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RP-HPLC METHOD DEVELOPMENT FOR THE QUANTITATIVE DETERMINATION OF DEXAMETHASONE IN HERBAL FORMULATION

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ABSTRACT

A simple, sensitive method for the determination of dexamethasone used as an adulterant in herbal formulation was developed using reverse phase high performance liquid chromatographic method (RP-HPLC). The analysis was performed on cyano (250 × 4.6mm, 5µm) column using 0.1% ortho phosphoric acid and acetonitrile (80: 20 v/v) as mobile phase at flow rate 1.2 mL/min. The eluents were monitored with UV detector at 242 nm. In the developed method dexamethasone elutes at a retention time of 8.8 min. The proposed method is having linearity in the concentration range from 5 to 60 µg/mL of dexamethasone. The present method was validated with respect to system suitability, linearity, precision, limit of detection (LOD) and limit of quantification (LOQ), accuracy (recovery) and robustness. The proposed method can be readily utilized for the detection of dexamethasone as an adulterant in herbal formulation.

KEYWORDS: Dexamethasone, Herbal formulation, RP-HPLC.

1.1 INTRODUCTION

Dexamethasone[1] is chemically known as 9-fluoro-11β,17-dihydroxy-16α-methyl-21-(phosphonooxy) pregna-1, 4-diene-3,20-dione (Fig 1). It is steroidal prescription drug used to treat inflammatory conditions like arthritis, allergies and skin problems like psoriasis and eczema.

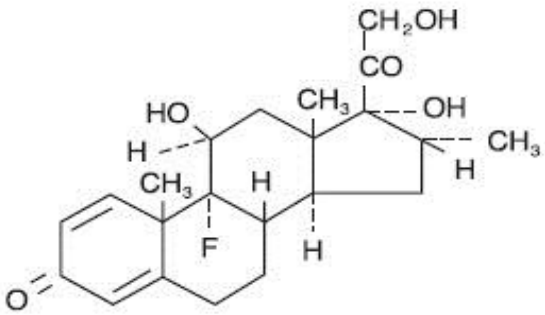

Figure 1: Structure of Dexamethasone.

Herbal medicines are increasingly being used in both preventative and treatment based medicines and tonics because consumers perceive herbals as natural, safe, harmless and free from adverse side effects. However, several reports[2,3] exist of adulteration of herbal formulations with synthetic drugs such as corticosteroids, steroidal/non-steroidal anti-inflammatory agents, anti-allergic, skin products etc. Herbal formulations intended for anti-inflammatory, anti-allergic, anti-asthmatic effect etc. are most likely to be adultered with corticosteroids for faster action. Adulteration[4-6] of herbal drugs with synthetic adulterants can lead to many complications including herb-drug interactions. Dexamethasone is one of the most frequently used adulterant in herbal formulation[7] because it gives immediate relief from allergic symptoms due to suppression of immune system.

Literature available for the identification and quantitative determination of dexamethasone in herbal formulation is scanty. Yoe-Ray Ku *et al*., carried out studies on systematic evaluation and comparision of the recoveries of eight steroids from spiked traditional chinese medicine (TCM) extract using silica gel solid-phase extraction (SPE) are reported. Yun Sik Nam *et al*., Monitoring of clobetasol propionate and betamethasone dipropionate as undeclared steroids in cosmetic products used for the treatment of eczema, seborrhoea and psoriasis, without any indication on the label of the cosmetic products.

The present study was aimed at developing a simple, sensitive, rapid, reproducible, precise and accurate HPLC method to identify and quantify dexamethasone in herbal formulation.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Dexamethasone obtained as gift sample from Strides Arco Labs, Banglore, India. HPLC grade water, HPLC grade acetonitrile were procured from Merck Ltd, Mumbai, India. All

other chemicals used are analytical reagent grade (AR grade) like ortho phosphoric acid procured from Loba Chemie, Mumbai, India.

2.2 Instrumentation and analytical conditions

Shimadzu HPLC (LC Solutions handling system) with LC- 2010A_{HT} Prominence liquid chromatograph and SPD-20A prominence UV/Visible detector was employed for present study. In addition, Shimadzu electronic balance, and micropore filtration assembly were used in this study.

