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Development and validation of a stability-indicating HPLC method for simultaneous determination of salicylic acid, betamethasone dipropionate and their related compounds in Diprosalic Lotion®

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ABSTRACT

Diprosalic Lotion® is an anti-inflammatory drug product that contains salicylic acid and betamethasone dipropionate as active pharmaceutical ingredients (APIs). A reversed-phase high performance liquid chromatography (RP-HPLC) method was developed for simultaneous determination of salicylic acid, betamethasone dipropionate, and their related compounds in Diprosalic Lotion®. A 150 mm × 4.6 mm I.D. YMC J'sphere ODS-H80 column at 35 °C and UV detection at 240 nm was used. A gradient elution was employed using 0.05% (v/v) methanesulfonic acid solution and acetonitrile as mobile phases. A total of thirty three compounds from Diprosalic Lotion® samples were separated in 38 min. The stability-indicating capability of this method has been demonstrated by the adequate separation of all the impurities and degradation products in expired stability samples of Diprosalic Lotion®. The method was validated as per the current ICH guidelines.

1. Introduction

Diprosalic Lotion® is an anti-inflammatory drug product that contains two APIs: betamethasone dipropionate (BD) and salicylic acid (SA) (Fig. 1). BD, known as the fifth-generation corticosteroid, is a potent compound that treats skin inflammation related to dermatoses and psoriasis [1,2]. To achieve better efficacy and reduce adverse effects [2,3], compounds with different pharmacological mechanisms such as SA are added to BD to form an effective fixed-dose combination therapy.

The synthetic process for the production of BD introduces precursor impurities such as betamethasone, betamethasone 17- and 21-monopropionates [4], which can also form when BD is exposed to acidic or basic conditions. In formulated drug products, BD can form other degradants such as betamethasone enolaldehydes [4–6]. Synthetic SA may contain impurities such as 4-hydroxybenzoic acid, 4-hydroxyisophthalic acid and phenol. In the presence of metal ions or EDTA, SA can degrade to a variety of related compounds via hydroxylation or free radical reactions [7,8]. For Diprosalic Lotion®, leachables (Table 1) that are reported from either high or low density polyethylene plastic container [9,10] also should be monitored throughout the product shelf life since certain leachables could be potentially hazardous to human health.

Most of reported RP-HPLC methods were for either BD [11,12] or SA [13,14] alone. A method that can simultaneously analyze BD and SA in an ointment product has been reported [15]. However, this method has a narrow linearity range and a poor sensitivity due to the use of THF in the mobile phase. Marini et al. reported a generic method for the assay of BD and SA in dermopharmaceutical forms [16]. However, this method cannot separate all the related compounds of BD and SA. So far, available literature methods are inadequate to be adapted as a stability-indicating method for Diprosalic Lotion®.

In this paper, the development of a stability-indicating RP-HPLC method for Diprosalic Lotion® is reported. This is the first known method that has the capability to simultaneously separate and quantitate the two APIs, separate and estimate all the potential impurities and degradation products including 5 leachables in Diprosalic Lotion®. This method was validated as per the current International Conference on Harmonization (ICH) guideline [17].

2. Experimental condition

2.1. Reagents

Salicylic acid, betamethasone dipropionate, and all betamethasone dipropionate related compounds (Table 1) were provided

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Table 1
List of known salicylic acid (SA) and betamethasone dipropionate (BD) related compounds and potential leachables in the Diprosalic Lotion® drug product.

Salicylic acid related compounds	
1	4-hydroxybenzoic acid
2	Catechol
3	2,5-dihydroxybenzoic acid
4	2,3-dihydroxybenzoic acid
5	4-hydroxyisophthalic acid
6	Phenol
7	Salicylic acid
Betamethasone dipropionate related compounds	
8	Betamethasone
9	Betamethasone 17-ketone
10	Betamethasone 17-propionate
11	Betamethasone-(*Z*)-enolaldehyde
12	Betamethasone-(*E*)-enolaldehyde
13	Betamethasone 21-propionate
14	Betamethasone 21-acetate, 17-propionate
15	Betamethasone dipropionate
16	Betamethasone 9,11-epoxide 17,21-dipropionate
17	Beclomethasone dipropionate
18	9-Bromo, betamethasone dipropionate[a]
19	Betamethasone 17-propionate, 21-butyrate
20	9,11-ene-betamethasone dipropionate[b]
21	Betamethasone-11,17,21-tripropionate
22	6-Bromo, betamethasone dipropionate[c]
Potential leachables	
23	Benzyl alcohol
24	Benzaldehyde
25	Diethyl phthalate
26	Benzophenone
27	2-hydroxy-4-methoxybenzophenone

[a] The fluorine atom at position 9 is replaced by a bromine atom.
[b] The fluorine atom at position 9 and OH group at position 11 are eliminated to form the double bond.
[c] The hydrogen atom at position 6 is replaced with a bromine atom.

