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A pharmaco-scintigraphy study of riboflavin 5′-phosphate sodium capsules (1 vs. 4)

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Abstract

The comparative in-vivo disintegration, gastric emptying and bioavailability of 41 mg radiolabeled riboflavin 5′-phosphate sodium capsules administered as a single hard gelatin capsule or as four capsules was evaluated in human subjects. A total of 41 mg of riboflavin 5′-phosphate sodium and 7 MBq of technetium sulfur colloid were incorporated in a single gelatin capsule or the same dose was administered in four capsules. A randomized crossover design was conducted in eight subjects under fasting conditions. Capsule disintegration and gastric emptying was measured by γ scintigraphy and the relative amount of riboflavin excreted in the urine was measured by HPLC. The in-vivo disintegration time and gastric emptying of the four capsules was significantly faster than for the one capsule. The bioavailability of the four capsule regimen was 16.5 versus 11.2% for the one capsule. The amount of riboflavin excreted in the urine in the 0–2 h interval was significantly greater for the four versus one capsule. The in-vivo performance of one 41 mg riboflavin 5′-phosphate sodium capsule is significantly different than the equivalent dose administered in four capsules. Further studies are needed to understand the underlying mechanism for the difference between the regimens. © 1997 Elsevier Science B.V.

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1. Introduction

The design and development of a pharmaceutical dosage form is a costly process with great penalties for progression into clinical trials with

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the wrong dosage form or formulation. γ Scintigraphy allows a formulator to understand and predict the performance of dosage forms early in the development process, before the initiation of more costly clinical or pharmacokinetic studies. Specifically, information on in-vivo disintegration and gastrointestinal transit time can be useful in assisting the formulator to design the most appropriate dosage form.

During early clinical testing of a new chemical entity, several capsules are often administered to constitute the desired dose and it is generally assumed that the number of orally administered capsules has no effect on drug bioavailability. However, Abolin recently studied the bioavailability of four 7.5 mg immediate release capsules with a single 30 mg capsule of temazopam (Abolin et al., 1993). The two formulations were bioequivalent in respect to extent of absorption, however the four 7.5 mg capsules reached peak plasma concentration significantly faster than the 30 mg capsule (mean $T_{max.}$ 1.18 and 1.73 h, respectively). This may be attributed to faster wetting and dissolution due to the greater surface area of the four capsules, however a hypothesis for the difference was not reported. Scintigraphy can be used to examine these types of formulation questions by providing the formulator with visual and quantitative information on the in vivo disintegration and gastric emptying of solid oral dosage forms.

This study combines scintigraphy with conventional bioavailability techniques to study the effect of the number of orally administered hard gelatin capsules on the in vivo disintegration, gastric emptying and bioavailability of riboflavin 5′-phosphate sodium.

2. Materials and methods

2.1. Drug substance selection

Riboflavin 5′-phosphate sodium was selected as the test drug because it does not have any readily observable pharmacological action following oral administration and the pharmacokinetic profile of riboflavin has been well characterized (Levy and Jusko, 1966; Jusko et al., 1970; Levy and Hewitt, 1971; Marcus and Coulson, 1990). It has a history of use as a tracer for drug compliance and has been used in the evaluation of drug interactions following oral administration (Levy et al., 1972; Feldman and Hedrich, 1983; Babiker et al., 1989).

2.2. Study design

The study was a randomized, crossover design in eight healthy male subjects (ages 21–38 years; weights 62–85 kg) conducted under a protocol approved by the Albany Medical Center Institutional Review Board. Subjects refrained from taking any drugs or vitamins from 1 week prior to the study to completion, were in good health and had no history of gastrointestinal tract surgery. Each subject provided written consent.

2.3. Formulation, dosing and sample collection

Subjects received 41 mg of riboflavin 5′-phosphate sodium (30 mg riboflavin) and 7 MBq technetium-99m sulfur colloid in one white opaque No. 1 hard gelatin capsule or the same dose administered in four No. 1 hard gelatin capsules. The capsules contained riboflavin 5′-phosphate sodium (Roche Vitamins and Fine Chemicals, Nutley, NJ), hydrous lactose (Sheffield, Norwich, NY), microcrystalline cellulose (Avicel PH 101, FMC Corporation, Philadelphia,

Table 1
Formulations of radiolabeled riboflavin 5′-phosphate sodium capsules

	One capsule (mg)	Four capsules (mg)
Riboflavin 5′- phosphate sodium dihydrate	41[a]	10.25[b]
Lactose, hydrous microcrystalline cellulose (Avicel PH 101)	194	223
Magnesium stearate	65.9	65.9
Tc-99m sulfur colloid	1 MBq	1 MBq

[a] Equivalent to 30 mg riboflavin.
[b] Equivalent to 7.5 mg riboflavin.

