

Liquid chromatography-based method for estimation of colour additives in fishery products

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Colour is the primary perception which plays a significant role in the purchase of food commodities. In fact, the consumer also associates the colour of a food product with its taste and their storage condition. These generate a certain product expectation for consumer acceptability (Derndrofer and Gruber, 2017). The artificial colour additives are not nature-identical but are chemically synthesized. The colour additives are added to food to compensate for the colour losses due to the effect of light, temperature, air and moisture. Artificial colour additives are generally preferred over their natural counterparts because of inherent advantages like stability, availability, cost, efficacy, etc. However, due to the consumer's health concerns such as allergenicity, behavioural disorders, neurotoxicity, genotoxicity, and carcinogenicity associated with their consumption (Corradini et al. 2018), synthetic food colours are governed by regulatory limits. Hence, their determination becomes very critical. Food Safety and Standards Regulations (FSSR, 2011) has authorized the use of the following colour compounds in fish and fishery products such as Allura Red, Brilliant Blue, Fast Green FCF, Indigo Carmine, Ponceau 4R and Sunset Yellow FCF. Petigara Harp et

al. (2013) developed a chromatographic method for the estimation of FDA-certified colour additives viz., Brilliant Blue FCF, Indigo Carmine, Fast Green FCF, Erythrosine, Allura Red AC, Tartrazine and Sunset Yellow FCF using a reversed-phase C18 column. The colour additives were resolved by mobile phase consisting of ammonium acetate in water and methanolic ammonium acetate in gradient elution mode. The detection was carried out at 420, 520 and 620 nm. The complete separation of colours took 47 minutes. Keeping this in mind, an attempt was made towards developing a liquid chromatography-based method for the estimation of FSSAI-approved food colour additives in fish and fishery products. The chromatographic separation of the colour in the standards was achieved by reversed-phase C-18 column using a mobile phase mix consisting of ammonium acetate and 90% methanol in gradient mode at a flow rate of 1 ml/min. The detection was carried out at a single wavelength of 254 nm. The protocol (Table 1) gave efficient separation of the colour additives present in the standard solution in less than 24 minutes (Fig. 1). The method developed is efficient in saving time as well as the amount of solvent used for separation. The method is being validated for parameters viz., linearity,

range, repeatability, reproducibility, limit of detection and quantitation, and the method developed shall be applicable for the

determination and quantitation of approved colours in fish and fishery products.

Table 1. The elution time of various colour additives

Sl No.	Analyte	Additive No.	Class of dye	Chemical Name	Retention Time (min)
1	Allura Red *	129	Azo	Disodium 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonate	15.1
2	Brilliant Blue *	133	Triarylmethane	Disodium salt of alpha 4-(N-ethylbetaulfobenzylamino)-phenyl] alpha [4-(N-ethyl-3 Sulfonatobenzylimino] cyclohexa-2, 5-dienylidene] toluene-2-sulfonate	17.8
3	Erythrosine	127	Xanthene	Disodium or dipotassium salt of 2',4', 5', 7', tetraiodo- fluorescein	23.3
4	Fast Green *	143	Triarylmethane	Disodium salt of 4-[4-(N-ethyl-p-sulfobenzylamino)-phenyl-(4-hydroxy-2-sulphonumphenyl)-methylene]- (N-ethyl-N-p-sulphobenzyl 2, 5-cyclohexadienimine)	17.5
5	Indigo carmine*	132	Indigoid	Disodium Salt of indigotine-5, 5'-Disulphonic acid	4.0
6	Ponceau 4R*	124	Azo	Trisodium salt of 1-(4-sulpho-1-naphtylazo) naphthol-6, 8-disulphonic acid	11.7
7	Sunset Yellow*	110	Azo	Disodium salt of 1(4-sulphophenylazo) 2-naphthol-6-sulphonic acid	13.4
8	Tartrazine	102	Azo	Trisodium salt of 5-hydroxy-1-p-sulphophenyl-4-(p-sulphophenylazo) pyrazol-3-carboxylic acid	8.4

*Approved for usage in fish and fishery products as per FSSR 2011

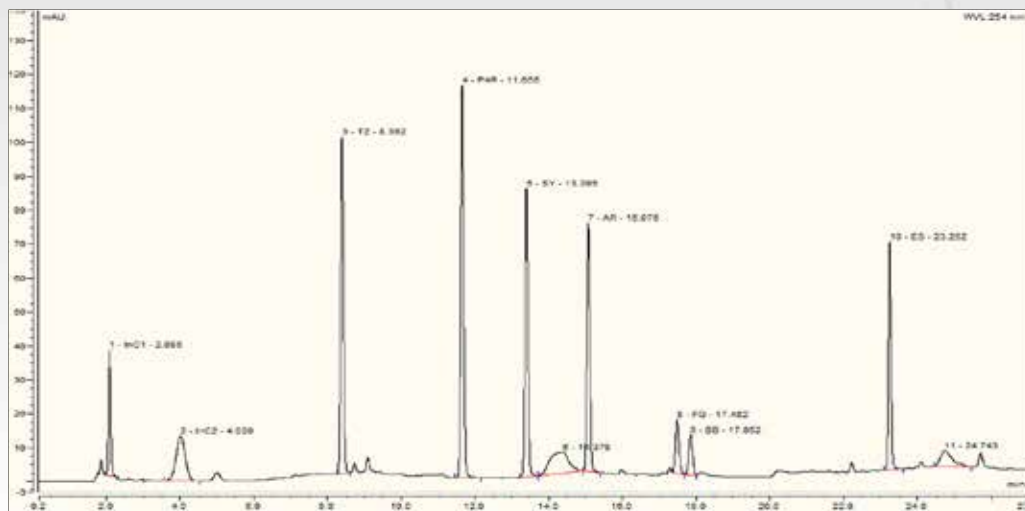


Fig 1. Chromatogram displaying the peaks for various colour additives at 254 nm (InC - Indigo carmine; TZ - tartrazine; P4R - Ponceau 4R; SY - Sunset yellow; AR - Allura Red; FG - Fast Green; BB - Brilliant Blue; ES - Erythrosine).

Reference

Petigara Harp, B., Miranda-Bermudez, E., & Barrows, J. N. (2013). Determination of seven certified colour additives in food products using liquid chromatography. *Journal of Agricultural and Food Chemistry*, 61(15), 3726-3736.

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