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pH effect on paraben stability for parenteral drug formulation

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ABSTRACT: The pH optimization and EDTA are widely used in pharmaceutical formulations. The aim of the research work was to evaluate the pH effect and develop a including stable paraben pharmaceutical product. pH of final product is most important critical quality attributes (CQA). Variability pH of formulation is affecting paraben stability. Therefore, chemical stability may affect paraben assay, so this CQA was be evaluated throughout parenteral formulation. A total of four formulations were designed to the stability study. To improve the stability of paraben formulations (T1-T4) were evaluated with different pH ranges and EDTA during stability period. Stability studies were performed to assay analysis of methyl paraben and propyl paraben. The rate of assay results was compared to T1-T4 formulations. As a result of paraben assay analysis for T2 formulation was found to be within in specification limit. The one of them were determined to the best formulations for paraben stability during stability periods. The research proposes a novel stable formulation and proper storage conditions for paraben parenteral solutions. The instability problem of paraben formulation was optimized with targeted pH modification.

KEYWORDS: Drug stability; excipient; methyl paraben; parenteral formulation; propyl paraben.

1. INTRODUCTION

Excipients are the component of a pharmaceutical formulation to achieve the stability and efficacy of final product. They play practical physical and chemical roles in different dosage forms, serving as preservative or pH agents to allow formulation of appropriately during stability period [1, 2].

Sterility of any pharmaceutical product is main final product specification for parenteral formulations. Meyer et al. reported that one-third of the 350 parenteral drugs on sale in international pharmaceutical market are multi-dose formulations which require the inclusion of an antimicrobial preservative [3].

For liquid formulations, parabens are well known antimicrobial agents and they are commonly used to prevent the growth of micro-organisms in the pharmaceutical products [4]. Among the paraben types are alkyl ester of parahydroxybenzoic acid that are called as parabens and respective their salts form. It includes methyl and propyl parabens that are best known preservative for drug formulation. These preservatives commonly used approved concentrations for parenteral formulation are as follow 0.10–0.18 % and 0.01–0.02 % for methyl and propyl parabens [5].

Due to chemical interaction of compounds in drug formulation some chemical factors such as pH, drug-drug interactions, temperature, oxidizing and reducing agents etc. can be affected stability and biological activity of drug ingredients. The researcher reported that parabens, are inactivated in the presence of non-ionic surfactants, and this chemical interaction may have serious problems for preservation of the final drug product [6].

Another important physicochemical factor is pH, and it could have a negative effect on the stability and activity of parabens for final product and throughout shelf life. Both pH value of solution and pKa of parabens are involved in excipient stability that related to antimicrobial activity [7]. For this reason, paraben stability in parenteral formulation is the important to protect the final product from microbial contamination. Despite the increasing different stability problem of paraben formulations, there have been limited studies on stability of preservative in pharmaceutical literature.

Therefore, the aim of this study was to optimize parabens stability of parenteral formulation and evaluated the pH effect for parabens stability during stability period. This contributes lead to novel parenteral

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formulation including parabens in terms of stable drug formulation. In this respect, this study could be the first investigation to parenteral formulation of pharmaceutical research.

2. RESULTS

Drug formulations have different critical quality attributes (CQA). The most important CQA is a pH for the drug formulation. Most of the formulation ingredients are characteristically weak acid or base compounds. Due to this common chemical characteristic, the parenteral drug formulation stability closely related pH value during shelf life. T1 to T4 formulations were different pH range adjusted 6.50 to 8.40 to evaluated paraben stability (Table 1). In addition, EDTA was used as chelating agent as an excipient in T3 formulation. Paraben stability studies were performed with different pH range for 6-months at three different storage conditions. The stability samples were analyzed for assay parameter end of stability period. The highest paraben decomposition was determined to 40 °C temperature for all formulation trails during 6-month stability period. Generally, propyl paraben is more stable than methyl paraben during all shelf-life condition and it is not stable in T1 or T4 formulations with accelerated stability period. Assay of parabens are shown during stability period in Table 1. T1 is an alkaline formulation for the paraben stability. Based on paraben assay results of T1, the amount of methyl paraben is out of specification, and it decreased about 46 % at end of accelerated stability condition due to negatively effect of alkaline pH. Although propyl paraben is stable at long term and intermediate term, the assay of propyl paraben decreased from 0.44 mg/vial to 0.37 mg/vial at 40°C ± 2°C/75% RH ± 5% RH condition.

