DETERMINATION OF ETHACIZINE IN BIOLOGICAL FLUIDS

Alikhodjaeva M., Boymuratov F.T.,
Ataxanov A., Jalilov F.

Alfraganus University Department of Pharmacyand chemistry Uzbekistan
e-mail: alikhodjaeva.madina@gmail.com

Abstract

Currently, there has been a significant increase in cardiovascular pathology, and due to this, the frequency of use of antiarrhythmic drugs has noticeably increased.In the process of using drugs of this group, severe side effects are more likely to occur. In general, patients with structural heart disease have a higher risk of developing ventricular arrhythmias and proarrhythmias when using antiarrhythmic drugs, especially belonging to the class IC.The drug etacizine belongs to this class. The purpose of this study is to develop methods for the determination of the antiarrhythmic drug etacizine in biological fluids suitable for the practice of chemical and toxicological laboratories.

The method is based on the detection, isolation and quantitative determination of etacizine from dosage forms and biological fluids using modern physicochemical methods of UV spectrophotometry, thermal desorption surface ionization spectroscopy. To purify extracts obtained from biological objects, the thin layer chromatography (TLC) method is used.

Keywords: thin layer chromatography (TLC), UV spectrophotometry, Termodesorbtion Surface-Ionization (TDSI) spectroscopy, etacizine, blood, urine.

Introduction.

Modern research raises questions about the safety of antiarrhythmic therapy, since it is the frequency of proarrhythmic effects that is the main factor currently limiting the use of any antiarrhythmic drug. In general, this problem seems to be very closely related to the problem of life-threatening ventricular tachyarrhythmias and sudden cardiac death [1].

Ethacizine is a class ICantiarrhythmic agent, related to moracizine. It is used for the treatment of severe and refractory ventricular and supraventricular arrhythmias, especially those accompanied by organic heart disease [2-4]. It is also indicated as a treatment of refractory tachycardia associated with Wolff–Parkinson–White syndrome. The risk connected to arrhythmogenic action of ethacizin increases when it combines with MAO inhibitors and antiarrhythmics (class IA) [5-6]. Therefore, there is a need for development of an analytical methodology for the identification and quantification of ethacizin in biological fluids (blood and urine).

Ethacizine - Ethyl 10-[3-(diethylamino)propionyl]phenothiazine-2-carbamate $C_{22}H_{27}N_3O_3S$MW =413.54 g/mol

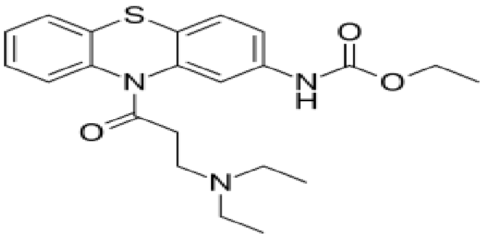


Figure 1. Chemical structure of Ethacizine.

Determination of ethacizin can be done via several analytical methods that are reported in literature:

HPLC method and UV spectrophotometry are considered as a time efficient analytical tools for determination of ethacizin in pharmaceutical dosage form [7-8]. There are some reports showing estimation of substance in biological samples especially from plasma [9]. This study has been done with the aim to develop precise, cost effective and simple method for determination of ethacizin in biological fluids.

Methods

Determination of Ethacizineby TLC method

The thin layer chromatography (TLC) method is used to purify extracts obtained from biological objects. With the aim to study the conditions of thin-layer chromatographic analysis of etacizine, chromatographic ready-made plates with a fixed layer of silicagel from Merck and Silufol UV-254 were used.Various solvent systems were used as the mobile phase. In addition, in order to develop the conditions for the chromatographic analysis of etacizine, standard solutions of the drug in ethanol of 1 mg/ml were prepared [10].

