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Development and validation of a stability-indicating RP-HPLC method for assay of betamethasone and estimation of its related compounds

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ABSTRACT

Betamethasone (9α-fluoro-16β-methylprednisolone) is one of the members of the corticosteriod family of active pharmaceutical ingredient (API), which is widely used as an anti-inflammatory agent and also as a starting material to manufacture various esters of betamethasone. A stability-indicating reverse-phase high performance liquid chromatography (RP-HPLC) method has been developed and validated which can separate and accurately quantitate low levels of 26 betamethasone related compounds. The stability-indicating capability of the method was demonstrated through adequate separation of all potential betamethasone related compounds from betamethasone and also from each other that are present in aged and stress degraded betamethasone stability samples. Chromatographic separation of betamethasone and its related compounds was achieved by using a gradient elution at a flow rate of 1.0 mL/min on a ACE 3 C18 column (150 mm × 4.6 mm, 3 μm particle size, 100 Å pore size) at 40 °C. Mobile phase A of the gradient was 0.1% methanesulfonic acid in aqueous solution and mobile phase B was a mixture of *tert*-butanol and 1,4-dioxane (7:93, v/v). UV detection at 254 nm was employed to monitor the analytes. For betamethasone 21-aldehyde, the QL and DL were 0.02% and 0.01% respectively. For betamethasone and the rest of the betamethasone related compounds, the QL and DL were 0.05% and 0.02%. The precision of betamethasone assay is 0.6% and the accuracy of betamethasone assay ranged from 98.1% to 99.9%.

1. Introduction

Betamethasone (9α-fluoro-16β-methylprednisolone) is a steroid active pharmaceutical ingredient (API) as well as a key intermediate which is used to manufacture other related APIs, such as betamethasone 17-valerate, betamethasone acetate, betamethasone dipropionate, and betamethasone sodium phosphate. To ensure the quality of drug products, which contains betamethasone (BM) API, an HPLC method capable of separating and quantifying BM and all the potential BM related compounds (refer to Fig. 1) is required.

Developing a stability-indicating HPLC method for BM is very challenging because there are many BM related compounds which have similar structures as BM. Previous reports of the HPLC analysis of BM was mainly focused on the separation of BM and one of its key isomers, namely dexamethasone [1–5]. The structures of these 2 isomers are only different from each other by the orientation of methyl group at the C16 position (Fig. 1). The Ph. Eur. HPLC method for BM related compounds cannot separate all the potential impurities and degradation compounds of BM [6]. However, the Ph. Eur. HPLC method can resolve several related compounds of BM, e.g., dexamethasone, BM-21-aldehyde, 1,2-dihydrobetamethasone Compounds A, E, H, J, M, and O (structures are shown in Fig. 1). Recently, a stability-indicating RP-HPLC method for separation of BM and its related compounds has been reported in the literature [7]. This method [7] can separate 13 betamethasone related compounds including dexamethasone, and Compounds B, E, H, J, M, N, S, U, V, W, X, Y (structures are shown in Fig. 1). Recent studies related to the degradation chemistry of BM [8,9] and unpublished data generated in our laboratory identified additional related compounds of BM (Compounds G, I, K, P, Q. See Fig. 1 for structures).

To the best of our knowledge, none of the currently available analytical methods (including the Ph. Eur. method) can separate and quantitate all the known related compounds of BM API. Furthermore, there is no stability-indicating HPLC method reported in the literature that can adequately separate and accurately quantitate BM and all of its 26 known related compounds (shown in Fig. 1).

In this paper, we report the very first stability-indicating RP-HPLC method for the assay of BM and estimation of all the known related compounds of BM. The new method is capable of separating twenty-six related compounds (including BM-21-aldehyde, dexamethasone, and 1,2-dihydrobetamethasone, as well as 7 newly identified degradants) of BM from BM and from each other. This method was successfully validated according to the International

