

STABILITY STUDIES OF THE CONSTITUTED SOLUTION OF AMPICILLIN FOR INJECTION

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1. Purpose

According to the storage condition and application method, to observe the clinical application concentration of ampicillin sodium for injection, and the stability for different period under 30℃, so as to provide a reference for clinical application.

2. Test preparation

2.1 Reagent:

Sterile water for injection

Batch No.: 1411263203

Source: Shijiazhuang No. 4 Pharmaceutical Co., Ltd.

0.9% sodium chloride injection

Batch No.: 1409143202

Source: Shijiazhuang No. 4 Pharmaceutical Co., Ltd.

Glucose

Batch No.: 20120321

Source: Tianjin Yongda Co., Ltd.

5% glucose solution: preparing when using

2.2 Dissolving process

Dissolve 0.5g of ampicillin sodium for injection in 2ml of sterile Water for Injection, the concentration is 250 mg/mL.

Dissolve 0.5g of ampicillin sodium for injection in 5ml of sterile Water for Injection, the concentration is 100 mg/mL.

Dissolve 3 vials of ampicillin sodium for injection 0.5g in 50ml of sterile Water for Injection, the concentration is 30 mg/mL.

Dissolve 3 vials of ampicillin sodium for injection 0.5g in 50ml of 0.9% sodium chloride Injection, the concentration is 30 mg/mL.

Dissolve 0.5g of ampicillin sodium for injection in 25ml of 5% glucose solution, the concentration is 20 mg/mL.

2.3 Storage condition

Temperature: 30°C

Sampling time point: 0 h, 2 h, 4 h, 8 h, 12 h, 24 h

2.4 Type of instrument

HPLC: Agilent 1100: DE23921115

Chromatographic column: C18 Batch No: 8018824 Source: Dikma

Particle analyzer: GWF-5J

pH meter: MP225

2.5 Sample

Source: NCPC

Batch No.: 150601, specification: 0.5g

Water: 0.8%

2.6 Reference standard

Ampicillin working standard

Source: NCPC

Batch No.: EU140914

Labeled amount: 85.8% (E) 867µg/mg (U)

3. Test basis

USP

4. Member and responsibility

Department	Name	Post
QC center	Zhang Yuge	Director of instrument office
	Zhao Cuili	Technical director of instrument office
	Luo Yuhan	Technician of instrument office
	Liu Gaiyun	Analyst of instrument office
	Li Xinjuan	Director of No. 1 chemical laboratory
	Wang Qingjia	Technician of No. 1 chemical laboratory
	Liu Shirong	Analyst of No. 1 chemical laboratory

Training evaluation: through the training of the validation protocol, the group members can understand the purpose and regulation of the test deeply, the training effect is good.

Evaluated by: Zhao Cuili

Evaluation time: Jun. 24, 2015

5. Procedure

5.1 Observation of assay stability

a) Chromatographic condition

Column: 250mm×4.6 mm, packing C18 5μm, Flow rate: 2.0ml/min; detection wavelength: 254nm; injection volume: 20 μl.

b) 12% acetic acid solution: measure 12ml glacial acetic acid, dilute to 100mL with purified water, mix well.

c) Mobile phase:

Mobile phase A: add 5ml of 12% acetic acid solution to 50ml of 1mol/L monopotassium phosphate solution (dilute 13.61g of monopotassium

phosphate to 100ml with purified water), add 400ml of acetonitrile, dilute to 4545ml with purified water, mix well, prepare for use after suction filtration.

Mobile phase B: acetonitrile

Mobile phase: mobile phase A – mobile phase B (98:2)

d) Uracil solution: dissolve and dilute uracil to 50ml with purified water, shake well.

e) Resolution solution: dissolve and dilute 50mg of ampicillin working standard and 6mg of caffeine to 50ml volumetric flask with purified water, shake well as resolution solution.

f) Reference solution: weigh two volumes of ampicillin reference, 50mg per volume, dilute with purified water and transfer to two-thirds of 50ml volumetric flask, dissolve to scale with purified water by ultrasound, shake well as reference solution 1 and reference solution 2.

g) Test solution:

250mg/ml sterile water for injection: take 100mg of the sample into 100ml volumetric flask, accurately weigh, dilute with 0.4ml sterile water for injection to the solution of 250mg/ml, store at 30°C, gradually dilute the solution at 0 h, 2 h, 4 h, 8 h, 12 h and 24 h respectively to reach the concentration of 1mg/ml.

