
**MATHEMATICS METHODS AND INFORMATION
SYSTEMS IN CHEMICAL TECHNOLOGY**

**МАТЕМАТИЧЕСКИЕ МЕТОДЫ И ИНФОРМАЦИОННЫЕ
СИСТЕМЫ В ХИМИЧЕСКОЙ ТЕХНОЛОГИИ**

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RESEARCH ARTICLE

Implementation of pharmaceutical development using multivariate analysis of multi-criteria optimization on the example of the stage of purification of oligohexamethyleneguanidine hydrosuccinate

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Abstract

Objectives. The study set out to use mathematical modeling, in particular the method of multifactorial analysis of multicriteria optimization (MAMO), in the development of a pharmaceutical product.

Methods. After carrying out experimental tests based on the proposed algorithmic sequence, the obtained data were interpreted using MAMO.

Results. The possibility of using MAMO to solve the applied problem of purifying oligohexamethyleneguanidine hydrosuccinate (OHMG-HS), considered as a pharmaceutical precursor for the creation of medicines, was demonstrated.

Conclusions. The expediency of using the proposed algorithm as a tool for pharmaceutical development is substantiated by identifying dependencies of the influence of purification conditions on the final content of admixtures in the target product.

Keywords: pharmaceutical development, mathematical modeling, antibiotic resistance, oligohexamethyleneguanidines

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НАУЧНАЯ СТАТЬЯ

Реализация фармацевтической разработки с применением многофакторного анализа многокритериальной оптимизации на примере этапа очистки гидросукцината олигогексаметиленгуанидина

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Аннотация

Цели. Данное исследование посвящено использованию математического моделирования, в частности метода многофакторного анализа многокритериальной оптимизации (МАМО), в фармацевтической разработке.

Методы. В ходе исследования была предложена алгоритмическая последовательность эксперимента и проведены необходимые испытания. Полученные данные были интерпретированы при помощи МАМО.

Результаты. Изучена возможность применения МАМО для решения прикладной проблемы очистки гидросукцината олигогексаметиленгуанидина (ОГМГ-ГС), рассматриваемого в качестве фармацевтической субстанции для создания лекарственных средств.

Выводы. Были выявлены зависимости влияния условий очистки на конечное содержание примесей в целевом продукте и доказана целесообразность использования предложенного алгоритма в качестве инструмента фармацевтической разработки.

Ключевые слова: фармацевтическая разработка, математическое моделирование, антибиотикорезистентность, олигогексаметиленгуанидины

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INTRODUCTION

With the number of deaths worldwide caused by drug-resistant microorganisms exceeding 50000 per year, antimicrobial resistance is widely perceived as a major problem. However, the complex and multifactorial nature of antimicrobial resistance remains poorly understood, especially in the context of human-, animal- and environmental interactions. The situation is exacerbated by a lack of reliable information, the slow development of new antimicrobial drugs, and high infection incidence rates. Thus, the emergence and spread of antimicrobial resistance requires immediate attention from both medical professionals and developers of new compounds that may exhibit antimicrobial activity [1].

As a consequence of the reduced effectiveness of current means of prevention and treatment of human infectious diseases due to the resistance of microorganisms to drugs and disinfectants, the World Health Organization expects antibiotic resistance to become the biggest threat to human health by 2050 [2]. In 2017, in order to implement the National Security Strategy of the Russian Federation and the State Policy for ensuring the chemical and biological safety of the Russia for the period up to 2025 and beyond, the Government of the Russian Federation approved the Strategy to Prevent the Spread of Antimicrobial Resistance to 2030 [3]. A direction implemented within the framework of this Strategy is related to the search for new ways of synthesizing substances with antimicrobial activity that are capable of overcoming identified resistance mechanisms.

Previously proposed methods for the synthesis of oligohexamethyleneguanidine (OHMG) salts [4, 5] showed sufficient efficiency against various pathogenic and opportunistic microorganisms, including fungi and viruses [6, 7]. In this regard, OHMG derivatives are currently actively used to create drugs based on them [8, 9].

The process for obtaining derivatives of poly- and oligohexamethylene guanides consists in the polycondensation of hexamethylenediamine (HMDA) and guanidine salts followed by conversion into the required OHMG salt. However, the main problem inherent in this process is the content of a sufficiently large quantity of residual impurities in the target compound. Although a recent study [10] showed that the use of microfluidic synthesis makes it possible to achieve a low content of monomer impurities compared to bulk synthesis, the obtained results do not meet the requirements of the State Pharmacopeia of the Russian Federation¹. Therefore,

the aim of the present study is to identify the optimal conditions for the purification of the target compound from impurities using mathematical modeling.

MATERIALS AND METHODS

The following reagents were used in the experiments: HMDA (99.5%, *Acros Organics*, Belgium), guanidine hydrocarbonate (GHC) (99.5%, *Sigma-Aldrich*, USA), chloroform (99.5%, *EKOS-I*, Russia), acetone (99.75%, *EKOS-I*, Russia), carbon tetrachloride (99.6%, *EKOS-I*, Russia), methylene chloride (99.5%, *EKOS-I*, Russia).

