European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 03, 2020

Quantitive Determination And Validation Of Cobafen (Lyophilizate 10mg For Preparation Of Solution For Injection)

Nilufar K. Abdullaeva[1], Raykhona A. Khusainova[2], Nilufar M. Rizaeva[3], Egor A. Pshenichnov[4]

[1,2,3,4]*Department of Pharmaceutical Chemistry, Tashkent Pharmaceutical Institute, Tashkent, Uzbekistan.*

Email: info@pharmi.uz

ABSTRACT

Research objective: to conduct a quantitative determination of sodium diclofenac and vitamin B-12 in new lyophilic drug Cobafen using modern physicochemical methods of analysis.

Materials and methods. During the research we used the samples of commercially available substances of mecobalamin chloride produced by Apex Medichem Ltd. (India), D-mannitol manufactured by Shandong Tianli Pharm. Co. Ltd. (China) and polyvinyl pyrrolidone (PVP) produced by Merck (Germany), as well as chemical reagents by Sigma-Aldrich (USA) and Hi-Media (India). For spectrophotometry analysis we applied a UV-1800 double-beam spectrophotometer (Shimadzu, Japan) and Agilent 8453E single-beam spectrophotometer (Agilent Technologies, Germany). Besides, the study was carried out under conditions of reverse phase HPLC (Agilent 1280 gradient HPLC chromatograph (Agilent Technologies, USA) and LC-20 (Shimadzu, Japan)).

Results. Conducted 5 independent experiments for each formulation and each freezing mode showed that D-mannitol is the additive agent of choice. Besides, lyophilizates produced using D-mannitol corresponded to the indicated quality attribute. When analyzing mecobalamin by HPLC, it was established that the method of choice was PP with methanol: buffer solution (26.5:73.5), the optimal concentration in the analysis of mecobalamin is 2 mg / ml.

Conclusion. It was established that sharp freezing condition is the most preferred due to the saving in production cycle time. The proposed HPLC method for quantitative determination of the active substance in the drug, validated by all validation parameters, is included in the pharmacopoeial monographs of JURABEK LABORATORIES JV LLC.

Key-words: Cobafen, quantitative determination, validation, lyophilizates, freezing.

1. INTRODUCTION

Liophilisation, also known as freeze drying method, is widely used in the pharmaceutical industry for stabilization and improvement of long-term storage stability of drugs, especially in the production of injectable dosage forms. The stability of the drug molecule is a key factor which impacts on the choice of drug form by the manufacturer: injectable drug in liquid or powder form. According to the research results, it was established that the molecules have a different structure and physicochemical properties. Depending on

this property, the scientists distinguish the injection forms into those containing large or small molecules. Non-biological drugs such as Omeprazole, Pantoprazole, Vancomycin, etc., belong to the small molecule category, and Rituximab, Etanercept, etc. are referred to the category of large molecules. Vaccines such as DTP, TABT vaccine (Typhoid), varicella vaccine are classified as large molecules category; as well as probiotics - lactic acid bacteria, bifidobacteria are also referred to large molecules.

In 2016, the US Food and Drug Administration (FDA) approved 104 injectable drugs, of which 43 were in the form of lyophilizate, including the NDA, BLA and ANDA categories. It can be assumed that these approved drugs are replicated or will be replicated around the world. Thus, in the future, lyophilized injections will become promising, and every manufacturing company using basic lyophilization method will achieve meaningful results [5].

At present time, drugs based on cyanocobalamin metabolites - Methylcobalamin and Mecobalamin are widely used in medical practice [1,2]. They present chemically active substances that enter the biochemical processes of the body's vital activity without initial metabolic transformations and have a more pronounced therapeutic effect [3].

However, the high reactivity of these substances allots a task for specialists to determine the optimal dosage form with a minimum of stabilizers and preservatives, which will preserve the therapeutic effect of the vitamin B12 metabolite.

One of these promising manufacturers is JURABEK LABORATORIES JV LLC (Uzbekistan). Today the company produces a lyophilic drug Cobafen, the main components of which are sodium diclofenac and cyanocobalamin. The studies showed that the combination of these medicinal substances with different mechanisms of action can increase the effectiveness of the treatment.

Currently pain is considered a serious factor in the quality of life. In particular, the problem of back pain is extremely urgent, since most of the population throughout the world suffer from it in different periods of their lives. Moreover, 70% of population at least once in life lose the ability to work for this reason. The peak of complaints of back pain falls on the prime of life - middle working age from 30 to 45. In most cases the treatment includes the administration of nonsteroidal anti-inflammatory drugs (NSAIDs) diclofenac sodium, which is a pathogenetic agent for acute and chronic pain associated with tissue damage and inflammation. Vitamin B 12 participates in a number of intracellular processes, providing the health of cells and the production of sufficient energy for the healthy nervous system [5].

RESEARCH OBJECTIVE

The aim of this paper is a quantitative determination of sodium diclofenac and vitamin B-12 in the new lyophilic drug Cobafen using modern physicochemical methods of analysis.

2. MATERIALS AND METHODS

The object of the research is Cobafen, lyophilizate 10 mg for the preparation of solution for injection in vials (with a solvent).

During the investigation we used the samples of commercially available substances of mecobalamin chloride produced by Apex Medichem Ltd. (India), D-mannitol manufactured by Shandong Tianli Pharm. Co. Ltd. (China) and polyvinyl pyrrolidone (PVP) produced by Merck (Germany), as well as chemical reagents by Sigma-Aldrich (USA) and Hi-Media (India). We also applied a UV-1800 double-beam spectrophotometer (Shimadzu, Japan) and Agilent 8453E single-beam spectrophotometer (Agilent Technologies, Germany). Spectra were determined in the wavelength range from 200 to 760 nm. The appropriate solvent was used as a compensation solution.

Besides, the study was carried out under conditions of reverse phase HPLC. We used Agilent 1280 gradient HPLC chromatograph (Agilent Technologies, USA) and LC-20 (Shimadzu, Japan). Column Zorbax (Agilent Technologies, USA) C18 (250 mm x 4.6 mm, 5 μm), Zorbax guard column (Agilent Technologies, USA) (14 mm x 4.6 mm, 5 μm). Column temperature 25 °C. The Table 20 shows the chromatographic conditions.

