CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1 INTRODUCTION :

Betablockers are competitive inhibitors of the effects of catecholamine at beta-adrenergic receptor sites. The principal effect of betablocker is to reduce cardiac activity by diminishing or preventing beta-adrenoceptor stimulation. By reducing the rate and force of contraction of the heart and decreasing the rate of conduction of impulses through the conducting system, the response of the heart to stress and exercise is reduced. These properties are used in the treatment of Angina pectoris to reduce the oxygen consumption and increase the exercise tolerence of the heart. They are used in the treatment of Cardiac Arrhythmias to block adrenergic stimulation of cardic pace maker potentially. Betablockers reduce the incidence of reinfarction and death after myocardial infarction, and if given sufficiently early may reduce infarct size and development.

The betablockers are also beneficial in the long term treatment of hypertension. Betablockers have been shown to reduce intraocular pressure following topical application to the eye[1].

Beta blocking drugs are rapidly gaining acceptance as primary agent for the treatment of hypertension, a trend that has rapidly accelerated over past few years and is likely to continue.

Prompted by this highest status of antihypertensive drugs in the modern therapeutic armamentarium, a few selected compounds were extensively reviewed from the literature. Appreciation of the basic fact that the pharmaceutical industry has an obligation

to design, test and produce dosage forms that provide the consumer with products having the attributes of quality, purity, uniformity of content, stability, safety and physiological availability clearly necessitate pre-formulation profile, stability testing by a stability indicating method and quality control process consistent with the modern concept of quality assurance and biopharmaceutics.

Consequently analytical abstracts and international Pharmaceutical abstracts were reviewed since 1970 to reveal the general methods of analysis and stability characteristics of several antihypertensive, betablockers like Propranalol, Atenolol, Pindolol, Timolol Maleate, Sotalol Hydrochloride and Nadolol.

Literature data on Pindolol an indole derivative was found to be rather scanty and this provoked interest to work on stability and analysis of the same. Nadolol, Sotalol hydrocloride and Timolol Maleate, selective betablockers also merited consideration on similar reasons. Hence all the four compounds viz. Pindolol, Nadolol, Timolol Maleate and Sotalol Hydrochloride were fully reviewed from chemical abstracts since 1970 with regard to their analysis and stability data.

A comprehensive account of literature review for four compounds adopted for the investigation viz. Pindolol, Nadolol, Timolol Maleate and Sotalol Hydrochloride are presented herewith. Literature relevant to the actual work presented in the ensuing chapters is described at length.

There are many excellent compilations (88-92) which deal with the chemical nature, preparation structure and therapeutic applications of these drugs.

1.2 PINDOLOL

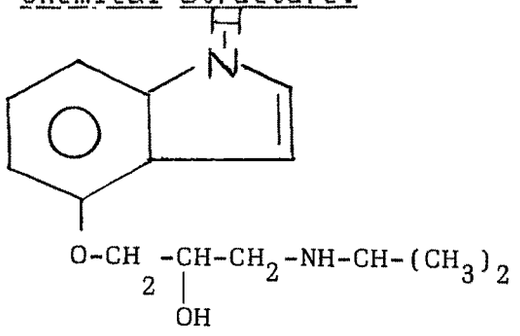
1.2.1 DESCRIPTION [4]

a. Chemical Name : 1-(Indol-4-Yloxy)-3-isopropylaminopropan-2-ol.

b. Molecular Formula : $C_{14}H_{20}N_2O_2$

c. Molecular weight : 248.3

d. Chemical Structure.



e. Appearance, colour, odour and taste :

A white or almost white, odourless or almost ordourless crystalline powder.

f. Official status and dosage forms :

British Pharmacopoeia [3] and United State Pharmacopoeia [4]

as tablets 5 mg/10 mg.

1.2.2 PHYSICAL PROPERTIES [1-4]

a. Solubility :

Practically in soluable in water, slightly soluble in dehydrated alcohol and chloroform. Sparingly soluble in methanol. It dissolves in mineral acids.

b. Melting range : 169° C --174° C

c. Dissociation constant :

$P^{a}K$ value was found to be 9.7 (24° C).

d. Ultraviolet Spectrum :

Characteristic absorption wavelengths in aqueous acid and

methanol being 264 nm and 287 nm. No alkaline shift observed.

e. Infrared Spectrum :

Characteristic absorption frequencies include 768, 1096, 1250, 1288, 1053, 890 wave numbers.

f. Mass spectrum :

Principal peaks at m/z 72, 133, 30, 116, 248, 134, 56, 41.

1.2.3 METHODS OF ANALYSIS :

Literature review for Pindolol revealed UV spectrophotometric, colorimetric, titrimetric, thin layer chromatographic, gas chromatographic, high performance liquid chromatographic methods of analysis as follows.

a. Uv Spectrophotometric methods :

1. British Pharmacopoeia[3] has prescribed a UV spectrophotometric method for the estimation of Pindolol tablets by extracting drug in methanol and measuring the absorbance at 264 nm against methanol as blank.

b. Colorimetric Methods :

1. Issa et al[5] have reported a colorimetric method for estimation of Pindolol as bulk drug based on its reaction with 2,3-dichloro 5,6-dicyano-p-benzoquinone. The absorbance was measured at 460 nm against appropriate reagent blank.
2. Mahrous et al[6] have reported colorimetric method for estimation of Pindolol based on its reaction with P-chloranilic acid to give a stable and intensively coloured ion pair salt having absorbance maxima at 522 nm Vs. a reagent blank. Beer's law was valid for 0.04-16 µg/ml.
3. A simple and rapid colorimetric method for the estimation of pindolol in tablets was reported by El-Yazbi et al[7]. The

drug was reacted with 3-Methyl-2-benzothiazolone hydrazone Hcl in presence of Ferric chloride and hydrochloric acid. The absorbance was measured at 550 nm. A linear correlation was observed between 3.0-15 µg/ml.

4. G. Raman Rao et al[8] have reported a colorimetric method for the estimation of pindolol as bulk drug and tablets using F-C reagent in presence of sodium carbonate solution. The absorbance was measured at 765 nm and Beer's law obayed in 1-10 ug/ml range.

5. Shastry C.S.P. et al[9] have used a colourimetric method for the assay of tablet and bulk durg. Pindolol was treated with Metol and Pottassium dichromate in the pH 3.0 phthalate buffer medium and absorbance was measured at 640 nm after 10 minutes. Beer's law was obayed in the range of 8-64 µg/ml.

c. Spectrophotofluorimetric Methods :

1. Mohamed et at[10] have reported spectrofluorometric determination of Pindolol and its dosage forms. Pindolol was determined in tablets at ambient temperature by spectrophotofluorimetric method in 96% v/v ethanol as the solvent. The intrinsic fluorescence of drug showed excitation and emission maximum at 263nm and 305 nm respectively. The calibration curve was linear in the range 1.0×10^{-7} to 1.5×10^{-6} M.

d. Thin layer Chromatographic Methods :

1. Spahn.H et al[11] have reported T.L.C. method for determination of pindolol in plasma and urine using precoated sillica gel plates developed in $CHCl_3$: MeOH : HoAc (15:20:5). The detection limit in plasma was 2 ng/ml and in urine was 20

ng/500 µl.

2. Zuo et al[12] have employed T.L.C. technique on silica gel for estimation of Pindolol from tablets using CH_2Cl_2 : MeoH : HCooH (75:23.5:1.5) as developing solvent and detection at 254 nm.

