and $AUC_{0-24\ h}$ (1944 ± 202 μg/h per dl), while the corresponding Fefol-Z® values were C_{max} (102 ± 20.7), T_{max} (4.93 ± 1.51) and $AUC_{0-24\ h}$ (1953 ± 200). Analysis of variance on log-transformed zinc data for C_{max}, T_{max} and $AUC_{0-24\ h}$ revealed lack of significant difference among the two formulations. The mean relative bioavailability of $AUC_{test}/AUC_{reference}$ was 0.98 (90% confidence interval range; 95–101%) and for $C_{max\ test}/C_{max\ reference}$ was 0.92 (90% confidence interval range: 89–96%). The results also indicate a possible inhibition of zinc absorption by iron content of both formulations. It is concluded that Folifer-Z® product is bioequivalent to Fefol-Z® product.

Keywords: Iron; Zinc; Bioequivalence; Pharmacokinetics

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1. Introduction

Iron and zinc are among the most important essential minerals in human body. There are about 3.5 g of iron present in the body of normal adults. Tissue iron is complexed to proteins (transferrin and ferritin) and hemes (hemoglobin, myoglobin and the heme of certain enzymes such as cytochromes). Hemoglobin contains approximately 65% of the total body iron. The basal daily iron requirement for an adult male is about 1 mg/day. Such daily requirements are higher in females and are increased during growth, menstruation, pregnancy and after bleeding or blood donations. Oral iron absorption from dosage forms is greatly affected by formulation factors. Other variables such as age, sex, food, beverages, antacids, tetracyclines, degree of anemia, stage of pregnancy, lactation, achlorhydria, gastrectomy and malabsorption significantly affect the bioavailability of iron. (Simon et al., 1981, 1984; Wallenburg and Van Eijk, 1984; Gordeuk et al., 1986). Iron, whether of dietary or medicinal sources, is absorbed mainly from the upper part of the small intestine by active and passive transport processes. In addition, absorption from the colon has also been documented (Chlou et al., 1997).

Zinc deficiency in human diets was first recognized in the early 1960s. It plays a key role in genetic expression, cell division, growth and is essential for the function of more than 200 enzymes. Manifestations of zinc deficiency include stunting, dermatitis, poor healing of wounds, congenital anomalies and neuropsychologic impairments (Fabris et al., 1991; Sandsteal, 1994). Recently, it has been demonstrated that zinc is essential for maintaining co-ordination between the major homeostatic networks, i.e. the nervous, neuroendocrine and immune systems during the organism's entire life (Fabris, 1992). This is supporting a new theoretical approach to explain ageing processes (Hoadley et al., 1985).

Zinc bioavailability is influenced by various minerals in the diet including iron, manganese, selenium and copper. Other food components, such as phytates and casein reduce absorption of zinc from intestines (Davies and Stewart, 1987).

Obstetricians in Jordan as in many parts of the world prescribe iron preparations for almost all pregnant and lactating women. This is probably due to high prevalence of malnutrition and frequent pregnancies. For this reason the Arab Pharmaceutical Manufacturing Company, APM, formulated Folifer-Z® tablets. Thus, this study was undertaken to compare iron and zinc pharmacokinetic parameters of Folifer-Z® tablets with Fefol-Z® capsules (Smith Kline and French, UK (SK and F)) as a reference pharmaceutical formulation.

2. Materials and methods

2.1. Formulations

Folifer-Z® are sustained-release tablets manufactured by the Arab Pharmaceutical, Sult, Jordan, batch No. 21896; manufacturing date: August 1996; expiry date: July 1999. Each tablet contains 150 mg ferrous sulfate(47 mg elemental iron), 61.8 mg zinc sulfate monohydrate (22.5 mg elemental zinc) and 500 μg folic acid. The iron is specially formulated for sustained-release over several hours while the zinc is formulated for release over 1–2 h.

