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Research Article

A Novel Analytical Method for Determining a Combination of Tetrahydrozoline and Antazoline HCl by Modulating Ratio Spectra

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Abstract

In this study, the combination of pharmaceutical formulation of tetrahydrozline HCl (TZ) and antazoline HCl (AN) was determined without separating them using smart analytical UV spectrophotometric methods. While the Extended Ratio Subtraction Method (EXRSM) is used to determine AN, the Ratio Subtraction Method (RSM) is utilized to determine TZ and is linked with the ratio subtraction technique. The calibration curves for AN and TZ are linear, ranging from 3.0 to 30.0 µg/mL and 5.0 to 45.0 µg/mL, respectively. Analyzing several laboratory-prepared combinations of the two medications allowed researchers to assess the specificity of the designed methods. The selected drugs' combined dosage form was determined with success using both approaches. Validation was carried out in accordance with ICH requirements, and it was found that repeatability, accuracy, and intermediate precision were all within acceptable limits. Statistical studies showed that both methods can be competitively applied in quality control laboratories. RSM and the EXRSM are complementary to one another, as shown by the determination of AN and TZ without pre-separation. Without any prior separation, the EXRSM was able to differentiate between substances with an extended spectrum using the same characteristics. Therefore, one alternative approach to liquid chromatography techniques is the combination of EXRSM and RSM.

Keywords

Antazoline, Tetrahydrozoline, Ratio Subtraction, Extended Ratio Subtraction, Spectrophotometry

1. Introduction

Being a first-generation antihistaminic drug with anti-arrhythmic properties [1]; antazoline HCl (AN) (Figure 1a) is a 4,5-dihydro-N-phenyl-N-(phenylmethyl)-1H-imidazole-2-methanamine hydrochloride. AN is an antagonist for the H1 receptor [2]; it causes reversible competition on the H1 receptor [3, 4]. Its anticholinergic effect raised it to be used in nasal congestion relief or the treatment of allergic conjunctivitis symptoms.

Tetryzoline (or tetrahydrozoline) (TZ) (Figure 1b) is 2-(1,2,3,4-tetrahydronaphthalen-1-yl)-4,5-dihydro-1H-imida

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zole; hydrochloride and was invented in 1954 to be used starting from 1959 as an over-the-counter eye/nasal drop [5]. TZ is mainly an agonist for both alpha-2 receptor and imidazoline receptor I-1 [6]. TZ's action is hailed by the sympathomimetic agent combined with the alpha-adrenergic activity [7]. For eye drops; it is used to constrict the blood vessels and hence to reduce the redness of the eye [8]. Commonly a combination between AN and TZ is employed in ocular use to relieve symptoms of conjunctivitis [9].

Using multiple techniques, including reversed-phase ion-pair high-performance liquid chromatography, HPLC, and HPTLC, both AN & TZ are simultaneously measured in their pharmaceutical preparation without pre-separation [10-15]. However, because TZ's absorption spectra lacks a significant peak, particularly at low concentrations, not many studies are reporting the spectrophotometric methods for the simultaneous preparation of these two molecules. Four spectrophotometric approaches were recently reported by our lab [4] for the simultaneous determination of the binary mixture of AN & TZ without the need for preparatory separation [4].

In this study, two novel spectrophotometric methods for the determination of the two components of the ocular pharmaceutical preparation: Antazoline HCl and Tetrahydrozyline HCl were investigated.

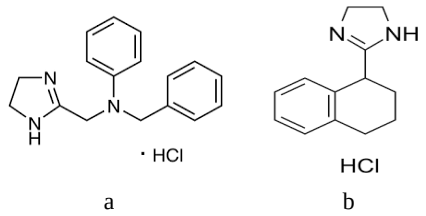

Figure 1. *Chemical structure of (a) Antazoline HCl and (b) Tetryzoline HCl.*

2. Materials and Methods

2.1. Instrumentation

The instrument utilized was a Shimadzu UV-2400 PC Series Spectrophotometer (Tokyo, Japan), which was supported by two identical 1 cm quartz cells. The gadget only had one fast scan mode, with 2 nm spectrum band and 2800 nm/min scanning speed at intervals of 0.1 nm. An IBM-compatible computer was connected to the spectrophotometer. Version 3.7 of the UVPC personal spectroscopic software was used, and an HP desk jet printer was included. This system was employed for absorbance measurements and treatment of data.