3. Jack D.B. et al[13] have used two different solvent systems for detection of pindolol in methanol extracts obtained from urine samples. The solvent systems were, $MeCO_2Et$: meoH (40:5) and $MeCO_2Et$: MeoH : concentrated NH_3 (40:5:5).

e. Gas chromatographic methods :

1. Susanto F. et al[14] have reported gas chromatographic method for assay of Pindolol in human plasma samples.

2. Guerret.M. et al[15] have reported an electron capture gas liquid chromatographic method for the estimation of Pindolol from plasma or urine.

3. Yamaguchi et al[16] has proposed a gas chromatographic method for the estimation of Pindolol in plasma and other biological samples.

4. Tawakol et al[17] have reported a gas chromatographic method for the estimation of Pindolol it self and in tablet formulation.

f. Titrimetric Method :

1. B.P.[3] AND U.S.P.[4] methods are same in principle involving non-aqueous titration against 0.1N perchloric acid for the assay of bulk drug using potentiometric end point detection.

g. High performance liquid chromatographic methods :

Reported in table 1.

1.2.4 PHARMACOLOGICAL PROFILE :

1. Pharmacokinetics : Pindolol is completely absorbed from the

Table 1

g ESTIMATION OF PINDOLOL BY HPLC

Sample	Interal std	Column	Mobile phase	Detector	Detection limit	Ref
Plasma		Lichcrosphere octadecyl silane	Methanol : perchloric acid 1:4	Amperio-metric	0.5 ng/ml	18
Plasma Urine		Nucleclosil C18	Methanol : perchloric acid 1:4	uv	50 ng/ml 5 ug/ml	19
Plasma		ODS	Perchloric acid : MeCN 3:2	Fluores-cence		20
Plasma Urine		ODS	99.62% Methanol	Fluores-cence	2 ng/ml	21
Plasma		Supercosil Lc 8	Propan 2-ol: Acetonitrile 0.3% H PO 1:10:9 3 4	Fluores-cence	2 ng/ml	22
Plasma		ODS	MeOH : 0.01M HClO 1:4 4	Ampero-metric	0.5 ng/ml	23
Tablet	Nor tript-yline	L16	Acetonitrile : methanol: ammonium carbonate: 475 : 475 : 50	uv		24

gastrointestinal tract and peak plasma concentrations are obtained about 1-2 hours after a dose. It is only partially metabolised and is excreted in the urine both unchanged and in the form of metabolites. Small amolunts are reported to be excreted in bile. About 40-60% is reported to be bound to plasma proteins. Pindolol is also excreted in breast milk.[1]

2. Adverse effects : the adverse effects observed after oral administration of Pindolol have been described.[1]

3. Uses : Pindolol is used in the treatment of hypertension and angina pectoris.[1]

1.3 TIMOLOL MALEATE[1-4,24]

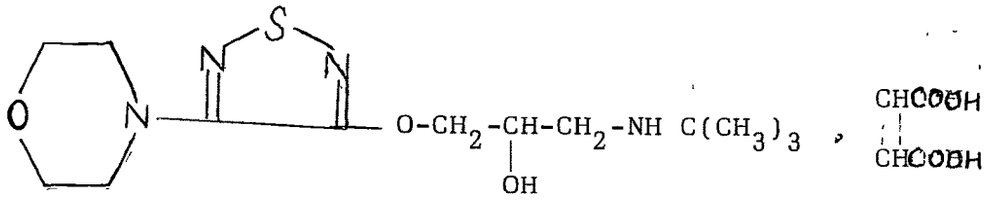
1.3.1 DESCRIPTION :

a. Chemical Name : (-)-(S)-1-(tert butylamino)-3-[(4-morpholino-1,2,5-thia diazol-d-Yl)oxy]-2-Propanol.

b. Molecular Formula : $C_{13}H_{24}N_4O_3S.C_4H_4O_4$.

c. Molecular Weight : 432.49

d. Chemical structure :



e. Appearance, color, odour and taste :

Timolol Maleate is a white, odourless, crystalline powder.

f. Official status and dosage forms :

British Pharmacopoeia[3] and united States Pharmacopoeia[4] as tablets and eye drops.

1.3.2 PHYSICAL PROPERTIES [1-4,24]

a. Solubility :

Soluable in water, ethanol, methanol. Sparingly soluble in chloroform, propylene glycol. Practically insoluble in ether, cyclohexane, iso octane.

b. Melting range : 201.5° C – 202.5° C

c. Dissociation constant : E.Obernoltzer[25] has reported Pka of timolol base by potentiometric titration in water at 25° C is approximately 9.2. The native pH of a saturated aqueous solution of timolol maleate is 4.

d. Ultraviolet spectrum :

Characteristic absorption wavelength in aqueous acid being 294 nm. No alkaline shift observed.

e. Infrared Spectrum :

Characteristic absorption frequencies include 1497, 1120, 1230, 1590, 1620 wave numbers.

f. Mass Spectrum :

Principal peaks at m/z 30, 86, 57, 129,74,56,41, 114.

1.3.3 STABILITY

R. Raman et al[26] reported solid state and solution stability. Timolol maleate is an extremely stable compound at room temperature in the solid state. Heating timolol maleate to above its melting point and keeping it molten for 10 minutes produced discoloration and 5 % loss in potency. Same results are obtained on heating timolol maleate at 95° C for 3 weeks in air atmosphere (100% RH). Timolol Maleate is extremely stable in aqueous solution (protected from light) with no decomposition being detected in injectable formulations stored at ambient temperature

for five years. Timolol maleate can be degraded in solution if exposed to elevated temperature or intense ultraviolet radiation. Maximum stablity occuring near pH4. It is recommended thataqqueous timolol maleate solutions be stored protected from light.

1.3.4 METHODS OF ANALYSIS

Literature review for Timolol Maleate revealed titrimetric analysis, spectrophotometric analysis, UV spectrophotometric, colorimetric, thin layer chromatographic, high performance liquid chromatographic, gas chromatographic methods of analysis.

a. Titrimetric Methods :

The British Pharmacopoeia[3] United States Pharmacopoeia[4] has prescribed nonaqueous titration method against perchloric acid for the assay of bulk drug using potentiometric end point detection.

b. UV Spectrophotometric Methods :

1. A direct spectrophotometric method has been proposed based on the extinction at 294 nm for determination of timolol maleate in eye drops or opthalmic solution[3,29] in tablets[3,27] in bull drugs and formulation[29].

2. Bigley et al[30] have reported a UV Spectrophotometric method via. flow injection analysis as the detection mode for timolol maleate determination.

c. Colorimetric Methods :

1. Guvena B. et al[31] have reported a sensitive colorimetric method for the estimation of timolol maleate in opthalmic solution by reaction with bromothymol blue. The absorbance of the yellow complex was measured at 400 nm. pH of the solution was maintained at 7.4. A linear relationship was obtained between

1-20 µg/ml.

d. Thin layer Chromatographic Methods :

1. The United States pharmacopoeia[4] prescribed a TLC method for detecting the timolol maleate using silicagel G. Developing solvent system was chloroform : methanol : 28% ammonia in water (80:20:1 v/v). Detection was made under visible light after exposure to iodine vapour for 2 hours.

2. Schulz et al[32] reported TLC method for estimation of Timolol maleate.

e. Gas chromatography methods :

1. Rao et al[33] have reported gas chromatographic method for the Timolol maleate opthalmic solution, chlorpheniramine maleate was used as internal standard.

