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MAZUR Ecaterina

**DEVELOPMENT OF A NEW COMBINED MEDICINAL
PRODUCT FOR USE IN HYPOKALEMIA**

316.01 PHARMACY

Abstract of the PhD Thesis in Pharmaceutical Sciences

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The thesis was carried out at the Drug Development Center and the Department of Pharmaceutical and Toxicological Chemistry, Faculty of Pharmacy, Nicolae Testemițanu State University of Medicine and Pharmacy.

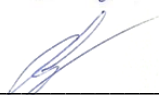
Scientific supervisor:

Uncu Livia, dr. hab. pharm. sci., associate professor



Co-supervisor:

Parii Sergiu, dr. hab. med. sci., associate researcher

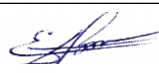


Members of the guidance committee:

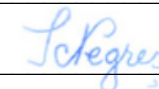
Valica Vladimir, dr. hab. pharm. sci., university professor



Diug Eugen, dr. hab. pharm. sci., university professor



Negreș Simona, dr. hab. pharm. sci., university professor



The defense will take place on *18.11. 2026*, at *14.00* in the premises of the Nicolae Testemițanu State University of Medicine and Pharmacy, str. Ștefan cel Mare și Sfânt, 165, office 205 in the meeting of the Public Defense of the PhD Thesis Committee, approved by the decision of the Scientific Council of the Nicolae Testemițanu State University of Medicine and Pharmacy of *16.09.2026*, decision no. 9/2_4.

The Committee Members of the Public Defense:

President:

Adauji Stela,

dr. hab. pharm. sci., associate professor



Members:

Uncu Livia,

dr. hab. pharm. sci., associate professor, supervisor,

Valica Vladimir,

dr. hab. pharm. sci., university professor, reviewer,

Ciurba Adriana-Daniela,

dr. hab. pharm. sci., university professor, reviewer,

Cibotari Silvia,

dr. pharm. sci., associate professor, associate researcher, reviewer,

Author: Mazur Ecaterina



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INTRODUCTION

Research relevance. Hypokalemia is one of the most common electrolyte disorders encountered in clinical practice, when the blood potassium level is lower than normal (3.5 - 5.5 mEq/L), being associated with an increased risk of cardiovascular, neuromuscular and metabolic complications. Potassium deficiency favors the occurrence of heart rhythm disorders and worsens the prognosis of patients with coronary artery disease, previous myocardial infarction, ventricular hypertrophy and heart failure, which highlights the need for prompt monitoring and correction of electrolyte imbalances [5]. The diagnosis of hypokalemia may be delayed because the clinical manifestations are often nonspecific and overlap with the symptoms of other conditions, such as headache, muscle weakness, fatigue and general weakness. More specific manifestations of hypokalemia are progressive muscle weakness, muscle cramps, paresthesia, hyporeflexia, cardiac arrhythmias, and, in severe forms, flaccid paralysis and respiratory failure. Confirmation of hypokalemia requires determination of serum potassium concentration and electrocardiogram [3, 4, 14, 16].

According to data, approximately 17% of cardiovascular diseases originate from hypokalemia. This electrolyte imbalance frequently occurs as a result of the administration of certain drugs, especially thiazide and loop diuretics, which promote renal potassium loss [1, 6]. In this context, the risks associated with hypokalemia are higher in patients with a history of cardiovascular disease, such as coronary artery disease, myocardial infarction, left ventricular hypertrophy, or congestive heart failure [5, 17]. In the Republic of Moldova, diseases of the circulatory system continue to hold first place in the mortality structure, with the number of deaths attributed to this pathology being 20 993 in 2022, 19 511 in 2023 and 19,316 in 2024 [2].

Correction of hypokalemia is performed according to the severity of the deficiency, by adjusting dietary intake and administering potassium supplements. However, existing therapy has certain limitations, such as insufficient elemental potassium content, non-optimized solubility and bioavailability, pharmaceutical forms insufficiently adapted to the patient's needs, reduced gastrointestinal tolerability and, in particular, the limited availability of optimized therapeutic combinations with complementary mechanisms of action. Therefore, the need has arisen to develop new medicinal products capable of simultaneously providing potassium supplementation, reducing potassium losses and correcting associated electrolyte imbalances [7, 11].

In this context, the development of a tetracomponent combined medicinal product, in the form of a powder dosed in sachets, which combines potassium aspartate (AspK), magnesium aspartate (AspMg), potassium orotate (OrK) and spironolactone (Spir), intended for use in hypokalemia, is justified. The proposed association aims to combine the intake of potassium and magnesium with the potassium-sparing action of spironolactone. The product development involves the components compatibility determination, optimization of the composition and manufacturing technology, development and validation of analytical methods, standardization and stability assessment, as well as preclinical investigation in order to obtain a standardized, stable combined medicinal product with the potential for the practical application.

The aim of the research was to develop, optimize, standardize and preclinically evaluate a combined medicinal product, in the form of a powder dosed in sachets, intended for use in hypokalemia.

The research objectives:

1. assessment of the market of medicines and food supplements containing potassium in the Republic of Moldova, intended for use in hypokalemia;
2. carrying out preformulation, formulation and biopharmaceutical evaluation studies in order to justify the composition and pharmaceutical form of the combined medicinal product;
3. development of technology for manufacturing dosed powder in sachets, under laboratory conditions;
4. development, optimization and validation of analytical methods for identification, dosage and quality control of active substances and the developed medicinal product;
5. conducting stability studies on the developed powder to determine its shelf life;

6. standardization of the medicinal product and development of analytical documentation for standardization;
7. conducting preclinical studies to determine acute toxicity, evaluate the hypokalemia correction effect and assess the antioxidant capacity of the developed medicinal product.

The scientific novelty and originality of the work consist in the substantiation and development of a new fixed-dose combination of the medicinal product, in the form of oral powder dosed in sachets, intended for use in hypokalemia. The innovative nature of the product is determined by the association of AspK, AspMg, OrK and Spir in a single pharmaceutical form, designed to ensure a multifactorial action through complementary mechanisms, contributing to potassium supplementation, reducing its losses, correcting the concomitant magnesium deficiency.

The originality of the research lies in the scientific substantiation of the formulation by using the SeDeM methodology to optimize this tetracomponent formula and identify the M13 composition and the factorial experimental design to optimize the composition and technological properties of the powder, as well as in the complex assessment of the compatibility of active pharmaceutical ingredient (API) and excipients (Ex) by thermoanalytical and spectroscopic methods. Within the framework of the research, analytical methods adapted to the powder matrix were developed and validated for the identification and dosing of the active components in the developed powder, stability studies and preclinical research were carried out on the acute toxicity, efficacy in correcting hypokalemia and antioxidant capacity of this fixed-dose combination, which gives the work a pronounced innovative and applied character.

Synthesis of research methodology and justification of research methods. The research was conducted in accordance with modern principles of pharmaceutical development and included studies of preformulation, formulation, optimization, standardization and preclinical evaluation of the medicinal product. The compatibility and stability of the active substances and excipients were assessed by differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and high-performance liquid chromatography (HPLC), complementary to detect molecular and thermal interactions. The optimization of the formulation composition of the final combined medicinal product was achieved by applying the SeDeM methodology for the integrated evaluation of technological properties and Design-Expert for quantifying the effects of formulation factors and optimizing responses, using pharmaceutical excipients that meet the requirements of the European Pharmacopoeia (Ph. Eur. 10).

In the formulation stage, 17 experimental formulations were developed, by qualitative and quantitative modification of the excipients, while the composition and doses of the active substances were kept constant in all models: without API (F1–F17) and with API (M1–M8, M13, M14, M16, M17), evaluated by determining the apparent and compacted density, Carr index, Hausner ratio, angle of repose and flow rate. Among the formulations, models M8, M13 and M17 presented favorable technological parameters, and based on the results of dissolution studies for the evaluation of pharmaceutical availability (*in vitro*), model M13 was selected as the optimal formulation for further research.

For quality control and product standardization, modern, selective and accessible chemical and physicochemical methods were used, such as thin layer chromatography (TLC), HPLC, UV-Vis spectrophotometry, atomic absorption spectrometry (AAS) and atomic emission spectrometry (AES) to verify the elemental content of Mg^{2+} and K^{+} . The development of the fixed-dose medicinal product was carried out in accordance with the recommendations of the ICH guidelines and the principles of Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP).

The storage, systematization and processing of experimental data, calculations and graphical representation of the results were performed using Microsoft Excel 2010, OriginLab 8.0 and IBM SPSS Statistics 27 programs. The statistical analysis included descriptive indicators (mean, SD, RSD, 95 % CI, IQR, minimum and maximum values).

Implementation of scientific results. The research results were applied through the development of the technical -analytical and methodological documentation necessary for the standardization and manufacture of the combined pharmaceutical product "Kaliumcell". For this

purpose, the Pharmacopoeia Monograph "Kaliumcell, powder for oral administration" was developed, which regulates the quality of the combined product MF MD-USMF 08/73 of 23.09.2025. At the same time, the Technological Regulation was developed, which regulates the production process of the tetracomponent combined powder "Kaliumcell", "Kaliumcell, powder for oral administration", no. 7/4 of 23.09.2025.

