Promoting the Quality of Medicines Plus (PQM+)

Product Information Report: Gentamicin

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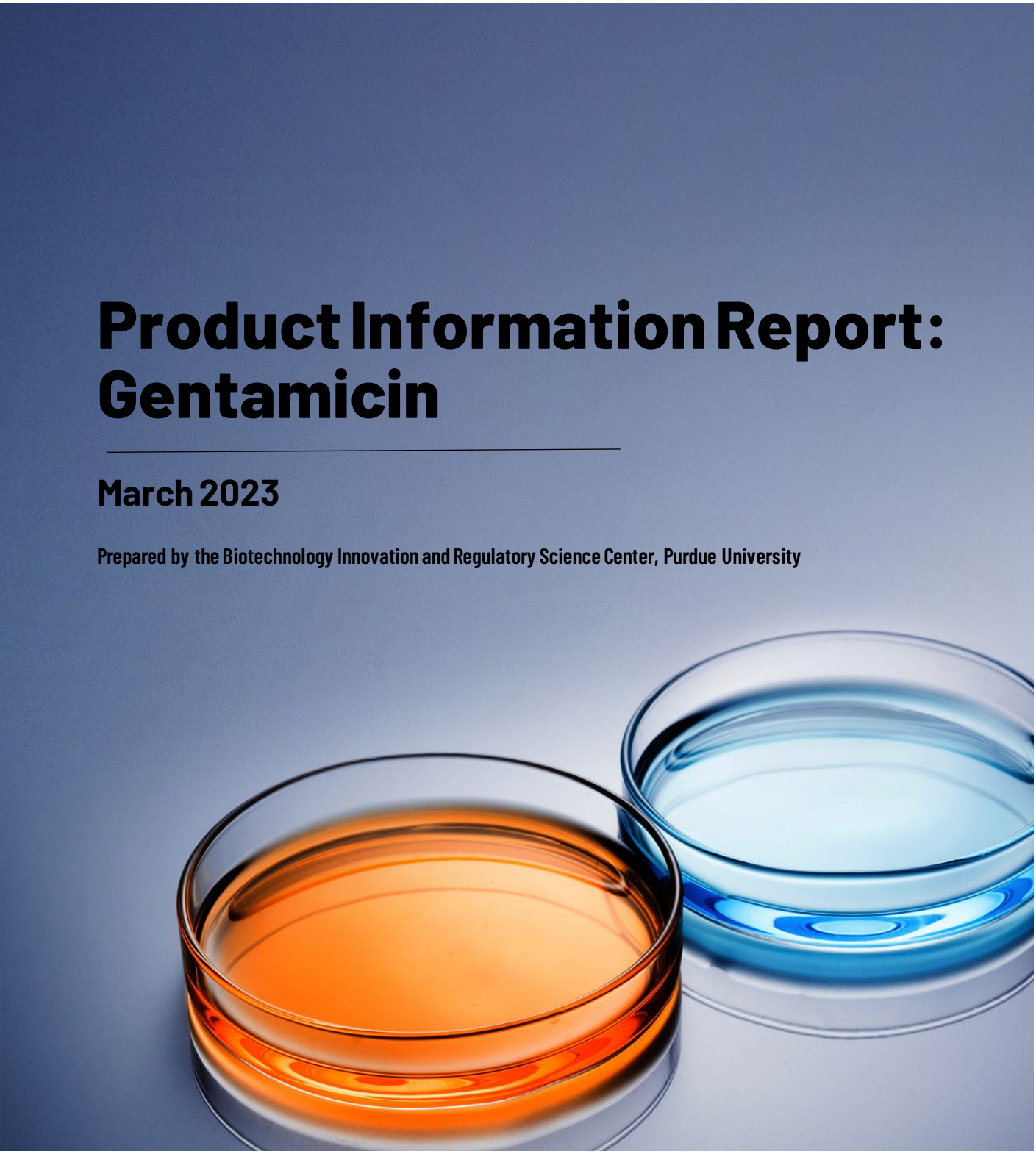
Prepared by the Biotechnology Innovation and Regulatory Science Center, Purdue University



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Acronyms

2-MCBT	2-mercaptobenzothiazole
ADE	acceptable daily exposure
ADJ PH	pH adjustment
ADME	absorption, distribution, metabolism, and excretion
ALS	antimicrobial lock solutions
API	active pharmaceutical ingredient
APP	aggregated production planning
Australian TGA	Australian Therapeutic Goods Administration
BET	bacterial endotoxins test
BIRS	Biotechnology Innovation and Regulatory Science
BP	British Pharmacopeia
CAD	corona-charged aerosol detector
CEP	certificate of appropriateness
CPP	critical process parameter
CQA	critical quality attribute
DCS	distributed control system
DOE	design of experiments
DSC	differential scanning colorimetry
EDQM	European Directorate for the Quality of Medicines & HealthCare
EDTA 2NA	ethylenediaminetetraacetic acid disodium salt
ELSD	evaporative light scattering detector
EMA	European Medicines Agency
FPP	full packaged product
FTIR	Fourier transform infrared spectroscopy
gm	gram
GMP	good manufacturing process
HCL	hydrogen chloride

HFBA	heptafluorobutyric acid
HPLC	high-performance liquid chromatography
IARC	International Agency for Research on Cancer
ICH	International Council for Harmonization
IID	Inactive Ingredient Database
InChI	International Chemical Identifier
IP-RPLC	ion-pairing reversed-phase liquid chromatography
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
l	liter
LC50	lethal concentration 50
LD50	lethal dose 50
LDH	lactate dehydrogenase
LDLo	lowest lethal dose
LOAEL	lowest observed adverse effect level
m^3	cubic meter
MDE	maximum daily exposure
MeSH	Medical Subject Headings
MF	modifying factor
mg	milligram
MHz	megahertz
mL	milliliter
mol	molar mass
MPPUD	maximum potency per unit dose
MS	mass spectrometer
NaOH	sodium hydroxide
NMR	nuclear magnetic resonance
NMT	not more than
NOAEL	no observable adverse effect level

NOEL	no observed effect level
OEL	occupational exposure level
PAD	pulsed amperometric detection
PFPA	pentafluoropropionic
pH	potential of hydrogen
PI	process intensification
PIR	product information report
PK	pharmacokinetics
PLC	programmable logic controller
ppm	parts per minute
PSI	pound-force per square inch
q.s.	quantity sufficient
QTPP	quality target product profile
RF	retention factor
RMP	revolutions per minute
RS	reference standard
SGOT	serum glutamic-oxaloacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
SmPC	summary of product characteristics
TFA	trifluoracetic acid
TGA	thermogravimetric analysis
TLC	thin-layer chromatography
UF_A	animal to human uncertainty factor
UF_C	composite uncertainty factor
UF_E	severity effect
UF_H	average human to sensitive human uncertainty factor
UF_R	reference effect level
UF_S	shorter term to longer term uncertainty factor
UK-MHFA	United Kingdom Mental Health First Aid

US FDA	United States Food and Drug Administration
USAN-USP	USP Dictionary of United States Adopted Names
USP	United States Pharmacopeia
UV	ultraviolet
UV/Vs	ultraviolet-visible spectrophotometry
w/v	weight per volume
WFI	water for injection
µg	microgram

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Executive Summary

Gentamicin is an aminoglycoside antibiotic. It is a bactericidal inhibitor of protein synthesis. Its primary use is to treat infections caused by aerobic gram-negative bacteria. Aminoglycosides are complex small molecules containing amino sugars linked to an aminocyclitol ring by glycosidic bonds.

This product information report (PIR) provides expert scientific analysis of the physicochemical, biopharmaceutics, and toxicological properties; analytical; formulation; and manufacturing of gentamicin. The PIR will provide critical information and guidance to manufacturers, as well as stakeholders concerned with access and supply of priority essential medicines.

Information in this PIR includes chemical structure/formula, IUPAC name, physico-chemical properties, moisture sorption, and solubility-related data. Gentamicin has been characterized through various spectroscopic techniques such as Fourier transform infra-red (FTIR), nuclear magnetic resonance (NMR), mass, and ultraviolet (UV) visible. All analytical data was measured at ambient temperature. The document summarizes these. Gentamicin is usually marketed as an injectable solution or in a topical ointment.

Gentamicin sulfate solution is a sterile injectable solution typically provided in vials or ampules. Gentamicin is a fermentation product that is provided as the sulfate salt. This salt is manufactured into a sterile injectable solution using standard formulation and fill/finish operations. The critical aspect of this manufacture is to maintain the sterility of the product.

The report also provides toxicology information. The major toxicities of gentamicin are its ototoxicity and nephrotoxicity. Unfortunately, the ototoxicity of gentamicin is in many cases irreversible. The nephrotoxicity is usually reversible. Precautions for safe handling include avoiding contact with concentrated solutions.

Formulation and Formulation Barriers to Entry

Formulation

Gentamicin Sulfate Injection

Gentamicin injection is a sterile solution of gentamicin sulfate in water for injection and mostly available in 2 mL vials or ampoules in two concentrations (10 mg/mL or 40 mg/mL) for parenteral administration. Gentamicin sulfate, a water-soluble antibiotic of the aminoglycoside group, is a sulfate salt of gentamicin fractions C_1, C_{1a} C_2 and C_{2a}, derived by the growth of *Micromonospora purpurea*, an actinomycete (Fresenius, 2022; USAID, 2019). It is a clear, colorless, and odorless solution (Panpharma, 2022; MSD, 2022) diluted in 0.9% sodium chloride or 5% glucose solution. It was patented in 1962 and approved for medical use in 1964. Gentamicin is one of the most frequently prescribed aminoglycosides, due to its spectrum of activity, low cost, and high availability. It is effective against both Gram-positive and Gram-negative organisms, but particularly useful to treat Gram-negative infections.

