BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **26**: 403–409 (2005)
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI**: 10.1002/bdd.469

Multiple Dose Pharmacokinetics of Caffeine Administered in Chewing Gum to Normal Healthy Volunteers

Shariq A. Syed[a], Gary H. Kamimori[b], William Kelly[b] and Natalie D. Eddington[a,*]
[a] *Pharmacokinetics-Biopharmaceutics Laboratory, Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland at Baltimore, AHB 540A, 100 Penn Street, Baltimore, MD 21201, USA*
[b] *Department of Neurobiology and Behavior, Walter Reed Army Institute of Research, 503 Robert Grant Road, 2W 97 Silver Spring, MD 20910-7500, USA*

ABSTRACT: The purpose of this study was to examine the pharmacokinetics of three doses of caffeine administered as *Stay Alert*® chewing gum in a multiple dose regimen.

Methods: A double-blind, parallel randomized, four-treatment study design was employed. The treatment groups were: 50, 100 and 200 mg caffeine and placebo. Subjects were 48 ($n = 12$ per group), healthy, non-smoking, males and females who had abstained from caffeine ingestion for at least 20 h prior to dosing, who were randomly assigned to the treatment groups. Caffeine was administered at 2400, 0200 and 0400 h depending on the treatment group. Blood samples were collected pre-dose and at 5, 15, 30, 45, 60, 75, 90 and 105 min after each caffeine dose. Samples were also collected at 7.5, 8.5 and 18 h after the last dose of caffeine. Plasma caffeine levels were analysed by a validated UV-HPLC method.

Result: The mean T_{max} after the third dosing ranged from 0.37 to 1.12 h. C_{max} for 50, 100 and 200 mg was 2.69, 3.45 and 6.33 mg/l, respectively. AUC_{inf} for 50, 100 and 200 mg group was 33.2, 46.94 and 86.94 mg/l * h, respectively. AUC_{inf} values suggested a dose proportionate increase. Dose normalized C_{max} and $AUC_{0-\tau}$ values across doses were not significantly different, suggesting linearity was maintained after multiple doses of the *Stay Alert*® chewing gum. There were no group related differences in elimination.

Conclusions: The results suggest that caffeine administered in the gum formulation (*Stay Alert*® chewing gum) via a multiple dosing regimen provides an effective and convenient means of maintaining effective concentrations of caffeine that would in some operational scenarios be desirable for maintaining alertness and performance in sleep deprived individuals. Copyright © 2005 John Wiley & Sons, Ltd.

Key words: caffeine chewing gum; caffeine multiple dose; pharmacokinetics; buccal absorption

Introduction

Sleep loss and fatigue are associated with degraded physical performance, cognitive impairment and disturbance of mood [1,2]. Caffeine is a commonly used stimulant, known to alleviate the effects of sleep deprivation and fatigue. The pharmacodynamics and pharmacokinetics of caffeine have been well characterized [3]. It is rapidly absorbed after oral dosing, and extensively metabolized by the liver (99%) to form three major metabolites 3,7-dimethylxanthine, 1,7-dimethylxanthine and 1,3-dimethylxanthine. At typical dose levels (e.g. 1 cup of coffee/70–100 mg), caffeine exhibits dose-independent or linear pharmacokinetics [3]. However, at

*Correspondence to: Department of Pharmacokinetics-Biopharmaceutics Laboratory, Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland at Baltimore, AHB 540A, 100 Penn Street, Baltimore, MD 21201, USA.
E-mail: neddingt@rx.umaryland.edu

Received 13 June 2003
Revised 8 June 2005
Accepted 16 June 2005

higher doses (e.g. 250–500 mg single dose), the clearance of caffeine is significantly reduced and its elimination half-life is prolonged, indicating non-linearity [1].

Caffeine has also been used to counteract the effects of sleep deprivation. Penetar *et al*. [4] showed that caffeine is effective for reversing performance, alertness and mood deficits produced by prolonged sleep deprivation. Other studies have demonstrated that the pharmacodynamic properties of caffeine are dose dependent [1]. Over the past few years, there has been increased interest in the pharmacodynamic effects of caffeine during sleep deprivation, reflecting concerns surrounding the ubiquitous and pervasive problem of sleep loss in military and other operational environments. Thus, studies have been conducted to compare various caffeine doses and modes of delivery (capsule, oral solution, gum), with the aim of determining which is the safest, most reliable and most rapidly absorbed. Gum formulations have been a particular focus, since such formulations have been used to enhance the rate of absorption for various other agents including aspirin [5,6], verampamil [7] and nicotine [8].

