

**DEVELOPMENT OF METHODS FOR IDENTIFICATION AND  
DETERMINATION OF BIOLOGICALLY ACTIVE SUBSTANCES IN SEDAVIT  
HERBAL TABLET PRODUCTS**

**Salomov Shokhabbos Nozimjon ugli**

Student of Andijan State Medical Institute, Uzbekistan

E mail: [salomovshoxabbos7@gmail.com](mailto:salomovshoxabbos7@gmail.com)

Phone number : +998978374147

**Annotation:** Bioactive groups were identified and methods for their identification and determination in the herbal preparation Sedavit, tablets, were developed. Methods were developed for high-performance liquid chromatography (HPLC) for the identification of polyphenolic compounds and the quantitative determination of pyridoxine and nicotinamide; gas chromatography (GC) for the determination of isovaleric acid, spectrophotometry (SP) for the quantitative determination of flavonoids.

**Keywords:** herbal medicines, Sedavit, identification, quantitative determination, control methods.

Herbal medicines consist of a complex of substances belonging to several chemical groups and under the influence of technological processes or internal enzymatic reactions, chemical transformations of these substances can occur. The task of standardizing medicinal plant raw materials and drugs based on them is often complicated by the lack of accurate control indicators and the existing indicators are not accepted for testing qualitative and quantitative properties. The main goal of our work is to develop a method for standardizing finished pharmaceutical products (PPF) on a plant basis, ensuring their effectiveness and safety for consumers, ensuring the development of appropriate technical parameters and quality control methods, among other things, for the identification of counterfeit drugs [1, 2]. Standardization includes the following interrelated stages of pharmaceutical product development:

- Select individual compounds, biologically active compounds (BAC) or markers to assess the quality of FMPs;
- select methods and develop techniques for the identification and/or quantification of individual compounds, BAS groups or markers in FMPs;
- selection and substantiation of standardization criteria taking into account the requirements of pharmacopoeial monographs, data from scientific literature and characteristics of production technology.

Let's look at each of these moments using the example of the development process of a finished herbal product, Sedavit tablets.

**MATERIALS AND METHODS**

The object of our study is the drug Sedavit, in tablet form, whose active ingredient is a concentrated extract containing 5 plant ingredients (valerian rhizome, hawthorn fruit, St. John's wort, peppermint leaves, hop cones), vitamins - nicotinamide (vitamin P) and pyridoxine hydrochloride (vitamin B6).

The study used gas chromatography (GC) and high performance liquid chromatography (HPLC). GC studies were performed on an Agilent 6890 N" gas chromatograph with a flame ionization detector (Agilent, USA). For HPLC studies, an Agilent 1200 liquid chromatograph with a diode array detector (Agilent, USA) was used. Quantitative determination of total flavonoids was performed on a Cary 100 spectrophotometer (Varian, Australia). The study used standards including chlorogenic, caffeic, ferulic and rosmarinic acids (Fluka), rutin, hypersaccharide, luteolin, quercetin, apigenin (Fluka). The reagents used for the study complied with the requirements of the European Pharmacopoeia (EPH) and the State Pharmacopoeia of Uzbekistan (SPU). The reaction solutions were prepared according to the requirements of EP/SPU [3, 4].

## RESULTS AND DISCUSSION

Isolation of individual compounds, groups of bioactive substances or markers. Each plant component of the complex extract contains a variety of bioactive substances, the composition and proportion of which may vary depending on the geographical and climatic conditions of growth and cultivation, harvesting, drying, etc. The complex extract is obtained by simultaneous extraction of a mixture of five plant materials. This complicates the development of a control strategy that includes the qualitative or quantitative determination of the bioactive substances or markers inherent in each medicinal plant material (MPM), since the extract will contain both substances common to different MPMs and substances characteristic of one MPM. In this regard, we conducted a literature review on BAS of the studied raw materials [5–7], thanks to which we selected groups of substances whose presence and content could be used for identification and quantitative determination (evaluation results are presented in the table).

*Table 1. Biologically active substances of the studied types of raw materials*

Raw materials	Compounds
Peppermint leaves	Essential oil (from 2.40 to 3.75%). Essential oil basically consists of menthol (41-65%), menthone and menthyl acetate. Carotene, flavonoids: apigenin, hesperidin, rutin, chlorogenic, rosmarinic, ursolic and oleanolic acids; microelements: copper, manganese, strontium, etc.
St. John's wort herb	Flavonoids: hypericin, hyperoside, rutin, quercitrin, quercetin, chlorogenic, caffeic acids, tannins, coumarins, carotenoids, vitamins C and PP
Hawthorn berries	Flavonoids: quercetin, hyperoside, rutin, etc.; chlorogenic, caffeic acids, glycosides, carotenoids, tannins, fatty oils, saponins, pectin substances, etc.
	Essential oil (from 0.5 to 2%), the main part of which is valerianic-borneol ester, various organic

Rhizomes with roots of valerian	acids: valerianic, isovaleric, formic, acetic, etc., valepotriates, alkaloids: valerine, chatinine, etc.
Hop cones	Bitter substances humulone, lupulone, humulenilic acid, humulinone isomers, essential oil, flavonoids rutin, quercitrin, tannins, etc.

