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Analytical Method Development and Validation of Prednisolone Sodium Phosphate by QbD Approach

Omprakash G. Bhusnure*, Gholve S.B., Bawage Manoj, Vinod Todkar, Padmaja S Giram,
Department of Quality Assurance, Channabasweshwar Pharmacy College, Maharashtra, India- 413512

Abstract: *According to ICH Q8 (R2) guidelines, an experimental work was planned for both spectroscopic and chromatographic method development and its validation. QbD approach was implemented for spectroscopic method development and its validation but chromatographic method development and validation was performed by conventional method. The research work demonstrated that the UV is valid for the determination of assay of Prednisolone sodium phosphate. It describes the materials and methods used in experimental work. For performing experimental work analytical grade chemicals and HPLC grade chemicals (methanol, water, ethanol & acetonitrile) was used. The spectrophotometric method development and validated on UV spectrophotometer by using suitable solvent (ethanol, methanol & water) and detection was performed at 246nm.QbD approach was carried out for spectroscopic method development by varying 17 parameters and critical parameters were extracted by using principal component analysis and by observation. For all the variable parameters as stated in Ishikawa diagram, the absorbance was recorded over the concentration range.*

Key words: *Prednisolone sodium phosphate, UV Spectrophotometry, ICH Q8 (R2), Ishikawa Diagram, Critical Parameters, Quality by Design (QbD).*

I. Introduction

Of late, Analytical Quality by Design (AQbD) has been gaining increased acceptance in the industrial, academic and regulatory circles. Considered as a science and risk-based approach, AQbD provides rational understanding of the critical method parameters (CMPs) affecting the critical analytical attributes (CAAs) of an analytical method. Quality by design is an essential part of the modern approach to pharmaceutical quality QbD has become the answer to assist both industry and FDA to move towards a more scientific, risk based, holistic and proactive approach to pharmaceutical development [1]. The first step started with the Process Analytical Technologies guideline, which was followed by the International Conference on Harmonization Q8, Q9, Q10 and Q11 guidelines[2]A Higher level of assurance of product quality with Improved product and process design and understanding Quality risk management in manufacturing, Monitoring, tracking and trending of product and process & Provide opportunities for continual improvement[3] In real time stability testing, the duration of test period should normally be long enough to allow significant product degradation under recommended storage conditions. Alternatively, if the product is essentially stable the test should be conducted for a long enough period to indicate clearly that no measurable degradation occurs.

Accelerated stability testing refers to storage of product under condition that accelerates degradation commonly by an increase in temperature. Stress conditions that generally accelerate changes fall under the general heading of temperature, light, moisture, agitation, gravity, pH, packaging, and method of manufacture. This may permit, in some circumstances the prediction of stability of product at ordinary shelf temperature from data obtained from stress testing.

One of the most important elements in most stability testing of marketed pharmaceutical products is an evaluation of retained stability samples. Stability samples are tested at predetermined intervals. Thus if the product has a five years shelf life, it is conventional to test at 3,6,9,12,18,24,36,48 and 60 months.

The available regulatory guidance provides useful definitions and general comments about degradation studies The International Conference on Harmonization (ICH) guidelines indicates that stress testing is designed to determine the intrinsic stability of the molecule by establishing degradation pathway in order to identify the likely degradation products and to validate the stability indicating power of the analytical procedure used ICH guidelines stability testing of new drug substances and products' Q1A (R2) and (Q1B) requires that stress testing should be carried out to elucidate the substance. It suggests that the degradation products that are formed under the variety of condition should include the effect of temperature, appropriate oxidation, photolysis and susceptibility [4].

The ICH guidelines indicates that stress testing is designed to help, "Determine the intrinsic stability of the molecule by establishing the degradation pathways in order to identify the likely degradation products and to validate the stability indicating power of analytical procedure used".

Acid/Base stress testing is performed to force the degradation of drug substance to its primary degradation products by exposure to acidic and basic conditions over time. Functional groups likely to introduce acid/base hydrolysis are amides (lactams), esters (lactones), carbamates, imides, imines, alcohols (epimerization for chiral centers) and aryl amines[5].

Oxidative studies are executed to force the degradation of drug substances to determine the primary oxidative degradation products. The 1987 Stability Guidelines state that a high oxygen atmosphere should be evaluated in stability studies on solutions or suspensions of the bulk drug substance. Drug substance functional groups that are susceptible to oxidation reactions include heteroatom (nitrogen: N-oxides and sulfur: sulfoxide and sulfones), benzylic sites, and aldehydes and ketones.

The goal of the photo stability studies is to force the degradation of drug substances via UV and fluorescent conditions over time to determine the primary degradation products. UV and visible lights are the most energetic electromagnetic radiation sources to which pharmaceutical drug substances and drug products are typically exposed. A molecule absorbs light when an absorption band exist that overlaps to some extent with the incident light energy and a valence electron in the relevant chromophore is raised to an excited state[6,7,8].