3. RESULTS AND DISCUSSION

*Preparation of samples for freezing***:** solutions in the amount of 1 ml were poured into 5 ml glass vials (glass class: HC-1) produced by Neo-Tech Plast (Republic of Uzbekistan). Lyophilization of samples and sealing of vials with bromine-butyl stoppers with combined aluminum caps was performed on the automated conveyor equipment Tofflon (China).
Conditions for lyophilization: the lyophilic dryer shelves were kept at -50 °C and pressure in chamber 0.01 mbar during 5 hours. The shelves were heated in the following modes:
- from –50 ° C to –10 ° C at the rate 10 °C/h;
- from -10 ° C to 0 ° C at the rate 3 ° C/h;
- from 0 ° C to +25 ° C at the rate 5 ° C/h.

After the drug reached the temperature +25 ° C (the pressure in the chamber - 0.01 mbar), it was kept at this temperature for 4 hours. In general lyophilization lasted 22 hours.
Physicochemical testing of the finished product and the quantitative determination of the active substance were determined in the chemical laboratory of the Quality Control Department of the pharmaceutical plant JURABEK LABORATORIES JV LLC (Republic of Uzbekistan) in accordance with the requirements of the European Pharmacopoeia [4].
*The appearance o*f the lyophilizate was determined visually.
The solubility of the lyophilizate was determined visually, recording the dissolution time with a stopwatch. A sufficient amount of water for injection was added to the weighed sample of the substance (1 g) or to the contents of the vial, and the mixture was shaken for 3 min.
Determination of weight loss on drying was carried out by drying the preparation in a vacuum drying oven over P_2O_5 at room temperature and residual pressure of 5 mm Hg. to constant weight. The weight loss in all studied samples did not exceed 3.0%.
The clarity of the solution was determined visually comparing the test liquid with the solvent.
The pH of the aqueous solution of the preparation was determined potentiometrically using a Mettler Toledo instrument (USA).
Quantitative determination of mecobalamin was performed by UV spectrophotometry (SP) and HPLC [6,7]
SP method. Despite the fact that to date the leading foreign pharmacopoeias usually apply IR spectroscopy and chromatographic methods to identify substances and preparations, the UV spectrophotometry is also widely used in pharmacopoeial analysis. For substances with good chromophores, this method allows to conduct the analysis on "identification" and "quantitative determination", and in some cases it can also be used in purity analysis by absorption at certain wavelengths. The complex conjugated system in the structure of mecobalamin allows to widely use UV spectrophotometry for the pharmacopoeial analysis.
The determination is performed by the method of absorption spectrophotometry in the UV area.
The optical density of the test solution and work standard of mecobalamin solution was measured on the spectrophotometer, wavelength 351 nm in a cuvette with a layer thickness of 10 mm, using a solvent as the reference solution.
The content of mecobalamin in one vial in percentages of the LC (X) is calculated by the formula:

$$X = \frac{D_1 * m_0 * 5 * 100 * 100 * P * G}{D_0 * m_1 * 100 * 25 * 100 * L} = \frac{D_1 * m_0 * P * G}{D_0 * m_1 * 5 * L}$$

where

D_1 - the optical density of the test solution;

D_0 - the optical density of the work standard of mecobalamin solution;

M_1 - weighed sample of the drug, mg;

M_0 - weighed sample of the mecobalamin work standard, mg;

P - the content of mecobalamin in the mecobalamin work standard, %;

G - the average mass of the vial contents, mg;

L - the label claim of mecobalamin in one vial, mg.

The drug must contain at least 90.0% and not more than 110.0% of the label clay of mecobalamin.

Note. *Solvent*. 10.9 g sodium acetate trihydrate in a 1000 ml volumetric flask is added 8 ml of glacial acetic acid. The mixture is dissolved and diluted to the mark with injection water.

Test solution. About 100 mg of the drug in a 100 ml volumetric flask is diluted with the solvent up to the mark. 25.0 ml of the resulting solution is taken to the 100 ml volumetric flask and diluted with the solvent to the mark.

Standard sample solution. About 50 mg (accurately weighed) of mecobalamin work standard in a 100 ml volumetric flask is dissolved in the same solvent to the mark. 5 ml of the resulting solution is placed into a 100 ml volumetric flask and dissolved with the solvent to the mark.

Quantitative determination of mecobalamin by HPLC. In modern pharmacopoeial analysis, the HPLC method is widely applied for identification, analysis of purity and quantitative determination of drugs. Besides, this method is used in the analysis of substances and medicinal preparations of vitamin B-12. In the pharmacopoeial analysis of mecobalamin, various sample preparation techniques, chromatographic conditions, and different eligibility criteria of the chromatographic system are used.

Quantative determination of mecobalamin using HPLC.

Buffer solution. 10 g of di-sodium hydrogen phosphate monosubstituted (Na_2HP0_4) is dissolved in 800 ml of injection water, the pH is adjusted to 3.5 with orthophosphoric acid, placed in a 1000 ml volumetric flask and diluted with water to the mark with.

Mobile phase. Methanol: buffer solution (26.5:73.5).

Test solution. About 400 mg of the drug is placed in a 100 ml volumetric flask, dissolved in the mobile phase and the volume is brought up to the mark with the same solvent.

Reference solution. About 40 mg of mecobalamin work standard is placed in a 100 ml volumetric flask, dissolved in the mobile phase and the volume of the solution is brought to the mark with the same solvent.

Chromatographic conditions:

Column	25.0 x 0.46 cm, Silica gel for chromatography, 5 μm;
Column temperature	35 ° C;
Flow rate	1.0 ml / min;
Spectrophotometric detector,	361 nm;
Sample volume	20 μL.

The content of mecobalamin in the drug in percentage (X) is calculated by the formula:

$$X = \frac{S_1 * m_0 * 100 * P * G}{S_0 * m_1 * 100 * L} = \frac{D_1 * m_0 * P * G}{D_0 * m_1 * L}$$

where

S_1 - the average value of the peak areas of mecobalamin, obtained from the test solution chromatograms;
S_0 - the average value of the areas peak of mecobalamin, obtained from mecobalamin work standard chromatograms;
M_1 - the sample of the drug, mg;
M_0 - weighted sample of mecobalamin work standard, mg;
P - the content of mecobalamin in mecobalamin work standard, %;
G - the average mass of one vial contents, mg;
L - the LC of mecobalamin in one vial, mg.
The drug must contain min. 90.0% and max. 110.0% of the label clay of mecobalamin.

