Time (min)	Mobile Phase A (per cent *V/V*)	Mobile Phase B (per cent *V/V*)
0 - 30	64 → 44	36 → 56
30 - 35	44 → 0	56 → 100
35 - 45	0	100
45 - 50	0 → 64	100 → 36
50 - 60	64	36

Flow rate: 1.2 ml/min.

Detection: spectrophotometer at 214 nm.

Injection: 100 µl of test solution (a), reference solutions (a) and (b).

System suitability: reference solution (b):

– *retention time*: molgramostin = about 22 min,
– *repeatability*: maximum relative standard deviation of 5.0 per cent after 4 injections,
– *resolution*: minimum 2 between the peaks due to albumin and molgramostim.

Limits:

– *any impurity*: for each impurity, maximum 1.5 per cent,
– *total of impurities eluting between 5 min and 30 min*: maximum 4 per cent.

Bacterial endotoxins (*2.6.14*): less than 5 IU in the volume that contains 1.0 mg of protein.

ASSAY

Protein. Liquid chromatography (*2.2.29*) as described in the test for related proteins.

Injection: 150 µl of test solution (b) and reference solution (b).

Calculate the content of molgramostim using the declared content of molgramostim in *molgramostim CRS*.

Potency. Determination of the biological activity of molgramostim concentrated solution based on the stimulation of proliferation of TF-1 cells by molgramostim.

The following method uses the conversion of tetrazolium bromide (MTT) as a staining method. Validated alternative stains such as Almar blue have also been found suitable.

TF-1 cells are incubated with varying dilutions of test and reference preparations of molgramostim. They are then incubated with a solution of MTT. This cytochemical stain is converted by cellular dehydrogenases to a purple formazan product. The formazan is then measured spectrophotometrically. The potency of the preparation to be examined is determined by comparison of the dilutions of the test preparation with the dilutions of the appropriate International Standard of molgramostim or with a reference preparation calibrated in International Units, which yield the same response (50 per cent maximal stimulation).

The International Unit is the activity contained in a stated amount of the appropriate International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Add 50 µl of dilution medium to all wells of a 96-well microtitre plate. Add an additional 50 µl of this solution to the wells designed for the blanks. Add 50 µl of each solution to be tested in triplicate (test preparation and reference preparation at a concentration of about 65 IU/ml, plus a series of 10 twofold dilutions to obtain a standard curve). Then add to each well 50 µl of a TF-1 cell suspension containing 3×10^5 cells per millilitre, maintaining the cells in a uniform suspension during addition.

Incubate the plate at 36.0-38.0 °C for a minimum of 24 h in a humidified incubator using 6 ± 1 per cent CO_2. Add 25 µl of a 5.0 g/l sterile solution of *tetrazolium bromide R* to each well. Reincubate for 5 h. Remove the plates from the incubator and add to each well 100 µl of a 240 g/l solution of *sodium dodecyl sulphate R* previously adjusted to pH 2.7 with hydrochloric acid. Reincubate overnight.

Determine the relative quantity of purple formazan product formed in each well by measuring the absorbance (*2.2.25*) using a 96-well microtitre plate reader. Read each plate at 570 nm and at 690 nm. Subtract the reading at 690 nm from the reading at 570 nm. Analyse the data by fitting a sigmoidal dose-response curve to the data obtained and by using a suitable statistical method, for example the 4-parameter model (see *5.3*).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits (P = 0.95) of the estimated potency are not less than 74 per cent and not more than 136 per cent of the stated potency.

STORAGE

In an airtight container, protected from light, at a temperature below – 65 °C.

LABELLING

The label states:

– the content, in milligrams of protein per millilitre,
– the potency, in International Units per milligram of protein.

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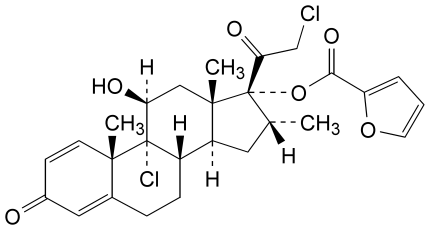
corrected 6.0

MOMETASONE FUROATE

Mometasoni furoas



$C_{27}H_{30}Cl_2O_6$ M_r 521.4
[83919-23-7]

DEFINITION

9,21-Dichloro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate.

