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COMPLEX COMBINATION OF ANALYTICAL METHODS FOR THE QUANTITATIVE DETERMINATION OF FLUACIZINE IN SOLUTIONS, BIOLOGICAL MATERIAL AND AIR

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Identification techniques of fluacizine by color reactions and thin layer chromatography were elaborated. The results of chromatographic analysis of fluacizine for two systems of solvents (methanol – ammonia and benzene – methanol – acetic acid) reveal that necessary interval for the chromatogram developing is 35 min, the detection limit is 1 μg, disappearance of spots on plates occurs in 30-40 min and the R_f value depends on purity and qualification of dissolvents. The fluacizine detection and determination has been fulfilled by UV-spectrophotometry in phosphate buffer solution at 256 nm. The kept up to date analysis methods of the spectrophotometric and gel-chromatographic analysis were used for techniques elaboration of the quantitative determination of fluacizine in solutions, biological material and air.

Keyword: fluacizine, color reactions, thin layer chromatography, UV-spectrophotometry and gel-chromatography, chemical and toxicological analysis.

КОМПЛЕКСНЕ ПОЄДНАННЯ АНАЛІТИЧНИХ МЕТОДІВ ДЛЯ КІЛЬКІСНОГО ВИЗНАЧЕННЯ ФТОРАЦИЗИНУ В РОЗЧИНАХ, БІОЛОГІЧНОМУ МАТЕРІАЛІ І В ПОВІТРІ

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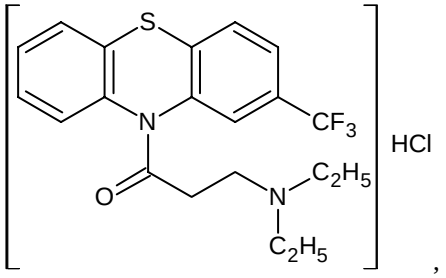
Розроблено методики ідентифікації фторацизину за допомогою кольорових реакцій та хроматографії в тонкому шарі сорбенту. Результати хроматографічного аналізу фторацизину для двох систем розчинників (метиловий спирт – амоніак і бензен – метиловий спирт – ацетатна кислота) показали, що час необхідний, для розгортання хроматограм становить 35 хв, границя відкриття становить 1 мкг, плями на пластинках зникають через 30-40 хв а на величину R_f суттєво впливає чистота і кваліфікація розчинників. Выявление и определение фторацизина также осуществляли методом спектрофотометрии в УФ-области спектра в фосфатном буферном растворе при 256 нм. Опрацьовані раніше методи спектрофотометричного і гель-хроматографічного аналізів були використані для розробки методик кількісного визначення фторацизину в розчинах, біологічному матеріалі і в повітрі.

Ключові слова: фторацизин, кольорові реакції, хроматографія в тонкому шарі сорбенту, УФ-спектрофотометрія, гель-хроматографія, хіміко-токсикологічний аналіз.

PROBLEM STATEMENT. In medical practice various chemicals are broadly used for treatment of different diseases. Among this multitude the considerable group of chemical agents belongs to psycho-stimulants. That is why fluacizine which uses in psychiatry for depression removal became the object of our study.

Fluacizine, I.U.P.A.C. name 3-(diethylamino)-1-[2-(trifluoromethyl)-10*H*-phenothiazin-10-yl] propan-1-one hydrochloride



, is white, crystal (melting point is 163–166°C), slightly soluble in water and well soluble in methanol, ethanol and even chloroform. The crystals darken on light. The tablets (0.025 g) or solution (1.25%) in ampoules (on 1 ml) produce on the basis of this substance. Fluacizine is acyl-derivative of phenothiazine, is officinal preparation and is in the register "B".

Presently, for identification and quantitative determination of fluacizine is known [1–3] to use various analytical methods. However information concerning fitness of these methods for the toxicological analysis is absent. Hence, the aim of the paper is to elaborate sensitive techniques of fluacizine identification and determination by color reactions and thin layer chromatography and also application of already known techniques of a spectrophotometric analysis in ultra-violet spectral region and a gel-chromatographic method for quantitative determination of fluacizine in solutions, a biological material and in air.

