CHAPTER III

STABILITY AND KINETIC STUDIES

This chapter describes the methodology and out come of the several preformulation and formulation stability and kinetic experiments designed to evaluate the effect of various parameters like pH, temperature and humidity and to study compatibility of the drugs in question with the excipients used in tablet formulation. Similar studies were made with the available market formulation also.

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

The published literature revealed inadequate data on stability of timolol maleate and nadolol as described in the section 1.2.2 and 1.2.3. No significant data on stability has been published in case of pindolol and sotalol hydrochiloride. In case of both these compounds no reference could be located dealing with stability as a function of pH. In view of scanty data on stability of these compounds as a function of pH and non availability of stability data of timolol maleate and nadolol at different pH stability of these drugs was investigated at different pH over a range of 2.2-8.

1. REAGENTS.

1. Standard samples of pindolol, timolol maleate, nadolol and sotalol hydrochloride.
2. Mc Ilvaine citrate-phosphate buffer pH 3.0-8.0[59].
3. Acid phthalate buffer pH 2.2, 3.0, 4.0, (5.0 neutralized), 6.0
   Phosphate and borate buffer 6.0, 7.0, 8.0, 9.0, pH
4. Acetyl acetone reagent: 30 gm of ammonium acetate and 1 ml of

acetyl acetone in 100 ml water.

5. 0.01M sodium periodate solution in water.

6. Methanol.

II EXPERIMENTAL PROCEDURE

One ml of drug solution (0.5% m/v) was added to each of 6 seperate 100ml volumetric flasks contaning buffer solution of pH 3.0, 4.0, 5.0, 6.0, 7.4 and 8.0 in respective order. The contents were mixed after making up the volume with respective buffer solution. The solution was divided in to three equal portion of about 33 ml and filled in amber glass round bottles. One of the bottles was stored at ambient temperature, another in refrigerator and the third in thermostatically controlled ovens at 50° C for stability study. The solutions were assayed for their initial concentration by acetyl acetone reagent as described in section 2.10. The samples were withdrawn at every week/15 days up to 90 days and studied for stability.

The concentration of drug solution obtained was converted in to percentage of intial concentration which was taken to be 100. The logarithm of the percentage of initial concentration was plotted against time in days. Time required for 50% and 90% degradation (t0.5 and t0.9) respectively were calculated from the slope of the respective regression line for each pH and compared with one another.

A similar set was prepared and 5ml sample solution was filled in 5 ml ampoules and sealed and kept at 60° C and 70° C for 24 hours in thermostatically controlled ovens and analysed initially and at the interval of 2 hours in the beginning for 8 hours and at the end of 24 hours.

II RESULTS AND DISCUSSION

Since the emphasis was to study stability of these drugs over a wide pH rage of 2.2 to 8 in solution form. The maximum concentration of the solute that could be studied was reduced to 0.500% m/v. Obviously, the concentration of solute and proportion of aqueous phase were limited by buffer pH 8.0.

Pindolol solution degraded at higher pH and higher temperature as can be seen from table 3.1, 3.2. The study of table 3.1 and 3.2 indicate practically negiligible degradation occurred at pH 5.0 even at tempareture 50° C. At other pH values drug tended to degrade noticeably.

Timolol Maleate showed good stability at pH4 at 50° C though the solution degraded at higher temperature at this pH. (Table 3.3 and 3.4)

Nadolol solution showed good stability at pH 7.4 at 50° C though the solution degraded at higher temperature at this pH (Table 3.5 and 3.6). It also showed good stability at ambient temperature at lower pH values.

Sotalol hydrochloride showed good stability at pH 5.0 at 50° C though the solution degraded at higher temperature at this pH (Table 3.7 and 3.8).

At refrigerator temperature all most all the solutions of all these three drugs, Timolol Maleate, Nadolol and Sotalol hydrochloride at different pH values remained stable.

The graphs of logarithm of percentage of initial concentration verses time in hours were rectilinear (figure 3.1 to 3.4). Hence the degradation of all these drugs at higher temperature 70° C followed first order kinetics.

TABLE 3.1

STABILITY OF 0.500 % m/v SOLUTION OF PINDOLOL IN PHTHALATE, PHOSPHATE AND BORATE BUFFER AT RT, FT AND 50° C

Storage Temperature	No. of days	Concentration of Pindolol expressed as percentage of initial concentration at different pH values.					
		3.0	4.0	5.0	6.0	7.4	8.0
Initial	0	100.00	100.00	100.00	100.00	100.00	100.00
ambient Temperature	90	99.60	99.71	99.36	100.65	100.38	98.57
50° C	90	97.76	97.24	99.71	98.05	96.89	96.00
Refrigerator Temperature	90	99.07	99.17	99.89	99.98	99.91	99.80

TABLE 3.2

STABILITY OF 0.500 % m/v SOLUTION OF PINDOLOL IN PHTHALATE, PHOSPPHATE AND BORATE BUFFER AT 60° C AND 70° C

Storage Temperature	Time in hours	Concentration of Pindolol expressed as percentage of initial concentration at different pH values						
		3.0	4.0	5.0	6.0	7.4	8.0	9.0
60° C	24	91.46	92.50	96.66	85.75	74.32	75.00	43.02
70° C	24	84.33	84.34	91.46	77.32	63.00	60.12	29.91

TABLE 3.3

STABILITY OF 0.500 % m/v SOLUTION OF TIMOLOL MALEATE IN PHTHALATE, PHOSPHATE AND BORATE BUFFER AT RT, FT AND 50° C

Storage Temperature	No. of days	Concentration of Timolol Maleate expressed as percentage of initial concentration at different pH values.					
		3.0	4.0	5.0	6.0	7.4	8.0
Initial	0	100.00	100.00	100.00	100.00	100.00	100.00
ambient Temperature	90	99.08	99.81	99.03	99.05	99.10	99.27
50° C	90	98.00	99.00	97.40	98.08	96.01	98.44
Refrigerator Temperature	90	99.70	99.89	99.73	99.60	99.81	99.64

TABLE 3.4

STABILITY OF 0.500 % m/v SOLUTION OF TIMOLOL MALEATE IN PHTHALATE, PHOSPPHATE AND BORATE BUFFER AT 60 C AND 70° C

Storage Temperature	Time in hours	Concentration of Timolol Maleate expressed as percentage of initial concentration at different pH values						
		3.0	4.0	5.0	6.0	7.4	8.0	9.0
60° C	24	93.20	95.60	91.36	89.28	75.10	72.35	67.53
70° C	24	89.72	90.46	84.22	79.43	61.25	60.89	49.77

TABLE 3.5

STABILITY OF 0.500 % m/v SOLUTION OF NADOLOL IN PHTHALATE, PHOSPHATE AND BORATE BUFFER AT RT, FT AND 50 °C

Storage Temperature	No. of days	Concentration of Nadolol expressed as percentage of initial concentration at different pH values.					
		3.0	4.0	5.0	6.0	7.4	8.0
Initial	0	100.00	100.00	100.00	100.00	100.00	100.00
ambient Temperature	90	99.10	99.51	99.65	98.16	99.59	99.45
50°C	90	98.23	98.73	98.81	97.21	99.60	97.32
refrigerator Temperature	90	99.08	99.20	99.90	99.98	99.91	99.90

TABLE 3.6

STABILITY OF 0.500 % m/v SOLUTION OF NADOLOL IN PHTHALATE, PHOSPPHATE AND BORATE BUFFER AT 60°C AND 70°C

Storage Temperature	Time in hours	Concentration of Nadolol expressed as percentage of initial concentration at different pH values.						
		3.0	4.0	5.0	6.0	7.4	8.0	9.0
60°C	24	89.30	91.40	87.75	93.20	96.65	85.75	72.35
70°C	24	79.45	85.20	78.80	89.70	91.40	77.30	60.70

TABLE 3.7

STABILITY OF 0.500 % m/v SOLUTION OF SOTALOL HYDROCHLORIDE IN PHTHALATE, PHOSPHATE AND BORATE BUFFER AT RT, FT AND 50° C

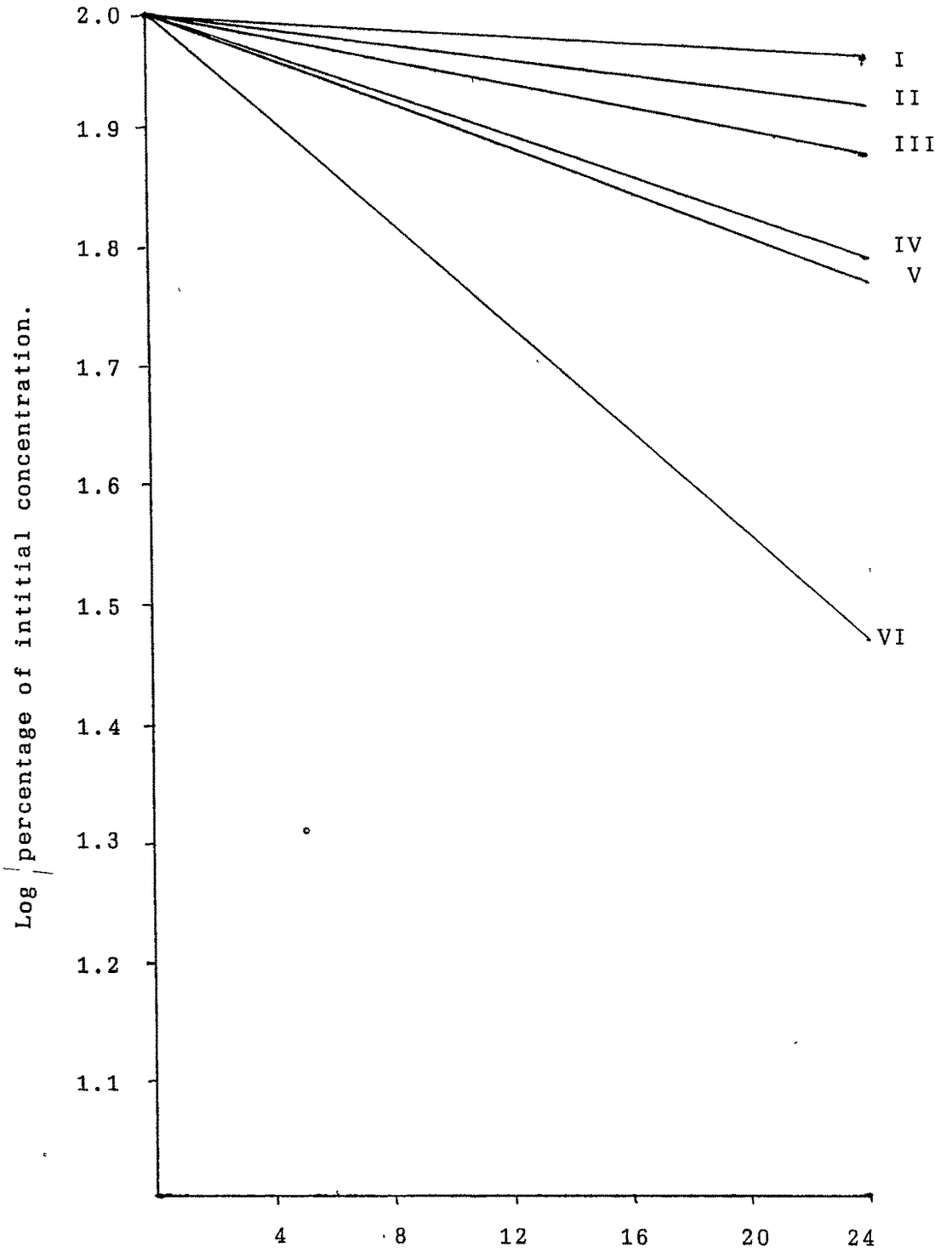
Storage Temperature	No. of days	Concentration of Sotalol hydrochloride expressed as percentage of initial concentration at different pH values.					
		3.0	4.0	5.0	6.0	7.4	8.0
Initial	0	100.00	100.00	100.00	100.00	100.00	100.00
ambient Temperature	90	99.22	98.66	99.58	98.63	98.57	98.57
50° C	90	96.87	97.31	99.40	98.16	97.76	98.17
refrigerator Temperature	90	100.38	100.03	99.93	100.86	100.05	99.98

