Istanbul J Pharm 50 (2): 89-94
DOI: 10.26650/IstanbulJPharm.2020.0003
Original Article

Evaluation of excipients effects on the impurity profile of lyophilized hydroxocobalamin formulation

Gökay Gün[1], İbrahim Karabacak[1], Müge Güleli[1], Şevki Kızılok[1], Sercan Semiz[1], Mahmut Özbek[1], Süleyman Özakın[1]

[1]World Medicine Pharmaceutical Company, Research and Development Department, Istanbul, Turkey

ORCID IDs of the authors: G.G. 0000-0002-4264-8273; İ.K. 0000-0002-0082-3088; M.G. 0000-0002-1539-3228; Ş.K. 0000-0002-2072-1310; S.S. 0000-0003-1944-2004; M.Ö. 0000-0002-6505-3811; S.Ö. 0000-0001-9536-9852

Cite this article as: Gun, G., Karabacak, I., Guleli, M., Kizilok, S., Semiz, S., Ozbek, M., & Ozakin, S. (2020). Evaluation of excipients effects on the impurity profile of lyophilized hydroxocobalamin formulation. *İstanbul Journal of Pharmacy, 50* (2), 89-94.

ABSTRACT

Background and Aims: The aim of the research work was to compare excipient effects and develop a stable pharmaceutical product in the form of lyophilized powder. Mannitol, lactose monohydrate, EDTA and glycine are widely used in pharmaceutical formulations and food products.

Methods: The production method consists of mainly four parts: raw material weighing process, preparation of bulk solutions, lyophilization and analytical determination. A total of five formulations were prepared and lyophilized to the stability study. To improve the stability of hydroxocobalamin formulations (F1-F5), they were evaluated with different excipients (mannitol, lactose monohydrate, EDTA and glycine) during the stability period. Stability studies were performed to check impurity and assay of hydroxocobalamin.

Results: The rate of impurity and assay results were compared to F1-F5 formulations. As a result of impurity and assay analysis for F3 and F4, the formulations were found to be within limit. Both of them were determined to the best formulations for impurity of hydroxocobalamin.

Conclusion: The research proposes a new stable formulation and proper storage conditions for lyophilized hydroxocobalamin parenteral solutions. The impurity problem of lyophilized hydroxocobalamin formulation was optimized with lactose monohydrate and lactose monohydrate + EDTA combination.

Keywords: Drug formulation, excipient, lyophilization, hydroxocobalamin, stability

INTRODUCTION

Cyanocobalamin and hydroxocobalamin are best known as a water-soluble vitamin, one of the B-vitamins involved in energy production and cellular functions (Kennedy, 2016; Edelmann, Chamlagain, Santin, Kariluoto, & Piironen, 2016). Many chemical and physical factors could have a negative effect on the stability of these compounds. Both of these two vitamins are prone to degradation in liquid environments, particularly when exposed to light (Monajjemzadeh, Ebrahimi, Milani & Valizadeh, 2014). B group vitamins are sensitive to factors such as: heat, light, moisture, oxidizing and reducing agents, acids and or bases (Shchavlinskii, Neiman, Lazareva, & Orlov, 1995; Ahmad, Ansari & Ismail, 2003; Kondepudi, 2016; Schnellbaecher, Binder, Bellmaine, & Zimmer 2019). Hydroxocobalamin is a derivative of cyanocobalamin and the cyano functional group attached to Co^{+3} in the tetrapyrrolic corrin macrocyclic ring in cyanocobalamin is replaced by a hydroxyl group in hydroxocobalamin (Ahmad, Ahmed, Anwar, Sheraz & Sikorski, 2016).

Address for Correspondence:
Süleyman ÖZAKIN, e-mail: suleyman.ozakin@worldmedicine.com.tr

This work is licensed under a Creative Commons Attribution 4.0 International License.

Submitted: 16.01.2020
Revision Requested: 15.04.2020
Last Revision Received: 23.04.2020
Accepted: 03.06.2020

Lyophilization (Freeze-drying) is a well-known method for formulating liquid or parenteral products to get stablity of active pharmaceutical ingredients during shelf life. In the lyophilization process, the water content of the final product is reduced to a low-level that does not support chemical reactions related impurity (Mishra, Saini, & Maurya, 2017).

