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Quantitative Analysis of Codelac Broncho Tablets and Syrup by High-Pressure Liquid Chromatography

G. B. Golubitskii[a] and V. M. Ivanov

Analytical Chemistry Department

e-mail: sandro@analyt.chem.msu.ru

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Abstract—A procedure was proposed for quantitative analysis of Codelac Broncho tablets and syrup, a new original drug, by high-pressure liquid chromatography. The active principles of the tablets were separated in 6 min with an efficient resolution of all component peaks. Preproduction tablet samples were analyzed. The results of the analysis meet the requirements of normative technical documentation and technologic loads. The adequacy of the results was validated by the analysis of model solutions that contained all active principles and adjuvants. For the syrup, two versions of analysis were proposed, in isocratic and gradient elution modes. These versions are virtually equivalent with respect to the analysis time and precision. The isocratic elution version is, however, easier to implement and is proposed for inclusion into the draft pharmacopoeic standards for commercial production.

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Codelac Broncho is a multicomponent cough suppressant in tablet and syrup forms containing synthetic and plant-derived active principles (see scheme). The major active principles are ambroxol hydrochloride (I) and trisodium glycyrrhizate (II). The tablets in addition contain a dry extract of thermopsis and several adjuvants and fillers. Nipagin (III) and Nipazol (IV) are conservants.

In parallel with the development of technology for tablet and syrup production, quantitative analyses were developed. Inasmuch as the drug has a complex formula, it is difficult to develop quantitative analyses for all of its components. A real task was set: to develop a procedure for the simultaneous determination of the two major active principles (I) and (II) in the tablets and syrup and the two conservants (III) and (IV) in syrup.

EXPERIMENTAL

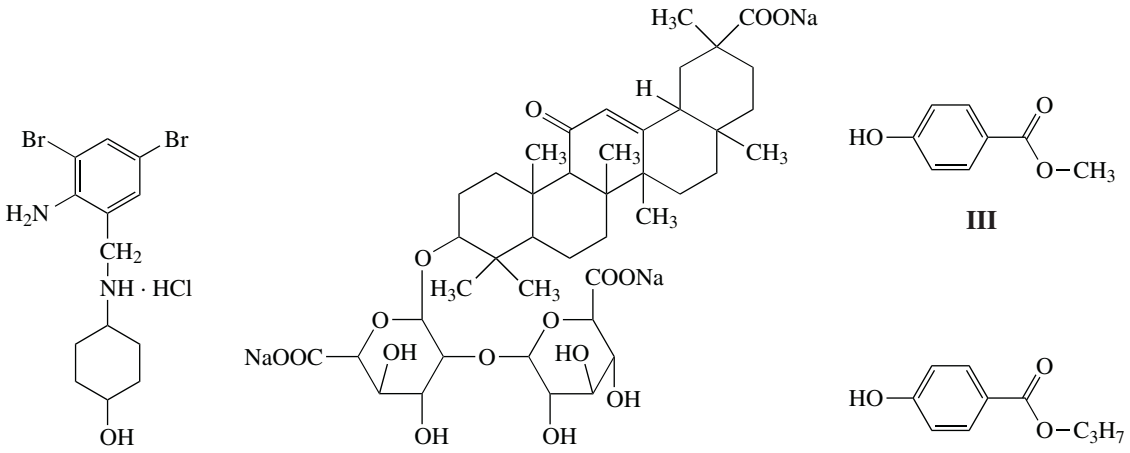
Reagents. Acetonitrile for gradient chromatography (Sigma) and ultrapure water (resistivity, 18.2 MΩ/cm; prepared on a Direct Q Millipore setup) were used to prepare eluents and to dissolve standards and test samples. The standards of the analyzed drugs were pharmaceuticals verified by quality control for compliance to their certificates. The other chemicals used were of at least pure for analysis grade.

Instruments. Chromatography was carried out on a Waters Alliance 2695 chromatograph equipped with a Waters 2996 diode-matrix detector. The certified dead volume of the chromatograph was 0.650 mL. A column 150×4.6 mm and a protective precolumn 12.5×4.6 mm were used, both packed with Zorbax SB C8 reversed-phase sorbent with the particle size 3.5 μm (Agilent Technologies) and thermostated at 40°C.

The eluent pH was monitored with a pH-673M pH-meter/millivoltmeter with a glass indicator electrode and a silver chloride reference electrode.

