Research Article

Optimization and Characterization of Methylcobalamin for Nasal Spray Technology in Management of Pernicious Anemia

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Abstract

The present investigation was to deliver methylcobalamin via nasal route for the effective treatment for management of pernicious anemia. It was hypothesized that drug delivery through the use of nasal sprays is a unique alternative to the more conventional oral or intravenous administration of drugs. The preliminary study was conducted to build up a formulation for management of pernicious anemia. Further, formulation was analyzed for its composition, purity and safeguard. Samples were tested by various chemical & analytical methods for calibration. Based on the studies and analysis, optimized composition for present formulation was derived. The parameters like pH, Osmolarity, drug content, appearance, transmission rate have been analyzed for characterization of optimized sample. Further, Nasal sprays was loaded with optimized drug which has shown a faster response to the blood stream or area of interest than oral applied drug drugs, and does not degrade the drug as it travels through the digestive system. Nasal sprays are designed to deliver a precise drug dose to the correct absorption site within the nasal mucosa. The spray pump produces the droplet-size distribution, which must be optimized to increase nasal deposition and minimize lung deposition.

Keywords: Pernicious anemia, calibration, droplet-size distribution, nasal mucosa

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Introduction

Pernicious Anemia is a chronic illness caused by impaired absorption of vitamin B12 because of lack of intrinsic factor (IF) in gastric secretion. It occurs as a relatively common Adult form of anemia that is associated with gastric atrophy and loss of IF production and as rare congenital autosomal recessive form in which IF production is lacking without gastric atrophy. The disease was given its common name because it was fatal before treatment became available, first as liver therapy and subsequently as purified vitamin B-12. Today, the term "pernicious" is no longer appropriate, but it is retained for historical reasons [1]. Many drugs impair cobalamin uptake in the ileum but are rarely a cause of symptomatic vitamin B-12 deficiency, because they are not taken for long enough to deplete body stores of cobalamin. Such agents include nitrous oxide, cholestyramine, *para* -aminosalicylic acid, neomycin, metformin, phenformin, and colchicine [2]. Pernicious anaemia presents insidiously, and many of the signs and symptoms are due to anemia itself, where anemia is present. However, in 20% of cases of cobalamin deficiency, anemia is not observed. Major symptoms of Pernicious Anemia includes fatigue, depression, low grade fevers, Nausea, weight loss, diarrhea, dyspepsia, neuropathic pain, jaundice, difficulty in walking, muscle weakness and clumsiness, high blood pressure and shortness of breath, tachycardia etc., [3]. The treatment of Pernicious anemia varies from country to country and from area to area. A permanent cure for Pernicious anemia is lacking, although repletion of B_{12} should be expected to result in a cessation of anemia-relatedsymptoms, a half in neurological deterioration, and (in cases where neurological problems are not advanced) neurological recovery complete and permanent remission of all symptoms, so long as B_{12} is supplemented. Repletion of B-12 can be accomplished in a variety of ways. [4].The formulated drug contains methylcobalamin issued in the pernicious anemia when oral and injectable fails to give therapeutic response. Methylcobalamin is commercially available in tablet dosage form but it fails to get desire bioavailability. For that intranasal route become promising route for optimum dose availability in the systemic circulation. [5-8]. The choice normally depending on factors such as clinical benefit, convenience, cost, the properties of the drug and the pharmacokinetic profile needed. In acute situations, an injectable form may be required, whereas for long-term therapy noninvasive procedures are preferred. Oral administration is usually the modality of choice. However, in some situations, the oral route may not be advantageous. [9]. An attempt was made to develop composite formulation of methylcobalamin with Benzalkonium chloride, Citric acid anhydrous, Hydroxy

propyl methyl cellulose.The solubility of composite drug was found to be highest in Glycofurol which was marked as carrier agent for preparation of nasal spray. Glycerine was added to inhibit the drying of mucous membrane of nasal mucosa.

The performance of developed spray formulation was optimized, characterized and optimized through chemical and histopathological analysis. In vitro studies shows that drug release study of methylcobalamin nasal spray showed excellent drug release from formulation with 97.37% drug released in 240min indicating that the sustained release nature of the optimized formulation. It was found that drug is beneficial for management of pernicious anaemia and suggested as an alternative and effective solution.

Material and Methods

The number of drugs introduced in to the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug in to the market to the date of its inclusion in pharmacopoeias. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs [10].

The drugs, excipients and chemicals were used are of analytical grades and checked for their analytical quality. The individual drug of admixture with respective suppliers has been shown in **Table 1**. The purity and quality of the constituents were found of analytical grades (**Figure 1**). The major equipments were highly calibrated and have high degree of precision (**Table 2**). Further, various combination has been intended for optimization of admixture.

Table 1 List of Drug, excipients, chemicals and solvents with their suppliers

Sr. No.	Drug/ Excipients/ Solvent	Supplier
1	Methylcobalamin	Torrent Research center, Bhat, Gandhinagar.
2	Water	Millipore distilled water, TRC, Bhat, Gandhinagar.
3.	Volumetric flask (100ml,10ml)	Vijay scientific and chemical agency, Nadiad.
4.	Pipette (1ml, 5ml,10ml)	Vijay scientific and chemical agency, Nadiad.

