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Modeling of solvent effects on phytocompounds' extraction from *glycyrrhizae* radix

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Abstract

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INTRODUCTION

Many plants from the *Fabaceae* family play an important role in human life due to their wide use in agriculture and medicine to obtain foods and drugs. A special place in this family belongs to plants of the *Glycyrrhiza* genus. Some plants of this genus accumulate polysaccharides, pectin substances, triterpene saponosides (in general as potassium and calcium salts of glycyrrhizic acid), tannins, as well as phenolic compounds (flavonoids, chalcones, coumarins, phenolcarbonic acids, etc.), organic acids, sugars, and some other compounds in their underground organs.[1,2,3,4,5]

The main biologically active substances from *Glycyrrhizae radix* are triterpene saponosides (glycyrrhizic acid), flavonoids, and chalcones.[1,3,4] These substances are responsible for some useful pharmacological effects, such as expectorative, anti-inflammatory, anti-allergic, healing, spasmolytic, antiviral, antibacterial.[6,7,8,9,10]

It should be noted that many scientific works all over the world are dedicated to the phytochemical analysis of different extracts from *Glycyrrhizae* radix.[4,5,8] However, the authors have found no systemic studies on the dependency between the qualitative and quantitative content of extracts from *Glycyrrhizae* radix and physical properties of the

solvent, which is an important step for substantiation and development of a rational technology of drugs from this type of plant raw material.

The aim of the article was to study modeling of solvent effects on phytocompounds' extraction from *Glycyrrhizae* radix for substantiation of rational choice of the extractant in the technology of drugs obtained from this type of plant raw material.

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MATERIALS AND METHODS

Plant raw material

For study purposes used plant raw material "*Glycyrrhiza* radices" by privately held corporation "Liktravy," Zhitomir, Ukraine, lot No. 50914, expiry date X/2019, and by privately held corporation "Krasnogorskleksredstva," Krasnogorsk, Russia, lot No. 30417, expiry date V/2020.

Preparation of extracts

Before extraction, the plant raw material was grinded to particle size of 0.1–0.5 mm. The process of extraction was carried out by simple maceration for 24 h at temperature 25°C ± 1°C and extractant/plant raw material ratio 5:1 (v/w). For this purpose used hydro-ethanolic solutions with ethanol concentration of 22%, 41%, 50%, 71%, 82%, and 96% ± 1% v/v, determined with an alcoholmeter and ethanol 55% wt., 2-propanol 55% wt., acetone 55% wt., and methanol 80% ± 1% wt., determined by density.

Chemicals and reagents

For the preparation of hydro-ethanolic solutions used distilled water and ethanol for pharmaceutical and medicinal purposes 96% v/v. For the preparation of 2-propanol 55% wt., acetone 55% wt., and methanol 80% wt. solutions used analytically pure solvents. Qualitative analyses of compounds were carried out by reference substances of licuroside and monoammonium glycyrrhizate (glycyram) (Ukrainian State Pharmacopoeia, Ukraine), and by their ultraviolet-spectra and retention times in the high-performance liquid chromatography (HPLC) fingerprint.

High-performance liquid chromatography analysis

After maceration, the extracts were decanted, centrifuged at 13,000 rpm for 5 min, and analyzed by reversed phase HPLC. A reversed phase HPLC

analysis was carried out on a chromatograph of “Agilent Technologies 1200 Infinity” type, the USA. The process of a reversed phase HPLC analysis was carried out under the following conditions: mobile phase (a): 1% water solution of formic acid, (b): ethanol 96% v/v in linear gradient elution; chromatographic column: Supelco Ascentis express C_{18}, sized 2.7 μm × 100 mm × 4.6 mm; mobile phase velocity: 0.5 ml/min; temperature of the chromatographic column: +35°C; sample volume: 1 μl.

Control of chromatographic system suitability

The chromatographic system can be considered suitable if the following conditions are reached: the peak's theoretical plates (N) has to be not <1000; the peak's asymmetry coefficient (T) has to be <2.0; the peak's separation coefficient (RS) not <1.5; and relative standard deviation (RSD) of peak's area RSD has to be <2.0.[11]

Statistical analysis

All the experiments were repeated four times. Statistical calculations were carried out using MS Excel 2010.

