Organic Process Research & Development **2003**, *7*, 896−903

Synthesis and Characterisation of Impurities of Manufacture in Support of Certificate of the European Pharmacopoeia Applications Part 1 (Clobetasone Butyrate)

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Abstract:

Established pharmaceutical products often have complex supply chains and regulatory histories. In Europe the European Directorate for the Quality of Medicine (EDQM) (information from European Pharmacopoeia website, http://www.pheur.org/) have promoted the use of certificates of suitability of the European Pharmacopoeia to ensure the quality of medicines by a commonly recognised set of standards, allowing the free movement of medicinal products in member states. This contribution describes the analytical and synthetic chemistry required to synthesise the impurities of manufacture of clobetasone butyrate.

Introduction

As established pharmaceutical products continue through their life cycle, the need to continue to comply with current regulatory requirements is essential. As part of this, adherence to ICH guidelines[2] on identification and control of impurities and use of appropriate analytical methodology has become a prerequisite. Regulatory strategy for these products has also developed as industries attempt to register their products in regions of the world rather than individual countries.

More and more the use of Certificates of the European Pharmacopoeia (CEP) is commonplace for these well-established actives. These CEP documents allow certification that the manufacture and analysis of pharmaceutical products adhere to the requirements of European Pharmacopoeia and are appropriate to the needs of the regulatory authorities.

Clobetasone butyrate, Figure 1, is the active ingredient in Eumovate, a potent corticosteroid formulation used in the treatment of mild-to-moderate inflammatory skin diseases such as eczema and dermatitis. It was first identified in the 1960s[3] and came to market in 1976, where it has remained as an integral part of the management of corticosteroid-responsive dermatoses.[4] It is approved in over 100 countries around the world and is an important part of GlaxoSmithKline's (GSK) portfolio of respiratory and dermatological medicines.

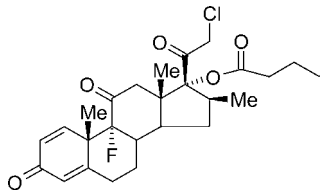

Figure 1. **Clobetasone-17-butyrate.**

As part of the strategy to certify clobetasone butyrate's manufacture as suitable for the European Pharmacopoeia it is essential to identify all impurities at a level of >0.10% using a validated analytical method.

Initial studies done within GSK identified a number of impurities that had to be synthesised in support of a CEP application. Their synthesis and characterisation to allow validation of a new analytical method is essential in supporting the application and defining the specification for the active ingredient.

Previous work[5] within GSK had provided a list of the impurities identified in clobetasone butyrate drug substance as postulated structures on the basis of spectral and chromatographic data. The isolation of these impurities either from enriched samples (often a laborious exercise by prep-LC) or by independent synthesis forms the basis of this discussion.

Discussion

Impurity Identification. The impurities in question were identified as follows:

1. Clobetasone-17-propionate. This impurity is likely to arise due to small amounts of *n*-propionic anhydride, or

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(1) Information from European Pharmacopoeia Website http://www.pheur.org/.

(2) International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Section Q3B: Guidelines on Impurities in New Drug Products via Food and Drug Administration (FDA); web-site - http://www.fda.gov/cder/guidance/1317fnl.pdf.

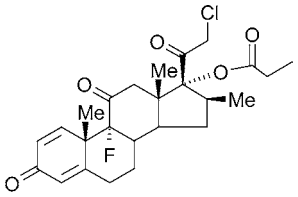
(3) Elks, J.; Phillipps, G. H. Ger Offen 1969, 87 pp.; German Patent DE 1902340.

(4) Eumovate (clobetasone butyrate 0.05%) cream: a review of clinical efficacy and safety. Goustas, P.; Cork, M. J.; Higson, D. *J. Dermatol. Treat.* **2003**, *14*, 71−85.

(5) Unpublished work GSK Ware.

Published on Web 11/06/2003
10.1021/op0341216 CCC: $25.00

mixed anhydrides, present in the *n*-butyric anhydride used for butyration of the 17-hydroxy position. Although controlled by specification, this impurity had been seen at levels greater than 0.10% in drug substance.



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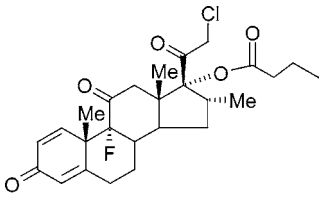
2. 16α-Methyl Clobetasone-17-butyrate. This impurity is derived from the isomeric starting material, again controlled by specification at the start of the manufacturing synthesis.



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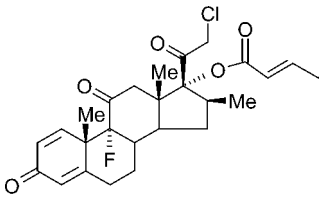
3. Clobetasone-17-*trans*-crotonate. This impurity was seen at very low levels, lower than 0.10% by HPLC. However, it was important to obtain a sample to prove control. Similar to impurity **1**, the origin again pointed to the butyric anhydride used in the synthesis. Careful control of the crotonic anhydride content was essential in ensuring routine levels of this impurity below 0.10%.



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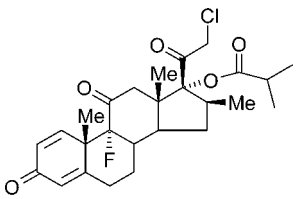
4. Clobetasone-17-isobutyrate. Similar to impurity **1**, the origin again pointed to the butyric anhydride used in the synthesis. Careful control of the isobutyric anhydride content is essential to ensure control of this impurity.



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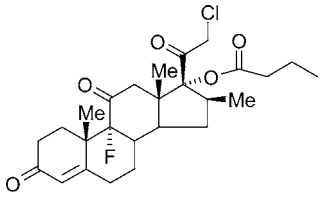
5. 1,2-Dihydroclobetasone-17-butyrate. This is likely to be derived from an impurity in the registered starting material for the synthesis of clobetasone butyrate.



