Formulation Development of Vitamin K1 (10 mg injection) and Accelerated Stability Study

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ABSTRACT

Vitamin K being a fat-soluble vitamin has two forms available naturally. Vitamin K is involved in the production of different proteins that are needed for blood clotting and the building of bones. Common indications of vitamin K include hemorrhagic disease of newborn, malabsorption syndrome, cystic fibrosis, biliary atresia, antidote to warfarin and hepatic failure. The problems associated with Vitamin K deficiency can significantly contribute to bleeding, poor bone development, osteoporosis, and increased risk of cardiovascular disease. Only 2 mg of Vitamin K injection is available in Pakistan, and if adults experience internal bleeding, other hemorrhagic diseases, or other vitamin K deficiency-related conditions, 5 injections of the typical 2 mg neonate dose are required to be given. Therefore, the primary goal of this study was to reduce the cost of injections and introduce the adult dose (10 mg) of injections, which had previously only been available internationally. Following many trials, an optimized adult dosage was created. A six-month stability study was performed using different instruments including HPLC, UV, FTIR etc. Different parameters like pH, assay, physical appearance, sterility and others are checked and verified according to British Pharmacopeia.

Keywords: Vitamin K, Injection, Formulation, Accelerated Stability

INTRODUCTION

Vitamin K is a nutrient that may be present in food and also as dietary supplements. It functions as a cofactor for several enzymes involved in carboxylation. The carboxylation of vitamin K enables the coagulation components to bind calcium ions, further enabling the cascade pathways. Numerous studies have linked osteoporosis and cystic fibrosis to vitamin K insufficiency. Vitamin K available in two diverse bioactive forms: K2 (menadiones) and K1 (phylloquinone). Plants produce phylloquinone. It is mostly found in green leafy vegetables due to its direct role in photosynthesis. Vitamin K1 may be metabolised by animals, which leads to the production of coagulation factors. It can also produce vitamin K2 in animals [1] [2].

As part of the synthesis of clotting factors, vitamin K2 adds carboxylic acid groups to glutamate residues (Glu) to create gamma-carboxyglutamate residues (Gla). The calcium ion chelation is made possible by the gamma-carboxyglutamate residue, which consists of one carbon atom and two carboxylic acid groups. The continuation of the clotting cascades is dependent on the binding of calcium ions to vitamin K- dependent clotting proteins. Furthermore, vitamin K helps to make prothrombin, factor VII, factor IX, and factor X. Giving vitamin K to infants, those with congenital hypoprothrombinemia, renal impairment, cases of severe heparin-induced anticoagulation, and those who are sensitive to vitamin K should be done with caution. Only 2 mg of Vitamin K injection is available in Pakistan, and 5 injections of the standard 2 mg neonate dose must be administered if adults suffer from internal bleeding, hemorrhagic disorders, or other vitamin K deficiency-related conditions [3] [4]. Thus, the primary goal of this study was not only to reduce Vitamin K injection cost but also to introduce its adult dose (10 mg), which was previously only available internationally.

MATERIALS AND METHODS

Chemicals

Phytomenadione (GT Pharma), Cremophore 40 (Lamberti Chemical), Dextrose (XiwangPharmaceutical China), Sodium acetate (Sigma Aldrich), Benzyl alcohol (Banchun Pure chemical korea), Glacial acetic acid (Merck), HPLC grade ethanol 96% (WR Chemical)

Equipment

FTIR (Agilent Technology, Germany), HPLC (Agilent Technology, Germany), pH meter (WTE, Germany), Sonication bath (Huanghua Faithful, China), Laminar flow hood (Technico Scientific, Pakistan), Analytical balance (Shimadzu, Japan), Stability Chamber (Technico Scientific, Pakistan).

Method of trial preparations

After autoclaving, benzyl alcohol was added in the cremophore and phytomenadione (Solution A). Then, distilled water was mixed with dextrose and sodium acetate anhydrous to dissolve (Solution B). Now combine these solutions and makeup volume with distilled water. Based on research trails, a composition was selected that is suitable for an optimized formulation of Vitamin K injection. Further, stability study was done and found satisfactory on the formulation.

Types of stability study performed

To guarantee steady production for the duration of the product's declared shelf life of 12+ months, stability studies were performed.