Excipients (inactive compounds) are the components of a pharmaceutical formulation to achieve the stability and efficacy of the final product. They are added to increase the solubility of bulk, improve stability, enhance drug delivery and targeting, and modify drug safety or pharmacokinetic profile (Mehmood & Farooq, 2015). According to statistical data, about 67% of the lyophilized marketed products of active molecules contain excipients in their formulation (Baheti, Kumar, & Bansal, 2010). Bulking agents are well-known excipients for a lyophilized product. Bulking agents in lyophilized formulations provide an adequate cake structure.

Although poor stability of hydroxocobalamin has been previously reported (Ahmad et al., 2014), some lyophilized products are commercialized in the pharmaceutical market (www.drugbank.ca). In spite of the increasing impurity problem of hydroxocobalamin formulations, there have been limited studies on stability of vitamins in literature.

Therefore, the aim of this study was to optimize impurity related hydroxocobalamin and proper choice of excipients for lyophilized vitamin drug products. In addition, lyophilized formulations were designed and made ready using some bulking, chelating and buffering agents to improve stability of formulations. In this respect, our study can be the first to investigate this pharmaceutical research.

MATERIAL AND METHODS

Materials

Hydroxocobalamin was produced from Interquim; EDTA, hydrochloric acid and glycine were purchased from Merck, lactose monohydrate was produced from Meggle, and mannitol was produced from Roquette. Vials were produced from Mefar (Istanbul, Turkey). 0.2 µm filter was purchased from Sartorius. The pharmaceutical grade sample of hydroxocobalamin hydrogen chloride was purchased from Ferrer. The lyophilized powder for injection containing hydroxocobalamin, diclofenac potassium and betamethasone sodium phosphate sample was produced by the World Medicine Pharmaceutical Industry and Trade Inc. (Istanbul, Turkey). Citric acid sodium salt was supplied from Acros, disodium hydrogen phosphate and methanol was purchased from Merck. The water (0.05 µc) was produced by the Sartorius Stedim Biotech system as HPLC grade.

Methods

The manufacturing method consists of mainly four parts: raw material weighing process, preparation of bulk solutions, lyophilization and analytical determination. All production steps are detailed below. The excipients that we used in our formulation are classic excipients used in pharmaceutical product formulations. In stability analysis, batch size was 100 vials for each formulation. Our finished product was produced using conventional production equipment.

Method of preparation for formulation trials

1. Take water for injection (WFI) into a proper production tank by 80 percentage of total volume (20-25°C).
2. Add excipient (bulking or chelating agents) to wfi slowly under continuous mixing at 600 rpm and mix it until it is completely dissolved.
3. To the solution add hydroxocobalamin under continuous mixing at 600 rpm and mix it until it is completely dissolved
4. Check pH and if it is necessary adjust pH with 1 M diluted HCl solution
5. Add water for injection up to total volume and mix the solution
6. Filter bulk solution through 0.2 µm filter
7. Lyophilization

Formulation trials

Lyophilized hydroxocobalamin powder for injection formulation development study is shown in Table 1. In order to obtain an optimum impurity profile for the final product with different excipients, the F1-F5 formulation trials were evaluated. A composition of each formulation is presented below. All samples

Table 1. Detailed formulation trials.

		Formulation Trials				
Function	**Ingredients**	**F1 (mg/vial)**	**F2 (mg/vial)**	**F3 (mg/vial)**	**F4 (mg/vial)**	**F5 (mg/vial)**
API	Hydroxocobalamin	13,00	13,00	13,00	13,00	13,00
Bulking agent	Mannitol	87,00	87,00	-	-	-
Chelating agent	EDTA	-	0,25	-	0,25	-
Bulking agent	Lactose Monohydrate	-	-	150,00	150,00	-
Bulking agent	Glycine	-	-	-	-	22,50
pH agent	1M HCl	q.s	q.s	q.s	q.s	q.s
Solvent	Water for injection	q.s to 1,50 ml	q.s to 1,50 ml	q.s to 1,50 ml	q.s to 1,50 ml	q.s to 1,50 ml
	pH adjusting	5,58	5,50	5,48	5,50	5,51

Table 2. Lyophilization set parameter for cycles.

