

Optimizing the Method for Obtaining Monoammonium Salt of Glycyrrhizic Acid from the Ural Licorice (*Glycyrrhiza uralensis* Fisher) Roots of Siberian Populations

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Abstract

A method was optimized for obtaining monoammonium salt of glycyrrhizic acid (glycyram) from roots of Ural licorice (*Glycyrrhiza uralensis* Fisher) of Siberian populations, including the following stages: the extraction treatment of roots with 0.5 % NH_4OH solution, the sedimentation of the sum of acids using concentrated H_2SO_4 , the subsequent re-extraction with 1 % H_2SO_4 solution in acetone, the precipitation of triammonium salt of glycyrrhizic acid with 25 % NH_4OH solution and its conversion into monoammonium salt (glycyram) via crystallization from glacial CH_3COOH . With the use of this method one could obtain the samples of glycyram with the purity of 85.2–87.5 % (according to HPLC data). The content of glycyrrhizic acid in the samples of the Ural licorice roots under investigation ranges within 2–4 %.

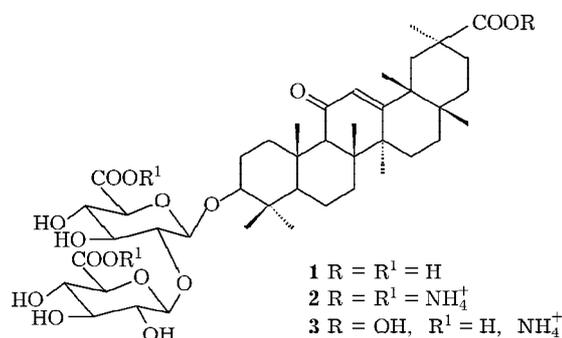
Key words: the Ural licorice (*Glycyrrhiza uralensis* Fisher), glycyrrhizic acid, glycyram

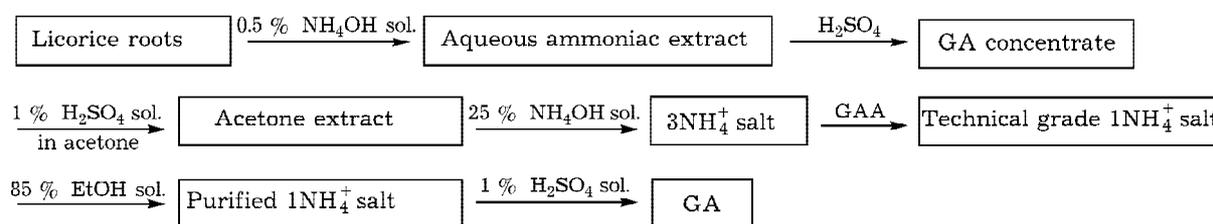
INTRODUCTION

The Ural licorice (*Glycyrrhiza uralensis* Fisher) represents a valuable herb from the legume family (Leguminosae) belonging to the section of true licorices *Euglycyrrhiza* Boiss and is allowed for use in Russian domestic official medicine alongside with another licorice species *Glycyrrhiza glabra* L. [1]. The Ural licorice is used in also traditional Chinese and Tibetan folk medicine [2]. The medicinal value of licorice root is determined by the presence of biologically active substances, namely, triterpene glycoside, or glycyrrhizic acid (GA) **1** which exhibit a high and manifold physiological activity (anti-inflammatory, antiulcerogenic, antiallergic, antidotal, antitumor, antiviral, etc. activities) [3].

The monoammonium salt of GA **3** is the basic substance of the domestic preparation glycyram that is used in medicine as anti-inflammatory and antiallergic remedy. Glycyram

is recommended for the treatment of bronchial asthma in children as a substitute of steroids, as well as for the treatment of allergic dermatitis, eczema and other diseases [4]. Glycyram can serve as a remedy for additional therapy in oncology [5]; it exhibits myelotropic and cardioprotective action [6, 7]. The monoammonium salt of GA is used in the food industry of many countries as a safe sweetener and a flavour additive [8].





