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Article

# Impurity Profiling and a Stability-Indicating UPLC Method Development and Validation for the Estimation of Related Impurities of Halobetasol Propionate in Halobetasol Propionate 0.05% (w/w) Cream

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**A simple, short and stability-indicating reverse phase-ultra-performance liquid chromatography method was developed and validated for the quantitative determination of related impurities of halobetasol propionate in halobetasol propionate 0.05% cream formulation. The proposed method was developed on an ACQUITY UPLC™ BEH Phenyl (2.1 × 100 mm, 1.7 μm) column at 40°C with a mobile phase containing a gradient mixture of potassium hydrogen phosphate buffer and acetonitrile and methanol as modifiers with a runtime of 13.0 min at a monitored wavelength of 242 nm. A simple preparative method and liquid chromatography–mass spectrometry-compatible UPLC method also were developed for the isolation and identification of impurities and degradation products. The drug was subjected to forced-degradation conditions and found to degrade significantly. The stability-indicating capability of the developed method is established by analyzing forced-degradation samples in which the spectral purity of halobetasol propionate is ascertained along with the separation of degradation products from the analyte peak. The developed method was validated as per International Conference on Harmonization guidelines. The developed method is precise (%relative standard deviation <2.0) and is capable of detecting and quantifying all the six impurities at a level of 0.01 and 0.03%, respectively, with respect to test concentration. The wide linearity range, sensitivity, accuracy, short retention time and simple mobile phase imply that the method is suitable for routine quantification of halobetasol propionate and its related substances.**

## Introduction

Halobetasol propionate is a synthetic corticosteroid for topical dermatological use with anti-inflammatory, antipruritic and vasoconstrictive properties (1, 2). The corticosteroids constitute a class of primarily synthetic steroids used topically as an anti-inflammatory and antipruritic agent. Chemically halobetasol propionate is 21-chloro-6α,9-difluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3-20-dione,17-propionate, $C_{25}H_{31}ClF_2O_5$ (Figure 1). Halobetasol propionate has the molecular weight of 485. It is a white crystalline powder insoluble in water. Each gram of halobetasol propionate cream 0.05% (w/w) contains 0.5 mg of halobetasol propionate.

Like other topical corticosteroids, halobetasol propionate has anti-inflammatory, antipruritic and vasoconstrictive actions. The mechanism of the anti-inflammatory activity of the topical corticosteroids, in general, is unclear. However, corticosteroids are thought to act by the induction of phospholipase A2 inhibitory proteins, collectively called lipocortins. It is postulated that these proteins control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes by inhibiting the release of their common precursor, arachidonic acid. Arachidonic acid is released from membrane phospholipids by phospholipase A2 (3).

Impurity profiling is the common name of a group of analytical activities, the aim of which is the detection, identification/structure elucidation and quantitative determination of identified and unidentified (organic and inorganic impurities, residual solvents) impurities in bulk drugs and pharmaceutical formulations (4, 5). Since this is the best way to characterize the quality, safety, efficacy as well as stability of bulk drugs and pharmaceutical formulations, this is the core activity in modern drug analysis. Impurity profiling is very crucial and critical during the synthesis of drug substances and manufacture of dosage forms, as it can provide crucial data regarding the quality, safety, efficacy, toxicity of drugs, various limits of detection (LODs) and limits of quantification (LOQs), structures of several organic and inorganic impurities, usually associated with bulk drugs and finished products.

Ultra-performance liquid chromatography (UPLC) is a relatively new technique that offers better separation capabilities when compared with high-performance liquid chromatography (HPLC), with added benefits of a shorter runtime and lower solvent consumption. One of the key developments that facilitate the new UPLC technology is sub-2 μm particles used as column packing material (6).The literature indicates that the UPLC system allows an ~9-fold decrease in analysis time when compared with the conventional HPLC system using 5 μm particle size analytical columns, and about a 3-fold decrease in analysis time in comparison with 3 μm particle size analytical columns, without compromise on overall separation (7–11). In the present study, this technique has been applied to the development of the stability-indicating method, method validation study and UPLC-time-of-flight mass identification of related substances of halobetasol propionate.

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, which enables recommendation of storage conditions, retest periods and establishing shelf life. The content of related substances of halobetasol propionate cream 0.05% (w/w) is required to be determined using a stability-indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines (12) and United State Pharmacopoeia (USP) (13).

