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Quantitative determination of clobetasone butyrate in bulk and cream formulation by a validated stability-indicating reversed-phase HPLC method

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RESEARCH ARTICLE

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ABSTRACT

A simple isocratic reversed-phase HPLC method for quantification of clobetasone in bulk and cream dosage forms has been developed. Chromatographic analysis was accomplished on an C18 column utilizing a mixture of methanol and water (84:16 *v:v*, pH = 6.0) as mobile phase. An effluent flow rate of 1 mL/min was adjusted and the detection was made at 240 nm wavelength. The method was evaluated according to ICH guidelines Q2 R1 for linearity, specificity, sensitivity, precision and accuracy. The method exhibited good linearity with correlation coefficient (r^2) of 0.9993 over the concentration range from 5 to 50 µg/mL. The recoveries of the test drug from the cream sample was found to be 98.56 to 99.51% and the limit of detection and quantification were calculated as 0.85 and 2.83 µg/mL, respectively, suggesting the accuracy and sensitivity of the developed method. The precision was demonstrated by a low percentage of relative standard deviation (<1%) from six independent assay analysis performed for the cream formulation. Stability indicating property of the proposed method was demonstrated by performing the analysis of forced degradation samples. The developed method can be used for estimation of the clobetasone butyrate in bulk and pharmaceutical formulations for routine analysis in the quality control laboratories.

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1. Introduction

Clobetasone butyrate is chemically, 21-chloro-9-fluoro-17-hydroxy-16b-methylpregna-1,4-diene-3,11,20-trione-17-butyrate (Figure 1), a potent corticosteroid and as butyrate ester, it is used topically for the treatment of skin inflammation associated with psoriasis, eczema and other dermatitis forms [1-3]. It is not a cure for the condition, but helps to relieve the symptoms. Although less potent topical steroids are often preferred for use in children. A short course of clobetasone butyrate may be prescribed for children with severe eczema on the arms or legs. Clobetasone butyrate may also be prescribed for the short courses treatment of psoriasis for areas like face or inside of elbows and knees [4]. Clobetasone butyrate (0.05%) cream formulation, classified as OTC (over the counter) product, has been available in the pharmacy stores and used to treat mainly acute eczema and allergic dermatitis in both adults and children. Comparative studies have reported that clobetasone butyrate (0.05%) was more effective than other topical steroids such as hydrocortisone butyrate (1.0%) and flurandrelone (0.0125%) for the treatment skin inflammatory conditions. Additionally, due to negligible systemic absorption, it has been considered to be systemically safe [5,6]. Apart from dermal conditions, clobetasone butyrate has also found its application in ophthalmic inflammatory conditions, where it is applied as 1.0% clobetasone butyrate eye drop [2,7].

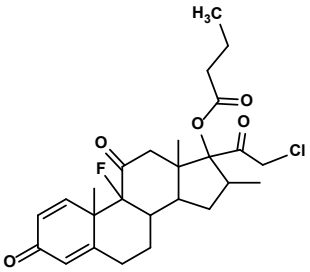

Figure 1. Chemical structure of clobetasone butyrate.

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An extensive literature survey revealed that, an insignificant amount of attention has been paid towards the determination of clobetasone butyrate both in bulk as well as in topical formulations. Only one method based on chromategraphic determination of clobetasone butyrate in ointment preparation could be found [8]. On the other hand considerable interest was shown to the analysis of propionate ester of clobetasone and hence, several analytical methods based on HPLC have been reported for its determination in pure and topical formulations, either in single or in combination with other drugs [4,9-13]. To the extent of our knowledge, no HPLC method for quantitative analysis of clobetasone butyrate in cream formulation has been reported so far. As a consequence, in the present study, a stability indicating liquid chromategraphic method for determination of clobetasone butyrate in bulk and cream formulation has been developed and validated in accordance with International Conference on Harmonization (ICH) guidelines Q2 R1 [14]. The method would be suitable for routine quantification of clobetasone cream samples in pharmaceutical quality control laboratories.

