UDC 615.07:543.544.5.068.7

A. A. Goncharova[1], I. I. Baranova[1], A. Y. Kulikov[2]

[1]*The National University of Pharmacy, Kharkov*

[2]*State Enterprise "Ukrainian Scientific Pharmacopoeial center for quality of drugs"*

IDENTIFICATION AND QUANTITATIVE DETERMINATION OF ACTIVE COMPONENTS OF "DERMALIPOIN" CREAM

The techniques of high performance liquid chromatography for the determination of α-lipoic acid and tea tree oil in the new medicament for use in the diabetic foot were developed. Offered modern techniques allow both the identification and quantitative determination of test substance in the cream.

Key words: α-lipoic acid, tea tree oil, identification, quantitative determination, high performance liquid chromatography.

STATEMENT OF THE PROBLEM

Diabetes mellitus disease gets threatening level in recent years [12, 13]. Worldwide, there is a rapid increase in the number of patients that increases the frequency of specific diabetes complications such as heart pathology, kidney failure, blindness, foot lesions, etc. [14]. We have developed a cream "Dermalipoin" for use in diabetic foot syndrome [4]. As active substances to the cream were added -lipoic acid and urea, that due to their properties effect anti-inflammatory and wound healing. As an antimicrobial component tea tree oil was selected and it was also proved that this component acts as a highly effective preservative [10].

According to the requirements State Pharmacopoeia of Ukraine in the analysis of soft dosage forms is necessary to conduct qualitative and quantitative determination of all active substances [5]. During previous studies methods for analyzing urea have been developed. I was determined to be optimal the biuret test and the reaction with concentrated nitric acid for the qualitative determination of urea in cream and bromatometry for the quantitative determination [3].

ANALYSIS OF RECENT RESEARCH AND PUBLICATIONS

For identification and definition of quantitative content of essential oils in medicines most often used method of gas chromatography (GC) according to SPU 2.2.28 and 2.2.46 (N) [5]. For pharmacopoeial monograph on tea tree oil for the identification of substances carried out by thin layer chromatography. Researchers of the National University of Pharmacy have developed GC test procedures for qualitative and quantitative analysis of tea tree oil in pharmaceutical dosage forms [1, 8, 9].

FORMULATION OF OBJECTIVES

The objective of this phase of the experiment was to develop techniques of qualitative and quantitative determination of α-lipoic acid and tea tree oil for standardization of the developed cream.

THE MAIN MATERIAL OF RESEARCH MATERIALS AND METHODS

The study was conducted jointly with the State Enterprise "Ukrainian Scientific Center Pharmacopoeial quality of drugs" (Kharkov).

The object of the study was the cream for use in diabetic foot syndrome following composition: olive oil, shea butter, emulsifier complex, α-lipoic acid, urea, tea tree essential oil, purified water.

Chromatographic measurements were performed on a liquid chromatograph Hewlett Packard 1050 (Agilent Technologies, Waldbronn, Germany) equipped with an integrator 3395 series.

As a standard (control) substances pharmaceutical substances of drugs containing the basic substance of at least 98% were used. The tests were performed according to the requirements of the State Pharmacopoeia of Ukraine 2.2.29 [5].

The method and conditions of chromatography are presented in research results.

RESULTS AND DISCUSSION

The work revealed that the most appropriate and modern method for qualitative and quantitative determination of α-lipoic acid and tea tree oil in the designed cream is high-performance liquid chromatography (HPLC) [2, 7, 11]. We have developed methods (procedures) for determining α-lipoic acid and tea tree oil in cream by this method.

Determination procedures consist of next stages: test solution preparation, comparison solution preparation, performance analysis, interpretation of results (for identification) and calculation of the quantitative content of the studied components (for the quantitative determination).

Determination procedure for α-lipoic acid

Preparation of the test solution. Approximately 1.00 g (exact sample) of drug is placed in a 100 ml capacity graduated flask, add 40 ml of acetonitrile, mix thoroughly to obtain a homogeneous mixture, complete with acetonitrile up to the mark and mix. Filter the resulting solution through a teflon membrane filter with 0.45 microns pore size.

