MINISTRY OF HEALTHCARE OF THE REPUBLIC OF UZBEKISTAN

THE TASHKENT PHARMACEUTICAL INSTITUTE

On the rights of manuscript

UDC 611.123:547.672:544.46

BEGIMOVA DILNORA ISMAILOVNA

Development of technology immunostimulator preparations based on St. John's Wort (*Hypericum scabrum* L.)

5A510602 – Technology of immubiological and microbiological preparations

D I S S E R T A T I O N
for the masters of academic degree

Supervisor:

Dr. M.G. Ismailova

Scientific co-supervisor:

PhD T.F. Ibragimov

Tashkent – 2016

CONTENT

ABBREVIATIONS

AC – Ante Christum

Al – Aluminium

API – Active pharmaceutical ingredient

approx. – approximately

BC – Before Christ

BP – British Pharmacopoeia

cm – cantimeter

CNS – Central nervous system

CRS – Common Reporting Standard

DMA – Dimethylformamide

EP – European Pharmacopoeia

Fig. – Figure

FV LLC – Foreign venture Limited liability company

g – gram

GC – Gas chromatography

GP – Government Pharmacopoeia

h – hour

HIV – Human immunodeficiency virus

HPLC – High performance liquid chromatography

HPMC – Hydroxipropyl methyl cellulose

i.e. – it est

IP – Initial process

JV LLC – Joint venture Limited liability company

l – liter

MCC – Microcrystalline cellulose

min – minute

mg – milligram

ml – milliliter

mm – millimeter

M_r – molecular weight

NLT – Not less than

nm – nanometer

NMT – Not more than

p. – page

PE – Polyethylene

PE – Private enterprise

ppm – Parts per million

PSPE – Privately science produsing enterprise

PVC – Polyvinyl chloride

PVDC – Polyvinylidene Chloride

R – Reagent

RH – Relative humidity

sec – second

TLC – Thin layer chromatography

TP – Technological process

USP – United States Pharmacopoeia

UV – Ultraviolet spectroscopia

V – Volume

μl – microliter

μl – micrometer

INTRODUCTION

Base of theme dissertation and importance of them. After obtaining of independence, the pharmaceutical industry in Uzbekistan was practically undeveloped, especially in the beginning of independence years. Population was supplied by only 10-15% of medicines by government yearly, other providers was foreign companies. That's why developing of pharmaceutical industry was planned carry out by means of modernization in force and creation of new pharmaceutical attended manufactures according to request of international standards. In order to promote emergence of new manufacturing companies, there were come to a lot of decisions, they are Resolution # PP-731 "On Programme of Modernization, Technical, and Technological Re-tooling of Companies in the Pharmaceutical Sector up to 2011" of the President of Uzbekistan dated 19.11.2007, Resolution # PP-1319 "On Measures for Streamlining Licensing of Pharmaceutical and Medical Practices" of the President of Uzbekistan dated 07.04.2010, the state Programme "On priorities of the industrial development of the Republic of Uzbekistan for 2011-2015" approved by the Resolution of the President of the Republic of Uzbekistan # PP-1442 dd. 15.12.2010, the Annex # 9 to the Resolution of the President of the Republic of Uzbekistan # PP-1668 dd. 27.12.2011 "On the Investment Programme of the Republic of Uzbekistan for 2012". The programs contained complex arrangements in developing pharmaceutical industry in Uzbekistan [1-4].

Nowadays pharmaceutical industry is developing with increased speed in Uzbekistan. Actual proof there is opening new local industrial pharmaceutical companies in the Republic: the FV LLC «NOBEL PHARMSANOAT», the PSPE «RADIKS», the JV LLC «NOVA PHARM», the JV LLC «REMEDY GROUP», the PE «DENTAFILL PLUS» and others. In addition, at the same time with increasing producers it is increasing number of new drugs. Many medicines of these factories are made base on imported substance or in-bulk medicines, practically there are more less factories, which manufactured active

substance for drugs. Foregoing aim of programs was to solve important problem which manufacturing domestic substances and medications. Besides on Uzbekistan is a rich natural plant country to produce original medications and biologically active additives using local raw materials. That's why one of the important our task to make new drugs with using herbal plants which learned by Abu Ali Ibn Sina and to raise assortments of herbal medicines based on local standardized dry extracts [5,6].

In recent years, interests for the herbal plant St. John's Wort have been increasing. The traditional uses of St. John's Wort include treatment for bacterial infections, respiratory conditions, skin wounds, peptic ulcers, and inflammation, immunomodulatory action and others. St John's Wort contains a wide range of different natural product classes. Best known are the naphthodianthrones, with hypericin and pseudohypericin as main compounds, and the prenylated phloroglucinols group containing hyperforin. Moreover, typical natural product groups comprise xanthones, flavonoids, and biflavonoids [7].

St. John's Wort boosts the immune and endocrine systems, which makes it an even more powerful antidepressant. Studies have shown that there is a link between how we feel emotionally and how well our immune system is functioning. A depressed person's immune system generally does not function well partly due to the overproduction of interleukins, an immune system messenger (communicator between cells). St. John's Wort decreases the production of interleukins. It calms an overactive immune system bringing back into balance and the symptoms clear up. St John's Wort acts on the brain and nervous system, boosts the immune system and relieves the physical symptoms associated with depression [8].

Determining object and subject of research. The research objects are two type of St. John's Wort, they are *Hypericum perforatum* L. and *Hypericum scabrum* L. Based on these plants we obtained at first dry extract of St. John's Wort *Hypericum perforatum* L. and *Hypericum scabrum* L., then we prepared

liquid extract, “St. John’s Wort” 300 capsules, “St. John’s Wort” 300 tablets only by type of St. John’s Wort *Hypericum scabrum* L. These preparations (liquid extract, “St. John’s Wort” 300 capsules, “St. John’s Wort” 300 tablets) are subjects of research.

The purpose of the study. Development of technology preparations based on dry extract of *Hypericum scabrum* L. which grows in Uzbekistan for stimulate immune system and for depression.

For achievement, setting purpose solves these tasks:

1. Researching of types of St John’s Wort *Hypericum perforatum* L. and *Hypericum scabrum* L. by chemical compositions and comparing them;
2. Development of technology obtaining dry extracts of St John’s Wort *Hypericum perforatum* L. and *Hypericum scabrum* L. and comparing them;
3. Standardization of the obtained dry extracts;
4. Development of technology obtaining liquid extract of “St John’s Wort” 300, capsules of “St John’s Wort” 300, tablets of “St John’s Wort” 300 based on *Hypericum scabrum* L.;
5. Standardization of the obtained preparations;
6. Researching of stability of capsules.

Scientific novelty. Development of technology dry extract of St. John’s Wort based on the type of *Hypericum scabrum* L. which grows in Uzbekistan has been carried out for the first time and has been compare with widely spread type of *Hypericum perforatum* L. In addition, these extracts have been standardized by sum hypericins, hyperfoorin and sum flavonoids for the first time with contemporary and exactly method of HPLC.

Developments of technology liquid extract of St. John’s Wort, “St. John’s Wort” 300 capsules, “St. John’s Wort” 300 tablets based on these dry extract of *Hypericum scabrum* L. have been carried out for the first time and these preparations have been standardized by sum hypericins, hyperforin and sum flavonoids for the first time with contemporary and exactly method of HPLC.

Stability of capsulated form “St. John’s Wort” 300 has been examined for the first time.

Primary decisions and suppositions of research: development of technology local substance dry extract of St. John’s Wort which grows in territory Uzbekistan for make preparations for stimulating immune systeme and for depression.

Materials and methods. Objects of research are two type of St. John’s Wort *Hypericum perforatum L.* and *Hypericum scabrum L.* and obtained dry extract, liquid extract, tablets and capsules based on *Hypericum scabrum L.* are subjects of research.

Different methods of extraction: solid-liquid extraction, liquid-liquid extraction, maceration, percolation, obtaining liquid extract by diluting dry extract; methods of solid drugs: direct compression, wet granulation, dry granulation; methods standardization: TLC, HPLC, GC; self-life stability.

The practical significance. Preparation of herbal forms (liquid extract, tablets and capsules) on the basis of St. John’s Wort type of *Hypericum scabrum* L. which grows and widespread in Uzbekistan are allow to improve assortments of herbal, immumodulatory and antidepression medicines in the national markets. Especially using domestic medicinal herbs is of the great practical importance for Republic of Uzbekistan.

Volume and structure of master dissertation. Dissertation material found on 77 pages of typescript. Text of work includes Introduction, Review of literature data (Chapter I), Main part (Chapter II), Development of technology and standardization of dry and liquid extracts, capsules and tablets basen on St. John’s Wort (Chapter III), Bioecvivalence of capsulated form “St. John’s Wort” 300 (Chapter IV), Stability of capsulated form of St. John’s Wort (Chapter V) and conclusions. The work contains 11 figures and 22 tables. Literature list consist of 22 domestic and 86 foreign sources of literature.

Chapter I. LITERATURE REVIEW

1. History of St. John's Wort plant

St. John's Wort is the one of the unique herbal plant, which is widely used in folk medicine and modern medicine for treatment of different diseases.

St. John's Wort has been used as an herbal remedy for its anti-inflammatory and healing properties since the Middle Ages [9]. *Hypericum perforatum L.* was the first known in antiquity as a plant with supernatural properties and later as a medicinal herbal drug. The generic name "hypericum" derives from Greek word "hyper" (above) and "eikon" (a figure, possibly an unwanted apparition) and means "over an apparition", which relates to the ancient use of the plant to exorcise evil spirits or influences. It have been placed over religious icons as a symbol of protection. The second term, "perforatum" is derived from the Latin "perforated" because the leaves, when held to the light, reveal translucent dots or small holes, giving the impression that the leaf is perforated. The popular name Saint John's Wort (old English "wort" means plant) is related to its yellow flowers traditionally gathered for the feast of St. John the Baptist (24th of June), and ''wort'' is the old English word for plant [10].

St. John's Wort is traditionally used both externally and internally with many therapeutic applications, including its uses as a vulnerary, diuretic, and in the treatment of euralgic conditions, sciatica and poisonous reptile bites. The use of its top flowering parts was originally documented by ancient Greek medical herbalists Hippocrates (ca. 460-377 B.C.), Theophrastus (ca. 372-287 B.C.), Dioscorides (first century), and Galen (ca. 130–200).

Theophrastus and Galen wrote about the medicinal properties of St. John's Wort, noting its use as a vulnerary (wound healing) and for treatment of neuralgic conditions such as sciatica and hip pain. Mattioli wrote about its usage as an emmenagogeu, diuretic and antimalarial. Dioscorides in his "Materia Medica" wrote that St. John't Wort is helped to burning wound.

Since the time of the Swiss physician Paracelsus (ca. 1493–1541), it has been used to treat psychiatric disorders, and was described as "arnica for the nerves", and it has been used in traditional European medicine for centuries to treat neuralgia, anxiety, neurosis, and depression [11].

In the nineteenth and twentieth centuries St. John't Wort was used to treat hysteria and nervous affections with depression. It was also prescribed externally to treat wounds, bruises, sprains, and other conditions [12,13].

In Central Asia one of the scientists which learned St. John's Wort was Avicenna (Abu Ali ibn Sina). The plant was included to "Al-Qanoon fi al-Tibb" ("The Canone of Medicine") by Avicenna in 11th AC. He called this plant Ivfarikun in his book - "The Canon of Medicine". He wrote: "St. John't Wort increases secretion of urine, if drink it during forty days contract, it cures inflammation of nerve. When use it in internal diseases, it stops for four days fever". In addition, he wrote about it properties and effects: "rarefying, tinning, and resolving". And about effect for tumor and acne: "Helped in cold tumor and large harden". About effects of wounds and ulcers: "Medicinal bandage formed from leaves helps for burn with fire and healed up large wounds and malignant ulcers. If pounded leaves to sprinkle to flabby and rotten ulcers, it will bring a benefit". In tool with joint: "Boiled St. John's Wort in wine, helped from hurt of leg and from inflammation of nerve, especially if drink it forty days contract". Ibn Sina learned side effects of this plant too: "If take those (seeds) more, they will kill". He recommended seeds, but a bit quantity and he said: "Seeds of St. John's Wort produces congestion, assigns and helps more to neck" [14-18].

More than sixteen of 50 species of *Hypericum* were widely used in Chinese traditional medicine to possess antimicrobial, antidotary, emenagogue properties, and ability to treat hepatitis and improve blood circulation. In Turkish folk medicine, an aqueous decoction prepared by boiling of flowering plants *Hypericum perforatum* L. which is used as a remedy to treat peptic ulcer and prostatitis.

In ancient Russia this herb was considered as a cure for 99 illnesses. According to Russian national doctors: “As without flour it is impossible to bake bread, so without St. John’s Wort it is impossible to treat many illnesses of people and animals”.

Traditionally, dry herbs used for preparation of teas as a tonic and to improve function of secretory glands, and gastrointestinal tract. The infusion of the herb was heavily utilized against cough, heart failure, headaches, flu, sore throat, rheumatism, bed-wetting, liver failure, and to treat wounds and ulcers. [19].

The American Indians traditionally used dried plant as a meal, and fresh leaves for their soothing effect. The herb was used to cure bronchitis and to overcome bed-wetting in children.

Gerard (1597), the famous English herbalist, referred to a St.John’s Wort salve, which he formulated using the herb, as the best and most precious natural wound-healing therapy available. In his own words: “Saint John’s Wort with his flowers and seed boiled and drunken, provoketh urine, and is right good against the stone in the bladder, and stoppeth the laske. The leaves stamped are good to be laid upon burnings, scaldings, and all wounds; and also forrotten and filthy ulcers”.

Among the first most effective and widely spread pharmaceutical uses of St. John’s Wort in Europe after the 16th century was the use of the distilled oil of the herb as a therapy for wounds and bruises. It was so effective that surgeons not only used it to clean wounds but also included it in the first official pharmacopoeia of London as Oleum Hyperici.

In 1633, Gerard’s Herbal recorded the plant’s use as a balm for burns, wounds, ulcers and bites and its oil was also popularly used during this time.

Culpeper (1652) pointed out the unique wound healing properties of the herbal ointment. He also claimed the beneficial properties of the herb against stings and bites of poisonous animals. According to him: “It is a singular wound herb, healing inward hurts or bruises, it opens obstructions, dissolves swelling

and closes up the lips of wounds. It is good for those who are bitten or stung by any venomous creature and for those that cannot make water" [20].

Pharmacological learning of St. John's Wort was begun by German scientist Buhner in 1830. He isolated one of the active substances from St. John's Wort plant and he called it red hypericin. Based on this substance it had been prepared medical oil, which saves in many illness. At decreasing content this oil, it occurs destruction of pathogenic microbe and on the contrary, useful bacteria's are kept. In 1911 this active substance was identified and called barely hypericin, and in 1953 it was finally interpreted its chemical structure. Hyperforin was evolved from St. John's Wort only at the end of 1960 by Russian chemist Kolosov M.N. St. John's Wort preparations were included in Russian Pharmacopoeia since 1968. Presently, the rediscovery of this very old herbal remedy takes place, proving the tremendous pharmacological properties of this plant [21].

2. Chemical composition of St. John's Wort (*Hypericum perforatum L. and Hypericum scabrum L.*) and their pharmacological properties

St. John's Wort contains a wide spectrum of different biological active components, including six major natural product groups: naphthodianthrones, phloroglucinols, flavonoids, biflavones, phenylpropanes, proanthocyanidins. Besides these there are another components included: either oils, other chemical compounds (xanthones (1,3,6,7-tetrahydroxyxanthone and kielcorin C, acids, hydroperoxycadiforin, bisanthraquinone glycosides) [22-36]. Chemical structure and their activities are shown in Table 1 and Fig.1 (annex, table 1, p.97, fig.1, 113).

Naphthodianthrones. Naphthodianthrones occur in St. John's Wort in concentrations of less than 0.1% to 0.15%. The best known naphthodianthrone components are hypericin and pseudohypericin (also emodin-anthranol and cyclo-pceudohypericin). Isohypericin and protohypericin are also present. The red pigments hypericin and pseudohypericin are found in a concentration

ranging from 0.3% to 0.33%, depending of harvesting period, drying process, and storage. Hypericin content also varies widely among growing regions and concentration varies among plant parts: flowers, buds, top leaves, and secondary stems yield the highest amount.

Taking internally, hypericin plays the specific role as a catalyst of some intracellular reactions, and important metabolic processes of the organism. Hypericin is believed to act as an antibiotic, antiviral and non-specific kinase inhibitor.

Recently, great interest has focused on the antiviral activity of hypericin and pseudohypericin, which affects enveloped viruses such as herpes simplex virus, cytomegalovirus, and human immunodeficiency virus type 1 and stabilizes immune system. Furthermore, the use of hypericin as an inactivator of retroviruses in blood products has been suggested, and it may also be useful as an anticancer drug. Thanks to the biological active compounds of St. John's Wort (mainly hypericin), the plant can use for complex treat some types of cancer. Hypericin is promoted kill cells of cancer.

Although antidepressive therapy with hypericin and pseudohypericin has been well established, little is known about their pharmacokinetics and their therapeutic indices even though preliminary studies on the antiviral efficiency in humans have been conducted.

The naphthodianthrones show a restricted solubility in almost all solvents; the pure compounds, especially hypericin, are almost insoluble in water at ambient temperature. Nevertheless, more than 40% of the naphthodianthrone amount is extractable from the crude drug when preparing a tea with water at 60 to 80°C (approx. 35% pseudohypericin and 6% hypericin). The increase in solubility suggests the presence of coeffectors in the drug material that modify the solubility of the naphthodianthrones. The potassium salts of hypericin and pseudohypericin have been identified as "soluble" pigments of the Hypericum species [37].

Hypericin is also a photosensitizing substance; i.e. produces singlet oxygen upon exposure to visible light. The toxic action of hypericin occurs from absorption the pigment from the intestine and concentration near the skin. After the exposure to direct sunlight, the photodynamic hemolysis takes place. Hypericin does not show particular toxicity in the absence of sunlight. No cases, involving human toxicity have been reported [38, 39].

Hyperican and Pseudohyoericin have been shown in clinical trials to have antiviral effects on Herpes simplex I and II, Influenza, and rabies virus. There tends to be blocking processes of the virus within a body cell and/or they may directly inactivate the maturing virus cells.

Because of these compounds, St. John's Wort extract is being tested in HIV treatment. St. John's Wort is taken in large doses so that the blood is saturated with hypericin and then begins to infiltrate other tissue. To activate the hypericin after it enters the body tissues requires sunlight. It is believed that the retrovirus will begin to diminish in the HIV infected blood.

A promising use of St. John's Wort is in the area if cancers and tumors due to its outstanding ability to work at the cellular level against destructive invaders like virus and bacteria as well as against cancerous cells and tumors of varying kinds. Hypericin has tumor targeting qualities in combating cancerous cells [40-44].

Phloroglucinols. Two closely related compounds found in St. John's Wort are hyperforin, at 2.0% - 4.5% of the main phloroglucin content and adhyperforin (0.2% - 1.9%) which contains an additional methyl group.

These are found only in the reproductive parts (about 2% in the flowers, 4.4% in the ripe fruits and 4.5% in the unripe fruit). However, traditional preparations such as teas and tinctures of the herb contain little or no hyperforin. Furohyperforin, the major oxidation product of hyperforin, occurs in the aerial parts at about 5% of the hyperforin concentration.

Although hyperforin is quite unstable – especially in aqueous solutions and when exposed to light and heat – it is present in many commercial extracts at concentrations of 0-6%.

The hyperforins of St. John's Wort present very interesting compounds from a pharmacological standpoint, although their pharmacological activities are as yet little known. This supports the possible role of hyperforin in the antidepressant activity of the herb. Hyperforin also possesses antimalarial activity and was found to be active against Plasmodium falciparum.

Hyperforin belongs to group of compound known as acylphloroglucinols. The hyperforin has also proved to be difficult to synthesize, making St. John's Wort, the only plant containing high concentrations of the chemical, its only commercially viable source [45-49].

Flavonoids. Flavonoids comprise the major group of biologically active compounds in St. John's Wort (2-4%). The flavonol aglycones identified so far include kaempferol, luteolin, myricetin and quercetin. Hyperoside (hyperin) and rutin usually dominate among the glycosides of St. John's Wort followed by quercitrin and isoquercitrin.

Flavonol glycosides were shown to possess spasmolytic activity. These compounds also inhibit monoamine oxidase A, the enzyme responsible for the catabolism of biogenic amines. The greatest activity for the flavonoid aglycones – quercetin, kaempferol and luteolin – whereas the glycosides were less active. However, the level of flavonoids present in St. John's Wort is too low to be responsible for the therapeutic efficacy of the crude herb. Rutin was shown to be essential for the antidepressant activity of St. John's Wort extracts [50].

Biflavones. Biflavones are a rare group of dimeric flavones found in some vegetable sources. Three biflavones detected in St. John's Wort are 3, 8-biapigenin (0.1-0.5%), amentoflavone (0.01-0.05%) and 6, 8-diquercetin. The therapeutic significance of these biflavones in St. John's Wort is still unknown.

Amentoflavone, however, was shown to possess anti-inflammatory and analgesic activities.

Phenylpropanes. These compounds mainly occur as esters of hydroxycinnamic acids, such as p-coumaric acid and caffeic acid. Chlorogenic acid has been detected in St. John's Wort extract at concentrations below 1%. Its role in the pharmacological effects of St. John's Wort is unknown.

Proanthocyanidins. These compounds are represented by tannins, catechin, epicatechin, procyanidin B_2, cyanidin, protocatechuic acid. Their total concentration ranges from 2-4% with a maximum concentration at the preflowering stage. The dimeric procyanidin has been isolated from the plant together with additional dimeric, trimeric, and tetrameric procyanidins.

The various biological effects of proanthoc yanidins include antioxidant, antiviral, and antimicrobial, but no antidepressant effect has ever been reported (annex, table 1, p.97).

Either oils. The major components of the essential oil of St. John's Wort contains aliphatic compounds (2-methyl octane, n-nonane, n-decane, n-undecane, n-tetradecanol, 2-methyl-decane, and 2-methyl-dodecane) along with terpenoids (α-pinene, β-pinene, geraniol, β-caryophyllene, β-farnesene, humulene, and germacrene D). Differences in the biosynthesis of sesquiterpene and aliphatic hydrocarbons, and in oxygenated aliphatics, in flowers and leaves have been indicated. The data has shown that concentrations of β-caryophyllene and caryophyllene oxide in essential oils from the leaves are higher than those from the flowers, whereas dodecanol, spathulenol, viridiflorol, carotol and tetradecanol are present in higher quantities in the flowers.

Other chemical constituents. Other typical constituents are xanthones (1,3,6,7-tetrahydroxyxanthone and kielcorin C (0.01%), acids (isovalerianic, nicotinic, myristic, palmitic, and stearic), carotenoids, choline, nicotinamide, pectin, β-sitosterol, pectin, fatty acids, amino acids, vitamin C, tannins, hydroperoxycadiforin (in stems and leaves) and bisanthraquinone glycosides.

The macro- and microelements composition is also well known. The micro-elements Ba, V, Li, Ag, Au, I, and Br were not detected. The plant accumulates Mo, Se, Cd, and, possibly, Mn. Historically, branches and flowers used for wool and fabric dyes. Depending on strength, colors of yellow, green, pink and red, were obtained [51-57].

It is known two types of St. John's Wort grow in Uzbekistan: *Hypericum perforatum* L. and *Hypericum scabrum* L. According to results of content active substances (sum hypericins, flavonoids, hyperforin) are the same in both types. Comparing chemical composition between *Hypericum perforatum* L. and *Hypericum scabrum* L. which grows in Uzbekistan are shown in Table 2 (annex, table 2, p.98).

3. Drug forms of St. John's Wort

For medicinal applications the plant of St. John's Wort used in the form of dry herb, different extracts (liquid, dry extracts), oils, infusions, teas, tablets and capsules. Some of these forms have been produced in Uzbekistan and other countries. In Uzbekistan, there is only tincture production, but in abroad there are different forms of St. John's Wort. All preparations of St. John's Wort are made only based on *Hypericum perforatum L.*

4. Liquid forms of St. John's Wort

There are many liquid medicinal forms of St. John's Wort: broths, infusions, liquid extracts, oils and tinctures.

