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Article

An RP-HPLC Method for the Stability-Indicating Analysis of Impurities of Both Fusidic Acid and Betamethasone-17-Valerate in a Semi-Solid Pharmaceutical Dosage Form

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A topical pharmaceutical cream containing the active pharmaceutical ingredients (APIs) betamethasone-17-valerate and fusidic acid has been developed for the treatment of inflammatory skin conditions and associated secondary infections. In this work, a novel stability-indicating RP-HPLC method has been developed for the simultaneous quantitation of impurities of both APIs present in this cream. The HPLC column was a 150 mm × 4.6 mm I.D. YMC-Pack Pro C18 column with 3 μm particles. The column-oven temperature was maintained at 40°C and UV detection at 235 nm was used. A gradient programme was employed at a flow rate of 0.7 mL/min. Mobile phase A comprised of a 16:21:21:42 (v/v/v/v) mixture of methanol, 10 g/L phosphoric acid, HPLC grade water and acetonitrile. Mobile phase B comprised of a 24:5:5:66 (v/v/v/v) mixture of methanol, 10 g/L phosphoric acid, HPLC grade water and acetonitrile. The method has been validated according to current International Conference on Harmonisation (ICH) guidelines and applied during formulation development and stability studies. The procedure has been shown to be stability-indicating for the topical cream.

Introduction

Dermatological disorders, such as eczema and psoriasis, can have a debilitating effect on the quality of life of affected patients due to their very obvious symptoms, including severe inflammation, itching, bleeding and excess skin growth. Further complications often arise due to secondary infections of open wounds caused by picking or scratching of the affected areas by the patient. A common treatment for these disorders is the use of topical glucocorticoids, such as hydrocortisone and betamethasone, together with antibiotic agents including fusidic acid (FA) and gentamicin sulfate. For this purpose a topical pharmaceutical preparation containing the active pharmaceutical ingredients (APIs) betamethasone-17-valerate (BV) and fusidic acid (Figure 1) has been developed.

Current international guidelines (1, 2) require that degradation products of APIs in finished pharmaceutical products are quantified at release and also during the shelf-life of the product. This is to guarantee an acceptable level of quality by ensuring that (1) the APIs do not degrade to such an extent that the efficacy of the product is diminished and (2) the levels of potentially toxic impurities, arising through degradation of these APIs, are maintained below well-defined limits. To minimize degradation, the APIs should be formulated in a suitably stable vehicle. This is the goal of formulation development and this process requires the employment of a stability-indicating analytical procedure.

None of the analytical methods reported in the literature are suitable for the simultaneous analysis of impurities of both BV and FA in a single chromatographic run. There are methods available for the analysis of the single APIs in topical formulations, e.g. Po *et al.* (3) developed an HPLC procedure for the analysis of BV and its degradation products in topical preparations. The available methods, however, are not suitable for the present analysis since they are not selective for all relevant impurities. With regard to FA, the vast majority of published methods are only suitable for the analysis of the main component, i.e. the API, and do not consider potential impurities. Furthermore, many of the published procedures are often poorly selective, being based on methods such as UV-Vis spectrophotometry (4) or atomic absorption spectrometry (5) which are not stability-indicating. Shaikh *et al.* (6) published a method for the simultaneous quantitation of chlorocresol, mometasone furoate and FA in creams. This method is capable of separating and quantifying the impurity 3-didehydrofusidic acid (3-ketofusidic acid), which is one of the main impurities of FA. However, 14 other impurities related to FA have been described, many of which are potential degradation products (7). These impurities have not been considered in any of the peer-reviewed literature methods found.

Consequently, it was necessary to develop a new procedure for the selective analysis of impurities of both active substances. The analysis of both APIs with the aid of a single HPLC run would significantly simplify laboratory analysis, thus saving time and reducing costs.

In this paper the development of a novel stability-indicating RP-HPLC method for the simultaneous quantitation of impurities of both BV and FA in a single chromatographic run is reported. The method has been validated according to current International Conference on Harmonisation (ICH) guidelines and applied to development formulations and stability samples of cream.

Materials and methods

Reagents

Fusidic acid hemihydrate and betamethasone-17-valerate were purchased from OJSC Biosintez (Penza, Russia) and Crystal Pharma (Valladolid, Spain), respectively. Both APIs were of Ph. Eur. Grade.

