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The Impact of Selected Preparations of Trace Elements – Magnesium, Potassium, Calcium, and Zinc on the Release of Diclofenac Sodium from Enteric Coated Tablets and from Sustained Release Capsules

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. In an aging society, many patients require long-term treatment. This fact is associated clearly with the simultaneous occurrence of lifestyle diseases such as hypertension, diabetes, and even osteoarthritis. Concomitant medications, which are a common practice, pose a major threat of an interaction between these drugs. Very popular now "fast way of life" that makes people have less and less time to prepare well-balanced meals of high nutritional value. The result of this lifestyle is an increased need for supplementation preparations necessary vitamins and minerals. Given the wide availability of dietary supplements (shops, kiosks, petrol stations) raises the question about the possibility of an interaction between the uncontrolled intake of dietary supplements and medications received in the most common diseases of civilization.

Objectives. The aim of this study was to investigate the effect of the most important minerals (magnesium, potassium, calcium, zinc) contained in the popular nutritional supplements, the release also often used as an anti-pain, anti-inflammatory, diclofenac sodium from the different formulations.

Results. Among the many as sodium diclofenac selected two most common: film-coated tablets and sustained release capsules. The study showed a significant effect of minerals on the release of diclofenac sodium and differences that impact, depending on the test form of the drug (**Adv Clin Exp Med 2014, 23, 2, 205–213**).

Key words: diclofenac, magnesium, potassium, calcium, zinc, release.

The phenomenon of polipharmacotherapy increases frequently, especially between patients who require chronic medication. This poses a risk of dangerous interactions. The known impact on the absorption of drugs is dependent on the type and quantity of food intake, as well as the time of drug intake. Influence of different factors on the absorption of drugs, their bioavailability, and pharmacological effect was studied in numerous works [1, 2]. The aim of this study was the assessment of the impact of commonly used, non-prescription, mineral supplements, on the release of diclofenac sodium from various dosage forms. Among the commercially available forms, two of the most commonly used forms were selected: enteric-coated tablets and sustained release capsules. Diclofenac sodium as a popular examplification of the medical substance was chosen due to the fact that it is one of the most commonly used anti-inflammatory preparations. The study involved simple dietary supplements containing magnesium, potassium, calcium, zinc, and a combination preparation, a mixture of the above mentioned elements and several other micronutrients. Diclofenac sodium belongs to the group of non-steroidal anti-inflammatory drugs (NSAIDs). This substance is rapidly and easily absorbed in the gastrointestinal tract; peak blood levels after oral administration are obtained after approximately 2 h [3–7]. Magnesium affects the sensitivity of nerve tissue and muscular tissue and entails the decrease of the muscle tension, including the heart muscle [7–11]. The combination of magnesium and vitamin B_6 is widely used, as the

vitamin increases the absorption of magnesium ions in the gastrointestinal tract [12–17]. Potassium and sodium ions with chloride co-ion influence the regulation of aqueous and mineral balance, as well as the acidic-basic balance within the body system. Potassium is marketed both in the form of simple formulas and in combination with other minerals and vitamins [8, 18, 19]. Zinc is a co-factor for approximately 80 enzymes from six classes: oxidoreductases, transferases, hydrolases, lipases, isomerase, ligases. Zinc affects humoral and cellular immunity and has immunomodulatory and antiviral activity. Zinc in simple preparation for oral use is available in the form of uncoated and coated tablets [8, 20–23]. Calcium ions enable the development of bones and teeth. Intracellular calcium ions provide universal factor of the second order, which affects the behavior of normal synaptic sensitivity of the neuromuscular system. Calcium also affects blood clotting [8, 24, 25]. The most usual calcium formulas are effervescent tablets, coated and uncoated tablets, as well as capsules and syrups. In the human body, chromium is involved in the metabolism of glucose, in the glucose tolerance factor – (GTF), responsible for removing glucose from the blood [8, 26]. Chromium is sold both in the form of simple and composited preparations [7, 26]. Selenium is involved in more than 20 enzymes of oxidative reactions [8]. On the market the selenium element is available in the form of simple formulas, as well as complex preparations, however mostly in the form of simple tablets [7]. Copper is included in several enzymes involved in iron metabolism, in inactivation of free radicals, in the nervous system activity, in the process of erythropoiesis and in the inflammatory occurrence [8]. The commercial preparations are composed of copper in conjunction with vitamins and other minerals, and the separate products with copper salts only are unavailable [7]. Manganese influences the metabolism of carbohydrates and lipids, affects the formation of connective tissue and the reproduction phenomena [8]. Manganese, and molybdenum, similarly to the copper, are present only in the form of composed preparations, with other supplements, vitamins or minerals [7]. Iron, as a component of hemoglobin and myoglobin, was one of the first supplemented elements. As a component of cytochromes is involved in electron transfer and oxygen reduction [8]. Commercial preparations contain iron both as simple drug forms, as well as complex preparations with vitamins and other minerals.