Broth. Avicenna wrote many different recipes of broths St. John's Wort for various illness in his book "The Canon of Medicine". He made broth from flowersof St. John's Wort for treat internal diseases, inflammation of nerve, tumor, spotty, broth from leaves of St. John's Wort for burn in fire, large wounds, malignant ulcers. He separately emphasized about broth from seeds, when making broth from seed, should be take a bit of seed, if take more it will be harmful for health.

Nowadays there are three broths of St. John's Wort in folk medicine.

1. Broth for cold (chill) and headache. To 200 ml boiling water add 1 table-spoon plant of *Hypericum perforatum L.*and boil on a bit fire about 15 min. Filter after cooling. Drink 3 times a day in 0.25 glass.

2. Broth for diseases of kidney and bladder. To 4 L of boiling water add 1 table-spoon plant of *Hypericum perforatum L.*and boil on a bit fire about 15 min. Then cool and filter. Drink 3 times a day in 0.5 glass. Preparing broth can drink for diseases of digestion.

3. Broth for depression. To 250 ml of boiling water add 1 table-spoon of *Hypericum perforatum L.* plant and boil on a bit fire about 10-15 min. Filter after cooling. Drink 3 times a day of 0.25 glass.

Tincture. Tincture of St. John's Wort is used in folk medicine for treat rheumatism, gout, tuberculosis, bleeding, haemorrhoids, furuncle and others. It may prepare with different concentration of alcohol:

1. Tincture for stomatitis and gingivitis. To 5 part of alcohol add 1 part of plant *Hypericum perforatum* L. and put to brew about week. Then filter. Use 3 times a day by 40-50 drops. For rinsing mouth and throat dilute 30-40 drops of tincture to 125 ml water.

2. Tincture 10:200 for using internal as astringent, resolvent and antiseptic in colitis, gastric ulcers and duodenum, in diseases of kidney and bladder, also styptic, antiwormal, in headache. Indicate in 0.25 glass 3 times a day.

3. Tincture 1:5 with 40% ethanol is used as astringent and resolvent in stomatology. 50 drops to 100 ml water. This recipe is prepared by M.D. Mashkovskiy in his book "The Medicinal substanses" [58].

Oil. Oil of St. John's Wort is for external application (in bedsore, burn, ulcer, rinsing mouth). To 200 ml sunflower-seed oil add 3 table-spoon plant of *Hypericum perforatum L.* and put to brew about 2 week, periodically shake, then filter. Indicate treatment for wounds and burns [59,60].

"Novoimanin" ***(Novoimaninum)*** – polyfenol complex preparation, which transparent resinous reddish-yellow mass with odour of honey. It is

manufactured in form of 1% solution in 95% ethanol. For external application, inhalation.

5. Solid forms of St. John's Wort

Briquettes of plant *Hypericum perforatum* L. – is used for preparing broths for rinsing mouth and in diarrhea and colitis. It is manufactured in packets by 75 g or 100 g. Broth is made to 200 ml water.

"St. John's Wort-Zerde", herbal-tea – is made with reduction plant of *Hypericum perforatum* L. in packets. It is used for treat diseases of gastrointestinal tract and metabolic diseases. Producer is "Zerde -Herbal", Region of South Kazakhstan.

"Hyflarin" *(Hyflarini)* – preparation of *Hypericum perforatum* L. after obtaining "Novoimanin". It possesses resolvent, antioxidant effects. Applied for treat sharp and chronic defects of nephritis.

"Imanin" *(Imaninum)* – herbal antibiotic, which picked out from plant of *Hypericum perforatum* L. by academic V.G. Drobotko in Kiev. It effects more than 40 types of microbes. Used in abscess, phlegmon, ulcer, burns II and III,

"Helarium Hypericum" - is dragee, which is made, based on standardized dry extract of *Hypericum perforatum* L., it contents 285 mg in one tablet. Active substance is sum hypericins, it effects on the central nervous system (CNS) and are used to treat mild depression-like mood disorders. Producer is "Bionorica", Germany [61].

"Laif" 900 – antidepressant herbal origin, containing standardized dry extract of *Hypericum perforatum* L. which obtaining by alcohol solution from plant material. Active substances of dry extract are naphthodianthrones (hypericin), phloroglucinols (hyperforin) and flavonoids [62].

"Relax" – nutritive dragee contents dry extract of *Hypericum perforatum* L. in optimal composition of hypericin, it is used for depression, fright, insomnia, hepatitis, cholecystitis, gastritis.

“Hyper” – nutritive complex preparation, which contents: *Hypericum perforatum* L, howthorn, vitamins C, B1, B6, B12 and magnesium. Base specialty of medicine “Hyper” is antidepressive action.

“Deprim” – tablets and capsules containing dry extract of *Hypericum perforatum* L., active components hypericin and hyperforin. Preparation improves mood, decreases sense of fright and effort, normalizes sleep and appetite, increases psychical activity, efficiently, stabilizes immunity.

All medicines of St. John’s Wort are made based on plant *Hypericum perforatum* L [63-66].

Imported drugs based on St. John’s Wort are shown in Table 3 (annex, table 3, p.99).

6. Pharmacopeia methods for standardization

St. John’s Wort powdered plant, dry extract of St. John’s Wort have been included into many Pharmacopeias such as Russian Pharmacopeia since 1968, the United States Pharmacopeia 2007 (USP 2007), Europe and British Pharmacopeias (EP, BP). All indicated abstracts figure only *Hypericum perforatum* L., but there is not any notification regarding *Hypericum scabrum* L.

Russian Pharmacopeia (GP XI, edition 2). Analytical methods of *Hypericum perforatum* L. plant are identification, sum flavonoids calculated as rutin (NLT 1.5%), water content (NMT 13%), total ash (NMT 8%), dissoluble ash (NMT 1%), organic impurity (NMT 1%), mineral impurity (NMT 1%) and analytical methods of *Hypericum perforatum* L. powdered plant are identification, sum flavonoids calculated as rutin (NLT 1.5%), water content (NMT 13%), total ash (NMT 8%), dissoluble ash (NMT 1%), organic impurity (NMT 1%), mineral impurity (NMT 1%) in GP XI. Assay method of sum flavonoids are standardized with ethanol (50%) at 415 nm by UV analyses. Definition other active compounds of *Hypericum perforatum* L. are not there [67-70].

USP. There are analytical methods for powdered plant *Hypericum perforatum L.* and for dry extract of *Hypericum perforatum* L. They are botanical characteristics, identification by TLC, total ash (NMT 5.0%), water content (NMT 10.0%), foreign organic matter (NMT 2.0%), pesticide residues, microbial enumeration, content of hypericin and pseudohypericin (NLT 0.04%), hyperforin (NLT 0.6%) by HPLC in powdered *Hypericum perforatum* L. and identification by TLC, water (NMT 5.0%), total ash (NMT 7.0%), microbial enumeration, pesticide residues, heavy metals (50 μg per g), organic volatile impurities, content of hypericin and pseudohypericin (90.0% to 110.0%), hyperforin (90.0% to 110.0%) by HPLC in the powdered *Hypericum perforatum L.* extract. Recently, USP reported a monograph of St. John's Wort in which both naphthodianthrone and phloroglucinol derivatives are evaluated by HPLC analysis. According to this monograph, the powdered St. John's Wort (*Hypericum perforatum* L.) should contain not less than 0.6% of hyperforin and not less than 0.04% of hypericin and pseudohypericin combined calculated as oxybenzone, also, the standardized extract should contain not less than 0.2% of hypericin and pseudohypericin combined and not less than 3.0% of hyperforin calculated as oxybenzone. This analytical method is more selective, accurate and reproducible than UV analysis.

But this method is difficult to understand which peak is belonging to hypericin, pseudohypericin, hyperforin, rutin, hyperoside and others. Besides this USP method BP and EP methods are still more reliable and easy (to find what is peak it) method and we can believe to results which obtained from its [71-74].

BP and EP. Also in the European and British Pharmacopeia analytical methods of herbal drug and powdered dry extract, quantified are the same, including definition, characters, identification by TLC, water (NMT 5.0%), foreign matter (NMT 3.0%), loss on drying (NMT 10.0%), total ash (NMT 10.0%), assay (total hypericins). The EP and BP require not less than 0.08% naphthodianthrones of the hypericin group (not less than 0.08%), calculated as

hypericin using UV spectrophotometry analysis for herbal drug and determining naphthodianthrones of the hypericin group, expressed as hypericin from 0.1% to 0.3%, total flavonoids, expressed as rutin (minimum 6.0%), hyperforin (maximum 6.0%) using HPLC analysis for powdered dry extract.

The pharmacopeial quantitative analytic methods for the drug are based on the determination of herbal drug St. John's wort by the colorimetric method that is, using the red colouring of St. John's Wort. The original colorimetric method (in the 1991 modification) extracts substances deemed "inert" using for the determination of hypericins. This treatment has been criticized because it is unreliable, therefore European Pharmacopeia, adopting the DAC method, has eliminated the pretreatment with dichloromethane and replaced methanol by tetrahydrophurane as the extraction solvent.

Nevertheless, the European and British Pharmacopoeia published in Pharmacopeia the following draft monograph, with a particular assay for the extract, which represents an innovation as it adopts HPLC methods.

The first method of HPLC analysis of the drug was published in 1987 by Holzl and Ostrowski, with remarkable results, and had the merit of acting as a basis for various other HPLC methods, mainly developed in Eastern Europe, where St. John's Wort was widely cultivated. This method highlighted, amongst other things, the possibility of accurately determining the percentages of hyperforin in the drug and the difficulties of revealing hypericins which show very long retention times in contrast with their polarity in TLC. These problems still arise with the more updated HPLC methods.

Comparing the analytical results obtained with the above HPLC method for the extracts, with the DAC ones, the latter are on average higher by 30% because the liquid chromatography succeeds in isolating "aspecific inert substances, present in the extract, which are involved in the quantitative spectrophotometric determination, increasing their values". The HPLC method, proposed for the drug and extracts by the Consortium of the German Producers, does not appear to have been adopted yet by any Pharmacopeia [75-81].

Chapter II. MAIN PART

1. Objects and subjects of research

The research objects are two type of St. John's Wort, they are *Hypericum perforatum* L. and *Hypericum scabrum* L. Based on these plants we obtained at first dry extract of St. John's Wort *Hypericum perforatum* L. and *Hypericum scabrum* L., then we prepared liquid extract, "St. John's Wort" 300 capsules, "St. John's Wort" 300 tablets only by type of St. John's Wort *Hypericum scabrum* L. These preparations (liquid extract, "St. John's Wort" 300 capsules, "St. John's Wort" 300 tablets) are our subjects of research as well.

St. John's Wort *Hypericum perforatum* L.

Family: Hypericaceae.

Geographical distribution: *Hypericum perforatum* L., (Hypericaceae, Clusiaceae, Guttiferae), commonly known as St. John's Wort, is an herbaceous perennial of Eurasian origin. The plant is abundant in wild throughout Europe, Russia, Caucasus, Iran, Central Asia, India, Mongolia, China and Japan, growing on grasslands, meadows, and on the edges of forests. The plant spreads rapidly and can invade pastures, disturbed sites, roads and sparse woods, and considered as an aggressive weed on pastures of New Zealand, Australia and North America.

This plant is distributed very wide in Republic of Uzbekistan. There are *Hypericum perforatum* L. as well as *Hypericum scabrum* L. in territory of Uzbekistan grow.

Botanical characteristics. St John's Wort is a perennial plant with extensive, creeping rhizomes. Its stems are 20-50 cm and erect, profusely branched in the upper section, two-edged and can grow to 1 m high. Root is slender, woody, with abundant fibrous secondary roots. The rhizomes often present giving rise to new shoots. It has opposing, stalkless, narrow, elliptical, or oblong-ovate, long or slightly larger leaves that longwise is 7-30 mm, width is 3-15 mm. The leaves are dark-green in color, with transparent dots throughout

the tissue and occasionally with a few black dots on the lower surface. Leaves exhibit obvious translucent dots when held up to the light, giving them a ‘perforated’ appearance, hence the plant's Latin name. Two special features, the transparent dots on leaves and two-raised lines down the stem, make identification of this plant easier.

Its bract is lancetic. Small cup is divided deep, small cup leaves are lancetic. Petals are yellow colored, often black-dotted, particularly at the margins.

Inflorescence is a cymose panicle with numerous delicate golden-yellow flowers. Flowers are 3 cm in diameter, have five green sepals, five golden-yellow petals, many stamens, associated to three tufts, and one pistil. Flowering occurs from June to August. Fruit is a three-celled capsule with many seeds. Seeds are small, ovale-cylindrical with rounded to pointed ends, dark brown, the surface pitted, 1 mm long. When flower buds (not the flowers themselves) or seedpods are crushed, a reddish-purple liquid is produced (annex, fig. 2, p.114).

Flowering: June-July.

Fruiting: July-August.

Harvest of the plant for medicinal purposes must occur in July and August, and it must be dried immediately to avoid loss of potency.

Traditionally using. Traditionally, dry herbs used for preparation of teas as a tonic and to improve function of secretory glands, and gastrointestinal tract. The infusion of the herb was heavily utilized against cough, heart failure, headaches, flu, sore throat, rheumatism, bed-wetting, liver failure, and to treat wounds and ulcers.

The infusion of the fresh florets kept in sunflower oil for 2-3 weeks was commonly used as an effective wound healing remedy. The plant was known to possess astringent, antiseptic, hemostatic, anti-inflammatory and stimulatory properties, and effectively used against ischialgia, gout, mastitis, pulmonary tuberculosis, excessive bleeding during menstruation, and boils. The American Indians traditionally used dried plant as a meal, and fresh leaves for their

soothing effect. The herb was used to cure bronchitis and to overcome bed-wetting in children [82-85].

Preparations: "Phytotea of St. John's Wort", "Tincture St. John's Wort", "Imanin" tablets, "Novoimanin" tablets, "Gelarium Gipericum" tablets, "Deprim" tablets and capsules, "Layf" 900 tablets, "St. John's Wort" tablets, "Novopassit" complex preparation, "Negrustin" complex preparation and others.

St. John's Wort *Hypericum scabrum* L.

Family: Hypericaceae.

Geographical distribution: *Hypericum scabrum* L., is distributed widely in Central Asia, Afghanistan, Iran, Iraqi, Syria, Turkey, Armenia, Azerbaijan, Grazes, Russia (Altai), Kazakhstan, Turkmenistan, Tajikistan, Uzbekistan, Pakistan and China.

This plant is distributed very wide in Republic of Uzbekistan. If all *Hypericums* which are grorn in Uzbekistan are 100%, *Hypericum scabrum* L.is grown about 85%.

Botanical characteristics. St John's Wort is a perennial plant with extensive, creeping rhizomes. Its stems are multiple, 17-35 cm and usually can grow to 10-15 cm high. Base of stems is wooden, many parts are rounded, ascending, rough, and violent or red colored.

Leaves are sitting, dark bluish-grey colored, lanceted or oblong-lanceted, longwise is 9-25 mm, and width is 25 mm. all leaves are bluntly, narrowing.

Flowers are multiple, are 3-6 cm in diameter, have five green sepals, five golden-yellow petals, many stamens, associated to three tufts, and one pistil. Flowering occurs from June to August. Fruit is a three-celled capsule with many seeds. Seeds are small, ovale-cylindrical, dark brown, the surface pitted, 1.5 mm long.

Its bract is oblong-linear. Small cup is divided deep, small cup leaves are lancetic. Petals are yellow colored, oblong-ovale.

Flowering: May-June.

Fruiting: July-September.

Traditionally using. Traditionally, *Hypericum scabrum* L. used for treat the most various diseases as *Hypericum perforatum.* This plant helps in illnesses of liver, heart, acorn, bowels, bladder, sore throat and others. Flowers of *Hypericum scabrum* L. are added to tea that drinks in jaundice. Prepared 10% tincture from *Hypericum scabrum* L. flowers are possessed bactericidal effect in diseases of staphylococci, intestinal bacillus, streptococci.

Preparations: Is not made.

Using all informations about *Hypericum perforatum* L. and *Hypericum scabrum* L. we composed comparing table of parameters them (annex, table 1, p.97). This table is shown that plants of *Hypericum perforatum* and *Hypericum scabrum* differs with size of stems, leaves and appearance of leaves, bracts, and time of flowering, fruiting. But color of flowers and seeds are the same, but flowers of *Hypericum perforatum* have with a few black dots on the lower surface, flowers of *Hypericum perforatum* are rough (annex, table 4, p.100).

Hypericum perforatum is distributed mainly in Europe, the States and Australia. *Hypericum scabrum* is spread principally in Central Asia [86-89].

Besides that from above mentioned informations similarity of these plant is traditionally using of them. However, all import and our local preparations are made based on *Hypericum perforatum.*

2. Methods of research

We used different methods to prepare dry extract of St. John's Wort and to development of liquid extract of "St. John's Wort" 300, capsulated and tableted form "St. John's Wort" 300 and to learn their standardization. They are:

1. Methods for obtaining dry extract;
2. Methods for obtaining liquid extract;
3. Methods for obtaining capsulated and tableted form;
4. Methods for standardization;
5. Methods for study stability.

Methods for obtaining dry extract

Extraction, as the term is used pharmaceutically, involves the isolation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts and powdered extracts. Such preparations popularly have been called galenicals, named after Galen, the second century Greek physician. There are many methods of extraction:

1) **Solid – liquid extraction.** Solid-liquid extraction allows soluble components be removed from plants using a solvent. Active substances from plants are isolated by this method, but content of active substance obtained extract based on this method maybe lesser.

2) **Liquid – liquid extraction.** Liquid-liquid extraction involves using a liquid solvent to remove a liquid component from a liquid mixture. The component dissolves preferably in the solvent. This method usually uses in clean extracts and isolating maximally active substance from liquid extracts.

3) **Maceration.** This simple widely used procedure involves leaving the pulverized plant to soak in a suitable solvent in a closed container .simple maceration is performed at room temperature by mixing the ground drug with the solvent (drug solvent ratio : 1:5 or 1:10) and leaving the mixture for several days with occasional shaking or stirring. The extract is then repeated from the plant particles by straining . The process is repeated for once or twice with fresh solvent .Finally the last residue of extract is pressed out of the plant particles using a mechanical press or a centrifuge.kinetic maceration differe from simple one by continuous stirring.

4) **Percolation.** The powdered plant material is soaked initially in a solvent in a percolator. Additional solvent is then poured on top of the plant material and allowed to percolate slowly (drop wise) out of the bottom of the

percolator. Additional filtration of the extract is not required because there is a filter at the outlet of the percolator.

From these methods of extractions, we used solid-liquid extraction, liquid-liquid extraction and percolation methods while process obtaining dry extract of St. John's Wort. Solid-liquid and percolation extraction methods are used for extraction plant in technological process of dry extract (TP 1.); liquid-liquid extraction is used for cleaning and isolating of extract.

Methods for obtaining liquid extract

Liquid extracts are prepared by methods as the same dry extracts: solid-liquid extraction, liquid-liquid extraction, maceration, percolation and others. Besides these methods there is the easiest method which obtained from dry or soft extracts (dissolution dry or soft extracts). We made liquid extract of St. John's Wort by the method of dissolution obtained St. John's Wort dry extract. This method is the easiest, cheaper, quickly, conventional method than others.

Methods for obtaining capsulated and tableted form

Compressed tablets are the most widely used solid dosage form so they must satisfy a number of physical requirements in terms of hardness, disintegration ability, friability and uniformity. To provide these tablet characteristics in accordance with the selected ingredients, manufacturers can use three different processing technologies: direct compression, dry granulation and wet granulation.

1) Direct compression. Direct compression is a popular choice because it provides the shortest, most effective and least complex way to produce tablets and capsules. The manufacturer can blend an API with the excipient and the lubricant, followed by compression, which makes the product easy to process. No additional processing steps are required.

2) Wet granulation. In wet granulation, granules are formed by the addition of a granulation liquid onto a powder bed which is under the influence of an impeller or air. The agitation resulting in the system along with the wetting of the components within the formulation results in the aggregation of the

primary powder particles to produce wet granules. The granulation liquid (fluid) contains a solvent, which must be volatile so that it can be removed by drying, and be non-toxic. Typical liquids include water, ethanol and isopropanol either alone or in combination. The liquid solution can be either aqueous based or solvent-based. Aqueous solutions have the advantage of being safer to deal with than solvents.

3) Dry granulation. The dry granulation process is used to form granules without using a liquid solution because the product granulated may be sensitive to moisture and heat. Forming granules without moisture requires compacting and densifying the powders. In this process, the primary powder particles are aggregated under high pressure. It can be passed through a mill and final blend before capsule and tablet compression [90,91].

We probed all methods of capsulated and tableted form for developing composition and technology capsules and tablets "St. John's Wort". Then we selected the most optimally composition and technology through using these methods.

Methods for standardization

After preparing dry extract, liquid extract, tablets and capsules we standardized these preparations by methods of TLC (identification), HPLC (identification, assay sum hypericins, sum flavonoids, and hyperforin), GC method for determine residual solvents.

1) TLC. Thin-layer chromatography is a method to isolate each ingredient by developing a mixture in a mobile phase, using thin-layer made of a suitable stationary phase, and is applied for identification, purity test, etc. of substances.

2) HPLC. Liquid chromatography is the method to develop a mixture injected into a column prepared with a suitable stationary phase by passing a liquid as a mobile phase through the column, in order to isolate the mixture into its components by making use of the difference of retention capacity against the stationary phase, and to determine the components. This method can be applied

to a liquid or soluble sample, and is used for identification, purity test, and quantitative determination.

3) GC. Gas chromatography is the method to develop a mixture injected into a column prepared with a suitable stationary phase by passing a gas (carrier gas) as a mobile phase through the column, in order to isolate the mixture into its components by making use of the difference of retention capacity against the stationary phase, and to determine the components. This method can be applied to a gaseous or vaporizable sample, and used for identification, purity test, and quantitative determination [92].

Methods for study stability

Stability of drugs is one the important property of drugs. We must define stability tests every new drugs. The purpose of stability testing is to provide evidence on how the quality of finally product varies with time under the influence of a variety of environmental factors, such as temperature, humidity, and light, and to establish a retest period for the drug product and recommended storage conditions. The choice of test conditions is based on an analysis of the effects of climatic conditions, which are described on the basis of the mean kinetic temperature derived from climatic data; thus, the world can be divided into four climatic zones, I–IV.

Data from stability studies should be provided on at least three primary batches of the drug product. The primary batches should be of the same formulation and be packaged in the same container closure system proposed for marketing. The testing should cover, as appropriate, the physical, chemical, biological, and microbiological attributes; preservative content (e.g., antioxidant, antimicrobial preservative); and functionality tests (e.g., for a dose delivery system).

There are three methods of stability:

1) Long-term stability: 25°C ± 2°C, 60% RH (relative humidity), ± 5% RH, 12 months. For long-term studies, frequency of testing should be sufficient

to establish the stability profile of the drug product. For products with a proposed shelf life of at least 12 months, the frequency of testing at the long-term storage condition should normally be every 3 months over the first year, every 6 months over the second year, and annually thereafter through the proposed shelf life.

2) Intermediate stability: 30°C ± 2°C, 60% RH, ± 5% RH, 6 months. At the accelerated storage condition, a minimum of three time points, including the initial and final time points (e.g., 0, 3, and 6 months), from a 6-month study is recommended. Where an expectation (based on development experience) exists that results from accelerated testing are likely to approach significant change criteria, increased testing should be conducted either by adding samples at the final time point or by including a fourth time point in the study design.

3) Accelerated stability: 40°C ± 2°C, 75% RH, ± 5% RH, 6 months. When testing at the intermediate storage condition is called for as a result of significant change at the accelerated storage condition, a minimum of four time points, including the initial and final time points (e.g., 0, 6, 9, and 12 months), from a 12-month study is recommended. Reduced designs (i.e., matrixing or bracketing), in which the testing frequency is reduced or certain factor combinations are not tested at all, can be applied if justified.

Intermediate and accelerated stability methods are used to foresee stability of drugs and speed up registration new drugs [93].

Chapter III. DEVELOPMENT OF TECHNOLOGY AND STANDARDIZATION OF DRY AND LIQUID EXTRACTS, CAPSULES AND TABLETS BASED ON ST. JOHN'S WORT

1. Development of technology and standardization of dry and liquid extracts.

There are many different methods of obtaining substances, pure substances and extracts. Most of all methods of synthetic and natural are widely used. Natural medicinal products are commonly used in medicine and their popularity and availability may significantly affect the health of patients. Traditionally, herbs for infusion or dosage forms containing extracts, oils, juices and recently solid dosage forms, tablets and capsules with powdered or micronized herbs are used. Similarly, to synthetic medicinal products, they are subject to extensive assessments on their quality, safety and therapeutic efficacy. However, unlike synthetic medicinal products are a pure substances, herbal medicinal products are a complex mixture of active substances. However, synthetic medicinal products create different side effects in spite of pure [94].