2. Experimental

2.1. Materials

The clobetasone butyrate reference standard (RS) was purchased from Sigma Aldrich, (Steinheim, Germany). The eumovate (Clobetasone butyrate 0.05%) cream marketed by Glaxo-SmithKline, USA was procured from local pharmacy shop (Jazan, Kingdom of Saudi Arabia). The HPLC grade methanol was purchased from Merck, Germany. Hydrochloric acid, orthophosphoric acid, 30% hydrogen peroxide and sodium hydroxide were purchased from Sigma Aldrich (Steinheim, Germany). The HPLC grade water used throughout the analysis was produced in our laboratory using Milli-Q water purification system (Millipore, Molsheim, France). All the analytical solutions have been filtered through 0.22 µ nylon syringe filter (Millipore, Molsheim, France) before injection into HPLC system.

2.2. Equipments and chromatographic conditions

All the chromatographic analysis was carried out on Shimadzu LC-20 AT HPLC system (Tokyo, Japan) equipped with photodiode array (PDA) detector covering a range of 190-400 nm (SPD-M20A), autosampler (SIL-20A) and column thermostat chamber (CTO-20A). Analytical balance (Metler Toledo, USA), hot air oven (SEL LAB, Sheldon Manufacturing Inc. USA), digital water bath (Daihan Labtech, Korea) and ultrasonicator (Wisd, Dathan Scientific, Korea) were used during the experiment. The chromatographic analysis was carried out on isocratic mode and separation was obtained by using Zodiac C18 column (50 mm × 4.6 mm, 5 µm) as stationary phase and a mixture of water and methanol (16:84, *v*:*v*, pH adjusted to 6.0 with *o*-phosphoric acid) was used as mobile phase. A column temperature of 27 °C was maintained, 1 mL/min effluent flow rate, 20 µL injection volume and 15 min runtime were set throughout the analysis. The data were acquired at a detection wavelength of 240 nm. The HPLC system was monitored and the data were processed using Shimadzu LC-Solution software. The mobile phase was used as diluent.

2.3. Preparation of standard solution

The stock standard solution was prepared by transferring an appropriate quantity of clobetasone butyrate reference standard in a volumetric flask, dissolved in mobile phase by sonicating for 5 min and volume was made up with mobile phase to achieve a solution of 1 mg/mL concentration. The working standard solution (25 µg/mL) for method development was prepared from stock standard solution using mobile phase as diluent. The standard stock solution was also diluted with mobile phase to prepare a series of working solutions of 5-50 µg/mL concentrations, which were used for linearity experiment. The above working standard and linearity solutions were injected in the system and analysed in six replicates.

2.4. Preparation of sample solution

An accurately weighed 500 mg of cream sample (equivalent to 10 mg of clobetasone butyrate) was taken into a 100 mL volumetric flask. Approximately, 60 mL of diluent was added to the flask and sonicated for 20 min with occasional shaking and then the final volume was adjusted using mobile phase as diluent and mixed thoroughly. Further dilutions were made to achieve required concentrations. Prior to injection, the solution were filtered using 0.45 µ nylon filter.

2.5. Method validation

The validation of the proposed method was performed in accordance with the ICH guidelines Q2 R1 [14]. The parameters such as system suitability, linearity, limit of detection and limit of quantitation, precision, accuracy, specificity-forced degradation of cream samples and solution stability were assessed.

2.5.1. System suitability

To make sure that the HPLC system is appropriate for proposed experiment, the system suitability parameters such as retention time, number of theoretical plates, tailing factor of analyte peak and percent RSD of area for six replicate injections of analyte were observed at the beginning of the experiment and monitored throughout the analysis. For this purpose, working standard solution (25 µg/mL) was analysed and chromatograms were recorded. The number of theoretical plate more than 3000, tailing factor (less than 1.5) and % RSD of analyte peak areas of six replicate injections less than 1% was considered to be the acceptance criteria.

2.5.2. Linearity

Linearity of the proposed method was evaluated at five concentration levels in the range 5 to 50 µg/mL. The linearity solutions were analysed in six replicates and the chromatograms were recorded. Calibration plot was prepared by plotting the peak area of analyte against respective concentrations and analysed by least-square regression; *y*-intercept, slope and correlation co-efficient were calculated.

2.5.3. Precision

Precision of the developed method was assessed by carrying out the assay analysis of six independent cream samples at 25 µg/mL concentration level. To assess the intra-day precision (repeatability), the % RSD of all six samples analysed on the same day on different times were calculated. Inter-day precision was assessed by analysing the cream samples at different days by different analyst of the same laboratory. The inter-day precision was expressed as % RSD of the assay analysis.

