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# DEVELOPMENT OF THE BASICS OF PRODUCTION TECHNOLOGY OF DACARBAZINE SUBSTANCE WITH ANTICANCER EFFECT

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**The basics of the technology for the production of dacarbazine, which is an active ingredient in medicines for the treatment of metastatic melanoma, Hodgkin's lymphoma, and soft tissue sarcoma, are presented. Based on the laboratory methodology, the production was scaled up as a periodic production with a capacity of 35 tons per year to produce 1 ton of the substance from commercially available reagents. Six main technological stages of production were selected, which allowed to obtain a product with a yield of 86 %. Based on material calculations, the required operating loads of components at each stage are determined. The choice of technological equipment for the process is carried out. The organization of the process is presented in the technological flowchart and the equipment and technological scheme.**

**Key words: melanoma, dacarbazine, 5-amino-1*H*-imidazole-4-carboxamide, diazotation, coupling reaction, dimethylamine, development of the technology basis, technological scheme.**

## Introduction

Malignant tumors are the second most common cause of death in the world, making the search for effective treatments a priority in medicine. The process of creating new anti-cancer drugs involves several stages of research, development, clinical trials, and industrial production, each of which is crucial to ensure the safety and efficacy of new drugs [1–3]. According to estimations by the company Visiongain, one of the leading analytical companies, more than 20 million new cancer cases are expected in 2025, compared to 12 million new cases in 2008. Also, according to the World Agency for Research on Cancer, the number of cancer patients is expected to increase by 1.5 times by 2030 [4]. The main problems in the fight against cancer in Ukraine are delayed diagnosis and expensive anti-cancer therapy [5]. The most common cancers include lung, breast, prostate, colon, skin, and leukemia [6]. The most dangerous of them is skin melanoma. According to the National Cancer Registry, 5051 cases of melanoma were registered in Ukraine in 2022–2023. In 2023, 31,217 people were registered with this disease [7]. Dacarbazide is one of the main chemotherapeutic drugs used in the monotherapy of melanoma and in the complex therapy of Hodgkin's lymphoma and soft tissue sarcoma.

Dacarbazine or 5-[(1*E*)-dimethyltriaz-1-en-1-yl]-1*H*-imidazole-4-carboxamide (Fig. 1) is a triazene derivative of imidazole carboxamide by chemical structure. According to the ATC classification, it belongs to the pharmacotherapeutic group L01AX04 Antineoplastic agents. Alkylating compounds. Its mechanism of action is associated with inhibition of cell growth and inhibition of DNA synthesis. It also has an alkylating effect and may be involved in other mechanisms of cancer tumor suppression. It is considered that dacarbazine itself has no antineoplastic effect, but as a result of microsomal N-demethylation, it is rapidly converted to 5-amino-imidazole-4-carboxamide and methyl cation, which causes the alkylating effect of dacarbazine [8]. It was first synthesized in 1959 at Southern Research Company in the USA [9]. In 1975, it was approved for use in medical practice by the FDA (USA) for the treatment of metastatic melanoma as a monotherapy and as part of the complex therapy of Hodgkin's lymphoma, mainly with vinblastine, bleomycin and doxorubicin [10],

as well as advanced soft tissue sarcoma (except for mesothelioma and Kaposi's sarcoma) in adults [8]. It is used only in the injectable form for intravenous administration. It is included in the World Health Organization's List of Essential Medicines. It is known worldwide under such trade names DTIC-Dome, Dacarbazin, Dacarbazina, Detimedac, Deticene, DIC, Oncocarbil, and Dacatic [11] and is available by prescription. Like other anticancer drugs, it has a number of serious side effects [8, 11].

