

lamotrigine (5 µg in the sample) and caffeine (10 µg in the sample) were applied at the distance of 1 cm. The chromatographic plate was placed into the chamber with the corresponding mixture of solvents and eluated. When the solvent front passed 8 cm from the starting line, the plate was removed from the chamber, air-dried, examined under UV-light at 254 nm and treated with Dragendorff's reagent.

Results and discussion. The elaboration of proposed conditions for the detection and identification of lamotrigine by TLC was performed with extracts obtained from liver tissues. For verifying the suitability of chromatographic systems, tested samples of lamotrigine were administered in parallel with caffeine. The chromatographic system was considered suitable, if clear visible spot of caffeine were on chromatogram. Obtained results are given in Table 1.

Table 1

Parameters of the chromatographic mobility of lamotrigine

№	Chromatographic system	Rf values	
		Lamotrigine	Caffeine
1	Butanol-glacial acetic acid-water (30:5:15)	0.43±0.02	0.31±0.02
2	Ethyl acetate-methanol-25% ammonium solution (17:2:1)	0.58±0.01	0.45±0.02
3	Chloroform-butanol-25% ammonium solution (70:40:5)	0.60±0.02	0.42±0.01
4	Chloroform-methanol (9:1)	0.33±0.02	0.24±0.02
5	Methanol-25% ammonium solution (100:1.5)	0.70±0.01	0.43±0.02
6	Butanol-glacial acetic acid-water (15:5:30)	0.66±0.02	0.45±0.01
7	Methanol-butanol (60:40)	0.25±0.02	0.13±0.01
8	Chloroform-ethanol (20:1)	0.88±0.02	0.53±0.02
9	Ethyl acetate-chloroform-water (9:3:2.5)	0.81±0.01	0.46±0.02

Conclusions. It has been found that lamotrigine shows the chromatographic mobility in all used solvent systems with Rf values from 0.25±0.02 to 0.88±0.02. Systems of butanol-glacial acetic acid-water (30:5:15) with values Rf=0.43±0.02 and ethyl acetate-methanol-25% ammonia solution with values Rf=0.58±0.01 has been considered as the most suitable for analytical diagnosis of lamotrigine acute poisonings.

ANTIOXIDANT ACTIVITY OF ANISE OIL AND ITS ANALYTICAL APPLICATION

Kutsanyan A. A.

Scientific supervisor: prof. Blazheyevskiy M. Ye.

National University of Pharmacy, Kharkiv, Ukraine

aka130999@gmail.com

Introduction. Anethole (1-methoxy-4-propenylbenzene) - the main component of the essential oils of Anise (*Anisum vulgare Gaeth.*) and Fennel (*Foeniculum vulgare Mill.*). Due to the reflex effect on the gastric and intestinal mucus receptors, it causes the expectorant and laxative action of the drugs from them. Anethole also shows an antimicrobial activity.

As anethole in essential oils in a mixture with other terpene compounds and does not contain strongly functional groups, quantitative determination of it, based on the application of classical chemical or physicochemical methods, is a rather difficult task.

For the medicinal plant raw material Anise and Fennel, as well as Liquor Ammonii anisatus, the Pharmacopeia provides for the definition of the total content of essential oils. During the analysis of essential oil of Fennel, indicators such as density, refractive index, etc. are determined, and then on the

basis of these data it is concluded about its quality. It is generally accepted that the main indicator of the quality of medicinal plant raw material and drugs of these is the content of biologically active substances.

Nowadays, to study the composition of Essential oils of Anise and Fennel is used the method of gas-liquid chromatography. However, in our opinion, the issue of quantitative determination of anethole in plant raw materials and medicines was not enough attention.

Aim. The purpose of the present work was to study the antioxidant activity of anise oil using as a model reaction of catalytic oxidation of luminol with hydrogen peroxide in the presence of hemoglobin, and then processing the method for quantitatively determining the content of anise oil in a *Liquor Ammonii anisatus* by the method of chemiluminescence.

Materials and methods. A sensitive and simple chemiluminescent method for the determination of Anise oil based on its inhibitory effect on the hemoglobin-catalyzed reaction of chemiluminescence oxidation of luminol with hydrogen peroxide was proposed. The chemiluminescent oxidation of luminol by hydrogen peroxide in the presence of hemoglobin is used. Chemiluminescence properties such as peak observed light intensity (I), area of emission (S) are studied, varying the concentration of all reactants using a multivariate factorial approach. Chemiluminescence intensity (I) was measured using an assembled chemiluminescence installation (Chemiluminometer - 01) including photoelectric multiplier (PEM) FEU-84-A, weak current meter IMT- 0.5 and fast-operating recording potentiometer LINE RECORDER TZ 4620 (Laboratorni pristroje, Czech Republic). The integral chemiluminescence (S) was recorded using the digital automatic integrator I-02 for 80 sec. The content of Anise oil was found in calibration graph.

Results and discussion. The stock 0.01 mol/L solution of Luminol (5-Amino-2,3-dihydro-1,4-phthalazinedione (H₂L), Sigma-Aldrich, 97%), additionally recrystallised from a saturated solution of sodium hydroxide) in a 0.01 mol/L solution of sodium hydroxide: 0.1772 g of Luminol are dissolved in a 100.0 ml 0.01 mol/L solution of sodium hydroxide. The resulting solution of Luminol is diluted with double distilled water exactly 10 times. Hemoglobin (human erythrocytes) solution was prepared by dissolving the certain amount of Hb (*Simko* Ltd. Lviv, Ukraine) in double distilled water.

In this communication, a new chemiluminescent method based on inhibitory effect on the hemoglobin-catalyzed reaction of chemiluminescence oxidation of luminol with hydrogen peroxide by Anise oil (or trans-Anethole) was proposed. The experimental conditions for the system of H₂L-H₂O₂-Hb-Anise oil (or trans-Anethole) was optimized, and Anise oil was detected by the decreased initial light intensity (ΔI) and/or area of emission (ΔS). A chemiluminescence assay for the determination of antioxidant capacity has been optimized and applied to analyses of model solutions in the present study. The optimal concentrations of reagents (Luminol, NaOH, H₂O₂, Hemoglobin) have been determined, as well as the optimal reaction conditions (mixing order, pH, temperature, sample volume). All of the measurements were performed at the emission maximum of the oxidized form of luminol (425 nm). The optimal concentrations of the reagents were determined as follows: 0.05 mmol/L luminol, 0.05 mol/L NaOH, 0.853 mmol/L H₂O₂ and 0.05 $\mu\text{g}/\text{mL}$ Hb in the reaction mixture. Under the optimal experimental conditions is, the decrease of the light intensity of system (ΔI) is in linear relationship with the concentration of Anise oil. The calibration graph is linear in the range ($w, \%$) from 1.50×10^{-3} to $1.5 \times 10^{-2} \%$ ($\Delta I = 1850.5 w - 1.68$ ($r = 0.985$)) with detection limit of $1.0 \times 10^{-3} \%$. This method can be used for the determination of Anise oil content in *Liquor Ammonii anisatus* with satisfactory results. Analyses were carried out in solutions, 0.05 mol/L NaOH, at room temperature. With the optimized chemiluminescence assay, the antioxidant capacities of various water and water-ethanol solutions were determined (Anise oil, trans-Anethole) and the results were compared. Was noted a significant difference in the antioxidant activity of the essential oil of Anise and its individual key component, trans-Anethole.

Conclusions. This optimized chemiluminescence assay is simple, rapid and reliable, and it represents a good alternative to classical method high performance liquid chromatography (HPLC) for the determination of antioxidant capacity of herbal extracts and other food samples.