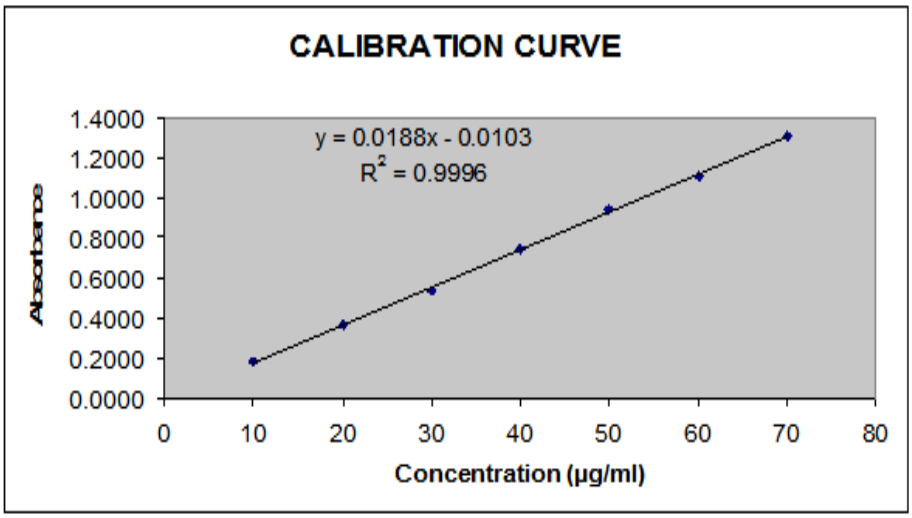

Figure 1 Calibration curve for methylcobalamin via UV-visible spectrophotometric analysis

Table 2 List of equipments with their manufacturer

Sr. No.	Equipments	Manufacture
1	Electronic balance	Model number: SL-234, DenverInstrument, Germany.
2	UV-spectrophotometer	Model number: Shimadzu UV-2450 Shimadzu corporation, Japan.
3	Graduated Pipettes 2, 5and 10 ml	Sun glasses, Kheteshwar trade link, Nadiad.
4	Graduated Volumetric flask 100ml	Sun glasses, Kheteshwar trade link, Nadiad.

The three different combinations (MC1, MC2 and MC3) have been studied for optimization as shown in **Table 3**. Further, parameters like pH, Osmolarity, appearance, drug content, transmission rate have been analyzed for characterization of optimized sample of methylcobalamin.

Table 3 Formulation chart of methylcobalamin for nasal spray technology

Ingredients	MC1	MC2	MC3
Methylcobalamin(mcg)	500	500	500
Benzalkonium chloride (mg)	0.02	0.02	0.02
Glycerin(mg)	223	223	223
Glycofurol (mg)	100	100	100
Sodium citrate dihydrate(mg)	0.32	0.36	0.38
Citric acid anhydrous(5%w/v)	-	-	-
Water(ml)	10	10	10

UV-visible spectrophotometric analysis for calibration curve of methylcobalamin

Accurately weighted 50mg of Methylcobalamin was transferred to the 100ml volumetric flask and volume was made with water. From this solution, 0.1ml was withdrawn and transferred to the 100ml volumetric flask. Finally, the wavelength was selected by scanning the 10 µg/mL of mecobalamin solution between 200 to 400 nm in a spectrophotometer. The scanned results proved maximum absorption in 353 nm (Figure 1), therefore 353 nm was selected as the λmax for estimation [11].

As per obtained standard curve of methylcobalamin, it was found that the sample is safe fail to use. Purity and quality was at par with competitive drugs. Further, characterization of drug was studied analyzing pH conditions matching to nasal cavity. Further, Osmolarity and drug content were observed for aptness of drug. The appearance and transmission rate was also examined to assure high-end clarity of methylcobalamin.

Results and Discussion

Appearance

Appearance of three batches (MC1-MC3) was tested against white & black background & turbidity were checked, the test was carried out. The results are shown below in **Table 4**. All the formulations were observed for the 24 hour for clarity and precipitation of the drug. The formulation is recognized as Transparent with bluish ting), non-clear (Turbid or milky), stable (no precipitation at the end of 24 hr) and unstable (phase separation or precipitate with24h). [12] The formulationsMC1, MC2 and MC3 and are found to be clear after 24 hour (Table 4). Here all the formulations were found to be stable showing clear and no drug precipitation.

