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SHORT COMMUNICATION

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# THE SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF TRIMAZOLIN AND PHENYLEPHRINE HYDROCHLORIDE IN NASAL PREPARATIONS

*This paper presents the experimental results for the simultaneous spectrophotometric determination of two active components in nasal solutions. The resolution of two-component mixtures of timazolin and phenylephrine has been accomplished by using partial least-squares. The method comprised of the absorptivity measurement in a nasal solution at wavelengths of 265 and 272 nm, respectively. Notwithstanding the presence of two components and their high degree of spectral overlap, they have been determined simultaneously with high accuracy and precision, with no interference, rapidly and without resorting to extraction procedures using non aqueous solvents. This method was tested and validated for various parameters according to ICH guidelines. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation <2 %), while being simple, cheap and less time consuming. The metod was apllied for the analysis of these drug substances in their commercial pharmaceutical formulations.*

*Key words: trimazolin hydrochloride; phenylephrine hydrochloride; validation.*

In practice, a great number of nasal preparations, which most often contain ephedrine and phenyleprine as adrenergic vasoconstrictors, are commercially present today [1]. The subject of this paper 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 hydrocloride ($C_{13}H_{19}N_2Cl$, $M_w$ = 238.76 g mol$^{-1}$) or (2-(2,4,6-trimethylbenzyl)-imidazoline hydrochloride or 2-(2,4,6-trimethylbenzyl)-1,3-diazacyclopenten-(2)-hydrochloride is an alpha-adrenergic (decongestant) agent which stimulates alpha-adrenergic receptors (Figure 1), producing pronounced vasoconstriction [2]. It is a white, crystalline, almost odourless, bitter substance, freely soluble in water, slightly soluble in ethanol, insoluble in ether and acetone with the melting point of 279-281 °C. A 10 % aqueous solution has a pH of about 5.5-6.5 [3].

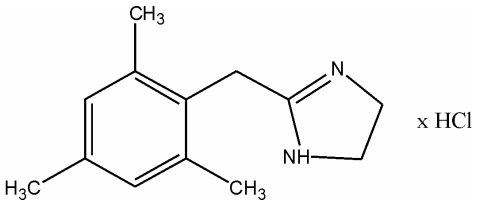


*Figure 1. Chemical structure of trimazolin hydrochloride.*

The trimazolin-phenylephrine mixture is not yet official in any pharmacopoeia. The patent literature has data on only the trimazolin and trimazolin hydrochloride synthesis procedure, without physicochemical, spectroscopic characterization and other investtigation methods [4]. To our knowledge, no analytical methods could be traced for the analysis of trimazolin hydrochloride or trimazolin-phenylephrine combination in a pharmaceutical dosage form. Therefore, a simple, rapid and reliable method for the simultaneous assay of both components in the mixture seemed to be necessary. The development of UV spectrophotometric methods can provide a very useful alternative for the routine analysis of nasal formulations. The objective of the present study was to develop simple, precise, accurate and economic analytical methods for the estimation of trimazolin hydrochloride and phenylephrine hydrochloride in a pure form and in phar-

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maceutical formulations. The developed analytical method was validated as per ICH guidelines [5,6], and Serbian requirements [7]. Statistical tests were performed on validation data [8,9].

## MATERIALS AND METHODS

### Apparatus

A double-beam Varian Cary-100 Conc UV-Vis spectrophotometer connected to the computer and loaded with Cary WinUV software was used for all absorbance measurements and data treatment. The instrument has an automatic wavelength accuracy of 0.1 nm and matched quartz cells of the10 mm cell path length.

### Chemicals

The standards of trimazolin hydrochloride (99.91 %) and phenylephrine hydrochloride (99.97 %) were obtained as gift samples from the Pharmaceutical and Chemical Industry Zdravlje-Actavis (Leskovac, Serbia), and used without further purification. All other chemicals and reagents used were of analytical grade (Merck Chem. Ind.).

### Pharmaceutical preparation

A commercial pharmaceutical preparation for children, Adrianol-T nasal drops, labeled to contain 0.5 mg $cm^{-3}$ of trimazolin hydrochloride and 0.5 mg $cm^{-3}$ of phenylephrine hydrochloride. Adrianol-T contains 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.

