SOP Title: **Kanamycin Residue Content Determination by LCMS**
SOP Number: **23023**
Revision: **00**

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1. PURPOSE

The determination of Kanamycin residue in biopharmaceutical products using liquid chromatography with mass spec detection. Biopharmaceutical products are extracted with acetonitrile, centrifuged and the supernatant is analyzed by liquid chromatography coupled with electrospray ionization MS/MS monitoring selected positive ion transition 485 m/z to 163 m/z. Peak areas are measured and quantified against a standard curve that is generated.

2. SCOPE

This Procedure applies to PA/QC personnel who performed the analysis.

3. RESPONSIBILITIES

3.1 The Director, Technical Operations, Process Analytics/Quality Control (PA/QC)

- Defines this procedure.

3.2 Process Analytics/Quality Control (PA/QC)

- Trains Laboratory personnel.
- Performs this procedure.
- Reviews data

3.3 Biopharmaceutical Quality Assurance (BQA)

- Provides quality oversight.

4. MATERIALS AND REAGENTS

Part Number	Description	BDP Approved Substitution Permitted?
21471	1 mL pipette tips	☒ YES ☐ NO
21470	200 µL pipette tips	☒ YES ☐ NO
21472	20 µL pipette tips	☒ YES ☐ NO
20335	0.1-10 µL pipette tips	☒ YES ☐ NO
20006	15 mL Centrifuge Tube	☒ YES ☐ NO
31214	Waters Acquity UPLC Glycan BEH Amide column	☒ YES ☐ NO
31065	Acetonitrile, HPLC grade	☐ YES ☒ NO
30881	0.1% FA (formic acid) in water	☐ YES ☒ NO
30880	0.1% FA (formic acid) in Acetonitrile	☐ YES ☒ NO
10028	Kanamycin	☐ YES ☒ NO
20595	1.5 mL Microcentrifuge tubes	☒ YES ☐ NO
N/A	High Quality water (which is deionized, reverse-osmosis, Milli-Q, WFI or other purified water)	☒ YES ☐ NO

5. EQUIPMENT

- Waters UPLC system.
- Waters Xevo-G2-XS QTOF system.
- Eppendorf Centrifuge.
- Pipettes 1 mL, 200 µL, 20 µL, 2 µL.

Document the equipment used on **Form 23023-01 Kanamycin testing – Reagents, Materials, and Equipment**

6. PROCEDURE

6.1 Sample Handling and Storge

6.1.1 Received frozen samples are stored at ≤ -70°C in PA/QC and thawed at ambient temperature prior to use.

6.1.2 Any remaining samples are stored at ≤ -70°C after use.

6.2 Sample Preparation

6.2.1 Preparation of Biopharmaceutical Sample

6.2.1.1 A 2x250 µL aliquot of sample is mixed with 750 µL of acetonitrile per sample in two 1.5 mL centrifuge tubes to produce a protein precipitate of the sample.

6.2.1.2 Allow mixture to sit at ambient temperature for 15 minutes.

6.2.1.3 Centrifuge the tubes of protein precipitate at 12,000 x g for 20 minutes.

6.2.1.4 Transfer approximately 900 µL of the supernatant from each tube to a 15 ml tube.

6.2.1.5 Preparation of samples will be documented on ***Form 23023-02 Kanamycin testing form.***

6.3 Instrument Operating Conditions

6.3.1 Waters Acquity UPLC System (following **SOP 22964**)

6.3.1.1 Column: Waters Acquity UPLC Glycan BEH Amide column, BDP PN 31214 or equivalent.

6.3.1.2 Mobile phase composition: A= 0.1% FA (formic acid) in Water and B = 0.1% FA (formic acid) in Acetonitrile

6.3.1.3 Document outlining the conditions that were used for the operation of LCMS system will be printed and included in the test report.

6.4 Calibration Curve Samples

6.4.1 Dilution 1: prepare fresh 10mg/mL solution of Kanamycin in water by dissolving 10.0 mg of Kanamycin in 1 mL of water to make Dilution 1.

