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ABSTRACT

of the dissertation for the degree
of Doctor of Philosophy

"DEVELOPMENT AND VALIDATION OF THE METHOD OF DETERMINATION OF KALINOL PLUS AND FAGOLIN WITH HIGH PERFORMANCE LIQUID CHROMATOGRAPHY"

Speciality:	3400.02 - Pharmaceutical chemistry, pharmacognosy
Field of science:	Pharmacy
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GENERAL CHARACTERISTICS OF THE WORK

Relevance and developing level of the topic

One of the important problems facing the science of pharmaceutical chemistry is the development of new and highly sensitive analytical methods for quality control of medicines, as well as the improvement of existing methods. In accordance with modern requirements of drug production, the application of validated analytical methods is important for both the development of new drugs and the control of their quality during use[1,2].

Analytical quality control of the raw materials and medicines is important in the system of Pharmaceutical Quality Assurance. Validation of the methods is carried out in order to ensure accurate and reliable results of the methods used for analytical control of implementation. Validation of analytical methods is carried out in quality control laboratories during both production and use of medicines[3].

Preperation of national standards on the quality of medicines on the basis of various methods, ensuring their effectiveness and safety[4]. By the use of different analytical methods, the final result on the quality of the medicine esentially depends on the reliability of the method. Special attention should be paid to the research, study and

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application of this field in our country as well. Validation of analytical methods is widely used in the production of medicines in the laboratories of research and quality control [4,5,6].

Validation of the analytical method is one of the important requirements in the documents of variety of international institutions and organizations regulating the production and use of medicines, including the principles of good manufacturing practice (GMP) and good laboratory practice (GLP)[7].

The effectiveness of drug analysis is associated with the use of modern physicochemical methods. Recently, chromatographic methods, including high performance liquid chromatography (HPLC), have assumed of great practical importance in pharmaceutical analysis. This method has important advantages such as high sensitivity, specificity and short-time analysis[8].

The main active ingredients in “Kalinol plus” are potassium bromide and thyme extract, and the main active ingredients in “Fagolin” are peanut oil and dexpanthenol. Currently for controling the quality of “Kalinol plus”, only analyzes are performed to determine the identity and quantity of potassium bromide, which is unsatisfactory. In the analysis of "Fagolin" product gas-liquid partition chromatography is used. It is important to develop a modern analytical method or to improve the method in use for the analysis of both drugs. Thus, the analysis of “Kalinol plus” and “Fagolin” products with high performance liquid chromatography and the validation of the method is prevailing [4,9].

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Object and subject of the research

“Kalinol plus” syrop (DQ: №15-00336) and “Fagolin” ointment (DQ: №11-00087) were used as the object of research. The subject of the study is the analysis of methods for determining the amount of drugs "Kalinol plus" and "Fagolin" by high-performance liquid chromatography.

The purpose and the tasks of the research

The purpose of the research was conducted for the development and validation of the method of determination of quantity of “Kalinol plus” and “Fagolin” products with high performance liquid chromatography.

The following **tasks** are planned to achieve this goal:

1. The study of optimal chromatographic conditions for the method of high performance liquid chromatography analysis of the main active components of "Kalinol plus" product;
2. To identify of optimal chromatographic parameters for the determination of the quantity of dexpanthenol in "Fagolin" product by high performance liquid chromatography-mass spectroscopy;
3. To develop the method for the determination of the quantity of thymol in "Kalinol plus" product by high performance liquid chromatography;
4. To improve the method of determining the quantity of dexpanthenol in "Fagolin" product by HPLC;
5. To identify the validation parameters of the method for the determination of thymol content in “Kalinol plus” by HPLC;
6. To assess the validation method of determination of the quantity of dexpanthenol in "Fagolin" product by HPLC-MS/MS.

Research methods. High-performance liquid chromatography-mass spectrometry/ mass spectrometry was used to determine the quantity of dexpanthenol in “Fagolin” preparation and high-performance liquid chromatography-ultraviolet was used to determine the quantity of thymol in “Kalinol plus” preparation.

Validation of the methods of quantification of the studied preparations by HPLC-MS/MS and HPLC-UV was carried out and

the obtained results were statistically processed by parametric and non-parametric methods.

