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Research article / Оригинальная статья

Development of a Gastro-retentive Dosage Form of a New Promising Anti-tuberculosis Drug Macozinone

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Abstract

Introduction. Due to increase in the frequency of detecting cases of tuberculosis caused by strains of mycobacteria with resistance not only to traditional, but also recently introduced into clinical circulation anti-tuberculosis drugs, it is urgent to search for and develop new drugs that can be effective against multidrug-resistant (MDR-TB) and extensively drug resistant (XDR-TB) strains. One of the most promising classes of such compounds are fluorine derivatives of benzothiazinones, and particularly compound PBTZ169 (INN macozinone). This antibiotic has a high specificity against mycobacteria tuberculosis (*M. tuberculosis*), inhibiting one of the key enzymes of cell wall synthesis. However, macozinone as an active pharmaceutical ingredient has significant features of physical and chemical properties that hinder the development of oral dosage forms based on it. It is classified as class IV by BCS and is characterized by a very low solubility and lipophilicity, a pronounced dependence of dissolution rate on the pH of the medium, and very low bioavailability when taken orally.

Aim. To substantiate the target profile, critical quality attributes and to develop a prototype of an oral dosage form with modified release of macozinone, allowing to maximize its pharmacological activity.

Materials and methods. Using pharmaceutical substance macozinone hydrochloride and various excipients, experimental tablets with a dosage of 500 mg macozinone were developed. The influence of the composition of the media and the added excipients on the solubility of macozinone in various biorelevant media, the degree of swelling in the liquid and the degree of mucoadhesion of the experimental tablets to the mucus of the pig stomach were evaluated. The HPLC method was used to evaluate the kinetics of the release of the active substance.

Results and discussion. In this work, the expediency of creating macozinone-containing gastro-retentive dosage forms with a slow release of the active substance, the delay mechanism of which is provided by swelling and increased adhesion to the gastric mucosa, has been substantiated. Various tablet samples were experimentally tested in which the modification of the release of the active substance and the degree of swelling and mucoadhesion were varied by introducing various excipients into the formulations, including known swelling and bioadhesive matrix agents.

Conclusion. According to the results of the experiments, samples of high-dose (500 mg) swellable and mucoadhesive tablets created by the technology of two-stage granulation with the inclusion of macozinone – hydroxypropyl-beta-cyclodextrin mixtures in the primary granules and introduction of combinations of soluble and insoluble hydrophilic matrix agents into the intergranular space were recognized as the most promising for subsequent pharmacokinetic studies.

Keywords: tuberculosis, macozinone, gastro-retentive tablet, modified release, mucoadhesion, cyclodextrin

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

Contribution of the authors. Vladimir G. Nesterenko, Boris A. Rudoy, Roman N. Bolgarin, Natalia A. Nikitina substantiated the actuality of the work. Boris A. Rudoy carried out scientific management of the entire research. Damir K. Salakhetdinov developed the compositions and produced experimental tablets. Yuri G. Kazaishvili, Victoria S. Scherbakova conducted research on the properties of tablets. Igor E. Shohin, Yuri V. Medvedev, Elizaveta N. Fisher, Evgeniya A. Malashenko investigated the dissolution of samples. Boris A. Rudoy, Damir K. Salakhetdinov, Yuri G. Kazaishvili prepared the text of the article. All authors participated in the discussion of the results.

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Разработка гастроретентивной лекарственной формы нового перспективного противотуберкулезного лекарственного средства макозинон

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Резюме

Введение. В связи с увеличением частоты выявления случаев туберкулеза, вызванных штаммами микобактерий, устойчивых не только к традиционным, но и недавно введенными в клинический оборот противотуберкулезным средствам актуальной является задача поиска и разработки новых лекарственных средств, способных эффективно подавлять мультирезистентные МЛУ и ШЛУ – штаммы *M. tuberculosis*. Одним из наиболее перспективных классов такого рода соединений являются трифтор-производные бензотиазинонов, и, в частности, соединение PBTZ169 (МНН макозинон). Однако макозинон обладает существенными особенностями физико-химических свойств, которые затрудняют разработку на его основе лекарственных форм для перорального применения. Он относится к классу IV по BCS и характеризуется низкой растворимостью, низкой липофильностью, выраженной зависимостью растворения от pH среды, очень низкой биодоступностью при приеме внутрь.

Цель. Обосновать целевой профиль, критические показатели качества и разработать прототип пероральной лекарственной формы с модифицированным высвобождением макозинона, позволяющей в максимальной степени реализовать его фармакологическую активность.

Материалы и методы. На основе фармацевтической субстанции макозинона гидрохлорида и различных вспомогательных веществ нарабатывали экспериментальные таблетки с дозировкой макозинона 500 мг. Оценивали влияние состава сред и добавляемых вспомогательных веществ на растворимость макозинона в различных биорелевантных средах, степень набухания в жидкости и степень мукоадгезии экспериментальных таблеток к слизистой желудка свиньи. Для оценки кинетики высвобождения активного вещества использовали метод ВЭЖХ.

Результаты и обсуждение. С учетом особенностей свойств макозинона обоснована целесообразность создания его гастроретентивных лекарственных форм с замедленным высвобождением активного вещества, механизм задержки которых в верхних отделах желудочно-кишечного тракта обеспечивается за счет набухания таблеток и повышенной адгезии к слизистой желудка. Экспериментально испытаны различные образцы таблеток, в которых модификация высвобождения активного вещества и степень набухания и мукоадгезии варьировали за счет введения в состав формуляций различных вспомогательных веществ, в том числе известных набухающих и биоадгезивных матричных агентов.