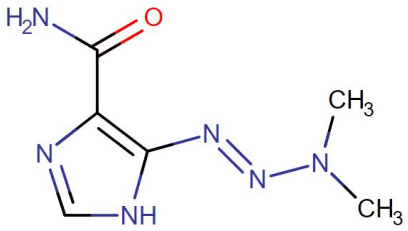



*Fig. 1. Structure of dacarbazine*

In the territory of Ukraine, according to the State Register of Medicinal Products of Ukraine [12], the following trade names with this substance are available to consumers on the pharmaceutical market by prescription, exclusively of foreign origin:

– "Dacarbazine Medak" in the form of powder for injection with a dosage of 100/200/500/1000 mg in vials (Medak Gesellschaft fur klinische Spezialpraparate m.b.H., Germany);

– "Dakarbazin" in the form of lyophilisate for injection solution with a dosage of 200/500 mg in vials (Venus Medicines Limited, India).

According to the *Tabletki.ua* website, the price of "Dacarbazine Medak" in June 2024 is quite high and ranges from UAH 5337.48 to 6816.11 UAH for 10 vials in a dosage of 200 mg/vial. It should be noted that this is currently the only drug available on the pharmaceutical market. The long duration of treatment, in particular for Hodgkin's disease (usually 6 cycles of ABVD combination therapy), as well as the availability of only one dosage on the Ukrainian pharmaceutical market, makes it a rather limiting factor for use by ordinary cancer patients in Ukraine. According to the State Register of Medicinal Products, the substance dacarbazine (manufactured by "Vuab Pharma Ltd", Czech Republic) is registered, but no domestic drugs based on it are not produced. It should also be noted that the current State Pharmacopoeia of Ukraine [13] does not have a monograph on the substance dacarbazine, but, for example, in the US Pharmacopoeia and the International Pharmacopoeia, one can easily find the relevant documents [11].

According to the *Chemical Book* information service [14], in June 2024, there are 300 manufacturers-suppliers of dacarbazine in the world, offering it on the markets of the USA, the United Kingdom, Canada, Germany, Czech Republic, Switzerland, France, Japan, China, and India. On average, the price of technical dacarbazine with a purity of 95–99 % on the Chinese market is about 250 UAH per 1 gram [15], and on the US market – 30–198 USD per 1 gram according to the *SciFinder-n* database [16]. However, a foreign technical product needs to be thoroughly purified to obtain the status of a pharmaceutical active ingredient. This requires additional financial costs, which increases the price of the substance.

Therefore, summarizing the abovementioned, it can be concluded that there is a need to design and develop a technology for the Ukrainian production of dacarbazine.

The purpose of work was to develop the basics of the production technology of this substance based on the selected laboratory method of production, determine the amount of raw materials required to produce 1 ton of product, select the necessary technological equipment, and develop a technological flowchart and a basic equipment and technological scheme of production.

## Materials and research methods

Data on the registration of pharmaceutical products with the active ingredient dacarbazine in the pharmaceutical market of Ukraine were obtained from the State Register of Medicines of Ukraine [13] in June 2024. The data from the *SciFinder-n* database [16] were used to analyze the methods of dacarbazine production. The results were processed using the methods of systematization, analysis, and comparison of data.

To develop the basics of dacarbazine production technology, material calculations were performed for each of the 6 technological stages to determine the amount of raw materials consumed.

Technological calculations made it possible to select the necessary types of technological equipment [17–19]. Based on this, a basic equipment and technological scheme of production was developed.

The technological flow chart is based on the division into technological stages of production, taking into account the parameters of process control and the introduction of the necessary reagents.