In order to determine if caffeine is also more rapidly absorbed from gum (*Stay Alert*® chewing gum) vs an immediate release capsule, the pharmacokinetics of these two formulations were recently compared in a single dose study [9]. A significantly faster absorption rate was evident for the gum formulation, as evidenced by its higher absorption rate constant (k_a), higher C_{max} and lower T_{max}. However, the extent of absorption (AUC_{inf}) for both formulations was comparable.

The present study constitutes the next step in determining the operational usefulness of caffeinated chewing gum—determination of whether enhanced absorption is also evident across a multiple dosing regimen, and characterization of other aspect of the pharmacokinetics of caffeine in *Stay Alert*® chewing gum.

Materials and Methods

Forty-eight young (18–35 years), healthy, non-smoking males ($n = 28$) and females ($n = 20$) who habitually consumed less than 300 mg of caffeine per day volunteered for this study. Females were not using nor had used any form of hormonal contraceptives in the 3 months prior to the study (such contraceptives are known to modify the metabolism of caffeine [10]). After signing an informed consent, the health status of the subjects was determined on the basis of medical history, physical examination and routine laboratory tests. The study was conducted at the Walter Reed Army Institute of Research (WRAIR) and the protocol for the study was approved by the Human Subjects Research Review Board of the Office of the Surgeon General of the Army.

Dosing and pharmacokinetic sampling

Subjects were randomly assigned to one of four treatment groups as follows: placebo, 50, 100 or 200 mg of caffeine as *Stay Alert*® chewing gum. The subjects were restricted from using caffeine, alcohol or any medications for 32 h prior to dosing. An indwelling catheter was inserted into the forearm vein of each subject and maintained with a saline drip prior to dosing. Each subject was administered two sticks of gum and was instructed to chew the gum for 5 min. Previous work has demonstrated that approximately 85% of the caffeine is delivered by 5 min of chewing [11]. Subjects were administered the *Stay Alert*® gum at 2400, 0200 and 0400 h, and after chewing for 5 min the subjects expectorated the gum. Blood samples were collected at the following times: pre- dose and at 5, 15, 30, 45, 60, 75, 90 and 105 min after each dose. In addition, blood samples were collected at 7.5, 8.5 and 18 h after the first dose of caffeine. Plasma was immediately separated by centrifugation and stored at −70°C until analysed. The subjects received a standardized lunch and dinner, and water was available *ad libitum*.

Analytical method

A valid specific high-performance liquid chromatography method was used to quantify caffeine in the plasma samples [12]. To 250 µl of plasma, 250 µl of 0.8 M perchloric acid containing 8 µg/ml of the internal standard, 8-chlorotheo-

phylline was added. The resulting solution was vortexed for 10 s and centrifuged at 6000 rpm for 5 min. Fifty μl of the supernatant was injected onto the chromatographic system. The analyte was eluted with a Phenomenex C_{18} analytical column (Phenomenex, CA) (15 cm × 4.6 mm). The mobile phase consisted of acetonitrile/tetrahydrofuran/acetic acid/H_2O (50:30:5:915 v:v:v:v) and pumped at a flow rate of 1 ml/min. Caffeine was detected using UV absorption at a wavelength of 274 nm. The LOQ was 100 ng/ml with a within-day variation of less than 5% and a between-day variation of less than 10%.

Pharmacokinetic and statistical analysis

Non-compartmental and compartmental modeling was used to estimate caffeine pharmacokinetic parameters after multiple dose administration. The caffeine concentration-time data were evaluated using Winnonlin® Professional (Pharsight Inc., Cary, NC, v 3.1). The maximum caffeine concentration measured for each subject was C_{max}. The time that C_{max} occurred was T_{max}. The area under the curve (*AUC*) from time 0 to the end of the dosing interval (τ), $AUC_{0-\tau}$, the *AUC* from 0 to the last concentration time point (AUC_{cplast}) was determined by the trapezoidal method. The AUC_{inf} was determined by the following equation

