Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(1):213-222
(http://derpharmachemica.com/archive.html)

Development and validation of UV-Spectrophotometric method for quantitative determination of inulin by specific absorbance

Olga A.Yevtifieieva, Kseniya I. Proskurina, Nataliya N. Smelova and Irina Yu. Petukhova*

Department of Analytical Chemistry, National University of Pharmacy, Pushkinska str., Kharkiv, Ukraine

---

ABSTRACT

The sensitive, accurate and precise spectrophotometry method by specific absorption of quantitative determination of the inulin substance after acid hydrolysis to the5-hydroxymethylfurfural(5-HMF) has been developed. The specific absorption of 5-HMF, which is equal to 258±2.86, has been determined experimentally. The validation characteristics of the proposed method (linearity, accuracy, precision, robustness) have been studied. The estimation of validation characteristics of the quantitative determination methods of inulin substance by the specific absorbance allows us to conclude that using the method is correct for routine analysis.

Keywords: Inulin, 5-hydroxymethylfurfural, spectrophotometry, specific absorption.

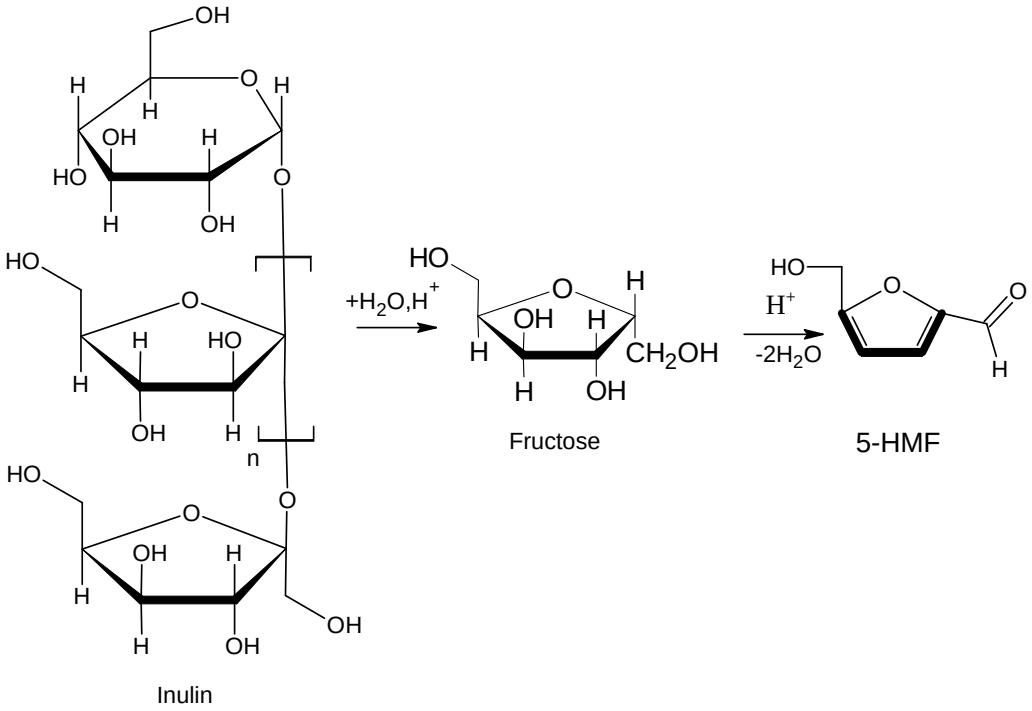
INTRODUCTION

Inulin is a heterogeneous combination of polymer of fructose, which are in the form of D-fructofuranose and connected by a β(2→1)-glycosides bonds [1,5,7,11,17]. Due to the fact that enzymes which able to decompose this bonds are absence in the human body, inulin transit passes through the gastro-intestinal tract, causing hypocholesteremic, hypoglycemic and prebiotic actions [3,4,5,10,17,18].

Currently, monographs of substance inulin and dosage forms with inulin are absence in the State Pharmacopoeia of Ukraine (SPhU). These are shown in the US and British pharmacopoeias, where the inulin is widely demanded in medical practice [2,9].

Methods of structural analysis and quality control of the inulin obtained from various plant sources have been devoted a significant amount of research of domestic and foreign scientists. The UV-/ visible spectrophotometry methods are the most cost-effective and easy-to-follow[17].

For the quantitative determination of inulin method by UV spectrophotometry by specific absorption was chosen [11]. This method based on ability of inulin to destruct with forming of monomers, in particular fructose, when heated with 5% hydrochloric acid solution during 2.5 hours. Further heating of the reaction medium leads to an intramolecular dehydration of obtained fructose in the product of acid-catalyzed conversion of polysaccharides - 5-hydroxymethylfurfural (5-HMF), which has the maximum absorption at the wavelength of 285 nm. The reaction is shown in Scheme 1 [10, 11, 20]. According to the scientific work [11] the content of inulin is calculated taking the specific absorbance to be 298.



