The nebulizing gas (N_2) flow rate was 1.8 L min^{-1}. The DAD detector was employed in the wavelength range from 200-400 nm wavelength.

The developed HPLC-DAD-ELSD method for the qualitative and quantitative evaluation of terpene lactones was validated according specificity, precision, accuracy, detection (*LOD*) and quantifcation (*LO*Q) limits, linearity and robustness**.**

Results and discussion. A dispersion analysis of the received data established that the growth location and harvesting time had a significant influence ($p<0.05$) on the amount of terpene lactones that were identified in *G. biloba* leaves. The research data revealed that the factors influence on individual compounds distributed as follows: time of harvest contributed to the majority of ginkgolide A, B, C and bilobalide variations at $\eta2=0.406$, $\eta2=0.475$, $\eta2=0.414$, $\eta2=0.235$, resp. The collection growth location showed lower impact to content of terpene lactones and the location × time of harvest interaction was not significant for all compounds.

In order to clarify the influence of collecting time on the composition of bioactive compounds in *G. biloba,* comprehensive studies on the regularity of compound accumulation in medicinal plant raw materials were conducted. The content of terpene lactones in *G.biloba* leaves distribute in following order: bilobalide >ginkgolide A>ginkgolide C>ginkgolide B. Due to the maximum concentration of ginkgolide A, B, C and bilobalide October is recommended as appropriate times to harvest leaves of *G. biloba* for industry.

The terpene lactone contents in *G. biloba* leaf samples that were collected in different phytogeographical regions (the northern and western parts of Lithuania) have been compared statistically. The application of Tukey's HSD post-hoc test has established that raw materials that are collected in northern Lithuania contain statistically significantly higher ($p<0.05$) amounts of ginkgolide A, B and C than do raw materials that are collected in other part of Lithuania. According to the results of this test, the raw materials that are collected in western part of Lithuania contain statistically significantly higher amounts of bilobalide than do raw materials that are collected in other part of the country. Our research data confirmed that geographical factors have a significant influence on the biosynthesis of terpene lactones in *G. biloba* leaves. Differences in the quantitative composition of compounds (according to the terpene lactones contents in raw materials) in the samples from the same or different regions may be related to the age of the plant. The results indicate that the content of the terpene lactones in *G. biloba* leaves from young trees are higher than in those from old trees.

Conclusion. Data on the contents of the accumulated terpene lactones in *G. biloba* growing in Lithuania will supplement the existing knowledge. Data on differences in quantitative composition of *G. biloba* plant material during the vegetative cycle can be used for rational planning of the collection of plant material rich in terpene lactones.

ANALYSIS OF CYTOTOXICITY OF EXTRACT OF THE WILLOW ON THE MODEL OF THE RAT BONE MARROW CELLS

*S*eyedeh Arshia Khadem
Scientific supervisor: Doc.candi.med.scie. Shatalova A.M.
National University of Pharmacy, Kharkiv, Ukraine
Arshiya.7050@yahoo.com

Introduction. Study toxicity is one of the earliest stages of a pharmacological analysis . In toxic pharmacological studies, toxicity assessment methods that are alternative to classical tests in experimental animals, precisely, models using cell cultures or native cells, are finding increasing use. These models, although not always, can accurately predict toxicity in vivo, but allow us to evaluate the cellular and molecular mechanisms of toxicity. Thus, the use of rat red bone marrow cells as a model in a trypan blue test makes it possible to assess damage to the cytoplasmic membrane or violation of its permeability mechanisms. By cytotoxicity is understood the appearance of pathological changes in cells or cell death under the action of various agents.

