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Forced degradation study to develop and validate stability-indicating RP-LC method for the determination of ciclesonide in bulk drug and metered dose inhalers

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ABSTRACT

A simple, selective and precise stability-indicating reversed-phase liquid chromatographic method was developed and validated for the determination of ciclesonide. Ciclesonide was subjected to acid and alkali hydrolysis, oxidation, thermal and photo-degradation. The degradation products were well separated from the pure drug. The method was based on isocratic elution of ciclesonide and its degradation products on reversed phase C18 column (250 mm × 4.6 mm, 10 μm) – Phenomenex using a mobile phase consisting of ethanol–water (70:30, v/v) at a flow rate of 1 mL min^{-1}. Quantitation was achieved with UV detection at 242 nm. Linearity, accuracy and precision were found to be acceptable over the concentration range of 5–200 μg mL^{-1}. Desisobutyryl-ciclesonide was prepared by selective alkaline hydrolysis of the ester and proved to be the main degradation product. The proposed method was successfully applied to the determination of ciclesonide in bulk and in its pharmaceutical preparation.

1. Introduction

Ciclesonide, 2-[(1S, 2S, 4R, 8S, 9S, 11S, 12S, 13R)-6-cyclohexyl-11-hydroxy-9,13-dimethyl-16-oxo-5,7-dioxapentacyclo[10.8.0.02,9.04,8.013,18]icosa-14,17-dien-8-yl]-2-oxoethyl 2-methylpropanoate (Fig. 1a) is a new, once-daily inhaled corticosteroid [1]. Inhaled corticosteroids (ICS) have been the most effective long-term control drugs available for the treatment of persistent asthma. The pharmacological characteristics desirable for an ideal ICS include low oral bioavailability, high lung deposition, potent activity in the lungs, potent glucocorticoid receptor binding affinity, prolonged pulmonary retention time, high plasma protein binding, a short physiologic half-life and rapid clearance from the circulation [2]. Ciclesonide possesses several of these properties. It has been administered as a pro-drug in an aerosol solution, and it is converted to its pharmacologically active metabolite, desisobutyryl-ciclesonide (Fig. 1b), in the lungs, the target organ, by endogenous esterase [2].

Ciclesonide has been determined in its rotacaps by spectrophotometry [3] and in biological fluids by LC–MS/MS [1,2]. However, there is no reported LC method for the determination of ciclesonide in its metered dose inhaler (MDI) dosage form. Besides, forced degradation of ciclesonide has not been published. Accordingly, the goal of the present work was to develop simple, sensitive and selective LC method for the determination of ciclesonide in its metered dose inhaler dosage form. Moreover, the present manuscript describes the degradation behavior of ciclesonide under acidic, basic, oxidative, thermal and photolytic conditions with the study of the main degradation product; desisobutyryl-ciclesonide which is also the active metabolite [2]. Optimization of LC conditions to separate the drug and its degradation products on a reversed phase C18 column, method validation and characterization of degradation products were discussed.

2. Experimental

2.1. Instrumentation

The HPLC (Younglin instrument Acme 9000, Korea) system was equipped with vacuum degasser, mixer, gradient pump and UV/Vis detector. Separation and quantitation were made on C18 column (250 mm × 4.6 mm, 10 μm) – Phenomenex. A Soniclean 120T ultrasonic processor (Australia) was used.

The MDI sampling apparatus (Copley Scientific Ltd, UK) with a critical flow controller model TPK (Copley Scientific Ltd, UK) was used. The final filter was a 25 mm AE fiber glass filter (Pall Corporation, USA). Vacuum flow through the apparatus was provided by a GAST pump (Brook Crompton, UK). The flow through the MDI was 28.3 L min^{-1} with a flow-duration of 8.5 s such that the inhalation volume was 4 L as recommended by the Pharmacopeial Methods

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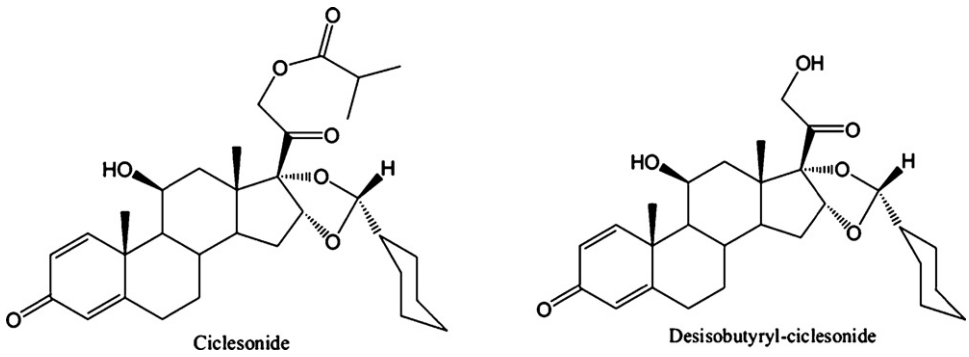

Fig. 1. Chemical structure of (a) ciclesonide and (b) desisobutyryl-ciclesonide.

[4–6]. The flow was measured by an electronic digital flow meter (MKS Instruments, USA). Parafilm M laboratory film (Pechiney Plastic Packaging, USA) was used to seal the apparatus.

