CHAPTER IV

SUMMARY

Attempts were made to develop several new analytical methods for estimation of bulk powder and common dosage forms of four selected β blocker (antihypertensives) drugs namely pindlol, timolol maleate, nadolol and sotalol hydrochloride.

Several preformulation experiments including stability as a function of pH and temperature and compatibility with several excipients at elevated temperature and / or humidity were designed for these drugs. Stability experiments were confined to the study of tablet formulation of all the four drugs and opthalmic formulation of timolol maleate at elevated temperature and / or humidity.

4.1 DEVELOPMENTAL ANALYSIS

4.1.1 COMPLEXATION WITH DIFFERENT METALS.

No chromogenic reaction was found to take place between nadolol and sotalol hydrochloride and all the metal solutions tried viz. nickel , manganese, ferric, lead, copper, mercuric, ceric , zinc, stannous, ferrous, cobalt and uranyl at extreme acid pH (1.0) and at pH 2.0 to 8.0 both in cold and with heating. when reacted in equimolar proportions. Timolol mealeate reacted with ceric metal solution and gave highly unstable blue colour.

Pindolol gave positive reaction with ferric and ceric metal solutions. ceric metal solution gave blue colour with it but it was found to be unstable. A blue green coloured complex was formed when pindolol was treated with ferric chloride solutions in presence of 0.1 N hydrochloric acid solution. The λ max of this solution was found to be at 620 nm, after the solution was heated for 10 minutes on boiling water bath. The calibration curve was rectilinear between 25- 120 µg/ml. The proposed spectrophotometric method is suitable for estimation of pindolol bulk powder and its dosge forms.

4.1.2 COUPLING WITH DIAZOTIZED PRIMARY AROMATIC AMINES.

No chromogenic reaction was found to occur between pindolol, timolol maleate, nadolol and sotalol hydrochloride and all the 12 primary aromatic amines /amine acids tried viz. Aniline, p-nitroaniline, p-chloro aniline, p-amino phenol. p-amino benzoic acid, o-phenylene diamine, p-toluidine, p-anisidine, sulfanilic acid, sulfanilamide 4-nitro-2-amino -phenol -6-sulfonic acid and 8-amino 1- napthol -3-6 disulfonic acid.

4.1.3 REACTION WITH p - DIMETHYL AMINO BENZALDEHYDE.

Out of four β blockers tried only pindolol reacted with p - dimethyl amino benzaldehyde and gave reddish purple coloured complex with λ max at 570 nm. p-dimethyl amino benzaldehyde reagent solution was prepared in 85: 15 v/v glacial acetic acid : hydrochloric acid and 1.5 minutes heating time in boiling waterbath was required for the mqximum colour intensity. The calibration curve was found to be rectilinear in the range of 0.5- 5.0 µg/ml. The method was found to be sensitive as well as precise.

4.1.4 REACTION WITH VANILLIN.

Out of four β blockers tried only pindolol reacted with vanillin in concentrated Hcl and gave reddish coloured complex with λ max at 520 nm. The calibration curve was observed rectilinear in the range of 0.2- 16.0 µg/ml. The usual tablet excipients did not interfere with the results. The method was found to be simple, rapid and sensitive.

4.1.5 Reaction with sodium periodate.

Of the four B blockers treated with sodium periodate only pindolol reacted with sodium periodate solution in presence of 0.1N hydrochloric acid solution with λ max at 540 nm. Calibration curve was rectilinear between 70-120 µg/ml. The method was not sensitive as compared to the method developed with p-dimethyl amino benzaldehyde or vanillin, though the results of analysis of market formulations were comparable with B.P. method.

4.1.6 REACTION WITH AMMONIUM METAVANADATE.

Of the four β blockers treated with ammonium metavanadate only pindolol reacted with ammonium metavanadate in 0.25N sulphuric acid with formation of blue colour. The maximum absorbance was observed at 635 nm. The change in pH of the medium did not improve the method. The calibration curve was rectilinear in the range of 4-64 µg/ml. The method was found to be sensitive as well as precise.

4.1.7 REACTION WITH 3,5 DINITROBENZOYL CHLORIDE.

Pindolol,Timolol Maleate,Nadolol.and Sotalol hydrochloride all the four β blockers reacted with 3.5 dinitrobenzoyl chloride in pyridine. DMF was found to be a suitable solvent rather then acetone. The absorbance maxima was observed at 520 nm. The calibration curve was found rectilinear in the range of 10-60 µg/ml, 4-24 µg/ml, 20-80 µg/ml and 2-30 µg/ml respectively for Pindolol. Timolol Maleate,Nadolol and sotalol hydrochloride. The method was found to be sensitive and rapid.

