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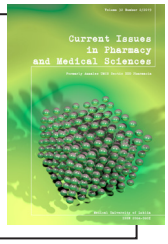

Comparative dissolution studies on granules with acetaminophen and caffeine using the basket and paddle methods with simultaneous spectrophotometric determination of active substances

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ABSTRACT

Acetaminophen and caffeine, popular therapeutic substances used to relieve pain or alleviate the symptoms of cold. The aims of the study were the comparison of granules, in terms of dissolution rate and moreover the development of spectrophotometric method to the simultaneous determination of both active pharmaceutical ingredients (APIs) in granules. The granules were tested by two pharmacopoeial methods of dissolution for solid dosage forms, and the dissolution profiles for each formulation were compared. A method of simultaneous determination of two medicinal substances by the double calibration method using derivative spectrophotometry was used. Considering the dissolution process carried out in the paddle apparatus, it was shown that more than 80% of acetaminophen and caffeine were released from each of the preparations in a clearly shorter time than 10 minutes. Carrying out the basket test, substances dissolved gradually, much slower than in the paddle method. The time required to release 80% of both active substances from majority of tested preparations was from 30 to 45 minutes. Application of the first derivative spectrophotometric method allows simultaneous determination of acetaminophen and caffeine in the mixture, without the need to separate them first.

INTRODUCTION

Granules are a solid dosage form composed of aggregates of particles of APIs and excipients. They have some advantages over powders: they are easier to dose, do not adhere to the mucosa of the palate and pharynx and do not cause choking [1]. The main aim of the study was the comparison of various granules, in terms of dissolution rate, containing acetaminophen and caffeine – popular therapeutic substances used to relieve pain or alleviate the symptoms of cold. Granules were tested by two pharmacopoeial methods of API dissolution from the dosage form. The dissolution profiles for each formulation were compared. In our previous paper, we described a method of preparing and testing the physical properties of granules containing acetaminophen and caffeine [2]. This current paper also presents a spectrophotometric method of simultaneous determination of two APIs in granules by the double calibration method, using derivative spectrophotometry.

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MATERIALS AND METHODS

For this experiment, granules were prepared using the following APIs: acetaminophen (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and caffeine anhydrous (Fluka Chemie AG, Buchs, Swiss). The excipients used in the preparation of granules include: lactose monohydrate (Pharma Cosmetic, Cracow, Poland), D-mannitol (Sigma-Aidrich Chemie GmbH, Steinheim, Germany), calcium hydrophosphate anhydrous (PPH POCH S.A., Gliwice, Poland), corn starch (Radix-Bis, Rotmanka, Poland), polyethylene glycol 6000 (PEG 6000) (Fluka Chemie AG, Buchs, Swiss), polyvinylpyrrolidone K30 (PVP K30) (Fluka Chemie AG, Buchs, Swiss), and ethyl alcohol 96% (v/v) (PPH POCH S.A., Gliwice, Poland). Hydrochloric acid analytical weighed amount 0,1M (Chempur, Piekary

Slaskie, Poland) was used as the dissolution medium in the dissolution test.

Spectrophotometer UV-Vis (Helios Omega Thermo Scientific, USA) was employed for the acquisition and storage of spectra. The dissolution test was carried out in a DT 600 HH Dissolution Tester (Erweka, Heusenstamm, Germany): USP method 1 – basket apparatus and USP method 2 – paddle apparatus.

Preparation of Granules. The granules were made by way of the wet granulation method, as described in our previous paper [2]. PEG 6000 was the binder in granules series 1-5 and PVP K30 had the same function in granules series 6-10 (Table 1).

Table 1. The composition of granules (amounts of the substances are given in per cent *m/m*)

Granules	Acetaminophen	Caffeine	Lactose	Mannitol	Calcium hydrophosphate	Corn starch	PEG 6000	PVP K30
Serie 1	1.67	0.33	88.00				10.00	
Serie 2	1.67	0.33		88.00			10.00	
Serie 3	1.67	0.33		44.00	44.00		10.00	
Serie 4	1.67	0.33			88.00		10.00	
Serie 5	1.67	0.33	52.80			35.20	10.00	
Serie 6	1.67	0.33	93.00					5.00
Serie 7	1.67	0.33		93.00				5.00
Serie 8	1.67	0.33		46.50	46.50			5.00
Serie 9	1.67	0.33			93.00			5.00
Serie 10	1.67	0.33	55.80			37.20		5.00

