Reference solution (b). Dissolve 5 mg of *chlordiazepoxide CRS* and 5 mg of *clonazepam CRS* in the mobile phase and dilute to 50 mL with the mobile phase. Dilute 1 mL of the solution to 100 mL with the mobile phase.

Reference solution (c). Dilute 1.0 mL of the test solution to 200.0 mL with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *acetonitrile R, water R* (40:60 *V/V*).

Flow rate: 1 mL/min.

Detection: spectrophotometer at 230 nm.

Injection: 20 µL.

Run time: 5 times the retention time of clobazam.

Retention time: clobazam = about 15 min.

System suitability: reference solution (b):

– *resolution*: minimum 1.3 between the peaks due to chlordiazepoxide and clonazepam.

Limits:

– *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

– *any other impurity*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent),

– *total of other impurities*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent),

– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

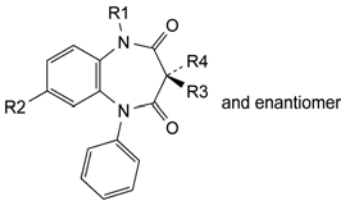
Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Dissolve 50.0 mg in *alcohol R* and dilute to 100.0 mL with the same solvent. Dilute 2.0 mL of the solution to 250.0 mL with *alcohol R*. Measure the absorbance (*2.2.25*) at the maximum at 232 nm.

Calculate the content of $C_{16}H_{13}ClN_2O_2$ taking the specific absorbance to be 1380.

IMPURITIES

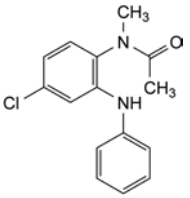


A. R1 = R3 = R4 = H, R2 = Cl: 7-chloro-5-phenyl-1,5-dihydro-3*H*-1,5-benzodiazepine-2,4-dione,

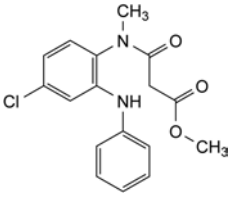
B. R1 = CH_3, R2 = R3 = R4 = H: 1-methyl-5-phenyl-1,5-dihydro-3*H*-1,5-benzodiazepine-2,4-dione,

C. R1 = R3 = CH_3, R2 = Cl, R4 = H: (3*RS*)-7-chloro-1,3-dimethyl-5-phenyl-1,5-dihydro-3*H*-1,5-benzodiazepine-2,4-dione,

D. R1 = R3 = R4 = CH_3, R2 = Cl: 7-chloro-1,3,3-trimethyl-5-phenyl-1,5-dihydro-3*H*-1,5-benzodiazepine-2,4-dione,



E. *N*-[4-chloro-2-(phenylamino)phenyl]-*N*-methylacetamide,

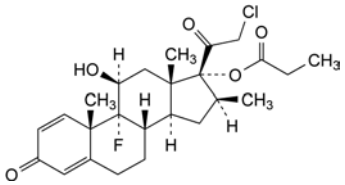


F. methyl 3-[[4-chloro-2-(phenylamino)phenyl]methylamino]-3-oxopropanoate.

01/2008:2127
corrected 6.0

CLOBETASOL PROPIONATE

Clobetasoli propionas



$C_{25}H_{32}ClFO_5$ M_r 467.0
[25122-46-7]

DEFINITION

21-Chloro-9-fluoro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate.

Content: 97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, freely soluble in acetone, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *clobetasol propionate CRS*.

TESTS

Specific optical rotation (*2.2.7*): + 112 to + 118 (dried substance).

Dissolve 0.500 g in *acetone R* and dilute to 50.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution (a). Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase.

Test solution (b). Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a). Dissolve 20.0 mg of *clobetasol propionate CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (b). Dissolve the contents of a vial of *clobetasol impurity J CRS* in 2.0 mL of the mobile phase. To 0.5 mL of this solution add 0.5 mL of test solution (b) and dilute to 20.0 mL with the mobile phase.

Reference solution (c). Dissolve the contents of a vial of *clobetasol for peak identification CRS* (containing impurities A, B, C, D, E, L and M) in 2 mL of the mobile phase.

Reference solution (d). Dilute 1.0 mL of test solution (a) to 50.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 20.0 mL with the mobile phase.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;

– *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm);

– *temperature*: 30 °C.

Mobile phase: mix 10 volumes of *methanol R*, 42.5 volumes of a 7.85 g/L solution of *sodium dihydrogen phosphate monohydrate R* adjusted to pH 5.5 with a 100 g/L solution of *sodium hydroxide R* and 47.5 volumes of *acetonitrile R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 240 nm.

Injection: 10 µL of test solution (a) and reference solutions (b), (c) and (d).

Run time: 3 times the retention time of clobetasol propionate.

Identification of impurities: use the chromatogram supplied with *clobetasol for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, D, E, L and M.

Relative retention with reference to clobetasol propionate (retention time = about 10 min): impurity A = about 0.4; impurity B = about 0.6; impurity C = about 0.9; impurity J = about 1.1; impurity D = about 1.2; impurity L = about 1.3; impurity M = about 1.6; impurity E = about 2.1.

System suitability:

– *resolution*: minimum 2.0 between the peaks due to clobetasol propionate and impurity J in the chromatogram obtained with reference solution (b);

– the chromatogram obtained with reference solution (c) is similar to the chromatogram supplied with *clobetasol for peak identification CRS*.

Limits:

– *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.6; impurity C = 1.5;

– *impurity E*: not more than 1.4 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.7 per cent);

– *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent);

– *impurities B, C*: for each impurity, not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.3 per cent);

– *impurities A, L, M*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.2 per cent);

– *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.10 per cent);

– *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (d) (2.0 per cent);

– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution (b) and reference solution (a).