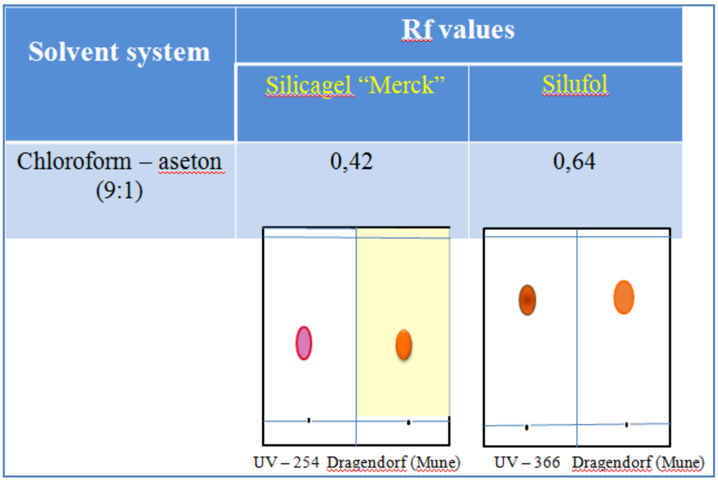
A sample of 5 µl of an ethanol 0.1% solution of etacizine is applied to the chromatographic plate using capillaries or a micro-syringe. After drying, the plates with the deposited samples are placed in a chromatographic chamber which is pre-saturated with solvent vapors indicated in Table 1[11]. When the solvent front reached a height of 10 cm from the starting line, the plate was removed from the chamber and dried at room temperature. To detect drug localization zones on chromatographic plates, the latter were first viewed under a chemiscope (UV lamp) at 254 nm and 366 nm, then sprayed with a reagent and observed for changes in background color and spot color. For this purpose, Dragendorff's reagent according to Mounier was used.In this case, the etacizin stain turns into orange-brown. Among the solvent systems, chloroform-aseton turned out to be the most optimal in a ratio of (9:1) with a value of Rf = 0.64.

Table 1.

The value of Rf etacizine in various solvent systems

Solventsystems	Merck ,Rf values	Silufol UV-254, Rf values
chloroform-aseton (9:1)	0.42	0.64
chloroform, acetone, 30% acid acetate, ethanol and in 18:2:1:7	0.45	0.48
chloroform, ethyl acetate and ethanol (10:3:1)	0.64	0.66

Mixture of chloroform-asetonin 9:1 (or a mixture of chloroform, acetone, 30% acid acetate, ethanol and in 16:4:1:9) ratio as mobile phase. UV irradiation at 254 nm and 366 nm and Dragendorff's reagent were used for visualization of the spots on the plate. Ethacizin was detected at Rf 0.64-0.66 by TLC method. The results of the analysis are presented in Figure 2.



Solvent system	Rf values	
	Silicagel "Merck"	Silufol
Chloroform – aseton (9:1)	0,42	0,64

Figure 2. The results of determination of ethacizin by TLC.

Quantitative analysis uses instrumental methods [18-20]. Among them, the spectrophotometric method is widely used. This is due to high specificity, versatility, comparative ease and high measurement accuracy [11-13].

Determination of Ethacizin by UV Spectroscopy

UV spectrophotometric analysis was performed with a spectrophotometer of the company «Agilent Technologies» Model 8453. To identify ethacizin, ethanolic solution of the standard sample with concentration of 20µg / ml was utilized. When measuring the absorbance of a standard solution of ethacizin, one absorption peak at a wavelength of 267 nm in the range of 200-400 nm were observed. UV absorption spectrum of ethanol solution of ethacizin is shown in Fig.3.

