Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, Tandoğan-Ankara,Turkey

# Rapid and sensitive HPLC method for the simultaneous determination of dorzolamide hydrochloride and timolol maleate in eye drops with diode-array and UV detection

N. Erk

*Received October 21, 2002, accepted January 20, 2003*

*Prof. Dr. Nevin Erk, Faculty of Pharmacy, Department of Analytical Chemistry, University of Ankara, Tandoğan Ankara, Turkey*
*erk@pharmacy.ankara.edu.tr*

*Pharmazie 58: 491–493 (2003)*

A rapid and sensitive HPLC method has been developed for the simultaneous determination of dorzolamide hydrochloride and timolol maleate. The drugs were monitored with a diode-array detector at two fixed wavelengths ($\lambda = 250.0$ nm for dorzolamide hydrochloride and 300.0 nm for timolol maleate). Liquid chromatography was performed on a RP-YMC pack ODS A-132 $C_{18}$ (5 μm, 15 cm × 6.0 mm) column and the mobile phase consisted of an acetonitrile : phosphate buffer (pH 2.5) : methanol (5 : 85 : 10 v/v/v) mix and a flow rate of 1.2 ml · min$^{-1}$. The linearity of the method ranged between 4.0–45.0 μg · ml$^{-1}$ for dorzolamide hydrochloride and and 2.0–20.6 μg · ml$^{-1}$ for timolol maleate in binary mixture. The procedure was successfully applied to the determination of these compounds in pharmaceutical preparations and gave a high recovery, good accuracy and precision without any interference by the excipients.

## 1. Introduction

[(−)-(*SS*)-4-Ethylamino-5,6-dihydro-6-methyl-7,7-dioxide-4*H*-thieno(2,3-b)thio-pyran-2-sulfonamide, dorzolamide (hydrochloride), a potent and selective inhibitor of human carbonic anhydrase, is topically used for reduction of elevated intraocular pressure [1, 2]. Timolol (maleate), (*S*)-3-*tert*-butylamino-1-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-2-propanol, is a nonspecific β-adrenergic blocker. It was the first β-blocker used as an antiglaucoma agent, and since then none of the newer β-blockers have been found to be more effective than timolol (maleate). The two drugs are often combined in eye drops for the therapy of glaucoma.

Literature methods for the determination of dorzolamide hydrochloride are mainly based individually on HPLC with UV detection under atmospheric pressure, chemical ionization tandem mass spectrometry in human serum and urine [3–5] and capillary electrophoresis [6]. There are several reports on the determination of timolol maleate, individually or in combination with pilocarpine, including GLC [7] and HPLC of plasma samples [8, 9], HPTLC [10] and, with dorzolamide hydrochloride, spectrophotometry [11–15].

More recently, dorzolamide hydrochloride has been marketed in combination with timolol maleate in eye drops. Dorzolamide hydrochloride is not yet offical in any pharmacopoeia. To the best of our knowledge, no HPLC method has been described for the simultaneous determination of both drugs in eye drops.

The aim of this work was to develop a rapid, sensitive and specific method for this purpose using HPLC, with simultaneous detection by a diode array detector. This method should be transferrable to quality control laboratories for the determination of both drugs in the presence of each other. The proposed method should require no separation of dorzolamide hydrochloride and timolol maleate before analysis.

## 2. Investigations, results and discussion

A RP-HPLC system with a YMC pack ODS A-132 $C_{18}$ analytical column and an eluant of acetonitrile : phosphate buffer (pH 2.5) : methanol (5 : 85 : 10 v/v/v) gave good separation of both drugs. The mobile phase was found to be essential to improve the shape of dorzolamide hydrochloride and timolol maleate peaks. The Fig. shows a typical HPLC chromatogram of the standard compounds. A binary mixture of dorzolamide hydrochloride and timolol maleate could be resolved from the co-formulated excipients using an ODS stationary phase and an eluant mixture of acetonitrile : phosphate buffer (pH 2.5) : methanol (5 : 85 : 10 v/v/v). The separations could be obtained in less than 3.9 min. The retention times for dorzolamide hydrochloride and timolol maleate were found to be 3.9 and 2.7 min, respectively. The spectra have been found for (a) dorzolamide hydrochloride (24.0 μg · ml$^{-1}$) with a maximum at 250.0 nm and for (b) timolol maleate (18.0 μg · ml$^{-1}$) with a maximum at 300.0 nm. Thus, the $\lambda_{max}$ of dorzolamide hydrochloride and timolol maleate were selected for quantitative work, and much better detector responses for the two drug were achieved. The analytical data for the calibration graphs are listed in Table 1. The linearity of the detector response for both drugs was determined by plotting peak area ratios vs concentration.