2.5.4. Accuracy

Accuracy of the proposed method was estimated by performing the recovery experiment by following standard addition procedure. The analysis was carried out by known

amount of clobetasone butyrate working standard added to pre-analyzed solution of the formulation in the test concentration range of 50, 100 and 150%. The recovery samples were run into the HPLC system in triplicate and mean recovery and % RSD were calculated.

2.5.5. Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) of the present method were determined by screening a series of diluted solutions of clobetasone butyrate reference standard. The solutions were prepared by sequentially diluting the standard solution in mobile phase. The LOD and LOQ values were calculated from signal-to-noise ratio (S/N). The LOD value for the analyte was considered when S/N = 3 and the LOQ value was evaluated at S/N =10 criterion.

2.5.6. Solution stability

The stability of the tested drug in its analytical solutions (standard and sample solutions) were tested by storing at laboratory temperature (~25 °C) for 24 hours and in the refrigerator (5±1 °C) for 72 hours and analysing at 12 h intervals. After every 12 h the samples were analysed into the HPLC system in triplicate and the assay results were calculated from the observed peak area.

2.5.7. Specificity-forced degradation studies

Force degradation studies on the clobetasone butyrate cream sample was conducted to reveal whether the developed method was possessing stability-indicating property and could unambiguously analyse the test drug in presence of degradation products and impurities. The clobetasone butyrate cream sample was exposed to stressed conditions including acid hydrolysis, base hydrolysis, oxidative stress, thermal degradation (dry and wet heat) and direct sunlight (photolytic degradation) to induce partial degradation of the drug.

Acid degradation: For acid degradation study the cream sample was dispersed in 10 mL of diluent and the mixture was subjected to acid hydrolysis by adding 1 mL of 4 N HCl. The resulting mixture was left in dark chamber at 37 °C for 1 h. The solution was then neutralized with dilute NaOH solution.

Base degradation: For base degradation study the cream sample was dispersed in 10 mL of diluent and the mixture was subjected to base hydrolysis by adding 1 mL of 1 N NaOH solution. The resulting mixture was kept in dark chamber at 37 °C for 6 h. The solution was then neutralized with dilute HCl.

Oxidative degradation: To examine the stability of the test drug towards oxidation, the weighed cream sample was dispersed with 10 mL of diluent and the oxidation was accomplished by addition of 1 mL of 30% hydrogen peroxide. The resulting mixture was kept in dark chamber for 6 h at 37 °C.

Thermal degradation: The thermal degradation was examined at wet heat conditions. The sample was dispersed in 5 mL ultra-pure water and heated on water bath (~100 °C) for 6 h.

Photolytic degradation: To examine the photostability of the clobetasone cream, the sample was exposed to direct sunlight for 6 h.

All the forced degradation experiments were carried out at a concentration of 25 µg/mL. In all the experiments clobetasone butyrate cream content equivalent to 5 mg of clobetasone butyrate was weighed and the samples solutions were prepared by following the same procedure as described in the sample preparation section. All the degradation experiments were performed in four replicates.

The sample solutions were injected in triplicate in the HPLC system using the developed method and chromatograms were recorded. Peak purity of the main analyte peak was checked by PDA detector and the assay results were calculated against the reference standard.

3. Results and discussion

3.1. Method development and optimization

The major objective of the current study was to develop a simple, sensitive and accurate reversed-phase HPLC method for determination clobetasone butyrate in bulk and cream formulation. The aim was also to separate the degradation products of the test drug in a short runtime. In the development process, various buffers and pH range of 4-8 and composition of aqueous and organic phases were tried. Two organic solvents, acetonitrile and methanol were tested. Methanol was found to be superior in terms of chromatographic characteristic of the tested drug. After several trials, a mixture of methanol and water at a ratio of 84:16 (*v*:*v*) (pH = 6.0) was found to be optimum for the developed method. At a mobile phase composition of methanol and water (50:50, *v*:*v*, pH = 6.0), no analyte peak was observed within 10 min runtime. Hence, the proportion of methanol was gradually increased until the drug has been eluted at about 4 min at 1 mL/min flow rate. A detection wavelength of 240 nm was selected on the basis of UV-spectral scanning of the standard solution. Based on results obtained from several trails during the method optimization step, C18 column (50 mm × 4.6 mm, 5 µm) was found to be optimum stationary phase. Other parameters such as injection volume of 20 µL and column oven temperature of 27 °C were also optimized. By applying the finalized chromatographic conditions, the standard and sample solutions were analysed. The representative chromatograms of clobetasone butyrate in bulk and cream formulation showing excellent peak shape have been depicted in Figure 2. The extraction and good recovery of the active drug from a semisolid matrix such as cream is one of the critical aspects of analysing semisolid dosage forms. Hence, considering the solubility of clobetasone butyrate, mobile phase (methanol:water, 84:16, *v*:*v*, pH = 6.0) was used as diluent for the preparation and dilution of all analytical solutions.