Spectrophotometric Determination of Spectra of Caffeine and Acetaminophen. Three solutions of active substances in hydrochloric acid 0.1M (HCl 0.1M) were prepared to determine the spectrum [3]. One contained caffeine, the other – acetaminophen, and the third – a mixture of both substances. The concentration of active substances in each was 10.0 µg/ml. The prepared solutions were tested in a spectrophotometer in the wavelength range from 205 to 310 nm, using 0.1M HCl as reference. The absorption spectrum of acetaminophen showed a maximum at wavelength $\lambda = 243$ nm, and in the case of caffeine, the maximum absorbance was at $\lambda = 271$ nm. Absorption spectra are given in Figures 1-3.

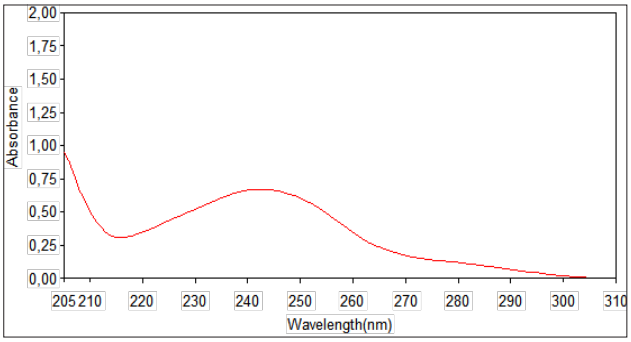

Figure 1. Absorption spectrum of acetaminophen in 0.1M HCl

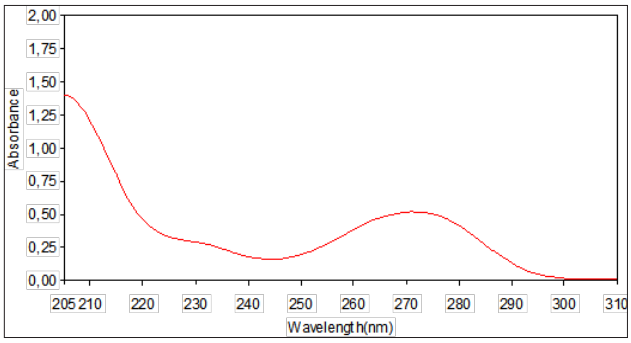

Figure 2. Absorption spectrum of caffeine in 0.1M HCl

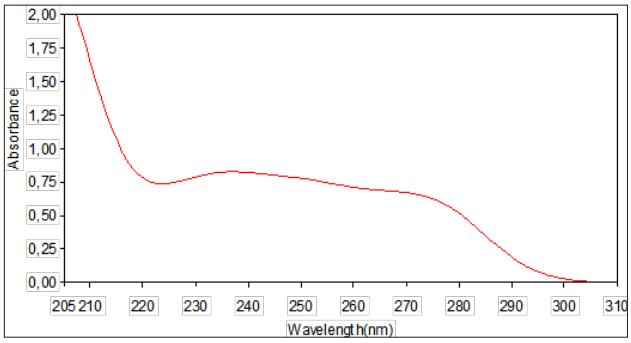

Figure 3. Absorption spectrum of mixture of acetaminophen and caffeine in 0.1M HCl

The graphs on Fig. 1-3 show that the spectra of acetaminophen and caffeine overlap. The simultaneous, quantitative determination of these substances in the mixture, without first separating them, causes difficulties. For this reason, derivative spectrophotometry was used to determine acetaminophen and caffeine side by side. The use of derivative spectroscopy is fully justified in this case, because there is no wavelength where one of compounds can be quantified without the interference of the second one

Determination of the Content of Active Substances in Granules Using Derivative Spectrophotometry. The content of caffeine and acetaminophen in the prepared granules was determined by the double calibration method using derivative spectrophotometry [4,5]. The first derivative was calculated using the VISION pro spectrophotometer software. To simultaneously determine the contents of the two active substances, assays were done at two selected wavelengths $\lambda 1 = 260$ and $\lambda 2 = 284$ nm. The spectrum of the first derivative of absorbance is given in Figure 4.

