A New HPLC Method for the Separation and Quantitative Determination of Loratadine

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The development of a HPLC method for determination of an antihistaminic H1 drug, last generation, loratadine, in different samples (pharmaceuticals, biological environments etc.). The method involved liquid – phase extraction of loratadine using methanol as solvent. In isocratic conditions as follows: type-C8 chromatography column (Zorbax Eclipse XDB-C8, 4.6 mm x 150 mm, particle size 5 µm, the limit temperature is 60 ° C, pH = 2-9); flow rate: 1.5 mL/min; Column temperature: 27°C; injection volume: 10µL; 280 nm. For the drug, standard curve was linear from 0.5 to 9 µg/mL. The method was validated and applied on pharmaceutical dosage forms and on biological samples as well. It was obtained optimal results. Thus, it can be used in the control of synthetic process and determination of loratadine in drug substance, pharmaceutical preparation and biological samples.

Keywords: loratadine, HPLC, precise method

The pharmaceutical industry has strict regulations on activity development and control analytical laboratories. Thus, special attention is given to optimize and use methods of analysis and control of raw materials, intermediates and finished products.

Loratadine (Ethyl 4-(8–chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridine-11-ylidene)piperidine -1-carboxylate) is a H1 receptor antagonist of second generation [1].

The aim of this paper is to provide a chromatographic method for the separation and quantitative determination of loratadine, a second generation antihistamine with applications both pharmaceutical and biological medium [2-4].

It was devised a method using XDB-C8 column and mobile phase, phosphate buffer *p*H = 2.9 with acetonitrile in the ratio 15:11 (v/v), with detection at 280 nm. For this purpose were taken into account the following aspects: Setting the detection wavelength, the optimum solution concentration of loratadine, mobile phase composition effect, influence of mobile phase *p*H and influence of chromatographic column temperature. The method will be validated [5-13].

Experimental part

Material and method

Chromatographic conditions are: HPLC Agilent 1200 quaternary pump, DAD, thermostat, degassing system, autosampler; type-C8 chromatography column (Zorbax Eclipse XDB-C8 double related ends, 4.6 mm x 150 mm, particle size 5 µm, the limit temperature is 60 °C, *p*H = 2-9); flow rate: 1.5 mL/min; Column temperature: 27°C; injection volume: 10µL; mobile phase: acetonitrile / (an aqueous solution of ammonium dihydrogen phosphate 10 g/L was added to 5 mL of phosphoric acid): 11/15 (v/v); detection wavelength: 280 nm.

Were used loratadine in methanol standard solution (0.6 mg / 100 mL) and working solutions of loratadine in methanol (various concentrations)

Results and discussions

I. Establishing detection wavelength

It was considered a sample of standard solution of loratadine in order to determine the wavelength of detection. After equilibration with mobile phase of the chromatography column for 60 min, injections of 10 µL sample were conducted at different wavelengths in both the UV and in the visible range (fig. 1). The analysis of chromatograms (fig. 1) shows that at the wavelength λ = 280 nm, loratadine presents optimal height and peak area for the proposed analyzes.

II. The optimum concentration of the solution of loratadine

Were processed several dilutions of the standard solution of loratadine. From each diluted solution were injected 10 µL each in chromatographic conditions mentioned. Peak area for each determination was recorded.. Linearity was verified on the 0.5 to 12.0 µg / mL (fig. 2). It was found that it is respected up to the concentration of 9.0 µg / mL.

The method was validated in field 0.5 µg/mL – 9.0 µg/mL (fig. 3).

III. The effect of the composition of the mobile phase

Were recorded loratadine chromatograms of standard solutions, using a mixture of solvent A (aqueous solution of monobasic ammonium phosphate 10 mg / mL with *p*H adjusted to 2.9 with phosphoric acid) and solvent B (acetonitrile) as mobile phase. The ratio of solvent A and solvent B mixture varied according to table 1.

IV. Mobile phase preparation

One mobile phase was prepared for each mixing ratio: by dissolving ammonium dihydrogen phosphate in bidistilled water, the *p*H adjusted to the value 2.9 potentiometer with phosphoric acid, was filled to the mark with acetonitrile and was homogenized. The resulting mixture was held 10 min ultrasonic bath for degassing.

