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Caffeine release from selected pharmaceutical preparations

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Reference drugs that are available on the Polish market were subjected to qualitative, quantitative and caffeine release analyses. The HPLC technique was used in all assays.

Determination of the pharmaceutical availability of caffeine showed the following values of release for the respective preparations: Apap Extra 97.2±2.4%; Guaranax 66.8±1.3%; Guarana (Herbapol) 94.1±1.1%; Guarana (Walmark) 99.9±5.0%, Panadol 86.9±0.2%, Diabolo Guarana 73.5±5.0%, Magne B-6 Active 55.4±13.6%, Drive Max 88.2±1.6%. For Guarana Caps, pharmaceutical availability was not determined because the substance was not released from the capsule in the acceptor fluid (HCl, pH=2).

The results of the analysis of the selected dietary supplements for their caffeine content confirmed earlier reports on instances of divergence between the actual concentration of a given active substance and the declared value. The analysis of caffeine release profiles for the selected pharmaceuticals shows significant differences between them. The analyses did not indicate the presence of a caffeine-tannins complex. No extended or retarded release of caffeine from preparations containing a guarana seed extract, which might otherwise be indicative of a gradual release of caffeine from such complex, was observed. Differences in the profiles of caffeine release from the test preparations are attributable to the properties of the respective drug forms, such as the presence of a coating or gelatin capsule. The test results are not indicative of the presence of a caffeine-tannins complex which, otherwise, might cause caffeine to be gradually released.

Keywords: caffeine, pharmaceutical preparations, release of active substance, caffeine-tannins complex, high-performance liquid chromatography

INTRODUCTION

The structure of derivatives of xanthines, such as caffeine (Fig. 1), was identified in the 19th century. The discovery is deemed to have been made by Hermann Emil Fischer (1852–1919), who demonstrated (in 1897) that caffeine and uric acid had similar structures before proving that the compound was trimethylxanthine. On the other hand, Fischer's research merely confirmed the structure proposed earlier by Ludwig Medicus (1847–1915) [1].

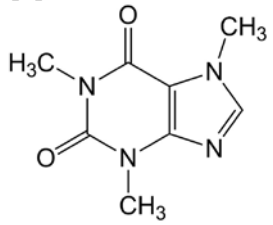

Fig. 1. Caffeine.

The effect of caffeine is observed in the cerebral cortex, the autonomous nervous system, and the cardiac muscles [2]. Nevertheless, a number of studies on the effect of caffeine fail to give an unambiguous answer about the effect itself and its intensity. This is explained by the occurrence of individual and environmental differences, affecting the metabolism of the compound [3]. Reasonable doses of caffeine (100–300 mg/day) stimulate the activity of the cerebral cortex by enhancing both intellectual and physical efficiency and helping fight drowsiness and fatigue. Different opinions are expressed on the effect of caffeine on the ability to concentrate [2]. Among purine group alkaloids, when compared with theobromine and theophylline (Fig. 2 and Fig. 3), caffeine has the strongest stimulating effect on the central nervous system [4].

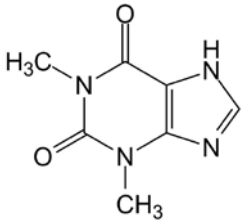

Fig. 2. Theobromine.

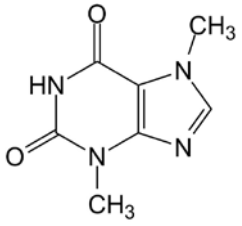

Fig. 3. Theophylline.

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The daily consumption of caffeine in Europe is in the range of 280–490 mg [5]. Caffeine is quickly and almost completely (99%) absorbed after oral administration. Its maximum blood concentration is observed after 50 to 75 min. Its pharmacokinetics after intravenous administration is comparable to that after oral administration [6]. Between 25–40% of the drug is bound with plasma protein, and its distribution volume is 0.52–1.05 l/kg. The compound readily passes through the placenta barrier, the blood-brain barrier, and into the milk of breast-feeding mothers [7].

