System suitability: reference solution (b):
- retention time: molgramostim = 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 Alamar 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 x 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 CO2. 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.

01/2005:1449 corrected

MOMETASONE FUROATE
Mometasoni furoas

C_{27}H_{30}Cl_{2}O_{6} \quad M, 521.4

DEFINITION
Mometasone furoate contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of 9,21-dichloro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate, calculated with reference to the dried substance.

CHARACTERS
A white or almost white powder, practically insoluble in water, soluble in acetone and in methylene chloride, slightly soluble in alcohol.

It melts at about 220 °C, with decomposition.

IDENTIFICATION
First identification: A, B.
Second identification; B, C, D.

A. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with mometasone furoate CRS. Examine the substances prepared as discs.

B. Examine by thin-layer chromatography (2.2.27), using a TLC silica gel F_{254} plate R.

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 beclometasone dipropionate CRS in reference solution (a) and dilute to 10 ml with the same solution.

Apply separately to the plate 5 µl of each solution. Prepare the mobile phase by adding a mixture of 1.2 volumes of water R and 8 volumes of methanol R to a mixture
Test solution. Dissolve 20.0 mg of the substance to be acetic acid R of Prepare a mixture of equal volumes of acetonitrile R and water R to which is added 0.1 volumes 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 solution. Reference solution (a). Dissolve 2 mg of mometasone furoate CRS and 6 mg of beclometasone dipropionate CRS in the solvent solution and dilute to 10.0 ml with the same solvent. Dilute 0.25 ml of the solution to 10.0 ml with the solvent solution. Reference solution (b). Dilute 1.0 ml of the test solution to 20.0 ml with the solvent solution. Dilute 1.0 ml of the solution to 10.0 ml with the solvent solution. The chromatographic procedure may be carried out using:

- a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with octadecysilysil silica gel for chromatography R (5 μm),
- as mobile phase at a flow rate of 1 ml/min a mixture of equal volumes of acetonitrile R and water R,
- as detector a spectrophotometer set at 254 nm.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with 20 μl of reference solution (b) is at least 50 per cent of the full scale of the recorder. Inject 20 μl of reference solution (a). When the chromatograms are recorded in the prescribed conditions, the retention times are: mometasone furoate about 17 min and beclometasone dipropionate about 22 min.
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 alcohol, 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.
Appearance of solution (2.2.1): clear and not more intensely coloured than reference solution GY6 or Y6 (2.2.2, Method II).

Related substances. Liquid chromatography (2.2.29). Carry out the test protected from light.

Test solution. Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.
Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.
Reference solution (b). Dilute 2.0 ml of reference solution (a) to 100.0 ml with the mobile phase.
Reference solution (c). Expose 10 ml of reference solution (a) to daylight for 15 min before injection.
Reference solution (d). Dissolve 15.0 mg of tartaric acid R in the mobile phase and dilute to 100.0 ml with the mobile phase.

Column:
- size: l = 0.25 m, Ø = 4.6 mm.
- stationary phase: base-deactivated end-capped octadecylsilyl silica gel for chromatography R (5 µm).

Mobile phase: to a mixture of 0.35 volumes of triethylamine R and 85 volumes of water R adjusted to pH 2.5 with phosphoric acid R, add 5 volumes of tetrahydrofuran R and 10 volumes of methanol R.
Flow rate: 0.75 ml/min.
Detection: spectrophotometer at 226 nm.
Injection: 20 µl.
Run time: twice the retention time of morantel.
System suitability: reference solution (c):
- resolution: minimum of 2 between the principal peak and the preceding peak ((Z)-isomer).