TIAMULIN HYDROGEN FUMARATE FOR VETERINARY USE

Tiamulini hydrogenofumaras ad usum veterinarium

\[
\text{C}_{32}\text{H}_{51}\text{NO}_8\text{S} \quad M, 610
\]

DEFINITION

(3aS,5R,5S,6S,8R,9aR,10R)-6-Ethenyl-5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro-3a,9-propano-3aH-cyclopentacycloocten-8-yl \[2-(diethylamino)ethyl]sulphanylacetate hydrogen butenedioate.

Semi-synthetic product derived from a fermentation product.

CHARACTERS

Appearance: white or light yellow, crystalline powder.

Solubility: soluble in water, freely soluble in anhydrous ethanol and soluble in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry (2.2.24).

Comparison: tiamulin hydrogen fumarate CRS.

TESTS

pH (2.2.3): 3.1 to 4.1.

Dissolve 0.5 g in carbon dioxide-free water R and dilute to 50 ml with the same solvent.

Related substances. Liquid chromatography (2.2.29).

Ammonium carbonate buffer solution pH 10.0. Dissolve 10.0 g of ammonium carbonate R in water R, add 22 ml of perchloric acid solution R and dilute to 1000.0 ml with water R. Adjust to pH 10.0 with concentrated ammonia R1.

Solvent mixture: ammonium carbonate buffer solution pH 10.0, acetonitrile R1 (50:50 V/V).

Test solution. Dissolve 0.200 g of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). Dissolve 0.200 g of tiamulin hydrogen fumarate CRS in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

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

Reference solution (c). Dissolve 40.0 mg of fumaric acid R in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (d). Dissolve 4 mg of tiamulin for peak identification CRS (tiamulin hydrogen fumarate containing impurities B, C, D, F, H and I) in the solvent mixture and dilute to 1 ml with the solvent mixture.

COLUMN: size: \( l = 0.15 \text{ m}, \phi = 4.6 \text{ mm}, \)

stationary phase: end-capped octadecysilyle silica gel for chromatography R (5 \( \mu \text{m} \)),

temperature: 30 °C.


Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 212 nm.

Injection: 20 \( \mu \text{l} \).

Run time: 3 times the retention time of tiamulin.

Identification of impurities: use the chromatogram supplied with tiamulin for peak identification CRS and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities B and H.

Relative retention with reference to tiamulin ( retention time \( = \) about 18 min): impurity G = about 0.2;

impurity A = about 0.22; impurity H = about 0.23; impurity I = about 0.3; impurity J = about 0.4;

impurity K = about 0.45; impurity B = about 0.5; impurity L = about 0.65; impurity C = about 0.66;

impurity F = about 0.8; impurity M = about 0.85;

impurity D = about 1.1; impurity S = about 1.4;

impurity T = about 1.6; impurity E = 2.4.

System suitability: reference solution (a):

— baseline separation between the peaks due to tiamulin and impurity D.

Limits:

— impurities B, H: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent),

— impurities A, C, D, E, F, G, I, J, K, L, M, S, T: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent),

— any other impurity: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),

— total: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent),

— disregard limit: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent); disregard any peak present in reference solution (c).

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

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

Injection: test solution and reference solution (a).

Calculate the percentage content of \( \text{C}_{32}\text{H}_{51}\text{NO}_8\text{S} \) from the declared content of tiamulin hydrogen fumarate CRS.

STORAGE

Protected from light.

IMPURITIES


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: N, O, P, Q, R.

A. \( R_1 = R_2 = H \): (3a\(S\),5\(S\),6\(S\),8\(R\),9\(R\),9\(a\)\(R\),10\(R\))-6-ethenyl-5,8-dihydroxy-4,6,9,10-tetramethyloctahydro-3\(a\),9-propano-3\(a\)H-cyclopentacycloocten-1(4\(H\))-one (mutilin),

B. \( R = \text{CH}_2\text{-C}_6\text{H}_5 \): 2-(benzylsulphonyl)-N,N-diethylthiocarbamate,

C. \( R = \text{S-C}_2\text{H}_4\text{N}(\text{C}_2\text{H}_5)\text{H}_2 \): 2,2'-disulphane-1,2-diylbis(N,N-diethylthiocarbamate),

D. (3\(a\)\(R\),4\(R\),6\(S\),8\(R\),9\(R\),9\(a\)\(R\),10\(R\))-6-ethenylhydroxy-4,6,9,10-tetramethyl-1,5-dioxodecahydro-3\(a\),9-propano-3\(a\)H-cyclopentacycloocten-8-yl [(2-(diethylamino)ethyl)sulphonyl]acetate,

E. (3\(a\)\(R\),4\(R\),6\(S\),8\(R\),9\(R\),9\(a\)\(R\),10\(R\))-6-ethenyl-4,6,9,10-tetramethyl-1,5-dioxodecahydro-3\(a\),9-propano-3\(a\)H-cyclopentacycloocten-8-yl [(2-(diethylamino)ethyl)sulphonyl]acetate (11-oxotiamulin),

F. impurity of unknown structure with a relative retention of about 0.8,

G. \( R_1 = \text{CO-CH}_3\text{-OH} \), \( R_2 = H \): (3\(a\)\(S\),5\(S\),6\(S\),8\(R\),9\(R\),9\(a\)\(R\),10\(R\))-6-ethenyl-5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro-3\(a\),9-propano-3\(a\)H-cyclopentacycloocten-8-yl hydroxyacetate (pleuromutilin),