Material and Methods

Within this study the modified pharmacopeial method was applied. Two tablets were placed in a vessel filled the buffer mentioned below: one diclofenac sodium formulation, and one mineral formulation. The environment of the small intestine was simulated in vessels intended for standard release device – Van Kel model 7025. The drug was released to a volume of 500 cc of phosphate buffer of pH 7.4. The speed of rotation was 50 rpm. A constant temperature of 37 ± 2°C was maintained in the vessels. The released drug was assessed in a spectrophotometer, where six repetitions were performed for every preparation. The samples were taken in specified intervals. In the case of enteric-coated tablets the samples were taken after: 3, 9, 15, 21, 27, 33, 39, 45, 51, and 57 min, while for the prolonged-release capsules the sampling points were as follows: 15, 45, 75, 105, 135, 165, 195, 225, 255, and 285 min. Two formulations of diclofenac sodium were assessed in the present work: 50 mg of diclofenac sodium in coated tablets, and 100 mg of diclofenac sodium in extended-release capsules. The following mineral supplements were involved in the study: magnesium chloride coated tablets, 64 mg of Mg^{2+}, capsules of calcium carbonate 400 mg, potassium chloride prolonged-release capsules, 315 mg, zinc sulphate coated tablets, 200 mg Zn^{2+}, multimineral dragees containing: 54 mg of Ca, 41.6 mg of P, 33.3 mg of Mg, 9 mg of Fe, Zn of 7.5 mg, 2.5 mg of Mn, 2.5 mg of K, 1 mg of Cu, 0.05 mg of I, 5 μg of Cr, Mo of 5 μg, 5 μg of Se.

Raw data was compared using a parametric ANOVA with post-hoc test NIR (least significant difference). The normality of distribution of raw data rated three different statistical tests: the Kolmogorov-Smirnov test, Lilleforsa and using the Shapiro-Wilk and Levene's test of homogeneity of variance and Brown-Forsyth. All statistical analyzes conducted assumed the confidence level $p = 0.05$. All measurements were repeated min. 6 times. Statistical analysis was performed based on the program STATISTISA UK version 10.2

Results

Important differences in the release curves may be observed in the course of the release of diclofenac sodium from its commercial form – gastro-resistant tablet – to the ambient medium, compared to the release in the presence of various micronutrients – as it is presented in Fig. 1. A cumulative graph, which presents the constant rates of release of diclofenac sodium from the gastro-resistant tablets with and without the presence of micronutrients is attached in Fig. 2. The release rate of diclofenac sodium to the standard environment was is in the range of 0.3134 h^{-1}. When magnesium ions were applied to the acceptor medium,

