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Determination of tetrahydrozoline hydrochloride and fluorometholone in pharmaceutical formulations by HPLC and derivative UV spectrophotometry

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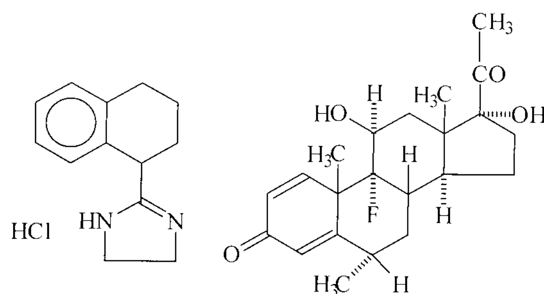
Two methods for the quantitative determination of tetrahydrozoline hydrochloride (**1**) and fluorometholone (**2**) in pharmaceutical eye drops (Efemoline®) are described. The procedures are based on derivative UV spectrophotometry and HPLC. In the former method, $d^2A/d\lambda^2$ values were measured in methanol at 226 and 282 nm for **1** and **2**, respectively. The relative standard deviations for the method were found to be 1.06% for **1** and 0.98% for **2**. The latter method based on a reversed phase HPLC system using a Partisil 5 ODS analytical column. The mobile phase used for the separation of **1**, **2** and internal standard (lidocaine) was methanol/acetonitrile/water (50 : 50 : 10 v/v) and the compounds in the eye drops were detected at 220 nm using an UV detector. The relative standard deviations for the HPLC method were determined to be 0.61% and 0.50% for **1** and **2**, respectively. The proposed methods, which give thoroughly comparable data, are simple, rapid, and allow precise and accurate results and could be used for commercial formulations containing tetrahydrozoline hydrochloride and fluorometholone in combination.

1. Introduction

Tetrahydrozoline hydrochloride [2-(1,2,3,4-tetrahydro-1-naphthyl)-2-imidazoline monohydrochloride (**1**)] a sympathomimetic agent with marked α-adrenergic activity has been introduced in some ophthalmic solutions to replace the more widely used naphazoline [1]. Fluorometholone [9α-fluoro-11β,17α-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione (**2**)] is used as an anti-inflammatory local steroid in pharmaceutical products [2].

Although several analytical procedures [3–13] have been developed for the determination of or stability of either **1** or **2** in pharmaceutical preparations separately or in combination with other drugs, no method has been reported to achieve the simultaneous quantification of the two components in mixtures.

This paper describes a RP-HPLC and second derivative UV spectrophotometric method that can be routinely used to assay **1** and **2** simultaneously in an ophthalmic solution.



Tetrahydrozoline Hydrochloride (**1**)

Fluorometholone (**2**)

2. Investigations, results and discussion

2.1. Analysis of tetrahydrozoline hydrochloride and fluorometholone by derivative UV spectrophotometry

Fig. 1 shows the zero-order UV spectra of **1**, **2**, and a mixture of **1** and **2**. Due to the extensive overlap of the spectral bands of two drugs, conventional UV spectrophotometry cannot be used for their individual determination in a binary mixture. Derivative spectra of different orders