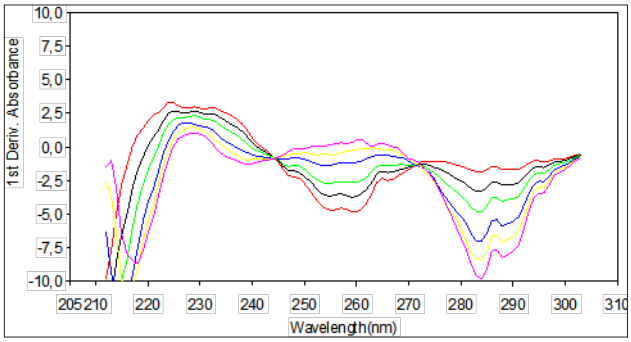

Figure 4. The spectrum of the first derivative of absorbance for the wavelength of the caffeine and acetaminophen

Table 2. Curve equations used to calculate the amount of caffeine and acetaminophen

Substance	λ1 = 260 nm	λ2 = 284 nm
Caffeine	y = 0.1317x + 0.0162 r^2 = 0.9999	y = -0.1863x – 0.012 r^2 = 1
Acetaminophen	y = -0.1672x – 0.0218 r^2 = 0.9998	y = -0.0341x + 0.0123 r^2 = 0.9988

r^2 – linearity factor

Equations of the curves in Table 2 were used to create calibration equations (eq.1, eq.2), that allow the calculation of the amount of active substances to be measured.

$$Cp = \frac{[A\lambda_1 + 0.0056 - (0.1317 \times C_c)]}{-0.1672} \quad \text{(eq.1)}$$

$$Cc = \frac{\{-0.0341 \times (A\lambda_1 + 0.0056) + [-0.1672 \times (0.0003 - A\lambda_2)]\}}{-0.03564} \quad \text{(eq.2)}$$

C_p – acetaminophen concentration,
C_c – caffeine concentration,
A – absorbance,
λ_1 = 260 nm,
λ_2 = 284 nm.

To determine acetaminophen and caffeine content in granules, HCl 0.1M solutions were prepared as follows: 1.0 g of the granules were placed into a 100 ml volumetric flask to which 15.0 ml of HCl 0.1M was added. The contents of the flask were shaken in a laboratory shaker (neoLab, Vortex mixer 7-2020, Munich, Germany) for 10 minutes and then supplemented with HCl 0.1M to 100 ml. After this, 1.0 ml of the resulting solution was withdrawn and diluted to 10.0 ml with HCl 0.1M. A filtration step followed. All samples were filtrated by single use syringe filters, pore size 0.20 µm, Sterile-EO Ophtalsart Sartorius. The contents of the substances were determined in the Helios Omega spectrophotometer, at wavelengths λ = 260 and 284 nm, according to the method described above. We also checked that the excipients did not interfere with the measurement of the absorbance of the active substances.

Dissolution of Caffeine and Acetaminophen from Granules. The dissolution test for granulates was carried out using the Erweka DT 600 Dissolution Tester, via two pharmacopoeial methods: the basket apparatus (method I) and the paddle apparatus (method II), according to European Pharmacopoeia 9th Edition (Ph. Eur.) [6].

HCl 0.1M was used as the dissolution medium, in an amount of 900 ml, heated to 37±0.5°C, and the rotation speed was set to 100 rpm. Granule samples with a weight of 5.0 g were tested, poured directly into a beaker filled with dissolution medium (paddle apparatus) or by using a dry basket (basket apparatus). Aliquots of 2.0 ml solution were taken after 1, 2, 5, 10, 15, 30, 45 and 60 minutes, measuring the time from the beginning of the study. Each time, the contents of the beaker were supplemented with an equivalent volume of fresh dissolution medium. The taken samples were then diluted with HCl 0.1M to a volume of 10 ml. Prior to the spectrophotometric test, the resulting solutions were filtered through 0.22 µm membrane filters to remove any powder particles.