The applicative value of the results obtained was confirmed by obtaining 12 implementation documents, of which 6 scientific-didactic, integrated into the curricula of the disciplines and in the methodological recommendations for the training of students of the Faculty of Pharmacy (no. 03-333 of 04.02.2025, no. 03-792 of 24.03.2025, no. 03-2321 of 07.07.2025, no. 03-2322 of 07.07.2025, no. 03-2323 of 07.07.2025 and no. 03-4398 of 24.10.2025), 5 innovator certificates, 2 of which as first author: "Application of the methodology for determining the compatibility of components in combined solid pharmaceutical forms by the infrared spectroscopy method with transformation Fourier (FTIR) and "Preparation of combined powder containing potassium aspartate, magnesium aspartate, potassium orotate and spironolactone ".

The research results were implemented in the scientific and practical activity of the Vasile Procopișin University Pharmaceutical Center (Implementation Act No. 2 of 03.03.2026) and the Drug Development Center within Nicolae Testemițanu University State of Medicine and Pharmacy (Implementation Act No. 1 of 04.03.2026), and an implementation act in practical process within SC "Balkan Pharmaceuticals" SRL (Implementation Act No. 158 of 24.04.2026), demonstrating the industrial applicability of the developed combined medicinal product and the possibility of its integration into the production process. The implementation confirms the practical value, innovative character and technological transfer prospects of the developed product.

Publications on the topic of the thesis. The research results were approved by scientific publications: articles -11, including articles in ISI journals, SCOPUS - 1 (IF 6.9), of which 2 articles as a single author; abstracts - 38, of which 4 abstracts as a single author, including in collections of international scientific events - 14; the presentation within the framework of 41 active participations in national and international scientific events, including plenary presentation - 1, oral presentations - 12, posters - 28; innovator certificates - 5 (of which 2 as first author), technological regulation - 1, pharmacopoeia monograph - 1, practical implementation documents - 12, including: in practice at SC "Balkan Pharmaceuticals" SRL - 1, in the scientific-practical process - 5, including at the Vasile Procopișin University Pharmaceutical Center within Nicolae Testemițanu State University of Medicine and Pharmacy; in the study process in the curriculum of the disciplines of the Faculty of Pharmacy - 6.

Thesis volume and structure. The thesis is presented on 141 pages of basic text and includes an introduction, 5 chapters, general conclusions and practical recommendations, a bibliography with 181 references and 18 appendices. The structure of the thesis includes a literature review chapter, the chapter "Materials and Methods", and three chapters devoted to the author's own research. The illustrative material is represented by 40 figures and 25 tables.

Keywords: hypokalemia, fixed-dose combination of the medicinal product, oral powder, potassium aspartate, magnesium aspartate, potassium orotate, spironolactone, formulation, standardization, validation, HPLC, UV-Vis spectrophotometry, FTIR, DSC, stability studies, preclinical studies.

THESIS CONTENT

1. HYPOKALEMIA AND CURRENT THERAPEUTIC APPROACHES

Potassium (K^+) is the main intracellular cation and an essential electrolyte for the normal physiological function. It participates in the transmission of nerve impulses, contraction of skeletal and cardiac muscles, maintenance of membrane potential, water and electrolyte and acid-base balance, as well as in energy and carbohydrate metabolism. Approximately 98% of the total amount of potassium is located in the intracellular compartment, and the physiological serum concentration is 3.5–5.5 mmol/L [3, 4].

Hypokalemia, defined as a decrease in serum potassium concentration below 3.5 mmol/L, is one of the most common electrolyte disorders encountered in clinical practice. The most common causes are diuretic therapy, gastrointestinal losses, insufficient potassium intake, hypomagnesemia, and some endocrine and renal disorders [5].

Clinical manifestations are nonspecific and include fatigue, muscle weakness, cramps, and paresthesia, and in severe cases, paralysis, respiratory failure, and serious cardiac arrhythmias may occur. Because the symptoms are similar to those of many other conditions, the diagnosis can only be confirmed by determining the serum potassium concentration and evaluating electrocardiographic changes. Hypokalemia is associated with an increased risk of cardiovascular complications, especially in patients with coronary artery disease, myocardial infarction, or heart failure, but it can also affect renal function, bone metabolism, and carbohydrate metabolism [5].

Treatment depends on the severity of the deficiency and its cause. In mild forms, correction of dietary intake and administration of oral potassium preparations (potassium chloride, citrate, orotate or aspartate, including combinations with magnesium) are recommended, while in severe forms, intravenous administration under electrocardiographic monitoring is necessary [3]. Potassium-sparing diuretics, such as spironolactone, are also used in diuretic-induced hypokalemia.

Although there are numerous potassium medicinal products available, they have limitations related to gastrointestinal tolerability, low elemental potassium content, the need for repeated administration, and the lack of combinations capable of simultaneously correcting electrolyte deficits and reducing potassium losses [6, 7]. These limitations justify the development of combination medicinal products with superior therapeutic efficacy and compliance.

On the pharmaceutical market, combined preparations intended to correct potassium deficiency are predominantly represented by associations of potassium salts with magnesium, an association justified by the fact that hypomagnesemia favors renal potassium losses and can cause or maintain hypokalemia. In order to diversify potassium sources and complete the action of the formula at the metabolic level, potassium orotate (OrK) was also included in the proposed combination. At the same time, in patients with a history of cardiovascular disease under chronic treatment with thiazide diuretics, the association of a potassium-sparing diuretic, such as Spir, can contribute to reducing renal potassium losses [1, 9]. Thus, the association of AspK, AspMg, OrK and Spir in a single medicinal product proposes a complex approach, based on complementary mechanisms, for the correction of hypokalemia [7].

2. MATERIALS AND METHODS

2.1. Research methodology for the development of the combined medicinal product

The study was conducted in the Laboratories of the Drug Development, Analysis and Standardization (LDDAS), Phytochemistry and Bioanalysis within the Drug Development Center (DDC) of Nicolae Testemițanu State University of Medicine and Pharmacy (SUMP). Preformulation, formulation, biopharmaceutical evaluation, development and validation of analytical methods, as well as stability studies of the combined powder with AspK, AspMg, OrK and Spir were performed, using methods such as CSS, HPLC, UV-Vis spectrophotometry, dissolution test and determination of technological parameters.

Complementary research was carried out in collaboration with the Institute of Chemistry of USM (FTIR spectroscopy, AAS, AES), the University of Debrecen, Hungary (spectroscopy FTIR, ICP-OES), University of Graz, Austria (CSS, HPLC) and Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania (DSC).

The preclinical studies were conducted in the Laboratory for Preclinical and Clinical Drug Evaluation of the DDC, with the approval of the Ethics Committee of the Nicolae Testemițanu SUMP (Minutes no. 57 of 12.04.2018). These included the assessment of acute toxicity on 60 white laboratory mice and the assessment of the potassium restoration process and antioxidant activity on 24 white laboratory rats, using an experimental model of furosemide -induced hypokalemia.

2.2. Materials used in the development of the pharmaceutical formulation process

The following reference standards were used in the research: potassium aspartate hemihydrate, magnesium aspartate dihydrate and spironolactone (European Pharmacopoeia), potassium orotate (Sigma- Aldrich). For the development of the experimental formulations, the medicinal substances AspK, AspMg, OrK and Spir were used, as well as excipients and auxiliary substances: microcrystalline cellulose, anhydrous and monohydrate lactose, magnesium stearate, stearic acid, citric acid, mannitol, saccharin, fructose and L-aspartic acid. The excipients used in the development of the formulations were of pharmacopoeia quality and admitted for pharmaceutical use in accordance with the requirements of Ph. Eur., and the reagents and solvents had a degree of purity corresponding to the applied analysis methods.

The pharmaceutical forms were developed and prepared as experimental laboratory batches: three batches of the four-component combined powder, dosed in sachets, containing AspK, AspMg, OrK and Spir.

2.3. Research methods and equipment used in the development of the medicinal product

The study applied modern instrumental methods for qualitative and quantitative analysis, with high sensitivity and accuracy. The compatibility between API and Ex was assessed by FTIR spectroscopy (Perkin Elmer Spectrum Two with ATR, USA) and DSC (Mettler Toledo DSC 822, Switzerland). Analytical studies were performed by applying the following methods: UV-Vis spectrophotometry (*Agilent Technologies 8453, Shimadzu 1800*), atomic emission spectroscopy (*Agilent Technologies 5100 ICP-OES*) and atomic absorption spectroscopy (*AAS-1 Carl Zeiss, Germany*), HPLC with UV detector (*Shimadzu LC-20A, Agilent 1100*) and fluorescence detector (*Agilent 1260*). The technological parameters of the powders were determined using equipment for the evaluation of the tapped density, fluidity and granulometry. *In vitro* dissolution test was performed with the Electrolab TDT-8 apparatus, and the assay of API released in the dissolution medium was performed by HPLC (*Shimadzu LC-20A, Agilent 1260*). The API dosage from the combined powder was performed by complexometry, formol titration and HPLC (*Shimadzu LC-20A*). For sample preparation, pH meter, analytical balance and muffle furnace were used. For the thin layer chromatography, pre-coated TLC sheets Xtra SIL G/UV₂₅₄ were used.

3. PREFORMULATION, FORMULATION, OPTIMIZATION AND COMBINED POWDER TECHNOLOGY

3.1. Analysis of the pharmaceutical market for potassium-containing products and substantiation of the development of a new combined medicinal product

Analysis of the pharmaceutical market in the Republic of Moldova (State Nomenclature of Medicines and State Register of Food Supplements, 27.12.2025) revealed that the number of medicines intended for the treatment of hypokalemia is relatively low, compared to food supplements. It was found that combination medicines represent approximately one third of the preparations used in general medical practice and one fifth of hospital therapy [11].