4.1.8 REACTION WITH 1-FLUORO 2,4 DINITRO BENZENE (DNFB).

Pindolol,Timolol Maleate,Nadolol and Sotalol hydrochloride reacted with DNFB. The method was modified for Timolol Maleate,Nadolol and Sotalol hydrochloride. All these three drugs reacted with DNFB in aqueous medium. Where as Pindolol reacted in alcoholic medium. For Pindolol benzene was found to be a suitable solvent and the maximum absorbance was observed at 415 nm. The calibration curve was found to be rectilinear in the range of 0.1-20 µg/ml. For Nadolol,Timolol Maleate and Sotalol hydrochloride maximum absorbance was observed at 370 nm, 370 nm and 400 nm respectively. The calibration curve was found to be rectilinear in the range of 0.8-48 µg/ml, 0.2-34 µg/ml and 0.6-24 µg/ml respectively for Timolol Maleate,Nadolol and Sotalol hydrochloride.

4.1.9 REACTION WITH FOLIN-CIOCALTEU REAGENT.

Only Sotalol hydrochloride reacted with F-C reagent and gave blue colour with absorbance maxima at 725 nm. In order to have maximum sensitivity addition of sodium carbonate preceding that of F -C reagent was necessary. The calibration curve was found to be reactilinear in the range of 0.8-40 µg/ml. The proposed method was found to be simple, rapid, accurate and suitable for routine analysis of Sotalol hydrochloride in bulk and dosage forms.

4.1.10 REACTION WITH ACETYL ACTONE REAGENT.

All the four drugs were reacted with acetyl actone reagent with maximum absorbance at 380 nm, 412 nm, 412 nm and 400 nm respectively for Pindolol, Nadolol, Timolol Maleate and Sotalol hydrochloride. The calibration curve was found rectilinear in the range of 1-14 µg /ml, 4-32 µg/ml,2-16 µg/ml and 8-40 µg/ml for Pindolol, Timolol Maleate.Nadolol and Sotalol hydrochloride respectively. The results were comparable and method was found to be sensitive and precise.

4.1.11 COMPLEXATION WITH DIFFERENT ACIDIC AND BASIC DYES.

Of the 13 acidic and 6 basic dyes employed claton yellow, fluorescein sodium, methyl red, erythrosine and tropaeolin 00 did not react with drug over a pH range of 1-8. The three dyes namely phenol red, sunset yellow and tartrazine did react with drug substances to form ionpairs. However the difference between methanolic solutions of blank and sample were not descrinible and colour of organic extract at 40 µg/ml concentration was very light. The remaining 5 dyes viz. Bromocresol green, bromothymol blue, eriochrome black T, methyl orange and thymol blue exhibited ion pair reactions. Pindolol, Nadolol and Sotalol hydrochloride reacted with bromothymol blue while Pindolol,Timolol Maleate and Sotalol hydrochloride reacted with bromocresol green. Benzene and chloroform were found to be suitable extraction solvents for the ion pairs. Ion pairs of Pindolol, Timolol Maleate and Sotalol hydrochloride with bromocresol green dye gave an absorption maxima at 412 nm. The pH of optimum complexation was found to be 1.0 for all the three compounds. The calibration curve was reactilinear between 4-56 $\overset{\mu}{\cancel{m}}$gc/ml, 2-32 $\overset{\mu}{\cancel{m}}$gc/ml, 2-40 µg/ml for Pindolol, Timolol Maleate and Sotalol hydrochloride with bromocresol green. While 4-64 µg/ml, 2-32 µg/ml, 5-60 µg/ml for Pindolol, Nadolol and Sotalol hydrochloride with bromothymol blue. Pindolol,Timolol Maleate and Sotalol hydrochloride gave ion pair complex with eriochrome black T with maximum absorbance at 470 nm, 520 nm and 540 nm respectively. Timolol Maleate also reacted with methyl orange with absorbance maxima at 412 nm..The pH of optimum complexation was found to be 2.2 for all compounds and chloroform was found to be the most suitable extraction solvent. The calibration curve was rectilinear in the range of 12-60 µg/ml in case of Pindolol and Timolol Maleate with eriochrome black T and 12-40 µg/ml in case of Sotalol hydrochloride with eriochrome black T. The calibration curve was rectilinear in the range of 2 40 µg/ml in case of Timolol Maleate with methyl orange. The proposed spectrophotometric method for the determination of these β blockers is simple, rapid, reliable and can be used for the routine analysis of these drugs in all their common dosage forms.

