Closantel sodium dihydrate for veterinary use

EUROPEAN PHARMACOPOEIA 6.0

— stationary phase: propylsilyl silica gel for chromatography R (5 µm);
— temperature: 40 °C.

Mobile phase:
— mobile phase A: dissolve 4 g of potassium dihydrogen phosphate R in 1000 ml of water for chromatography R, and adjust to pH 4.0 with phosphoric acid R;
— mobile phase B: mobile phase A, acetonitrile R 1 (25:75 V/V);

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent V/V)</th>
<th>Mobile phase B (per cent V/V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 15</td>
<td>90 → 30</td>
<td>10 → 70</td>
</tr>
<tr>
<td>15 - 15.1</td>
<td>30 → 90</td>
<td>70 → 10</td>
</tr>
<tr>
<td>15.1 - 20</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

Flow rate: 1.5 ml/min.

Detection: spectrophotometer at 210 nm.

Injection: 5 µl.

System suitability: reference solution (b):
— resolution: minimum 5 between the peaks due to clonidine and impurity B.

Limits:
— unspecified impurities: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— total: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— disregard limit: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (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.

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

ASSAY
Dissolve 0.200 g in 70 ml of ethanol (96 per cent) R. Titrate with 0.1 M ethanolic sodium hydroxide determining the end-point potentiometrically (2.2.20).

1 ml of 0.1 M sodium hydroxide is equivalent to 26.66 mg of C₉H₁₀Cl₃N₃.

CLOSANTEL SODIUM DIHYDRATE
FOR VETERINARY USE

Closantelum natricum dihydricum
ad usum veterinarium

C₂₂H₁₃Cl₂I₂N₂NaO₂·2H₂O

Mₗ, 721

[61438-64-0]

DEFINITION
N-[5-Chloro-4-[(RS)-(4-chlorophenyl)cyanomethyl]-2-methylphenyl]-2-hydroxy-3,5-diiodobenzamide sodium salt dihydrate.

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

CHARACTERS
Appearance: yellow powder, slightly hygroscopic.

Solubility: very slightly soluble in water, freely soluble in ethanol (96 per cent), soluble in methanol.

It shows polymorphism (5.9).

IDENTIFICATION
A. Infrared absorption spectrophotometry (2.2.24).

Preparation: discs without recrystallisation.

Comparison: closantel sodium dihydrate CRS.

B. Dissolve 0.1 g in 2 ml of ethanol (96 per cent) R. The solution gives reaction (a) of sodium (2.3.1).

TESTS

Appearance of solution. The solution is clear (2.2.1) and not more intensely coloured than reference solution GY₄ (2.2.2, Method II).

Dissolve 0.50 g in ethanol (96 per cent) R and dilute to 50 ml with the same solvent.

1584 See the information section on general monographs (cover pages)
Related substances. Liquid chromatography (2.2.29). Prepare the solutions immediately before use and protect from light.

Test solution. Dissolve 0.100 g of the substance to be examined in methanol R and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve 10 mg of closantel for system suitability CRS (containing impurities A to J) in methanol R and dilute to 1.0 ml with the same solvent.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with methanol R. Dilute 5.0 ml of this solution to 25.0 ml with methanol R.

Column:
- size: l = 0.10 m, Ø = 4.6 mm,
- stationary phase: base-deactivated octadecylsilyl silica gel for chromatography R (3 µm),
- temperature: 35 °C.

Mobile phase:
- mobile phase A: to 100 ml of a 7.7 g/l solution of ammonium acetate R previously adjusted to pH 4.3 with acetic acid R, add 50 ml of acetonitrile R and 850 ml of water R;
- mobile phase B: to 100 ml of a 7.7 g/l solution of ammonium acetate R previously adjusted to pH 4.3 with acetic acid R, add 50 ml of water R and 850 ml of acetonitrile R;

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent V/V)</th>
<th>Mobile phase B (per cent V/V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 2</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>2 - 22</td>
<td>50 → 20</td>
<td>50 → 80</td>
</tr>
<tr>
<td>22 - 27</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>27 - 28</td>
<td>20 → 50</td>
<td>80 → 50</td>
</tr>
<tr>
<td>28 - 32</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Flow rate: 1.5 ml/min.