2.2. Chemicals and Reagents

chemicals and solvents utilized were of analytical grade; methanol was supplied from Fisher Scientific, UK. Sodium hydroxide was supplied from ADWIC [Cairo- Egypt].

2.3. Authentic Samples

Pure samples of AN and TZ were supplied from Shanghai Yurui Pharmaceutical Co., Ltd, China, with purity of 99.78% ± 1.007 and 99.79% ± 1.487 for AN and TZ, respectively. Benzalkonium chloride was supplied from Orchidia Co. Pharma (Cairo, Egypt).

2.4. Pharmaceutical Dosage Form

The pharmaceutical formulation was supplied by Orchidia Co. Pharma through their product: "Trillerg" ophthalmic drops.

It is claimed that each mL contains 0.5 mg of AN, 0.4 mg of TZ and 0.05 mg of benzalkonium chloride.

2.5. Stock Solutions

Transfer 10 mg of each authentic drug to a separate 100-mL volumetric flask followed by the addition of 50.0 mL of 0.1 N methanolic NaOH. The volume was then completed by the same solvent to get stock solutions 0.1mg/mL of AN and TZ

2.6. Procedure

2.6.1. The Spectral Properties of AN and TZ

Two aliquots equivalent to 300.0 µg of both AN & TZ were separately transferred from their stock solutions (0.1 mg/mL) into two 10-mL volumetric flasks. After dilution with 0.1 N methanolic NaOH; the zero-order (D_0) absorption spectra were measured in range of 200 – 400 nm using 0.1 N methanolic NaOH as blank (Figure 2).

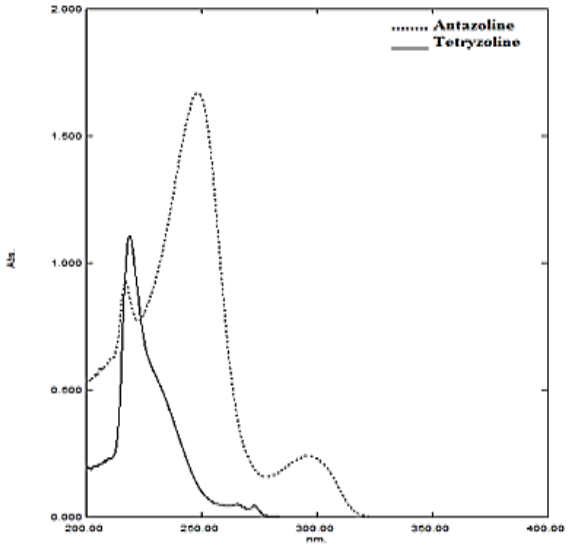

Figure 2. *Zero order absorption spectra of AN (30.0 µg/mL) (- - -) and TZ (30.0 µg/mL) (—) in 0.1 N methanolic NaOH.*

2.6.2. Construction of Calibration Curves

Different aliquots equivalent to 30.0 – 300.0 µg and 50.0 – 450.0 µg of AN and TZ, respectively were accurately transferred from their stock solutions (0.1 mg/mL) into two separate sets of 10-mL volumetric flasks then completed to the volume with 0.1 N methanolic NaOH. The spectra of the standard solutions were scanned from 200 to 400 nm and saved in the computer.

In case of extended ratio subtraction method (EXRSM) coupled with the RSM; the calibration curves represented the absorbance of zero-order spectra of AN at 248.0 nm and TZ at 219.0 nm against the corresponding concentrations and the regression equations were measured.

2.6.3. Application to Laboratory Mixtures

To get different ratios of the two drugs (including the commercial formulation), aliquots corresponding to 50.0–250.0 µg/mL of AN and 250.0–50.0 µg/mL of TZ were transferred from their stock solutions (0.1 mg/mL) into a set of 10-mL volumetric flasks. The volume was then finished using 0.1 N methanolic NaOH. The computer was used to store the spectra of the created mixes, which were scanned between 200 and 400 nm.