2.2. Reagents and reference samples

Standard ciclesonide (certified to contain 100.30%) and ciclohale metered dose inhaler (labeled as a nominal dose of 80 μg ciclesonide per puff) were supplied by Cipla Ltd, India. HPLC grade ethyl alcohol was supplied by Sigma–Aldrich, Germany. Bi-distilled water was produced in-house (Aquatron Water Still, A4000D, UK). Membrane filters 0.45 μm (Teknokroma, Barcelona, Spain) were used. All other chemicals and reagents used were of analytical grade unless indicated otherwise.

2.3. Chromatographic conditions

Chromatographic separation was achieved on a C18 column (250 mm × 4.6 mm, 10 μm) – Phenomenex® applying isocratic elution based on a mobile phase consisting of ethanol–water (70:30, v/v). The mobile phase was pumped through the column at a flow rate of 1 mL min^{-1}. Analyses were performed at ambient temperature and detection was carried out at 242 nm. The injection volume was 30 μL.

2.4. Standard stock solution preparation

Standard stock solution of ciclesonide (1.0 mg mL^{-1}) was prepared by dissolving 100 mg of the drug in methanol, sonicated and completed to volume in a 100 mL volumetric flask. The required concentrations were prepared by serial dilutions.

2.5. Sample preparation

The total emitted dose from three ciclohale MDI was determined using MDI sampling apparatus mentioned in Section 2.1. Each inhaler mouthpiece was inserted tightly into the mouthpiece adaptor of the dose sampling unit and aligned along the horizontal axis. The emitted dose from the MDI was measured by collecting 10 individual doses at 28.3 L min^{-1} with an inhaled volume of 4 L each time. Three determinations were made for each dose emission ($n = 3$). Some doses were tested and some were discharged to waste according to the randomization method listed in Table 1. The MDI was shaken and primed by firing 2 doses to waste before use. The discharge of the dose was done in co-ordination with switching on the vacuum pump. For each dose emission procedure, the critical flow controller system was used to produce sonic flow conditions, as recommended by the Pharmacopeial Methods [4–6]. Following dose emission into the apparatus, the sampling unit was washed with methanol HPLC grade (Sigma, UK) and the filter was completely submerged in methanol and then sonicated for 3 min (preliminary analysis revealed that this procedure removes all drug entrained on the filter). All the solutions were collected and made up to a volume of 10 mL for the MDI then chromatographed. The total dose emitted was the amount deposited in the plastic dose sampling apparatus and the final filter.

2.6. Procedure

2.6.1. Calibration curve of ciclesonide

Accurately measured aliquots of stock standard solutions equivalent to 50–2000 μg ciclesonide were transferred into a series of 10 mL volumetric flasks. The solutions were completed to volume with methanol. A volume of 30 μL of each solution was injected in triplicates into the chromatograph. The conditions including the mobile phase at flow rate 1 mL min^{-1} and detection at 242 nm were adjusted. A calibration curve was obtained by plotting area under the peak (AUP) against concentration (C).

2.6.2. Assay of ciclesonide in bulk and ciclohale MDI

The procedure mentioned in Section 2.6.1 was repeated using concentrations equivalent to 20–180 μg mL^{-1} ciclesonide in bulk. For the determination of the total emitted dose of ciclesonide in ciclohale MDI (Cipla, India), the sample solution prepared in Section 2.5 was serially diluted and then injected in triplicates. The concentrations of ciclesonide were calculated using calibration equation.

2.7. Forced degradation of ciclesonide

Forced degradation studies of bulk drug included appropriate solid state and solution state stress conditions in accordance with the ICH regulatory guidance [7]. The stock solution was used for the forced degradation study to provide an indication of the stability-indicating property and specificity of proposed method.

Prior to injection, samples were withdrawn at appropriate time, neutralized (in case of acid and alkali hydrolysis) and the solutions were diluted with methanol. The total chromatographic run time was about two times the retention of the drug peak.

Table 1
Table of randomization method for doses emitted from the MDI to measure the emitted dose, aerodynamic characterization and those emitted to waste.

Dose emission	Aerodynamic diameter	Waste
Device (1)		
1–10	50–60	11–50
111–120		61–110
Device (2)		
11–20	1–10	
Device (3)		
41–50	101–110	1–40
		51–100

2.7.1. Acid- and base-induced degradation

To 5 mL of methanolic stock solution, an appropriate volume of 2 M HCl was added and the mixture was diluted with water to 10 mL to reach molarities of 0.1 M, 0.5 M and 1 M HCl, separately. The mixtures were kept at room temperature for 8 h. Alkaline degradation studies were carried out in a similar manner with molarities of 0.1 M, 0.5 M and 1 M NaOH for 8 h. These experiments were repeated at higher temperature of 75 °C for 0.5 h while keeping all other conditions constant. For study in neutral condition, drug was held in methanol at room temperature for 24 h. The forced degradation in acidic and basic media was performed in the dark in order to exclude the possible degradative effect of light. Thirty microlitres of the resultant solutions were injected onto column and the chromatograms were run as described in Section 2.3.

