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**Food Safety and Standards Authority of India**

(A statutory Authority established under the Food Safety and Standards Act, 2006)

(Quality Assurance Division)

**FDA Bhawan, Kotla Road, New Delhi - 110002**

Dated, the <sup>14</sup>12 March, 2019

**ORDER**

**Subject: Methods for analysis of fortificants in food products - reg.**

The Scientific Panel on Methods of Sampling and Analysis, Scientific Committee and Food Authority has approved the methods for analysis of fortificants **(Annexure - I)** in various food products.


2. The food testing laboratories are hereby requested to use the aforesaid methods for analysis of fortificants.

3. Any issue related to these methods may be forwarded to the Scientific Panel on Methods of Sampling & Analysis for its consideration at email: sp-sampling@fssai.gov.in

*Encl: Methods*

To:

1. All FSSAI Notified Laboratories
2. All State Food Testing Laboratories

  
(Bhaskar N.)  
Advisor (QA)

## Instrumentation based methods for analysis of Fortificants in Foods

S. No.	Commodity	Parameter	Test Method	Brief of Sample Size & extraction process, Mobile Phase solvent	Instrument & Detector/Method
1	Salt	Iodine Content	AOAC 925.56 (Titrimetric)	Microwave digestion with nitric acid. pH adjustment with ammonium hydroxide. Alkalization helps to convert volatile iodine to more stable iodide or Iodate. Sodium Iodide used as intermediate standard.	
		Iron Content	AOAC 2011.14 (ICP), AOAC 985.35 and AOAC 999.10 (AAS), AOAC 2015.06 (ICP-MS)	Microwave assisted Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)	AAS / ICP-OES/ICP-MS (wave length 259.94)
2	Milk	Vitamin A	AOAC 20 <sup>th</sup> Ed. 2016 2001.13	Use of pyrogallic acid, saponify. Neutralize with acetic acid dilute with THF:ethanol. Mobile phase: Methanol:water, isocratic) Gradient : Acetonitrile and Dichloromethane	HPLC- UV
		Vitamin D <sub>2</sub>	AOAC 2016.05		Liquid Chromatography-Tandem Mass Spectrometry
		Vitamin D <sub>3</sub>	AOAC 2002.05	Sample Size : 5-20g Ext Solvent : Alcohol (Methanol/Isopropyl alcohol/Ethanol), Iso-octane etc Mobile phase: Gradient (Water with Acetonitrile &Methanol) Gradient : Isopropanol-n-heptane (20 + 80). Dilute 200 mL isopropanol to 1 L with n-heptane.	HPLC-PDA/UV/VWD
3	Vegetable Oil	Vitamin A	AOAC 20 <sup>th</sup> Ed. 2016 2001.13	Use of pyrogallic acid, saponify. Neutralize with acetic acid	HPLC- UV

				dilute with THF:ethanol. Mobile phase: Methanol:water, isocratic) Gradient : Acetonitrile and Dichloromethane	
		Vitamin D <sub>2</sub>	AOAC 2016.05		Liquid Chromatography-Tandem Mass Spectrometry
		Vitamin D <sub>3</sub>	AOAC 2002.05	Sample Size : 5-20g Ext Solvent : Alcohol (Methanol/Isopropyl alcohol/Ethanol), Iso-octane etc Mobile phase: Gradient (Water with Acetonitrile &Methanol) Gradient : Isopropanol-n-heptane (20 + 80). Dilute 200 mL isopropanol to 1 L with n-heptane.	HPLC-PDA/UV/VWD
4	Atta	Iron	AOAC 2011.14 (ICP), AOAC 985.35 and AOAC 999.10 (AAS), AOAC 2015.06 (ICP-MS)	Microwave assisted Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)	AAS / ICP-OES/ICP-MS (wave length 259.94)
		Vitamin B <sub>12</sub>	AOAC 2014.02, AOAC 2011.10 & 2011.09 - safety precaution is to be taken while using KCN.	Sample extracted in KCN buffer (pH 4.5). Post autoclave and centrifugation, supernatant was syringe filtered prior to RP-HPLC using methanol - water gradient to resolve Vit. B12 peak. Sample concentration and purification using SPE further improved peak resolution and removal of interfering peaks. Use of Immuno-affinity cartridges help in selective concentration and extraction. Mobile phase: A) 0.025% TFA in water B) Acetonitrile	HPLC / UPLC UV (DAD) detector at 361 nm  Use of Immunoaffinity column, NaCN followed by UPLC-UV  HPLC, requiring potassium cyanide
		Vitamin A	AOAC 20 <sup>th</sup> Ed. 2016 2001.13	Use of pyrogalllic acid, saponify. Neutralize with acetic acid dilute with THF:ethanol. Mobile phase: Methanol:water, isocratic)	HPLC- UV

				Gradient : Acetonitrile and Dichloromethane	
		Thiamine	AOAC 20 <sup>th</sup> Ed. 2016, 957.17	Sample Size : 5-10g, Ext Process: 0.1 - 0.5 N HCl/NaOH/H <sub>2</sub> SO <sub>4</sub> /Phosphate buffer, Enzyme treatment: 0.1 - 1g takadiastase, papain, protease etc, Mobile phase: Gradient (Phosphate buffer with Acetonitrile & Trifluoroacetic acid)	HPLC-PDA/FLD & LC-DAD/MS (J.AOAC Int.2009; 680-687)
		Riboflavin	AOAC 20 <sup>th</sup> Ed. 2016, 970.65 & J. Chro A, 2005, 49	Sample Size : 5-10g, Ext Process: 0.1 - 0.5 N HCl/NaOH/H <sub>2</sub> SO <sub>4</sub> /Phosphate buffer, Enzyme treatment: 0.1 - 1g takadiastase, papain, protease etc, Mobile phase: Gradient (Phosphate buffer with Acetonitrile & Trifluoroacetic acid)	HPLC-PDA/FLD & LC-DAD/MS (J.AOAC Int.2009; 680-687)
5	Maida	Same as Atta	Same as Atta	-do-	-do-
6	Rice	Same as Atta	Same as Atta	-do-	-do-