Preparation of α-lipoic acid reference solution. Approximately 0.05 g (exact sample) of α-lipoic acid standard sample is placed in a 50 ml capacity graduated flask, dissolve with 20 ml of acetonitrile, complete with acetonitrile to the mark and mix. 2.0 ml of the resulting solution is placed in a 10 ml capacity graduated flask, complete with mobile phase up to the mark and mix.

Analysis procedure. The reference solution and the test solution 20 ml each chromatographed on a liquid chromatograph with a spectrophotometric detector receiving at least 3 chromatograms under the following conditions [15]:

- Column Supelcosil LC-18, size 150 mm x 4.6 mm, filled with sorbent particle size 3 microns or equivalent;
- Mobile phase: acetonitrile - 0.05 M solution of potassium dihydrogen phosphate, pH brought to 2.5 with concentrated phosphoric acid potentiometrically (45:55 by volume), degassed in convenient way;
- The temperature of the column thermostat 30.0 °C;
- The rate of mobile phase 0.8 ml/min;
- Detection by wavelength of 332 nm.

Chromatographic system is considered suitable if the following conditions are fulfilled:

• The efficiency of the chromatographic system calculated for a peak of α-lipoic acid should be not less than 1500 tt;

• Peak symmetry factor of α-lipoic acid should not exceed 2.0;

• The relative standard deviation of peak areas α-lipoic acid must meet the requirements 2.2.46 (SPU 1 ed., Suppl. 2) [6].

Identification of α-lipoic acid. In the chromatogram obtained with test solution (Fig. 2) retention time of peak of α-lipoic acid must meet the retention time of the peak of α-lipoic acid in the chromatogram obtained with reference solution (Fig. 1).

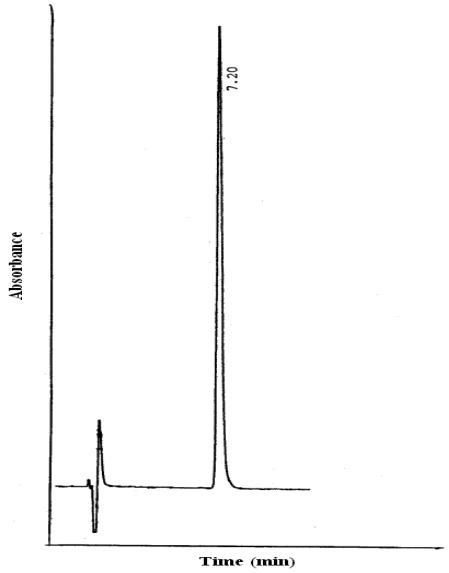

Fig. 1. *Chromatogram obtained with α-lipoic acid reference solution*

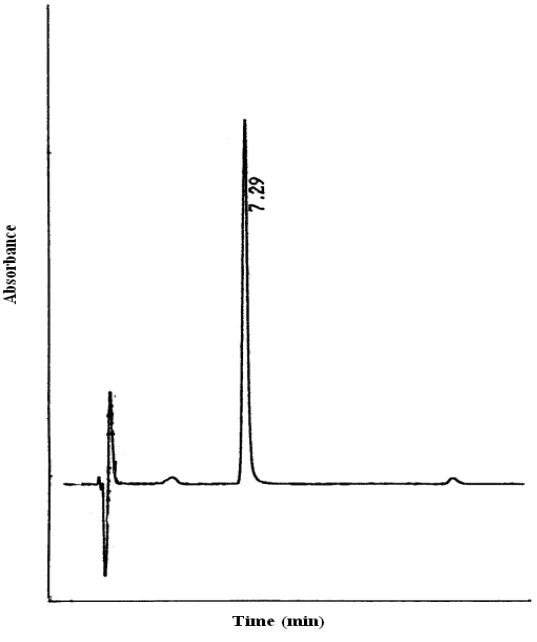

Fig. 2. *Chromatogram obtained with the test solution of cream "Dermalipoin" for α-lipoic acid identification*