Fefol-Z® are sustained-release capsules manufactured by Smith Kline and French, Herfordshire, UK, batch No. 395950; manufacturing date: April 1995; expiry date: March 1998. Each capsule contains 150 mg ferrous sulfate (47 mg elemental iron), 61.8 mg zinc sulfate monohydrate (22.5 mg elemental zinc) and 500 μg folic acid. The iron is specially formulated for sustained-release over several hours while the zinc is formulated for release over 1–2 h.

2.2. Subjects

Thirty young healthy male volunteers with a mean ($\pm$S.E.M.) age of 27 ± 4.8 years, body weight of 77.2 ± 7.95 kg, and height of 172.8 ± 5.82 cm participated in this study. Female volunteers were excluded to avoid variability in physiological status peculiar to females like menstruation, pregnancy, lactation, etc. Also, the de-

sign of the study includes a donation of one unit of blood before starting the study. We thought this will be inconvenient for female volunteers. All subjects were in good health as determined by a medical history, physical examination and laboratory investigation which included blood chemistry, haematology and urine analysis. The study protocol, conforms with the 1964 Declaration of Helsinki, was approved by the Institutional Review Board of the Islamic Hospital. Each volunteer signed an informed consent form. Housing of the volunteers and blood sampling were conducted at the Islamic Hospital in Amman under medical supervision.

2.3. Study design

A randomly balanced two-way crossover design was carried out in this investigation. One unit of blood was donated by each volunteer 4 days prior to the first week of the study. All volunteers were admitted to the Islamic Hospital at 19:00 h, one night before the study day and given a light dinner. After dinner no food or xanthine containing beverages were allowed. On assigned study days, at 07:00 h, each volunteer took a small piece of bread (200 g) and an indwelling catheter was placed in his forearm antecubital vein and a baseline (0 h) blood sample was withdrawn. At 08:00 h each subject was given either Fefol-Z® or Folifer-Z® preparation with 200 ml water. Blood sampling was continued for 24 h at the following time points: 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 20 and 24 h. Blood was collected in lithium-heparinized tubes, quickly centrifuged for 5 min and plasma was immediately aspirated and stored at −20°C till time of analysis of iron and zinc. A washout period of 1 week was given for all subjects before crossover into the next iron preparation. On the study days food and fluid intake were served as detailed in Table 1 and subjects were not allowed to smoke. Adverse reactions exhibited by the subjects during study periods were reported.

2.4. Iron determination

The analysis of plasma iron concentrations was performed by the automated Ferene S method (Artiss et al., 1981; Eskelinen et al., 1983) using Optima–Kone spectrophotometer (Kone Instruments Corporation Espoo, Finland) in the laboratories of the Islamic Hospital. In this method, iron is released from its binding proteins by guanidine buffer. Ascorbic acid reduces ferric iron to ferrous iron which then forms a colored product with Ferene-S reagent. The intensity of the color is measured at 600 nm. This method is linear up to 1000 μg/dl with a sample: reagent ratio of 20:100. Iron standard samples were run before, in the middle and at the end of every

Table 1
Scheduled food and fluid intake of participants during the study days of iron and zinc bioequivalence

Day	Time approximate	Meal/service
Wednesday	19:00	Hospital admission: a piece of bread (200 g), soup (300 ml), potato chips (100 g), an apple and a cup of tea (200 ml).
Thursday	07:30	Breakfast: a piece of bread (200 g) only.
	08:00	Drug administration with 200 ml water.
	11:00	Water (200 ml).
	13:00	Water (200 ml).
	16:30	Lunch: a piece of bread (200 g), soup (300 ml), potato chips (100 g), an apple, orange juice (200 ml) and one banana.
	19:30	Dinner: a piece of bread (200 g), soup (300 ml), potato chips (100 g), an apple and orange juice (200 ml).
Friday	08:00	Breakfast: a piece of bread (200 g) chick peas with ground meat, tea (200 ml).
	08:30	Volunteers were discharged.

working analysis session. The inter- and intra-coefficient variations of the assay were 7.73 and 4.19%, respectively.

