DEVELOPMENT AND VALIDATION OF HPLC/UV-PROCEDURE FOR EFAVIRENZ QUANTITATIVE DETERMINATION BY THE METHOD OF CALIBRATION CURVE

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Introduction. Efavirenz is a synthetic compound from the group of non-nucleoside reverse transcriptase inhibitors used for treatment of HIV infection. There are cases of acute poisoning due to administration of efavirenz, including cases of suicide attempts.

Aim. To develop HPLC/UV-procedure of efavirenz quantification using the system of HPLC-analyzer MiLiChrome® A-02 and carry out its step-by-step validation in the variant of the method of calibration curve to prove the acceptability for further application in analytical toxicology.

Materials and methods. Efavirenz was of pharmacopoeial purity. The reference, stock and model solutions of secnidazole were prepared using ethanol as a solvent.

The HPLC/UV-analyses conditions: high pressure liquid chromatograph MiLiChrome® A-02 (EcoNova, Russia); *Eluent A* (0.2 M $LiClO_4$ – 0.005 M $HClO_4$) and *Eluent B* (acetonitrile) were used as the mobile phase components; HPLC microcolumn of Ø2×75 mm dimension and reversed phase ProntoSIL 120-5-C18 AQ, 5 μm (BISCHOFF Analysentechnik und -geräte GmbH, Germany) were used as an analytical column; all analysis was carried out at 40°C and flow rate of 100 μl/min.; the mobile phase was run in gradient elution mode, namely from 5% to 100% *Eluent B* for 40 min, then 100% *Eluent B* for 3 min.; detection was performed at 247 nm.

Results and discussion. The specificity of the used chromatographic conditions has been confirmed in relation to other antiretroviral medicine. Retention time for efavirenz is 11.95 min. To prove the possibility of the proposed procedure application in further analysis its step-by-step validation has been carried out in the variant of the method of calibration curve. Such validation parameters as in process stability, linearity/calibration model, accuracy and precision (repeatability) have been estimated by model solutions.

Conclusions. New procedure of efavirenz quantitative determination by the method of HPLC/UV has been developed; its acceptability for application has been shown.

EVALUATION OF POLYPHENOL COMPOUNDS AND ANTIOXIDANT ACTIVITY IN IVY (*Hedera Helix L.*) LEAVES

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Introduction. In folk and traditional medicine ivy leaves (Hedera helix L.) are well known and widely used for its pharmacological effects, such as relief of expectoration, reduction of inflammation, promotion of blood supply to the pelvic organs. The ivy leaves contain saponins, flavonoids, phenolic acids, emetine alkaloid, aminoacids, sterols, proteins, vitamins, polyacetylenes. Mainly saponins, due to their high concentration, followed by dicaffeoylquinitial acids and the flavonol derivatives. It is known that most of the compound responsible for medical effects are triterpenic saponins, it is therefore intended to determine the amount of phenolic compound and antioxidant activity in this work.

Aim. To evaluate the antioxidant activity and the amount of polyphenolic compounds in ivy leaves (Hedera helix L.) collected in different European countries by spectrophotometric method.

Material and methods. The total content of phenolic compounds has been evaluated by Folin-Ciocalteu method and calculated by gallic acid equivalent. Total content of flavonoids have been

evaluated using spectrophotometer, results calculated and expressed in equivalent of rutin. The antioxidant activity has been measured with CUPRAC assay using spectrophotometer.

Results and discussions. The total content of phenolic compounds and flavonoids collected in Europe were different. Maximum content of phenolic compounds was determined in the samples of Naujoji akmenė, Lithuania (1,970 g/ml) and the minimum was in Vienna (0,372 g/ml). Maximum content of flavonoids were determined also in Naujoji akmenė, Lithuania (0,180 g/ml) and the minimum were identificated in Bratislava (0,049 g/ml). Moreover, in this research was measured antioxidant activity of ivy leaves. Maximum antioxidantical activity show off leaves from Naujoji akmenė, Lithuania (0,357 mg/g) and the minimum activity were in leaves from Vienna (0,077 mg/g).

Conclusion. The quantity of these active compounds is different depending from plant growth conditions and climate. Larger amounts of active substances found in the ivy leaves obtained from Lithuania than in other European countries. This means that Lithuania has the right conditions for growing ivy leaves from which medical devices with high efficacy are produced.

IDENTIFICATION OF NAPHAZOLINE IN NOSAL WASHES

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Introduction. Naphazoline, 2-(1-naphthylmethyl)-2-imidazoline hydrochloride, is a relatively long-lasting vasoconstrictor, which acts on the alpha receptors of smooth vascular muscle. Clinically, patients poisoned with naphazoline may exhibit miosis, mydriasis, palpitations, hypertension or hypotension, bradycardia, pallor, cyanosis, diaphoresis, anxiety, insomnia, tremor, agitation, hallucinations, seizures, lethargy, obtundation, and coma. Cases of naphazoline poisoning by accidental over dose have also been reported. There are several analytical methods to determine the naphazoline content; the most common among these are the chromatographic methods, namely, gas chromatography–mass spectrometry, high performance liquid chromatography. Some chromatographic methods have been also reported for the separation or quantitative measurement of naphazoline, e.g., sequential injection chromatography, and capillary electrophoresis methods. This sensitive method was employed for toxicological analysis of naphazoline in poisoned patients. Unlike naphazoline does not cause heart toxicity.

Aim. Development of isolation method from biological material. Development a UV spectrophotometric method for qualitative determination of naphazoline in nosal washes.

Material and methods. All used solvents and reagents were qualified "pure chemical". In conducting the study, naphazoline isolated from the dosage form — drops, 0.1% naphthyzine solution, the authenticity and purity of which were confirmed by chemical and physico-chemical methods of analysis, was used as a working standard sample. A spectrophotometer ULAB S131UV (China) was used to study the UV spectra of naphazoline.

Results and discussion. The working standard sample was evaporated in a water bath to a dry residue. 5 ml of 0.1 M HCl was added to the residue and the optical density was measured at a wavelength of λ=270 nm. The length of the cuvette is 10 mm. The reference solution was 0.1 mol / dm^3 HCl. The spectral characteristics of naphazoline (working standard sample) in a 0.1 mol / dm^3 solution of hydrochloric acid are presented in table 1.

Table 1

Spectral characteristics of naphazoline in 0.1 mol / dm^3 solution of hydrochloric acid

Wavelength, λ nm	270	280	287	291
Absorption	0,84	0,53	0,47	0,51

As can be seen from table 1. Ehe UV spectra in a solution of hydrochloric acid 0.1 mol / dm^3 absorption maxima, naphazoline were detected at 270, 280, 287 and 291 nm. The obtained spectra were identical to the spectra given in the literature. The maximum absorption was observed at a wavelength of 270