

NEW MODIFIED RELEASE TABLETS WITH PENTOXIFYLLINE BASED ON LIPOPHILIC MATRIX. NOTE 2. STABILITY STUDY OF THE ACTIVE SUBSTANCE FROM THE PROPOSED FORMULATIONS

ELEONORA MIRCIA^{1*}, SILVIA IMRE², EMESE SIPOS³, VERONICA AVRIGEANU¹, GABRIEL HANCU⁴, BARNA IANTOVICS⁵, TEODORA BALACI⁶

University of Medicine and Pharmacy Târgu-Mureș, Faculty of Pharmacy, Gh. Marinescu 38, 540139, Târgu-Mureș, Romania

¹*Organic Chemistry Department*

²*Drug Analysis Department*

³*Pharmaceutical Technology Department*

⁴*Pharmaceutical Chemistry Department*

⁵*“Petru Maior” University, Faculty of Science and Letters, Department of Mathematics and Informatics, Târgu-Mureș, Romania*

⁶*University of Medicine and Pharmacy “Carol Davila”, Faculty of Pharmacy, Pharmaceutical Technology Department, Bucharest, Romania*

**corresponding author: nmircia@yahoo.com*

Abstract

The objective of this study was to test the stability of pentoxifylline in experimental formulations based on lipophilic matrix.

In order to achieve this, we studied the influence of heat stress on the stability of the proposed formulations, by keeping the product samples at high temperatures and humidity, in a stability chamber, during periods lasting from one to six months, sampling at different periods of time and then analyzing the test samples.

Experimental results show that all the proposed formulations can be considered stable.

Rezumat

Obiectivul acestui studiu, l-a constituit testarea stabilității pentoxifilinei din formulările experimentale pe bază de matriță lipofilă.

În acest sens, s-a studiat influența stresului termic asupra stabilității formulărilor propuse, prin păstrarea unor mostre de produs la valori de temperatură și umiditate mai ridicate, în camera de stabilitate, timp de 1-6 luni. S-au prelevat probe la diferite perioade de timp, acestea fiind analizate.

Rezultatele obținute experimental indică faptul că toate formulările analizate prezintă stabilitate optimă.

Keywords: pentoxifylline, lipophilic matrix, stability

Introduction

Pentoxifylline is a xanthine derivative, which improves blood flow in the microcirculation [7].

Our objective was to investigate the stability of pentoxifylline from the proposed formulations and its compatibility with the excipients used to obtain the lipophilic molds. To achieve this goal, the modified release formulated tablets were submitted to heat stress [8], by keeping samples at high temperatures [11], in a stability chamber, for periods from one to six months, sampling at different periods of times and then analyzing the samples by applying a quantitative determination method for the active substance [10] or for its degradation products.

As lipophilic matrix former we used Precirol AT 05 [1,3]. The controlled release tablets with pentoxifylline in the hydrophobic molds contained different concentrations of Precirol (10%, 15%, 20%, 25%, 30% and 0%) and were obtained by granulation followed by compression of the granulated. The studied formulations were kept in the stability chamber at a temperature of $40\pm 2^{\circ}\text{C}$ and at a relative humidity of $75\pm 5\%$. At different time intervals (one, three, six months), samples were prelevated to accomplish the dissolution test and the assay of pentoxifylline.

Materials and Methods

For the formulation of tablets we used pentoxifylline pure substance (Merck). Tablets were formulated with 400 mg pentoxifylline and contained different ratios of Precirol AT (Table I).

Table I

The composition of the formulations with pentoxifylline incorporated into lipophilic matrix

Ingredients (mg)	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆
Pentoxifylline	400.00	400.00	400.00	400.00	400.00	400.00
Precirol AT 05	80.00	120.00	160.00	200.00	240.00	-
Compritol	50.00	50.00	50.00	50.00	50.00	50.00
Lactose	270.00	230.00	190.00	150.00	110.00	350.00

During the assay we used: acetonitrile (Merck), purified water (Direct Q-5 Millipore, Millipore SA, Molsheim, France). All the materials were of analytical grade.