Quantitative determination. The content of α-lipoic acid in grams in 1 g of the drug calculated with the formula (1):

$$Y = \frac{S \cdot m_0 \cdot 2 \cdot P \cdot 100}{S_0 \cdot 50 \cdot 10 \cdot 100 \cdot m} = \frac{S \cdot m_0 \cdot P}{S_0 \cdot m \cdot 500} \quad (1);$$

where:

S — the average peak areas of α-lipoic acid calculated from the test solution chromatogram;

S_0 — the average peak areas of α-lipoic acid calculated from the reference solution chromatogram;

m_0 — sample weight of α-lipoic acid standard sample, g;

m — sample weight of the drug, g;

P — the fraction of the main substance in α-lipoic acid standard sample.

The content of α-lipoic acid in 1 g of the drug should be from 0,018 to 0,022 g.

Determination procedure for tea tree oil

Preparation of the test solution. Approximately 4.0 g (exact sample) of drug is placed in a 25 ml capacity graduated flask, add 10 ml of methanol, mixed thoroughly to obtain a homogeneous mixture, complete with methanol up to the mark, mix and centrifuged at a speed of rotation of 7000 rpm for 10 minutes. Filter the resulting supernatant liquid through a teflon membrane filter with 0.45 microns pore size, discarding the first 5 ml of filtrate.

Preparation of tea tree oil reference solution. Approximately 0.5 g (exact sample) of tea tree oil standard sample is placed in a 100 ml capacity graduated flask, add 50 ml of methanol, mixed thoroughly to dissolve and complete up to the mark with methanol and mix. Filter the resulting solution through a teflon membrane filter with 0.45 microns pore size, discarding the first 5 ml of filtrate.

Analysis procedure. Reference solution and the test solution 10 ml each chromatographed on liquid chromatograph with a spectrophotometric detector receiving at least 3 chromatogram of the following conditions:

- Column Symmetry C18, size 150 mm x 3.9 mm, filled with sorbent particle size 5 microns or equivalent;
- Pre-column: Symmetry C18 60 mm × 4.6 mm, filled with sorbent particle size 5 microns or equivalent;
- Mobile phase: methanol – water (90:10 v/v), degassed in convenient way;
- The temperature of the column thermostat 30.0 °C;
- rate of mobile phase 0.8 ml/min;
- Detection by wavelength 254nm.

Chromatographic system is considered suitable if the following conditions are fulfilled [15]:

• The efficiency of the chromatographic system calculated for peak of tea tree oil should be at least 1,000 tt;

• Factor of peak symmetry of tea tree oil should not exceed 2.0;

• The relative standard deviation of peak areas of tea tree oil must meet the requirements 2.2.46 (SPU 1 ed., Supl. 2) [6].

Identification of tea tree oil. In the chromatogram obtained with test solution (Fig. 4) retention time peak corresponding to the tea tree oil must meet the retention time of the main peak of tea tree oil in the chromatogram obtained with reference solution (Fig. 3).

Quantitative determination. The content of tea tree oil in grams in 1 g of the drug is calculated by the formula:

$$Y = \frac{S \cdot m_0 \cdot 25}{S_0 \cdot 100 \cdot m} = \frac{S \cdot m_0 \cdot P \cdot 0.25}{S_0 \cdot m} \quad (2);$$

where:

S — the average peak areas of tea tree oil calculated from the test solution chromatogram;

S_0 — the average peak areas of tea tree oil calculated from the reference solution chromatogram;