2.5. Zinc determination

The analysis of zinc concentrations was performed in the laboratories of the Arab Pharmaceutical Manufacturing Co., Sult, Jordan using atomic absorption. Briefly; 200 μl aliquot of plasma sample was placed into 5-ml plastic tube, 800 μl of deionized water was added and vortexed for 30 s. Then, 100 μl aliquots of the sample, blank and a standard were mixed with aliquot sampling kit and the zinc concentration was determined using Unicam 929 Atomic Absorption Spectrophotometer. This method is linear upto 250 μg/dl with excellent zinc recovery ($>99\%$). The inter- and intra coefficient variations of the assay were 6.33 and 3.14%, respectively.

2.6. Pharmacokinetic analysis

The maximum plasma concentration of iron or zinc (C_{max}) and time to reach C_{max}(T_{max}) after the oral administration were determined from concentration-time curves. The area under the plasma concentration-time curve (AUC) was computed by using the linear trapezoidal rule upto 24 h ($AUC_{0-24\,h}$). All results were expressed as mean ± S.D. and the coefficient of variation (CV) was calculated for the obtained pharmacokinetic parameters.

2.7. Statistical analysis

Standard statistical methods for bioequivalence studies using a two-treatment crossover design were used (WHO, 1994; US Pharmacopeia XXIII, 1995). Untransformed and Log_{10}-transformed data of iron and zinc were used in the statistical analysis of pharmacokinetic parameters. T_{max}, C_{max} and AUC values were compared using two-way analysis of variance (ANOVA). The two one-sided hypotheses at the $\alpha = 0.05$ level of significance was used to test for AUC and C_{max} by constructing the 90% confidence interval for the ratio between the test (Folifer-Z®) and reference(-Fefol-Z)® averages (Hauschke et al., 1990). The Student's t-test was used to determine whether there was any significant difference among means. A difference between two related means was considered statistically significant for a p-value equal to or less than 0.05.

3. Results

3.1. Iron

In order to achieve a relative iron deficiency in the study, each subject was asked to donate one unit of blood 4 days prior to participation in the study. Such donation resulted in a significant decrease of plasma iron level by 22.4% ($p < 0.05$). The mean values of plasma iron level before and 4 days after donation of blood were 83.93 ± 31.1 and 65.33 ± 22.97 μg/dl, respectively.

The mean ± S.E.M. of plasma iron concentration-time curves for both preparations are presented in Fig. 1. As exhibited in Fig. 1 there was a significant ($p < 0.05$) gradual increase in plasma iron levels after administration of both preparations which started 2 h after treatment and continued till reaching a maximum level approximately 6 h after treatment. Then, a gradual decrease in plasma iron level was observed till 16 h post-treatment. Further significant increases in iron levels ($p > 0.05$) were observed, with both preparations, at 20 and 24 h after treatment as compared to zero time or preceding values. Fefol-Z® achieved significantly greater ($p < 0.05$) plasma iron levels at 5 and 6 h post-drug administration (Fig. 1). While Folifer-Z® preparation achieved greater plasma iron levels at 1, 2, 3, 12, 14, 16, 20 and 24 h post-drug administration (Fig. 1).

The mean ± S.D. of C_{max} values for Folifer-Z® and Fefol-Z® were 103 ± 46.2 and 109 ± 41.5 μg/dl, respectively (Table 2). Paired Student's t-test statistical analysis indicated that the C_{max} values were significantly greater ($p > 0.001$) for Folifer Z® and Fefol-Z® as compared to their corresponding zero time values (Fig. 1). This significant increase is a strong indication for absorbing iron from both products after oral administration.

