Pharmaceutical Chemistry Journal, Vol. 44, No. 12, March, 2011 (Russian Original Vol. 44, No. 12, December, 2010)

DEVELOPMENT OF A CHROMATOGRAPHIC METHOD FOR QUANTITATIVE ANALYSIS OF PHENIBUT IN BIOLOGICAL SAMPLES

I. N. Tyurenkov,[1,*] V. N. Perfilova,[2] L. A. Smirnova,[2] A. F. Ryabukha,[2] E. A. Suchkov,[2] and S. A. Lebedeva[2]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 44, No. 12, pp. 53 – 55, December, 2010.

Original article submitted March 19, 2009.

An ion-pair chromatography method is developed using a reversed-phase C18 column with UV detection for the quantitative analysis of phenibut. The proposed method possesses sufficient selectivity and high sensitivity that enable its effective use during pharmacokinetic studies of phenibut in addition to other GABA derivatives in biological samples.

Key words: phenibut, quantitative analysis, HPLC.

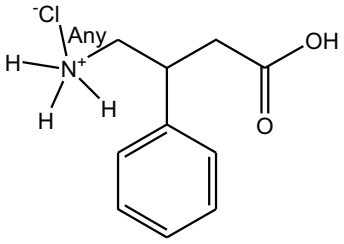
Biological fluids are complicated samples to analyze because they are multi-component mixtures that include a large number of inorganic and organic compounds of various chemical structures. Therefore, the selection of the method for analyzing biopharmaceuticals is very important in pharmacokinetic studies. The selected method should be highly sensitive and capable of working with small sample volumes. It should be very specific, selective, reliable, reproducible, and general [1 – 3]. HPLC methods, which are some of the principal analytical methods for carrying out pharmacokinetic studies, satisfy these requirements [4].

Existing methods for quantitative analysis of phenibut in biological fluids were developed using GC at the end of the 1980s [5]. They have several serious drawbacks typical of this analytical method such as a high analytical temperature, an unstable stationary phase, and out-dated equipment. The existing methods also cannot be adapted to modern analytical standards.

We developed an HPLC method using diode-array detection in order to carry out pharmacokinetic studies of phenibut and other GABA derivatives. The use of a diode-array detector makes it possible to record the full absorption spectrum at each time point. This enables a conclusion to be made quickly regarding the optimum detection wavelength for each peak in the chromatogram, simplifies the identification of the sample components, and increases the selectivity or reduces the detection limit.

[1] Volgograd State Medical University, Volgograd, Russia.
[2] Research Institute of Pharmacology, Volgograd State University, Volgograd, Russia;
[*] e-mail: fibfuv@mail.ru

EXPERIMENTAL PART



The phenibut content in biological fluids was determined by HPLC on a Shimadzu (Japan) liquid chromatograph with a diode-array detector and Supelcosil LC-18 column (5 μm, 100 mm × 4.6 mm).

The mobile phase consisted of acetonitrile (UV 210, Russia) and a buffer consisting of monobasic potassium phosphate (50 mM, pH 2.7, Russia). The capacity coefficient of phenibut was increased by adding to the eluent the ion-pair reagent sodium heptanesulfonate (0.12%).

Phenibut drug substance was detected at wavelength 205 nm. The sensitivity of the method was 1 μg/mL; retention time, 12 – 14 min. The compound was identified and the concentrations calculated using the absolute standard method [6, 7].

Phenibut was extracted and proteins were simultaneously precipitated from biological samples of plasma, serum and whole blood in addition to aqueous homogenates of organs (20%) and rat tissues (10%) using TFA in a 1:0.5 ratio.

Samples were irradiated for 10 min in an ultrasonic bath to precipitate proteins and were centrifuged for 15 min at 3,000 rpm on an Eppendorf centrifuge (Germany). Then, the

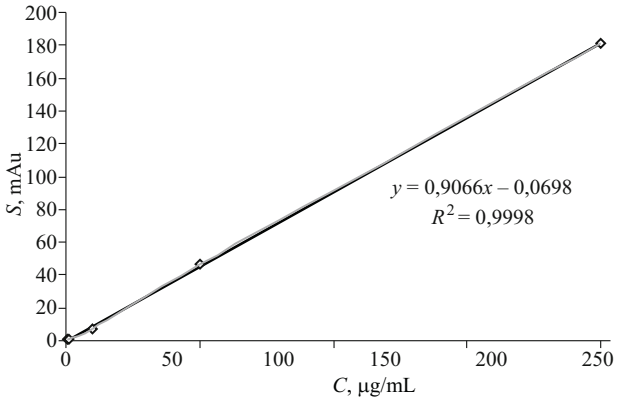


Fig. 1. Area (S) under chromatographic peak as a function of phenibut concentration (C).