Content: 97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white powder.

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

mp: about 220 °C, with decomposition.

IDENTIFICATION

First identification: A, B.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs.

Comparison: *mometasone furoate CRS*.

B. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Reference solution (a). Dissolve 20 mg of *mometasone furoate CRS* in *methylene chloride R* and dilute to 20 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of *anhydrous beclometasone dipropionate CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate: *TLC silica gel F_{254} plate R*.

Mobile phase: add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application: 5 µl.

Development: over a path of 15 cm.

Drying: in air.

Detection A: examine in ultraviolet light at 254 nm.

Results A: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B: spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool; examine in daylight and in ultraviolet light at 365 nm.

Results B: the principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability: reference solution (b):

– the chromatogram shows 2 spots which, when examined in ultraviolet light at 365 nm, may not be completely separated.

C. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 15 min a light yellow colour develops. When examined in ultraviolet light at 365 nm, no fluorescence is seen. Add this solution to 10 ml of *water R* and mix. The colour fades and there is no fluorescence.

D. Mix 80 mg with 0.30 g of *anhydrous sodium carbonate R* and ignite in a crucible until an almost white residue is obtained. Allow to cool and dissolve the residue in 5 ml of *dilute nitric acid R*; filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*): + 50 to + 55 (dried substance).

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 10.0 ml with the same solvent.

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

Solvent mixture. Mix 50 ml of *acetonitrile R* and 50 ml of *water R*, then add 0.1 ml of *acetic acid R*.

Test solution. Dissolve 20.0 mg of the substance to be examined in 4.0 ml of *acetonitrile R* and dilute to 20.0 ml with the solvent mixture.

Reference solution (a). Dissolve 2 mg of *mometasone furoate CRS* and 6 mg of *anhydrous beclometasone dipropionate CRS* in the solvent mixture, then dilute to 10.0 ml with the solvent mixture. Dilute 0.25 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the test solution to 20.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

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

Mobile phase: *acetonitrile R, water R* (50:50 *V/V*).

Flow rate: 1 ml/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µl.

Run time: twice the retention time of mometasone furoate.

Retention time: mometasone furoate = about 17 min; beclometasone dipropionate = about 22 min.

System suitability: reference solution (a):

– *resolution*: minimum 6 between the peaks due to mometasone furoate and beclometasone dipropionate; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

– *impurities A, B, C, D, E, F, G, H, I*: for each impurity, not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

– *total*: not more than 1.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent);

– *disregard limit*: 0.1 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.

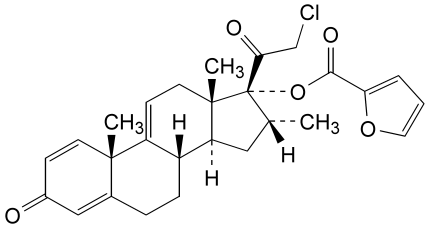
ASSAY

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 249 nm.

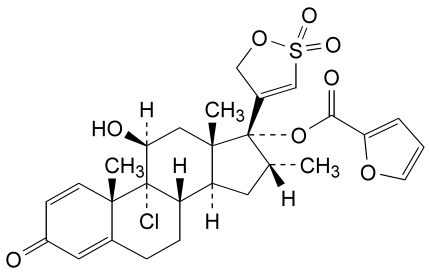
Calculate the content of $C_{27}H_{30}Cl_2O_6$ taking the specific absorbance to be 481.

IMPURITIES

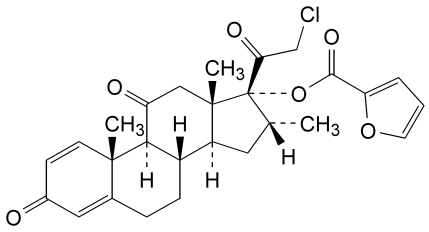
Specified impurities: *A, B, C, D, E, F, G, H, I*.



