ABSTRACT

Background: Today, there are some unresolved issues and discussions concerning inulin quantitative determination in medicinal plant raw materials (MPRM). MPRM containing polyfructans or fructosans (inulin and others) are rather complex multicomponent matrices with many interacting compounds. The article discusses the prospects for further standardization of inulin-containing pharmacopoeial MPRM that include, in addition to polysaccharides (inulin), other biologically active compounds with pharmacological activity.

Materials and Methods: Different types of search tools such as Google scholar, Google, scientific literature, normative documentation of Russian Federation (State Pharmacopoeia of Russian Federation IV edition and others) electronic databases such as e-Library, Scopus, Web of Science, Pubmed had been searched and data obtained.

Results: The pharmacopoeial spectrophotometric procedures of inulin determination in the Russian Federation are approved in a version that does not fully satisfy modern standardization criteria. Regulatory changes required in the near future.

Conclusion: Undoubtedly, to determine inulin quantitatively, it is necessary to modify the existing spectrophotometric procedures and introduce an alternative additional, more specific HPLC-RID (or similar) ones.

Key words: Inulin quantitative determination, Polyfructans, Fructosans, HPLC-RID.

INTRODUCTION

Nowadays, such diseases as diabetes mellitus, atherosclerosis, dysbiosis, and obesity occupied a prominent position despite the significant successes of medicine and pharmacy, the improvement of living conditions and nutrition of the population. These diseases lead to disability, and, as a result, to a high mortality rate. Phytotherapy is of great importance in the prevention and comprehensive treatment of such patients. Phytotherapy has several advantages over treatment with synthetic drugs. These advantages are: high pharmacotherapeutic efficacy of herbal remedies, low toxicity or lack thereof, mild action, which determines the possibility of their long-term use without the risk of adverse reactions.1,2

Inulin and fructooligosaccharides (fructosides of various polymerization degrees) are reserve polysaccharides of some plants, actively used in both the pharmaceutical and food industries; they are utilized in the production of biologically active additives and drugs. They can positively affect the metabolism, activate the pancreas function, stabilize blood glucose levels, promote the excretion of heavy metals salts and the absorption of calcium, iron, as well as provide hypcholesterolemic, immunomodulating and prebiotic effects. Currently, interest in polysaccharides, and particular inulin, has increased significantly; whereas previously polysaccharides were mainly used as excipients in the production of various dosage forms, in recent years they have been more widely regarded as biologically active compounds (BAC). Thereby, the standardization of inulin-containing medicinal plant raw materials (MPRM) seems to be very relevant and necessary.1,4

MATERIALS AND METHODS

Different types of search tools such as Google scholar, Google, scientific literature, normative documentation of Russian Federation (State Pharmacopoeia of Russian Federation IV edition and others) electronic databases such as e-Library, Scopus, Web of Science, Pubmed had been searched and data obtained.

RESULTS AND DISCUSSION

Characterization of polyfructans

Fructooligosaccharides (FOS) (oligofructose) – a mixture of oligomers, which contain from 1 to 7 moieties of D-fructose, and as a rule, terminal D-glucose. FOS is obtained in two ways: by splitting inulin and by enzymatic transfer of fructose to sucrose (transfructosylation). Oligofructose, like inulin, belongs to water-soluble dietary fiber and is prebiotic, and a mixture of these compounds, which is also often called fructooligosaccharides, has more effective prebiotic properties. Polyfructans (fructosans, Pfrus) are carbohydrate polymers formed by a sucrose molecule, an extended chain of fructose moieties. In nature, Pfrus are found in bacteria, fungi, and plants in which they perform various functions.