Qualitative and Quantitative Composition

- Gentamicin injection 10 mg/mL: each vial (2mL) contains gentamicin sulfate equivalent to 20 mg gentamicin base
- Gentamicin injection 40 mg/mL; each vial (2mL) contains gentamicin sulfate equivalent to 80mg gentamicin base (USAID, 2019)

An example of a typical formulation for gentamicin 40mg/mL contains the following excipients:

- Gentamicin sulfate equivalent to 40 mg gentamicin,
- Methylparaben 1.8 mg (preservative)
- Propylparaben 0.2 mg as (preservative)
- Sodium metabisulfite 3.2 mg (antioxidant)
- Edetate disodium 0.1 mg (chelating agent)
- Water for injection q.s.
- Sodium hydroxide and/or sulfuric acid may have been added for pH adjustment (Fresenius, 2022)

Formulation Barriers to Entry

Gentamicin sulfate injection should be sterile and therefore manufactured under aseptic conditions, which require that the drug product, container, and closure are rendered sterile. The release of the final product is contingent to sterility. Hence, a quality system should be adequately established to prevent microbial contamination and particulate content of the product during the different processing steps. This implies that the preparation steps, prefiltration, sterile filtration, filling, and sealing of ampoules will occur in an environment that is designed to maintain product sterility. In addition, during the manufacturing process, critical process parameters and critical quality control parameters should be defined through process validation and performed at each stage during the manufacturing process.

Formulation Challenges

Finished product manufacturers of gentamicin sulfate injection are required to control each active pharmaceutical ingredient (API) used during the formulation and provide regulatory authorities with information regarding their compliance to predetermined specifications adopted from the manufacturers of the APIs. Peptone from non-vegetable sources can be used in the manufacture of gentamicin. In that case, the source of peptone should be adequately documented and declared by the manufacturer. The dossier must document the name and address of the suppliers of peptone; where the source of the API changes, the finished product manufacturer and regulatory authorities must be notified.

Notably the sources of peptone are vegetable or animal protein. Using fish peptone raw material during the upstream fermentation process may result in elevated levels of histamine, as the bacteria in the fish produce histamine through enzymatic conversion of free histidine. Consequently, control of residual histamine as a specified impurity forms part of the API specifications, particularly where the fermentation process is adopted (EMA, 2018). Manufacturers are advised to use peptone from vegetable origin during the fermentation process rather than animal origin to prevent this risk.

A list of excipients in some gentamicin solutions for injection approved by stringent regulatory authorities (U.S. FDA, UK MHFA and Australian TGA) is provided in the tables below. Also indicated are the limits, as stated in the FDA's Inactive Ingredient Database (IID) for the excipients (data through October 1, 2022; database last updated: October 19, 2022).

Table 1. *List of excipients and their proposed function with IID limits for gentamicin injection, 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)	ADJ PH* (*ADJ PH: pH Adjustment)	N/A
Sulfuric Acid (UNII: O40UQP6WCF)	pH adjustment	(Fresenius Kabi, 2021); (FDA, 2022)	ADJ PH*	N/A

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 MDE* (*MDE: Maximum daily exposure)	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%w/v MPPUD** Intramuscular (**MPPUD: Maximum potency per unit dose) 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

Ingredients	Function	Reference	IID Limit	Usual Recommended Concentration
Sodium Hydroxide (UNII: 55X04QC32I)	pH Adjustment	(Hospira, 2021); (FDA, 2022)	ADJ PH	N/A
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
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/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/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 Paediatric 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%

Ingredients	Function	Reference	IID Limit	Usual Recommended Concentration
2M Sodium Hydroxide	pH Adjustment	(Zentiva, 2022)		N/A
1M Sulphuric 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 BP 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. EMA has not developed database of excipients in approved drug products like the US FDA IID (Elder & Faïs, 2019).

Stability

Storage, Stability, and Degradation

Gentamicin sulfate injection is stable at room temperature and thus has 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 shows shelf life and storage conditions of some approved products by the U.S. FDA, EMA, and Australian TGA.

Table 10. *Shelf life and storage condition of some approved products by U.S. FDA, EMA, and Australian TGA. (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).

Impact of Storage Conditions on Gentamicin Sulfate Stability

Gentamicin, as an API, is reported to be stable when stored at standard conditions, even after autoclaving. Gentamicin sulfate has been shown in studies to exhibit excellent stability under normal conditions as well. The influences of environmental factors, such as light, humidity, heat, and atmospheric oxidation, were not significant as liquid chromatography and mass spectrometry analysis produces C1, C1a, and C2 (the main degradation products of gentamicin). Forced degradation produces no impurities or unexpected degradants in the study (Xu et al., 2002). Physical and chemical in-use stability has been demonstrated for gentamicin sulfate for 24 hours at 25ºC. The recommended storage for gentamicin is 15ºC-25ºC, negating the need to refrigerate or implement cold chain storage.

Impact of Other Antibiotics Used in Combination with Gentamicin Sulfate Stability

Studies have shown gentamicin sulfate stability in various pharmaceutical dosage forms, for parenteral solutions, ophthalmic sterile solutions, and antimicrobial lock solutions (ALS). Stability of gentamicin in ALS was conducted for 12 months in vials at 25ºC±2ºC, 60%±5% RH, and at 40ºC±2ºC,75%±5% RH and for 24 hours and 72 hours in totally implantable venous access ports. Physicochemical stability results confirmed that the stability of ALS was maintained for 12 months and 24 hours and 72 hours in totally implantable venous access ports (Fiolet et al., 2018). Stability studies in accelerated conditions of gentamicin-glyceryl monooleate-water based gel used in the treatment of chronic osteomyelitis showed that gentamicin sulfate in the gel was stable at zero, three, and six months at 60%RH and 25ºC, with only 11% decrease from the 110% after six months of exposure (Sombie et al., 2014). However, the recent summary of product characteristics for gentamicin sulfate 10 mg/mL, 40 mg/mL, and 20 mg/mL solutions for injection report that physico-chemical inactivation of gentamicin occurs when mixed in solution with certain drugs (Wockhardt, 2022). These include beta-lactam

antibiotics (penicillin, cephalosporins), erythromycin, diazepam, furosemide, flecainide acetate, or heparin sodium, as well as lipiphysan (a special oil-in-water emulsion for parenteral nutrition).

Impact of Intravenous Fluids on Gentamicin Sulfate Stability

Gentamicin sulfate injections are usually administered into tubing of intravenous infusions during treatment. Studies have shown that the gentamicin injection is stable in ringers dextrose infusion. The gentamicin sulfate was administered at room temperature (27ºC) and cold temperature (4ºC) for 24 hours. Reverse-phase HPLC was used to determine the concentration of gentamicin sulfate for 0, 1, 2, 3, 4, 5, 6, and 24 hours after administration into the infusion and gentamicin concentration after the exposure was significant (Saptarini et al., 2015). In another study, physical and chemical stability evaluation of gentamicin sulfate was carried where it was admixed in 0.9% sodium chloride injection and packaged in Autodose Infusion System Bags. The samples were stored, protected from light, and evaluated at appropriate intervals for seven days at 23°C and up to 30 days at 4°C. Physical stability was assessed by means of a multistep evaluation procedure that included both turbidimetric and particulate measurement, as well as visual inspection and at appropriate intervals during the study period. Chemical stability was assessed using HPLC analytical method to determine concentrations of the gentamicin sulfate. The results showed that gentamicin sulfate remained stable 30 days at 4°C and for seven days at 23°C (Xu et al., 2002).

Efficacy and Adverse Effects

Efficacy

Gentamicin is the only aminoglycoside derived from the genus *Micromonospora purpurea*, a group of antibiotics that presents bactericidal activity by permanently binding to prokaryotic ribosomal proteins, thus causing inhibition of protein synthesis (Cox, 1970; Fitzgerald & Newquist, 2013; Mathews & Bailie, 1987; Pfizer New Zealand Limited, 2022). Gentamicin exhibits induced cross-resistance with other aminoglycosides derived from streptomyces (Cox, 1970) and has shown activity against the following organisms: Gram-positive staphylococcus species, *Listeria monocytogenes*; Gram-negative *Campylobacter coli, Campylobacter jejuni*, *Citrobacter koseri*, *Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli, Francisella tularensis, Klebsiella oxytoca, Klebsiella pneumonia, Proteus vulgaris, Salmonella enterica subsp. Enterica, Serratia marcescens, Yersinia enterolitica, Yersinia pseudotuberculosis* (Moore et al., 1984b; Panpharma UK Ltd, 2022; Wockhardt UK Ltd, 2022). Resistance to gentamicin develops slowly (Cox, 1970; Fresenius Kabi USA, LLC, 2013). The following organisms are known to develop resistance to gentamicin: (1) most streptococcal species (including *Streptococcus pneumoniae* and the Group D streptococci), enterococcal species (including *Enterococcus faecalis, E. faecium*, and *E. durans*); (2) anaerobic organisms (such as *Bacteroides species, Clostridium species*) (Fresenius Kabi USA, LLC, 2013; Panpharma UK Ltd, 2022; Pfizer New Zealand Limited, 2022; Wockhardt UK Ltd, 2022); (3) aerobic Gram-positive micro-organisms (such as *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis, Staphylococcus haemolyticus Staphylococcus hominis)*; (4) aerobic Gram-negative micro-organisms such as (such as *Acinetobacter spp. Citrobacter freundii, Morganella morganii, Proteus mirabilis, Pseudomonas aeruginosa Burkholderia cepacia, Legionella pneumophila, Stenotrophomonas maltophilia)*; and (5) atypical pathogens *(*such as *Chlamydia spp. Chlamydophila spp. Mycoplasma spp. Ureaplasma urealyticum)* (Panpharma UK Ltd, 2022; Wockhardt UK Ltd, 2022). In addition, some strains of salmonella are resistant to gentamicin (Hospira, 2022).

