# OPTIMIZATION OF THE EXTRACTION PROCESS WHEN RECEIVING THE SUM ALKALOIDS OF THE CRAMBE KOTSCHYANA

R.A. Botirov, U.T. Karimov, S.F. Aripova, Sh.Sh. Sagdullaev

Institute of the chemistry of plant substances AS Uz*, 100170, Mirzo Ulugbek st., 77, Tashkent, fax: (998 71) 262 -73-48, e-mail: ulugbek_chem@mail.ru*

**Resume.** Using the method of mathematical planning experiments on Box-Wilson, to develop a technological scheme for obtaining the substance of the drug found the optimal conditions for the extraction process: row material size - 5 mm, solvent -80% ethanole, temperature - 20°C, extraction time - 6 hours.

**Keywords:** *Crambe kotschyana*, Cruciferae, goitrine, goitridine, alkaloids, extraction, technology, optimization process, Box-Wilson method, *Crambe kotschyana*

**Introduction**. The plants of the Crambe genus (Brassicaceae (Cruciferae)) – is the perennial herbs, widespread in Central Asia, have 12 species. In Uzbekistan there are 4 species: *C. Kotschyana* Boiss., *C. edentula* F., *C. schugnana* Korsh., *C. Gordjadinii* Spryg. Et Pol., *C. orientalis* (*C. amabilis*) Butk. Et Majlun, which grow mainly in Namangan (Kasan-Sai), Ferghana (Fedchenko-Gorchakovo) areas [1]. *Crambe kotschyana* Boiss. - Krambe Kochi (Qatron) - a perennial endemic forage plant, growing 2.5 meters in height. Habitat and distribution at the outcrops of variegated colored rocks, along gravelly and fine-grained slopes of the lower and middle mountain belts in Uzbekistan (Tashkent, Andijan, Fergana, Samarkand, Kashkadarya and Surkhandarya regions; Karakalpak Autonomous Regions), Central Asia, Iran, Afghanistan, Western Tibet.

According to the literature, the fruits contain vegetable oil to 17.5%, seeds - from 30.0 to 38.0%. In the aerial part identified coumarins, vitamin C, β-carotene. The seeds in Central Asian folk medicine are used in catarrh of the upper respiratory tract. The roots and stems are edible and are used by the local population for food, as well as for animal feed. In the roots the presence of carbohydrates: starch, disaccharides and monosaccharides. Some species of Crambe in Uzbekistan are introduced, the reaches yield 16-20 c/ha of dry mass [2]. The aerial part of C. kotschyana, collected in April 2009 in the Jizzakh region were studied. The content of alkaloids in the aerial part is 0.2% [3].

The *C. kotschyana* Boiss. herb is the source for the preparation of a crambinine drug of an antithyroid action. The sum of alkaloids contains alkaloids of goitrin and goitridine [4].

Extraction of biologically active substances is the main stage of processing the medicinal raw materials plant and animal origin [5].

In pharmaceutical technology water, organic solvents and their mixtures, as well as aqueous solutions of acids and alkalis are used as selective solvents in during extraction [6].

The efficiency of the extraction process depends on many factors, the main of which are: hydrodynamic conditions, the interface, the difference in concentration, the duration of the process, the viscosity of the extractant, and the temperature. Therefore, the study of the extraction of biological active substances from plant materials is the determining factor in the technological process.

To develop the technology of extract of plant raw materials, it is necessary to select the optimal extraction conditions.

The aim of this work is optimization of the extraction process for the production of Krambinin from the aerial part of *Crambe kotschyana*.

To optimize the extraction process, the method of mathematical experiment planning, which has a wide application in pharmacy [7], was used.

**Experimental.** However, it is known that the extraction of natural compounds depends on many factors, each of them has a greater or lesser effect on the yield of the final product.

The studies were carried out on the basis of one-factor experiments to collect a priori information, i.e. in each experiment the parameters of only one of the factors affecting the process were changed, the others remained unchanged.

