

IVANA M. SAVIĆ¹
GORAN S. NIKOLIĆ¹
VLADIMIR M. BANKOVIĆ²
IVAN M. SAVIĆ¹

¹Faculty of Technology, University
of Niš, Leskovac, Serbia

²Pharmaceutical and Chemical
Industry Zdravlje-Actavis,
Leskovac, Serbia

SCIENTIFIC PAPER

UDK 615.2:543.544.5:661.12

DOI: 10.2298/HEMIND0902087S

QUANTITATIVE ESTIMATION OF TRIMAZOLIN HYDROCHLORIDE IN PHARMACEUTICAL PREPARATION BY RP-HPLC METHOD

A sensitive and selective RP-HPLC method was developed and validated for the quantitative determination of trimazolin hydrochloride in nasal drops formulations. The mobile phase composition was water-acetonitrile (50:50, v/v) and the UV detection was carried out at 270 nm. Linearity range in the concentration range of 10 to 110 µg cm⁻³. The method was tested and validated for various parameters according to ICH guidelines. The detection and quantization limits were found to be 1.45 and 4.8 µg cm⁻³, respectively. The results demonstrated that the procedure for estimation of trimazolin hydrochloride in nasal drops formulations was accurate, precise and reproducible.

Nasal stuffiness or congestion occurs as a result of swelling of the nasal membranes. Histamine opens the blood vessels and encourages the fluid leakage from them, thereby causing the tissues to become “congested”. This reaction reduces the space inside the nose through which we breathe and results in the typical “blocked” or stuffy nose. While antihistamines can control many symptoms of allergic rhinitis, they are not very helpful for treating nasal congestion once it has already occurred [1]. At this point, decongestants can be a very useful addition. Decongestants act on a receptor on the blood vessels. The blood vessels thereby shrink, which in turn reduces the blood flow to the area and lessens the leakage of the fluid into the tissues. The result is a nasal passage that feels more “open”. It is important to remember that decongestants do not help with an itchy, sneezing, and runny nose.

Nasal preparations are liquid, semi-solid or solid preparations intended for administration to the nasal cavities obtain a systemic or local effect. Nasal preparations are as far as possible non-irritating and do not adversely affect the functions of the nasal mucosa and its cilia. Aqueous nasal preparations are usually isotonic and may contain excipients, for example, to adjust the viscosity of the preparation, to adjust or stabilize the pH, to increase the solubility of the active substance, or to stabilize the preparation [2]. Unless otherwise justified and authorized, aqueous nasal preparations supplied in multidose containers comprise a suitable antimicrobial preservative in appropriate concentration, except where the preparation itself has adequate antimicrobial properties.

In practice, a great number of this type of preparations, which most often contain ephedrine and phenylephrine as adrenergic vasoconstrictors, are commercially

present today [3]. Various methods have been reported in literature for the analysis of phenylephrine hydrochloride including spectrophotometry [4–8], spectrophotometry with chromogenic reagent [9], fluorometry [10], chromatography [11,12]. High-performance liquid chromatography [13–16], micellar liquid chromatography [17], micellar electrokinetic chromatography [18], capillary zone electrophoresis [19,20], spectro-fluorimetric and derivative spectrophotometric methods [21], are also reported for determination of phenylephrine hydrochloride.

As pharmaceutically active substances, ephedrine and phenylephrine can act alone or can be combined with other substances of the similar effect, thus increasing the preparation efficiency. The object of this investigation is a decongestive preparation of Adrianol drops (Zdravlje-Actavis, Leskovac, Serbia). Besides phenylephrine, adrianol contains trimazolin hydrochloride as an adrenergic vasoconstrictor. The efficiency of the preparation is increased by the use of trimazolin hydrochloride.

Trimazolin hydrochloride ((2-(2,4,6-trimethylbenzyl)-imidazoline hydrochloride) is an alpha-adrenergic (sympathomimetic) (Fig. 1) [22].

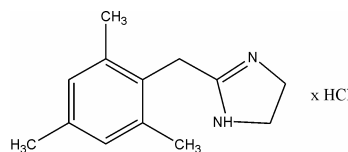


Figure 1. Chemical structure of trimazolin hydrochlorid.
Slika 1. Hemijska struktura trimazolin-hidrohlorida.