Method of Validation. The precision of the spectrophotometric method was determined by repeatability: intra-day and intermediate precision: inter-day and expressed as a relative standard deviation (RSD). Intra-day precision was assessed by analysing three different concentrations in triplicate of both active substances (acetaminophen: 8,16, 20 µg/ml, caffeine: 4, 12, 20 µg/ml). This was done at three different intervals, within the same study and in the same day. Inter-day precision was determined by triplicate determination of given concentrations of acetaminophen and caffeine over three following days as per ICH guidelines. They are included in Table 3

Table 3. Precision of the system for assay of acetaminophen and caffeine in standard solutions

Substance	Concentration [µg/ml]	Precision (n = 3) Response factor* (mean± SD)	RSD [%]	Intermediate precision (n = 3) Response factor* (mean± SD)	RSD [%]	(n=9) Total RSD [%]
Acetaminophen	8	-0.1376±1.59E-03	-1.16	-0.1363±3.22E-03	-2.36	-1.72
	16	-0.1793±6.30E-05	-0.04	-0.1827±3.85E-03	-2.11	
	20	-0.2269±5.63E-04	-0.25	-0.2278±1.19E-03	-0.52	
Caffeine	4	-0.2046±2.42E-03	-1.18	-0.2123±8.23E-03	-3.88	-2.77
	12	-0.0914±7.49E-04	-0.82	-0.0925±7.20E-04	-0.78	
	20	-0.0371±2.88E-04	-0.78	-0.0361±1.04E-03	-2.89	

* Relationship between absorbance and concentration of the drug

RESULTS AND DISCUSSION

In the dissolution test six measurements were made for each granule and the averages of obtained results are given in Figures 5-8.

The dissolution test of caffeine and acetaminophen was carried out by two methods, while maintaining the same process conditions. These included the type of dissolution medium, its volume, or the rotation speed. Similar studies on ibuprofen pellets were carried out by Chevalier *et al*. [7]. They compared the dissolution of the pelleted drug in three apparatuses: a paddle, a reciprocating cylinder and a flow-through. While the results of the study were similar in the paddle and flow-through apparatus, the dissolution in the reciprocating cylinder was faster. According to the authors, this was caused by the more rapid pellet disintegration in the moving cylinder. Both the basket and the paddle methods are the pharmacopeial methods of testing the dissolution of active substances from the dosage form. Nevertheless, different dissolution profiles were obtained in studies on granulates with acetaminophen and caffeine (Fig. 5-8). Thus, the observations of Chevalier *et al*. [7] showing the influence of the test method on the dissolution of active substances were confirmed.

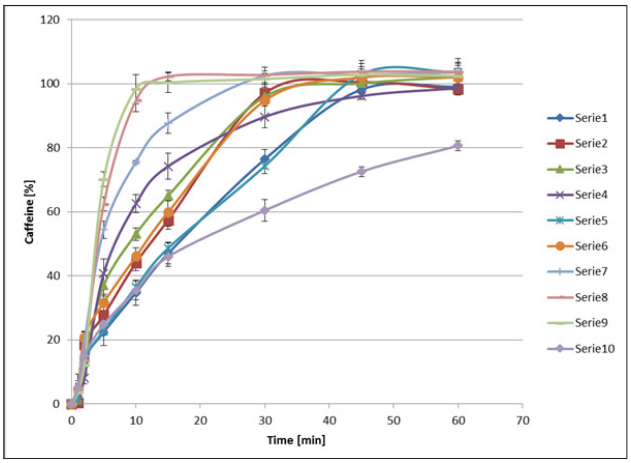

Figure 5. Dissolution profiles of caffeine from granules in 0.1M HCl (basket apparatus) with respect to time (n = 6)

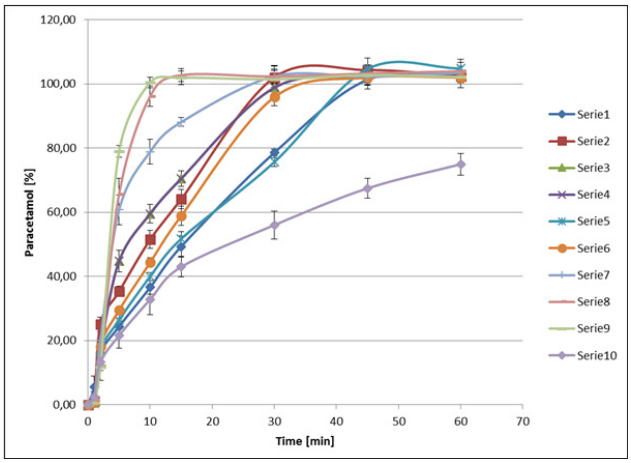

Figure 7. Dissolution profiles of acetaminophen from granules in 0.1M HCl (basket apparatus) with respect to time (n = 6)

