Readily carbonisable substances. To 0.20 g of the powdered substance to be examined add 10 ml of *sulphuric acid R* and heat in a water-bath at 90 ± 1 °C for 60 min. Cool rapidly. The solution is not more intensely coloured than reference solution Y_2 or GY_2 (*2.2.2, Method II*).

Chlorides (*2.4.4*): maximum 50 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Oxalates: maximum 300 ppm.

Dissolve 0.50 g in 4 ml of *water R*, add 3 ml of *hydrochloric acid R* and 1 g of *zinc R* in granules and heat on a water-bath for 1 min. Allow to stand for 2 min, decant the liquid into a test-tube containing 0.25 ml of a 10 g/l solution of *phenylhydrazine hydrochloride R* and heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 ml of *potassium ferricyanide solution R*. Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 4 ml of a 0.05 g/l solution of *oxalic acid R*.

Sulphates (*2.4.13*): maximum 150 ppm.

To 10 ml of solution S add 2 ml of *hydrochloric acid R1* and dilute to 15 ml with *distilled water R*.

Heavy metals (*2.4.8*): maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Sodium: maximum 0.30 per cent.

Atomic emission spectrometry (*2.2.22, Method II*).

Test solution. To 10 ml of solution S add 1 ml of *dilute hydrochloric acid R* and dilute to 100 ml with *distilled water R*.

Reference solutions. Prepare the reference solutions using a solution of *sodium chloride R* containing 1 mg of Na per millilitre diluted as necessary with *distilled water R*.

Wavelength: 589 nm.

Water (*2.5.12*): 4.0 per cent to 7.0 per cent, determined on 0.500 g. After adding the substance to be examined, stir for 15 min before titrating.

ASSAY

Dissolve 0.150 g in 20 ml of *anhydrous acetic acid R*, heating to about 50 °C. Allow to cool. Titrate with *0.1 M perchloric acid* using 0.25 ml of *naphtholbenzein solution R* as indicator until a green colour is obtained.

1 ml of *0.1 M perchloric acid* is equivalent to 10.21 mg of $C_6H_5K_3O_7$.

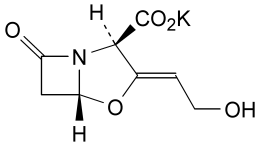
STORAGE

In an airtight container.

01/2008:1140

POTASSIUM CLAVULANATE

Kalii clavulanas



$C_8H_8KNO_5$ M_r 237.3

[61177-45-5]

DEFINITION

Potassium (2*R*,3*Z*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate, the potassium salt of a substance produced by the growth of certain strains of *Streptomyces clavuligerus* or obtained by any other means.

Content: 96.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder, hygroscopic.

Solubility: freely soluble in water, slightly soluble in alcohol, very slightly soluble in acetone.

PRODUCTION

The method of production, extraction and purification are such that clavam-2-carboxylate is eliminated or present at a level not exceeding 0.01 per cent.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *Ph. Eur. reference spectrum of potassium clavulanate*.

B. It gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S. Dissolve 0.400 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

pH (*2.2.3*): 5.5 to 8.0.

Dilute 5 ml of solution S to 10 ml with *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*): + 53 to + 63 (anhydrous substance), determined on solution S.

Absorbance (*2.2.25*): maximum 0.40 at 278 nm.

Dissolve 50.0 mg in *0.1 M phosphate buffer solution pH 7.0 R* and dilute to 50.0 ml with the same solution. Measure immediately the absorbance of this solution.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use*.

Test solution. Dissolve 0.250 g of the substance to be examined in mobile phase A and dilute to 25.0 ml with mobile phase A.

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

Reference solution (b). Dissolve 10 mg of *lithium clavulanate CRS* and 10 mg of *amoxicillin trihydrate CRS* in mobile phase A and dilute to 100 ml with mobile phase A.

Column:

– *size*: *l* = 0.10 m, Ø = 4.6 mm,

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),

– *temperature*: 40 °C.

Mobile phase:

– *mobile phase A*: a 7.8 g/l solution of *sodium dihydrogen phosphate R* adjusted to pH 4.0 with *phosphoric acid R* and filtered through a 0.5 µm filter,

– *mobile phase B*: a mixture of equal volumes of mobile phase A and *methanol R*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	100	0
4 - 15	100 → 50	0 → 50
15 - 18	50	50
18 - 24	50 → 100	50 → 0
24 - 39	100	0

Flow rate: 1 ml/min.

Detection: spectrophotometer at 230 nm.

Injection: 20 µl.

System suitability: reference solution (b):

– *resolution*: minimum 13 between the first peak (clavulanate) and the second peak (amoxicillin).