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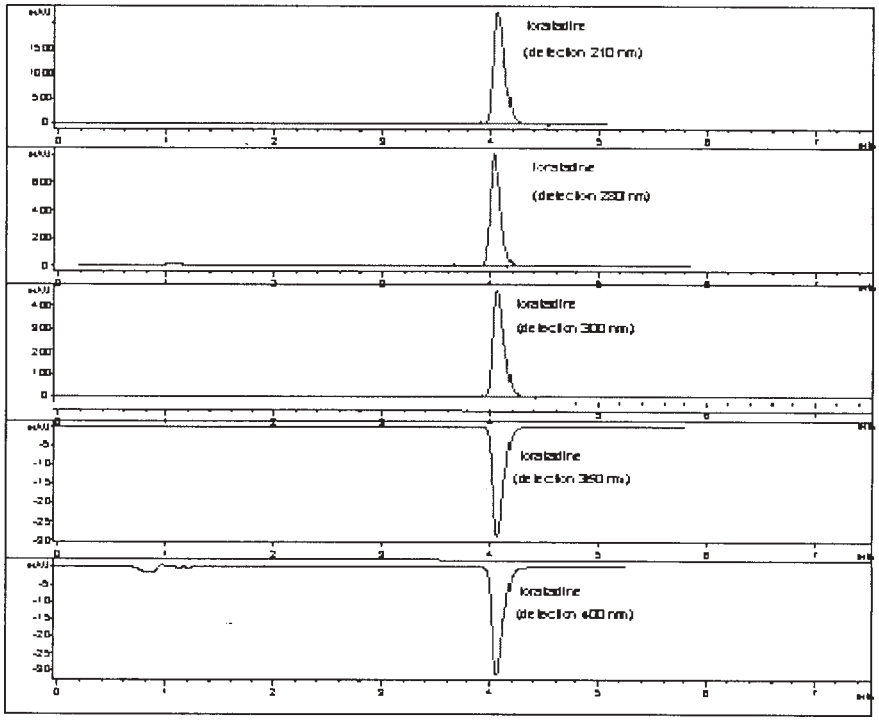

Fig.1. Comparative chromatogram of the standard solution of loratadine at different wavelengths

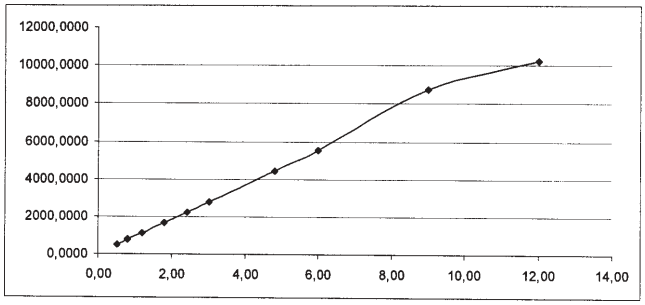

Fig. 2. Determination of the concentration area of the loratadine solution

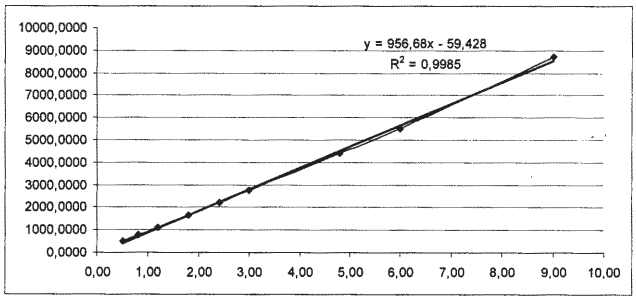

Fig. 3. The regression line for the concentration range 0.5 μg/mL – 9.0μg/mL

Table 1

VARIATION OF THE RATIO SOLVENT A / SOLVENT B FOR ADJUST THE COMPOSITION OF THE MOBILE PHASE

No.	Mixing ratio (v/v)	
	Solvent A	Solvent B
1	13	13
2	14	12
3	15	11
4	16	10
5	18	8

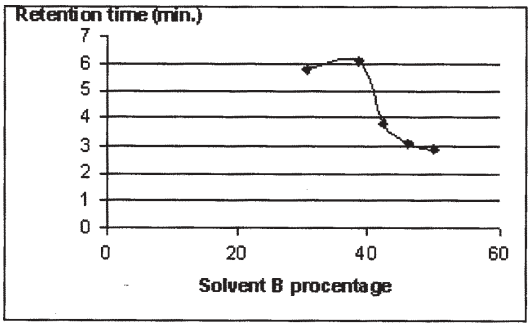

Fig. 4. Graphic representation of retention time according to the percentage of acetonitrile in the mobile phase composition

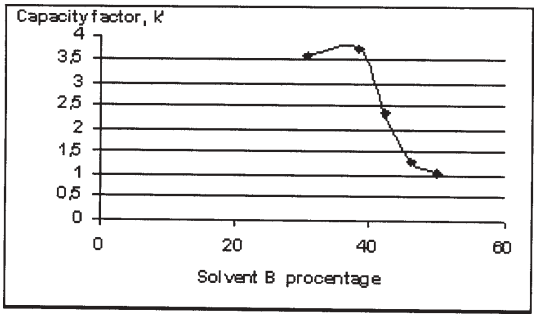

Fig. 5. Graphic representation of the capacity factor based on the percentage of acetonitrile in the mobile phase composition

Injections of 10 μL standard solution loratadine were performed, in chromatography conditions mentioned, chromatograms were recorded.