The main provisions of the defense

- The thymol in “Kalinol plus” preparation was developed on the basis of optimal chromatographic conditions for the analysis of HPLC, the method for determining the quantity allows to control the quality of the product;
- An improved and developed method for determining the quantity of dexpanthenol by HPLC-MS/MS in "Fagolin" ointment allows to control quality of product during the production and use of it;
- The validation parameters of thymol in "Kalinol plus" preparation determined by the method of determining of the quantity by HPLC indicates that the method has accuracy, precision, specificity, linearity, system suitability and robustness;
- An assessment validation of the method for determining the quantity of dexpanthenol in "Fagolin" ointment by HPLC-MS/MS indicates that the method has accuracy, precision, specificity, linearity, system suitability and robustness.

The scientific novelty of the research

For the first time, optimal chromatographic conditions for the analysis of thymol in “Kalinol plus” preparation by HPLC were determined and a method for determining the quantity was developed.

Optimal conditions for the analysis of dexpanthenol with HPLC-MS/MS in the preparation "Fagolin", as well as the suitability of the chromatographic system were determined and a method for determining the amount was developed.

For the first time, the validation parameters of the method for determination of the quantity of thymol in “Kalinol plus” preparation by HPLC were determined and the method was found to have satisfactory accuracy, precision, specificity, linearity and durability.

For the first time, assessment validation of the method for determination of the quantity of dexpanthenol in “Fagolin” ointment

by HPLC-MS/MS was carried out and the method was found to have satisfactory accuracy, precision, specificity, linearity and durability.

A patent (I 2019 0090) was obtained for the method of determining the amount of dexpanthenol in “Fagolin” by HPLC.

Practical and theoretical significance of the research

The method for determining the quantity of thymol in “Kalinol plus” preparation by HPLC which was developed for the first time, allows to control its quality during the production and sale of the product. The proposed method is being used in the production of the product at the "Azerfarm" pharmaceutical plant, as well as in quality assessment procedures of product in the Analytical Expertise Center of the Ministry of Health of the Republic of Azerbaijan.

An improved method for determining the quantity of dexpanthenol by HPLC-MS/MS in "Fagolin" preparation is being used in quality assessment procedures of product at the "Azerfarm" pharmaceutical plant, as well as in the Analytical Expertise Center of the Ministry of Health of the Republic of Azerbaijan.

An assessment validation of the method for determining the quantity of thymol in "Kalinol plus" preparation by HPLC was carried out and the obtained results are being used in the production and State registration process of the product.

The validation indicators of the method for determining the quantity of dexpanthenol in "Fagolin" preparation by HPLC-MS/MS was determined and the obtained results are being used in the production and State registration process of the product.

The practical recommendations on the development of the method of determining the quantity of "Kalinol plus" and "Fagolin" products by HPLC are being used in the education of relevant pharmaceutical faculties at the Department of Pharmaceutical Chemistry, Azerbaijan Medical University.

Approbation and application

The results obtained in separate parts of the dissertation materials was presented I and II Science Festival of Azerbaijan Medical University (2014, 2016), at the final scientific conference “Actual problems of medicine” of Azerbaijan Medical University (2015), II International scientific and practical Internet-conference

“Analytical chemistry in pharmacy” (2016, Kharkov), XXIII Russian National Congress "Man and Medicine" (2016, Russia), “International scientific review of the problems and prospects of modern science and education” (2016, Boston/USA), Scientific-practical conference held at AMU on the occasion of meeting devoted to the "120th anniversary of Aziz Aliyev's birth" (2017), Actual Problems of Medicine" dedicated to the 25th anniversary of the restoration of the state independence of Azerbaijan (2017), Scientific-practical conference dedicated to the 100th anniversary of the Azerbaijan Democratic Republic (2018), II All-Russian Interuniversity GxP Summit with international participation “Choosing the best. Time forward" (2018, Sochi), III International Scientific and Practical Internet Conference (2018, Kharkiv), and at the international scientific-practical conference dedicated to the "100 th Anniversary of the founding of the Faculty of Medicine" of the Azerbaijan Medical University. Presented at the V International Scientific Congress of Pharmacists of Azerbaijan (2020) on "Modern problems of pharmacy".

21 scientific works (9 articles and 11 abstracts), 1 patent were published on the basis of dissertation materials.

Results the obtained were used in the teaching of relevant departments of medicines of the Department of Pharmaceutical Chemistry of the Azerbaijan Medical University, at the “Azerfarm” LLC factory and at the stage of state registration of medicines in the Analytical Expertise Center of the Ministry of Health of the Republic of Azerbaijan, as well as for the purpose of implementation of quality control of medicines.