The following equipments were used for the stability tests: Sanyo stability chamber. Testing conditions: temperature: $40\pm 2^{\circ}\text{C}$, relative

humidity 75 ± 5 %. For the dissolution test we used: AB 54S balance (Mettler-Toledo, Switzerland), T 700H ultrasound bath (Elma Transsonic), Agilent 1100 HPLC system (Agilent, USA) with quaternary pump, automatic injector, thermostat column. Water purifying system: Direct Q-5 (Millipore, Millipore SA, Molsheim, France).

Methods

To determine the stability of the prepared formulations, we performed the dissolution test and the assay of pentoxifylline [2,13].

In vitro dissolution studies: The test was carried out regarding the stipulations of USP [14,15]: we used the apparatus nr.2 (with paddles), as dissolution medium 1000 mL water [13], temperature $37 \pm 0.5^\circ\text{C}$, rotation speed - 50 rpm. The tests were carried out on 6 tablets for each of the studied formulations ($n = 6$) [2,6,12]. For the assay of pentoxifylline released from the pharmaceutical form, in each experiment 0.2 mL solution samples were prelevated at intervals of 20, 30, 60 minutes in the first hour; from 30 to 30 minutes for the next three hours and then from hour to hour [4,9]. The tests were performed for a period of 8 hours. The volume of solution taken was replaced with an equivalent volume of distilled water to maintain a constant volume of the dissolution medium. Samples (0.2 mL) were diluted with distilled water to 10 mL. Pentoxifylline was determined by UV spectrophotometric analysis at 274 nm using as blank distilled water.

Quantitative determination: We developed and applied a method for the quantitative determination of the active substance from the analyzed lipophilic molds. We used: an Extend C18 column [5], 250mm X 46 mm; 5 μm (Zorbax, Agilent Technologies, USA); a mobile phase containing water (A) and acetonitrile (B), A: B = 70: 30 (V/V) [10]. Before use, the mobile phase was degassed on an ultrasound bath and filtered through a 0.45 μm Millipore filter. The mobile phase flow rate was 1 mL/min. Separation occurred at 30°C , at a wavelength of 280 nm. The injection volume was 10 μL . The analysis time was five minutes.

Preparation of standard solutions: For the quantitative determination of pentoxifylline from the formulated tablets, we chose the external standard method. Every day we prepared a pentoxifylline stock solution in water containing 200 $\mu\text{g/mL}$ pentoxifylline, by dissolving on an ultrasound bath 10 mg pentoxifylline in 25 mL distilled water, in a 50 mL calibrated flask and completing to volume with distilled water. From the stock solution, we prepared five standard working solutions in water with a concentration of 10 $\mu\text{g/mL}$ by diluting in a calibrated flask 0.5 mL stock solution to 10 mL with

distilled water. The calibration standard solution was prepared five times in each analysis day.

Preparation of the test solutions: The determinations for each formulation were performed on a number of five tablets. The interpretation were made taking in consideration the stipulation of British Pharmacopoeia 2005 [12], which establishes limits of acceptance regarding the active substance content depending on the different number of tablets we worked on.

Five tablets were weight, the average mass of each was calculated and the mixture was pulverized and homogenized. 20 mg of powder (equivalent to 10 mg pentoxifylline) were brought quantitatively in a 100 mL flask and were stirred up with 50 mL water on an ultrasound bath for 10 minutes. After completing the volume with water (100 µg/mL pentoxifylline concentration), the samples were filtered through a 0.45 µm Millipore filter, after that 1mL of the filtered solution was transferred in a 10 mL flask and was completed with water to 10 mL.

Pentoxifylline extraction was achieved in the same way for all the studied formulations, working with five tablets. To verify the interferences caused by excipients, blank tablets (without active substance) were prepared corresponding to the studied formulations. Blank tablets were prepared and extracted in the same way as the analyzed ones. A 10 µg/mL concentration solution in water was obtained.

Results and Discussion

The dissolution tests results from samples taken after one month, three months and six months are presented in table II.

The quantitative determination of pentoxifylline from modified release tablets subjected to thermal stress was made after sampling at one month, three and respectively six months. The calculations were made by comparing the peak areas obtained from the sample solutions with the ones of the standard solutions, every day of the analysis. The values obtained at the standard solutions analysis are mentioned in table III and the results obtained following the quantitative determination of pentoxifylline from the modified release tablets studied in table IV.