H. (2\(E\)E)-4\((2R)\)-2\((\text{[3}a\text{\(S\)},5\text{\(S\)},6\text{\(S\)},8\text{\(R\)},9\text{\(R\)},9\text{\(a\)}\text{\(R\)},10\text{\(R\)}\))-8\((\text{[2-(diethylamino)ethyl]sulphonyl})\text{acetyl})\text{oxy})\text{5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro-3a,9-propano-3aH-cyclopentacycloocten-6-yl-2-hydroxyethyl}y1-4-oxobut-2-enoic acid (19,20-dihydroxytiamulin 20-fumarate),

I. (2\(E\)E)-4\((2R)\)-2\((\text{[3}a\text{\(S\)},5\text{\(S\)},6\text{\(S\)},8\text{\(R\)},9\text{\(R\)},9\text{\(a\)}\text{\(R\)},10\text{\(R\)}\))-8\((\text{[2-(diethylamino)ethyl]sulphonyl})\text{acetyl})\text{oxy})\text{6-ethenyl-1,5-dihydroxy-4,6,9,10-tetramethyldecahydro-3a,9-propano-3aH-cyclopentacycloocten-2-yl}\text{oxy}y1-4-oxobut-2-enoic acid (2,3-dihydroxytiamulin 2-fumarate),

J. \( R = \text{CH}_2\text{-C}_6\text{H}_5 \): 2-(benzylsulphonyl)-N,N-diethylthiocarbamate,

K. \( R_1 = H \), \( R_2 = \text{CO-CH}_3 \): (3\(a\)\(S\),5\(S\),6\(S\),8\(R\),9\(R\),9\(a\)\(R\),10\(R\))-6-ethenyl-5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro-3\(a\),9-propano-3\(a\)H-cyclopentacycloocten-8-yl acetate (mutilin 11-acetate),

L. \( R_1 = \text{CO-CH}_3\text{-O-SO}_2\text{-C}_6\text{H}_5 \), \( R_2 = H \): (3\(a\)\(S\),5\(S\),6\(S\),8\(R\),9\(R\),9\(a\)\(R\),10\(R\))-6-ethenyl-5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro-3\(a\),9-propano-3\(a\)H-cyclopentacycloocten-8-yl [[(4-methylphenyl)sulphonyl]oxy]acetate (pleuromutilin 22-tosylate),

M. \( R_1 = R_2 = \text{CO-CH}_3 \): (3\(a\)\(S\),5\(S\),6\(S\),8\(R\),9\(R\),9\(a\)\(R\),10\(R\))-6-ethenyl-4,6,9,10-tetramethyl-1-oxodecahydro-3\(a\),9-propano-3\(a\)H-cyclopentacycloocten-5,8-diyi diacetate (mutilin 11,14-diacetate),

N. \( R_1 = \text{CO-CH}_3\text{-O-C}_2\text{H}_5 \), \( R_2 = H \): (3\(a\)\(S\),5\(S\),6\(S\),8\(R\),9\(R\),9\(a\)\(R\),10\(R\))-6-ethenyl-5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro-3\(a\),9-propano-3\(a\)H-cyclopentacycloocten-8-yl [[2-(diethylamino)ethyl]sulphonyl]acetate,

O. \( R = H \): 2-(diethylamino)ethanethiol,
Tianeptine sodium

DEFINITION
Sodium 7,[(11RS)-3-chloro-6-methyl-6,11-dihydrodibenzo[cd, f][1,2]thiazepin-11-yl]amino]heptanoate 5,5-dioxide.

Content: 99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance: white or yellowish powder, very hygroscopic.
Solubility: freely soluble in water, in methanol and in methylene chloride.

IDENTIFICATION
A. Infrared absorption spectrophotometry (2.2.24).


B. It gives reaction (a) of 2.3.1.

TESTS
Impurity A. Gas chromatography (2.2.28).

Internal standard solution. Dilute 1 ml of ethyl 5-bromovalerate R in ethanol R and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 250.0 ml with ethanol R.

Test solution. Dissolve 0.1000 g of the substance to be examined in the internal standard solution and dilute to 2.0 ml with the same solution.

Reference solution. Dissolve 10.0 mg of tianeptine impurity A CRS in the internal standard solution and dilute to 200.0 ml with the same solution.

Column:
- material: fused silica,
- size: l = 25 m, Ø = 0.25 mm,
- stationary phase: poly(cyanopropyl) disiloxane R (film thickness 0.2 µm).

Carrier gas: helium for chromatography R.
Linear velocity: 26 cm/s.

Split ratio: 1:100.

Temperature:
- column: 150 °C,
- injection port and detector: 210 °C.

Detection: flame ionisation.

Injection: 1 µl.

Run time: twice the retention time of ethyl 5-bromovalerate.

System suitability: reference solution:
- elution order: ethanol, ethyl 5-bromovalerate, impurity A,
- resolution: minimum 10 between the peaks due to ethyl 5-bromovalerate and impurity A,
- signal-to-noise ratio: minimum 20 for the peak due to impurity A.

Limit:
- impurity A: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent).

Related substances. Liquid chromatography (2.2.29).

Solvent mixture. Mix 50 volumes of methanol R and 50 volumes of water for chromatography R.

Test solution. Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.