Theoretical part

For mathematical modeling of the influence of solvent's dielectric constant on phytocompounds’ equilibrium concentration in the extract, the authors used theoretical fundamentals of physical chemistry related to Van-der-Waals forces and Gibbs energy.

Herewith, Gibbs energy is related to the equilibrium constant of the phytocompound at a first approximation by the equation, (1) and energy of Van-der-Waals forces can be described by equations (2)– (6), all of which are related to the dielectric constant of the solvent:[12]

$$\Delta G = -RT\ln K = -RT\ln C \qquad (1)$$

$$\Delta G = \Delta G_{solid} + \Delta G_{solv} + \Delta G_{unpred} \qquad (2)$$

$$\Delta G_{solid} = N_A \cdot \left(\frac{\mu_1 \mu_2}{(4\pi \cdot \varepsilon_0 \cdot \varepsilon_y) \cdot r^3} + \frac{3 \cdot \alpha_1 \cdot \alpha_2}{(4\pi \cdot \varepsilon_0 \cdot \varepsilon_y)^2 \cdot 2 \cdot r^6} \cdot \left(\frac{I_1 \cdot I_2}{I_1 + I_2} \right) \right) \quad (3)$$

$$\Delta G_{solv} = -N_A \cdot \left(\frac{\mu_3 \cdot \mu_2}{(4\pi \cdot \varepsilon_0 \cdot \varepsilon_x) \cdot r^3} + \frac{2 \cdot \mu_3^2 \cdot \mu_2^2}{(4\pi \cdot \varepsilon_0 \cdot \varepsilon_x)^2 \cdot 3 \cdot k \cdot T \cdot r^6} + \frac{3 \cdot \alpha_3 \cdot \alpha_2}{(4\pi \cdot \varepsilon_0 \cdot \varepsilon_x)^2 \cdot 2 \cdot r^6} \cdot \left(\frac{I_3 \cdot I_2}{I_3 + I_2} \right) \right) \quad (4)$$

$$\Delta G_{solid} = N_A \cdot \left(\frac{(e_2 z_2) \cdot \mu_1}{(4\pi \cdot \varepsilon_0 \cdot \varepsilon_y) \cdot r^2} + \frac{(e_2 z_2)^2 \cdot \alpha_1}{(4\pi \cdot \varepsilon_0 \cdot \varepsilon_y)^2 \cdot 2 \cdot r^4} \right) \quad (5)$$

$$\Delta G_{solv} = -N_A \cdot \left(\frac{(e_2 z_2) \cdot \mu_3}{(4\pi \cdot \varepsilon_0 \cdot \varepsilon_x) \cdot r^2} + \frac{(e_2 z_2)^2 \cdot \mu_3^2}{(4\pi \cdot \varepsilon_0 \cdot \varepsilon_x)^2 \cdot k \cdot T \cdot r^4} + \frac{(e_2 z_2)^2 \cdot \alpha_3}{(4\pi \cdot \varepsilon_0 \cdot \varepsilon_x)^2 \cdot 2 \cdot r^4} \right) \quad (6)$$

$$\varepsilon_y = \phi_1 \cdot \varepsilon_1 + (1 - \phi_1) \cdot \varepsilon_x \quad (7)$$

where ΔG is Gibbs energy, J/mol; R is gas constant, J/(K·mol); T is absolute temperature, K; K is equilibrium constant; C is equilibrium concentration of the phytocompound in the extract, mol/l; ΔG_{solid} is cohesive energy of biologically active substances and plant raw material matrix molecules, J/mol; ΔG_{solv} is interaction energy of phytocompound and solvent molecules, J/mol; ΔG_{unpred} is unpredicted energy processes, J/mol; N_A is Avogadro number, mol^{-1}; π is mathematical constant; ε_0 is electrical constant, F/m; ε_1 ε_x are dielectric constants of the plant raw material matrix and solvent; μ_1 μ_2 μ_3 are dipole moments of molecules for plant raw material matrix, phytocompound, and solvent, respectively, C·m; α_1 α_2 α_3 are polarizability of molecules for plant raw material matrix, phytocompound, and solvent, respectively, m^3; I_1 I_2 I_3 are ionization energy of molecules for plant raw material matrix, phytocompound, and solvent, respectively, J; e_2, z_2 are phytocompound's valency and charge; r is the distance between the molecules, m; and φ_1 is the volume part of plant raw material matrix.

