Promoting the
QUALITY OF MEDICINES Plus

Amoxicillin Job Aid to Assist with Laboratory Testing

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The Promoting the Quality of Medicines Plus (PQM+) Program is a five-year cooperative agreement between USAID and USP to sustainably strengthen medical product quality assurance systems in low- and middle-income countries. The program works to improve medical product quality through cross-sectoral and systems strengthening approaches and the application of international quality assurance standards across the pharmaceutical system. By sharing scientific expertise and providing technical support and leadership, PQM+ helps create resilient and robust local health systems that address diseases such as HIV/AIDS, tuberculosis, malaria, and neglected tropical diseases, as well as improve maternal, newborn, and child health.

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Acronyms

API	active pharmaceutical ingredients
Bacterial Endotoxin Test	BET
BPCRS	The British Pharmacopoeia Chemical Reference Substance
BP	British Pharmacopeia
EPCRS	The European Pharmacopoeia Chemical Reference Substance
cS	concentration of sample solution
cU	concentration of standard solution
DT	dispersible tablet
FPP	finished pharmaceutical product
F	conversion factor
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
NLT	no less than
NMT	no more than
ODS	Octadecyl-silica
P	Potency
PQM+	Promoting the Quality of Medicines Plus
PQR	product quality review
Q	quantity of dissolved active
rS	peak response from sample solution
rU	peak response from standard solution
Rf	retention factor
RF	response Factor
RS	reference standard
SOP	standard operating procedure
TLC	thin layer chromatography
TS	test solution
USAID	U.S. Agency for International Development
USP	U.S. Pharmacopeial Convention
UV	Ultraviolet
V	Volume

This document is a compilation of information to assist in the testing of amoxicillin active pharmaceutical ingredient (API) and finished pharmaceutical products (FPPs).

Amoxicillin Dispersible Tablet (DT)

Tests	USP
Identification	**By thin layer chromatography (TLC):** – Apply 5 µL of a 4mg/ml solution of USP Amoxicillin RS in 0.1 N HCl and an aqueous dispersion of the tablet that contains 4mg/ml of Amoxicillin in 0.1 N HCl within 10 minutes on a 0.25-mm layer of chromatographic silica gel mixture. – Use a solvent system of methanol, chloroform, pyridine, and water (90:80:1:30). **Acceptance criteria**: – The *RF* value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution.*
Assay by HPLC	**Assay by high performance liquid chromatography (HPLC)** **Mobile phase preparation:** – Acetonitrile and *Diluent* (1:24) – Decrease the acetonitrile concentration to increase the retention time of amoxicillin. **Sample preparation:** – Prepare a dispersion of 20 tablets for oral suspension using a suitable aliquot of water. – Dilute a portion of the dispersion with *Diluent* to obtain a solution containing 1.2 mg/mL of amoxicillin. – Pass a portion of the solution through a filter of 1-µm or finer pore size. – Use this solution within 6 hours. **Standard preparation:** – Prepare a 1.2 mg/mL of USP Amoxicillin RS in *Diluent*. – Use this solution within 6 hours. **Diluent preparation:** – Prepare a 6.8 g/L of monobasic potassium phosphate in water. – Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of 5.0 ± 0.1. **Test** **Chromatography system:** – Mode: LC – Detector: UV 230 nm – Column: 4-mm × 25-cm – Packing L1

Tests	USP
	– Flow rate: 1.5 mL/min – Injection volume: 10 µL **System suitability:** – Sample: *Standard solution* **Suitability requirements:** – Capacity factor: 1.1–2.8 – Column efficiency: 1700 theoretical plates – Tailing factor: NMT 2.5 – Relative standard deviation: NMT 2.0% **Analysis:** Calculate the percentage of the labeled amount of amoxicillin (C16H19N3O5S) in the portion of tablets for oral suspension taken: **Result = (*rU*/*rS*) × (*CS*/*CU*) × *P* × *F* × 100** – Acceptance criteria: 90.0–110.0%
Disintegration	– Use a suitable disintegration test apparatus with water heated at 20 ± 50C. – Acceptance criteria: NMT 3 minutes
Dissolution	**Mobile phase preparation:** – Acetonitrile and *Buffer* (10:390). – Pass through a filter of 0.5-µm or finer pore size. **Standard solution preparation:** – 0.05 mg/mL of USP Amoxicillin RS in *Buffer*. – Use this solution within 6 hours. **Sample solution preparation:** – Pass a portion of the sample through a filter of 0.5-µm or finer pore size. – Dilute a suitable aliquot of the filtrate with water to obtain a concentration of 0.045 mg/mL of amoxicillin. – Use this solution within 6 hours. **Test:** – Use apparatus 2 on RPM of 75 with 900 ml water and run for 30 minutes. **Chromatographic system:** – Use an HPLC model – Detector: UV 230 nm – Columns-guard: 2-mm × 2-cm – Packing L2

Tests	USP
	Analytical: – 3.9-mm × 30-cm – Packing L1 – Column temperature: 40 ± 1° – Flow rate: 0.7 mL/min – Injection volume:10 µ **System suitability:** Sample: *Standard solution* **Suitability requirements:** – Capacity factor: 1.1–2.8 – Column efficiency 1700 theoretical plates – Tailing factor: NMT 2.5 – Relative standard deviation: NMT 1.5% **Analysis**: **Result = (*rU*/*rS*) × *CS* × *V* × *D* × *P* × *F* × (1/*L*) × 100** **Tolerances:** – NLT 80% (*Q*) of the labeled amount of amoxicillin (C16H19N3O5S) is dissolved.
Dispersion fineness	– Place 2 tablets for oral suspension in 100 mL of water and stir until completely dispersed. A smooth dispersion that passes through a No. 25 sieve is obtained.
Uniformity of dosage units	– Should meet with USP ⟨905⟩

Amoxicillin Tablet

Test	USP	Minilab
Identification	**Retention time from the HPLC assay** **Acceptance criteria:** – The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.	**Stock sample preparation**: Transfer a powdered tablet to a bottle and shake for 3 minutes by adding 22.5 ml water followed by 2ml of conc ammonia and stele. **Working sample preparation**: Pipette 1 ml of the stock sample solution into a 10-ml vial and add 3 ml of methanol. Close and shake the vial and label as *Amoxicillin Working Sample Solution*.

