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Bioanalytical method development and validation of prednisolone in rat plasma using RP-HPLC method

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Abstract
A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed and validated for quantitative determination of Prednisolone in plasma. Metformin is used as an internal standard. The method was carried out with Anaytical technologies Lts. Model no. 3000Series. The optimized chromatographic conditions were optimized using a mobile phase methanol: water in the ratio of 70:30 at flow rate 0.9ml/min. Stationary phase was used as Grace C18 column (250mm x 4.6ID, partical size:5 micron). Detection was carried out at 238nm.The method was developed and tested for linearity range of 100ng/ml to 1600ng/ml. The developed method was validated in terms of selectivity, accuracy, precision, linearity and stability study. Proposed developed method can be used in bioanalytical, bioequivalence & pharmacokinetic studies with desired precision and accuracy.

Keywords: HPLC, vortex mixer, prednisolone

Introduction
Methods of measuring drugs in biological media are increasingly important for the study of bio availability and bioequivalence studies, new drug development study, clinical pharmacokinetics and therapeutic drug monitoring. Liquid-liquid extraction is probably the most widely used technique because the analyst can remove a drug or metabolite from larger concentrations of endogenous materials that might interfere with the final analytical determination and also the technique is simple, rapid, and has a relatively small cost factor per sample. Literature survey revealed that validated RP-HPLC method for the quantification of Prednisolone in rat plasma is not reported earlier. For estimation of the drugs present in biological fluid, HPLC method is considered to be more suitable. In this study we have developed compatible RP-HPLC method with liquid-liquid extraction process for determination of Prednisolone in plasma and the developed method is validated as per regulatory requirements.

Materials and Methods
Chemicals
API of Prednisolone was gifted by Zydus cadila, Ahmadabad. Solvents used are water for HPLC grade (Millie Q or equivalet), Ethyl acetate (HPLC grade), Diethyl ether, Chloroform and Dichloromethane.

Standard Solution Preparation
Standard solutions were prepared by using HPLC grade methanol and water in the ratio 1:1. Initially 10 mg of drug was weighted and transferred into the standard flask; the combined solvent (methanol and water) added and finally made the volume with the same up to 100ml to get 100ppm stock solution. The stock solution further seriely diluted was used for the analysis. The stock solution was maintained refrigerated at 8ºC.

Extraction Method
In this process first take 1 ml of plasma from sample which is previously stored at 5-7^{0}C. In this add 0.0125 mililitre of 1ppm of drug (prednisolone) which is prepared in methanol: water combination & 0.125 mililiter of 1ppm of internal standard. After this vortex the above prepared mixture for 3 mins. also in this add 0.200 mililiter of 1% of hydrochloric acid to provide acidic nature to the plasma. Again vortex the above mixture for 3-5 mins. In this add ethyl acetate which act as a extracting solvent & again vortex the mixture for 3-5 mins. Now withdraw 2ml of ethyl acetate in which drug is extracted in fresh tube & finaly allow to evaporate the solvent which will leave dried drug in tube & dilute it with 0.500 mililiter of mobile phase.

Method Validation
The method performance was evaluated for selectivity, accuracy, precision, linearity, stability at various conditions including bench top stability, freeze thaw stability and recovery.

Results and Discussion
Chromatographic Optimization
The chromatogram was developed initially using separation condition such as mobile phase (methanol: water in the ratio of 10:90 increasing order). The system was used Anaytical technologies Lts. Model no. 3000Series. The optimized chromatographic conditions were optimized using a mobile phase methanol: water in the ratio of 70:30 at flow rate 0.9ml/min respectively with the stationary phase was used as

Grace C18 column (250mm x 4.6ID, partical size: 5 micron). The chromatograms of Prednisolone with IS have been shown in fig.1.

