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STABILITY OF CYANOCOBALAMIN IN PARENTERAL PREPARATIONS

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ABSTRACT

The stability of sewn commercial parenteral preparations of cyanocobalamin (vitamin B_{12}) on storage under normal laboratory conditions for a period of twelve months has been studied using a two component spectrophotometric method for the simultaneous determination of eyanocobalamin and the degradation product, hydrosocobalamin, at 550 and 525 rim. The single ingredient vitamin H_{12} preparations have been found to be stable and the potency lies within the B.P. limits. In multi-ingredient (B_1 + B_6 + B_{12}) preparations cyanocobalamin is unstable and degrades from 28% to 37% with concomitant formation of hydroxocobalamin (1.7% to 25.5%) and oxidation products amounting to 56.4% ± 9.3. Thus more than half of the vitamin content is lost during storage and these preparations are not suitable for parenteral use.

INTRODUCTION

Cyanocobalamin (vitamin B_{12}) injection is a sterile aqueous solution of cyanocobalamin containing sufficient acetic acid or hydrochloric acid to adjust the pH to about 4 using a suitable buffering agent (B.P., 1988). The official requirements for the pH of cyanocobalamin injections are between 3.8 and 55 (B.P., 1988) and 4.7 to 7.0 (U.S.P., 1990). Commercial cyanocobalamin parenteral preparations are available either as vitamin BI2 alone or in combination with B vitamins such as thiamine (B_1) and pyridoxine (B_6).

Sterile aqueous solutions of vitamin B_{12} are stable at pH 4.5-5. However, the presence of reducing agents even in trace quantities can markedly influence potency as a result of irreversible oxidation (Macek, 1960). Cyanocobalamin is converted to hydroxocobalamin (B_{12b}) at pH 35-65 on exposure to light (Baxter *et al,* 1953) as indicated by the decrease in absorbance at 361 nm (Bayer, 1964). The photolysis follows zero-order kinetics (Vogler *et al,* 1976) and cyanocobalamin has maximum stability in the pH range 6-7 (Ahmad *et al.,* 1992). In parenteral solutions, cyanocobalamin is unstable in the presence of thiamine and nicotinamide (Blitz *et al.*, 1954, 1956; Mukherjee and Sen, 1959; Dale and Booth, 1976) as well as their degradation products (Feller and Macek, 1955; Mukherjee and Sen, 1959; Heathcote and Wills, 1962). Amide cyclization and amide hydrolysis in acidic and basic media also contribute to the overall stability of B_{12} solutions (Kirschbaum, 1981; Connors *et al.,* 1986).

The study of the stability of cyanocobalamin is complicated by the fact that in degraded solutions the official methods (B.P., 1988; U.S.P., 1990) for the assay of cyanocobalamin (361 nm) and hydroxocobalamin (351 nm) are not applicable due to their mutual interference. The presence of even up to about 10% of Bum in degraded solutions may not show any shift in absorption at 361 nm and preservatives absorbing in this region may also interfere with the assay (Hasluni, 1973). Other methods (Bader *et al.*, 1953; Heathcote and Duff, 1954; Bayer, 1964; DeWitt and Muck, 1973; Horvath and Szepesi, 1978) for the determination of B_{12} and B_{12b} are time consuming and give only approximate results. In the present work a newly developed spectrophotometric method (Ahmad *et al.*, 1992) has been applied to investigate the stability of cyanocobalamin in parenteral preparations by simultaneous determination of B_{12} and B_{12b} at 550 and 525 nm. Similar specific methods (Fareedi, 1976; Husain, 1987; Ahmad and Hussain, 1992) have been used to study the photolysis of cyanocobalamin in presence of other vitamins.

MATERIAL AND METHODS

Cyanocobalamin and hydroxocobalamin (Eur.P.) were obtained from Fluka (Switzerland) and were found to be chromatographically pure. All solvents and reagents *were* analytical grade or of the purest form available from BDH/Merck. Parenteral preparations of cyanocobalamin made by different manufacturers were procured from the market (Table 1) and stored in the original containers and packaging at room temperature (25°-28°C) for a period of 12 months under normal laboratory light conditions.

Cyanocobalamin and hydroxocobalamin in fresh and degraded solutions were determined according to the method of Ahmed *et al* (1992) by a two-component spectrophotometric assay at 550 and 525 nm. All determinations were carried out in duplicate and the results are presented as averages. When the duplicate results showed a variation of more than five percent, the assays were repeated until satisfactory data obtained.

RESULTS AND DISCUSSION

All commercial samples of vitamin B_{12} preparations, on storage for 12 months, showed the presence of hydroxocobalamin as a degradation product. This was confirmed by thin layer chromatography on silica gel using the previously described solvent systems (Ahmad *et al.*, 1992). The pH of the samples varied from 3.25 to 5.50 (fresh) and from 3.53 to 5.96 (stored) indicating a slight increase in the pH value during storage (Table 2). This is probably due to the formation of hydroxocobalamin [Co^{3+} OH] which exists as an equilibrium mixture with aquocobalamin (pKa 7.8; Hayward *et al.*, 1965).