The main methods of polymer purification consist of several dissolution cycles followed by precipitation and washing with various solvents [11]. For washing poly- and oligoguanidines [12], chloroform, carbon tetrachloride, and similar solvents are used. However, in order to establish the optimal time for the purification process, as well as the ratio of components and dependencies between the initial parameters and the values of residual impurities, it becomes necessary to conduct many experiments involving different variations and combinations of the initial values. In this case, significant increases in reagent consumption and time preclude the rapid achievement of satisfactory results. In this regard, mathematical modeling techniques such as multifactorial analysis of multicriteria optimization (MAMO) can become useful tools for improving process parameters and saving resources [13]. Taking into account the application of MAMO methods, we propose the following algorithmic sequence:

- 1) search for information in foreign and local literary sources;
- 2) conducting preliminary experiments in the absence of reliable literature data;
- 3) formulating a hypothesis of criteria dependence on various factors and determining parameters for verifying hypothesis validity;
- 4) obtaining an approximating function in accordance with paragraph 3 according to the experimental data;
- 5) search for optimal values;
- 6) conducting verification experiments for compliance with the verification parameters defined in clause 3;
- 7) selection of the most appropriate time-solvent ratio from those calculated.

The application of mathematical modeling can be described according to the algorithmic sequence

¹ State Pharmacopeia of the Russian Federation, 14th ed. OFS.1.1.0006.15 Pharmaceutical substances. URL: <https://minzdrav.gov.ru/poleznye-resursy/xiv-izdanie-gosudarstvennoy-farmakopei-rossiyskoy-federatsii>. Accessed February 15, 2023 (in Russ.).

proposed above on the example of purification of oligohexamethyleneguanidine hydrosuccinate (OHMG-HS) (Fig. 1).

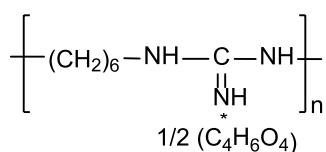


Fig. 1. Formula of oligohexamethyleneguanidine hydrosuccinate (OHMG-HS).

Based on the literature data [5], the following solvents were chosen for the purification process: chloroform, carbon tetrachloride, methylene chloride, and acetone. The most important and controllable factors were the amount of solvent added and the residence time of the sample in the chosen solvent.

The content of related impurities—HMDA and GHC, sulfate ash, heavy metals, as well as residual solvents (acetone, chloroform, methylene chloride, carbon tetrachloride)—were chosen as acceptance criteria for the target product. The corresponding data, which are taken from the State Pharmacopeia

of the Russian Federation, 14th edition, are given in Table 1 (see Footnote 1).

Since no information about the mutual influence of factors was obtained, we assumed that there is mutual influence—that is, a nonlinear dependence on the factors expressed in quadratic terms taking the form xy . Therefore, to expand the range, we built experiments according to a full factorial design (containing all possible combinations of all factors at a certain number of levels, an equal number of times) (Table 2). To check hypothesis validity, the extraction coefficient (R) was used, which should lie within 10% for the test experimental points [14]. Having chosen the optimal purification method, the relative standard deviation for the quality indicators of OHMG-HS (Table 1) obtained during the measurements of 5 samples should not exceed 5%.

During the experiment, 20% aqueous solutions of the OHMG-HS salt were prepared, followed by the addition of the required amount of one of the solvents in accordance with Table 2 at room temperature (25°C). The solutions were thoroughly mixed and left to settle. After that, the target solutions were decanted and evaporated on a Laborota 4000 rotary evaporator (*Heidolph*, Germany) at 100°C

Table 1. Quality criteria for the OHMG-HS target product

Indicator	Requirement for the content of residual admixtures, no more %
Hexamethylenediamine (HMDA) admixture	0.0500
Guanidine hydrocarbonate (GHC) admixture	0.0500
Sulfate ash	0.1000
Heavy metals	0.0010
Chloroform	0.0060
Acetone	0.5000
Carbon tetrachloride	0.0004
Methylene chloride	0.0600

Table 2. Conditions of the salt purification process of OHMG-HS

No.	Solvent	Added, mL	Settling time, h
1	Chloroform	30	8
2		35	12
3		40	18
4		45	24
5	Carbon tetrachloride	30	8
6		35	16
7		40	24
8		45	28
9	Methylene chloride	30	16
10		35	24
11		40	28
12		45	36
13	Acetone	40	1.5
14		45	1
15		50	2
16		55	2.5

RESULTS AND DISCUSSION

During the preliminary experiments, the following data were obtained on the content of impurities in samples of OHMG-HS (Table 3).