Sch. 1.Reaction of acid hydrolysis of inulin with the generation of 5-HMF

Experimental evaluation of the metrological characteristics of the proposed method has shown that the method has a large error. According to the method, the content of inulin in terms of fructose is 76.94%, the standard deviation - 0.89% and the relative confidence interval - 1.90% [19]. In our opinion, too low results of analysis can be explained by the formation side of humic substances, as well as dehydration of 5-HMF to levulinic and formic acid [20], which are very weakly absorbing compounds in the UV region. The study found that the temperature and time of the acid hydrolysis has a significant influence on the formation of 5-HMF.

Spectrophotometry by the specific absorbance is pharmacopoeial analysis method and its application does not require a standard samples [13, 14, 15].Given the above, the aim of our work is to optimize the conditions of the method (hydrolysis time), the experimental determination of the specific absorption score 5-HMF and evaluation of metrological characteristics of the developed method.

MATERIALS AND METHODS

The substance of inulin manufactured by Alfa Aesar No. A18425 batch number H5597was used in the experimental researches. According to the certificate, the moisture of the substance is 7.67%, optical rotation -36.7°, sulphate ash <0.01%.

The analytical balance AV 204 S/A METTLER TOLEDO was used. Reagents, measuring glass-ware of class A (first class) and excipients meeting the requirements of the SPhU were used for the work [16].

Thin-layer chromatography (TLC)

The TLC was performed on silica gel plates “Silufol” (Merk, Germany) with the mixture of 70 volumes of glacial acetic acid, 60 volumes of chloroform and 10 volumes of water as the mobile phase.

1µl of the following solutions were applied separately to the plate. The solutions of inulin (Inu), glucose (Glc), fructose (Frc) and sucrose (Scr) each of them with concentration 10mg/ml were used as standards. For TLC analysis of the hydrolysis products of inulin substance five solutions were prepared under the following procedure. Transfer about 6.25 g of the inulin substance, accurately weighed, to a 25-ml volumetric flask, dissolve in a water, dilute with water to volume, and mix. Transfer this solution to a round-bottom flask with ground glass joint neck, add 50.0 ml of 5% hydrochloric acid solution. Connect the flask to backflow condenser and place in a boiling water bath. Pipet 1.0 ml of this solution after 30 min (Frc-30), 60 min (Frc-60), 90 min (Frc-90), 120 min (Frc-120), 150 min (Frc-150), into separate 25-ml volumetric flask, dilute with water to volume, and mix.

The chromatograms in a continuous elution tank for about 4 hours were developed. After removal of the plate, evaporate the solvent in a current of warm air and spray with a solution in acetone containing 1% v/v of diphenylamine, 1% v/v of aniline and 1% v/v of orthophosphoric acid and heat for 10 minutes at 130°.

High performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD)
Structural analysis by HPAE-PAD was performed on an ion chromatograph Dionex DX 500 IC with amperometric detector and analytical column Carbo Pac PA1® (9x250 mm). The sample (injection volume: 50 µl) was analyzed with the use of eluent from 0.20 M (t=15 min) to 0.50 M (t=55 min) CH_3COONa in 0.1 M NaOH; the speed of the mobile phase is 1.5 ml/min, analysis time is further 0-70 min. Detection was performed under the following conditions: E_1=0,40 V (t_1=400 ms), E_2=1,0 V (t_2=200 ms), E_3=0,25 V (t_3=400 ms) [1,6,7,8].

The content of fructose (X, %) in inulin substance is calculated by the formula:

$$\omega\% = \frac{\sum S}{\sum S_{total}} \times 100,$$

in which

$\sum S$ - area of the peak due to the fructose;

$\sum S_{total}$ – sum of the areas of the peaks due to all component in the chromatogram obtained with test solution.

UV Spectrophotometry
The spectrophotometer "SPECORD 200" was used in the work. The developed spectrophotometric method is shown in Figure 1. The measurements were performed with 1-cm cells at (20±1)°C. The content of inulin (X, %) in terms of fructose is calculated by the formula:

$$\mathrm{X} = \frac{\mathrm{A}\cdot \mathrm{V_1}\cdot \mathrm{V_2}\cdot 100}{\mathrm{A}_{1\mathrm{cm}}^{1\%}\cdot \mathrm{m}\cdot \mathrm{V_p}\cdot(100-W)} = \frac{\mathrm{A}\cdot 50\cdot 25\cdot 100}{\mathrm{A}_{1\mathrm{cm}}^{1\%}\cdot \mathrm{m}\cdot 2\cdot(100-7.67)},$$

in which

A – absorbance of the solution B;
V_1 – the volume of the volumetric flask for preparation the solution A;
V_2 – the volume of the volumetric flask for preparation the solution B;
$\mathrm{A}_{1\mathrm{cm}}^{1\%}$ – specific absorption of 5-HMF;
m - the amount, in g, of inulin substance;
V_p – the volume of the pipette;
W – moisture percentage of the analyzed inulin substance, % (% is 7.67);

The statistical processing of the experimental data was carried out according to the Article of the SPhU "Statistical analysis of the chemical experiment" [N][16].

