CHAPTER IV

RESULTS AND DISCUSSION

1. Methods of quantitative analysis of fluconazole

1.1 Tests for methods of quantitation of fluconazole solution by UV spectroscopy at 260 nm

Validation of analytical methods for quantitative determination of fluconazole solution by UV spectroscopy.

1.1.1 Accuracy

These experiments were conducted to verify that the methods used for fluconazole in solvents and mixed solvents were sufficiently accurate and precise.

1.1.1.1 Analysis of fluconazole in ethanol

Table 5 shows percent analytical recovery at each fluconazole concentration. The mean percent analytical recovery was 99.5 which was sufficiently high with a low % CV (1.35). This indicates that the UV spectroscopy method was accurate for quantitative analysis of fluconazole in ethanol.

Table 5 Percent analytical recoveries of fluconazole in ethanol by UV spectrophotometry.

Fluconazole concentration (mg/ ml)	Concentration Calculated from calibration curve	% Analytical recovery
0.100	0.100	100.00
	0.101	101.00
	0.101	101.00
0.120	0.119	99.12
	0.119	99.55
	0.119	99.55
0.150	0.148	98.47
	0.149	99.15
	0.147	97.78
0.200	0.199	99.53
	0.199	99.53
	0.200	100.04
0.240	0.247	103.06
	0.246	102.63
	0.245	101.99
0.300	0.296	98.67
	0.296	98.67
	0.297	99.00
0.400	0.399	99.84
	0.401	100.35
	0.400	100.09
		Mean = 99.95
		SD = 1.35
		%CV = 1.35

1.1.2 Precision

Tables 6 and 7show %CV for each fluconazole concentration in the range of 0.0980-1.2840. This indicates that the UV spectrophotometry method are sufficiently precise for quantitation of fluconazole solution and that sufficient precision would be obtained when the concentrations were in the range of 0.1-0.4 mg/ml.

1.1.3 Linearity

Linear regression analysis of the absorbance against concentration was performed with a correlation coefficient (r) of 0.9995. Data for the calibration curve of fluconazole is presented in Table 8 and Figure 4.

1.1.4 Specificity

Under the conditions selected for solubility studies, the peaks of various solvents and mixed solvents did not interfered with the peak of fluconazole. This validation was made by comparing the spectra from UV spectrophotometer between solvent systems and the mixed solvent systems with fluconazole in ethanol. The spectra from UV spectrophotometer are showed in Figures 5 and 6.

Table 6 Within run precision data by UV spectrophotometry.

Concentration	Absorbance			Mean	SD	% CV
(mg/ ml)	No. 1	No. 2	No. 3			
0.10	0.208	0.210	0.209	0.209	0.001	0.478
0.12	0.244	0.245	0.245	0.245	0.006	0.236
0.15	0.300	0.302	0.298	0.300	0.002	0.667
0.20	0.400	0.400	0.402	0.400	0.001	0.289
0.24	0.494	0.492	0.489	0.492	0.003	0.512
0.30	0.588	0.588	0.590	0.589	0.001	0.196
0.40	0.790	0.794	0.792	0.792	0.002	0.253

Table 7 Between run precision data by UV spectrophotometry.

Concentration	Absorbance			Mean	SD	% CV
(mg/ ml)	Day 1	Day 2	Day 3			
0.10	0.209	0.205	0.204	0.2060	0.003	1.2840
0.12	0.243	0.245	0.242	0.243	0.002	0.6290
0.15	0.300	0.304	0.303	0.302	0.002	0.6890
0.20	0.401	0.400	0.402	0.401	0.001	0.2490
0.24	0.490	0.492	0.492	0.491	0.002	0.2350
0.30	0.587	0.588	0.587	0.587	0.006	0.0980
0.40	0.790	0.793	0.792	0.792	0.002	0.1930

Table 8 Data for calibration curve of fluconazole in ethanol by UV spectrophotometry.

Fluconazole concentration	Absorbance			Mean
(mg/ ml)	No. 1	No. 2	No. 3	
0.10	0.208	0.210	0.209	0.209
0.12	0.244	0.245	0.245	0.245
0.15	0.300	0.302	0.298	0.300
0.20	0.400	0.400	0.402	0.400
0.24	0.494	0.492	0.489	0.492
0.30	0.588	0.588	0.590	0.589
0.40	0.790	0.794	0.792	0.792

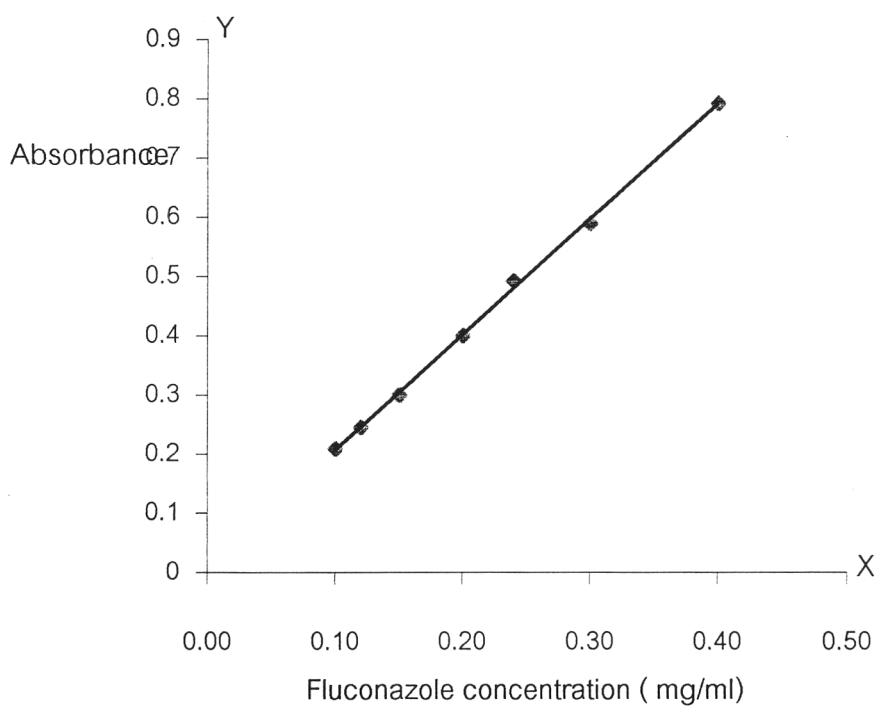


Figure 4 The calibration curve of fluconazole in ethanol by UV spectrophotometry.

$$Y = 1.9472 X + 0.0124$$

$$R = 0.9995$$

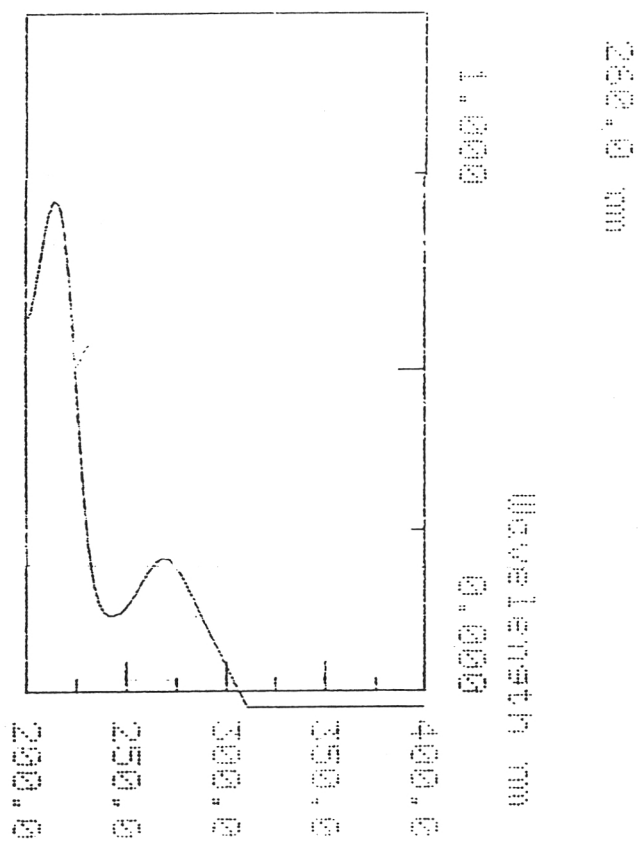


Figure 5 Spectra of fluconazole in ethanol from UV spectrophotometer.

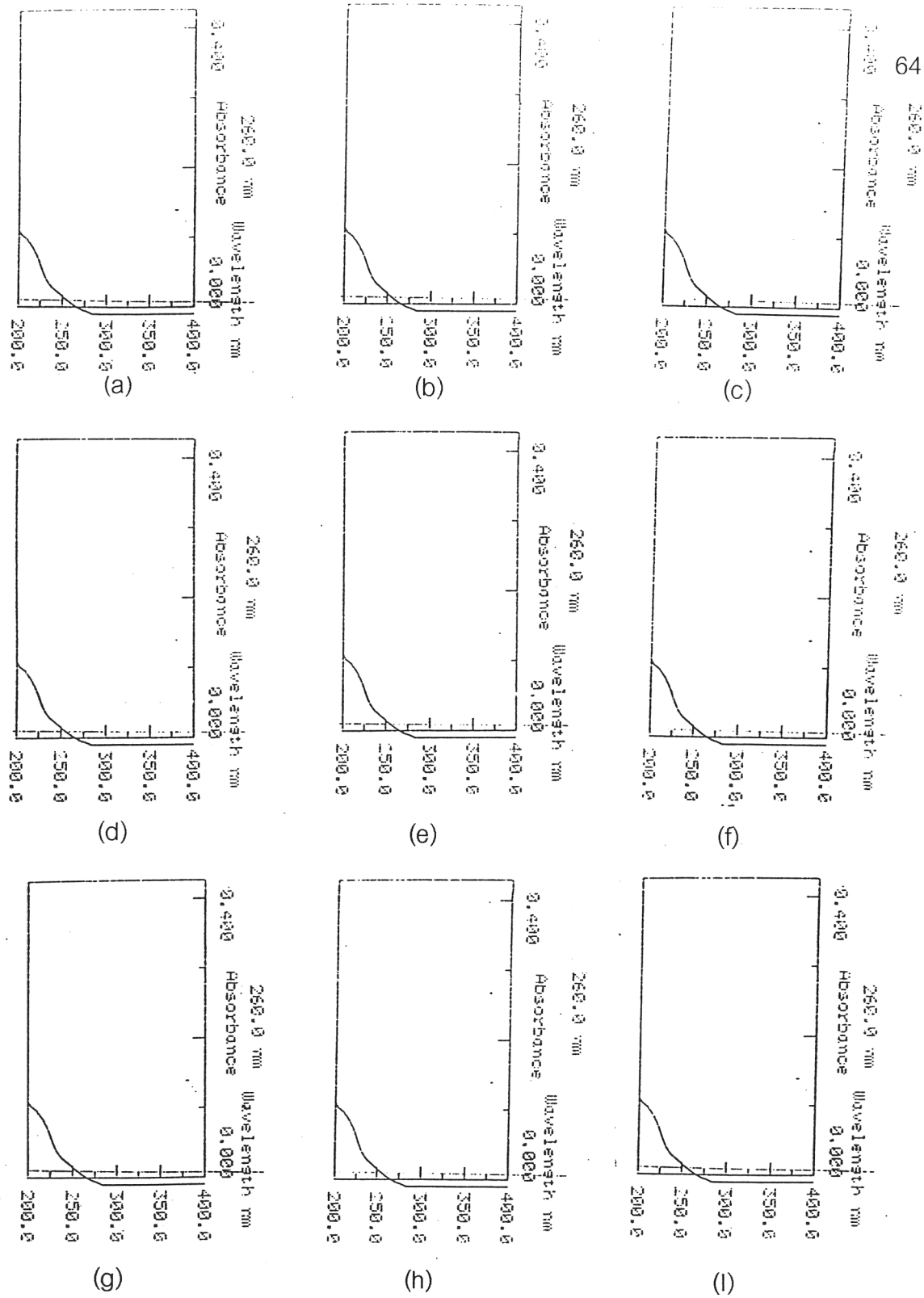


Figure 6 Spectra of various solvents from UV spectrophotometer. ethanol, (a); propylene glycol, (b); polyethylene glycol 400, (c); water, (d); ethanol-water, (e); propylene glycol-water, (f); polyethylene glycol 400-water, (g); polyethylene glycol 4000-water, (h); polyethylene glycol 4000-ethanol-propylene glycol-water, (I).