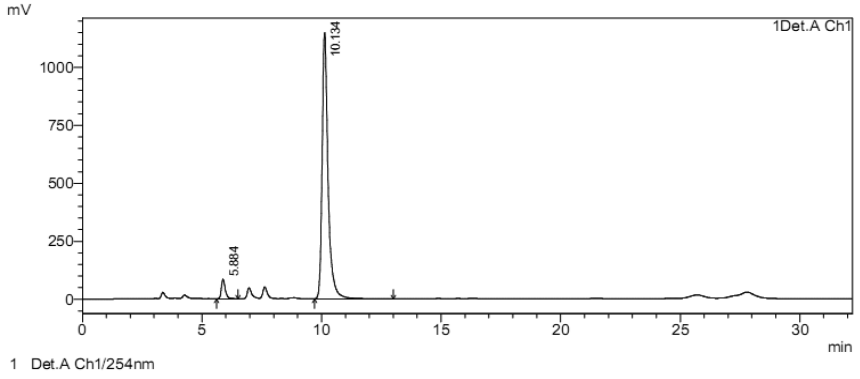

Fig. 3. *Chromatogram obtained with tea tree oil reference solution*

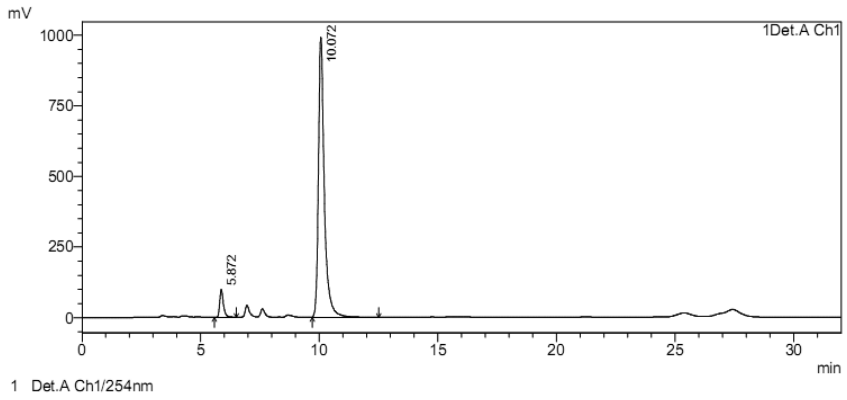

Fig. 4. *Chromatogram obtained with the test solution of cream «Dermalipoin» for tea tree oil identification*

m_0 — sample weight of standard sample of tea tree oil, g;

m — sample weight of the drug, in grams.

The content of tea tree oil in 1 g of the drug should be from 0,027 to 0,033 g.

CONCLUSIONS AND PROSPECTS FOR FURTHER RESEARCH

The methods of qualitative and quantitative determination of α-lipoic acid and tea tree oil by high performance liquid chromatography in a new emulsion drug for use in diabetic foot syndrome were developed. Content of α-lipoic acid and tea tree oil in 1 gram of the developed cream was calculated. The proposed modern test procedures meet the requirements of the State Pharmacopoeia of Ukraine and can simultaneously conduct quantitative detection and identification of these substances in multicomponent cream.

The data will later be used in the design of quality control methods for the developed cream "Dermalipoin" for use in diabetic foot syndrome.

REFERENCES INFORMATION

1. Баранова И. И. Количественное определение эфирного масла чайного дерева в геле / И. И. Баранова, О. Г. Башура, В. А. Лебединец // Физиологически активные вещества. – 2000. - №2 (30). – С. 55-58.
2. Высокоэффективная жидкостная хроматография в контроле качества лекарственных средств / Г. И. Барам, Е. Д. Гольдберг, Д. В. Рейхарт и др. // Фарматека. — 2005. — №2. — С. 12-15.
3. Гончарова А. А. Ідентифікація та кількісне визначення сечовини в кремі для застосування при синдромі діабетичної стопи / А. А. Гончарова, І. І. Баранова, М. Є. Блажеєвський // Проблеми екологічної та медичної генетики і клінічної імунології : зб. наук. пр. – 2014. – № 4 (124). – С. 147–153.
4. Гончарова А. А. Обґрунтування складу крему для застосування при синдромі діабетичної стопи / А. А. Гончарова, І. І. Баранова, Ю. В. Ковтун // Косметологія : сьогодення та майбутнє : наук.–практ. конф. з між нар. участю, м. Харків, 15 лист. 2013 р. – Х., 2013. – С. 60–61.
5. Державна Фармакопея України / Держ. п-во «Науково-експертний центр». — 1–е вид. – Х. : РІРЕГ, 2001. – 556 с.
6. Державна Фармакопея України / Держ. п-во «Науково-експертний фармакопейний центр». – 1–е вид., 2 допов.– Х. : РІРЕГ, 2008. – 620 с.
7. Куликов А. Ю. Высокоэффективная жидкостная хроматография в фармацевтическом анализе. Тест «Проверка пригодности хроматографичес-кой системы» / А. Ю. Куликов, А. Г. Верушкин. // Вісник Харк. Нац. Ун-ту. № 549, Серія Хімія. – 2002.– Вип. 9 (31). – С. 57-65.
8. Лебединець О. В. Ідентифікація та кількісне визначення ефірних олій чайного дерева та евкаліпту в складі стоматологічного гелю / О. В. Лебединець, О. А. Зінченко, І. І. Баранова // Сучасні досягнення фармацевтичної технології : матеріали ІІ наук.-практ. конф. з міжнар. участю, м. Харків, 17-18 листоп. 2011 р. – Х.: Вид-во НФаУ, 2011. – С. 109.
9. Левачкова Ю. В. Розробка методик визначення ефірних олій у песаріях «фітовагін» методом газової хроматографії [Електронний ресурс] / Ю. В. Левачкова, В. М. Чушенко //Управління, економіка та забезпечення якості в фармації . - 2012. - № 1. - С. 33-36.
10. A review of applications of tea tree oil in dermatology / N. Pazyar, R. Yaghoobi, N. Bagherani,