With purpose to determine the specific absorbance of 5-HMF five solutions with the concentration 2.56-3.84·10^{-5}g/ml were prepared according to the procedure which is shown in Figure 1. The measurements were performed with 1-cm cells at (20±1)°C. The values of the specific absorbance were calculated by the formula:

$$A_{1cm}^{1\%} = \frac{A\cdot V_1\cdot V_2\cdot 100}{m\cdot(100-W)\cdot V_p\cdot 100} = \frac{A\cdot 50\cdot 25\cdot 100}{m\cdot(100-7.67)\cdot 2\cdot 100},$$

in which

$A_{1cm}^{1\%}$ -specific absorption of 5-HMF;
A – absorbance of the solution;
V_1 – the volume of the volumetric flask for preparation the solution A;
V_2 – the volume of the volumetric flask for preparation the solution B;
V_p – the volume of the pipette;
W – moisture percentage of the analyzed inulin substance, % (% is 7.67);
m - the amount, in g, of inulin substance taken.

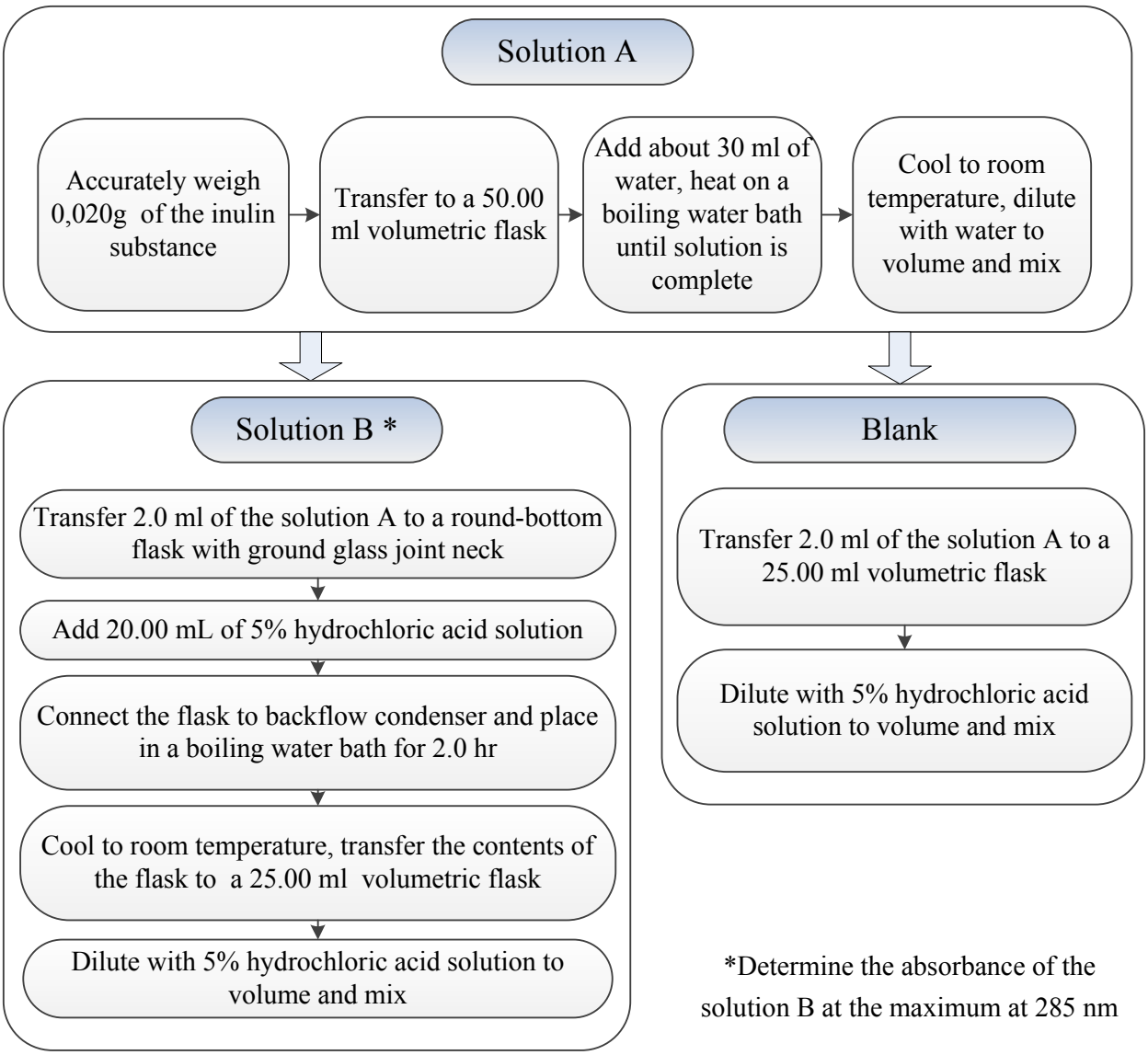


Fig. 1. The developed method of quantitative determination of the inulin substance by spectrophotometry by specific absorbance

RESULTS AND DISCUSSION

In the absence of national normative documentation, foreign standard Official Monographs "Inulin" (the US Pharmacopeia 36) was usedfor evaluating of the results of quantitative determination inulin substance. According to these monograph the fructose content in inulin should not be less than 94% and not more than 102% calculated on the driedbasis. Requirements for the complete uncertainty analysis results $\max_{\Delta As}$, expressed as a one-sided confidence interval of 95% is associated with high (BH) and low (BL) content tolerances (% of nominal) expressed by the relation [2,9]:

$$\Delta_{As} \leq B = \frac{B_H - B_L}{2};; \ \Delta_{As} \leq \max \Delta As = \frac{102 - 94}{2} = 4.0\%$$

Beforehand the thin layer chromatography (TLC) of the hydrolysis products of the analyzed inulin substance were conducted to establish the conditions of hydrolysis, under which a greatest amount of 5-HMF are formed. The analysis were carried out according to the requirements of the British Pharmacopoeia monograph [2]. The results of the chromatography are shown in Figure1.

