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Original Article

RELATIVE COMPARISON OF STABILITY AND DEGRADATION OF METHYLCOBALAMIN TABLETS OF DIFFERENT BRANDS AT DIFFERENT STORAGE SETTINGS

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ABSTRACT

Objective: To assess relative comparison of stability and degradation of Methylcobalamin tablets of different brands at various storage circumstances.

Methods: The comparative *in vitro* study of Methycobal (innovator brand) with its other 5 different brands Cobalamin, Neuromet, Incobal, Qbal and Mecobal was organized for evaluation of physicochemical features of hardness, thickness, friability, weight variation, disintegration time and accelerated stability at 3 temperatures, 25 °C, 30 °C±65 % and 40 °C±75 % respectively for 3 mo. Later all brands were passed through HPLC for checking the extent of degradation of drug products.

Results: All tablet brands were within the weight variation specified limits except Mecobal with a relative standard deviation of 6.83%. The weight variation values of Methycobal, Cobalamin, Neuromet, Incobal, Qbal and Mecobal were 0.29%, 0.11%, 0.09%, 0.13%, 0.09% and 0.14% after friability test respectively as per standard limits**.** The average thickness of Cobalamin, Incobal and Mecobal were not within specified limits. The average hardness of all trades was within limits except Cobalamin and Mecobal exceeding 6kp. The disintegration time of all companies was as per specifications.

Conclusion: Qbal was found economical and cost-effective. However, study facts unveiled no noteworthy variety in the Q. C assessments of Methylcobalamin brands.

Keywords: Stability, Degradation, Methylcobalamin, Storage

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INTRODUCTION

Methylcobalamin, Mecobalamin, MeCbl, and MeB12 all are declared terms used to identify vitamin B12 [1]. MeCbl is a therapeutically active segment of vitamin B_{12}, also applied in the management of Alzheimer's sickness, rheumatoid arthritis [2]. Vitamin B12 is necessary for cellular DNA production and therefore playing role in variety of functions of tissues in the body, development of myelin sheath, systemic circulation and gastro epithelial linings [3, 4]. Vitamin B12 is critical for actions of neurons, erythrocytes (RBCs) manufacturing, and is a cofactor for three foremost reactions; the transfer of methylmalonic acid to succinyl coenzyme A; the changing of homocysteine to methionine and the change of 5-methyltetrahydrofolate to tetra hydro folate [5, 6]. Maternal vitamin B12 shortage while pregnancy or during lactation can progress to neural tube faults, malfunction to flourish, hypotonic state, abnormalities in movement, and anemic situation. Females at higher hazard or with recognized deficit need to be supplemented with vitamin B12 throughout pregnancy or during breastfeeding or giving nourishment [7, 8].

There are various preparations applied to lessen the risk of scarcity of vitamin B12 like mucoadhesive buccal tablets, microencapsulated formulations, lozenges, liposomes, buccal films, nose sprays, intra-nasal drops, transdermal solubilized emulsions, mouth spray, gelatin parenteral dose shape, inhaler/pen, buccal mucoadhesion hydrogelic films and toothpastes etc [9, 10].

MATERIALS AND METHODS

Study design

Comparative *in vitro* quality control parameters amongst the commercially available tablet brands of Methylcobalamin innovator brand compared with it's five other different brands produced in Pakistan.

Chemicals

Methylcobalamin tablets of innovator brand Innov-B and other five Brands Cobalamin, Neuromet, Incobal, Qbal and Mecobal were chosen for the study project. 200 Tablets of each brand were purchased from local medicine market Quetta, Pakistan. Methylcobalamin was gifted by Martin Dow marker. All brands of Methylcobalamin contain 500 mcg.