1.2 Tests for methods of quantitation of fluconazole syrup by HPLC

Validation of analytical methods for quantitative determination of fluconazole syrup by HPLC.

1.2.1 Accuracy

These experiments were conducted to verify that the methods used for fluconazole syrup analysis were sufficiently acculate and precise.

a) Analysis of fluconazole in solution

Table 9 shows percent analytical recovery at each fluconazole concentration in solution. The mean percent analytical recovery was 100.21 which, sufficiently high with a low % CV (1.87), which indicates that the HPLC method was accurate for quantitative analysis of fluconazole in solution.

b) Analysis of fluconazole in syrup

Table 10 shows percent analytical recovery of each concentration of fluconazole syrup. The results show that the mean percent recovery was 100.37 which was sufficiently high with a low %CV (1.35). These results indicate that satisfactory quantitation of fluconazole in syrup was achieved by using HPLC.

Table 9 Percent analytical recoveries of fluconazole in solution by HPLC.

Fluconazole concentration (mcg/ ml)	Concentration Calculated from calibration curve	% Analytical recovery
250	257.38	102.95
	256.94	102.78
	257.82	103.13
275	278.51	101.28
	278.95	101.44
	279.83	101.76
300	295.68	98.56
	296.99	98.99
	295.68	98.56
500	486.69	97.34
	487.13	97.43
	488.45	97.69
700	700.60	100.09
	700.60	100.09
	701.92	100.274
900	899.54	99.95
	903.06	100.34
	910.54	101.17

Mean = 100.21

SD = 1.84

% CV = 1.87

Table 10 Percent analytical recoveries of fluconazole in syrup by HPLC.

Fluconazole concentration (mcg/ ml)	Concentration Calculated from calibration curve	% Analytical recovery
250	257.50	103.00
	260.30	104.12
	253.11	101.24
275	277.00	100.73
	275.55	100.20
	275.65	100.24
300	295.65	98.55
	295.87	98.62
	297.60	99.20
500	499.58	99.92
	498.88	99.78
	498.65	99.73
700	703.45	100.49
	703.30	100.47
	699.52	99.93
900	903.12	100.35
	902.89	100.32
	898.32	99.81

Mean = 100.37

SD = 1.35

%CV = 1.35

1.2.2 Precision

Tables 11 and 12 show %CV for each fluconazole concentration in the range of 0.124-1.109. This indicates that the HPLC method was sufficiently precise for quantitation of fluconazole solution and that sufficient precision would be obtained when the concentrations were in the range of 250 – 900 μg/ml. Thus, the samples were diluted accordingly before subjected to the HPLC analysis.

1.2.3 Linearity

Linear regression analysis of the peak area ratio against concentration was performed with a correlation coefficient (r) of 0.9996. Data for the calibration curve of fluconazole are presented in Table 13 and Figure 7, chromatograms are presented in Figure 8.

1.2.4 Specificity

Figure 9 shows typical chromatograms of fluconazole in syrup and in solution. Fluconazole in both syrup and solution was eluted as a distinct peak with a retention time of 6.71 min. These peaks were not interfered by the peaks of other pharmaceutical components. Thus, the complete separation of fluconazole and prednisolone peaks could be achieved. The separate chromatograms of phosphate buffer solution (PBS), syrup USP, sorbitol 70% w/v cosolvent and disodium edeate, sodium bisulfite, propyl gallate, peppermint, sodium saccharin, paraben concentrate in PBS are shown in Figures 9 and10. All of these chromatograms show a distinct peak at the same retention time of 2.05 min except for that of paraben concentrate which shows a distinct peak with retention time of

Table 11 Within run precision data by HPLC.

Concentration (mcg/ ml)	Peak Area Ratio			Mean	SD	% CV
	No. 1	No. 2	No. 3			
250	0.231	0.230	0.232	0.231	0.001	0.467
275	0.279	0.280	0.282	0.280	0.002	0.669
300	0.318	0.321	0.318	0.319	0.001	0.398
500	0.752	0.753	0.756	0.753	0.002	0.247
700	1.238	1.238	1.241	1.239	0.002	0.157
900	1.690	1.698	1.715	1.701	0.001	0.744

Table 12 Between run precision data by HPLC.

Concentration (mcg/ ml)	Peak Area Ratio			Mean	SD	% CV
	Day 1	Day 2	Day 3			
250	0.229	0.228	0.227	0.228	0.001	0.617
275	0.270	0.274	0.276	0.274	0.003	1.109
300	0.322	0.322	0.321	0.322	0.004	0.124
500	0.745	0.741	0.752	0.746	0.006	0.764
700	1.241	1.238	1.238	1.239	0.002	0.157
900	1.716	1.716	1.720	1.717	0.002	0.130

Table 13 Data for calibration curve of fluconazole in ethanol by HPLC.

Fluconazole concentration	Peak area ratio			Mean
(mcg/ ml)	No. 1	No. 2	No. 3	
250	0.231	0.230	0.232	0.231
275	0.279	0.280	0.282	0.280
300	0.318	0.321	0.318	0.319
500	0.752	0.753	0.756	0.753
700	1.238	1.238	1.241	1.239
900	1.690	1.698	1.715	1.701

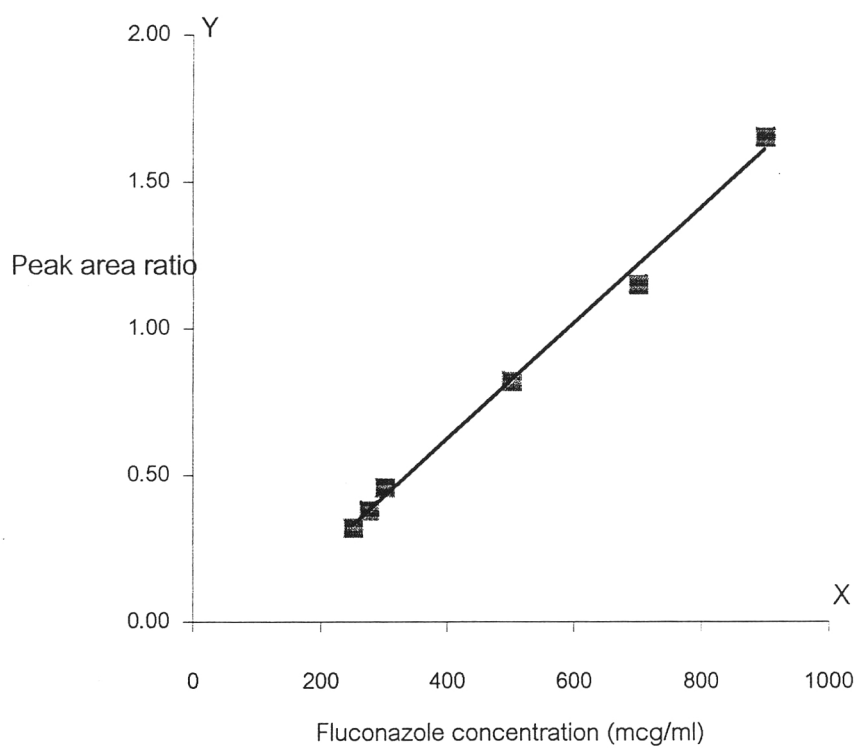


Figure 7 The calibration curve of fluconazole by HPLC.

$$Y = 0.0023X - 0.3538$$

$$R = 0.9996$$

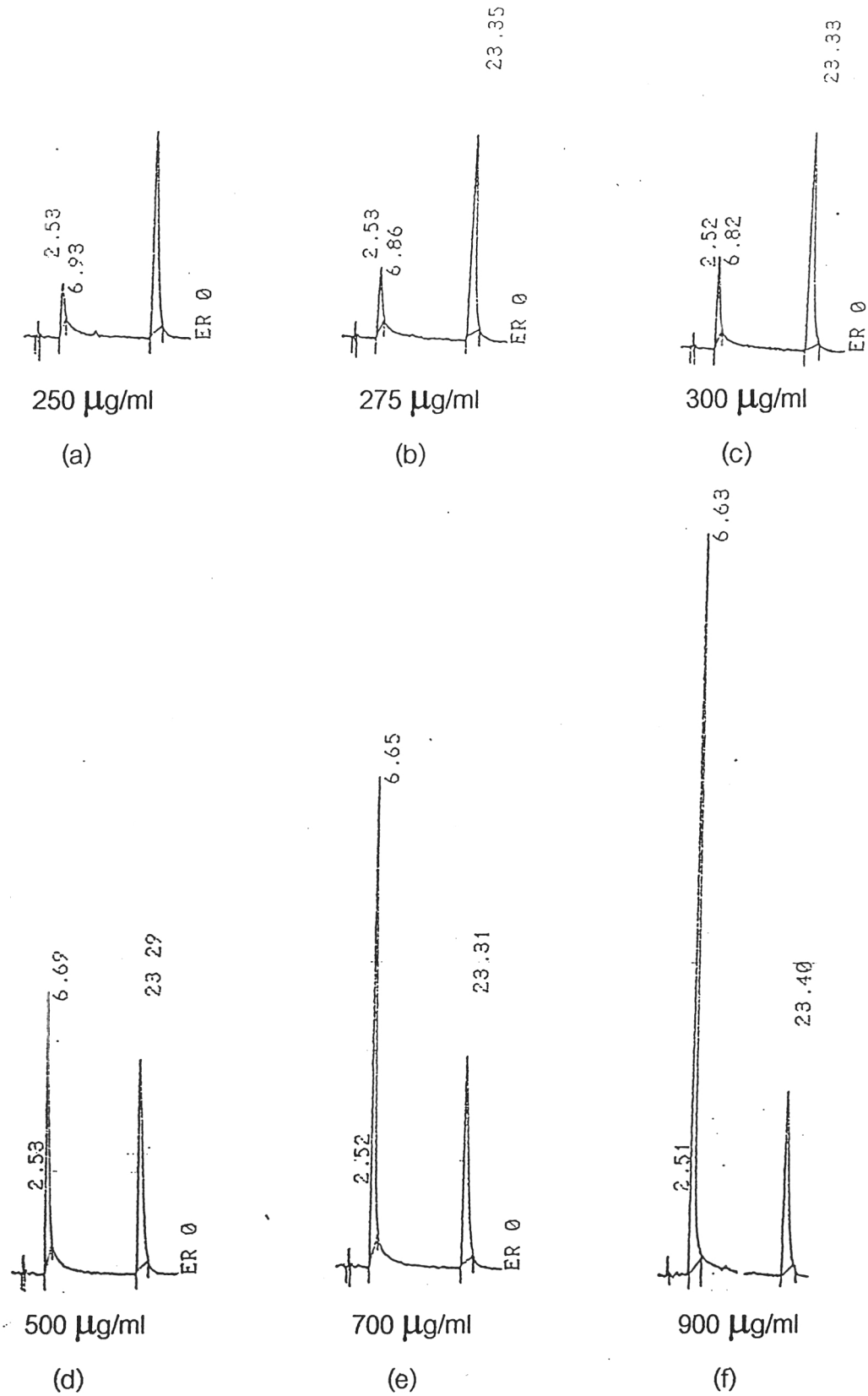


Figure 8 Chromatograms of standard solutions of fluconazole at concentrations of 250 µg/ml, (a); 275 µg/ml, (b); 300 µg/ml, (c); 500 µg/ml, (d); 700 µg/ml, (e); 900 µg/ml, (f). Retention time of fluconazole and prednisolone are at 6.50 – 7.50 and 23.00 – 24.00, respectively.