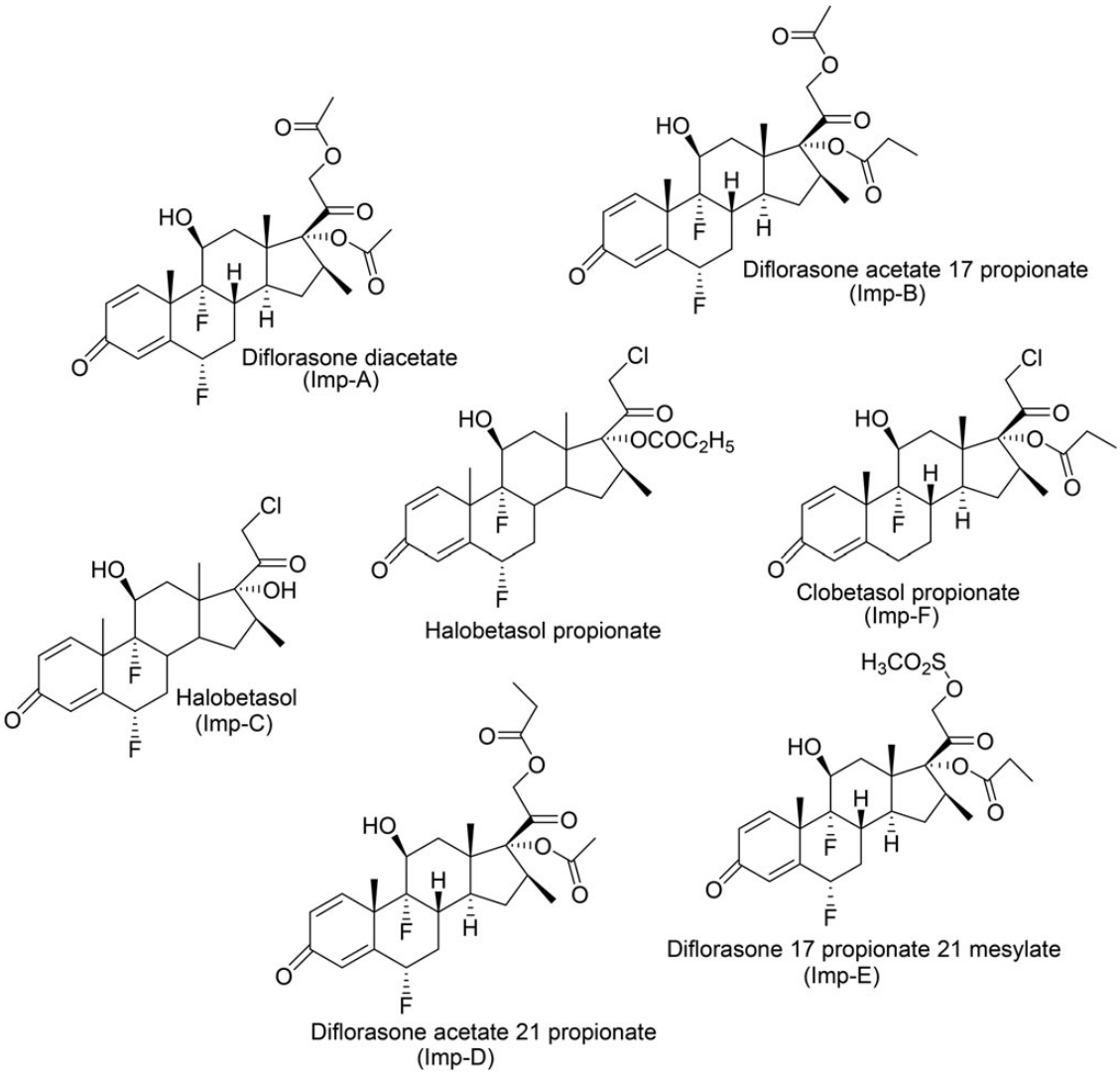


**Figure 1.** Chemical structures of halobetasol propionate and its impurities.

A literature survey revealed a few stability-indicating analytical methods for the quantification of related substances of halobetasol propionate based on HPLC (14, 15), but all impurities were not included and there was no liquid chromatography–mass spectrometry (LC–MS)-compatible method for preparative isolations and mass identification. The literature review showed that no single stability-indicating reverse phase-ultra-performance liquid chromatography (RP-UPLC) method with a short runtime was reported for the estimation of related compounds of halobetasol propionate in topical cream. Thus the paper describes the development and validation of a stability-indicating RP-UPLC method to monitor the related compounds of halobetasol propionate in topical cream as well as preparative impurity isolation and LC–MS identification of degradation products of halobetasol propionate.

## Experimental

### *Chemicals and reagents*

Active pharmaceutical ingredient standards and samples were supplied by Dr Reddy's Laboratories Limited, IPDO, Hyderabad, India. HPLC-grade acetonitrile, methanol, tetrahydrofuran, potassium dihydrogen phosphate, *ortho*–phosphoric acid and trifluoroacetic acid were procured from Merck. Milli Q water used was obtained by using a Millipore Milli Q Plus water purification system.

### *Equipment*

Analytical separation trials were carried out in an ACQUITY UPLC System (Waters, Milford, MA, USA) consisting of a binary solvent manager, sample manager and photo diode array (PDA) detector. The output signal was monitored and processed using the Empower2 software. A Cintex digital water bath was used for hydrolysis studies. Photostability studies were carried out in a photostability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (Cintex, Mumbai, India). The preparative isolation study was performed on an Agilent 1200 series preparative HPLC system that was equipped with an automated fraction collector and photodiode array detector. The data were collected and processed

using the Chemstation software. The mass identification study was carried out by UPLC–ToF. The UPLC–ToF MS system consisted of an ACQUITY, UPLC system and a Micro mass LCT Premier XE Mass Spectrometer (high-sensitivity orthogonal time-of-flight instrument, Waters) equipped with a lock mass sprayer, operating in either the positive- or negative-ion mode.

### *Chromatographic conditions*

The chromatographic column used was an ACQUITY UPLC BEH Phenyl (2.1 × 100 mm, 1.7 μm) column and the separation was achieved by a short runtime gradient method. Mobile phase A contains a mixture of 10 mM potassium dihydrogen phosphate buffer with pH adjusted to 3.0 with 5% *ortho*-phosphoric acid, acetonitrile and methanol in the ratio of 80:15:5 (v/v); and Mobile phase B contains a mixture of 10 mM potassium dihydrogen phosphate buffer with pH adjusted to 3.0 with 5% *ortho*-phosphoric acid, acetonitrile and methanol in the ratio of 20:70:10 (v/v). The gradient program (time (min)/%B) was set as 0.01/40, 3.75/45, 7.75/50, 11.75/55, 12.00/40 and 13/40 with a flow rate of 0.2 mL/min and injection volume of 4.0 μL. The column temperature was maintained at 40°C and the peaks were monitored at 242 nm. Diluent A is tetrahydrofuran and Diluent B is a mixture of water and methanol in the ratio of 50:50 (v/v) used for sample preparation.

### *Preparative HPLC conditions*

The preparative column used was XBridge Prepshield RP 18 (10 × 250 mm, 5.0 μm). Mobile phase A contains 0.1% trifluoroacetic acid (TFA) in Milli Q water and Mobile phase B contains the mixture of Mobile phase A and acetonitrile in the ratio of 10:90 (v/v). The gradient program (time (min)/%B) was set as 0.01/45, 20/65, 25/75, 27/45 and 30/45 with a flow rate of 5.0 mL/min and injection volume of 500 μL. The column temperature was maintained at 40°C and the peaks were monitored at 242 nm. A mixture of water and methanol in the ratio of 50:50 (v/v) was used as the diluent for sample preparation.

### *LC–MS/MS conditions*

The chromatographic column used was an ACQUITY UPLC BEH Phenyl (2.1 × 100 mm, 1.7 μm) column. Mobile phase A contains a mixture of 0.1% TFA in Milli Q water, acetonitrile and methanol in the ratio of 80:15:5 (v/v), and Mobile phase B contains a mixture of 0.1% TFA in Milli Q water, acetonitrile and methanol in the ratio of 20:70:10 (v/v). The gradient program (time (min)/%B) was set as 0.01/40, 3.75/45, 7.75/50, 11.75/55, 12.00/40 and 13/40 with a flow rate of 0.2 mL/min and injection volume of 1.0 μL. The column temperature was maintained at 40°C and the peaks were monitored at 242 nm. A mixture of water and methanol in the proportion of 50:50 (v/v) was used as the diluent for sample preparation.

### *Preparation of solutions*

#### *Preparation of standard solution*

A stock solution of halobetasol propionate (500 μg/mL) was prepared with Diluent A. From the above stock solution 0.25 μg/mL of standard solution was prepared with Diluent B and used as diluted standard for the determination of related substances. Individual stock solutions (0.5 μg/mL) of the impurities (denoted as Impurity (Imp)-A to Imp-F (Figure 1)) were prepared with the diluent and used for validation.

