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Identification and quantification determination of the main components of medicinal plants in the dental gels "Rotrin - Dent "

Dental diseases, such as inflammatory illnesses of the oral mucosa, are the inflammatory diseases of gums and are the important problems of stomatology [1,2]. At the base of National University of Pharmacy it was made the gel for the treatment of inflammatory diseases of the mucous membranes of the oral cavity [3,4,5]. In the gel as a biologically active substance it was added the alcohol complex of herbs: chamomile flowers, calendula flowers, yarrow (2:1:1) at the composition of gel as the biological active substance.

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[1] Данилевский Н. Ф. Мониторинг состояния гигиены полости рта взрослого населения Украины как медицинское обоснование планирования региональных программ профилактики (Сообщение 1) / Н. Ф. Данилевский, М. Ю. Антоненко, Л. Ф. Сидельникова // Современная стоматология. – 2005. – № 2 (30). – С. 164 – 168.

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Biologically active substances of plants that are fond in the complex have the local anti-inflammatory effect , aggravate and accelerate the repair processes of the mucous membranes (improvement of trophic processes) and have certain hemostatic characteristics [1].

The objective of this phase of the experiment is to develop a method of qualitative and quantitative determination of the complex of plant substances in the founded gel for its standardization.

These researches were carried out with the State Enterprise "Scientific and expert pharmacopoeia center of quality control of drugs" in Kharkiv. It was found that the method of high efficiency liquid chromatography is the best method for the qualitative and quantitative determination of components in the gel. [2,3,4]

We have developed a method of determining of the proposed components of the gel with the help of the method of liquid chromatography.

The method consists in determining of the basic components of an alcoholic solution of herbs which are found in the gel : luteolin -7- glycoside , apigenin -7- glycoside , rutin , isorhamnetin , luteolin and apigenin (see Table 1.) .

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Table 1

Content of basic components of medical herbs:

Complex of medical herbs in the ratio 2:1:1	Total amount of alcohol solution, g.	Approximate content of active materials, in mg:
chamomile flowers calendula flowers yarrow	10,0	Apigenin glycoside 0.08 Luteolin glycoside 0.04 isorhamnetin 0.04 luteolin 0.02 apigenin 0.02 rutine 0.10

Experimental part

Tests on the identification of selected components of medicinal plants were carried out in accordance with the requirements of HFC. In the chromatogram of the test solution the retention time of peaks of luteolin-7-glycoside, apigenin-7-glycoside, rutin, isorhamnetin, luteolin and apigenin correspond to the retention time of peaks of luteolin-7-glycoside, apigenin-7-glycoside, rutin, isorhamnetin, luteolin and apigenin on the chromatogram of the reference solution.

Quantitative determination of the main components of medicinal plants consists of the following steps:

Preparation of test solution

Approximately 1.00 g (exact weight) of the gel is placed into a flask of 25 ml. We add 10 ml of 80% ethanol, we mix thoroughly to obtain a homogeneous mixture and we dilute the volume of solution with 80% ethanol to the mark, we mix and centrifuge at a speed of 7000 rev/min. during 10 min. We filter the obtained solution through a Teflon membrane filter with a orifice of 0.45 microns.

[1] Державна Фармакопея України / Держ. п-во “Науково-експертний фармакопейний центр”. – 1-е вид., 1. доп. – Х. : РІРЕГ, 2004. – 520 с.

Preparation of the comparative solution

10.0 mg (exact weight) of the standard sample of luteolin, about 10.0 mg (exact weight) of the standard sample of apigenin, about 50.0 mg (exact weight) of the standard sample of rutine, about 20.0 mg (exact weight) of the standard sample of isorhamnetin, about 20.0 mg (exact weight) of the standard sample of luteolin-7-glycoside and about 40.0 mg (exact weight) of the standard sample of apigenin-7-glycoside is placed in a flask of 50 mL, add 20 ml of 80% ethanol we mix to dissolve (it is acceptabel to use the ultrasound), the solution volume was adjusted with 80% ethanol to the mark and mix. 1.0 ml of this solution was placed into a flask of 25 ml and dilute the solution with 80% ethanol to the mark and mix. The obtained solution is filtered through a Teflon membrane filter with an orifice of 0.45 microns.

Accomplishment of the analysis

50 mcl of the comparative solution and the test solution are chromatographed on a liquid chromatograph with a spectrophotometric detector , obtaining at least three chromatograms at the following conditions:

- Column Hypersil BDS-C18, with the size of 250 mm x 4.6 mm, filled with a sorbent with the size of particles of 5 microns or the analogous;
- Pre-column : Hypersil BDS-C18 60 mm x 4.6 mm, filled with a sorbent with the size of particles of 5 microns or analogous;
- Mobile phase : concentrated phosphoric acid - water (1:99 by volume) , degassed by the convenient manner ;
- Mobile phase B: concentrated phosphoric acid - acetonitrile (1:99 by volume) degassed by the convenient manner ;
- Temperature of column thermostat 30,0 ° C;
- Mobile phase speed 1.0 ml / min;
- Detection in wavelength 340 nm.