To carry out mathematical calculations, it is necessary to normalize the obtained data. Data on the quantity of solvents (x) and the settling time of the mixture (y) are normalized according to the formula (1):

$$x_{\text{norm}} = \frac{x - x_{\min}}{x_{\max} - x_{\min}}, y_{\text{norm}} = \frac{y - y_{\min}}{y_{\max} - y_{\min}}. \quad (1)$$

Data on residual impurities (z) are normalized in such a way (2) that the value of 0 coincides with the actual value of 0 and that the value of 1 corresponds to the maximum permissible concentration (in the graphs, everything below the line $y = 1$ is acceptable):

$$z_{\text{norm}} = \frac{z}{z_{\text{maximum allowed}}}. \quad (2)$$

Normalized data are presented in Table 4.

Next, the response surface was constructed (approximation). In accordance with the full factorial design, the dependence of criteria on factors has the form (3):

$$F(x,y) = A + Bx + Cy + Dxy, \quad (3)$$

where x is the amount of solvent; y is settling time A , B , C , and D are regression coefficients. The term Dxy corresponds to the mutual influence of factors.

According to the experimental data, the value of the coefficients A , B , C , and D can be accurately determined. For the $F(x,y)$ dependence, the standard deviation was considered, after which it was differentiated by each of the coefficients. The

Table 3. Quantitative values of quality indicators after cleaning

No.	Solvent	Amount after cleaning, %	HMDA, %	GHC, %	Sulfate ash, %	Heavy metals, %
1	Chloroform	0.016	0.212	0.150	0.02	0.0017
2		0.007	0.138	0.076	0.04	0.0011
3		0.008	0.094	0.048	0.03	0.0008
4		0.006	0.066	0.091	0.03	0.0009
5	Carbon tetrachloride	0.076	0.212	0.149	0.03	0.0013
6		0.059	0.178	0.110	0.04	0.0012
7		0.043	0.121	0.076	0.04	0.0009
8		0.032	0.177	0.092	0.14	0.0010
9	Methylene chloride	0.094	0.146	0.171	0.02	0.0014
10		0.059	0.112	0.057	0.03	0.0008
11		0.061	0.060	0.054	0.05	0.0005
12		0.058	0.051	0.046	0.02	0.0006
13	Acetone	0.067	0.092	0.05	0.02	0.0009
14		0.024	0.062	0.048	0.04	0.0006
15		0.015	0.047	0.051	0.03	0.0008
16		0.013	0.049	0.046	0.03	0.0007

resulting system was equated to zero and solved with respect to the coefficients A , B , C , and D . Thus, an approximation was constructed for the dependence of the amounts of each of the residual impurities on the normalized factors x and y . The plot of this fitting function comprises the response surface.

Processing of experimental data and mathematical modeling by multivariate analysis of multicriteria optimization was carried out using Wolfram Mathematica software (Wolfram Research, USA).

Carbon tetrachloride

According to the calculations for the carbon tetrachloride solvent, the following dependencies (F) of the impurity residues on the amount of added solvent (x) and settling time (y) were obtained. Figure 2 shows the level lines for each impurity.

The solid line indicates the limit value, while the dotted line indicates the permissible values. Optimal points should be located in the area of intersection of lines of all colors:

$$1. F_{\text{solvent}}(x,y) = (341.25 - 191.25x - 109.375y + 39.375xy) \cdot 0.0004\%;$$

$$2. F_{\text{HMDA}}(x,y) = (-18.19 + 40.77x - 11.795y - 7.245xy) \cdot 0.05\%;$$

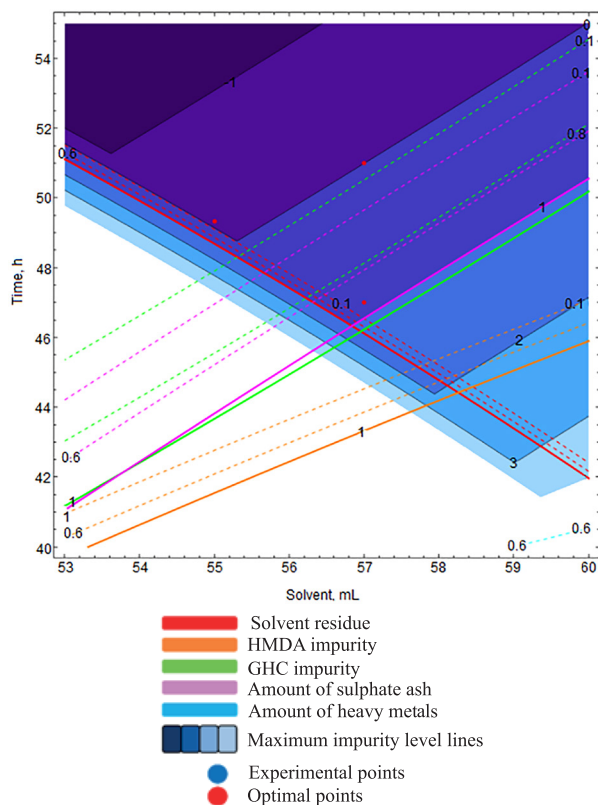
$$3. F_{\text{GHC}}(x,y) = (-1.49 + 9.63x - 7.875y + 1.575xy) \cdot 0.05\%;$$

$$4. F_{\text{sulfate ash}}(x,y) = (-11.5 + 20.25x - 6.125y - 1.575xy) \cdot 0.1\%;$$

$$5. F_{\text{heavy metals}}(x,y) = (-3.8 + 9x - 1.05y - 3.15xy) \cdot 0.001\%.$$

