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Validation of UV molecular absorption spectrometric method for loratadine determination

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Abstract The objective of this study was the validation of a developed spectrophotometric method in order to determine loratadine, a miscellaneous and "nonsedating" antihistamine agent. Loratadine and potassium tetraiodomercuriate form a macromolecule which can be detected by molecular absorption spectrometry in well-determined working conditions: reaction environment, the optimal amount of reagent, time reaction. The following parameters were studied: linearity, precision (repeatability of the detection, repeatability of the method and intermediate precision), exactness, limit of detection, limit of quantification. The method will be used to analyze biological and pharmaceutical samples for application in pharmacokinetic or bioequivalence studies.

Keywords: loratadine, potassium tetraiodomercuriat, linearity, precision, exactness

1. Introduction

In allergic manifestations, the effects of histamine obtained by H1 receptor activation are very important. H1 antihistamines control these effects [1].

Loratadine and its metabolites, descarboetoxiloratadine, cetirizine (racemic mixture) and levocetirizine (active isomer) are antihistamines of latest generation [1].

Loratadine $C_{22}H_{23}ClN_2O_2$ contains 98.5 to 101.5% dry substance. It is a white or almost white powder, crystalline, practically insoluble in water, slightly soluble in acetone and methanol [2].

The published methods for determining loratadine are spectrometric or chromatographic [3, 4].

Our previous study presented the development of a method for determination of loratadine by UV spectrometry: loratadine methanolic solution and complex ion tetraiodomercuriat $[HgI_4]^{2-}$ form a compound in the presence of hydrochloric acid. Detection wavelength was established at λ = 380 nm (at this wavelength the compound shows maximum absorption in methanol in comparison with maximum absorption loratadine, which is at 288 nm). The optimum concentration of potassium tetraiodomercuriat was found to be 1.9428 mM, the acidity of the reaction was acquired by adding 1 mL 0.05 M HCl solution, and absorbance can be read 10 minutes after reagents are added [5].

The purpose of this study was to validate this analytical method by establishing the following parameters: linearity, precision, repeatability, exactness, limit of detection, limit of quantification [6,7,8].

2. Experimental

Spectrometric measurements were done using a UV-VIS spectrophotometer Jasco V-630.

All used reagents were of the highest purity: Loratadine, reference substance, purity 98.5%, (Sigma); Methanol (Merck); Hydrochloric acid 37% (Tunic Prod Bucharest); Potassium iodide (Merck); Mercury (II) chloride (Merck).

The following solutions were prepared:

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- loratadine standard solution in methanol (1 mg/mL): 100 mg loratadine (reference substance) is dissolved in methanol in 100 mL volumetric flask; for experiments, a working solution of 0.1mg/mL loratadine was prepared by diluting standard solution 1/10 with methanol;
- aqueous solution of potassium tetraiodimercuriat 0.017 M: 30 mL 0.1 N $HgCl_2$, (13.6 g $HgCl_2$ dissolved in distilled water to one liter) was mixed with 57 mL solution KI 0.1 N (16.6 g KI dissolved in distilled water to one liter) added drop by drop and stirring continuously; the working solution (1.9428 mM) was prepared by diluting the standard solution 2/15 (v/v) in distilled water; the following reaction took place:

$$HgCl_2\ (aq) + 2\ KI\ (aq) \rightarrow 2KCl\ (aq) + HgI_2\ (pp)$$
$$HgI_2\ (pp) + 2KI\ (aq) \rightarrow K_2\ [HgI_4]\ (aq)$$

- aqueous solutions 0.1 M and 0.05 M HCl.

In principle, loratadine in methanol solution forms with complex ion tetraiodomercuriate $[HgI_4]^{2-}$, in the presence of hydrochloric acid, a hardly soluble compound that shows maximum absorbance at 380 nm, proportional to its concentration in loratadine [5].

One mL of loratadine working solution was mixed with 1 mL of each reagent and filled up with distilled water to 5 mL. Each sample was analyzed, measuring the absorbance at wavelength λ = 380 nm.

To assess **the linearity**, the regression line has been plotted. The results of analytical method were mathematically processed using the method of least squares [9, 10] and then it was determined the concentration range for which the variation is linear.