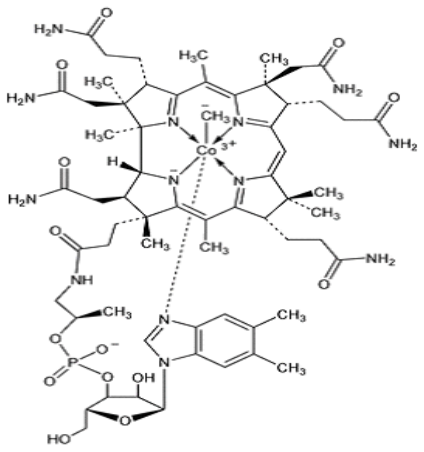

Fig. 1: Structure of methylcobalamin [11]

Reagents for HPLC sampling

Acetonitrile for Chromatographic process (Lichrosolv), Buffer solution pH 3.0+/-0.02 (20 °C), (Citrated Hcl), Mecobalamin reference sample, LiChrospher 100 RP-8 end-capped (10 micrometers), Disposable filter 0.45 um.

Instruments

Digital Electronic Balance, Vernier caliper, Disintegrator (Pharma tester), Hardness tester (Pharma tester), Roche Friabilator (Pharma

tester), Stability chamber (Binder II-KBF720), HPLC apparatus (Shimadzu, LiChroCART 250-4 HPLC Cartridge) of analytical grade.

Methodology

The relative examination of Methylcobal with other five diverse companies including Cobalamin, Neuromet, Incobal, Qbal and Mecobal was systematized for valuation of physical and chemical attributes such as hardness, Thickness and Diameter, Friability, weight variation and accelerated steadiness at 3 temperatures, lab temperature, 30 °C and 40 °C by keeping relative humidity±65% and±75% correspondingly for 3 mo prior to stability testing as well as subsequently. Then innovator brand along with 5 other trades were passed through HPLC test for knowing the degradation of tablets of brands.

Physical evaluation of methylcobalamin tablet brands

Weight variation test

200 tablets of each brand were weighed in isolation with the above-mentioned Digital analytical weighing balance and average weight and the percentage of variance was unveiled for every brand [12]. The equation for calculation of percentage weight variation is given below;

Percentage weight variation= (average weight-individual weight)/individual weight x 100 %.

Thickness test

20 tablets of each brand were employed for thickness determination by Vernier caliper in mm.

Hardness test

Tablet hardness is typically expressed as the load necessary to crush a tablet positioned on its perimeter and hardness is occasionally called tablet crushing force. The appropriateness of the tablet believed to mechanical stability at some point in wrapping and consignment would typically be forecasted on the criteria of hardness. The crushing potency was determined with a tablet hardness tester (Monsanto). Ten tablets were arbitrarily elected from each brand for this test [13].

Friability test

The trial was initiated by weighing 10 tablets overall that is measured as the initial weight, Wi. All the tablets being kept in the drum of friability tester and apparatus was revolved at 100 rpm for 4 min (25 rpm for 1 min). Then tablets were deducted, and re-weighed (just the intact ones). This is estimated as the ultimate weightage, Wf. Then the % age loss of weight of tablets was computed by utilizing the equation given under [13].

Percentage friability= {(wi-wf)/wi)} x100

Disintegration test

Tablet disintegration was determined in the tablet disintegration Apparatus. 6 tablets from each brand were subjected to distilled water at 37 °C. The disintegration time was taken to be the time no particle remained on the basket of the system [14].

Stability studies

All selected tablets of Methylcobalamin brands were targeted to constancy studies at 3 temperatures (lab temperature, 30 °C+65% and 40 °C+75%) for three months in stability chamber as per International Conference on Harmonization (ICH) guidelines [15].

Chemical assay

Mobile phase

Acetonitrile LiChrosolve–H_2O-buffer solution pH 3, 180 ml+800 ml+20 ml).

Because of the tremendous light sensitiveness of Methylcobalamin, the model solution and reference solution ought to be synthesized in a dark room. Cover the glass equipment used tightly in aluminium foil; darken the sample compartment of the autosampler with black paper.