Calculate the percentage content of $C_{25}H_{32}ClFO_5$ using the chromatogram obtained with reference solution (a) and the declared content of *clobetasol propionate CRS*.

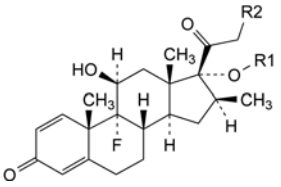
STORAGE

Protected from light.

IMPURITIES

Specified impurities: A, B, C, D, E, L, M.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *F, G, H, I, J, K.*



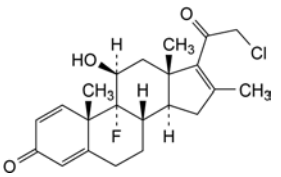
A. R1 = CO-C_2H_5, R2 = OH: 9-fluoro-11β,21-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate (betamethasone 17-propionate),

G. R1 = H, R2 = Cl: 21-chloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione (clobetasol),

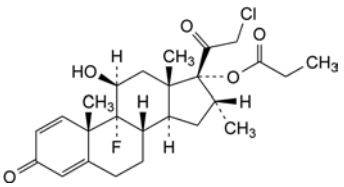
H. R1 = CO-C_2H_5, R2 = H: 9-fluoro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate,

I. R1 = CO-C_2H_5, R2 = O-SO_2-CH_3: 9-fluoro-11β-hydroxy-16β-methyl-21-[(methylsulfonyl)oxy]-3,20-dioxopregna-1,4-dien-17-yl propanoate,

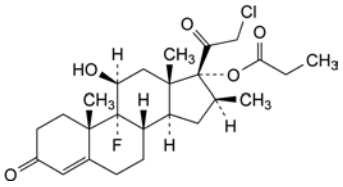
K. R1 = H, R2 = O-CO-C_2H_5: 9-fluoro-11β,17-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-21-yl propanoate (betamethasone 21-propionate),



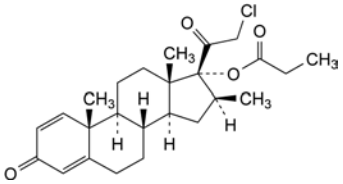
B. 21-chloro-9-fluoro-11β-hydroxy-16-methylpregna-1,4,16-triene-3,20-dione,



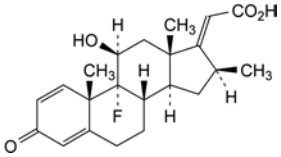
C. 21-chloro-9-fluoro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate,



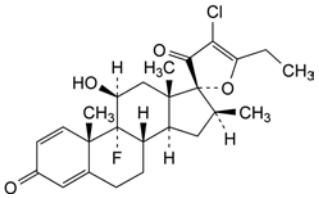
D. 21-chloro-9-fluoro-11β-hydroxy-16β-methyl-3,20-dioxopregn-4-en-17-yl propanoate (1,2-dihydroclobetasol 17-propionate),



E. 21-chloro-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate,



F. 9-fluoro-11β-hydroxy-16β-methyl-3-oxopregna-1,4,17(20)-trien-21-oic acid,



J. (17*R*)-4′-chloro-5′-ethyl-9-fluoro-11β-hydroxy-16β-methylspiro[androsta-1,4-diene-17,2′(3′*H*)-furan]-3,3′-dione (17α-spiro compound),

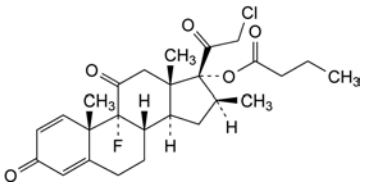
L. unknown structure,

M. unknown structure.

01/2010:1090
corrected 6.7

CLOBETASONE BUTYRATE

Clobetasoni butyras



$C_{26}H_{32}ClFO_5$ M_r 479.0
[25122-57-0]

DEFINITION

21-Chloro-9-fluoro-16β-methyl-3,11,20-trioxopregna-1,4-dien-17-yl butanoate.

Content: 97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: practically insoluble in water, freely soluble in acetone and in methylene chloride, slightly soluble in ethanol (96 per cent).

mp: about 178 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *clobetasone butyrate CRS*.

TESTS

Specific optical rotation (*2.2.7*): + 131 to + 138 (dried substance).

Dissolve 0.250 g in *ethanol R1* and dilute to 25.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture: *anhydrous formic acid R, acetonitrile R, water R* (0.1:43:57 *V/V/V*).

Test solution. Dissolve 65 mg of the substance to be examined in 5.0 mL of *acetonitrile R* and dilute to 25.0 mL with the solvent mixture.

Reference solution (a). Dissolve 13 mg of *clobetasone butyrate for system suitability CRS* (containing impurity F) in 1 mL of *acetonitrile R* and dilute to 5.0 mL with the solvent mixture.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3.5 μm);
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A*: *anhydrous formic acid R, water R* (0.1:99.9 *V/V*);
- *mobile phase B*: *anhydrous formic acid R, acetonitrile R* (0.1:99.9 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	57	43
3 - 26	57 → 43	43 → 57

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 241 nm.

Injection: 10 μL.

Identification of impurities: use the chromatogram supplied with *clobetasone butyrate for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity F.

Relative retention with reference to clobetasone butyrate (retention time = about 14 min): impurity F = about 0.9.

System suitability:

- *resolution*: minimum 3.5 between the peaks due to impurity F and clobetasone butyrate in the chromatogram obtained with reference solution (a);
- *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.