EXPERIMENTAL PART AND RESULTS OBTAINED. *Fluacizine testing by color reactions.* Fluacizine is revealed to color under the influence of the common alkaloid reagents. A certain coloring of aqueous fluacizine solution (3 drops) appears immediately after addition of solution (2 drops) of corresponding alkaloid reagent. Preparation of the reagents is described in work [4]. All given reactions are sensitive and reliable. Determination ranges, coloring of aqueous fluacizine solution and the common alkaloid reagents are given in Table 1.

Fluacizin identification by thin layer chromatography. The choice for fluacizin identification of thin layer chromatography method is caused by its high sensitivity and fulfillment rapidity [5].

Silica gel CSM has been used for investigations. It was washed from impurity, then was dried and kept in a glass vessel. Concurrently, the glass plates (13×18 cm) were washed, dried, wiped with ethanol and covered by thin layer of sorbent (silica gel CSM). Sorbent was prepared as follows: silica gel (4.68 g) and gypsum (0.24 g) mixed and added the distilled water (14 ml). The obtained mix put upon a glass plate and evenly distributed on all surface. The prepared plates were placed in a desiccator and held over anhydrous sodium sulfate.

Table 1 – The color test reactions

Alkaloid reagent				H_2SO_4 (conc.)
Erdmann's	Froede's	Mandelin's	Marquis'	
–	red 1:3000	pink 1:3000	pink 1:3000	pink-violet 1:3000

Footnote: the sign "–" means that alkaloid reagent does not give coloring with fluacizine

Simultaneously, fluacizine separation was carried out in presence of other energizers. A driblet of fluorine solution in ethanol (20 μg) was put to the start line, which is located from bottom edge of plates at distance of 2,5 cm, and on the right side from it (at distance of 2 cm) other driblet of solution mixture energizers was put too. Two systems of dissolvents (methanol – ammonia (200:3) and benzene – methanol – acetic acid (14:4:1)) were selected. Spots on CSM plates identify by means of Dragendorf's reagent modified by Mounier [6]. Thus spots become yellow-orange.

Fluacizine and other energizers as well were chromatographed on finished "Sylufol" plates (Sweden) which were activated previously. For that, the aqueous solution of potassium permanganate (0.1%) was used as an identifier. Spots of fluacizine, azaphenum, damilene maleinate, indopan were the yellow against a pink background of the "Sylufol" plate, it of melipramin and nialamide were blue and white respectively. Spots of sydnocarb did not identify by aqueous solution of potassium permanganate. Revealing reagent of sydnocarb spots was prepared as follows: bromophenol blue indicator (0.05 g) was dissolved in acetone (10 ml); it was filled with solution of silver nitrate (1 %) up to 100 ml in acetone and water system (1:3). Sydnocarb spots were blue against a primrose background. The retardation factor (R_f) was determined by the following ratio:

$$R_f = \frac{\text{migration distance of substance}}{\text{migration distance of solvent front}}.$$

The results of thin layer chromatography analysis of fluacizin on "Silufol" and CSM plates in corresponding systems of dissolvents are given in Table 2. The data of the Table 2 shows that proposed systems of dissolvents successfully can be used for detection and separation of fluacizin and other energizers. It was determined that necessary interval for the chromatogram developing in this systems of dissolvents are 35 min. The detection limit of fluacizine and azaphenum are 1 μg, it of damilene maleinate and melipramin are 3 μg, it of nialamide and indopan are 5 μg and it of sydnophen and sydnocarb are 10 μg. The disappearance of spots on plates occurs in 30-40 min and the R_f value depends on purity and qualification of dissolvents.

Table 2 – Fluacizine separation in a mix with other energizers by thin layer chromatography method on "Silufol" and CSM plates

No.	Mixing ratio	System of dissolvents	R_f value	
			Sylufol	CSM
1	*Azph*	methanol – ammonia (200 : 3)	0.20	0.55
	DM		0.48 and 0.85	0.67
	Mlp		0.40	0.42
	Flcs		0.68	0.82
2	*Indp*	methanol – ammonia (100 : 1,5)	0.21	0.40
	Mlp		0.39	0.40
	Nlm		0.80	0.77
	Sdcb		0.92	0.80
	Flcs		0.67	0.82
3	*Azph*	benzene – methanol – acetic acid (14 : 4 : 1)	0.00	0.03
	Sdph		0.21	0.41
	Flcs		0.32	0.52
4	*Indp*	benzene – methanol – acetic acid (14 : 4 : 1)	0.10	0.12
	Nlm		0.41	0.31
	Flcs		0.30	0.45
	Sdcb		0.64	0.84

Footnote: *Azph* is azaphenum; *DM* is damilene maleinate; *Indp* is indopan; *Flcs* is fluacizine; *Mlp* is melipramin; *Nlm* is nialamide; *Sdcb* is sydnocarb; *Sdph* is sydnophen.