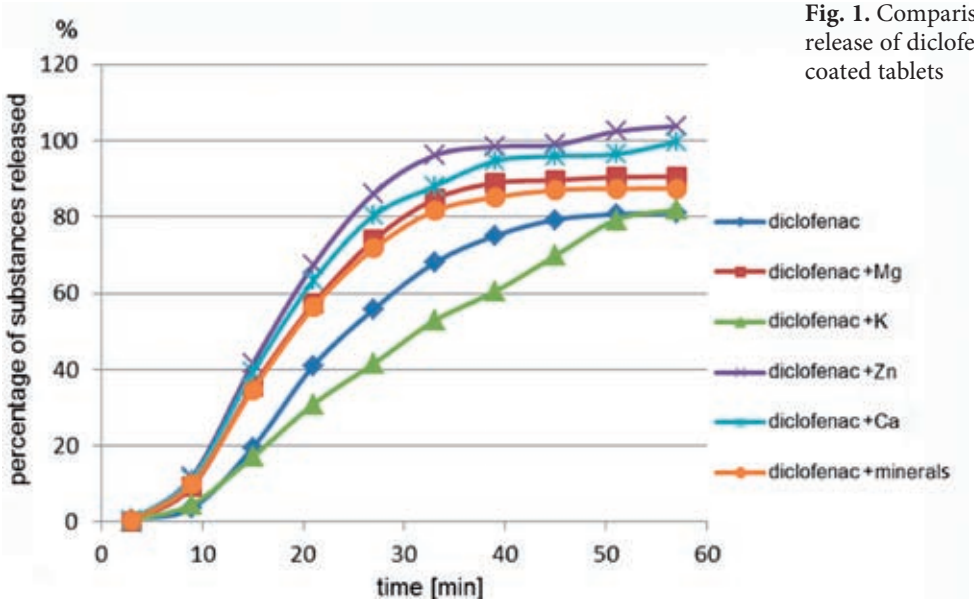

Fig. 1. Comparison of the curves of the release of diclofenac sodium enteric-coated tablets

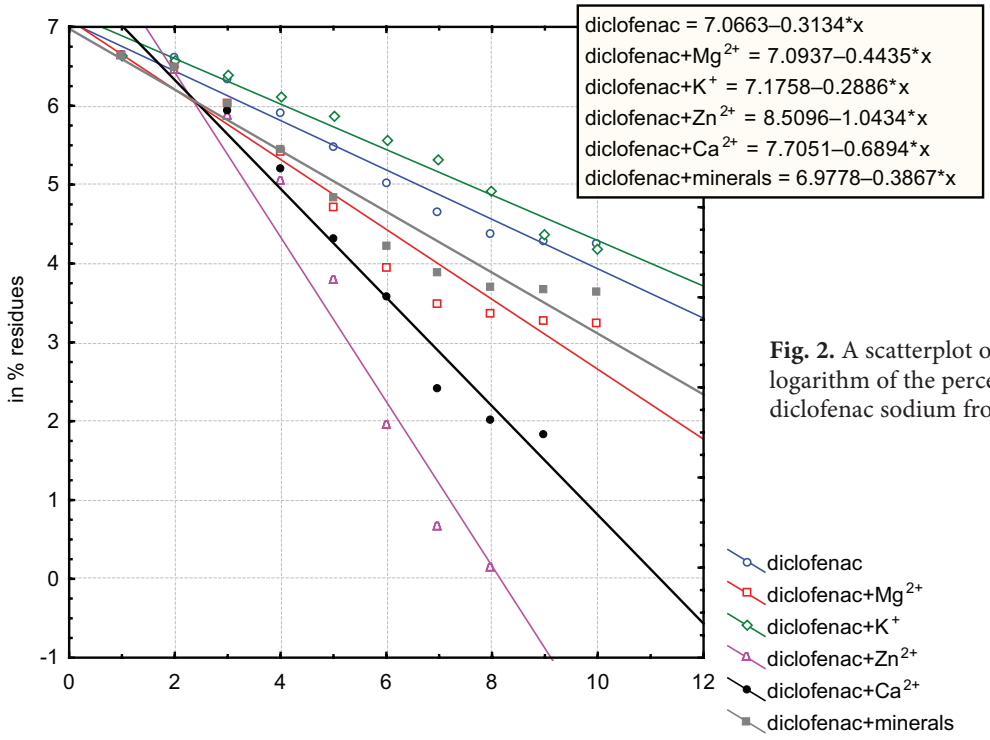

Fig. 2. A scatterplot of the natural logarithm of the percentage of residual diclofenac sodium from enteric tablet

the level of doclofenac was higher than that of diclofenac alone. The release rates of diclofenac sodium alone (k_d) and with the ions of magnesium (Mg_k) are 0.3134 h^{-1} and 0.4435 h^{-1} respectively. Increased value of the release rate for the sample with magnesium ions confirms the observed differences in evaluated release profile. In the case of potassium ions, the curve of the release of diclofenac potassium in the initial stage of the process is rather flattened, compared to the other release profiles; the release of the active substance in the presence of potassium ions is on the lowest level in all the assessed samples. Consequently, the amount of released drug substance in the presence of the mineral was 15.16% lower than in the sample with pure diclofenac, at the 35th min of the release process. At a later stage, the gap narrows, and the release of the final point comes to compensation, so a slight increase in the quantity of the released active substance in the sample with potassium ions is observed. Finally the amount of released diclofenac has the value of 81.94%, responding to the 0.93% of diclofenac assessed alone. The rate of release of diclofenac sodium in the presence of potassium ions was calculated to be 0.2886 h^{-1}. Our study revealed clear differences

