

diluting 5 ml of a 200 g/l solution of *sodium hydroxide R* to 20 ml with *water R* and adding 1.5 ml of 0.01 M *hydrochloric acid*, 5 ml of *nitric acid R* and diluting to 50 ml with *water R*.

**Related substances.** Liquid chromatography (2.2.29).

**Test solution (a).** Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 ml with the mobile phase.

**Test solution (b).** Dilute 1.0 ml of test solution (a) to 20.0 ml with the mobile phase.

**Reference solution (a).** Dissolve 50.0 mg of *crotamiton CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

**Reference solution (b).** Dissolve 15.0 mg of *crotamiton impurity A CRS* in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

**Reference solution (c).** Dilute 1.0 ml of test solution (a) to 100.0 ml with the mobile phase.

**Reference solution (d).** Dissolve 15 mg of *crotamiton impurity A CRS* in the mobile phase and dilute to 100 ml with the mobile phase. Dilute 1 ml of this solution to 10 ml with test solution (a).

**Column:**

- size:  $l = 0.25$  m,  $\varnothing = 4$  mm;
- stationary phase: silica gel for chromatography R (5  $\mu$ m).

**Mobile phase:** tetrahydrofuran R, cyclohexane R (8:92 V/V).

**Flow rate:** 1.0 ml/min.

**Detection:** spectrophotometer at 242 nm.

**Injection:** 20  $\mu$ l of test solution (a) and reference solutions (b), (c) and (d).

**Run time:** 2.5 times the retention time of the *E*-isomer.

**Relative retention** with reference to the *E*-isomer:  
*Z*-isomer = about 0.5; impurity A = about 0.8.

**System suitability:** reference solution (d):

- resolution: minimum 4.5 between the peaks due to impurity A and the *E*-isomer.

**Limits:**

- **impurity A:** not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (3 per cent);
- **sum of impurities other than A:** not more than the sum of the areas of the peaks due to the *Z*- and *E*-isomers in the chromatogram obtained with reference solution (c) (1 per cent);
- **disregard limit:** 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (c); disregard any peak due to the *Z*-isomer.

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

## ASSAY

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

**Injection:** test solution (b) and reference solution (a).

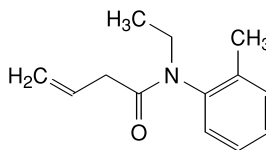
Calculate the percentage content of  $C_{13}H_{17}NO$  from the sum of the areas of the peaks due to the *Z*- and *E*-isomers in the chromatograms obtained. Calculate the content of the *Z*-isomer, as a percentage of the total content of the *E*- and *Z*-isomers, from the chromatogram obtained with test solution (b).

## STORAGE

Protected from light.

## IMPURITIES

**Specified impurities:** A.

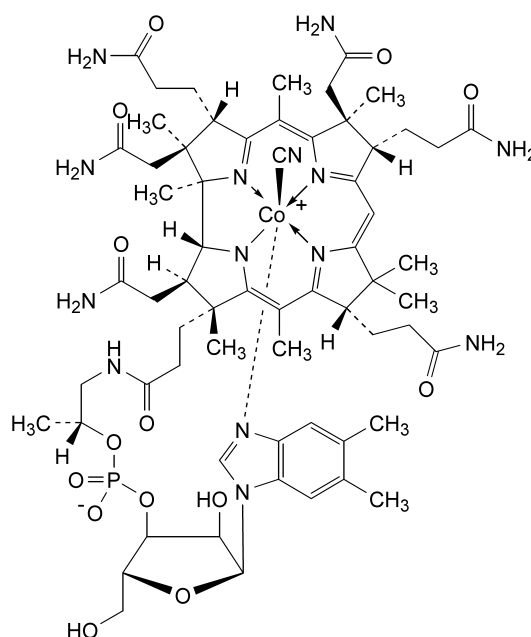


A. *N*-ethyl-*N*-(2-methylphenyl)but-3-enamide.

01/2008:0547  
corrected 6.0

# CYANOCOBALAMIN

## Cyanocobalaminum



$C_{63}H_{88}CoN_{14}O_{14}P$   
[68-19-9]

$M_r$  1355

## DEFINITION

$\alpha$ -(5,6-Dimethylbenzimidazol-1-yl)cobamide cyanide.

**Content:** 96.0 per cent to 102.0 per cent (dried substance).

This monograph applies to cyanocobalamin produced by fermentation.

## CHARACTERS

**Appearance:** dark red, crystalline powder or dark red crystals.

**Solubility:** sparingly soluble in water and in ethanol (96 per cent), practically insoluble in acetone.

The anhydrous substance is very hygroscopic.

## IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry (2.2.25).

**Test solution.** Dissolve 2.5 mg in *water R* and dilute to 100.0 ml with the same solvent.

**Spectral range:** 260-610 nm.

**Absorption maxima:** at 278 nm, 361 nm and from 547 nm to 559 nm.

**Absorbance ratio:**

- $A_{361} / A_{547.559} = 3.15$  to  $3.45$ ;
- $A_{361} / A_{278} = 1.70$  to  $1.90$ .