Name of the organization where the dissertation work is performed.

The dissertation work was carried out at Department of Pharmaceutical Chemistry of Azerbaijan Medical University and in laboratories of the Analytical Expertise Center of the Ministry of Healthcare of the Republic of Azerbaijan.

Volume and structure of the dissertation.

The work consists of 187 pages compiled on a computer, introduction, literature review, research materials and methods, and 3

chapters of personal research, conclusions, results, practical recommendations, and literature indexes of 11 domestic and 223 foreign scholars, and 5 websites. The reserach work is illustarted with 45 tables, 28 figures and 32 formuls. The volume of the structural sections of the dissertation consists of introduction 11369 Chapter I 53762, Chapter II 30645, Chapter III 20828, Chapter IV 25702, Chapter V 34551, results 2473, final part 19784, practical recommendations 531 and the list of abbreviations consists of 1437 characters. The total volume of the dissertation contains 201092 signs.

MATERIALS AND METHODS OF RESEARCH

For satisfying dissertation's objectives during the determination of the quantity different reagents and equipment were used.

The quantity of dexpanthenol in "Fagolin" ointment was determined on chromatograph of Agilent 1100 series HPLC UV-detector (USA). A Zorbax SB-C18 (4.6 x 250 mm) column with a particle size of 5 μm was used as the stationary phase.

In the preparation of systems used in chromatography to determine the quantity of dexpanthenol in "Fagolin" ointment 85% orthophosphate acid water in a 9:1 ratio (Merck/Germany, serial number: K44422173316) and acetonitrile (HPLC Grade, Merck/Germany, serial number 1674930 308), dexpanthenol standard, 98.9% purity (USP/Rockville, reference standard, serial number: K0I270), matrix (Azerfarm LTD; east peanut oil, Vaseline oil, distilled monoglyceride, glycerin, nipagin, nipasol, purified water, aerosil), model mixture and blank solution were used.

The quantity of thymol in "Kalinol plus" syrup was determined on chromatograph of Agilent 1100 series HPLC UV-detector (USA). A Zorbax SB-C18 (4.6 x 250 mm) column with a particle size of 5 μm was used as the stationary phase.

In the preparation of systems used in chromatography to determine the quantity of thymol in "Kalinol plus" syrup 1: 1 ratio of water and acetonitrile (HPLC Grade, Merck/Germany, serial number:

I674930 308), thymol standard (KRKA/Slovenia, serial number: UG1456), acetone (HPLC Grade, Merck/Germany, serial number: I674930 308), matrix (sugar syrup, potassium bromide, ethyl alcohol of 80%), model mixed, moving phase was used.

A titration method was also used to determine the quantity of potassium bromide in “Kalinol plus” syrup. In titration, for the determination of potassium-bromide content in “Kalinol plus” syrup liquid nitric-acid, potassium-bromide standard (SigmaAldrich), 0.1 M silver-nitrate (Vecton), Ammonium-iron alum (Vecton), 0.5% potassium-permanganate (Ekpos), 0.1 M ammonium-rhodanide, model mixture (sugar syrup, thyme extract, ethyl alcohol of 80% and active substance (potassium bromide)), matrix (sugar syrup, thyme extract and ethyl alcohol of 80% (Azerfarm LTD) were used.

RESEARCH RESULTS AND THEIR DISCUSSION

Among medicines ointments have a leading position in medical practice in the treatment of purulent inflammatory disease of the skin. “Fagolin” ointment (State registration number: DV №11-00087) is used in the treatment of dermatitis, pyoderma caused by staphylococci, minor and moderate burns, soft tissue wounds, as well as postoperative wounds, difficult-to-heal skin transplants and skin cracks.

Ingredients of "Fagolin" ointment (100 g):

East peanut oil	– 3,0 g
Dexpanthenol	– 5,0 g
Vaseline oil	– 15,0 g
Emulsifier №1	– 8,0 g
Polyethylene oxide -400	– 30,0 g
Nipagin	– 0,1 g
Nipasol	– 0,2 g
Purified water	– till 100 g

"Fagolin" ointment is produced at "Azerfarm LTD" pharmaceutical plant and the analysis of fatty oils, one of the main active ingredients of the product, is carried out by gas-liquid

chromatography. One of the main requirements of local and international regulatory agencies at the stage of registration as well as re-registration of drugs, is to implement the validation of the analytical method. The validation of the analyzed analytical method is necessary for ensuring the accuracy and precision of the analysis results.