For this interval the regression line was plotted and the obtained data were evaluated statistically, determining the correlation coefficient (r), regression coefficient (r^2), standard deviation of regression slope (RSD), standard error of regression line (SE) and equation of absorbance line = f (c):

$$Absorbance = a\ x\ concentration + b$$

(a = slope; b = intercept)

The precision, expressed by relative standard deviation (RSD%) should not exceed 5% [11].

In order to evaluate the system's **precision**, the following parameters have been assessed: repeatability of the detection, repeatability of the method and intermediate precision.

For **repeatability of detection**, ten determinations of the same sample (0.08 mg/mL) were done, under the same experimental conditions, measuring the values of absorbance.

Repeatability of the method is a measure of variability when the same analyst works in the same conditions. The results were expressed by the standard deviation of repeatability and the coefficient of variation of multiple determinations for a single sample, in one run test (relative standard deviation).

$$RSD = standard\ deviation\ /\ average\ x100$$

The coefficient of variation should not exceed 2%. Repeatability conditions include: (i) constant measurement method; (ii) same operator; (iii) same measuring equipment used in the same conditions; (iv) same location; (v) occurrence of measurements on short periods during the day.

For determining **intermediate precision** of the method, the entire experiment was performed the following day, with newly prepared reagents, the process being the same as for the repeatability method of determination.

Exactness expresses the degree of agreement between the result of a test and the accepted (real) reference value of the measuring device. The exactness was determined on nine samples of loratadine.

The method for calculating the standard error and slope of the regression line was used to determine **limit of detection** and **limit of quantification.**

Limit of detection (LOD) is the lowest analyte concentration that can be detected.

$$LOD = \frac{3 \times SE}{slope}$$

Limit of quantification

(LOQ) is the lowest analyte concentration that can be detected quantitatively, at an acceptable level of uncertainty.

$$LOQ = \frac{10 \times SE}{slope}$$

3. Results and Discussions

3.1. Linearity

Four series of working solutions were prepared under described conditions, with concentration in loratadine from 0.01 to 0.1 mg/mL

The variation of average absorbance it was plotted versus concentration.

The equation of calibration line calculated by mathematical regression is:

Absorbance = 7.5789 x concentration - 0.0113

Statistic data are synthesized in **Table 1.**

Table 1 – Statistics calculation for linearity

Correlation coefficient (r)	0.9998
Regression coefficient (r^2)	0.9997
Standard error of the regression line (SE)	0.005
Intercept (b)	-0.0113
Slope (a)	7.5789

Analysing the value obtained by Fisher test [9,10], we can see that calculated value ($F_{calculated}$ =8974.140) is much higher than value tabled for a 5% risk and 8 rank of liberty (F_{tabled}=5.317), which entitles us to reject the variant of „null hypothesis", i.e., factorial variable (concentration) influences significantly the behaviour of resultant variable (absorbance). Thus, correlation ratio is significant.

The correlation between theoretical concentration and the calculated one, using the equation of calibration line, is appropriate.

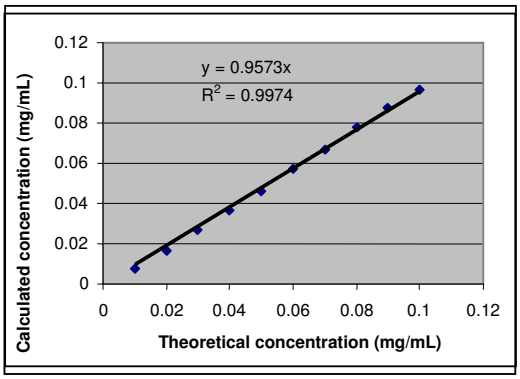

Fig. 1 - Linearity results using the optimized UV spectrometric method

3.2. Precision

Precision of the method was tested by assessing repeatability of the detection, repeatability of the method and intermediate precision.

3.2.1. Repeatability of detection

The results of ten absorbance measured values of the same sample (0.08 mg/mL) were statistically evaluated and the average value, the standard deviation and relative standard deviation were calculated (**Table 2**).

Table 2 – Statistical results for repeatability of detection

Average	0.6137
SD	0.0017
RSD	0.2807

Considering the value of relative standard deviation, RSD = 0.2807, the study demonstrates that UV spectrometric method for determination of loratadine is repeatable.

3.2.2. Repeatability of the method

In order to determine the repeatability of this method, nine samples covering specific range of concentration (3 determinations for 3 different values of concentration) were prepared and analyzed. Using the calibration equation, the

concentration and recovery for each sample have been established (**Table 3**).