The chromatographic determination was performed using 0.1% ortho phosphoric acid and acetonitrile in the ratio of 80:20 (v/v) as mobile phase and a stationary phase of stainless steel column phenomenex- cyano column (250 × 4.6mm, 5µm). The column oven temperature is maintained at 40°C.The chromatography run time was maintained up to 12.0 min with flow rate at 1.2 mL/min with injection volume 10 µL and the eluent was monitored at 242 nm.

2.3 Preparation of stock solutions

2.3.1 Preparation of standard stock solution

Accurately weighed 100.0 mg of dexamethasone (bulk drug) was dissolved in 50.0 mL of mobile phase in 100 mL volumetric flask and sonicated for five minutes until it dissolves the material and the volume was made up with mobile phase. From this 10.0 mL of solution was taken and diluted to 100 mL (stock solution). Solution was filtered through 0.45 µm filter paper.

2.3.2 Preparation of calibration curve

Aliquots of dexamethasone ranging from 0.5 – 6.0 mL were pipetted into a series of 10 Ml volumetric flask and diluted to the mark with mobile phase. The linearity range of dexamethasone was found to be 5.0 – 60.0 µg/mL. The calibration curve was constructed by plotting peak area against concentration.

2.3.3 Preparation of sample extract

A known amount of dexamethasone was spiked into herbal medicine (GOUTEX found to contain no dexamethasone by proposed method). 1.25 gm of sample powder was accurately weighed and dissolved in 100 mL water, then subjected to sonication for 20 min and filtered. Mark was repeatedly extracted with 100 mL water thrice. The total water extract was

collected and extracted with chloroform thrice (125 mL x 3) and discarded chloroform extract. Finally volume of water extract is made up to 500 mL with water.

2.4 Optimization of the method

The optimization of the analytical procedure has been carried out by varying the stationary phase, mobile phase composition, flow rate of the mobile phase. In the present investigation the best separation of dexamethasone from interferences in formulation was achieved using a stationary phase of phenomenex cyano column (250mm X 4.6mm, 5µm) in comparision with that of phenomenex C18 column(250mm X 4.6mm, 5µm). With C18 column, the drug peak was eluted at a lower retention time which was merging with the formulation peaks (Fig 4 & 5). Various solvent systems such as acetonitrile: water, methanol: water, 0.1% ortho phosphoric acid: acetonitrile were tried in different ratios to get good chromatogram. Best results were obtained with mobile phase of acetonitrile, 0.1% orthophosphoric acid (20:80 v/v) (Fig 2).

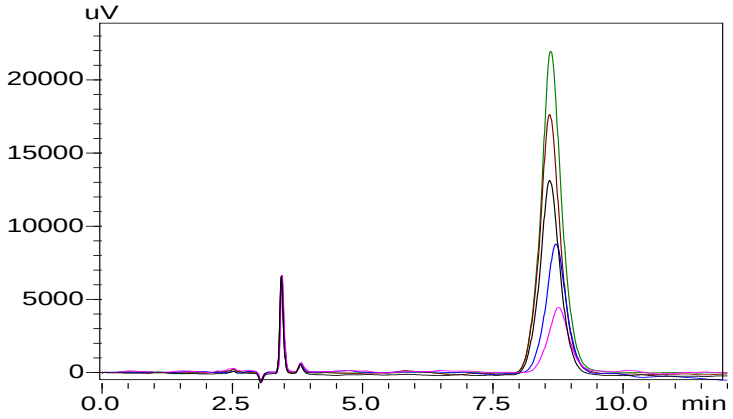

Fig 2: chromatogram for standard drug (Dexamethasone).

3. METHOD VALIDATION

The method was validated for the parameters like system suitability, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, ruggedness and robustness in accordance with International Conference of Harmonization (ICH) Guidelines.[8, 9]

3.1 System suitability testing

System suitability study was carried out by six replicate injections of the drug containing 5mcg/mL of concentration. System suitability of the method was evaluated by analyzing the tailing factor, theoretical plates of the column, mass distribution ratio (capacity factor), column efficiency (N), HETP and relative retention are tabulated in (Table 1).