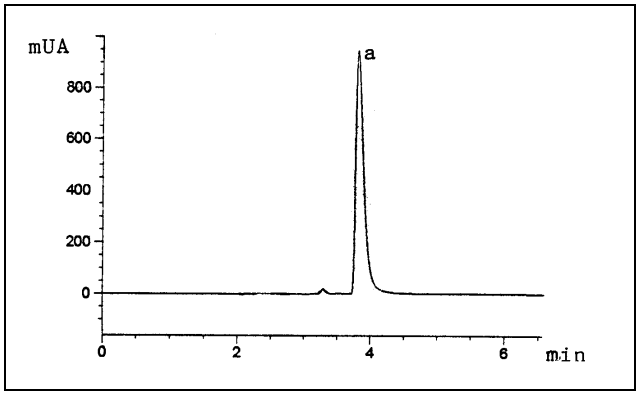


Fig. a: Chromatogram of a standard solution containing 20.0 $\mu g \cdot ml^{-1}$ dorzolamide hydrochloride channel 1 from detector set at $\lambda = 250.0$ nm

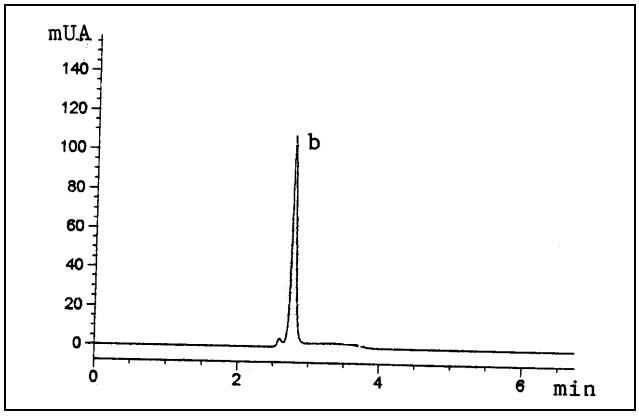


Fig. b: Chromatogram of a standard solution containing 5.0 $\mu g \cdot ml^{-1}$ timolol maleate channel 2 from detector set at $\lambda = 300.0$ nm

Linearity range for dorzolamide hyrochloride and timolol maleate was of 4.0–45.0 $\mu g \cdot ml^{-1}$ and 2.0–20.6 $\mu g \cdot ml^{-1}$, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) of dorzolamide hydrochloride and timolol maleate were calculated on the peak area using the following equations:

$$LOD = 3 \times N/B \qquad LOQ = 10 \times N/B$$

where N, the noise estimate, is the standard deviation of the peak areas (three injections) of the dugs, B is the slope of the corresponding calibration curve. The limit of quantification and the limit of detection of dorzolamide hydrochloride and timolol maleate were found to be 3.10 $\mu g \cdot ml^{-1}$ and 2.30 $\mu g \cdot ml^{-1}$ and 1.00 $\mu g \cdot ml^{-1}$ and 0.82 $\mu g \cdot ml^{-1}$, respectively.

Day-to-day precision and accuracy were evaluated by analyzing five samples of three different concentrations at low, medium and high concentrations, which were prepared and analyzed on the same day (Table 2). Sample-to-sample variability was assessed using five samples of three different concentrations at low, medium and high concentrations analyzed on five different days over a period of two week. These results show the accuracy and reproducibility and the assay. Thus, it was concluded that there were no significant intra-day and inter-day differences for the assay. To establish the validity and applicability of the proposed method, ten synthetic binary mixtures in the concentration range reported in Table 3 were assayed by the present procedures. The data indicate that the method has good accuracy and precision.