3.2. Method validation

After finalizing the optimum chromatographic conditions, the method was subjected to validation according to ICH guidelines Q2 R1 [14]. The developed method has been evaluated for system suitability, linearity, precision, accuracy, sensitivity, solution stability and specificity.

The system suitability and column performance were demonstrated by injecting standard solution at the beginning of the experiment and the system suitability parameters were recorded form the chromatograms. The tailing factor for clobetasone butyrate peak was 0.99 and number of theoretical plates was recorded to be 4986, which has verified the column efficiency. The % relative standard deviation (RSD) of the peak area of six replicate injections was 0.42, which has indicated that the HPLC system was precise. The acceptable system suitability parameters have suggested that the system was suitable to conduct the experiment. The system suitability parameters were monitored throughout the experiment.

To demonstrate the linearity of the developed HPLC method, the standard solutions over the concentration range of 5 to 50 µg/mL at five point levels were analysed. The linear calibration graph was obtained between the observed peak areas of analyte and the concentration of the respective linearity solutions (Figure 3).

Table 1. Linearity results of clobetasone butyrate by the proposed HPLC method *.

Parameters	Clobetasone butyrate
Linearity range (µg/mL)	5-50
Correlation coefficient (r^2)	0.9993
Slope (a)	150.2
Intercept (b)	2151.3
Regression equation ($y = ax + b$)	$y = 150.2x + 2151.3$

* The linearity solutions were injected in six replicates

Table 2. Standard deviation and % relative standard deviation of the peak area of recorded in linearity experiment (n = 6).

Concentrations (µg/mL)	Standard deviation (SD)	% Relative standard deviation (%RSD)
5	30.97	1.08
10	33.19	0.92
20	35.49	0.67
25	31.03	0.53
50	40.72	0.42

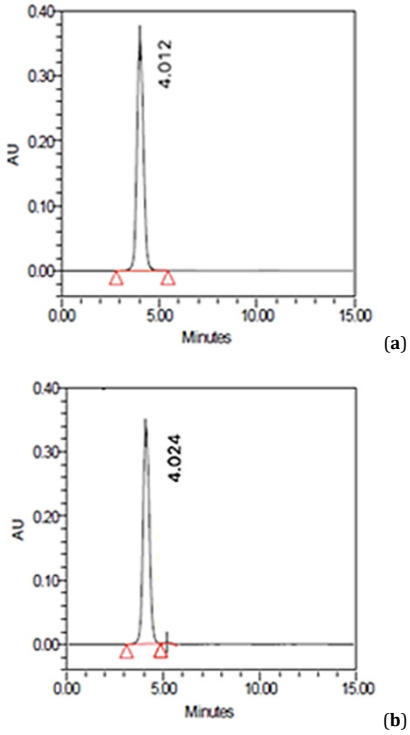

Figure 2. Representative chromatograms of clobetasone butyrate in standard (**a**) and cream sample (**b**).

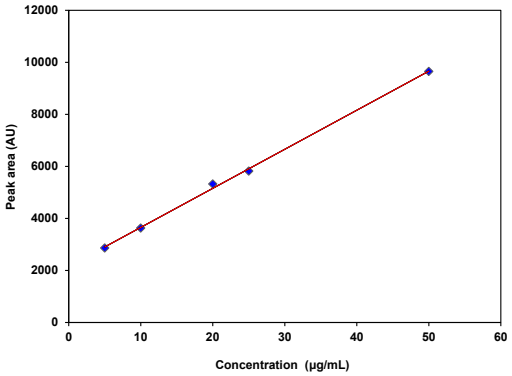

Figure 3. Calibration graph of clobetasone butyrate.