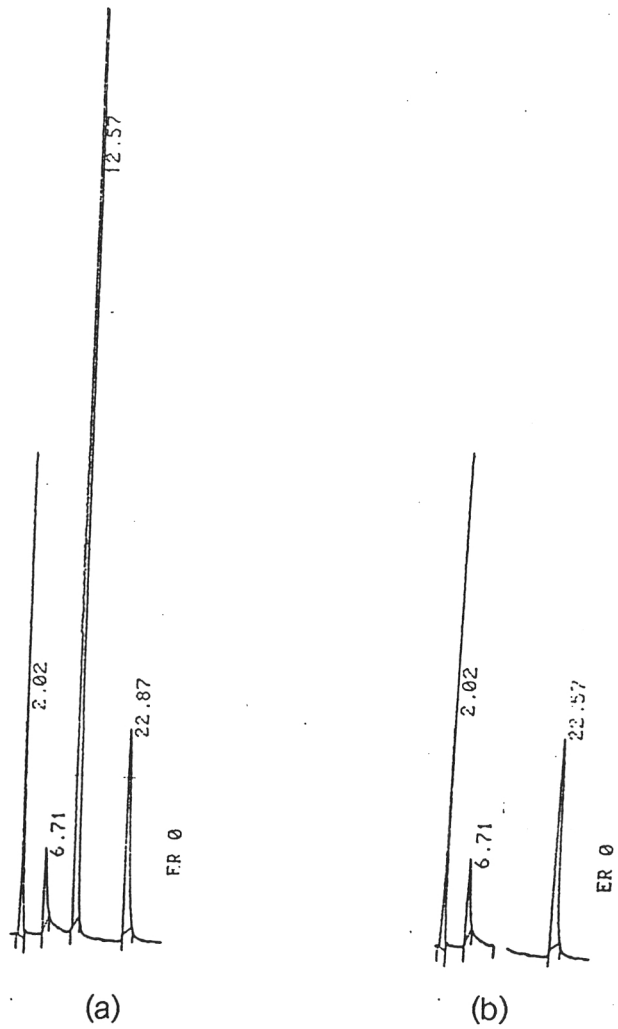


Figure 9 Chromatograms of fluconazole in fluconazole syrup, (a); and in the solution (b).

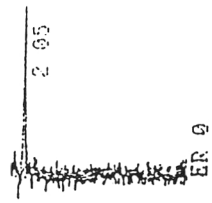
The retention time of phosphate buffer is 2.02 min.

The retention time of fluconazole in fluconazole syrups is 6.71 min.

The retention time of fluconazole in solution is 6.71 min.

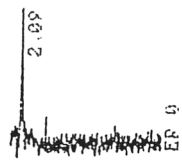
The retention times of prednisolone (the internal standard) are 22.87 and 22.57 min in syrups and in the solution, respectively.

The retention times of paraben concentrate is at 12.57min.



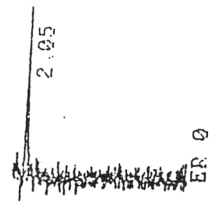
phosphate buffer

(a)



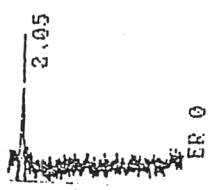
syrup USP

(b)



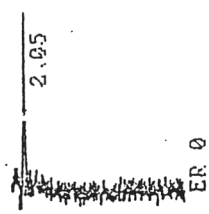
sorbital

(c)



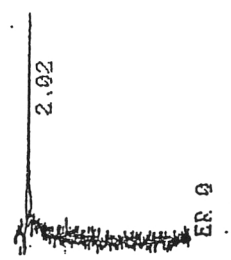
cosolvent

(d)



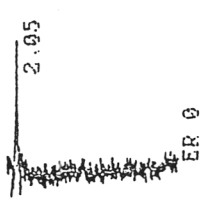
disodium edetate

(e)



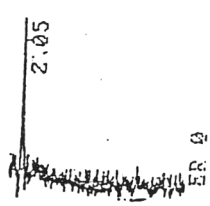
sodium bisulfite

(f)



peppermint

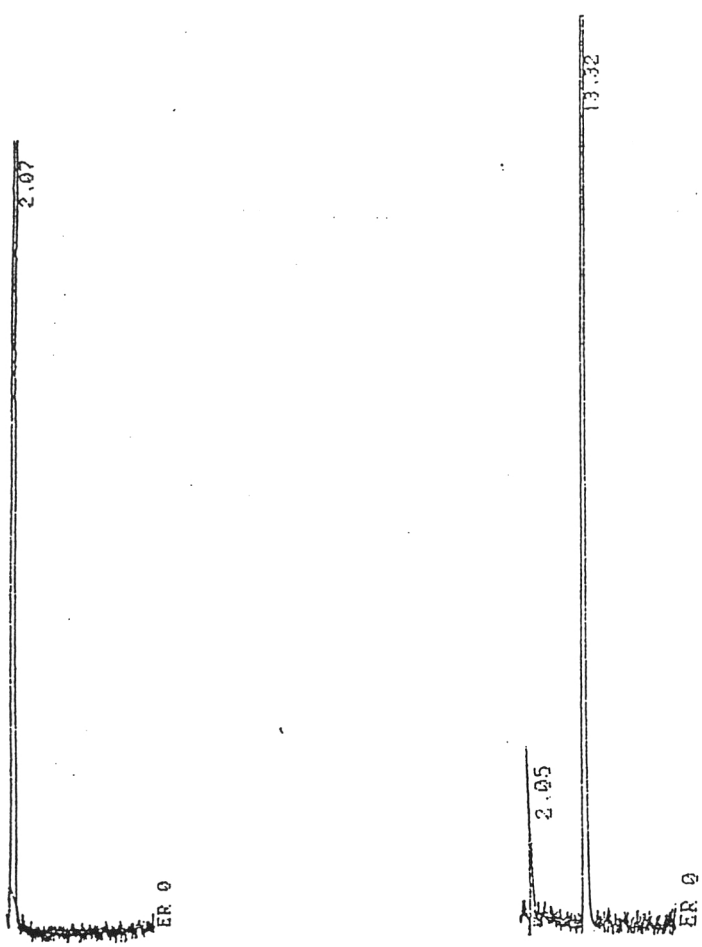
(g)



propyl gallate

(h)

Figure 10 Chromatograms of phosphate buffer (a), syrup USP (b), sorbital (c), cosolvent (d), disodium edetate (e), sodium bisulfite (f), peppermint(g), propyl gallate (h) in phosphate buffer.



sodium saccharin (a)

paraben concentrate (b)

Figure 11 Chromatograms of sodium saccharin (a) and paraben concentrate (b) in PBS.

13.32 min. These peaks did not interfered with the peak of fluconazole. In conclusion, this method had high specificity for analysis of fluconazole

1.2.5 Stability Indication of fluconazole and other pharmaceutical components in formula

A stability indicating assay is an important methodology to ensure that the capability of the method used in the stability studies is high enough to separate the parent drug from its decomposition products but this experiment could not separate the decomposition products of fluconazole. Following the peak area of fluconazole was used instead for this experiment. Figure 12 shows that there was no degradation product from the solution containing mixed solvent of ethanol-propylene glycol-polyethylene glycol 4000-water, disodium edetate, sodium bisulfite, propyl gallate, PBS, sorbitol, peppermint, sodium saccharin and paraben concentrate. There were the degradation products of sucrose in syrup (Figure 13) corresponding to the work of D.R. Heidemann (Heidemann 1978) which indicated that dosage form containing sugar was degraded to 5-hydroxymethylfurfural which can be detected by HPLC. Figure 13 shows that there were 2 peaks at retention times of 3.27 min and 5.17 min when syrup containing fluconazole was injected and compared with the solution containing fluconazole. The peak of degradation products of sugar did not interfere with the peak of fluconazole. Therefore, this method could be used for assay of fluconazole in the presense of degradation products of other pharmaceutical components in formula.

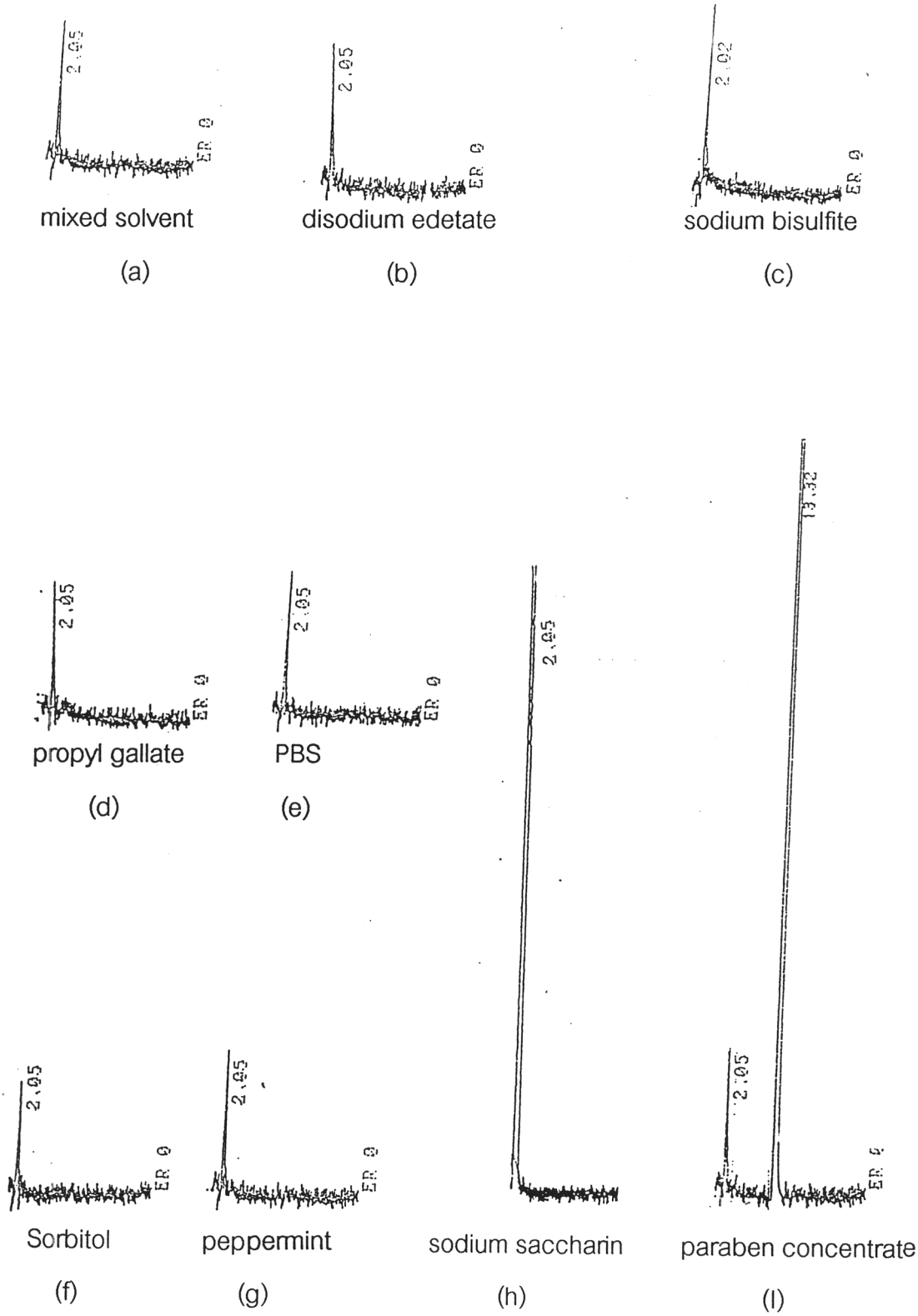
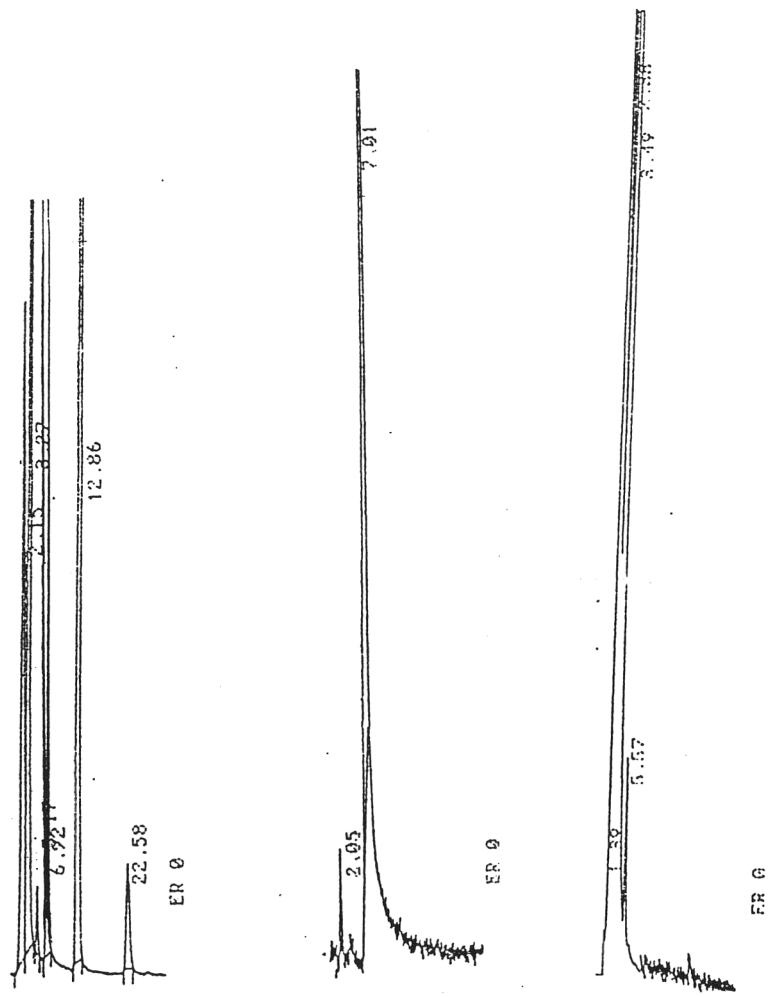


Figure 12 Chromatograms of decomposed mixed solvent of ethanol-propylene glycol-polyethylene glycol 4000-water (a), disodium edetate (b), sodium bisulfite (c), propyl gallate (d), PBS (e), sorbitol (f), peppermint(g), sodium saccharin (h), paraben concentrate (I) in PBS



fluconazole syrup (no antioxidant) (a)

fluconazole in PBS (b)

syrup USP (c)

Figure 13 Chromatograms of decomposed fluconazole syrups at 60 ° C and exposed to light for 90 days (a); fluconazole, (b) ; syrup USP in PBS(c).

