Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

J. Med. Sci., 3 (2): 163-168
March-April, 2003

A Convenient Colorimetric Assay Method for Determination of Vitamin B_{12} content in Pharmaceutical Preparations

Firoj Ahmed, [1]Rebecca Banoo, [1]Ghazi Muhammad Sayedur Rahman and [1]Md. Omar Faruk Khan

A convenient colorimetric assay method for the determination of vitamin B_{12} content in pharmaceutical preparations has been developed. The proposed method is based on the decomposition of vitamin B_{12} by HNO_3 followed by subsequent formation of a stable colored complex (λ_{max} 435 nm) between the liberated cobalt ion and Nitroso-R-salt. Commercial injectable preparations containing vitamin B_{12} of three different manufacturers (two were single component injection and one was multi-component injection containing vitamin B_1, B_6 and B_{12}) were analyzed by the proposed method. All of them were found to meet USP limit. A recovery test was also performed which was in close agreement with that of the USP method. Different statistical parameters such as standard deviation, co- efficient of variation and confidence interval were also determined to validate the method.

Key words: Colorimetric assay, vitamin B_{12}, pharmaceutical preparations

Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh
[1]Department of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publish original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued six times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Firoj Ahmed
Pharmacy Discipline
Khulna University
Khulna-9208
Bangladesh

e-mail: firog@khulna.bangla.net

ANSInet
Asian Network for Scientific Information

Introduction

Vitamin B_{12}, a precursor of co-enzyme B_{12} is one of the most important and essential members of vitamin B complex family. It plays an important role in hematopoeitic system and said to have functions on nervous system. Because of micro level presence of vitamin B_{12}, it necessitates a simple and easy method for its potency determination in different Pharmaceutical preparations and food products. However the determination of vitamin B_{12} in Pharmaceutical preparations is usually done either directly by measuring the absorbance of its water solutions at 361 nm (USP, 2000) or indirectly by a method which involves the determination of cobalt formed by the decomposition of vitamin B_{12} as it contains 4.34% cobalt (The MERCK Index). The direct method is applied to Pharmaceutical preparations that give clear water solutions, whereas in presence of other components interfering with its determination, various methods have been suggested for separation of vitamin B_{12} prior to its determination (USP, 2000).

For indirect determination of vitamin B_{12} as cobalt, various methods have been proposed. They are based on the decomposition of the samples and subsequent colorimetric determination of cobalt by complexing with some suitable color forming legand. Rodica ***et al.*** (1969) developed a method of photometric determination of B_{12} as cobalt by complexing with 1-benzoyl-4-phenyl-thiosemicarbazide in ethanol. Eldawy ***et al.*** (1976) developed a method of forming colored complex with Na-7-nitroso-8-hydroxyquinoline-5-sulfonate for colorimetric determination of cobalt. Vasilikiotis ***et al.*** (1979) described a method for colorimetric determination of B_{12} as cobalt by complexing with 5,5/-dipyridyl-2-pyridylhydrazone in solution containing 20% H_2SO_4. Bruno ***et al.*** (1982) developed a new spectrophotometric method for determination of vitamin B_{12} as cobalt, complexing with hexamethyl-phosphoramide and thiocyanate. Zuting ***et al.*** (1985) described a sensitive spectrophotometric method of micro amount determination of cobalt in vitamin B_{12}. Sakai ***et al.*** (1991) developed an indirect spectrophotometric method of determining B_{12} using 3-(2-pyridyl)-5, 6-diphenyl-1, 2, 4-triazine:cobalt complex formation. More ***et al.*** (1992) developed a spectrophotometric method of determining trace amount of cobalt using iso-nitroso-4-methyl-2-pentanone. Zheng ***et al.*** (1995) developed color reaction of 1-(2-imidazolylazo)-2-napthol-4-sulfonic acid with cobalt.

Although a number of methods have been proposed for the determination of vitamin B_{12} but none of them were found to be suitable in terms of simplicity, rapidity and accuracy. The present study was to develop a simple, precise and sensitive method with a high recovery range that can be applied successfully for determination of vitamin B_{12} from any type of commercial pharmaceutical preparations of both single and multi-component.

