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A STUDY ON DESIGN & DEVELOPMENT OF BETAMETHASONE ACETATE AND BETAMETHASONE SODIUM PHOSPHATE EXTENDED-RELEASE SUSPENSION

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ABSTRACT
Background: The development of an injectable composition of betamethasone sodium phosphate and betamethasone acetate with an equivalent drug release profile to the marketed reference drug product, "Celestone Soluspan®," is highly challenging. To overcome this drug development problem, there is a need for a practical methodology for the preparation and evaluation of injectable compositions. **Methodology:** Different sterilization methods (Dry Heat Sterilization and Autoclave) and phase methods (two- or three-phase methods) are used for the preparation of the injectable composition of betamethasone sodium phosphate and betamethasone acetate. Two-phase or three phase methods and order of addition of excipients during the preparation of the formulation are the unique methodology of the present study and plays an important role in the stability of the composition. The release profile of the developed formulations is determined by using a USP type IV dissolution apparatus (STF buffer pH 7.4 as dissolution medium, 6.0 ml/min flow rate for 120 min), and stability study is also performed. **Results and Discussion:** As per the results of the present study Trial no. 3 shows betamethasone freebase 2.68% and total impurities 3.52% at 40°C /75% RH for 90 days and also gives similar release profile (f2 value 95%) as compared to the marketed formulation/RLD (Reference Listed Drug) i.e. Celestone Soluspan®. **Conclusion:** Present study concludes that injectable suspension of betamethasone sodium phosphate and betamethasone acetate using dry heat sterilisation of betamethasone acetate and three-phase method shows superior results or equivalent release profile as compared to the RLD and the key features of the present study.

INTRODUCTION

Synthetic corticosteroids are derivatives of prednisolone and analogs of cortisol. The synthetic corticosteroids include methylprednisolone, betamethasone sodium phosphate, betamethasone acetate, dexamethasone, and triamcinolone. Corticosteroids are mainly used to reduce inflammation and suppress the immune system [1-2]. The corticosteroids are grouped into two categories based on their particle size or

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aggregation with red blood cells, particulates, and non-particulates [3]. Particulates having larger particle sizes or aggregates (10-100 µm) are less water-soluble, whereas non-particulate corticosteroids are smaller (0-10 µm) and are freely water-soluble [3-4]. Non-particulate corticosteroids are appropriate for parenteral use and are theoretically safer for epidural use. With epidural administration, there is a risk of accidental intravascular injection. Still, since the particle size of non-particulate corticosteroids is smaller than red blood cells, "this would eliminate the risk of embolic infarction in the event of inadvertent intravascular injection" [5]. The particulate corticosteroids are theoretically supposed to be more efficacious because of their large particle size and longer retention in the epidural space [3,5].

The particle size of the drug plays a crucial role, affecting the in vivo behavior of the injected drug suspension. Smaller drug particles provide fast dissolution, wider spreading, lower infiltration, and milder fibrosis. In contrast, larger particles result in slower dissolution and cause more severe fibrous encapsulation at the injection site over 4 weeks [6]. Large microspheres showed a slower initial drug release, followed by a more rapid drug release in comparison with small microspheres. The high burst release of small microspheres may induce side effects, while slow release at a late stage may be therapeutically ineffective. In conclusion, it was essential to control the fraction of small microspheres in microsphere formulations to obtain the desired drug release behavior [7].

Betamethasone acetate is commonly used in combination with Betamethasone sodium phosphate. Betamethasone sodium phosphate has a short half-life of 36 to 72 hours, providing immediate activity, while betamethasone acetate has a longer half-life, resulting in sustained activity [8]. Betamethasone injectable suspension is a sterile aqueous suspension containing betamethasone sodium phosphate 3mg/mL and betamethasone acetate 3mg/mL [9]. This combination is approved by the USFDA [United States Food and Drug Administration]. It is commercially available under the brand name "Celestone Soluspan®" in injectable dosage, administered intramuscularly to relieve pain and inflammation (swelling) in many different conditions [10]. Corticosteroids, the primary drug therapy for inflammatory diseases, are frequently used in treating inflammatory conditions. When oral therapy is not possible, the intramuscular use of betamethasone sodium phosphate and betamethasone acetate injectable suspension is employed for allergic states, dermatologic diseases, endocrine disorders, rheumatic disorders, and other conditions [11-17].