2. Determination of fluconazole solubility

The solubilities of fluconazole in pure solvents and mixed solvents are shown in Table 14. Comparison of solubilities of fluconazole in various mixed solvents and their plots versus percent of cosolvents in water were shown in Figure 14.

Solubility of fluconazole in water was approximately 6.59 mg/ml. The approximate solubilities of fluconazole in ethanol, polyethylene glycol 400, propylene glycol were 107.76, 114.5, 173.5 mg/ml, respectively. The required solubility of fluconazole in formulation was 10 mg/ml. Therefore the method for increasing solubility of fluconazole was cosolvency by mixing cosolvent (i.e., ethanol, propylene glycol, polyethylene glycol 400) with solvent (i.e., water). From these data the solubility of fluconazole in ethanol-water 1:9, propylene glycol-water 2:8 and polyethylene glycol 400 – water 2:8 could be used as a mixed solvent for fluconazole solution or syrup. For reduction of the alcoholic taste of alcohol or unpleasant taste of propylene glycol and polyethylene glycol 400 in formulation, the system of polyethylene glycol 4000-water and of polyethylene glycol 4000-water in mixed solvent with different concentrations were investigated as shown in Tables 15 and 16. Increasing the concentration of polyethylene glycol 4000 resulted in increasing the solubility of fluconazole. Increasing of other cosolvents (i.e., ethanol, propylene glycol) also increase the solubility of fluconazole. From Table 16 polyethylene glycol 4000 4%w/v ethanol 7% v/v propylene glycol 7% v/v in water gave an approximate solubility equal to 10 mg/ml and the texture of this solvent had suitable viscocity for preparation of fluconazole syrups.

Table 14 Observed solubilities of fluconazole in pure and mixed solvents at 30°C

Solvents	Solubility of fluconazole (mg/ml)
Water	6.59 ± 0.01
Ethanol	107.76 ± 0.03
Propylene glycol	173.50 ± 0.10
Polyethylene glycol 400	114.50 ± 0.03
Ethanol-water (1:9)	11.45 ± 0.14
Ethanol-water (2:8)	19.71 ± 0.75
Ethanol-water (3:7)	37.16 ± 0.04
Ethanol-water (4:6)	77.54 ± 0.55
Ethanol-water (1:1)	131.77 ± 1.02
Propylene glycol-water (1:9)	8.74 ± 0.39
Propylene glycol-water (2:8)	11.75 ± 0.82
Propylene glycol-water (3:7)	17.06 ± 0.28
Propylene glycol-water (4:6)	26.26 ± 1.55
Propylene glycol-water (1:1)	49.46 ± 3.17
Polyethylene glycol 400-water (1:9)	8.54 ± 0.16
Polyethylene glycol 400-water (2:8)	11.11 ± 0.97
Polyethylene glycol 400-water (3:7)	15.45 ± 0.36
Polyethylene glycol 400-water (4:6)	22.28 ± 0.52
Polyethylene glycol 400-water (1:1)	32.65 ± 0.31

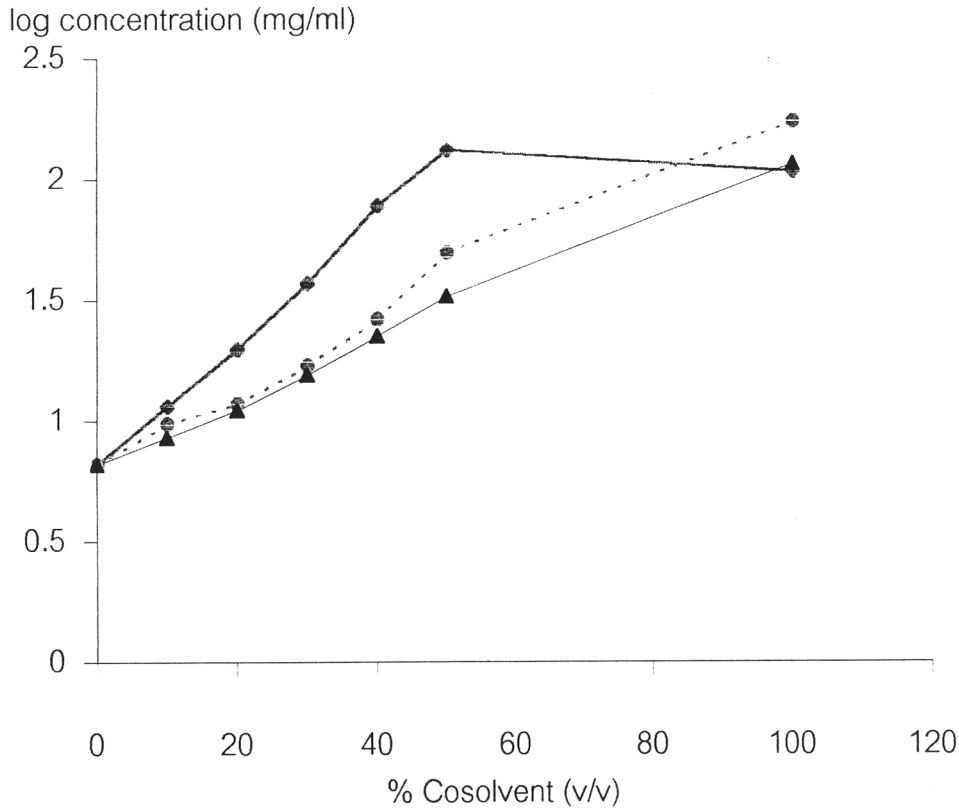


Figure 14 Comparison of solubilities of fluconazole in various mixed solvents.

Table 15 Observed solubilities of fluconazole in polyethylene glycol 4000-water at 30°C.

Polyethylene glyco 4000-water	Solubility of fluconazole (mg/ml)
Polyethylene glyco 4000-water (1:9)	6.49 ± 0.03
Polyethylene glyco 4000-water (2:8)	6.57 ± 0.39
Polyethylene glyco 4000-water (3:7)	6.77 ± 0.29
Polyethylene glyco 4000-water (4:6)	6.98 ± 0.11
Polyethylene glyco 4000-water (1:1)	7.61 ± 0.20

Table 16 Observed solubilities of fluconazole in cosolvent (PEG 4000-ethanol-PG-water) at 30°C.

PEG 4000-ethanol-PG-water	Solubility of fluconazole (mg/ml)
PEG 4000-ethanol-PG-water (1-2-2-95)	6.81 ± 0.10
PEG 4000-ethanol-PG-water (1-4-4-91)	7.89 ± 0.03
PEG 4000-ethanol-PG-water (1-7-7-85)	8.16 ± 0.25
PEG 4000-ethanol-PG-water (2-2-2-94)	7.26 ± 0.00
PEG 4000-ethanol-PG-water (2-4-4-90)	8.12 ± 0.10
PEG 4000-ethanol-PG-water (2-7-7-84)	9.13 ± 0.01
PEG 4000-ethanol-PG-water (3-2-2-93)	7.81 ± 0.05
PEG 4000-ethanol-PG-water (3-4-4-89)	8.60 ± 0.01
PEG 4000-ethanol-PG-water (3-7-7-83)	9.72 ± 0.21
PEG 4000-ethanol-PG-water (4-2-2-92)	7.77 ± 0.19
PEG 4000-ethanol-PG-water (4-4-4-88)	8.31 ± 0.30
PEG 4000-ethanol-PG-water (4-7-7-82)	9.75 ± 0.38

3. Preparation of fluconazole syrups

From Table 16 mixed solvent of polyethylene glycol 4000 : ethanol : propylene glycol 4 g : 7 ml : 7 ml was chosen for dissolving fluconazole 1 gm. Other ingredients were dissolved in distilled water. Paraben concentrate, sorbitol and syrup USP were added. The solution was mixed. The final volume was adjusted to 100 ml with distilled water.

4. Stability testing

4.1 Physical stability of fluconazole in fluconazole syrups

4.1.1 Heating cooling cycle

Physical characteristics such as pH, color and clarity of all formulation of fluconazole syrups after storage in an incubator at controlled temperature of 45 ^{o}C for 24 hours and in a refrigerator at temperature of 4 ^{o}C for 24 hours for 6 cycles were not changed.

From Table 17, fluconazole syrups were stored at 60 ^{o}C in the presence of light, the pH was decreased from 5.03 to 3.57. Table 18, fluconazole syrups were stored at 60 ^{o}C, the pH was changed from 5.03 to 3.52. So that only the temperature at 60 ^{o}C had an effect on pH of fluconazole syrups.

Table 17 Effect of temperature and light on pH of fluconazole syrups (stored at 60°C, presence of light).

pH / Formula	Time (days)						
	0	6	15	28	43	73	100
1.No antioxidant	5.07	4.91	4.79	4.85	3.83	3.90	3.89
2.Propyl gallate (0.001%w/v)	5.09	5.05	4.78	4.42	4.34	3.90	3.85
3.Propyl gallate (0.005%w/v)	5.09	5.02	4.73	4.24	4.00	3.95	3.74
4.Propyl gallate (0.010%w/v)	5.07	5.07	4.67	4.26	4.10	3.91	3.77
5.Sodium bisulfite (0.050% w/v)	5.08	4.97	4.61	4.09	4.00	3.85	3.64
6. Sodium bisulfite (0.075% w/v)	5.09	4.77	4.52	3.88	3.78	3.63	3.57
7. Sodium bisulfite (0.100% w/v)	5.07	4.76	4.46	3.91	3.87	3.73	3.57
8.Disodium edetate (0.005%w/v)	5.06	5.03	4.94	4.40	4.31	4.02	3.90
9.Disodium edetate (0.010%w/v)	5.03	4.92	4.70	4.05	3.94	3.72	3.59
10.Disodium edetate (0.050%w/v)	5.06	4.87	4.53	3.95	3.84	3.81	3.75

Table 18 Effect of temperature on pH of fluconazole syrups (stored at 60°C, absence of light).

pH / Formula	Time (days) 0	6	15	30	44	74	107
1.No antioxidant	5.07	4.97	4.76	4.25	4.37	4.02	3.64
2.Propyl gallate (0.001%w/v)	5.09	4.93	4.80	4.56	4.33	4.29	3.92
3.Propyl gallate (0.005%w/v)	5.09	4.88	4.70	4.31	4.01	3.89	3.68
4.Propyl gallate (0.010%w/v)	5.07	4.92	4.74	4.51	4.42	4.22	3.73
5.Sodium bisulfite (0.050% w/v)	5.08	4.81	4.52	4.27	4.25	3.99	3.80
6. Sodium bisulfite (0.075% w/v)	5.09	4.76	4.49	4.02	3.95	3.77	3.56
7. Sodium bisulfite (0.100% w/v)	5.07	4.78	4.54	4.18	4.15	3.98	3.52
8.Disodium edetate (0.005%w/v)	5.06	4.99	4.80	4.39	4.38	4.29	3.96
9.Disodium edetate (0.010%w/v)	5.03	4.93	4.58	3.99	3.88	3.75	3.62
10.Disodium edetate (0.050%w/v)	5.06	4.99	4.83	4.39	4.24	4.00	3.55

Table 19 Effect of temperature and light on color change of fluconazole syrups (stored at 60°C, presence of light).