Заключение. Наиболее перспективными для последующих фармакокинетических исследований признаны образцы высокодозированных (500 мг) набухающих и мукоадгезивных таблеток, созданных по технологии двухстадийной грануляции с включением в состав первичных гранул смеси макозинона и гидроксипропил-бета-циклодекстрина и последующим внесением в межгранульное пространство комбинаций растворимого и нерастворимого гидрофильных набухающих и мукоадгезивных матричных агентов (ГПМЦ, ГЭЦ, ПЭО).

Ключевые слова: туберкулез, макозинон, гастроретентивная таблетка, модифицированное высвобождение, мукоадгезия, циклодекстрин

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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ABBREVIATIONS

CT – clinical trial; MDR – multidrug resistance; BDR – broad drug resistance; HPMC – hydroxypropyl methylcellulose; HPCD – hydroxypropyl beta-cyclodextrine; HEC – hydroxyethylcellulose; PEO – polyethylene oxide; MCC – microcrystalline cellulose; S – surfactants; CD – cyclodextrine.

INTRODUCTION

Nowadays, the problem of the increase of treatment efficacy of tuberculosis and other mycobacterioses has become especially challenging due to the more widespread resistance of infectious agent strains to known drug products. According to the WHO, about 500 thousand of new cases of rifampicin-resistant tuberculosis were recorded in 2019, 78 % of them had multidrug resistance (MDR). The Russian Federation is among the three countries (together with India and China) with the greatest spread of antibiotic-resistant forms of tuberculosis infection [1, 2]. Recently, more and more cases of tuberculosis caused by strains of mycobacteria having become resistant to the drugs lately introduced to clinical practice – bedaquiline and delamanid have been found [3–6]. Due to that, the search of anti-tuberculosis drugs with new modes of action is a challenging and priority task for development of effective antibiotics [7–9].

So far, clinical and preclinical studies have shown that a new class of chemical compounds is prospective for treatment of tuberculosis – 2-amino substituted-1.3-benzothiazine-4-ons. Compounds of the class have a unique and mycobacterium-specific inhibition mechanism of microorganism growth and propagation – suppression of activity of enzyme decaprenil-phosphoribose-2'-epimerase 15 (DprE1) necessary for the synthesis of a component of mycobacterial cell wall. The representative of the class – 2-[4-(cyclo-5-hexylmethyl)piperazine-1-yl]-8nitro-6-(trifluoromethyl)-4H-1.3-benzothiapine-4-one (PBTZ169, INN macozinone) [10, 11] – is one of the most well-studied candidates for development of an anti-tuberculosis drug with a wide range of action [12–15]. Therefore, the development of an effective dosage form of the compound is an up-to-date task.

It is known that the first and second line antituberculosis drugs are nowadays exclusively oral solid forms (tablets, capsules) [2, 16].

Meanwhile macozinone as a chemical compound has significant particularities which complicate the development of an effective oral drug on its basis. It is poorly soluble in water and is at the same time characterized with low lypophility. Macozinone is a weak base (pKa = 5.9) with a prominent dependence between solubility and pH values: it is initially poorly soluble in water at pH 1-2 which is dramatically lowered with the decrease of acidity, at pH > 5, the substance loses solubility and is crystallized which prevents its transport through intestinal mucosa.

Based on the tests *in vitro* (in the system of Caco-2 cells), macozinone refers to drugs with a low permeability [17, 18]. Due to the low systemic bioavailability of macozinone (according to various estimates, 1 to 8 %) found in preclinical and clinical studies on oral dosage forms with immediate release of active substance (capsules or suspensions), the administration of maximal drug dosages (up to 640–1280 mg) did not provide a reliable achievement of a target level of effective therapeutic macozinone concentration in plasma [18, 19].

With regards to the data, macozinone refers to pharmacologically active substances of class IV according to the generally accepted BCS classification (poorly soluble, poorly permeable).

The aim of the work was to justify the target profile, critical quality values and development of a prototype of oral dosage form with the modified release of macozinone allowing to implement maximally its pharmacological activity.

MATERIALS AND METHODS

Materials

Pharmaceutical substance of macozinone hydrochloride batch 030918 ("BION" LLC, Russia).

Reference sample (RS) of macozinone hydrochloride batch 300-51 ("BION" LLC, Russia).

Reference samples in the test of mucoadhesive ability – "Gralise" (gabapentine) swelling tablets, 600 mg (Almatica Pharma, USA); "Fromilid" UNO (clarithromycin) coated tablets, 400 mg (KRKA, Slovenia).

Excipients: MCC – microcrystalline cellulose type 101 (JRS Pharma, Germany); HPBCD – (2-hydroxypropyl-β-cyclodextrine, hydroxypropylbetadex) (Roquette, France); HPMC – (hydroxypropyl methylcellulose) hypromellose K100M type 2208 (Colorcon / DOW, USA); HEC – (hydroxyethyl cellulose) hyethellose 250 HHX (Ashland, США); carbopol 71G, 974P (Lubrizol, USA / BASF, Germany; CMC – carmellose sodium CMC 7 HF PF (Ashland, USA); colloid silicone dioxide (Aerosil® 200) (Wacker, Germany);

croscarmellose (JRS Pharma, Germany); crospovidone XL-10 (Ashland, USA); magnesium stearate ST-v (Nitika, India); PEG – macrogol 6000, 8000 (Croda, Great Britain); sodium stearyl-fumarate (JRS Pharma, Germany); povidone K25, K30, K90 (BASF, Germany); polysorbate-80 (Croda, Great

Britain); PEO – polyethylene oxide WSR 303 (Colorcon / DOW, USA).

