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Riga Stradins University

Department of Dosage Forms Technology

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TECHNOLOGY AND INVESTIGATION OF PINE NEEDLES THICK EXTRACT CAPSULATED DOSAGE FORM

Summary of the doctoral thesis

Speciality – Pharmacy

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The doctoral thesis was elaborated at the Department of Dosage Forms Technology of Riga Stradins University.

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The doctoral thesis is available at the library of Riga Stradins University.

The presentation of the doctoral thesis will take place at the open meeting of the Promotion Council of Pharmacy disciplines of Riga Stradins University on June 7, 2011, at 14.00 in RSU Hippocrates lecture-hall, in Riga, Dzirciema Str. 16.

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ABBREVIATIONS USED IN THE TEXT

λ – wavelength, nm
Al/PVC blisters – blisters from aluminium foil and polyvinylchloride film
GMO – glycerol monooleate
HDPE – high density polyethylene
LDPE – low density polyethylene
n – number of analyzed samples
NLT – not less than
NMT – not more than
pH – hydrogen ion concentration in an aqueous solution
Ph. Eur. – European Pharmacopoeia
PNTE – pine needles thick extract
$PNTE_1$ – pine needles thick extract with content of moisture and volatile substances NMT 40 %
$PNTE_2$ – pine needles thick extract with content of moisture and volatile substances NMT 10 %
r – correlation coefficient
R^2 – determination coefficient
R_f – retardation factor
RH – relative humidity
RSD – relative standard deviation
SD – standard deviation
SGC – soft gelatin capsule
TLC – thin-layer chromatography
UV/VIS – ultraviolet and visible part of light spectrum

TOPICALITY OF THE STUDY

Herbal preparations are used extensively for the prevention and treatment of different diseases. This preparations contain substances with explicit pharmacological action, as well as vitamins, minerals, unsaturated fatty acids, antioxidants and other biologically active substances, which are necessary for the human body and which have a positive influence on the trophic and reparative processes in the organism.

The herbal preparations have unique properties, which are not inherent to synthetically derived preparations. The nature provides a variety of complicated substances, complexes of biologically active substances, ensuring a therapeutic activity or preventive properties, which can't be expressed when the components are used separately.

Among trees which have healing properties a significant place belongs to ordinary pine *(Pinus sylvestris, L.)*. As an evolutionary oldest plant of a seed group, the pine was able to survive and adapt to untoward conditions by producing a unique complex of biologically active substances, which is widely used in scientific and popular medicine.

With the progress of forest biochemistry, the technologies of processing of coniferous trees greenery – the waste of wood production, were developed. These technologies allow obtain valuable natural substances for their use in medicine, cosmetology, agriculture, industry and other fields.

Forests are one of the most important national wealth of Latvia. In accordance with the forests statistical inventory data, the forests in Latvia take 3.22 million ha or 49.9 % of the state territory. The pine stands take 29 % of the whole forest stands.

Latvian forests are considered to be an ecologically clean source of herbal raw material. From pine needles and young browses JSC „Biolat" produces a thick extract, which contains chlorophylls, carotenoids, polyprenols, vitamins E and K, unsaturated fatty acids, phytosterols and other biologically active substances.

In a modern society an incorrect life style is an actual problem. This incorrect life style includes a low diet, lack of mobility, stress, pollution of the environment and as a result – gastrointestinal diseases, lowering of the immunity and other disorders. Herbs can help to fight against these problems.

Extensively are investigated biologically active substances of plant origin, which possess a complex influence on gastric and duodenal ulcer, and simultaneously show reparative and antioxidant effect, especially in cases when ulcer disease and *Helicobacter pylori* infection are diagnosed.

The pine needles thick extract shows such a complex influence in the treatment of gastrointestinal diseases. Non-clinical investigations showed that the pine needles thick extract (PNTE) has gastro-protective action, reparative influence on experimental gastric ulcer, bactericidal effect on grampositive bacteria and gramnegative bacteria *Pseudomonas aeruginosa*, antioxidant effect and a weak immune modulating effect. PNTE has not irritable, allergic effect, cancerogenic, teratogenic and mutagenic properties, and it is non-toxic for warm-blooded animals.

Clinical investigations showed that PNTE is well bearable in spite of the specific organoleptic properties. The effect of PNTE is linked to the stimulation of secretory immune globulin-A excretion in saliva, enhancement of microcirculation and stimulation of mucosal secretion in stomach and duodenum. Indications for the use of PNTE are chronic active gastritis (mainly type B), subsidiary therapy in the case of chronic gastroduodenal ulcer, ulcerous and non-ulcerous dyspepsia. PNTE can supplement and optimize the therapy of patients with post-radiation syndrome.

PNTE with the name "Fitestens" in Latvia was used as a gastro-protector since 1995 till 2005. As a dosage form it was filled into amber glass jars in an amount of 50 g or 100 g, and a spoon was used for dosing. In spite of the high therapeutic effect and sufficient amount of the raw material, the sales of this medicine were not large. Possibly, it was partly related to the fact that the packaging of PNTE was not enough convenient for the use. PNTE was not dosed, and when used orally, a specific bitter taste was tangible.

At present in the drug register and in the food supplement register in Latvia there are no analogue products. In the food supplement register there are products containing chlorophylline and carotenoids. Considering the previously mentioned, it can be concluded that the development of a dosage form of PNTE is actual problem.

For the prevention and treatment of gastrointestinal diseases, oral dosage forms are mostly used. Capsules are one of the most optimal of them. Capsules are precisely dosed, convenient in the use, they mask the unpleasant taste and odour of preparations, provide the stability of active

substances. That is the reason why capsules are chosen as a dosage form of PNTE.

Developing a dosage form of a preparation, it is important to investigate the properties of the active substance, to find optimal excipients, to stimulate the introduction of the technology in the industry, to develop the product quality assessment methods, to choose an appropriate packaging. This study is dedicated to solve of all of these tasks.

AIM AND TASKS OF THE STUDY

The aim of the study is to develop a capsulated dosage form of pine needles thick extract (PNTE) and to investigate its quality and stability.

The tasks of the study:

1. To investigate the properties of carotenoids and chlorophylls in PNTE as quality markers.
2. To develop a powder formulation with PNTE for filling into hard gelatin capsules and to assess its quality and stability.
3. To develop a lipophilic formulation with PNTE, using appropriate excipients and technological methods, for filling into soft and hard gelatin capsules, and to evaluate its quality and stability.
4. To evaluate the possibility to apply different encapsulating methods for preparation of PNTE dosage form.
5. To elaborate quality control methods for PNTE capsulated dosage form.
6. To investigate the stability of PNTE capsulated dosage form in different types of packaging during long term and accelerated stability testing.

NOVELTY AND PRACTICAL SIGNIFICANCE OF THE STUDY

1. An oral dosage form of PNTE was developed and its stability was investigated.
2. The developed PNTE soft gelatin capsules were registered under the name of "Fitesten" in the food supplement register of Latvia Food Centre.

3. A project of the description of the manufacturing process of PNTE soft gelatin capsules was prepared, the quality control methods and a project of the quality specification of PNTE soft gelatin capsules were elaborated.

STRUCTURE AND SIZE OF THE THESIS

The thesis is written in Latvian. It consists of introduction, review of the literature, characteristic of the used material and methods, layout of results, discussion, conclusions and practical recommendations, list of literature and other sources of information, and appendix. The size of the thesis is 137 pages and the size of the appendix – 23 pages. The thesis contains 30 tables and 50 figures. The list of the literature and other sources of information contains 273 references.

APPROBATION OF THE STUDY RESULTS

The results of the study have been discussed at the united meeting of the Department of Dosage Forms Technology and the Department of Pharmaceutical Chemistry of RSU on September 23, 2010.

The results of the study were reported in poster presentations at 10 international congresses, conferences and local conferences.

The results of the study were published in 3 revised scientific publications, 2 patents, 6 abstracts of international congresses and conferences and 4 abstracts of local conferences.

MATERIALS AND METHODS USED IN THE STUDY

Materials. Pine needles thick extract (PNTE) was prepared from greenery (needles, young browses and sprigs which are not lignified) of pines growing in forests of Latvia. PNTE contains extractives from pine greenery soluble in non polar solvents. $PNTE_1$ with content of moisture and volatile substances NMT 40 % and $PNTE_2$ with content of moisture and volatile substances NMT 10 % were used in the study. $PNTE_1$ and $PNTE_2$ were received from JSC „Biolat" (Latvia). The quality of $PNTE_1$ complies

with the requirements of the Pharmacopoeia monograph FP 95-0002/42-26-95 „Fitestens", the quality of $PNTE_2$ complies with the requirements of the document LV UTN 000312820-18-2008 „*Skuju biezais ekstrakts*" (JSC Biolat 2008.02.19).

The raw material for the preparation of $PNTE_1$ and $PNTE_2$ are the pine needles and young browses under 6 mm in diameter. The raw material is cut into small particles in the size under 6 – 10 mm, and extracted during 3.5 h with petrol BR-1 (or n-hexane, or Nephrase C2 – 80/120) in proportion 1 : 5. The petrol extract is settled for 12 h at (14 – 18) °C temperature for the sedimentation of wax. After that the petrol is distilled in rotor evaporator IR-1M2 at (60 – 70) °C temperature. Acidic part of the soft residue is neutralized with sodium hydroxide solution at (70 – 80) °C temperature until pH 8.0 – 9.0 is obtained.

For the preparation of the experimental samples of hard gelatin capsules containing $PNTE_1$, the following active substances and excipients were used: calcium carbonate; magnesium oxide, light; aluminium oxide, hydrated; bismuth subnitrate, heavy; talc; calcium stearate; stearic acid; magnesium stearate; sodium metabisulphite; lactose, monohydrate; tween-80; liquorice dry extract; food bran, wheaten; ethanol 96 % (V/V); purified water; hard gelatin capsules No. 0, 00.

For the preparation of the experimental samples of soft gelatin capsules containing $PNTE_1$ and $PNTE_2$ the following emulsifiers were used: tween-20, tween-40, tween-60V, tween-65, tween-80, Sorbital T40P (sorbitan monopalmitate), Multec Soral MS (sorbitan monostearate), Multec Soral MO (sorbitan monooleate), emulsifier T-2, emulsifier pentol, distilled monoglycerides, Rylo™ MD 50 Pharma (glycerol monostearate), Rylo™ MG 20 Pharma (glycerol monooleate), Rylo™ AC 19 Pharma (diacetylated monoglycerides).

For the preparation of the soft gelatin capsules containing $PNTE_1$ and $PNTE_2$ the following excipients were used: arachis oil, sunflower oil, soya-been oil, olive oil, gelatin, glycerol, nipagin (metil-p-hydroxybenzoate), salicylic acid, sodium metabisulphite, isopropyl alcohol, purified water, hard gelatin capsules No. 0.

Reagents: petroleum solvent Nephrase C2-80/120; ethanol 96 % (V/V); petroleum ether 40 – 80; diethyl ether; chloroform; acetone; n-hexane; ethyl acetate; benzene; thin-layer chromatography plates Al SIL G/UV 254; aluminium oxide, active y-Al_2O_3 *Brockmann* II type for chromatography, neutral; β-carotene reference standard, purity ≥ 97 %;

phosphomolybdic acid; sodium carbonate, anhydrous; sodium sulphate, anhydrous; potassium dichromate; purified water.

Methods. Appearance, colour, odour and taste of PNTE and preparations containing PNTE were tested by organoleptic method.

For the identification of PNTE and powder containing $PNTE_1$, chlorophylls and carotenoids were proved. Chlorophyll was identified by the colour of ethanol solution and by the fluorescence in UV light at 254 nm wavelength. Carotenoids and non saturated hydrocarbons were proved by the colour reaction with phosphomolybdic acid 10 % ethanol solution. The identification of carotenoids in PNTE and in powder containing $PNTE_1$ was performed by a thin-layer chromatography (TLC) method using a stationary phase – silica gel and a mobile phase – solvent systems (see Table 1), reference standard – β-carotene. The chromatograms were examined in day light and in UV light at 254 nm wavelength. For the development of the carotenoids zones the chromatography plates were treated with phosphomolybdic acid 10 % ethanol solution, heated at (60 – 80) °C temperature for 5 min and examined in day light.

