In suggested conditions the investigated organic acids have the following retentions times (as shown at the Figure 1): Levulic acid (12,39 min), Heptanoic acid (12,85 min), Malic acid (13,05 min), Lactic acid (14,28 min), Glycolic acid (14,51), Oxalic acid (15,06 min), Nonanoic acid (15,53), Maleic (2-Butenedioic) acid (17,49 min), Succinic (Butanedioic) acid (17,63), Stearic acid (24,72), Citric acid (25,81).

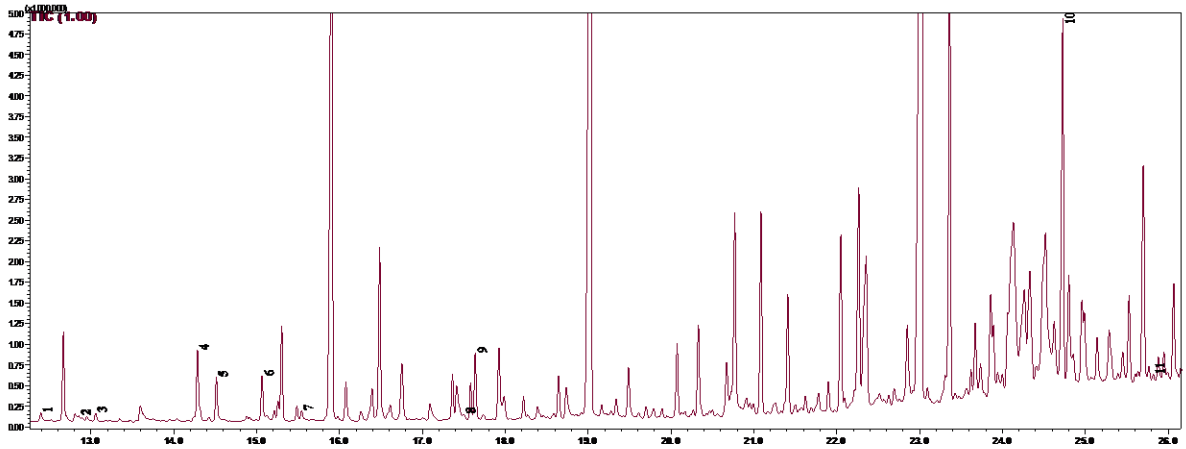

Fig. 1 Typical chromatogram of mitochondria sample

Conclusion. According to received data, the most optimal derivatization conditions were as the following: as derivatization agent was MTBSTFA, as the solutions for dissolution and reaction – water and acetonitrile, respectively. We detected the most effective chromatographic conditions for analysis of organic acids, the results suggest that the method could be usefully integrated in analysis of organic acid.

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR DETERMINATION OF BIOLOGICAL ACTIVE SUBSTANCES OF *CROCUS SATIVUS* L.

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Introduction. *Crocus sativus* L. is a parennial plant of Iridaceae family. Its usege for medical purpose is good known from ancient time. It contains many biological active substances (such as phenolic acids, flavonoids, isoflavonoids,). *Crocus sativus* L. is used for treatment inflammatory, depressive, cardiac, hepatic, diabetic and other diseases. Besides, earlier studies reported that *Crocus sativus* L. was safe at various doses and did not show any severe side effects. Different growing conditions like humidity, amount of sunlight or soil composition in various geographic areas can effect impact for plant qualitative and quantitative consist. Therefore it is important to have more data from different locations more often in order to have comprehensive knowledge about main components of plant. Stigma is main part of this herb, it is most widely exploitable, although, leaves, stems, tepals or corms also could be quite good source of some bioactive compounds. Thus would reduce plant material wasting and have added value.

Aim of our work was determination and comparing of bioactive components that contains in raw material of *Crocus sativus* L., collected from different locations.

Matherial and methods. We used high performance liquid chromatography (HPLC) for the qualitative and quantitative analysis. Method was carried out by using a C18 reversed-phase column (250*4.6 mm with particle size 5µm) with temperature 25°C. The binary solvent system of the mobile phases were used, 0,1 % acetic acid in water as mobile phase A and acetonitrile as a mobile phase B at the flow rate 1.0 ml/min with following gradient program: 0-8 min, 5-15% B; 8-30 min, 15-20% B; 30-48 min, 40-50% B; 58-65 min, 50% B; 65-66 min, 50-95% B. Extraction by methanol was selected as optimal solvent for raw material. We analyzed different parts of *C.sativus* L. such as pistils, tepals, stems

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