Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(6): 21-23
Received: 04-09-2016
Accepted: 03-10-2016

Dmitriy Moiseev
Vitebsk State Medical University, Vitebsk, Belarus

Correspondence
Dmitriy Moiseev
Vitebsk State Medical University, Vitebsk, Belarus

Stress testing of drug substances from herbal origin is a new way of stability investigations of medicinal preparations

Dmitriy Moiseev

Abstract
The article presents data on effect of destructive agents (acid and alkaline hydrolysis, oxidation, exposure of metal ions) on the preservation of flavonoids in the extracts of meadowsweet flowers.

Keywords: Meadowsweet, HPLC, flavonoids, spiraeoside, quercetin, stress testing

1. Introduction

To assess the impact of external factors on the stability of synthetic pharmaceutical substances stress tests (acidic and alkaline hydrolysis, refluxing, oxidation, effect of cations of iron and copper, irradiation) are used [1]. The impurities what are degradation products of active pharmaceutical ingredient don't influence on the results of assay, which is necessary to prove the specificity of analytical method for validation. In addition, knowledge of the basic ways of destruction helps to make right decisions for the package of medicinal products, more defensing the drug from external factors [2].

For the standardization of herbal substances one of the most important parameter is the quantitative content of biologically active substances (BAS). In addition, pharmacopoeial monographs usually indicate only the lowest limit (minimum) of BAS amount instead of their possible content (min-max) of values that is used for most of synthetic medicines and pharmaceutical substances. Currently, this approach is tried to change. In the guidance documents of the Committee on medicinal plants to the European Medicines Agency (EMEA/HPMC) there is considered acceptable deviation in assay of substances under standardization (of marker), ±10% (analytical marker) and ±5% (pharmacological marker) from the initial value over the estimated shelf life [3]. Consequently, the primary task of the manufacturer for meeting herbal substances to requirements of the pharmacopoeial monograph throughout the shelf life is minimization of biological, physical and chemical processes in herbal substances during the processing and storage. By regulating the temperature of the drying of the herbal substances it can be achieved not only removal of water, but also inactivation of enzymes in plant cells, which can cause degradation of the active substances. In the storage throughout the shelf life the destruction of some groups of biologically active substances (essential oils, alkaloids), which is used for standardization, can reach 50%. The degradation of the substances can be reduced by primary processing of the raw materials and package, ensuring maximal safety of biologically active substances during the storage. In fact, physical and chemical degradation of the marker compounds during the storage is reduced under the action of external factors: the processes of oxidation and reduction, hydrolysis, interaction with packaging materials.

Stress testing of stability of extracts from herbal substances is complicated by the presence in them compounds similar on chemical structure to markers (initially presenting or degradation products of markers) which are not captured in the assay. According to the recommendations for stress testing, the stability of the substances in solutions under the action of acids and alkalis is tested at room temperature for 2 weeks, or by refluxing with acids and alkalis with various concentrations (0,01 M...5 M) from 2 to 24 hours [1, 2]. Resistance to the action of oxidizing agents is verified in the interaction of substance with hydrogen peroxide concentration from 0,1 to 2% within 24 hours at room temperature or with hydrogen peroxide concentration from 1 to 30% by refluxing. The effect of metal cations (Cu^{2+} and Fe^{3+}) on the process of destruction of matter is checked too. A substance is considered to be resistant to the destructive agent action if not more than 10–15% of original content is destroyed during testing.

2. Materials and Methods

Three series of the herbal substances *Filipendula ulmaria* L. are used as test objects. They were harvested in the Vitebsk region (Belarus) in its natural habitat in 2014 and 2015 years throughout the period of accordance with the recommendations of the GACP (Good agricultural practice for the production of medicinal plants). Before analysis the samples were stored in the leaky package. In early works we studied the safety of flavonoids in the herbal substances of the meadowsweet flowers under the influence of different conditions (temperature, humidity and tightness of packing) for solid and powdered materials [4].