Process	Temperature °C	Gradient/min	Holding/min	Vacuum/ mbar
Freezing	-40	60	400	-
1st Drying	-20	60	200	0.120
	-10	50	300	0.120
	0	50	250	0.120
	5	40	80	0.100
2nd Drying	20	60	120	0.090
	30	40	230	0.090

were loaded into the lyophilizer and the system was started by setting parameters. The set parameters for lyophilization cycle are recorded in Table 2. After the cycles were completed, the vials were removed from the lyophilizer and were stored at room temperature for analytical determination.

Lyophilization

Vials filled with hydroxocobalamin are put in the lyophilizer (Tofflon-Lyo 0,5 L), homogenously distributed and the system is started (Table 2).

Preparation of formulations for stability test

Stability studies were performed for long-term (25°C ± 2°C/60% RH ± 5% RH), intermediate term (30°C ± 2°C/65% RH ± 5% RH) and accelerated (40°C ± 2°C/75% RH ± 5% RH) test conditions using ICH guidelines [Q1A (R2)]. Samples were stored in stability rooms until the end of the stability period for assay and impurity analysis. These studies were investigated to increase the rate of chemical degradation of lyophilized product using HPLC analysis. Each stability evaluation was performed at least 3 times.

HPLC chromatographic conditions of hydroxocobalamin related substances

The HPLC method was carried out on Kromasil 100 C8 (250 mm × 4.6 mm, 5 µm) column with 20 µL injection volume at a wavelength of 351 nm on a Waters Alliance E2695 separation module equipped with a Waters 2489 photodiode array (PDA) detector and 2998 UV detector, an Empower-pro data handling system (Waters Corporation, Milford, MA, USA). Column and sample temperatures were 25°C. The separation was employed using isocratic elution, and the flow rate was maintained at 1.5 mL/min. Buffer solution was prepared by dissolving 16.7 g citric acid monosodium salt and 8.1 g disodium hydrogen phosphate in 1000 mL purified water. Mobile phase was prepared by mixing the buffer solution and methanol at the ratio of 805:195 (v:v) and filtering through 0.45 µm filter (Atici & Yazar, 2015; Atici, Yazar, Agtas, Ridvanoglu, & Karlıga, 2017).

Preparation of standard solution

10.3 mg hydroxocobalamin HCl equivalent to 10.0 mg hydroxocobalamin was weighed into a 100 mL amber volumetric flask, dissolved in an ultrasonic bath for 5 minutes adding approximately 40 mL mobile phase and then completed to volume with mobile phase. 1.0 mL of this solution was transferred into a 10 mL amber volumetric flask and completed to volume with mobile phase and filtered through 0.45 µm PTFE filter (Eldawy, Mabrouk, & El-Barbary, 2002).

Preparation of sample solution

1 vial content equivalent to 10.0 mg hydroxocobalamin was dissolved with mobile phase and transferred into 10 mL amber volumetric flask, washing it carefully, and diluted to volume. Filtered through 0.45 µm PTFE filter.

RESULT AND DISCUSSION

F1 to F5 were formulated with different excipients. Lyophilization was carried out with an optimized recipe and setting parameter (Table 2). After lyophilization process, the vials obtained from the five different formulations cake structure were intact and elegant. Hydroxocobalamin stability studies were performed with different excipients for 3-months in different storage conditions. The lyophilized samples were analyzed for assay and impurity parameters after reconstitution with solvent at the end of the stability period. The analytical data relating to the samples are reported in Tables 3 and 4.

Table 3. Assay results of hydroxocobalamin formulations during 3-months stability period.