The corticosteroids (betamethasone) in extended-release formulations have demonstrated various fetal benefits for prenatal use. In the United States, betamethasone is typically administered as a 1:1 mixture of betamethasone acetate and betamethasone phosphate. Betamethasone phosphate is rapidly metabolized by dephosphorylation, while betamethasone acetate undergoes a slower deacetylation process. This allows for the rapid effect of betamethasone phosphate, with a delayed metabolism and extended release effect from betamethasone acetate. The extended-release effect of betamethasone acetate provides a prolonged pharmacokinetic profile, resulting in extended relief. Due to this combination of rapid and delayed-onset actions, betamethasone is typically administered at 24-hour intervals [18]. The extended-release formulation offers patient compliance through a once-daily dosage regimen, compared to the immediate-release formulations, which are administered several times a day.

The development of the extended-release injectable composition of betamethasone sodium phosphate and betamethasone acetate has posed various challenges, including the formation of impurities generated due to excipient-excipient or drug-excipient interactions, and the degradation of betamethasone acetate to betamethasone free base or other related substances, which are not addressed in any of the published references. The challenges, as mentioned above, can be overcome by proper optimization of the development process. The two-phase or three-phase method for the preparation of injectable composition is developed to minimize the excipient-excipient or drug-excipient interaction, and degradation of betamethasone acetate can be minimized by using an optimized sterilization method (dry heat & autoclave methods) of betamethasone acetate in slurry or alone. The objective of the present study is to develop a stable extended release injectable composition of betamethasone sodium phosphate & betamethasone acetate and compare the release of the drug with the release of the drug from the RLD "Celestone Soluspan®". Another objective of the study was to assess the impact of various process attributes, such as drug excipient compatibility, order of excipient addition, and type of sterilization, on the stability and release profile of the composition.

MATERIALS AND METHODS

Materials

Instruments, facility, and all reagents and materials used in the present study were provided by the Mankind Research Centre, IMT, Manesar, Gurgaon (Haryana), India. Liquid chromatographic system comprising the Waters HPLC 2695 Alliance system equipped with a Zorbax Eclipse XDB-C18 3.5 mm, 75 x 4.6 mm column, or a Hypersil ODS 3 µm (250 x 4.6) mm column, or a Silversil (150 x 4.6) mm, 5µm column used for the chromatographic study. The Lab India dissolution test apparatus, equipped with a six-paddle assembly and a double-beam UV spectrophotometer (Shimadzu, Japan), was used for the dissolution test. IKA T25 basic homogenizer was used for homogenization, and Genist SS 304 Rectangular Autoclave (Horizontal), Working pressure 30 (PSI), Genist^{R} was used for autoclaving. A hot air Oven was used for dry heat sterilization. Malvern Mastersizer 3000 was used for particle size measurement.

Particle Size Measurement

The particle size measurement of the suspension product is performed using a Malvern Mastersizer 3000 by the wet slurry method, with HPLC-grade water used as the dispersant.

Analysis Procedure

The suspension was used as a sample for testing. The sample was maintained under continuous mixing during the analysis to ensure its homogeneous nature. The sample was added to the dispersant tank using a plastic dropper. The Hydro MV accessory was filled with dispersant, and the background was measured. After completion of the background measurement, the sample was added to the Hydro MV accessory. The obscuration was observed, and it should be between 5% and 20%. Three measurements for the sample were taken, and the average of these measurements was reported. The data was reported as Dv10, Dv50, Dv90 [19-20].

Preparation of BA & BSP Suspension for Injection

The following methods prepared an injectable suspension of betamethasone acetate and betamethasone sodium phosphate:

a) Two-Phase Method b) Three-Phase Method

The following methods were used to sterilize betamethasone acetate used in the methods above:

a) Dry Heat Sterilization (DHS Method)
b) Autoclave Sterilization Method

Detailed methods of sterilization are described below:

Sterilization of Betamethasone Acetate (BA): Betamethasone acetate was sterilized by two methods:

a) Dry Heat Sterilization (DHS Method): Betamethasone acetate was placed inside the dry heat sterilizer chamber and sterilized at 165 °C ± 10 °C for 3 hours. After 3 hours, a suspension dosage form was prepared using sterilized betamethasone acetate.

b) Autoclave Sterilization Method: A betamethasone acetate slurry was prepared using excipients and water for injection. The slurry was placed in an autoclave at 121°C ± 10°C for 15 minutes. The sterilized betamethasone acetate slurry was allowed to cool to room temperature for further processing.

