

Determination of active substances of the “Propolis-Derma” pharmaceutical compositions by reverse-phase high-performance liquid chromatographic method

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Abstract

Aim: This study presents the material devoted to working out the methods of identification and quantitative determination of active substances of medicated products “Propolis-Derma” (“Propolis-PNH,” “Propolis-PSC,” and “Propolis-PCD”) with antifungal, antimicrobial, and keratolytic activity, created for the treatment of dermatomycoses, pityriasis versicolor, as well as diseases caused by yeast-like fungi of *Candida albicans*.

Materials and Methods: As a result, the conditions, methods, and opportunity have presented for the quantitative determination of the content of active pharmaceutical ingredients of “Propolis-Derma” pharmaceutical compositions by the method of the reverse-phase high-performance liquid chromatography on the basis of milichrom A-02 chromatograph. **Results and Discussion:** By comparing the retention times and the values of spectral ratios of the peaks obtained with such data in the database of chromatograph, the presence of naftifine hydrochloride has been confirmed in the composition of the medicated product “Propolis-PNH,” chlorquinaldol and salicylic acid – in the composition of “Propolis-PSC,” and chlorhexidine digluconate – in “Propolis-PCD” composition. In addition, in each of five series of experimental samples, the presence of phenolic propolis compounds which are part of the propolis tincture has been established as one of the main active components of the pharmaceutical compositions offered. **Conclusions:** It has been defined that experimental data on the content of biologically active substances of pharmaceutical compositions “Propolis-PNH,” “Propolis-PSC,” and “Propolis-PCD” are well correlated with the composition of the medicated preparations developed and the literary data on the chemical structure and composition of the basic substances, and the method offered gives the possibility to carry out standardization of the composition of the medicine developed, namely, “Propolis-Derma.”

Key words: Antifungal, antimicrobial, and keratolytic activity, pharmaceutical compositions “Propolis-Derma,” propolis, reverse-phase high-performance liquid chromatography

TOPICALITY

At the present time, fungal skin lesions are one of the key issues of dermatology and modern medicine, in general, among the diseases of infectious pathologies characterized by the incidence of dermatomycoses, onychomycoses, and candidomycoses, mainly caused by infection with spores of fungal bacteria of the genus

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Microsporum, *Trichophyton*, or *Candida albicans* in individuals with reduced resistance of the organism while certain diseases and lesions of skin appendages or mucous membranes of internal organs, etc.^[1,2]

Development of this disease infection is also facilitated by primary or acquired immunodeficiency (HIV-infection, acceptance of glucocorticoids, cytostatics, and immunosuppressants), adverse environmental background, malignancy, diabetes, tuberculosis, chronic stress, and other factors that lead to the depletion of the body defenses and development of associated pathological processes.^[3,4]

It should be noted that diagnostics of dermatomycoses and onychomycoses has recently shown a tendency to detect several types of pathogenic microorganisms, what gives grounds to consider this fact as an existence of mixed infection of these diseases. Therefore, a physician faces a rather complicated task of choosing a high effective systemic antimycotic with a wide range of antifungal activity and a minimal risk of side effects development, taking into account the length of this pathology treatment.^[5]

Considering the results of researches conducted on the pharmaceutical market of Ukraine,^[6-9] which indicates the diversity of antimicrobial drugs is mainly represented by preparations of synthetic origin, and therefore, the relevance of new drugs creation based on substances of natural origin has been confirmed.^[10]

The composition has been developed by us and new pharmaceutical compositions have been proposed for introduction into medical practice under the conventional name “Propolis-Derma” (“Propolis-PNH,” “Propolis-PSC,” and “Propolis-PCD”) with antifungal, antimicrobial, and keratolytic activity created for the treatment of dermatomycoses, pityriasis versicolor, as well as of diseases caused by yeast-like fungi of *C. albicans*.^[11-14]

Subsequently, the results of microbiological studies have established that test-samples of preparations “Propolis-PNH,” “Propolis-PSC,” and “Propolis-PCD” exhibit significant antagonistic properties against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), selective antibacterial capacity – to *Escherichia coli*, and expressed antifungal activity in reference to fungi strains of *C. albicans* genus,^[12] that meets the requirements of the State Pharmacopoeia of Ukraine^[15] and can be applied in the treatment of mycoses in conditions of the high fungi resistance to conventional antifungal medicines.