Formulation	Limit (mg/vial)	25°C ± 2°C/60% RH ± 5% RH Assay (mg/vial)	30°C ± 2°C/65% RH ± 5% RH Assay (mg/vial)	40°C ± 2°C/75% RH ± 5% RH Assay (mg/vial)
F1	9-14,3	11,04	10,96	10,35
F2	9-14,3	10,93	10,84	9,70
F3	9-14,3	12,19	11,82	11,99
F4	9-14,3	14,11	14,05	13,76
F5	9-14,3	7,59	6,78	7,01

Table 4. Total impurity results of hydroxocobalamin formulations during 3-months stability period.

Formulation	Limit (%)	25°C ± 2°C/60% RH ± 5% RH Total Impurity (%)	30°C ± 2°C/65% RH ± 5% RH Total Impurity (%)	40°C ± 2°C/75% RH ± 5% RH Total Impurity (%)
F1	Max 10	8,72	11,21	16,19
F2	Max 10	9,58	10,44	15,28
F3	Max 10	6,59	6,96	7,14
F4	Max 10	5,56	5,57	5,71
F5	Max 10	28,66	30,03	32,10

The assay of hydroxocobalamin is shown during the stability period in Table 3. The presence of lactose monohydrate and lactose monohydrate + EDTA in formulation (F3-F4) positively affected the assay of the vitamin in storage conditions. In addition, the assay results of F1 and F2 were in the limits under all stability conditions. Additionally, based on HPLC analysis, the assay of hydroxocobalamin of F5 decreased to 6,78 mg/vial and 7,01 mg/vial in the intermediate term and accelerated stability period (Table 3). According to the F5 stability results, the active compound is not stable in glycine solution form. Due to the negative effect of glycine, the amount of hydroxocobalamin decreased by about 30% in each stability condition.

Based on impurity analysis, the formulation of F3 and F4 are compatible with an active substance and total impurity limits are lower than the other three formulations during the stability period. The results of 3-month impurity test for F5 formulation in 40 °C is the highest among all formulation and storage conditions. Moreover, both F1 and F2 formulations exceed the impurity limit. According to the data obtained from the formulation of F3 and F4 impurity analysis, excipients of lactose monohydrate and EDTA improve hydroxocobalamin stability and formulation impurity. Meanwhile, glycine negatively altered impurity and stability of formulation (Table 4).

It is observed that the highest rate of impurity is determined at about 30% in each of the three stability conditions with F5. F1 and F2 both have acceptable impurity values, and can be stabilized in stability condition (25°C) but they exceed limits in other conditions for impurity. According to analysis results, degradation of hydroxocobalamin and rate of impurity are related to each other, it is clear that there is a correlation between F5 impurity and assay results.

The HPLC method was validated according to ICH guidelines [Q2 (R1)]. The validation parameters included system specificity, suitability, linearity, accuracy, precision (system, method and intermediate precision) and robustness.

In specificity test, no another peak was observed in dilution and placebo solution chromatograms at the retention time of hydroxocobalamin, which were all separated from each other and found spectrally pure (purity angle < purity threshold). Linearity test standard solutions were prepared at concentration levels ranging from LOQ to 140% of the specification level. The results are given in Table 5.

The accuracy was determined by measuring recovery at known concentrations of the hydroxocobalamin (80%, 100% and 120%) and analyzed. 95% Confidence interval limits of recoveries were calculated (Table 5).

Table 5. Results of validation parameters.

Parameter		Results
Linearity	Range	LOQ-140.0%
	Equation	y=12117723.7107x - 333.0610
	Correlation coefficient	r^2=1.0000
Accuracy	Range	80.0%-120.0%
	Average	99.72
	95% Confidence Interval Limits	99.29-100.15
Precision	System Precision	RSD=0.31%
	Method Precision	Total impurity RSD=0.95%
	Intermediate precision	Total impurity RSD=7.02%

System precision was conducted with six repeated injections of standard solutions prepared at 100% concentration and RSD of peak areas was found below 10.0%. Method precision was performed by preparing 6 sample solutions described in section preparation of sample solution. Relative standard deviations (RSD) were calculated and the results were found below 10.0%. Intermediate precision was studied by different analysts and with different devices. Each analyst prepared 1 standard solution and 6 sample solutions. All the results were compared and RSD was calculated (Table 5).