Dose prediction for gentamicin injection is complicated due to individual variation (Siber et al., 1975). It is therefore recommended for the treatment of serious infections caused by susceptible strains, in treatment of bacterial neonatal sepsis (bacterial septicaemia), and for serious bacterial infections of the central nervous system (meningitis), urinary tract, respiratory tract, gastrointestinal tract, peritonitis, skin, bone, and soft tissue. However, in severe infection treatment can begin before establishing susceptibility. Successful patient outcomes have been linked to early aggressive therapy. The loading dose plays an important role in disease management (Fresenius Kabi USA, LLC, 2013; Moore et al., 1984a, 1984b). Mortality has been associated with antibiotic failure in patients receiving doses lower than 5µg/mL post-infusion (Moore et al., 1984a).

Dosage

Gentamicin is recommended to be used in combination with other antibiotics to minimize the development of resistance and potential overgrowth on the non-susceptible organisms (Pfizer New Zealand Limited, 2022). Initially, favorable outcomes were observed with gentamicin administered 4.5-7.5 mg/kg body weight/day in 2-4 divided doses, with mean peak levels greater than 7µg/ml (Cox, 1970; Moore et al., 1984b; Panpharma UK Ltd, 2022; Ramlakhan et al., 2014; Siber et al., 1975; Wockhardt UK Ltd, 2022). Currently, gentamicin dosing recommendations are shifting toward a once-daily administration using both AUC and target peak levels, although no concrete evidence proves its advantage over frequent dosing (Barclay et al., 1995; Hayward et al., 2018; Hoff et al., 2009; McDade et al., 2010). A study (McDade et al., 2010) suggests improved efficacy due to faster peak concentrations in once-daily dosing, which agrees with successful outcomes associated with the initial loading dose (Hoff et al., 2009; Moore et al., 1984a). Dose adjustments are recommended in patients with impaired kidney function (Cox, 1970; Moore et al., 1984b; Panpharma UK Ltd, 2022; Ramlakhan et al., 2014; Siber et al., 1975; Wockhardt UK Ltd, 2022)

Adverse Effects

The two major adverse reactions of gentamicin are ototoxicity and nephrotoxicity. Damage to the sensory cells of the ear can lead to hearing loss, balance problems, and tinnitus. Gentamicin damages cells in the proximal tubule, which causes kidney injury because of acute tubular necrosis. Renal function should be measured regularly; in the case of renal impairment, the interval between doses should increase or the dose should decrease. It is contraindicated in patients with myasthenia gravis and parkinsonism, considering its curare-like effect on neuro-muscular function (Fresenius Kabi USA, LLC, 2013; Ghadially & Ramsay, 1988; Pfizer New Zealand Limited, 2022; Ramlakhan et al., 2014).

Several studies have demonstrated systemic contact dermatitis occurring within 24 hours of administering the drug (Ghadially & Ramsay, 1988; Paniagua et al., 2002). Other adverse reactions include: respiratory depression, lethargy, confusion, depression, visual disturbances, decreased appetite, weight loss, hypotension and hypertension, rash, itching, urticaria, generalized burning, laryngeal edema, anaphylactoid reactions, fever and headache, nausea, vomiting, increased salivation and stomatitis, purpura, pseudotumor cerebri, acute organic brain syndrome, pulmonary fibrosis, alopecia, joint pain, transient hepatomegaly, and splenomegaly (Fresenius Kabi USA, LLC, 2013; Panpharma UK Ltd, 2022; Wockhardt UK Ltd, 2022). Laboratory abnormalities reported in association with gentamicin treatment include increased levels of serum transaminase (SGOT, SGPT), serum LDH, and bilirubin; decreased serum calcium, magnesium, sodium, and potassium; anemia, leukopenia, granulocytopenia, transient agranulocytosis, eosinophilia, increased and decreased reticulocyte counts, and

thrombocytopenia. Hypomagnesemia, hypocalcemia, and hypokalemia may be symptomatically diagnosed with muscle weakness in patients receiving gentamicin treatment (Pfizer New Zealand Limited, 2022; Wockhardt UK Ltd, 2022). Cross-allergenic reactions resulting in eczematous eruptions have been reported in patients who have received other aminoglycosides within 24 hours of receiving gentamicin (Ghadially & Ramsay, 1988; Paniagua et al., 2002; Pfizer New Zealand Limited, 2022).

Bioavailability, Pharmacokinetics, ADME

Absorption

Gentamicin sulfate is poorly absorbed following oral administration, presenting with low bioavailability (Cox, 1970; Recchia et al., 1995). It is classified on the Biopharmaceutics Classification System (BCS) as a class III, highly water-soluble compound. Gentamicin sulfate is available in a 40 mg/mL solution for injection/infusion in varying vial/ampoule sizes up to 800 mg/20 mL. A lower concentration formulation is also available (10 mg/mL) for use in dosing pediatric populations (Fresenius Kabi USA, LLC, 2013; Panpharma UK Ltd, 2022; Pfizer New Zealand Limited, 2022; Wockhardt UK Ltd, 2022). The drug is rapidly absorbed following intramuscular administration to reach peak levels in 30 to 60 minutes and immediately upon a 30- or 60-minute IV infusion. Following recommended dosing, mean peak serum concentrations are achieved between 4µg/mL and 7µg/mL; mean peak concentrations <5µg/mL were sub-therapeutic (Cox, 1970; Moore et al., 1984a). Significant variability in peak concentrations and half-life of gentamicin occurs in patients with normal renal function, as observed in various studies; a shorter half-life has been associated with fever and anemia whereas a long half-life is linked to low creatinine clearance. A marked age-related variation occurred in dose-response, which diminished when the dose was calculated on body surface area (Siber et al., 1975). Serum creatinine concentrations have been reported to have a high correlation with gentamicin half-life; therefore, appropriate dose intervals can be calculated based on a creatinine test and doses can be adjusted for patients with impaired renal function (Barclay et al., 1995; Fresenius Kabi USA, LLC, 2013).

Distribution

Gentamicin is well distributed in all extracellular fluids, with serum concentrations affected by the temperature of the body. The serum levels remain measurable for eight to 10 hours (Fresenius Kabi USA, LLC, 2013; Siber et al., 1975). Gentamicin protein binding is not clinically significant, reported between 0-35%, allowing for free clearance through glomerular filtration (Cox, 1970; Wockhardt UK Ltd, 2022).

Metabolism and Elimination

Gentamicin is not metabolized by the liver. Following glomerular filtration, gentamicin is excreted unchanged in microbiologically active form by the kidney with 80-90% recovered in the urine within 24 hours. Caution should be exercised when the drug is used in patients with impaired kidney function. The drug may be reabsorbed causing accumulation and toxicity (Fresenius Kabi USA, LLC, 2013; Hayward et al., 2018). The dominant elimination half-life in patients with normal renal function is around two to three hours. Elderly patients eliminate gentamicin more slowly than younger adults do (Panpharma UK Ltd, 2022; Pfizer New Zealand Limited, 2022; Wockhardt UK Ltd, 2022).

Process Equipment

Solution Preparation

Most of the equipment required to manufacture gentamicin comprises 300 series austenitic stainless steel, with tantalum or glass-lined vessels employed for preparation of formulations sensitive to iron and other metal ions. The vessels can be equipped with external jackets for heating and/or cooling and various types of agitators, depending on the mixing requirements of the individual formulation. In many facilities, various tank sizes are available. Larger facilities may have the high-capacity tanks permanently installed and permanently connected to process utilities. Smaller vessels are generally mobile and positioned in individual processing booths or rooms as needed (CoxGad, 2008).

Pre-Filtration and Sterile Filtration

Certain solutions and liquids that cannot be sterilized in the final container can be filtered through a sterile filter of nominal pore size, 0.22 micron (or less), or with at least equivalent microorganism-retaining properties, into a previously sterilized container. Such filters can remove bacteria and molds, but not all viruses or mycoplasmas. Consideration should be given to complementing the filtration process with some degree of heat treatment. A double-filter layer, or second filtration, through a further sterilized microorganism-retaining filter immediately prior to filling may be advisable. The final sterile filtration should be carried out as close as possible to the filling point (WHO, 2011). The ultra-filtration technology is applied at the washing machine's filter. The clean and sterile washing water and compressed air are obtained through a terminal filter, which can improve the washed bottle's clarity (Intertech Technologies PVT LTD).

Filling

The blow/fill/seal units are purpose-built machines with containers formed from a thermoplastic granulate, filled, and then sealed in a single continuous operation by the one automatic machine (WHO, 2011). The desirable equipment for this is an ampoule injectable liquid filling production line, which includes an ultrasonic washing machine, sterilizing tunnel, and ampoule filling and sealing machine. It is divided into washing zone, sterilizing zone, and filling and sealing zone, which can work together as well as independently. The compact line realizes single-linkage, continuous operation from washing, sterilizing, filling, and sealing. The whole production process realizes the cleaning operation, protects products from contamination, and meets the GMP production standard (Intertech Technologies PVT LTD).