Table 4. Normalized experimental data

No.	Solvent	Normalized added volume	Normalized settling time
1	Chloroform	0.67	0.33
2		0.78	0.50
3		0.89	0.75
4		1.00	1.00
5	Carbon tetrachloride	0.67	0.29
6		0.78	0.57
7		0.89	0.86
8		1.00	1.00
9	Methylene chloride	0.68	0.44
10		0.78	0.67
11		0.89	0.78
12		1.00	1.00
13	Acetone	0.73	0.60
14		0.82	0.40
15		0.91	0.80
16		1.00	1.00


Fig. 2. Results of OHMG-HS optimization with carbon tetrachloride.

As can be seen from the traces of residual impurities, the amount of residue after cleaning can fall below the maximum allowable values given the correct solvent ratio and sufficient time. This area on the chart marked in dark blue and purple is bounded by red, pink and green solid charts. Within this allowable area, 3 optimal points for the reaction were found (red dots on the graph) with integer values of the added amount of solvent (mL) and settling time (h, min):

1. $x = 57 \text{ mL}$, $y = 47 \text{ h}$;
2. $x = 55 \text{ mL}$, $y = 49 \text{ h } 20 \text{ min}$;
3. $x = 57 \text{ mL}$, $y = 51 \text{ h}$.

Methylene chloride

Similar calculations were carried out for the methylene chloride solvent (Fig. 3):

1. $F_{\text{solvent}}(x,y) = (4.1 - x - 8.3y + 6.17xy) \cdot 0.06\%$
2. $F_{\text{HMDA}}(x,y) = (13.6 - 19x + 0.67y + 5.8xy) \cdot 0.05\%$;

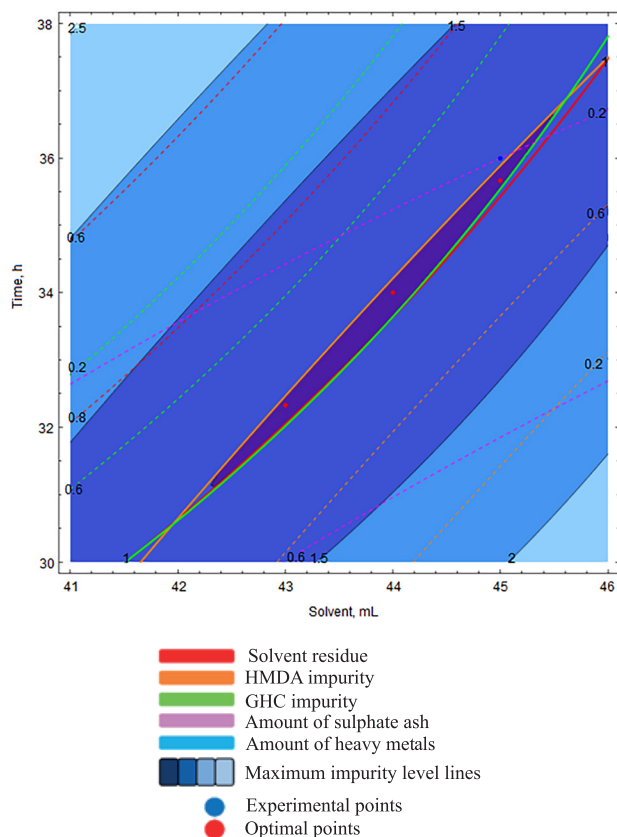


Fig. 3. Results of OHMG-HS optimization with methylene chloride.

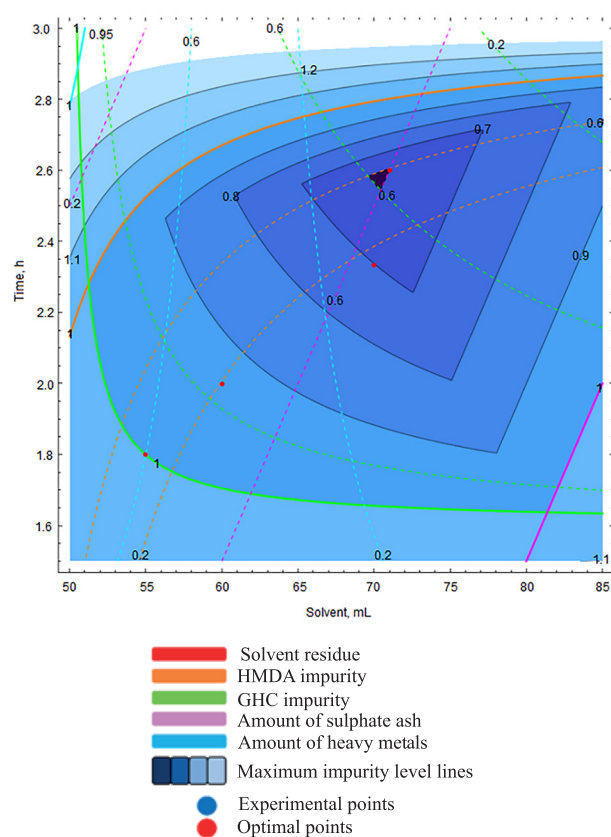


Fig. 4. Results of OHMG-GS optimization with chloroform.