The market structure assessment demonstrated the predominance of medical products containing potassium aspartate in combination with magnesium aspartate (38.5%), followed by preparations based on potassium chloride (23.1%) and those containing potassium bicarbonates in combination with magnesium salts (15.4%). The other forms of potassium salts were identified in lower proportions, 7.7% each. Products with L-aspartate contain lower amounts of elemental potassium, they have a higher bioavailability compared to inorganic salts. At the same time, the analysis demonstrated that on the market of the Republic of Moldova there is no medicinal product, combining AspK, AspMg, OrK and Spir, which justified the development of the combined medicinal product "Kaliumcell".

3.2. Compatibility assessment of active substances and excipients by spectroscopy FTIR and DSC

The compatibility of API (AspK, AspMg, OrK and Spir), both among themselves and with the excipients, was evaluated by spectroscopy FTIR and DSC. The FTIR spectra of the individual substances, of the API-API and API-Ex binary mixtures (ratio 1:1, m/m), as well as the corresponding DSC thermograms were recorded (figure 1).

The FTIR spectra of the individual API confirmed their identity by the presence of characteristic bands. For AspK, the bands corresponding to the $\nu(\text{NH}_3^+)$ vibrations in the range of 3286–3010 cm^{-1} , $\nu_{\text{as}}(\text{COO}^-)$ around 1600 cm^{-1} and $\nu_{\text{s}}(\text{COO}^-)$ at approximately 1400 cm^{-1} were revealed. AspMg showed characteristic bands at 3034 and 2643 cm^{-1} ($\nu(\text{NH}_3^+)$), 1571 cm^{-1} ($\nu_{\text{as}}(\text{COO}^-)$) and 1392 cm^{-1} ($\nu_{\text{s}}(\text{COO}^-)$). For OrK, the lactam carbonyl $\nu(\text{C}=\text{O})$ bands were identified around 1690 cm^{-1} , the $\nu(\text{COO}^-)$ bands in the range of 1560–1400 cm^{-1} and the $\nu(\text{N}-\text{H})$ band around 3100 cm^{-1} . The spectrum of spironolactone (Spir) was characterized by the intense carbonyl $\nu(\text{C}=\text{O})$ bands at 1764, 1689 and 1672 cm^{-1} , as well as the bands at 1282–1220 cm^{-1} corresponding to the C–H and C–O vibrations specific to the steroid structure. The spectra obtained corresponded to reference standards and data from the literature.

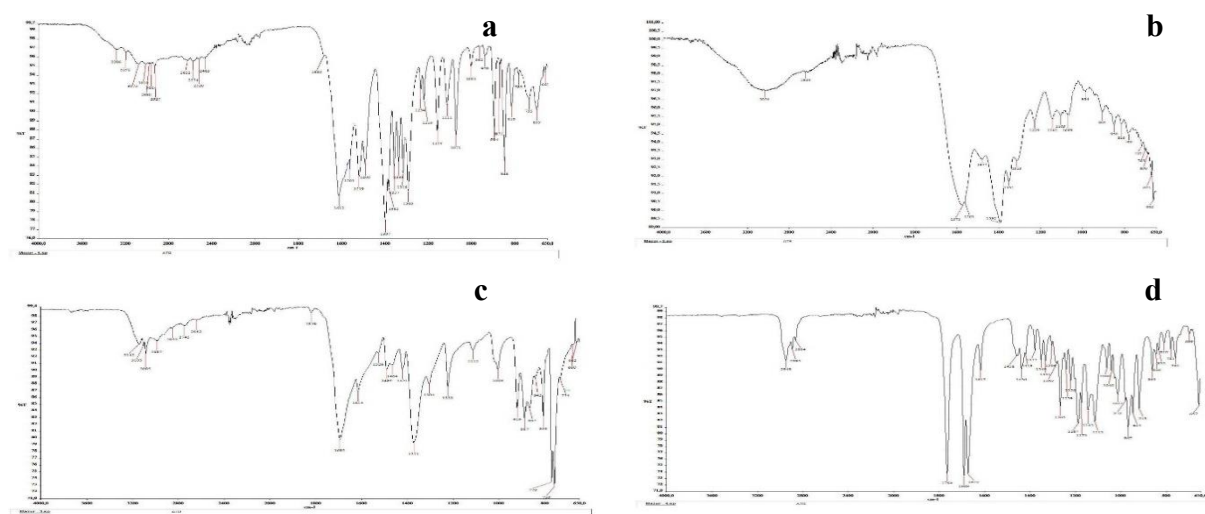


Figure 1. FTIR spectra of individual active substances: a – potassium aspartate (AspK); b – magnesium aspartate (AspMg); c – potassium orotate (OrK); d – spironolactone (Spir)

The analysis of the binary API-API mixtures revealed the preservation of the characteristic bands of all components. For the AspK–AspMg and AspMg–OrK systems, broadening of the bands in the range of 3300–3000 cm^{-1} , shifts of the carboxylate bands (1600–1370 cm^{-1}) and the appearance of a weak band at 500–600 cm^{-1} , attributed to Mg–O interactions, were observed. In the AspK–OrK, AspMg–Spir, AspK–Spir and OrK–Spir mixtures, only insignificant shifts of the C=O, COO^- and N–H bands were recorded, without the appearance of new bands, which confirms the predominance of physical interactions (hydrogen bonds and electrostatic interactions) and the absence of chemical reactions between API.

The compatibility with excipients was evaluated for 17 excipients by analyzing 68 binary API-Ex mixtures, of which microcrystalline cellulose and stearic acid showed only minor overlaps and shifts of the characteristic bands, confirming the physicochemical compatibility with all API. Lemon flavoring caused more pronounced changes in the bands in the range 3295–3142 cm^{-1} , without the appearance of new bands, indicating weak intermolecular interactions. In contrast, citric acid and sodium saccharin showed significant spectral changes and were excluded from the formulation stage.

DSC analysis confirmed the characteristic thermal behavior of the API and their compatibility in binary mixtures. AspK showed two characteristic endothermic effects, the main one at 164 °C ($\Delta H = 159.2 \text{ J/g}$), associated with the formation of polyaspartic acid and subsequent transformation into polysuccinimide, with degradation above 280 °C, indicating good thermal stability. AspMg showed a similar profile, preceded by a dehydration process and with the onset of degradation at approximately 260 °C. OrK showed two endothermic peaks at 416.90 °C ($\Delta H = 124.65 \text{ J/g}$) and 429.41 °C ($\Delta H = 269.55 \text{ J/g}$), attributed to the melting of two polymorphic forms

and characteristic of superior thermal stability. Spir exhibited an endothermic melting peak at 207.44 °C ($\Delta H = 51.25$ J/g), followed by thermal decomposition at temperatures above 250 °C, characteristic of moderate thermal stability (figure 2).

Thermograms of binary API-API mixtures revealed the preservation of the main thermal effects of the components, with minor shifts in temperatures and enthalpies. For the systems containing AspK and AspMg, the characteristic processes of polyaspartic acid formation (150–167 °C; $\Delta H = 19$ –115 J/g) and polysuccinimide (230–252 °C; $\Delta H = 49$ –210 J/g) were observed. In the mixtures with OrK, the endothermic effects specific to melting at 406–437 °C ($\Delta H = 65$ –364 J/g) were maintained, and in the systems containing Spir, the characteristic melting peak at 200–208 °C ($\Delta H = 17$ –29 J/g) was preserved, without the appearance of new thermal transitions. Comparative analysis of DSC thermograms of binary API-API mixtures revealed the preservation of the main thermal effects characteristic of SA.

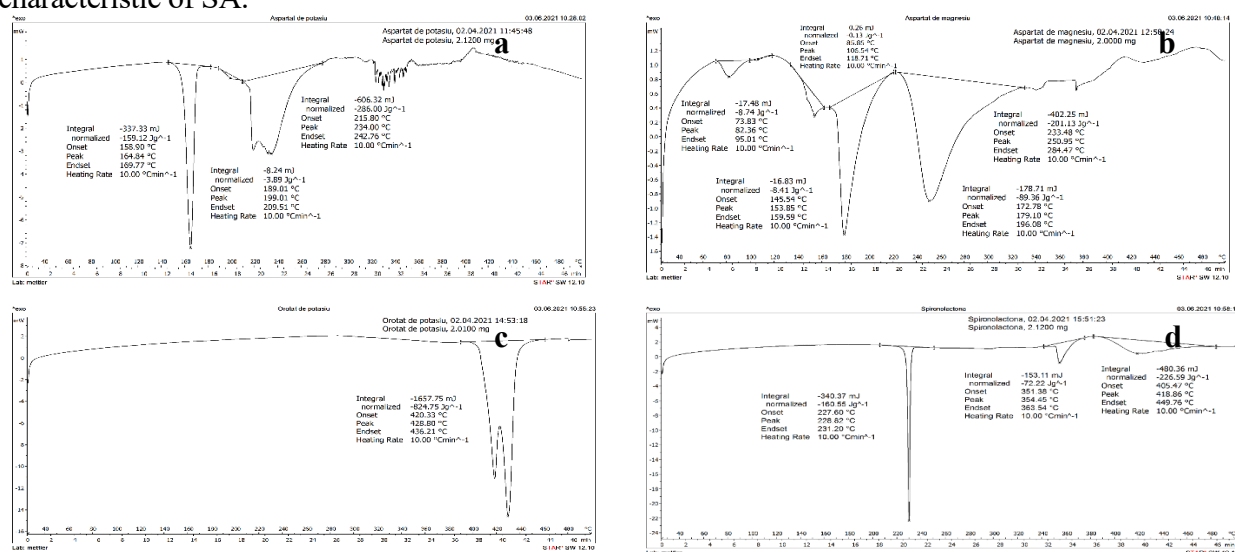


Figure 2. DSC thermograms of individual active substances: a – potassium aspartate (AspK); b – magnesium aspartate (AspMg); c – potassium orotate (OrK); d – spironolactone (Spir)

