Stability of emamectin benzoate in methanol stock solution at three different temperatures

Bioassays are increasingly being used as a tool to predict sensitivity or to detect changes in sensitivity of sea lice to available sea lice treatments; methods are available for conducting bioassays with pyrethroids (deltamethrin [Alphamax®] and cypermethrin [Excis®]), azimethiphos [Salmosan®] and emamectin benzoate [SLICE®].

Several methods for determining sea lice sensitivity to emamectin benzoate are available, including the SEARCH protocol[1] and a protocol by Wescott, *et al*.[2] Both of these published methods utilize methanol as the solvent for preparation of emamectin benzoate stock solutions. A similar bioassay method using propylene glycol as the solvent for stock solutions has been used since 2000 at the University of Stirling and is in use by the Fish Vet Group (Scotland).[3]

To facilitate rapid bioassays, MSD Animal Health has determined that stock solutions of emamectin benzoate should be available for immediate dilution and use. In support of this effort, it is necessary to understand the stability of emamectin benzoate in stock solutions under typical storage conditions. Consequently, the following study was conducted at the Institute of Marine Research, Bergen, Norway, to examine the stability of emamectin benzoate stored in methanol, in dark bottles, at three different temperatures: 20° C, 4° C and -20° C (68° F, 39.2° F and -4° F, respectively).

Materials and methods

Emamectin benzoate standard was obtained from MSD Animal Health. Methanol was HPLC-grade from VWR International, LLC. Dark bottles (25 mL) were from Schott.

PREPARATION OF STANDARDS

- 3 x 50 mL stock solution (5 mg emamectin benzoate + 50 mL methanol) was prepared.
- Each of the three bottles was split into 5 aliquots of 10 mL each.
- One series of five stock solutions was stored at room temperature, another series at 4° C (39.2° F) and the third series at -20° C (-4° F).
- Samples from all three storage temperatures were checked for stability after 1, 2 and 4 weeks.
- In addition, the samples stored at 4° C (39.2° F) and -20° C (-4° F) were checked for stability after 8 and 12 weeks.

Analysis

The HPLC system used consisted of a Spectra-Physics SP 8800 ternary HPLC-pump connected to

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TABLE 1

	Initiation of study	End of study
Room temperature	94.2140 ± 1.6270 (peak area)	93.9147 ± 0.3570 (4 weeks)
4° C (39.2° F)	95.2729 ± 1.5061 (peak area)	96.0942 ± 0.5560 (12 weeks)
-20° C (-4° F)	97.1420 ± 1.4100 (peak area)	94.5513 ± 1.4000 (12 weeks)

Table 1. Concentrations of emamectin benzoate measured as peak areas in the solutions stored at different temperatures. The numbers in Table 1 are the mean of five samples.

a Gilson 234 Autoinjector and a Spectra-Physics UV-detector operating at a wavelength of 260 nm. The detector output was coupled to a computerized data system consisting of a Dionex UCI-50 Universal Chromatography Interface, Dionex Chromeleon Version 6.80 and a PP04X Dell computer for storage and integration of the chromatograms. The analytical column was a 150 x 4.6 mm ACE-3, C-18, 3 µm connected to a short C-18 pre-column (10 x 4.6 mm). The column was operated at room temperature. The mobile phase used contained methanol — deionized water (98 - 2 v/v). The solution was filtered through a 0.2 µm Millipore filter and degassed using helium and 6 min of sonication. The flow rate was 0.8 mL min^{-1} giving an elution time of approximately 3.3 min.

The stock solutions (20 µl) were injected directly into the HPLC without any pre-treatment.

Results

No significant reductions in emamectin benzoate concentrations were detected at any of the temperatures within the time range of this study (Table 1).

In conclusion, emamectin benzoate is stable when stored in methanol in dark bottles for at least 4 weeks at room temperature and 12 weeks when stored at 4° C (39.2° F) and -20° C (-4° F).

References

[1] *Sealice Resistance to Chemotherapeutants — A handbook in resistance management, Search Project* (QKK2-CT-00809), from: http://www.rothamsted.bbsrc.ac.uk/pie/search-EU/index.php.

[2] Westcott, JD, H Stryhn, JF Burka, KL Hammell. 2008. Optimization and field use of a bioassay to monitor sea lice *Lepeophtheirus salmonis* sensitivity to emamectin benzoate. *Diseases of Aquatic Organisms.* 79:119–131.

[3] Correspondence on file.

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