Theoretical and experimental approaches to the development of spectrophotometric methods of analysis of phenolic compounds in herbal raw material and preparations based on it.

A.I. Marakhova[1], E.V. Sergunova[2], A.A. Sorokina[2]

[1]Peoples' Friendship University of Russia, 117198, Moscow, Russia,

Miklukho-Maklaya str., 6.

[2] I.M. Sechenov First Moscow medical state university.

Abstract

The features of spectrophotometric analysis of phenolic compounds in herbal raw material considering the impact of concomitant compounds of metabolome. From generalization of our own research and literature data compiled algorithm of development of spectrophotometric analysis methodology of biologically active compounds of medicinal herbal raw material and drugs based on it. The article describes the features of the spectrophotometric analysis of flavonoids, anthraquinones, phenologlycosides, phenolcarbonic acids. Developed a methodology for spectrophotometric analysis of biologically active compounds in herbal raw vaterial and preparations based on it.

Keywords: spectrophotometry, flavonoids, anthraquinons, phenologlycosids, phenolcarbonic acids, standard sample.

Introduction

Medicinal plants remain source of drugs that have a wide spectrum of pharmacological activity. Interest in phytotherapy due to the possibility of using medicinal vegetative raw materials and products based on it in pediatrics in the treatment and prevention of various diseases with minimal side effects.

All drugs, including herbal preparations should be subjected to the certification means that they conduct quality control. Adequate quality of medicinal vegetative raw materials is largely dependent on the level of the requirements laid down in the normative documentation and analysis methods used.

Methodological part

The most widely used modern methods of analysis of the vegetative raw material is photometry, spectrophotometry in particular. This method is used for the qualitative and quantitative determination of the various groups of biologically active substances (BAS): flavonoids, anthraquinons, saponins, phenologlykosids, phenolcarbonic acids, and others. The important features of the method are: objective assessment of the quantitative content of BAS, simplicity and speed of analysis; wide and accessible park of spectrophotometers.

Features of spectrophotometric analysis of medicinal vegetative raw materials are associated with the presence of a large number of BAS. Most scientific publications devoted to physical and

chemical methods in the analysis of vegetable raw materials has narrowly focused. Therefore the purpose of this paper is to generalize research on the analysis of herbal drugs containing phenolic compounds by spectrophotometric method (both own and reported in the literature), consider the possibility of "through" standardization medicinal vegetative raw materials and drugs based on it using spectrophotometry. The "through standardization" means quality control of medicines vegetable raw material and drugs obtained from it by by one group of pharmacologically active compounds by the same method.

Class of phenolic compounds is widely represented in medicinal herbs. For the analysis of most of them are used spectrophotometry. However, each specific type of medicinal plants has its own features in the analysis due to the presence of concomitant compounds. Currently, the normative documentation for the analysis of flavonoids often used spectrophotometry of colored complex with aluminum chloride due to the wide availability of the reagent and the stability of the complex. To suppress self dissociation of flavonoids added acetic or hydrochloric acid. Complexes formed between a small excess of aluminum chloride and ortho-hydroxyl groups of the rings A and B, are destroyed in the presence of an acid [6]. Aluminum chloride complexes formed between the keto group at the C4 position or 3- and 5- hydroxyl group at C3 or C5 are stable in the presence of an acid [3,6].

It has been shown that the absence of acid affects the position of the maximum absorption and the possibility of precise definition. Some natural bioactive compound promotes the formation of a buffer solution in the extract, such as in the leaves of Urtica. When adding an acid to an alcohol or aqueous extract of of Urtica leaves, pH after some time shifts to the alkaline side. This practically eliminates definition flavonoids spectrophotometrically by the formation of sediment in the reaction mixture. The solution to this problem was the addition of acetate buffer that supports the pH at 4-5. The method of differential spectrophotometry in the analysis of flavonoids shows versatility in «through» standardization of medicinal vegetative raw material and drugs based on it [4].

