Quantitative determination of glycyrrhizinic acid by square-wave voltammetry and high-pressure liquid chromatography

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Abstract

Novel adsorptive stripping square-wave voltammetric method as well as a new high-pressure liquid chromatographic method for direct determination of glycyrrhizinic acid in dosage pharmaceutical preparation, used against virus infections, have been developed. Glycyrrhizinic acid is an electrochemically active compound, which undergoes irreversible reduction on a mercury electrode surface in an aqueous medium. Its redox properties were studied thoroughly by means of square-wave voltammetry, as one of the most advanced electroanalytical technique. The voltammetric response depends mainly on the pH of the medium, composition of the supporting electrolyte, as well as the parameters of the excitement signal. It was also observed that the voltammetric properties strongly depend on the accumulation time and potential, revealing significant adsorption of glycyrrhizinic acid onto the mercury electrode surface. Upon this feature, an adsorptive stripping voltammetric method for quantitative determination of glycyrrhizinic acid was developed. A simple, sensitive and precise reversed phase HPLC method with photodiode array UV detection has also been developed, mainly for comparison and conformation of the results obtained with the voltammetric method.

Key words: Glycyrrhizinic acid, square-wave voltammetry, high-pressure liquid chromatography

Introduction

Glycyrrhizinic acid (GA) is a saponin extracted from Glycyrrhiza glabra root. According to its chemical structure, it belongs to penthacyclical triterpenoid saponins, products of β-amyrin (1). GA and its aglycon 18β glycyrrhetinic acid are widely used against bacterial and virus infections, also as antiflogistics, expectorants, corrigens and as components with mineralocorticoid effect (2).

Many analytical methods have been employed for the determination of GA, such as: chromatographic techniques (high-pressure liquid chromatography, thin-layer chromatography, micellar electrokinetic chromatography, gas chromatography) (3-10); electrochemical techniques (oscillopolarography, single-sweep polarography) (11, 12); spectroscopic methods (13, 14). Immunoassays (ELISA and RIA techniques) were also used for determination of the glycyrrhizinic acid (15).

The general purpose of the work was to develop simple, precise and accurate methods for quantification of glycyrrhizinic acid in pharmaceutical dosage forms.

Adsorptive stripping voltammetry is a valuable and well-known analytical method for trace analysis of electroactive compounds capable to adsorb onto the working electrode surface (16).

In the family of pulse techniques square-wave voltammetry appears to be one of the most advanced electroanalytical techniques (17). This technique is attributed with unique advantages, such as: high frequency and amplitude, solitary approach in the current-sampling procedure, as well as the effective ability to discriminate the capacity current. Thus, for developing a particular absorptive stripping voltammetric method for determination of miscellaneous species, square-wave voltammetry is most frequently a technique of choice.

In addition to the current voltammetric method, the paper also describes a new chromatographic method for determination of GA serving mainly for comparison and conformation of the results obtained with the voltammetric method.

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Experimental

The analyzed sample was a pharmaceutical dosage in a form of spray. As declared by the manufacturer, the sample contains 0.1 % (w/w) glycyrrhizinic acid, while other components are maleic acid, fumaric acid, ascorbic acid, folic acid, polysorbate 80, propylen glycol and purified water. Glycyrrhizinic acid, standard substance, was a product of "Chemonova International, S. A." from Spain.

Voltammetric method

All used chemicals were of analytical reagent grade. KI, Na_2SO_4, $NaNO_3$, $KClO_4$, KBr, citric acid, and Na_2HPO_4 were all Merck products. Double distilled water was used. The buffer solutions were prepared by mixing of appropriate quantities of citric acid and Na_3PO_4. The stock solution of glycyrrhizinic acid was prepared by dissolving in redistilled water.

The oxygen removal from the electrolyzed solutions was performed by purging with highly purified nitrogen for ten minutes prior each measurement, and a nitrogen blanket over the solution was maintained thereafter. The measurements were carried out at room temperature.

All voltammograms were recorded with multi-mode polarograph Princeton Applied Research Model 384 B equipped with static mercury drop electrode Model PAR 303A. The reference was Ag/AgCl (sat. KCl) and a platinum wire was used as a third electrode. A small mercury drop with a surface of 0.0147 cm^2 was used as a working electrode.

The other experimental conditions are indicated in the caption of the figures.