2. Yamaji et al[34] and Earlam et al[35] have prescribed gas chromatographic method for the estimation of Timolol meleate.

f. Mass fragmentographic method :

1. Mass fragmentographic determination of Timolol Maleate in plasma and urine was reported by Fourillan J.B. et al[40].

g. High performance liquid chromatographic methods :

Reported in table 2

1.3.5 PHARMACOLOGICAL PROFILE

a. Pharmacokinetic :

Timolol maleate is completely absorbed from the gastro intestinal tract but is subjected to moderate first pass metabolism. Peak plasma concentration occur about 1-2 hours after a dose. It is extensively metabolised in the liver and metabolites being excreted in the urine together with some unchanged timolol. It crosses the placenta and appears in breast mill. Protein binding

Table 2

g ESTIMATION OF TIMOLOL MALEATE BY HPLC

Sample	Interal std	Column	Mobile phase	Detector	Detection limit	Ref
Plasma urine		R sil CN	Water : Acetonitrile : 0.1 M NaH_2PO_4 6 : 3 : 1 pH3	uv	4 - 27 ng/ml	36
Plasma urine		Hypersil -5-ODS	99.13% Aceto-nitrile containing triethyl amine pH 3	uv	2 ng/ml	37
Plasma breast milk	Propranalol	Partisil ODS	MeOH:0.2M NaH_2PO_4 88% H_3PO_4 H_2O 500 : 200 : 3 : 297	electrochem thinlayer cell	2 ng/ml	38
Bulk powder		ODS	Methanol:0.005M hexanesulfonic acid 1 : 2	uv		39

is reported to be low[1].

Some Timolol meleate is absorbed systemically following instillation of the eye drops, administred directly to the eye as an opthalmic drop appears to be rapidly absorbed producing a decrease in intraocular pressure within three hours[1].

b. Adverse effects :

A number of adverse reactions like respiratory reactions and cardiac reactions, including death due to bronchospasm in patients with asthma and rarely death in association with cardiac failure have been reported.

c. Use :

Timolol meleate is effective in lowering intraocular pressure and is widely used in patients with open angle glaucoma and aphakic glaucoma. It is also indicated both for the treatment of hypertension and to reduce cardiovascular mortality and the risk of reinfarction in patients who have survived the acute phase of myocardial infarction and who are clinically stable.

1.4 NADOLOL

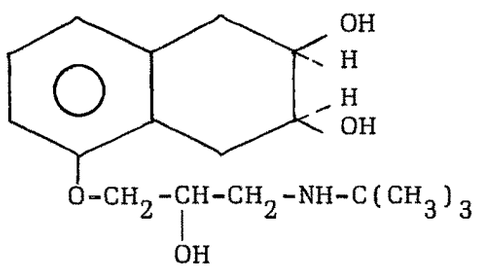
1.4.1 DESCRIPTION[1,2,4]

a. Chemical Name : (2r,3s)-5-(3-tert butylamino-2-hydroxy propoxy)-1,2,3,4 tetra hydronaphthalene-2,3-diol.

b. Molecular Formula : $C_{17}H_{27}NO_4$

c. Molecular weight : 309.4

d. Chemical strucutre :



e. Appearance, color, odour and taste :

A white crystalline odourless powder.

f. Official status and dosage form :

United States Pharmacopoeia[4] include tablets 40 mg/80 mg and in combination with bendrofluazide 5 mg.

1.4.2 PHYSICAL PROPERTIES

a. Solubility :

Soluble in water, freely soluble in ethanol, slightly soluble in chloroform, practically insoluble in 1,1,1 trichloro ethane.

b. Melting range : 124° C - 136° C.

c. Polymorphism :

No polymorphism has been reported for Nadolol. Dwadke[42] has observed that an amorphous form of Nadolol obtained by lyophilizing an aqeous solution of the compound.

d. Ultraviolet Spectrum :

Characteristic absorbance wavelengths in aqueous acid and methanol is 269 nm and 276nm. No alkaline shift was observed.

e. Infrared Spectrum :

Characteristic absorbance frequencies include 1092, 1070, 1248, 1262, 1582, 1217 wave numbers (KBr disc)

f. Mass Spectrum :

Principal peaks at m/z 30,86,57,294,71,310,70,87.

g. Nuclear Magnetic resonance :

NMR spectrum of Nadolol has been described[41].

1.4.3 STABLITY :

1. Solid State Stability[41]

Nadolol exhibits excellent stability as a solid[43]. No apparent degradation of the bulk samples held at high temperature for

prolonged periods.

2. Solution stablity :

Lyophilized sterile solution of Nadolol in 0.1M pH 7.4 sodium phosphate buffer showed no evidence of decomposition when held at ambient temperature for 51 days[43] In unbuffered solution samples prepared at various pH were stable after three months storage at 50° c.[44] A very slight discoloration was noted in some samples after 3 months at 50° C storage. Nadolol solution at 80° C for two months produce degradation and discoloration at most pH values. Exposure to intense light results in discoloration of solution at pH 2,2.92,9.8 after 2 weeks of storage.

1.4.4 METHODS OF ANALYSIS :

Literature review for Nadolol revealed several conventional and modern methods of analysis viz. Uv spectrophotometric, colorimetric, titrimetric, Thin layer chromatographic, gas chromatographic, high performance liquid chromatographic and fluorimetric methods of analysis.

a. Uv spectrophotometric methods :

1. C. papastephanou et al[45] have reported spectrophotometric analysis of Nadolol in the UV region at about 218nm, 270nm and 278 nm. The molar absorptivity of Nadolol was quite low but useful to study dissolution rates of Nadolol tablets.

b. Colorimetric Methods :

1. Ozden S. et al[46] and Pvalatin[47] have reported a sensitive colorimetric method for the estimation of Nadolol in tablets by reaction with bromophenol blue. The pH was maintained at 3.2 (with acetate buffer) and absorbance of the chloroform extract was measured at 412 nm. A linear calibration was obtained

between 60-600 µg/ml.

2. E.Ivashkiv[48] has reported a colorimetric method for the estimation of Nadolol in tablets. Nadolol was oxidized with periodic acid and the resulting aldehyde was reacted with 2,4-dinitro phenly hydrazine to form the corresponding hydrazone. Excess reagent was removed with cupric chloride soultion. The hydrazone was extracted into chloroform and its absorbance was measured at the 352nm and Beer's law obeyed between 0-200 µg/ml.

c. Fluorescence Spectrophotometric method:

Ivashkiv[49] has developed a fluorescence spectrophotometric method for the Nadolol in human serum and urine. Nadolol was oxidised with periodic acid to the corresponding dialdehyde and coupled with o-phenylene diamine produced a fluorescent compound. The excitation and emission wavelengths were 305 nm and 445 nm respectively. 0.01 µg of the sample was measured by this method.

d. Titrimetric Method :

1. E.Ivashkiv[50] has reported titrimetric method for the estimation of Nadolol in tablets. Nadolol was oxidized with chloramine T and the excess reagent was reacted with potassium idodide. The liberated iodine was titrated with sodium thiosulphate.

2. J.Alicino[51] has reported a non-aquenous titrimetric method for the estimation of Nadolol bulk and formulation. Perchloric acid was used as titrant and end point monitored potentiometrically or with an internal indicator quinaldine red or crystal violet.

3. United States Pharmacopoeia[4] has prescribed nonaqueous

titration method against perchloric acid for the assay of bulk drug.

e. Gas Chromatographic/mass Spectrometric methods :

1. Funke P.T. et al[52] have reported selected ion monitoring and gas chromatographic/mass spectrometric method for the determination of Nadolol from the serum.

