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Bio analytical Method Development and Validation of Selected Corticosteroid 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 Betamethasone in plasma. Paracetamol is used as an internal standard. The method was carried out with Analytical technologies Lts. Model no. 3000Series. The optimized chromatographic conditions were optimized using a mobile phase methanol: water in the ratio of 60:40 at flow rate 0.9ml/min. Stationary phase was used as Grace C18column (250mm x 4.6ID, partical size: 5 micron). Detection was carried out at 242nm.the method was developed and tested for linearity range of 10ng/ml to 160ng/ml. The developed method was validated in terms of selectivity, accuracy, precision, linearity and stability study. Proposed developed method can be used in bio analytical, bioequivalence & pharmacokinetic studies with desired precision and accuracy.

Keywords: HPLC, Vortex mixer, Betamethasone

Introduction
Methods of measuring drugs in biological media are increasingly important for the study of bioavailability 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 Betamethasone 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 Betamethasone in plasma and the developed method is validated as per regulatory requirements.

Materials and Methods
Chemicals
API of Betamethasone 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 was 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 (Betamethasone) 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 60:40 at flow rate 0.9ml/min with the stationary phase was used as Grace

C18column (250mm x 4.6ID, partical size: 5 micron).
The chromatograms of Betamethasone with IS have been shown in fig.1.

Selectivity
The desired method used RP-HPLC method for separation of betamethasone from Paracetamol (IS) and was shown to be selective for the analyte and its IS (retention times for betamethasone and Paracetamol were 5.80 and 7.20 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 10.0-160 ng/ml.fig.4 shows calibration curve of betamethasone. The calibration curve includes 6 calibration standards which are distributed 0.758 with goodness of fit.

Accuracy and Precision
Accuracy and Precision was evaluated by analyzing 3 bathches. Each batch consist of three replicates of LQC, MQC and HQC. The interday and intraday precision and accuracy of the method for each concentration levels are represented in Table 1.

Table 1: Intraday and Inter day Precision and Accuracy of Betamethasone.

			Standard Deviation		Accuracy	Precision
Conc.	**Conc.**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
LQC	0.1875	3.0972	3.0692	0.03857	1.2568	1.2568
	0.1875	3.0252				
	0.1875	3.0852				
MQC	1.5	0.0662	0.066	0.00385	5.8345	5.8345
	1.5	0.0621				
	1.5	0.0698				
HQC	2.5	0.0325	0.036	0.003325	9.2266	9.2266
	2.5	0.0365				
	2.5	0.0391				

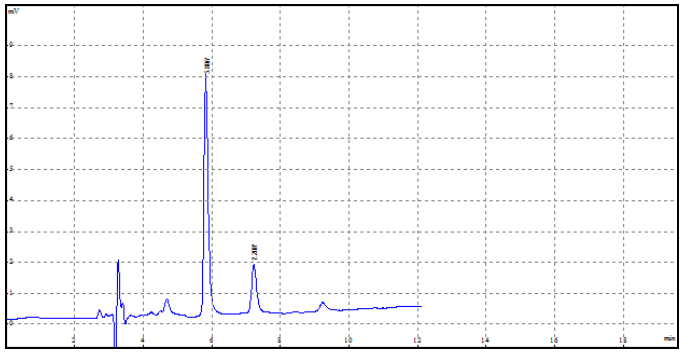

Fig 1: Typical chromatogram of Betamethasone with Paracetamol.

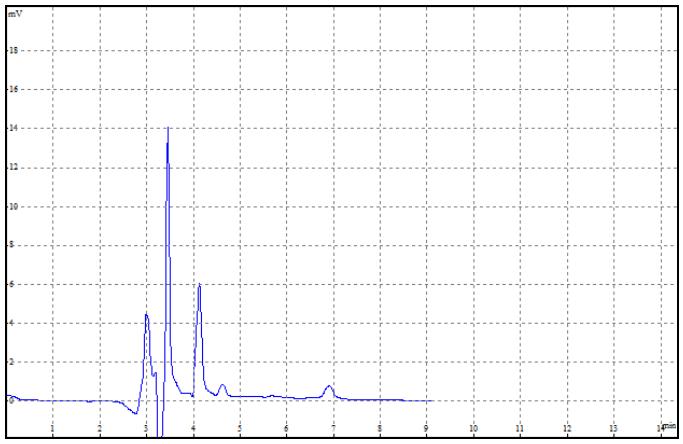

Fig 2: Typical chromatogram of blank plasma sample.

