Development of the methods for spectrophotometric determination of the flavonoid concentration in solution to study bioavailability of the amount of bioactive substances in capsules containing the powder of *Scutellaria baicalensis* roots and rhizomes

Recently, the test "Dissolution", which allows evaluating bioavailability of active ingredients, has become one of the most important tools in the quality control of new and generic drugs development ("bio waiver" procedure). However, for some drugs the use of classical pharmacopoeia buffer solutions *in vitro* does not always completely adequately reflect their behavior *in vivo*. As a solution of this problem the group of biorelevant media, which allow simulating dissolution and adsorption of drugs in the gastrointestinal tract of a patient, has been developed.

**Aim.** To develop the methods of spectrophotometric determination of the flavonoid concentration in 0.1 M hydrochloric acid solution and biorelevant media FaSSIF and FeSSIF with pH 6.5 and 6.8 calculated with reference to baicalin for further study of bioavailability of hard gelatine capsules containing a finely divided powder of *Scutellaria baicalensis* roots and rhizomes.

**Materials and methods.** For the studies on bioavailability determination of pharmacologically active substances of a finely divided powder of *Scutellaria baicalensis* roots and rhizomes the method of absorption spectrophotometry in the ultraviolet and visible regions of the spectrum calculated with reference to baicalin was chosen.

**Results and discussion.** Adsorption spectra of baicalin solutions in 0.1 M hydrochloric acid solution and biorelevant media FaSSIF (pH 6.5) and FeSSIF (pH 6.8) in the range from 230 to 370 nm consist of two absorption bands. The band with the maximum at 276 nm is more intense, while the band in the near ultraviolet region is less intensive, but broader. The use of this maximum for quantification will allow increasing the specificity of the analysis. The analysis of adsorption spectra of the solutions obtained during extraction of the powder from *Scutellaria baicalensis* roots and rhizomes with 0.1 M hydrochloric acid solution and biorelevant media FaSSIF and FeSSIF with pH 6.5 and pH 6.8 has shown that their structure is virtually the same as the spectral structure of baicalin solutions. In order to test the possible impact of the dissolution media on the total optical density at the analytical wavelength the adsorption spectra of the biorelevant media FaSSIF and FeSSIF with pH 6.5 or 6.8 have been studied. The analysis of the data obtained has shown that the media are really nontransparent in ultraviolet light and have quite intensive absorption in the range of 230-290 nm. The absorbance testing of baicalin solutions in 0.1 M hydrochloric acid solution and biorelevant media FaSSIF and FeSSIF with pH 6.5 and pH 6.8 has shown that the dependence is linear and obeys the Beer-Lambert law in the range of the concentration from 0.4 to 3.6-4.0 · 10⁻³ %.

**Conclusions.** To develop the method for determining the concentration of solutions of pharmacologically active substances while studying bioavailability of capsules containing a finely divided powder of *Scutellaria baicalensis* roots and rhizomes the adsorption spectra of baicalin in 0.1 M hydrochloric acid solution and biorelevant media FaSSIF and FeSSIF with pH of 6.5 and 6.8 have been studied.

**Key words:** adsorption spectroscopy; baicalin; flavonoids; capsules; *Scutellaria baicalensis*; biorelevant media

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Розробка методики спектрофотометричного визначення концентрації розчинів flavонодів для вивчення біодоступності суми біологічно активних речовин у капсулах з порошком коренів та кореневищ шоломниці байкальської

Останнім часом тест «Розчинення», який дозволяє оцінити біодоступність діючих речовин, став одним з найважливіших інструментів у галузі контролю якості розробки нових і відтворення патентованих лікарських засобів (ЛЗ) (процедура «Біовейвер»). Однак для деяких ЛЗ застосування класичних фармакопейних буферних розчинів *in vitro* не завжди повністю адекватно відображає їх поведінку *in vivo*. Тому для моделювання процесу розчинення у шлунково-кишковому тракті доцільно використовувати групу біорелевантних середовищ (biorelevant media).

**Мета роботи.** Розробити методику спектрофотометричного визначення концентрації розчинів flavонодів у 0.1 М розчинні кислоти і біорелевантних середовищах FaSSIF і FeSSIF з pH 6.5 і 6.8 у перерахунку на байкалін для подальшого вивчення біодоступності твердих желатинових капсул з подрібненим порошком коренів та кореневищ шоломниці байкальської.
Материалы и методы. Для проведения исследования биодоступности фармакологически активных веществ подрібненого порошку корней и корневищ шлемника были изучены адсорбционные спектры растворов байкаліну в 0,1 М растворе соляной кислоты и биорелевантных середовищ FaSSIF и FeSSIF с рН 6,5 и 6,8.

Результаты и их обсуждение. Адсорбционные спектры растворов байкаліну в 0,1 М растворе соляной кислоты и биорелевантных сред FaSSIF и FeSSIF с рН 6,5 и 6,8 показывают, что их структура практически повторяет структуру спектров растворов байкаліну. З метою проверки возможного влияния середовищ на суммарную оптическую плотность при аналитической длине волны 276 нм была проведена адсорбционная спектрофотометрия в области спектра 230-290 нм. Процедура включает в себя измерение суммарной оптической плотности при аналитической длине волны 276 нм.