$$3. F_{\text{GHC}}(x,y) = (15.1 - 7.82x - 30.88y + 24.53xy) \cdot 0.05\%;$$

$$4. F_{\text{sulfate ash}}(x,y) = (-4.17 + 7.84x + 1.16y - 4.63xy) \cdot 0.1\%;$$

$$5. F_{\text{heavy metals}}(x,y) = (7.8 - 9x - 6.3y + 8.1xy) \cdot 0.001\%.$$

Residual impurities plots show that, given the correct solvent ratio and sufficient time, the amount of residue after cleaning can fall below the acceptable limits (purple area bordered by orange, green, and red solid graphs). Within this allowable area, 3 red dots characterize the optimal (integer) values of the added amount of solvent and the settling time for carrying out the reaction:

1. $x = 44 \text{ mL}, y = 34 \text{ h};$
2. $x = 43 \text{ mL}, y = 32 \text{ h } 20 \text{ min};$
3. $x = 45 \text{ mL}, y = 35 \text{ h } 40 \text{ min}.$

Chloroform

Figure 4 shows the dependencies (F) of impurity residues on the amount of added solvent (x) and settling time (y) for the chloroform solvent in accordance with the calculations:

$$1. F_{\text{solvent}}(x,y) = (26 - 48x + 32y - 9xy) \cdot 0.006\%;$$

$$2. F_{\text{HMDA}}(x,y) = (17.28 - 21.24x - 0.48y + 5.76xy) \cdot 0.05\%;$$

$$3. F_{\text{GHC}}(x,y) = (16.4 - 17.1x - 23.04y + 25.56xy) \cdot 0.05\%;$$

$$4. F_{\text{sulfate ash}}(x,y) = (-3.6 + 8.1x - 6y + 1.8xy) \cdot 0.1\%;$$

$$5. F_{\text{heavy metals}}(x,y) = (6.9 - 7.2x - 6y + 7.2xy) \cdot 0.001\%.$$

In Fig. 4, the blue triangle (the darkest of those present), bounded by orange, green, and pink solid graphs, indicates the area where the amount of residue after cleaning can fall below the limit values at the desired ratio of solvent and time. Within this allowable range, the optimal (integer) values for the solvent amount added and the settling time are indicated by a red dot:

$$x = 41 \text{ mL}, y = 15 \text{ h } 20 \text{ min}.$$

Acetone

Similar calculations for the acetone solvent are shown in Fig. 5:

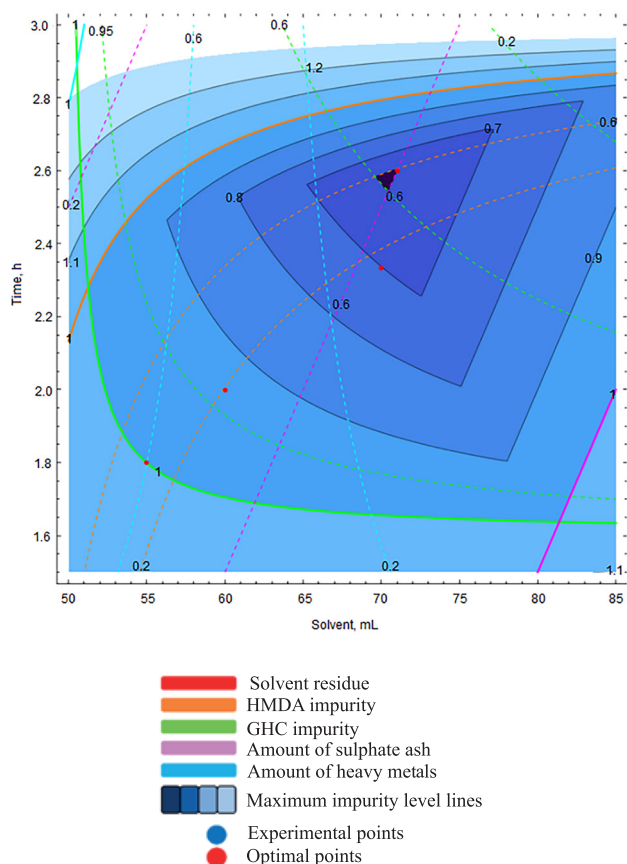


Fig. 5. Results of optimization of OHMG-GS with acetone.