Materials and Methods

Instruments

(a) Electronic Balance, model: BA 210 S, Sartorius, Germany (b) pH meter, model: PHI 32 Beckman, USA © UV-Visible Spectrophotometer, model: UV-VIS-160 A Shimadzu, Japan (d) Hot Spot furnace, model: Tactical 308 Gallenkamp, UK.

Reagents

Nitric acid was obtained from Merck, Germany as were the conc. Hydrochloric acid, Nitroso-R-salt and Cobaltous Nitrate. Sodium acetate trihydrate was purchased from BDH, UK. Standard vitamin B_{12} (88%) was obtained from BASF, Germany.

Preparation of sample

Pharmaceutical preparations containing 230.4 µgm of vitamin B_{12} (equivalent to 10 µgm of Co^{+2}) was taken in a porcelain crucible. The sample was burnt to ashes, first on a Bunsen flame then in an oven at 600°C. After burning to ashes, the residue was dissolved in 1 ml of conc. HNO_3 and was evaporated to dryness on a boiling water bath. Then 5 ml of combined acid solution (5 ml conc. HCl+1 ml conc. HNO_3 diluted to 200 ml with distilled water) was added to it and heated to dissolve completely. 0.5 ml of 0.2% nitroso-R-salt and 1 gram of sodium acetate crystal was added and again heated on a boiling water bath for 1 min followed by the addition of 1 ml of conc. nitric acid, boiled again for 1 min and finally cooled to room temperature. Nitroso-R-salt formed red purple colored complex with cobalt. Lastly the volume of the solution was adjusted to 10 ml with distilled water to give the concentration 1.7×10^{-5}(M) as B_{12} and 1.696×10^{-5} (M) as cobalt.

Preparation of blank

Five ml of combined acid solution was taken in a 25 ml beaker. 0.5 ml of 0.2% Nitroso-R-salt and 1 g of sodium acetate crystal was added and heated on a boiling water bath for 1 min. Then 1 ml of conc. nitric acid was added, boiled again for 1 min followed by cooling to room temperature. Finally the yellow colored solution was adjusted to a volume of 10 ml with distilled water.

Preparation of vitamin B_{12} stock solution

26.2 mg of standard vitamin B_{12} was dissolved in distilled water and made upto 100 ml in a volumetric flask. One ml of this solution contained 0.23 mg vitamin B_{12} (or 10 µgm of Cobalt). The concentration of vitamin B_{12} stock solution is 1.7×10^{-4}(M) as vitamin B_{12} and 1.696×10^{-4}(M) as cobalt.

Determination of λmax and stability profile of the colored complex

Absorbance was scanned from 200 to 800 nm and the absorption maxima was determined. The absorbance of the solution (10 µgm of cobalt ml^{-1}) at 435 nm was measured up to 16 at 1 h interval.

Preparation of standard curve

Absorbances (λ_{max} 435 nm) at different concentrations (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0 µgm ml^{-1} of cobalt) were plotted against their respective concentration and a straight line was obtained passing through the zero ordinates. Regressed values were calculated from the obtained absorbances and the "plot of regressed absorbances ***versus*** concentration" were also plotted.

Potency determination by proposed method

Samples from three different companies (S_F and S_G to be single component injection where as S_M is a multi-component one) were collected randomly from retail pharmacy and analyzed by the proposed method. For the purpose of comparison, potency of B_{12} in sample S_F, S_G and S_M were also determined by USP method (Table 1).

Recovery Experiment by standard addition

A recovery experiment was performed to check the accuracy and reproducibility of the proposed method. Four different known amounts of standard vitamin B_{12} (equivalent to 2.5, 5.0, 7.5 and 10.0 µgm of cobalt, respectively) were added to a fixed amount of vitamin B_{12} sample solution (equivalent to 5 µgm of cobalt). And the contents were determined each time using the proposed method. Simultaneously recovery test was also done by USP method. Four different known amount of standard vitamin B_{12} (10, 20, 30 and 40 µgm B_{12}, respectively) were added to a sample (equivalent to 50 µgm of B_{12}) and test was carried out (Table 2).

