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STUDY OF STABILITY AND DETERMINATION OF RESIDUAL QUANTITY OF ORGANIC SOLVENT IN LIPOPHILIC EXTRACT OF PUMPKIN

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Abstract

Important indicators of medicines' quality are stability and shelf life, that is, the time during which no negative changes in the physico-chemical, pharmacological and consumer characteristics of the product are observed. When developing the composition of a new substance or a drug, the shelf life is determined experimentally, by periodically evaluating all indicators included into the methods of quality control. To establish the qualitative characteristics of the pumpkin extract, the appearance, solubility, identification, microbiological purity, and quantitative content of active substances in the extract were evaluated. To determine the residual amount of organic solvent in the extract, the method of gas-liquid chromatography was used.

Keywords: Stability, lipophilic extract, residual solvent, gas-liquid chromatography.

Introduction

Back in 1959, British scientists Russell W.M.S and Burch Standardization and quality control of medicines in our time remains an important task of modern pharmacy. One of the conditions ensuring the effectiveness and safety of finished drugs is the high quality of active and auxiliary substances used in their manufacture.

Currently, in accordance with the rules of Good Manufacturing Practice 42-4.0: 2008 (GMP), it is the responsibility of manufacturers of pharmaceutical products to conduct a stability study, as a result of which the expiration date and storage conditions of active substances are established. Stability studies should be carried out at the stage of development of pharmaceutical substances or drugs. According to GMP requirements at the development of specifications for raw materials a maximum storage period before re-inspection should be set, and in the specification for finished products there should be shelf life specified. Stability tests are based on obtaining data on the quality change of a substance under the influence of various environmental factors: temperature, light, humidity, etc. The Common Technical Document (CTD) adopted in the EU, USA and Japan establishes the basic requirements for conducting research on the stability of pharmaceutical products (Guidance, 2014; Mitkina *et al* ., 2015; Medvetsky *et al* ., 2014; Lyapunov *et al* ., 2016; Ryzhikova *et al* ., 2014; Sakaeva *et al* ., 2013).

International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has adopted resolution for the regulation of residual amounts of organic solvents, which establishes the limits of their contents in substances, auxiliary substances and finished pharmaceuticals.

Residual solvents in drugs are volatile organic substances that are used and not completely removed in the process of substances, excipients or finished drugs production.

Since residual solvents have no therapeutic effect, they must be removed to meet the requirements of the specification, good manufacturing practice (GMP) or other quality requirements. Medicines should not contain residual solvents in quantities exceeding the established limit .In the production of active pharmaceutical substances, excipients and drugs use solvents of the following classes of toxicity: having high toxicity - class 1 (benzene, carbon tetrachloride, etc.), less toxic solvents - class 2 (acetonitrile, hexane, methanol, formamide, toluene and others), the safest are solvents of class 3 (anisole, acetone, heptane, etc.).

Thus, all substances and finished drugs should be controlled for the content of those solvents that may be present in them. The norms of the content of residual organic solvents in substances must be justified taking into account the degree of toxicity of each of them to humans and the environment.

In addition, information about the solvent used in the production process of the substance, as well as data on stability should be indicated in the registration dossier of the substance manufacturer, which is submitted during state registration to obtain a certificate of compliance with the Pharmacopoeia (Sabirzyanov *et al* ., 2017; Egorova *et al* . , 2014; Sadchikova *et al* .2004).

To control residual solvents of class 1 or 2 (or class 3 with a content of more than 0.5%), if possible, use the procedure described in the general pharmacopoeial monograph or use a suitable validated procedure. In the quantitative determination of residual solvents, the result obtained is taken into account in the quantitative analysis of the substance, except for the cases when the determination of the mass loss during drying is carried out. The concentration limit of hexane in pharmaceutical substances in (ppm) is 290.

In previous works, we have developed a technology for producing a lipophilic extract from pumpkin meal pulp (*Cucurbita pepo* L. and *Cucurbita moschata* (Duch) Poir.), which was a homogeneous, oily, resinous mass with a specific odour, orange colour, insoluble in water, ethanol and highly soluble in chloroform, hexane, and ether (Vishnevska *et al* ., 2014).

