LOLA Quality Standards and MOA

Test		Specification	Methods
Appearance		White crystal or crystalline powder	DAB
Identication	A.Optical Rotation,°	+26.5 ~ +29.0 °	Ph.Eur. 2.2.7
	B.IR	corresponds to standard	Ph.Eur.2.2.24
Purity	Clarity of the solution	≤No. 1 Turbidity Standard	Ph.Eur.2.2.1,
	Color of solution	≤ B9	Ph.Eur.2.2.2(II)
	pH	6.0 ~ 7.0	Ph.Eur. 2.2.3
	Related substance (TLC)	Spots other than the main spots in the test solution are not larger than the spots obtained in the standard solution	Ph.Eur. 2.2.27
	Chloride	≤ 300 ppm	Ph.Eur.2.2.4
	Sulfate	≤ 200 ppm	Ph.Eur.2.4.13
	Ammonium	≤ 400 ppm	DAB N 2.4.1
	Iron	≤ 30 ppm	Ph.Eur.2.4.9
	Heavy metals	≤ 10 ppm	Ph.Eur.2.4.8
	Water	≤ 7.0 %	Ph.Eur.2.5.12
	Sulfated ash	≤ 0.2 %	Ph.Eur.2.4.14
	Arsenic	≤ 2ppm	In-house
Assay		98.0 ~ 102.0 %	Ph.Eur.2.2.20
Residual solvent(Methanol)		≤ 3,000 ppm	In-house
bacterial endotoxin（EU/mg）		＜0.006	In-house
Total plate counts（cfu/g）		⩽ 1000	ChP General chapter 1105
Yeast and Mould（cfu/g）		⩽100	ChP General chapter 1105
E.coli（cfu/g）		Absence	ChP General

		chapter 1106

检验方法

1) Appearance: White crystal or crystalline powder

Take 1 g of sample in a watch glass placed on a white paper or a white paper.

2）Identification

2.1) A. Specific rotation: +26.5 ～ +29.0 °

Dissolve 2.0 g of sample (as anhydrous) in 25 mL of 6 mol / L HCl solutionand measure the D line of the sodium (589.3 nm) at 20 ±0.5℃ and100 mm length. (Ph. Eur. 2.2.7)

2.2) B. IR:The IR Spectrum of sample corresponds to L-ornithine-L-Aspartate standard.

Sample and Standard have the same absorption spectrum as Standard when measured according to the potassium bromide method (KBr) of the infrared spectral method. (Ph. Eur. 2.2.24)

3) Purity

Test solution: Dissolve 2.5 g of sample in 100 mL ofwater

3.1) Clarity and coloration:

Using identical test-tubes of colourless, transparent, neutral glass with a flat base and an internal diameter of 15-25 mm, compare Test solution to be examined with a reference suspension freshly prepared as described below. Ensure that the depths ofthe layers in the 2 test-tubes are the same (about 40 mm). Compare the liquids in diffused daylight 5 min after preparation of the reference suspension, viewing vertically against a black background. Test solution is considered clear if its clarity is the same as that of water R or of the solvent used, or if its opalescence is not more pronounced than that of reference suspension I (Ph. Eur.2.2.1)

Using identical tubes of colourless, transparent, neutral glass with a flat base and an internal diameter of 15 mm to 25 mm, compare Test solution to be examined with water R or the solvent or the reference solution (see Tables of reference solutions) the depth of the layer being 40 mm. Compare the colours in diffused daylight viewing vertically against a white backg

round. Test solution is colourless if it has the appearance of water R or the solvent or is not more intensely coloured than reference solution B9 (Ph. Eur. 2.2.2(II))

3.2) pH: 6.0 ~ 7.0

Weigh 2.5 g of the sample, dissolve in water to make 100 mL, and measure the pH. (Ph. Eur. 2.2.3)

3.3) Related substance: Spots other than the main spots in the test solution are not larger than the spots obtained in the standard solution

Precisely weighed 2.5 g of this solution into a 100mL volumetric flask, add 100 mL of water and dissolve. Use this solution as the sample solution. Take exactly 2 mL of the sample solution and dilute it with 100 mL of water. Use this solution as the standard solution. Dissolve 5 μL of the above sample solution and standard solution on a thin plate made of silica gel for thin layer chromatography according to European Pharmacopoeia Thin Layer Chromatography. Next, it is developed at about 10 cm using water: acetic acid (98%): 1-butanol = 25: 25: 50 as a developing solvent, and then dried at 110 °C for 15 minutes. When the ninhydrin solution is evenly sprayed and dried at 110 °C for 10 minutes.

* Ninhydrin solution: 0.2 g of ninhydrin is completely dissolved in 94 mL of n-butanol and 6 mL ofacetic acid. Shade to keep

3.4) Chloride: ≤ 300 ppm

▪Sample solution: Take 6.7 mL ofTest solution and dilute with 15 mL of water.