Finished dry mixtures for composition of biorelevant media FaSSIF / FeSSIF / FaSSGF (Biorelevant.com, Great Britain).

Equipment

- moisture analyzer AND MX-50 (A&D, Japan);
- texture analyzer (TA.XTplus, Stable Micro Systems, Great Britain);
- laboratory balance Vibra HT-224RCE (Vibra, Japan);
- vibro sieve CISA RP 200N (CISA, Spain);
- laboratory mixer – granulator with an overhead stirrer, mixing device EUROSTAR 20 high sped digital (IKA, Germany);
- HPLC system Agilent 1100 (Agilent Technologies, USA;
- tablet press Futorque X-1 (NORTEC Industrial Solutions, Poland);
- friability tester ERWEKA TAR 220 (ERWEKA GmbH, Germany);
- durability tester ERWEKA TBH 425 TD (ERWEKA GmbH, Germany);
- solubility tester RC-6D (SaintyCo Group, China);
- drying cabinet Memmert UF110 (Memmert, Germany).

Test methods

Evaluation of compatibility of excipients with the active substance. To evaluate applicability of excipients to be used in developed tablets, mixtures of equal amounts of the pharmaceutical substance with each of the selected excipients were placed to glass vials kept in controlled conditions (climatic chamber) at temperature 70°C and relative humidity 80 %. In parent mixtures and samples kept for 7 and 14 days, identity (preservation of the form and size of macozinone peak), assay of the active substance and presence of impurities were controlled with HPLC. Moreover, possible changes of physical parameters of mixture powders were controlled visually with evaluation of color and homogeneity preservation (absence of visible aggregates).

Production of granulate powders with inclusion of cyclodextrine. Dry mixtures of substances were prepared with the intensive mixing of dry powders of the pharmaceutical substance and hydroxypropyl beta-cyclodextrine in molar ratios 1:0.5 (6.54 g of macozinone equivalent to the base + 10 g of HPCD); 1:1 (3.26 g of macozinone + 10 g of HPCD); 1:2 (1.63 g of macozinone + 10 g of HPCD) in a laboratory mixer for 5 minutes.

Using the prepared mixtures, granulated powders were prepared with the method of wet granulation with addition of a small amount of purified water (ratio mixture:moisturizer – 2.5:1) and granulation in the conditions of a high shear force. The obtained granulates were dried in a drying cabinet at temperature 50–55 °C up to residual moistness 1–2 %.

Production of experimental tablets with a modified release of macozinone. To achieve main target quality parameters (ability to swelling and mucoadhesion) and regulation of the extent and kinetics of release of the active substance from tablets, known excipients forming hydrophilic matrixes and simultaneously having mucoadhesive properties were introduced into experimental tablets. For production of experimental tablets, three technological approaches differing by the principles of introduction of matrix components into tablets were introduced.

Variant 1. To produce tablets in which matrix agents were introduced immediately into granules, dry powders of the pharmaceutical substance and excipients were intensively mixed mechanically with further addition of a moisturizer and production of granules using the mixer-granulator. They were mixed for 2 minutes till a fine flowable granulate was formed (visual control).

Considering a very low solubility of macozinone in water and unsatisfactory technological characteristics of substance macozinone hydrochloride (a very low flowability, poor wetting ability, increased particle coadhesion) in individual variants of the technology, isopropyl alcohol (composition F1, moisturizer – 7 % solution of povidone K-30 in isopropanol) or ethanol (composition F3, moisturizing mixture water:ethyl alcohol in ratio 30:70 with introduction of ½ amount of povidone K-90 and polysorbate-80), correspondingly, were introduced into a moisturizing fluid.

The granulate was dried in a drying cabinet up to residual moistness not above 3 % and calibrate through the sieve with cell size 0.710 mm.

To prepare tablet mixture, a calibrated granulate was introduced to the mixer, granules were powdered with the lubricant (magnesium stearate or sodium stearyl fumarate) mixing till it became homogeneous for 5 minutes (visual control).

Variant 2. In the technology variant in which matrix agents were introduced both to granules and intergranule space, granules were prepared as in variant 1, and matrix agents added above granules were introduced to the mixture as dry powders were mixed for 4–5 minutes with further powdering of granules with the lubricant.

Variant 3. In the technology variant, the method for a separate preparation of components was used. In this cases, "primary" granules based on macozinone hydrochloride were developed with the addition of hydroxypropyl-beta cyclodextrine with a short-term (not more than 1 minute) intensive mixing of dry powders with further stage of wet granulation in purified water (ratio mixture:moisturizer – 2.5:1). Granules were dried in a drying cabinet up to residual moistness not above 2 % and calibrated through the sieve with cell size 05 mm. On the second stage, "secondary" granules were produced mixing dry powders of "primary" granules in the granulator with other excipients selected for a certain composition, with further introduction of the moisturizer to the system (aqueous solution of polysorbate-80), and short-term (not more than 2–3 minutes) mixed till a fine flowable granulate was formed. The "secondary" granulate was dried up to residual moistness not above 2 % and calibrated through the sieve with cell size 0.71 mm. On the stage of tablet mixture preparation, the prepared "secondary" granules were powdered with the lubricant.

