Acta Pharmaceutica Turcica
XLII (2,3) (98-100) 2000

DETERMINATION OF DORZOLAMIDE IN OPHTHALMIC SOLUTIONS USING SOLID-PHASE EXTRACTION BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

DORZOLAMİD'İN KATI-FAZ EKSTRAKSİYONU KULLANILARAK YÜKSEK PERFORMANSLI SIVI KROMATOGRAFİSİ İLE OFTALMİK SOLÜSYONLARDA TAYİNİ

SERAP SAĞLIK

Department of Analytical Chemistry, Faculty of Pharmacy, University of İstanbul-TURKEY

Dorzolamide was separated from ophthalmic solutions by using solid-phase extraction (SPE) technique and analysed by HPLC and FTIR. The assay of dorzolamide was made by high-performance liquid chromatography technique. The limit of assay was 25 μg/mL..

Dorzolamid oftalmik çözeltilerden katı-faz ekstraksiyon tekniği kullanılarak ayrıldı ve HPLC ve FTIR ile analiz edildi. Dorzolamid miktar tayini yüksek performanslı sıvı kromatografisi tekniği ile yapıldı. Tayin limiti 25 μg/mL' dir

Keywords: *Dorzolamide; Solid-phase extraction; Highperformance liquid chromatography; Ophthalmic solution*

Aualitar kelimeler: *Dorzolamid; Katı–faz ekstraksiyonu; Yüksek peformanslı sıvı kromatografisi; Oftalmik slüsyon.*

Introduction

Dorzolamide is (4*S*-trans)-4-(ethyl-amino)-5, 6-dihydro -6-methyl -4H-thieno [2,3-b] thiopyran-2- sulfonamide 7,7-dioxide. It is a new member of this class of carbonic anhydrase inhibitors and used as a potential antiglaucoma agent(1).

Dorzolamide hydrochloride is stable in solution form(2). Different HPLC systems have been used of dorzolamide for purity control (3,4), determination in biological fluids (5,6) and eye-drops (7).

In this paper; a HPLC technique using SPE column for determination of dorzolamide in ophthalmic solutions is proposed.

Materials and Methods

All solvents and reagents were Merck (Germany) products, ethanol 96% (Tekel, İstabul). Methanol and acetonitrile were HPLC grade and orthophosphoric acid 88%, hydrochloric acid 37%, ethyl acetate, potassium bromide, potassium chloride and sodium hydroxide were analytical grade.

Buffer solution was prepared by adding 25 mL of 0.2M potassium chloride to 3.9 mL of 0.2M hydrochloric acid and diluting to 100 mL with distilled water.

Trusopt (Merck Sharp&Dohme, USA): Dorzolamide ophthalmic solution, 2%.

SPE column: C_{18}, 500 mg, 3 mL (J.T. Baker).

HPLC: HP 1100 (Hewlett-Packard).

IR: FTIR-8201PC (Shimadzu).

Extraction from ophthalmic solutions

Before extraction, SPE column was conditioned by eluting through the column 3 mL of 96% ethanol, followed by 3 mL of buffer solution. 1 mL dorzolamide solution was diluted to 3 mL with buffer solution and passed through the column. The column was subsequently washed with 3 mL of buffer followed by 3 mL of distilled water. Elution of dorzolamide was accomplished by passing 10 mL of 0.01M sodium hydroxide solution (stock solution). 0.125, 0.25, 0.375, 0.5, 0.75, 1.125, and 1.5 mL were drown from the stock solution and diluted to 10 mL with distilled water to give a series of working standards with concentrations of 25, 50, 75, 100, 150, 225, and 300 μg/mL.

Chromatographic conditions

HPLC experiments were conducted using a Nucleosil 100-7-C_8 column (25 cm length, 4.0 mm i.d., HiChrom, England) with a flow rate of 1.5 mL/min at ambient temperature. HP 1100 series Diode-Array detector set at 254 nm was used together with HP 1100 ChemStation software programme. The mobile phase consisted of

0.017M orthophosphoric acid (pH 4)-methanol-acetonitrile (85:10:5, v/v/v) and the injection volume was 20 µL.

UV spectrum of dorzolamide was taken by HP 1100 HPLC after chromatografic separation.

IR spectrum

For the IR spectrum of dorzolamide, 0.5 mL stock solution was extracted with 5 mL ethyl acetate, the organic phase was separated and evaporated under nitrogen. The residue was analysed in FTIR-8201PC.

Results and Discussion

The UV spectrum of dorzolamide isolated from ophthalmic solutions by SPE technique in HPLC is shown in Fig.1. The UV maximum absorbance is 254.16 nm, through the literature is 255 nm (8).