It is a white, crystalline, almost odorless, bitter substance, freely soluble in water, slightly soluble in ethanol, insoluble in ether and acetone. The melting point is in the range of 279–281 °C. A 10% aqueous solution has a pH of about 5.5–6.5 [23].

Various methods have been reported in literature for the analysis of phenylephrine hydrochloride. However, in domestic and foreign pharmacopoeias, as well as in scientific literature, there are no data on trimazolin and the methods of its investigation [24–27]. The patent

Corresponding author: I. M. Savić, Department of Pharmaceutics, Faculty of Technology, Bulevar oslobođenja 124, 16000 Leskovac, Serbia.

E-mail: ici86@info-net.rs

Paper received: 5 February, 2009.

Paper accepted: 24 March, 2009.

literature has data on only the trimazolin and trimazolin hydrochloride synthesis procedure, without physico-chemical, spectroscopic characterization and other investigation methods [25].

For the routine analysis, a simple and rapid HPLC method is suggested in the paper. A literature survey of literature has not revealed any simple validated RP-HPLC method for estimating trimazolin hydrochloride in nasal drops formulations. The development of simple and accurate RP-HPLC methods can provide a very useful alternative for the routine analysis of nasal formulations and dissolution samples. The object of the present study was to develop simple, precise, accurate and validated, economic analytical methods for the estimation of trimazolin hydrochloride in a pure form and in pharmaceutical nasal formulations. In order to be useful for the drug testing, the proposed method should be validated according to the ICH regulations [29–30], and Ph. Yug. V [24]. Statistical tests were performed on validation data [31–32].

EXPERIMENTAL

Apparatus

The method development was performed with an Agilent 1100-Series HPLC system consisting of an Agilent 1100-Series variable wavelength UV detector and an Agilent 1100-Series autosampler using a 50 μL sample loop (Faculty of Technology, Leskovac). The system was controlled and data analyses were performed with the Agilent HPLC Data Analysis software. The assays (repeatability) were performed with another LC system consisting of a Agilent 1100-Series binary pump and Agilent 1100-Series DAD detector (Zdravlje-Actavis, Leskovac). The detector was set at 270 nm and the peak areas were integrated automatically by the computer using the Agilent HPLC Data Analysis software program. The separation was carried out at ambient temperature using a ZORBAX Eclipse XDB-C18 column, (4.6 mm \times 250 mm, 5 μm). All the calculations concerning the quantitative analysis were performed with the external standardization by measuring the peak areas.

Chromatographic conditions

RP-HPLC analysis was performed by isocratic elution with a flow rate of 0.8 $\text{cm}^3 \text{min}^{-1}$. The mobile phase composition was water–acetonitrile (50:50 v/v). All solvents were filtered through a 0.45 μm millipore filter. Volumes of 50 μL of the solutions and samples prepared were injected into the column. Quantification was effected by measuring at 270 nm as established from the two-dimensional chromatogram. Throughout the study, the suitability of the chromatographic system was monitored the efficiency column and peak asymmetry.

Chemicals

The standard of trimazolin hydrochloride (99.96%) and the standard of phenylephrine hydrochloride (99.98%) were kindly donated by the Pharmaceutical and Chemical Industry Zdravlje-Actavis (Leskovac, Serbia) and used without further purification.

Pharmaceutical preparation

Commercial pharmaceutical preparations Adrianol-T nasal drops (for children) and Adrianol nasal drops (for adults), containing trimazolin hydrochloride, were kindly donated by Zdravlje-Actavis. Adrianol-T nasal drops were labeled to contain 0.5 mg cm^{-3} of trimazolin hydrochloride and 0.5 mg cm^{-3} of phenylephrine hydrochloride. Adrianol nasal drops were labeled to contain 1.5 mg cm^{-3} of trimazolin hydrochloride and 1 mg cm^{-3} of phenylephrine hydrochloride. Adrianol formulations contain excipients like disodium hydrophosphate dihydrate, citric acid monohydrate, methyl cellulose M.H.B. 10000, glycerol, phenyl–mercury(II) borate, ammonium hydroxide, ethanol 96% and pure water. All other chemicals and reagents used were of analytical grade (Merck Chem. Ind.).