Table 1. Assay results of parabens formulations during 6-months stability period

Formulation		Limit	Initial	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)
T1	Methyl paraben	2.40 mg/vial (± 10 (2.16-2.64)	2.31 mg/vial	1.83 mg/vial	1.65 mg/vial	1.07 mg/vial
	Propyl paraben	0.45 mg/vial (± 10 (0.41-0.49)	0.44 mg/vial	0.42 mg/vial	0.40 mg/vial	0.37 mg/vial
T2	Methyl paraben	2.40 mg/vial (± 10 (2.16-2.64)	2.39 mg/vial	2.35 mg/vial	2.29 mg/vial	2.18 mg/vial
	Propyl paraben	0.45 mg/vial (± 10 (0.41-0.49)	0.47 mg/vial	0.45 mg/vial	0.44 mg/vial	0.42 mg/vial
T3	Methyl paraben	2.40 mg/vial (± 10 (2.16-2.64)	2.30 mg/vial	2.20 mg/vial	2.01 mg/vial	1.03 mg/vial
	Propyl paraben	0.45 mg/vial (± 10 (0.41-0.49)	0.44 mg/vial	0.43 mg/vial	0.43 mg/vial	0.42 mg/vial
T4	Methyl paraben	2.40 mg/vial (± 10 (2.16-2.64)	2.33 mg/vial	1.81 mg/vial	1.62 mg/vial	1.03 mg/vial
	Propyl paraben	0.45 mg/vial (± 10 (0.41-0.49)	0.43 mg/vial	0.41 mg/vial	0.39 mg/vial	0.35 mg/vial

The pH range of T2 formulation is between 7.40 – 7.80. According to the data obtained from the formulation of T2 stability analysis, assay result of T2 is in limit at three stability condition. It is observed that the highest rate of assay is determined to during each three-stability period with T2 formulation. The results of a 6-month assay test for methyl paraben is the highest among all formulation and shelf-life conditions. It is observed that the lowest rate of degradation for methyl paraben is determined with T2 formulation about 8% during stability period.

Based on HPLC analysis, the presence of EDTA in T3 formulation is not positively affected to assay of both methyl paraben and propyl paraben at 25°C ± 2°C/60% RH ± 5% RH 30°C ± 2°C/65% RH ± 5% RH 40°C ± 2°C/75% RH ± 5% RH stability conditions but the formulation has acceptable assay values for methyl paraben at 25°C after 6 month stability. Moreover, the formulation of T3 assay results is lower than T2 formulation during stability period when compared to each two formulations. For this reason, EDTA is not improve to paraben stability with T3 formulation. According to T4 formulation which is pH 6.50 – 7.00 stability results parabens are not stable end of stability period. It is determined that the worst assay result for methyl and propyl parabens were obtained with this formulation. In addition, assay results two of these parabens are out of specification at tested stability period. In this case, pH dependent paraben decomposition was performed to all storage condition. It is clear that there is correlation with pH values and assay results.

The HPLC method was validated according to ICH guidelines [Q2 (R1)] [8]. The validation parameters included system specificity, suitability, linearity, accuracy, precision (system, method, and intermediate precision) and robustness. In specificity test, not another peak was detected on dilution and placebo solution chromatogram at the retention time of methyl paraben and propyl paraben 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 50 % to 150% of the specification level. The accuracy was determined by measuring recovery at known concentrations of the methyl paraben and propyl paraben (50%, 100% and 150%) and analyzed. 95% Confidence interval limits of recoveries were calculated (Table 2).

Table 2. Results of validation parameters

Parameter	Specification	Result	
		Methyl paraben	Propyl Paraben
System suitability	The RSD value for six repeated injections should not be greater than 2.00%.	0.36 %	0.39 %
	The tailing factor for standard injection should not be greater than 2.00 %.	1.00 %	1.00 %
	The theoretical number of plates for standard injection should be greater than 2000.	197474	387350
Linearity	R≥ 0.998	0.999980	0.999977
Accuracy	% Recovery between 97 % and 103 %		
	50%	101.76 %	100.78 %
	100%	102.13 %	100.44 %
	150%	101.26 %	99.58 %
Precision System precision	RSD % <2.00	0.36%	0.39%
Precision Method precision	RSD % <2.00	0.39%	0.32%
Precision Intermediate precision	RSD % <2.00	0.83%	0.99%
	<3.00%	0.93%	2.80%

System precision was conducted with two preparation of standards solution and six repeated injections of standard-I solution and twice injections of standard-II solution prepared at 100% concentration and RSD of peak areas was found below 2%. The compliance between standard-I solution and standard -II solution should be 98%-102%. 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 2 %. Intermediate precision was studied by different analysts and with different

devices. Each analyst prepared 2 standard solution and 6 sample solutions. All the results compared, and RSD was calculated. To validation of the developed method, parameters were verified, and standard solutions were tested. Column temperature by ±5 °C, Flow ratio by ±0.1 ml/min and different wavelength by ±2 nm was changed 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 different hours during in 48 hours after at 25 °C. The obtained data were compared and % variations were calculated, and all results were below 2 %.