TABLE 3.8

STABILITY OF 0.500 % m/v SOLUTION OF SOTALOL HYDROCHLORIDE IN PHTHALATE, PHOSPPHATE AND BORATE BUFFER AT 60° C AND 70° C

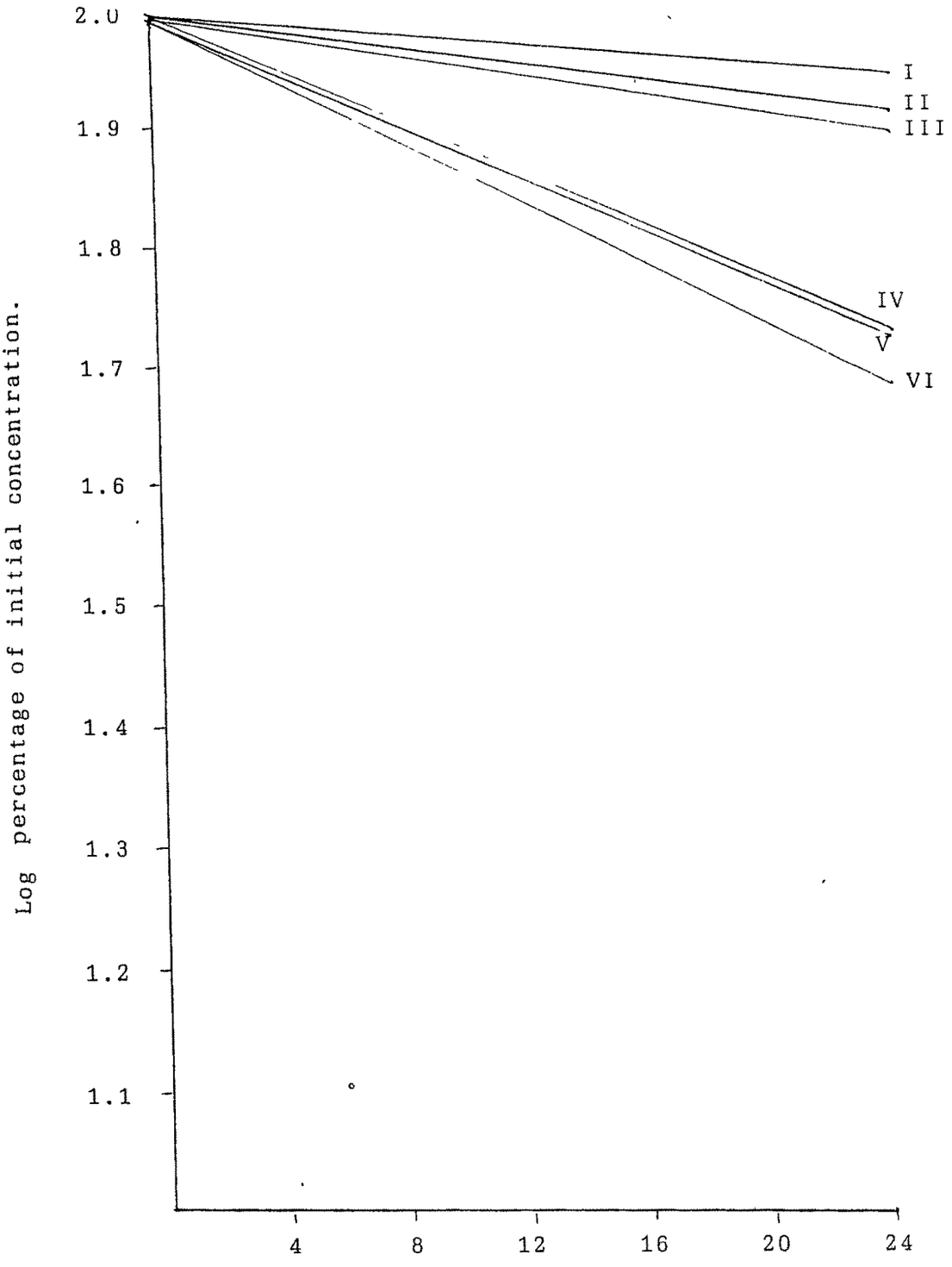
Storage Temperature	Time in hours	Concentration of Sotalol hydrochloride expressed as percentage of initial concentration at different pH values						
		3.0	4.0	5.0	6.0	7.4	8.0	9.0
60° C	24	91.36	94.10	96.32	87.75	79.25	67.53	50.47
70° C	24	86.09	89.25	92.54	78.88	67.50	49.77	33.33

Fig.



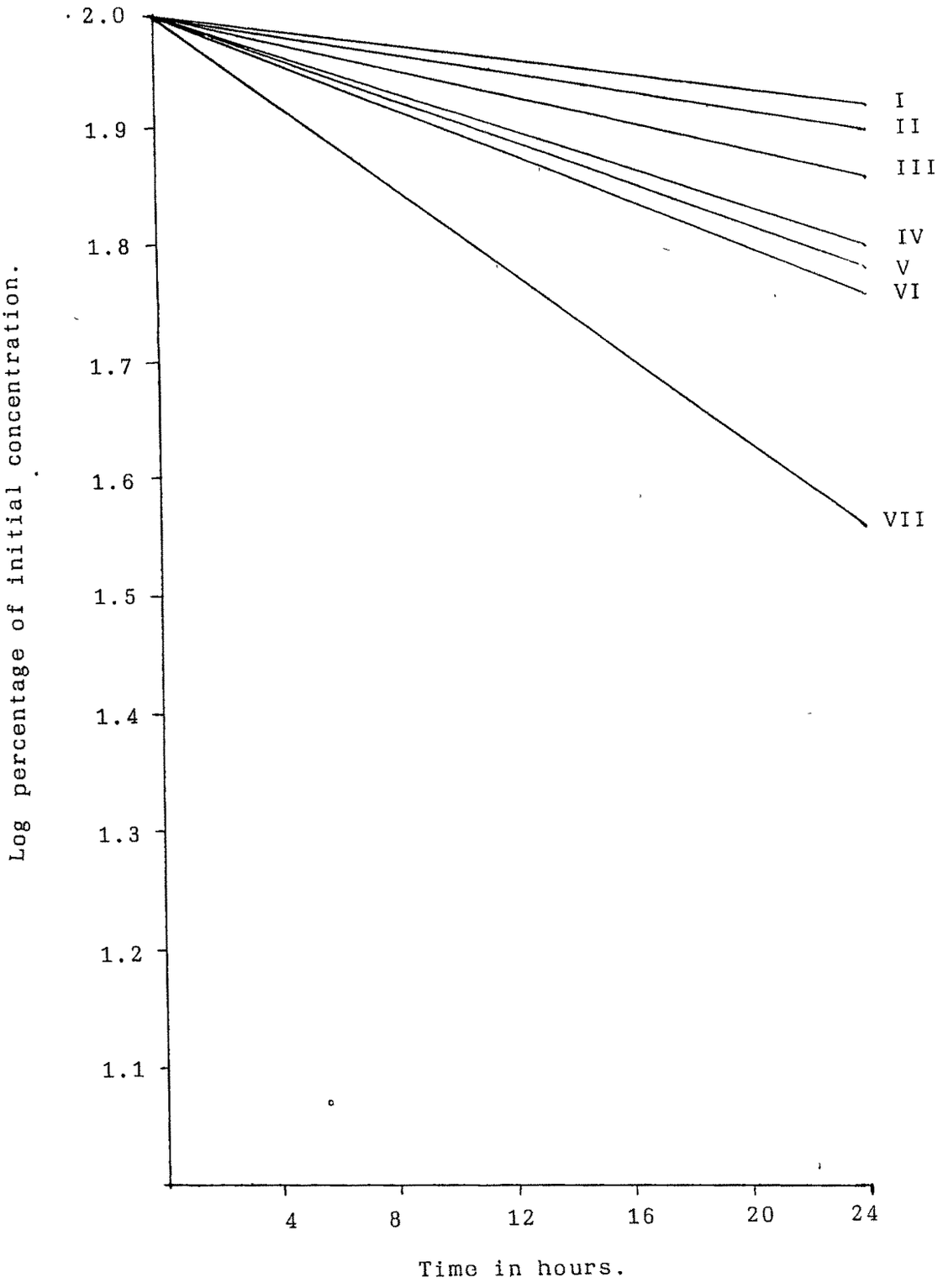
Degradation of 0.5% m/v solution of Pindolol at 70° at (I) pH 5.0 (II) pH 3.0 & 4.0 (III) pH 6.0 (IV) pH 7.4 (V) pH 8.0 (VI) pH 9.0

Fig. 3



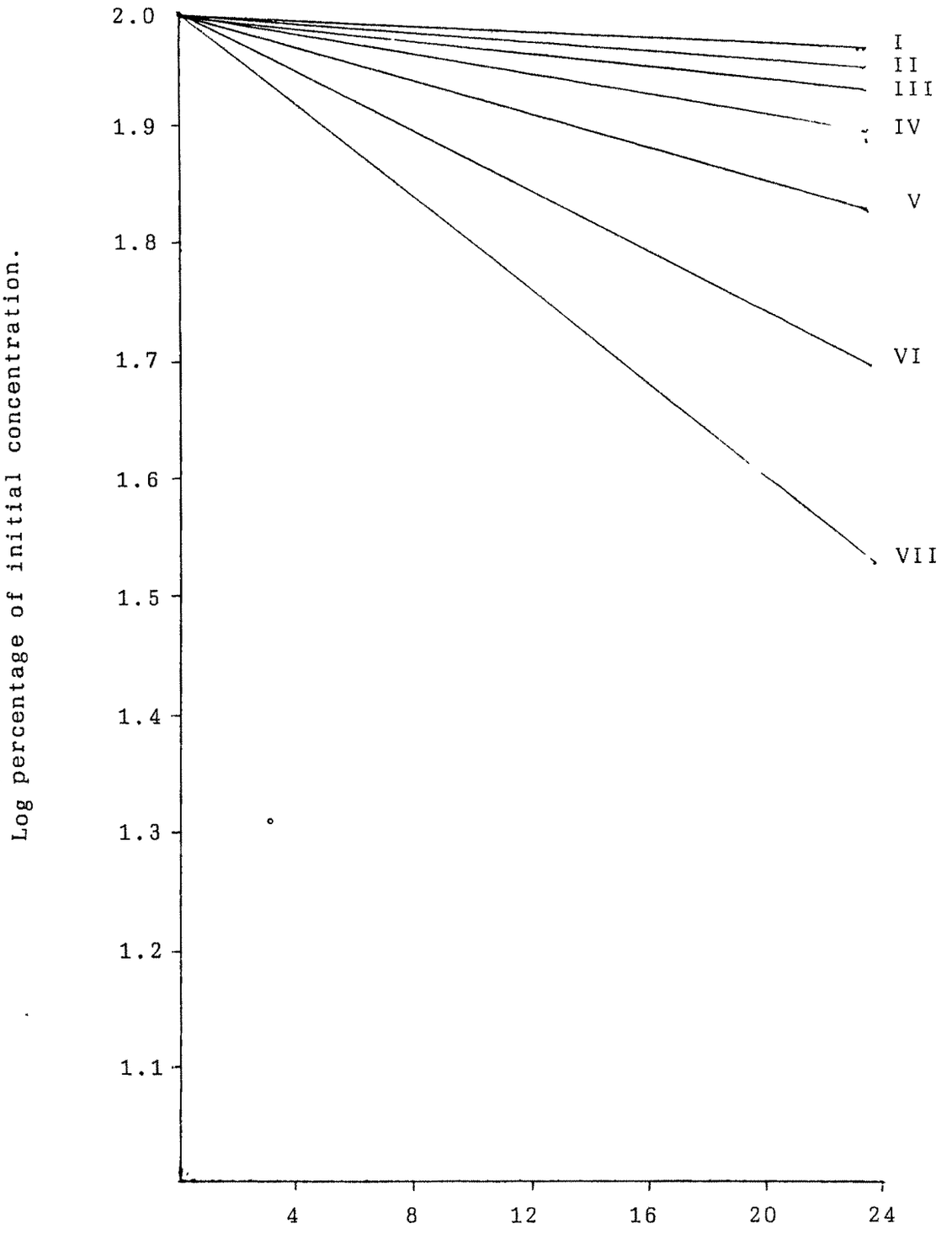
Degradation of 0.5% m/v solution of Timolol Maleate at 70° at (I) pH 3.0 & 4.0 (II) pH 5.0 (III) pH 6.0 (IV) pH 7.4 (V) pH 8.0 (VI) pH 9.0

Fig.