2.7.2. Hydrogen peroxide-induced degradation

To 5 mL of methanolic stock solution, 5 mL of 6% (v/v) H_2O_2 and 30% (v/v) H_2O_2 were separately added to reach final concentrations of 3% and 15% (v/v) H_2O_2, respectively. The prepared mixtures were kept at room temperature for 12 and 5 h, respectively. Thirty microlitres of the resultant solutions were injected onto column and the chromatograms were run as described in Section 2.3.

2.7.3. Thermal and photolytic degradation

The dry powder of the drug was placed in oven at 55 °C for 72 h to study dry heat degradation. The photochemical stability of the drug was also studied by exposing the dry powder to UV light for 24 h. Powder was then dissolved and diluted with methanol. Thirty microlitres of the resultant solutions were injected onto column and the chromatograms were run as described in Section 2.3.

2.8. Preparation of desisobutyryl-ciclesonide

Ciclesonide (200 mg) was dissolved in a mixture of methylene chloride:methanol (9:1) containing sodium hydroxide with a final molarity of 0.5 M NaOH. The solution was stirred and followed up by injection onto the column using the same chromatographic conditions till complete disappearance of ciclesonide peak (1.5 h). The solution was acidified using 2 M HCl. The organic layer was separated and the aqueous layer was washed with methylene chloride (3× 10 mL). The combined organic layer and washings were evaporated to dryness.

3. Results and discussion

3.1. Method development

During the optimization cycle, several chromatographic conditions were attempted using C18 column (250 mm × 4.6 mm, 10 μm) – Phenomenex. Various mobile phase compositions like methanol with water, or acetonitrile with water, in different proportions, were tried in an isocratic mode. Detection was carried out at 242 nm to obtain sufficient peak intensity for both drugs and degradants. It was found that, at least 70% of organic modifier was needed to elute all peaks within 10 min due to high lipophilicity of ciclesonide and its degradation products. Neither methanol nor acetonitrile gave sufficient resolution. During the development cycle, a mobile phase consisting of ethyl alcohol:water (70:30, v/v) at a flow rate of 1.0 mL min^{-1} and UV detection at 242 nm, in an isocratic mode, gave good separation of the drug and its degradation products. In literature, two mobile phases were applied for ciclesonide: gradient elution with acetonitrile (containing 10 mM of acetic acid and 10% of acetone) [1] and isocratic elution with a mixture of 0.1% formic acid solution and methanol [2]. In comparison, the mobile phase developed in this work is simpler. The retention time of ciclesonide was found to be 5.7 min, Fig. 2.

Table 2
System suitability tests for the proposed LC method.

Parameter	Data
N	9704
T	1.04
%R.S.D. of 6 injections of	
Peak area	0.15
t_R (min)	0.64

N, number of theoretical plates; T, tailing factor; %R.S.D., %relative standard deviation; t_R, retention time.

3.2. System suitability tests

System suitability tests are an integral part of liquid chromatographic methods in the course of optimizing the conditions of the proposed method [8]. They are used to verify that the resolution and reproducibility were adequate for the analysis performed. The parameters of these tests are column efficiency (number of theoretical plates), tailing of chromatographic peak, repeatability as %R.S.D. of peak area for six injections of a solution of a 80 μg mL^{-1} (100% concentration) and reproducibility of retention as %R.S.D. of retention time. The results of these tests for the proposed method are listed in Table 2.

3.3. Method validation

3.3.1. Linearity

In this study, eight concentrations were chosen. Each concentration was analyzed three times. Good linearity of the calibration curve was verified by the high correlation coefficient. The analytical data of the calibration curve including standard deviations for the slope and intercept (S_b, S_a) are summarized in Table 3.

3.3.2. Accuracy

Accuracy of the results was calculated by % recovery of 5 different concentrations (injected in triplicates) of ciclesonide in bulk. The results obtained including the mean of the recovery and standard deviation are displayed in Table 3.

3.3.3. Precision

The repeatability of the method was assessed by six determinations for each of the three concentrations (64, 80, 96 μg mL^{-1}) representing 80, 100, 120%, respectively. The repeatability of sample application and measurement of peak area for active compound

Table 3
Results obtained by the proposed LC method for the determination of ciclesonide.

Parameter	Data
Retention time (min)	5.70
Wavelength of detection (nm)	242
Range of linearity (μg mL^{-1})	5–200
Regression equation	Area = 50,021 $C_{\mu g\ mL^{-1}}$ −26,524
Regression coefficient (r^2)	0.9999
LOD (μg mL^{-1})	1.05
LOQ (μg mL^{-1})	3.50
S_b	152.09
S_a	15,999.37
Confidence limit of the slope	50,021 ± 358.93
Confidence limit of the intercept	−26,524 ± 37,758.51
Standard error of the estimation	29,696.28
Inter-day (%R.S.D.)	0.57–0.69
Intra-day (%R.S.D.)	0.22–0.35
Drug in bulk	99.94 ± 0.92
Drug in dosage form	101.75 ± 2.89%