The tested experimental tablet compositions were given in table 2.

To produce tablets, a laboratory rotation tablet press with the matrix 20 × 10 mm in dimensions allowing to get capsule-shaped tablets ("oblong") of the assigned size was used. Press force was regulated for provision of the breaking strength of tablets (in the range of 180 to 220 N).

Produced tablets were also controlled by the parameters: geometric dimensions, weight, breaking force in accordance with State Pharmacopeia XIV.

Swelling ability of tablets was evaluated by the change of geometric dimensions (figure 1) and change of their weight after being placed to baskets (device USP I) which were immersed to medium 0.01 N HCl with temperature 37.0 ± 0.5 °C.

Swelling index (SI) was calculated by the equation

$$SI = \left(\frac{W_h - W_i}{W_i} \right) \cdot 100,$$

where W_h – weight of a tablet after being swelled in the medium (0.01 n HCl); W_i – baseline tablet weight.

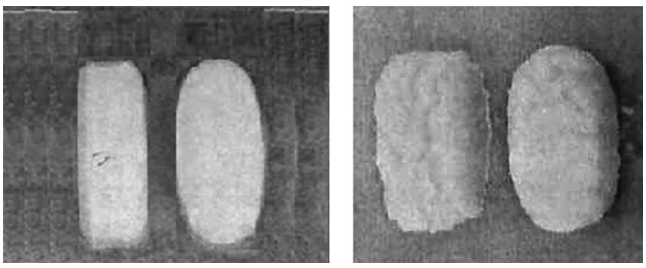

Figure 1. Swelling and partial erosion of the tablets

When compatibility of pharmaceutical ingredients, release extent of the active substance were evaluated, the validated HPLC method with reference sample of macozinone hydrochloride was used for the assay of macozinone in solutions. Aliquots withdrawn from vessels with dissolution media were diluted with methanol up to estimated final concentrations, ultrasonified and filtered through a teflon membrane filter 0.22 µm.

Test conditions:

- HPLC equipment Agilent 1100 (Agilent technologies, USA).
- Column YMC-Triat C18 250 × 4.6 mm; 5 µm.
- Mobile phase: buffer solution pH 4.0 – acetonitrile (30:70).
- Flow rate: 1 ml/min.
- Column temperature: 25 °C.
- Detector: UV 254 nm.
- Injection volume: 10 µl.
- Run time: 30 min.
- Typical retention time of macozinone: about 14–16 min.

Extent of dissolution of the pharmaceutical substance, mixtures and granulates of macozinone hydrochloride and CD mixtures was evaluated in the conditions:

- Device: magnetic mixer.
- Rotation rate: 150 rot/min.
- Mixing time: 60 minutes.
- Medium volume: 250 ml.
- Medium temperature: 37 ± 0.5 °C.
- Dissolution medium: 0.01 H HCl with the addition of 0.001 % benzethonium chloride; acetate buffer solution (pH 5.0); medium FaSSIF (pH 5.0); medium FeSSSIF (pH 6.5).

Solutions simulating fasted (FaSSIF) and fed condition (FeSSIF) of the gastrointestinal environment were prepared from commercially available concentrates in accordance with the manufacturer's instruction (pH value of FaSSIF medium was made up with hydrochloric acid to

5.0 which corresponded to a larger extent to acidity of the upper duodenal contents).

The release of the active substance in the test of modified release tablets was evaluated in the conditions:

- Dissolution tester: type II paddle mixer.
- Rotation speed: 50 rot/min.
- Injection volume: 500 ml.
- Medium temperature: 37 ± 0.5 °C.
- Dissolution medium: 0.01 M HCl with the addition of 0.001 M benzetonium chloride.

Samples of 300 ml were withdrawn in 1, 2, 3, 4, 6 h, after the sampling, volume of fluid in vessels were substituted with equivalent amount of the medium. Samples were filtered through a membrane filter, kept at room temperature in a place protected from light for not more than 8 hours. Macozinone concentrations in withdrawn samples were determined with HPLC method.

The amount of macozinone released from a tablet (M_i) and its total accumulation (ω_i) in % of tablet contents in each time point were calculated by the equations:

$$M_i = 500C_i + 300\sum_{j=1}^{j=i-1} C_i,$$

$$\omega_i = M_i/D,$$

where i – sampling point (time) (= 1, 2, 3...6 час); 300 – volume of a sample taken; C_i – macozinone (mg/l) in a sample taken (based on the assay results with the method of HPLC control); D – macozinone contents in a tablet (mg) (= 500).

In the experiments evaluating mucoadhesive ability of tablets, fresh pig stomachs (within 24 h after sampling, freezing was not allowed) rinsed with saline ("Argoferma" LLC, Russia) were used.