Caffeine is considered to be the most frequently used psychoactive substance. According to the American Psychiatric Association (APA), a substance is regarded as having an addictive effect if at least three of the seven proposed criteria are satisfied. Caffeine satisfies four of the seven criteria: tolerance to the effect of the compound is observed, withdrawal symptoms are shown, patients have problems in reducing its consumption, and the substance is used even though it is known to have undesirable effects and being harmful to the body [8].

MATERIALS AND METHODS

Test material

The test material included dietary supplements and drugs with caffeine, available in Poland. Their compositions and forms are shown in Table 1.

Reagents

- methanol for HPLC (from Merck)
- caffeine (from Sigma-Aldrich)
- water for HPLC (from J. T. Baker)

Equipment

The analyses were performed using the following equipment:

- Liquid chromatograph HP 1050 from Hewlett Packard, with UV detector;
- Paddle apparatus for analysis of active substance release from solid drug forms, set up as described in the Polish Pharmacopoeia IX – Apparatus 2.

Chromatographic conditions

- analytical wave length: 280 nm.
- stationary phase – Waters Symmetry column C-18, 5 μm (4.6 × 250 mm).
- mobile phase – methanol : water 40:60.

Table 1. Composition of test preparations with caffeine.

Preparation	Composition, as declared by manufacturer	Form of preparation
	Dietary supplements	
Diabolo Guarana	Guarana seed extract 380 mg (45 mg caffeine)	capsules
Magne – B6 Active	Guarana seed extract 232 mg (min. 51 mg caffeine)	coated tablets
Drive Max	Guarana seed extract 10% (30 mg caffeine)	capsules
Guaranax	Guarana extract 400 mg (20% caffeine)	capsules
Guarana	Guarana seed extract (min. 27 mg caffeine)	capsules
Guarana	Guarana 800 mg, (10.5 mg caffeine)	tablets
Guarana Caps	Guarana extract 450 mg (100 mg caffeine)	capsules
	Drugs	
Etopiryna	Acetylsalicylic acid 300 mg Etenzamid 100 mg Caffeine 50 mg	tablets
Coffepirine	Acetylsalicylic acid 450 mg Caffeine 50 mg	tablets
Panadol Extra	Paracetamol 500 mg Caffeine 65 mg	coated tablets
Apap Extra	Paracetamol (500mg), Caffeine (65mg)	coated tablets

Table 2. Statistical parameters for the calibration curve y = ax + b.

Statistical parameters	Peak height curve	Peak area curve
a (gradient)	1.8589	0.5561
b (abscissa)	0.3023	-0.6191
R	0.9989	0.9991
R^2	0.9978	0.9983
S	1.8120	0.4721
av. Y	99.26	28.98
RSD [%]	1.8	1.6

R – regression coefficient; S – deviation of curve; RSD – relative standard deviation

Preparation of calibration curve for caffeine solutions

The calibration curve for caffeine was prepared by consecutive dilutions using a stock solution of concentration of 1 mg/ml. Solutions with concentrations of 5, 10, 25, 50, 100, 200 μg/ml were prepared using the mobile phase. Three injections were made for the standard solution. The calibration curve is shown in Fig. 4 and its parameters are shown in Table 2.

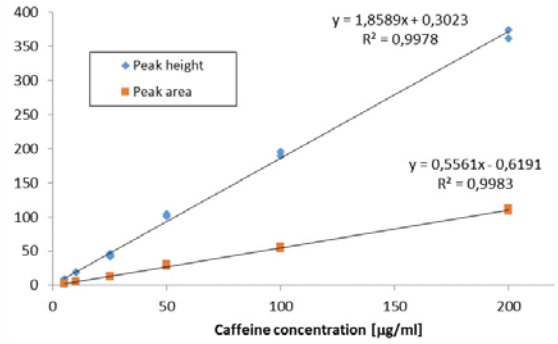


Fig. 4. Calibration curves for caffeine

Preparation of the pharmaceuticals to testing

The selected pharmaceutical preparations with caffeine were in the form of tablets or capsules. The tablets were dissolved in 50 ml measuring flasks filled with a 40% methanolic solution. The content of the capsules was quantitatively transferred into 50 ml measuring flasks and then dissolved in a 40% methanolic solution.