Color / Formula	Time (days)						
	0	6	15	28	43	73	100
1.No antioxidant	PY	Y	Y	RB	DB	DB	DB
2.Propyl gallate (0.001%w/v)	PY	Y	Y	Y	Y	DB	DB
3.Propyl gallate (0.005%w/v)	PY	Y	YB	RB	DB	DB	DB
4.Propyl gallate (0.010%w/v)	PY	Y	Y	RB	DB	DB	DB
5.Sodium bisulfite (0.050% w/v)	PY	Y	Y	RB	DB	DB	DB
6. Sodium bisulfite (0.075% w/v)	PY	Y	YB	RB	DB	DB	DB
7. Sodium bisulfite (0.100% w/v)	PY	Y	YB	RB	DB	DB	DB
8.Disodium edetate (0.005%w/v)	PY	Y	Y	B	DB	DB	DB
9.Disodium edetate (0.010%w/v)	PY	Y	B	RB	DB	DB	DB
10.Disodium edetate (0.050%w/v)	PY	Y	B	RB	DB	DB	DB

PY = Pale yellow

Y = Yellow

YB = Yellow brown

B = Brown

RB = Red brown

DB = Dark brown

Table 20 Effect of temperature on color change of fluconazole syrups (stored at 60°C, absence of light).

Color / Formula	Time (days)						
	0	6	15	30	44	74	107
1.No antioxidant	PY	Y	Y	RB	RB	DB	DB
2.Propyl gallate (0.001%w/v)	PY	Y	Y	B	B	DB	DB
3.Propyl gallate (0.005%w/v)	PY	Y	YB	B	DB	DB	DB
4.Propyl gallate (0.010%w/v)	PY	Y	Y	B	B	DB	DB
5.Sodium bisulfite (0.050% w/v)	PY	Y	Y	B	B	DB	DB
6. Sodium bisulfite (0.075% w/v)	PY	Y	Y	B	DB	DB	DB
7. Sodium bisulfite (0.100% w/v)	PY	Y	Y	Y	Y	DB	DB
8.Disodium edetate (0.005%w/v)	PY	Y	Y	Y	Y	DB	DB
9.Disodium edetate (0.010%w/v)	PY	Y	YB	RB	DB	DB	DB
10.Disodium edetate (0.050%w/v)	PY	Y	Y	RB	RB	DB	DB

PY = Pale yellow

Y = Yellow

YB = Yellow brown

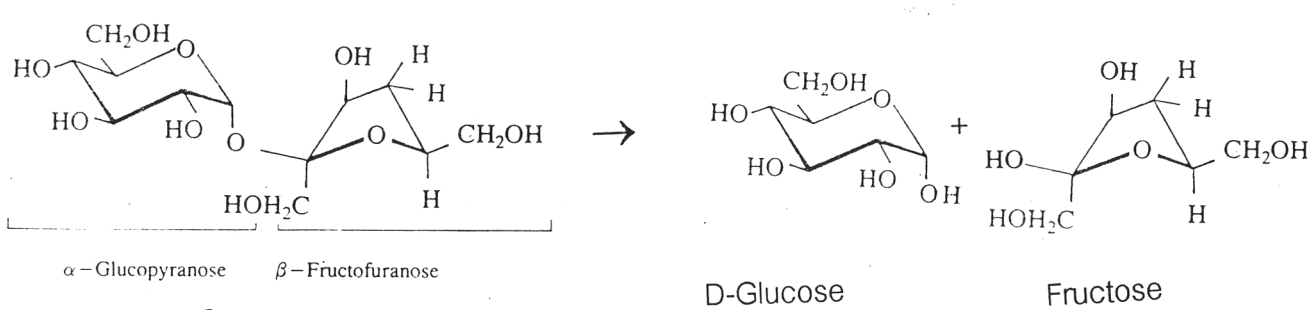
B = Brown

RB = Red brown

DB = Dark brown

Table 19, Fluconazole syrups were stored in the same temperature at 60 ^{o}C in the presence of light, shows that the color was changed from pale yellow to yellow and dark brown the same as table 20, The color was change from pale yellow to yellow and dark brown. But the preparations in table 20 were not exposed to light. So that only temperature had an effect on color change of fluconazole syrups.

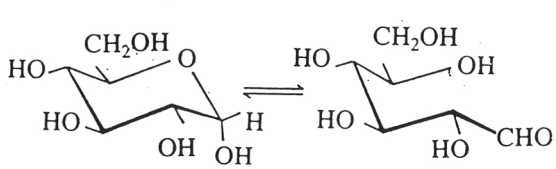
When syrup stored at 60 ^{o}C in the presence of light for 90 days, pH was changed from 5.24 to 3.59. This result is decribed as follows:



Sucrose

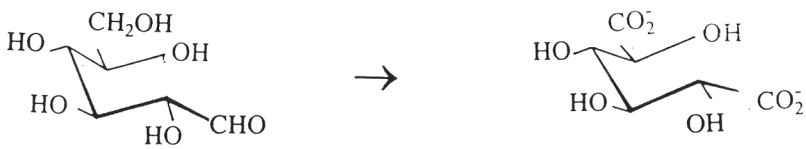
D-Glucose

Fructose



D-Glucose

D-Glucose (open ring)



D-Glucose (open ring) Dicarboxylic acid

When sucrose is hydrolyzed by diluted aqueous acid, it yields equal amounts of glucose and fructose. Glucose was oxidized by H^+ in the solution to form dicarboxylic acid resulted in changing pH of the solution from 5.09 to 3.55 (Morrison and Boyd, 1987).

Table 21 shows the physical change of solution containing each additive individual component after storage at 60 ^{o}C, presence of light for 90 days. In this table only sorbitol and syrup caused changing in color from pale yellow to yellow and dark brown color. So that the pH and color will change in all formulas which contain sorbitol and syrup. Syrup was prepared by dissolving sucrose in the water. When sucrose is hydrolyzed by diluted aqueous acid, it yields equal amounts of glucose and fructose. Degradation products of sucrose on heating by Maillard reaction were 5-hydroxymethylfurfural, furfural, 2,5-dimethyl-4-hydroxy-3-(2H)-furanone, 2-furoic acid, 2-acethylfuran and furfuryl alcohol (Yuan and Chen., 1999). The colorless compounds from degradation products of glucose were identified as 5-(hydroxymethyl) furfural and 2,5-dimethyl-4-hydroxy-3(2H)-furanose (Ames, Bailey and Mann., 1999). The study demonstrated that furan group generated color of browned glucose in the Maillard reaction (Hofman, Bors and Stettmaier., 1999). Therefore the color change of fluconazole syrups in these investigation were come from furan group which decomposed from syrup in the presence of acid and heat.

Table 21 Physical characteristics of each component in solution before and after storage at 60°C for 90 days.

Component in phosphate buffer	pH		color	
	Before	After	Before	After
Sodium saccharin	5.39	5.35	PY	PY
Paraben concentrate	5.36	5.33	PY	PY
Sorbital	5.31	5.30	PY	Y
Syrup USP	5.24	3.59	PY	B
Peppmint	5.34	5.28	PY	PY
Fluconazole in cosolvent	5.55	5.39	PY	PY
Phosphate buffer	5.41	5.42	PY	PY
Cosolvent	5.56	5.48	PY	PY
Disodium edetate (0.005 % w/v)	5.42	6.01	PY	PY
Disodium edetate (0.010 % w/v)	5.30	5.82	PY	PY
Disodium edetate (0.050 % w/v)	5.30	5.78	PY	PY
Sodium bisulfite (0.050 % w/v)	5.34	5.29	PY	PY
Sodium bisulfite (0.075 % w/v)	5.23	5.23	PY	PY
Sodium bisulfite (0.100 % w/v)	5.34	5.31	PY	PY
Propyl gallate (0.001% w/v)	5.32	5.31	PY	PY
Propyl gallate (0.005% w/v)	5.41	5.40	PY	PY
Propyl gallate (0.010% w/v)	5.42	5.35	PY	PY

PY = paie yellow

Y = yellow

B = brown

Chemical stability of fluconazole syrup

The chemical stability of fluconazole syrups stored at 60°C was studies for 107 days. Table 22 shows percent drug remaining in various formulations after exposure to light. Table 23 shows percent of fluconazole remaining under light protection conditions. From these data formulation 2 in table 22 which containing 0.001 % propyl gallate as an antioxidant shows trendency to have high percentage of drug remaining in formula.

Determination of the order of the reaction

To determine the order of the reaction, if a plot of concentration of fluconazole remaining versus time is a straight line, the reaction kinetic is said to be zero-order. The reaction kinetics is first-order when a plot of ln (concentration) versus time gives a straight line whereas the second-order is the result of the straight line of the plot of 1/(concentration) versus time. Linear regression was used to determine the correlation coefficients (r) which were then compared as shown in Appendix III. The results indicated that the correlation coefficients of zero, first second-order were not different from one another. However, the degradation of fluconazole syrups followed zero-order reaction, because percent of fluconazole decomposition were not high enough to indicate the order of reactions and zero-order reaction was safety to determine the shelf-life of formulas. The plots of fluconazole concentration versus time of all formulations studied are shown in Appendix IV.

Table 22 Percent of fluconazole remaining in fluconazole syrups as a function of time (stored at 60°C, presence of light).

Time (Days) / Formula	% Drug Remaining[a]						
	0	6	15	28	43	73	100
1.No antioxidant	100.00	92.28	91.85	90.64	88.29	82.13	79.38
2.Propyl gallate (0.001%w/v)	100.00	97.69	96.57	94.23	97.75	93.98	87.73
3.Propyl gallate (0.005%w/v)	100.00	100.63	99.09	97.34	92.68	94.49	78.84
4.Propyl gallate (0.010%w/v)	100.00	98.23	98.06	97.35	95.32	86.01	79.21
5.Sodium bisulfite (0.050% w/v)	100.00	97.65	95.94	94.71	94.09	91.79	79.51
6. Sodium bisulfite (0.075% w/v)	100.00	91.35	94.36	92.08	91.19	89.00	72.44
7. Sodium bisulfite (0.100% w/v)	100.00	95.40	95.89	92.79	90.80	90.48	79.28
8.Disodium edetate (0.005%w/v)	100.00	101.02	96.44	95.15	96.33	93.05	70.14
9.Disodium edetate (0.010%w/v)	100.00	100.66	101.66	94.93	92.96	91.99	74.34
10.Disodiumedetate (0.050%w/v)	100.00	95.35	91.93	92.02	91.03	86.50	71.07

a = mean (n =3)

Table 23 Percent of fluconazole remaining in fluconazole syrups as a function of time (stored at 60°C, absence of light).

Time (Days) / Formula	% Drug Remaining[a]						
	0	6	15	30	44	74	107
1.No antioxidant	100.00	99.81	96.11	95.19	96.00	88.63	77.63
2.Propyl gallate (0.001%w/v)	100.00	97.14	96.06	97.09	94.71	94.71	84.72
3.Propyl gallate (0.005%w/v)	100.00	98.82	98.59	94.89	96.27	92.19	63.95
4.Propyl gallate (0.010%w/v)	100.00	105.53	100.11	98.47	99.39	93.34	77.96
5.Sodium bisulfite (0.050% w/v)	100.00	99.16	97.94	95.98	94.03	90	85.59
6. Sodium bisulfite (0.075% w/v)	100.00	97.01	92.52	92.26	88.66	84.08	78.05
7. Sodium bisulfite (0.100% w/v)	100.00	97.79	92.11	93.13	90.32	89.50	77.39
8.Disodium edetate (0.005%w/v)	100.00	98.40	95.92	95.68	93.33	92.52	85.52
9.Disodium edetate (0.010%w/v)	100.00	104.67	101.09	99.92	100.57	91.15	82.66
10.Disodiumedetate (0.050%w/v)	100.00	95.35	93.18	92.25	88.51	88.51	73.49

a = mean (n =3)

Chemical stability of fluconazole syrup

The chemical stability of fluconazole syrups stored at 60°C was studies for 107 days. Table 22 shows percent drug remaining in various formulations after exposure to light. Table 23 shows percent of fluconazole remaining under light protection conditions. From these data formulation 2 in table 22 which containing 0.001 % propyl gallate as an antioxidant shows trendency to have high percentage of drug remaining in formula.

