CHAPTER II

This chapter describes the various analytical methods proposed, the equipments and reagents involved therein and critically discusses the scope, merits and demerits of the proposed methods vis a vis analytical methods published so far.

2.1 COMPLEXATION WITH DIFFERENT METALS :

The published literature did not reveal any analytical method involving metal complexation for the determination of β-blockers in question . The efforts were thus made to develop complexometric methods of estimation of these drugs using metal complexation .

I) REAGENTS :

1) 0.0025 M standard solution of pindolol in a mixture of methanol and water (1:3).
2) 0.0025 M standard solution of timolol maleate in water
3) 0.0025 M standard solution of nadolol in water.
4) 0.0025 M standard solution of acebutolol hydrochloride in water.
5) 0.1 N hydrocholoric acid.

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6) Mc Ilvaine citrate - phospate buffer pH 2.0 - 8.0

7) 0.0025 M Nickel chloride solution in water .

8) 0.0025 M Manganese chloride solution in water.

9) 0.0025 M Ferric chloride solution in water.

10) 0.0025 M Lead nitrate solution in water.

11) 0.0025 M Copper sulphate solution in water.

12) 0.0025 M Mercuric chloride solution in water.

13) 0.0025 M Ammonium ceric sulphate solution in water.

14) 0.0025 M Zinc sulphate solution in water.

15) 0.0025 M Stannous chlorid solution in water.

16) 0.0025 M Ferrous sulphate (exhiccated) in water.

17) 0.0025 M Cobalt nitrate solution in water.

18) 0.10% m/v Uranyl zinc acetate solution in water.

II) EXPERIMENTAL PROCEDURE :

To separate tubes cointaining 2ml of Pindolol / Timolol maleate / Nadolol / Sotalol hydrochloride solution 2ml of 0.1 N hydrochloric acid or 2ml of buffer pH 3.0 were added. To each tube 2ml of different metal soloution was added and the colour formed if any was compared with an appropriate reagent blank prepared simultaneously both when cold and after heating on a boiling water bath for 2 - 5 minutes .

III) RESULT AND DISCUSSION :

No chromogenic reaction was found to take place between Nadolol and Sotalol hydrochloride and all the metal solution 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 when cold and with heating. No

difference in appearance of sample and reagent blank solutions were indicated when they were mixed in equimolar proportion.

Pindolol gave positive reaction with ferric and ceric metal solutions. With ceric metal solution it gave blue colour which was found to be unstable. With ferric metal solution it gave blue green colour on heating and the reaction was studied in detail and reported in 2.1.1.

Timolol maleate also reacted with ceric metal solution and gave highly unstable blue colour.

2.1.1 **REACTION OF PINDOLOL WITH FERRIC IONS :**

a) EQUIPMENT :

Systronics UV visible spectrophotometer model 108.

b) REAGENTS :

1) 0.10 % m/v pindolol solution in 0.1 N hydrochloric acid.

2) 0.0025 M ferric chloride solution in water.

3) Mc Ilvaine citrate - phosphate buffer pH 2.2 - 8 [59]

(refer table 2.1)

4) 0.1 N hydrochloric acid.

c) EXPERIMENTAL PROCEDURE :

1) Determination of optimum pH.

To separate tubes containing 2ml of pindolol solution 2ml of 0.1 N hydrochloric acid (pH 1.0) or 2ml of buffer(pH 2.2-8) was added followed by 2ml of ferric metal solution. The contents were heated on boiling water bath for 5 minutes. Cooled and diluted to volume with water. Extinction of blue green color was measured at 620nm against appropriate reagent blank prepared simultaneously.

Table 2.1

Composition of citrate Phosphate buffer (Mc Ilvaine)[59]

pH	Sodium Phasphate $Na_2HPO_4.12.H_2O$ g/L	Citric acid $C_6H_8O_7.H_2O$ g/L
2.2	1.4	20.6
3.0	14.7	16.7
4.0	27.6	12.9
5.0	36.9	10.2
6.0	45.2	7.7
7.0	59.0	3.7
8.0	69.7	0.58

2. DETERMINATION OF OPTIMUM HEATING TIME :

2ml of standard pindolol solution was added to 5 seperate 50ml calibrated flasks. To each of these flask 2ml of 0.1N hydrochloric acid solution followed by 2ml of ferric chloride solution were added. One flask was kept in cold and the remaining flasks were heated for 5, 10, 15 and 20 minutes on boiling water bath, cooled and diluted to volume. Absorbance was measured at 620nm against appropriate reagent blank initially and then at intervals of 5 minutes.

3. PREPARATION OF CALIBRATION CURVE :

Different aliquots of standard pindolol solution (0.2 to 5.0ml) were transfered to seperate 50ml calibrated flask 2ml of 0.1N hydrochloric acid was added followed by 2ml of ferric chloride solution. The flasks were then loosely stoppered and heated on boiling water bath for 10 minutes and rapidly cooled to room temperature and diluted to volume. Absorbance of the blue green

colour formed was measured at 620nm against the appropriate reagent blank. The calibration curve was prepared by ploting absorbance versus concentration of pindolol (µg/ml).

4. ESTIMATION IN TABLETS :

A quantity of the mixed contents of 20 tablets equivalent to 10mg of pindolol was shaken with 25 ml of 0.1 N hydrochloric acid for 10 minutes. The solution was filtered and volumed made up to 50ml. Aliquots of this solution after dilution were taken and the colour developed and measured as described under calibration curve.

5. REASULT AND DISCUSSION :

When pindolol solution was heated with ferric chloride solution a blue green colour complex was formed . The λ max of this solution was found to be 620nm (figure 2.1). The reagent blank was found to be almost colourless. The maximum absorbance was obtained with 0.1 N hydrochloric acid solution.

The results (table 2.2) showed that maximum colour development occured after 10 minutes heating with 0.1 N hydrochloric acid which remained stable up to 30 minutes (table 2.3), however heating on a boiling water bath greatly facilitated the colour development with colour and absorbance increasing up to 10minutes of heating. Increase of the heating time beyond this decreased the colour intensity.

Calibration curve was rectilinear between 25-120 µg/ml (figure 2.2) at 620nm. Extinction was measured at this wavelength because the absoption maxima was found to be well defined. It was found that recovery data obtained in case of bulk powder and dosage forms varied between 99.2 and 101.0% . Reproducibility of results was checked by analysis of each tablet formulation 5 times.

Fig. 2.1

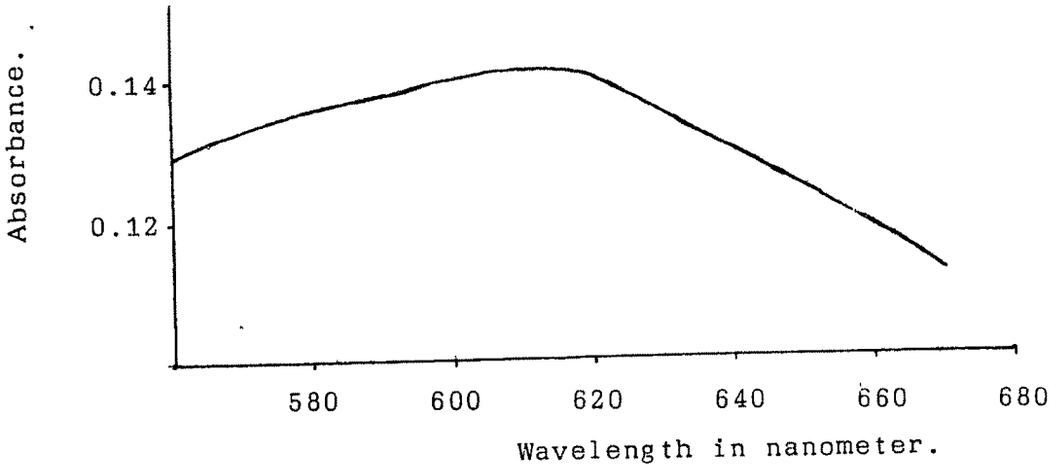


Fig. 2.2

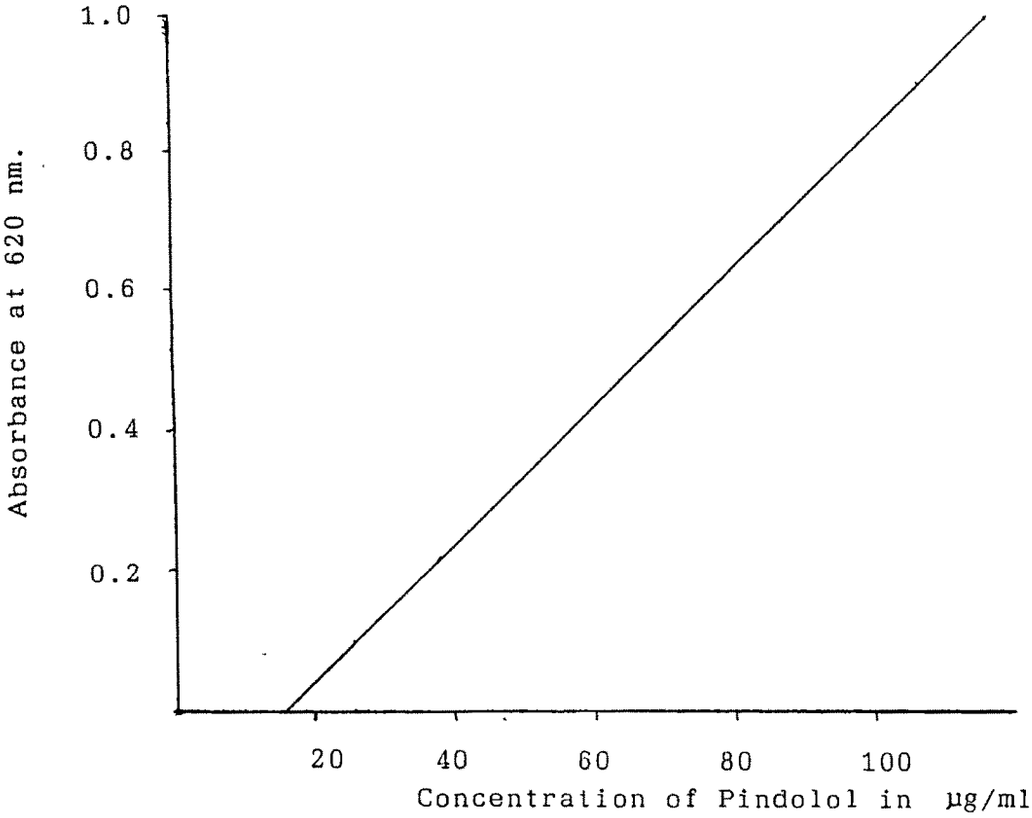


Fig. 2.1 - Absorption spectrum of complex of Pindolol with ferric chloride reagent.

Fig. 2.2 - Calibration curve of complex of Pindolol with ferric chloride reagent.

The study of table 2.4 and 2.5 indicates that the proposed spectrophotometric method is suitable for estimation of pindolol bulk powder and its dosage forms. The results obtained are comprable to B.P.[3] method in terms of accuracy and precision. The method is simple rapid and reliable and can be adopted for the purpose of routine analysis of pindolol and its dosage forms. The usual tablet excipients did not interfere with the procedure.

Table 2.2

Effect of heating time on complexation of pindolol with ferric ion.

Heating time in minutes	Absorbance at 620 nm
0	0.195
5	0.312
10	0.350
15	0.340
20	0.310

Table 2.3

Determination of stability of pindolol - Ferric complex at 620 nm

Time in minutes	Absorbance
0	0.350
5	0.350
10	0.349
15	0.350
20	0.351
25	0.350
30	0.350
35	0.348

40	0.345
50	0.300
60	0.250

Table 2.4

Estimation of pindolol and its dosage forms by ferric ion complexation method

Sample	Proposed method		B.P. method	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	99.80	±0.50	99.30	±0.69
Tablet 1,5 mg	100.52	±0.56	100.10	±0.65
Tablet 2,5 mg	100.15	±0.50	99.80	±0.64

Table 2.5

Optical characteristic, precision and accuracy of the proposed method for pindolol.

Data	Result
λ max (nm)	620
Calibration curve limit(µg/ml)	25-120
Molar Absorptivity ($l.mole^{-1}.cm^{-1}$)	2.48×10^3
Sandell's sensitivity ($\mu g/cm^2$ /0.001 absorbance unit)	0.025
Regression equation (mx+b) = y	
slope = m	0.01
intercept = b	0.125
Correlation Coefficient (r)	0.998

2.2 COUPLING WITH DIAZOTIZED PRIMARY AROMATIC AMINES

The published literature did not reveal any analytical method

involving coupling of these drug substances with diazotized primary aromatic amines. This stimulated the interest for investigating of this aspect.

I) REAGENTS :

1. 0.10% m/v solution of pindolol in 0.1M hydrochloric acid.
2. 0.10% m/v solution of timolol mealeate in water.
3. 0.10% m/v solution of nadolol in water.
4. 0.10% m/v solution of sotaolol hydrochloride in water.
5. 1.00% m/v solution of Sodium nitrite.
6. 15.00% m/v solution of Sodium hydroxide.
7. 0.10% v/v solution of Aniline in N hydrochloric acid.
8. 0.10% m/v solution of P-Nitro aniline in N hydrochloric acid.
9. 0.10% m/v solution of P-Chloraniline in N hydrochloric acid.
10. 0.10% m/v solution of P-Aminophenol in N hydrochloric acid.
11. 0.10% m/v solution of P-Aminobenzoic acid in N hydrochloric acid.
12. 0.10% m/v solution of O-Phenylene diamine in N hydrochloric acid.
13. 0.10% m/v solution of P-Toluidine in N hydrochloric acid.
14. 0.10% m/v solution of P-Anisidine in N hydrochloric acid.
15. 0.10% m/v solution of Sulfanilic acid in N hydrochloric acid.
16. 0.10% m/v solution of Sulfanilamide in N hydrochloric acid.
17. 0.10% m/v solution of 4-nitro-2-amino-phenol-6-sulfonic acid in N hydrochloric acid.
18. 0.10% m/v solution of 8-amino-1-napthol-3,6-disulfonic acid in N hydrochloric acid.

II) EXPERIMENTAL PROCEDURE

5 ml of Sodium Nitrite solution was added to separate tubes

containing 5 ml of respective amine solution, cooled in an ice bath for 10 minutes to facilitate diazotization. To this was added 5 ml of each of the drug solution and colur found if any was compared with an appropariate reagent blank prepared simultaneously, before and after addition of 5 ml of Sodium Hydroxide solution, added slowly and under continuous observation.

III) RESULT AND DISCUSSION

No chromogenic reaction was found to occur between pindolol/timolol maleate/nadolol/sotalol hydrochloride and all the 12 primary aromatic amines/amine acids tried as indicated by no difference in apperance of sample and reagent blank solutions. Before the addition of Sodium Hydroxide, the colour of the sample and balnks were either colorless or yellow depending upon the colour of diazotized amine under test. When Sodium Hydroxide solution was added to render the solution alkaline to facilitate any probable coupling reaction with the drug substance, no colour change occured until about 3ml addition, but when the volume of alkali exceeded 3ml, hydrochloric acid in the medium was neutralized and drug samples were precipitated resulting in white/yellow - orange turbid sample as against colourless/yellow - orange coloured blank solutions.

2.3 REACTION WITH P-DIMETHYL AMINO BENZALDEHYDE

The published literature did not reveal any assary method for pindolol/timolol maleate/nadolol/sotalol hydrochloride involving the p-dimethyl amino benzaldehyde or such other aldehydes. Experiments were designed to explore the possiblity of any such reaction.

I) REAGENTS

1. 0.10% m/v solution of pindolol in 0.1N hydrochloric acid.
2. 0.10% m/v solution of timolol maleate in water.
3. 0.10% m/v solution of nadolol in water.
4. 0.10% m/v solution of sotalol hydrochlorid in water.
5. P-dimethyl amino benzaldehyde (PDAB) reagent:

   10 mg of P-dimethyl amino benzaldehyde in 150 ml mixture of glacial acetic acid : hydrochlorid acid (6:5 v/v).
6. 5.00% m/v solution of ferric chloride.
7. 0.1 N hydrochloric acid.

II) EXPERIMENTAL PROCEDURE

6 ml of PDAB reagent was added to seperate tubes containing 2 ml of the respective drug solution. The contents were then heated on boiling water bath for two minutes, cooled and diluted with 0.1N hydrochloric acid solution. The tubes were examined as such and after addition of 0.2ml of ferric chloride solution.

III) RESULT AND DISCUSSION

The contents in each tube were colourless except that of pindolol before ferric cloride addition and remained colourless or turned to just light yellow green both in sample and blank tubes on addition of ferric cloride solution. In case of pindolol raddish purple colour was observed before addition of ferric cloride and after addition of ferric cloride no change was observed in the colour. The reaction of pindolol with P-dimethyl amino benzaldehyde was promising and a sensitive method was developed based on it described below.

2.3.1 **REACTION OF PINDOLOL WITH P-DIMETHYL AMINO BENZALDEHYDE.**

I) EQUIPMENT : Bausch and lomb "spectronic 20" spectrophotometer.

II) REAGENTS :

1. 10 mg of pindolol in 50 ml of 0.1N hydrochloric acid solution (standard solution).
2. 10 mg of P-dimethyl amino benzaldehyde in 150 ml mixture of glacial acetic acid : Hydrochloirc acid (85:15 v/v).
3. 0.1N hydrochloric acid.

III) EXPERIMENTAL PROCEDURE :

Preparation of calibration curve

An aliquot of standard solution of pindolol (5 ug to 50 ug) was transfered in to 10 ml volumetric flask and 6 ml of PDAB reagent was added. The countents were then heated on a boling water bath for 1.5 minutes, cooled and diluted to the mark with 0.1N hydrochloric acid. The absorbance was measured at 570 nm against the reagent blank prepared in a similer manner by replacing the pindolol solution with an equal volume of 0.1N hydrochloric acid.

IV) DETERMINATION OF OPTIMUMM VOLUME OF PDAB REAGENT

1 ml of standard pindolol solution was added to 6 separate 10 ml volumetric flasks. Different volumes of PDAB reagent(3 to 8 ml) were added and the contents were heated on a boiling water bath for 1.5 minutes, cooled and diluted to the mark with 0.1N hydrochloric acid. Absorbance was measured as described under experimental procedure.

V) DETERMATION OF OPTIMUM HEATING TIME

1ml of standard pindolol solution was added to 6 sperate 10 ml

volumetric flasks. 6 ml of PDAB reagent was added and the contents were heated for 1, 1.5, 2, 2.5, 3, 4 minutes on a boiling water bath and the same procedure was followed as described under experimental procedure.

VI) ESTIMATION IN TABLETS

Aquantity of the mixed contents of 20 tablets equivalent to 10 mg of pindolol was shaken with 25 ml of 0.1N hydrochloric acid for 10 minutes. The solution was filtered and volume was made up to 50 ml. Aliquots of this solution affter dilution were taken and the colour developed and measured as described earlier.

VII) <u>REASULT AND DISCUSSION</u>

The absorption spectrum of raddish purple coloured complex developed due to reaction of pindolol with P-dimethyl amino benzaldehyde is shown in figure 2.3. The maximum absorbance was observed to be at 570 nm. The volume ratio of glacial acetic acid : hydrochloric acid was optimised and found to be 85 : 15 v/v. The higest coloured intensity was obtained in the presence of 6.0 ml of PDAB reagent (Table 2.6). On increasing or decreasing the volume of reagent, the coloured intensity decreased. The maximum colour intensity was obtained with 1.5 minutes heating on a boiling water bath and the developed colour remained stable for three hours (Table 2.8). The usual tablet excipients did not interfere with the estimation. Pindolol tablets obtained from different commercial sources were analysed and the results compaired with the official method[3] (Table 2.9). The calibration curve was found to be rectilinear in the range of 0.5 - 50 µg/ml (Figure 2.4). The method was found to be sensitive as well as precise. (Table 2.10).

Fig. 2.7

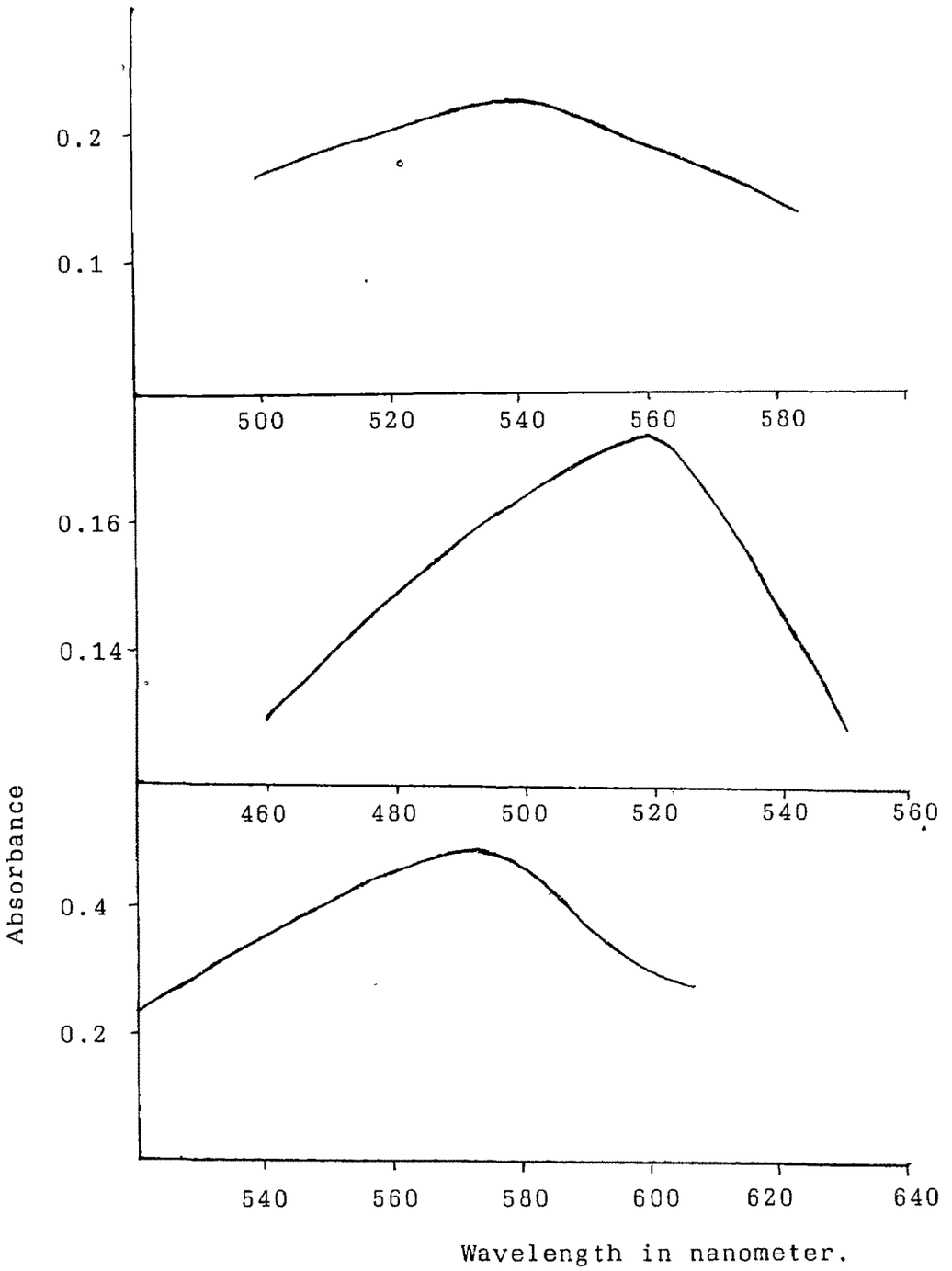


Fig. 2.5

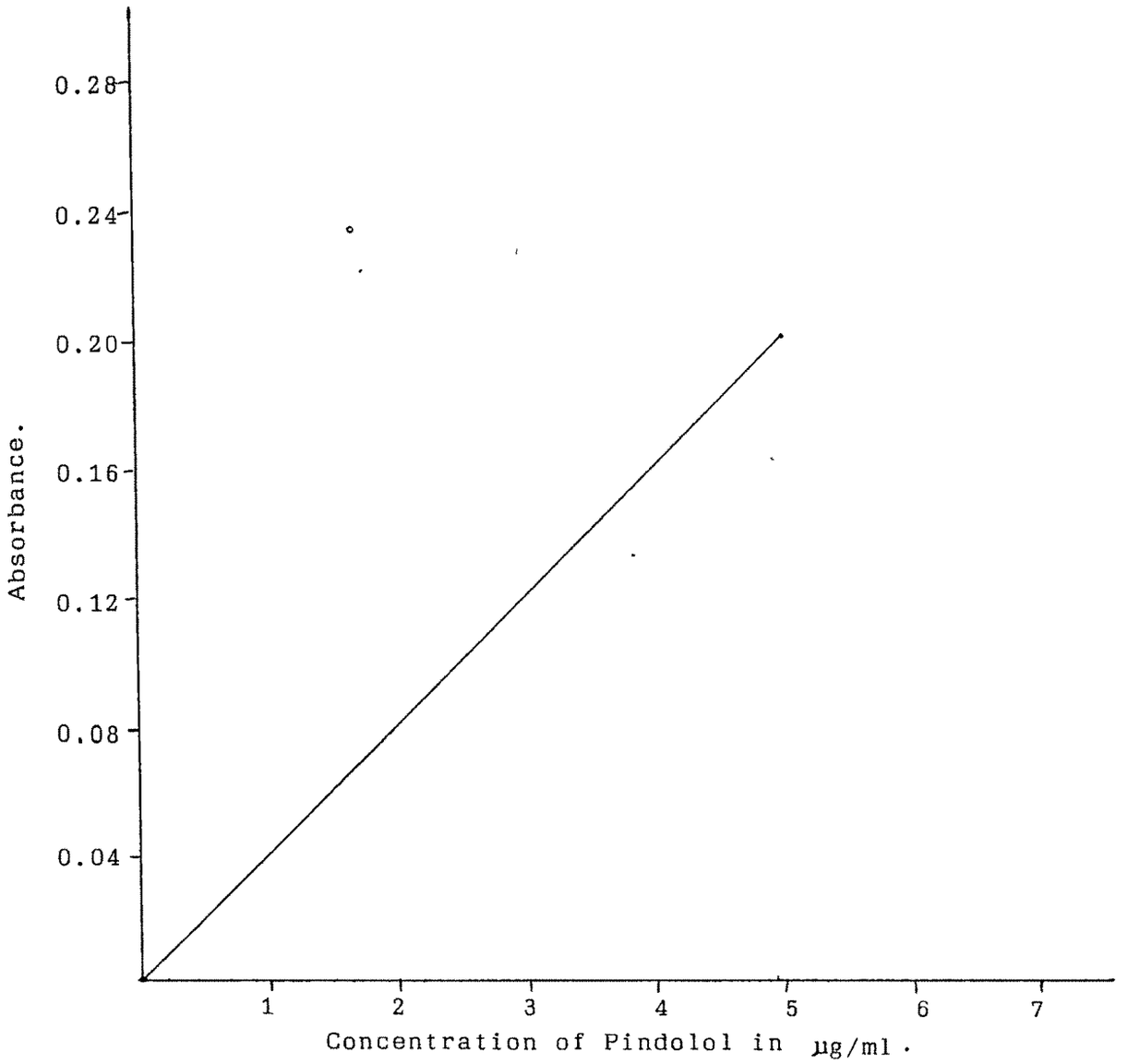
Fig. 2.3

Fig. 2.7 - Absorption spectrum of Pindolol with sodium per iodate.

Fig. 2.5 - Absorption spectrum of Pindolol with vanillin.

Fig. 2.3 - Absorption spectrum of Pindolol with P-dimethyl amino benzaldehyde.

Fig. 2.4



Calibration curve of Pindolol with P-dimethyl amino benzaldehyde.

Table 2.6 DETERMINATION OF OPTIMUM VOLUME OF PDAB REAGENT

Volume of reagent in ml	Absorbance at 570 nm
3	0.280
4	0.291
5	0.300
6	0.316
7	0.301
8	0.293

Table 2.7
DETERMINATION OF OPTIMUM HEATING TIME

Time in minutes	Absorbance at 570 nm
0.0	0.04
1.0	0.305
1.5	0.315
2.0	0.250
2.5	0.210
3.0	0.190
4.0	0.150

Table 2.8
STABILITY OF THE COLOURED DEVELOPED WITH PINDOLOL AND PDAB REAGENT

Time in minutes	Absorbance at 570 nm
0.0	0.314
30.0	0.315
60.0	0.314
90.0	0.314
120.0	0.314

150.0	0.312
180.0	0.310
210.0	0.300

Table 2.9

RESULTS OF DETERMINATION OF PINDOLOL BY PDAB METHOD

Sample	PDAB method		B.P. method	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	99.89	±0.051	99.77	±0.15
Tablet 1	101.44	±0.044	99.80	±0.13
Tablet 2	101.56	±0.064	100.58	±0.19

Table 2.10

OPTICAL CHERACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHOD FOR PINDOLOL

Data	Result
λmax (nm)	570
Beer's law (µg/ml)	0.5 - 5.0
Molar absorptivity ($l.mole^{-1}.cm^{-1}$)	19.86×10^3
Sandell's Sensitivity ($\mu g/cm^2$ /0.001, absorbance unit)	0.001
Regression equation (mx + b) =y	
slope = (m)	0.08
intersept = (b)	0.00
correlation coefficient (r)	0.999

2.4 REACTION WITH VANILLIN

The published literature did not reveal any assay method using vanillin. Experiments were designed to explore the possibility of

such a reaction.