1. $F_{\text{solvent}}(x,y) = (1.05 - 1.27x - 0.62y + 0.86xy) \cdot 0.5\%$;
2. $F_{\text{HMDA}}(x,y) = (11.32 - 12.41x - 8.3y + 10.37xy) \cdot 0.05\%$;
3. $F_{\text{GHC}}(x,y) = (-0.71 + 1.92x + 2.7y - 2.99xy) \cdot 0.05\%$;
4. $F_{\text{sulfate ash}}(x,y) = (-0.3 + 1.1x - 0.5y) \cdot 0.1\%$;
5. $F_{\text{heavy metals}}(x,y) = (-0.29 + 0.63x + 3.5y - 3.14xy) \cdot 0.001\%$.

As can be seen from the graphs of residual impurities, acetone is the best solvent, as it requires less settling time compared to chloroform, carbon tetrachloride, and methylene chloride. Four optimal points were proposed and approximated:

1. $x = 55 \text{ mL}, y = 108 \text{ min}$;
2. $x = 60 \text{ mL}, y = 120 \text{ min}$;
3. $x = 71 \text{ mL}, y = 156 \text{ min}$;
4. $x = 70 \text{ mL}, y = 140 \text{ min}$.

Table 5 indicates the values of the acceptance criteria calculated for points that meet the criteria.

From the data obtained by MAMO, it can be seen that the optimal points for OHMG-HS along with those for chloroform and methylene chloride solvents are on the border of the acceptance criteria in terms of HMDA and GHC impurities. From the location of the remaining proposed points for carbon tetrachloride and acetone solvents, it can be seen that the settling time of the mixture is reduced several times when using acetone in comparison with the use of carbon tetrachloride, which belongs to hazard class 1—highly toxic solvents (according to the State Pharmacopeia of the Russian Federation of the Russian Federation, carbon tetrachloride is used in pharmaceutical production in exceptional cases, when it is impossible to refuse its use). In this connection, the optimal method for purifying OHMG-HS is reprecipitation with acetone at the appropriate ratios of added solvent and mixture settling time:

1. 55 mL – 108 min;
2. 60 mL – 120 min;
3. 71 mL – 156 min;
4. 70 mL – 140 min.

After carrying out mathematical calculations, it was decided to reproduce control experiments that check the correctness of the obtained data (optimal points) (Figs. 6 and 7). The obtained experimental data are presented in Table 6.

Thus, based on a comparison of the experimental data with the MAMO data, the advanced hypothesis can be confirmed as valid, since for the methods of quantitative determination, the recovery factor (R) corresponds to an interval from 90% to 110%.

The precision of the technique was determined by the parameter of convergence (repeatability). For OHMG-HS, after selecting a single optimal point and performing the required number of experiments, the standard deviation and dispersion values were calculated for the results of HMDA, GHC, sulfate ash, and heavy metal impurities (Table 7).

The characteristics presented in the table indicate the compliance of the obtained results with the established acceptance criteria (Table 1) and the reproducibility of the technological stage of purification of OHMG-HS under the conditions selected using MAMO.

CONCLUSIONS

The obtained results were interpreted using MAMO according to the described algorithm.

Table 5. Acceptance criteria at the optimal points for the OHMG-HS

Solvent	Amount of solvent, mL, and settling mixture time	Amount of solvent after cleaning, %	HMDA, %	GHC, %	Sulphate ash, %	Heavy metals, %
Chloroform	41 mL, 15 h 20 min	0.0000	0.0490	0.0490	0.0990	0.0007
Methylene chloride	44 mL, 34 h	0.0590	0.0480	0.0700	0.0320	0.0005
	43 mL, 33 h 20 min	0.0590	0.0490	0.0470	0.0390	0.0005
	45 mL, 35 h 40 min	0.0590	0.0480	0.0490	0.0230	0.0006
Carbon tetrachloride	57 mL, 47 h	0.0000	0.0100	0.0430	0.0870	0.0001
	55 mL, 49 h 20	0.0000	0.0160	0.0210	0.0170	0.0003
	57 mL, 51 h	0.0000	0.0110	0.0110	0.0120	0.0004
Acetone	55 mL, 108 min	0.0000	0.0200	0.0500	0.0440	0.0006
	60 mL, 120 min	0.0000	0.0090	0.0470	0.0500	0.0005
	71 mL, 156 min	0.0000	0.0290	0.0280	0.0600	0.0000
	70 mL, 140 min	0.0000	0.0050	0.0350	0.0630	0.0000

Table 6. Experimental (practical) and calculated (theoretical) data obtained during the OHMG-HS purification of in acetone

Specifications	No. 1	No. 2	No. 3	No. 4	Recovery factor <i>R</i> theor./pract.
Volume of added solvent (mL) and settling time (min)	55 mL, 108 min	60 mL, 120 min	71 mL, 156 min	70 mL, 140 min	—
Amount of solvent after cleaning, theor., %	0.0000	0.0000	0.0000	0.0000	100%
Amount of solvent after cleaning, pract., %	0.0000	0.0000	0.0000	0.0000	
HMDA, theor., %	0.0200	0.0090	0.0050	0.0290	91.3%
HMDA, pract., %	0.0210	0.0070	0.0090	0.0320	
GHC, theor., %	0.0500	0.0470	0.0280	0.0350	98.2%
GHC, pract., %	0.0410	0.0390	0.0420	0.0410	
Sulphate ash, theor., %	0.0440	0.0500	0.0600	0.0630	96.4%
Sulphate ash, pract., %	0.0460	0.0510	0.0630	0.0650	
Heavy metals, theor., %	0.0006	0.0005	0.0000	0.0000	100%
Heavy metals, pract., %	0.0005	0.0003	0.0002	0.0001	