Statistical analysis

Data obtained from the results of recovery experiment for proposed method were analyzed using standard deviation, co-efficient of variation, standard error and confidence interval at two different level of significance (Table 3).

Results and Discussion

The absorption maxima of the colored complex formed from liberated cobalt ions after decomposition of vitamin B_{12} with Nitroso-R-salt was observed at 435 nm. From the “plot of` absorbance versus time” (Fig. 1) it is evident that the colored complex remained stable for 12 h with slight decrease of its absorbance after 16 h (≅ 5%).

From the “plot of absorbance ***versus*** varied concentration of colored complex” to fit both standard (Fig. 2) and regressed curve (Fig. 3), it was evident that straight line fitted in the concentration ranges from 0.00 to 17.5 µgm ml^{-1} and the molar absorptivity was calculated from the curve in different concentrations and the average value was found to be 2343.27 M^{-1} and 2329.72 M^{-1} respectively with an standard deviation of ± 9.57 (approx. 0.408%).

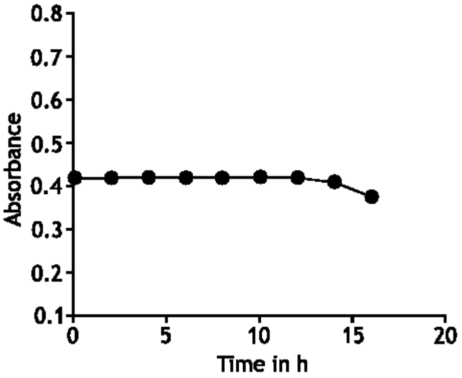

Fig. 1: Time dependent stability curve of the colored complex

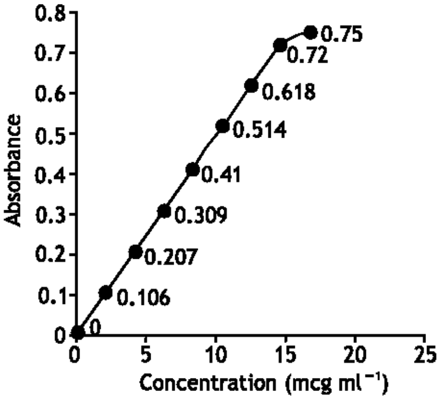

Fig. 2: Standard curve

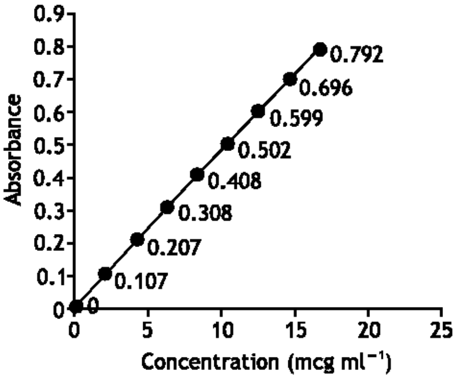

Fig. 3: Regression curve

Table 1: Determination of potency by the proposed method

Product code	Sample in (ml) (equivalent to 10 μmg of cobalt ml^{-1})	Standard solution (ml)	Amount of B_{12} declared ml^{-1} (μgm)	Av. Abs.	Average amount found ml^{-1} (μgm)	% potency B_{12}
S_F	0.23	1.0	1000	0.402	980	98%
S_G	0.23	1.0	1000	0.406	990	99%
S_M	0.23	1.0	1000	0.402	980	98%

Table 2: Determination of % recovery in proposed method

Serial no.	Addition of standard (μgm)	amount of sample (μgm)	Absorbances 1	2	3	Av. Abs.	Amount declared (μgm)	Amount found (μgm)	% recovery
1	0.00	5.00	0.210	0.207	0.209	0.208	5.00	5.024	99.71%
2	2.50		0.308	0.312	0.310	0.310	7.50	7.524	
3	5.00		0.409	0.408	0.410	0.409	10.0	9.980	
4	7.50		0.516	0.515	0.512	0.514	12.5	12.50	
5	10.0		0.620	0.619	0.617	0.618	15.0	15.00	