3. DISCUSSION

Excipients may have different functional groups to interact with other formulation ingredients. They even trace amounts can adversely affect the stability and efficacy of drug product. Oxidation, hydrolysis, photolysis, polymerization, and isomerization reactions are well known excipient related interactions [9]. Oxidative decomposition reaction may cause many changes in the drug formulation such as active compound or excipient. This reaction led to instability of the formulation component, and it tends to decrease assay [10]. According to literature EDTA is the commonly used as chelating agent for parenteral drug formulations to improve formulation ingredients stability [11]. The results showed that the parenteral formulation included EDTA enhance to hydroxocobalamin stability for stability studies [12]. In contrast to literature, we detected that using EDTA in parenteral formulation is not enhanced to paraben stability during shelf-life periods. It is clear that paraben instability problem is not directly oxidation reaction.

The important determination for liquid formulations is the effect of pH on the stability of the solution [13]. It is hypothesized that the pH value of solution is chemically effective on the active moiety in the paraben molecules [14]. For this reason, pH dependent stability is studied over a range of pH values for paraben. Based on the literature, the optimal paraben stability observed with the widely pH range [15]. Our results showed that methyl and propyl paraben are stable narrower a range of the pH between 7.40-7.80 in solution. These range is specially prevented to methyl paraben decomposition during shelf life for parenteral formulation. According to our formulation trails we reported that methyl paraben is much more stable with pH 7.40-7.80 than studied other pH ranges. Besides, the rate of decomposition of methyl paraben is low adjustment the pH this value when compared other conditions. Indeed, the main problem of paraben stability could be related with ester hydrolysis that it may be undergo reaction of acid or base catalyzed. Miloud et al. have demonstrated that methyl and propyl paraben degradation take place induced different pH values. The content of paraben during stability period may cause degradation and instability of drug formulation [16]. In the previous study, the researcher showed that both methyl and propyl parabens are degraded with pH ranges over the 6.50 in the liquid formulation. In addition, it is a well-known that hydrolysis of parabens is occurred under alkaline condition [17]. By determining the best pH range of the paraben was evaluated with our formulation studies. In this respect our study result lead to contribute pH dependent paraben stability for parenteral formulations. Minhui et al. have established the connection between functional groups of sugar alcohols or sugars can also react with methylparaben it may lead to transesterification reaction with methylparaben as well as degradation of paraben [18]. This chemical interaction is the important to paraben instability problem and incompatibilities in liquid pharmaceutical dosage forms. By comparing the alteration of pH, it could be noticed that reaction kinetics and degradation mechanism different type of parabens [19[. For this reason, pH of drug formulation is important the efficacy and stability of paraben in the final products.

CQAs are crucial to ensure the safety and effectiveness of the drug product. It includes parameters such as assay, stability, microbial contamination, pH, appearance, clarity and sterility for parenteral product. One of them CQAs could be affected by any other parameters. For example, paraben stability directly is related to microbial contamination of drug product and it ensures the sterility of the formulation. In addition, active pharmaceutical ingredient or excipient degradation can lead to alteration of appearance and clarity limits during stability period. CQAs provides understanding and controlling drug product quality for this reason the first step is to identify and define the CQAs in terms of the safety and effectiveness of the drug product [20[. Based upon the physicochemical properties of the drug substance and parabens are to impact the CQA for parenteral formulation. The impurity, assay, pH are well-known CQAs of the finished product. The assessment of parabens attributes on drug product CQAs may affect the final product specifications. For this reasons, pH of drug product could be evaluated to critical formulation variables.

To the best of our knowledge, there is not so much research that is performed pH optimization and using EDTA as stabilizing additives in paraben parenteral drug formulations. Herein, we reported that a novel parenteral drug formulation was designed for paraben stability during shelf-life conditions.

4. CONCLUSION

The present research study was designed to develop a liquid dosage form of paraben formulation during the stability period. Based on the physicochemical properties of paraben stability was optimized with formulation pH modification and EDTA combination formulations. We also determined that methylparaben and propyl paraben are incompatibility with out of specific pH range for pharmaceutical research. Our finding of this research proposes a new stable formulation and proper storage conditions for parenteral solutions with paraben preservatives. Therefore, most important finding of this study is to scientific report for specifically for paraben stability related pharmaceutical literature.

5. MATERIALS AND METHODS

Nonsteroidal anti-inflammatory active ingredient (Diclofenac sodium) and corticosteroid active ingredient (Betamethasone sodium phosphate) were produced from Crystalpharma (India); EDTA, hydrochloric acid, sodium phosphate and sodium hydroxide were purchased from Merck, benzyl alcohol was produced from Scharlau, propylene glycol was produced from BASF, methyl paraben and propyl paraben were produced from Hebei Guanlang Biotechnology. Vials were produced from Schoot. 0.2 µm filter was purchased from Sartorius. The sample of active pharmaceutical ingredients were purchased from Ferrer. The water was produced by the Sartorius Stedim Biotech system as HPLC grade. Parenteral drug manufacturing method consists of mainly four parts such as raw material weighing process, preparation of bulk solutions, filtration, and analytical analysis. Whole manufacturing steps of bulk solution is detailed below. The ingredients of drug product are pharma grade excipients to use in pharmaceutical product formulations. A total 150 vials from each formulation were stored for stability analysis. The manufacturing process was performed with using conventional liquid production equipment.

