Type of Manuscript:
Research / Review/ Original/ Short Communication/ News / Case Study **(Select any one)**

Title (Times New Roman front 14 **Bold**)

IDENTIFICATION OF TIMOLOL AND VALSARTAN IN TABLET DOSAGE FORM BY SIMULTANEOUS SPECTROPHOTOMETRIC METHOD

Author (S) (Times New Roman front 12 **Bold)**

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Running title (Times New Roman front 12)

Running Title: Spectroscopic estimation of Tablet.

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ABSTRACT: (Times New Roman front 12 **Bold Capital**)
Content (Times New Roman front 12)
Aim: The accurate, precise, sensitive and economical Spectrophotometric method was developed and validated for simultaneous estimation of Timolol and Valsartan in tablet dosage form. Method: The UV method employed was Simultaneous Equation Method. The method employs 274 nm as λ1 and 250 nm as λ2 for formation of equations. Result: Timolol and Valsartan obeys Beer's law in the concentration range 10-160 µg/mL^{-1} (r2=0.9959) and 10-160 µg/mL^{-1} (r^2=0.9979). The mean recovery for Timolol and Valsartan were found to be 100.1±0.3578% and 99.99±0.1852% respectively. Discussion: The developed method were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. Conclusion: Thus the proposed methods were successfully applied for simultaneous determination of Timolol and Valsartan in routine industrial work.

KEYWORDS: (Times New Roman front 12 **Bold Capital**) Content (Times New Roman front 12 [at least 6-8 key words]) Timolol, Valsartan, Validation, Simultaneous Equation Method.

INTRODUCTION: (Times New Roman front 12 **Bold Capital**)
Content (Times New Roman front 12)
Drugs play a vital role in the progress of human civilization by curing diseases. Analytical chemistry is divided into two branches qualitative and quantitative[1]. Timolol is a β_1 receptor specific antagonist, chemically (RS)-4-(2-hydroxy-3-isopropylaminopropoxy) phenylacetamide[2], Valsartan is 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[2′-(1*H*-te-trazol-5-yl)(biphenyl-4-yl)methyl]imidazole, potassium salt[3]. Timolol and Valsartan combination is used in treatment of hypertension.

Content (Times New Roman front 12) Several methods are available in the literature for the determination of Timolol and Valsartan. Most of these methods are for the determination of Timolol and Valsartan separately or in combination with other drug. Analytical methods reported for quantitative determination of Timolol individually in pharmaceutical formulations or biological fluids are HPLC[3-6] and UV[7-9]. Analytical methods reported for quantitative determination of Valsartan individually in pharmaceutical formulations or biological fluids are HPLC[10,11] and UV[12,13]. (Model citation in text)

MATERIAL AND METHODS: (Times New Roman front 12 **Bold Capital**)

Chemicals and reagents: (Times New Roman front 12 **Bold**)

Content (Times New Roman front 12) Timolol and Valsartan potassium were procured from Unichem Laboratories Ltd. (Sikkim). Commercial pharmaceutical preparation Nusar Timolol tablets, manufactured by Emcure Pharma. Ltd., containing 50 mg of Timolol and 50 mg of Valsartan was collected from local market. Acetonitrile, methanol and water used were of analytical grade (Qualigens Fine Chemicals, Mumbai, India). A 0.45 µm nylon filter (Pall life Sciences, Mumbai, India) was used. All other chemicals and reagents used were analytical grade unless otherwise indicated.

Instrumentation: (Times New Roman front 12 **Bold**)

Content (Times New Roman front 12) The proposed work was carried out on a Shimadzu UV-visible spectrophotometer (model UV-1800 series), which possesses a double beam double detector configuration with a1 cm quartz matched cell. All weighing was done on electronic balance (Sansui-vibra DJ-150S-S). A Fast clean ultrasonicate cleaner (India) was used for degassing the mobile phase.

Selection of Solvents: (Times New Roman front 12 **Bold**)

On the basis of solubility study methanol was selected as the solvent for dissolving Timolol and Valsartan.

Preparation of Standard Stock Solutions of Timolol and Valsartan: (Times New Roman front 12 **Bold**)

Timolol Stock Solution: (Times New Roman front 12 **Bold**)

Content (Times New Roman front 12) An accurately weighed quantity of TIMOLOL (50 mg) was taken in 50 ml volumetric flask and dissolved in methanol (20 ml) with the help of ultrasonication for about 10 min. Then the volume was made up to the mark using methanol to get Timolol standard stock solution (1 mg / ml).

Timolol Working Standard Solution: (Times New Roman front 12 **Bold**)

Content (Times New Roman front 12) Timolol standard stock solution 5 mL was diluted to 50 mL using 77% v/v methanol to get working standard solution 100 µg / mL

Valsartan Stock Solution: (Times New Roman front 12 **Bold**)

Content (Times New Roman front 12) An accurately weighed quantity of valsartan (50 mg) was taken in 50 mL volumetric flask and dissolved in methanol (20 mL) with the help of ultrasonication for about 10 min. Then the volume was made up to the mark using methanol to get Valsartan standard stock solution (1 mg/mL).

