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Research Article

NOVEL STABILITY-INDICATING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY METHOD FOR SIMULTANEOUS ESTIMATION OF HYDROCORTISONE AND TETRACYCLINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: A combination of hydrocortisone and tetracycline as topical ophthalmic ointment is used for skin irritations, eye infections, inflammation, skin infections, acne, and rashes. The objective of the current work is to a simple, rapid, accurate, and precise, stability-indicating reverse-phase liquid chromatographic method was developed for the simultaneous estimation of hydrocortisone and tetracycline in bulk and pharmaceutical dosage form.

Methods: The separation was carried out in Discovery C18 column (250 × 4.6 mm, 5 µm) using mobile phase ratio of water (pH 2.2 adjusted with orthophosphoric acid):acetonitrile (40:60 v/v) in an isocratic elution mode with a flow rate of 1.0 ml/min at detection wavelength of 244 nm. The injection volume was 10 µl and the column temperature was set at 30°C.

Results: The retention time for hydrocortisone and tetracycline was found to be 2.214 ± 0.001 min and 3.497 ± 0.001 min, respectively. Calibration curves were linear (r^2=0.999) at a concentration range of 2.5–15 mg/ml for both hydrocortisone and tetracycline. The percentage recoveries were found to be 99.13–99.67% for hydrocortisone and 99.39–99.61% for tetracycline. Relative standard deviation was found to be 0.3% for both the drugs. Limit of detection and limit of quantification values of hydrocortisone and tetracycline were found to be 0.09 µg/ml and 0.27 µg/ml and 0.17 µg/ml and 0.52 µg/ml, respectively. The drugs were subjected to various stress conditions and found no interference of degraded products peak at the retention times of analyte peaks.

Conclusion: A rapid and accurate reverse-phase high-performance liquid chromatographic method was developed for simultaneous estimation of hydrocortisone and tetracycline, and the method was validated as per the International Council for Harmonization guidelines. Hence, the developed method can be successfully applied for the simultaneous estimation of hydrocortisone and tetracycline in bulk and ointment formulation.

Keywords: Hydrocortisone, Tetracycline, Stability-indicating reverse-phase high-performance liquid chromatographic, Method development, Method validation.

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INTRODUCTION

Hydrocortisone (Fig. 1) is chemically (8S,9S,10R,11S,13S,14S,17R)-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-2,6,7,8,9,11,12,14,15,16-decahydro-cyclopenta[a]phenanthren-3-one. It is a synthetic corticosteroid that can be administered into the eyes to decrease the inflammation. It works by acting within cells to decrease the release of these substances in a particular area, thereby reducing swelling, redness, and itching. Tetracycline (Fig. 2) is chemically (4S,4aS,5aS,6S,12aR)-4-(dimethylamino)-1,6,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carboxamide. It is a broad-spectrum naphthacene antibiotic, binds to 30S subunit of bacterial ribosome [1]. It interferes with the binding of aminoacyl-tRNA to the mRNA-ribosome complex, thereby inhibiting protein synthesis.

Extensive literature survey revealed that there were ultraviolet (UV) spectroscopic methods for the estimation of hydrocortisone and with other combinations [2,3]. Few reverse-phase high-performance liquid chromatographic (RP-HPLC) methods were reported for the estimation of hydrocortisone alone and with other combinations [4-7]. A high-performance liquid chromatographic tandem mass spectrometric method for the estimation of hydrocortisone in human urine has been reported [8] and a high-performance thin-layer chromatographic method has been reported for quantification of hydrocortisone and clotrimazole [9]. Along with these RP-HPLC methods have reported for the estimation of tetracycline [10,11]. However, no RP-HPLC method has been reported for the simultaneous assay of specified drugs in bulk and pharmaceutical formulation. Therefore, it was thought propitious to develop HPLC procedure that serves as a rapid, accurate, and simple method for the simultaneous estimation of hydrocortisone and tetracycline in bulk and pharmaceutical formulation.

METHODS

Chemicals and reagents

Reference standards of hydrocortisone and tetracycline were obtained as gift samples from Spectrum Pharma Research Solutions (Hyderabad, India). HPLC grade acetonitrile, water (Milli-Q), and chemicals of AR grade were procured from Merck (Mumbai). Pharmaceutical dosage form (Cortecycline ointment) was obtained as a gift sample from Syntho Pharmaceuticals Pvt. Ltd. (India).

Instrumentation

Waters HPLC 2695 system equipped with quaternary pumps and photodiode array detector, Discovery C18 column (4.5 × 250 mm × 5 µm) was employed for the study. The data acquisition and integration were performed using Empower 2 software.

