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Formulation and evaluation of Prednisolone Sodium Phosphate injection

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Abstract

Prednisolone Sodium Phosphate (PSP) is a synthetic corticosteroid exerting potent glucocorticoid and weak mineralocorticoid activity.The present study was undertaken with an intention to develop a stable and effective parenteral formulation, containing the high concentration of drug PSP. PSP is heat sensitive and water soluble drug but unstable upon standing at room temperature or at elevated temperature in water. So the effects of various cosolvents in the solubility of PSP have been evaluated.PSP was tried with different cosolvents such as Ethanol, Glycerine and Propylene glycol. The drug was made into injection formulation for administered as a SVP. The influence of heat, light and atmospheric oxygen on the stability of the drug was studied. Out of all trials, formulation containing propylene glycol (16.5%w/v) and glycerine (5%w/v) in combination was found to be more stable and passed all tests satisfactorily. Accelerated stability study was conducted for 3 months at 45^0 C / 75% RH, formulation containing propylene glycol (16.5%w/v) and glycerine (5%w/v) in combination was found to pass all tests satisfactorily.

Key-Words: Prednisolone Sodium Phosphate, Glucocorticoid, Parenteral formulation, SVP, Accelerated stability

Introduction

Injections include a wide variety of therapeutic agents e.g., for the treatment of cancer, infections, cardiovascular diseases, arthritis, inflammatory diseases, diabetes, hormonal deficiencies and many other disease states including life threatening emergency conditions. There are more than 400 injections products listed in the USP and, because of the huge number of biotechnology molecules in clinical study, this number will continue to grow rapidly over the next several years. About 80% or greater of all SVPs commercially available are prepared by aseptic processing. LVPs usually involve intravenous infusion, dialysis, or irrigation fluids containing electrolytes, sugar, amino acids, blood, blood products, and fatty lipid emulsions. SVP formulations are simple formulations compared with other pharmaceutical dosage forms, composed of active ingredients, solvent system (preferably aqueous), minimal number of excipients, in the appropriate container and closure packaging system. Formulation scientists have severe restrictions in number and choice of added substances because of safety considerations.

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Prednisolone sodium phosphate is a synthetic adrenocortical steroid derivative with predominantly glucocorticoid properties possessing anti-inflammatory and immunosuppressive action.. It is used in the treatment of inflammation, immunosuppression, asthma, arthritis. The aim of the present study is to formulate mixed solvent system solution of high concentration of prednisolone sodium phosphate injection and evaluate the parenteral dosage form containing PSP and to carry out the accelerated stability study at 40^0 C/ 75 % RH.

Material and Methods[2-5]

Preformulation Studies

Solubility studies of PSP in different solvents (saturation solubility method)

Excess of PSP was added to screw capped 10 ml amber colored glass bottles containing fixed volumes (2 ml) of the DW, cosolvent water blends, separately. These bottles were shaken mechanically at 25 ± 2^0 C in a constant temp. Bath for 24 h. These mixtures were allowed to equilibrate for the next 12 h and then centrifuged for 3 min at 2000 rpm at 37 ± 2^0 C. The supernatant was filtered through 0.45 µ membrane. An aliquot of the filtrate was diluted with water and the resulting solutions were analyzed spectrophotometrically at 247 nm.

Effect of temperature on stability of drug

3.0(3.3) % w/v PSP solution in WFI is filled into vials. The vials were sealed and placed at refrigeration, room-temperature, 40 °C, 50°C and 75°C for 4 weeks and weekly observed for colour change and crystal growth. The samples placed at refrigeration and room temperature served as controls.

Effect of sunlight on drug degradation

One set of colourless vials containing 1%w/v of prednisolone sodium phosphate solution in DW were exposed to sunlight to estimate the effect of light on the stability of formulation. Other set of colourless vials containing 1%w/v of prednisolone sodium phosphate solution in DW were wrapped with aluminium foil (controlled) and stored in dark. Both sets of vials were stored at RT. One vial of each set was withdrawn every 1 week up to 4 weeks and observes for colour change or precipitation.