Table 2
In-vitro test results for the radiolabeled riboflavin 5′-phosphate sodium capsules

	Method	Target	Results (%)
10.25 mg capsule	Content uniformity	85–115%	98
		RSD <6.0%[a]	3.0
	Dissolution at 15, 30 min (water apparatus 2)	80% in 30 min	95 in 15 min
41 mg capsules	Content uniformity	85–115%	97.5
		RSD <6.0%	3.5
	Dissolution at 15, and 30 min. (water, apparatus 2)	80% in 30 min	97 in 15 min

[a] RSD, relative standard deviation.

PA), magnesium stearate (Samrak, Bronxville, NY). The capsules were labeled with technetium Tc-99m sulfur colloid prepared from technetium Tc-99m sodium pertechnetate obtained from a technetium-99m generator (Mallinckrodt Medical, St. Louis, MO) and a kit for the preparation of technetium Tc-99m sulfur colloid injection (CIS-US, Bedford, MA). The capsule blends were prepared in a mortar and pestle using geometric dilution and hand packed into No.1 hard gelatin capsules (Elanco, Indianapolis, IN). The quantitative formulations are listed in Table 1. A total of ten individual capsules were assayed for riboflavin (US Pharmacopeia Convention, 1995) and dissolution was performed using apparatus 2 in 900 ml of distilled water.

The riboflavin absorption study was carried out as a modification of the method described by Feldman and Hedrich (1983). After an overnight fast, subjects consumed a hard roll, a sweet roll and 50 ml of tap water, 30 min prior to administration of the riboflavin 5′-phosphate sodium-Tc sulfur colloid capsule(s). The capsule(s) were administered with 50 ml of water at room temperature. Total urine samples were collected at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 24, 30 and 36 h. A 50 ml volume of water at room temperature was given after each urine sample for the first 3 h after which fluids were permitted ad libitum. Glacial acetic acid (3 ml/100 ml urine) was added to the urine as a stabilizer and samples were stored in a freezer at -20°C until analysis. The blank urinary excretion was obtained from a 12 h urine sample collected on the day before the capsules were administered.

2.4. Riboflavin analysis

Riboflavin urinary concentrations were measured using a reversed-phase, high-performance liquid chromatographic (HPLC) method with fluorescence detection developed in our laboratory. A mobile phase consisting of 13% acetonitrile and 87% phosphate buffer (potassium phosphate monobasic [20 mM]; tetrabutylammonium hydroxide [40% w/w, 5 mM] adjusted to pH 3.0 with 85% phosphoric acid) was pumped through a 5 μm, reversed-phase C_{18} column (150×4.6 mm; Ultrasphere; Beckman Instruments, Fullerton, CA) at a flow rate of 1 ml/min. The signal was recorded by fluorescence detection with excitation and emission wavelengths of 450 and 535 nm, respectively.

A 1 ml aliquot of each standard, control and unknown subject specimen was passed through a syringe filter (0.22 μm $\times$ 3 mm; Corning Laboratories, Corning, NY) before injection. The injection volume was 25 μl and each sample was assayed in duplicate. The retention time for riboflavin was 4.7 min. The limit of quantitation was 0.098 μg/ml; this represents a signal to noise ratio of five. Peak area of riboflavin (Riboflavin USP, Sigma, St. Louis, MO) spiked urine was linear over the range of 0.098–50 μg/ml. Correlation coefficients for each subject's standard curve ($n = 11$ concentrations per curve) was >0.99. Within-day and between-day coefficients of variation for high (50 μg/ml), medium (6.25 μg/ml) and low (0.781 μg/ml) controls bracketing every four subject unknowns was less than 8%. A priori acceptance criteria of unknown concentration de-