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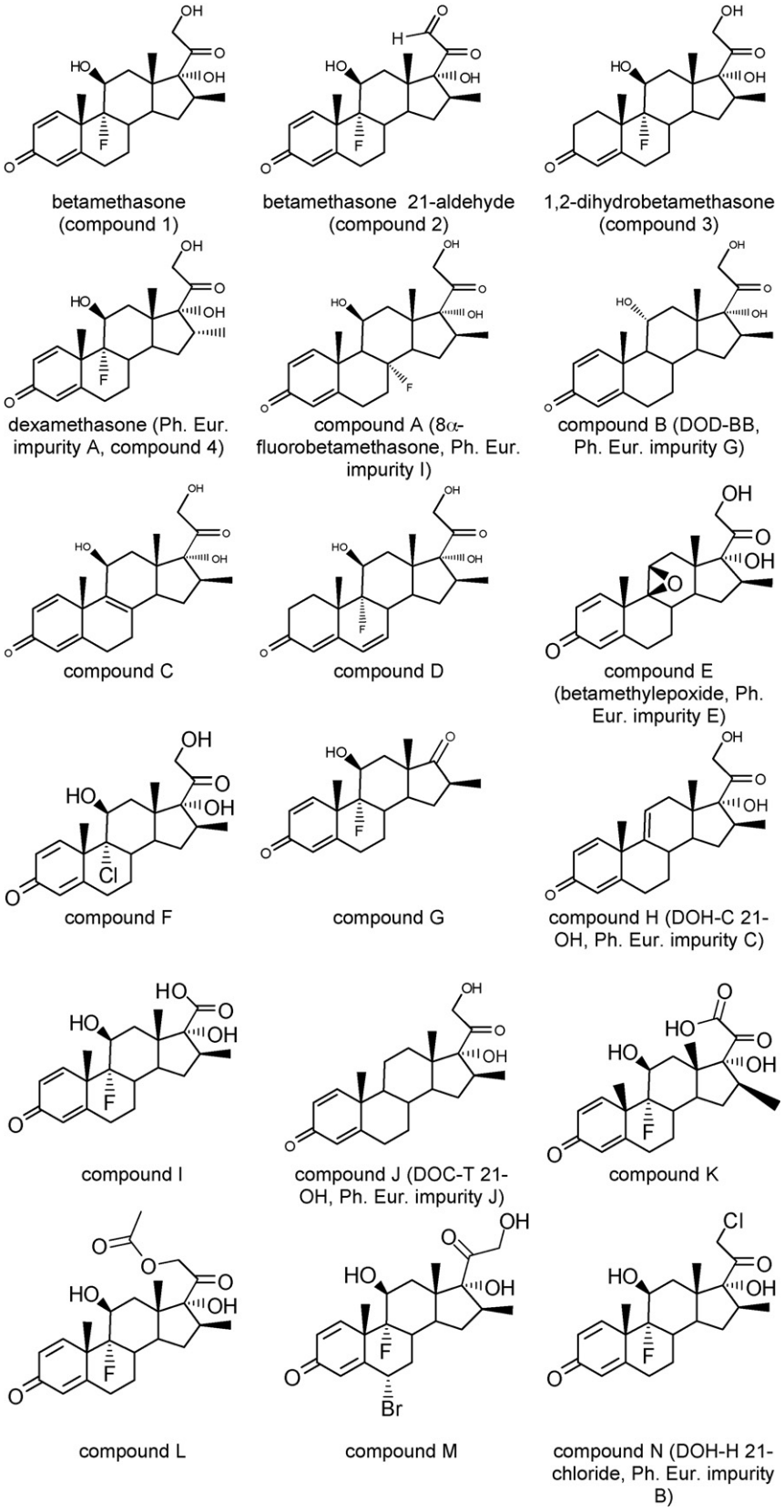

Fig. 1. Chemical structures of betamethasone and its related compounds.

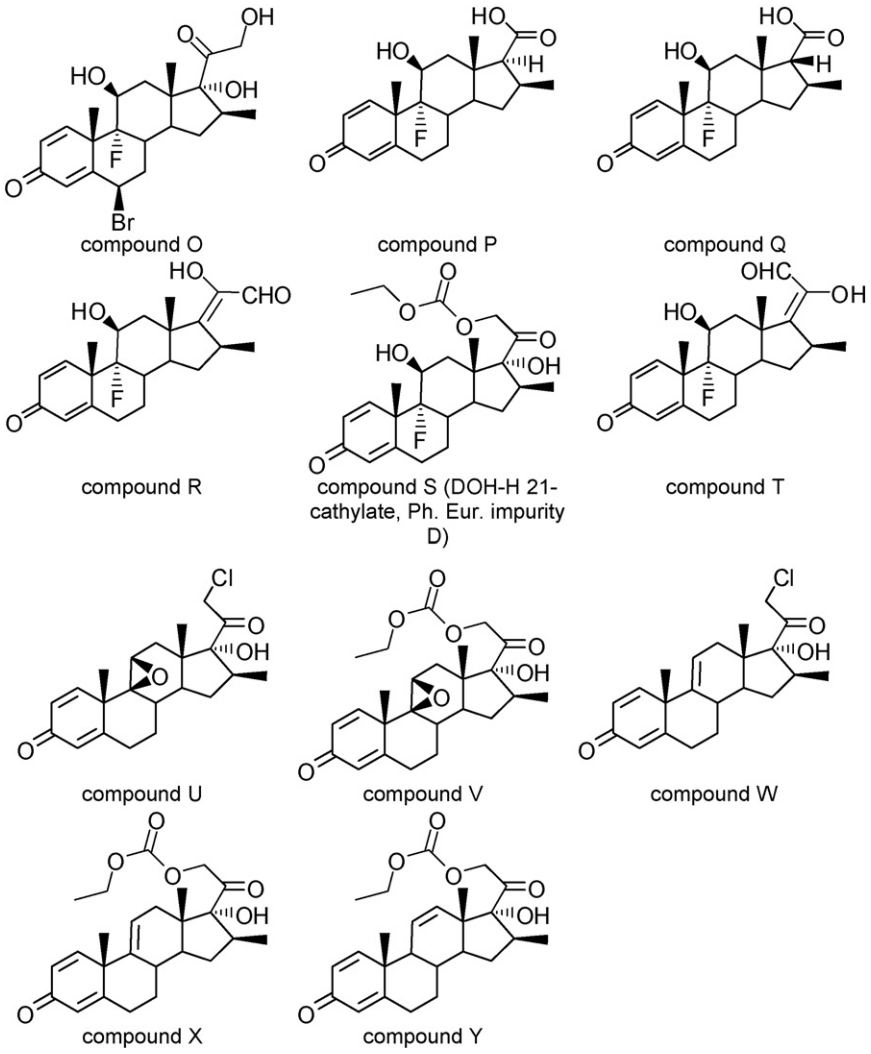

Fig. 1. (*Continued*).

Conference Harmonization (ICH) guidelines (Validation of Analytical Procedures: Test and Methodology Q2).