2. Solmon et al[53] have developed a gas chromatographic method for the quantitative measurement of Nadolol in solution, chlorphenaramine maleate was used as an internal standard.

3. Cohen et al[54] have reported a gas chromatographic method with selected ion monitoring mass spectrometric method for the determination of Nadolol in human serum and urine.

4. Ribick et al[55] have reported high sensitive gas chromatographic/mass selective detection of Nadolol in plasma.

f. Thin layer chromatographic :

Targos F.P.[56] has reported a TLC separation was achieved on silicagel G plates using the solvent system acetone : chloroform : 2N ammonium hydroxide (80:10:10). The plates were observed under ultraviolet light maximum at 254 nm.

g. High performance liquid chromatographic methods :

1. B. Patel[57] has reported HPLC method for estimation of Nadolol.

Column : Reverse phase ethylsilance.

Mobile phase : 35% methanol : 65% aqueous 0.0005 M Hcl (0.05M NaCl solution).

Detection : Uv detector, fixed wavelength (254 nm) or variable wavelength (220 nm).

2. Jane et al[58] have reported HPLC method for the estimation of

Nadolol.

Column : 5 WSpherisorb (silica).

Mobile Phase : Methanolic NH Clo (10 mM pH 6.7)

Detection : Low wavelength UV and fluorescence.

Sample Preparation : Derivatization with o-phthaldialdehyde.

4. The United States Pharmacopoeia[4] has prescribed HPLC method for the estimation of Nadolol in tablets plain and also combined tablet with bendroflumethiazide.

1.4.5 PHARMACOLOGICAL PROFILE[1,41]

a. Pharmacokinetics : Nadolol is incompletely absorbed from gastrointestinal tract to give peak plasma concentration about 3 or 4 hours after a dose. It does not appear to be metabolised and is excreated unchanged in urine and the bile. It is only about 30% bound to plasma proteins and is reported to be dialysable. Nadolol is excreted in breast milk.

b. Adverse effects : The adverse effects observed after an administration of nadolol have been reported.

c. Uses : Nadolol is used in the treatment of hypertension and in the prophylaxis of migraine. It is also used in the management of tyrotoxicosis.

1.5 SOTALOL HYDROCHLORIDE

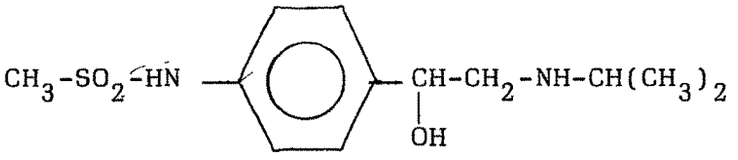
1.5.1 DESCRIPTION[1]

a. Chemical Name : 4'-(1-hydroxy-2 isopropylamino ethyl) methane sulphananilide.

b. Molecular Formula : $C_{12}H_{20}N_2O_3S$

c. Molecular weight : 272.4

d. Chenical structure :



e. Appearance, colour, odour and taste :

An offwhite to pale cream crystalline powder.

f. Official status and dosage form :

Not official in any of the phaomacopoeia. Available as tablets 40 mg., 80 mg. and 200 mg., injection 10 mg/ml in ampoules of 4 ml. and tablets in combination with hydrochlorthiazide.

1.5.2 PHYSICIAL PROPERTIES

a. Soluability : Freely soluable in water, sparingly soluable in chloroform, insoluable in solvent ether.

b. Melting range : 206° C to 207° C

c. Dissociation constant : pKa values 8.3, 9.8

d. Ultraviolet spectrum : Chalacteristic absorption wavelength in aqueus acid is 269 nm and in aqueous alkali is 249 nm.

e. Infrared Spectrum : Characteristic absolption freequencies include 992, 1160, 785, 1512, 1230, 1585 wave number (kbr disk)

f. Mass spectrum : Principal peaks at m/z 72, 30, 43, 122, 73, 41, 106, 121.

1.5.3 METHODS OF ANALYSIS:

The conmprehensive literature survey revealed a tritrimetric, TLC, few HPLC and GC methods for the estimation of sotalol hydeochloride.

a. Titrimetric Methods: The private circulation of manufacturer of sotalol products [59] reported a non-aqueous titrimetric method for the estimation of sotalol Hydorchloride. 0.1N perchloric acid was used as titrant and solvent blue 19 solution as indicator.

b. Thin layer chromatographic methods:

I. The private circulation of manufacturee of sotalol products [59] reported a TLC methos for the estimation of Sotalol Hydrochloride. Acetone: 25% Ammonia solution in the proportion of 9:1 was used as mobile phase.

II. Moll et al [60] have reported a thin layer chromatographic method for the determination of Sotalol Hydrochloride from human urine samples, Phme: iso ProH: 25% NH OH (3:6:1) was used as a mobile phase. [4]

c. NMR Spectroscopic methods:

Lorio et al [61] reported a NMR Spectroscopic method for the estimation of Sotalol Hydrochlroide in bulk and tablets. The method was based on peaks of the Me groups attached.

d. High pressure liquid chromatographic methods:

I. Gluth et al [62] reported HPLC method for the analysis of Sotalol Hydrochloride in plasma and urine.

Column: Hypersil ODS

Mobile phase: MecN : water : a.a. PH(2.5) (20:79:1)

Detection: Fluorometric.

Ion Pairing reagent: Heptane sulfonic acid

II. Karkkainen et al[63] reported HPLC method for the estimation of Sotalol Hydrochloride in serum and urine.

Internal standard: Atenolol standard solution.

Mobile phase: 0.01 M phosphate buffer pH 3.2: MecN. 20:80 (containing 3 mM, n octyl sodium sulfate).

Detection: UV at 226 nm.

III. Jane et al[64] also reported HPLC method for the estimation of Sotalol Hydrochloride on un-modified silica column together with non aqueous ionic eluents.

IV Bartek et al[65] have determined HPLC method for estimation of Sotalol Hydrochloride.

Column: Bonda Pack C 18

Mobile phase: Eto Ac : MecN (1:2)

Detection: Fluorescence excitation and emission wavelength 240 nm and 310 nm respectively.

V Poirier et al[66] have described HPLC mehtod for the estimation of Sotalol Hydrochloride from blood serum.

Column: Octadecyl silane.

Mobile phase: H_2O : Methanol:a.a.: Methane sulfonic acid (91:8.5:0.5:0.25) pH 3.3

Detection: UV at 235 nm.

VI Hoyer et al[67] also described HPLC determination of Sotalol Hydrochloride in blood serum using octadecyl silane as a column and with UV detection at 235 nm.

VII Poey et al[68] have reported HPLC method for the Sotalol

Hydrochloride using Rsil CN/RP8 column with H_2O:MeoH:Et NH (30:70:0.05) / H_2O: MecN:pH3.0 Phosphate buffer (40:50:10) as mobile phase with UV detection.

VIII Gluth et al[69] have reported another HPLC method for the estimation of Sotalol Hydrochloride in human plasma and urine.

Internal standard: Atenolol

Column: Hypersil ODS

Mobile phase : H_2O : HoAC (20:79:1)

IX Morris et al[70] have reported liquid chromatographic fluoresence mehtod for the estimation of Sotalol Hydrochloride from plasma.

X Dyde et al[71] have reported HPLC method for the determination of Sotalol Hydrochloride from human plasma using C18 column using MecN : 0.05M H_3PO_4 (12:88) and excitation and emission at 235 and 315 nm respectively.