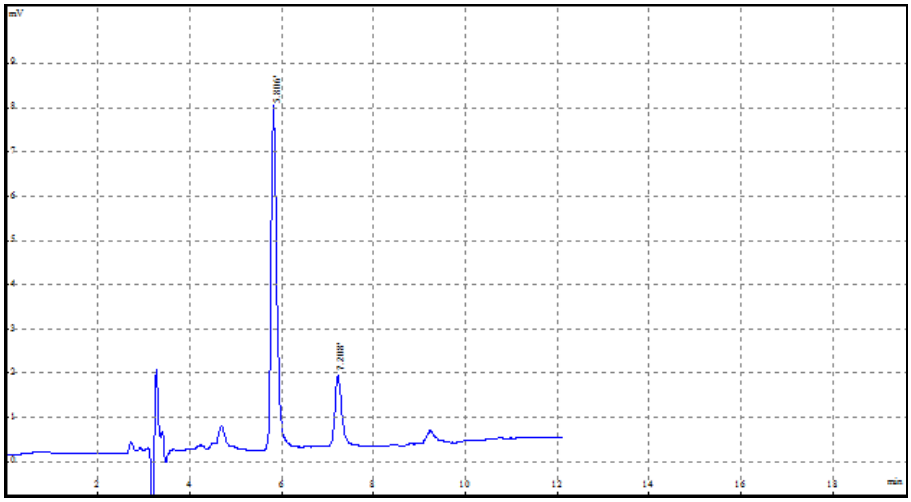

Fig 3: Typical chromatogram of plasma sample spiked with Betamethasone and Paracetamol.

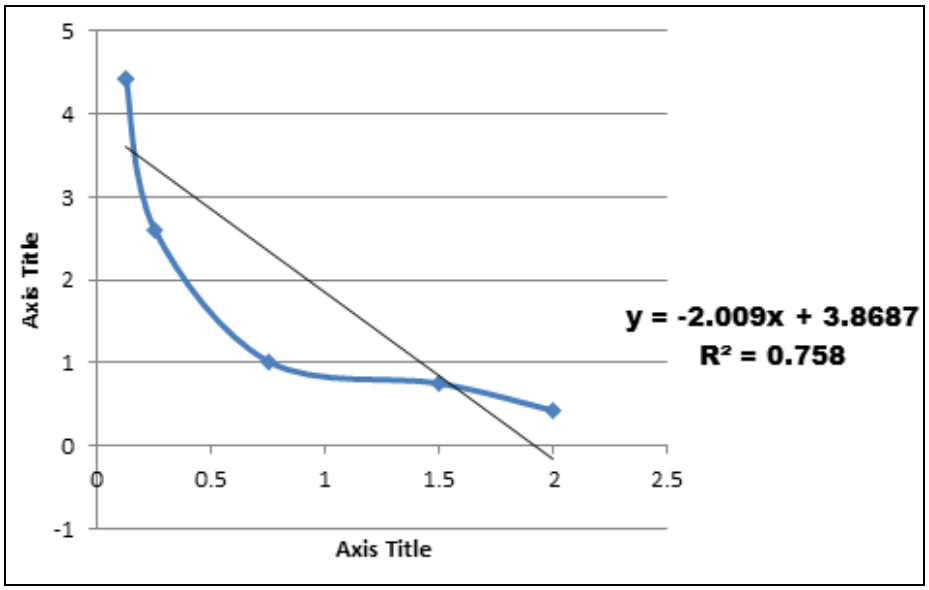

Fig 4: Calibration curve of Betamethasone.

Recovery

The recovery was evaluated by comparing response of extracted and unextracted samples. The average recovery for Betamethasone in plasma was ranged from 85.2 to 88.6% for the low, medium and high quality control samples with an average of 87.2%.

Stability Studies

Stability studies were performed to evaluate the stability of Betamethasone 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. Betamethasone was found to be stable for three freeze and thaw cycles.

Table 2: Validation Parameters of Betamethasone by HPLC method.

Sr. No	Parameters	Results
01	Selectivity	Pass
02	System suitability	Pass
03	Accuracy & precision	Pass
04	Linearity	Betamethasone- R^2 = 0.758, Dexamethasone- R^2 = 0.721 Prdnisolone- R^2 = 0.725
05	Recovery	Pass
06	Bench top stability	Short term stock stability-(2hrs, 12hrs, 24 hrs) Long term stock stability-(10days, 20days, 30 days)
07	Freeze thaw stability	Pass (3 cycles)

Conclusion

The current validated bio analytical HPLC method for Betamethasone 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 above selected corticosteroid 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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