HPLC method

All used chemicals were HPLC grade. The chromatograms were recorded on Waters HPLC system, equipped with Pump Waters 600 E, sample injector Rheodyne 7725i with 20 µl loop, Photodiode Array Detector 996 and column oven. Glycyrrhizinic acid was separated on a 5 mm Symmetry C18 column (25 cm x 4.6 mm i.d.) operated at 35 ^{0}C, using 25 mM potassium dihydrogen phosphate (pH 2.2) / acetonitril (60: 40 v/v) as a mobile phase (and solvent) at a flow rate of 1.6 ml/min and detection at 251 nm. The chromatographic system was controlled by software program Waters Millennium 32.

Results and discussion

*Determination of glycyrrhizinic acid
by voltammetric method*

Glycyrrhizinic acid is both electroactive and surface-active compound. Its undergoes totally irreversible reduc-

tion onto the mercury electrode surface. Under conditions of square-wave voltammetry, the response consists of a single well-defined peak at potential of about -1.50 V as represented in Fig. 1. The SW voltammograms were analyzed for the peak position, height, and half-peak width. All these properties of the voltammetric responses are strong function of pH and chemical composition of the medium, accumulation time and potential, as well as the parameters of the excitement signal.

The redox reaction of GA could be measured only within the pH interval from 2.0 to 8.0. Beyond these boundaries, no voltammetric response could be detected. In strong acidic medium pH ≤ 2, the hydrolysis of GA occurs at appreciable rate (18) preventing its electrochemical detection. As pH increases from 2.0 to 4.9, the SW peak shifts slightly towards potential that is more negative (see data in Table 1). At the same time, the peak current depends non-linearly on pH, crossing through maximum value at about pH = 3.8. Within the interval 5.4 ≤ pH ≤ 7.5, the SWV response of GA splits in two peaks indicating existence of two different redox reactions. For analytical purposes, this phenomenon is strongly unfavorable.

The complexity of the pH effect upon the voltammetric response arises from the complexity of the redox reaction of GA as well as the complexity of its adsorption process onto the working electrode surface. Nevertheless, the electrolyte solution with 3.8 ≤ pH ≤ 5.0 provides a save medium for analytical application, as the SWV response in those solutions consists of a well-defined single peak. Moreover, the ratio $I_p/\Delta E_{p/2}$ (where I_p and $\Delta E_{p/2}$ is a peak current and half-peak width, respectively) should serve as a main criterion for selecting an optimal response for analytical purposes. This quantity reaches a maximum value for pH = 4.7 revealing that this medium is the most suitable for quantitative determination of GA.
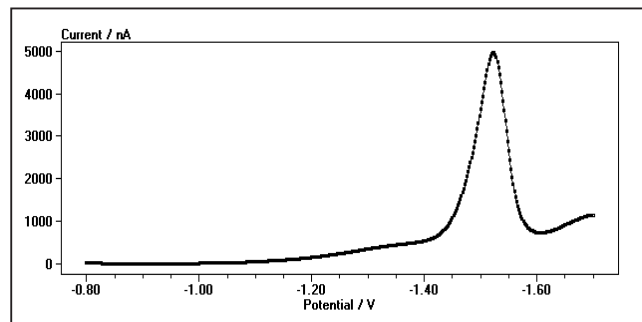

Fig. 1. Square-wave voltammogram of 1×10^{-5} mol/L glycyrrhizinic acid solution recorded in 0.1 mol/L KI electrolyte solution buffered to pH 4.7. Other experimental conditions are: initial potential E_s = -0.3 V, final potential E_f = -1.7 V, accumulation time $t_{acc.}$ = 100 s, accumulation potential $E_{acc.}$ = -0.3 V, SW frequency f = 100 Hz, SW amplitude E_{sw} = 25 mV and increment dE = 4 mV

Table 1. Dependence of peak potential and peak current of the voltammetric response of 5×10^{-6} mol/L glycyrrhizinic acid solution on pH recorded in citrate buffers. The other experimental conditions were: $f = 100$ Hz, $E_{SW} = 25$ mV, $dE = 2$ mV, $E_S = -0.8$ V and Ef = -1.7.