Ключевые слова: адсорбционная спектрофотометрия; байкалін; флавоноїди; капсули; шлемник байкальський; біорелевантні середовища.

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Recently, the test “Dissolution”, which allows evaluating bioavailability of active ingredients, has become one of the most important tools in the quality control of new and generic drugs development (“biowaiver” procedure) [1-4]. However, for some drugs the use of classical pharmacopoeia buffer solutions in vitro does not always completely adequately reflect their behavior in vivo. As a solution of this problem the group of biorelevant media, which allow simulating dissolution and adsorption of drugs in the gastrointestinal tract of a patient, has been developed [5-8].

The aim of the work was to develop the methods of spectrophotometric determination of the flavonoid concentration in 0.1 M hydrochloric acid solution and biorelevant media FaSSIF and FeSSIF with pH 6.5 and 6.8 calculated with reference to baicalin for further study of bioavailability of hard gelatine capsules containing a finely divided powder of Scutellaria baicalensis roots and rhizomes.

To achieve this goal it was necessary to study the adsorption spectra of baicalin and the extracts obtained from the powder of Scutellaria baicalensis roots and rhizomes to identify the analytical absorption band – a considerably intense peak without absorption of other components of the analytical system and to set limits of absorbance subordination to Bouguer-Lambert-Beer law in order to conduct further research on studying bioavailability of biologically active substances in the capsules containing the powder of Scutellaria baicalensis roots and rhizomes [9, 10].

Materials and methods

The biological activity of the plant raw material is usually caused not by one active ingredient, but a group of pharmacologically active compounds of the similar structure. Determination of the content of such substances is carried out by the non-specific method based on the common fragments of the structure, and the determination results are recalculated for the most decisive marker compound. For the studies on bioavailability determination of pharmacologically active substances of a finely divided powder of Scutellaria baicalensis roots and rhizomes the method of absorption spectrophotometry in the ultraviolet and visible regions of the spectrum calculated with reference to baicalin was chosen.

In order to select the analytical absorption peak the absorption spectra of baicalin in 0.1 M hydrochloric acid solution simulating the acidic medium of the gastric juice were studied, and then in biorelevant media FaSSIF and FeSSIF with pH 6.5 and 6.8 modeling the processes in the intestine. The accurately weighed quantity of 0.0501 g of baicalin standard sample (SS) was placed in a 50 ml volumetric flask, then 1 ml of dimethylformamide was added. The content of the flask was mixed to dissolution and diluted to the volume with 96% ethanol (solution A). In 100 ml volumetric flask 1 ml of solution A was placed, diluted to the volume with 0.1 M hydrochloric acid solution or buffer solution of FaSSIF with pH 6.5 or FeSSIF with pH 6.8 and thoroughly mixed. Adsorption spectra of the resulting solution were recorded using an Evolution 60-S spectrophotometer in the cells with the layer thickness of 10 mm. As a control solution the corresponding solvent was used.

In order to test the effect of all extractives on the total extract absorption spectrum and the possibility of quantitative determination of flavonoids in the study of solubility of biologically active substances of the powder from Scutellaria baicalensis roots and rhizomes the adsorption spectra of the solutions obtained by extraction of the powder with 0.1 M hydrochloric acid solution or biorelevant media FaSSIF and FeSSIF with pH 6.5 and 6.8 were investigated. Approximately 0.297 g of the test samples of the powder from Scutellaria baicalensis roots and rhizomes (accurate weight) differed by powder fineness were quantitatively transferred to a 250 ml conical flask with a ground glass stopper, then 100.0 mL of 0.1 M hydrochloric acid solution or biorelevant media FaSSIF and FeSSIF with pH 6.5 or 6.8 were added. The flask was placed in a thermostat with the temperature of 37 ± 2 °C on a device for shaking and stirred for one hour. The resulting extracts were filtered through a “blue ribbon” paper filter; the first portions of filtrate were discarded. As a control solution 0.1 M hydrochloric acid solution or biorelevant media FaSSIF and FeSSIF of pH 6.5 or 6.8 were used.

Biorelevant media were prepared by the formulations given in the literature [4].

Surfactants lecithin and sodium taurocholate in the composition of the media can be nontransparent to ultraviolet light, their maxima interacting with extractive substances can shift, affect the total absorption of solutions and distort the results of the research. Adsorption spectra of the media were observed under the same conditions compared to water.

One of the conditions that must be met for quantitative measurements in spectrophotometry is absorbance subordination of the solutions analyzed to Beer-Lambert law. The required amount of solution A was placed in a 25 ml volumetric flask, diluted to the volume with the corresponding buffer solution and mixed. The absorbance of the resulting solution was measured using an Evolution 60-S spectrophotometer at a wavelength of $\lambda_{\text{max}} = 317$ nm in the cells with the layer thickness of 10 mm. As a control solution the corresponding solution of 0.1 M hydrochloric acid or biorelevant media FaSSIF and FeSSIF with pH 6.5 or 6.8 was used.