2. Experimental

2.1. Materials

Betamethasone and its related compounds were provided by Schering-Plough Corporation (Union, NJ). The HPLC grade 1,4-dioxane was purchased from three different suppliers namely, Alfa Aesar (26 Parkridge Road, Ward Hill, MA), Acros Organics (part of Thermo Fisher Scientific International Inc., Liberty Lane Hampton, NH), and Sigma–Aldrich (3050 Spruce St., St. Louis, MO). The anhydrous *tert*-butanol (≥99.5%) and methanesulfonic acid (≥99.5%) was purchased from Sigma–Aldrich. HPLC grade water, acetonitrile, methanol, ethanol, isopropanol, tetrahydrofuran (THF), 1,4-dimethoxy ethane, acetic acid, formic acid and phosphoric acid were purchased from Fisher Scientific. Milli-Q Water (18.2 MΩ cm) was obtained using a Milli-Q system (Millipore, Billerica, MA).

2.2. Instrumentation

An Agilent Technologies 1100 Series HPLC system (Santa Clara, CA) and Waters 2695 Alliance HPLC systems equipped with 2996 photodiode-array detector (PDA) and/or 2487 dual wavelength UV detectors (Milford, MA) were used for method development. All HPLC systems were equipped with a column compartment with temperature control and an on-line degasser. Data acquisition, analysis, and reporting were performed by Empower2 (Waters) chromatography software. Different Waters 2695 HPLC systems were used for method validation.

2.3. Chromatographic conditions and solution preparations

The chromatographic conditions used during method development are provided in Section 3. The validated method uses a primary HPLC column ACE 3 C18 (15 cm × 4.6 mm I.D., 3 μm particle size, 100 Å pore size) purchased from Mac-Mod Analytical Inc., and the alternate column was Develosil ODS-UG-3 (15 cm × 4.6 mm I.D., 3 μm particle size, 140 Å pore size) purchased from Phenomenex. The chromatographic separation of betamethasone and its related compounds was achieved using a gradient elution at a flow rate of 1.0 mL/min using the primary or the alternate column at 40 °C using mobile phase A (0.1% methanesulfonic acid aqueous solution) and mobile phase B (*tert*-butanol and 1,4-dioxane (7:93, v/v)), and UV detection at 254 nm. The sample injection volume was 50 μL. The gradient program is shown in Table 1. The total chromatographic run time is 68 min followed by a wash and re-equilibration of the column to initial mobile phase conditions.

Table 1
HPLC gradient of the finalized method.

Run time (min)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)	Gradient curve
0.0	1.0	80	20	Linear
40.0	1.0	75	25	Linear
65.0	1.0	48	52	Linear
68.0	1.0	48	52	Linear
Column wash and equilibration to initial conditions				
68.1	1.0	80	20	Linear
77.0	1.0	80	20	Linear

Table 2
HPLC gradient of the preliminary method.

Run time (min)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)	Gradient curve
0.0	1.5	75	25	Linear
15.0	1.5	75	25	Linear
35.0	1.5	25	75	Linear
39.0	1.5	10	90	Linear
Column wash and equilibration to initial conditions				
40.0	1.5	75	25	Linear
45.0	1.5	75	25	Linear

Sample solutions were prepared by dissolving appropriate amounts of betamethasone and its related compounds into acetic acid–acetonitrile (0.1:100, v/v) by sonication followed by filling to the volume with acetic acid aqueous solution (0.1%). The analytical concentration of betamethasone was 0.4 mg/mL in acetic acid–acetonitrile–water (0.1:24:76, v/v/v).

To determine linearity for betamethasone, triplicate samples of betamethasone were prepared at 0.05% (QL), 0.2%, 0.5%, 1%, 2%, 50%, 75%, 90%, 100%, 110%, and 125% of the betamethasone analytical concentration (0.4 mg/mL). The linearity of betamethasone 21-aldehyde was determined using triplicate preparations at 0.02% (QL), 0.05%, 0.2%, 0.5%, 1.0%, and 2.0% of betamethasone analytical concentration. The linearity of ten other betamethasone related compounds were determined using triplicate preparations of each compound at 0.05% (QL), 0.2%, 0.5%, 1.0%, and 2.0% of betamethasone analytical concentration. The detection limit (DL) of betamethasone 21-aldehyde was 0.01% of the betamethasone analytical concentration. The DL of all the other compounds studies was 0.02% of the betamethasone analytical concentration.

3. Results and discussions

3.1. HPLC method development

3.1.1. Development of a preliminary HPLC method for BM and related compounds

The current European Pharmacopoeia (Ph. Eur.) betamethasone related compound method [6] cannot separate all the known potential related compounds of BM. However, it can separate a few key related compounds of BM such as BM-21-aldehyde and 1,2-dihydrobetamethasone. In addition, it was observed that the reproducibility of Ph. Eur. Method for some BM impurities (particularly BM-21-aldehyde) was variable [10]. The preliminary HPLC method uses a gradient elution at 1.0 mL/min flow rate on a YMC-Pack ODS-AQ column (YMC, 15 cm × 4.6 mm I.D., 3 μm particle size, 100 Å pore size, from Waters) at 55 °C using mobile phase A [glyoxal–ammonium acetate 20 mM (0.04:100, v/v)] and mobile phase B (acetonitrile). The gradient table is shown in Table 2. The detection is UV at 240 nm and the sample is prepared using methanol as the diluent. The details of this method are described