Chemically Prednisolone Sodium Phosphate is glucocorticoid and its IUPAC name is Disodium [2-[(8S,9S,10R,11S,13S,14S,17R)-11,17-dihydroxy-10,13-dimethyl-3-oxo-7,8,9,11, 12,14,15,16-octahydro-6H-cyclopenta[a]phenanthren-17-yl]-2-oxoethyl]- phosphate. It is mainly used for the treatment of a wide range of inflammatory and auto-immune disease such as asthma multiple sclerosis, rheumatoid arthritis, autoimmune hepatitis etc. Prednisolone is also known as 'disease modifying anti-arthritic drugs because of its anti-inflammatory action by inhibiting gene transcription for COX-2, cytokines, cell adhesion molecules, and inducible NO synthetase. It is soluble in water; soluble in methanol, Ethanol, slightly soluble in alcohol and in chloroform; and very slightly soluble in acetone and in dioxane

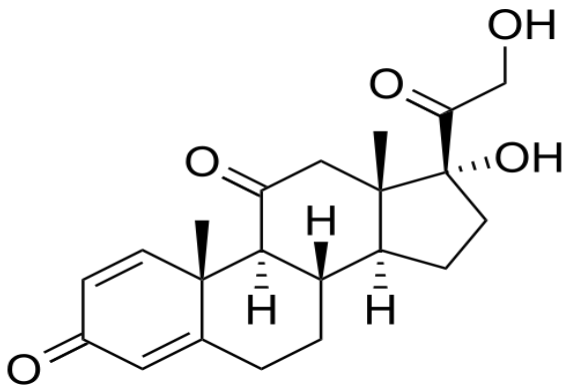

Fig. 1 Prednisolone Sodium Phosphate ($C_{21}H_{27}Na_2O_8P$)

Prednisolone irreversibly binds with glucocorticoid receptors (GR) alpha and beta for which they have a high affinity. Alpha GR and Beta GR are found in virtually all tissues with variable numbers between 3000 and 10000 per cell, depending on the tissue involved. Prednisolone can activate and influence biochemical behavior of most cells. The steroid/receptor complexes dimers and interact with cellular DNA in the nucleus, binding to steroid-response elements and modifying gene transcription. They induce synthesis of some proteins, and inhibit synthesis of others. Not all metabolic actions on genes are known. Most mediator proteins are enzymes, e.g., cAMP-dependent kinase[9].

The present work aims at systematic development of a simple, rapid and highly sensitive HPLC method for the analysis of Prednisolone by QbD approach.

II. Materials And Method

All chemicals used during the project work were either AR or HPLC grade. The various reagents and chemicals used during experimental work are as follows;

Table 1: Reagent and chemicals

Sr No	Name of reagent used	Make
1	Water	HPLC grade
2	Methanol	HPLC grade
3	Acetonitrile	HPLC grade
4	Ammonium acetate buffer	Prepare in HPLC grade
5	Phosphate Buffer	Prepare in HPLC grade

Instruments:

Table 2: List of instruments

Sr No	Name of Equipment	Source
1	HPLC	Agilent 1220 Infinity LC
2	UV	Shimadzo, Model: UV-1800
3	Detector	Variable wavelength detector
4	Electronic weighing balance	Shimadzo BL- 220 H
5	Hot air oven	Nisco company
6	Sonicator	The ultrasonics PCi Analytics sonicator
7	Digital pH Meter	Lab India

Methods

Preliminary solubility study of drug:

Solubility of the drug was determined at 28±1 C. A small quantity of standard drug was dissolved in different solvents like distilled water, ethanol, methanol, acetonitrile, alcohol, chloroform, acetone, dioxane.

Preparation of Stock solution:

Preparation of standard stock solution of Prednisolone Sodium Phosphate:

10 mg of Prednisolone Sodium Phosphate accurately weighted by electronic balance and dissolved in 80ml of double distilled water in 250ml conical flask. Content of flask was kept for stirring on magnetic stirrer for 10 min and transferred in 100ml volumetric flask. Conical flask was rinsed by 20ml of double distilled water and this water was used to make up volume 100ml of volumetric flask to give conc. of 100µg/ml.

Prepration of working standard solution of Prednisolone Sodium Phosphate:

The working solution of Prednisolone sodium phosphate was prepared by further diluting the stock solution. Then pipette out 0.2ml, 0.4ml, 0.6ml , 0.8ml , 1.0ml & 1.2ml of solution and make up to 10ml leads to 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml & 12 µg/ml concentration solution. This solution was estimated by UV spectrophotometer by using H_2O as blank at 246nm.

Fixing of wave length

After selecting the suitable solvent, the fixing of the λmax for the proposed method is very important. This can be done by scanning the drug sample (Prednisolone Sodium Phosphate) solution in H_2O in the range of 400nm-200nm and the most repeated maximum absorbance with linearity and repeatability can be fixed as λmax for the drug. And in the proposed method for Prednisolone sodium phosphate drug shows maximum 246 nm. With more linearity, repeatability (ruggedness) and the λmax was fixed as 246 nm.