Gradient grade methanol and acetonitrile were purchased from VWR International GmbH (Darmstadt, Germany). Purified water was obtained from the in-house purification system at mibe GmbH Arzneimittel (Brehna, Germany). Phosphoric acid (85%, analysis grade, d = 1.71 g/mL) was purchased from Merck (Darmstadt, Germany). All cream samples were provided by mibe GmbH Arzneimittel (Brehna, Germany). 3-Ketofusidic acid, betamethasone-21-valerate, "fusidic acid for peak identification CRS" and "fusidic acid impurity mixture CRS" were purchased from the European Directorate for the Quality of

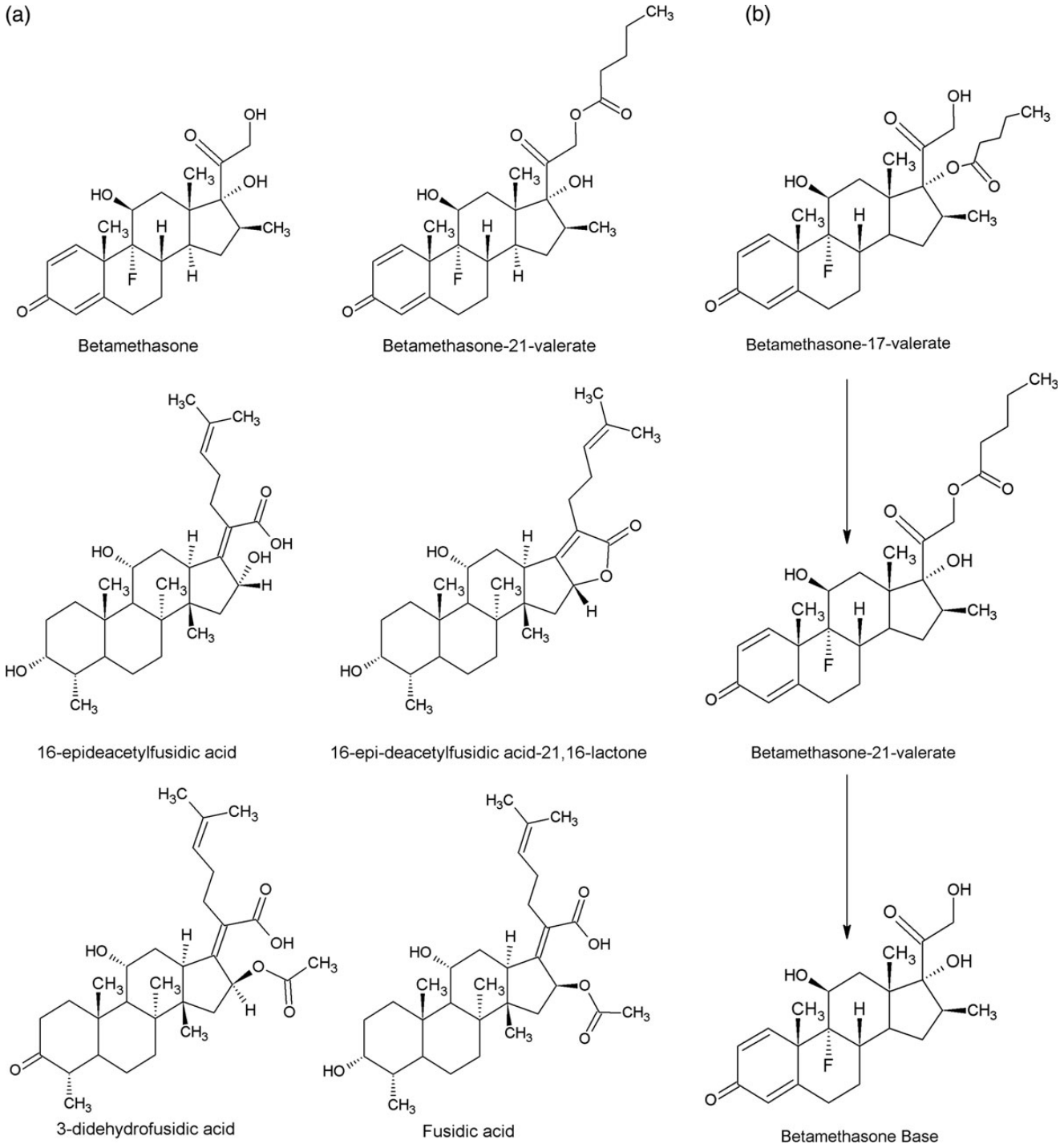

Figure 1. (a) Chemical structures of the main degradation products of the APIs found in the cream. (b) Degradation pathway of betamethasone-17-valerate.