Test	USP	Minilab
		Standard stock preparation: Powder a reference std tablet in 45 ml water and 5 ml conc ammonia. **Working standard preparation**: Pipette 1 ml of the stock standard solution into a 10-ml vial and add 3 ml of methanol (100%). Pipette 1 ml of the stock standard solution into a 10-ml vial and add 4 ml of methanol (80%). **Development solution:** Pipette 15 ml of ethyl acetate, 5 ml of glacial acetic acid, and 5 ml of water into the jar being used as TLC developing chamber. **Spotting:** Mark an origin line parallel to and about 1.5 cm from the bottom edge of the chromatoplate and apply 2 µl of each test and standard solution prepared as shown in the picture opposite, using the microcapillary pipettes supplied. **Detection:** Dry off all residual solvent and observe the chromatoplate under UV light of 254 nm before and after iodine staining. Use these methods of detection for amoxicillin identification and quantification purposes. **Acceptance criteria**: Rf value of the sample and standard solutions should be the same.
Assay	**Buffer preparation:** – 6.8 g/L of monobasic potassium phosphate in water. – Adjust with 45% potassium hydroxide TS to a pH of 5.0 ± 0.1. **Mobile phase preparation:**	**Acceptance criteria**: The spot for amoxicillin in the chromatogram obtained with the test solution must correspond in terms of colour, size, intensity, shape, and travel distance to that in the chromatogram obtained with the lower and higher standard solution.

Test	USP	Minilab
	– Prepare a mixture of Acetonitrile and *Buffer* (1:24). **Standard solution preparation:** – Prepare a 1.2 mg/mL of USP Amoxicillin RS in *Buffer*. – Use this solution within 6 hours. **Sample solution preparation:** – Place NLT 5 tablets in a high-speed glass blender jar containing *Buffer* sufficient to yield a concentration of 1 mg/mL of anhydrous amoxicillin. – Blend for 4 ± 1 min, allow to stand for 5 min, and centrifuge a portion of the mixture. **Test** **Chromatographic system:** – Mode: LC – Detector: UV 230 nm – Column: 4-mm × 25-cm 10-µm packing L1 low rate: 1.5 mL/min and injection volume: 10 µL **System suitability:** Sample: *Standard solution* **Suitability requirements:** – Tailing factor: NMT 2.5 – Relative standard deviation: NMT 2.0% **Analysis:** **Result = (*rU*/*rS*) × (*CS*/*CU*) × *P* × *F* × 100** **Acceptance criteria:** – 90.0–120.0%	
Dissolution	**Buffer preparation:** – Place 27.2 g of monobasic potassium phosphate in 3 L of water. – Adjust with 45% potassium hydroxide TS to a pH of 5.0 ± 0.1. – Dilute with water to obtain 4 L of solution. **Mobile phase preparation:** – Acetonitrile and *Buffer* (10:39).	

Test	USP	Minilab
	– Pass through a filter of 0.5-µm or finer pore size. **Standard solution preparation:** – 0.05 mg/mL of USP Amoxicillin RS in *Buffer*. – Use this solution within 6 hours. **Sample solution preparation:** – Pass a portion of the sample through a filter of 0.5-µm or finer pore size. – Dilute a suitable aliquot of the filtrate with water to obtain a concentration of 0.045 mg/mL of amoxicillin. –Use this solution within 6 hours. **Test:** – Use apparatus 2, rpm of 75, with 900 ml water and run for 30 minutes. **Chromatographic system:** – Use an HPLC Model – Detector: UV 230 nm – Columns-guard: 2-mm × 2-cm – Packing L2 **Analytical**: 3.9-mm × 30-cm – Packing L1 – Column temperature: 40 ± 1° – Flow rate: 0.7 mL/min and injection volume: 10 µ **System suitability:** Sample: *Standard solution* **Suitability requirements:** – Capacity factor: 1.1–2.8 – Column efficiency 1700 theoretical plates – Tailing factor: NMT 2.5 – Relative standard deviation: NMT 1.5% **Analysis**: **Result = (*rU*/*rS*) × *CS* × *V* × *D* × *P* × *F* × (1/*L*) × 100**	

Test	USP	Minilab
	Tolerances: – NLT 75% (*Q*) of the labeled amount of amoxicillin (C16H19N3O5S) is dissolved. – For products labeled as chewable tablets, proceed as directed above. — For chewable Tablets labeled to contain 200 or 400 mg Time: 20 min **Tolerances:** – NLT 70% (*Q*) of the labeled amount of amoxicillin (C16H19N3O5S) is dissolved. – For chewable tablets labeled to contain 125 or 250 mg Time: 90 min **Tolerances:** – NLT 70% (*Q*) of the labeled amount of amoxicillin (C16H19N3O5S) is dissolved. – For veterinary products, proceed as directed above, except use *Apparatus 2* at 100 rpm.	
Disintegration	**NA**	1. Place one tablet or capsule into a 100–150 mL wide-neck bottle containing 100 mL water at close to 37 ± 2° C. 2. Stir or shake the liquid by swirling the bottle periodically; continue for 30 minutes. [Note: For data documentation/record, you may continue until complete disintegration is observed.] 3. Read and record the time (in minutes) when disintegration is complete. Repeat the test on 5 additional tablets or capsules. **Acceptance criteria: NMT 30 minutes**