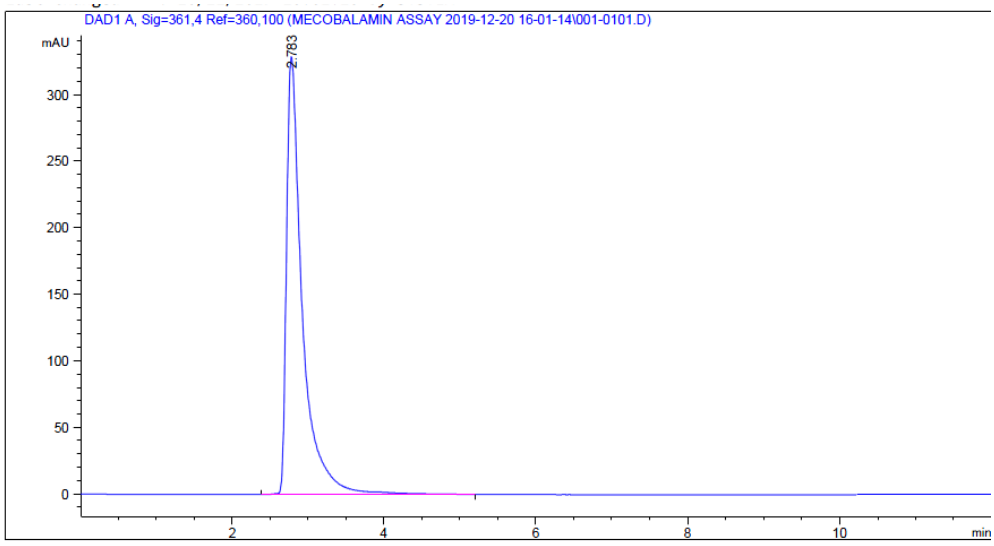


Fig. 1. Chromatogram of mecobalamin standard solution

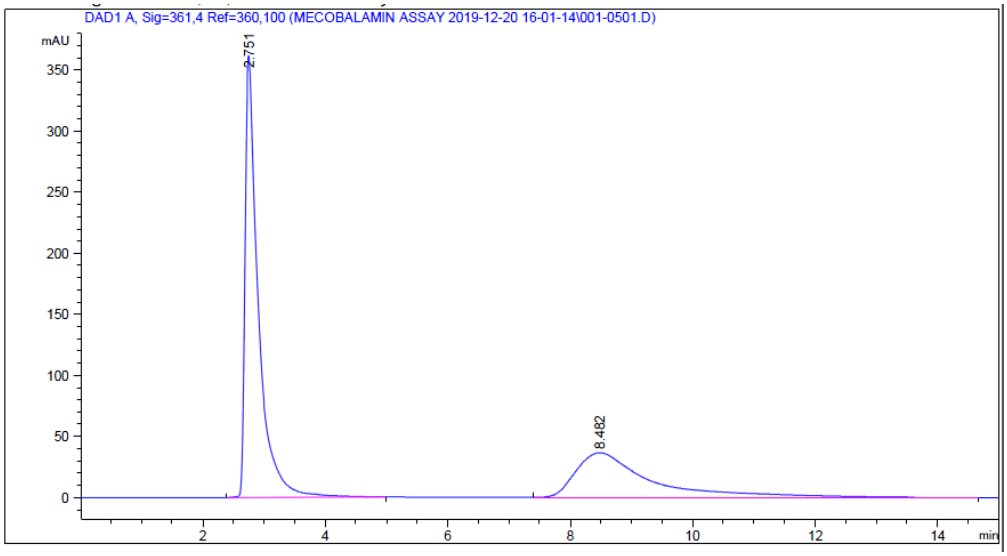


Fig. 2. Chromatogram of mecobalamin test solution in the drug Cobafen

Analytical method validation for the quantitative determination of methylcobalamin in Cobafen, lyophilizate 10 mg for the preparation of a solution for injection.
Analytical method validation is the experimental proof that the method is applicable for its intended purpose.

This paper regulates the characteristics of analytical methods, determined for validation, as well as corresponding criteria for the applicability of validated technologies intended for quality control of drugs and pharmaceutical substances.

Quantitative determination methods, including methods for determining impurities and limit concentration, are subject to validation. Identification methods of drugs are subjected to validation if it is necessary to confirm their specificity.

Validation assesses the analytical method according to the characteristics listed below, selected according to the typical recommendations given in the table:

- specificity;
- detection limit;
- quantitation limit;
- analytical area (range);
- linearity;
- trueness;
- precision;
- robustness.

The specificity of the method is determined by comparing the values of the active ingredient in the analysis of solvent, placebo and drug.

Table 1

Validation parameter	Acceptance criteria
Specificity	Neither the solvents, nor the reagents used in sample preparation, nor the placebo components should not distort the results.
Linearity	Correlation coefficient ≥0.99
Within-lab precision	t≤ 2.228 F≤ 5.05
Trueness	Response factor: average 97.5 - 102.5%. Coefficient of Variance ≤ 2.0%. Confidence interval should include 100% of values.

Besides, we conducted analysis and measurement of the placebo (reference medicinal product, mixture of all drug ingredients without active ingredient), active ingredient and drug. The Table 2 presents the data obtained:

Table 2

Sample	Optical density
Placebo	0,000
Active ingredient (101 mg / 100ml)	0,512
Medicinal product	0,506

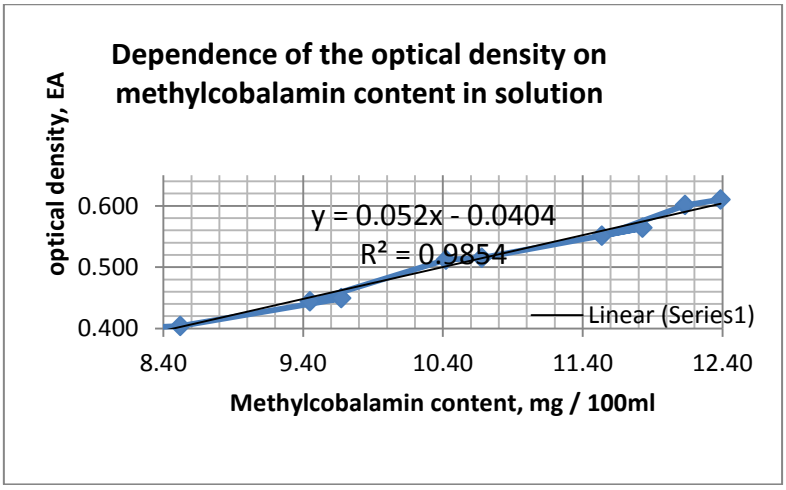
The linearity of the method was determined on min. 5 different dilutions of the test solution in the application range of 80 - 120% concentration of the analyte in the test solution.

