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## Simultaneous identification of synthetic and natural dyes in different food samples by UPLC-MS

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**Abstract.** Fast foods and variety food items are populating among the food lovers. To improve the appearance of the food product in surviving gigantic competitive environment synthetic or natural food dyes are added to food items and beverages. Although regulatory bodies permit addition of natural colorants due to its safe and nontoxic nature in food, synthetic dyes are stringently controlled in all food products due to their toxicity by regulatory bodies. Artificial colors are need certification from the regulatory bodies for human consumption. To analyze food dyes in different food samples many analytical techniques are available like high pressure liquid chromatography (HPLC), thin layer chromatography (TLC), spectroscopic and gas chromatographic methods. However all these reported methods analyzed only synthetic dyes or natural dyes. Not a single method has analyzed both synthetic and natural dyes in a single run. In this study a robust ultra-performance liquid chromatographic method for simultaneous identification of 6 synthetic dyes (Tartrazine, Indigo carmine, Brilliant blue, Fast green, malachite green, sunset yellow) and one natural dye (Na-Cu-Chlorophyllin) was developed using acquitic UPLC system equipped with Mass detector and acquity UPLC HSS T3 column (1.8  $\mu$ m, 2.1x50 mm, 100Å). All the dyes were separated and their masses were determined through fragments' masses analyses.

### 1. Introduction

A food color additives are named as pigments or dyes which, when added to food, is capable of improving color of the food. There are synthetic and semi-synthetic color additives, semi-synthetic dyes or natural dyes which mainly originate from plants or animals. Saffron and turmeric are two examples of this category. Color additives are added to food to maintain the color loss due to long term storage conditions in correcting the natural variations in color and provide color to colorless foods in some cases. In beverages and food products the additives like colorants, sweeteners, stimulants, preservatives are frequently added. Nonnutritive food additives are nowadays unavoidable in food products. Most of these additives are in markets not harm to use in food at significant level but when the color additives added into food exceed the permitted limit it potentially causes hyperactivity in children, due to that in developed countries these color additives are restricted strictly. Hence it's necessary to ensure the food quality the amount of additives



individually added in the food products might be quantified and monitored. Food and Drug Administration (FDA) has controlled and monitored the permitted food colors usage in food items [1-2].

The European Food Safety Authority (EFSA) has recently undertaken the safety of food additives on a sequence of re-evaluations, including colors which are approved in the European Union. Also EFSA has adopted a first opinion which raised potential safety concerns on food color Red 2G (EFSA, 2007). Already in 2002, Sasaki et al. revealed that at the level of 10 mg/kg b.w. two food dyes tartrazine and amaranth started induced dose related DNA damage in the mouse colon for 3 h after oral administration. For tartrazine at 2000 mg/kg the DNA damage was observed after 24 h administration but sunset yellow did not yield any significant increase in DNA damage in colonic cells [3].

For more than 35 years child behavior has been examined for the influence of artificial food colors (AFCs) with mass of evidence from imperfect studies. To understand the attention deficit hyperactivity disorder (ADHD), AFC literature explains that ADHD is a quantitative analysis, such as hypertension and for some individuals near the threshold might push over it by significant symptom variation. The disadvantages of many studies are non-standardized outcome measures, non-standardized diagnosis, imperfect blinding, and questionable sample selection. Recent studies revealed that a small but significant harmful effect of AFCs on children's behavior with diagnosable ADHD was not confined. ADHD appear to be less of a public health problem than a AFCs problem. AFCs may not be a major problem of ADHD per se, however whether or not they have ADHD it seems to affect children regardless, and if most of the children in the class suffer a small behavioral change with color additives or synergistic effects, they may have a combine effect on class climates. In March 2011, the Drug Administration (FDA) Food Advisory Committee and United States (U.S.) Food conducted a study to collect data on the behavioral effect of synthetic food dyes, specifically for artificial food colors (AFCs).