Amoxicillin Capsule

Test	USP	Minilab
Identification	**Retention time from the HPLC assay**	Same as the tablet form

Test	USP	Minilab
	Acceptance criteria: – The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.	
Assay	**Buffer preparation:** – 6.8 g/L of monobasic potassium phosphate in water. – Adjust with 45% potassium hydroxide TS to a pH of 5.0 ± 0.1. **Mobile phase preparation:** – Prepare a mixture of Acetonitrile and *Buffer* (1:24). **Standard solution preparation:** – Prepare a 1.2 mg/mL of USP Amoxicillin RS in *Buffer* – Use this solution within 6 hours. **Sample solution preparation:** – Place NLT 5 tablets in a high-speed glass blender jar containing *Buffer* sufficient to yield a concentration of 1 mg/mL of anhydrous amoxicillin. – Blend for 4 ± 1 min, allow to stand for 5 min, and centrifuge a portion of the mixture. **Test** **Chromatographic system:** – Mode: LC – Detector: UV 230 nm – Column: 4-mm × 25-cm – 10-µm packing L1 – Flow rate: 1.5 mL/min – Injection volume:10 µL **System suitability:** Sample: *Standard solution* **Suitability requirements:** – Tailing factor: NMT 2.5 – Relative standard deviation: NMT 2.0% **Analysis** **Result = (*rU*/*rS*) × (*CS*/*CU*) × *P* × *F* × 100** **Acceptance criteria:**	

Test	USP	Minilab
	90.0–120.0%	
Dissolution	**Buffer preparation:** – Place 27.2 g of monobasic potassium phosphate in 3 L of water. – Adjust with 45% potassium hydroxide TS to a pH of 5.0 ± 0.1. – Dilute with water to obtain 4 L of solution. **Mobile phase preparation:** – Acetonitrile and *Buffer* (10:39). – Pass through a filter of 0.5-µm or finer pore size. **Standard solution preparation:** – Place 0.05 mg/mL of USP Amoxicillin RS in *Buffer*. – Use this solution within 6 hours. **Sample solution preparation:** – Pass a portion of the sample through a filter of 0.5-µm or finer pore size. – Dilute a suitable aliquot of the filtrate with water to obtain a concentration of 0.045 mg/mL of amoxicillin. – Use this solution within 6 hours. **Test:** – Use apparatus 2, rpm of 100, with 900 ml water and run for 90 minutes. **Chromatographic system:** – Use an HPLC model – Detector: UV 230 nm – Columns-guard: 2-mm × 2-cm – Packing L2 **Analytical**: 3.9-mm × 30-cm – Packing L1 – Column temperature: 40 ± 1° – Flow rate: 0.7 mL/min and injection volume: 10 µ **System suitability:** Sample: *Standard solution* **Suitability requirements:** – Capacity factor: 1.1–2.8	

Test	USP	Minilab
	– Column efficiency 1700 theoretical plates – Tailing factor: NMT 2.5 – Relative standard deviation: NMT 1.5% **Analysis**: **Result = $(rU/rS) \times CS \times V \times D \times P \times F \times (1/L) \times 100$** **Tolerances:** – NLT 80% (Q) of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) is dissolved.	
Uniformity of dosage units	– Should meet with USP ⟨905⟩	

Amoxicillin Oral Suspension

Tests	USP
Identification	– Shake a portion of oral suspension with a mixture of acetone and 0.1 N hydrochloric acid (4:1) to obtain a solution containing about 1 mg of amoxicillin per mL. The solution responds to the *Identification* test under Amoxicillin Capsules.
Water determination	– Not more than 2.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol.
Assay	**Iodometry:** **Standard preparation:** – Prepare as directed for Standard Preparation under Iodometric Assay—Antibiotics ⟨425⟩, using USP Amoxicillin RS. **Sample preparation:** – Using the dosing pump, deliver a number of doses of oral suspension, equivalent to about 250 mg of amoxicillin, to a separator containing 100 mL of hexanes and shake vigorously. Add 140 mL of water and shake for 5 minutes. Allow the layers to separate and drain the lower, aqueous layer into a 250-mL volumetric flask. Repeat the extraction with two 50-mL portions of water. Combine the aqueous extracts in the volumetric flask, dilute with water to volume, and mix. **Test:** – Proceed with Oral Suspension as directed for Procedure under Iodometric Assay—Antibiotics ⟨425⟩, using USP Amoxicillin RS. Calculate the quantity, in mg, of amoxicillin ($C_{16}H_{19}N_3O_5S$) in each dose of oral suspension taken by the formula. **Analysis** **Result = $(250/N)(F/2000)(B - I)$** in which N is the number of doses taken, and the other terms are as defined therein.

Amoxicillin Sodium for Injection

Tests	British Pharamacopeia (2020)	Remarks
Identity	**Test A:** The infrared absorption spectrum, Appendix II A (refer to BP), is concordant with the reference spectrum of amoxicillin sodium (RS 010). **Test B:** Carry out the method for TLC, Appendix III A (refer to BP), using the following solutions: (1) Dissolve a quantity of the contents of a sealed container in sufficient sodium hydrogen carbonate solution to produce a solution containing the equivalent of 0.25% w/v of amoxicillin. (2) 0.25% w/v of amoxicillin trihydrate BPCRS in sodium hydrogen carbonate solution. (3) 0.25% w/v of each of amoxicillin trihydrate BPCRS and ampicillin trihydrate BPCRS in sodium hydrogen carbonate solution. **Chromatographic conditions:** (a) Use a TLC silica gel F254 silanised plate (Merck silanised silica gel 60 F254s (RP-18) plates are suitable). (b) Use the mobile phase described below. (c) Apply 1 µL of each solution. (d) Develop the plate to 15 cm. (e) After removal of the plate, allow it to dry in air, expose it to iodine vapour until spots appear, and examine in daylight. **Mobile phase:** 10 volumes of acetone and 90 volumes of a 15.4% w/v solution of ammonium acetate adjusted to pH 5.0 with glacial acetic acid. **System suitability:** The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots. **Confirmation:** The principal spot in the chromatogram obtained with solution (1) is similar in position, colour, and size to that in the chromatogram obtained with solution (2). **Test C:** Yields reaction B characteristic of sodium salts, Appendix VI. (refer to BP). – The area of any other secondary peak is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%). – The sum of the areas of all the secondary peaks is not greater than 9 times the area of the principal peak in the chromatogram obtained with solution (2) (9%).	