We prepared and carried out measurements of drug solutions with the active substance concentration in the interval from 80 to 120%: 2 solutions with the active substance concentration 80%, 2 solutions with the active substance concentration 90%, 2 solutions with the active substance concentration 100%, 2 solutions with the active substance concentration 110% and 2 solutions with the active substance concentration 120%.

Table 3

№	Concentration level,%	Weighted sample, mg	Sod. methylcobalamin in weighted sample, mg	Y (Optical density, EA)
1	80	98,8	8,3200	0,402
2	80	101,2	8,5221	0,404
3	90	103,2	9,6750	0,449
4	90	100,8	9,4500	0,444
5	100	101,1	10,4227	0,512
6	100	103,6	10,6804	0,515
7	110	105,4	11,8306	0,564
8	110	102,8	11,5388	0,551
9	120	100,10	12,1333	0,601
10	120	102,2	12,3879	0,610
average			10,4961	0,5052

Based on the data obtained, the dependence of the optical density on the concentration of methylcobalamin in the solution was determined. The program also draws a trend line and determines the regression equation.



Precision is carried out by testing min. 6 prepared samples at 100% concentration of the active substance in the test solution.

6 solutions of the drug at 100% concentration of the active substance were prepared and taken measurements. The results are shown in the Table 4:

Analyte No. 1. Weighted sample of St 50.2mg, G 100mg, Optical density St 0.504EA

Table 4

No. of sample	Weighted sample, mg	Optical density, EA	Specified content of methylcobalamin%	Xi-Xav	$(Xi-Xav)^2$
1	100,4	0,491	97,4	0,15143348	2,29E-02
2	102,6	0,501	97,3	0,00407434	1,66E-05
3	105,1	0,509	96,5	-0,79343384	6,30E-01

4	100,8	0,491	97,0	-0,23515634	5,53E-02
5	102,9	0,503	97,4	0,10766314	1,16E-02
6	101,6	0,500	98,0	0,76541923	5,86E-01
			$\overline{X} = 97{,}27$		Σ 1,31E+00

Table 5

Analyte No. 2. Weighed sample of St 51.3mg G 101mg, Optical density St 0.513EA

No. of sample	Weighted sample, mg	Optical density, EA	Specified content of methylcobalamin%	Xi-Xav	$(Xi-Xav)^2$
1	101,3	0,495	98,71	0,283252	8,02E-02
2	98,6	0,482	98,75	0,322891	1,04E-01
3	100,4	0,491	98,79	0,363293	1,32E-01
4	103,1	0,504	98,75	0,323288	1,05E-01
5	102,8	0,496	97,46	-0,960525	9,23E-01
6	100,7	0,489	98,09	-0,332199	1,10E-01
		$\overline{X} = 98{,}42$		Σ 1,45E+00	

Table 6

Statistical characteristics,%	Results Analyte No. 1	Results Analyte No. 2
Minimum value	96,5	97,46
Maximum value	98,0	98,79
Mean value	97,3	98,42
Standard deviation	0,511	0,539
Coefficient of Variance	0,525	0,548
The lower limit of confidence interval (P = 95%)	96,7	97,86
The upper limit of confidence interval (P = 95%)	97,8	98,99
$F(95\% f_1=N_1-1;f_2=N_2-1) \leq 5.05$	1,11	
$t_{kp}(95\%$ and N1+N2-k=10) ≤ 2.228	1,660	
Minimum value	96,5	97,46

Note: No statistically significant differences

The trueness of the method determines the response of the active substance in the placebo. The placebo concentration is 100% of the sample specified in the control method. The active substance is added to the model mixture in accordance with the required concentration level (lower limit - max. 80, 100%; upper limit - min. 120%).

We prepared and carried out measurements of drug solutions with the active substance concentration in the interval from 80 to 120%: 3 solutions with the active ingredient concentration 80%, 3 solutions with the active ingredient concentration 90%, 3 solutions with

the active ingredient concentration 100%, 3 solutions with the active ingredient concentration substances 110% and 3 solutions with the active substance concentration 120%.

Weighted sample St 50.2mg G 100mg, Optical density St 0.504 EA
Table 7

Concentration level, %	Weighed sample of methylcobalamin, mg	Sod. methylcobalamin in the sample	Optical density, EA	Determination of content methylcobalamin, %	Specified content of methylcobalamin,%	Response ,%
80%	98,8	8,3200	0,402	81,1	80,0	101,32
80%	101,2	8,5221	0,404	79,5	80,0	99,41
80%	100,1	8,4295	0,402	80,0	80,0	100,00
100%	101,1	10,4227	0,512	100,9	100,0	100,88
100%	103,6	10,6804	0,515	99,0	100,0	99,03
100%	99,10	10,2165	0,497	99,9	100,0	99,90
120%	100,10	12,1333	0,601	119,6	120,0	99,67
120%	102,2	12,3879	0,610	118,9	120,0	99,08
120%	100,8	12,2182	0,608	120,2	120,0	100,13
Mean value						99,94

Table 8
Statistical processing of the results

Statistical characteristics, %	Results
Mean value	99,94
Standard deviation	0,771
Coefficient of Variance	0,77
Lower limit of the confidence interval (P = 95%)	99,34
Upper limit of the confidence interval (P = 95%)	100,53

4. CONCLUSIONS

1. Obtained data of 5 independent tests for each formulation and each mode of freezing showed that D-mannitol the additive agent of choice.
2. Lyophilizates using D-mannitol as a cryoprotector at concentrations 0.080; 0.100; 0.120 g/vial corresponded to the indicated quality attribute. Lyophilizate reconstitution time containing 0.080 g/vial of additive agent was minimum. In this regard, a composition of hydroxocobalamin chloride with D-mannitol at concentration of 0.080 g/vial is proposed for further research.
3. The freezing condition does not influence on the quality of the lyophilizate. Sharp freezing condition is most preferred due to the saving in production cycle time.
4. UV spectrometric method has been developed to determine the "Quantification" indicator of the drug Cobafen.
5. When analyzing mecobalamin by HPLC, it was established that the method of choice was PP with methanol: buffer solution (26.5:73.5), the optimal concentration in the analysis of mecobalamin is 2 mg / ml.

6. The proposed HPLC method for quantitative determination of the active substance in the drug, validated by all validation parameters, is included in the pharmacopoeial monographs of JURABEK LABORATORIES JV LLC.

