and tubers, that we obtained from Lithuanian botanical garden. Besides, we measured the amount of main compounds in the stigmas samples, which we received from Morocco, Spain, India, Iran, Ukraine, Azerbaijan, Germany, Italy, Australia and Lithuania.

Result and discussion. Method was validated in terms of linearity, precision, accuracy and specifity. for the identification of active compounds. Validation data met all requirments of the International Conference on Harmonization (ICH). Ten diferrent flavonoids and five phenolic acids were identified and quantified by HPLC method. Analysis showed that 6,7-dihydroxyflavone, rutin, luteolin, apigenin-7-glucoside were dominant compounds from flavonoids. While ferulic and chlorogenic acids were dominant from phenolic acids. The highest amount of 6,7-dihydroxyflavone (2.004 mg/g) was determined in sample from Italy, rutin (17.695 mg/g) in Spanish sample, liuteolin (1.367 mg/g) in Indian sample, apigenin-7-glucoside (6.944 mg/g) in Lithuanian stigmas sample. The highest amount of ferulic acid (0.564 mg/g) was estimated in sample from India and Uttar Pradesh region, chlorogenic acid (0.404 mg/g) in Lithuanian region. In The Lithuanian *Crocus sativus* L. tepals, pistils, stems and tubers also were detected some dominants compounds. In all Lithuanian samples was identified apigenin-7-glucoside with an average quantity of 3.351 mg/g. While rutin was detected just in Lithuanian petals sample (2.822 mg/g). From non-dominant compounds such as apigenin, kaempherol, isoquercitrin and p-coumaric acid the highest amounts were found in Italian stigmas (6.322 mg/g), Spanish stigmas (1.05 mg/g), Lithuanian pistils (3.934 mg/g) and Indian (Maharashtra) stigmas samples, respectively. 5-7-dihydroxy-4-methoxyisoflavone was the only one compound determined just in one sample (from Spain, 0.192 mg/g).

Conclusion. We developed and validated HPLC method that can be used for quantitative and qualitative measurements of phenolics acids, flavonoids, isoflavonoids in *Crocus Sativus* L. raw materials and other plants. Also we compared *Crocus sativus* L. raw materials from different countries and found out that most of the dominant compunds were in India (Uttar Pradesh) and Italy samples. According to obtained results, *Crocus sativus* L. is a promising plant and can be used as sourse of phenolic acids and flavonoids.

DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC PROCEDURE FOR DETERMINATION OF UREA IN MILK

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Introduction. Urea is the final metabolite of nitrogen-containing compounds of ruminants. Typically, the urea content in milk varies in the range of 0.15 – 0.70 mg/mL. Determination of milk urea content values is important for different fields: veterinary, cattle feeding, dairy processing, food quality control etc.

Aim. To develop UV-spectrophotometric procedure for determination of urea in milk and carry out its step-by-step validation in the variants of the method of calibration curve, method of standard and method of additions.

Materials and methods. All spectrophotometric measurements were carried out using a single beam VIS-spectrophotometer UNICO S2100 (UNICO, USA) with wavelength scanned from 1000 nm to 325 nm.

Analysis is carried out in two stages: 2 aliquots of milk (in 10.00 mL each) are taken from the sample to be analysed. 1.00 mL of phosphate buffer solution (pH = 7) is added to the first aliquot and 1.00 mL of urea additive is added to the second aliquot. The mixtures are vortexed for 5 minutes and processed with 10.00 mL of trichloroacetic acid solution (stage 2), then centrifuged for 30 minutes at 8000 rpm. The supernatant is separated and filtered. 5.00 mL of *p*-dimethylaminobenzaldehyde reagent are added to 5.00 mL of the obtained supernatant and the solution to be analysed is ready.

Results and discussion. Development of the procedure has been carried out by its step-by-step validation by such validation parameters as range, in process stability, linearity/calibration model, accuracy and precision, limit of detection and limit of quantification, specificity/selectivity, and also total uncertainty. The measurement of absorbance of solutions to be analysed is optimal not earlier than 10 minutes, and no later than 30 minutes after their preparation; the acceptable uncertainty of the procedure

(10%) with allowable value of the correlation coefficient (0.99) can be achieved with a number of concentration levels more than 8. The procedure is effective in the range of 0.14 – 0.70 mg/mL, the detection limit is 0.05 mg/mL. The accuracy of procedure is characterized by satisfactory indicators.

Using the proposed procedure the quantitative determination of urea in commercially available milk samples has been carried out.

Conclusions. Application of the proposed procedure allows to quantify the urea content in milk samples and obtain correct and accurate results.

DEVELOPMENT OF ASSAY METHOD OF TRANS-10-HYDROXY-2-DECENOIC ACID (10-HDA) IN ROYAL JELLY AND FOOD SUPPLEMENTS BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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Introduction. Royal jelly (RJ) is a yellowish-white secretion from the hypofaringeal and mandibular glands of nurse bees. It is a complex compound, which consists of amino acids, carbohydrates, proteins, sugars, lipids, also minerals and vitamins. The unique feature of RJ is fatty acid, (2E)-10-hydroxydec-2-enoic acid (10-HDA). Since only RJ from bee products contains it. Thus, this compound is a biomarker of RJ, and amount of 10-HDA shown the quality of its commercial products. In the past few years, consumption of RJ as a dietary supplement is consistently growing across the world. That's why the determination of 10-HDA is very important. TLC method was developed to detect the 10-HDA content in royal jelly, lyophilized tablets and capsules.

Aim. The aim of this study is to develop method of quantitative and qualitative determination of 10-HDA in fresh royal jelly and in different food supplements, such as lyophilized tablets and capsules, which contains royal jelly.

Materials and methods. Standard solution was prepared by diluting of 10-HDA weight in methanol. Test solutions were prepared by using to different techniques. Weight of lyophilized tablets, which contains 70 mg of royal jelly, was sonicated with usage of methanol, as an extractor.

The content of capsules, which contains 60 mg of royal jelly, was extracted with a portion of diethyl ether (15 ml), then non-soluble material was extracted with methanol (3x15ml), duration of each extraction was 15 min. Raw material of RJ was extracted using the same method. Extracts were evaporated with liquid nitrogen and were diluted in 1 ml of the same solvent. The separation was performed on silica gel plates (HPTLC Silica gel 60 F_{254}) using a mixture of chloroform: methanol: water (65:35:7) as mobile phase, migration distance was over path of 80 mm. The standard and test samples were spotted in the form of bands of width 6 mm. The determination was performed after post-chromatographic treatment with a 5% solution of potassium dichromate in sulfuric acid, then plate was heated at 150 ˚C and examined with using CAMAG TLC Visualizer 2 at 366 nm.

Results and discussion. The photo of TLC plate separation obtained at 366nm (Figure 1) shown the presence of 10-HDA in all of the analyzed extracts. The intensity of the bands separated on the silica gel plate is different in analyzed samples, it shows that it can be differences between concentration of 10-HDA in different forms of supplements. Spots of test samples were coresponded to the spot obtained from standard solution and all of them had Rf=0,77. We obtained good separation in tablets, capsules and raw material tests samples. (Fig. 1) The average amount of 10-HDA in capsules is 0,88 mg per average mass of capsule, while in tablets is 1,93 mg. The amount of 10-HDA in raw material was 0.80%.The regression coefficient of main substance calibration curve ($R2$) was 0.9991 (Fig.1). The precision of the method met all requirement of ICH guidelines, since all the obtained relative standard deviation (RSD) values were lower than 2.0%.