in the dissolution profile of diclofenac in the presence of calcium ions, compared to the diclofenac. The release rate of diclofenac from the donor compartment with calcium ions (Ca_k = 0.6894 h^{-1}) is more than twice than the rate constant of diclofenac alone (k_d). The release of diclofenac occurred in the presence of calcium ions much faster and more intensely. Similarly in the case of zinc ions the release of diclofenac was enhanced. Value of the release rate (k_{Zn}) for the sample with zinc ions is 1.0434 h^{-1}. High value of the release rate for the sample with zinc ion indicates that the release occurred faster, and the patient may absorb a high dose of diclofenac, when applying parallel calcium supplement to his diet. The next stage of this study was to compare the effect of the minerals gathered simultaneously in one tablet. The resulting release curve is similar to the curve of pure diclofenac. Initially, very slight increase in quantity of released diclofenac is observed when multi-mineral preparation is applied. The difference in the quantity of released diclofenac during the process – comparing to the pure diclofenac compartment – was observed after ca. 30 min, and was 15.96%. Then, the difference between the release in the presence of minerals and without the presence of any element gradually decreases. The difference at the endpoint is in the range of 6.48% of substances released comparing to the reference curve. The amount at the endpoint was calculated to be 87.50%. The release rates of diclofenac sodium (k_{multi}) in the presence of the multi-mineral preparation was evaluated at 0.3867 h^{-1}. The release rates are similar, what explains the slight differences in the release profile of individual cases and consequently the final quantity of substances released. The last and yet most important step is the calculation of statistical comparison of the percentage of substances released 57 min after the time for all cases. For this purpose LSD test was used. Despite the proven impact of all minerals on the degree of drug substance release, only in the case of calcium ions, these differences are significant in terms of statistics (Fig. 3). Comparing the percentage of substances released after 57 min and a constant rate of release was found that calcium and zinc ions increase the amount of released diclofenac sodium enteric coated tablets.

Prolonged-Release Capsules

Figure 4 presents the summarized release curves of diclofenac sodium from extended release capsules, in the presence of co-administered minerals. For a detailed analysis of the collected data the release rate was evaluated (Fig. 5). The release rate ($k_{d\text{-}ret}$) in the case of the capsules was assessed as 0.1966 h^{-1}. Magnesium ions have a complex effect on the release of diclofenac. Initially, the increase of release was observed. After 45 min the difference between the percentage of substances released from the reference sample, and from the sample assessed in the presence of mineral was highest and to the value was 2.63%. However, later the release of diclofenac was slower. In the chamber containing diclofenac in the presence of magnesium ions the average concentration of diclofenac was 14% lower, compared to that in the chamber with pure diclofenac. At the last measurement point,

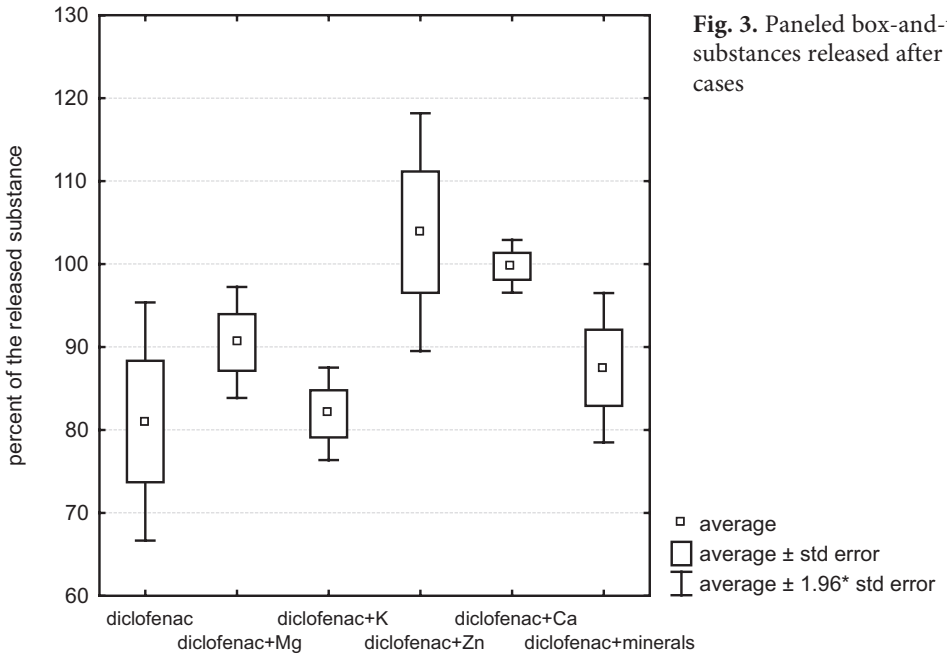

Fig. 3. Paneled box-and-whisker diagram percent substances released after time 57 min, for all the cases