Valsartan Working Standard Solution: (Times New Roman front 12 **Bold**)

Content (Times New Roman front 12) Valsartan standard stock solution 5 mL was diluted to 50 mL using 77% v/v methanol to get working standard solution 100 µg / mL

Determination of λ Max of Individual Component: (Times New Roman front 12 **Bold**)

Content (Times New Roman front 12) An appropriate aliquot portion of Timolol (0.8 mL) and Valsartan (0.2 mL) were transferred to two separate 10 mL volumetric flasks, the volume was made up to the mark using 77 %v/v methanol to obtain Timolol (80 µg/mL) and Valsartan (20 µg/mL). Drug solutions were scanned separately between 200 nm to 400 nm. Timolol shows λ_{max} at 274 nm while Valsartan at 250 nm, respectively (Fig 1).

Overlay Spectra of Timolol and Valsartan: (Times New Roman front 12 **Bold**)

Content (Times New Roman front 12) The overlain spectrum of both drugs was recorded (Fig 1) and two wavelengths 274.0 nm (λ_{max} of Timolol) and 250.2 nm (λ_{max} of Valsartan) were selected for further study.

Linearity Study for Timolol: (Times New Roman front 12 **Bold**)

Content (Times New Roman front 12) An accurately measured aliquot portion of working standard solution of TIMOLOL was transferred to seven separate 10 mL volumetric flasks. The volume was made up to the mark using 77% v/v methanol to obtain concentrations (10-160

µg/mL). Absorbance of these solutions was measured at 274 nm, (Table1) Calibration curve was plotted, absorbance Vs concentration as shown in (Fig 2).

Linearity Study for Valsartan: (Times New Roman front 12 **Bold**)

Content (Times New Roman front 12) Accurately measured aliquot portions of working standard solution of VALSARTAN were transferred to seven separate 10 mL volumetric flasks. The volume was made up to the mark using 77% v/v methanol to obtain concentrations (10-160 µg/mL). Absorbance of these solutions was measured at 250 nm, (Table 1). Calibration curve was plotted, absorbance Vs concentration as shown in (Fig 3).

Estimation of Laboratory Mixture by Proposed Method: (Times New Roman front 12 **Bold**)

Content (Times New Roman front 12) In order to see the feasibility of proposed method for simultaneous estimation of TIMOLOL and VALSARTAN in marketed pharmaceutical formulations, the method was first tried for estimation of drugs in standard laboratory mixture. Accurately weighed TIMOLOL (50 mg) and VALSARTAN (50 mg) were taken in 100 mL volumetric flask, dissolved in methanol (60 mL) with the help of ultrasonication for about 10 min and the volume was made up to mark using the same. Appropriate aliquot portion (1 mL) was transferred to 10 mL volumetric flask and further diluted using 77% v/v methanol to get Timolol (50 µg/ mL) and VALSARTAN (50 µg/ mL). The absorbance was recorded at 274 nm and 250 nm against solvent as blank.

Amount of each drug was estimated using following equations,

$$C_x = \frac{A_2 \times ay_1 - A_1 \times ay_2}{ax_2\, ay_1 - ax_1\, ay_2}$$

$$Cy = \frac{A_1 \times ax_2 - A2 \times ax_1}{ax_2\, ay_1 - ax_1\, ay_2}$$

Where; A1 and A2 are the absorbance of diluted mixture at λ_1 and λ_2, Cx and Cy are the concentration of X and Y respectively. ax1 and ax2 are absorptivities of X at λ_1 and λ_2 respectively. ay1 and ay2 are absorptivities of Y at λ_1 and λ_2 respectively.

Application of the Proposed Method for Estimation of Drugs in Tablets: (Times New Roman front 10 **Bold**)

Content (Times New Roman front 10) Twenty 'Nusar-Timolol' Tablets containing Timolol (50 mg) and Valsartan (50 mg) were weighed and ground to fine powder. A quantity of sample equivalent to Timolol (50 mg) and Valsartan (50 mg) was transferred into 100 mL volumetric flask containing methanol (60 ml), sonicated for 15 min and the volume was made up to the mark and filtered through Whatman filter paper (No. 45). This solution was (1 ml) transferred to 10 ml volumetric flaks, dissolved and volume was adjusted to the mark. The absorbance of the solutions was measured at 274 nm and 250 nm against blank. The concentrations of two drugs in sample were determined by using simultaneous equations.

Validation of Proposed Method: (Times New Roman front 10 **Bold**)

The Proposed method was validated as per the ICH guidelines.

Accuracy [Recovery Study]: (Times New Roman front 10 **Bold**)

Content (Times New Roman front 10) Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. A known amount of standard drug solutions were added to the tablet powder to make final concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method. The absorbance recorded and the % recoveries were calculated using formula. % Recovery = [A - B/ C] X 100

Where, A = Total amount of drug estimated, B = Amount of drug found on preanalysed basis and C = Amount of Pure drug added.