Proposed composition of BA and BSP Injectable Suspension: Two injectable suspension formulations were prepared to study the effect of the order of addition of excipients and sterilization method on the injectable suspension of BA and BSP. The qualitative and quantitative details of the formulations are presented in Table 1.

Drug-Excipient Phase Addition Order Study: Based on the sterilization method and the number of phases used in preparing the suspension, four methods were developed to investigate the effect of excipient addition order. The no. of phases used for the preparation of the composition is as follows:

TWO-PHASE PROCESS (BA PHASE & BSP PHASE)

Method 1: BA sterilized by DHS method: 3 mg of betamethasone acetate was taken and sterilized by the dry heat sterilization method at 165°C ± 10°C for 3 hours. Sterilized betamethasone acetate was taken, and slurry was prepared by mixing betamethasone acetate with 8.9 mg (for formulation 1) or 7.5 mg (for formulation 2) of dibasic sodium phosphate dihydrate buffer, 3.8 mg (for formulation 1) or 5.2 mg (for formulation 2) of monobasic sodium phosphate dihydrate buffer, 0.1 mg (for formulation 1) or 0.15 mg (for formulation 2) of EDTA, and 0.2 mg (for formulation 1) or 0.15 mg (for formulation 2) of benzalkonium chloride. The slurry was heated at 80°C to obtain the betamethasone acetate phase. Further, the betamethasone sodium phosphate phase was prepared by mixing 3.95 mg of betamethasone sodium phosphate and water for injection, and the mixture was sterilized by filtration through a 0.2-micron filter. The betamethasone acetate phase and betamethasone sodium phosphate phase were mixed aseptically to obtain the final injectable suspension.

Method 3: BA sterilized by the autoclave method: Non-sterilized 3 mg of betamethasone acetate was taken. A slurry of betamethasone acetate was prepared by mixing betamethasone acetate with 8.9 mg (for formulation 1) or 7.5 mg (for formulation 2) of dibasic sodium phosphate dihydrate buffer, 3.8 mg (for formulation 1) or 5.2 mg (for formulation 2) of monobasic sodium phosphate dihydrate buffer, 0.1 mg (for formulation 1) or 0.15 mg (for formulation 2) of EDTA, and 0.2 mg (for formulation 1) or 0.15 mg (for formulation 2) of benzalkonium chloride. The slurry was autoclaved at 121°C ± 10°C for 15 minutes to obtain the betamethasone acetate phase. Further, the betamethasone sodium phosphate phase was prepared by mixing 3.95 mg of betamethasone sodium phosphate and water for injection and sterilized by filtration through a 0.2-micron filter. The betamethasone acetate phase and betamethasone sodium phosphate phase were mixed aseptically to obtain the final injectable suspension.

Three-phase process (BA Phase, BSP Phase & Buffer Phase)

Method 2: BA sterilized by DHS method: 3 mg of betamethasone acetate was taken and sterilized by the dry heat sterilization method at 165 °C ± 10 °C for 3 hours. Sterilized betamethasone acetate was taken, and slurry was prepared by mixing betamethasone acetate with 0.2 mg (for formulation 1) or 0.15 mg (for formulation 2) of benzalkonium chloride and water for injection. The slurry was heated at 80°C to obtain the betamethasone acetate phase. Furthermore, the betamethasone sodium phosphate phase was prepared by mixing 3.95 mg of betamethasone sodium phosphate with water for injection and sterilized by filtration through a 0.2 µm filter. The buffer phase was also prepared by mixing 8.9 mg (for formulation 1) or 7.5 mg (for formulation 2) of dibasic sodium phosphate dihydrate buffer, 3.8 mg (for formulation 1) or 5.2 mg (for formulation 2) of monobasic sodium phosphate dihydrate buffer, and 0.1 mg (for formulation 1) or 0.15 mg (for formulation 2) of EDTA with water for injection and sterilized by filtration through 0.2 micron filter. The betamethasone acetate phase, betamethasone sodium phosphate phase, and buffer phase were mixed aseptically to obtain the final injectable suspension.