Sample solution

Weigh 10 coated tablets in 50 ml volumetric flask (equal to almost. 10 mg of Mecobalamin) with 50 ml of H2O and shake mechanically for 60 min, build up the volume to the spot with H2O and blend. Pass fraction of mixture via a disposable filter with the help of a disposable syringe.

Standard solution

Melt 50 mg of Mecobalamin, correctly weigh, in water for making 100 ml. Dilute 10 ml of solution (alike to 5milli g of Mecobalamin) in 50 milli liters V. flask, setup the volume up to the sign with distilled water.

Chromatographic conditions

The Apparatus of Liquid chromatograph with mechanical injection system, Detector (Ultraviolet Spectrophotometer) with Wave Length of 351 nm having Sample Volume of 50 ul passed through a Column (LichroCART 250-4, LiChrospher 100 RP-8) end-capped (10 um) at a flow rate of 1.5 ml/minute at a temperature of 40 °C along with running time of 20 min and retention time around about 4 min.

Method and assessment

Inject the sample solution and standard solution each twofold. Find out the separate peak areas by integration.

RESULTS AND DISCUSSION

Label information of tablet brands

The Label known information about all brands are listed in table 1.

Table 1: Labeling information regarding samples

B # code	B # No	Pr. (10) Tabs.	Mfg Date	Exp Date	Manufacturer
Methycobal	129890	168	Mar-2019	Feb-2022	Hilton Pharma
Cobalamin	9006	96.51	Apr-2019	Apr-2024	Macter International
Neuromet	Q596	133.90	Feb-2020	Feb-2023	Martin Dow Marker
Incobal	444	78.08	Feb-2019	Feb-2022	Indus Pharma
Qbal	19692	64.09	May19	Apr.22	Bosch Pharmaceuticals
Mecobal	003	82.69	Feb-19	Feb-22	Nabiqasim Industries

***Abbreviations:** B # No (Batch Number), Pr. (Price), Mfg (Manufacturing Date), Exp (Expiry Date), of the total batches (n=3).

Table 2: Physical manifestations of various brands

B-Code	Color	Coating
Methycobal	White	Sugar Coated
Cobalamin	White	Sugar Coated
Neuromet	White	Sugar Coated
Incobal	White	Sugar Coated
Qbal	White	Sugar Coated
Mecobal	White	Sugar Coated

*Abbreviation: B-code (Batch code), of the total n=3 all batches show same color and coating.

Table 3: Weight uniformity of various brands

B-codes	Average weight (g)	Variation (RSD) NMT (6%)
Methycobal	0.154±0.01	3.09±0.01
Cobalamin	0.208±0.02	4.72±0.02
Neuromet	0.157±0.01	3.98±0.02
Incobal	0.196±0.11	5.791±0.01
Qbal	0.101±0.01	2.981±0.01
Mecobal	0.018±0.11	6.83±0.21

The values are expressed as mean±RSD = Relative Standard Deviation,n=3. NMT= Not more than 6% # Acceptance Criteria<6%.

Weight uniformity of tablet samples

All the selected tablet brands were within the weight variation specified limits except Mecobal with a relative standard deviation over 6.83% as affirmed in table 3.

Thickness of various tablet brands

The average thickness of Cobalamin, Incobal, and Mecobal were not within limits of standard specifications as revealed in table 4.

Table 4: The average thickness of different batch brands

Batch codes	Average thickness (mm)
Methycobal	3.753±0.02
Cobalamin	4.374±0.11
Neuromet	3.574±0.01
Incobal	4.002±0.11
Qbal	2.78±0.02
Mecobal	4.295±0.02

All results values are mean of,(n=3)±SD, # Acceptance criteria<3.10

Hardness of tablet brands

The average hardness of all brands was within the limits except Cobalamin (10.49 kp) and Mecobal (10.462 kp) above 6kp as described below in table 5.