#### *Preparation of sample solution*

Ten gram of sample was weighed and transferred into a 250-mL glass beaker; 25 mL of Diluent A was added and sonicated for about 15 min with intermediate swirling; the content was transferred completely into a 50-mL volumetric flask and 20 mL of Diluent A was added and this was sonicated for 10 min, cooled and diluted to volume with Diluent A and mixed well. Five milliliters of the above solution was diluted to 10 mL with Diluent B. A portion of the sample solution was filtered through a 0.45 μm membrane polytetrafluoroethylene (PTFE) filter. The solution obtained was analyzed by UPLC.

#### *Preparation of sample solution for preparative isolation*

Ten grams of sample were dissolved in 50 mL of tetrahydrofuran and heated up to 40°C to get clear solution. To this solution, 50 mL of Diluent B was added. The solution was filtered through a 0.45-μm membrane PTFE filter and concentrated by rotaevaporation. The obtained concentrated solution was subjected to impurity isolation by preparative HPLC.

### *Stress studies*

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. Stress studies were performed at an initial concentration of 50 μg/mL of halobetasol propionate solution to prove the stability-indicating property of the proposed method. Intentional degradation was attempted by the stress conditions of photolytic sunlight for ∼ 1.2 million lux h and ultra violet (UV) light, both at shorter and longer wavelengths for ∼200 $Wh/m^3$, heat (60°C for 6 h), acid (1 M HCl at 60°C for 30 min), base (1 M NaOH at 60°C for 30 min), hydrolysis (60°C for 30 min) and oxidation (6.0% $H_2O_2$ at 60°C for 30 min) to evaluate the ability of the proposed method to separate halobetasol propionate from its degradation products.

The purity of the peaks obtained from the stressed samples was verified by using a PDA detector. The purity angle was within the purity threshold limit obtained in all stressed samples and demonstrates the analyte peak homogeneity. Assay of stressed samples was performed by comparison with reference standards and the mass balance (% assay + % sum of all impurities + % sum of all degradation products) for stressed samples was calculated.

### *Method validation*

The proposed test method has been validated by UPLC as per ICH guidelines (16).

#### *Precision*

The repeatability of the test method was proved by injecting six individual preparations of the halobetasol propionate sample spiked with 1.0% of its six impurities (1.0% of impurities with respect to 50 μg/mL halobetasol propionate). The intermediate precision of the method was also evaluated using a different analyst and different instrument (Waters ACQUITY UPLC System),

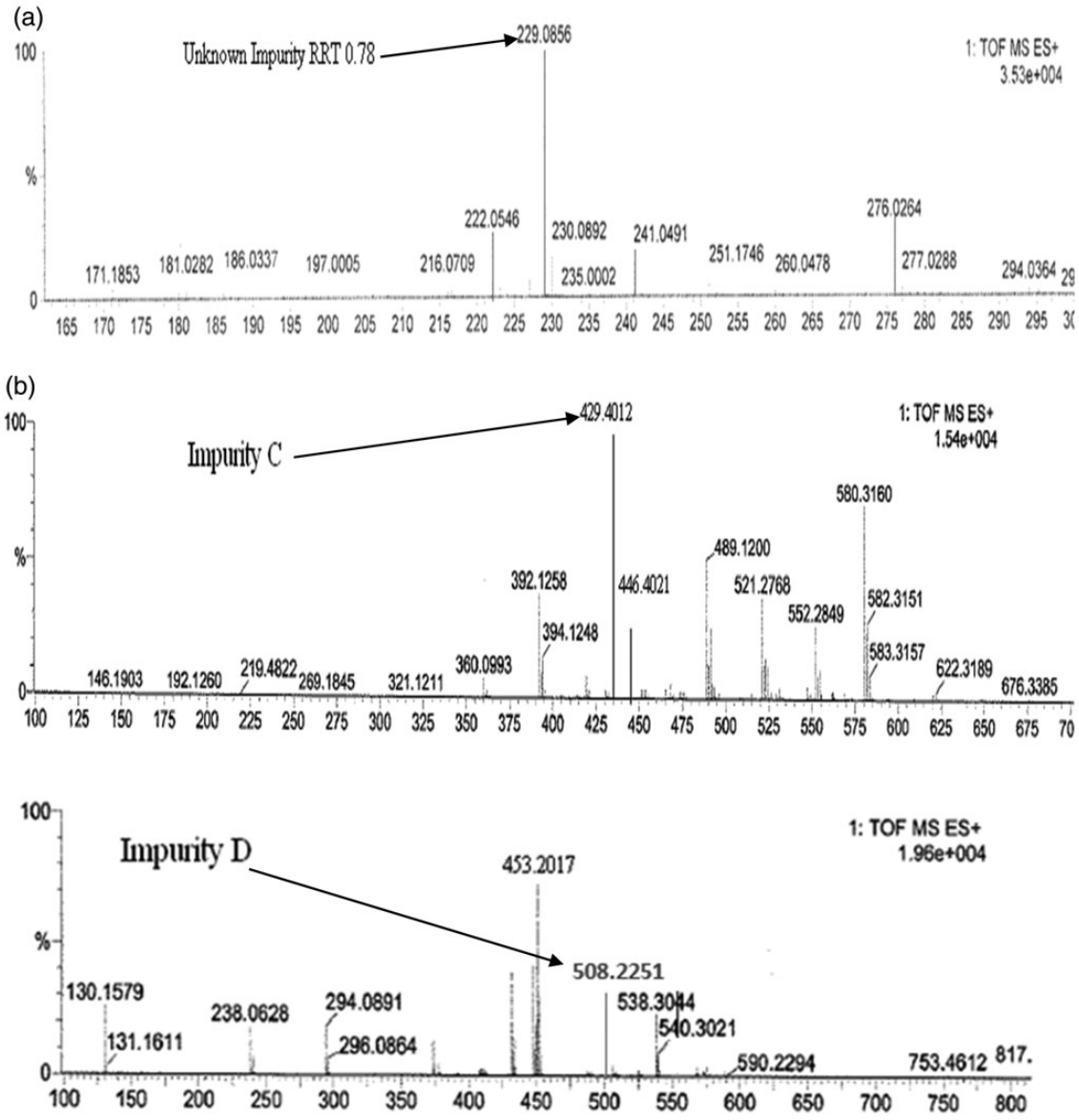


**Figure 2.** LC–MS data of stressed samples: (a) mass spectral data of unknown impurity, (b) mass spectral data of degradation products found in oxidation and (c) mass spectral data of degradation products found in acid hydrolysis.

and performing the analysis on a different day. The %RSD (relative standard deviation) of peak area of each impurity was calculated for both precision and intermediate precision.

