

Simultaneous quantitation of antibiotics in vaccine formulations using LCMS-8045

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1. Overview

Antibiotics are used to prevent bacterial contamination during vaccine manufacture. Most of the antibiotics are removed during the vaccine purification process, but trace quantities remain in some vaccines. The amount of antibiotics that remain in the final vaccine formulation needs to be mentioned in the vaccine excipient summary. The antibiotics in the final vaccine formulation can cause severe allergic reactions and hence needs to be checked during the final batch release

This poster describes an LC-MS/MS method for the simultaneous quantification of Polymyxin B1, Polymyxin B2, Streptomycin, Gentamycin C1, Gentamycin C2, Neomycin, Hygromycin, Kanamycin, and Tobramycin antibiotics from vaccine formulations.

2. Introduction

Vaccines have had a dramatic effect on the prevalence of communicable diseases. They represent the most effective measures in public health by controlling and preventing the spread of infectious diseases. However, vaccines may cause adverse events which can range from trivial and inconvenient to severe and life-threatening.^[1]

A vaccine contains materials in addition to the active immunogen. Some of these materials are added by the manufacturer for a specific purpose - for example, stabilizers and adjuvants. Others are residual materials from the manufacturing process. Many vaccines contain little amounts of antibiotics such as neomycin, streptomycin, polymyxin, and the fungicide amphotericin B, to prevent bacterial and fungal contamination respectively during the manufacturing process. To date, one single case of anaphylaxis has been linked to a neomycin-containing vaccine. It is a general agreement that patients with anaphylactic reactions to topical or systemic neomycin should not be vaccinated with neomycin-containing vaccines. Other antibiotics like streptomycin, gentamycin, and polymyxin B sulphate have been reported to trigger mild to life-threatening allergic reactions caused by topical and/or systemic clinical use. Usually, proven anaphylaxis or allergy to one or more antibiotics contained in vaccines is considered an absolute contraindication to those vaccines.

A manufacturer need not specifically test for antibiotics in the final container, however, the calculated amount of residual antibiotics (based on dilutions of the amount that was added) must be noted on the package label. Although not all the results of all final releases and inprocess testing are contained in the labelling that accompanies a product, the FDA does specify which information should be included.^[3] As the antibiotics in the final vaccine formulation can cause severe allergic reactions, their concentrations need to be checked against the label claim during the final batch release.

Table 1. Content of antibiotics in vaccines as per label claim

	Bevac	Biopolio	Infanrix	Influvac	Jenvac	Nexipox	Priorix	Rotavac	Tetraxim
Hygromycin B	×	×	×	×	×	×	×	×	×
Streptomycin	×	×	×	×	×	×	×	×	Trace
Kanamycin	×	15 μg/0.1 ml	×	×	×	×	×	15 μg/0.5 ml	×
Tobramycin	×	×	×	×	×	×	×	×	×
Gentamicin C2	×	×	×	Trace	×	×	×	×	×
Gentamicin C1	×	×	×	Trace	×	×	×	×	×
Neomycin	×	15 μg/0.1 ml	Trace	×	×	Trace	Trace	15 μg/0.5 ml	Trace
Polymyxin B2	×	×	Trace	×	×	×	×	×	Trace
Polymyxin B1	×	×	Trace	×	×	×	×	×	Trace

Here we describe a MRM based LC-MS/MS method for the simultaneous quantification of polymyxin B1, polymyxin B2, streptomycin, gentamycin C1, gentamycin C2, neomycin, hygromycin, kanamycin, and tobramycin antibiotics in the above mentioned vaccine formulations.

3. Methods

3-1. Sample preparation method

The different vaccine samples (mentioned in table no. 1) were procured from the pharmacy for analysis. The freeze-dried vaccines were reconstituted in 0.5 ml diluent [5:5:90 v/v/v Formic acid : Isopropanol (IPA) : 0.15% Heptafluorobutyric acid (HFBA)]. Vaccines were filtered using a 0.22 µm nylon filter. All vaccines except Biopolio and Rotavac were diluted in 1:1 proportion and injected for analysis. The Biopolio vaccine was diluted 1000 times, while Rotavac was diluted 100 times before injection.