For the determination of arbutin in the samples liquid chromatograph Agilent 1260 was used, in complete with feed and degassing system for four solvents, diode-array detector, a column thermostat, automatic device for input samples (sampler). For the separation the chromatographic column Zorbax SB C-18 was used (particle size 5 µm, length 250 mm, diameter 4, 6 mm, manufacturer Agilent Technologies). The solvent system: 0, 01 M solution of potassium dihydrophosphate (Kh h), brought to pH=3,0 by concentrated phosphoric acid (H. h.) and acetonitrile (for liquid chromatography, "Merck") (the ratio of 80:20 by volume), column temperature 30 °C. The wavelength of detection 370 nm was corresponded to maxima of the peaks, it was chosen after recording the absorption spectra at the wavelengths of 200–400 nm. For assay of flavonoids in extracts the standard samples of isoquercitrin (CAS [482-35-9], secondary HPLC (SH) > 90%), spiraeoside (CAS [20229-56-5], SH >95%) and quercetin (CAS [117-39-5], SH > 98%) were used [5].

Quercetin is one of the main products of quercetin glycosides hydrolysis, therefore its quantitative content in the extracts was considered.

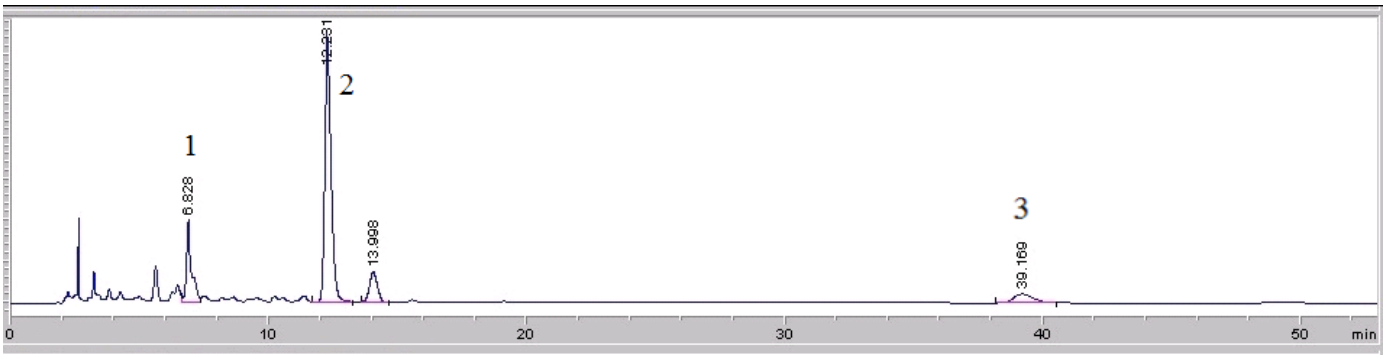

Fig 1: Chromatogram of extract of meadowsweet flowers at absorption wavelength of 370 nm (1 – isoquercitrin; 2 – spiraeoside; 3 – quercetin)

The separation of the substances from herbal materials was conducted as shown below. The extraction by 40% ethanol from the sample of crushed meadowsweet flowers was performed in tightly sealed glass container in boiling water bath in the ratio of raw materials and extractant of 1:50. The extracts were centrifuged and placed in the vials (glass containers) for chromatography in volume 900 µl with addition of 100 µl of reagent (0,1 M NaOH; 0,1 M HCl; 3% H_2O_2; 0,5 M salts Cu^{2+} and Fe^{3+}; H_2O). These samples were chromatographed through 10–15 minutes after preparing, hermetically sealed and subjected to further periodic monitoring after 24, 96, 216, and 360 hours after preparation of solutions.

3. Results and Discussion

The original content of flavonoids for three series of meadowsweet flowers is presented in table 1.