6.4.1.1 Dilution 2: prepare a 1mg/mL of Kanamycin in water by diluting 100 µL of Dilution 1 with 900 µL of water.

6.4.1.2 Dilution 3: Prepare a 100 µg/mL of kanamycin in 30% v/v Acetonitrile by diluting 100 µL of Dilution 2 with 600 µL of water and 300 µL of Acetonitrile.

6.4.1.3 Dilution 4: prepare a 10 µg/mL stock solution of Kanamycin in 30% v/v Acetonitrile by diluting 100 µL of Dilution 3 900 µL of 30% v/v Acetonitrile in water.

6.4.1.4 Preparation of calibration standards will be documented on **Form 23023-02 Kanamycin testing form.**

Prepare Calibration Standards in duplicate from Dilution 4

Standard ID	**Volume Of Dilution 4 (µL)**	**Volume Of 30% v/v Acetonitrile in water (µL)**	**Concentration Of Kanamycin (µg/mL)**
Blank	0	1000	0
1	100	900	1.0
2	50	950	0.5
3	25	975	0.25
4	10	990	0.1
5	7.5	992.5	0.075
6	5.0	995	0.05
7	2.5	997.5	0.025
8	1	999	0.01

6.4.2 Preparation of Spike Sample in duplicate

Sample ID	Volume Of Dilution 4 (µL)	Volume Of 30% v/v Acetonitrile in water (µL)	Sample From Step 6.2.1.4	Concentration Of Kanamycin (µg/mL)
Sample A	0	750	250	0
Sample A + Spike 1	2.5	747.5	250	0.025
Sample A + Spike 2	5	745	250	0.05

6.4.3 Analyze calibration standards in duplicate by LCMS. Remove the peak area value of blank solution from the standard samples. Prepare a calibration curve of concentration in µg/mL Kanamycin versus peak area in Excel. Use Excel to fit the points and determine the equation of the fit and R^2 value. If the R^2 value is ≥ 0.95 it would be accepted for the validity of the test. If R^2 value is not within the limits, then the highest and/or lowest concentrations may be removed, and remaining points may be fit using Excel. If removing of some data points provide an acceptable R^2 value, then the equation from those points may be used. If removal of some points does not provide a R^2 value within limits the assay needs to be reperformed. This will be documented in the form to specify if some points are not used in the analysis.

6.4.4 Analyze biopharmaceutical sample solution plus spikes from section 6.4.2 in duplicate by LCMS. Calculate the concentration of Kanamycin in sample and sample plus spikes using the equation generated in 6.4.3 to determine sample concentration of Kanamycin. If the spike recovery is within 100 ± 30% of spiked amount, then the assay is accepted as working, if not it needs to be reperformed.

6.4.4.1 Peak areas will be documented on **Form 23023-03 – Peak Readings for the determination of Kanamycin concentration.**

6.4.4.2 Kanamycin in undiluted sample can be calculated as following.

Kanamycin (µg/mL) in undiluted sample = Calculated concentration * 16 (Dilution factor)

6.4.5 Results for the determination of Kanamycin will be documented on ***Form 23023-04 Determination of Kanamycin Concentration***.

7. DOCUMENTATION AND RECORDS

Document on **Form 23023-01**, **23023-02**, **23023-03** and **23023-04**. Mass spec operating conditions will be printed and included as part of the documentation.

8. REFERENCES AND RELATED DOCUMENTS

Document Number	Title
22955	Routine Calibration of the Waters Xevo G2-XS QToF (QToF) Mass Spectrometer
22964	Operating the Waters Acquity UPLC Using MassLynx
22971	Guidelines for Analyses Conducted on the UPLC/QTOF Mass Spectrometer
23023-01	Kanamycin testing – Reagents, Materials, and equipment.
23023-02	Kanamycin testing form
23023-03	Kanamycin testing – Peak area readings for determination of Kanamycin concentration
23023-04	Determination of Kanamycin Concentration