The ampoules are sterilized by the hot air laminar flow sterilization principle. The heat distribution is more even. The ampoules under the high-temperature sterilization condition meets the GMP standard (Intertech Technologies PVT LTD). Blow/fill/seal equipment used to produce products that are terminally sterilized should be installed in at least a Grade D environment (WHO, 2011).

Sealing of Ampoules

The equipment requires a negative-pressure sealing principle to seal the high-efficiency filter, which is used to purify the tunnel. The filter is easy to install and ensures that the class 100 clean room conditions are maintained. The equipment design should include a chain conveying belt with flank. The conveying belt should not be off track and the maintenance of the equipment should be convenient and laborsaving. Equipment with advanced technology, such as multi-needle filling,

front and rear nitrogen charging, and wire drawing sealing, can meet various product type standards. Equipment fittings and services should be designed and installed so that operations, maintenance, and repairs can occur outside the clean area. Equipment that must be dissembled for maintenance should be resterilized after complete reassembly when possible (CoxGad, 2008).

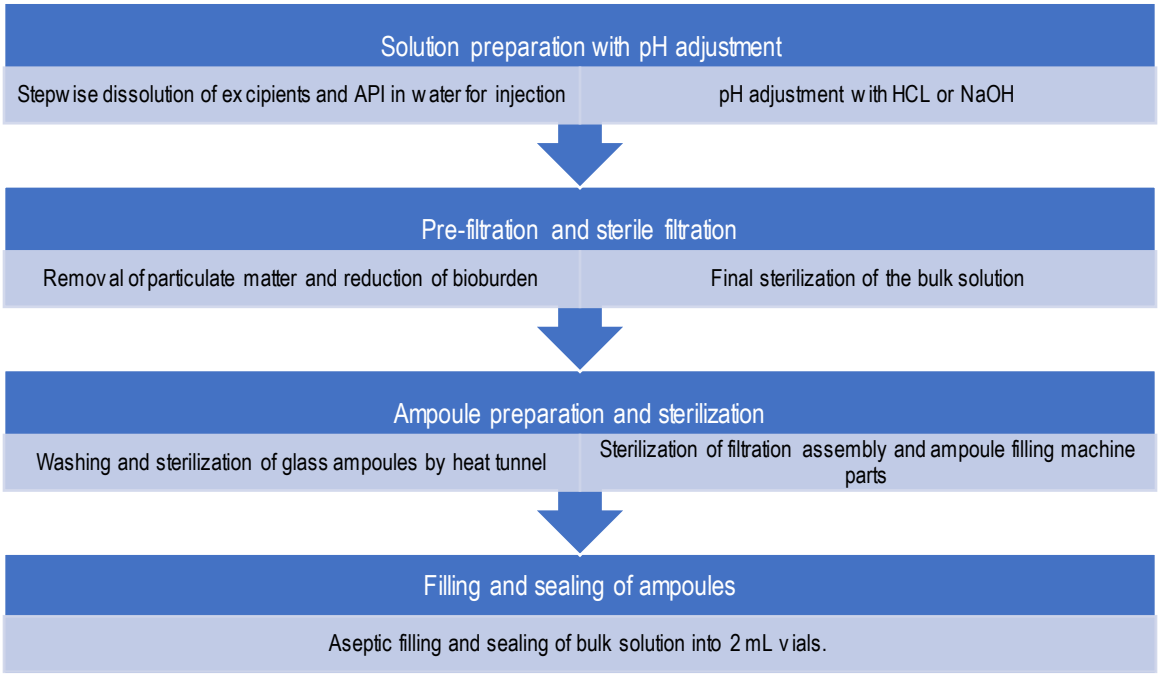
Packaging

The terminal sterilization of the finished product containers may be performed in the same sterilizers utilized to supply the aseptic processing operations. The differing process needs of terminal sterilization will sometimes dictate the use of sterilizers specifically designed for it, incorporating air overpressure systems, internal fans, and spray cooling. Where this is the case, the terminal sterilizer is located proximate to the crimping/sealing areas. A double-door sterilizer design is preferred, with staging areas for filled containers to be sterilized and a separate area for containers that have completed the process.

Manufacturing Process

The key quality concerns of the gentamicin manufacturing process are the final sterilization process and the sterility of the facility. The four major steps in the gentamicin injection manufacturing process are: 1) preparation of solution with pH adjustment; 2) pre-filtration; 3) sterile filtration; and 4) filling and sealing of ampoules, as Figure 1 illustrates (Im-Amornphong & Tomazzini, 2019).

Figure 1. *Process Flow Chart for the Manufacture of Gentamicin Sulfate Injection.*



Solution Preparation with pH Adjustment: This stepwise process dissolves excipients and drug API in water for injection (WFI). The final step of this stage is pH adjustment with HCL or NaOH to a pH of 3.0-5.5. During this stage, the solution is purged with nitrogen twice to remove dissolved oxygen. This occurs at the initial step when water for injection is added into the preparation tank and after pH adjustment. The nitrogen also helps prevent product oxidation (Chénglìxiá et al., 2010). Samples are collected for assay.

Pre-Filtration and Sterile Filtration: This stage of the process involves sterilization of the bulk solution and removal of particulate matter using 0.22-micron filters. The bulk solution is filtered through a 0.22-micron filter to remove microbes and particulate matter. Filter integrity tests take place before and after the filtration process.

Sterilization of Ampoules and Filling Machine: Glass ampoules are washed and sterilized using dry heat. The filtration assemblies 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. Environmental control of this area is important in preserving product sterility. Pre-filters and particulate air filters control the particulate content of air in this clean room.

Other inventions to improve the manufacturing process include adding sodium sulfite, EDTA-2Na as an antioxidant in the liquid preparation. This method addressed the problem of color disparity due to oxidation, which affects the stability of the product (Chénglìxiá et al., 2010).

At every level of the production process, operating in-process controls are defined. The pore size, compatibility with the product, absence of extractables, and absence of adsorption of the API or any of the components should all be verified for the filters used in sterile filtration.

A manufacturing process validation protocol should be submitted to the regulatory body for the first three production-scale batches. Also required are the finished process validation reports for the three cycles/runs of the sterile processes. The manufacturer must submit the complete validation data to produce at least three consecutive production-scale batches, if they are already producing batches at production scale (USAID, n.d.).

Another method consists of the following steps: 1) installing a nitrogen distributor in a dispensing tank; 2) taking water for injection amounting to 50–60% of the total volume; 3) adding excipients and a specified amount of water for injection; 4) adjusting the pH value of the dispensed solution; and, following the test demonstrating the qualification of the medicine content, 5) decarbonizing and performing aseptic filter-pressing. The gentamicin sulfate injection created using this preparation method cannot change color, and the color of the same lot is consistent, according to the acceleration test and sample observation. When stored in accordance with the recommended storage conditions within the expiration date, the product's color-grade qualifying rate can approach 100% (Chénglìxiá et al., 2010).

The formulation in this second method includes 40 mg of gentamicin sulfate powder, methyl parahydroxybenzoate 1.6-2.0 mg, propyl p-hydroxybenzoate 0.1-0.3 mg, sodium sulfite 3.0-3.2 mg, ethylenediaminetetraacetic acid disodium salt (EDTA 2Na) 0.05-0.1 mg, and water for injection adds to 1 mL.

This method addressed the problem of color disparity due to oxidation which impacted the stability of the product. In addressing this problem, sodium sulfite, EDTA 2Na antioxidant is added in the liquid preparation. The nitrogen also helps to prevent the product oxidation stain during sealing (Chénglìxiá et al., 2010).

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 USP standards.
  - Composition of the rubber stopper, along with a declaration from the supplier that the material is free of 2-mercapto benzothiazoles (2-MCBT) 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 or
    - Routine leak testing performed as part of product manufacture.
- Compatibility
  - Extractables/leachables 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 sorption.
  - Compatibility of the full packaged product (FPP) with diluents (such as 5% dextrose injection or 0.9% sodium chloride, 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 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).

CQAs, CPPs

Critical Quality Attributes (CQAs)

Table 11. *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 (TLC)	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>

CQA	Acceptance Criteria	Justification
Ph	3.0–5.5	USP<791>
Bacterial Endotoxins	Not more than 0.71 USP endotoxin unit/mg of gentamicin	USP<85>
Particulate Matter	Meet the requirements for small-volume injections	USP<788>
Extractable Volume	Comply	USP<1>
Sterility	Sterile	USP <71>

Critical quality attributes (CQAs) are physical, chemical, biological, or microbiological properties or characteristics that must fall within a certain limit, range, or distribution to guarantee the required product quality. The unpredictability of a process parameter, known as a critical process parameter (CPP), might affect the CQA. To guarantee that the CPP generates goods that meet specified quality standards, the process needs careful monitoring and management. The multidimensional combination and interplay of input factors, such as material qualities, and process parameters that have been shown to offer assurance of quality is known as the design space. A change is not deemed to have occurred when working within this design space. The essential actions in quality by design include developing a quality target product profile (QTPP), specifying CQAs, and comprehending risk management throughout the lifecycle (USAID, n.d.).