Experimental Part

Considering the fact, that the most effective method for identifying and quantitative determining the ingredients content of multi-component drugs is a high-performance

liquid chromatography (HPLC); the aim of this work was to work out the method of quantitative determination of the active substances of the “Propolis-Derma” pharmaceutical compositions by this method exactly.

As opposed to the other methods for determining biologically active compounds, the advantage of HPLC method is exactly an ability to identify and quantify substances of different volatility degrees in one sample under carefully selected chromatographic conditions. The presence of such conditions assures the use of so-called “HPLC-analyzer” – an approach the very core of which is analysis of compounds from a certain list (from 20 to 500 substances) with the use of a single chromatographic system. Herewith, the database for the array of standard substances is compiled, and further identification of peaks on chromatograms of the test samples is carried out, by comparison, their retention times, as well as spectral ratios with this database.^[16-18] One of the variants of such a system was developed on the basis of Milichrome A-02 chromatograph (“EcoNova” JSC, Novosibirsk, Russian Federation) are among them.^[19,20]

Therefore, in this research, the results of quantitative determination of the active substances of “Propolis-Derma” preparations and their certain ingredients have been presented with the use of the HPLC-analyzer system exactly.

As the objects of the research, the following samples of the active pharmaceutical ingredients (API) of medicated preparations “Propolis-Derma” were considered: Propolis tincture (the registration certificate № UA/5422/01/01, Order № 700 of the Ministry of Health of Ukraine of 26.10.2011, pharmacopeia monograph 42Y-34-19-95, series 10115) manufactured by “Vitamins” PJSC (Uman, Ukraine); naftifine hydrochloride ($C_{21}H_{21}N \cdot HCl$, USP-39, p. 4978-4979, series B252564) of “SandozSyntekIlaçHammaddeler»production (Turkey) w=99.92%; chlorquinaldol ($C_{10}H_7Cl_2NO$, registration certificate № UA/12467/01/0, Order № 658 of the Ministry of Health of Ukraine of 23.08.2012, series 14/1143/F) manufactured by “All’chemS.A.S.” (France), applicant “Lekhim-Kharkiv” JSC (Kharkiv, Ukraine) w=99.1%; salicylic acid ($C_7H_6O_3$, SPbU 2.0, p. 581-582, series S5670131), manufactured by “MerckKGaA” (Germany) w=99.6%; chlorhexidine gluconate 20% solution ($C_{34}H_{54}Cl_2N_{10}O_{14}$, Eph 7.0., p. 1660-1662, series A-150034, w=100.0%) manufactured by “MEDICHEM S.A.,” Spain.

Based on the substances mentioned above, we have prepared experimental samples of the medicated preparations “Propolis-Derma,” which declared composition is presented in Table 1.

The ready solutions were put into drug markers according to the worked-out technology^[11,14] and subjected to further investigations.

The determination was carried out by the method of reverse-phase HPLC (RPHPLC) with means of microcolonial liquid

Table 1: The composition of “Propolis-Derma” pharmaceutical compositions with the antifungal, antimicrobial, and keratolytic action

№	The name of the sample	Composition of the sample	Amount, %
1.	“Propolis-PNH”	Naftifine hydrochloride	0.1
		Tincture of propolis	99.9
2.	“Propolis-PSC”	Chlorquinaldol	0.5
		Tincture of propolis	77.2
		Salicylic acid	0.3
		96% ethanol	22
3.	“Propolis-PCD”	Chlorhexidine digluconate 20% solution	3.0
		Tincture of propolis	72.0
		96% ethanol	20.0
		Water	5.0

chromatograph Milichrom A-02 produced by “EcoNova” JSC, Novosibirsk. The solutions applied for the trials have been prepared by mass-volume method using analytical balances of the 2nd class of accuracy “RADWAG” manufactured by the “RADWAG Wagi Elektroniczne” company (Radom, Poland). For the production of solutions samples of “Propolis-Derma” pharmaceutical compositions, the propolis tincture and 96% ethanol were taken as solvents.