In systems containing AspK and/or AspMg, specific processes involving the formation of polyaspartic acid and its subsequent conversion to polysuccinimide were identified, with maximum temperatures ranging 149.54–166.56 °C and 229.87–251.69 °C, respectively. In the mixtures containing Spir, the endothermic effect corresponding to its melting was maintained in the range of 199.59–208.14 °C, and in those containing OrK, the two endothermic peaks characteristic of the polymorphic forms were preserved, located at approximately 413–418 °C and 430–437 °C. The reduced temperature shifts and enthalpy changes are attributed to the physical interactions between the components and the dilution effect in the mixture, without the appearance of new thermal transitions or the disappearance of those characteristic of API. These results demonstrate the thermal compatibility of the investigated combinations and support their use in the development of the combined medicinal product.

DSC analysis of the API-Ex binary mixtures (40 thermograms) revealed differences in the thermal behavior of the investigated systems. In the mixtures with microcrystalline cellulose (MCC), the main thermal transitions of API were preserved. For the OrK/MCC system, the melting of the excipient was observed at $T_{max} = 345.58$ °C ($\Delta H = 198.87$ J/g), and the polymorphic forms of potassium orotate were maintained at 420.15 °C ($\Delta H = 90.66$ J/g) and 438.45 °C ($\Delta H = 120.69$ J/g), confirming the thermal compatibility of the components.

In the magnesium stearate (MgStear) systems, the characteristic AspK, AspMg and OrK processes were preserved. For AspK, the transformation to polyaspartic acid and the formation of polysuccinimide were recorded at 163.48 °C ($\Delta H = 106.31$ J/g) and 237.75 °C ($\Delta H = 156.53$ J/g), and for AspMg at 160.90 °C ($\Delta H = 17.07$ J/g) and 251.15 °C ($\Delta H = 63.22$ J/g). In the OrK/ MgStear system, the characteristic endothermic effects were maintained at 411.97 °C ($\Delta H = 115.71$ J/g) and 434.72 °C ($\Delta H = 109.29$ J/g). In contrast, in the Spir/ MgStear mixture, the melting point of

spironolactone shifted to 180.79 °C ($\Delta H = 28.97$ J/g), suggesting physical interactions between the components.

Overall, DSC analysis demonstrated the absence of significant physicochemical incompatibilities under the investigated conditions in most of the combinations studied and contributed to the selection of excipients for the formulation of the combined medicinal product. The changes in the thermal profile highlighted for the Spir/ MgStear system indicated possible interactions between the components, which is why magnesium stearate was replaced by stearic acid in the final formulation. The use of microcrystalline cellulose, lactose and stearic acid ensured the thermal compatibility of the API and the stability of the developed formulation.

The results of FTIR spectroscopy were confirmed by DSC analysis, which demonstrated the preservation of the characteristic thermal effects of the components and the absence of new thermal transitions. Based on compatibility studies, the following compatible excipients were selected: microcrystalline cellulose (MCC), stearic acid and limonene -based flavoring, subsequently used in the development of experimental formulations.

3.3. Development of combined powder and determination of technological parameters

Based on the results of the compatibility studies, 17 experimental formulations (F) were developed with different types and proportions of excipients, initially in the absence of API, to optimize the technological properties of the mixtures. These were evaluated by the SeDeM method, and the formulations that met the SeDeM and processability criteria were subjected to the determination of technological parameters, in order to select the optimal formulation. The selected formulations were subsequently supplemented with API, obtaining 12 experimental models (M) of the combined medicinal product, and re-evaluated by determining the technological parameters (table 1).

Table 1. Percentage composition of experimental models M1–M17 of the combined powder

composition	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17
SA, %	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6
MCC, %	10	25	50	0	66.4	10.0	25	50	10	25	50	0	75	10	25	50	25.4
Lactose, %	56.4	41.4	16.4	66.4	0.0	66.4	51.4	26.4	65.4	50.4	25.4	75.4	0.0	55.4	35.4	5.4	0
Flavoring, %	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Saccharin, %	10	10	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0
Stearic acid, %	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
Fructose, %	0	0	0	0	0	0	0	0	0	0	0	0	0	10	15	20	50

Table 2. Comparative evaluation matrix of pharmacotechnical parameters of M models

Acceptance criteria: IC < 26%; HR < 1.35; α < 46°; flow time ≤ 25 s/100 g

Parameter	Criterion	M1	M2	M3	M4	M5	M6	M7	M8	M13	M14	M16	M17
Carr Index (CI, %)	CI < 26%	34.74	32.97	26.14	33.29	18.70	32.77	30.91	26.14	23.03	28.52	19.00	19.10
Hausner ratio (HR)	IH < 1.35	1.53	1.49	1.35	1.50	1.23	1.49	1.45	1.35	1.30	1.40	1.23	1.24
Angle of repose (α , °)	α < 46°	46	44	40	44	34	43	42	42	34	41	32	36
Flow time at 100 g (s)	≤ 25 seconds	61.06	32.53	35.46	43.50	18.33	99.72	91.63	91.12	15.55	33.32	19.34	23.83
Models selected for dissolution test (S)									S	S			S

Legend:

Compliant Borderline* Non-compliant

* The "borderline" category is used exclusively as a visual aid for values in the immediate vicinity of the threshold ($\pm 5\%$); the actual acceptance criteria remain those indicated in the header.

Note: M8, M13 and M17 are highlighted according to the selection made in the study. The matrix presents the four pharmacotechnical parameters comparatively and does not replace the integrated assessment evaluation used for the selection of formulations.

The evaluation of the technological properties was carried out for the experimental formulations of type F (17 formulations) and M (12 formulations), by determining the initial and

tapped densities, Carr index (IC), Hausner ratio (HR), angle of repose (α) and flow rate (t''). The results were interpreted according to the accepted criteria for powders intended for oral administration, considering the values IC <26%, HR <1.35, α <46° and flow time ≤ 25 s/100 g as appropriate (table 2).

Based on the results obtained, three optimal models (M8, M13 and M17) were selected for determining the release parameters through the dissolution test and subsequent development of the medicinal product.

3.4. *In vitro* evaluation of the pharmaceutical availability of the combined powder using the dissolution test

The *in vitro* pharmaceutical availability of the developed combined powders was evaluated by determining of the *in vitro* dissolution profile, using the Electrolab TDT-08L dissolution apparatus. The study was carried out in dissolution media at pH = 2 and pH = 6.8, at the temperature of 37 ± 0.5 °C, in a volume of 500 mL and at a stirring rate of 75 rpm, according to the recommendations of ICH Q6A and ICH Q4B. Samples were taken at intervals of 5, 10, 20, 30, 45 and 60 min, and the concentration of released API was determined by the HPLC. The acceptance criterion was the release of at least 75% of the amount of API within 45 min. The obtained results are presented in the table 3.

Table 3. Results of API release from the powder by the dissolution test in the acid and neutral media

The API release in the acid medium									
Time, min	Spir, %			OrK, %			AspK Mg, %		
	M8	M13	M17	M8	M13	M17	M8	M13	M17
0'	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
5'	63.79%	29.03%	46.55%	96.90%	76.63%	89.35%	91.21%	88.65%	85.46%
10'	74.34%	47.51%	54.57%	97.45%	89.29%	90.35%	88.55%	88.94%	93.91%
20'	76.75%	61.66%	62.26%	97.07%	97.58%	92.20%	89.76%	87.30%	92.29%
30'	77.46%	64.39%	68.55%	95.79%	97.38%	93.93%	88.26%	84.60%	88.12%
45'	81.28%	75.42%	70.97%	96.26%	97.69%	93.59%	85.66%	88.02%	87.01%
60'	91.33%	75.16%	75.54%	97.29%	95.91%	93.64%	83.77%	86.96%	87.69%
The API release in the neutral medium									
Time, min	Spir, %			OrK, %			AspK, AspMg, %		
	M8	M13	M17	M8	M13	M17	M8	M13	M17
0'	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
5'	29.13%	36.60%	25.14%	27.50%	57.32%	36.82%	88.94%	86.94%	89.52%
10'	36.34%	43.47%	25.04%	39.23%	66.28%	44.05%	83.19%	83.39%	86.33%
20'	40.11%	41.01%	29.07%	61.46%	67.17%	55.38%	81.94%	82.04%	84.98%
30'	39.59%	40.77%	32.99%	60.07%	64.13%	58.24%	82.47%	82.97%	86.67%
45'	39.95%	43.66%	30.52%	60.41%	61.49%	58.11%	86.14%	83.14%	85.32%
60'	39.96%	40.37%	34.86%	59.52%	61.41%	61.59%	82.71%	79.71%	89.76%

The analysis of the dissolution profiles revealed differences between the M8, M13 and M17 formulations. In acid medium (pH = 1-2), the M8 and M13 formulations demonstrated a suitable dissolution profile, while the M17 formulation showed a lower release of spironolactone, indicating the need for technological optimization. In the medium with pH = 6.8, AspK and AspMg showed a fast and constant release, OrK - moderate release, and Spir - the lowest release, determined by its limited solubility in this medium (figure 3).

