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Simultaneous Determination of Dorzolamide hydrochloride and Timolol maleate in ophthalmic solutions using HPLC

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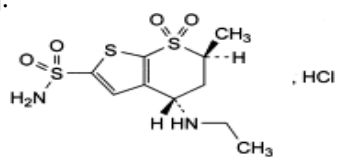
ABSTRACT

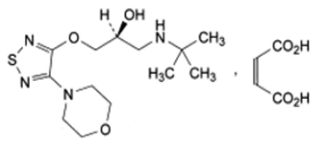
A simple high performance liquid chromatographic (HPLC) method for the simultaneous determination of dorzolamide hydrochloride and timolol maleate in eye drops formulation is presented. The HPLC separation was undertaken on a Eurospher 100 C-18 25*4.6 (5µm) column using a mobile phase of acetonitrile, tetrahydrofuran (THF) and 1% phosphoric acid aqueous solution,(15:3:82 v/v). System suitability was assessed through measurement of factors affecting column efficiency i.e. peak symmetry, capacity factor and resolution. Analytes concentrations were calculated utilizing peak area and peak height. The linearity range (r value > 0.99) was 120-480 µg/ml and 360-1440 µg/ml for timolol maleate and dorzolamide hydrochloride respectively. The limit of detection and limit of quantification for timolol maleate were 5.95µg/ml and 18.03µg/ml. The corresponding values for dorzolamide hydrochloride were 25.18µg/ml and 76.31µg/ml.

Introduction

Dorzolamide hydrochloride is a carbonic anhydrase inhibitor; used for the treatment of glaucoma and ocular hypertension [1].



Timolol maleate is a β-adrenoceptor antagonist which is formulated as eye drops for the treatment of glaucoma [1].



A number of methods have been reported for the determination of timolol maleate and dorzolamide hydrochloride separately [1, 2], and in combination using spectrophotometric methods [3, 4].

One HPLC method has been cited for the determination of dorzolamide and timolol. The method employed monitoring the drugs with a diode-array detector at two fixed wavelength (250nm and 300nm for dorzolamide and timolol respectively) [5].

The present work describes an HPLC method for the simultaneous determination of timolol maleate and dorzolamide hydrochloride in binary mixtures or in eye drops formulation.

Experimental:

Materials:

Reference materials: timolol maleate (manufactured by M/s Lavallee, France) and dorzolamide hydrochloride (manufactured by M/s Elkton, VA Virginia, and USA) were purchased from Peshawar, Pakistan, and were used as received without further treatment.

The preparation used was Xolamol oph.® solution, Jamjoom Pharma, Kingdom of Saudi Arabia purchased from local pharmacies in Khartoum, Sudan. Acetonitrile and methanol were of HPLC grade (Scharlau, India). All other chemicals were analytical grade reagents: glacial acetic acid (Scharlau, India), sodium acetate, (E., MERCK, Darmastadt), ammonia (BDH chemicals, England), orhtophosphoric acid (BDH chemicals, England).

Chromatographic conditions:

HPLC was performed on a KNAUER HPLC system software ChromGate™; the column used was Eurospher 100 C-18 25*4.6 (5µm).

The mobile phase used was 15:3:82v/v acetonitrile: tetrahydrofuran: 1%v/v aqueous phosphoric acid, which was degassed by ultrasonication.

The detector was UV, model K-2501 set at 275nm. Injection volume was 20 µl and the pump was K-120/501with a flow rate of 1.5ml/min.

Preparation of solutions:

(a)Timolol maleate stock solution: Timolol maleate (0.05 g) was accurately weighed and transferred into a 50-ml volumetric flask, dissolved in about 30 ml water, then volume completed with water.

Dorzolamide hydrochloride stock solution: Dorzolamide hydrochloride (0.15g) was accurately weighed and transferred into a 50-ml volumetric flask, dissolved in about 30 ml and the volume completed with water.

(b)Mixed standard working solutions: Four serial dilutions were prepared by transferring aliquots of 3, 6, 9,12ml of the standard stock solution of both timolol maleate and dorzolamide hydrochloride into 25-ml volumetric flasks. The volume was completed with water.

The standard mixture was prepared in the ratio of 1:3 timolol maleate to dorzolamide hydrochloride, to simulate the ratio of both in the eye drops.

(c)Preparation of the sample:

One ml of the eye drops was accurately delivered into 25-ml volumetric flask, and the volume completed with water.

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Procedures:

Precision

Six replicate measurements were made by using the following solutions in water: (i) 136.8 µg of timolol maleate per ml, and (ii) 445.2µg of dorzolamide hydrochloride per ml. they were injected separately.

Linearity

Four different concentrations of timolol maleate, and dorzolamide hydrochloride were prepared in water; 20 µl of each concentration was injected into the HPLC column.