Determination of the order of the reaction

To determine the order of the reaction, if a plot of concentration of fluconazole remaining versus time is a straight line, the reaction kinetic is said to be zero-order. The reaction kinetics is first-order when a plot of ln (concentration) versus time gives a straight line whereas the second-order is the result of the straight line of the plot of 1/(concentration) versus time. Linear regression was used to determine the correlation coefficients (r) which were then compared as shown in Appendix III. The results indicated that the correlation coefficients of zero, first second-order were not different from one another. However, the degradation of fluconazole syrups followed zero-order reaction, because percent of fluconazole decomposition were not high enough to indicate the order of reactions and zero-order reaction was safety to determine the shelf-life of formulas. The plots of fluconazole concentration versus time of all formulations studied are shown in Appendix IV.

Statistical consideration

The differences in degradation rate constant values indicated the effect of each factor studied. Analysis of covariance was used to compare the differences of degradation rate constants. The null hypothesis means that all of the degradation rate constant values were not different from the other ones but an alternative hypothesis was that at least one pair of the degradation rate constant values was not equal. The p-value is always related to a hypothesis test; it is the probability of obtaining a result as extreme as or more extreme than the one observed, if the null hypothesis is true. If the p-value was more than 0.05, then the null hypothesis was accepted and the differences in the degradation rate constants were said to be statistically insignificant. If the p-value was less than 0.05, then the null hypothesis was rejected and the alternative hypothesis was accepted. The analysis of covariance were performed by using the Statistical Package for the Social Sciences (SPSS).

4.2 Effect of light

Tables 24, 25 and Figures 15, 16 show drug concentration remaining and linear plot of drug concentration remaining vs. time of fluconazole syrup in the presence of light and the absence of light at 60 ^{o}C, respectively.

Table 24 Stability data of fluconazole syrups stored at 60 °C (Formulation 1 , no antioxidant).

Time (Day)	pH	Color of Syrup	Concentration of fluconazole remaining (mg/ 5ml)			
			No. 1	No. 2	No. 3	Average conc ± SD
0	5.07	Clear	50.490	49.329	50.799	50.206 ± 0.776
6	4.91	Yellow	46.821	46.047	46.112	46.327 ± 0.429
15	4.79	Yellow	46.639	46.169	45.528	46.112 ± 0.558
28	4.85	Red brown	45.600	45.950	44.95	45.500 ± 0.507
43	3.83	Dark brown	44.324	44.300	44.347	44.324 ± 0.024
73	3.90	Dark brown	41.360	41.507	40.819	41.229 ± 0.362
100	3.89	Dark brown	40.509	37.232	41.814	39.852 ± 2.361

Zero - order : $C = C_o + kt$

conc = 48.1463 - 0.0886 time

$C_o = 48.1463$, $k = -0.0886$, $r = 0.9500$

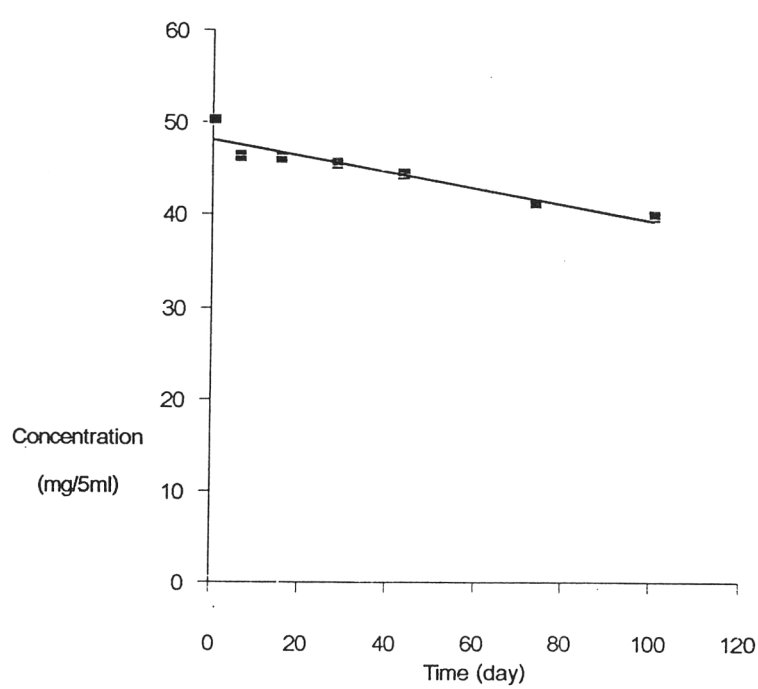


Figure 15 Linear plot of drug concentration remaining v.s time of fluconazole syrup (no antioxidant) in the presence of light at 60 ° C.

Table 25 Stability data of fluconazole syrup when stored at 60 °C (Formulation 1 , no antioxidant).

Time (Day)	pH	Color of Syrup	Concentration of fluconazole remaining (mg/ 5ml)			
			No. 1	No. 2	No. 3	Average conc ± SD
0	5.07	Clear	50.490	49.329	50.809	50.209 ± 0.779
6	4.97	Yellow	51.002	50.113	49.222	50.112 ± 0.890
15	4.76	Yellow	48.116	48.983	47.664	48.254 ± 0.670
30	4.25	Red brown	47.797	47.782	47.795	47.791 ± 0.082
44	4.37	Red brown	48.936	47.459	48.197	48.197 ± 0.739
74	4.02	Dark brown	44.406	43.756	45.056	44.406 ± 0.650
107	3.64	Dark brown	37.483	38.469	40.969	38.974 ± 1.797

Zero - order : $C = C_o + kt$

$conc = 50.7099 - 0.0979\ time$

$C_o = 50.7099$, $k = -0.0979$, $r = 0.9630$

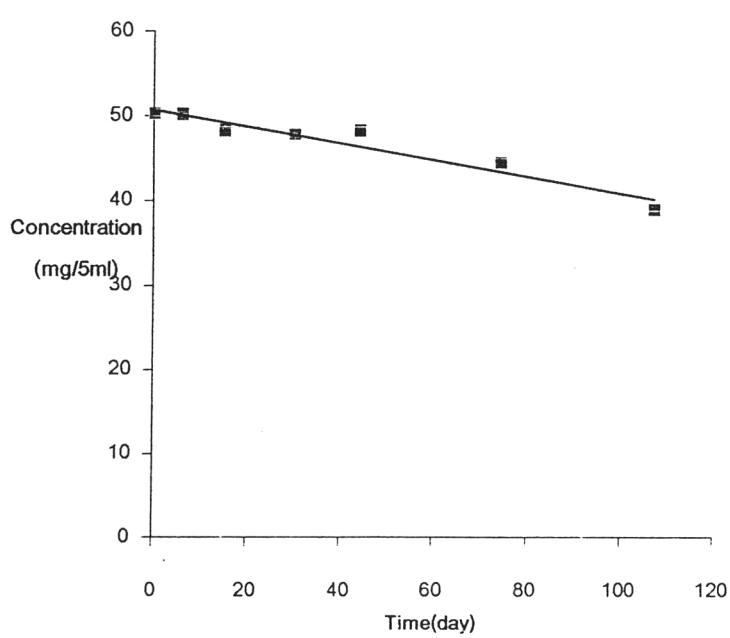


Figure 16 Linear plot of drug concentration remaining v.s time of fluconazole syrup (no antioxidant) in the absence of light at 60 °C.

The degradation rate constant of fluconazole syrup (no antioxidant) stored in the absence of light (k = 0.0979 day^{-1}) and the degradation rate constant of fluconazole syrup (no antioxidant) stored in the presence of light (k = 0.0847day^{-1}). However, the degradation rate constant in each storage condition are not statistically significant. The p-value, which were used to indicate the differences in rate constants are more than 0.05.

4.3 Effect of antioxidants

Antioxidants are divided broadly into three groups. Three groups of antioxidants were free radical inhibitors, oxygen scavengers and chelating agents. Analysis of covariance was performed to examine the differences in the values of degradation rate constants and the p-value was used to indicate the differences with the significant level of 0.05 as shown in Appendix VII.

4.3.1 Effect of free radical inhibitor

Propyl gallate is widely used as a free radical inhibitor. Three concentration levels of propyl gallate, 0.001, 0.005, 0.01% w/v, were studied. The concentration of fluconazole remaining and a plot of drug concentration vs. time of fluconazole syrup containing different concentrations of propyl gallate were shown in in Appendix IV.

Effect of propyl gallate

Three concentration levels of propyl gallate of 0.001, 0.005, 0.01 %w/v were studied. When 0.001 %w/v propyl gallate was added to the fluconazole syrup,

which was stored in the absence of light , it retarded the degradation rate constant but fluconazole syrup containing 0.05, 0.01 %w/v seemed that propyl gallate accelerated the degradation rate of fluconazole instead of rate retardation. When 0.001, 0.01 %w/v propyl gallate was added to the fluconazole syrup, which were stored in the presence of light, their retarded the degradation rate constants but fluconazole syrup containing 0.05 %w/v seemed that propyl gallate accelerated the degradation rate of fluconazole instead of rate retardation. In Figure 17 shows the plots of rate constant vs. concentration of propyl gallate. The rate is independent of concentration of the reactant. However, the changes in degradation rate constants in each condition are not statistically significant since all the p-values, which were used to indicate the differences in rate constants within a storage condition, are more than 0.05. In general, free radical inhibitors can retard or accelerate the oxidation reaction. When they accelerate the reaction, they are called prooxidants (Chipault, 1962). When 0.001 %w/v propyl gallate was added to the fluconazole syrup, propyl gallate at concentration of 0.001% w/v shows a tendency to act as an inhibitor of free radicals in all storage conditions that may be explained by itself form radicals which are stable and incapable of continuing the propagation chain cycle. While the fluconazole syrup containing 0.005% w/v propyl gallate gave more accelleration rate than the fluconazole syrup containing 0.001, 0.01 % w/v propyl gallate. This explained that 0.005% w/v propyl gallate shows a tendency to act as a prooxidant. At higher concentrations, propyl gallate can generate its original form by abstracting a hydrogen atom from compound (RH) and induces the formation of new free radical (R•) present in equation 38 (Duval and Poelman ,1995). The kinetic behaviors are described as follows :

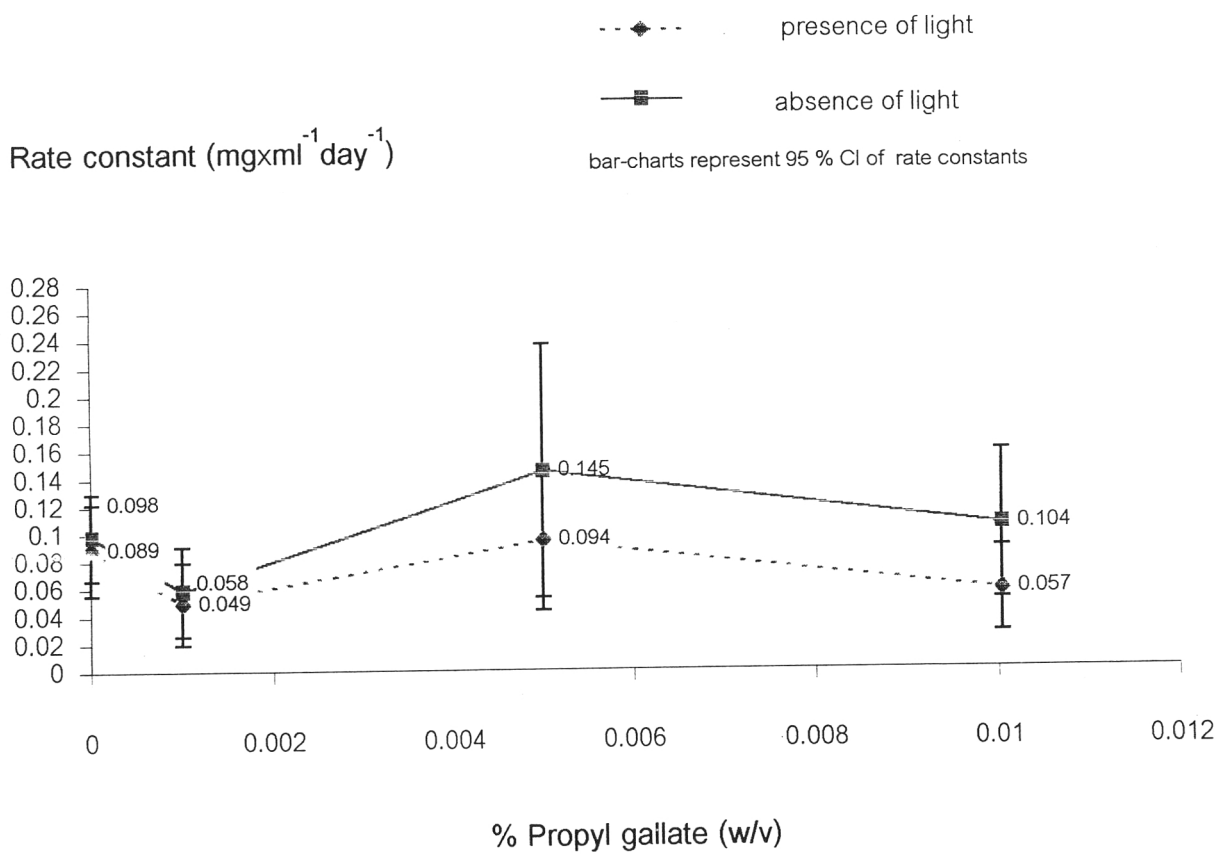


Figure 17 Plots of rate constant vs concentration of propyl gallate.