The identification of API on chromatograms of the preparations examined was performed by the method of external standard. The production samples of the API substances were applied as standard ones. Within the analysis, the standard solutions of API substances were prepared, and the trials were conducted analogically to the ones with solutions of examined samples of preparations. For the analysis, 2 µl samples of standard solutions of active compounds, solutions of pharmaceutical compositions “Propolis-PNH,” “Propolis-PSC,” “Propolis-PCD,” and propolis tincture have been selected and chromatographed on the liquid chromatography with ultraviolet (UV)-detector with receiving at least five chromatograms.

Conditions of the Chromatography

- Column: Ø2 × 75 mm with reverse phase ProntoSIL-120-5-C18AQ (“Bischoff Analysentechnik und Geräte GmbH,” Germany);
- Eluent in the pump “A” of chromatograph—[0.2 M LiClO₄–0.005 M HClO₄];
- Eluent in the pump “B” of chromatograph—acetonitrile “for HPLC;”
- A linear gradient from 10 to 80% of acetonitrile for 40 min;
- Flow rate (eluent loss)—100 µl/min;
- Temperature of the column thermostat –35°C;

- Detection: A detector – UV-spectrophotometer with 6 wavelengths (240, 250, 260, 270, 280, and 300 nm);
- Mode: Single-beam;
- Accuracy-normal;
- Time constant –0.08 s.

Based on the results of experiments on the chromatographic system suitability for the analysis by the RP HPLC method, it was established that the system meets the requirements of 2.2.46 “methods of chromatographic separation” (SPH 2.0, Vol. 1, pp. 126-135).^[15]

Subsequently, samples were prepared for each pharmaceutical preparations “Propolis-Derma.”

For Pharmaceutical Preparation “Propolis-PNH”

- To prepare a solution of a standard sample (SSS) of the substance of naftifine hydrochloride at a concentration of 1 mg/ml: 50 mg (precise weight) of the substance of naftifine hydrochloride was placed into a 50 ml flask, and the volume was increased to the marked point by 80% ethanol;
- To prepare the examined solution of the preparation “Propolis-PNH” sample: 10 ml of “Propolis-PNH” preparation was placed into a 100 ml flask, and the volume was increased to the marked point by 80% ethanol;
- To prepare a solution of propolis tincture: 10 ml of Propolis tincture was placed into a 100 ml flask and adjusted to the marked point by 80% ethanol.

For Pharmaceutical Preparation “Propolis-PSC”

- To prepare an SSS of salicylic acid at a concentration of 1 mg/ml: 50 mg (precise weight) of salicylic acid was placed into a flask of 50 ml capacity, and the volume ethanol was adjusted to the mark by 96% ethanol;
- To prepare a solution of standard sample of chlorquinaldol at a concentration of 1 mg/ml: 50 mg (precise weight) of chlorquinaldol was placed into a flask of 50 ml, and the volume was adjusted to the mark by ethanol 96%;
- To prepare the examined solution of “Propolis-PSC”: 25 µl of “Propolis-PSC” was placed into a 50 ml flask, and the volume was increased to the mark by 96% ethanol;
- To prepare a solution of standard sample of propolis tincture with 96% ethanol: 20.0 g of 96% ethanol was added to 77.2 g of propolis tincture. The obtained solution of propolis tincture was 2-fold diluted by 96% ethanol.

For Pharmaceutical Preparation “Propolis-PCD”

- To prepare a solution of standard sample of chlorhexidine digluconate at a concentration of 0.1%: 5 g of 20% solution of chlorhexidine digluconate (w = 100.0%) was

placed into a 100 ml flask, and the volume was adjusted to the mark by 96% ethanol. The solution obtained was 10-fold diluted by 96% ethanol;

- To prepare the examined solution of “Propolis-PCD” sample: 10 ml of “Propolis-PCD” was placed into a 100 ml flask, and the volume was increased to the mark by 96% ethanol;
- To prepare a solution of propolis tincture: To 72 g of propolis tincture, 20 g of 96% ethanol and 8.0 g of water were added; the solution of propolis tincture was 10-fold diluted by 96% ethanol.