1. Accelerated Stability Study:

2. Ongoing Stability Study (Real Time)

To comply with regulatory and GMP requirement, the Accelerated stability study program has been performed for this product in the following condition.

Storage: $30^oC \pm 2^oC$ 65% ± 5% RH

Testing frequency: 0^{th}, 1^{st}, 2^{nd}, 3^{rd},4^{th}, 6^{th} month

Stability indicating tests, including physical appearance, pH, sterility, endotoxin test and assys performed according to the British Pharmacopoeia.

Drug and excipients compatibility studies

The aim of the drug-excipient compatibility study was to examine if a medicine was compatible with excipients frequently used in injectable dosage forms. Distinctly, all excipient was joined with the drug, and the samples were sustained at enhanced (30°C 2°C/65% 5% RH/ 30 days' time period) and real-time (30°C 2°C/75% 5% RH/ 30 days) temperatures. After 30 days onwards, the samples were collected and examined for any changes physically (Table 1 and 2).

Table 1. Drug and excipients compatibility (accelerated study)

Active Ingredient	**Excipients**	**Drug-Excipientnets Ratio**	**Initial observation**	**Condition - 40ºC±2ºC/ 75%±5 RH**			
				1 week	**2 Week**	**3 Week**	**4 Week**
Phytomenadione	--------	------	Yellow color clear liquid	No Change	No Change	No Change	No Change
	WFI	01:100	Very Sparingly Soluble	Phase separation	Phase separation	Phase separation	Phase separation
	Tween 20 + WFI	20:20:100	Slightly yellow solution	No Change	No Change	No Change	Hazy solution

Table 2. Drug and excipients compatibility (real-time study)

Active Ingredient	Excipients	Drug-Excipients Ratio	Initial observation	Condition - 30ºC±2ºC/ 65%±5 RH			
				1 week	2 Week	3 Week	4 Week
Phytomenadione	--------	------	Yellow colour clear liquid	No Change	No Change	No Change	No Change
	WFI	01:100	Very Sparingly Soluble	Phase separation	Phase separation	Phase separation	Phase separation
	Tween 20 + WFI	20:20:100	slightly yellow solution	No Change	No Change	No Change	Hazy solution
	Creamophore: Benzylalcohal	10.91:69 .87:9.0	Clear yellow oily Solution	No Change	No Change	No Change	No Change

pH analysis

Digital pH meter was used to check pH of the solutions.

FTIR analysis

In 1st step, sample was placed in the FTIR spectrometer. The spectrometer exposures the sample to Infrared beams, which it then processes to regulate how much of the beam of IR and at what frequencies the sample absorbs IR beam.

HPLC analysis

HPLC analysis is performed under following conditions:

Column: Octadecylsilyl silica gel

Dimension: (15cm × 4mm) 5 µm

Flow rate: 1.5 ml/min.

Detector: 254 nm

Injection volume: 50 µl

RESULTS

Formulation development

The data is compliant following a six-month expedited stability examination. Four experiments are made and kept in a stability chamber with different formulations. At subsequent periods, the result is consistent. Three of the early tests came up empty. The first effort was given up after a month owing to a cloudy solution. The second effort was given up after one month due to pH drop and test failure. Trial #3 was dismissed as a result of pH decisions that were rendered after two months. The identical formulation was used for three trials, and they were effective since trial four is still chemically and physically stable (Table 3).

Table 3. Formulation of Vitamin K1 Injection

Sr. No.	Material Description	Unit	Standard Quantity	
			38.5 L	For 5 L
1	Phytomenadione	g	420	54.545
2	Tween 80	kg	2.69	0.349
3	Glycerin	kg	1.444	0.187
4	Benzyl Alcohol	g	346.5	45
6	NaoH	mL	Qs.	Qs.
7	Distilled Water	L	Qs.	Qs.

Accelerated stability studies

The results of the accelerated stability studies are presented in table 4 and 5. All values were found to be in range as recommended by British Pharmacopoeia 2022.