The regression analysis showed the existence of an excellent correlation between the observed peak area and concentration of the analyte, which has proved the linearity of the present method. The correlation coefficient (r^2) was found to be >0.999. The linearity results are presented in Table 1 and the standard deviation (SD) and % RSD of the peak area at each concentration has been shown in Table 2.

The method precision experiment was carried out to check the variation among the analysis of multiple sampling of the same sample at laboratory condition.

Table 3. Intra-day and inter-day precision results of clobetasone butyrate cream (0.05%, *w:w*) by the proposed method.

Experiment	Assay %	Average % assay ± %RSD
Intra-day assay (n = 6)	100.97	100.19±0.94
	100.84	
	99.11	
	100.91	
	100.43	
	98.90	
Inter-day assay (n = 6)	100.17	99.21±0.88
	100.30	
	99.01	
	98.67	
	98.04	
	99.06	

Table 4. Accuracy (recovery) results of clobetasone butyrate cream.

Amount of drug present in pre-analysed sample (µg/mL)	Amount of standard drug (RS) added (µg/mL)	Percentage of concentration level added	Amount of drug recovered (µg/mL)	% Recovery ± SD; %RSD *
25	12.5	50%	37.32	99.51±1.46; 1.47
25	25.0	100%	49.58	99.15±1.13; 1.14
25	37.5	150%	61.60	98.56±0.68; 0.69

* Values for three determinations.

Table 5. Summary of force degradation results of clobetasone butyrate cream *.

Stress conditions	Peak purity check of the main peak	Average % assay ± SD
Acid hydrolysis (4 N HCl, RT, 60 min, n = 4)	Passed	99.022±0.772
Base hydrolysis (1 N NaOH, RT, 6 h, n = 4)	Passed	99.213±0.843
Thermal degradation (on water bath, ~100 °C, 6 h, n = 4)	Passed	82.117±1.113
Oxidative degradation (30% H_2O_2, RT, 6 h, n = 4)	Passed	98.467±1.032
Photolytic degradation (direct sunlight, 6 h, n = 4)	Passed	78.764±1.213

* SD is standard deviation; RT = Room temperature.

The intra-day method precision was established by performing six independent assay analysis of clobetasone butyrate cream at different times of the same day. Whereas, the reproducibility (intermediate precision) was evaluated by performing the assay experiments by different analyst of the same laboratory at different day. The average % assay was calculated to be 100.19 and 99.21% for intra-day and inter-day analysis, with %RSD less than 1. The low %RSD values have indicated the current HPLC method is precise. The method precision data has been given in Table 3.

The accuracy of the developed method was evaluated by adding the standard clobetasone butyrate into the pre-analysed sample of cream formulation at 50, 100 and 150% levels of the target concentration (25 µg/mL). The experiment was conducted in triplicate and the average % recovery at each level was found to be in the acceptable limit (±2%). The observed recovery results have demonstrated the accuracy of the developed HPLC method. Percent recovery values along with standard deviation and % relative standard deviation has been depicted in Table 4.

The sensitivity of the developed HPLC method was proved by low LOD and LOQ values, determined at S/N of 3:1 and 10:1 respectively. The LOD and LOQ values of clobetasone butyrate by the present study were found to be 0.85 and 2.83 µg/mL. The sample and standard solutions were found to be stable when stored at normal laboratory temperature for at least 24 h (bench-top stability) and in the refrigerator for 48 h. The solutions were assayed at 12 h interval and the % RSD of the assay results was calculated as 0.73 and 0.89% at laboratory temperature and refrigerator, respectively. The solution stability results have indicated that the analysis can be performed for at least 24 h at the laboratory conditions using the same sample and standard solution without any loss of the active drug component.