Loss on drying of PNTE and preparations containing PNTE was determined according to Ph. Eur. 6.0, p. 2.8.17, by heating at (100 – 105) °C temperature for 3 h.

Hydrogen ion concentration (pH) of PNTE aqueous solution was determined according to Ph. Eur. 6.0, p. 2.2.3.

Content of carotenes in PNTE and in preparations containing PNTE was determined by spectrophotometry method at 450 nm wavelength after isolation from PNTE or preparations containing PNTE by column chromatography method (sorbent – aluminium oxide, eluting solvent – Nephrase). Potassium dichromate aqueous solution was used as a reference solution.

For the quantitative determination of carotenes in $PNTE_1$, $PNTE_2$ and in preparations containing $PNTE_1$, the precise weight of sample was mixed with sodium carbonate and acetone and filtered into a separating funnel. The acetone solution was mixed with Nephrase, than acetone was extracted with water and water from Nephrase solution was removed by anhydrous sodium sulphate. The Nephrase solution was eluted through the aluminium oxide column till carotenes, separated as an orange-yellow zone from other pigments, were transferred to a 100 ml measuring flask, and the eluted solution became colourless. The absorbance of the obtained solution

was determined spectrophotometrically at 450 nm wavelength in a 1 cm cell using UV-Visible spectrophotometer Nicolet Evolution 100 (Thermo Electron Corporation). The absorbance of the potassium dichromate reference solution was determined concurrently.

For the determination of the content of carotenes in soft gelatin capsules (SGC) containing $PNTE_2$ the previously described method was modified. The precise weight of the filling mass of one capsule was mixed with Nephrase and filtered. The Nephrase solution was eluted through the column as previously described. The eluted solution was collected in a 50 ml measuring flask.

The content of carotenes, calculated to β-carotene, in the dry preparation ($PNTE_1$ or $PNTE_2$) or in the capsule fill (taking into account the content of moisture and volatile substances of $PNTE_2$) should be NLT 30 mg %. The content of carotenes in the soft gelatin capsule should be NLT 80 μg.

Flowability of powders containing $PNTE_1$ was determined using a device VA-12-A with a vibratory funnel. The method is based on the determination of velocity of powder falling out of a funnel. The powder flowability should be in the range from 6.6 to 12.0 $g \cdot s^{-1}$.

Uniformity of mass of SGC containing $PNTE_2$ was determined according to Ph. Eur. 6.0, p. 2.9.5. Electronic balance type ABS 220-4 (KERN & Sohn GmbH, Germany) was used. The deviation of filling mass of each capsule from the average filling mass should not exceed ± 7.5 %.

Disintegration time of SGC containing $PNTE_2$ was determined according to Ph. Eur. 6.0, p. 2.9.1. The capsules should disintegrate in 30 min in water or in simulated intestinal fluid.

Stability studies of the powder formulation containing $PNTE_1$ in hard gelatin capsules were carried out: long term stability studies at (25 ± 2) °C temperature and accelerated stability studies at (40 ± 2) °C temperature (primary packaging: amber glass jars). Long term stability studies of SGC containing $PNTE_2$ were carried out: for the laboratory batch and for the pilot batches at (25 ± 2) °C temperature (in thermostat), for the manufacturing batches at (25 ± 2) °C / (60 ± 5) % RH (in climatic chambers). Accelerated stability studies of SGC containing $PNTE_2$ were carried out: for the laboratory batch and for the pilot batches at (30 ± 2) °C temperature (in thermostat), for the manufacturing batches at (30 ± 2) °C / (65 ± 5) % RH (in climatic chambers). Capsules were packed in two types of packaging: 1) high density polyethylene (HDPE) containers with low

density polyethylene (LDPE) caps; 2) transparent, colourless PVC film and aluminium foil (Al/PVC) blisters. The batch analysis were performed after manufacturing, as well as after each 3 months in the first year of the stability testing and after each 6 months in the second year according to the guidelines of the European Medicines Agency (EMA) CPMP/ICH/2736/99.

Data statistical analysis was performed using MS Office Excel 2007 program. The central tendency indices of findings – the arithmetical mean, and dispersion indices – the standard deviation (SD) were calculated. The linear regression analysis was used for the stability study.

RESULTS AND DISCUSSION

Investigation of PNTE properties, evaluation of quality and development of carotenes assay method in PNTE dosage form

Pigments chlorophylls and carotenoids can be mentioned as one of the mainly investigated compounds in pine needles. These substances can serve as markers in the PNTE complex, indicating in general the quality of the extract and its dosage forms.

Estimation of solubility of PNTE

$PNTE_1$ and $PNTE_2$ solubility in polar and non-polar solvents was determined. It was found that $PNTE_1$ and $PNTE_2$ are freely soluble in chloroform, partly soluble in water, ethanol, acetone, petroleum ether, Nephrase.

Qualitative composition of PNTE determination by thin-layer chromatography method

Colour reactions were used for the qualitative analysis of $PNTE_1$ and $PNTE_2$, and TLC method was used for the qualitative analysis of $PNTE_1$. In our study the TLC method was examined and specified for the qualitative evaluation of $PNTE_2$.

For the identification of carotenes in $PNTE_2$, solvent systems, given in literature, were examined, chromatograms were developed in upward vertical direction on silica gel. $PNTE_2$ 1 % chloroform solution was prepared and β-carotene 1 % chloroform solution was used as a reference solution.

The solvent systems No. 4, 7, 9, 10, 11 (Table 1) are considered to be suitable for the separation of carotene from other pigments in PNTE.

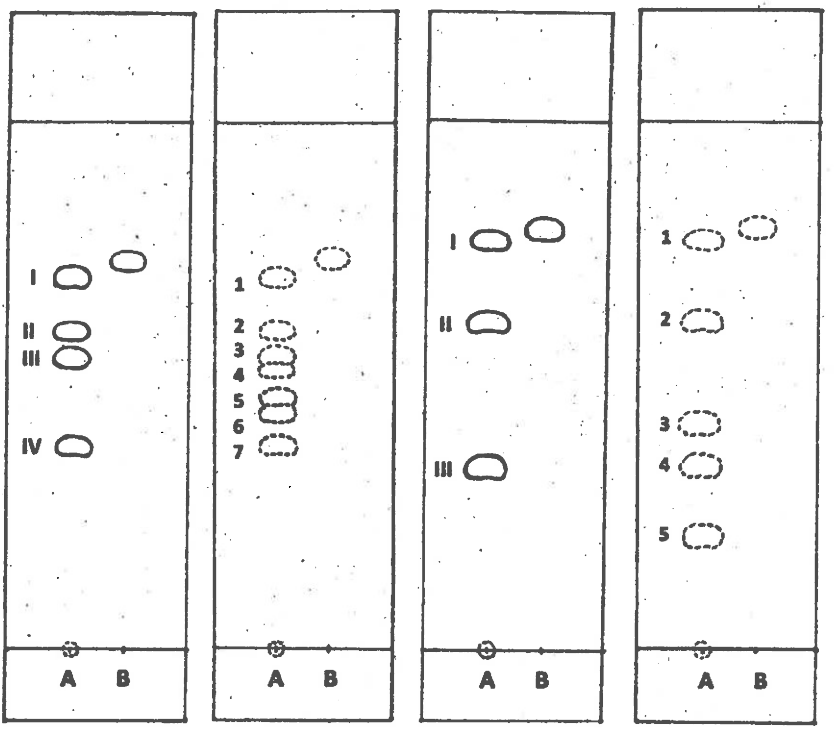
Table 1. Identification of carotene in $PNTE_2$ by TLC method

No.	Solvents	Ratio (V/V)	R_f (mean ± SD, n = 2)	
			$PNTE_2$ solution in chloroform	β-carotene reference solution in chloroform
1.	Petrol	100 %	0.03±0	0.03±0
2.	Benzene – ethyl acetate	77 : 23	0.71±0.01	0.74±0
3.	n-Hexane – ether	30 : 70	1.00±0	1.00±0
4.	n-Hexane – ether	80 : 20	0.78±0	0.79±0
5.	Chloroform – ethanol	99 : 1	0.77±0.01	0.79±0.02
6.	Petroleum ether (40 – 80)	100 %	0.13±0	0.12±0.01
7.	Petroleum ether – acetone	94 : 6	0.60±0	0.60±0
8.	Petroleum ether – benzene	98 : 2	0.16±0	0.16±0
9.	Petroleum ether – ether	90 : 10	0.73±0.01	0.73±0
10.	Petroleum ether – ether	95 : 5	0.67±0.02	0.67±0.03
11.	Petroleum ether – chloroform	75 : 25	0.90±0	0.89±0.01

Out of all pigments in these systems only carotene moves forward, but chlorophylls and other carotenoids remain on the start line. Examining the chromatograms in day light, orange-yellow colour zone, corresponding to the β-carotene position in the chromatogram, obtained with the reference solution, is visible. In this position a dark brown colour zone is visible in UV light.

Treatment of chromatograms with 10 % ethanol solution of phosphomolybdic acid for the development of carotenoid zones, as mentioned in literature, in our opinion is not necessary, because this reaction is not specific. The carotenes are well identified by colour, during examination of the chromatograms in day light.

The solvent systems No. 2 and 5 can be used for separation of $PNTE_2$ components (Figure 1).



1. 2. 3. 4.

Figure 1. Chromatograms of $PNTE_2$ in the solvent system benzene-ethyl acetate (77 : 23) (picture 1, 2) and in the solvent system chloroform-ethanol (99 : 1) (picture 3, 4):
A – $PNTE_2$, B – β-carotene reference standard; 1 and 3 are zones visible in day light; 2 and 4 are zones visible in UV light.

In the chromatogram obtained in the solvent system benzene-ethyl acetate (77 : 23) 4 zones are visible in day light:

I orange-yellow colour zone in the position of β-carotene, $R_f = 0.71 \pm 0.01$ (n = 2);

II green colour zone (identified as chlorophyll), $R_f = 0.61 \pm 0.01$ (n = 2);

III green-yellow colour zone, $R_f = 0.55 \pm 0$ (n = 2);

IV yellow colour zone, $R_f = 0.39 \pm 0$ (n = 2).

7 zones are visible in UV light:

1. dark brown colour zone in the position of β-carotene, $R_f = 0.71 \pm 0.01$ (n = 2);

2. bright red fluorescence zone (identified as chlorophyll), $R_f = 0.61 \pm 0.01$ (n = 2);

3. pink-violet fluorescence zone, $R_f = 0.55 \pm 0$ (n = 2);

4. red fluorescence zone, $R_f = 0.49 \pm 0.03$ (n = 2);

5. violet fluorescence zone, $R_f = 0.47 \pm 0.01$ (n = 2);

6. pink-violet fluorescence zone, $R_f = 0.44 \pm 0.01$ (n = 2);

7. dark brown colour zone, $R_f = 0.39 \pm 0$ (n = 2).

To avoid the use of benzene, the solvent system chloroform-ethanol (99 : 1) can be used. In the chromatogram obtained in this system 3 zones are visible in day light:

I orange-yellow colour zone in the position of β-carotene, $R_f = 0.77 \pm 0.01$ (n = 2);

II green colour zone (identified as chlorophyll), $R_f = 0.63 \pm 0.01$ (n = 2);

III yellow colour zone, $R_f = 0.37 \pm 0.03$ (n = 2).

5 zones are visible in UV light:

1. dark brown colour zone in the position of β-carotene, $R_f = 0.77 \pm 0.01$ (n = 2);

2. bright red fluorescence zone (identified as chlorophyll), $R_f = 0.63 \pm 0.01$ (n = 2);

3. light blue fluorescence zone, $R_f = 0.46 \pm 0.02$ (n = 2);

4. pink-violet fluorescence zone, $R_f = 0.37 \pm 0.03$ (n = 2);

5. violet fluorescence zone, $R_f = 0.25 \pm 0.04$ (n = 2).

Determination of the PNTE qualitative composition by column chromatography and UV/VIS spectrophotometry methods

For the quantitative analysis of $PNTE_1$ and $PNTE_2$ determination of carotenes content includes the isolation of carotenoids from the extract or from the preparations containing the extract by column chromatography on a sorbent aluminium oxide, using Nephrase as eluting solvent, and following spectrophotometry at 450 nm wavelength is used. At the same time the column chromatography and spectrophotometry can be used for the identification of pigments (chlorophylls and carotenoids).