Table 4. Accelerated stability studies for different parameters

Sr. No.	Parameter	Specification	12-01-22	14-02-22	18-03-22	20-04-22	12-05-22	07-07-22
			0 Months	1 Months	2 Months	3 Months	4 Months	5 Months
1.	Description	A clear Slightly yellow liquid.	Complies	Complies	Complies	Complies	Complies	Complies
2.	pH	5.0 – 7.5	6.29	6.26	6.13	6.21	6.29	6.12

3.	Identification	RT for sample and standard solution should be same	+ve	+ve	+ve	+ve	+ve	+ve
4.	Sterility Test	Must be sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile
5.	Endotoxin Test	NMT 0.25 EU/mg	Complies	Complies	Complies	Complies	Complies	Complies
6.	Assay Each 1 ml contains Phytomenadione…2mg	90.0 – 115.0 %(B.P Specs)	106.82 %	108.90 %	107.42 %	104.24 %	104.64 %	102.49 %

Table 5. Accelerated studies for stability

Product Name	K-Lot Injection 10 mg/ml (Trial)	Batch No.	TKKL004
Mfg. Date	01-2022	Exp. Date	01-2024
Storage Condition	This Simulated Trial packs are kept in stability chamber at 30 ◦C ± 2 ◦C	Reference	BP Specifications
		QC No.	$TAst_{22}$-001
		Date of Commence	12-01-2022
Type of Study	Accelerated Stability Study	Date of Completion	07-07-2022

FTIR studies

FTIR analysis is performed for verification of excipients and active ingredient. The results verified the compounds use for the formulation development, including benzyl alcohol (Figure 1), sodium acetate anhydrous (Figure 2), dextrose (Figure 3), cremophore (Figure 4), Vitamin K (Figure 5) and Vitamin K injection (Figure 6).

