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QUANTITATIVE ANALYSIS OF POLYSACCHARIDES FROM *Plantago major* LEAVES USING THE DREYWOOD METHOD

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A method for determining with a relative uncertainty of less than 3% the total content of polysaccharides in Plantago major *leaves that were converted to galacturonic acid was developed using the Dreywood method. The polysaccharide content in* Plantago major *leaves determined by the developed method was 1.44-1.52%.*

Key words: Dreywood method, *Plantago major* L., polysaccharides, quantitative analysis.

Leaves of *Plantago major* L. (plantain) are an official medicinal raw material. The biological activity of this type of plant material is due to a complex of biologically active compounds, among which the water-soluble polysaccharides (Psa) are particularly noteworthy [1].

The USSR State Pharmacopeia, XI Ed., art. 20 [2] suggests standardization by gravimetry of the biologically active substances (BAS) from *P. major* leaves using total content of PSa in the aqueous extract. However, this method is labor-intensive and poorly reproducible. Belyakov suggested standardizing this type of medicinal raw material by spectrophotometry using the abililty of reducing sugars to reduce picric acid to picramic [3] and converting the PSa content to glucose. However, this does not reflect the actual composition of the complex of water-soluble PSa from *P. major* leaves.

Herein we propose a method for quantitative determination of the PSa content using the Dreywood method [4] for standardizing active substances from *P. major* leaves.

Absorption spectra of the products from reaction of the PSa complex from *P. major* leaves and galacturonic acid (GalUA) with anthrone in conc. H_2SO_4 in ethanol showed that they exhibit a common maximum at 424 nm that was selected as the analytical wavelength (Fig. 1).

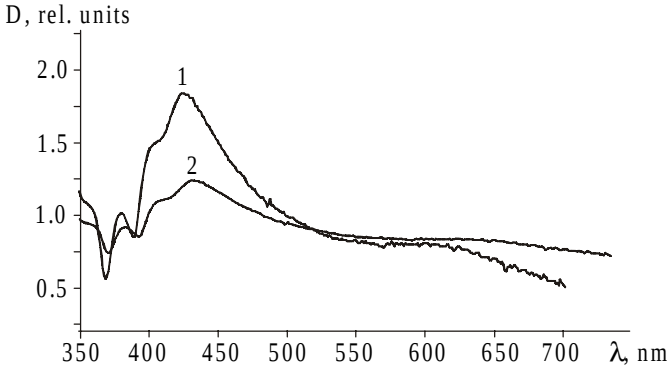

Fig. 1. Absorption spectra of galacturonic acid (1) and PSa complex from *P. major* leaves (2).

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TABLE 1. Accuracy of the Developed Method Determined by the Added—Found Method

PSa content in aliquot, μg GalUA	Added GalUA, μg	Theoretical PSa content, μg GalUA	Found PSa, μg GalUA	Uncertainty	
				abs., μg	rel., %
315	80	395	384	-11	2.75
315	160	475	480	+5	1.05
315	240	555	542	-13	2.34

TABLE 2. Analysis of a Standard Sample of Galacturonic Acid

GalUA in aliquot, μg	Found GalUA, μg	Relative uncertainty, %	
		single determination	average
254	251/256/249	1.18/0.79/1.97	1.31
279	275/271/272	1.43/2.86/2.51	2.27
345	338/348/354	2.03/0.87/2.61	1.84