The extent of mucoadhesion of tablet samples to gastric stomach mucosa was tested with texture analyzer (TA.XTplus, Stable Micro Systems, Great Britain) with the load sensor 50 H [20]. A flap of the pyloric mucosa 25–25 mm in dimensions was separated from the muscular layer of the stomach, fixed on a microscope slide with cyanoacrylate glue. A tablet of the test product (sample) was fixed to a cylindrical test with a two-sided adhesive tape. After the mucosa was moistened with saline, a tube with tenso sensor and fixed tablet was lowered till it got in touch with the mucosa with the rate 0.5 mm/sec, the tablet was pressed to the mucosa with a 100 g weight for 300 sec, and the tube was risen to a baseline position with rate 0.5 mm/sec. The device indications recorded in automatic regime were used for plotting of "tension-deformation" curves with the device software. Force parameters (in H), adhesion tension and rupture work of an adhesive layer were calculated ($W_{adh.}$).

Mucoadhesive properties of tablets were additionally evaluated with the wash-off method (see, for example, [21]) using a standard dissolution tester of type II (paddle mixer). The mucosal fragments 25 × 25 mm in dimensions was fixed with cyanacrylate glue to rectangular metal plates. A tablet was placed to the mucosa slightly moistened with saline and pressed with force 0.01 N for 300 sec. The plates with the test sample attached with clips were fixed to the mixer paddles, immersed to the test medium (750 ml 0.1 n hydrochloric acid, 37 ± 0.5 °C), and tablet breaking from the gastric mucosa was controlled at the rate of mixer rotation 25 rot/min (figure 2).

Figure 2. Control of swelling rate and duration of mucoaghesion of the tablets in dissolution type II apparatus

RESULTS AND DISCUSSION

Justification of a target profile of the product quality

Due to the particularities of macozinone properties, a dosage form with a rather high dosage providing a prolonged release of the active substance predominantly in the upper gastrointestinal regions (stomach, upper small intestine) appears to be a promising dosage form for a necessary therapeutic effect. A gastroretentive (retaining in the stomach) dosage form meets the requirements to the greatest extent [22, 23].

When the requirements to the composition of the dosage form were developed, it was appropriate to test some or other methods for the increase of solubility values of the pharmaceutical substance as part of the pharmaceutical development. Therefore both for the inclusion to dissolution testing media and inclusion to formulations, some known solubilizers and moisturizers (surfactants, alcohols, Tween, PEG, cyclodextrines) should be tested.

It was also necessary to provide possibility to retard release of the active substance from a high dose form for the prevention of achievement of its solubility and crystallization limits in the limited medium volume. With regards to the particularities of macozinone pharmacokinetics found in humans which is characterized with short periods of maximal plasma concentrations (T_{max} = 60–90 min) and half-life ($T_{1/2}$ = 6–8 hours) [18], it was appropriate to increase prolongation of release of the active substance at least up to 3–4 T_{max} periods (i. e. at least up to 6–8 hours).

Among known methods for the achievement of a gastroretentive effect [22] for poorly soluble macozinone, with regards to necessary high dosage in a unit of a dosage form, variants of dosage forms able to swell and have mucoadhesion were considered the most feasible.

The main parameters for swelling delivery systems providing a necessary gastric retention are their geometric sizes and form. It is considered that a minimal geometric size of a tablet by the largest dimension should be about 2 cm, and an ellipsoid form (capsule-shaped) is optimal for product retention [24–26].

Moreover, unlike floating gastroretentive systems, making a dosage form mucoadhesive, allows to reduce probability of its premature "forced" evacuation during the most intensive phase III of the migration motor complex, as well as in patients with accelerated gastric movements or in the period after food is delivered into the stomach [27, 28].

To justify target values of mucoadhesive ability of the developed dosage form, mucoadhesive properties of three reference samples similar in form (capsule-shaped tablet) and dimensions (analogous to sizes of experimental tablets) were preliminarily evaluated experimentally after development of appropriate methods: gastroretentive swelling – floating tablet "Gralise" containing bioadhesive agents HPMC and PEO, "Fromilid" tablets largely coated with another mucoadhevie agent – carboxymethylcellulose, as well as tablets of the same size and form produced from epoxide preparation of the simulator (model) with hydrophobic properties. Based on the test results of the samples (table 1), target values of the adhesive ability of the developed tablets were selected: breaking force – at least 1.0 H (appropriate destruction activity of the adhesive compound – at least 5 J/m^2) and duration of tablet fixation on the mucosa in wash-off conditions for at least 300 min.

Table 1. Mucoadhesion characteristics of model tablets

Characteristic	Samples		
	Epoxy model	"Fromilide"	«Gralise»
Detachment force, N	0.21	0.84	0.337
Work of adhesion, J/m^2	0.73	3.71	1.12
Duration of adhesion, min	< 1	> 300	> 300

Note. * The average value of three measurements.

One of the key requirements to parameters of the achieved dosage form is the achievement of a necessary release rate of the active substance. Concentration of the substance dissolved in gastric and intestinal juice should not exceed the upper solubility limit recorded for macozinone. The calculations based on the selected target parameters of retention time of the dosage form evacuation in the stomach (at least 6 hours), macozinone dosage in a tablet (500 mg), maximal macozinone solubility in the acidic gastric environment (not above 200 μg/ml), known mean evacuation rates of liquid contents from the stomach, as well absorption rate (absorption constant) of macozinone recorded in clinical trials have shown that acceptable release rate of the active substance in the acidic gastric environment should be in the range 30–40 mg/hour.

