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Reverse phase HPLC method for the simultaneous estimation of lidocaine HCl, Prednisolone acetate and Dimethylsulfoxide in a pharmaceutical gel formulation

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ABSTRACT

A simple, fast and reliable reverse-phase high-performance liquid chromatographic (HPLC) method was developed for the assay of Lidocaine HCl, Prednisolone acetate and Dimethylsulfoxide in a pharmaceutical gel formulation. Separation was achieved in a PrincetoneSPHRE 100 C18 column (250mm X 4.6mm, 5µ), using a mobile phase consisting of Acetonitrile: Potassium dihydrogen phosphate (0.01M) adjusted to pH 7.0 with triethylamine, in the ratio 54:46 (v/v) and a flow rate of 1.0mL/min. The detection was made with a UV detector measuring at the maximum for the compound. The validation study demonstrated that the method was precise, accurate and linear over the concentration range of analysis with a limit of detection for Dimethylsulfoxide, Prednisolone acetate and Lidocaine HCl was 5.0µg/ml, 10.5µg/ml and 50.0µg/ml respectively. The limit of quantification for Dimethylsulfoxide, Prednisolone acetate and Lidocaine HCl was found to be 20.0µg/ml, 35.0µg/ml and 180.00µg/ml respectively. Linear regression analysis for Dimethylsulfoxide, Prednisolone acetate and Lidocaine HCl was found in the range of 25-200µg/ml, 30-230µg/ml and 100-1000µg/ml gave correlation coefficients higher than 0.995 for all the three analytes. The method developed was applied to the analysis of Lidocaine HCl, Prednisolone acetate and Dimethylsulfoxide in a pharmaceutical gel formulation.

KEYWORDS

DMSO Dimethyl sulfoxide (DMSO);
Prednisolone acetate (PA) and Lidocaine HCl (LH);
HPLC-UV;
Assay;
Method validation.

INTRODUCTION

The product is an effective topical preparation for the treatment of musculoskeletal conditions in horses[15] (all types of horses like racehorse, sports and leisure horse). It is easily absorbed, providing rapid and effective local anti-inflammatory action and pain relief.It is used for the treatment of musculoskeletal injuries[18,19] in target animals like synovitis, tendonitis[21], osteitis, arthritis[20], os-

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teoarthritis, arthrosis, soft tissue swellings, hard lumps, callus and corns, muscle strains and pulled muscles.

Prednisolone Acetate[1-7,21-28] is a white or almost white crystalline powder belongs to glucocorticoids[13] having molecular formula C23H30O6 and molecular weight 402.5. Practically insoluble in water, slightly soluble[12] in ethanol (96 per cent) and in methylene chloride. It is also called as 11b, 17-Dihydroxy-3, 20-dioxopregna-1, 4-dien-21-yl acetate.

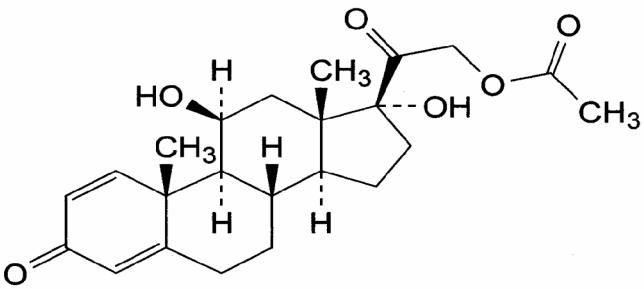

Figure 1 : Chemical structure of prednisolone acetate.

Lidocaine Hydrochloride[1-7,11,14] is local anaesthetic; Class I antiarrhythmic it is a white or almost white, crystalline powder having molecular formula as C14H22N2O, HCl, H2O and molecular weight as 288.8.It is very soluble in water, freely soluble in ethanol (96 per cent). Melting point is 74 °C to 79 °C.

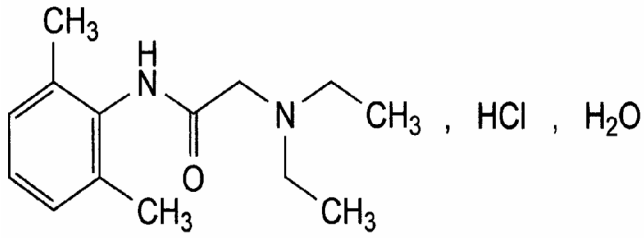

Figure 2 : Chemical structure of lidocaine hydrochloride.

Dimethyl Sulfoxide[1-8,15-19] is a colorless liquid or colorless crystal it is hygroscopic in nature; miscible with water and with ethanol (96 per cent). It has relative density1.100 to 1.104 with a refractive index 1.478 to 1.479 and freezing point 18.3°C.