1.5.4 PHARMACOLOGICAL PROFILE:

a Pharmacokinetics:

Sotalol Hydrochloride completely absorbed form the gastrointestinal tract and peak plasma concentrations are obtained about 2 or 3 hours after the administration of a dose. It is not metabolised and is excreted unchanged in the urine. It is not bound to plasma proteins. It crosses the placenta and found in breast milk. However only small amounts are reported to cross the blood brain barrier and enter the crebrospinal fluid.

b Adverse Effect:

The adverse effect observed after the administration of Sotalol AHydrochloride have been reported[1,42].

c Use:

Sotalol Hydrochloride is used in the treatment of Hypertension, Angina Pectoris and for the emergency treatment of cardiac arrhythmias.

1.6 REAGENTS USED FOR THE PROPOSED METHODS:

1.6.1 P-Dimethyl aminobenzaldehyde and vanillin [72-75]

Certain amines condense with various aldehydes in strongly acid media to give products that are oxidizable to give a colour. Among the many aldehydes that have been shown to react are P-dimethyl amino benzaldehyde, vanillin, formaldehyde, benzaldehyde, salicyladehyde, piperonal, paraldehyde, p-acetyl amino benzaldehyde, m & p - nitrobenzaldehyde, m-amino benzaldehyde and metaldehyde. Of the foregoing aldehydes best results have been obtained with P N-dimethylamino benzaldehyde. The most common oxident used is atmospheric oxygen but the process has been hastened by the addition of hydrogen peroxide, nitrites, nitrates, ferric ion and several other metal ion catalysts.

The reaction of P-dimethyl aminobenzaldehyde with indoles and pyrroles has been used qualitatively and quantitatively for many years [76,80,81,88]. The reaction with aromatic amines to give schiff bases is equally well documented.

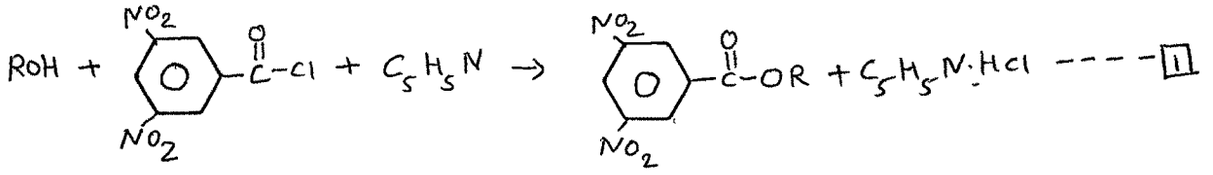
In hydrochloric acid indole reacts with aldehyde to yield red products. The reaction involves the condensation of the aldehyde with two molecules of Indole. The intial condensation products being oxidised by oxygen from the air.

1.6.2 3,5 Dinitro benzoyl chloride:

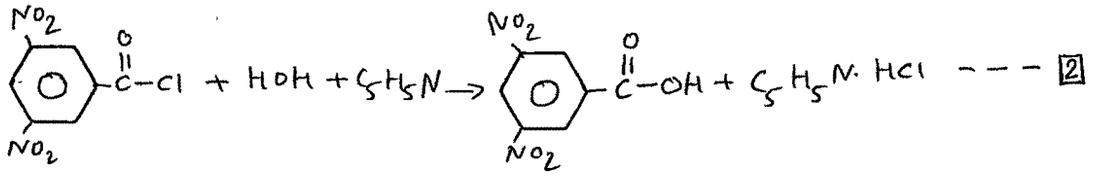
3,5 dinitro benzoyl chloride has been used for many years to prepare derivatives of alchols for identification purposes. 3,5

dinitrobenzoyl chloride with quantitatively determine some stubbon hydroxyl groups more readily [76].

The reaction of 3,5 dinitro benzoyl chloride with an alcohol in pyridine solution is shown in equation 1.



The excess dinitrobenzoyl chloride is hydrolysed by water as indicated by equation 2.



Since alcholos show no significant absorption in UV and visibile region, all colorimetric methods for these compounds require their conversion to an absorbing derivative. Primary and secondary alcohols undergo acylation by 3,5 dinitrobenzoyl chloride in pyidine solutionto form the corresponding 3,5 dinitro benzoate esters. The ester appear to form colored quinoidal ions in basic medium and the intensity of the colour can be measured to provide an analysis of the original amount of alcohol. The ability of the 3,5 dinitro benzoylchloride to form coloured quinoidal ions provide a useful analytical tool for determining low concentration of substances

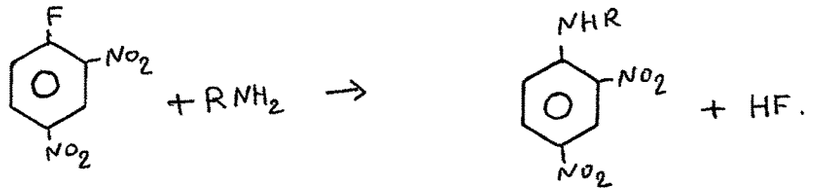
1.6.3 Sodium periodate and potassium dichromate:

Sodium metaperiodate is a specific oxidant for compounds with vicinal diols [78,79]. The reaction is quite specific and clear cut [72]. Secondary alcohols can be oxidized to ketones with potassium dichromate [90]. The excess of dichromate is eliminated

with hypophosphrous acid and ketone is determined easily[83].

1.6.4 1-Fluoro 2,4 dinitro benzene 73,88 (FDNB)

Primary and seconday amines have been analysed colorimetrically by reaction with FDNB[81,84,85].



Seconday amines may give analogous reactions.

1.6.5 Complexation with dyes[72].

Complex formation or the formation of neutral ion pairs is a method frequently used for the analysis of amines. In this approach the amine in its protonated state forms a complex with an anionic species to form a neutral ion pair which can be extracted in to an organic solvent by using a pairing agent that produces a colored ion pair. The amine in the organic phase can be measured colorimetically. It is also possible however ot use a non absorbing anion to form the ion pair. In this case the native absorptivity of the amine is measured in the organic phase usually chloroform or dichloromethane.

1.6.6 1,2 naphthoquinone-4 sulfonate:

Sodium 1,2 naphthoquinone-4 sulfonate, the orthoquinone give coloured compounds with primary amino compounds[86,87,88] and compounds which contains two removable hydrogen atoms attached to one carbon atom or nitrogen atom. Deeply coloured para quinoid condensation products results in case of primary aromatic amine. An active CH_2 group can also be formed through isomerisation. Pertinent examples are resorcinal, Phloroglucinol, indole, pyrrole and nitro methane.

1.7 DEVELOPMENT OF ANALYTICAL METHODS:

The modern mehods of choice HPLC, GLC, NMR and mass spectrometric for purity assays depend on sophisticated equipments which are very costly and cause problems of maintenance. Hence they ar not within the range of most laboratories and small scale industries. The visible spectrophotometric and fluorimetric techniques are very simple and do not involve high cost. They are easy to carry out and the instruments need very little maintenance. No specially trained persons are necessary. The limitations of many of the visible spectrophotometric or fluorimetric methods of analysis, lie in the chemical reactions upon which these procedures are based rather than the instrumental sophistication. Many chemical reactions resulting in the formation of coloured or fluoroscent species involving any particular drug molecule are quite selective or can be rendered selective through the introduction of masking agents, control of pH, use of solvent extraction techniques, adjustment of oxidation states or by prior removal of interfering substances by resorting to chromatographic techniques[98-102].