Tests	British Pharamacopeia (2020)	Remarks
	– Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).	
Alkalinity test	**TESTS for** **Alkalinity:** pH of a solution containing the equivalent of 10% w/v of amoxicillin, 8.0 to 10.0, Appendix V L (refer to BP).	
Water content	**Water:** Not more than 4.0% w/w, Appendix IX C (refer to BP). Use 0.3 g.	
Bacterial endotoxin test	**Bacterial endotoxins:** – Carry out the test for bacterial endotoxins, Appendix XIV C (refer to BP). – Dissolve the contents of the sealed container in water BET to give a solution containing the equivalent of 10 mg per mL of amoxicillin (solution A). – The endotoxin limit concentration of solution A is 2.5 IU of endotoxin per mL.	
Related substances	**Related substances** Carry out the method for liquid chromatography, Appendix III D (refer to BP), using the following solutions: (1) Add 80 mL of mobile phase A to a quantity of the contents of a sealed container containing the equivalent of 0.15 g of amoxicillin and shake for 15 minutes. Mix with the aid of ultrasound for 1 minute, add sufficient mobile phase A to produce 100 mL, mix and filter. (2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A. (3) Add 1 mL of water to 0.2 g of amoxicillin trihydrate BPCRS, shake, and add, dropwise, dilute sodium hydroxide solution to obtain a solution. (The pH of the solution is about 8.5.) Store the solution at room temperature for 4 hours and dilute 0.5 mL to 50 mL with mobile phase A. (4) 0.0004% w/v of cefadroxil BPCRS and 0.003% w/v of amoxicillin trihydrate BPCRS in mobile phase A. **Chromatographic conditions:** (a) Use a stainless steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 µm) (Hypersil 5 ODS is suitable). (b) Use gradient elution and the mobile phase described below. (c) Use a flow rate of 1 mL per minute. (d) Use an ambient column temperature.	

Tests	British Pharamacopeia (2020)	Remarks
	(e) Use a detection wavelength of 254 nm. (f) Inject 50 µL of each solution. **Mobile phase** **Mobile phase A**: Mix 1 volume of acetonitrile and 99 volumes of a pH 5.0 buffer solution prepared in the following manner: to 250 mL of 0.2m potassium dihydrogen orthophosphate, add 2m sodium hydroxide until the pH reaches 5.0 and add sufficient water to produce 1000 mL. **Mobile phase B:** Mix 20 volumes of acetonitrile and 80 volumes of the pH 5.0 buffer solution. Equilibrate the column with a mobile phase ratio A:B of 92:8. Inject solutions (1) and (2) and start the elution isocratically with the chosen mobile phase. Immediately after elution of the amoxicillin peak, start a linear gradient elution to reach a mobile phase ratio A:B of 1:100 over a period of 25 minutes. Continue the chromatography with mobile phase B for 15 minutes; then equilibrate the column for 15 minutes with the mobile phase chosen originally. Inject mobile phase A and use the same elution gradient to obtain a blank. Inject solution (3). The three main peaks eluted after the principal peak correspond to amoxicillin diketopiperazine, amoxicillin dimer, and amoxicillin trimer. The retention times of these peaks relative to that of the principal peak are about 3.4, 4.1, and 4.5 respectively. **System suitability:** The test is not valid unless, in the chromatogram obtained with solution (4), the resolution factor between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the composition of the mobile phase. **Limits:** In the chromatogram obtained with solution (1): The area of any peak corresponding to amoxicillin dimer is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%).	
Uniformity of dosage unit	Determine the weight of the contents of 10 containers as described in the test for uniformity of weight, Appendix XII C1 (refer to BP), Powders for Parenteral Administration.	
Assay by HPLC	Carry out the method for liquid chromatography, Appendix III D (refer to BP), using the following solution:. (1) Add 80 mL of mobile phase A to a quantity of the mixed contents of the 10 containers containing the equivalent of 60 mg of amoxicillin and shake for 15 minutes. Mix with the aid of ultrasound for 1 minute, add sufficient mobile phase A to produce 100 mL, mix and filter (Whatman GF/C filter paper is suitable).	Specification: 90.0–105.0% of the stated amount

Tests	British Pharamacopeia (2020)	Remarks
	(2) 0.070% w/v of amoxicillin trihydrate BPCRS in mobile phase A. (3) 0.0004% w/v of cefadroxil BPCRS and 0.003% w/v of amoxicillin trihydrate BPCRS in mobile phase A. **Chromatographic conditions:** (a) Use a stainless steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 µm) (Hypersil 5 ODS is suitable). (b) Use isocratic elution and the mobile phase described below. (c) Use a flow rate of 1 mL per minute. (d) Use an ambient column temperature. (e) Use a detection wavelength of 254 nm. (f) Inject 50 µL of each solution. **Mobile phase:** A mixture of 8 volumes of mobile phase B and 92 volumes of mobile phase A. **Mobile phase A:** Mix 1 volume of acetonitrile and 99 volumes of a 25% v/v solution of 0.2m potassium dihydrogen orthophosphate adjusted to pH 5.0 with 2m sodium hydroxide. **Mobile phase B:** Mix 20 volumes of acetonitrile and 80 volumes of a 25% v/v solution of 0.2m potassium dihydrogen orthophosphate adjusted to pH 5.0 with 2m sodium hydroxide. **System suitability:** The Assay is not valid unless, in the chromatogram obtained with solution (3), the resolution factor between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the composition of the mobile phase to achieve the required resolution. **Determination of content:** Calculate the content of C16H19N3O5S in a container of average content weight from the chromatograms obtained and from the declared content of C16H19N3O5S in amoxicillin trihydrate BPCRS.	