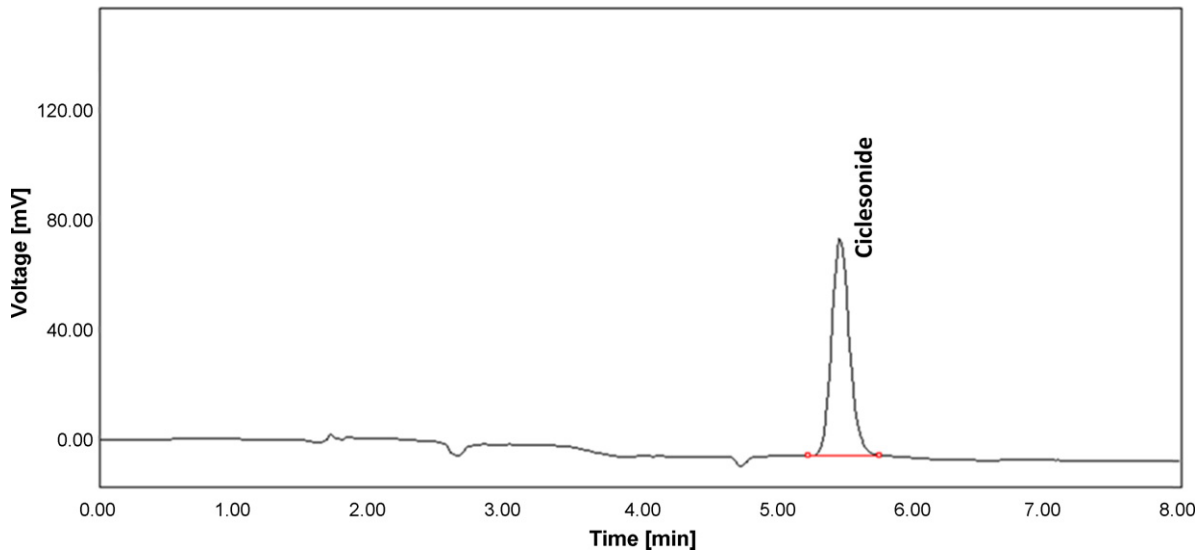

Fig. 2. HPLC chromatogram of ciclesonide (80 μg mL^{-1}).

Table 4
Results for the determination of repeatability for ciclesonide by the proposed LC method.

Conc. (%claim)	Mean	Standard deviation	%R.S.D.
64 (μg mL^{-1}) (80%)	3,132,860.833	4873.350	0.156
80 (μg mL^{-1}) (100%)	3,899,616.500	8892.708	0.228
96 (μg mL^{-1}) (120%)	4,676,216.333	10,059.898	0.215

were expressed in terms of percentage relative standard deviation (%R.S.D.) and found to be less than 1% in the three concentrations. Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc. A second analyst carried out experiments described in repeatability. All experiments described in repeatability were repeated in the second day by the same analyst to evaluate day to day ruggedness. Results for the determination of repeatability and intermediate precision (day to day and analyst to analyst) are displayed in Tables 4–6.

3.3.4. Specificity

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences. Good resolution and absence of interference from any of the degradation products are shown in Figs. 3 and 4. Besides, the chromatogram of the pharmaceutical formulation samples was checked for the appearance of any extra peaks (Fig. 5). The chromatogram of ciclesonide in the sample solution was found to be identical to the chromatogram obtained by the standard solution. Moreover, results close to 100% were obtained for the determination of ciclesonide in dosage form, Table 3. These results confirm the specificity of the proposed method.

3.3.5. Limit of detection and limit of quantitation

Limit of detection (LOD) which represents the concentration of analyte at S/N ratio of 3 and limit of quantification (LOQ) at which S/N ratio is 10 were determined experimentally for the proposed methods and results are given in Table 3.

Table 5
Results for the determination of intermediate precision (analyst to analyst) for ciclesonide by the proposed LC method.

Conc.		Replicate peak area			Mean	Standard deviation	%R.S.D.
		1	2	3			
64 (μg mL^{-1}) (80%)	Analyst 1	3,123,216	3,134,597	3,136,096	3,136,069	6988.038	0.223
	Analyst 2	3,138,779	3,142,938	3,140,788			
80 (μg mL^{-1}) (100%)	Analyst 1	3,883,623	3,900,363	3,911,078	3,911,157	16,549.54	0.423
	Analyst 2	3,922,884	3,923,688	3,925,308			
96 (μg mL^{-1}) (120%)	Analyst 1	4,658,134	4,683,457	4,685,605	4,691,377	19,689.1	0.420

Table 6
Results for the determination of intermediate precision (day to day) for ciclesonide by the proposed LC method.

Conc.		Replicate peak area			Mean	Standard deviation	%R.S.D.
		1	2	3			
64 (μg mL^{-1}) (80%)	Day 1	3,136,096	3,173,310	3,184,958	3,167,343	22,062.52	0.697
	Day 2	3,142,938	3,184,272	3,182,486			
80 (μg mL^{-1}) (100%)	Day 1	3,883,623	3,940,727	3,942,106	3,929,692	23,872.81	0.607
	Day 2	3,923,688	3,942,339	3,945,671			
96 (μg mL^{-1}) (120%)	Day 1	4,658,134	4,723,886	4,730,380	4,710,150	26,756.89	0.568
	Day 2	4,705,699	4,722,658	4,720,140			