Comparative analysis of dissolution profiles and kinetic parameters demonstrated that the M13 formulation exhibits the most favorable release behavior, being selected as the optimal formulation for further development of the combined powder.

The composition of the selected formulation includes 0.140 g of potassium aspartate hemihydrate, active substance and source of potassium; 0.158 g of magnesium aspartate dihydrate, active substance, source of magnesium and is essential for enzymatic activity and neuromuscular function; 0.150 g of potassium orotate, active substance and source of potassium and contributes

to the intracellular transport of potassium and supports cellular metabolism; 0.024 g of spironolactone, active substance with diuretic and potassium-sparing action; 1.508 g of microcrystalline cellulose, used as a diluent to ensure uniformity of the mixture and improve the flow properties of the powder; 0.020 g of stearic acid, with a lubricant role, to reduce adhesion and facilitate powder processing, and 0.0002 g of lemon flavoring, intended to improve organoleptic properties and acceptability.

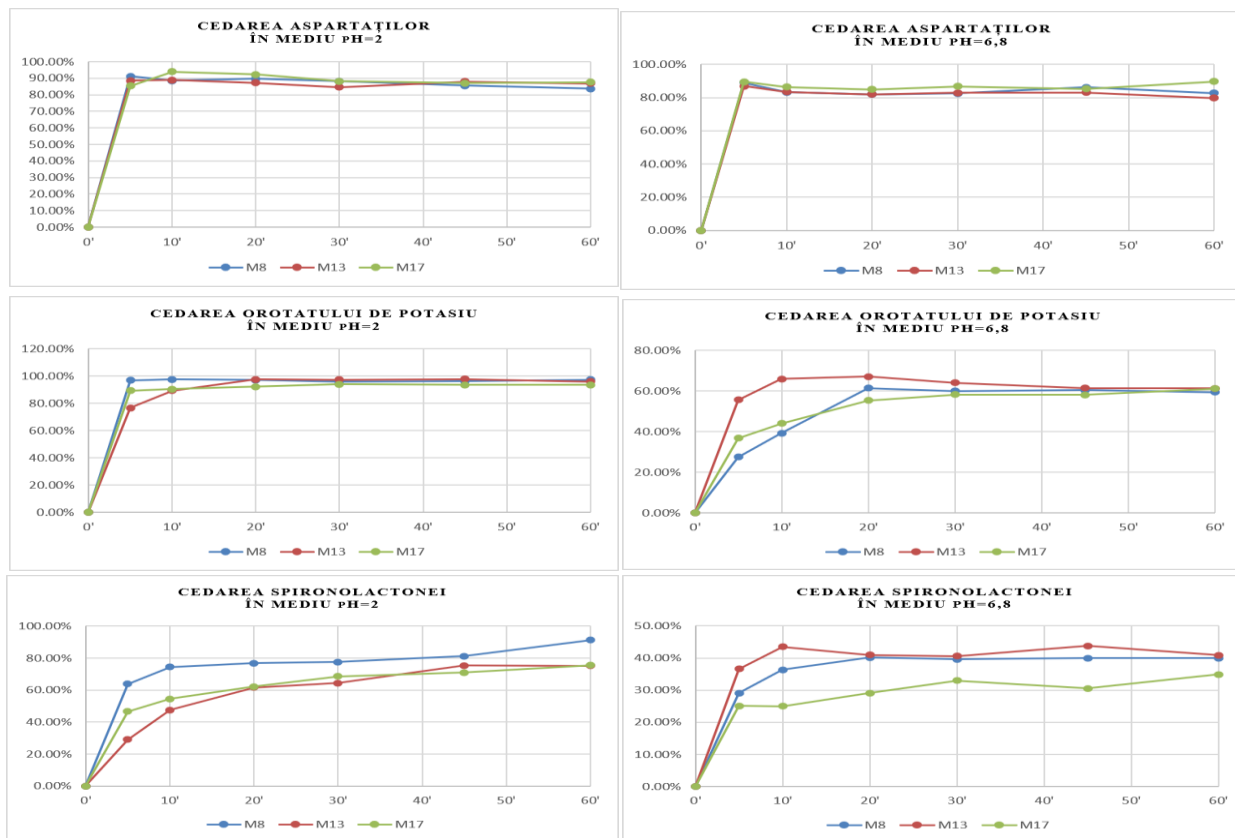


Figure 3. Dissolution kinetics of the powder models in acid (pH=2) and neutral (pH=6.8) media

The optimized composition of the combined powder was established by optimization using the Design Expert program. The results confirmed the M13 formulation as the optimal composition, consistent with the preformulation studies (table 4).

Table 4. Independent variables (factors) and investigated ranges

Factor (code)	Name	Unit	Low level	High level	Mediate	SD
A	MCC	%	70.00	80.00	75.00	5.00
B	Stearic acid	%	0.50	1.50	1.00	0.50
C	C (technological factor)	-	0.00	1.00	0.50	0.50

3.5. Technology for the production of the combined powder containing AspK, AspMg, OrK and Spir

The development of the technology for obtaining the combined powder aimed at optimizing the processing processes of API and Ex, so as to ensure the homogeneity, stability and quality of the pharmaceutical product. The technological stages were developed in accordance with the requirements of the European Pharmacopoeia and the principles of Good Manufacturing Practices (GMP). The raw materials are weighed according to the composition and sieved through a sieve with a mesh size of 160–500 μm . Microcrystalline cellulose is introduced into the mixer, after which stearic acid, spironolactone, potassium orotate, potassium aspartate, magnesium aspartate and flavoring are successively added. Mixing is carried out until a homogeneous mixture is obtained, which is subjected to quality control, after which it is dosed into sachets and packaged.

4. DEVELOPMENT OF METHODS FOR ANALYSIS AND STANDARDIZATION OF COMBINED POWDER. STABILITY STUDIES

4.1. Chemical and physicochemical methods used for the identification of API

The development of analysis methods for the combined powder was carried out using a systematic approach and quality control principles, based on the study of the physicochemical properties of API, methods for identification, dosage and control of their purity. Three experimental batches (S01, S02 and S03) of the combined powder were prepared in the LDDAS within DDC and used for the identification, development and validation of analytical methods.

The identification of API was carried out on pure substances, mechanical mixture (1:1) and pharmaceutical form, using chemical reactions specific to their functional groups and instrumental methods of analysis. The potassium ion (K^+) was identified by the formation of a white crystalline precipitate of potassium hydrogen tartrate (after scraping the walls of the test tube with a glass rod and cooling under a stream of cold water), as well as the appearance of a yellow crystalline precipitate characteristic of the $K_2Na[Co(NO_2)_6]$ salt, confirming the presence of K^+ in the analyzed samples. The magnesium ion was confirmed by the formation of a white crystalline precipitate of magnesium ammonium phosphate $MgNH_4PO_4$, insoluble in acetic acid, soluble in mineral acids. The aspartate ion was identified by the reaction with ninhydrin, which generated an intense blue-violet color, characteristic of aliphatic amino acids. The orotate ion was confirmed by the appearance of an intense red coloration in the presence of Fe^{3+} ions, indicating the formation of ferric salts specific to orotate. Spir was identified by the appearance of a yellow-green fluorescence, followed, after heating, by the appearance of the intense red coloration and the release of H_2S , a reaction specific to thio-derived fragments, formed following mineralization.

The identification of API in the combined powder was also performed by UV-Vis spectrophotometry and HPLC. The UV-Vis spectra of API, mechanical mixtures and pharmaceutical form were recorded in different solvents and dissolution media: alkaline solutions (0.01 M, 0.1 M, 1 M NaOH, KOH, $Ca(OH)_2$, $CaCO_3$ and $Mg(OH)_2$), as well as in organic solvents (ethanol, methanol, acetonitrile, dimethylformamide and dimethylsulfoxide). The best results were obtained using the methanolic solution of spironolactone (10 $\mu g/mL$) and the 0.1 M NaOH solution for potassium orotate (20 $\mu g/mL$), which were selected for the development of the UV-Vis spectrophotometric method (figure 4).

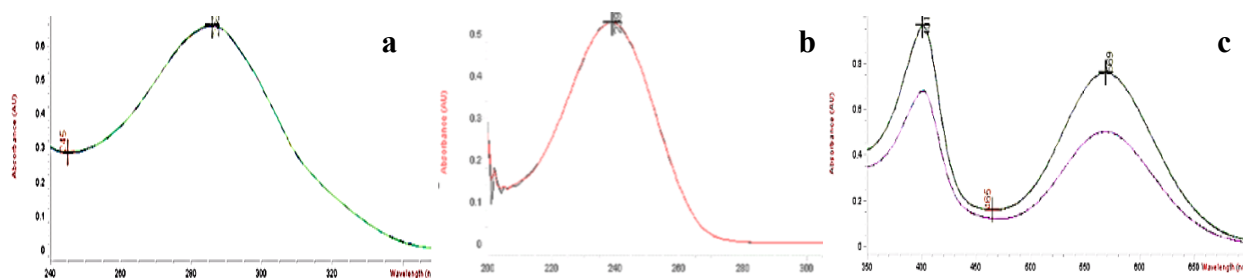


Figure 4. UV-Vis absorption spectra of sample solutions from the combined powder: OrK in 0.1 M NaOH (a), Spir in methanol (b) and Aspartates (c)

By UV-Vis spectrophotometry, the identification of API was confirmed by comparing the spectra of the sample solution with those of the standard solutions, which showed characteristic absorption maxima at 239 ± 2 nm for Spir in methanol (10 $\mu g/mL$), 286 ± 2 nm for OrK in 0.1 M NaOH (20 $\mu g/mL$) and 402 ± 2 nm for aspartates (10 $\mu g/mL$), after reaction with ninhydrin.