The obtained values for repeatability of the method were statistically processed (**Table 4**).

According to experimental data obtained, the relative standard deviation (RSD = 4.632%) is less than 5%, which proves that the proposed analysis method is precise.

3.2.3. Intermediate precision

The absorbance values obtained by processing the samples of intermediate precision are presented and recovery was established using equation of calibration curve to calculate concentration (**Table 5)**.

Table 4 – Statistical results for repeatability of the method

Average	102.464
SD	4.746
RSD	4.632%

According to statistical data (**Table 6**), the relative standard deviation (RSD = 3.81%) has a value up to 5%, which shows that the method is precise.

Table 3 – Results to assess the repeatability of UV spectrometric method for determination of loratadine

N°.	Theoretical concentration (mg/mL)	Absorbance	Calculated concentration (mg/mL)	Recovery %
1	0.06	0.50112	0.064629	107.71
2		0.51220	0.066091	110.15
3		0.50289	0.064863	108.10
4	0.07	0.53244	0.068762	98.23
5		0.54285	0.070136	100.19
6		0.53424	0.068999	98.57
7	0.08	0.61453	0.079593	99.49
8		0.61235	0.079306	99.13
9		0.62115	0.080467	100.58

Table 5. Results to assess the intermediate precision of UV spectrometric method for loratadine determination

N°.	Theoretical concentration (mg/mL)	Absorbance	Calculated concentration (mg/mL)	Recovery %
1	0.06	0.49102	0.063297	105.49
2		0.5092	0.065696	109.49
3		0.48277	0.062208	103.68
4	0.07	0.54111	0.069906	99.86
5		0.5394	0.06968	99.54
6		0.53459	0.069046	98.63
7	0.08	0.60453	0.078274	97.84
8		0.65235	0.084584	105.72
9		0.6309	0.081753	102.19

Table 6 – Statistical results for intermediate precision of the method

Average	102.4974
SD	3.906451
RSD	3.81%

Confidence interval for each individual value for 8 degree of liberty and a precision of 95% (t= 2.31) is [11]:

$$X - (t.SD) < \mu < x + (t.SD)$$
$$102.464 - (2.31 \times 4746) < \mu < 102.464 + (2.31 \times 4746)$$
$$91.50\% < \mu < 113.43\%$$

3.3. Exactness

Nine samples were prepared for determining exactness. The concentrations of loratadine were ranged from 0.06 to 0.08 mg/mL. For each level of concentration, three samples were done and analyzed, measuring absorbance (**Table 7).**

Using equation of calibration line, the concentration has been calculated. The recovery of calculated concentration against the theoretical one was statistically processed (**Table 8).**

Table 8 – Statistical processed of recovery in determining of exactness

Average	100.5978
SD	1.117124
RSD	1.11%

The evaluation of exactness for UV spectrometric method of loratadine determination has a recovery of 100.59%. (ranged from 98.53 to 102.05%).

3.4. Limit of detection and limit of quantification

For the quantification limit LOQ, which usually is the lowest point on the standardization curve, we obtained a value of 0.006 mg/mL which is higher than the value obtained by for the limit of detection LOD of 0.002 mg/mL, being in agreement with the method's purpose.

4. Conclusions

The following experimental data for the validation of the new developed analytical method of loratadine spectrometric determination in UV have been obtained: (i) RSD was <5% (between 0.28 and 3.81%) (ii) the correlation coefficient of regression line (r^2 = 0.9997) was higher than 0,995 (iii) the method exactness was between 98.59% and 102.05% (the mean recovery is 100.59%) (iv) the LOD was 0.002 mg/mL and (v) the LOQ was 0.006 mg/mL.

All this issues demonstrate that the method is suitable to be used in drugs and pharmaceutical preparations control.

Table 7. Results for exactness of spectrometric method for determining loratadine

No. Det.	Theoretical concentration (mg/mL)	Absorbance	Calculated concentration (mg/mL)	Recovery %
1	0.06	0.47109	0.060667	101.11
2		0.46902	0.060394	100.65
3		0.47277	0.060889	101.48
4	0.07	0.54155	0.069964	99.94
5		0.55004	0.071084	101.54
6		0.53403	0.068972	98.53
7	0.08	0.61453	0.079593	99.49
8		0.63005	0.081641	102.05
9		0.62100	0.080447	100.55

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