### Calibration graphs

The first series of calibrated flasks contained a varying concentration of trimazolin hydrochloride (10--60 μg $cm^{-3}$) and a constant concentration of phenylephrine hydrochloride. The second series contained a varying concentration of phenylephrine hydrochloride (10-60 μg $cm^{-3}$) and a constant concentration of trimazolin hydrochloride. Trimazolin hydrochloride and phenylephrine hydrochloride were estimated at 265 and 272 nm in aqueous medium.

### Sample preparation

The aliquot (1 $cm^3$) of Adrianol T equivalent to 0.5 mg trimazolin hydrochloride and 0.5 mg of phenylephrine hydrochloride was taken and suitably diluted with water to get a 50 μg $cm^{-3}$ concentration and the sample was analyzed using proposed analytical methods.

### Spectrophotometric measurements

*Absorbance ratio method*

Such a method of analysis is based on the linear relationship between the absorbance value of a binary mixture and the relative concentration of such a mixture. The quantification analysis of trimazolin hydrochloride and phenylephrine hydrochloride in the binary mixture is performed by using following equations (1) and (2):

$$A_1 = f_{11}c_1 + f_{12}c_2 \quad (1)$$

$$A_2 = f_{21}c_1 + f_{22}c_2 \quad (2)$$

where: $A_1$ and $A_2$ denotes the absorbances of the nasal solution (Adrianol T) measured at $\lambda_1$ (265 nm) and $\lambda_2$ (272 nm), $c_1$ and $c_2$ denotes the concentrations of trimazolin hydrochloride and phenylephrine hydrochloride, respectively. $f_{11}$ and $f_{12}$ denotes the slopes of regression equations of trimazolin hydrochloride and phenylephrine hydrochloride at $\lambda_1$. $f_{21}$ and $f_{22}$ denotes the slopes of regression equations of trimazolin hydrochloride and phenylephrine hydrochloride at $\lambda_2$.

## RESULTS AND DISCUSSION

The UV spectra of trimazolin and phenylephrine hydrochloride in the aquous medium and UV spectrum of Adrianol T are shown in Figure 2.

In particular, the absorbance at 265 nm for trimazolin hydrochloride and at 272 nm for phenylephrine hydrochloride were considered as the optimum working wavelengths for their determination. The best linear response to analyte concentrations is achieved by using these wavelengths. The regression curve was calculated by the least-squares method. Beer's law was obeyed in the concentration range from 10 to 60 μg $cm^{-3}$ of trimazolin hydrochloride and phenylephrine hydrochloride.

The absorption spectra of trimazolin hydrochloride and phenylephrine hydrochloride were not changed in the presence of common excipients used in the pharmaceutical preparations. The calculated *t*-values were found to be (0.814 for trimazolin hydrochloride at 265 nm and 1.45 for phenylephrine hydrochloride at 275 nm) less than that of the tabulated *t*-values (2.225). Therefore, the proposed analytical method is specific and selective for the drug.

The linearity range for trimazolin hydrochloride at 265 nm estimation was found to be 10-60 μg $cm^{-3}$ ($r$ = 0.9992) and 10-60 μg $cm^{-3}$ ($r$ = 0.9993) for phenylephrine hydrochloride at 275 nm. Goodness of the fit of the regression equations was supported by high regression coefficient values. The accuracy ranged

from 40 to 60 μg cm$^{-3}$. The excellent mean percenttage recovery values, close to 100 %, and their low standard deviation values (*SD* < 1.0) represent high accuracy of the analytical methods. The validity and reliability of the proposed methods was assessed by the recovery studies. The mean percenttage recoveries (*RSD*) for lower, intermediate and higher concentrations were found to be 99.63 (40 μg cm$^{-3}$), 99.50 (50 μg cm$^{-3}$) and 100.08 (60 μg cm$^{-3}$) for trimazolin hydrochloride and 100.57 (40 μg cm$^{-3}$), 99.60 (50 μg cm$^{-3}$) and 100.33 (60 μg cm$^{-3}$) for phenylephrine hydrochloride, respectively. The validity and reliability of the proposed methods was further assessed by recovery studies *via* the standard addition method. The mean percenttage recoveries for the concentration from 50 μg cm$^{-3}$ were found to be 99.74 (0.58), 100.50 (0.64) and 99.84 (0.53) for trimazolin hydrochloride and 100.53 (0.61), 100.87 (0.49) and 99.04 (0.73) for phenylephrine hydrochloride, respectively. These results revealed that any small change in the drug concentration in the solutions could be accurately determined by the proposed analytical methods.