The light absorption spectrum of $PNTE_2$ 0.5 % chloroform solution at 300 – 700 nm wavelengths showed a typical view of green and yellow plant pigments with main maximums λ_{max} at 415 nm and 666 nm (Figure 2). It is known that light absorption of plant pigments chlorophylls *a* and *b*,

their derivatives and carotenoids takes place in the diapason of 400 – 700 nm wavelengths. Chlorophylls have two absorption diapasons: I at 400 – 500 nm and II at 600 – 700 nm and carotenoids have one absorption diapason at 400 – 500 nm. Therefore these pigments should be separated by the column chromatography.

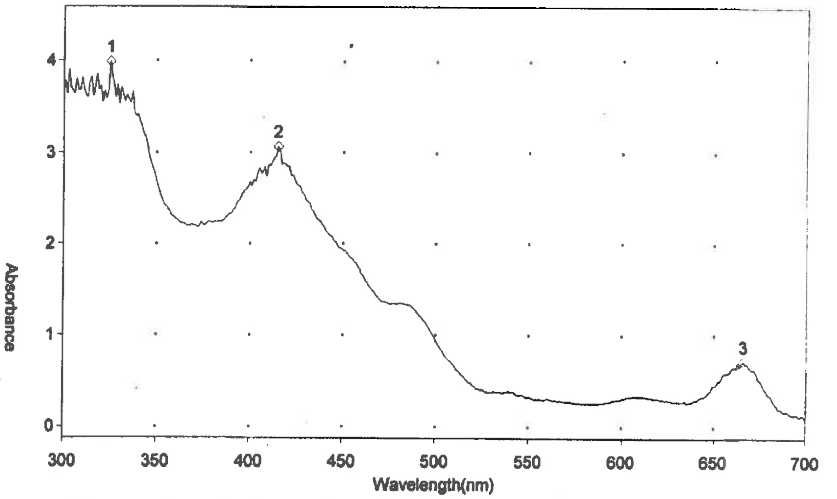


Figure 2. Light absorption spectrum of $PNTE_2$ 0.5 % chloroform solution in the diapason of 300 – 700 nm wavelengths

As it is known from literature, in open-column chromatography the adsorption affinity depends on the number of conjugated double bonds, cyclization, and the presence of oxygen substituents. The wavelength of maximum absorption and the shape of the spectrum (spectral fine structure) are characteristic of the chromophore. Using solvents with increasing polarity for the elution of $PNTE_2$ pigments, 5 fractions were obtained, and their light absorption spectra were picked up.

Eluting the carotenoids with Nephrase, the orange-yellow colour carotenes (including β-carotene) were eluted first, then they were followed by bright yellow colour carotenes, but bright light yellow colour zone remained at the top of the column under chlorophylls. As it is known from the literature data, carotenes are eluted through the alumina open column

as an emulsifier. Using this method for the analysis of SGC containing $PNTE_2$, a very stable emulsion develops because the filling mass of the capsules contains an emulsifier. Compared with $PNTE_1$, the content of moisture in $PNTE_2$ is reduced to 10 %. This factor, as well as the presence of vegetable oil and emulsifier in the capsule filling mass makes this filling mass more hydrophobic. It allows the sample to dissolve in Nephrase without the acetone application, and avoids the use of water for the removal of acetone from Nephrase. The exclusion of the acetone solution from the sample preparation procedure allows to accelerate and to facilitate the analysis and to reduce the loss of carotenes during the preparation of the sample.

Modifying the method, it was necessary to ascertain that making changes in the existent assay method of carotenes the equivalent components are extracted and by chromatography through the column the equivalent eluted solution is obtained. For this purpose the light absorption spectra of the eluted solutions of the samples prepared by both methods were picked up. The light absorption spectrum of the SGC with $PNTE_2$ eluted solution containing carotenes (λ_{max} 447 and 477 nm, shoulder at 425 nm; %III/II = 37.5) obtained by the approved method and the spectrum of the eluted solution (λ_{max} 448 and 477 nm, shoulder at 425 nm; %III/II = 35.7) obtained by the modified method show analogous number of maxima and one main maximum approximately at 450 nm wavelength. It indicates that the eluted solutions of the samples prepared by both methods are equivalent.

Validation of the carotenes assay method in SGC containing PNTE

The specificity, precision (repeatability of results), accuracy and linearity of the method were determined. It was established that the method is specific while the absorbance of *placebo* solution at 450 nm wavelength is negligible: $A_{mean} \approx 0.00275$; the method is enough precise and repeatable: RSD = 1.58 %; the recovery of the active substance average value is (103.72 ± 1.43) %, RSD = 1.37 %; the linear correlation between absorbance and concentration of the active substance in the solution is not casual: $R^2 = 0.995$, $r = 0.998$.

Evaluation of the quality of PNTE batches used in the study

The quality of PNTE batches used in the study was tested according to the requirements of Pharmacopoeia manuscript FP 95-

0002/42-26-95 „Fitestens" (Table 2) and LV UTN 000312820-18-2008 „*Skuju biezais ekstrakts*" (Table 3).

Table 2. Quality indices of $PNTE_1$ batches used in the study

Tests	Specification requirements	1st batch	2nd batch
Appearance, colour, odour, taste	Thick mass of dark green colour, with pine needles odour and bitter taste	Conform	Conform
Identification: - chlorophylls - carotenoids	 Conforms Conforms	 Conforms Conforms	 Conforms Conforms
Loss on drying (%) [mean ± SD, n = 3]	NMT 40.0	38.71±0.49	31.93±0.55
Content of carotenes in dry product (mg %) [mean ± SD, n = 3]	NLT 30.00	45.47±1.09	32.34±1.57

Table 3. Quality indices of $PNTE_2$ batches used in the study

Tests	Specification requirements	1st batch	2nd batch	3rd batch	4th batch
Appearance, colour, odour, taste	Thick mass of olive green to dark green colour, with faint, specific pine needles odour and bitter taste	Conform	Conform	Conform	Conform
Identification: - chlorophylls - carotenoids and non-saturated hydrocarbons	 Conforms Conforms	 Conforms Conforms	 Conforms Conforms	 Conforms Conforms	 Conforms Conforms
Loss on drying (%) [mean ± SD, n = 3]	NMT 10.0	8.54±0.65	8.90±0.30	10.86±0.44	10.81±0.67
pH of $PNTE_2$, 1 % water solution [mean ± SD, n = 3]	8.0 – 9.0	8.88±0.03	8.47±0.03	8.87±0.06	8.70±0.05
Content of carotenes in dry product (mg %) [mean ± SD, n = 3]	NLT 30.00	34.76±0.46	39.45±0.93	44.35±0.93	38.34±1.14

Investigation of possibilities to apply excipients and technological methods for the development of PNTE dosage form

Development of the technology of powder formulation containing $PNTE_1$ for filling into hard gelatin capsules

PNTE as an active substance doesn't possess optimal technological properties. Poor solubility of PNTE in water, ethanol, and oil, high viscosity of PNTE make difficult the technological process of preparation of a dosage form with this substance. It is not possible to obtain a dry extract from PNTE because it contains waxy and resinous substances.

For the development of PNTE dosage form, the possibility to prepare powder formulations for filling into hard gelatin capsules has been investigated. For the preparation of the powder formulations PNTE with the content of moisture and volatile substances NMT 40 % – $PNTE_1$ was used. Two variants of the preparation of the powder formulation were proved in the study: variant I – mixing the extract with inorganic active substances, plant extracts and excipients; variant II – mixing the extract with food wheat bran.

Preparation of variant I of powder formulations containing $PNTE_1$

27 powder formulations of $PNTE_1$ were prepared and evaluated organoleptically for the appearance and consistence. In the table 4, 10 formulations, showing comparatively better properties after visual evaluation and their consistence evaluation, are presented.

The optimal properties showed the formulation No. 9 containing 1 part of $PNTE_1$, 1.5 parts of magnesium oxide and 1.5 parts of calcium carbonate. A dry, flowable powder was obtained. Furthermore it contained components with antacid properties. This formulation was used in further study, and at the suggestion of gastroenterologists it was supplemented with bismuth subnitrate for the improvement of antacid, antiseptic and antiulcer properties (formulation No. 10).

Table 4. Variant I of powder formulations containing $PNTE_1$

Ingredient, amount (mass part)	Formulation No.									
	1	2	3	4	5	6	7	8	9	10
$PNTE_1$	1	1	1	1	1	1	1	1	1	1
Lactose, monohydrate	6	6	-	-	-	-	-	-	-	-
Liquorice dry extract	-	1	-	-	-	-	-	-	-	-
Aluminium oxide, hydrated	-	-	-	-	-	1	-	-	-	-
Magnesium oxide	-	-	1.5	2	-	1	1	1.25	1.5	1.5
Calcium carbonate	-	-	-	-	5	-	1	1.25	1.5	1.5
Bismuth subnitrate	-	-	-	-	-	-	-	-	-	1

To facilitate the mixing of $PNTE_1$ with powders, the extract was diluted with ethanol 96 % (V/V) in proportions 1 : 0.5 and 1 : 1, with purified water in proportion 1 : 1, and with tween-80 1 % water solution in proportion 1 : 1. For the preparation of powder formulations, mixer with three paddles (mixing speed 120 rpm) and heated reservoir were used. Out of the 4 prepared samples, one formulation obtained by diluting $PNTE_1$ with tween-80 1 % water solution was chosen, because the use of the surface active substance facilitated the mixing process of $PNTE_1$ with powders.

Checking the content of carotenes in the prepared variant I batches of powder formulations containing $PNTE_1$, it was established that the content of carotenes was reduced during storage period (Table 5).

Table 5. Content of carotenes in the powder formulation No. 10, variant I containing $PNTE_1$ during the stability study

Time of storage	Content of carotenes (mg %) [mean ± SD (n = 3)]			
	Storage conditions (25 ± 2) °C		Storage conditions (40 ± 2) °C	
	1st batch	2nd batch	1st batch	2nd batch
Before storage	37.28±0.55	36.17±0.43	37.28±0.55	36.17±0.43
1 month	16.09±0.67	12.25±0.68	9.45±0.34	8.36±0.74
2 months	5.64±0.97	4.64±1.08	0	0

The visual control showed that the powder formulation No. 10, containing $PNTE_1$ and the powder formulations No. 1 – 9 showed in table 4, changed their colour from green to grey. Probably it happened due to the

oxidation process of carotenes and chlorophylls and possible interaction between $PNTE_1$ components and other formulation ingredients.

For the development of a stable dosage form containing $PNTE_1$ powder formulations with antioxidant were prepared (Table 6).

Table 6. Variant I of powder formulations containing $PNTE_1$ with antioxidant

Ingredient, amount (mass part)	Formulation No.			
	11	12	13	14
$PNTE_1$	10	10	10	10
Magnesium oxide	15	15	15	20
Calcium carbonate	15	15	15	-
Bismuth subnitrate	10	-	-	-
Tween-80	0.1	0.1	-	-
Sodium metabisulphite	0.25	0.2	0.2	0.2
Content of carotenes after preparation (mg %) [mean ± SD (n = 3)]	35.33±0.65	37.74±0.32	37.85±0.25	38.07±0.42

Standardizing the prepared formulations, it was established that after the 6 months storage of powders the carotenes could not be assessed in all formulations. The colour of powders changed from green to gray. It can be concluded that the addition of the antioxidant (sodium metabisulphite) in concentration under 0.5 % has not improved the stability of powders containing $PNTE_1$.

Preparation of variant II of powder formulations containing $PNTE_1$

For the development of the powder formulation containing $PNTE_1$: 1) the particle size of the excipient – food wheat bran (bran, non sieved, or bran, sieved through the sieve with 1 mm orifice diameter), 2) the proportion of $PNTE_1$ and bran (1 : 5 or 1 : 10) were defined experimentally. As the visual quality and the consistence of obtained powders were similar, the fractioned composition with the particle size of 1 mm and the combination with the smallest amount of bran was chosen (1 : 5).