One of the active substance of St. John's Wort is hypericin (sum hypericins). It can be prepared by the synthetic method and by the natural method of extraction [95]. Mainly it can be obtaining by the method of extraction [96]. Some extracts of St. John's Wort was standardized to flavonoids, tannins, soponins, essential oils and others [97,98]. Effects of this all extracts are actually different.

For isolating sum hypericins we use the method of extraction and take aerial parts of the plants St. John's Wort kind of *Hypericum perforatum* L. and *Hypericum scabrum* L. for extraction. Cause of taking two types of plant that comparing sum hypericins in these plants. Because in some references was written that in sum hypericins in *Hypericum scabrum* L. is absent or very few [99]. For this reason all medicinal products from St. John's Wort is made based on type of *Hypericum perforatum* L.

After comparing sum hypericins of two dry extracts of St. John's Wort we prove that *Hypericum scabrum* L. which is grown in Uzbekistan contains sum hypericins like *Hypericum perforatum* L. Then we made different preparations which consists of 0.9 mg of sum hypericins (hypericin and pseudohypericin) only based on standardized extract of St. John's Wort (*Hypericum scabrum* L.):

1) liquid extract of St. John's Wort (*Hypericum scabrum* L.),
2) "St. John's Wort" 300 capsules and
3) "St. John's Wort" 300 tablets.

2. Selection of extractants for the extraction

We take aerial parts of the *Hypericum perforatum* L. and *Hypericum scabrum* L. (annex, fig.3, p.114) grown in Uzbekistan and dried. The plant material was combined by the Laboratory of Glycosides, Institute Chemical Plants Substance. The aerial parts of the plant were dried in shadow at the room temperature and cut into small particles.

Selection of the best reagents played important role in process extraction to isolate maximum content of API. We have tried eight different eluents to isolate sum of hypericins from the aerial parts of *Hypericums*. They are:

1) Purified water,
2) Ethanol 40%,
3) Ethanol 60%,
4) Ethanol 80%,
5) Ethanol 96%,
6) Ethyl acetate 98%,
7) Butanol 98%,
8) Acetone 98%.

We weighted about 1,0 grams of powdered two type of plants to 16 flasks and each was extracted separately by above mentioned 8 reagents (in ratio 1:7) during one day at room temperature by the method of percolation. After then we

evaporated all reagents using rotary evaporator till dry extract observing. This 16 preparing dry extracts are analyzed by the method of HPLC and the receiving results are shown in Table 5 (annex, table 5, p.101).

Table 5 shows that aqueous ethanol 80% isolated maximally sum of hypericins from *Hypericum perforatum* L. (57.5 mg dry extract (5.75%) containing 0,29% sum hypricins) and *Hypericum scabrum* L. (56.4 mg dry extract (5.64%) containing 0.31% sum hypricins).

So, we have selected the most of effective reagent for extraction of hypericins sum from *Hypericum perforatum* L. and *Hypericum scabrum* L. by ethanol 80% which isolates more API than others and the dry extract preparing by mentioned reagent, which gave us a high yield of extractives with highest content of hypericins.

3. Technology of dry extract

Technology of obtaining dry extract of St. John's Wort consists of following processes:

Initial processes (IP):

IP 1.1. Preparing personals.

IP 1.2. Equipment, air and manufacturing rooms preparation.

IP 1.3. Preparing raw materials: 1. Drying plants in shadow at the room temperature. 2. Crushing plants.

IP 1.4. Preparing excipients.

Technological processes (TP):

TP 1. Extraction of St. John's Wort.

Extraction (solid-liquid extraction) is carry out by aqueous ethanol 80% in ratio 1:7 during 5 days at the room temperature by the method of percolation. Forms red colored liquid extracts (annex, fig. 4.a., p.115). Extraction finishes when the last liquid extract will be colorless. Prepared liquid extract consists of different biological active substances: naphtodianthrones (sum hypericins), flavonoids, alkaloids, tannins, soponins, essential oils and others.

TP 2. Thickening.

Prepared liquid extract is evaporated with helping rotor extractor at 45°C. Forms red colored thick extract (annex, fig. 4.b., p.115).

TP 3. Cleaning of extract.

For the isolation of the sum of hypericins from excessive matters (alkaloids, essential oils) and maximally isolate sum hypericins thick extract is cleaned with chloroform in ratio 1:2 (liquid-liquid extraction) with helping isolating funnel (annex, fig. 4.c., p.115). Mix 10 min., and after dividing into two parts (upper part is water, bottom part is chloroform), pour off parts of chloroform. Cleaning finishes when part of chloroform will be colorless. All parts of chloroform collect into another bottle. Part of colorless water is removed. Obtained part of chloroforms is evaporated with helping rotor extractor at temperature 45°C.

TP 4. Obtaining dry extract of St. John's Wort

Received thick extract is dried at temperature 45°C in spray drier. Then this powder is ground up in mill and sieved though 0.1 mm sieve. Forms dry extract of St. John's Wort (annex, fig. 4.d., p.115). Obtained dry extract will used for make different medical forms (liquid extract, tablets, capsules). Technological chart of obtaining dry extract of St. John's Wort is listed in Fig. 5 (annex, fig.5, p.116).

4. Standardization of dry extract St. John's Wort

The introduction of the monograph "dry extract quantified of St. John's Wort herb 07/2008:1874" into the European and British Pharmacopoeias (EP, BP), the analytical methods used to determine sum hypericins, flavonoids and hyperforin in St. John's wort by HPLC [100,101]. We selected for standardization our dry extract requirements of the EP and BP.

Appearance: Brownish-grey powder.

Identification: ***A. Thin-layer chromatography (2.2.27).***

Test solution: Disperse 0.25 g of the extract to be examined in 5 ml of methanol R. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Reference solution: Dissolve 5 mg of rutin R and 5 mg of hyperoside R in methanol and dilute to 10 ml with the same solvent. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Plate TLC silica gel plate R (5-40 μm) [or *TLC silica gel plate R* (2-10 μm)].

Mobile phase anhydrous formic acid R, water R ethyl acetate R (6:9:90 V/V/V).

Application: 10 μl [or 5 μl] as bands of 10 mm [or 8 mm].

Development: Over a path of 10 cm [or 7.5 cm].

Drying: At 100 - 105ºC for 10 min.

Detection: Spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in methanol R and then with a 50 g/l solution of *macrogol 400 R* in methanol R. examine after about 30 min in ultraviolet light at 365 nm.

Results: See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other fluorescent zones may be present in the chromatogram in the obtained with the test solution (annex, table 6, p.102).

Obtained TLC chromatogram of dry extract of St. John's Wort is shown in Fig. 6 (annex, fig.6, p.117).

B. HPLC. Examine the chromatograms obtained in the test for assay.

Results: The principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the reference solution (St. John's Wort dry extract CRS).

C. Alternative method. HPLC. Examine the chromatograms obtained in the test for assay.

Results: The principal peak (hypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peak (hypericin) in the chromatogram obtained with the reference solution (hypericin).

Loss on drying (2.2.32). Not more than 4.0 per cent, determined on 1.000 g of the powdered extract by drying in an oven at 100°C to 105°C for 3 h.

Calculation:

$$\% = \frac{W_1 - W_2}{W_1 - W_0} \cdot 100$$

W_0 – weight of a weighing bottle in gram;

W_1 – weight of a weighing bottle with substance in gram;

W_2 – weight of a weighing bottle with substance after drying in gram.

Total ash (2.4.16). Not more than 10.0 per cent. Heat a platinum dish to redness for 10 minutes, allow cooling in a desiccator and weighing. Unless otherwise specified in the monograph, place 1 g of the substance being examined in the dish, ignite at about 600°C. Cool, weigh again, ignite for 15 minutes and repeat this procedure until two successive weighings do not differ by more than 0.5 mg.

Calculation:

$$\% = \frac{W_2 - W_0}{W_1 - W_0} \cdot 100$$

W_0 – weight of a platinum dish in gram;

W_1 – weight of a platinum dish with substance in gram;

W_2 – weight of a platinum dish with substance after igniting in gram.

Heavy metals (2.4.8). Maximum 10 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of lead standard solution (10 ppm Pb) R.

Residual solvents. Ethanol not more than 5000 ppm, chloroform more than 5000 ppm.

Method GC.

Column: Innowax 30 m * 0.53 mm * 1 μm;

Flow rate helium/nitrogen: 7.3 ml/min;

Pressure: 5.0 psi;

Average rate: 49 cm/sek;

Detector: FID:

Oven: is shown in Table 7 (annex, table 7, p.102).

Temperature: 250°C;

Flow hydrogen: 40 ml/min;

Flow air: 400;

Flow carrier: 30 ml/min;

Inlet:

Carrier gas: helium or nitrogen;

Oven: 180°C;

Pressure: 5.0 psi;

Total flow: 46.5;

Split ratio: 5:1;

Flow split: 36.4;

Solvent and blank: Dimethylacetamid (DMA);

Reference solution of ethanol: Weight 500 mg of ethanol R in DMA and dilute to 100 ml with the same solvent. Dilute 1.0 ml of this solution to 50 ml with the same solvent. Filter the solution through a membrane filter with a porosity of 0.45 mm (0.1 mg/ml).

Reference solution of chloroform: Weight 500 mg of chloroform R in DMA and dilute to 100 ml with the same solvent. Dilute 1.0 ml of this solution to 50 ml with the same solvent. Filter the solution through a membrane filter with a porosity of 0.45 mm (0.1 mg/ml).

Test solution: Weight 200 mg of substance in DMA and dilute to 10 ml with the same solvent. Filter the solution through a membrane filter with a porosity of 0.45 mm (20 mg/ml).

Injection: 1 μl.

Note: Add the blank, reference solution ethanol, reference solution chloroform and test solution into head-space bottles over 5 ml all solution.

Calculation:

$$ppm = \frac{S_1 \cdot C_{st} \cdot 1000000}{S_{st} \cdot C_1}$$

S_{st} – area of the ethanol (chloroform) in the chromatogram obtained with the reference solution ethanol (chloroform);

S_1 – area of the ethanol (chloroform) in the chromatogram obtained with the test solution;

C_{st} – concentration of reference solution ethanol (chloroform), (mg/ml);

C_1 – concentration of test solution, (mg/ml).

Assay: Total hypericins, expressed as hypericin ($C_{30}H_{16}O_8$; M_r 504.5): 0.10 per cent to 0.30 per cent (dried extact).

Liquid chromatography (2.2.29).

Method 1.

Test solution: Dissolve 70.0 mg of the extract to be examined in 25 ml of methanol R. Sonicate and centrifuge the solution. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Reference solution: Dissolve a quantity of *St. John's Wort standardized dry extract CRS* corresponding to 0.15 mg of hypericin in 25 ml of methanol R. Sonicate and centrifuge. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Column:

- size: l=0.15 m, Ø=4.6 mm;
- stationary phase: octadecylsilyl silica gel for chromatography R (5 μm);
- temperature: 40°C.

Mobile phase: Mix 39 volumes of ethyl acetate R, 41 volumes of a 15.6 g/l solution of sodium dihydrogen phosphate R adjusted to pH 2.0 with phosphoric acid R and 160 volumes of methanol R.

Flow rate: 1.0 ml/min.

Detection: Spectrophotometer at 590 nm.

Injection: 20 µl.

Run time: 15 min.

Identification of peaks: Use the chromatogram supplied with *St. John's Wort standardized extract CRS* and the chromatogram obtained with the reference solution to identify the peaks due to pseudohypericin and hypericin.

System suitability: reference solution:

- the chromatogram obtained is similar to the chromatogram supplied with *St. John's Wort standardized extract CRS;*

- *resolution:* minimum 2 between the peaks due to pseudohypericin and hypericin.

Calculate the percentage content of total hypericins, expressed as hypericin, using the following expression:

$$X = \frac{(S_1 + S_2) \cdot m_0 \cdot 25 \cdot P}{S_0 \cdot 25 \cdot m_1} = \frac{(S_1 + S_2) \cdot m_0 \cdot P}{S_0 \cdot m_1}$$

S_0 – area of the peak due to hypericin in the chromatogram obtained with the reference solution;

S_1 – area of the peak due to pseudohypericin in the chromatogram obtained with the test solution;

S_2 – area of the peak due to hypericin in the chromatogram obtained with the test solution;

m_0 – mass of *St. John's Wort standardized extract CRS* used to prepare the reference solution, in grams;

m_1 – mass of the extract to be examined used to prepare the test solution, in grams;

P – percentage content of hypericin in *St. John's Wort standardized extract CRS*, in %.

Method 2. Alternative method. HPLC. Condition of chromatography and test solution are the same with *"Method 1"*, only reference solution is another.

Reference solution: Dissolve 1.0 mg of *hypericin CRS* to be examined in 100 ml of methanol R. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Calculate the percentage content of total hypericins, expressed as hypericin, using the following expression:

$$X = \frac{(S_1 + S_2) \cdot m_0 \cdot 25 \cdot P}{S_0 \cdot 100 \cdot m_1} = \frac{(S_1 + S_2) \cdot m_0 \cdot P}{S_0 \cdot m_1 \cdot 4}$$

S_0 – area of the peak due to hypericin in the chromatogram obtained with the reference solution;

S_1 – area of the peak due to pseudohypericin in the chromatogram obtained with the test solution;

S_2 – area of the peak due to hypericin in the chromatogram obtained with the test solution;

m_0 – mass of *hypericin CRS* used to prepare the reference solution, in grams;

m_1 – mass of the extract to be examined used to prepare the test solution, in grams;

P – percentage content of hypericin in *hypericin CRS*, in %.

Assay: Flavonoids, expressed as rutin ($C_{27}H_{30}O_{16}$; M_r 610.5): minimum 6.0 per cent (dried extact), hyperforin ($C_{35}H_{52}O_4$; M_r 536.8): maximum 6.0 per cent (dried extact) and not more than the content stated on the label.

Liquid chromatography (2.2.29). *Carry out the assay protected from light.*

Solvent mixture: water R, methanol R (20:80 V/V).

Test solution: Dissolve 70.0 mg of the extract to be examined in 20 ml of solvent mixture. Sonicate and centrifuge. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Reference solution (a): Dissolve 70.0 mg of *rutoside trihydrate CRS* in 200 ml of solvent mixture. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Reference solution (b): Dissolve 75.0 mg of *St. John's Wort standardized extract CRS* in 20 ml of solvent mixture. Sonicate and centrifuge. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Column:

- size: l=0.15 m, Ø=4.6 mm;
- stationary phase: octadecylsilyl silica gel for chromatography R (3 μm);
- temperature: 40°C.

Mobile phase:

- *mobile phase A:* phosphoric acid R, water R (3:1000 V/V);
- *mobile phase B:* phosphoric acid R, acetonitrile R (3:1000 V/V);

Detection: Spectrophotometer at 360 nm, then at 275 nm after the elution of biapigenin (about 22 min).

Injection: 10 μl.

Condition of gradient for HPLC is shown in Table 8 (annex, table 8, p.102).

Identification of peaks: Use the chromatogram supplied with *St. John's Wort standardized extract CRS* and the chromatogram obtained with the reference solution (b) to identify the peaks due to rutin, hyporoside, isoquercitroside, quercitroside, quercetin, biapigenin, hyperforin and adhyperforin.

System suitability: reference solution (b):

- the chromatogram obtained is similar to the chromatogram supplied with *St. John's Wort standardized extract CRS;*
- *resolution:* minimum 2 between the peaks due to rutin and hyporoside, and minimum 2.0 between the peaks due to hyperforin and adhyperforin.

Calculate the percentage content of total flavonoids, expressed as rutin, using the following expression:

$$X = \frac{(S_4 + S_5 + S_6 + S_7 + S_8 + S_9) \cdot m_2 \cdot 20 \cdot P}{S_3 \cdot 200 \cdot m_3} =$$

$$= \frac{(S_4 + S_5 + S_6 + S_7 + S_8 + S_9) \cdot m_2 \cdot P}{S_3 \cdot m_3 \cdot 10}$$

S_3 – area of the peak due to rutin in the chromatogram obtained with the reference solution (a);

S_4 – area of the peak due to rutin in the chromatogram obtained with the test solution;

S_5 – area of the peak due to hyporoside in the chromatogram obtained with the test solution;

S_6 – area of the peak due to isoquercitroside in the chromatogram obtained with the test solution;

S_7 – area of the peak due to quercitroside in the chromatogram obtained with the test solution;

S_8 – area of the peak due to quercetin in the chromatogram obtained with the test solution;

S_9 – area of the peak due to biapigenin in the chromatogram obtained with the test solution;

m_2 – mass of *rutoside trihydrate CRS* used to prepare the reference solution, in grams;

m_3 – mass of the extract to be examined used to prepare the test solution, in grams;

P – percentage content of rutin in *rutoside trihydrate CRS*, in %.

Calculate the percentage content of hyperforin, using the following expression:

$$X = \frac{S_{10} \cdot m_2 \cdot 20 \cdot P \cdot 2.3}{S_3 \cdot 200 \cdot m_3} = \frac{S_{10} \cdot m_2 \cdot P \cdot 2.3}{S_3 \cdot m_3 \cdot 10}$$

S_3 – area of the peak due to rutin in the chromatogram obtained with the reference solution (a);

S_{10} – area of the peak due to hyperforin in the chromatogram obtained with the test solution;

m_2 – mass of *rutoside trihydrate CRS* used to prepare the reference solution, in grams;

m_3 – mass of the extract to be examined used to prepare the test solution, in grams;

2.3 – correction factor for hyperforin with respect to rutin;

P – percentage content of rutin in *rutoside trihydrate CRS,* in %.

Storage: Store protected from light at the temperature 15-25°C in a well closed container.

Dry extract of St. John's Wort were standardizated according to the requirements of the BP and EP, which all parameters: appearance, identification (A), assay of sum hypericins, sum flavonoids and hyperforin. We have added parameters of determining identification (B, C), loss on drying, total ash, heavy metals, residual solvents and assay of sum hypericins (Method 2). Obtained dry extract of St. John's Wort conformed to all tests required for the dry extracts. Results of standardization dry extract of St. John's Wort is shown in Table 9 (annex, table 9, p.103). Other chromatograms of analysis are shown Fig.7. (annex, fig.7, p.117) and in supplement.

5. Technology of liquid extract

After preparation of two dry extracts from *Hypericum perforatum* and *Hypericum scabrum,* it was known that chemical contents of the dry extract of *Hypericum scabrum* is similar with the dry extracts of *Hypericum perforatum.* That's why next stage of our work was made liquid extract of St. John's Wort only based on *Hypericum scabrum* [102].

We used active substance of standardized dry extract of St. John's Wort, which dissolved in ethanol 70% to obtain liquid extract of St. John's Wort.

Composition of liquid extract of St. John's Wort (to 1 bottle) is following in Table 10 (annex, table 10, p.103):

Technology of obtaining liquid extract of St. John's Wort consists of following processes:

Initial processes (IP):

IP 1.1. Preparing personals.

IP 1.2. Preparing equipments, air and manufacturing rooms.

IP 1.3. Preparing raw material:

Weight sufficient supply of active substance (standardized dry extract of St. John's Wort).

IP 1.4. Preparing excipient:

Preparing solvent of ethanol 70%. Prepare this solvent according to GP XI, edition 1 [1]: ethanol 95% : purified water (737 ml : 288 ml).

Technological processes (TP):

TP 1.1. Mixing.

Dissolve standardized dry extract of St. John's Wort in ethanol 70% at permanent mixing.

TP 1.2. Cleaning of liquid extract and settling.

Obtained solution is settled not less than 1 day at the dark place at the temperature is not high 10°C until obtaining colorless liquid.

TP 1.3. Filtering.

Filtering is made under vacuum using nutch-filter. Wash remaining sediment with a little quantity of ethanol 70%, filtrate and add to main solution. Carefully mix filtrated solution (liquid extract) and make standardization.

Packing.

Pour off over 100 ml liquid extract into brown-glass bottles.

We called prepared liquid extract "St. John's Wort" 300 liquid extract (annex, fig.8, p.117). 5 ml of this liquid extract contains 300 mg of standardized dry extract of St. John's Wort (0.9 mg sum hypericins).

Technological chart of obtaining liquid extract of "St. John's Wort" 300 is listed in Fig. 9 (annex, fig.9, p.118).

6. Standardization of "St. John's Wort" 300 liquid extract

There is only tincture which is the fluid forms of drug from plant St. John's Wort. This tincture consists of flavonoids and it isn't exact dozed to active substance that isn't standardized.

We standardized liquid extract of "St. John's Wort" 300 which obtained based on standardized dry extract of St. John's Wort (*Hypericum scabrum*) according to BP and EP, as well as dry extract. Besides, we added other parameters, which determined in liquid extracts.

Appearance: Appearance of prepared liquid extract should be colorless and a brownish-red liquid.

Identification: ***A. Thin-layer chromatography (2.2.27).***

Test solution: Obtained liquid extract. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Preparing reference solution, mobile phase, TLC plate, spraying solutions and other conditions are made as dry extract of St. John's Wort.

B. HPLC. Examine the chromatograms obtained in the test for assay.

Results: The principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the reference solution (St. John's Wort dry extract CRS).

C. Alternative method. HPLC. Examine the chromatograms obtained in the test for assay.

Results: The principal peak (hypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peak (hypericin) in the chromatogram obtained with the reference solution (hypericin).

Filling up volume: Determining of filling up volume is made at temperature 25°C with using 10 bottles of liquid extract. Transfer the contents into a dry measuring cylinder of such a capacity that the volume to be

determined occupies at least 40 per cent of the nominal volume of the cylinder (over 150 ml). Measure the volume transferred. The volume should be from 97 ml to 103 ml (OST 64-492-85).

Density of liquid extract. Density should be from 0.85 g/ml to 0.87 g/mg (GP XI, edition I, p.24, method 1) [103].

The density is determined by dividing the weight in air of the quantity of the liquid being examined that fills a pycnometer at temperature 20°C by the weight in air of water required to fill the pycnometer after making allowance for the thrust of the air.

The density is calculated from the expression:

$$\rho_{20} = \frac{(m_2 - m) \cdot 0.99703}{m_1 - m} + 0.0012$$

m – mass of empty pycnometer in gram;

m_1 – mass of pycnometer with purified water in gram;

m_2 – mass of liquid extract in gram;

0.99703 – the density of water at temperature 20°C in g/cm^3 (taking into account density of air);

0,0012 – density of air at temperature 20°C and at barometric pressure 1011 GPa.

Assay: Total hypericins, expressed as hypericin ($C_{30}H_{16}O_8$; M_r 504.5): 0.89 mg to 0.99 mg in 5 ml liquid extract.

Liquid chromatography (2.2.29).

Method 1.

Test solution: Weight 5 ml of the liquid extract and dissolve to be examined in 100 ml of methanol R. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Reference solution: Dissolve a quantity of *St. John's Wort standardized dry extract CRS* corresponding to 0.9 mg of hypericin in 100 ml of methanol R. Sonicate and centrifuge. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Column:

- size: l=0.15 m, Ø=4.6 mm;

- stationary phase: octadecylsilyl silica gel for chromatography R (5 µm);

- temperature: 40°C.

Mobile phase: Mix 39 volumes of ethyl acetate R, 41 volumes of a 15.6 g/l solution of sodium dihydrogen phosphate R adjusted to pH 2.0 with phosphoric acid R and 160 volumes of methanol R.

Flow rate: 1.0 ml/min.

Detection: Spectrophotometer at 590 nm.

Injection: 20 µl.

Run time: 15 min.

Identification of peaks: Use the chromatogram supplied with *St. John's Wort standardized extract CRS* and the chromatogram obtained with the reference solution to identify the peaks due to pseudohypericin and hypericin.

System suitability: reference solution:

- the chromatogram obtained is similar to the chromatogram supplied with *St. John's Wort standardized extract CRS;*

- *resolution:* minimum 2 between the peaks due to pseudohypericin and hypericin.