To analyze synthetic and natural food colors the development of instrumental and chemical method of separation, identification, quantification have become highly necessary for the academic and government institutional, regulatory authorities, food and beverages industries in assessing the quality of food products. Most of the food colors are artificial and natural food colors are soluble in water to use in UPLC analysis. Food beverages are not compatible for UPLC due to the complication of sample matrices. Generally samples are not suitable for inject directly and pretreatment process needs to do for injection which differs sample to sample types and it depends on the food product [4-7].

Normally food sample contains sugar, oil, fat and these components give negative impact on column. Therefore the filtration is important for UPLC analysis, specifically samples like juice, iced coffee are milk added product and these samples contain proteins which should be removed by protein precipitation technique using organic solvents prior to filtration. The loss of dyes may occur while sample undergoes filtration process. The sample loss is due to interaction between filter membrane and analytes. Chemical structure, physical properties, molecular weight and product formation as well as ionization properties also may affect the interaction. The membrane property also contributes to the interactions; like membrane polymer's chemical structure, polymer formulation, purity and hydrophilicity among others influence interactions among them. Analytes may bind nonspecifically to membrane during unwanted interaction. Improper solubility also leads to sample loss as undissolved will be blocked by membrane filtration. For low polar solvents the target impurities in the sample will impact greatly in filtration recoveries [8-10].

Therefore the sample loss induced by the sample medium, the compound solubility and membrane wettability can directly affect. Based on the sample medium the membrane filter will be selected but it's difficult to predict the interaction of membrane and target impurities. Therefore by considering the sample medium the membrane filter will be selected. Usually for aqueous based samples hydrophilic type membranes are used like regenerated cellulose (RC), polyethersulfone (PES), cellulose acetate (CA). Polytetrafluoroethylene (PTFE) filters is used for organic-solvent based samples, especially for aggressive solvents. For mixtures of organic and aqueous samples, PES, PP, PTFE, RC and nylon (polyacrylamide, PA) filters can be used. Normally it depends on solvent composition. It is highly recommended for preliminary tests on filtration recovery to avoid unwanted interactions.

In this study, we analyzed 6 synthetic food colors and one natural food color within 3 min: This paper describes an analytical method for detection of 6 synthetic food colors in drinks and candies by UPLC equipped with a Mass detector using a short analytical column [11-13].

## 2. Materials and method

### 2.1. Reagents

The permitted food colors Tartrazine, Indigo carmine, Brilliant blue, Malachite green, Sunset yellow and Chlorophyllin sodium copper salt were purchased from SRL and caramosine, fast green were purchased from LOBA chem., Acetonitrile (HPLC grade), Hydrochloric acid (HCL), Sodium hydroxide (NaOH), formic acid and water were of HPLC grade. Other chemicals were obtained from commercial sources.

#### 2.1.1. Preparation of Mobile phase A

1mL of formic acid was transferred to 500mL standard flask using micropipette and diluted to the mark with Mili-Q water. Buffer solution pH was adjusted to 8.6 and filtered with 0.22 micro membrane filter.

#### 2.1.2. Preparation of mobile phase B

1mL of formic acid was transferred to 500mL standard flask using micropipette and diluted to the mark with acetonitrile. Solution pH was adjusted to 8.6 and filtered with 0.22 micro membrane filter.

#### 2.1.3. Colorant standard solution

Standard stock solutions of indigo carmine, tartrazine, sunset yellow FCF, fast green FCF, Brilliant blue, malachite green, Na-Cu-chlorophyllin were prepared individually by taking 20 mg of each standard in a 10 mL volumetric standards flask. A 300  $\mu$ L amount of acetonitrile was added to each flask and a premixed solution of mobile phase A and B (80:20, v.v) was used as diluent. Sonication was used when required.

#### 2.1.4. Mixed standard solution

About 100  $\mu$ L of each standard was diluted to get a solution of 200 mg/L each using premixed mobile phases.