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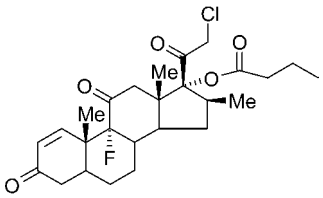
6. 4,5-Dihydroclobetasone-17-butyrate. The analogous impurity to impurity **5** above, this impurity also derives from the starting material used in the synthesis of clobetasone butyrate.



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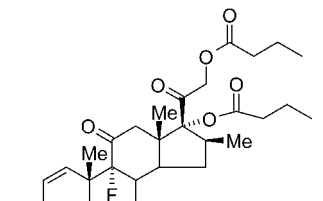
7. Clobetasone-17,21-dibutyrate (11-Dehydrobetamethasone-17,21-dibutyrate). An impurity of synthesis, control of this impurity is achieved purely by control of reagent excess and reaction completion during the clobetasone synthetic pathway.



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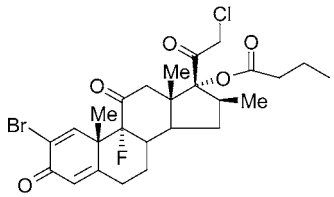
8. 2-Bromo-clobetasone-17-butyrate. This impurity derives from the starting material for the synthesis of clobetasone butyrate.



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Impurity Synthesis. The significant quantities required for these impurities for confirmation of structure, validation, and use as an analytical standard precluded the use of prep-LC. Thus, a synthetic route was required for each of the impurities which could be used to cross-validate the impurity versus the postulated structure and against the impurity seen in the analytical method (allowing for confirmation of retention time, relative response factor, etc.).

Of the impurities thought to derive from analogous impurities in the synthetic starting material, the obvious route would be from that analogue in the starting material itself. However, without a separate exercise in isolation of these impurities, available starting materials for this project were limited.

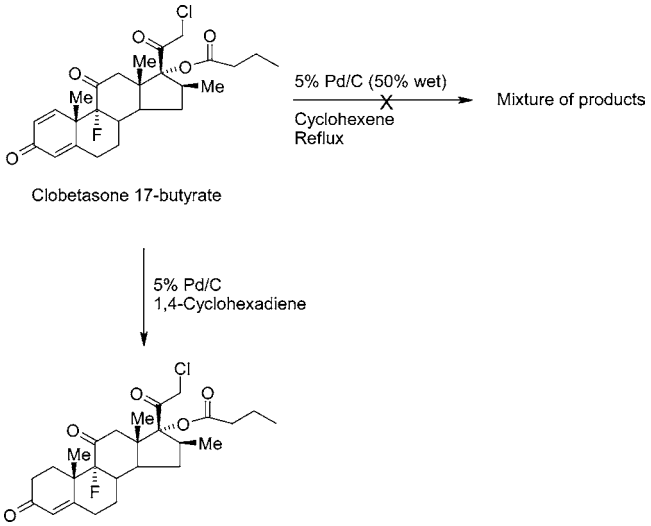
Thus, in the interest of speed, alternative methods were identified to allow routes into impurities **2**, **5**, **6**, and **8**.

Impurity **2** was, of course, the simplest, utilising the readily available 16-α isomer of the fluorohydrin intermediate as starting material, dexamethasone. Manipulation of this impurity through the clobetasone butyrate synthetic route allowed the dexamethasone derivative of clobetasone butyrate to be isolated in good yield and high purity. Due to constraints on disclosure of the relevant intellectual property this impurity will not be discussed further.

Impurity **5**, 1,2-dihydro-clobetasone-17-butyrate, was afforded by known reduction techniques of the dienone A ring of clobetasone butyrate.[6]

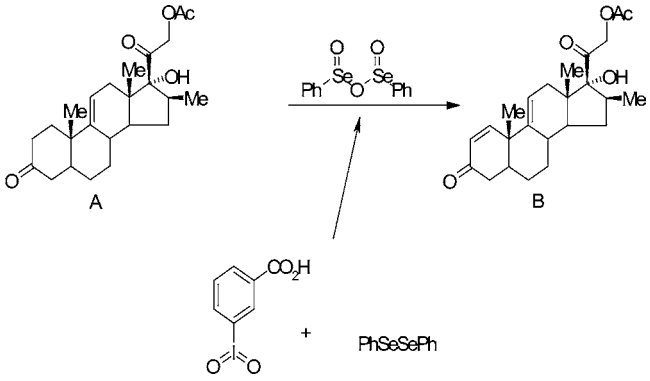
Direct synthesis of this impurity from the drug substance was considered reasonable,[7] as per Scheme 1. However, the attempted selective hydrogenation of clobetasone 17-butyrate using water-wet 5% palladium on charcoal and cyclohexene as a hydrogen source failed. The reaction was very slow, even at reflux, producing more than one product. It was necessary to use dry 5% Pd/C with associated change to the more active 1,4-cyclohexadiene and lowering of the reaction temperature to give better selectivity. The impurity was formed in 85% yield in situ by HPLC analysis (isolated yield after purification 42%).

Scheme 1



The regioisomeric compound, impurity **6**, was less favoured by this reduction approach and was difficult to purify from the reaction mixture. However, an alternative literature approach appeared more selective, although it presented more hazards by the use of an iodoxy compound, which is potentially unstable on heating.[8] Thus, manipulation of a readily available A-ring saturated starting material equivalent, Δ-9-dihydro, **A**, in Scheme 2, using benzene selenenic anhydride (generated in situ from diphenyl diselenide and 3-iodoxy benzoic acid[9]) as the oxidant, gave the analogous impurity, B, as starting material for the clobetasone impurity as a crude mixture (~50% in situ by HPLC).

