DEFINITION
Fenbendazole for veterinary use contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of methyl [5-(phenylsulphanyl)-1H-benzimidazol-2-yl]carbamate, calculated with reference to the dried substance.

CHARACTERS
A white or almost white powder, practically insoluble in water, sparingly soluble in dimethylformamide, very slightly soluble in methanol.

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

TESTS
Related substances. Examine by liquid chromatography (2.2.29).

Test solution. Dissolve 50.0 mg of the substance to be examined in 10.0 ml of hydrochloric methanol R.

Reference solution (a). Dissolve 50.0 mg of fenbendazole CRS in 10.0 ml of hydrochloric methanol R. Dilute 1.0 ml of this solution to 200.0 ml with methanol R. Dilute 5.0 ml of this second solution to 10.0 ml with hydrochloric methanol R.

Reference solution (b). Dissolve 10.0 mg of fenbendazole impurity A CRS in 100.0 ml of methanol R. Dilute 1.0 ml of this solution to 10.0 ml with hydrochloric methanol R.

Reference solution (c). Dissolve 10.0 mg of fenbendazole impurity B CRS in 100.0 ml of methanol R. Dilute 1.0 ml of this solution to 10.0 ml with hydrochloric methanol R.

Reference solution (d). Dissolve 10.0 mg of fenbendazole CRS and 10.0 mg of mebendazole CRS in 100.0 ml of methanol R. Dilute 1.0 ml of this solution to 10.0 ml with hydrochloric methanol R.

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 octadecysilyl silica gel for chromatography R (5 µm),
- as mobile phase at a flow rate of 1 ml/min:
  Mobile phase A. Mix 1 volume of anhydrous acetic acid R, 30 volumes of methanol R and 70 volumes of water R.
  Mobile phase B. Mix 1 volume of anhydrous acetic acid R, 30 volumes of water R and 70 volumes of methanol 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>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 10</td>
<td>100 → 0</td>
<td>0 → 100</td>
<td>linear gradient</td>
</tr>
<tr>
<td>10 - 40</td>
<td>0</td>
<td>100</td>
<td>isocratic</td>
</tr>
<tr>
<td>40 - 50</td>
<td>0 → 100</td>
<td>100 → 0</td>
<td>re-equilibration</td>
</tr>
</tbody>
</table>

When the chromatograms are recorded in the prescribed conditions, the retention time for fenbendazole is about 19 min. Inject separately 10 µl of each solution. The test is not valid unless, in the chromatogram obtained with reference solution (d), the resolution between the peaks corresponding to fenbendazole and mebendazole is at least 1.5.

In the chromatogram obtained with the test solution: the area of the peaks corresponding to impurity A and impurity B is not greater than 2.5 times the area of the corresponding peak in the chromatograms obtained with reference solution (b) and reference solution (c) (0.5 per cent); the area of any peak, apart from the principal peak and the peaks corresponding to impurity A and impurity B respectively, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent); the sum of the areas of all the peaks, apart from the principal peak, is not greater than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent). Disregard any peak with an area less than 0.2 times that of the principal peak in the chromatogram obtained with reference solution (a).

Heavy metals (2.4.8). 1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of lead standard solution (10 ppm Pb) R.

Loss on drying (2.2.32). Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 100-105 °C for 3 h.

Sulphated ash (2.4.14). Not more than 0.3 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g in 30 ml of anhydrous acetic acid R, warming gently if necessary. Cool and titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.2.20). 1.0 ml of 0.1 M perchloric acid is equivalent to 29.94 mg of C_{15}H_{13}N_{3}O_{2}S.

STORAGE
Protected from light.

IMPURITIES
A. R = H: methyl (1H-benzimidazol-2-yl)carbamate,  
B. R = Cl: methyl (5-chloro-1H-benzimidazol-2-yl)carbamate.

01/2005:1209

FENBUFEN
Fenbufenum

C_{17}H_{15}O_{3}

DEFINITION
Fenbufen contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 4-(biphenyl-4-yl)-4-oxobutanoic acid, calculated with reference to the dried substance.

CHARACTERS
A white, fine, crystalline powder, very slightly soluble in water, slightly soluble in acetone, in alcohol and in methylene chloride.
IDENTIFICATION

First identification: B.
Second identification: A, C.