Medicines and Healthcare (EDQM). Betamethasone was purchased from Sigma-Aldrich.

Instrumentation

Shimadzu Prominence HPLC Systems (Shimadzu, Japan) were used for method development and validation. The HPLC systems were equipped with a binary pump (LC-20AD), a temperature-controlled auto-sampler (SIL-20AC$_{HT}$), a temperature-controlled column compartment (CTO-20AC) as well as an on-line degasser (DGU-20A$_5$). A (SPD-M20A) DAD detector was employed. The software packages used were SHIMADZU LCsolution version 1.24 SP1 and SHIMADZU Class-VP version 6.14 SP2A.

Chromatographic conditions and sample preparation

The employed HPLC method is described in Table I. Samples were prepared by weighing ~5.00 g of cream into a 50.0 mL

volumetric flask, adding 30 mL of acetonitrile and suspending by shaking in a water bath at 65°C. The increased temperature allows for complete suspension of the cream by facilitating the melting of the fatty components, e.g. Vaseline. Thorough mixing was ensured with the help of a vortexing machine. The APIs were extracted into solution by shaking the suspension on a flat-bed shaker at ~200 shakes per minute. The mixture was allowed to cool to room temperature before being made up to volume with acetonitrile. Unwanted fatty components were "frozen-out" by placing the suspension in a freezer (−20°C) for 20 min. A portion of the cold suspension was then centrifuged at 3000 rpm for 5 min. The resulting supernatant was removed and allowed to warm to room temperature before dilution. This prevents any temperature related dilution errors.

A portion of the supernatant was diluted 1 : 2 (v/v) with purified water. This served to further aid the precipitation of unwanted matrix components and also to improve peak symmetry by reducing sample solvent strength. Finally, the solution was filtered through a 0.45 μm regenerated cellulose filter (Macherey-Nagel, Germany) into a suitable HPLC vial. The concentrations of the APIs were 1000.0 and 60.70 μg/mL for FA and BV, respectively. The impurities were quantified against a 0.5% external standard prepared by dissolving 40.0 mg of FA and 2.428 mg of BV in 30 mL of acetonitrile in a 50.0 mL volumetric flask before making up to 50.0 mL with the same solvent. This solution was then diluted appropriately with a 50 : 50 (v/v) mixture of acetonitrile and water, giving concentrations of 5.000 and 0.3035 μg/mL for FA and BV, respectively. The impurities of FA were quantified against the 0.5% FA standard and the impurities of BV against the 0.5% BV standard.

Results and conclusions

Method development: column, gradient, injection volume and oven temperature

Fusidic acid hemihydrate has a single carboxylic acid functional group with a pK_a of 5.3 (8). Consequently, the retention time of FA will be influenced by the pH of the mobile phase. A suitable mobile phase should have a pH of 2 units below the pK_a of the acidic group, in order to ensure that the molecule remains completely protonated in solution. For this reason a mobile phase acidified with phosphoric acid was chosen. BA, on the other hand, is a neutral compound and its retention on the analytical column will not be affected by pH.

As starting conditions mobile phase A comprised of a 20 : 40 : 40 (v/v/v) mixture of methanol, acetonitrile and a 10 g/L solution of phosphoric acid (85%). Mobile Phase B comprised of a 10 : 20 : 70 (v/v/v) mixture of the same solvents. The gradient programme started with 100% mobile phase A held for 3 min followed by an increase to 100% mobile phase B over the next 25 min. 100% B was then held for a further 10 min. The initial flow rate was 1.0 mL/min.

Through column screening experiments it was found that an YMC-Pack Pro C18, 150 mm × 4.6 mm column with 3.5 μm particles provided the best overall separation between all impurities. However, several impurities were still not satisfactorily separated. In order to achieve an acceptable separation the gradient programme, flow-rate and mobile phase composition were altered.