Materials and methods. The qualitative and quantitative assessment of the cytotoxicity of a heavy extract of white willow bark was made according to the Shrek method in a modification with

trypan blue. The object of the research was the native rat bone marrow red cells obtained by washing it out of the tubular bones with cold saline. Native cells were exposed to an aqueous willow bark extract at an initial concentration of 40%. The researched solutions were introduced into the tablet with the help of a dispenser, and the dose was reduced by 2, 4, 8, 16, 32 by rolling, and the bone marrow suspension was added to each cell with the same dispenser. Native rat bone marrow cells in suspension with saline were used as negative controls. The control of the cytotoxic effect was carried out after 15, 45, 90 minutes. 0.1% solution trypan blue was used to determine live and dead cells. Cells with damaged cytoplasmic membrane, which were stained in blue, were assessed as dead. In each experiment, 100 cells were counted. The number of viable / non-viable cells in the hemocytometer was counted, and the results were expressed as a percentage of non-viable cells of their total number.

Result. Evaluating the results of cytotoxicity of an aqueous extract of white willow bark on the native red bone marrow cells in comparison with the control, a significant cytotoxic effect was observed only when the cells came into contact with the maximum concentration of the extract upon exposure to 45 minutes. Observations obtained against a background of other concentrations in the range of 0.625, 1.25, 2.5, 5, 10, 20% showed no significant effect on cell viability. These results indicate the relative safety of the researched extract.

A NEW ENZYME-KINETIC PHOTOMETRIC METHOD FOR DETERMINATION OF THE DEQUALINIUM CHLORIDE

Shapovalenko D. D., Kovalska O.V.
Scientific supervisor: prof. Blazheyevskiy M. E.
National University of Pharmacy, Kharkiv, Ukraine
lena05021985@ukr.net

Introduction. A novel enzyme-kinetic method for the determination Dequalinium chloride (DCl) in aqueous solutions by means of H_2O_2 – *4*-Ethoxyaniline (*p*-Ph) detection system has been proposed. In the presence of cholinesterase (ChE), acetylcholine (ACh) was hydrolyzed to choline and acetic acid. After that H_2O_2 could interact with unreacted ACh and could formed CH_3CO_3H which oxidize *p*-Ph to Azoxyphenetole (APh), resulting in a light brown color developing and an increase of the absorbance at 350 nm. A new kinetic photometric method developed for estimating ChE activity using ACh as substrate measures the rate APh formation and assay of the DCl is highly sensitive with a LOQ of $0.24 \cdot 10^{-7}$ mol·L^{-1}. The obtained assay is fairly simple, inexpensive, which may be used for the screening trace amount of DCl.

Aim. A new sensitive kinetic photometric method for Dequalinium chloride determination has been proposed.

Material and methods. For light absorbance of solutions "photoelectric concentration colorimeter («CPC-2»)" was used (Zagorsky Optical & Mechanical Plant, Russia). Using the filter №2 and quartz cell of 1.0 cm. pH value was measured at Ionomer I – 160M laboratory (Belarus) by using EGL 43-07. For research reagents were used: *p*-Phenetidine (4 – ethoxyaniline – 98%) (SIGMA – ALDRICH). Pharmacopoeia acetylcholine chloride medicine – 0.2 g per amp/5 ml (manufactured by "VECTOR" – State Science Center of virology and biotechnology in Russian Federation" (Russia). Dry protein drug of cholinesterase from horse serum was taken – 80 mg / fL (VI class), 22 AE/mg. "Stabilized Hydrogen Peroxide 30-40%" (LLC "Inter – Synthes", Boryslav, Ukraine).

Results and discussion. The content of Dequalinium chloride was calculated using the calibration graph. The calibration curve was linear in the concentration range of $(2.12\text{-}6.33) \cdot 10^{-7}$ mol·L^{-1} of DCl with a correlation coefficient r=0.999. The LOQ was $0.24 \cdot 10^{-7}$ mol·L^{-1}.

Conclusions. A new sensitive and specific enzyme-kinetic method for determination of Dequalinium chloride was presented. The method has satisfactory reproducibility and accuracy. The RSD ≤3.34% (δ = +1.42% ... – 0.47%) at determining the concentration of Dequalinium chloride in model solutions.