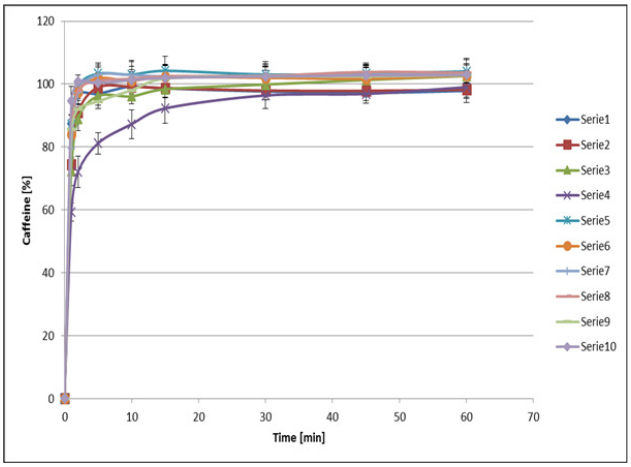

Figure 6. Dissolution profiles of caffeine from granules in 0.1M HCl (paddle apparatus) with respect to time (n = 6)

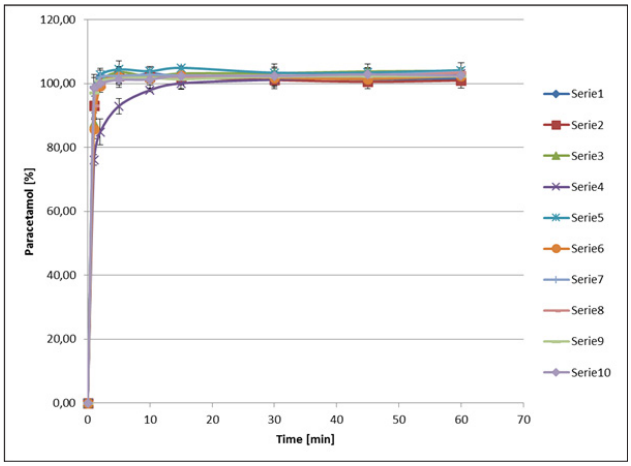

Figure 8. Dissolution profiles of acetaminophen from granules in 0.1M HCl (paddle apparatus) with respect to time (n = 6)

Considering the dissolution process carried out in the paddle apparatus, we showed that all tested granulates met the pharmacopoeial requirements for the dissolution of APIs. In our work, more than 80% of the acetaminophen and caffeine contents were released from each of the preparations in a clearly shorter time than 10 minutes (Fig. 5-8). Moreover, the required amount of acetaminophen was released during the first minute of the test from most granulates. The time required to release 80% of the active substance was only extended to 2 minutes in the case of series 4 (calcium hydrogen phosphate and PEG 6000). In considering the dissolution of caffeine, we noted that the indicated amount of the substance passed into the dissolution medium during the first 2 minutes from granules 2 (mannitol and PEG 6000), 3 (mannitol, calcium hydrogen phosphate and PEG 6000) and 7 (mannitol and PVP K30), and with series 4, only after 5 minutes of testing. Kraciuk and Sznitowska [8] demonstrated that a dissolution profile is strongly influenced by the filler type and, in the case of calcium hydrogen phosphate, a very slow dissolution is normally observed. In contrast, mannitol allowed for very fast dissolution of active substance and, hence, Kraciuk and Sznitowska recommend the use of this filler [8]. The requirements for the dissolution of caffeine were met in the first minute of the process for the other formulations.