Linearity and range:

For linearity study from the working standard at different concentration 2, 4, 6, 8, 10 and 12 µg/ml of drug solution were placed in 6 different 10ml volumetric flask volume was made up to the mark with water. Absorbance was measured at 246nm. Then obtained data were used for the linearity calibration plot.

Accuracy and recovery study:

This study was carried out using the stock solution (100µg/ml). Take three concentrations 2 µg/ml, 6µg/ml, and 12µg/ml. And take six reading of these concentrations. Calculate the % RSD of the concentration. Results within the range of ensure an accurate method as well as indicate non-interference with the excipients of formulation.

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of prednisolone sodium phosphate solution of the drug to pre-analyzed tablet solutions. The resulting solutions were then reanalyzed by proposed methods.

Intra-day precision (repeatability) and inter-day precision study(intermediate precision):

The standard stock solution of prednisolone sodium phosphate was Prepared. Prepare the three concentration of (2, 6, and 12 µg/ml), by using mobile phase water. Take λ_{max} at the intraday and inter day. Calculate the % RSD. Variation of results within the day (intra-day), Variation of result between days (inter day) were analyzed. Intraday precision was analyzing Prednisolone Sodium Phosphate for three times in the same day at 246nm. Inter-day precision was determined by analyzing the drug different day for three days at 246nm. Precision data for Prednisolone Sodium Phosphate at 246nm.

Reproducibility:

Reproducibility is assessed by mean of an inter laboratory trial. The absorbance readings were measured at 246nm at different laboratory using another spectrophotometer and the value obtained were evaluate using t-test to verify their reproducibility data for prednisolone sodium phosphate at 246nm is recorded.

Specificity and selectivity:

Specificity and selectivity of Prednisolone sodium phosphate is as given Table 27.

Limit of Detection & Limit of Quantitation:

The limit of detection and quantification of drug are calculated with the standard deviation and slop.

$$LOD = \frac{3.3\sigma}{S} \quad , \quad LOQ = \frac{10\sigma}{S}$$

Where,

- σ =Standard deviation
- σ S= slope of the calibration curve

Stability of Sample:

Samples prepared for repeatability study were preserved for 24 hour at room temperature and analyzed on the following day to test for short-term stability. The sample of 4µg/ml drug solution was prepared by suitable dilution with diluents and absorbance were taken at 246nm against the blank. The stability of sample was found to be more than 10 hrs.

Acid degradation:

The preparation of 0.01N hydrochloric acid (HCl) was done by diluting 0.085 ml of conc. HCL to 100 ml of distilled water. Etoricoxib was accurately weighted and was transferred to a labeled round bottomed flask. Reflux the sample for 2 hrs. And pipette out 1ml to 10 ml volumetric flask and adjust with mobile phase. . Volume of 20µl was injected into the system for chromatographic analysis and results of all chromatograms were compared to see whether degradation occurred or not. The degradation of drug is not more than 30%.

Base degradation:

The 0.01N Sodium Hydroxide (NaOH) was prepared by dissolving 0.04 gm of sodium hydroxide pellets in 100 ml of distilled water. The solution was standardized with 0.01 N HCl as per Indian Pharmacopoeia (I.P).

Etoricoxib was accurately weighted and was transferred to a labeled round bottomed flask. . Reflux the sample for 2 hrs.And pipette out 1ml to 10 ml volumetric flask and adjust with mobile phase. Volume of 20µl was injected into the system for chromatographic analysis and results of all chromatograms were compared to see whether degradation occurred or not. The degradation of drug sample is not more than 22%.

Neutral condition:

Weight accurately 10 mg drug and transferred in to100 ml water in round bottom flask. Reflux it for 2 hours. Pipette out 1ml in to 10 ml volumetric flask and adjust with mobile phase.

Photo stability study:

Photo stability was performed by placing 10 mg of etoricoxib in daylight for 24 hours. The samples were diluted with methanol up to 100ml in a volumetric flask. Pipette out 1 ml sample diluted up to 10 ml by mobile phase. Volume of 20µl was injected into the system for chromatographic analysis.

Dry heat:

Standard etoricoxib was placed in an oven at 60°C for 2 hours to study dry heat degradation. 10 mg drug samples were diluted with methanol up to 100ml in a volumetric flask. Pipette out 1 ml and were diluted up to 10 ml by mobile phase. Volume of 20µl was injected into the system for chromatographic analysis and results of both chromatograms were compared to see whether degradation occurred or not.

Assay Procedure -

Take weight of 10 tablet of any brand of Prednisolone Sodium Phosphate tablet. Crush the tablet in the motor pestle. Accurately weigh the quantity of powder equivalent to 10mg of drug in 100 ml volumetric flask and add ethanol to adjust the volume up to 100 ml. Pipette out the 1 ml in to 10 ml volumetric flask make the volume with mobile phase to get conc. 10µg/ml and analyse the reading on HPLC. Calculate the percentage

purity of tablet.

III. Observations and Result

Preliminary solubility study of drug:

Solubility of the drug was determined at 28±1 C. A small quantity of standard drug was dissolved in different solvents.