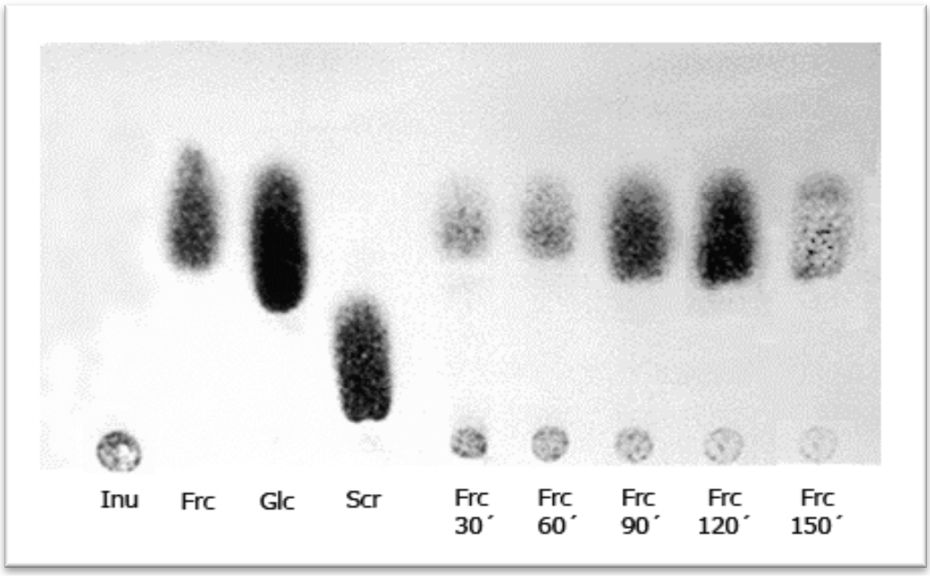


Fig. 2. The chromatogram of hydrolysis stages of insulin substance after 30 min., 60 min., 90 min., 120 min., 150 min

The largest amount of 5-HMF is formed through 90-120 minutes after initiation hydrolysis, as indicated by the intensity of the chromatographic spots (Frc-90´ Frc-120´) (Fig. 2).

Monosaccharides and oligosaccharides in the inulin substance and the standard fructose were determined by high performance anion exchange chromatography with pulsed amperometric detector (HPAE-PAD) [1,6,7,8] (Figure3, 4).