I) REAGENTS

1 0.10% m/v solution of pindolol in 0.1N hydrochloric acid.

2 0.10% m/v solution of timolol maleate in water.

3 0.10% m/v solution of nadolol in water.

4 0.10% m/v solution of sotalol hydrochloride in water.

5 distilled water.

6 1.00% w/v solution of vanillin in concentrated hydrochloric acid.

II) EXPERIMENTAL PROCEDURE

2 ml of vanillin reagent solution was added to seperate tubes contianing 2 ml of the respective drug solutions. Contents were mixed and diluted with distilled water. The absorbance of the solution was recorded spectrophotmetrically against the reagent blank.

III) RESULT AND DISCUSSION

The colour of the solution in each tube ranged from colurless to very light yellow except that of pindolol sample tube. Pindolol gave red coloured complex with vanillin and results were promising. A sensitive method was developed based on this reaction described below :

2.4.1 REACTION OF PINDOLOL WITH VANILLIN

I) EQUIPMENT : Bausch and Lomb "spectronic 20" spectrophotmeter.

II) REAGENTS

1 10 mg of standard pindolol sample in 50 ml of 0.1N hydrochloric acid (solution further diluted for experiment).

2 1.0% w/v solution of vanillin in concentrated hydrochloric acid.

III) PREPARATION OF STANDARD CURVE

An aliquot of standared solution of pindolol was transfered into 10ml volumetric flask and 2ml of vanillin reagent was added. The contents of the flask were mixed and diluted to the mark with distilled water. The absorbance was measured at 520 nm against the reagent blank.

IV) DETERMATION OF OPTIMUM VOLUME OF VANILLIN REAGENT

To the 10 ml volumetric flasks containing 1 ml of standared pindolol solution in each different volumes of vanillin reagent (1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3ml and 4 ml) was added and the contents were mixed and diluted to the mark with distilled water. The absorbance was measured at 520 nm against the reagent blank.

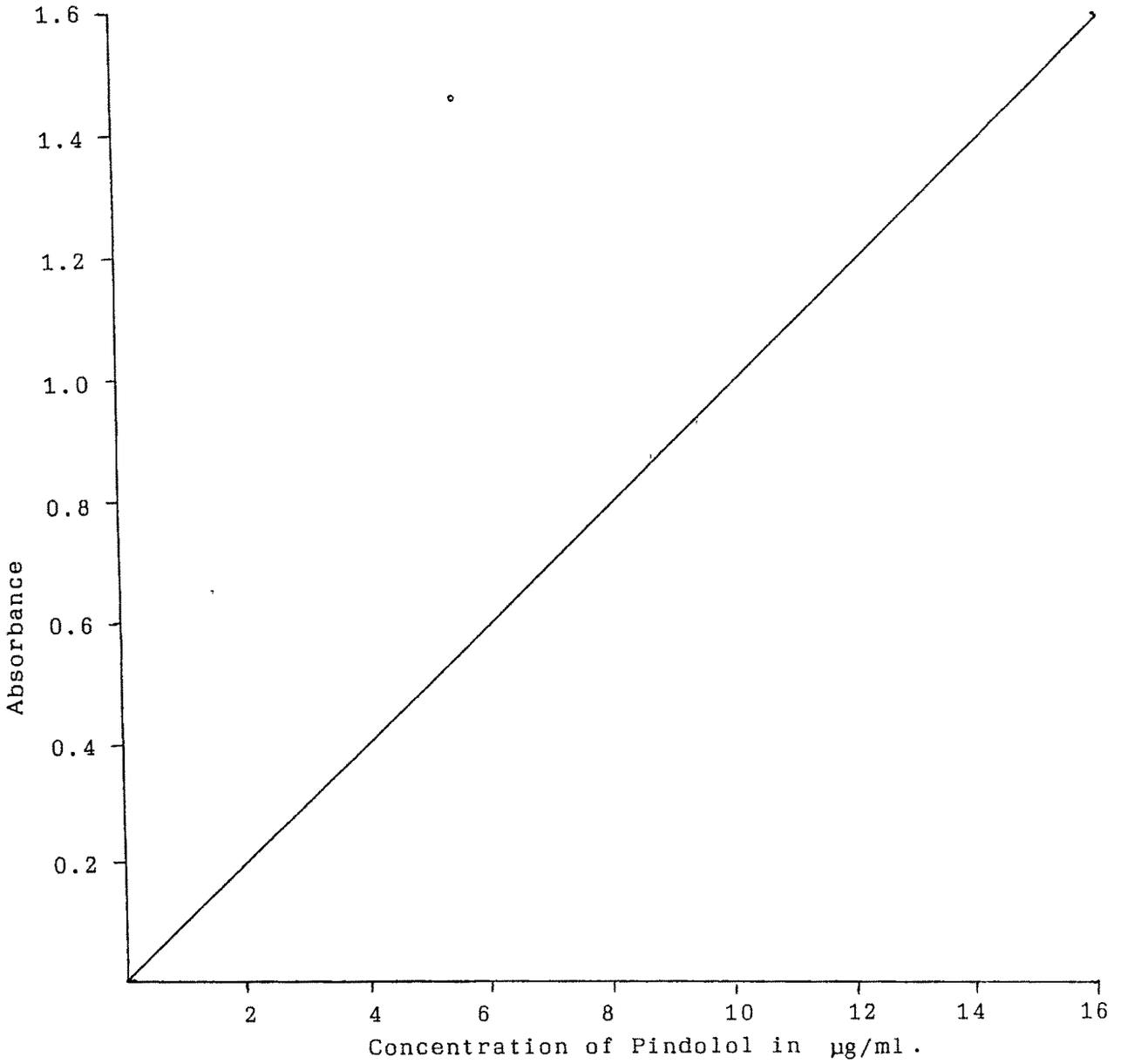
V) ESTIMATION IN TABLETS

A quantity of the mixed contents of 20 tablets equivalent to 10 mg of pindolol was shaken with 25 ml of 0.1N hydrochloric acid for 10 minutes. The solution was filtered and volume was made upto 50 ml. Aliquots of this solution after dilution were taken and the colour developed and measured as described earlier.

VI) REASULT AND DISCUSSION

The absorption spectrum of red colored complex of the pindolol and vanillin has been shown in figure 2.5. The maximum absorbance was observed at 520 nm. The highest colour intensity was observed with 2ml of vanillin reagent (Table 2.11) and the colour remained stable for 30 minutes (Table 2.12). The calibration curve was observed rectilinear in the range of 0.2 - 16.0 µg/ml (Figure 2.6). The usual tablet excipients did not interfere with the results. Pindolol tablets obtained from different commercial

Fig. 2.6



Calibration curve of Pindolol with vanillin.

sources were analysed and the results compared with the official method [3] (Table 2.13). The method was found to be simple, rapid, and sensitive (Table 2.14).

Table 2.11

DETERMINATION OF OPTIMUM VOLUME OF VANILLIN REAGENT

Volume of reagent	Absorbance at 520 nm
1.0	0.18
1.5	0.30
2.0	0.43
2.5	0.41
3.0	0.37
4.0	0.33

Table 2.12

STABILITY OF THE RED COLOURED COMPLEX OF PINDOLOL-VANALLIN

Time in minutes	Absorbance at 520 nm
0.0	0.43
5.0	0.43
10.0	0.42
20.0	0.43
30.0	0.43
40.0	0.41
50.0	0.39
60.0	0.35

Table 2.13

RESULTS OF DETERMINATION OF PINDOLOL BY VANILLIN METHOD

Sample	Vanillin method		B.P. method[3]	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	100.9	±0.065	100.10	±0.15
Tablet 1	101.0	±0.066	100.05	±0.13
Tablet 2	100.8	±0.064	99.70	±0.12

Table 2.14

OPTICALLY CHARACTERISTIC, PRECISION AND ACCURACY OF THE PROPOSED METHOD FOR PINDOLOL

Data	Result
λmax (nm)	520
Beer's law limits (µg/ml)	0.2 - 16.0
Molar absorptivity ($l.mole^{-1}.cm^{-1}$)	24.83×10^3
Sandell's sensitivity ($\mu g/cm^2$ /0.001 absorbance unit)	0.003
Regression equation (mx + b) = y	
slope = (m)	0.1
intercept = (b)	0.0
Correlation coefficient (r)	0.998

2.5 REACTION WITH SODIUM PERIODATE

The published literature did not reveal any assay method using sodium periodate and attempts were made to explore the possibility of any such reaction.

I) REAGENTS

1 NO.1-4 as describe in 2.4 reagents.

2 0.1N sodium periodate solution in water.

3 Mc Ilvaine citrate - phosphate buffer pH 2.0 - 8.0[59]

4 0.1N hydrochloric acid.

II) EXPERIMENTAL PROCEDURE

1ml of 0.1N sodium periodate solution was added to seperate tubes containing 2 ml of respective drug solutions, diluted with distilled water and observed before and after heating for 5 minutes on boiling water bath.

III) RESULT AND DISCUSSION

The samples of timolol maleate, nadolol and sotalol hydrochloride remained coloureless before and after heating on boiling water bath with sodium periodate. While the sample of pindolol gave reddish purple colour with sodium periodate without heating. There was no change in colour intensity observed after heating. This colour reaction led to the study in detail about the chromogenic reaction conditions.

2.5.1 REACTION OF PINDOLOL WITH SODIUM PERIODATE

I) REAGENTS

1 0.10% m/v solution of pindolol in 0.1N hydrochloric acid.

2 0.1N sodium periodate solution in water.

3 0.1N hydrochloric acid.

4 Mc Ilvaine citrate phosphate buffer pH 2.2 to 8.0[59].

II) PREPARATION OF STANDARED CURVE

Aliquot of standared solution of pindolol (0.2 ml to 4 ml) was transfered in to 25 ml volumetric flask and 2 ml of 0.1N hydrochloric acid and 2 ml of 0.1N sodium periodate solution was

added. After 10 minutes the constents were diluted to the mark with distilled water and the absorbance was measured at 540 nm against the reagent blank.

III) DETERMINATION OF OPTIMUM pH

To the 25 ml volumetric flask containing 2 ml of standared pindolol solution 2ml 0.1N hydrocloric acid and 2 ml of buffer solution (0.1N Hcl pH 2 to 8) respectively and 2 ml of sodium periodate solution was added and volume was madeup after 10 minutes. Absorbance was measured as mentioned earliar.

IV) DETERMINATION OF OPTIMUM VOLUME OF SODIUM PERIODATE SOLUTION

2 ml of 0.1N hydrochloric acid was added to the 25 ml volumetric flasks each containing 2 ml of standared drug solution. Different volumes (1 ml to 4 ml) of sodium periodate solution was added and volume was made up after 10 minutes. The absorbance was measured as mentioned earlier.

V) ESTIMATION IN TABLETS

A quantity of the mixed contents of 20 tablets equivalent to 10 mg of pindolol was shakern with 25 ml of 0.1N Hcl for 10 minutes. The solution was filtered and volume was made up to 50 ml. Aliquots of this solution after dilution were taken and the colour developed and measured as described earlier.

VI) RESULT AND DISCUSSION

The absorption spectrum of the coloured complex of pindolol - sodium periodate is shown in Figure 2.7 with maximum absorbance at 540 nm. The addition of 0.1N Hcl to pindolol solution gave reddish purple colour. The addition of buffer solution of pH 2 to 7 and

pH8 change to the colour to pale yellow colour with no sharp absorbance maxima and therefore the 0.1N hydrochloric acid solution was selected for the experiment. 2 ml solution of sodium periodate was found optimum for coloure developement (Table 2.15) but the colour developed was found to be stable for only 10 minutes (Table 2.16). Calibration curve was rectilinear between 70 - 120 µg/ml. The method was not sensitive as compared with the method developed with P-dimethyl amino benzaldehyde or vanimillin, though the results of analyses of market formulation were comparable with B.P.[3] method (Table 2.17) but the method is simple and rapid.

Table 2.15

DETERMINATION OF OPTIMUM VOLUME OF SODIUM PERIODATE SOLUTION

Volume in ml	Absorbance at 540 nm
1.0	0.222
2.0	0.300
3.0	0.284
4.0	0.250

Table 2.16

STABILITY OF PINDOLOL PERIODATE COMPLEX

Time in minutes	Absorbance at 540 nm
0.0	0.300
5.0	0.300
10.0	0.300
15.0	0.295
20.0	0.290
30.0	0.280

60.0		0.269

Table 4.17

RESULTS OF ESTIMATION OF PINDOLOL BY PERIODATE METHOD

Sample	Periodate method		B.P. method	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	99.10	±0.091	100.10	±0.15
Tablet 1	99.55	±0.088	100.03	±0.13
Tablet 2	98.90	±0.090	99.82	±0.12

2.6 REACTION WITH AMMONIUM METAVANADATE

The published literature did not reveal any reaction using ammonium metavanadate and attempts were made to investigate any possibility of such a reaction.

1) REAGENTS

1 NO 1-4 as described in 2.4 reagent.

2 0.1M solution of ammonium metavanadate (amv) in sulphuric acid of following strength 0.1M, 0.25M and 0.5M

II) EXPERIMENTAL PROCEDURE

1 ml of ammonium metavandate reagent was added to seperate tubes containing 1 ml of respective drug solution. The contants were diluted with distilled water.

III) RESULT AND DISCUSSION

The sample of timolol maleate, nadolol and sotalol hydrochloride remained clolurless on addition of ammonium metavanadate solution. The tube containing sample of pindolol gave blue colour without heating. The reaction condition of pindolol leading to this colour development were there fore

investigated further.

2.6.1 REACTION OF PINDOLOL WITH AMMONIUM METAVANADATE

I) REAGENTS

1 No 1 – 5 as given in 2.6 reagents.

II) PREPARATION OF STANDARED CURVE

Aliquots of standared solution of pindolol was transfered in to 25 ml volumeteric flask and 1 ml of 0.1M ammonium metavanadate solution was added and the contents of the flask were diluted with distilled water. The absorbance of the blue colour formed was measured at 635 nm against the reagent blank prepared in simillar manner.

III) DETERMINATION OF OPTIMUM CONCENTRATION OF SULPHURIC ACID

To the 25 ml volumetric flask containing 1 ml of standared pindolol solution 1 ml each of ammonium metavanadate in 0.1N sulphuric acid, in 0.25N sulphuric acid and in 0.5N sulphuric acid was added and the volume was made upto mark with distilled water and absorbance was measured as explained in standared curve.

IV) DETERMATION OF OPTIMUM VOLUME OF AMMONIUM METAVANADATE REAGENT

To the 25 ml volumetric flask containing 1 ml of standared drug solution different volumes of (0.5 ml, 1 ml, 1.5 ml, 2 ml and 3 ml) ammonium metavanadate in 0.25N sulphuric acid was added and the volume was made up with distilled water. The absorbance was measured by the procedure mentioned earlier.

V) RESULT AND DISCUSSION

The absorption spectrum of the coloured complex of pindolol - vanadium was shown in Figure 2.9 with maximum absabance at 635 nm. The change in pH of the system did not imporve the method. 1 ml solution of ammonium metavanadate in 0.25N sulphuric acid was found optimum for stable colour development (30 minutes). (Table 2.19, 2.20). Pindolol tablets obtained from different commercial sources were analysed and the results compared with the official method [3] (Table 2.21). The method was found to be sensitive as well as precise. The colour was found to be stable for more than half an hour (Table 2.22). Calibration curve was rectilinear in the range of 4 to 64 µg/ml (Figure 2.10). The result are summarised in Table 2.23.

Table 2.19

DETERMINATION OF CONCENTRATION OF SULPHURIC ACID

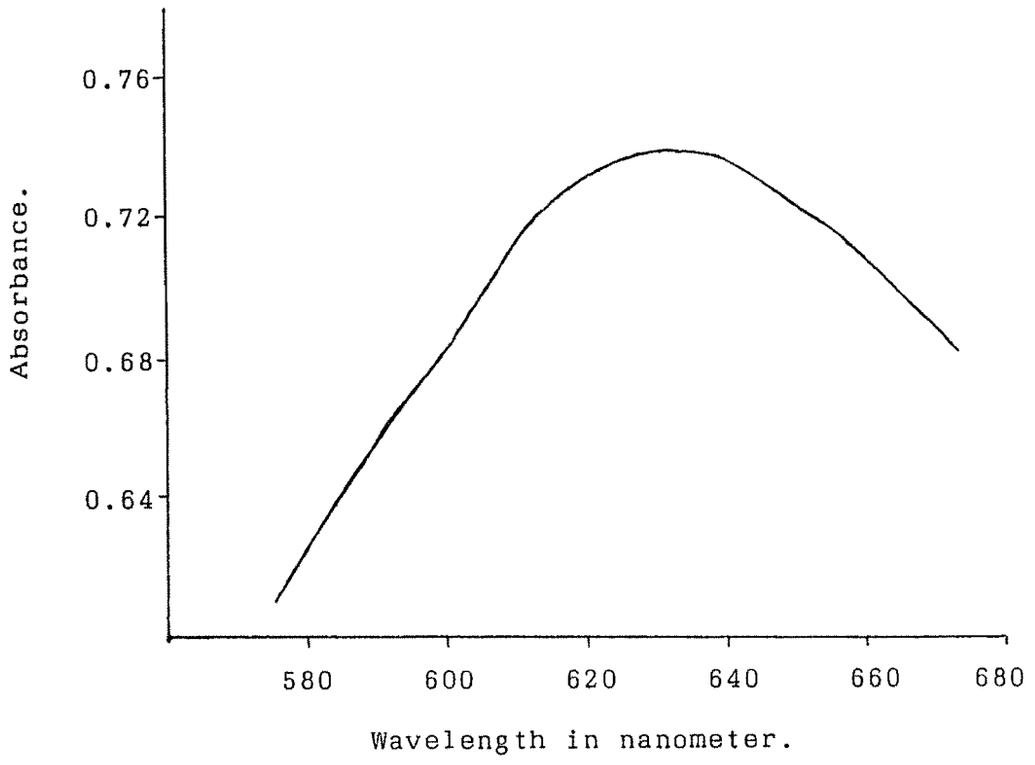
Concentration of sulpheric acid	Absurbance at 635 nm
0.10N	0.280
0.25N	0.325
0.50N	0.310

Table 2.20

DETERMATION OF VOLUME OF AMMONIUM METAVANDATE REAGENT

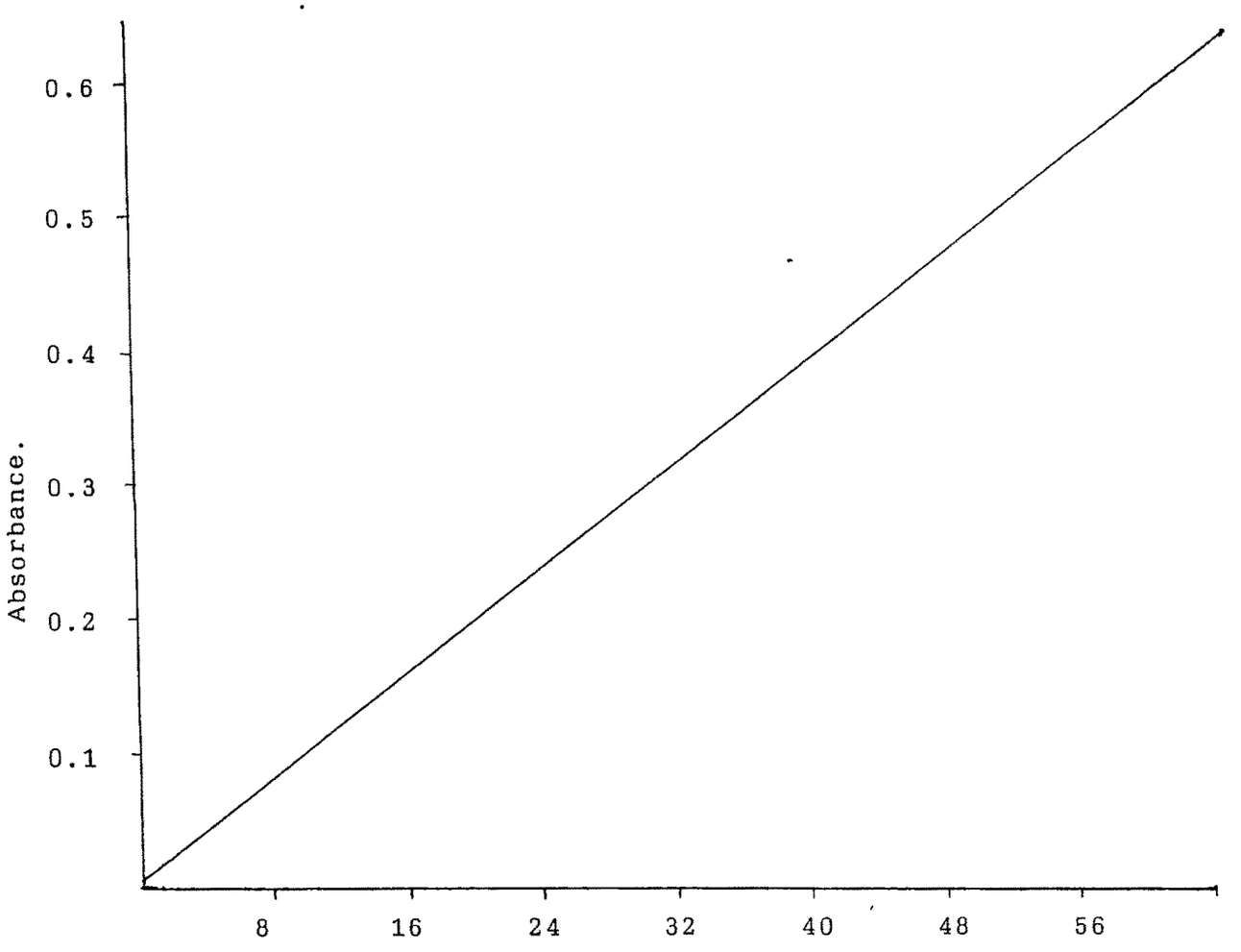
Volume in ml	Absorbance at 635 nm
0.5	0.315
1.0	0.325
1.5	0.320
2.0	0.310
3.0	0.300

Fig. 2.9



Absorption spectrum of Pindolol with Ammonium metavanadate.

Fig. 2.10



Calibration curve of Pindolol with ammonium metavanadate.

Table 2.21

STABILITY OF PINDOLOL – VANADIUM COMPLEX

Time in minutes	Absorbance at 635 nm
0.0	0.325
10.0	0.325
20.0	0.324
30.0	0.325
40.0	0.320
50.0	0.310
60.0	0.300
70.0	0.280

Table 2.22

RESULTS OF ESTIMATION OF PINDOLOL BY AMMONIUM METAVANADATE METHOD

Sample	Metavanadate method		B.P. method[3]	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	98.90	±0.065	99.70	±0.12
Tablet 1	99.68	±0.064	99.82	±0.13
Tablet 2	100.10	±0.061	100.90	±0.15

Table 2.23

OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHOD FOR PINDOLOL

Data	Result
λ max (nm)	635
Calibration curve limits (µg/ml)	4 - 64

Molar absorptivity ($l.mole^{-1}.cm^{-1}$)	20.56×10^3
Sandell's Sensitivity ($\mu g/cm^2$ /0.001, absorbance unit)	0.048
Regression equation (mx + b) = y	
slope = (m)	0.0083
intersept = (b)	- 0.001
correlation coefficient (r)	0.998

2.7 REACTION WITH 3,5 DINITROBENZOYL CHLORIDE

The published literature did not reveal any reaction using 3,5 dinitro benzoyl chloride therefore attempts were made to investigate any possiblity of such a reaction.

1) REAGENTS

1 1.0 gm 3,5 dinitrobenzoyl chloride (3,5D) in 10 ml of redistilled pyridine. (using a hot water bath to maintain solution and prepared fresh immediately prior to use.)

2 0.10% m/v solution of pindolol in pyridine.

3 0.10% m/v solution of timolol maleate in pyridine.

4 0.10% m/v solution of nadolol in pyridine.

5 0.10% m/v solution of sotalol hydrochloride in pyridine.

6 2.00N hydrochloric acid.

7 Pyridine redistilled.

8 2.0N solution of sodium hydroxide in water.

9 Dimethyl formamide (DMF).

10 Hexane.

II) EXPERIMENTAL PROCEDURE

1 ml of 3,5 D was added to seperate 100 ml volumetric flasks containing 2 ml each of drug sample in pyridine. The sample were allowed to react for 15 minutes at ambient temperature. 25 ml of

2.ON hydrochloric acid was added and samples were extracted in 20 ml of hexane. The contents were shaken for 30 seconds and allowed the two phases to seperate completely. Aliquots of the top layer was pipetted in to another 25 ml volumetric flask. 10 ml of dimethyl formamide and 0.3 ml of 2N NaOH solution was added. The contents were shaken and allowed to stand for 3 to 5 minutes. The absorbance of the samples were determined at 520 nm against the reagent blank.

Since the results were promising a sensitive colorimetric method for each drug was developed.

III) OPTIMISATION OF VARIOUS CONDITIONS OF THE EXPERIMENT

To the samples different volumes of reagents was added and rest of the procedure was followed as explained in above paragraph (experimental procedure). After determining the volume of reagent volumes of 2N Hcl and 2N NaOH there after attempts were made for the selection of a suitable solvent for extraction of the chromogenic substance. Pyridine, acetone and dimethyl formamide were tried for extraction conditions for the stability of the colour was established and the method was compared with officeial B.P. method's for pindolol, timolol maleate and nadolol and for sotalol hydrochloride results were compared with reported method since drug is not official in any of the pharmacopoeias.

IV) PROCEDURE FOR TABLETS/TIMOLOL MALEATE EYE SOLUTION

A quantity of the mixed content of 20 tablets/5 ml of timolol maleate eye solution of each drug sample equivalent to 10 mg of drug sample has shaken with 25 ml of pyridine for 10 minutes. The solution was filtered with glass wool and volume made up to 50 ml with pyridine. Aliquots of these solutions were taken and colour

developed and measured as described in experimental procedure.

V) RESULT AND DISCUSSION

Pindolol, timolol maleate, nadolol and sotalol hydrochloride reacted with 1 ml of 3,5 D in pyridine. 1 ml of 3,5 D reagent was optimum for colour development. 25 ml of 2N Hcl and 0.3 ml of 2N NaOH solution was required for maintaining the condition of reaction. DMF was found to be a suitable solvent, rather than acetone. The absorbance maxima was observed at 520 nm (Figure 2.11). The colour was found to be stable for only 15 minutes. The calibration curve was found rectilinear in the range of 10-60 µg/ml 4-24 µg/ml 20-80 µg/ml and 5-30 µg/ml respectively for pindolol, timolol maleate, nadolol and sotalol hydrochloride (figure 2.12). The methods were compared with B.P.[3] methods for pindolol, nadolol and timolol maleate and a published method[66] for sotalol hydrochloride and representated in the Table 2.24, 2.25, 2.26, 2.27. The results are summarised in Table 2.28.