▪To 15 mL of the Sample solution add 1 mL of dilute nitric acid R and pour the mixture as a single addition into a test-tube containing 1 mL of silver nitrate solution R2. Prepare a standard in the same manner using 10 mL of chloride standard solution (5 ppm Cl) R and 5 mL of water R. Examine the tubes laterally against a black background. After standing for 5 min protected from light, any opalescence in the Sample solution is not more intense than that in the standard. (Ph. Eur. 2.4.4)

3.5) Sulfate: ≤ 200 ppm

▪Sample solution: Weigh 0.75 g of the sample, dissolve in water to make 15 mL.

▪All solutions used for this test must be prepared with distilled water R. Add 3 mL of a 250 g/L solution of barium chloride R to 4.5 mL of sulfate standard solution (10 ppm SO4) R1. Shake and allow to stand for 1 min. To 2.5 mL of this suspension add 15 mL of the Sample solution and 0.5 mL of acetic acid R. Prepare a standard in the same manner using 15 mL of sulfate standard solution (10 ppm SO4) R instead of the prescribed solution. After 5 min, any opalescence in the test solution is not more intense than that in the standard. (Ph. Eur. 2.4.13)

3.6) Ammonium: ≤ 400 ppm

▪Sample solution

25mg of this product is placed on a watch glass with a diameter of 60 mm and dissolved or suspended by adding 0.5 ml of water R. 0.30 g heavy magnesium oxide R is added to the solution or suspension.

▪Standard solution

In the same way, a reference mixture of 0.10 ml ammonium solution (100 ppm) R, 0.5water R and 0.30 g heavy magnesium oxide R is prepared at the same time.

▪Immediately after mixing, a second watch glass with a diameter of 60 mm, on the inner surface of which a red litmus paper R moistened with a drop of water R had previously been attached, is placed edge by edge on the first watch glass. The test and reference mix are warmed to 40 °C for 15 minutes. The litmus paper over the test mixture must not turn blue more intensely than the litmus paper over the reference mixture. (DAB N 2.4.1)

3.7) Iron: ≤ 30 ppm

▪Sample solution

Dissolve 0.33 g of sample in 10 mL of dilute hydrochloric acid, add 10 mL of methyl isobutyl ketone each time, and extract it strongly (3 times) for 3 minutes. Separately, separate the methyl isobutyl ketone layer, add 10 mL of water, extrude strongly for 3 minutes, and use the aqueous layer as the Sample solution.

▪Add 2 mL of a 200 g/L solution of citric acid monohydrate R and 0.1 mL of thioglycollic acid R. Mix, make alkaline with ammonia R and dilute to 20mL with water R. Prepare a standard in the same manner, using 10 mL of iron standard solution (1 ppm Fe) R. After 5 min, any pink colour in the test

solution is not more intense than that in the standard. (Ph. Eur. 2.4.9)

3.8) Heavy metals: ≤ 10 ppm

.Test solution: Place the 2 g of sample to be examined in a silica crucible with 4 mL of a 250 g/L solution of magnesium sulfate R in dilute sulfuric acid R. Mix using a fine glass rod. Heat cautiously. If the mixture is liquid, evaporate gently to dryness on a water-bath. Progressively heat to ignition and continue heating until an almost white or at most greyish residue is obtained. Carry out the ignition at a temperature not exceeding 800 °C. Allow to cool. Moisten the residue with a few drops of dilute sulfuric acid R. Evaporate, ignite again and allow to cool. The total period of ignition must not exceed 2 h. Take up the residue in 2 quantities, each of 5 mL, of dilute hydrochloric acid R. Add 0.1 mL of

phenolphthalein solution R, then concentrated ammonia R until a pink colour is obtained. Cool, add glacial acetic acid R until the solution is decolorised and add 0.5 mL in excess. Filter if necessary and wash the filter. Dilute to 20 mL with water R.

.Reference solution (standard).

Prepare as described for the Test solution, using the 2 mL of lead standard solution (10 ppm Pb) R instead of the substance to be examined. To 10 mL of the solution obtained add 2 mL of the Test solution.

.Monitor solution. Prepare as described for the Test solution, adding to the substance to be examined the volume of lead standard solution (10 ppm Pb) R prescribed for preparation of the reference solution. To 10 mL of the solution

obtained add 2 mL of the test solution.

.Blank solution.

A mixture of 10 mL of water R and 2 mL of the test solution,.To 12 mL of each solution, add 2 mL of buffer solution pH 3.5 R. Mix and add to 1.2 mL of

thioacetamide reagent R. Mix immediately. Examine the solutions after 2 min.

.System suitability:

The reference solution shows a slight brown colour compared to the blank solution, the monitor solution is at least as intense as the reference.

.Result: any brown colour in the test solution is not more intense than that in th

e reference solution. If the result is difficult to judge, filter the solutions thro ugh a
suitable membrane filter (nominal pore size 0.45 um). Carry out the filtration slowly and uniformly, applying moderate and constant pressure to the piston. C ompare the
spots on the filters obtained with the different solutions. (Ph. Eur. 2.4.8)

3.9) Water: ≤ 7.0 %

Take 0.200g sample and test with Karl Fischer method. (Ph. Eur. 2.5.12) The sample is dissolved in 10mL of formamide at 50° C, the solution is mixe d with 20mL of methanol(anhydrous) and cooled to room temperature. Perfor m a blank test.