Solution preparation. For analysis, carefully ground tablets (exact weights, ~0.275 g) were placed into a volumetric flask 100 mL in capacity, CH_3CN–H_2O (1 : 4 vol/vol, 40 mL) was added, stirred for 3 min, brought to the volume with the same solvent mixture, and stirred. To prepare the solution of standards (SS), exact weights of ambroxol hydrochloride (about 0.100 g) and trisodium glycyrrhizate (about 0.300 g) were placed into a volumetric flask 100 mL in capacity, the same CH_3CN–H_2O mixture (40 mL) was added, stirred until dissolution, brought to the required volume with the same solvent mixture, and stirred (solution A). Then, an aliquot of solution A (5.0 mL) was transferred to a volumetric flask 100 mL in capacity, brought to the volume with the same CH_3CN–H_2O mixture, and stirred. Model solutions for checking the adequacy of the results were prepared in the same manner as SS, but diluted with 4.0–6.0 mL of solution A and the corresponding amount of placebo (a mixture of all components except for the substances to be determined).

[a] *OAO Farmstandart-Leksredstva.*



The analysis was carried out as follows. To a volumetric flask 100 mL in capacity, an analyzed syrup (5.0 mL) was placed, a CH_3CN–H_2O mixture (3 : 7 vol/vol, 40 mL) was added, stirred for 3 min, brought to the volume with the same solvent mixture, and stirred. To prepare SS, to a volumetric flask 100 mL in capacity, placed were ambroxol hydrochloride (~0.100 g), trisodium glycyrrhizate (~0.300 g), Nipagin (~0.038 g), and nipazol (~0.013 g); the same CH_3CN–H_2O mixture (40 mL) was added, stirred until dissolution, brought to the volume with the same solvent mixture, and stirred (solution A). Then, an aliquot of solution A (10.0 mL) was transferred to a volumetric flask 100 mL in capacity, brought to the volume with the same solvent mixture, and stirred. To check the adequacy of the results, model solutions were prepared as SS, but diluted with 8.0–12.0 mL of solution A and the corresponding amount of placebo.

All solutions used in the analysis of the tablets and syrup were filtered through a hydrophilic membrane filter with the 0.45 μm pore size (fluoroplastic filters are preferred because of their stability in water–acetonitrile solutions).

Table 1. Programmed variation in the eluent composition in the gradient elution mode

Time, min	Eluent composition, vol %	
	CH_3CN : 0.025 M KH_2PO_4 (pH 2.5) = 3 : 2	0.025 M KH_2PO_4 (pH 2.5)
0	30	70
16	90	10
17	90	10
18	30	70
21	30	70

Analytical procedure. A test solution of the tablets and the SS were chromatographed. The eluent used was CH_3CN–0.025 M KH_2PO_4 (2 : 3; pH 2.5) at a flow rate of 1.0 mL/min. The injected sample volume was 20.0 μL; the detection wavelength was 250 nm.

In the analysis of the syrup, the test solution and the SS were chromatographed. In the isocratic elution mode, the eluent used was a CH_3CN–0.025 M KH_2PO_4 (3 : 7; pH 2.5) with a flow rate of 1.0 mL/min. In the gradient elution mode, the composition of the eluent was changed in accordance with the program described in Table 1. The injected sample volume was 20.0 μL; the detection wavelength was 254 nm.

Data processing. For the tablets, the peak areas for the analyzed components were determined, and the content of each component in the tablets was found from

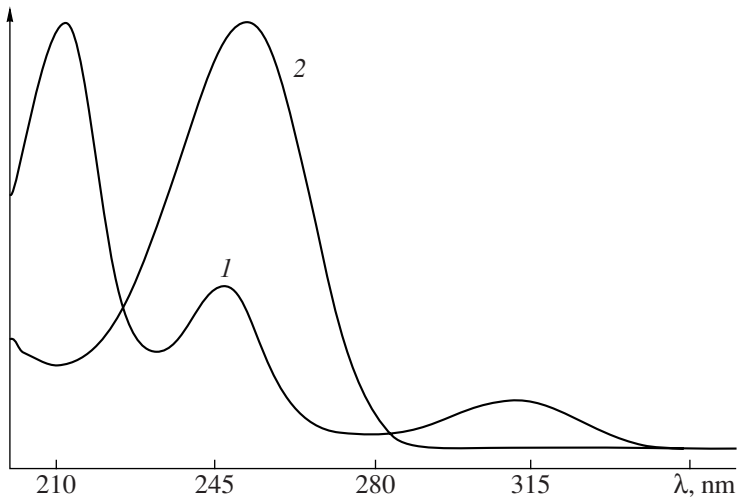
$$X = (S_{test} m_{st} m_{av})/(S_{st} m_{test}),$$

where S_{test} and S_{st} are the average peak areas for the analyzed component in the chromatograms of the test solution and the SS, respectively; m_{st}, m_{av}, and m_{test} are, respectively, the weight of the reference in the SS, the average tablet weight, and the weight of ground tablets taken for preparing the test solution (g).