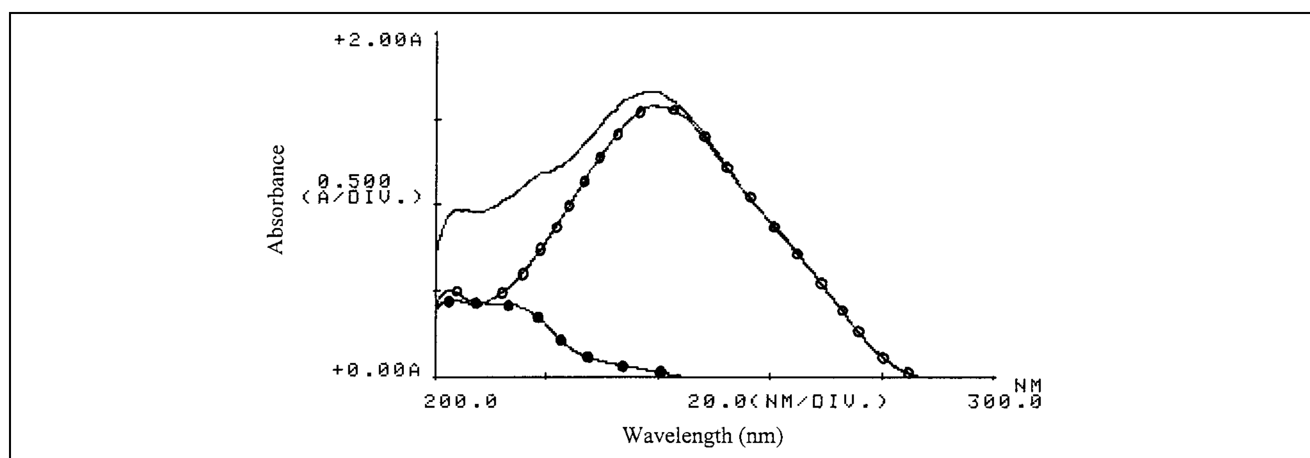


Fig. 1: Zero-order UV spectra of 10 μg/ml tetrahydrozoline hydrochloride (●), 40 μg/ml fluorometholone (○), and its binary mixture (–), in methanol