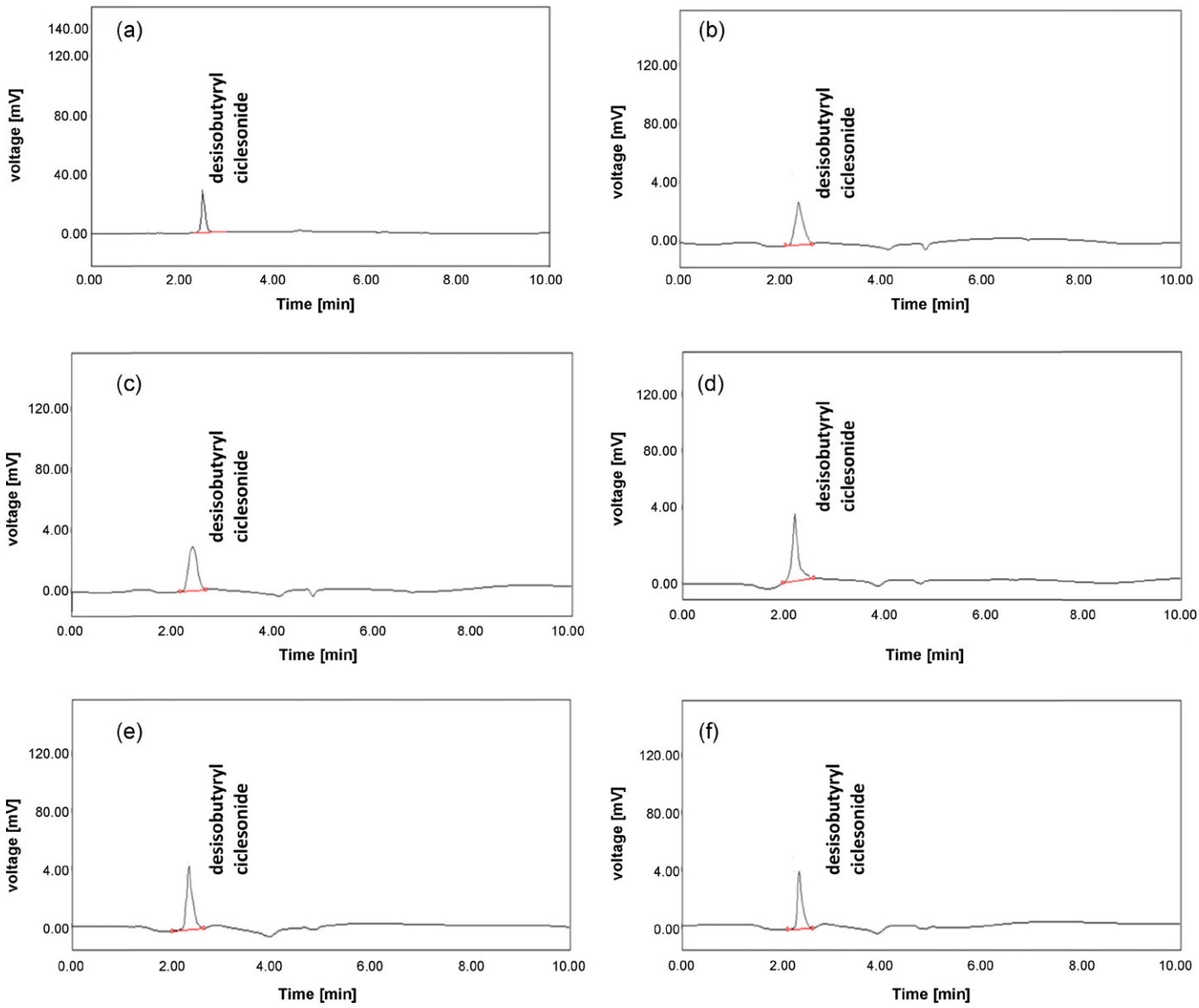

Fig. 3. HPLC chromatogram of degraded ciclesonide in (a) 0.1 M NaOH at RT; (b) 0.5 M NaOH at RT; (c) 1 M NaOH at RT; (d) 0.1 M NaOH at 75 °C; (e) 0.5 M NaOH at 75 °C; and (f) 1 M NaOH at 75 °C.

3.3.6. Robustness

Robustness was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 mL min^{-1} to 0.8 mL min^{-1} and 1.2 mL min^{-1}. The organic strength was varied by ±2%. These variations did not have significant effect on chromatographic resolution by the proposed LC method for ciclesonide and its degradation products in alkali or acid conditions.

3.4. Degradation behavior

3.4.1. Acid- and base-induced degradation

The chromatograms of the alkaline degraded samples for ciclesonide using different molarities of NaOH, either on cold or on hot, showed complete disappearance of the intact drug and appearance of an additional peak at retention time of 2.35 (Fig. 3). This reflects that ciclesonide is alkali-labile due to ester hydrolysis and formation of desisobutyryl-ciclesonide. Acidic degradation studies showed additional peaks at 2.35 and 3.40 min as shown in Fig. 4. The peak at 3.40 min is suggested to be due to an intermediate product obtained only in acidic conditions, which could not be identified. Complete acid degradation of ciclesonide to the degradation product, previously suggested to be desisobutyryl-ciclesonide, was only obtained when the drug was heated in 1 M HCl. This result is in accordance with the fact that base catalyzed ester hydrolysis is preferred over acid catalyzed hydrolysis [9]. The peaks of degraded products were well resolved from the drug peak. Under neutral condition, methanolic solution of ciclesonide did not show any additional peaks.