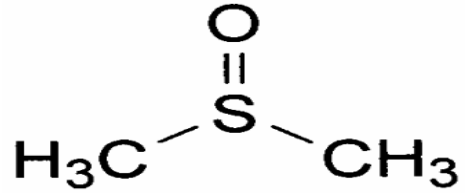

Figure 3 : Chemical structure of dimethyl sulfoxide.

MATERIAL AND METHODS[6,10,11,13]

The formulation is stable throughout the experiment and remained stable for 2years.

Acetonitrile (HPLC grade) and Potassium dihydrogen phosphate were procured from Merck''.

Instrumentation and chromatographic conditions

The method was performed on a JASCO system consisting of solvent delivery module PU-2089, Ultraviolet-visible spectrophotometric detector module UV-2075 plus, and system controller module as Borwin with a rheodyne injection valve with a 20µl loop attached. Isocratic Chromatographic separation were carried out in a stainless steel PrincetoneSPHRE 100 C18 column (250mm X 4.6mm, 5µ), with Acetonitrile: Potassium dihydrogen phosphate (0.01M) adjusted to pH 7.0 with triethylamine, in the ratio 54:46 (v/v) and a flow rate of 1.0mL/min. The mobile phase was filtered through 0.45µm Millipore membrane filter and degassed. The determination was performed with UV-Vis detector set at 261nm.

Preparation of standard solution

Stock solution for prednisolone acetate and dimethyl sulfoxide

Weigh accurately about 46 mg prednisolone acetate and transfer to 50 ml volumetric flask, add 20 ml Dimethyl sulfoxide and make up the volume with purified water.

Standard solution

Weigh accurately about 23 mg Lidocaine Hydrochloride and transfer to 100 ml volumetric flask, add 50 ml purified water and 5ml stock solution and make up the volume to mark with purified water.

Sample solution

Take about 2.5 g sample in 100 ml volumetric flask, add 50 ml water and shake. If foam is produced allow it to settle and make up the volume with purified water and shake well.

Procedure

Filter both Sample and Standard Solution with 0.2 micron filter paper and inject 20 microlitres. Calculate the result by comparing peak area ratio from the sample with that from the standard

preparation. Determine the weight/ml and calculate the result accordingly.

Method validation

The method was validated in accordance with International Conference on Harmonization guidelines (ICH) for validation[9,10,11,13] of analytical procedure.

Analysis of sample (in house sample) was carried out using the above method and the result are tabulated in TABLE 1.

TABLE 1 : Analysis of sample (in house sample) was carried out using the above method and the result are tabulated.

Contents	Label claim %w/w	Sample* Found
Dimethyl Sulfoxide	88.0%v/v	89.25%v/v
Prednisolone Acetate	0.2%w/v	0.208%w/v
Lidocaine HCl	1.0%w/v	0. 96%w/v

Linearity

Linearity is studied to determine the range over which analyte response is a linear function of concentration. This study was performed by preparing standard solutions at different concentrations and analysis was performed in triplicate. The calibration plots for the proposed method were obtained over the range of 25-250µg/ml(25, 50, 100, 150, 200, and 250) for DMSO, 30-230µg/ml (30, 60, 90, 120, 150, 180, and 230) for Prednisolone acetate and 100-1000µg/ml (100, 200, 400, 600, 800, and 1000) for Lidocaine HCl. The responses were measured as peak area. The calibration curves were obtained by plotting peak area against concentration.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection and limit of quantification of the developed method were determined by injecting progressively low concentration of the standard solutions using the developed RP-HPLC method. The LOD of DMSO, Prednisolone acetate and Lidocaine HCl was found to be 5.0µg/ml, 10.5µg/ml and 30.0µg/ml respectively. The LOQ is the smaller concentration of the analyte response that can be quantified accurately the LOQ was 20.0µg/ml, 35.0µg/ml and 180.00µg/ml respectively.

Recovery

To study the accuracy and reproducibility of the proposed method recovery experiments were carried out. A fixed amount of pre-analysed sample was taken and standard drug was added at 50%, 80% and 100% levels. Each level was repeated three times. The contents of DMSO, Prednisolone acetate and Lidocaine HCl per gram found by proposed method is shown in the TABLE 2.. The mean recoveries of DMSO, Prednisolone acetate and Lidocaine HCl were in the range of 99.49%, 99.74% and 99.93% respectively which shows there is no interference from excipient.

Precision

Precision of method was studied by analysis of multiple sampling of homogeneous sample. The precision of analytical procedure expresses

TABLE 2 : Results of accuracy experiment.