To manufacture a product with the appropriate quality attributes, the packaging process must be devised. Understanding the effects of packaging process factors and material properties on product CQAs is necessary during this procedure. Understanding the variability in the materials used and the procedures performed, as well as how they affect the performance and quality of the final product, is crucial (USAID, n.d.).

The QTPP describes the design criteria for the product and should therefore form the basis for development of the CQAs, CPPs, and control strategy (see Table 12).

Table 12. *Quality target product profile for gentamicin sulfate injection (gentamicin sulfate, n.d.).*

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

Critical Process Parameters (CPPs)

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). CPPs, when varied beyond the acceptable limit range, 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).

During the addition of the water and stirring stage, the water should be added to 50-60% of the volume of the tank and the water temperature should be controlled between 40°C and 60°C (Cheng et al., 2010). The mixing speed and time should be monitored (Lopes, 2014). The pH should be maintained between 5.5 and 6.0 as the gentamicin powder is added and the solution should be stirred evenly (Cheng et al., 2010; Lopes, 2014). The oxygen should be removed from the headspace; it would oxidize the gentamicin, causing a color change (Cheng et al.,

2010). During nitrogen purging, dissolved oxygen should be controlled to a minimum level (Lopes, 2014). An antioxidant and nitrogen gas are added to prevent product oxidation, a major degradation pathway for gentamicin (Cheng et al., 2010; Im-Amornphong & Tomazzini, 2019). Prior to filtration, the appearance, bioburden, density, and assay of the solution should be within established limits (Lopes, 2014). Filter and pre-filter tests should confirm that the pore sizer is not more than 0.22um (Lopes, 2014; WHO, 2011). The filtration pressure and the time taken to filter the solution of a known volume should be validated and controlled (WHO, 2011). The vials to be used should be sterilized in an autoclave with the pressure, temperature, and time controlled. Capping quality should be visually inspected to ensure that the caps are tightly crimped onto the vials (Lopes, 2014). Filling should occur in an aseptic condition that is validated using media fills (WHO, 2011). The fill volume of the vials should be controlled (WHO, 2011). The identified CPPs can be used in manufacturing to ensure that the manufactured gentamicin will meet the desired CQAs. Table 13 summarizes the CPPs of manufacturing process for gentamicin solution for injection.

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

Manufacturing step	Operations involved	CPP
Bulk Solution Preparation	• Dissolving drug substance and excipients to form the bulk solution • Purging the solution with nitrogen to remove dissolved oxygen • pH adjustment	• Temperature of water/solution • Mixing speed and time • Dissolved oxygen NMT 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)

Scale-Up Challenges

Scale-up refers to the intentional efforts to increase output (WHO, 2009). In the context of gentamicin injection, scale-up implies an increase in amount or production capacity. During drug development, small sizes, generally referred to as laboratory scale or developmental batches, are typically used due to failure risks. During drug development, lead molecules are initially synthesized as small batches called laboratory or developmental batches to minimize losses in case of failure. After these batches have undergone experimental studies, including preclinical and clinical testing, and are found to meet the set criteria, the need to scale up production to serve a wide population arises (scaling up from laboratory to commercial batch sizes). The process of scale-up follows the need for the lead molecule along the drug development process flow that is typically characterized by an increase in the number of subjects. The yields from the fermentation of gentamicin are typically low and dependent on process parameters (Lee & Deway, 1979). Despite the relevance of process scale-up, several challenges may be faced.

Variation in the Biomass Formed: During scale-up, it is difficult to maintain consistent quality of drug product, which may be attributed to large composite of ingredients used (Sarkis et al., 2021).

These variabilities may include changes in the quantities of the active ingredient, challenges in identifying the active ingredients, and incompatibilities/interactions between the ingredients. Due to the large composites of ingredients used, it is often difficult to maintain the consistent quality of the drug product during scale-up (Sarkis et al., 2021). The fermentation process of gentamicin requires different ingredients and process parameters, such as the media, level of aeration, pH, and the inoculum, among others, which must be proportionately scaled up to result in the proper yield and quality of the gentamicin.

This challenge can be overcome using technological advancements, such as process intensification (PI) and electronic production execution systems (Pathak & Thassu, 2010). Quality by a design approach that involves a thorough study of the design space that has the capability of providing simulations during scale-up, such as design of experiments (DOE), is critical to minimize waste and losses at the pharmaceutical developmental stage level.

Production Planning and Scheduling: There is a challenge of lead time management since there is often a hold time for the bulk products as they await processing/quality control to be moved downstream. This is typical in a discrete manufacturing process where the bulk product needs to wait for quality assurance clearance before moving to the next stage. Also, bulk products may often need to wait for the next process to be free and the line to be cleared before taking on the incoming product (Sarkis et al., 2021). Gentamicin sulfate, being a sterile product, requires minimal holding time—typically less than 24 hours (Preparations & Organization, 2012).

This challenge is worse in industries that manufacture multiple products that may share equipment, facilities, and personnel with increased risks of mix-ups and cross-contamination. However, this challenge can be overcome by using aggregated production planning (APP) techniques and embracing continuous manufacturing techniques that employ online quality assurance and advancement in technology with online commands.

Equipment Capacity: Optimum utilization of equipment involves capacity limits, beyond which risks that can arise from their usage will increase exponentially (Sarkis et al., 2021). Proper use of equipment involves the maximum capacity/load. In the production of gentamicin sulfate, equipment such as bioreactors have capacity limits beyond which their usage may be risky and unsafe.

In the pharmaceutical industry, the equipment used for developmental stages may not have the capacity to process commercial batch sizes. This calls for investment in manufacturing equipment that can meet the market demand. The bottlenecks of equipment can be overcome by improving production throughout, along with proper equipment maintenance.

Analytical Method Transfers and Validation: Method transfer and validation during scale-up is a regulatory requirement. Most regulatory agencies require analytical method transfer to be conducted and that the entire scale-up process be validated to ensure consistency in analyses and quality of the products. These activities of analytical method transfer and validation require resources to be conducted (Kamravamanesh et al., 2019).

Critical process may include:

- Large-scale inoculum development
- medium sterilization
- aeration
- agitation

- heat removal
- pH control

Method transfers, qualifications, and validations are regulatory requirements, as they assure consistency in outcomes. The scale-up process should be validated to ensure consistency in analyses and the quality of the products (Kamravamanesh et al., 2019). The challenge can be bridged through early consultation with the regulatory agency to establish the requirements and any waivers, if applicable.

Contamination Control: The introduction of unwanted particles or organisms in the formulation is considered to be fatal due to the mode of administration of gentamicin sulfate. During scale-up, the risk of contamination increases tenfold (Lonsane et al., 1992). As the materials, equipment, and people increase, so does the risk of contamination—especially if the processes are discrete. This increases the costs of sterilization. However, it can be overcome by adopting continuous and/or automated processing.

Waste Management: During scale-up, process consumables increase and the waste increases proportionately. Some techniques used in management of bio-waste include composting, landfilling, ethanol production after enzymatic saccharification, and nucleic acid recovery from the spores present in the residue (Lonsane et al., 1992). The challenge of waste management can be overcome by contracting specialized service providers, especially for active or biological waste, and treatment to reduce environmental harm.

Toxicity

Gentamicin toxicity (also called gentamicin poisoning) is known to cause kidney damage, renal failure, nerve damage, ototoxicity (damage to the ear, such as hearing loss, vertigo or ringing in the ears), balance problems, oscillopsia (bouncing vision),Commercial gentamicin is a complex of several compounds comprising the major compounds (C1, C2, C1a) and some minor compounds. The C2 compound has the strongest ototoxic effects, while the C1a compound is more vestibulotoxic than ototoxic (Kobayashi et al., 2008).

Neurotoxicity, manifested as both bilateral auditory and vestibular ototoxicity, has been reported. Nephrotoxicity has occurred in both patients with normal renal function and those with pre-existing renal damage if treated at higher doses and/or for periods longer than recommended (Saleh et al., 2016). Nephrotoxicity has also been reported in patients with impaired renal function and in those who receive high doses or prolonged therapy. When gentamicin accumulates in the renal proximal tubular cells, it can cause damage leading to proteinuria and reduction of the glomerular filtration rate (Balakumar et al., 2010). Neuromuscular blockade and respiratory paralysis have been reported following parenteral injection, topical instillation (as in orthopedic and abdominal irrigation or local treatment of empyema), and oral use of aminoglycosides, especially when given soon after anesthesia or muscle relaxants. If blockage occurs, calcium salts may reverse these phenomena, but mechanical respiratory assistance may be necessary (Saleh et al., 2016). Anaphylaxis, hypersensitivity, and allergic reactions due to gentamicin have not been frequently reported. However, non-immediate, cutaneous reactions are the most commonly reported (Childs-Kean et al., 2019).

Strategies to overcome hypersensitivity reactions, such as desensitization, have been utilized with successful outcomes (Childs-Kean et al., 2019). In cases of toxicity or overdose, the medication should be discontinued immediately. Hemodialysis may be initiated to lower

gentamicin serum concentrations. During administration, due to the potential for ototoxicity and nephrotoxicity, monitoring of vestibule, cochlea, and renal function was recommended before, during, and shortly after treatment (*Gentamicin 40mg_ml Solution for Injection_Infusion - Summary of Product Characteristics (SmPC) - (Emc). Google search Accessed November 8th 2022*). Concurrent administration of gentamicin and other potentially ototoxic or nephrotoxic drugs should be avoided (Triggs & Charles, 1999). Aspirin use may also attenuate this ototoxicity risk (Chen et al., 2007). Gentamicin should be prescribed with the utmost care considering factors such as the patient's age, height, weight, and kidney function. No acute toxicity was observed by infusion over 30 minutes (Loewenthal & Dobson, 2010). A once daily dosing early in the infection also maximized benefits with reduced toxicity (Modi et al., 1998).