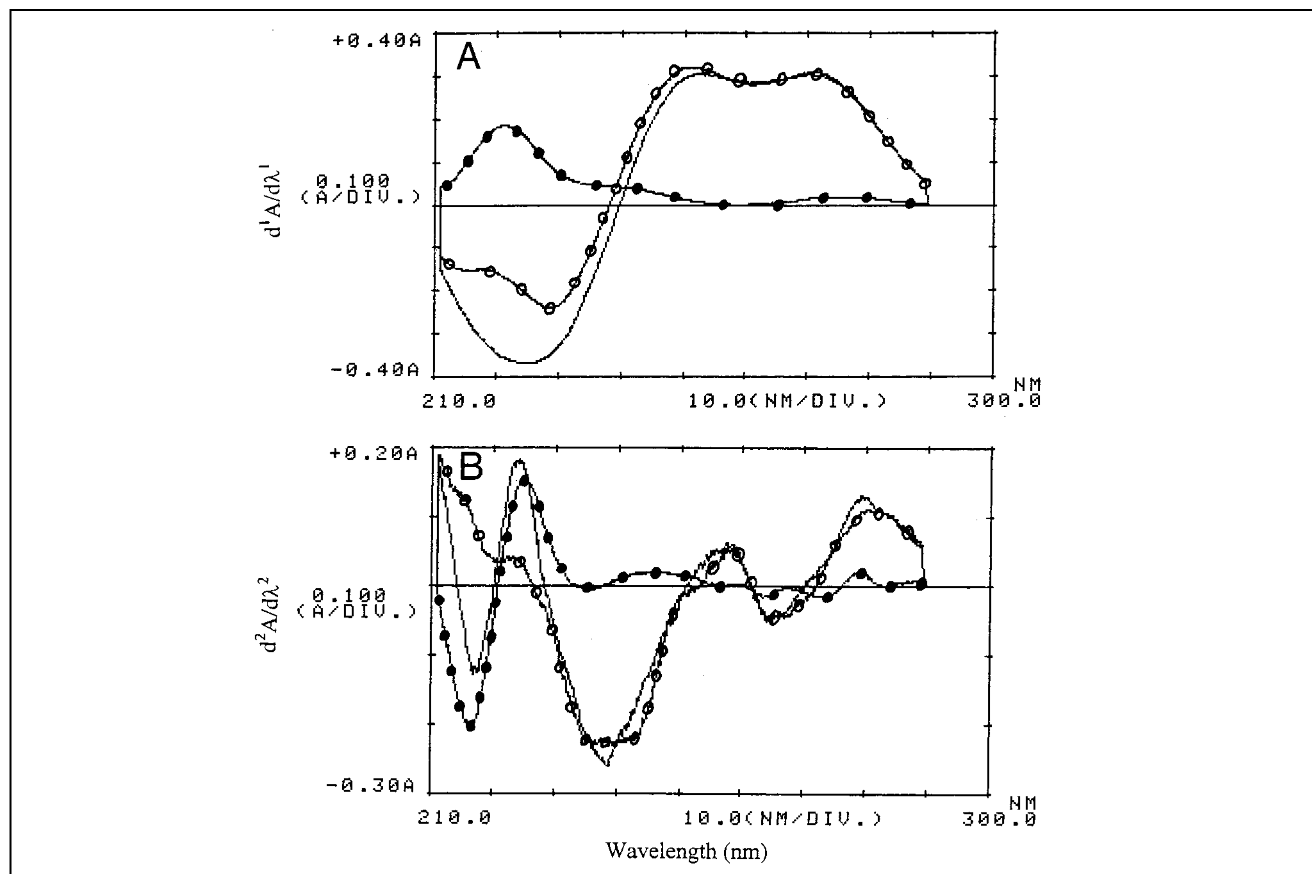


Fig. 2.: First-order (A), second-order (B) derivative UV spectra of 10 μg/ml tetrahydrozoline hydrochloride (●), 40 μg/ml of fluorometholone (○), and its binary mixture (−), in methanol ($\Delta\lambda = 12.6$ nm)

were obtained using smoothed spectra using **1**, **2** and their binary solutions (Fig. 2). First derivative spectra was not found to be resolved as can be seen in Fig. 2. However, zero-crossing second derivative spectrophotometry permits a more selective identification and determination of the two drugs in a mixture comparing first derivative spectrophotometry (Fig. 2). The zero-crossing method involves measurement of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelengths of the derivative spectra of the individual component. The second derivative spectrum of **1** exhibits a maximum at 226 nm, while **2** reads zero and **2** exhibits an absorption at 282 nm while **1** reads zero (Fig. 2B).

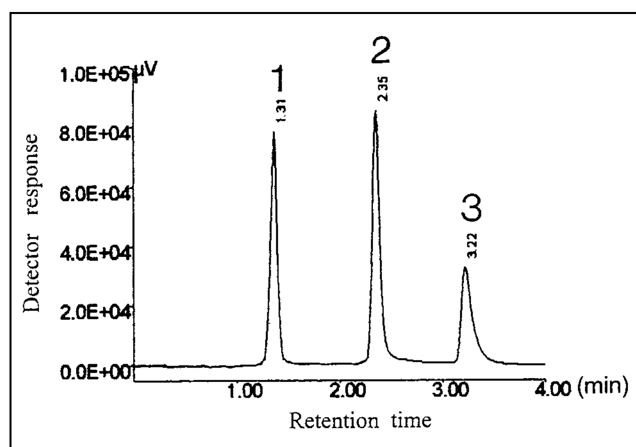


Fig. 3: Typical chromatogram obtained by RP-HPLC analysis of the standard compounds (1) tetrahydrozoline hydrochloride (10 μg/ml), (2) fluorometholone (40 μg/ml), (3) internal standard (lidocaine, 8 μg/ml)

Quantitative investigations using regression analysis have established that the drug concentrations correlate well with the measured second derivative peaks. The regression equations were $y = 0.175 + 0.410x$ ($r = 0.9980$) for **1** at 226 nm and $y = 0.089 + 0.363x$ ($r = 0.9986$) for **2** at 282 nm (where y is the $d^2A/d\lambda^2$ value, x is the concentration of drug in μg/ml).

The recovery test was performed from synthetic mixtures containing various amounts of **1** and **2** (Table 1). The results show a mean recovery of 100.97% for **1** and 100.60% for **2** using the proposed second derivative spectrophotometric method. The relative standard deviation (RSD) for **1** and **2** was 1.06% and 0.98%, respectively. The results are reproducible and precise as the RSD values are very low. This method has been successfully applied to a commercial pharmaceutical eye drop solution and the results obtained from commercial eye drops shown in Table 2. There was no interference observed with the excipients in the eye drop.