The solutions obtained were filtered and analyzed under the conditions stated above, obtaining at least 5 chromatograms with a sample volume of 2 mcl.

For the identification and quantification of the content of API in “Propolis-Derma” pharmaceutical compositions, before the trials, UV spectra of active substances were previously removed on the milichrom A-02 chromatograph in the range of interval beginning/end—from 190 to 362 nm, the step of interval was equal to 2 nm.

For the quantitative determination of the content of naftifine hydrochloride in the preparation “Propolis-PNH” as analytical length the wave of 260 nm was selected (maximum of naftifine hydrochloride absorption in a solution of standard solution). UV-spectrum of light absorption of naftifine hydrochloride is presented in Figure 1.

The concentration of naftifine hydrochloride (X_1), mg/ml, was calculated according to the formula:

$$X_1 = \frac{S \times C_{CT} \times w}{S_0 \times 100} \times 10$$

Where: S – peak area of naftifine hydrochloride in absorbance units (AU) with the use of chromatograph “Propolis-PNH” at 260 nm;

S_0 – peak area of naftifine hydrochloride in AU in chromatograms of SS solution at 260 nm;

w – active substance content in a naftifine hydrochloride, %;

C_{st} – concentration of the standard sample solution of naftifine hydrochloride, mg/ml.

Identification of determinable compounds was carried out according to retention time (t_R), considering level of values of spectral ratios.

For the quantitative determination of the salicylic acid content in the “Propolis-PSC” as analytical length, the wave of 240 nm was selected (absorption maximum of salicylic acid in the standard sample solution). UV-spectrum of salicylic acid absorption is presented in Figure 2.

The concentration of salicylic acid (X_2), mkg/ml, was calculated according to the formula:

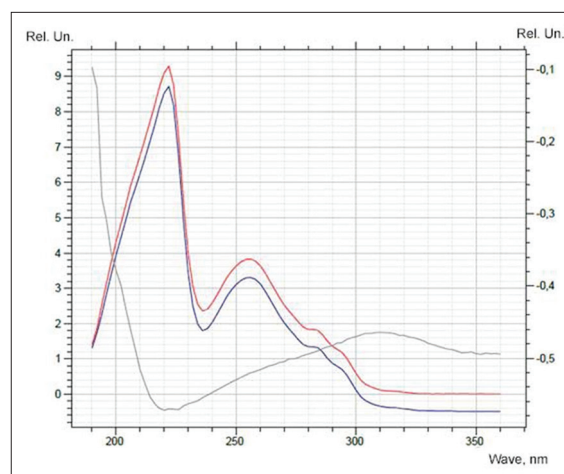


Figure 1: Ultraviolet -spectrum of absorption of naftifine hydrochloride substance

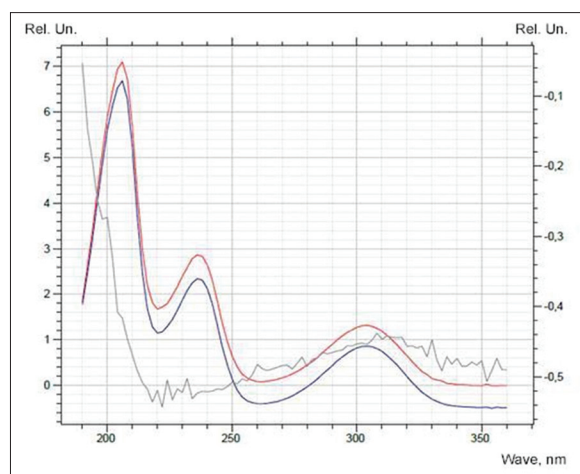


Figure 2: Ultraviolet-spectrum of salicylic acid absorption

$$X_2 = \frac{S \times C_{CT} \times w}{S_0 \times 100} \times 2$$

Where: S – peak area of salicylic acid in AU in chromatogram of the solution of “Propolis-PSC” preparation;

S_0 – peak area of salicylic acid in AU in chromatogram of the standard sample solution;

w – active substance content in the standard sample of salicylic acid, %;

C_{st} – concentration of the SS solution of salicylic acid, mg/ml.