All the four β blockers gave ion pair complex with crystal violet especially in buffer solution pH 3.0. Chloroform was found to be suitable for extration. The absorption maxima was observed at 580 nm for all the four drugs. Calibration curve was rectilinear in the range of 20-200 µg/ml of all the four drugs.

All the four β blockers behaved identically so far as chloroform extraction was concerned as well as their λmax.

4.1.12 SPECTROPHOTOFLUORIMETRIC METHOD FOR THE ESTIMATION OF β BLOCKERS.

Sotalol hydrochloride gave intrinsic fulorescence in pure methanol at 280 nm excitation wavelength and 310 nm as emission wavelength. In 0.5 M sulphuric acid medium it gave excitation wavelength 275 nm and 310 nm as emission wavelength. Fluorescence intensity could be linearly correlated with concentration of Sotalol hydrochloride from 1-40 ng/ml.

Attempts to chelate the drugs with cupric ions in methanol at an acidic pH with a view to induce fluorescence gave negative results. Pindolol, Nadolol and Sotalol hydrochloride with potassium ferricyanide in methanolic medium gave fluorescence at 270 nm, 275 nm and 280 nm as excitation wavelength respectively and 310 nm as emission wavelength in case of all the three drugs. Fluorescence intensity could be linearly correalated with concentration of Pindolol from 10-60 ng/ml for Nadolol from 5-50 ng/ml and for Sotalol hydrochloride from 4-48 ng/ml. The presence of any of the four drugs tried at the concentration of 40 µg/ml did not affect the fluorescence of 0.025% m/v solution of quinine sulphate.

Timolol Maleate exhibited remerkable quanching property on the narutal fluorescence of fluorescein in methanol, when measured with 470 nm and 510 nm as the excitation and emission wavelength. Interestingly enough Pindolol, Nadolol and Sotalol hydrochloride exhibited increase in the intensity of fluorescence of fluorescein, when mesured with the 470 nm and 510 nm as optimum excitation and emission wavelength for Pindolol and Sotalol hydrochloride where as optimum excitation and emission wavelength for Nadolol were 470 nm and 530 nm, besides increase in fluorescence intensity the presence of Nadolol brought about qualitative change in the excitation specturm of fluorescein. Excitation maxima retained at 470 nm where as emission maxima was shifted from 510 nm to 530 nm when containing 40 ng/ml quantity of Nadolol. The graphs of logaritham of percentage of fluorescence of fluorescein vs concentration of Timolol Maleate was rectilinear from 0.1 - 4.0 µg/ml. The graphs of percentage of fluorescence of fluorescein or percentage increase of fluorescence of fluorescin vs concentration of respective drugs pindolol, Nadolol and Sotalol hydrochloride gave ractilinear relation from 1-20 ng/ml.2-25 ng/ml and 2-25 ng/ml. The method worked satisfactorilly for the estimation of all the commercially available dosage forms of the respective drugs.

4.1.13 REACTIONS OF PINDOLOL.

Pindolol in acidic medium when treated with sodium nitrite and then with N-1-napthyl ethylene diamine gave purple colour with absorbance maxima at 540 nm. The calibration curve was found to be rectilinear in the range of 0.64-40 µg/ml.

0.1% m/v solution of Pindolol in sulphuric acid reacted with potassium dichromate in sulphuric acid and gave blue colour with absorbance maxima at 626 nm. The calibration curve was rectilinear in the range of 4-36 µg/ml.

Pindolol reacted with 1,2 Napthoquinone 4-sulfonic acid after heating but the colour was not stable and no sharp absorbance maxima was observed.

Pindolol also gave pink colour in the presence of mixture of glacial acetic acid: hydrachloric acid 30:10 on heating for 8 minutes on boiling water bath . Stable pink colour with absorbance maxima at 580 nm was obtained and rectilinear calibration curve was obtained in the range of 4-40 µg/ml.