Equations (3) and (4) can be used for polar molecules, and equations (5) and (6) for ions. After substitution of equations (1), (3), and (4) into (2), we can obtain final equation (8) that represents the dependency between polar phytocompound concentration and dielectric constant of the solvent:

$$\frac{R \cdot T}{N_A} \cdot \ln C = \frac{1}{\varepsilon_x^2} \left((D + E) - \frac{\varepsilon_x^2}{\varepsilon_y^2} B \right) + \frac{1}{\varepsilon_x} \left(G - \frac{\varepsilon_x}{\varepsilon_y} A \right) + \Delta G_{unpred} \quad (8)$$

$$D = \frac{2 \cdot \mu_3^2 \cdot \mu_2^2}{(4\pi \cdot \varepsilon_0)^2 \cdot 3 \cdot k \cdot T \cdot r^6},$$

$$E = \frac{3 \cdot \alpha_3 \cdot \alpha_2}{(4\pi \cdot \varepsilon_0)^2 \cdot 2 \cdot r^6} \cdot \left(\frac{I_3 \cdot I_2}{I_3 + I_2} \right), \quad B = \frac{3 \cdot \alpha_1 \cdot \alpha_2}{(4\pi \cdot \varepsilon_0)^2 \cdot 2 \cdot r^6} \cdot \left(\frac{I_1 \cdot I_2}{I_1 + I_2} \right),$$

$$G = \frac{\mu_3 \cdot \mu_2}{(4\pi \cdot \varepsilon_0) \cdot r^3}, \quad A = \frac{\mu_1 \cdot \mu_2}{(4\pi \cdot \varepsilon_0) \cdot r^3}$$

where

Equation (8) is relatively difficult for mathematical manipulation, and to simplify it, the authors supposed that relations $\varepsilon_x^2/\varepsilon_y^2$ and $\varepsilon_x/\varepsilon_y$ are equal to the constant. In this case, the dependency will be defined in general by the balance of energy coefficients *(D + E)-const·B* and *(G-const·A)* and can predict both, extremum and plateau, in a certain range of solvent dielectric constant values.

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RESULTS AND DISCUSSION

Figure 1 presents HPLC fingerprint chromatogram at 268.4 nm for the extract from *Glycyrrhizae radix* of “liktravy” based on hydro-ethanol extractant with ethanol concentration of 71% v/v. Extracts obtained with ethanol 22%, 41%, 50%, 82%, and 96% v/v had the same type of chromatograms with differences in the peak amplitude/area of the main components. It should be noted that glycyrrhizic acid derivatives were practically not detected in ethanol solution 96% v/v, while these compounds were detected in high quantity in more diluted solutions of ethanol, starting from 82% v/v and below.