2.2 Analysis of tetrahydrozoline hydrochloride and fluorometholone by HPLC

For HPLC analysis, initially various mobile phase compositions were tried in attempts to separate drugs and internal standard. RP-HPLC system using an ODS analytical column and methanol/acetonitrile/water (50 : 50 : 10 v/v) gave good separation of drugs and internal standard (lidocaine). Fig. 3 shows a typical HPLC chromatogram of the standard compounds. Chromatographic investigations revealed that a mixture of **1** and **2** could be resolved from

Table 1: Recovery of tetrahydrozoline hydrochloride (1) and fluorometholone (2) in synthetic mixtures by proposed second derivative UV spectrophotometric (A) and HPLC (B) methods

Amount added (µg/ml)		Found (µg/ml)				Recovery (%)			
1	2	1		2		1		2	
		A	B	A	B	A	B	A	B
10	20	9.98	10.07	20.06	19.85	99.80	100.70	100.30	99.25
12	20	12.20	12.05	20.41	20.25	101.67	100.42	102.05	101.25
14	20	14.01	13.97	20.03	20.11	100.07	99.79	100.15	100.55
16	20	16.28	16.07	20.06	20.08	101.75	100.44	100.30	100.40
18	20	17.91	18.10	19.81	20.09	99.50	100.55	99.05	100.45
10*	40*	10.16	9.95	40.74	40.25	101.60	99.50	101.85	100.62
10	45	10.28	10.15	45.29	45.07	102.80	101.50	100.64	100.15
10	50	10.04	10.07	49.84	50.09	100.40	100.70	99.68	100.18
10	55	10.16	10.09	55.15	55.13	101.60	100.90	100.27	100.24
10	60	10.05	9.97	61.03	60.35	100.50	99.70	101.72	100.58
						$\bar{x}$ = 100.97	$\bar{x}$ = 100.42	$\bar{x}$ = 100.60	$\bar{x}$ = 100.37
						RSD = 1.06	RSD = 0.61	RSD = 0.98	RSD = 0.50

* The amount of drug (at appropriate dilution) in the commercial pharmaceutical eye drops

Table 2: Results of the simultaneous determination of tetrahydrozoline hydrochloride and fluorometholone in pharmaceutical eye drops

Drug	Labelled (mg/ml)	n	Found ($\bar{x}$) (mg/ml)		Recovery (%)		RSD (%)	
			A	B	A	B	A	B
1	0.250	10	0.254	0.248	101.60	99.20	1.09	0.83
2	1.000	10	1.012	1.003	101.20	100.30	1.20	0.69

1: Tetrahydrozoline hydrochloride, **2**: Fluorometholone, A: Second derivative UV spectrophotometric method, B: HPLC method

the co-formulated excipients using an ODS stationary phase and a mixture of methanol/acetonitrile/water (50 : 50 : 10 v/v). The separations could be obtained in less than 4 min. The retention times for **1**, **2** and internal standard were 1.31, 2.35 and 3.22 min, respectively (Fig. 3). The peak area ratios of **1** and **2** to internal standard shows a linear relationship with their concentrations. The regression equations for **1** and **2** were $y = 0.354 + 0.210x$ and $y = 0.238 + 0.156x$, respectively (where y is peak area ratio and x is the concentration of drug in µg/ml). The correlation coefficient of the calibration curves were found to be 0.9998 for **1** and 0.9989 for **2**.

In the HPLC method, the RSD was 0.61% for **1** and 0.50% for **2** (Table 1). Commercially available eye drops were analysed by the HPLC method (Table 2). No significant differences in RSD were found between the results obtained by the HPLC from synthetic mixtures and the commercial solution.

It can be concluded that the reported methods for the simultaneous determination of **1** and **2** in pharmaceutical eye drops (Efemoline®) are simple and rapid. Although there was no significant differences between the methods applied, the results indicate that the HPLC method could be considered for routine analysis. The method is accurate, precise, stability indicating and reproducible. Additionally, the sensitivity and reliability of the HPLC method over a wide concentration range will extend the use of this method.