Selectivity

The desired method used RP-HPLC method for separation of Prednisolone from Metformin (IS) and was shown to be selective for the analyte and its is (retention times for prednisolone and metformin were 5.24 and 6.11 minutes respectively).No interfering peaks were observed with the same retention time of the analyte when different plasma samples were analysed.fig.2 and fig.3 represent the chromatograms of blank plasma and plasma sample spiked with drugs respectively.

Linearity

Linearity was demonstrated from 100-1600 ng/ml.fig.4 showes calibration curve of Prednisolone. The calibration curve includes 6 calibration standards which are distributed 0.999 with goodness of fit.

Accuracy and Precision

Accuracy and Precision was evaluated by analyzing 5 bathches, each batch consist of five replicates of LQC, MQC and HQC. The precision and accuracy of the method for each concentration levels are represented in Table 1.

Table 1: Intraday and Interday Precision and Accuracy of Prdnisolone

			Standard Deviation		Accuracy	Precision
Conc.	**Conc.**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
LQC	0.187	3.164	3.374	0.162158873	4.80613138	4.80613137
	0.187	3.525				
	0.187	3.41				
	0.187	3.25				
	0.187	3.521				
MQC	1.5	0.5295	0.555	0.039515351	7.11296242	7.112962423
	1.5	0.5982				
	1.5	0.5025				
	1.5	0.5621				
	1.5	0.5854				
HQC	2.5	0.3244	0.33466	0.011809234	3.52872577	3.528725774
	2.5	0.3521				
	2.5	0.3254				
	2.5	0.3302				
	2.5	0.3412				