1.7.1 Classification of Functional Group in Drugs:

A distinguisthing feature of organic drug molecules is the presence of functional groups in them. Based on the reactions of these functional groups, one can easily analyse any organic drug however, complicated the rest of the structure may be. These functional groups can be classified into three categories acidic, basic and neutral.

1.7.2 Selection of reagents for organic analysis[103]

several papers are being published evry year on the

reactions and possible applications of new and old organic reagents for organic analysis. The selection of an appropriate reagent for a particular qualitatical situation is still a challenging problem. The choice of a particualr reagent depends on careful consideration such factors as the scale and economics of the reactions the presence of other functional groups that might be adversely affected by reagent, the deactivation of the reaction centre by steric and electronic effects, the instability or high reactivity of the desired product, the rate of reaction, position of equilibrium (in the face of a reversible reaction) and other related factors. The selection of a reagent for the determination of a particular compound is made after a literature search for methods that have been used in parallel situations elsewhere of that show reasonable promise for the compound under consideration. If adequate information is not available in this way, then the reagents that act most rapidly and stoichiometrically can be chosen after investigation of the performance of several possibly selected ones on a pure samples of the compound. Reagent selectively for a particular functional group is normally the minimum requirement. Specifictiy of the reagent for a simple compound containing that functional group is often desirable, not only to isolate it from other compounds containing the same functional group but also to eliminate the effets of interfering compounds.

1.7.3 Systematic study in Visible Spectrophotometry (104)

Among the several physico-chemical methods available, the author employed the simple visible spectrophotometric techniqes in her investigations recorded in this thesis. In visible

spectrophotometric (which coveres wavelength region 370-800 nm). electronic transitions in energy levels is possible by the absorption of radiations due to π-π^* and n-π^* transition, where π^* is antibonding atomic orbital while n stands for non-bonding orbital having energy in between bonding and antibonding orbitals. An absorption spectrum is a graphical representation of light absorbed by a substance at definite wavelength. To plot a characteristic absoption curve, the values of the wavelength (λ) are laid of along the axis of abscissas and the value of the absurbance (A) along the axis of ordinates. A characterisic of an absorption spectrum is the position of the peaks of light absorption by the substance and also the intesisty of absorption. The colour internsisty i.e. absorbance of the coloured analyte solution is measured by spectrophotometer in the visible region of the spectrum.

1.7.4 DEVELOPMENT OF A METHOD

In developing a quantitative method for determining an unknown concenteration of a given species by absorption spectrophotometry, the first step is the choice of the absorption band at which absorbance measurements are made. An ultraviolet or visible absorption spectrum of the specise to be detrmined is obtained either from the literature or experimentally by means of scanning spectrophotometer. When several absorption bands of suitable absorptivity are present, the band selected should fevour wavelength regions that correspond to relatively high output of the light source and high spectral sensitivty of the detector(usually λmax). The absorption band should not overlap with band of the solvent or contaminants, including excess reagents that might be present in the sample. It is necessary that along the wavelength axis, the absroption spectrum of an analyte or reagent ion added should be well sperated in at least one place from the absorption spectrum of the reagent it self, although it generally matters little it it is generally separeted from that of the ion or not because the instensity of the absorption band due to the latter is low.

Both the colour developing reagent and the absorbing product must be stable for a reasonable period of time (stability period) and it is always advisable to prepare standards and unknow on a definite time schedule.

1.7.5 ESTABLISHMENT OF OPTIMUM CONDITION OF THE METHOD[104]

The basis of most spectrophotometric methods is usually (a) complex formation (b) an oxidation reduction process or (c) a catalytic effect. In each type of reaction the yield of coloured

species whose absorbance is measured and thus the sensitivity of the method, rate of colour formation and stability is effected by the concerations of the reagent in the solution, the nature of the solvent, the temperature, the pH of the medium, order of addition of reagents and waiting period. It is necessary to establish the optimum condition in the procedure to be developed through control experiments by varying one among them above parameters and keeping other constant at a time and measuring the absorbance at λ max. The ranges of different parameters within which attainment of high λ max coupled with maximum intensity and stability of the coloured species is achieved are known as the optimum condition.

1.7.6 SELECTIVITY OF THE METHOD

The selectivity (or specificity) of the method is ascertained by studying the effect of a wide range of excipients and other additives usually present is the pharmaceutical formulation on the determination under optimum conditions.

In the initial interference studies a fixed concentration of the drug is determined several times by the optimum procedure in the presenence of a suitable (1-100 fold) molar excess of the foreign compound under investigation and its effect on the absorbance of solutions is noticed. The foreign compould is considered to be non interfering if at these concentration it constantly produces an error less then 3% in the absorbance observed with the pure drug.

1.7.7 LINEARITY AND SENSITIVITY OF THE METHOD

A knowledge of the sensitivity of the colour is important and the following terms are commonly employed for expressing the sensitivity.

Lambert's law states that the absorbance is propertional to the thickness of the solution. Beer's law states that the abserbance is proportional to the concentration. The combination of the two laws is Beer - Lambert's law. It can be expressed mathematically as

$$A = \frac{\log \text{Intensity of incident radiation}}{\text{Intensity of transmitted light}} = a.b.c.$$

The basorbance (A) is proportional to the concentration (c) of absorbing species if absorptivity (a) and thickness of the medium (b) are constant when c is in moles per liter, the constant is called the moler absorptivity and has the symbol € Beer's law limits and € max values are expressed as µg/ml and $1.mole^{-1}.cm^{-1}$ respecitvely.

Sandell's sensitivity[105] refers to the number of ug of the drug determined converted to the coloured product, which in a column solution of cross section $1cm^2$ shows an absorbance of 0.001 (expressed as µg cm^{-2}).

1.7.8 VALIDITY OF BEER'S LAW

The laws is not applicable for highly concentrated solution. solution exhibiting flurescence or suspensions may not strictly adher to Beer's law. If a dilute solutions during measurement undergoes chemical reaction such as oxidation, reduction, hydrolysis,association, dissociation or polymerization then the law is not valid.

$y = mx + b$ m = slope b = intercept

$$\text{slope} = m = \frac{\Sigma xy - \frac{(\Sigma x.\Sigma y)}{n}}{c}$$

$$b = \frac{\Sigma y - m\Sigma x}{n}$$

Correlation coefficient : It is used to measure the ability of the regression line to explain veriation in the independent variable and is calculated from the following formula.

$$r = \frac{\Sigma(x - \bar{x}) \cdot (y - \bar{y})}{\sqrt{\Sigma(x-\bar{x})^2 \cdot \Sigma(y - \bar{y})^2}}$$

where $\bar{x}$, $\bar{y}$ are arithmatic mean of x and y respectively.

Recovery : A known amount of the constitunt being determine is added to the sample, which is analyses for the total amount of constituents present. The difference between the analytical result for sample with and without the added constituents gives the recovry of the amount of added constituent. If the recovery is satisfactory confidance in the accuracy of the procedure is enhanced.

standard deviation :

$$s = \sqrt{\frac{(x1 - \bar{x})^2 + (x2 - \bar{x})^2 + \quad (xn - \bar{x})^2}{n-1}}$$

$$= \sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}$$

1.8 SYSTEMATIC STUDY

Drugs are rerely administrered in their pure form and more often then not, they have to be necessarly admixed with various kinds of adjuvants resulting in their transformation to the so called "dosage form". Hence each and every dosage from irrespective of its final form and nature is a combination of drug component and an assortment of different kinds of non-drug components collectively known as additives and each destined to

93-96

serve a specific purpose .