## 3. UPLC Parameters

UPLC system: Aquatic UPLC

Run Time: 3 min

Column: Aquatic UPLC HSS T3 100 A, 1.8 $\mu$ m, 2.1mm\*100mm

Mobile phase A: 0.1% HCOOH in water

Mobile phase B: 0.1% HCOOH in CAN

Flow rate: 1.0ml/min

Injection volume: 5  $\mu$ L

### 3.1. MS CONDITIONS

MS System: Waters Quattro Premier™ XE Ionization

Mode: Positive electrospray (ESI+) Multiple reaction monitoring

**Table 1. Gradient program used for separation of dyes by UPLC-MS**

S.NO	Time(min)	Flow rate (ml/min)	Mobile phase A (%)	Mobile phase B (%)
1	0-2	1.0	95	5
2	2-2.5	1.0	5	95
3	2.5-3.0	1.0	65	5

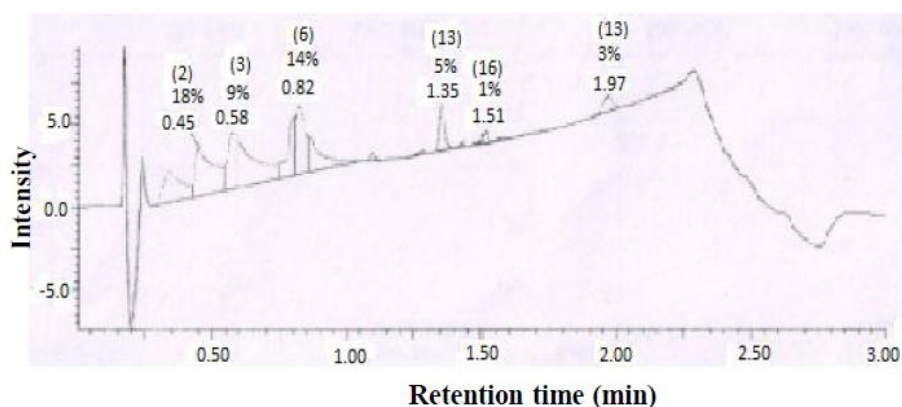
**Table 2. Fragment ions observed in the MS-MS analysis**

Pigments	Retention time	Molecular formula	Molecular weight	Ion 1	Ion 2
Indigo carmine	0.50	C <sub>16</sub> H <sub>8</sub> N <sub>2</sub> O <sub>8</sub> S <sub>2</sub>	420.37	421.0	-
Sunset yellow	0.64	C <sub>16</sub> H <sub>10</sub> N <sub>2</sub> O <sub>7</sub> S <sub>2</sub>	406.38	409.1	-
Fast green	0.79	C <sub>37</sub> H <sub>34</sub> N <sub>2</sub> O <sub>10</sub> S <sub>3</sub>	762.86	765.2	766.1
Brilliant blue	0.89	C <sub>37</sub> H <sub>37</sub> N <sub>2</sub> O <sub>9</sub> S <sub>3</sub>	749.89	749.2	750.1
Malachite green	1.32	C <sub>23</sub> H <sub>25</sub> N <sub>2</sub>	329.45	329.3	330.3
Na-Cu-Chlorophyllin	1.96	C <sub>34</sub> H <sub>31</sub> CuN <sub>4</sub> N <sub>3</sub> O <sub>6</sub>	667.14	667.1	660.3