To determine the quantitative content of chlorquinaldol in the “Propolis-PSC” preparation, a wave 250 nm of length was chosen (maximum is on the spectrum of chlorquinaldol). The UV-spectrum of chlorquinaldol absorption is presented in Figure 3.

The concentration of chlorquinaldol (X_3), mkg/ml, was calculated in accordance with the formula:

$$X_3 = \frac{S \times C_{CT}}{S_0} \times 2$$

Where: S – peak area of chlorquinaldol in AU;
 S_0 – peak area of chlorquinaldol AU of the standard solution;
 C_{st} – concentration of the standard solution of chlorquinaldol, mg/ml.

For the quantitative determination of the content of chlorhexidine digluconate in the “Propolis-PCD” preparation, as analytical, the wave was chosen which length corresponded to the maximum absorption of chlorhexidine digluconate in a standard solution of 260 nm. The spectrum of absorption of chlorhexidine 0.1 g digluconate solution is presented in Figure 4.

The concentration of chlorhexidine digluconate (X_4), mg/ml in the preparation “Propolis-PCD” was calculated by the formula:

$$X_4 = \frac{S}{S_0 \cdot 100} \times C_{CT} \times 10 \times P \times 5$$

Where: S – average value of peak area of chlorhexidine digluconate in AU in the “Propolis-PCD” preparation according to the data of five chromatograms;

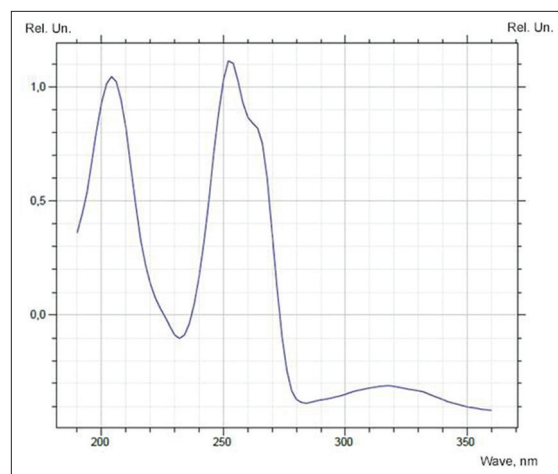


Figure 3: Ultraviolet-spectrum of chlorquinaldol absorption in interval 190–362 ± 2 nm

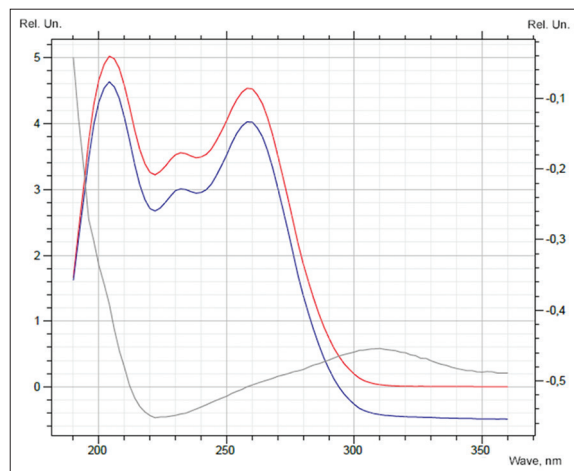


Figure 4: Ultraviolet-spectrum of absorption of 0.1% chlorhexidine digluconate solution

S_0 – average value of peak area of chlorhexidine digluconate absorbance of the standard solution according to the data of five chromatograms;

C_{st} – concentration of the standard solution of chlorhexidine digluconate, %.

To verify the results of quantitative determination of propolis tincture, naftifine hydrochloride, chlorquinaldol, salicylic acid, and chlorhexidine digluconate in “Propolis-Derma” pharmaceutical compositions, 75 mg of SS solution of naftifine hydrochloride was dissolved in 25 ml of the examined drug “Propolis-PNH” with the content of naftifine hydrochloride previously known. The obtained solution was 10-fold diluted with 80% ethanol and chromatographed, and then, the concentration of naftifine hydrochloride was calculated.