Stock and standard solution

One stock solution of 0.5 mg cm^{-3} trimazolin hydrochloride was prepared by dissolving 25 mg of trimazolin hydrochloride in a 50 cm^3 mobile phase. The stock solution of 0.5 mg cm^{-3} phenylephrine hydrochloride was prepared by dissolving 25 mg of phenylephrine hydrochloride in a 50 cm^3 mobile phase.

Procedure for calibration curve

For preparing different concentrations, aliquots of the stock solution were transferred into a series of 10 cm^3 standard volumetric flasks and the volumes were made with the respective media. Ten different concentrations were prepared in the range of 10–110 $\mu\text{g cm}^{-3}$ of trimazolin hydrochloride in mobile phase for a standard curve. In a similar way, five different concentrations were prepared in the range of 100–500 $\mu\text{g cm}^{-3}$ of trimazolin hydrochloride considering the declaration value. Triplicate 50 μL injections were made for each solution. The final concentrations of trimazolin hydrochloride in the samples were calculated by comparing the sample and standard peak obtained with the average of three injections of standard solutions.

Sample preparation

Aliquots (1 cm^3) of nasal drop preparations (Adrianol-T or Adrianol) equivalent to 0.5 mg and 1.5 mg of trimazolin hydrochloride, respectively, were taken and suitably diluted with the mobile phase in order to get a 50 $\mu\text{g cm}^{-3}$ concentration and the samples were injected into the chromatograph.

Validation

For specificity and selectivity of method, trimazolin hydrochloride solutions ($50 \mu\text{g cm}^{-3}$) were prepared in the mobile phase along with and without common excipients, separately. All the solutions were injected into the XDB-C18 RP-HPLC column. In this assay, it was tested by running solutions containing the placebo of the specialties in the same quantities and in the conditions in which the samples to show that there is no peak in the retention times corresponding to the analytes. Paired *t*-test at 95% level of significance was performed to compare the area of peak ($n = 10$) (Table 1).

Table 1. Statistical data of the regression equations and validation parameters for trimazolin hydrochloride ($n = 10$)
Tabela 1. Statistički podaci regresione jednačine i potvrđnih parametara za trimazolin-hidrohlorid ($n = 10$)

Parameter	Value
Slope (SE) ^a	2842.4163 (165.43)
Intercept (SE) ^a	87.8169 (10.61)
Regression coefficient (<i>r</i>)	0.9884
Specificity and selectivity, <i>t</i> ^b	1.94
Linearity ($\mu\text{g cm}^{-3}$)	10–110
Limit of detection (<i>LOD</i> , $\mu\text{g cm}^{-3}$)	4.8
Limit of quantification (<i>LOQ</i> , $\mu\text{g cm}^{-3}$)	12.3

^aStandard error of mean; ^btheoretical values at 95% confidence limits $t = 2.225$

The linearity study verifies that the sample solutions are in a concentration range where analyte response is linearly proportional to the concentration. To establish linearity of the proposed methods, eleven separate series of trimazolin hydrochloride solutions (10 – $110 \mu\text{g cm}^{-3}$ in the mobile phase) were prepared from the stock solutions and analyzed. Least square regression analysis was done for the data obtained.

The accuracy of the method is the closeness of the measured value to the true value for the sample. To determine the accuracy of the proposed method, different levels of drug concentrations – lower concentration (LC, 80%), intermediate concentration (IC, 100%) and higher concentration (HC, 120%) were prepared from independent stock solutions and analyzed ($n = 10$). Accuracy was assessed as the percentage relative error and mean% recovery (Table 2). To provide an additional support to the accuracy of the developed assay method, the standard addition method was employed, which involved the addition of different concentrations of pure drug (10, 20 and $30 \mu\text{g cm}^{-3}$) to a known preanalyzed formulation sample and the total concentration was determined using the proposed methods ($n = 10$). The recovery of the added pure drug was calculated as $\% \text{Recovery} = ((c_t - c_s)/c_a) \times 100$, where c_t is the total drug concentration measured after standard addition; c_s , drug concentration

in the formulation sample; c_a , drug concentration added to formulation (Table 3).

