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# Timolol Form Some Prostagland in Analoges BTRP-HPLC Method A Review

M.Rama Krishna(1), S. Sai Latha(2), B.Siva Kumar(3), K.Priyanka(4), P.Nirmal Kumar(5), J.N. Suresh Kumar(6)

*(1) Research Scholar,CPU, Rama Krishna, Department of Pharmaceutical Analysis, Narasaraopet Institute of Pharmaceutical Sciences, Narasaraopet*

*(2,3,4,5) Research Scholar, B. Pharmacy, Narasaraopet Institute of Pharmaceutical Sciences, Narasaraopet*

*(6) Professor and Principal, Department of Pharmaceutics, Narasaraopet Institute of Pharmaceutical Sciences, Narasaraopet, Kottapakonda Road, Guntur(District)*

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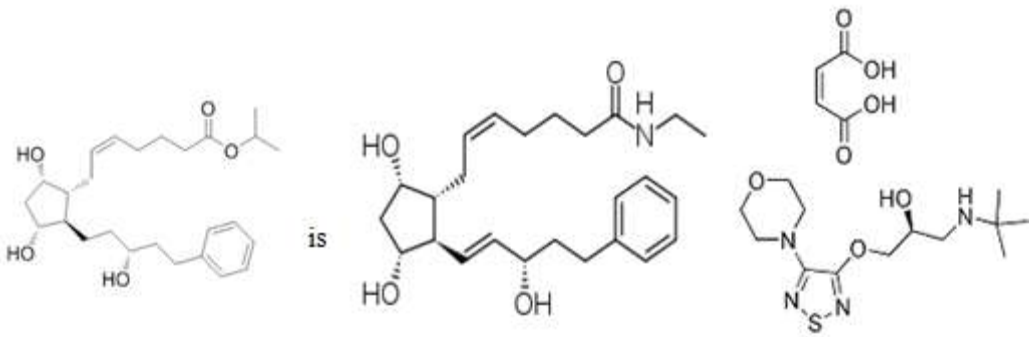
**ABSTRACT**

Timolol (TIM), along with some prostaglandin analogues, they are Bimatoprost, Latanoprost and Travoprost are used in treatment of glaucoma by decreasing intraocular pressure. The chromatography separation was carried out using gradient elution mode with a mobile phase consisting of 25 mM phosphate buffer (Ph 3-+0.1), acetonitrile and methanol at flow rate of 1Ml /min on a stationary phase composed of Thermo hypersil BDSC 18 ( 4.6 X 250 mm,5um column . UV detection was carried out at 295 nm and 210 nm wavelength at 25℃ The method was carried validated with respect to accuracy, Precission , linearity and robustness as per ICH guidelines were over the concentration ranges of 10.00-600.00 ug /ml for Timolol, 0.99995 for Bimatoprost, Latanoprost and 0.99996 for Travoprost the method was successfully applied for the determination of Timolol in its synthetic mixture and co –formulated eyedrops with each of the prostaglandin analog combination with Timolol

**KEY WORDS:** Timolol (TIM), Bimatoprost (BIM), Travoprost (TRV), High Performance liquid Chromatography (HPLC).

## I. INTRODUCTION

Fixed –combination eyedrops for treating glaucoma were developed to improve patient compliance. Timolol is a non –selective adrenergic receptor blocking agent. It blocks beta receptors that are found on the ciliary body. this action reduces the amount of aqueous humour that is secreted into the eyeball by cillary body. Timolol also blocks beta receptors found on the blood vessels that supply the ciliary body. This causes the blood vessels to constrict and reduces the amount of watery fluid that filters out of the blood vessels to form aqueous humour. Bimatoprost(1(c)) is synthetic prostaglandin analog chemically 7-[3-hydroxy -5-phenyl]-pent-1enyl)-cyclopentyl]-N-ethyl-hept-5-enamide.Bimatoprost also lower the rate of aqueous formation in eye . both these effects the decrease the pressure with in the eye. So the availability of the prostaglandin, timolol fixed combination has simplified adjunctive medication regimens. These combination reduces the intraocular pressure in the treatment of open angle glaucoma and ocular hypertension(Fig.1).