Method 4: BA sterilized by the autoclave method: Non-sterile 3 mg of betamethasone acetate was used. A slurry of betamethasone acetate was prepared by mixing 3 mg of betamethasone acetate with 0.2 mg (for formulation 1) or 0.15 mg (for formulation 2) of benzalkonium chloride and water for injection. The slurry was autoclaved at 121°C ± 10 °C for 15 minutes to obtain the betamethasone acetate phase. Furthermore, the betamethasone sodium phosphate phase was prepared by mixing 3.95 mg of betamethasone sodium phosphate with water for injection and sterilized by filtration through a 0.2 µm filter. The buffer phase was prepared by mixing 8.9 mg (for formulation 1) or 7.5 mg (for formulation 2) of dibasic sodium phosphate dihydrate buffer, 3.8 mg (for formulation 1) or 5.2 mg (for formulation 2) of monobasic sodium phosphate dihydrate buffer, and 0.1 mg (for formulation 1) or 0.15 mg (for formulation 2) of EDTA with water for injection & sterilized by filtration through 0.2 micron filter. The BA phase, BSP phase, & buffer phase were mixed aseptically to obtain the final injectable suspension.

Stability of prepared BA & BSP injectable suspension

The composition in trials was prepared using methods and formulations (as listed in Table 3) and subjected to a stability study. Compositions were stored in stability testing chambers at 25 °C and 40% RH for 3 months, and at 40°C and 75% RH for 3 months. The stability of compositions was measured in terms of free betamethasone (BA) and total impurity at the initial time and after 3 months. The method used for preparing the formulation, its composition, and the stability of the trial under different conditions is summarized in Table 2. The stability of BA and BSP injectable suspensions was determined using the HPLC method.

Table 1: Composition of Injectable Suspension of BA And BSP

Name of Pharmaceutical Ingredient	Role	Formulation 1	Formulation 2
Betamethasone Sodium Phosphate	API	3.95 mg	3.95 mg
Betamethasone Acetate	API	3 mg (D90: 15-20µ)	3 mg (D90: 15-20µ)
Dibasic sodium phosphate dihydrate	Buffer	8.9 mg	7.5mg
Monobasic sodium phosphate dihydrate	Buffer	3.8 mg	5.2mg
Edetate disodium dihydrate	Chelating Agent	0.1 mg	0.15mg
Benzalkonium Chloride	Preservative	0.2 mg	0.15mg
Water for Injection	Solvent	q.s. to 1 ml	q.s. to 1 ml

Table 2: BA & BSP injectable suspension & its Stability analysis

Column	Silversil (150 x 4.6) mm, 5µm		
Column Oven Temp.	45°C		
Sample Temperature	10°C		
Flow Rate	1.5 mL/min		
Detector Wavelength	254 nm		
Seal wash	Water: ACN (90:10 %V/V)		
Needle wash	Water: ACN (10:90 %V/V)		
Injection volume	20 µL		
Run time	30minutes		
Mobile Phase	Mobile Phase A: 2.7g Potassium Dihydrogen Phosphate in 1L water		
	Mobile Phase B: Acetonitrile and Tetrahydrofuran (4:1)		
Mode	Gradient		
Gradient Program	**Time (min)**	**Mobile Phase A(%)**	**Mobile Phase B(%)**
	00	90	10
	15	55	45
	18	30	70
	23	30	70
	25	90	10
	30	90	10

RESULTS AND DISCUSSION

Drug-Excipient Compatibility Study & Phase Selection

Betamethasone sodium phosphate (BSP) and betamethasone acetate (BA) were mixed with different excipients in different combinations for the study of drug-excipient interactions (as given in Table 2). Prepared samples were stored under various conditions at 25°C for 15 days and at 60°C for 15 days, and the total impurity was determined. Further, the samples were heated at 121°C for 30 minutes, and total impurity was determined. The details of the experiment are presented in Table 3. Two injectable suspension formulations were prepared to investigate the effect of the order of excipient addition and sterilization method on the injectable suspension of BA and BSP. The impurity and stability data at various conditions are presented in Table 3. Based on the results from the drug-excipient compatibility study, it is clear that the order of addition of the excipient and the combination of drug-excipient phase selection play an essential role in the stability of the prepared suspension. Results, as presented in Table 2, clearly indicate that BA and BSP should not be in the same mixture (phase) because when BA and BSP are combined in the same phase, the total impurities increase by more than 12% under autoclave conditions at 121°C for 30 min. Similarly, BSP + BKC + WFI, BSP + DSP + WFI, BSP + MSP + WFI, and BSP + EDTA + WFI mixtures were used in the same phase; the total impurities after autoclaving at 121°C for 30 minutes increased to 19.99%, 64.31%, 64.76%, and 68.31%, respectively