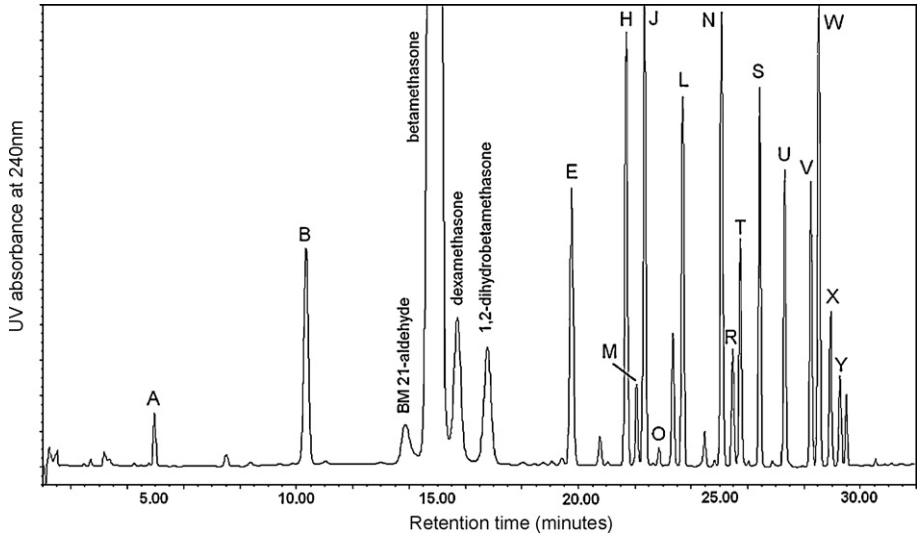

Fig. 2. Representative chromatogram of a mixture of BM and its related compounds using the preliminary HPLC method.

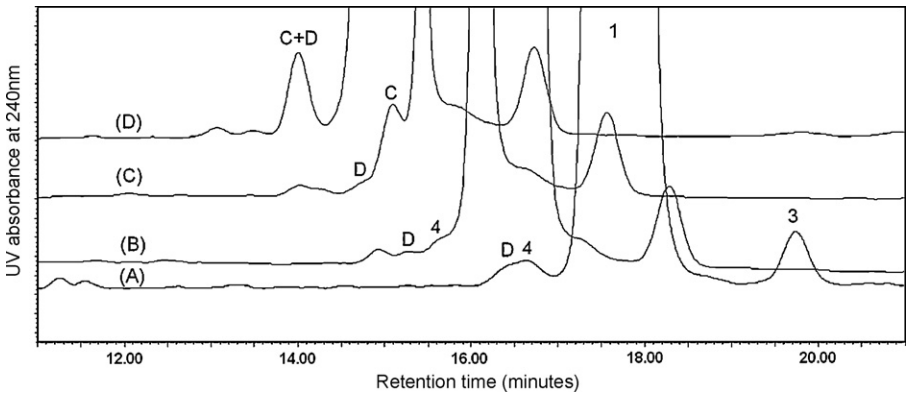

Fig. 3. Chromatograms showing the two new peaks (Compounds C and D). Chromatograms were acquired using preliminary HPLC method with variations in mobile phase B: (A) acetonitrile, (B) THF–acetonitrile (4:96, v/v), (C) THF–acetonitrile (8:92, v/v), and (D) THF–acetonitrile (12:88, v/v). BM-21-aldehyde (labeled as '4') merged with betamethasone in the Chromatograms (C) and (D).

elsewhere [10]. A representative chromatogram using these HPLC conditions is shown in Fig. 2 which includes the gradient program details. As shown in Fig. 2, this method is capable of separating BM-21-aldehyde, dexamethasone, and 1,2-dihydrobetamethasone from BM. Additionally, two degradants (Compounds R and T) were also separated.

When this method was used to analyze BM API samples, one additional peak with UV_{max} at 285 nm was found co-eluting with BM-21-aldehyde (Fig. 3A). The peak was later determined to be Compound D. Another unknown peak co-eluting with betamethasone (Compound C) was also discovered when a small amount of THF was added to the mobile phase B (Fig. 3B–D). Therefore, additional HPLC method development was required in order to separate all the peaks that are co-eluting or eluting near BM peak.

3.1.2. Separation of seven critical peaks—evaluation of mobile phase B

Although there are 26 known BM related compounds (Fig. 1), the method development activities initially focused on BM and the six related compounds that eluted immediately before

Table 3
Method development chromatographic conditions and resolution results for the seven critical peaks.

HPLC condition	Retention time (min) and comment on key resolution						
	BM (Compound 1)	Dexamethasone (Compound 2)	1,2-Dihydro-betamethasone (Compound 3)	BM-21-aldehyde (Compound 4)	Compound C	Compound D	Compound E
1a	21.83	23.30	24.84	22.48, co-eluted with BM	18.93	Co-eluted with BM	26.54
1b	19.00	18.71, co-eluted with BM	20.36	19.27, co-eluted with BM	18.00	16.82	25.19
1c	24.10	25.43	26.44	22.43	Co-eluted with BM	23.11	35.38
1d	18.12	18.05, co-eluted with BM	19.76	15.92	Co-eluted with BM	16.75	21.22
1e	29.95	32.71	32.23, co-eluted with '2'	28.18	Co-eluted with BM	26.72	42.01
2	19.70	20.57, co-eluted with BM	21.71, overlapped with BM	27.71, significantly peak broadening	18.68	17.18	26.16
3	14.97	15.20, co-eluted with BM	16.18, overlapped with BM	25.65, significantly peak broadening	18.95	13.60	12.98
4	11.39	11.18, co-eluted with BM	12.32, overlapped with BM	15.40, significantly peak broadening	10.43	10.15	15.56
5	23.83	26.59	28.36	26.01, overlapped with '2'	18.41	25.87, overlapped with '4'	23.52, co-eluted with BM
6	25.47	27.66	29.90	30.79	22.16	Co-eluted with BM	28.94
7a	43.08	47.90	51.42	53.00	41.01	Co-eluted with BM	55.33
7b	30.76	30.76	34.18	37.37	28.48	29.30	38.82