Determination of caffeine release profiles for selected preparations

The rate of release of caffeine from the selected preparations was found by the method described in the Polish Pharmacopoeia IX. The rate of release of a medicinal substance, also termed pharmaceutical availability, was found for *in vitro* conditions, using a paddle apparatus.

The samples, drawn from the apparatus, were analyzed by HPLC.

Test conditions for determination of the rate of caffeine release

The test apparatus was a 1000 ml thermostated three-necked flask. A hydrochloric acid solution (pH = 2) was used as the acceptor fluid. The volume of the acceptor fluid was 500 ml. The material in the flask was continuously stirred during the test using a mechanical paddle stirrer. The acceptor fluid was maintained at a constant temperature of 37.0±0.5°C.

The test procedure and drawing samples for analysis

The volume and the temperature of the acceptor fluid were controlled before starting the test. As soon as the temperature was stable at 37.0±0.5°C, a single form of the selected preparation was introduced, while commencing time measurement. Samples (1ml) of the acceptor fluid were drawn using an automatic pipette at specified intervals, selected depending on the drug form. Every time after drawing a sample, the volume of the acceptor fluid was supplemented by adding 1ml of the hydrochloric acid solution at pH=2. The fluid volume drawn for testing was transferred to Eppendorf test tubes. The samples for testing were drawn until the results of chromatographic analysis indicated no increment in the caffeine contents in three consecutive portions of the test solution. Three independent release rate tests were performed for each test preparation.

METHOD VALIDATION

Precision

The precision of the method was assessed with repeatability and intermediate precision (intra-day and inter-day).

The repeatability of the method to assay caffeine was found by injecting a 10 μg/ml caffeine standard solution in the chromatographic column 10 times. From the obtained analyte retention times, the mean value of retention was found to be 4.653±0.008

min. The resulting peaks were characterized by symmetry, good repeatability and very good precision, as indicated by their low RSD of 0.178%.

The resulting peak areas were used to determine their corresponding concentrations. The results, as well as the values of standard deviations and RSD, are shown in Table 3.

Intra-day precision was determined by injecting three different concentrations (10, 25 and 50 μg/ml) for six times on the same day. Peak areas were measured; their corresponding analyte concentrations, standard deviations and % RSD's were calculated.

Inter-day precision was determined by injecting three different concentrations (10, 25 and 50 μg/ml) six times for three days in a week. Peak areas were measured; their corresponding analyte concentrations, standard deviations and % RSD's were calculated.

The interday and intraday precisions of caffeine are presented in Table 4. The low % RSD values of repeatability (0.189%), intra-day (0.21% - 0.27%) and inter-day (0.21% – 0.39%) variations indicate that the precision of the proposed method is good.

Limit of detection and limit of quantification

The limit of detection (LOD) under the present chromatographic conditions is defined by the concentration of analyte giving a signal to noise ratio of 3:1. The limit of quantification (LOQ) is defined as the lowest value of the analyte concentration that is determinable with the appropriate precision and accuracy. From 6 parallel results, obtained for the respective concentrations of the standard solution, the values of the relative standard deviation were calculated and the value of RSD vs. caffeine concentration was used for finding LOQ for RSD = 10%.

For the test caffeine: LOD = 3 μg/ml and LOQ = 9 μg/ml.

Accuracy

Accuracy was found by determining the amount of the caffeine standard added to the previously analyzed product. The analyte standard was added at three different concentrations (50, 100 and 150%) of caffeine in the preparations. The analysis was carried out three times for the selected products having different declared concentrations of caffeine (Table 5).

