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Empirical Calculation of Fatty Acid and Glycerol Composition in Evaluating Drug Quality

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Abstract—Based on empirical indices of the composition and quality of drugs prepared from oil-bearing plant material, an algorithm for calculation of the fatty acid and glycerol composition is developed that can be used for screening evaluation of potential counterfeits and identification of poor-quality products.

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The increase in the share of counterfeit drugs is a problem associated, first, with a threat to population health and, second, with losses to the state treasury and private producers, including lost profits and expenses for combating counterfeit products and for protection of intellectual property rights. According to data of independent studies, the share of counterfeit drugs on the Russian pharmaceutical and parapharmaceutical market is 12% [1].

Various international organizations participate in developing measures for combating counterfeiting of drugs and dietary supplements; the World Health Organization (WHO) is especially active in this area [2]. According to the WHO definition, a counterfeit drug is understood to be a pharmaceutical product that is intentionally and in a misleading manner supplied with false labeling in regard to its authenticity and/or source of origin. A counterfeit can pertain to either brand-name or reproduced drugs (generics); classified as counterfeit products are products with the proper ingredients or with incorrect ingredients, not containing active ingredients, or with an amount of an active ingredient or drug not corresponding to the label in a counterfeit package [3]. A considerable quantity of counterfeit and poor-quality products are identified among drugs and dietary supplements of natural origin, in particular, prepared from fat- and oil-bearing medicinal plant material. The main types of counterfeits of these drugs of natural origin are either products containing other active ingredients not corresponding to those shown on the label or products containing active ingredients whose source of origin differs from the source shown on the label.

ANALYSIS

The above factors, as well as the large number of samples requiring testing, with the comparatively high cost of pharmacopoeial analysis, necessitate changes in the system of drug quality control. In connection with this, WHO recommends the use of simplified tests, or so-called methods of screening evaluation. Methods of screening evaluation of potential counterfeits, not replacing pharmacopoeial methods of testing, enable rather rapid and less expensive identification of counterfeit drugs and dietary supplements.

If an oil-bearing medicinal plant material has a nonspecific composition of fatty acid acylglycerols and is characterized by the absence of any specific accompanying substances, the discovery in the drug or dietary supplement of an admixture of other fatty oils that have a similar fatty acid acylglyceride composition is a laborious task not always leading to unambiguous solutions.

The discovery of an admixture of sunflower seed oil in drugs and dietary supplements with pumpkin seed oil and wheat germ oil is especially difficult even with the use of chromatographic methods of identification of fatty acids since the fatty acid composition of acylglycerols of these oils is mainly represented by nonspecific fatty acids: $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$.

In this study, on the basis of empirical composition and quality indices (CQIs) of drugs and dietary supplements prepared from oil-bearing medicinal plant material, an algorithm for calculation of the fatty acid and glycerol composition is developed that can be used for screening evaluation of potential counterfeits and identification of poor-quality products among this group of medicines.

METHODS

As objects of study, we chose the dietary supplements pumpkin seed oil [4], Tykvin [5], Vitadioil [6], and wheat germ oil [7] and the drug Tykveol [8], all having a nonspecified fatty acid acylglycerol composi-

Table 1. Composition and quality indices of the studied series of drugs prepared from oil-bearing medicinal plant material having a nonspecific content of fatty acid acylglycerides

Index \ Object	Tykveol	Tykvin	Pumpkin seed oil	Vitadioil	Wheat germ oil
Saponification number	223.0 ± 3.1	223.0 ± 3.1	214.0 ± 3.2	208.6 ± 3.1	210.4 ± 3.2
Acid number	1.05 ± 0.02	0.39 ± 0.01	0.62 ± 0.01	1.16 ± 0.02	3.21 ± 0.05
Content of unsaponifiable substances, %	0.77 ± 0.01	0.65 ± 0.01	0.75 ± 0.01	0.62 ± 0.01	0.55 ± 0.01
Content of free fatty acids, %	0.450 ± 0.007	0.170 ± 0.003	0.280 ± 0.005	0.530 ± 0.008	1.450 ± 0.032

tion and characterized by the absence of any specific accompanying substances. The study was performed on five series of each of the objects.

For calculation of the composition of fatty acids and glycerols, the following CQIs of the objects of study were determined: saponification number, acid number, content of unsaponifiable substances, and free fatty acid content. The saponification number and acid number were determined according to the methods of the State Pharmacopoeia (ninth edition). In determination of the acid number, potentiometric control of end point titration was used [9]. The content of unsaponifiable substances was determined according to the method of the International Pharmacopoeia [10]. The content of free fatty acids in the objects of study was determined by the method proposed by Tyutyunnikov [11]. In addition, the content of free fatty acids in the objects of study was calculated on the basis of two empirical CQIs—the saponification number and the acid number. The results of the determination of the main CQIs of the objects of study are presented in this work with an indication of the calculated confidence interval ($f = 5$; $P = 0.95$).

The calculation of the composition of fatty acids and glycerols was carried out on the basis of three empirical CQIs of drugs prepared from oil-bearing medicinal plant material, namely, the saponification number, the acid number, and the content of unsaponifiable substances.

1. The average relative molecular mass of acylglycerols (MMG) prepared from oil-bearing medicinal plant material is calculated according to the formula

$$MMG = (3 \times 56110)/SN,$$

where 3 is the coefficient of conversion to triacylglycerols (TAGs), 56110 is the relative molecular mass of potassium hydroxide, and SN is the saponification number of the sample.

2. The average relative molecular mass of fatty acids (MMFA) is calculated according to the formula

$$MMFA = (MMG - 38)/3,$$

where MMG is the average relative molecular mass of acylglycerols, 38 is the relative molecular mass of the hydrocarbon part of glycerol, and 3 is the coefficient of conversion to TAGs.