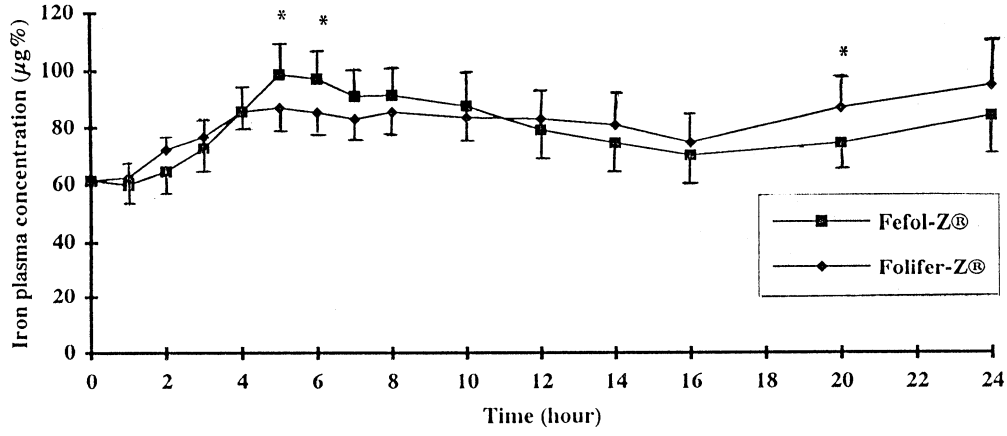

Fig. 1. Mean plasma iron concentration-time curves for 28 subjects following oral administration of Fefol-Z® or Folifer-Z®. Vertical bars represent S.E.M. $*p < 0.05$ compared to corresponding point value.

The time required to reach the C_{max}, the T_{max} was 5.93 ± 2.94 and 6.64 ± 2.54 h for Folifer-Z® and Fefol-Z®, respectively (Table 2, Fig. 1). This difference is significant ($p < 0.05$) in the log-transformed data. T_{max} is a parameter of the release mechanism of drug formulation. Thus, the tablet formulation of the test drug, Folifer-Z® is significantly faster in releasing iron than the reference capsule Fefol-Z®. The area under the concentration time curve ($AUC_{0-24\ h}$) values were 1937 ± 706 and 1865 ± 699 μg/h per dl for Folifer-Z® and Fefol-Z®, respectively (Table 2). A two-way analysis of variance (ANOVA) performed on linear and log-transformed data revealed no statistical significant differences among values of C_{max} and $AUC_{0-24\ h}$ of Folifer-Z® and Fefol-Z® preparations (Table 2). The two one-sided hypotheses at the $\alpha = 0.05$ level of significance was tested for AUC and C_{max} by constructing the 90% confidence interval for the ratio between the Folifer-Z® and Fefol-Z® (Table 3). Regarding the AUC-ratio, mean value was 1.07 and the confidence interval range was 99–115%. Meanwhile, the C_{max}-ratio mean value was 0.96 and the confidence interval range was 88–105%. Thus the 90% confidence intervals of AUC-ratio and C_{max}-ratio lie within the acceptable bioequivalence range of 80–120%. The 90% confidence interval test revealed that two subjects (no. 20 and 34) were outliers on the basis that their AUC-ratios were more than 2 S.D. above the mean, (Table 3) therefore, all their values were shown but not included in data calculations of the iron study. Further statistical analysis using Student's t-test for C_{max}, T_{max} and $AUC_{0-24\ h}$ of both formulations were found to be not significant.

In summary, based on the insignificant differences in iron pharmacokinetic data, AUC, C_{max} and on their relative bioavailability ratios, it is concluded that the two products are bioequivalent.