The powder mixture of $PNTE_1$ and bran in proportion 1 : 5 was tested for the flowability before filling into the hard gelatin capsules. The powder formulation containing $PNTE_1$ (with the particle size 1 mm) had

low flowability (0.96 $g \cdot s^{-1}$). It was necessary to increase the flowability of the powder to make it possible for filling into the hard gelatin capsules. For this reason, the powder containing $PNTE_1$ was supplemented with glidants and mixture of glidants extensively used in the technology of tablets and granules (Table 7). With the help of glidants the flowability of the powder formulation containing $PNTE_1$ and bran was improved. The best flowability was showed by the formulation No. 10 (Table 7).

Table 7. Flowability of variant II of powder formulations containing $PNTE_1$ and bran (1 : 5), with glidants

Glidant, amount (%)	Formulation No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Magnesium stearate	-	0.5	1	-	-	-	0.5	0.5	1	1	1	-
Calcium stearate	-	-	-	1	-	-	-	-	-	-	-	1
Stearic acid	-	-	-	-	1	-	-	-	-	-	-	-
Talc	-	-	-	-	-	2	1	2	1	2	3	1
Flowability ($g \cdot s^{-1}$), mean	0.96	6.81	6.71	6.91	5.51	6.22	6.38	7.02	7.31	7.40	7.23	6.35
SD (n=5)	0.04	0.10	0.24	0.11	0.03	0.07	0.19	0.17	0.18	0.10	0.24	0.23

The content of carotenes in the powder formulation containing $PNTE_1$ and bran (1 : 5) with glidants (formulation No. 10) immediately after the preparation was (30.74 ± 0.45) mg %. It was established that the carotenes and chlorophylls could not be detected in the formulation after 6 months storage at (25 ± 2) °C and at (40 ± 2) °C temperature. Probably the oxidation process of carotenoids and chlorophylls or other chemical changes took place.

Technologically it is possible to prepare a powder formulation containing $PNTE_1$ for filling into hard gelatin capsules. However, the changes observed during the storage period testified the lack of stability of the powder formulation filled into the capsules. The results of the study show that it is not efficient to prepare powder formulations containing $PNTE_1$ with magnesium oxide, calcium carbonate, bismuth subnitrate, and wheat bran.

Development of the technology of the preparation of soft gelatin capsules containing PNTE

The second direction of the study of PNTE dosage forms was the development of soft gelatin capsules (SGC). The optimal content of moisture in PNTE was specified, appropriate excipients and their quantity were defined, and different methods for the preparation of SGC were approbated in the technology development process of SGC containing PNTE.

Dipping method for the preparation of SGC

The optimal composition of gelatin solution for the preparation of SGC by dipping method, consisting of gelatin 31.14 %, glycerol 22.64 %, purified water 46.08 %, and nipagin 0.14 % as a preservative, was found experimentally. For the preparation of the gelatin solution, glycerol was mixed with purified water and heated till 70 °C temperature. Nipagin was dissolved in the mixture. Gelatin was added, and the mass was heated at (80 – 85) °C temperature and kept in a thermostat at 45 °C temperature for 24 h.

Metal moulds were used for the preparation of SGC by dipping method. The obtained gelatin shells were filled with the formulations containing $PNTE_1$ and sealed with the soldering iron. The capsules were washed with isopropyl alcohol and dried at room temperature.

Preparation of formulations containing $PNTE_1$ for filling into SGC

$PNTE_1$ without excipients was tried to fill in SGC. It was found that a deformation of capsule shells occurred in the next day after preparation of capsules, because the high content of moisture in $PNTE_1$ negatively affected the capsule shells.

For minimizing the effect of moisture on the capsule shells, the possibility to prepare a homogenous mixture of $PNTE_1$ and vegetable oil for filling into SGC was examined. Sunflower or arachis oil was mixed with $PNTE_1$ in ratio 1 : 1, and surface active substances (emulsifiers) in amount of 3 %, 5 %, and 7 % were added. The quality of the prepared formulations was evaluated by homogeneity and consistency (Table 8).

Preparation of the formulations containing $PNTE_1$ and vegetable oil (1 : 1) with emulsifiers: the emulsifier was melted and mixed with vegetable oil at (45 – 50) °C temperature. $PNTE_1$ was heated till (45 – 50) °C and added to the vegetable oil and emulsifier mixture, stirred till a homogenous mass was achieved.

Formulations of $PNTE_1$ with arachis oil in ratio 1 : 1 and 5 % of emulsifier: tween-20, tween-60V, tween-80, Sorbital T40P, Multec Soral MS, and Multec Soral MO, which were homogenous and of liquid consistence, were filled in SGC prepared by dipping method. The capsules were stored at (25 ± 2) °C and at (30 ± 2) °C temperature. It was found that the deformation of capsules started after 1 week due to the high moisture content in the capsule filling mass.

Table 8. Quality of formulations of $PNTE_1$ with vegetable oil (1 : 1) and emulsifiers

Emulsifier		Result*	Emulsifier		Result*	Emulsifier		Result*
name	%		name	%		name	%	
Tween-20	3	±	Sorbital T40P	3	±	Rylo MG 20 Pharma	3	–
	5	+		5	+		5	±
							7	t/c
Tween-40	3	–	Multec Soral MS	3	±	Rylo MD 50 Pharma	3	–
	5	–		5	+		5	±
	7	±					7	t/c
Tween-60V	3	±	Multec Soral MO	3	±	Rylo AC 19 Pharma	3	-
	5	+		5	+		5	-
							7	t/c
Tween-65	3	–	Emulsifier T-2	3	–	Distilled monoglycerides	3	–
	5	±		5	–		5	±
	7	±		7	t/c		7	t/c
Tween-80	3	±	Pentol	3	–	* Result: + homogenous mass; ± mass, separating in layers; – non miscible mass; t/c – mass of thick consistence		
	5	+		5	±			
				7	±			

Preparation of formulations containing dried $PNTE_1$ for filling into SGC

It was not rational to reduce the moister content of the capsule fill by the dilution of $PNTE_1$ with vegetable oil more than 50 %, because the dosage of the active substance then would be too low, or the size of the dosage form would be too large. Therefore, the content of moisture and volatile substances in $PNTE_1$ was reduced by drying. At the laboratory the dried $PNTE_1$ was obtained with the loss of moisture and volatile substances in the range of (35.0 – 41.6) %, i.e., almost a dry mass.

$PNTE_2$, arachis oil and GMO (30 : 65 : 5) prepared by the dipping method are shown in the table 10.

Table 10. Quality of SGC containing 300 mg of $PNTE_2$, prepared by the dipping method

Tests	Specification requirements	Results	n
Appearance	Soft gelatin capsules of oval shape with visible sealing spot, containing thick mass of dark green colour with pine needles odour and bitter taste	Conforms	20
Identity: absorption maxima of UVNIS spectrum of eluted solution containing carotenes: λ_{max} (nm); characteristic position of chlorophylls and carotenoids in the chromatography column	(shoulder 425); 450 ± 3; 477 ± 2 after eluting of carotenes, other carotenoids remain at the top of the column under chlorophylls	Conforms	3
Average mass (g)	1.0000	0.9728	20
Uniformity of mass (g) (%)	0.9250 - 1.0750 ± 0.0750 ±7.5	0.9120 - 1.0455 -0.0608 + +0.0727 -6.25 + +7.47	20
Disintegration time (min)	Not more than 30	8 - 10	6
Content of carotenes (mean± SD) in preparation (mg%) in capsule (mg)	 Not less than 30 Not less than 80	 38.94±0.58 104.1±1.6	3
Primary packaging	HDPE containers with LDPE caps		

The quality of the prepared capsules was within the required limits, only the uniformity of mass of capsules slightly exceeded the defined ranges. It can be explained by the fact that the capsules were prepared and filled manually.

The technological process of preparation of the laboratory batch of SGC containing $PNTE_2$ and in-process control outline is shown in the figure 4.

Suitable excipients were chosen to develop the formulations of the dried $PNTE_1$ for filling into SGC. The dried $PNTE_1$ with vegetable oil without emulsifiers did not form the homogenous mixture. Formulations of the dried PNTE1 with arachis or sunflower oil and emulsifiers in ratio 30 : 65 : 5 were prepared. The quality of the formulations was evaluated by the homogeneity and consistency (Table 9). The homogenous emulsion of PNTE with vegetable oil was prepared using emulsifier T-2, Rylo MG 20 Pharma, and distilled monoglycerides.

Table 9. Quality of formulations of dried $PNTE_1$ with vegetable oil and emulsifiers (30 : 65 : 5)

Emulsifier	Result*	Emulsifier	Result*	Emulsifier	Result*
Tween-20	±	Sorbital T40P	±	RyloMG20 Pharma	+
Tween-40	-	Multec Soral MS	±	RyloMD50 Pharma	±
Tween-60V	-	Multec Soral MO	±	Rylo AC 19 Pharma	±
Tween-65	-	Emulsifier T-2	+	Distilled monoglycerides	+

* Result: + homogenous mass; ± mass, separating in layers; - non miscible mass

Preparation of laboratory batch of SGC containing $PNTE_2$ by dipping method

It was not possible to minimize the content of moisture and volatile substances of $PNTE_1$ for less than 10 % in the Iarge-scale manufacturing. Therefore, further in the study the industrially manufactured PNTE with the content of moisture and volatile substances NMT 10 % - $PNTE_2$ was used. The optimal dosage of $PNTE_2$ is 300 mg.

For the preparation of $PNTE_2$ and arachis oil emulsion for filling in SGC, emulsifier Rylo MG 20 Pharma (glycerol monooleate, GMO) was used, because its quality complies with the requirements of European Pharmacopoeia.

The formulation of $PNTE_2$ with arachis oil and GMO (30 : 65 : 5) in the amount of 1 g (containing 300 mg of $PNTE_2$) was filled into SGC prepared by the dipping method. Quality indices of SGC containing

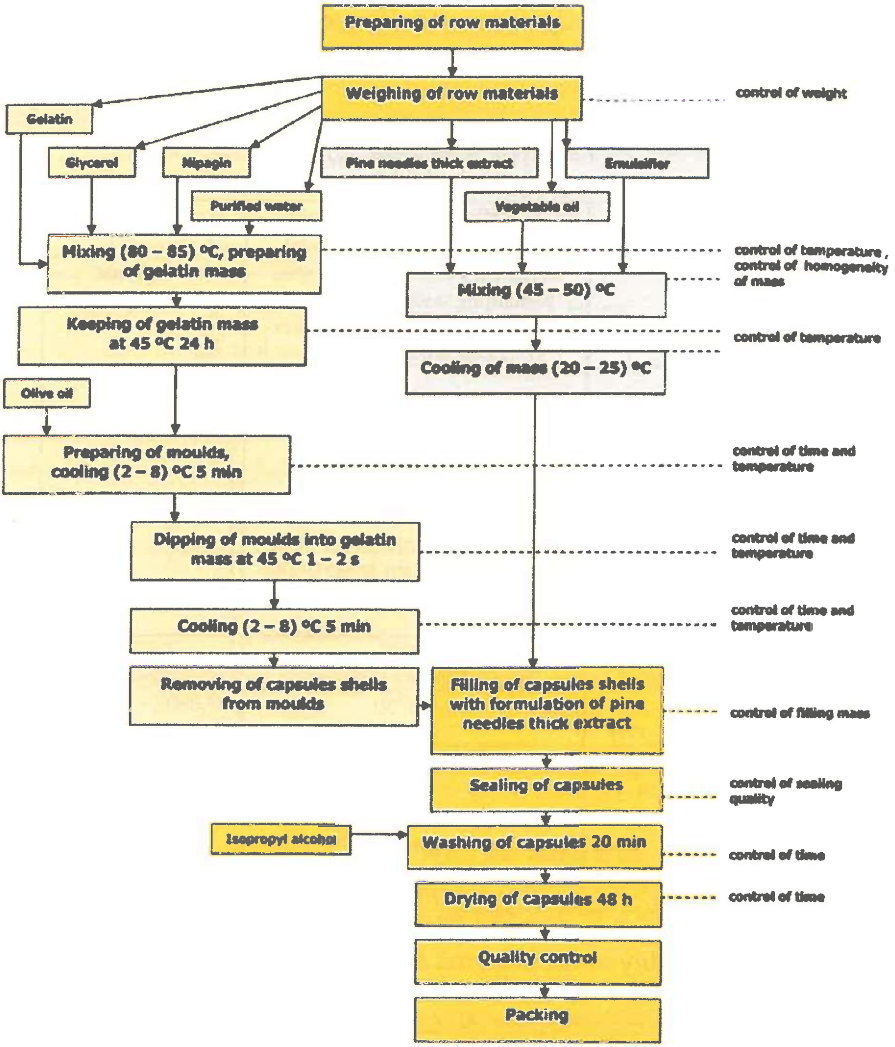


Figure 4. Outline of the technological process of the preparation of the laboratory batch of SGC containing $PNTE_2$

Filling of formulation containing $PNTE_2$ into two-piece hard capsules

A possibility to fill liquid and soft masses into two-piece hard capsules exists in the industrial manufacturing. Therefore the formulation containing $PNTE_2$ was tried to fill into hard capsules in the laboratory. The formulation was prepared as described above and filled with a syringe into hard gelatin capsules of size No. 0 in the amount of 500 mg (containing 150 mg of $PNTE_2$).