Stability of Prepared Injectable Suspension

Several trials (8 batches) are prepared using two different formulations and two different sterilization methods. The said trials (batches) are kept in stability for 0 day and 90 days at 25°C/40% RH & 40°C/75% RH; data are given in Table 3. Free betamethasone & total impurities of the trial 1 at 25°C/40% RH are 2.10 and 2.40, respectively, & at 40°C/75% RH are 2.91 and 3.92, respectively. Free betamethasone & total Impurities of the trial 3 at 25°C/40% RH are 2.03 and 2.26, respectively, and at 40°C/75% RH are 2.68 and 3.52, respectively, as per the results presented in Table 4.

Release Profile of Prepared Composition of BA & BSP Suspension

For all dissolution experiments, the "USP apparatus IV" (open loop) dissolution apparatus was used. A dissolution medium of STF (Shear Thickening Fluid) buffer pH 7.4 was chosen. The flow rate of 6.0 ml per minute for 120 minutes was selected. The medium, which was vacuum degassed under a degasser, was maintained at 37 ± 0.5°C. Samples were drawn at 1, 3, 5, 7, 10, 15, 20, 30, 45, 60, 90, and 120 minutes, and the release of the reference drug (RLD) and prepared samples (Trials 1-8) weres determined. Statistical methods are used for comparison of dissolution profiles using two factors, f1 and f2. The factor f2, known as the similarity factor, measures the closeness between the two profiles using the following formula:

$$f2 = 50 \cdot \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\}$$

Where n is the number of time points, Rt and Tt are the dissolution values of the reference and test product at time t, respectively.

Several FDA and EMEA guidances adopt the f2 comparison as a criterion for estimating the similarity between in vitro dissolution profiles. When the two profiles are identical, f2 = 100, and an average variation of 10% at all determined time points contributes to an f2 value of 50. The FDA and EMEA have established a public standard for the f2 value, between 50 and 100, to ensure the sameness of the two dissolution profiles

[21]. The drug release profile results of the prepared formulations (Trials 1-8) were compared with the release profile of the RLD (Celestone Soluspan®), calculated as per the above formula. As evident from Table 5, Trials 1 to 4 show better release profile as compared to Trials 5 to 8. Further, Trial 3 shows a more similar drug release profile as compared to RLD, which is clearly evident from the f2 value (95). The drug release profile results of the prepared formulations (Trials 1-8) were compared with the release profile of the RLD (Celestone Soluspan®) calculation as per the above formula. As evident from Table 5, Trials 1 to 4 show better release profile as compared to Trials 5 to 8. Further, Trial 3 shows more similar drug release profile as compared to RLD which is clearly evident from the f2 value (95).

Table 3: Drug-Excipient Compatibility Study & Their Stability

Batch No.	Composition	Drug: Excipient Ratio	Total Impurities (Initial at 25°C)	Total Impurities (25°C for 15days)	Total Impurities (60°C for 15 days)	Total Impurities (Autoclave at 121°C for 30min.)
1	BA	01:00	0.18	0.21	0.06	0.04
2	BA + WFI	01:00	0.34	0.47	0.72	0.23
3	BA + BSP+ WFI	01:01	0.82	0.99	5.2	12.95
4	BA + BKC+ WFI	01:05	0.56	0.63	0.78	0.89
5	BA+ DSP + WFI	01:05	0.2	0.39	0.38	1.33
6	BA+MSP + WFI	01:05	0.51	1.92	0.52	1.51
7	BA+ EDTA + WFI	01:05	5.95	7.31	7.4	9.1
8	BSP	01:00	0.57	0.62	3.71	16.32
9	BSP + WFI	01:00	0.77	0.99	3.63	13.75
10	BSP + BA+ WFI	01:01	0.98	3.17	3.27	14.14
11	BSP + BKC+ WFI	01:05	3.11	3.6	8.71	19.99
12	BSP+DSP + WFI	01:05	1.39	1.0	21.82	64.31
13	BSP+MSP + WFI	01:05	1.27	1.5	22.81	64.76
14	BSP+ EDTA + WFI	01:05	4.2	4.37	22.7	68.31

BA-Betamethasone Acetate; BSP- Betamethasone Sodium phosphate; WFI- Water for injection, BKC- Benzalkonium chloride; DSP- Dibasic sodium phosphate dihydrate; MSP- Monobasic sodium phosphate dihydrate; EDTA- Edetate disodium dihydrate; API: Active Pharmaceutical Ingredient

Table 4: Stability data of different batches (Trials) using different formulations and methods.