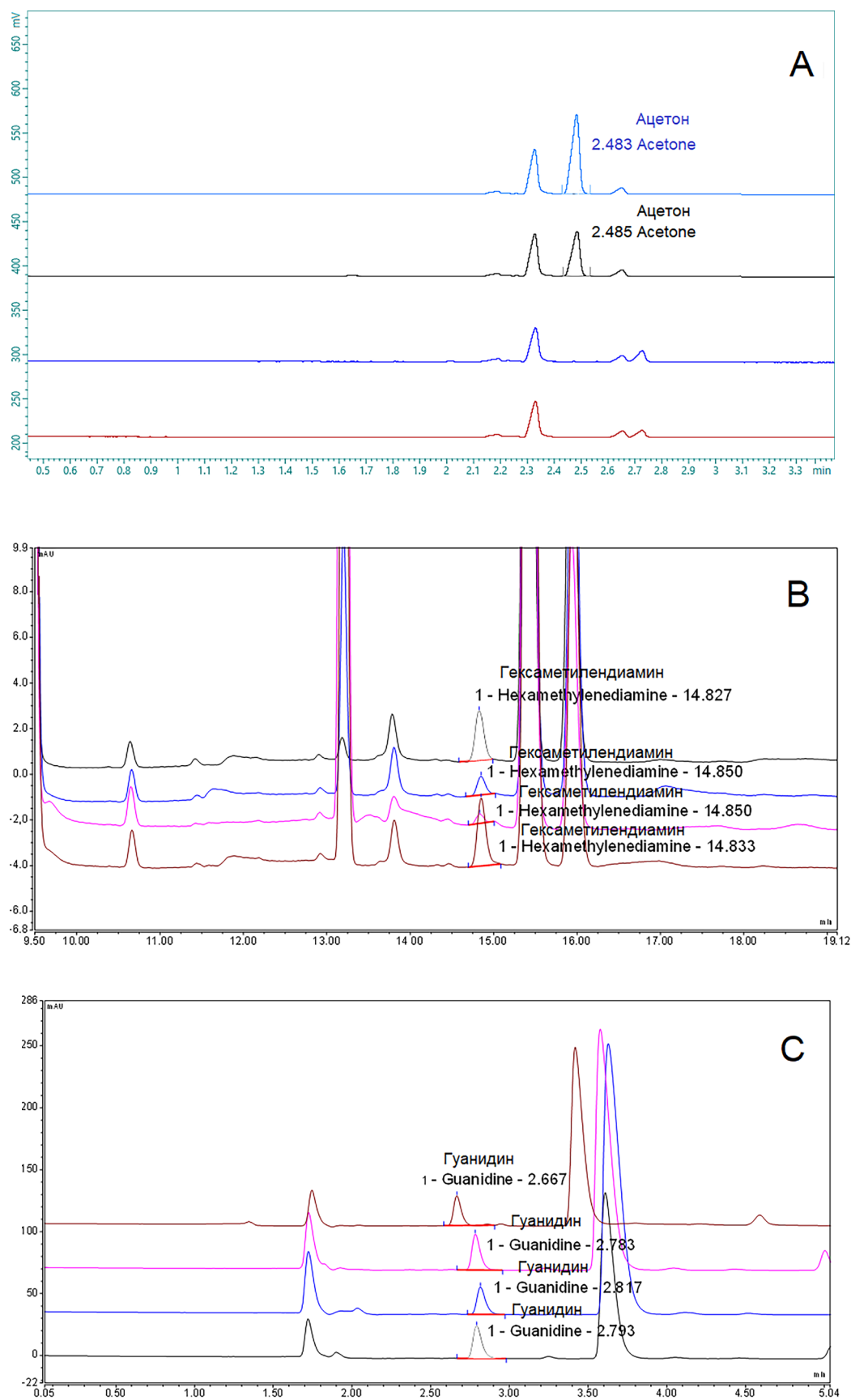


Fig. 6. HPLC analysis results for the content of acetone (A), HMDA (B), and GHC (C) admixtures for OHMG-HS samples No. 1–2.