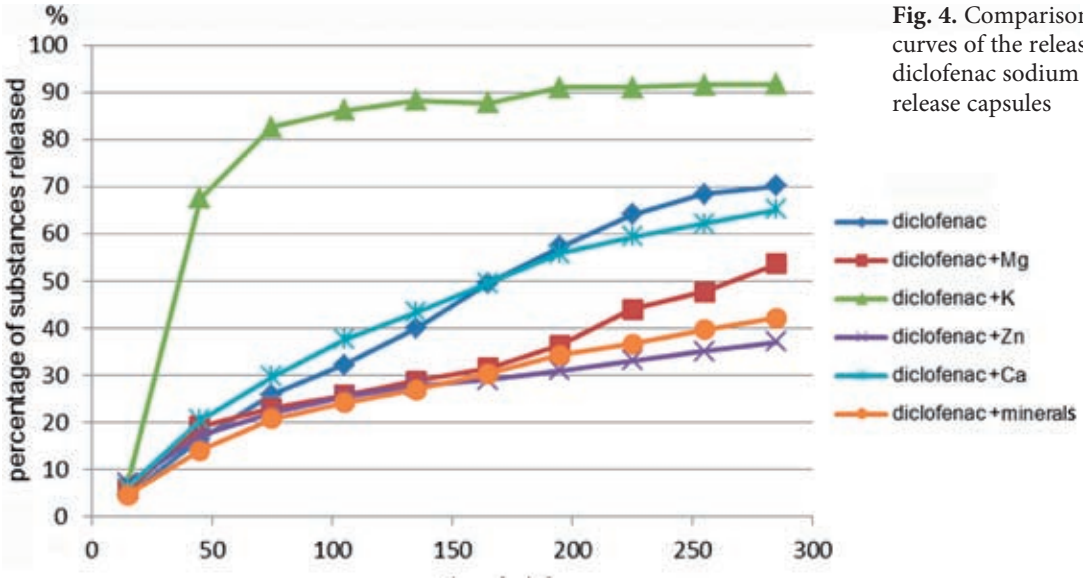

Fig. 4. Comparison of the curves of the release of diclofenac sodium extended release capsules

