Improved SLICE® (emamectin benzoate) medicated-feed assay method

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SLICE® (emamectin benzoate, 0.2% w/w) medicated premix is a parasiticide indicated for the treatment and prevention of sea lice infestations in salmonids. Preformed fish pellets are top-coated with the premix, and then an overcoat of fish oil is applied to ensure even distribution of the premix on pellets.

Analysis of medicated feeds is essential for successful sea lice treatment because it can confirm proper fish dosing, which helps mitigate the risk of re-infestations and treatment resistance.

A high performance liquid chromatography (HPLC) method for assaying emamectin benzoate in fish feed, developed and validated by analytical chemists Farer and Hayes of Schering-Plough Animal Health (now MSD Animal Health), was published in 2005. This method has been used to analyze feed medicated with SLICE at many of the laboratories that support feed mills in salmon-producing countries.

Recently, however, the HPLC assay method has undergone minor modifications, specifically regarding sample preparation, to improve the recovery of emamectin benzoate and ensure reproducible results. This modified method has been fully validated.

The purpose of this bulletin is to summarize the changes and their impact on assay results, and provide guidance on interpretation of feed analysis results. The target audience includes laboratories that support testing of medicated fish-feed samples, manufacturing feed mills or commercial operations, as well as veterinarians and farm managers.

Overview

The HPLC method is validated to assay feed medicated with a concentration ranging from 2 mg to 20 mg emamectin benzoate per kilogram of feed. The method involves three steps:

- Liquid extraction of emamectin benzoate from feed
- Cleanup by solid phase extraction to remove matrix interferences inherent in the feed
- Quantitation by HPLC analysis

Modifications

Modifications to the method involve the liquid extraction and cleanup steps and are intended to improve the recovery of emamectin benzoate during the extraction process.

With the original method, liquid extraction was performed with duplicate rounds of shaking for 60 minutes, followed by sonication for 15 minutes. Immediately following initial sample extraction,

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cleanup by solid phase extraction would be performed. This method has been revised to include the following minor changes:

STEP ONE

Liquid extraction

- A 10-mL aliquot of water is added to ground feed and allowed to penetrate the feed for 60 minutes prior to the addition of the extraction solvent.
- Sonication of the liquid extract is performed in a water bath heated to 50° C (122° F).
- A 40-mL (previously 50-mL) aliquot of water is added following sonication to compensate for water added to wet the feed prior to the addition of the extraction solvent.
- The final liquid extract is allowed to settle overnight.

STEP TWO

Cleanup by solid phase extraction

- The solid phase extraction cartridge conditioning wash volumes are increased from 5 mL to 10 mL.
- The liquid extract is added by gravity alone, without the use of vacuum.
- A 10-mL aliquot of wash solvent is used to rinse the filtration column.
- The storage stability of two wash solvents has been shortened from 2 weeks to 1 week.

STEP THREE

Quantitation by HPLC

No changes were made to this part of the process. The sample preparation modifications do not affect chromatography, and the procedures described for quantitation in the original method are used.

Other recommendations

We recommend preparing fortified control feed to assess method performance. Unmedicated fish feed is fortified with a known amount of emamectin benzoate and processed by the assay method along with the samples. The expectation is that fortified control-feed recovery be within 85% to 110% of the added amount. If the fortified control-feed samples fail to meet this specification, then the medicated-feed samples that are concomitantly prepared are invalidated.

We have provided guidance with the new method on sample, standard and mobile phase stability to allow stopping points during the analysis in case an unexpected interruption occurs in sample preparation.

Validation

Validation experiments were performed by multiple analysts on different days to assess the impact of changes on the method's accuracy and precision over a range from 50% of the lowest to 150% of the highest incorporation rates (the range recommended by the United States Food and Drug Administration). As summarized in Table 1, recovery of emamectin benzoate has been improved by approximately 10% compared to the originally published method.

Figure 1 demonstrates the variability in percent recovery as a function of concentration. Lower levels of emamectin benzoate had more-variable recoveries. However, the results for fortified control feed are within the defined acceptance criteria of 85% to 110%.