Degradation of 0.5% m/v solution of Nadolol at 70° at. (I) pH 7.0 (II) pH 6.0 (III) pH 4.0 (IV) pH 3.0 (V) pH 5.0 (VI) pH 8.0 (VII) pH 9.0

Fig.



Degradation of 0.5% m/v solution of Sotalol Hydrochloride at 70° at (I) pH 5.0 (II) pH 4.0 (III) pH 3.0 (IV) pH 6.0 (V) pH 7.4 (VI) pH 8.0 (VII) pH 9.0

The calculations of slope, rate constant (k) half life (t1/2) ie time required for 50% degradation of the drug and t 0.9 ie time required for 10% degradation of the Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride at pH 5, pH 4, pH 7.4 and pH 5 respectively are summerized in table 3.9 to 3.12.

Higher temperature and acidic pH are extremely detrimental to the stability of Nadolol. The temperature dependency of degradation was observed more explicitly from the Arrhenius plot obtained by plotting logerithm of ($k \times 10^{3}$) verses (1/Absolute temperature $\times 10^{3}$). In consonance with the Arrhenius equation:-

$$\log k = \frac{Ha}{2.303R} \cdot \frac{1}{T} + \log s$$

k : specific rate of degradation

R : gas constant (1.987 calories degree^{-1}.mole^{-1})

T : absolute temperature

S : frequency factor

Heat of activation (ΔH_a) which represents the energy of the reacting molecules must acquire in order to undergo reaction were calculated from slope of these graphs and were summerised in table 3.13 to 3.16 (the arrhenius plot commprised of 2 points 60° C, 70° C (figure 3.5, 3.6).

Lachman and De Luca[93] have reported that preparations that degraded through solvolytic processes e.g. reaction in solution generally have heats of activation in the range of 10 to 30 K cal mole^{-1}. Quite interestingly the heat of activation obtained for these drugs (Table 3.13 to 3.16) were found in the same region (10 - 25 K cal mole^{-1}) signifying the degradation through solvolytic process.

TABLE 3.9

KINETICS OF DEGRADATION OF 0.50% m/v SOLUTION OF PINDOLOL AT 60°C AND 70°C

Temperature	pH	Slope of regression line -K/2.303	Rate constant $K\ Hours^{-1}$	t1/2 hours	t0.9 hours
60°	3.0	-1.62×10^{-3}	3.72×10^{-3}	186.29	28.22
	4.0	-1.41×10^{-3}	3.25×10^{-3}	213.30	32.32
	5.0	-6.15×10^{-4}	1.42×10^{-3}	486.51	74.17
	6.0	-2.78×10^{-3}	6.40×10^{-3}	108.16	16.38
	7.4	-5.37×10^{-3}	1.24×10^{-2}	56.03	8.49
	8.0	-5.26×10^{-3}	1.19×10^{-2}	57.80	8.76
	9.0	-1.50×10^{-2}	3.50×10^{-2}	19.71	2.99
70°	3.0	-3.08×10^{-3}	7.10×10^{-3}	97.57	14.78
	4.0	-3.08×10^{-3}	7.10×10^{-3}	97.57	14.78
	5.0	-1.62×10^{-5}	3.72×10^{-3}	186.29	28.22
	6.0	-4.65×10^{-3}	1.07×10^{-2}	64.65	9.79
	7.4	-8.36×10^{-3}	1.92×10^{-2}	3.60	0.55
	8.0	-9.20×10^{-3}	2.12×10^{-2}	32.68	4.9
	9.0	-2.20×10^{-2}	5.03×10^{-2}	13.77	2.08

TABLE 3.10

KINETICS OF DEGRADATION OF 0.50% m/v SOLUTION OF TIMOLOL MALEATE AT 60° C AND 70° C

Temperature	pH	Slope of regression line -K/2.303	Rate constant K^{-1} Hours	t1/2 hours	t0.9 hours
60° C	3.0	-1.27×10^{-3}	2.93×10^{-3}	236.14	35.77
	4.0	-8.14×10^{-4}	1.87×10^{-3}	369.56	55.99
	5.0	-1.64×10^{-3}	3.77×10^{-3}	184.06	27.88
	6.0	-2.05×10^{-3}	4.73×10^{-3}	146.65	22.21
	7.4	-5.18×10^{-3}	1.19×10^{-2}	58.07	8.79
	8.0	-5.85×10^{-3}	1.35×10^{-2}	51.38	7.78
	9.0	-7.10×10^{-3}	1.63×10^{-2}	42.35	6.41
70°	3.0	-1.96×10^{-3}	4.52×10^{-3}	153.29	23.22
	4.0	-1.81×10^{-3}	4.18×10^{-3}	165.85	25.12
	5.0	-3.11×10^{-3}	7.18×10^{-3}	96.82	14.67
	6.0	-4.17×10^{-3}	9.60×10^{-3}	72.20	10.94
	7.4	-8.87×10^{-3}	2.04×10^{-2}	33.92	5.14
	8.0	-8.97×10^{-3}	2.06×10^{-2}	33.51	5.08
	9.0	-1.26×10^{-3}	2.90×10^{-2}	23.89	3.62

TABLE 3.11

KINETICS OF DEGRADATION OF 0.50% m/v SOLUTION OF NADOLOL AT 60° C AND 70° C

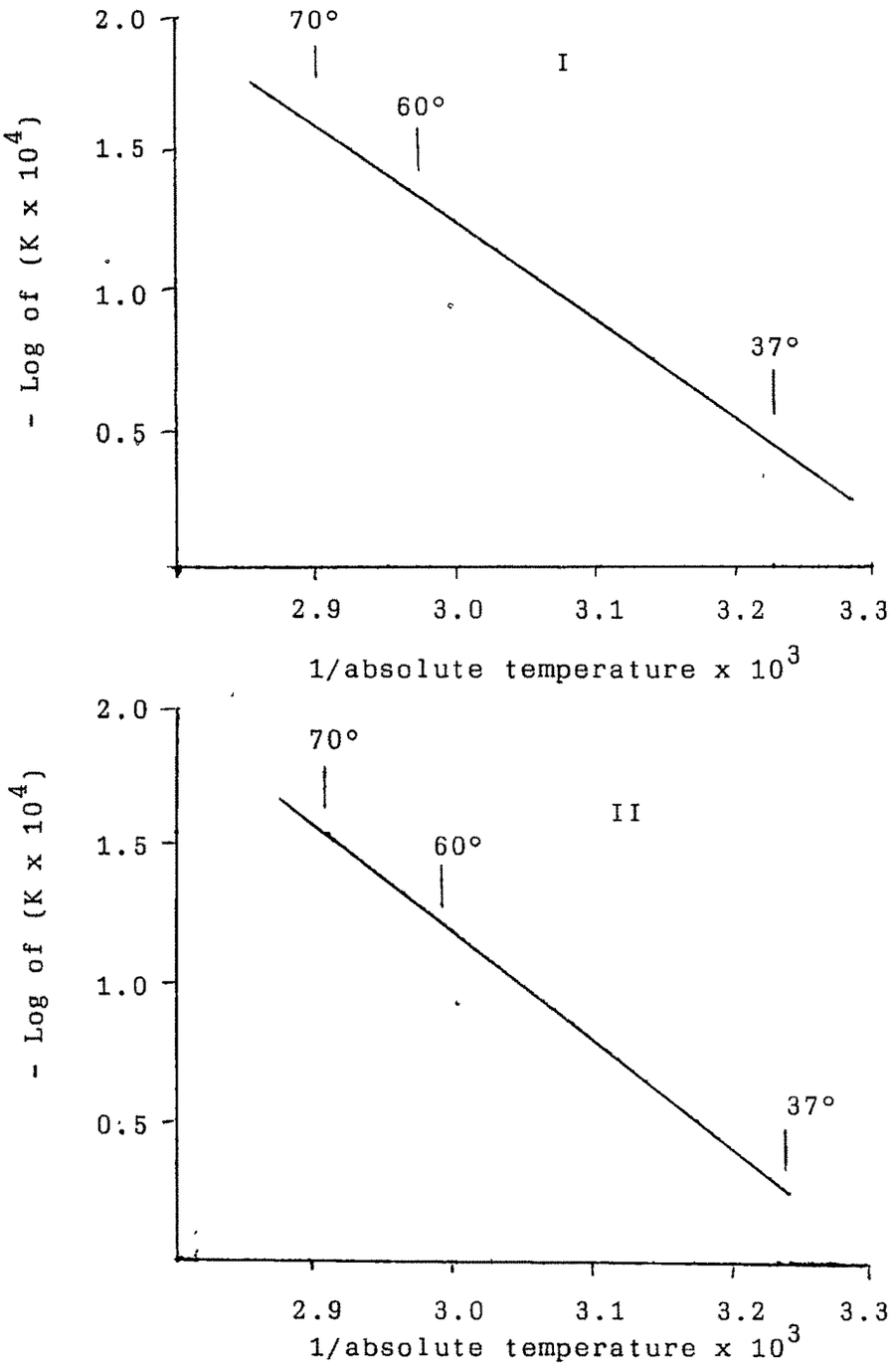
Temperature	pH	Slope of regression line -K/2.303	Rate constant K Hours^{-1}	t1/2 hours	t0.9 hours
60°	3.0	-2.05×10^{-3}	4.71×10^{-3}	146.94	22.26
	4.0	-1.63×10^{-3}	3.74×10^{-3}	184.92	28.01
	5.0	-2.36×10^{-3}	5.45×10^{-3}	126.94	19.28
	6.0	-1.27×10^{-3}	2.93×10^{-3}	236.14	35.77
	7.4	-6.17×10^{-4}	1.42×10^{-3}	488.03	73.94
	8.0	-2.78×10^{-3}	6.40×10^{-3}	108.17	16.38
	9.0	-5.86×10^{-3}	1.35×10^{-2}	51.38	7.78
70°	3.0	-4.16×10^{-3}	9.58×10^{-3}	72.28	10.95
	4.0	-2.89×10^{-3}	6.67×10^{-3}	103.82	15.73
	5.0	-4.31×10^{-3}	9.92×10^{-3}	69.79	10.57
	6.0	-2.17×10^{-3}	4.99×10^{-3}	138.68	21.01
	7.4	-1.62×10^{-3}	3.74×10^{-3}	184.92	28.01
	8.0	-4.65×10^{-3}	1.07×10^{-2}	64.64	9.79
	9.0	-9.03×10^{-3}	2.08×10^{-2}	33.31	5.05