Limits:

– *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),

– *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent),

– *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Aliphatic amines. Gas chromatography (*2.2.28*).

The method shown below can be used to determine the following aliphatic amines: 1,1-dimethylethylamine; diethylamine; *N,N,N′,N′*-tetramethylethylenediamine; 1,1,3,3-tetramethylbutylamine; *N,N′*-diisopropylethylenediamine; 2,2′-oxydi(*N,N*)dimethylethylamine.

Internal standard solution: dissolve 50 µl of *3-methylpentan-2-one R* in *water R* and dilute to 100.0 ml with the same solvent.

Test solution. Weigh 1.00 g of the substance to be examined into a centrifuge tube. Add 5.0 ml of the internal standard solution, 5.0 ml of *dilute sodium hydroxide solution R*, 10.0 ml of *water R*, 5.0 ml of *2-methylpropanol R* and 5 g of *sodium chloride R*. Shake vigorously for 1 min. Centrifuge to separate the layers.

Reference solution. Dissolve 80.0 mg of each of the following amines *1,1-dimethylethylamine R*; *diethylamine R*; *tetramethylethylenediamine R*; *1,1,3,3-tetramethylbutylamine R*; *N,N′-diisopropylethylenediamine R* and *2,2′-oxybis(N,N-dimethylethylamine) R* in *dilute hydrochloric acid R* and dilute to 200.0 ml with the same acid. Introduce 5.0 ml of this solution into a centrifuge tube. Add 5.0 ml of the internal standard solution, 10.0 ml of *dilute sodium hydroxide solution R*, 5.0 ml of *2-methylpropanol R* and 5 g of *sodium chloride R*. Shake vigorously for 1 min. Centrifuge to separate the layers.

Column:

– *material*: fused silica,

– *size*: *l* = 50 m, Ø = 0.53 mm,

– *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 5 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 8 ml/min.

Split ratio: 1:10.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 7	35
	7 - 10.8	35 → 150
	10.8 - 25.8	150
Injection port		200
Detector		250

Detection: flame ionisation.

Injection: 1 µl of the upper layers obtained from test solution and reference solution.

Relative retention with reference to 3-methylpentan-2-one (retention time = about 11.4 min): impurity H = about 0.55; impurity I = about 0.76; impurity J = about 1.07; impurity K = about 1.13; impurity L = about 1.33; impurity M = about 1.57.

Limit:

– *aliphatic amines*: maximum 0.2 per cent.

2-Ethylhexanoic acid (*2.4.28*): maximum 0.8 per cent.

Water (*2.5.12*): maximum 0.5 per cent, determined on 1.00 g.

Bacterial endotoxins (*2.6.14*): less than 0.03 IU/mg if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use*.

Test solution. Dissolve 50.0 mg of the substance to be examined in a 4.1 g/l solution of *sodium acetate R* previously adjusted to pH 6.0 with *glacial acetic acid R*, and dilute to 50.0 ml with the same solution.

Reference solution (a). Dissolve 50.0 mg of *lithium clavulanate CRS* in a 4.1 g/l solution of *sodium acetate R* previously adjusted to pH 6.0 with *glacial acetic acid R* and dilute to 50.0 ml with the same solution.

Reference solution (b). Dissolve 50.0 mg of *lithium clavulanate CRS* and 50.0 mg of *amoxicillin trihydrate CRS* in a 4.1 g/l solution of *sodium acetate R* previously adjusted to pH 6.0 with *glacial acetic acid R* and dilute to 50.0 ml with the same solution.

Column:

– *size*: *l* = 0.3 m, Ø = 4.6 mm,

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mix 5 volumes of *methanol R1* and 95 volumes of a 15 g/l solution of *sodium dihydrogen phosphate R* previously adjusted to pH 4.0 with *dilute phosphoric acid R*.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 230 nm.

Injection: 10 µl.

System suitability: reference solution (b):

– *resolution*: minimum 3.5 between the first peak (clavulanate) and the second peak (amoxicillin).

1 mg of clavulanate ($C_8H_9NO_5$) is equivalent to 1.191 mg of $C_8H_8KNO_5$.

STORAGE

In an airtight container, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

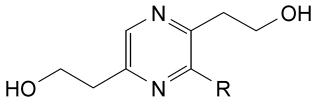
IMPURITIES

Specified impurities: A, B, C, D, G, H, I, J, K, L, M.

Other detectable impurities: E, F.

By liquid chromatography: A, B, C, D, E, F, G.

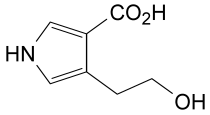
By gas chromatography: H, I, J, K, L, M.