Additional validation parameters that were evaluated include range, proof of performance, robustness, extraction efficiency and sample stability. The results demonstrate that the method is suitable for the quantitation of

emamectin benzoate in medicated fish feed over a range of 2 ppm to 20 ppm.

Method reproducibility was confirmed by a recent inter-laboratory study. Samples from two commercial-scale batches were analyzed independently, with results showing excellent agreement between the two labs.

TABLE 1

Emamectin benzoate-level replicate	Percent recovery	
	Analyst 1	Analyst 2
1 ppm -1	90	95
1 ppm -2	90	97
1 ppm -3	92	100
1 ppm -4	93	97
1 ppm -5	88	91
Analyst average (n=5)	91	96
Analyst RSD* (n=5)	2.2	3.5
Pooled average (n=10)	93	
Pooled RSD (n=10)	4.1	
15 ppm -1	96	96
15 ppm -2	95	95
15 ppm -3	96	98
15 ppm -4	95	96
15 ppm -5	96	93
Analyst average (n=5)	96	96
Analyst RSD (n=5)	0.6	1.9
Pooled average (n=10)	96	
Pooled RSD (n=10)	1.3	
30 ppm -1	95	95
30 ppm -2	95	96
30 ppm -3	95	95
30 ppm -4	95	96
30 ppm -5	94	93
Analyst average (n=5)	95	95
Analyst RSD (n=5)	0.5	1.3
Pooled average (n=10)	95	
Pooled RSD (n=10)	0.9	

*RSD is the calculated Relative Standard Deviation

Table 1. Accuracy, precision repeatability and intermediate precision for the emamectin benzoate in medicated-feed assay

Data analysis and interpretation

The results of fortified control samples (with recovery of 85% to 110%) should serve to demonstrate that the method has been properly performed. If a single sample gives unacceptable results, repeat analysis of duplicate preparations should be considered to confirm the original result.

Even with improvements to the method, there is an approximate 5% loss in recovery from the sample preparation itself, as demonstrated in Table 1. Therefore, one should expect sample results to be within ±10% of the recovered value for the fortified control-feed samples. The ±10% range for recovery is acceptable for a medicated feed. Many regulatory agencies provide specifications that are even wider, given the variability in manufacturing, sampling and complexity of the assay of medicated feeds.

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FIGURE 1

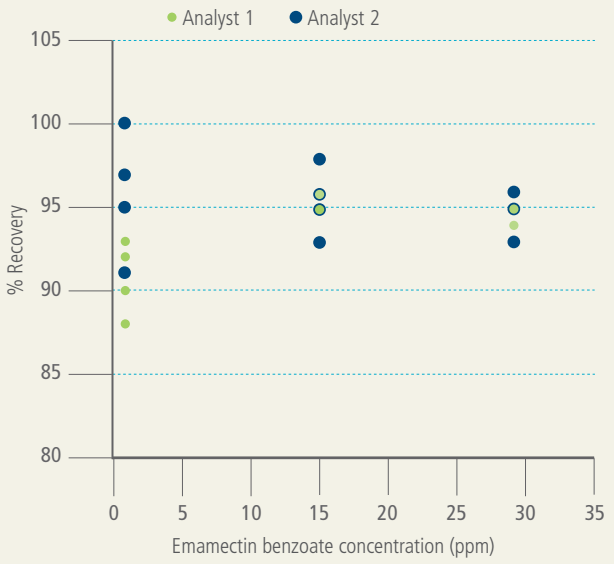

Figure 1. Percent recovery of emamectin benzoate from medicated salmonid feed as a function of concentration

Some regulatory authorities may not accept correcting assay results of samples for recovery percentage; we advise following local, applicable guidelines regarding this practice.

Conclusions

The previous method used to quantitate emamectin benzoate in medicated fish feed has been modified to improve recovery, accuracy and precision. The modified method averages 90% to 100% recovery, which is an improvement of 10% over the previously published method, as demonstrated by a series of validation experiments. The updated method recommends the preparation of fortified control samples to provide additional assurance that the method was performed properly.

References

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