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DEVELOPMENT & VALIDATION OF AN *IN VITRO* DISSOLUTION METHOD WITH HPLC ANALYSIS FOR BETAMETHASONE ACETATE AND BETAMETHASONE SODIUM PHOSPHATE IN FORMULATED DOSAGE FORM

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ABSTRACT

The intended purpose of this work is to develop and validate a dissolution test for betamethasone acetate and betamethasone sodium phosphate injectable suspension using a reverse-phase liquid chromatographic method. After testing sink conditions, dissolution medium and stability of the drug, the best conditions were: flow through cell of diameter 22.6 mm, 2.0 ml per minutes flow rate for 120 minutes, 500mL of sodium dihydrogen ortho phosphate buffer pH 7.4 with 2.5 % of sodium lauryl sulphate as dissolution medium, using a USP Apparatus IV (A flow through cell dissolution apparatus with seven cells dissolution tester Sotax CE7). The method was validated to meet requirements for a global regulatory filing and this validation included specificity, precision, linearity and accuracy. Release of more than 85% of the label amount was achieved over 60 min in the medium through out the study. The dissolution test developed was adequate for its purpose and could be applied for quality control of betamethasone acetate and betamethasone sodium phosphate formulation dosage form.

INTRODUCTION: Dissolution test has emerged in the pharmaceutical field as a very important tool to characterize drug product performance [1]. It provides measurements of the bioavailability of a drug as well as demonstrating bioequivalence from batch-to-batch. Besides, dissolution is a requirement for regulatory approval for product marketing and is a vital component of the overall quality control program [2-4]. Betamethasone sodium phosphate (**Fig. 1**) and Betamethasone acetate (**Fig. 2**) are corticosteroids and are chemically known as Pregna-1, 4-diene-3, 20-dione, 9-fluoro-11, 17-dihydroxy-16-methyl-21-(phosphonooxy)-disodium salt, (11β, 16β)-.9-Fluoro-11β, 17, 21-trihydroxy-16 β -methylpregna-1, 4-diene-3, 20-dione 21-(disodium phosphate) [151-73-5] & Pregna-1, 4-diene-3, 20-dione, 9-fluoro-11, 17-dihydroxy- 16- methyl- 21- (acetyloxy)-, (11β, 16β)-.9-Fluoro-11β, 17, 21-trihydroxy-16β- methylpregna-1, 4-diene-3, 20-dione 21-acetate [987-24-6].

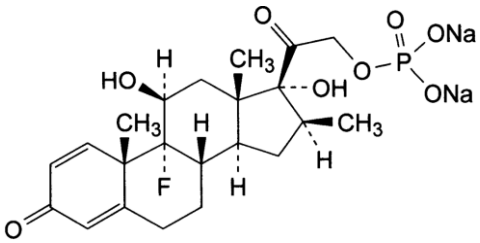

FIG. 2: CHEMICAL STRUCTURE OF BETAMETHASONE SODIUM PHOSPHATE

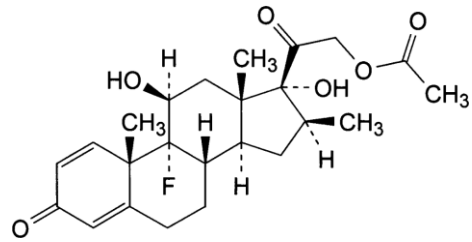

FIG. 2: CHEMICAL STRUCTURE OF BETAMETHASONE ACETATE

Betamethasone Phosphate and Betamethasone Acetate are prodrugs used to treat corticosteroid-responsive disorders [5]. As a combination product they achieve their pharmacological effects through the immediate and slow release of Betamethasone [6-7].

Literature search revealed that as such there is a lack of method by which *in vitro* dissolution rate can be accurately quantified [8]. Although there are methods available for determination of betamethasone in human plasma by tandem mass spectrometry [9], assay determination by HPLC [10], with different combinations [11], also the data of stabilizations are available [12].

This study, describes the development of a fast, accurate and precise HPLC method with isocratic elution for determination of betamethasone in pharmaceutical formulations and in dissolution media for drug quality control purposes. The dissolution method was also developed and validated according to USP guidelines [13].

Experimental:

Materials & reagents: All experiments were performed using ‘A class’ volumetric glassware, and an in-house standard of pharmaceutical grade betamethasone acetate and betamethasone sodium phosphate. Analytical reagent grade monobasic potassium phosphate (Spectrochem, India), HPLC grade methanol (Spectrochem, India) and highly pure HPLC grade Milli-Q water (Millipore, Bedford, MA, USA) were used in mobile phase preparation.