Trial no.	Method No.	Formulation No.	25°C/40% RH & 40°C /75% RH(0 days)		25°C/40% RH(90 days)		40°C /75% RH(90 days)	
			FB	TI	FB	TI	FB	TI
1	1	1	0.14	0.47	2.10	2.40	2.91	3.92
2	3	1	0.29	0.72	2.80	2.90	3.10	4.49
3	2	1	0.06	0.29	2.03	2.26	2.68	3.52
4	4	1	0.23	0.64	2.60	2.80	2.95	4.40
5	1	2	1.30	1.60	2.90	3.40	3.50	4.79
6	3	2	1.50	1.80	3.10	3.70	3.80	4.99
7	2	2	1.20	1.70	2.94	3.60	3.72	4.92
8	4	2	1.60	1.90	3.30	3.80	3.99	5.10

FB= Free betamethasone, TI= Total Impurities

DISCUSSION

The injectable composition of betamethasone acetate (insoluble) and betamethasone sodium phosphate (soluble) is developed to provide the desired long-term action of the drug. Betamethasone sodium phosphate provides an immediate therapeutic effect due to its higher solubility, whereas betamethasone acetate has a

longer half-life, which provides a sustained therapeutic effect. Injectable suspension of betamethasone acetate and betamethasone sodium phosphate was sterilized by two methods, i.e. the DHS method and the autoclave method. Alone, sterilization of betamethasone acetate helps minimize the total impurity, while betamethasone sterilized in the slurry or in the mixture with excipient tends to increase the total impurities. Available literature teaches sterilisation of wet mass of API & excipients for use in the final composition [22]. Literature also teaches the combined sterilization of betamethasone acetate, betamethasone sodium phosphate, and excipients [23]. In the current study, sterilization of betamethasone acetate is done separately using the DHS or autoclave method, and sterilization of betamethasone sodium phosphate is done using sterile filtration. Sterilization of betamethasone acetate plays an important role and is majorly responsible for impurity formation, especially free betamethasone formation, which reduces the therapeutic effect. As per the stability results shown in Table 3 & 4, the total impurities are minimised in the DHS method as compared to the autoclave method.

Table 5: Release profile of the injectable suspension composition

Time/Trial	**RLD**	**Trial no.1**	**Trial no.2**	**Trial no.3**	**Trial no.4**	**Trial no.5**	**Trial no.6**	**Trial no.7**	**Trial no.8**
Time(Min)	Release %	Release %	Release %	Release %	Release %	Release %	Release %	Release %	Release %
1	3	4	1	3	2	0	1	0	1
3	11	13	7	11	8	2	5	3	5
5	20	23	14	19	16	10	6	7	11
7	29	32	20	28	24	17	12	14	18
10	42	45	32	41	36	26	21	23	28
15	57	62	48	57	49	42	35	39	44
20	68	73	61	68	65	54	45	49	57
30	82	88	70	83	76	62	54	57	68
45	93	97	79	94	83	70	63	66	74
60	97	101	85	98	89	78	70	72	81
90	100	103	91	101	92	83	78	80	87
120	101	104	95	101	97	89	85	87	92
f2 value(%)	100	70.6	52.2	95	60.6	41	34.2	36.7	45.6

HPLC was performed to assess any compatibility between the drug and the excipients. The data obtained from the study (presented in Table 3) suggest that there was an increase in formulation impurities, including free betamethasone and other impurities, when BSP was directly added with other excipients such as phosphate buffers, BKC, and EDTA. There is also an increase in the impurity level when BSP is directly mixed with the BA & water for injection. The reason for the increase in impurity levels appears to be the chemical interaction between BSP and BA, as well as with excipients. The chemical interaction leads to the displacement of sodium ions from the BSP in aqueous suspension, thereby increasing the amount of impurities in the composition. Furthermore, it is also depicted that when BA was directly mixed with EDTA, it led to an increase in impurities. When BA was mixed with the buffers and further mixed with other excipients, it resulted in the generation of a very small amount of impurities and yielded a stable suspension composition. The mixing of BA with buffer prevents the partial dissociation/hydrolysis of BA due to the maintenance of pH during the formulation process and hence leads to the generation of a very small amount of impurities. Based on the drug-excipients compatibility study, two injectable suspension compositions (Formulations 1 & 2, given in Table 1) were developed that were different w.r.t. excipients quantitatively. The stability and release profile of the BA & BSP injectable suspension are dependent on various process parameters, such as the type of sterilization method used and the order of addition of excipients. To study the effect of process parameters on stability and release profile, we have developed four methods (Methods 1 to 4). Methods are different in terms of:

