DETERMINATION OF CAFFEINE CONTENT FROM DIFFERENT PHARMACEUTICAL AND NATURAL PRODUCTS

Andreia Corciova[a] and Bianca Ivanescu[b]*

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The present study focuses on the quantification of caffeine from 11 pharmaceutical and natural products. Pharmaceutical products were purchased from pharmacy and include 4 types of tablets and 1 suppository type product. Natural products were purchased from specialty stores and comprise green tea, black tea and different kinds of coffee. Some of the quality parameters like friability and disintegration time for tablets, melting behavior, deformation temperature and time of deformation for suppositories were determined. The caffeine content was determined using UV spectrophotometric methods by measuring the absorbance at 272 nm, after separating the caffeine from the others active ingredients and excipients. The results showed that caffeine content in pharmaceutical products purchased from pharmacies is in the range recommended by European and Romanian Pharmacopoeia. In the case of natural products, the caffeine content decreases in the following order: bulk coffee beans freshly ground > ground coffee packed > black tea > green tea > decaffeinated coffee.

* Corresponding Authors
Fax: +40.232.211.820
E-Mail: biancaivanescu@yahoo.com
[a] "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Faculty of Pharmacy, Department of Drugs Analysis, 16 Universitatii Street, RO-700115 Iasi, Romania
[b] "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Faculty of Pharmacy, Department of Pharmaceutical Botany, 16 Universitatii Street, RO-700115 Iasi, Romania

Introduction

Caffeine ($C_8H_{10}N_4O_2$, 1,3,7-trimethylxanthine) (Figure 1) is a naturally occuring alkaloid that can be found in *Coffeae semen*, *Theae folium*, *Cacao semen*, *Colae semen*, *Mate folium* and *Paulliniae cupanae semen.* Due to its pharmacological effects, caffeine can also be found in different pharmaceutical products, associated for example with aspirin for treatment of headaches, with ergotamine for the antimigraine effect, with paracetamol and propyphenazone for pain relief or it can be used alone in the treatment of mild respiratory depression. Caffeine is a stimulant of central nervous system that reduces fatigue, increases attention and generates a faster and clearer flow of thoughts. When is consumed in moderate amounts, caffeine is good for health. It can reduce the risk of hepatocellular and endometrial cancer, and the risk of type 2 diabetes. Caffeine improves physical performance and sometimes is used as a doping substance by athletes. Furthermore, it has a bronchodilator effect attenuating asthma symptoms and a diuretic effect on the kidney.[1-6]

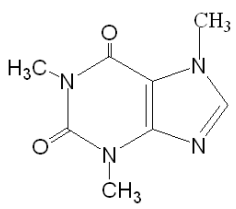

Figure 1. Caffeine structure

On the other hand, caffeine stimulates the stomach to produce large amounts of acid and aggravates peptic ulcers of stomach and duodenum. If it is consumed in large amounts can produce constriction of blood vessels, increase the blood pressure and provoke arrhythmia because of the cardiac stimulation. Also, caffeine elevates the levels of free fatty acids in the plasma and, when is overused, can exacerbate premenstrual symptoms. Caffeine crosses the placenta and if it is consumed during lactation may cause irritability and wakefulness in breastfed babies. When caffeine is consumed in quantities exceeding 250 mg per day can lead to nervousness, irritability, restlessness, insomnia, headaches and heart palpitations. This condition is named as caffeinism.[1-6]

For the determination of caffeine content from different matrices, various modern methods can be used: HPLC,[7-9] FTIR-ATR,[10] sequential perturbation caused by different amounts of caffeine on the oscillating chemical system in a continuous stirred-tank reactor (CSTR),[11] electrochemical detection of caffeine that uses a single-walled carbon nanotube on carbon-ceramic electrode (SWCNT/CCE).[12] Most of these methods are very complex and expensive, so we chose UV-VIS spectrophotometric method because is cheaper, rapid, simple and approachable in scientific teaching labs of school and universities.[3, 13-16]

The purpose of this paper was to determine the amount of caffeine in 5 pharmaceutical products and 6 natural products, using UV spectrophotometric methods.