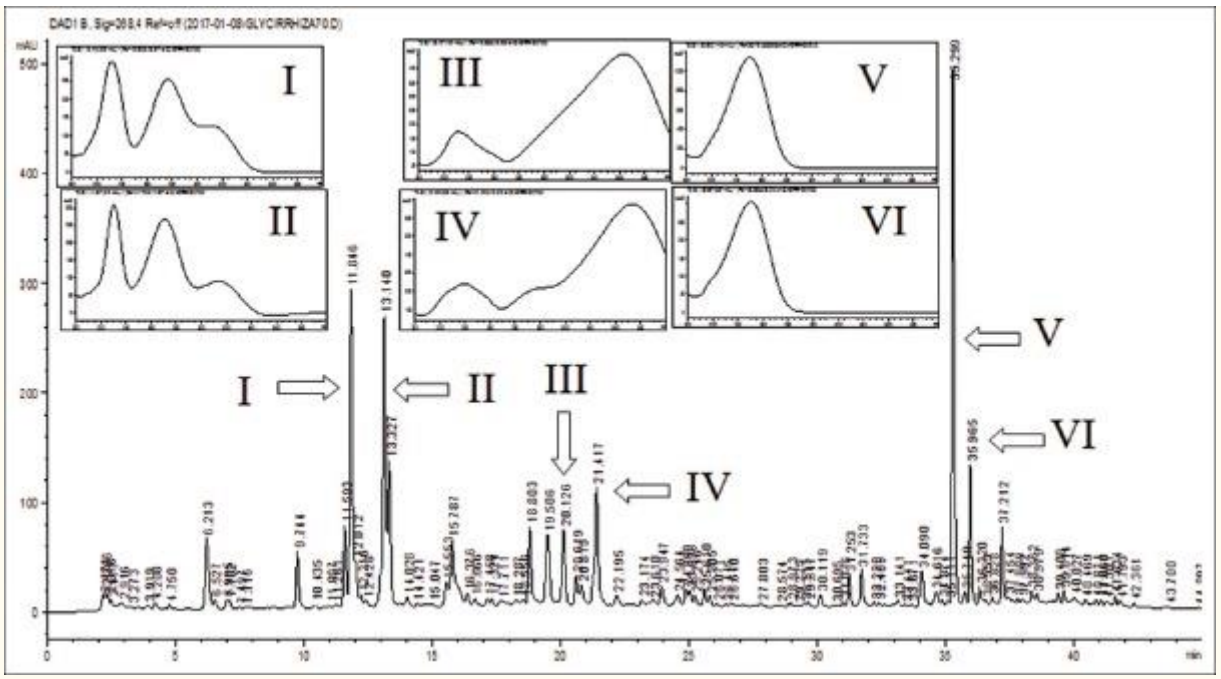

Figure 1

High performance liquid chromatography fingerprint of the extract based on 71% v/v ethanol at 268.4 nm with UV-spectra of the main compounds: I and II are flavonoids, III and IV are chalcones, V and VI are glycyrrhizic acid derivatives

As it can be seen from Figure 1, dominative compounds in the extract based on ethanol solution 71% v/v from *Glycyrrhizae radix* are flavonoids (Compounds I and II), chalcones (Compounds III and IV), and glycyrrhizic acid derivatives (Compounds V and VI).

The data obtained correlate well with other works.[1,4] Using reference standards, we identified three compounds. Compound IV is chalcone licuroside, and glycyrrhizic acid derivatives V and VI are constituents of Glycyram. Chromatographic system suitability parameters for these compounds are presented in Table 1.

Table 1

Chromatographic system suitability parameters for licuroside ana glycyram compounds

Compound	Retention time (t_R), min	Asymmetry coefficient (T)	Separation coefficient (Rs.)	Theoretical plates number (n)	RSD of peak's area RSD, %
Licuroside (IV) at 360.4 nm	21.7±0.6	0.80	2.31	169803	1.7
Glycyrrhizic acid derivative (V) at 258.4 nm	35.3±0.1	0.68	1.65	699649	1.9
Glycyrrhizic acid derivative (VI) at 258.4 nm	35.9±0.1	0.72	1.50	888042	1.9
Condition	-	≤2.0	≥1.5	≥1000	≤2.0

*The mean value and its CI (mean±SEM) are calculated with repeat counts n=4 and significance level P=0.95. RSD: Relative standard deviation, CI: Confidence interval, SEM: Standard error of mean[11]

As it can be seen from Table 1 all parameters for compounds in this chromatography system are satisfy the conditions and these compounds can

be determined by HPLC analysis in our conditions. To evaluate the quantitative content of licuroside and glycyram in extracts, a HPLC analysis method was developed using absolute calibration. The standard solutions of Licuroside (from 9.00 to 0.103 mmol/l) and Glycyram (from 21.4 to 0.101 mmol/l) were analysis under the given above conditions, and the absolute calibration linear equations were obtained for sum of glycyrrhizic acid derivatives, it is $C = (2.11 \pm 0.06) \cdot 10^{-6} \cdot S$, (mol/l), with determination coefficient equal to $R^2 = 0.999$ and for Licuroside, it is $C = (6.10 \pm 0.18) \cdot 10^{-7} \cdot S$, (mol/L), with determination coefficient equal to $R^2 = 0.999$.