Table 1: The quantitative content of flavonoids for three series of meadowsweet flowers, mg/ml

	1 series	2 series	3 series
Isoquercitrin	32,4	26,3	22,1
Spiraeoside	175	161	152
Quercetin	5,35	4,71	3,24

The data on the safety of flavonoids in the extracts of meadowsweet flowers under the action of the destructively agents are shown in table 2.

Table 2: Degradation of flavonoids (% of initial content) in *Filipendula ulmaria* L. flowers under the action of the destructively agents after 15 days, mg/ml

	0,3% H_2O_2	H_2O	0,01M HCl	0,01M NaOH	Cu^{2+}	Fe^{3+}
Isoquercitrin	101,7±1,3	102,5±1,2	106,0±3,2	89,2±1,7	80,9±1,6	71,8±19,3
Spiraeoside	95,5±1,2	97,3±3,0	99,3±0,6	19,5±5,8	45,5±9,3	53,1±13,3
Quercetin	102,3±2,4	104,2±1,5	102,6±0,8	1,6±0,5	56,4±20,5	16,5±5,3

In the extracts from *Filipendula ulmaria* L. flowers all investigated flavonoids were stable (more than 85% of initial level) in acidic and neutral conditions, as well as in the oxidation by 0, 3% hydrogen peroxide during the observation period (table 1). Isoquercitrin was stable in alkaline conditions. The influence of metal cations (Cu^{2+} and Fe^{3+}) led to degradation of flavonoids, quercetin was destroyed more rapidly.

For the processes leading to the significant degradation of flavonoids (over 15%) the rate constants were calculated. The zero, first and second reaction orders were tested by graphical methods. At the same time the correlation coefficients were higher in reactions of second order for all reagents.

Table 3: The rate constants and correlation coefficients of the reactions of flavonoids degradation in the meadowsweet flowers (the second reaction order)

	0,01M NaOH		Cu^{2+}		Fe^{3+}	
	Rate constant	**Correlation coefficient**	**Rate constant**	**Correlation coefficient**	**Rate constant**	**Correlation coefficient**
Isoquercitrin	Stable		0,66	0,985	1,11	0,988
Spiraeoside	11,1	0,987	3,28	0,995	2,49	0,985
Quercetin	158	0,983	2,08	0,997	14,5	0,991

These data indicate the sufficient specificity of the technique (good separation of the chromatographic peaks of the marker components and possible impurities in the chromatograms).
In the stress testing of meadowsweet flowers extracts high stability of the marker components (isoquercitrin, spiraeoside and quercetin) during hydrolysis and oxidation is shown. Therefore, the unsealed package is possible to use for the storage of the raw materials and medicines on its basis, but the contour-cell package can be used for dosed preparations on the basis of the finely ground raw materials (capsules and tablets). In the production it should avoid the contact of plant material with cations of iron and copper.

4. References

1. Klick S. Toward a generic approach for stress testing of drug substances and drug products. Pharmaceutical Technology. 2005; 2:48-66.
2. International conference on harmonization, ICH Q1A (R2): Stability testing of new drug substances and products. Step 5 (2003), EMEA, 2006, 20.
3. Guideline on quality of herbal medicinal products/ traditional herbal medicinal products EMA/CPMP/QWP/2819/00. Rev.2, London, 2011, 13.
4. The State Pharmacopoeia of the Republic of Belarus (under ed. Marchenko SI). 2nd ed., Pobeda, Molodechno. 2016; 2:1368.
5. Moiseev DV. Development and validation the method of quantification of flavonoids in meadow sweet flowers by liquid chromatography. Vestnik Farmacii. 2011; 4:36-42.
6. Moiseev DV. Stress testing of drug substances of herbal origin – a new ways of investigation stability of medicinal preparations. The international scientific and practical conference «Biological characteristics of medicinal and aromatic plants and their role in medicine», dedicated to the 85th anniversary of FGBNU VILAR and the 65th anniversary of the botanical garden of aromatic plants. Scherbinskaya tipografiya, Moscow, 2016, 505-507.