Phenolic compounds of herbal origin have anti-inflammatory, bactericidal, and fungicidal, as well as antioxidant properties because of their ability to neutralize free radicals. Their number is large and achieves $10^5$ [1]. The composition of phenolic compounds in medicinal herbs has not been investigated enough despite the great variety of the procedures providing their determination. Russian State Standard GOST 24027.2-80 sets the determination of the total of tannins based on their titration with potassium permanganate [2]. However, because of the oxidation of the other substances, this procedure can lead to overestimated results. For the spectrophotometric determination of phenolic compounds, spectral characteristics of individual substances should be known and some preliminary separation of the components of the analyzed mixture should be carried out because of the close positions of the absorption maxima [3]. The major drawbacks of fluorimetric and polarographic methods used to determine phenolic compounds include low precision and reproducibility of the results, as well as the interference of the sample matrix of the herbal material [4].

All the procedures listed above provide the determination of total phenolic substances; however, in most cases in pharmacology it is important to know the composition of the components. Chromatographic methods are employed in such cases; however, they also have some disadvantages. Thin layer chromatography is not sufficiently precise, while gas chromatography does not provide the determination of the majority of phenolic compounds. Taking into account the properties of phenolic compounds, high-performance liquid chromatography (HPLC) can be considered the most preferable method.

The aim of the present study was to develop a procedure for the determination of individual phenolic compounds in groups of phenolcarboxinic, cinnamic acids, and flavonoids in medicinal herbs by reversed-phase HPLC.

**EXPERIMENTAL**

**Reagents.** The following phenolcarboxinic acids were used in the study: gallic, protocatechuic, 4-hydroxybenzoic, salicylic, mandelic, $p$-anisic, vanillic, syringic acid, cinnamic acids: cinnamic, $trans$-ferulic, $trans$-caffeic acid, rutin, quercetin, dihydroquercetin, and $(-)$-epicatechin for 30 min is studied. The lower limit of quantification of phenolic compounds is $1–2.5 \mu g/L$. The procedure was applied to the determination of phenolic compounds in aqueous extracts of *Hypericum perforatum*; its sample was found to contain protocatechuic acid, $(-)$-epicatechin, and also rutin.

**Keywords:** phenolic compounds, flavonoids, medicinal herbs, reversed-phase HPLC

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The study was made using a Shimadzu LC 20 Prominence chromatograph (Japan) with a SPD M20A diode-array detector. The column 150 × 2.1 mm Zorbax SB C18, 5 µm (Agilent, the USA) protected with pre-column 20 × 2.1 mm Zorbax SB C8, 5 µm (Agilent, United States) was used.

The compositions of the mobile phase were as follows: eluent A, acetonitrile; eluent B, 0.04 M KH₂PO₄ acidified by 35% H₃PO₄ to pH 2.8. The flow rate of the mobile phase was 0.25 mL/min.

RESULTS AND DISCUSSION

The selection of conditions of chromatographic separation. Reversed-phase version of HPLC using columns with chemically attached octadecylsilane groups and eluents of different compositions are usually employed in analytical practice for the separation of phenolic compounds of herbal origin. The main components of the eluent are usually isopropanol, methanol [5–7], acetonitrile [8–11]. However, when isopropanol or methanol is used, the sufficient separation of the phenolic compounds is not achieved; therefore, it is preferable to introduce acetonitrile into the mobile phase.

To reduce peak broadening due to the dissociation of compounds, eluent B (0.04 M potassium dihydrogen phosphate) was acidified with ortho-phosphoric acid to pH 2.8. The improvement of the separation of the considered phenolic substances was achieved using gradient elution. It is known [12] that the separation of catechins is maximal if the mobile phase is a water–acetonitrile mixture with linearly increasing concentration of acetonitrile from 10 to 30 vol. %. On the other hand, for some phenolcarboxylic acids the eluting strength of the mobile phase containing 10 vol. % of acetonitrile is high and they are not retained in the column. At the same time, the introduction of even 30 vol. % of acetonitrile is not sufficient for the elution of quercetin. The conditions for separation and determination of the maximal amount of compounds were optimized by varying the composition of the eluent in a wide range of acetonitrile concentrations (from 3 to 40 vol. %).

The optimal gradient mode was chosen using a model mixture of phenolic compounds that contained gallic, protocatechuic, caffeic, ferulic acids, rutin, quercetin, dihydroquercetin and (-)-epicatechin.

**Fig. 1.** Structures of the considered phenolic compounds

I: salicylic acid (R₁=OH, R₂=R₃=R₄=H), 4-hydroxybenzoic acid (R₁=H, R₂=R₃=R₄=H), protocatechuic acid (R₁=R₂=R₃=H, R₄=OH), gallic acid (R₁=R₂=R₃=H, R₄=OH), vanillic acid (R₁=R₂=H, R₃=OCH₃, R₄=OH), p-anisic acid (R₁=R₂=R₃=H, R₄=OCH₃), syringic acid (R₁=H, R₂=R₃=OH, R₄=OH); II: mandelic acid; III: cinnamic acid (R₁=R₃=R₂=R₄=H), p-coumaric acid (R₁=R₂=R₃=H, R₄=OH), trans-cafeic acid (R₁=R₂=H, R₃=OCH₃), trans-ferulic acid (R₁=R₂=H, R₃=OH, R₄=OCH₃), sinapic acid (R₁=H, R₂=R₃=OH, R₄=OCH₃); IV: dihydroquercetin; V: quercetin (R₁=R₂=OH), rutin (R₁=R₂=OH, R₃=rutinose), naringin (R₁=R₂=H, R₃=rutinose), hesperidin (R₁=H, R₂=OCH₃, R₃=rutinose); VI: (-)-epicatechin.