Table 4 Visual observation of methylcobalamin for nasal spray technology

Sr.	Batches	Precipitation	Clarity
1	MC 1	NO	YES
2	MC 2	NO	YES
3	MC 3	NO	YES

pH value of Methylcobalamin during nasal spray

The pH values of methylcobalamin nasal spray (MC1-MC3) were determined using pH meter (CystronicSystem361, Ahmadabad), calibration of pH meter was conducted before tablets of pH 4 & pH 7. Each measurement was carried out in triplicate and results were presented as mean ± standard deviation. [13]

The stability Nasal spray is greatly affected by pH. The excipients used in the formulation would decide the pH of the final product. The change in pH finally leads to instability of formulation and not turn to lead maximum nasal absorption of drug. Here for maximum nasal absorption pH should be in range of 6 to 6.7. [14]

All the formulations have shown similar pH values in the range of 6.38 to 6.68. Thus stability of formulation was not affected by pH and formulations remain stable. pH of all the formulations is showed in the **Table 5**.

Table 5 pH of Methylcobalamin for nasal spray technology

Batch	pH of the formulation			Mean*
	(I)	(II)	(III)	
MC 1	6.68	6.73	6.67	6.693 ± 0.032
MC 2	6.41	6.56	6.46	6.476±0.076
MC 3	6.53	6.61	6.57	6.570±0.040
*Values are mean ± S. D. (n=3)				

Osmolarity of methylcobalamin nasal spray

The change in the Osmolarity of methylcobalamin nasal spray (MC1-MC3) was determined using osmometer (Cryscopic osmometer System 3250, Delhi). The machine were 1st of all calibrated and then samples were placed one by one. The machine will automatically tell the osmolarity of formulation on the principal of freezing point of depression. Each measurement was carried out in triplicate and results were presented as mean ± standard deviation. [15]

To maintain the tonicity of the nasal formulation is most important factor. All formulation checked for the osmolarity. Here optimum osmolarity is maintained to achieve the desired isotonicity of the nasal solution. Here solution which are hypertonic or hypotonic lead to Sevier nasal irritation. So it is necessary to maintain the isotonicity. Here for nasal solution osmolarity in the range of 250-300 mosm/kg. The results of Osmolarity are shown in **Table 6**. In the present study formulation MC3 shown the osmolarity in nasal appropriate range compared to other formulations.

Table 6 Osmolarity of Methylcobalamin for nasal spray technology:

Batch	Osmolarity of the formulation			Mean*
	(I)	(II)	(III)	
MC 1	103	108	110	106.3 ± 0.042
MC 2	134	138	141	137.4±0.076
MC 3	136	138	139	137.5.±0.040
*Values are mean ± S. D. (n=3)				

Determination of drug content Methylcobalamin in the nasal spray

Assay of weighed amount of formulations were carried out to determine the drug content. The weighed samples were dissolvedin10mlofwaterandthesolutions were filtered, using Whatman filter paper. The content was estimated spectrophotometricallyat353nm using standard curve [14].

The drug content of Methylcobalamin nasal spray was measured by UV spectrophotometric method. As shown in **Table 7**, the percentage drug content was determined by considering 5mg of methylcobalamin as a 100%. All the formulations are within the 97.60 to 99.00% of drug content.

Table 7 Drug content of Methylcobalamin for nasal spray technology

Batch	% Drug content			Mean*
	(I)	(II)	(III)	
MC 1	99.1	98.9	99.0	99.00 ± 0.81
MC 2	98.0	96.6	98.2	97.60 ± 0.87
MC 3	98.6	98.8	97.7	98.36 ± 0.58
*Values are mean ± S. D. (n=3)				

Determination of percentage transmission of methylcobalamin loaded nasal spray

The percentage transmittance of three batches (MC1-MC3) was measured at 650 nm using UV-spectrophotometer (UV-2450, Shimadzu Corporation. Japan) keeping distilled water as blank. Each measurement was carried out in triplicate and results were presented as mean± standard deviation as shown in **Table 8** [16].

Table 8 Percentage (%) Transmission of methylcobalamin for nasal spray technology

Batch	%Transmission			Mean*
	(I)	(II)	(III)	
MC 1	98.5	98.0	98.7	98.4±0.36
MC 2	97.4	97.1	96.9	97.13±0.25
MC 3	97.8	97.5	98.2	97.83±0.35
*Values are mean ± S. D. (n=3)				

The clarity of nasal spray can be observed by transparency, measured in form of Percentage transmittance (% T). Formulations having %T greater than 96% indicated high clarity of nasal spray. While formulations having %T less than 96% suggested less clarity of nasal spray. This may due to bigger droplet size of the formulation. Because of the

greater droplet size, globules may reduce transparency of nasal spray and thereby values of %T were decreased. The results of %T are shown. In the present study formulation MC1 showed highest transmittance compared to other formulations.

Conclusion

The present study was an attempt made to develop methylcobalamin based nasal spray in which admixture was optimized and characterized using critical parameters of quality considering their responses. The studies about this formulation suggested that this formulation might be suitable as an alternative formulation for effective management of pernicious anaemia. The efficacy of this formulation has some of the critical aspects that demand the focused investigation are: real time stability analysis, establishment of in vitro in vivo correlation, extensive pre-clinical and clinical evaluation supported with toxicological studies and bio availability studies in volunteers etc. need to be established.

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