Therefore, to evaluate the extent of their effect on extraction, as well as to determine the conditions for the maximum yield of the sum of alkaloids from the aerial part of the plant, we applied the method of mathematical planning for the Box-Wilson experiment.

The optimization parameter was the yield of the sum of alkaloids at the first contact of the phases. In all experiments, the amount of raw material and the method of isolation were identical. In the experiments 0.5 kg of air-dry raw material was used in static conditions.

To determine the maximum yield of extractive substances and alkaloids, it is necessary to select the optimal extractant. As an extractant, ethyl alcohol of various concentrations (60, 70, 80%) was used. The extract was obtained by percolation.

The variable factors affecting the yield of alkaloids were: concentration of extractant, degree of grinding of raw materials, duration of extraction and infusion temperature. To find the optimal values of variable factors, the method of mathematical experiment planning was used.

Based on the theoretical principles of extraction, under the following equilibrium conditions, the following restrictions on the levels of variable factors were introduced: the extractant concentration ($x_1$) from 60 to 80; degree of crushing of raw materials ($x_2$) from 3 to 7 mm; duration of extraction .000000 ($x_3$) from 3 to 9 hours; extraction temperature ($x_4$) from 20 to 60 ºC. On the basis of a priori information (in this case, the results of one-factor experiments), the factors most influencing the extraction were chosen and established for them the following basic levels and variation intervals (Table 1).

**Table 1**

**Factors and variation intervals**

| Factors | Variation levels | | | variation intervals | Unit of measurement |
|---|---|---|---|---|---|
| | Low | Middle | Upper | | |
| $X_1$ | 60 | 70 | 80 | 10 | % |
| $X_2$ | 3 | 5 | 7 | 2 | mm |
| $X_3$ | 3 | 6 | 9 | 3 | h |
| $X_4$ | 20 | 40 | 60 | 20 | $^oC$ |

There are two levels of four factors, i.e. complete factor experiment of type $2^4$. We used a fractional replica 2, replicas from the full factorial experiment $2^4$ with the use of planning type $2^{4-1}$ with generating relations $X_4 = X_1*X_2$.

The experimental planning matrix and the obtained results are shown in Table 2. Each of the eight experiments was performed in accordance with the matrices formed, using the selected levels of each factor encoded in the matrix by the signs "+" or "-" (respectively, upper and lower levels of variation).

The results of the experiments are presented in the form of a regression equation:

$Y=b_0+b_1x_1+b_2x_2+b_3x_3+b_4x_4$

The $b_0$, $b_1$, $b_2$, $b_3$, $b_4$, are the regression coefficients of the incomplete quadratic equation.

Using the formula the values of the regression coefficients were calculated:

$b_0$= 4,38; $b_1$= 7,35; $b_2$= 0,76; $b_3$= 4,46; $b_4$= 2,42;

Substituting the calculated values of the "b" coefficients in the equation, we obtained the following first-order regression equation: $Y = 4{,}38+ 7{,}35\ X_1 + 0{,}76\ X_2 + 4{,}46\ X_3 + 2{,}42\ X_4$

To ensure the correctness of the experiment, the adequacy of the obtained model, statistical processing of the data was carried out.