The antibiotics were analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera XS coupled with the LCMS-8045, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan (Figure 1) using conditions described in table 2.

LCMS-8045, sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), an ultra-fast scanning speed of 30,000 u/sec (UFscanning), and a polarity switching speed of 5 msec (UFswitching). This system ensures the highest quality of data, with a very high degree of reliability.



Figure 1 Nexera XS with LCMS-8045 system

3-2. Analytical conditions

Table 2. Instrument parameters

UHPLC condition (Nexe	era XS system)			
Column	Raptor ARC-18 (150 mm x 3.0 mm, 2.7 micron) (S/N :19052059)			
Mobile phase	A: 10mM HFBA in water B: Acetonitrile			
Flow rate	0.3 mL/min			
Gradient program (B%)	0-6 min \rightarrow 10-95 (%); 6-7.5 min \rightarrow 95 (%); 7.5-8 min \rightarrow 95-10 (%); 12 min \rightarrow Stop.			
Injection vol.	40 μL			
Column temperature	35°C			
Diluent Formic acid:IPA:0.15% HFBA (5:5:90 v/v/v)				
MS Parameters (LCMS-	8045)			
MS interface	ESI			
Nitrogen gas flow	Nebulizing gas – 3 L/min; Drying gas – 10 L/min			
MS temperatures	Desolvation line - 250°C; Heating block - 400°C; Interface - 280°C			

Table 3. MRM transitions of antibiotics

Sr. No.	Antibiotic	MRM (quantifier)	MRM (qualifier)
1	Hygromycin B	528>352	
2	Streptomycin	582>263	582>246
3	Kanamycin	485>163	
4	Tobramycin	468>163	
5	Gentamicin C2	464>322	464>160
6	Gentamicin C1	478>157	478>322
7	Neomycin	615>161	615>293
8	Polymyxin B2	595>101	595>202
9	Polymyxin B1	602>101	602>120

4. Results

4-1. Linearity of the antibiotics

Nine-point linearity for the antibiotics was prepared and analyzed. Calibration curves for respective antibiotics were plotted. A representative chromatogram of nine antibiotics is given in figure 2. Calibration curves for 9 antibiotics are given in figure 3.