The quality indices of the prepared two-piece hard gelatin capsules are shown in the table 11. The quality of the capsules was within the required limits, only the uniformity of mass of capsules exceeded the defined ranges. It can be explained by the fact that the capsules were filled manually.

Table 11. Quality of hard gelatin capsules filled with formulation containing $PNTE_2$ 150 mg

Tests	Specification requirements	Results	n
Appearance	Hard gelatin capsules containing thick mass of dark green colour with pine needles odour and bitter taste	Conforms	20
Identity: absorption maxima of UV/VIS spectrum of eluted solution containing carotenes: λ_{max} (nm); characteristic position of chlorophylls and carotenoids in the chromatography column	(shoulder 425); 450 ± 3; 477 ± 2 after eluting of carotenes, other carotenoids remain at the top of the column under chlorophylls	Conforms	3
Average mass (g)	0.5000	0.5479	20
Uniformity of mass (g) (%)	0.4625 – 0.5375 ± 0.0375 ± 7.5	0.4789 – 0.6303 -0.0690 ÷ +0.0824 -7.44 ÷ +7.03	20
Disintegration time (min)	Not more than 30	4 – 6	6
Content of carotenes (mean ± SD) in preparation (mg %)	Not less than 30	33.91±1.18	3
Primary packaging	HDPE containers with LDPE caps		

Possibility to apply different encapsulating methods used in industrial manufacturing for preparation of $PNTE_2$ dosage form

Preparation of SGC with formulation containing $PNTE_2$ by a droplet method

The droplet method for preparation of SGC with formulation containing $PNTE_2$ was approbated at JSC „RealCaps" Moscow, Russia.

The formulation for encapsulation containing $PNTE_2$ 30 %, emulsifier GMO 5 %, and soya-bean oil 65 % was prepared. The gelatin solution prepared at the enterprise was used. Equipment „Capsulator" (produced in Belarus) for preparation of SGC was used.

The technology of the preparation of capsules

The capsules filling mass from the tank under pressure passes to the dosing device, from which it simultaneously with the gelatin solution, heated till 60 °C temperature in the tank, is pumped into the nozzle head. The capsules are formed with the help of pulsing pump and pass into circulating cooling system (soya-bean oil cooled till +10 °C temperature), in which the gelatin capsule shells solidify. Then the capsules pass into a container with soya-bean oil cooled till +10 °C temperature. The capsules are kept in the cooling chamber, the cooling oil is removed, and the capsules are dried and washed with isopropyl alcohol.

At the beginning of the encapsulating process, capsules of spherical shape were formed: the fill drop was surrounded with gelatin solution drop. But coming down to the lower part of cooling tube, the fill formulation frequently penetrated through the capsules shells. The mixing of the cooling oil with the capsules filling mass made difficult the further preparation of capsules. After the change of cooling oil it was possible to obtain a small amount of capsules, but as soon as capsules broke, it was necessary to change the cooling oil again.

It was tried to prepare capsules with the formulation diluted with oil half-and-half (contained $PNTE_2$ 15 %, GMO 2.5 % and soya-bean oil 82.5 %), as well as to prepare capsules with the filling mass of 0.6 g and 0.3 g. In all attempts during the encapsulating process a lot of defective capsules were obtained. The capsules differed in size, shape and mass.

Preparation of SGC with formulation containing $PNTE_2$ by stamping method

SGC with formulation containing $PNTE_2$ were prepared by stamping method at the enterprise „Minskintercaps", Belarus, using rotary

die equipment GIC (USA). Three pilot batches and two manufacturing batches were prepared.

The formulation containing $PNTE_2$ 50 %, sunflower oil 45 %, and GMO 5 %, was prepared for filling into capsules. The $PNTE_2$ content in the formulation was increased with the aim to minimize the capsule fill mass and the size of capsule. Each capsule contained the amount of 600 mg (300 mg of $PNTE_2$).

The technological process of preparation of SGC with $PNTE_2$ included the preparation of raw materials, preparation of gelatin solution, and preparation of capsule filling mass, stamping of capsules, drying and purification of capsules, quality control of unpacked capsules, packing and quality control of the finished product.

For the preparation of the formulation for filling into capsules, the speed for mixing the components for the 1st pilot batch was 300 – 430 – 500 rpm, $PNTE_2$ was mixed 10 min with the emulsifier solution in oil. For the 2nd and the 3rd pilot batches the components were mixed 15 min slowly with a spatula. For the manufacturing batches the components were mixed 30 min in mixer at low speed – 25 – 30 rpm.

Primary packaging used for $PNTE_2$ SGC was:

- Al/PVC blisters for the 1st pilot batch, the 3rd pilot batch and I manufacturing batch;
- HDPE containers with LDPE caps for the 2nd pilot batch and II manufacturing batch.

The appearance of SGC of all batches complied with the specification requirements (Table 12). The initial quality of the 1st pilot batch was not satisfactory: the content of carotenes was under the required level. The quality of the 2nd and the 3rd pilot batches, and I and II manufacturing batches was satisfactory. Such difference in the quality of capsules may be explained by the mixing rate of the filling mass. Probably during the mixing process of the 1st pilot batch capsule filling mass in the high-speed mixer, the oxygen molecules entered the mass and caused the oxidation process of carotenes. Therefore SGC of other pilot batches and manufacturing batches were prepared using the slow mixing of capsule filling mass.

Table 12. Quality of SGC containing 300 mg of $PNTE_2$, prepared by the stamping method

Tests	Specification requirements	n	Results				
			1st pilot batch	2nd pilot batch	3rd pilot batch	I manufacturing batch	II manufacturing batch
Appearance	Soft gelatin capsules of oval shape with visible seal, containing thick mass of dark green colour with pine needles odour and bitter taste	20	Conforms	Conforms	Conforms	Conforms	Conforms
Identity: absorption maxima of UV/VIS spectrum of eluted solution containing carotenes: λ_{max} (nm); characteristic position of chlorophylls and carotenoids in the chromatography column	(shoulder 425); 450 ± 3; 477 ± 2 after eluting of carotenes, other carotenoids remain at the top of the column under chlorophylls	3	Conforms	Conforms	Conforms	Conforms	Conforms
Average mass (g)	0.6000	20	0.6087	0.5997	0.5809	0.5885	0.5823
Uniformity of mass (g)	0.5550 – 0.6450 ± 0.0450	20	0.5842 – 0.6314 -0.0245 ÷ +0.0227	0.5811 – 0.6140 -0.0186 ÷ +0.0143	0.5707 – 0.5878 -0.0102 ÷ +0.0069	0.5780 – 0.5987 -0.0105 ÷ +0.0102	0.5727 – 0.5908 -0.0096 ÷ +0.0085
(%)	± 7.5		-4.03 ÷ +3.73	-3.09 ÷ +2.39	-1.75 ÷ +1.19	-1.78 ÷ +1.74	-1.65 ÷ +1.46
Disintegration time (min)	Not more than 30	6	18 – 20	18 – 20	17 – 19	17 – 20	18 – 20
Content of carotenes (mean ± SD)							
in preparation (mg %)	Not less than 30	3	22.03±0.86	41.89±1.13	36.36±0.43	36.78±0.52	36.45±0.93
in capsule (µg)	Not less than 80		58.9±2.3	112.1±3.0	97.3±1.2	98.4±1.4	97.5±2.5

Investigation of the stability of a dosage form containing $PNTE_2$

The stability studies were performed with all prepared batches of SGC and with hard capsules containing $PNTE_2$. The capsules were estimated for the appearance, uniformity of mass, disintegration time and content of carotenes during the stability testing. According to the requirements of European Medicines Agency (EMA) guidelines, for the content of carotenes as markers in $PNTE_2$ and dosage forms containing $PNTE_2$ a variation in marker content during the proposed shelf life of ± 10 % of the initial assay value can be accepted, because the therapeutic activity of carotenes in $PNTE_2$ is unknown.

The assay is the most important index that testifies the quality of a pharmaceutical product. Monitoring the content of carotenes in each batch of capsules after manufacturing and during stability studies in definite time intervals, it was found that the quality of the laboratory batches of SGC and hard gelatin capsules, two pilot batches, and two manufacturing batches during the stability testing was satisfactory (the content of carotenes was NLT 30 mg %). In our study one pilot batch showed the initial content of carotenes under the required level. (See tables 13 – 19, figures 5, 7, and 9).

The appearance is one of the indices showing the changes in the quality of pharmaceutical product. During the stability testing of $PNTE_2$ SGC, it was ascertained that the appearance of capsules depended on the primary packaging (HDPE containers with LDPE caps or Al/PVC blisters) and storage conditions.

In the stability studies, were the temperature was controlled in thermostats, during long term stability testing at (25 ± 2) °C and accelerated stability testing at (30 ± 2) °C, no changes were observed in the appearance of capsules. The quality of $PNTE_2$ SGC of the laboratory batch prepared by the dipping method and packed in HDPE containers maintained during the period of the long term stability testing for 24 months and accelerated stability testing for 12 months. No changes were observed in the capsules shape, shell or fill. The quality of the hard gelatin capsules containing $PNTE_2$ formulation, stored at (25 ± 2) °C temperature, maintained during 24 months. Also the appearance of capsules of all prepared pilot batches packed in both types of packaging complied with the required standards for 9 months (for one batch 12 months). The stability studies of the 2nd and the 3rd pilot batches are in progress.

The manufacturing batches of $PNTE_2$ SGC were stored in the climatic chambers for a long term at (25 ± 2) °C / (60 ± 5) % RH and accelerated stability testing at stress conditions at (30 ± 2) °C / (65 ± 5) % RH. It allowed evaluate the influence of moisture and the type of packaging on the stability of the dosage form. The results of the stability studies of two manufacturing batches of $PNTE_2$ SGC stored for 6 months were obtained. The stability studies of these batches are in progress.

It was found that the stability of the manufacturing batches of capsules packed in HDPE containers maintained during all test period of long storage term and accelerated stability studies. The appearance and the uniformity of mass of capsules were in the limits of the regulated standards.

The appearance of the capsules of manufacturing batches packed in Al/PVC blisters after 3 months of storage in the climatic chambers was satisfactory, only the capsule shells became softer. After 6 months of storage more pronounced changes were observed in the appearance of capsules: the shells of capsules became very soft and swollen. Particularly it was related to capsules stored at stress conditions. These observations indicate the insufficient protection from moisture for capsules packed in Al/PVC blisters. The uniformity of mass of capsules remained in the limits of the regulated standards.

Also the colour of capsules shells was different, depending on the type of packaging and storage conditions for $PNTE_2$ SGC of the manufacturing batches. The shells of just prepared capsules were transparent and light brownish-yellow. During storage they became darker and greenish-brown. The colour intensity was greater in the shells of capsules stored at stress conditions at (30 ± 2) °C / (65 ± 5) % RH, and the darkest shells were from the capsules packed in Al/PVC blisters. It is linked to the fact that Al/PVC blister has greater moisture permeability compared with HDPE container.