Detection: spectrophotometer at 240 nm.

Injection: 10 µl.

Relative retention with reference to closantel (retention time = about 16 min): impurity A = about 0.07;
impurity B = about 0.48; impurity C = about 0.62;
impurity D = about 0.65; impurity E = about 0.82;
impurity F = about 0.89; impurity G = about 0.93;
impurity H = about 1.13; impurity I = about 1.16;
impurity J = about 1.55.

System suitability: reference solution (a):
- resolution: baseline separation between the peaks due to impurity G and closantel,
- the chromatogram obtained is similar to the chromatogram supplied with closantel for system suitability CRS.

Limits:
- correction factors: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.5;
impurity B = 1.3;
impurity C: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
impurities F, H, I: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
impurities A, B, C, D, E, J: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
any other impurity: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
total: not more than 7.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);
disregard limit: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (2.5.12): 4.8 per cent to 5.8 per cent, determined on 0.250 g.

Use a mixture of 1 volume of dimethylformamide R and 4 volumes of methanol R as the solvent.

ASSAY
Dissolve 0.500 g in 50 ml of a mixture of 1 volume of anhydrous acetic acid R and 7 volumes of methyl ethyl ketone R. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.2.20).

1 ml of 0.1 M perchloric acid is equivalent to 68.5 mg of C₂₂H₁₃Cl₂I₂N₂NaO₂.

STORAGE
In an airtight container, protected from light.

IMPURITIES
Specified impurities: A, B, C, D, E, F, G, H, I, J.
Clotrimazole contains not less than 98.5 per cent and not more than the equivalent of 100.5 per cent of 1-[2-chlorophenyl]diphenylmethyl]-1H-imidazole, calculated with reference to the dried substance.

CHARACTERS
A white or pale yellow, crystalline powder, practically insoluble in water, soluble in alcohol and in methylene chloride.

IDENTIFICATION
First identification: B.
Second identification: A, C, D.
A. Melting point (2.2.14): 141 °C to 145 °C.
B. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with clotrimazole CRS.
C. Examine before spraying in ultraviolet light at 254 nm, the chromatograms obtained in the test for (2-chlorophenyl)diphenylmethanol. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).
D. Dissolve about 10 mg in 3 ml of sulphuric acid R. The solution is pale yellow. Add 10 mg of mercuric oxide R and 20 mg of sodium nitrite R. Allow to stand with occasional shaking. An orange colour develops, becoming orange-brown.

TESTS
Appearance of solution. Dissolve 1.25 g in alcohol R and dilute to 25 ml with the same solvent. The solution is clear (2.2.1) and not more intensely coloured than reference solution BY6 (2.2.2, Method II).
(2-Chlorophenyl)diphenylmethanol. Examine by thin-layer chromatography (2.2.27), using silica gel GF254 as the coating substance.
Test solution (a). Dissolve 0.50 g of the substance to be examined in alcohol R and dilute to 5 ml with the same solvent.
Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with alcohol R.
Reference solution (a). Dissolve 50 mg of clotrimazole CRS in alcohol R and dilute to 5 ml with the same solvent.
Reference solution (b). Dissolve 10 mg of (2-chlorophenyl)diphenylmethanol CRS in alcohol R and dilute to 5 ml with the same solvent. Dilute 1 ml of the solution to 10 ml with alcohol R.
Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 0.5 volumes of concentrated ammonia R1, 10 volumes of propanol R and 90 volumes of toluene R. Allow the plate to dry in air. Spray with a 10 per cent V/V solution of sulphuric acid R in alcohol R and heat at 100 °C to 105 °C for 30 min. Any spot corresponding to (2-chlorophenyl)diphenylmethanol in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).
Imidazole. Examine by thin-layer chromatography (2.2.27), using silica gel G R as the coating substance.
Test solution. Dissolve 0.50 g of the substance to be examined in alcohol R and dilute to 10 ml with the same solvent.