## METHOD SPECFICATION

#### Faculty of Biosciences, NMBU

# Method name: Astaxanthin BIOVIT no.: msp1017

## 1. Method of analysis / Principle / Main instrument

Pigments are important ingredients in feed to achieve a desired coloring of salmonids, where it represents an important cost of the feeds. Astaxanthin (AX) is the natural main carotenoid in the meat of wild salmonids which absorbs the pigment upon ingestion of zooplankton or krill. Shellfish products contain AX and was used to pigment salmonids in the early 1970s. Later synthetic, industrial produced astaxanthin and canthaxathin has been used as a pigment source for farming salmon and trout. Today, there is a growing interest in the use of pigment additives in feed from natural microbiological sources like microalgae, shrimp and krill. Such natural sources will also leave traces of other carotenoids such as  $\beta_i\beta$ -carotene, canthaxanthin, echinenone and idoxanthin. Therefore, the accurate determination of AX forms in this kind of biological matrices are necessary which involves extraction prior to their determination. By using an HPLC method instead of more traditional methods like color cards and spectrometry, it is possible to quantify the levels of AX (and canthaxanthin), but also to distinguish between some of the isomers of AX [1,2].

Main instrument: Ultimate 3000 UHPLC with UV detector (Thermo Scientific).

# 2. Reference and any modifications

CEN/TS 16233-1:2011 (E) - HPLC method for the determination of xanthophylls in fish flesh. Part 1: Determination of astaxanthin and canthaxanthin

#### **3.Requirements for the degree of grinding**

Feed and feces analysis: 0.2 g sample is required. Degree of grinding 1 mm. Fish muscle analysis: 1-2 g homogenized sample is required. A control sample containing 3.2 mg/kg should be run with each batch.

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## 4. Contact person:

Lab leader: Hanne Kolsrud Hustoft Responsible for analysis: Elin Follaug Johnsen

## 5. Additional literature

- 1) Østerlie, Marianne. Sluttrapport: Utvikling av metode for analyse av pigment i muskel hos laksefisk. Høgskolen i Sør-Trøndelag (2010)
- 2) Darias Hernández, Tania. "Astaxanthin determination in marine biological samples: an overview." (2013)

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