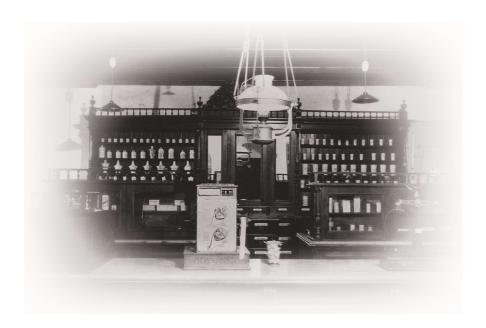# The 8th International Conference on Pharmaceutical Sciences and Pharmacy Practice

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# ABSTRACTS OF POSTERS

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## Development of the HPLC and spectrophotometric methods for quantitative determination of methylcobalamin in multi-component capsules

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Methylcobalamin is coenzyme form of vitamin B12. It plays an important role in maintaining health of people. Methylcobalamin deficiency causes many diseases, such as blood deficiency, depression, irritability, psychosis, hyperhomocysteinemia and finally cardiovascular disorder [1]. It is chemically Co$\alpha$-[$\alpha$-(5,6-dimethylbenz-1*H*-imidazolyl)]-Co$\beta$methylcobamide. Since methylcobalamin is an active metabolite of cyanocobalamin, thereby allowing patients get effect of the medicine faster than when using cyanocobalamin [2]. Methylcobalamin used in the treatment of trigeminal neuralgia, megaloplastic anemia, diabetic neuropathy and facial paralysis in Bell's pasly syndrome [3].

According to the requirements of State Pharmacopoeia of Ukraine for each substance method for quantitative determination in medicines should be developed [4].

HPLC analysis was performed on "ProStar" Varian liquid chromatograph. The chromatography was carried out in the following conditions: Hydrosphere C18 column (150 mm×4.6 mm, 3 μm particle size); mobile phase – *acetonitrile : water : trifluoroacetic acid* in the ratio (80:920:1); flow rate – 1 ml/min; column temperature 35 ºC; spectrophotometer detection at 220 nm; injection volume – 20 μL; run time – 20 min. In the specified conditions the retention time of methylcobalamin is about 12 minutes.

In the assay of methylcobalamin by developed method a low content of the substance in the capsules was systematically observed. It can be explained by the adsorption of methylcobalamin with capsule excipients and it also settles down on the membrane filter. In the development of sample preparation of test solution for HPLC method this problem wasn't resolved.

For this reason, we decided to use method of spectrophotometry instead HPLC method. UV method has some advantages over HPLC method: specific wavelength of methylcobalamin (526 nm) where other components don't interference for its determination, as well that method is faster and economic. In the process of sample preparation, we propose using centrifugation instead of filtration with further selection of supernatant.

Spectrophotometry analysis was performed on "Specord 200" spectrophotometer. A phosphate buffer solution with a pH of 6.8 was used as the solvent. According to the literature data acceptable pH range for methylcobalamin is 4.5-7. The use of phosphate buffer allowed avoiding the influence of other capsules components on the position of the methylcobalamin maximum (526 nm), which was observed when water was used as a solvent.

The UV spectrophotometric method developed is a new, simple, precise, accurate and fast for quantitative estimation of methylcobalamin in the capsules.

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# Development of the HPLC method for related impurities determination of active pharmaceutical ingredients in the process of the industrial synthesis

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Epilepsy is the disease, toward recurrent seizures unprovoked by any systemic or acute neurological insults. The treatment of this symptom involves taking anticonvulsant. The dibenzylamide of malonic acid (Dibamk) is a promising biologically active substance, has shown the activity with regard to arresting muscle cramps of different origin. The substance is attractive in terms of industrial synthesis, as it can be obtained in one stage with high yields from available chemicals [1, 2].

Providing of the population with safe medicine is the main aim of the Pharmaceutical Industry. Quality of medicines depends from many factors. One of the main and defining is quality of its components and first of all active pharmaceutical ingredients (API). One of the factors that effect on active pharmaceutical product is its purity. Presence of impurity in drug substances or drug products is inevitable. Therefore, manufacturers who synthesize their own

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Active Pharmaceutical Ingredients have to monitor level of impurities. As it can change activities of medicines and cause adverse reaction in consequence of which to threaten human health. The impurities observed in drug substances can be formed during synthesis or also can be derived from intermediates, reagents, solvents, reaction by-products and other reason [3].

