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Promoting the Quality of Medicines Plus (PQM+)

Gentamicin Injection Job Aid to Assist with Dossier Preparation

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usp®

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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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Contents

Acronyms

ADJ PH	pH adjustment
API	active pharmaceutical ingredient
CPP	critical process parameter
CQA	critical quality attributes
EMA	European Medicines Agency
FDA	U.S. Food and Drug Administration
IID	Inactive Ingredient Database
MDE	maximum daily exposure
MPPUD	maximum potency per unit dose
PQM+	Promoting the Quality of Medicines Plus
UNII	unique ingredient identifier
USAID	U.S. Agency for International Development
USP	U.S Pharmacopeial Convention
w/v	weight in volume
WHO	World Health Organization

Overview

This document is a job aid to enable quick access to technical information by those preparing dossiers for gentamicin. The information consists of guidance on physicochemical and toxicological properties; analysis, formulation, and manufacturing stability; storage; and controls of gentamicin. The information herein is adapted from the Promoting the Quality Medicines + program's Gentamicin Product Information Report (January 2023).

Product formulation is described in the Product Information Report.

Aid for Common Technical Document* Module 2.3.P.4 – Control of Excipients (Gentamicin Sulfate Injection)

* Common Technical Document (CTD)

Table 1
List of excipients and their proposed function with Inactive Ingredient Database (IID) limits for Gentamicin injection, U.S. Pharmacopeial Convention (USP) pediatric 20 mg per 2 mL by Fresenius Kabi, USA.

Ingredients	Function	Reference	IID Limit	Usual Recommended Concentration
Sodium hydroxide (UNII†: 55X04QC32I)	pH adjustment	(Fresenius Kabi, 2021); (FDA^, 2022)	pH adjustment (ADJ) PH*	N/A
Sulfuric acid (UNII: O40UQP6WCF)	pH adjustment	(Fresenius Kabi, 2021); (FDA, 2022)	ADJ PH*	N/A

† Unique Ingredient Identifier (UNII)
^ U.S. Food and Drug Administration (FDA)

Table 2.
List of excipients and their proposed function with IID limits for gentamicin sulfate injection, USP 80 mg/2 mL by Hospira, USA.

Ingredients	Function	Reference	IID Limit	Usual Recommended Concentration
Sodium metabisulfite (UNII: 4VON5FNS3C)	Antioxidant	(Hospira, 2021); (FDA, 2022). USAID. (n.d.) p. 18	40 mg maximum daily exposure (MDE)	2.9 mg in 1 mL
Edetate disodium anhydrous (UNII: 8NLQ36F6MM)	Chelating agent	(Hospira, 2021); (FDA, 2022); (Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 255	0.01% weight in volume (w/v) maximum potency per unit dose (MPPUD)** Intramuscular **MPPUD: 3 mg MDE* Intravenous	0.1 mg in 1 mL
Methylparaben (UNII: A2I8C7HI9T)	Preservatives	(Hospira, 2021); (FDA, 2022)	14 mg MDE* Intramuscular 5%w/v MPPUD** Intravenous	1.8 mg in 1 mL
Propylparaben (UNII: Z8IX2SC1OH)	Preservatives	(Hospira, 2021); (FDA, 2022)	2 mg MDE* Intramuscular, Intravenous	0.2 mg in 1 mL
Sodium hydroxide (UNII: 55X04QC32I)	pH adjustment	(Hospira, 2021); (FDA, 2022)	ADJ PH	N/A

Ingredients	Function	Reference	IID Limit	Usual Recommended Concentration
Sulfuric acid (UNII: O40UQP6WCF)	pH adjustment	(Hospira, 2021); (FDA, 2022)	315 mg MDE* Intramuscular, Intravenous	N/A
Water (UNII: 059QF0KO0R)	Solvent	(Hospira, 2021); (Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 802		

Table 3.
List of excipients and their proposed function for Cidomycin 80 mg/2 mL solution for injection by SANOFI UK

Ingredients	Function	Reference	IID Limit	Usual Recommended Concentration
Sodium chloride	Tonicity agent	(FDA, 2022); (Sanofi, 2022); (Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 671. (FDA, 2022)	0.86%w/v MPPUD** Intramuscular 340 mg MDE* Intramuscular 1080 mg MDE* Intravenous	≤0.9%
Water for Injection	Solvent	(Sanofi, 2022); (Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 802		
2N Sodium hydroxide (10%)	pH adjustment	(Sanofi, 2022)		N/A
Sulfuric acid	pH adjustment	(Sanofi, 2022)		N/A

Table 4.
List of excipients and their proposed function for gentamicin 10mg/mL solution for injection or infusion by Wockhardt UK, Ltd.