"Kalinol plus" syrup (State registration: №15-00336) is being produced at "Azerfarm LTD" pharmaceutical plant, and is used in medical practice as a expectorant in acute and chronic inflammation of the respiratory tract.

Ingredients of Kalinol plus syrup (100 q):

Thyme extract	– 12 g
Potassium bromide	– 1 g
Sugar syrup	– 82 g
Alcohol 80%	– 5 g

In accordance with the plan of the dissertation, in order to control the quality of Fagolin ointment and Kalinol plus syrup, research was carried out on the development of methods for their determination with HPLC.

In order to select the optimal method for determining the quantity of dexpanthenol in *"Fagolin"* ointment, studies were conducted on the volume of injection, temperature, flow rate and various parameters of the tube.

In order to select the optimal injection volume condition for the determination of quantity, different volumes were taken:10 μl, 20 μl, 30 μl, and the optimal peak area was considered optimal for observation at 20 μl.

In order to find temperature optimal condition, tests are carried out at different degrees: 28°C, 30°C, 32°C and the optimal peak area is considered optimal for observation at 30°C.

In order to select the optimal flow rate condition, different flow levels were taken: 0.8 ml/min, 1.0 ml/min, 1.2 ml/min and the optimal peak area was observed at 1.0 ml/min.

In order to select the optimal mobile phase condition, different ratios were taken: 50/950, 100/900, 150/850 and the optimal ratio was 100/900.

In order to develop the method, Zorbax Eclipse XDB-C18 (3.0 x 75 mm) with a particle size of 3.5 μm, Novapak C18 (3.9 x 150 mm) with a particle size of 4 μm and Zorbax SB-C18 were used to select the optimal column for chromatographic separation. (4.6 x 100 mm) particle size columns of 3.5 μm were used. However, the Zorbax SB-C18 (4.6 x 100 mm) particle size recorded a symmetrical peak when using a 3.5 μm speaker, which was considered optimal.

As a result of the research, it was determined that the injection volume was 20 μl, the temperature 30 °C, the flow rate 1.0 ml/min, mobile phase ratio 100/900 and the “Zorbax SB-C18” (4.6 x 250 mm) tube was optimal.

So, the suitability of the chromatographic system for the analysis of dexpanthenol in "Fagolin" product by HPLC-MS/MS was determined and a method for determining the quantity was developed. A patent has been obtained on the determination of the quantity of dexpanthenol in "Fagolin" product.

Taking the above mentioned into consideration, an assessment validation of the method for determining the quantity of dexpanthenol in “Fagolin” ointment by HPLC was carried out.

For the validation assessment, first of all, solutions of a standard sample of Fagolin ointment and dexpanthenol were prepared.

200 mg of a standard sample of dexpanthenol is added to a 100 ml volumetric flask, about 20 ml of the active phase is added to it and shaken until completely dissolved, then it is made up to volume with the moving phase. Take 5 ml of the standard solution and add it to a 100 ml flask, add about 20 ml of the moving phase and shake, then make up to volume with the moving phase to obtain a solution with a concentration of 0.1 mg / ml.

4.0 g of Fagolin ointment is added to a 100 ml volumetric flask, about 20 ml of the moving phase is added to it and shaken until it is dissolved, then it is made up to volume with the moving phase. Degassed in an ultrasonic bath for 15 minutes. Take 5 ml of the solution and add it to a 100 ml volumetric flask, add about 20 ml of the active phase, then make up to volume with the mobile phase and obtain a solution with a concentration of 0.1 mg / ml. The resulting

solution is filtered through a fluoroplastic filter with a pore size of 0.45 μm.

An assessment validation of the method of determining the quantity of dexpanthenol in “Fagolin” ointment by HPLC was carried out, and specificity, accuracy, precision, linearity, system compatibility, limit of detection, detection limit and stability were determined. The studied method is characterized by high efficiency and specificity. The accuracy limits range of the method is from 99.28% to 100.89%, the coefficient of variation is 1.04, and the standard deviation is up to 1.05; standard deviation of accuracy index 0.41%, coefficient of variation 0.41, reliability interval of average value ± 0.43; the linearity index is 0.06-0.14, correlation coefficient in viscosity interval range 0.99, angle coefficient is 10703, and coefficient of cut-off point is 86.981. Durability indicators: flow rate 1ml/min; moving phase 90:10 ratio; temperature 30 °C; injection volume of 20 μl is considered optimal for observation. It was also determined that the detection limit of dexpanthenol is 0.000025 mg/ml, limit of detection is 0.0001 mg/ ml, the relative compatibility of the peak area of the system compatibility is not more than 2, the relative balance of the peak area is 1.06, and the number of theoretical layers is 13747.