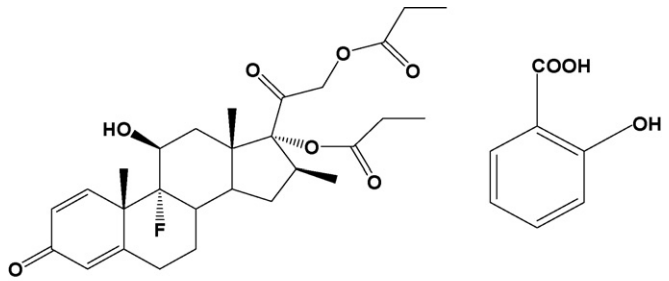

Fig. 1. Chemical structures of betamethasone dipropionate (BD) and salicylic acid (SA).

by Schering-Plough (Kenilworth, NJ). All salicylic acid related compounds and leachables (Table 1) were purchased from Sigma–Aldrich (St. Louis, MO). All HPLC grade solvents and chemicals were obtained from Fisher Scientific (Hampton, NH). Milli-Q Water (18.2 MΩ cm^{-1}) was obtained from an in-house Milli-Q System (Millipore, Billerica, MA). All Diprosalic Lotion® samples were provided by Schering-Plough.

2.2. Instrumentation

A Hitachi LaChrom Elite HPLC system (Hitachi High Technologies America, Inc., San Jose, CA) equipped with the ChromSword® software (Merck KGaA, Darmstadt, Germany) and a Waters 2695 Alliance HPLC system (Milford, MA) were used for method development. Both HPLC instruments were equipped with a quaternary pump system, a column compartment with temperature control, an on-line degasser, and a UV detector. Data acquisition, analysis and reporting were performed by Hitachi EZChrom Elite, Waters Millennium32, and SAS System JMP® version 4, respectively.

2.3. Chromatographic conditions and sample preparation

The HPLC column was the YMC J'sphere ODS-H80, 150 mm × 4.6 mm I.D., 4 μm particle size (YMC, Milford, MA). The mobile phases consisted of *A*: 0.05% (v/v), methanesulfonic acid in water and *B*: 100% acetonitrile. The gradient program is in Table 2. The flow rate was 2.0 mL/min and column temperature was 35 °C. The detection wavelength was set at 240 nm and the sample injection volume was 50 μL.

The sample diluent was 100 mM sodium monobasic phosphate in water/acetonitrile mixture (70/30, v/v). The pH of the sample diluent was adjusted to about 2.7 by 1 M hydrochloric acid. The assay concentrations of SA and BD standard solutions were 0.06 and 0.077 mg/mL, respectively. All standard and sample solutions were protected from light to prevent photo degradation. The specificity test mixture solution was prepared by spiking an expired Diprosalic Lotion® sample solution with BD and SA related compounds and potential leachables at ~1% level. Two Diprosalic Lotion® sample solutions are required due to the significant difference [18] on label strength between BD and SA (0.65 and 20 mg/mL, respectively). One is prepared by diluting 3.0 ± 0.3 g Diprosalic Lotion® to 25 mL for the assay of betamethasone dipropionate and the estimation of API-related compounds as well as potential leachables. Another is prepared by diluting 750 ± 75 mg lotion to 250 mL for the assay of salicylic acid only.

