Spectrophotometric method for quantitative measuring essential oil in aromatic water and distillate with rose smell

E Semenova¹, V Presnyakova², D Goncharov³, M Goncharov³, E Presnyakova⁴, S Presnyakov⁵, I Moiseeva¹, S Kolesnikova⁶

¹ Penza State University, Ministry of Education and Science of the Russian Federation, 440026, Penza, Russian Federation
² Academic gymnasium № 1534, 117036, Moscow, Russian Federation
³ Province boarding school for gifted children, 440046, Penza, Russian Federation
⁴ State Commission of Russian Federation for Selection Achievements Test and Protection, 107139, Moscow, Russian Federation
⁵ Bauman Moscow State Technical University, 105005, Moscow, Russia
⁶ National Research Nuclear University MEPhI (Moscow Engineering Physics Institute), 115409, Moscow, Russian Federation

E-mail: sef1957@mail.ru

Abstract. In this connection, we improved the express methods of determining the mixture of volatile aromatic substances by the spectrophotometry of aromatic water and steam distillate of essential oil raw materials (traditional or biotechnological with rose smell). Direct spectrophotometry of distillation water is impossible because it is a colloid of liquid oil and law is not observed. Therefore, it is necessary to dissolve 1 ml of distillate in ethanol in the ratio 1:4, in this case we take real solution with no lipophilic fall-out on the walls of cuvette, also the light absorption law is observed. There are stable maximums in spectrums of studied oils. Optical density of these maximums is a result of summary absorption of terpenoid components (aromatic and monoterpenic alcohols, its ethers). Optical density of tested and standard solutions is measured in appropriate wavelengths. Spectrophotometric method of determination of essential oil quantity in aromatic water with rose smell differs with high sensitivity (10⁻⁵-10⁻⁶ gmol/l) and allows to determine oil concentration from 0.900 to 0.008 mg with an error less than 1%. At that, 1 ml is enough for analysis. It’s expedient to apply this method while operating with small quantity of water distillate in biochemical and biotechnological researches and also as express control for extraction and hydrodistillation of essential oil raw material (rose petals and flowers from different origin, eremothecium cultural liquid etc.).

1. Introduction
The production rose of oil is extremely popularly, as more than 50% of world perfumery brands are fabricated on its basis. Its output is an average of 0.025%, and to produce 1 kg of oil is necessary to manually collect and process about 4 tons of petals. After distillation of the oil remains is rose water. In it the share of oil constitutes about 0.2%. A prime vendor of pink water on the world market is Iran, at the same time oil in this country does not produce [1]. On the world market supply rose oil of the highest quality, the amount of which is currently around 600 kg/year, total 4 countries: Saudi Arabia,
Bulgaria, Turkey and Uzbekistan [2-6]. Until 1992 the production of rose oil by steam distillation in the Soviet republics (Ukraine, Moldova, etc.) was about 4 tons/year. Now it has decreased due to the economic crisis in the CIS countries [7]. In 2005 in the Crimea had produced 600 kg of essential oil (extract) of rose, which is 2 times less than the maximum achieved by the essential oil industry in this region of parameters [8].

The world produces several types of valuable aromatic products pink directions: smell of rose, geranium, palmarosa, rosewood [9]. Currently, on the basis of fungal synthesis developed by biotechnology eremothecium oil, similar in composition with rose oil [10, 11]. As a rule, quantitative determination of essential oils and individual compounds aroma-forming conduct gravimetric and chromatography (using a gas-liquid analyzer) methods [12, 13]. They differ in complexity and duration. In this regard, we have attempted to improve the Express method of determining the mixture of volatile aromatic substances by spectrophotometry flavored waters and hydrodistillation essential raw materials with the smell of roses.

2. Materials and methods

As material for a research served 14 samples of aromaprodukt, including 6 – pink water (the aromatic water received from petals fines herbes), 2 – hydrosedistillates of leaves of aromatic wild roses; 2 biotechnological semi-products – cultural liquids an eremothecium. For preparation of serial double cultivations of standard solutions used 2 samples of the Crimean rose attar (essential oil and absolutes), a sample of rose oil of the Egyptian origin, 2 samples of eremothecium oil (biotechnological "pink" oil), 2 samples received from chemically synthesized components: pink essence and aromakompozition "A tea rose".