Experimental

Materials

Caffeine standard was purchased from Sigma-Aldrich (Germania). All the chemicals used were analytical grade reagents. The pharmaceutical products used in the analysis were purchased from pharmacy and they correspond to 3 types of uncoated tablets containing, in addition to caffeine, aspirin and paracetamol (Sample 1); paracetamol and

propyphenazone (Sample 2); paracetamol, phenacetin and codeine (Sample 3); a type of effervescent tablet containing indomethacin and prochlorperazine (Sample 4) and a type of suppository containing indomethacin and prochlorperazine (Sample 5).

The natural products used for this analysis were purchased from specialty shops. The products used are green tea (Sample 6), black tea (Sample 7), bulk coffee beans which was freshly ground (Sample 8), ground coffee from producer 1 (Sample 9), ground coffee from producer 2 (Sample 10) and bulk decaffeinated coffee (Sample 11)

Equipment

A Jasco V 530 double beam UV-Vis spectrophotometer was used. All the measurements were made in 1.0 cm quartz cells at a scan speed of 1000 nm min^{-1} and scan range of 200 – 400 nm, fixed slit width of 2 nm.

Methods

First, caffeine was extracted using special conditions in order to separate it from other active ingredients or excipients that the product may contain. Determination of caffeine content in all samples was carried out by using a UV spectrophotometric method. The specific absorbance of caffeine was used for calculation of caffeine content in tablets while the external standard method was applied for suppositories and the calibration curve method was used for natural products.

Separation of caffeine from the samples

Sample 1 and 2 - To a quantity of powder corresponding to one tablet, 40 mL of water were added in a separatory funnel. The mixture was stirred for 5 minutes, then 5 mL of H_2SO_4 (0.05 mol) were added and caffeine was extracted three times with 20 mL of chloroform by shaking each time for 5 minutes. All chloroform phases were combined in another separatory funnel and were extracted again thrice using for each extraction 20 mL of NaOH (0.1 mol) and shaking for 5 minutes. The chloroform phase was filtered through anhydrous sodium sulfate and evaporated. The residue was quantitatively transferred into a 100 mL flask using HCl (0.1 mol). 2 mL of this solution was diluted with HCl (0.1 mol) to 100 mL, and the absorbance was measured at 272 nm using HCl (0.1 mol) as blank.[17]

Sample 3 - The same method was used to obtain the residue as for the previous samples. To the residue we added 0.1 g sodium benzoate and a small quantity of water. The solution containing the caffeine adduct was made up to a 100 mL flask using 0.1 M HCl. 2 mL from this solution was diluted with 0.1 M HCl to 100 mL, and the absorbance was measured at 272 nm using 0.1 M HCl as blank.

Sample 4 - To a quantity of powder corresponding to one tablet, 25 mL of neutralized alcohol were added. Then 2 drops of phenolphthalein were added and the titration was started using 0.1 M NaOH until a persistent pink colour was obtained. After the addition of an extra 5 mL of 10 % NaOH solution, the caffeine was extracted four times with 20 mL of chloroform. The chloroform phases were filtered through anhydrous sodium sulfate and evaporated. The residue was quantitatively transferred in a 100 mL flask using 0.1 M HCl. 2 mL from this solution were diluted with 0.1 M HCl to 100 mL, and the absorbance was measured at 272 nm using the solvent as blank.

For each pharmaceutical product presented as tablets, we crushed 20 tablets to a fine powder, after having first determined their average weight. Also, we determined some of the quality parameters like friability and disintegration time.[18,19] The caffeine content of tablets was calculated using a formula that includes: the absorbance of the sample, the specific absorbance of caffeine at 272 nm (470), the mass of powder used and the average weight of 20 tablets.

Sample 5 - The average weight of 20 suppositories was determined and after that the suppositories were pulverized and homogenized. An amount of powder equivalent to one suppository mass was extracted at high temperature using 10 mL of water and 2 g of paraffin. Next, the solution was cooled and filtered into a 50 mL volumetric flask. The procedure was repeated four more times using also the filter covered with residue; the flask was filled to the mark and the solution was homogenized. 1 mL from the final solution was diluted 50 mL with 0.1 M HCl and the absorbance was measured at 272 nm. A solution of 1 mg mL^{-1} caffeine was used as a standard. The caffeine content of suppositories was calculated using a formula that includes: the absorbance of the sample, concentration and absorbance of standard solution and the average weight of a suppository.