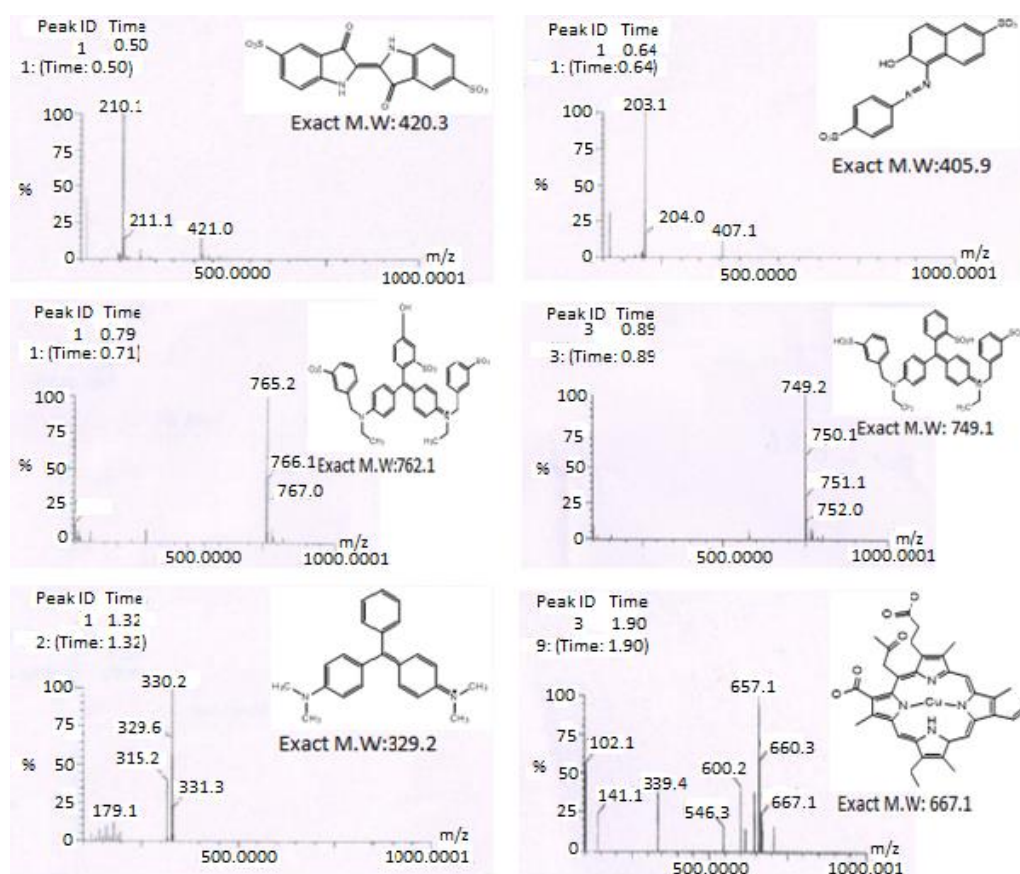
#### 4. Results and discussion

The aim of this study was to determine the synthetic dyes and natural dyes using UPLC-MS techniques. The newer developments in dye synthesizing technology are to prepare different complex dyes, some of which contain three or more  $-\text{SO}_3^-$  groups. UPLC-MS was performed and the information obtained is summarized in Table 2. From the data summarized in Table 2 under TS condition it shows [M-Na] ion. The dye structure shows three negative sulphonated groups saturated with Na<sup>+</sup>, and one protonable positive aminic group (Fig. 1). The characterization study was carried out in negative ion-mode due to the presence of three anionic groups in the molecule. Based on the no of Na atoms in the molecules it loses one or two Na<sup>+</sup> atoms and also replaces those atoms with hydrogen atoms. Dyes like Na-Cu-chlorophyllin, Acid yellow which has carboxylic group, showed the [M-x, Na-COOH]<sup>2-</sup> ion and lost this group. In other dyes fragmentation ions related to cleavage of the azo group near the naphthalic ring, were captured.