The aim of our work was to study the stability and the shelf life of the lipophilic extract, as well as to determine the residual amount of organic solvent in the lipophilic extract of pumpkin.

Methods

Stability studies were carried out in accordance with the manual 42-3.3: 2004 " Guide to quality. Medicines. Stability study" (Lyapunov *et al*., 2004).

To establish the shelf life, we observed the samples of the extract for 27 months of storage in dark glass bottles in a dry place protected from light, at temperature conditions 5 ± 3 °C (in the refrigerator) and 25 ± 2 °C. Considering the consistency of the studied samples, as packaging we have chosen glass bottles used for liquid dosage forms in the pharmaceutical industry and ensuring tightness during long-term storage.

The quality control of plant extracts is regulated by the Pharmacopoeia according to the following indicators: organoleptic indicators, identification, quantification, microbiological purity, etc.

In the study of the quality of the lipophilic extract, organoleptic indicators (description) were primarily evaluated. For this, the appearance and characteristic organoleptic properties of the samples (colour, odour, texture), as well as signs of physical instability and solubility, were controlled.

In previous works, we have developed methods for qualitative (identification) and quantitative determination of active substances in the extract, studied the microbiological purity of the extract (Degtyarova *et al* ., 2014; Degtyarova *et al* ., 2015; Degtyarova, 2015; Degtyarova *et al* ., 2015).

Identification of carotenoids was performed by thin layer chromatography (TLC) according to the requirements of State Pharmacopoeia of Ukraine (SPU) 2, art. 2.2.27. Quantitative determination of the number of carotenoids in terms of β-carotene, was performed by the method of absorption spectrophotometry in the visible region (SPU 2, art. 2.2.25). Quantitative determination of the amount of phytosterols in terms of cholesterol was performed by high performance liquid chromatography (HPLC) (SPU 2, Art. 2.2.29). Microbiological purity study was carried out according to the method SPU 2, Art.2.6.12. To determine the antimicrobial activity, we used the method of diffusion into agar with reference strains of microorganisms: P. aeruginosa ATCC 9027, S. aureus ATCC 6538 (State Pharmacopoeia of Ukraine, 2015).

To determine the residual amount of organic solvent in the extract, gas-liquid chromatography was used (Cunha *et al* ., 2011; Cicchetti *et al* ., 2008; Egazaryants, 2009; Hadjmohammadi *et al* ., 2016; Hadjmohammadi and Ghoreishi, 2011; Hadjmohammadi and Ghoreishi, 2011; Pervova *et al* ., 2016; Marriott *et al* ., 2009; Usova *et al* ., 2017).

Conditions for analysis:

1. Instrument requirements and chromatographic conditions.

Gas chromatograph with a flame ionization detector, in which the following conditions were set up:

- column: quartz capillary, 60 x 0.32 mm in size, 1.8 μm DB-624 or equivalent, for which the requirements of the "Chromatographic System Suitability Test" are fulfilled;
- the column thermostat temperature was programmed from 40 ° C (delay 5 min) to 200 ° C (delay 20 minutes), temperature rise - 5 ° C / min;
- evaporator unit temperature - 230 ° C; flow separation - 1: 5;
- detector temperature - 290 ° C;
- velocity of carrier gas (helium) - 2 ml / min.

2 Preparation of working standard sample solution (WSS).

5 ml of the internal standard solution, 12.7 μl of hexane were placed in a 100 ml volumetric flask. The volume was adjusted with the internal standard solution to the mark and mixed (solution 1).

3.0 ml of solution 1 placed in a vessel that was tightly closed (3 samples) with a capacity of 20.0 ml, 0.5 ml of water P, 0.5 g of sodium chloride P and 1.0 g of substance were added. The vessels were immediately sealed with a rubber gasket with a fluoroplastic coating. Then they were alternately placed in a thermostat - a device for analysing the equilibrium vapor phase - and kept at a temperature of 120 ° C for 30 minutes.

3 Preparation of the internal standard solution.

100 mg of n-butanol P placed in a 10.0 ml volumetric flask. Brought the volume with a solution of dimethylacetamide (DMAc P) to the mark and stirred. 5.0 ml of the resulting solution placed in a volumetric flask with a capacity of 500.0 ml. Brought the volume of the DMAc P solution to the mark and mixed.