Modification of the initial part of the gradient programme from 100% B for 3 min to 95% A: 5% B for 20 min and reducing the flow rate to 0.7 mL/min was found to increase resolution between all of the peaks eluting in this portion of the chromatogram. It was also found that reducing the quantity of methanol in mobile phase B from 200 to 165 mL/L enabled a better separation between the impurities betamethasone-21-valerate and 26-oxofusidic acid as well as between the impurities (24*R*)-24,25-dihydroxyfusidic acid-21,24-lactone and 26-hydroxyfusidic acid. An injection volume of 50 μL was chosen because of the requirement to achieve a high level of sensitivity for the impurities of BV due to its low concentration in the drug product (0.64 mg/g) when compared with fusidic acid (20.0 mg/g). Fifty microliters were also the maximum possible injection volume of the system.

The available impurities of BV were identified by dissolving an appropriate quantity of each compound in the sample solvent and injecting them into the HPLC. Their respective retention times and relative retention times were recorded and compared with the peaks in the sample solution. For the known impurities of fusidic acid, which were not available as pure substances, the EP standards *fusidic acid for peak identification CRS* and *fusidic acid impurity mixture CRS* were used together with information about the relative retention times of each impurity as provided in the draft PHARMEUROPA monograph for fusidic acid monohydrate API (9). See Figure 2 for an example chromatogram of a cream sample.

Table I
Description of HPLC Method

Column description	–	YMC-Pack Pro C18, 150 × 4.6 mm, 3 μm particles			
Guard column	–	Gemini C18, 4.0 mm L × 3.0 mm ID (Phenomenex)			
Flow rate	mL/min	0.7			
Mobile Phase	A	16 : 21 : 21 : 42 (v/v/v/v) Methanol/10 g L^{-1} phosphoric acid/HPLC grade water/acetonitrile			
	B	24 : 5 : 5 : 66 (v/v/v/v) Methanol/10 g L^{-1} phosphoric acid/HPLC grade water/acetonitrile			
Wavelength	nm	235			
Column temperature	°C	40			
Auto-sampler temperature	°C	25			
Gradient	–	Time (min)	Event	% A	% B
		START	Pump A B.	95	5
		20.00	Pump A B.	95	5
		45.00	Pump A B.	0	100
		50.00	Pump A B.	95	5
		60.00	Pump A B.	STOP	–
Injection volume	μl	50			

Method validation

Selectivity

In order to demonstrate the selectivity of the analytical method, samples of cream which had been stored under accelerated stability conditions (40°C/75% relative humidity) for 12 months as well as samples stressed under oxidative- (exposure to air, 3 days) and pH stress (pH 7, 40°C, 6 months) were measured. Experience gathered during analytical and formulation developed had shown that these samples contained all of the relevant degradation products of the APIs at concentration levels significantly higher than would be expected over the normal shelf-life of the pharmaceutical product (24 months at 25°C/60% relative humidity). These samples were therefore considered to be