Limit of detection and limit of quantification were calculated from calibration curve results using the following formulas:

$$L.O.D = \frac{3.3 \times Sa}{b}$$

$$L.O.Q = \frac{10 \times Sa}{b}$$

where Sa is the standard deviation for the intercept, b is the slope [6].

Assay:

Triplicate samples of each of the mixed standard working solutions (b) were analyzed by HPLC. Calibration curves for each of timolol maleate and dorzolamide hydrochloride were constructed using peak area and peak height against the corresponding concentration of each drug. The calibration curves were used for the assay of the drugs.

Results and discussion:

Selection of mobile phase:

HPLC has become one of the most widely used instrumental analytical systems. This is perhaps due to the wide range of available columns (normal phase, reversed phase and ion exchange), along with the variety of selectivity introduced by change in mobile phase composition.

HPLC provides selectivity, simplicity of separation methods, and reproducibility.

These properties lead to the wide variety of applications of HPLC, ranging from separation of aromatic hydrocarbons, pharmaceuticals, and pesticides to applications in bioanalysis. Irrespective of the type of analysis, the goal is to provide information about the composition of the sample, purity, content, and possible available impurities [7].

In this study an HPLC method for the simultaneous determination of timolol maleate and dorzolamide hydrochloride is presented.

The separation was undertaken under isocratic conditions. In order to affect the simultaneous elution of the two components under isocratic conditions, factors like mobile phase composition, and pH were investigated. Table (1) summarizes the preliminary trials carried out to optimize the chromatographic conditions which can give satisfactory resolution for these compounds.

The assessment of good separation was judged from good capacity factor, peak symmetry and resolution factor with reproducible retention time.

These criteria were obtained when using 15:3:82v/v acetonitrile: tetrahydrofuran: 1%v/v aqueous phosphoric acid, at a flow rate of 1.5 ml/min.

The elution order was dorzolamide hydrochloride at 2.52min (K' value 1.1), and timolol maleate at 4.48 (K' value 2.73) fig (1).

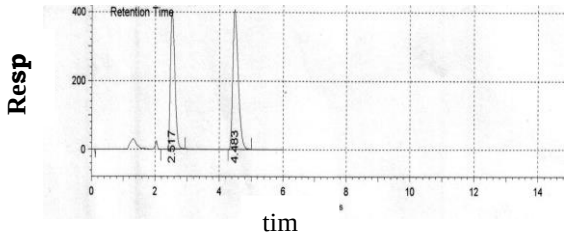

Fig (1): A typical chromatogram of the eye drops solution injected showing dorzolamide hydrochloride at 2.52min retention time and timolol maleate peak at 4.48min retention time

Linearity:

A calibration curve was prepared using a mixture of the drugs at concentration of 120-480 µg/ml for timolol maleate and 360-1440 µg/ml for dorzolamide hydrochloride. The obtained correlation coefficient values (r) for timolol maleate were (0.9992) and for dorzolamide hydrochloride were (0.999). The regression analysis data was calculated at 95% confidence level for the slope ($b \pm ts_b$) and the intercept ($a \pm ts_a$) using the formula

$$P = (b \pm ts_b)\, x + (a \pm ts_a) \qquad [6]$$

where P is the peak area, b the slope, s_b the standard deviation for the slope, the t-value at 95% confidence level for (n – 2), s_a the standard deviation for the intercept, and *x* the concentration (µg/ml)

The results for dorzolamide hydrochloride were

$P = (342868 \pm 8439.72)x + (84586557 \pm 8320777.026)$

The results for timolol maleate were

$P = (1386224.6 \pm 1709.005)x + (61886441 \pm 7949064.352)$

These results indicated good distribution of points along the linearity range.

Assay:

The method was applied for the determination of these compounds in eye drops formulation marketed in Sudan (Xolamol oph. solution®) which contains timolol maleate and dorzolamide hydrochloride in a ratio of 1:3.

Both timolol maleate and dorzolamide hydrochloride are good UV-absorbing compounds with λ_{max} at about 315.8 and 250.3nm respectively. However, in this study it was found that using λ_{max} at 275nm gave good response for both drugs according to their ratio in the eye drops.

Both peak areas and peak heights were utilized in the analytes concentration determination. As can be seen from table (2) either peak area or peak height can be utilized for the determination of the analytes concentrations (SD values less than 0.7). The stability of the eye drops constituents in their original packing was assessed by exposing the container to sunlight (1 day) or after being left at room temperature for seven days. No change of concentration or change of retention times was observed.

The effect of the eye drops matrix on the assay was checked by the recovery addition method. The results showed good recovery: 100.67±1.29 n=3 for timolol maleate (added concentration was 136.8µg/ml) and 100.24±0.99 n=3 for dorzolamide hydrochloride (added concentration was 445.2µg/ml). These results showed clearly that the eye drops matrix did not affect the assay results obtained by the present method.