3. Results and discussion

3.1. Method development

3.1.1. Optimization of mobile phase, stationary phase, and HPLC parameters

SA and some of its related compounds (Table 1) contain one or two carboxylic groups, which tend to deprotonate and result in rapid elution under neutral or basic HPLC conditions. The pK_as of SA and its related compounds are around 3.0 [20]. Therefore, the pH of the mobile phase needs to be less than 3 to prevent deprotonation [21]. Trifluoroacetic acid, phosphoric acid, formic acid, acetic acid, propionic acid and methanesulfonic acid were investigated as aqueous phase additives. Among them, methanesulfonic acid generated a relatively flat baseline and no interfering peaks. The low concentration (0.025%) of methanesulfonic acid yielded a pH of ~3.3, which is higher than the pK_a of SA. High concentrations at 0.1% or above decreased the pH values below pH 2, which can be detrimental to the HPLC column. At 0.05% methanesulfonic acid in water, the pH is approximately 2.7, which is sufficient to protonate

Table 2
The optimized HPLC gradient profile.

Time (min)	Flow rate (mL/min)	*A*: Methanesulfonic acid aqueous solution (0.05%, v/v)	*B*: Acetonitrile	Gradient curve
0	2.0	90	10	
7.5	2.0	75	25	Linear
37.5	2.0	35	65	Linear

Table 3
Results of preliminary stationary phase screening.

Column brand	Column properties	Column performance on specificity mixture	Evaluation of resolution of specificity mixture after ChromSword optimization
MAC-MOD ACE 3	3 μm, 15% carbon loading, monomeric bonded fully endcapped	Best peak symmetry for BD and its related compounds, peak overlapping for several late-eluted analytes	Co-elution for betamethasone 21-acetate, 17-propionate and 2-hydroxy, 4-methoxy benzophenone, beclomethasone and 9-bromo betamethasone dipropionate
J'sphere ODS-L80	4 μm, 9% carbon loading, monomeric bonded well endcapped	The minimal peak tailing for salicylic acid, co-elution of several BD related compounds and leachables	Co-elution for betamethasone 21-acetate, 17-propionate and 2-hydroxy, 4-methoxy benzophenone, Z, E-enolaldehyde and betamethasone 21-monopropionate
J'sphere ODS-M80	4 μm, 14% carbon loading, monomeric bonded well endcapped	Co-elution of late-eluted BD related compounds	Co-elution for beclomethasone and 9-bromo betamethasone dipropionate, need 80 min for baseline separation of all compounds
J'sphere ODS-H80	4 μm, 22% carbon loading, monomeric bonded well endcapped	A slight tailing for salicylic acid, good resolution for all BD and SA related compounds	Good resolution for all analytes
Develosil UG C18	3 μm, 15% carbon loading, monomeric bonded surface bonded with a monochlorosilylating reagent	A slight tailing for salicylic acid, co-elution of several BD related compounds	Co-elution for betamethasone 21-acetate, 17-propionate and 2-hydroxy, 4-methoxy benzophenone, beclomethasone and 9-bromo betamethasone dipropionate
SymmetryShield RP18	3.5 μm, 15% carbon loading, monomeric bonded polar-embedded stationary phase	Serious peak tailing for salicylic acid	Good resolution for all analytes, inaccurate integration for betamethasone due to the tailing of salicylic acid peak
YMC Pro-Pack C_{18}	3 μm, 15% carbon loading, monomeric bonded Lewis acid-base endcapped surface	Co-elution for betamethasone and salicylic acid, co-elution of BD related compounds	Co-elution for beclomethasone and 9-bromo betamethasone dipropionate
Synergi Fusion-RP	4 μm, 14% carbon loading, monomeric bonded, hydrophilic endcapped	Serious peak tailing for salicylic acid, co-elution for several late-eluted compounds	Co-elution for Z, E-enolaldehyde and betamethasone 21-monopropionate

SA and its related compounds and within the vendor recommended pH range.

Several 150 mm × 4.6 mm reverse-phase C_{18} columns with various properties (Table 3) were evaluated by using the optimized mobile phase composition. Each RP-HPLC column was tested by the specificity test mixture solution in at least three gradient programs. The simulation procedure was performed similar to the previously described steps [19].

Due to its high concentration (20 mg/mL) in Diprosalic Lotion®, the SA in the specificity test mixture solution appeared as an overloaded tailing peak. Therefore, the resolution between SA and its adjacent peaks was maximized in the simulation by varying the ratio of mobile phase A and B. The simulated results indicated that a two-step gradient (Table 2) can be adapted. The first steep gradient was to separate early-eluted SA related compounds. The second shallow gradient was needed to resolve all BD related compounds and leachables at a high acetonitrile ratio. Among those tested columns (Table 3), the J'sphere ODS-H80 and SymmetryShield RP 18 exhibited adequate resolution for all analytes. However, the peak tailing of SA on SymmetryShield RP 18 column was more serious than that on J'sphere ODS-H80 column. This tailing effect significantly affected the quantitation of betamethasone, a process impurity and degradation product of BD. Therefore, the J'sphere ODS-H80 column was selected as the primary stationary phase.