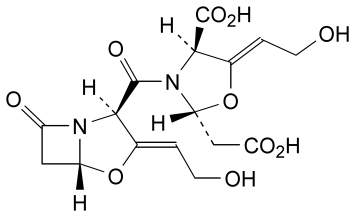
A. R = H: 2,2′-(pyrazine-2,5-diyl)diethanol,

B. R = CH_2-CH_2-CO_2H: 3-[3,6-bis(2-hydroxyethyl)pyrazin-2-yl]propanoic acid,

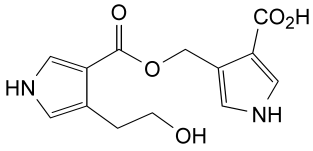
C. R = CH_2-CH_3: 2,2′-(3-ethylpyrazine-2,5-diyl)diethanol,



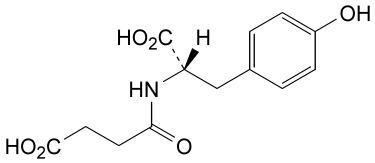
D. 4-(2-hydroxyethyl)pyrrole-3-carboxylic acid,



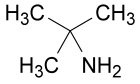
E. (2*R*,4*R*,5*Z*)-2-(carboxymethyl)-5-(2-hydroxyethylidene)-3-[[(2*R*,3*Z*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabibyclo[3.2.0]hept-2-yl]carbonyl]oxazolidine-4-carboxylic acid,



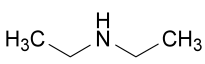
F. 4-[[[[4-(2-hydroxyethyl)-1*H*-pyrrol-3-yl]carbonyl]oxy]methyl]-1*H*-pyrrole-3-carboxylic acid,



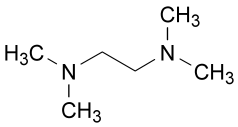
G. 4-[[(1*S*)-1-carboxy-2-(4-hydroxyphenyl)ethyl]amino]-4-oxobutanoic acid (*N*-succinyltyrosine),



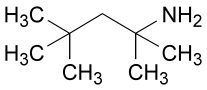
H. 2-amino-2-methylpropane (1,1-dimethylethylamine),



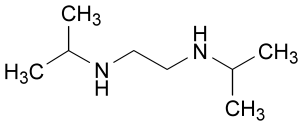
I. diethylamine,



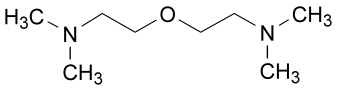
J. 1,2-bis(dimethylamino)ethane (*N*,*N*,*N*′,*N*′-tetramethylethylenediamine),



K. 2-amino-2,4,4-trimethylpentane (1,1,3,3-tetramethylbutylamine),



L. *N*,*N*′-bis(1-methylethyl)-1,2-ethanediamine (*N*,*N*′-diisopropylethylenediamine),

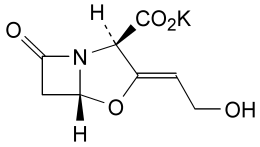


M. bis(2-dimethylamino)ethyl ether [2,2′-oxybis(*N*,*N*-dimethylethylamine)].

01/2008:1653

POTASSIUM CLAVULANATE, DILUTED

Kalii clavulanas dilutus



$C_8H_8KNO_5$ M_r 237.3

DEFINITION

Dry mixture of *Potassium clavulanate (1140)* and *Cellulose, microcrystalline (0316)* or *Silica, colloidal anhydrous (0434)* or *Silica, colloidal hydrated (0738)*.

Content: 91.2 per cent to 107.1 per cent of the content of potassium clavulanate stated on the label.

CHARACTERS

Appearance of diluted potassium clavulanate: white or almost white powder, hygroscopic.

Solubility of potassium clavulanate: freely soluble in water, slightly soluble in alcohol, very slightly soluble in acetone.

The solubility of the diluted product depends on the diluent and its concentration.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

B. It gives reaction (b) of potassium (*2.3.1*).

ERRATA

In the following monographs, after the heading ‘Other detectable impurities’ in the Impurities section, read:

‘(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*)’

Articaine hydrochloride (1688)
Biperiden hydrochloride (1074)
Caffeine (0267)
Caffeine monohydrate (0268)
Ibuprofen (0721)
Ifosfamide (1529)
Metformin hydrochloride (0931)
Naphazoline hydrochloride (0730)
Norethisterone acetate (0850)
Oxaliplatin (2017)
Potassium clavulanate (1140)
Potassium clavulanate, diluted (1653)
Testosterone propionate (0297)
Thiamine hydrochloride (0303)
Thiamine nitrate (0531)
Tranexamic acid (0875)