For the test preparations, mean recovery for three different analyzed amounts of the standard added was in the range from 100.04 to 100.41%.

RESULTS AND DISCUSSION

Assay of caffeine in the selected preparations

The test samples were analyzed by HPLC to find the actual content of caffeine in the test preparations. The results are shown in Table 6.

Analysis of test samples and obtaining of release profiles

The test samples were analyzed one by one, using HPLC. Increments in the caffeine peak heights were referred to the time after which a sample was collected. The percentages of caffeine release from the respective preparations were calculated from the peak heights, using values for standard solutions of caffeine. Using the above information, the caffeine release profiles were plotted for the test preparations (Fig. 5A-H).

Release profiles, showing the percentage release of an active substance from a given drug form vs. time, are an important source of information about the properties of pharmaceutical preparations and their quality. The pharmaceutical availability tests of the selected preparations with caffeine, performed by the present authors, were intended to verify the theory that the release of caffeine from drugs containing guarana extract was extended in time. In the literature, the phenomenon is accounted for by the gradual decomposition of guaranine complexes in the presence of gastric juice. Information on the mild, long-lasting effect of caffeine originating from guarana seeds is habitually provided in leaflets for drugs having a stimulating effect.

Table 3. Results of repeatability (n=10).

	Retention time (min)	Assay of caffeine (μg/ml)
	4.655; 4.649; 4.666; 4.649; 4.659; 4.667; 4.646; 4.651; 4.648; 4.643	10.02; 9.98; 10.01; 10.03; 10.04; 9.99; 10.02; 10.03; 10.03; 10.02
Mean	4.653	10.02
SD	0.008	0.02
RSD [%]	0.178	0.189

n – number of repetitions, SD – standard deviation, RSD – relative standard deviation

Table 4. Intermediate precision (n=6).

Concentration of caffeine (μg/ml)		Precision	
		Intra-day	Inter-day
		10.02; 10.00; 10.05; 10.01; 10.00; 10.04	Day 1. 10.02; 10.00; 10.05; 10.01; 10.00; 10.04
	Mean	10.02	10.02
	S.D.	0.02	0.02
	RSD	0.21	0.21
			Day 2. 10.02; 9.99; 10.04; 9.98; 10.03; 10.04
10	Mean		10.02
	S.D.		0.03
	RSD		0.26
			Day 3. 10.04; 10.01; 10.05; 9.97; 10.04; 10.03
	Mean		10.02
	S.D.		0.03
	RSD		0.29
		25.09; 25.10; 24.95; 24.96; 25.05; 25.06	Day 1. 25.09; 25.10; 24.95; 24.96; 25.05; 25.06
	Mean	25.04	25.04
	S.D.	0.06	0.06
	RSD	0.26	0.26
			Day 2. 25.12; 24.91; 24.97; 25.06; 25.07; 25.12
25	Mean		25.04
	S.D.		0.08
	RSD		0.34
			Day 3. 25.16; 24.90; 24.99; 25.12; 25.09; 25.12
	Mean		26.06
	S.D.		0.10
	RSD		0.39
		50.22; 49.90; 50.14; 49.92; 50.05; 50.18	Day 1. 50.22; 49.90; 50.14; 49.92; 50.05; 50.18
	Mean	50.07	50.07
	S.D.	0.14	0.14
	RSD	0.27	0.27
			Day 2. 50.21; 50.00; 49.85; 50.18; 50.10; 50.20
50	Mean		50.09
	S.D.		0.14
	RSD		0.28
			Day 3. 50.25; 50.12; 49.90; 49.92; 50.05; 50.22
	Mean		50.08
	S.D.		0.15
	RSD		0.29

Table 5. Results of recovery study (n=3).