Scheme 2



Purification of the combined outputs from several reactions was achieved by column chromatography. This material was transformed into the desired impurity using the normal sequence of reactions of clobetasone butyrate with some minor modifications.

In the case of impurity **8**, 2-bromo-clobetasone-17-butyrate, it was known[10] that the forming reaction of the clobetasone starting material, triene, Figure 2, involves the production of a 2,4-dibromide and subsequent elimination of two moles of HBr to insert two double bonds into the molecule (the dienone ring A moiety).

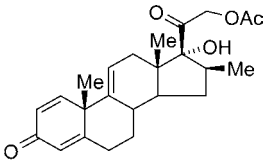
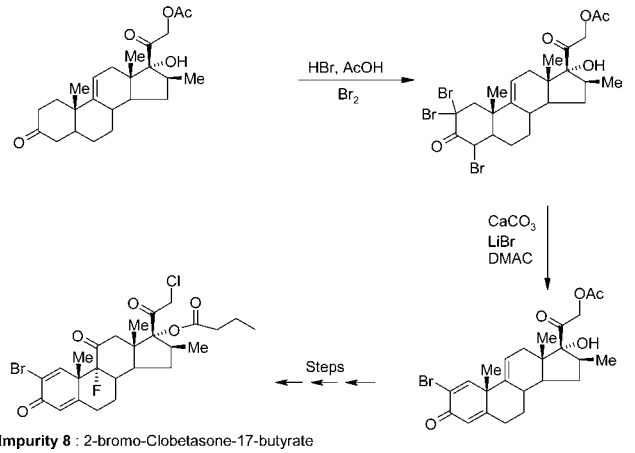


Figure 2. **"Triene".**

If over-bromination occurs, a 2,2,4-tribromide may be produced, elimination of which will yield a 2-bromo analogue, which undergoes all subsequent transformations to give the derivative impurity in clobetasone butyrate (Scheme 3).

Scheme 3



(6) Chen, C. *Tetrahedron* **1958**, *3*, 43.
(7) Angell, R. M.; Biggadike, K.; Farrell, R. M.; Flack, S. S.; Hancock, A. P.; Irving, W. R.; Lynn, S. M.; Procopiou, P. A. *J. Chem. Soc., Perkin Trans. 1*, **2002**, 831−839.
(8) Kazmierczak, P.; Skulski, L.; Kraszkiewicz, L. *Molecules* **2001**, *6*, 881−891.
(9) Barton, D. H. R.; Godfrey, C. R. A.; Morzycki, J. W.; Motherwell, W. B.; Ley, S. V. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1947.
(10) Djerassi, C.; Sholz, C. *J. Am. Chem. Soc.* **1947**, *69*, 2404.

A wide variety of conditions was tried to replicate this chemistry, but optimisation gave only a 10% yield of 2-bromotriene, mainly due to problems with the physical form during workup (poor physical form of impure product). Nonetheless, this provided sufficient amounts of the 2-bromo analogue of triene to convert to the desired clobetasone butyrate impurity by using normal synthetic process.

Impurities controlled by specification of reagents are commonplace within pharmaceutical manufacture. Often control of these impurities at very low levels is essential to ensure that the derivative impurity in API is truly under control. That is similar in the case of impurities **1**, **3**, and **4**, all of which are produced due to low levels of impurities seen in *n*-butyric anhydride. These impurities, which can be present as pure anhydride or mixed anhydride products, are controlled by specification and closely monitored by GC. This does put a specific requirement on the supply and purity of butyric anhydride.

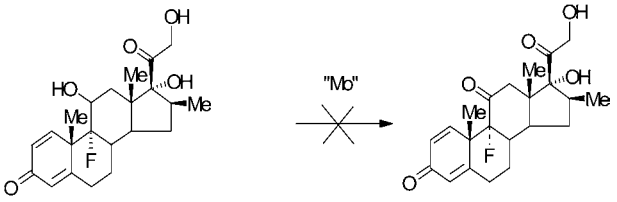
The impurities themselves were relatively easy to produce in significant quantities by use of the derivative anhydride in place of butyric anhydride in the standard synthetic scheme. Thus *n*-propionic anhydride was used for impurity **1**, crotonic anhydride for impurity **3**, and isobutyric anhydride for impurity **4**, respectively.

The remaining impurity is the only one that is truly an impurity of synthesis, directly controlled by the close control of reagent excess in the synthesis. Impurity **7**, clobetasone-17,21-dibutyrate (11-dehydrobetamethasone-17,21-dibutyrate), can be formed due to reaction of reagent excess on a process impurity following incomplete conversion at a preceding stage.

Strict control of the specification of the intermediate is required to control this impurity.

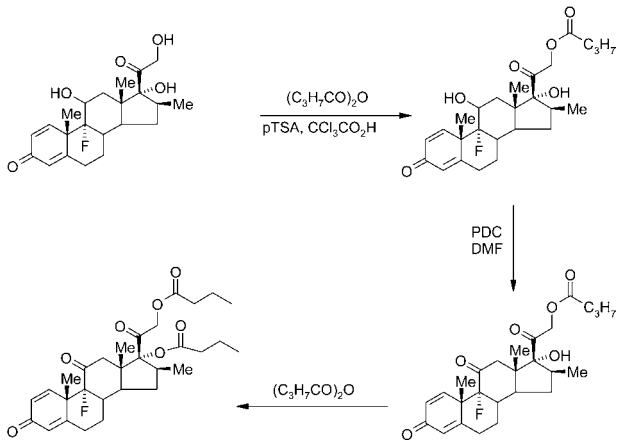
The attempted direct synthesis of this impurity highlighted that an alternative mechanism may actually be more likely than that thought. Thus, direct oxidation of betamethasone followed by butyration at the 17- and 21-hydroxyls proved difficult, as the attempted formation of "11-dehydrobetamethasone" from betamethasone alcohol using a molybdenum reagent[11] was fruitless (Scheme 4).