Co-Amoxiclav DT

Tests	British Pharmacopeia (2020)	Remark
Identification	Carry out the method for TLC, Appendix III A (refer to BP), using the following solutions: (1) Shake a quantity of the powdered tablets containing the equivalent of 0.4 g of clavulanic acid in 100 mL of a mixture of 4 volumes of	

Tests	British Pharmacopeia (2020)	Remark
	methanol and 6 volumes of 0.1m mixed phosphate buffer pH 7.0 and filter. (2) 0.4% w/v of lithium clavulanate EPCRS and 0.8% w/v of amoxicillin trihydrate BPCRS in a mixture of 4 volumes of methanol and 6 volumes of 0.1m mixed phosphate buffer pH 7.0. **Chromatographic conditions:** (a) Use a silica gel F254 precoated plate (Merck silica gel 60 F254 plates are suitable). Impregnate the plate by spraying it with a 0.1% w/v solution of disodium edetate in mixed phosphate buffer pH 4.0 and allow to dry overnight. Activate the plate by heating at 105° for 1 hour just prior to use. (b) Use the mobile phase described below. (c) Apply 1 µL of each solution. (d) Develop the plate to 15 cm. (e) After removal of the plate, dry in air and examine under ultraviolet light (254 nm). **Mobile phase:** 1 volume of butan-1-ol, 2 volumes of a 0.1% w/v solution of disodium edetate in mixed phosphate buffer pH 4.0, 6 volumes of glacial acetic acid and 10 volumes of butyl acetate. **Confirmation:** The principal spots in the chromatogram obtained with solution (1) correspond in position and colour to those in the chromatogram obtained with solution (2).	
Assay by HPLC	Weigh and powder 20 tablets. Carry out the method for liquid chromatography, Appendix III D (refer to BP), using the following solutions: (1) Dissolve, with shaking, a quantity of the powdered tablets containing the equivalent of 0.25 g of amoxicillin in 400 mL of water, add sufficient water to produce 500 mL, mix and filter. (2) 0.05% w/v of amoxicillin trihydrate BPCRS and 0.02% w/v of lithium clavulanate EPCRS in water. **Chromatographic conditions:**	Content of amoxicillin, $C_{16}H_{19}N_3O_5S$ 90.0–110.0% of the stated amount Content of clavulanic acid, $C_8H_9NO_5$ 90.0–110.0% of the stated amount

Tests	British Pharmacopeia (2020)	Remark
	(a) Use a stainless-steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 µm) (Hypersil ODS 5 µm is suitable). (b) Use isocratic elution and the mobile phase described below. (c) Use a flow rate of 2 mL per minute. (d) Use an ambient column temperature. (e) Use a detection wavelength of 220 nm. (f) Inject 20 µL of each solution. **Mobile phase:** 5 volumes of methanol and 95 volumes of a 0.78% w/v solution of sodium dihydrogen orthophosphate monohydrate, adjusted to pH 4.4 with orthophosphoric acid. **System suitability:** The assay is not valid unless, in the chromatogram obtained with solution (2), the resolution factor between the peaks due to amoxicillin and lithium clavulanate is at least 3.5 and the symmetry factor of the peak due to lithium clavulanate is at most 1.5. **Determination of content:** Calculate the content of C16H19N3O5S and of C8H9NO5 in the tablets using the declared content of C16H19N3O5S in amoxicillin trihydrate BPCRS and the declared content of C8H8LiNO5 in lithium clavulanate EPCRS. Each mg of C8H8LiNO5 is equivalent to 0.9711 mg of C8H9NO5.	
Disintegration	Comply with the requirements for **Dispersible Tablets**.	
Related substance	**Clavulanate polymer and other fluorescent impurities:** Carry out the method for fluorescence spectrophotometry, Appendix II E (refer to BP), using the following freshly prepared solutions: (1) To a quantity of the finely powdered tablets containing the equivalent of 0.1 g of clavulanic acid add 50 mL of a 0.1m phosphate buffer solution pH 5.0, prepared as described below, stir until the sample is evenly dispersed, and add	

Tests	British Pharmacopeia (2020)	Remark
	sufficient of the buffer solution to produce 100 mL. Shake the solution vigorously for 1 minute, shake mechanically for 5 minutes, and then with the aid of ultrasound for 5 minutes and filter through a 0.45-µm filter. To prepare the buffer solution, dissolve 15.6 g of sodium dihydrogen orthophosphate in 800 mL of water, adjust the pH to 5.0 using 1m sodium hydroxide, and add sufficient water to produce 1000 mL. (2) Prepare a solution containing 0.42 µg per mL of quinine sulfate BPCRS in 0.5m sulfuric acid. [Note: The fluorescence of quinine sulfate is 118 times more intense than that of an equivalent concentration of clavulanate polymer.] **Procedure:** Measure the fluorescence of the solutions using an excitation wavelength of 360 nm and an emission wavelength of 440 nm, using the phosphate buffer solution in the reference cell. **Limits:** The fluorescence obtained with solution (1) is not more intense than that obtained with solution (2) (5% w/w, calculated with respect to the content of clavulanic acid). **Related substances:** Carry out the method for liquid chromatography, Appendix III D (refer to BP), using the following solutions: (1) Disperse a quantity of the powdered tablets containing the equivalent of 30 mg of amoxicillin in 15 mL of mobile phase A with the aid of ultrasound for 20 minutes, with occasional shaking. Add sufficient mobile phase A to produce 20 mL and filter through a 0.45-µm membrane filter. (2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A. (3) 0.0004% w/v of cefadroxil BPCRS and 0.003% w/v of amoxicillin trihydrate BPCRS in mobile phase A. (4) 0.075% w/v of lithium clavulanate EPCRS in mobile phase A. **Chromatographic conditions:**	