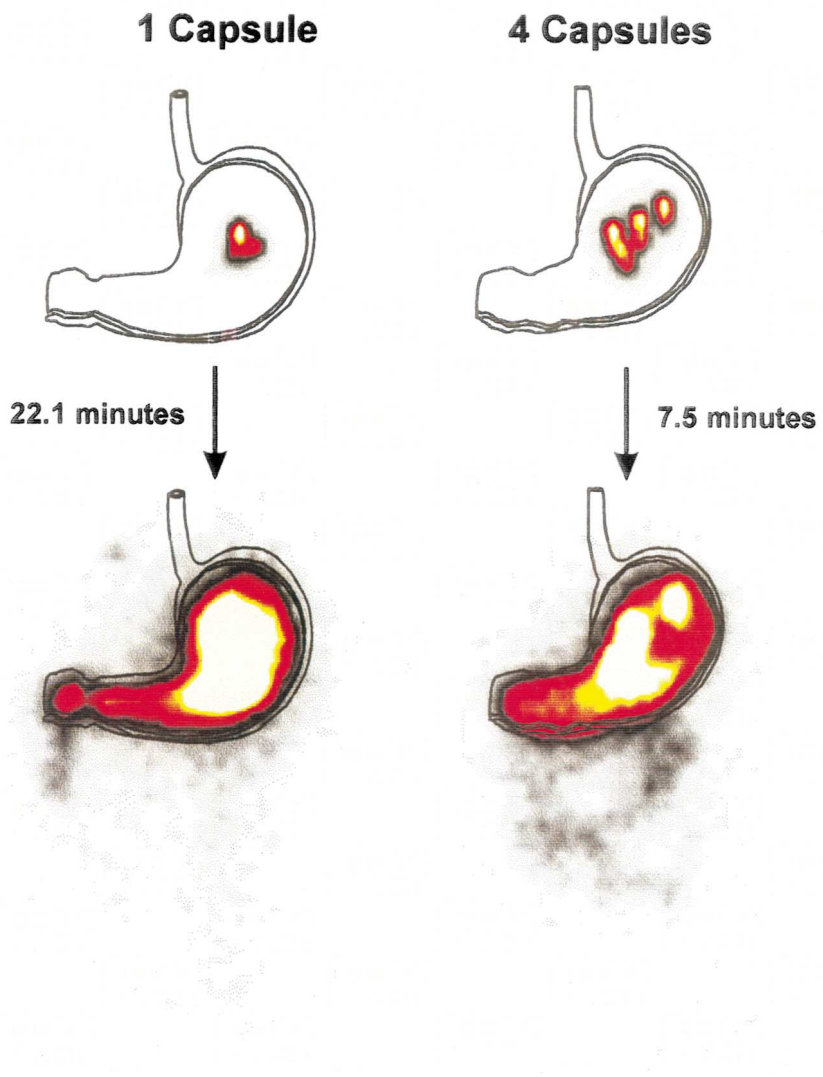

Fig. 1. Scintigraphic images depict the typical disintegration process in the stomach of one (left) versus four (right) hard gelatin capsules. The times shown are mean interval to disintegration.

terminations were that control samples be within 15% of theoretical values and that each subject specimen be within 15% of the mean value from duplicate measurements.

2.5. Imaging protocol and analysis

Imaging was performed in the supine position with large field of view gamma cameras (Siemens Medical Systems, Hoffman Estates, IL) each fitted with a low energy all purpose collimator. Serial 2.5 min scintiphotos were obtained immediately after ingestion of the capsules through 3.0 h post dosing. Digitized scans were transferred to an IBM compatible personal computer for region of interest (ROI) analysis. For each subject ROIs were drawn around the stomach. Decay corrected time-activity curves for the stomach were plotted. From these curves the time to one half and one quarter of the maximum activity and the AUC

were calculated. The disintegration time was determined by visual inspection of the image data and was defined as the time at which radioactivity was uniformly distributed throughout the stomach. Uniform distribution was defined as the complete filling of the stomach volume with a homogeneously intense activity and the focal intensity due to capsule activity was indistinguishable from the remainder of the stomach contents as determined by a nuclear radiologist.

3. Results and discussion

3.1. In vitro capsule evaluation

The in-vitro results for both capsule formulations are shown in Table 2. The content uniformity was within the guidelines established in the USP XXIII. The dissolution profile in water for both formulations was rapid and completed by the 15 min interval. This was expected for a highly soluble (43 mg/ml, pH 3.8) drug formulated in a rapidly disintegrating and dissolving capsule formulation.