Scheme 1.

The methods for GA and its monoammonium salt isolation from licorice roots are being improved for decades. For example, the authors of [9, 10] proposed the methods for obtaining glycyram from a concentrated extract of licorice roots, whereas the authors of [11] offered to use the roots of *Gl. glabra* L. These methods include the use of ion-exchange resins in the process of glycyram isolation, which technologically complicates the process of target product isolation, since this case requires for the application of columns with a great volume of resin and the use of a significant volume of solvents for glycoside absorption and desorption from the columns. In addition, for monitoring the purity of the target product, glycyram, earlier only spectrophotometric methods were used which resulted in overestimating of the data concerning the content of target products [9–11].

A well-known method described in [11], allows one to obtain technical grade glycyram from roots of *Gl. glabra* L., wherein the content of GA can reach 24 % [12]. This method includes the following stages: the fermentation of grinded licorice roots with water at 35–40 °C during 22–26 h, the subsequent extraction treatment of the roots with 0.25 % NH_4OH solution, acidifying with concentrated H_2SO_4 up to pH 1–3, drying of the sum of acids at 50–60 °C under vacuum in drying chamber during 20–25 h, the extraction with anhydrous acetone in three stages at 60–70 °C and the precipitation of triammonium GA salt **2** with 25 % NH_4OH solution. The treatment of latter by a triple amount of glacial acetic acid (GAA) results in the formation of technical grade glycyram **3** with the yield amounting to 12.3 g per 100 g of roots (50 % as calculated for roots with the content of GA about 24 %).

TABLE 1

Experimental conditions and the yield of glycyram from roots of the Ural licorice (100 g)

Exp. No.	Habitat	GA content, %	NH_4OH volume, mL	Yield, g	
				Dry concentrate (% GA)	3NH_4^+ salt of GA
1	Karauok District	3.7	800	8.7 (42.5)	5.3
2	Buryatia	2.0	1500	5.2 (38.9)	3.9
3	Novosibirsk Region	2.0	1000	3.8 (52.0)	3.0
4	« «	2.0	1500	5.4 (37.0)	2.3
5	« «	n/d	1500	6.8 (n/d)	4.2
6	« «	2.0	1500	4.8 (41.5)	3.0
7	« «	3.9	1500	6.7 (58.1)	3.5
8	« «	3.8	1500	6.4 (60.2)	3.2
9	« «	3.7	1500	7.2 (51.1)	5.6
10	« «	4.0	1500	6.9 (58.0)	5.2

Notes. 1. In the exp. No. 1 the extraction was carried out with 2.0 % NH_4OH solution, in the exp. Nos. 2–10 with an extracting agent (1 % H_2SO_4 solution in acetone) (in mL) and dry concentrate (in g) amounted to 5 : 1, in the other and salts **2** amounted to 10 : 1, in the other experiments the ratio was equal to 3 : 1. 4. In the exp. Nos. 7, 8 the yield of in the other experiments it was determined after recrystallizing from 85 % EtOH solution. 5. n/d – not determined.

As it was demonstrated in our studies [13], the content of the basic substance in technical grade glycyram, does not exceed $(75 \pm 2) \%$, according to HPLC data. Besides, the authors of [12] offered to use roots of *Glycyrrhiza glabra* L. as a raw material with a high GA content whose resources are concentrated outside Russia (Afghanistan, Kazakhstan, Turkmenistan, Uzbekistan, etc.). Over the territory of Russia (the Siberian and Ural regions) such species as the Ural licorice widely occurs with a lower content of GA. So, according to the authors of [14], the content of GA in the Ural licorice widespread in West Siberia can reach 9.2–10.9 %.