Tests	British Pharmacopeia (2020)	Remark
	(a) Use a stainless steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 µm) (Hypersil ODS is suitable). (b) Use gradient elution and the mobile phase described below. (c) Use a flow rate of 1 mL per minute. (d) Use an ambient column temperature. (e) Use a detection wavelength of 254 nm. (f) Inject 50 µL of each solution. **Mobile phase:** Mobile phase A1 volume of acetonitrile and 99 volumes of a pH 5.0 buffer solution prepared in the following manner: To 250 mL of 0.2m potassium dihydrogen orthophosphate, add 2m sodium hydroxide until the pH reaches 5.0 and then add sufficient water to produce 1000 mL. Mobile phase B20 volumes of acetonitrile and 80 volumes of the pH 5.0 buffer solution. Use the following gradient conditions: Equilibrate the column with the mobile phase ratio established during system suitability. Inject freshly prepared solution (1) and immediately after the elution of the amoxicillin peak, start a linear gradient elution to reach a mobile phase ratio A:B of 0:100 over 25 minutes. Continue the chromatography with mobile phase B for a further 15 minutes. Equilibrate the column for 15 minutes with the starting mobile phase ratio established during system suitability before the next injection. Inject mobile phase A using the same elution gradient to obtain a blank. **System suitability:** Equilibrate the column with a mobile phase ratio A:B of 92:8. The test is not valid unless, in the chromatogram obtained with solution (3), the resolution factor between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the ratio A:B of the mobile phase. **Limits:**	

Tests	British Pharmacopeia (2020)	Remark
	In the chromatogram obtained with solution (1): The area of any peak with a retention time relative to amoxicillin of about 4.1 (amoxicillin dimer) is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%). The area of any other secondary peak is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%). Disregard any peak corresponding to the principal peak in the chromatogram obtained with solution (4).	

Co-Amoxiclav Oral Suspension

Tests	British Pharmacopeia (2020)	Remark
Identity	Carry out the method for TLC, Appendix III A (refer to BP), using the following solutions: (1) Disperse, with shaking, a quantity of the oral suspension containing the equivalent of 0.4 g of clavulanic acid in 100 mL of a mixture of 4 volumes of methanol and 6 volumes of 0.1m mixed phosphate buffer pH 7.0 and filter. (2) 0.4% w/v of lithium clavulanate EPCRS and 0.8% w/v of amoxicillin trihydrate BPCRS in a mixture of 4 volumes of methanol and 6 volumes of 0.1m mixed phosphate buffer pH 7.0. **Chromatographic conditions:** (a) Use a silica gel F254 precoated plate (Merck silica gel 60 F254 plates are suitable). Impregnate the plate by spraying it with a 0.1% w/v solution of disodium edetate in mixed phosphate buffer pH 4.0 and allow to dry overnight. Activate the plate by heating at 105° for 1 hour just prior to use. (b) Use the mobile phase described below. (c) Apply 1 µL of each solution. (d) Develop the plate to 15 cm. (e) After removal of the plate, dry in air and examine under ultraviolet light (254 nm). **Mobile phase:** 1 volume of butan-1-ol, 2 volumes of a 0.1% w/v solution of disodium edetate in mixed phosphate buffer pH 4.0, 6 volumes of glacial acetic acid and 10 volumes of butyl acetate. **Confirmation:**	

Tests	British Pharmacopeia (2020)	Remark
	The principal spots in the chromatogram obtained with solution (1) correspond in position and colour to those in the chromatogram obtained with solution (2).	
Acidity or alkalinity	**TESTS for** **Acidity or alkalinity** pH of a solution containing the equivalent of 2.5% w/v of amoxicillin, 4.0 to 7.0, Appendix V L (refer to BP).	
Assay	**ASSAY:** Carry out the method for liquid chromatography, Appendix III D (refer to BP), using the following solutions: (1) Disperse, with shaking, a quantity of the oral suspension containing the equivalent of 0.25 g of amoxicillin in 400 mL of water, add sufficient water to produce 500 mL, mix and filter. (2) 0.05% w/v of amoxicillin trihydrate BPCRS and 0.02% w/v of lithium clavulanate EPCRS in water. **Chromatographic conditions:** (a) Use a stainless steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 µm) (Hypersil ODS is suitable). (b) Use isocratic elution and the mobile phase described below. (c) Use a flow rate of 2 mL per minute. (d) Use an ambient column temperature. (e) Use a detection wavelength of 220 nm. (f) Inject 20 µL of each solution. **Mobile phase:** 5 volumes of methanol and 95 volumes of a 0.78% w/v solution of sodium dihydrogen orthophosphate monohydrate, adjusted to pH 4.4 with orthophosphoric acid. **System suitability:** The assay is not valid unless, in the chromatogram obtained with solution (2), the resolution factor between the peaks due to amoxicillin and lithium clavulanate is at least 3.5 and the symmetry factor of the peak due to lithium clavulanate is at most 1.5. **Determination of content:** Determine the weight per mL of the oral suspension, Appendix V G (refer to BP), and calculate the content of C16H19N3O5S and of C8H9NO5, weight in volume, using the declared content of C16H19N3O5S in amoxicillin trihydrate BPCRS and the declared content of C8H8LiNO5 in lithium clavulanate EPCRS.	When freshly constituted, not more than 120.0% of the stated amount. When stored at the temperature and for the period stated on the label during which the oral suspension may be expected to be satisfactory for use, not less than 80.0% of the stated amount