3. Experimental

3.1. Materials

Tetrahydrozoline hydrochloride (**1**), fluorometholone (**2**) and a commercial preparation (Efemoline® eye drops) were gifts from Ciba-Geigy Co. (Istanbul, Turkey). The labelled content in 1 ml eye drop was as follows: Tetrahydrozoline hydrochloride 0.25 mg; Fluorometholone 1.00 mg. HPLC grade acetonitrile and methanol were purchased from J. T. Baker Inc. (Phillipsburg, NJ, USA). Lidocaine was obtained from Merck Co. (Darmstadt, Germany).

3.2. Spectrometric equipment and conditions

A Shimadzu UV-160 double beam spectrophotometer with a fixed slit with 2 nm was used. The derivative UV spectra of standard and test solutions were recorded in 1 cm quartz cells over the range 210–300 nm ($\Delta\lambda = 12.6$ nm). The scan speed was 10 nm/min.

3.3. Chromatographic system and conditions

The HPLC (Jasco International Co., Ltd., Tokyo, Japan) consisted of a model PU-980 solvent delivery system, and a model 970/975 UV-VIS detector connected to a Panasonic model KX-P1150 multimode integrator (Matsushita Electric Industrial Co., Ltd., Japan). A model 7125 sample injector (Rheodyne, Cotati, CA, USA) equipped with a 20 µl loop was used.

The separation was performed on a Partiisil 5 ODS (3) analytical column (250 × 4.60 mm i.d.) (Phenomenex Ltd., Cheshire, UK). The mobile phase consisted of a mixture of methanol/acetonitrile/water (50 : 50 : 10 v/v). The mobile phase was prepared daily, filtered, sonicated before use, and delivered at a flow rate of 1.5 ml/min. The detector wavelength was set at 220 nm.

3.4. Derivative UV spectrophotometric method

Stock solutions of **1** and **2** were prepared by dissolving approximately 100 mg, accurately weight, in 100 ml of methanol. Dilutions from stock solutions of **1** and **2** were prepared in methanol in a range of 5–20 µg/ml and 20–60 µg/ml, respectively. The calibration curves for second derivative spectrophotometry were constructed by plotting the drug concentration versus the absorption of $d^2A/d\lambda^2$ at 226 nm and 282 nm for **1** and **2**, respectively. To study the accuracy and precision of the proposed methods, recovery experiments were carried out by standard addition technique. Working standard solutions of drug mixtures in methanol (containing 20 µg/ml of **2** and increasing concentrations of **1** ranging from 10–18 µg/ml; and containing 10 µg/ml of **1** and increasing concentrations of **2** ranging from 40–60 µg/ml) were prepared. The second derivative spectra of these solutions were recorded at 226 and 282 nm for **1** and **2**, respectively. The solutions described above were also used for HPLC analysis.

3.5. HPLC method

Stock solutions of **1** (100 µg/ml) and **2** (100 µg/ml) in methanol were prepared. Mixtures containing **1** and **2** were prepared by dilution with mobile phase. The concentrations of **1** and **2** were in the range of 3–20 and 10 to

60 µg/ml, respectively. A constant concentration of the internal standard (lidocaine, 8 µg/ml in methanol) was added to the mixture of **1** and **2**. The mixtures (20 µl) were then chromatographed on the reversed phase ODS column. A calibration curve was obtained by plotting the peak area ratio of the drug to internal standard against the drug concentration.

3.6. Analysis of eye drops

An accurately measured volume (1 ml) of eye drop solution was transferred into a 25 ml volumetric flask and internal standard (8 µg/ml) was added.

The contents were diluted to volume with mobile phase. The solution (20 µl) was chromatographed by HPLC. The amounts of **1** and **2** were calculated from the linear regression equations of the calibration curves or using a reference standard solution injected under the same conditions.

Second derivative UV spectrophotometric analysis was carried out on the above solution, without internal standard, using the corresponding calibration curves.

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