Table 3: Statistical analysis of data from the recovery experiment for proposed method

						(CI)	
Serial no.	Amount of cobalt (μm)*	Corresponding amount of B_{12} (μm)	(SD)	(CV)	(SE)	With 95% level of significance (μm)	With 99% level of significance (μm)
1	5.024	115.76	0.761	0.657	0.932	114.72-116.80	114.17-117.34
2	7.524	173.36	0.894	0.515	1.09	172.14-174.57	171.50-175.21
3	9.980	229.95	0.466	0.202	0.570	229.10-280.80	228.48-281.42
4	12.50	288.01	0.788	0.273	0.965	286.93-289.10	286.37-289.65
5	15.00	345.62	0.785	0.227	0.961	344.50-346.10	343.98-347.25

* = From Table 2 (column 9), SD = St. deviation, CV = Coefficient of variation, SE = Standard error, CI = Confidence interval

The proposed method was successfully employed for determination of vitamin B_{12} in single and multi-component commercial preparations. All the samples (the potency of S_F, S_G and S_M were found to be 98, 99 and 98%, respectively) tested by the proposed method (Table 1) were in close agreement with the potency found using the USP method (the potency of S_F and S_G was found to be 98.83 and 99.2%, respectively). The percentage recovery value (Table 2) in proposed method (99.7%) was also in agreement with that for the USP method (100.51%).

Validity of the method was confirmed by the determination of standard deviation, co-efficient of variation, standard error and confidence interval at 95 and 99% level of significance.

The proposed method suffered no interference by other components and the formed colored complex was stable (for 12 h) at room temperature. Besides present method was simple, rapid and accurate over other methods and can be applied successfully for the analysis of vitamin B_{12} in pharmaceutical preparations.

Acknowledgments

The authors acknowledge to the authority of Beximco Infusions Ltd. for their cooperation in using their facilities of UV-Visible spectrophotometers and other equipment.

References

Bruno, P., 1982. A new spectrophotometric method for determination of vitamin B_{12} as cobalt, Anal. Lett., 14: 1493-500.

Eldawy, M.A. and A.S. Tawfiq, 1976. A rapid and sensitive colorimetric determination of cobalt (II), J. Pharm. Sci., 65: 664-6.

More, P.S. and A. Sawant, 1992. Determination of trace amount of cobalt by extraction and spectrophotometry using iso-nitrosomethyl pentanone, Anal. Chem., 31: 984-5.

Rodica, C. and E. Florean, 1969. Photometric determination of cyanocobalamin, Pharmagie, 24: 462-64.

Sakai, T., N. Ohno and H. Sasaki, 1991. Indirect spectrophotometric determination of B_{12} using 3-(2-pyridyl)-5, 6-diphenyl-1, 2, 4 triazine-cobalt complex formation, Bunseki Kagaku, 40: 305-8.

The Merck Index: MERCK and CO., INC. Whitehouse Station, NJ, USA, 7th Ed., pp: 1098.

USP, 2000. United States Pharmacopoeial Convention Inc., Rockville, MD 20852, Asian Ed., pp: 480.

Vasilikiotis, G.S., T.H.A. Kouimtzis and A. Voulgaropoulos, 1979. Spectrophotometric determination of cyanocobalamin (as cobalt), Microchem. J., 22: 479-483.

Zheng, E. and S. Zhang, 1995. Study of color reaction of 1-(2-imidazolylazo)-2-napthol-4-sulfonic acid with cobalt, Fenxi Huaxue, 23: 610.

Zuting, P., X. Nengyong and X. Mianyi, 1985. Spectrophotometric determination of cobalt in vitamin B_{12}, Wuhan Daxue Xuebao, 1: 85.