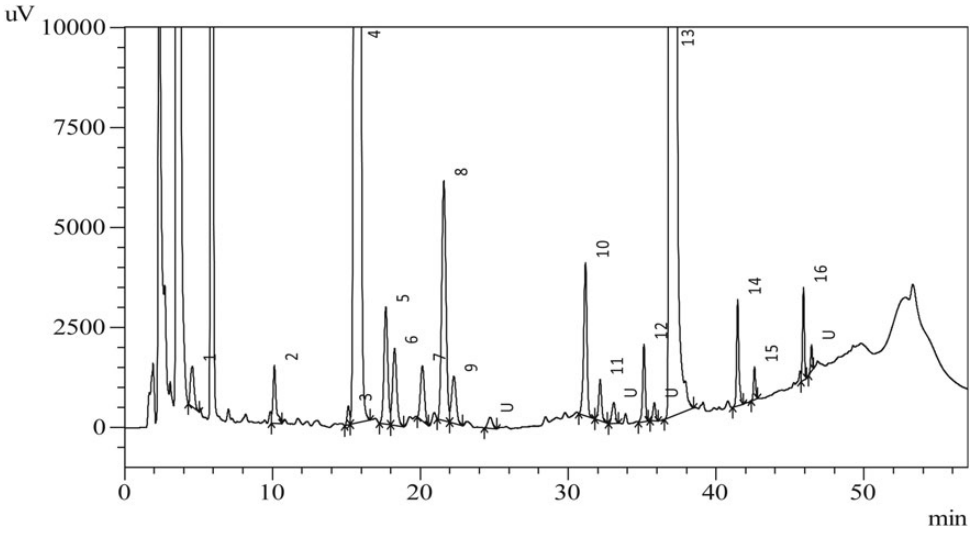

Figure 2. Chromatogram of cream sample measured shortly after manufacture. HPLC conditions as provided in Table I. Peak assignment as follows: 1. Betamethasone. 2. 24,25-Dihydro-24,25-dihydroxyfusidic acid. 3. 24,25-Dihydro-24,25-dihydroxyfusidic acid 21,25-lactone. 4. Betamethasone-17-valerate. 5. (24*R*)-24,25-dihydro-24,25-dihydroxyfusidic acid-21,24-lactone. 6. 26-Hydroxyfusidic acid. 7. (24*S*)-24,25-dihydro-24,25-dihydroxyfusidic acid-21,24-lactone. 8. Betamethasone-21-valerate. 9. 26-Oxofusidic acid. 10. 3-Didehydrofusidic acid. 11. 11-Didehydrofusidic acid. 12. 16-Epideacetylfusidic acid. 13. Fusidic acid. 14. 16-epideacetylfusidic acid-21,16-lactone. 15. Deacetylfusidic acid-21,16-lactone. 16. 11-Deoxyfusidic acid. U, unknown impurity.

worst case. Stress tests as well as accelerated stability studies had shown that the impurities betamethasone and betamethasone-21-valerate are the predominant degradation products of BV which are formed in the cream under normal storage conditions. The degradation scheme of BV is described in Figure 1b.

The data also showed that the impurities 16-epideacetylfusidic acid, 16-epideacetylfusidic acid-21,16-lactone, 3-didehydrofusidic acid and 26-hydroxyfusidic acid are the main degradants of FA observed. 16-Epideacetylfusidic acid is the most abundant degradant of fusidic acid seen in stress studies. It is generated by hydrolysis of the acetyl group at position C16. Further reaction of the resulting free hydroxyl group with the neighboring carboxyl group at C21 forms the cyclic-lactone 16-epideacetylfusidic acid-21,16-lactone, the second most abundant degradant of FA observed. The structures of the above-mentioned impurities are provided in Figure 1a. The purity of the main API peaks and relevant impurities was evaluated using a photodiode array scan from 200 to 400 nm, whereby spectra were recorded and compared across the entire peak. The peaks were found to be pure with this method. No interferences from solvent or placebo components were observed.

Linearity

The linearity of the detector response for betamethasone, betamethasone-21-valerate, BV, 3-didehydrofusidic acid and FA was checked using a 13-point calibration over a suitable range as detailed in Table II. These substances were chosen because they were the only impurities which were commercially available as pure substances. The APIs FA and BV were used as surrogates for those impurities which were not available. This is common practice in analytical chemistry. The correlation coefficients of the best-fitting lines obtained by least-squares regression were all >0.999. The residual values demonstrated no particular trend.

Table II
Data from the Linearity and LOD/LOQ Experiments

Substance	Range (%)	*r*	Residuals	LOD (%)	LOQ (%)
Betamethasone	0.1–8.7	0.9999	No trend	0.008	0.029
Betamethasone-21-valerate	0.1–5.8	0.9999	No trend	0.033	0.112
Betamethasone-17-valerate	0.1–8.7	0.9999	No trend	0.029	0.097
3-Didehydrofusidic acid	0.1–6.1	0.9999	No trend	0.003	0.010
Fusidic acid hemihydrate	0.1–7.8	0.9999	No trend	0.002	0.008

Precision and accuracy

The repeatability (reproducibility) of the analytical procedure was checked by preparation and measurement of six sample solutions by Analyst A using HPLC Machine A on Day 1. The sample had been stressed at 50°C for 8 weeks and contained all relevant degradation products. The relative standard deviation (rsd) of the content values for each of the impurities was $<5.0\%$. The intermediate precision was determined by preparation and measurement of a further 6 samples by Analyst B using HPLC Machine B on Day 2. The rsd values of a total of 12 measurements were also $<5.0\%$.