Table 2. Accuracy and the precision data for the developed method ($n = 10$)

Tabela 2. Tačnosti i preciznost podataka za korišćeni metod ($n = 10$)

Level	Predicted concentration $\mu\text{g cm}^{-3}$		Mean recovery \pm SD %
	Mean \pm SD	%RSD	
LC ($40 \mu\text{g cm}^{-3}$)	40.23 ± 0.326	0.812	100.57 ± 0.326
IC ($50 \mu\text{g cm}^{-3}$)	50.21 ± 0.337	0.672	100.42 ± 0.337
HC ($60 \mu\text{g cm}^{-3}$)	59.92 ± 0.321	0.533	99.87 ± 0.321

Table 3. Standard addition of trimazolin hydrochloride for accuracy ($n = 10$)

Tabela 3. Standardni dodatak trimazolin hidrohlorida u cilju tačnosti ($n = 10$, $c = 50 \mu\text{g cm}^{-3}$)

Pure drug added $\mu\text{g cm}^{-3}$	Total drug found \pm SD $\mu\text{g cm}^{-3}$	Recovery \pm RSD %
0	50.21 ± 0.34	100.42 ± 0.67
10	59.92 ± 0.32	99.87 ± 0.58
20	70.35 ± 0.45	100.50 ± 0.64
30	79.87 ± 0.42	99.84 ± 0.53

The repeatability was determined by using different levels of drug concentrations (the same concentration levels taken in the accuracy study), prepared from independent stock solutions and analyzed ($n = 10$) (Table 2). Inter-day, intra-day and inter-instrument variation were studied to determine the intermediate precision of the proposed analytical methods. Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intra-day variation. The same procedure was followed for three different days in order to study the inter-day variation ($n = 10$). One set of different levels of the concentrations was re-analyzed using another HPLC Agilent 1100-Series system, by proposed methods to study inter-instrument variation ($n = 10$). The relative standard deviation (%RSD) of the predicted concentrations from the regression equation was taken as precision (Table 4). The precision studies were also carried out by using the real samples of trimazolin nasal drops preparation (0.5 mg cm^{-3}) in a similar way to a standard solution to prove the usefulness of method.

The limit of detection (*LOD*) was calculated by comparison of the three-fold variation of signal to noise ratio (3 S/N) obtained from analysis of the standards.

The limit of quantification (*LOQ*) was defined as the lowest measured quantity above which the analyte can be quantified at a given statistical level of (10 S/N). *LOD* and *LOQ* were experimentally verified by ten injections of trimazolin hydrochloride at the *LOD* and *LOQ* concentrations ($n = 10$) (Table 1).

To determine the robustness of the developed method, experimental conditions were purposely altered. The flow rate of the mobile phase was $0.8 \text{ cm}^3 \text{ min}^{-1}$. To study the effect of flow rate on the resolution, it was changed by 0.2 units from 0.6 to $1 \text{ cm}^3 \text{ min}^{-1}$, while the other mobile phase components were held constant as per method. The effect of column temperature on resolution was studied at 20 and $30 \text{ }^\circ\text{C}$ instead of $25 \text{ }^\circ\text{C}$, while the other mobile phase components were held constant as per method.

Comparative method

The employed procedure for the comparative method (UV spectrophotometry) is described in the other paper [33].

RESULTS AND DISCUSSION

HPLC method optimization

A RP-HPLC method was developed for trimazolin hydrochloride and it can be conveniently employed for routine quality control in pharmaceutical dosage forms. The chromatographic conditions were optimized in order to provide a good performance of the assay. During the optimization of the method different stationary phases like C18, C8 and cyano, different mobile phases and using organic modifiers like methanol in the mobile phase. The chromatographic separation was achieved on a ZORBAX Eclipse XDB-C18 column ($4.6 \text{ mm} \times 250 \text{ mm}$), with a particle size of $5 \text{ }\mu\text{m}$. Satisfactory separation of used standards was obtained with a mobile phase consisting of water–acetonitrile ($50:50 \text{ v/v}$). If methanol was used instead of acetonitrile, the separation of trimazolin hydrochloride and phenylephrine was also with unsatisfactory resolution in nasal formulations. The maximum absorption of trimazolin hydrochloride was detected at 270 nm and this wavelength was chosen for the analysis. The retention times for the standard solution of trimazolin hydrochloride (0.5 mg cm^{-3}) and trimazolin hydrochloride in Adrianol-T nasal drops preparation were observed to be 4.5 min and 4.570 min , respectively (Fig. 2a and 2d).