Effect of atmospheric oxygen on stability

To assess the effect of oxygen, 1%w/v of PSP solution in DW (2ml) was filled into 5 ml vials. The air in one of two 5 ml vial sets was not displaced before sealing (condition 'A'), whereas the air present in another 5 ml vial sets was replaced by flushing with nitrogen and sealed (condition 'B'). samples from both sets of vials were withdrawn every 1 week upto 4 weeks and observed for colour change.

Formulation development

Attempts were made to develop a stable parenteral formulation using cosolvent/s along with other excipients. The dose selected for formulation was 55mg of PSP in 1ml solvent. The prepared formulations contain the following ingredients along with their concentrations are given in Table 5.

Sterilization Studies

The injection samples were taken in glass syringe, the membrane filter holder was attached to the syringe. A prefilter of 1.5 micrometers was placed in this holder, after which filters of 0.22,0.45, 1.2 and 1.5 micrometers were placed successively and tested whether the injection sample could pass through these membranes or not.

Sterility testing

Direct transfer method

Aliquots of the samples are transferred aseptically into fluid thioglycolate medium and soyabean casein digest medium.The inoculated thioglycolate medium is incubated at 32^0 C and soyabean casein digest samples at 22^0 C for 7 days. Likewise negative and positive controls are prepared.

Evaluation of formulation batches

Physical appearance

For the large scale of production, 10-liter batch size was considered on the tentative basis for further study. It takes almost 4-5 hrs for processing a batch. Therefore, prepared formulations were visually observed for their physical appearance at initially and after 4 weeks of time interval.

pH measurement

The pH of the prepared formulations was measured using Spectralab pH meter at 25 $\pm 1^{0C}$.

Particulate matter

The particulate matter can be determined by the visual inspection by naked eye under direct light beam.

Assay content for Prednisolone sodium phosphate

Carried out by the method for liquid chromatography (HPLC) using the following solution. Solution (1) Take injection and make dilution of injection which contains 0.001% w/v of prednisolone phosphate in mobile phase. Refer ence solution (a). Weigh accurately about 10 mg of prednisolone sodium phosphate RS, dissolve in sufficient water to produce 100.0 ml (solution A) and dilute 10.0 ml of the solution to 100.0 ml with water.Reference solution (b). Add 10 ml of a 0.01 per cent w/v solution of betamethasone sodium phosphate RS in water to

10 ml of solution A and dilute to 100 ml with water.. Mobile phase: a mixture of 45 volumes of methanol and 55 volumes of citro-phosphate buffer pH 5.0,. Column: a stainless steel column 20 cm x 4.6mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (10 μm). Flow rate: 2 ml/min Spectrophotometer set at 247 nm. A 10μl loop injector. Inject reference solution (b). The test is not valid unless the resolution between the peaks due to betamethasone sodium phosphate and prednisolone sodium phosphate is at least 2.5.Inject alternatively the test solution and reference solution (a).Calculate the content of C21H29O8P in the injection.

Osmolarity

The concentration of osmotically active particles expressed in terms of osmoles of solute per liter of solution. Osmolarity is calculated from experimentally determined osmolality of a solution. Theoretical osmolarity is calculated as per the following formula;

Osmolarity = (Concentration/mol.wt X 1000) X No. of dissociable ions

The osmolarity of formulations of Prednisolone sodium phosphate for Injection was measured using Osmomat Auto Osmometer.

Stability Studies

The protocol of the stability studies was in conformity with the recommendations given in WHO document pertaining to stability testing of products intended and ICH guidelines. To assess the accelerated stability, the sealed ampoules of the formulations were stored in

ICH certified stability chambers (Thermolab) at 40±2^{0}C and 75%±5% RH for three months. The samples were withdrawn monthly and evaluated for change in physical appearance (color, precipitation), pH change, particulate matter, sterility and percent drug content, if any.