1(a) Timolol maleate 1(b) Latnoprost 1(C) Bimatoprost

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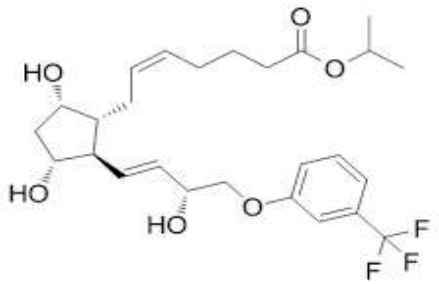


1(d) Travoprost

**Fig.1 The Structural Formulae for the Studied Drugs**

## Experimental

### Materials and Reagents

All the chemicals used were of analytical re-agentgrade,andthesolventswereofHPLCgrade.TIM maleate (certifiedtocontain 99.9%)and BIM (certified to contain 99 %) were sup-pliedbyAllergan-Cairo-Egypt.LAT(certifiedto contain 100.1 %) was supplied by Orchidia,Egypt. TRV (certified to contain 100 %) waskindly supplied by clearsynth, Egypt. Potassiumdihydro-genorthophosphate,methanolandaceto-nitrile were obtained from (Sigma-Aldrich, MO,U.S.A). Ganfort®eye drops solution (each 1 mlof solution labeled to contain 0.3 mg BIM and 5mg TIM (as 6.8 mg of TIM maleate)); batch #E74596waskindlysuppliedby(AllerganPharma-ceuticals Ireland, Ireland). Xalacom®eye dropssolution(each1mlofsolutionlabeledtocontain50gLATand5mgTIM);batch#J69719(PfizerManufacturingBelgiumNVpuurs,Belgium),DuoTrav®eyedrops solution (each 1 mlof solutionlabeledtocontain40gTRVand5mgTIM);

### Instrumentation

TheLCsystemconsistedofThermoScientific™UltiMate™ 3000 Rapid Separation QuaternarySystem (UnitedStates).Separationandquantitationwere madeonThermoHypersilBDS $C_{18}$ (4.6 x 250 mm, 5m) column. Dionex™Chromeleon™7.2Chromatography DataSystem wasusedfortheinstrumentcontrol,data acquisition and analysis. Jenway 3510 pH-meterwas used for pH measurements. Deionized waterwaspreparedusingMilli-Q®Direct8/16sys-tem - Millipore (France). The mobile phase wasfiltered through 0.2m Anatop 25 Whaman in-organicmembranefilter(Maidstone,England)anddegas sedusingElmasonicPdegasser(Germany)

### Chromatographicconditions

Amixtureofpotassiumdihydrogen orthophosphate buffer solution (pH 3±0.1, 25mM)(PBS),acetonitrile and methanol in gradient elu-tionmodeasshownin(Table2a)wasprepared.

| DosageForm | ActiveIngredient |
|---|---|
| Ganfort®eyedrops | TimololandBimatoprost |
| Xalacom® eye drops | TimololandLatanoprost |
| DuoTrav®eyedrops | TimololandTravoprost |
| Timoptol®eyedrops | Timolol |
| Lumigan®eyedrops | Bimatoprost |
| Xalatan®eyedrops | Latanoprost |
| Travatan®eyedrops | Travoprost |

**table1a.Someofthemostcommonlyprescribedanti-glaucomaeyedropsavailableinthemarket**

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| Time(min) | PBS% | Acetonitrile | % | Methanol | % |
|---|---|---|---|---|---|
| 0 | 50 | 45 | 5 | | |
| 6 | 15 | 70 | 15 | | |
| 8 | 15 | 70 | 15 | | |
| 8.1 | 50 | 45 | 5 | | |
| 10 | 50 | 45 | 5 | | |

**Table2a.Timetableofthevalidatedgradientmethod**

**Standard stock solutions preparation**

136.68 mg of TIM maleate (equivalent to 100mgTIM)aswellas10mgofeachofBIM,LATand TRV were accurately weighed and transferredinto100mlambervolumetricflasksseparately.Then,theyweredissolvedinasolventcom-posed of acetonitrile and water (50:50, v/v) andthe solutions were made up to volume with thesamesolventtogivefinalconcentrationsof1000.00 mg/ml of TIM as well as 100.00 mg/mlforBIM,LATandTRV.