For the lipophilic suppositories we determined some of the quality parameters, such as melting behaviour, deformation temperature and also time of deformation .[18, 19]

Samples 6-11 - An exactly weighed amount of tea samples was infused for 30 minutes. For coffee samples decoctions were made by boiling the coffee for 5 minutes. The solutions were cooled and filtered. To 20 mL of each solution, 10 mL of 0.1 M HCl and 2 mL of basic lead acetate solution were added, and diluted with water to 250 mL. The resulting solution was filtered and 50 mL of it was mixed with 0.2 mL of 4.5 M H_2SO_4 and diluted to 100 mL with water. After mixing and filtration, the absorbance was measured at 272 nm.[3] The caffeine content of samples 6-11 was calculated using the calibration curve equation.

Results and Discussions

Table 1 shows the quality parameters for the analyzed tablets and Table 2 the quality parameters for suppositories.

Table 1. Quality parameters for the analyzed tablets.

Samples	Disintegration time (min)	Friability	Average weight (g)
1	9.0	0.2658 %	0.5161
2	2.0	0.2054 %	0.6496
3	5.0	0.4122 %	0.6497
4	1.5	0.512 %	1.8017

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According to the European and Romanian Pharmacopoeia, the disintegration time in water, for uncoated compressed tablets must not exceed 15 minutes and for effervescent tablets 5 minutes. The friability should not be higher than 1 %. As shown by the results in table 1, the samples comply with the quality parameters.

Table 2. Quality parameters for suppositories

Samples	Melting behavior	Softening Temp.	Softening Time	Average Wt. (g)
5	20	38 ℃	3.45 min	1.5271

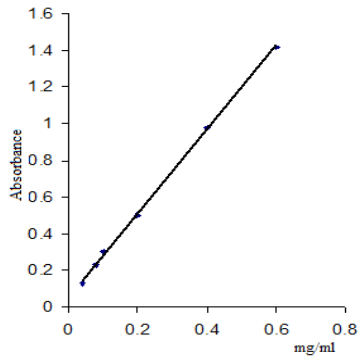

Figure 2. Calibration curve for caffeine.

The suppositories have to melt within 30 minutes, the softening temperature should be 37 ± 2 ^{0}C and the softening time should be at least 3 minutes. For Sample 5, the quality parameters are fulfilled.

The caffeine content of samples 6-11 was calculated using the calibration curve equation. In order to plot the calibration curve (Figure 2), a standard solution of caffeine 1 mg mL^{-1} was prepared and different solutions with concentrations between 0.04 – 0.6 mg mL^{-1} were made by dilution. Table 3 presents the statistical parameters for the determination of caffeine.

Table 3. Statistical data regarding caffeine determination.

Statistical Parameter	Value
Correlation Coefficient (r^2)	0.9993
Standard Error	0.014544
Intercept	0.0503
Slope	2.2943
Limit of detection	0.043834
Limit of quantification	0.132831

For each sample, the determinations were repeated three times, on three consecutive days. Table 4 presents the results for determination of caffeine content in pharmaceutical products expressed in mg per tablet and mg per suppository. Table 5 shows the caffeine content from natural products, expressed in g caffeine per 100 g tea or coffee.