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functions. This class of natural polysaccharides (PSh) is produced by more than 15% of plant species belonging to different families. Pfrus are most commonly found in Liliaceae, Asteraceae, Campanulaceae, and Polemoniaceae. Structurally, Pfrus are divided into three classes, which differ in the type of bond between the moieties of fructose: the inulin group (Figure 1) – β-(2 → 1)-link; levans group – β-(2 → 6)-link; graminan group – β-(2 → 1) and β-(2 → 6)-link. Pfrus inulin groups are used to produce several dietary supplements that can positively affect metabolism, stabilize blood glucose levels and, in connection with this, partially replace antidiabetic drugs. Inulin helps to remove heavy metal salts from the body and assimilate calcium and iron, as well as activate the pancreas and affect the carbohydrate metabolism in the liver. There is evidence of the presence of inulin probiotic and immunomodulating properties.3,5

Procedures for inulin determination

Nowadays, there are many analytical methods for determining inulin, both as an individual pharmaceutical substance, and as part of medicinal plant materials and complex preparations. These methods include high performance liquid chromatography, high-performance anion exchange chromatography with pulsed amperometric detection, spectrophotometric and photocolorimetric determination methods. These methods are analyzed in great detail in the works6–9, so we will not dwell on their analysis here.

Most often, methods are used to determine the quantitative content of inulin in terms of the dominant product of its hydrolysis – fructose. However, the problems that arise during the determination are related to the selection of the optimal conditions for the process (temperature, time), the degree of hydrolysis, as well as the presence of by-products or low molecular weight fractions, which cause a significant error in most existing methods. An important role is played by the high cost of the reagents and equipment involved in the analysis, as well as the considerable complexity of the determination process.10–13

Pharmacopoeial methods of inulin analysis in MPRM

The spectrophotometric method is based on the Seliwanoff’s reaction for free fructose (Figure 2). Fructose interacts with resorcinol in concentrated hydrochloric acid when heated; the liquid becomes red gradually. The reaction is caused by an unstable compound – 5-(hydroxymethyl)furfural (HMF), forming during the oxidation of fructose. Under the influence of concentrated hydrochloric acid, the latter condenses with resorcinol, giving a colored compound. The reaction is based on the property of HMF to be formed from ketoses quite easily.14

In Russia, several types of plant material containing Pfrus of inulin-type are official: burdock roots, elecampane rhizomes and roots, dandelion roots. Next, we discuss two pharmacopoeial procedures for determining Pfrus in burdock and elecampane MPRM; only extractives are determined in dandelion MPRM.15

The structures that inulin forms (inulin clumps) are well defined by microscopic analysis (Figure 3). Dandelion root parenchyma cells are filled with colorless lumps (inulin mass forming specific bodies) and clumps (small lumps) of inulin, which dissolve easily when the slide mount is heated (cross-section of the root), and inulin is also contained in the wood parenchyma cells. Parenchymal cells of the inner part of the cortex, wood and the core rays of the burdock root contain inulin clumps (slide mount of a cross-section of the root without heating). The elecampane rhizome with roots parenchyma cortex cells contain inulin in the form of shapeless, colorless, highly refracting light lumps (the slide mount is viewed without heating).

Burdock is a herbaceous biennial plant. It is widely used in folk and official medicine; more commonly used burdock root, which has a rich chemical composition (polysaccharides, essential oils, fatty oils, tannins) and pharmacological properties. According to pharmacopeial monograph (PM) of State Pharmacopoeia of Russian Federation 2.3.0025.15 MPRM “Arctii radices – Burdock roots” are presented by collected in autumn or early spring, peeled from the remains of stems, leaves, thin roots, washed from the ground, cut into pieces and dried roots of biennial herbaceous plants of greater burdock (Arctium lappa L.), woolly burdock (Arctium tomentosum Mill.), little burdock (Arctium minus (Mill.) Bernh.) Asteraceae family (Figure 4). A decoction of the burdock roots has a diuretic, moderate choleretic and diaphoretic effects, improves mineral metabolism; it has a local anti-inflammatory and wound healing effects.15–23