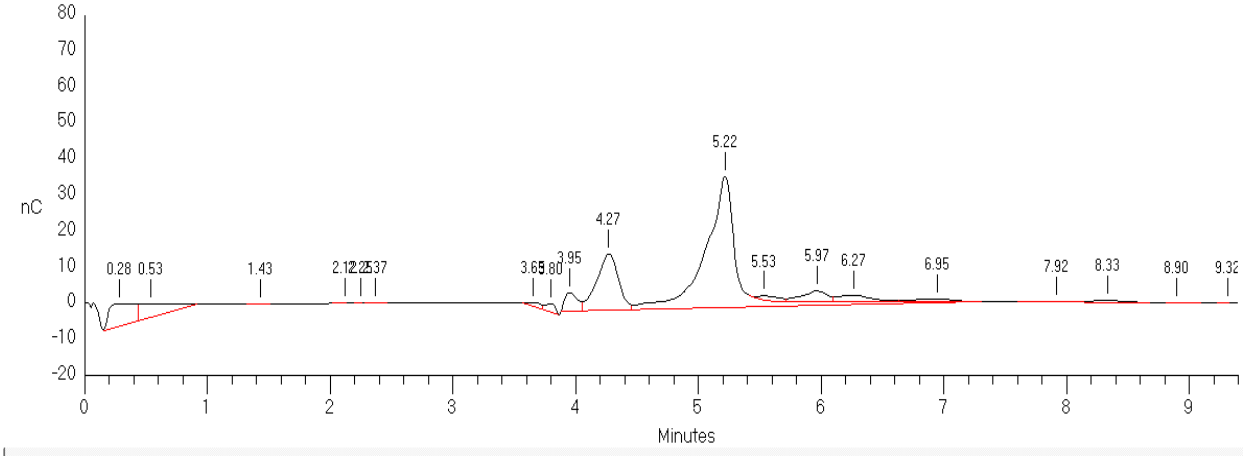


Fig. 3. The HPAE-PAD chromatogram of standard fructose

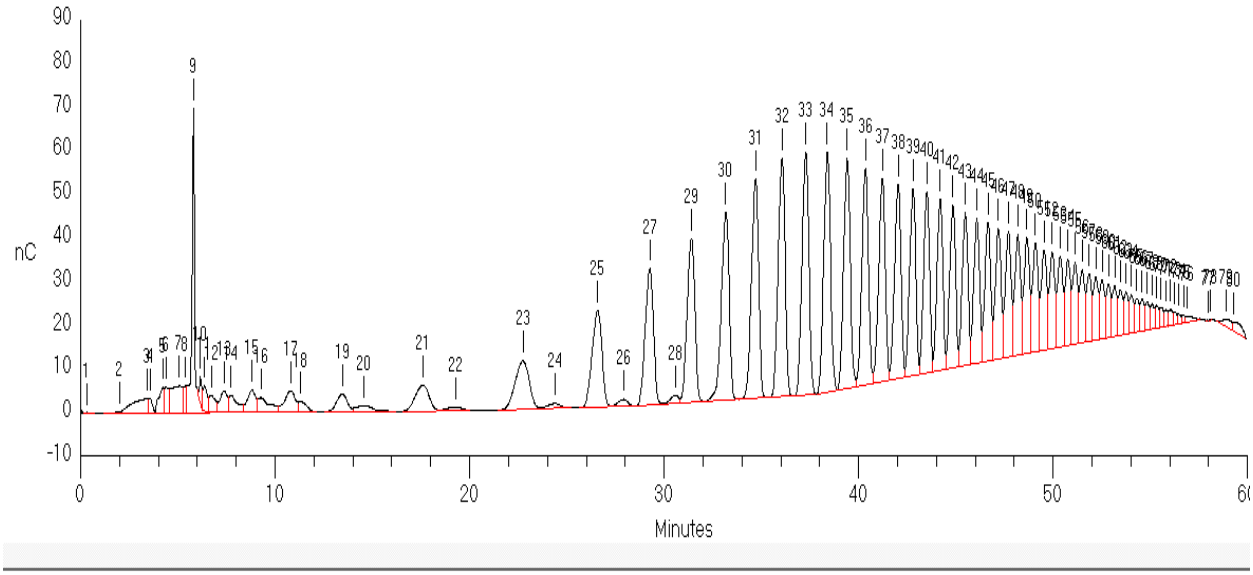


Fig. 4. The HPAE-PAD chromatogram of the substance inulin Alfa Aesar

According to the results of structural analysis (Figure3, 4) inulin substance before hydrolysis contains a free fructose (retention time tR = 5.22 (Figure3)) in the amount of 22.60%, as indicated by the first peak detecting №9 (Figure4). Order to optimize the spectrophotometry method the specific absorbance of 5-HMF obtained in the course of hydrolysis has been determined experimentally. The absorption maximum of 5-HMF is at the wavelength of 285 nm (Figure5), which is consistent with literature data[18].

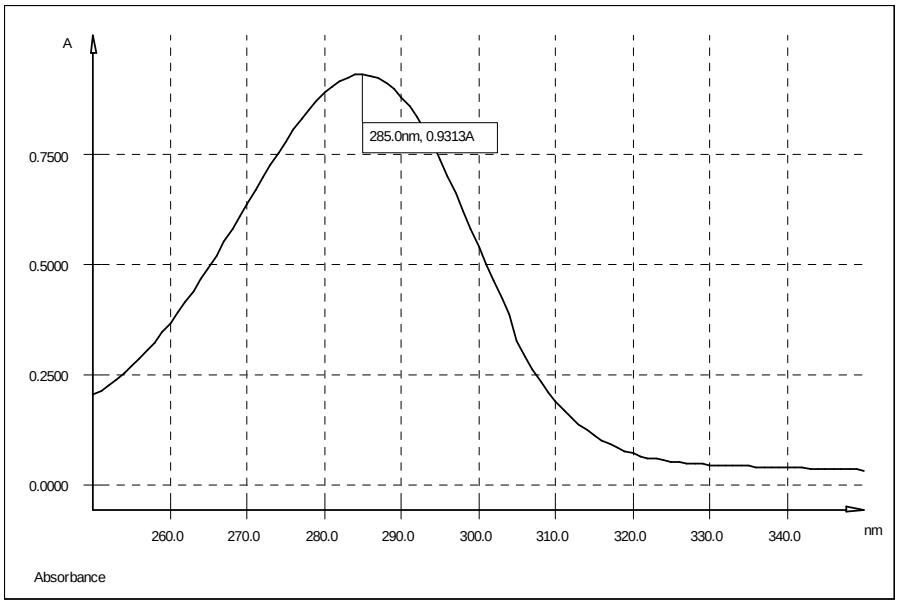


Fig. 5.The UV absorption spectrum of 5-HMFobtained in the course of hydrolysis

The results of determination of the specific absorption ($\mathbf{A_{1см}^{1\%}}$)of 5-HMF are shown in Table 1 and Figure 6.