Table 14. *Toxicity of gentamicin in several species*

Classification of Toxicity	Details of Toxicity
Acute oral toxicity	**LD50 (Rat):** 8,000 - 10,000 mg/kg **LD50 (Mouse):** 10,000 mg/kg
Acute inhalation toxicity	**LC50 (Rat):** > 0.2 mg/l; Exposure time: 4 h; Test Atmosphere: dust/mist; Remarks: No mortality observed at this dose.
Acute toxicity (other routes of administration)	**LD50 (Rat):** 67 - 96 mg/kg Application Route: intravenous **LD50 (Rat):** 371 - 384 mg/kg Application Route: intramuscular **LDLo (Monkey):** 30 mg/kg Application Route: intravenous
Chronic toxicity	**Species: Dog** LOAEL: 3 mg/kg; Application Route: intramuscular; Exposure time: 12 months; Target Organs: Kidney; Symptoms: vomiting, salivation **Species: Monkey** LOAEL: 50 mg/kg; Application Route: Subcutaneous; Exposure time: 3 weeks; Target Organs: kidney, inner ear **Species: Monkey** LOAEL: 6 mg/kg; Application Route: intramuscular; Exposure time: 3 weeks; Target Organs: blood, kidney, inner ear, liver **Species: Rat** NOAEL: 5 mg/kg; LOAEL: 10 mg/kg; Application Route: intramuscular; Exposure time: 52 weeks; Target Organs: kidney, blood **Species: Rat** NOAEL: 12.5 mg/kg; LOAEL: 50 mg/kg; Application Route: intramuscular; Exposure time: 13 weeks; Target Organs: kidney
Genotoxicity	**Genotoxicity in vitro**: Test Type: in vitro mammalian cell gene mutation test; Result: negative; Test Type: chromosome aberration test in vitro; Result: equivocal **Genotoxicity in vivo:** Test Type: mammalian erythrocyte micronucleus test (in vivo cytogenetic assay); Species: mouse; Application Route: intravenous injection; Result: negative
Human ingestion	**Ingestion:** Target Organs: kidney; Target Organs: inner ear; Symptoms: dizziness, vertigo, hearing loss, tinnitus, fetal deafness

Source: (*Gentamicin (10_pct) Injection Formulation_AH_MX - Google Search*, Accessed November 8th 2022)

Carcinogenic, Reproductive, and Developmental Hazards

Gentamicin is not listed as a known human carcinogen or for causing genotoxicity in any International Agency for Research on Cancer (IARC) groups (Wockhardt, 2022). The limited nonclinical literature mentions that prenatal or postnatal administration of gentamicin to rodents and guinea pigs produces developmental toxicity of the kidney and/or inner ear in fetuses and offspring (*PanPharma, 2022*).

Gentamicin crosses the placenta barrier and the fetal concentrations can be 30% of the maternal plasma concentrations; a third of the maternal plasma volume has been reported to be excreted in breast milk; and concentration in fetal kidney tissue and damage to the eighth cranial nerve has been reported. Therefore, gentamicin is contraindicated in pregnancy, and

breastfeeding should be discontinued during therapy (Panpharma UK Ltd, 2022; Pfizer New Zealand Limited, 2022; Wockhardt UK Ltd, 2022).

Gentamicin studies in humans, or investigational or post-marketing data, have demonstrated fetal risk. Nevertheless, potential benefits from the use of the drug may outweigh the potential risk. The product is classified as Pregnancy Category D. Fetal auditory and vestibular nerve damage may occur. The fetus is at greatest risk during the second and third trimesters. Pregnant rats treated with intramuscular injection of 75 mg/kg gentamicin for 12 days from day 10 of gestation delivered low-birth-weight pups 15 hours later than controls. The administration of gentamicin to pregnant rats caused focal tubular lesions in the developing kidney, a reduced rate of early nephrogenesis, and general growth retardation (Gilbert et al., 1991). In guinea pigs, intramuscular doses of 4 mg/kg bw/day given on gestation days 48 to 54 did not induce teratogenic effects. In rabbits, after intramuscular administrations of gentamicin at doses of 0.8 and 4 mg/kg bw/day on gestation days 6 to 16, no teratogenic effects were reported. Gentamicin sulfate was studied for its effect on embryo/fetal development in rats. The LOAEL was noted to be 375 mg/kg/day. For prenatal and postnatal development in rats with subcutaneous application, a 660 mg/kg/day LOAEL was observed, developmental toxicity was also noted. With injection in prenatal and postnatal development in rats at subcutaneous 660 mg/kg/day LOAEL, neonatal toxicity was observed (Pfizer Pharmaceuticals Group, 2023).

While most genotoxicity studies showed no indication of a genotoxicity potential of gentamicin, some limited in vitro mutagenicity tests showed genotoxic potential of gentamicin. However, the study designs of these in vitro assessments were deemed inadequate to evaluate the genotoxic potential of gentamicin. Following well-conducted genotoxicity tests (two in vitro tests including a chromosomal aberration assay in CHO-K1 cells and a CHO/HGPRT gene mutation assay; one in vivo mouse micronucleus test), it was concluded that gentamicin is unlikely to be genotoxic (The European Agency for the Evaluation of Medicinal Products Veterinary Medicines and Information Technology, 2000).

Occupational Exposure Levels (OEL) Calculation

Utilizing the NOAEL (Hospira, 2021) and (MSD, 2022) and uncertainty/safety factor for determining occupational exposure limits as presented by Ku (2000). Consideration of uncertainty factors was as discussed by Naumann (Dankovic et al., 2015) and (Lovsin Barle et al., 2016). An OEL for gentamicin was calculated as follows:

OEL = NOEL (mg/kg/day) x BW (kg) / V (m^3/day) x S x UF x MF x α

OEL = 660 mg/kg/day x 70 kg / 10 m^3/day x 2 x 900 x 10 x 1

= 0.256 mg/m^3

= 256 µg/m^3

NOAEL= No Observable Adverse Effect Level.

UF=uncertainty factors (6 for rat to human extrapolation, 10 for inter-human variation, 3 for sub-chronic to chronic extrapolation, 5 for available pre-clinical toxicity data)

MF= Modifying factor of 10 for fatal anaphylactic reactions that may happen due to gentamicin

S= steady state based on elimination half-life = 2

α = pharmacokinetic factor based on bioavailability=1

V = volume of air breathed in a shift = 10 m^3

This OEL was designed to be a 12-hour-per-day, 40-hour-per-week airborne concentration, with nearly all workers repeatedly exposed day after day without adverse health effects, based on currently available information. It did not consider hypersensitive or otherwise unusually responsive individuals or persons with hypersensitivity to gentamicin, which may be exacerbated by exposure to this drug.

Control Band Assignment

According to the International Labor Organization, control banding is a complementary approach to protecting worker health by focusing resources on exposure controls. Since it is not possible to assign a specific occupational exposure limit (OEL) to every chemical in use, a chemical is assigned to a "band" for control measures, based on its hazard classification according to international criteria, the amount of chemical in use, and its volatility/dustiness. The outcome is one of four recommended control strategies:

1. Employ good industrial hygiene practices
2. Use local exhaust ventilation
3. Enclose the process
4. Seek the advice of a specialist

Gentamicin was assigned as a Category 2 (0.1-1 mg/m^3) substance in the four-band control banding system (Niosh, n.d.).

Table 15. *Control bands for exposures to hazardous chemicals.*

Band No.	Target Range of Exposure Concentration	Hazard group	Control
1	>1 to 10 mg/m^3 dust >50 to 500 ppm vapor	Skin and eye irritants	Use good industrial hygiene practice and general ventilation
2	>0.1 to 1 mg/m^3 dust >5 to 50 ppm vapor	Harmful on single exposure	Use local exhaust ventilation Engineering controls
3	>0.01 to 0.1 mg/m^3 dust >0.5 to 5 ppm vapor	Severely irritating and corrosive	Enclose the process Containment, strict engineering controls
4	<0.01 mg/m^3 dust <0.5 ppm vapor	Very toxic on single exposure, reproductive hazard, sensitizer (exposure to any concentration of a sensitizer requires expert advice)	Seek expert advice

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, avoiding 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 (UV/Vs) technique, different types of detection techniques like corona-charged aerosol detector (CAD) and electrochemical detector (ECD) are used as a most powerful and versatile technique for the demonstration of these molecules in the analytical field. Analytical methods help ensure the quality of the drug products. Ion-pairing reversed-phase liquid chromatography (IP-RPLC) is widely used to analyze aminoglycosides via volatile perfluorinated carboxylic acids, such as trifluoroacetic acid (TFA), pentafluoropropionic PFPA), and heptafluorobutyric acid (HFBA), as pairing ions in the mobile phase. This helps retain the aminoglycosides on the column and improve the separation.

Due to the lack of a suitable chromophore, experimental aminoglycosides and their related compounds cannot be detected by UV or fluorescence detection without extensive derivatization. Therefore, alternatives such as corona charged aerosol detectors (CAD), 2 evaporative light scattering detectors (ELSD), mass spectrometers (MS)1,3 and electrochemical detectors (e.g., PAD) 4,5 are frequently used to detect these compounds (Al-Amoud et al., 2002).