The retention time for the standard solution of phenylephrine hydrochloride (0.5 mg cm^{-3}) and phenylephrine hydrochloride in Adrianol-T nasal drops preparation were observed to be 2.255 and 2.321 min , respectively (Fig. 2a and 2d). Total time of the analysis was less than 10 min .

The chromatographic parameters, such as efficiency column and peak asymmetry were reconsidered for the trimazolin hydrochloride standard. According to the obtained value Number of Theoretical Plates ($N = 324$), the conclusion is that the efficiency column is satisfactory ($HETP = 0.771$). The 0.41 asymmetry peak value indicates that the peak is not ideally symmetric, that is, it is not a Gauss peak. Having in mind that $W_{ab} < W_{bc}$, this

means that there is a certain interaction between the stationary phase and the investigated component. A similar effect was noticed in the analysis of Adrianol preparation which explains the movement of the peak from 4.500 to 4.570 min (Fig. 2d).

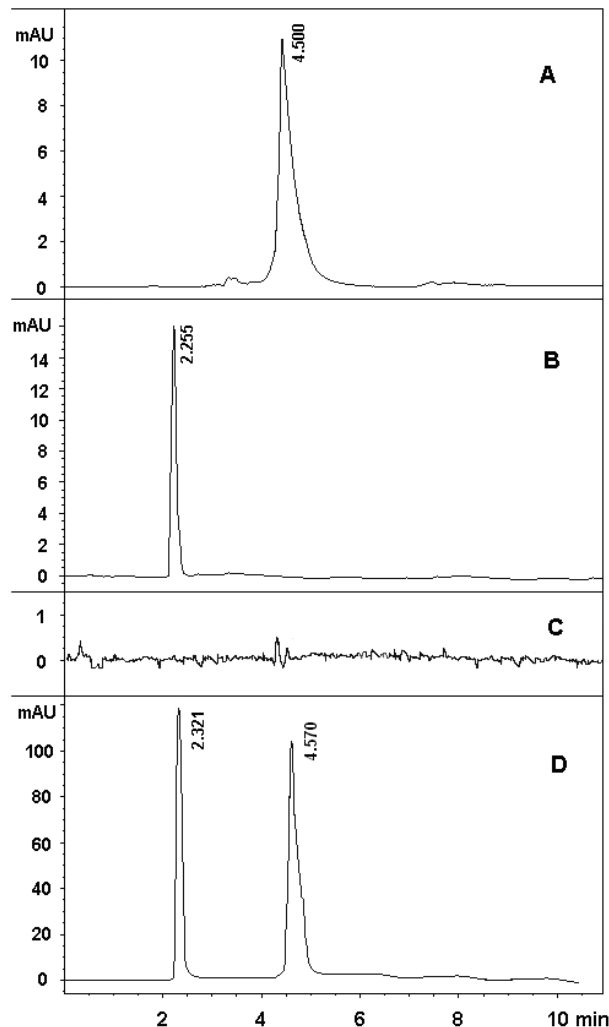


Figure 2. The HPLC chromatograms at 270 nm of trimazolin hydrochloride standard (A), phenylephrine standard (B), placebo (C) and Adrianol-T nasal drops preparation (D). Slika 2. HPLC hromatogrami pri 270 nm za trimazolin hidrohlorid standard (A), fenilefrin standard (B), placebo (C) i adrianol-T (D).

Calibration curve

Excellent linearity was obtained between the peak areas and the concentrations. The linear regression equation obtained with a regression coefficient, r of 0.9884 and standard deviation, SD of 18.0621 was: $A_{270} = (2842.4163 \times c \text{ (mg cm}^{-3}\text{)} + 87.8169)$. Linearity range was not obeyed in the range of $100\text{--}500 \text{ }\mu\text{g cm}^{-3}$, considering a declaration value of Adrianol-T preparation, but linearity range was obeyed in the concentration range of $10\text{--}110 \text{ }\mu\text{g}\cdot\text{cm}^{-3}$.

Validation

The chromatogram of trimazolin hydrochloride was not changed in the presence of common excipients used in the formulation of nasal drops. The chromatogram of the pure drug sample was matched with the formulation samples in mobile phase (Fig. 2c). The calculated *t*-values were found to be less than that of the tabulated *t*-values (Table 1). Therefore, the proposed analytical method is specific and selective for the drug.