Table 1: System suitability parameters of the proposed analytical method for Dexamethasone

Parameter	Dexamethasone
Theoretical plates/meter	2875
HETP	79.9
Symmetric factor	0.914
Retention time in minutes (Rt)	8.8
Tailing factor	1.1
Capacity factor	0.798

3.2 Linearity

The calibration curve was established by plotting the peak areas of dexamethasone versus the concentrations of dexamethasone samples. Linear correlations were found between peak areas and dexamethasone concentration (Fig. 3) and are described by the regression equations:

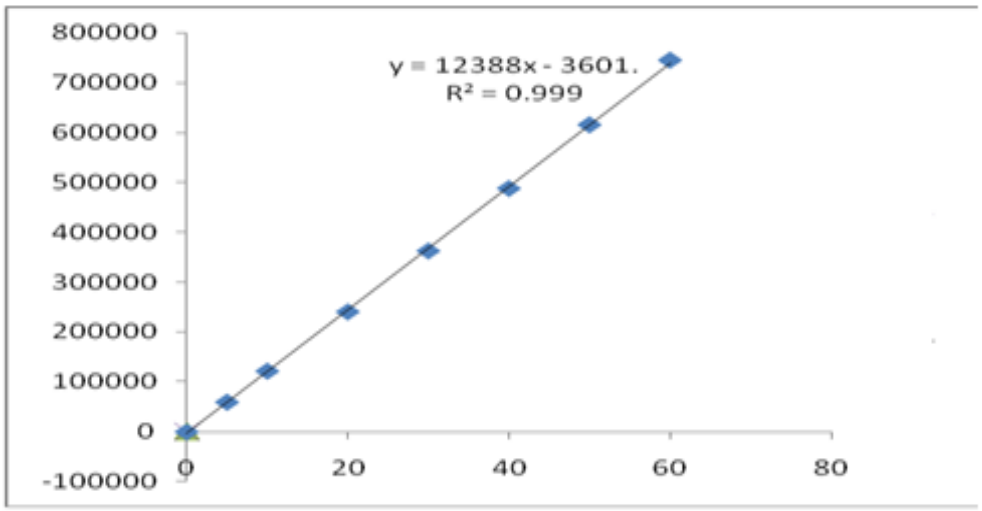

Figure 3: Calibration graph of Dexamethasone.

Y= 12388x + 3601; r^2 0.999, where r^2 is the correlation coefficient. The Beer's law is obeyed in the concentration range of 5-60 µg/mL. The results of the study are shown in (Table 2).

Table 2: The regression characteristics of the proposed method

Parameter	Values
Range (µg/mL)	5-60
Regression Equation	Y= 12388x - 3601
Regression coefficient (r^2)	0.999
Slope	12388
Intercept	3601

3.3 Accuracy (Recovery study)

Accuracy of the method was studied by recovery experiments. The recovery was performed at three levels 80, 100 and 120% as per ICH guide lines. The present recovery experiments were performed at three levels concentrations (18, 20, 22 µg/mL) by adding known amount of pure drug to the previously analyzed herbal sample solution (sample solution of known concentration 10 µg/mL). The results of the study are shown in (Table 3).

Table 3: Percent recovery studies of the method

Name of the Drug	Sample ID	Amount added (µg/ml)	Amount recovered (µg/ml) n=3	Percentage recovery	Average Percentage recovery
Dexamethasone	80%	18	17.10	94.96	95.67
	100%	20	18.93	94.86	
	120%	22	21.40	97.26	

3.4 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines .The limit of detection was found to be 129 ng and limit of quantification was found to be 388ng.

3.5 Precision

Precision of the method was evaluated in terms of intra-day and inter- day precision. 5µg/mL concentration of dexamethasone was analyzed in six replicates on the same day (intra-day precision) and in five consecutive days (inter-day precision). The peak-area based intra-day RSD value was 1.0 %. The inter-day precision showed higher RSD values of 1.12 %. The results of the study are shown in (Table 4).