A. Kazerouni // International Journal of Dermatology. – 2013. – Vol. 52, № 7. – P. 784–790.
11. Handbook of Ion Chromatography / [ed. by J. Weiss]. – New York : John Wiley & Sons, 2005. – 931 p.
12. IDF Diabetes Atlas: global estimates of the prevalence of diabetes for 2011 and 2030 / D. Whiting, L. Guariguata, C. Weil, J. Shaw // Diabet Res. Clin. Pract. – 2011. – Vol. 94, № 3. – P. 311–321.
13. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country–years and 2.7 million participants / G. Danaei, M. Finucane, Y. Lu et al. // The Lancet. – 2011. – Vol. 378, № 9785. – P. 31–40.
14. Turns M. Diabetic foot ulcer management : the podiatrist's perspective / M. Turns // Br. J. Community. Nurs. – 2013. – Suppl. 14. – P. 16–9.
15. Validating chromatographic methods: a practical guide / D. Bliesner. – New York : John Wiley & Sons, 2006. – 304 p.

УДК 615.07:543.544.5.068.7

А. А. Гончарова, И. И. Баранова, А. Ю. Куликов

ИДЕНТИФИКАЦИИ И КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ АКТИВНЫХ КОМПОНЕНТОВ КРЕМА «ДЕРМАЛИПОИН»

Разработаны методики высокоэффективной жидкостной хроматографии для определения α-липоевой кислоты и масла чайного дерева в новом лекарственном средстве для применения при синдроме диабетической стопы. Предложенные современные методики позволяют одновременно проводить идентификацию и количественное определение исследуемых веществ в креме.

Ключевые слова: α-липоевая кислота, масло чайного дерева, идентификация, количественное определение, высокоэффективная жидкостная хроматография

УДК 615.07:543.544.5.068.7

А. А. Гончарова, І. І. Баранова, А. Ю. Куліков

ІДЕНТИФІКАЦІЯ ТА КІЛЬКІСНЕ ВИЗНАЧЕННЯ АКТИВНИХ КОМПОНЕНТІВ КРЕМУ «ДЕРМАЛІПОІН»

Розроблено методики високоефективної рідинної хроматографії для визначення α-ліпоєвої кислоти і олії чайного дерева в новому лікарському засобі для застосування при синдромі діабетичної стопи. Запропоновані сучасні методики дозволяють одночасно проводити ідентифікацію та кількісне визначення досліджуваних речовин в кремі.

Ключові слова: α-ліпоєва кислота, олія чайного дерева, ідентифікація, кількісний визначення, високоефективна рідинна хроматографія

Адреса для листування:
61118, м. Харків, вул. Блюхера, 4
Кафедра товарознавства НФаУ
E-mail: aromafarm@mail.ru

Надійшла до редакції:
07.10.2014