Precision: (Times New Roman front 10 **Bold**)

Content (Times New Roman front 10) Precision was determined as intra-day and inter-day variations. Intra-day precision was determined by analyzing Timolol (19.2, 25.6, and 32 µg/mL) and Valsartan (19.2, 25.6, and 32 µg/mL) for three times on the same day. Inter-day precision was determined by analyzing the same concentration of solutions for three different days over a period of week.

Ruggedness: (Times New Roman front 10 **Bold**)

Content (Times New Roman front 10) Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by two different analyst using same operational and environmental conditions.

LOD: (Times New Roman front 10 **Bold)**

Content (Times New Roman front 10) Limit of detection of Timolol and Valsartan were found to be 0.00407 µg/mL and 0.00913 µg/mL respectively.

LOQ: (Times New Roman front 10 **Bold)**

Content (Times New Roman front 10) Limit of Quantitation of Timolol and Valsartan were found to be 0.01233 µg/mL and 0.02769 µg/mL respectively.

ACKNOWLEDGEMENT: (Times New Roman front 12 **Bold Capital)**

The authors are grateful to the authorities of T.V.E.S.'s Hon. L. M. C. College of Pharmacy, Faizpur for the facilities.

CONFLICT OF INTEREST: (Times New Roman front 12 **Bold Capital)**

The authors declare no conflict of interest.

REFERENCES: (Times New Roman front 12 **Bold Capital)**

Content (Times New Roman front 12)

1. Jeffery GH, Bassett J, Mandham J, Denny RC, Editors. Vogel's Text Book of Quantitative Chemical Analysis. UK: Longman Scientific and Technical Pub; 1994. pp. 3-4.

2. Gaur R, Azizi M, Gan J, Hansal P, Harper K, Editors. British Pharmacopoeia. London: Medicines and Healthcare products Regulatory Agency (MHRA); 1988. pp. 49-58.
3. Bhusari VK, Dhaneshwar SR. Validated HPTLC Method for Simultaneous Estimation of Timolol, Hydrochlorothiazide and Amlodipine Besylate in Bulk Drug and Formulation. Int J Anal Bioanal Chem, 2011; 1(3): 70-76. (Model Journal reference)
4. Chheta N, Gandhi SP, Rajput SJ. Development and Validation of a Stability indicating High Performance Liquid Chromatographic (HPLC) Method for Timolol and Hydrochlorthiazide in Bulk Drug and Tablet Formulation. Int J Chem Tech Res, 2009; 1(3): 654-662.
5. Krishna R, Gupta A, Wadodkar R, Wadodkar SG. Validated Reverse Phase HPLC Method for Simultaneous Estimation of Atorvastatin and Timolol in Tablets. Der Pharmacia Lettre, 2011; 3(4): 393-403.
6. Jaina N, Jaina R, Thakura N, Jaina S and Jain DK. Simultaneous Spectrophotometric Estimation of Lercanidipine Hydrochloride and Timolol in Tablet Dosage Form. Eurasian J Anal Chemistry. 2011; 6(2): 84-90.

Table 1. Standard curve data of Timolol and Valsartan. (Times New Roman front 12 **Bold**)

Sl. No.	Concentration (µg/ml)	Absorbance	
		Timolol	Valsartan
1	2	0.022	0.234
2	4	0.067	0.441
3	6	0.085	0.511
4	8	1.02	0.824
5	10	1.05	1.101
6	12	1.08	1.231

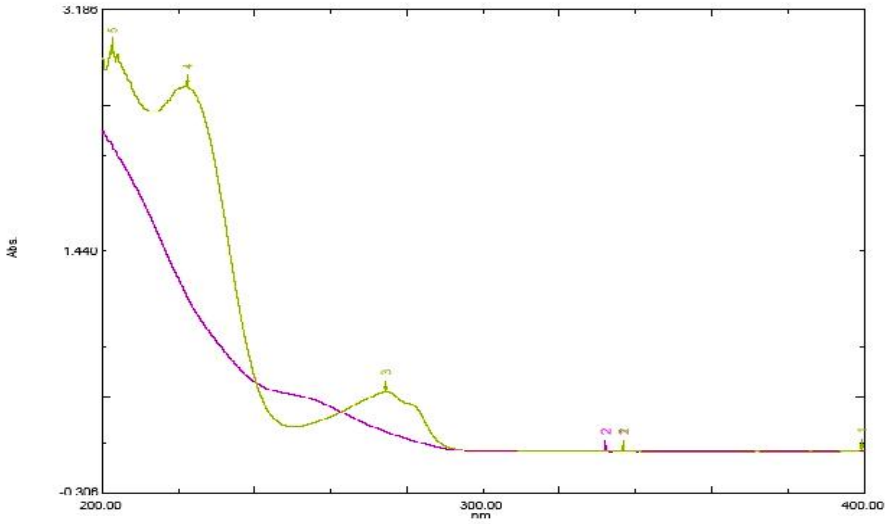

Fig 2. Overlay Spectra of Timolol and Valsartan. (Times New Roman front 12 **Bold**)