4. Preparation of the solution to verify the suitability of the chromatographic system. 5 ml of the DMAc P solution placed in a 200 ml volumetric flask, 1.5 g (accurate weight) of methanol P, 1.5 g (accurate weight) of acetone P, 0.3 g (accurate weight) of methylene chloride P, 1.5 g (accurate weight) of ethyl ether P, 0.445 g (accurate weight) of toluene P, 0.1 g (accurate weight) of pyridine P were added. Diluted the volume of the DMAc P solution to the mark and mixed.

3.0 ml of the obtained solution placed in a vessel, tightly closed, with a capacity of 20.0 ml, 0.5 ml of water P, 0.5 g of sodium chloride were added. The vessels were immediately sealed with a rubber gasket with a fluoroplastic coating. Then they were alternately placed in a thermostat — a device for analysing the equilibrium vapor phase — and kept at a temperature of 120 °C for 30 minutes.

5. The technique.

1.0 g of the substance placed in a vessel (3 samples) with a capacity of 20.0 ml, tightly closed, 0.5 ml of water P, 0.5 g of sodium chloride and 3.0 ml of an internal standard solution (n-butanol P) were added. The vessels were immediately sealed with a rubber gasket with a fluoroplastic coating. Then they were alternately placed in a thermostat - a device for analysing the equilibrium vapor phase - and kept at a temperature of 120 °C for 30 minutes.

Chromatographed the gas phase over a solution of WSS, obtaining from 2 to 6 chromatograms. The injection volume was 1.0 ml. For the peak areas of the solvents from the chromatograms obtained, the relative standard deviation (RSD) was calculated. The preparation of parallel chromatograms (n0) was stopped when the requirements of the suitability of the chromatographic system were reached (Fig. 4.1, 4.2).

The results were considered reliable if the requirements of the "Test of the suitability of the chromatographic system" were met.

The quantitative content of hexane (X), in ppm, in the sample of the drug was calculated according to the formula:

$$X = \frac{B_i \cdot 3 \cdot 10^6 \cdot \rho \cdot V_o}{(B_0 - B_i) \cdot m \cdot 100 \cdot 1000}$$

Where:

B_i - the average value of the peak area of hexane in the chromatogram of the test solution;

B_0 - the average value of hexane peak area in the chromatogram of WSS;

V_o - the volume of hexane, µl;

ρ is the density of hexane (0.6548);

m is the mass of the sample of the drug in grams.

The residual content of hexane in the extract should not exceed 290 ppm.

6. Testing the suitability of the chromatographic system.

The chromatographic system was considered suitable if the following conditions were met:

- the degree of peaks separation, calculated by the peaks of solvents from chromatograms of the solution, to check the suitability of CS, should have been at least 1.5;

- the relative standard deviation calculated for the peak areas of the solvents from chromatograms of SS solution should have been no more than 6.0%;

- the coefficient of peaks symmetry, calculated by the peaks of the solvent from the chromatograms of the solution to check the suitability of CS, should have been from 0.8 to 1.5

Chromatograms of the WSS solution and the test sample solution of the lipophilic pumpkin pulp extract are presented in Figures 1, 2. The data of the peaks shown on the chromatograms of the WSS and the test sample of the lipophilic extract are presented in table 1.

Results and Discussion

As a result of studies, hexane was found in a lipophilic extract - a solvent of class 2 toxicity. The results of the residual solvent content in the extract (in ppm) in the lipophilic extract do not exceed the regulated norms. Thus, the presence in the extract of hexane in an acceptable amount indicates the possibility of its use as a solvent in the technology of obtaining lipophilic pumpkin pulp extract.

When stored for 27 months and at a temperature of 25 ± 2 ° C and relative humidity (60 ± 5) %, the extract samples did not change their appearance, but after 6 months of storage the quantitative content of carotenoids and phytosterols in the extract did not meet the requirements of quality control methods.

Based on the obtained results, the stability of the extract for 24 months was proved and the shelf life was determined - 2 years in dark glass bottles at temperatures of 5 ± 3 ° C (in the refrigerator) (Table 2, Table 3).