Results and Discussion

Solubility of PSP was checked in distilled water, ethanol, 10% & 20%glycerine, 15% PEG 300 & 15%PEG 400, Propylene Glycol (10%, 20%, 30%, 40%, 50%). The results are shown in Table 1.PSP shows maximum solubility in water and it is 250 mg/ml. It has solubility about 125.23 mg/ml in 30% propylene glycol. The solubility decreases in propylene glycol after 30% concentration.Various stress tests are performed on solid and solution samples to establish the effect of heat, light and oxygen on the drug substance stability.

As from Table 2 it is observed that there is color change as well as formation of precipitate after 1,2,3,4 weeks of storage at 40, 50 & 75^0 C. Thus PSP is sensitive to heat. Therefore heat sterilization method is not used for sterilization. From the literature it was found that filtration can be used for sterilization. As from Table 3 it is observed that there is no turbidity formed in the solution after 1,2,3,4 weeks of storage. Thus PSP is stable in light and thus the injection can be packaged in flint type or amber glass vials or ampoules. As from Table 4 it is observed that there is change in color in air sealed vials while there is no change in color in purged vials after 1 week of storage at 30 & 40^{0}C. Hence PSP is sensitive to oxygen & therefore sodium bisulphite is added as antioxidant in the formulation and disodium EDTA is added as chelating agent. Disodium EDTA acts as synergists and thus increases the activity of sodium bisulphite.

A stable parenteral formulation of water soluble drug PSP was formulated after performing trials with various solvents as shown in Table 5. Thus prepared formulations were subjected for various tests. All the formulations were found to be easily passing through all the pore size filters as shown in Table 6 and hence 0.22 µm pore size filter was selected to filter all the prepared formulations separately. None of the formulations showed turbidity or signs of microbial growth (except the positive control) as observed in Table 7 at the end of incubation period, indicating all the formulations were sterile and thus all the formulations were subjected to further evaluations.

Physical appearance (color, transparency, precipitation, etc.) as shown in Table 8 of prepared formulations was studied. Formulation F3, F6 appeared colorless and clear initially as well as after 4 week of time interval at 25^0 C/60% RH and 40^0 C/75% RH. The pH of all the formulations was set initially in the range of 7.0 to 8.0.pH of the prepared solution initially and after 4 week of time interval was measured which are shown in Table 9. Formulation F6 shows good stability after 4th week as shown in Table 10. Assay content for Prednisolone sodium phosphate after initial & 4th week when stored at 25 & 40^{0}C is shown in Table 11. Osmolarity data of the formulations are presented in Table 12. Stability study results are shown in table 13.

Conclusion

From the various cosolvents studied, it was found that propylene glycol (16.5%w/v) and glycerine (5%w/v) in combination provides an excellent stability for preparation of parenteral dosage form of Prednisolone sodium phosphate.

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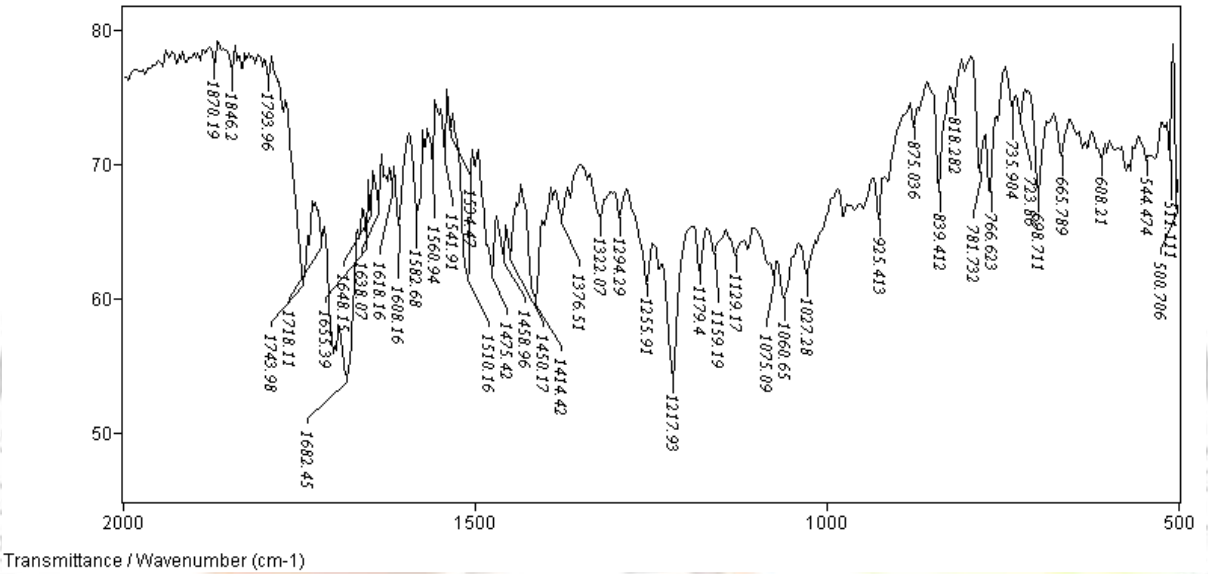

