

## ANNEX

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Identification number of the feed additive	Name of the Additive	Composition, chemical formula, description, analytical method.	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	End of period of authorisation
					mg active substance/kg of complete feed with a moisture content of 12%			
Category: Nutritional additives. Functional group: Vitamins, provitamins and chemically well-defined substances having similar effect								
Sub classification: vitamin D								
3a670a	25-Hydroxy cholecalciferol	<b>Additive composition:</b>	Salmonids			0,800	1. The additive shall be incorporated in feedingstuffs via the use of a premixture.	[10 years from the date of entry into force of this
		Preparation with a maximum content of 1.25% of 25-hydroxycholecalciferol  Solid form	Finfish species other than salmonids.			0,075		

		<b>Characterisation of the active substance:</b>  25-Hydroxycholecalciferol . Its precursor compound, 5,7,24-cholestatrienol, is produced with <i>Saccharomyces cerevisiae</i> CBS 146008. After extraction, the precursor is converted chemically to 25-hydroxy-pro-vitamin D <sub>3</sub> , which is further transformed photochemically to 25-hydroxycholecalciferol  Chemical formula: C <sub>27</sub> H <sub>44</sub> O <sub>2</sub> .H <sub>2</sub> O  CAS number: 63283-36-3  <u>Purity criteria</u> <ul style="list-style-type: none"> <li>– 25-hydroxycholecalciferol &gt;94%</li> <li>– other sterol derivatives ≤ 1% each</li> <li>– erythrosine &lt; 5 mg/kg</li> </ul> <b>Analytical method<sup>1</sup></b>  For the determination of 25-hydroxycholecalciferol in the feed additive: Ultra Performance Liquid Chromatography coupled to	Ornamental fish			0,075	2. In the directions for use of the additive and premixtures, the storage conditions and the stability to heat treatment shall be indicated.  3. Maximum content of the combination of 25-hydroxycholecalciferol with cholecalciferol (vitamin D <sub>3</sub> ) per kg of complete feedingstuff: <ul style="list-style-type: none"> <li>– ≤ 1, 500 mg<sup>2</sup>(equivalent to 60 000 IU of cholecalciferol) for salmonids ,</li> <li>– ≤ 0,075 mg equivalent to 3 000 IU of cholecalciferol) for fish species other than salmonids and for ornamental fish,</li> <li>– ≤ 0,050 mg (equivalent to 2 000 IU of cholecalciferol) for other categories of</li> </ul>	Regulation – Precise date to be completed by the OP]
			Other species and categories of animals except pigs, poultry and ruminants			0,050		

<sup>1</sup>Details of the analytical methods are available at the following address of the Reference Laboratory: [https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports\\_en](https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en).

<sup>2</sup> 40 IU cholecalciferol (vitamin D<sub>3</sub>) = 0,001 mg cholecalciferol (vitamin D<sub>3</sub>).

		<p>spectrophotometric detection (UPLC-UV)</p> <p>For the determination of 25-hydroxycholecalciferol in premixtures: High Performance Liquid Chromatography coupled to spectrophotometric detection (HPLC-UV)</p> <p>For the determination of 25-hydroxycholecalciferol in compound feed and in low concentrated premixtures: High Performance Liquid Chromatography coupled to tandem mass spectrometry (HPLC-MS/MS)</p>					<p>animals except poultry, pigs and ruminants.</p> <p>4. For users of the additive and premixtures, feed business operators shall establish operational procedures and organisational measures to address the potential risks resulting from their use. Where those risks cannot be eliminated by such procedures and measures, the additive and premixtures shall be used with personal breathing and skin protective equipment.</p>	
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