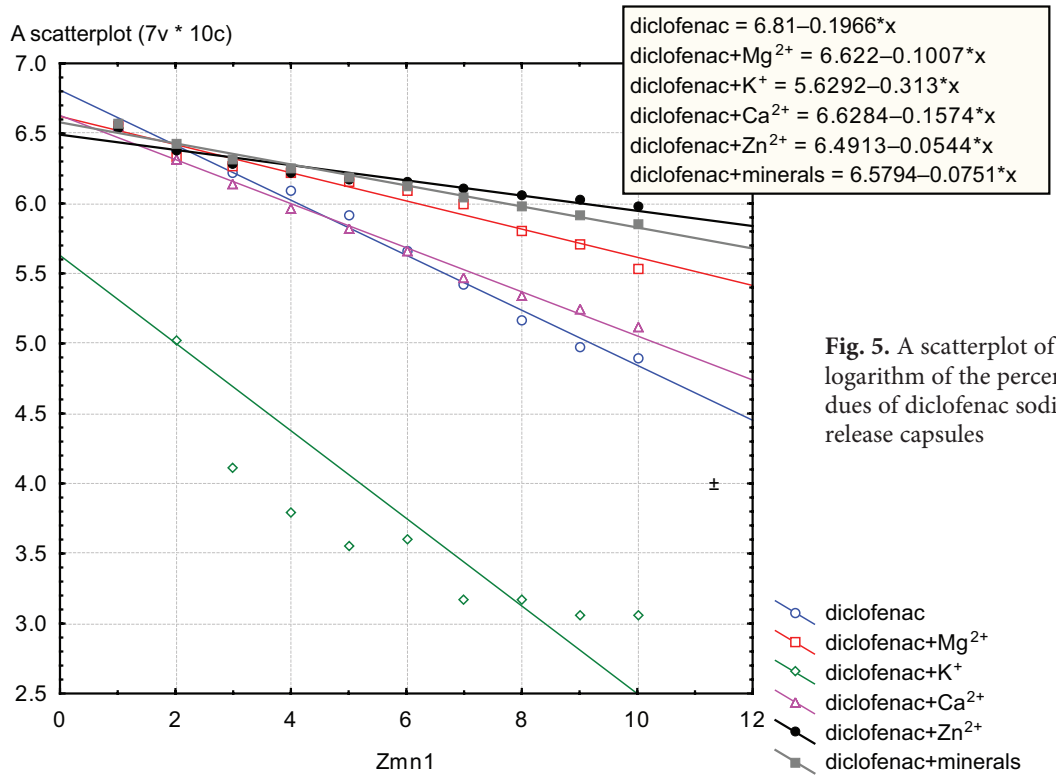

Fig. 5. A scatterplot of the natural logarithm of the percentage of residues of diclofenac sodium sustained-release capsules

the percentage of released drug substance reached a value of 53.54%, which is about 16.65% less than in the case of diclofenac alone. In order to confirm the results, the release rates were calculated for the release of diclofenac ($k_{d\text{-}ret}$) and diclofenac in the presence of magnesium ($Mg_{k\text{-}ret} = 0.1007\ h^{-1}$. The potassium ions strongly enhance the release of the drug substance. Already in the first minutes of the release, fast drug release in the sample with the mineral can be observed. Over time, the difference between a good amount of released diclofenac potassium and diclofenac from fast-growing, reaching a maximum after about 1 h: 56.82%. Then the curve stabilizes, the differences gradually decrease, but are unable to compensate the quantity of substances released in both trials. In the last measurement point, the percentage of the released diclofenac sodium is 91.69% and is about 21.50% higher than in the chamber with diclofenac alone. Release rate for diclofenac sodium in the presence of potassium ions ($K\text{-}k_{ret} = 0.313\ h^{-1}$) is much higher than the constant ($k_{d\text{-}ret}$). The effect of calcium ions is more complicated. Initially, a clear increase in the release of drug substance is observed in the

presence of micronutrient. This impact is not long and after about half the time of release process, there is no difference in the quantity of substances released between samples. In the further stage of the process, inhibition of release of diclofenac sodium is observed in the presence of calcium ions. Ultimately, the final amount of the substance released from the mineral compartment is 65.15% and is about 5.04% lower than in the sample with diclofenac alone. For the diclofenac, is the calculated release rate reaches 0.1966 h^{-1}, whereas for the sample with calcium ions the release rate ($k_{Ca\text{-}ret}$) is 0.1574 h^{-1}. These values are close to each other, but the slightly lower release rate in the presence of calcium ions explains the low final amount of released drug substance, compared to the sample with diclofenac only. The curve of release of diclofenac sodium in the presence of zinc ions has a different course than the curve plotted from data of the release of diclofenac alone. Initially on the graph a small, short-term increase of release is visible, but definitely important is the subsequent gradual, systematic reduction of the percent of substance released with the passing time. The release curve of diclofenac in the presence of Zn is much lower and therefore the amount of released diclofenac sodium is also lower. The end-point percentage of released drug substance was 37.00%. It is about 33.16% less than in the reference sample. This may be explained by the inhibition of the release of diclofenac sodium from the dosage form, when zinc in present in the sample. Release rate of diclofenac in the presence of zinc ions (Zn-k_{ret} = 0.0544 h^{-1}) is approximately four times lower than the rate constant $k_{d\text{-}ret.}$. The effect of minerals on the release process, from the very beginning of the release is rather uniform. On the graph the clear inhibition of the release of drug substance in the presence of a multi-mineral dietary supplement from the first minutes of the process was observed (Fig. 4). The highest difference between the released amounts of the diclofenac and diclofenac in the presence of multi-mineral preparation was visible after 255 min, and reached 28.78% less substance. At the endpoint, the difference was 28.00%. Release rate for diclofenac sodium in the presence of minerals (multi-ret_k) is 0.0751 h^{-1}, while the $k_{d\text{-}ret}$ is equal to 0.01966 h^{-1}. Such a large difference in the values of constants is the cause of significant changes in the release curves.