Tab. 1. The results of determining of the specific absorption ($A_{1см}^{1\%}$) of 5-HMF

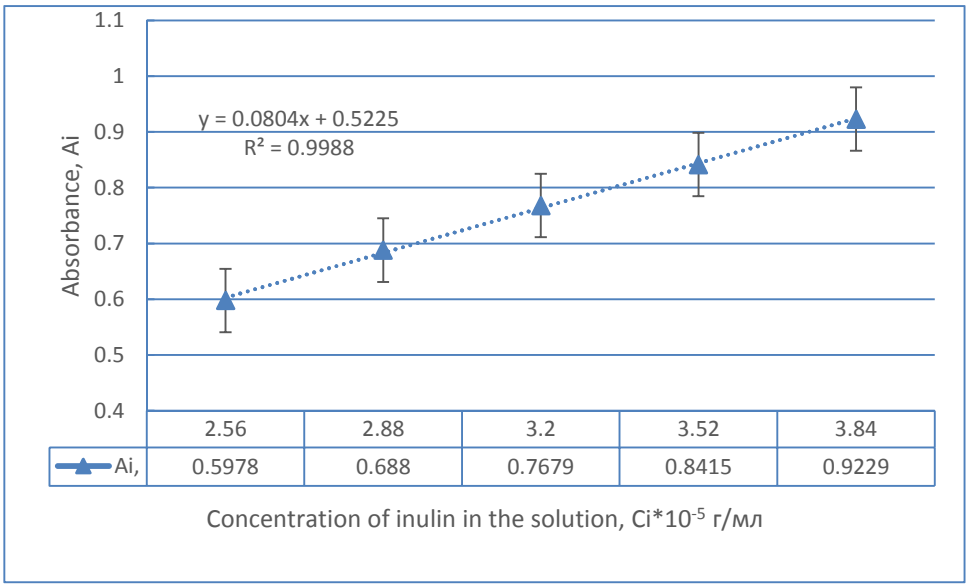
№	*Introduced inulin, g/ml·10^{-5}*	*The optical density, A**	$A_{1см}^{1\%}$	*Metrological characteristics*
1	2.56	0.5978	252.92	$\overline{X}$=258.16
2	2.88	0.6880	258.72	S = 3.00
3	3.20	0.7679	259.91	$S_{\bar{X}}$=1.34
4	3.56	0.8415	258.93	$\Delta\bar{X}$=2.86
5	3.84	0.9229	260.31	$\bar{\varepsilon}_{\bar{X}}$=1.11%

** – the average value of three measurements*

According of the results of study of the dependence of absorbance on the concentration of inulin substance in the solution the specific absorption of 5-HMF for substance inulin is 258±2.86.

Based on the national developments in the field of methods validation of drugs optimization of the quantitative determination method of the inulin substance by UV spectrophotometry by specific absorbance was carried out [12-16]. The acceptance criteria of the assay method for inulin substance were calculated considering of the determined value of specific absorption of 5-HMF and the permissible limits (table. 2).

Fig. 6.The plot of the dependence of absorbance on the concentration of inulin substance in the solution



Tab. 2. The critical values of the for the systematic error (max δ_{tot}), total uncertainty of the analysis (max Δ_{As}) and the parameters of the linear dependence Yi=b•Xi+a *

Parameter	***The calculation of the parameter***	***The value***
λ, nm	–	285
X_{nom}	$X_{nom} = (B_H+B_L)/2$, B_H– the upper permissible limit; B_L – the lower permissible limit	94.00-120.00
$A_{1см}^{1\%}$	–	258
C_{nom}mg/100 ml	$C_{nom}=[m_н \cdot (100-W) \cdot 100] \cdot Dil \cdot (X_{nom}/100)$	2.895 mg/100 ml
A_{nom}	$A_{nom}= A_{1см}^{1\%} \cdot C_{nom}$	0.7499
max Δ_{As}%	max Δ_{As} = B	4.00
maxδ_{tot} = maxΔ_{prec}%	maxδ_{tot} = maxΔ_{prec} = $(\sqrt{2}/2) \cdot$ max Δ_{As}	2.83
RSD_o %	RSD_o = maxΔ_{prec} / ($t_{95\%}$;g-2)	1.20
RSD_{range} %	$RSD_{range} = \sqrt{\frac{(X_i-\bar{X})^2}{g-1}}$	15.81
min R_c^2	min R_c^2= 1-$\sqrt{\frac{RSD_0^2}{RSD_{range}^2}}$	0.9942

**the number of points 5, the permissible limits94-102% (±4,0%), for the range of 80 -120%*

For the preliminary assessment of the quantitative determination method of inulin the comparison of the A_{nom} with the minimum nominal absorbance min $A_{nom}$was performed:

$$A_{nom} \geq \frac{2}{\max \Delta As} = 0.5000.$$

Considering that the A_{nom} value exceeds min A_{nom} (0.7499 > 0.5000), the evaluation of the metrological parameters for the substance inulin could performed without correction nominal optical density and/or permissible limits.

Uncertainty of the sample preparation

The requirements to maximum permissible error of volumetric glassware and instruments were used for estimation of uncertainty of sample preparation Δ_{Sp} (Table 3), which should be insignificant compared to the maximum permissible uncertainty of the analysis results (max Δ_{As}).

Tab. 3. The assessment of uncertainty of sample preparation of the quantitative determination method of the substance inulin

Operation of sample preparation	*Parameter*	*Uncertainty, %*
Weighing on the analytical balance, mg	20	0.2 mg/20 mg·100% = 1.00
Volumetric dilution, ml	50.00	0.17
Taking an aliquot, ml	2.00	0.50
Volumetric dilution, ml	25.00	0.23
		$\Delta_{SP} = \sqrt{1^2 + 0.17^2 + 0.5^2 + 0.23^2} = 1.15 \leq 1.28\%$.

The uncertainty of sample preparation is insignificant compared to the maximum permissible uncertainty of the analysis results:

$\Delta_{SP} \leq 0.32 \cdot \max \Delta_{As} = 0.32 \cdot 4.0 = 1.28\%$.