Based on the calculation, the approximate target curve of macozinone release from the dosage form in the stomach (figure 3), with regards to which reference time points for the control were selected during the test in vitro using the developed method – 1, 3 and 6 hours, and the allowable variation ranges of the parameter of cumulative release of the active substance were determined in each point (see table 3). Due to rather low macozinone solubility, the method for control of macozinone release with standard type II device (paddle mixer) should be modified so that solubility limits of the active substance were not achieved in the solubility period between two sampling points.

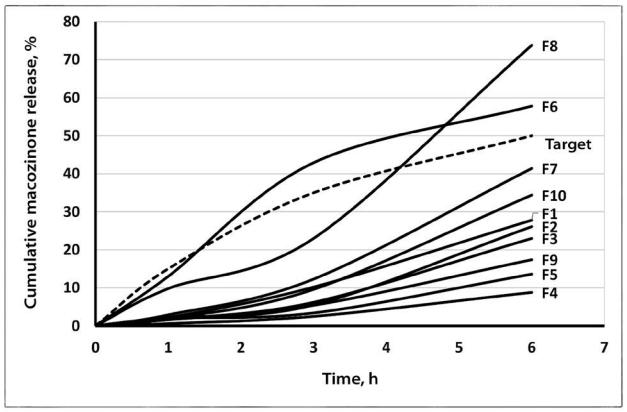

Figure 3. Cumulative curves of macozinone release from the experimental tablets

With the regards to the abovementioned, as critical quality values of the developed dosage form – gastroretentive tablet with dosage by the active substance 500 mg – the following were selected: geometric sizes (initial and after swelling in dissolution medium) and form (capsule-shaped), swelling degree (index), degree of mucoadhesion (to pig gastric mucosa), degree and rate of the active substance rate in the dissolution test (in reference time points 1, 3, 6 hours). The established values of critical quality parameters are given in table 4.

Moreover, pressing effort and strength of produced tablets (breaking tests) were also controlled.

Compatibility of components for experimental compositions

The study of all variants having stress exposure to paired mixture composed of the pharmaceutical substance (macozinone hydrochloride) and each of excipients selected for the development of experimental tablets with HPLC method did not show any changes in HPLC characteristics of the active substance, as well as, no accumulation of extra impurities of the main substances was found – products of component interaction. The fact largely may be explained by a high chemical and hydrolytic stability of substance macozinone hydrochloride almost insoluble in water.

Evaluation of pharmaceutical substance macozinone and its complexes with CD

The study results on dissolution of pharmaceutical substance macozinone hydrochloride (table 2) have confirmed that it was adequately referred to the class of substances almost in water.

The prominent relationship between macozinone hydrochloride dissolution and pH values of the medium shown both the for substance itself and its complexes with cyclodextrine, as well as in biorelevant media indicates that a narrow bioavailability window is typical for the drug product, and the main amount of the drug substance delivered orally is absorbed in the stomach and upper small intestines. The evident increase of macozinone solubility in medium FeSSIF at value pH 6.5 compared to acetate buffer and intestinal juice simulator in fasting condition (FaSSIF, pH 5.0) may be related to additional emulsifiers present in medium FeSSIF as gall acid salts.

It is evident that the pharmacokinetic particularities recorded in I phase clinical trials are explained with the properties of macozinone as well as the previous data on its low permeability through cell barriers [17, 18] – a rapid (in 1–1.5 hour) achievement of maximum plasma concentration, a very short half-life period (less than 8 hours) and the increase of a relative bioavailability of a drug administered during meals [19].

Table 2. Relative solubility of macozinone and macozinone-hydroxypropyl-beta-cyclodextrin granules in different mediums (%)

Sample*	0,01 M HCl, pH 1,2**	Acetic buffer solution pH 5,0	FaSSIF pH 5,0	FeSSIF pH 6,5
Macozinone HCl	100***	1.9	0.8	35
Granules of Macozinone HCl + HPCD (2:1)	138	3.1	1.1	48
Granules of Macozinone HCl + HPCD (1:1)	116	7.1	1.1	31
Granules of Macozinone HCl + HPCD (1:2)	120	2.9	0.9	39

Note. * For granulates designated molar ratio of components.
** Medium contain 0,001 % benzetonium chloride.
*** The value of solubility of Macozinone HCl in 0,01 M HCl, pH 1,2 is accepted as 100 %.

As the results show, the additional increase of macozinone solubility, especially profound in a weakly acidic medium, is achieved when tested as granulation powders of mixtures with hypdroxypropyl-beta-cyclodextrine. It is known that modes of action of cyclodextrines may vary for dissolution of poorly soluble substances. The phenomenon of formation of inclusion complexes (clathrates) of amphiphilic cyclodextrines

with hydrophobic lipophilic molecules is most fully described in the literature. Such complexes can be produced wit various methods – with intensive mixing or grinding of dry powders, mixing in solutions, on the margins of phase separation, co-precipitation, mixture lyophilization, etc. [29]. As a rule, the most effective formation of such complexes occurs in comparable molar ratios of CD and host substance which complicates the use of such approach for high dose products. Nevertheless, being mixed with substances with marked hydrophobic properties, their some amount may form true inclusion complexes with preservation of excess crystalline forms. I.e. such forms represent combined mixtures of crystals and clathrate formulations.