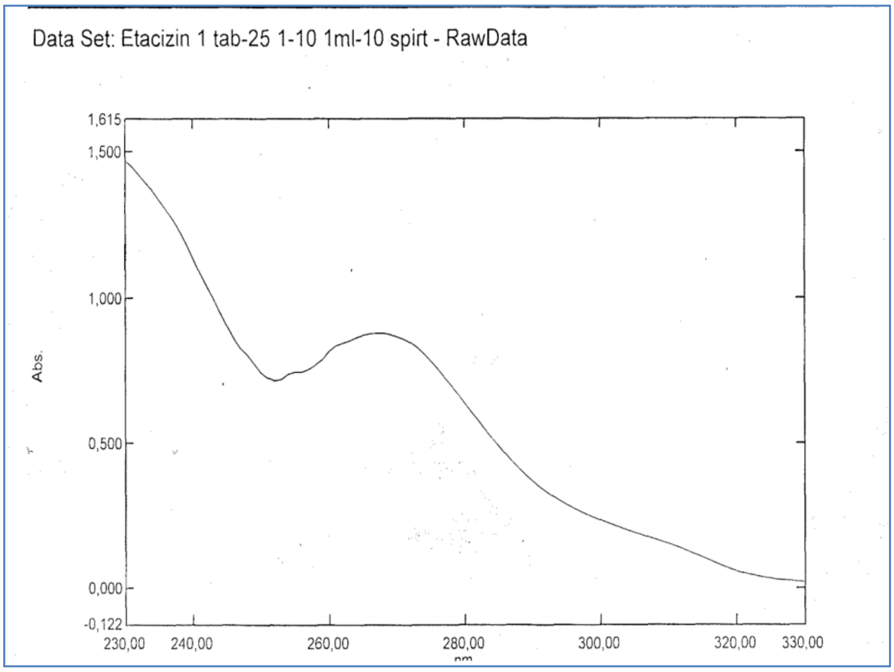


Figure 3. UV-spectrum of ethacizin'sstandard solution in ethanol ethacizin.

In order to conduct quantitative determination of ethacizin via UV spectrophotometry using the following procedure: For quantitative determination of ethacizin, ethanol solution containing 5.0; 10.0; 15.0; 20.0; 25.0 µg/ml ethacizin were prepared. The absorbance of each solution in the UV spectrophotometer was measured. As a comparative solution, the ethanol solution was used. The results were compliant with Beer's law in the concentration range of 3-30 µg/ml. The gained results can be used to calculate the specific and molar absorption coefficient, which are shown in Table 2.

Table 2.

The results of determination of specific and molar absorption coefficients of ethacizin in ethanol

Concentration, µg/ml	D	E	ε
5	0.221	442.0	19890
10	0.441	441.0	19845
15	0.663	442.0	19890
20	0.878	439.0	19755
25	1.096	438.4	19728

The following formula can be used for the quantitative determination of ethacizin:

$$X = \frac{D \cdot V_1 \cdot 1000}{E_{1cm}^{1\%} \cdot 100 \cdot V_2}$$

where, D– absorbance of the solution;

$E_{1cm}^{1\%}$ -Specific absorption rate of ethacizin;

V_1 – volume of the researched solution, in ml;

V_2 – volume of isolation, in ml.

Determination of Ethacizin by Termodesorbtion Surface-Ionization (TDSI) spectroscopy

To study etacizine in medicines and biological fluids by TDPI spectroscopy, an analysis was performed under the following conditions.

TDSI-spectrum of ethacizin was measured on apparatus PII-N-S "Iskovich", supplied with the detector of superficial ionization.Ethanol (3 ml) was used as a solvent for TDSIS analysis.TDSIS Parameters:

-emitter – oxidized molybdenum, containing iridium;
-emitter's voltage – 405 V;
-emitter's temperature – 390-420ºC;
-temperature of the evaporation – 20-505 ºC;
-air flow rate – 50 l/h (the compressor's voltage – 12V);
-test sample volume taken for analysis – 1,0 µl;
-time of the analysis – 3 min;
-record of spectra is carried out by means computer program directly [14,16-17].

Standard solutions of the drug were prepared to identify the etacizinedrug.For this reason, an exact weight of 0.001 g of etacizine was weighed, which was dissolved in 95% ethanol solution in a 10 ml volumetric flask. The solution was brought to the mark with the same solvent.A working standard solution of 100 µl/ml was prepared from this solution. A sample of 1 µl was taken from the working solution using a micro-syringe and injected into the cylindrical cavity of the vapor-forming tape (evaporator) of the PII-N-S “Iskovich” apparatus, and Termodesorbtion Surface-Ionization spectr was obtained.