5.1. Preparation for Parenteral Formulation Trials

- Weighing of raw materials.
- Take water for injection (wfi) into a manufacturing vessel (25±2 °C).
- Add slowly the preservatives (methyl and propyl paraben) to vessel under continuous mixing and mix it until it is completely dissolved.
- Add propylene glycol, benzyl alcohol, sodium phosphate into manufacturing vessel and mixing to get clear solution.
- To solution this step adds Diclofenac sodium and Betamethasone sodium phosphate under continuous mixing and the solution until it is completely dissolved.
- Check pH of bulk solution and if it is necessary adjust pH with 1M diluted NaOH solution or 1M HCl solution.
- Add water for injection up to total volume and mix the solution.
- Filter bulk solution through 0.2 µm filter and filling of filtered solution to vials.

5.2. Formulation Trials

The parenteral formulation development study is shown in Table 3. To obtain an optimum assay result, different formulation trials (T1-T4) were performed to evaluate shelf stability of methyl and propyl paraben. A composition of each formulation is presented below.

Table 3. Detailed parenteral formulation trials

Function	Ingredients	Formulation Trials			
		T1 (vial)	T2 (vial)	T3 (vial)	T4 (vial)
Active	Diclofenac sodium	2.00-3.00 %	2.00-3.00 %	2.00-3.00 %	2.00-3.00 %
Active	Betamethasone sodium phosphate	0.08-0.09 %	0.08-0.09 %	0.08-0.09 %	0.08-0.09 %
Preservative	Methyl paraben	2.40 mg	2.40 mg	2.40 mg	2.40 mg

		Formulation Trials			
Preservative	Propyl paraben	0.45 mg	0.45 mg	0.45 mg	0.45 mg
Buffering agent	Sodium phosphate	5.00 mg	5.00 mg	5.00 mg	5.00 mg
Co-solvent	Propylene glycol	575.00 mg	575.00 mg	575.00 mg	575.00 mg
Co-solvent	Benzyl alcohol	60.00 mg	60.00 mg	60.00 mg	60.00 mg
Chelating Agent	EDTA	-	-	0.50 mg	-
pH agent	1M NaOH or 1M HCI	q.s	q.s	q.s	q.s
Solvent	Water for injection	q.s to 3.00 ml	q.s to 3.00 ml	q.s to 3.00 ml	q.s to 3.00 ml
pH adjusting		8.40 – 8.60	7.40 – 7.80	7.40 – 7.80	6.50 – 7.00

5.3. Preparation of Formulations for Stability Test

Stability studies were performed to 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 by ICH guidelines [Q1A (R2)] [21]. Samples were stored to stability rooms until the end of the stability period for assay analysis. These studies were investigated to stability of parabens by using HPLC analysis. Each stability evaluation has been performed at least 3 times.

5.4. HPLC Chromatographic Conditions of Paraben Assay

The HPLC method was carried out on Kinetex EVO C18 (150 mm x 4,6mm; 5µm) column with 20 µl injection volume at a wavelength of 272 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 ml/min. Mobile phase A was prepared by dissolving 2.72 g potassium dihydrogen phosphate salt with 1000 ml purified water. The pH is adjusted to 7.50 with 1N sodium hydroxide. Filtered through a 0.45 µm NY filter. Mobile phase B was prepared by mixing the acetonitrile and water at the ratio of 65:35 (v: v) and filtering through 0.45 µm filter [22]. Mobile phase was prepared by mixing the Mobile phase A and Mobile phase B at the ratio of 805:195.

5.5. Preparation of Standard Solution

40 mg methyl paraben working standard was weighed into a 20 ml volumetric flask, dissolved in an ultrasonic bath for 5 minutes by adding approximately 20 ml mobile phase and then completed to volume with mobile phase. In addition, 50 mg propyl paraben was weighed into a 100 ml volumetric flask dissolved in an ultrasonic bath for 5 minutes by adding approximately 500 ml mobile phase and then completed to volume with mobile phase. 4 ml methyl paraben and 3 ml propyl paraben standard stock solutions were transferred into 100 ml volumetric flask and completed to volume with mobile phase and filtered through 0.45 µm RC filter [23,24].

5.6. Preparation of Sample Solution

1 ml test sample transferred into 10 ml volumetric flask diluted to volume. Filtered through 0.45 µm RC filter.

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