4.2. Analytical methods for the determination of active pharmaceutical ingredient

Complexometric titration of AspMg and formol titration of AspK.

The AspMg content was determined by complexometric titration with 0.05M disodium edetate solution (Na_2EDTA), and the AspK content by formol titration with 0.1 mol/L NaOH, calculated by the difference. The determinations were carried out on 3 batches of powder dosed in sachets, with 5

replicate determinations for each batch. The statistical evaluation of the results demonstrated that the values obtained for AspK varied between 0.1339 - 0.1424 g ($p = 0.129$), and for AspMg between 0.1502 - 0.1600 g ($p = 0.086$), falling within the allowed limits, with $RSD < 2.0\%$, confirming the precision and reproducibility of the dosage methods.

Potassium and magnesium dosage by AES and AAS

The potassium and magnesium content was determined by atomic emission/absorption spectrometry (AES/AAS) on 3 batches of powder dosed in sachets, using calibration curves in the ranges 2.5–10.0 mg/L for potassium and 1.0–4.0 mg/L for magnesium. The method demonstrated adequate linearity, with determination coefficients $R^2 = 0.9946$ for potassium and $R^2 = 0.9994$ for magnesium. The statistical evaluation of the results showed an elemental potassium content ranged from 0.1339 to 0.1424 g ($p = 0.129$), while the AspMg content ranged from 0.1502 to 0.1600 g ($p = 0.086$). All values were within the specified acceptance limits, with $RSD \leq 2.0\%$, confirming the precision and reproducibility of the assay methods.

UV-Vis spectrophotometric method.

UV-Vis spectrophotometric method of the combined powder was performed on 3 batches of powder dosed in sachets, performing 5 replicate determinations for each batch. Statistical evaluation of the dosing results, performed on samples prepared to ensure concentrations of 20 $\mu\text{g/mL}$ OrK in 0.1M NaOH and 10 $\mu\text{g/mL}$ Spir in methanol, demonstrated that the values obtained for OrK (0.1475–0.1537 g; $p = 0.175$) at 286 ± 2 nm and Spir (0.0234–0.0243 g; $p = 0.235$) at 238 ± 2 nm fall within the accepted limits, with $RSD \leq 2.0\%$.

The UV-Vis spectrophotometric method was developed and validated for OrK and Spir in accordance with the recommendations of the ICH Q2(R1/R2) guideline, by evaluating the parameters of specificity, linearity, precision, accuracy, limits of detection and quantification, as well as robustness.

The linearity of the spectrophotometric method was demonstrated for the OrK standard solutions in the concentration range of 5–50 $\mu\text{g/mL}$ ($y = 0.0331x + 0.0138$, $R^2 = 0.9998$), confirming compliance with the Bouguer–Lambert–Beer law (figure 5). The sensitivity of the method was demonstrated by the values $LOD = 0.119$ $\mu\text{g/mL}$ and $LOQ = 0.361$ $\mu\text{g/mL}$.

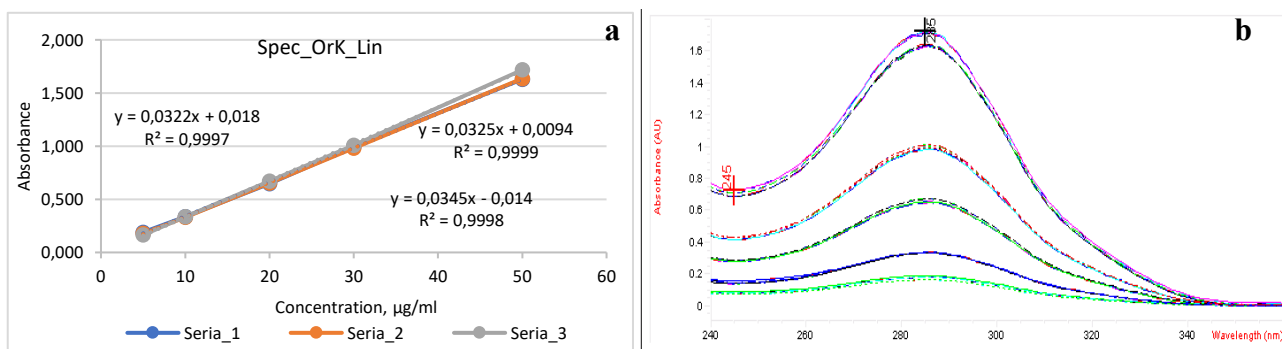


Figure 5. Linearity for three experimental batches: a - Calibration curves and b - UV spectra of potassium orotate standard solutions at different concentration levels (5–50 $\mu\text{g/mL}$)

The accuracy assessment, by the standard addition method at the levels of 80%, 100% and 120%, showed recoveries ranging between 94.55% and 102.71 %, with RSD values $\leq 2\%$, falling within the acceptance criteria. The precision (intra-day and inter-day) and robustness of the method were also confirmed by RSD values $\leq 2\%$, and the specificity study demonstrated the absence of excipient interferences at the wavelength of 286 ± 2 nm.

The linearity of the spectrophotometric method for Spir was shown in the concentration range 5–30 $\mu\text{g/mL}$ ($y = 0.0549x + 0.0169$; $R^2 = 0.997$), confirming its applicability for the quantitative determination of Spir (figure 6). The sensitivity of the method was demonstrated by the values $LOD = 0.14$ $\mu\text{g/mL}$ and $LOQ = 0.44$ $\mu\text{g/mL}$.

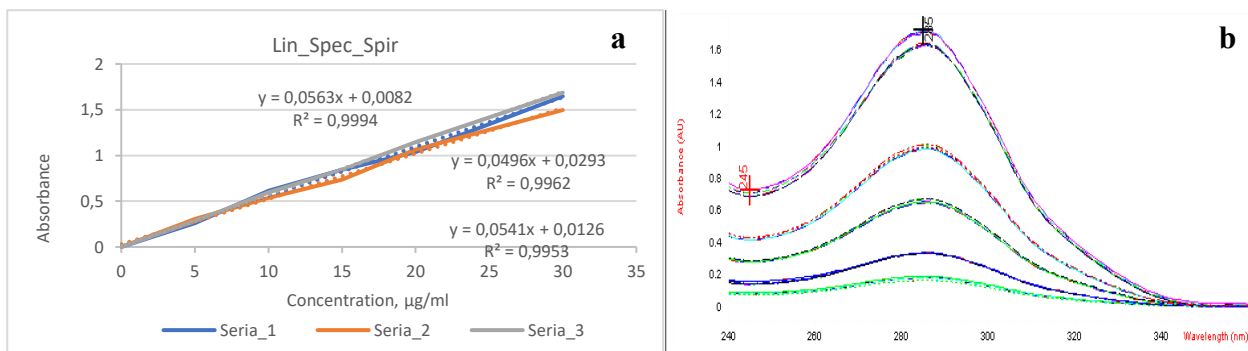


Figure 6. Linearity for three experimental batches: a – Calibration curves and b – UV spectra of spironolactone standard solutions at different concentration levels (5–30 µg/mL)

The accuracy assessment, performed by the standard addition method at levels of 80%, 100% and 120%, showed recoveries ranging from 96.17% to 103.66 %, with RSD values $\leq 2\%$, falling within the acceptance criteria. The precision (intra-day and inter-day), specificity and robustness of the method were also confirmed by RSD values $\leq 2\%$, and the excipients in the formulation did not present interferences at the characteristic wavelength of 239 ± 2 nm.

The validation results demonstrate that the developed spectrophotometric methods meet all the criteria set out in ICH Q2(R1/R2), being specific, linear, precise, accurate, robust and sufficiently sensitive for the identification and quantitative determination of potassium orotate and spironolactone in combined powder in sachet form.

HPLC method.

HPLC method of the combined powder was performed on 3 batches of powder dosed in sachets, performing 5 replicate determinations for each batch, using a Teknokroma C18 column (250 × 4.6 mm; 5 µm), mobile phase methanol: phosphoric acid 0.1% (60:40, v/v), in isocratic mode, with a flow rate of 1.0 mL/min, at 30 °C, injection volume of 20 µL and UV detection at 279 ± 2 nm for OrK and 240 ± 2 nm for Spir.

The statistical evaluation of the dosing results, performed on samples prepared at concentrations of 100 µg/mL OrK and 15 µg/mL Spir, demonstrated that the values obtained for OrK ($t_R = 2.38$ min) varied between 0.1484 and 0.1537 g ($p = 0.554$), and for Spir ($t_R = 7.18$ min) between 0.0237 and 0.0240 g ($p = 1.554$), falling within the accepted limits, with RSD $\leq 2.0\%$ (figure 7).