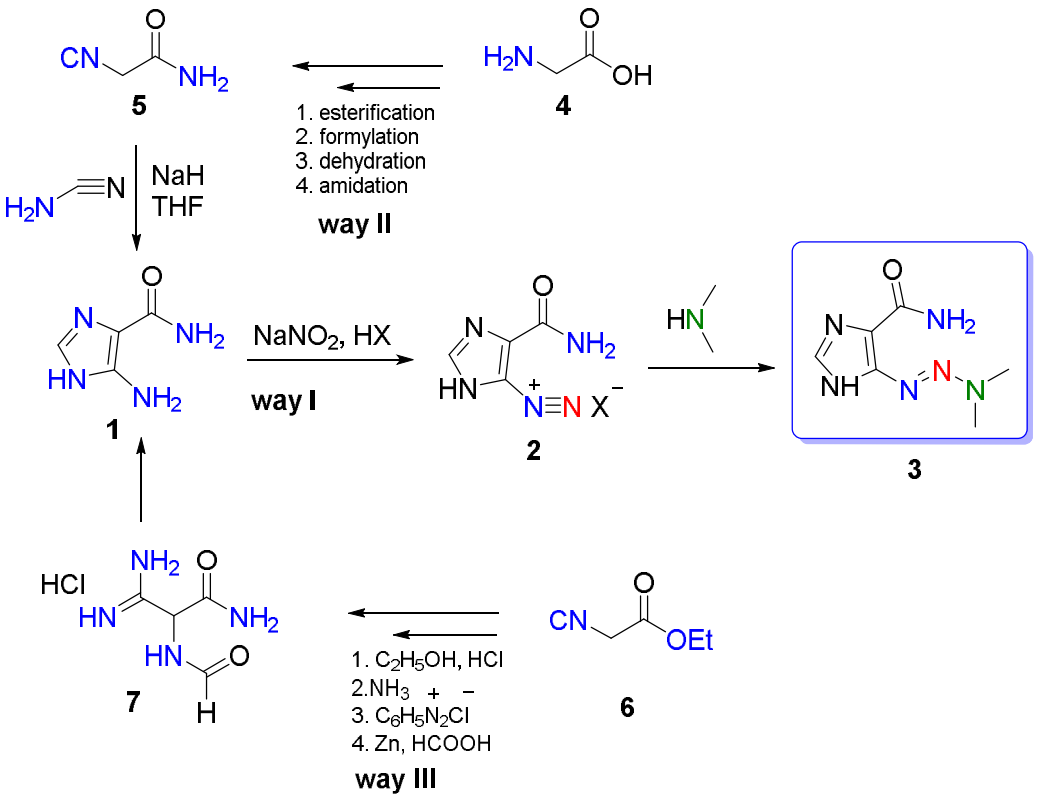
**Research results and discussion**

Several methods for the preparation of dacarbazine are known in the literature. The simplest method of its synthesis is the diazotation reaction of 5-amino-1*H*-imidazole-4-carboxamide **1** followed by azo coupling with dimethylamine (Scheme 1, way I) [11]. The yields of dacarbazine **3** from this transformation are 76–86 %. The second synthesis way was developed by Chinese scientists [20]. Glycine **4** was used as a starting compound for the synthesis of dacarbazine, which gave 2-isocyanoacetamide **5** through a series of chemical transformations (Scheme 1, way II). The latter, when reacted with cyanamide in the presence of sodium hydride, forms a cyclic product for further transformation by diazotation and coupling reactions. The total yield of this method was 22.3 %.

In the third synthetic approach (Scheme 1, way III), ethyl cyanoacetate **6** is used as the starting compound, which is converted first to imino ethyl ether. Further, it is converted to an amide, which reacts with benzene diazonium chloride to form an azo compound. The reduction of the latter in the presence of formic acid leads to the formation of the formamide product **7**, which is cyclized to 5-amino-1H-imidazole-4-carboxamide **1** [21].

Dacarbazine can also be obtained from other starting reagents involved in the second and third synthesis ways, including 6–7 conversion steps, which leads to a low total product yield (~ 22–30 %) [20, 22].

The analysis of synthetic approaches to the preparation of dacarbazine shows that the first way based on 5-amino-1*H*-imidazole-4-carboxamide **1** is the most optimal. The laboratory method reported in the literature was chosen as the basis [23].




*Scheme 1. Synthetic approaches of dacarbazine obtaining*

Thecompound **1** is dissolved in cold water in the presence of hydrochloric acid and sodium nitrite to form diazonium salt. The diazonium chloride is then filtered off and dissolved in methanol. Then, under cooling in the presence of a dimethylamine solution, a coupling reaction takes place, leading to the formation of dacarbazine. The yield of the product after drying is 86 %.