Scheme 4



An alternative consideration that trans-esterification can take place was supported by the successful synthetic pathway used. Thus, selective butyration of the primary 21-hydroxyl of betamethasone, using one equivalent of butyric anhydride at ambient temperature, gave betamethasone 21-butyrate. This compound was oxidised at the 11-position using pyridinium dichromate as a neutral oxidant. Finally, a second butyration reaction gave the desired impurity as per Scheme 5.

Scheme 5



As a result of the synthesis of these impurities of manufacture, their validation versus postulated structures could be completed in support of the application of the Certificate of the European Pharmacopoeia.

A typical chromatogram of potential impurities is seen in Figure 3.

Experimental Section

Characterisation. Characterisation was, in most cases, achieved by NMR using a Varian Unity Plus 400 MHz instrument and LC−MS using an Agilent HP1100 MSD system. NMR spectra were typically acquired over 64 scans for proton spectra and in excess of 2000 scans for carbon spectra. The samples were dissolved in d_6-DMSO or $CDCl_3$, containing 0.03% v/v TMS in 5-mm NMR tubes. TMS was used as an internal reference standard for the proton experiment. The central lines of the solvents were used as a reference for ^{13}C spectra. All experiments were conducted at 25 °C, and no shift relaxation agents were employed.

LC−MS analysis employed the following conditions: *Gradient method using the following timetable:

column	Luna C18, 50 mm × 2 mm, 3 μm, AH016
mobile phase A*	0.05% (v/v) TFA in H_2O
mobile phase B*	0.05% (v/v) TFA in MeCN
oven temp (°C)	40
flow rate (mL/min)	1.0

time (min)	% B
0.0	0
8.0	95
9.0	95
9.1	0
10.0	0

MSD mode	APCI $^+$ve
scan range (daltons)	100−750

Impurity 1: Synthesis of Clobetasone-17-propionate. Due to constraints on disclosure of the relevant intellectual property, the synthesis of impurity **1** will not be discussed in detail. Its structure was confirmed by ^1H NMR.

(11) Trost, B. M.; Matsuyama, Y. *Isr. J. Chem.* **1984**, *24*, 134.

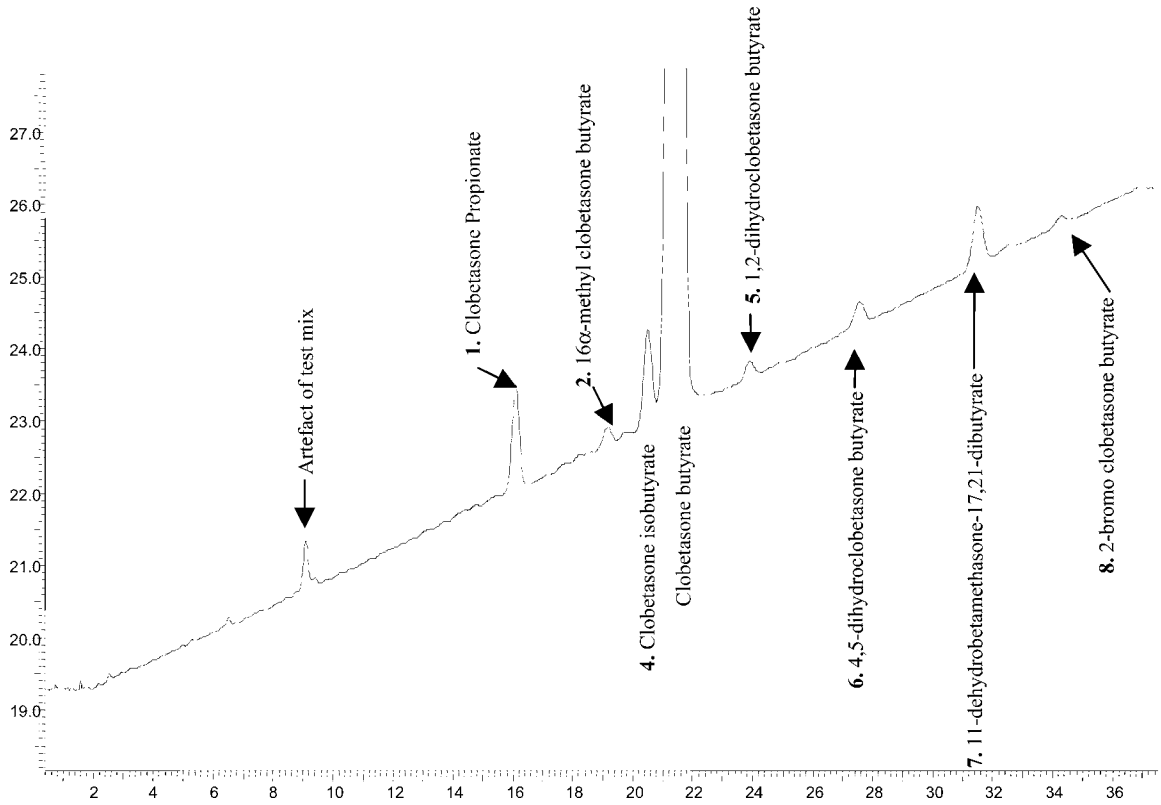


Figure 3. **Chromatogram showing elution order of potential impurities. Impurity 3: clobetasone-17-*trans*-crotonate is not typically seen at levels >0.1% w/w and is therefore not included in the chromatogram above or the test mix for clobetasone butyrate.**

^{1}H NMR (C_2D_6SO, 400 MHz) δ 0.65 (3H, s, 13-C**H**$_3$), 1.05 (3H, d, *J* 7, CH_2C**H**$_3$), 1.15 (1H, m, 15′-**H**), 1.25 (3H, d, *J* 7, 16-C**H**$_3$), 1.5 (4H, s over m, 10-C**H**$_3$, 7′-**H**), 1.9 (1H, m, 14-**H**), 2.0 (1H, m, 6′-**H**), 2.2−2.7 (8H, m, not assigned), 3.35 (1H, m, 12-**H**), 4.15 (1H, d, J_{gem} 14, C**H**′$_2$Cl), 4.35 (1H, d, J_{gem} 14, C**H**″$_2$Cl), 6.1 (1H, s, 4-**H**), 6.2 (1H, d × d, *J* 10,2, 2-**H**), 7.3 (1H, d, *J* 10, 1-**H**).