Drug name	Amount added mg/tablet	Amount recovered mg/tablet	Recovery% ± SD	Mean recovery% ± SD
Apap Extra	32	32.3	100.94 ± 0.03	100.35 ± 0.77
	65	65.4	100.62 ± 0.02	
	97	96.5	99.48 ± 0.03	
Coffepirine	25	25.1	100.40 ± 0.04	100.04 ± 0.62
	50	50.2	100.40 ± 0.05	
	75	74.5	99.33 ± 0.04	
Guarana Caps	50	50.3	100.60 ± 0.05	100.41 ± 0.25
	100	100.5	100.50 ± 0.06	
	150	150.2	100.13 ± 0.07	
Guarana	5	4.95	99.00 ± 0.05	100.28 ± 1.93
	10	10.25	102.5 ± 0.06	
	15	14.90	99.33 ± 0.05	

Table 6. Content of caffeine in the selected preparations.

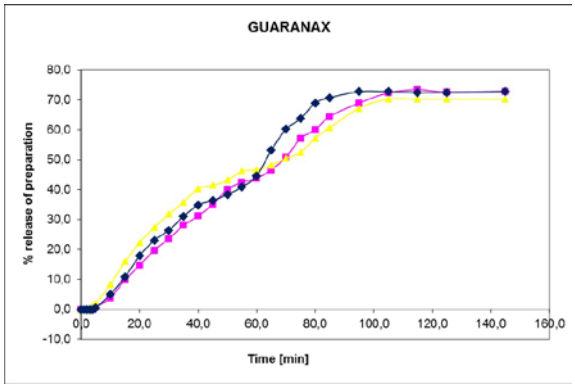
Preparation	Declared content of caffeine [mg]	Assay of caffeine [mg]	SD
Diabolo Guarana	45	64.1	6.9
Magne – B6 Active	min. 51	92.2	1.8
Drive Max	30	39.3	1.6
Guaranax	80	107	5
Guarana	min. 27	54.7	5.2
Guarana	10.5	11.0	0.7
Guarana Caps	100	105	5
Etopiryna	50	51.4	3.0
Coffepirine	50	50.6	2.5
Panadol Extra	65	64.4	3.1
Apap Extra	65	65.7	2.5

For drugs with a non-modified release, the duration of pharmaceutical availability tests is preferably 30, 45 or 60 minutes. After that time, a minimum of 80% of their active substance will have been released [9].

In the case of dietary supplements with caffeine originating from guarana, the drug forms are not ones with modified release. The process may be extended because the caffeine-tannins complex is decomposed. Moreover, differences in the active substance release for different forms of pharmaceutical preparations must be taken into account. The test preparations in question were in the form of tablets and capsules.

The pharmaceutical availability tests indicate that the release of caffeine from the preparations was as follows: Apap Extra 97.2±2.4% (release time 25.0±0.0 min); Guaranax 66.8±1.3% (101.7±1.3 min); Guarana (Herbapol) 94.1±1.1% (20.0±0.0 min); Guarana (Walmark) 99.9±5.0% (release time 20.0±0.0 min), Panadol 86.9±0.2% (20.0±0.0 min), Diabolo Guarana 73.5±5.0% (release time 38.3±2.9 min), Magne B-6 Active 55.4±13.6% (43.3±5.8 min), Drive Max 88.2±1.6% (23.3±2.9 min). For Guarana Caps, pharmaceutical availability was not determined because the dietary supplement failed to decompose in the acceptor fluid (HCl, pH=2).

The test results did not indicate the presence of a caffeine-tannins complex in the dietary supplements containing a guarana seed extract, or any extended or retarded release of caffeine from preparations with a guarana seed extract, which might otherwise be indicative of a gradual release of caffeine from the complex. Differences in the caffeine release profiles for the test preparations are attributable to the properties of the respective drug forms, such as the presence of a coating or gelatin capsule. A research model for *in vitro* conditions does not encompass all the processes taking place in the living body. Therefore, the potential effect of other chemical compounds existing in the guarana seeds on the absorption processes taking place *in vivo* should be considered.