Table 2.24

RESULTS OF ESTIMATION OF PINDOLOL BY 3,5 D METHOD

Sample	3,5 D method		Official method B.P.[3]	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	99.78	±0.045	99.82	±0.13
Tablet 1	100.05	±0.04	100.10	±0.14
Tablet 2	99.10	±0.043	100.90	±0.15

Table 2.25

RESULTS OF ESTIMATION OF TIMOLOL MALEATE BY 3,5 DMETHOD

Sample	3,5 D method		Official method B.P.[3]	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	99.25	±0.052	99.63	±0.25

Tablet 1	99.58	±0.050	99.10	±0.27
Tablet 2	99.99	±0.054	99.32	±0.26
Eye solution	99.70	±0.052	99.40	±0.26

Table 2.26

RESULTS OF ESTIMATION OF NADOLOL BY 3,5 DMETHOD

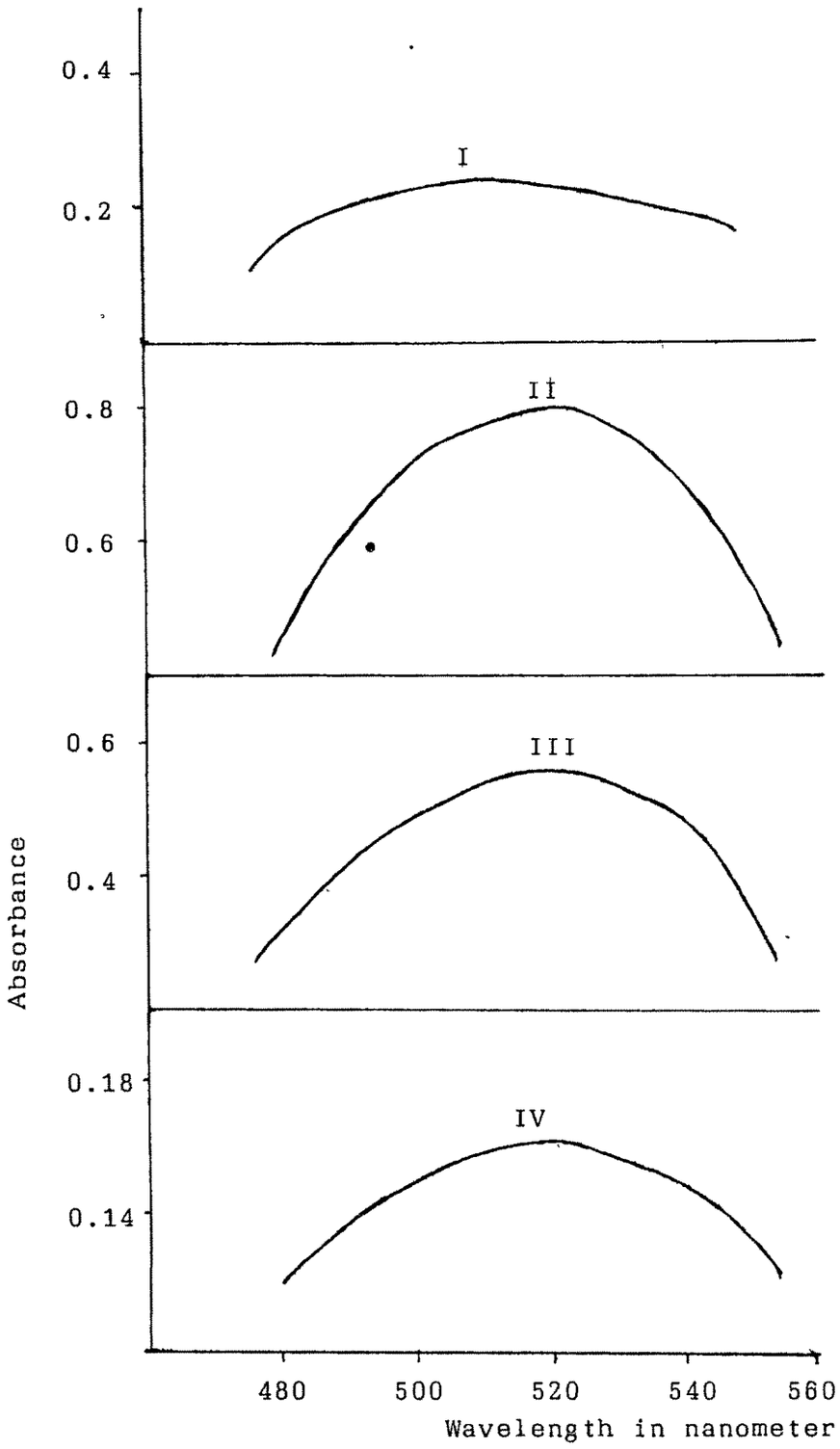
Sample	3,5 D method		Official method B.P. 83	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	99.12	±0.023	99.83	±0.33
Tablet 1	99.35	±0.020	99.30	±0.34
Tablet 2	100.06	±0.021	99.86	±0.33

Table 2.27

RESULTS OF ESTIMATION OF SOTALOL HYDROCHLORIDE BY 3,5 DMETHOD

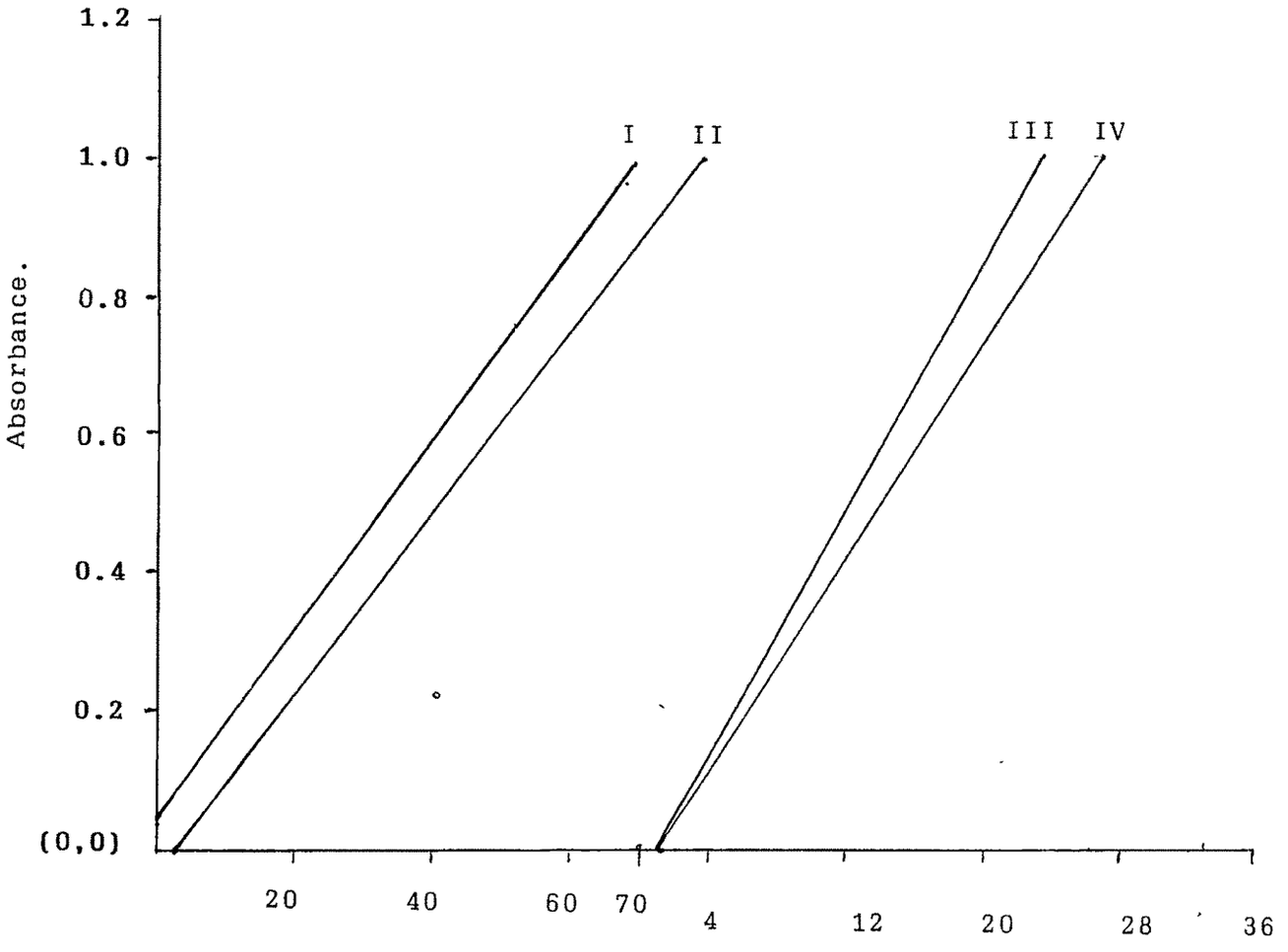
Sample	3,5 D method		Published method	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	100.10	±0.041	99.75	±0.52
Tablet 1	99.89	±0.042	99.60	±0.51
Tablet 2	99.75	±0.043	100.00	±0.50

Fig. 2.11



Absorption spectrum of (I) Pindolol (II) Sotalol Hydrochloride (III) Timolol Maleate (IV) Nadolol with 3,5Dinitro benzoyl chloride reagent.

Fig. 2.12



Calibration curve of (I) Pindolol (II) Nadolol (III) Timolol - Maleate (IV) Sotalol Hydrochloride with 3,5Dinitro Benzoyl Chloride.

Table 2.28

OPTICAL CHARACTERISTIC, PRECISION AND ACCURACEY OF THE PROPOSED METHOD FOR PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE

Data	Result			
	Pindolol	Timolol maleate	Nadolol	Sotalol hydrochloride
λmax (nm)	520	520	520	520
Calibration curve limit(μg/ml)	10-60	4-24	20-80	2-30
Molar absorptivity ($l.mole^{-1}.cm^{-1}$)	3.97×10^3	16.21×10^3	4.02×10^3	9.53×10^3
Sandell's sensitivity ($\mu g/cm^2$ /0.001 absorbance unit)	0.038	0.023	0.013	0.021
Regression equation (mx + b) = y				
Slope = m	0.016	0.0375	0.130	0.035
Intercept = b	- 0.050	0.0500	0.030	0.010
Correlation coefficient = r	0.999	0.9990	0.999	0.999

2.8 REACTION WITH 1-FLUORO- 2,4 DINTROBENZENE

The published literature did not reveal any reaction using 1-fluoro 2,4 dintro benzene. Attempts were made to investigate any possibility of such a reaction.

1) REAGENTS

1 0.10% m/v solution of pindolol in eathanol

2 4.80% v/v solution of 1-fluoro 2,4 dinitro benzene in eathanol (DNFB).

3 Sodium bicarbonate/carbonate buffer containing 4.2% sodium bicarbonate and 5.3% sodium carbonate.

4 0.80% w/v solution of NaOH.

5 Benzene.

6 1.00% v/v DNFB in acetone and 2.5% w/v borax in water mixed immediately in the proportion of 1:9 before experiment.

7 5.00% v/v Hcl in 1,4 dioxane.

8 0.10% m/v solution of nadolol, Timolol maleate and sotalol hydrochloride in water.

II) EXPERIMENTAL PROCEDURE (FOR PINDOLOL)

0.5 ml of fresh DNFB No 6 reagent was added to seperate 50 ml volumetiric flasks containing 2 ml of standard solution of pindolol. 1 ml of buffer solution was added and contents were heated at 60 C for 10 minutes, there after 0.3 ml of 0.8% NaOH solution was added to this mixture and heating was continued for another 10 minutes. The mixture was then cooled and extracted with 20 ml benzene. The absorbance of the benzene layer was measured at 415 nm.

III) EXPERIMENTAL PROCEDURE FOR TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE

1 ml of DNFB reagent no 6 was added to 10 ml of volumetric flasks containing 1 ml of either drug solution. Heated at 75-80° C for 30 minutes, cooled to ambient temperature and 4 ml of Hcl dioxane reagent was added. Samples were diluted to mark with distilled water. The absorbance was measured at 370 nm for nadolol and timolol maleate, 400 nm for sotalol hydrochloride.

IV) RESULT AND DISCUSSION

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. The method was optimised and 0.5 ml of DNFB reagent was optimum for the reaction. The heating time of 10 minutes was found to be suitable before and after adding 0.3 ml 0.8% NaOH solution is sufficient for maximmum color development in case of pindolol and benzene was found to be a suitable solvent. Maximum absorbance was observed at 415 nm (Figure 2.13). The market formulations were analysed with this method and results were compared with B.P. method (Table 2.29). The calibration curve was found to be rectiliner in the range of 0.1 µg/ml - 20 µg/ml (Figure 2.14). The method was found to be sensitive and precise.

For nadolol, timolol maleate and sotalol hydrochloride 1 ml of DNFB reagent prepared by adding borex was suitable for the reaction. 30 minutes heating time was necessary for maximum

colour development and 4 ml of hydrochloric acid dioxane reagent was required for stable colour formation. Maximum absorbance was observed for timolol maleate and nadolol at 370 nm (Figure 2.15) and for sotalol hydrochloride at 400 nm (Figure 2.15). The colour was found to be stable for more than half an hour. The market formulations of these drugs were analysed by this method and results were compared with official method for nadolol (Table 2.31) and timolol maleate (Table 2.30) and for sotaolol hydrochloride (Table 2.32) results are compared with published method. The calibration curve was found to be reactiliner in the rage of 0.8 - 18 µg/ml, 0.2 - 34 µg/ml, 0.6 - 24 µg/ml (Figure 2.16) respectively for timolol maleate, nadolol and sotalol hydrochloride. The results were summarised in table 2.33.

Table 2.29

RESULTS OF ESTIMATION OF PINDOLOL BY DNFB METHOD

Sample	DNFB method		Officeial method[3]	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	99.23	±0.056	99.82	±0.12
Tablet 1	98.99	±0.053	100.10	±0.13
Tablet 2	99.63	±0.055	100.90	±0.15

Table 2.30

RESULTS OF ESTIMATION OF TIMOLOL MALEATE BY DNFB METHOD

Sample	DNFB method		Officeial method[3]	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	99.79	±0.046	99.63	±0.25
Tablet 1	100.04	±0.051	99.10	±0.27
Tablet 2	99.86	±0.049	99.32	±0.26

Table 2.31

RESULTS OF ESTIMATION OF NADOLOL BY DNFB METHOD

Sample	DNFB method		Officeial method[3]	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	98.99	±0.076	99.83	±0.33
Tablet 1	100.03	±0.073	99.30	±0.34
Tablet 2	99.75	±0.075	99.86	±0.33

Table 2.32

RESULTS OF ESTIMATION OF SOTOLOL HYDROCHLORIDE BY DNFB METHOD

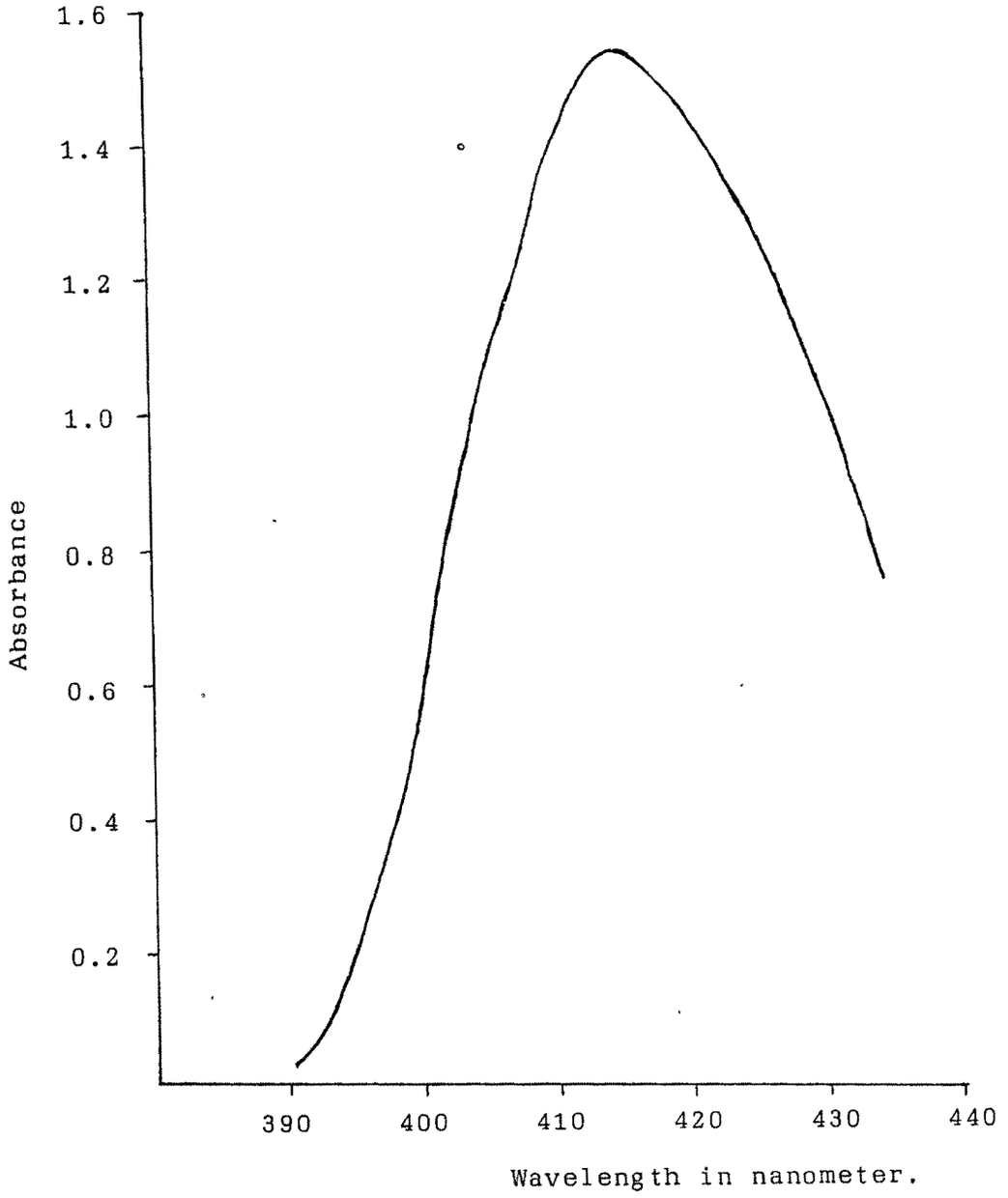
Sample	DNFB method		Reported method[66]	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	99.99	±0.025	99.75	±0.52
Tablet 1	100.12	±0.024	99.60	±0.51
Tablet 2	100.01	±0.025	100.00	±0.50

Table 2.33

OPTICAL CHARACTERISTIC, PRECISION AND ACCURACEY OF THE PROPOSED METHOD FOR PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE

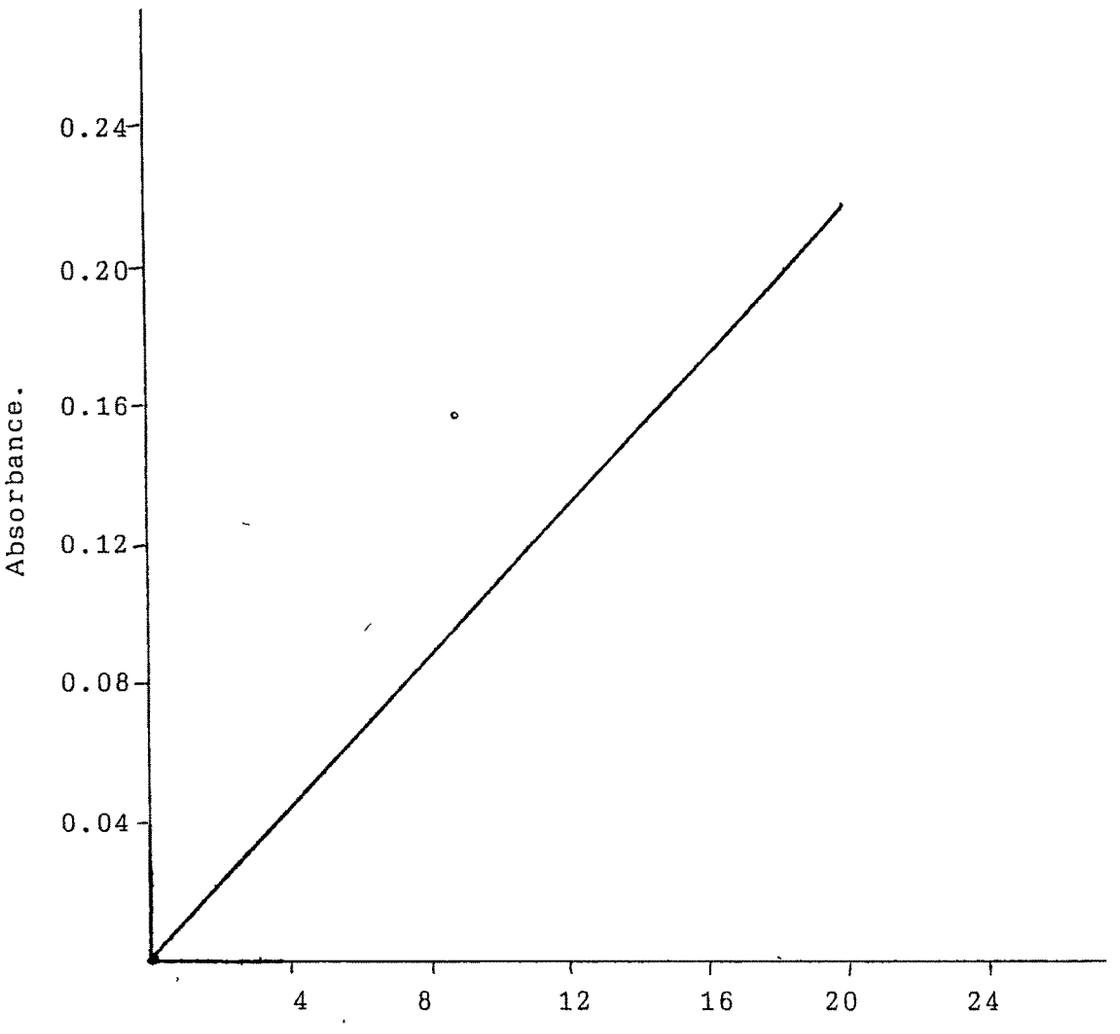
Data	Result			
	Pindolol	Timolol maleate	Nadolol	Sotalol hydrochloride
λmax (nm)	415	370	370	400
Calibration curve limit(µg/ml)	0.1-20	0.8-48	0.2-34	0.6-24
Molar absorptiovity ($l.mole^{-1}.cm^{-1}$)	2.98×10^3	12.54×10^3	7.73×10^3	22.60×10^3
Sandell's sensitivity ($\mu g/cm^2$ /0.001 absorbance unit)	0.017	0.013	0.032	0.012
Regression equation (mx + b) = y				
Slope = m	0.012	0.029	0.025	0.083
Intercept = b	0.0	0.0	0.0	0.0
Correlation coefficient = r	0.999	0.999	0.999	0.999

Fig. 2.13



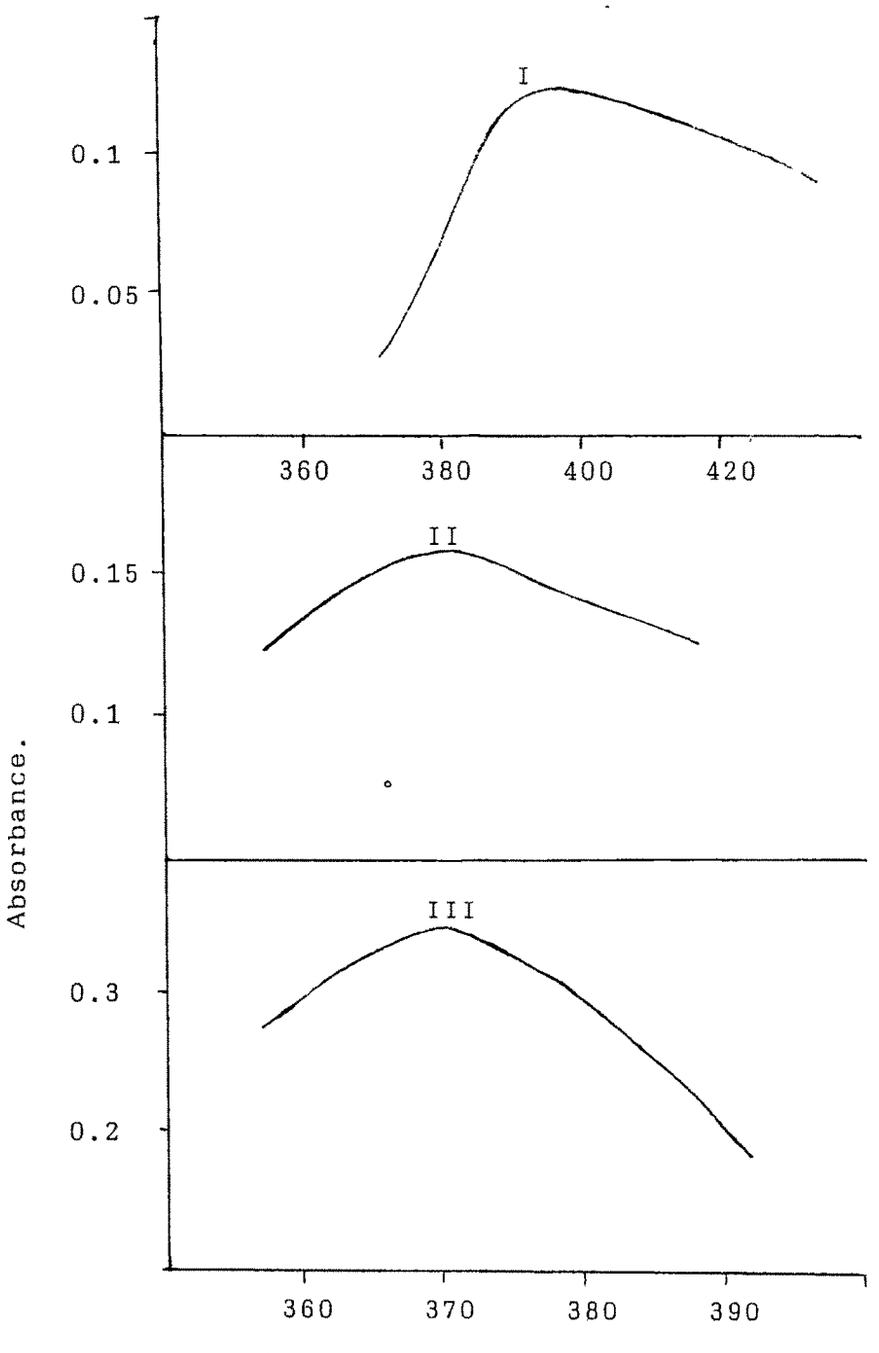
Absorption spectrum of Pindolol with DNFB.

Fig. 2.14



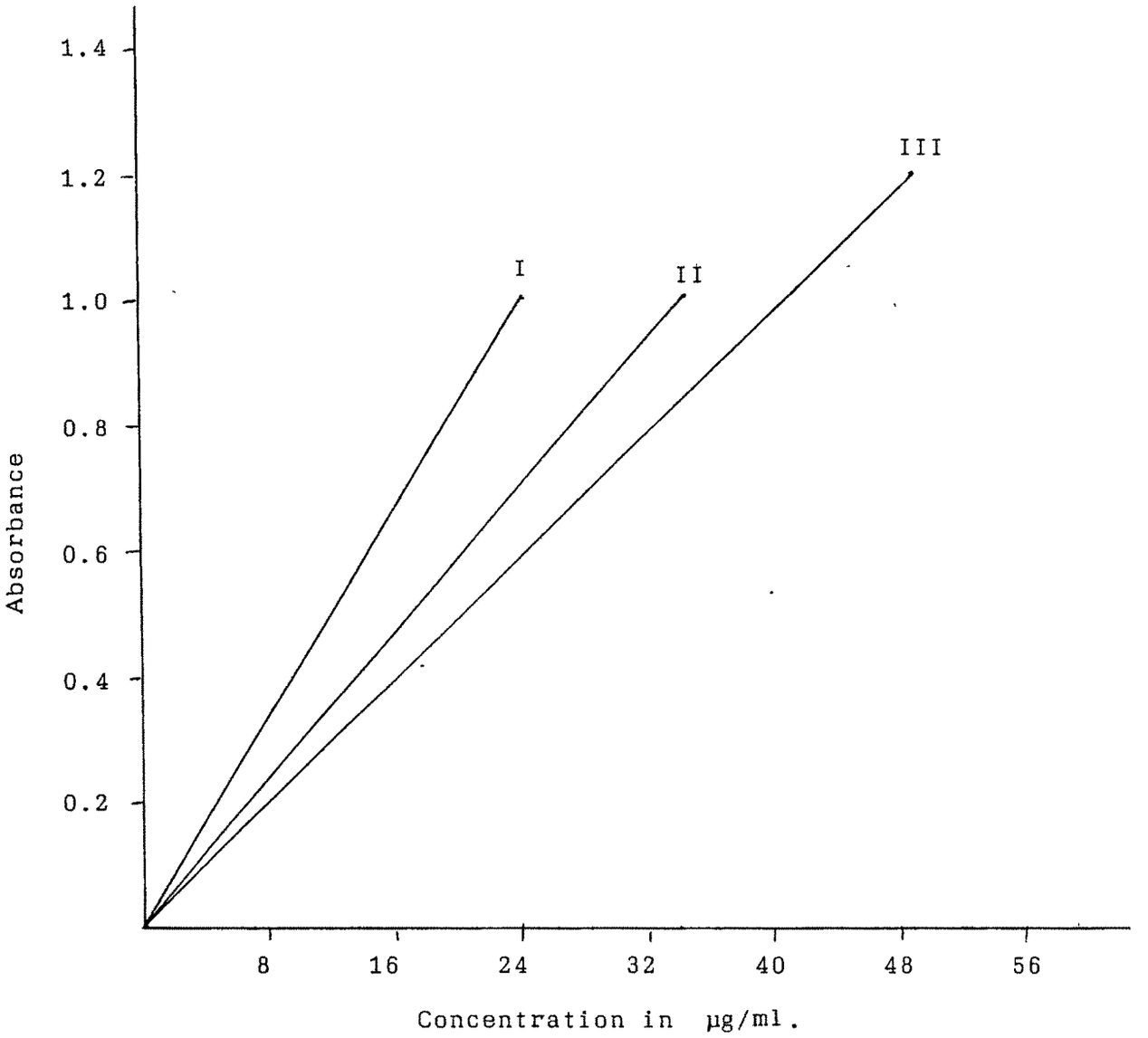
Calibration curve of Pindolol with 1,Fluro 2-4 dinitro benzene.

Fig. 2.15



Absorption spectrum of (I) Sotalol Hydrochloride (II) Timol Maleate (III) Nadolol with DNFB.

Fig. 2.16



Calibration curve of (I) Sotalol Hydrochloride (II) Nadolol (III) Timolol Maleate with 1,Fluro 2-4 dinitro benzene.

2.9 REACTION WITH F-C REAGENT

The published literature reveled one such method of analysis for the estimation of pindolol while the literature did not reval any information regarding rest of the drug viz. timolol maleate nadolol and sotalol hydrochloride for such a reaction. Attempts were made to investigate any such reaction for these drugs.