Ingredients	Function	Reference	IID Limit	Usual Recommended Concentration
Sodium metabisulfite (E223)	Antioxidant	(FDA, 2022). (USAID, n.d.) p.18; (Wockhardt, 2022)	40 mg MDE*	2.9 mg in 1 mL
Sodium hydroxide	pH adjustment	(Wockhardt, 2022)		N/A

Ingredients	Function	Reference	IID Limit	Usual Recommended Concentration
Sulfuric acid (10%)	pH adjustment	(Wockhardt, 2022)		N/A
Water for injections	Solvent	(Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 802. (Wockhardt, 2022)		

Table 5.
List of excipients and their proposed function with IID limits for gentamicin 40 mg/mL solution for injection or infusion by Noridem Enterprises, Ltd.

Ingredients	Function	Reference	IID Limit	Usual Recommended Concentration
Disodium edetate	Chelating agent	(FDA, 2022); (Noridem, 2021); (Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 255	0.01%w/v MPPUD** Intramuscular 3 mg MDE* Intravenous	0.005 and 0.1% w/v
Sodium metabisulfite (E223)	Antioxidant	(FDA, 2022); (Noridem, 2021). (USAID. n.d.) p. 18	40 mg MDE*	2.9 mg in 1 mL
Sodium hydroxide 1 N	pH adjustment	(Noridem, 2021);		N/A
Sulfuric acid 0.5 M	pH adjustment	(Noridem, 2021);		N/A
Water for injections	Solvent	(Noridem, 2021); (Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 802		

Table 6.
List of excipients and their proposed function for gentamicin 40 mg/mL solution for injection or infusion by Panpharma UK, Ltd.

Ingredients	Function	Reference	IID Limit	Usual Recommended Concentration
Disodium edetate	Chelating agent	(FDA, 2022); (Panpharma, 2022); (Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 255	0.01%w/v MPPUD** Intramuscular 3 mg MDE* Intravenous	0.005 and 0.1% w/v
Sodium chloride	Tonicity agent	(FDA, 2022); (Panpharma, 2022); (Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 671	0.86%w/v MPPUD** Intramuscular 340 mg MDE* Intramuscular 1080 mg MDE* Intravenous	≤0.9%
Sulfuric acid	pH adjustment	(Panpharma, 2022)		N/A
Water for injections	Solvent	(Panpharma, 2022); (Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 802		

Table 7.
List of excipients and their proposed function for gentamicin pediatric 20mg/2 mL by Zentiva UK

Ingredients	Function	Reference	IID Limit	Usual Recommended Concentration
Sodium chloride	Tonicity agent	(FDA, 2022); (Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 671; (Zentiva, 2022)	0.86%w/v MPPUD** Intramuscular 340 mg MDE* Intramuscular 1080 mg MDE* Intravenous	≤0.9%
2M Sodium hydroxide	pH adjustment	(Zentiva, 2022)		N/A
1M Sulfuric acid	pH adjustment	(Zentiva, 2022)		N/A
Water for injections	Solvent	(Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 802; (Zentiva, 2022)		

Table 8.
List of excipients and their proposed function for Genticin (gentamicin) 40 mg/mL injectable by ADVANZ Pharma UK.

Ingredients	Function	Reference	IID Limit	Usual Recommended Concentration
Water for injection	Solvent	(Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 802		
Sulfuric acid	Acidifying Agent	(Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 758		N/A

Table 9.
List of excipients and their proposed function for Pfizer (Australia) gentamicin 80 mg/2mL (as sulfate) injection British Pharmacopeia ampoule (11376) by Pfizer Australia Pty, Ltd.

Ingredients	Function	Reference	IID Limit	Usual Recommended Concentration
Disodium edetate	Chelating agent	(FDA, 2022); (Pfizer, 2022); (Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 255	0.01%w/v MPPUD** Intramuscular 3 mg MDE* Intravenous	0.005 and 0.1% w/v
Water for injections	Solvent	(Pfizer, 2022); (Rowe, Sheskey, Owen, & American Pharmacists Association, 2006) p. 802		
Sodium hydroxide	pH adjustment	(Pfizer, 2022)		N/A
Sulfuric acid	pH adjustment	(Pfizer, 2022)		N/A

Note. Unlike the FDA IID, the European Medicines Agency (EMA) has not developed a database of excipients in approved drug products (Elder & Faïs, 2019).