The goal of the present work consisted in the development of an improved method for obtaining monoammonium GA salt **3** from roots of the Ural licorice harvested in various regions of Siberia, as well as the investigation of various samples of roots for GA content using HPLC method. Scheme 1 displays the sequence of operations we offered whereby the samples of monoammonium GA salt (1NH_4^+ salt) **3** were obtained meeting the requirements of Pharmacopoeial Standard VFS 42-419-75 (the content of GA > 87 %).

Grinded roots of the Ural licorice were extracted with 0.5 % aqueous solution of NH_4OH at 20–22 °C, a sum of acids was precipitated with concentrated H_2SO_4 (pH 1–2). The sediment obtained was filtered, washed

with water so as to obtain neutral pH value of washing waters, then it was dried and a dry concentrate of GA was obtained. The latter was repeatedly extracted with 1 % H_2SO_4 solution in acetone at 20–22 °C, the ratio between the liquid and solid phases (expressed in mL/g) being equal to 10 : 1 or 5 : 1. Then it was extracted with pure acetone under boiling within 30 min. The acetone extracts were joined and then a triammonium GA salt **2** was precipitated using 25 % NH_4OH solution (pH 7–8) (see Scheme 1).

The triammonium salt **2** was filtered, dried and recrystallized from GAA. The sediment of technical grade monoammonium GA salt **3** formed in this process was filtered, washed with acetone and dried. According to HPLC data, the content of the basic substance in the glycyram samples obtained amounted to 65.5–69.6 %. After a single-stage recrystallization from 85 % EtOH we obtained purified glycyram with the content of the basic substance amounting to 82.5–87.5 (± 0.8) %.

The results of experiments are presented in Table 1. One can see that the maximal yield of the GA concentrate is observed under extraction of grinded licorice roots with 0.5 % NH_4OH solution in two stages (1000 and 500 mL) and re-extraction of dry GA concentrate with 1 % H_2SO_4 solution in acetone at a

Technical grade glycyram	Recrystallized glycyram	Purity according to HPLC (± 0.8 %)	Melting point, °C
27	1.8	85.2	218–221
16	0.8	85.6	224–226
09	0.5	87.0	221–223
12	0.6	85.2	223–225
25	1.2	87.5	223–225
14	0.7	85.5	218–220
22	0.8	83.8	228–232
23	1.0	82.5	230–234
27	1.3	87.0	220–222
26	1.3	87.6	223–225

0.5 % NH_4OH solution was used for the extraction. 2. In the exp. Nos. 1, 2, 4, 5 the ratio experiments the ratio was equal to 10 : 1. 3. In the experiment No. 3 the ratio of GAA (in mL) recrystallized glycyram was determined after recrystallizing from 70 % EtOH solution,