$$AUC_{inf} = AUC_{cplast} + C_{plast}/\lambda_z \quad (1)$$

The elimination rate constant (λ_z) was determined by linear regression of the linear portion of the ln (conc) vs time profile. Typically, four to five time points were used to determine the terminal elimination rate constant. A one-compartment model assuming first-order oral absorption and first-order elimination provided the best fit for individual patient concentration-time data. The choice of PK model was based on the standard goodness of fit criteria which included weighted sum of squares of residuals (WSSR), Akaike's information criteria (AIC), Schwarz criteria (SC), residual plots, and plots of observed and model-predicted concentration vs time. The model with the smallest values for AIC, SC and WSSR was chosen as the best model. Absorption rate constant (K_a) was estimated from the absorption phase by compartmental modeling. Additional pharmacokinetic parameters determined by compartmental modeling were V_d/F, Cl/F. The accumulation factor was determined by comparison of the third dose vs the first dose as defined below in the following equation

$$\frac{(1 - e^{-n\lambda z\tau})}{(1 - e^{-\lambda z\tau})} \quad (2)$$

where n is the number of doses, τ is the dosing interval and λ_z is the terminal elimination rate constant. A parametric general linear model was applied to each of the aforementioned pharmacokinetic parameters. Inferential statistical analyses consisted of one-way ANOVA with a Tukey's post-hoc test. The significance level was $p < 0.05$.

Results

Forty-eight normal healthy volunteers completed the study with no serious adverse effects. The mean (± SD) age, weight and height of subjects were 25 (± 5.1) years, 158.45 (± 27.39) lbs and 58 (± 4.25), respectively. The first pre-dose concentrations of caffeine were below the LOQ of the assay. This indicates that the subjects did not take caffeine prior to the start of the study. Figure 1 depicts the geometric mean caffeine plasma concentration versus time profile after the 50, 100 and 200 mg multiple dose gum treatments obtained after dosing at 2400, 0200 and 0400 h. As expected, the plasma caffeine concentrations displayed a rapid rise after each administration and C_{max} levels were achieved within 15 min after administration. Furthermore, the pharmacokinetic profile of the three-dosage groups appears to be mono-exponential. Figure 2 illustrates the mean (± SD) C_{max} and $AUC_{0-\tau}$ for the three dosing intervals. In general, this shows a steady increase in both the C_{max} and *AUC* with each successive dose.

As stated, the subjects were randomly assigned to one of the four groups: placebo, 50, 100 or 200 mg of caffeine as *Stay Alert*® chewing gum. Subjects were administered the 50 mg dose of the gum at time 2400, 0200 and 0400 h. Samples were

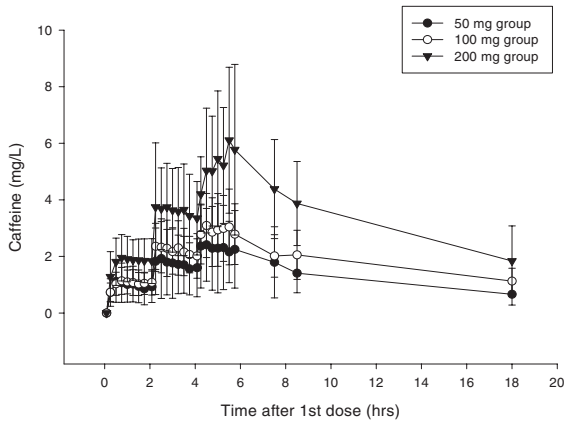

Figure 1. Mean (± SD) caffeine plasma concentration profiles following a 50, 100 and 200 mg multiple dose of caffeine as gum formulation to healthy volunteers. Caffeine was administered at 0, 2 and 4 h. Twelve subjects were enrolled in each treatment group