For the syrup, the peak areas for the analyzed components were determined and the content of each component (g) in one dose of the syrup (5 mL) was determined from

$$X = (S_{test} m_{st})/S_{st},$$

where S_{test} and S_{st} are the average peak areas for the analyzed component in the chromatograms of the test solution and SS, respectively; and m_{st} is the weight of the standard in the SS (g).



Normalized absorption spectra of (*1*) ambroxol and (*2*) trisodium glycyrrhizate.

RESULTS AND DISCUSSION

Optimum analysis parameters for the syrup. The figure shows absorption spectra for the analyzed components recorded on-line with a diode-matrix detector. From these spectra, the detection wavelength was established equal to 250 nm, near the absorption peak of component II and one of the absorption peaks of component I.

Rapid optimization was performed to determine the optimum parameters for the separation of the components. Rapid optimization means studying some factors that influence the chromatographic parameters of compounds [1]. The other factors were evaluated proceeding from the key physical-chemical properties of the components. In order for the optimum retention of glycyrrhizic acid and a satisfactory shape of its peak to be achieved for purposes of quantitative analysis, pH in the eluent should be less than three (the molecular form of the compound). At this pH, an ambroxol molecule is protonated, its retention decreases, but the peak shape remains acceptable. A satisfactory resolution of the peaks of the analyzed and other components is also achieved under these conditions. To determine the required concentration of the organic modifier in the eluent, the CH_3CN concentration was varied from 30 to 50 vol %. To determine the elution time of an irretainable substance, chromatograms of water were recorded. The results (Table 2) demonstrate that the optimum resolution and the minimum analysis time were provided by 40 vol % CH_3CN. The retention time of ambroxol and glycyrrhizate was 2.022 and 3.747 min, respectively.

Optimum analysis parameters for the syrup. The detection wavelength was 254 nm. Previously [2], we developed an analysis method for Codelac Phyto syrup, a multicomponent drug. This drug contains codeine phosphate as the active principle. To achieve an efficient resolution of the codeine peak from the peaks of the plant-derived components, we exploited the property of codeine to be irreversibly retained by some reversed-phase sorbents if salts are absent in the eluent. Gradient elution was carried out with the use of acetonitrile–water and acetonitrile–phosphate buffer solution mixtures; the second eluent was introduced after all components, except for codeine, were eluted. Because of the absence of codeine phosphate in Codelac Broncho syrup and because of a comparatively lower content of plant-derived substances, we decided to use a simpler elution version with phosphate buffer solution introduced in the very beginning of the analysis. With the starting 0.025 M KH_2PO_4 (pH 4.7), the result was unsatisfactory: the shape of the glycyrrhizic

Table 2. Effect of the CH_3CN concentration on the retention parameters

$c(CH_3CN)$, vol %	30	35	40	50
t_{gl}, min	21.567	7.319	3.747	2.212
t_{ambr}, min	2.854	2.304	2.022	1.772
t_0, min	1.347	–	–	–
$\ln k_{gl}$	3.006672	1.787082	0.875469	–0.14503
$\ln k_{ambr}$	0.410121	–0.04395	–0.39304	–0.85567

Table 3. Metrological characteristics for the determination of ambroxol hydrochloride and trisodium glycyrrhizate in Codelac Broncho tablets

Component	S_{max}, g	S^2_{max}	ε_{max}, %	$e_{r\,av}$, %	Δe_r, %	$e_{r\,max}$, %
Ambroxol hydrochloride	0.00007	5.39×10^{-9}	3.15	–0.073	0.137	–1.05
Trisodium glycyrrhizate	0.00018	3.14×10^{-8}	5.08	–0.028	0.168	1.69

Table 4. Metrological characteristics for the determination of ambroxol hydrochloride, trisodium glycyrrhizate, Nipagin, and Nipazol in Codelac Broncho