Early-eluted SA related compounds such as 4-hydroxybenzoic acid, catechol, and 2,5-dihydroxybenzoic acid did not exhibit sufficient resolution on J'sphere ODS-H80 column under the optimized gradient. Thus, the separation of specificity mixture was further optimized by varying the column temperature since the impact of temperature on selectivity can be pronounced on compounds with polar-substituted groups [21–23]. From 30 to 45 °C, the resolution of 4-hydroxybenzoic acid/catechol (RS1) and catechol/2.5-dihydroxybenzoic acid (RS2) exhibited linear changes as the function of column temperature (Fig. 2) with a slope value at 0.0457 resolution unit per degree Celsius for 4-hydroxybenzoic acid/catechol and at −0.0988 resolution unit per degree (°C) for catechol/2.5-dihydroxybenzoic acid, respectively. Data in Fig. 2 showed that 35 °C is the optimum temperature for resolving these three compounds.

Due to the relatively low back pressure (~1700 psi) of J'sphere ODS-H80 column at 1.5 mL/min, the flow rate was increased to 2.0 mL/min (Table 2). All known and unknown analytes in the Diprosalic Lotion® sample were adequately resolved in 38 min (Fig. 3) followed by a 9-min column wash and re-equilibration to initial conditions.

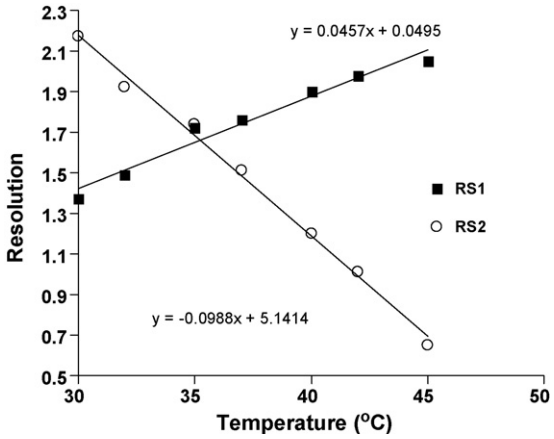

Fig. 2. Effect of column temperature on the resolution of adjacent salicylic acid related compounds (RS1: resolution between 4-hydroxybenzoic acid and catechol; RS2: resolution between catechol and 2, 5-dihydroxybenzoic acid). The HPLC conditions are the same as those indicated in Section 2.

Table 4
Summary of column screening results for the alternate HPLC column.