100mg/ml sterile water for injection: take 100mg of the sample into 100ml volumetric flask, accurately weigh, dilute with 1ml sterile water for injection to the solution of 100mg/ml, store at 30°C, gradually dilute the solution at 0 h, 2 h, 4 h, 8 h, 12 h and 24 h respectively to reach the concentration of 1mg/ml.

30mg/ml sterile water for injection: take 1500mg of the sample, accurately weigh, dilute to 50ml with sterile water for injection to the solution of 30mg/ml, store at 30°C, draw two portions of 3.3ml at 0 h, 2 h, 4 h, 8 h, 12 h and 24 h respectively, dilute to 100ml volumetric flask with purified water to reach the concentration of 1mg/ml.

30mg/ml 0.9% sodium chloride injection: take 1500mg of the sample,

accurately weigh, dilute to 50ml with 0.9% sodium chloride injection to the solution of 30mg/ml, store at 30°C, draw two portions of 3.3ml at 0 h, 2 h, 4 h, 8 h, 12 h and 24 h respectively, dilute to 100ml volumetric flask with purified water to reach the concentration of 1mg/ml.

20mg/ml 5% glucose solution: take 500mg of the sample, accurately weigh, dilute to 25ml with 5% glucose solution to the solution of 20mg/ml, store at 30°C, draw two portions of 5ml at 0 h, 2 h, 4 h, 8 h, 12 h and 24 h respectively, dilute to 100ml volumetric flask with purified water to reach the concentration of 1mg/ml.

h) Analysis procedure

Inject the resolution solution once, record the chromatogram, select the resolution mode from the report template and print the chromatogram, record the resolution between ampicillin peak and caffeine peak, should not less than 2.0.

Inject the reference solution 1 once, record the chromatogram, calculate the tailing factor of ampicillin peak should not more than 1.4.

Inject the uracil solution once, record the retention time of uracil and reference solution, calculate the capacity factor should not more than 2.5.

Inject the reference solution 1 continuously 5 times, record the chromatogram, calculate the relative standard deviation of major peak area should not more than 1.0%.

Inject the reference solution 2 once, take the fifth injection chromatogram of reference solution 1 and injection chromatogram of reference solution 2, calculate the correction factor with chromatographic work station and obtain the average value.

Inject the test solution respectively once, record the chromatogram, retention time of the major peak of test solution should be correspond with that of the reference solution, calculate the assay of ampicillin with external method by the chromatographic work station, obtain the average value,

observe the changes of component of the test sample.

5.2 Observation of solution clarity and color

a) Dissolution of test sample

250mg/ml sterile water for injection: take 18 vials of test sample (0.5g), add 36ml of sterile water for injection.

100mg/ml sterile water for injection: take 6 vials of test sample (0.5g), add 30ml of sterile water for injection.

30mg/ml sterile water for injection: take 3 vials of test sample (0.5g), add 50ml of sterile water for injection.

30mg/ml 0.9% sodium chloride injection: take 3 vials of test sample (0.5g), add 50ml of 0.9% sodium chloride injection.

20mg/ml 5% glucose solution: take 2 vials of test sample (0.5g), add 50ml of 5% glucose solution.

b) Analysis of test sample

Place the test solution dissolved with the above-mentioned method at 30°C, take 5ml at 0h, 2h, 4h, 8h, 12h and 24h, test the solution clarity and color.

5.3 Observation of particulate matter

a) Dissolution of test sample

250mg/ml sterile water for injection: take 90 vials of test sample (0.5g), add 180ml of sterile water for injection.

