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Study of Sedative Tea Phytocomplex within the Framework of Studies Aimed at Creation of a Rectal Dosage Form with Antihistaminic Effect

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We designed a new complex drug with antiallergic effect containing, in addition to the main component loratadine, a phytocomplex for an extra therapeutic effect. A collection of plants with sedative activity is chosen and the optimal agent for extraction of bioactive compounds (40% ethanol) and optimal degree of plant fragmentation are determined. Chemical composition of the sedative tea is evaluated by reverse phase HPLC. The marker components of the species are detected: xanthohumol and isoxanthohumol — *Humulus lupulus* cone components, *Mentha piperita* rosmarinic acid, and scutellareine, *Menyanthes trifolia* element — quercetin-3-rutinoside, and caffeic acid. Standardization of the species by the absolute graduation method in conversion to quercetin-3-rutinoside is suggested.

Key Words: *antihistaminic drugs; suppositories; medicinal plants; fragmentation degree; high pressure liquid chromatography (HPLC)*

Numerous rectal suppositories with various therapeutic effects, local and total systems, are available. High-molecular compounds, such as insulin and heparin, can also be administered per rectum. Suppositories are used for painless premedication, induction anesthesia and basal narcosis; rectal administration of drugs is used in cardiological practice [3,7]. In some countries (France, Italy, and Ukraine) drugs in rectal suppositoria dosage form are used for the treatment of allergic conditions.

As a rule, the composition of antihistaminic drugs is not supplemented with substances relieving the clinical symptoms of allergy [9]. Phytocomplexes of some medicinal plants with soft target pharmacological activity seem to be useful for the purpose [5].

The rectal dosage form with antihistaminic activity (loratadin) is supplemented with an active component (phytocomplex), relieving the negative symptoms of allergic disease [8]. Together with loratadine, a thick extract from medicinal plant species of the following composition is added into the suppositoria mass: *Mentha piperita* L. leaves, *Menyanthes trifolia* L. (buckbean) leaves, *Valeriana officinalis* L. rhizome with roots, and *Humulus lupulus* L. cones [1,5,9]. The predicted pharmacological response is based on the proven characteristics of the active components of the phytocomplex. These plants contain polyphenol complexes with sedative, anti-inflammatory, antioxidant, and antihistaminic effects [2].

The aim of this part of the study is the development of analytical instruments for complex support of the process of innovation dosage form creation. The innovation in the pharmaceutical technology of this dosage form is the use of mechanochemistry approach [4]. Realization of our target includes the following problems: to find the optimal extractant for attaining the maximum output of bioactive substances from sedative complex; to determine the acceptable degree of sedative plant fragmentation, at which the extraction of the active components would be the maximum; to detect the chemical composition and suggest a me-

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thod for standardization of the sedative species by the marker components.

MATERIALS AND METHODS

Valerian rhizome with root (FS.2.5.0009.15), peppermint leaves (FS.2.5.0029.15), hops cones (FS.2.5.0046.15), buckbean leaves (State Pharmacopoeia XI), ethanol 40.70, 95% (State Pharmacopoeia X, GOST), and pure water (FS 42-0324-09) were used in the study. The test object was separated by reverse phase HPLC.

Chromatographic studies were carried out on a device (Agilent Technologies 1200 Infinity) with an automated sample selector Agilent 1200, vacuum microdegasifier, gradient pump, and a thermostat of the same series, and diode matrix spectrophotometric detector Agilent 1200 (λ=190-195 nm) with a 2-nm scanning step. Spectral data and chromatograms were recorded and processed using Agilent Chem Station software.

Chromatographic column Supelco Ascentis express C_{18} 2.7 μ×100 mm×4.6 mm was used; mobile phase velocity 0.5 ml/min, temperature 35°C, sample volume 1 μl. Mobile phase: 1.0% water solution of formic acid (A) — 95% ethanol (B) in the gradient elution mode. The mobile phase composition varied from 90 to 10% phase A over 40 min [6]. Flavonoids were detected at λ=350 nm, hydroxycinnamic acids and flavones at λ=325 nm, and chalcones at λ=370 nm.