**Table 2**

**Experimental Design Matrix and Results**

| № exp. | Factor code | | | | | $Y_1$ | $Y_2$ | $Y_{aver}$ | ΔYi | $\Delta Y_i^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | $X_0$ | $X_1$ | $X_2$ | $X_3$ | $X_4=X_1X_2$ | | | | | |
| 1 | + | + | + | + | + | 55,1 | 54,9 | 55 | 0,10 | 0,01 |
| 2 | + | + | - | + | - | 24,5 | 24,1 | 24,3 | 0,20 | 0,04 |
| 3 | + | - | + | + | - | 29,1 | 26,3 | 27,7 | 1,40 | 1,96 |
| 4 | + | - | - | + | + | 26,2 | 22,9 | 24,5 | 1,65 | 2,72 |
| 5 | + | + | + | - | + | 46,8 | 36,5 | 41,6 | 5,15 | 26,5 |
| 6 | + | + | - | - | - | 25,3 | 24,9 | 25,1 | 0,20 | 0,04 |
| 7 | + | - | + | - | - | 36,6 | 30,5 | 33,5 | 3,05 | 9,30 |
| 8 | + | - | - | - | + | 29,6 | 20,7 | 25,1 | 4,45 | 19,80 |

A dispersion calculated by the formula use to determine the variation of the values of repeated experiments:

$$S_i^2 = \frac{2\Delta Y^2}{1}$$

$Y_q$ – result of individual experiment, $Y_{cp}$ – arithmetic mean; (n – 1) – the number of degrees of freedom equal to the number of repeated experiments minus one.

The homogeneity of the dispersion was calculated by the Cochran test:

$$G_{exp} = \frac{S^2 \max}{\sum_{i=1}^{N} S^2{}_i} \leq G_{cr}$$

$G_{cr}$ = 0,6798

$G_{exp}$= 0,4391

The result obtained corresponds to the conditions of the formula. The dispersion is homogeneous.

To verify the adequacy of the model obtained, a dispersion of adequacy was first determined.

$$S_{ad}^2 = \frac{n\sum(Yav - Yac)^2}{N - q}$$

$S_{ad}^2 = 36{,}6$

$$S_y^2 = \frac{\sum_{i=1}^{N}\sum_{q=1}^{n}\left(Y_{iq} - Y\right)^2}{N(n-1)}$$

$S_y^2 = 15{,}1$

The adequacy of the model was checked by the Fisher criterion:

$$F_{exp} = \frac{S_{ad}^2}{S_y^2} = 2{,}42$$

$F_{tab}$ (2.8) = 4,5

In this case
$F_{exp} < F_{tab}$; 2,42 < 4,5
следовательно, модель адекватна.

To check the significance of the regression coefficients, a dispersion of the regression coefficients was found:

$$S_{bi}^2 = \frac{S_y^2}{N} = 1{,}88$$

$$S_{bi} = \pm\sqrt{S_{bi}^2} = 1{,}37$$

Confidence interval is determined: $\Delta b_i = t\, S_{bi}$ ($\Delta b_i = 0{,}045$).

t - is the table value of the Student's test for the number of degrees of freedom with which $S_y^2$ was determined at the selected significance level (Δtкр = 3,182);

$S_{bi}$ – quadratic error of the regression coefficient.

The coefficient is significant if its absolute value is greater than the confidence interval. According to the quantitative contribution, the factors were arranged in the following order:

$X_1 > X_3 > X_4 > X_2$

Table 3

The significance of the coefficients

| $b_i$ –value | sign | $\Delta b_i$ –value | Results |
|---|---|---|---|
| 32,12 | > | 4,371 | The coefficient is significant. |
| 4,38 | > | 4,371 | The coefficient is significant. |
| 7,35 | > | 4,371 | The coefficient is significant. |
| 0,76 | < | 4,371 | The coefficient is not significant. |
| 4,46 | > | 4,371 | The coefficient is significant. |
| 2,42 | < | 4,371 | The coefficient is not significant. |

As can be seen from Table. 3, the factors $X_1$, $X_2$, $X_3$ were significant, which is quite understandable.

**Conclusion**. One of the tasks of optimization of extraction by the method of mathematical planning of the experiment is to quantify the contribution of each of the selected factors to the extraction result. According to the quantitative contribution, the factors are arranged in the following order: : $X_1>X_3>X_4>X_2$.