**Procedure**

**Construction of Calibration Graphs**

AliquotsofTIM,BIM,LATandTRVfromtheir stocksolutionsweretransferredseparatelyinto4series of10mlambervolumetricflaskstogetthefinalconcentrationsintherangeof10.00-600.00 □ g/mlforTIMand1.00-60.00□ g/mlforBIM, LAT and TRV. The volume was diluted with asolventcomposedofPBS,acetonitrileandmethanol (50:45:5, v/v/v). The solution was injected intriplicate and chromatographed under the chromatographicconditions.Thepeakareaswereplotted versus the concentration of each drug in □ g/ml to get the calibration graphs and the correspondingregressionequationswerederived.

**Preparation of the laboratory prepared syntheticmixtures**

AccuratealiquotsofTIM,BIM,LATandTRV stock solutions were transferred into a series of10mlambervolumetricflaskskeepingthepharmaceuticalratiosof500:30:5:4□ g/ml,250:15:2.5:2 □g/ml, 600:36:6:4.8 □ g/ml, 300:30:5:4 □ g/ml,400:15:2.5:2 □ g/ml for TIM/BIM/LAT/TRV respectivelyandthendilutedtovolumewithasolventcomposedofPBS,acetonitrileandmethanol(50:45:5, v/v/v). The percentage recoveries werecalculatedusingthecorrespondingregressionequations.

## II. RESULTS AND DISCUSSION

Theaimofthisworkistodevelopandvalidateasimpleandfast RP-LCmethodforthesimultaneousdeterminationofTIM,alongwithsomeofthemostcommonlyprescribedprostaglandinana-logs. The four drugs are separated in a reasonableruntimelessthan8min.asshowninIt also permitted the assay of these drugs in theirsyntheticmixturesandinpharmaceuticaleyedropssolution.

**Table3.SystemsuitabilitydatafortheRP-LCmethod**

| Item | TIM | BIM | LAT | TRV |
|---|---|---|---|---|
| N | 10454 | 15045 | 42896 | 48455 |
| K‘ | 10.57 | 12.99 | 27.68 | 28.83 |
| Tailingfactor(T) | 0.89 | 0.98 | 0.92 | 0.92 |
| %RSDof6injections | 0.4045 | 0.565 | 0.426 | 0.32 |
| □ | 1 | 1.22 | 2.51 | 2.61 |
| R | 5.9 | 29.3 | 2.22 | |

N:numberoftheoreticalplates;

K‘:capacityfactor; R: resolution factorT:tailingfactor; □ relativeretentiontime

separation of the four analytes in a shorter runtime. Using buffer in mobile phase was necessarytoinfluencetheionizationoftheanalytesandhelp

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their co-elution. Different pH values wereexamined and the best separation was achievedusingpH=3.Gradientelutionpermitshorterti me(<8min)forseparationofthefourdrugswithgoodreso lution between all peaks especially betweenLAT and TRV (Fig. 2). The retention times forTIM,BIM,LATandTRV were2.85,3.52,7.26 and7.58minrespectively.Thesystemsuitabilitytestsw ereusedtoverifythattheconditionsof the chromatographic system were adequate fortheseparation(Table3).