3.2. Zinc

The mean ± S.E.M. of plasma zinc concentration-time curves for both preparations are presented in Fig. 2. This figure demonstrates an initial significant decrease ($p > 0.05$) in plasma zinc level which persisted for 3 h. Afterwards, zinc levels started to rise to a peak value at 6 h then declined before rising again to the initial base line. The zigzagging pattern of zinc absorption curve could be due to many reasons, such as variations among subjects, circadian rhythm and or iron-zinc interactions (Section 4). Plasma zinc values of Fefol-Z® achieved significantly ($p < 0.05$) greater plasma zinc values at 4, 5, 6, 7 and 24 h (Fig. 2) compared to Folifer-Z®. However both formulations exhibited a fluctuating plasma zinc curve pattern. In general, the plasma zinc levels in both

preparations were less than the corresponding values at zero time (Fig. 2). The mean C_{max} values were 101 ± 20.7 and 102 ± 20.7 μg/dl for Folifer-Z® and Fefol-Z®, respectively (Table 4). The time required to reach the C_{max} values, T_{max} was 4.86 ± 1.53 and 4.93 ± 1.51 h for Folifer-Z® and Fefol-Z®, respectively (Table 4). In addition, the area under the concentration-time curve ($AUC_{0-24\ h}$) were 1944 ± 202 and 1953 ± 200 μg/h per dl for Folifer-Z® and Fefol-Z®, respectively. The differences between values of both preparations, were insignificant for T_{max}, C_{max} and $AUC_{0-24\ h}$ (Table 4). Furthermore, the statistical analysis based upon variance homogenity, the two-way analysis of variance was performed on linear and $\log_{10}$-transformed data for C_{max}, T_{max}, and $AUC_{0-24\ h}$ of both preparations. Such analysis exhibited no significant differences (Table 4). Regarding the AUC-ratio, the mean value was 0.98 and the 90% confidence interval range was 95–101% (Table 5). The C_{max}-ratio mean value was 0.92 with a 90% confidence interval range 89–96% (Table 5). Thus, the 90% confidence interval test of AUC-ratio and C_{max}-ratio lie within the acceptable bioequivalence range of 80–120%. The 90% confidence interval test revealed that the subject no. (34) was outlier (on the basis of AUC-ratio which was more than 2 S.D. above the mean), therefore, all his values were shown but not included in data calculations of the zinc study (Table 5). Further statistical analysis using Student's *t*-test for C_{max}, T_{max} and $AUC_{0-24\ h}$ of both formulations were found to be not significant.

Based on the zinc pharmacokinetic data, AUC, C_{max}, T_{max} and on their relative bioavailability ratios, it is concluded that the two products are bioequivalent.

3.3. Side effects

Both products were well tolerated since only seven (23%) subjects who received Fefol-Z® complained of mild headache that lasted for 2 h following drug administration and one subject complained of mild heartburn that persisted throughout the study. With respect to Folifer-Z®, four subjects (13%) complained of mild headache that lasted for 2 h following drug administration and one subject complained of mild abdominal pain. No other serious side effects were observed.

4. Discussion

The objective of this study was to establish the pharmacokinetic parameters of Folifer-Z®, a new formulation of zinc, iron and folic acid, by comparing it to Fefol-Z® as a reference formula. In order to achieve this goal, the following two procedures were taken in consideration. First, the subjects were asked to donate one unit of blood 4 days prior to their participation in the study. Such blood donation resulted in a significant decrease ($p < 0.05$) in their plasma iron levels. This significant iron decrease is in agreement with what has been reported by Milman and Sondergaard (1984). These authors found that at each phlebotomy of 500 ml, an average of 253 mg of iron was lost. According to the calculation of Finch et al. (1977), an individual donation of one unit of

Table 2
A comparison of the mean ($\pm$S.D.) pharmacokinetic values of iron derived from the concentration-time curves of orally administered Fefol-Z® and Folifer-Z® to 28 subjects

Pharmacokinetic parameter	Fefol-Z®	Folifer-Z®	*p* value*	
			Untransformed data	Log_{10}-transformed data
$AUC_{0-24\ h}$ (μg/h per dl)	1865 ± 699	1937 ± 706	0.363	0.267
C_{max} (μg/dl)	109 ± 41.5	103 ± 46.2	0.267	0.165
T_{max} (h)	6.64 ± 2.54	5.93 ± 2.94	0.052	0.038**

* Two-way analysis of variance was performed for statistical significance.

