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RESEARCH ARTICLE

Development and Validation of a Simple Liquid Chromatography Method for the Quantification of Degradation Products of Beclomethasone Dipropionate from a Respirator Suspension Formulation

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ABSTRACT

Beclomethasone dipropionate is a corticosteroid used extensively for the treatment of allergic rhinitis and asthma. Being a steroid prepared synthetically, it has quite a few known related substances and degradation products which are structurally similar to Beclomethasone. Pharmacopeial methods available for the determination of the impurities of Beclomethasone dipropionate utilize harsh conditions and a long analytical run time. In the present work a simple method has been developed for the quantification of the process impurities and the degradation products of Beclomethasone dipropionate from a respirator suspension formulation. The separation has been achieved using an ultra-performance liquid chromatographic method. The method utilizes a Waters Acquity BEH C18 UPLC column using gradient elution with mobile phase consisting of acetonitrile and distilled water. The separation of 10 known process impurities and degradation products was achieved in about 20 minutes. The method was found to be sensitive, selective, precise, accurate and robust under the conditions tested. The method was utilised for the stability monitoring of the respirator suspension formulation and was found to provide accurate and consistent results.

KEYWORDS

Beclomethasone, Degradation, Respirator Formulation

INTRODUCTION

Beclomethasone dipropionate chemically is (8S,9R,10S,11S,13S,14S,16S,17R)-9-chloro-11-hydroxy-10,13,16-trimethyl – 3 – oxo-17-[2-(propionyloxy)acetyl]-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-17-yl propionate (Figure 1). It is a potent glucocorticosteroid having molecular formula $C_{28}H_{37}ClO_7$. It is an anti-inflammatory steroid diester of Beclomethasone and is chemically related to Dexamethasone.

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It demonstrates its anti-inflammatory effect by inhibiting both the inflammatory cells and the release of inflammatory mediators. It is a prodrug which is converted to the free form i.e. Beclomethasone base[1]. It is available in various pharmaceutical presentations, such as creams, metered dose inhaler, dry powder inhaler and respirator suspension etc.

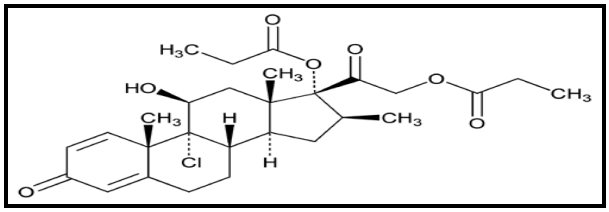

Figure 1: Chemical structure of Beclomethasone dipropionate

Beclomethasone is an old molecule hence significant amount of work is published related to the quantification of Beclomethasone alone or in combination from different matrixes. Various analytical methods using techniques such as HPLC[2,3], LC-MS/MS[4,5], HPTLC[6], etc. have been reported. For Since Beclomethasone is a synthetic corticosteroid, many process impurities are formed during its manufacture. These impurities are controlled by purification process. European Pharmacopoeia has a high performance liquid chromatography (HPLC) method for the determination of the process impurities and degradation products for the Beclomethasone Dipropionate API[7]. The method utilizes chromatographic conditions for the separation of the impurity peaks from the Drug peak which are harsh for the chromatographic column utilised for the separation. The separation is carried out using highly acidic buffer with a pH of 2.35 while corrosive organic solvent such as tetrahydrofuran is used as organic modifier. The column needs to be maintained at a very high temperature of 50 ºC. These conditions are not suitable for the delicate silica based HPLC columns used for the analysis, which may result in the rapid deterioration of column packing material and thereby the column performance. The analytical run time for the method is also about 70-75 minutes. For, an in- process check this analysis time means the batch will need to be kept in quarantine for more than 2 hours, resulting is overall reduction in the output of Quality control laboratory at the micro level and the manufacturing unit as a whole on a macro level.

UPLC or Ultra Performance Liquid Chromatography is a novel instrumentation chromatography technique developed by Waters Corporation, US. The UPLC utilises sub 2 micron size silica particles for achieving selectivity never before seen in column chromatography involving very short separation time. The different chemistries available also assist in developing methods for analytes requiring modified silica phases. The better selectivity demonstrated by UPLC columns provides a wide area of application to methods involving long HPLC analytical run time.