REFERENCES

[1] Kant, N., Saralch, S., & Singh, H. (2011). Ponderomotive self-focusing of a short laser pulse under a plasma density ramp. *Nukleonika*, *56*, 149-153.
[2] Patyar, S., & Patyar, R. R. (2015). Correlation between sleep duration and risk of stroke. *Journal of Stroke and Cerebrovascular Diseases*, *24*(5), 905-911.
[3] Khamparia, A., & Pandey, B. (2015). Knowledge and intelligent computing methods in e-learning. *International Journal of technology enhanced learning*, *7*(3), 221-242.
[4] Singh, A., Lin, Y., Quraishi, M. A., Olasunkanmi, L. O., Fayemi, O. E., Sasikumar, Y., ... & Kabanda, M. M. (2015). Porphyrins as corrosion inhibitors for N80 Steel in 3.5% NaCl solution: Electrochemical, quantum chemical, QSAR and Monte Carlo simulations studies. *Molecules*, *20*(8), 15122-15146.
[5] Singh, S., Kumar, V., Upadhyay, N., Singh, J., Singla, S., & Datta, S. (2017). Efficient biodegradation of acephate by Pseudomonas pseudoalcaligenes PS-5 in the presence and absence of heavy metal ions [Cu (II) and Fe (III)], and humic acid. *3 Biotech*, *7*(4), 262.
[6] Mia, M., Singh, G., Gupta, M. K., & Sharma, V. S. (2018). Influence of Ranque-Hilsch vortex tube and nitrogen gas assisted MQL in precision turning of Al 6061-T6. *Precision Engineering*, *53*, 289-299.
[7] Prakash, C., Singh, S., Pabla, B. S., & Uddin, M. S. (2018). Synthesis, characterization, corrosion and bioactivity investigation of nano-HA coating deposited on biodegradable Mg-Zn-Mn alloy. *Surface and Coatings Technology*, *346*, 9-18.
[8] Feng, X., Sureda, A., Jafari, S., Memariani, Z., Tewari, D., Annunziata, G., ... & Sychrová, A. (2019). Berberine in cardiovascular and metabolic diseases: from mechanisms to therapeutics. *Theranostics*, *9*(7), 1923.
[9] Bashir, S., Sharma, V., Lgaz, H., Chung, I. M., Singh, A., & Kumar, A. (2018). The inhibition action of analgin on the corrosion of mild steel in acidic medium: A combined theoretical and experimental approach. *Journal of Molecular Liquids*, *263*, 454-462.
[10] Sidhu, G. K., Singh, S., Kumar, V., Dhanjal, D. S., Datta, S., & Singh, J. (2019). Toxicity, monitoring and biodegradation of organophosphate pesticides: a review. *Critical Reviews in Environmental Science and Technology*, *49*(13), 1135-1187.
[11] Nanda, V., & Kant, N. (2014). Enhanced relativistic self-focusing of Hermite-cosh-Gaussian laser beam in plasma under density transition. *Physics of Plasmas*, *21*(4), 042101.
[12] Kotla, N. G., Gulati, M., Singh, S. K., & Shivapooja, A. (2014). Facts, fallacies and future of dissolution testing of polysaccharide based colon-specific drug delivery. *Journal of Controlled Release*, *178*, 55-62.
[13] Farooq, R., & Shankar, R. (2016). Role of structural equation modeling in scale development. *Journal of Advances in Management Research*.
[14] Singh, S., Ramakrishna, S., & Gupta, M. K. (2017). Towards zero waste manufacturing: A multidisciplinary review. *Journal of cleaner production*, *168*, 1230-1243.
[15] Mahla, S. K., Dhir, A., Gill, K. J., Cho, H. M., Lim, H. C., & Chauhan, B. S. (2018). Influence of EGR on the simultaneous reduction of NOx-smoke emissions trade-off under CNG-biodiesel dual fuel engine. *Energy*, *152*, 303-312.

[16] Nanda, V., Kant, N., & Wani, M. A. (2013). Self-focusing of a Hermite-cosh Gaussian laser beam in a magnetoplasma with ramp density profile. *Physics of Plasmas*, *20*(11), 113109.
[17] Kaur, P., Singh, S. K., Garg, V., Gulati, M., & Vaidya, Y. (2015). Optimization of spray drying process for formulation of solid dispersion containing polypeptide-k powder through quality by design approach. *Powder Technology*, *284*, 1-11.
[18] Sharma, D., & Saharan, B. S. (2016). Functional characterization of biomedical potential of biosurfactant produced by Lactobacillus helveticus. *Biotechnology Reports*, *11*, 27-35.
[19] Wani, A. B., Chadar, H., Wani, A. H., Singh, S., & Upadhyay, N. (2017). Salicylic acid to decrease plant stress. *Environmental Chemistry Letters*, *15*(1), 101-123.
[20] Mishra, V., Patil, A., Thakur, S., & Kesharwani, P. (2018). Carbon dots: emerging theranostic nanoarchitectures. *Drug discovery today*, *23*(6), 1219-1232.
[21] Kumar, V., Pitale, S. S., Mishra, V., Nagpure, I. M., Biggs, M. M., Ntwaeaborwa, O. M., & Swart, H. C. (2010). Luminescence investigations of Ce3+ doped CaS nanophosphors. *Journal of alloys and compounds*, *492*(1-2), L8-L12.
[22] Pudake, R. N., Swaminathan, S., Sahu, B. B., Leandro, L. F., & Bhattacharyya, M. K. (2013). Investigation of the Fusariumvirguliformefvtox1 mutants revealed that the FvTox1 toxin is involved in foliar sudden death syndrome development in soybean. *Current genetics*, *59*(3), 107-117.
[23] Kapoor, B., Singh, S. K., Gulati, M., Gupta, R., & Vaidya, Y. (2014). Application of liposomes in treatment of rheumatoid arthritis: quo vadis. *The scientific world Journal*, *2014*.
[24] Haldhar, R., Prasad, D., & Saxena, A. (2018). Myristica fragrans extract as an eco-friendly corrosion inhibitor for mild steel in 0.5 M H2SO4 solution. *Journal of Environmental Chemical Engineering*, *6*(2), 2290-2301.
[25] Bordoloi, N., Sharma, A., Nautiyal, H., & Goel, V. (2018). An intense review on the latest advancements of Earth Air Heat Exchangers. *Renewable and Sustainable Energy Reviews*, *89*, 261-280.
[26] Sharma, P., Mehta, M., Dhanjal, D. S., Kaur, S., Gupta, G., Singh, H., ... & Chellappan, D. K. (2019). Emerging trends in the novel drug delivery approaches for the treatment of lung cancer. *Chemico-biological interactions*, *309*, 108720.
[27] Goga, G., Chauhan, B. S., Mahla, S. K., & Cho, H. M. (2019). Performance and emission characteristics of diesel engine fueled with rice bran biodiesel and n-butanol. *Energy Reports*, *5*, 78-83.
[28] Umashankar, M. S., Sachdeva, R. K., & Gulati, M. (2010). Aquasomes: a promising carrier for peptides and protein delivery. *Nanomedicine: Nanotechnology, Biology and Medicine*, *6*(3), 419-426.
[29] Sharma, A., Shree, V., & Nautiyal, H. (2012). Life cycle environmental assessment of an educational building in Northern India: A case study. *Sustainable Cities and Society*, *4*, 22-28.
[30] Kaur, T., Kumar, S., Bhat, B. H., Want, B., & Srivastava, A. K. (2015). Effect on dielectric, magnetic, optical and structural properties of Nd–Co substituted barium hexaferrite nanoparticles. *Applied Physics A*, *119*(4), 1531-1540.
[31] Datta, S., Singh, J., Singh, S., & Singh, J. (2016). Earthworms, pesticides and sustainable agriculture: a review. *Environmental Science and Pollution Research*, *23*(9), 8227-8243.
[32] Vij, S., & Bedi, H. S. (2016). Are subjective business performance measures justified?. *International Journal of Productivity and Performance Management*.