The linearity range for trimazolin hydrochloride estimation was found to be 10–110 $\mu\text{g cm}^{-3}$ ($r = 0.9884$) (Table 1).

Goodness of the fit of the regression equations was supported by high regression coefficient values.

The accuracy was determined in range from 20 to 60 $\mu\text{g cm}^{-3}$ (Table 2).

The excellent mean %recovery values, close to 100%, and their low standard deviation values ($RSD < 1.0$) represent high accuracy of the analytical methods. The validity and reliability of the proposed methods was further assessed by recovery studies *via* standard addition method. The mean %recoveries (%*RSD*) for concentration of 50 $\mu\text{g cm}^{-3}$ showed in Table 3.

These results revealed that any small change in the drug concentration in the solutions could be accurately determined by the proposed analytical methods.

Precision was determined by studying the repeatability and the intermediate precision. Repeatability (%*RSD*) ranged from 40 to 60 $\mu\text{g cm}^{-3}$, at all three levels of trimazolin hydrochloride concentrations (Table 4).

Table 4. System precision study ($n = 10$)

Tabela 4. Preciznost sistema ($n = 10$)

$c / \mu\text{g cm}^{-3}$	Intra-day repeatability % <i>RSD</i> ($n = 10$)			Intra-instrument repeatability % <i>RSD</i> ($n = 10$)
	Day 1	Day 2	Day 3	
40	39.65 (0.812)	39.94 (0.672)	40.48 (0.512)	38.96 (0.889)
50	49.85 (0.05)	50.32 (0.212)	49.88 (0.08)	50.35 (1.408)
60	60.55 (0.183)	60.51 (0.842)	60.15 (0.083)	60.34 (1.547)

The repeatability results indicated the precision under the same operating conditions over a short interval of time and the inter-assay precision. The intermediate precision expresses within-laboratory variations in different days and in different instruments. In the interme-

mediate precision study, %*RSD* values were not more than 2.0% in all the cases (Table 4). *RSD* values found for the proposed analytical method were well within the acceptable range indicating that the method had excellent repeatability and the intermediate precision. %*RSD* values for the precision studies with real samples of nasal drops were found to be less than 2.

LOD and *LOQ* were found to be 1.45 and 4.8 $\mu\text{g}\cdot\text{cm}^{-3}$ for trimazolin hydrochloride (Table 1).

Robustness

In all the deliberately varied chromatographic conditions (flow rate and column temperature) no significant change in the assay value was observed. The system suitability parameters like tailing and the *RSD* values were well within the limits. Tailing was 1.0 and 0.9 and *RSD* was 0.8 and 0.6% for flow rate and column temperature variations, which confirms the robustness of the developed method.

Applicability of the proposed method

The imposed method was applied for the determination of trimazolin hydrochloride in pharmaceutical formulations using the direct calibration curve. As it can be seen in Table 5, the results obtained for this method are in accordance with the UV spectrophotometric method. The results of the imposed method were statistically compared with those of the UV spectrophotometric method using a point hypothesis test [34,35]. Table 5 shows that the calculated *F* and *t* values at the 95% confidence level are less than the theoretical ones, confirming no significant differences between the performance of the proposed and the UV spectrophotometric method.

Estimation of formulations

The assay value of trimazolin hydrochloride in preparations ranged from 98.40 to 101.20% with standard deviation not more than 0.0091% (Table 6). Assay values of formulations were the same as mentioned in the label claim indicating that the interference of excipient matrix is insignificant in the estimation of trimazolin hydrochloride by the proposed analytical methods. The estimated drug content with low values of standard deviation established the precision of the proposed method. The calculated Student's *t*-values did not exceed the tabulated values (Table 6).