Implementation of QbD approach for the Spectrophotometric method developement as per ICH Q8(R2) guidelines for estimation of Prednisolone Sodium Phosphate by varying various parameters and these variable parameters were designed as per Ishikawa. Critical parameters for the development of zero order spectrophotometric method are considered as various solvent, sample preparation of tablet, wavelength at 244 & 246 nm, slit width as 1, scan speed and sampling interval (0.05, 0.1, 0.2, 0.5, 1.0, 2.0)

Table 3: Solubility study in different Solvent by UV-absorbance

Solvent	Concentration	Absorbance at	Absorbance at
Methanol	2	0.091	0.092
	4	0.177	0.179
	6	0.285	0.284
	8	0.35	0.354
	10	0.458	0.464
	12	0.529	0.534
Ethanol	2	0.101	0.102
	4	0.201	0.202
	6	0.289	0.291
	8	0.393	0.397
	10	0.472	0.477
	12	0.569	0.575
Water	2	0.085	0.086
	4	0.192	0.194
	6	0.261	0.265
	8	0.36	0.364
	10	0.446	0.452
	12	0.547	0.555
Water (With stirring)	2	0.141	0.143
	4	0.286	0.29
	6	0.425	0.41
	8	0.532	0.54
	10	0.675	0.685
	12	0.786	0.798

Table 4: Effect of Stirring on absorbance at (λ_{max}-246nm) As per Stirring time

olvent	Conc. µg/ ml	Effect of Stirring on absorbance at (λ_{max}-246) As per Stirring time							
		0 min		2 min		4 Min		6 Min	
		244 nm	246 nm	244 nm	246 nm	244 nm	246 nm	244 nm	246 nm
Water (Stirring tech.)	**2**	0.085	0.086	0.102	0.104	0.109	0.113	0.105	0.106
	4	0.192	0.194	0.191	0.194	0.199	0.205	0.213	0.216
	6	0.261	0.265	0.282	0.286	0.292	0.3	0.299	0.299
	8	0.36	0.364	0.377	0.383	0.391	0.402	0.417	0.41
	10	0.446	0.452	0.468	0.474	0.48	0.49	0.508	0.516
	12	0.547	0.555	0.576	0.585	0.59	0.6	0.632	0.641

Solvent	Conc. µg/ ml	Effect of Stirring on absorbance at (λ_{max}-246) As per Stirring time							
		8 min		10 min		12 min		14 min	
		244 nm	246 nm	244 nm	246 nm	244 nm	246 nm	244 nm	246 nm
Water (Stirring tech.)	**2**	0.127	0.129	0.141	0.143	0.141	0.143	0.141	0.143
	4	0.245	0.249	0.286	0.29	0.286	0.29	0.286	0.29
	6	0.357	0.363	0.425	0.41	0.425	0.41	0.425	0.41
	8	0.463	0.47	0.532	0.54	0.532	0.54	0.532	0.54
	10	0.585	0.59	0.675	0.685	0.675	0.685	0.675	0.685
	12	0.73	0.741	0.786	0.798	0.786	0.798	0.786	0.798

Table 5: UV Absorbance as per Instrumental Parameter (C.Q.A.) Con. 2ppm

Time interval		Fast mode		Medium mode		Slow mod		Very Slow Mode	
		244	246	244	246	244	246	244	246
0.05	**Abs.**	0.142	0.143	0.142	0.144	0.141	0.142	0.142	0.144
	Time Req.	2.4 min		10.5 min		27 min		53.1 min	
0.1	**Abs.**	0.142	0.143	0.143	0.145	0.141	O.145	0.143	O.145
	Time Req.	1.4 min		6 min		12 min		21 min	
0.2	**Abs.**	0.141	0.144	0.141	0.143	0.140	0.542	0.142	0.145
	Time Req.	1 min		3 min		7 min		12 min	
0.5	**Abs.**	0.140	0.142	0.142	0.144	0.143	0.145	0.141	0.143
	Time Req.	0.45 min		1.23 min		3.2 min		8 min	
1.0	**Abs.**	0.140	0.542	0.142	0.142	0.143	0.142	0.142	0.144
	Time Req.	0.25 min		0.45 min		2 min		4.40 min	
2.0	**Abs.**	0.142	0.143	0.142	0.144	0.141	0.142	0.142	0.144
	Time Req.	0.10 min		0.22 min		0.45 min		2.30 min	