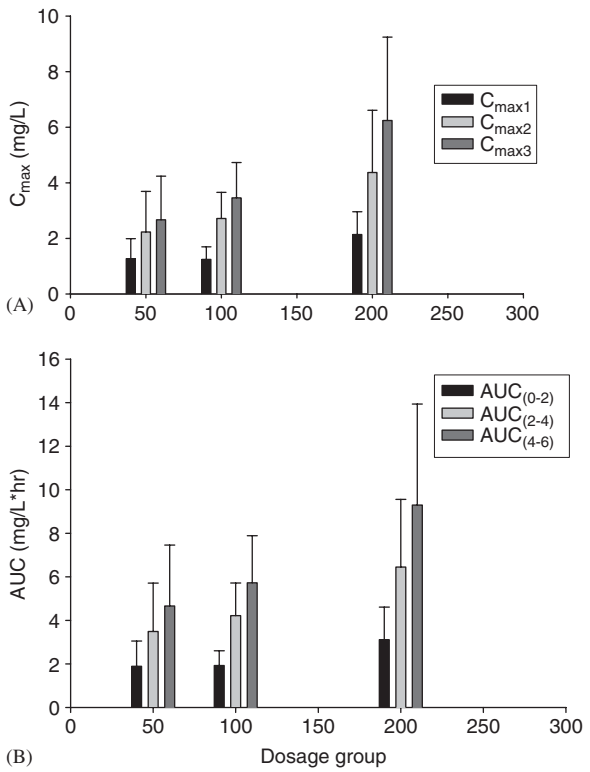

Figure 2. Mean (± SD) C_{max} (A) and *AUC* (B) following a 50, 100 and 200 mg multiple dose of caffeine as gum formulation to healthy volunteers. Caffeine was administered at 0, 2 and 4 h. Twelve subjects were enrolled in each treatment group

also collected at 7.5, 8.5 and 18 h after the first dose. The pharmacokinetic parameters obtained after administration of caffeine are summarized in Tables 1 and 2. After the third 50 mg dose of the chewing gum, the mean C_{max} associated with the 50 mg dose group was 2.609 (+1.481) mg/l. The time to reach peak concentration (T_{max}) was consistent across dosings, suggesting that the rapid absorption seen with the previous single dose study is maintained after successive doses. The extent of absorption, AUC_{inf} over the three doses was found to be 33.2 mg/l * h. The mean $AUC_{(4-6)}$ after the third dose (4.66 mg/l * h) displayed an accumulation of 2.308 compared with the first dose (1.88 mg/l * h). Figure 2A, B displays the C_{max} and $AUC_{0-\tau}$ observed with each dose and highlights the accumulation achieved with multiple doses of the *Stay Alert*® gum.

The 100 mg treatment group also displayed accumulation after the second and third doses. Caffeine plasma concentrations displayed an increase in C_{max} with every subsequent dose as illustrated in Figure 2A. The C_{max} after the third dose (3.46 mg/l) was significantly higher ($p < 0.05$) than the first dose (1.25 mg/l). T_{max} values (T_{max1}: 0.937, T_{max2}: 2.781, T_{max3}: 4.841 h) at each dosing interval indicate rapid caffeine absorption after each dosing. The extent of caffeine absorption (AUC_{inf}) over the three doses was 46.95 mg/l * h. Again as was evident with C_{max}, the AUC_{4-6} (5.73 mg/l * h) displayed an accumulation ratio of 2.467 compared with the first dose ($AUC_{0-2} = 1.92$ mg/l * h).

The plasma concentration profile of subjects in the highest dose group showed a similar pattern of drug accumulation, with C_{max} for the third dosing interval being the highest at 6.24 mg/l. The mean C_{max} associated with three dosing intervals were 2.14 (C_{max1}), 4.37 (C_{max2}) and 6.24 (C_{max3}) mg/l (Figure 2). T_{max} values (T_{max1}: 1.028, T_{max2}: 2.77, T_{max3}: 5.125 h) for the highest dosing group continued to show a similar pattern of rapid caffeine absorption after each dosing. The mean AUC_{inf} was 86.93 mg/l * h (Table 2) and partial *AUC* for the three dosing intervals were AUC_{0-2}: 3.11, AUC_{2-4}: 6.45 and AUC_{4-6}: 9.2 9 mg/l * h (Figure 2). Partial areas in this maximum dose group also displayed a consistent dose dependent increase.