It was plotted the variation of the retention time (fig. 4) and the variation of the capacity factor (fig. 5) according to the percentage of solvent B in mobile phase composition.

It is noted that varying the amount of acetonitrile in the mobile phase composition has a significant influence on the retention of loratadine.

The mixing ratio of solvent A / solvent B, chosen for subsequent determinations is 15/11 (v / v) whereas such give an optimum capacity factor and loratadine retention time of about 4 min ensuring avoidance of interference of excipients present in pharmaceutical products or in biological samples analyzed.

To sum, the composition of the mobile phase acetonitrile / ($NH_4H_2PO_4$ aqueous 10g/L which was added 5 mL H_3PO_4):

11/15 (v / v) is used for the application of the present method.

V. Effect of pH mobile phase

The effect of the mobile phase *p*H on retention was followed, by analyzing a standard solution of loratadine (6 µg / mL), three times injected. It have performed three series of determinations. For each series one mobile phase was prepared as described in research modifying the *p*H of the aqueous solution.

Considering the basic function of loratadine and for maintaining it in a protonated form, the *p*H of the mobile phase was potentiometric adjusted to 2.0 - 3.5. It is found that varying the *p*H of the aqueous component of the mobile phase determines modification of retention time (fig. 6).

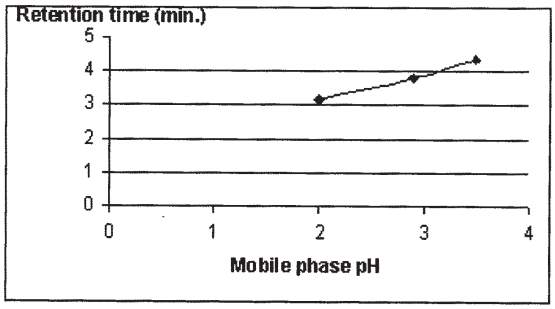

Fig. 6. Variation of the retention time depending on the changing *p*H of the mobile phase

Changing the *p*H value determines modify the capacity factor, the peak area of loratadine and time of analysis.

Therefore, a *p*H of 2.9, leading to the maximum area and loratadine retention time of about 4 min is suitable for the method (fig. 7).

The temperature influence

Temperature was selected by recording a series of chromatograms for the standard solution of loratadine using the mobile phase mixture: acetonitrile / ($NH_4H_2PO_4$ aqueous solution 10 g / L which was added 5 mL H_3PO_4): 11/15 (v / v). The column temperature varied between 27-45 ° C (fig. 8).

It is noted that an increase in the operating temperature increases the retention time and hence the capacity factor (fig. 9 and fig. 10). Thus, for the HPLC analysis of loratadine, was chosen working temperature of 27°C, for which the loratadine has a retention time of about 4 minutes, and its peak is symmetrical.

Bringing together the results presented, it was established working mode: equilibrate chromatographic column with mobile phase for 60 min; Inject 10 µL sample and record the chromatogram with UV detection.

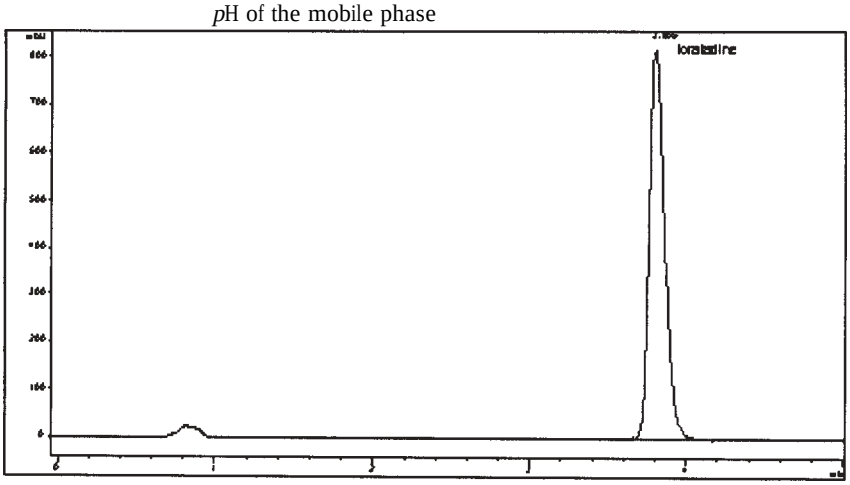

Fig. 7. Chromatogram of loratadine solution in an injection volume of 10 µl at *p*H 2.9 of mobile phase