The specificity and stability-indicating property of the developed method was evaluated by performing forced degradation study of clobetasone cream samples. The specificity of an analytical method is the ability of the method to detect the analyte in the presence of potential impurities and other components present in the analytical solution. In this study, the forced degradation was performed by exposure of cream samples to various stressed conditions such as acid and base hydrolysis, thermal degradation (wet heat), peroxide oxidation and photolytic degradation. The stressed samples were analysed and assay results were calculated from the observed peak area of the main analyte. The forced degradation results suggested that clobetasone butyrate in cream sample showed highest sensitivity towards photolytic degradation; the sample has been exposed to direct sunlight for 6 h. The assay result was calculated to be 78.76% indicating that 21.24% drug was degraded. The major degradation product peak was observed at 9.0 min. After photolytic degradation the test drug was remarkably degraded by thermal degradation and the assay was calculated as 82.12%, indicating the decomposition of 17.88% of clobetasone butyrate. The two degradation products were eluted at 1.5 and 9.9 min. The test drug has exhibited no degradation against acidic and basic hydrolysis, while insignificant decomposition was observed when exposed to oxidative degradation by hydrogen peroxide (1-2% degradation). The specificity (selectivity) was also demonstrated by the peak purity of the analyte peak. The chromatograms from the sample and standard solutions were compared with the blank chromatograms and no interference was observed at the retention time of the test drug, which has further proved the specificity of the method. The chromatograms of forced degradation study were represented in Figures 4 and 5 and the degradation results have been summarized in Table 5.

4. Conclusion

A simple and specific reversed-phase HPLC method for the determination of clobetasone butyrate in bulk and cream dosage forms has been successfully developed. The retention time of the analyte was short (~4 min), the peak shape was excellent and the system suitability parameters were acceptable. The method was evaluated according to ICH guidelines Q2 R1 and proved to be sensitive, linear, precise and accurate for detection and estimation of clobetasone butyrate. The method has been subjected to forced degradation study, which has demonstrated the specificity for the analyte.

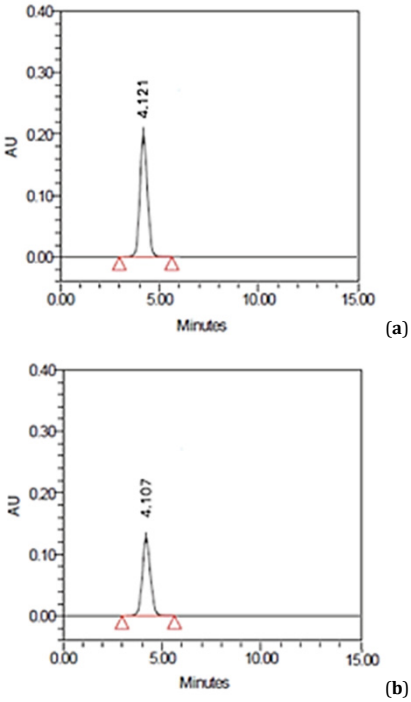

Figure 4. Representative chromatograms of forced degradation samples: (**a**) acid degradation and (**b**) alkali degradation.

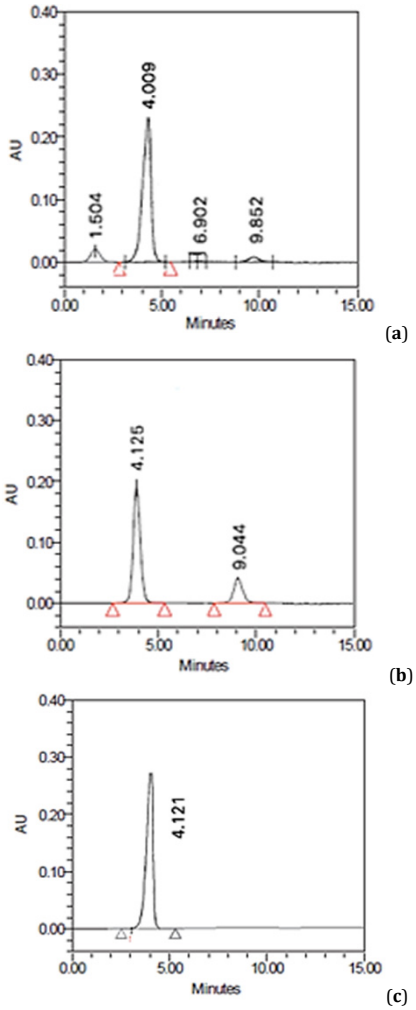

Figure 5. Representative chromatograms of forced degradation samples: (**a**) wet heat degradation; (**b**) photolytic degradation and (**c**) oxidative degradation.

The specificity was further proved by the absence of any interference from blank and placebo at the retention time of the analyte. The test drug was found to be stable in the analytical solutions for at least 24 h. The current method can be used for stability indicating quantification of the test drug in routine analysis and quality control of pharmaceutical products.

Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
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