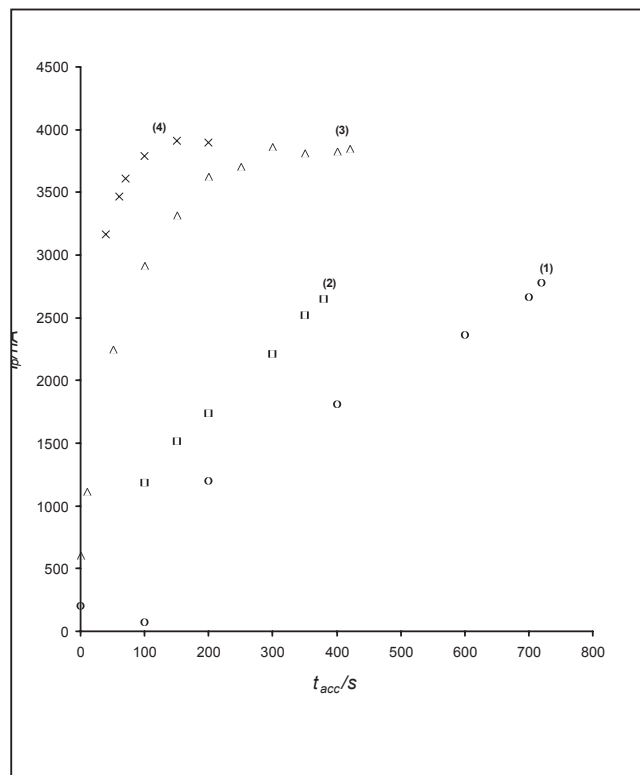| pH	$E_{p,1}$/V	$E_{p,2}$/V	$I_{p,1}$/nA	$I_{p,2}$/nA
2.0	No voltammetric response			
3.6	-1.476		603.7	
3.8	-1.478		1183	
4.1	-1.486		343.4	
4.3	-1.476		443.6	
4.5	-1.502		255.5	
4.7	-1.496		309.5	
4.9	-1.506		372.4	
5.4	1.414	-1.494	556.7	773.7
6.1	-1.37	-1.45	159	172
6.8	-1.358	-1.464	273.7	306.4
7.5	-1.382	-1.456	144.6	153.9
8.0	No voltammetric response		No voltammetric response	

Fig. 2. Dependence of the peak currents on the accumulation time for different glycyrrhizinic acid concentration recorded in 0.1 mol/L KI buffered to pH = 4.7. Experimental conditions were: $E_{acc} = -0.8$ V, $f = 100$ Hz, $E_{sw} = 25$ mV, $dE = 2$ mV and c (glyc.) $= 5 \times 10^{-7}$ (1), 1×10^{-6} (2), 5×10^{-6} (3) 1×10^{-5} mol/L (4).

Generally speaking, the adsorption of an electroactive compound onto the electrode surface is affected primarily by the chemical composition of the medium and the accumulation potential (16). The accumulation of GA on the mercury electrode surface was studied in four diverse supporting electrolytes. The results for the peak currents are listed in Table 2.

As it can be seen, the highest currents were obtained in 0.1 mol/L solution of KI indicating that in this medium the adsorption occurs at highest rate. Keeping in mind that the iodide ions are themselves adsorbed on the electrode surface, one can conclude that GA exhibits substantial tendency for adsorption. The data presented in Fig. 2 support this assumption. The Figure 2 illustrates the effect of the accumulation time upon the peak currents of GA investigated at various concentrations of the analyte. By increasing the accumulation time, the peak current becomes higher and the entire I_p-t_{acc} relationship obeys a low of an isotherm. The appear plateaus

of the curves 1 and 2 corresponds to the saturation of the electrode with electroactive material. For analytical purposes, the electrode should be covered by a monomolecular layer of deposited GA molecules, which is fulfilled within the linear portions of the isotherms.

The influence of the accumulation potential on the peak current is presented in Fig. 3. The accumulation potential affects considerably the entire accumulation process through its influence upon the structure of the electric double layer adjacent to the electrode surface (16). The peak current depends sigmoidally on the accumulation potential in a form of a pseudopolarogram.

Table 2. Dependence of peak current of 5×10^{-6} mol/L glycyrrhizinic acid solution on the accumulation time recorded in diverse supporting electrolytes. Experimental conditions were: $f = 100$ Hz, $E_{sw} = 25$ mV, $dE = 2$ mV, $E_{acc} = -0.8$ V. All supporting electrolytes were buffered with citrate buffer to pH = 4.7.