All spectral measurement were made on "Hitachi 2000" spectrophotometer.

I) REAGENTS

1 0.10% m/v solution of timolol maletate in water.

2 0.10% m/v solution of nadolol in water.

3 0.10% m/v solution of sotalol hydrchlorid in water.(SH)

4 5.00% w/v solution of sodium carbonate in water.

5 Folin - ciocalteu reagent (F-C reagent) dilute in the ratio 1:3 with distilled water [8].

II) EXPERIMENTAL PROCEDURE

8 ml of 5% sodium carbonate solution was added to separate tubes containing 1 ml of drug sample. The maxiture was thoroughly shaken and F-C reagent was added to each. The solutions were kept at ambient tempeture for 30 minutes. The contents were diluted with distilled water and absorbance was measured against the reagent blank.

III) RESULT AND DISCUSSION

Only sotalol hydrochloride reacted with F-C reagent and gave blue colour. While timolol maleate and nadolol gave faint bluo colour. A sensitive colorimetric method was developed for the estimation of sotalol hydrchloride as described below.

2.9.1 REACTION OF SOTALOL HYDROCHLORIDE WITH F-C REAGENT

I) REAGENT

1 No 3, 4 and 5 given in 2.9.

II) PREPRATION OF STANDARED CURVE

Aliquot of standard solution of SH was transfered into 25 ml volumetric flasks and 5 ml of 5% sodium carbonate solution was added to the flasks, while shaking 2 ml of F-C reagent was added to the flask. The mixture was kept for 30 minutes. The solutions were made upto mark with distilled water the absorbance was measured at 725 nm against a reagent blank.

III) DETERMATION OF OPTIMUM VOLUME OF 5% SODIUM CARBONATE SOLUTION

To 25 ml volumetric flasks containing 1 ml of standared SH solution in each different volumes of 5% sodium carbonate solution (6 ml, 7 ml, 8 ml, 9 ml, 10 ml) was added and same procedure as described in standared curve was followed and absorbance was measured agianst reagent blank.

IV) DETERMATION OF OPTIMUM VOLUME OF F-C REAGENT

To 25 ml volumetric flasks containing 1 ml of standared SH solution and 8 ml of 5% sodium carbonate solution different volumes of F-C reagent (0.5 ml, 1 ml, 2 ml, 3 ml, 4 ml) were added and same procedure as described in standared curve was followed and absorbance was measured against reagent blank.

V) DETERMATION OF REACTION TIME.

To 25 ml volumetric flasks containing 1 ml of standared SH solution and 8 ml of 5% sodium carbonate solution and while shaking 2 ml of F-C reagent was added to each flask and the solutions were allowed to stand for 15 minutes, 30 minutes, 45

minutes and 60 minutes. The same procedure was fallowed as in standared curve.

VI) ESTIMATION IN TABLETS

A quantity of the mixed content of 20 tablets eqivalent to 10 mg of SH was dissolved in distilled water in 50 ml calibrated flask and made up to the mark with distilled water and then filtered. Aliquots of this solution were taken, the colour developed and measured as described earlier. The results obtained by the proposed method was compared with the reported method.[66]

VII) RESULT AND DISCUSSION

The absorption spectrum of SH with F-C reagent has been shown in figure 2.17. It shows maximum absorbance at 725 nm. 8 ml of 5% sodium carbonate solution was sufficient for maintaining the optimum condition (Table 2.34). 2 ml solution of F-C reagent was necessary for maximum colour development (Table 2.35). The order of the addition of reagents played an emportant role. For maximum sensitivity, addition of sodium carbonate should precede that of F-C reagent. The colour obtained with the drug was found to be stable for one hour. The calibration curve was found to be reactiliner in the rage of 0.8 - 40 µg/ml (figure 2.18). None of the usual excipients employed in the formulation of dosage forms intefered in the proposed method. The tablets of SH available from the market were analysed by this method and results were compared with the reported method[66] (Table 2.36).

Molar absorptivity ($l.mole^{-1}.cm^{-1}$) and sandell's sensitivity ($\mu g/cm^2$ /0.001 absorbance) were found to be 1.99×10^4, 0.016 respectively. The method was checked for precision by repeating the experiments nine times with same quentity of the drug. The SD

Fig. 2.17

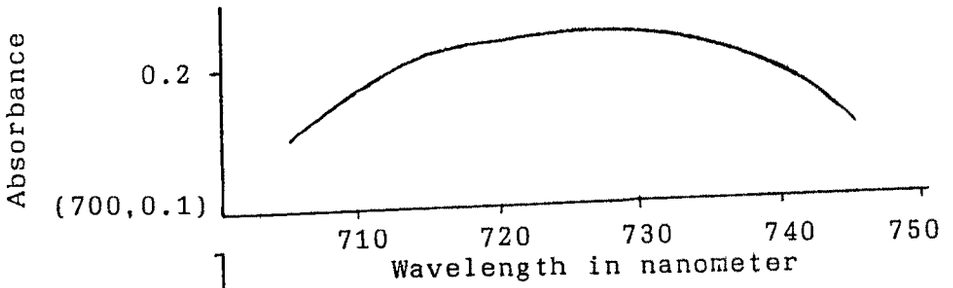


Fig. 2.18

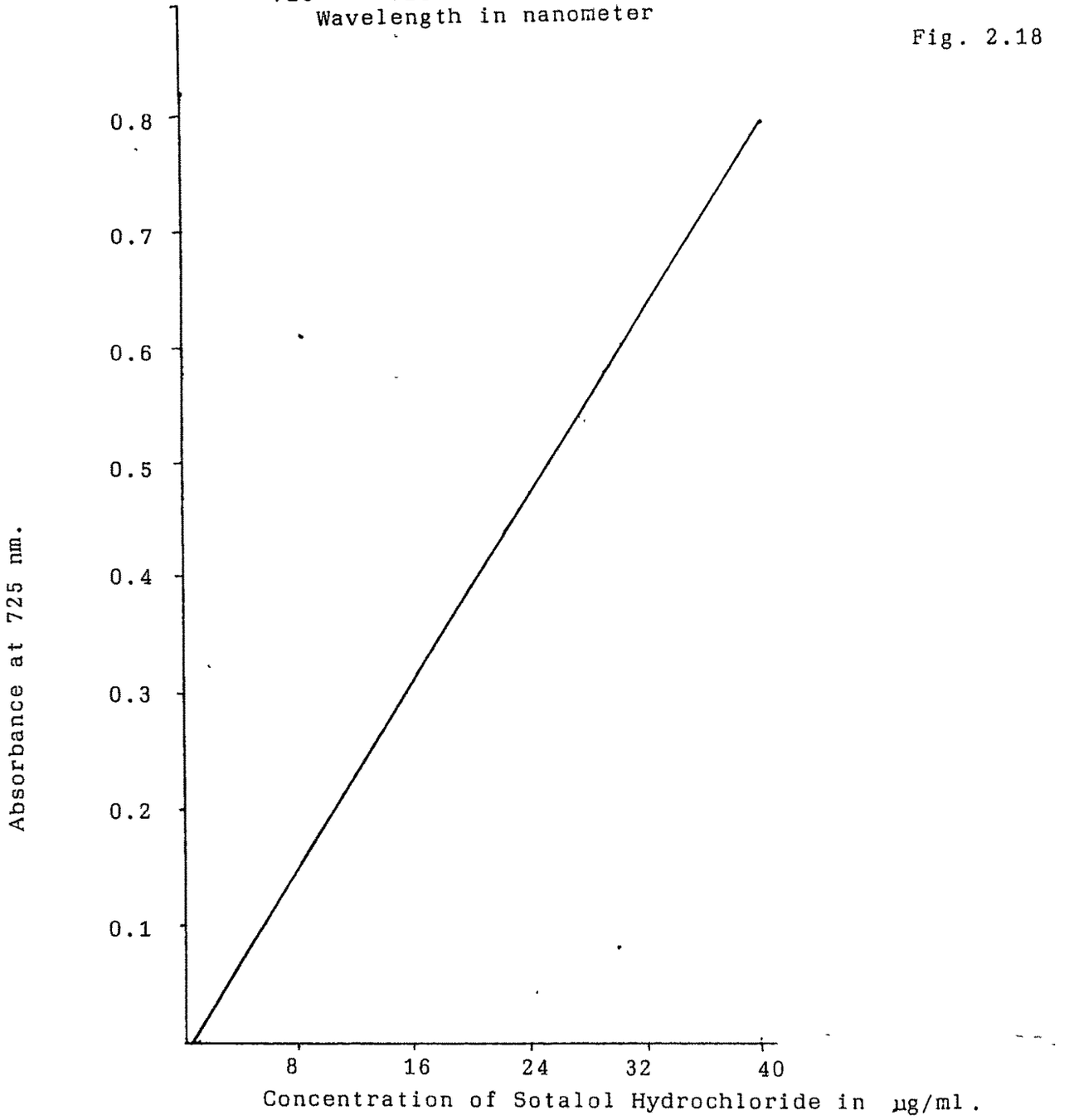


Fig. 2.17 - Absorbance spectrum of Sotalol Hydrochloride with F-C reagent.

Fig. 2.18 - Calibration curve of complex of Sotalol Hydrochloride with F-C reagent.

and % RSD were found to be ±0.0340 and ±0.430 respectively. The precision of the method was ensured by taking the observations with replicate samples. The coefficient of variation was found to be 0.999. The proposed method was found to be simple, rapid, precise and suitable for routine analysis of SH in bulk and dosage forms.

Table 2.34

DETERMATION OF OPTIMUM VOLUME 5% SODIUM CARBONATE SOLUTION

Volume in ml	Absorbance at 725 nm
6	0.351
7	0.422
8	0.464
9	0.443
10	0.422

Table 2.35

DETERMATION OF OPTIMUM VOLUME OF F-C REAGENT

Volume of F-C reagent in ml	Absorbance at 725 nm
0.5	0.261
1.0	0.392
2.0	0.464
3.0	0.392
4.0	0.353

Table 2.36

RESULTS OF DETERMINATION OF SH BY F-C METHOD

66

Sample	Proposed method		Reported method	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	99.83	±0.035	99.75	±0.52

Tablet 1	99.99	±0.034	99.60	±0.51
Tablet 2	100.03	±0.034	100.00	±0.50

2.10 REACTION WITH ACETYL ACETONE REAGENT

Published literature did not reveal any reaction with acetyl acetone reagent and there fore attempts were made to find out any such reaction.

I) REAGENTS

1 0.10% m/v solution of pindolol in 0.1N hydrochloric acid.

2 0.10% m/v solution of nadolol in water.

3 0.10% m/v solution of timolol maleate in water.

4 0.10% m/v solution of sotalol hydrochloride in water.

5 0.01N sodium periodate in water.

6 Acetyl acetone reagent : 30 gm of ammonium acetate and 1 ml acetyl acetone in water diluted to 100 ml with water.

II) EXPERIMENTAL PROCEDURE

1 ml of sodium periodate solution was added to separate 25 ml volumetric flasks containing 1ml of drug solution. The flasks were heated on boiling water bath for 15 minutes. After cooling 5 ml of acetyl acetone reagent was added. The contents were again heated for 10 minutes, cooled and volume was made up with water. The absorbance was measured against the reagent blank.

III) DETERMINATION OF OPTIMUM VOLUME OF SODIUM PERIODATE SOLUTION

To separate flask containing 1 ml of drug solution in each different volumes of sodium periodate solution (0.5 ml, 1ml, 2ml,3 ml) was added and the procedure described earlier was followed.

IV) DETERMINATION OF OPTIMUM VOLUME OF ACETYL ACTONE REAGENT

To separate 25 ml volumetric flasks containing 1 ml of

respective drug solution and 1 ml of sodium periodate solution, different volumes of acetyl acetone reagent were added (3 ml, 4 ml 5 ml, 6 ml, 7 ml and 8 ml) and the procedrue described earlier was followed.

V) DETERMATION OF HEATING TIME

The reaction mixtures before and after addition of acetyl acetone reagent were heated for different time intervals and optimum heating time was determined and same procedure followed as described earlier.

VI) ESTIMATION IN TABLETS

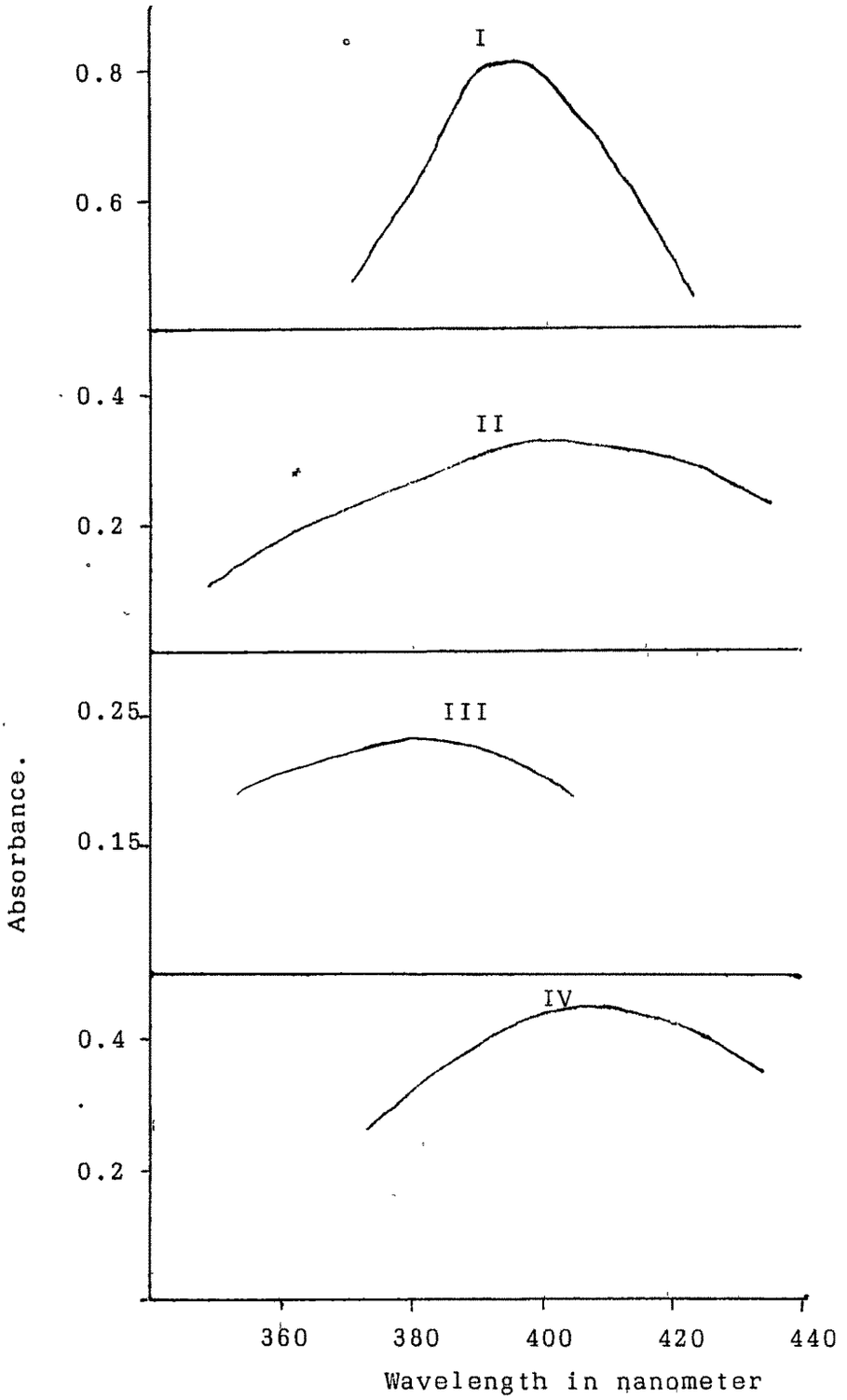
A quantity of the mixed contents of 20 tablets equivalent to 10 mg of pindolol was shaken with 25 ml of 0.1N Hcl while that of 10 mg of timolol maleate, nadolol and sotalol hydrochloride was shaken with distilled water and the solutions were filtered and volume was made up to 50 ml. Aliquots of these solutions were analysed in the same manner as described earlier.

VII) RESULT AND DISCUSSION

The absorption spectra of all the four drugs with acetyl acetone reagent was indicated 380 nm, 412 nm, 412 nm and 400 nm λ max for pindolol, nadolol, timolol maleate and for sotalol hydrchloride respectively(figure 2.19). The 15 minutes heating time before addition of acetyl acetone reagent and 10 minutes heating time after addition of acetyl acetone reagent was found to be sufficient for maximum coloure development. 1 ml of sodium periodate solution and 5 ml of acetyl acetone reagent was necessary for the colour development. The colour was found to be stable for more than two hours.

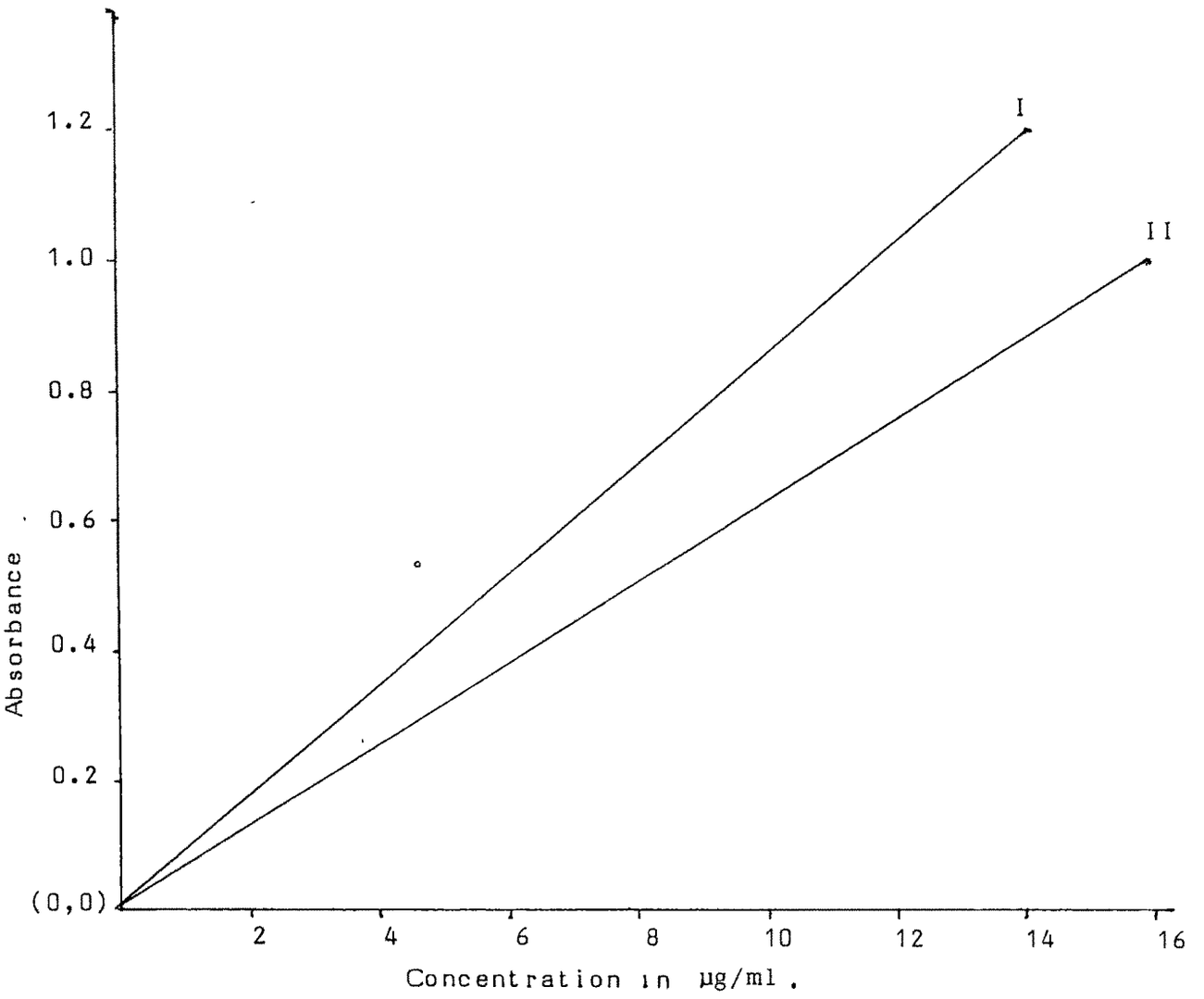
The calibration curve was found to be rectiliner in the range of

Fig. 2.19



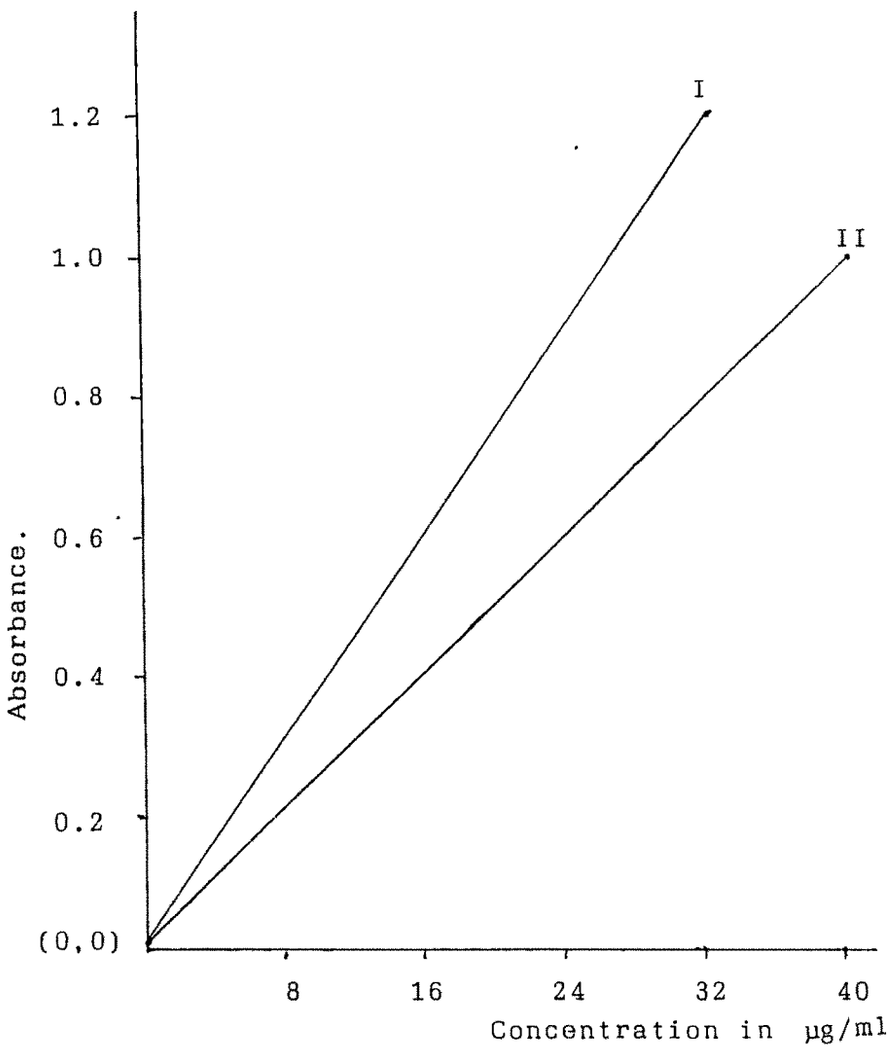
Absorption spectrum of (I) Sotalol Hydrochloride (II) Nadolol (III) Pindolol (IV) Timolol Maleate with acetyl acetone reagent.

Fig. 2.20



Calibration curve of (I) Pindolol (II) Nadolol with acetyl acetone reagent.

Fig. 2.21



Calibration curve of (I) Timolol Maleate (II) Sotalol Hydrochloride with acetyl acetone reagent.

1-14 µg/ml, 4-32 µg/ml, 2-16 µg/ml and 8-40 µg/ml for pindolol (figure 2.20), timolol maleate (figure 2.21), nadolol (figure 2.20) and for sotalol hydrochloride (figure 2.21) respectively. The method was applied for the marketed formulations of each drug and compared with official method for pindolol, timolol maleate and nadolol and that of reported method for sotalol hydrochloride. The results were comparable and the method was found to be sensitive and precise (Table 2.41).

Table 2.37

RESULT OF DETERMINATION OF PINDOLOL BY ACETYL ACTONE REAGENT

Sample	Proposed method		Official method[3]	
	Assay	Std.dev	Assay	STd.dev
Bulk powder	99.76	±0.043	99.82	±0.12
Tablet 1	99.31	±0.042	100.10	±0.13
Tablet 2	100.09	±0.043	100.90	±0.14

Table 2.38

RESULTS OF DETERMINATION OF TIMOLOL MALEATE BY ACETYL ACTONE REAGENT

Sample	Proposed method		Official method[3]	
	Assay	Std.dev	Assay	STd.dev
Bulk powder	99.73	±0.032	99.63	±0.25
Tablet 1	99.88	±0.034	99.10	±0.27
Tablet 2	99.96	±0.033	99.32	±0.26

Table 2.39

RESULTS OF DETERMINATION OF NADOLOL BY ACETYL ACTONE REAGENT

Sample	Proposed method		Official method[3]	
	Assay	Std.dev	Assay	STd.dev
Bulk powder	100.03	±0.061	99.83	±0.33

Tablet 1	100.00	±0.062	99.30	±0.34
Tablet 2	99.87	±0.063	99.86	±0.33

Table 2.40

RESULTS OF DETERMINATION OF SOTALOL HYDROCHLORIDE BY ACETYL ACTONE REAGENT

Sample	Proposed method		Reported method [66]	
	Assay	Std.dev	Assay	STd.dev
Bulk powder	99.98	±0.039	99.75	±0.52
Tablet 1	99.12	±0.038	99.60	±0.51
Tablet 2	99.32	±0.032	100.00	±0.50

2.11 COMPLEXATION WITH DIFFERENT ACIDIC AND BASIC DYES

The published literature revealed one analytical method each for the determination of timolol maleate and nadolol by forming ion pair in aqueous solution with bromothymol blue and bromophenol blue respectivley, extracting this ion pair into an orgainc solvent and determining the concentration of the extracted ion pair spectrophotometrically. The pH of the aqueous phase was critical for the success of these methods. The dye must be present in its ionic form so that it can form the ion pair with drug sample and excess of reagent will remain in aqueous solution unextracted by the solvent. This type of reaction was explored in depth by attempting the reaction of these 4 beta blockers with 13 different acidic and 5 different basic dyes in a bid to explore a better assay method with the necessary attributes.

a. PRELIMINARY EXPERIMENT

I) REAGENTS

1 0.10% m/v solution of pindolol, timolol maleate nadolol and

Table 2.41

OPTICAL CHARACTERISTIC, PRECISION AND ACCURACEY OF THE PROPOSED METHOD FOR PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE

Data	Result			
	Pindolol	Timolol maleate	Nadolol	Sotalol hydrochloride
λmax (nm)	380	412	412	400
Calibration curve limit(µg/ml)	1-14	4-32	0.2-16	8-40
Molar absorptiovity (1.mole^{-1}.cm^{-1})	31.35×10^3	16.43×10^3	19.18×10^3	6.81×10^3
Sandell's sensitivity (µg/cm^2 /0.001 absorbance unit)	0.014	0.022	0.021	0.016
Regression equation (mx + b) = y				
Slope = m	0.086	0.038	0.062	0.025
Intercept = b	- 0.01	- 0.0235	- 0.0125	- 0.02
Correlation coefficient = r	0.999	0.999	0.999	0.999

sotalol hydrochloride in methanol.

2 0.10% m/v solution of bromocresol green in methanol.

3 0.10% m/v solution of bromothylmol blue in methanol.

4 0.10% m/v solution of claton blue in water.

5 0.10% m/v solution of eriochrome black T in water.

6 0.10% m/v solution of erythrosine in water.

7 0.10% m/v solution of fluorescein sodium in water.

8 0.10% m/v solution of methyl orange in water.

9 0.10% m/v solution of methyl red in methanol.

10 0.10% m/v solution of phenol red in methanol.

11 0.10% m/v solution of sunset yellow in water.

12 0.10% m/v solution of tartrazine in water.

13 0.10% m/v solution of tropaeolin OO in water.

14 0.10% m/v solution of thymol blue in methanol.

15 0.1M and 1 M hydrochloric acid.

16 Mc Ilvaine citrate-phosphate buffer pH 2.2, 3.0 - 8.0[59].

17 Methanol.

18 Solvent for extraction : amyl alcohol, benzene, chloroform, carbon tetrachloride, ethyl acetate , iso butanol, iso octanol and ether.

19 0.10% m/v solution of crystal violet in water.

20 0.10% m/v solution of malachite green in water.

21 0.10% m/v solution of methylene blue in water.

22 0.10% m/v solution of oracel blue B in methanol.

23 0.10% m/v solution of safranine in water.

24 0.10% m/v solution of napthol benzene in methanol.

II) EXPERIMENTAL PROCEDURE

To one of the test tubes of 5 separate sets of two testtubes

each was added 1 ml of respective drug solution where as 1 ml of methanol was added to the other test tube to serve as reagent blank. To these 5 sets were added one of the following solutions :- 1 ml of M hydrochloric acid, 1 ml of 0.1M hydrochloric acid. 1 ml of buffer pH 3.0, 1 ml of buffer pH 5.0 and 1 ml of pH 8 respectively. To each test tube was added 1 ml of respective dye solution and colour of sample and blank solution was compared both in cooled and after heating.

