Chapter III

Results and Discussions

I. Upon analysing the results obtained by determination of vitamin B_{12} as summarized in Table 1.

The various concentrations of vitamin B_{12} were determined by column chromatography and spectrophotometric methods, using the absorbance at 361 nm and 550 nm. showing that :

- The % recovery of vitamin B_{12} at 361 nm. was between
  96.90 %-- 100.01 % S.D. = 1.058
  C.V. = 1.073
- The % recovery of vitamin B_{12} at 550 nm. was between
  96.81% - 103.70 % S.D. = 2.081
  C.V. = 2.084

The ratio of absorbance of vitamin B_{12} fraction as showed in Table 2, the ratio of absorbance at 361/550 was between 3.13-3.37

The mixture of dioxane -HCl is an eluating solution.

Cyanocobalamin gives the maximum absorbance at 361 and 550 nm.[25], the value of $E^{1\%}_{1cm}$ is

$E^{1\%}_{1cm}$ = 207 at 361 nm.
= 63 at 550 nm.

At 550 nm. the result of vitamin B_{12} may be interferred by other red pigments, so that, the method of assay of vitamin B_{12} by Strohecker[22] was recommended to be used only at 361 nm. but the absorbance at 550 nm. was determined in order to confirm the result at 361 nm. and prove for the purity of the fraction from the column chromatography.

II. Table 3 shows the percentage of vitamin B_{12} in vitamin B-complex containing vitamin C Injection with various stabilizers kept at room temperature in different periods of time. The stabilizers added in the sample solutions are as follow :-

1. Glycine 2 % and histidine 2% in sample 1 and 2

The amino portion of glycine and the imidazole portion of histidine are the functional portions as the same as those consisting of some parts in the vitamin B_{12} molecule. On considering to their chemical structures, the two amino acids; glycine and histidine are selected to study for stabilization in this experiment. The 2 % solution of them were proposed to use for preparing the parenteral solution to obtain the iso-osmotic solution[24]. It has been shown that some investigations observed the fact that the haemolysis may occur in the body system if the strength was reduced to 1 % solution.It appears therefore that the 2 % solution should be reasonable to use as an experimental concentration.

2. Mannitol, sorbitol and xylitol 5 % solution in sample of 3,4 and 5

These sugar alcohols are selected to study, according to their properties as commonly used solvents for injectable preparations with non-toxic agents and should not react chemically with ingredients in the formulation.(9)

The 5 % solution was proposed to be used in experiments because the 5.48 % solution of sorbitol is obtained as iso-osmotic.(24) Therefore sugar alcohols group could be suitable for studying the stabilization of vitamin B_{12} in parenteral solutions and at the same time, also avoiding to form too viscous solution caused by their concentration higher than that level.

3. Caffeine and theophylline 0.2 % solutions in sample of 6 and 7

Xanthines were selected as stabilizers for vitamin B_{12} in this study because according to their structure, they are composed of two parts of pyrimidine and imidazole portions which also are to be found in some parts of vitamin B_{12} and thiamine molecules. The concentration of xanthines should be low to prevent or inhibit the decomposition of vitamin. The required quantity of xanthines would minimize the action of physiological side effects that may occur to humans, while still keeping the stabilized effectiveness to the vitamins too.

III. Table 4 indicates that the purity of the vitamin B_{12} fraction from the column chromatography by the given method, the ratio of the absorbance at 361/550 from experimental data are agreed to the given limit 3.0 - 3.5

IV. Table 5 illustrates the accumulated rate of change of the percentage of vitamin B_{12} by each of the stabilizers in this order in the eight week :

at 361 nm.

Mannitol, Sorbitol, Xylitol > Glycine, Histidine > Theophylline, Caffeine

at 550 nm.

Mannitol, Sorbitol, Xylitol > Glycine, Histidine > Theophylline, Caffeine.

The above results indicated that the caffeine and theophylline from xanthine group give the lowest rate of change at both 361 nm. and 550 nm. and for this reason, the xanthines : caffeine and theophylline can stabilize the vitamin B_{12} in the vitamin B-complex containing vitamin C Injections better than both of the sugar alcohols : sorbital, mannitol, xylitol and the amino acids : glycine and histidine.

The mechanism of preventing the decomposition of vitamin B_{12} in this experiment has not been proved. It may be caused by the function of pyrimidine and imidazole portions of caffeine molecule react as a competitive prevention thiamine decomposition

and at the sametime to prevent the reducing power of the ascorbic to the molecule of vitamin B_{12}. It is also shown the effeciency of amino acids and sugar alcohols as the stabilizers for vitamin B_{12} in comparison with caffeine.

Theophylline is an expensive substance in comparison to caffeine, it is recommend to use caffeine for stabilizing agent in preparation of the parenteral solution in pharmaceutical manufacturing and it has been selected to use for study in this experiment.

V. Table 6 shows the percentage of vitamin B_{12} in vitamin B-complex containing vitamin C Injection using caffeine as a stabilizer in various concentrations (0.1 %, 0.2 % and 0.3%) kept at room temperature.