**B. Thin-layer chromatography (2.2.27). Carry out the test protected from light.**

**Test solution.** Dissolve 2 mg of the substance to be examined in 1 ml of a mixture of equal volumes of *ethanol* (96 per cent) *R* and *water R*.

**Reference solution.** Dissolve 2 mg of *cyanocobalamin CRS* in 1 ml of a mixture of equal volumes of *ethanol* (96 per cent) *R* and *water R*.

**Plate:** *TLC silica gel G plate R*.

**Mobile phase:** *dilute ammonia R1, methanol R, methylene chloride R* (9:30:45 *V/V/V*).

**Application:** 10 µl.

**Development:** in an unsaturated tank, over a path of 12 cm.

**Drying:** in air.

**Detection:** examine in daylight.

**Results:** the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

**Limits:**

- **total:** not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (3 per cent);
- **disregard limit:** the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

**Loss on drying (2.2.32):** maximum 12.0 per cent, determined on 20.00 mg by drying *in vacuo* at 105 °C for 2 h.

**ASSAY**

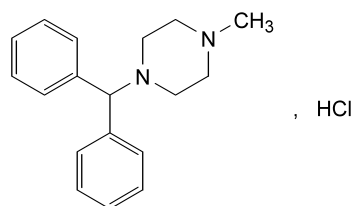
Dissolve 25.00 mg in *water R* and dilute to 1000.0 ml with the same solvent. Measure the absorbance (2.2.25) at the absorption maximum at 361 nm.

Calculate the content of  $C_{63}H_{88}CoN_{14}O_{14}P$  taking the specific absorbance to be 207.

**STORAGE**

In an airtight container, protected from light.

01/2008:1092  
corrected 6.0

**CYCLIZINE HYDROCHLORIDE****Cyclizini hydrochloridum**

$C_{18}H_{23}ClN_2$   
[305-25-3]

$M_r$  302.8

**DEFINITION**

Cyclizine hydrochloride contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 1-(diphenylmethyl)-4-methylpiperazine hydrochloride, calculated with reference to the dried substance.

**CHARACTERS**

A white or almost white, crystalline powder, slightly soluble in water and in alcohol.

**IDENTIFICATION**

**First identification:** B, E.

**Second identification:** A, C, D, E.

**A.** Dissolve 20.0 mg in a 5 g/l solution of *sulphuric acid R* and dilute to 100.0 ml with the same acid solution (solution A). Examined between 240 nm and 350 nm (2.2.25), solution A shows two absorption maxima, at 258 nm and 262 nm. The ratio of the absorbance measured at the maximum at 262 nm to that measured at the maximum at 258 nm is 1.0 to 1.1. Dilute 10.0 ml of solution A to 100.0 ml with a 5 g/l solution of *sulphuric acid R* (solution B). Examined between 210 nm and 240 nm, solution B shows an absorption maximum at 225 nm. The specific absorbance at the maximum is 370 to 410. Verify the resolution of the apparatus (2.2.25); the test is not valid unless the ratio of the absorbances is at least 1.7.

**TESTS**

**Related substances.** Liquid chromatography (2.2.29).

**Test solution.** Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase. *Use within 1 h.*

**Reference solution (a).** Dilute 3.0 ml of the test solution to 100.0 ml with the mobile phase. *Use within 1 h.*

**Reference solution (b).** Dilute 5.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase. *Use within 1 h.*

**Reference solution (c).** Dissolve 25 mg of the substance to be examined in 10 ml of *water R*, warming if necessary. Allow to cool and add 5 ml of a 1.0 g/l solution of *chloramine R* and 0.5 ml of 0.05 *M hydrochloric acid*, then dilute to 25 ml with *water R*. Shake and allow to stand for 5 min. Dilute 1 ml of this solution to 10 ml with the mobile phase and inject immediately.

**Column:**

- **size:**  $l = 0.25$  m,  $\varnothing = 4$  mm;
- **stationary phase:** *octylsilyl silica gel for chromatography R* (5 µm).

**Mobile phase:** mix 26.5 volumes of *methanol R* and 73.5 volumes of a 10 g/l solution of *disodium hydrogen phosphate R* adjusted to pH 3.5 with *phosphoric acid R* and use within 2 days.

**Flow rate:** 0.8 ml/min.

**Detection:** spectrophotometer at 361 nm.

**Injection:** 20 µl.

**Run time:** 3 times the retention time of *cyanocobalamin*.

**System suitability:**

- the chromatogram obtained with reference solution (c) shows 2 principal peaks;
- **resolution:** minimum 2.5 between the 2 principal peaks in the chromatogram obtained with reference solution (c);
- **signal-to-noise ratio:** minimum 5 for the principal peak in the chromatogram obtained with reference solution (b).