The accuracy of the analytical procedure was determined by preparation and measurement of nine solutions comprising of placebo which had been spiked with each of the available impurities over the range 0.1–8.0% of the theoretical sample concentration. The recovery rates for each impurity lay between 99.0 and 111.2%. The recovery rates of both APIs were evaluated in the same fashion and over the same range, with values of 99.0 and 102.9% obtained for BV and FA, respectively.

Solution stability and robustness of HPLC parameters

Solution stability was determined by preparation of standard and sample solutions and analyses of these solutions at 4 hourly intervals over a period of 48 h. The solutions were stored at 25°C and protected from light. The active substance BV proved to be stable

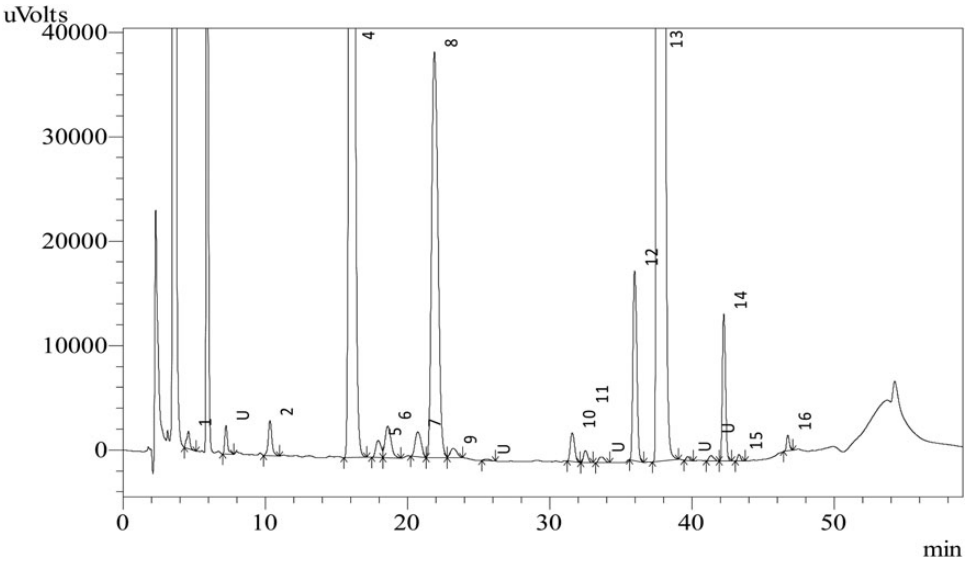

Figure 3. Chromatogram of a degraded cream sample analyzed after stressing under the conditions of 40°C/75% RH at pH 7 for 2 months. The sample demonstrated a significant degradation. HPLC conditions as provided in Table I. Peak 3 was not present in this particular sample. 1. Betamethasone. 2. 24,25-Dihydro-24,25-dihydroxyfusidic acid. 3. 24,25-Dihydro-24,25-dihydroxyfusidic acid-21,25-lactone. 4. Betamethasone-17-valerate. 5. (24*R*)-24,25-dihydro-24,25-dihydroxyfusidic acid 21,24-lactone. 6. 26-Hydroxyfusidic acid. 7. (24*S*)-24,25-dihydro-24,25-dihydroxyfusidic acid 21,24-lactone. 8. Betamethasone-21-valerate. 9. 26-Oxofusidic acid. 10. 3-Didehydrofusidic acid. 11. 11-Didehydrofusidic acid. 12. 16-Epideacetylfusidic acid. 13. Fusidic acid. 14. 16-Epideacetylfusidic acid-21,16-lactone. 15. Deacetylfusidic acid-21,16-lactone. 16. 11-Deoxyfusidic acid. U, unknown impurity.

over the measurement period with the content values of relevant degradation products remaining unchanged. The FA samples showed a slight increase of 0.05% of the impurity 16-epideacetylfusidic acid over 48 h. This increase was considered negligible. The content values of all other known impurities remained unchanged. No new unknown impurities were observed for either of the APIs.