1a. Acetonitrile–THF–H_3PO_4 aqueous solution (0.1%) (12:6:82, v/v/v), 1.5 mL/min, YMC Pro-C18 column, 55 °C.
1b. Acetonitrile–*tert*-butanol–H_3PO_4 aqueous solution (0.1%) (7.5:7.5:85, v/v/v), 1.5 mL/min, YMC Pro-C18 column, 50 °C.
1c. Acetonitrile–H_3PO_4 aqueous solution (0.1%) (20:80, v/v/v), 1.4 mL/min, Luna 3u PFP (2) column (3 μm, Phenomenex), 50 °C.
1d. Acetonitrile–H_3PO_4 aqueous solution (0.1%) (20:80, v/v/v), 1.5 mL/min, Luna CN column (3 μm, Phenomenex), 30 °C.
1e. Acetonitrile–H_3PO_4 aqueous solution (0.1%) (20:80, v/v/v), 1.4 mL/min, Luna C8 column (3 μm, Phenomenex), 30 °C.
2. Methanol–20 mM ammonium acetate (40:60, v/v), 1.5 mL/min, YMC-Pack Pro-C18 column, 50 °C.
3. Ethanol–20 mM ammonium acetate (25:75, v/v), 1.5 mL/min, YMC-Pack Pro-C18 column, 50 °C.
4. Isopropanol–20 mM ammonium acetate (18:82, v/v), 1.5 mL/min, YMC-Pack Pro-C18 column, 50 °C.
5. THF–H_3PO_4 aqueous solution (0.1%) (14:86, v/v), 1.3 mL/min, ACE 3 C18 column, 50 °C.
6. 1,2-Dimethyoxyethane–H_3PO_4 aqueous solution (0.1%) (25:75, v/v), 1.0 mL/min, ACE 3 C18 column, 45 °C.
7a. 1,4-Dioxane–H_3PO_4 aqueous solution (0.1%) (25:75, v/v), 1.0 mL/min, YMC-Pack Pro-C18 column, 40 °C.
7b. 1,4-Dioxane–H_3PO_4 aqueous solution (0.1%) (25:75, v/v), 1.0 mL/min, ACE 3 C18 column, 40 °C.

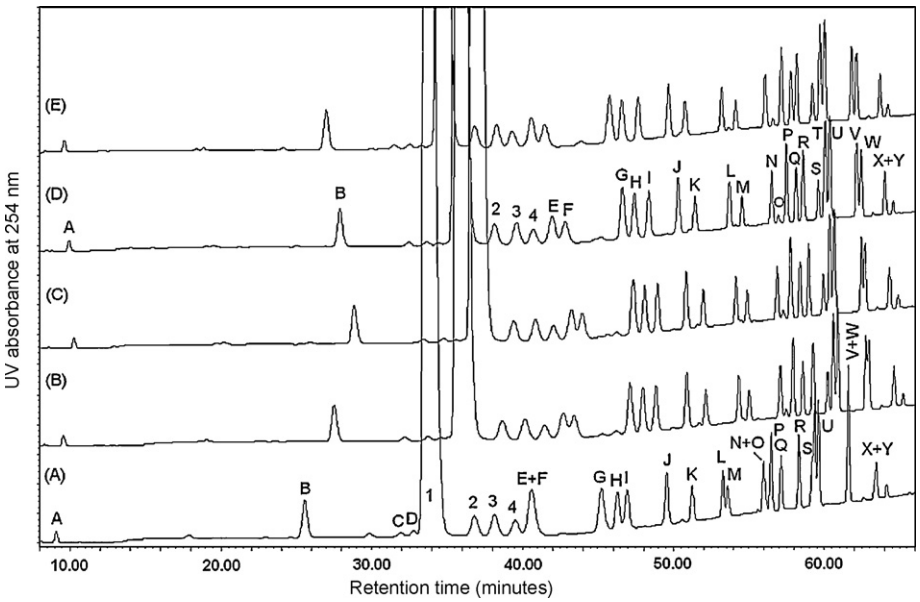

Fig. 4. Chromatograms of a mixture of BM and its related compounds (spiked at 0.2–0.5% level) using different percentage of *tert*-butanol in 1,4-dioxane as mobile phase B. Mobile phase B: (A) 0%, (B) 5%, (C) 6%, (D) 7%, (E) 8% *tert*-butanol in 1,4-dioxane. In Chromatogram A, Compound T (not labeled) coeluted with Compound S.