There is necessity of developing a simple chromatographic method which is having a higher throughput and does not cause deterioration of the column. The present work describes the development and validation of a new UPLC- UV method for the quantification of related substances and degradation products from Beclomethasone dipropionate in a respirator suspension formulationas per ICH Q2 (R1) guidelines[8].

MATERIALS AND METHOD

Standards and Chemicals

Beclomethasone dipropionate and the impurity standards were provided by Cipla Ltd., India. Beclomethasone dipropionate respules (each respule containing Beclomethasone dipropionate 0.4 mg/ml) were prepared for the study purpose. Acetonitrile used was of the chromatography grade (HPLC) and distilled water was prepared in-house using the TKA water purification system. The mobile phase solvents were sonicated 5 minutes and filtered through 0.20 µm teflon filter. Ultra Performance Liquid Chromatography (UPLC) chromatography system from Waters Corporation, USA was used for the development and validation. Clenil respules (each respules containing Beclomethasone dipropionate 0.8 mg/2 ml, manufactured by Cheisi Pharma, Italy) were procured and used for testing the method performance with unknown samples.

Selection of Analytical Wavelength

The UV absorption response of Beclomethasone and its impurities was checked at 238 and 254 nm. The analytical method published in European Pharmacopoeia[7] utilises 254 nm as the detection wavelength. However, no difference in the total number of impurity peaks or the impurity %age was found between the two wavelengths. Chromatography at 238 nm provided better response for the peaks of interest, hence the quantification of the

impurities in the present method was performed at 238 nm.

Chromatographic Conditions

The separation of the impurities was achieved using a gradient elution program consisting of Distilled water (Mobile phase A) and Acetonitrile (Mobile phase B). The gradient program is provided in Table 1. The peaks were separated on a Waters Acquity BEH C18 column (100 x 2.1 mm, 1.7 µm) UPLC column. The mobile phase was pumped at 0.4 ml/ min and the column oven was maintained at 25 ºC. The injection volume was 5 µL.

Table 1: Mobile phase gradient program

Time	Mobile Phase A %	Mobile Phase B %
0.01	60	40
1.00	60	40
10.00	55	45
13.00	53	47
17.00	45	55
17.30	60	40
20.00	60	40

Preparation of Standard and Sample Solutions

The stock solution of Beclomethasone dipropionate (BDP) standard was prepared by dissolving 20 mg of Beclomethasone dipropionate standard in acetonitrile to give a 200 µg/ml standard stock solution. This solution was successively diluted to give a working solution of 0.4 µg/ml of BDP.

The ten known related substances and degradation products (Table 2) were available as solutions containing 50 µg/ml of each impurity. These solutions were diluted to give a final solution containing 0.4 µg/ml of each impurity for individual peak identification. A resolution solution containing 0.4 µg/ml of each impurity and 200 µg/ml of BDP was prepared considering 0.2% as the limit to which each impurity was to be controlled in the formulation.

Table 2 Degradation products and the relative retention time obtained in the method

Peak No	Impurity name	RRT
1	Beclomethasone base	0.15
2	Beclomethasone 21 propionate	0.36
3	Beclomethasone 21 acetate	0.50
4	Beclomethasone 17 propionate	0.75
5	Epoxide impurity	0.95
6	Beclomethasone dipropionate	1.00
7	Beclomethasone 9 bromo analog	1.06
8	Beclomethasone 21 butyrate	1.23
9	Beclomethasone delta 9 analog	1.32
10	6 alpha chloro Beclomethasone	1.36
11	6 alpha bromo Beclomethasone	1.43

Each respule contains 0.8 mg of BDP. For sample preparation, five respule was opened by twisting the cap, the inside of the cap was washed with acetonitrile using syringe and needle in a 20 ml volumetric flask. The complete contents of the respule were transferred to the same 20 ml volumetric flask. The insides of the respule were rinsed twice with small aliquots of acetonitrile and the washings were collected in the same flask. The volume was made up with acetonitrile to 20 ml and the flask was shaken thoroughly to dissolve all the contents of the respule. The resultant sample solution contained 200 µg/ml of BDP. Placebo solution was prepared by diluting 2.5 ml of placebo to 5 ml using acetonitrile.

Validation

Validation was performed as per the requirements of ICH guidelines. The following experiments were performed as part of the validation.