Fluacizine UV-spectrophotometric detection. The light-absorption measurement is put into practice by means of spectrophotometer SF-26 (cuvet is 1 cm).

Fluacizine ultra-violet spectrums were studied in various dissolvents (water, solution (0.02 N) of sulfuric acid (pH is 1.9) phosphate buffer solution (pH is 7.6) acetate buffer solution (pH is 4.6) methanol, chloroform) and the values of molar and specific absorption coefficient are presented in article [7]. Hence, fluacizine in acid and neutral aqueous solutions possesses peak light-absorption at 256 nm while it in methanol and chloroform have maximum at 260 nm.

Furthermore, the relationship between light-absorption and concentration of phosphate buffer solution at 256 nm was studied too. Preparation of buffer solutions is described in work [8]. Phosphate buffer solution was used for comparison. The light-absorption spectrum exclusively in phosphate buffer solution was used for the quantitative fluacizine determination.

The results of UV-spectrophotometric detection of fluacizine are given in Table 3. It is not difficult to see that obtained value of the optical density of fluacizine in phosphate buffer solution at 256 nm ($E_{1cm}^{1\%}$ = 192) obey Beer–Lambert–Bouguer law in the concentration range of 5 to 30 μg/ml.

Table 3 – Value of specific absorption coefficient of fluacizine in phosphate buffer solutions

Fluacizine concentration, μg/ml	Optical density, *D*	$E_{1cm}^{1\%}$
5	0.120	189
10	0.191	190
15	0.290	193
20	0.392	195
30	0.574	190
		$\overline{X}$ =192

Gel-chromatographic analysis of fluacizine. Selected conditions of UV-spectrophotometric detection of fluacizine was used for technique elaboration of fluacizine gel-chromatography in biological material. The basic principles of gel-chromatography is described by G. Determan in work [9]. In this method use agar or polyacrylamide gel, sephadex gel *etc* [10].

Earlier, the dependence of fluacizine distribution from sephadex type and composition of eluting liquid was studied. The sephadex types such as G-10 (40-120 μm), G-25 (50-150 μm) and G-25 (100-300 μm) were used in our investigations. The solution (0.02 N) of sulfuric acid with pH 1.9, the solution (1%) of acetate acid with pH 2.5, phosphate buffer solution (pH 7.6), the solution (0.8%) of chloric acid with pH 1.8 were used as the eluents. We have been checking the solutions for the presence of fluacizine by UV-spectrophotometry. It was ascertained that sephadex G-25 (100-300 μm) is the best gel type for investigations. Moreover, in process of elution by solution of phosphate buffer mixture, fluacizine comes out of the chromatography column later than in case of elution by acid solutions [11-13].

The elaborated method of the gel-chromatographic analysis of fluacizine was applied for the purification of an extracts out of biological material from admixtures. The admixture from such extraction, as a rule, contains much more massive molecules than the studied, toxicological important substances. That is why at a passing of such extracts through columns, filled with gel sephadex, the studied substance elutes from a column later, than admixture.

Table 4 – The distribution of admixtures and fluacizine in the eluates fractions

Fraction number	Dilution rate	Optical density at 256 nm	
		for fractions without fluacizine	for fractions with fluacizine
1–12	2	∞	–
13	2	0.64	0.07
14	2	0.60	0.10
15	2	0.58	0.14
16	2	0.55	0.18
17	2	0.52	0.22
18	2	0.42	0.30
19	2	0.38	0.24
20	2	0.32	0.20
21	2	0.30	0.16
22	2	0.22	0.07
23	2	0.15	0.05
24	2	0.08	0.00
25	2	0.04	0.00
26	2	0.00	0.00
27	2	0.00	0.00

In order to use a gel-chromatography method for separation of fluorine from admixture, it is necessary to know, where (that is, in what fractions of eluate) the admixture collects. For that, the series of experiments were carried out. Phosphate buffer solution (200 ml) was added to the crushed liver (100 g) not containing fluacizine and it all was left for two hours. The obtained extracts were separated off biological material and were centrifuged during 20 min (10,000 rpm). This centrifugate (10 ml) was brought in into column filled with gel sephadex G-25 (100-300 μm). Elution was carried out by phosphatic buffer solution. Eluate was collected up with fractions (in 10 ml) by means of automated fraction collector. Optical density at 256 nm was measured for solutions of each test-tube. It was ascertained that most admixtures were eluted from a column earlier than fluacizine (down to 13 fraction), but small amount of proteins and other admixtures were eluted at the same time with fluacizine (of 13 to 22 fractions). Therefore quantitative determination of fluacizine, extracted from biological material, is not possible by using direct UV-spectrophotometry analyses. That is why there was a need to apply an extraction method to additional purification of the extracts.