To validation of the developed method, parameters were verified and standard solutions were tested. Column temperature by ±2˚C, mobile phase ratio by ±5.0 ml was changed and column with different lot number was used. Variations (%) were calculated and no significant difference was found between initial and altered conditions.

Solution stability was also evaluated by monitoring the peak area response. Standard and sample solutions were analyzed right after its preparation 6, 24, and 48 hours after at 5˚C and 25˚C. Results were compared and % variations were calculated and all results were below 10.0%.

According to literature, mannitol, trehalose, sucrose, lactose, glucose, and dextran glycine, are the most commonly used bulking agents for lyophilized products (Cappola, 2000). Also, the moisture ratio of lyophilized powder showed better stability with mannitol than with lactose as bulking agents (Korey & Schwartz, 1989). Lactose is a well-known reducing sugar. Although it may undergo Maillard Reaction with an amine group leading to instability of the formulation and it tends to increase impurity (Frank, 2004), we reported that hydroxocobalamin is much more stable with lactose than other excipients according to our formulation studies. Heathgote et al. demonstrated that hydroxocobalamin forms a complex with glycine more easily than with other amino acids (Heathgote, Moxon, & Slifkin, 1970). We detected that impurity level is the highest formulation with glycine. Glycine is an organic compound that contains amine and carboxyl groups. Related functional groups may lead to Maillard Reaction with hydroxocobalamin as well as degradation of the active ingredient. In contrast, the formulations included lactose monohydrate and EDTA enhance hydroxocobalamin stability. In the previous study, Herman et al. reported that the rate of decomposition of sodium methylprednisolone succinate with both mannitol and lactose as excipients, and marked that formulation which is mannitol as excipient showed a faster degradation in comparison to with lactose (Herman, Sinclair, Milton, & Nail, 1994). It is well-known that mannitol is a crystallization of the bulking agent unlike lactose. It is hypothesized that the product stability with lactose is better than mannitol. Crystallization of mannitol consists of δ-mannitol and mannitol hemihydrate during lyophilization. Release of water molecules from hemihydrate structure during stability period may cause degradation and instability of humidity sensitive drug products (Liao, Krishnamurthy, & Suryanarayanan, 2007; Gressl et al., 2017). Dubost et al. established the connection between a cyclic peptide drug and mannitol interaction in a lyophilized formulation. The results showed that degradation takes place with mannitol induced oxidation in a lyophilized injection (Dubost et al., 1996) Many stability and impurity problems during development and commercialization may be encountered in matching the inappropriate ingredients in pharmaceutical dosage forms (Carstensen, Osadca, & Rubin, 1969). Excipients that may have different functional groups interact with active pharmaceutical ingredients. These compounds, even in trace amounts, can adversely affect the stability and efficacy of formulation. Excipient related functional group associated with drug-excipient interaction such as Schiff base formation in formulation. Oxidation, hydrolysis, photolysis, polymerization and isomerization reactions are well known active compound-active compound interactions or active compound-excipient interactions (Fatima, Mamatha, Qureshi, Anitha, & Rao, 2011). These chemical drug-excipient interactions are important in drug impurity problems and incompatibilities in drug formulation (Hotha, Roychowdhury, & Subramanian, 2016; Vranic, 2004). Unwanted chemicals in formulation, called impurity profile, take place in some chemical interactions. For this reason, impurity profile of drug formulation is important for the efficacy and safety of the final products (Tegeli et al., 2011).

To the best of our knowledge, there is little research that has been done using lactose monohydrate, EDTA and glycine as stabilizing additives in hydroxocobalamin mixed parenteral solutions. Herein, we report novel lyophilized hydroxocobalamin formulations.