Among the tested granules, the fastest dissolution of both acetaminophen and caffeine was demonstrated for preparations containing a mixture of lactose and corn-starch (series 10 with lactose, corn-starch and PVP K30 and series 5 with lactose, corn-starch and PEG 6000). During the first minute, 98.7% of the acetaminophen and 94.7% of the caffeine from granules series 10 and 97.8% of the acetaminophen and 87.3% of the caffeine from series 5 were released (Fig. 6 and 8). This granule behaviour can be explained by the low mechanical strength of these formulations. The disintegrant properties of starch and its swelling capacity promote the formation of capillaries inside the granules. The dissolving liquid easily penetrates through these capillaries, hence, accelerating the dissolution of APIs [1]. The slowest dissolution of active substances was found in the case of granules series 4, of which 76.0% of all acetaminophen and 59.3% of all caffeine were released in the first minute of the study (Fig. 6 and 8). The binding of calcium hydrogen phosphate to PEG 6000 (series 4) is stronger than in other preparations, and resulted in the reduction of intermolecular spaces and the impeded penetration of the dissolution medium into the granules structure. This led to a slower dissolution of APIs from this preparation. During the first two minutes, a fairly rapid dissolution of the substances was observed, after which the dissolution rate was slowed down. However,

the dissolution profiles of active substances from all granules were generally similar. A slightly higher variation depending on the type of the filler was observed for formulations containing polyethylene glycol 6000 (PEG 6000, series 1-5). Hori *et al*. [9] also demonstrated that the paddle method gave faster dissolution profiles at all rotation speeds than did any of the other methods tested. The paddle method, thus, gives satisfactory drug dissolution at any rotation speed tested.

On carrying out the basket test, significant changes were noticed during the active substances dissolution process, compared to the paddle method (Fig. 5 and 7). Herein, substances dissolved gradually and more slowly than in the paddle method. This was indicated by smaller amounts of acetaminophen and caffeine released in the first minutes of the study and, therefore, longer times needed to dissolve 80% of all active substances. Within 10 minutes, only the granules of series 8 and 9 released into the dissolution medium, respectively: 96.2% and 100.0% of all acetaminophen and 94.8% and 98.4% of all caffeine (Fig. 5 and 7). The time required to release 80% of both active substances from the majority of tested preparations was from 30 to 45 minutes. Incomplete dissolution of active substances was found in the time of the study in the case of series 10 granules alone. This contained lactose, corn-starch and polyvinyl pyrrolidone K30 (PVP K30). Herein, less than 75.0% of all acetaminophen and 80.6% of all caffeine were released into the dissolution medium within 60 minutes. A remnant of the introduced granules was also noted in the basket. In this, undissolved amounts of active substances remained at the end of the test. We also noticed that granules with lactose (series 1 and 6) and with a mixture of lactose and maize starch (series 5 and 10) were characterized by similar dissolution profiles (Fig. 5 and 7). A quite intensive increase in the dissolution was observed, in all granules during the first few minutes of the study, after which the amount of substances dissolved increased almost proportionally. In addition, the basket method saw greater divergences in the process of dissolution from granules differing in excipients, compared to the paddle method, with clearest changes observed in formulations with PVP K30 series 6-10.

Considering the type of binder used, we revealed that regardless of the method of testing, granules obtained using the ethanolic PVP K30 solution (series 6-10) were characterized by a faster dissolution, compared to formulations containing PEG 6000 (series 1-5). However, the observed impact was mild. The dependence of the dissolution process on the type of binder is more noticeable in the basket method. The acceleration of dissolution due to the replacement of PEG 6000 with PVP K 30 is probably due to the lower binding of the powder mixture by PVP K 30, which is also indicated by the shorter disintegration time of granulates containing this type of binder [2].

Comparing the course of the dissolution processes depending on the method of conducting the study, we demonstrated that in using the paddle method, the drug substances were dissolved much faster. The weaker dissolution of acetaminophen and caffeine in the basket method was probably due to the presence of a mechanical barrier in the form of a mesh with apertures of 0.40 mm diameter inside the rotating basket. Skripnik *et al*. [10] showed, that compared to apparatus 1, the paddle provides important advantages, especially concerning the possibility of excipients from the formulation becoming stuck in the basket mesh.

The granules placed in the basket also occupy much smaller volumes than if these were poured loosely into a beaker with a dissolution medium, as in the paddle method. Furthermore, the dissolution medium penetrates more easily into the space between the particles because of the more loose grain packing of granules than in the basket. This caused faster dissolution and increases the dissolution rate of the active ingredients. The dissolution profiles of the active substances obtained by the basket method were the closest to those obtained in the paddle method in the case of calcium hydrogen phosphate containing granules (series 4 and 9). Granules with lactose (series 1 and 6) and formulations containing a mixture of lactose and maize starch (series 5 and 10) showed the largest differences in the dissolution processes.