Aid for CTD Module 3.2.P.8 – Stability (Gentamicin Sulfate)

Gentamicin sulfate injection is stable at room temperature. Therefore, there is no requirement for cold chain storage. The shelf life of the product from different manufacturers varies from two to four years. The storage conditions are: “Do not store above 25°C. Do not refrigerate or freeze. Protect from light.” Table 10, below, shows shelf life and storage conditions of some approved products by the FDA, EMA, and Australian Therapeutic Goods Administration.

Table 10.
Shelf life and storage condition of some approved products by US FDA, EMA and Australian Therapeutic Goods Administration (USAID, n.d.).

Product manufacturer	Shelf Life	Storage Condition	Reference
Fresenius Kabi, USA	Not specified	Store at 20–25˚C. [See USP, Controlled room temperature.]	(USAID, n.d.); (Fresenius Kabi, 2021).
Hospira, USA.	Not specified	Store at 20–25˚C. [See USP, Controlled room temperature.]	(USAID, n.d.); (Hospira, 2021).
SANOFI UK	3 years	Do not store above 25°C. Do not refrigerate or freeze. Store in the original package in order to protect from light.	(USAID, n.d.); (Sanofi, 2022).
Wockhardt UK Ltd	2 years	Do not store above 25ºC. Do not refrigerate or freeze. Store in the original package in order to protect from light.	(USAID, n.d.); (Wockhardt, 2022).
Noridem Enterprises Ltd.	3 years	This medicinal product does not require any special storage conditions. Do not refrigerate or freeze.	(Noridem, 2021).
Panpharma UK Ltd.	3 years	Store below 30ºC	(Panpharma, 2022).
Zentiva UK.	2 years	Do not store above 25ºC. Do not refrigerate or freeze.	(Zentiva, 2022).
ADVANZ Pharma UK	4 years	Do not store above 25ºC. Do not freeze.	(ADVANZ, 2021).
Pfizer Australia Pty Ltd.	2 years	Store below 25°C. Protect from light.	(USAID, n.d.); (Pfizer, 2022).

Aid for CTD Module 2.3. P.5 Control of Drug Product (Gentamicin Sulfate Injection)

Table 11.
Standard quality requirement of gentamicin sulfate injection
Critical process parameter (CPP)
Critical quality attributes (CQAs) of gentamicin sulfate injection (USAID, n.d.)

CQA	Acceptance Criteria	Justification
Appearance	Clear, colorless solution, free from visible particulate matter	Visual Inspection USP <1>
Identification (thin-layer chromatography)	The intensities and Rf values of the three principal spots obtained from the test solution correspond to those obtained from the standard solution	USP<621>
Assay	90.0–125.0%	USP<81>
pH	3.0–5.5	USP<791>
Bacterial endotoxins	Not more than 0.71 USP endotoxin unit/mg of gentamicin	USP<85>

CQA	Acceptance Criteria	Justification
Particulate matter	Meet the requirements for small-volume injections	USP<788>
Extractable Volume	Comply	USP<1>
Sterility	Sterile	USP <71>

Although the manufacturing procedure of gentamicin injection is simple, the key quality concern is the sterilization process, as well as the sterility of the facility where it is manufactured (Im-Amornphong & Tomazzini, 2019). When varied beyond the acceptable range limit, CPPs have an impact on the CQAs and therefore should be controlled to ensure that the process produces the desired quality of gentamicin solution (Lopes, 2014). The environment should meet Grade C cleanliness during solution preparation and Grade A for high-risk operations, such as vial filling, while the background environment for Grade A should be Grade B (WHO, 2011). The CPPs are derived from the unit operations for an injectable manufacturing process of aseptic processing by sterile filtration (Lopes, 2014; WHO, 2011).