*LOD and LOQ*

The LOD and the LOQ for the six impurities and halobetasol propionate were estimated at the concentrations for which signal-to-noise ratios were 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentrations. Precision was also determined at the LOQ level by analysis of six individual preparations of the six impurities and calculating the RSD (%) of the peak area for each impurity.

*Accuracy*

The accuracy of an analytical procedure expresses the closeness of agreement between the true value and the observed value. For all impurities, the recovery was determined in triplicate for 50, 75, 100, 125 and 150% of the analyte concentration (50 μg/mL) of the drug product. The percentage of recoveries for six impurities was calculated.

*Linearity*

To establish the linearity of the related substance method, solutions were prepared by diluting the standard stock solution and impurity stock solution to obtain the required concentrations at seven different levels ranging from LOQ to 200% (i.e., LOQ, 50, 75, 100, 125, 150 and 200%) to the specification level, 1.0%. The correlation coefficient, slope and $y$-intercept of the calibration curve were calculated (i.e., the LOQ to 200% for halobetasol propionate and its impurities).

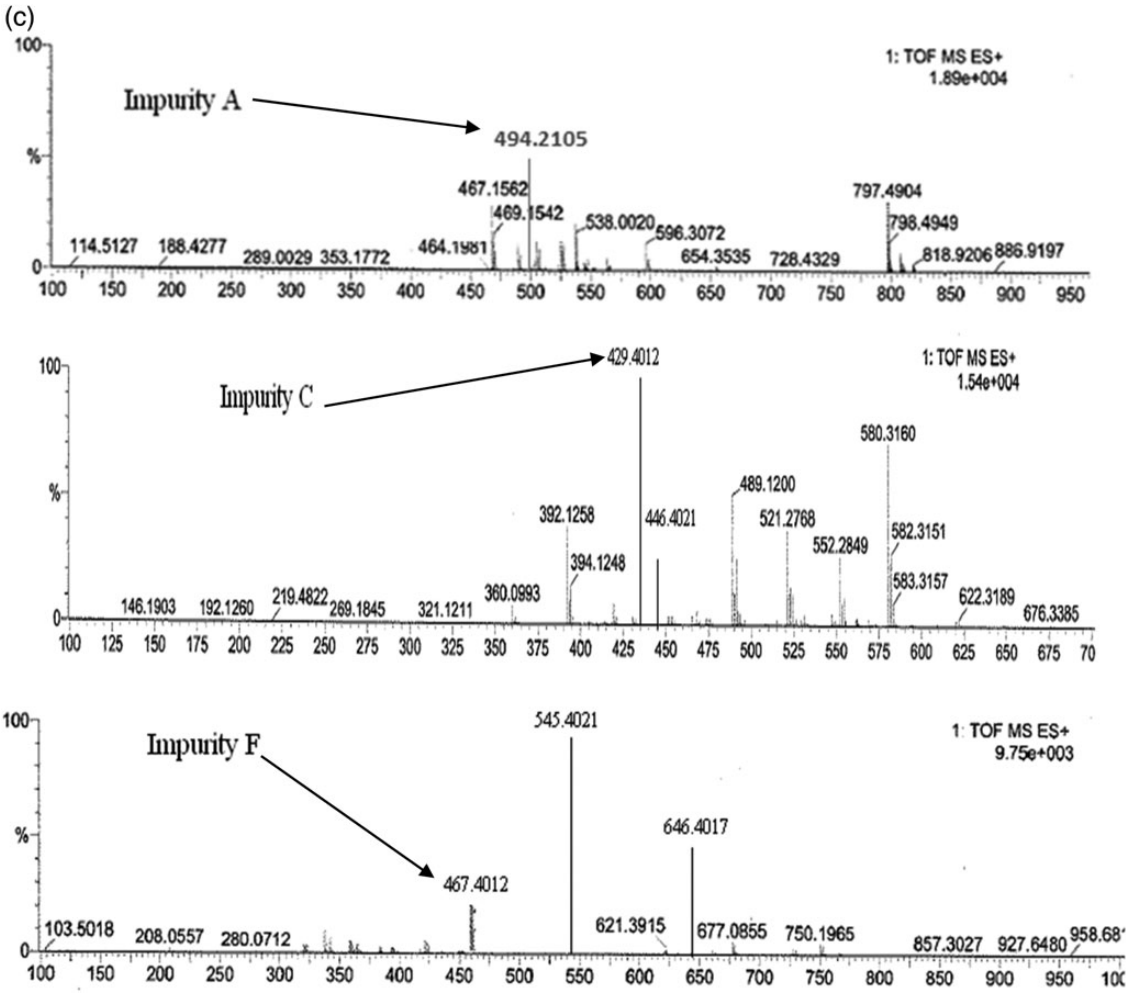


**Figure 2.** *Continued*

**Table I**
Summary of Forced-Degradation Results

| Degradation condition | Time (h) | Assay (%, w/w) | Mass balance (% assay + % deg. products) | % Net degradation[a] | Observation |
|---|---|---|---|---|---|
| 1 M HCL (acid hydrolysis) | 0.5 | 93.8 | 95.0 | 1.2 | Imp-A, -C, -F formed |
| 1 M NaOH (base hydrolysis) | 0.5 | 85.9 | 96.2 | 10.3 | Imp-B, -C, -E, -F formed |
| Oxidation by 6% peroxide | 0.5 | 95.2 | 96.6 | 1.4 | Imp-C, -D formed |
| Water hydrolysis | 0.5 | 94.4 | 95.5 | 1.1 | Imp-B, -C, -E, -F formed |
| Thermal degradation | 6 | 94.9 | 96.1 | 1.2 | Imp-B, -C, -E, -F formed |
| UV light | 19 | 91.04 | 95.0 | 3.96 | Imp-A, -B, -C, -E, -F formed |

[a]% Net degradation = % degradation in stressed sample − % degradation in unstressed sample.

*Robustness*

The robustness study was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions, which have been described in the Chromatographic conditions section. The factors chosen for this study, which were critical sources of variability in the operating procedures such as temperature of the column ($\pm 5^{\circ}$C), mobile phase pH ($\pm 0.2$) and flow rate ($\pm 0.2$ mL/min), were identified. In all these experiments, the mobile-phase components were not changed. The effect of the percentage of organic strength on resolution was studied by varying the strength of organic modifiers (acetonitrile and methanol) by $-10$ to $+10\%$, while other mobile-phase components were held constant as stated in the Chromatographic conditions section. The resolution between halobetasol propionate and impurities was evaluated in the deliberately altered experimental conditions.