Calculate the percentage content of total hypericins, expressed as hypericin, using the following expression:

$$X = \frac{(S_1 + S_2) \cdot m_0 \cdot 100 \cdot \rho \cdot 5 \cdot P}{S_0 \cdot 100 \cdot m_1 \cdot 100} = \frac{(S_1 + S_2) \cdot m_0 \cdot \rho \cdot 5 \cdot P}{S_0 \cdot m_1 \cdot 100}$$

S_0 – area of the peak due to hypericin in the chromatogram obtained with the reference solution;

S_1 – area of the peak due to pseudohypericin in the chromatogram obtained with the test solution;

S_2 – area of the peak due to hypericin in the chromatogram obtained with the test solution;

m_0 – mass of *St. John's Wort standardized extract CRS* used to prepare the reference solution, in grams;

m_1 – mass of the liquid extract to be examined used to prepare the test solution, in grams;

ρ – density of the liquid extract, in g/ml;

5 – 5 ml liquid extract, in ml;

P – percentage content of hypericin in *St. John's Wort standardized extract CRS*, in %.

Method 2. Alternative method. HPLC. Condition of chromatography and test solution are the same with *"Method 1"*, only reference solution is another.

Reference solution: Dissolve 1.0 mg of *hypericin CRS* to be examined in 100 ml of methanol R. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Calculate the percentage content of total hypericins, expressed as hypericin, using the following expression:

$$X = \frac{(S_1 + S_2) \cdot m_0 \cdot 100 \cdot \rho \cdot 5 \cdot P}{S_0 \cdot 100 \cdot m_1 \cdot 100} = \frac{(S_1 + S_2) \cdot m_0 \cdot \rho \cdot 5 \cdot P}{S_0 \cdot m_1 \cdot 100}$$

S_0 – area of the peak due to hypericin in the chromatogram obtained with the reference solution;

S_1 – area of the peak due to pseudohypericin in the chromatogram obtained with the test solution;

S_2 – area of the peak due to hypericin in the chromatogram obtained with the test solution;

m_0 – mass of *hypericin CRS* used to prepare the reference solution, in grams;

m_1 – mass of the extract to be examined used to prepare the test solution, in grams;

ρ – density of the liquid extract, in g/ml;

5 – 5 ml liquid extract, in ml;

P – percentage content of hypericin in *hypericin CRS*, in %.

Assay: Flavonoids, expressed as rutin ($C_{27}H_{30}O_{16}$; M_r 610.5): minimum 18.0 mg in 5 ml liquid extract, hyperforin ($C_{35}H_{52}O_4$; M_r 536.8): maximum 18.0 mg in 5 ml liquid extract.

Liquid chromatography (2.2.29). *Carry out the assay protected from light.*

Solvent mixture: water R, methanol R (20:80 V/V).

Test solution: Weight 5 ml of the liquid extract and dissolve to be examined in 100 ml of solvent mixture. Sonicate and centrifuge. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Reference solution (a): Dissolve 70.0 mg of *rutoside trihydrate CRS* in 200 ml of solvent mixture. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Reference solution (b): Dissolve 75.0 mg of *St. John's Wort standardized extract CRS* in 20 ml of solvent mixture. Sonicate and centrifuge. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Column:

- size: l=0.15 m, Ø=4.6 mm;
- stationary phase: octadecylsilyl silica gel for chromatography R (3 μm);
- temperature: 40°C.

Mobile phase:

- *mobile phase A:* phosphoric acid R, water R (3:1000 V/V);
- *mobile phase B:* phosphoric acid R, acetonitrile R (3:1000 V/V);

Condition of gradient for HPLC is shown in Table 8 (annex, table 8, p.102).

Detection: Spectrophotometer at 360 nm, then at 275 nm after the elution of biapigenin (about 22 min).

Injection: 10 μl.

Identification of peaks: Use the chromatogram supplied with *St. John's Wort standardized extract CRS* and the chromatogram obtained with the reference solution (b) to identify the peaks due to rutin, hyporoside,

isoquercitroside, quercitroside, quercetin, biapigenin, hyperforin and adhyperforin.

System suitability: reference solution (b):

- the chromatogram obtained is similar to the chromatogram supplied with *St. John's Wort standardized extract CRS;*

- *resolution:* minimum 2 between the peaks due to rutin and hyporoside, and minimum 2.0 between the peaks due to hyperforin and adhyperforin.

Calculate the percentage content of total flavonoids, expressed as rutin, using the following expression:

$$X = \frac{(S_4 + S_5 + S_6 + S_7 + S_8 + S_9) \cdot m_2 \cdot 100 \cdot \rho \cdot 5 \cdot P}{S_3 \cdot 200 \cdot m_3} =$$

$$= \frac{(S_4 + S_5 + S_6 + S_7 + S_8 + S_9) \cdot m_2 \cdot \rho \cdot 5 \cdot P}{S_3 \cdot m_3 \cdot 2}$$

S_3 – area of the peak due to rutin in the chromatogram obtained with the reference solution (a);

S_4 – area of the peak due to rutin in the chromatogram obtained with the test solution;

S_5 – area of the peak due to hyporoside in the chromatogram obtained with the test solution;

S_6 – area of the peak due to isoquercitroside in the chromatogram obtained with the test solution;

S_7 – area of the peak due to quercitroside in the chromatogram obtained with the test solution;

S_8 – area of the peak due to quercetin in the chromatogram obtained with the test solution;

S_9 – area of the peak due to biapigenin in the chromatogram obtained with the test solution;

m_2 – mass of *rutoside trihydrate CRS* used to prepare the reference solution, in grams;

m_3 – mass of the extract to be examined used to prepare the test solution, in grams;

ρ – density of the liquid extract, in g/ml;

5 – 5 ml liquid extract, in ml;

P – percentage content of rutin in *rutoside trihydrate CRS,* in %.

Calculate the percentage content of hyperforin, using the following expression:

$$X = \frac{S_{10} \cdot m_2 \cdot 100 \cdot P \cdot \rho \cdot 5 \cdot 2.3}{S_3 \cdot 200 \cdot m_3} = \frac{S_{10} \cdot m_2 \cdot P \cdot \rho \cdot 5 \cdot 2.3}{S_3 \cdot m_3 \cdot 2}$$

S_3 – area of the peak due to rutin in the chromatogram obtained with the reference solution (a);

S_{10} – area of the peak due to hyperforin in the chromatogram obtained with the test solution;

m_2 – mass of *rutoside trihydrate CRS* used to prepare the reference solution, in grams;

m_3 – mass of the extract to be examined used to prepare the test solution, in grams;

ρ – density of the liquid extract, in g/ml;

5 – 5 ml liquid extract, in ml;

2.3 – correction factor for hyperforin with respect to rutin;

P – percentage content of rutin in *rutoside trihydrate CRS,* in %.

Storage: Store protected from light at the temperature 15-25°C in a well closed container.

Results of the liquid extract of "St. John's Wort" 300 is shown in Table 11 (annex, table 11, p.104).

Obtained the liquid extract of "St. John's Wort" 300 is made at first time by us and obtaining time of the liquid extract is very short. We developed method of dry extract of St. John's Wort from BP and EP for the liquid extract of "St. John's Wort" 300 by the methods of TLC, HPLC. We added parameters appearance of liquid extract, HPLC methods of identification, filling of volume,

density of liquid extract. Advantage side of this liquid extract is, at first, technology of obtaining the liquid extract is easy, at second, taking this liquid extract is convenient for all people.

7. Development of technology and standardization capsulated form based on the dry extract of *Hypericum scabrum*

Dry extract of St. John's Wort is a hydroscopic powder which is freely dissolved in ethanol, methanol and but insoluble in water. We had learned the main technological properties of dry extract of St. John's Wort before selected excipients for capsulated form "St. John's Wort" 300, they are fractional composition, flowability, bulk density, compressibility, compression rate and residual moisture. Results defined technologic parameters are given in Table 14 (annex, table 14, p.106).

In Table 12 (annex, table 12, p.105) shown to some technological parameters of dry extract, do not have proper for formulation capsulated form: flow ability and compressibility properties. We did research to make better these parameters by using different excipients and methods. There are some excipients which are improved technological parameters of active substance without using wet granulation. Therefore, we tested direct compression method and wet granulation method.

We have developed the drug formulation of "St. John's Wort" 300 capsules using standardized dry extract of St. John's wort as active substance. Other ingredients (excipients): microcrystalline cellulose (MCC) is added to the formulation as filling agents, silica colloidal anhydrous (aerosil 200) is used as glidant, magnesium stearate as lubricant, ethanol 50% as solvent and empty transparent - transparent hard gelatine capsules #00. Using these excipients we made three compositions in different ratio of them (annex, table 13, p.105).

Microcrystalline cellulose. It is a pure, tasteless, odorless, partially depolimerised cellulose derivative which has a porous structure. Different types of microcrystalline cellulose possessing different properties such as

hydrophillicity, flowability and particle size are suitable for direct compression, wet granulation suspension manufacture. In the formulation, 41.4% microcrystalline cellulose was used as filling and direct compression agent in order to enhance flow ability.

Silica; Colloidal Anhydrous (Aerosil 200). Silica; Colloidal Anhydrous (Aerosil 200) is added to capsule form in order to improve the flow properties of powder and reduce the friction of particles. Light, loose, bluish-white, odorless, tasteless, non-gritty, amorphous powder with a particle size of 15 nm. It is commonly used as glidant Silica; Colloidal Anhydrous (Aerosil 200) is selected as glidant and used in a ratio of 1.0% in the finished product formulation.

Magnesium stearate. Magnesium stearate provides easy remove from the seal. It also prevents adhesion to punch and it shows lubricant properties since it reduces friction constant for powder particles flowing over each other. It is insoluble in water, alcohol and ether, slightly soluble in hot alcohol and benzene. It is a powder which is cohesive and with poor flowability properties. It is incompatible with acidic and alkaline materials and ferric salts. It should be avoided to mix with strong oxidants. It should be used carefully with the active substances, which are incompatible with alkaline materials. It may prolong the solubility time of the drugs due to its hydrophobic property. Thus, lower concentrations are preferred. It is used in the finished product formulation in 1.0%.

Ethanol 50%. It is solvent which dissolved ethanol 96% with water, and it is a light, volatile, and colorless, with a distinctive odor. Daily dose unit is 5000 ppm. It is used for wet granulation solvent, especially for extracts.

For preparing composition 1 we used direct compression, which economical, i.e., does not require additional equipments, reduces energy consumption, raises productivity. As a result, we took unsatisfactory results; we could not improve flow ability of capsule mass.

Composition 2 was based on wet granulation method. After wet granulation we improved flow ability of capsule mass, but filling capsules was more i.e. bulk density was a bit more.

Then to make better bulk density we reduced 2 times (from 2.0% to 1.0%) content of aerosil 200 with wet granulation method. Obtaining composition 3 was wholly satisfactory in technological parameters of capsulated mass.

Moreover, we selected solvents for wet granulation when we were researching wet granulation method. We took three solvents: purified water, ethanol 96% and ethanol 50%. Powders didn't connect when wet granulation with water, so when wet granulation with ethanol 96% solvent flies very quickly and then we probed with solvent which the same per cent of water and ethanol 96% (ethanol 50%), the powders connected with each other, and we obtained granules for capsulated form "St. John's Wort" 300.

Preparing capsules with composition 3 conform to requirements with GP XI, edition II and BP, EP. Next researches were determination of technological parameters capsulated masses (annex, table 14, p.106) [104].

8. Technology of "St. John's Wort" 300capsules

Composition of "St. John's Wort" 300 capsules (for 1 capsule) is following in Table 13 (annex, table 13, p.105):

Technology of obtaining "St. John's Wort" 300 capsules consists of following processes:

Initial processes (IP):

IP 1.1. Preparing personals.

IP 1.2. Preparing equipment, air and manufacturing rooms.

IP 1.3. Preparing raw material:

Weight sufficient supply of active substance (standardized dry extract of St. John's Wort).

IP 1.4. Preparing excipient:

Weighting excipients (microcrystalline cellulose (MCC), aerosil 200, magnesium stearate and empty transparent - transparent hard gelatine capsules #00) and solvents (etanol 96% and water).

Technological processes (TP):

TP 1. Wet granulation.

TP 1.1. Preparing solvent for wet granulation.

Preparing solvent of ethanol 50%. Prepare this solvent according to GP XI, edition 1: ethanol 96%: purified water (526 ml : 504 ml).

TP 1.2. Mixing.

Sieving standardized dry extract of St. John's Wort, microcrystalline cellulose (MCC), aerosil 200 through appropriate size of sieve and then mix them.

TP 1.3. Wetting.

Wet granulation is carry out with prepared solvent of ethanol 50%. Wetting is made with a little at a time to doesn't form clods.

TP 1.4. Wet granulation.

After wetting, mix all ingredients well. Wet granules are sieved through appropriate size of sieve.

TP 2. Drying.

TP 2.1. Wet granules are spread in the trays and put in the oven. Dried in the oven at 45°C till necessary residual wet.

TP 3. Dry granulation.

TP 3.1. Dry granulation.

Dried granules are sieved through appropriate size of sieve.

TP 3.2. Powdering.

Powdering is made by addition of magnesium stearate till obtaining homogeneous mixture.

TP 4. Capsule filling and dedusting.

TP 4.1. Capsule filling.

Capsule filling is carry out with machine PTK.

TP 4.2. Dedusting**.**

P 1. Packing.

Finally, products are packed into the bottles or blister over 30 capsules [105,106].

Technological chart of obtaining dry extract of St. John's Wort is listed in Fig. 10 (annex, fig.10, p.119).

9. Standardization of "St. John's Wort" 300 capsules

Appearance: Hard gelatine transparent - transparent capsules #00 colorless, transparent capsules #00, containing brownish-grey powder.

Identification: ***A. Thin-layer chromatography (2.2.27).***

Test solution: Disperse 441.7 mg of the capsules content to be examined in 5 ml of methanol R. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Preparing reference solution, mobile phase, TLC plate, spraying solutions and other conditions are made as dry extract of St. John's Wort.

B. HPLC. Examine the chromatograms obtained in the test for assay.

Results: The principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the reference solution (St. John's Wort dry extract CRS).

C. Alternative method. HPLC. Examine the chromatograms obtained in the test for assay.

Results: The principal peak (hypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peak (hypericin) in the chromatogram obtained with the reference solution (hypericin).

Average weight and uniformity of mass. Average weight capsules should be 530 mg ±10% (from 477.00 mg to 583.00 mg).

Weight individually 20 units taken at random, the contents of 20 units, and determine the average mass. Not more than two of the individual masses deviate from the average mass by more than 10% and none deviates by more than twice that percentage (20%).

Weigh an intact capsule. Open the capsule without losing any part of the shell and remove the contents as completely as possible. For soft shell capsules, wash the shell with a suitable solvent and allow standing until the odour of the solvent is no longer perceptible. Weigh the shell. The mass of the contents is the difference between the weighings. Repeat the procedure with another 19 capsules.

Water content. Not more than 6.0% (Karl-Fischer Method).

Add about 20 ml of methanol R the titration vessel and titrate to the electrometric end-point with the *Karl Fischer reagent VS*. Quickly transfer the prescribed amount of the substance (about 500 mg) to be examined to the titration vessel. Stir for 1 minute and titrate again to the electrometric end-point.

Calculate the water content using the formula:

$$water\ (\%) = \frac{V \cdot F \cdot 100}{M}$$

V – volume of the *Karl Fischer reagent VS* expending for titration;

T – factor of the *Karl Fischer reagent VS*;

M – the mass in milligrams of the substance to be examined.

Disintegration time. Capsules should be disintegrate not more than 30 minute.

Tested over 6 capsules at 37°C in purified water.

Residual solvents. Ethanol not more than 5000 ppm.

Method GC.

Column: Innowax 30 m * 0.53 mm * 1 μm;

Flow rate helium/nitrogen: 7.3 ml/min;

Pressure: 5.0 psi;

Average rate: 49 cm/sec;

Detector: FID:

Oven: is shown in Table 7 (annex, table 7, p.102).

Temperature: 250°C;

Flow hydrogen: 40 ml/min;

Flow air: 400;

Flow carrier: 30 ml/min;

Inlet:

Carrier gas: helium or nitrogen;

Oven: 180°C;

Pressure: 5.0 psi;

Total flow: 46.5;

Split ratio: 5:1;

Flow split: 36.4;

Solvent and blank: Dimethylacetamid (DMA);

Reference solution of ethanol: Weight 500 mg of ethanol R in DMA and dilute to 100 ml with the same solvent. Dilute 1.0 ml of this solution to 50 ml with the same solvent. Filter the solution through a membrane filter with a porosity of 0.45 mm (0.1 mg/ml).

Test solution: Weight 353.3 mg of capsules content in DMA and dilute to 10 ml with the same solvent. Filter the solution through a membrane filter with a porosity of 0.45 mm (20 mg/ml).

Injection: 1 µl.

Note: Add the blank, reference solution ethanol and test solution into head-space bottles over 5 ml all solution.

Calculation:

$$ppm = \frac{S_1 \cdot C_{st} \cdot 1000000}{S_{st} \cdot C_1}$$

S_{st} – area of the ethanol in the chromatogram obtained with the reference solution ethanol;

S_1 – area of the ethanol in the chromatogram obtained with the test solution;

C_{st} – concentration of reference solution ethanol, (mg/ml);

C_1 – concentration of test solution, (mg/ml).

Microbial limit: Total Bacteria: NMT 1000 cfu/g, fungi: NMT 100 cfu/g, E.Coli Absent/g: Absent/g.

Assay: Total hypericins, expressed as hypericin ($C_{30}H_{16}O_8$; M_r 504.5): 0.81 mg to 0.99 mg.

Liquid chromatography (2.2.29).

Method 1.

Test solution: Dissolve 123.7 mg of the capsules content to be examined in 25 ml of methanol R. Sonicate and centrifuge the solution. Filter the solution through a membrane filter with a porosity of 0.45 mm (0.0084 mg/ml).

Reference solution: Dissolve a quantity of *St. John's Wort standardized dry extract CRS* corresponding to 0.15 mg of hypericin in 25 ml of methanol R. Sonicate and centrifuge. Filter the solution through a membrane filter with a porosity of 0.45 mm (0.0084 mg/ml).

Column:

- size: l=0.15 m, Ø=4.6 mm;

- stationary phase: octadecylsilyl silica gel for chromatography R (5 μm);

- temperature: 40°C.

Mobile phase: Mix 39 volumes of ethyl acetate R, 41 volumes of a 15.6 g/l solution of sodium dihydrogen phosphate R adjusted to pH 2.0 with phosphoric acid R and 160 volumes of methanol R.

Flow rate: 1.0 ml/min.

Detection: Spectrophotometer at 590 nm.

Injection: 20 μl.

Run time: 15 min.

Identification of peaks: Use the chromatogram supplied with *St. John's Wort standardized extract CRS* and the chromatogram obtained with the reference solution to identify the peaks due to pseudohypericin and hypericin.

System suitability: reference solution:

- the chromatogram obtained is similar to the chromatogram supplied with *St. John's Wort standardized extract CRS;*

- *resolution:* minimum 2 between the peaks due to pseudohypericin and hypericin.

Calculate the content of total hypericins, expressed as hypericin, calculated in one capsule content, using the following expression:

$$X = \frac{(S_1 + S_2) \cdot m_0 \cdot 25 \cdot m_a \cdot P}{S_0 \cdot 25 \cdot m_1 \cdot 100} = \frac{(S_1 + S_2) \cdot m_0 \cdot m_a \cdot P}{S_0 \cdot m_1 \cdot 100}$$

S_0 – area of the peak due to hypericin in the chromatogram obtained with the reference solution;

S_1 – area of the peak due to pseudohypericin in the chromatogram obtained with the test solution;

S_2 – area of the peak due to hypericin in the chromatogram obtained with the test solution;

m_0 – mass of *St. John's Wort standardized extract CRS* used to prepare the reference solution, in milligrams;

m_1 – mass of the test to be examined used to prepare the test solution, in milligrams;

m_a – average weight of capsules, in milligrams;

P – percentage content of hypericin in *St. John's Wort standardized extract CRS*, in %.

Method 2. Alternative method. HPLC. Condition of chromatography and test solution are the same with *"Method 1"*, only reference solution is another.

Reference solution: Dissolve 1.0 mg of *hypericin CRS* to be examined in 100 ml of methanol R. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Calculate the content of total hypericins, expressed as hypericin, calculated in one capsule content, using the following expression:

$$X = \frac{(S_1 + S_2) \cdot m_0 \cdot 25 \cdot m_a \cdot P}{S_0 \cdot 100 \cdot m_1 \cdot 100} = \frac{(S_1 + S_2) \cdot m_0 \cdot m_a \cdot P}{S_0 \cdot m_1 \cdot 4 \cdot 100}$$

S_0 – area of the peak due to hypericin in the chromatogram obtained with the reference solution;

S_1 – area of the peak due to pseudohypericin in the chromatogram obtained with the test solution;

S_2 – area of the peak due to hypericin in the chromatogram obtained with the test solution;

m_0 – mass of *hypericin CRS* used to prepare the reference solution, in milligrams;

m_1 – mass of the test to be examined used to prepare the test solution, in milligrams;

m_a – average weight of capsules, in milligrams;

P – percentage content of hypericin in *hypericin CRS*, in %.

Assay: Flavonoids, expressed as rutin ($C_{27}H_{30}O_{16}$; M_r 610.5): minimum 18.0 mg, hyperforin ($C_{35}H_{52}O_4$; M_r 536.8): maximum 18.0 mg.

Liquid chromatography (2.2.29). *Carry out the assay protected from light.*

Solvent mixture: water R, methanol R (20:80 V/V).

Test solution: Dissolve 123.7 mg of the capsules content to be examined in 20 ml of solvent mixture. Sonicate and centrifuge. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Reference solution (a): Dissolve 70.0 mg of *rutoside trihydrate CRS* in 200 ml of solvent mixture. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Reference solution (b): Dissolve 75.0 mg of *St. John's Wort standardized extract CRS* in 20 ml of solvent mixture. Sonicate and centrifuge. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Column:

- size: l=0.15 m, Ø=4.6 mm;

- stationary phase: octadecylsilyl silica gel for chromatography R (3 μm);

- temperature: 40°C.

Mobile phase:

- *mobile phase A:* phosphoric acid R, water R (3:1000 V/V);

- *mobile phase B:* phosphoric acid R, acetonitrile R (3:1000 V/V);

Detection: Spectrophotometer at 360 nm, then at 275 nm after the elution of biapigenin (about 22 min).

Injection: 10 μl.

Condition of gradient for HPLC is shown in Table 8 (annex, table 8, p.102).

Identification of peaks: Use the chromatogram supplied with *St. John's Wort standardized extract CRS* and the chromatogram obtained with the reference solution (b) to identify the peaks due to rutin, hyporoside, isoquercitroside, quercitroside, quercetin, biapigenin, hyperforin and adhyperforin.

System suitability: reference solution (b):

- the chromatogram obtained is similar to the chromatogram supplied with *St. John's Wort standardized extract CRS;*

- *resolution:* minimum 2 between the peaks due to rutin and hyporoside, and minimum 2,0 between the peaks due to hyperforin and adhyperforin.

Calculate the content of total flavonoids, expressed as rutin, calculated in 1 capsule content, using the following expression:

$$X = \frac{(S_4 + S_5 + S_6 + S_7 + S_8 + S_9) \cdot m_2 \cdot 20 \cdot m_a \cdot P}{S_3 \cdot 200 \cdot m_3 \cdot 100} =$$

$$= \frac{(S_4 + S_5 + S_6 + S_7 + S_8 + S_9) \cdot m_2 \cdot m_a \cdot P}{S_3 \cdot m_3 \cdot 10 \cdot 100}$$

S_3 – area of the peak due to rutin in the chromatogram obtained with the reference solution (a);

S_4 – area of the peak due to rutin in the chromatogram obtained with the test solution;

S_5 – area of the peak due to hyporoside in the chromatogram obtained with the test solution;

S_6 – area of the peak due to isoquercitroside in the chromatogram obtained with the test solution;

S_7 – area of the peak due to quercitroside in the chromatogram obtained with the test solution;

S_8 – area of the peak due to quercetin in the chromatogram obtained with the test solution;

S_9 – area of the peak due to biapigenin in the chromatogram obtained with the test solution;

m_2 – mass of *rutoside trihydrate CRS* used to prepare the reference solution, in milligrams;

m_3 – mass of the test to be examined used to prepare the test solution, in milligrams;

m_a – average weight of capsules, in milligrams;

P – percentage content of rutin in *rutoside trihydrate CRS*, in %.