** Statistically significant ($p < 0.05$).

Table 3
Individual relative bioavailability of iron ($AUC_{0-24\ h}$ and C_{max} ratios) of Folifer-Z® to Fefol-Z®

Subject number	$AUC_{0-24\ h}$ (μg/h per dl)			C_{max} (μg/dl)		
	Reference	Test	Ratio (T/R)	Reference	Test	Ratio (T/R)
2	1731	2379	1.4	79	122	1.5
4	1680	1843	1.1	87	94	1.1
6	1090	1803	1.7	53	84	1.6
8	2110	2258	1.1	113	127	1.1
10	1726	1995	1.2	109	113	1.0
12	801	902	1.2	47	42	0.9
14	2043	2078	1.0	155	98	0.6
16	1929	1849	1.0	135	79	0.6
18	1334	1783	1.3	78	91	1.2
20[a]	1227	2452	2.0	84	85	1.0
22	1915	1946	1.0	118	103	0.9
24	2120	2033	1.0	142	48	0.8
26	2208	2399	1.1	140	139	1.0
28	1410	1968	1.4	91	102	1.1
30	1756	2058	1.2	109	88	0.8
32	1762	1897	1.1	121	89	0.78
34[a]	872	1852	2.1	53	93	1.8
36	1827	1513	0.8	108	68	0.6
31	2230	2161	1.0	125	136	1.1
33	1384	1392	1.0	80	77	1.0
37	880	1040	1.2	47	37	0.8
39	1782	1361	0.8	95	72	0.8
41	2759	2305	0.8	164	116	0.7
43	1486	1363	0.9	75	58	0.8
45	1520	1048	0.7	115	78	0.7
47	1459	1136	0.8	66	58	0.9
49	1036	1667	1.6	56	79	1.4
50	3698	4575	1.2	200	229	1.1
51	3212	2902	0.9	173	240	1.4
52	3340	2589	0.8	182	141	0.8
Mean			1.07			0.96
Number			28			28
C.V. (%)			22.7			28.5
90% C.I. range			99–115			88–105

[a] Outlier values not included in calculations.

blood resulted in an increase in the daily iron requirement by 0.65 mg/day in the male and 0.58 mg/day in the female. Second, we adopted a two-way crossover design to study the bioavailability of iron and zinc. This method is widely used as a measure of iron absorption by several investigators and was proved to be comparable with the whole body radioactive iron method (Ekenved et al., 1972; Dietzfelbinger, 1987). Although, serum iron is not only dependent on the amount of iron absorbed per unit time, but also on the internal flow of iron to and from serum. Still it might be possible to compare the relative bioavailability of iron from different preparations by studying the increase in serum iron in the same subject (Ekenved et al., 1972).

In this study, Fefol-Z® and Folifer-Z® products exhibited typical sustained-release characteristics. Both formulations showed no sharp rises nor drops in plasma iron levels during the 24 h of the