Table II
Pentoxifylline quantities (%) dissolved from the lipophilic matrix after one, three and six months

No.	Time (minutes)	F ₁			F ₂			F ₃			F ₄			F ₅			F ₆		
		1 month	3 months	6 months	1 month	3 months	6 months	1 month	3 months	6 months	1 month	3 months	6 months	1 month	3 months	6 months	1 month	3 months	6 months
1.	20	5.01	4.99	4.97	5.12	5.11	5.10	5.09	5.08	5.06	5.03	5.02	5.01	5.54	5.53	5.51	42.52	42.51	42.50
2.	30	9.80	9.78	9.77	9.70	9.70	9.68	9.65	9.63	9.60	9.42	9.40	9.38	8.92	8.91	8.90	56.25	56.22	56.21
3.	60	14.60	14.58	14.56	15.30	15.28	15.26	15.90	15.88	15.86	13.05	13.04	13.02	12.98	12.96	12.94	80.13	80.11	80.10
4.	90	26.30	26.29	26.28	28.25	28.23	28.22	25.30	25.29	25.27	20.85	20.83	20.80	19.30	19.30	19.28	90.10	90.10	90.08
5.	120	31.12	31.11	31.09	32.12	32.10	32.09	33.20	33.19	33.18	31.56	31.55	31.53	30.01	30.00	30.00	97.13	97.10	97.09
6.	150	44.20	44.20	44.18	42.20	42.17	42.15	41.30	41.28	41.25	39.83	39.81	39.80	38.05	38.03	38.01	98.90	98.88	98.86
7.	180	48.30	48.28	48.26	48.02	48.01	48.01	47.90	47.88	47.87	47.28	47.26	47.24	45.21	45.20	45.19	99.92	99.91	99.90
8.	240	59.22	59.21	59.20	58.15	58.13	58.10	57.25	57.24	57.22	50.80	50.78	50.75	48.31	48.30	48.28	99.93	99.92	99.91
9.	360	69.50	69.48	69.45	64.13	64.10	64.08	65.10	65.08	65.07	66.07	66.07	66.06	60.12	60.11	60.10	99.98	99.96	99.94
10.	480	70.25	70.24	70.22	70.80	70.78	70.75	71.35	71.33	71.31	72.51	72.50	72.47	78.22	78.21	78.20	99.99	99.97	99.96

Table III
Standard solution analysis prepared for pentoxifylline quantitative determination after maintaining for different periods under heat stress conditions

No.	Period of time (months)	Standard concentration [$\mu\text{g/mL}$]	Medium area $\pm$ SD CV%, n = 5
1.	1	10.30	206.90 $\pm$ 0.23, 0.11
2.	3	10.00	176.84 $\pm$ 0.33, 0.20
3.	6	10.00	176.80 $\pm$ 0.33, 0.19

Table IV
Results obtained following the quantitative determination of pentoxifylline (PTX) from modified release tablets after one month, three and six months

No.	Formulation	0 month		1 month		3 months		6 months	
		Found concentration [mg/tablet]	PTX quantity (%)	Found concentration [mg/tablet]	PTX quantity (%)	Found concentration [mg/tablet]	PTX quantity (%)	Found concentration [mg/tablet]	PTX quantity (%)
1.	F ₁	373.44	93.36	373.44	93.36	373.19	93.30	373.10	93.27
2.	F ₂	369.96	92.49	369.96	92.49	369.66	92.41	369.43	92.35
3.	F ₃	400.87	100.21	400.87	100.21	400.64	100.16	400.34	100.08
4.	F ₄	359.97	89.99	359.97	89.99	359.67	89.91	359.50	89.87
5.	F ₅	365.82	91.45	365.82	91.45	365.75	91.43	365.63	91.40
6.	F ₆	370.86	92.71	370.86	92.71	370.00	92.50	369.98	92.49

The quantities of pentoxifylline dissolved at different sampling times remained constant after one month of storage in heat stress conditions and their values are identical to those obtained immediately after the formulation of tablets. The values obtained after three and six months have very small variations in the pentoxifylline quantities of dissolved samples.

The data analysis contained in table IV shows that for the same pharmaceutical formulation, the absolute differences registered at the quantitative determination in time are less than 0.23%.

The concentrations and percentages of pentoxifylline are between the accepted limits stipulated in the pharmacopoeias, which is between 356.8-444mg (89.2% -111%), $n = 5$ tablets.