Optimized chromatographic conditions

Separation was achieved on a Discovery C18 (250 × 4.6 mm, 5 µm) column at wavelength of 244 nm, using mobile phase water (pH 2.2

adjusted with orthophosphoric acid [OPA]):acetonitrile (40:60) in an isocratic elution mode with an injection volume 10 µl at a flow rate of 1.0 ml/min. The column temperature was set at 30°C.

Diluent

Acetonitrile and water in the ratio of 50:50 were used as diluent.

Preparation of standard solution

The standard stock solution was prepared by accurately weighing 2.5 mg of hydrocortisone and 2.5 mg of tetracycline standards and transferred into 25 ml clean dry volumetric flask, 10 ml of diluent was added, sonicated for 10 min, and made up to the final volume with diluent. 1 ml from the above stock solution was taken into a 10 ml volumetric flask and made up to the volume with diluent to get a final concentration of 10 µg/ml of hydrocortisone and 10 µg/ml of tetracycline.

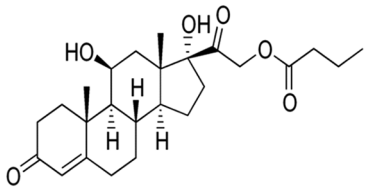

Fig. 1: Chemical structure of hydrocortisone

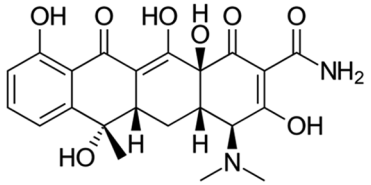

Fig. 2: Chemical structure of tetracycline

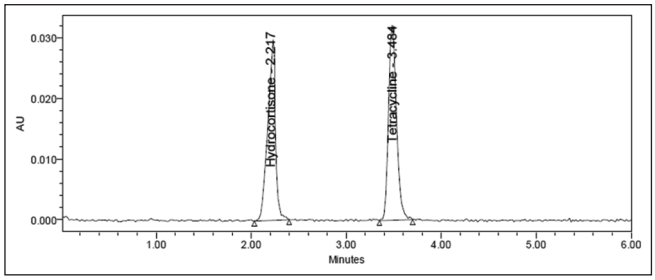

Fig. 3: Standard chromatogram of hydrocortisone and tetracycline

Table 1: Results of system suitability

Parameters	Hydrocortisone	Tetracycline	Limits
Retention time	2.214±0.001	3.497±0.001	-
Resolution	-	8.2	NLT 2
Theoretical plates	3496.4	8592.7	NLT 2000
Tailing factor	0.9	1.2	NMT 2

NLT: Not less than; NMT: Not more than

Preparation of sample preparation

The sample solution was prepared by taking 3 g of ointment, transferred into motor, triturated for 5 min, from this, 1 g of ointment was weighed and transferred to a 100 ml volumetric flask. To this, 10 ml of glacial acetic acid was added and stirred for 40 min on a magnetic stirrer and made up to mark with methanol and then it was centrifuged for 20 min. The supernatant was collected and filtered using 0.45 µm filters. From this, 1 ml of filtered stock solution was taken, transferred to 10 ml of volumetric flask, and made up with diluent.

Method validation

Linearity

Linearity of the developed method was established at concentration levels from 50% to 150% of target assay concentration. The corresponding peak areas were noted and the data were subjected to least square regression analysis. From this, limit of detection (LOD) and limit of quantification (LOQ) values were calculated using the formula method as given in International Council for Harmonization (ICH) guidelines [12].

Precision

The precision of the assay method was determined by repeatability which was evaluated at 100% concentration level. Six determinations were performed and the percentage relative standard deviation (RSD) was calculated.

Accuracy

The accuracy of the assay method was evaluated by recovery studies in triplicate at three concentration levels (50, 100, and 150%) by the following standard addition method and the percentage recoveries were calculated.

Robustness

The robustness of the assay method was established by introducing small but deliberate alterations in the optimized chromatographic conditions such as flow rate (±0.1 ml/min), temperature (±2°C), and mobile phase ratio (±5%) and system suitability parameters were evaluated.