Table 4: Intraday and interday precision

Replicates	Interday	Intraday
1	60239	59639
2	62784	60286
3	63729	63694
4	60482	59299
5	63523	60491
6	61329	61014
Average	62372	60237
Standard deviation	695.4	605.8
% RSD	1.12	1.0

3.6 Ruggedness

Method ruggedness was checked by varying the lot number and manufactures of reagents Orthophosphoric acid), solvents (HPLC grade water, acetonitrile) procured from Qualigens Fine chemicals (Mumbai, India) and column (phenomenex Cyano -250 x 4.6 mm; 5 µm). The effect of changes was observed on chromatographic parameters like retention time, tailing factor, resolution, peak area and theoretical plates. The results obtained show no significant variation in above parameters indicating ruggedness of method.

3.7 Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. Robustness is done by slightly varying the experimental conditions like the proportions of the mobile phase (±2% on total proportion), column temperature (40ºC ± 5ºC), flow rate (1.2 mL/min ± 0.2 mL) and wavelength (242 nm ± 2 nm) of detection. The effect of changes was observed on chromatographic parameters like retention time, tailing factor, resolution, peak area and theoretical plates. The results obtained show no significant variation in above parameters indicating robustness of method.

3.8 Application of method for estimation of dexamethasone by spiking into herbal formulation

A known amount of standard drug was added to herbal formulation and then the sample is extracted by liquid-liquid extraction (extraction of sample powder (formulation) with water followed by extraction with chloroform). Water extract is collected and analysed in HPLC. The drug peak is obtained at a retention time of 8.8 min (Fig 6). The values are compiled and reported in (Table-5).

Table 5: Recovery of Dexamethasone added to herbal medicine

S. NO	Amount added(mg)	Dexamethasone found(mg)	Recovery
1	0	0	-
2	1	0.9452	94.52
3	2	1.942	97.22
4	4	3.8268	95.67

Similarly without spiking the drug, the herbal medicine is extracted in the same manner as above and water extract is analysed in HPLC. This shows the blank chromatogram (Fig 5).

4. RESULTS AND DISCUSSION

The objective of present study was to develop a rapid and sensitive HPLC method for the quantitative determination of dexamethasone in herbal formulation using most commonly employed HPLC method. In the proposed method cyano column was used and eluents were monitored at 242 nm. Mobile phase consisting of 0.1% ortho phosphoric acid, acetonitrile in the ratio of 80:20 (v/v) with a flow rate of 1.2 mL/min, run time 12.0 min and retention time was 8.8 min had given immaculate results when compared to other methods. The system suitability parameters have been determined and values were tabulated in (Table I). A plot of ratio of peak area against concentration (µg/mL) gave a linear regression (r^2=0.999) over the concentration range 5 - 60 µg/mL.

The proposed method was also validated for intra and inter day variation and %CV was found to be 1.0 %. The lowest level of quantification was observed to be 388 ng/mL which indicates the sensitivity of method. The accuracy of HPLC method was assessed by adding known amount of the drug to pre analyzed sample solution.

A known amount of dexamethasone was added to herbal medicine and the overall recovery was estimated by standard addition method. The % recovery (95.67%) indicating that proposed method is accurate. Although the presence of interference in herbal medicine did not obviously interfere with the identification of dexamethasone, those interferences could be moved efficiently with Liquid Liquid Extraction (LLE). Robustness results obtained showed no significant variation in parameters (retention time, tailing factor, resolution, peak area and theoretical plates) indicating robustness of method. In comparison with other published methods for determination of dexamethasone the advantages of this method are ease of operation, short analysis time (total run time < 12 minutes, which is important for routine analysis) and satisfactory limit of quantification.