According to the PM total content of polysaccharides in terms of fructose should be at least 8% in burdock roots (whole/crushed/powder). For analysis, the MPRM analytical sample is crushed to the size of the particles passing through a 1-mm sieve. About 1.0 g (accurately weighed) of the crushed MPRM is placed in a 250 ml flask with a thin section, 60 ml of water is added and heated on a tile for 30 minutes. The resulting extract is cooled to room temperature and filtered through a paper filter into a 200 ml volumetric flask, avoiding the ingress of MPRM on the filter. The extraction is repeated twice more, each time using 30 ml of water: the first time for 30 minutes, and the second for 15 minutes. The MPRM is transferred to a paper filter, the flask is washed, and then the residue on the filter is washed, using 10 ml of water each time. To the resulting extract 2 ml of 10% lead acetate solution is added, mixed and left for 10 minutes. Then 2 ml of 5% sodium phosphate disubstituted solution is added, mixed and left for 5 minutes. Then the volume of the solution is adjusted to the mark with water and mixed. The solution is filtered through a paper filter, discarding the first 10-15 ml of the filtrate (solution A). 5.0 ml of solution A is placed in a 100 ml volumetric flask, the volume of the solution is adjusted to the mark with water and stirred (solution B). In a 25 ml volumetric flask, 5 ml of 0.1% resorcinol alcohol solution, 5.0 ml of solution B are added, the volume is adjusted to the mark with 30% hydrochloric acid solution and stirred (solution C). In a 25 ml volumetric flask 5 ml of 0.1% resorcinol alcohol solution, 5 ml of water are added, the volume is adjusted to the mark

Figure 1: Structural formula of inulin.

Figure 2: Seliwanoff’s reaction.
with 30% hydrochloric acid solution and stirred (reference solution). The flasks with the reference solution and solution B are heated in a water bath at a temperature of 80 °C for 20 minutes, cooled; the volume of extracts in the flasks is adjusted with the same solvent to the mark. The optical density of solution B is measured on a spectrophotometer at a wavelength of 482 nm in a cuvette with a 10 mm layer thickness in comparison to the reference solution. The total polysaccharides content in terms of fructose is calculated using the specific absorption coefficient of the reaction products of fructose with resorcinol in an acidic medium, in absolutely dry MPRM (X) according to the formula:

\[ X = \frac{A_{\text{opt}} \cdot 200 \cdot 100 \cdot 25 \cdot 100}{A_{15\%} \cdot a \cdot 5 \cdot 5 \cdot (100 - W)} \]

where \( A_{\text{opt}} \) – the optical density of solution B; \( A_{15\%} \) – specific absorption coefficient of the reaction products of fructose with resorcinol in an acidic medium at a wavelength of 482 nm, equal to 298; \( a \) – sample mass of MPRM, g; \( W \) – moisture content in MPRM, %.\(^{15}\)

Elecampane is a perennial herb with yellow flowers up to 1.5 meters high. In official medicine, rhizomes and roots are used; it contains polysaccharides (inulin), essential oils, bitter glycosides, saponins, etc. According to the PM “Elecampane rhizomes and roots – Inulae helenii rhizomata et radices”, the MPRM is presented by collected in autumn, peeled from the remains of aerial parts and ground, dried rhizomes and roots of a wild-growing and cultivated perennial herbaceous plant of Elecampane (Inula helenium L.), Asteraceae family (Figure 5).

The infusion of elecampane rhizomes and roots has an expectorant effect.\(^{15,16,24-28}\) The PM includes the section "Determination of the main groups of biologically active substances", where a qualitative reaction for inulin is presented. Orange-red coloring (inulin) should be observed when applying specific reagent to a cross-section of elecampane MPRM (whole or powder), Figure 6. It includes 0.1 ml of 20% thymol alcohol solution and 0.05 ml of concentrated sulfuric acid. Blue staining should not be observed (absence of starch) when applying specific reagent (0.1 ml of iodine solution) to elecampane MPRM (whole or powder).