Specificity

The specificity of the method was evaluated by chromatographing the individual solution of BDP, the impurity solutions and the placebo solution. The resolution solution containing BDP and the impurities was also chromatographed to demonstrate the resolution between the critical pair of peaks. The resolution chromatogram is presented in Figure 2. The chromatogram for the diluent blank and placebo are presented as Figure 3 and 4 respectively.

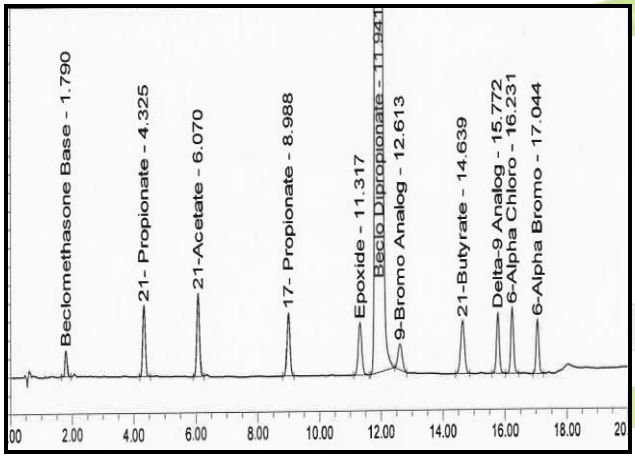

Figure 2: Typical chromatogram for Resolution solution showing Beclomethasone dipropionate and the impurity peaks

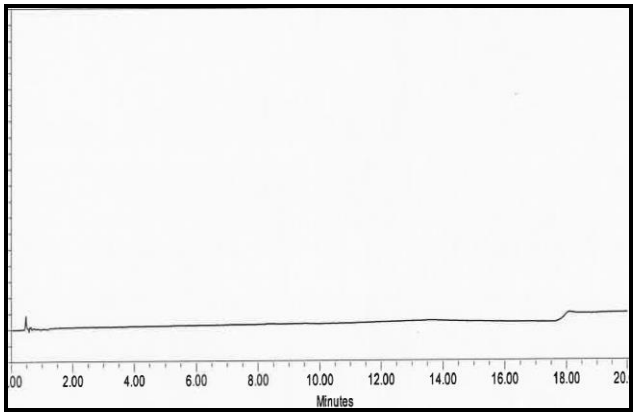

Figure 3: Typical chromatogram for diluent blank

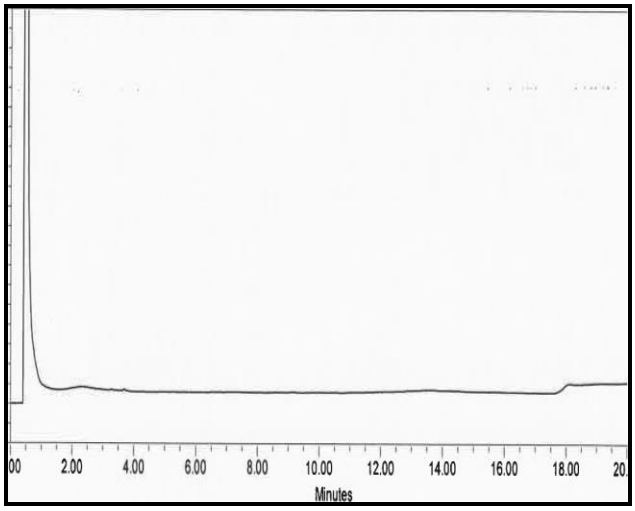

Figure 4: Typical chromatogram for the placebo solution

Sensitivity

Sensitivity of the method was evaluated by determining the limit of detection (LOD) and the limit of quantification (LOQ) for the BDP peak. The LOD and LOQ were evaluated by chromatographing concentrations of 0.05%, 0.04%, 0.03%, 0.02% and 0.01% of the concentration of BDP in the sample solution. LOQ was defined as the concentration with signal to noise ratio of at least 10, while LOD was defined as the concentration with signal to noise ratio of at least 3. The chromatogram of the working level standard solution (0.4 µg/ml) is presented as Figure 5.