Table 4. Caffeine concentration in pharmaceutical products

Conc. declared in per unit sample	Day 1		Day 2		Day 3	
	Conc. found	% Recovery	Conc. found	% Recovery	Conc. found	% Recovery
Sample 1	20.49	102.45	19.89	99.45	20.23	101.15
20 mg	20.56	102.8	20.18	100.9	19.97	99.85
	20.23	101.15	19.99	99.95	20.34	101.7
Average ± SD	20.42 ± 0.17	102.13 ± 0.86	20.02 ± 0.14	100.1 ± 0.73	20.18 ±0.19	100.9 ±0.95
Sample 2	51.02	102.04	49.96	99.92	50.12	100.24
50 mg	50.99	101.98	49.99	99.98	50.29	100.58
	50.97	101.94	50.01	100.02	50.07	100.14
Average ± SD	50.99 ± 0.025	101.98 ± 0.050	49.98 ± .025	99.97 ± 0.050	50.16 ± 0.11	100.32 ± .23
Sample 3	24.88	99.52	24.87	99.48	24.68	99.52
25 mg	24.79	99.16	24.92	99.68	24.23	99.16
	24.66	98.64	24.75	99	24.76	98.64
Average ± SD	24.77 ± 0.11	99.10 ± 0.44	24.84 ± 0.08	99.38 ± 0.35	24.55 ± 0.28	99.10 ± 0.44
Sample 4	76.01	101.34	76.4	101.86	76.35	101.8
75 mg	76.25	101.66	76.39	101.85	75.98	101.30
	76.35	101.8	76.28	101.70	76.71	102.28
Average ± SD	76.20 ± 0.17	101.60 ± 0.23	76.35 ± 0.06	101.80 ± 0.08	76.34 ± 0.36	101.79 ± .48
Sample 5	148.27	98.84	147.99	98.66	148.28	98.85
150 mg	148.1	98.73	147.29	98.19	148.66	99.10
	148	98.66	147.64	98.42	148.41	98.94
Average ± SD	148.12 ± 0.13	98.74 ± 0.09	147.64 ±0.35	98.42 ± 0.23	148.45 ± .19	98.96 ± 0.12

As seen in table 4, the recovery varies between 100.1 – 102.13 % (Sample 1), 99.97 – 101.98 % (Sample 2), 99.10 – 99.38 % (Sample 3), 101.60 – 101.80 % (Sample 4), 98.42 – 98.96 % (Sample 5). The limits established by Romanian Pharmacopoeia for tablets, where deviation depends on the caffeine content in each sample, are ± 7.5 %. For suppositories the permissible deviation is ± 5 %. So the variations are within the permissible range.

Table 5. Caffeine concentration in natural products (g $100g^{-1}$)

Sample No.	Concentration found, average ± SD		
	Day 1	Day 2	Day 3
6	1.016 ± 0.015	1.027 ± 0.004	1.005 ± 0.0223
7	1.08 ± 0.017	1.071 ± 0.004	1.076 ± 0.03
8	1.410 ± 0.009	1.408 ± 0.33	1.421 ± 0.19
9	1.373 ± 0.21	1.376 ± 0.08	1.369 ± 0.01
10	1.272 ± 0.039	1. 286 ± 0.59	1.277 ± 0.38
11	0.451 ± 0.040	0.442 ± 0.008	0.449 ± 0.04

The caffeine content in tea and coffee depends on the type of tea leaves, on the type of coffee beans, on the type of treatment and the method of preparation used. Generally, there is a higher content of caffeine in the tea leaves (3-5 %) than in coffee beans (1.35 - 2 %),[20, 21] but after the preparation of beverages there is a smaller content in tea than in coffee. That is probably because the coffee was boiled and the tea was infused. Another study compared the caffeine content of tea leaves and showed that the green tea has a caffeine content of 10-20 mg g^{-1} dried herb, while the black tea has a caffeine content of 22-28 mg g^{-1} dried herb.[22]

In our samples, the caffeine content of black tea is 1.071-1.08 % natural products, while the green tea has 1.005 – 1.016 % natural product. For the coffee samples, the content of caffeine varies in the range 1.272 - 1.421 % natural product, depending on the type of coffee used. Higher caffeine content was obtained when using bulk coffee beans freshly ground.

For decaffeinated coffee, the method for decaffeination is very important because in this process up to 97 % of the caffeine content is lost. The caffeine content in our sample is between 0.442 and 0.451 %.

Conclusions

All the analyzed pharmaceutical products conform to the requirements of European and Romanian Pharmacopoeia. There is a bigger quantity of caffeine content in the coffee samples than in the tea samples and in black tea than in green tea.

For the coffee samples, the caffeine content decreases in the following order: bulk coffee beans freshly ground > ground coffee from producer 1 > ground coffee from producer 2 > decaffeinated coffee.

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