



U.S. Fish & Wildlife Service

Aquatic Animal Drug Approval Partnership**DRUG RESEARCH INFORMATION BULLETIN****Stability of Eugenol in Solutions of AQUI-S®20E**

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Various compounds are commonly used by fisheries professionals and others to sedate or anesthetize fish for procedures such as measuring weight or length, collecting blood samples, surgical implantation of tags or tracking devices, and transport. Some of these compounds, e.g., clove oil or carbon dioxide gas, are not typically considered drugs by end-users. However, according to the U.S. Food and Drug Administration (FDA), the definition of animal “drug” includes any article (other than feed) intended to change the structure or function of an animal’s body. As such, any compound used to tranquilize, sedate, or anesthetize fish is considered a drug and must be approved by the FDA before it can be used (other than fish used in laboratory research). Fisheries professionals and others need legal access to safe and effective sedatives that can be used under a variety of environmental conditions. In addition, a fish sedative should (1) be easy to use, (2) offer some analgesia, (3) be inexpensive, and (4) allow the user to release or harvest treated fish after sedation.

Tricaine methanesulfonate products available as Finquel® (Argent Chemical Laboratories, Inc., Redmond, Washington) and Tricaine-S® (Western Chemical, Inc. Ferndale, Washington) are currently the only products approved by the FDA for the temporary immobilization of fish and other aquatic, cold-blooded animals. Although these products are generally considered safe and effective and are widely used by fisheries professionals and others, their use is restricted to four families of fishes (Ictaluridae, Salmonidae, Esocidae, and Percidae) and requires a 21-d withdrawal period for fishes intended for human consumption. For certain fisheries applications, holding fish after sedation and recovery is not practical, particularly in field settings. To avoid these complications, an FDA-approved, immediate-release sedative is urgently needed.

AQUI-S®20E (10% eugenol) is a fish sedative developed by AQUI-S New Zealand, Ltd. (Lower Hutt, NZ), and it has become the lead candidate for approval in the U.S. as an immediate-release sedative. AQUI-S NZL is working with a variety of public-sector data-generating partners to establish that the product is efficacious; safe to fish, humans, and the environment; and can be manufactured consistently according to FDA criteria. Research study protocols have been developed to generate efficacy and target animal safety data in support of a U.S. approval. Such studies require the concentration of exposure baths be analytically verified to confirm the actual sedative concentration tested is acceptably close to the target concentration.

A simple UV-Vis spectrophotometric method has been developed to measure the concentration of eugenol in water. Ideally during a study, samples of AQUI-S®20E solutions could be collected throughout the day, but analyzed only after the last sample had been collected. However, for this approach to work, the eugenol concentration in a solution of AQUI-S®20E must be stable over time. As such, I conducted a study at the U.S. Fish and Wildlife Service Bozeman Fish Technology Center (BFTC) in February 2011 to evaluate the stability of eugenol in solutions of AQUI-S®20E prepared from a single water source and repeatedly analyzed over a 144-h period.

Methods

One-gallon solutions of 10, 50, and 100 mg/L eugenol (100, 500, and 1,000 mg/L AQUI-S®20E, respectively) were prepared with BFTC spring water in plastic buckets. Five water samples (~2-4 mL) were collected from each bucket immediately following preparation and then periodically during the next 144 h. Solutions were left uncovered under ambient conditions (~22°C, overhead lights on for approximately 8 h/weekday and supplemented by natural light through a window). Each time samples were collected, the eugenol concentrations in samples were measured by UV-Vis spectrophotometry (279.0 nm, Genesys®2; Thermo Electron Corp., Madison, Wisconsin). At each collection time (T=0, 2, 4, 6, 8, 12, 24, 28, 32,

48, 102, and 144 h), a fresh 500-mg/L eugenol stock standard was prepared by dissolving 0.5 g eugenol ($\geq 99.5\%$, PT Indesso Aroma, Jakarta, Indonesia) in 50 mL 100% ethyl alcohol (Fisher Scientific, Pittsburgh, Pennsylvania) and diluted with BFTC spring water to 1,000 mL in a volumetric flask. A set of five eugenol working standards (5, 10, 25, 40, and 60 mg/L) was then prepared by transferring appropriate volumes of stock standard into 100-mL volumetric flasks and again diluting with BFTC spring water. The absorbance of each working standard was measured, and the resulting data pairs (x = stock standard concentration, y = absorbance) were used to create a standard curve. Standard curves were deemed acceptable at R^2 values ≥ 0.99 . The eugenol concentration of the highest working standard (60 mg/L) was less than the nominal eugenol concentration of the 100-mg/L eugenol solution; consequently, samples collected from the 100-mg/L eugenol solution were diluted 50:50 v/v with BFTC spring water before analysis. Slope and y-intercept values for each standard curve were used in the following equation to determine the concentration of samples collected from the AQUIS®20E solutions: eugenol concentration (mg/L) = (absorbance – y-intercept)/slope.

Results and Discussion

No trend was apparent to suggest that the concentration of eugenol in any of the working solutions decreased over time (Figure 1). The greatest difference between analytically verified and target eugenol concentrations in the 10, 50, and 100 mg/L eugenol solutions were 5.9% (at $T=32$ h), 5.8% (at $T=24$ h), and 7.6% (at $T=12$ h), respectively. Concentrations of eugenol in the 10-, 50-, and 100-mg/L eugenol solutions at the end of the 144-h storage period differed from the nominal concentrations by 4.3%, 4.3%, and 4.7%, respectively. The concentrations of eugenol measured in each working solution were considered accurate, in part, because the R^2 value for each standard curve was ≥ 0.9991 .

In conclusion, it appears that eugenol concentrations in AQUIS®20E solutions are stable up to 144 h when the solutions are stored uncovered at room temperature. Consequently, in fish sedation studies conducted in laboratories or fish culture facilities, there should be ample time to collect and analyze AQUIS-S®20E samples. Moreover, in fish sedation studies conducted with wild fish captured from streams, rivers, or lakes, there should be sufficient time to collect AQUIS-S®20E samples in the field and return those samples to the laboratory for analysis.

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Figure 1. Relative difference that each sample collected over a 144-h period was from the target concentration of the 10-, 50-, or 100-mg/L eugenol solutions prepared at time = 0 h. Note that each data point on the graph represents the mean of five samples analyzed from each working solution at each sampling time.