As described in the European and U.S. pharmacopoeias, the analysis of gentamicin is based on an HPLC-PAD method using a C18 silica-based column. 4,5 The mobile phase contains TFA, HFBA, and acetonitrile. Its pH is adjusted to 2.6 by sodium hydroxide (NaOH) to avoid the silica bonded phase hydrolysis when exposed to lower pH conditions.

Acceptable Daily Exposure (ADE) Calculation

Health-based limits for active pharmaceutical ingredients (API), referred to as acceptable daily exposures (ADEs), are necessary to the pharmaceutical industry and used to derive acceptance limits for cleaning validation purposes and evaluating cross-carryover. ADEs represent a dose of an API unlikely to cause adverse effects if an individual is exposed, by any route, at or below this dose every day over a lifetime.

According to EMEA:

The NOEL was established as 10 mg/kg/day, in dogs given oral doses of gentamicin sulphate at doses of 0, 2, 10, 60 and 120mg/kg bw/day over a period of 14 weeks. (EMEA, 2001)
In rats, that were orally given gentamicin for a period of 3 months, the NOEL was 19 mg/kg/day. (EMEA, 2001).

Dogs are closest for extrapolation to humans {F1 = 5 for extrapolation from rats to human and 2 for dogs – ICH, 2022} and the study was longest. Therefore, the NOEL derived in dogs was chosen for the ADE calculation.

$$\textbf{ADE} = \left(NOAEL \left(\frac{\frac{mg}{kg}}{day} \right) \right) xBW/(UFc\ x\ MF\ x\ \alpha)$$

$$\text{ADE} = \left(10 \left(\frac{\frac{mg}{kg}}{day} \right) X\ 50kg\right)/(600\ x\ 1\ x\ 0.01)$$

$$\textbf{ADE}_{\textbf{oral}} \textbf{= 83.33 mg/day}$$

In calculating the ADE value for gentamicin, a composite uncertainty factor (UF_C) of 600 was used. The choice was made to account for the following factors:

$UF_C = (UF_A \times UF_H \times UF_S \times UF_E \times UF_R)$

where

UF_A = Interspecies

UF_H = Intraspecies variability

UF_S = Length of study

UF_E = Severity of effect

UF_R = Reference effect level

MF = Modifying Factor

PK = Pharmacokinetic Factor

Choice of Uncertainty and Modifying Factors

1. The NOEL in dogs (10 mg/kg/day) was selected as the point of departure, and this is based on the data in dogs (EMEA, 2001); therefore, a factor of **2** was applied to UF_A. (ICH, 2022)
2. UF_H- Considering specific intraspecies variability of data, of **10;** default factor for human variability in population (ASTM E3219 – 20, 2020);
3. UF_S The data reviewed was based on 14 weeks' studies less 2 years in dogs; therefore, an uncertainty factor of **10** (ICH, 2022)
4. Gentamicin is non-genotoxic carcinogenicity, nor teratogenicity. Because of its moderate toxicity an uncertainty factor of **3** was applied to UF_E .(ICH, 2022)
5. UF_L – 1. Default factor when NOAEL is established.
6. UF_D The database of information was complete; therefore, a modifying factor of **1** was used to account for mild adverse effects other than hypersensitivity produced by gentamicin.
7. The bioavailability of Gentamycin is low approximately 1%. (McAdams et.al, 2020) The POD from the study was established from the oral route yet the ADE being established is for the IV route. Therefore, Absorption Factor (α, PK-ABS) is 0.01.

$$\alpha = \frac{\%\ \text{systemic bioavailability (route of exposure - oral – 1\%)}}{\%\ \text{system bioavailability (route of administration at the PoD – IV-100\%)}}$$

(Barle et. al, 2017)

Analytical Profile

Active Pharmaceutical Ingredient (API)

Gentamicin sulfate contains 20 to 40% of gentamicin C1, 10-30% of gentamicin C1a. The sum of gentamicins C2, C2a, and C2b is 40%-60%. The content of gentamicin C1 is between 25% and 50%; the content of gentamicin C1a is between 10% and 35%; and the sum of the contents of gentamicin C2a and gentamicin C2 is between 25% and 55% (Brettler, 2020). Gentamicin is freely soluble in water and 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 (United States Pharmacopeia, 2022).

Chemical Structure/Formula

Table 16. *Chemical Structure/Formula.*

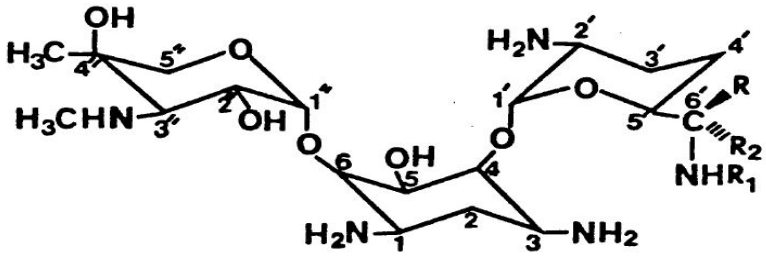
Name	CAS No.	Formula	Molecular Weight
Gentamicin Sulfate	1405-41-0	$C_{60}H_{125}N_{15}O_{25}S$	1488.8g/mol
Gentamicin C1	25876-10-2	C21H43N5O7	477.6 g/mol
Gentamicin C1a	26098-04-4	C19H39N5O7	449.5 g/mol
Gentamicin C2	25876-11-3	C20H41N5O7	463.6 g/mol

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; 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 2.



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, 463b 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 (NMR) studies showing 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 and gentamicin C_1 had both this and an additional N-methyl. Lastly, optical rotation studies confirmed that each of the gentamicin C components is dextrorotary (Kraisintu, 1981).

IUPAC Name

Gentamicin Sulfate: (2*R*,3*R*,4*R*,5*R*)-2-[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-diamino-3-[(2*R*,3*R*,6*S*)-3-amino-6-[(1*R*)-1-aminoethyl]oxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-5-methyl-4-(methylamino)oxane-3,5-

diol;(2*R*,3*R*,4*R*,5*R*)-2-[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-diamino-3-[(2*R*,3*R*,6*S*)-3-amino-6-(aminomethyl)oxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-5-methyl-4-(methylamino)oxane-3,5-diol;(2*R*,3*R*,4*R*,5*R*)-2-[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-diamino-3-[(2*R*,3*R*,6*S*)-3-amino-6-[(1*R*)-1-(methylamino)ethyl]oxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-5-methyl-4-(methylamino)oxane-3,5-diol;sulfuric acid

CHEMBL515827(PubChem, 2022).

InChI

InChI=1S/C21H43N5O7.H2O4S/c1-9(25-3)13-6-5-10(22)19(31-13)32-16-11(23)7-12(24)17(14(16)27)33-20-15(28)18(26-4)21(2,29)8-30-20;1-5(2,3)4/h9-20,25-29H,5-8,22-24H2,1-4H3;(H2,1,2,3,4)(PubChem, 2022)

(USAN-USP)

Gentamicin C1a: 0-3-Deoxy-4-C-methyl-3-(methylamino)-β-L-arabinopyranosyl-(1→6)-0-[2,6-diamino-2,3,4,6-tetradeoxy-α-D-erythro-hexopyranosyl-(1→4)]-2-deoxy-D-streptamine (Chambers, 2022).

EDQM

Gentamicin Sulfate**:** 4,6-diamino-3-{[3-deoxy-4-C-methyl-3-(methylamino) pentopyranosyl]oxy}-2-hydroxycyclohexyl2-amino-2,3,4,6,7-pentadeoxy-6-(methylamino)heptopyranoside.

MeSH Synonyms

G Myticin, G-myticin, Garamycin, Gentacycol, Gentamicin, Gentamicin Sulfate, Gentamicin Sulfate (usp), Gentamicin, Gentamicin, Gentavet, Genticin, Gmyticin, Sulfate, Gentamicin etc. (Chambers, 2022).

Physical Properties

Table 17. *Physical properties of gentamicin.*

Parameter	Specification
Appearance	BP and European Pharmacopoeia: White or almost white, hygroscopic powder (2020). International Pharmacopoeia: A white to cream-colored odorless powder.
Color	White
Melting point	Melts with decomposition between 218ºC and 237ºC
Density	1.000 g/cm3
Optical Activity	International Pharmacopoeia: 0.10 g/mL sample solution, with reference to the anhydrous substance: $[\alpha]_D^{20\,°C}$ = +107° to +121° (2020). USP: For 10 mg/mL sample solution, analyzed per USP General Chapter <781> OPTICAL ROTATION: +107° to +121° (Pharmacopeia, n.d.) BP: Test done per Appendix V F (Determination of Optical Rotation and Specific Optical Rotation), (Ph. Eur. method 2.2.7): + 107° to + 121° (anhydrous basis) (*Gentamicin Sulfate - British Pharmacopoeia*, n.d.) Merck| Sigma-Aldrich: $[\alpha]^{25}{}_d$ = 102° (water)
Solubility	Soluble 50 mg/mL
pH	Its pH in an aqueous solution containing 40 milligrams per milliliter is not less than 3.5 and not more than 5.5 (Chemical book, 2022)

Parameter	Specification
Pka	DrugBank: Strongest Acidic, 12.55 Strongest Basic, 10.12 (*Gentamicin Sulfate | DrugBank Online*, n.d.)
Pkb	PubChem: 9.0 (amine moieties)
LogP	DrugBank: -1.6 -3.1 (*Gentamicin Sulfate | DrugBank Online*, n.d.)