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References

1. J.O. Simultaneous determination of bisphenol A and bisphenol B in beverages and powdered infant formula by dispersive liquid – liquid microextraction and heartcutting multidimensional gas chromatography - mass spectrometry. Food Add. Contam, 2011; No. 4 (28): 513-519.
2. Cicchetti E., Merle P., Chaintreau Cicchetti A. Quantitation in gas chromatography: usual practices and performances of a response factor database. Flavor and Fragrance Journal, 2008; 23 (6): 450–459. doi: 10.1002 / ffj.1906
3. Degtyarova K. O., Vishnevska L. I., Bisaga Ye. I. Determination of lipophilic substances content in pumpkin pulp extracts. Phytotherapy. Digest, 2014; 1: 74-77.
4. Degtyarova K.O., Grudko V.O., Vishnevska L.I., Bisaga Ye. I. Determination of biologically active compounds in lipophilic extracts of pumpkin by the method of thin-layer chromatography. Topical issues of pharmaceutical and medical science and practice, 2015; 2 (18): 45-48.
5. Degtyarova E. A. Development of phytosterols quantitation method by HPLC. "The introduction of the achievements of medical science into clinical practice. ": materials of the X scientific-practical conference for young students and students of the TSMU named after Abu Ali ibni Sino with international participation, Dushanbe, 2015; 340-341.
6. Degtyarova K. O., Gerasimova I. V., Oproshanska T.V. Study of phisico-chemical properties of suppositories on the basis of a lipophilic extract of pumpkin meal. Management, economics and quality assurance in pharmacy, 2016; 2 (46): 14-18.
7. Degtyarova E. A., Grudko V. O., Vishnevskaya L. I., Bisaga Ye. I. Determination of biologically active substances in lipophilic extracts of pumpkin by thin layer chromatography. Topical issues of pharmaceutical and medical science and practice, 2015; 2 (18): 45-48.
8. Yegazaryants S.V. Chromatographic methods for the analysis of petroleum products. Bull. Mosk. University, 2009; No. 2 (50): 75-99.
9. Egorova A.V., Fedosenko A.A., Vityukova Ye.O., Kashutsky S.N., Maltsev G.V. Validation of the HPLC technique for determining residual quantities of clofeline hydrochloride from pharmaceutical equipment surfaces. Bulletin of Odessa National University. Chemistry, 2014; 3 (51): 40-51.
10. EudraLex. – The Rules Governing Medicinal Products in the European Union. – Volume 4. EU Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use. http://ec.europa.eu/health/documents/eudralex/vol-4/index en.htm
11. Guidelines for the examination of medicines. The study of stability and the establishment of expiration dates of drugs M.: POLYGRAPH-PLUS, 2014; 3: 224.
12. Hadjmohammadi M. R., Mousavi Kiasari Z., Nazari S.S.S.J. Separation of some phenolic acids in micellar liquid chromatography using design of experiment-response surface methodology. Journal of Analytical Chemistry, 2016; 6 (71): 639-645.
13. Hadjmohammadi M. R., Ghoreishi S. S. Determination of oestrogens in water samples using dispersive liquid-liquid microextraction and high-performance