Table 12.
Quality target product profile (QTPP) for gentamicin sulfate injection

QTPP elements	Target
Dosage form	Parenteral
Dosage strength	40 mg in 1 mL
Route of administration	Intramuscular and Intravenous
Drug product quality attributes	See CQA

Table 13:
Summary of CPPs of manufacturing process for gentamicin solution for injection

Manufacturing Step	Operations Involved	CPP
Bulk solution preparation	• Dissolve drug substance and excipients to form the bulk solution. • Purge the solution with nitrogen to remove dissolved oxygen. • pH adjustment	• Temperature of water/solution • Mixing speed and time • Dissolved oxygen no more than 0.5 mg/l • pH of solution (3.5–5.0)
Preparation of ampoules	Sterilization of ampoules by dry heat tunnel	Temperature (330°C) and time
Equipment sterilization	Sterilization of the filtration assemble and ampoule filling machine parts	• Time and temperature • 120°C for 30min
Pre-filtration	Preparation of the filtration assembly	Filter integrity tests – should not be more than 0.22 micrometers
Sterile filtration	Filtration	• Filtration pressure • Filtration time
Filling and sealing of ampoules	Filling and sealing of ampoules	• Clean room: Grade A • Line speed • Fill volume (2.1–2.2 mL)

Aid for CTD Module 2.3.P.3 – Manufacturing Process

Manufacturing Process Steps	Description
Solution preparation with pH adjustment	Dissolve excipients and active pharmaceutical ingredient (API) in WFI and adjust the pH with hydrochloric acid or sodium hydroxide to a pH of 3.0–5.5.
Pre-filtration and sterile filtration	Process involves sterilization of the bulk solution and removal of particulate matter using 0.22-micron filters.
Sterilization of ampoules and filling machine	Glass ampoules are washed and sterilized using dry heat. The filtration assembles and ampoule filling machine parts are also sterilized prior to the filling stage, using dry heat.
Filling and sealing of ampoules	This is the final stage of the manufacturing process. The bulk sterile solution of the drug product is filled into sterile, dry glass ampoules and sealed in a clean room of class 100. The fill volume is adjusted to 2.15 mL. for each ampoule.

Packaging

Neutral Type I glass vials should be used. The suitability of the container should be demonstrated, including the following properties:

- Safety
  - Glass vials and rubber stoppers must meet standard requirements, such as from USP.
  - Composition of the rubber stopper, along with a declaration from the supplier that the material is free of 2-mercapto benzothiazoles and nitrosamines, should be provided.
  - If applicable, washing and sterilization/depyrogenation should be supported by process validation data.
- Protection
  - Container integrity regarding microbial contamination should be demonstrated by microbial or dye ingress or other methods:
    - One-time test reported as part of product development
    - Routine leak testing performed as part of product manufacture
- Compatibility
  - Extractable/leachable data of the rubber stoppers should be provided.
  - Accelerated and long-term stability data on vials stored in inverted orientation should be submitted to further support absence of leachables as well as absorption.
  - Compatibility of the full packaged product (finished pharmaceutical product) with diluents (such as 5% dextrose injection or 0.9% sodium chloride as per the label instruction), if relevant, over the proposed dilution range (label) in specified containers may also need to be demonstrated (USAID, n.d.).

The manufacturing process development program or process improvement program should identify any critical process parameters that should be monitored or controlled to ensure that the product is of the desired quality. For those products intended to be sterile, an appropriate method of sterilization for the drug product and primary packaging material should be chosen and the choice justified (Tietje & Brouder, 2010).

Aid for CTD Module 3.2.S.4 – Control of Drug Substance

Analytical Profile

Active Pharmaceutical Ingredient

Gentamicin sulfate contains 20 to 40 percent of gentamicin C1, 10–30% of gentamicin C1a. The sum of gentamicins C2, C2a, and C2b is 40–60 percent. The content of gentamicin C1 is between 25 and 50 percent; the content of gentamicin C1a is between 10 and 35 percent; and the sum of the contents of gentamicin C2a and gentamicin C2 is between 25 and 55 percent (Brettler, 2020). Gentamicin is freely soluble in water but insoluble in alcohol, acetone, chloroform, ether, and benzene. Gentamicin sulfate API has been shown in studies to exhibit excellent stability under normal conditions, which can be an advantage during formulation.

Gentamicin sulfate has a potency equivalent to NLT 590 µg/mg of gentamicin, calculated on the dried basis (USP, 2022).