The purity of vitamin B_{12} fraction by using caffeine as a stabilizer is summarized in Table 7. The ratio of 361/550 is between 3.0 - 3.5

The accumulated rate of change of vitamin B_{12} using caffeine as a stabilizer and during different periods of time at 361 nm and 550 nm. are summerized in Table 8 in the following order ;

In the eight week at 361 nm. :

Caffeine 0.1 % $\rangle$ Caffeine 0.2 % $\rangle$ Caffeine 0.3 %

The accumulated rate of change of the percentage of vitamin B_{12} by using 0.1 % caffeine gives the more change than when using the caffeine 0.2 % and 0.3 %

In the eighth week at 550 nm.

Caffeine 0.1 % $\rangle$ Caffeine 0.2 % $\rangle$ Caffeine 0.3 %

The outcome agreed to the result at 361 nm. as indicates that caffeine 0.2 % and caffeine 0.3 % gave almost the same results.

By using caffeine as stabilizer in Table 6 . give the percentage of vitamin B_{12} left in the eighth week;

Caffeine 0.1 %	Vitamin B_{12} left	=	95.72 %
Caffeine 0.2 %	Vitamin B_{12} left	=	97.51 %
Caffeine 0.3 %	Vitamin B_{12} left	=	98.22 %

The comparison of these concentrations of caffeine, the percentage of vitamin B_{12} by using caffeine 0.2 % and 0.3 % are almost the same and are better than using caffeine 0.1 %.

To prevent the decomposition of vitamin B_{12}, it is recommended to use caffeine at the lowest concentration that can keep the potency of vitamin B_{12} in the limit of percentage labelled amount, so that the minimum concentration of caffeine should be 0.2 % .

Table 1

Method of Assay for Vitamin B_{12} (Raw Material) by Spectrophotometric Method Using Column Chromatography.

No.	Vitamin B_{12} concentration (mg./ml.)	% Recovery of Vitamin B_{12}	
		at 361 nm.	at 550 nm.
1.	0.273	98.97	98.49
2.	0.273	99.91	101.61
3.	0.300	98.28	99.58
4.	0.311	97.31	97.43
5.	0.369	98.91	98.31
6.	0.378	99.66	101.61
7.	0.378	96.90	101.61
8.	0.400	97.42	98.16
9.	0.359	97.69	99.86
10.	0.432	98.62	99.79
11.	0.432	99.23	101.75
12.	0.391	98.99	103.70
13.	0.391	97.99	99.33
14.	0.313	100.01	99.54
15.	0.313	99.18	96.81
Mean ($\bar{X}$)		98.61	99.84
Standard Deviation (S.D.)		1.058	2.081
% Coefficient of Variation (C.V.) (26)		1.073	2.084

Table 2 Ratio of Absorbance of Eluate from Column in Table 1.

No.	Vitamin B_{12} concentration (mg./ml.)	Absorbance		Ratio 361/550
		at 361 nm.	at 550 nm.	
1.	0.273	0.208	0.063	3.30
2.	0.273	0.210	0.065	3.23
3.	0.300	0.227	0.070	3.24
4.	0.311	0.233	0.071	3.28
5.	0.369	0.281	0.085	3.31
6.	0.378	0.290	0.090	3.22
7.	0.378	0.282	0.090	3.13
8.	0.400	0.300	0.092	3.26
9.	0.359	0.270	0.084	3.21
10.	0.432	0.328	0.101	3.25
11.	0.432	0.330	0.103	3.20
12.	0.391	0.298	0.095	3.14
13.	0.391	0.295	0.091	3.24
14.	0.313	0.241	0.073	3.30
15.	0.313	0.239	0.071	3.37

Table 3 Potency of Vitamin B_{12} in Vitamin B-Complex Containing Vitamin C Injection with VariousStabilizers kept at Room Temperature.

No.	Stabilizers	% Vitamin B_{12} at 361 nm.*				% Vitamin B_{12} at 550 nm.*			
		1 week	3 week	4 week	8 week	1 week	3 week	4 week	8 week
1.	Glycine 2%	92.27	83.68	82.24	80.82	96.06	87.85	84.34	82.01
2.	Histidine 2%	92.98	84.04	81.53	80.46	96.96	89.03	85.52	80.83
3.	Mannitol 5%	85.11	80.46	79.02	77.25	91.37	79.66	78.48	76.15
4.	Sorbitol 5%	86.90	82.25	80.09	78.68	92.55	85.52	80.83	78.78
5.	Xylitol 5%	89.40	82.97	79.02	77.95	94.89	83.17	77.31	77.31
6.	Caffeine 0.2%	98.76	97.62	97.42	97.27	99.57	99.57	97.23	97.23
7.	Theophylline 0.2%	100.13	99.42	97.42	97.27	101.92	99.57	98.40	97.23
8.	Control (No Stabilizer)	83.68	75.09	72.17	68.67	85.52	77.31	73.80	71.46