Calculate the percentage content of hyperforin, calculated in one capsule content, using the following expression:

$$X = \frac{S_{10} \cdot m_2 \cdot 20 \cdot m_a \cdot P \cdot 2.3}{S_3 \cdot 200 \cdot m_3 \cdot 100} = \frac{S_{10} \cdot m_2 \cdot m_a \cdot P \cdot 2.3}{S_3 \cdot m_3 \cdot 10 \cdot 100}$$

S_3 – area of the peak due to rutin in the chromatogram obtained with the reference solution (a);

S_{10} – area of the peak due to hyperforin in the chromatogram obtained with the test solution;

m_2 – mass of *rutoside trihydrate CRS* used to prepare the reference solution, in grams;

m_3 – mass of the test to be examined used to prepare the test solution, in milligrams;

m_a – average weight of capsules, in milligrams;

2.3 – correction factor for hyperforin with respect to rutin;

P – percentage content of rutin in *rutoside trihydrate CRS,* in %.

Results of standardization capsules "St. John's Wort" 300 is shown in Table 15 (annex, table 15, p.107).

10. Development of technology and standardization tableted form based on the dry extract of *Hypericum scabrum L.*

We studied the tableted form "St. John's Wort" 300 at the same time when we were developing capsulated form "St. John's Wort" 300. We probed three compositions for tableted form such as capsulated form by using excipients MCC, aerosil, and magnesium stearate (annex, table 16, p.108). Flowability was problem in composition 1. Then we made composition 3 of capsulated form, which we selected for capsules, but thickness of tablets was very highest. That is why we reduced average weight of tablet than capsule, i.e. by reducing content of MCC in composition 3. Composition 3 was very optimal composition for tablets.

Microcrystalline cellulose. In the formulation, 33.1% microcrystalline cellulose was used as filling in order to enhance flowability.

Silica; Colloidal Anhydrous (Aerosil 200). Silica; Colloidal Anhydrous (Aerosil 200) is selected as glidant and used in a ratio of 0.95% in the finished product formulation.

Magnesium stearate. It is used in the finished product formulation in 0.95%.

Ethanol 50%. It is used for wet granulation solvent, especially for extracts.

Film Coating Material #16. The film coating material selected for "St. John's Wort" 300 film coated tablet formulation is Opadry #16 which is composed of HPMC 2910/Hypromellose 15 cP, titanium dioxide, ethyl cellulose 10 cP, quinoline yellow aluminum lake, diethyl phthalate, FD&C Yellow #6 / sunset yellow FCF aluminum lake, ponceau 4R aluminum lake. 12% (w/w) dispersion of Opadry #16 prepared with purified water is used in the formulation. At the end of coating process, light green colored film coated tablets are obtained.

Selection solvents for wet granulation were learned at the same time capsules too. Preparing tablets by composition 3 conform to requirements with GP XI, edition II and BP, EP. Next researches were determination of technological parameters capsulated masses (annex, table 17, p.108).

11. Technology of tablets "St. John's Wort" 300

Composition of "St. John's Wort" 300 tablet (for 1 tablet) is following in Table 16 (annex, table 16, p.108):

Technology of obtaining "St. John's Wort" 300 tablets consists of following processes:

Initial processes (IP):

IP 1.1. Preparing personals.

IP 1.2. Preparing equipment, air and manufacturing rooms.

IP 1.3. Preparing raw material:

Weight sufficient supply of active substance (standardized dry extract of St. John's Wort).

IP 1.4. Preparing excipient:

Weighting excipients (microcrystalline cellulose (MCC), aerosil 200, magnesium stearate and empty transparent - transparent hard gelatine tablets #00) and solvents (etanol 96% and water).

Technological processes (TP):

TP 1. Wet granulation.

TP 1.1. Preparing solvent for wet granulation.

Preparing solvent of ethanol 50%. Prepare this solvent according to GP XI, edition 1: ethanol 96% : purified water (526 ml : 504 ml).

TP 1.2. Sieving.

Sieving standardized dry extract of St. John's Wort, microcrystalline cellulose (MCC), aerosil 200 through appropriate size of sieve and then mix them.

TP 1.3. Wetting.

Wet granulation is carry out with prepared solvent of ethanol 50%. Wetting is made with a little at a time to does not form clods.

TP 1.4. Wet granulation.

After wetting, mix all ingredients till obtaining homogeneous mass. Wet granules are sieved through appropriate size of sieve.

TP 2. Drying.

TP 2.1. Wet granules are spread in the trays and put in the oven. Dried in the oven at 45°C till since humidity value reaches 3%.

TP 3. Dry granulation.

TP 3.1. Dry granulation.

Dried granules are sieved through appropriate size of sieve.

TP 3.2. Dry mixing.

Dry mixing is made with adding magnesium srearate till obtaining homogeneous mixture.

TP 4. Compressing and dedusting.

TP 4.1. Compressing.

After preparing homogeneous mixture is given to next process (compressing). This process is made in tablet machine.

TP 4.2. Dedusting**.**

TP 5. Film coating.

TP 5.1. Preparing solvent for coating tablets. Film coating solution is prepared in a proper stainless steel pan. Film coating matter Opadry #16 and purified water are mixed until a homogenous solution is obtained.

TP 5. Film coating of tablets.

Tablets are taken into film coating pan and turned. The coating process is continued until the solution is finished.

P 1. Packing.

Finally products are packed into the bottles or blister over 30 units.

Technological chart of obtaining "St. John's Wort" 300 tablets is listed in Fig. 11 (annex, fig.11, p.120).

12. Standardization of tablets "St. John's Wort" 300

Appearance: Light green colored, oval, biconvex, film coated tablets, one face is notched.

Tablet Dimensions. Diameter: 14.90 mm ± 0.2 mm, thickness 5.30 mm ± 0.4 mm, width 6.15 mm ± 0.3 mm.

Identification: ***A. Thin-layer chromatography (2.2.27).***

Test solution: Disperse 416.7 mg of the tablet mass to be examined in 5 ml of methanol R. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Preparing reference solution, mobile phase, TLC plate, spraying solutions and other conditions are made as dry extract of St. John's Wort.

B. HPLC. Examine the chromatograms obtained in the test for assay.

Results: The principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the reference solution (St. John's Wort dry extract CRS).

C. Alternative method. HPLC. Examine the chromatograms obtained in the test for assay.

Results: The principal peak (hypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peak (hypericin) in the chromatogram obtained with the reference solution (hypericin).

Average weight and uniformity of mass. Average weight tablets should be 500 mg ±5% (from 475.00 mg to 525.00 mg).

Weight individually 20 units taken at random, the contents of 20 units, and determine the average mass. Not more than 2 of the individual masses deviate from the average mass by more than 5% and none deviates by more than twice that percentage (10%).

Water content. Not more than 6.0% (Karl-Fischer Method).

Add about 20 ml of methanol R the titration vessel and titrate to the electrometric end-point with the *Karl Fischer reagent VS*. Quickly transfer the prescribed amount of powdered tablets (about 500 mg) to be examined to the titration vessel. Stir for 1 minute and titrate again to the electrometric end-point.

Calculate the water content using the formula:

$$water\ (\%) = \frac{V \cdot F \cdot 100}{M}$$

V – volume of the *Karl Fischer reagent VS* expending for titration;

T – factor of the *Karl Fischer reagent VS*;

M – the mass in milligrams of the substance to be examined.

Disintegration time. Tablets should be disintegrate not more than 30 minute.

Tested over 6 tablets at 37°C in purified water.

Residual solvents. Ethanol not more than 5000 ppm.

Method GC.

Column: Innowax 30 m * 0.53 mm * 1 μm;

Flow rate helium/nitrogen: 7.3 ml/min;

Pressure: 5.0 psi;

Average rate: 49 cm/sek;

Detector: FID:

Oven: is shown in Table 7 (annex, table 7, p.102).

Temperature: 250°C;

Flow hydrogen: 40 ml/min;

Flow air: 400;

Flow carrier: 30 ml/min;

Inlet:

Carrier gas: helium or nitrogen;

Oven: 180°C;

Pressure: 5.0 psi;

Total flow: 46.5;

Split ratio: 5:1;

Flow split: 36.4;

Solvent and blank: Dimethylacetamid (DMA);

Reference solution of ethanol: Weight 500 mg of ethanol R in DMA and dilute to 100 ml with the same solvent. Dilute 1.0 ml of this solution to 50 ml with the same solvent. Filter the solution through a membrane filter with a porosity of 0.45 mm (0.1 mg/ml).

Test solution: Weight 333.3 mg of powdered tablets in DMA and dilute to 10 ml with the same solvent. Filter the solution through a membrane filter with a porosity of 0.45 mm (20 mg/ml).

Injection: 1 µl.

Note: Add the blank, reference solution ethanol and test solution into head-space bottles over 5 ml all solution.

Calculation:

$$ppm = \frac{S_1 \cdot C_{st} \cdot 1000000}{S_{st} \cdot C_1}$$

S_{st} – area of the ethanol in the chromatogram obtained with the reference solution ethanol;

S_1 – area of the ethanol in the chromatogram obtained with the test solution;

C_{st} – concentration of reference solution ethanol, (mg/ml);

C_1 – concentration of test solution, (mg/ml).

Microbial limit: Total Bacteria: NMT 1000 cfu/g, fungi: NMT 100 cfu/g, E.Coli Absent/g: Absent/g.

Assay: Total hypericins, expressed as hypericin ($C_{30}H_{16}O_8$; M_r 504.5): 0.81 mg to 0.99 mg.

Liquid chromatography (2.2.29).

Method 1. *Test solution:* Dissolve 116.7 mg of powdered tablets to be examined in 25 ml of methanol R. Sonicate and centrifuge the solution. Filter the solution through a membrane filter with a porosity of 0.45 mm (0.0084 mg/ml).

Reference solution: Dissolve a quantity of *St. John's Wort standardized dry extract CRS* corresponding to 0.15 mg of hypericin in 25 ml of methanol R. Sonicate and centrifuge. Filter the solution through a membrane filter with a porosity of 0.45 mm (0.0084 mg/ml).

Column:

- size: l=0.15 m, Ø=4.6 mm;

- stationary phase: octadecylsilyl silica gel for chromatography R (5 μm);

- temperature: 40°C.

Mobile phase: Mix 39 volumes of ethyl acetate R, 41 volumes of a 15.6 g/l solution of sodium dihydrogen phosphate R adjusted to pH 2.0 with phosphoric acid R and 160 volumes of methanol R.

Flow rate: 1.0 ml/min.

Detection: Spectrophotometer at 590 nm.

Injection: 20 μl.

Run time: 15 min.

Identification of peaks: Use the chromatogram supplied with *St. John's Wort standardized extract CRS* and the chromatogram obtained with the reference solution to identify the peaks due to pseudohypericin and hypericin.

System suitability: reference solution:

- the chromatogram obtained is similar to the chromatogram supplied with *St. John's Wort standardized extract CRS;*

- *resolution:* minimum 2 between the peaks due to pseudohypericin and hypericin.

Calculate the content of total hypericins, expressed as hypericin, calculated in 1 tablet, using the following expression:

$$X = \frac{(S_1 + S_2) \cdot m_0 \cdot 25 \cdot m_a \cdot P}{S_0 \cdot 25 \cdot m_1 \cdot 100} = \frac{(S_1 + S_2) \cdot m_0 \cdot m_a \cdot P}{S_0 \cdot m_1 \cdot 100}$$

S_0 – area of the peak due to hypericin in the chromatogram obtained with the reference solution;

S_1 – area of the peak due to pseudohypericin in the chromatogram obtained with the test solution;

S_2 – area of the peak due to hypericin in the chromatogram obtained with the test solution;

m_0 – mass of *St. John's Wort standardized extract CRS* used to prepare the reference solution, in milligrams;

m_1 – mass of the test to be examined used to prepare the test solution, in milligrams;

m_a – average weight of tablets, in milligrams;

P – percentage content of hypericin in *St. John's Wort standardized extract CRS*, in %.

Method 2. Alternative method. HPLC. Condition of chromatography and test solution are the same with *"Method 1"*, only reference solution is another.

Reference solution: Dissolve 1.0 mg of *hypericin CRS* to be examined in 100 ml of methanol R. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Calculate the content of total hypericins, expressed as hypericin, calculated in 1 tablet, using the following expression:

$$X = \frac{(S_1 + S_2) \cdot m_0 \cdot 25 \cdot m_a \cdot P}{S_0 \cdot 100 \cdot m_1 \cdot 100} = \frac{(S_1 + S_2) \cdot m_0 \cdot m_a \cdot P}{S_0 \cdot m_1 \cdot 4 \cdot 100}$$

S_0 – area of the peak due to hypericin in the chromatogram obtained with the reference solution;

S_1 – area of the peak due to pseudohypericin in the chromatogram obtained with the test solution;

S_2 – area of the peak due to hypericin in the chromatogram obtained with the test solution;

m_0 – mass of *hypericin CRS* used to prepare the reference solution, in milligrams;

m_1 – mass of the test to be examined used to prepare the test solution, in milligrams;

m_a – average weight of tablets, in milligrams;

P – percentage content of hypericin in *hypericin CRS*, in %.

Assay: Flavonoids, expressed as rutin ($C_{27}H_{30}O_{16}$; M_r 610.5): minimum 18.0 mg, hyperforin ($C_{35}H_{52}O_4$; M_r 536.8): maximum 18.0 mg.

Liquid chromatography (2.2.29). *Carry out the assay protected from light.*

Solvent mixture: water R, methanol R (20:80 V/V).

Test solution: Dissolve 116.7 mg of powdered tablets to be examined in 20 ml of solvent mixture. Sonicate and centrifuge. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Reference solution (a): Dissolve 70.0 mg of *rutoside trihydrate CRS* in 200 ml of solvent mixture. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Reference solution (b): Dissolve 75.0 mg of *St. John's Wort standardized extract CRS* in 20 ml of solvent mixture. Sonicate and centrifuge. Filter the solution through a membrane filter with a porosity of 0.45 mm.

Column:

- size: l=0.15 m, Ø=4.6 mm;

- stationary phase: octadecylsilyl silica gel for chromatography R (3 μm);

- temperature: 40°C.

Mobile phase:

- *mobile phase A:* phosphoric acid R, water R (3:1000 V/V);

- *mobile phase B:* phosphoric acid R, acetonitrile R (3:1000 V/V);

Detection: Spectrophotometer at 360 nm, then at 275 nm after the elution of biapigenin (about 22 min).

Injection: 10 μl.

Condition of gradient for HPLC is shown in Table 8 (annex, table 8, p.102).

Identification of peaks: Use the chromatogram supplied with *St. John's Wort standardized extract CRS* and the chromatogram obtained with the reference solution (b) to identify the peaks due to rutin, hyporoside, isoquercitroside, quercitroside, quercetin, biapigenin, hyperforin and adhyperforin.

System suitability: reference solution (b):

- the chromatogram obtained is similar to the chromatogram supplied with *St. John's Wort standardized extract CRS;*

- *resolution:* minimum 2 between the peaks due to rutin and hyporoside, and minimum 2.0 between the peaks due to hyperforin and adhyperforin.

Calculate the content of total flavonoids, expressed as rutin, calculated in 1 tablet, using the following expression:

$$X = \frac{(S_4 + S_5 + S_6 + S_7 + S_8 + S_9) \cdot m_2 \cdot 20 \cdot m_a \cdot P}{S_3 \cdot 200 \cdot m_3 \cdot 100} =$$

$$= \frac{(S_4 + S_5 + S_6 + S_7 + S_8 + S_9) \cdot m_2 \cdot m_a \cdot P}{S_3 \cdot m_3 \cdot 10 \cdot 100}$$

S_3 – area of the peak due to rutin in the chromatogram obtained with the reference solution (a);

S_4 – area of the peak due to rutin in the chromatogram obtained with the test solution;

S_5 – area of the peak due to hyporoside in the chromatogram obtained with the test solution;

S_6 – area of the peak due to isoquercitroside in the chromatogram obtained with the test solution;

S_7 – area of the peak due to quercitroside in the chromatogram obtained with the test solution;

S_8 – area of the peak due to quercetin in the chromatogram obtained with the test solution;

S_9 – area of the peak due to biapigenin in the chromatogram obtained with the test solution;

m_2 – mass of *rutoside trihydrate CRS* used to prepare the reference solution, in milligrams;

m_3 – mass of the test to be examined used to prepare the test solution, in milligrams;

m_a – average weight of tablets, in milligrams;

P – percentage content of rutin in *rutoside trihydrate CRS*, in %.

Calculate the percentage content of hyperforin, calculated in one tablet, using the following expression:

$$X = \frac{S_{10} \cdot m_2 \cdot 20 \cdot m_a \cdot P \cdot 2.3}{S_3 \cdot 200 \cdot m_3 \cdot 100} = \frac{S_{10} \cdot m_2 \cdot m_a \cdot P \cdot 2.3}{S_3 \cdot m_3 \cdot 10 \cdot 100}$$

S_3 – area of the peak due to rutin in the chromatogram obtained with the reference solution (a);

S_{10} – area of the peak due to hyperforin in the chromatogram obtained with the test solution;

m_2 – mass of *rutoside trihydrate CRS* used to prepare the reference solution, in grams;

m_3 – mass of the test to be examined used to prepare the test solution, in milligrams;

m_a – average weight of tablets, in milligrams;

2.3 – correction factor for hyperforin with respect to rutin;

P – percentage content of rutin in *rutoside trihydrate CRS*, in % [107,108].

Results of standardization tablets “St. John’s Wort” 300 is shown in Table 18 (annex, table 18, p.109).

Chapter IV. STABILITY OF CAPSULES "ST. JOHN'S WORT" 300

Obtained dry extract is obtained out at the Institute of Chemistry of Plant Substances (ICPS) and liquid extract, "St. John's Wort" 300 tablets and capsules based on this dry extract are carried out by FV "Nobel Pharmsanoat" as a part of the innovative project # 6-KX-0-18571. Among these FV "Nobel Pharmsanoat" selects capsulated form for registration. For this reason we have been studying stability only for capsulated form.

Stability study is carried out as self-life stability. The stability conditions and data are presented in the dossier. The analytical procedures used for stability tests are done according to finished product specification of "St. John's Wort" 300 capsules. General test methodology is followed and justified during the stability studies.

The purpose of the stability tests was to verify possible variation of the finished product regarding the parameters given in the finished product specifications.

According to the stability data obtained, it is shown that "St. John's Wort" 300 capsules maintain its identity, strength, purity and quality for 2 years, when stored in the described package.

The stability of "St. John's Wort" 300 capsules was carried out including long –term and according to the CPMP – ICH Guidelines "Stability testing guidelines: stability testing of new drug substances and products".

Self-life stability studies: 25°C ± 2°C and 60%RH ± 5%, 24 months;

Test parameters according to shelf life specification of "St. John's Wort" 300 capsules in Chapter 3.2.2. for self-life stability studies are given below:

a) Appearance;
b) Average weight and uniformity of mass;
c) Water content;
d) Disintegration time;
e) Residual solvents;
f) Content of sum hypericins;

g) Content of sum flavonoids, hyperforin;

h) Microbial limit.

There is not recommend make accelerated stability because of they are made based on herbal plants.

1. Self-life stability of capsulated forms

Self-life stability studies are carried out at temperature 25^{o}C± 2°C and 60% ± 5% RH period of 24 months in transparent PVC/PE/PVDC-Al blister packaging. The stability studies are followed at intervals of initial, 3, 6, 9, 12, 18 and 24 months.

We started self-life stability tests in September 2015 and we are continuing other month. We finished until 12 month.

Tested batches of "St. John's Wort" 300 capsules for self-life stability studies and the remarks are given in the Table 19 (annex, table 19, p.109).

Obtaining results of self-life stability "St. John's Wort" 300 capsules for three batches during 9 month are shown in Table 20-22 (annex, table 20-22, p.110-112).

Results of tests: The results of the stability during 9 month checks can be summarized and interpreted as follows with reference to the investigated specifications of the finished product.

a) Physical and pharmaceutical characteristics:

Appearance of the capsules, average weight and uniformity of mass, disintegration and water content remained practically unchanged during 9 month.

b) Chemical characteristics:

The content of the sum hypericins, the contents of the sum flavonoids and hyperforin remained in the limits of acceptability; the test results proved the good chemical stability of the active ingredients in the finished product and the good physical stability of the capsules during 9 month.

c) Microbiologic characteristics:

Microbial limits remained within limits during 9 month stability studies.

d) Characteristics of the packaging:

No interactions of the immediate container with the pharmaceutical dosage form could be found out during this investigation, providing that the primary package is suitable for the pharmaceutical product during 9 month.

Conclusion. From the test results, we can see that no obvious variation on appearance, average weight and uniformity of mass, water content, disintegration time, residual solvents, assays (sum hypericins, sum flavonoids and hyperforin) and microbial limit occurred during 9 month.

After finishing self-life stability (after 24 month), if obtained data will be good physical, pharmaceutical, chemical, microbiological and packaging characteristics, we can store these “St. John’s Wort” 300 capsules under stability condition (transparent PVC/PE/PVDC-Al blister) during 2 years.

Chapter V. BIOEQUIVALENCE OF CAPSULETED FORM "ST. JOHN'S WORT" 300

1. Results of bioequivalence of capsulated form

The capsulated form "St. John's Wort" 300 was given to the Department of Researches of Pharmacology and Toxicology at the Institute of Chemistry and Plant Substances (ICPS) to conduct a bioequivalence of obtained preparation. There was comparison analyses of obtained preparation "St. John's Wort" 300, batch number of S010815, expiry date until 09.2017, producer "Nobel Pharmsanoat", Republic of Uzbekistan with original preparation of "Helarium Hypericum", coating tablets, batch number of 067975, expiry date until 09.2016, producer "Bionorica", Germany.

Active substance of in both preparations is dry extract of St. John's Wort, content of dry extract the same. Difference of them is form of medicines ("St. John's Wort" 300 – capsules, "Helarium Hypericum" - tablets).

Purpose of this research is researching bioequivalence (comparative sharp toxics and active specifics of activity of two preparations.

Study of sharp toxics and active specifics of activity (antidepressant and immunostimulation) carried out in white rats and experiment made together.

General conclusions of bioequivalenty.

1. Active substances of capsules of "St. John's Wort" 300, batch number of S010815, expiry date until 09.2017, producer "Nobel Pharmsanoat", Republic of Uzbekistan with imported preparation of "Helarium Hypericum", coating tablets, batch number of 067975, expiry date until 09.2016, producer "Bionorica", Germany are identified. Both preparations are contented dry extract of St. John's Wort.

2. Domestic capsules of "St. John's Wort" 300 by the characters toxics is not differenced with imported coating tablets of "Helarium Hypericum". Both preparations are included to group IV less toxics.

3. By the activity (antidepressant and immunostimulation) of both preparations isn't differenced.

4. Test preparation “St. John’s Wort” 300 capsules manufactured by “Nobel Pharmsanoat” are bioequivalent by parameter of sharp toxics with comparing preparation of “Helarium Hypericum”, coating tablets, manufactured by “Bionorica” (annex, p.121).

CONCLUSIONS

The study “Development of technology of immunostimulator preparations based on St. John’s Wort (*Hypericum scabrum L.*)” we come to the following conclusions:

1. Chemical compositions of *Hypericum perforatum L.* and *Hypericum scabrum L.* were compared and proved that chemical composition of *Hypericum scabrum L.* are much the same as *Hypericum perforatum L.* which grows in Uzbekistan.

2. Optimal technology was selected for obtaining dry extract of *Hypericum perforatum L.* and *Hypericum scabrum L.* and they were standardized by sum hypericins, hyperforin and sum flavonoids. Prepared dry extract of *Hypericum perforatum L.* contents 0.31% sum hypericins, 25.51% sum flavonoids and 4.36% hyperforin, dry extract of *Hypericum scabrum L.* contents 0.30% sum hypericins, 24.14% sum flavonoids and 4.21% hyperforin.

3. Based on obtained dry extract of *Hypericum scabrum L.* we developed optimal composition and optimal technology of preparing liquid extract of *Hypericum scabrum L.*, tablets and capsules “St. John’s Wort” 300. Then these preparations were standardized and analyzed by all physical and chemical parameters, all parameters were conformed to requirements of in-house specification.

4. From three preparations we selected to produce (in much quantity) capsulated form with collaboration FV “Nobel Pharmsanoat” and stability test are carrying out. Self-life stability is carrying out and not finished yet.