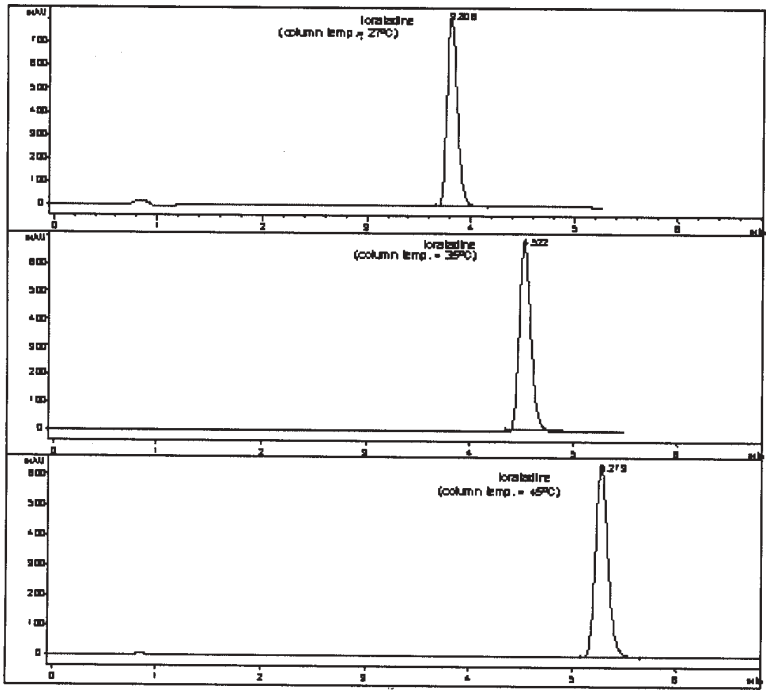

Fig. 8. Comparative chromatogram of the standard solution of loratadine at different temperatures

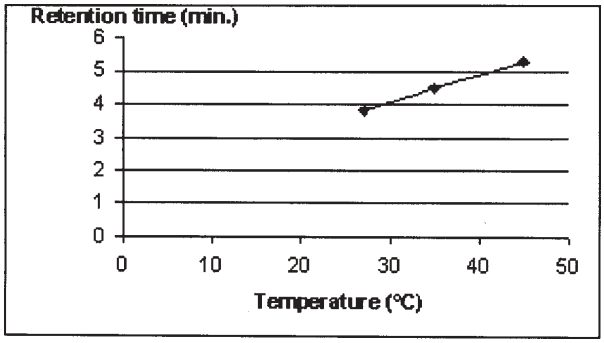

Fig. 9. Variation of the retention time depending on the operating temperature

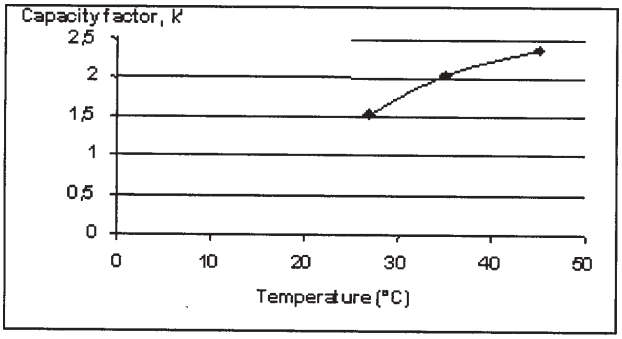

Fig. 10. Variation of capacity factor depending on operating temperature

Conclusions

Has been described a method for the determination of loratadine by high performance liquid chromatography, isocratic conditions, by detecting it in the ultraviolet at 280 nm.

The analysis method was optimized on the 0.5 µg/mL – 9.0 µg/mL range. It was established the detection wavelength (280 nm), the composition of mobile phase: acetonitrile / ($NH_4H_2PO_4$ aqueous solution 10 g / L which was added 5 mL H_3PO_4): 11/15 (v / v) and the *p*H of the mobile phase (2.9). It has been established the optimum working temperature of 27 ° C, temperature at which the loratadine has a retention time of about 4 min , and its peak is symmetrical.

The method was validated and applied to loratadine dosage of pharmaceuticals and biological samples.

The method presented in the present work has proved useful in the analysis and control of drug studies and in bioequivalence and pharmacokinetic studies.

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