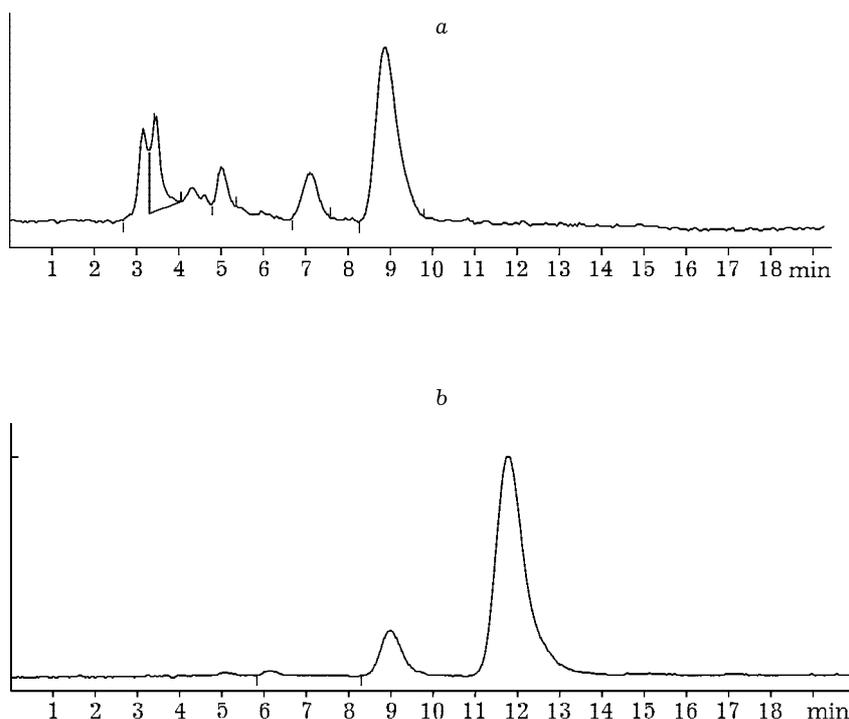


Fig. 1. Chromatographic profile of GA concentrate (a) and purified glycyram (b) obtained from roots of the Ural licorice.

ratio $S : L = 10 : 1$ (10 mL of the extracting agent per 1 g of the concentrate). According to HPLC data, the maximal yield of GA concentrate extracted from roots of Siberian species of the Ural licorice amounts to 6.4–7.2 g for two-stage extraction with 0.5% NH_4OH solution having GA content of 51.1–60.2% (Fig. 1, a). This corresponds to GA content in roots, equal to 3.7–4.0% (for the roots harvested over the territory of the Novosibirsk Region). The minimum content of GA in the samples of licorice root under investigation amounts to 2.0%, whereas the content of GA in the concentrates obtained from these root samples varies within the range of 37.0–41.5%.

A more complete GA extraction level can be reached using the extraction treatment of roots with 0.5% NH_4OH solution instead of 0.25% NH_4OH solution according to the method described in [11], as well as with the use of 1% H_2SO_4 solution in acetone as the second extraction agent. Moreover, we have excluded the stage of licorice root preliminary fermentation with water and the operation of drying the sum of acids in a drying chamber. Due to this fact, the process of the target product extraction

from plant raw material proceeds much faster at lower thermal energy inputs.

The yield of technical grade monoammonium salt **3** with the content of the basic substance of 68.2–69.6% with respect to 100 g of roots for the plants growing over the territory of the Novosibirsk Region and Buryatia, amounts to 0.9–2.7 and 1.6 g, respectively and depending on licorice habitat and extraction conditions. The yield of glycyram depends also on the amount of GAA used in the operation of transforming 3NH_4^+ salt **2** into 1NH_4^+ salt **3**. The optimum variant for the operation mentioned consists in carrying out the crystallization of salt **2** from the GAA (on heating GAA up to 90–95 °C) at a ratio between the volume of acid and the mass of salt **2** equal to 3 : 1. The quantitative content of the basic substance in glycyram samples obtained after single-stage recrystallization from 85% ethanol according to HPLC data amounted to 85.2–87.6 (± 0.8)% (see Table 1).

The samples with GA content equal to 87% and higher (see Fig. 1, b) meet the requirements of VFS 42-419-75. The total yield of purified glycyram as calculated with respect to dry roots amounts 32–35%. The samples of glycyram

obtained via recrystallization from 70 % EtOH solution are characterized by a lower content of the basic substance (82.2–83.8 %).

The processing of purified monoammonium GA salt with 1 % H₂SO₄ solution and chloroform resulted in obtaining the samples with GA content of (87.5±0.8) %. Taking into account the crystallization loss of 3NH₄⁺ GA salt from GAA, the total content of GA in root samples of the Ural licorice from Siberian populations under investigation ranges within 2.0–4.0 %. These populations of licorice are appropriate to use as a plant raw material for obtaining the basic substance of glycyram according to the process flowsheet we have suggested.