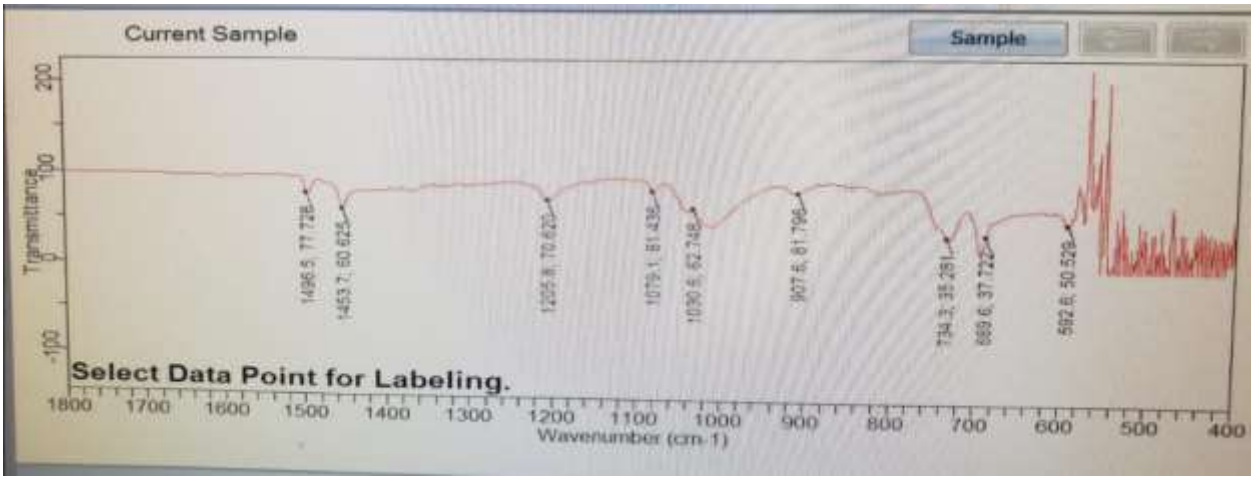

Figure 1. FTIR spectrum of benzyl alcohol

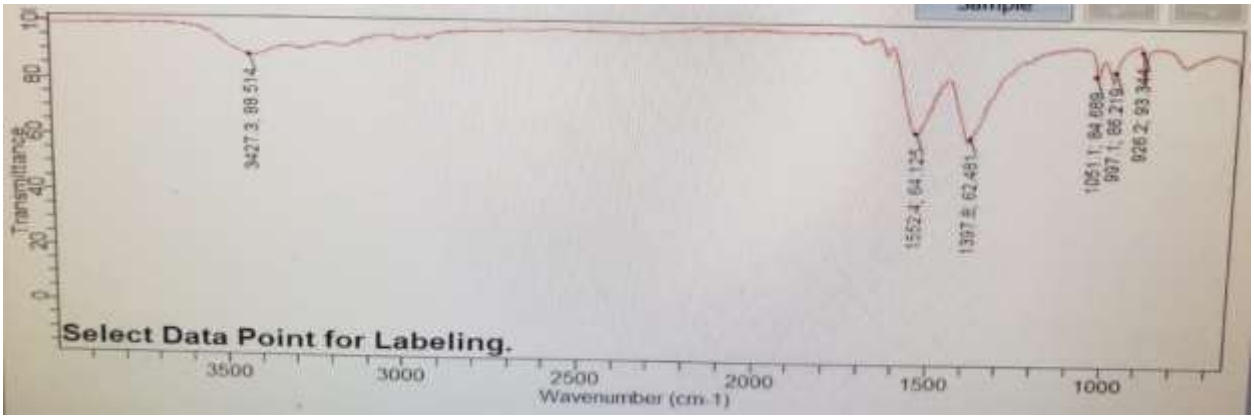

Figure 2. FTIR spectrum of sodium acetate anhydrous

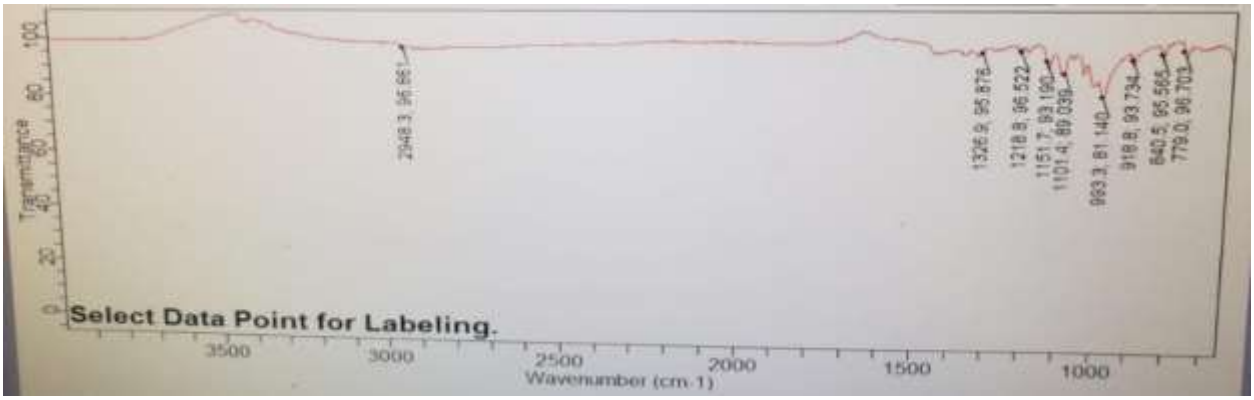

Figure 3. FTIR spectrum of dextrose

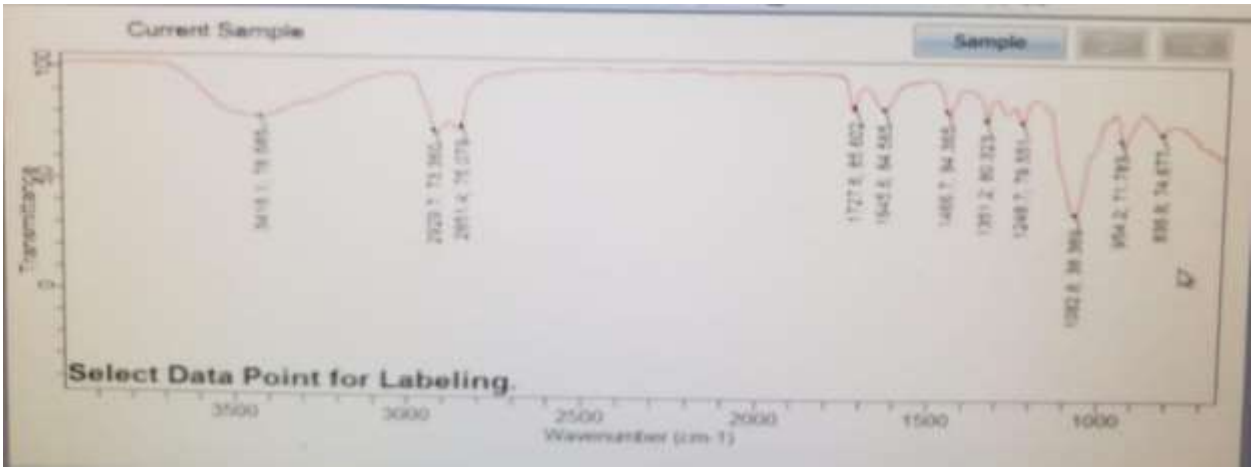

Figure 4. FTIR spectrum of cremophore