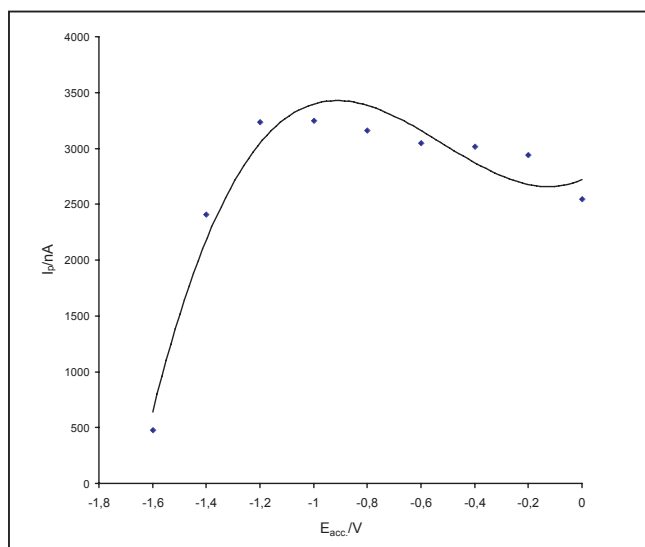| $t_{acc.}$/s	I_p/nA Na$_2$SO$_4$	I_p/nA NaNO$_3$	I_p/nA KClO$_4$	I_p/nA KI	I_p/nA KBr	I_p/nA Citrate buffer
0	431.9	395.5	436.4	562.9	425.9	446
50						2323
60	2329	2099	2272	2839	2485	
100						3298
120	3396	3190	3264	3862	3272	

Fig. 3. Dependence of the peak current of glycyrrhizinic acid on the deposition potential. Experimental conditions were: $f = 100$ Hz, $E_s = -0.8$ V, $E_{sw} = 25$ mV, $t_{acc} = 15$ s, d$E = 2$ mV, c (glyc.) $= 5 \times 10^{-6}$ mol/L, supporting electrolyte 0.1 mol/L KI buffered to pH 4.7.

The peak current decreases considerably as the accumulation potential becomes more negative than -1.0 V. The most effective accumulation was observed under accumulation potential of about $E_{acc} = -0.80$ V and hence, this value was used in the further measurements.

As mentioned previously, the sensitivity of the method depends markedly on the properties of the potential waveform applied in the course of the stripping step. Accordingly, an attempt was made to optimize the properties of the SW excitement signal such as SW amplitude E_{sw} (the height of the potential pulses), SW frequency f (the duration of single potential pulse), and scan increment dE (the step of the staircase potential ramp at which potential pulses of square shape are superimposed).

The peak current increases strongly by increasing the SW amplitude from 2 to 50mV. This phenomenon has already been predicted by the theory of SWV regarding a totally irreversible redox process, which proceeds from an immobilized state of the reactant.

As the amplitude becomes higher than 50mV, the peak current reaches a maximum value forming a plateau on the I_p-E_{sw} function (see curve 1 in Fig. 4). The peak potential shifts in positive direction within the interval 2mV $\leq E_{sw} \leq$ 150mV. However, the amplitude also influences the shape of the voltammetric response, causing the SW peak to widen by the increase of the amplitude. The ratio $I_p/\Delta E_{p/2}$ gains a maximum value for Esw = 25mV, consequently this value was selected as an optimal for analytical measurements.

The signal frequency was varied over the interval from 10 to 120 Hz. It was observed that the frequency

affects both the position and height of the SW response. A linear relationship between E_p (peak potential) and logarithm of the frequency was observed with a constant slope of $\Delta E_p/\Delta \log(f) = -30.1$ mV. At the same time, the peak current increases linearly with the SW frequency, which is typical for redox reaction occurring on the electrode surface via adsorbed reactant. The half-peak width is insensitive on the signal frequency. Thus, SW frequency of $f = 120$Hz was considered as an optimal value for further quantitative analysis.
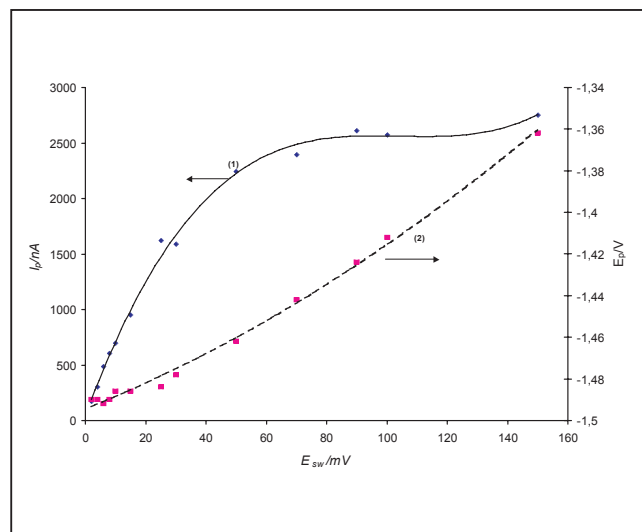

Fig. 4. Dependence of the peak currents (curve 1) and peak potentials (curve 2) on the signal amplitude. Experimental conditions were: $f = 100$ Hz, d$E = 2$ mV, $E_{acc} = -0.8$ V, $t_{acc} = 15$ s, c (glyc.) $= 5 \times 10^{-6}$ mol/L, supporting electrolyte 0.1 mol/L KI buffered to pH 4.7.