Forced degradation studies

Stress degradation was carried out to check the specificity of the developed method. The drugs were subjected to various stress conditions such as acid, alkali, oxidative, photolytic, and thermal conditions recommended by ICH guidelines. The degraded products were generated and analyzed. For acid degradation studies, 1 ml of 2 N HCl was added to 1 ml of stock solution and refluxed for 30 min at 60°C. To this, 1 ml of 2 N NaOH was added to neutralize the resultant solution and was diluted to 10 ml with diluent. For alkali degradation studies, 1 ml of 2 N NaOH was added 1 ml of stock solution and refluxed for 30 min at 60°C. To this, 1 ml of 2 N HCl was added to neutralize the resultant solution and was diluted to 10 ml with diluent. For oxidative degradation, 1 ml of 20% hydrogen peroxide was added to 1 ml of

Table 2: Results of linearity

% level	Hydrocortisone		Tetracycline	
	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
25	2.5	34,761	2.5	36,433
50	5	68,112	5	70,737
75	7.5	102,414	7.5	104,963
100	10	136,463	10	139,559
125	12.5	169,209	12.5	175,085
150	15	201,466	15	210,225
Correlation coefficient (r^2)	0.999		0.999	

standard stock solution. The solution was kept for 30 min at 60°C. The resultant solution was diluted to 10 ml with diluent. For thermal degradation, the standard stock solution was taken in a beaker and placed in oven at 105°C for 1 h and 1 ml from the resultant solution was diluted to 10 ml with diluent. For photo stability studies, the standard drug solution was exposed to UV light by keeping the beaker in UV chamber for 1 day, from the resultant solution 1 ml was taken and diluted up to 10 ml with diluent.

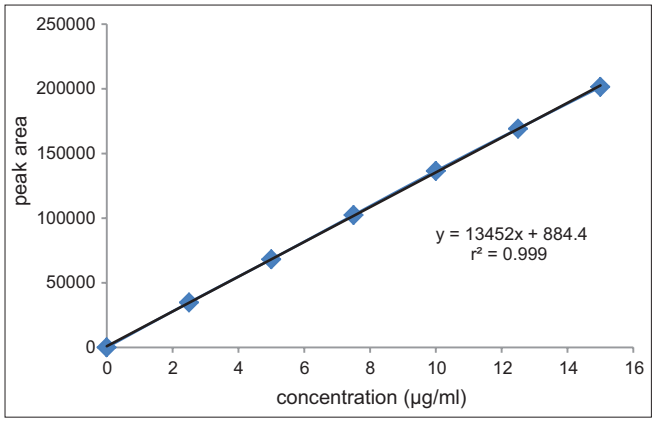

Fig. 4: Calibration curve of hydrocortisone

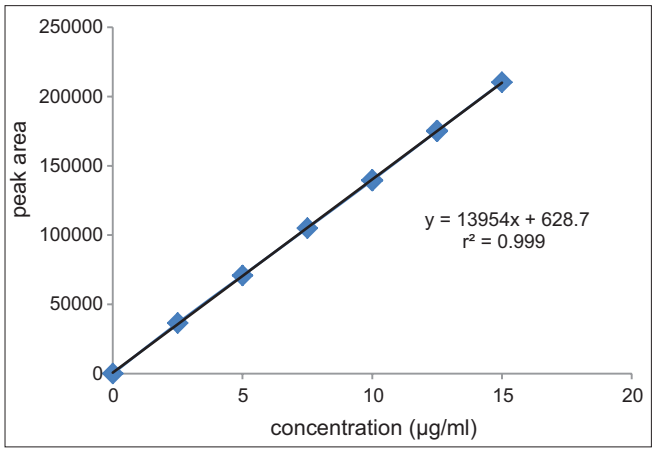

Fig. 5: Calibration curve of tetracycline

Table 3: Results of accuracy

Level (%)	Hydrocortisone	Tetracycline
	Mean recovery*	Mean recovery*
50	99.67	99.39
100	99.13	99.61
150	99.19	99.44

*Represents mean of three determinations

Assay of formulation

Applicability of the developed method can be done by assaying the formulation. For this, standard preparations were made from the reference standard and sample preparations were from formulation. Both sample and standards are injected 6 times into the chromatographic system. Amount of drug present in the formulation was estimated by taking the standard as the reference. The percentage assay was calculated.

RESULTS AND DISCUSSION

Method development

A novel analytical method was established for the simultaneous estimation of hydrocortisone and tetracycline. Sharp and highly resolved peaks were obtained using Discovery C18 column (250 mm × 4.6 mm, 5 µm) at detector wavelength of 244 nm. The mobile phase containing water (pH adjusted to 2.2 with OPA):acetonitrile (40:60% v/v) was pumped at a flow rate of 1 ml/min. The column temperature was set at 30°C. The standard and sample chromatograms were presented (Fig. 3). All the system suitability parameters such as theoretical plates, resolution, and tailing factor were found to be within the limits and are summarized in Table 1.