For the first time, assessment validation of the method for determination of the quantity of dexpanthenol in “Fagolin” ointment by HPLC-MS/MS was carried out and the method was found to have satisfactory accuracy, precision, specificity, linearity and durability.

The method of determination of quantity of “Kalinol plus” was carried out by chemical method – by titrimetry method according to potaccium-bromide. The novelty of the method developed by us is that the standardization of the product was carried out as per thymol by HPLC, which is the main active ingredient of thyme extract. Thyme extract itself has a multi-component composition. There are no analogues of the product. The product is being produced in Ukraine and Russia and titrimetry method is being used to control quality of it.

Gas chromatography, spectrophotometry, GC/MS methods are also widely used for the analysis of thymol or carvacrol in essential oils derived from plants. Homewer, we analyzed thymol by HPLC/

In order to select the optimal method for determining the quantity of thymol in "Kalinol plus" syrup, studies were conducted on the volume of injection, temperature, flow rate and various parameters of the tube.

In order to select the optimal injection volume condition for the determination of quantity, different volumes were taken:10 μl, 8μl, 12 μl, and the optimal peak area was considered optimal for observation at 10 μl.

In order to find temperature optimal condition, tests are carried out at different degrees: 27°C, 30°C, 33°C and the optimal peak area is considered optimal for observation at 30°C.

In order to select the optimal flow rate condition, different flow levels were taken: 0.8 ml/min, 1.0 ml/min, 1.2 ml/min and the optimal peak area was observed at 1.0 ml/min.

In order to select the optimal mobile phase condition, different ratios were taken: 47/53, 50/50, 53/47 and the optimal ratio was 50/50.

In order to develop the method, Zorbax Eclipse XDB-C18 (3.0 x 75 mm) with a particle size of 3.5 μm, Novapak C18 (3.9 x 150 mm) with a particle size of 4 μm and Zorbax SB-C18 were selected to select the optimal column for chromatographic separation. (4.6 x 250 mm) particle size 5 μm columns were used. However, the Zorbax SB-C18 (4.6 x 250 mm) particle size recorded a symmetrical peak when using a 5 μm column, which was considered optimal.

As a result of the research, it was determined that the injection volume was 10 μl, the temperature 30 °C, the flow rate 1.0 ml/min, mobile phase ratio 50/50 and the “Zorbax SB-C18” (4.6 x 250 mm) tube was optimal.

For the first time, the optimal chromatographic conditions for the analysis of thymol in “Kalinol plus” product by HPLC were determined and a method for determining the quantity was developed

An assessment validation of the method of determining the quantity of thymol in “Kalinol plus” syrup by HPLC was carried out,

and specificity, accuracy, precision, linearity, range of application and system compatibility were determined. The studied method is characterized by high efficiency and specificity. The accuracy limits range of the method is from 98.06% to 100.77%, the coefficient of variation is 0.99, and the standard deviation is up to 0.99; standard deviation of accuracy index 0.85%, coefficient of variation 0.85, reliability interval of average value (p = 95%) ± 0.89; the linearity index is 0,0032-0,0048, correlation coefficient in viscosity interval range 0.99, angle coefficient is 7318.3, and coefficient of cut-off point is 1.5767. Including durability indicators: flow rate 1ml/min; moving phase 50:50 ratio; temperature 30 °C; injection volume of 10 µl is considered optimal for observation. Also the relative compatibility of the peak area of the system compatibility is not more than 2, the relative balance of the peak area is 1.03, and the number of theoretical layers is 20199.

For the first time, the validation parameters of the method for determination of the quantity of thymol in “Kalinol plus” product by HPLC were determined and the method was found to have satisfactory accuracy, precision, specificity, linearity and durability

The main active ingredients in “Kalinol plus” product are thyme extract and potassium bromide. Until the present, for controling the quality of product, only the method of determination of quantity of potassium bromide was carried out. Therefore, an assessment validation of the method of determination of potassium bromide titration in “Kalinol plus” syrup was carried out.