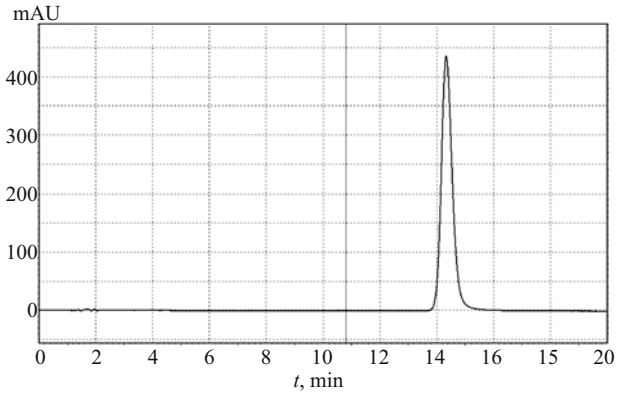


Fig. 2. Chromatogram of phenibut in aqueous solution.

supernatant liquid was collected and injected into a 20-μL loop. The degree of extraction of the test compound was 90%.

RESULTS AND DISCUSSION

The absolute standard method was used for quantitative analysis of the compound. The peak areas as functions of phenibut concentration were analyzed by regression analysis in the concentration range 1 – 200 μg/mL. It was found that the calibration curves were linear with regression coefficient (R^2) 0.99 (Fig. 1). The daily percent variations (repeatability of the method) were determined and were less than 20% in the studied concentration range. The day-to-day percent variations (reproducibility of the method) for the test compound were generally less than 10% (Table 1).

The average absolute percent variations upon repeated analysis after storing aqueous solutions of the compound for 72 h were within the same limits, indicating that the studied compound was stable. A study of the effect of freeze/thaw cycles showed that the average absolute percent variations for phenibut were within the same limits. This indicated that the compound was stable under the influence of these factors. The sensitivity of the method for the test compound was 1 μg/mL. The average measurement error was generally less than 10%.

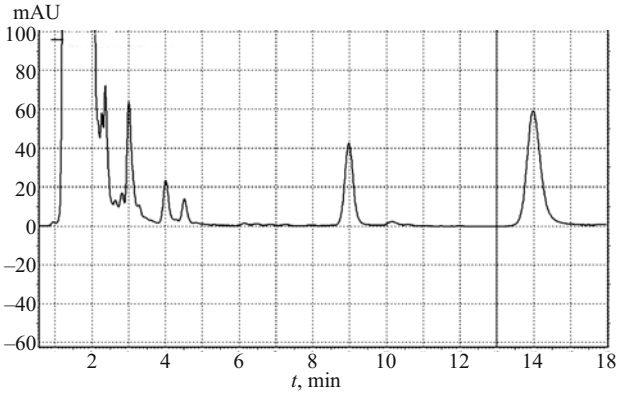


Fig. 3. Chromatogram of phenibut in rat blood plasma.

Figures 2 and 3 show chromatograms of phenibut in aqueous solution and blood plasma.

Thus, an ion-pair chromatography method using a C-18 reversed-phase column and UV detector was developed for analysis of phenibut drug substance. It was sufficiently sensitive and selective for use with biological samples.

The optimum method for phenibut extraction was selected and had practically no effect on the average measurement error of the chromatographic quantitative analysis method.

TABLE 1. Reproducibility and Repeatability Parameters for HPLC Quantitative Analysis Method for Phenibut in the Linear Range of Chromatographic Peak Area vs. Solution Concentration ($M \pm m$)

Concentration, μg/mL	Daily concentration variations, μg/mL			Repeatability, ± Δ%			Reproducibility (measurement error), %
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	
1	0.99 ± 0.13	0.99 ± 0.09	0.83 ± 0.11	–1	–1	–17	6.33
10	8.25 ± 1.37	8.12 ± 1.27	7.36 ± 1.03	–17.5	–18.8	–26.4	20.9
50	51.96 ± 7.04	51.99 ± 6.75	53.91 ± 2.8	3.92	3.98	7.82	5.24
200	199.59 ± 17.06	202.53 ± 21.43	199.15 ± 20.84	–0.21	1.27	–0.43	0.64

Thus, the developed method for quantitative analysis was highly selective and sensitive. This enabled it to be used effectively to study the pharmacokinetics of phenibut and other GABA derivatives in biological samples.

REFERENCES

1. A. Henschen, K.-P. Hupe, F. Lottspeich, W. Voelter, et al., *High Performance Liquid Chromatography in Biochemistry*, VCH, Weinheim, Deerfield Beach, Florida (1985).
2. K. E. Geckeler and H. Eckstein, *Analytische und preparative Labormethoden*, Vieweg, Braunschweig (1987).
3. *Determination of Chemical Compounds in Biological Media* [in Russian], MUK 4.1.763-4. 1779-99 (1999).
4. *USSR State Pharmacopoeia*, XIth Ed., Meditsina, Moscow (1987).
5. N. Karkishchenko, V. Khoron'ko, S. Sergeeva, and V. Karkishchenko, *Pharmacokinetics* [in Russian], Feniks, Rostov-on-Don (2001).
6. K. Doerffel, *Statistik in der analytischen Chemie*, Deutscher Verlag fur Grundstoffindustrie, Leipzig (1990).
7. W. Rolf, *J. Chromatogr. B*, **729**, 43 – 53 (1999)