Table 2 presents peak area values for the six compounds that dominate in the extracts.

Table 2

Peak area and concentration values for compounds identified in the extracts from *Glycyrrhizae* radix of "Liktravy", obtained from ethanol-water solutions with different concentrations

Compound (λ, nm)	Retention time, min*	Compound peak area, mAU·s*					
		Ethanol, % v/v					
		22	41	50	71	82	96
1. Flavonoid I (268.4)	12.0±0.4	1376±41	2345±70	2380±71	1747±52	1245±37	769±23
2. Flavonoid II (268.4)	13.4±0.4	813±24	1255±37	1400±42	1372±41	1123±34	555±16
3. Chalcone III (360.4)	20.4±0.6	2600±78	3022±90	3337±100	3619±108	3052±92	1315±39
4. Chalcone IV (360.4), Licuroside	21.7±0.6	2910±87	3205±96	3955±119	5047±151	4444±133	1992±59
5. Chalcone IV (360.4), licuroside concentration, mmol/l		1.78±0.05	1.96±0.06	2.41±0.07	3.08±0.09	2.71±0.08	1.22±0.04
6. Glycyrrhizic acid derivative V (258.4), Glycyram	35.3±0.1	6665±199	6974±209	7100±213	6614±198	4620±139	49.9±1.5
7. Glycyrrhizic acid derivative VI (258.4), Glycyram	35.9±0.1	1583±47	1663±49	1685±51	1579±47	1127±34	0
8. Glycyram concentration, mmol/l	-	17.4±0.5	18.2±0.5	18.5±0.6	17.3±0.5	12.1±0.4	0.105±0.003

*The mean value and its CI (mean±SEM) are calculated with repeat counts n=4 and significance level $P=0.95$. RSD: Relative standard deviation, CI: Confidence interval, SEM: Standard error of mean.[11]

As it can be seen from the data in Table 2, maximum peak areas for flavonoids (Compounds I and II) are observed for ethanol solution 50% v/v. Chalcones (Compounds III and IV) have maximum content in ethanol solution with concentration of 71% v/v. Glycyrrhizic acid derivatives (Compounds V and VI) have maximum peak area values in ethanol solution with concentration 50% v/v, but these values differ insignificantly from those in ethanol solution with concentration 41 and 71% v/v.

It should be noted that ethanol solution with concentration 96% v/v accumulated approximately 1.8-fold less flavonoids, 2.3-fold less chalcones, and 115-fold less glycyrrhizic acid in comparison with ethanol solution 82% v/v.

The fact of phenolic compounds' selective extraction into ethanol solution with concentration 96% v/v provides us with a tool for development of a step

by step treatment package technology for *Glycyrrhizae* radix processing and separate production of extracts rich in phenolic compounds (flavonoids and chalcones) and glycyrrhizic acid derivatives.

From the data in Table 2, we can also see that for extraction of all kinds of phytocompounds, the optimal range of ethanol concentration falls within the range of 40%–70% v/v. These results reveal easonability of development technology to obtain extracts (*sicca*, *fluida* and *spissa*) or a tincture from *Glycyrrhizae radix* in the territory of the Russian Federation and Ukraine using ethanol solution 55% ±15% v/v. In this case, an extract or a tincture will have maximum content of not only phenolic compounds but glycyrrhizic acid derivatives, too. In addition, we will see significant energy reduction at the stage of evaporation in comparison with water extracts.