An assessment validation of the method of determination of potassium bromide by titrimetry in “Kalinol plus” syrup was carried out and the precision (102.83 ± 0.95%, 104.53 ± 0.95%), the accuracy (0.49%), linearity (0.08-0.12 mg/ml) and range of application (80% -20%) were identified. The studied method is characterized by specificity and allows to control the quality of "Kalinol plus" syrup during the production and sale of it.

As part of an assessment of the validation parameters of the analytical method validation protocol should be developed. The validation protocol defines how the process of the analytical method is carried out and the parameters given for the test, as well as shows

how the assessment will be carried out by covering the moment of decision that made on confirmation of acceptance of test results.

The protocol is a comprehensive form of instruction which describes the details of a case planned to investigate a new procedure.

The validation protocol describes the system of work with validation documents and the scope of validation work. Documents collected during the qualification are collected in folders and registered under the name "Validation Protocols".

The validation protocol consists of the following annexes:

- list of persons involved in the validation work (indicating their degree of responsibility),
- Copies of used equipment verification / calibration certificates,
- research procedures (methodologies),
- table of research results, deviation pages and content of deviation pages

According to GMP rules, it is necessary to use a series in the protocol that contains all the necessary information related to the testing process. The copies of the approved protocols on the series should be attached to the validation protocol

The validation protocol should be numbered, signed and dated. The protocol should also include other information - objectives, validation staff, type of validation (prospective, direct, retrospective, re-validation), the list of all equipment to be used. As well as, the calibration requirements for all measuring instruments, the copies of the main documentations of product, statistical parameters used in the analysis data, specifications, forms and diagrams used to document the results should be included.

In accordance with the dissertation work plan, a validation protocol of the method of determining the quantity of dexpanthenol in "Fagolin" product by HPLC was prepared.

The main purpose of the protocol was to determine the validation indicators of the method of determining the quantity of dexpanthenol in "Fagolin" ointment by high-performance liquid chromatography

All equipment and devices have been qualified and tested before to use for validation of the analytical method.

The head of the analytical laboratory plans the research, controls conducting of research, checks the completeness of the working documents. Before starting validation studies the "Quality Control Unit" checks and confirms the accuracy of the protocol layout, as well as checks the information specified in the validation act of method and gives a positive opinion.

Important analytical effect parameters (specificity, accuracy, precision, linearity, system compatibility, range of application and sustainability) are reflected in the protocol.

The describtion and principles of analytical process, materials, equipment and parameters of equipment and the main stages of the method were given in the protocol.

Validation results are calculated with % and decimal systems/

Criteria for acceptance of specificity - the peak area of dexpanthenol should not appear in the blank solution and matrix, for the peak area of dexpanthenol - the purity angle <purity limit, purity index> the purity limit should be followed; criteria for acceptance of linearity - correlation and determination coefficient should be ≥0.95, angle coefficient should be specified, cross-section coefficient should be 100% ± 2% of the standard area; criteria of acceptance for accuracy - recovery rate and average value for each concentration should be calculated individually, the sample analyzed at each level should be between 95% and 105%, the relative standard deviation should not exceed 2% for each concentration; criteria of acceptance for precision - the average value (in%) and the variation coefficient of 6 samples prepared from the standard solution should be calculated, the standard deviation of 6 analyzed samples should be calculated, the reliability interval should be 95%±5%; criteria of acceptance for the stability indicator - relative standard deviation should not exceed 2%, standard deviation should be calculated; criteria of acceptance for the system compliance indicator - chromatograms of the solution prepared from "Fagolin" product and dexpanthenol standard are taken 6 straight times and the relative standard deviation of the average value of the peak area should be calculated (≤2.0%), the number of theoretical

layers of peaks taken from the “Fagolin” product and dexpanthenol standards should be ≥2000, the relative balance of the peak area taken from the “Fagolin” product and dexpanthenol standards should be ≤ 2.0.

In accordance with the dissertation work plan, a report was prepared on the validation of the method of determining the quantity of thymol in "Kalinol plus" product by HPLC.

The validation report is a document covering the assessment of the validation, results, collected and summarized protocols. Suggestions for the improving of the process or equipment also included here.

The validation report includes the approved validation protocol, the results of the tables or graphs, the process procedure, and all the analytical results of the validation indicators.