[33] Chawla, R., & Sharma, S. (2017). Molecular dynamics simulation of carbon nanotube pull-out from polyethylene matrix. *Composites Science and Technology*, *144*, 169-177.
[34] Prakash, C., & Uddin, M. S. (2017). Surface modification of β-phase Ti implant by hydroaxyapatite mixed electric discharge machining to enhance the corrosion resistance and in-vitro bioactivity. *Surface and Coatings Technology*, *326*, 134-145.
[35] Saxena, A., Prasad, D., & Haldhar, R. (2018). Investigation of corrosion inhibition effect and adsorption activities of Cuscuta reflexa extract for mild steel in 0.5 M H2SO4. *Bioelectrochemistry*, *124*, 156-164.
[36] Prabhakar, P. K., Kumar, A., & Doble, M. (2014). Combination therapy: a new strategy to manage diabetes and its complications. *Phytomedicine*, *21*(2), 123-130.
[37] Wheeler, K. C., Jena, M. K., Pradhan, B. S., Nayak, N., Das, S., Hsu, C. D., ... & Nayak, N. R. (2018). VEGF may contribute to macrophage recruitment and M2 polarization in the decidua. *PLoS One*, *13*(1), e0191040.
[38] Singh, A., Lin, Y., Ansari, K. R., Quraishi, M. A., Ebenso, E. E., Chen, S., & Liu, W. (2015). Electrochemical and surface studies of some Porphines as corrosion inhibitor for J55 steel in sweet corrosion environment. *Applied Surface Science*, *359*, 331-339.
[39] Gill, J. P. K., Sethi, N., Mohan, A., Datta, S., & Girdhar, M. (2018). Glyphosate toxicity for animals. *Environmental Chemistry Letters*, *16*(2), 401-426.
[40] Kumar, V., Singh, S., Singh, J., & Upadhyay, N. (2015). Potential of plant growth promoting traits by bacteria isolated from heavy metal contaminated soils. *Bulletin of environmental contamination and toxicology*, *94*(6), 807-814.
[41] Patel, S. (2012). Potential of fruit and vegetable wastes as novel biosorbents: summarizing the recent studies. *Reviews in Environmental Science and Bio/Technology*, *11*(4), 365-380.
[42] Srivastava, G., Das, C. K., Das, A., Singh, S. K., Roy, M., Kim, H., ... & Philip, D. (2014). Seed treatment with iron pyrite (FeS 2) nanoparticles increases the production of spinach. *RSC Advances*, *4*(102), 58495-58504.
[43] Nagpal, R., Behare, P. V., Kumar, M., Mohania, D., Yadav, M., Jain, S., ... & Henry, C. J. K. (2012). Milk, milk products, and disease free health: an updated overview. *Critical reviews in food science and nutrition*, *52*(4), 321-333.
[44] Vaid, S. K., Kumar, B., Sharma, A., Shukla, A. K., & Srivastava, P. C. (2014). Effect of Zn solubilizing bacteria on growth promotion and Zn nutrition of rice. *Journal of soil science and plant nutrition*, *14*(4), 889-910.
[45] Lin, Y., Singh, A., Ebenso, E. E., Wu, Y., Zhu, C., & Zhu, H. (2015). Effect of poly (methyl methacrylate-co-N-vinyl-2-pyrrolidone) polymer on J55 steel corrosion in 3.5% NaCl solution saturated with CO2. *Journal of the Taiwan Institute of Chemical Engineers*, *46*, 214-222.
[46] Mahesh, K. V., Singh, S. K., & Gulati, M. (2014). A comparative study of top-down and bottom-up approaches for the preparation of nanosuspensions of glipizide. *Powder technology*, *256*, 436-449.
[47] Singh, G., Gupta, M. K., Mia, M., & Sharma, V. S. (2018). Modeling and optimization of tool wear in MQL-assisted milling of Inconel 718 superalloy using evolutionary techniques. *The International Journal of Advanced Manufacturing Technology*, *97*(1-4), 481-494.
[48] Chauhan, C. C., Kagdi, A. R., Jotania, R. B., Upadhyay, A., Sandhu, C. S., Shirsath, S. E., & Meena, S. S. (2018). Structural, magnetic and dielectric properties of Co-Zr substituted M-type calcium hexagonal ferrite nanoparticles in the presence of α-Fe2O3 phase. *Ceramics International*, *44*(15), 17812-17823.

[49] Sharma, A., Shahzad, B., Kumar, V., Kohli, S. K., Sidhu, G. P. S., Bali, A. S., ... & Zheng, B. (2019). Phytohormones regulate accumulation of osmolytes under abiotic stress. *Biomolecules*, *9*(7), 285.

[50] Balakumar, P., Chakkarwar, V. A., Kumar, V., Jain, A., Reddy, J., & Singh, M. (2008). Experimental models for nephropathy. *Journal of the Renin-Angiotensin-Aldosterone System*, *9*(4), 189-195.

[51] Singh, A., Lin, Y., Liu, W., Kuanhai, D., Pan, J., Huang, B., ... & Zeng, D. (2014). A study on the inhibition of N80 steel in 3.5% NaCl solution saturated with CO2 by fruit extract of Gingko biloba. *Journal of the Taiwan Institute of Chemical Engineers*, *45*(4), 1918-1926.