Pseudomolecular ion $[M + H]^+$ observed at *m*/*z* 465.2, consistent with the theoretical monoisotopic mass of 464.18 Da. Associated M + 2 and M + 3 peaks correspond with the theoretical for chemical formula $C_{25}FClO_5H_{30}$. Fragmentation pattern indicated loss of the propionate group.

Impurity 2: Synthesis of 16α-Methylclobetasone 17-Butyrate. Due to constraints on disclosure of the relevant intellectual property, the synthesis of impurity **2** will not be discussed in detail. Its structure was confirmed by ^{1}H NMR.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.7 (3H, s, 13-C**H**$_3$), 0.9−1.05 (6H, m, CH_2C**H**$_3$ + 16-C**H**$_3$), 1.4 (1H, m, 15′-**H**), 1.55 (3H, s, 10-C**H**$_3$), 1.65−1.9 (5H, m, includes C**H**$_2CH_3$) 2.1−2.5 (5H, m, includes C**H**$_2CH_2CH_3$), 2.55−2.75 (2H, m, unassigned), 3.35 (2H, m, includes 12-**H**), 4.05 (2H, s, C**H**$_2$-Cl), 6.15 (1H, s, 4-**H**), 6.3 (1H, d × d, *J* 10,2, 2-**H**), 7.4 (1H, d, *J* 10, 1-**H**).

Impurity 3: Synthesis of Clobetasone 17-*trans*-Crotonate. Due to constraints on disclosure of the relevant intellectual property, the synthesis of impurity **3** will not be discussed in detail. Its structure was confirmed by ^{1}H, ^{13}C NMR, and MS.

^{1}H NMR (C_2D_6SO, 400 MHz) δ 0.6 (3H, s, 13-C**H**$_3$), 1.05 (1H, m, 15′-**H**), 1.2 (3H, d, *J* 6, 16-C**H**$_3$), 1.45 (4H, s over m, 10-C**H**$_3$, 7′-**H**), 1.8 (1H, m, 14′-**H**) 1.85 (3H, d × d, =CH−C**H**$_3$, *J* 6, 2), 1.95 (1H, m, 6′-**H**), 2.1−2.65 (6H, m, not assigned) 3.3, (1H, m, 12-**H**), 4.05 (1H, d, J_{gem} 15, C**H**′$_2$-Cl), 4.3 (1H, d, J_{gem} 15, C**H**″$_2$Cl), 5.95 (1H, d × d, C**H**=CH−CH_3 *J* 15, 2), 6.1 (1H, s, 4-**H**), 6.15 (1H, d × d, *J* 10, 2, 2-**H**), 6.95 (1H, d × q, *J* 15, 6, CH=C**H**-CH_3), 7.25 (1H, d, *J* 10, 1-**H**).

^{13}C NMR (C_2D_6SO, 100 MHz) δ 16.0, 18.7, 20.0 21.7, 27.4, 30.0, 34.2, 37.6 (d, *J* 20), 43.2, 46.6 (d, *J* 23), 47.5, 47.7, 48.2, 50.8, 93.4 (C−OCO), 100 (d, *J* ∼180 C−F), 122.3 (C=C−CO), 126.2 (alkene), 129.9 (alkene), 148.8 (C=C−CO), 151.9 (alkene), 164.7 (alkene), 166.6 (O−C=O), 185.5 (CO−CH_2Cl), 198.0 (C_3 ketone), 207.0 (d, *J* ∼25, CO−CF).

Pseudomolecular ion $[M + H]^+$ observed at *m*/*z* 477.4, consistent with the theoretical monoisotopic mass of 476.18 Da. Associated M + 2 and M + 3 peaks ratios corresponded with theoretical ratios for chemical formula $C_{26}FClO_5H_{30}$. Fragmentation pattern indicated loss of a butyrate isomer group.

Impurity 4: Synthesis of Clobetasone 17-Isobutyrate. Due to constraints on disclosure of the relevant intellectual property the synthesis of impurity **4** will not be discussed in detail. Its structure was confirmed by ^{1}H NMR and MS.

^{1}H NMR (C_2D_6SO, 400 MHz) δ 0.6 (3H, s, 13-C**H**$_3$), 1.1 (6H, d, *J* 7,2, CH(C**H**$_3$)$_2$), 1.1 (1H, m, 15′-**H**), 1.2 (3H, d, *J* 7, 16-C**H**$_3$), 1.45 (4H, s over m, 10-C**H**$_3$, 7′-**H**), 1.85 (1H, m, 14-**H**), 1.95 (1H, m, 6′-**H**), 2.1−2.6 (6H, m, not assigned) 2.65 (1H, sept, *J* 7, C**H**(CH_3)$_2$), 3.25 (1H, m, 12-**H**), 4.05 (1H, d, J_{gem} 14, C**H**′$_2$Cl), 4.3 (1H, d, J_{gem} 14, C**H**″$_2$Cl), 6.1 (1H, s, 4-**H**), 6.15 (1H, d × d, *J* 10, 2, 2-**H**), 7.25 (1H, d, *J* 10, 1-**H**).