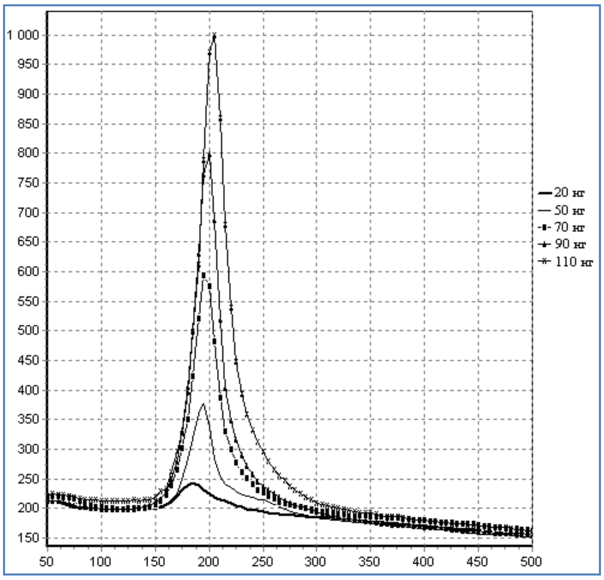


Figure 4. TDSI-spectrum of ethacizin’s various solutions

At a temperature of ~198 ± 5°C, the appearance of characteristic peaks for etacizine was observed. The spectrum of etacizine is shown in Figure 5. Standard etacizinesolutions containing 20, 50, 70, 90 and 110 ng/ml were prepared for quantitative determination. A sample of 1 μl was taken from these solutions, which were sequentially introduced into the evaporator. Then the surface ionization spectra of the studied samples were taken. The obtained spectra were recorded as standards in a computer data bank. The sensitivity of the method is in the range of 1·10-7. Quantitative determination was carried out according to a calibration graph based on the exact concentration of the standard nominal solution. The results of the analysis are shown in Figure 5 and Table 3.

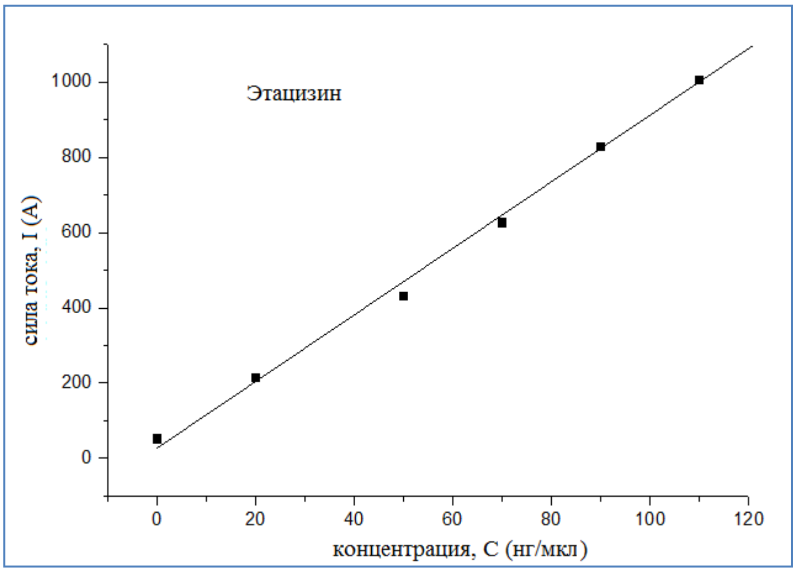


Figure 5. Calibration curve for quantitative analysis of Ethacizin

Table 3.

Results of quantitative analysis of Ethacizin via TDSIS methodology

C (x) Concentration, ng/ µl	I (y) Current intensity
0	50
20	212
50	430
70	625
90	827
110	1005

Method of isolation of ethacizin from biological fluids

For this purpose, the blood and urine of volunteers were used as objects. First, the experiments were carried out on model samples and then the developed method of analysis was tested on the biofluids of volunteers who took etacizine. [15].