Total uncertainty of the analysis results(Δ_{As})

The prognosis of the total uncertainty of the analysis was conducted according to the data of the Table 2 (the uncertainty of the analytical operationsΔ_{FAO} = 0.49) and the formula:

$$\Delta_{As} \leq \sqrt{max\delta_{tot}^2 + \Delta_{SP}^2 + \Delta_{FAO}^2} = \sqrt{2.84^2 + 1.28^2 + 0.49^2} = 3.15 \leq 4.0\%$$

The total uncertainty of the analysis results not exceeds the maximum permissible uncertainty.

Stability of solutions

The assessment of the stability of solutions with concentration $3.20 \cdot 10^{-5}$ g/ml was performed during one hour (every 15 minutes), determining the absorbance of the test solution. The results of the research are shown in Table 4.

Tab. 4. The results of the stability study of solution

	The term of stability studyn$_t$, min (A*)					$A_{r,t}$	$S_{r,t}$	RSD_t,%	Δ_t,%	maxδ_{tot}
	0	15	30	45	60					
A	0.7489	0.7476	0.7485	0.7483	0.7511	0.7489	0.11	0.18	0.38	2.83

** The values of the absorbance is the average of three measurements of the solution.*

The results presented in Table 4 confirm that solution of inulin is characterized by stability for 1 h. The uncertainty of the absorbance of the analytical solution is Δ_t = 0.38%, and it is insignificant (Δ_t = 0.38% ≤ 2.83% = max δ_{tot}).

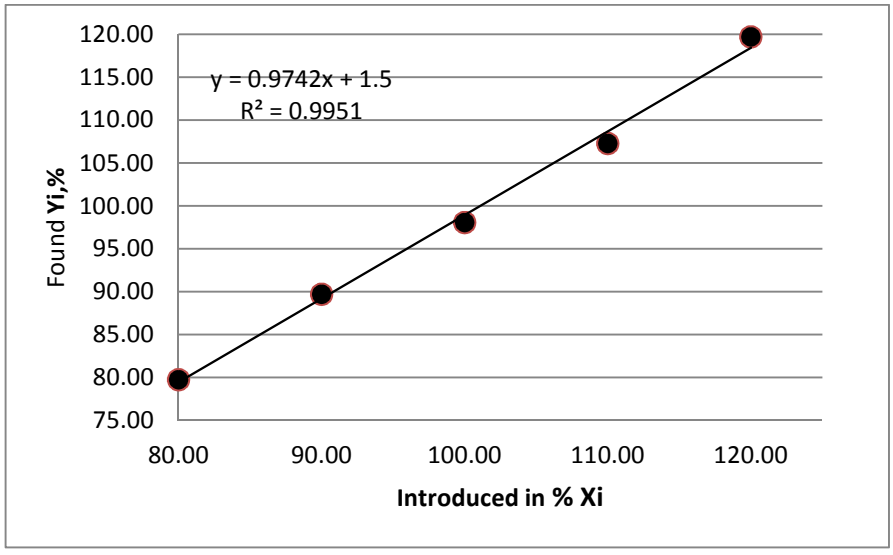


Fig. 6.The plot of linear dependence of absorbance on the concentration of inulin in the normalized coordinates

Linearity

Using 5 model solutions with the concentration $2.56\text{-}3.84 \cdot 10^{-5}$g/ml (according to the range 80% - 120%) the evaluation of linearity was performed. Absorbance of the model solutions was measured three times with removing the cuvette. Due to the fact that the concentrations and analytical signals are advisable to give in the normalized

coordinates the following values were calculated: $Y_i = (A_i*/A_{nom})\cdot 100\%$, $X_i = (C_i/C_{nom})\cdot 100\%$. The linear dependence of Y_i from X_i ($Y = b\cdot X+a$) shown in Figure 6 was plotted.

The calculated statistical values b, S_b, a, S_a, RSD_0 and r (Table 5) shows that the requirements for the parameters of the linear dependence are performed.

Tab. 5. Metrological characteristics of the linear dependence

Parameter	*Value*	*Criteria (for permissible limits of 94-102%)*	*Conclusion*
b	0.9742	–	–
S_b	0.9870	–	–
a	1.5000	1) statistical significance≤ $t(95\%;g-2)\cdot S_a = 2.37\cdot S_a = 2.90$ 2) acceptable value≤5.84	satisfied
S_a	1.2247	–	–
RSD_0	1.07	2.83	satisfied
r^2	0.9951	≥0.9942	satisfied

**The range of 80-120%, the number of points is 5.*

Accuracy

The results (Table 6) shows that the method is characterized by accuracy (the systematic error $\delta = 1.04\%$ meets the requirements of $\delta \leq 1.79\%$) and by convergence (the relative confidence interval $\Delta_{prec} = 2.27\%$does not exceed the critical value for convergence of the results $\Delta_{prec} \leq 2.83\%$).