REFERENCES

1. Resolution of the President of the Republic of Uzbekistan № PP-731 on 19.11.2007 “On Programme of Modernization, Technical, and Technological Re-tooling of Companies in the Pharmaceutical Sector up to 2011”.
2. Resolution of the President of the Republic of Uzbekistan № PP-1319 on 07.04.2010 “On Measures for Streamlining Licensing of Pharmaceutical and Medical Practices”.
3. Resolution of the President of the Republic of Uzbekistan № PP-1442 on 15.12.2010 “On priorities of the industrial development of the Republic of Uzbekistan for 2011-2015”.
4. Resolution of the President of the Republic of Uzbekistan № PP-1668 on 27.12.2011 “On the Investment Programme of the Republic of Uzbekistan for 2012”.
5. Dusmuratov M., Imamnazarov J., Hakimov F., Sharipova Kh. Investment guide to the pharmaceutical industry Uzbekistan. – T.: UNDP, 2013. - p. 4-16.
6. Dusmuratov M. Investment guide to the pharmaceutical industry Uzbekistan. – T.: UNDP, 2011. - p. 1-25.
7. Joanne Barnes, Linda A. Anderson and J. David Phillipson. St. John's Wort (Hypericum perforatum L.): a review of its chemistry, pharmacology and clinical properties // Journal of Pharmacy and Pharmacology. - London, 2001. - P. 583-600.
8. O. Grundmann, Y. Lva, O. Kelber, V. Butterweck. Mechanism of St. John's wort extract (STW3-VI) during chronic restraint stress is mediated by the interrelationship of the immune, oxidative defense, and neuroendocrine system // Neuropharmacology. - USA, 2010. - P. 767-773.

9. Wolters Kluwer. The Review of Natural Products, The most complete source of national product information, 5th edition, USA, 2008. - p. 1217-1228.
10. Vikas Kumar, P.N. Singh, A.V. Muruganandam, S.K. Bhattacharya. Hypericum perforatum: Nature's mood stabilizer // Indian Journal of Experimental Biology. - India, Vol. 38. - November 2000. - P. 1077-1085.
11. Anna Rita Bilia, Sandra Gallori, Franco F. Vincieri. St. John's Wort and depression Efficacy, safety and tolerability-an update // Life Sciences. - Italy, 2002. - P. 3077-3096.
12. Miroslav Dostalek, Anna Katarina Stark. St. John's Wort (Hypericum perforatum L.) // Metabolism of Drug and Other Xenobiotics, first edition. - Wiley, 2012. - P. 583-610.
13. Veronika Butterweck. Mechanism of Action of St John's Wort in Depression // CNS Drugs, 17(8). - Germany, 2003. - P. 539-562.
14. Farrukh Sharopov. Combined Faculties for Natural Sciences and for Mathematics of Ruperto-Carola University of Heidelberg, Germany: Dissertation for the degree of Doctor of Natural Sciences. - Tajikistan, 2015. - p. 1-18.
15. Abu Ali inb Sino. The Canon of Medicine (Канон врачебной науки), Academy of Sciences Republic of Uzbekistan. - Abu Ali ibn Sino, Volume II, Tashkent, 1996. - P. 110, 202-203.
16. Abu Ali inb Sino, The Canon of Medicine (Канон врачебной науки), Academy of Sciences Republic of Uzbekistan, - Abu Ali ibn Sino, Volume III, Tashkent, 1996. - P. 204-205, 214-215.
17. Abu Ali inb Sino, The Canon of Medicine (Канон врачебной науки), Academy of Sciences Republic of Uzbekistan, - Abu Ali ibn Sino, Volume IX, Tashkent, 1996. - P. 158-159.
18. Abu Ali inb Sino, The Canon of Medicine (Канон врачебной науки), Academy of Sciences Republic of Uzbekistan, - Abu Ali ibn Sino, Volume X, Tashkent, 1996. - P. 16-21, 58-59, 66-74.

19. Kapranova E.G. St. John’s Wort for the ninety-nine disease. - p. 1-60.
20. Zeb Saddiqe, Ismat Naeem, Alya Maimoona. A review of the antibacterial activity of Hepericum perforatum L. // Journal of Ethnopharmacology. - Ireland, 2010. - P. 511-521.
21. Nazim Mametov. Pharmacological properties of Hypericum perforatum L. // International symposium ethnobotany medicinal plants, folk traditions history, pharmacology ethnomedicine. – Universida Para La Paz Costa Rica, 1999. - P. 1-16.
22. Ute Wolfle, Gunter Seelinger, Christoph M. Schempp. Topical Application of St. John’s Wort (Hypericum perforatum) // Planta Med. - New York, 2013. - P. 109-120.
23. Veronika Butterweck. St. John’s Wort: Quality Issues and Active Compounds // Botanical Medicine: From Bench to Bedside. - Florida, 2009. - P. 69-91.
24. Jinous Asgarpanah. Phytochemistry, pharmacology and medicinal properties of Hypericum perforatum L. // African Journal of Pharmacy and Pharmacology. - Vol. 6(19), 2012. - P. 1387-1394.
25. Jeffrey M. Greeson, Britt Sanford, Daniel A. Monti. St. John’s wort (Hypericum perforatum): a review of the current pharmacological, toxicological, and clinical literature // Psychopharmacology. – 2001. - P. 402–414.
26. Silvio Sosa, Roberto Pace, Anna Bornancin, Paolo Morazzoni, Antonella Riva, Aurelia Tubaro and Roberto Della Loggia. Topical anti-inflammatory activity of extracts and compounds from Hypericum perforatum L. // Journal of Pharmacy and Pharmcology. - Italy, 2007. - P. 703-709.
27. И.О.Ломовский. Влияние условий механохимической обработки на экстракцию гиперицина из травы зверобоя // Химия растительного сырья. - 2012. - №3. - С. 93-99.

28. Mario Wurglics and Manfred Schubert-Zsilavecz. Hypericum Perforatum: A ‘Modern’ Herbal Antidepressant Pharmacokinetics of Active Ingredients // Clin Pharmacokinet. - Germany, 2006. - P. 449-468.
29. Alex Sparreboom, Michael C. Cox, Milin R. Acharya, and William D. Figg. Herbal Remedies in the United States: Potential Adverse Interactions with Anticancer Agents // Journal of clinical oncology. – 2003. - P. 2496-2497.
30. Motavalizadehkakhky Alireza. Antimicrobial activity and chemical composition of essential oils of four Hypericum from Khorasan, Iran // Journal of Medicinal Plants Research. - Vol. 6(12). – 2012. - P. 2478-2487.
31. Farukh S. Sharopov, Isomiddin S. Gulmurodov, William N. Setzer. Essential oil composition of Hypericum perforatum L. and Hypericum scabrum L. growing wild in Tajikistan // Journal of Chemical and Pharmaceutical Research. - USA, 2010. - P. 284-290.
32. Swati Saxena. Obesity associated colon tumorigenesis: An assessment of tumor phenotype. A thesis presented to the University of Waterloo in fulfillment of the thesis requirement for the degree of Master of Science in Biology, Waterloo, Ontario. - Canada, 2006. - P. 41-66.
33. Jiři Patočka. The chemistry, pharmacology, and toxicology of the biologically active constituents of the herb Hypericum perforatum L. // Journal of Applied Biomedicine. – 2003. - P. 61-70.
34. Hasan Cetin Ozen, Mehmet Bashan. The Composition of Fatty Acids in Hypericum scabrum, H. scabroides and H. Amblysepalum // Turk J Chem. - Tubitak, 2003. - P. 723 - 725.
35. Агабалаев А.А., Каранкевич Е.Г., Попова О.П., Кураева З.И. Экстракция гиперицина из зверобоя продырявленного Hypericum perforatum // Известия национальной академии наук Белорусы. – Белорусия, 2011. - №4. - С. 40-42.
36. Варданян Ануш Петросовна. Культивирование Hypericum perforatum L. сопряженным методом in vitro и гидропоники // Автореферат диссертации на соискание ученой степени кандидата биологических

наук по специальности 03.00.14. – «Биотехнология», Ереван. – 2013. - С. 3-6.

37. Reinhold Kerb, Jurgen Brockmoller, Beate Staffeldt, Michael Ploch, and Ivar Roots. Single-Dose and Steady-State Pharmacokinetics of Hypericin and Pseudohypericin // Antimicrobial agents and chemotherapy. - USA, 1996. - P. 2087–2093.
38. Abhishek D. Garg, Damage-associated molecular patterns: revealing the molecular communication between dying cancer cells and the immune system. - Leuven University Press, 2012. - p. 32-105.
39. M.K. AI-Rifaee, T. Aburjai and N. Haddad. Hypericin from Hypericum triquetrifoliu11l in wild and under cultivation: variation revealed by genetic distance // 6'h Conference on Aromatic and Medicinal Plants of Southeast European Countries. - P. 829-847.
40. Gerassim M. Kitanov. Hypericin and pseudohypericin in some Hypericum species // Biochemical Systematics and Ecology. - №29. – 2001. - P. 171-178.
41. Opinion of the Scientific Committee on Food on the presence of hypericin and extracts of Hypericum sp. in flavourings and other food ingredients with flavouring properties. -Scientific Committee on Food, 2002. - p. 2-19.
42. В.В.Пунегов, Р.Л.Сычев, Н.В.Портнягина, Э.Э.Эчишвили. Изменчивость содержания нафтодиантроновых пигментов в сырьевой фитомассе дикорастущих образцов Hypericum maculatum в среднетаежной поднозе Республики Коми // Химии растительного сырья. - 2011. - №3. - С. 139-143.
43. Е.А. Юшкова, О.А. Старцева, Л.К. Рочева, В.Г. Зайнуллин, В.В. Пунегов, Г.Г. Зайнуллин, И.С. Боднарь. Эффективность водорастворимой формы гиперицина при радиационном воздействии на Drosophila melanogaster // Известия Самарского научного центра Российской академии наук. - 2014. - Т. 16, - № 5(5). - С. 1785-1789.

44. Edward C. Bell. The effects of St. John's Wort on the pharmacokinetics of corticosteroid and non-steroidal drug preparations. A Dissertation Submitted to The Graduate Faculty of Auburn University for Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy. - Auburn University, Alabama. - 2005. P. 35-56.
45. Lala Ram Jat. Hyperforin: a potent anti-depressant natural drug // International Journal of Pharmacy and Pharmaceutical Sciences. - Vol. 5. - Suppl 3, 2013. - P. 9-13.
46. Ayman EL-Meghawry EL-Kenawy. Apoptotic and anti-angiogenic activity of Hypericumperforatum L on 4-(methylnitrosamino)- 1-(3-pyridyl)-1-butanone-induced lung cancer in rats // International Journal of Advanced Research. - Volume 2, Issue 1. - 2014. - P. 1057-1065.
47. Robert J. Wolf, Ralf A. Hilger, Jörg D. Hoheisel, Jens Werner, Frank Holtrup. In Vivo Activity and Pharmacokinetics of Nemorosone on Pancreatic Cancer Xenografts // Plos one. - 2013. - P. 1-8.
48. Grigorov L., Pankova S. Hyperforin – benefit on oncology // Interventional oncology sans frontiers. - Italy, 2013. - P. 3-20.
49. Katja Karppinen. Biosynthesis of hypericins and hyperforins in Hypericum perforatum L. (St. John's Wort). Precursors and genes involved. – Universityy of OULU, 2010. - p. 13-21.
50. Omar M.E. Abdel-Salam. Anti-Inflammatory, Antinociceptive, and Gastric Effects of Hypericum perforatum in Rats // The Scientific World Journal. - Egypt, 2005. - P. 585–596.
51. Bernd L. Fiebich, Rainer Knorle, Kurt Appel, Thomas Kammler, Gabriele Weiss. Pharmacological studies in an herbal drug combination of St. John's Wort (Hypericum perforatum) and passion flower (Passiflora incarnata): In vitro and in vivo evidence of synergy between Hypericum and Passiflora in antidepressant pharmacological models // Fitoterapia. - Germany, 2011. - P. 474-480.

52. Karen Pilkington, Anelia Boshnakova, Janet Richardson. St. John's wort for depression: Time for a different perspective? // Complementary Therapies in Medicine. – UK, 2006. - P. 268—281.
53. Katharina Schroecksnadel, Birgit Fischer, Harald Schennach, Guenter Weiss, Dietmar Fuchs. Antioxidants Suppress Th1-type Immune response In Vitro // Drug Metabolism Letters. - Bentham Science Publishers Ltd., 2007. - P. 166-171.
54. Irma Meijerman, Jos H. Beijnen, Jan H.M. Schellensa. Herb–Drug Interactions in Oncology: Focus on Mechanisms of Induction // The Oncologist. - №11. – 2006. - P. 742–752.
55. Marta Kubera, Beata Grygier, Beatriz Arteta, Krystyna Urbanska, Agnieszka Basta-Kaim, Bogusawa Budziszewska, Monika Leoekiewicz, Elbieta Koaczkowska, Michael Maes, Marian Szczepanik, Monika Majewska, Wadysaw Lason. Age-dependent stimulatory effect of desipramine and fluoxetine pretreatment on metastasis formation by B16F10 melanoma in male C57BL/6 mice // Pharmaceutical reports. - Institute of Pharmacology Polish Academy of Sciences. - P.1124.
56. Chung-Hsuen Wu, Access factors associated with the use of St. John's Wort among adults with depressive symptoms. A thesis submitted in partial fulfillment of the requirement for the degree of master of health policy and administration. - Washington, State University. Department of Health Policy and Administration, 2006. - p. 7-35.
57. A.Vandenbogaerde, P.Zanoli, G.Puia, C.Truzzi, A.Kamuhabwa, P.De Witte, W.Merlevede and M.Baraldi. Evidence That Total Extract of Hypericum perforatum Affects Exploratory Behavior and Exerts Anxiolytic Effects in Rats // Pharmacology Biochemistry and Behavior. - Vol. 65. - No. 4. - 2000. - P. 627–633.
58. М.Д. Машковский. Лекарственные средства. Том 1. - Москва, «Новая Волна». 2002. - с. 300.

59. The ABC Clinical Guide to Herbs. - American Botanical Council, 2002. - p. 303-316.
60. St John’s wort (Hypericum perforatum L.). - Materia Medica. - p. 542-552.
61. Абдуллаева Д.Д. Эффективность препарата Гелариум^{R} Гиперикум у женщин, перенесших тяжелую форму преэклампсии и перинатальной потерей // Рецепт. - №2(64). - 2009. - С. 221-224.
62. Steigerwald Arzneimittelwerk. Лайф 900 (LAIF® 900) Hypericum perforatum* N06A X20 // Международный неврологический журнал. - №3 (37). - 2013. - С. 143-144.
63. Kerry Bone. MediHerb. - Product Catalog, 2015. - p. 71.
64. Sisjagin P.N. (RU), Redzhepova G.R. (RU), Nikulin D.M. (RU), Vtjurin S.V. (RU). Patent: Preparation applied for farm animals youngsters being of immunostimulating action, and method for its application. – Russian, 2005. - p. 1-6.
65. О.В. Товчига, С.Ю. Штыголь. Влияние лекарственных растений на выделительную функцию почек // Экспериментальная и клиническая фармакология. - 2009. - С. 50-59.
66. Куркин В.А., Правдицева О.Е. Сравнительное исследование содержания суммы фловоноидов и антраценпроизподных в препаратах травы зверобоя // Химико-фармацевтический журнал. - Москва, 2008. - №10. – С. 39-42.
67. Государственная фармакопея XI, вып.2. Общие методы анализа. – М.: - Медицина, 1990. - с. 156-159.
68. Куркин В.А., Правдицева О.Е. Сравнительное исследование содержания суммы фловоноидов и антраценпроизподных в препаратах травы зверобоя // Химико-фармацевтический журнал. - Москва, 2008. - №10. - С. 39-42.
69. Загурская Ю. В.1, Баяндина И. И. Спектрометрический подход к изучению содержания фенольных соединений в некоторых лекарственных растениях // Лекарственные растения: фундаментальные

и прикладные проблемы Материалы I Международной научной конференции (21—22 мая 2013 г., г. Новосибирск). – Новосибирск, 2013. - С.156-159.

70. О.Е. Правдивцева, В.А. Куркин. Исследования по обоснованию новых подходов к стандартизации сырья и препаратов зверобоя продырявленного // Химия растительного сырья. - 2008. - №1. - С. 81–86.
71. United States Pharmacopeia National Formulary. St. John’s Wort. Powdered St. John’s Wort. Powdered St. John’s Wort Extract. - 2006. - P. 978-980
72. Rocco Longo, Volker Schulz. Hypericum the last science discovery // Natural 1. – 2002. - P. 74-79.
73. Neerja Tewari, Hema Menghani, P. D. Hamrapurkar, Tukaram Mane, Ashish Suthar, Vijay Singh Chauhan. Development of a High-Performance Liquid Chromatographic Method for the Quantitative Determination of Hyperforin // International Journal of Applied Science and Engineering. - 2008. - P. 39-46.
74. Габрук Н. Г., Шутеева Т. А., Метелев А. В. Биологически активные вещества зверобоя продырявленного (Hypericum perforatum L.) // Лекарственные растения: фундаментальные и прикладные проблемы Материалы I Международной научной конференции (21—22 мая 2013 г., г. Новосибирск). – Новосибирск, 2013. - С. 145-148.
75. British Pharmacopoeia. St. John’s Wort. St. John’s Wort Quantified Dry Extract. - Volume IV. - 2015. - P. 3866-3869.
76. European Pharmacopoeia. St. John’s Wort. St. John’s Wort dry extract, quantified. - 7th edition. Monograph 2013. - P. 1241-1243., 4878-4880.
77. Malgorzata Anyoewska, Anna Kowalczuk, Anna Lozak, Renata Jablczyska, Zbigniew Fijalek. Determination of total hypericins in St. John’s Wort and herbal medicinal products // Acta Poloniae Pharmaceutica. - Drug Research, Vol. 67, №6. - Polish Pharmaceutical Society, 2010. - P. 587-593.

78. Gerlie C. De Los Reyes and Robert T.Koda. Determining hyperforin and hypericin content in eight brands of St. John's wort // American Society of Health-System Pharmacists. - Vol. 59. - 2002. - P. 545-547.
79. Л.Н. Зимина, В.А. Куркин, В.М. Рыжов. Исследование флавоноидного состава травы зверобоя пятнистого методом высокоэффективной жидкостной хроматографии // Фармация. – Медицинский альманах, 2012. - С. 227-229.
80. Marcello Nicoletti. HPTLC fingerprint: a modern approach for the analytical determination of Botanicals // Revista Brasileira de Farmacognosia Brazilian Journal of Pharmacognosy. - 21(5). - 2011. - P. 818-823.
81. S. Puri, G. Handa, A.K. Kalsotra, V.K. Gupta, A.S. Shawl, O.P. Suri, and G.N. Qazi. Preparative High-Performance Liquid Chromatographic Separation of Naphthodianthrones from St. John's Wort // Journal of Chromatographic Science. - Vol. 44. - 2006. - P. 177-180.
82. В.П. Махлаюк. Лекарственные растения в народной медицине. - Москва. - с. 23-25.
83. В.В. Карпук. Фармакогнозия. - МИНСК БГУ, 2011. - с. 251-252.
84. Климова Т. С. Семенная продуктивность Hypericum perforatum L. в разных растительных сообществах // Лекарственные растения: фундаментальные и прикладные проблемы Материалы I Международной научной конференции (21—22 мая 2013 г., г. Новосибирск). – Новосибирск, 2013. - С. 55-57.
85. П.П. Гамбарян, М.С. Мусаелян, Е.А. Меликян / Определение запасов сырья рода Hypericum L. в Армении // Биологический журнал Армении. - 1-2 (60). – 2008. - С. 28-33.
86. Ali Kemal Ayan, Oguzhan Yanar, Cuneyt Cirak, Mahmut Bilgener / Morphogenetic and diurnal variation of total phenols in some Hypericum species from Turkey during their phonological cycles // Bangladesh J. - Bot. 36(1). - 2007. - P. 39-46.

87. Nicolai M.N., Sara L.C. Morphological and Phytochemical Diversity Among Hypericum Species of the Mediterranean Basin // Medicinal and Aromatic Plant Science and biotechnology. - Global Science Books, 2011. - P. 14-28.
88. Холматов Х.Х., Ахмедов У.А. Фармакогнозия. – Т.: Ибн Сина, 1995. - с. 137-138.
89. Nicolai M. Nurk. Phylogenetic analyses in St. John's wort (Hypericum) Inferring character evolution and historical biogeography. Dissertation zur Erlangung des akademischen Grades des Doktors der Naturwissenschaften (Dr. rer. nat.) eingereicht im Fachbereich Biologie, Chemie, Pharmazie der Freien Universitat Berlin. 2011. - p. 1-16.
90. Махкамов С.М., Махмуджонова К.С. Тайёр дори турлари технологияси. - Тошкент, «EXTREMUM PRESS», 2010. - 232-282 б.
91. Чуешев В.И., Чернов Н.Е., Хохлова Л.Н. Промышленная технология лекарств, Том 2, - Харков, 2002. - с. 102.
92. The Japanese Pharmacopoeia. General tests, Processes and Apparatus. - 2006. - P. 33-37.
93. Raymond C Rowe, Paul J Sheskey, Marian E Quinn. Handbook of Pharmaceutical Manufacturing Formulations Semisolid Products. Stability Testing of Drug Substances and Drug Products. - Volume 4 of 6. - P. 25-30.
94. Ipek Pesin Suntara, Esra Kupeli Akkola, Demet Yılmazerb, Turhan Baykala, Hasan Kırmızıbekmezc, Murat Alperb, Erdem Yesilada. Investigations on the in vivo wound healing potential of Hypericum perforatum L. // Journal of Ethnopharmacology. - Turkey, 2010. - P. 468-477.
95. Ban Khalid Victor, Wafaa M.A. Al Shaikh Hamed. Preliminary phytochemical screening and evaluation of in vitro antioxidant activity of Iraqi species of Hipericum perforatum aerial part // International research journal of pharmacy. - 2014. - P. 369-373.
96. Sang Wook Kim, Jeong Hoon Park, Seung Dae Yang, Min Goo Hur, Yu-Seok Kim, Jong-Seo Chai, Young Soon Kim, and Kook Hyun Yu. Facile

Synthesis and Radioiodine Labeling of Hypericin // Bull. Korean Chem. Soc. – 2004. - Vol. 25, №8. - P. 1147-1150.

97. О. В. Стасевич, Н. А. Коваленко, Г. Н. Супиченко, В. Н. Леонтьев. Экстракционное выделение гиперицинов из некоторых сортов травы зверобоя продырявленного // Химия, технология органических веществ и биотехнология. - Труды БГТУ, 2012. - №4. - С. 180-182.
98. Е.Г. Кольцова, А.И. Годлевская. Изучения условий экстракции биологически активных компонентов Hypericum perforatum // Вопросы химии и химической технологию. - 2012. - №4. - С. 31-34.
99. Anastasia Karioti, Franco F. Vincieri, Anna R. Bilia. Rapid and efficient purification of naphthodianthrones from St. John's wort extract by using liquid–liquid extraction and SEC // J. Sep. Sci. - 2009. – 32. - P. 1374 – 1382.
100. Jaime Tortoriello, Alejandro Zamilpa, Armando Herrera, Ofelia Romero-Cerecero / Determination of hypericin and hyperforin content in different phytopharmaceuticals and dietary supplements containing Hypericum perforatum extract // Salud Mental. - Vol. 26. - №4. - 2003. - P. 59-63.
101. Т.Ф. Ибрагимов, Д.И. Бегимова, Б.А. Абдурахманов, М.Г. Исмаилова / Стандартизация лекарственной субстанции на основе Hypericum scabrum // Материалы конференции молодых ученых «Актуальные проблемы химии природных соединений», посвященной памяти акад. С.Ю.Юнусова. - Ташкент, 2015. - С. 159.
102. Бегимова Д.И., Ибрагимов Т.Ф., Исмаилова М.Г. Разработка технологии жидкого экстракта зверобоя // Материалы Республиканской научно-практической конференции (с международным участием) «Актуальные вопросы образования, науки ии производства в фармации». - Ташкент, 2015. - С. 172-173.
103. Государственная фармакопея XI, вып.1. Общие методы анализа. – М.: -Медицина, 1987. - с. 24.

104. Raymond C Rowe, Paul J Sheskey, Marian E Quinn. Handbook of Pharmaceutical Excipients. Sixth edition. - USA, 2009. - p. 17, 134, 185, 404.

105. Бегимова Д.И., Ибрагимов Т.Ф. Технологический процесс производства капсул «Зверобой» 300 // Материалы Республиканской научно-практической конференции (с международным участием) «Актуальные вопросы образования, науки ии производства в фармации». - Ташкент, 2015. - С. 172-174.