liquid chromatography. Acta Chim. Slov, 2011; 58: 765-780.
14. Hadjmohammadi M. R., Ghoreishi S.S. Determination of oestrogens in water samples using dispersive liquid-liquid microextraction and high-performance liquid chromatography. Acta Chim. Slov, 2011; 58: 765-780.
15. Lyapunov M., Bezugla O., Takhtaulova N. Guideline 42-4.0: 2016.Medicines. Good Manufacturing Practice. Kiev, Ministry of Health of Ukraine, 2016.
16. Lyapunov M., Georgiyivsky V., Bezugla O. Guideline 42-3.3: 2004. Quality guide. Medicines. Stability study Kiev, Ministry of Health of Ukraine, 2004: 16.
17. Mitkina L.I., Kovaleva E.L., Prokopov I.A. Stress studies and photostability as part of data on pharmaceutical drug development. News of NCESMP 2015; 2: 9-12.
18. Medvetsky A.I., Shcherbakova L.I., Kompantsev V.A., Gokzhaeva L.P., Vasina TM. Study of stability and determination of expiry dates for alprazolam microparticles based on Poly-d, l-lactide-co-glycolide. Pharmacy and Pharmacology, 2014; 4 (5): 64-68.
19. Pervova M. G., Plotnikova K. A., Chizhov D. L., Pestov A. V., Saloutin V. I. Determination of glycols in glycol-containing oligomers using reaction gas-liquid chromatography methods. Journal of Analytical Chemistry, 2016; 6 (71): 660-666.
20. Marriott P. J., Graham T. E., Dufour Marriott J.-P., Emerging P. J. Opportunities for Flavor Analysis through Hyphenated Gas Chromatography. Journal of Agricultural and Food Chemistry, 2009; 57 (21): 9962–9971. doi: 10.1021 / jf9013845
21. Ryzhikova V. A., Kuznetsova E. G., Belov V. Yu., Salomatina L. A., Sevastyanov V. I. Determination of stability of the transdermal therapeutic system of the local anesthetic bromocaine. The Bulletin of Transplantology and Artificial Organs , 2014; 4 (14): 89-95.
22. Sadchikova, NP, Belov, A. B., Karmanova, T. M. Determination of residual amounts of organic solvents in sodium diclofenac substances. VSU Bulletin. Series: Chemistry.Biology. Pharmacy, 2004; 1: 184-188.
23. Sabirzyanov D. R., Tumilovich E. Yu., Karpenko Yu. N. Analysis of residual solvents in anilocaine substance. The Journal of scientific articles "Health and Education Millennium, 2017; 10:359-362.
24. Sakaeva I. V., Butyanian N. D., Kovaleva E. L., Sakanyan E. I., Mitkina L. I., Prokopov I. A., Shelekhina E. S., Mitkina Yu. V. Main approaches to the study of the stability of drugs: domestic and international experience. News of Scientific Centre for Expertise of Medical Products, 2013; 3: 8-11.
25. State Pharmacopoeia of Ukraine // SE "Ukrainian Scientific Pharmacopoeia Centre for Quality of Medicines. 2nd ed.Ukraine. Kharkiv, 2015; 1: 1128.
26. Usova S. V., Vershinin V. I., Mamontova A. V. Chromatographic determination of the total content of aromatic hydrocarbons: analysis of model mixtures. Bull. of Omsk University, 2017; 1: 59–64.
27. Vishnevska L. I., Degtyarova K.O. Studies on development of lipophilic pumpkin extract technology. Collection of scientific works of P. L. Shupik's NMAPO employees, 2014; 23 (4): 231–237.

Table 1. The data of the peaks shown on the chromatographs of the WSS and the test sample of the lipophilic extract

Object of study	Retention time	Peak area
Hexane	9,516	98635
Lipophilic extract	9,523	93129

Table 2. Stability indicators of lipophilic extract during storage in dark glass bottles at 5 ±3 ° C in the refrigerator

Indicator		Storage period, months						
	Start	3	6	9	12	18	24	27
1	2	3	4	5	6	7	8	9
Appearance	Orange oily resinous mass with a specific odour							
Solubility	Soluble in chloroform, hexane, ether							
Identification								
carotenoids								
With a solution of *phosphomolybdic acid P*	Carotenoids - blue staining							
With a solution of *Stibium (III) chloride P*	Carotenoids - green staining							
TLC (after treatment with a 5% alcohol solution of *phosphomolybdic acid P)*	On the chromatogram of the test solution, a stain appears at the level of the stain on the chromatogram of the β-carotene SS solution and the stain with a lower Rf value							
Maximum absorption at λ = 450 (absorption spectrophotometry method)	Complies							
Quantitative content								
Carotenoids in terms of β-carotene, mg (from 9.66 to 10.96 mg)	10.23 ± 0.01	10.24 ± 0.02	10.27 ± 0.01	9.89 ± 0.01	9.85 ± 0.02	9.77 ± 0.01	9.71 ± 0.01	8.51 ± 0.01
Phytosterols in terms of cholesterol, % (from 1.973 to 2.025%)	2.017 ± 0.001	2.021 ± 0.001	2,005 ± 0,001	2,022 ± 0,001	2,013 ± 0,001	1,986 ± 0,001	1,984 ± 0,001	1,887 ± 0,001
Microbiological purity: aerobic fungi and anaerobic fungi (total)	$<10^2$							
P. aeruginosa, S. Aureus	No growth							