The scan increment exhibits rather similar effect to the voltammetric response as that of SW amplitude and consequently those results will be not repeated hereafter. The value of d$E = 8$ mV was selected as most relevant for quantitative measurements.

Therefore, the following set of experimental conditions are proposed for quantitative determination of GA: supporting electrolyte 0.1 mol/L KI buffered with citrate buffer to pH = 4.7, deposition potential $E_{acc} = -0.8$ V, SW frequency $f = 120$ Hz, SW amplitude $E_{sw} = 25$ mV, and scan increment d$E = 8$ mV. The duration of the deposition step depends on the analyte concentration range.

A calibration curve of GA was recorded in 0.1 KI buffered to pH = 4.7, over the concentration range from 5×10^{-7} mol/L to 7×10^{-6} mol/L. The other experimental conditions were: $f = 100$Hz, d$E = 8$mV, $E_{sw} = 25$ mV, $E_{acc} = -0.8$V and $t_{acc} = 15$s. The line is associated with a linear regression correlation coefficient of R = 0.9992.

To estimate the accuracy of the proposed adsorptive voltammetric method, a recovery analysis was undertaken and the corresponding results are listed in Table 3.

Table 3. Results of recovery analysis obtained by standard addition method. Experimental conditions were: f = 100 Hz, E_{sw} = 25 mV, dE = 2 mV, supporting electrolyte 0.1 mol/L KI buffered to pH 4.7.

Sample	Added (mol/L)	Found (mol/L)	Recovery (%)
Glycyrrhizinic acid -	4.97×10^{-6}	4.80×10^{-6}	96.58
spray	5.9×10^{-6}	6.06×10^{-6}	102.71
	9.95×10^{-6}	10.40×10^{-6}	104.50

For recovery analysis, the standard addition method and the Hon equation were utilized. The Hon formula for determination of the sample concentration reads:

$$c_s = \frac{c_{st} \cdot V_{st} \cdot I_{p,s}}{I_{p,st} \cdot (V_{cell} + V_{st}) - I_{p,s} \cdot V_{cell}}$$

where c_s is sample concentration, c_{st} is concentration of the standard addition, V_{st} is volume of the standard addition, $I_{p,s}$ is current associated with the sample, $I_{p,st}$ is current of the standard addition, and V_{cell} is volume of the electrolyzing solution.

Determination of glycyrrhizinic acid by HPLC method

A reversed phase HPLC procedure was developed as a comparative method for determination of GA in pharmaceutical preparation. GA was well resolved from the other formulation components using the previous established selective chromatographic conditions. The retention time of glycyrrhizinic acid was found to be approximately 4.8 minutes (Fig. 5).

Peak purity of glycyrrhizinic acid in the sample has been established with software program Waters Millennium 32 by comparing the purity angle (0.119) with purity threshold (0.269).

The dependence of the GA peak area on the concentration of the injected solution was linear over the investigated range of 10 to 500 µg/ml with a linear regression correlation coefficient of R = 0.9994.

The accuracy of the chromatographic procedure was confirmed by the method of standard addition (Table 4).

The recovery values of GA determinations range from 99.1 to 101.77 % confirming that HPLC method is both quantitative and accurate.

The results of GA determination in pharmaceutical preparation obtained by square-wave voltammetry and high-pressure liquid chromatography are given in Table 5.