100mg/ml sterile water for injection: take 36 vials of test sample (0.5g), add 180ml of sterile water for injection.

30mg/ml sterile water for injection: take 12 vials of test sample (0.5g), add 200ml of sterile water for injection.

30mg/ml 0.9% sodium chloride injection: take 12 vials of test sample (0.5g), add 200ml of 0.9% sodium chloride injection.

20mg/ml 5% glucose solution: take 8 vials of test sample (0.5g), add 200ml of

5% glucose solution.

b) Analysis of test sample

Place the test solution dissolved with the above-mentioned method at 30°C, take 5ml at 0h, 2h, 4h, 8h, 12h and 24h and test for 4 times, the data for the first time is ignored, calculate the average value for the last 3 times results, check the number of particulate matter greater than 10µm and 25µm every vial of test sample at every time point.

5.4 Observation of pH

a) Dissolution of test sample

250mg/ml sterile water for injection: take 18 vials of test sample (0.5g), add 36ml of sterile water for injection.

100mg/ml sterile water for injection: take 6 vials of test sample (0.5g), add 30ml of sterile water for injection.

30mg/ml sterile water for injection: take 3 vials of test sample (0.5g), add 50ml of sterile water for injection.

30mg/ml 0.9% sodium chloride injection: take 3 vials of test sample (0.5g), add 50ml of 0.9% sodium chloride injection.

20mg/ml 5% glucose solution: take 2 vials of test sample (0.5g), add 50ml of 5% glucose solution.

b) Analysis of test sample

Place the test solution dissolved with the above-mentioned method at 30°C, take 5ml at 0h, 2h, 4h, 8h, 12h and 24h, test pH value.

5.5 Acceptance criteria

Ref. No.	Item	Specification
1	Assay (calculated on the	845µg/mg~988µg/mg of ampicillin

	anhydrous basis)	
2	Clarity and colour of solution	Clarity: not more pronounced than that of reference suspension I. Color: not more than that of reference solution YG ₅
3	Particulate matter	≥10μm: ≤ 6000 particles/vial ≥25μm: ≤ 600 particles/vial
4	pH	8.0~10.0

6. Work table

Content	Description	Quantity
Work table 1	Observation table for Ampicillin sodium for injection 250mg/ml (sterile water for injection)	1
Work table 2	Observation table for Ampicillin sodium for injection 100mg/ml (sterile water for injection)	1
Work table 3	Observation table for Ampicillin sodium for injection 30mg/ml (sterile water for injection)	1
Work table 4	Observation table for Ampicillin sodium for injection 30mg/ml (0.9% sodium chloride injection)	1
Work table 5	Observation table for Ampicillin sodium for injection 20mg/ml (5% glucose solution)	1

Work table 1 Observation table for Ampicillin sodium for injection (0.5g)

Test results of Ampicillin sodium for injection (0.5g) after being dissolved by sterile water for injection

Concentration / temperature	Time (h)	Assay (anhydrous basis) (μg/mg)	Clarity	Color	pH	Particulate matter	
						≥10μm	≥25μm
250mg/ml 30℃	0	899	0.25	2	9.13	293	14
	2	894	0.25	3	9.06	135	3
	4	890	0.25	3	8.72	93	3
	8	894	0.25	3	8.61	68	3
	12	888	0.25	3	8.53	108	11
	24	887	0.25	3	8.43	148	14

Conclusion: from the work table 1, under the condition of 30℃, the test results of ampicillin sodium for injection 0.5g after being dissolved by sterile water for injection meet the specification at 24h, therefore, the solution is stable in 24h.

Work table 2 Observation table for ampicillin sodium for injection (0.5g)

Test results of ampicillin sodium for injection (0.5g) after being dissolved by sterile water for injection

Concentration / temperature	Time (h)	Assay (anhydrous basis) (μg/mg)	Clarity	Color	pH	Particulate matter	
						≥10μm	≥25μm
100mg/ml 30℃	0	893	0.25	1	9.00	413	10
	2	894	0.25	2	8.91	160	6
	4	890	0.25	2	8.61	85	3
	8	891	0.25	2	8.50	96	11
	12	890	0.25	2	8.44	223	14
	24	888	0.25	2	8.38	337	44

Conclusion: from the work table 2, under the condition of 30℃, the test results of ampicillin sodium for injection 0.5g after being dissolved by sterile water for injection meet the specification at 24h, therefore, the solution is stable in 24h.