TABLE 3.12

KINETICS OF DEGRADATION OF 0.50% m/v SOLUTION OF SOTALOL HYDROCHLORIDE AT 60° C AND 70° C

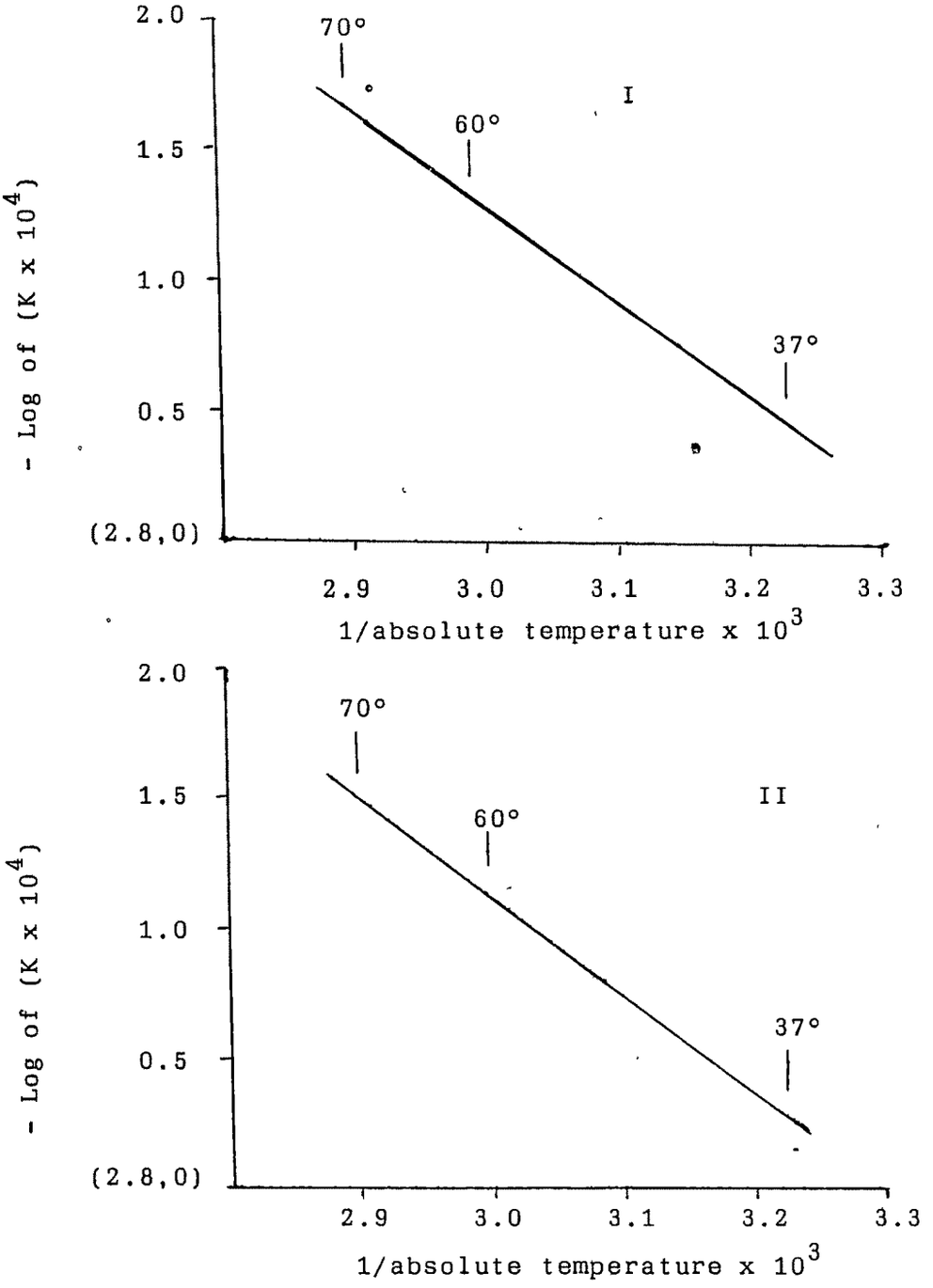
Temperature	pH	Slope of regression	Rate constant	t1/2 hours	t0.9 hours
60° C	3.0	-1.64×10^{-3}	3.76×10^{-3}	184.06	27.88
	4.0	-1.10×10^{-3}	2.53×10^{-3}	273.45	41.43
	5.0	-6.78×10^{-4}	1.56×10^{-3}	443.52	67.20
	6.0	-2.36×10^{-3}	5.45×10^{-3}	127.25	19.28
	7.4	-4.21×10^{-3}	9.69×10^{-3}	71.51	10.83
	8.0	-7.11×10^{-3}	1.64×10^{-2}	42.30	6.41
	9.0	-1.20×10^{-2}	2.84×10^{-2}	24.32	3.68
70° C	3.0	-2.71×10^{-3}	6.24×10^{-3}	111.03	16.82
	4.0	-2.08×10^{-3}	4.74×10^{-3}	146.21	22.15
	5.0	-1.40×10^{-3}	3.23×10^{-3}	214.49	32.49
	6.0	-4.29×10^{-3}	9.89×10^{-3}	70.10	10.61
	7.4	-7.11×10^{-3}	1.64×10^{-2}	42.30	6.41
	8.0	-1.26×10^{-2}	2.90×10^{-2}	23.89	3.62
	9.0	-1.98×10^{-2}	4.57×10^{-2}	15.14	2.29

Fig. 3.5



Arrhenious plot indicating temperature dependency of degradation of (I) Timolol Maleate at pH 4.0 (II) Pindolol at pH 5.0

Fig. 3.6



Arrhenious plot indicating temperature dependency of degradation of (I) Sotalol Hydrochloride at pH 5.0 (II) Nadolol at pH 7.4

On the basis of t 0.9 values in case of Pindolol solutions at pH 5, 60° C its shelf life is expected to be approximately 74 hours and at 70° C its self life is expected to be 28 hours (Table 3.9).

On the basis of t 0.9 values incase of Timolol Maleate solutions at pH 4, 60° C its self life is expected to be approximately 56 hours and at 70° C its shelf life is expected to be 25 hours (Table 3.10).

On the basis of t 0.9 values incase of Nadolol solutions at pH 7.4, 60° C its shelf life is expected to be approximately 74 hours and at 70° C its shelf life is expected to be 28 hours (Table 3.11).

On the basis of t 0.9 values in case of Sotalol hydrochloride solutions at pH, 5 60° C its shelf life is expected to be approximately 67 hours and at 70° C its shelf life is expected to be 32 hours (Table 3.12).

At higher temperature and high acid pH Pindolol gave pink colour the samples were extracted in chloroform and then analysed. Kinetics of degradation of 0.5% m/v solution of Pindolol, Timolol Maleate, Nadolol, Sotalol hydrochloride at pH 5, pH 4, pH 7.4 and pH 5 respectively at 60°, 70° is shown in table 3.13 to 3.16.

3.2 STABILITY OF PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE WHEN EXPOSED TO U.V. RADIACTION

The published litature did not reveal any data on the stability of these drugs in solution when exposed to UV light. The experiments were designed to study the effect of uv light on the solution of these drugs at their respective table pH values viz

TABLE 3.13

HEATE OF ACTIVATION FOR SOLVOLYTIC DEGRADATION OF PINDOLOL AT DIFFERENT pH

pH	Heate of activation KCal ΔHa from arrhenius plot : slope X 2.303 X R	Heate of activation Kcl ΔHa from $60^{\circ}C/70^{\circ}C$ Rate constant
3.0	14.270	14.282
4.0	17.265	17.274
5.0	21.250	21.336
6.0	11.360	11.366
7.4	9.670	9.776
8.0	12.560	12.592
9.0	7.90	7.906

TABLE 3.14

HEATS OF ACTIVATION FOR SOLVOLYTIC DEGRADATION OF TIMOLOL MALEATE AT DIFFERENT pH

pH	Heats of activation KCal ΔH_a from arrhenius plot : slope X 2.303 X R	Heats of activation Kcl ΔH_a from 60° C/70° C Rate constant
3.0	9.40	9.549
4.0	17.50	17.696
5.0	14.15	14.175
6.0	15.64	15.643
7.4	11.86	11.871
8.0	12.50	9.434
9.0	12.70	12.701

TABLE 3.15

HEATS OF ACTIVATION FOR SOLVOLYTIC DEGRADATION OF NADOLOL AT DIFFERENT pH

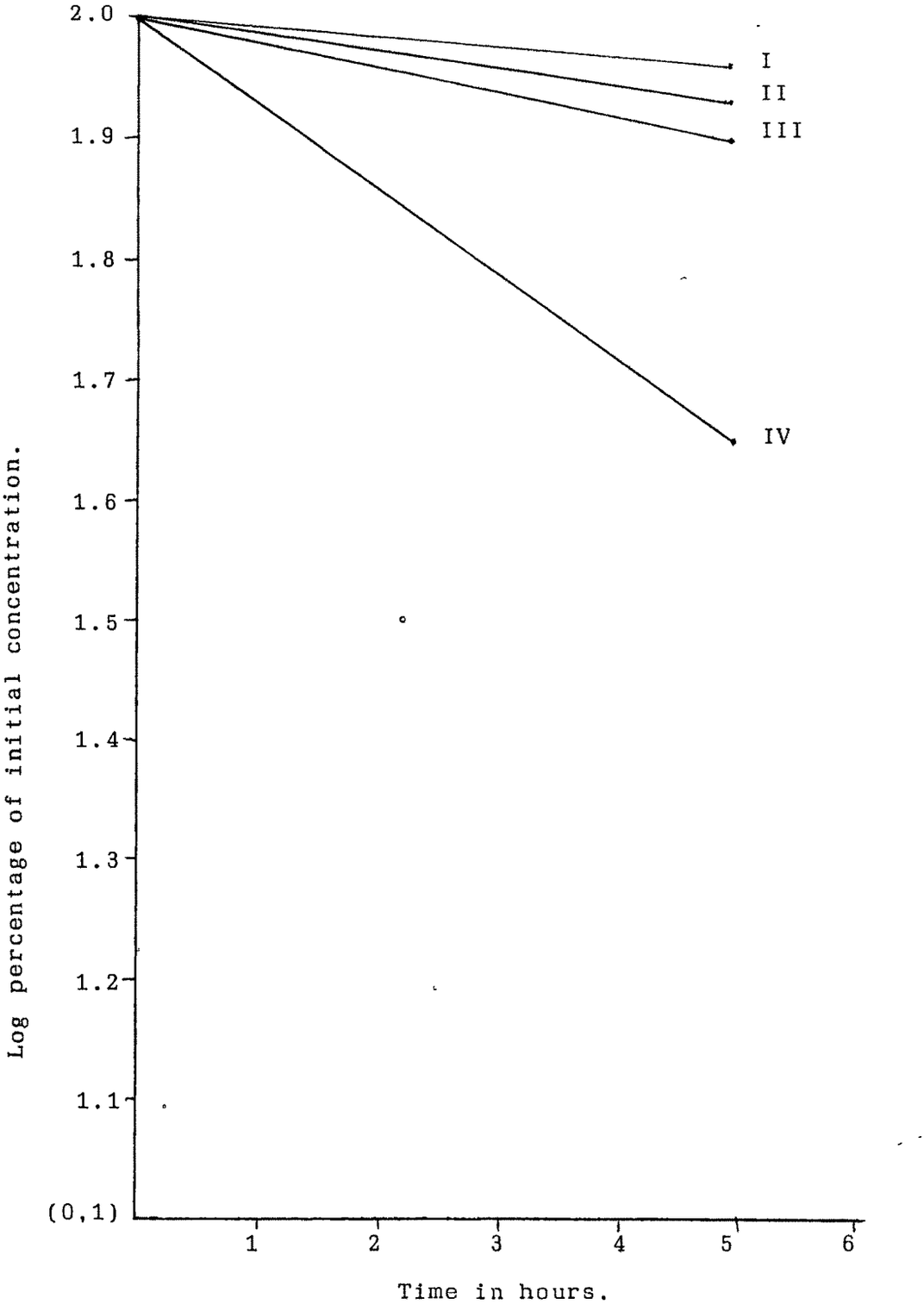
pH	Heate of activation KCal ΔH_a from arrhenius plot : slope X 2.303 X R	Heate of activation Kcl ΔH_a from 60°C/70°C Rate constant
3.0	15.500	15.665
4.0	12.650	12.749
5.0	13.220	13.261
6.0	11.730	11.753
7.4	21.500	21.429
8.0	11.400	11.367
9.0	9.600	9.569

TABLE 3.16

HEATE OF ACTIVATION FOR SOLVOLYTIC DEGRADATION OF SOTALOL HYDROCHLORIDE AT DIFFERENT pH

pH	Heate of activation KCal ΔH_a from arrhenius plot : slope X 2.303 X R	Heate of activation Kcl ΔH_a from 60° C/70° C Rate constant
3.0	11.400	11.389
4.0	13.720	13.819
5.0	16.000	16.044
6.0	13.000	13.169
7.4	11.500	11.592
8.0	22.650	22.670
9.0	10.470	10.474

Fig. 3.7



Degradation of 0.5% m/v solution of (I) Nadolol at pH 7.4 (II) Timolol Maleate at pH 4.0 (III) Sotalol Hydrochloride at pH 5.0 (IV) Pindolol at pH 5.0 under Uv radiation.

Pindolol at PH 5, Timolol maleate at PH 4, Nadolol at PH 7.4. and Sotalol hydrochloride at pH 5.

I EQUIPMENT

1. "Hitachi 2000-U" spectrophotometer.
2. Closed cabinet with near uv readiation (254 nm) output.

II REAGENTS

1. 0.50% m/v solution of Pindolol, Timolol Maleate, Nadolol AND Sotalol hydrochloride in methanol.
2. Phosphate and borate buffer pH 4, 5 and 7.6[60].
3. Methanol, Sodium sulphite, methyle paraben.