Weight variation before and after friability tests

The weight variation values 0.009(0.29%), 0.005(0.11%), 0.03(0.09%), 0.003(0.13%), 0.002(0.09%) and 0.004(0.14%) of Innovator brand, Cobalamin, Neuromet, Incobal, Qbal and Mecobal after friability test respectively were within the standard limits of Specifications as given table 6**.**

Table 5: The average hardness of various brands

Batch codes	Average hardness (kp)
Methycobal	5.78±0.02
Cobalamin	10.49±0.01
Neuromet	5.55±0.11
Incobal	6.4±0.01
Qbal	4.9±0.11
Mecobal	10.462±0.21

***Abbreviation:** kp (kilopond), All results values are mean of, (n=3), ±SD# Acceptance criteria=<8

Disintegration time of tablet brands

The disintegration time of all brands was in the range 2-20 min as per the standard specifications as stated under in table 7.

Table 6: Weight values and variation before and after friability tests

B # codes	Wt. before friability	Wt. after friability (g) Variation (g) (%)
Methycobal	3.089±0.01	3.080±0.02 0.09(0.29%)
Cobalamin	4.173±0.02	4.168±0.01 0.05(0.11%)
Neuromet	3.176±0.01	3.173±0.02 0.03(0.09%)
Incobal	2.244±0.01	2.241±0.01 0.03(0.13%)
Qbal	2.014±0.02	2.012±0.01 0.02(0.09%)
Mecobal	3.502±0.01	3.497±0.02 0.004(0.14%)

The values are expressed as weight before friability-weight after friability±= Variance from the mean, of the total n=3

Table 7: Average disintegration times of different sample brands

Batch code	Disintegration time (Average)
Methycobal	10 min
Cobalamin	20 min
Neuromet	10-11 min
Incobal	4 min
Qbal	2 min
Mecobal	5-6 min

All the values of total (n=3) are mentioned as average disintegration time, # Acceptance Criteria= 60 min.

Stability studies of tablet brands

Stability of a biopharmaceutical product can be described as the capacitance of a specific preparation in a definite container/closure system to persist within its physical, chemical, microbiological, hazardous, defensive and informational specifications [16].

The objective of stability is to offer substantiation on how the superiority of a formulation differs with time under the effect of a diversity of environmental aspects

Like temperature, moisture, and light. Dilapidation is probably to arise under steamy environment of higher ambient temperature and humidity [17]. Therapeutic product stability is a multifarious

collective procedure which needs substantial time, expenditure, utilization, and methodical skills to synthesize therapeutically efficient formulations, efficiency, excellence and safe nature [18].

The results of accelerated stability at 3 temperatures, lab temperature, 30 °C±65%and 40 °C±75% respectively for a duration of 3 mo were within the specified limits as revealed in table 8.

Table 8: Stability studies of chosen brands

Brands	Conditions	Stability Results
Methycobal	25 °C	99.13%
	30 °C±65%	98.16%
	40 °C±75%	98.02%
Cobalamin	25 °C	93.03%
	30 °C±65%	93.86%
	40 °C±75%	93.39%
Neuromet	25 °C	101.02%
	30 °C±65%	101.72%
	40 °C±75%	100.33%
Incobal	25 °C	111.03%
	30 °C±65%	111.94%
	40 °C±75%	109.71%
Qbal	25 °C	96.51%
	30 °C±65%	96.88%
	40 °C±75%	97.32%
	25 °C	93.71%
Mecobal	30 °C±65%	94.85%
	40 °C±75%	94.32%

Total (n=3) showed stability results at temp 25 °C30 °C±65% RH (Relative Humidity)and 40 °C±75% RH (Relative Humidity).

HPLC sampling results and calculations

The photosensitive material, mecobalamin, emerges at retention time of approximately 3.68 min. Usual changing in the chromatography scheme can essentially cause changeable investigational surroundings. The endeavor of this measurement is to uphold a constant extrication performance. The chromatogram retention time is given in fig. 2.

The results of HPLC of tablet brands are mentioned in table 9 and calculations are given underneath the table 9.