The developed method was applied to the recovery of dorzolamide hydrochloride and timolol maleate in three batches of commercial eye drop formulations. The results presented in Table 4 are in good agreement with the labelled content. All data represent the average of five determinations. Low values of relative standard deviation indicate very good reproducibility of the measurement.

In conclusion, the proposed procedure was successfully applied to the determination of the studied compounds in pharmaceutical dosage forms. The proposed method gives good resolution between of dorzolamide hydrochloride and timolol maleate with a short analysis time (<5 min). The method is simple and rapid and does not involve

**Table 1: Statistical analysis of calibration curves in the HPLC determination of dorzolamide hydrochloride and timolol maleate**

| Parameters | Dorzolamide hydrochloride | Timolol maleate |
|---|---|---|
| Range ($\mu g \cdot ml^{-1}$) | 4.0–45.0 | 2.0–20.6 |
| Detection limits ($\mu g \cdot ml^{-1}$) | 1.00 | 0.82 |
| Quantitation limits ($\mu g \cdot ml^{-1}$) | 3.10 | 2.30 |
| Regression equation (Y)[a] | | |
| Slope (b) | $3.67 \times 10^{-4}$ | $4.95 \times 10^{-4}$ |
| Intercept (a) | $1.20 \times 10^{-3}$ | $7.91 \times 10^{-3}$ |
| Correlation coefficient (r ) | 0.9999 | 0.9996 |
| Rel. std. dev. (%)[b] | 0.94 | 1.09 |
| % Range of error[b] (% 95 confidence limit) | 0.68 | 0.85 |

[a] $Y = a + bC$ where C is concentration in $\mu g \cdot ml^{-1}$ and Y in peak areas
[b] Five replicate samples

**Table 2: Sample-to-sample (intraday) and day-to-day (interday) precision of dorzolamide hydrochloride and timolol maleate standards analyzed by HPLC**

| Theoretical Concentration ($\mu g \cdot ml^{-1}$) | Dorzolamide hydrochloride | | Dorzolamide hydrochloride | | Timolol maleate | | Timolol maleate | |
|---|---|---|---|---|---|---|---|---|
| | Intraday | Measured* | Interday | Measured** | Intraday | Measured | Interday | Measured |
| | Concentration ($\mu g \cdot ml^{-1}$) | | Concentration ($\mu g \cdot ml^{-1}$) | | Concentration ($\mu g \cdot ml^{-1}$) | | Concentration ($\mu g \cdot ml^{-1}$) | |
| | Mean | RSD % | Mean | RSD % | Mean | RSD % | Mean | RSD % |
| 4.0 | 3.9 | 0.48 | 3.6 | 1.04 | – | – | – | – |
| 25.0 | 24.9 | 0.85 | 24.6 | 1.53 | – | – | – | – |
| 45.0 | 44.5 | 0.64 | 44.4 | 1.96 | – | – | – | – |
| 2.0 | – | – | – | – | 1.8 | 0.68 | 1.7 | 1.47 |
| 11.0 | – | – | – | – | 11.1 | 0.89 | 10.9 | 1.91 |
| 20.6 | – | – | – | – | 20.2 | 0.97 | 20.0 | 2.13 |

* Mean values represent five different sample standards for each concentration
** Intraday reproducibility was determined from five different runs over a 2 weeks period