Table 5. Determination of trimazolin hydrochloride by the HPLC and the UV spectrophotometry

Tabela 5. Određivanje trimazolin-hidrohlorida HPLC i UV spektrometrijom

Pharmaceutical preparation	Taken μgcm^{-3}	Trimazolin hydrochloride found by HPLC ^a ($x \pm SD$, μgcm^{-3})	<i>RSD</i> ^a %	Recovery ^a %	<i>F</i> ^b	<i>t</i> ^b	UV spectrophotometry $x \pm SD$, $\mu\text{g cm}^{-3}$
Trimazolin hydrochloride	50	50.21 ± 0.34	0.67	100.42	1.36	1.03	49.75 ± 0.29

^aData are based on the average obtained from five determinations; ^btheoretical *F* value ($\nu_1 = 4$, $\nu_2 = 4$) and *t* value ($\nu = 8$) at the 95% confidence level are 6.39 and 2.31, respectively

Table 6. Assay results for the determination of trimazolin hydrochloride in commercial nasal solution (Adrianol-T and Adrianol)

Tabela 6. Rezultati određivanja trimazolin-hidrohlorida u komercijalnim rastvorima adrianol-T i adrianol

Sample (preparation)	Adrianol-T
Total trimazolin hydrochloride found ($\pm SD$), mg cm ⁻³	0.506 \pm 0.0091
Accuracy ^a , %	1.20
<i>t</i> ^b	1.98
Sample	Adrianol
Total trimazolin hydrochloride found ($\pm SD$), mg cm ⁻³	0.494 \pm 0.0086
Accuracy ^a , %	-1.60
<i>t</i> ^b	2.09

^aAccuracy is given in % relative error (100×(predicted concentration - nominal concentration)/nominal concentration); ^btheoretical values at 95% confidence limits *t* = 2.225

CONCLUSION

The proposed RP-HPLC method is simple, sensitive, rapid and specific and can be used for in a drug manufacturing quality control of trimazolin hydrochloride in nasal drops formulations. The method described in this study was suitable to determine concentrations in the range 0.01 to 0.11 mg cm⁻³ for trimazolin hydrochloride, precisely and accurately. Limits of detection and quantization for trimazolin hydrochloride with lower concentration were 1.45 and 4.8 µg·cm⁻³, respectively, values which are under the lowest expected concentrations in the sample. The sample recovery from the formulation was in good agreement with its respective label claim, which suggested non-interference of formulation excipients in the estimation.

Acknowledgements

This work was supported by the Ministry of Science and Technological Development of the Republic of Serbia, project TR-19035. The authors are grateful for the financial support provided by this Ministry.