According to literature data [5–7], the BAS composition of four MPMs (hawthorn berries, St. John's wort, peppermint leaves, hop cones) included phenolic compounds, which were selected based on their qualitative and quantitative properties. To demonstrate the presence of valerian rhizomes in MPMs, isovaleric acid was chosen as a marker.

Selection of criteria and methods for the identification and/or quantification of individual compounds, groups of biologically active substances or markers in the finished medicinal product. When choosing a control method, it is necessary to take into account the selectivity, sensitivity of the method, and suitability for quality control in the medicinal plant raw material - extract - concentrate - finished product chain. Highly selective chromatographic control methods best meet these requirements and are suitable for the standardization of herbal preparations [8]. Liquid chromatography is proposed for the identification and quantification of the synthetic components nicotinamide (vitamin P) and pyridoxine hydrochloride (vitamin B6). The conditions for conducting the analysis allow for simultaneous identification and quantitative determination. Liquid chromatography was chosen for the determination of phenolic compounds in pharmaceutical products. The substances were identified by comparing the retention times of the peaks in the chromatograms of the test solutions with those of the standards. During the development of the method, samples of chlorogenic, caffeic, ferulic and rosmarinic acids, rutin, hypersaccharide, luteolin, quercetin and apigenin were used as standards. For routine control, chlorogenic, rosmarinic, caffeic and quercetin acids were chosen as the most specific representatives of the biologically active substances of the plant components contained in the drug. Chromatography was performed on a liquid chromatograph with a UV detector using a C18 chromatographic column; mobile phase A: 0.6 g/l sodium dihydrogen phosphate monohydrate solution with pH 2.5 (using phosphoric acid), mobile phase B: acetonitrile; mobile phase flow rate - 1.0 ml/min; detection at 330 nm. Typical chromatograms of the comparison solution and the test solution are shown in Figures 1.1 and 1.2. Gas chromatography was used to determine isovaleric acid in the rhizomes and roots of Valerian in FPP. The determination was carried out by comparing the retention time of the main peak in the chromatogram of the test solution with the retention time of the isovaleric acid peak, a substance characteristic of the rhizome and root of valerian. The chromatography was carried out on a gas chromatograph with a flame ionization detector, using a 60 m capillary column with an internal diameter of 0.53 mm, the stationary phase was macrogol 20000 P with a layer thickness of 1 µm; the carrier gas was helium for chromatography P, the carrier gas flow rate was 5 ml/min, the flow distribution was 1:1. Typical chromatograms of isovaleric acid comparison solution and Sedavit tablet test solution are shown in Figures 2.1 and 2.2.



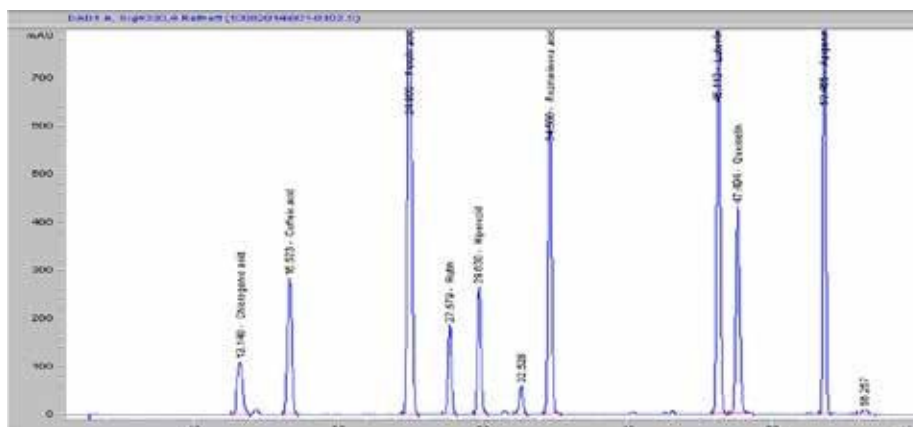


Figure 1.1 Typical chromatogram of the reference solution obtained during the identification of phenolic compounds