A. Melting point (2.2.14): 186 °C to 189 °C.

B. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with fenbufen CRS.

C. Examine by thin-layer chromatography (2.2.27), using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

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 10 mg of fenbufen CRS in methylene chloride R and dilute to 10 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of ketoprofen CRS in methylene chloride R and dilute to 10 ml with the same solvent. To 5 ml of the solution, add 5 ml of reference solution (a).

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of anhydrous acetic acid R, 25 volumes of ethyl acetate R and 75 volumes of hexane R. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. 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). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

TESTS

Related substances. Examine by liquid chromatography (2.2.29).

Test solution. Dissolve 50.0 mg of the substance to be examined in a mixture of 40 volumes of dimethylformamide R and 60 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a). Dilute 0.5 ml of the test solution to 50.0 ml with a mixture of 40 volumes of dimethylformamide R and 60 volumes of mobile phase A. Dilute 1.0 ml of the solution to 10.0 ml with a mixture of 40 volumes of dimethylformamide R and 60 volumes of mobile phase A.

Reference solution (b). Dissolve 25 mg of fenbufen CRS and 6 mg of ketoprofen CRS in a mixture of 40 volumes of dimethylformamide R and 60 volumes of mobile phase A and dilute to 10 ml with the same mixture of solvents. Dilute 1 ml of the solution to 100 ml with a mixture of 40 volumes of dimethylformamide R and 60 volumes of mobile phase A.

The chromatographic procedure may be carried out using:
- a stainless steel column 0.125 m long and 4.0 mm in internal diameter packed with octadecylsilyl silica gel for chromatography R (5 µm),
- as mobile phase at a flow rate of 2 ml/min the following solutions:
  - Mobile phase A. 32 volumes of acetonitrile R and 68 volumes of a mixture of 1 volume of glacial acetic acid R and 55 volumes of water R,
  - Mobile phase B. 45 volumes of acetonitrile R and 55 volumes of a mixture of 1 volume of glacial acetic acid R and 55 volumes of water R,

Mobile phase B

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent V/V)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>0 – 15</td>
<td>100</td>
<td>0</td>
<td>isocratic</td>
</tr>
<tr>
<td>15 – 20</td>
<td>100 → 0</td>
<td>0 → 100</td>
<td>linear gradient</td>
</tr>
<tr>
<td>20 – 35</td>
<td>0</td>
<td>100</td>
<td>isocratic</td>
</tr>
<tr>
<td>35 – 40</td>
<td>0 → 100</td>
<td>100 → 0</td>
<td>linear gradient</td>
</tr>
<tr>
<td>40 – 45</td>
<td>100</td>
<td>0</td>
<td>re-equilibration</td>
</tr>
</tbody>
</table>

— as detector a spectrophotometer set at 254 nm.

Inject 20 µl of reference solution (a) and 20 µl of reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (a) is at least 50 per cent of the full scale of the recorder. The test is not valid unless, in the chromatogram obtained with reference solution (b), the resolution between the peaks corresponding to ketoprofenand to fenbufen is at least 5.0.

Inject 20 µl of the test solution. In the chromatogram obtained with the test solution: the area of any peak, apart from the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); the sum of the areas of any peaks, apart from the principal peak, is not greater than five times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Disregard any peak due to the solvent and any peak with an area less than 0.2 times that of the principal peak in the chromatogram obtained with reference solution (a).

Heavy metals (2.4.8). 1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of lead standard solution (10 ppm Pb) R.

Loss on drying (2.2.32). Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 100-105 °C for 3 h.

Sulphated ash (2.4.14). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 75 ml of acetone R previously neutralised with phenolphthalein solution R1 and add 50 ml of water R. Add 0.2 ml of phenolphthalein solution R1 and titrate with 0.1 M sodium hydroxide. Carry out a blank titration. 1 ml of 0.1 M sodium hydroxide is equivalent to 25.43 mg of C16H14O3.

IMPURITIES

A. 3-(4-chlorophenyl)-3-oxopropanoic acid,

B. R = CO-CH=CH-CO2H, R′ = H: 4-(biphenyl-4-yl)-4-oxobut-2-enoic acid,

C. R = R′ = H: biphenyl,

D. R = CO-CH2-CH2-CO2H, R′ = OH: 4(4′-hydroxybiphenyl-4-yl)-4-oxobutanoic acid.