3.2. In vivo capsule performance

One subject was excluded from the analysis because he took fluoxetine which is known to increase gastric motility (Olin, 1994). Fig. 1 is a representative gastric scintigraphic image of the disintegration process of one versus four capsules in the stomach. A summary of the formulations in-vivo disintegration and gastric emptying times is listed in Table 3. The mean in-vivo disintegration time was 22 min for one capsule as compared to 7.5 min for four capsules. The paired difference comparison for the mean disintegration time is statistically significant ($P < 0.05$). The $T_{1/2}$ mean gastric emptying time was 78.9 min for the one capsule and 28.2 min for the four capsules. The paired difference test comparison for (AUC), $T_{1/2}$ max. and $T_{1/4}$ max. are statistically significant ($P < 0.05$). As shown in Fig. 2, the difference between the individual gastric emptying profiles of one versus four capsules is consistently different.

Table 3
In-vivo disintegration and gastric residence times for one versus four capsules

	One capsule	Four capsules	P-value
T_{dis}[a]	22.1 (10.8)[b]	7.5 (2.9)	<0.05
AUC[c]	2623.9 (726.8)	1406.5 (513.1)	<0.05
$T_{1/2}$ Max[d]	78.9 (32.8)	28.2 (12.2)	<0.05
$T_{1/4}$ Max[e]	116.1 (37.9)	59.6 (23.6)	<0.05

[a] Time (min) to dosage form disintegration.
[b] Mean $\pm$ S.D.
[c] Area under the time versus activity curve.
[d] Time (min) to half the maximal gastric activity.
[e] Time (min) to 1/4 of the maximal gastric activity.

Lagas et al. studied the in-vivo disintegration time of pure benotylate versus a lyophilized version in a single hard gelatin capsule (Legas et al., 1980). The superior wetting of the lyophilized drug resulted in significantly faster in vivo disintegration. Digenis reported the in vivo disintegration time of a water soluble formulation containing etidronate sodium dosed on an empty stomach (Digenis, 1982). The initial disintegration was rapid with the onset of disintegration in 6 min, complete disintegration occurred in 30 min. Fell reported an in-vivo disintegration time of 15 min for acetylsalicylic acid granules in one capsule (Fell and Degenis, 1984). In this study the disintegration time for one capsule is similar to the results reported by previous researchers, however there was significantly faster disintegration time for four riboflavin 5′-phosphate sodium capsules as compared to one. This finding was unexpected for highly soluble, rapidly dissolving capsule formulations.

3.3. Riboflavin bioavailability

Riboflavin is absorbed in the proximal intestinal tract, and not from the colon (Levy and Jusko, 1966). It is absorbed by a saturable, specific transport mechanism that is not saturated following oral administration of 30 mg riboflavin when the riboflavin is administered with a meal (Campbell and Morrison, 1963; Levy and Jusko, 1966). Levy and Jusko studied normal males in whom riboflavin was administered orally immedi-