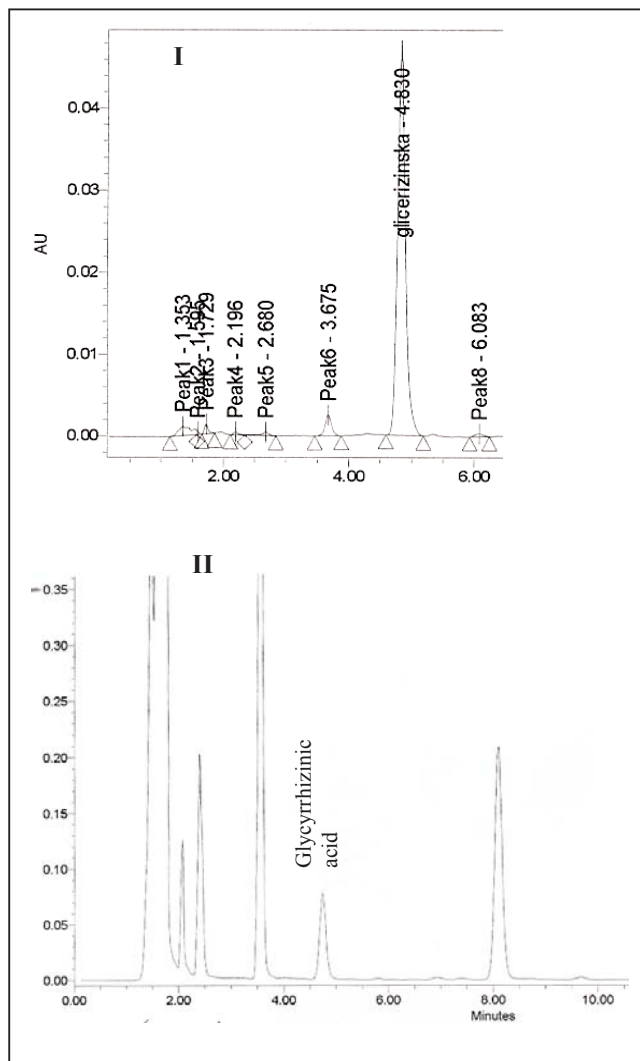As it can be seen, the results obtained by voltammetric and HPLC method are precise, accurate and reproducible.

Fig. 5. Chromatograms of: I - glycyrrhizinic acid, standard substance (100 µg/ml), II – sample (glycyrrhizinic acid ~ 100 µg/ml).

Table 4. Results of glycyrrhizinic acid determination by method of standard addition, for estimating the accuracy of the HPLC method

Sample	Added (mg/ml)	Found (mg/ml)	Recovery (%)
Glycyrrhizinic acid -	-	0.9953	-
	0.25	1.2341	99.10
spray	0.50	1.4733	98.53
	1.00	2.0306	101.77

Table 5. Results of glycyrrhizinic acid determination in sample (spray) by square-wave voltammetry and high-pressure liquid chromatography

Method	Declared (mg/ml)	Determined (mg/ml)	RSD (%)	Recovery (%)
SWV	1.0	1.037	4.84	103.7
HPLC		0.993	1.54	99.3

Conclusion

A new adsorptive stripping square-wave voltammetric method for quantitative determination of glycyrrhizinic acid in pharmaceutical preparations is proposed. The method is simple, fast and precise. The voltammetric measurement itself takes a few seconds. Moreover, it is important to emphasize that the proposed method could be applied without previous treatment of the original preparation. The proposed method is also attractive due to the low cost of the electrochemical instrumentation. The conditions for quantification of GA applying high-pressure liquid chromatography are also established. The precision, accuracy and reproducibility of the voltammetric method are comparable to those of HPLC method and both may be applied for routine analysis and determination of glycyrrhizinic acid in pharmaceutical formulations.

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Резиме

Квантитативно определување на глициризинска киселина со квадратно-бранова волтаметрија и течна хроматографија под висок притисок

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Клучни зборови: глицеризинска киселина, квадратно-бранова волтаметрија, течна хроматографија под висок притисок

Развиен е метод на квадратно-бранова волтметрија и течна хроматографија под висок притисок за директно определување на глициризинската киселина во дозиран фармацветски препарат, што се користи против вирусни инфекции. Глициризинската киселина е електрохемиски активна суспстанца, што подлежи на иреверзибилна редукција на површината на живината електрода, во воден медиум. Редокс својствата на глициризинската киселина беа целосно проучени со примена на квадратно-брановата волтаметрија, како една од најефикасните електроаналитички техники. Волтаметрискиот одговор главно зависи од pH на средината, составот на основниот електролит, како и од параметрите на екситирачкиот сигнал. Исто така, утврдено е дека волтаметрискиот одговор е силно зависен од времето на акумулација и од потенцијалот, кои укажуваат на значителна адсорпција на глициризинската киселина на површината на живината електрода.

Методот на реверзно-фазна течна хроматографија под висок притисок беше развиен, главно за споредба и потврдување на резултатите добиени со волтаметрискиот метод.