Table 3. Stability indicators of the lipophilic extract of pumpkin pulp during storage in dark glass bottles at a temperature of 25 ± 2 ° C and relative humidity (60 ± 5) %

Indicators	Storage time, months							
	Start	3	6	9	12	18	24	27
1	2	3	4	5	6	7	8	9
Appearance	Orange oily resinous mass with a specific odour							
Solubility	Soluble in chloroform, hexane, ether							
Identification								
Carotenoids								
With a solution of *phosphomolybdic acid P*	Carotenoids - blue staining							
With a solution of *Stibium (III) chloride P*	Carotenoids - green staining							
TLC (after treatment with 5% alcoholic solution of *Phosphomolybdic acid P*)	On the chromatogram of the test solution, a stain appears at the level of the stain on the chromatogram of the β-carotene SS solution and the stain with a lower Rf value							
Maximum absorption at λ = 450 (absorption spectrophotometry method)	Complies							
Quantitative content								
Carotenoids in terms of β-carotene, mg (from 9.66 to 10.96 mg)	10.23 ± 0.02	9.38 ± 0.01	8.92 ± 0.01	8.49 ± 0.01	8.13 ± 0.02	7.85 ± 0.01	7.62 ± 0.01	6.57 ± 0.01
Phytosterols in terms of cholesterol,% (from 1.973 to 2.025%)	2,105 ± 0,001	2,010 ± 0,001	1,987 ± 0,001	1,902 ± 0,001	1,803 ± 0,001	1.624 ± 0.001	1.624 ± 0.001	1.624 ± 0.001
Microbiological purity: aerobic fungi and anaerobic fungi (total)	$<10^2$							
P. aeruginosa, S. Aureus	No growth							

Figure 1. Chromatogram of the SS solution: 1 - system peak; 2 - hexane; 3 - internal standard; 4 - DMAc P; 5 - system peak.

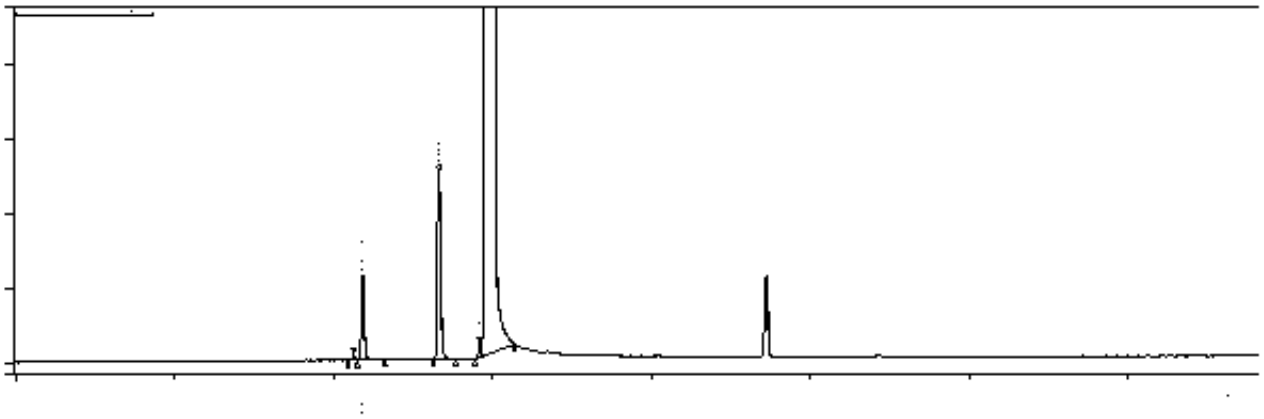

Figure 2. Chromatogram of the sample solution of the pumpkin pulp lipophilic extract: 1 - system peak; 2 - hexane; 3 - internal standard; 4 - DMAc P; 5 - system peak.