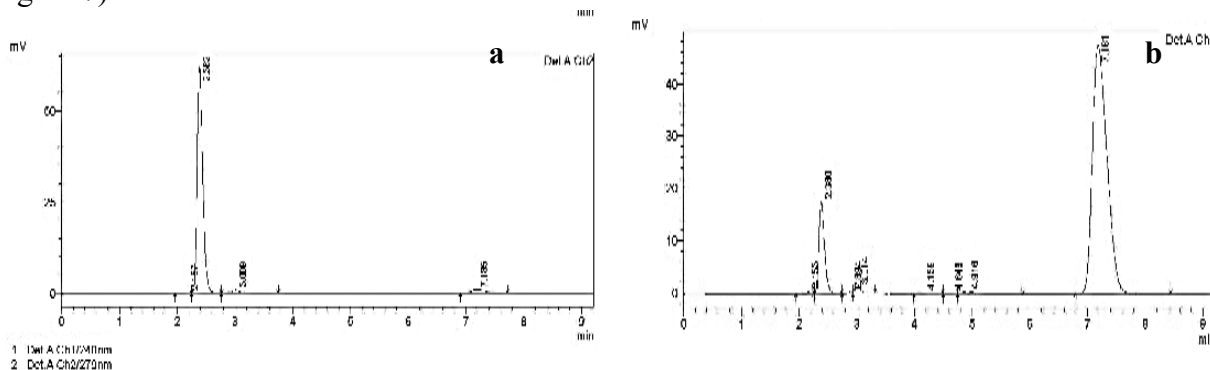


Figure 7. HPLC-UV chromatograms of the sample solutions of the spironolactone 15 µg/mL (a) and potassium orotate 100 µg/mL (b).

The HPLC method for the simultaneous determination of OrK and Spir from combined powder was developed and validated. During the development stage, different mobile phase compositions and chromatographic conditions were investigated, in order to obtain an efficient separation of the two active substances.

Linearity was demonstrated in the range of 10–150 µg/mL for both APIs, with coefficients of determination of $R^2 = 0.994$ for OrK and $R^2 = 0.999$ for Spir (Figure 8). The sensitivity of the

method was confirmed by LOD/LOQ values of 1.28/3,88 µg/mL for OrK and 1.03/3.12 µg/mL for Spir.

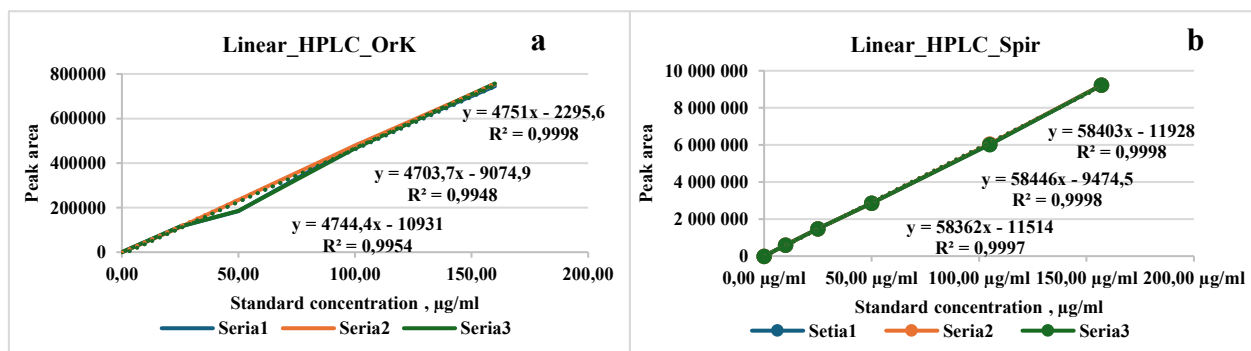


Figure 8. Calibration curves of standard solutions at different concentration levels (10-150 µg/mL) for OrK (a) and Spir (b) by HPLC

The accuracy assessment, by the standard addition method at levels of 80%, 100% and 120%, presented recoveries of 98.25–102.76% for OrK and 93.90–101.02 % for Spir, with RSD values $\leq 2\%$. Also, the precision (intra-day and inter-day), specificity and robustness of the method were confirmed, all determinations falling within the acceptance criteria ($RSD \leq 2\%$).

The results obtained demonstrate that the developed HPLC method is specific, linear, precise, accurate, robust and sufficiently sensitive for the simultaneous determination of potassium orotate and spironolactone in the combined medicinal product. For the first time, an HPLC method was developed for the simultaneous determination of the two active substances in a combined powder intended for the treatment of hypokalemia. The method was used *in vitro* dissolution studies and included in the Pharmacopoeia Monograph of the combined medicinal product "Kaliumcell".

4.3. Stability studies and standardization of combined powder

The stability of the combined powder was evaluated in accordance with ICH Q1A recommendations, through forced degradation studies under conditions of acid and alkaline hydrolysis, oxidation, high temperature and exposure to natural light and UV radiation. Analysis of changes was performed by UV-Vis spectrophotometric methods at 0, 3 and 24 hours [8]. The forced degradation studies showed that spironolactone is the most sensitive to acid, alkaline hydrolysis and oxidative conditions, while potassium orotate demonstrated superior stability. Exposure to light and high temperature caused less changes. Determination of potassium and magnesium content by AES and AAS methods confirmed the stability of these components under all experimental conditions investigated.

The stability of the powder under accelerated and real-time conditions for long-term was evaluated on three experimental batches. Accelerated stability studies were performed at 40 °C and 60 °C, while long-term real-time stability studies were conducted at 25 °C over a period of 24 months. At each control point, the organoleptic aspect and API content were evaluated by analytical methods. The results obtained demonstrate that the combined powder retains its quality characteristics under the recommended storage conditions, and based on the statistical processing of the experimental data, a shelf life of at least 24 months was established for the combined "Kaliumcell" powder.

The results of the technological, analytical and stability studies constituted the basis for the standardization of the medicinal product. The criteria for identification and dosing of the API and the parameters necessary for the quality control of the dosed powder were established. The developed chemical, spectrometric, spectrophotometric and chromatographic methods were integrated into an analytical strategy adapted to the characteristics of each component of the formulation.

Based on these results, the Pharmacopoeia Monograph for the combined medicinal product "Kaliumcell", powder for oral administration dosed in sachets, containing potassium aspartate,

magnesium aspartate, potassium orotate and spironolactone, was developed and approved. The document establishes the quality requirements, identification and dosage methods, acceptance criteria, storage conditions and shelf life of the product. The development of the Pharmacopoeia Monograph confirms the completion of the standardization stage and provides the necessary analytical support for reproducible quality control of the developed formulation.

5. EVALUATION OF THE TOXICITY AND HYPOKALEMIA CORRECTION EFFECT OF THE COMBINED POWDER

The preclinical investigations of the combined medicinal product "Kaliumcell" were approved by the Research Ethics Committee of the Nicolae Testemițanu SUMP (Minutes no. 57 of 12.04.2018). The studies were conducted in accordance with the recommendations of OECD TG 423 (Acute Toxic Class Method) [12, 13].

The acute toxicity of the preparation was evaluated in 60 mice, after intraperitoneal and intragastric administration, in doses of 100, 300, 500 and 1000 mg/kg. With intraperitoneal administration, one death (17%) was recorded at doses of 500 mg/kg and 1000 mg/kg, and with intragastric administration, only one death (17%) was recorded, only at the dose of 1000 mg/kg. The results obtained demonstrated that the combined medicinal product Kaliumcell falls into the IV toxicity class, characterized by low acute toxicity.

The preclinical pharmacological study was performed on 24 female rats (*Rattus albicans*), of which 18 were included in the experimental groups, and 6 constituted the control group. Hypokalemia was induced by the administration of furosemide (32 mg/kg), and the efficacy of the combined preparation "Kaliumcell" (100 mg/kg, oral administration) was compared with the standard treatment. The animals were monitored for 28 days under standardized conditions. The obtained data were statistically processed by Post- Hoc analysis performed using the Tukey HSD test. Potassium loss was observed in the groups with induced hypokalemia: by 46.86% ($p=0.0001$) for Lot II; by 32.51% ($p=0.001$) - Lot III and by 25.99% ($p=0.001$) - Lot IV compared to control Lot I. Statistically significant differences were identified between Lot II - Lot III, as well as Lot II - Lot IV, which indicates an increase in potassium levels in Lot III by 26.99% ($p=0.033$), and in Lot IV by 39.26% ($p=0.022$), compared to Lot II. In contrast, between Lot III - Lot IV, statistically significant differences were not found ($p=0.998$), which indicates a similar effect of these on potassium levels.

The obtained results demonstrate that the combined medicinal product "Kaliumcell" effectively corrects experimentally induced hypokalemia and contributes to the restoration of electrolyte balance, confirming its therapeutic potential for use in hypokalemia.

In parallel with the evaluation of the potassium-restoring effect, oxidative stress markers and antioxidant system parameters were analyzed. Administration of "Kaliumcell" resulted in the reduction in catalase activity (CAT), the increase in the level of advanced protein oxidation products (PPOA) and free thiol groups (SH). For the other investigated markers, no statistically significant differences were observed ($p>0.05$), suggesting modulation of some parameters of the redox status and a moderate antioxidant effect, without significant changes in the redox status.