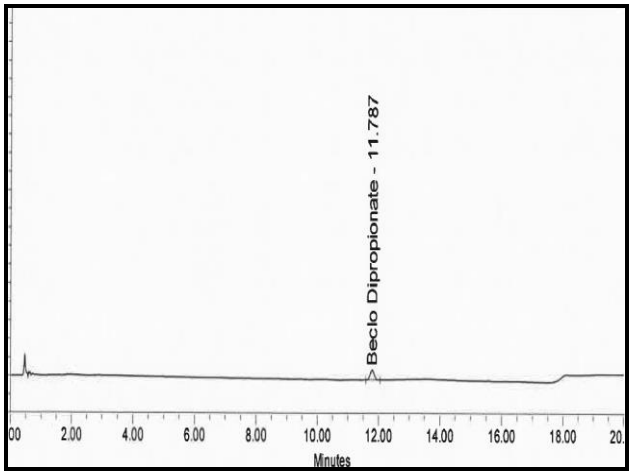

Figure 5: Typical chromatogram for the Beclomethasone dipropionate standard solution (0.4 µg/ml) for impurity calculation

Linearity and Range

Linearity and range was evaluated by chromatographing the solutions having concentrations from LOQ till 150% of the working concentration for each of the peaks.

The response factor for each of the impurity peaks was identified with respect to the BDP peak. The detector response against BDP concentration was plotted and the regression analysis on the line plot was performed.

Precision and Accuracy

Precision was evaluated as system precision and method precision. In system precision, the working level solution of BDP standard was chromatographed six times and the %RSD of the replicate injections was evaluated. The method was considered to meet system precision if the % RSD for area response for the replicate injections was NMT 5.0%.

For the method precision six individual sample preparations were prepared using the sample preparation method. The six sample solutions were chromatographed and the % RSD for each impurity peak was evaluated.

The method was considered to meet the method precision criteria if the % RSD for the % impurity from the six unique sample preparations for each impurity was NMT 10.0%. System precision was evaluated on each day of validation and analysis to determine the suitability of the chromatographic system for analysis.

Ruggedness

Ruggedness was evaluated by performing the analysis on two different days by different analysts and comparing the results of the two analyses. On both days the standard, placebo and six fresh samples were prepared using the sample preparation method and chromatographed using the chromatographic conditions specified in the method.

The method was considered to be rugged if the % RSD for the % impurity from the two analyses for each impurity was NMT 10.0%.

Robustness

Robustness of the method was evaluated by demonstrating the minor changes in the method parameters did not affect the results obtained from the analysis. The changes were made to the flow rate (change from 0.4 ml to 0.38 and 0.42 ml) and the column oven temperature (change from 25 ºC to 23 ºC and 27 ºC). The impact of the changes on the resolution and the % of impurities found was evaluated. The method was considered to be robust if the cumulative % RSD for the % impurity between the original method parameter and the altered method parameter two analyses for each impurity was NMT 10.0%.

Application of Method

The developed method was utilised for the determination of impurities in the Clenil respirator suspension samples under controlled condition and samples which were exposed to heat sterilization cycles at 121 ºC. For preparation of the sample for analysis the contents of 5 respules were pooled together in a 20 ml volumetric flask. The insides of respules were washed twice with small portions of acetonitrile and the washings were added to the volumetric flask. The volume was made up with acetonitrile and the contents mixed thoroughly to provide a clear solution. This solution was used for the estimation of the degradation products.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions

The UPLC system provides unparalleled advantages over the conventional HPLC analysis due to the sub two micron particle size. The specific chemistry of the BEH (Ethylene bridged hybrid) bonding provides additional specificity for separating compounds with closely related structures. Modification of the gradient composition of distilled water and acetonitrile provided separation between the impurity and BDP peaks. The ambient temperature of 25 ºC was found to be suitable to achieve the separation. The mobile phase

gradient program was found to provide the required separation between the BDP peak and the impurity peaks.

The method was found to be specific, as all of the impurity peaks and placebo peaks were well resolved from each other and the principal peak of BDP. The resolution between any two peak pairs was found to be at least 2.66. The % RSD for the system precision experiment were found to be 0.97%. The results of the specificity and precision experiment are summarised in Table 3.