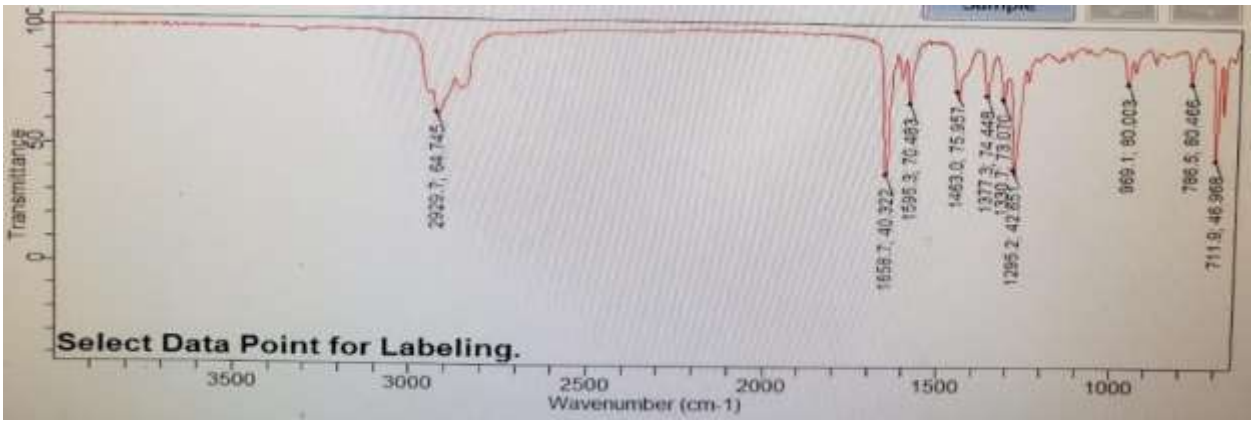

Figure 5. FTIR spectrum of Vitamin K

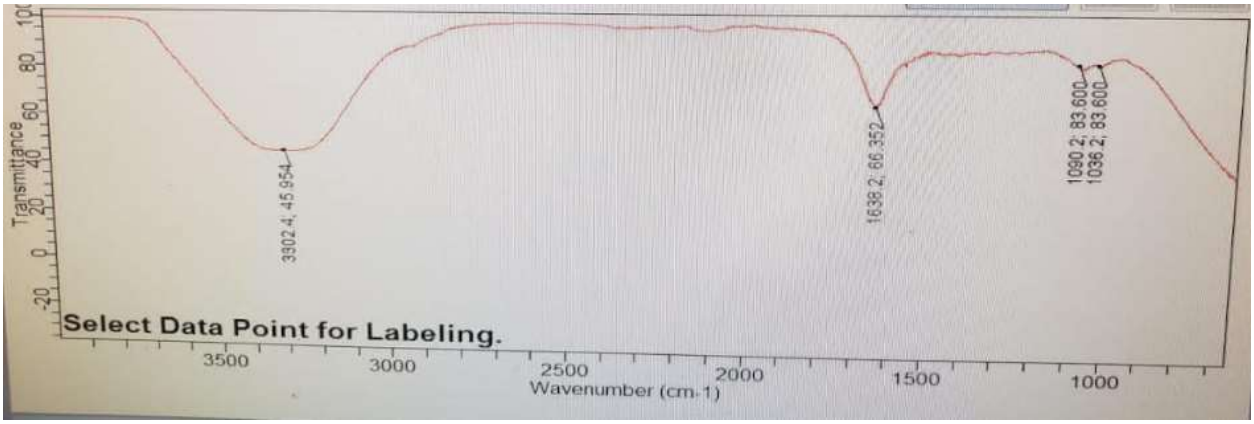

Figure 6. FTIR spectrum of Vitamin K injection

HPLC analysis

The results of the Vitamin K injection HPLC analysis is shown in Figure 7. Retention time (RT) at 7.706 (min) with 85.70 area (%) indicating the presence of phytomenadione (Vitamin K1).