Sodium dihydrogen ortho phosphate buffer pH 7.4 with 2.5 % of sodium lauryl sulphate was used as a dissolution medium. The mobile phase was filtered through a 0.45 µm membrane filter (Millipore, Barcelona) and degassed under vacuum by filtering assembly, prior to use. The pharmaceutical preparation, declaring to contain betamethasone acetate and betamethasone sodium phosphate was obtained from Strides Arcolab, Bangalore, India for analysis.

Dissolution (instrumentation & conditions): For all dissolution experiments, USP Apparatus IV (A flow through cell dissolution apparatus with seven cells dissolution tester Sotax CE7) dissolution apparatus was used, while in the preparation of the mobile phase and sample & standard aliquots, analytical balance (Sartorius, CP225D) was used.

Dissolution testing was performed in compliance with USP {711} using apparatus 4 with flow through cell. A dissolution medium of sodium dihydrogen ortho phosphate buffer pH 7.4 with 2.5 % of sodium lauryl sulphate was chosen based on the solubility profiles obtained and its benefits compared to others. Flow rate of 2.0 ml per minutes for 120 minutes was selected with media volume of 500 mL. The medium, which was vacuum degassed under degasser (Electrolab), was maintained at 37 ± 0.5°C. Samples were drawn at 5, 10, 30,45,60,90,120 mins for early validation work.

Chromatographic method: An HPLC method with UV detection was selected for the method of analysis. The reversed-phase procedure utilized a Waters Symmetry, C18, 150mm x 4.6 mm, 5µ and UV detection at 254 nm. This wavelength was selected because it is a UV maximum and provides enough sensitivity needed for quantitation of this drug concentration in the dissolution samples. The column temperature was maintained at 35°C.

The mobile phase contained methanol and $10.2 gl^{-1}$ solution of potassium dihydrogen phosphate buffer (700 : 500 v/v, respectively). The flow rate was 1.2 mL min^{-1} for 12 min with an injection volume of 20 µL. A standard solution of active pharmaceutical ingredient (API) was prepared first in mobile phase, and subsequently diluted down to the appropriate concentration with dissolution medium.

RESULTS & DISCUSSION:

Chromatographic Method Development: Drug solubility and solution stability are important properties to be considered when selecting the dissolution medium [14]. In this study, the first approach was to compare buffers of different pH commonly used for solid dosage forms. As per the concentration of betamethasone acetate and betamethasone sodium phosphate into the formulation, sink conditions in water demonstrated the suitability of the dissolution medium and also the compatibility with dissolution medium was checked.

The results obtained in the preliminary studies were satisfactory. After setting dissolution parameters, the chromatographic parameters were optimized. For some of the parameters the reference of related substances method from betamethasone API in United States Pharmacopoeia [15] was considered during development.

During development the major problem observed is of the peak response and the peak shape. There were different trials taken for optimization of the HPLC column, the Waters µ Bonda pack column is selected because of its larger surface area (220 $m^2 g^{-1}$) and medium carbon loading (6.2%), and as a result a good peak shape with tailing of about 1.2–1.3 was achieved, which was well within the acceptance criteria of <1.5 as per various pharmacopoeia. The concentration of sample solution was such as 1.0mg mL^{-1}.

During spectrum scanning the wavelength where maxima observed was 254nm which is a magnifying wavelength, even though the desired response was not achieved, to overcome this difficulty the injection volume is increased. Initially a few trials are taken with different injection volumes *e.g.* 5µL *etc.* which has not satisfied the requirement of method by mean of peak response, and considering the probable very low concentration of initial time points during performance of dissolution profile the injection volume optimized is 10 µL, wavelength is 254 nm.

After optimizing all the parameters, the method was checked for quality control purpose successfully.

Evaluation of Validation Data:

Specificity: The aim of the Specificity study is to assess unequivocally analyte in presence of components that may be expected to be present. The specificity of the method was checked for diluent interference as well as all the other excipients interference. Diluent and subsequently placebo mixture (in triplicate) were injected to check any interference. Purity factor of analyte peak is found greater than purity threshold and no peak due to placebo was detected at retention time of analyte peak. The study proves that test method is specific for quantification of dissolution of analyte without interference of any other excipients.

Precisions:

Instrument Precision (suitability of system): System suitability shall be checked for the conformance of suitability & reproducibility of chromatographic system for analysis. Systems suitability was checked by injecting six replicate injections of standard solution. For conformance of suitability, % RSD for standard peak shall not be more than 2.0. The results revealed that the % RSD was 1.0% and which proved the suitability of system (**fig. 3**).