- Gradient elution with the following program:

Time, min.	Mobile phase A	Mobile phase B
0-10	80	20
10-30	80→20	20→80
30-40	20	80
40-41	20→80	80→20
41-50	80	20

Under such conditions the peaks of luteolin -7- glycoside , apigenin -7- glycoside , the rutine isorhamnetin , luteolin and apigenin are completely separated from each other and from the supporting components of the gel.

Before the quantitative analysis it was chromatographed 10 mkl of the separate solutions luteolin -7- glycoside , apigenin -7- glycoside , rutin , isorhamnetin , luteolin and apigenin (concentration 5 mg / ml) to determine their retention times and to identify the correspond biologically active components.

The chromatographic system is considered suitable if the following conditions are fulfilled:

- Efficiency of the chromatographic system , calculated from the peak of apigenin -7- glycoside should be not less than 2000 tt ;
- Symmetry factor of the peak of rutine must be not more than 2.0 ;
- The comparative standard deviation of the peak areas of luteolin -7- glycoside , apigenin -7- glycoside , rutin , isorhamnetin , luteolin and apigenin must comply with the requirements of 2.2.46 (HFC 1.2). [1]

[1] Державна Фармакопея України / Держ. п-во “Науково–експертний фармакопейний центр”. – 1-е вид., 2. доп. – Х. : РІРЕГ, 2008. – 620 с.

Contents of luteolin-7-glycoside, apigenin-7-glycoside or rutin or isorhamnetin or luteolin, or apigenin, in milligrams per 1 g of gel is calculated by the formula (1):

$$Y = \frac{S \cdot m_0 \cdot 1 \cdot P \cdot 25}{S_0 \cdot 50 \cdot 25 \cdot 100 \cdot m} = \frac{S \cdot m_0 \cdot P}{S_0 \cdot m \cdot 5000}, \quad (1)$$

where

- S - is the average value of the peak areas of luteolin-7-glycoside, or apigenin-7-glycoside and rutin or isorhamnetin or luteolin and apigenin, calculated from the chromatogram of the test solution;
- S_0 - The average value of the peak areas of luteolin-7-glycoside and apigenin-7-glycoside or rutine or isorhamnetin or luteolin and apigenin, calculated from the chromatogram of the comparative solution;
- M_0 - Mass of weight CO luteolin-7-glycoside and apigenin-7-glycoside or routine or isorhamnetin or luteolin and apigenin, mg
- P- the content of the basic substance in CO luteolin-7-glycoside and apigenin-7-glycoside and rutin or isorhamnetin or luteolin, or apigenin, in percentage.

1 g of the gel shall contain:

luteolin-7-glycoside not less than 0.03 mg
apigenin 7-glycoside not less than 0.06 mg
rutine of not less than 0.08 mg
isorhamnetin not less than 0.03 mg
luteolin not less than 0.015 mg
apigenin not less than 0.015 mg.

The specificity of the methods is confirmed by the placebo of chromatograms and components, which are determined. It is shown that in the chromatogram of placebo solution (Fig. 1) there is no any peaks with retention times that coincide with the retention times of the components which are determined. [1,2]

Also, the chromatogram of comparative solution (fig. 2) and at the typical chromatogram of the test solution (Fig. 3) the specificity is confirmed by the fulfillment of the requirements for the spectral purity of peaks of the components

which are determined. Cleanliness of the peaks is obtained using detektor photodiode. [3,4]

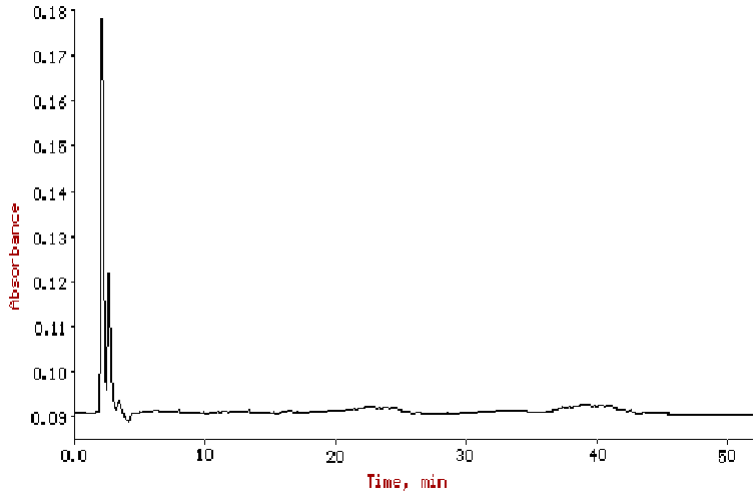

Fig 1. Chromatogram of the solution of drug doesn't contain the alcohol complex of medical herbs.