**Table 3: Recovery experiments obtained for different binary mixtures of dorzolamide hydrochloride (Dor) and timolol maleate (Tim) analyzed by HPLC**

| Added ($\mu g \cdot ml^{-1}$) | | Found ($\mu g \cdot ml^{-1}$) | | Recovery (%) | |
|---|---|---|---|---|---|
| Dor | Tim | Dor | Tim | Dor | Tim |
| 20.00 | 3.00 | 19.75 | | 98.75 | |
| 20.00 | 4.00 | 19.96 | | 99.80 | |
| 20.00 | 6.00 | 19.85 | | 99.25 | |
| 20.00 | 7.00 | 20.08 | | 100.40 | |
| 20.00 | 8.00 | 19.99 | | 99.95 | |
| 10.00 | 5.00 | | 4.95 | | 99.00 |
| 15.00 | 5.00 | | 4.99 | | 99.80 |
| 20.00 | 5.00 | | 4.93 | | 98.60 |
| 25.00 | 5.00 | | 4.90 | | 98.00 |
| 30.00 | 5.00 | | 4.86 | | 97.20 |

| | $\overline{X}$ (%) | RSD (%) |
|---|---|---|
| Dor | 99.63 | 0.64 |
| Tim | 98.52 | 0.98 |

**Table 4: Results obtained in determination of dorzolamide hydrochloride and timolol maleate in pharmaceutical dosage forms[a]**

| | Mean (mg) ± SD[b] | |
|---|---|---|
| | Dorzolamide hydrochloride | Timolol maleate |
| Batch 1 | 20.2 ± 0.8 | 5.2 ± 0.9 |
| Batch 2 | 19.7 ± 1.1 | 4.8 ± 1.5 |
| Batch 3 | 20.5 ± 1.7 | 5.1 ± 0.6 |

[a] Cosopt® eye drops were labeled to contain 20.0 mg dorzolamide hydrochloride, 5.0 mg timolol maleate per drop respectively
[b] Each value is the mean of ten experiments; SD: standard deviation

complex instrumentation or complicated sample preparation. A high recovery shows that the method is free from the interferences of the co-formulated excipients used in the formulations. This method has been found suitable for the routine analysis of the eye drops in quality control laboratories for products of similar type and composition.

## 3. Experimental

### *3.1. Apparatus*

A chromatographic system consisted of a HP 1100 series mode quaternary pump with a HP 1100 series manual injector 20 µl fixed loop, equipped with a diode array and multiple wavelength UV/VIS detectors. The detector was set at $\lambda = 250.0$ nm and 300.0 nm and peak areas were integrated automatically by computer using Agilent Chem-Station software programme. pH was measured with a Radiometer NEL pH 890 digital pH meter equipped with a combined glass-calomel electrode and an ultrasound generator.

### *3.2. Chemicals*

Dorzolamide hydrochloride and timolol maleate were kindly donated by MSD Pharm. Ind. Analytical grade phosphoric acid and HPLC grade methanol, and acetonitrile were purchased from Merck Chem. Ind. All other chemicals were of analytical-reagent grade.

### *3.3. Pharmaceutical preparation*

A commercial pharmaceutical preparation (COSOPT® eye drops MSD Pharm. Ind. Turkey, containing 20.0 mg of dorzolamide, 5.00 mg of timolol, 0.00075% benzalkonium chloride and water q.s per drop) was assayed.

### *3.4. Standard solutions and calibration curves*

Stock solutions of 100.0 $\mu g \cdot ml^{-1}$ of dorzolamide hydrochloride and timolol maleate in methanol were prepared. Mixtures containing dorzolamide hyrochloride and timolol maleate were prepared by dilution with mobile phase. The concentrations of dorzolamide hydrochloride and timolol maleate were in the range of 4.0–45.0 $\mu g \cdot ml^{-1}$ and 2.0–20.6 $\mu g \cdot ml^{-1}$, respectively. The mixtures (20 µl) were chromatographed on the reversed phase RP-YMC pack ODS A-132 $C_{18}$ (5 µm, 15 cm × 6.0 mm) column and a flow rate of 1.2 $ml \cdot min^{-1}$. Fresh stock standard solutions were prepared each day.

### *3.5. Analysis of eye drops*

One milliliter of eye drop solution was transferred into a volumetric flask. The contents were diluted with the mobile phase. The solution was chromatographed by HPLC. The amounts of dorzolamide hydrochloride and timolol maleate were calculated from the linear regression equations of the calibration curves or using a reference standard solution injected under the same conditions.