It is interesting to note that European pharmacopeia contains a monograph describing licorice ethanolic liquid extract, standardized.[11] According to requirements of this monograph, the extract is obtained from the Licorice root by a suitable method using ethanol solution of 70% v/v. Furthermore, the extract should contain glycyrrhizic acid from 3 to 5% w/w, and ethanol content in the extract should be within the range of 52–65% v/v, which almost completely agrees with our results.

To model dependence between phytocompound concentration and solvent dielectric constant according to equation (8), the authors used peak area values presented in Table 2, the absolute calibration linear equations and reference data about the dielectric constant for ethanol-water solutions presented in work.[13]

The relationship between the natural logarithm of concentration (ln*C*) of Licuroside and glycyrrhizic acid derivatives in the extracts and dielectric constant of ethanol-water solutions are presented in Figure 2. Values and their confidence interval (mean ± standard error of the mean) are calculated with repeat counts $n = 4$ and significance level $P = 0.95$.

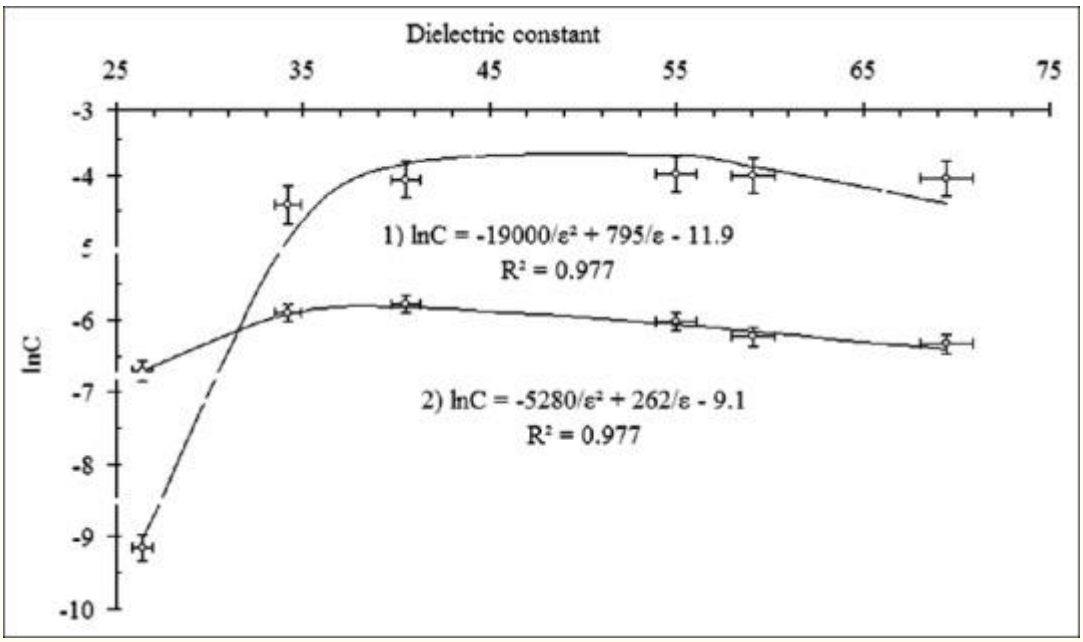

Figure 2

Dependency between phytocompounds' natural logarithm of concentration in extracts and dielectric constant of ethanol-water solutions. (1) Glycyram (sum of glycyrrhizic acid derivatives, compound V and VI); (2) chalcone (Licuroside), compound IV

As it can be seen from Figure 2, the relationship between the natural logarithm of glycyrrhizic acid derivatives (equation 1) and Licuroside (equation 2) concentration and ethanol-water dielectric constant has good approximation by equation (8) with determination coefficient R^2 =0.977.

The equations obtained predict the maximum content of glycyrrhizic acid derivative accumulation in solvent-water solutions with the range of dielectric constant values of 48 ± 5 units, and for licuroside 40 ± 4 units.

Based on these data, it is reasonable to expect that solvent-water solution should have a range of dielectric constant values of 44 ± 4 units to reach simultaneously maximum concentration of these compounds in the extract.