The results obtained in the paddle method of the dissolution test were consistent to the results obtained in the disintegration test, due to similar conditions – lack of a mechanical barrier. Each of the series of granules disintegrated in a time much shorter than 10 minutes, which suggests that the dissolution of active substances also took place in a relatively short time [2]. However, in the diagrams showing dissolution profiles, larger differences between individual series are visible in the case of the basket test method (Fig. 5-8). It is difficult to directly compare apparatuses in terms of the anticipated effects on dissolution, as dosage form, location and behaviour during a dissolution test can also vary between paddle, basket, and flow-through apparatuses [11]. The best dissolution method is that which reflects the behaviour of the formulation, regardless of whether the dissolution rate is high or low. In the case of granules, the basket method gave greater formulation differentiation behaviour than did the paddle method.

The study showed that the paddle method of testing the dissolution of active substances from granules is more consistent to the results obtained in the disintegration test, because acetaminophen and caffeine are released much faster than when using the basket method. During the first 10 minutes, there was almost complete dissolution of APIs from all the tested formulations. This result is compatible with the rapid disintegration time of the preparations. Still, the best dissolution method is that which provides the most discriminant conditions, and that which is more capable of detecting differences between formulations when they are present, and that which is more relevant for dissolution performance. So the basket method is more suitable for testing granules.

The course of the dissolution process of the active substances depends on the type of excipients. The slowest dissolution of the active substances was observed in utilizing the paddle method, from granules containing calcium hydrogen phosphate and PEG 6000. The most rapid dissolution was from preparations containing a mixture of lactose and corn-starch, regardless of the binder used or the method applied.

To conclude, application of the first derivative spectrophotometric method allows simultaneous determination of acetaminophen and caffeine in the mixture, without the need to separate them first.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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REFERENCES

1. Sznitowska M. (ed.) *Farmacja stosowana. Technologia postaci leku*. Warsaw: PZWL; 2017:229-51 (in Polish).
2. Szumilo M, Belniak P, Swiader K, Holody E, Poleszak E. Assessment of physical properties of granules with acetaminophen and caffeine. *Saudi Pharm J.* 2017;25(6):900-5.
3. *The Polish Pharmacopeia*. 6th ed., The Minister of Health (PL), Warsaw; 2002:323-4,554 (in Polish).
4. Sokol A, Karpinska J, Talecka R, Starczewska B. Quantification of ranitidine hydrochloride in the presence of its decomposition product by spectrophotometric methods. Application for kinetic study. *Acta Pol Pharm Drug Res.* 2011;68(2):169-77.
5. Vichare V, Mujgond P, Tambe V, Dhole SN. Simultaneous spectrophotometric determination of paracetamol and caffeine in tablet formulation. *Int J PharmTech Res.* 2010;2(4):2512-6.
6. *The European Pharmacopoeia*. 9th ed., The Council of Europe (EU), Strasbourg; 2017:429-34.
7. Chevalier E, Viana M, Artaud A, Chomette L, Haddouchi S, Devidts G, et al. Comparison of three dissolution apparatuses for testing calcium phosphate pellets used as ibuprofen delivery systems. *AAPS PharmSciTech.* 2009;10(2):597-605.
8. Kraciuk R, Sznitowska M. Effect of different excipients on the physical characteristics of granules and tablets with carbamazepine prepared with polyethylene glycol 6000 by Fluidized Hot-Melt Granulation (FHMG). *AAPS PharmSciTech.* 2011;12(4):1241-7.
9. Hori S, Kawada T, Kogure S, Yabu S, Mori K, Akimoto M. Comparative release studies on suppositories using the basket, paddle, dialysis tubing and flow-through cell methods I. Acetaminophen in a lipophilic base suppository. *Pharm Dev Tech.* 2017;22(1):130-5.
10. Skripnik K, Riekes M, Pezzini B, Cardoso S, Stulzer H. Investigation of the dissolution profile of gliclazide modified-release tablets using different apparatuses and dissolution conditions. *AAPS PharmSciTech*. 2017;18(5):1785-94.
11. Todaro V, Persoons T, Grove G, Healy A, D'Arcy D. Characterization and simulation of hydrodynamics in the paddle, basket and flow-through dissolution testing apparatuses – a review. *Diss Tech*. 2017;24(3):24-36.