106. D.I. Begimova, M.A. Kim, T.F. Ibragimov, M.G. Ismailova. Technology and standardization of capsulated form based on the dry extract of Hypericum scabrum // 11th International Symposium on the Chemistry of Natural Compounds. - Antalya, 2015. - P. 129.

107. T.F. Ibragimov, D.I. Begimova, M.G. Ismailova. Development of technology and standardization for tablets based on the dry extract of Hypericum scabrum // 11th International Symposium on the Chemistry of Natural Compounds. - Antalya, 2015. - P. 130.

108. Д.И. Бегимова, Т.Ф. Ибрагимов, М.Г. Исмаилова. Стандартизация таблеток на основе сухого экстракта зверобоя шероховатого (Hypericum scabrum L.) // Фармацевтический журнал. - №4. - Ташкент, 2015. - С. 74-79.

ANNEXES

Table 1

Chemical composition of St. John's Wort and them activity

Component group	Components	Activity
Naphthodianthrones	Hypericin, pseudohypericin, protoohypericin, protopseudohypericin	Antiviral, antidepressant, anti-inflammatory, immunostimulity
Phloroglucinols	Hyperforin, adhyperforin	Antibacterial activity against Gram-positive bacteria, wound-healing, neurotransmitter inhibitor, antidepressant, potential, anticarcinogenic, angiogenesis inhibition, and antimalarial
Flavonoids	Rutin, hyperoside (hyperin), quercitin, isoquercetin, methyhesperidin, iso-quercetrin, quercitrin	Capillary - strengthening, anti-inflammatory, diuretic, cholagogic, dilates coronary, arteries, sedative, tumor inhibition, antitumor, antidiarrheal
Biflavones	3, 8-biapigenin, amentoflavone, 6, 8-diquercetin.	Sedative, antiphlogistic
Phenylpropanes	Hydroxycinnamic acids, p-coumaric acid, caffeic acid, chlorogenic acid	Spasmolytic activity
Proanthocyanidins	Tannins and dimeric, trimeric, and tetrameric procyanidins	Antiphlogistic, antioxidant
Volatile oils	Xanthones	Antidepressant, antimicrobial, antiviral, diuretic, cardiotonic, MAOA inhibitor

Table 2

Comparing chemical composition between *Hypericum perforatum L.* and *Hypericum scabrum L.* which grows in Uzbekistan

Components	***Hypericum perforatum L.* (uzbek)**	***Hypericum scabrum L.* (uzbek)**
Naphthodianthrones (hypericin and pseudohypericin)	0.01 – 0.04%	0.01 – 0.037%
Flavonoids (hyperoside, rutin and quercetin)	0.7%	9 - 11%
Tannins	10 – 12.8%	13.5 - 15%
Essential oils	0.1 – 0.33%	0.2 – 0.29%
Carotin, xolin	до 55%	4 - 7%
Ascorbic acid, organic acids.	+	+

Table 3.

Imported drugs based on St. John's Wort

Name of drug (brand name)	Standardization	Pharmaceutical form	Distributor
Esbericum®	hyperforin 1.47%	capsules containing 60 mg of St John ' s Wort dried extract	Schaper & Bruemmer, Germany
Hypericum 2000 plus®	hypericin 0.055%, hyperforin 0.75%	capsules containing St. John's Wort dried extract equivalent to 2 g dry herb and *Ginkgo biloba* equivalent to 100 mg dry leaf	Nutra - Life, New Zealand
Jarsin 300®	hypericin 0.28%	tablets containing 300 mg of St John ' s Wort dried extract (LI 160)	Lichtwer Pharma AG, Germany
Kira®	hypericin 0.28%	tablets containing 100 mg of St. John's Wort dried extract (LI 160)	Lichtwer Pharma AG, Germany
LI 160®	hypericin 0.12 – 0.28%	Dried extract of St. John's Wort flowers and leaves	Lichtwer Pharma AG, Germany
Neuroplant®	hypericin 0.12 – 0.28, hyperforin 3.0 – 6.0%	tablets containing 300 mg WS 5570 extract	Dr Willmar Schwabe Pharmaceuticals, Germany
Neuroplant® AKTIV	hypericin 0.12 – 0.28, hyperforin 3.0 – 6.0%	tablets containing 600 mg WS 5570 extract	Dr Willmar Schwabe Pharmaceuticals, Germany
Movina®	hyperforin 3.0 – 6.0%	capsules containing 300 mg of St. John's Wort dried extract	Boehringer Ingelheim AB, Sweden
WS® 5570	hypericin 0.12 – 0.28%, hyperforin 3.0 – 6.0%	soft gel containing 300 mg of St. John's Wort dried extract	Dr Willmar Schwabe Pharmaceuticals, Germany
ZE 117®	hypericin 0.12 – 0.28, hyperforin < 0.5%	dried extract of St. John's Wort flowers and leaves	Zeller AG, Switzerland

Table 4.

Comparing plants of *Hypericum perforatum* and *Hypericum scabrum*

Hypericum perforatum	***Hypericum scabrum***
	
Botanical characteristics	
Family: *Hypericaceae*	
- a perennial plant, - stems are 20-50 cm, - leaves are elliptical or oblong-ovate, - bracts are lancetical, - flowers are yellow colored, - seeds are smell, dark brown colored, - flowering: June-July, - fruiting: July-August.	- a perennial plant, - stems are 17-35 cm, - leaves are lancetical or oblong-lancetical, - bracts are oblong-linear, - flowers are yellow colored, - seeds are smell, dark brown colored, - flowering: May-June, - fruiting: July-September.
Geographical distribution	
- Europe, - Russia, - Caucasus, - Iran, - Central Asia, - India, - Mongolia, - China, - Japan, - New Zealand, - Australia, - North America and others.	- Central Asia, - Afghanistan, - Iran, - Iraqi, - Siriy, - Turkey, - Armenia, - Azerbaijan, - Grazes, - Russia (Altai), - Kazakhstan, - Turkmenistan, - Tajikistan, - Uzbekistan, - Pakistan, - China and others.

Table 5.

Yield of dry extracts which obtaining with different reagents and contents of sum hypericins theirs.

Hypericum perforatum L.			
Reagents	**Ratio**	**Yield of dry extracts**	**Content of sum hypericins (%)**
Purified water	1:7	28.3 mg (2.83%)	0.09
Ethanol 40%	1:7	36.1 mg (3.56%)	0.12
Ethanol 60%	1:7	39.4 mg (3.94%)	0.15
Ethanol 80%	**1:7**	57.5 mg (5.75%)	0.29
Ethanol 96%	1:7	49.1 mg (4.91%)	0.23
Ethyl acetate 98%	1:7	27.9 mg (2.79%)	0.18
Butanol 98%	1:7	26.7 mg (2.67%)	0.18
Acetate 98%	1:7	18.5 mg (1.75%)	0.16
Hypericum scabrum L.			
Reagents	**Ratio**	**Yield of dry extracts**	**Content of sum hypericins (%)**
Purified water	1:7	27.4 мг (2.74%)	0.06
Ethanol 40%	1:7	35.6 мг (3.56%)	0.11
Ethanol 60%	1:7	38.1 мг (38.1%)	0.16
Ethanol 80%	**1:7**	**56.4 мг (5.64%)**	**0.27**
Ethanol 96%	1:7	50.7 мг (5.07%)	0.22
Ethyl acetate 98%	1:7	26.6 мг (2.66%)	0.19
Butanol 98%	1:7	29.5 мг (2.95%)	0.18
Acetate 98%	1:7	16.3 мг (1.63%)	0.13

Table 6.

TLC chromatogram of reference and test solutions

Top of the plate	
	A yellowish-orange fluorescent zone 2 red fluorescent zones (hypericin and pseudohypericin) ----------
----------	3 yellowish-orange fluorescent zones
----------	----------
Hyperoside: a yellowish-orange fluorescent zone	A yellowish-orange fluorescent zone (hyperoside)
	Yellow and blue possibly superimposed fluorescent zones
Rutin: a yellowish-orange fluorescent zone	A yellowish-orange fluorescent zone (rutin)
Reference solution	**Test solution**

Table 7.

Condition of oven for GC

	°C/min	**°C**	**Hold, min**	**Flow time**
Start		40	5	5
Ramp I	15	220	6	23.00
Ramp II		50		23.00

Table 8.

Condition of gradient for HPLC

Time (min)	**Mobile phase A (per cent V/V)**	**Mobile phase B (per cent V/V)**	**Flow rate (ml/min)**
0 – 8	82	18	0.8
8 – 18	82 → 47	18 → 53	0.8
18 – 18.1	47 → 3	53 → 97	0.8
18.1 – 19	3	97	0.8 → 1.2
19 – 29	3	97	1.2
29 – 30	3 → 82	97 → 18	1.2

Table 9.

Results of standardization dry extract of St. John's Wort

Parameters	Limits	Results (*Hypericum perforatum L.*)	Results (*Hypericum scabrum L.*)
Appearance	Brownish-grey powder	**Brownish-grey powder**	**Brownish-grey powder**
Identification	A. TLC B. HPLC. The principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the reference solution (St. John's Wort dry extract CRS) C. HPLC. The principal peak (hypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peak (hypericin) in the chromatogram obtained with the reference solution (hypericin)	**A. TLC: Confirm** **B. Confirm** **C. Confirm**	**A. TLC: Confirm** **B. Confirm** **C. Confirm**
Loss on drying	Not more than 4.0%	**3.23%**	**3.37%**
Total ash	Not more than 10%	**4.45%**	**4.12%**
Heavy metals	Not more than 10 ppm	**Менее 10 ppm**	**Менее 10 ppm**
Residual solvents **Ethanol** **Chloroform**	 Not more than 5000 ppm Not more than 5000 ppm	 **1120.8 ppm** **129.7 ppm**	 **1213.5 ppm** **141.2 ppm**
Content of sum hypericins expressed as hypericin	0.1% to 0.3%	**0.31%**	**0.30%**
Content of sum flavonoids expressed as rutin	Minimum 6.0%	**25.51%**	**24.14%**
Content of hyperforin	Maximum 6.0%	**4.36%**	**4.21%**

Table 10.

Composition of liquid extract of St. John's Wort (to 1 bottle)

Name of raw materials	Content
Active substance:	
Standardized dry extract of St. John's Wort (equivalent to 0.9 mg sum hypericins)	6000 mg;
Exipient:	
Ethanol 70%	to 100 ml

Table 11.

Results of standardization liquid extract of "St. John's Wort" 300

Parameters	Limits	Methods	Results
Appearance	Appearance of prepared liquid extract should be colorless and a brownish-red liquid.	Visual	Appearance of prepared liquid extract is colorless and a brownish-red liquid.
Identification	A. TLC B. HPLC. The principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the reference solution (St. John's Wort dry extract CRS) C. HPLC. The principal peak (hypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peak (hypericin) in the chromatogram obtained with the reference solution (hypericin)	A. TLC. B. HPLC. C. HPLC.	A. TLC: Confirm B. The principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the reference solution (St. John's Wort dry extract CRS) C. The principal peak (hypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peak (hypericin) in the chromatogram obtained with the reference solution (hypericin)
Filling of volume	100 ml ± 3% From 97 ml to 103 ml	OST 64-492-85	99.8 ml
Density of liquid extract	From 0.85 g/ml to 0.87 g/ml	GP XI, edition 1, p.24, method 1	0.863 g/ml
Content of sum hypericins expressed as hypericin in 5 ml liquid extract	0.9 mg / 5 ml From 0.89 mg to 0.99 mg	BP, EP	0.92 mg / 5 ml
Content of sum flavonoids expressed as rutin in 5 ml liquid extract	Minimum 18 mg / 5 ml	BP, EP	72.63 mg / 5 ml
Content of hyperforin in 5 ml liquid extract	Maximum 18 mg / 5 ml	BP, EP	13.2 mg / 5 ml

Table 12.

Technological parameters of dry extract St. John's Wort (*Hypericum scabrum*)

Technological parameters	Unit	Obtained results
Fractional composition +1000 -1000 +850 -850 +500 -500 +250 -250 +75	μm, %	0 1.5 8.4 74.2 15.9
Flowability	$kg/s \cdot 10^{-3}$	1.9
Bulk density	kg/m^3	542.3
Compressibility	N	88
Compression rate		2.2
Residual moisture (at the tenperature 70^0C)	%	2.45

Table 13.

Three different compositions for obtaining "St. John's Wort" 300 capsules

Name of ingredients	Composition 1	Composition 2	Composition 3
	Unit, mg	Unit, mg	Unit, mg
Dry extract St. John's Wort	300	300	300
Microcrystalline cellulose (MCC)	165.5	214.1	219.4
Aerosil 200	4.75	10.6	5.3
Magnesium stearate	4.75	5.3	5.3
*Ethanol (60%)	-	0.32	0.32
Total weight:	475	530	530
Empty transparent - transparent hard gelatine capsules №00			

* Not added to the total weight due to evaporation during manufacturing process.

Table 14.

Technological parameters of three compositions for "St. John's Wort" 300 capsules (*Hypericum scabrum*)

Technological parameters	Unit	Composition 1	Composition 2	Composition 3
Fractional composition +1000 -1000 +850 -850 +500 -500 +250 -250 +75	μm, %	0 0.9 8.9 81.6 8.6	0 1.1 8.7 77.5 12.7	0 1.5 8.4 74.2 15.9
Flowability	$kg/s \cdot 10^{-3}$	1.2	1.7	1.9
Bulk density	kg/m^3	701.3	812.7	542.3
Compressibility	N	33	91	88
Compression rate		3.1	2.6	2.2
Residual moisture (at the tenperature 70^0C)	%	1.85	2.56	2.45

Table 15.

Results of standardization capsules "St. John's Wort" 300

Parameters	Limits	Results
Appearance	Hard gelatine transparent - transparent capsules №00 colorless, transparent capsules №00, containing brownish-grey powder.	**Hard gelatine transparent - transparent capsules №00 colorless, transparent capsules №00, containing brownish-grey powder.**
Physical parameters		
Average weight and uniformity of mass	From 477.0 mg to 583.0 mg ±10.0%	**519.07 мг (+) 8.04%, (-) 4.93%**
Disintegration time	NMT 30 min	**8 min**
Chemical parameters		
Identification	A. TLC B. HPLC. The principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the reference solution (St. John's Wort dry extract CRS) C. HPLC. The principal peak (hypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peak (hypericin) in the chromatogram obtained with the reference solution (hypericin)	**A. TLC: Confirm** **B. The principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the reference solution (St. John's Wort dry extract CRS)** **C. The principal peak (hypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peak (hypericin) in the chromatogram obtained with the reference solution (hypericin)**
Water content	NMT 6.0%	**4.82%**
Residual solvents **Ethanol** **Chloroform**	 Not more than 5000 ppm Not more than 5000 ppm	 **1213.5 ppm** **74.2 ppm**
Content of sum hypericins expressed as hypericin	From 0.81 mg to 0.99 mg	**0.90 mg**
Content of sum flavonoids expressed as rutin	Minimum 18.0 mg	**72.57%**
Content of hyperforin	Maximum 18.0 mg	**12.68%**

Table 16.

Three different compositions for obtaining "St. John's Wort" 300 tablets

Name of ingredients	Composition 1	Composition 2	Composition 3
	Unit, mg	Unit, mg	Unit, mg
Dry extract St. John's Wort	300	300	300
Microcrystalline cellulose (MCC)	165.5	219.4	165.5
Aerosil 200	4.75	5.3	4.75
Magnesium stearate	4.75	5.3	4.75
*Ethanol (60%)	-	0.3	0.3
Total weight (without filmcoat):	**475**	**530**	**475**
Film Coating Material: Opadry №16	25	25	25
*Purified water	0.5	0.5	0.5
Total weight:	**500**	**555**	**500**

* Not added to the total weight due to evaporation during manufacturing process.

Table 17.

Technological parameters of three compositions for "St. John's Wort" 300 tablets (*Hypericum scabrum*)

Technological parameters	Unit	Composition 1	Composition 2	Composition 3
Fractional composition +1000	μm, %	0	1.5	5.7
-1000 +850		0.9	10.8	37.6
-850 +500		8.9	28.7	31.1
-500 +250		81.6	49.2	22.8
-250 +75		8.6	9.8	2.8
Flowability	$kg/s \cdot 10^{-3}$	3.2	2.7	4.5
Bulk density	kg/m^3	701.3	412.7	612.7
Compressibility	N	33	91	76
Compression rate		3.1	2.6	2.74
Residual moisture (at the tenperature 70^0C)	%	1.85	2.56	2.86

Table 18.

Results of standardization tablets "St. John's Wort" 300

Parameters	Limits	Results
Appearance	Light green colored, oval, biconvex, film coated tablets, one face is notched.	**Light green colored, oval, biconvex, film coated tablets, one face is notched.**
Physical parameters		
Average weight and uniformity of mass	From 475.0 mg to 525.0 mg ±5.0%	**498.74 мг (+) 0.73%, (-) 0.74%**
Disintegration time	NMT 30 min	**10 min**
Chemical parameters		
Identification	A. TLC B. HPLC. The principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the reference solution (St. John's Wort dry extract CRS) C. HPLC. The principal peak (hypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peak (hypericin) in the chromatogram obtained with the reference solution (hypericin)	**A. TLC: Confirm** **B. The principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peaks (hypericin and pseudohypericin) in the chromatogram obtained with the reference solution (St. John's Wort dry extract CRS)** **C. The principal peak (hypericin) in the chromatogram obtained with the test solution is similar in position and size to the principal peak (hypericin) in the chromatogram obtained with the reference solution (hypericin)**
Water content	NMT 6.0%	**4.73%**
Residual solvents **Ethanol** **Chloroform**	 Not more than 5000 ppm Not more than 5000 ppm	 **1245.7 ppm** **90.6 ppm**
Content of sum hypericins expressed as hypericin	From 0.81 mg to 0.99 mg	**0.92 mg**
Content of sum flavonoids expressed as rutin	Minimum 18.0 mg	**72.63 mg**
Content of hyperforin	Maximum 18.0 mg	**12.67 mg**

Table 19.

Obtaining information of self-life stability "St. John's Wort" 300 capsules

Batch № of finished product	Batch size	Production Date	Long-term stability period	Remarks
S010816	575 capsules	August 2015	24 months	Product is stable (during 9 month)
S020816	1150 capsules	August 2015	24 months	Product is stable (during 9 month)
S030816	2130 capsules	August 2015	24 months	Product is stable (during 9 month)

Table 20.

Self-life stability studies of "St. John's Wort" 300 capsules

Product name: "St. John's Wort 300" capsules	**Stability starting date:** 09.2015
Batch №: S010815	**Stability period:** 24 months
Production place: FV, "Nobel Pharmsanoat"	**Test condition:** 25°C± 2°C, 60 % ± 5% RH
Production date: 08.2015	**Active ingredient:** Dry extract St.John's Wort
Batch size: 575 capsules	**Active ingredient batch №:** 020615
Packaging: Transparent PVC/PE/PVDC-Al Blister	**Active ingredient source:** ICPS, Uzbekistan

Tests	Specifications	Initial	3 months	6 months	9 months	12 months	18 months	24 months
Appearance	Hard gelatine transparent - transparent capsules №00 colorless, transparent capsules №00, containing brownish-grey powder	Conforms	Conforms	Conforms	Conforms	Ongoing	Ongoing	Ongoing
Average weight and uniformity of mass	477.0 mg to 58.,0 mg ±10.0%	519.07 мг (+) 8.04%, (-) 4.93%	521.30 мг (+) 7.51%, (-) 5.01%	523.24 мг (+) 6.18%, (-) 3.86%	526.78 мг (+) 7.55%, (-) 6.05%	Ongoing	Ongoing	Ongoing
Disintegration time	NMT 30 min	8 min	8 min	8 min	9 min	Ongoing	Ongoing	Ongoing
Water content	NMT 6.0%	4.82%	4.97%	5.04%	5.27%	Ongoing	Ongoing	Ongoing
Residual solvents **Ethanol** **Chloroform**	 NMT 5000 ppm NMT 5000 ppm	 1213.5 74.2	 1211.1 72.6	 1208.7 71.8	 1203.9 70.6	Ongoing	Ongoing	Ongoing
Content of sum hypericins expressed as hypericin	0.81 mg to 0.99 mg	0.90 mg	0.90 mg	0.89 mg	0.88 mg	Ongoing	Ongoing	Ongoing
Content of sum flavonoids expressed as rutin	Minimum 18.0 mg	72.57 mg	72.50 mg	72.41 mg	72.37 mg	Ongoing	Ongoing	Ongoing
Content of hyperforin	Maximum 18.0 mg	12.68 mg	12.53 mg	12.44 mg	12.40 mg	Ongoing	Ongoing	Ongoing
Microbial limit	Total bacteria: NMT: 1000 cfu/g, Fungi: NMT 100 cfu/g, E.coli: Absent.	Conforms	-	-	-	Ongoing	Ongoing	Ongoing

Table 21.

Self-life stability studies of "St. John's Wort" 300 capsules

Product name: "St. John's Wort 300" capsules	**Stability starting date:** 09.2015
Batch №: S020815	**Stability period:** 24 months
Production place: FV, "Nobel Pharmsanoat"	**Test condition:** 25°C± 2°C, 60 % ± 5% RH
Production date: 08.2015	**Active ingredient:** Dry extract St.John's Wort
Batch size: 1150 capsules	**Active ingredient batch №:** 020615
Packaging: Transparent PVC/PE/PVDC-Al Blister	**Active ingredient source:** ICPS, Uzbekistan

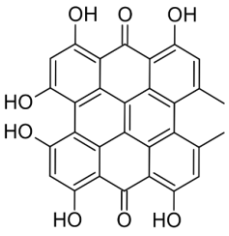
Tests	Specifications	Initial	3 months	6 months	9 months	12 months	18 months	24 months
Appearance	Hard gelatine transparent - transparent capsules №00 colorless, transparent capsules №00, containing brownish-grey powder	Conforms	Conforms	Conforms	Conforms	Ongoing	Ongoing	Ongoing
Average weight and uniformity of mass	477.0 mg to 583.0 mg ±10.0%	520.68 мг (+)7.18%, (-)4.74%	522.76 мг (+)7.23%, (-)5.59%	522.92 мг (+)6.73%, (-)4.59%	524.75 мг (+)7.41%, (-)5.68%	Ongoing	Ongoing	Ongoing
Disintegration time	NMT 30 min	8 min	8 min	8 min	9 min	Ongoing	Ongoing	Ongoing
Water content	NMT 6.0%	4.78%	4.88%	5.04%	5.27%	Ongoing	Ongoing	Ongoing
Residual solvents Ethanol	NMT 5000 ppm	1379.9 92.7	1374.6 90.5	1370.4 89.1	1368.2 86.1	Ongoing	Ongoing	Ongoing
Content of sum hypericins expressed as hypericin	0.81 mg to 0.99 mg	0.89 mg	0.89 mg	0.88 mg	0.88 mg	Ongoing	Ongoing	Ongoing
Content of sum flavonoids expressed as rutin	Minimum 18.0 mg	72.55 mg	72.47 mg	72.40 mg	72.37 mg	Ongoing	Ongoing	Ongoing
Content of hyperforin	Maximum 18.0 mg	12.62 mg	12.54 mg	12.50 mg	12.47 mg	Ongoing	Ongoing	Ongoing
Microbial limit	Total bacteria: NMT: 1000 cfu/g, Fungi: NMT 100 cfu/g, E.coli: Absent.	Conforms	-	-	-	Ongoing	Ongoing	Ongoing

Table 22.