- Sterilization method
- Order of sterilization

- Order of addition of excipients
- Method of preparation of composition (Two-phase or Three-phase)

Available literature describes the process of preparing betamethasone acetate/betamethasone sodium phosphate suspension by the single-phase method, wherein excipients are added one by one in a single container until the final formulation is formed [20]. We have developed eight different compositions (Trial Nos. 1-8, Table 4) using two different quantitative compositions, two different sterilization methods, and two different methods of adding phases (Table 4). Trials no. 3, 4, 7, and 8 are prepared by the three-phase method, whereas trials no. 1, 2, 5, & 6 are ready by the two-phase method. The prepared formulations are subjected to stability studies for up to 3 months. The results of the stability study are given in Table 3.

The stability is measured based on impurity levels, which include free betamethasone and total impurities. Trials no. 5 to 8 show higher impurity levels after 3 months of stability at 40°C/75% RH (4.79, 4.99, 4.92 & 5.10, respectively, % area by HPLC) when compared with trials no. 1 to 4, which show lower total impurity levels (3.92, 4.49, 3.52 & 4.40, respectively, % area by HPLC). This is due to the amount of BKC in the composition. Trials 5 to 8 have a lower amount of BKC (0.15 mg) as compared to trials 1 to 4 (0.2 mg). The dry heat sterilization (DHS) method was used for the preparation of trials no. 1 and 3, which have lower total impurity levels after 3 months at 40°C/75% RH (3.92 & 3.52% area by HPLC, respectively) in comparison with trials no. 2 and 4 (4.49 & 4.40% area by HPLC), which are sterilized by the autoclave method. According to the study, the composition sterilized by the autoclave sterilization method contained more impurities than the composition sterilized by the DHS method. Data as shown in Table 4. Therefore, it is clearly indicated that the three-phase process exhibits better stability compared to the stability of the composition prepared using the two-phase process. This is because the three-step process minimizes the direct contact of drugs, i.e., BA & BSP, with excipients like EDTA & buffers. The prepared formulations are subjected to various studies to assess their suspension properties, including particle size, redispersibility, and content uniformity. The study depicts that the reproduced formulation of Trials 1 and 3 shows better suspension properties. In vitro, release studies are essential for ensuring the long-duration action performance. The reproducibility of the rate and duration of drug release was carried out in STF buffer at pH 7.4 at 37 ± 0.5°C. From the *in vitro* drug release study, it was revealed that the composition of trials 1 and 3 exhibited the best release profile when compared with other compositions. Trials no. 1 and 3 release at least 50% of drugs after 15 minutes, at least 90% of medicines are released after 45 minutes, and approximately all drugs are released after 90 minutes (Table 5 & Figure 1). A prior published reference shows a slower release of betamethasone (100% release in 9 hours) from the formulation compared to the RLD, as observed in the current study (100% release in 90 minutes), which is undesirable according to the US FDA's bioequivalence requirements [20]. The f2 value is a measurement of the similarity between the dissolution profiles of two accurate profiles (test and RLD). The similarity factor (f2) has been calculated for each batch. The f2 value of Trial No. 3 is 95%, and the f2 value of Trial No. 1 is 70.6%, which are higher than those of other Trials. The value indicates that the composition of Trial No. 3 shows more similarity than that of Trial No. 1, as shown in Figure 2 (Celestone Soluspan).