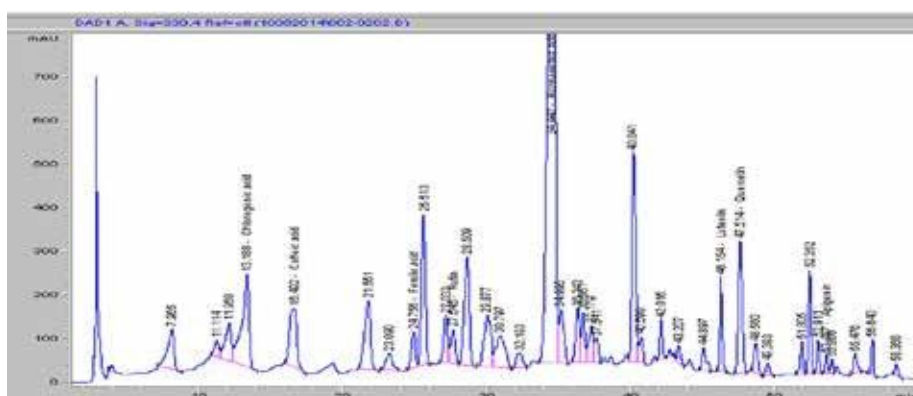


Figure 1.2 Typical chromatogram of the test solution of Sedavit, tablets, obtained during the identification of phenolic compounds

When selecting quantitative determination criteria, the content of biologically active substances in plant raw materials, semi-finished products and technological features should be taken into account. The requirements should reflect the actual level of biologically active substances, which characterizes the effectiveness of the

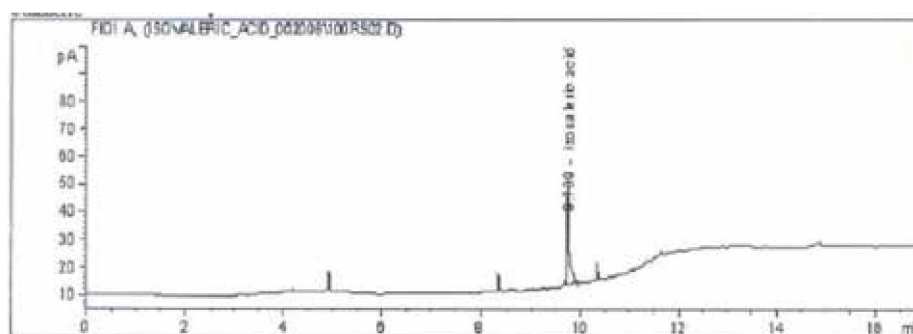


Figure 2.1 Typical chromatogram of the comparison solution of isovaleric acid obtained during its identification

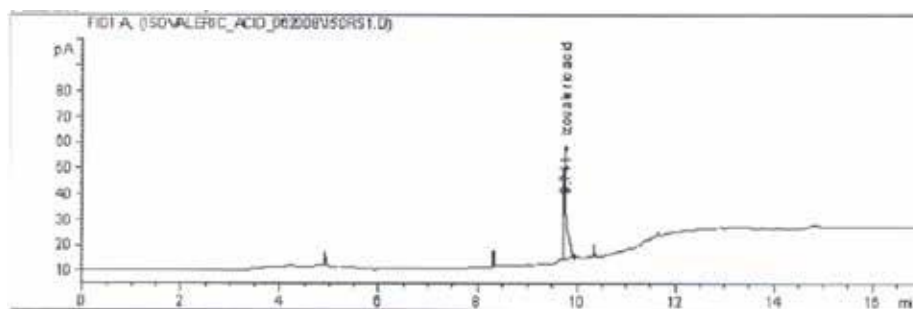


Figure 2.2 Typical chromatogram of the test solution of Sedavit, tablets, obtained during the identification of isovaleric acid

finished medicinal product. In this case, if the finished medicinal product contains a negligible amount of biologically active substances or markers, the specification should only include identification requirements and during quantitative testing the amount of biologically active substances should be determined; in this case, tests can be carried out, for example, by spectrophotometric methods. Sedavit tablets from the UK contain a complex extract consisting of five medicinal plant raw materials. Its therapeutic effect is determined by a complex of biologically active substances isolated from plant raw materials. In this regard, when quantitatively