Stability of $PNTE_2$ SGC and hard gelatin capsules of the laboratory batches

Table 13. Stability of $PNTE_2$ SGC of the laboratory batch

Time of storage	Long term stability study (25 ± 2) °C		Accelerated stability study (30 ± 2) °C	
	Content of carotenes (mg %) [mean ± SD, n = 3]	Disintegration time (min) [n = 6]	Content of carotenes (mg %) [mean ± SD, n = 3]	Disintegration time (min) [n = 6]
Specification requirements	NLT 30	NMT 30	NLT 30	NMT 30
Before storage	38.94±0.58	8 – 10	38.94±0.58	8 – 10
3 months	36.57±0.42	8 – 11	42.14±0.82	9 – 12
6 months	35.64±0.52	9 – 11	36.07±0.74	12 – 15
9 months	35.12±0.87	9 – 12	35.80±0.69	14 – 17
12 months	34.52±0.94	10 – 12	35.34±0.85	20 – 24
18 months	31.83±1.73	13 – 16	-	-
24 months	30.12±0.80	15 – 18	-	-

Table 14. Stability of $PNTE_2$ hard gelatin capsules of the laboratory batch

Time of storage	Long term stability study (25 ± 2) °C	
	Content of carotenes (mg %) [mean ± SD, n = 3]	Disintegration time (min) [n = 6]
Specification requirements	NLT 30	NMT 30
Before storage	33.91±1.18	4 – 6
3 months	33.43±0.56	4 – 7
6 months	33.56±0.75	5 – 7
9 months	32.88±0.97	5 – 8
12 months	32.27±1.68	6 – 10
18 months	29.46±1.55	8 – 11
24 months	28.10±1.25	9 – 13

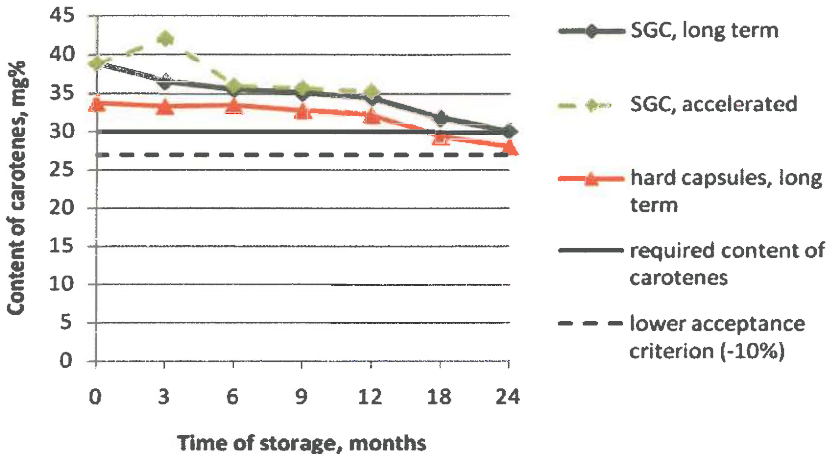


Figure 5. Content of carotenes of $PNTE_2$ SGC and hard gelatin capsules of the laboratory batches during the stability study

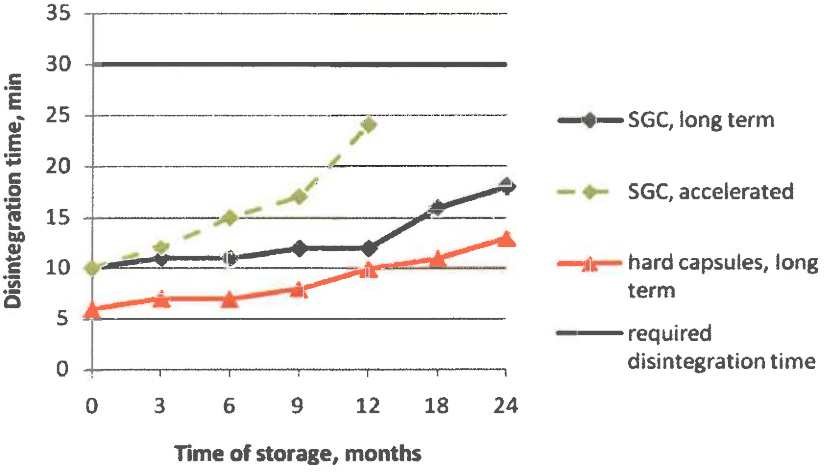


Figure 6. Disintegration time of $PNTE_2$ SGC and hard gelatin capsules of the laboratory batches during the stability study

Stability of PNTE$_2$ SGC of the pilot batches

Table 15. Stability of PNTE$_2$ SGC of the 1st pilot batch

Time of storage	Long term stability study (25 ± 2) °C		Accelerated stability study (30 ± 2) °C	
	Content of carotenes (mg %) [mean ± SD, n = 3]	Disintegration time (min) [n = 6]	Content of carotenes (mg %) [mean ± SD, n = 3]	Disintegration time (min) [n = 6]
Specification requirements	NLT 30	NMT 30	NLT 30	NMT 30
Before storage	22.03±0.86	18 – 20	22.03±0.86	18 – 20
3 months	21.91±0.29	19 – 22	23.45±0.46	22 – 24
6 months	19.48±0.50	23 – 26	20.73±0.24	26 – 30
9 months	16.85±0.34	26 – 28	19.88±1.56	28 – 30
12 months	16.22±0.19	26 – 30	11.33±0.23	29 – 32

Table 16. Stability of PNTE$_2$ SGC of the 2nd pilot batch

Time of storage	Long term stability study (25 ± 2) °C		Accelerated stability study (30 ± 2) °C	
	Content of carotenes (mg %) [mean ± SD, n = 3]	Disintegration time (min) [n = 6]	Content of carotenes (mg %) [mean ± SD, n = 3]	Disintegration time (min) [n = 6]
Specification requirements	NLT 30	NMT 30	NLT 30	NMT 30
Before storage	41.89±1.13	18 – 20	41.89±1.13	18 – 20
3 months	42.77±1.38	20 – 22	42.64±1.73	22 – 24
6 months	43.43±1.16	20 – 23	43.58±1.36	23 – 25
9 months	43.39±1.39	21 – 24	43.80±2.63	25 – 27

Table 17. Stability of PNTE$_2$ SGC of the 3rd pilot batch

Time of storage	Long term stability study (25 ± 2) °C		Accelerated stability study (30 ± 2) °C	
	Content of carotenes (mg %) [mean ± SD, n = 3]	Disintegration time (min) [n = 6]	Content of carotenes (mg %) [mean ± SD, n = 3]	Disintegration time (min) [n = 6]
Specification requirements	NLT 30	NMT 30	NLT 30	NMT 30
Before storage	36.36±0.43	17 – 19	36.36±0.43	17 – 19
3 months	35.59±0.43	20 – 22	35.89±0.41	21 – 23
6 months	35.46±0.84	21 – 23	36.01±0.31	23 – 26
9 months	35.45±0.68	22 – 25	36.44±0.15	26 – 28

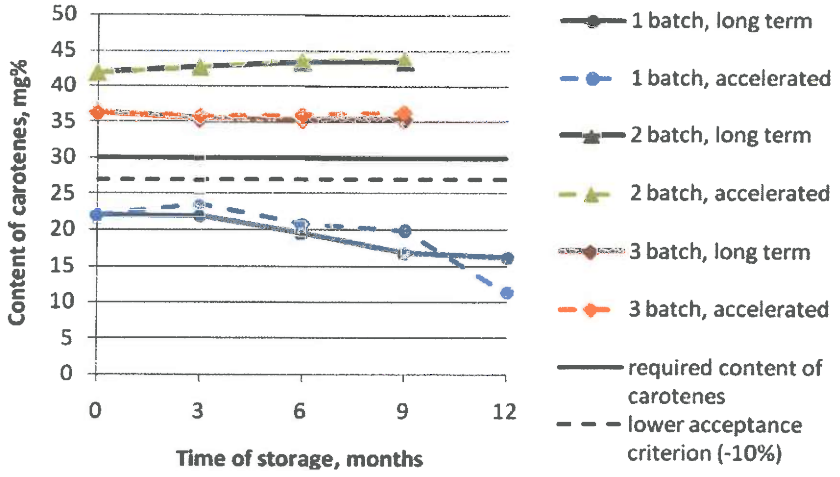


Figure 7. Content of carotenes of $PNTE_2$ SGC of the pilot batches during the stability study

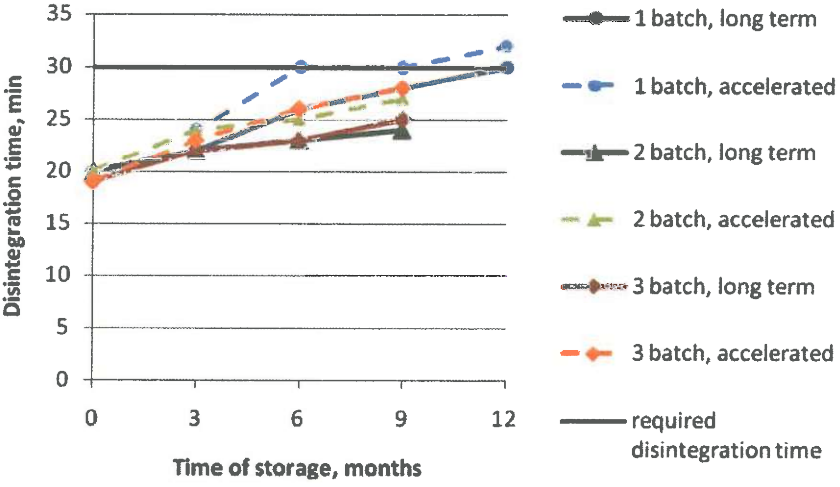


Figure 8. Disintegration time of $PNTE_2$ SGC of the pilot batches during the stability study

Stability of PNTE$_2$ SGC of the manufacturing batches

Table 18. Stability of PNTE$_2$ SGC of I manufacturing batch

Time of storage	Long term stability study (25 ± 2) °C / (60 ± 5) % RH		Accelerated stability study (30 ± 2) °C / (65 ± 5) % RH	
	Content of carotenes (mg %) [mean ± SD, n = 3]	Disintegration time (min) [n = 6]	Content of carotenes (mg %) [mean ± SD, n = 3]	Disintegration time (min) [n = 6]
Specification requirements	NLT 30	NMT 30	NLT 30	NMT 30
Before storage	36.78±0.52	17 – 20	36.78±0.52	17 – 20
3 months	36.45±0.90	20 – 22	36.75±1.20	24 – 26
6 months	34.83±0.71	21 – 23	34.96±0.09	28 – 35

Table 19. Stability of PNTE$_2$ SGC of II manufacturing batch

Time of storage	Long term stability study (25 ± 2) °C / (60 ± 5) % RH		Accelerated stability study (30 ± 2) °C / (65 ± 5) % RH	
	Content of carotenes (mg %) [mean ± SD, n = 3]	Disintegration time (min) [n = 6]	Content of carotenes (mg %) [mean ± SD, n = 3]	Disintegration time (min) [n = 6]
Specification requirements	NLT 30	NMT 30	NLT 30	NMT 30
Before storage	36.45±0.93	18 – 20	36.45±0.93	18 – 20
3 months	36.71±0.80	20 – 22	36.36±0.37	23 – 25
6 months	35.66±0.74	20 – 22	37.02±0.26	24 – 28

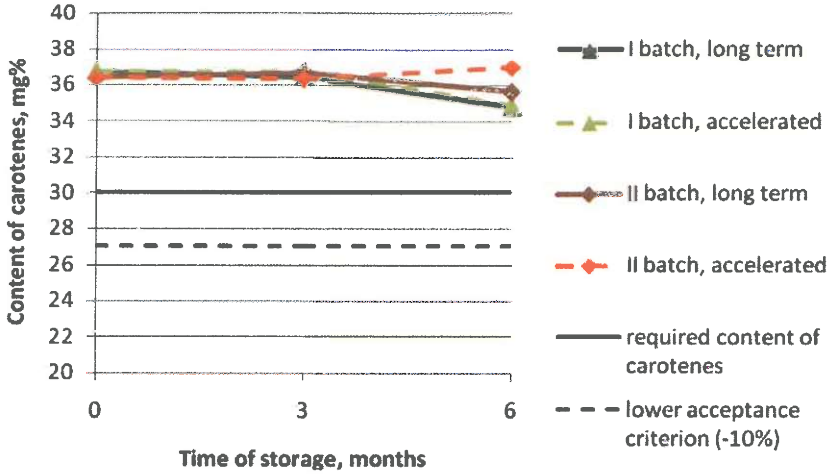


Figure 9. Content of carotenes of $PNTE_2$ SGC of the manufacturing batches during the stability study