In developing these methods a systamatic study of the effects of verious parameters were underlaken by varying one parameter at a time and controlling all the other parameters which is the standard practice in this type of work.

1.8.1 SPECTRAL CHARACTERIESTICS

In order to ascertain the wavelength of maximum absorption (λ max) of the coloured species formed in each of the method specified amounts of drug was taken and the colour were developed seperately following the procedure of methods. The absorption spectrum of each coloured species was scanned on a spectrophotmeter in the wavelength region of 400 - 800 nm against the corresponding reagent blank.

The results are graphically presented by plotting a graph of absorbance vs. wavelength.

1.8.2 OPTICAL CHARACTERISTICS

In order to test whether the coloured species formed in the method adhere to Beer's law, the absorbances at appropriate wavelength of the set of solutions contianing graded amounts of drug and specified amounts of reagents were measrued against the corresponding reagent blanks in the method. Concentration-absorbance corelations are recorded grephically to determine the Beer's law adherence. Beer's law limit, molar absorptivity, sandell's sensitivity for drug with each of the mentioned reagents were calculated and recorded in tables.

Regression analysis using the method of liner least squares of the absorbance value obtained with drug and each of the reagent system gave slope (m) intercept (b) and correlation coefficient (r) for

each system. These are recorded in tables.

1.8.3 ANALYSIS OF FORMULATION

To find out the sutability of the proposed method for the assay of formulations of different pharmaceutical products containing drugs were analysed by the proposed methods and the U.S.P/BP/reported method. The results obtained by each of the proposed methods were compaired statistically with those obtained by the U.S.P/BP/reported methods.

1.8.4 RECOVERY STUDIES

Recovery studies were conducted by analysing each pharmaceutical formulation for the active ingredient in the first instance by the proposed method 10 mg of the pure drug was then added to each one of the drug previously analysed formulations and the total amount of the drug was then once agian determined.

1.8.5 INTERFERENCE STUDIES

The excipients and additives usually found in the commercial formulations of drug were found not to interfere with colour development by the procedures of methods. Analysis of model formulations of these drug prepared in the author's labouratory revealed that the normally used additives did not interfere as indicated with carbohydrate components such as starch, glucose, lactose, glyceryl monostearate, polyethylene glycols and propylene glycols.

1.8.6 PRECISION

To test the precision of each proposed method absorbance values of a set of six replicates of a fixed amount of drugs were recorded in the usual manner follwing the recommmended procedures. The percent relative standard deviation and the

percent range of error calculated from this data are recorded in the tables.

1.8.7 ACCURACY

To confirm the accuracy of the methods different quantities of bulk samples of the drug within Beer's law limit were taken and estimated by each of the proposed methods. The percent error calculated with this data for each method is recorded in tables.

1.9 RESEACH ENVISAGED

Experimental work has been confined to stability and analysis of four important β-blockers namely Pindolol, Nadolol, Timolol maleated and Sotalol hydrochloride.

A : DEVELOPMENTAL ANALYSIS

1.9.1 COMPLEXATION WITH DIFFERENT METALS

The published literature did not reveal any analytical method involving metal complexation for the estimation of B-blockers in question. The efforts were then made to develop some colorimetric method of estimation of these drugs using metal complexation.

Reaction of Pindolol, Nadolol, Timolol maleate and Sotalol hydrochloride at pH ranging from 3.0 to 10.0 has been studied with 12 different metal ions viz. nickel, manganese, feeric, lead, cupric, meceuric, ceric, zinc, stanous, ferrous, uranyl zinc acetate and cobalt in equimolar proportions. The reaction of Pindolol with ferric and ceric metal solution held good promice. With feric metal solution it gave blue green colour on heating having λmax at 620 nm. The colour was found to be stable for an hour at pH 1.0. With ceric it gave blue colour which was found to be unstable.

Timolol maleate also reacted with ceric meatl solution and gave

highly unstable blue colour Nadolol and Sotalol hydrochloride do not react with any of the metals.

1.9.2 COUPLING WITH DIAZOTIZED PRIMARY AROMATIC AMINES

The published literature did not reveal any analytical method involving coupling of these B-blocker drugs with diazotized primary aromatic amines. This prompted the experiments in search of possible colorimetric method of estimation. 12 different primary aromatic amines and amine acids such as o-phenylene diamine, p-aminophenol, 8-amino-1-naphtol-3-6 disulfonic acid, 4-nitro-2-amino-phenol-6 sulfonic acid , aniline, p-cloro aniline, p-amino benzoic acid, o-phenylene diamine, p-toluidine, p-anisidine, sulfanilic acid and sulfanilamide, were tried for this reaction. None of the diazotised amines reacted with Pindolol, Timolol maleate, Nadolol and Sotalol hydrochloride as indicated by no difference in appearance of sample and reagent blank solution.

1.9.3 REACTION WITH P-(DIMETHYL AMINO) BENZALDEHYDE

The published literature did not reveal any analytical method for the estimation of these drugs involiving p-dimethyl amino benzaldehyde or other aldehydes were as Pindolol has been reported to react with p-dimethyl amino benzaldehyde, used as an identification test[2]. Cousequently reaction of Pindolol, Timolol maleate, Nadolol and Sotolol hydrochloride has been attempted with p-dimethyl amino benzaldehyde with and without ferric ions. Pindolol reacted with p-dimethyl amino benzaldehyde in glacial acetic acid : hydrochloric acid mixture (85 : 15 v/v) with a maximum absabance at 570 nm[p1]. None of the other drugs reacted with p-dimethyl amino benzaldehyde.

1.9.4 REACTION WITH VANILLIN

Pindolol also reacted with Vanillin (1% w/v) in concentrated hydrochloric acid with a maximum absorbance at 520 nm. A simple selective and sensitive method has been developed based on this reaction. Maximum colour intensity was obtained with two ml of Vanillin reagent (1% w/v) the calibration curve was found to be rectilinear between 0.2-16.0 µg/ml concentration [p2].

1.9.5 REACTION WITH SODIUM PERIODATE

Reaction characteristic of these drugs has been studied with sodium periodate. Pindolol reacted with 0.1N sodium periodate solution and the reaction was studied in detail for the effect of pH and termperature.

1.9.6 REACTION WITH AMMONIUM METAVANADATE

All the drug samples were attempted for reaction with ammonium metavanadate with sulphuric acid. Only Pindolol reacted with ammonium metavanadate and gave blue colour with a λmax at 635 nm. The method was studied in detail.

1.9.7 REACTION WITH 3,5 DINITROBENZOYL CHLORIDE

Since all the four drugs possess secondary -oH group in the side chain and since secondary -oH group reacts with 3,5 dinitrobenzoyl chloride attempts were made with all the four drugs for the reaction with 3,5 dinitrobenzoyl chloride. Drug samples in pyridine reacted with 3,5 dinitrobenzoyl chloride (10% w/v in pyridine) in presence of 2N Hcl. The absorbance spectrum and various experimental conditions were optimised and methods developed for the routine analysis of all the drugs.

1.9.8 REACTION WITH 1-FLUORO - 2,4 DINTROBENZENE (DNFB)

All the four drugs were attempted for reaction with 1-fluoro-

2,4 dinitrobenzene. Pindolol seemed to react with 1-fluoro -2,4 dintrobenzene in ethanol in presence of sodium bicarbonate/Carbonate buffer on heating. The method modified for Timolol maleate, Nadolol and Sotalol hydrochloride. 1-Fluoro 2,4 dintitrobenzene in acetone (1% v/v) and 2.5% w/v borex in water were mixed in the proportion 1:9 immediately before experiment and drug sample after heating with this reagent were treated with 5% v/v Hcl in 1,4 dioxame. All the methods were studied in detail and optimised.