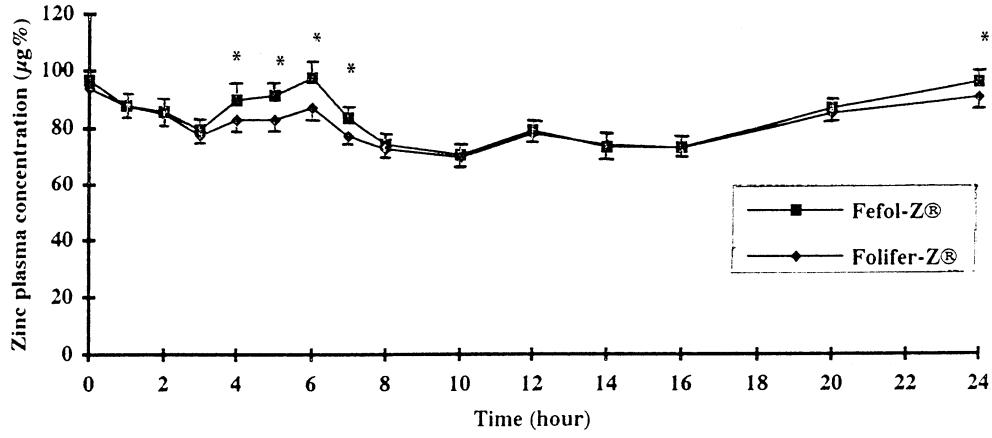

Fig. 2. Mean plasma zinc concentration-time curves for 28 subjects following oral administration of Fefol-Z® or Folifer-Z®. Vertical bars represent S.E.M. $*p < 0.05$ compared to corresponding point value.

study period. The observed rise in plasma iron levels which occurred at 16, 20 and 24 h post-drug administration may be due to the absorption of iron from a distal site of the gastrointestinal tract, e.g. colon (Chlou et al., 1997). Sustained-release preparations are designed to release iron in the lower, rather than, the upper small intestine. Therefore, patients who cannot tolerate conventional oral iron dosage forms because of their side-effects, may obtain at least as much iron with fewer unpleasant symptoms if they use a sustained-release formulation (Nielsen et al., 1976; Cook et al., 1982).

Regarding the zinc data obtained from this study, both products Folifer-Z® and Fefol-Z®, yielded similar zinc absorption profiles. Similar to iron, zinc absorption from the gastrointestinal tract is regulated via two mechanisms, a non-saturable process, which is not affected by zinc intake, and a saturable process, which is stimulated by zinc depletion and is likely due to a specific carrier-mediated high-affinity mechanism (Gordon et al., 1982). In contrast to iron, zinc has no stores in the human body and its plasma concentration is hormonally regulated (Couturier et al., 1988). Circadian variations in plasma zinc in man has been reported (Couturier et al., 1988). The authors indicated that the time course of daily plasma cortisol fluctuations were parallel to that of zinc. Such circadian rhythm of zinc was independent of zinc intake and was closely related to the circadian rhythm of cortisol (Couturier et al., 1988). The zinc data of our study exhibited a large fluctuation in plasma zinc profiles with both products. The data shows an initial phase of decline ($p < 0.05$) in plasma zinc concentration of Folifer-Z® and Fefol-Z® after 1 h which lasted for 3 h post-drug administration (Fig. 2). Such initial de-

Table 4
Comparison of mean ($\pm$S.D.) phamacokinetic values of zinc derived from the concentration-time curves of orally administered Fefol-Z® and Folifer-Z® to 28 subjects

Pharmacokinetic parameter	Fefol-Z®	Folifer-Z®	p-value*	
			Untransformed data	Log_{10}-transformed data
$AUC_{0-24\ h}$ (μg/h per dl)	1953 ± 200	1944 ± 202	0.428	0.601
C_{max} (μg/dl)	102 ± 20.7	101 ± 20.7	0.602	0.492
T_{max} (h)	4.93 ± 1.51	4.86 ± 1.53	0.994	0.933

* Two-way analysis of variance was performed for statistical significant.

Table 5
Individual relative bioavailability of zinc ($AUC_{0-24\ h}$ and C_{max} ratios) of Folifer-Z® to Fefol-Z®