The development of statistical results is presented in Table 1. Both the analysis of variance was adopted, because the p value is less than 0.05.

The last step is the calculation of a statistical comparison of the percentage of substances released after 285 min for all cases, which is used to test the LSD. Comparing data from Table 1, it can be concluded that the investigated ions have a significant effect on the release rate of diclofenac sodium extended release capsules. As many as 4 cases of statistically significant result were obtained: for the ions of magnesium, potassium, zinc and minerals, only change the release curve in the presence of calcium ions is not confirmed statistically (Fig. 6). Taking into account the percentage of substances released in 285 min and the constant rate of release, it was found that potassium ions increase the amount of released diclofenac sodium, and magnesium ions, zinc and mineral mixture reduces the amount of released diclofenac.

The release rates of all combinations of diclofenac with various selected trace elements with most popular minerals are showed in Table 2.

Discussion

In the study the main outcome is connected to the evaluation of the fact that the minerals contained in food supplements affect the pharmaceutical availability of diclofenac sodium from its preparation. It was also noted that the impact of the same type of ions may vary depending on the dosage form, resulting in the inhibition or acceleration of the release. In the presence of magnesium ions, when the release was performed from the gastroresistant tablets, after 285 min 90.55% of the total amount of diclofenac sodium was released, which is about 9.52% more than in the reference sample. In the case of prolonged-release capsules, the final amount of the substance released was 53.54%,

Table 1. Statistical analysis of variance on the percentage of substances released during 4 h 45 min of prolonged-release capsules

The sum of squared deviations between the groups	The number of degrees of freedom between groups	Variance between groups	The sum of squared deviations within groups	The number of degrees of freedom within groups	Variance within groups	P
12130.04	5	2426.008	657.4584	30	21.91528	$p < 0.000001$

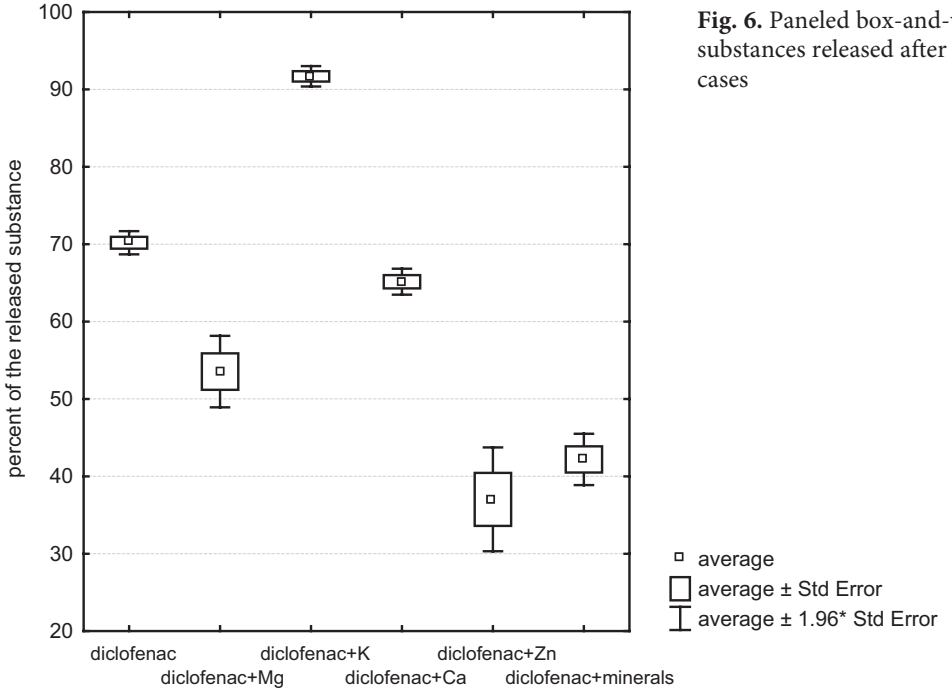

Fig. 6. Paneled box-and-whisker diagram percent substances released after time 285 min, for all the cases

Table 2. Release rates k [h^{-1}]