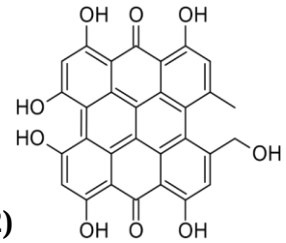
Self-life stability studies of "St. John's Wort" 300 capsules

Product name: "St. John's Wort 300" capsules	**Stability starting date:** 09.2015
Batch №: S030815	**Stability period:** 24 months
Production place: FV, "Nobel Pharmsanoat"	**Test condition:** 25°C± 2°C, 60 % ± 5% RH
Production date: 08.2015	**Active ingredient:** Dry extract St.John's Wort
Batch size: 2310 capsules	**Active ingredient batch №:** 020615
Packaging: Transparent PVC/PE/PVDC-Al Blister	**Active ingredient source:** ICPS, Uzbekistan

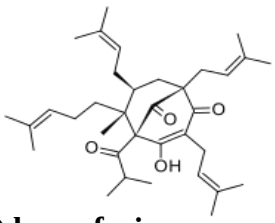
Tests	Specifications	Initial	3 months	6 months	9 months	12 months	18 months	24 months
Appearance	Hard gelatine transparent - transparent capsules №00 colorless, transparent capsules №00, containing brownish-grey powder	Conforms	Conforms	Conforms	Conforms	Ongoing	Ongoing	Ongoing
Average weight and uniformity of mass	477.0 mg to 583.0 mg ±10.0%	518.60 мг (+)7.25%, (-)5.07%	521.12 мг (+)6.41%, (-)5.56%	523.46 мг (+)6.88%, (-)4.73%	525.69 мг (+)7.16%, (-)5.84%	Ongoing	Ongoing	Ongoing
Disintegration time	NMT 30 min	8 min	8 min	8 min	8 min	Ongoing	Ongoing	Ongoing
Water content	NMT 6.0%	4.81%	4.86%	4.93%	5.02%	Ongoing	Ongoing	Ongoing
Residual solvents Ethanol	NMT 5000 ppm	1298.3 80.8	1296.1 78.8	1293.7 77.0	1290.7 75.4	Ongoing	Ongoing	Ongoing
Content of sum hypericins expressed as hypericin	0.81 mg to 0.99 mg	0.89 mg	0.89 mg	0.88 mg	0.87 mg	Ongoing	Ongoing	Ongoing
Content of sum flavonoids expressed as rutin	Minimum 18.0 mg	72.52 mg	72.49 mg	72.42 mg	72.37 mg	Ongoing	Ongoing	Ongoing
Content of hyperforin	Maximum 18.0 mg	12.60 mg	12.56 mg	12.51 mg	12.48 mg	Ongoing	Ongoing	Ongoing
Microbial limit	Total bacteria: NMT: 1000 cfu/g, Fungi: NMT 100 cfu/g, E.coli: Absent.	Conforms	-	-	-	Ongoing	Ongoing	Ongoing



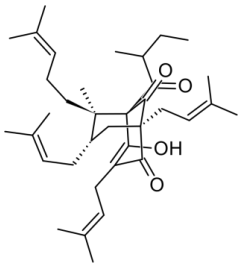
1) hypericin



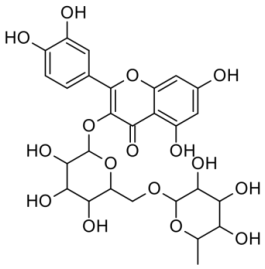
2) pseudohypericin



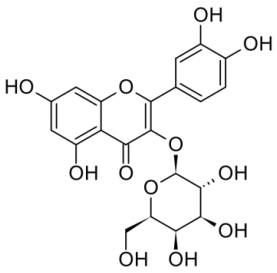
3) hyperforin



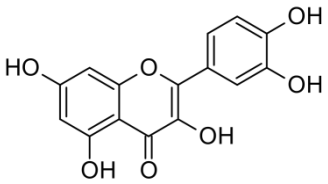
4) adhyperforin



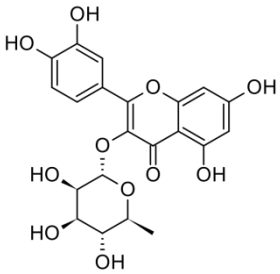
5) rutin



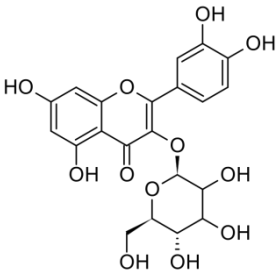
6) hyperoside



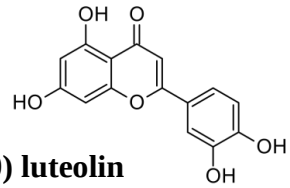
7) quercetin



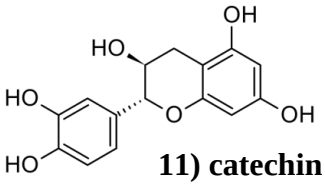
8) quercetrin



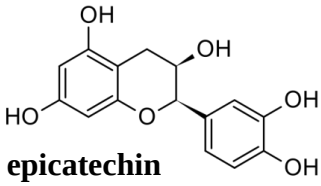
9) isoquercetrin



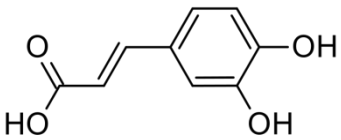
10) luteolin



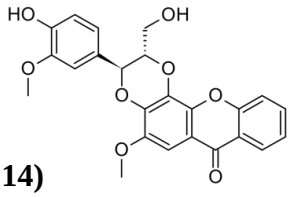
11) catechin



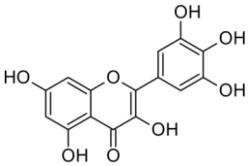
12) epicatechin



13) caffeic acid



14) kielcorin



15)myricetin

Fig. 1. Chemical structures biologically active compounds of St. John's Wort: 1) hypericin, 2) pseudohypericin, 3) hyperforin, 4) adhyperforin, 5) rutin, 6) hyperoside, 7) quercetin, 8) quercetrin, 9) isoquercetrin, 10) luteolin, 11) catechin, 12) epicatechin, 13) caffeic acid, 14) kielcorin, 15) myricetin.

Figure 1. *Hypericum perforatum* (St. John's Wort).

Figure 3. *H. perforatum* flowers.

Figure 2. *H. perforatum* leaves.

Figure 4. *H. perforatum* fruits.

Fig. 2. *Hypericum perforatum L.*: 1) *Hypericum perforatum L.*, *2) Hypericum perforatum L.* leaves, 3) *Hypericum perforatum L.* flowers, 4) *Hypericum perforatum L.* fruits.

Fig. 3. Raw material

a) Process of solid – liquid extraction (aerial parts of St. John's Wort + 80% ethanol)

c) Process of liquid – liquid extraction (water's part + chloroform's part)

b) Process of evaporating of liquid extract

d) Obtained dry extract of St. John's Wort

Fig.4. Aerial Parts of *Hypericum scabrum L.* which grown in Uzbekistan

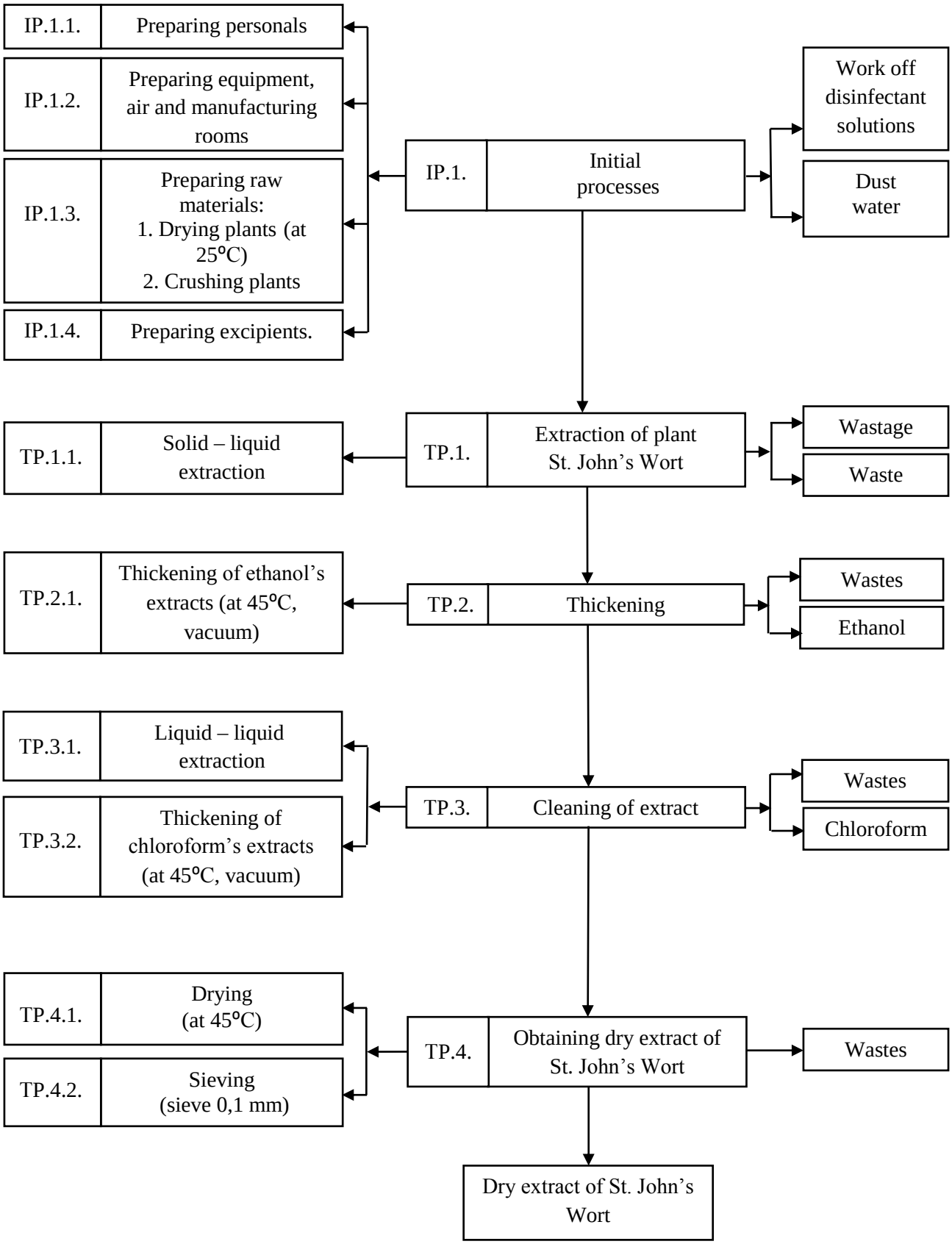


Fig. 5. Technological chart of obtaining dry extract of St. John's Wort

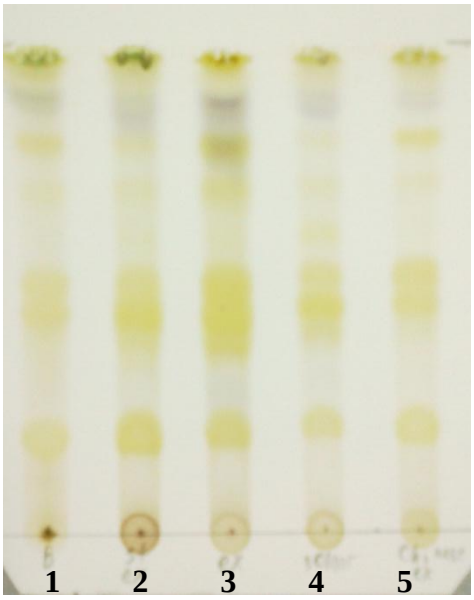
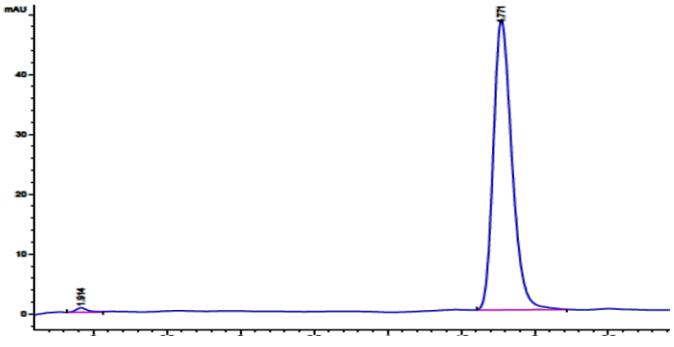
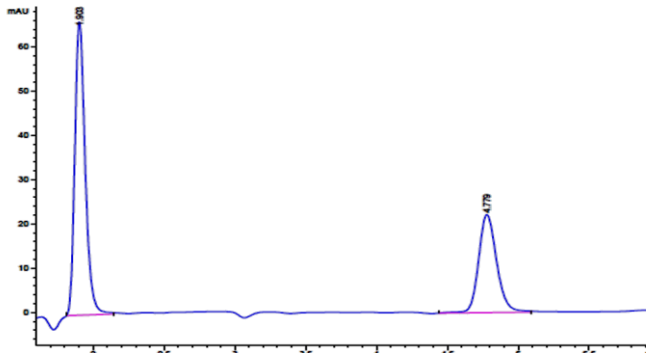


Fig. 6. Obtained TLC chromatogram. 1-standard; 2-our dry extract H.scabrum; 3-our dry extract H.perforatum; 4-original preparation "Helarium Hypericum"; 5-dry extract from China.



1



2

Fig. 7. Obtained TLC chromatogram. 1 - HPLC chromatogram of standard: RRT (hypercin) = 4.771; 2- HPLC chromatogram of our dry extract H.scabrum: RRT (hypericin) = 4.779.

Fig.8. obtained liquid extract of "St. John's Wort" 300

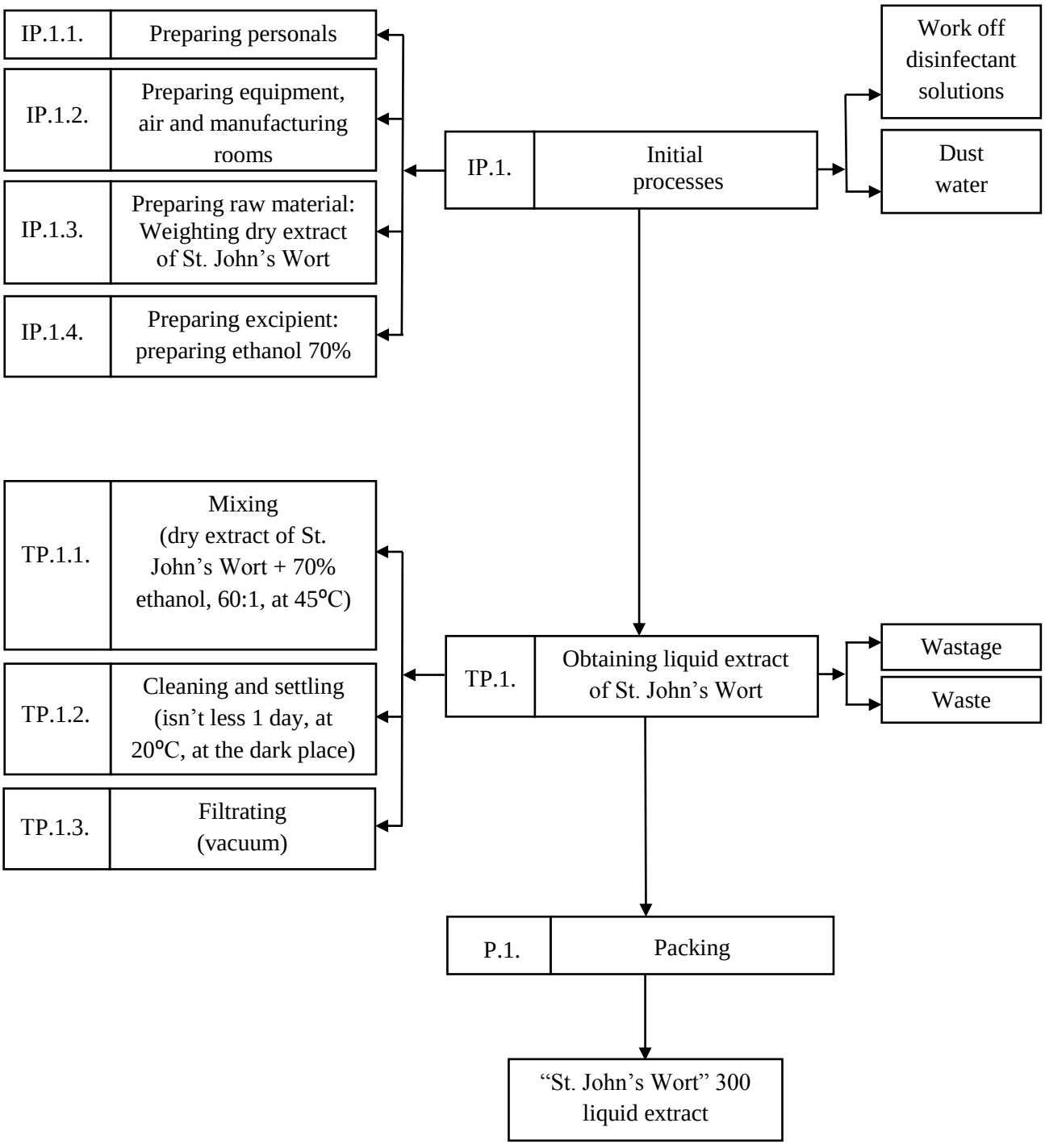


Fig. 9. Technological chart of obtaining liquid extract of "St. John's Wort" 300 (*Hypericum scabrum*)

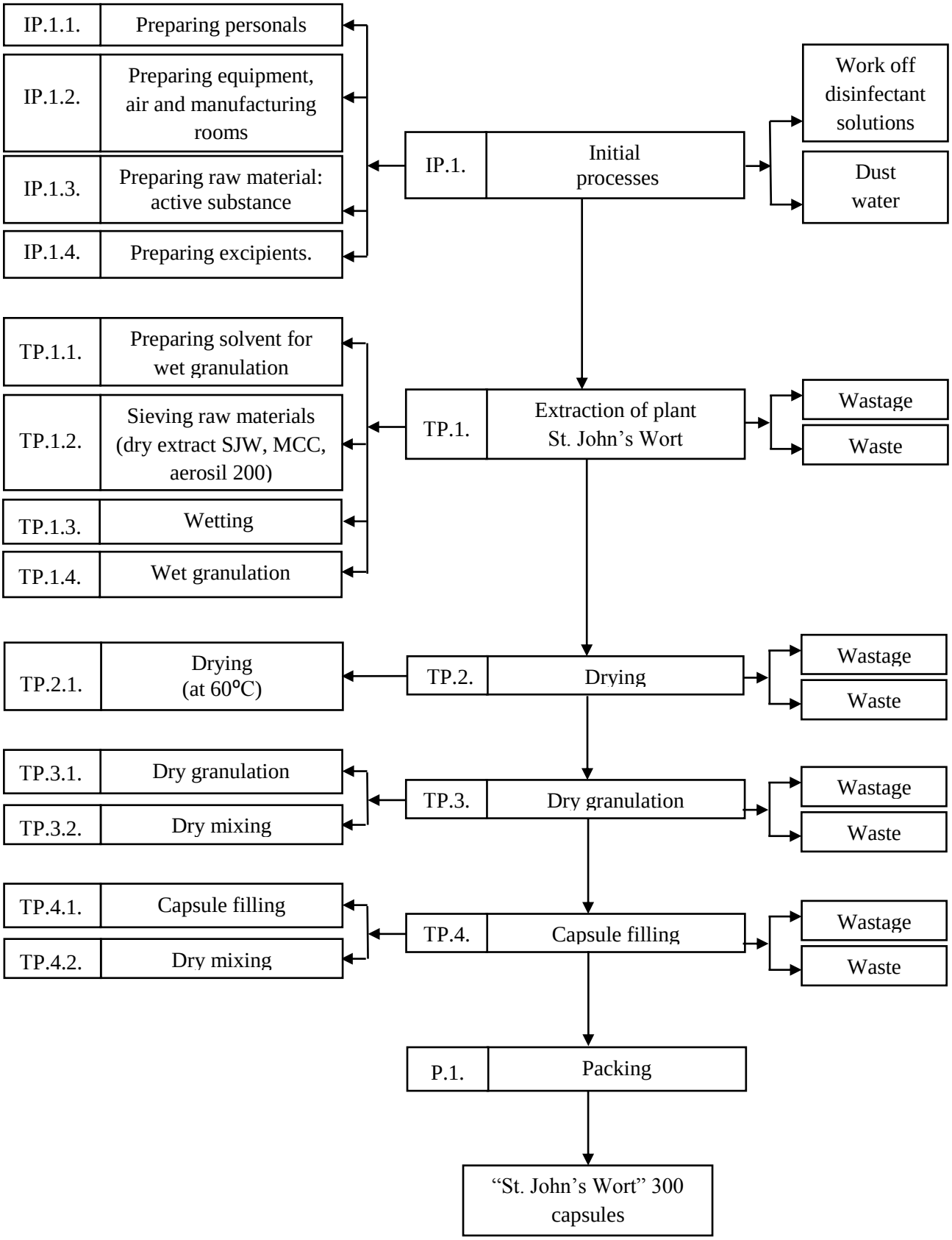


Fig. 10. Technological chart of obtaining "St. John's Wort" 300 capsules

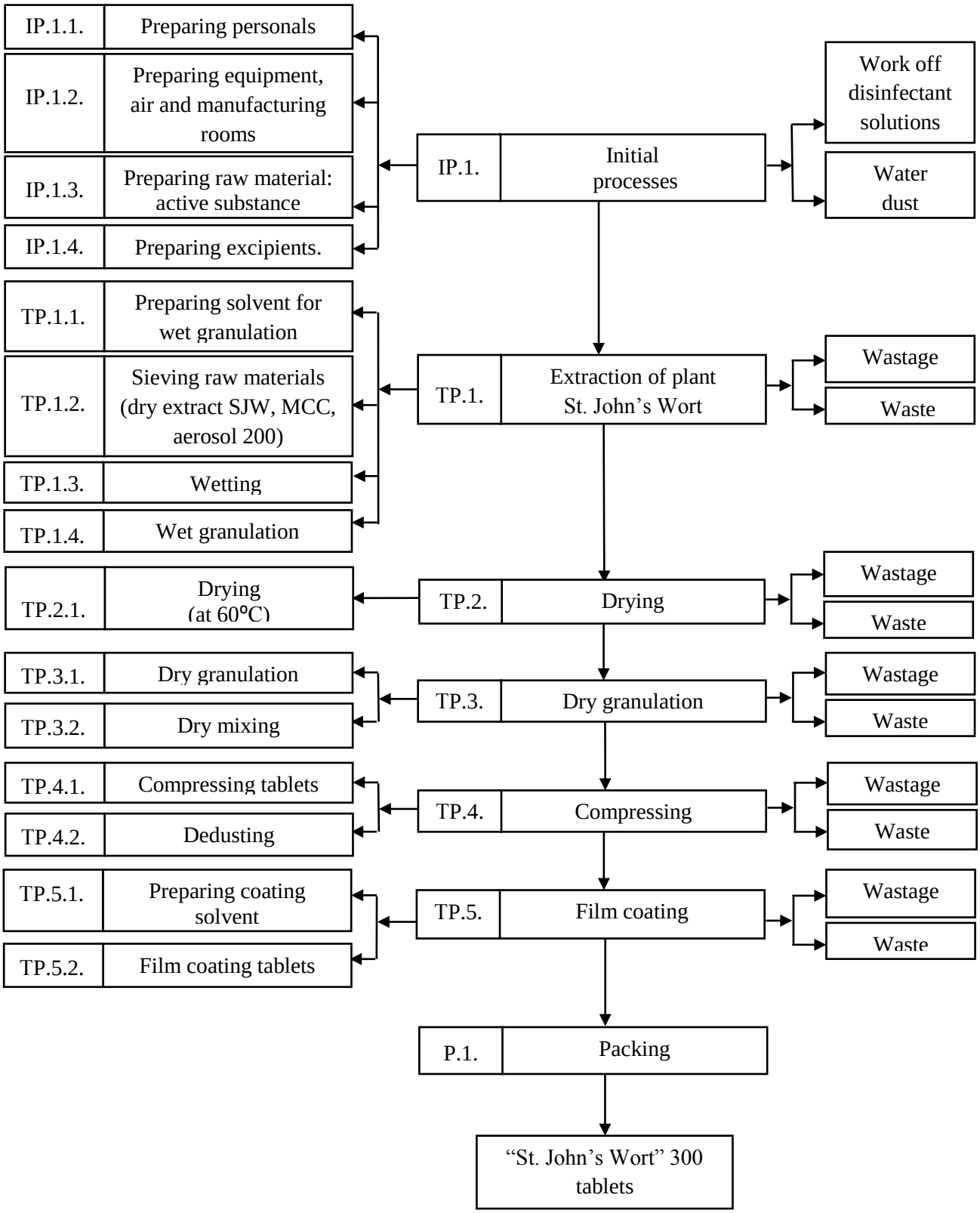


Fig. 11. Technological chart of obtaining "St. John's Wort" 300 tablets