III EXPERIMENTAL PROCEDURE

50 ml 0.5% m/v solution of Pindolol in buffer pH 5, Timolol Maleate in pH 4, Nadolol in pH 7.4 and Sotalol hydrochloride in pH 5 contained in a 100 ml bicker was placed in the uv cabinet immediately, below the lamp with a maxium out put at 254 nm radiation. The beakers were kept in ice-water in a plastic bowl to minimize the change in volume by evaporation due to rise in temperature. 0.5 ml was trasnffered at 1/2 hours intervals of time and diluted with the same buffer and samples were scanned in the region of 220 nm to 350 nm uv light. The concentration was calculated at their absorbance maxima against the reagent blank.

The same sets of experiments were repeated by adding 0.1% m/v concentration of methyl paraben and sodium sulphite. The concentration of drug solution obtained was converted into percentage of initial concentration which was taken to be 100.

IV RESULT AND DISCUSSION

Of the four betablocker drugs tried all the samples were affected by uv light and they were degraded. In case Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride 44%, 15%, 9% and 10%

Table 3.17

STABILITY OF PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE IN UV RADIATION (254 nm) AFTER 5 HOURS EXPOSURE EXPRESED AS PERCENTAGE OF INITIAL CONCENTRATION

Sample	Pindolol pH 5	Timolol Maleate pH 4	Nadolol pH 7.4	Sotalol hydrochloride pH 5
Pure drug	56.21	84.91	90.98	89.80
Drug + methyl paraben	94.18	92.89	92.68	94.95
Drug + sodium sulphite	Due to blue colour experiment suspended	95.12	95.86	98.37

(figure 3.7) degradation respectively was observed. The presence of methyl paraben prevented the effect of uv light on drug samples and only 2-4% degradation was observed. The presence of sodium sulphite turned solution of Pindolol blue after 3 hours exposeure but the colour faded vere fast on removing it from uv chamber. the presence of sodium sulphite produced less degradation.

V CONCLUSION

From these observations it can be concluded that the presence of sodium sulphite prevents the degradation of these drugs in presence of uv radiation more then methyl paraben or sodium sulphite acts as a better preservative then methyl paraben in presence of uv radiation in case of Timolol Maleate,Nadolol and Sotalol hydrochloride but in case of Pindolol it produces colour formation.

3.3 STABILITY OF PINDOLOL, TIMOLOLMALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE WITH DIFFERENT TABLET EXCIPIENTS

The liturature survey reveled that no reference pertaining to stability of these drugs with different formulation excipients was published. This provoked interest to investigate the stability of these drugs with different excipients used in tablet formulation at elevated temperature and / or humidity.

I. EQUIPMENT

1. Carl.zeiss, Jena, spectrophotometer with 10 mm matched cells.
2. Perkin Elimer infrared spectrophotometer model 221 (observation were made as KBr pallets)
3. Thin layer chromatographic essembly.
4. uv cabinet.

II. REAGENTS

1. Standared samples of Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride.

2. Excipients used in tablet formulation : lactose, dicalcium phosphate, micro crystalline cellulose, maize starch, polyvinyl pyrrolidone, collidal sillicon dioxide, sodium lauryl sulphate, magneshium stearate and talc.

3. Silica gel G

4. Iodine

5. Solvent for TLC : i) methanol : strong ammonia solution (100:1.5) ii) cyclohexane : toluene : diethyl amine (75:15:10) iii) chloroform methanol (90:10)

6. Methanol

7. Acetyl acetone reagent as in 2.1 reagent

8. Sodium periodate 0.01M solution in water

III. EXPERIMENTAL PROCEDURE

a. Planning and conditions of storage.

Each drug substance was intimately mixed with 7 tablet excipients viz lactose, dicalcium phosphate, micro crystalline cellulose, maize starch, polyvinyl pyrrolidone, colloidal sillicon dioxide and sodium lauryl sulphate in the ratio of 1:5 (0.5 + 2.5) by separately triturating the mixture in a pastle and mortar. Each drug substance was also mixed with magnesium sterate and talc in the ration of 20:1 (1+ 0.050) in the same way. The uniform mixture of each drug excipient combination was distributed into 5 approximately equal parts and filled into white glass vials and closed with rubber stoppers except those to be stored at RH 75%. The vials were labelled appropirately wrapped with brown

papers and stored at ambient temperature,ambient temperature RH 75% 45°, 60° C and 60° C RH 75%, in thermostatially controlled ovens upto 150 days. Samples of excipients plain were also kept separately for the purpose of comparision. The condition of RH 75% was obtained by exposing the vials in the closed dessicator filled with saturated sodium chloride solution and maintained at the requisite temperature[59]. The samples were observed every fortinightly and their appearance was compared with those stored at ambient temperature and also with plain excipient stored at respective temperature to serve as control.

b. Method for Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride.

The samples stored in all the five conditions were analysied every 30 days. To 25 ml calibrated flask 10 mg sample of respective drug substances (standared) accurately weighed and dissolved using methanol to yield 400 µg/ml. Mixtures of the drugs with the excipients were finely powdered where ever needed and a quantity of mixture equvialent to 10 mg of respective drug (60 mg or 10.5 mg as the case may be) were transferred to 25 ml separate calibrated flask. To this was added methanol and contents shaken for 90 seconds using a vortex mixer. The solvent was added to volume and mixed to yield 400 µg/ml concentration and filtered through a fine pore fillter paper when not clear. To a separate set of 25 ml calibrated flasks 50 mg of respective excipients stored concurrently and the process was repeated to yield corresponding reagent blank solution.

The absorbance of clear solution of samples were measured at 264 nm, 295 nm, 269 nm and 269 nm,respectively in case of Pindolol,

Timolol Maleate, Nadolol and Sotalol hydrochloride. All the samples were also analysed using acetyl acetone reagent (section 2.10) and in addition to this the samples of Pindolol were also estimated by the method described in section 2.2 and 2.4.

c. Qualitative study of degradation by thin layer chromatography. Glass plates were coated uniformely with a layer of 0.25 mm of silica gel G and air dried and. The plats were activated and sparyed with 0.1 M potassium hydroxide in methanol and dried.

In 4 different sets, 3 plates each were spotted with 50 mcl solution of the following in the methanol containing the equivalent of 1% m/v of respective drug using a lambdar pipette.

1. Respective drug kept at RT for 150 days.
2. Respective drug kept at 60 C RT 75% for 150 days.
3. Respective drug + carboxy methyl cellulose kept at 60 C RH 75% for 150 days
4. Respective drug + polyvinyl pyrrolidone kept at 60 C RH 75% for 150 days.
5. Respective drug + sodium lauryl sulphate kept at 60 C RH 75% for 150 days.

The plates were separately kept in a chamber saturated with the following mobile phases and developed to a height of about 13 - 15 cm.

I. Methanol : strong ammonia solution (100:1.5)

II. Cyclohexane : toluene : diethyl amine (75:15:10)

III. Chloroform :methanol (90:10)

The plates were dried and subjected to detection under uv lamp equipped with 254 nm output and also to iodine vapour.

IV. RESULT AND DISCUSSION

a. Physical observations

Samples of Pindolol, Nadolol and Sotalol hydrochloride with different excipients behaved identically so far as their physical stability is concerned (Tablet 3.18, 3.20, 3.21). Sample of Timolol Maleate with different excipients also yielded nearly similar observations and in same case the interaction was even less (Table 3.19).

1. Sample of pure drug and their mixture in 1:5 proportion with dicalcium phosphate, lactose and colloidal silicon dioxide showed no signs of physical deterioration even after 150 days of storage at RT , RT RH 75%, 45° C, 60° C and 60° C RH 75%.

2. Mixture Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride showed noticeable physical deterioration with maize starch, talc and magnesim sterate at 60° C and 60° C RH 75% when compared with the excipients and drug samples stored alone under identical conditions. It is worth mentioning that megnesium sterate and talc though present in much less propertion, degraded the drug substance which were otherwise quite stable at 60° C and 60° C RH 75%.

3. Mixture of drug substances with carboxy methyl cellulose degraded at 60° C and 60° C RH 75 % turned soft / hard / sticky cake. While sample of carboxy methyl cellulose alone gave only slight change.

4. Mixture of drug substance with polyvinyl pyrrolidone appeared to interact rapidly and started forming yellow/ black colour semisolid cake in just one week time of storage. Which darkened

TABLE 3.18

PHYSICAL APPEARANCE OF PINDOLOL WITH DIFFERENT EXCIPIENTS AT ELEVATED TEMPERATURE AND /OR HUMITY OF AFTER 150 DAYS OF STORAGE

Storage condition / sample	RT	RT RH 75%	45° C	60° C	60° C RH 75%
P1	White free flowing powder	same as RT	same as RT	White-off white free flowing powder	same as 60 C
P2	White free flowing powder	same as RT	same as RT	White-off white free flowing powder	same as 60 C
P3	White free flowing powder	same as RT	same as RT	White-off white free flowing powder	same as 60 C
P4	White free flowing powder	White-light yellow moist cake	Off white light-yellow hard cake	Gray-yellow hard cake	Deep yellow-brown moist hard cake
P5	White free flowing powder	same as RT	same as RT	White-off white powder tends to agglomerate	White-off white powder tends to Agglomerate
P6	White free flowing powder	White-light yellow moist cake	Off white light-yellow hard cake	Grey yellow hard cake	Deep yellow-brown moist hard cake
P7	White free flowing powder	Tends to agglomerate	White-off white free flowing powder	same as 45 C	Yellow clear transparent solution
P8	White free flowing powder	same as RT	white-off white free flowing powder	same as 45 C	Grayish powder adhering to the bottom
P9	White free flowing powder	same as RT	white-off white free flowing powder	Off white,light greyish powder adhering to the bottom	Greyish powder adhering to the bottom
P10	White free flowing powder	same as Rt	same as RT	white-off white free flowing powder	same as 60 C

P1 = Pindolol pure
P2 = Pindolol + lactose (1:5)
P3 = Pindolol + dicalcium phosphate (1:5)
P4 = Pindolol + microcrystalline cellulose (1:5)
P5 = Pindolol + maize starch (1:5)
P6 = Pindolol + polyvinyl pyrrolidone (1:5)
P7 = Pindolol + sodium lauryl sulphate (1:5)
P8 = Pindolol + magnesium stearate (20:1)
P9 = Pindolol + talc (20:1)
P10 = Pindolol + silicone dioxide (1:5)

TABLE 3.19

PHYSICAL APPEARANCE OF TIMOLOL MALEATE WITH DIFFERENT EXCIPIENTS AT ELEVATED TEMPERATURE AND /OR HUMIDITY AFTER 150 DAYS OF STORAGE

Storage condition sample	RT	RT RH 75%	45° C	60° C	60° C RH 75%
T1	White free flowing powder	same as RT	same as RT	White-off white free flowing powder	same as 60 C
T2	White free flowing powder	same as RT	same as RT	White-off white free flowing powder	same as 60 C
T3	White free flowing powder	same as RT	same as RT	White-off white free flowing powder	same as 60 C
T4	White free flowing powder	same as RT	same as RT	Off white yellow hard cake	Brown moist cake
T5	White free flowing powder	Tends to agglomerate	Tends to agglomerate	Off white-light greyish powder flowes in lumps	Off white white powder adhering to bottom
T6	White free flowing powder	White,light yellow moist cake	Light yellow some what hard cake	Yellow extermely hard cake	Deep yellow light brown semi transperent exteremly hard mass
T7	White free flowing powder	Tends to agglomerate	Tends to agglomerate	Off white very light grey powder forming small agglomerates	Yellow clear transperant liquid
T8	White free flowing powder	same as RT	same as RT	same as RT	White-off white powder adhering to bottom
T9	White free flowing powder	same as RT	same as RT	same as RT	Light yellow-grey powder adhering to bottom
T10	White free flowing powder	same as RT	same as RT	White-off white free flowing powder	Off white powder forming small agglomerates

T1 = Timolol maleate pure
T2 = Timolol maleate + lactose (1:5)
T3 = Tindolol + dicalcium phosphate (1:5)
T4 = Timolol maleate + microcrystalline cellulose (1:5)
T5 = Timolol maleate + maize starch (1:5)
T6 = Timolol maleate + polyvinyl pyrrolidone (1:5)
T7 = Timolol maleate + sodium lauryl sulphate (1:5)
T8 = Timolol maleate + magnesium stearate (20:1)
T9 = Timolol maleate + talc (20:1)
T10 = Timolol maleate + silicone dioxide (1:5)

TABLE 3.20

PHYSICAL APPEARANCE OF NADOLOL WITH DIFFERENT EXCIPIENTS AT ELEVATED TEMPERATURE AND /OR HUMITY AFTER 150 DAYS OF STORAGE