Table 1. Mean (± SD) pharmacokinetic parameters following multiple doses of 50, 100 and 200 mg of *StayAlert*® gum to normal healthy volunteers using compartmental analysis

Treatment group	V_d/F (l/kg)	Cl/F (l/h kg)	λ_z (h^{-1})	K_a (h^{-1})	$T_{1/2}$ (h)
50 mg	0.820 (± 0.324)	0.116 (± 0.067)	0.145 (± 0.053)	4.388 (± 1.669)	5.631 (± 2.715)
100 mg	1.197 (± 0.376)	0.128 (± 0.064)	0.105 (± 0.029)	4.854 (± 1.935)	7.23 (± 2.83)
200 mg	1.273 (± 0.236)	0.120 (± 0.063)	0.09 (± 0.032)	3.294 (± 1.270)	8.531 (± 3.0)

To determine if multiple dosing altered the pharmacokinetics of caffeine, dose normalized values of C_{max} and AUC were also examined. Neither the dose-normalized values for C_{max} nor the AUC_{inf} differed across the treatment groups. The mean volume of distribution ranged over 0.82–1.27 l/kg, consistent with earlier observations that caffeine distributes rapidly with no specific binding to tissues [10]. Likewise, elimination parameters λ_z and Cl/F did not differ across the treatment groups. This suggests that at higher treatment doses there is no saturation of metabolic pathways, i.e. pharmacokinetics in humans at levels of normal exposure (< 250 mg) are not dose dependent. The elimination rate constant and Cl/F values showed no statistical differences across the treatment groups. These results suggest that linearity is maintained in a multiple dose regimen.

Discussion

The objective of this study was to evaluate the pharmacokinetics of caffeine extruded from a gum, across multiple doses. A steady accumulation of drug after each dosing interval was evident, and linear kinetics was suggested by the dose normalized AUC and C_{max} values. In a previous study the rate of absorption and relative bioavailablity of caffeine administered were compared in chewing gum versus capsules [9]. The C_{max} levels achieved were 0.7, 1.2 and 3.7 mg/l for the 50, 100 and 200 mg caffeine gum groups. The C_{max} levels achieved in the present study for the first dosing interval are similar to levels achieved in the previous study for each caffeine gum group. The elimination rate constants obtained in that prior single dose study ranged over 0.144–0.17 for the 50–200 mg dose groups, and were not significantly different from those obtained in the present study.

These findings suggest that the faster rate and extent of absorption seen with the single dose study is essentially maintained across repeated doses. Also, the pharmacokinetic parameters seem to be dose independent. These findings suggest that both the physical and mental performance deficits that result from sleep loss or fatigue could be reversed quickly by caffeine administered in a gum formulation, and benefits might be maintained over an extended period of time with repeated dosing.

In a gum formulation, caffeine absorption occurs primarily through the buccal mucosa—a site at which drug absorption is known to be rapid [13]. The buccal mucosa has a rich vascular supply resulting in a favorable rate of absorption for many drugs, especially for lipophilic agents such as caffeine. However, it is likely that with the gum formulation some portion of the caffeine was also swallowed with saliva, and absorbed in the gastrointestinal tract. The likelihood of this is supported by that fact that multiple peaks in the plasma profiles were evident for a number of subjects, suggesting multiple sites of absorption (i.e. buccal mucosa for the early peak, and GI tract for the later peak). This most likely contributed to the high variability of the pharmacokinetic parameters, indicated by high standard deviations.

Another potential source of increased variability was the 'mastication rate.' Differential

Table 2. Mean (± SD) pharmacokinetic parameters following multiple doses of 50, 100 and 200 mg of *StayAlert*® gum to normal healthy volunteers using non-compartmental analysis

Treatment group	C_{max1} (mg/l)	C_{max2} (mg/l)	C_{max3} (mg/l)	$AUC_{(0-2)}$ (mg/l*h)	$AUC_{(2-4)}$ (mg/l*h)	$AUC_{(4-6)}$ (mg/l*h)	$AUC_{(inf)}$ (mg/l*h)	T_{max1} (h)	T_{max2}(h)	T_{max3}(h)	Accumulation factor
50 mg	1.219 (± 0.698)	2.12 (± 1.4)	2.609 (± 1.48)	1.887 (± 1.16)	3.491 (± 2.225)	4.662 (± 2.806)	33.22 (± 17.87)	0.681 (± 0.297)	2.75 (± 0.512)	4.375 (± 0.954)	2.308
100 mg	1.245 (± 0.448)	2.717 (± 0.935)	3.460 (± 1.271)	1.925 (± 0.683)	4.217 (± 1.50)	5.726 (± 2.179)	46.947 (± 24.04)	0.937 (± 0.438)	2.781 (± 0.489)	4.841 (± 0.54)	2.467
200 mg	2.14 (± 0.82)	4.37 (± 2.24)	6.24 (± 3.00)	3.11 (± 1.5)	6.45 (± 3.11)	9.29 (± 4.64)	86.93 (± 49.78)	1.028 (± 0.49)	2.77 (± 0.45)	5.125 (± 1.30)	2.531

mastication rates, both within and between subjects, could have contributed to variability in the data, by affecting the rate at which the caffeine was extruded from the gum. Variability resulting from this and other factors may have been reduced had the study utilized a crossover design.

