

Vitellogenin Analysis and the use of Aprotinin

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ABSTRACT

Endocrine activity of the test substance may be indicated by measuring the glycoprotein, vitellogenin (VTG), a pre-cursor of yolk proteins produced by the liver of female fish in response to circulating endogenous oestrogen.

The OECD 229 guideline recommends that when blood is sampled from the caudal vein/artery, the separated plasma should be stored with aprotinin (a protease inhibitor) prior to VTG analysis. However, some evidence is available in the literature [1] which indicates that the addition of protease inhibitor may reduce the amount of VTG in the sample. [1] Brodeur JC, Woodburn KB, Zhang F, Bartels MJ, Klecka GM. Plasma sampling and freezing procedures influence vitellogenin measurements by enzyme-linked immunoassay in the fathead minnow (*Pimephales promelas*). Environmental Toxicology and Chemistry, 2006 February 25(2):337-48

METHODS

Blood samples were taken from the caudal vein/artery from six sexually mature, phenotypic adult female fathead minnow (*Pimephales promelas*). The plasma from each sample was separated by centrifugation and divided into two samples one of which was stored with 0.13 units of aprotinin and one without. The samples were stored deep frozen prior to analysis by Enzyme-Linked Immunosorbent Assay (ELISA) to determine the concentration of VTG in the samples. Each sample was analysed in duplicate and the mean VTG concentration calculated.

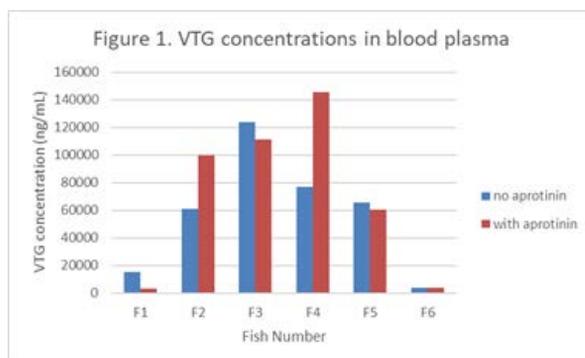
RESULTS

The results are presented in Table 1 and graphically in Figure 1. Three out of the six samples showed comparable levels of VTG between the two samples indicating that there was no difference in VTG concentrations in samples stored with or without aprotinin. A single sample showed a lower VTG concentration in the sample stored with aprotinin whereas two out of the six samples showed higher VTG concentrations in samples stored with aprotinin.

Addition of protease inhibitor might not increase stability of vitellogenin in fathead minnow plasma samples

Table 1: Blood plasma VTG concentrations after storage without and with aprotinin

Sample ID	Mean concentration (ng/mL x 10 ³)		%CV between samples
	Without aprotinin	With aprotinin	
F1	1.55	0.34	90.5
F2	6.11	9.75	33.8
F3	12.4	11.1	7.6
F4	7.67	14.6	43.8
F5	6.58	6.04	6.1
F6	0.39	0.38	2.2



CONCLUSIONS

Although the number of fish sampled was small, the results indicated that the use of the protease inhibitor, aprotinin, may not be required for the storage of caudal vein/artery blood plasma samples.



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