To 2 separate 25 ml of calibrated flasks were added 1 ml of respective drug solution and 1 ml of methanol (to serve as reagent blank) respectively. To each flask 2.5 ml of respective dye solution was added. Some quantity of methanol was needded to keep the reaction product in solution in case of bromo cresol green and bromo thymol blue and volume was made up with water. Aliquots of 2 ml sample mixture and reagent blank solution were transfered to 8 sets of 2 test tubes each and extraction was attempted after addition of 10 ml of respective organic solvent by shaking thoroughly. The color of the organic extract of sample solution relative to that of reagent blank was carefully noted.

III) RESULT AND DISCUSSION

Of the 13 acidic and 6 basic different dyes employed, claton yellow, fluorescein sodium methyl red, erythrosine and tropaeolin OO. did not react with drugs over a pH ragne of 1 - 8 as indicated by no difference between the appearance of the sample and reagent blank solution. The extraction into 8 different organic solvents was attempted. The there dyes namely phenol red, sunset yellow and tartrazine did react with the drug substances to form ionpairs as shown by the colour of organic

extract. However the difference between the sample and blank in aqueous methanolic solution was not discernible and colour of organic extract at 40 µg/ml was very light. The remaining 5 dyes viz. Bromocresol green, bromothymol blue, erichrome black T,methyl orange and thymol blue exhibited apparent difference between the sample and blank solutions from pH 1 to 5 indicating the possibility of ionpair reaction with the drugs in question. It was interesting to note that chloroform was the only solvent suitable for extraction of ionpairs of drugs with all the dyes found to react. The drug substances in question viz. Pindolol, timolol maleate, nadolol and sotalol hydrochloride behaved identically from the point of view of ionpair extraction. The colour of orgainc extract with suitable solvents at 40 µg/ml concentration was rather too light in case of thymol blue. Hence this dye was not studied further. The reaction with four remaining dyes viz. Bromocresol green, bromothymol blue, methyl orange and eriochrome black T were investigated further in abid to develop a sensitive analytical method.

Out of 5 different basic dyes tried 3 of them viz. methylene blue. oracet blue B and malachite green did not react with these drugs over a pH range of 1 – 10 as indicated by no differance between the appearance of respective sample and blank solutions and unsuitablity of extraction in to different organic solvents. Crystal violet and safranine were the basic dyes found to react with these drugs. It formed and an ionpair with these drugs at pH 1 – 3. The complex could be extracted in chloroform. This reaction was studied further with a view to develop an assay method for the estimation of these drugs.

IV) REACTION WITH BROMOTHYMOL BLUE AND BROMOCRESOL GREEN

I REAGENTS

1 0.10% m/v solution of pindolol, timolol maleate, nadolol and sotalol hydrochloride in methanol.

2 0.10% m/v solution of bromothymol blue in methanol.

3 0.10% m/v solution of bromocresol green in methanol.

4 0.10M and 1 M hydrochloric acid.

5 Mc - Ilvaine citrate - phosphate buffer pH 2.2, 3.0, 4.0, 5.0.

6 Chloroform, methanol benzene.

II) EXPERIMENTAL PROCEDURE

a. DETERMATION OF ABSORPTION SPECTRA IN DIFFERENT SOLVENTS

To 25 ml calibrated flasks each containing 1 ml of respective drug solution 2.5 ml of 1 M hydrochlorid acid, 2.5 ml of respective dye solution were added, the volume was made up with water. An aliquot of 10 ml was extracted with 10 ml of benzene/ chlorform and absorption spectrum of the organic extract was scanned against the extract of the reagent blank solution to determine the absorption maxima. The absorbance maxima for chloroform extract was 412 nm and for benzene extract was 405 nm. The absorbance of the chloform extract was measured at 412 nm and that of benzene extract at 405 nm upto 60 minutes to determine the stability of extinction.

b. DETERMINATION OF OPTIMUM pH

To one of the 2 calibrated flasks (25 ml) of 6 separate sets containing 2 flasks each 1 ml of drug solution was added and to the other 1 ml of methanol was added to serve as reagent blank. To each set was added one of the following in respective order. 2.5 ml of 1 M Hcl, 2.5 ml of 0.1 M Hcl. 10 ml of buffer pH 2.2, 10

ml of buffer pH 3.0, 10 ml of buffer pH 4.0 and 10 ml of buffer pH 5.0. To each flask were added 2.5 ml of dye solution and water to volume and mixed. An aliquot of 10 ml of extracted with 10 ml of benzene/chloroform and absorbance of the organic extract was measured at 405 nm/ 412 nm against the respective reagent blank. after centrifugation for clarification of the extract.

c. PREPARATION OF CALIBRATION CURVE

An aliquot of standared drug solution was transfered into 25 ml volumetric flask, 2.5 ml of 0.1M hydrochloric acid and 2.5 ml of dye solution were added and water to make up the volume. The colour was extracted with 10 ml benzene/chloroform and absorbance was measured at 405 nm/ 412 mnm against the reagent blank.

d. ESTIMATION IN TABLETS

A quantity of the mixed contents of 20 tablets equivalent to 10 mg of respective drug was shaken with 25 ml of methanol for 10 minutes. The solution was then filtered and aliquots of this solution after dilution were treated as described under calibration curve.

e. METHOD FOR OPTHALMIC SOLUTION (TIMOLOL MELEATE)

To the sample equivalent to 2.5 mg of timolol maleate 10 ml of buffer solution (pH 9.7)[34] was added and the solution was extracted with chloroform (2 X 10 ml portions). To the aliquots of this solution 2 ml of methanol was added and treated as described under calibration curve.

f. RESULT AND DISCUSSION

Preliminary experiment described earlier held high promise for the method with the discernible difference in the apperance of sample blank solution at an extreme acid pH (1.0) Table 2.42.

Pindolol nadolol and sotalol hydrochloride reacted with bromothymol blue while pindolol. timolol maleate and sotalol hydrochloride reacted with bromocresol green. Extraction with different organic solvents from aqueous methanolic solution indicated benzene and chloroform as the suitable extraction solvents for the ionpairs.

Direct scanning of ionpair in aqueous methanolic phase against the corrosponding blank from 340 nm - 520 nm held no promise what so ever. However chloroform and benzene extracts displayed well defined absorption peaks when scanned against the appropriate reagent blanks. Ionpairs of pindolol, timolol maleate and sotaolol hydrochloride with the bromocrosol green dye gave an absorption maxima at 412 nm (figure 2.22). Pindolol, nadolol and sotalol hydrochloride also gave absorption maxima at 417 nm with bromothymol blue dye in chloroform extract (figure 2.23).

Absorption of chloroform extract at 412 nm exhibited good colour stability for about one hour. Benzene extract all though gave stable colour, consiberable less absorbance then chlororform extract. The pH of optimum complexation was found to be 1.0 for all the three compounds as can be seen from the data presented in Table 2.42

Heating the reaction product in aqueous methanolic medium on steam bath for 2 minutes prior to extraction in benzene increased the absorbance only marginally which did not further increase with additional heating.

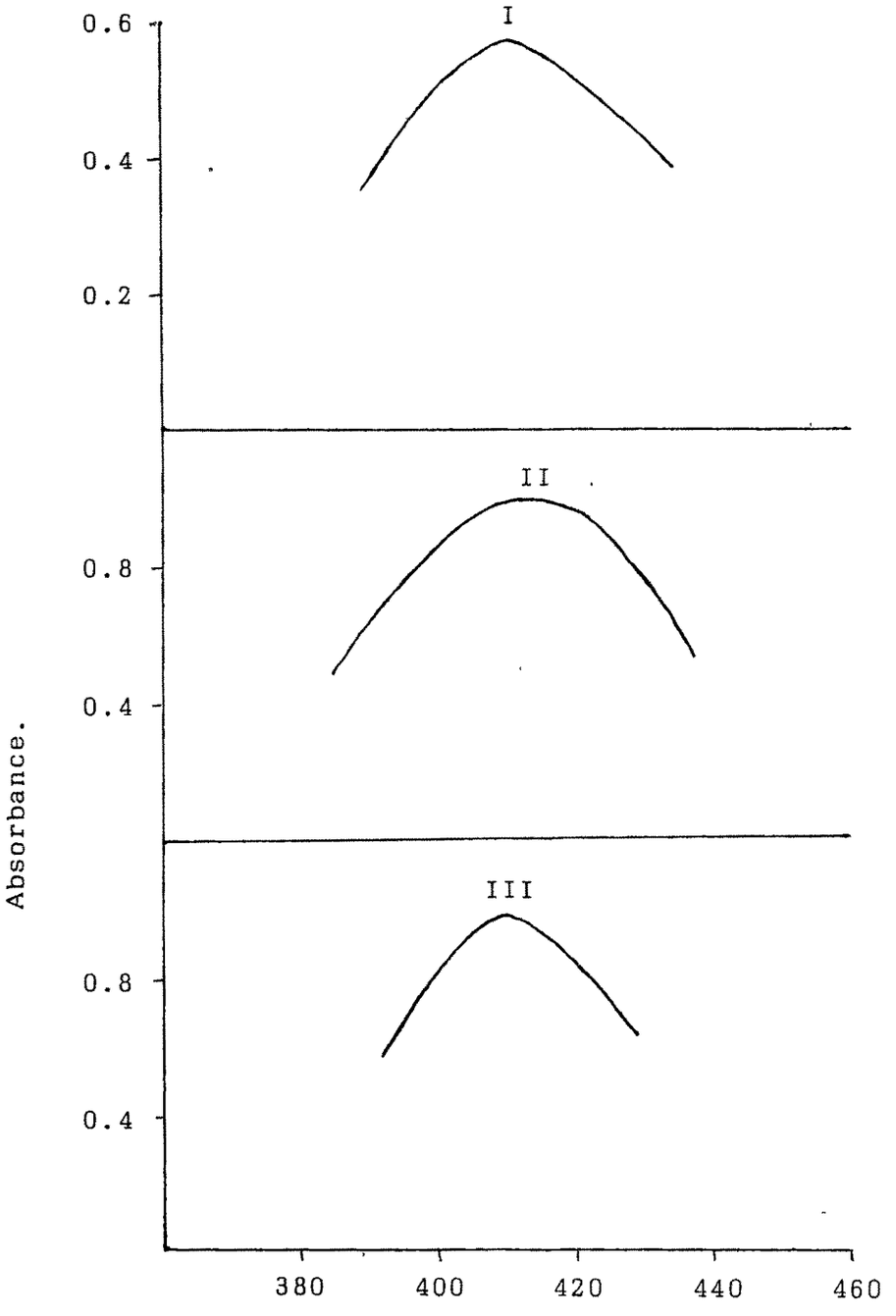
The calibration curve was rectilier between 4 - 56 µg/ml, 2 - 32 µg/ml, 2 - 40 µg/ml for pindolol, timolol maleate and sotalol hydrochloride with bromicrosol green (figure 2.24). While 4 - 64

Table 2.42

EFFECT OF pH ON SOLVENT (CHLOROFORM) EXTRACTION OF PINDOLOL, NADOLOL, AND SOTALOL HYDROCHLORIDE WITH BROMOTHYMOL BLUE AND PINDOLOL, TIMOLOL MALEATE AND SOTALOL HYDEROCHLORIDE WITH BROMOCRASOL GREEN

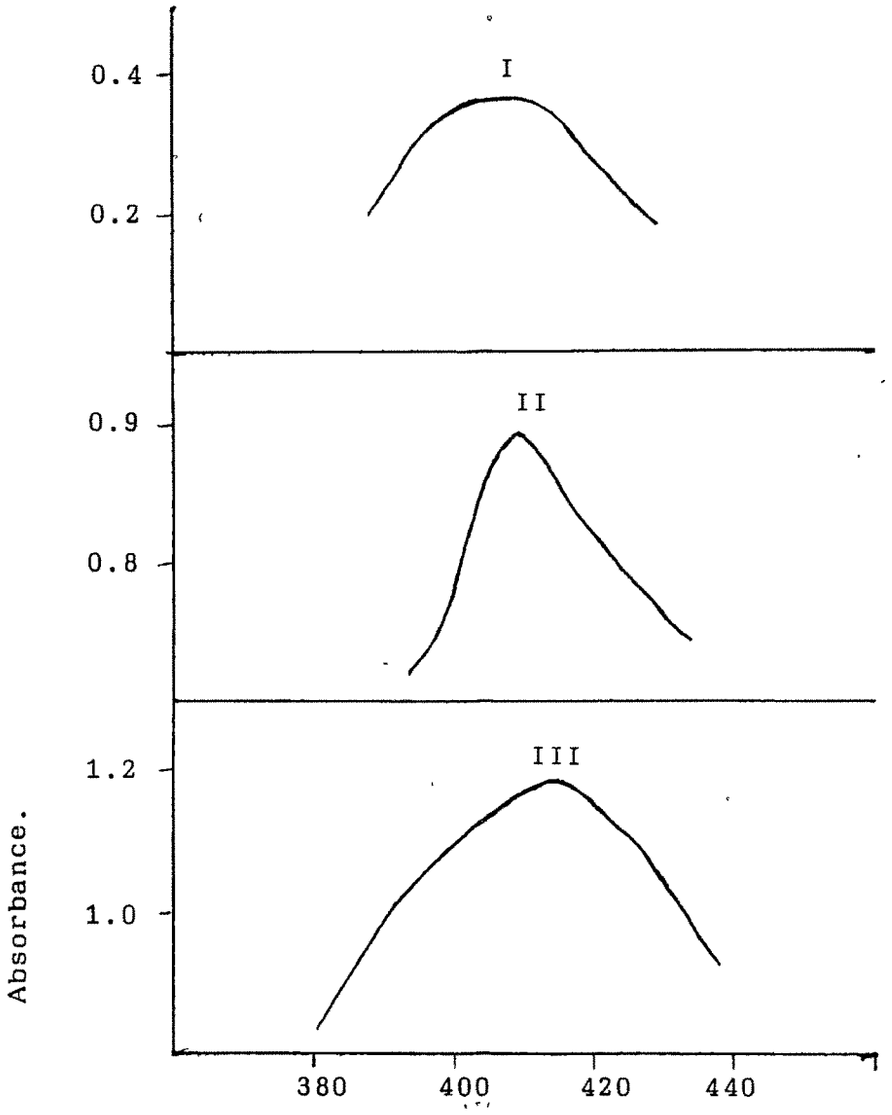
Medium	pH	Absorbance at 412 nm					
		with bromocrosol green			with bromothymol blue		
		pindolol	timolol maleate	sotalol hydrochloride	pindolol	nadolol	sotalol hydrochloride
0.1 M Hcl	1.0	0.702	0.708	0.875	0.725	0.780	0.805
0.01M Hcl	2.0	0.471	0.474	0.700	0.600	0.580	0.605
Buffer 1	2.2	0.320	0.520	0.610	0.430	0.410	0.325
Buffer 2	3.0	0.194	0.273	0.372	0.205	0.190	0.180

Fig. 2.22



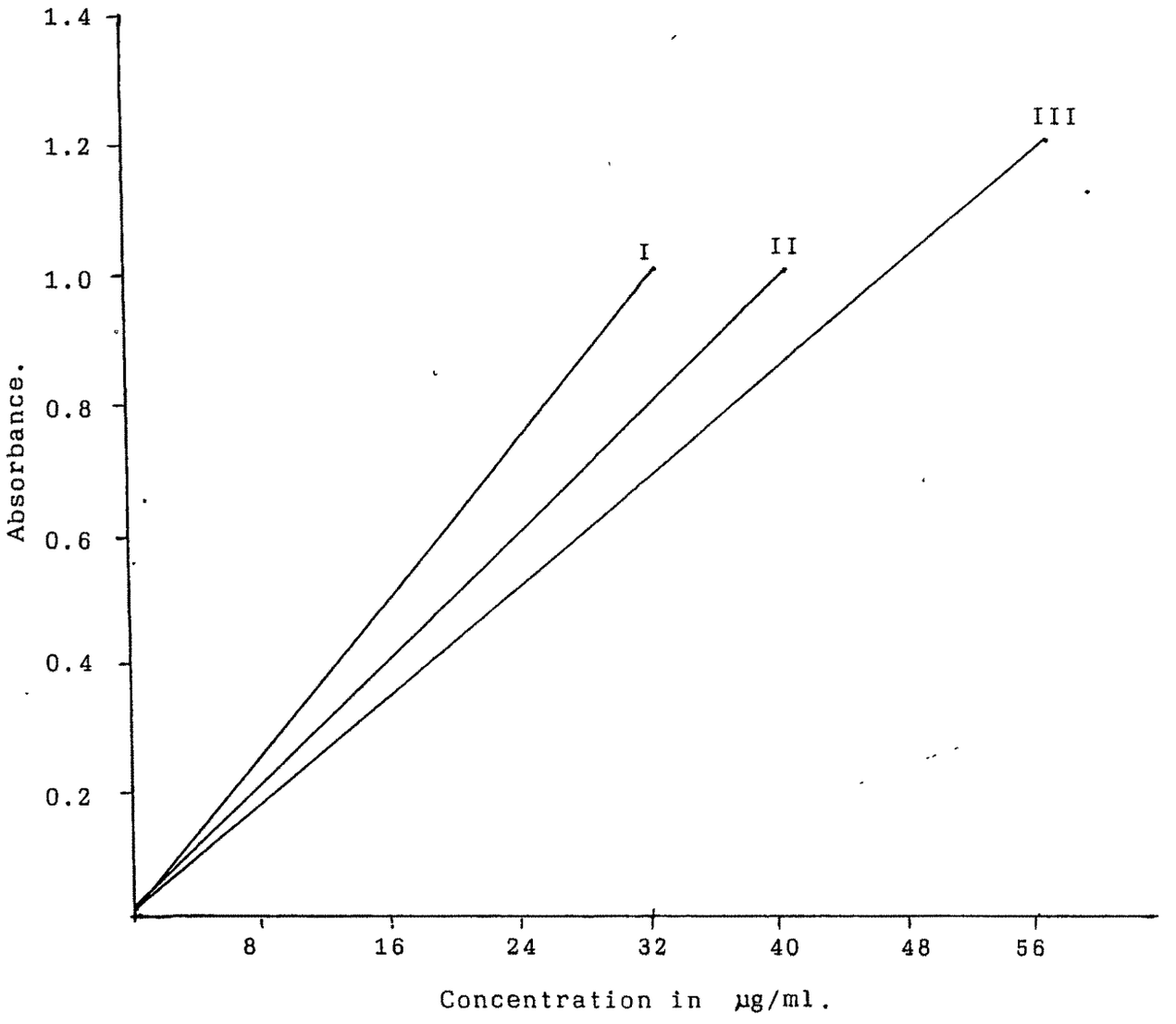
Absorption spectrum of ion pair of (I) Sotalol Hydrochloride (II) Timolol Maleate (III) Pindolol with bromo cresol green.

Fig. 2.23



Absorption spectrum of ion pair of (I) Timolol Maleate (II) Sotalol Hydrochloride (III) Nadolol with bromo - thymol blue.

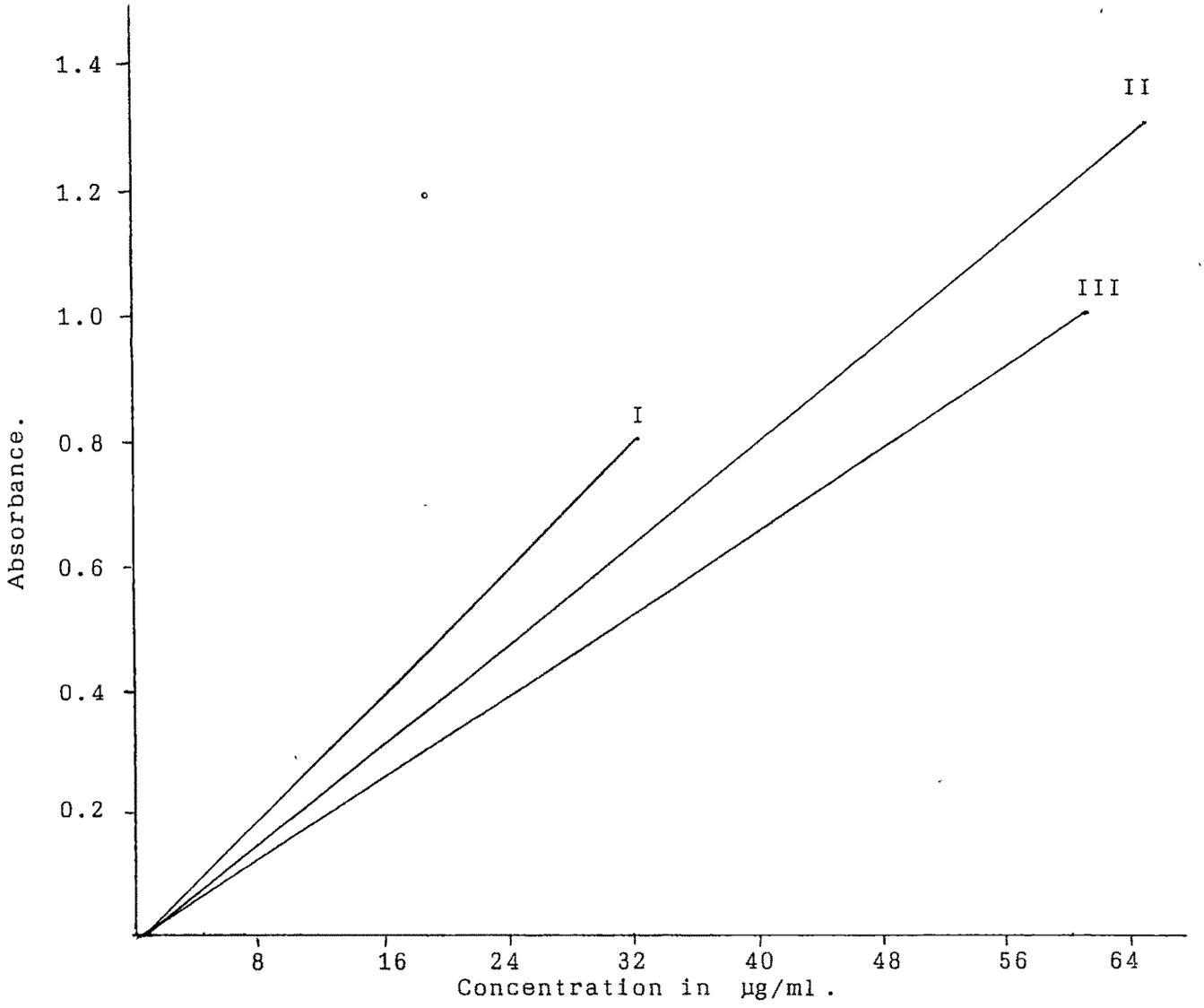
Fig. 2.24



Calibration curve of ion pair of (I) Timolol Maleate (II) Sotalol Hydrochloride (III) Pindolol with bromocresol green.

µg/ml, 2 - 32 µg/ml and 5 -- 60 µg/ml for pindolol, nadolol and sotalol hydrochloride with bromithymol blue (figure 2.25).

Fig. 2.25



Calibration curve of (I) Nadolol (II) Pindolol (III) Sotalol Hydrochloride with bromothymol blue.

The result of the estimation of marketed prepration of Pindolol, Nadolol and Sotalol hydrochloride with Bromothymol blue where as Pindolol, Timolol, Sotalol hydrochloride with Bromochrol green reported in Table 2.43 to 2.48.

Table 2.43

RESULTS OF ESTIMATION OF PINDOLOL BY BROMOCRESOL GREEN

Sample	Proposed method		Official method[3]	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	99.91	±0.099	99.82	±0.12
Tablet 1	99.84	±0.098	100.10	±0.13
Tablet 2	99.89	±0.097	100.90	±0.14

Table 2.44

RESULTS OF ESTIMATION OF TIMOLOL MALEATE BY BROMOCRESOL GREEN

Sample	Proposed method		Official method[3]	
	Assay	Std.dev	Assay	Std.dev
Bull. powder	99.94	±0.083	99.63	±0.25
Tablet 1	99.87	±0.082	99.10	±0.27
Tablet 2	99.99	±0.80	99.32	±0.26

Table 2.45

RESULTS OF ESTIMATION OF SOTALOL HYDROCHLORIDE BY BROMOCRESOL GREEN

Sample	Proposed method		Reported method[66]	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	99.86	±0.089	99.75	±0.52
Tablet 1	99.77	±0.090	99.60	±0.051
Tablet 2	99.95	±0.089	100.00	±0.50

Table 2.46
RESULTS OF ESTIMATION OF PINDOLOL BY BROMOTHYMOL BLUE

Sample	Proposed method		Official method[3]	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	99.83	±0.032	99.83	±0.12
Tablet 1	100.50	±0.039	100.09	±0.13
Tablet 2	100.05	±0.034	100.89	±0.14

Table 2.47
RESULTS OF ESTIMATION OF NADOLOL BY BROMOTHYMOL BLUE

Sample	Proposed method		Official method[3]	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	99.89	±0.062	99.82	±0.33
Tablet 1	99.73	±0.056	99.30	±0.34
Tablet 2	99.92	±0.063	99.86	±0.33

Table 2.48 RESULTS OF ESTIMATION OF SOTALOL HYDROCHLORIDE BY BROMOTHYMOL BLUE

Sample	Proposed method		Reported method[66]	
	Assay	Std.dev	Assay	Std.dev
Bulk powder	99.79	±0.50	99.75	±0.52
Tablet 1	99.86	±0.51	99.60	±0.51
Tablet 2	99.93	±0.52	100.00	±0.50

V) REACTION WITH ERIOCHROME BLACK T AND METHYL ORANGE

1. REAGENTS

1. 0.10% m/v solution of Eriochrome black T in water.
2. 0.10% m/v solution of methyl orange in water.
3. 0.10% m/v solution of Pindolol, Timolol maleate and sotalol hydrochloride in methanol.

4. Mc - Ilvaine citrate phosphate buffer pH 2.2, 3.0. 5.0, 8.0.

5. 0.1M and 1 M hydrochloric acid.

6. Choroform, benzene, methanol.

II. EXPERIMENTAL PROCEDURE

a. DETERMINATION OF SUITABLE EXTRACTION SOLVENTS :

Preliminary experiments indicated the possibility of selective extraction of the ionpairs of drug substances with the dye into 3 different organic solvents necessitating further experiments to screen out the best among them.

To 50 ml calibrated flasks 1.0 ml of drug solution, 20 ml of buffer pH 2.2. 2 ml of either dye solution, 14 ml of methanol were added and water to volume. An aliquot of 10 ml containing 20 ug/ml of drug substance was extracted with 10 ml each of benzene, chloroform and carbon tetrachloride and the absorbance of organic layer after centrifugation was measured at 470 nm. 520 nm and 540 nm respectively for Pindolol, Timolol maleate and sotalol hydrochloride with eriochrome black T and at 412 nm for Timolol maleate with methyal orange against the appropriate reagent blank prepared simaltameously to determine the intensity of absorbance and stability of the colour.

b. DETERMINATION OF OPTIMUM pH

An experiment similar to one designed in case of Bromocresol green and Bromothymol blue (sec. 2.11.1) was performed to determine the pH of optimum complexation. Extraction using 0.05% m/v drug solution. 0.10% m/v dye solution and 7 ml of extra quantity of methanol prior to volume making was necessary. The absorbance of chloroform extact was read as described in above paragraph.

c. DETERMINATION OF STOCHIOMETRY OF COMPLEX BY CONTINUOUS VARIATION PLOT

To a set of 9 separate 25 ml volumetric flasks 1-9 ml aliquots of 0.05 M drug solution and aliquots of 0.05 M erichrome black T / methyl orange solution in complimentary proportition were added to make 10 ml. To each flask was added 10 ml of buffer solution pH 2.2 and water to volume. An aliquot of 10 ml was extracted with 10 ml of chloroform and absorption was measured at 470 nm, 520 nm and 540 nm respectively for Pindolol, Timolol maleate and Sotalol hydrochloride with eriochrome black T and at 412 nm for Timolol maleate with methyl orange against the appropriate reagent blank prepared simultaniously.

d. PREPRATIN OF CALIBRATION CURVE

Aliquots of 0.050% m/v solution of respctive drug (0.125, 0.25, 0.5, 1.2, 3.4 and 5.0 ml) was added to a set of 8 separate 25 ml calibrated flasks and aliquots of methanol in complimentry proportion to make 8 ml. To each flask 10 ml of buffer solution of pH 2.2, 2.0 ml of erichromo black T / methyl orange and water was added to volume and mixed to yield 2.5 to 100 mg/ml concentration of drug. An aliquot of 10 ml was transfered to a 50 ml separator and extracted twice with 10 ml portion of chloroform. The yellow extract in case of Timolol Maleate with methyl orange and reddish coloured complex of Pindolol, Timolol Maleate and Sotalol hydrochloride with eriochrome black T were collected into 25 ml calibrated flasks and volume was made up with chloroform to yield 2.5 - 100 mg/ml concentration of the drug. The Absroption was measured as described in above paragraph against reagent blank extract. The absorbance of the complex was plotted against

concentration of respective drug in ug/ml.

e. ESITMATION IN TABLETS

A quentity of mixed contents of 20 tablets equivalent to 10 mg of respective drug sample was shaken with 25 ml methanol and contents were shaken for 10 minutes. Methanol was added to volume and mixed. The solution was then filletered and aliquots of this was transferred to a 25 ml calibrated flask and estimation was carried out following the procedure as described earliar under calibration curve.