It should be noted that in addition to implementation of true inclusion complexes, solubility of hydrophobic molecules in the presence of cyclodextrines may also increase due to other types of physical chemical interactions, for example, stabilization of such molecules in solutions as a result of non-covalent interaction with cyclical oligosaccharides [30], as well as susceptibility of hydrophobic molecules to aggregation and precipitation from solutions. In the latter case, the interaction of oligosaccharides and hydrophobic drug molecules allows to achieve stabile "oversaturating" concentrations of poorly soluble drug substances in biological fluids [31].

With regards to the obtained data, as one of the task in construction of dosage form was to use methods and tablet components promoting the increase of solubility, permeability and decrease of dependence between substance solubility and change of pH of biological media. One of the possible methods for solution of the task is to develop a high-dose gastroretentive form with modified release of active substance including the variant with inclusion of hydrophilic cyclodextrines within.

Development and evaluation of properties of experimental tablets

Compositions of developed experimental tablets and results of evaluation of their properties are given in tables 3, 4 and on figure 3.

As table 4 and figure 3 showed at the control point of 6 hours, that tablets with compositions F6, F7, F10 were the closest to assigned release parameters of the active substance, however the two latter had the excessively retarded release kinetics of macozinone. At the same time, composition F1 initially reviewed as the potential prototype which was similar by the principles of the technology used (aqueous alcohol one-stage granulation) in the development of the swelling and mucoadhesive acyclovir tablet described in the literature [32], had the highest swelling and adhesive ability, however, in tests, the tablet splitting was recorded by the longitudinal axis during the swelling. The attempt to correct the disadvantages with the introduction of additional amount of the binder and substitution of mucoadhesive components (composition F3) led to the excessive decrease of release value of the active substance.

Tablets produced by variant 2 of the technology with introduction of mucoadhesive agents both within granules and intergranule space (compositions F4, F5) had rather high mucoadhesion rates but were characterized with a low release rate of the active substance.

Tablets with composition F6 produced by the technology of the two-stage manufacture of granules in which "primary" granules were prepared with mixing of the pharmaceutical substance and hydroxylpropyl-beta-cyclodextrine (in ratio 2:1 by weight) in conditions of a negligible moistening with water, were most compatible with the combination of target parameters (see figures 1–3). In this variant of the technology, the stage of introduction of matrix swelling and highly mucoadhesive polymers as dry powders to the intergranule space was implemented. One of the matrix components is water-insoluble carbopol, and the second one is water-soluble polymer – hydroxyethylcellulose (F6). It is known that for poorly soluble substances, their release from matrix dosage forms is determined mainly the rate of matrix erosion [22, 23]. In tablets with compositions F6, F7, F8, matrix agents being swollen form a peculiar viscous adhesive, gradually eroding membrane around granules containing the active substance. The selected combination of insoluble and soluble polymers provides the necessary rate of the matrix destruction. Moreover, it should be considered that swelling ability of carbopols significantly increases with the increase of pH value of the medium above 5.5–6.0 which it is found in vivo as a tablet moves to the lower gastric regions and the duodenum. It should be also stated that when high molecular PEO is used as a matrix agent instead of GEC (composition F7), the retardation of the active substance release from tablets is shown after they are place to the dissolution medium, with further increase of the value almost up to minimal target values in 6 hours. The data indicates that, in principle, it is possible to achieve target parameters of macozinone tablets to be developed with a modified release of the active substance by variation of quantitative ratios and type of matrix agents introduced between granules.

Table 3. **Experimental formulations of the tablets with modified release of macozinone (mass %)**

Name of the component	Function	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Macozinone HCl	Pharmaceutical substance	49.09	49.09	49.09	49.09	49.09	49.09	49.09	49.09	49.09	49.09
Primary granules (wet granulation)											
Hydroxypropyl-beta-cyclodextrin, HPBCD	Complexing / solubilizing agent / Permeability enhancer	–	–	–	–	–	24.91	24.91	24.91	–	–
Crosspovidone XL10	Filler / disintegration agent	–	–	–	–	–	–	–		24.91	–
Microcrystalline cellulose 101	Filler / disintegration agent										32.90
Secondary granules (wet granulation)											
Microcrystalline cellulose 101	Filler / disintegration agent	34.51	24.91	24.91	13.41	24.91	–	–			
Polysorbate-80	Solubilizing agent	–	1.00	1.00	–	1.00	1.00	1.00	1.00	1.00	1.00
Povidone K90	Binder	–	6.00	6.00	–		–	–	–	–	6.00
Povidone K30	Binder	3.10	–	–	–	–	–	–	–	–	–
Povidone K25	Binder	–	–	–	6.00	–	–	–	–	–	–
PEG-6000	Solubilizing agent / plasticizer	–	–	–	–	5.00	5.00	5.00	5.00	5.00	–
PEG-8000 (dry)	Solubilizing agent / plasticizer	–	–	–	15.00	–	–	–		–	–
Carmellose sodium (Na-CMC)	Solubilizing agent / plasticizer	–	6.00	6.00	–	4.00	4.00	4.00	4.00	4.00	–
Carbopol® 974P	Mucoadhesive / disintegration agent	9.00	–	–	–	5.00	–	–		–	–
PEO (Polyox WSR 303)	Mucoadhesive / disintegration agent	3.00	–	–	5.00	–	–	–		–	–
HPMC K100M	Mucoadhesive / disintegration agent	–	12.00	12.00	–	–	–	–		–	–
Dry mixing/dusting											
PEO (Polyox WSR 303)	Matrix / mucoadhesive gent	–	–	–	–	10.00	–	5.00	10.00	5.00	10.00
HPMC K15M	Matrix / mucoadhesive gent	–	–	–	–	–	–	–	5.0	–	–