The process of hydrodistillation of essential oil raw (air-dried and fresh petals and leaves of roses and wild roses, the culture fluid of eremothecium) was used for receiving fragrant water. The flask was placed petals or leaves, filled with water, the culture fluid (2/3 volume) and boiled for 2-6 hours. Steam through a tube came to the refrigerator which was cooled with flowing water. The cooled condensate skapyvat in an accumulative flask [12]. Stored water distillate (fragrant water) for the subsequent researches in the refrigerator at 4 ±1 °C.

Distillation (fragrant) waters with the pink direction of a smell represent colloidal solution of oil, their direct spectrophotometry is impossible as at the same time Ber's law isn't observed [14]. Therefore, dissolved 1 ml of the distillate in ethanol in the ratio 1:4, while it is a true solution, eliminates the deposition of lipophilic compounds on the walls of the cuvette and observed the law of light absorption.

The oil concentration was determined in 2 ways: from the calibration graph and by comparison with a known sample. To construct the calibration curve prepared a series of standard solutions, and then 1 ml each of them was dissolved in 4 ml of ethanol and spectrophotometrically.

Since it is known that individual components of the rose essential oil (phenylethanol, monoterpenic alcohols, their ethers) have different absorption rates, for the preparation of standard solutions used samples of oils having a different origin and the same composition as the investigated samples, as the mismatch of the component composition of the oils in the studied and standard solutions may lead to inaccurate measurement results.

The optical density of test and standard solutions was measured on the spectrophotometer SF-103 at the respective wavelengths were experimentally determined in the range of 200-300 nm, cuvette thickness of 1 cm, was used as control 80 % ethanol. After measuring the optical density of the investigated solution, according to the schedule found the concentration of oil in 1 ml of distillate.

Determination of the concentration of oil is an ideal method was carried out by measuring the optical density of the investigated solution \(D_x\) and compare it with the standard optical density of a saturated solution of oil \(D_{st}\) the content of 0.4-0.5 mg/ml. Further calculated by the formula: \(C_x = C_{st} * \frac{D_x}{D_{st}}\) [15]. The statistical analysis of results of a research was carried out with use of programs of Excel (Microsoft, the USA) and a Statistica 6 package [16, 17].
3. **Results and discussion**

In the spectrum of studied oils are stable maximum in the region of 250-280 nm, the optical density which is the result of the total absorption of the terpenoid components of nature (aromatic and monoterpane alcohols, their ethers). The absorption spectra of pink and eremothecium natural oils, synthetic aroma compositions (Figure 1) and the dependence of optical density of samples from the oil content (Figure 2) in the sample shown in the figures below.
Figure 1. Absorption spectrum aromaproduct And Crimean rose oil (0.125 mg/ml), B - pink essence (1 mg/ml), In - aromacomposition "Tea rose" (0.125 mg/ml), G - eremothecium oil (0.25 mg/ml).
Figure 2. The dependence of optical density of samples from the oil content in the sample.

The carried-out comparative determination of amount of oil in samples of various origin (table 1) spectrophotometric and gravimetric by methods has shown that metrological characteristics are reproduced and the relative error of a technique doesn't exceed 1%, in this regard it is enough to carry out the analysis no more than in three repetition.

Table 1. Comparative determination of the amount of oil gravimetric and spectrophotometric methods of aromatic waters and distillates of different origin.