Column brand	Column properties	Column performance on specificity mixture	Suitable (yes/no)
Atlantis C_{18}	3 μm, 15.5% carbon loading	Inadequate resolution for betamethasone 21-monopropionate and benzophenone, beclomethasone and 9-bromo betamethasone dipropionate	No
Intersil C_{18}	3 μm, 18% carbon loading, low purity silica	Co-elution for betamethasone 21-acetate, 17-propionate and 2-hydroxy, 4-methoxy benzophenone, beclomethasone and 9-bromo betamethasone dipropionate	No
Hydrosphere C_{18}	3 μm, 12% carbon loading, hydrophilic endcapped	Co-elution for beclomethasone and 9-bromo betamethasone dipropionate. Partial resolution for Z, E-enolaldehyde and betamethasone 21-monopropionate	No
Hypersil C_{18}	3 μm, 13% carbon loading	Co-elution for Z, E-enolaldehyde and betamethasone 21-monopropionate	No
Gemini C_{18}	4 μm, 14% carbon loading, polar-embedded surface	Partial resolution for betamethasone 21-acetate, 17-propionate and 2-hydroxy, 4-methoxy benzophenone, beclomethasone and 9-bromo betamethasone dipropionate	No
Kromasil C_{18}	3.5 μm, 19% carbon loading	Baseline separation for all compounds except for the switch order of diethyl phthalate and betamethasone 17-monopropionate	No
LiChrospher C_{18}	3 μm, 22% carbon loading, no endcapping	Co-elution for betamethasone 21-acetate, 17-propionate and 2-hydroxy, 4-methoxy benzophenone, beclomethasone and 9-bromo betamethasone dipropionate	No
Luna C_{18}	3 μm, 18% carbon loading	Partial resolution of diethyl phthalate and betamethasone 17-monopropionate	No
Necleosil	3 μm, 20% carbon loading	Co-elution for betamethasone 21-acetate, 17-propionate and 2-hydroxy, 4-methoxy benzophenone, beclomethasone and 9-bromo betamethasone dipropionate	No
Prodigy ODS3	3 μm, 16% carbon loading	Co-elution for betamethasone 21-acetate, 17-propionate and 2-hydroxy, 4-methoxy benzophenone, beclomethasone and 9-bromo betamethasone dipropionate	No
Sunfire C_{18}	3 μm, 15.5% carbon loading	Co-elution of beclomethasone and 9-bromo betamethasone dipropionate	No
Symmetry C_{18}	3.5 μm, 19% carbon loading, endcapped	Co-elution for betamethasone 21-acetate, 17-propionate and 2-hydroxy, 4-methoxy benzophenone, beclomethasone and 9-bromo betamethasone dipropionate	No
UltraCarb C_{18}	3 μm, 22% carbon loading, no endcapping	Co-elution for betamethasone 21-acetate, 17-propionate and 2-hydroxy, 4-methoxy benzophenone, beclomethasone and 9-bromo betamethasone dipropionate	No
YMC Pro C_{18}	3 μm, 16% carbonloading with Lewis acid-base endcapping	Co-elution of beclomethasone and 9-bromo betamethasone dipropionate	No
YMC Pro C18 RS	3 μm, 22% carbonloading with Lewis acid-base endcapping	Baseline separation for all compounds, identical selectivity to J'sphere ODS-H80	Yes
YMC-Pro-AM	3 μm, 17% carbon loading, well endcapped	Co-elution for betamethasone 21-acetate, 17-propionate and 2-hydroxy, 4-methoxy benzophenone, beclomethasone and 9-bromo betamethasone dipropionate	No
YMC-Pro-AQ	3 μm, 14% carbon loading with hydrophilic endcapping	Partial resolution for betamethasone 21-acetate, 17-propionate and 2-hydroxy, 4-methoxy benzophenone, Peak overlapping for beclomethasone and 9-bromo betamethasone dipropionate	No

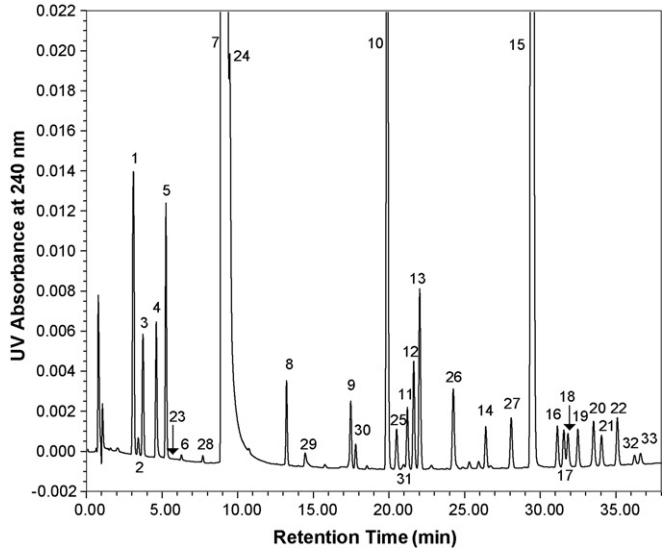

Fig. 3. The chromatogram of a specificity mixture solution. The HPLC conditions are listed in Section 2. Peaks 1–27 are the same as those listed in Table 1. Peak 28–33 are unknown peaks found in Diprosalic Lotion®.

3.1.2. Screening of alternate column

Seventeen different C_{18} RP-HPLC columns (Table 4) were screened under the optimized conditions to identify the alternate column. Most of the screened columns were unable to resolve two impurities: beclomethasone dipropionate and 9-bromo-betamethasone dipropionate. Some columns were unable to resolve either the *Z* and *E* isomers betamethasone enolaldehydes or the betamethasone 21-acetate-17-propionate and 2-hydroxy-4-methoxybenzophenone. Among them, the YMC-Pack Pro C18 RS that achieved the similar selectivity to J'sphere ODS-H80 was selected as the alternate column.