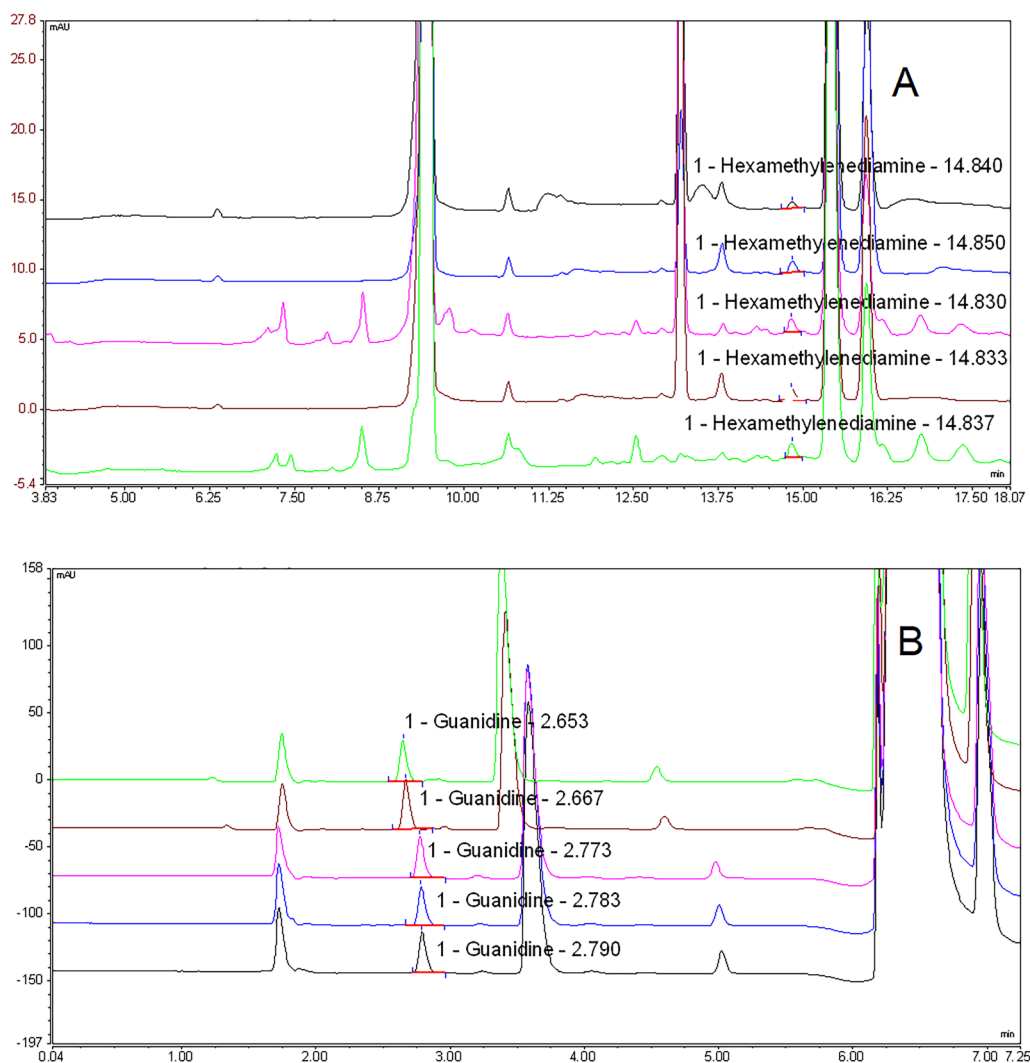


Fig. 7. HPLC analysis results for the content of HMDA (A) and GHC (B) admixtures for OHMG-HS obtained in the convergence study (5 repeats).

Table 7. Results of the convergence method checking

No.	Cleaning conditions		Quality criteria			
	solvent, mL	Settling time, min	HMDA, %	GHC, %	Sulphate ash, %	Heavy metals, %
1	70	140	0.011	0.045	0.063	0.0002
2			0.012	0.044	0.059	0.0001
3			0.007	0.043	0.062	0.0003
4			0.008	0.042	0.063	0.0001
5			0.009	0.041	0.061	0.0002
$\bar{A} \pm \sigma$			0.009 ± 0.002	0.043 ± 0.002	0.061 ± 0.002	0.0002 ± 0.0001

* $\bar{A} \pm \sigma$ – Standard deviation and dispersion.

As a result, dependencies of the influence of the ratio of the added amount of solvent and the settling time of the mixture on the final content of impurities in the target product were revealed. For the highest quality purification of OHMG-HS, the use of acetone solvent is advisable, since this reduces the process time to several hours while minimizing the quantity of impurities. Using statistical methods, the validity and repeatability of the proposed algorithmic model was substantiated. The use of MAMO for predicting the results and plotting the dependencies of the parameters and criteria of the reaction confirmed its feasibility as a means of reducing the time and material costs involved in experiments.

Authors' contributions

D.O. Shatalov – study concept;
K.N. Trachuk – writing and editing the text of the article, implementation of the analytical stage in the experimental studies, literature review;
A.V. Aydakova – conducting experiments;
D.A. Akhmedova – conducting experiments, writing and editing the text of the article;
I.S. Ivanov – conducting experiments;
D.S. Minenkov – processing experimental data;
I.Yu. Blazhevich – processing experimental data;
S.A. Kedik – developing a technological base for research.

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