Method: Determination of Ivermectin in Animal Feed by LC-MS/MS

SOP: SOP-IVM Feed Assay (Office of the Texas State Chemist, Rev. 1)

Guidance: FDA CVM Guidance for Industry #135

Laboratory: Office of the Texas State Chemist (OTSC)

Date: 01/12/2026

1. Purpose

The purpose of this validation study was to demonstrate that the LC-MS/MS method for the determination of ivermectin in animal feed is suitable for its intended use and meets the validation characteristics recommended in FDA CVM Guidance for Industry #135 for Type C medicated feeds.

2. Method Summary

Animal feed samples were extracted with methanol, diluted, and analyzed by LC-MS/MS using matrix-matched calibration standards and ivermectin-d_2 as an internal standard. Quantitation was based on peak area ratios. (**Attachment 1**)

3. Validation Design

Two representative feed matrices (cattle and deer feed) were evaluated. Validation samples were fortified at 5, 50, 150, and 500 ppm. Multiple validation sets were analyzed on different days by different analysts to assess repeatability and intermediate precision. (**Attachment 2**)

4. Specificity

No interfering peaks were observed at the retention time of ivermectin in matrix blanks. Identification was confirmed by retention time and MRM transitions. (**Attachment 3**)

5. Linearity and Range

Matrix-matched calibration curves demonstrated linearity with $R^2 \geq 0.995$ across the range of 0.5–25 µg/g. (**Attachment 1 and 3**) The validated method range was established as 0.5–625 ppm. (**Attachment 1**)

6. Accuracy and Precision

Mean recoveries across all matrices and levels were within 90–110% of nominal concentrations. Repeatability and intermediate precision met acceptance criteria, with RSDs within recommended limits for medicated feed assays. (**Attachment 4 and Table 1**)

Table 1 Accuracy and Precision of the fortification validation study

Cattle Feed	Fortified Level (ppm)	Within Run			Between Run
		Day 1	Day 2	Day 3	Overall
Recovery (%)	5	103%	97%	104%	101%
	50	104%	97%	104%	102%
	150	103%	104%	102%	103%
	500	103%	100%	104%	102%
RSD (%)	5	1.65%	2.70%	1.21%	3.52%
	50	1.36%	5.28%	2.44%	4.65%
	150	1.72%	2.23%	1.27%	1.82%
	500	1.88%	4.12%	0.66%	2.96%
Deer Feed	Fortified Level (ppm)	Within Run			Between Run
		Day 1	Day 2	Day 3	Overall
Recovery (%)	5	104%	97%	104%	102%
	50	102%	97%	105%	101%
	150	104%	103%	102%	103%
	500	104%	95%	102%	100%
RSD (%)	5	2.25%	3.81%	2.29%	4.30%
	50	1.42%	2.45%	1.62%	3.82%
	150	1.05%	4.01%	2.45%	2.62%
	500	1.37%	3.01%	1.82%	4.22%

7. LLOD and LLOQ

LLOD and LLOQ were determined using the calibration-curve approach described in CVM GFI #135 using ten replicates of the fortified samples at 0.1 ppm. (**Attachment 5**)

LLOD = 0.034 ppm

LLOQ = 0.104 ppm

8. Proof of Performance

Four batches of medicated feed were prepared to evaluate method performance. The target concentrations were 20 ppm and 75 ppm for deer feed, and 200 ppm and 400 ppm for cattle feed. (**Attachment 6**)

Ten replicate samples at each concentration level were analyzed using the developed method on different dates (**Attachment 7**). The resulting data were evaluated to assess the accuracy and precision of the method. (**Attachment 8 and Table 2**)

Table 2 Accuracy and Precision of the analysis of medicated feed

Sample Matrix	Assigned Value of IVM (ppm)	Average (ppm)	Recovery (%)	SD (ppm)	RSD (%)
Cattle Feed	200	203.65	101.82%	9.25	4.54%
Cattle Feed	400	387.05	96.76%	7.98	2.06%
Deer Feed	20	20.76	103.80%	0.67	3.24%
Deer Feed	75	73.90	98.53%	2.56	3.46%

9. Conclusion

The method meets all applicable FDA CVM GFI #135 validation requirements and is suitable for routine regulatory analysis of ivermectin in animal feed.

10. Statement of Compliance

This validation study was conducted in accordance with FDA CVM Guidance for Industry #135. All validation characteristics met acceptance criteria.