3.1.3. Diluent optimization for sample preparation

During method development, it was found that BD standard solution is only stable between pH 2.5 to neutral pH. Initially the mobile phase A (0.05% methanesulfonic acid aqueous solution, v/v, pH 2.7) was used to prepare the Diprosalic Lotion® sample solution. However, SA and several its related compounds in the sample solution showed peak splitting (Fig. 4). A review of the product formula revealed that 0.1 M sodium hydroxide is included to neutralize the SA and form a buffer system. The sample prepared with 0.05% methanesulfonic acid resulted in a solution with pH ~4.0, which keeps SA and its related compounds in deprotonated forms. After

Table 5
The Assay results of API, quantifiable degradation products/impurities and total mass balance of 7 representative Diprosalic Lotion® drug product samples from batch testing data.

Age of sample (month)	SA (%)	BD (%)	4-Hydroxybenzoic acid (%)	4-Hydroxyisophthalic acid (%)	Betamethasone 17-monopropionate (%)	Betamethasone-(E)-enolaldehyde (%)	Betamethasone 21-monopropionate (%)	Total mass balance of BD	Total mass balance of SA
32	102.29	94.55	0.07	0.03	6.98	0.49	0.76	104.58	102.36
26	103.44	98.17	0.04	0.04	3.87	0.26	0.32	103.21	102.63
22	101.18	98.22	0.04	0.03	4.61	0.34	0.37	104.72	101.18
19	101.85	97.38	0.04	0.03	3.55	0.25	0.21	102.23	101.85
16	100.91	98.41	0.04	0.03	3.56	0.27	0.29	103.60	100.91
10	100.25	100.39	0.07	0.04	2.22	0.11	0.06	102.25	102.00
4	101.76	99.75	0.07	0.04	0.94	0.06	0.18	102.37	101.87

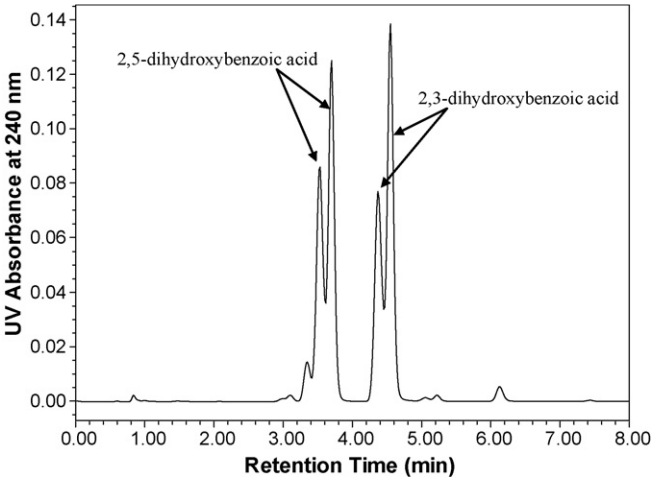

Fig. 4. The chromatogram of the specificity mixture dissolved in 0.05% methanesulfonic acid diluent. The HPLC conditions are listed in Section 2.

sample was injected into the column, the significant difference in pH between mobile phase (pH 2.7) and sample solution (buffered at pH 4.0) causes the incomplete conversion of SA and its related compounds from the deprotonated form to the protonated form. Therefore, the peak splitting was observed. To prevent the peak splitting of SA related peaks, the diluent must break the sample buffer system and bring the pH of sample solution close to 2.7 but above pH 2.5 to prevent the degradation of BD. Sample diluents with various buffer capacities were explored. Acetonitrile/water (30/70, v/v) with ~100 mM of sodium phosphate buffer at pH 2.7 ± 0.2 was able to break the sample buffer system and bring the pH of the sample solution to pH ~3.0. With this sample diluent, the BD standard solution was stable for at least 7 days; SA and its related compounds in sample solution were quickly converted to protonated forms on column by mobile phase and no peak splitting phenomenon was observed.

3.2. Method validation

3.2.1. Specificity

Diprosalic Lotion® has been commercially available for many years. The degradation pathway of BD and SA were also well characterized [4–8]. Expired Diprosalic Lotion® samples and an expired batch (age of 32 months) spiked with all related compounds of BD and SA (Table 1) should provide a true reflection of degradation products under the worst case scenario. The chromatogram of specificity mixtures (Fig. 3) showed that SA, BD, and their related compounds as well as potential leachables and unknowns in Diprosalic Lotion® were all adequately resolved from each other. Both SA and BD peaks were demonstrated as pure by evaluating the photodiode array scan from 200 to 400 nm at intervals across each peak.