CONCLUSION

The present research study was designed to develop a lyophilized dosage form of a hydroxocobalamin formulation during the stability period. Based on the physicochemical properties of hydroxocobalamin and excipients, the impurity parameter was optimized with lactose monohydrate and lactose monohydrate + EDTA combination formulations. We also showed that the hydroxocobalamin vitamin is incompatibility with glycine for pharmaceutical research. The finding of this research proposes a new stable formulation and proper storage conditions for lyophilized hydroxocobalamin parenteral solutions. Thus, the most important aspect of this study is being the first scientific report specifically for hydroxycobalamin related pharmaceutical literature.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- Ö.S., G.G., İ.K.; Data Acquisition- Ö.S., G.G., İ.K.; Data Analysis/Interpretation- G.M., K.Ş., S.S.; Drafting Manuscript- Ö.S.; Critical Revision of Manuscript- Ö.S., G.G.; Final Approval and Accountability-G.G., İ.K., M.G., Ş.K., S.S., M.Ö., S.Ö.; Technical or Material Support- İ.K.; Supervision- Ö.S., Ö.M.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: Authors declared no financial support.

Acknowledgement: This paper was supported by World Medicine Pharmaceutical Company. The authors are thankful to Rovshan TAGIYEV who is founder and chairman of the World Medicine Pharmaceutical Company. We also thank Metin KARABULUT who is product development manager and R&D department of World Medicine Pharmaceutical Company.