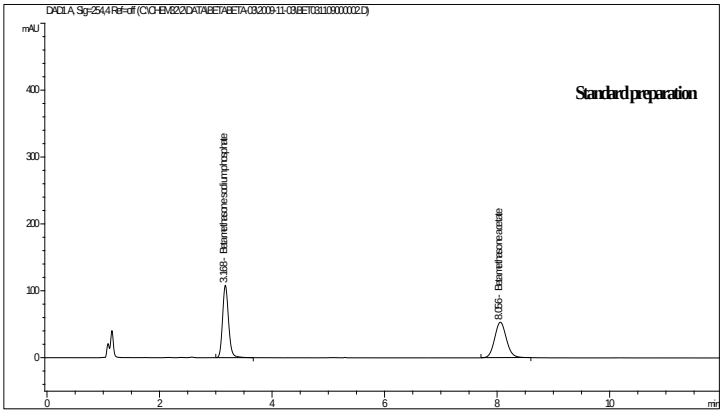

FIG. 3: STANDARD CHROMATOGRAM

Method Precision: The purpose of this experiment is to prove the repeatability of the results obtained by this quantification methodology. To conform the repeatability six sets of sample solution were injected and % RSD of the results was observed. To comply this parameter the value of % RSD shall not exceed 6.0% the resulting RSD was within the acceptance limit and showed the method is precise.

TABLE 1: METHOD PRECISION DATA

Set	Betamethasone acetate	Betamethasone sodium phosphate
1	104.1	91.6
2	107.0	89.2
3	104.7	94.3
4	106.7	96.0
5	107.1	93.6
6	99.2	95.2
Mean	**104.8**	**93.3**
RSD	**2.9%**	**2.7%**

Intermediate Precision (Ruggedness): To demonstrate reliability of results obtained by the dissolution test method with day to day, analyst to analyst, system to

system and column to column variability intermediate precision of dissolution test method was demonstrated by conducting method precision done by different analyst on different chromatographic system using different column (from same make of column but with different serial number), different dissolution system and on different day.

For acceptance of results, % RSD of individual analyst shall not exceed 6.0% and difference between two analysis shall not be more than 5.0%. The experiment yielded the % RSD of 3.2% and 2.5% and the difference of %RSD was 2% which shown that the method is rugged.

Linearity of Detector Response: Linearity of the detector response of the dissolution test method was demonstrated in the range of 20.0% to 150.0% (20.0%, 50.0%, 75.0%, 100.0%, 125.0% and 150.0%) of target concentration of analyte. Prepared solutions were injected in duplicate and linearity graphs of concentration in ppm (*X*-axis) *versus* average area (*Y*-axis) were plotted.

Correlation coefficient, square of correlation coefficient, slope of regression, relative standard deviation of response factor, *Y*-intercept and *Y*-intercept bias at 100.0% linearity level were calculated for analyte peak. The correlation coefficient was found to be 0.9999 for Betamethasone acetate and 1.0000 for Betamethasone sodium phosphate which was far better than the acceptance criteria of 0.9900.

The *Y*-intercept and *Y*-intercept bias were calculated and *Y*-intercept bias was found lying between 0.5 and the acceptance limit was set at ±5.0%. These results showed the method linear for the given wide range of concentrations (**Table 2, fig. 4 & 5**).

TABLE 2: LINEARITY DATA

Linearity level	Concentration (ppm) of betamethasone acetate	Concentration (ppm) of betamethasone sodium phosphate
20.0%	1.0052	0.0375
50.0%	10.0517	0.1800
75.0%	25.1292	0.3600
100.0%	100.5168	0.9599
125.0%	251.2920	1.3499
150.0%	351.8088	3.5997
Correlation coefficient (*r*)	**0.9990**	**1.0000**
Square of correlation coefficient (r^2)	**0.9970**	**0.9990**
Slope of regression	**17.021**	**12.709**
RSD of response factor	**1.0%**	**1.0%**
***Y*-intercept**	**0.073**	**0.168**