To analyze an object containing etacizine, 5 ml of blood was taken and placed in a 50 ml conical flask while adding 5 ml of acetate buffer solution and 5 ml of saturated sodium chloride. After wards solution alkalinized with 25% ammonium hydroxide to create pH 8,0-9,0 was added. Then extraction proceeded with chloroform three times. Chloroform-water layer is centrifuged. Then chloroformed extracts were combined and evaporated till the appearance of dry residue which was analyzed by methods of TLC. In addition, the chloroformed extracts were cleaned by TLC method. Zone of substance accumulation was eluted with ethanol and elute was investigated by the methods of UV spectroscopy and TDSI spectroscopy.

Table 4.

Results of quantitative determination of etacizin in blood

AddedEtacizine, mg	Determined in blood, mg/ml	Determinedintheblood, %	Metrologicalcharacteristics
0,5	0,334	66,8	X_{cp}= 0,3482; S_2=0.0001 S=0.0116; S_x=0.0052 T=5.84 Eps=19,4294% Eps_cp=8,6891%
0,5	0,357	71,4	
0,5	0,348	69,6	
0,5	0,340	68,0	
0,5	0,362	72,4	

Method for isolating etacizin from urine

A model mixture of urine (10 ml) containing a certain amount of etacizine is placed in a 50 ml conical flask , and 5 ml of acetate buffer solution, 5 ml of saturated sodium chloride solution was added. Similarly, solution alkalinized with 25% ammonium hydroxide to create pH 8,0-9,0 was added, and extracted with chloroform three times (10 ml each). The mixture is transferred to a separatory funnel and left to separate the layers. The organic solvent layer is passed through a filter containing 3.0 g of anhydrous sodium sulfate. The chloroform extracts are combined, and the organic solvent is distilled off in a rotary vacuum evaporator at 400C to a dry residue. The dry residue is dissolved in 5.0 ml of ethanol and analyzed. The chloroformed extracts were cleaned by TLC method. Zone of substance accumulation was eluted with ethanol and elute was investigated by the methods of UV spectroscopy and TDSI spectroscopy.

Table 5.

Results of quantitative determination of etacizin in urine

AddedEtacizine, mg	Determined in blood, mg/ml	Determinedintheblood, %	Metrologicalcharacteristics
0,5	0,372	74,4	X_{cp}= 0,3808; S_2=0.0001 S=0.0114; S_x=0.0051 T=5.84 Eps=17.5329% Eps_cp=7,8410%
0,5	0,384	76,8	
0,5	0,398	79,6	
0,5	0,369	73,8	
0,5	0,381	76,2	

Conclusion

In order to detect ethacizin in biological samples TDSIS method was used. TDSI-spectrum illustrates that ethacizin had peaked at ~198±5˚C (sensitivity 0,5µg/ml). The calibration curve for ethacizin was prepared in the concentration range of 10-110 ng/µl. The wavelength maximum for ethacizin was found to be 267 nm. The results were compliant with Beer's law in the concentration range of 3-30 µg/ml.

The method of determination of ethacizininbiologicalfluids has been worked out. The optional conditions of ethacizin have been investigated for TLC, TDSI- and UV- spectroscopy, which are very accurate and sensitive for determination of ethacizin. The offered methods have been approved as a useful at the analysis of blood and urine volunteers and recommended for the worked in the chemical laboratory of Forensic medicine department.

References

[1]. Tsaregorodtsev D.A., Okisheva E.A., Gracheva E.I., Sedov A.V., Sulimov V.A. A new approach to evaluating the effectiveness and safety of etacizine in patients without structural pathology of the heart // Cardiology and cardiovascular surgery, 2, 2014-pp.89-96

[2]. Effect of the antiarrhythmic agent moricizine on survival after myocardial infarction. The Cardiac Arrhythmia Suppression Trial II Investigators. N Engl J Med 1992; 327: 4: 227—233.

[3]. Radzevich A.E., KonyakhinA.Yu., Kameneva T.R. and others. The place of etacizine in the therapy of atrial fibrillation.// Cardiology.-2005; 1: 206—213.