### *3.6. Assay validation*

A two week validation of the analysis was performed, and evaluated statistically. The statistical analyses were done. The acceptance criteria used to validate the assay have been published or are available as guidelines [16].

### *3.7. Linearity*

Triplicate calibration curves were generated each day for three consecutive days. The concentrations of dorzolamide hydrochloride and timolol maleate were in the range of 4.0–45.0 $\mu g \cdot ml^{-1}$ and 2.0–20.6 $\mu g \cdot ml^{-1}$, respectively.

### *3.8. Recovery*

In order to establish the reliability, suitability, accuracy, reproducibility and to check the interference from co-formulated excipients used in the formulation, recovery experiments were carried out. Known amounts of the pure sample solutions were added to the preanalysed formulations of each drug, and the binary mixtures were analysed by the proposed method. From the total amount of drug found, the percentage recovery was calculated. After five repeated experiments, the recoveries were calculated.

### *3.9. Precision, accuracy and limit of quantitation*

The accuracy and sample-to-sample and day-to-day precision of the method were estimated by assaying three replicate quality control samples at five different concentrations for each drug in the three analytical runs. The assays were determined by comparing the means of the measured concentrations with the theoretical concentrations expressed as percent deviation (%). The overall mean precision was defined by the relative standard deviation (RSD) of five quality control samples at five different concentrations analysed over five days. The lower limit of quantitation was the lowest non-zero concentration level, which could be accurately (relative error <20%) and reproducibly (C.V. 20%) quantitated [17].

**References**


1 Baldwin, J. J.; Ponticello, G. S.; Anderson, P. S.; Mercko, M. A.; Randall, W. C.; Schwan, H.; Sugrue, M. F.; Gautheron, P. S.; Grove, J.; Mallorga, P.; Viader, M. P.; McKever, B. M.; Navia, M. A.: J. Med. Chem., **32**, 2513 (1989)
2 Blacklock, T. J.; Sohar, P.; Buthcher, J. W.; Lamanec, T.; Grabowski, E. J. J.: J. Org. Chem. **58**, 1672 (1993)
3 Matuszewski, B. K.; Constanzer, M. L.; Woolf, E. J.; Au, T.; Haddix, H.: J. Chromatogr. B. **653**, 77 (1994)
4 Constanzer, M. L.; Chavez, C. M.; Matuszewski, B. K.: J. Pharm. Biomed. Anal. **15**, 1001 (1997)
5 Satuf, M. L.; Robles, J. C.; Goicoechea, H. C.; Oliveri, A. C.: Anal. Lett. **32**, 2019 (1999)
6 Tim, R. C.; Kautz, R. A.; Karger, B. L.; Electrophoresis **21**, 220 (2000)
7 Carlin, J. R.; Walkar, R. W.; Davies, R. O.; Ferguson, R. K.; Vandenheuvel, W. J. A.: J. Pharm. Sci. **69**, 1111 (1980)
8 Lennard M. S.; Parkin, S.: J. Chromatogr. B. **338**, 249 (1985)
9 Kubota, K.; Nakamura, H.; Koyama, E.; Yamada, T.; Kikuchi, K.; Ishizaki, T.: J. Chromatogr. B. **533**, 255 (1990)
10 Kulkarni, S. P.; Amin, P. D.: J. Pharm. Biomed. Anal. **23**, 983 (2000)
11 Erk, N.: J. Pharm. Biomed. Anal. **28**, 391 (2002)
12 Bebawy, L. I.: J. Pharm. Biomed. Anal. **27**, 737 (2002)
13 Patel, B. R.; Krischbaum J. J.; Poet, R. B.: J. Pharm. Sci. **70**, 336 (1981)
14 Mazzo D. J.; Snyder, P. A.: J. Chromatogr. **438**, 85 (1988)
15 Mazzo, D. J.: J. Chromatogr. **299**, 503 (1984)
16 Ng, L. L.: Reviewer Guidance: Validation of chromatographic Methods from the Centre for Drug Evaluation and Research, November, 1994
17 Shah, V. P.; Midha, K. K.; Dighe, S.: J. Pharm. Sci. **81**, 309 (1992)