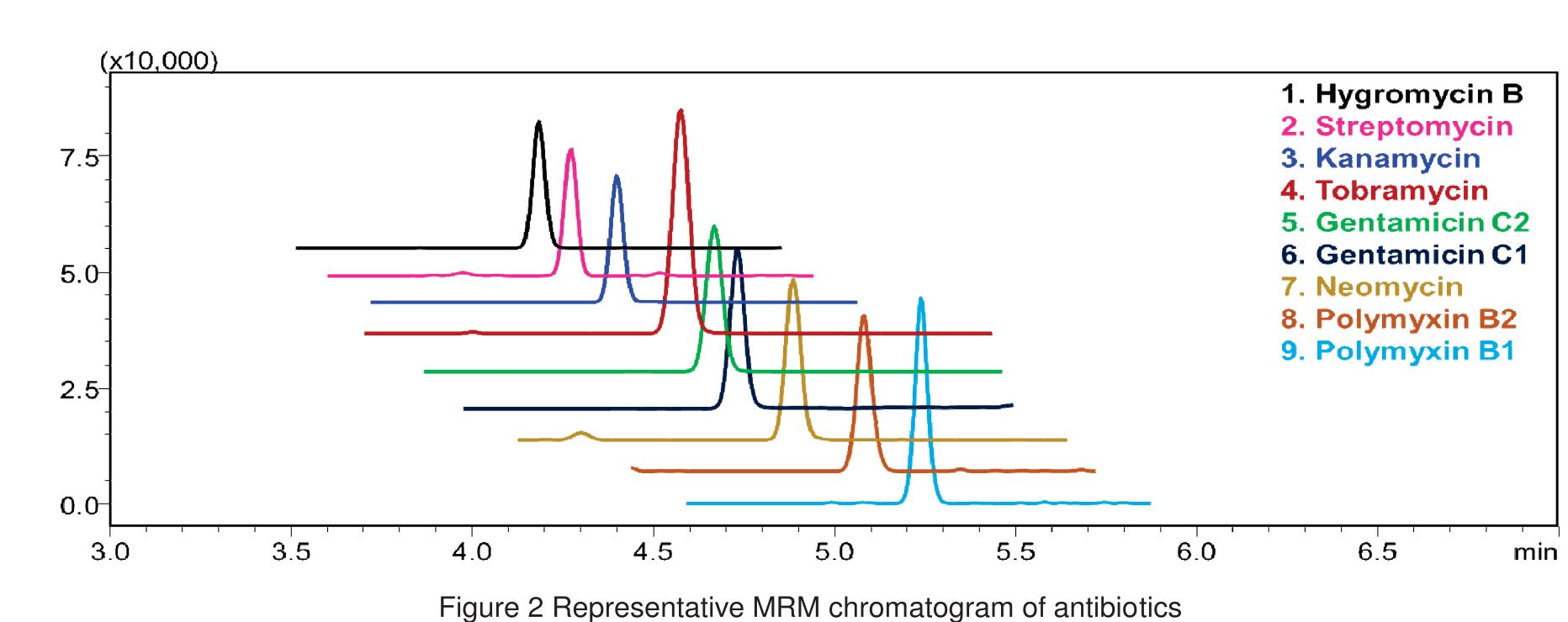


Table 4. Summary of linearity & LOQ results

	Colibration range		LOQ				
Compound	Calibration range (ppb)	r ²	Conc. (ppb)	%RSD (n=6)	Avg. S/N (n=6)		
Hygromycin B	35 to 2000	0.983	35	6	116		
Streptomycin	35 to 3000	0.984	35	6	49		
Kanamycin	30 to 2500	0.991	30	4	26		
Tobramycin	30 to 2000	0.986	30	3	41		
Gentamicin C2	30 to 2000	0.981	30	3	17		
Gentamicin C1	35 to 2000	0.985	35	3	25		
Neomycin	30 to 2000	0.981	30	5	17		
Polymyxin B2	30 to 2000	0.987	30	6	63		
Polymyxin B1	40 to 2000	0.980	40	4	32		

The results for correlation coefficient, repeatability and S/N at LOQ level are shown in table 4. The results of vaccine sample analysis is shown in table 5.