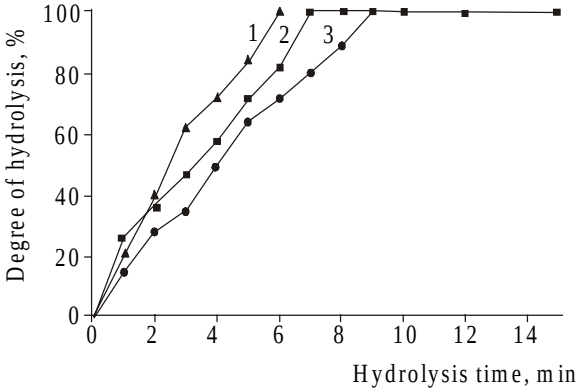

Fig. 2

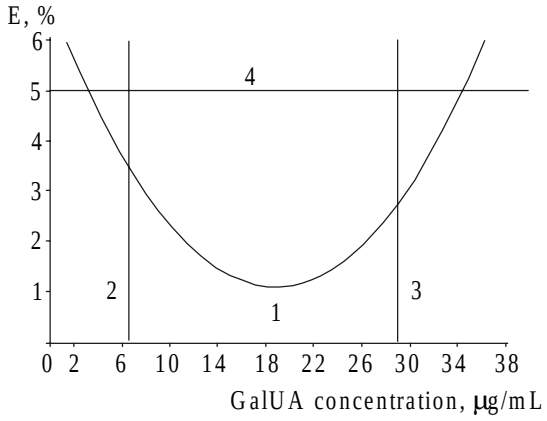

Fig. 3

Fig. 2. Hydrolysis kinetics of glucomannan (1), arabinogalactan (2), and galacturonan (3) from *P. major* leaves.

Fig. 3. Method of increasing sample: Relative uncertainty (E) as a function of galacturonic acid solution concentration (c) (polynomial regression equation $E = 0.016025 \cdot c^2 - 0.6020947 \cdot c + 6.7409664$) (1); lines defining the region of working concentrations (2, 3), and 5% relative uncertainty level (4). Cecil CE 2011 (K_{instr} 0.98).

The optimal extraction parameters were determined during the development of the analytical method. The extraction of PSa is greatest if purified water is used as the extractant. Equilibrium is attained in 90 min at a raw material:extractant ratio of 1:70-80 and boiling. Performing four 0.5-h extractions affords the highest (95%) amount of PSa in the first two extractions. The third extracts 4% of the total; the fourth, about 1%. Some researchers [3] proposed performing two extractions for 30 min. However, we found that only 58% of the PSa were extracted after the first 30 min; 25%, after the second. Therefore, the raw material as a whole retains about one quarter of them. An attempt to replace the two extractions (1:70 and 1:50) by one at a raw material: extractant ratio of 1:120-150 turned out to be ineffective. The extract after 1.5 h contained 1.4 times less PSa than for the multiple extraction.

We added a hydrolysis coefficient to the conversion formula to take into account the fact that a molecule of water was added to each polymer unit during the PSa hydrolysis. As indicated above, the principal monomer of the *P. major* PSa complex was GalUA. The molecular weight (MW) of the polymer unit was 176 g/mol. The MW of GalUA formed by hydrolysis was 194 g/mol. The increase in the MW made it necessary to introduce a correction coefficient, the value of which was ~0.91 [5].

An investigation of the hydrolysis rate of the components of the *P. major* PSa complex found that arabinogalactan, galacturonan, and glucomannan are completely hydrolyzed in conc. H_2SO_4 in 8-10 min (Fig. 2).

TABLE 3. Reproducibility of the Method Using Independent Determinations

PSa content converted to GalUA, %	Average, %	Deviation from average, %
1.42/1.41/1.40	1.41	+0.71/0.00/-0.71
1.47/1.51/1.48	1.49	-1.34/+1.34/-0.67
1.37/1.40/1.38	1.38	-0.73/+1.45/0.00

TABLE 4. Metrological Properties of the Developed Method [n = 11, P = 95%, t(p,f) = 2.23]

Raw material	Property				
	$\bar{x}$, %*	S^2	S_x	±Δx, %	E, %
P. major leaves	2.04	$4.68 \cdot 10^{-3}$	$2.06 \cdot 10^{-2}$	0.05	2.25
"Plantaglucid"	7.37	$4.30 \cdot 10^{-2}$	$6.25 \cdot 10^{-2}$	0.14	1.89
"Plantain juice"	0.085	$1.60 \cdot 10^{-5}$	$1.21 \cdot 10^{-3}$	0.003	3.17

$\bar{x}$, %, is the average; S^2, standard deviation; S_x, mean square deviation; ±Δx, %, absolute uncertainty of the arithmetic mean; E, %, relative uncertainty.

The systematic uncertainty of the method (evaluation of its correctness) was determined by performing experiments using varying sample sizes, the added—found method, and analysis of standard GalUA.

Varying the sample sizes showed that the modified Dreywood method contained a constant systematic uncertainty that decreased with increasing weight of the analyte. Figure 3 shows the change of correctness of the GalUA determination in the aqueous extract of *P. major* leaves. At the working concentrations, the relative uncertainty of the method was less than 3.5%. The systematic uncertainty was lowest for GalUA solutions with concentrations of 16-22 μg/mL.