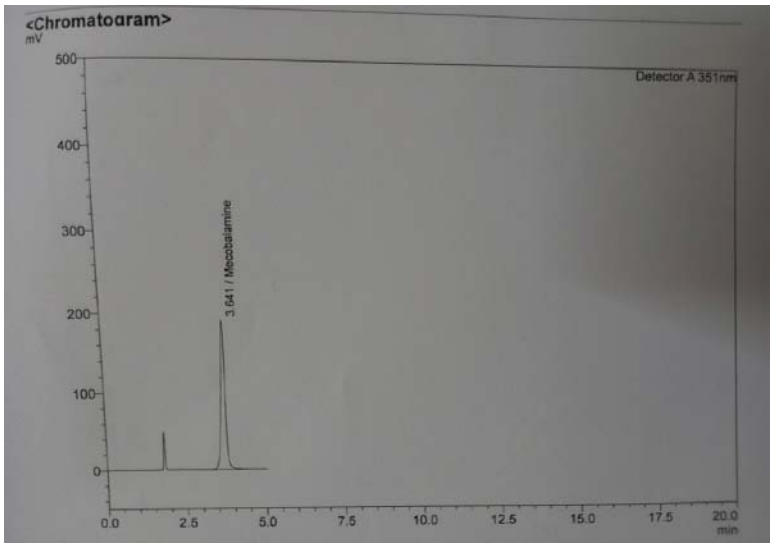

Fig. 2: HPLC results chromatogram of mecobalamin retention time 3.64

Table 9: HPLC facts about different brands

Product-B	B. No	Conditions	Mcg/Tablet Result	
Methycobal	129890	30 °C±65%40 °C±75%	490.78490.12	98.16%98.02%
Cobalamin	9006	30 °C±65%40 °C±75%	469.29466.96	93.86%93.39%
Neuromet	Q596	30 °C±65%40 °C±75%	508.58501.67	101.72%100.33%
Incobal	444	30 °C±65%40 °C±75%	559.72548.57	111.94%109.71%
Qbal	19692	30 °C±65%40 °C±75%	483.34477.23	97.88%92.32%
Mecobal	003	30 °C±65%40 °C±75%	474.26471.62	94.85%94.32%

*Abbreviations: B. No (Batch number), Mcg (Microgram) at temp 25 °C30 °C±65% RH (Relative Humidity)and 40 °C±75% RH (Relative Humidity) =. Results

Calculations

A_{st} x wtstd x 10 x 50 x P x Av. wt x 1000 = mcg Mecobalamin/tablet

A_s x 100 x 50 x wtsp x 100

Where,

A_s =Peak area of the sample solution

A_{st} = Peak area of the standard solution P= Purity of the standard, in %

Wtstd = Weight of standard taken, in mg Wtsp= Weight of the sample taken, in mg

Av. Wt =Average weight of the tablets

CONCLUSION

It was noticed that a larger discrepancy in worth in the identical generic brands of Methylcobalamin. Qbal with cost 64.09 Pakistani rupees/ten tablets was observed commercially efficient. Nevertheless, as per the consequences of present readings disclosed no significant diversity in the qualitative analysis of Methylcobalamin brands. The superiority in stipulations of weight, thickness, disintegration, Friability and chemical evaluation (HPLC) were appraised, compared and found equivalent to one and other. Stability testing indicated every brand is comparable to other. Consequently, this is demonstrating that less rated medicines also offer excellent biopharmaceutical beneficial outcomes. Hence, it was summed up that the worth effectual drug ought be employed and may be recommended.

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AUTHORS CONTRIBUTIONS

We put forward a thesis topic entitled “Relative Comparison of Stability and Degradation of Methylcobalamin Tablets of Different Brands at Different Storage Settings” by Abdul Raziq, Dr. Syed Umer Jan, Dr. Rahman Gul, Yousaf Khan. Abdul Raziq, Dr. Rahman Gul carried out the industrial practical work, have interpreted the data and wrote the paper. All authors have examined the manuscript and are the guarantors.

CONFLICT OF INTERESTS

The Author of this article has no conflict of interest.

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