EXPERIMENTAL

UV spectra were registered using a Specord UF-400 spectrophotometer. The optical activity was measured with the use of a Perkin-Elmer 241 MC polarimeter in a tube with a length of 1 dm. The TLC assay investigation was performed using Silufol plates (Czechia). The spots of substances were visualized with 20 % phosphotungstic acid solution in ethanol on heating up to 110–115 °C. The HPLC analysis was carried out using a DuPont chromatograph with a UV detector ($\lambda = 254$ nm), the columns being packed with μ -Bondapak C-18 (3.9 × 300 mm) reverse phase, with the use of MeOH–CH₃COOH–H₂O mixture with 60 : 35 : 5 volume ratio as a mobile phase (MP); the MP flow rate being equal to 1 mL/min.

General technique for obtaining monoammonium GA salt (glycyram) from roots of the Ural licorice

Grinded roots of the Ural licorice (100 g) were covered with 1000 mL of 0.5 % aqueous solution of NH₄OH, shaken within several minutes and the mixture was held for 10–12 h at 20–22 °C. The ammoniac extract obtained was decanted, roots were additionally covered with 500 mL of 0.5 % NH₄OH solution and the mixture was held for 14–15 h. The extracts were joined together and then filtered. The filtrate was added with concentrated H₂SO₄, the acidity of the solution being brought up to pH 1–2.

The precipitate formed was subjected to sedimentation, filtered using a Buechner funnel, washed with distilled water (pH ~ 7) and dried first in air and further at the temperature of 110–120 °C during 6–7 h. The sediment obtained (GA concentrate) was extracted with 1 % H₂SO₄ solution in acetone (with a ratio between the liquid and solid phases amounting to 5 : 1 or 10 : 1) during 1 h at 20–22 °C. The acetone extract was drained *via* decantation; a residue was added with acetone and boiled then within 30 min using a water bath. Both the extracts were joined together, and 25 % NH₄OH solution was added under stirring to gain the value of pH 7–8. Triammonium GA salt **2** was filtered, washed with acetone and dried. Dry salt **2** was dissolved in GAA on heating at 90–95 °C and was then left to crystallize at 20–22 °C. The crystals of monoammonium GA salt **3** formed were filtered, washed with acetone, dried. Thus we obtained technical grade glycyram with the purity of 65.5–69.6 %.

By means of the recrystallization from 85 % ethanol we obtained purified glycyram. The content of the basic substance therein, according to HPLC data, amounted to 85.2–87.6 (±0.8) % (μ -Bondapak C-18 column, 3.9 × 300 mm); mobile phase: MeOH–H₂O–CH₃COOH with a ratio 60 : 35 : 5 (in volume); flow rate being equal to 1.0 mL/min; UV detector ($\lambda = 254$ nm). The yield values are presented in Table 1. The content of water in the samples obtained samples ranged within 6.0–7.5 %. UV spectrum (25 % EtOH solution): $\lambda_{\text{max}} = 253$ –256 nm, $\log \varepsilon = 3.92$ –4.12; $[\alpha]_D^{20} + (45 \pm 5)^\circ$ (s 0.04, 25 % EtOH solution). The melting point for the samples obtained depends on the content of impurities and water (see Table 1). According to the authors of [15], m.p. 220–222 °C (decomp.).

Glycyrrhizic acid obtaining from glycyram

Purified monoammonium GA salt **3** ((87.0±0.8) %) in amounts of 0.6 g was processed with 6 mL of 1 % H₂SO₄ aqueous solution on heating by means of a water bath during 10 min, then it was cooled and held in a refrigerator during 14–15 h. White GA sediment was filtered, washed with ice-bath water, dried and extracted with chloroform (5 mL) under stir-

ring. According to HPLC data, the yield of GA amounted to 0.42–0.45 g, the content of basic substance being equal to $(87.5 \pm 0.8) \%$. UV spectrum (MeOH): $\lambda_{\max} = 249 \text{ nm}$, $\log \varepsilon = 4.07$; $[\alpha]_D^{20} + (60 \pm 2)^\circ$ (s 0.04, MeOH), cf. [16]: $[\alpha]_D^{20} + 62^\circ$ (s 0.04, MeOH).

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