Tests	British Pharmacopeia (2020)	Remark
	Each mg of C8H8LiNO5 is equivalent to 0.9711 mg of C8H9NO5. Repeat the procedure using a portion of the oral suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.	
Related substance	**Clavulanate polymer and other fluorescent impurities:** Carry out the method for fluorescence spectrophotometry, Appendix II E (refer to BP), using the following freshly prepared solutions: (1) Disperse, with shaking, a quantity of the oral suspension containing the equivalent of 0.1 g of clavulanic acid with 50 mL of a 0.1m phosphate buffer solution pH 5.0, prepared as described below, shake vigorously for 1 minute and then shake with the aid of ultrasound for 5 minutes; add sufficient of the buffer solution to produce 100 mL and filter through a 0.45-µm filter. To prepare the buffer solution dissolve 15.6 g of sodium dihydrogen orthophosphate in 800 mL of water, adjust the pH to 5.0 using 1m sodium hydroxide and add sufficient water to produce 1000 mL. (2) Prepare a solution containing 0.42 µg per mL of quinine sulfate BPCRS in 0.5m sulfuric acid. [Note: The fluorescence of quinine sulfate is 118 times more intense than that of an equivalent concentration of clavulanate polymer.] **Procedure:** Measure the fluorescence of the solutions using an excitation wavelength of 360 nm and an emission wavelength of 440 nm, using the phosphate buffer solution in the reference cell. **Limits:** The fluorescence obtained with solution (1) is not more intense than that obtained with solution (2) (5% w/w, calculated with respect to the content of clavulanic acid). **Related substances:** Carry out the method for liquid chromatography, Appendix III D (refer to BP), using the following solutions: (1) Disperse, with shaking, a quantity of the oral suspension containing the equivalent of 30 mg of amoxicillin in 15 mL of mobile phase A. Add sufficient mobile phase A to produce 20 mL and filter through a 0.45-µm membrane filter. (2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase A.	

Tests	British Pharmacopeia (2020)	Remark
	(3) 0.0004% w/v of cefadroxil BPCRS and 0.003% w/v of amoxicillin trihydrate BPCRS in mobile phase A. (4) 0.075% w/v of lithium clavulanate EPCRS in mobile phase A. **Chromatographic conditions:** (a) Use a stainless steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 µm) (Hypersil ODS is suitable). (b) Use gradient elution and the mobile phase described below. (c) Use a flow rate of 1 mL per minute. (d) Use an ambient column temperature. (e) Use a detection wavelength of 254 nm. (f) Inject 50 µL of each solution. **Mobile phase:** Mobile phase A1 volume of acetonitrile and 99 volumes of a pH 5.0 buffer solution prepared in the following manner: To 250 mL of 0.2m potassium dihydrogen orthophosphate, add 2m sodium hydroxide until the pH reaches 5.0 and then add sufficient water to produce 1000 mL. Mobile phase B20 volumes of acetonitrile and 80 volumes of the pH 5.0 buffer solution. Use the following **gradient** conditions: Equilibrate the column with the mobile phase ratio established during system suitability. Inject freshly prepared solution (1) and immediately after the elution of the amoxicillin peak, start a linear gradient elution to reach a mobile phase ratio A:B of 0:100 over 25 minutes. Continue the chromatography with mobile phase B for a further 15 minutes. Equilibrate the column for 15 minutes with the starting mobile phase ratio established during system suitability before the next injection. Inject mobile phase A using the same elution gradient to obtain a blank. **System suitability:** Equilibrate the column with a mobile phase ratio A:B of 92:8. The test is not valid unless, in the chromatogram obtained with solution (3), the resolution factor between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the ratio A:B of the mobile phase. **Limits:** In the chromatogram obtained with solution (1):	

Tests	British Pharmacopeia (2020)	Remark
	The area of any peak with a retention time relative to amoxicillin of about 4.1 (amoxicillin dimer) is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%). The area of any other secondary peak is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%). Disregard any peak corresponding to the principal peak in the chromatogram obtained with solution (4) and any peaks due to excipients.	

Co-Amoxiclav for Injection

Identity	**Test A:** Carry out the method for TLC, Appendix III A (refer to BP), using the following solutions: (1) Shake a quantity of the contents of the sealed container containing the equivalent of 0.4 g of clavulanic acid in 100 mL of a mixture of 4 volumes of methanol and 6 volumes of 0.1m mixed phosphate buffer pH 7.0 and filter. (2) 0.4% w/v of lithium clavulanate EPCRS and 0.8% w/v of amoxicillin trihydrate BPCRS in a mixture of 4 volumes of methanol and 6 volumes of 0.1m mixed phosphate buffer pH 7.0. **Chromatographic conditions:** (a) Use as the coating silica gel F254 (Merck silica gel 60 F254 plates are suitable). Impregnate the plate by spraying it with a 0.1% w/v solution of disodium edetate in mixed phosphate buffer pH 4.0 and allow to dry overnight. Activate the plate by heating at 105° for 1 hour prior to use. (b) Use the mobile phase as described below: (c) Apply 1 µL of each solution. (d) Develop the plate to 15 cm. (e) After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm). **Mobile phase:** 1 volume of butan-1-ol, 2 volumes of a 0.1% w/v solution of disodium edetate in mixed phosphate buffer pH 4.0, 6 volumes of glacial acetic acid and 10 volumes of butyl acetate. **Confirmation:** The principal spots in the chromatogram obtained with solution (1) are similar in position and colour to those in the chromatogram obtained with solution (2). Test B: In the Assay, the retention time of the two principal peaks in the chromatogram obtained with solution (1) correspond to those in the chromatogram obtained with solution (2).	
Alkalinity test	**TESTS for** **Alkalinity:** pH of a solution containing the equivalent of 10% w/v of amoxicillin, 8.0 to 10.0, Appendix V L (refer to BP).	
Related substance	**Clavulanate polymer and other fluorescent impurities:**	