3.10) Sulfated ash: ≤ 0.2 %

Ignite a suitable crucible (for example, silica, platinum. porcelain or quartz) at 600 ± 50 °C for 30 min, allow to cool in a desiccator over silica gel or other suitable desiccant an weigh. Place the 2.0 g of sample to be examined in the crucible and weigh. Moisten the substance be examined with a small amount of sulfuric acid R (usually 1 mL) and heat gently at as low a temperature as u ntil the sample is thoroughly
charred. After cooling, moisten the residue with a small amount of sulfuric a cid R (usually 1 mL), heat gently until white fumes are no longer evolved an d ignite at 600 + 50 °C until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Allow th e crucible to cool in a
desiccator over silica gel or other suitable desiccant, weigh it again and cal culate the percentage of residue. If the amount of the residue so obtained exc eeds the
prescribed limit, repeat the moistening with sulfuric acid R and ignition, as previously, for 30min periods until 2 consecutive weighings do not differ by more
than 0.5 mg or until the percentage of residue complies with the prescribed li mit. (Ph. Eur. 2.4.14)

3.11）Arsenic≤ 2ppm

(1) Sample bottle: Take 1 g of the sample, dissolve it in 23ml of water, and a dd

5ml of hydrochloric acid.

(2) Standard bottle: Precisely measure 2 ml of standard arsenic solution, add 5 ml of hydrochloric acid and 21ml of water.

(3) Add 5ml of potassium iodide test solution and 5 drops of acidic stannous chloride test solution to each of the two bottles. After leaving at room tempera ture for 10 minutes, add 2g of zinc particles, immediately plug the filled airwa y (containing 60mg

of lead acetate cotton) tightly onto a triangular flask, and place the arsenic me asuring device in a 25-40 ℃ water bath for 45 minutes of reaction. Take out the mercury

bromide test strip to obtain it. The spots in the sample bottle should not be d eeper

than the spots in the standard bottle (2ppm).

3.12）Bacterial endotoxins

Take an appropriate amount of this product and dilute it with BET water to prepare a solution with a concentration of 21mg/ml. Using TAL (sensitivity: 0.125EU/ml) for testing, the bacterial endotoxin content in every 1mg of sample should be less than 0.006EU.

3.13)Microbial limit

Take this product and check it according to the microbial limit of non-sterile p roducts: microbial count method, controlled bacteria test method and the micro bial limit standard of non-sterile drugs. The Total plate counts shall not exceed 100 cfu/g, the Yeast and Mould shall not exceed 10 cfu/g, E.coli shall not be detected (cfu/g).

4) Assay (Anhydrous): 98.0 ~ 102.0 %

Precisely weigh 70 mg of the sample, dissolve in 5 mL of formic acid, add 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid. (Potentiometric titration)0.1 mol/L Perchloric acid 1 mL = 8.84 mg $C_9H_{19}N_3O_6$

5）Residual solvent(Methanol（≤3000ppm）

▪Test solution: Take 0.2 g of this product, weigh it precisely, and transfer it to a 20 ml headspace bottle. Take 1 ml of water precisely and dissolve it.

▪ Sample storage solution:

Accurately weigh 4g of this product into a 10ml volumetric flask, add water t o dissolve to the mark, shake well to obtain.

▪ Reference solution

Reference solution: Take approximately 0.6g to 100ml of methanol (accurate to 0.0002g) into a volumetric flask, weigh accurately, add water to the mark, shake well, and obtain the reference stock solution. Accurately measure 1ml of reference stock solution and 5ml of test stock solution into the same 10ml volumetric flask, dilute with water to the mark, shake well, and transfer 1ml into a 20ml headspace bottle, seal.

▪blank solution: Place in a 20 mL headspace vial and add 1 mL of water to use as a blank test solution.

▪System suitability: When the standard solution is injected repeatedly six times, it is appropriate that the RSD (%) of the retention time and area value of the methanol peak is 5.0% or less.

▪Operation

Gas chromatography		Condition
Detector		Flame ionization(FID)
Column		DB-624 Capillary(G43), 0.25 mm x 30 m, 1.4㎛
Temp.	Detector	280 ℃
	Oven	40 ℃(5 min)→240 ℃(Rate 20℃/ min)(10 min)
Carrier gas		nitrogen
Split Ratio		20 : 1
Flows		1) Hydrogen : 40 mL/min, 2) Air : 400.0 mL/min

Head-space	Condition
Sample equilibration temperature	80 ℃
Quantitative loop temperature	120 ℃
Transmission line temperature	120 ℃
Equilibrating Time	20 min

Injection Time	1 min(mL)
GC cycle time	25 min

▪Calculation

$$\text{Residue of MeOH (ppm)} = \frac{\mathbf{At \times Ws \times 1 \times 10}}{\mathbf{As \times 1000 \times Wt}} \times \frac{\mathbf{Ps}}{\mathbf{100}}$$

At : Peak area of residual solvent in the sample solution

As : Peak area of residual solvent in standard solution

1: Dilution factor of sample

1000 : Dilution of standard solution

Wt : Weight of Sample (mg)

Ws : Weight of Standard (mg)

Ps : Purity of Standard