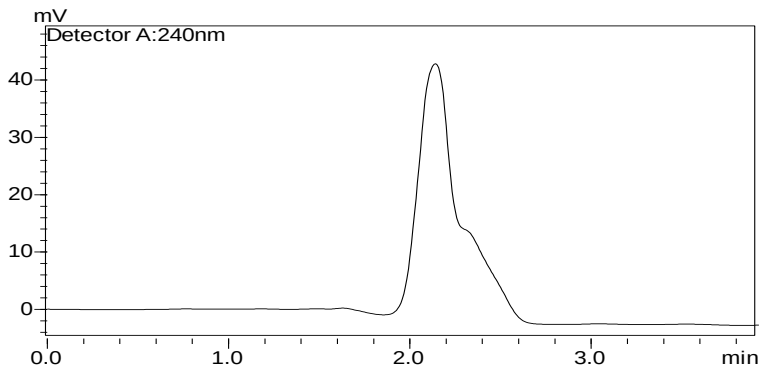

Fig 4: Chromatogram of Dexamethasone in C18 column.

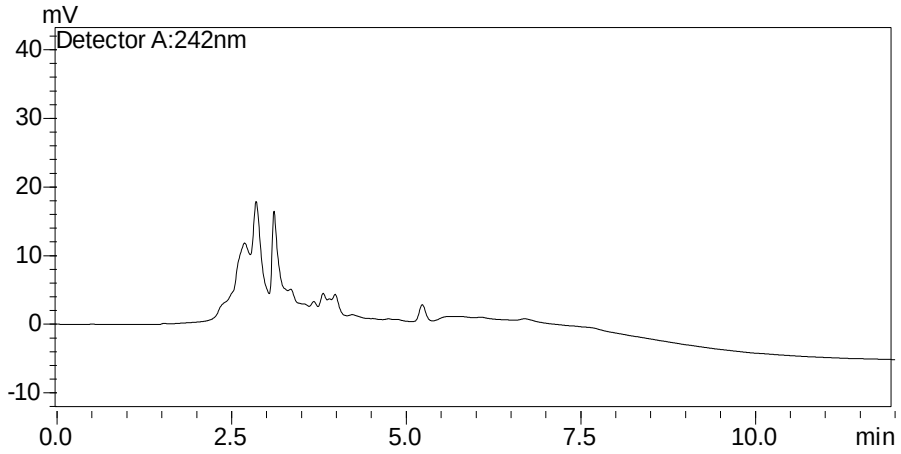

Fig 5: chromatogram of herbal extract without Dexamethasone.

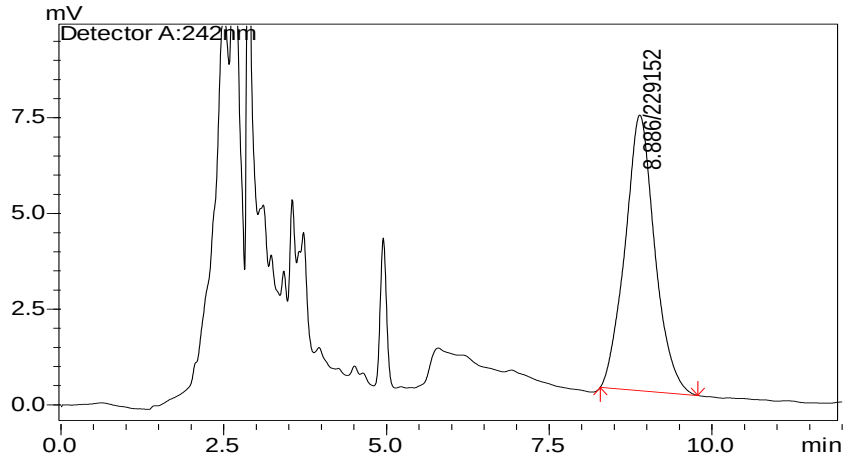

Fig 6: Chromatogram of herbal extract with Dexamethasone using cyano column.

5. CONCLUSION

It is important to develop a fast and efficient method to detect dexamethasone in herbal medicine. In this study we have developed a method combining LLE and HPLC. The developed method was found to be simple, sensitive, accurate, precise and reproducible. It can be used for routine quantitative determination of dexamethasone as adulterant in herbal formulations.

6. ACKNOWLEDGEMENT

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