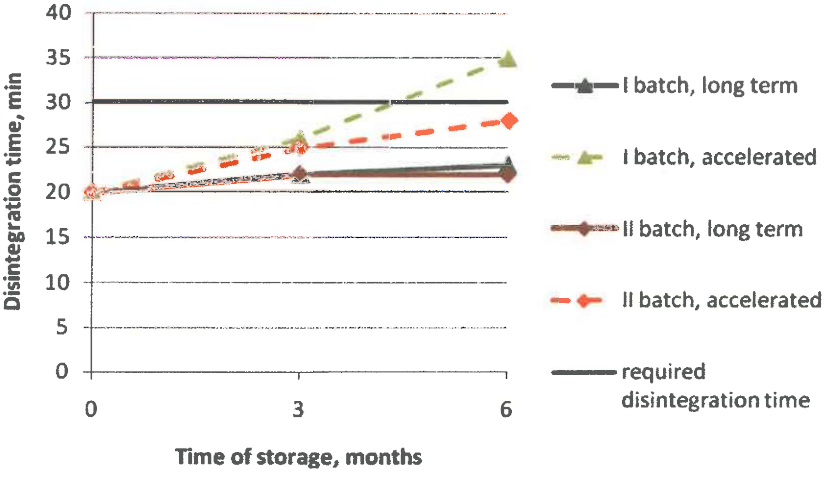


Figure 10. Disintegration time of $PNTE_2$ SGC of the manufacturing batches during the stability study

Disintegration peculiarities of $PNTE_2$ SGC

For the absorption and effect in the organism the active substances must firstly release from the dosage form, therefore it is important to estimate the disintegration time of the dosage form. SGC prepared by the dipping method disintegrate faster, compared with the capsules prepared by the stamping method (Figures 6, 8, and 10). It can be explained by the fact that the shells of capsules prepared by the dipping method are thin and have a sealing spot opening first when the capsule gets into the disintegration medium. SGC prepared by the stamping method have thicker shells due to the technological process.

The disintegration test of $PNTE_2$ SGC showed that the disintegration time of capsules in water depended on the primary packaging and storage conditions and time. The disintegration process resulted in the formation of swollen, rubbery water-insoluble membranes known as pellicles during disintegration testing. The pellicles surround the fill or the capsules and prevent the fill from being released, in spite of the fact that the capsules in water at 37 °C temperature became swollen and deformed. The observed phenomenon is described in literature as gelatin cross-linking, which is common to gelatin as a polymer. Aldehydes (also in the PNTE) react with ε-amino groups (mainly lysine) and built cross sites in the gelatin molecules. This process is accelerated at high temperature and humidity.

Figure 11 shows the disintegration stages in water of $PNTE_2$ SGC stored in the climatic chambers for 6 months.

The disintegration time was greater for the capsules packed in Al/PVC blisters and stored at stress conditions at (30 ± 2) °C / (65 ± 5) % RH.

The disintegration of $PNTE_2$ SGC stored in Al/PVC blisters in the climatic chamber at (30 ± 2) °C / (65 ± 5) % RH for 6 months was tested in simulated intestinal fluid (phosphate buffer solution with pH 6.8 and with pancreas powder) at (37 ± 0.5) °C temperature. It was found that in this medium the capsules disintegrate faster and completely: the capsule fill disintegrates in 15 min, and the capsule shells disintegrate completely in 20 min.

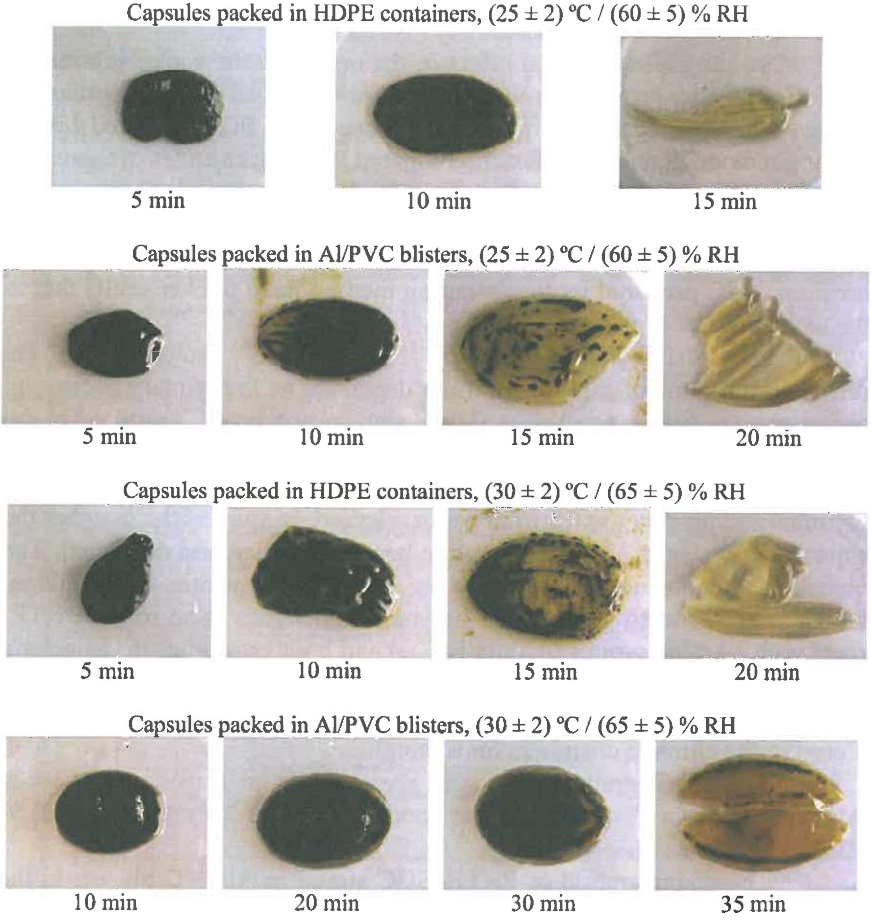


Figure 11. Disintegration of $PNTE_2$ SGC in water at 37 °C after storage in climatic chambers for 6 months

Prediction of the shelf life of $PNTE_2$ SGC

As the quality of $PNTE_2$ SGC of the 1st pilot batch was not satisfactory, but the stability studies of other pilot batches and manufacturing batches are in progress, the results of the completed stability study for 24 months of the $PNTE_2$ SGC laboratory batch were used as a

shelf life estimation model. The capsules were evaluated by the content of carotenes and disintegration time (Figures 12 and 13).

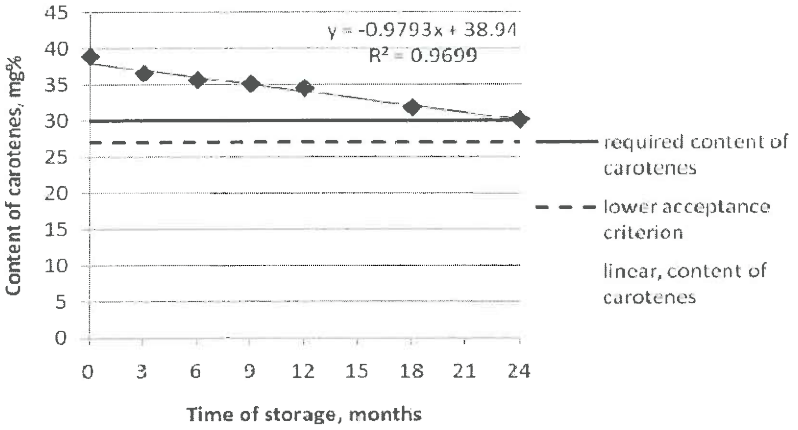


Figure 12. Content of carotenes of $PNTE_2$ SGC of the laboratory batch during the long term stability study

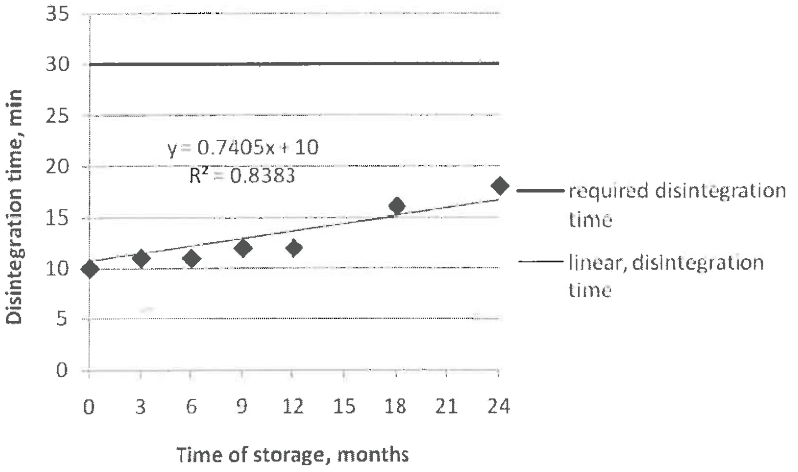


Figure 13. Disintegration time of $PNTE_2$ SGC of the laboratory batch during the long term stability study

A linear regression equation was used for calculation of the carotene content in the active substance – $PNTE_2$ for the preparation of a qualitative finished product:

$a = y - b \cdot x = 27 - (-0.9793 \cdot 8) = 34.83$ mg % (~35 mg %).

The quality of the 2nd and the 3rd pilot batches and the manufacturing batches was in the required limits (the content of carotenes was in the range 36.36 – 41.89 mg %).

I manufacturing batch, the stability study of which is in progress, was analyzed with the use of extrapolation method. The content of carotenes of this batch decreased faster, than of the 2nd and the 3rd pilot batches and II manufacturing batch, after 6 months. The content of carotenes and the disintegration time of capsules of I manufacturing batch after 24 months of storage was predicted.

The content of carotenes of I manufacturing batch was extrapolated, and at the end of the shelf life has been expected:

$y = a + b \cdot x = -0.465 \cdot 8 + 36.78 = 33.06$ mg %,

and the disintegration time has been expected:

$y = a + b \cdot x = 0.9286 \cdot 8 + 20 = 27.43 \approx 27$ min (Figures 14 and 15).

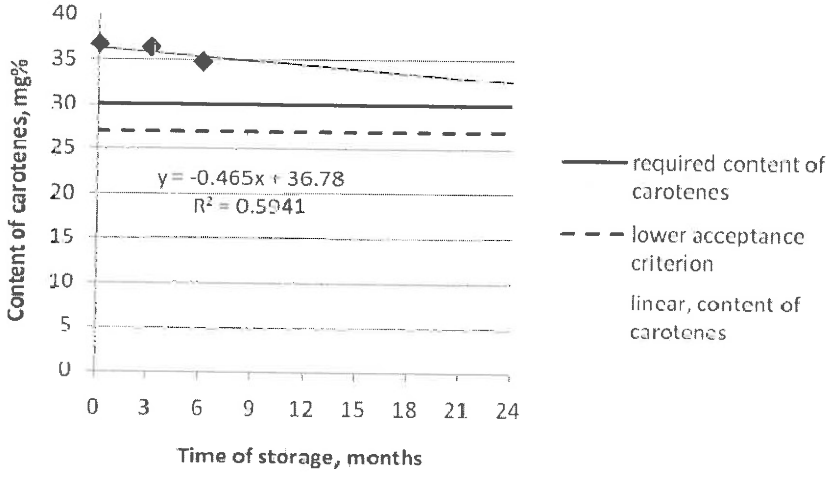


Figure 14. Content of carotenes of $PNTE_2$ SGC of I manufacturing batch during the long term stability study

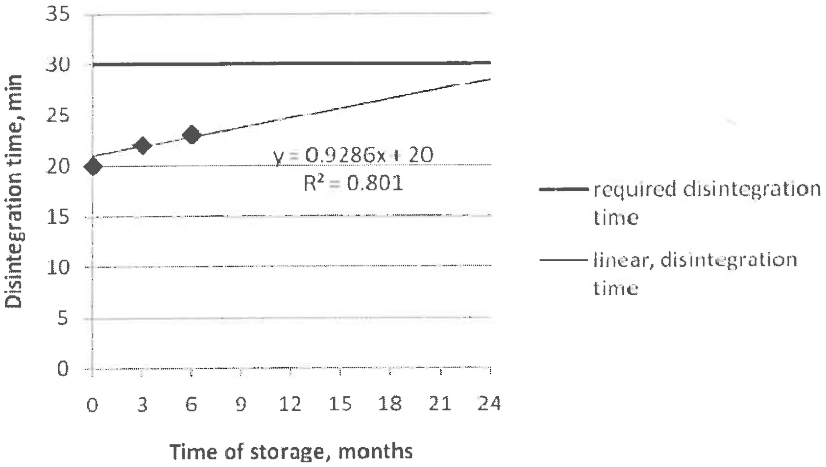


Figure 15. Disintegration time of $PNTE_2$ SGC of I manufacturing batch during the long term stability study

It can be concluded that the quality of the 2nd and the 3rd pilot batches and of the manufacturing batches during stability studies remained in the limits of the regulated standards, and it is expected that the shelf life of $PNTE_2$ SGC will be 24 months.