GENERAL CONCLUSIONS

1. The analysis of the physiological role of the potassium ion in maintaining electrolyte homeostasis and the clinical implications of potassium deficiency on the cardiovascular, neuromuscular and renal systems justifies the need to develop a combined drug, capable of ensuring the effective and safe correction of potassium deficiency. Hypokalemia is one of the most common electrolyte disorders, often underdiagnosed but associated with severe complications, further emphasizing the need for modern, effective, and safe pharmaceutical formulations for the correction of this pathological condition.
2. The analysis of the pharmaceutical market for potassium-containing medicinal products and food supplements in the Republic of Moldova and other countries revealed the predominance of multicomponent preparations and the limited availability of combination medicinal products with

multiple mechanisms of action intended for the treatment of hypokalemia. A novel four-component medicinal combination containing potassium aspartate, magnesium aspartate, potassium orotate, and spironolactone was developed to provide potassium supplementation at both plasma and intracellular levels and to address the pathophysiological mechanisms responsible for potassium loss.

3. Following preformulation, compatibility and formulation studies, carried out by FTIR, DSC methods and evaluation of pharmacotechnical properties, the compatibility of the active principles with each other and with the selected excipients was demonstrated, as well as the possibility of their formulation in the form of dosed powder in sachets. 17 experimental models were prepared and investigated, and the M8, M13 and M17 formulations presented the best technological and biopharmaceutical parameters, being selected for further studies. The dissolution test demonstrated that the M8 and M13 formulations present the most favorable release profiles of the active substances and the best pharmaceutical availability *in vitro*.
4. The application of modern optimization methods such as: factorial design and the SeDeM method allowed the establishment of a technology for manufacturing combined powder dosed in sachets, under laboratory conditions, based on the principles of GMP and GLP. The significant influence of excipients on the flow properties and dissolution rate was demonstrated, establishing that increasing the concentration of stearic acid and microcrystalline cellulose reduces the release rate of active substances. The M13 formulation was identified as the closest to the optimal variant, ensuring a stable balance between pharmacotechnical and biopharmaceutical parameters.
5. The developed and validated analytical methods meet the validation requirements regarding specificity, linearity, precision, accuracy and robustness, confirming their applicability for quality control and standardization of the developed drug. Modern methods of analysis and standardization have been developed and validated, using chemical and physico-chemical methods. For potassium orotate and spironolactone, UV-Vis spectrophotometric and HPLC methods have been developed and validated, for magnesium aspartate - the complexometric method, and for potassium aspartate - the formol titration method, for magnesium and potassium in the combined powder - the AAS and AES methods.
6. Stability studies conducted under stress, accelerated and real-time conditions confirmed the physicochemical stability of the developed combined powder and allowed determining the shelf life of the product. It was found that the quality parameters of the active substances were maintained under the recommended storage conditions for 24 months, allowing the development of the Pharmacopoeia Monograph and the Technological Regulation for the tetracomponent combined medicinal product "Kaliumcell", constituting the necessary documentary basis for the standardization of the product.
7. Preclinical studies have demonstrated a favorable profile of safety and pharmacological efficacy in experimental models. The determination of acute toxicity revealed that the combined powder belongs to a low acute toxicity class, and the experimental evaluation of electrolyte balance confirmed the hypokalemia correction effect by significantly increasing the serum potassium concentration. At the same time, the moderate antioxidant capacity of the product was demonstrated, which completes the pharmacological profile and supports the therapeutic applicability of the developed combination.
8. The research results were capitalized by developing the Pharmacopoeia Monograph, the Technological Regulation, obtaining innovator certificates and implementing the results both in scientific and practical activity and in the university teaching process. Implementation documents were carried out at the Laboratory for Drug Development, Analysis and Standardization, at the Vasile Procopiu University Pharmaceutical Center, as well as at the SC "Balkan Pharmaceuticals" SRL, which confirms the applicative value, innovative character and real technological transfer prospects of the developed medicinal product.

PRACTICAL RECOMMENDATIONS

1. It is recommended to continue advanced preclinical pharmacological research and initiate clinical trials for the tetracomponent combined medicinal product "Kaliumcell", in order to fully evaluate the therapeutic efficacy and safety of administration in patients with hypokalemia.
2. The developed and validated analytical methods (HPLC, UV-Vis spectrophotometry, AAS, AES, complexometry and formalin titration) are recommended for implementation in drug quality control laboratories, in university teaching laboratories and in pharmaceutical research units for the standardization and analysis of potassium-containing combination drugs.
3. The Pharmacopoeia Monograph and Technological Regulations developed for the combined medicinal product "Kaliumcell" can serve as the basis for technological transfer to the local pharmaceutical producer and for initiating the industrial production process of the fixed-dose combination.
4. Research results are recommended for use in the university and postgraduate teaching process, especially in the disciplines of pharmaceutical chemistry, magistral pharmaceutical technology, drug analysis, drug development and pharmaceutical research.

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- **Patents, registration certificates, materials for invention fairs**
 1. **Mazur E**, Uncu L, Valica V, Diug E, Parii S. **Innovator certificate no. 6429 of 12.01.2026**. Preparation of combined powder containing potassium aspartate, magnesium aspartate, potassium orotate and

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ACTIVE PARTICIPATION IN SCIENTIFIC FORUMS

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✓ International

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ABSTRACT

Ecaterina Mazur. DEVELOPMENT OF A NEW COMBINED MEDICINAL PRODUCT FOR USE IN HYPOKALEMIA

PhD Thesis in Pharmaceuticals Scenes, Chisinau, 2026

Research relevance: Hypokalemia is one of the most common electrolyte disturbances encountered in clinical practice and is frequently associated with cardiovascular disorders. Potassium is involved in the generation of action potentials, nerve impulse transmission, and the contraction of cardiomyocytes, smooth muscle, and skeletal muscle.

Aim: The aim of the research was to develop, optimize, standardize, and preclinically evaluate a combined medicinal product in the form of an oral powder in unit-dose sachets intended for use in hypokalemia.

Objectives: The research objectives included the evaluation of the market for potassium-containing products; conducting compatibility, preformulation, formulation, and biopharmaceutical evaluation studies; developing the manufacturing technology; developing and validating analytical methods; conducting stability studies and establishing the shelf life; developing analytical and quality standardization documentation; and performing the preclinical evaluation of acute toxicity, the hypokalemia-correcting effect, and antioxidant capacity.

Scientific novelty and originality consist in the substantiation and development of a fixed-dose four-component combination comprising potassium aspartate, magnesium aspartate, potassium orotate, and spironolactone in a single pharmaceutical dosage form – a unit-dose oral powder. For the first time for this formulation, the compatibility of the components and the influence of excipients on the technological and biopharmaceutical properties were evaluated, and analytical methods adapted to the multicomponent matrix were developed.

Fundamentally new results for science and practice: The compatibility of the active substances and excipients was evaluated by FTIR and DSC. Seventeen experimental compositions were developed and evaluated by determining their technological parameters, applying the SeDeM methodology, and performing factorial optimization using Design-Expert. Biopharmaceutical performance was assessed by dissolution testing in media with different pH values. TLC, UV-Vis spectrophotometry, HPLC, AAS, AES, complexometric titration, and formol titration were used for the identification and assay of the components. The analytical methods were validated in accordance with ICH requirements. The preclinical studies included the evaluation of acute toxicity and efficacy in an experimental model of furosemide-induced hypokalemia. The optimal formulation was selected based on SeDeM evaluation, experimental design, and dissolution profiles. Chemical and instrumental methods for the assay of the components were developed and validated, including an HPLC method for the simultaneous determination of potassium orotate and spironolactone. Stability studies enabled the establishment of storage conditions and shelf life. The preclinical evaluation demonstrated a favorable safety profile and a significant effect.

Theoretical significance of the study: The research contributes to the development of the scientific basis for the development, formulation, and standardization of a combined medicinal product intended for the correction of hypokalemia. The study expands the existing knowledge regarding component compatibility, composition optimization, the manufacturing technology of unit-dose powder in sachets, and the development of analytical methods.

Practical value: is confirmed by the development and approval of the Pharmacopoeia Monograph and the Manufacturing Technological Regulation for the medicinal product “Kaliumcell”, the granting of innovator certificates, and the implementation of the results in scientific and practical activities, the pharmaceutical industry, and the university educational process.

Implementation of scientific results: The scientific results obtained were implemented through the development of the combined medicinal product in the form of a unit-dose powder in sachets, its standardization, and the development of analytical and regulatory documentation for quality standardization – the Pharmacopoeia Monograph and the Manufacturing Technological Regulation. The research results were implemented in the curricula of academic disciplines and in methodological guidelines intended for the educational process of students of the Faculty of Pharmacy, in the scientific and practical activities of the “Vasile Procopișin” University Pharmaceutical Center, as well as in the laboratories of the Center for Drug Development of Nicolae Testemițanu State University of Medicine and Pharmacy.

Structure of the thesis: The thesis comprises 141 pages of main text and includes an introduction, five chapters, general conclusions, practical recommendations, a bibliography containing 181 references, and 18 annexes. The illustrative material comprises 40 figures and 25 tables. The research results are reflected in 49 scientific and scientific-methodological publications, including 11 scientific articles and 38 abstracts, and were presented through 41 active participations in national and international scientific events.

Keywords: hypokalemia, combined medicinal product, oral powder, potassium aspartate, magnesium aspartate, potassium orotate, spironolactone, preformulation, optimization, standardization, HPLC, UV-Vis spectrophotometry, FTIR, DSC, stability, preclinical studies.