**Table4.CharacteristicsandresultsoftheproposedRP-LCmethod**

| Item | TIM | BIM | LAT | TRV |
|---|---|---|---|---|
| Linearityrange(□ g/mL) | 10-600 | 1-60 | 1-60 | 1-60 |
| Limitofdetection,LOD(□ g/mL) | 0.027 | 0.0203 | 0.1507 | 0.134 |
| Limitofquantitation,LOQ(□ g/mL) | 0.082 | 0.0617 | 0.4567 | 0.407 |
| Intercept(a) | 364.37 | 3.5462 | 0.0702 | 1.8235 |
| Slope(b) | 28.227 | 33.926 | 22.175 | 18.963 |
| Correlationcoefficient($R^2$) | 0.9965 | 0.99995 | 0.99998 | 0.99996 |
| S.D.ofintercept(Sa) | 202.26 | 2.884 | 0.988 | 1.417 |
| S.D.ofslope(Sb) | 0.635 | 0.091 | 0.031 | 0.0445 |

[a]Y=a+bC,whereCistheconcentrationin□ gm$L^{-1}$andYisthepeakarea

**Table5a.Recoveryexperiments obtainedfor different binarymixturesofTimolol(TIM)andBimatoprost(BIM)intheirpharmaceuticaldosageformanalyzedbythede velopedHPLCmethod**

| Ganforteye | | Added | | Found | | Recoverydrops(□g.ml$^{-1}$) | | (□g.ml$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|
| | % | | | | | | | | |
| Timolol | Bimatoprost | Timolol | Bimatoprost | Timolol | Bimatoprost | Timolol | Bimatoprost | | |
| 200 | 12 | 50 | 5 | 50.703 | 5.041 | 101.406 | 100.829 | | |
| 250 | 15 | 250 | 25 | 250.327 | 10.145 | 100.131 | 101.454 | | |
| 300 | 18 | 150 | 10 | 148.162 | 15.176 | 98.775 | 101.175 | | |
| 400 | 24 | 100 | 15 | 101.649 | 20.082 | 101.649 | 100.410 | | |
| 500 | 30 | 50 | 20 | 50.367 | 25.474 | 100.734 | 101.897 | | |
| | | | **X %** | **RSD%** | | | | | |
| | | Timolol | 100.5388 | 1.145 | | | | | |
| | | Bimatoprost | 101.153 | 0.564 | | | | | |

**Table5b.Recoveryexperimentsobtainedfordifferentbinarymixturesof Timolol(TIM)andTravoprost(TRV)i ntheirpharmaceutical dosageformanalyzedbythedevelopedHPLCmethod**

| DuoTraveye | | Added (□g.ml$^{-1}$) | | Found(□g.ml$^{-1}$) | Recovery% | drops(□g.ml$^{-1}$) | | (□g.ml$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | % | | | | | |
| Timolol | Travoprost | Timolol | Travoprost | Timolol | Travoprost | Timolol | Travoprost | | |
| 200 | 1.6 | 50 | 3 | 50.5817 | 3.0182 | 101.1635 | 100.606 | | |
| 250 | 2 | 300 | 1 | 98.0634 | 0.998 | 98.0634 | 99.83125 | | |
| 300 | 2.4 | 250 | 1.5 | 151.176 | 1.4766 | 100.784 | 98.4408 | | |
| 400 | 3.2 | 150 | 2 | 245.325 | 2.01075 | 98.130 | 100.5378 | | |
| 500 | 4 | 100 | 4 | 294.387 | 4.0527 | 98.129 | 101.318 | | |
| | | | **X %** | **RSD%** | | | | | |