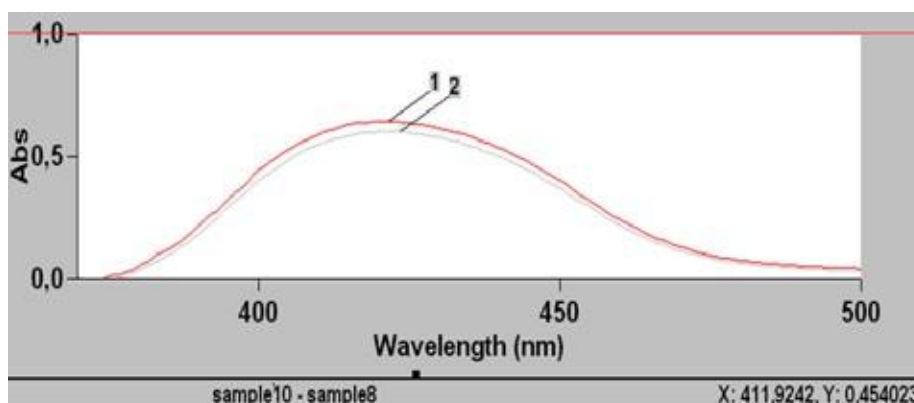


Figure 3 Differential electronic absorption spectra of the aluminum chloride complex with flavonoids of the studied Sedavit solution, tablets (1) and standard rutin solution (2)

analyzing active substances, it is unreasonable to determine each component individually by chromatography and then calculate the total peak area. For these purposes, it is sufficient to use spectrophotometric methods to determine the total optical density of the components determined under the analytical conditions [9]. When choosing a standard sample for recalculating the active substance content, we took into account its availability for routine control, the inclusion of the selected substance in the determined compound group and the need to use conversion factors [9]. French rutin was chosen as the standard, which under analytical conditions gave a maximum absorbance at 415 nm and did not show a reliable difference in spectrum compared to the indicator of the analyzed solution. For routine control, it is proposed to use the absorption index value of rutin with aluminium chloride, which simplifies and reduces the cost of the method for quantitative determination of flavonoids, a typical group for medicinal plant raw materials used. The optical density of the

test solution was determined after the formation of a colored complex of flavonoids extracted with ethyl acetate and aluminium chloride. The initial solution (ethyl acetate extract) without adding aluminium chloride reagent was used as a compensation solution, which avoids the influence of the accompanying substances extracted with ethyl acetate on the analytical results. In parallel, the optical density of the rutin standard sample solution with aluminium chloride was measured using the initial rutin standard solution without adding aluminium chloride reagent as a compensation solution. Typical differential absorption spectra of the aluminium chloride complex with flavonoids of the test sample (complex extract) and the rutin standard are shown in Figure 3.

## CONCLUSION

As a result of this work, a herbal medicine standardization algorithm was proposed using Sedavit tablets as an example, including conditions for selecting bioactive groups and markers, identification and quantitative determination methods, qualitative and quantitative methods, and quantitative criteria for drug evaluation.

## REFERENCES

1. Myagkie lekarstvennye formi: uchebno-metodicheskoe posobie / V. F. Dzyuba [i dr.] pod red. V. F. Dzyubi. – Voronej : Izd-vo VGU 2015. – 108 s.
2. Nikolaev, N. P. Tekhnologiya mazey / N. P. Nikolaev. – Moskva: MORION, 1998. – S. 250–261.
3. Slivkin, A. I. Kontroli kachestva ekstemporalinix lekarstvennix form / A. I. Slivkin [i dr.] pod red. A. I. Slivkina. – Voronej: Izd-vo VGU 2003. – S. 21–28, 46.
4. Validasiya analiticheskix metodov / A. P. Arzamashev [i dr.] // Farmasiya. – 2006. – № 4. – S. 8–12.
5. Sur, S. V. Metodologiya osenki kachestva rastitelinix lekarstvennix sredstv na osnovanii rezul'tatov, poluchennix s pomosh'yu sovremennix analiticheskix metodov / S. V. Sur // Farmasevticheskij jurnal. – 2002. – № 6. – S. 64 – 71.
6. Guidelines for the assessment of herbal medicines / WHO Expert Committee on Specification for Pharmaceutical Preparation. – Thirty-fourth Report. – Geneva: World Health Organization, 1996. – (WHO Technical Report Series, No. 863).
7. Farmasevticheskaya ensiklopediya Ukraini / Golova red. radi V. P. Chernix. –2-e izd. – K.: «MORION», 2010. – 1632 s.
8. Sur, S. V. Problemi i perspektivi razrabotki i vnedreniya sovremennix lekarstvennix sredstv rastitelinogo proisxojdeniya / S. V. Sur, O. M. Grisenko // Liki Ukraïni. – 2002. – № 4. – S. 47–49.
9. Rastitelinie lekarstvennye sredstva / N. P. Maksyutina [i dr.] // K.: Zdorov'ya, 1985. – 280 s.