Flavonoids constitute the hydrophilic fraction and have a wide spectrum of pharmacological activity. Therefore, standardization can be carried out on the content of flavonoids for medicinal vegetative raw material from which the infusions, decoctions and aqueous-alcohol extracts are manufactured. For example, methodic of analysis of total flavonoids have been developed in raw materials, infusions and tinctures of Chamomile, Calendula and Thymus [1,2,3,4]. It was found that the wave lengths corresponding to maxima absorption in the determination of the flavonoid in raw material and dosage forms differ. This fact should be taken into account when the new methodic is developed or when certain methodic is transferred to the new object [4].To avoid errors in the photometric analysis of flavonoids in the raw material should be obtained from the absorption spectra in the wavelength range of 250-600 nm of the sample solution, the sample solution with the

aluminum chloride and the differential spectrum with aluminum chloride. Such an approach helps to identify the analytical wavelength, to choose the standard sample and note the influence of concomitant compounds. Differential spectrophotometry has advantages over the direct method especially for medicinal vegetative raw material containing flavonoids together with significant amount phenolcarbonic acids. An example of such raw materials are flowers of Helichrysum [2].

In cases of the joint presence of phenolcarbonic acids and flavonoids some methods of extraction are used for them separate determination.
For natural flavonoid compounds such as 5-hydroxy-flavone, 7-hydroxyflavone, 3-hydroxyflavone, metoksiflavon-3, 4'-hydroxyflavone, 4'-metoksiflavon, 5,4'- and 5,7-dihidroksiflavons distribution coefficient is in ranging from 3.3 to 4.17. This allows to develop the conditions for separate extraction of flavonoids and phenol carbonic acids. Joint extraction of phenolcarbonic acids and flavonoids from the raw material is carried out with ethyl alcohol, then extract evaporated and the residue is dissolved in a buffer solution of pH = 2. Then follows the extraction with ethyl acetate, allows you to select phenol carbonic acids [7].

However, flavonoids do not always affect the analysis of phenol carbonic acids. For example, it is shown that in the raspberry fruits, in which flavonoid compounds are represented mainly anthocyanins, sum of phenolcarbonic acids can be successfully determined by direct spectrophotometry in alcoholic extract [4].

Flavonoids may also have an influence on spectrophotometric analysis phenologlycosids. For the separation of flavonoids from phenologlycosids suggested to use purification by column chromatography with sorbent - aluminum oxide. Flavonoids trapped on aluminum oxide, when phenologlycosids eluted by water-alcohol mixture [9].

The problem of a standard sample choosing is particularly importance in the photometric analysis antraquinones. In the existing methods of the Russian Pharmacopoeia [10] recount of content of sum antraquinones proposed to conduct on istisin - a compound which is not a component of the chemical composition of raw materials. The calculation is made from the calibration graph constructed from the solutions of cobalt (III) chloride hexahydrate. However, studies have shown that the absorption maximum alkaline- ammonia solution of anthraquinones of frangula bark corresponds to 526 ± 2 nm, while the $CoCl_2 \cdot 6H_2O$ solution absorption maximum falls on the 510 ± 2 nm [4].A similar approach is used in the European Pharmacopoeia [8].

In multicomponent systems such as the collection for the treatment of hemorrhoids, which includes five components, in the absorption spectrum of ammonium salts anthraquinones contribute concomitant compounds and the absorption maximum is observed at 516 ± 2 nm. In this case, the standard sample may be used glucofrangulin A maximum absorption of ammonium salt which is at a wavelength 515 nm ± 2 [4].

Literature

1. A.A. Sorokina, A.I. Marakhova, N.N. Fedorov, T.A. Belous. Pharmacy, 2012, 4, 43-45.
2. A.I. Marakhova, N.N. Fedorovsky, A.A. Sorokina, P.V. Baurin in the book. The modern pharmaceutical science and practice: traditions, innovations, priorities, Samara, 2011, 41-45.
3. A.I. Marakhova, N.N. Fedorov, A.A. Sorokina Pharmacy, 2007, 3, 10-11.
4. A.I. Marakhova, N.N. Fedorovsky, T.A. Skalozubova, A.S. Avrach, A.A. Sorokina, E.V. Sergunova. Electronic scientific journal "Health and education in Siberia, 2011, 5.
5. A.I. Marakhova, N.N. Fedorov, A.A. Sorokina, M.N. Galko. Pharmacy, 2013, 5, 14-17.
6. G.V. Vasiliev. Chemistry of Natural Compounds, 2008, 5, 512-513.
7. E.V. Avdeeva. PhD Pharm. Sciences. Samara Medical University, Samara, 1996, 159.
8. European Pharmacopeia 6th 2009.
9. T.Yu. Kovaleva, I.A. Samylina, V.A. Ermakova. Traditional Medicine, 2012, 5, 241-243.
10. State Pharmacopoeia of the Russian Federation, Moscow, 2008.