Desisobutyryl-ciclesonide was prepared by a very mild and rapid procedure for the efficient alkaline hydrolysis of esters in non-aqueous conditions [10]. Ester hydrolysis was confirmed using mass spectroscopy which showed molecular ion peak at 472 (M+1). As ciclesonide is a labile ester, desisobutyryl degradant proved to be the main degradation product by comparison of its retention time with that of the main peak of acid and alkaline degradation. Besides, it is the active metabolite of the drug [2]. HPLC chromatogram of the hydrolytic product showed a single peak at the same retention time (t_R = 2.35), Fig. 6.

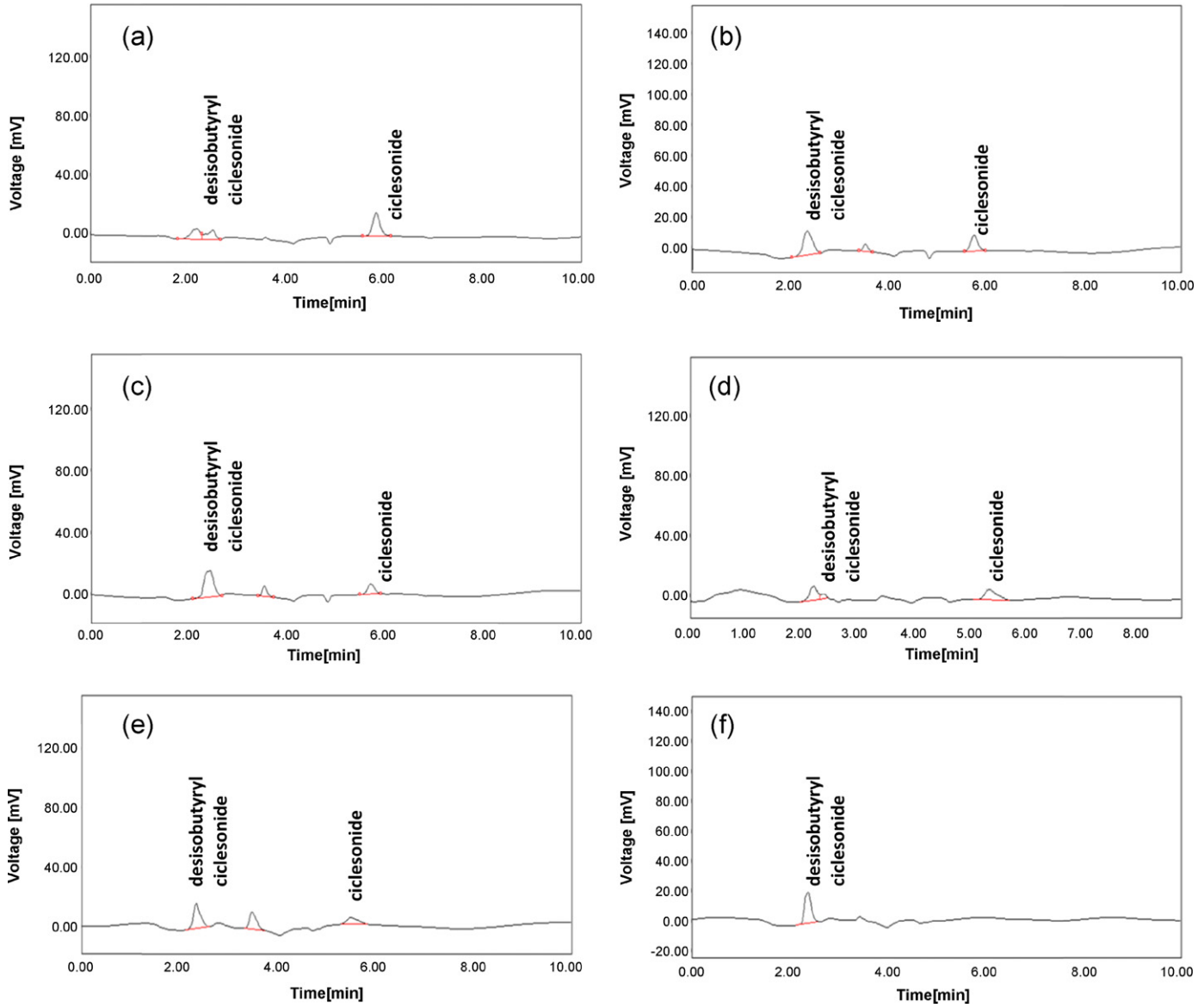

Fig. 4. HPLC chromatogram of degraded ciclesonide in (a) 0.1 M HCl at RT; (b) 0.5 M HCl at RT; (c) 1 M HCl at RT; (d) 0.1 M HCl at 75 °C; (e) 0.5 M HCl at 75 °C; and (f) 1 M HCl at 75 °C.