For analysis of TZ by RSM, zero-order absorption spectrum of each laboratory prepared mixture was divided by the absorption spectrum of standard AN' (30.0 µg/ mL) (Figure 3). Next, the amplitude in the plateau region at λ 278.0–311.0 nm (the constant) was recorded and subtracted from the resultant ratio spectra (Figure 4).

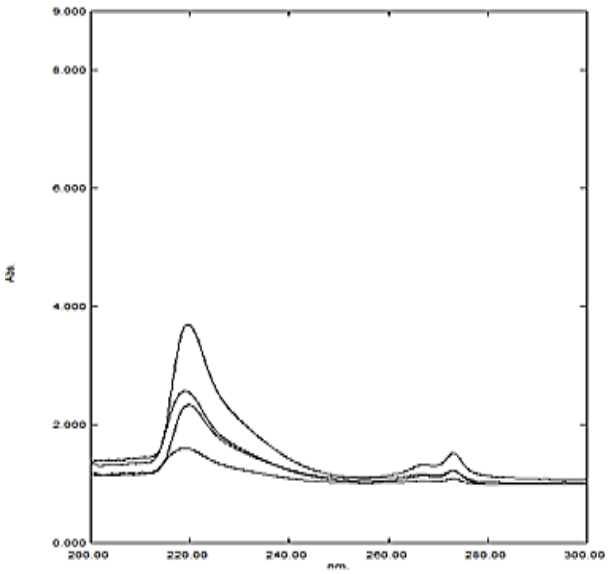

Figure 3. *Ratio spectra of laboratory mixtures of TZ (X) and AN (Y) using 30.0 µg/mL AN (Y') as a divisor.*

Then obtained ratio spectra was then multiplied by the spectra of AN' (30.0 µg/ mL), to yield the zero spectra of TZ (Figure 5). Accordingly, the concentration of TZ was calculated using the corresponding regression equation at its λ_{max}.

For determination of AN by EXRSM, Zero-order absorption spectrum of each obtained TZ was divided by the absorption spectrum of TZ' (45.0 µg/mL as a divisor to determine the constant values at plateau region (223.0–270.0 nm) (Figure 6), then zero-order absorption spectrum of each laboratory prepared mixture was divided by the absorption spectrum of TZ' (45.0 µg/mL) as a divisor (Figure 7).

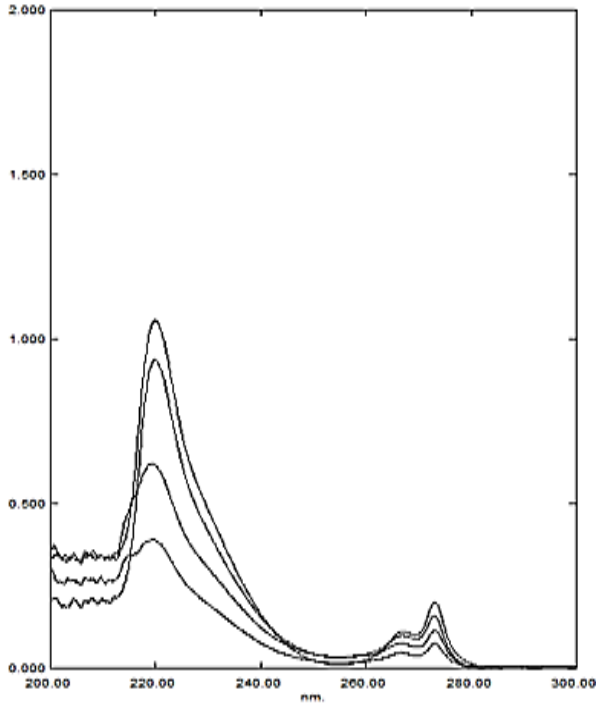

Figure 4. *Ratio spectra of laboratory prepared mixtures of TZ (X) and AN (Y) using 30.0 µg/mL AN (Y') as a divisor after subtraction of the constant.*