Method validation

The developed method was validated for parameters such as system suitability, specificity, linearity, accuracy, precision, LOD, LOQ, and robustness according to ICH guidelines for analytical procedures.

Specificity

Specificity of the method was determined by injecting blank, placebo, and degraded samples under similar chromatographic conditions. No interfering peaks were found at retention times of analyte peaks.

Linearity and range

The linearity was developed at six concentration levels from 25% to 150% within the concentration range of 2.5–15 µg/ml for hydrocortisone and tetracycline. The results of linearity of are tabulated in Table 2. The correlation coefficients were found to be 0.999 for both drugs and the representative calibration plots were shown (Fig. 4 and 5) which met the method validation acceptance criteria, and hence, the method was said to be linear for both the drugs in the specified concentration range.

Accuracy

For the evaluation of accuracy, the percentage recoveries were calculated for both the drugs and presented in Table 3. The recovery values indicate that the developed method was found to be accurate.

Precision

The results of precision are tabulated in Table 4. RSD was calculated and reported. RSD values were found to be <2% clearly assured that the developed method was found to be precise.

Table 4: Results of precision studies

Number of injections	Hydrocortisone		Tetracycline	
	Retention time	Peak area	Retention time	Peak area
1	2.201	136,355	3.484	138,455
2	2.209	136,725	3.495	138,525
3	2.214	135,808	3.496	138,308
4	2.214	135,799	3.496	138,599
5	2.214	135,790	3.497	139,190
6	2.215	135,783	3.498	139,083
Mean*±SD		136,043±402.2		139,693±358.1
RSD		0.3		0.3

*Mean of six determinations. SD: Standard deviation, RSD: Relative standard deviation

Table 5: Results of robustness

Parameter	Hydrocortisone		Tetracycline	
	Tailing	Plate count	Tailing	Plate count
Less flow rate (0.9 ml/min)	0.90	3434	0.90	7838
More flow rate (1.1 ml/min)	1.10	3335	1.10	7641
Less column temperature (28°C)	0.91	3426	1.20	8170
More column temperature (32°C)	0.89	3137	1.22	8229
Less organic phase (45:55)	0.87	2665	1.21	8002
More organic phase (35:65)	0.93	3849	1.18	7959

Table 6: Results of forced degradation studies

Type of degradation	Hydrocortisone		Tetracycline	
	Purity angle	Purity threshold	Purity angle	Purity threshold
Acid	0.695	1.495	0.172	1.257
Base	0.695	1.495	0.172	1.257
Peroxide	1.075	1.694	1.383	1.533
Thermal	2.258	3.572	0.542	0.868
UV	1.150	3.273	0.654	0.874

UV: Ultraviolet

LOD and LOQ

LOD and LOQ were evaluated from the standard deviation of the Y intercepts and slope of the calibration curve of hydrocortisone and tetracycline by formula method calibration curves. The LOD and LOQ values were found to be 0.09 µg/ml and 0.27 µg/ml and 0.17 µg/ml and 0.52 µg/ml for hydrocortisone and tetracycline, respectively. The results indicate the sensitivity of the developed method.

Robustness

Robustness of the developed method was determined by varying conditions such as flow rate, temperature, and organic phase ratios; system suitability parameters were compared with that of method. The results are tabulated in Table 5. Small changes in the conditions have no significant effect on tailing, plate count, and retention time of specified drugs. Hence, the developed method was found to be robust.

Forced degradation studies

The purity angle of the analyte components under various stress conditions was less than the threshold values, indicating spectral homogeneity across the peak. The results of forced degradation studies are presented in Table 6. The purity angle values were less than that of purity threshold values. The results specify that there was no interference of degraded peaks with analyte peaks. Hence, the method has successfully resolved the degraded component peaks.

Assay of formulation

The applicability of developed method can be done by assaying the formulation. Cortecycline ointment was assayed and the mean percentage recovery was found to be 99.35% and 99.48% for hydrocortisone and tetracycline, respectively, and hence, the method was successfully employed for the assay of available formulation.

CONCLUSION

The presented RP-HPLC method was proved to be simple, precise, accurate, and stability indicating for the simultaneous estimation of hydrocortisone and tetracycline pharmaceutical dosage form. The results of the study indicate that the proposed method of analysis can be used in quality control departments with respect to routine analysis for the assay of the ointment containing hydrocortisone and tetracycline**.**

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AUTHORS' CONTRIBUTIONS

All authors have contributed equally for this research article.

CONFLICTS OF INTEREST

Authors declare that no conflicts of interest exist in this research work.

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