Precision:

The validity of the present method was assessed by calculating the accuracy from the assay results. The t-values

were calculated considering the actual content as 100% for each (known mean) according to the formula

$$t = (x - \mu)\sqrt{n}/s$$

where x is the mean, μ is the known mean, n is the number of determinations, s is standard deviation [6].

Table (3) shows the t-values obtained, which ascertained accuracy of the developed method.

Limit of detection and limit of quantification:

Limit of detection and limit of quantification were calculated from the calibration curve results. They were found to be 1.85μg/ml and 5.61μg/ml for timolol maleate, and 25.18μg/ml and 76.31μg/ml for dorzolamide hydrochloride. The low levels indicate that the method is sensitive and suitable for the simultaneous determination of timolol maleate and dorzolamide hydrochloride.

Only one HPLC method has been reported for the determination of both dorzolamide and timolol in combined form [5]. In this method, the drugs were monitored with a diode-array detector at two fixed wavelengths (250nm and 300nm for dorzolamide hydrochloride and timolol maleate respectively). It is evident that the authors worked out the concentration of each drug using simultaneous equations. The linearity of the method ranged between 4.0–45.0 μg • ml–1 for dorzolamide hydrochloride and 2.0–20.6 μg • ml–1 for timolol maleate in binary mixture, indicating sensitivity of the method.

In the currently presented method, a UV-VIS fixed wavelength detector was used for the determination of the two drugs. It is thus evident that both drugs can be measured easily and simultaneously in one HPLC run and utilizing one λ (275nm). This advantage is believed to overweigh the relatively high concentrations range covered in linearity testing.

Conclusion:

The presented, developed HPLC method for determination of dorzolamide hydrochloride and timolol maleate is considered simple, linear, accurate, sensitive and reproducible. Thus, the method can be successfully used for the routine quality control of dorzolamide hydrochloride and timolol maleate within a short analysis time (less than 5 minutes). The method can also be utilized for the assay of each drug alone using either compound as internal standard for the other.

Acknowledgement:

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Table (1): Results of system optimization trials

Mobile phase	Flow rate	T_0*	Dorzolamide hydrochloride			Timolol maleate			resolution
			Tr**	K'***	Peak sym	Tr	K'	Peak sym	
18:82 Acetonitrile:5%v/v acetic acid	1.50	1.50	2.40	0.60	1.20	5.45	2.60	1.69	1.04
18:82 Methanol:5% acetic acid	1.50	1.50	2.20	0.46	1.00	9.95	5.64	1.64	3.69
25:75 Acetonitrile:5%v/v acetic acid	1.50	1.60	2.10	0.31	1.14	3.43	1.14	1.44	0.99
25:75 Acetonitrile:Na phosphate 0.01 M pH3.6	1.50	1.25	2.83	1.26	1.60	4.65	2.72	1.86	0.33
25:75 Acetonitrile:5%v/v acetic acid	1.80	1.45	1.90	0.31	1.00	2.83	0.95	1.44	0.72
18:5:77 acetonitrile:THF:5%v/v acetic acid	1.50	The peak of timolol maleate showed splitting							
10:90 THF:5%v/v acetic acid	1.50	2.00	3.17	0.58	1.08	3.72	0.86	1.47	1.00
22:78 acetonitrile:1%v/v ammonia	1.50	The peaks were tailing badly							
5:5:90 Acetonitrile:THF:2.5% v/v acetic acid	1.50	1.60	2.80	0.75	1.18	4.47	1.79	1.64	1.07
5:5:90 Acetonitrile:THF:2.5% v/v acetic acid + 2ml phosphoric acid	1.50	1.90	3.00	0.57	1.17	5.14	1.71	1.31	2.30
15:85 Acetonitrile:1%v/v phosphoric acid	1.50	2.00	2.93	0.47	1.20	8.42	3.21	1.66	2.85
15:3:82 Acetonitrile:THF:1%v/v phosphoric acid	1.50	1.20	2.52	1.10	1.10	4.48	2.73	1.08	1.63
0.01 M KH_2PO_4 in 50:50 methanol:water	1.50	2.20	2.72	0.24	1.00	4.57	1.08	1.30	2.85

*T_0 unretained peak
**Tr retention time
***K' capacity factor

Table (2): Assay results (± SD) of Xolamol oph. solution® utilizing peak areas and peak heights for both drugs

Drug	Utilizing peak area	Utilizing peak height
Dorzolamide hydrochloride	100.25 ± 0.62 n=6	99.67 ± 0.38 n=6
Timolol maleate	100.08 ± 0.24 n=6	99.79 ± 0.51 n=6

Table (3): The calculated t values compared to the tabulated ones

Drug	t-values (n – 1)	
	Utilizing peak area	Utilizing peak height
Dorzolamide hydrochloride	0.78(cal.) 2.57(tab.)	1.31(cal.) 2.57(tab.)
Timolol maleate	0.04(cal.) 2.57(tab.)	0.72(cal.) 2.57(tab.)