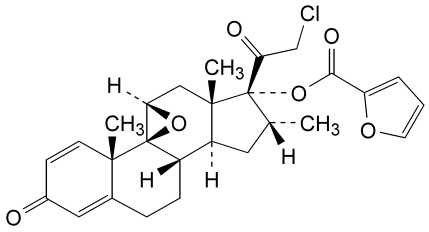
A. 21-chloro-16α-methyl-3,20-dioxopregna-1,4,9(11)-trien-17-yl furan-2-carboxylate,



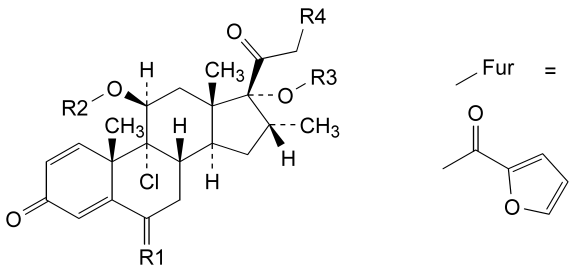
B. 4-[9-chloro-17-[(furan-2-ylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17β-yl]-5*H*-1,2-oxathiole 2,2-dioxide,



C. 21-chloro-16α-methyl-3,11,20-trioxopregna-1,4-dien-17-yl furan-2-carboxylate,



D. 21-chloro-9,11β-epoxy-16α-methyl-3,20-dioxo-9β-pregna-1,4-dien-17-yl furan-2-carboxylate,

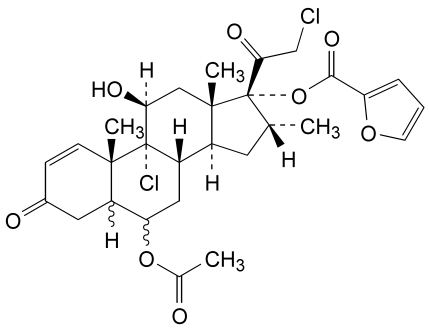


E. R1 = H_2, R2 = R3 = Fur, R4 = Cl: 9,21-dichloro-16α-methyl-3,20-dioxopregna-1,4-diene-11β,17-diyl bis(furan-2-carboxylate),

F. R1 = O, R2 = H, R3 = Fur, R4 = Cl: 9,21-dichloro-11β-hydroxy-16α-methyl-3,6,20-trioxopregna-1,4-dien-17-yl furan-2-carboxylate,

G. R1 = H_2, R2 = R3 = H, R4 = Cl: 9,21-dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione (mometasone),

H. R1 = H_2, R2 = H, R3 = Fur, R4 = OH: 9-chloro-11β,21-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate,

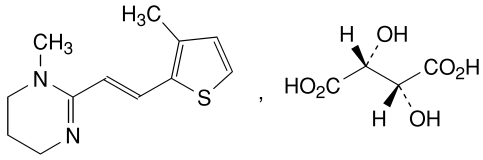


I. 9,21-dichloro-11β-hydroxy-16α-methyl-3,20-dioxo-5ξ-pregn-1-ene-6ξ,17-diyl 6-acetate 17-(furan-2-carboxylate).

01/2008:1546
corrected 6.0

MORANTEL HYDROGEN TARTRATE FOR VETERINARY USE

Moranteli hydrogenotartras ad usum veterinarium



$C_{16}H_{22}N_2O_6S$ M_r 370.4
[26155-31-7]

DEFINITION

1-Methyl-2-[(*E*)-2-(3-methylthiophen-2-yl)ethenyl]-1,4,5,6-tetrahydropyrimidine hydrogen tartrate.

Content: 98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance: white or pale yellow, crystalline powder.

Solubility: very soluble in water and in ethanol (96 per cent), practically insoluble in ethyl acetate.

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 167 °C to 172 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *morantel hydrogen tartrate CRS.*

C. Dissolve about 10 mg in 1 ml of a 5 g/l solution of *ammonium vanadate R*. Evaporate to dryness. Add 0.1 ml of *sulphuric acid R*. A purple colour is produced.

D. Dissolve about 10 mg in 1 ml of *0.1 M sodium hydroxide*. Transfer to a separating funnel and shake with 5 ml of *methylene chloride R*. Discard the organic layer. Neutralise the aqueous layer with a few drops of *dilute hydrochloric acid R*. The solution gives reaction (b) of tartrates (*2.3.1*).

TESTS

Solution S. Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Monographs M