1.9.9 REACTION WITH FOLIN-CIOCALTEU REAGENT

All the drug samples were attempted to react with F-Creagent. Sotalol hydrochloride reacted with Folin - Ciocalteu reagent in presence of sodium carbonate solution with a maximum absorbance at 725 nm [p3]. Reaction of Pindolol with F-C reagent is allready reported [8] in the liturature.

1.9.10 REACTION WITH ACETYL ACETONE REAGENT

All the samples of four drug were treated with sodium periodate, heated and allowed to react with acetyl acetone reagent. A sensitive method for the estimation of these drugs were developed.

1.9.11 COMPLEXATION WITH DIFFERENT ACIDIC AND BASIC DYES

Published literature reveled one analytical method each for the determination of Timolol maleate in opthalmic solution and nadolol in pharmaceutical preparation by forming a ion pair complex with bromothymol blue [31] and bromophenol blue [45] respectively. This characteristic property of these drugs were investigated in detail in a bid to devise better analytical methods of estimation.

Reaction of Pindolol, Timolol maleate, Nadolol and Sotalol

hydrochloride has been extensively studied with 13 different acidic dyes and 6 different basic dyes at pH 1.0 to 10.0 both in cold and after heating.

The basic dyes used included crystal violet, malachite green, methylene blue, oracet blue B and safranine. The acid dyes tried included bromocresol green, bromothymol blue, clatan yellow, eriochrome black T, erythrosine, fluorescein sodium, methyl orange, methyl red, phenol red, sunset yellow, tartrazine, tropeoline OO and thymol blue. The reaction of Pindolol with bromocresol green, bromothymol blue, eriochrome black T and crystal violet, reaction of Timolol maleate with bromocresol green, methyl orange, eriochrome black T and crystal violet, reaction of Nadolol with bromothymol blue and crystal violet and reaction of Sotalol hydrochloride with bromocresol green eriochrome black T and crystal violet were positive and were therefore investigated extensively.

1.9.12 SPECTROPHOTOFLUORIMETRIC METHOD

The published literature reveled one analytical method each for the estimation of Pindolol [11] and nadolol [48]. Samples of timolol maleate and nadolol showed negligible fluorescence in Uv region as such in ethanol. Hence experiments were designed based on fluorimetric method for the estimation of these drugs.

The effect of pH, solvent, oxidizing agents and metal complexation on natural fluorescence was studied in depth. In view of their ultraviolet absorption characteristics attempts were made to use these drug as quenchers for the fluorescence of two well known fluorophores, quinine sulphate and fluorescein. The qualitative and quantitative aspects of these effect on the

fluorescence of the fluorophores were fully investigated.

1.9.13 REACTIONS WITH OTHER REAGENTS

The reactions of these drugs with 1,2 Naptho quinone 4 sulfonic acid, N-1-naphthyle ethlene diamine, potassim dichromate, metol and with $FeCl_3$ in presence of ammonium thiocyanate were investigated and studied in detail. Reaction of pindolol with concentrated Hcl and acetic acid mixture was also studied. Some of the above colorimetric methods mentioned above were applied for the estimation of these drugs in biological fluid like blood and urine.

STABILITY AND KINETIC STUDIES

1.9.14 STABILITY OF PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE TABLET AND OPTHALMIC SOLUTION OF TIMOLOL MALEATE AT ELEVATED TEMPERATURE AND /OR HUMIDITY.

The published literature reveled satisfactory physico - chemical stability data incase of solution and solid state stability of timolol maleate [25]. However corresponding data was not available in case of pindolol,nadolol and sotatol hydrochloride and their formulations.

Stability of tablets of all the four drugs were studied by storing them at ambient temperature, ambient temperature RH 75%,45° C, 60° C and 60° C - RH /5% up to 160 - 180 days and follwoing any degradation visually. UV spectropholometricaly and by reaction with acetyl acetone reagent. For pindolol, the proposed method of estimation, with vanillin and p-dimethyl amino benzaldehyle were also used for the study.

Stability of timolol maleate opthalmic solution was studied by storing them at ambient temperature, 45° C and 60° C upto 120-180 and

following any degradation visually, Uv spectrophotmetrically and colourimetrically using acetyl acetone reagent.

1.9.15 STABILITY OF PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE AT ELEVATED TEMPERATURE AT DIFFERENT pH

The published literature revealed no data regarding the stability of pindolol and sotalol hydrochloride as a function of pH. Although stability of timolol maleate [25] and nadolol [42] has been reported as a function of pH and temperature in aqueous solution. Such data has not been published in case of injectable solvent actually used for injection formulations.

Hence stability of pindolol, timolol maleate, nadolol and sotalol hydrochloride was studied at different temperature as a function of pH by storing 0.50% m/v solution in a mixture of Mc Ilvaine citrate, phospate buffer [59] ranging from pH 2.2 to 8.0 at ambient temperature refrigerator temperature and 50°C u to 120-150 days and following and degradation by UV spectrophotometrically and colorimetrically using acetyl acetone reagent.

1.9.16 STABILITY OF PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE IN ULTRAVIOLET RADIATION

The published literature did not reveal any data regarding the stability of these drugs under UV light consequently stability of these drugs were studied at pH 5, pH 4, pH 7.4 and pH 5 respectively under ultraviolet radiation. Studies were also carried out in alcoholic medium and in presence of methyl paraben and sodium sulphite in aqueous buffer system.

1.9.17 STABILITY OF PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE WITH DIFFERENT EXCIPIENTS AT ELEVATED TEMPERATURE AND/OR HUMIDITY.

The published literature did not reveal any data regarding the compatibility of these B blocker drugs with different excipients used in formulations. Consequently stability of pindolol, timolol maleate, nadolol and sotalol hydrochloride was studied as such and in combination with different excipients used in tablet formulation by storing the mixture of these drugs with lactose, dicalcium phosphate, microcrystalline cellulose, starch, polyvinyl pyrrolidone, colloidal silicon dioxide, sodium lauryl sulphate in (1 + 5) proportion and magnesium stearate and talc in (20 + 1) proportion at ambient temperature, ambient temperature RH 75%, 45° C, 60° C and 60° C - RH 75% up to 150 days and following any degradation visually and by Uv spectrophotometrically. Samples stored with lactose, polyvinyl pyrrolidone and sodium lauryl sulphate at 60° C , 60° C RH 75% were subjected to thin layer chromatography reveal the likely degradation products qualitatively.

1.9.18 STABILITY OF PINDOLOL, TIMOLOL MEALEATE,NADOLOL AND SOTALOL HYDROCHLORIDE WITH HYDROCHLORTHIAZIDE AT ELEVATED TEMPERATURE

The published literature did not reveal any data regarding the stability of these drugs with hydrochlorthiaze diuretic commanly used in combination in tablet formulations. The experiments were then designed to study the stability of these drugs with hydrochlorthiazide diuretic at ambient temperature and at 60° C.

1.9.19 HPLC METHOD FOR THE STABILITY OF TIMOLOL MALEATE

The published literature revealed few HPLC methods for the estimation of timolol maleate in dosage forms and bodyfluids. None of these methods reported were utilized for stability studies. Keeping in view the high efficiency of the HPLC methods of estimation a method was designed using methanol : acetonitrile (50:50) solvent system and a C18 column for the stability of timolol maleate.