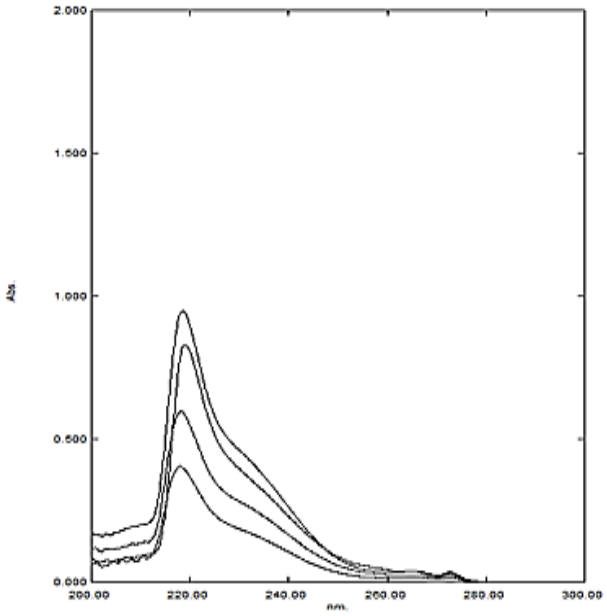

Figure 5. *The zero order absorption spectra of TZ by the proposed ratio subtraction method after multiplication by the divisor (Y').*

Then subtract the spectra obtained in figure 6 from spectra in Figure 7. Later, the same process as RSM were followed using TZ' (45.0 µg/mL) as a divisor. The concentration of AN in all mixtures is calculated using the corresponding regression equation at its λmax.

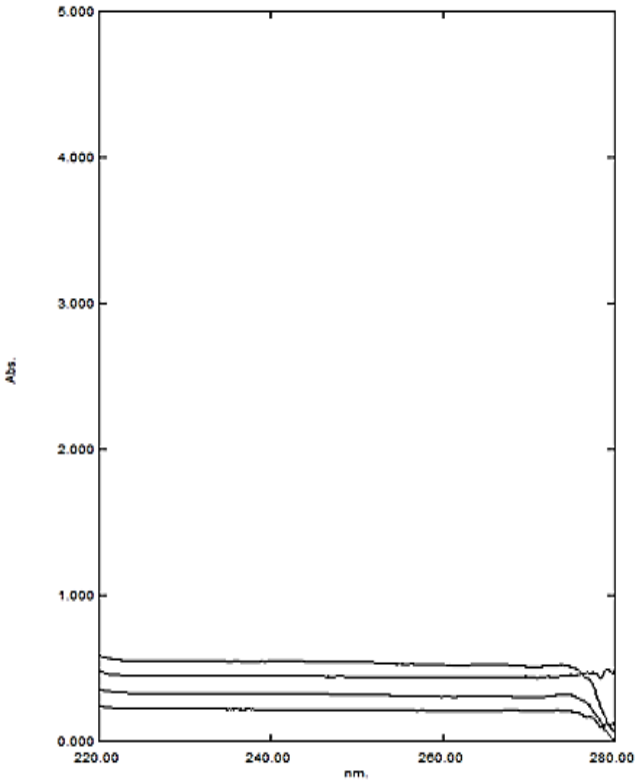

Figure 6. *Ratio spectra of obtained spectra of TZ (X) using 45.0 µg/mL TZ (X') as a divisor and 0.1 N methanolic NaOH as a blank.*

2.6.4. Application to a Pharmaceutical Dosage Form

To analyze the concentration of AN and TZ in ophthalmic drops (labelled to contain 0.5 mg AN and 0.4 mg TZ in each mL); ten eye drops solution were mixed and an accurate volume equivalent to 10.0 mg AN and 8.0 mg TZ was transferred to a 100-mL volumetric flask, then the volume was completed with 0.1 N methanolic NaOH to get concentration of 0.1 mg/mL of AN and 0.08 mg/mL of TZ. From the prepared solution, further dilutions were prepared to get final concentration of 10.0 µg/ mL AN & 8.0 µg/mL TZ.

As described before, the general procedures were followed to determine the concentration of both drugs in their pharmaceutical dosage form. The concentrations of AN and TZ in the prepared samples were calculated from the corresponding regression equations. Using the standard addition approach, the eye drop's solution content was combined with varying amounts of authentic AN and TZ before carrying out the aforementioned procedure.