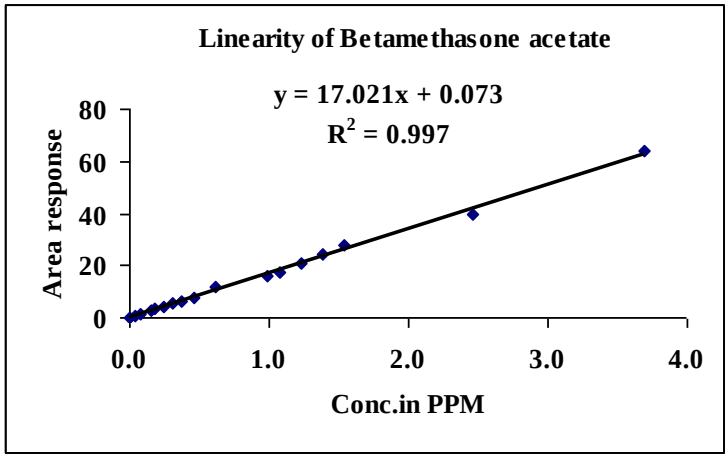

FIG. 4: LINEARITY CURVE OF BETAMETHASONE ACETATE

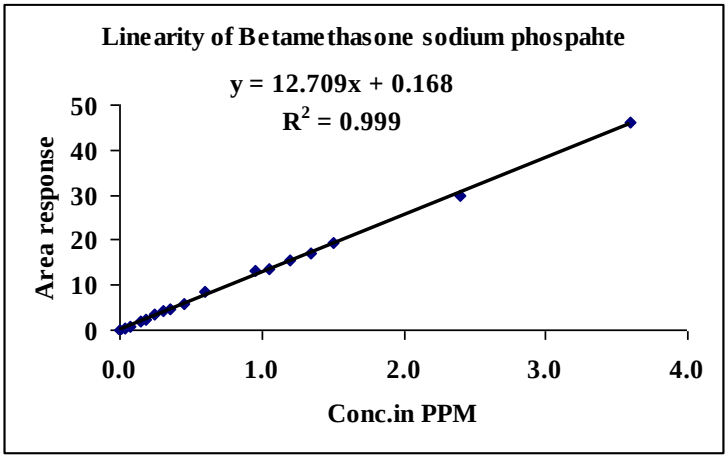

FIG. 5: LINEARITY CURVE OF BETAMETHASONE SODIUM PHOSPHATE

Accuracy (by recovery): Accuracy of test method was performed in the range of 50.0% to 300.0% (50.0%, 100.0%, 200.0% and 300.0%) of target concentration of analyte. Triplicate sets of samples at each concentration were prepared and injected by single injection into the liquid chromatography system and chromatograms were recorded. The two acceptance criteria were placed for conformance of accuracy, the % RSD of all the sets shall not exceed 5.0% and the % recovery shall be within 95.0% to 105.0% for all the sets (**Table 3**).

TABLE 3: ACCURACY DATA OF BETAMETHASONE ACETATE

Accuracy level	Set no.	Theoretical concentration of analyte (mg)	Practical concentration of analyte(mg)	Recovery (%)	Average recovery (%)	RSD
50.0%	Set 1	0.0298	0.0285	95.6	95.3	1.6
	Set 2	0.0298	0.0279	93.6		
	Set 3	0.0298	0.0288	96.6		
100.0%	Set 1	0.0595	0.0614	103.2	101.9	1.5
	Set 2	0.0595	0.0596	102.5		
	Set 3	0.0595	0.0609	102.4		
200.0%	Set 1	0.1190	0.1136	95.5	97.9	2.2
	Set 2	0.1190	0.1173	98.6		
	Set 3	0.1190	0.1185	99.6		
300.0%	Set 1	0.1785	0.1794	100.5	99.6	1.1
	Set 2	0.1785	0.1754	98.3		
	Set 3	0.1785	0.1784	99.9		

TABLE 4: ACCURACY DATA OF BETAMETHASONE SODIUM PHOSPHATE

Accuracy level	Set no.	Theoretical concentration of analyte (mg)	Practical concentration of analyte (mg)	Recovery (%)	Average recovery (%)	RSD
50.0%	Set 1	0.0298	0.0289	97.0	97.6	0.5
	Set 2	0.0298	0.0291	97.7		
	Set 3	0.0298	0.0292	98.0		
100.0%	Set 1	0.0596	0.0604	101.3	102.5	1.6
	Set 2	0.0596	0.0607	101.8		
	Set 3	0.0596	0.0622	104.4		
200.0%	Set 1	0.1192	0.1191	99.9	100.3	0.8
	Set 2	0.1192	0.1188	99.7		
	Set 3	0.1192	0.1206	101.2		
300.0%	Set 1	0.1788	0.1881	105.2	104.5	1.1
	Set 2	0.1788	0.1878	105.0		
	Set 3	0.1788	0.1846	103.2		

CONCLUSION: The possibility to obtain with a dissolution test reliable results on the pharmaceutical to be tested is essential to ensure the quality, safety and efficacy of the developed drug product. A dissolution method with HPLC analysis for Betamethasone acetate and Betamethasone sodium phosphate in injectable suspension has been fully validated to meet global regulatory requirements. The methodology was evaluated for specificity, linearity, precision and accuracy in order to establish the suitability of the analytical method. The conditions that allowed the dissolution profile determination were flow through cell (USP apparatus 4) and 2.0 ml flow rate. The method was demonstrated to be adequate for quality control of betamethasone acetate and betamethasone sodium phosphate dosage form.

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