Thus, the scheme for the production of dacarbazine in a laboratory can be represented in two steps as follows (Scheme 2):

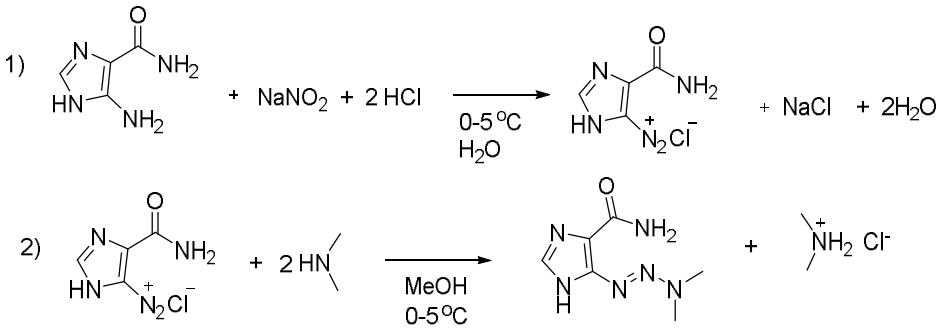



*Fig. 2. Scheme of the laboratory method for the synthesis of dacarbazine*

Based on the two-step synthesis process of dacarbazine, its production can be projected from the following six technological steps with a total yield of 86 %.

Stage 1: Preparation of diazonium salt of 5-amino-1*H*-imidazole-4-carboxamide, stage yield 99 %.

Stage 2. Filtration of diazonium salt, stage yield 91 %.

Stage 3: Obtaining of dacarbazine, stage yield 99 %.

Stage 4. Filtration of dacarbazine precipitate, stage yield 98 %.

Stage 5. Washing the precipitate with water, stage yield 99 %.

Stage 6. Drying of dacarbazine precipitate, stage yield 99.4 %.

To determine the amount of starting materials consumed to produce 1 tonne of the finished product according to the chemism (Fig. 2), a material calculations [17–19] were carried out for the proposed production. As a result, we determined the amounts of the following reagents: 5-amino-1*H*-imidazole-4-carboxamide, sodium nitrite, hydrochloric acid, and dimethylamine, as well as water and methanol (Table 1).

Dacarbazine production is assumed to be as periodic with a capacity of 35 tons per year. The powder components are added manually. The auxiliary equipment is calculated for the supply of liquid components [17–19]. The main equipment (reactors, pressure filters, and a dryer) is calculated according to the volume of reaction mixtures at each technological stage [17–19]. As a result of technological calculations, the required amount of each type of equipment and its main characteristics were selected [17] (Table 2). It is proposed that all equipment for the production of dacarbazine should be produced of AISI-306 stainless steel, which is widely used in the production of pharmaceutical equipment.

*Table 1*

**Amounts required to produce 1 tonne of dacarbazine**

| Name of the compound (purity %) | Mass, kg | | Volume, $m^3$ | Density, $kg/m^3$ |
|---|---|---|---|---|
| | technical product | product, 100 % | | |
| 5-Amino-1H-imidazole-4-carboxamide (99 %) | 813.0015 | 804.8715 | – | – |
| Sodium nitrite (99 %) | 445.2156 | 440.7634 | – | – |
| Concentrated hydrochloric acid (37 %) | 1335.6468 | 494.1893 | 2.7 | 1190 |
| Water to dilute the reaction mixture | – | 645.4574 | 0.4 | 1000 |
| Water to form a sodium nitrite solution | – | 2796.04 | 2.8 | 1000 |
| Methanol | – | 1533.5204 | 1.9 | 792 |
| 40% Aqueous dimethylamine solution | 1285.763 | 514.3052 | 1.4 | 890 |
| Water for washing precipitate at Stage 5 | – | 2000.244 | 2 | 1000 |