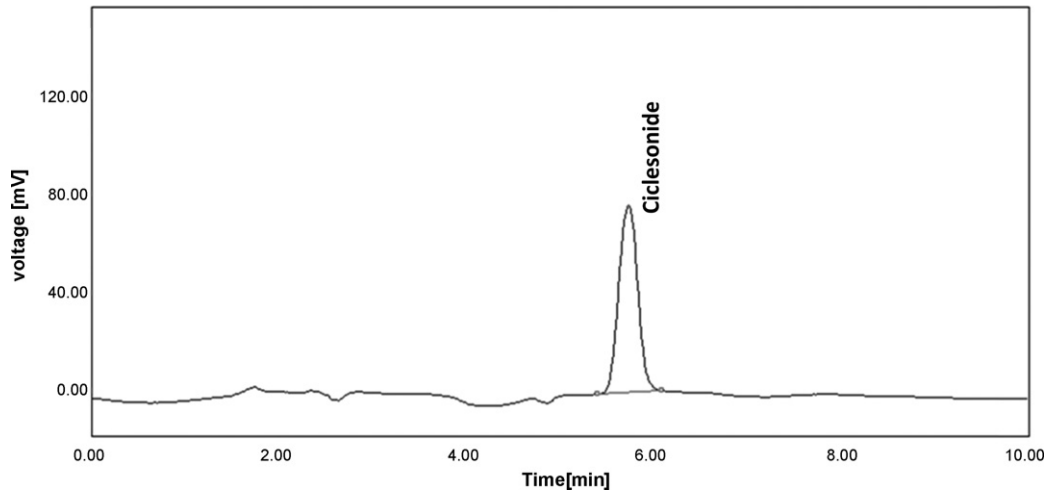

Fig. 5. HPLC chromatogram of ciclohale inhaler containing 80 μg mL^{-1}.

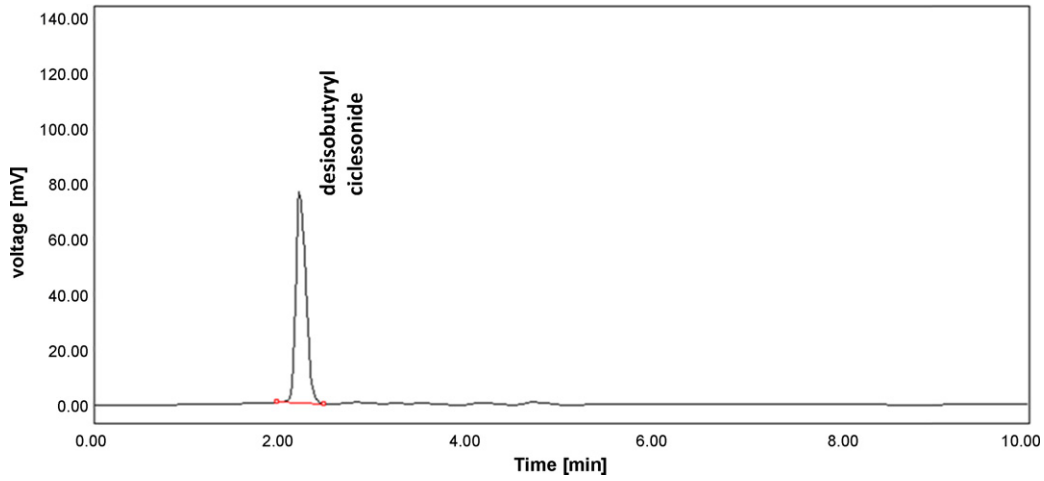

Fig. 6. HPLC chromatogram of desisobutyryl-ciclesonide (80 μg mL^{-1}).

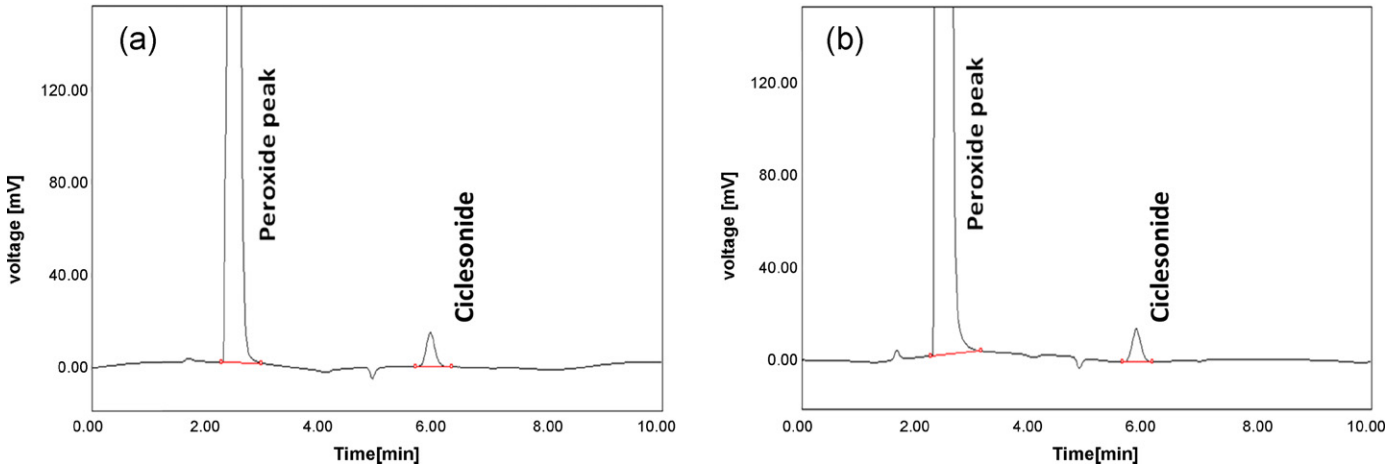

Fig. 7. HPLC chromatogram of degraded ciclesonide in (a) 3% H_2O_2 and (b) 15% H_2O_2.