and after BM (dexamethasone, 1,2-dihydrobetamethasone, BM-21-aldehyde, Compound C, Compound D, and Compound E). Establishing the preliminary chromatographic conditions to adequately resolve these seven critical pairs would then be used to optimize the separation of the other 19 BM related compounds. Depending on the mobile phase conditions, the peaks merged with one another or with BM. Various chromatographic parameters were evaluated including %acetonitrile, organic additives, column temperature and different types of HPLC columns. The highlights of the results including experimental parameters are summarized in Table 3. The preliminary mobile phase conditions (HPLC conditions 1a–1e in Table 3) used in the study were unable to provide complete separation for the seven critical peaks. Therefore, several additional mobile phase B organic solvents were also evaluated, such as, methanol, ethanol, isopropanol, THF, 1,2-dimethoxyethane and 1,4-dioxane (HPLC conditions 2–7 in Table 3). As expected, the chromatographic separation and elution order of the seven critical peaks were different when different organic solvents were used in mobile phase B. Among the solvents tested, only 1,4-dioxane showed the most promising results. All the seven critical peaks were separated using ACE 3 C18 column (40 °C) at 1.0 mL/min when 1,4-dioxane–H_3PO_4 aqueous solution (0.1%) (25:75, v/v) was used as mobile phase B.

3.1.3. Optimization of separation using tert-butanol as additive

After the organic solvents for the mobile phase was determined, a gradient program was developed for the separation of all other BM related compounds with the help of Chromsword software (Merck KGaA, Darmstadt, Germany) (Fig. 4A). The gradient is for t = 0–40 min, a linear gradient from A–B (77:23, v/v) to A–B (73:27, v/v); for t = 40–65 min, a linear gradient from A–B (73:27, v/v) to A–B (45:55, v/v); for t = 65–68 min, a constant composition of A–B (45:55, v/v). Mobile phase A is 0.1% methanesulfonic acid in water and mobile phase B is dioxane.

As shown in Fig. 4A, there were several compounds co-eluting or partially overlapping: Compound E and Compound F; Compound L and Compound M; Compound N and Compound O; Compound S and Compound T; Compound X and Compound Y. In order to improve the separation of these overlapping compounds, a small amount of acetonitrile and *tert*-butanol was added into mobile phase B to improve the solvent selectivity [11]. Adding 5% acetonitrile in 1,4-dioxane did not affect the peak separation.

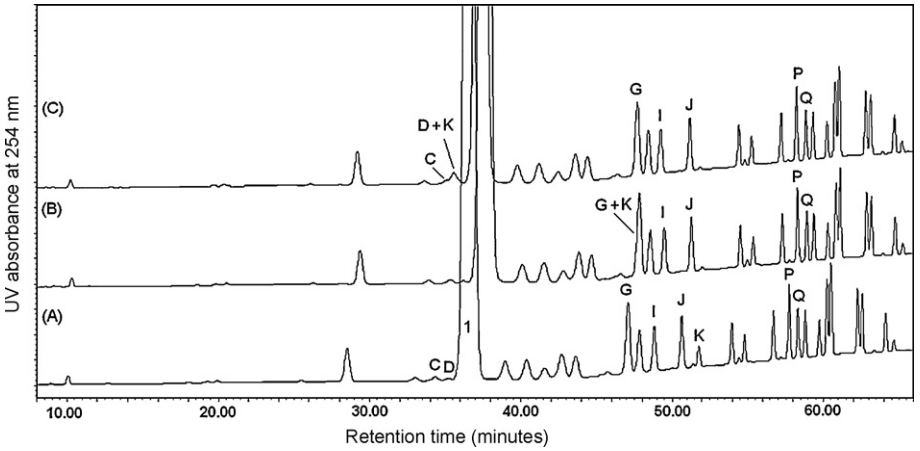

Fig. 5. Chromatograms of a mixture of BM and its related compounds using different acid additives in mobile phase A: (A) 0.1% methanesulfonic acid, (B) 0.1% H_3PO_4, and (C) 0.1% formic acid.

Table 4
HPLC columns (15 cm × 4.6 mm) screened for alternate column selection.