Pseudomolecular ion $[M + H]^+$ observed at *m*/*z* 479.2, consistent with the theoretical monoisotopic mass of 478.19 Da. Associated M + 2 and M + 3 peak ratios correspond with theoretical ratios for chemical formula $C_{26}FClO_5H_{32}$. Fragmentation pattern indicated loss of a butyrate isomer group.

Impurity 5: Synthesis of 1,2-Dihydroclobetasone 17-Butyrate. *1,2-Dihydroclobetasone 17-Butyrate.* 5% Palladium-on-C (5.0 g) was slurried in a mixture of ethanol (210 mL) and 1,4-cyclohexadiene (4.0 mL). Pure clobetasone 17-butyrate (9.58 g, 20 mmol) was added and the solution stirred at room temperature. 1,4-Cyclohexadiene (3 × 2.0 mL) was added periodically over the next 48 h. After 70 h there was no starting material present. The solution was filtered through a Celite bed and washed through with further ethanol (3 × 60 mL). The solvents were removed by rotary evaporation to give the crude product as a colourless oil (~10 g) which was purified by flash column chromatography on silica (EtOAc/hexane, 1:3→1:2→2:3→1:1, eluant) to give the product as a thick foam. This was triturated with hexane (20 mL) and cooled to 4 °C overnight. The solid produced was filtered and dried in a vacuum oven at ambient temperature overnight to give *1,2-dihydroclobetasone 17-butyrate* as a white powder (4.03 g, 42%).

^{1}H NMR (C_2D_6SO, 400 MHz) δ 0.55 (3H, s, 13-C**H**$_3$), 0.85 (3H, t, *J* 7, CH_2C**H**$_3$), 1.1 (1H, m, 15′-**H**), 1.2 (3H, d, *J* 7, 16-C**H**$_3$), 1.4 (3H, s, 10-C**H**$_3$), 1.45−1.6 (3H, m, C**H**$_2$-CH_3, 7′-**H**), 1.7 (1H, m, 14-**H**), 1.9−2.55 (13H, m, not assigned) 3.3 (1H, m, 12-**H**), 4.1 (1H, d, J_{gem} 15, C**H**′$_2$Cl), 4.3 (1H, d, J_{gem} 15, C**H**″$_2$Cl), 5.7 (1H, s, 4-**H**).

Pseudomolecular ion $[M + H]^+$ was observed at *m*/*z* 481.5, consistent with the theoretical monoisotopic mass of 480.21 Da. Associated M + 3 peak ratio corresponded with the theoretical ratio for chemical formula $C_{26}FClO_5H_{34}$.

Impurity 6: Synthesis of 4,5-Dihydroclobetasone 17-Butyrate. *4,5-Dihydrotriene (1,9-Diene): Method A.* Δ^{9-}Dihydro (15 g, 37.3 mmol) was dissolved in a mixture of acetic acid (220 mL) and ethyl acetate (180 mL). The solution was cooled to −1 °C. HBr in acetic acid (~1 mL) was added, followed by a solution of bromine (2 mL, 39 mmol) in acetic acid (30 mL) over ca. 5 min. The resultant solution was stirred at −1 °C for 80 min. The reaction mixture was added dropwise (over ca. 20 min) to rapidly stirred water (400 mL) before further water (800 mL) was added. The white solids formed were filtered, washed with water until the pH of the washings approached neutrality and dried for 1 h.

This crude monobromide was dissolved in DMAC (300 mL). Calofort U (calcium carbonate) (13.9 g) and lithium bromide (9.0 g) were added, and the resultant mixture was heated to 110 °C overnight. After cooling, the reaction mixture was added slowly to a solution of concentrated HCl (47 mL) in water (240 mL). More water (500 mL) was added, and the resultant precipitate was filtered and washed with water until the pH of the washings approached neutrality. This material was dried in a vacuum oven overnight to give the crude product which contained ~40% *4,5-dihydrotriene* by HPLC.

4,5-Dihydrotriene (1,9-Diene): Method B. 3-Iodylbenzoic acid (prepared by a literature method[8]) (9.97 g, 36 mmol, 1.7 equiv) and diphenyl diselenide (1.31 g, 4.2 mmol, 0.2 equiv) were dissolved in toluene (421 mL). The solution was stirred at 75 °C overnight, under a blanket of nitrogen. A solution of Δ^9-dihydro (8.41 g, 20.9 mmol) in toluene (100 mL) was added and the mixture heated to ~97 °C for 72 h. The resultant mixture was cooled, and the white solid produced was filtered off. The filtrate was washed with saturated sodium bicarbonate solution (3 × 210 mL) and saturated brine (210 mL). The solution was dried over magnesium sulfate and filtered, and the solvents were evaporated to give a thick brown oil. This was triturated with hexane in a refrigerator, and the solid produced was filtered and dried to give crude *4,5-dihydrotriene* as an orange powder (10.6 g) containing ~50% of the desired product.

A pure sample of *4,5-dihydrotriene* was obtained by flash column chromatography on silica (EtOAc:hexane, 3:7, eluant). Approximately 17 g of crude material yielded pure *4,5-dihydrotriene* as a yellow, crystalline solid (2.91 g).

Due to constraints on disclosure of the relevant intellectual property the remainder of the synthesis of impurity **6** will not be discussed in detail. Its structure was confirmed by ^{1}H NMR and MS.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.7 (3H, s, 13-C**H**$_3$), 1.0 (3H, t, *J* 7, CH_2C**H**$_3$), 1.2 (1H, m, 15′-**H**), 1.3 (3H, s, 10-C**H**$_3$), 1.35 (3H, d, *J* 7, 16-C**H**$_3$), 1.5−2.7 (16H, many peaks, not assigned), 3.5 (1H, m, 12′-**H**), 3.95 (2H, s, C**H**$_2$Cl), 5.9 (1H, d, *J* 10, 2-**H**), 7.3 (1H, d, *J* 10, 1-**H**).

