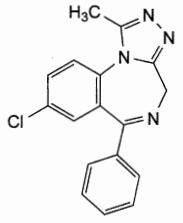
of *salicylic acid* in place of the solution being examined and beginning at the words 'add 4 mL of *iron(III) chloride solution...*'. Each g of salicylic acid is equivalent to 1.305 g of $C_9H_8O_4$.

Alprazolam

(Ph. Eur. monograph 1065)



$C_{17}H_{13}ClN_4$ 308.8 28981-97-7

Action and use
Benzodiazepine.

Ph Eur

DEFINITION
8-Chloro-1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in acetone and in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION
First identification B.

Second identification A, C.

A. Dissolve the substance to be examined in the smallest necessary quantity of *ethyl acetate R* and evaporate to dryness on a water-bath. Thoroughly mix 5.0 mg of the substance to be examined with 5.0 mg of *alprazolam CRS*. The melting point (*2.2.14*) of the mixture does not differ by more than 2 °C from the melting point of the substance to be examined.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison alprazolam CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethyl acetate R*, evaporate to dryness on a water-bath and record new spectra using the residues.

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

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

Reference solution (a) Dissolve 10 mg of *alprazolam CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *alprazolam CRS* and 10 mg of *midazolam CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate TLC silica gel GF_{254} plate R.

Mobile phase glacial acetic acid R, water R, methanol R, ethyl acetate R (2:15:20:80 *V/V/V/V*).

Application 5 µL.

Development Over a path of 12 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability: reference solution (b):
— the chromatogram shows 2 clearly separately spots.

Results 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).

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Buffer solution Dissolve 7.7 g of *ammonium acetate R* in 1000 mL of *water R* and adjust to pH 4.2 with *glacial acetic acid R*.

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

Reference solution (a) Dissolve 2 mg of *alprazolam CRS* and 2 mg of *triazolam CRS* in *dimethylformamide R* and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dilute 5.0 mL of the test solution to 100.0 mL with *dimethylformamide R*. Dilute 0.5 mL of this solution to 10.0 mL with *dimethylformamide R*.

Column:
— *size*: l = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *phenylsilyl silica gel for chromatography R1* (5 µm).

Mobile phase:
— *mobile phase A*: buffer solution, *methanol R* (44:56 *V/V*);
— *mobile phase B*: buffer solution, *methanol R* (5:95 *V/V*);
— *temperature*: 40 °C;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	98	2
15 - 35	98 → 1	2 → 99
35 - 40	1	99

Flow rate 2 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL; inject *dimethylformamide R* as a blank.

Retention time Triazolam = about 9 min; alprazolam = about 10 min.

System suitability: reference solution (a):
— *resolution*: minimum 1.5 between the peaks due to triazolam and alprazolam.

Limits:
— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
— *disregard limit*: 0.2 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.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

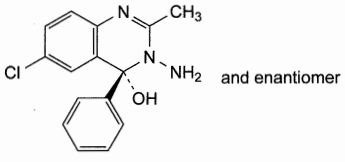
Dissolve 0.140 g in 50 mL of a mixture of 2 volumes of *acetic anhydride R* and 3 volumes of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Titrate to the 2nd point of inflexion.

1 mL of *0.1 M perchloric acid* is equivalent to 15.44 mg of $C_{17}H_{13}ClN_4$.

STORAGE

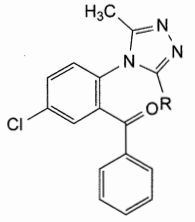
Protected from light.

IMPURITIES



and enantiomer

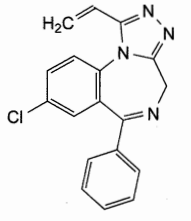
A. (4*RS*)-3-amino-6-chloro-2-methyl-4-phenyl-3,4-dihydroquinazolin-4-ol,



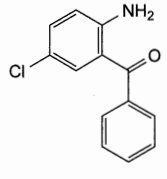
B. R = CH_2OH: [5-chloro-2-[3-(hydroxymethyl)-5-methyl-4*H*-1,2,4-triazol-4-yl]phenyl]phenylmethanone,

C. R = H: [5-chloro-2-[3-methyl-4*H*-1,2,4-triazol-4-yl]phenyl]phenylmethanone,

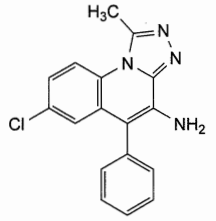
F. R = CH_2Cl: [5-chloro-2-[3-(chloromethyl)-5-methyl-4*H*-1,2,4-triazol-4-yl]phenyl]phenylmethanone,



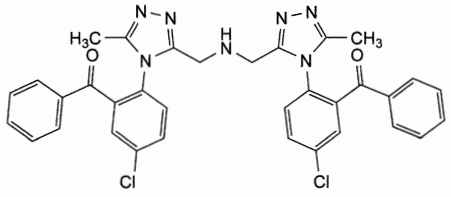
D. 8-chloro-1-ethenyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine,



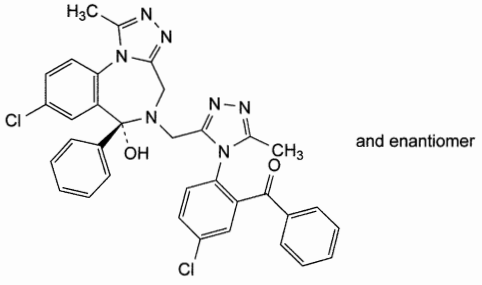
E. (2-amino-5-chlorophenyl)phenylmethanone,



G. 7-chloro-1-methyl-5-phenyl[1,2,4]triazolo[4,3-*a*]quinolin-4-amine,

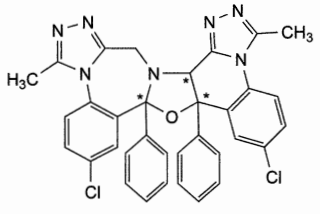


H. bis[[4-(2-benzoyl-4-chlorophenyl)-5-methyl-4*H*-1,2,4-triazol-3-yl]methyl]amine,



and enantiomer

I. [5-chloro-2-[3-[[(6*RS*)-8-chloro-6-hydroxy-1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-5(6*H*)-yl]methyl]-5-methyl-4*H*-1,2,4-triazol-4-yl]phenyl]phenylmethanone,



J. 2,17-dichloro-6,13-dimethyl-18b,19a-diphenyl-8b,19adihydro-10*H*,18b*H*-[1,2,4]triazolo[4‴,3‴:1″,2″]quinolo[3″,4″:4′,5′]oxazolo[3′,2′-*d*]-1,2,4-triazolo[4,3-*a*][1,4]benzodiazepine.