*Table 2*

**Specification of main and auxiliary equipment for the production of dacarbazine**

| Equipment / symbols | Calculated number of of equipment, pcs. | Volume, $m^3$ | Dimensions of the equipment, mm | | Characteristics of the equipment |
|---|---|---|---|---|---|
| | | | height | length | |
| Reactor / R-1 | 1 | 0.25 | 600 | 1000 | shell, $F = 2.10$ $m^2$, anchor stirrer |
| Reactor / R-2 | 1 | 0.25 | 600 | 1000 | shell, $F = 2.10$ $m^2$, anchor stirrer |
| Filter / F-1 | 1 | 0.063 | 670 | 850 | $F = 0.19$ $m^2$ |
| Filter / F-2 | 1 | 0.16 | 820 | 1100 | $F = 0.38$ $m^2$ |
| Dryer / Dr-1 | 1 | 2.0 | 1185×1410×2050 | | $F = 2.4$ $m^2$ |
| Storage / St-1 | 1 | 2.0 | 1200 | 1900 | for concentrated hydrochloric acid |
| Storage / St-2 | 1 | 0.25 | 600 | 1000 | for methanol |
| Storage / St-3 | 1 | 0.8 | 1000 | 1150 | for dimethylamine solution |
| Measuring tank / MT-1 | 1 | 0.063 | 400 | 550 | for concentrated hydrochloric acid |
| Measuring tank-mixer / MT-2 | 1 | 0.125 | 400 | 1050 | blade stirrer, for sodium nitrite solution |
| Measuring tank / MT-3 | 1 | 0.1 | 400 | 850 | for methanol |
| Measuring tank / MT-4 | 1 | 0.1 | 400 | 850 | for dimethylamine solution |
| Collector / Col-1 | 1 | 2.0 | 1200 | 1900 | for filtrate |
| Collector / Col-2 | 1 | 0.8 | 1000 | 1150 | for filtrate |

To visualize the organization of the dicarbazine production process, a technological flowchart has been developed that allows planning, process staging, and control of the required process parameters at each technological stage (Fig. 3).

We have developed an equipment and technology scheme showing the principle of technology realization (Figs. 4, 5), taking into account the step-by-step process of dacarbazine production (Fig. 3) and the technological equipment selected for it (Table 2).

At *Stage 1* purified water is input into the reactor R-1, which is made of stainless steel and equipped with a jacket and anchor stirrer. After that, 5-amino-1*H*-imidazole-4-carboxamide is added through a hatch. The mixture is cooled to a temperature of +5 °C. Observe the dissolution of carboxamide. Without stopping the cooling, a solution of concentrated hydrochloric acid is added from the storage tank St-1 through the measuring tank MT-1 using compressed nitrogen. After that, an aqueous solution of sodium nitrite is added to the reactor R-1. The reaction mixture is stirred at +5 °C and the formation of a yellow suspension of diazonium salt is observed for half an hour with constant stirring.

At *Stage 2* the reaction mixture with diazonium salt from reactor R-1 is passed to the filter F-1. The filtrate is collected in the collector Col-1 for regeneration. The diazonium salt from the filter is transferred to the reactor R-2.

At *Stage 3* methanol is added to the reactor R-2 from storage St-2 through the measuring tank MT-3 using compressed nitrogen. Diazonium salt is added through the hatch from the filter F-1 and dissolved at stirring and cooling to + 5 °C. Then, a 40 % aqueous solution of dimethylamine is added to the reactor R-2 from storage St-3 through the measuring tank MT-4. During 2 h of stirring with constant cooling and stirring of the reaction mixture, the formation of dacarbazine precipitate is observed.

At *Stage 4* the reaction mixture from the reactor R-2 is filtered on the filter F-2. The filtrate is collected in the collector Col-2 for regeneration.