In order to test the robustness of the method, the HPLC parameters were deliberately varied from normal conditions as follows: column temperature (± 5°C), flow rate (± 0.2 mL/min.), volume of acetonitrile in mobile phase A (± 20 mL/L), volume of methanol in mobile phase A (± 20 mL/L), volume of the acidic aqueous phase in mobile phase A (± 20 mL/L), detection wavelength (± 2 nm) and proportion of mobile phase B at begin of gradient programme (± 3%). Some of the conditions tested, e.g. increasing the column-oven temperature by 5°C, lead to a co-elution of the impurities (24*S*)-24,25-dihydroxyfusidic acid-21,24-lactone and betamethasone-21-valerate. As a result, a visual examination of the resolution between critical peak pairs has been adopted as a system suitability requirement before sample measurement.

Limits of detection (LOD) and quantitation (LOQ)

The limits of detection and quantitation were defined as 3 times and 10 times the signal-to-noise ratio and were calculated using a mixed standard solution containing the available impurities at a suitably low concentration level (ca. 0.1%). The calculated LOD and LOQ values are summarized in Table II. For impurities which were not available for purchase, the respective active substances were used as a surrogate. This is a standard approach used in analytical chemistry.

Response factors for known impurities

The response factors for the available impurities betamethasone, betamethasone-21-valerate and 3-didehydrofusidic acid were calculated by preparing solutions of equal concentration of each of the impurities together with their respective APIs and comparing the resulting peak areas. For the remaining known impurities of FA, which were not available for purchase, the prescribed response factors were taken directly from the EP monograph for fusidic acid (7). In cases where no response factor was available, the impurities were presumed to have the same response as the API, i.e. factor 1.000. The approach taken is justified since the impurities cannot be purchased as pure substances and the purity of the cream preparation is being measured at the same wavelength as prescribed in the EP monograph for the API. The relevant response factors are provided in Table III.

Analysis of development and stability samples

Thirty-eight trial batches of topical cream were analyzed with the above-detailed purity procedure during formulation development. It was observed that betamethasone-17-valerate was isomerizing rapidly to its corresponding 21-valerate isomer in some of the formulations and that this process was both temperature and pH dependent. Furthermore, the isomerization rate was also shown to be significantly influenced by the concentration of the primary emulsifier in the cream formulation. At higher concentrations of emulsifier, e.g. 5%, the isomerization occurs rapidly with ca. 5% betamethasone-21-valerate being formed at pH 5.0 ± 0.2 after 3 months storage under the conditions 25°C/60% relative humidity. Reducing the emulsifier concentration to an appropriate level lead to a significant reduction of the isomerization rate, with only 0.1% betamethasone-21-valerate being observed, under the same storage conditions and duration of storage as above. Betamethasone-21-valerate and betamethasone were the only degradants of BV observed in the cream samples, the latter only being observed under suitable pH stress conditions ($pH > 7$). In the final cream product only betamethasone-21-valerate is observed.

Table III
Accelerated Stability Data and Mass-Balance Values for the Topical Cream

