DEVELOPMENT OF METHODS OF ANALYTICAL DIAGNOSTICS OF LAMOTRIGINE POISONING

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Introduction. Epilepsy is one of the most common diseases of the nervous system with more than 40 million people worldwide. Population studies conducted in developed countries have shown that this disease is annually detected in 40-70 people per 100,000 population, and the incidence rates with a life expectancy of up to 3%. Today, about 100,000 patients with a diagnosis of epilepsy are registered in Ukraine, and the actual picture is no less than 500,000 with its manifestations.

For the treatment of epilepsy a wide group of anticonvulsants has been used. The specificity of pharmacotherapy of epilepsy is the long-term treatment of patients, sometimes throughout life, which causes a high likelihood of creating a toxicological situation. According to FDA and patientsville.com websites, more than 30 countries have reported lethal poisoning as a result of lamotrigine, which is one of the most common anticonvulsant medicines.

Aim. The aim of this work is to develop methods for analytical diagnosis of acute lamotrigine poisoning.

Materials and methods. For the development of optimal conditions for isolation of lamotrigine from biological objects model samples of fresh pig liver (20 g) were prepared, which saturated 10 mg of lamotrigine.

Isolation of lamotrigine from a biological matrix was performed with acetonitrile, acidified with HCl. For this, 20 g of ground pork liver, pre-saturated for 24 h with ethanol solution of lamotrigine (10 mg), was placed in a flask, was added 50 ml of acetonitrile, acidified with 6 M HCl solution to pH 2.0-2.5, insisted for 30 min and filtered. The resulting extract was basified with 30% NaOH to pH = 9 and extracted with chloroform. Then it was evaporated to dryness, dissolved in 10 ml of ethanol and examined.

The identification and quantification of lamotrigine was performed by HPLC method with UV detection on liquid chromatograph "Milichrome-A-02" (Ekonova, Novosibirsk). For separation of substances, a Prontosil-120-5-C18-AQ reversed-phase column of size Ø2×75 mm, a grain size of 5 μm (Bischoff Analysetechnik und Geräte GmbH, Germany) was used. The speed of the mobile phase is 100 μl/min. The temperature of the column thermostat is 35 °C. UV spectrophotometric detection is carried out simultaneously for 8 wavelengths of 210-300 nm. Chromatogram analysis and processing were performed using the Analytics-Chrom program.

Results and discussion. To develop the conditions for the identification of lamotrigine in the HPLC extractions obtained, its basic chromatographic parameters were investigated under the above conditions (Table 1).

Table 1. Results by definition of major chromatographic parameters of lamotrigine

Parameter	Results		
	x_i	$\bar{x}$	RSD, %
Retention time (t_R), min	10.10	10.10	0.14
	10.01		
	10.19		
Retention volume (V_R), μl	998.0	1000	0.13
	1004.0		
	1001.0		

The results obtained indicate that the proposed conditions can be used to identify and quantify the toxicant in the extracts obtained from biological objects.

In order to develop a method for the quantitative determination of lamotrigine, a series of solutions with different concentrations were prepared. Further, the peak area of each sample was determined and a plot of the peak area versus concentration was plotted. Chromatography of the test solutions was performed under the above conditions. A series of ethanol solutions of lamotrigine with concentrations of 0.1–20.0 μg/ml were prepared to construct the calibration graph. The tested ethanol solutions were chromatographed three times. The sample volume was 20 μl. The linearity of the constructed calibration graph in the coordinates (S, units) – (C, μg/ml) was observed in the interval of 0.1–20.0 μg/ml. The linear regression method shows the equation of the calibration dependence of the peak area (y) on the concentration (x) of the general form: y = *b*x+*a*. Metrological characteristics are given in table 2.

Table 2. Metrological characteristics of the calibration dependence of the lamotrigine peak area on the concentration

r	b	a	S_b	S_a	Δb	Δa
0.9996	0.010228	0.001219	$9.66272 \cdot 10^{-5}$	0.000749	0.001951	0.000249

Based on the data obtained, the equation of the calibration dependence is as follows:

$$S = 0.0102\ C + 0.0012$$

where S – peak area, units; C – concentration of the substance, μg/ml.

The proposed conditions have been used in studies on the identification and quantification of lamotrigine in extractions from biological objects. It was found that up to 84% of the toxicant can be isolated by isolating lamotrigine from liver tissues with acidified acetonitrile.

Conclusions. Thus, the developed methods of isolation, identification and quantification make it possible to reliably identify and quantify lamotrigine in extractions from biological objects, which can be used in chemical-toxicological research in drug poisoning.