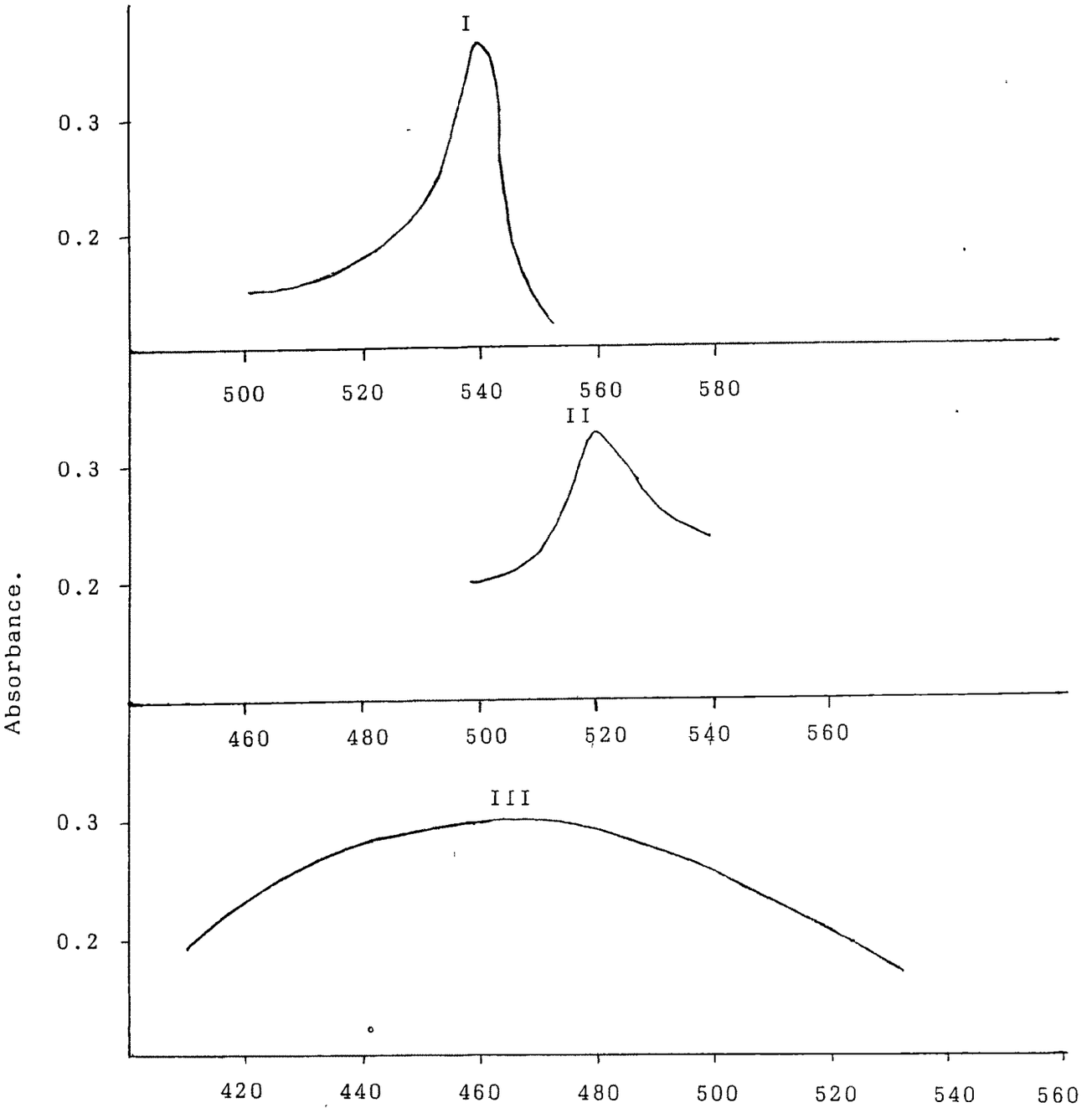
f. METHOD FOR OPTHAMIC SOLUTION (Timolol Maleate) :

To the sample equivalent to 5 mg of Timolol Maleate 10 ml of buffer solution (pH 9.7) was added and the mixture was extracted with chloroform (2 X 10 ml portion)[34] aliquots of this solution 2 ml of methanol was added and treated as described under calibration curve.

g. RESULT AND DISCUSSION

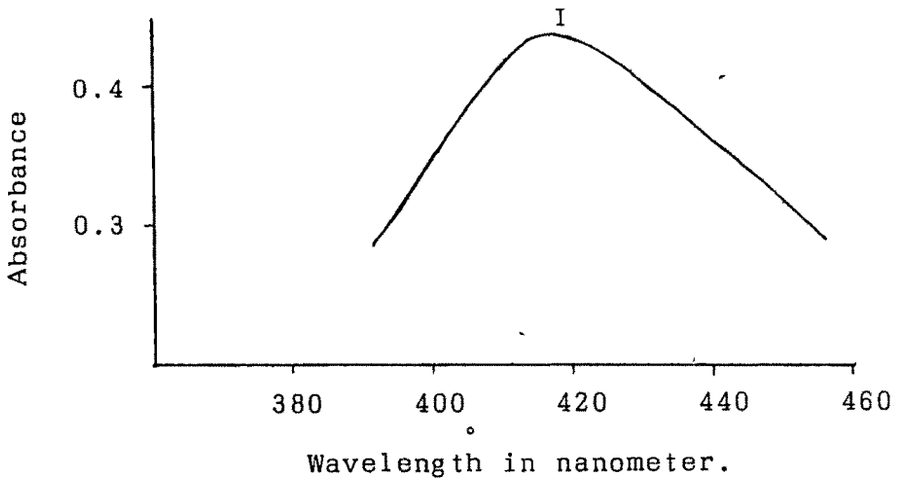
Preliminary experiments described earlier high very hig promise for the method with discernible difference in the appearance of sample and balnk solutions over a pH range of 1 - 5. Benzene, chloroform and carbon tetracloride were found to be suitable solvents for the selective extraction of the ionpair at pH 2.2. Experiment to determine the best extraction solvent revealed chloroform extract most suitable one with maximum stable colour development. The absorbance maxima was found to be 470 nm, 520 nm and 540 nm (figure 2.26) respectively for Pindolol, Timolol Maleate and Sotalol hydrochloride with eriochrome black T and at 412 nm for Timolol Maleate with methyl orange against the appropriate reagent blank prepared simultaneously (figure 2.27).

Fig. 2.26



Absorption spectrum of ion pair of (I) Sotalol Hydrochloride (II) Timolol Maleate (III) Pindolol with eriochrome black T.

Fig. 2.27



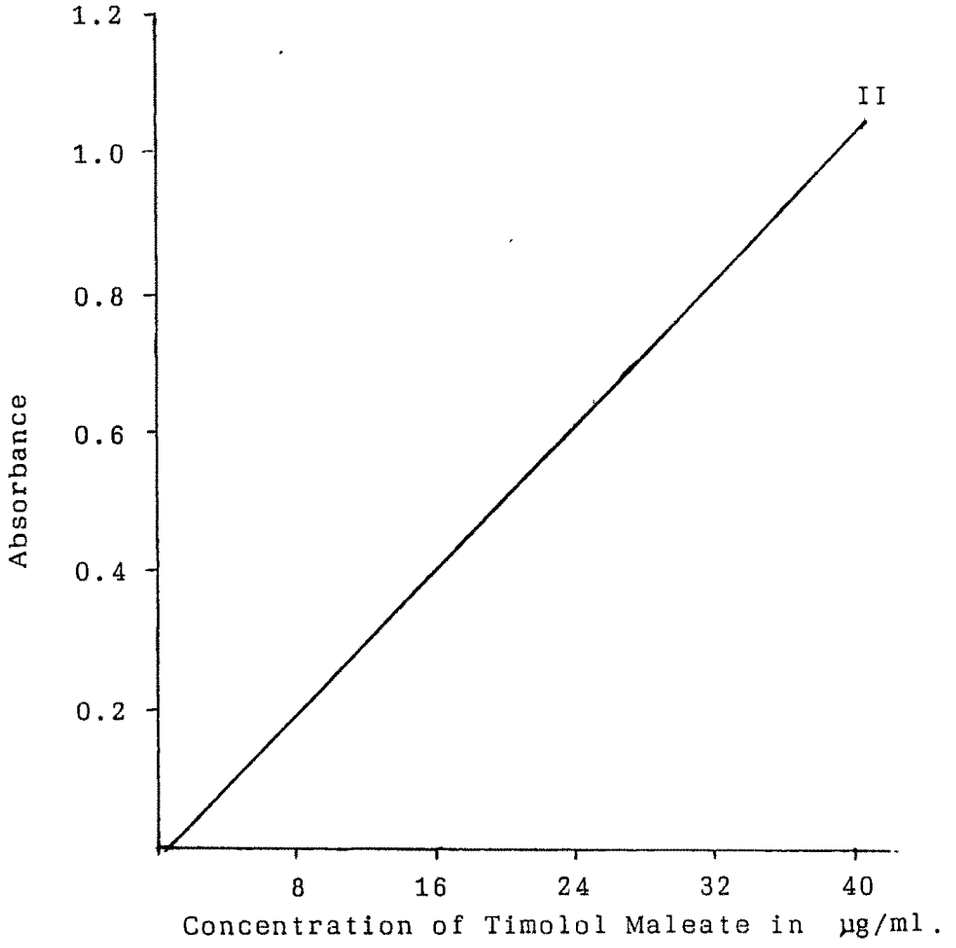


Fig. 2.27(I) - Absorption spectrum of ion pair of Timolol Maleate with methyl orange.

Fig. 2.27(II)- Calibration curve of Timolol Maleate with methyl orange.

The pH of optimum complexation was found to be 2.2 for all the compounds.

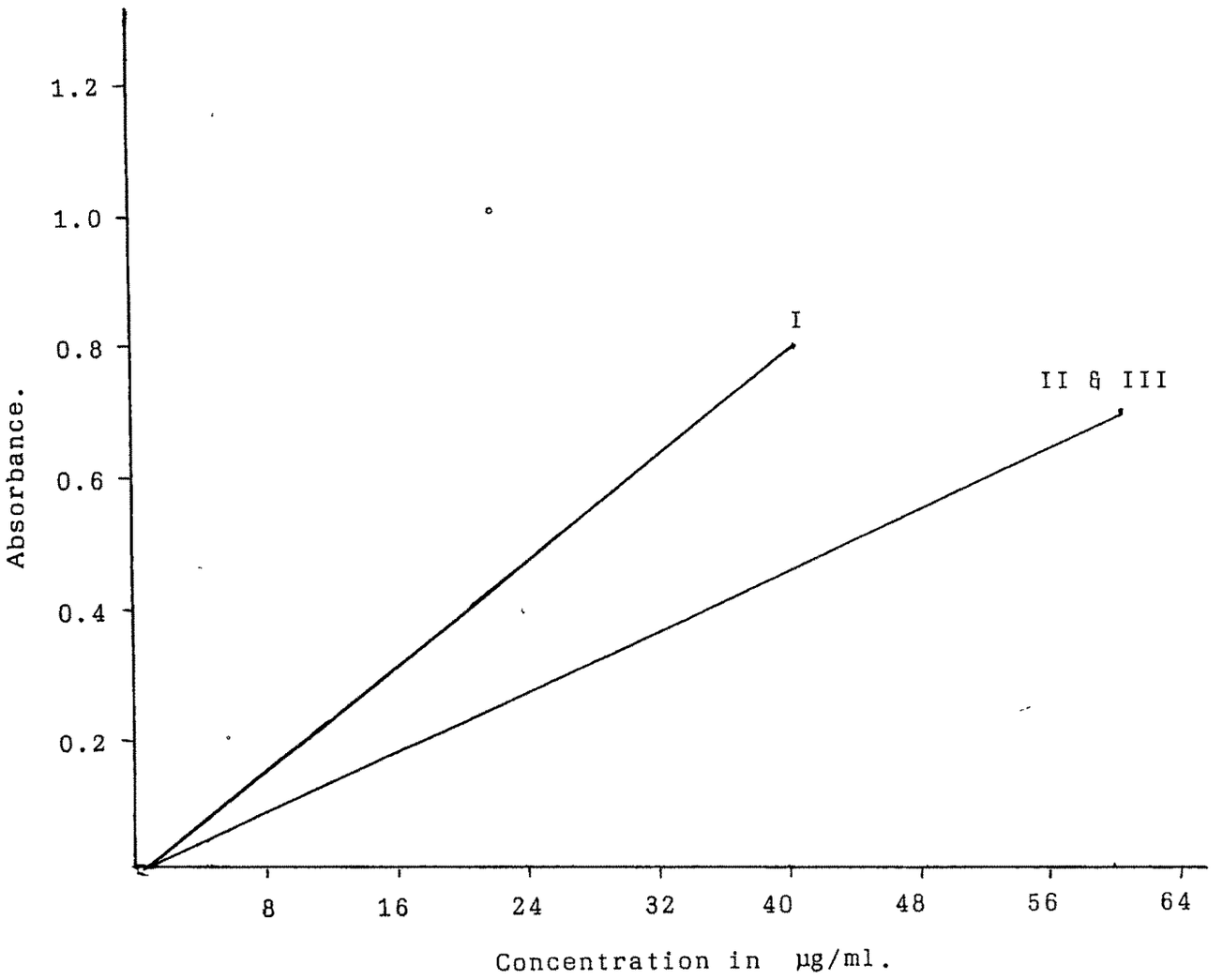
The stoichiometry of the complex was found to be 1:1 in case of all the drugs viz. Pindolol, Timolol Maleate and Sotalol hydrochloride with eriochrome black T and Timolol Maleate with methyl orange when determined by countinuous variation plot method at the total concentration of the drug and dye at 2×10^{-4} M by extracting the ion pair from aqueous methanolic solution into chloroform and measuring the absorbance at respective absorption maximum (figure 2.29, 2.30). However the drug to dye ratio of about 1:3 was employed in the subsequent experiment and analysis for complet complexation and extraction.

The calibration curve was rectilinear in the region 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 (figure 2.28). The calibration curve was rectilinear in the range of 2 - 40 µg/ml in case of Timolol Maleate with methyl orange (figure 2.27).

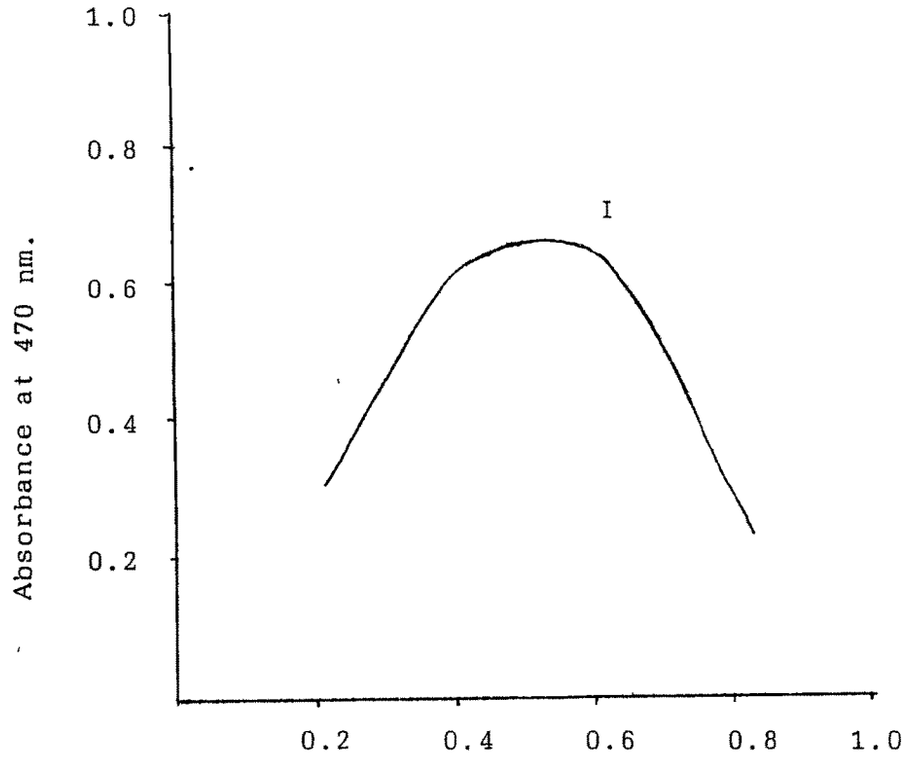
The method could be successfully applied for the estimation of all the commercially available dosage forms viz tablet and opthmalic solution (Timolol Maleate). The results obtained are comparable with B.P.[3] method for Pindolol, Timolol Maleate and with published method[66] for Sotalol hydrochloride for estimation of dosage forms in terms of accuracy and precision.

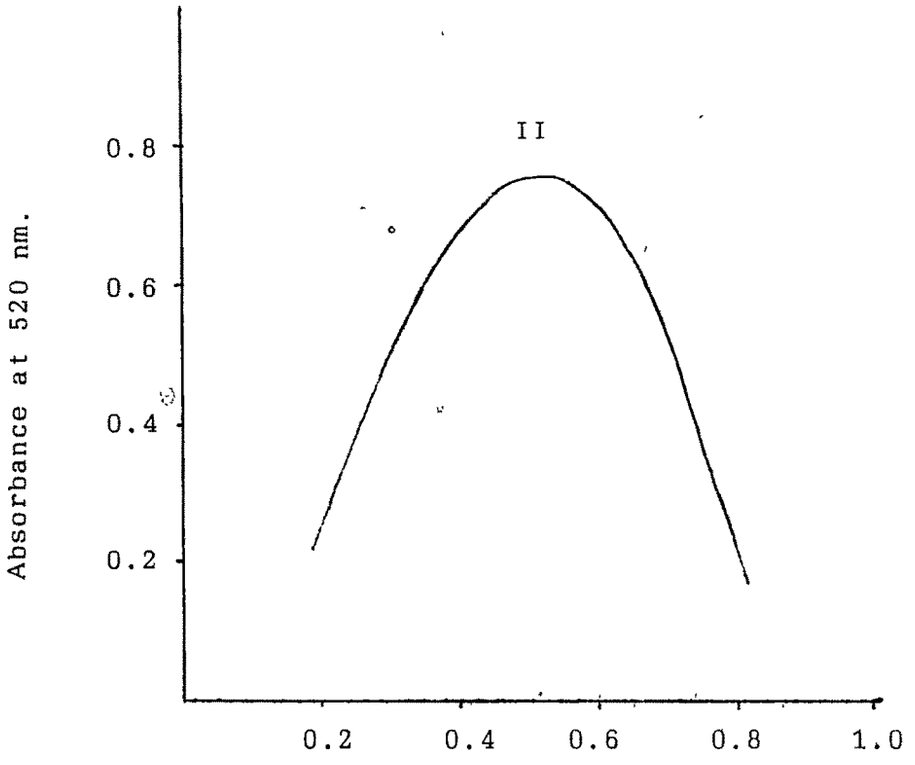
The proposed spectrophometric method for determination of these B blockers is simple, rapid and reliable and can be used for the routine abalysis of these drugs in all their common dosage forms.

Fig. 2.28



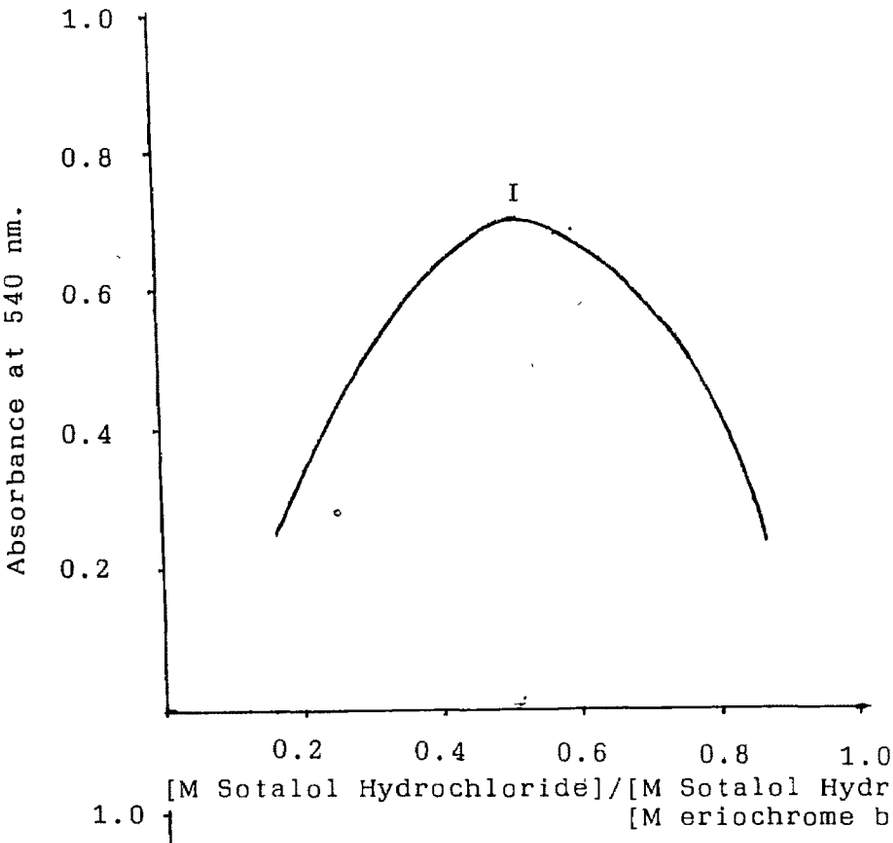
Calibration curve of (I) Sotalol Hydrochloride (II) Pindolol (III) Timolol Maleate with erichrome black T.

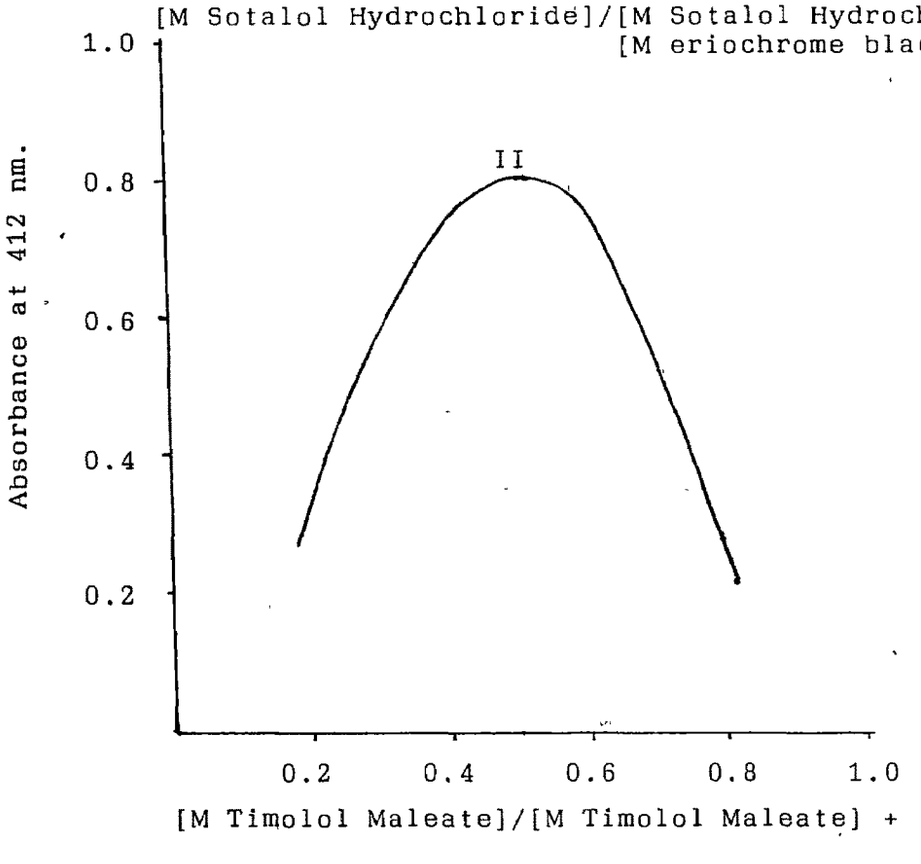




Continuous variation plot of ion pair (I) Pindolol (II) Timolol Maleate with eriochrome black T.

Fig. 2.30





Continuous variation plot of (I) Sotalol Hydrochloride (II) Timolol Maleate with eriochrome black T.

h. COMPERATIVE EVALUATION

The results obtained with bromothymol blue were identical to those obtained with bromocresol green in many respects and expectedly so because of the close structural resembalance between the two dyes.

The proposed spectrophotometric methods with bromothymol blue,bromocresol green, methyl orange and erichrome black T was simillar to the two published methods in two striking features. First the proposed method is based upon extraction of the ionpair of drug with dye from aqueous methanolic medium set to required pH with chloroform simillar to the published method, second-the range of rectilinearity obtained in the proposed method is much broder than the published methods without sacrificing the sensitivity of the method.

After compairing with the published methods it was found that the estimation of Pindolol, Timolol maleate and Sotalol hydrochloride with bromocresol green and the estimation of Pindolol. Nadolol and Sotalol hydrochloride with bromothymol bluegave better results so far as the precision and accuracy are concerned. But the range of concentration of drug determinable by the proposed method is definately better than the published ones.

3. REACTION WITH CRYSTAL VIOLET

I. REAGENTS:

1. 0.10% m/v solution of Pindolol, Timolol maleate, Nadolol and Sotalol hydrochloride in methanol.
2. 0.10% m/v solution of crystal violet in water.
3. Mc Ilvaine citrate-phosphate buffer pH 2.2 – 8 [59].
4. Methanol, benzene, chloroform.

11. EXPERIMENTAL PROCEDURE:

(PREPARATION OF CALIBRATION CRUVE).

To a set of 8 separate 25 ml calibrated flasals aliquits of standered solution of respective drug, 10 ml of buffer solution pH 3.0 and 2 ml of crystal violet dye solution were added and the volume was made up with water. An aliqauot of 10 ml was transfered to a 50 ml separator and extracted twice with 10 ml portion of chloroform. The violet extract was clolected in to25 ml calibrated flask and volume was made up with chloroform to yield 1-40 µg/ml concentration of the drug. Absorbance was measured at 580 nm against the reagent blank prepared in the simillar manner.

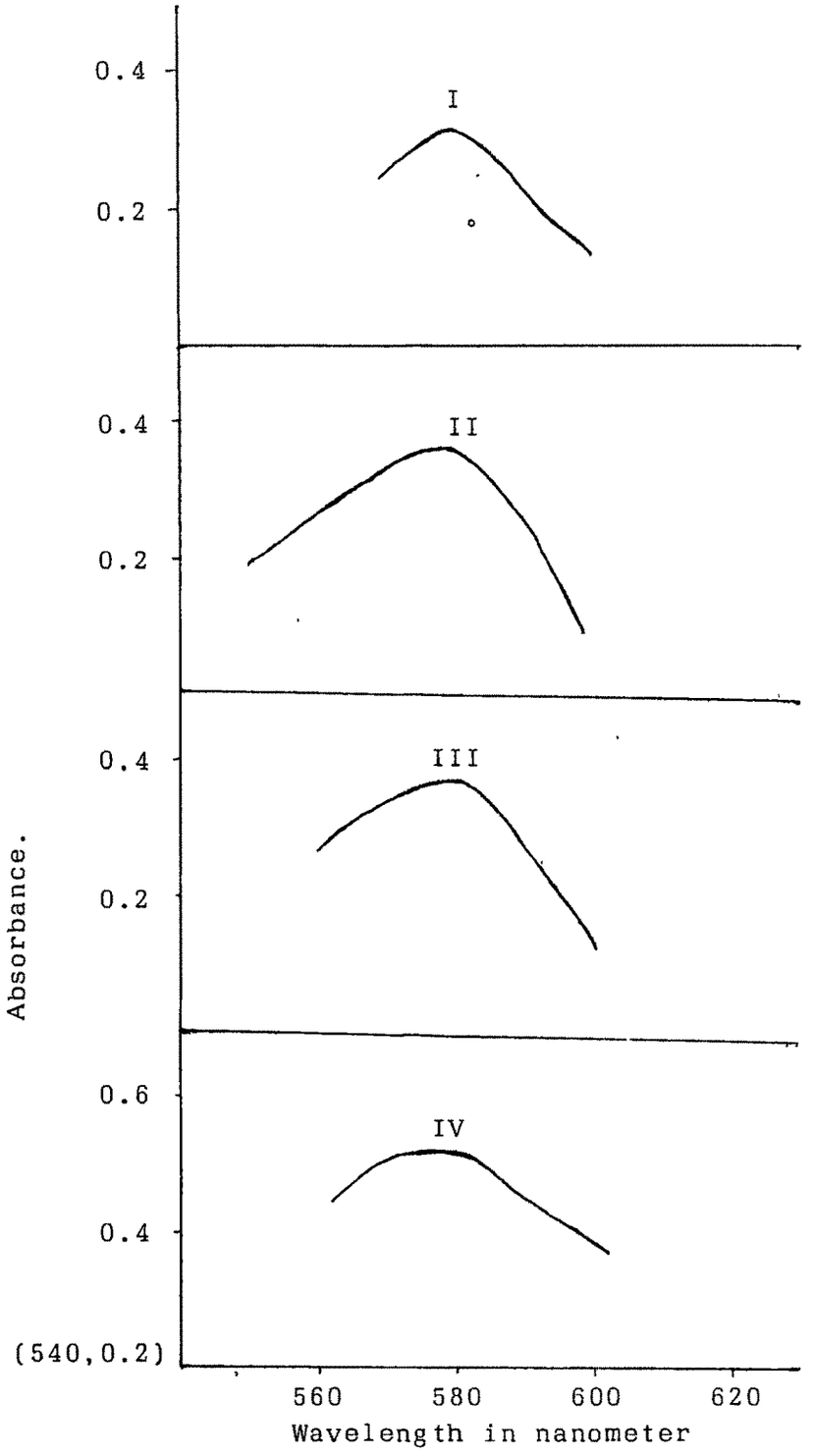
111 RESULT AND DISCUSSION

Preliminary experiments described earlier showed good promise for the reaction between all the four B-blockers and crystal violet especially in buffer solution of pH 3. Chloroform was found to be suitable for extraction of any possible ionpair from buffered solution of pH 3 giving stable violet colour. Scanning of sample extracts against blank extracts gave absorption maxima at 580 nm (figure 2.33). Calibration curve was rectiliner in the range of 20-200 µg/ml of all the four drugs (figure 2.32). All the 4 B-blockers behaved identically so far as chloroform extraction was concerned as well as in the λmax.