Tab. 6. The results of the statistical evaluation of the metrological characteristics of methods of quantitative determination of inulin substances by spectrophotometry after acid hydrolysis product is 5-HMFby themethod of specific absorbance

No. of the model solution	*Accurately weighed quantity of inulin (m=0.0201g) Taking an aliquot, ml*	*Introduced in % to the concentration, X_i*	*The absorbance, A_i**	*Found in % to the concentration,Yi% ($A^{1\%}_{1cm}$=258)*	*Found in % to the introduced $Z = (Y_i/X_i)\cdot 100\%$*
1	1,60	80.00	0.5978	77.59	96.96
2	1,80	90.00	0.6880	89.28	99.21
3	2,00	100.00	0.7679	99.66	99.72
4	2,20	110.00	0.8415	109.21	99.27
5	2,40	120.00	0.9225	119.78	99.79
Mean, Z%					98.96
Relative standard deviation, RSD_x.%					1.07
Relative confidence interval, $\Delta_{prec}\% = t(95\%,4)\cdot RSD_x$					2.27
Critical value for convergence of results,$\Delta_{prec} \leq 2.83\%$					satisfied
Systematic error,$\delta = \lvert X - 100 \rvert$					1.04
Criterion of the systematic error insignificance 1) $\delta \leq \Delta_{As}/\sqrt{5} = 1.79$ 2) if it is not satisfied 1), then $\delta \leq \max\delta_{tot} = 2.83$					satisfied
The overall conclusion of the method					correct

** The values of the absorbance is the average of three measurements of the solution*

CONCLUSION

The optimal interval 90-120min of hydrolysis has been established under which a greatest amount of 5-HMF are formed.

The value of specific absorption - 258±2.86 of 5-HMF for substance inulin has been experimentally defined.

The prognosis total uncertainty of the analysis results of the assay method for inulin not exceeds the maximum permissible uncertainty $\Delta_{As} = 3.15 \leq 4.0\%$. The stability of the absorbance of the solutions of 5-HMF ($\Delta_t = 0.38\% \leq 2.83\% = \max \delta_{tot}$) at the wavelength of 285 nm for one hour has been experimentally proven. The percentage of inulin using the developed method in terms of the fructose is98.99%.

The assessment of validation characteristics obtained experimentally of the developed quantitative determination method of the inulin substance by the specific absorbance allows us to conclude that the method is correct and can be used for routine analysis, considering the simplicity of the experiment and no need for using a standard samples.

REFERENCES

[1] M.S. Alistair, O.P. Glyn, A.V. Peter, Food Polysaccharides and Their Applications, CRC Press, Taluor & Francis Group, **2014**, 752.
[2] British Pharmacopiea, British Pharmacopoeia Commission, London, Stationery Office, **2009**, Vol.3, 9160.
[3] B. Kleessen , B. Sykura, H.J. Zunft, M. Blaut, *The American Journal of Clinical Nutrition*, **1997**, 65(5), 1397.

[4] D. Özer, S. Akin, B. Özer, *Food Sci. and Technol. International*, **2005**, 11 (1), 19.
[5] M. B. Roberfroid,*J. Nutr*, **2007**, 137, 2493.
[6] J. Rohrer, Analysis of Carbohydrates by High- Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD),Thermo Fisher Scientific, Technical Note, **2012**, 20.
[7] S. N. Ronkart, C. S. Blecker, H. Fourmanoir, *Analytica Chimica Acta*, **2007**, 604 (1), 81.
[8] B. Quemener, J. F. Thibault, P. Coussement, *Lebensmitt. Wissench Technol*, **1994**, 27, 125.
[9] United States Pharmacopoeia and National Formulary, Rockvilla MD, USP Convention, **2013**, Vol. 36, 3923.
[10] A. V. Yanitskaya, I. Yu. Mitrofanova, *Journal of VolgSMU*, **2012**, 3(43), 24.
[11] N. A. Anan´ina, O. A. Andreeva, L. P. Mycots, E. T. Oganesyan, *Pharmaceutical Chemisty Journal*, **2009**, 3 (43),157.
[12] A. I. Grizodub,*Pharmacom*, **2002,** 3, 42.
[13] A. I. Grizodub, O. A. Ievtifieieva, K. I. Proskurina, *Pharmacom*, **2014**, 1, 29.
[14] A. I. Grizodub, O. A. Ievtifieieva, K. I. Proskurina, *Pharmacom*, **2014**, 2, 45.
[15] A. I. Grizodub,*Pharmacom*, **2006,** 1/2, 35.
[16] State Pharmacopoeia of Ukraine, Ukrainian scientific Pharmacopoeial center for quality of medicines, Kharkiv, Vol. 1-2. **2004**, **2008**.
[17] O. A. Ievtifieieva, K.V. Dynnik, N. N. Smelova, *Collection of scientific works of staff member of P. L. Shupyk NMAPE*, **2015**, 24, 72.
[18] O. A. Ievtifieieva, K. I. Proskurina, N. N. Smelova, Prospects for the development of scientific research in the 21st century : proceedings of the 6th International Scientific and Practical Conference, Makhachkala, **2014**, 180.
[19] O. A. Ievtifieieva, N. N. Smelova, U.M. Datkhaev, K. I. Proskurina, *Vestnik KazNMU*, **2014**, 5, 54.
[20] V. E. Tarabanko, M. A. Smirnova, Yu. V. Chelbina, M. Yu. Chernyak, *Chemistry of plant raw material*, **2011,** 1, 87.