The validation report includes the name and purpose of the work, reference to the protocol, details of the material, equipment, programs used, details of the procedure and test methods, results (compared to the acceptance criteria).

A validation report on the method of the determining of quantity of thymol in “Kalinol plus” product by HPLC has been prepared.

The validation report summarizes the results of the research, describes the deviations identified as a result of the research (see the “notes” section in the deviation pages and protocols) and the results of their elimination. The validation process for production conditions and technical processes was carried out in the pilot-scale batch of the product. During the experiment, separate tests were conducted for the criteria of acceptance of the parameters of the analytical method.

The validation report contains information on the implementation of the process, evaluation, deviation parameters, preparation and approval of the validation act.

Criteria for acceptance of specificity was as follows:

a. The peak area of dexpanthenol did not appear in the blank solution and matrix;
b. for the peak area of dexpanthenol - the purity angle <purity limit, purity index> the purity limit was followed

Criteria for acceptance of linearity was as follows:

- correlation and determination coefficient was ≥ 0.95
- angle coefficient was 7318.3;
- cross-section coefficient of standard area was 1.5767.

Criteria of acceptance for accuracy indicators was as follows:

- limits of accuracy indicators were 98.06% - 100.77%;
- Relative standard deviation was 0.99%.

Criteria of acceptance for precision was as follows:

- Average value of 6 samples prepared from the standard solution calculated as 100.42%, and coefficient of variation as 0.85%;
- the standard deviation of 6 analyzed samples calculated as 0.85%;
- The confidence interval was 0.89% within 95%±5%.

Criteria of acceptance for the stability indicator is as follows:

- The relative standard deviation did not exceed 2% on each indicator;
- The standard deviation is also calculated for each indicator

Criteria of acceptance for the system compliance indicator is as follows:

- chromatograms of the solution prepared from “Kalinol plus” product and thymol standard were taken 6 straight times and the relative standard deviation of the average value of the peak area was calculated as 0.69% and 0.39%;
- the number of theoretical layers of peaks taken from the “Kalinol plus” product and thymol standard was 20199 and 19948 within the interval of ≥ 2000;
- the relative balance of the peak area taken from the “Kalinol plus” product and thymol standard was 1.03 and 0.99 within the interval of ≤ 2.0;

The method for determining the quantity of thymol in “Kalinol plus” product by HPLC which was developed for the first time, allows to control its quality during the production and sale of the product. The proposed method is being used in the production of the product at the "Azerfarm" pharmaceutical plant, as well as in quality assessment procedures of product in the Analytical Expertise Center of the Ministry of Health of the Republic of Azerbaijan.

An improved method for determining the quantity of dexpanthenol by HPLC-MS/MSin "Fagolin" product is being used in quality assessment procedures of product at the "Azerfarm LTD" pharmaceutical plant, as well as in the Analytical Expertise Center of the Ministry of Health of AR.

An assessment validation of the method for determining the quantity of thymol in "Kalinol plus" product by HPLC was carried out and the obtained results are being used in the production and State registration process of the product.

The validation indicators of the method for determining the quantity of dexpanthenol in "Fagolin" product by HPLC-MS/MSwas determined and the obtained results are being used in the production and State registration process of the product.

The practical recommendations on the development of the method of determining the quantity of "Kalinol plus" and "Fagolin" products by HPLC are being used in the education of appropriate pharmaceutical faculties at the Department of Pharmaceutical Chemistry of Azerbaijan Medical University

RESULTS

1. In order to develop a method for determining the quantity of thymol by high-performance liquid chromatography in "Kalinol plus" product optimal chromatographic conditions were prepared and it was determined that the flow rate was 1 ml/min, injection volume 10 μl, temperature 30°C, wave length 274 nm, mobile phase 50:50 ratios and the “Zorbax SB-C18” (4.6 x 250 mm) tube was optimal [6;7].
2. For the first time, a method for determining the quantity of thymol by high-performance liquid chromatography in “Kalinol plus” product was developed. The method is characterized by high selectivity, optimal chromatographic separation, as well as with efficiency [3;8;17].
3. Optimal condition was selected for the method of determination of the improved quantity of dexpanthenol by HPLC-MS/MS in

“Fagolin” product and it was determined that the flow rate was 1 ml/min, injection volume 20 µl, temperature 30°C, wave length 206 nm, mobile phase 9:1 ratio and the “Zorbax SB- C18” (4.6 x 250 mm) tube was optimal [12;15].