3. The content of free fatty acids (CFA) of drugs prepared from oil-bearing medicinal plant material is calculated according to the formula

$$CFA = (100MMFA \cdot AN)/56110,$$

where MMFA is the average relative molecular mass of fatty acids, AN is the acid number of the sample, and 56110 is the relative molecular mass of potassium hydroxide.

4. The content of acylglycerols (CAG, %) in drugs prepared from oil-bearing medicinal plant material is calculated according to the formula

$$CAG = (100 - CFA - CUS),$$

where CFA is the content of free fatty acids (%) and CUS is the content of unsaponifiable substances in the sample (%).

RESULTS AND DISCUSSION

As a result of the study, the values of the saponification number, acid number, content of unsaponifiable substances, and content of free fatty acids in the drugs prepared from oil-bearing medicinal plant material shown in Table 1 were determined.

On the basis of the CQIs determined, the composition of fatty acids and glycerols of the objects of study was calculated (Table 2) according to the above calculation method. It is known that the composition of fatty acids in the lipophilic fractions of oil-bearing medicinal plant material is constant. At the same time, the quantitative content of individual fatty acids in different samples of the same lipophilic fraction (fatty oil) obtained from a particular medicinal plant material can vary, but most often within small ranges. Differences in the composition and quantity of fatty acids of lipophilic fractions of plant material are reflected in the specific consumption of reagents used in the corresponding reactions. When a high-quality drug is obtained from oil-bearing medicinal plant material, the specific consumption of reagents expressed by the corresponding indices (saponification number, acid number, content of unsaponifiable substances, content of free fatty acids) varies in comparatively small, constant ranges. In addition, the values of the saponification number and acid number are directly related with the fatty acid composition of drugs prepared from oil-bearing medicinal plant

Table 2. Theoretical content of fatty acids and glycerols of drugs prepared from oil-bearing medicinal plant material having a nonspecific content of fatty acid acylglycerols

Index \ Object	Tykveol	Tykvin	Pumpkin seed oil	Vitadioil	Wheat germ oil
Average relative molecular mass of acylglycerols	755	755	787	807	800
Average relative molecular mass of fatty acids	239	239	250	256	254
Content of acylglycerols, %	99	99	99	99	98

material. Consequently, the aggregate of such CQIs as saponification number, acid number, and content of unsaponifiable substances enables identification of drugs prepared from oil-bearing medicinal plant material.

The calculation of fatty acid and glycerol composition performed on the basis of CQIs showed the identity of the drug Tykveol and the dietary supplement Tykvin with regard to the following indices: average relative molecular mass of acylglycerols, average relative molecular mass of fatty acids, and content of acylglycerols (Table 2). Thus, on the basis of this calculation of the fatty acid and glycerol composition, the drug Tykveol and the dietary supplement Tykvin can be identified as the fatty oil of pumpkin seeds, which is stated by the manufacturer in the corresponding quality standards [5, 8].

The results of the calculation of the fatty acid and glycerol composition of the dietary supplement pumpkin seed oil [4] allow the supposition of a potential counterfeit, related with the presence in it of sunflower seed oil. This hypothesis agrees with the results of the preliminary screening evaluation of the studied series of the dietary supplement pumpkin seed oil with regard to the index *description.* According to the screening evaluation performed, this dietary supplement of the studied series is a greenish brown oily liquid with the characteristic taste of pumpkin seed oil having the specific odor of sunflower seed oil, which does not meet the requirements of the quality standard for this dietary supplement [4]. It is known that the average molecular mass of fatty acids of sunflower seed oil is 283 [11]. As shown in Table 2, the average relative molecular mass of acylglycerols of the dietary supplement pumpkin seed oil of the studied series exceeds the corresponding index for the drug Tykveol and the dietary supplement Tykvin by 4.2% and the average molecular mass of fatty acids is correspondingly 4.6% higher.

According to the technical specifications, the dietary supplement Vitadioil is a mechanical mixture of pumpkin seed oil and wheat germ oil. In the manufacture of this dietary supplement, a content of pumpkin seed oil of not more than 80% and of wheat germ oil of not less than 20% by volume is regulated [6]. Pumpkin seed oil and wheat germ oil have practically identical fatty acid compositions. In addition, in these oils there are no accompanying substances typical for only one of the oils. Organoleptic peculiarities in mixtures of fatty oils are very difficult to recognize, especially if they are not clearly manifested and if one of the fatty oils is in the mixture in a small quantity. However, the comparison of the calculated composition of fatty acids and glycerols of the dietary supplement Vitadioil with the drug, the dietary supplement pumpkin seed oil, and the dietary supplement wheat germ oil allows Vitadioil to be identified as a mixture of the corresponding fatty oils (Table 2).

The expedience of increasing the number of tests in identification of counterfeit and poor-quality products among drugs and dietary supplements prepared from oil-bearing medicinal plant material is determined by the need to decrease the growth of costs. At the same time, costs related with performing screening evaluation must be compared with the probability of the appearance of iatrogenic pathology, i.e., with the possibility of the appearance of more significant medical, social, and economic problems due to the use of counterfeit or poor-quality drugs and dietary supplements. In this aspect, calculation of the fatty acid and glycerol composition can be viewed as method of screening evaluation of potential counterfeit drugs prepared on the basis of oil-bearing medicinal plant material having a nonspecific composition of fatty acid acylglycerols. Calculation of the fatty acid and glycerol composition of this group of drugs is performed on the basis of three empirical CQIs—the saponification number, the acid number, and the content of unsaponifiable substances—and makes it possible to reliably and objectively identify the corresponding drug or dietary supplement, i.e., to identify a potential counterfeit or a poor-quality product.

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