

QUANTITATIVE DETERMINATION OF *trans*-ANETHOLE IN “GRIPE WATER” BY CHEMILUMINESCENCE METHOD

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Introduction. Fennel (*Foeniculum vulgare* Mill.) is well-known plant for its essential oil which has been widely used for many years as a constituent in many pharmaceutical products. It is a main constituent in “Gripe Water” given to infants in case of colic and gastrointestinal discomfort [1]. Essential oil of fennel has carminative, insecticidal, antioxidant and antimicrobial effects [2,3]. According to the British Pharmacopoeia, two varieties are known for this plant species; bitter and sweet fennel. Bitter fennel oil contains not less than 60.0 per cent of *trans*-anethole and sweet fennel oil contains not less than 80.0 per cent of *trans*-anethole [4].

It is important to develop simple and rapid analytical methods for determination of essential oil components in pharmaceutical products. Gas chromatography (GC) is the mostly used analytical technique for quantitative determination of the marker components in essential oils. It is used for analysis of commercial samples of fennel essential oil depending on *trans*-anethole content [4]. Although GC is characterized by high sensitivity, it suffers from several drawbacks such as time consumption, need of column selection, problems associated with chromatographic peaks, and need of calibration curve using authentic reference compounds.

Aim. Purpose the aim of this work was to study the antioxidant activity of *trans*-Anethole and fennel oil using as a model the chemiluminescent reaction of catalytic oxidation of Luminol with hydrogen peroxide in the presence of Hemoglobin, and then propose of the new method determination of the content of *trans*-Anethole in “Gripe Water”.

Materials and methods. Object of the study was “Gripe Water”, oral solution in 100 ml bottles produced by "Leda" (Ukraine, Harkiv). In 100 ml solution - Fennel oil 0.005 g. Fennel essential oil 5 ml Aromatics.

The stock 0.01 mol/L solution *trans*-Anethole was prepared using *trans*-Anethole ALDRICH/117870 - 99%. Synonym: 4-Propenylanisole; *trans*-1-Methoxy-4-(1-propenyl)benzene. CAS Number: 4180-23-8.

The stock 0.01 mol/L solution of Luminol (5-Amino-2,3-dihydro-1,4-phthalazinedione (H₂L), Sigma-Aldrich, 97%), additionally recrystallized 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, Hb) solution was prepared by dissolving the certain amount of Hb (Simko Ltd. Lviv, Ukraine) in double distilled water. A sensitive and simple chemiluminescent method for the determination of Fennel oil (or *trans*-Anethole) on the basis of its inhibitory effect on the Hb-catalyzed oxidation reaction of luminol chemiluminescence with hydrogen peroxide has been proposed. Chemiluminescent oxidation of luminol with hydrogen peroxide in the presence of Hb is used. The properties of chemiluminescence such as the peak intensity

of the observed light (I_{CL}), which is changed from the concentrations of all reagents using a multifactor approach, are investigated. The intensity of chemiluminescence (I_{CL}) was measured using an assembled chemiluminescent unit (chemiluminometer-01), which includes a photoelectric multiplier (PEM) FEU-84-A, low current meter IMT-0.5 and high-speed recording potentiometer LINE RECORDER TZ 4620, Laboratory Czech Republic). The anise oil content was determined by means of a calibration graph.

Results and discussion. In this communication, a new chemiluminescent method based on inhibitory effect on the Hb-catalyzed reaction of chemiluminescence oxidation of luminol with hydrogen peroxide by fennel oil (or *trans*-Anethole) was proposed. The experimental conditions for the system of $H_2L-H_2O_2-Hb$ -fennel oil (or *trans*-Anethole) was optimized, and fennel oil was detected by the decreasing initial light intensity (ΔI_{CL}). 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, H_2O_2 , Hb) 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 (425 nm). The optimal concentrations of the reagents were determined as follows: 0.05 mmol/L H_2L , 0.05 mol/L NaOH, 0.853 mmol/L H_2O_2 and 0.05 $\mu\text{g}/\text{mL}$ Hb in the reaction mixture. Under optimal experimental conditions, the reduction of the light intensity of the system (ΔI_{CL}) is linearly dependent on the concentration of *trans*-anethole. The calibration graph is linear in the range ($w, \%$) from $1.96 \cdot 10^{-3}$ to $1.5 \cdot 10^{-2}\%$ ($\Delta I_{CL} = (833,7 \pm 50) w$ ($r = 0.999$)) with a detection limit (3S) of $5,9 \cdot 10^{-4}\%$ (Fig. 1). This method was used to determine the content of *trans*-Anethole in "Gripe Water" with satisfactory results. The analyzes were performed in solutions of 0.05 mol/L NaOH at room temperature. The antioxidant capacity of various aqueous-ethanolic solutions (fennel oil, *trans*-anethole) was determined by optimized chemiluminescence analysis and the results were compared. There was a significant difference in the antioxidant activity of fennel essential oil and its separate key component, *trans*-anethole. Therefore, we analyzed the dosage form containing the essential oil using standard *trans*-Anethole additives. This method can be used for the determination of *trans*-Anethole content in anise oil in pharmaceutical preparation "Gripe Water" with satisfactory results. The content of *trans*-Anethole was $0,004 \pm 0,0001\%$. $RSD=2,0 \%$ ($n=5$; $P=0,95\%$).

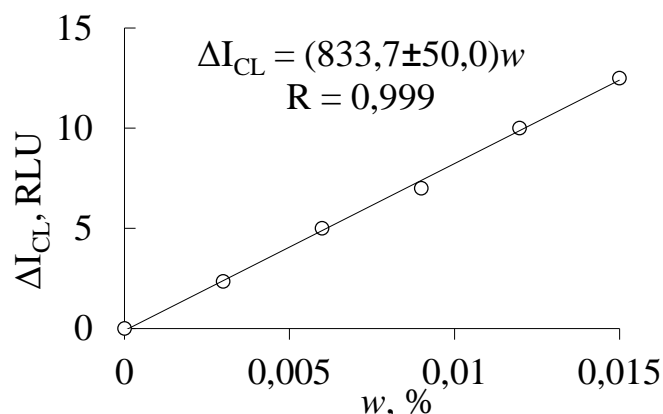


Fig. 1 Calibration graph for quantification of *trans*-Anethole

Conclusions. A simple and rapid method was developed for determination of the amount of *trans*-Anethole in the essential oil of fennel based on inhibitory effect on the Hb-catalyzed reaction of chemiluminescence oxidation of luminol with hydrogen peroxide. The developed method allows to determination of content of *trans*-Anethole in the pharmaceutical preparation “Gripe Water”.

References

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