2.12 SPECTROPHOTOFLUORIMETERIC METHOD

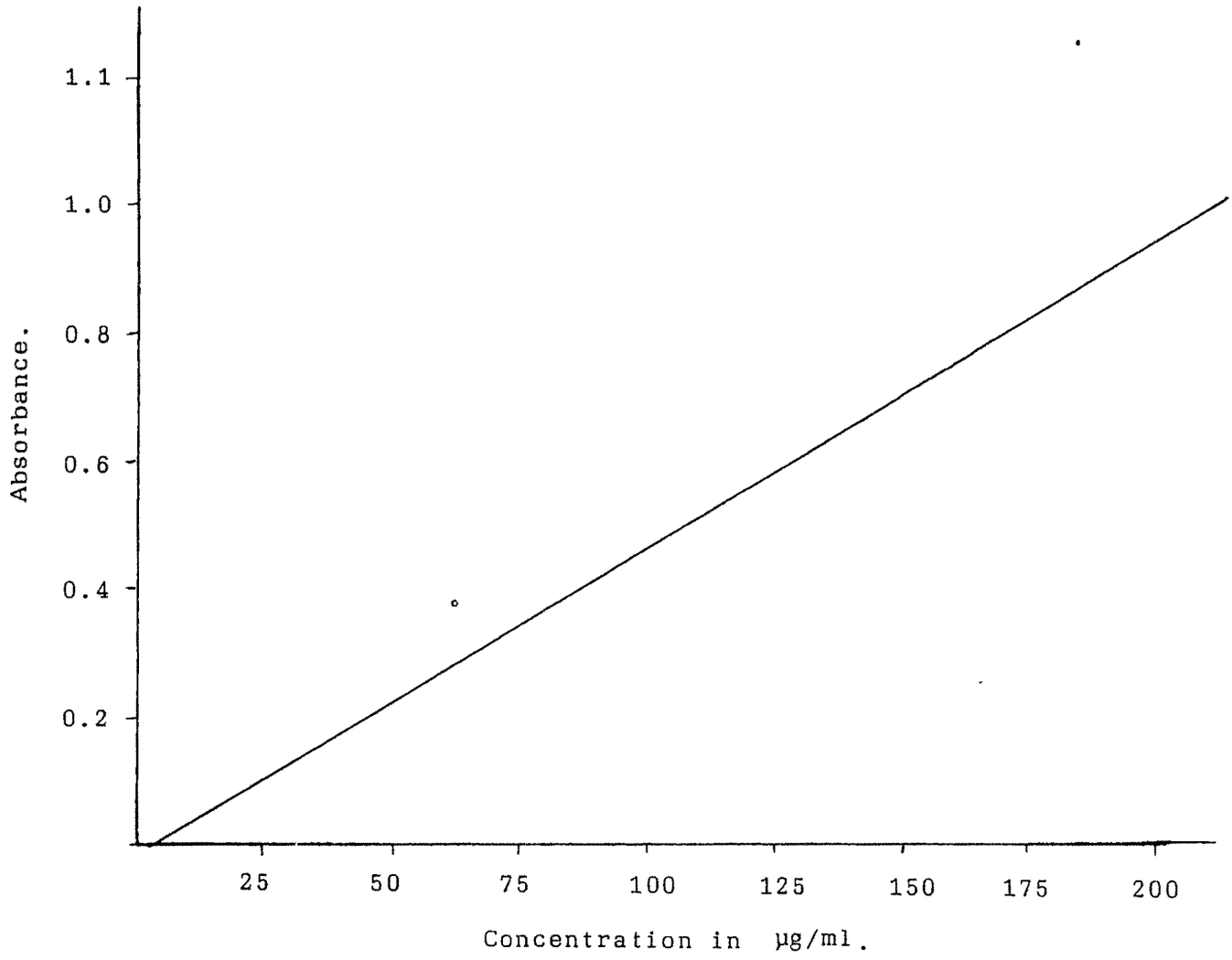
The published liturate on Pindolol and Nadolol roveled one method each for the estimation of these drugs by spectrophotofluorimetric method. Pindolol in ethanol medium gave fluorescence at 263 nm and 305 nm as excitation and emission

Fig. 2.32



Absorption spectrum of (I) Timolol Maleate (II) Pindolol (III) Sotalol Hydrochloride (IV) Nadolol with crystal violet.

Fig. 2.33



Calibration curve of Pindolol, Nadolol, Sotalol Hydrochloride and Timolol Maleate with crystal violet.

wavelength respectively. Nadolol after treatment with sodium periodate was again treated with o - phenylene diamine gave fluorescence at 305 nm and 445 nm as the excitation and emision wavelength respectively. No publication could be traced in the litature on spectrophotofluorimetric method for the estimation of Timolol Maleate and Sotalol hydrochloride. This could either be due to their negligible native fluorescence in the ultraviolet region or due to their photodegradation when exposed to Uv radiations.

Prompted by this lacuna, attempts were made to develop some spectrophotofluorimetric methods of analysis by designing different experiments consistent with the chemical structure of these B-blockers that could be conducive to fluorescence development.

2.12.1 PRELIMINARY EXPERIMENT

I. EQUIPMENT

Shimadzu spectrpholofluorimeter RF 540 and fused quartz sqare 10 mm cells having and 5 ml volume were used.

11. REAGENTS

1. 0.10% m/v solution of Pindolol,Timolol Maleate, Nadolol and Sotalol hydrochloride in methanol.
2. 0.1M and 1M hydrochloric acid.
3. 0.1M aquous perchloric acid solution (1.1% v/v in water).
4. 0.1M and 1M sulphuric acid.
5. Mc Ilvaine citrate phosphate buffer pH 2.2, 3.0, 4.0, 5.0 and 6.0 [59].
6. 0.1M soldium bicarbounate solution.
7. 0.1% m/v solution of potassium ferricyanide in water.

8. 0.001M copper sulphat solution.

9. Methanol, chloroform, polyethylene glycol - 200.

10. 0.025% m/v solution of quinine sulphate in 0.1M sulphuric acid.

11. 0.025% m/v solution of fluorescein in methanol.

III. DETERMINATION OF EXPERIMENTAL FLUORESCENCE

The following general procedure was adopted for determining the optimum excitation and emission wavelength.

1. Excitation wavelength was fixed at one of the known absorption maxima in Uv region and emission wavelength was varied to determine the optimum wavelength.

2. Optimum emission wavelength found in step 1. was fixed and excitation wavelength was varied to determine the effect of the latter on fluorescence intensity (excitation spectrum uncorrected) and optimum excitation wavelength corresponding to maximum fluorescence was also determined.

Optimum excitation wavelength found in step 2 was fixed and emission wavelength was varied to determine the effect of the latter on fluorescence intensity (emission spectrum uncorrected) and optimum emission wavelength corresponding to maximum fluorescence was also determined.

IV ESTIMATION OF NATURAL FLUORESCENCE

The following solution were prepared for each drug with 40 µg/ml concentration .

1. To a 25 ml calibrated flask 1 ml of respective drug solution was added diluted to volume with methanol.

2. To 25 ml calibrated flask 1 ml of respective drug solution, 2.5 ml of 0.1M hydrochloric acid were added and methanol

to volume.

3. To a 25 ml calibrated flask 1 ml of respective drug solution, 2.5 ml 0.1M perchloric acid were added and methanol to volume.

4. To 7 separate 25 ml calibrated flasks containing 1 ml of respective drug solution one of the following in respective order was added. 2.5 ml of 1 M sulphuric acid, 2.5 ml of 0.1M sulphuric acid, 10 ml of buffer pH 2.2, 10 ml of buffer pH 3.0, 10 ml of buffer pH 4.0, 10 ml of buffer pH 5.0 and 10 ml of buffer pH 6.0 to each flask were added and methanol to volume.

5. To 2 separate 25 ml calibrated flasks 1 ml of 0.1 % m/v respective drug solution in chloroform/polyethylene glycol 200 were added and the respective solvent to volume.

The optimum excitation and emission wavelengths were determined for each of them and fluorescence intensity obtained at the optimum excitation and emission wavelength was recorded for the sample and corresponding blank solution. The difference between the two gave corrected fluorescence intensity.

V. DETERMINATION OF EFFECT OF METAL COMPLEXATION ON FLUORESCENCE

To a 25 ml calibrated flask 1 ml of respective drug solution, 2.5 ml of 0.1M hydrochloric acid, 1 ml copper sulphate solution were added and methanol to volume. The fluorescence spectra and intensity of fluorescence were determined as in the previous experiments.

VI. DETERMINATION OF EFFECT OF OXIDIZING AGENTS ON FLUORESCENCE

The following solutions were prepared for spectrophotofluorimetric study at 40 µg/ml concentration of

respective drug substances.

1. To a 25 ml calibrated flask 1 ml of respective drug solution, 1 ml of potassium ferricyanide solution were added and methanol to volume.

2. To a 25 ml calibrated flask 1 ml of respective drug solution, 1 ml of potassium ferricynide solution, 1 ml of sodium bicarbonate solution were added and methanol to volume.

3. To a 25 ml calibrated flask 1 ml of respective drug solution, 2 ml of hydrogen peroxide solution, 2.5 ml of water and were added methanol to volume.

4. To a 25 ml calibrated flask 1 ml of respective drug solution, 2 ml of hydrogen peroxide solution, 2.5 ml of 0.1 M sulphuric acid were added and methanol to volume.

The fluorescence spectra and intensity of fluorescence were determined as in the previous experiments.

VII DETERMINATION OF THE EFFECT OF DRUGS ON FLUORESCENCE OF QUININE SULPHATE

1 ml of respective drug solution was added to a 25 ml calibrated flask containing 1 ml of quinine sulphate solution and methanol to volume. The standared solution of fluorophore was prepared by diluting 1 ml of (0.025% m/v) quinine sulphate to 25 ml in a calibrated flask to serve as a control flask for comparision. The fluorescence spectrum of standared solution was scanned and the fluorescence of these solution was measured against the appropriate reagent blank with 352 nm and 443 nm as excitation and emission wavelength found to be optimum with the instrument used.

VIII. DETERMINATION OF EFFECT OF DRUGS ON FLUORESCENSE OF FLUORESCEIN

To a 25 ml calibrated flask 1 ml of fluorescein solution (0.025 m/v), 1 ml of respective drug solution were added and methanol to volume. The standared solution of fluorophore was prepared by diluting 1 ml of fluorescein solution to 25 ml in a clibrated flask to serve as a control for comparison. The fluorescence spectrum of standared solution was scanned and the fluorescence of these solution was measured against methanol and with 470 nm and 510 nm as excitation and emission wavelength found to be optimum with the instrument used. The excitation and emission spectra of fluorescein solution containing Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride were scanned and compared with that of standared fluorescein spectrum.

In modification of the above experiment 2.5 ml of 0.1 M hydrochloric acid/2.5 ml of 0.1 M sodium bicarbonate was added to study the effect of extreme acid and alkaline pH on the fluorescence spectrum of fluorescein and effect of drugs in question on such modified spectrum of fluorescnin.

The fluorescence of fluorescein was measured as such and when containg Timolol Maleate at 470 nm excitation wavelength and 510 nm emission wavelength in pure methanol medium. When containing Pindolol and Sotalol hydrochloride at 470 nm excitation and 510 nm emission wavelength and when containing Nadolol solution at 470 nm excitation and 530 nm emission wavelength in pure methanol medium.

Fluorescein in methanol was scanned as such and also when containing 4 µg/ml concentration of either drug solution.

IX. PREPARATION OF CALIBRATION CURVE

To separate 25 ml calibrated flasks containing 1 ml of fluorescein solution 0.25, 0.50, 1, 2, 3, 4, 6, 8, 10 and 12 ml of standard solutions of either of the drug were added. Mehtanol was added to volume and mixed. The standard solution of fluorophore was prepared by diluting 1 ml of fluorescein solution to 25 ml in a calibrated falsk to serve as a control for comparision. The fluorescence of these solution was measured against methanol with 470 nm and 510 nm as excitation and omission wavelength found to be optimum. The fluorescence of each solution was expressed as percentage of fluorescence of fluorescein (%F). The substraction of (%F) from 100 gave the precentage fluorescence quenching (%FQ) or increase in fluorescence (%FR). Logarithm of (%F) and (%FQ)/(%FR) was also determined. The following graphs were drawn in a bid to established if linearity existed between fluorescence intensity and concentration of respective drug.

1. Percentage of fluorescence of fluorescein (%F) vs. concentration of respective drug in ng/ml (C).
2. Percentage of fluorescence quenching (%FQ)/(%FR) vs. concentration of respective drug in ng/ml.
3. Log percentage of fluorescence of fluorescein (log %F) vs. concentration of respective drug in ng/ml.
4. Log percentage of fluorescence quenching(log %FQ)/(log %FR) vs. concentration of respective drug in ng/ml.
5. Log percentage of fluorescein (log %F) vs. Log concentration of respective drug in ng/ml (log c).
6. Log percentage of fluorescence quenching (log %FQ)/(log %FR)

vs. log concentration of respective drug in ng/ml (log c).

X. ESTIMATION IN TABLET

A total of 20 tablets were weighed and average weight was determined. An accurately weighed portion of finely ground tablets equivalent to 10.0 mg of either drug was transffered toa 50 ml calibrated flask. To this about 30 ml of methanol was added and contents shaken for 10 minutes. Methanol was added to volume and mixed. The solution was then filtered and 1 ml aliquot of the filtrate was diluted to 10 ml in a calibrated flask.

To a 25 ml calibrated flask 1 ml of fluorescein solution, 1 ml of test solution as above were added and methanol to volume. The fluorescence of this solution was measured as described under the calibration curve. The attenuated fluorescence intensity was expressed as percentage of fluorescence of fluorescein and the content of the respective drug in mg per tablet was calculated using the liner calibration curve of logarithm of fluorescence of fluorescein (log %F) vs. concentration of the drug in ng/ml (C).

XI. ESTIMATION IN OPTHALMIC SOLUTION (TIMOLOL MALEATE)

To the sample equivalent to 5.0 mg of Timolol Maleate 10 ml of buffer solution (pH 9.7) was added and the solution was extracted with chloroform (2 X 10 ml). A 10 ml portion of the combined extract was evaporated under vacuum and residue was dissolved in 25 ml of methanol and the procedure as described above for the estimation of tablets was followed.

XII. RESULTS AND DISCUSSION

Excitation and emission spectra and fluorescence intensity in particular obtained there after with the different solutions

enlisted above gave very unstable and imprecise fluorescence intensity in most cases except Sotalol hydrochloride.

Sotalol hydrochloride gave intrinsic fluorescence with 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 emission wavelength (figure 2.34) Fluorescence intensity could be linearly correlated with concentration of Sotalol hydrochloride from 1 - 40 ng/ml (figure 2.35). Change of solvent from methanol to chloroform retarded the fluorescence readings Where as viscus solvent like poly ethylene glycol 200 some what enhanced the fluorescence.

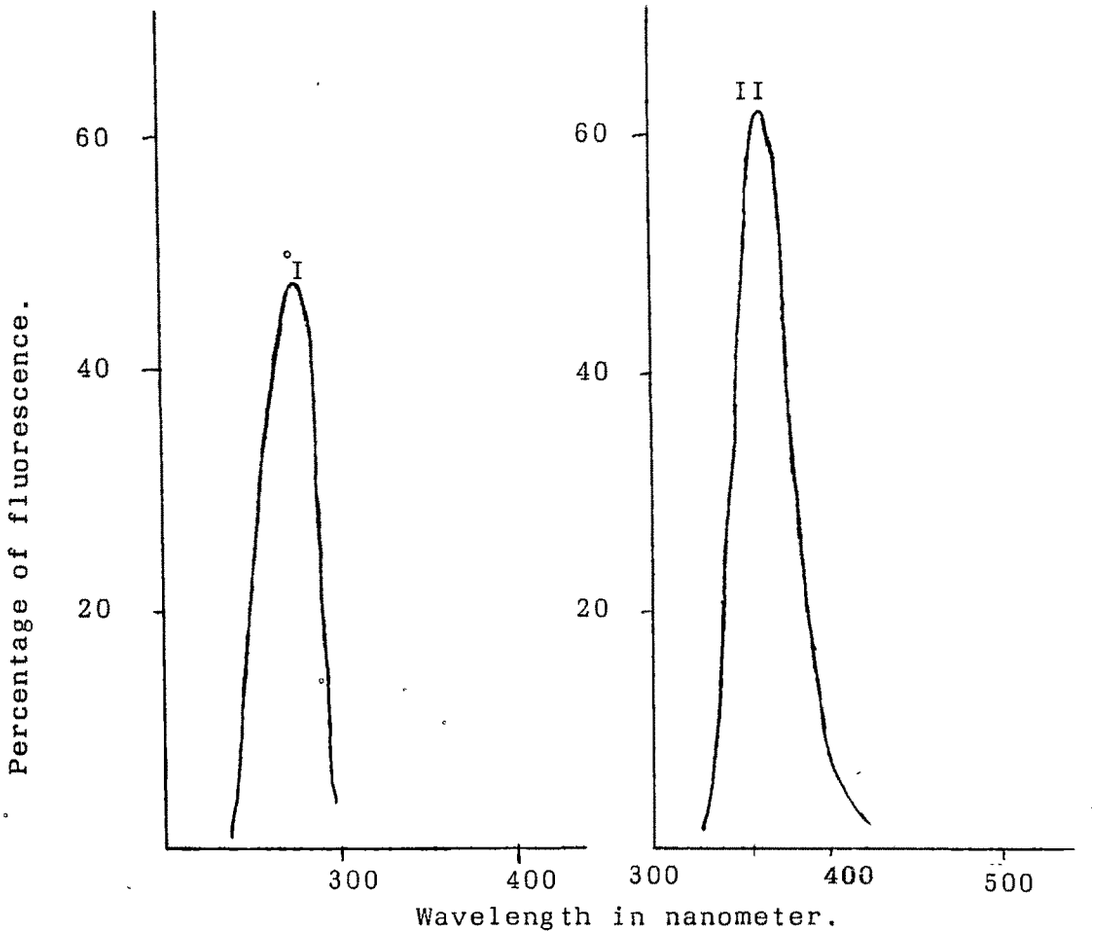
Attempts to chelate the drugs with cupric ions in methanol at an acidic pH with view to induce fluorescence gave negative results.

Attempts to oxidise the drug compounds with neutral and alkaline potassium fericyanide and neutral and acidic hydrogen peroxide gave promissing results. The results with neutral potassium ferricyanide were more prescise and were studied in detail 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 durgs.

Fluorescence intensity colud be linearlly correlated 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 (figure 2.36).

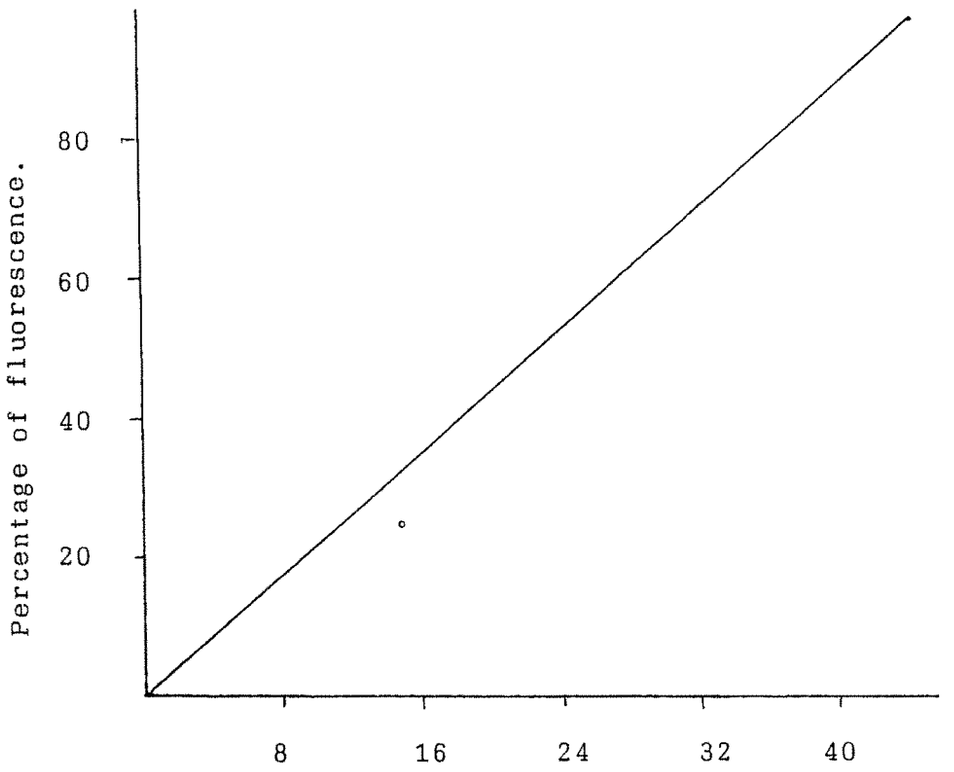
Attempts were made to use these drugs as quenchers for the fluorescence of two well known fluorophores. Experiments of this

Fig. 2.



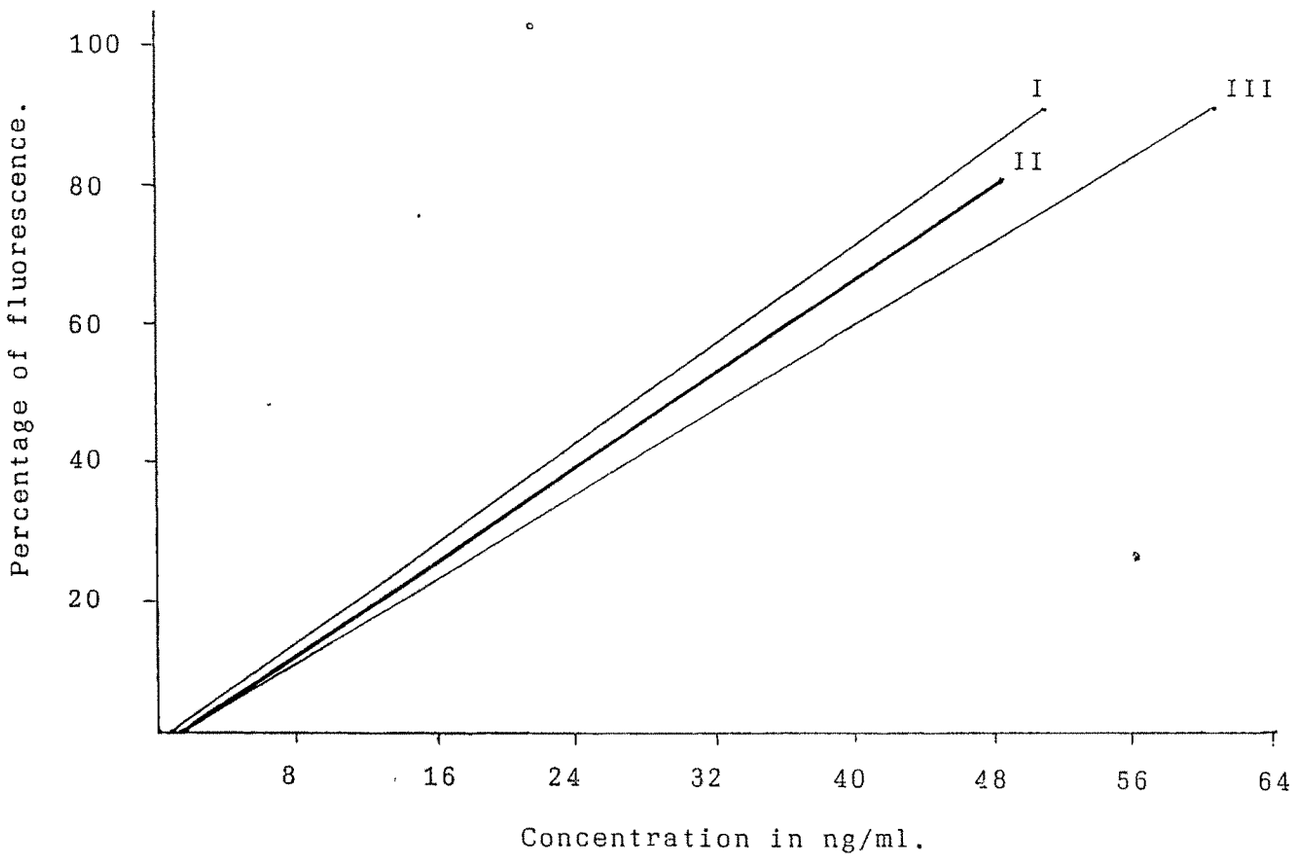
Exicitation (I) and Emission (II) spectrum (uncorrected) respectively of 40 ng/ml solution of Sotalol Hydrochloride in sulphuric acid.

Fig. 2.35



Calibration curve of fluorescence of Sotalol Hydrochloride in methanol or sulphuric acid.

Fig. 2.36



Calibration curve of fluorescence of (I) Naddlol (II) Sotalol Hydrochloride (III) Pindolol with potassium ferricyanide.

type were planned in view of the absorption characteristics of these drugs and possibilits of some reaction between the drug and the fluorophore.

Under the experimental condition described quinine sulphate exhibited strong fluorescence in 0.1 M sulphuric acid and when fluorescence spectra was scanned, optimum excitation and emission wavelength were found to be 352 nm and 442 nm corresponding to maximum fluorescence intensity of about 450 nm at 10 ug/ml concentration. The pesence of any of the drugs under study at 40 ug/ml concentration did not affect the fluorescence reading at all and any possible quenching effect of the drugs on the fluorescence of quinine sulphate was ruled out.

In sharp contrast to the preceding experiment one β blocker timolol maleate exhibited remarkable quenching property on the natural fluorescence of fluorescein in methanol when measured with the 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 fluorescien when measured with 470 nm and 510 nm as optimum excitation wavelength for Pindolol and Sotalol hydrochloride were as optimum excitation and emission wavelength for Nadolol were 470 nm and 530 nm.

Besides increase in fluorescence intenisty, presence of Nadolol brought about qualitative change in the excitation spectrum of fluorescencein. Excitation maxima retained at 470 nm where as emission maxmima was shifted from 510 nm to 530 nm when containing 40 ng/ml quantity of Nadolol. Fluorescence excitation and emission wavelength of 10 ng/ml solution of fluorescein were

almost retained at 470 nm and 510 nm respectively in presence of 40 ng/ml concentration of Pindolol, Timolol Maleate and Sotalol hydrochloride in methanol. The use of 0.1 M hydrochloric acid increased the fluorescence intensity noticeably. The optimum excitation maxima of fluorescein shifted from 470 nm in methanol to 442 nm in acidic methanol were as the emission maxima was retained at 510 nm. The use of a mixture of 0.1 M sodium bicarbonate and methanol (1+9) as the medium instead of methanol greatly enhanced the ionization of fluorescein and hence fluorescence but quanching property of Timolol Maleate was completely lost. The graph of percentage of fluorescence of fluorescein (%F) or percentage of fluorescence quanching (%FQ) vs concentration of Timolol Maleate (C) gave curve as drawn in figure 2.36b. The graph of logarithm of percentage of fluorescence of fluorescein (log %F) vs concentration of Timolol Maleate was rectilinear from 0.1 - 4.0 ug/ml (figure 2.36a) and this graph was used for the purpose of quantitative analysis of the dosage forms. The logarithm of percentage of fluorescence of fluorescein and concentration of Timolol Maleate gave rectilinear relationship.

The graphs of percentage of fluorescence of fluorescein (%F) or percentage of increase of fluorescence of fluorescein (%FR) vs concentration of respective drug (C) (Pindolol, Nadolol and Sotalol hydrochloride) gave rectilinear relation from 1-20 ng/ml concentration of Pindolol and 2 to 25 ng/ml concentration of Nadolol and Sotalol hydrochloride (figure 2.37). The method worked satisfactorily for the estimation of all the commercially available dosage forms of respective drugs.