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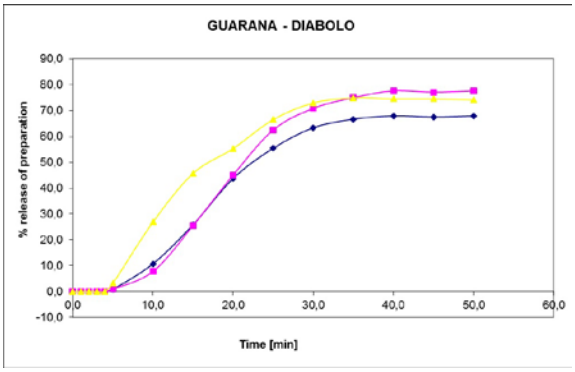

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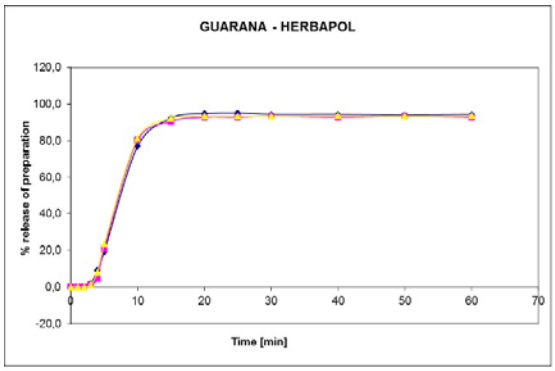

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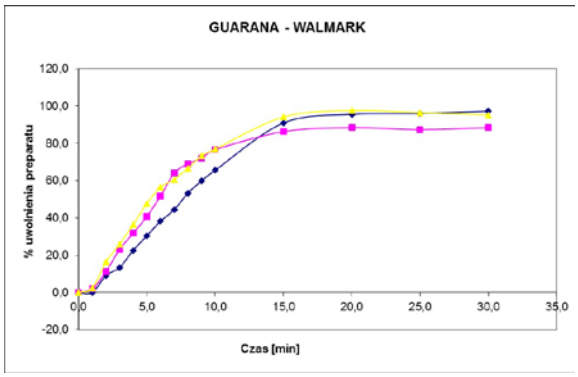

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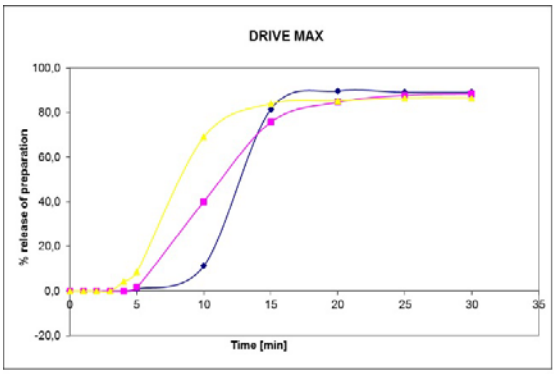

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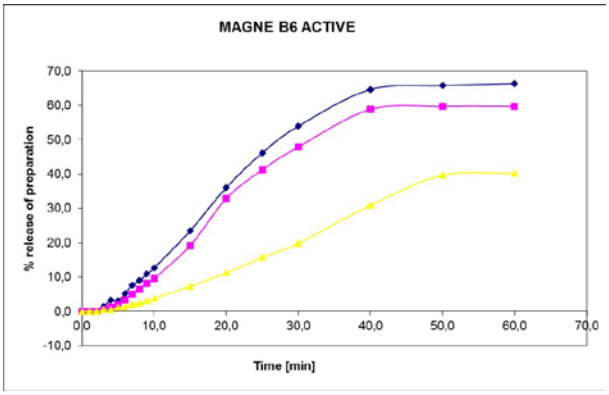