*Solution stability and mobile-phase stability*

Solution stability was established by injecting the same standard and spiked sample at time intervals of Days 1, 2 and 5. Standard and spiked sample solution were placed on bench top during the study and the results with those obtained from freshly prepared

reference standard solutions were compared. Mobile-phase stability was established by injecting the freshly prepared standard and spiked sample at time intervals of Days 1, 2 and 5, without changing the mobile phase.

## Results

### *Results of forced-degradation studies of halobetasol propionate*

All forced-degradation samples were analyzed at an initial concentration of 50 μg/mL of halobetasol propionate with the aforementioned UPLC conditions using a PDA detector to monitor the homogeneity and purity of the halobetasol propionate peaks. Significant degradation was observed when the drug was subjected to acid, base, oxidative, water hydrolysis, thermal and UV–visible conditions. The purity angle was within the purity threshold limit obtained in all stressed samples and demonstrates the analyte peak homogeneity. In stability conditions and in all the above degradations one unknown impurity formed at relative retention time (RRT) 0.78. The same was isolated by preparative HPLC with the experimental conditions given in the Preparative HPLC conditions section. Preparative LC is a versatile, robust and widely used dominant purification technique for the isolation of pharmaceutical impurities. The objective of an analytical HPLC run is the qualitative and quantitative determination of a compound. For a preparative HPLC run it is the isolation and purification of a valuable product. The objective of the present study is to isolate the degradants of halobetasol propionate formed during stability and under stressed conditions. All impurity fractions were collected separately from 100 injections and pooled together separately. The pooled samples were rotaevaporated separately at 25°C under high vacuum to remove the organic solvents and solidified through the lyophilization process.

The degradation products that were formed during the stress studies were identified by LC–MS. LC–MS analysis was performed as per experimental conditions given in the LC–MS-MS conditions section. The unknown impurity-eluting RRT 0.78 exhibited molecular ions at $m/z$ 229.08 amu $(M+H)^+$. The stressed sample by oxidation exhibited molecular ions at $m/z$ 429.40 amu $(M+H)^+$ and $m/z$ 554.60 amu $(M+H)^+$ (methanol adduct) corresponding to Imp-C and Imp-D, respectively. The stressed sample of acid hydrolysis exhibited molecular ions at $m/z$ 528.40 amu $(M+H)^+$ (methanol adduct), 429.40 amu $(M+H)^+$ and 467.40 amu $(M+H)^+$ corresponding to Imp-A, Imp-C and Imp-F, respectively. Mass spectral data of unknown impurity and degradation products formed during stressed conditions of oxidation and acid hydrolysis are shown in Figure 2a–c.

**Table II**
LOD, LOQ, Regression and Precision Data

| Compound | LOD (%) | LOQ (%) | Regression equation ($y$) | | Correlation coefficient | Precision (%RSD) | Intermediate precision (%RSD) |
|---|---|---|---|---|---|---|---|
| | | | Slope ($b$) | Intercept ($a$) | | | |
| Imp-A | 0.011 | 0.032 | 121,391 | 286 | 0.999 | 2.0 | 2.1 |
| Imp-B | 0.009 | 0.026 | 154,015 | −32 | 0.999 | 1.6 | 1.5 |
| Imp-C | 0.017 | 0.050 | 93,252 | 6 | 0.999 | 0.6 | 0.5 |
| Imp-D | 0.009 | 0.026 | 171,786 | 137 | 0.999 | 0.5 | 0.6 |
| Imp-E | 0.011 | 0.032 | 117,256 | 19 | 0.999 | 0.4 | 0.5 |
| Imp-F | 0.015 | 0.046 | 120,471 | 383 | 0.999 | 0.2 | 0.3 |
| Halobetasol propionate | 0.013 | 0.039 | 126,109 | 953 | 0.999 | 0.2 | 0.3 |

**Table III**
Evaluation of Accuracy

| Compound | % of recovery at each level[a] | | | | | |
|---|---|---|---|---|---|---|
| | LOQ (%) | 50% | 75% | 100% | 125% | 150% |
| Imp-A | 101.5 ± 0.9 | 107.0 ± 0.9 | 105.4 ± 0.1 | 103.0 ± 0.3 | 107.5 ± 0.9 | 103.0 ± 0.3 |
| Imp-B | 96.1 ± 1.4 | 111.2 ± 0.9 | 107.4 ± 0.5 | 104.7 ± 0.3 | 103.2 ± 0.1 | 102.4 ± 0.3 |
| Imp-C | 100.8 ± 0.3 | 106.2 ± 0.2 | 107.0 ± 0.9 | 104.2 ± 0.2 | 102.7 ± 0.3 | 101.8 ± 0.2 |
| Imp-D | 103.0 ± 2.1 | 111.2 ± 0.5 | 107.4 ± 0.5 | 101.3 ± 0.4 | 103.2 ± 0.2 | 99.3 ± 0.2 |
| Imp-E | 108.7 ± 3.2 | 106.0 ± 0.1 | 97.2 ± 0.2 | 101.2 ± 1.2 | 99.7 ± 0.5 | 101.9 ± 1.8 |
| Imp-F | 104.5 ± 0.1 | 107.3 ± 0.1 | 103.7 ± 0.5 | 101.7 ± 0.5 | 101.2 ± 0.2 | 102.0 ± 0.4 |
| Halobetasol propionate | 105.5 ± 2.5 | 97.5 ± 0.3 | 105.0 ± 0.7 | 102.4 ± 0.3 | 101.8 ± 0.4 | 98.9 ± 0.7 |

[a]Mean ± %RSD of three determinations.

**Table IV**
Results of Robustness Study (Variation in USP Resolution)

| Compound | Flow (mL/min) | | Temp. (°C) | | Buffer pH | | Acetonitrile[a] | | Methanol[b] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.18 | 0.22 | 35 | 45 | 2.8 | 3.2 | 90% | 110% | 90% | 110% |
| Imp-A | – | – | – | – | – | – | – | – | | |
| Imp-B | 6.72 | 6.73 | 6.73 | 6.77 | 6.76 | 6.74 | 6.70 | 6.78 | 6.72 | 6.76 |
| Imp-C | 1.65 | 1.64 | 1.64 | 1.67 | 1.67 | 1.65 | 1.62 | 1.68 | 1.65 | 1.67 |
| Imp-D | 3.10 | 3.10 | 3.10 | 3.12 | 3.12 | 3.11 | 3.08 | 3.13 | 3.10 | 3.12 |
| Imp-E | 21.35 | 21.36 | 21.35 | 21.38 | 21.35 | 21.35 | 21.32 | 21.39 | 21.33 | 21.36 |
| Imp-F | 3.26 | 3.27 | 3.27 | 3.30 | 3.26 | 3.27 | 3.25 | 3.31 | 3.26 | 3.29 |
| Halobetasol propionate | 2.37 | 2.36 | 2.36 | 2.40 | 2.36 | 2.37 | 2.33 | 2.40 | 2.36 | 2.38 |

[a]The results are more or less same for the variation of acetonitrile in Mobile phases A and B.
[b]The results are more or less same for the variation of methanol in Mobile phases A and B.