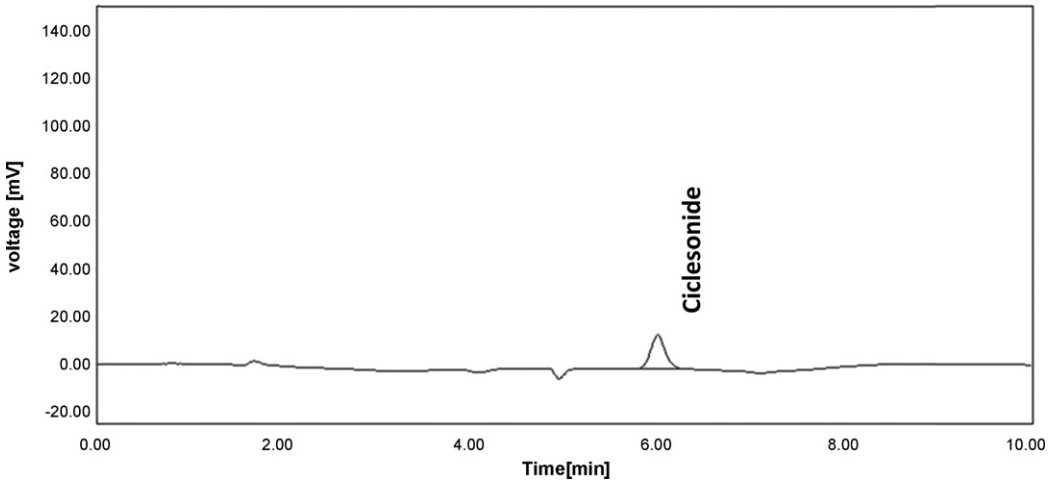

Fig. 8. HPLC chromatogram of dry heat degraded ciclesonide.

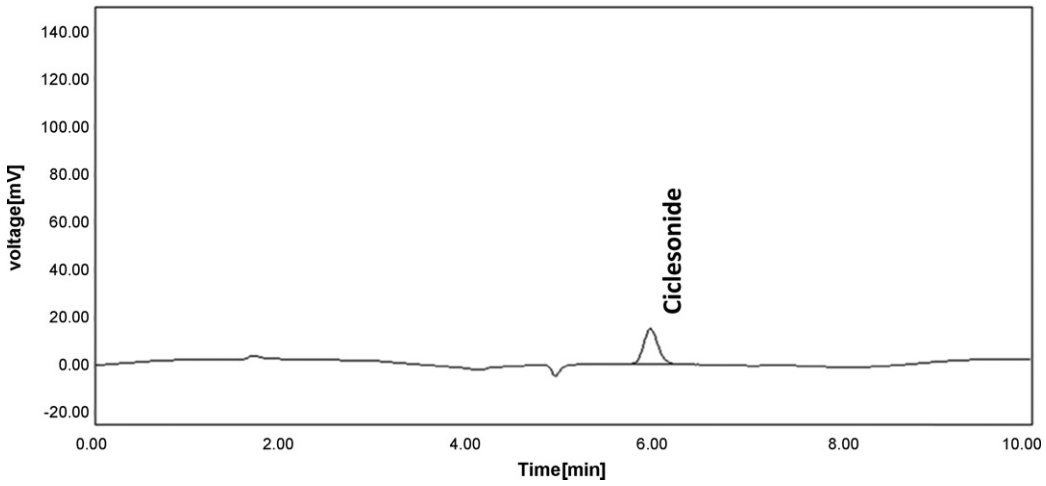

Fig. 9. HPLC chromatogram of UV degraded ciclesonide.

3.4.2. Hydrogen peroxide-induced degradation

The sample degraded with 3% and 15% (v/v) hydrogen peroxide (Fig. 7) showed no additional peaks. This was confirmed by good percentage recovery of the intact drug.

3.4.3. Thermal and photolytic degradation

The samples degraded under dry heat conditions (Fig. 8) showed no additional peaks. The photo-degraded sample showed no additional peak when drug solution was exposed to UV light for 24 h, Fig. 9.

From the aforementioned data, the drug was found to be susceptible to acid and base hydrolysis but resistant to oxidation, dry heat degradation, and photodegradation.

4. Conclusion

A validated stability-indicating LC method was developed to study the degradation behavior of ciclesonide under acid, alkali, oxidation, thermal and photolysis conditions. The drug was found to be degraded in alkaline and acidic conditions due to the presence of ester linkage, which is susceptible to hydrolysis, and found to be stable to oxidation, thermal and photolysis conditions. The proposed LC method has the advantages of simplicity, precision, accuracy and convenience for the separation and quantification of ciclesonide either alone or in the presence of its desisobutyryl-derivative as the major degradation product. Hence, the proposed LC method can be used for the quality control of the cited drug.

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