The yield is 54.9%, which is quite acceptable for the first contact of the phases. From the regression coefficients of the equation, after calculating the confidence interval ($\Delta_{bi}$ = 4,371), it was established that the main factors affecting the process include the degree of milling of raw materials, the concentration of alcohol and the duration of extraction. Statistical analysis ($F_{ex}$= 2.42 <$F_{tab}$ = 4.5) showed that the mathematical model is adequate.

A steep ascent was not carried out, as a further increase in temperature adversely affected the quality of the amount of alkaloids, and also led to additional costs in obtaining the target product.

At Department of Pharmacology and Toxicology of the Institute of Chemistry of the Plant Substances of the Academy of Sciences of the Republic of Uzbekistan on the model of experimental thyrotoxicosis in rats the antithyroid activity of the sum alkaloids of *C. kotschyana* was studied and determined. The revealed activity is expressed in the normalization of the condition and behavior of the experimental animals, as well as in the leveling of thyroid hormone levels in the serum and is confirmed by morphological analysis data were established.

Recovery of cell and tissue structural organization of the thyroid in experimental animals when administered amount *C. kotschyana* sum of alkaloids were shown. This served as an incentive for the development on its basis of a drug for use in endocrinological practice in the treatment of patients with manifestations of thyrotoxicosis [8]. The patent of the Republic of Uzbekistan No. IAP 01.17.2012 was received on "Anti-thyroid agent" [9].

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**Р.А. Ботиров, У.Т. Каримов, С.Ф. Арипова, Ш.Ш. Сагдуллаев.**

## ОПТИМИЗАЦИЯ ПРОЦЕССА ЭКСТРАКЦИИ ПРИ ПОЛУЧЕНИИ СУММЫ АЛКАЛОИДОВ *CRAMBE KOTSCHYANA*

Институт химии растительных веществ им. акад. С. Ю.Юнусова АН РУз, г. Ташкент, ул. Мирзо Улугбека, 77. тел: +998 71 262 59 13 e-mail: botiroovr@mail.ru

Для разработки технологической схемы получения субстанции предполагаемого лекарственного средства, названного условно крамбинин с помощью метода математического планирования эксперимента по Боксу-Уильсону найдены оптимальные условия процесса экстракции: это экстракция измельченного растительного сырья с размерами частиц 5 мм 80%-ным этиловым спиртом при температуре 20°C, продолжительность экстракции 6 ч. При этом выход суммы алкалоидов составил 95% от содержания в сырье.

**Ключевые слова:** гоитрин, гоитридин, алкалоиды, экстракция, технология, процесс оптимизации, метод Бокса–Уильсона, *Crambe kotschyana*.

**Р.А. Ботиров, И.И. Охунов, У.Т. Каримов, С.Ф. Арипова, Ш.Ш. Сагдуллаев.**

## КРАМБИНИН ДОРИ ВОСИТАСИНИ АЖРАТИБ ОЛИШДА ЭКСТРАКЦИЯ ЖАРАЁНИНИ МАТЕМАТИК РЕЖАЛАШТИРИШ

ЎзР ФА акад. С. Ю. Юнусов номидаги Ўсимлик моддалари кимёси институти, Тошкент ш., Мирзо Улугбек кўч. 77, тел: +998 71 262 59 13 e-mail: botiroovr@mail.ru

Крамбинин дори воситасини ажратиб олиш технологиясини ишлаб чиқиш учун экстракция жараёнини оптимал шароитларини ўрганишда, тажрибаларни математик режалаштириш усулидан фойдаланилди. Бунда эритувчининг концентрацияси 80%, хом ашёнинг майдалик даражаси 5 мм, экстракция жараёнини бориши 6 соат ва ҳарорат 20°C бўлганда алкалоидларни чиқиши 95% ташкил этиши аниқланди.

**Калит сўзлар:** *Crambe kotschyana*, гоитрин, гоитридин алкалоидлар, экстракция, технология, жараён, оптимал, босқич, ўсимлик, хом ашё, математик режалаштириш, Бзокс-Уильсон усули.