Table 6: UV Absorbance as per Instrumental Parameter (C.Q.A.) Con. 4ppm

Time interval		Fast mode		Medium mode		Slow mode		Very Slow Mode	
		244	246	244	246	244	246	244	246
0.05	**Abs.**	0.288	0.290	0.286	0.291	0.287	0.291	0.288	0.290
	Time Req.	2.40 min	-	10.45 min	-	27 min	-	53.1 min	-
0.1	**Abs.**	0.287	0.291	0.289	0.292	0.286	0.290	0.287	0.291
	Time Req.	1.40 min	-	6 min	-	12 min	-	21 min	-
0.2	**Abs.**	0.287	0.291	0.286	0.290	0.286	0.291	0.288	0.290
	Time Req.	1 min	-	3 min	-	7 min	-	12 min	-
0.5	**Abs.**	0.286	0.291	0.286	0.291	0.287	0.291	0.288	0.290
	Time Req.	0.45 min	-	1.23 min	-	3.20 min	-	8 min	-
1.0	**Abs.**	0.287	0.291	0.289	0.292	0.288	0.290	0.287	0.291
	Time Req.	0.25 min	-	0.45 min	-	2 min	-	4.4 min	-
2.0	**Abs.**	0.289	0.292	0.287	0.291	0.288	0.290	0.288	0.290
	Time Req.	0.10 min	-	0.22 min	-	0.45 min	-	2.3 min	-

Table 7: UV Absorbance as per Instrumental Parameter (C.Q.A.) Con. 6ppm

Time interval		Fast mode		Medium mode		Slow mode		Very Slow Mode	
		244	246	244	246	244	246	244	246
0.05	**Abs.**	0.409	0.410	0.413	0.414	0.412	0.414	0.411	0.412

	Time Req.	2.40 min	-	10.45min	-	27 min	-	53.10 min	-
0.1	**Abs.**	0.411	0.412	0.412	0.414	0.412	0.414	0.411	0.412
	Time Req.	1.40 min	-	6 min	-	12 min	-	21 min	-
0.2	**Abs.**	0.409	0.410	0.409	0.410	0.412	0.414	0.412	0.414
	Time Req.	1 min	-	3 min	-	7 min	-	12 min	-
0.5	**Abs.**	0.411	0.412	0.413	0.414	0.412	0.413	0.409	0.410
	Time Req.	0.45 min	-	1.23min	-	3.20 min	-	8 min	-
1.0	**Abs.**	0.409	0.410	0.412	0.413	0.413	0.414	0.411	0.412
	Time Req.	0.25 min	-	0.45 min	-	2 min	-	4.40 min	-
2.0	**Abs.**	0.413	0.414	0.412	0.414	0.413	0.412	0.414	0.414
	Time Req.	0.10 min	-	0.22min	-	0.45 min	-	2.30 min	-

Table 8: UV Absorbance as per Instrumental Parameter (C.Q.A.) Con. 8ppm

Time interval		**Fast mode**		**Medium mode**		**Slow mode**		**Very Slow Mode**	
		244	**246**	**244**	**246**	**244**	**246**	**244**	**246**
0.05	**Abs.**	0.533	0.541	0.532	0.541	0.534	0.542	0.531	0.541
	Time Req.	2.40 min	-	10.45 min	-	27 min	-	53.10 min	-
0.1	**Abs.**	0.534	0.542	0.535	0.543	0.534	0.542	0.531	0.542
	Time Req.	1.40 min	-	6 min		12 min	-	21 min	-
0.2	**Abs.**	0.534	0.541	0.535	0.543	0.534	0.542	0.533	0.541
	Time Req.	1 min	-	3 min	-	7 min	-	12 min	-
0.5	**Abs.**	0.534	0.542	0.532	0.541	0.533	0.541	0.534	0.543
	Time Req.	0.45 min	-	1.23 min	-	3.2 min	-	8 min	-
1.0	**Abs.**	0.533	0.543	0.534	0.543	0.534	0.542	0.533	0.542
	Time Req.	0.25 min	-	0.45 min	-	2 min	-	4.40 min	-
2.0	**Abs.**	0.532	0.541	0.533	0.541	0.534	0.543	0.534	0.543
	Time Req.	0.10 min	-	0.22 min	-	0.45 min	-	2.30 min	-

Table 9: UV Absorbance as per Instrumental Parameter (C.Q.A.) Con. 10ppm

Time interval		Fast mode		Medium mode		Slow mode		Very Slow Mode	
		244nm	246nm	244nm	246nm	244nm	246nm	244nm	246nm
0.05	Abs.	0.680	0.690	0.679	0.690	0.678	0.688	0.675	0.685
	Time Req.	2.40 min	-	10.45 min	-	27 min	-	53.10 min	-
0.1	Abs.	0.679	0.690	0.678	0.688	0.678	0.688	0.680	0.690
	Time Req.	1.40 min	-	6 min	-	12 min	-	21 min	-
0.2	Abs.	0.679	0.690	0.675	0.685	0.680	0.690	0.678	0.688
	Time Req.	1 min	-	3 min	-	7 min	-	12 min	-
0.5	Abs.	0.680	0.690	0.676	0.684	0.678	0.688	0.680	0.690
	Time Req.	0.45 min	-	1.23 min	-	3.20 min	-	8 min	-
1.0	Abs.	0.679	0.690	0.676	0.684	0.679	0.690	0.676	0.684
	Time Req.	0.25 min	-	0.45 min	-	2 min	-	4.40 min	-
2.0	Abs.	0.679	0.690	0.676	0.684	0.680	0.690	0.680	0.690
	Time Req.	0.10 min	-	0.22 min	-	0.45 min	-	2.30 min	-