Fig. 2.36(a)

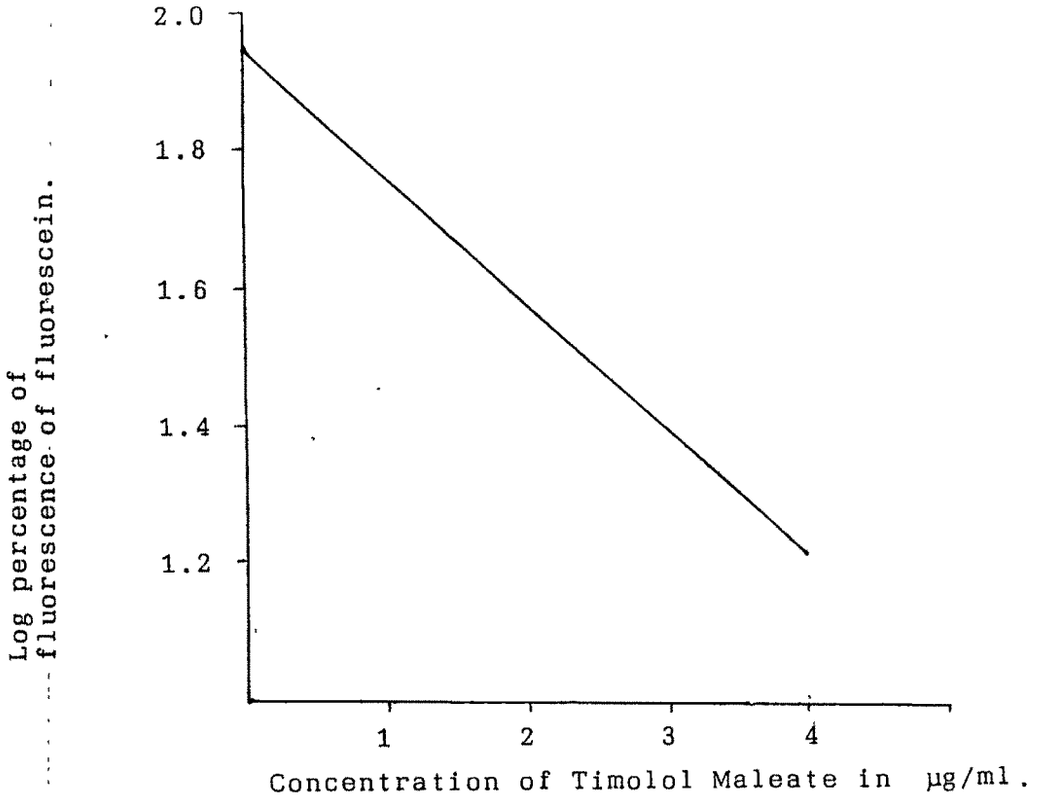


Fig. 2.36(b)

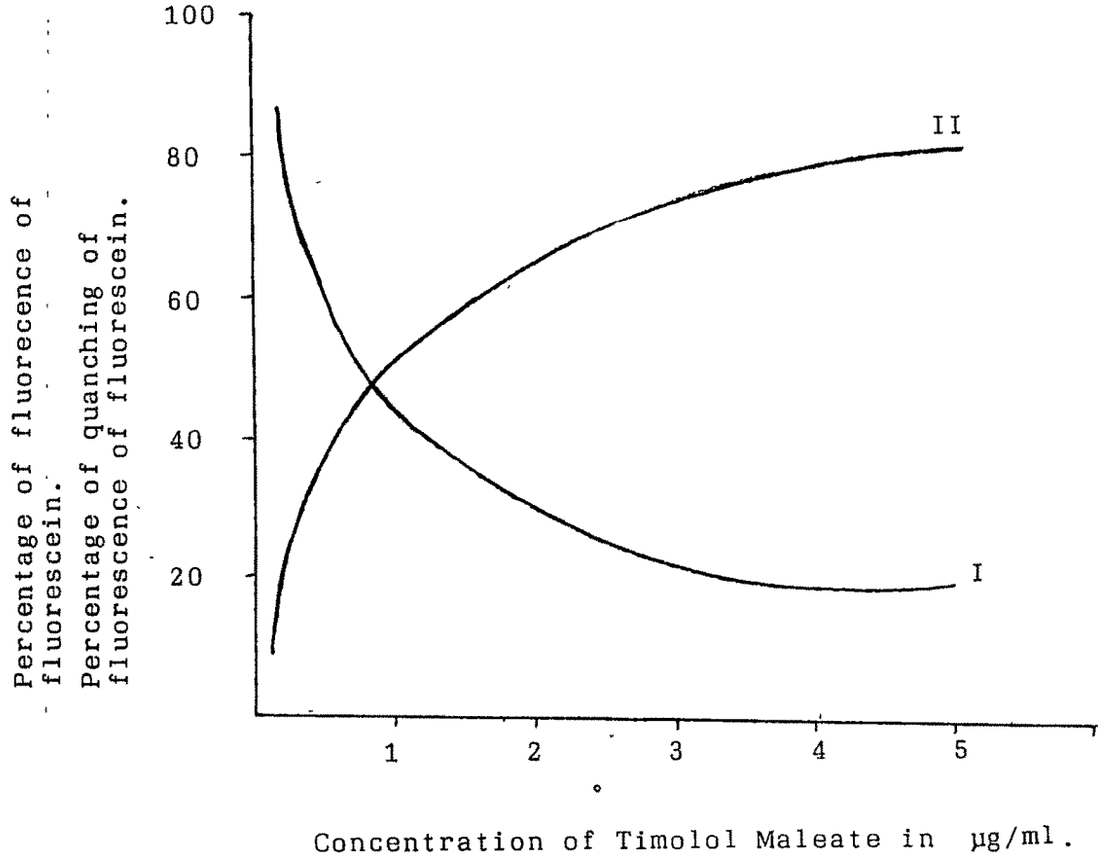
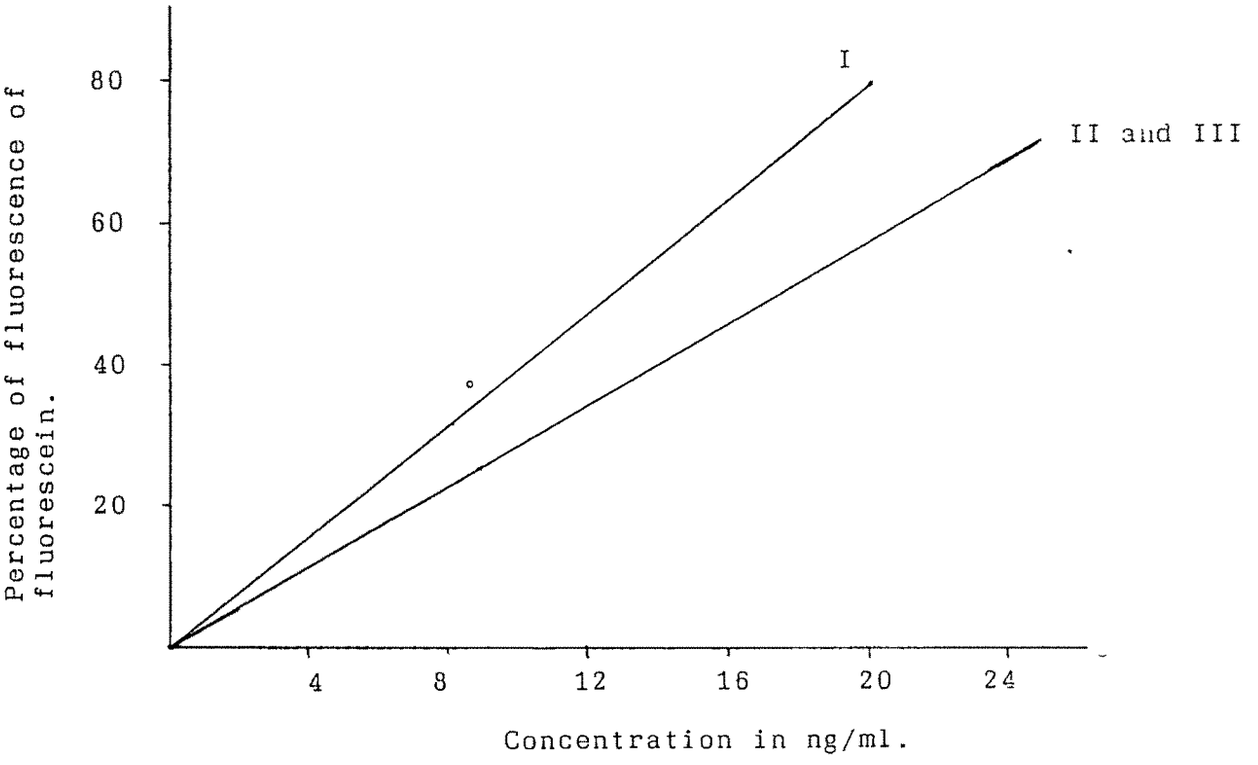


Fig. 2.37



Increase in the fluorescence intensity of fluorescein by (I) Pindolol (II) Nadolol (III) Sotalol Hydrochloride.

The results obtained are comparable to B.P.[3] method for Pindolol, Timolol Maleate and Nadolol but the method for Sotalol hydrochloride in terms of accuaracy and precision was found to be comparable with the published method[66]. The proposed spectrophotofluorimetric method for the determination of Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride is simple,rapid and reliable and can be used for routin analysis of dosage form of these drugs. In view of the specificity of the method for these drugs and its high sensitivity the method seems to be promising for pharmacokinetic studies as well. The addition of sodium bicarbonate increase in the fluorescence of fluorescein there fore the contribution of these drugs Pindolol, Nadolol and Sotalol hydrochloride to the inhancement of fluorescence of fluorescein was not easy to determine.

2.13 REACTIONS OF PINDOLOL

Only Pindolol was observed to react few other reagents like 1,2 napthoquinone - 4 sulfonic acid, N-1-napthyl ethylene diamine and potassium dichromate which is being discuss as follows :-

I. REAGENTS

1. 0.10% m/v solution of Pindolol in 0.1N hydrochloric acid and 0.1% m/v Pindolol in 0.1 N sulphuric acid.
2. 0.1N hydrochloric acid.
3. 4N sulphuric acid.
4. 1.0% w/v solution of ammonium suphamate in water.
5. 0.10% w/v solution of sodium nitrite in water.
6. 0.10% w/v N-1-napthyl ethylene diamine.
7. 100 mg potassium diachromate in 100 ml water :sulphuric acid. (80 : 20)

8. 0.10% w/v solution of 1,2 napthoquinone - 4 sulfonic acid sodium salt in water.

II. EXPERIMENTAL PROCEDURE

1. N-1-NAPTHYL ETHYLENE DIAMINE REACTION

Aliquots of standard Pindolol solution was added to the 25 ml calibrated flask containing 6 ml of 4 N sulfuric acid. 1 ml of 0.1% sodium nitrite solution was added and the mixutre was allowed to react for 1 minutes. Then 2 ml of 1 % ammonium sulphamate solution was added and mixture was allowed to stand for another 2 minutes followed by then 0.8 ml of 0.1% N-1-napthyl ethylene diamine. The mixture was diluted to volume with water and absorbance was measured at 540 nm against the reagent blank after 10 minutes.

2. POTASSIUM DICHROMATE REACTION

To the 10 ml calibrated flask, aliquots of standard Pindolol solution in sulfuric acid 0.2 ml of potassium dichromate solution ware added and contents were diluted to volume with water. The absorbance was measured at 626 nm against reagent blank.

3. 1,2 NAPTHOQUINONE 4 - SULFONIC ACID REACTION.

Several aliquots of standard Pindolol solution were taken in 50 ml calibrated flask. Then 20 ml of water and 2.0 ml of 1.2 napthoquinone 4 sulfonic acid sodium salt solution was added. Contents were heated on boiling watabath for 10 minutes cooled and volume was made up with water and the absorbance was measured at 620 nm against reagent blank.

III RESULTS AND DISCUSSION

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 method was optimised for the volume of each reagent. 6 ml of 4N sulfuric acid, 1 ml of sodium nitrite, 2 ml of ammonium sulphamate and 0.8 ml of N-1-napthyl ethylene diamine were found optimum for colour development. The colour was found to be stable for one hour and the calibration curve found to be rectilinear in the range of 0.64 - 40 µg/ml (figure 2.38). The method was compared with B.P.[3] method. The proposed method was found to be simple, accurate and precise.

Sulphuric acid solution of Pindolol (0.1% m/v) reacted with potassium dichromate in sulphuric acid medium gave blue colour with absorbance maxima at 626 nm. The 0.2 ml of dichromate reagent was found optimum for the development of colour. The colour was found to be stable for 45 minutes . Calibration curve was rectilinear in the rage of 4 - 36 µg/ml (figure 2.39). The method was compared with B.P.[3] method.

Pindolol reacted with 1, 2 napthoquinone - 4 sulfonic acid after heating but the colour was not stable and there was no sharp absorbance maxima observed and therefore method was not studied in detail.

Pindolol also gave pink colour in the presence of mixture of glacial acetic acid : hydrochloric acid 30:10. On heating for 8 minutes on boling 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 (figure 2.40).

2.14 APPLICATION OF PROPOSED SPECTROPHOTMETRIC METHODS FOR THE ESTIMATION OF BETABLOCKER IN BIOLOGICAL FLUIDS LIKE BLOOD AND

Fig. 2.38

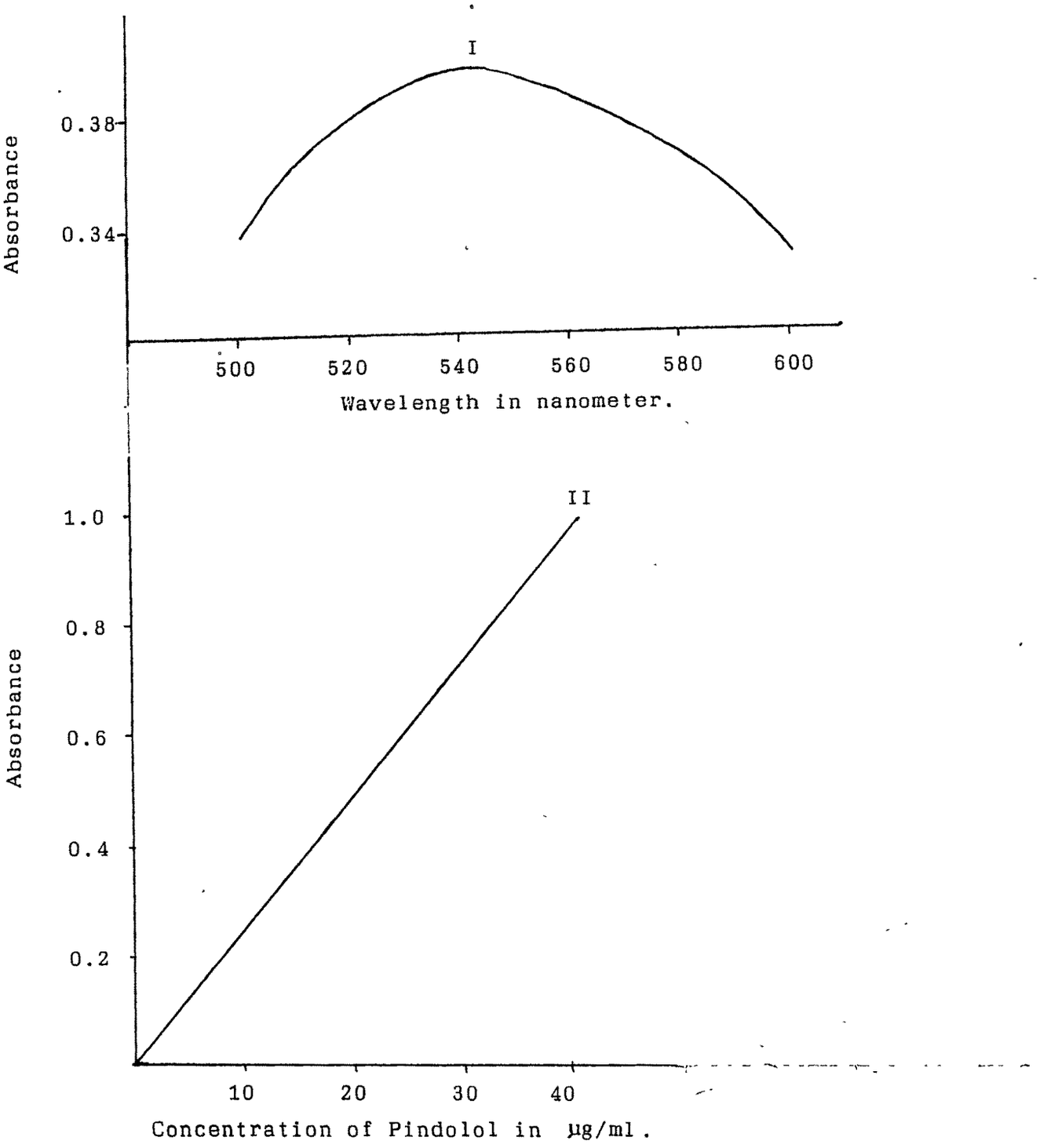
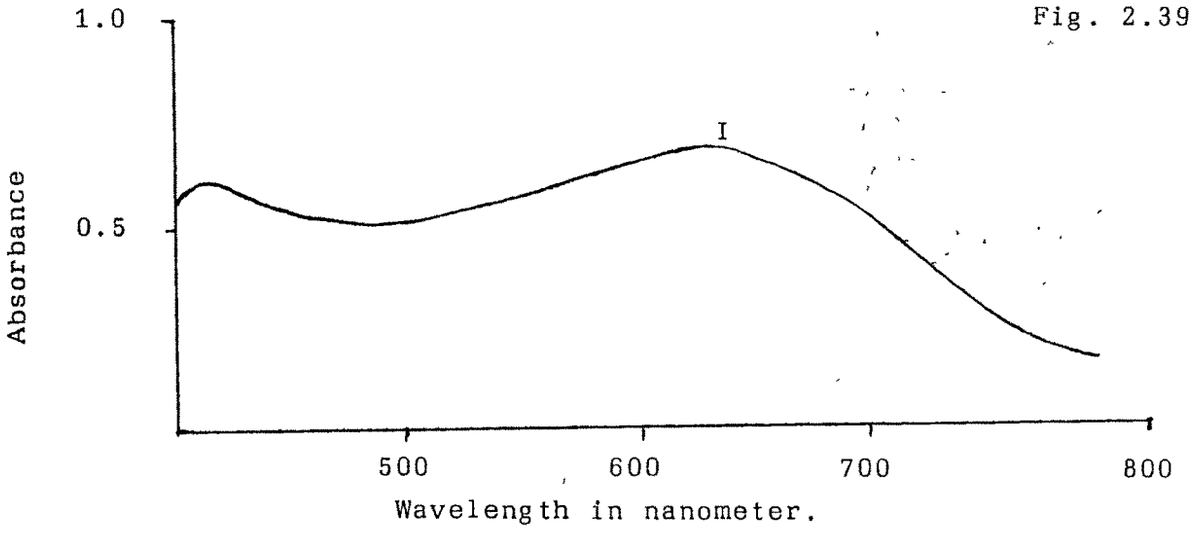


Fig. 2.38(I) - Absorption spectrum of Pindolol with N-1-Napthyl ethylene diamine.

Fig. 2.38(II)- Calibration curve of Pindolol with N-1-Napthyl ethylene diamine.

Fig. 2.39



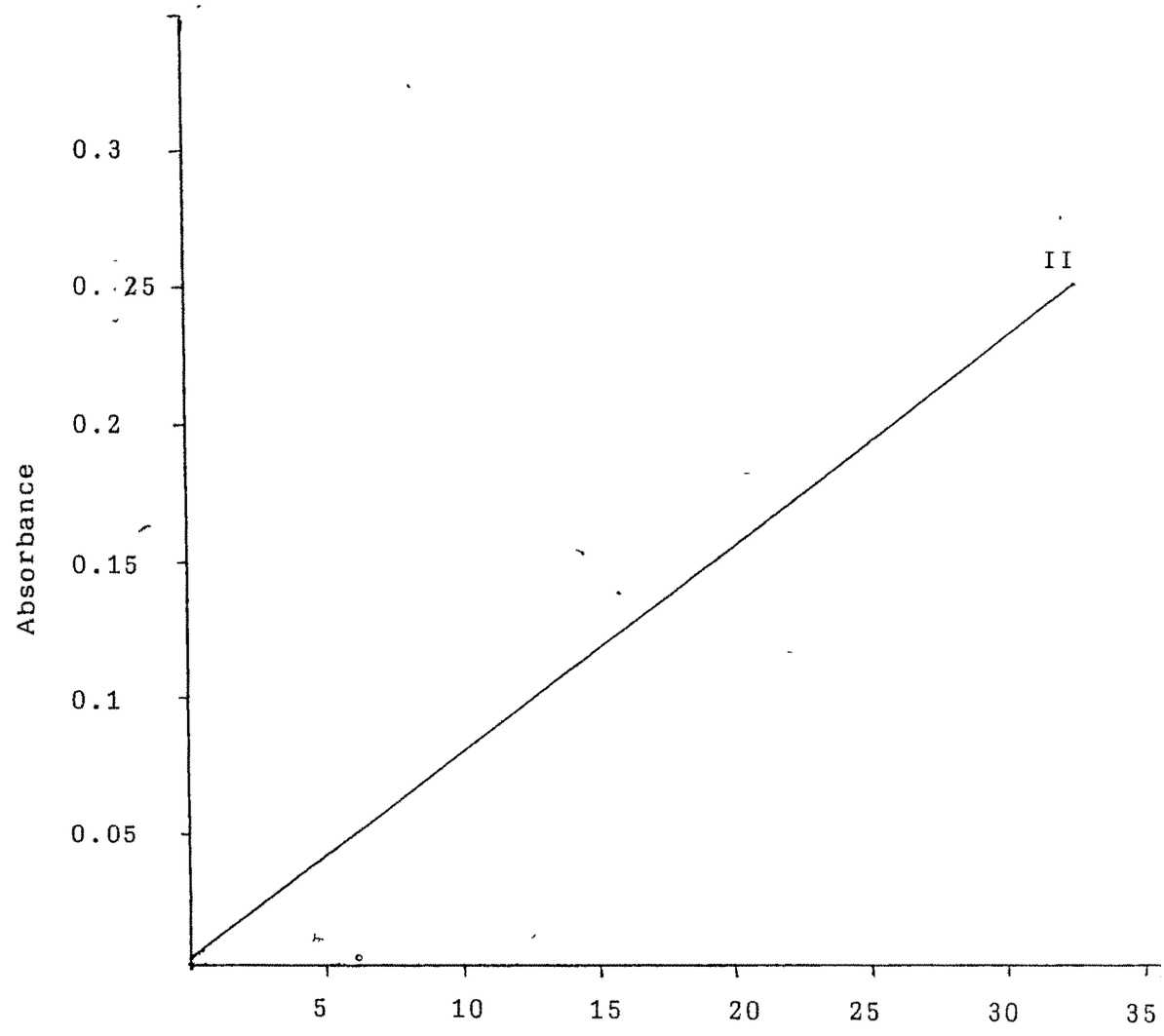


Fig.2.39(I) - Absorption spectrum Pindolol with potassium dichromate.

Fig.2.39(II)- Calibration curve of Pindolol with potassium dichromate.

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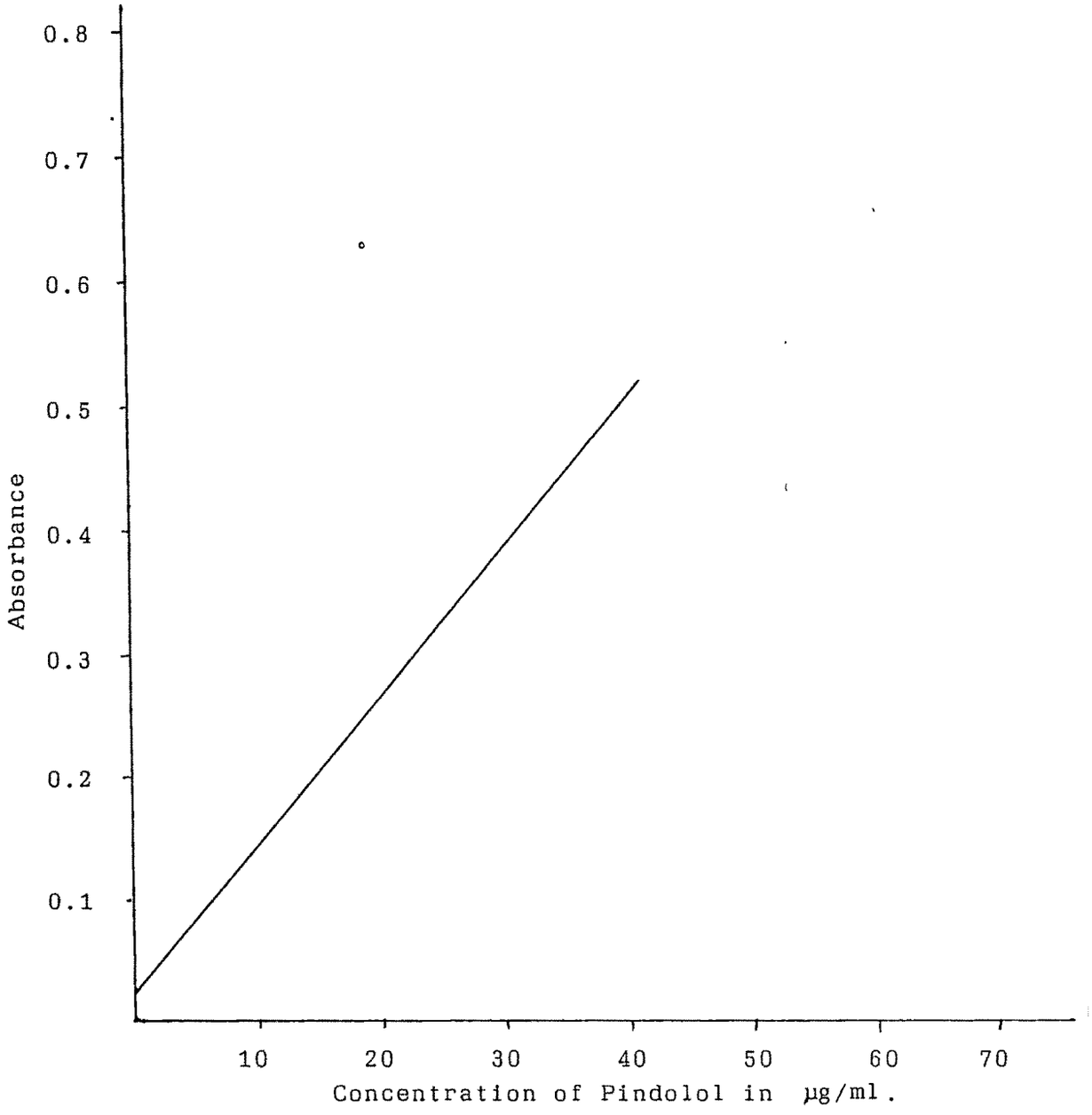
Fig. 2.40



Calibration curve of Pindolol in glacial acetic acid Hydrochloride acid (30:10).

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. It seems very little work has been reported on spectrophotometric determination of these drugs in biological fluids. In the present investigation an attempt has been made to use the proposed spectrophotmetric methods (section 2.1 to 2.13) for the determination of small quantities of these drugs in biological fluids.

I. REAGENTS

1. These were prepared freshly from time to time in the respective solvents as described in section 2.1 to 2.13.

2. Sulphuric acid 0.666N.

3. 10% w/v solution of sodium tungstate in water.

4. Standard drug solutions as described under section 2.1 - 2.13.

5. Human blood samples collected from medical college of Baroda.

II. EXPERIMENTAL PROCEDURE

PROCEDURE FOR DETERMINATION OF DRUG IN SERUM :

In determining the drug content in the serum, the blood was centrifuged and serum (2.0 ml) was pipetted into a test tube. sulfuric acid solution (2.0 ml) and sodium tungstate solution (2.0 ml) were added and mixed well. This treatement resulted in the precipitation of proteins from serum. Precipitated fluid was centrifuged at 3000 r.p.m. for 15 minutes and a clear supernatant was obtained, further centrifugation was necessary if clear supernatant was not obtained within the specified time.

The clear supernatent was poured in to test tube. 3.0 ml aliquot of the clear fluid equivalent to 1.0 ml of original blood serum was pipetted in to a 100 ml separatory funnel. To this standard drug solution (1.0 ml) was added and the contents were extracted with suitable solvent, filtered through anhydrous sodium sulphate and the extract was evaporated to dryness. The reside of drug so obtained was dissolved in the respective solvent and calibration curve was prepared as described in section 2.1 -2.13 for each drug. This calibration curve was used subsequently for the determination of drug in the biological fluids under investigation.

The blank was prepared in a similar manner except that the standared drug solution was replaced by the serum (3.0 ml).

PROCEDURE FOR DETERMINATION OF DRUGS IN URINE

Urine sample (1.0 ml) was taken and amount of drug was estimated in the spiked samples in a similar manner as described for serum.

III RESULT AND DISCUSSION

The recovery of Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride in serum and urine was found to be satisfactory by the proposed methods. (99.25,99.90, 99.35, 99.90 % respectively).