* % average from n = 2

Table 4 Ratio of Absorbance of Eluate from Column in Table 3

No.	Stabilizers	1 week			3 week			4 week			8 week		
		Absorbance		Ratio	Absorbance		Ratio	Absorbance		Ratio	Absorbance		Ratio
		361nm.	550nm.	361/550	361nm.	550nm.	361/550	361nm.	550nm.	361/550	361nm.	550nm.	361/550
1.	Glycine 2%	0.258	0.082	3.15	0.234	0.075	3.12	0.230	0.072	3.19	0.226	0.070	3.23
2.	Histidine 2%	0.260	0.082	3.17	0.235	0.076	3.09	0.228	0.073	3.12	0.225	0.069	3.26
3.	Mannitol 5%	0.238	0.078	3.05	0.225	0.068	3.31	0.222	0.067	3.22	0.216	0.065	3.32
4.	Sorbitol 5%	0.243	0.079	3.08	0.230	0.073	3.15	0.225	0.069	3.26	0.220	0.067	3.28
5.	Xylitol 5%	0.250	0.081	3.09	0.232	0.071	3.27	0.222	0.066	3.36	0.218	0.066	3.30
6.	Caffeine 0.2%	0.275	0.085	3.24	0.273	0.085	3.21	0.270	0.083	3.25	0.272	0.083	3.25
7.	Theophylline 0.2%	0.280	0.087	3.22	0.278	0.085	3.27	0.270	0.084	3.21	0.272	0.083	3.25
8.	Control (No Stabilizer)	0.234	0.073	3.21	0.210	0.066	3.18	0.200	0.063	3.18	0.192	0.061	3.15

Table 5 Accumulated Rate of Change of the Potency of Vitamin B_{12} from Table 3.

No.	Stabilizers	361 nm.				550 nm.			
		1 week	3 week	4 week	8 week	1 week	3 week	4 week	8 week
1.	Glycine 2%	7.73	16.32	17.75	19.18	3.94	12.15	15.66	17.99
2.	Histidine 2%	7.02	15.96	18.47	19.54	3.04	10.97	14.48	19.17
3.	Mannitol 5%	14.89	19.54	20.98	22.75	8.63	20.34	21.52	23.85
4.	Sorbitol 5%	13.10	17.75	19.91	21.32	7.45	14.48	19.17	21.52
5.	Xylitol 5%	10.60	17.03	20.98	22.05	5.11	16.83	22.69	22.69
6.	Caffeine 0.2%	1.24	2.38	2.58	2.73	0.43	2.43	2.77	2.77
7.	Theophylline 0.2%	0	0.58	2.58	2.73	0	0.43	1.60	2.77
8.	Control (No Stabilizer)	16.32	24.91	27.83	31.33	14.48	22.69	26.20	28.54

Table 6 Potency of Vitamin B_{12} in Vitamin B-Complex Containing Vitamin C Injection Using Caffeine as Stabilizer in Various Concentrations kept at Room Temperature.

No.	Stabilizers	% Vitamin B_{12} at 361 nm.*				% Vitamin B_{12} at 550 nm.*			
		1 week	3 week	4 week	8 week	1 week	3 week	4 week	8 week
1.	Caffeine 0.1%	97.86	97.15	96.44	95.72	97.92	94.42	93.26	93.26
2.	Caffeine 0.2%	98.58	98.22	97.86	97.51	99.09	99.09	97.92	97.92
3.	Caffeine 0.3%	99.28	98.93	98.93	98.22	99.09	99.09	99.09	97.92
4.	Control (no caffeine)	84.70	78.91	72.95	67.96	83.93	81.60	73.43	71.11

* % average from n= 2

Table 7 Ratio of Absorbance of Eluate from Column in Table 6

No.	Stabilizers	1 week			3 week			4 week			8 week		
		Absorbance		Ratio 361/550	Absorbance		Ratio 361/550	Absorbance		Ratio 361/550	Absorbance		Ratio 361/550
		361nm.	550nm.		361nm.	550nm.		361nm.	550nm.		361nm.	550nm.	
1.	Caffeine 0.1%	0.275	0.084	3.27	0.273	0.081	3.37	0.271	0.080	3.39	0.269	0.080	3.36
2.	Caffeine 0.2%	0.277	0.085	3.26	0.276	0.085	3.25	0.275	0.084	3.27	0.274	0.084	3.26
3.	Caffeine 0.3%	0.279	0.085	3.28	0.278	0.085	3.27	0.278	0.085	3.27	0.276	0.084	3.29
4.	Control (no caffeine)	0.238	0.072	3.31	0.222	0.070	3.10	0.205	0.063	3.25	0.190	0.061	3.13

Table 8 Accumulated Rate of Change of the Potency of Vitamin B_{12} from Table 6

No.	Stabilizers	361 nm.				550 nm.			
		1 week	3 week	4 week	8 week	1 week	3 week	4 week	8 week
1.	Caffeine 0.1%	2.14	2.28	3.56	4.28	2.08	5.58	6.74	6.74
2.	Caffeine 0.2%	1.42	1.78	2.14	2.49	0.91	0.91	2.08	2.08
3.	Caffeine 0.3%	0.72	1.07	1.07	1.78	0.91	0.91	0.91	2.08
4.	Control (no caffeine)	15.30	21.09	27.05	32.04	16.07	18.40	26.57	28.89