Aid for CTD Module 3.2.S.4 – Control of Drug Substance

Industrial Hygiene, Sampling, and Analytical Methods

Industrial hygiene is the science of keeping people safe and healthy at work and in their communities. Precautions for safe handling should be taken to avoid contact with skin and eyes. Formation of dust and aerosols should be avoided. HEPA terminated local exhaust ventilation should be considered at the point of generation of dust, fumes, or vapors. Standard measures for preventive fire protection should be undertaken.

Analytical test method

Various chromatographic techniques like liquid chromatography, gas chromatography, and mass spectrometry are used for the detection of aminoglycosides antibiotics. However, due to limitation of the ultraviolet-visible spectrophotometry technique, different types of detection techniques like corona-charged aerosol detector and electrochemical detector are used as the most powerful and versatile technique for the demonstration of these molecules in the analytical field. Analytical methods help to ensure the quality of the drug products. Ion-pairing reversed-phase liquid chromatography is widely utilized to analyze aminoglycosides by using volatile per fluorinated carboxylic acids, such as trifluoroacetic acid, pentafluoro propionic, and heptafluorobutyric acid as pairing ions in the mobile phase. This helps to retain the aminoglycosides on the column and improve the separation.

As described in European and U.S. Pharmacopeia, the analysis of gentamicin is based on a high-performance liquid chromatographic-photodiode array detector method using a C18 silica-based column. 4,5 The mobile phase contains trifluoroacetic acid, heptafluorobutyric acid, and acetonitrile. Its pH is adjusted to 2.6 by sodium hydroxide to avoid the silica bonded phase hydrolysis when exposed to lower pH conditions.

Chemical Structure/Formula

Chemical Structure/Formula

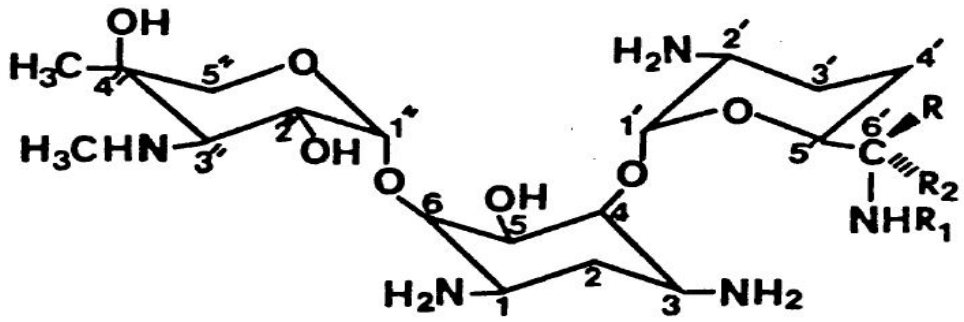
Name	CAS No.	Formula	Molecular Weight
Gentamicin sulfate	1405-41-0	$C_{60}H_{125}N_{15}O_{25}S$	1488.8g/mol
Gentamicin C1	25876-10-2	C21H43N5O7	477.6 g/mol
Gentamicin C1a	26098-04-4	C19H39N5O7	449.5 g/mol
Gentamicin C2	25876-11-3	C20H41N5O7	463.6 g/mol

Stereochemistry

Isolation and preliminary chemical studies showed that gentamicin is a complex of aminoglycoside antibiotics containing the aminocyclital 2-deoxystreptamine and two additional amino sugars (Kraisintu, 1981). The two amino sugars joined in a glycosidic linkage to a hexose nucleus. The hexose mentioned here is 2-deoxystreptamine, hence the compound is an aminoglycosidic aminocyclitol (INCHEM, 1994).

The complex is isolated as an optically active amorphous powder. It is readily soluble in water, pyridine, and dimethylformamide; moderately soluble in methanol, acetone, and ethanol; and insoluble in diethyl ether, benzene, and halogenated hydrocarbons. Chromatographic separation of the gentamicin complex shows it consists of three major components, designated as C_1, C_2 and C_{1a}.