40°C/75% relative humidity	Relative retention time*	Response factor, ¢	0 months	3 months	6 months	Units
Assay of fusidic acid	–	–	100.0	99.5	99.2	The assay values are in % relative to the start value
Purity of fusidic acid						*rrt values are relative to the fusidic acid peak
24,25-Dihydro-24,25-dihydroxyfusidic acid	0.28	1.000	<0.1	<0.1	0.05	¢ Response factors should be divided
24,25-Dihydro-24,25-dihydroxyfusidic acid 21,25-lactone	0.41	1.000	<0.1	<0.1	<0.1	
(24*R*)-24,25-dihydro-24,25-dihydroxyfusidic acid 21,24-lactone	0.49	1.429	<0.1	<0.1	<0.1	
26-Hydroxyfusidic acid	0.50	1.000	0.07	0.17	0.31	
(24*S*)-24,25-Dihydro-24,25-dihydroxyfusidic acid 21,24-lactone	0.56	1.429	0.05	0.08	0.13	
Impurity N	0.57	1.000	<0.1	<0.1	<0.1	
26-Oxofusidic acid	0.63	3.333	<0.1	<0.1	0.06	
3-Didehydrofusidic acid	0.85	1.074	0.16	0.20	0.23	
11-Didehydrofusidic acid	0.87	1.000	<0.1	<0.1	<0.1	
16-Epideacetylfusidic acid	0.96	1.667	<0.1	0.05	0.12	
16-Epideacetylfusidic acid 21,16-lactone	1.12	1.000	<0.1	<0.1	0.17	
Deacetylfusidic acid	1.14	1.000	<0.1	<0.1	<0.1	
Deacetylfusidic acid 21,16-lactone	1.15	1.667	<0.1	0.05	<0.1	
9,11-Anhydrofusidic acid	1.20	1.000	<0.1	<0.1	<0.1	
11-Deoxyfusidic acid	1.24	1.000	<0.1	<0.1	<0.1	
Unknown impurities, single	–	–	<0.1	<0.1	<0.1	
Total impurities	–	–	0.28	0.55	1.07	
Mass-balance	–	–	100.3	100.1	100.3	
Assay of Betamethasone-17-valerate	–	–	100.0	96.0	92.9	
Purity of betamethasone-17-valerate						
Betamethasone	0.12	1.154	0.12	0.13	0.09	
Betamethasone-17-valerate	0.60	0.930	0.34	3.82	10.01	
Unknown impurities, single	–	–	<0.1	<0.1	<0.1	
Total impurities	–	–	0.46	3.95	10.10	
Mass-balance	–	–	100.5	100.0	103.0	

Fusidic acid proved stable in all of the development formulations tested. Under accelerated (40°C/75% RH) conditions the final formulation showed an increase of the contents of the impurities 16-epideacetylfusidic acid, 16-epideacetylfusidic acid-21,16-lactone as well as the impurities 3-didehydrofusidic acid and 26-hydroxyfusidic acid. Of these impurities 16-epideacetylfusidic acid and 16-epideacetylfusidic acid-21,16-lactone appear to be the major degradation products of fusidic acid as demonstrated by Figures 2 and 3. The results of the accelerated stability studies are presented in Table III. The product shows significant degradation after 6 months storage. The mass-balance values were considered acceptable (generally, 95–105% is acceptable). This further underlines the stability-indicating nature of the procedure for the topical cream.

Conclusion

An RP-HPLC method was developed for the simultaneous quantitation of impurities of betamethasone-17-valerate and fusidic acid in a topical cream preparation. The method was validated according to current ICH guidelines and was demonstrated to be selective, linear, precise, accurate, robust and sufficiently sensitive within the validated range. The mass-balance values of degraded samples are acceptable, demonstrating the stability-indicating power of the procedure for the topical cream. The method is suitable for employment in the analysis of trial formulations, release batches as well as during ICH stability testing.

References

1. Specifications: Test procedures and acceptance criteria for new drug substances and new drug products: chemical substances. ICH, Q6A, (1999).
2. Impurities in New Drug Products. ICH, Q3B(R2), (2006).
3. Po, A.L.W., Irwin, W.J., Yip, Y.W.; High-performance liquid chromatographic assay of betamethasone-17-valerate and its degradation products; *Journal of Chromatography*, (1979); 176: 399–405.
4. Vladimirov, S., Fiser, Z., Agbaba, D., Zivanov-Stakic, D.; Spectrophotometric determination of fusidic acid and sodium fusidate in dosage forms; *Journal of Pharmaceutical and Biomedical Analysis*, (1995); 13: 675–678.
5. Al-Shaalan, N.H.; Atomic absorption spectrometric determination of fusidic acid in bulk powder and in pharmaceutical dosage form; *Journal of Chemical and Pharmaceutical Research*, (2010); 2: 135–143.
6. Shaikh, S., Muneera, M.S., Thusleem, O.A., Tahir, M., Kondaguli, A.V.; A simple RP-HPLC method for the simultaneous quantitation of chlorocresol, mometasone furoate and fusidic acid in creams; *Journal of Chromatographic Science*, (2009); 47: 178–183.
7. Fusidic Acid Monograph 01/2012:0798. European Pharmacopoeia 7.3.
8. Godtfredsen, W.O., Jahnsen, S., Lorck, H., Roholt, K., Tybring, L.; Fusidic acid: a new antibiotic; *Nature*, (1962); 193: 987.
9. Draft Monograph Fusidic acid monohydrate. PHARMEUROPA, Vol. 20, No. 1.