Table 10: UV Absorbance as per Instrumental Parameter (C.Q.A.) Con. 12ppm
Fixing of wave length

Time interval		Fast mode		Medium mode		Slow mode		Very Slow Mode	
		244	246	244	246	244	246	244	246
0.05	Abs.	0.788	0.799	0.789	0.800	0.785	0.798	0.785	0.796
	Time Req.	2.40 min	-	10.45 min	-	27 min	-	53.10 min	-
0.1	Abs.	0.789	0.800	0.785	0.798	0.789	0.800	0.788	0.799
	Time Req.	1.40 min	-	6 min	-	12 min	-	21 min	-
0.2	Abs.	0.788	0.799	0.786	0.798	0.785	0.796	0.789	0.800
	Time Req.	1 min	-	3 min	-	7 min	-	12 min	-
0.5	Abs.	0.789	0.800	0.785	0.796	0.789	0.800	0.788	0.799
	Time Req.	0.45 min	-	1.23 min	-	3.2 min	-	8 min	-
1.0	Abs.	0.788	0.799	0.786	0.798	0.785	0.796	0.789	0.800
	Time Req.	0.25 min	-	0.45 min	-	2 min	-	4.40 min	-
2.0	Abs.	0.789	0.800	0.785	0.796	0.786	0.798	0.785	0.798
	Time Req.	0.10 min	-	0.22 min	-	0.45 min	-	2.30 min	-

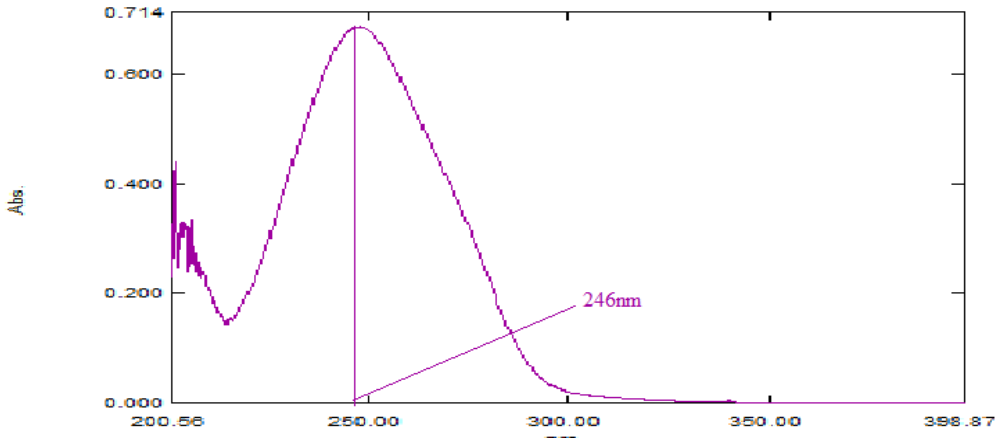

Figure 12: Fixing of wavelength for Prednisolone sodium phosphate

Linearity and range

The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the samples in the range of 2 to 12 mg/ml was linear with a correlation coefficient (R2) 0.999.

Table 11: Linearity and range for Prednisolone at 246nm

Sr. No.	Concentration (µg/ml)	Absorbance
1.	2	0.143
2.	4	0.290
3.	6	0.410
4.	8	0.540
5.	10	0.685
6.	12	0.798