Figure 1.



a) R = R_1 = CH_3; R_2= H Gentamicin C_1

b) R = CH_3 R_1 =R_2= H Gentamicin C_2

c) R = R_2 = R_1 =H Gentamicin C_{1a}

Mass spectrometry gave M^+ peaks at m/e 477, 463 and 449 for gentamicin C_1, C_2 and C_{1a}, respectively corresponding to molecular formulae of $C_{21}H_{43}N_5O_7$, $C_{20}H_{41}N_5O_7$ and $C_{19}H_{39}N_5O_7$. The difference of 14 mass units suggests that the three compounds differ in their degree of methylation. This was confirmed by Nuclear Magnetic Resonance Spectra studies, which showed that all three components contained a tertiary C-methyl group and an N-methyl group but that gentamicin C_2 had an extra secondary C-methyl while gentamicin C_1 had both this and

an additional N-methyl. Lastly, optical rotations studies carried out confirmed that each of the gentamicin C components is dextrorotary (Kraisintu, 1981).

Aid for CTD Module 3.2.P.5 Control of Drug Product

Method of analysis

The chemical structure of gentamicin reveals the lack of chromophore in the molecule, making the direct detection of the antibiotic difficult. This means that like other aminoglycosides, gentamicin shows no ultraviolet absorbance; thus, spectrophotometry cannot be used for the analysis of this antibiotic. In addition, the problem of spectrophotometric analysis is complicated by the fact that auxiliary constituents present in drugs make direct spectrophotometric measurements practically impossible due to interference (Krzek, Woltyńska & Hubicka, 2009). For this reason, and the difficulty involved in separating its different components, USP and European Pharmacopeia both specify that the composition of gentamicin C should be determined by liquid chromatography with pulsed electrochemical detection (Rodriquez et al., 2015).

Gentamicin injection contains an amount of gentamicin sulfate equivalent to not less than 90 percent and not more than 125 percent of the labeled amount of gentamicin. It may contain suitable buffers, preservatives, and sequestering agents, unless it is intended for intrathecal use, in which case it contains only suitable tonicity agents (USP, 2018).

Methods of analysis of some gentamicin test parameters

Test	Method of Analysis (USP, 2018)
Identification	Method: Thin Layer Chromatography Procedure: Apply separately a volume of injection equivalent to 20 µg of gentamicin and the same volume of a similar preparation of USP gentamicin sulfate RS to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel having an average pore size of 6 nm. Dilute the injection with water, if necessary, to obtain a test solution containing 1000 µg of gentamicin per mL. Where the injection contains less than 1000 µg per mL, apply a volume equivalent to 20 µg of gentamicin to the chromatographic plate in separate portions of not more than 20 µL each, each application being allowed to dry before the next is applied. Place the plate in a suitable chromatographic chamber and develop the chromatogram in a solvent system consisting of the lower phase of a mixture of chloroform, methanol, and ammonium hydroxide (20:13:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and expose the plate to vapors of iodine in a detection jar containing iodine crystals: the intensities and RF values of the three principal spots obtained from the test solution correspond to those obtained from the standard solution.

Test	Method of Analysis (USP, 2018)
Assay	Notes: For substances like gentamicin, which are not easily quantified by chemical or physical means, it is still necessary to express quantities of biological activity in units of biological potency, each defined by an authoritative reference standard. The potency of the antibiotic is designated in either units (U) or µg of activity. Two general techniques are employed: the cylinder-plate (or plate) assay and the turbidimetric (or tube) assay. The cylinder-plate technique is used for gentamicin. Method: Cylinder-Plate Assay The cylinder-plate assay depends on diffusion of the antibiotic from a vertical cylinder through a solidified agar layer in a petri dish or plate. The growth of the specific microorganisms inoculated into the agar is prevented in a circular area or "zone" around the cylinder containing the solution of the antibiotic.
Bacterial endotoxins test	Notes: The bacterial endotoxins test detects or quantifies endotoxins from Gram-negative bacteria using amoebocyte lysate from the horseshoe crab (Limulus polyphemus or Tachypleus tridentatus). Method: There are three techniques for this test: the gel-clot technique, which is based on gel formation; the turbidimetric technique, based on the development of turbidity after cleavage of an endogenous substrate; and the chromogenic technique, based on the development of color after cleavage of a synthetic peptide-chromogen complex. Any of the three techniques for the test is recommended for gentamicin. In the event of doubt or dispute, the final decision is made based upon the gel-clot limit test. The test is carried out in a manner that avoids endotoxin contamination.
pH	Notes: By definition, pH is equal to $-\log_{10}[H+]$. Where, H+ is the activity of the hydrogen (H+) or hydronium ion (H_3O+), and the hydrogen ion activity very closely approximates the hydrogen ion concentration. Method: pH is the value given by a suitable, properly calibrated, potentiometric sensor and measuring system. The measuring system has traditionally been referred to as the "pH meter." While the pH meter is still in common use, the measuring system can also be embedded inside the pH sensor, and the pH signal can be transmitted digitally to an external device such as a computer, programmable logic controller, distributed control system, data acquisition system, terminal, or another microprocessor-controlled device.
Particulate Matter in Injections	Notes: Particulate matter in injections and parenteral infusions consists of extraneous mobile undissolved particles, other than gas bubbles, unintentionally present in the solutions. Method: For the determination of particulate matter, Light Obscuration Particle Count Test or Microscopic Particle Count Test are usually used. When examining injections and parenteral infusions for subvisible particles, Light Obscuration Particle Count Test is preferably applied. Generally, it may be necessary to test some preparations by the Light Obscuration Particle Count Test followed by the Microscopic Particle Count Test to reach a conclusion on conformance to the requirements.