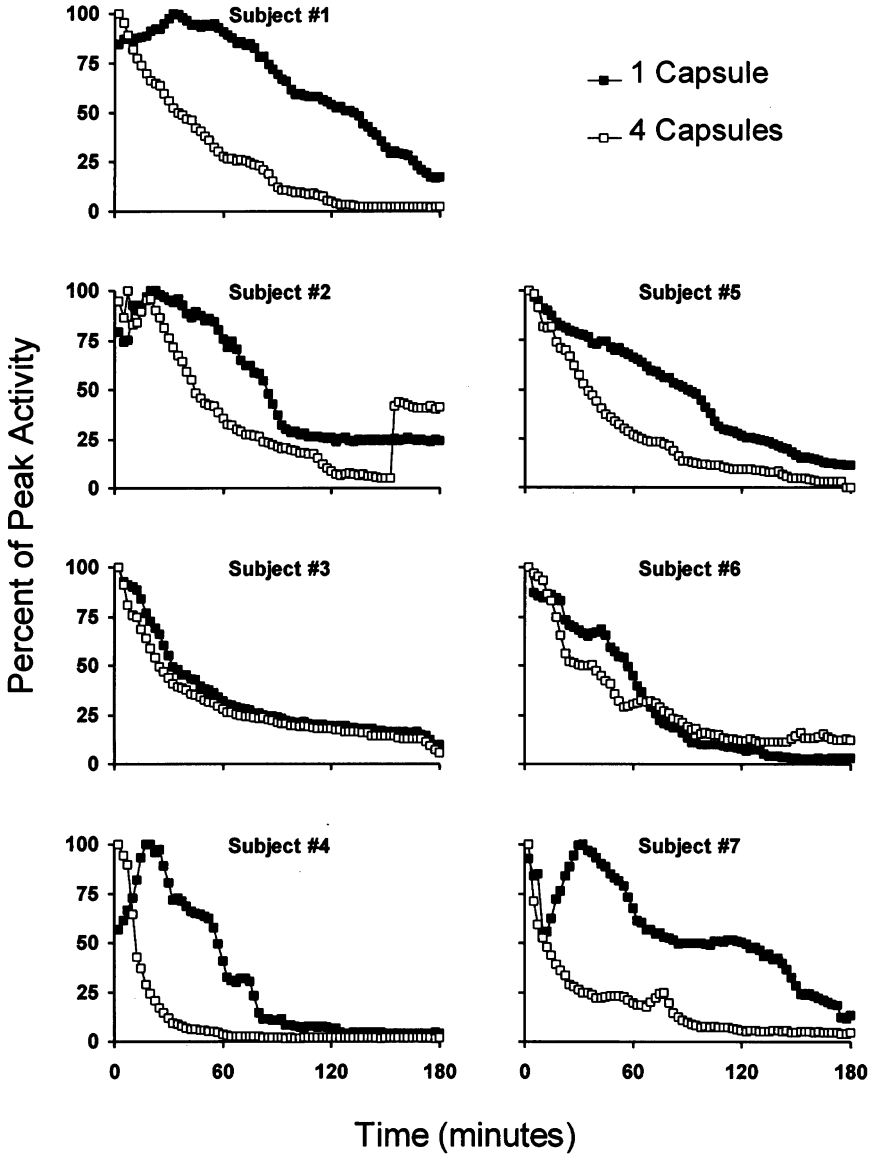

Fig. 2. Individual gastric emptying profiles comparing the performance of one versus four capsules. Percent of maximum radioactivity in the stomach versus time (min).

ately after a standard breakfast (Levy and Jusko, 1966). They reported a linear relationship between the dose and the amount recovered in the urine for doses up to 30 mg. The average recovery after administration of 30 mg or riboflavin was 62%. Under similar conditions Morrison and Campbell also found a linear relationship between dose and amount recovered in the urine for doses up to 20 mg (Fell and Degenis, 1984). They reported recoveries of 62% after administration of 30 mg of riboflavin. Urinary recoveries in the fasting state are drastically reduced. After a 30 mg dose of riboflavin the urinary recovery is reported to be 15.7, 19.3 and 21.4% (Levy and Jusko, 1966; Feldman and Hedrich, 1983; Levy and Hewitt, 1971).

