Meinertz, J.R. and T.M. Schreier. 2006. Evaluation of a method to determine concentrations of isoeugenol in the edible fillet tissue from rainbow trout, *Oncorhynchus mykiss*. Submitted to the UMESC archives September 12, 2006 and to the Association of Fish and Wildlife Agencies September 21, 2006. 1681 pages.

Summary

There is a critical need for an anesthetic/sedative with zero withdrawal time in U.S. public aquaculture and fishery management. AQUI-S^{TM} is a fish anesthetic/sedative approved for use in Australia, Chile, and New Zealand. Approval of AQUI-S^{TM} use in the United States is also being pursued. Among the data required to receive U.S. Food and Drug Administration approval for use in the United States are data validating an analytical method for the AQUI-S^{TM} marker residue.

Data from study number CAP-04-AQUIS-03, "Isoeugenol total residue depletion in the edible fillet tissue of rainbow trout (*Oncorhynchus mykiss*)", indicated that isoeugenol is the primary residue in extracts from rainbow trout skin-on fillet tissue after fish were exposed to AQUI-S^{TM}. Since isoeugenol is the primary residue, isoeugenol was believed to be the compound that will be selected as the marker residue for AQUI-S^{TM}. The method developed for determining isoeugenol concentrations in rainbow trout fillet tissue uses relatively common procedures and equipment. The method procedures include extracting isoeugenol from tissue with acetonitrile, evaporating the acetonitrile from the extract with rotary evaporation techniques, changing the polarity of the extract by adding water, concentrating the isoeugenol with solid phase extraction (spe) procedures, and determining concentrations with high pressure liquid chromatography. The method is specific, i.e., there were no chromatographic interferences in extracts from control rainbow trout fillet tissue and no interferences from other chemicals used in aquaculture. The method is sensitive, i.e., the method detection and quantitation limits are 0.007 and 0.02 µg/g, respectively. The method is accurate, i.e., the percentage of isoeugenol recovered from samples fortified at 1, 50, and 100 µg/g was always >82%. The method is repeatable, i.e., the within-day precision was always <10% and the day-to-day precision was <4% with samples fortified at 1, 50, and 100 µg/g. The method precision with tissue containing biologically incurred isoeugenol was <2.5%. The method is rugged, or robust, i.e., relatively minor changes in the method procedures did not affect the outcome of the analyses. The following method modifications did not result in unacceptable method performance (i.e., accuracy <80% and precision >10%): a 1-g increase in sample weight, a 5-mL decrease in the volume of acetonitrile used to extract the sample, a 3-mL increase in the sample volume after evaporation, a 5-mL increase or decrease in the volume of water used to hydrate the sample after evaporation, use of Chrom Tech, Inc. (Apple Valley, MN) brand frits to collect tissue debris in the spe reservoir, use of four different brands or types of spe columns to concentrate isoeugenol, failing to dry the spe column and eluting the column with 4 mL of eluting solvent, a 5% increase and decrease of methanol percentage in the eluting solvent, and use of Agilent and Alltech brand syringe filters to filter the final extract. Method performance was not grossly affected when the method was applied to tissue from rainbow trout cultured in different regions of the country. Degradation of isoeugenol in 90:10 methanol:water was significant in 1-µg/mL solutions but insignificant in 100-µg/mL solutions. Degradation of isoeugenol in tissue

extract was notable in extracts from tissue fortified at 1 µg/g but less notable in extracts from tissue fortified at 50 and 100 µg/g. Degradation of isoeugenol in tissue was affected by storage time, i.e., isoeugenol concentrations in fillet tissue with incurred residues decreased through time.