Storage condition sample	RT	RT RH 75%	45°C	60°C	60°C RH 75%
N1	White free flowing powder	same as RT	same as RT	White off white free flowing powder	White off white free flowing powder
N2	White free flowing powder	same as RT	same as RT	White off white free flowing powder	White off white free flowing powder
N3	White free flowing powder	same as RT	same as RT	White off white free flowing powder	White off white free flowing powder
N4	White free flowing powder	White-off white moist cake	Off white-light yellow hard cake	same as 45 C	Deep yellow brown moist cake
N5	White free flowing powder	same as RT	same as RT	White off white powder tends to agglomerate	Off white powder adhering to bottom
N6	White free flowing powder	White off white moist cake	Off white light yellow hard cake	same as 45 C	Deep brown hard mass
N7	White free flowing powder	Tends to agglomerate	Tends to agglomerate	Off white powder adhering to bottom	same as 60 C
N8	White free flowing powder	same as RT	same as RT	same as RT	White off white powder adhering to bottom
N9	White free flowing powder	same as RT	same as RT	same as RT	Light yellow powder adhering to bottom
N10	White free flowing powder	same as RT	same as RT	White off white free flowing powder	same as 60 C

N1 = Nadolol pure
N2 = Nadolol + lactose (1:5)
N3 = Nadolol + dicalcium phosphate (1:5)
N4 = Nadolol + microcrystalline cellulose (1:5)
N5 = Nadolol + maize starch (1:5)
N6 = Nadolol + polyvinyl pyrrolidone (1:5)
N7 = Nadolol + sodium lauryl sulphate (1:5)
N8 = Nadolol + magnesium stearate (20:1)
N9 = Nadolol + talc (20:1)
N10 = Nadolol + silicone dioxide (1:5)

TABLE 3.21

PHYSICAL APPEARANCE OF SOTALOL HYDROCHLORIDE WITH DIFFERENT EXCIPIENTS AT ELEVATED TEMPERATURE AND /OR HUMITY AFTER 150 DAYS OF STORAGE

Storage condition sample	RT	RT RH 75%	45° C	60° C	60° C RH 75%
S1	White free flowing powder	same as RT	same as RT	White off white free flowing powder	White off white free flowing powder
S2	White free flowing powder	same as RT	same as RT	White off white free flowing powder	White off white free flowing powder
S3	White free flowing powder	same as RT	same as RT	White off white free flowing powder	White off white free flowing powder
S4	White free flowing powder	White-light yellow moist cake	Off white-light yellow hard cake	Gray yellow hard cake	Deep yellow brown moist hard cake
S5	White free flowing powder	same as RT	same as RT	White off white powder flows in lumps	Off white powder adhering to bottom
S6	White free flowing powder	White-light yellow moist cake	Off white light yellow hard cake	Yellow exteremly hard cake	Deep brown semi transperent hard mass
S7	White free flowing powder	Tends to agglomerate	Tends to agglomerate	White-off white powder adhering to bottom	same as 60 C
S8	White free flowing powder	same as RT	same as RT	same as RT	White-off white powder adhering to bottom
S9	White free flowing powder	same as RT	same as RT	same as RT	Light yellow powder adhering to bottom
S10	White free flowing powder	same as RT	same as RT	White-off white free flowing powder	Off white powder forms small agglomerates

S1 = Sotalol hydrochloride pure
S2 = Sotalol hydrochloride + lactose (1:5)
S3 = Sotalol hydrochloride + dicalcium phosphate (1:5)
S4 = Sotalol hydrochloride + microcrystalline cellulose (1:5)
S5 = Sotalol hydrochloride + maize starch (1:5)
S6 = Sotalol hydrochloride + polyvinyl pyrrolidone (1:5)
S7 = Sotalol hydrochloride + sodium lauryl sulphate (1:5)
S8 = Sotalol hydrochloride + magnesium stearate (20:1)
S9 = Sotalol hydrochloride + talc (20:1)
S10 = Sotalol hydrochloride + silicone dioxide (1:5)

on further storage. However samples of polyvinly pyrrolidone stored alone alos tended to yield smilar observations through a bit less rapidly and the drugs in questions seemed to interact with polyvinyl pyrrolidone.

b. Quntitative degradation study by uv spectrophotometric assay

The recoveries of Pindolol, Timolol Maleate maleate Nadolol and Sotalol hydrochloride with different excipients at elevated temperature and /or humidity after 150 days of storage and also every month as measured by uv spectrophotmetric assay prescribed in experimental procedure. The assa values refer to 150 days of storage unless specified otherwise in the ensuing discussion (Table 3.22, 3.23, 3.24, 3.25).

1. Drug substance alone remined quite stable at RT RH 75% and 45° C. About 3-5% and 6- 8 % degradation was observed at 60° C and 60° C RH 75% respectively.

2. In testimony of the physical degradation mentioned above samples of Pindolol. Timolol Maleate, Nadolol and Sotalol hydrochloride stored with lactose showed some degradation at 60° C and 60° C RH 75% althoug physical stability was good. About 5.0 % and 6.0% degradation was observed in case of Pindolol. 3.5% and 5.5% in case of Timolol Maleate, 5.0% and 7.0% in case of Nadolol and 3.0% and 5.0% degradation was observed in case of Sotalol hydrochloride. The samples stored at 45° C were however quite stable.

3. Mixtures of all the four drugs in question displayed excellent stability at RT RH 75%, 45° C and 60° C with dicalcium phosphate where as oabout 4.5% degradation occured at 60° C RH 75% in case of Pindolol.

Table 3.22

UV SPECTROPHOTOMERIC ASSAY OF PINDOLOL WITH DIFFERENT EXCIPIENTS AT ELEVATED TEMPERATURE AND / OR HUMIDITY EXPRESSED AS PERCENTAGE OF INITIAL CONCENTRATION

Sample Storage Condition	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Initial	100	100	100	100	100	100	100	100	100	100
60°C RH 75% 60 DAYS	-	98.32	-	98.02	-	98.48	100.21	0-C	—	—
60°C 90 days	101.12	97.40	—	94.50	99.21	93.40	—	—	-	98.44
60°C RH 75% 90 days	—	96.10	—	95.81	—	94.60	92.86	—	93.90	91.64
RT RH 75% 150 days	99.27	96.60	99.15	93.40		91.50		99.05	99.00	
45°C 150 days	98.40	97.70	99.00	93.76	100.90	92.50	92.00	98.30	98.60	101.50
60°C 150 days	97.50	95.15	99.23	91.50	100.02	90.00	89.40	97.50	97.41	94.37
60°C RH 75% 150 days	94.56	94.31	96.38	92.10	90.62	88.00	88.66	94.50	94.56	91.60

Blank columns represents non assayable conditions
- Not assyed

P1 = Pindolol pure
P2 = Pindolol + lactose (1:5)
P3 = Pindolol + dicalcium phosphate (1:5)
P4 = Pindolol + microcrystalline cellulose (1:5)
P5 = Pindolol + maize starch (1:5)
P6 = Pindolol + polyvinyl pyrrolidone (1:5)
P7 = Pindolol + sodium lauryl sulphate (1:5)
P8 = Pindolol + magnesium stearate (20:1)
P9 = Pindolol + talc (20:1)
P10 = Pindolol + silicone dioxide (1:5)

Table 3.23

UV SPECTROPHOTOMERIC ASSAY OF TIMOLOL MALEATE WITH DIFFERENT EXCIPIENTS AT ELEVATED TEMPERATURE AND / OR HUMIDITY EXPRESED AS PERCENTAGE OF INITIAL CONCENTRATION

Sample Storage Condition	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Initial	100	100	100	100	100	100	100	100	100	100
60° C RH 75% 60 days	98.91	—	100.12	99.02	—	97.91	—	—	—	—
60° C 90 days	100.40	98.77	97.35	96.50	98.56	94.10	—	97.61	—	—
60° C RH 75% 90 days	—	93.95	96.85	97.81	—	94.12	—	—	—	—
RT RH 75% 150 days	98.70			95.50		92.00	98.60	98.60	98.70	100.12
45° C 150 days	99.83	101.12	100.02	95.90	101.00	93.20	91.95	99.74	100.10	98.15
60° C 150 days	96.50	96.41		93.50	99.40	91.10	91.08	96.50	96.31	90.00
60° C RH 75% 150 days	95.00	94.50		93.50	91.04	89.32		94.30	94.50	

Blank columns represents non assayable conditions

- not assayed

T1 = Timolol maleate pure
T2 = Timolol maleate + lactose (1:5)
T3 = Tindolol maleate + dicalcium phosphate (1:5)
T4 = Timolol maleate + microcrystalline cellulose (1:5)
T5 = Timolol maleate + maize starch (1:5)
T6 = Timolol maleate + polyvinyl pyrrolidone (1:5)
T7 = Timolol maleate + sodium lauryl sulphate (1:5)
T8 = Timolol maleate + magnesium stearate (20:1)
T9 = Timolol maleate + talc (20:1)
T10 = Timolol maleate + silicone dioxide (1:5)

Table 3.24

UV SPECTROPHOTOMERIC ASSAY OF NADOLOL WITH DIFFERENT EXCIPIENTS AT ELEVATED TEMPERATURE AND / OR HUMIDITY EXPRESED AS PERCENTAGE OF INITIAL CONCENTRATION

Sample Storage Condition	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10
Initial	100	100	100	100	100	100	100	100	100	100
60° C RH 75% 60 days	—	—	—	—	—	88.48	—	—	98.61	—
60° C 90 days	100.40	—	99.80	98.50	97.56	93.40	99.31	—	95.20	95.52
60° C RH 75% 90 days	—	—	—	91.70	—	84.60	—	—	100.00	97.00
RT RH 75% 150 days	101.25	99.64	99.50			91.50		99.60	99.54	
45° C 150 days	98.50	100.98		101.15	99.21	92.10	97.75	98.45	98.41	98.49
60° C 150 days	96.70	95.11	98.86	94.20	99.38	81.80	89.20	96.02	96.50	
60° C RH 75% 150 days	95.00	93.50		91.00	89.10	78.00	88.62	95.00	95.24	94.85

Blank columns represents nonassayable conditions
- Not assayed
N1 = Nadolol pure
N2 = Nadolol + lactose (1:5)
N3 = Nadolol + dicalcium phosphate (1:5)
N4 = Nadolol + microcrystalline cellulose (1:5)
N5 = Nadolol + maize starch (1:5)
N6 = Nadolol + polyvinyl pyrrolidone (1:5)
N7 = Nadolol + sodium lauryl sulphate (1:5)
N8 = Nadolol + magnesium stearate (20:1)
N9 = Nadolol + talc (20:1)
N10 = Nadolol + silicone dioxide (1:5)

Table 3.25

UV SPECTROPHOTOMERIC ASSAY OF SOTALOL HYDROCHLORIDE WITH DIFFERENT EXCIPIENTS AT ELEVATED TEMPERATURE AND / OR HUMIDITY EXPRESED AS PERCENTAGE OF INITIAL CONCENTRATION

Sample Storage Condition	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Initial	100	100	100	100	100	100	100	100	100	100
60° C RH 75% 60 days	99.91	—	—	—	—	86.58	97.50	100.21	101.35	100.12
60° C 90 days	100.20	100.00	99.75	98.30	97.50	94.35	—	99.61	99.51	99.15
60° C RH 75% 90 days	97.00	98.81	—	91.50	—	82.30	—	97.93	97.73	—
RT RH 75% 150 days	98.79	98.50	99.50			89.52		98.49	98.60	
45° C 150 days	99.73			100.10	98.21	90.10	95.70	99.70	99.70	99.00
60° C 150 days	99.50	97.30	98.85	95.00	98.00	79.80	92.10	99.67	99.87	95.00
60° C RH 75% 150 days	95.00	95.00		92.00	91.25	76.00		95.00	95.09	

Blank columns represents non assayable conditions
- Not assayed

S1 = Sotalol hydrochloride pure
S2 = Sotalol hydrochloride + lactose (1:5)
S3 = Sotalol hydrochloride + dicalcium phosphate (1:5)
S4 = Sotalol hydrochloride + microcrystalline cellulose (1:5)
S5 = Sotalol hydrochloride + maize starch (1:5)
S6 = Sotalol hydrochloride + polyvinyl pyrrolidone (1:5)
S7 = Sotalol hydrochloride + sodium lauryl sulphate (1:5)
S8 = Sotalol hydrochloride + magnesium stearate (20:1)
S9 = Sotalol hydrochloride + talc (20:1)
S10 = Sotalol hydrochloride + silicone dioxide (1:5)

4. Mixtures of Timolol Maleate, Nadolol and Sotalol hydrochloride displayed good stability with silicon dioxide except at 60° C RH 75% where about 6% degradation occured in case of Nadolol Samples of Pindolol stored at 60° C and 60° C RH 75% however showed 6% degradation.