According to the PM, the total content of fructosans and fructose in terms of inulin should be at least 25% in elecampane MPRM (whole/crushed/powder). For analysis, an analytical sample of the MPRM is crushed to the size of the particles passing through a 1 mm sieve. About 1.0 g (accurately weighed) of the crushed material is placed in a 250 ml flask, 60 ml of water is added and heated in a boiling water bath for 45 minutes, then cooled at room temperature for 5 minutes. The resulting extract is filtered through cotton wool into a 200 ml volumetric flask avoiding the ingress of MPRM on the filter. The flask is washed with 10 ml of water and flushing water is filtered into the same volumetric flask. The extraction with water is repeated twice more (the first time it is heated for 45 minutes with 30 ml of water, the second time – 15 minutes with 30 ml of water), the extract is filtered into the same volumetric flask. Then the MPRM is transferred to cotton wool, the flask is washed with 10 ml of water, flushing water is filtered through the cotton with another 10 ml of water. Cotton wool with MPRM is squeezed. 1 ml of 10% lead (II) acetate solution is added to the resulting flask in a volumetric flask, stirred and left for 10 minutes. Then 2 ml of 5% disodium hydrogen phosphate anhydrous solution is added to the flask, stirred and left for 10 minutes. Then the volume of the solution in the flask is adjusted to the mark with water and mixed. The contents of the flask are filtered through a paper filter, discarding the first 10-15 ml of the filtrate. 2 ml of the filtrate is placed in a 100 ml volumetric flask, the volume of the solution is adjusted to the mark with water and stirred (solution A of the test solution). 5 ml of 0.1% resorcinol alcohol solution and 10 ml of 30% hydrochloric acid are placed in each of two 50 ml conical flasks. Then, 5 ml of solution A of the test solution is...
added to the first flask and 5 ml of water to the second flask (reference solution A). Both flasks are heated in a water bath at a temperature of 80 °C for 20 minutes, then cooled to room temperature. The contents of the flasks are quantitatively transferred to the 25 ml volumetric flasks and the volume of the solutions in them is adjusted to the mark with 30% hydrochloric acid, mixed (solution B of the test solution, reference solution B). After 15 minutes, the optical density of solution B is measured on a spectrophotometer at a wavelength of 483 nm in a cuvette with a 10 mm layer thickness in comparison to the reference solution B. The total content of fructosans and fructose in terms of inulin in absolutely dry MPRM (X) is calculated by the formula:

\[
X = \frac{A_{1\%} - \alpha \cdot 2 \cdot 5 \cdot (100 - W)}{A_{1\%} \cdot \alpha \cdot (100 - W)} = \frac{A \cdot 5000000}{A \cdot 10000000}
\]

where \( A_{1\%} \) is the optical density of solution B of the test solution, \( A_{1\%} \) is the specific absorption coefficient of the reaction products of the interaction of inulin with resorcinol in an acidic medium, equal to 498; \( \alpha \) – sample mass of MPRM, g; \( W \) – moisture content in MPRM, %.

Dandelion is a widespread weed; it is a perennial herbaceous weed, up to 50 cm high. The dandelion has a short rhizome and a fleshy, spindle-shaped root. In official medicine, roots containing polysaccharides (inulin), bitter glycosides, triterpene compounds, and other BAC are used. According to PM "Dandelion roots – Taraxaci radices", MPRM is presented by collected in the autumn (August-September), peeled from the collet, washed from the ground and dried roots of the wild perennial herb of the dandelion (Taraxacum officinale Wigg.), Asteraceae family (Figure 7). A decoction of dandelion roots has a choleretic effect, increases appetite, enhances the secretion of gastric juice, increases appetite, enhances the secretion of gastric juice.

The PM includes the section “Determination of the main groups of biologically active substances”, where a qualitative reaction for inulin is presented. When applying the iodine solution to the root cortex or powder, there should be no blue color (absence of starch). A root scraping or powder should be colored in a purple-pink color (inulin) or powder, there should be no blue color (absence of starch). A root is presented. When applying the iodine solution to the root cortex.

The determination of inulin in mixture herbal products (herbal teas)

The determination of inulin was also carried out in the mixture herbal products (MHP). An example is a recipe for a new expectorant collection “Lorpolphyt” MHP (species “Lorpolphytum”) which includes the aerial parts of medicinal plants. It contains plantain leaves, chamomile and calendula flowers, horsetail, yarrow, and Saint-John’s-wort herbs, elecampane rhizomes and roots. The authors established the optimal conditions for the extraction of the total content of fructosans and fructose in terms of inulin in MHP: MPRM fineness – 1 mm, the extractant – hot purified water, the ratio of raw material and extractant – 1:50, the extraction time – 60 and 30 minutes in a boiling water bath with reflux condenser.