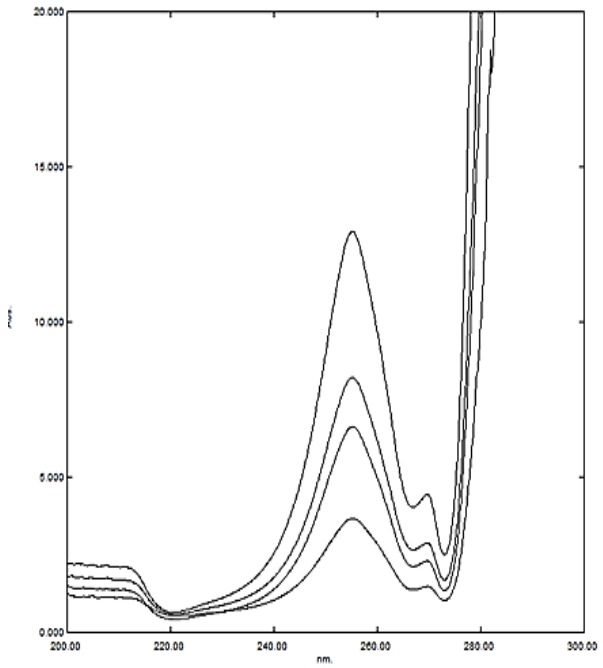

Figure 7. *Ratio spectra of laboratory mixtures of AN (Y) and TZ (X) using 45.0 µg/mL TZ (X') as a divisor.*

3. Results

3.1. UV-Spectrophotometric Multicomponent Analysis

Commonly, when a multicomponent analysis takes place using spectrophotometric analysis; a bias happens due to the interference between the interested analyte and the co-formulated compounds which may show absorbance in the same spectral range [16, 17]. This spectrum overlap necessitates using mathematical techniques for resolution [17, 18]. In this work, the authors investigated the application, validation and comparison of two spectrophotometric methods to determine AN & TZ in their dosage form: "Trillerg". In this formulation, benzalkonium chloride is added as a preservative which may cause spectral interference. In consequence, we investigated numerous mixtures with the same ratio as mentioned in the formulation and even up to 10 times of benzalkonium chloride concentration. Luckily, the preservative showed no absorbance in the UV-spectrum at low concentration as mentioned in Clarke's Analysis of Drugs [19] and British Pharmacopoeia (the absorbance of 10 µg is 0.0049 and 0.0058 at 256 nm and 262 nm, respectively). As seen in (Figure 2), the zero order absorption spectra of pure drugs exhibited overlap, making it difficult to determine them directly. Consequently, the chosen techniques demonstrate easy to use and capable of resolving the interfering component over the entire spectrum. Furthermore, the ratio spectra were returned to their initial zero order one (RSM and EXRSM) by the final mathematical operations.

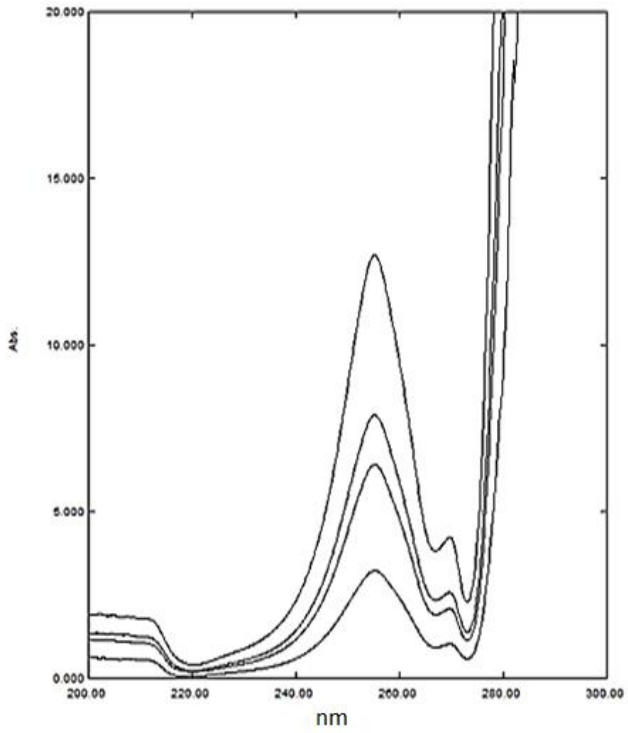

Figure 8. *Ratio spectra of laboratory mixtures of AN (Y) and TZ (X) using 45.0 µg/mL TZ (X') as a divisor after subtraction of the constant.*