Amount of Sample			Amount of drug added			Amount recovered			% Recovery		
DMSO µg/ml	PA µg/ml	LH µg/ml	DMSO µg/ml	PA µg/ml	LH µg/ml	DMSO µg/ml	PA µg/ml	LH µg/ml	DMSO µg/ml	PA µg/ml	LH µg/ml
20	40	250	50	50	50	69.89	89.69	299.89	99.84	99.65	99.96
20	40	250	50	50	50	68.99	88.99	299.91	98.55	98.88	99.97
20	40	250	50	50	50	69.56	89.99	299.64	99.37	99.98	99.88
20	40	250	80	80	80	98.99	119.81	329.96	98.99	99.84	99.98
20	40	250	80	80	80	98.98	119.68	329.54	98.98	99.73	99.86
20	40	250	80	80	80	99.99	119.99	329.90	99.99	99.99	99.96
20	40	250	100	100	100	119.89	139.79	349.92	99.90	99.85	99.94
20	40	250	100	100	100	119.91	139.90	349.88	99.92	99.92	99.96
20	40	250	100	100	100	119.86	139.82	349.56	99.88	99.87	99.87
				Average					99.48	99.74	99.93

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the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision was investigated by the analysis of six separately prepared samples of the same batch. From this six separate sample solutions was injected and the peak areas obtained used to calculate mean and percentage RSD values. Precision Expressed as % RSD is given in table which should be less than 2%.(TABLE 3).

TABLE 3 : Precision for DMSO, prednisolone acetate and lidocaine HCl.

Sample	DMSO (%)	PA (%)	LH (%)
Sample 1	102.3	103.2	101.6
Sample 2	99.80	100.8	102.2
Sample 3	102.9	101.6	101.0
Sample 4	100.8	103.1	101.3
Sample 5	102.3	101.9	102.7
Sample 6	101.5	102.9	102.5
Average	101.6	102.4	101.9
%RSD	1.12%	0.95%	0.67%

Robustness and ruggedness of the method

Robustness

Robustness is a measure of its capacity to remain unaffected by small but deliberate variations in the chromatographic method parameters and provides an indication of its reliability. This was done by small deliberate changes in the chromatographic conditions at 3 different levels and retention time of DMSO, Prednisolone Acetate and Lidocaine HCl was noted. The factor selected were flow rate, pH and % Acetonitrile in the mobile phase. It was observed that there were no deliberate changes in the chromatogram, which demonstrated that the RP-HPLC method developed, are robust. Results describe in TABLE 4.

Ruggedness

The USP guideline defines ruggedness as "the degree of reproducibility" of the test result obtained by the analysis of the same samples under a variety of normal test condition such as; different Laboratory, different analyst, different instrument etc. Here this was done by changing the instrument and analyst. A result, presented in the TABLE 5 indicates that the selected factors are remained unaffected by small variations of these parameters.

TABLE 4 : Robustness of the method.

Factor	Level	Retention time		
		DMSO	PA	LH
Flow rate ml/min				
0.9	-0.1	2.182	6.095	10.298
1.0	0	2.233	6.153	10.345
1.1	+0.1	2.424	6.281	10.432
pH of the mobile phase				
5.8	-1		6.099	10.278
6.8	0	2.233	6.153	10.345
7.8	+1		6.215	10.462
% Acetonitrile in the mobile phase				
53	-1	2.199	6.089	10.235
54	0	2.233	6.153	10.345
55	+1	2.456	6.243	10.452

TABLE 5 : Ruggedness of the method.

Parameter	DMSO Average of six values	Prednisolone AcetateAverage of six values	Lidocaine HCl Average of six values
Between Instrument I and II			
Instrument	% Content	% Content	% Content
I	103.0%	102.3%	97.2%
II	101.0%	103.9%	98.8%
Difference	2.0%	1.6%	1.6%
Between Instrument I and II			
Analyst	% Content	% Content	% Content
I	102.2%	103.3%	99.6%
II	103.5%	101.6%	98.5%
Difference	1.3%	1.7%	1.1%

CONCLUSION

The proposed RP-HPLC method allows for accurate, precise and reliable estimation of Diclofenac and Lidocaine in combined semi solid dosage form. The developed RP-HPLC method was found to be simple, rapid, selective, accurate and precise for the concurrent estimation of drugs in respective of three component dosage form of Dimethylsulfoxide, Prednisolone and Lidocaine HCl. The developed method can be used for routine quantitative simultaneous estimation of Di-

clofenac and Lidocaine in pharmaceutical preparation.

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