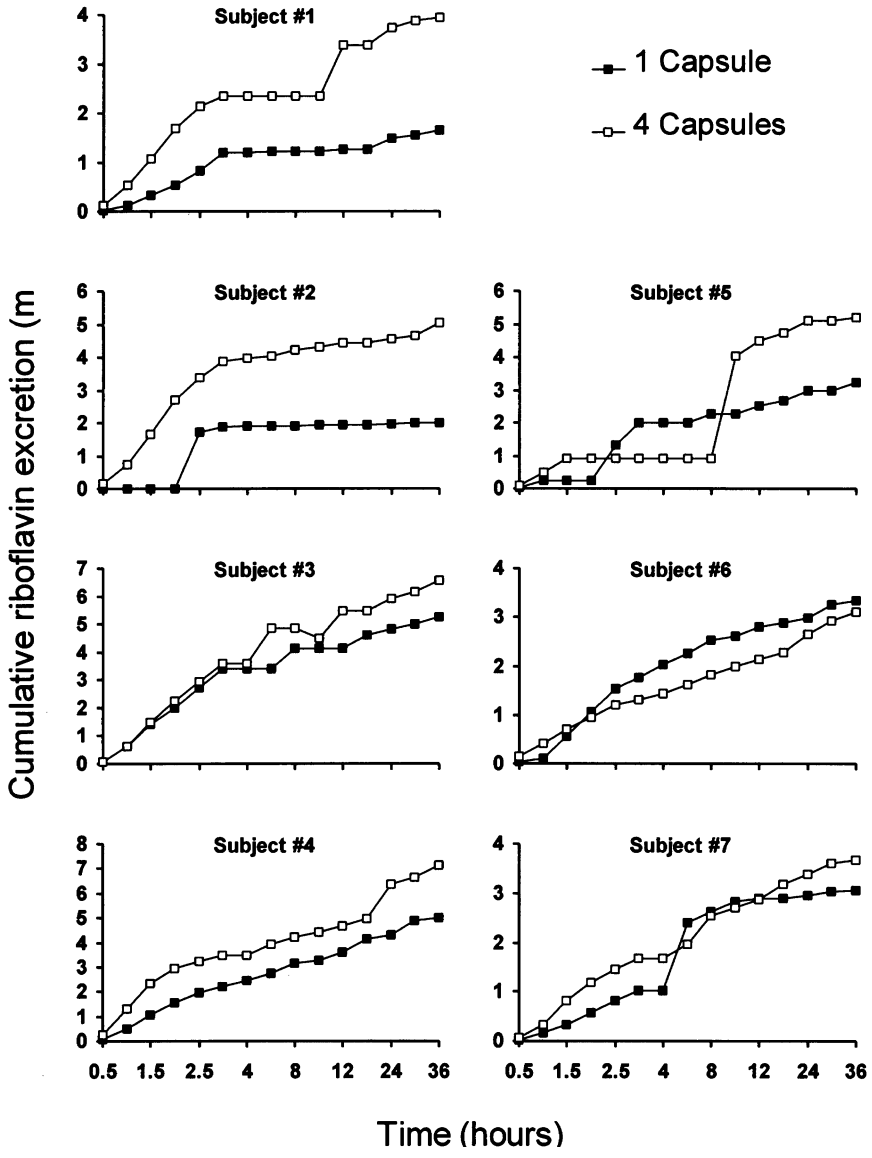

Fig. 3. Individual cumulative urinary riboflavin excretion profiles of one versus four capsules.

In this study, the relative extent of riboflavin excretion over the 36 h collection period was significantly greater for the four versus one capsule regimen (4.96 ± 1.51 mg versus 3.36 ± 1.36 mg, $P = 0.009$). This corresponds to an average fraction excreted in the urine of $16.5 \pm 4.66\%$ and $11.2 \pm 4.21\%$ for the four and one capsule regimens, respectively. Fig. 3 depicts the cumulative amount of riboflavin excreted in the urine, for each subject, from 0 to 36 h for both regimens. In addition to the overall extent of riboflavin excretion being greater, the four capsule regimen also resulted in a significantly greater cumulative drug excretion over the 0–2 h interval (1.66 ± 0.734 mg for four capsules versus 0.860 ± 0.719 mg for one capsule; $P = 0.015$). The faster rate of drug excretion over the 0–2 h interval is attributed to the faster disintegration and gastric emptying of the

four versus the one capsule regimen. The urinary recovery of riboflavin for both regimens was similar to the results of previous studies under fasting conditions. In this study, both regimens were dosed 30 min after a light breakfast. It appears that the light breakfast does not convert the gastric emptying profile to that of the fed condition resulting in bioavailability consistent with dosing in the fasting state.

The faster disintegration and gastric emptying time of the four capsules may be attributed to enhanced wetting and subsequent disintegration as a result of the higher lactose content in the 10.25 mg capsules. This hypothesis seems highly unlikely due to the high solubility of the drug substance and the rapid in-vitro dissolution profile of the two formulations. The enhanced emptying of the four capsules may be due to a chemical stimulant effect of the gelatin itself. This also seems unlikely because the total gelatin load of the four capsules is only 320 mg, as compared to 80 mg for one capsule and there is no published literature indicating the chemical stimulant effect of gelatin. Another possibility is a direct mechanical stimulation of the vagal receptors in the antrum stimulated by the coadministration of four relatively large capsules. In this situation, the four capsules disintegrate rapidly and the enhanced motor activity may more rapidly propel the disintegrated contents into the duodenum. Further studies are needed to confirm this propose mechanism responsible for the enhanced emptying of four hard gelatin capsules compared to one capsule.

4. Conclusion

Capsule disintegration time and gastric emptying rates are observed to be faster and the fraction of riboflavin excreted in the urine is greater when the dose of riboflavin is subdivided into four capsules relative to one capsule. These results suggest that bioavailability is not only affected by the capsule formulation, but also may be affected by the number of gelatin capsules used to constitute the drug dose. The clinical practice of dosing multiple capsules to constitute the therapeutic dose may result in unexpected bioavailability differences.

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