Aid for CTD Module 3.2.S.7 Stability

The API of gentamicin is its inorganic salts (i.e., gentamicin hydrochloride and gentamicin sulfate, the latter being the most used in formulation of finished products). When the aqueous solution of gentamicin was heated 100ºC for 30 minutes across a pH range of 2 to 12, its activity was not significantly altered (Luedemann & Weinstein, 1963). This simply implies that the compound is relatively stable in both acid and alkaline media. Among the four gentamicin substituent—C1, C1a, C2, and C2a—C2 is reported to be more adversely affected by heat (Mullins et al., 2016). It is worth noting that reconstituted gentamicin aliquots were stable for a

period of 1 year at -20ºC and 15 days at 37ºC across a wide range of pH (*Gentamicin Sulfate - CAS 1405-41-0 - Calbiochem | 345814*, n.d.). Gentamicin sulfate is resistant to heat degradation (Wang et al., 2004), and reported to be autoclavable, further indicating that gentamicin is thermostable beyond 120ºC (*Gentamicin Sulfate - CAS 1405-41-0 - Calbiochem | 345814*, n.d.). The available finished product of injectable gentamicin sulfate has a shelf life of two to four years when stored below 25ºC; and the reconstituted solution can remain stable for 24 hours at 25°C, and longer than 24 hours at 2–8°C when diluted with the infusion fluids—that is, 0.9 percent sodium chloride or 5 percent glucose solution (European Medicines Agency, 2019).

References

Al-Amoud, A. I., Clark, B. J., & Chrystyn, H. (2002). Determination of gentamicin in urine samples after inhalation by reversed-phase high-performance liquid chromatography using pre-column derivatisation with o-phthalaldehyde. *Journal of Chromatography* B, 769(1), 89-95.

Bym, K. Clase, Z. Ekeocha, and F. Masekela. (2022). Gentamicin product information report. Biotechnology Innovation and Regulatory Science Center, Purdue University. Retrieved from https://engineering.purdue.edu/BIRS/news/purdue-birs-gentamicin-pir-for-united-states-pharmacopeia/Final%20PIR%20BIRS%2012-1-22.pdf

Chénglìxiá et al. (2010). Preparation method of gentamicin injection, Chinese Patent No. CN101897664A.

Cox, C. E. (1970). Gentamicin. *Medical Clinics of North America*, 54(5), 1305–1315.

CoxGad, E. S. (2008). *Pharmaceutical Manufacturing Handbook: Production and Processes*.

European Agency for the Evaluation of Medicinal Products Veterinary Medicines and Information Technology. (2000). Committee for Veterinary Medicinal Products, Gentamicin Summary Report 2. Retrieved from https://www.ema.europa.eu/en/documents/mrl-report/gentamicin-summary-report-2-committee-veterinary-medicinal-products_en.pdf

Fresenius Kabi. (2021). Gentamicin injection, USP. Pediatric 20mg per 2ml. Highlights of prescribing information. Retrieved from https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=8bdeef7f-ad8e-46e0-a872-e945eae51d68

Fresenius Kabi USA, LLC. (2013). Gentamicin Injection. U.S. Food and Drug Administration. https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/062366s033lbl.pdf

Hospira, Inc. (2022, October). *GENTAMICIN SULFATE IN SODIUM CHLORIDE- gentamicin sulfate injection, solution.* Retrieved 30 January 2023, from https://labeling.pfizer.com/ShowLabeling.aspx?id=4471.