Subject number	$AUC_{0-24\ h}$ (μg/h per dl)			C_{max} (μg/dl)		
	Reference	Test	Ratio (T/R)	Reference	Test	Ratio (T/R)
2	1779	1743	1.0	77.5	75.8	1.0
4	1775	1990	1.1	80.0	88.5	1.1
6	1653	1944	1.2	73.8	86.7	1.2
8	2054	1593	0.8	94.8	68.2	0.7
10	2048	1684	0.8	108	89.9	0.8
12	1670	1747	1.0	83.3	94.0	1.1
14	1939	1923	1.0	97.5	84.5	0.9
16	2196	2074	0.9	109	91.5	0.8
18	2064	1815	0.9	102	93.3	0.9
20	2225	2255	1.0	112	99.5	0.9
22	2089	1956	0.9	113	105	0.9
24	2072	1924	0.9	115	90.8	0.8
26	2078	2111	1.0	129	97.3	0.8
28	2049	1795	0.9	102	77.8	0.8
30	2370	2262	1.0	115	113	1.0
32	2001	2019	1.0	107	100	0.9
34[a]	1695	2164	1.3	79.5	99.3	1.2
36	1977	1988	1.0	103	96.0	0.9
31	2312	2085	0.9	111	92.8	0.8
33	1809	1699	0.9	80.5	78.3	1.0
37	1900	1772	0.9	86.7	77.3	0.9
39	1837	1797	1.0	94.0	86.3	0.9
41	1676	1947	1.2	80.0	83.0	1.0
43	1843	1860	1.0	86.0	82.0	1.0
45	1702	1679	1.0	80.0	76.8	1.0
47	1624	1855	1.1	78.1	80.8	1.0
49	1937	1896	1.0	140	122	0.9
50	2089	1878	0.9	159	126	0.8
51	1922	1809	0.9	126	127	1.0
Mean			0.98			0.92
Number			28			28
C.V. (%)			9.62			12.3
90% C.I. range			95–101			89–96

[a] Outlier values not included in calculation.

cline could be due to circadian rhythm and/or the presence of high levels of ferrous sulfate delivered by both formulations in the subjects intestine. Dursun and Aydogan (1995) reported that the absorption of zinc is inhibited in the presence of high levels of iron. An interesting study conducted by Solomons and Jacob (1981) on iron-zinc interaction in human subjects, demonstrated a progressive reduction in the AUC of plasma zinc concentration when zinc (25 mg) was administered concurrently with increasing doses of ferrous iron. In other reports, iron deficiency induced by bleeding did not enhance zinc absorption from the gastrointestinal tract (Flanagan et al., 1980). Therefore, it was concluded by Solomons that the interaction of zinc and iron in the human intestine occurred in both the lumen and at some intracellular location distal to the site of regulation of iron absorption (Solomons, 1983). The results of our study also support the possibility of a significant zinc–iron interaction. A change in the formulation of such products may

be required. Such changes should be directed to enhance zinc release in the first hours after ingestion and then the iron content is released later. Afterwards, further clinical studies of such suggested formulations are recommended in order to ensure maximal absorbtion of both elements. Many commercial preparations contain zinc along with iron and other ingredients which should have a great potential of interaction. Therefore, the promotion of such products is warranted.

Although headache, as a side effect of oral iron preparation administration, is not known, yet 13% and 23% of subjects complained of mild headache following Folifer-Z® and Fefol-Z® administration, respectively. This headache could be due to food restrictions (such as xanthine containing beverages) or abstinence from smoking or due to the strict procedure of the protocol, i.e. hospitalization, venous canulation and blood drawing. Anyhow, the experienced headache was transient and lasted for 2 h after the administration of the formulations. Other side effects such as mild heartburn observed in one subject following Fefol-Z® administration and abdominal pain in another subject following Folifer-Z® administration are known genuine side effects of iron preparation. Gastrointestinal side effects such as nausea and epigastric discomfort commonly occur in patients on conventional oral iron therapy such as ferrous sulfate tablets. These symptoms are believed to correlate with a high concentration of ferrous ions in the gastric mucosa. Sustained-release iron medications are intended to slowly release their contents in the gastrointestinal tract and thus produce less gastrointestinal side effects (Levy et al., 1978; Cook et al., 1982). In general, both products, Fefol-Z® and Folifer-Z® were well tolerated.

In conclusion, the present study has shown that both Folifer-Z® and Fefol-Z® pharmacokinetic parameters such as AUC, and C_{max} for iron and AUC, C_{max} and T_{max} for zinc were similar. No statistical differences were observed. Thus, although the preparations are two different formulations of a sustained-release preparation, they are bioequivalent.

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