The added—found method indicated that the relative uncertainty was less than 2.80% (Table 1). The experiment with standard GalUA showed that the relative uncertainty of a single measurement was in the range 0.79-2.86% and could be either positive or negative (Table 2).

The mean uncertainty of the three determinations for the three samples lay within rather narrow (1.31-2.27%) limits. The deviation from the mean from three independent determinations was less than 1.5%. This indicated that the method had satisfactory reproducibility (Table 3).

We examined the possibility of using this method to analyze the preparations of *P. major* leaves "Plantain juice" (PJ) and "Plantaglucid" (PG). Table 4 gives the metrological properties of the developed method for the studied materials.

The developed method was used to determine the PSa content in nine samples of raw material and several industrial batches of PJ and PG. It fell in the range 1.5-3.2, 0.084-0.120, and 6.65-8.76%, respectively.

EXPERIMENTAL

Plant samples were *P. major* leaves acquired through a pharmacy chain (Krasnogorskleksredstva, ST-Medifarm, Russia), PJ (Vifitekh, Russia), and PG (Zdorov′e, Ukraine). The standard was D-(+)-galacturonic acid (Fluka BioChemica, monohydrate, ≥93%). Absorption spectra were recorded on Cecil CE 2011 and Agilent 8453E UV-Vis spectrophotometers in quartz cuvettes with a 10-mm pathlength.

The isolation and separation of the PSa complex has been described previously [4].

The hydrolysis kinetics of the components of the PSa complex were studied as follows. A weighed portion of the substance was dissolved in purified water. Concentrated H_2SO_4 was added. The mixture was heated on a boiling-water bath. Aliquots of the reaction mixture were taken after certain time intervals. The polymeric components were precipitated by 95% ethanol, centrifuged, and reacted with anthrone. The optical density of the solution was determined.

Quantitative Method for Determining Total PSa Content. An analytical sample of raw material was ground to a particle size that passed through a sieve with 1.0-mm spacings. A portion (2.0 g, accurately weighed) of ground raw material was placed in a 100-mL conical flat-bottomed flask with a ground-glass stopper. Distilled water (60 mL, FS 42-2619-89) was added. The contents of the flask were heated on a boiling-water bath for 1 h, cooled, and filtered into a 200-mL volumetric flask. The extraction was repeated under the same conditions. The volume of the combined filtrate was adjusted to the mark with purified water (Solution A).

Solution A (2 mL) was placed in a centrifuge tube, treated with 95% ethanol (4 mL), stirred, heated on a water bath for 10 min, cooled, and centrifuged at 3000 rpm for 10 min. The supernatant liquid was decanted. The precipitate was suspended in 95% ethanol (5 mL) and centrifuged again under the same conditions. The supernatant liquid was decanted. Hot air was blown over the precipitate to remove traces of ethanol. Anthrone in conc. H_2SO_4 (4 mL, 0.2%) was added to the precipitate. The mixture was heated on a boiling-water bath for 10 min, cooled and placed in a 25-mL volumetric flask with 95% ethanol. The volume was adjusted to the mark with the same solvent (Solution B).

The optical density of solution B was measured on a spectrophotometer at 424 nm in a cuvette with a 10-mm pathlength. The reference solution was anthrone in conc. H_2SO_4 (4 mL, 0.2%) kept under the same conditions as the test mixture.

The PSa content (X, %) was converted to GalUA and the absolute dry raw material was calculated using the formula

$$X = (D \times k^{\nu} \times 0.91/m \times E)\,[100/(100-W)],$$

where D is the optical density of the studied solution; k^{ν}, the dilution factor (2500); 0.91, the hydrolysis coefficient; E, the conversion factor for GalUA (214); m, the mass of raw material in g; and W, the mass loss upon drying the raw material in %.

The preparations PJ (2 mL) and PG (0.5 g) were analyzed using the described method.

The systematic uncertainty in the measurements was determined as before [6]. The results were treated metrologically using the literature recommendations [7]. The instrumental uncertainty of the spectrophotometer (K_{instr}) was calculated using potassium bichromate [8]. Regression analysis was carried out using the Advanced Grapher ver. 2.07 program set (Alentum Software Inc.).

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