CONCLUSIONS

1. Investigating the pine needles thick extract (PNTE) composition, it was found that carotenoids and chlorophylls can serve as markers, indicating the quality of the extract and its dosage forms in general.
   Solvent systems for the use in thin layer chromatography method, suitable for the separation of β-carotene from other pigments of PNTE were found, as well as solvent systems for the separation of PNTE components.
   It was ascertained that with the help of a column chromatography method it is possible to separate successfully carotenes from other carotenoids, chlorophylls, their derivatives and other components of PNTE, to perform the quantitative determination of the carotenes with the UV/VIS spectrophotometry method.

2. It was established that technologically it is possible to prepare a powder formulation containing PNTE for filling into hard gelatin capsules. However, changes observed during storage period testified the lack of stability of the powder formulation filled into the capsules.

3. A technology for preparation of a lipophilic formulation containing PNTE for filling into soft and hard gelatin capsules was developed.
   It was ascertained that content of moisture and volatile substances of PNTE in capsule fill must not exceed 10 %.
   Suitable excipients for the obtaining of a disperse system of the capsule fill: vegetable oil and emulsifier (glycerol monooleate) providing the preparation of a stable dosage form were found.

4. Evaluating the possibility for the use of different encapsulating methods for the preparation of the dosage form of PNTE, it was ascertained that small scale experimental batches of the soft gelatin capsules can be prepared in the laboratory by a dipping method. The lipophilic formulation containing PNTE also can be filled into the hard gelatin capsules.
   A rotary die method has to be used for the large scale manufacturing of the soft gelatin capsules containing PNTE. The lipophilic

formulation containing PNTE is not suitable for the preparation of the soft gelatin capsules by a droplet method.

5. Quality control methods of the soft gelatin capsules containing PNTE were developed. The samples preparation process for the separation of the carotenes by the column chromatography method for the assay of the carotenes was modified.
Validating the carotenes assay method in the soft gelatin capsules containing PNTE, it was established that the method is specific, enough precise and repeatable, the linear correlation between light absorption and concentration of the active substance in the solution is not casual.

6. Investigating stability of the soft gelatin capsules containing PNTE, it was ascertained that laboratory batch capsules maintained quality during long term stability study for 24 months and during accelerated stability study for 12 months.
The influence of type of packaging and storage conditions on the soft gelatin capsules containing PNTE was established. Evaluating the appearance and the disintegration time of capsules, it was established that the stability of the soft gelatin capsules containing PNTE is greater when stored in high density polyethylene containers, than in blisters made from aluminium foil and polyvinylchloride film. It refers mainly to the storage at stressed conditions at high temperature and moisture.
It is expected that the quality of the pilot batches and the manufacturing batches of the soft gelatin capsules containing PNTE will remain for 24 months.

PRACTICAL RECOMMENDATIONS

1. For the identification of carotenoids and chlorophylls in the soft gelatin capsules containing PNTE, the method for quantitative evaluation of carotenes – the column chromatography and sequential spectrophotometry analysis of carotenes – can be used. The carotenoids and chlorophylls can be identified by the characteristic position in the chromatography column.

2. For the assessment of the content of carotenes in the soft gelatin capsules containing PNTE, a modified method with simplified procedure for the preparation of the samples can be used. This method allows to accelerate and facilitate the analysis process, as well as to minimize the loss of carotenes.

3. For the evaluation of the disintegration time of the soft gelatin capsules containing PNTE, water medium has to be used. If the disintegration time exceeds the required limit of 30 min, phosphate buffer solution with pH 6.8 and with pancreas powder has to be used.

4. For the preparation of the soft gelatin capsules containing PNTE the active substance with content of carotenes NLT 35 mg % has to be used.

5. High density polyethylene containers with low density polyethylene caps, or blisters from aluminium foil and polyvinylchloride film are the proposed types of primary packaging for the soft gelatin capsules containing PNTE.

6. The shelf life of the soft gelatin capsules containing PNTE is 2 years. The dosage form should be stored at the temperature below 25 °C, protected from moisture and light.

REVISED SCIENTIFIC PUBLICATIONS

1. I. Daberte, I. Barene, J. Rubens, M. Daugavietis. Quality of pine needles thick extract used in phytotherapy // Rīga Stradiņš University. Collection of Scientific Papers: Research articles in medicine & pharmacy, 2008. Internal Medicine. Surgery. Medical Basic Sciences. Stomatology. Pharmacy. – Rīga: RSU, 2009. – P. 141–145.

2. I. Daberte, I. Bārene. Skuju biezā ekstrakta dozētas zāļu formas pagatavošanas un kvalitātes noteikšanas pētījumi // RSU Zinātniskie raksti. 2009. gada medicīnas nozares pētnieciskā darba publikācijas. Internā medicīna. Ķirurģija. Medicīnas bāzes zinātnes. Stomatoloģija. Farmācija. / Rīgas Stradiņa universitāte. – Rīga: RSU, 2010. – 547.– 555. lpp.

3. I. Daberte, I. Bārene. Priežu skuju biezā ekstrakta kapsulētas dozētas formas stabilitātes izpēte // The manuscript is accepted for publication in the RSU collection of scientific papers „Zinātniskie raksti: 2010. gada medicīnas nozares pētnieciskā darba publikācijas" in the Pharmacy section (RSU Scientific department reference No. 7-15/71, 28.09.2010).

PATENTS

1. Patents LV 13566 B, 20.08.2007. Skuju biezo ekstraktu saturošs sastāvs un tā iegūšanas metode. I. Daberte, I. Bārene, L. Štāle, J. Rubens, N. Saženova. (Pieteikums P-07-21 no 22.02.2007.).

2. WO/2010/064882, 10.06.2010. Thick pine needle extract composition for capsulation. Rubens, J., Daberte, I., Barene I., Daugavietis M.
   International application No.: PCT/LV2009/000009. Filling date: 01.10.2009.
   Priority data: P-08-205 04.12.2008.
   Patents LV 13888 B, 20.06.2009. Skuju biezo ekstraktu saturošs sastāvs iekapsulēšanai. J. Rubens, I. Daberte, I. Bārene, M. Daugavietis. (Pieteikums P-08-205 no 04.12.2008.).

ABSTRACTS OF THE CONFERENCES AND CONGRESSES

1. I. Bārene, I. Daberte, V. Eniņa. Skuju ekstrakta zāļu formu izstrādes iespējas // AML / RSU 2002. gada Medicīnas nozares zinātniskās konferences tēzes. (Rīgā, 2002. gada 15. februāris) – Rīga: AML/RSU, 2002. – 145. lpp.

2. I. Barene, I. Daberte. Quality of pine needles thick extract as herbal product // PSWC 2007 FIP Pharmaceutical Sciences World Congress, Amsterdam the Netherlands 22 – 25 April 2007. Abstracts on CD-ROM. Abstract PSWC07L_968.

3. I. Barene, I. Daberte, J. Rubens, M. Daugavietis. Producing and determination of quality of pine needles thick extract dosage form // Abstract Book. World Congress of Pharmacy and Pharmaceutical Sciences 2007. 67th International Congress of FIP. 31 August – 6 September 2007, Beijing, China. Industrial Pharmacy Section – Posters. P. 159.

4. I. Daberte, I. Barene, J. Rubens, M. Daugavietis. Producing and determination of qualitative indices of ordinary pine needles thick extract // European Journal of Pharmaceutical Sciences. Supplement "Abstracts of the 2nd BBBB Conference on Pharmaceutical Sciences, September 13 – 15, 2007, Tallinn-Tartu, Estonia". Vol. 32, Issue 1, Suppl. P. S32–S33.

5. I. Daberte, I. Bārene. Skuju biezā ekstrakta dozētas zāļu formas izstrādes pētījumi // Rīgas Stradiņa universitāte. 2008. gada zinātniskās konferences tēzes (Rīgā, 2008. gada 13. un 14. martā). – Rīga: RSU, 2008. – 25. lpp.

6. I. Daberte, I. Barene, J. Rubens, M. Daugavietis. Design and development of dosage form of pine needles thick extract // Abstract Book. World Congress of Pharmacy and Pharmaceutical Sciences 2008. 68th International Congress of FIP. 29 August – 4 September 2008, Basel, Switzerland. Natural substances – Posters. P. 234.

7. I. Daberte, I. Bārene, J. Rubens, N. Saženova. Skuju biezā ekstrakta kapsulētās zāļu formas iegūšanas iespējas // Rīgas Stradiņa universitāte. 2009. gada zinātniskās konferences tēzes (Rīgā, 2009. gada 2. un 3. aprīlī). – Rīga: RSU, 2009. – 221. lpp.

8. I. Barene, I. Daberte, J. Rubens, M. Daugavietis, N. Sazhenova. Possibility of producing of encapsulated dosage form of pine needles thick extract // Abstract Book. World Congress of Pharmacy and Pharmaceutical Sciences 2009. 69th International Congress of FIP. 3 – 8 September 2009, Istanbul, Turkey. Natural substances – Posters. P. 182.

9. I. Daberte, I. Bārene, J. Rubens, N. Saženova. Priežu skuju biezā ekstrakta kapsulētās zāļu formas stabilitātes izpēte // Rīgas Stradiņa universitāte. 2010. gada zinātniskās konferences tēzes (Rīgā, 2010. gada 18. un 19. martā). – Rīga: RSU, 2010. – 311. lpp.

10. I. Daberte, I. Barene, J. Rubens, M. Daugavietis, N. Sazhenova. Investigation of stability of soft gelatin capsules containing pine needles thick extract // World Congress of Pharmacy and Pharmaceutical Sciences 2010. 70th International Congress of FIP. 28 August – 2 September 2010, Lisbon, Portugal. Abstracts on USB. Abstract FIP10L_NP-P-013.

PRESENTATIONS AT THE CONFERENCES AND CONGRESSES

1. AML/RSU conference of medical sciences, 15 February 2002, Riga (oral presentation).

2. PSWC 2007 FIP Pharmaceutical Sciences World Congress, Amsterdam the Netherlands 22 – 25 April 2007 (poster presentation).

3. World Congress of Pharmacy and Pharmaceutical Sciences 2007. 67th International Congress of FIP. 31 August – 6 September 2007, Beijing, China (poster presentation).

4. 2nd BBBB Conference on Pharmaceutical Sciences, September 13 – 15, 2007, Tallinn-Tartu, Estonia (poster presentation).

5. Riga Stradins University scientific conference, 13 and 14 March 2008, Riga (poster presentation).

6. World Congress of Pharmacy and Pharmaceutical Sciences 2008. 68th International Congress of FIP. 29 August – 4 September 2008, Basel, Switzerland (poster presentation).

7. Riga Stradins University scientific conference, 2 and 3 April 2009, Riga (poster presentation).

8. World Congress of Pharmacy and Pharmaceutical Sciences 2009. 69th International Congress of FIP. 3 – 8 September 2009, Istanbul, Turkey (poster presentation).

9. Riga Stradins University scientific conference 18 and 19 March 2010, Riga (poster presentation).

10. World Congress of Pharmacy and Pharmaceutical Sciences 2010. 70th International Congress of FIP. 28 August – 2 September 2010, Lisbon, Portugal (poster presentation).