Table 3: Summary of the specificity and precision experiment parameters

Specificity	1. No interference at the retention time of the drug or impurity peaks. 2. Resolution between any peak pair ≥ 2.66	
Sensitivity	1. LOD = 0.08 µg/ml 2. LOQ = 0.12 µg/ml	
Precision	System precision (% CV for BDP peak)	Area= 0.97% Retention time= 0.12%
	Method precision (% CV for impurity)	Single max imp. = 0. 24% Total impurity = 0.35%

The method was found to be linear between 0.12 µg/ml to 0.6 µg/ml for BDP, with the regression coefficient r^2 to be 0.9996. The response factor for all the impurities was found to be within 0.8 to 1.2 compared to the response of BDP peak. The results of the accuracy and linearity & range experiment are summarised in Table 4. The linearity experiment graph is provided in Figure 6.

Table 4: Summary of the method validation parameters

Accuracy	LOQ = 104.23% 50% = 100.45% 100% = 99.79% 150% = 99. 58%
Linearity	Range: 0.12 µg/ml to 0.6 µg/ml Regression coefficient (r^2): 0.9994 Line equation: y = 12532x-44.61

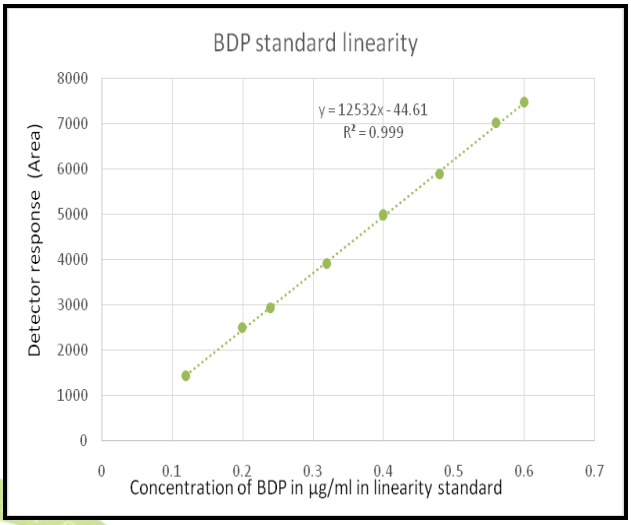

Figure 6: Linearity plot for Beclomethasone dipropionate

Table 5: Results of the ruggedness and robustness experiments

Ruggedness (Cumulative % CV)	Single max imp. = 2.45% Total impurity = 1.28%	
Robustness (Cumulative % CV)	Flow rate 0.38 ml/min	Single max imp. = 0.74% Total impurity = 0.48%
	Flow rate 0.42 ml/min	Single max imp. = 0.81% Total impurity = 0.33%
	Column oven 23ºC	Single max imp. = 1.25% Total impurity = 1.12%
	Column oven 27ºC	Single max imp. = 1.88% Total impurity = 0.68%

The method was found to provide consistent results and demonstrated significant degradation occurs when BDP is exposed to heat and the main route of degradation is epoxidation, evident from the increase in the epoxide impurity content. The typical chromatogram of sample solution is provide as Figure 7.

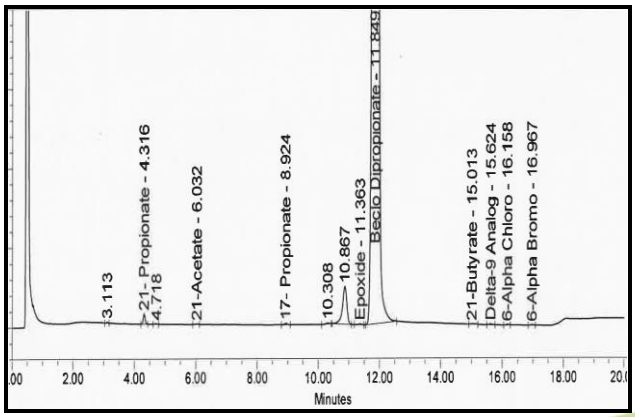

Figure 7: Typical chromatogram of samples solution of Beclomethasone dipropionate respirator suspension formulation

CONCLUSION

A rapid analytical method utilising the UPLC technique for the determination of process impurities and degradation products was developed and suitably validated. Comparison of the results between the pharmacopeial method and the UPLC method demonstrated similar results and hence equivalent performance. The analysis of marketed formulation and samples exposed to stress conditions has provided evidence that the method can be utilised as a quality control tool. The short analytical run time added with the sensitivity and reliability would be an efficient tool for the analytical chemist.

ACKNOWLEDGEMENTS

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