HPLC column	RT (BM) (min)	Particle size, pore size, carbon loading, surface area	Surface property	Comments
ACE 3 C18	35.6	3 μm, 100 Å, 15.5%, 300 m^2/g	Monomeric bonded fully endcapped	Good separation except Compound X and Y co-eluted.
ACE 3 C8	35.9	3 μm, 100 Å, 9.0%, 300 m^2/g	Monomeric bonded fully endcapped	BM 21-aldehyde, 1,2-dihydrobetamethasone and Compound E co-eluted. Compound J and K, Q and R overlapped. Compound F eluted 4 min later than Compound E.
Symmetry C18	38.0	3.5 μm, 100 Å, 19%, 335 m^2/g	Monomeric bonded well endcapped	Good separation except Compound D merged with BM.
Symmetry Shield RP18	37.4	3.5 μm, 100 Å, 17%, 335 m^2/g	Monomeric bonded polar-embedded stationary phase	Compound D and BM, 1,2-dihydrobetamethason and Compound E emerged. BM 21-aldehyde eluted between dexamethasone and 1,2-dihydrobetamethasone after Compound F. Compound F eluted 4 min later than Compound E. Many later eluting compounds overlapped.
Xbridge C18	30.6	3.5 μm, 135 Å, 18%, 185 m^2/g	Trifunctional bonded with end capping	Compounds C and D, Q and R, V and W partially overlapped and Compounds T and U completely merged.
Xbridge Shield RP18	30.5	3.5 μm, 135 Å, 17%, 185 m^2/g	Monomeric bonded polar-embedded stationary phase	Compound D and BM, 1,2-dihydrobetamethason and Compound E emerged. BM 21-aldehyde eluted between dexamethasone and 1,2-dihydrobetamethasone. Compound F eluted 4 min later than Compound E. Many later eluting compounds overlapped.
Acclaim C18	43.2	3 μm, 120 Å, 18%, 300 m^2/g	Proprietary	BM eluted during the 2nd gradient
YMC-Pack J'Sphere ODS-H80	37.9	4 μm, 120 Å, 22%, 500 m^2/g	Monomeric bonded well endcapped	1,2-Dihydrobetamtehasone and BM 21-aldehyde merged. Many later eluting compounds overlapped
YMC-Pack Pro-C18	40.1	3 μm, 120 Å, 16%, 320–350 m^2/g	Monomeric bonded Lewis acid-base endcapped surface	BM eluted during the 2nd gradient
Zorbax Eclipse Plus C18	37.6	3.5 μm, 95 Å, 9%, 160 m^2/g	Monomeric bonded surface with trimethylsilyl end capping	BM 21-aldehyde and 1,2-dihydrobetamethasone, Compound E and BM, Compound L and M, S and T merged
Develosil ODS-UG-3	37.4	3 μm, 140 Å, 18%, 300 m^2/g	Monomeric bonded surface with end capping	Good separation except Compound E and F co-eluted, X and Y overlapped
Develosil ODS-HG-3	37.3	3 μm, 140 Å, 18%, 300 m^2/g	Polymeric bonded surface with end capping	Compounds E and F, G and H, V and W overlapped; Compounds T and U, X and Y merged
Prodigy 3u ODS(3)	43.4	3 μm, 100 Å, 15.5%, 450 m^2/g	Monomeric bonded surface with trimethylsilyl end capping	BM eluted during the 2nd gradient

However, the separation was significantly improved when *tert*-butanol was added. As shown in Fig. 4B–E, 5–8% *tert*-butanol was added into 1,4-dioxane. The gradient programs were slightly adjusted to retain the similar retention time of BM. All BM related compounds were separated from betamethasone and from each other except Compound X and Compound Y. Based on the results, 7% *tert*-butanol in 1,4-dioxane was chosen for mobile phase B because it gave the best overall separation. Unlike the primary and secondary alcohol, the *tert*-butanol additive does not lead to peak broadening of the BM-21-aldehyde compound. The final HPLC conditions for method validation were set as described in Section 2.3.

3.1.4. Selection of the acid additive to mobile phase A

Different acid additives in mobile phase A were tested to optimize the separation of the four acid degradants of betamethasone (Compounds I, K, P, and Q). The representative chromatograms are shown in Fig. 5. As shown in Fig. 5, the retention time of Compound K changed significantly with the acids in mobile phase A and 0.1% methanesulfonic acid in mobile phase A gave the best separation of Compound K from other BM related compounds.

3.1.5. Selection of an alternate column

During the life-cycle of an analytical method, the primary column of the validated HPLC method may no longer be commercially

Table 5
Linearity, accuracy/recovery, precision and intermediate precision data for betamethasone and the related compounds.

Compound	Linearity (r)	Accuracy (%)	Precision-repeatability (%)	Intermediate precision[a]
BM	1.000	98.1–99.9[b]	0.6[b]	99.36 ± 0.26 (0.3)
		98.6–99.8	0.4	
Compound B	1.00	102.4–103.7	0.4	0.55 ± 0.02 (3.8)
Dexamethasone	1.00	103.6–116.3	3.8	0.71 ± 0.03 (4.9)
1.2-Dihydrobetamethasone	1.00	99.3–103.5	1.8	0.46 ± 0.02 (3.5)
BM-21-aldehyde	1.00	92.6–103.1	4.4	0.58 ± 0.02 (2.9)
Compound E	1.00	92.4–102.6	4.4	0.50 ± 0.02 (3.0)
Compound H	1.00	102.7–103.6	0.3	0.52 ± 0.01 (1.8)
Compound J	1.00	103.3–103.6	0.1	0.55 ± 0.02 (3.0)
Compound N	1.00	103.1–103.9	0.2	0.52 ± 0.00 (0.8)
Compound R	1.00	102.7–104.5	0.5	0.50 ± 0.02 (3.4)
Compound S	1.00	101.4–103.6	0.8	0.52 ± 0.01 (2.7)
Compound T	1.00	102.5–104.0	0.6	0.53 ± 0.01 (1.9)

[a] Average %assay or %RC ± SD (%RSD).
[b] % Accuracy and precision-repeatability at 75–125% range. The accuracy and precision-repeatability data without labeling are at 0.2–2.0% range.

available. Therefore, an alternate column should be identified which would be used in this situation. Twelve additional HPLC columns were screened (Table 4) using the optimized HPLC gradient conditions to identify the alternate column. Among the columns tested (Table 4), only Develosil ODS-UG-3 gave separation of betamethasone and related compounds that were comparable to the separation of the primary column. Hence, Develosil ODS-UG-3 was selected as the alternate column for the new method.

3.2. Method validation

3.2.1. The linearity, accuracy/recovery, precision, quantitation limit (QL), detection limit (DL)

The results of the linearity/accuracy validation studies for betamethasone and ten BM related compounds are shown in Table 5. The precision-repeatability was evaluated by the %RSD of the recoveries obtained from nine samples prepared as triplicates at the 3 concentration levels. Intermediate precision is demonstrated by performing a matrix experimental design in which day of testing, analysts, instruments, and analytical columns were varied during the analysis of a related compounds spiked API solution (Betamethasone Reference Standard spiked with the eleven validated related compounds at 0.5% level).