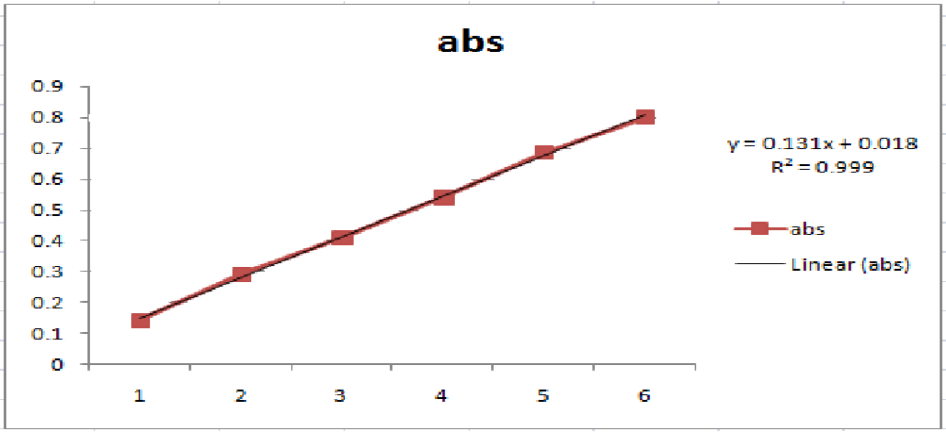

Figure 13: Linearity and range for Prednisolone sodium phosphate

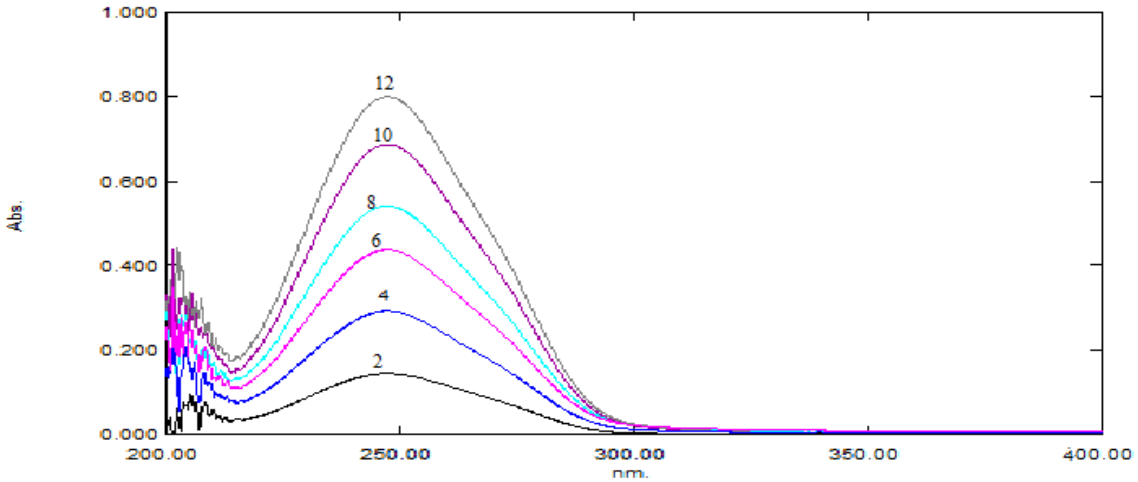

Figure 13: Accuracy sample overlay for Prednisolone sodium phosphate

Table 12: Linearity Parameter

Parameter	Data
Range	2 µg/m to 12 µg/m
Correlation coefficient	0.999
Slope	0.131
Intercept	0.018

Intra-day precision (repeatability) and inter-day precision study (intermediate precision):

Table 13: Precision data for Prednisolone at 246nm (Intra-Day)

Conc. (µg/ml)	Absorbance						Avg.	SD	% RSD
2	0.145	0.147	0.147	0.145	0.146	0.147	0.146	0.000983	0.673287
6	0.413	0.412	0.413	0.415	0.413	0.412	0.413	0.001095	0.265133
12	0.799	0.797	0.8	0.801	0.799	0.798	0.799	0.001414	0.176971

Table 14: Precision data for Prednisolone at 246nm (Inter-Day)

Conc. (µg/ml)	Absorbance						Mean	SD	% RSD
2	0.141	0.141	0.143	0.142	0.142	0.142	0.141	0.000753	0.533881
6	0.409	0.409	0.408	0.409	0.408	0.409	0.410	0.002683	0.65439
12	0.798	0.796	0.798	0.798	0.798	0.799	0.796	0.000983	0.123517

Reproducibility:

Table 15: Reproducibility data for Prednisolone at 246nm

Instrument I SHIMADZU	**Instrument II JASCO**	**Result of t-test**	**Inference**
0.295 ± 0.000516	0.296 ± 0.000616	0.99	Not significant difference

Limit of Detection & Limit of Quantitation:

$$LOD = \frac{3.3\sigma}{S}, \quad LOQ = \frac{10\sigma}{S}$$

Where,

σ =Standard deviation

S= slope of the calibration curve

Table 16: Limit of Detection & Limit of Quantitation

LOD	LOQ
0.0538	0.1631

Stability of Sample:

Table 17: Stability of Sample

Sr. No	**Concentration of drug solution** (µg/ml)	**Time** (min)	**Absorbance at 246nm.**
1	4	0	0.236
2	4	30	0.236
3	4	60	0.236
4	4	90	0.237
5	4	120	0.235
6	4	150	0.233
7	4	180	0.232
8	4	210	0.231
9	4	240	0.231
10	4	360	0.230
11	4	480	0.230
12	4	600	0.230

Assay Result of Marketed Formulation

Table 18: Assay Result of Marketed Formulation

Formulation	**Label claim mg/tablet**	**Amount found mg**	**% Label claim**
Tablet	5mg	4.96	99.20%
Tablet	5mg	4.98	99.60%
Tablet	5mg	4.97	99.40%
Tablet	5mg	4.98	99.60%

Degradation Studies

Table 19: Results of Stress Degradation Studies

Condition	**Time**	**%Degradation**
0.1N NaOH(1ml)	60min 90min	10.07% for 60min 13.95% for 90min
3N HCl(1ml)	60min 90min	11.75% for 60min, 14.79% for 90 min
30% H_2O_2 (1ml)	15min	15.65%for 15 min
Dry Heat 70°	48hrs	25.94% for 48 hrs.
Photolytic	3hrs 6hrs	11.44% for 3hrs 15.66% for 6hrs.