Based on the values obtained experimentally, we can conclude that in terms of the quantities of pentoxifylline dissolved at different times of sampling the formulations studied and kept in the heat stress at $40 \pm 2^\circ\text{C}$ and relative humidity $75 \pm 5\%$ for six months are stable.

Conclusions

To determine the analyzed tablets stability, *in vitro* dissolution studies were performed. The experimental data show that the pentoxifylline quantities dissolved at different sampling times remained constant after one month storage under heat stress conditions and exhibited the same values to those obtained immediately after formulation. The values obtained after three respectively six months exhibited very small variations of the pentoxifylline quantities dissolved from the analyzed samples.

For the quantitative determination of the active substance from the studied modified release tablets we have developed a high performance liquid chromatography method (HPLC). The proposed method is simple and allows the rapid determination of pentoxifylline from modified release tablets.

The present stability study carried out over a period of six months under heat stress conditions, showed after three respectively six months an insignificant decrease of pentoxifylline concentration in the studied formulations. The experimental values are within the values accepted by Pharmacopoeias [12-15], in the case where a limited number of pharmaceutical units are used, which indicates the stability of the proposed formulations.

References

1. Mircioiu C., Ionica G., Danilceac A., Miron D., Mircioiu I., Radulescu F., Pharmacokinetic and mathematical outliers for drugs with active metabolites. Note I. Model independent analyses for pentoxifylline, *Farmacia*, 2010, 58(3), 264-278

2. Byndu U., Pandiau K., Narayanan S.R., Determination of pentoxifylline in pharmaceutical preparations, *Pharmazie*, 1993, 48: 621-622.
3. Madgulkar A.R., Bhalekar M. et al., Compritol and Precirol: innovative pharmaceutical excipients, *Asian J. Chem.*, 2007, 19(1): 454-458.
4. Mircia E., Contribuții la cedarea retard a pentoxifilinei din forme farmaceutice solide, Teză de doctorat, U.M.F. Tg. Mureș, 2007.
5. Musch G., Hamoir T., Massart D.L., Determination of pentoxifylline (oxpentifylline) and its 5-hydroxy-metabolite in human plasma by solid-phase extraction and high-performance liquid chromatography with ultraviolet detection, *J. Chromatog. Biomed. Appl.*, 1989, 87: 368-374.
6. Özsoy Y., Tunçel T., Terzioğlu N., Özkirimli S., Formulation and *in vitro* release of pentoxifylline lipophilic matrix tablets, Proc. 4th World Meeting of APGI/APV, Florence, April 2002, Summary book, 2002, 105-106.
7. Prasad K., Lee P., Suppression of hypercholesterolemic atherosclerosis by pentoxifylline and its mechanism, *Atherosclerosis*, 2007, 192(2): 313-322.
8. Tomuța I., Leucuța S.E., Influența tratamentului termic asupra cedării substanței medicamentoase din pelete acoperite cu Eudragit RS30D, *Revista de Medicină și Farmacie Tg.Mureș*, 2004. 50(supl.II): 121-124.
9. Popa G., Ochiuz L., Stoleriu I., Popovici I., Binder and superdisintegrant influence on the properties of orally disintegrating high doses acetaminophen tablets, *Farmacia*, 2010, 58(3), 303-307.
10. Vrečer F., Kramar A., Study of pentoxifylline release from matrix tablets cellulose ethers, Proc. 1st World Meeting of APGI/APV, Budapest, May 1995, Summary book, 1995, 249-250.
11. Yuen K.H., Wong J.W., Peh K.K., Julianto T., Choy W.P., Comparative bioavailability study of two controlled-release pentoxifylline tablet preparations, *Drug. Dev. Ind. Pharm.*, 2000, 26(7): 803-807.
12. ^{xxx}British Pharmacopoeia, HMSO Publication, London, 2005.
13. ^{xxx}Farmacopeea Română, ediția a X-a, Editura Medicală, București, 1993.
14. ^{xxx}United States Pharmacopeia, USP 24, The United States Pharmacopeia Convention Inc., 1997.
15. ^{xxx}United States Pharmacopeia, USP 27, United States Pharmacopeia, Convention Inc., Rockville Md., 2004.

Manuscript received: December 11th 2010