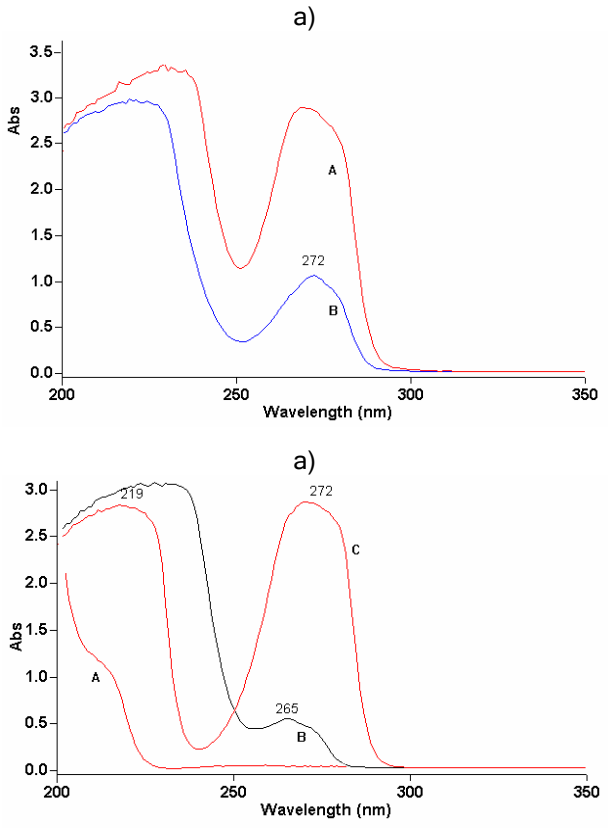


*Figure 2. UV-absorption spectra of a) Adrianol (A) and Adrianol-T (B) nasal drops preparation and b) excipiens of Adrianol T (A), trimazolin hydrohloride (B) and phenylephrine (C).*

The precision was determined by studying the repeatability and the intermediate precision. The repeatability (*RSD*) ranged from 40 to 60 μg cm$^{-3}$. 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 intermediate precision study, *RSD* values were not more than 2.0 % in all the cases. *RSD* values found for the proposed analytical method were well within the acceptable range indicating that the method has an excellent repeatability and the intermediate precision.

*LOD* and *LOQ* were found to be 2.758 and 8.276 μg cm$^{-3}$ for trimazolin hydrocloride at 265 nm, and 2.54 and 7.62 μg cm$^{-3}$ for phenylephrine hydrocloride at 272 nm.

By measuring the absorbance values at 265 nm ($\lambda_{max}$ for trimazolin hydrochloride ) and 272 nm ($\lambda_{max}$ for phenylephrine hydrochloride) in the nasal preparation (Adrianol T), the analysis of the binary mixture containing trimazolin hydrochloride and phenylephrine hydrochloride were made by using formulas (Eqs. (1) and (2)).

The assay values of trimazolin hydrochloride and phenylephrine hydrochloride in synthetic mixtures were found 100.50 and 100.12 %, respectively. The standard deviation was not more than 0.41 % for trimazolin hydrochloride and 0.09 % for phenylephrine hydrochloride.

The assay values of formulations were the same as mentioned in the label claim indicating that the interference of the excipient matrix is insignificant in the estimation of trimazolin hydrochloride and phenylephrine hydrochloride by proposed analytical methods. The estimated drug content with low values of the standard deviation established the precision of the proposed method. The calculated student's *t*-values (1.38 of trimazolin hydrochloride and 1.09 of phenylephrine hydrochloride) did not exceed the tabulated values (theoretical values at 95 % confidence limits is $t$=2.225).

## CONCLUSIONS

The method proposed is simple, rapid and direct. 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.This paper demonstrates the potential of the spectroscopy method as an analytical technique and its usefulness to accurately, rapidly, simply and simultaneously quantitate the active ingredients

in multicomponent pharmaceuticals. Moreover, the present method is fast with respect to the analysis time as compared to sophisticated chromatographic techniques.

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