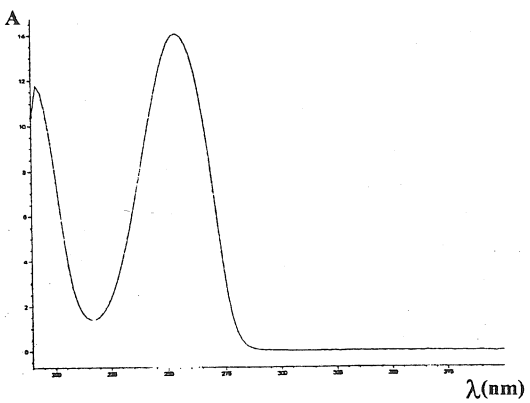

Fig. 1. Ultraviolet absorption spectrum of dorzolamide

Infrared spectrum of dorzolamide in KBr showed peaks at (cm^{-1}): 3372 (NH), 3040 (C-H), 2300-2800, 2689 (NH_2^+), 1589, 1536 (C=C), 1345, 1306, 1159, 1132 (SO_2). These results are similar with Quint *et al.* findings (8).

HPLC chromatogram of dorzolamide is shown in Fig.2. The retention time was 3.5 min in chromatographic conditions described above.

Standard curve of dorzolamide in HPLC is shown in Fig.3. Limit of assay was 25 µg/mL.

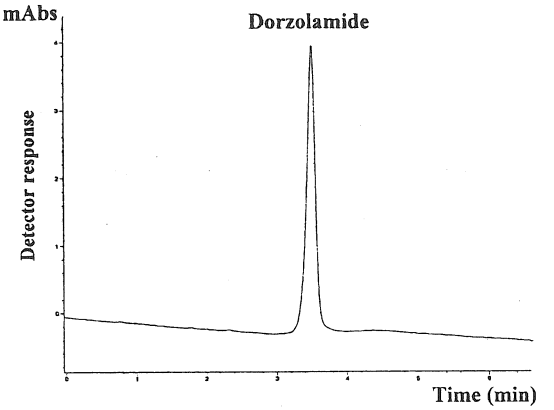

Fig.2. High performance liquid chromatogram of dorzolamide

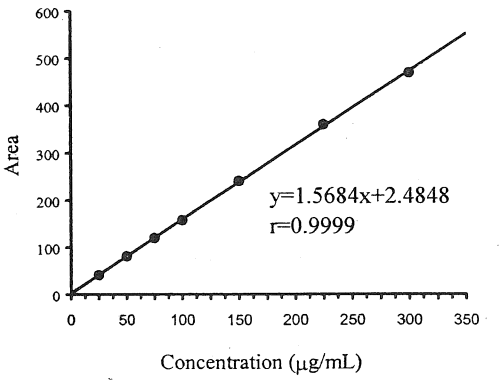

Fig. 3. Standard curve of dorzolamide

For chiral purity after derivatization dorzolamide has been determined in a Zorbax silica column at 254 nm with HPLC (3). Four reversed-phase HPLC columns have been used to analyse dorzolamide for purity and the chosen columns were packed with Perkin-Elmer CR C_8 and detection at 254 nm (4). The assay of dorzolamide in plasma has been developed using HPLC Beckman RP-8 column with UV detection at 252 nm (5).

An indirect chiral separation method of dorzolamide has been described and the procedure contains the chemical derivatization of the secondary amino group of the inhibitor (6). Dorzolamide has been also determined in eye-drops with HPLC Zorbax RX-C_8 column at 253 nm (7). In this work; a new assay based on HPLC technique is proposed using SPE of dorzolamide in ophthalmic solutions. The extracted dorzolamide was analysed by UV and IR methods. UV and IR spectrums of dorzolamide were similar with literatures (8). A new mobile phase system was used in this work.

Application of solid-phase extraction technique is the first for determination of dorzolamide in ophthalmic solutions. The proposed method is also easy and rapid.

References

1. Blacklock, T.J., Sohar, P., Butcher, J.W., Lamanec, T., Grabowski, E.J.J.: J. Org. Chem. 58, 1672 (1993)
2. Baldwin, J.J., Ponticello, G.S.,Anderson, P.S., Christy, M.E., Murcko, M.A., Randall, W.C., Schwam,H., Sugrue, M.F., Springer, J.P., Gautheron, P., Grove, J., Mallorga, P., Viader, M.P., McKeever, B.M., Navia, M.A.: J. Med. Chem. 32, 2510 (1989)
3. Matuszewski, B.K., Constanzer, M.L.: Chirality 4, 515 (1992)
4. Dovletoglou, A., Thomas, S.M., Berwick, L., Ellison, D.K., Tway, P.C., J. Liquid Chromatog. 18, 2337 (1995)
5. Matuszewski, B.K., Constanzer, M.L., Woolf, E.J., Au, T., Haddix, H.: J. Chromatog. B. 653, 77 (1994)
6. Matuszewski, B.K., Constanzer, M.L., Kiganda, M.: Pharm. Res. 11, 449 (1994)
7. Hasegawa, T., Hara, K., Hata, S.: Drug Metabol. Dispos. 22, 377 (1994)
8. Quint, M.P., Grove, J., Thomas, S.M.Dorzolamide Hydrochloride. In: Brittain, G. and Florey, K. (Eds), Analytical Profiles of Drug Substances and Excipients, Vol. 26, pp. 283-317, Academic Press, San Diego, 1999

Accepted: 07.03.2000