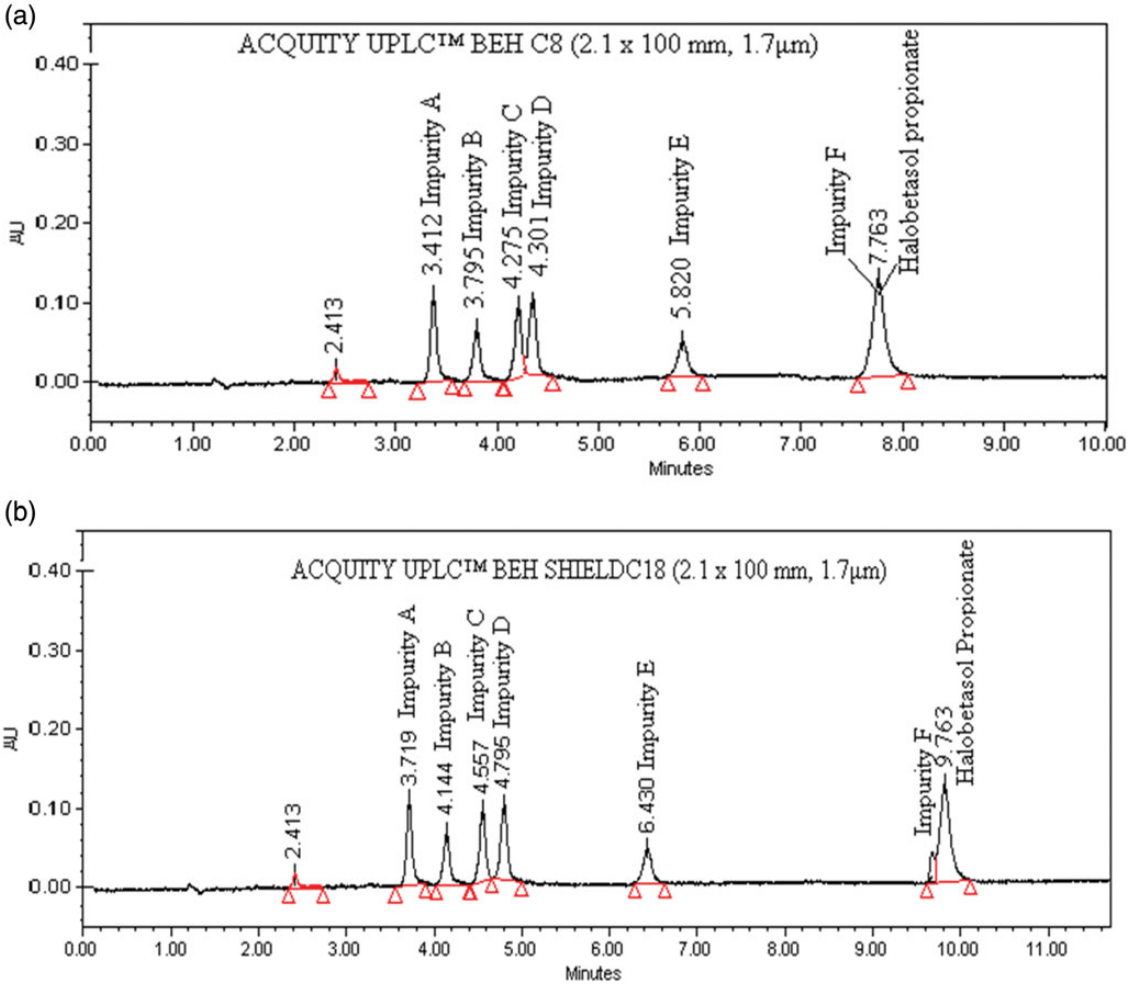


**Figure 3.** Typical chromatograms of halobetasol propionate spiked with its six impurities from method development trials: (a) typical chromatogram on ACQUITY UPLC BEH C8 (2.1 × 100 mm, 1.7 µm), (b) typical chromatogram on ACQUITY UPLC BEH SHIELD C18 (2.1 × 100 mm, 1.7 µm), (c) typical chromatogram on ACQUITY UPLC BEH Phenyl (2.1 × 100 mm, 1.7 µm)-isocratic method and (d) typical chromatogram on ACQUITY UPLC BEH Phenyl (2.1 × 100 mm, 1.7 µm)-gradient method.

Assay of stressed samples has been performed by comparison with the reference standard and the mass balance also calculated by the summation of % assay of the stressed sample, % known impurities and % degradation products found in the stressed sample. Results from forced-degradation studies are presented in Table I. The purity of halobetasol propionate was unaffected by the presence of its impurities and degradation products and thus confirms the stability-indicating power of the developed method.

## *Results of validation*

### *Precision*

The %RSD for peak areas of the six impurities, namely Imp-A, Imp-B, Imp-C, Imp-D, Imp-E and Imp-F, in the study of repeatability was within 2.0. Results for intermediate precision were within 2.0%. These results demonstrate that the method is precise and robust (Table II).

### *LOD and LOQ*

The determined LOD and LOQ values for halobetasol propionate and its six impurities are reported in Table II.

### *Accuracy*

The percentage of recoveries for halobetasol propionate and six impurities ranged from 97.2 to 110.2%. The results are reported in Table III.

### *Linearity*

For all six impurities of halobetasol propionate, the linear calibration curve was obtained in the concentrations ranging from LOQ to 200% (LOQ: 50, 75, 100, 125, 150 and 200%) of the nominal sample concentration (0.5 mg/mL) for halobetasol propionate and its impurities. The correlation coefficient obtained was $>0.999$ for all the components. The slope and $y$-intercept values are also provided in Table II, which confirmed good linearity between peak areas and concentration.