pH Degradation Studies

Table 20: Preparation of sample solution of pH 0-14 for pH stability

pH	Amount of Drug added (10 µg/ml) (ml)	Amount of 0.1N NaOH solution added (ml)	Amount of HCl/ NaOH added (ml)
1	4	4	0.7 ml of 2N HCl
2	4	4	3 mlof 0.2N HCL
3	4	4	21ml of 0.02N HCl
4	4	4	22ml of 0.002N HCl
5	4	4	31ml of 0.0002N HCl
6	4	4	21ml of 0.0002N HCl
7	4	0	-
8	4	1.1	-
9	4	2.5	-
10	4	3.2	-
11	4	4	-
12	4	4	3 mlof 0.2N NaoH
13	4	4	0.7 ml of 2N NaoH
14	4	4	7 ml of 2N NaoH

Table 21: pH Degradation Results

pH	**Absorbance** (at 230 nm)	**Concentration** (µg/ml)	**% Drug Degraded**
1	0.270	10 µg	60.86%
2	0.425	10 µg	38.40%
3	0.510	10 µg	26.08%
4	0.610	10 µg	11.59%
5	0.650	10 µg	5.79%
6	0.680	10 µg	1.44%
7	0.690	10 µg	0%
8	0.675	10 µg	2.17%
9	0.625	10 µg	9.42%
10	0.575	10 µg	16.66%
11	0.470	10 µg	31.88%
12	0.380	10 µg	44.92%
13	0.298	10 µg	56.81%
14	0.280	10 µg	59.42%

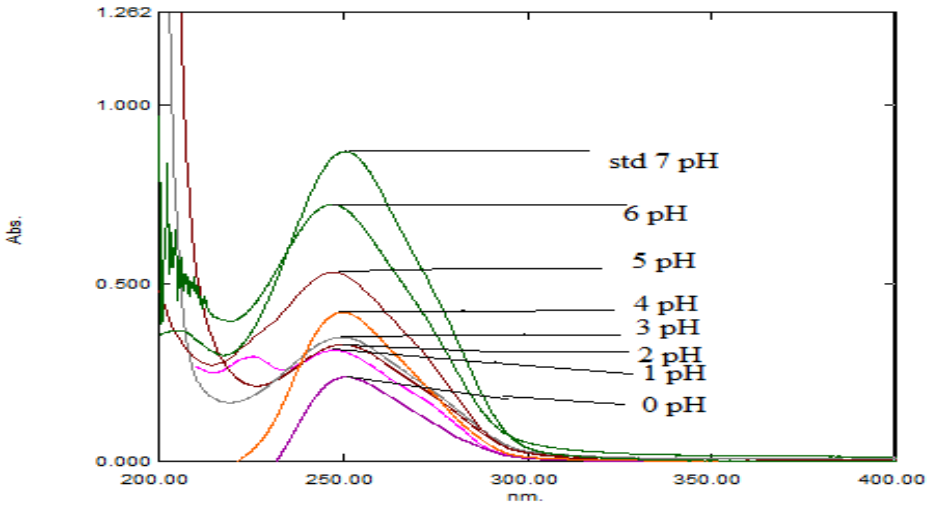

Figure15: pH Degradation Results (0pH to 7pH)

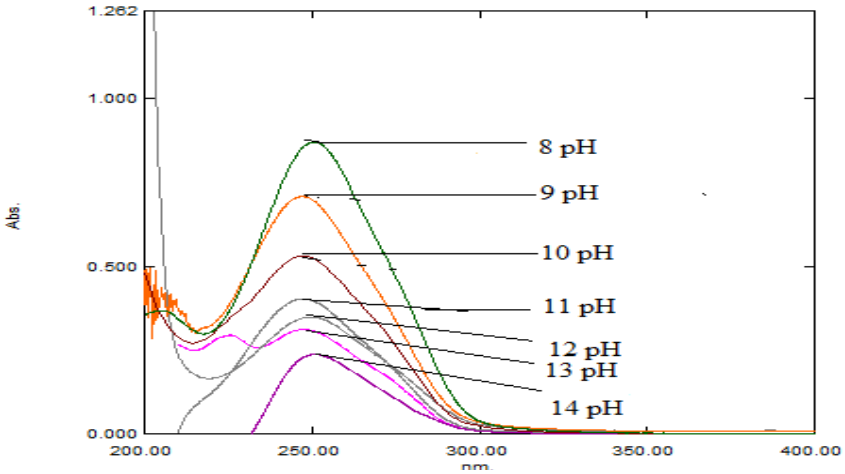

Figure16: pH Degradation Results (8pH to 14pH)

IV. Conclusion

The proposed methods were found to be accurate, precise, and economical and can be applicable for routine quality control analysis of Prednisolone sodium phosphate on pharmaceutical dosage form. Implementation of QbD approach resulted in more robust methods which can produce consistent, reliable, and quality data throughout the process and also save time and money. pH Degradation study shown that, degradation of Prednisolone takes more in more acidic and more basic environment. The pH 7.0 & 8.0 is more protective for degradation of Prednisolone.

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Conflict Of Interest

There is no any competing interest among the authors. Authors declare no conflict of interest.

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