To confirm this statement, an additional experiment was carried out using water solutions of ethanol 55% wt., 2-propanol 55% wt., acetone 55% wt., and methanol 80% wt. by virtue of the fact that these solutions have dielectric constant values within the range of 44 ± 2 units. The results obtained are presented in Table 3.

Table 3

Peak area and concentration values for compounds identified in extracts from *Glycyrrhizae* radix of "Krasnogorskleksredstva", obtained from ethanol-water solutions with different concentrations

Parameter	Retention time, min*	Solvent			
		Methanol 80% wt.	2-propanol 55% wt.	Acetone 55% wt.	Ethanol 55% wt.
1. Chalcone IV (360.4), Licuroside peak area, mAU·s*	21.5±0.7	2258±68	2798±84	2889±87	2669±80
2. Chalcone IV (360.4), Licuroside concentration, mmol/l		1.38±0.04	1.71±0.05	1.76±0.05	1.63±0.05
3. Glycyrrhizic acid derivative V (258.4), peak area, mAU·s	35.3±0.1	3551±107	6784±204	5441±163	6390±192
4. Glycyrrhizic acid derivative VI (258.4), peak area, mAU·s	35.9±0.1	646±19	1266±38	1007±30	1188±36
5. Glycyram concentration, mmol/l	-	8.8±0.3	17.0±0.5	13.6±0.4	16.0±0.5

*The mean value and its CI (mean±SEM) are calculated with repeat counts n=4 and significance level P=0.95. SEM: Standard error of mean, CI: Confidence interval

As it can be seen from the data of Table 3, peak area values for licuroside in the extracts with 2-propanol 55% wt., acetone 55% wt., ethanol 55% wt. are comparable to each other (maximum deviation is 8%), but for methanol 80% wt., this value has a stronger deviation (equal to 22%).

For glycyrrhizic acid derivatives, the sum of their areas is comparable only for 2-propanol 55% wt., and ethanol 55% wt., for acetone 55% wt. this deviation is equal to 20%, and for methanol 80% wt., it is 48%.

Thus, it can be concluded that the most suitable solvents for simultaneous extraction of both, chalcones and glycyrrhizic acid derivatives, are water solutions of 2-propanol 55% wt., and ethanol 55% wt. Furthermore, acetone and methanol solutions rank below, by 20% and 48%, respectively.

The data obtained reveal a good correlation between the experiment and theory, but further studies are necessary to investigate and explain the abnormal behavior of methanol solution.

In addition, one interesting fact has been also found. For construction and analysis of phytocompound's content dependency in the extract on ethanol-water solution's dielectric constant, it is not compulsory to use absolute values of the ln$C$, but the logarithm of the ratio of peak area value to a maximum value (ln$S$/S_{max}) can be used. In this case, we obtain correlation equations almost identical to those presented in Figure 2. For example, for Glycyram we get: $\ln S/S_{max} = -7.9 + 795/\varepsilon - 19004/\varepsilon^2$, with $R^2 = 0.977$, and for Licuroside: $\ln S/S_{max} = -3.3 + 263/\varepsilon - 5293/\varepsilon^2$, with $R^2 = 0.977$. As it can be seen, the constants at the dielectric constant are identical (within the error) and different for the absolute term in the equation, which, however, does not interfere calculations of the optimal value of the dielectric constant. This fact discovered allows using the results of HPLC analysis without necessity to buy and use of the standard compound, which significantly simplifies data acquisition and their manipulation in studies of the kind.

Thus, according to this study, the following general conclusions can be made:first, solvent's dielectric constant plays a key role in the distribution process of phytocompounds between the phases; second, there is a certain

range of dielectric constant values of the solvent-water solution, within which maximum phytocompound concentration in the extract can be observed; third, at a first approximation, the dependency between phytocompound concentration in the extract and dielectric constant of the solvent-water solution can be described by equation $\ln C = a + b/\varepsilon + d/\varepsilon^2$.