$AH \longrightarrow A^{\cdot} + H^{\cdot}$ ------ (eq .36) ; AH = propyl gallate

$R^{\cdot} + H^{\cdot} \longrightarrow RH$ ------ (eq .37) ; RH = fluconazole

$RH \longrightarrow R^{\cdot} + H^{\cdot}$ (slow)- -(eq .38)

$R^{\cdot} + O_2 \longrightarrow ROO^{\cdot}$ ------ (eq .39) this steps faster than

$ROO^{\cdot} + RH \longrightarrow ROOH + R^{\cdot}$ - - (eq .40) $R^{\cdot} + H^{\cdot} \longrightarrow RH$

The reaction from equations 39 and 40 were faster than reaction from equation 38. Because of faster reactions from equations 39 and 40 , there were more ROO• and R• . The free radical R• would react with O_2 to produce ROO• and ROO• would react with drug so R• and ROO• from equations 39 and 40 would increase the degradation rate constant. In the presence of light, propyl gallate might be generate $H^{\cdot}$ and from compound (RH) faster than in the absence of light ; therefore, the reaction might have more $A^{\cdot}$ to form radicals that are stable. The result showed that, the formula containing 0.005% w/v propyl gallate in the presence of light showed a tendency to has the degradation rate constants lower than in the absence of light. In 0.01% w/v propyl gallate the degradation rate constants were lower than fluconazole syrup containing 0.005% w/v propyl gallate because it might has remaining AH, which could react with $R^{\cdot}$ or $ROO^{\cdot}$.

4.3.2 Effect of oxygen scavengers

Sodium bisulfite was the widely used oxygen scavenger. Three concentration levels of sodium bisulfite, 0.05, 0.075, 0.1% w/v, were studied. The concentration of fluconazole remaining and a plot of drug concentration vs. time of fluconazole syrup containing different concentrations of sodium bisulfite are shown in Appendix IV.

Effect of sodium bisulfite

When 0.05 %w/v of sodium bisulfite was added to fluconazole syrup, the degradation rate constant of fluconazole was decreased when the light was absent in the system, but the degradation rate constant was increased when light was present. When 0.075 %w/v sodium bisulfite was added, the degradation rate constant of fluconazole was decreased more than the degradation rate constant of fluconazole containing 0.075 %w/v sodium bisulfite in all storage conditions. In Figure 18 shows the plots of rate constant vs. concentration of sodium bisulfite. The rate is independent of concentration of the reactant. The degradation rate constants in each storage condition are not statistically significant since all p-values, which were used to indicate the differences in rate constants within a storage condition, are more than 0.05 as shown in Appendix VII. Figure 18, fluconazole syrup containing 0.05 % w/v sodium bisulfite showed a tendency to has the low value of degradation rate constant. This result agreed with many reports showing that sodium bisulfite is best effective in optimum lower concentration levels. However, the degradation rate constants increased in the presence of light ; light might decrease the antioxidant effect of sodium bisulfite (Wade and Weller, 1994; Brawley, Bhatia and Karp 1998). The dotted line in

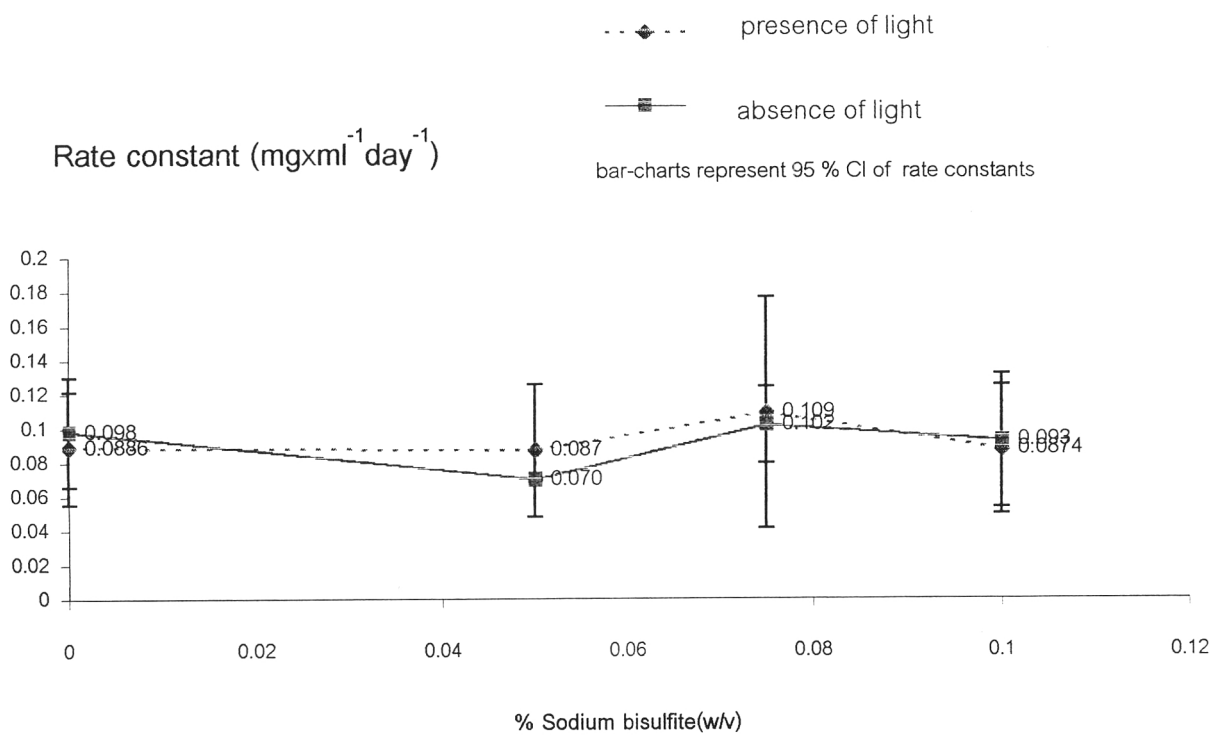


Figure 18 Plots of rateconstant vs. concentration of sodium bisulfite.

figure 18 shows that the rate constant (k) of the same formula which stored in the presence of light had high k value. When sodium bisulfite concentration is high, it can accelerate the degradation rate of many drugs and pharmaceuticals. Hussain, Wahner and Triplett (1978) reported that physostigmine degradation increases rapidly when the concentration of sodium bisulfite is increased from 0.001 M to 0.4 M. Malkki-Laine and coworkers (1995) found that the best stabilizing effect was obtained with 0.1 %w/v sodium bisilfite. The concentration of 0.05 %w/v sodium bisulfite was too low to stabilize the solution and that of 0.5 %w/v sodium bisulfite accelerated the decomposition of sulbutamol. Sodium bisulfite can react reversibly or irreversibly with various functional groups in drug molecules such as aldehyde, ketone and alkene. The inactivation of epinephrine and other drug molecules by bisulfite and the addition of bisulfite to a carbon-carbon double bond in uracil-type molecules (Munson, Hussain and Bilous, 1977). For example, Fluorouracil, which sodium bisulfite added to its double bond to yield 5-fluoro-5,6 dihydrouracil-6-sulfonate (Rork and Pitman,1975). Besides fluorouracil, the addition reaction of sodium bisulfite with alpha-beta unsaturated double bond of morphine, which have a strong electron attracting group on the alpha position (Yeh and Lach, 1971).

4.3.3 Effect of chelating agents

Disodium edetate was the widely used chelating agent studied. Three concentration levels of disodium edetate of 0.005, 0.01 and 0.05% w/v were studied. The concentration of fluconazole remaining and a plot of drug concentration vs. time of fluconazole syrup containing different concentration of disodium edetate were shown in Appendix IV.

Effect of disodium edetate

When 0.005 %w/v of disodium edetate was added to fluconazole syrups, the degradation rate constant of fluconazole was decreased when the light was absent in the system, but the degradation rate constant was increased when light was present. When 0.01 %w/v of disodium edetate was added, the degradation rate constants of fluconazole stored in the absence of light was increased but it was lower than the degradation rate constant of fluconazole in fluconazole syrup (no antioxidant). When 0.01 %w/v of disodium edetate was added to fluconazole syrup stored in the presence of light, the degradation rate constant of fluconazole was decreased from the degradation rate constant of fluconazole in fluconazole syrup containing 0.005 % w/v disodium edetate. When 0.05 % w/v of disodium edetate was added, the degradation rate constant of fluconazole were relatively unchanged in all storage conditions. Figure 19 shows the plots of degradation rate constant of fluconazole syrup vs. time in the presence and absence of light. The change in degradation rate constants in each storage condition are not statistically significant since all p-values are more than 0.05 as shown in Appendix VII. In the absence of light, fluconazole syrup containing 0.005% w/v disodium edetate showed tendency to retard the degradation rate of fluconazole because it acted as the chelating agent. In the presence of oxygen, disodium edetate at higher concentrations does not simply inhibit the oxidation process by acting as a chelating agent, it can also generate hydroxyl radical ($OH^{\cdot}$) in the presence of transition metal ions such as iron via a metal-catalyzed Habber-Weiss reaction which can be depicted as follows (Li et at., 1993) :

$$Fe^{3+} - EDTA + O_2^{\cdot-} \longrightarrow Fe^{2+} - EDTA + O_2 \quad \text{(eq. 41)}$$

$$2O_2^{\cdot-} + 2H^{+} \longrightarrow H_2O_2 + O_2 \quad \text{(eq. 42)}$$

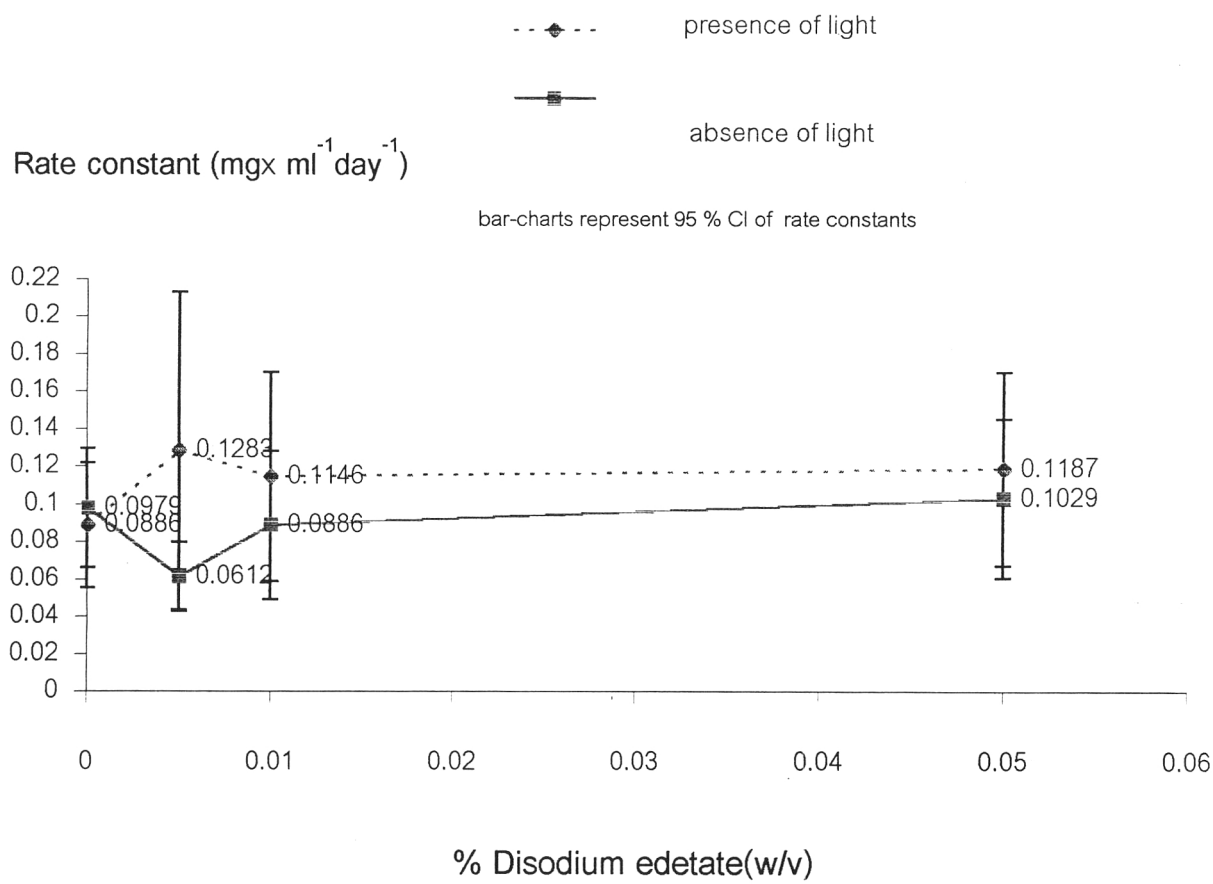


Figure 19 Plots of rate constant vs concentration of disodium edetate.