| The name of the sample                                      | The amount of oil specified (%) | Relative deviation, % |
|------------------------------------------------------------|--------------------------------|------------------------|
| Air-dried aromatic rose petals (Baikal, 2016)               | 0,081                          | 1,2                    |
| Fresh petals decorative fragrant rose (Penza, 2016)         | 0,117                          | 0,63                   |
| Air-dry the petals essential oil of roses (Crimea, 2013)    | 0,207                          | 0,38                   |
| Air-dry the petals essential oil of roses (Penza, 2014)     | 0,185                          | 0,74                   |
| Air-dry the petals essential oil of roses (Iran, 2013)      | 0,220                          | 0,36                   |
| Air-dry the petals of r essential oil roses of the variety Lada (Russia, 2013) | 0,241                          | 0,59                   |
| Fresh leaves of *Rosa pendulina* (Penza, 2016)             | 0,029                          | 0,98                   |
| Air-dry leaves of the aromatic rose (Baikal, 2016)          | 0,061                          | 0,85                   |
| Culture liquid *Eremothecium ashbyi*                        | 0,085                          | 1,0                    |
| Culture liquid *Eremothecium gossypii*                      | 0,056                          | 0,95                   |
The spectrophotometric method for the determination of the number of aromaproduct with the smell of roses has a number of advantages. In particular, the use of the absorption of monochromatic light, and this greatly increases the accuracy of the analysis. The method is applicable to mixtures and colorless solutions. Measurements are taken with sufficient speed and with negligible instrument error; the analysis requires a small amount of the solution.

4. Conclusion
Advanced us technique has a high sensitivity (10-5-10-6 gmol/l) and allows to determine the concentration of oil from 0.900 to 0.008 mg with an error of less than 1 %, while for the analysis of 1 ml is sufficient. It is expedient to use when working with small quantities of aqueous distillate in biochemical and biotechnological studies, and for express control of processes of extraction and hydrodistillation Attar of raw materials (petals of rose flowers of different origin culture fluid of eremothecium, etc.).

Acknowledgments
This work was supported by Competitiveness Program of National Research Nuclear University MEPhI.

References
[1] Haghighi M, Tehranifar A, Nikbakht A, Kafi M 2008 Research and current profile of Iranian production of damask rose (Rosa damascena Mill.) Acta horticulturae 769 499-455
[2] Erdogan G 2005 Turkey rose oil production and marketing: a review on problem and opportunities 5(10) 1871-1875
[3] Rusanov K, Kovacheva N, Stefanova K, Atanassov A, Atanassov I 2009 Rosa damascena – genetic resources and capacity building for molecular breeding Biotechnol.&Biotechnol. Eq. 23 1436-1439
[4] Dubal S A, Tilkari Y P, Momin S A, Borkar I V 2008 Biotechnological routes in flavour production Advanced Biotech March 3 20-31
[5] Serra S, Fuganti C, Brenna E 2005 Biocatalytic preparation of natural flavours and fragrances TRENDS in Biotechnology 23(4) 193-198
[6] Kovacheva N, Rusanov K, Atanassov I 2010 Industrial cultivation of oil bearing rose and rose oil production in Bulgaria during 21st century, directions and challenges Biotechnol. &Biotechnol. Eq. 2 1793-1798
[7] Voytkevich S A 1999 Essential oils for perfumery and aromatherapy M.: Food industry 284 p.
[8] Shlyapnikov V A, Afonin A V, Pehova O A, Suchkova V M 2006 Conception of Essential oils development Crimea industry Essential oil bearing and herb plants / Scientific reports Essential oil bearing and herb plants institute UAAS 26 12-18
[9] Semenova E F, Shpichka A I, Moiseeva I Y 2012 About essential oils biotechnology on the base of microbial synthesis European Journal of Natural History 4 29-31
[10] Semenova E F, Shpichka A I, Moiseeva I Y 2012 About explanation of elaboration of essential Eremothecium oil biotechnology International journal of experimental education 3 35-36
[11] Semenova E F, Shpichka A I, Presnyakova E V, Mezhennaya N A 2016 Processes of essential oil accumulation in petals of Rosa (Rosaceae) and Mycelium Eremothecium (Eremotheciaceae) Bull. of the State Nikit. Botan. Gard 118 25-33
[12] State pharmacopeia RF, XIII edition 2015 (http://femb.ru/feml, Ministry of Health RF) 1-3
[13] ISO 9842 2003 Rose oil
[14] Bershteyn I Y, Kamensky Y L 1975 Spectrophotometric analise in organic chemistry Chemistry 7 16
[15] Melnikov V N, Bugorsky P S 1975 Spectrophotometric method for quantitative measuring rose oil in distillates Bull.VNII essential oil plants 8 69-72
[16] Truhachova N V 2012 Mathematics statistics in biological-medical researchs with use Statistica
[17] Yakovlev V B 2005 Statistics. Microsoft Excel calculations M.: KolosS 350 p.