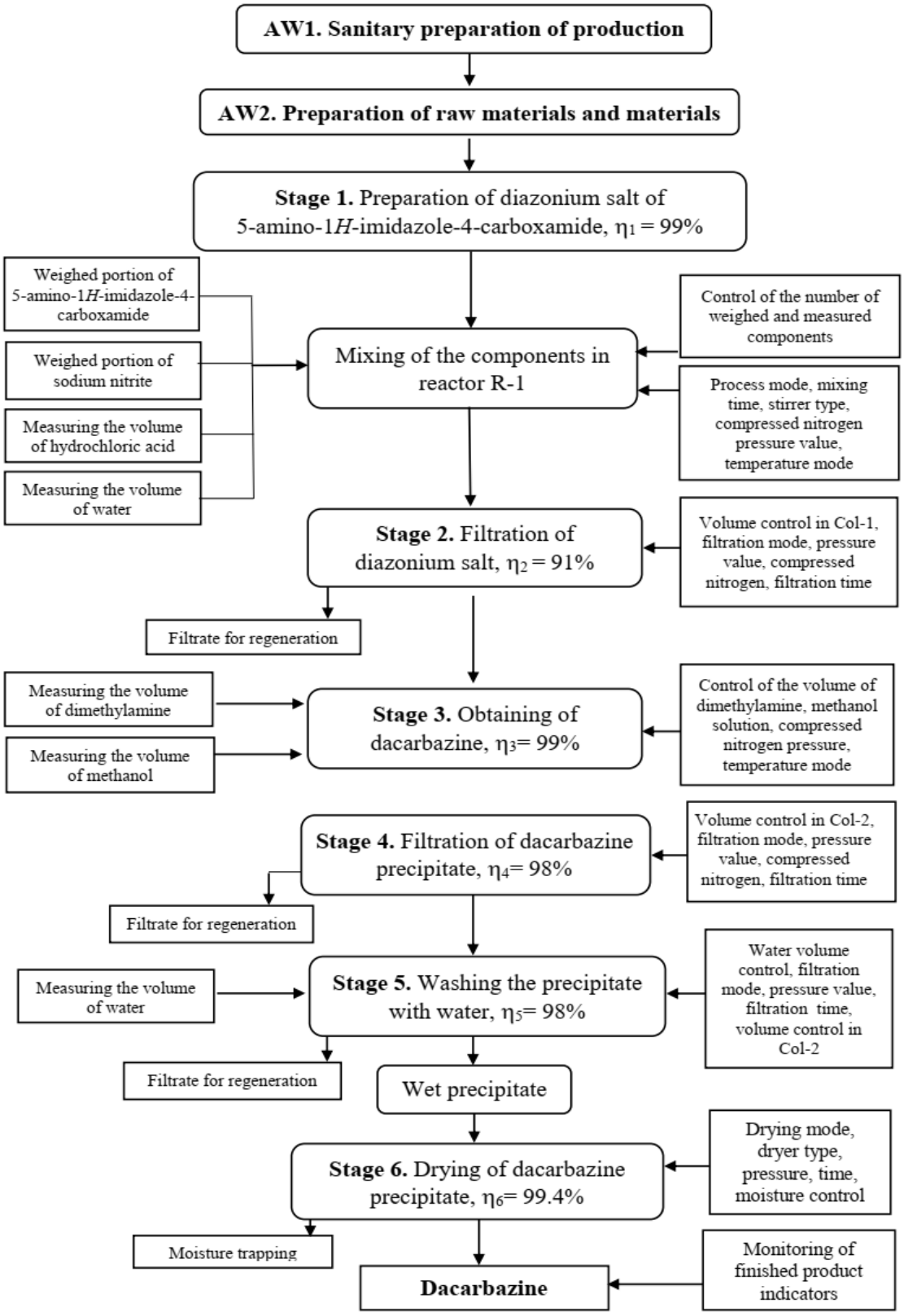



*Fig. 3. Flow chart of the technological process of dacarbazine production*

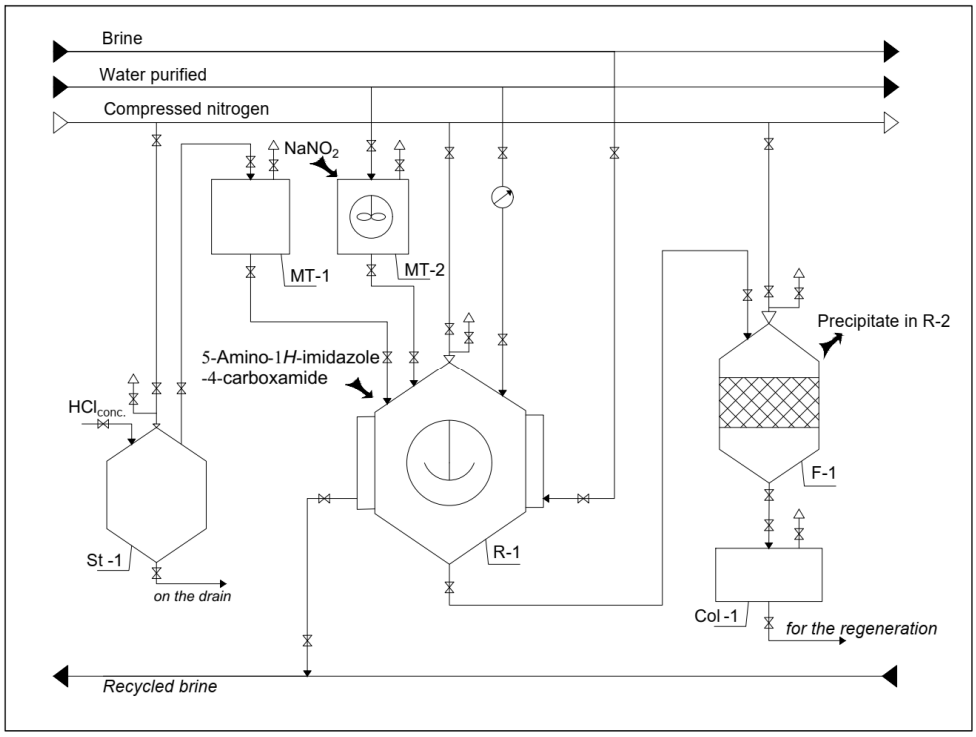



*Fig. 3. Principal equipment and technological scheme of dacarbazine production*

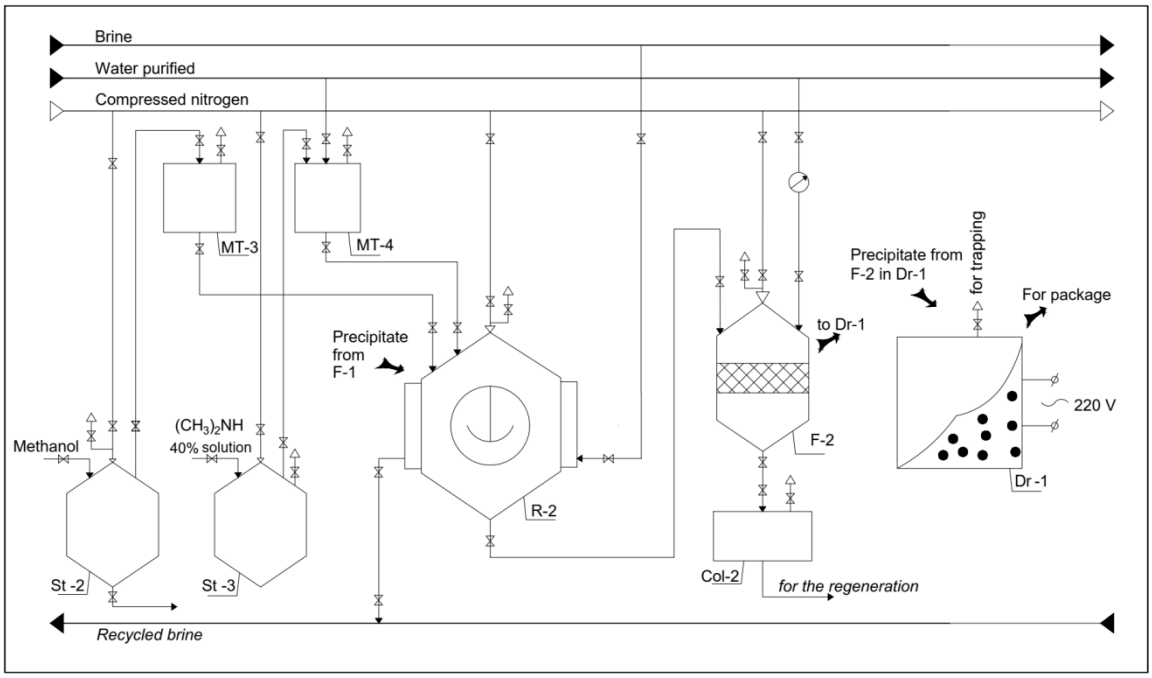



*Fig. 4. Principal equipment and technological scheme of dacarbazine production (continuation)*

At *Stage 5* the dacarbazine precipitate is washed with water on the filter F-2. The filtrate is collected in the collector Col-2 for regeneration. The precipitate from the filter is transferred to the drying stage.

At *Stage 6* the wet dacarbazine precipitate is dried in a dryer Dr-1 to a moisture content of 0.5 %. Upon completion of the drying process, the dry precipitate is sent for packaging and quality control of the finished product.

## Conclusions

It is determined that the dacarbazine-based drug currently available on the Ukrainian pharmaceutical market is the only one and is highly expensive. Dacarbazine, produced in the Czech Republic, is imported into Ukraine and is registered in the State Register of Medicines. However, there