Therefore, developing simple and effective method of impurities determination in API is necessary and actual. We propose to use the method of liquid chromatography, because it is sensitive and selective instrumental method of analysis and also it is proposed by the SPhU for determination of related impurities. The "ProStar" Varian liquid chromatograph was used in the study. The chromatography was conducted in the following conditions: Waters XBridge® C18 column with the size of 150mm × 4.6 mm, with the particle size of 3.5 µm; mobile phase A – 0,1% water solution of trifluoroacetic acid and mobile phase B – 0,1% acetonitrile solution of trifluoroacetic acid; the gradient program for chromatography was as follows: time (min) /%; mobile phase A: 0/90; 5/90→65; 15/65; 25/65→90; 30/90; flow rate – 1 ml/min; column temperature –25 ºC; detection was performed at the wavelength of 210 nm; injection size – 50µL; run time was 35 min.

In the specified conditions the retention time of main substance (Dibamk) is about 21,5 minutes. The retention times of impurity A (Benzylamin) and impurity B (Diethyl malonate) are about 3,4 and 17,0 minutes respectively.

The specified conditions provide the separation of the active substance and peaks of related impurities and that method is promising for determining the purity level of the Dibamk substance.

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# Determination of proanthocyanidins in *Pelargonium sidoides* DC root dry extract and the research on releasing of these compounds from the polymeric films

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# ABSTRACTS OF POSTERS

## Development the HPLC method for the determination of related substances in active pharmaceutical ingredient uridine 5'-monophosphate disodium salt

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Uridine is playing an important role in the pyrimidine metabolism of the brain. It supplies nervous tissue with the pyrimidine ring, and in turn, participates in a number of important metabolic pathways. Uridine and its nucleotide derivatives may also have an additional role in the function of the central nervous system as signaling molecules [1]. Also uridine promotes the restoration of damaged myelin sheaths of nerves and participates in the transport of protein. It is well known that uridine 5'-monophosphate (UMP) has some abilities to stimulate synthesis lipids in the membrane nerve cells, support cellular division and take a part in the regulation of metabolism [2]. The safety of a drug is dependent not only on the toxicological properties of the active substance itself, but also on its pharmaceutical impurities, which can be produced during synthesis of active pharmaceutical ingredient (APhI) or formed during the manufacturing process and/or storage of APhI. Therefore, the presence of such impurities and their levels in products are indicators of medicine quality, which can impose a risk to patient safety.

The aim of our work was the development of the HPLC method for the determination the related substances in APhI uridine 5'-monophosphate disodium salt.

Study was carried out on the basis of the State scientific-research laboratory of NUPh for medicinal substances quality control. For the investigation analytical chromatograph ProStar Varian was used. Chromatographic conditions were: YMC Hydroshere C18 column, 4.6×150mm, 3μm, with the precolumn; the mobile phase: dissolve 8.25 g of potassium dihydrogen phosphate and 2.40 disodium phosphate dodecahydrate in 900 mL of water, adjust to pH 5.5 with dilute phosphoric acid R and dilute to 1000 mL with water; flow rate – 0.7 mL/min; column temperature – 35 °C; detection – spectrophotometry at the $\lambda$=262 nm; injection volume – 20 μL; run time is 20 min. The test solution was prepared in the concentration 2 mg/mL; reference solutions (a and b) were prepared by dissolving the test solution in the concentrations 1% and 0.1% of the initial concentration; water was used as solvent. Relative retention with reference to uridine 5'-monophosphate (retention time = about 4,7 min): cytidine 5'-monophosphate (impurity A) – 0,8; cytosine (impurity B) – 0,9; UMP – 1,0; uridine (impurity C) – 2,2. Limit for specified impurities (A, B, C)

is not more than 0,15%; for unspecified impurities is not more than 0,1% and sum of impurities is not more 1,0% of the substance content.

According to the SPhU requirements, each method for determination of related impurities should be validated. We studied the main validation characteristics such as: specificity, robustness and the detection limit. The specificity of the methods is confirmed by the absence of the excipients effects. The detection limit of the given method was determined on the model mixtures with the known content of related impurities. According to our study, the detection limit for each unspecified impurities is 0,032% (0,64μg/mL) of the substance content (2 μg/mL). The study of robustness showed that temperature and flow rate did not affect on degree of separation and relative time of substance. The HPLC method for determination of related substances has been developed. The validation study for the "Related substances" test performed confirms the compliance of such characteristics as specificity, robustness and the detection limit.

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# Secondary metabolites from *Angelica archangelica* L. synergism with enilconazolum

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Secondary metabolites high structural diversity are important tools of plants needed against microbes (bacteria, fungi) and viruses. Disease resistance in plants depends on the activation of coordinated, multicomponent defence mechanisms. Bioactive plant-derived secondary metabolites are useful leads to synthesize new and more active antimicrobial agents as well as substances with new pharmacological effects by repeated structural modification. It is expected that structurally modified natural products will exhibit increased potency, selectivity, duration of action, bioavailability and reduced toxicity [1]. *Trichophyton* spp. are fungal species that known as tinea infections in various areas in humans and animals. Enilconazolum is used antifungal drug for the treatment of both

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