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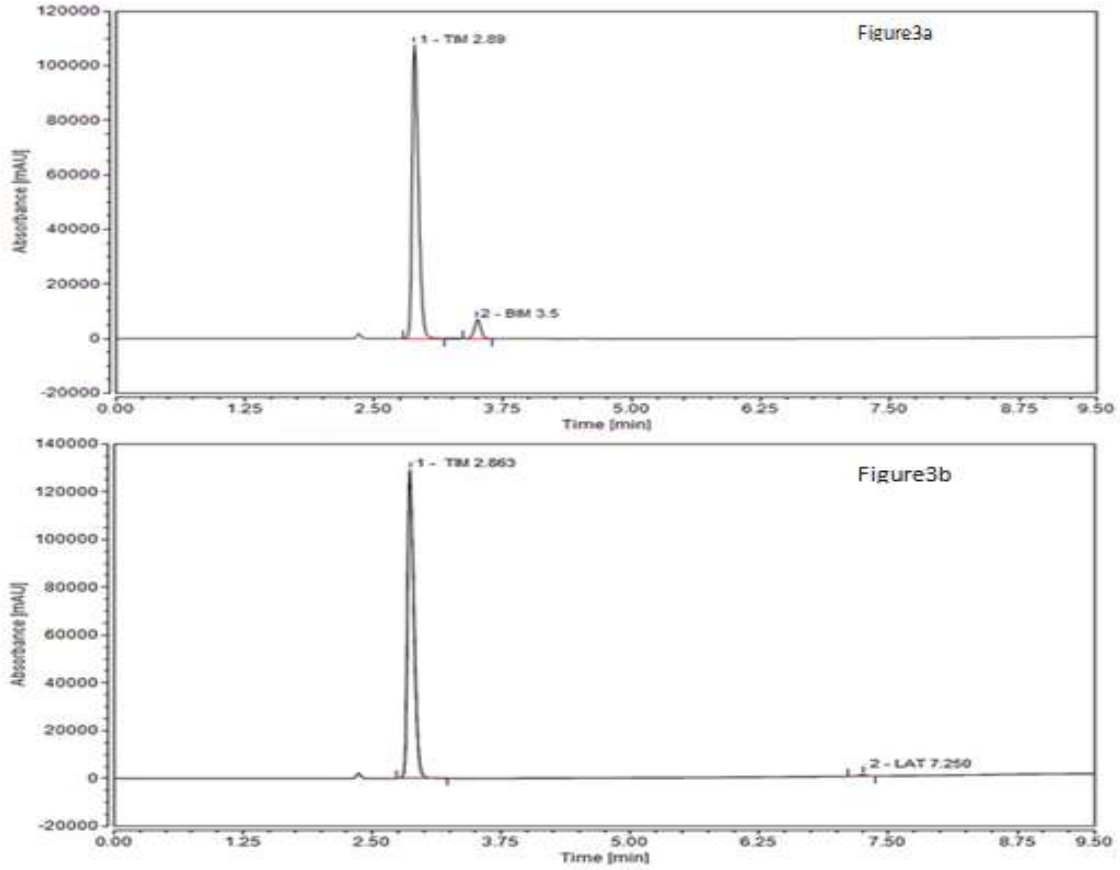
| | | |
|---|---|---|
| Timolol | 99.254 | 1.5877 |
| Travoprost | 100.147 | 1.0877 |

**Table5c.Recoveryexperimentsobtainedfordifferentbinarymixturesoftimolol(TIM)andlatanoprost(LAT)intheirpharmaceutical dosageformanalyzedbythedevelopedHPLCmethod**

| Xalacomeye | | Added(□g.ml$^{-1}$) (□g.ml$^{-1}$) | | Found(□g.ml$^{-1}$) % | | Recoverydrops(□g.ml$^{-1}$) | | (□g.ml$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| Timolol | Latanoprost | Timolol | Latanoprost | Timolol | Latanoprost | Timolol | Latanoprost | |
| 200 | 2 | 300 | 3 | 294.48205 | | 2.976 | 98.1606 | 99.2048 |
| 250 | 2.5 | 250 | 2.5 | 252.56807 | | 2.5375 | 101.027 | 101.5017 |
| 300 | 3 | 100 | 1 | 98.71867 | | 1.0077 | 98.7186 | 100.771 |
| 400 | 4 | 125 | 2 | 126.7079 | | 2.01713 | 101.366 | 100.8568 |
| 500 | 5 | 50 | 2.5 | 50.840 | | 2.5234 | 101.680 | 100.9371 |
| | | | **X %** | **RSD%** | | | | |
| | Timolol | | 100.190 | 1.6239 | | | | |
| | Latanoprost | | 100.6543 | 0.8537 | | | | |