Fig.1: FTIR spectra of Prednisolone Sodium Phosphate

Table 1: Solubility profile of PSP in different solvents

Medium	Concentration (mg/ml)
DM water	250.00
5%Ethanol	101.00
10% glycerine	49.920
20% glycerine	34.329
15%PEG 300	24.625
15%PEG 400	19.023
10%Propylene Glycol	95.676
20%Propylene Glycol	110.076
30%Propylene Glycol	125.236
40%Propylene Glycol	83.293
50%Propylene Glycol	52.062

Table 2: Heat stability profile of PSP

Temperature (°C)	Duration (weeks) for colour change				Duration (weeks) for precipitation			
	1	2	3	4	1	2	3	4
Refrigeration	–	–	–	–	N	N	N	N
Room temperature	–	–	–	–	N	N	N	N
40	+	+	+	+	Y	Y	Y	Y
50	+	+	+	+	Y	Y	Y	Y
75	+	+	+	+	Y	Y	Y	Y

+ Colour change, - No colour change
(Y) Precipitate, (N)No Precipitate

Table 3: Light stability study of PSP

Withdrawal week	Vial stored in dark	Colourless vial exposed to sunlight
1	–	+
2	–	+
3	–	+
4	–	+

- Clear, + Turbidity

Table 4: Test for colour change after a week

Withdrawal week	Vials in which Oxygen is present (not flushed with nitrogen)	Vials in which Oxygen is removed (flushed with nitrogen)
1	+	–
2	+	–
3	+	–
4	+	–

+ Colour change, - No colour change

Table 5: Concentration of different ingredients used in various trial formulations

Ingredients	Formulation					
	F1	F2	F3	F4	F5	F6
Prednisolone Sodium Phosphate	5.50gm	5.50gm	5.50gm	5.50gm	5.50gm	5.50gm
Propylene Glycol	–	–	16.5%w/v	–	–	16.5%w/v
Glycerine	–	5%w/v	–	–	5%w/v	5%w/v
Ethanol	–	–	–	5%w/v	5%w/v	–
Sodium bisulphite	0.1%w/v	0.1%w/v	0.1%w/v	0.1%w/v	0.1%w/v	0.1%w/v
Disodium EDTA	0.05%w/v	0.05%w/v	0.05%w/v	0.05%w/v	0.05%w/v	0.05%w/v
Nitrogen purged WFI	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml
Citric acid	q.s. to pH 7.0	q.s. to pH 7.0	q.s. to pH 7.0	q.s. to pH 7.0	q.s. to pH 7.0	q.s. to pH 7.0

Table 6: Filter pore size and filterability of the formulations of PSP

Formulation	Filter pore size (µm)	Observation
F1	0.22	+
	0.45	+
	1.2	+
	1.5	+
F2	0.22	+
	0.45	+
	1.2	+
	1.5	+
F3	0.22	+
	0.45	+
	1.2	+