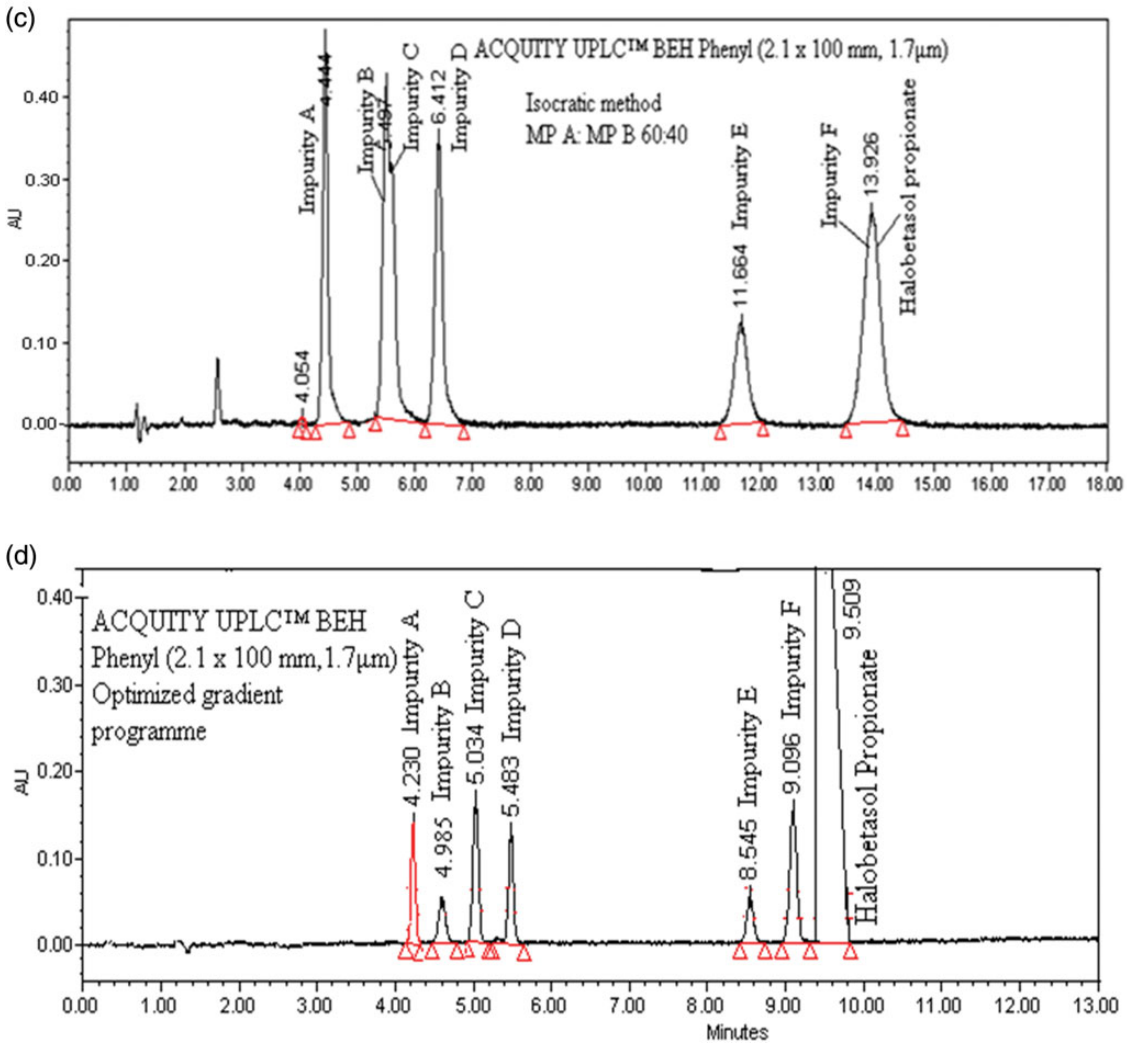


**Figure 3.** *Continued*

*Robustness*

In all the deliberately varied chromatographic conditions (flow rate, column temperature and pH of buffer, composition of organic solvent), all analytes were adequately resolved and elution orders remained unchanged, which indicate that the method is robust. Results of the robustness of the study are provided in Table IV.

*Solution stability and mobile-phase stability*

A mobile-phase and solution stability study was performed as a part of ruggedness. During the solution stability experiments, it was observed that the solution was stable for 24 h at room temperature and for 48 h when stored at 5 ± 2°C. The RSD of the assay of halobetasol propionate during the mobile-phase stability experiments was within 0.60%. No significant changes were experienced in the content of any of the impurities during the mobile-phase stability experiments. The mobile-phase stability experimental data confirm that the mobile phase used was stable up to 48 h. Hence from the above study, we can conclude that the prepared samples and mobile phase used for estimation of related substances were stable up to 48 h.

## Discussion

### *Method development and optimization*

The main criteria for development of a successful UPLC method for halobetasol propionate in 0.05% (w/w) cream were as follows: the method should be able to separate and determine the impurities of the drug in a single run and should be accurate, reproducible, robust, stability-indicating, free of interference from blank/placebo/impurities/degradation products and straightforward enough for routine use in the quality control laboratory. The spiked solution of related compounds with halobetasol propionate was subjected to separation by RP-UPLC. As part of the preliminary study, separation was attempted on ACQUITY UPLC BEH C8 (2.1 × 100 mm, 1.7 µm) (Figure 3a) and ACQUITY UPLC BEH shield RP18 (2.1 × 100 mm, 1.7 µm) (Figure 3b) columns with 0.05 M potassium dihydrogen phosphate in aqueous solution as buffer and acetonitrile as organic in different gradient programs at a flow rate of 0.2 mL $min^{-1}$. After thorough screening of different compositions of organic modifiers, buffer pH and column oven temperatures, separation was achieved within 18 min of runtime. The drawback on these columns was that the Imp-F and halobetasol propionate were co-eluting at the