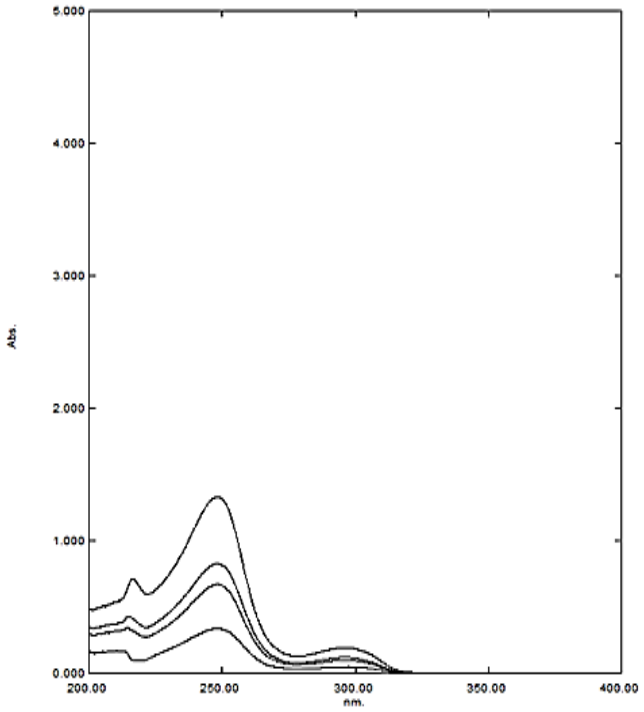

Figure 9. *The zero order absorption spectra of AN (Y) obtained by the proposed extended ratio subtraction method for the analysis of laboratory prepared mixtures after multiplication.*

3.2. Ratio Subtraction Method and Extended Ratio Subtraction Method Coupled with Ratio Subtraction Method

Simply, the RSM is based on determining TZ in a mixture of AN and TZ (AN is more extended) (Figure 2) through scanning the zero-order absorption spectra of mixtures of AN and TZ, then dividing them by chosen concentration of standard AN' (30.0 µg/mL) as a divisor. Notably, the authors select the concentration of the divisor that gives the best regression in the proposed concentration range. Consequently, ratio spectra obtained represents TZ/AN + constant, (Figure 3). Later, the constants (AN/AN') will be subtracted in the plateau region (278.0–311.0 nm) (Figure 4), then multiply the spectra obtained by the divisor AN' (30 µg/mL) to get the original zero-order spectra of TZ, (Figure 5). Through the corresponding regression equation at 219.0 nm; the concentration of TZ will be finally concluded. Furthermore, extended ratio subtraction method (EXRSM) can detect the concentration of AN in the mixture. For instance, the obtained TZ spectra are divided by a discreetly selected concentration of standard TZ' (45.0 µg/mL) to get a ratio spectrum that represent the constants TZ/TZ' in the plateau (223.0–270.0 nm); (Figure 6). The previously scanned zero-order absorption spectra of mixtures (AN and TZ) are divided by standard TZ' (45.0 µg/mL) as a divisor to get new ratio spectra which represent AN/TZ' + constant as shown in (Figure 7). Later, the values of constants TZ/TZ' are subtracted; (Figure 8). Next step is multiply the obtained spectra by the divisor TZ' (45.0 µg/mL) to get zero-order spectra of AN, as represented in (Figure 9). Finally, the obtained spectra could be used to determine AN and calculate its concentration from the corresponding regression equation at 248.0 nm.

4. Discussion

Starting with ratio subtraction method (RSM); that is described by dividing the spectrum of the overlapped mixture of X and Y by a certain concentration of the extended drug (Y) as a divisor Y' [17, 18, 20]. The resulting new curve represents X/Y' + constant. If this constant is calculated (the constant is parallel to the wavelength axis in the region of extended Y); we will have a new curve after subtracting the constant. By multiplying the obtained ratio spectrum with the divisor Y', we will get zero-order spectrum of component X.

