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Quantitative determination of *trans*-Anethole in Italian aniseed drink «Sambuca» by chemiluminescence method

Oleg Kryskiv*, Mykola Blazheyevskiy, Valeriy Moroz, Oleg Shpychak

National University of Pharmacy, Kharkiv, Ukraine

E-mail: oleg.kryskiw@gmail.com

Introduction. *trans*-Anethole (1-methoxy-4-[(E)-prop-1-enyl]benzene, t-ANE) is a major constituent of the essential oils of many plants, including anise, fennel, and thyme, and is widely used in the food, beverage, and fragrance industries for its sweet, anise-like flavor and scent. It's a natural flavoring and is also known for its potential pharmacological properties, such as anti-inflammatory and antioxidant effects. *trans*-Anethole is the principal volatile compound of aniseed spirits.

Aim. Purpose the aim of this work was to study the antioxidant activity of *trans*-Anethole 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 Italian flavored liqueur “Sambuca”.

Materials and methods. The stock solution *trans*-Anethole was prepared using *trans*-Anethole ALDRICH/117870 – 99%. *trans*-Anethole standard working solutions and Italian aniseed spirit, namely Sambuca were used in the study. 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. Hemoglobin (human erythrocytes, Hb) solution was prepared by dissolving the certain amount of Hb (Simko Ltd. Lviv, Ukraine) in double distilled water. *trans*-Anethole (t-ANE) was determined in commercially available Italian aniseed drink, namely «Sambuca». According to Regulation (EU) 2019/787, Sambuca contains 40% ethanol and 350 g per liter of sugars. Sambuca has a natural *trans*-Anethole content of between 1g and 2g per liter.

The intensity of chemiluminescence 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 integral chemiluminescence (S_{CL}) was recorded using the digital automatic integrator I-02 for 80 sec. Chemiluminescence measurements were performed in discrete mode using a 10 mL cuvette and a 0.5 mL pipette dispenser. All of the measurements were performed at the emission maximum (425 nm). The content of *trans*-Anethole was found by standard addition method.

Results and discussion. A new chemiluminescent method based on inhibitory effect on the Hb-catalyzed reaction of chemiluminescence oxidation of luminol with hydrogen peroxide by *trans*-Anethole was proposed. The experimental conditions for the system of H₂L-H₂O₂-Hb-*trans*-Anethole was optimized (0.05 mmol/L H₂L, 0.05 mol/L NaOH, 0.853 mmol/L H₂O₂ and 0.05 µg/mL Hb in the reaction mixture), and *trans*-Anethole was detected by the decreasing initial of the area of emission of the (ΔS , relative units). Under optimal experimental conditions, the reduction of the area of emission of the (ΔS) is linearly dependent on the concentration of *trans*-Anethole. The calibration graph is linear in the range (w/v , %) from $0.97 \cdot 10^{-3}$ to $1.5 \cdot 10^{-2}$ % ($\Delta S_{CL} = (10,42 \pm 0,33) \times 10^3 w$ ($r = 0.999$)) with a LOQ (10S) of $0,97 \cdot 10^{-3}$ %. This method was used to determine the content of *trans*-Anethole in “Sambuca” with satisfactory results. The content of *trans*-Anethole in Italian aniseed drink “Sambuca” was $0,12 \pm 0,001$ %. $RSD=2,5\%$ ($n=5$; $P=0,95\%$).

Conclusions. A simple and rapid method was developed for determination of the amount of *trans*-Anethole in Italian aniseed drink “Sambuca” based on inhibitory effect on the Hb-catalyzed reaction of chemiluminescence oxidation of luminol with hydrogen peroxide. This optimized chemiluminescent assay is simple, fast, and reliable, and is a good alternative to the standard GC technique for quantitative determination of *trans*-Anethole in aniseed drinks.