	Diclofenac	**Diclofenac + Mg^{2+}**	**Diclofenac + K^{+}**	**Diclofenac + Zn^{2+}**	**Diclofenac + Ca^{2+}**	**Diclofenac + multiminerals**
Enteric tablets	0.3134	0.4435	0.2886	1.0434	0.6894	0.3867
Sustained release capsules	0.1966	0.1007	0.3130	0.1574	0.0544	0.0751

and is 16.65% less than in the reference sample. The presented data suggests that magnesium ions strongly inhibit the release of diclofenac sodium from prolonged release tablets, as confirmed by statistical analysis. The effect of potassium ions is different, compared to other minerals evaluated in the work. In the case of the enteric-coated tablets, potassium significantly slows the release; however, the final amount of the substance released is approximately equal to the diclofenac released without the presence of potassium, and reaches the value of 81.94%. When sustained release form of diclofenac is applied, a completely different effect of potassium is observed. In the first few minutes there is a clear increase of the amount of diclofenac released, resulting in a final amount higher by 21.50% from the reference results. In the case of prolonged-release capsules statistical analysis confirms the significance of the obtained results. Calcium ions have various effects, which depend on the form of the assessed drug. When the enteric-coated tablets were evaluated, a visible increase in the release of diclofenac sodium was observed in the presence of mineral. The impact was uniform over the entire length of the curve. As a result, 18.71% more of the substance was released from the drug form in the presence of calcium ions, compared to the diclofenac enteric-coated tablets alone. The kinetic curve in the case of sustained-release capsules is significantly modified by the presence of calcium ions: in the initial stage of the process the release of diclofenac sodium increases by about 5% comparing to the reference, and is followed by inhibition of release by about 5% less than in the sample of reference diclofenac sodium in the form of sustained-release capsules. The analysis confirms the statistical significance of the effect of calcium ions on the release of diclofenac from enteric tablets. The change of the release profile of the drug from the prolonged-release capsules, comparing to the tablets, under the influence of calcium ions is not statistically significant. The effect of zinc ions on the release of both forms of the drug is clear, but definitely different. In the case of the enteric form, a strong increase in the release of diclofenac sodium was observed, when compared to the reference. This difference in the final measurement point was 22.83%. The zinc ions exhibit the strongest influence on the sustained-release capsules of diclofenac sodium, compared to other mineral preparations.

Finally, the concentration of diclofenac sodium in the last measurement is 33.16% lower than in the case of the reference sample of diclofenac sodium capsule. Differences in the release profile for both cases are very large, and both were considered statistically significant. We also examined the effect of micronutrients that occur simultaneously in one tablet. Also in this case, different results were obtained, depending on the dosage form. The release of diclofenac sodium enteric coated tablets is increased along the entire length of the process in the presence of minerals. Ultimately, the amount of drug substance released is 6.48% higher in comparison with the reference – tablets with diclofenac sodium. The reverse effect can be observed in the case of prolonged-release capsules. The inhibition of the release of the drug was observed and the amount of released diclofenac sodium was reduced to 28.00% compared to diclofenac preparation without the minerals. The impact of multi-mineral preparation on the release of diclofenac sodium from enteric-coated tablets is not statistically significant, whereas the same statistical analysis recognizes the significant differences in the release of diclofenac sodium from sustained-release capsules in the presence and without the presence of multi-mineral preparation.

The conducted research confirm that mineral supplements affect the release profile of enteric coated tablets and sustained-release capsules with diclofenac sodium. Preparation of magnesium chloride increases the release of the drug from the gastro-resistant tablets, and inhibits the release from prolonged-release capsules. The presence of potassium chloride inhibits the release of diclofenac sodium from the enteric coated tablets and increases in the process carried out by means of prolonged-release capsules. Calcium carbonate, when co-solving in the presence of commercial forms of diclofenac sodium, increases the release of the drug from the gastro-resistant tablets, and inhibits the release from the prolonged-release capsules. Zinc sulphate influences strongly the release of diclofenac sodium from enteric coated tablets, and the zinc sulphate mineral preparation inhibits the release of diclofenac sodium from sustained-release capsules. Multi-mineral preparation increases the release of diclofenac from enteric coated tablets, and inhibits the release from prolonged-release capsules. These differences in the effects of micronutrients on the release of diclofenac from both of assessed forms of the drug may be connected to the different physicochemical conditions prevailing in different parts of the gastrointestinal tract. It should be noted that the therapeutic approach may involve uncontrolled poly-pharmacotherapy observed more frequently among patients. The results may be valuable for the design of new multi-component supplementary preparations.

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