Component	S_{max}, g	S^2_{max}	ε_{max}, %	$e_{r\,av}$, %	Δe_r, %	$e_{r\,max}$, %
Ambroxol hydrochloride	0.00011	1.15×10^{-8}	5.76	–0.032	0.213	–1.53
Trisodium glycyrrhizate	0.00022	4.92×10^{-8}	3.04	–0.034	0.152	–1.48
Nipagin	0.00002	5.96×10^{-10}	2.61	–0.005	0.142	1.75
Nipazol	0.00002	3.70×10^{-10}	4.97	0.062	0.248	2.88

Table 5. Results of the analysis of Codelac Broncho tablets (three preproduction samples)

Component	Standard content, g	Contained in a tablet, g ($n = 9$; $P = 0.95$)				
		X_{av}	S	Sx_{av}	Δx_{av}	ε, %
Ambroxol hydrochloride	0.0185–0.0215	0.0196	0.0011	0.00037	0.0009	4.43
		0.0204	0.0009	0.00030	0.0007	3.49
		0.0201	0.0007	0.00023	0.0006	2.75
Trisodium glycyrrhizate	0.0277–0.0323	0.0299	0.0013	0.00043	0.0010	3.43
		0.0287	0.0009	0.00030	0.0007	2.48
		0.0308	0.0011	0.00037	0.0009	2.82

Table 6. Results of the analysis of Codelac Broncho syrup (three preproduction samples); reduced absorption spectra of (1) ambroxol and (2) trisodium glycyrrhizate

Component	Standard content, g	Contained in 5 mL, g ($n = 9$; $P = 0.95$)				
		X_{av}	S	Sx_{av}	Δx_{av}	ε, %
Ambroxol hydrochloride	0.009–0.011	0.00975	0.00034	0.00011	0.00027	2.74
		0.00988	0.00028	0.00009	0.00022	2.23
		0.01012	0.00036	0.00012	0.00028	2.80
Trisodium glycyrrhizate	0.0277–0.0323	0.03211	0.00122	0.00041	0.00096	2.99
		0.03020	0.00112	0.00037	0.00088	2.92
		0.02890	0.00098	0.00033	0.00077	2.67
Nipagin	0.0034–0.0041	0.00364	0.00012	0.00004	0.00009	2.59
		0.00354	0.00008	0.00003	0.00006	1.78
		0.00388	0.00014	0.00005	0.00011	2.84
Nipazol	0.0011–0.0014	0.00122	0.00006	0.00002	0.00005	3.87
		0.00118	0.00004	0.00001	0.00003	2.67
		0.00129	0.00009	0.00003	0.00007	5.49

acid peak was unacceptable for quantitative analysis. Likely, this was for the following reason: glycyrrhizic acid is contained in the eluent with a certain pH, and it should be converted to a molecular form in order for its peak to be symmetric. After the acidity of phosphate buffer was adjusted to pH 2.5 by addition of H_3PO_4, this assumption was verified: all peak shapes were satisfactory, indicating the efficient resolution. Because the change in the concentration of the organic modifier of the eluent upon the component separation in the gradient elution mode was as little as 36 vol %, we suggested that separation in the isocratic elution mode is also possible. The use of a CH_3CN–0.025 M KH_2PO_4 mixture (3 : 7; pH 2.5) gave satisfactory separation. The isocratic elution version has no advantages with regard to the analysis time, but is preferred for its easier implementation.

Analysis of model mixtures. Seventeen solutions were prepared, in which the contents of the analyzed compounds were ±20% of the values specified by the formula. The solutions were analyzed as described in the section **Analyical procedure**, and the results were processed as added/found using Excel worksheets. Tables 3 and 4 display the results obtained for the tablets and syrup, respectively. Comparing the mean relative errors of component determinations (e_{rcp}) with the corresponding confidence ranges Δe_r, we find no systematic errors ($e_{rcp} < \Delta e_r$). The peak areas for the analyzed compounds are linear functions of their concentrations in the specified range (with the correlation coefficient $K_{corr} > 0.99$).

Analysis of test samples. The procedure developed was used to analyze preproduction tablet samples (Table 5). The results of these analyses satisfied the normative documentation and technologic parameters. Preproduction syrup samples were analyzed by the developed procedure in the isocratic and gradient elution versions. The results obtained for the isocratic version are listed in Table 6. The results for the gradient elution version were virtually the same.

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