According to the procedure, MPRM analytical sample is crushed to the particle size passing through a 1 mm sieve. About 2.0 g (accurately weighed) of the crushed MHP is placed in a 100 ml round bottom flask with a thin section, 60 ml of water is added, heated in a boiling water bath for 1 h with the reflux condenser. Then it is cooled at room temperature for 5 min; the resulting extract is filtered through cotton wool into a 100 ml volumetric flask avoiding the ingress of MPRM on the cotton wool. The extraction is repeated with 30 ml of water, heating for 30 minutes, then cooled at room temperature for 5 minutes and filtered through the same filter into the same flask. MPRM and filter are washed with 5 ml of water; the cotton wool with raw materials is squeezed. To the resulting extract, 2 ml of a 10% lead acetate solution is added in a 100 ml flask; the solution is mixed and left for 10 minutes. Then 4 ml of 5% sodium phosphate solution is added, mixed, left for 5 minutes. The volume of the solution in the flask is adjusted to the mark with water and mixed. It is filtered through a paper filter, discarding the first 10-15 ml of the filtrate. 5 ml of the subsequent filtrate is placed in a 100 ml volumetric flask, the volume of the solution is adjusted to the mark with water and stirred (solution A).

In 2 conical 50 ml flasks, 5 ml of a 0.1% resorcinol alcohol solution and 10 ml of 30% hydrochloric acid solution are added. 5 ml of solution A (test solution) is added to the first flask, 5 ml of water (reference solution) – to the second. Both flasks are heated in a water bath at a temperature of 80 °C for 20 minutes, cooled to room temperature. The contents of the flasks are quantitatively transferred to 25 ml volumetric flasks. The volumes of the solutions in the flasks are adjusted to a mark with a 30% hydrochloric acid solution, and mixed. The optical density of the test solution is measured using a spectrophotometer at a wavelength of 483 nm (Figure 8) in a cuvette with a 10 mm layer thickness in comparison to the reference solution. The total content of fructosans and fructose (X) in terms of inulin and absolutely dry MPRM is calculated by the formula:

\[
X = \frac{A \cdot 100 \cdot 100 \cdot 25 \cdot 100}{498 \cdot m \cdot 5 \cdot 5 \cdot (100 - W)} = \frac{A \cdot 1000000}{498 \cdot m \cdot (100 - W)}
\]

where \( A \) – optical density of the analyzed solution; 498 – specific absorption coefficient of the reaction products of inulin with resorcinol in an acidic media; \( m \) – mass of a MPRM sample, g; \( W \) – moisture content in MPRM, %.

The total content of fructosans and fructose in the collection samples ranged from 3.63 to 4.13%.

Determination of indicator for the BAC content in MPRM

What is interesting is that there is no single terminology and methodology in the pharmacopoeial monographs of the State Pharmacopoeia of the Russian Federation. Two methods are completely identical (there are differences) in their presentation, but there are various terms for this indicator: “total content of fructosans and fructose in terms of inulin” and “total polysaccharides content in terms of fructose”. The spectrophotometric method is rather nonspecific, to a greater extent it is not an absolute, but a relative indicator. The total content of PSh in terms of inulin and the content of the inulin substance itself are significantly different. Nevertheless, it is not possible to refuse this procedures in pharmacopoeial analysis at this stage of development of science and technology due to the low availability of instrumentation for control and analytical laboratories in the Russian Federation. It is necessary to determine what is sufficient for standardization purposes, the total content of all carbohydrates or the inulin content. It should be mentioned that low molecular weight compounds can be removed by