When checking the validity of the results of quantitative determination of salicylic acid and chlorquinaldol in the “Propolis-PSC” preparation, 60 mg (precisely weighted amount) of salicylic acid and 65 mg (precisely weighted amount) of chlorquinaldol were dissolved in 25 ml of the studied preparation. The solution obtained with a concentration of salicylic acid 5.2 mg/ml and 7.46 mg/ml of chlorquinaldol were 2-fold diluted with 96% ethanol, chromatographed, and the concentration of salicylic acid and chlorquinaldol was determined in preparation with addition.

The similar experiments have been conducted to confirm the accuracy of the data of the quantitative determination of the chlorhexidine digluconate content in “Propolis-PCD” preparation: 1.2 ml of 20% solution of chlorhexidine digluconate was dissolved in 25.00 ml of the preparation examined. The solution obtained with a concentration 1.5% was 10-fold diluted with 96% ethanol and chromatographed, and then, concentration of chlorhexidine digluconate was determined in preparation with addition.

RESULTS AND DISCUSSION

In Tables 2 and 3, the results of identifying the API in the “Propolis-Derma” pharmaceutical compositions are presented, and the main chromatographic parameters of the identified compounds are determined in reference to the retention time (t_R), and the values of spectral ratios in the conditions for analysis we have proposed.

In accordance with the results of experimental studies, by comparing the retention times and the spectral ratios of the peaks obtained, a presence of naftifine hydrochloride and propolis tincture in the medicated preparation “Propolis-PNH” has been confirmed; as well as the presence of chlorquinaldol, salicylic acid, and propolis tincture presence – in the composition of the medicated drug “Propolis-PSC;” and chlorhexidine digluconate with propolis tincture presence – in the medicated drug “Propolis-PCD.”

Table 2: Comparing of chromatographic and spectral parameters of API in the standard solutions and “Propolis-Derma” pharmaceutical compositions

№	The name of the sample	Compound	Retention time, t_R , min	Absorption peak, λ , nm	Spectral ratios					
					240 nm 240 nm	250 nm 240 nm	260 nm 240 nm	270 nm 240 nm	280 nm 240 nm	300 nm 240 nm
1	“Propolis-PNH”	Naftifine hydrochloride	25.4	260	1	1.41	1.41	0.98	0.71	0.22
	Standard solution		25.4	260	1	1.23	1.34	1.1	0.81	0.59
2	“Propolis-PSC”	Chlorquinaldol	25.5	250	1	2.16	1.76	1.08	0.17	0.17
		Salicylic acid	13.0	240	1	0.25	0.04	0.06	0.17	0.48
		Chlorquinaldol	25.6	250	1	2.06	1.72	1.07	0.23	0.24
		Salicylic acid	13.09	240	1	0.24	0.04	0.67	0.18	0.4
3	“Propolis-PCD”	Chlorhexidine digluconate	25.6	260	1	1.17	1.31	1.02	0.535	0.05
	Standard solution		25.6	260	1	1.04	1.23	1.11	0.79	0.54

API: Active pharmaceutical ingredients

Table 3: Comparing of chromatographic and spectral parameters of the standard solutions of propolis tincture and “Propolis-Derma” pharmaceutical compositions

№	The name of the sample	Compound	Retention time, t_R , min	Absorption peak, λ , nm	Spectral ratios					
					240 nm 240 nm	250 nm 240 nm	260 nm 240 nm	270 nm 240 nm	280 nm 240 nm	300 nm 240 nm
1	“Propolis-PNH”	Propolis tincture	24.9	290	1	0.745	1.028	1.293	1.376	1.613
	Standard solution		25.0	290	1	0.741	1.011	1.249	1.342	1.600
2	“Propolis-PSC”	Propolis tincture	24.8	290	1	0.750	1.027	1.252	1.287	1.588
	Standard solution		24.8	290	1	0.740	1.001	1.227	1.300	1.600
3	“Propolis-PCD”	Propolis tincture	25.2	290	1	0.870	0.942	1.212	1.389	1.517
	Standard solution		25.0	290	1	0.851	0.951	1.253	1.459	1.560