The following equations can summarize this process:

$$(X+Y)/Y' = X/Y' + Y/Y' = X/Y' + \text{constant}$$

$$X/Y' + \text{constant} - \text{constant} = X/Y'$$

$$X/Y' \times Y' = X$$

As for extended ratio subtraction method, it aims to quantify the drug with more extended spectrum; Y [18, 19]. It

depends on dividing the D_0 spectrum of X (less extended component) by the known concentration of X' to get X/X' for each concentration in the mixtures. The later steps follow the same procedure of the ratio subtraction.

$$Y/X' \times X' = Y$$

Through the regression equations (by plotting the absorbance of the zero order spectra of each drug at its λ_{max} against its corresponding concentrations); so we get the concentration of both X & Y.

Both methods are complementary to each other due to the ability to determine each drug in binary mixture [16]. Advantageously, the extended ratio subtraction method can determine the extended drug in mixture at its λ_{max} which was not possible by the RSM [17]. Nevertheless, there are some challenges in the proposed methods as selecting the suitable divisor and the plateau region for the constant calculation [18]. Hence, it is advised to try different divisors' concentration to have the most suitable results. It should be noted that the linear response were obtained between the absorbance and the corresponding concentrations in the range of 3.0 – 30.0 µg/mL and 5.0 – 45.0 µg/mL for AN at 248.0 nm and TZ at 219.0 nm, respectively. The regression equations were computed and found to be:

$$AAN = 0.0552C+0.0047 \quad r = 0.9998$$

$$ATZ = 0.0338C+0.0706 \quad r = 0.9992$$

where A is the absorbance, C is the concentration in µg/mL and r is the correlation coefficient. The mean percentage recoveries were 100.09% ±0.815 and 100.11% ±1.21, for AN and TZ, respectively.

Method validation

Accuracy

Methods accuracies were checked by applying the proposed methods for the determination of different concentrations of pure AN and TZ within the linearity range. The concentrations were calculated from the regression equations, then calculate mean recovery percentage was (Table 1).

Precision

Repeatability

Using the previously mentioned procedure under linearity, three concentrations (10.0, 20.0 and 30.0 µg/mL) of AN and TZ were separately analyzed three times each, intra-day, and relative standard deviation was then calculated (Table 1).

Reproducibility (Intermediate Precision)

The above mentioned AN and TZ samples were analyzed on three successive days using the procedure stated under linearity. Relative standard deviation was then calculated (Table 1).

Table 1. *Parameters and validation sheet for determination of the cited compounds by the proposed methods.*

Parameters	AN	TZ
Slope [a]	0.0552	0.0338
Slope S.E.	0.000324	0.000495
Intercept [a]	0.0047	0.0706
Intercept S.E.	0.006043	0.013929
Correlation coefficient	0.9998	0.9992
Concentration range µg/mL	3 - 30	5 – 45
Accuracy Average (%)	100.09	100.11
S.D.	0.816	1.211
R.S.D. %	0.815	1.210
Repeatability [b] R.S.D %	0.431	0.293
Intermediate precision [c] R.S.D %	0.287	0.673
LOD µg/mL	0.70	1.30
LOQ µg/mL	2.10	3.90

[a] Results of five determinations
[b] = 3 concentrations ×3 replicates
[c] = 3 concentrations ×3 replicates

Selectivity

To assess the selectivity and applicability of the proposed methods; recovery studies were performed by analyzing laboratory prepared mixtures of the two drugs in different ratios including the commercial product ratio as shown in Table 2.

Table 2. *Results of analysis of AN and TZ in laboratory prepared mixtures both drugs in pure powder form by the proposed methods.*

Ratios	Antazoline HCl (Recovery * % ±SD)	Tetryzoline HCl (Recovery * % ±SD)
	ERSM at 248.0 nm	RSM at 219.0 nm
2:1	100.02 ±0.231	99.36 ±0.933
1:1	100.28 ±0.491	101.49 ±0.783
1.25:1**	99.19 ±0.534	101.15 ±0.124

Ratios	Antazoline HCl (Recovery * % ± SD) ERSM at 248.0 nm	Tetryzoline HCl (Recovery * % ± SD) RSM at 219.0 nm
1:2	100.77 ± 1.201	98.29 ± 0.942
MEAN	100.07	100.07
SD	0.661	1.512
RSD%	0.660	1.510

* Five determinations average
**The ratio in Trillerg Eye drop.