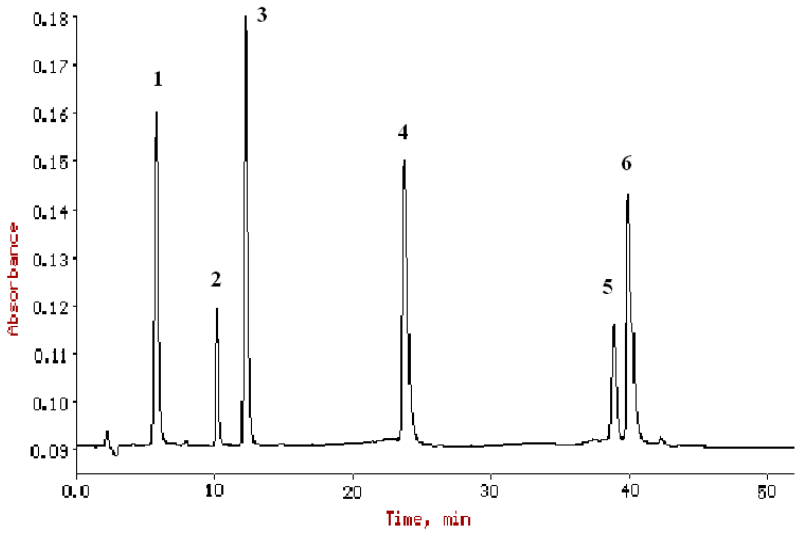

Fig. 2 Chromatogram of the comparative solution where the peaks: 1 - rutin, 2 - luteolin-7-glycoside, 3 -apigenin-7-glycoside, 4 - isorhamnetin, 5 - luteolin, 6 – apigenin

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[1] Cazes J. , Scott R.P.W. Chromatography theory. – New York–Basel : Marcel Dekker, Inc., 2002. – 476 p.

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[3] Fernandez Alba A.R. Chromatographic-Mass Spectrometric Food Analysis for Trace Determination of Pesticide Residues. – New York, London: Academic Press, 2004. – 510 p.

[4] Handbook of Ion Chromatography / [ed. by J. Weiss]. – New York : John Wiley & Sons, 2005. – 931 p

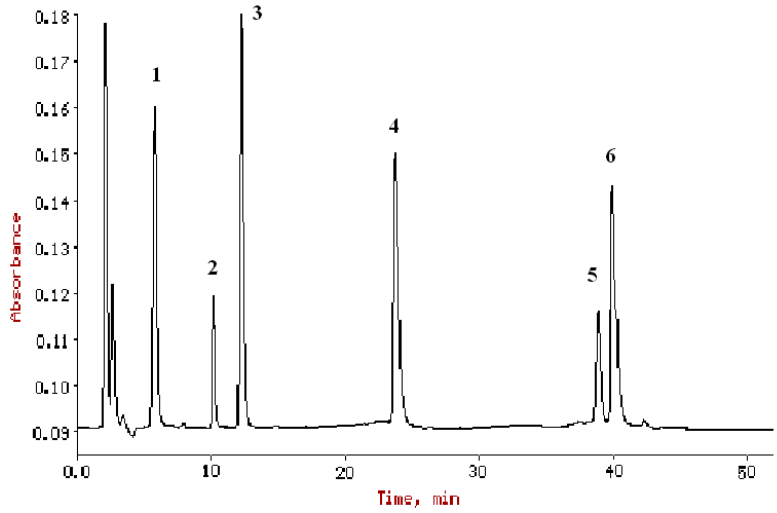

Fig. 3 Chromatogram of the comparative solution where the peaks: 1 - rutin; 2 - luteolin-7-glycoside 3 - apigenin -7-glycoside; 4 - isorhamnetin; 5 - luteolin; 6 - apigenin

CONCLUSIONS:

1. It was developed high efficiency liquid chromatography method for the quantitative determination of an alcoholic solution of the components of herbal raw materials (luteolin-7-glycoside, apigenin-7-glycoside, rutin, isorhamnetin, luteolin and apigenin) in the new dental gel of local action.
2. It was calculated and received the information of content of luteolin-7-glycoside, apigenin-7-glycoside, rutin, isorhamnetin, luteolin and apigenin in 1 gram of gel.
3. The specificity of the technique is confirmed by the chromatograms of placebo, by the comparative solution and a typical chromatogram of the test solution components
4. It was selected a modern high efficiency liquid chromatography method that ensured the specificity, accuracy and reproducibility of results, which allowed at the same time to carry out the quantification identification and determination of the test substance in the gel.

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