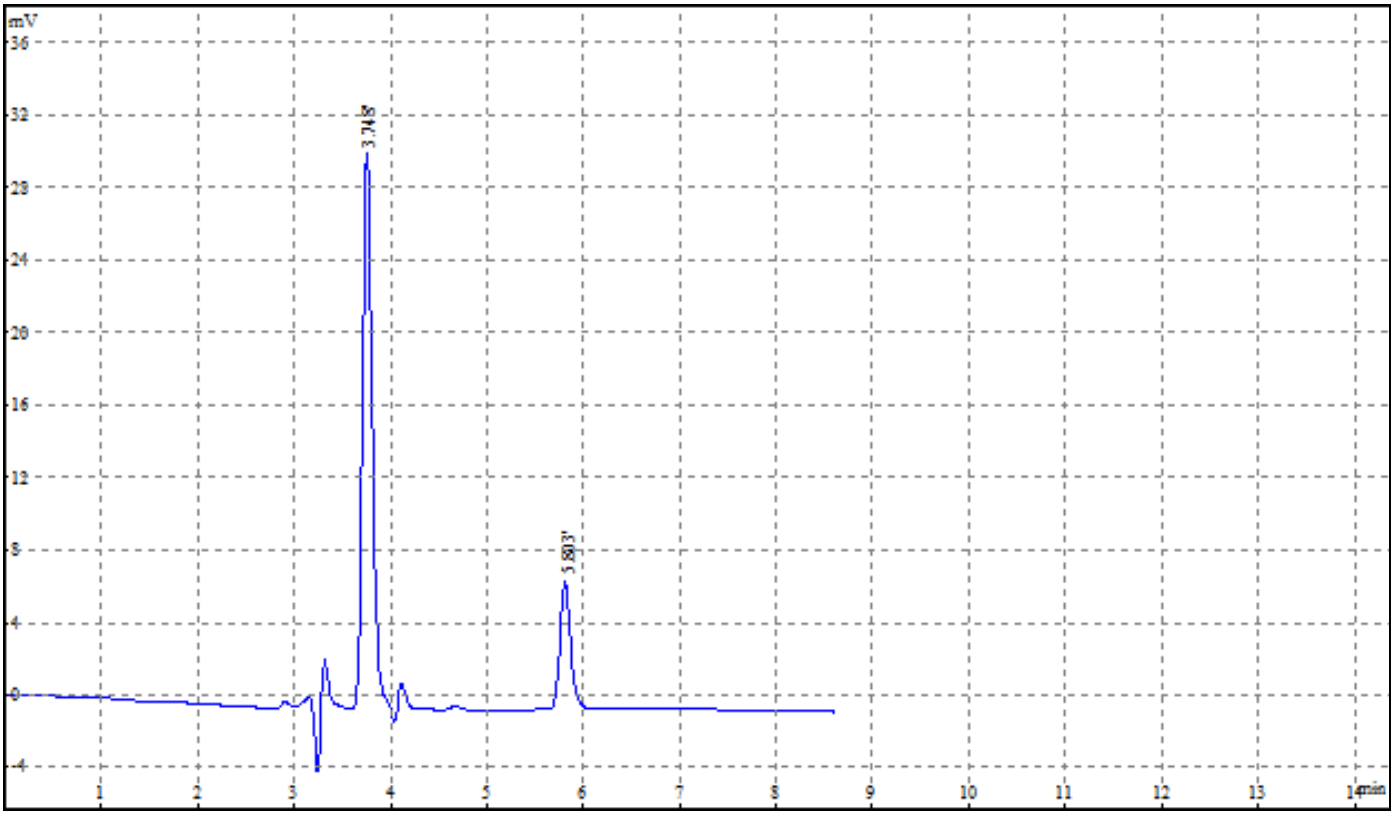

Fig 1: Typical chromatogram of Prednisolone with Metformin

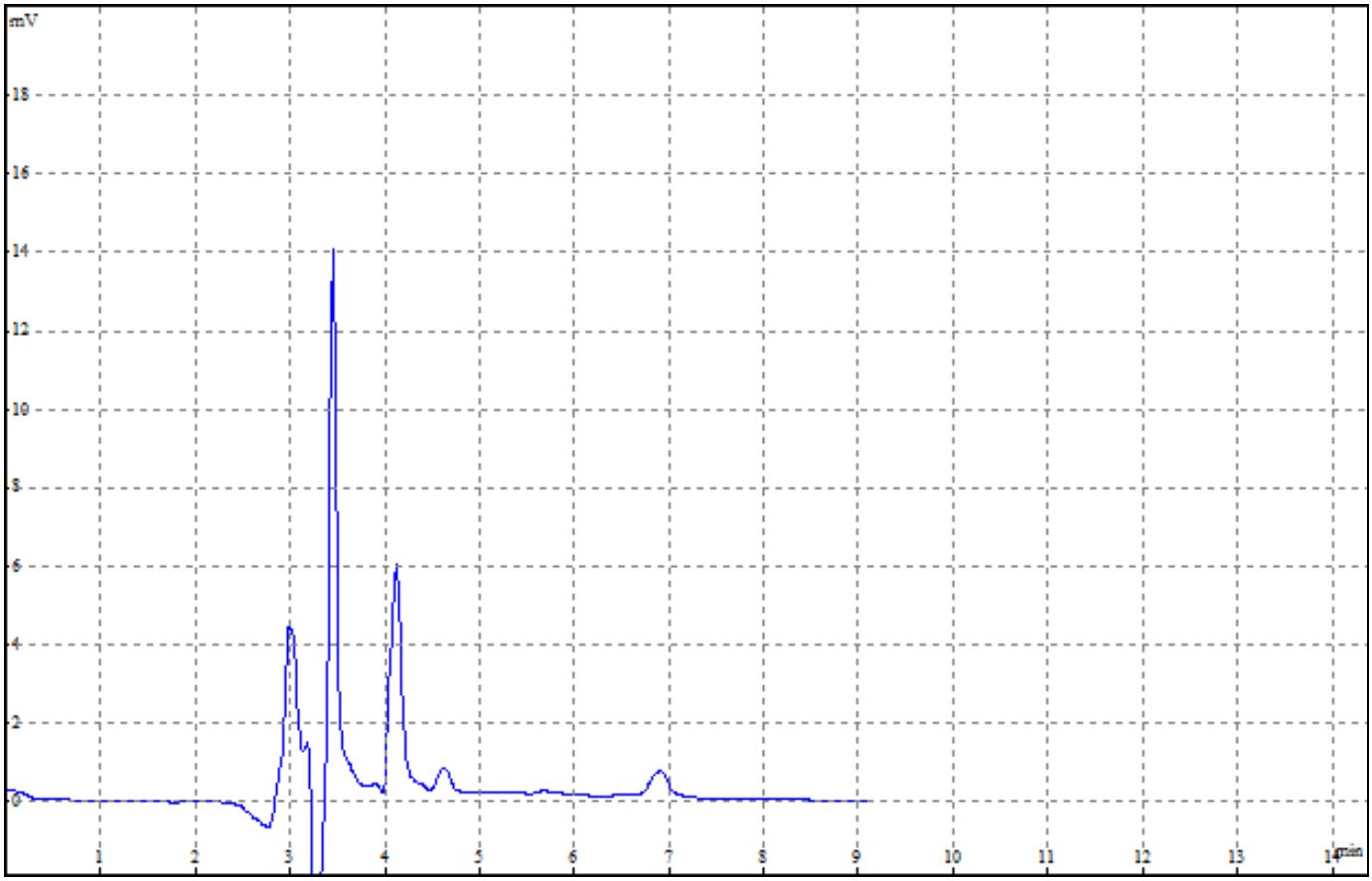

Fig 2: Typical chromatogram of blank plasma sample

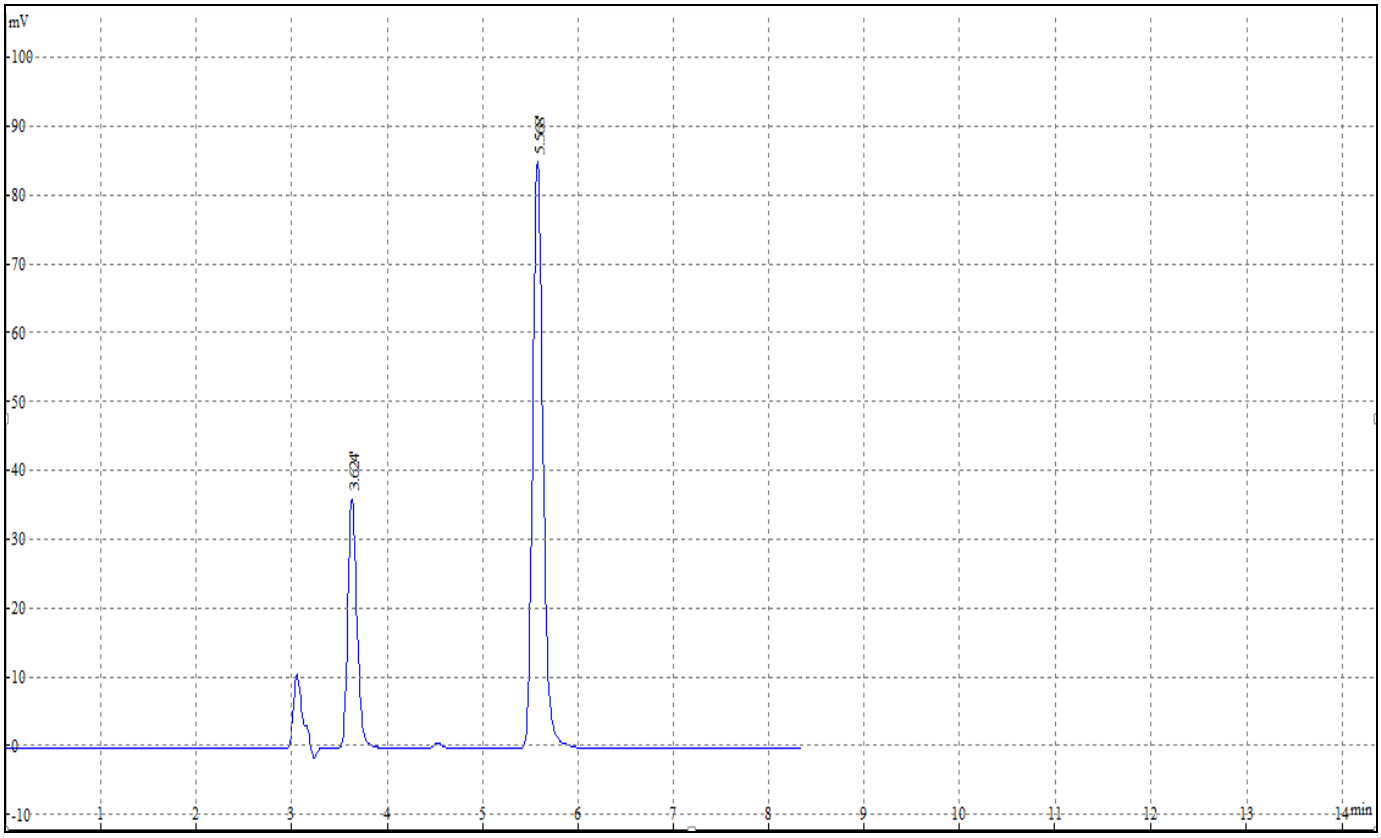

Fig 3: Typical chromatogram of plasma sample spiked with Prednisolone and Metformin

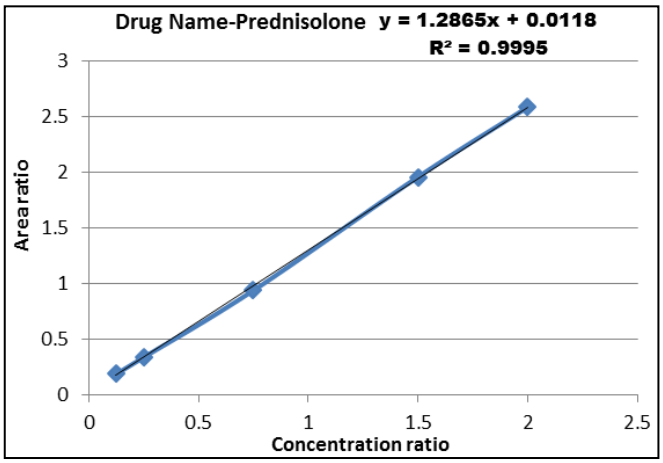

Fig 4: Calibration curve of Prednisolone

Recovery

The recovery was evaluated by comparing response of extracted and un-extracted samples. The average recovery for Prednisolone in plasma was ranged from 72.2 to 77.6% for the low, medium and high quality control samples with an average of 74.2%.

Stability Studies

Stability studies were performed to evaluate the stability of Prednisolone both in aqueous solution and in plasma after exposing to various stress conditions. The stability studies performed include bench top stability, freeze thaw stability, long term and short term stock stability. Prednisolone was found to be stable for three freeze and thaw cycles.

Table 2: Validation Parameters of Prednisolone by RP- HPLC method

S. No	Parameters	Results
1.	Selectivity	Pass
2.	System suitability	Pass
3.	Accuracy & precision	Pass
4.	Linearity	Prdnisolone-R^2 = 0.999
5.	Recovery	Pass
6.	Bench top stability	Short term stock stability-(2hrs,12hrs,24 hrs) Long term stock stability-(10days,20days,30 days)
7.	Freeze thaw stability	Pass (3 cycles)

Conclusion

The current validated bio analytical RP-HPLC method for Prednisolone offers good accuracy and significant advantages in terms of linearity, stability & selectivity. The separation method developed produce acceptable values of recovery. The chromatograms developed has well resolved peaks of Prednisolone without any interference. From the results we conclude that the developed method can be used in bio analytical, bioequivalence & pharmacokinetic studies with desired precision and accuracy.

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