Work table 3 Observation table for ampicillin sodium for injection (0.5g)

Test results of ampicillin sodium for injection (0.5g) after being dissolved by sterile water for injection

Concentration / temperature	Time (h)	Assay (anhydrous basis) (µg/mg)	Clarity	Color	pH	Particulate matter	
						≥10µm	≥25µm
30mg/ml 30℃	0	898	0.25	1	8.88	700	17
	2	896	0.25	1	8.79	209	9
	4	895	0.25	1	8.54	141	5
	8	892	0.25	2	8.41	184	10
	12	890	0.25	2	8.32	189	17
	24	880	0.25	2	8.23	303	24

Conclusion: from the work table 3, under the condition of 30℃, the test results of ampicillin sodium for injection 0.5g after being dissolved by sterile water for injection meet the specification at 24h, therefore, the solution is stable in 24h.

Work table 4 Observation table for ampicillin sodium for injection (0.5g)

Test results of ampicillin sodium for injection (0.5g) after being dissolved by
0.9% sodium chloride injection

Concentration / temperature	Time (h)	Assay (anhydrous basis) (µg/mg)	Clarity	Color	pH	Particulate matter	
						≥10µm	≥25µm
30mg/ml 30℃	0	897	0.25	1	8.60	657	10
	2	896	0.25	1	8.50	111	0
	4	893	0.25	1	8.38	82	10
	8	891	0.25	1	8.24	30	2
	12	898	0.25	1	8.16	64	12
	24	877	0.25	1	8.06	164	22

Conclusion: from the work table 4, under the condition of 30℃, the test results of ampicillin sodium for injection 0.5g after being dissolved by 0.9% sodium chloride injection meet the specification at 24h, therefore, the solution is stable in 24h.

Work table 5 Observation table for ampicillin sodium for injection (0.5g)

Test results of ampicillin sodium for injection (0.5g) after being dissolved by
5% glucose solution

Concentration / temperature	Time (h)	Assay (anhydrous basis) (µg/mg)	Clarity	Color	pH	Particulate matter	
						≥10µm	≥25µm
20mg/ml 30℃	0	899	0.25	1	8.70	745	15
	2	898	0.25	1	8.56	250	10
	4	889	0.25	1	8.35	110	10
	8	894	0.25	1	8.15	30	3
	12	897	0.25	1	8.04	265	20
	24	831	0.25	1	7.91	475	78

Conclusion: from the work table 5, under the condition of 30℃, the test results of ampicillin sodium for injection 0.5g after being dissolved by sterile water for injection meet the specification at 12h, assay and pH are out of specification at 24h, therefore, the solution is stable in 12h.

7. Summary

According to the above test results, the recommended storage condition for ampicillin sodium for injection 0.5g is listed as follows:

- 7.1 The constituted solution of ampicillin sodium for injection after being dissolved by sterile water for injection (250mg/ml) remains stable at 30°C for 24 hours.
- 7.2 The constituted solution of ampicillin sodium for injection after being dissolved by sterile water for injection (100mg/ml) remains stable at 30°C for 24 hours.
- 7.3 The constituted solution of ampicillin sodium for injection after being dissolved by sterile water for injection (30mg/ml) remains stable at 30°C for 24 hours.
- 7.4 The constituted solution of ampicillin sodium for injection after being dissolved by 0.9% sodium chloride injection (30mg/ml) remains stable at 30°C for 24 hours.
- 7.5 The constituted solution of ampicillin sodium for injection after being dissolved by 5% glucose solution (20mg/ml) remains stable at 30°C for 12 hours.

Date of completion: Jun. 30, 2015