4.1.14 APPLICATION OF THE PROPOSED SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF BETA BLOCKERS IN BIOLOGICAL FLUIDS LIKE BLOOD AND URINE.

The proposed spectrophotometric methods have been applied to estimate the quantity of Pindolol,Timolol Maleate,Nadolol and

Sotalol hydrochloride in the biological fluids such as blood and urine. The recovery of Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride in blood serum and urine was satisfactory (99.25%,99.90%, 99.35% and 99.90%) with the proposed method.

4.2 PREFORMULATION AND STABILITY STUDIES:-

4.2.1 STABILITY OF PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE AT ELEVATED TEMPERATURE AS A FUNCTION OF pH.

The pH dependant stability was investigated by subjecting the 0.500% m/v solution of respective drugs within the pH range 3-8. Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride gave excellent stability at pH [illegible] pH4, pH7.4 and pH5 respectively. High temperature and acidic pH are extremely detrimental to the stability of Nadolol.

4.2.2 STABILITY OF PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE WHEN EXPOSED TO UV RADIATION.

Of the four betablockers drug tried all the samples were affected by uv light exposure and were degraded. In case of Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride 44%, 15%, 9% and 10% degradation respectively was observed. The presence of sodium sulphite prevented the degradation of the drug when exposed to uv radiation more then methyl paraben but in case of Pindolol it (sodium sulphite) produces colour formation.

4.2.3 STABILITY OF PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE WITH DIFERENT TABLET EXCIPIENTS.

Sample of pure drug and their mixture in 1:5 proportion with dicalcium phosphate, lactose and colloidal silicon dioxide showed no signs of physical deterioration even after 150 days of storage at RT, RT RH75% , 45° C, 60° C and 60° C RH75% . All the four drug samples showed noticeable physical deterioration with maize starch, talc and magnesium stearate at 60° C and 60° C RH 75%.

The thin layer chromatogram developed with 3 different mobile phases and visualized with exposure to iodine vapour revealed that the drugs did react with carboxy methyl cellulose and sodium lauryl sulphate giving elongated bands as against the single spot obtained in case of pure drug substances. These findings were in conformity with uv spectrophotometric assay values which showed significant reduction in assay values in case of mixtures with these excipients.

4.2.4 STABILITY OF PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE WITH HYDROCHLORTHIAZIDE DIURETIC.

Each drug samples with hydrochlorthiazide in the proportion 4:1 were mixed and studied for stability. The samples of Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride with hydrochlorthiazide showed no signs of deteriotation even after 150 days of storage at RT and 45° C. The samples showed degradation to the extent of 8%, 6%, 6% and 5.5% with Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride respectively at 60° C RH 75% after 150 days of storage.

4.2.5 STABILITY OF PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE TABLET AND SOLUTIONS OF TIMOLOL MALEATE AT ELEVATED TEMPERATURE AND/OR HUMIDITY.

The tablets of Timolol Maleate, Nadolol and Sotalol hydrochloride displayed excellent physical stability and tablets stored at RT, RT RH75%, 45° C and 60° C remained white, intact tablets for 160 days of observation. The table of Pindolol remained physically white intact after 90 days at RT RH 75%,RT,45°C and 60°C. Tablets stored at 60 C RH 75% however showed noticeable physical deterioration. At RT RH 75% 45°C and 60°C about 16%, 10% and 14% degradation occurred respectively at the end of 160 days observation. The tablets of Timolol Maleate, Nadolol and Sotalol hydrochloride showed 2%, 6% 4.5% and 7%. 16%, 15% degradation at 60°C and 60°C RH 75% respectively. The solution of Timolol Maleate showed about 2% and 5% degradation at 45°C and 60°C respectively after 100 days. The sample gave 12% and 14.5% degradation in low density polyethylene containers at respective storage conditions. However products displayed very good stability up to 180days of storage at ambient temperature.

4.2.6 HPLC METHOD FOR THE STABILITY STUDY OF TIMOLOL MALEATE.

A satisfactory HPLC method was developed for the estimation of Timolol Maleate using methanol : acetonitrile 50:50 as the solvents. A plot of peak area [illegible] of standard Timolol Maleate solution vs concentration of the injected drugs was linear up to 1-5 μg/ml. The retention time was found to be 3.09 minutes. The stability results obtained by this method were comparable with uv method. This type of experiments could not be performed for other drugs due to only limited availability of the instrument.