is currently no domestic drug on the market. Considering the rapid growth of melanoma in Ukraine, there is an urgent need to develop technology for the domestic production of anti-tumor agents, including dacarbazine.

The analysis of known synthetic approaches in the literature to the preparation of dacarbazine allowed to choose a laboratory method using commercially available reagents. The scale-up of the laboratory method to production requirements has enabled the development of the basic technology for the production of dacarbazine substance with a capacity of 35 tonnes per year and a yield of 86 %, consisting of six main technological steps. In this project of production, "green" solvents – water and methanol – are used. The proposed production does not require a large amount of technological equipment to produce 1 tonne of finished substance in a periodic process. The main material and technological aspects calculated for this dacarbazine production project, as well as the proposed technological flowchart and equipment and process scheme, create the prospect of further development, approbation, and implementation of the domestic technology for obtaining active pharmaceutical ingredients.

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## РОЗРОБЛЕННЯ ОСНОВ ТЕХНОЛОГІЇ ВИРОБНИЦТВА СУБСТАНЦІЇ ДАКАРБАЗИНУ ІЗ ПРОТИРАКОВОЮ ДІЄЮ

**Викладено основи технології виробництва субстанції дакарбазину, яка є діючою речовиною лікарських засобів для лікування метастазної меланоми, лімфоми Hodgkin's та саркоми м'яких тканин. На основі лабораторної методики здійснено масштабування виробництва як періодичного з потужність 35 тонн за рік для одержання 1 тонни субстанції з комерційно доступних реагентів. Визначено шість основних технологічних стадій виробництва, що дають змогу одержати продукт з виходом 86 %. На основі матеріальних розрахунків визначено необхідні операційні завантаження компонентів на кожній стадії. Вибрано технологічне обладнання для здійснення процесу. Організацію процесу наведено у технологічній блок-схемі та апаратурно-технологічній схемі.**

**Ключові слова: меланома, дакарбазин, 5-аміно-1*H*-імідазол-4-карбоксамід, діазотування, реакція сполучення, диметиламін, розроблення основ технології, технологічна схема.**