[4]. Kukes V.G. Undesirable effects of medicines.- M.:Publishing house "Russian doctor", 2006.- 262 p.

[5]. Fang M.C. et al. National trends in antiarrhythmics and antithrombotic medications use in atrial fibrillation. Arch. Intern. Med., 2004; 164: 55–60.

[6]. Randall C. Baselt.Disposition of toxic drugs and chemicals in man.-2002. Biomedicalpublications, California, USA.-P. 84-85.

[7]. Prokofieva V.I., Chernova S.V., Kashtanova V.V., Shavratsky V.H., Gneushev E.I. The use of high-performance chromatography in the evaluation of etacizine purity // Chemico-pharmaceutical Journal, 1990. -No.4.-pp. 82-83.

[8]. Vasina V.V., Avdyunina N.I., Pyatin B.M., Arzamassev A.P. The influence of various factors on the stability and quality of etacizine // Chemico-pharmaceutical Journal, 1987. No.2. — pp. 226-228.

[9]. Comparative study of the experimental pharmacokinetics of etacizine, etmosine and etacizine // Chemico-pharmaceutical Journal, 2001.-No.6.-pp. 5-9.

[10]. Alikhodjaeva M.I., AtakhanovA.Sh., Jalilov F.S. Determination of some antiarrhythmic drugs by chromatographic screening methods // Universum: chemistry and biology : electron. scientific Journal 2024. 1(115).

[11]. Alikhodjaeva.M.I., Ikramov L.T. // Pharmacy, 1990. No. 1. pp. 80-81.

[12]. Alikhodjaeva, M., Atahanov, A., &Xamdamov, M. (2015). Development of Methodology for the Isolation and Determination of Propafenone in Blood Samples via HPLC. Journal of US-China Medical Science, 12, 163-171.

[13]. Yunuskhodzhaev, A. N., Shoislomov, B. Sh., &Alikhodzhaeva, M. I. (2014). Complications of drug therapy. Pharmaceutical Journal, (3), 365.

[14]. Alikhodjaeva M. I. Investigation of some synthetic diuretics by thermodesorption surface ionization spectroscopy //Issues of biological, medical and pharmaceutical chemistry. - 2009. – No. 1. – pp. 61-63.

[15]. Alikhodjaeva M. I. et al. Chromato-mass spectrometric determination of certain diuretics in biological fluids //Forensic medical examination. - 2009. – vol. 52. – No. 5. – pp. 27-29.

[16]. Batirov, Z., Toirov, I., Boymuratov, F., & Sharipov, S. (2021). Layered application of mineral fertilizers with the coulter ripper of a combined unit. In *IOP Conference Series: Materials Science and Engineering* (Vol. 1030, No. 1, p. 012168). IOP Publishing.

[17]. Mamatov, F., Mirzaev, B., Batirov, Z., Toshtemirov, S., Tursunov, O., & Bobojonov, L. (2020, July). Justification of machine parameters for ridge forming with simultaneous application of fertilizers. In *IOP Conference Series: Materials Science and Engineering* (Vol. 883, No. 1, p. 012165). IOP Publishing.

[18]. Khamidov, M., & Khamraev, K. (2020, July). Water-saving irrigation technologies for cotton in the conditions of global climate change and lack of water resources. In *IOP Conference Series: Materials Science and Engineering* (Vol. 883, No. 1, p. 012077). IOP Publishing.

[19]. Abdurakhmanov, U., Boitmuratov, F. T., Mukhamedov, G. I., Fionov, A. S., & Yurkov, G. Y. (2010). Electric conductivity of composite materials based on phenylon matrices and nickel particles. *Journal of Communications Technology and Electronics*, *55*, 221-224.

[20]. Maaroufi, A., Haboubi, K., El Amarti, A., & Carmona, F. (2004). Electrical resistivity of polymeric matrix loaded with nickel and cobalt powders. *Journal of Materials Science*, *39*, 265-270.