3.2.2. Quantitation limit (QL), linearity, precision, recovery

The, QL in pharmaceutical industry normally should be equal to or greater than 0.05% of the label strength. In this method, the 0.05% of the label strength of BD and SA standard solution and standard solution-spiked placebo generated S/N ratios about 20–30. The linear regression curves from 0.05 to 150% showed a coefficient of determination (R^2) of 1000 for both BD and SA with y-intercepts less than 0.2% of their assay concentrations. Recoveries of both BD and SA were between 99 and 102% from 50 to 150% of their assay concentrations. For all degradants, the linear regression curves obtained from 0.05 to 15% showed a coefficient of determination (R^2) of 1.00 with y-intercepts less than 45% of the QL response. Recoveries of

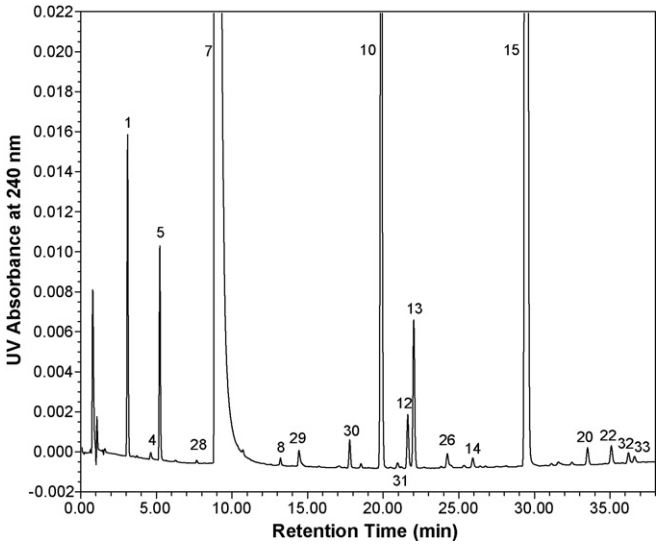

Fig. 5. A chromatogram of a Diprosalic Lotion® sample after storage at room temperature in the darkness for 32 months as a measure of sample stability. The HPLC conditions are the same as those indicated in Section 2. Unknown peaks 28–33 are the same as those in Fig. 3. The rest peaks are the same as those listed in Table 1.

all degradants were between 99 and 109% from 0.05 to 15% of the assay concentration.

3.2.3. Solution stability and robustness of HPLC parameters

Solution stability was assessed with BD and SA standard solution, BD and SA QL solution, Specificity test mixture solution and Diprosalic Lotion® sample solutions at room temperature and under refrigeration (2–8 °C). The solution stability results indicated that solutions are stable for at least 7 days at both conditions.

The HPLC parameters were deliberately varied from normal procedural conditions including gradient slope (±10%), flow rate (±0.2 mL), injection volume (±5 μL), column temperature (±5 °C), detection wavelength (±2 nm), column batch (3 lots), methanesulfonic acid content (±0.01%) in mobile phase A and HPLC instrument (two different vendors) to test the robustness of the method. Under these variations, all analytes were adequately resolved and elution orders remained unchanged. The assay variability of BD and SA was within ± 1%. The variability in the estimation of BD and SA related compounds was within ±10%. The QL solution maintained a signal-to-noise ratio over 10 in all varied conditions.

3.3. Analysis of Diprosalic Lotion® samples

Thirty five Diprosalic Lotion® samples (shelf life of 18 months) at different ages (4–32 months) were tested to determine the stability profile (Fig. 5). The data of representative batches (Table 5) indicated the degradation of BD into betamethasone 17-monopropionate via hydrolysis as the betamethasone 17-monopropionate concentration increases with ages. The results clearly demonstrated that the method is stability-indicating for Diprosalic Lotion®.

4. Conclusion

A reversed-phase HPLC method was developed to simultaneously determine BD, SA and their related compounds in Diprosalic Lotion®. It adequately separated and quantitated the two APIs, 26 API-related compounds (knowns and unknowns) and 5 potential leachables. This method was validated per current ICH guidelines and demonstrated to be stability-indicating for the Diprosalic Lotion®.

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