RESULTS

In order to select the optimal concentration of the extractant, a series of extractions from the studied sedative tea was carried out with the solvent (ethanol) in concentrations of 40, 60, 70, and 90%. The resultant extracts were chromatographied. The output of bioactive compounds extracted from the species was evaluated by comparing the areas of peaks in chromatograms. The bioactive compounds output was the highest after extraction by 40% ethanol.

Stage two of the study was selection of the optimal degree of fragmentation. The sedative tea was fragmented on a ball vibratory mill during 15, 30, or 45 min. Fragmented objects were then subjected to extraction in 40% ethanol and the resultant extracts were fractionated by chromatography. The output of bioactive compounds extracted from fragmented objects was evaluated by the sum of peaks of bioactive compounds. The highest output of the complex of substances was attained after the phytocomplex fragmentation for 45 min.

The next step of our study was detection of the marker components of the species, by which it could be standardized.

The studied composition includes peppermint leaves, buckbean leaves, valerian rhizome with root, and hops cones. The chromatogram shows the marker components characteristic of these plants. Figure 1 presents the chromatogram of 40% ethanol extraction of the species, recorded at λ=350 nm. The main elements of the species are xanthohumol and isoxanthohumol — peppermint constituents, rosmarinic acid and scutellareine — peppermint components, quercetin-3-rutinoside — buckbean constituent, and caffeic acid present in all these plants (Fig. 2).

These data suggest standardization of the composition by the above components. However, not all the standard specimens of these components are commercially available, and hence, we suggest standardization by one element of the composition — by quercetin-3-rutinoside.

The absolute graduation method was used for standardization of the composition. Calibration curve was plotted by the standard sample of quercetin-3-rutinoside in concentrations of 0.064-0.800 mg/ml. The curve demonstrated a direct relationship between the standard sample concentrations and the analytical signal (peak area).

The area of the peak corresponding to rutin was measured for the analyzed object, and the quantity was calculated from the calibration curve according to the formula:

$$x = \frac{C_{CT} \times W \times 100}{m \times (100-B)},$$

where C_{CT} was the quantity of flavonoids found in the calibration curve (%), *m* — weight of the sample (g), W — dilution of the sample, and D — weight loss after the sample drying (g).

The results of quantitative evaluation of flavonoids in conversion to quercetin-3-rutinoside were presented in Table 1. The content of rutin in the sedative tea specimen was 1.830±0.052%. The error of a single measurement was no more than 5%, which was within the range allowed by the State Pharmacopoeia.

TABLE 1. Results of Rutin Measurements in Sedative Tea

Parameter	Value
f	5
$\bar{x}$	1.83
s	0.02132
P, %	95
t(P, f)	2.57
Δ	0.05225
ε, %	2.86

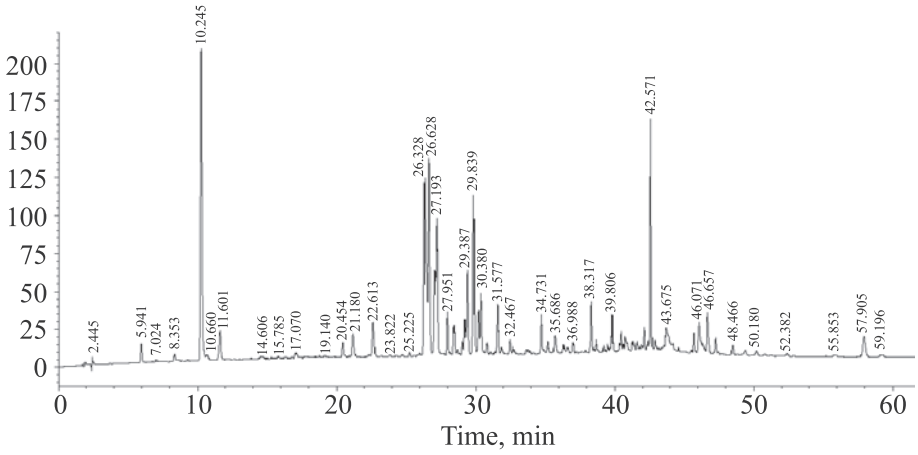

Fig. 1. Chromatogram of sedative tea polyphenol separation (diode matrix detection, λ=350 nm).