Results and discussion

Adsorption spectra of baicalin in 0.1 M hydrochloric acid solution and biorelevant media FaSSIF (pH 6.5) and FeSSIF (pH 6.8) in the wavelength range from 230 to 370 nm consist of two absorption bands (Fig. 1).

The band with the maximum at 276 nm is more intense; in this region most aromatic compounds absorb. The band in the near ultraviolet region is less intensive, but broader. Its maximum at 317 nm is in the range where typically flavonoids absorb, while other aromatic compounds do not absorb already, or their absorbance is not substantial. The use of this maximum for quantification will allow increasing the specificity of the analysis.

The analysis of adsorption spectra of the solutions (Fig. 2) obtained during extraction of the powder from Scutellaria
Fig. 1. Adsorption spectra of baicalin in 0.1 M hydrochloric acid solution and biorelevant media FaSSIF (pH 6.5) and FeSSIF (pH 6.8)

Fig. 2. Adsorption spectra of solutions obtained during extraction of the powder from Scutellaria baicalensis roots and rhizomes with 0.1 M hydrochloric acid solution and biorelevant media FaSSIF and FeSSIF with pH 6.5 and pH 6.8 has shown that their structure is virtually the same as the spectral structure of baicalin solutions.

In all spectra in the range from 230 nm to 430 nm there are also two bands – the intensive absorption band of aromatic compounds with the maximum in the range of 274-276 nm and the less intensive, but broader band with the maximum in the range of 315-318 nm. This band becomes less distinct due to the presence of the amount of compounds with the similar structure in the solution, but it is broad enough and can be used to measure the optical density in quantitative determination.

Thus, as a result of the experiment conducted to develop the spectrophotometric method for quantitative determination of the concentration of active ingredients of the powder from Scutellaria baicalensis roots and rhizomes the band in the near ultraviolet region with the maximum at 317 nm characterized by sufficient intensity and specificity was selected as the analytical absorption band.

In order to test the possible impact of the dissolution media on the total optical density at the analytical wavelength the adsorption spectra of biorelevant media FaSSIF and FeSSIF with pH 6.5 or 6.8 were studied (Fig. 3). The analysis of the data obtained has shown that the media are really not transparent in ultraviolet light and have...
quite intensive absorption in the range of 230-290 nm. However, at 317 nm they have not any individual peaks. The proper choice of the analytical absorption band in the near ultraviolet region and the use of dissolution media as control solutions allow neutralizing their impact on the total optical density.

The absorbance testing of baicalin solutions in 0.1 M hydrochloric acid solution and biorelevant media FaSSIF and FeSSIF with pH 6.5 and pH 6.8 has shown that the dependence is linear and obeys the Beer-Lambert law in the range of the concentration from 0.4 to 3.6·10^{-3} % (Fig. 4).

The specific absorption rate $A_{1\%}$ in the concentration range mentioned is from 366 to 379.

CONCLUSIONS
1. To develop the method for determining the concentration of solutions of pharmacologically active substances while studying bioavailability of capsules containing a finely divided powder of *Scutellaria baicalensis* roots and rhizomes the adsorption spectra of baicalin in 0.1 M hydrochloric acid solution and biorelevant media FaSSIF and FeSSIF with pH of 6.5 and 6.8 have been studied. It has been found that the adsorption spectra of baicalin in the range from 230 to 370 nm consist of two absorption bands with the maxima at 276 and 317 nm. The band in the near ultraviolet region is rather broad, intensive and can be promising for development of the spectrophotometric quantification method.

2. The study of the effect of co-extractive substances on the total spectrum of powder extracts from *Scutellaria baicalensis* roots and rhizomes has shown that although the band with the maximum at 317 nm becomes less expressive, it can be used to quantify the amount of flavonoids in the extracts calculated with reference to baicalin using the one-component single-wave spectrophotometry by standard.

3. Biorelevant media FaSSIF and FeSSIF with pH 6.5 and 6.8 have not absorption maxima in the near ultraviolet region; they will not distort the results of quantitative determination of flavonoids in extracts at 317 nm.

4. Determination of absorbance of baicalin solutions in 0.1 M hydrochloric acid solution and biorelevant media FaSSIF and FeSSIF with pH 6.5 and pH 6.8 has shown
that the dependence is linear and obeys the Beer-Lambert law in the range of the concentration from 0.4 to 3.6-4.0·10^{-3} \% . The specific absorption rate $A_{vis}$ in is in the range from 366 to 379.

5. As a result, the possibility of spectrophotometric determination for the concentration of biologically active substances of the powder of Scutellaria baicalensis roots and rhizomes in solutions calculated with reference to baicalin when studying bioavailability of the capsules developed has been proven.

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