The proposed spectrophotometric methods were successfully applied for the determination of AN and TZ in their pharmaceutical dosage form (Trillerg eye drop) as described in Table 3. Furthermore, the validity of the methods was checked by applying the standard addition technique (Table 3). It shows that the developed methods are accurate and specific for determination of the cited drugs in formulated dosage form without the interference of the pharmaceutical excipients.

Table 3. *Determination of AN and TZ in pharmaceutical dosage form and application of standard addition technique by the proposed methods.*

Dosage form	Antazoline HCl (ERSM at 248.0 nm)			
	Found%* ± S.D.	Conc. taken	Standard added	Recovery % of added**
Trillerg Eye drop	100.40% ± 0.566	10.0 µg/mL	5.0 µg/mL	99.76
			10.0 µg/mL	99.04
			20.0 µg/mL	99.95
Mean	99.58			
S.D.	0.480			
R.S.D.	0.482			
	Tetryzoline HCl (RSM at 219.0 nm)			
	Found %* ± S.D.	Conc. taken	Standard added	Recovery % of added**
	100.30% ± 0.953	8.0 µg/mL	5.0 µg/mL	100.64
			10.0 µg/mL	99.64
			20.0 µg/mL	99.11
Mean	99.79			
S.D.	0.777			
R.S.D.	0.779			

*Six determinations average
**Three determinations average

Results of the suggested methods for determination of AN and TZ were statistically compared with those obtained by applying the reported HPTLC method. The calculated t-and F-values were found to be less than the corresponding theoretical ones (Table 4), confirming good accuracy and excellent precision [21].

Table 4. *Statistical analysis between the results obtained for the determination of AN and TZ in pure samples by the proposed methods and those obtained by the reported method [9].*

Parameters	Developed Method		Reported Method**	
	AN	TZ	AN	TZ
Mean	100.09	100.11	99.78	99.79
S.D	0.816	1.211	1.008	1.487
R.S.D.%	0.815	1.210	1.007	1.487
Variance	0.6659	1.4665	1.0160	2.2111
n	6	6	6	6
Student's t	0.5855 (2.228) *	0.4086 (2.228) *		
F test	1.5258 (5.05) *	1.508 (5.05) *		

* Values in parenthesis are theoretical values of t and F at (p = 0.05). ** HPTLC method using aluminum plates precoated with silica gel 60 F254. The solvent system consisted of ethylacetate: methanol: ammonia (10:10:1, v/v/v). Analysis of drugs was carried out in the absorbance mode at 216 nm.

5. Conclusion

In conclusion, we confirm that RSM is a straightforward and authentic method without a need for complicated apparatus nor advanced software. It is characterized by high sensitivity and accuracy as it measures the drug directly at its characteristic maximum wavelength. Nevertheless, it is limited by inefficiency to determine the compounds with extended spectrum. Through the determination of AN and TZ without previous separation, we can state that RSM and the EXRSM are complementary for each other. With the same features, the EXRSM could distinguish compounds with extended spectrum without prior separation. Hence, the EXRSM coupled with RSM can be an alternative method for separation chromatographic techniques.

Abbreviations

ICH	International Council of Harmonization
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
UV	Ultraviolet
LOD	Limit of Detection
LOQ	Limit of Quantification

Author Contributions

Lamia Mohammed Abd Elhalim: Data curation, Formal Analysis, Methodology, Resources, Software, Validation, Writing – original draft

Nesrin Khamis Ramadan: Conceptualization, Project administration, Supervision, Writing – review & editing

Mohammed Khaled Abd El Rahman: Conceptualization, Data curation, Investigation, Project administration, Software, Supervision, Visualization

Maha Mohammed Galal: Data curation, Investigation, Visualization, Writing – review & editing

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Data Availability Statement

The data is available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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