$$Fe^{2+} - EDTA + H_2O_2 \longrightarrow Fe^{3+} - EDTA + HO^{\cdot} + HO^{-} \quad \text{(eq. 43)}$$

$HO^{\cdot}$ from equation 40 might react with $H^{\cdot}$ in the initiation phase of oxidative reaction of fluconazole which can be depicted as follows:

$$RH \text{ (fluconazole)} \longrightarrow R^{\cdot} + H^{\cdot} \quad \text{(eq. 44)}$$

$$HO^{\cdot} + H^{\cdot} \longrightarrow H_2O \quad \text{(eq. 45)}$$

When $HO^{\cdot}$ react with $H^{\cdot}$, reaction in the equation 44 will shift to the right. The result showed that, fluconazole might degrade more than fluconazole syrup containing 0.005 %w/v disodium edetate. Therefore, EDTA in the presence of oxygen and metal ions might act as a catalyst when its concentration increased. Its catalytic effect could be promoted by light, so the degradation rate constant was increase in fluconazole syrup containing disodium edetate 0.005% w/v in the presense of light. Because this concentration of disodium edetate in fluconazole syrup was high enough for act as a catalyst when light, oxygen and metal ion were presented. When 0.05 %w/v of disodium edetate was added, the degradation rate constant of fluconazole were relative unchanged from when 0.01 %w/v of disodium edetate was added to fluconazole syrup in all storage condition. Because all formulations had quantity of transition metal and oxygen closed to one another. Transition metal ions such as iron via a metal-catalyzed Habber-weiss reaction might limit the change of degradation rate constant of fluconazole when disodium edetate at higher concentrations.

5. Kinetic studies on the stabiltiy of fluconazole in fluconazole syrup

Fluconazole syrup containing 0.001% w/v propyl gallate showed tendency to have the lowest degradation rate constant when stored at 60 ° C throughout 107 days was chosen for prediction of shelf-life by Arrhenius method.

5.1 Arrhenius relationship

The stabiltiy of fluconazole in fluconazole syrup containing 0.001% w/v propyl gallate was determined by accelerated stability testing method and compared to the results obtained at ambient temperature (30±3°C) storage. The formulation was incubated at 45°, 55°, 65°, 70° C and ambient temperature. The first sample taken at zero time was referred to as 100 percent of initial concentration. The concentrations of fluconazole remaining in subsequent samples were calculated as percentage of initial concentration. The percent of fluconazole after incubation at different temperature was shown in Table 26, 27, 28, 29 and 30. The specific rate constants (k) were calculated from the slope of linear regression line at specified temperature as shown in Table 26, 27, 28, 29 and 30.

In this study, the specific rate constants of fluconazole in fluconazole syrup containing 0.001% w/v propyl gallate at temperature 45°C, 55°C, 65°C and 70°C were plotted according to Arrhenius relationship. The Arrhenius plot of natural logarithm of specific rate constants (lnk) versus the corresponding reciprocal of degree kelvin (1/T) exhibited linearity (r = 0.9811) as shown in Figure 20. The linearity of Arrhenius plot indicated proper selection of the temperature range studied.

Table 26 Stability of fluconazole in fluconazole syrup containing 0.001% w/v propyl gallate at 30° C.

Percent amount [a] of fluconazole after incubation at 30 ° C [b]	
Days	Percent
0	100.00
31	99.30
74	99.07
k	0.012
t_{90}	810

conc = 99.88 – 0.012 time ; r = 0.930

a = mean value of 3 experiments

b = average room temperature

Table 27 Stability of fluconazole in fluconazole syrup containing 0.001% w/v propyl gallate at 45 ^{o} C.

Percent amount of fluconazole after incubation at 45 ^{o} C	
Days	Percent
0	100.00
13	99.06
74	97.52
k	0.031
t_{90}	315

conc = 99.76 – 0.031 time ; r = 0.976

a = mean value of 3 experiments

Table 28 Stability of fluconazole in fluconazole syrup containing 0.001% w/v propyl gallate at 55° C.

Percent amount [a] of fluconazole after incubation at 55° C	
Days	Percent
0	100.00
13	97.43
31	96.17
38	96.65
54	92.50
74	95.30
k	0.073
t_{90}	122

conc = 98.90 – 0.073 time ; r = 0.795

a = mean value of 3 experiments

Table 29 Stability of fluconazole in fluconazole syrup containing 0.001% w/v propyl gallate at 65 $^{\circ}$ C.

Percent amount of fluconazole after incubation at 65 $^{\circ}$ C	
Days	Percent
0	100.00
13	94.50
31	93.42
38	91.15
54	90.86
74	92.29
k	0.098
t_{90}	73

conc = 97.13 – 0.098 time ; r = 0.779

a = mean value of 3 experiments

Table 30 Stability of fluconazole in fluconazole syrup containing 0.001% w/v propyl gallate at 70 $^{\circ}$ C.

Percent amount of fluconazole after incubation at 70 $^{\circ}$ C	
Days	Percent
0	100.00
13	88.19
31	93.50
38	94.10
54	88.19
74	84.47
k	0.155
t_{90}	44

conc = 96.85 – 0.155 time ; r = 0.752

a = mean value of 3 experiments

Table 31 Arrhenius relation of fluconazole degradation of fluconazole syrup containing 0.001%w/v propyl gallate.

Temperature			Specific rate constant ($mg \times ml^{-1} day^{-1}$)	
^{o}C	K	$1/T \times 10^{3}$ k	$k \times 10^{-2}$	lnk
45	318.15	3.14	3.10	- 3.47
55	328.15	3.05	7.31	- 2.62
65	338.15	2.96	9.78	- 2.32
70	343.15	2.91	15.54	- 1.86

Arrhenius Equation $\ln k = 17.18 - (6550.40)\frac{1}{T}$

Correlation coefficient (r) 0.981

Heat of activation (kcal/mol) 13.02

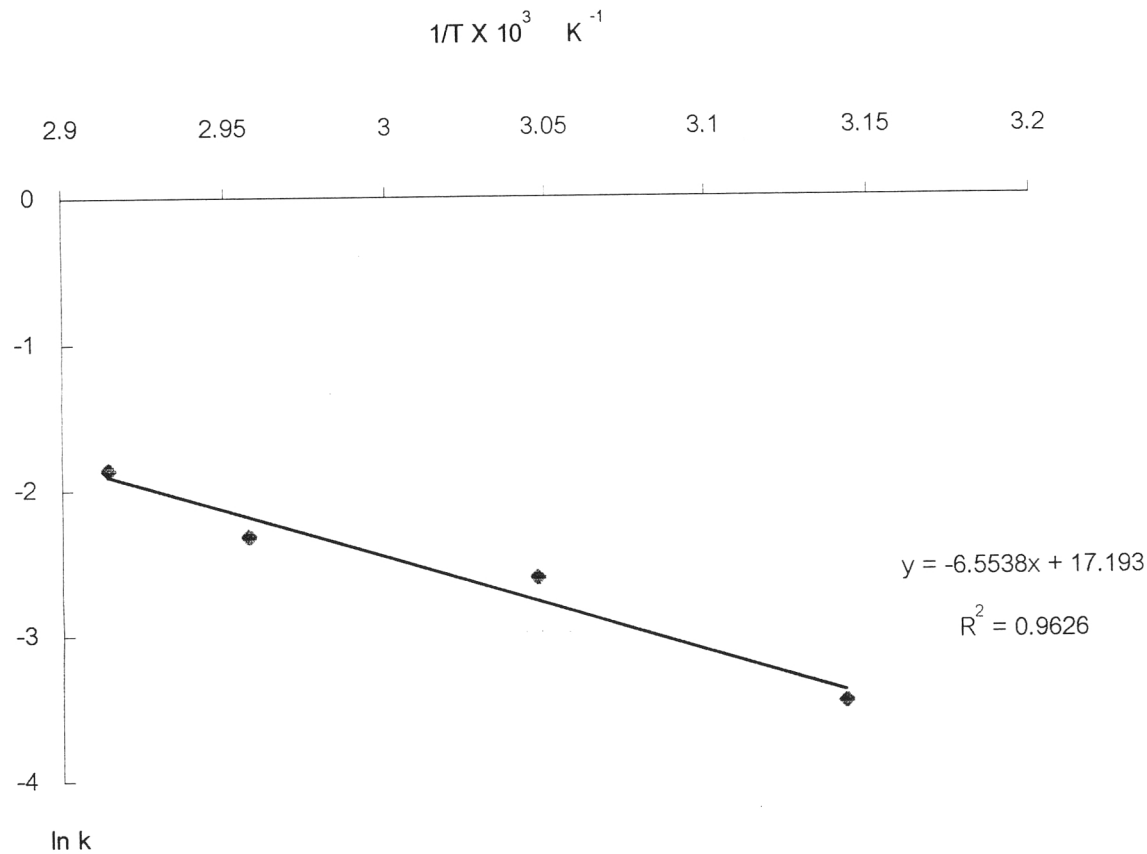


Figure 20 Arrhenius plot of the natural logarithm of specific rate constant (k) vs. the reciprocal of degree kelvin (1/T) of fluconazole in fluconazole syrup containing 0.001% w/v propyl gallate.

and possible prediction of degradation rate at lower temperature could be obtained by extrapolation.

Predicted degradation rates were obtained from extrapolation of Arrhenius plot to room temperature at 30°C ($1/T = 3.3003 \times 10^{-3}\ K^{-1}$) as shown in a typical plot in Figure 20. The predicted degradation rates at 30°C of fluconazole syrup containing 0.001% w/v propyl gallate was 0.0119 $(mg/ml)(day^{-1})$. This result of predicted degradation rate was closed to that as in calculated from actual incubation at 30 °C (Table 26).

5.2 Heat of activation

The apparent heat of activation (Ea) of fluconazole syrup containing 0.001% w/v propyl gallate was calculated from the slope of the Arrhenius plot (equation 32). The heat of activation was 13.02 kcal/mole. The heat of activation has been used in identification of reaction mechanisms. For example, formulations that degraded through solvolytic processes had heat of activation in the range of 10-30 kcal/mole. These heat of activation supported conduction accelerated temperature studies as there was marked increase in reaction rates at elevated temperatures. However, if diffusion or photolysis was the rate-determining step, the heat of activation was only in the magnitude of 2-3 kcal/mole, the temperature effect was small, and little advantage was gained by accelerated temperature studies in prediction. For reactions such as pyrolysis, the heat of activation was in the magnitude of 50-70 kcal/mole. Though rate of degradation would be high at elevated temperatures, it might not be of any practical significance at the temperature of marketing or storage (Pope, 1980).

In this study, fluconazole syrup containing 0.001% w/v propyl gallate had the heat of activation of 13.02 kcal/mole. The reaction rate constant at any temperature could be estimated from rate obtained at one elevated temperature (equation 35). This method provided some advantage in reducing the number of experiments required for prediction of product stability.

5.3 Shelf-life

Shelf-life of fluconazole is the time required for fluconazole degraded from 100% concentration to reach 90%. As the degradation of fluconazole was previously assumed to be a zero-order. Therefore, the shelf-life (t_{90}) of fluconazole in fluconazole syrup at ambient temperature could be calculated from the zero-order reaction rate (equation 30).

In this study, shelf life (t_{90}) was calculated by using predicted rate. This predicted shelf – life calculated from Arrhenius plot was 834 days (28 months).