REFERENCES

- Ahmad, I., Ansari, I. A., & Ismail, T. (2003). Effect of nicotinamide on the photolysis of cyanocobalamin in aqueous solution. *Journal of Pharmaceutical and Biomedical Analysis, 31*, 369–374.
- Ahmad, I., Qadeer, K., Zahid, S., Sheraz, M. A., Ismail, T., Hussain, W., & Ansari, I. A. (2014). Effect of ascorbic acid on the degradation of cyanocobalamin and hydroxocobalamin in aqueous solution: A kinetic study. *American Association of Pharmaceutical Scientists, 15*, 1324–1333.
- Ahmad, I., Ahmed, S., Anwar, Z., Sheraz, M. A., & Sikorski, M. (2016). Photostability and photostabilization of drugs and drug products. *International Journal of Photoenergy, 1*,1–19.
- Atici, B., & Karlıga, B. (2015). Identification, synthesis and characterization of process related impurities of benidipine hydrochloride, stress-testing/stability studies and HPLC/UPLC method validations. *Journal of Pharmaceutical Analysis, 4*, 256-268.
- Atici, B., Yazar, Y., Agtas, C., Ridvanoglu, N., & Karlıga, B. (2017). Development and validation of stability indicating HPLC methods for related substances and assay analyses of amoxicillin and potassium clavulanate mixtures. *Journal of Pharmaceutical and Biomedical Analysis, 136*, 1–9.
- Baheti, A., Kumar, L., & Bansal, A. K. (2010). Excipients used in lyophilization of small molecules. *Journal of Excipients and Food Chemistry, 1*, 41–54.
- Cappola, M. L. (2000). Freeze-Drying Concepts. The Basics, in McNally EJ (ed): *Protein formulation and delivery*, (pp 159-199), Marcel Dekker, New York.
- Carstensen, J. T., Osadca, M., & Rubin, S. H. (1969). Degradation mechanisms for water-soluble drugs in solid dosage forms. *Journal of Pharmaceutical Sciences, 58*, 549–553.
- DRUGBANK website, retrieved from https://www.drugbank.ca/drugs/DB00200.
- Dubost, D. C., Kaufman, M. J., Zimmerman, J. A., Bogusky, M. J., Coddington, A. B., & Pitzenberger, S. M. (1996). Characterization of a solid state reaction product from a lyophilized formulation of a cyclic heptapeptide. A novel example of an excipient-induced oxidation. *Pharmaceutical Research, 13*, 1811–1814.
- Edelmann, M., Chamlagain, B., Santin, M., Kariluoto, S., & Piironen, V. (2016). Stability of added and in situ-produced vitamin B12 in breadmaking. *Food Chemistry, 204*, 21–28.
- El-dawy, M. A., Mabrouk, M. M., & El-Barbary, F. A. (2002). Liquid chromatographic determination of fluoxetine. *Journal of Pharmaceutical and Biomedical Analysis, 3*, 561–571.
- Fatima, N., Mamatha, T., Qureshi, H. K., Anitha, N., & Rao, J. V. (2011). Drug-excipient interaction and its importance in dosage form development. *Journal of Applied Pharmaceutical Science, 1*(6), 66–71.
- Frank, K. (2004). Understanding Lyophilization formulation development. *Pharmaceutical Technology Lyophilization,* 10–18.
- Gressl, C., Brunsteiner, M., Davis, A., Landis, M., Pencheva, K., Scrivens G, Sluggett, G.W … Paudel, A. (2017). Drug–excipient interactions in the solid state: The role of different stress factors. *Molecular Pharmaceutics, 14*, 4560-4571.
- Heathgote, J. G., Moxon, G. H., & Slifkin, M. A. (1970). Ultraviolet, visible and infrared spectroscopic studies of the interaction of hydroxocobalamin with α-amino acid and peptides. *Spectrochimica Acta, 27*,1391–1408.
- Herman, B. D., Sinclair, B. D., Milton, N., & Nail, S. L. (1994). The Effect of bulking agent on the solid-state stability of freeze dried methylprednisolone sodium succinate. *Pharmaceutical Research, 11*, 1467–1473.
- Hotha, K. K., Roychowdhury, S., & Subramanian, V. (2016). Drug-Excipient interactions: Case studies and overview of drug degradation pathways. *American Journal of Analytical Chemistry,* İ, 107–140.
- Kennedy, D. O. (2016). B Vitamins and the brain: Mechanisms, dose and efficacy. *Nutrients, 8*, 2–29.
- Kondepudi, N. (2016). Stability of vitamins in pharmaceutical preparations *International Journal for Research in Applied Science & Engineering Technology, 4*, 499–502.
- Korey, D., & Schwartz, J. B. (1989). Effects of excipients on the crystallization of pharmaceutical compounds during lyophilization. *Journal of Parenteral Science and Technology, 43*, 80–83.
- Liao, X., Krishnamurthy, R., & Suryanarayanan, R. (2007). Influence of processing conditions on the physical state of mannitol- implication in freeze-drying. *Pharmaceutical Research, 24*, 370–376.
- Mehmood, Y., & Farooq, U. (2015). Excipients use in parenteral and lyophilized formulation development. *Open Science Journal of Pharmacy and Pharmacology, 3*, 19–27.
- Mishra, A., Saini, T. R., & Maurya, V. K. (2017). Process validation of lyophilization process. *World Journal of Pharmacy and Pharmaceutical Sciences, 7*, 365–397.
- Monajjemzadeh, F., Ebrahimi, F., Milani, P. Z., & Valizadeh, H. (2014). Effects of formulation variables and storage conditions on light protected vitamin B12 mixed parenteral formulations. *Advanced Pharmaceutical Bulletin, 4*, 329–338.
- ICH Guidelines. (2005). Stability testing of new drug substances and products, In Proceedings of International Conference on Harmonization Topic Q1A (R2), Geneva, Switzerland, http://www.ich.org/.
- ICH Guidelines. (2005). Validation of analytical procedures: Text and methodology, In Proceedings of International Conference on Harmonization Topic Q2 (R1), Geneva, Switzerland, http://www.ich.org/.
- Shchavlinskii, A. N., Neiman, A. V., Lazareva, N. P., & Orlov, S.V. (1995). Analytical methods for control of cyanocobalamin quality and its stability in drug dosage forms. *Pharmaceutical Chemistry Journal, 29*, 51–60.
- Schnellbaecher, A., Binder, D., Bellmaine, S., & Zimmer, A. (2019). Vitamins in cell culture media: Stability and stabilization strategies. *Biotechnology and Bioengineering,* 1–19.
- Tegeli, V. S., Gajeli, G. B., Chougule, G. K., Thorat, Y. S., Shivsharan, U. S., & Kumbhar, S. T. (2011). Significance of impurity profiling: A review. *International Journal of Drug Formulation and Research, 2*, 174–195.
- Vranic, E. (2004). Basic principles of drug-excipients interactions. *Bosnian Journal of Basic Medical Science, 4*(2), 56–58.