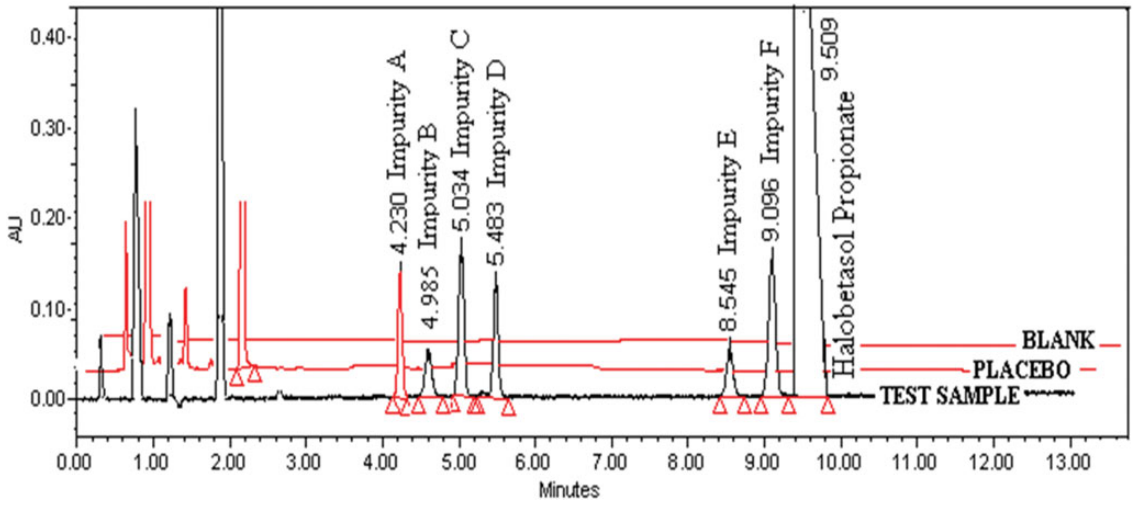


**Figure 4.** Typical overlaid chromatogram of blank, placebo and test sample.

**Table V**
System Suitability Parameters

| Compound | RT (min)[a] | RRT[b] ($n = 6$)[a] | USP resolution[c] ($n = 6$)[a] | USP tailing factor ($n = 6$)[a] |
|---|---|---|---|---|
| Imp-A | 4.23 | 0.44 ± 0.00 | – | 1.03 |
| Imp-B | 4.98 | 0.52 ± 0.01 | 6.75 | 0.59 |
| Imp-C | 5.03 | 0.53 ± 0.01 | 1.66 | 0.88 |
| Imp-D | 5.48 | 0.58 ± 0.00 | 3.11 | 1.02 |
| Imp-E | 8.54 | 0.90 ± 0.01 | 21.36 | 1.00 |
| Imp-F | 9.09 | 0.96 ± 0.01 | 3.28 | 1.01 |
| Halobetasol propionate | 9.50 | 1.00 ± 0.00 | 2.37 | 1.02 |

[a]Mean ± SD ($n = 6$ preparations).
[b]RRT was calculated against the retention time (RT) of halobetasol propionate.
[c]Resolutions were calculated between two adjacent peaks.
USP plate counts = 21,047.

same retention time and there is no optimum resolution between Imp-C and Imp-D. So the stationary phase was changed to the ACQUITY UPLC BEH Phenyl (2.1 × 100 mm, 1.7 µm) column, with isocratic (Figure 3c) and gradient conditions. Further changing the gradient program, Imp-F and halobetasol propionate were separated with good resolution and base to base separation was achieved between Imp-C and Imp-D; moreover, the runtime also shortened to 13 min (Figure 3d). Based upon these optimized chromatographic conditions, the LC–MS compatible UPLC method was also developed and employed for mass identification of halobetasol propionate and related impurities. Diluent optimization trials were carried out for the complete extraction of active pharmaceutical ingredient from the formulated cream by the thorough screening of different compositions of organic solvents and buffers. The optimized conditions are discussed in the Chromatographic conditions section. Interference with diluent and excipients (preservatives used in cream) was also checked and no interference was observed at impurity peaks and the halobetasol propionate peak. A typical overlaid chromatogram of blank, placebo and test sample is shown in Figure 4.

The relative response factor for all the six impurities was determined with respect to halobetasol propionate. The system suitability parameters were evaluated for halobetasol propionate and its six impurities (Table V). Under optimized conditions, halobetasol propionate and its six impurities were well separated.

## Conclusion

The rapid gradient RP-UPLC method developed for quantitative analysis of halobetasol propionate and related substances in halobetasol propionate 0.05% (w/w) cream is precise, accurate, linear, robust and specific. Satisfactory results were obtained from validation of the test method. This method exhibited excellent performance in terms of sensitivity and speed. The method is stability-indicating and can be used for routine analysis in quality control and also to test the stability of samples. A short gradient preparative method for impurity isolation and an LC–MS compatible method for impurity identification were also developed and employed successfully.

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## References

1. Drugs.com. Halobetasol Propionate, Pharmacology. http://www.drugs.com/ppa/halobetasol-propionate.html (accessed July 17, 2013).
2. Rxlist. Ultravate, halobetasol Propionate cream, 0.05%. http://www.rxlist.com/ultravate-drug.htm (accessed July 17, 2013).
3. Drug Bank. Halobetasol propionate. http://www.drugbank.ca/drugs/DB00596 (accessed July 17, 2013).
4. ICH Q3B (R2). Impurity in new drug products (2000).
5. ICH Q3A. Impurity in new drug substances (2000).
6. Swartz, M.E.; UPLC: an introduction and review; *Journal of Liquid Chromatography and Related Technologies*, (2005); 28: 1253–1263.
7. Russo, R., Guillarme, D., Nguyen, D.T., Bicchi, C., Rudaz, S., Veuthey, J.L.; Pharmaceutical applications on columns packed with sub-2 µm

particles; *Journal of Chromatographic Science*, (2008); 46: 199–208.
8. Nguyen, D.T., Guillarme, D., Rudaz, S., Veuthey, J.L.; Fast analysis in liquid chromatography using small particle size and high pressure; *Journal of Separation Science*, (2006); 29: 1836–1848.
9. Mazzeo, J.R., Neue, U.V., Marianna, K., Plumb, R.S.; Advancing LC performance with smaller particles and higher pressure; *Analytical Chemistry*, (2005); 77: 460A–467A.
10. Villiers, A.D., Lestremau, F., Szucs, R., Gelebart, S., David, F., Sandra, P.; Evaluation of ultra performance liquid chromatography: Part I. Possibilities and limitations; *Journal of Chromatography A*, (2006); 1127: 60–69.
11. Wren, S.A.C., Tchelitcheff, P.; Use of ultra-performance liquid chromatography in pharmaceutical development; *Journal of Chromatography A*, (2006); 1119: 140–146.
12. ICH Q1A (R2). Stability testing of new drug substances and products, (2003).
13. *The United States Pharmacopoeia*, 26th ed., US Pharmacopoeia Convention, MD, (2003); pp. 1151–1154.
14. Ankam, R., Mukkanti, K., Durgaprasad, S., Naidu, P.V.L.; Simultaneous determination of halobetasol and salicylic acid related substances by reversed phase high performance liquid chromatographic method; *Asian Journal of Chemistry*, (2010); 22: 3369–3375.
15. Ankam, R., Mukkanti, K., Durgaprasad, S., Naidu, P.V.L.; Simultaneous determination of halobetasol propionate and fusidic acid related substances by reversed phase high performance liquid chromatographic method; *Asian Journal of Chemistry*, (2010); 22: 3376–3380.
16. ICH Q2 (R1). Validation of Analytical Procedures: Text and Methodology. In: International Conference on Harmonization, (2005).