The studies conducted allow to conclude that for identification of the active substances in medicated preparations “Propolis-Derma” (“Propolis-PNH,” “Propolis-PSC,” and “Propolis-PCD”) same method may be applied, as despite the fact that retention time of the API such as naftifine hydrochloride, chlorhexidine digluconate, and chlorquinaldol is the same approximately, they are significantly different in terms of in their spectral ratios: Maximum point of naftifine hydrochloride and chlorhexidine digluconate absorption is 260 nm, of chlorquinaldol-250 nm, and salicylic acid is reliably identified by retention time.

The results of experiments having presented in Figures 1-4 indicate that on the naftifine hydrochloride spectrum [Figure 1], specific bands are evident at the maximum points of absorption 224 nm, 254 nm; on the spectrum of chlorhexidine digluconate [Figure 2] – maximum is at 205 nm, 232 nm, and 258 nm; on the spectrum of salicylic acid [Figure 3] – at 205 nm, 238 nm, and 330 nm; and on the spectrum of chlorquinaldol [Figure 4] – at 205 nm, 252 nm, and 320 nm.

Hereby, to confirm that taking into account the spectra obtained of the medicated preparations “Propolis-PNH,” “Propolis-PSC,” and “Propolis-PCD,” it is necessary to choose the following wavelengths: 240 nm, 250 nm, 260 nm, 270 nm, 280 nm, and 300 nm to create a unified method.

The typical chromatograms of solutions of investigated preparations are given in Figures 5-7.

The results of the quantitative determination of API which are part of the “Propolis-Derma” pharmaceutical compositions are presented in Table 4.

As is evident from the data in Table 4, the value of RSD does not exceed 2.3%. Reliability of the results obtained according to the worked out methods is confirmed by the addition technique [Table 5].

As can be seen from the data in Table 5, δ does not exceed 3.3% (relatively), that is, results are reliable.

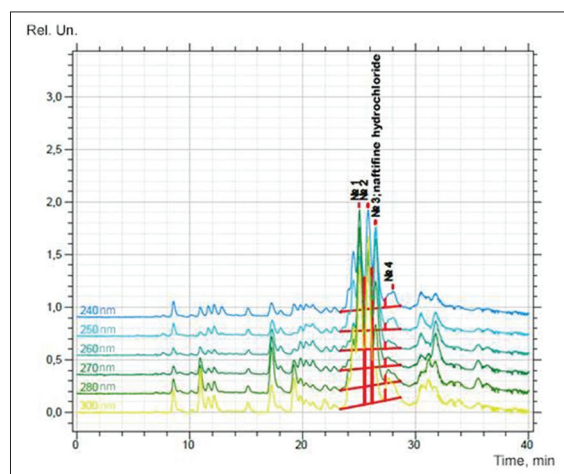
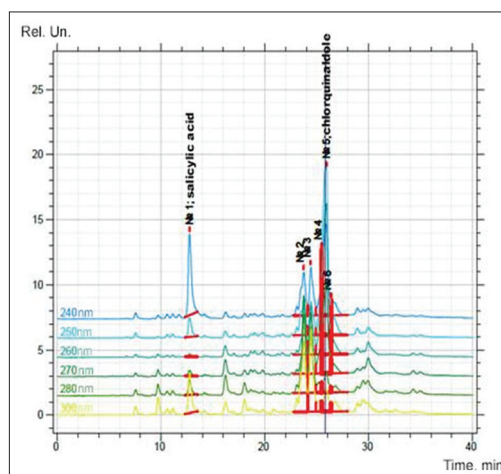
Table 4: Results of quantitative determination of APhI in the “Propolis-Derma” pharmaceutical compositions