	1.5	+
F4	0.22	+
	0.45	+
	1.2	+
	1.5	+
F5	0.22	+
	0.45	+
	1.2	+
	1.5	+
F6	0.22	+
	0.45	+
	1.2	+
	1.5	+

+ Injection passes through. - Injection does not pass through

Table 7: The growth of bacteria in soya bean casein digest medium and fluid Thioglycollate medium after seven days

Formulation	Soyabean-casein digest medium (SCDM)	Fluid thioglycollate medium (FTM)
F1	–	–
F2	–	–
F3	–	–
F4	–	–
F5	–	–
F6	–	–

- Clear; + Turbid

Table 8: Observation for physical appearance

Formulation Code	Time Point	Storage Conditions	
		25^0C	40^0C
F1	Initial	Clear, colorless solution	Clear, colorless solution
	4^{th} week	Discoloration	Discoloration
F2	Initial	Clear, colorless solution	Clear, colorless solution
	4^{th} week	Discoloration	Discoloration
F3	Initial	Clear, colorless solution	Clear, colorless solution
	4^{th} week	Clear, colorless solution	Clear, colorless solution
F4	Initial	Clear, colorless solution	Clear, colorless solution
	4^{th} week	Clear, pale yellow color	Clear, pale yellow color
F5	Initial	Clear, colorless solution	Clear, colorless solution
	4^{th} week	Discoloration	Discoloration
F6	Initial	Clear, colorless solution	Clear, colorless solution
	4^{th} week	Clear, colorless solution	Clear, colorless solution

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Table 9: Results of pH measurement

Formulation Code	Time Point	Storage Conditions	
		25^0C	40^0C
F1	Initial	7.01	7.01
	4th week	8.01	8.36
F2	Initial	7.12	7.12
	4th week	7.99	8.01
F3	Initial	7.34	7.34
	4th week	7.89	7.95
F4	Initial	7.01	7.01
	4th week	7.67	7.83
F5	Initial	7.43	7.43
	4th week	7.89	7.93
F6	Initial	7.02	7.02
	4th week	7.06	7.09

Table 10: Results of particulate matter

Formulation Code	Time Point	Storage Conditions	
		25^0C	40^0C
F1	Initial	–	–
	4th week	++	+++
F2	Initial	–	–
	4th week	++	+++
F3	Initial	–	–
	4th week	+	++
F4	Initial	–	–
	4th week	++	++
F5	Initial	–	–
	4th week	++	++
F6	Initial	–	–
	4th week	–	–

Key: (–) = Absent
(+)= Very few colloidal particulates, fibres or filling artifacts
(++)=Evidence of physical instability under light
(+++)=Physical instability readily observable with the naked eye

Table 11: Results of assay content of Prednisolone sodium phosphate

Formulation Code	Time Point	Storage Conditions	
		25^0C	40^0C
F1	Initial	98.9%	98.9%
	4th week	95.5%	92.7%
F2	Initial	100.15%	100.15%
	4th week	98.2%	97.1%
F3	Initial	100.1%	100.1%

	4th week	99.2%	97.3%
F4	Initial	99.3%	99.3%
	4th week	98.3%	97.71%
F5	Initial	100.47%	100.47%
	4th week	98.9%	97.1%
F6	Initial	101.3%	101.3%
	4th week	100.9%	100.53%

Table 12: Osmolarity data of prednisolone sodium phosphate

Formulation	Osmolality (mOsm/kg)
F1	266
F2	287
F3	256
F4	279
F5	302
F6	301

Table 13: Accelerated stability study

Test parameters		Time point (Storage condition 40^{0}C/75% RH)			
		Initial	After 30 days	After 60 days	After 90 days
Physical appearance		Clear, colorless solution	Clear, colorless solution	Clear, colorless solution	Clear, colorless solution
pH		7.01	7.09	7.07	7.12
Particulate matter		–	–	–	–
Assay		100.01%	99.92%	99.6%	98.3%
Sterility	Fluid thyoglycollate medium	Sterile	Sterile	Sterile	Sterile
	Soyabean casein digest medium	Sterile	Sterile	Sterile	Sterile

Key:(–)=Absent