Additional Characterization

Infrared spectrum: The infrared spectrum technique can be used to differentiate Gentamicin Sulfate from similar aminoglycoside antibiotics.

Nuclear magnetic resonance spectra (NMR): Proton Magnetic Resonance Spectrum: An 80 MHz proton NMR spectrum of a solution of Gentamicin Sulfate USP Reference Standard 15% w/v in D20 was obtained using a Varian CFT-20 spectrometer at ambient temperature and sodium 2,2-dimethylY 2-silapentane-5-sulfonate (DSS) as the internal reference.

Nuclear magnetic resonance spectra (NMR): The carbon-13 magnetic resonance was obtained using a Varian XL-100 spectrometer at ambient temperature and dioxane as the internal reference.

Mass spectrum: The mass spectrum of gentamicin free base, prepared by neutralization of gentamicin sulfate USP Reference Standard was obtained using a Varian MAT CH-5 medium resolution single focusing spectrometer at a probe temperature of 170^0C and a source temperature of 250°C.

Thermal properties (TGA, DSC)

Thermogravimetric analysis (TGA): TGA is used to show a loss of water at ~12% from ambient to 125°C and decomposition from 220°C – 330°C. A thermogravimetric analysis curve was obtained for gentamicin sulfate USP Reference Standard using a DuPont Nodel 950 thermogravimetric analyzer equipped with a Model 900 programmer-recorder. The analysis was performed at a heating rate of 10°C/minute, under a nitrogen atmosphere.

Differential scanning colorimetry (DSC): The DSC method can show a broad endothermic peak around 75°C due to water loss and large endotherm at 250°C, corresponding to melting decomposition. A differential scanning calorimetry curve was obtained for gentamicin sulfate USP Reference Standard using a DuPont Model 990 thermal analyzer equipped with a Model 910 cell base. The scan was performed at a temperature program rate of 10°C /minute, under a nitrogen atmosphere against aluminum sample pan (Abdulrahman Al-Majed, 2022).

Synthetic profile: The important strains micromonospora for producing gentamicin include *M. purpurea, M. echinospora, M. echinospora var. pallida* and *M. echinospora var. ferruginea*. *M. purpurea* can be sufficiently grown under aerobic conditions in an aqueous nutrient medium containing a source of digestible carbon like sugars, dextrose, and starch, together with digestible nitrogen like peptones and soya bean meal. The gentamicin-producing capacity of *M. purpurea* can be increased by adding a water-soluble salt of cobalt to the nutrient medium in the concentrations of 2.5x10-9 grams per milliliter to less than 1.25x10-5 grams per milliliter. Commercial production of gentamicin is possible if cobalt in quantities of at least 0.01 microgram as $CoCl_2 \cdot 6H2O$ per milliliter of medium is added during the fermentation process and water soluble ionizable cobalt salts can enhance gentamicin production. There is a limit to the

amount of cobalt to be added in the fermentation medium, depending on whether the medium is of natural origin or synthetic. Gentamicin production in is enhanced by adding 5 micrograms per milliliter calculated as $CoCl_2 \cdot 6H2O$.

According to the literature, gentamicin production was enhanced in several ways by fermenting *M. purpurea* in media containing varying concentrations of cobalt. A lyophilized culture of *M. purpurea* is added to a 300 mL shake flask, having 100 mL sterile medium consisting of Bacto beef extract (3 gm), tryptose (5 gm), dextrose (1 gm), soluble starch (24 gm), yeast extract (5 gm), and tap water (1000 mL). This is the germination stage, whereby the flask and its contents are incubated at 37°C on a rotary shaker at 280 RPM, 2-inch stroke for five days.

Approximately 50-gallon batches of inoculum are prepared at the inoculum phase. From the germination stage, 25 mL of inoculum is transferred to each of four 2-liter flasks containing 500 mL of the sterile medium utilized for germination. At 28°C, the flasks are incubated for five days on a rotary shaker at 280 RPM, 2 stroke. To each of the 20 2-liter flasks with 500 mL sterile medium of soya bean meal (30 gm), dextrose (40 mL), calcium carbonate (1 gm), and tap water (1000 mL), 25 mL of inoculum is added from the pooled contents of the flask. Contents in the flasks are incubated for three to five days at 28°C on a rotary shaker at 280 RPM.

Aseptically, the pooled broth is put into a 10-liter inoculum flask with a side arm. The inoculum of approximately 10 liters is transferred aseptically to a 65-gallon fermenter containing a sterile medium of 50 gallons consisting of Bacto beef extract (600 gm), bacto-tryptose (1000 gm), dextrose (200 gm), soluble starch (4800 gm), yeast extract (1000 gm), tap water (50 gallons), and antifoamer GE 60 (General Electric Co. brand of silicone defoamer) (100 mL). pH is adjusted to 6.9-7.0 before sterilization and aerobic fermentation is in effect for 24 hours until the packed cell volume is about 10%-15% at 37°C, with sterile air input at 54 cubic ft/min, pressure 7 PSI, and agitation 180 RPM.

One 50-gallon batch of inoculum during fermentation stage is aseptically put to a 675-gallon fermenter containing soybean meal (54 kg), calcium carbonate (9 kg), cerelose (72 kg), antifoamer (GE 60) (300 mL), soft water (450 gallons), and CoCl2. 6H2O and fermentation is done at 35°C with air input of 7 PSI, while shaking at 120 RPM and 15cu.ft/min. Larger quantities of gentamicin were yielded due to the presence of cobalt (William, 1964).

Impurity Profile

To assure the safety and effectiveness of a drug, it is critical to describe its substance's purity by identifying and quantifying the impurities (Hu & Rohrer, n.d.). Gentamicin sulfate is described in the major pharmacopeias. For instance, the U.S. Pharmacopeia includes methanol as an impurity. Bacterial endotoxins should also be included as a test parameter where the drug substance is to be used in sterile injectable dosage forms.

Histamine as an Impurity in Gentamicin

Patients with histamine intolerance represent approximately 1% of the population. The use of gentamicin-containing products with elevated levels of histamine residue may be associated with limited adverse events such as increased gastric secretion, increased heart rate, headache, and flush; or even moderate adverse events, such as fall in arterial pressure, urticaria and pruritus, and a less-likely—albeit real—possibility of causing life-threatening adverse events such as bronchospasm and cardiac arrest (EMA, 2018).

The production of the gentamicin API involves a typical fermentation process. However, histamine is present in the FPP and is known to cause adverse reactions even at very low

concentrations. As a result, a direct link has been made between the presence of histamine in the drug product and adverse drug reactions. Considering manufacturing capacity and batch data, the gentamicin sulfate certificate of appropriateness (CEP) should be lowered as much as is practically possible. Based on current batch data, a limit of 8 ppm is deemed within both the validated range of the analytical method and the current manufacturing capabilities of the active substance manufacturer (EMA, 2018).

Pharmacopeial Impurities

Gentamicin sulfate is described in major pharmacopeia including the BP, USP, and Ph. Eur. The pharmacopeias include details of impurities/related substances that should be controlled in gentamicin sulfate. The manufacturers of both the drug substance and the drug product should use the latest version of the selected pharmacopeia to control the drug substance. For example, the BP includes the following impurity limits: Impurity A (not more than 3.0%), impurity B (not more than 3.0%), any other impurity (not more than 3.0%), and total impurities (not more than 10.0%). These limits are higher than the usual ICH Q3B thresholds because gentamicin is a product of fermentation, for which higher levels of related substances are permitted.

Stability studies

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 implies that the compound is relatively stable in both acid and alkaline media. Among the four gentamicin substituents (C1, C1a, C2, and C2a), C2 is reported to be more adversely affected by heat (Mullins et al., 2016). Reconstituted gentamicin aliquots were stable for a period of one 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 (i.e., 0.9% sodium chloride or 5% glucose solution) (European Medicines Agency, 2019)

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 UV 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, the U.S. and European pharmacopeias 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%, and not more than 125%, 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).

Table 18. *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 of it, equivalent to 20 µg of gentamicin to the chromatographic plate in separate portions of not more than 20 µL each; allow each application 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 *R*F values of the three principal spots obtained from the test solution correspond to those obtained from the standard solution.
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 (BET) 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 $-\log_{10}[aH+]$. Where; aH+ 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 (PLC), distributed control system (DCS), 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:** Determination of particulate matter usually uses the light obscuration particle count test or microscopic particle count test. When examining injections and parenteral infusions for subvisible particles, the light obscuration particle count test is preferable. 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.

Conclusion

Gentamicin is an aminoglycoside antibiotic used for the treatment of a variety of bacterial infections including complicated infections. Gentamicin should for all indications, except complicated urinary tract infections, only be used in combination with other relevant antibiotics (predominantly together with a beta-lactam antibiotic or with an antibiotic effective against anaerobic bacteria). Appropriate design and maintenance of the sterile manufacturing facilities requires major investment, and this becomes a significant barrier to entry for most pharmaceutical manufacturers. These manufacturing considerations, in addition to others described in this document, directly impact the global supply and access to this life-saving product. This product information report summarizes the available literature and provides expert scientific analysis of the physicochemical, biopharmaceutics, pharmacokinetics and toxicological properties, API synthesis, analytical, formulation, and manufacturing of gentamicin. It is expected that the report will provide critical information and guidance to manufacturers, as well as stakeholders concerned with access and supply of priority essential medicines.

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