4. An improved method for determining the quantity of dexpanthenol by HPLC-MS/MSin "Fagolin" product was developed. The method is characterized by high selectivity and sensitivity, allows to control its quality during the production and use of the product [1;2].
5. For the first time the validation of high-performance liquid chromatography of thymol, as well as the determination of potassium bromide by titrimetry in “Kalinol plus” were carried out. The accuracy limits range of the HPLC method is from 98.06% to 100.77%, the coefficient of variation is 0.99, and the standard deviation is up to 0.99; standard deviation of accuracy index 0.85%, coefficient of variation 0.85, reliability interval of average value (p = 95%) ± 0.89; the linearity index is 0,0032-0,0048, correlation coefficient in viscosity interval range 0.99, angle coefficient is 7318.3, and coefficient of cut-off point is 1.5767. It was also determined that the relative compatibility of the peak area of the system compatibility should not exceed 2, the relative balance of the peak area is 1.03, and the number of theoretical layers is 20199. The method has optimal durability indicators [3;6].
6. For the first time, assessment validation of the method for determining the quantity of dexpanthenol in high-performance liquid chromatography in "Fagolin" product was conducted and it was determined that the method has high specificity and effectiveness. The accuracy limits range of the method is from 99.28% to 100.89%, the coefficient of variation is 1.04, and the standard deviation is up to 1.05; standard deviation of accuracy index 0.41%, coefficient of variation 0.41, reliability interval of average value (p = 95%) ± 0.43; the linearity index is 0.06-0.14, correlation coefficient in viscosity interval range 0.99, angle coefficient is 10703, and coefficient of cut-off point is -86.981. It was also determined that the detection limit of dexpanthenol is

0.000025 mg/ml, limit of detection is 0.0001 mg / ml, the relative compatibility of the peak area of the system compatibility is not more than 2, the relative balance of the peak area is 1.06, and the number of theoretical layers is 13747. The method has optimal durability indicators [1;2;5;17].

PRACTICAL RECOMMENDATIONS

1. The method of analysis of dexpanthenol by HPLC in "Fagolin" ointment can be used in the analysis of other medicines and food supplements containing dexpanthenol.
2. The method of analysis of thymol by HPLC in “Kalinol plus” syrup can be used in the development of various medicines containing essential oils, including thymol.
3. The results of the validation of analytical methods for the quantification of *Fagolin* and *Kalinol plus* with YEMX can be used in the validation of analytical methods for other drugs with similar composition.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANSI	- American National Standards Institute
AMU	- Azerbaijan Medical University
ICH	- International Council for Harmonisation
DD	- Diode detector
EPA	- Environmental Protection Agency
EOS	- Essential oil supplies
RP-HPLC	- Reversed-phase high performance liquid chromatography
FDA	- Food and Drug Administration
CFR	- Code of Federal Regulations
GC/MS	- Gas chromatography mass spectrometry
GLP	- Good laboratory practice
GMP	- Good manufacturing practice
ICH	- International Council for Harmonisation
IQ	- Instalation qualification
IUPAC	- International Union of Pure and Applied Chemistry
IUPAC/WELAC	- Western European Laboratory Accreditation Conference
GC	- Gas chromatography
GC/MS	- Gas chromatography / mass spectrometry
LC	- Liquid chromatography
LC-MS/MS	- Liquid chromatography - mass spectrometry / mass spectrometry
ES-MC	- Negative ion electrospray-mass spectrometry
EQI	- Essential quality indicators
LOD	- Limit of detection
OQ	- Operation qualification
PQCP	- Process quality control protocol
CPP	- Critical process parameters
RIM	- Reference Information Model
RP-HPLC	- Reversed-phase high-performance liquid chromatography
SPL	- Structured product labeling

DL	- Detection Limit
UHPLC–MC/MC	- Ultra high performance liquid chromatography mass spectrometry / mass spectrometry
USP	- United States Pharmacopeia
VASEDLLMHPLC	- Vortex-assisted surfactant-enhanced dispersive liquid-liquid microextraction - High-performance liquid chromatography
VMP	- Validation Master Plan
WHO	- World Health Organization
HPTLC	- High-performance thin-layer chromatography
HPLC-MS/MS	- High-performance liquid chromatography mass spectrometry/mass spectrometry
HPLC-RI	- High-performance liquid chromatography-refractive index
HPLC-UV	- High-performance liquid chromatography-ultraviolet

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