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CONCLUSION

Solvent effects on phytocompounds extraction from *Glycyrrhizae* radix have been studied and modeled for substantiation of rational choice of the extractant in the technology of drugs obtained from this type of plant raw material. It is shown that the solvent's dielectric constant plays a key role in the distribution process of phytocompounds between the phases; there is a certain range of dielectric constant values of the solvent-water solution, within which maximum phytocompound concentration in the extract can be observed; the dependency between phytocompound concentration in the extract and dielectric constant of the solvent-water solution can be described by equation $\ln C = a + b/\varepsilon + d/\varepsilon^2$. It is determined that ethanol solution 96% v/v extracts predominantly flavonoids and chalcones, but does not extract glycyrrhizic acid derivatives, which provides a reason for the development of a step by step treatment package technology for this type of plant raw material. An optimal range of ethanol concentration in hydro-ethanolic solutions for simultaneous extraction of phenolic compounds and glycyrrhizic acid derivatives has been found, and it falls within the range of 62.5% ±7.5% v/v. Other the most suitable solvents for simultaneous extraction of both, phenolic compounds and glycyrrhizic acid derivatives, are water solutions of 2-propanol, and acetone (55% wt.).

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Conflicts of interest

There are no conflicts of interest.

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REFERENCES

1. Litvinenko VI, Georgievskiy VP, Fursa NS, Ammosov AS, Popova TP. Yaroslavl: Avers Plus; 2014. Licorice: Taxonomy, Chemistry, Technology, Standardization, Pharmacology, Clinic. [Google Scholar]

2. Popova NV, Litvinenko VI, Kucanjan AS. Kharkov: Disa Plus; 2016. Medicinal Plants of the World: Encyclopedic Handbook. [Google Scholar]

3. Tolstikov GA, Baltina LA, Grankina VP, Kondratenko RM, Tolstikova TG. Novosibirsk: Academic publishing House Geo; 2007. Licorice: Biodiversity, Chemistry, Application in Medicine. [Google Scholar]

4. World Health Organization. Vol. 1. Geneva: World Health Organization; 1999. Monographs on Selected Medicinal Plants. [Google Scholar]

5. Zhang Q, Ye M. Chemical analysis of the Chinese herbal medicine gan-cao (licorice) J Chromatogr A. 2009;1216:1954–69. [PubMed] [Google Scholar]

6. Asl MN, Hosseinzadeh H. Review of pharmacological effects of *Glycyrrhiza* sp. And its bioactive compounds. Phytother Res. 2008;22:709–24. [PMC free article] [PubMed] [Google Scholar]

7. Baltina LA, Kondratenko RM, Baltina LM, Jr, Plyasunova OA, Pokrovskii AG, Tolstikov GA. Prospects for the creation of new antiviral drugs based on glycyrrhizic acid and its derivatives (a review) Pharm Chem J. 2009;43:3–12. [Google Scholar]

8. Delbò M. London: European Medicines Agency; 2013. Assessment report on *Glycyrrhiza glabra* L. and/or *Glycyrrhiza inflate* Bat. and/or *Glycyrrhiza uralensis* Fisch Fisch., radix, Report No: EMA/HMPC/571122/2010, Committee on Herbal Medicinal Products (HMPC) [Google Scholar]

9. Lakshmi T, Geetha R. *Glycyrrhiza glabra* Linn. Commonly known as licorice: A therapeutic review. Int J Pharm Pharm Sci. 2011;3:20–5. [Google Scholar]

10. Langer D, Czarczynska-Gpslinska B, Goslinski T. Glycyrrhetinic acid and its derivatives in infectious diseases. Curr Issues Pharm Med Sci. 2016;29:118–23. [Google Scholar]

11. European Pharmacopoeia. 8th ed. Strasbourg: European Directorate for the Quality of Medicines and HealthCare; 2014. [Google Scholar]

12. Hiemenz PC, Rajagopalan R. Revised and Expanded. 3rd ed. New York: Marcel Dekker Inc; 1997. Principles of Colloid and Surface Chemistry. [Google Scholar]

13. Wohlfarth C. Berlin: Springer-Verlag Berlin Heidelberg; 2015. Static Dielectric Constants of Pure Liquids and Binary Liquid Mixtures. [Google Scholar]