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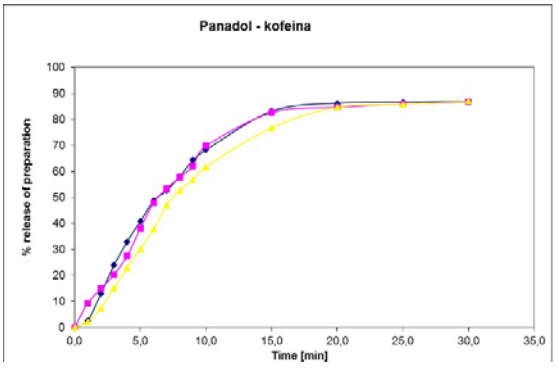

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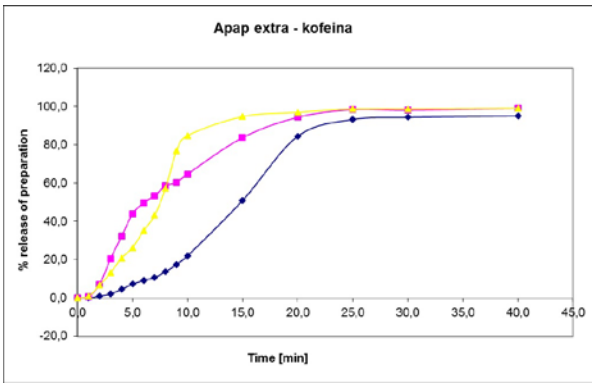

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Fig. 5A-H. Caffeine release profiles for the test preparations.

CONCLUSIONS

The HPLC method for determination of caffeine is characterized by very good precision and accuracy, as well as very good repeatability. The limits of detection and quantification are 3 and 9 μg/ml, respectively.

The pharmaceutical availability test results indicated that caffeine release from the test preparations was from 99.9±5.0% for Guarana (Walmark) (release time 20.0±0.0 min) to 55.4±13.6% for Magne B-6 Active (43.3±5.8 min). For Guarana Caps, pharmaceutical availability was not determined because it failed to decompose in the acceptor fluid (HCl, pH=2).

Findings for the test dietary supplements with a guarana seed extract do not indicate the presence of a caffeine-tannins complex, or any extended or retarded release of caffeine from such preparations, which might otherwise be indicative of the gradual release of caffeine from the complex.

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ОСВОБОЖДАВАНЕ НА КОФЕИН ОТ ИЗБРАНИ ФАРМАЦЕВТИЧНИ ПРЕПАРАТИ

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(Резюме)

Референтните лекарства на полския пазар са обект на качествен и количествен анализ, както и на анализ по отношение отделянето на кофеин. За тези анализи са използва високоефективна течна хроматография.

Определянето на фармацевтичната достъпност на кофеина показва следните стойности на освобождаване за различните препарати: Apap Extra 97.2±2.4%; Guaranax 66.8±1.3%; Guarana (Herbapol) 94.1±1.1%; Guarana (Walmark) 99.9±5.0%, Panadol 86.9±0.2%, Diabolo Guarana 73.5±5.0%, Magne B-6 Active 55.4±13.6%, Drive Max 88.2±1.6%. For Guarana Caps фармацевтичната достъпност не е определена поради факта, че субстанцията не се освобождава от капсулите в приемния флуид (HCl, pH=2).

Резултатите от анализите на тези хранителни добавки за кофеиновото им съдържание потвърждават предишни съобщения за различия между реалната концентрация на дадена активна субстанция и декларираната стойност. Анализите на кофеиновите профили на освобождаване показват значителни различия. Анализите не показват наличие на кофеин-танинов комплекс. Не се наблюдава ускорено или забавено освобождаване от препарата, съдържащ екстракт от семена на guarana, което може да е показателно за постепенното освобождаване от такъв комплекс. Разликите в профилите на освобождаване на кофеина от тестовите препарати са показателни за свойствата на лекарствените форми, като филм-таблетки или капсули. Тестовите резултати не са показателни за наличието на кофеин-танинови комплекси, които иначе могат да причинят постепенното освобождаване на кофеина.