	Carry out the method for fluorescence spectrophotometry, Appendix II E (refer to BP), using the following freshly prepared solutions: (1) To a quantity of the contents of a sealed container containing the equivalent of 0.1 g of clavulanic acid add 50 mL of a 0.1m phosphate buffer solution pH 5.0, prepared as described below, shake vigorously for 1 minute and then shake with the aid of ultrasound for 5 minutes; add sufficient of the buffer solution to produce 100 mL and filter through a 0.45-µm filter. To prepare the buffer solution, dissolve 15.6 g of sodium dihydrogen orthophosphate in 800 mL of water, adjust the pH to 5.0 using 1m sodium hydroxide, and add sufficient water to produce 1000 mL. (2) Prepare a solution containing 0.42 µg per mL of quinine sulfate BPCRS in 0.5m sulfuric acid. [Note: The fluorescence of quinine sulfate is 118 times more intense than that of an equivalent concentration of clavulanate polymer.] **Procedure:** Measure the fluorescence of the solutions using an excitation wavelength of 360 nm and an emission wavelength of 440 nm, using the phosphate buffer solution in the reference cell. **Limits:** The fluorescence obtained with solution (1) is not more intense than that obtained with solution (2) (5% w/w, calculated with respect to the content of clavulanic acid) **Related substances:** Carry out the method for liquid chromatography, Appendix III D (refer to BP), using the following freshly prepared solutions: (1) Dilute a quantity of the contents of a sealed container containing the equivalent of 0.1 g of amoxicillin in sufficient mobile phase A to produce 200 mL. (2) 0.0057% w/v of amoxicillin trihydrate BPCRS in mobile phase A. (3) 0.05% w/v of amoxicillin impurity standard BPCRS in mobile phase A. **Chromatographic conditions:** (a) Use a stainless steel column (5 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (3 µm) (Spherisorb S3 ODS2 is suitable). (b) Use gradient elution and the mobile phase described below: (c) Use a flow rate of 1.5 mL per minute. (d) Use a column temperature of 20°. (e) Use a detection wavelength of 230 nm. (f) Inject 20 µL of each solution.	

	Mobile phase: **Mobile phase A:** Dissolve 15.6 g of sodium dihydrogen orthophosphate in 1000 mL of water and adjust the pH to 4.2 with orthophosphoric acid. **Mobile phase B:** Mix 10 volumes of mobile phase A with 90 volumes of methanol. When the chromatograms are recorded under the prescribed conditions, the retention times relative to Amoxicillin (retention time, about 3 minutes) are: impurity D, about 0.8; impurity J, about 3.0 and 3.2. **System suitability:** The test is not valid unless the chromatogram obtained with solution (3) resembles the chromatogram supplied with amoxicillin impurity standard BPCRS and the resolution between the peaks due to amoxicillin and impurity D is at least 2.0. **Limits:** In the chromatogram obtained with solution (1): The area of any peak due to impurity D is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (5%). The area of any peak due to impurity J (the peak may appear as a doublet) is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (5%). The area of any other secondary peak is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (3%). The sum of the areas of all the secondary peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (15%).	
Water content	**Water:** Not more than 3.5% w/w, Appendix IX C (refer to BP). Use 0.5 g.	
Bacterial endotoxin test	**Bacterial endotoxins:** Carry out the test for bacterial endotoxins, Appendix XIV C (refer to BP). Dissolve the contents of the sealed container in water BET to give a solution containing the equivalent of 10 mg per mL of amoxicillin (solution A). The endotoxin limit concentration of solution A is 2.5 IU of endotoxin per mL.	
Assay	Determine the weight of the contents of 10 containers as described in the test for uniformity of weight, Appendix XII C1 (refer to BP), Powders for Parenteral Administration. Carry out the method for liquid chromatography, Appendix III D (refer to BP), using the following solutions:	Content of amoxicillin, $C_{16}H_{19}N_3O_5S$

	(1) Dissolve, with shaking, a quantity of the mixed contents of the 10 containers containing the equivalent of 0.1 g of amoxicillin in sufficient water to produce 100 mL, mix and filter. (2) 0.11% w/v of amoxicillin trihydrate BPCRS and 0.02% w/v of lithium clavulanate EPCRS in water. **Chromatographic conditions:** (a) Use a stainless steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 µm) (Hypersil ODS is suitable). (b) Use isocratic elution and the mobile phase described below. (c) Use a flow rate of 1 mL per minute. (d) Use an ambient column temperature. (e) Use a detection wavelength of 230 nm. (f) Inject 10 µL of each solution. **Mobile phase:** 80 volumes of methanol and 920 volumes of a 1.56% w/v solution of sodium dihydrogen orthophosphate in water, the pH of which has been adjusted to 4.0 with orthophosphoric acid. **System suitability:** The Assay is not valid unless, in the chromatogram obtained with solution (2), the resolution between the peaks due to amoxicillin and lithium clavulanate is at least 8.0. **Determination of content:** Calculate the content of C16H19N3O5S and C8H9NO5 in a container of average content weight using the declared content of C16H19N3O5S in amoxicillin trihydrate BPCRS and the declared content of C8H8LiNO5 in lithium clavulanate EPCRS. Each mg of C8H8LiNO5 is equivalent to 0.9711 mg of C8H9NO5.	90.0–107.5% of the stated amount Content of clavulanic acid, C8H9NO5 90.0–107.5% of the stated amount.

References

United States Pharmacopeia – National Formulary (USP 43–NF 38)

British Pharmacopeia (BP 2020)