[52] Kaur, T., Kaur, B., Bhat, B. H., Kumar, S., & Srivastava, A. K. (2015). Effect of calcination temperature on microstructure, dielectric, magnetic and optical properties of Ba0. 7La0. 3Fe11. 7Co0. 3O19 hexaferrites. *Physica B: Condensed Matter*, *456*, 206-212.

[53] Singh, P., Singh, A., & Quraishi, M. A. (2016). Thiopyrimidine derivatives as new and effective corrosion inhibitors for mild steel in hydrochloric acid: Electrochemical and quantum chemical studies. *Journal of the Taiwan Institute of Chemical Engineers*, *60*, 588-601.

[54] Anand, A., Patience, A. A., Sharma, N., & Khurana, N. (2017). The present and future of pharmacotherapy of Alzheimer's disease: A comprehensive review. *European journal of pharmacology*, *815*, 364-375.

[55] Saxena, A., Prasad, D., Haldhar, R., Singh, G., & Kumar, A. (2018). Use of Sida cordifolia extract as green corrosion inhibitor for mild steel in 0.5 M H2SO4. *Journal of environmental chemical engineering*, *6*(1), 694-700.

[56] Ahmadi, M. H., Ghazvini, M., Sadeghzadeh, M., Alhuyi Nazari, M., Kumar, R., Naeimi, A., & Ming, T. (2018). Solar power technology for electricity generation: A critical review. *Energy Science & Engineering*, *6*(5), 340-361.

[57] Kant, N., Wani, M. A., & Kumar, A. (2012). Self-focusing of Hermite–Gaussian laser beams in plasma under plasma density ramp. *Optics Communications*, *285*(21-22), 4483-4487.

[58] Gupta, V. K., Sethi, B., Upadhyay, N., Kumar, S., Singh, R., & Singh, L. P. (2011). Iron (III) selective electrode based on S-methyl N-(methylcarbamoyloxy) thioacetimidate as a sensing material. *Int. J. Electrochem. Sci*, *6*, 650-663.

[59] Mehta, C. M., Srivastava, R., Arora, S., & Sharma, A. K. (2016). Impact assessment of silver nanoparticles on plant growth and soil bacterial diversity. *3 Biotech*, *6*(2), 254.

[60] Gupta, V. K., Guo, C., Canever, M., Yim, H. R., Sraw, G. K., & Liu, M. (2014). Institutional environment for entrepreneurship in rapidly emerging major economies: the case of Brazil, China, India, and Korea. *International Entrepreneurship and Management Journal*, *10*(2), 367-384.

[61] Singh, A., Lin, Y., Obot, I. B., Ebenso, E. E., Ansari, K. R., & Quraishi, M. A. (2015). Corrosion mitigation of J55 steel in 3.5% NaCl solution by a macrocyclic inhibitor. *Applied Surface Science*, *356*, 341-347.

[62] Ansari, K. R., Quraishi, M. A., Singh, A., Ramkumar, S., & Obote, I. B. (2016). Corrosion inhibition of N80 steel in 15% HCl by pyrazolone derivatives: electrochemical, surface and quantum chemical studies. *RSC advances*, *6*(29), 24130-24141.

[63] Jnawali, P., Kumar, V., & Tanwar, B. (2016). Celiac disease: Overview and considerations for development of gluten-free foods. *Food Science and Human Wellness*, *5*(4), 169-176.

[64] Saggu, S., Sakeran, M. I., Zidan, N., Tousson, E., Mohan, A., & Rehman, H. (2014). Ameliorating effect of chicory (Chichorium intybus L.) fruit extract against 4-tert-octylphenol induced liver injury and oxidative stress in male rats. *Food and chemical toxicology*, *72*, 138-146.

[65] Bhatia, A., Singh, B., Raza, K., Wadhwa, S., & Katare, O. P. (2013). Tamoxifen-loaded lecithin organogel (LO) for topical application: development, optimization and characterization. *International Journal of Pharmaceutics*, *444*(1-2), 47-59.

[66] Singh, A., Lin, Y., Liu, W., Yu, S., Pan, J., Ren, C., & Kuanhai, D. (2014). Plant derived cationic dye as an effective corrosion inhibitor for 7075 aluminum alloy in 3.5% NaCl solution. *Journal of Industrial and Engineering Chemistry*, *20*(6), 4276-4285.

[67] Raza, K., Thotakura, N., Kumar, P., Joshi, M., Bhushan, S., Bhatia, A., ... & Katare, O. P. (2015). C60-fullerenes for delivery of docetaxel to breast cancer cells: a promising approach for enhanced efficacy and better pharmacokinetic profile. *International journal of pharmaceutics*, *495*(1), 551-559.

[68] Prabhakar, P. K., Prasad, R., Ali, S., & Doble, M. (2013). Synergistic interaction of ferulic acid with commercial hypoglycemic drugs in streptozotocin induced diabetic rats. *Phytomedicine*, *20*(6), 488-494.

[69] Chaudhary, A., & Singh, S. S. (2012, September). Lung cancer detection on CT images by using image processing. In *2012 International Conference on Computing Sciences* (pp. 142-146). IEEE.

[70] Mishra, V., Bansal, K. K., Verma, A., Yadav, N., Thakur, S., Sudhakar, K., & Rosenholm, J. M. (2018). Solid lipid nanoparticles: Emerging colloidal nano drug delivery systems. *Pharmaceutics*, *10*(4), 191.

[71] Singh, A. (2012). Hydroxyapatite, a biomaterial: its chemical synthesis, characterization and study of biocompatibility prepared from shell of garden snail, Helix aspersa. *Bulletin of Materials Science*, *35*(6), 1031-1038.

[72] Arora, S., & Anand, P. (2019). Binary butterfly optimization approaches for feature selection. *Expert Systems with Applications*, *116*, 147-160.

[73] Chhikara, N., Kushwaha, K., Sharma, P., Gat, Y., & Panghal, A. (2019). Bioactive compounds of beetroot and utilization in food processing industry: A critical review. *Food Chemistry*, *272*, 192-200.

[74] Singh, S., Kumar, V., Chauhan, A., Datta, S., Wani, A. B., Singh, N., & Singh, J. (2018). Toxicity, degradation and analysis of the herbicide atrazine. *Environmental chemistry letters*, *16*(1), 211-237.

[75] Baranwal, T., & Pateriya, P. K. (2016, January). Development of IoT based smart security and monitoring devices for agriculture. In *2016 6th International Conference-Cloud System and Big Data Engineering (Confluence)* (pp. 597-602). IEEE.