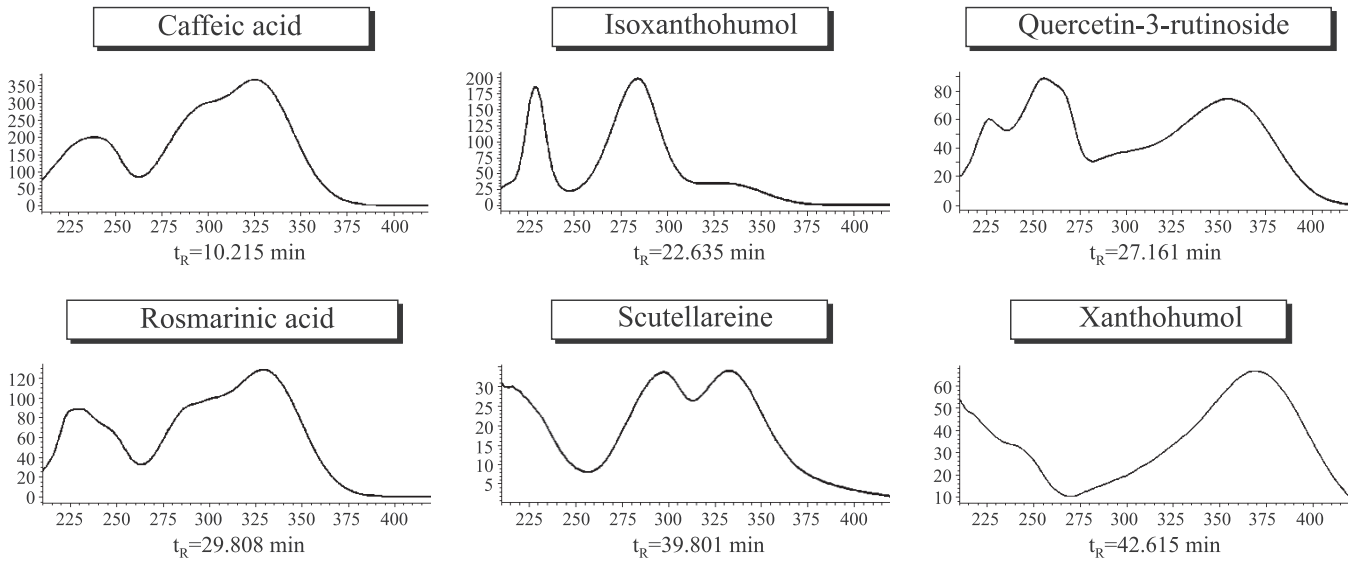

Fig. 2. Diode matrix detection of sedative tea components. t_R: time of the component retention in chromatogram.

The studies have determined the optimal extractant for extraction of the complex of bioactive compounds from the sedative tea: 40% ethanol. The optimal degree of the phytocomplex fragmentation is determined. The best output of the complex of bioactive compounds is attained after fragmentation of the species during 45 min. The chemical composition of the sedative species is detected. The marker components of the species are determined: xanthohumol and isoxanthohumol — hops cone components, rosmarinic acid and scutellareint — peppermint constituents, quercetin-3-rutinoside — buckbean element, and caffeic acid. A method for quantitative evaluation of the species is suggested: absolute graduation method in the course of chromatography with conversion to quercetin-3-rutinoside. The content of this component in the composition is 1.830±0.052%, with the single measurement error of 2.86%.

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