The quantitation limit (QL) and the detection limit (DL) were verified at the concentration level that generated a signal-to-noise (S/N) greater than 10 (QL) and the concentration level that generates S/N ≥ 3 (DL). For BM-21-aldehyde, the QL and DL were verified at 0.02% and 0.01%, respectively relative to the API analytical concentration. For betamethasone and the rest of the betamethasone related compounds, the QL and DL was verified at 0.05% and 0.02% level respectively relative to the API analytical concentration.

3.2.2. Method specificity

The method specificity was demonstrated through the peak purity analysis (photodiode-array detection) of five representative expired BM batches as well as the analysis of a specificity mixture solution containing about 0.5% of all other available related compounds spiked to one API batch. The studies demonstrated that the method is capable of resolving BM and its key related compounds from each other. BM and all spiked BM related compounds (except between Compound X and Compound Y) were adequately resolved from each other in the chromatogram of the specificity mixture solution and the expired API batches. The peak purity was verified as the purity angle of photodiode-array (PDA) scan from 200 nm to 400 nm for the 0.5% spiked API sample and these 5 expired batches were all much less than the purity threshold.

3.2.3. Solution stability and HPLC parameter robustness

BM and its related compounds were first dissolved using acidified acetonitrile, then diluted with acidified water. Commercial HPLC grade acetonitrile can contain trace levels of free hydrogen cyanide which can react with chemical entities such as 21-aldehyde

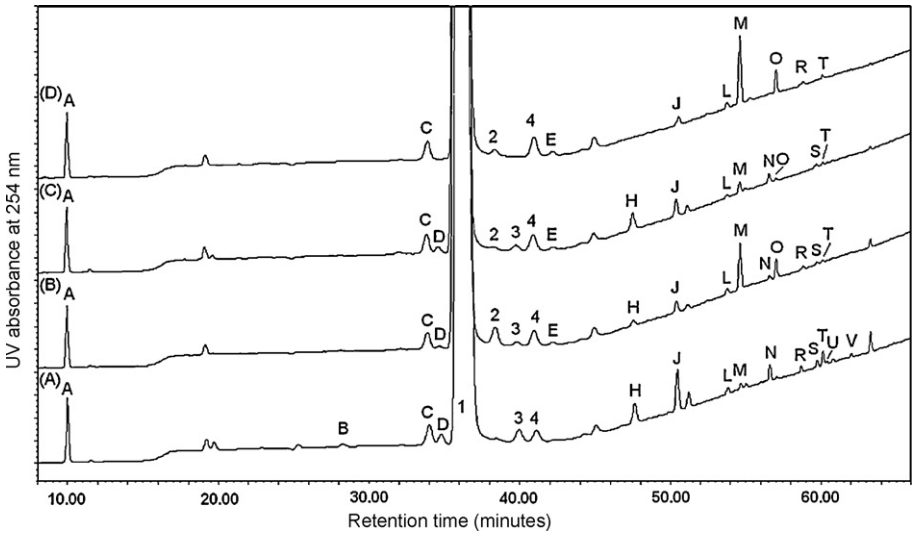

Fig. 6. Chromatograms of four representative commercial BM batches.

analogue of BM at neutral or sub-neutral pH conditions. Therefore, acetic acid was added in the diluent to retain an acidic pH which would eliminate the possibility of any reaction between BM-21-aldehyde and the residual hydrogen cyanide that can present in the acetonitrile. Our test results showed that BM and related compounds were stable for up to seven days either at ambient room temperature (RT) or under refrigeration (at 2–8 °C), when protected from regular lab lighting.

The HPLC parameters were deliberately varied from normal procedural conditions including gradient slope (±10%), flow rate (±0.05 mL/min), injection volume (±5 μL), column temperature (±2 °C), detection wavelength (±2 nm), column batch (3 lots), methanesulfonic acid content (±0.1%) in mobile phase A, *tert*-butanol content in mobile phase B (±0.5%), HPLC grade 1,4-dioxane from 3 vendors and HPLC instrument (Waters and Agilent) to test the robustness of the method. Under these variations, all analytes were adequately resolved and elution orders remained unchanged. The QL solution maintained a signal-to-noise ratio over 10 in all varied conditions. The peak resolution between betamethasone and dexamethasone were all larger than 1.5 under each variation.

3.3. Analysis of betamethasone API samples

Thirty-two BM API samples (shelf life of 39 months) at different ages (6–49 months) were tested to establish the specifications for the BM API commercial batches. The chromatograms of four representative batches were shown in Fig. 6. BM, BM related compounds and unknown peaks are well separated and can be accurately quantified.

4. Conclusions

The analytical method described in this paper is suitable for assay of Betamethasone and its related compounds in betamethasone API. The method has been demonstrated to be accurate, linear, precise, repeatable, specific, and robust, and therefore suitable for routine analysis of betamethasone. This method is a stability-indicating method because it can separate all known degradation products from betamethasone (API) and also from each other. To the best of our knowledge, this is the first stability-indicating HPLC method that can separate betamethasone, dexamethasone, BM-21-aldehyde, 1,2-dihydrobetamethasone including another twenty plus related compound peaks from each other via single HPLC run. As the method is successfully validated using ICH guidelines, it can be readily implemented in quality control laboratories for the purpose of lot release and stability testing of betamethasone API.

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