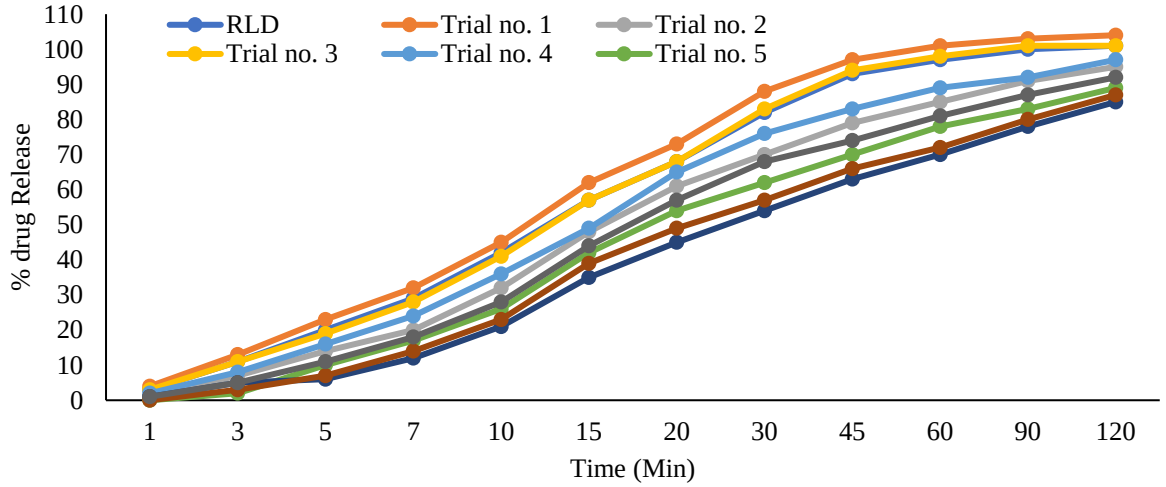

Figure 1: % Overlay of Drug Release Profile of Trial 1 to 8 and RLD

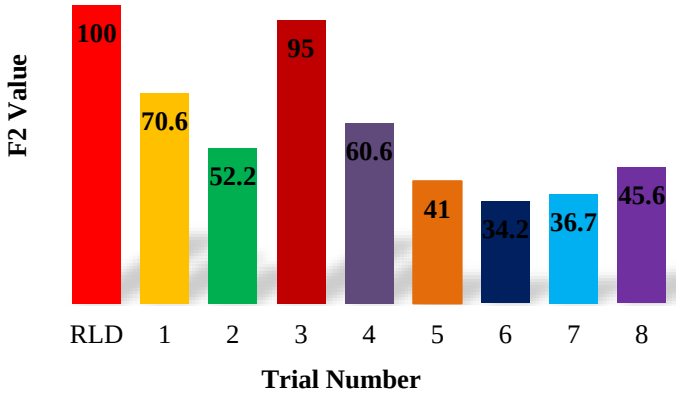

Figure 2: F2 Value of RLD & Trials 1 to 8

CONCLUSION

Betamethasone acetate and betamethasone sodium phosphate injectable suspensions were successfully developed using a two-phase or three-phase method, followed by sterilization via the DHS and autoclave methods. Separate sterilization of betamethasone acetate using the DHS method minimizes impurity formation, a notable finding of the present study. The DHS sterilization method was found to be superior to the autoclave sterilization method in terms of composition development, particularly in terms of stability and impurity formation. Furthermore, the three-phase development method was found to be superior to the two-phase development process because it minimizes the interaction of BSP with other excipients. The development of two-phase and three-phase methods is a key feature of the present study. The *in vitro* drug release (Trial 1 & 3) from the BA & BSP injectable suspension through the dissolution apparatus shows synchronous release of the BA & BSP suspension after 15 minutes (at least 50%) with sustained release after 60 minutes (atleast 90%), and cumulative drug release of BA & BSP suspension after 90 minutes was found to be atleast 99%. Furthermore, the drug release profile of the developed composition (Trial 3) is similar to that of the reference drug formulation (RLD). The betamethasone acetate and betamethasone sodium phosphate are helpful in various indications. Due to the complexity of the development of this product, the present study provides insight into the various critical process attributes (order of addition of excipients, sterilisation methods, and drug excipient compatibility studies) that need to be considered for the development of stable extended release injectable compositions, which in turn will be helpful for future development. The robust process developed in the present study will ease the burden on the pharmaceutical scientists and, in turn, on the patients with respect to the availability of affordable medicines.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Amit Bansal, Satish Sardana, and Tarun Wadhwa were involved in study design, data analysis, and manuscript drafting. Amit Bansal was engaged in experimental work & data collection. Satish Sardana was involved in manuscript correspondence with the editor. All authors read and approved the final manuscript, confirming agreement with the content and conclusions presented.

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