Pseudomolecular ion $[M + H]^+$ observed at *m*/*z* 481.5, consistent with the theoretical monoisotopic mass of 480.21 Da. Associated M + 3 peak ratio of corresponded with the theoretical ratio for chemical formula $C_{26}FClO_5H_{34}$.

Impurity 7: Synthesis of Clobetasone 17,21-Dibutyrate (11-Dehydrobetamethasone 17,21-Dibutyrate). *Betamethasone 21-Butyrate.* Betamethasone alcohol (11.75 g, 30 mmol) was dissolved in DCM (100 mL). Dried pTSA (950 mg, 5 mmol) and trichloroacetic acid (8.2 g, 50 mmol, 1.6 equiv) were added, followed by butyric anhydride (4.9 mL, 30 mmol, 1 equiv), and the reaction was stirred at room-temperature overnight. Sodium bicarbonate solution (6 g in 80 mL water) was added and the reaction left to stir for 10 min. The organic layer was separated and washed with more bicarbonate solution (11 g in 120 mL water). The aqueous layers were extracted with DCM (50 mL), and the combined organic layers were washed with water (2 × 70 mL). These aqueous washings were extracted with more DCM (50 mL), and the combined organic layers were distilled to a residual volume of 30 mL under atmospheric pressure. This solution was cooled to room temperature, methanol (75 mL) was added, and the reaction mixture was distilled to a residual volume of 45 mL The solution was cooled to room temperature, and methanol (75 mL) was added. Charcoal (0.75 g) was added, the mixture was refluxed under nitrogen for 45 min and filtered through Celite and left to stir overnight at 0 °C. The resulting precipitate was filtered, washed with chilled methanol (~6 mL), and dried in an air oven for 24 h at 40 °C to give the crude product as a white

powder. This was purified by flash column chromatography on silica (3% MeOH in DCM, eluant) to give *betamethasone 21-butyrate* as a white powder (6.77 g, 49%). The structure was confirmed by NMR.

11-Dehydrobetamethasone 21-Butyrate. Betamethasone 21-butyrate (5.02 g, 10.85 mmol) was dissolved in dry DMF (65 mL) and the solution cooled to 0 °C under a nitrogen atmosphere. Pyridinium dichromate (2.60 g, 6.9 mmol) was added, and the mixture was stirred at 0 °C overnight. Water (200 mL) was added dropwise over ca. 15 min, and the resultant mixture was left to stir at room temperature for 1 h. The precipitate was filtered, washed with water (4 × 100 mL), and dried in an air oven at 40 °C overnight to give *11-dehydrobetamethasone 21-butyrate* as a white powder (3.58 g, 71%). The structure was confirmed by NMR.

11-Dehydrobetamethasone 17,21-Dibutyrate. 11-Dehydrobetamethasone 21-butyrate (4.02 g, 8.7 mmol) was dissolved in DCM (55 mL). Dried pTSA (0.95 g, 5 mmol), trichloroacetic acid (8.16 g, 50 mmol), and *n*-butyric anhydride (1.5 mL, 9.2 mmol) were added, and the mixture was heated to reflux overnight. The solution was cooled to ambient temperature, and sodium bicarbonate solution (6 g in 70 mL water) was added. The reaction was then left to stir for 10 min. The organic layer was separated and washed with more bicarbonate solution (2 × 1.5 g in 35 mL water). The aqueous layers were extracted with DCM (25 mL), and the combined organic layers were washed with water (2 × 35 mL) and saturated brine (30 mL). The solution was distilled to a residual volume of 10 mL on the rotary evaporator, and methanol (25 mL) was added. The solvents were removed by rotary evaporation, and the solid produced was washed on a filter with ice-cold methanol (5 mL) and dried in a vacuum oven overnight at 40 °C to give *11-dehydrobetamethasone 17,21-dibutyrate* as a white powder (2.33 g, 50%).

^{1}H NMR (C_2D_6SO, 400 MHz) δ 0.6 (3H, s, 13-C**H**$_3$), 0.85 (6H, 2 × t, 2 × CH_2C**H**$_3$), 1.1 (1H, m, 15′-**H**), 1.15 (3H, d, *J* 7, 16-C**H**$_3$), 1.45 (3H, s, 10-C**H**$_3$), 1.45 (1H, m, unassigned), 1.6 (4H, m, 2 × C**H**$_2$CH$_3$) 1.85 (1H, m, 14-**H**), 1.9 (1H, m, 6′-**H**), 2.2−2.65 (10H, m, unassigned), 3.3 (1H, m, 12-**H**), 4.3 (1H, d, J_{gem} 16, C**H**′$_2$O), 4.7 (1H, d, J_{gem} 16, C**H**″$_2$O), 6.05 (1H, s, 4-**H**), 6.15 (d × d, *J* 10,2, 2-**H**), 7.25 (d, *J* 10, 1-**H**).

^{13}C NMR (C_2D_6SO, 100 MHz) δ 14.0, 14.1, 15.0, 18.1, 18.6, 19.6, 21.8 (d, *J* 5), 27.5, 31.0, 34.2, 35.5, 36.5, 37.6 (d, *J* ∼20), 43.0, 46.6 (d, *J* ∼25), 47.0, 47.7, 51.3, 68.3 (CH_2O), 93.2 (17-C), 100.0 (d, *J* ∼180), 126.2 (alkene), 129.9 (alkene), 151.9 (alkene), 164.7 (alkene), 173.1, 174.4 (2 × $OCOC_3H_7$), 185.5 (21-C), 199.2 (3-C), 205.2 (d, *J* ∼25, 11-C).

Pseudomolecular ion $[M + H]^+$ observed at *m*/*z* 531.3, consistent with the theoretical monoisotopic mass of 530.26 Da. Associated M + 2 and M + 3 peaks ratios corresponded with the theoretical ratios for the chemical formula $C_{30}FO_7H_{39}$.