Figure 7: Dandelion roots.
reaction were also selected; studies were carried out for optimization of
the total content of polyfructans in terms of fructose in absolutely dry
optical density of the solution is determined at a wavelength of 480 nm;
hydrochloric acid are added to an aliquot of the obtained extraction
solution, 1% resorcinol solution, 95% ethyl alcohol, concentrated
is repeated under the same conditions 2 more times. A 0.01% thiourea
heated in a boiling water bath for 60 minutes. The extraction of MPRM
conditions 2 more times. Water is added to the purified MPRM and
extract is filtered. The extraction of MPRM is repeated under the same
ethyl alcohol in a boiling water bath for 60 minutes. After cooling, the
MPRM analytical sample is crushed to the size of the particles passing
purification with high concentration alcohols (ethanol 90-95%).
Based on the foregoing, it seems appropriate to unify the procedures
for the quantitative determination of polysaccharides (polyfructans). It
is necessary to suggest several methods for their determination: one
is spectrophotometric, the other is HPLC-RID (or similar). In the
monograph on inulin-containing MPRM, it is recommended to include
two quantitative determinations on polysaccharides (inulin) and the
target group of BAC (essential oil, bitter glycosides, etc.).
Development of modern spectrophotometric methods
for the analysis of inulin in MPRM
It is important to note that the MPRM of inulin-containing plants
includes other resorcinol-positive components: fructose, sucrose
and several oligosaccharides, which can interfere with the PSh
determination. To eliminate this drawback, the authors proposed a
preliminary extraction of the raw material with 95% ethyl alcohol, in
which high molecular weight fructans are insoluble. For a quantitative
analysis of official MPRM – burdock roots, a spectrophotometric
method for determining the content of Pfrus using a modified resorcinol
method for determining ketoses is proposed. The metrological analysis
of the procedure showed that it is reproducible and has a linearly
varying error with a value of no more than 2% in the field of working
concentrations.38
Similar experiments were carried out for the roots of burdock and
elecampane. The optimal parameters for sample preparation of
burdock roots are established. Preliminary extraction with 95% ethyl
alcohol is carried out three times at 100 ° C, the target extraction is
carried out with water, the finesse degree of MPRM is 0.5-1.0 mm.
The procedure of quantitative determination of the total content
of Pfrus in terms of fructose in the burdock roots is as follows. An
MPRM analytical sample is crushed to the size of the particles passing
through a 1 mm sieve. A portion of the MPRM is extracted with 95%
ethyl alcohol in a boiling water bath for 60 minutes. After cooling, the
extract is filtered. The extraction of MPRM is repeated under the same
conditions 2 more times. Water is added to the purified MPRM and
heated in a boiling water bath for 60 minutes. The extraction of MPRM
is repeated under the same conditions 2 more times. A 0.01% thioareua
solution, 1% resorcinol solution, 95% ethyl alcohol, concentrated
hydrochloric acid are added to an aliquot of the obtained extraction
and heated in a boiling water bath. The mixture is cooled, diluted, the
optical density of the solution is determined at a wavelength of 480 nm;
the total content of polyfructans in terms of fructose in absolutely dry
MPRM is calculated by the same principle as described above.
For elecampane MPRM, the optimal conditions for the analytical
reaction were also selected; studies were carried out for optimization of
the reaction mixture composition. It should include 15% hydrochloric
acid, 52% ethanol to achieve maximum optical density. The addition
of thiourea slows down the process of destruction of the analytical
component. Inulin has partial solubility in aqueous solutions of ethanol
of various concentrations; 50% solubility is already observed for 61-
65% ethanol solutions. Therefore, for MPRM preparation, it is correct
to use only 95% extractant solutions, which, with 3-fold extraction, can
almost completely remove low molecular weight carbohydrates.29
CONCLUSION
Preparations obtained from inulin-containing plants have a very
diverse effect on the human body. Inulin is an integral part of water
e xtracts obtained from the raw materials of dandelion, elecampane,
burdock, which are pharmacopeia plants in the Russian Federation
and other countries. It is impossible to overestimate the relevance of
expanding research on inulin taking into account all the areas of this
polyfructosan application in modern medicine and pharmacy. In this
regard, it seems appropriate to justify the possibility of medical use of
these plants MPRM as sources of inulin-containing biologically active
complexes. Inulin is a pharmacologically active component of the
raw material and can expand the indications for the use of dandelion,
burdock and elecampane preparations. This also directly relates to
quantification methods. Unfortunately, pharmacopeia analysis of raw
materials containing inulin does not fully satisfy all the contemporary
requirements for herbal drugs quality control. This means that the
development of new or improvement of existing methods remains
an urgent task of modern medicine and pharmacy in the Russian
Federation.
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CONFLICTS OF INTEREST
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