The pharmacodynamic effects of caffeine are dependent on its pharmacokinetic properties. Caffeine improves performance and alertness in sleep-deprived subjects, and in individuals who are required to work long hours. With caffeine administered in a gum formulation, a quick onset of action is obtained (within 5–10 min of administration). The dose can be repeated every 2 h or as needed to have sustained levels of caffeine, and thus sustained performance for extended periods of time.

In summary, caffeine administered in the gum formulation (*Stay Alert*® chewing gum) via a repeated dosing regimen provides a viable means of sustaining blood concentrations of caffeine for prolonged periods, i.e. at levels necessary to achieve continuous, effective pharmacodynamic responses in sleep deprived individuals. Further studies are underway to discover and examine the potential PK-PD relationship.

Acknowledgements

We would like to acknowledge the assistance of the staff of the Division of Neuropsychiatry, Walter Reed Army Institute of Research for their assistance and support in the completion of this study and the preparation of this manuscript. Human volunteers participated after giving their free and informed written consent. This research was conducted in conformity with AR 70-25 and USAMRDC Reg 70-25 on the use of human volunteers in research and the Guiding Principles for Research Involving Animals and Human Beings. Use of trade names does not constitute endorsement of product. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the opinions of the Department of the Army or the Department of Defense.

References

1. Kaplan GB, Greenblatt DJ, Ehrenberg BL, *et al*. Dose-dependent pharmacokinetics and psychomotor effects of caffeine in humans. *J Clin Pharmacol* 1997; **37**: 693–703.
2. Babkoff H, Sing HC, Thorne DR, Genyes SG, Hegge FW. Perceptual distortions and hallucinations reported during the course of sleep deprivation. *Percept Mot Skills* 1989; **68**(3 Pt 1): 787–798.
3. Bonati M, Latini R, Galletti F, Young JF, Tognoni G, Garattini S. Caffeine disposition after oral doses. *Clin Pharmacol Ther* 1982; **32**: 98–106.
4. Penetar DM, McDonough JH Jr. Effects of cholinergic drugs on delayed match-to-sample performance of rhesus monkeys. *Pharmacol Biochem Behav* 1983; **19**: 963–967.
5. Woodford DW, Lesko LJ. Relative bioavailability of aspirin gum. *J Pharm Sci* 1981; **70**: 1341–1343.
6. Bousquet E, Tirendi S, Bonina FP, Montenagro L, Bianchi A, Cianpini N. Bioavailability of two formulations of acetylsalicylic acid gums. *Pharmazie* 1992; **47**: 607–609.
7. Christrup LL, Angelo HR, Bonde J, Kristensen F, Rasmussen SN. Relative bioavailability of methadone hydrochloride administered in chewing gum and tablets. *Acta Pharm Nord* 1990; **2**: 83–88.
8. Benowitz NL, Jacob P 3rd, Savanapridi C. Determinants of nicotine intake while chewing nicotine polacrilex gum. *Clin Pharmacol Ther* 1987; **41**: 467–473.
9. Kamimori GH, Kanyekar CS, Otterstettes R, *et al*. The rate of absorption and relative bioavailability of caffeine administered in chewing gum versus capsules to normal healthy volunteers. *Int J Pharm* 2002; **234**: 159–167.
10. Dews PB and International Life Sciences Institute. *Caffeine: Perspectives from Recent Research*. Springer-Verlag: Berlin, New York, 1984; 260.
11. Novum. *Relative Bioavailablity of Caffeine Chewing Gum Pieces vs. No-Doz Tablets*. Amurol Confections Company: USA, 1998.
12. Richard L, Leducq B, Baty C, Jambon J. [Plasma determination of 7 common drugs by high performance liquid chromatography]. *Ann Biol Clin* (*Paris*) 1989; **47**: 79–84.
13. Shargel L, Yu ABC. *Applied Biopharmaceutics and Pharmacokinetics*, 4th edn. Appleton & Lange: Stamford, CT, 1999; 768.