5. In agreement with the physical degradation mentioned above samples of all the four drugs. with polyvinyl pyrrolidone showed degradation at all the temperature and humidity conditions viz about 8-10.5%, 7-10%, 9-20%,and 11-24% at RT RH 75%,45° C, 60° C and 60° C RH 75% respectively for Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride.

6. Mixture of Pindolol and Timolol Maleate with micro crystalline cellulose showed about 8 - 12% degradation at 60° C and 60° C RH 75%. Where as samples of Nadolol and Sotalol hydrochloride yielded about 5% and 8% degradation at 60° C and 60° C RH 75%.

7. Quite expectedly samples of Pindolol, Timolol Maleate and Nadolol reacted with sodium lauryl sulphate and yielded about 8,8 and 2.5 % degradation at 45° C and 10.5, 9 and 11% degradation at 60° C. However samples of Sotalol hydrochloride shood 4% and 8% degradation at 45° C and 60° C respectively.

8. Mixture of all the four drugs showed negligible degradation with maize starch except at 60° C RH 75%, where as 9 - 11% degradation occured at 60° C RH 75%.

9. Mixture of all the four drugs in question with magnesium stearate showed degradation pattern same as those of pure drugs although the mixtures were found to degrade physically at 60° C and 60° C RH 75%.

10. Mixture of drug substances with talc showed degradation

pattern same as of pure drugs althoug the mixtures were found to degrade physically at 60° C and 60° C RH 75%.

In conclusion, degradation of drug substances occured with following excipients at different storage conditions:-

1. RT RH 75% polyvinyl pyrrolidone.
2. 45° C polyvinyl pyrrolidone, sodium lauryl sulphate.
3. 60° C lactose, sodium lauryl sulphate.
4. 60° C RH 75% lactose, maize starch, polyvinyl pyrrolidone, colloidal silicon dioxide, sodium lauryl sulphate.

The degradation profile as stated above is based on uv spectrophotometric assay method which was adopted in view of its simplicity for analysis.

c. Qualitative degradation study by thin layer chromotography

The calculation of Rf values of Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride kept at RT and 60° C RH 75% for 150 days and as mixture with polyvinyl pyrrolidone in 1:5 proportion kept at 60° C RH 75% for 150 days when using 3 different solvent sysytems are described in Table 3.26. The exposure to uv radiations was not found to be suitable mode of detection. Exposing to iodine vapours suited well for detecting the spots. In all three system tried, samples of respective drug kept at RT, 60° C RH 75% and admixture with polyvinyl pyrrolidone kept at 60° C RH 75% gave only one spot at the nearly same Rf value. This did not augur well with the uv spectrophotmetric assay values which showed a significant reduction in case of mixture with polyvinyl pyrrolidone.

The calculations of Rf values of Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride kept at RT and their mixture

Table 3.26

TLC DATA (RF VALUE) OF PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE WITH POLYVINYL PYRROLIDONE AT 60° C

Solvent system	Rf X 100							
	1	2	3	4	5	6	7	8
		**		**		*		*
Methanol : strong ammonia solution (100:1.5)	91.0	50.0	93.0	46.0	89.0	35.0	95.0	76.0
		**		**		*		*
Cyclohexane: toluene : diethyl amine (75 : 15 : 10)	82.0	31.0	90.0	37.0	70.0	28.20	71.0	52.0
		**		**		**		*
Cloroform : Methanol (90 : 10)	90.0	41.0	90.0	39.0	70.0	25.0	90.0	58.0

1. Pindolol at RT
2. Pindolol + polyvinyl pyrrolidone (1:5)
3. Timolol Maleate at RT
4. Timolol Maleate + polyvinyl pyrrolidone (1:5)
5. Nadolol at RT
6. Nadolol + polyvinyl pyrrolidone (1:5) at 60 C
7. Sotalol hydrochloride at RT
8. Sotalol hydrochloride + polyvinyl pyrrolidone (1:5)

Detection by Iodine vapour
* Band < 50 mm
** Ellongated band >= 50 mm

with carboxy methyl cellulose and sodium lauryl sulphate kept at 60° C for 150 days when using 3 different solvent system are described in Table 3.27

The chromatogram developed with first solvent system comprising of methanol : strong ammonia solution (100:1.5) revealed that drug substances did interact with carboxymethyl cellulose and sodium lauryl sulphate giving elongated bands compared to single spot in case of pure drug substances. Interestingly enough, an additional spot was seen at about 0.5 - 0.55 Rf in case of mixture with soidium lauryl sulphate.

The chromotram developed with second solvent system comprising of cyclohexame : toluene:diethyl amine (75:15:10) also displayed elongated bands in case of mixture with carboxy methyl cellulose and sodium lauryl sulphate as against single spot in case of pure drug substances. In case of second solvent system tried pure drug substances travelled as a single spot showing satisfactory Rf values and the mixtures with carboxymethyl cellulose and sodium lauryl sulphate travelled as an elongated band.

In essence, the drug substances in question were found to react with polyvinyl pyrrolidone and soidum lauryl sulphate giving elongated bands as compared to single spot in case of pure drug substances. Besides an extra spot at Rf value different from the pure drugs was observed in case of mixture with sodium lauryl sulphate. These findings augur well with the uv spectrophotometric assay values which showed a significant reduction in assay values in case of mixture with polyvinyl pyrrolidone and soidum lauryl sulphate.

Table 3.27

TLC DATA (Rf VALUE) OF PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE WITH SODIUM LAURLY SULPHATE AT 60° C

Solvent system	Rf X 100							
	1	2	3	4	5	6	7	8
Methanol : strong ammonia solution (100 : 1.5)	91.0	45.0	93.0	50.0	89.0	45.0	95.0	72.0 *
Cyclohexane : toluene : diethyl amine (75:15:10)	82.0	31.0	90.0	41.0 **	70.0	30.0 *	71.0	53.0 *
Chloroform : methanol (90 : 10)	90.0	33.0	92.0	42.0 **	70.0	30.0 *	90.0	64.0 *

1. Pindolol at RT
2. Pindolol + sodium lauryl sulphate
3. Timolol Maleate at RT
4. Timolol Maleate + sodium lauryl sulphate
5. Nadolol at RT
6. Nadolol + sodium lauryl sulphate
7. Sotalol hydrochloride at RT
8. Sotalol hydrochloride + sodium lauryl sulphate

Detection Iodine vapour

* Band < 50 mm

** Ellongated band >= 50 mm

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

Since there was no published literature available on the data of stability of these drugs in question with the hydrochlorthiazide diuretic which occures generally as a componant in several formulations along with some of the betablocker drugs. Experiments were designed to study the stability of these drugs with hydrochlor thiazide in synthetic mixtures.

I. REAGENTS

1. Sample of Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride.
2. Sample of hydrochlorthiazide.
3. methanol.

II. EXPERIMENTAL PROCEDURE

Each drug substance was intimately mixed with hydrochlorthiazide in the ratio (4:1). The uniform mixture of each drug with hydrochlor thiazide was distributed in to 5 appropriately equal parts and filled in to colourless glass vials and closed with rubber stoppers except those to be stored at RH 75%. The vials were labelled appropriately wrapped with brown or black papers and stored at ambient temperature, ambient temperature RH 75%, 45° C, 60° C and 60° C RH 75% in thermostatically controlled ovens up to 150 days. Samples of the bulk drugs were also kept separately as controls. The samples were analysed at the end of 150 days by uv method as described in section 3.3.

III. RESULT AND DISCUSSION

The samples of Pindolol, Timolol Maleate, Nadolol and Sotalol

Table 3.28

UV SPECTROPHOTOMETRIC ASSAY OF PINDOLOL, TIMOLOL MALEATE, NADOLOL AND SOTALOL HYDROCHLORIDE WITH HYDROCHLORTHIAZIDE DIURETIC AT ELEVATED TEMPERATURE AND / OR HUMIDITY

Storage condition	Amount recovered as percentage of initial assay							
	Pindolol		Timolol Maleate		Nadolol		Sotalol hydrochloride	
Initial	100		100		100		100	
	1	2	1	2	1	2	1	2
RT RH 75 % 150 days	99.27	98.81	98.70	97.90	101.25	100.00	98.79	98.50
45° C 150 days	98.40	97.90	99.83	98.40	98.50	98.30	99.73	99.00
60° C 150 days	97.50	96.30	96.50	95.25	96.70	96.00	99.50	99.00
60° C RH 75% 150 days	94.56	92.25	95.00	93.71	95.00	94.00	95.00	95.50

1. Control
2. With diuretic combination

hydrochloride with hydrochlorthiazide showed no signs of deterioration even after 150 days of storage at RT and 45° C. The samples showed degradation upto the extent of 8%, 6%, 6% and 5.5% observed in case of Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride respectively (table 3.28)at 60° C RH 75% after 150 days storage.

3.5 HPLC METHOD FOR THE STABILITY STUDY OF TIMOLOL MALEATE

Interestingly 75% of the HPLC methods of analysis published for the analysis of Timolol Maleate is based on reverse phase partition chromatography. (Section 1.2f).

Many of these showed the following features in common. Octadecyl silanized silica gel column, mixture of water and organic solvent (methanol in most cases) as mobile phase containing one of the following to bring the pH to acidic range. Phosphoric acid, sulfonic acid, sodium phosphate, flow rate 1-2 ml/minute and detection by uv at 215 nm/280 nm.

In view of all these methods published the experimetns were designed adopting the reverse phase partition chromatography using simple solvent system giving satisfactory results.

I. EQUIPMENT

Waters 590 High pressure liquid chromatograph with a C18 Bonda-Pack column and variable uv detector 484 model (waters 745 / 45 B datamodel)

II. REAGENTS

1. Sample of Timolol Maleate.

2. Methanol, acetonitrile (HPLC grade solvents)

III EXPERIMENTAL PROCEDURE

a. preparation of sample solution

0.1% m/v solution of Timolol Maleate was prepared in methanol.

b. Preparation of Mobile phase

mixture of methanol : Acetonitrle (50:50) was prepared for the use as mobile phase.

c. Operation conditions

Chromatograph was run observing the following specifications.

Injection : 10 ul of each solution was injected.

Mobile phase : As described above

Flow rate : 0.7 ml/minute

uv detector : 300 nm

Chart speed : 5/10 mm/minute

d. Preparation of stability samples of Timolol Maleate with different excipient for analysis.

Mixture with different ecipient as mentitioned in 3.5, equivalent to 10 mg of Timolol Maleate shaken with HPLC grade methanol and filtered.

IV RESULT AND DISCUSSION

In the experiment methanol: acetronitrile mixture (50:50) was used as a mobile phase in view of the fact that none of the published method involve the use of this common solvent system in the revered phase chromotrography. It was observed that the simple methanol : acetonitrile solvent system gave satisfactory results. The operation conditions described suited the experiment. A plot of peal area of standard Timolol Maleate vs concentration of the drug injected was linear upto 5 ug.