Table 1

Commercial parenteral preparations of cyanocobalamin used in the present investigation

Sample No.	Product	Labelled amount per ml(μg)	Container/ Capacity	Preservative/Additive stated on label
1	Vitamin B_{12}	1000	Amber glass 10 ml	Methyl p-hydroxybenzoate 0.15%
2	Vitamin B_{12}	1000	Amber glass 10 ml	Methyl p-hydroxybenzoate 0.15%
3	Vitamin B_{12}	1000	Amber glass 30 ml	
4	Compound injection* (B_1 + B_6 + B_{12})	1000	Amber glass 3 ml	
5	Compound injection* (B_1 + B_6 + B_{12})	1000	Amber glass 3 ml	
6	Compound injection* (B_1 + B_6 + B_{12})	1000	Amber glass 3 ml	
7	Compound injection* (B_1 + B_6 + B_{12})	1000	Colourless glass 3 ml	EDTA, benzyl alcohol, sodium bicarbonate

*B_1 and B_6 concentrations in sample 4-7 : 100 mg of each vitamin.

Table 2

Assay of cyanocobalamin and hydroxocobalamin in parenteral preparations stored at room temperature (25°-28°C) for 12 months.

Sample No.	Labelled* amount per ml (μg)	pH** Change	Cyanocobalamin content					Hydroxocobalamin (B_{12b}) μg/ml
			μg/ml B_{12}	% of labelled amount	overall loss,%	loss in terms of B_{12b},%	loss in terms oxidation products, %	
1	1000 (1103)	5.36 (5.78)	990	99.0	1.0	2.5	-	25
2	1000 (1042)	5.50 (5.96)	1026	102.6	-	1.2	-	12
3	1000 (1012)	4.45 (4.85)	950	95.0	5.0	2.8	-	27
4	1000 (870)	3.78 (4.15)	370	37.0	63.0	12.4	50.6	52.7
5	1000 (714)	3.25 (3.65)	369	36.9	63.1	1.7	61.4	6.5
6.	1000 (816)	3.27 (5.58)	278	27.8	72.8	25.5	46.7	95
7.	1000 (720)	3.40 (3.53)	280	28.0	72.0	5.3	66.7	20

*The values in parenthesis indicate the concentrations determined on 1-2 month old samples before storage and may represent any loss in B_{12} content during that period.

**pH values determined in fresh samples, and those in parenthesis after 12 months storage.

***The percentage of oxidation products was calculated from the overall loss of B_{12} - loss in terms of B_{12b}; Mean $\pm$ S.D. = 56.4% $\pm$ 9.3.

Among the stored samples of vitamin Biz the single ingredient (B_{I2}) samples 1,2 and 3 (1000 µg/ml), contained 99.0, 102.6 and 95.0% cyanocobalamin respectively of the labeled amount, and 25,12 and 27 µg/ml (2.5,1.2 and 2.8%) respectively of hydroxocobalamin as a degradation product (Table 2). Since the official limits for the content of anhydrous cyanocobalamin in B_{12} injections are 95.0 to 115.0% (B.P., 1988) and 95.0 to 115.0% (U.S.P., 1990) of the labelled amount, the cyanocobalamin content of single ingredient samples (1-3) lies within the pharmacopoeia) limits and, therefore, storage for 12 months under normal laboratory conditions does not appear to affect the potency of these preparations.

The multi-ingredient (B_1+ B_6 + B_{12}) samples 4-7 (B_{12} 1000 µg/ml), on storage for 12 months, showed 37.0, 36.9, 27.8 and 28.0% cyanocobalamin respectively of the labelled amount and 52.7, 6.5, 95 and 20 µg/ml (12.4, 1.7, 25.5 and 5.3%) respectively of hydroxocobalamin (Table 2). In all the samples, vitamin B_{12} loss is much greater than the prescribed pharmacopoeial limits and, therefore, these samples are not suitable for clinical use. Since the loss of cyanocobalamin is not in accordance with the formation of hydroxocobalamin, the possibility of further degradation to irreversible oxidation products (Macek, 1960) can not be excluded. An estimate of the degradation products indicated that more than half of the vitamin content (56.4% ± 9.3) is converted to the oxidation products and about one-tenth (11.2% 3 10.8) to hydroxocobalamin. The large deviation in hydroxo-cobalamin value (1.7 to 25.5%) may be due to variations in the degree of interaction of $_{B12}$ with other vitamins in the preparations and subsequent loss of Rim to oxidation products. Vitamin B_1 and its decomposition products, which possess reducing properties, are known to destabilize cyanocobalamin solutions (Blitz *et at*, 1954; Mukherjee and Sen, 1955) and cause degradation of B_{12} to B_{12b} and other products as observed in the compound preparations of vitamin $B_{12.}$ Appropriate stabilizing agents (Linter, 1973; Kirschbaum, 1981; Martin, 1983) may be used to preserve the potency of cyanocobalamin in the presence of other vitamins in parenteral and other liquid preparations. Such preparations should be protected from light during storage to prevent the formation of B_{12b}, which leads to the destruction of B_{12} to inactive products.

On the basis of the data obtained in this study it may be suggested that the manufacturer should use reliable stability- indicating methods to determine the intact drug content during storage testing and ensure that the product would maintain its labelled potency within the prescribed pharmacopoeial limits until its expiration date. Due consideration should be given to drug interactions in multi-ingredient preparations and suitable measures taken to minimise them during storage.

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