Name of the component	Function	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Carbopol® 71G NF	Matrix / mucoadhesive gent	–	–	–	10.00	–	–	10.00		10.00	–
Carbopol® 974P	Matrix / mucoadhesive gent	–	–	–	–	–	5.00	–		–	–
Hydroxyethylcellulose HEC 250 HHX	Matrix / mucoadhesive gent	–	–	–	–	–	10.00	–		–	–
Aerosil® 200	Glidant	0.50	–	–	1.00	–	–	–		–	–
Magnesium stearate	Lubricant	0.80	–	–	–	–	–	–		–	1.00
Sodium stearyl fumarate	Lubricant	–	1.00	1.0	0.50	1.00	1.00	1.00	1.00	1.00	–
Av mass of tablet, mg		1103	1109	1109	1102	1107	1115	1106	1103	1107	1101

Table 4. **Properties of the experimental tablets**

Characteristic	Target value	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Size, (L × W), mm	20 × 10	20 × 10	20 × 10	20 × 10	20 × 10	20 × 10	20 × 10	20 × 10	20 × 10	20 × 10	20 × 10
Height, mm	–	7.01	6.88	6.54	6.41	6.86	6.38	6.54	6.24	6.77	6.51
Size after swelling (L × W), mm	(> 22) × (> 12)	Splitting after 4 h	24 × 13	25 × 13	22 × 12	24 × 13	24 × 14	23 × 13	24 × 13 loss of form	24 × 15	21 × 11
Swelling index, %	> 80	–	105.1	114.8	71.9	101.4	99.3	104.3	124.9	135.9	39.3
Hardness, N	180–220	205	188	245	219	145	218	209	217	200	225
Cumulative Macozinone release (after 1 h), $\omega_{\Sigma 1}$, %	10–15	2.2	2.1	1.7	0.6	2.0	13.0	2.9	9.8	1.6	1.9
Cumulative Macozinone release (after 3 h), $\omega_{\Sigma 3}$, %	20–40	10.2	5.5	6.2	2.5	3.4	42.9	12.3	23.0	5.4	9.6
Cumulative Macozinone release (after 6 h), $\omega_{\Sigma 6}$, %	50–60	27.8	26.1	23.0	8.8	13.6	57.8	41.4	73.8	17.4	34.4
$W_{adh.}$, (work of destruction of adhesive junction), J/m^2	> 5	13.2	7.0	4.5	5.7	9.2	6.7	6.6	5.6	н/о*	2.2
Duration of adhesion (wash-off test), min	> 300	н/о*	> 300	н/о*	н/о	н/о*	> 300	> 300	н/о*	н/о*	н/о**

Note. * The determination was not carried out due to unsatisfactory values of the parameters of the release of the active substance.
** The determination was not carried out due to the unsatisfactory values of the detachment force parameter of mucoadhesion.

It should be also stated that as matrix agents introduced between granules are substituted with the combination of two water-soluble mucoadhesive polymers – PEO + HPMC (composition 8) within the implementation of the technology, the degree of erosion of the dosage form and, correspondingly, the release of the active substance become excessive.

When we tried to substitute soluble cyclodextrine within "primary granules" to insoluble capillary uncoupler crospovidone used in pharmaceutical compositions as well as for stabilization and increase of solubility of powders of hydrophobic drug substances tending to aggregation [33, 34] (composition F9), the degree of tablet swelling was significantly increased, and the release rate of the active was considerably decreased. When cyclodextrine was substituted to insoluble uncoupler MCC which can stabilize crystalline systems and improve solubility of substances [35–37] (composition F10), release of the active substance was higher but a tablet lost the ability to swelling and mucoadhesion.

CONCLUSION

Therefore, the results of evaluation of tablet variants with different composition and production methods showed that the samples containing preliminarily prepared separately granules based on powders of the pharmaceutical substance and hydroxypropyl-beta-cyclodextrine closest to the assigned target values of quality parameters of macozinone gastroretentive tablet to be produced. Such granules have provided a significantly higher solubility of macozinone in all tested media which allows to predict a higher bioavailability of the active substance. Evaluating applicability of developed dosage forms for the increase of bioavailability of the active substance and provision of the assigned therapeutic effect, along with a positive effect on solubility of hydrophobic substances, it should be considered that cyclodextrines are known to induce the increase permeability of biological barriers for hydrophobic molecules. The effect is related, in particular, to their ability to influence structure of biological barriers (phospholipid membranes, mucine). Cyclodextrines (as some other mono- and disaccharides) show the properties of chaotropic agents exposing to highly viscous systems, their ability to increase permeability of biologically membranes due to extraction of some lypophilic or amphiphilic components, in particular cholesterol, from membranes is also described [38–40].

With regards to the obtained data, a swelling and mucoadhesive tablet produced with the developed technology for two-stage granulation, containing complex of pharmaceutical substance macozinone hydrochloride and cyclodextrine within "primary" granules, with further intergranule introduction of combination of a soluble and insoluble polymeric highly bioadhesvie and swelling matrix agents (composition F6) is most prospective as a potential prototype of a gastroretentive tablet for further bioavailability studies.

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