The retention time was found to be 3.09 minutes. The stability results obtained by this method were comparable with the uv method described in section 3.3 (Table 3.29). This type of

Table 3.29

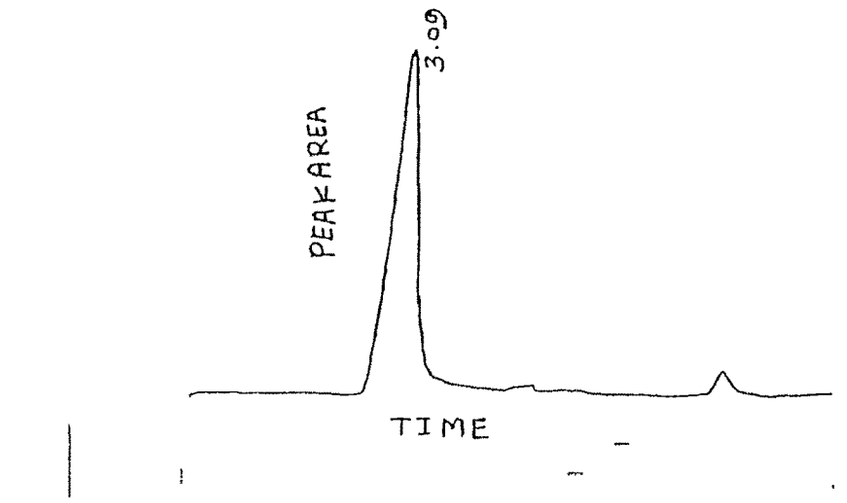
RESULTS OF ASSAY OF TIMOLOL MALEATE WITH DIFFERENT EXCIPIENTS AT ELEVATED TEMPERATURE AND / OR HUMIDITY EXPRESSED AS PERCENTAGE OF INITIAL CONCENTRATION

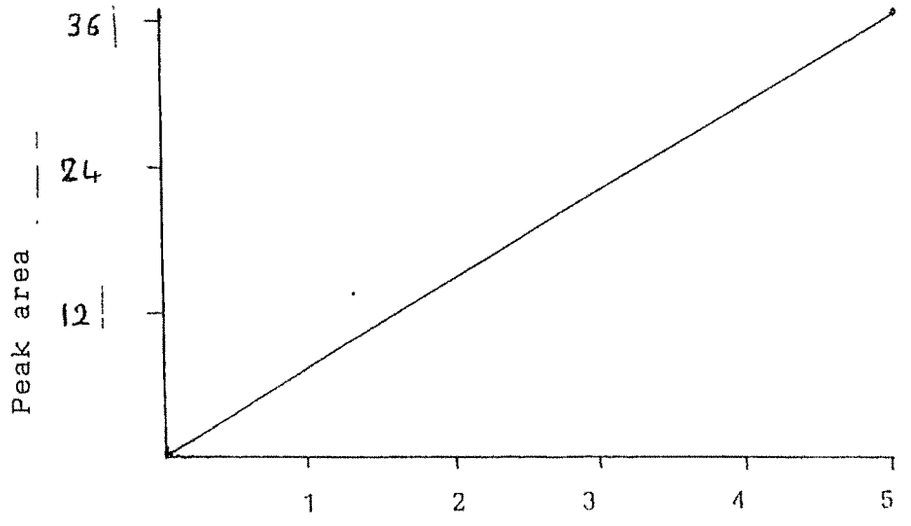
Sample		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Storage condition		amount recovered as percentage of initial assay									
Initial		100	100	100	100	100	100	100	100	100	100
RT RH 75%	1	98.70			95.50		92.0	98.60	98.60		100.12
150 days	2	98.50			95.30		92.10	98.50	98.50		100.00
45° C	1	99.83	101.12	100.02	95.90	100.00	93.20	91.95	99.69	101.10	98.12
150 days	2	99.70	100.00	100.02	95.50	100.05	93.10			100.50	98.20
60° C	1	96.50	96.41		93.50	99.40	91.10	90.08	96.50	96.31	90.00
150 days	2	95.00	96.00		93.00	99.20	91.00	90.30	96.60	96.50	90.25
60° C RH 75%	1	95.00	94.50		93.50	91.04	89.32		94.30	94.30	
150 days	2	94.50	94.50		93.50	90.00	89.50		94.10	94.10	

1. uv method
2. HPLC method

T1 = Timolol maleate pure
T2 = Timolol maleate + lactose (1:5)
T3 = Tindolol maleate + dicalcium phosphate (1:5)
T4 = Timolol maleate + microcrystalline cellulose (1:5)
T5 = Timolol maleate + maize starch (1:5)
T6 = Timolol maleate + polyvinyl pyrrolidone (1:5)
T7 = Timolol maleate + sodium lauryl sulphate (1:5)
T8 = Timolol maleate + magnesium stearate (20:1)
T9 = Timolol maleate + talc (20:1)
T10 = Timolol maleate + silicone dioxide (1:5)

Fig. 3.8





Concentration of Timolol Maleate in µg/ml.

Calibration curve of Timolol Maleate by HPLC.

experiment could not be performed for other drugs due to only limited avaliablity of the instrument.

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

The published literature did not reveal adequate stability data of the drugs in question and there for the experiments were designed to study the stability of the tablet formulation for all the four drugs viz Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride and opthalmic solution formulation of Timolol Maleate at elevated temperature and /or humidity.

I. REAGENTS

1. Standard samples of Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride.
2. 30 gm of ammonium acetate and 1 ml acetyl acetone in water, diluted to 100 ml with water : acetyl acetone reagent.
3. 0.01M sodium periodate solution in water.
4. Methanol.

II. EXPERIMENTAL PROCEDURE

To 5 separate HDPE bottles (opaque) 10 tablets each of respective drug substance were transfered and stored at ambient temperature (RT), ambient temperature RH 75%, 45° C, 60° C and 60° C RH 75% in thermostatically controlled ovens upto 160 days. The relative humidity of 75% was obtained by filling in the dessicatior[59] saturated sodium chloride solution . Closing the lid tighlly and storing the dessicator at respective temperature. The samples of tablets were examined visually at the end of every week and analysed intermittently using acetyl acetone reagent (Section

2.10) method, uv spetrophotmetric method, and by B.P.[3] method.

Commerically available Timolol Maleate 0.5% solution was stored at RT, 45° C and 60° C in the original containers. The samples of opthalmic solution were examined visually at the end of every week and analysed intermittently for Timolol content using spectrophotometric method with acetyl acetone reagent and uv spectrophotometric method as referred to above. In a separate experiment designed to study the compatibility of drug substance with packing meteritial. The samples Timolol Maleate were studied for stability at ambient temperature, 60° C and 70° C simultaneously in original containers and by transffering the formulated product from the original pack to low density polyethylene container. The samples were examined viually at the end of every week at 60° C and at end of every 4 days at 70° C and analysed as refered to above.

III. RESULT AND DISCUSSION.

The tablets of Timolol Maleate, Nadolol and Sotalol hydrochloride displayed excellent physical stability and tablets stored at RT, RT RH 75%, 45 C and 60 C remained white, intact tablets through out the 160 days of observation. The tablets of Pindolol remained physically white intect after 90 days at RT RH 75%, RT, 45° C and 60° C. Tablets stored at 60 C RH 75% however showed noticeable physical deterioration. Tablets of Pindolol absorbed quite large quantity of moisture and were semiliquified after about 20 days of storage and hence could not be studied for chemical stability. AT RT RH 75%, Rt, 45° C and 60° C and 60° C RH 75% about 16%, 10% and 14% and 19% degradation occured respectively at the end of 160 days of observation (Table 3.30). The tablets of Timolol Maleate stored at 60° C did not degrade,

Table 3.30

STABILITY OF PINDOLOL TABLET AT ELEVATED TEMPERATURE AND / OR HUMIDITY

No of days	Assay of Pindolol : As percentage of labelled amount (A) and as percentage of initial concentration (B)									
	RT		RT RH 75%		45°C		60°C		60°C RH 75%	
	A	B	A	B	A	B	A	B	A	B
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
80	99.81	100.00	95.35	96.00	97.81	98.00	94.78	94.98	93.70	92.32
160	100.10	99.74	84.10	84.84	90.20	90.00	86.25	86.89	81.23	80.70

Table 3.31

STABILITY OF TIMOLOL MALEATE AT ELEVATED TEMPERATURE AND / OR HUMIDITY

No of days	Assay of Timolol Maleate : As percentage of labelled amount (A) and as percentage of initial concentration (B)									
	RT		RT RH 75%		45°C		60°C		60°C RH 75%	
	A	B	A	B	A	B	A	B	A	B
0	100.25	100.00	100.25	100.00	100.25	100.00	100.25	100.00	100.25	100.00
80	101.01	100.85	99.71	99.41	99.23	99.00	98.23	98.00	96.55	96.33
160	99.87	99.64	99.10	99.00	99.10	98.91	98.52	98.29	93.24	93.00

about 2% degradation occured after 160 days of storage. Where as degradation at higher temperature and humidity ie 60° C RH 75% was rather significant ie about 7% degradation with change in colour from original white to yellowish at the end of 160 days observation. Negligible degradation was observed at RT, RT RH 75% and 45° C (Table 3.31).

The tablets of Nadolol showed negligable degradation at RT, RT RH 75% and 45° C.About 6% and 15% degradation occured at 60° C and 60° C RH 75%. The tablets turned light yllow at 60° C RH 75% after 160 days of storage. Negligible degradation was observed at RT, RT RH 75% and 45° C (Table 3.32).

The tablets of Sotalol hydrochloride showod 4.5 % and 15% degradation occured at 60° C and 60° C 75% RH respectively after 160 days of observation rather high as compared with other drugs. Negligible degradation was observed at RT, RT RH 75% and 45° C (Table 3.33).

The solution of Timolol Maleate remained colourless at 45° C and 60° C. Above 2 % and 5% degradation occured at 45° C and 60° C respectively after 100 days (Table 3.34). The prepration showed negligible degradation was observed at RT after 100 days. ?

Negligible degradation was observed in glass container stored at 45° C and RT after 100 days. In low densition polyethylene container at 45° C with respect to percentage of initial concentration the degradation was observed to be higher then the labelled amount as similar peturn in degradation was observed at 60° C. Negligible degradation was observed at RT in both the storage conditions. This speaks of some incompatibility between

Table 3.32

STABILITY OF NADOLOL AT ELEVATED TEMPERATURE AND / OR HUMIDITY

No of days	Assay of Nadolol : As percentage of labelled amount (A) and as percentage of initial concentration (B)									
	RT		RT RH 75%		45° C		60° C		60° C RH 75%	
	A	B	A	B	A	B	A	B	A	B
0	99.32	100.00	99.32	100.00	99.32	100.00	99.32	100.00	99.32	100.00
80	100.25	100.45	98.99	99.09	98.23	98.75	94.98	96.50	86.39	87.78
160	99.97	99.74	98.80	98.90	98.52	98.90	94.30	95.81	85.34	86.71

Table 3.33

STABILITY OF SOTALOL HYDROCHLORIDE AT ELEVATED TEMPERATURE AND / OR HUMIDITY

No of days	Assay of Sotalol hydrochloride : As percentage of labelled amount (A) and as percentage of initial concentration (B)									
	RT		RT RH 75%		45° C		60° C		60° C RH 75%	
	A	B	A	B	A	B	A	B	A	B
0	99.71	100.20	99.71	100.23	99.71	100.23	99.71	100.23	99.71	100.23
80	101.04	101.05	98.00	98.23	98.81	98.48	83.93	84.00	86.79	87.03
160	100.04	99.64	98.55	98.30	98.10	98.53	95.25	95.90	85.21	85.45

Table 3.34

STABILITY OF TIMOLOL MALEATE 0.50 % SOLUTION AT AMBIENT AND ELEVATED TEMPERATURE

No of days	Assay of Timolol Maleate as percentage of the labelled amount (A) and as percentage of initial concentration (B)					
	RT		45°C		60°C	
	A	B	A	B	A	B
0	99.25	100.00	99.25	100.00	99.25	100.00
40	99.00	99.75	99.00	99.20	98.45	98.53
60	98.90	99.63	98.60	98.80	97.83	97.90
100	98.83	99.60	98.26	98.73	94.70	94.90

Table 3.35

STABILITY OF TIMOLOL MALEATE 0.50% SOLUTION AT AMBIENT AND ELEVATED TEMPERAURES IN LOW DENSITY POLYETHYLENE VIS A VIS GLASS CONTAINER

No of days	Assay of Timolol Maleate as percentage of the labelled amount (A) and as percentage of initial concentration (B)					
	Low density polyethylene container					
	RT		45°C		60°C	
	A	B	A	B	A	B
0	99.25	100.00	99.25	100.00	99.25	100.00
100	98.00	98.17	90.00	87.83	87.66	85.55
	Glass container					
0	99.25	100.00	99.25	100.00	99.25	100.00
100	98.83	99.60	98.26	98.73	94.70	94.90

the preparation and low density polyethylene container resulting in diminution of assay value at 45° C and especially at 60° C. Such an interaction is rather unlikely at ambient temperature and hence the product displayed very good stability after 100 days of storage at ambient temperature (table 3.35).

In conclusion the marketed preparation of Pindolol, Timolol Maleate, Nadolol and Sotalol hydrochloride bore good stability at ambient temperature.