[76] Trukhanov, S. V., Trukhanov, A. V., Salem, M. M., Trukhanova, E. L., Panina, L. V., Kostishyn, V. G., ... & Sivakov, V. (2018). Preparation and investigation of structure, magnetic and dielectric properties of (BaFe11. 9Al0. 1O19) 1-x-(BaTiO3) x bicomponent ceramics. *Ceramics International*, *44*(17), 21295-21302.

[77] Singh, S., Singh, N., Kumar, V., Datta, S., Wani, A. B., Singh, D., ... & Singh, J. (2016). Toxicity, monitoring and biodegradation of the fungicide carbendazim. *Environmental chemistry letters*, *14*(3), 317-329.

[78] Bhyan, B., Jangra, S., Kaur, M., & Singh, H. (2011). Orally fast dissolving films: innovations in formulation and technology. *Int J Pharm Sci Rev Res*, *9*(2), 9-15.

[79] Saxena, A., Prasad, D., Haldhar, R., Singh, G., & Kumar, A. (2018). Use of Saraca ashoka extract as green corrosion inhibitor for mild steel in 0.5 M H2SO4. *Journal of Molecular Liquids*, *258*, 89-97.

[80] Panghal, A., Janghu, S., Virkar, K., Gat, Y., Kumar, V., & Chhikara, N. (2018). Potential non-dairy probiotic products–A healthy approach. *Food bioscience*, *21*, 80-89.
[81] Kumar, D., Agarwal, G., Tripathi, B., Vyas, D., & Kulshrestha, V. (2009). Characterization of PbS nanoparticles synthesized by chemical bath deposition. *Journal of Alloys and Compounds*, *484*(1-2), 463-466.
[82] Ansari, K. R., Quraishi, M. A., & Singh, A. (2015). Corrosion inhibition of mild steel in hydrochloric acid by some pyridine derivatives: an experimental and quantum chemical study. *Journal of Industrial and Engineering Chemistry*, *25*, 89-98.
[83] Singh, P. S., Singh, T., & Kaur, P. (2008). Variation of energy absorption buildup factors with incident photon energy and penetration depth for some commonly used solvents. *Annals of Nuclear Energy*, *35*(6), 1093-1097.
[84] Ansari, K. R., Quraishi, M. A., & Singh, A. (2015). Isatin derivatives as a non-toxic corrosion inhibitor for mild steel in 20% H2SO4. *Corrosion Science*, *95*, 62-70.
[85] Singh, A., Lin, Y., Ebenso, E. E., Liu, W., Pan, J., & Huang, B. (2015). Gingko biloba fruit extract as an eco-friendly corrosion inhibitor for J55 steel in CO2 saturated 3.5% NaCl solution. *Journal of Industrial and Engineering Chemistry*, *24*, 219-228.
[86] Dey, A., Bhattacharya, R., Mukherjee, A., & Pandey, D. K. (2017). Natural products against Alzheimer's disease: Pharmaco-therapeutics and biotechnological interventions. *Biotechnology Advances*, *35*(2), 178-216.
[87] Ansari, K. R., Quraishi, M. A., & Singh, A. (2015). Pyridine derivatives as corrosion inhibitors for N80 steel in 15% HCl: Electrochemical, surface and quantum chemical studies. *Measurement*, *76*, 136-147.
[88] Patel, S. (2012). Threats, management and envisaged utilizations of aquatic weed Eichhornia crassipes: an overview. *Reviews in Environmental Science and Bio/Technology*, *11*(3), 249-259.
[89] Mia, M., Gupta, M. K., Singh, G., Królczyk, G., & Pimenov, D. Y. (2018). An approach to cleaner production for machining hardened steel using different cooling-lubrication conditions. *Journal of Cleaner Production*, *187*, 1069-1081.
[90] Kondrateva T.S. Biopharmaceutical studies of children's suppositories with phosphothiamine. Pharmacy.-Moscow, 1990.-No.5.-P.14-15.
[91] Maksudova F.Kh., Karieva E.S., Tursunova M.Kh. Study of the pharmacological properties of the combined gel of sodium diclofenac and benzketozone ./Infection, immunity and pharmacologists I.- Tashkent.-2015.-№5.C.160-163 /
[92] Maksudova F. Kh., Karieva E. S. In vitro equivalence evaluationce of dic-
lofenac sodium generic medicinal preparation. // Pharmacy, a scientific and practical journal, special issue, St. Petersburg, 2016, pp. 461-464.
[93] Piotrovsky V.K. Model and model-independent methods for describing pharmacokinetics: advantages, disadvantages and interrelation. // Antibiotics and medical biotechnology. -Moscow, 1997.-№7.P.492-497.
[94] Kukes V.G., Sychev D.A. Clinical pharmacology. 5th ed ., Moscow, 2017, p. 478.
[95] Tillaeva U. M., Azizov U. M. Development of a methodology for isolating the amount of fensulcal determination from a biological object. Materials of the scientific-practical conference "Actual issues of education, science and production in pharmacy. Tashkent, 2009.-P.172 .
[96] Tillaeva U.M. Standardization and quality control of fensulcal in soft dosage forms. // Authors' dissertation for the study of the academician of the candidate of pharmaceuticals. Sciences . Tashkent. 2011.23 s.
[97] Golovkin V.A. On the importance of pharmacokinetics modeling for increasing the efficiency of biopharmaceutical research. // Optimization of drug supply and ways to

increase the effectiveness of pharmaceutical science : Sat. Tez.dokl.-Kharkov, 1986.-P.61-62.

[98] Stefanova A.V. Preclinical studies of medicines. Kiev. -2002. -650 p.

[99] Andersson, H.C. and Shapira, E. Biochemical and clinical response to hydroxocobalamin versus cyanocobalamin treatment in patients with methylmalonicacidemia and homocystinuria (cblC). // Journal of Pediatrics 132, 121-124, 1998.

[100] Kuwabara S., Nakazawa R., Azuma N. et al. Intravenous methylcobalamin treatment for uremic and diabetic neuropathy in chronic hemodialysis patients. // Internal Medicine, vol. 38, no. 6, pp. 472–475, 1999.

[101] Hall C A , Begley J/ A., Green-Colligan .PD. The availability of therapeutic hydroxocobalamin to cell. // Blood.Feb, 63(2), 335-41.1984

[102] European Pharmacopoeia (Eur.Ph.) 9.0 Edition.

[103] "Pharmaceutical industry" No. 4 (63), August 2017. SP XI, Issue 1, P. 110.SP XI, Issue 1, P.34.