Impurity 8: Synthesis of 2-Bromoclobetasone 17-Butyrate. *2-Bromotriene.* Δ^9-Dihydro (30 g, 74.5 mmol) was dissolved in a mixture of acetic acid (450 mL) and ethyl acetate (360 mL). The solution was cooled to −1 °C, and HBr in acetic acid (∼12 mL) was added. Bromine (11.9 mL, 230 mmol, 3.09 mol equiv) in acetic acid (30 mL) was then added over ca. 10 min at an increasing rate. The reaction was then left to stir at −1 °C for 80 min. The reaction mixture was added to water (2000 mL at 38 °C) with vigorous stirring, at an increasing rate, over ca. 20 min. More water (800 mL at 37 °C) was added over 5 min. This resulted in the formation of crude Δ^9-tribromide as an off-white, sticky gum. The crude Δ^{9-}tribromide was scraped off of the beaker, baffle, stirrer, etc., washed with water (until the pHs of the washings were approximately pH 5), and finally dried for ∼1 h under suction (∼24 g).

This material was dissolved in ethyl acetate (225 mL), Celite (1.5 g) was added, and the reaction mixture was left to stir for 5 min. The mixture was then filtered through Celite and added to a separatory funnel that contained a sodium acetate solution (2 g in 23 mL of water). The funnel was shaken well and left to settle for 5 min. The aqueous layer was run off into a second funnel, which contained ethyl acetate (40 mL). The resulting aqueous layer from the second funnel was run off and discarded, and the organic layer was added to the first separating funnel. This washing procedure was repeated using sodium bicarbonate solution (6 g in 75 mL) and finally sodium chloride solution (0.35 g in 27 mL of water). The organic layers were combined and the solvents removed under vacuum (keeping the temperature below 35 °C) to yield a thick, yellow paste (∼21 g) which was stored in the freezer overnight.

The crude Δ^9-tribromide (∼21 g) was added to DMAC (130 mL) and left to stir for 5 min. Calcium carbonate (Calfort U, 11 g, 110 mmol) and lithium bromide (8.59 g, 99 mmol) were added, and the reaction was heated at 109 °C (not lower than 105 °C) for 16 h. The mixture was cooled to 25 °C and added slowly to a solution of concentrated HCl (43 mL) in water (480 mL). Water (375 mL) was then added to the solution, and a brown precipitate formed. This was filtered, washed with water until the pH of the washings were approximately pH 5, and sucked as dry as possible under vacuum to give crude 2-bromotriene as a brown powder (∼10.1 g, ∼28% weight recovery). This was 21% 2-bromotriene by LC. The crude 2-bromotriene was purified by flash column chromatography on silica (EtOAC:hexane, 1:1, eluant) to give *2-bromotriene* as a yellow powder (1.34 g, 4% overall yield); ^{1}H NMR (C_2D_6SO, 400 MHz) δ 0.7−2.8 (22H, unassigned), 4.9 (1H, d, J_{gem} 16, CH_2O), 5.0 (1H, d, J_{gem} 16, CH_2O), 5.5 (1H, s, 17-OH), 5.65 (1H, m, 11-H) 6.2 (1H, s, 4-H), 8.05 (1H, s, 1-H).

Due to constraints on disclosure of the relevant intellectual property the remainder of the synthesis of impurity **8** will not be discussed in detail. Its structure was confirmed by NMR and MS.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.75 (3H, s, 13-C**H**$_3$), 0.95 (3H, t, *J* 7, CH_2C**H**$_3$), 1.25 (1H, m, 15′-**H**), 1.4 (3H, d, *J* 7, 16-C**H**$_3$), 1.6 (3H, s, 10-C**H**$_3$), 1.65−1.75 (3H, m, C**H**$_2$CH$_3$, 7′-H), 1.95 (1H, m, 14-**H**), 2.05 (1H, m, 6′-**H**), 2.15−2.65 (8H, m, not assigned), 3.45 (1H, m, 12-**H**), 3.95 (2H, s, C**H**$_2$-Cl), 6.25 (1H, s, 4-**H**), 7.8 (1H, s, 1-**H**).

Pseudomolecular ion $[M + H]^+$ observed at *m*/*z* 557.0, consistent with the theoretical monoisotopic mass of 556.10 Da. Relative ratios of M + 1 and M + 3 peaks corresponded with the theoretical ratios for chemical formula $C_{26}FBrClO_5H_{31}$.

Analytical Methodology. The impurities synthesised were used to validate the liquid chromatography method below. The validation performed was carried out in line with the International Conference on Harmonisation (ICH) requirements.

		%A	%B
analytical column	15 cm x 4.6 mm (i.d.) packed with Xterra RP18 (or equivalent), 3.5 μm		
flow rate	1.5 mL/min		
temperature	40 °C		
detection	UV at 241 nm		
injection volume	10 μL		
run time	approximately 40 min		
gradient	T0	100	0
	T35	0	100
	T36	100	0
	T40	100	0
	A = 60:40 water:acetonitrile, 0.1% formic acid		
	B = 50:50 water:acetonitrile, 0.1% formic acid		

Summary

The moving regulatory requirements in the pharmaceutical industry do not focus only on new chemical entities, but are especially relevant to established therapies and their active ingredients. Given that millions of doses of these active materials have been administered over the past decades it is as important to understand and show control over the impurities of synthesis. In summary, the requirements to synthesise and confirm the structures of proposed impurities of synthesis of clobetasone butyrate have been fulfilled.

Acknowledgment

We thank the wider Aladdin project team within GSK for their help and the Montrose site steering committee for the guidance in providing a robust regulatory package in support of the CEP application.

Received for review August 27, 2003.

OP0341216