Hospira. (2021). Gentamicin sulfate injection, USP 80mg/2ml. Highlights of prescribing information. Retrieved from https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=bb2e10d2-1500-4dde-8281-8ab44abf28f0

Millipore Sigma. (n.d.). Gentamicin Sulfate—CAS 1405-41-0—Calbiochem. Retrieved 13 October 2022, from https://www.merckmillipore.com/INTL/en/product/Gentamicin-Sulfate-CAS-1405-41-0-Calbiochem,EMD_BIO-345814

United States Agency for International Development (USAID). (n.d.). *GENTAMICIN – Manual for Procurement & Supply of Quality-Assured MNCH Commodities*. Retrieved from https://www.ghsupplychain.org/sites/default/files/2019-02/MNCH%20Commodities-Gentamicin.pdf

United States Agency for International Development (USAID). (n.d.). *Manual for Procurement & Supply of Quality-Assured Maternal, Newborn and Child Health Commodities*. Retrieved from https://www.americanpharmaceuticalreview.com/Featured-Articles/119645-Quality-by-Design-QbD-of-Sterile-Dosage-Form-Packaging/

United States Agency for International Development (USAID) Global Health Supply Chain

Program. (2019). *Manual for Procurement and Supply of Quality Assured Maternal Newborn and Child Health Commodities*. In U. G. H. S. C. Program (Ed.), (Vol. Module III, pp. 4). Retrieved from https://www.ghsupplychain.org/sites/default/files/2019-07/MNCH%20Commodities-FinalCombined.pdf

United States Food and Drug Administration. (2022). Inactive Ingredient Search for Approved Drug Products. Data Through: 1 October 2022. Database Last Updated 19 October 2022. Retrieved from https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm?event=BasicSearch.page

United States Pharmacopeia. (2023). General Chapter ⟨1⟩ Injections and Implanted Drug Products (Parenterals)—Product Quality Tests. USP-NF. Rockville, MD: United States Pharmacopeia. Retrieved from https://doi.usp.org/USPNF/USPNF_M98730_03_01.html

United States Pharmacopeia. (2022). Gentamicin Sulfate. USP-NF. Rockville, MD: United States Pharmacopeia. Retrieved from https://doi.org/10.31003/USPNF_M34850_04_01

William, C. (1964). Manufacture of Gentamicin, U.S. Patent No. 3,136,704. Washington, DC: U.S. Patent and Trademark Office.

Wockhardt UK Ltd. (EMA) (2022). Gentamicin 10mg/ml Solution for Injection or Infusion. Highlights of prescribing information. Retrieved from https://www.medicines.org.uk/emc/product/2407/smpc

Wockhardt UK Ltd. (EMA) (2022). Gentamicin 10mg/ml Solution for Injection or Infusion Summary of Product Characteristics. Retrieved from https://www.medicines.org.uk/emc/product/2407#PHARMACOKINETIC_PROPS

Wockhardt UK Ltd. (EMA) (2019). Gentamicin 40mg/ml Solution for Injection/Infusion: Summary of Product Characteristics. Retrieved from https://www.medicines.org.uk/emc/product/12877/smpc#INCOMPATIBILITIES

Wockhardt UK Ltd. (EMA) (2018). Assessment report for gentamicin (solution for infusion/solution for injection). Committee for Medicinal Products for Human Use. Retrieved from: https://www.ema.europa.eu/en/documents/referral/assessment-report-article-53-procedure-gentamicin_en.pdf

Wockhardt UK Ltd (EMA) (n.d.). Gentamicin 40mg/ml Solution for Injection or Infusion—Summary of Product Characteristics. Retrieved 18 November 2022 from https://www.medicines.org.uk/emc/product/2394/smpc

World Health Organization. (2011). WHO good manufacturing practices for sterile pharmaceutical products. Retrieved from https://www.who.int/docs/default-source/medicines/norms-and-standards/guidelines/production/trs961-annex6-gmp-sterile-pharmaceutical-products.pdf?sfvrsn=61682f0c_0

World Health Organization. (2009). Practical guidance for scaling up health service innovations. World Health Organization. Retrieved from https://apps.who.int/iris/bitstream/handle/10665/44180/9789241598521_eng.pdf;jsessionid=748C8434D4A61D790296FD900E2FB023?sequence=1