Further, the first 12 fractions (120 ml) were thrown off whereas the next fractions of 13 to 22 were unified (100 ml). This eluates was moved in the separating funnel, were acidulated by solution (0.02 N) of the sulphuric acid to pH 1.9 and were extracted by chloroform three times on 30 ml. These extracts with chloroform unify and evaporate. Concurrently, solution of sodium hydroxide was added in the separating funnel to aqueous phase for making alkaline medium (pH 9-10) and it all was shaken up with chloroform three times. The late extracts were evaporated too.

Quantitative determination of fluacizine in the solid residual was performed by spectrophotometric analysis. For that, the solid residuals were dissolved in ethanol (0.5 ml) then phosphate buffer solution (15 ml) was added there. Whereupon optical density (at 256 nm) of this prepared solution was measured. The done investigations showed that use of the proposed technique enables to isolate more of fluacizine from the alkaline medium.

Besides, we put into practice fluacizine detection in air. Saturated air with fluacizine (100 L) was passed (volumetric rate was 10 l/min) through the filter (AFA–WP–10). The filter was steeped into the ethanol and squeezed, the degree of desorption is 93 %. The ethanolic extract was evaporated at 60°C. The obtained dry residual was again dissolved in ethanol (0.5 ml) and it was analyzed for fluacizine content by means of thin layer chromatography (methanol – ammonia system (100:1.5)) on CSM plates (Dragendorf's reactant) or "Sylufol" plates (aqueous solution of potassium permanganate (0.1%)) as well as by using the above color reactions. The detection limit of fluacizine in the assay is 2 μg; the standard deviation is ±20 %; the interval of experimentation including choice of samples is one hour and a half.

The results of the proteins detection and other admixtures in the eluates obtained after passing of the extracts through sephadex gel G-25 (100-300 μm) with or without fluacizine are given in Table 4.

CONCLUSIONS. So, the thin layer chromato-graphy on silica gel CSM or on “Silufol” plates, the color reactions under common alkaloid reactants can be used for fluacizine identification. The thin layer chromatography technique of the detection and of the separation of fluacizine from the mixture energizers was proposed. Detection and determination of fluacizine too can be performed by UV-spectrophotometry in phosphate buffer solution at 256 nm. The separation conditions of mixture of fluacizine and protein in extracts from a biological material were elaborated by using gel-chromatographic analysis. The proposed technique of separation of fluacizine and biological material as well as its detection in solutions or in air can be recommended into practice of forensic-chemical laboratory.

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КОМПЛЕКСНОЕ СОЧЕТАНИЕ АНАЛИТИЧЕСКИХ МЕТОДОВ ДЛЯ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ФТОРАЦИЗИНА В РАСТВОРАХ, БИОЛОГИЧЕСКОМ МАТЕРИАЛЕ И ВОЗДУХЕ

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Разработано методики идентификации фторацизина с помощью цветных реакций и хроматографии в тонком слое сорбента. Результаты хроматографического анализа фторацизина для двух систем растворителей (метанол – аммиак и бензол – метанол – фцетатная кислота) показали, что время, необходимое для розвертки хроматограмм составляет 35 мин., граница открытия составляет 1 мкг, пятна на пластинах исчезают через 30-40 мин., а на величину R_f существенно вплияет чистота и квалификация растворителей. Выявление и определение фторацизина осуществляли методом спектрофотометрии в УФ-области спектра в фосфатном буферном растворе при 256 нм. Отработанные ранее методики спектрофотометрического и гель-хроматографического анализов были использованы для разработки методик количественного определения фторацизина в растворах, биологическом материале и воздухе.

Ключувые слова: фторацизин, цветные реакции, хроматография в тонком слое сорбента, УФ-спектрофотометрия, гель-хроматография, химико-токсикологический анализ.

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