№	APhI and their standardized content, %	Found, $x \pm \Delta x$, %	Metrological characteristics	RSD, %
“Propolis-PNH”				
1.	Naftifine hydrochloride 0.09–0.11	0.108±0.003	$x=0.108$ $S=0.0025$ $\Delta x=0.003$ $RSD=2.32\%$ $\varepsilon=2.89\%$	2.32
“Propolis-PSC”				
2.	Chlorquinaldol 0.45–0.55	0.486±0.004	$x=0.486$ $S=0.003$ $\Delta x=0.004$ $RSD=0.62\%$ $\varepsilon=0.82\%$	0.62
	Salicylic acid 0.27–0.33	0.277±0.004	$x=0.277$ $S=0.002$ $\Delta x=0.003$ $RSD=0.80\%$ $\varepsilon=1.00\%$	0.80
“Propolis-PCD”				
3.	Chlorhexidine digluconate 20% solution 0.50–0.60	0.627±0.005	$x=0.628$ $S=0.004$ $\Delta x=0.005$ $RSD=0.64\%$ $\varepsilon=0.80\%$	0.64

Table 5: Verification of the obtained results of analysis by the addition technique

№	Content of APhI,%	Added, %*	Found total, $x\pm\Delta x$, %	Accuracy δ^{**} , %
“Propolis-PNH”				
1.	Naftifine hydrochloride 0.108	0.297	0.401±0.003	−1.35
“Propolis-PSC”				
2.	Chlorquinaldol 0.486	0.257	0.736±0.004	−2.7−0.8
3.	Salicylic acid 0.277	0.240	0.515±0.004	
“Propolis-PCD”				
4.	Chlorhexidine digluconate 0.627	0.960	1.600±0.005	+1.35

$$^{**} \delta = \frac{\bar{x} - \mu}{\mu} \times 100\%, \text{ where } \mu^* - \text{actual content, \%}$$

**Figure 5:** Chromatogram of the “Propolis-PNH” preparations (dilution 1:10 by 80 % ethanol)**Figure 6:** Chromatogram of the “Propolis-PSC” preparation (dilution 1:2 by 96% ethanol)

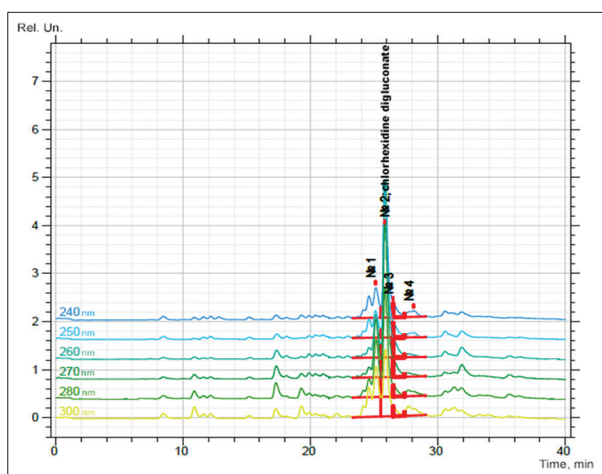


Figure 7: Chromatogram of the “Propolis-PCD” preparation (dilution 1:10 by 96 % ethanol)

Thus, the obtained results of the conducted studies testify to the suitability of the developed by us chromatography methods for identification and quantitative determination of active pharmaceutical ingredients (API) in the proposed medical and prophylactic agents “Propolis-Derma” (“Propolis-PNH”, “Propolis-PSC” of and of “Propolis-PCD”), which were studied do clinical pharmaceutical research which have therapeutic activity in doses of amniotic fluid index, which are included in Table 1.^[12,14]

The complex of experimental research allows implementing further standardization of the composition of medicated preparations “Propolis-PNH,” “Propolis-PSC,” and “Propolis-PCD” which have been developed by us.

CONCLUSIONS

1. The certain conditions are recommended and the possibility of identifying the ac API in the pharmaceutical compositions “Propolis-Derma” (“Propolis-PNH,” “Propolis-PSC,” and “Propolis-PCD”) according to the chromatographic (RP HPLC) and spectral parameters are shown.
2. The certain conditions are proposed, the method is developed, and the opportunity is represented for the quantitative determination of the API in the medicated preparations “Propolis-PNH,” “Propolis-PSC,” and “Propolis-PCD” by the RP HPLC.
3. The reliability (correctness) of the results of quantitative determination is proved by the method of additions.

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