**Specificity**

The specificity of the method was confirmedby observing any interference encountered fromcommonexcipients.Itwasfoundthattheexcipients did not interfere with the signals of the analytes. As shown in (Fig. 3 a, b, c). By compar-ing the chromatograms of the combined formula-tions and that of the synthetic mixtures, no addi-tional peak.



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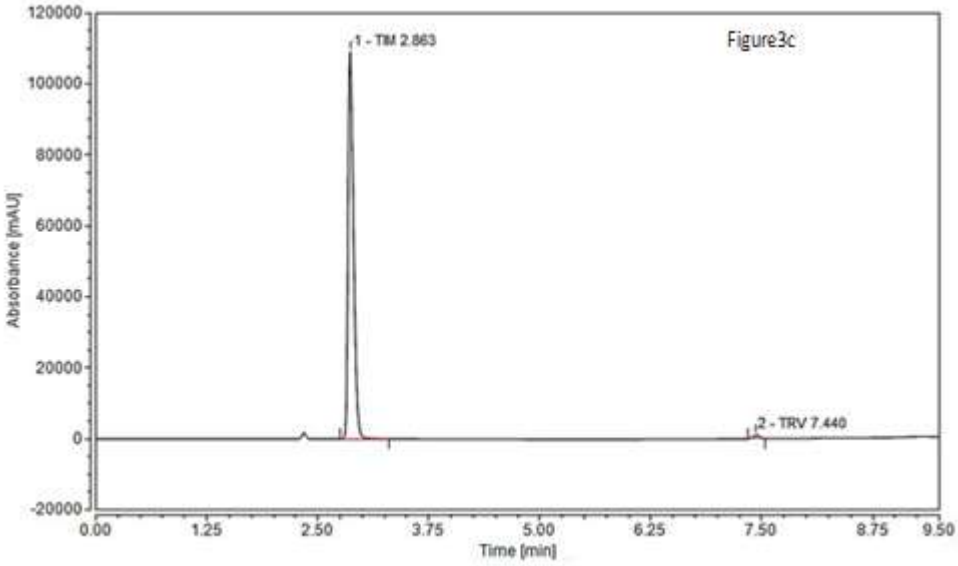


**Robustness**

Therobustnessofthemethodwasconfirmedb ychangingindifferentexperimentalparameters suchaspH(±0.3)andTemperature(±2ºC).Theobservat ionwasthatthereisnosignificanteffectontheresolution oftheanalyzedcompoundswhichindicatethatthepropos edmethodisrobust.

**Selectivity**

The proposed RP-LC method was applied tothesimultaneousdeterminationofTIMwithBIM, LAT and TRV in their synthetic mixturesin the medicinally recommended ratios of 500/30/5/4mg/ml,250/15/2.5/2mg/ml,600/36/6/ mg/ml, and in different ratios of 300/30/5/4mg/ml, 400/15/2.5/2mg/ml for TIM/BIM/LAT/TRV.

**Figure 2.** LC chromatograms of the analyzed pharmaceutical formulations using the describedchromatographic conditions where; (a) Ganfort®eye drops: TIM 250 g/ml, BIM15 g/ml (b)Xalacom®eyedrops:TIM250g/ml,LAT3g/ml(c)Duo trav®eyedrops:TIM250g/ml,TRV1.6 g/ml.

## III. CONCLUSION

A validated RP-HPLC method for the simultaneous determination of TIMOLOL Maleate along with mostcommonly prescribed prostaglandin analogues,namely; Bimatoprost, Latanoprost, Travoprost. This method issimple,accurate,preciseanditcanbeusedforroutinea nalysisofthecited drugsseparately orincombinations inmanydosageforms.

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