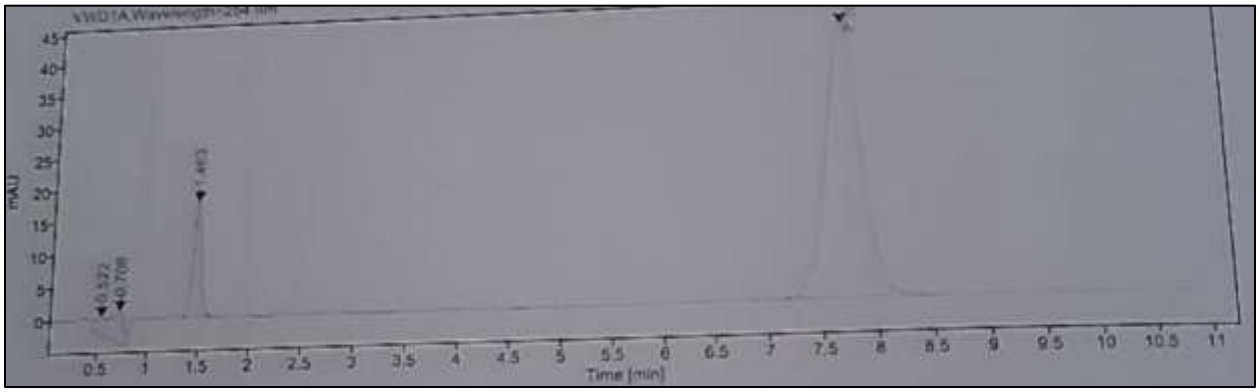

Figure 7. HPLC analysis of Vitamin K injection

DISCUSSION

We use drugs nowadays due to the myriad ailments induced by our luxurious and demanding lifestyles. There are several dosing types for different sickness conditions. Medication administration can be done orally, transdermally or parenterally. First pass metabolism undermines the effectiveness of the oral route. One benefit of transdermal medication administration over oral drug delivery is its first- pass effect. Yet, in transdermal drug delivery, the stratum corneum is the main barrier to drug penetration [5]. According to various studies from Asia, there are several reasons to be concerned about the region's use of dosage form in injections. One-third of patients who attended private medical practitioners had one or more injections, according to a survey performed in India [6]. Vitamin K belongs to the group of fat-soluble vitamins. For the production of a number of proteins involved in calcium and coagulation homeostasis in humans, they are regarded as essential cofactors. The phrase "vitamin K" originally comes from the Germanic word koagulation, which denotes the ability to halt bleeding or cause blood to clot [7]. Only babies in Pakistan are eligible for injections of vitamin K1. Adult dosage instructions weren't provided. As a result, this study was designed to create a formulation and concentrated on the adult dosage [8] [1].

Injectable formulations consider physical appearance very carefully. It ought to be clean and devoid of any impurities or foreign matter. Clear-cut answers were not acceptable. Throughout the trial, the vitamin K1 infusion remained steady, and no physical changes were noticed.The stability of injectables is significantly influenced by pH. As a function of pH, the stability of vitamin K1 in aqueous solutions was evaluated. The pH value at which vitamin K1 was determined to be the most stable was 6.2. (pH 5 – 7.5). Sterility test is used to check the any viable and extraneous microorganisms in the formulation. The sterility of Vitamin K1 product remained stable throughout the study

Every product being injected intravenously into a human body must undergo endotoxin testing. Endotoxin testing is required for all pharmaceuticals intended for injection and all medical devices intended for implantation. Endotoxins can cause a pyrogenic reaction (fever) or signs of septic shock if they are present. Examination of vitamin K1 injection demonstrated that all experiments were endotoxin-free, which was supported by the results [9]. The testing of a product to determine and measure the amount of its active medicinal components. Using HPLC technology, the formulation of vitamin K1 was tested, and the findings were confirmed to be adequate by compendial standards. Any modification, such as an increase or reduction in the assay, will have a direct impact on the product's stability. Results from trials 4, 5, and 6 were all determined to be between 90 to 110% [10].

CONCLUSION

The objective of the study was to achieve the stable, compatible and cost effective adult dose of vitamin k1 injection i.e. 10 mg/ml. The product meets all standard British Pharmacopoeia 2022 testing parameters (appearance, pH, identification, sterility, endotoxin and assay) and found safe and stable.

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