REFERENCES

- [1] J. Karamčić, Z. Dizdarević, S. Dizdarević, Lj. Cupać-Vujčić, Klinička imunologija, XX poglavlje: Alergijski rinitis, Svjetlost, Sarajevo, 2007, p. 393.
- [2] A. Goth, Medical Pharmacology: Principle and Concepts, 10th Ed., C.V. Mosby, St. Louis, Toronto, 1981.
- [3] Available at (<http://en.wikipedia.org/wiki/Phenylephrine>).
- [4] M. Knochen, J. Giglio, Talanta **64** (2004) 1226-1232.
- [5] S.A. Shama, J. Pharm. Biomed. Anal. **30** (2002) 1385-1392.
- [6] P. Solich, C.K. Polydorou, M.A. Koupparis, C.E. Efstathiou, J. Pharm. Biomed. Anal. **22** (2000) 781-789.
- [7] N. Erk, J. Pharm. Biomed. Anal. **23** (2000) 1023-1031.
- [8] M.S. Collado, V.E. Mantovani, H.C. Goicoechea, A.C. Olivieri, Talanta **52** (2000) 909-920.
- [9] I.S. Ahmed, A.S. Amin, J. Mol. Liq. **130** (2007) 84-87.
- [10] M.A. Martin, B. del Castillo, P. Prados, Talanta **40** (1993) 1719-1723.
- [11] J.O. De Beer, C.V. Vandenbroucke, D.L. Massart, J. Pharm. Biomed. Anal. **12** (1994) 1379-1369.
- [12] N. Okamura, H. Miki, T. Harada, S. Yamoshita, Y. Ma-saoka, Y. Nakamoto, Y. Nakamoto, M. Tsuguma, A. Yoshitomi, A. Yagi, J. Pharm. Biomed. Anal. **20** (1999) 363-372.
- [13] A. Marin, E. Garcia, A. Garcia, C. Barbas, J. Pharm. Biomed. Anal. **29** (2002) 701-714.
- [14] B. Olmo, A. Garcia, A. Marin, C. Barbas, J. Chromatogr. B **817** (2005) 159-165.
- [15] M.J. Galmier, A.M. Frasey, S. Meski, E. Beyssac, J. Petit, J.M. Aiache, C. Lartigue, Biomed. Chromatogr. **14** (2000) 202-204.
- [16] N. Erk, M. Kartal, Farmaco **53** (1998) 617-622.
- [17] M. Gil-Agusti, L. Monferrer-Pons, M. Celia Garcia-Alvarez-Coque, J. Esteve-Romero, Talanta **54** (2001) 621-630.
- [18] J. Maria Lemus Gallego, J. Perez Arroyo, J. Sep. Sci. **26** (2003) 947-952.
- [19] A. Marin, C. Barbas, J. Pharm. Biomed. Anal. **35** (2004) 769-777.
- [20] Z. Wang, Y.L. Sun, Z.P. Sun, J. Chromatogr. A **735** (1996) 295-314.
- [21] S.M. Sabry, M.H. Abdel-Hay, M.H. Barary, T.S. Belal, J. Pharm. Biomed. Anal. **22** (2000) 257-266.
- [22] R. Glennon, H. Law, U.S. Patent 6,048,862, 2000.
- [23] G. Nikolić, I. Savić, V. Banković, Hem. ind. **62** (2008) 69-77.
- [24] Jugoslovenska Pharmacopoeia, 5th Ed., Savremena administracija, Beograd, 2000.
- [25] British Pharmacopoeia, Version 2, The Stationeri Office Ltd., Norwich, 1998.
- [26] European Pharmacopoeia, 3th Ed., Council of Europe, Strasbourg, 1996.
- [27] United States Pharmacopoeia, USP-NF, USA, 2000.
- [28] S. Stanković, N. Stojanović, YU Pat. 46648, 1993.
- [29] The European Agency for the Evaluation of Medicinal Products, ICH Topic Q2B Note for Guideline on Validation of Analytical Procedures: Methodology GPMP/ICH/281/95, 1996.
- [30] United States Pharmacopoeia, Validation of Compendial Methods, 26th Ed., Pharmacopoeial Convention Inc., Rockville, MD, 2003.
- [31] S. Bolton, Pharmaceutical Statistics: practical and clinical application, 3th Ed., Marcel Dekker, New York, 1997, p. 21.
- [32] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, 2th Ed., Wiley, New York, 1984, p. 83.
- [33] I. Savić, G. Nikolić, I. Savić, V. Banković, CI&CEQ **14** (2008) 261-264.
- [34] C. Hartmann, J. Smeyers-Verbeke, W. Penninckx, Y. Heyden, P. Vankeerberghen, D. Massart, Anal. Chem. **67** (1995) 4491-4499.
- [35] D. Skoog, D. West, F. Holler, Fundamentals of Analytical Chemistry, Saunders College Publishing, Philadelphia, 1996, p. 51.

IZVOD**KVANTITATIVNO ODREĐIVANJE TRIMAZOLIN HIDROHLORIDA U FARMACEUTSKIM PREPARATIMA PRIMENOM RP-HPLC METODE**Ivana M. Savić¹, Goran S. Nikolić¹, Vladimir M. Banković², Ivan M. Savić¹¹Tehnološki fakultet, Bulevar oslobođenja 124, Leskovac²Farmaceutska i hemijska industrija, Zdravlje-Actavis, Vlajkova 199, Leskovac

(Naučni rad)

Selektivna i specifična RP-HPLC metoda razvijena je i potvrđena za kvantitativno određivanje trimazolin hidrohlorida u nazalnim preparatima. Mobilna faza sastoji se od vode i acetonitrila (50:50 v/v), a UV detekcija je vršena na 270 nm. Linerni opseg važio je u oblasti koncentracija od 10 do 110 $\mu\text{g cm}^{-3}$. Metoda je testirana i potvrđena za različite analitičke parametre prema ICH upustvu. Vrednosti granice dokazivanja i kvantifikacije iznosile su 1,45, odnosno 4,8 $\mu\text{g cm}^{-3}$. Rezultati eksperimenta pokazali su da je predložena metoda precizna, tačna i reproduktivna za određivanje trimazolin-hidrohlorida u nazalnim preparatima.

Ključne reči: Trimazolin-hidrohlorid

• HPLC • Validacija

Key words: Trimazolin hydrochloride

• HPLC • Validation