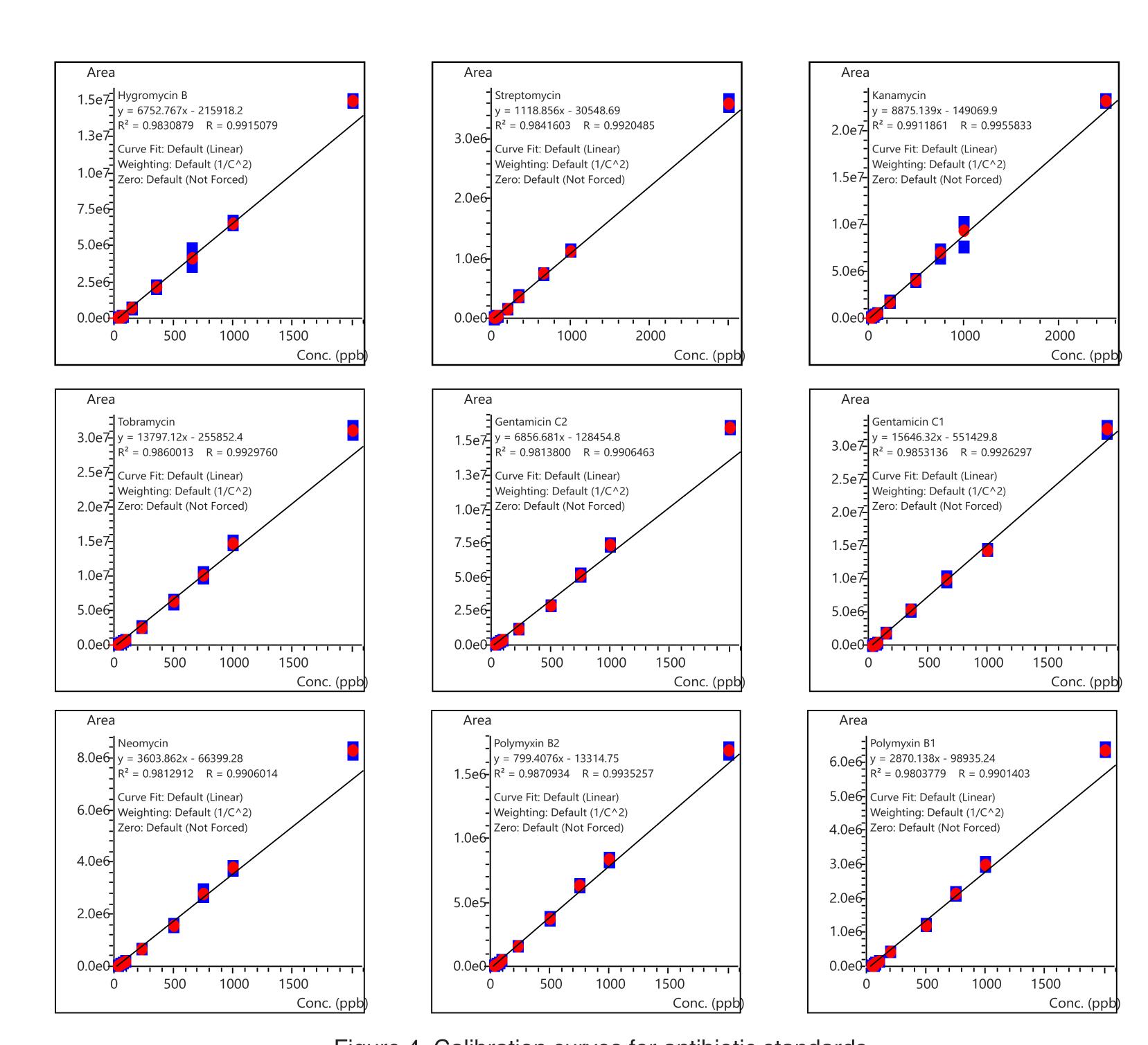


Figure 4 Calibration curves for antibiotic standards

Table 5. Content of antibiotics in vaccine samples (ppm)

	Bevac	Biopolio	Infanrix	Influvac	Jenvac	Nexipox	Priorix	Rotavac	Tetraxim
Hygromycin B									
Streptomycin									
Kanamycin		152.63						31.35	
Tobramycin									
Gentamicin C2				0.07					
Gentamicin C1				0.04					
Neomycin		153.92	0.04			0.04	3.15	29.46	0.04
Polymyxin B2									
Polymyxin B1									

Note: (--) Below limit of quantitation

5. Conclusion

- A single MRM based LC-MS method for the simultaneous quantification of nine antibiotics in nine different vaccines was successfully developed on the Shimadzu LCMS-8045 system.
- The linear response was observed for all the antibiotics with correlation coefficient greater than 0.98. The repeatability (n=6) at the LOQ level was found to be less than 10% RSD proving the ruggedness of the method.
- The content of neomycin and kanamycin in vaccines Biopolio and Rotavac matched the label claim. The traces of neomycin were found in Infanrix Hexa, Nexipox, Priorix and Tetraxim; while gentamycin C1 and C2 were found in Influvac added during the manufacturing.

6. References

For Research Use Only. Not for use in diagnostic procedures.

[1] Franceschini, Fabrizio, et al. "Vaccination in children with allergy to non active vaccine components." Clinical and translational medicine 4.1 (2015): 1-8.

[2] Fritsche, Philipp J., Arthur Helbling, and Barbara K. Ballmer-Weber. "Vaccine hypersensitivity-update and overview." Swiss medical weekly 140.17-18 (2010): 238-46.

[3] Finn, Theresa M., and William Egan. "Vaccine additives and manufacturing residuals in vaccines licensed in the United States." Plotkin's Vaccines. Elsevier, 2018. 75-83.