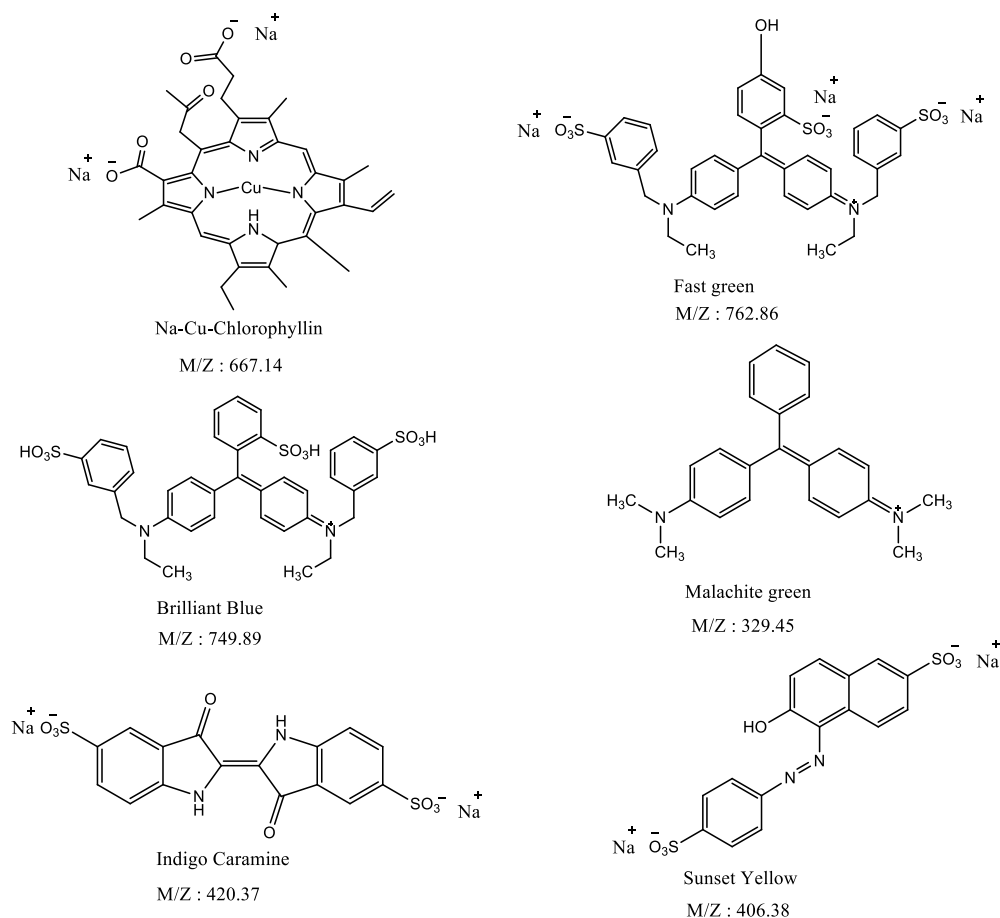
The molecular mass of the Na-Cu-Chlorophyllin is 667.14amu. This molecule provides well recognizable signals corresponding to three negative ions at  $m/z$  660.3, 657.1, and 667.1 (Fig. 3). The peak at  $m/z$  657.1 is due to the [M-2Na]<sup>2-</sup>, whereas the peaks at  $m/z$  660.3 and 667.1 represent [M-Na]<sup>-</sup> and [M-2Na+H]<sup>-</sup>, respectively. For synthetic dyes Indigo carmine, sunset yellow, malachite green, Brilliant blue, Fast green respectively the peaks at 421.0, 409.1, 329.3, 765.2, 749.2, 329.3 are due to the [M-2Na+H]<sup>-</sup>. In the characterization study the peaks corresponding to monocharge structures appear at higher temperatures, whereas the peak of bi-charge structure appears at lower temperatures. The characteristic product ions after fragmentation of each precursor ion peak by MS<sup>n</sup> analysis are summarized in Table 2. The mass to charge range was 200-1000  $m/z$  in negative ion full scan mode and MS<sup>n</sup> mode [14-19].



**Figure 1.** UPLC chromatograms of mixed food color standard solutions of synthetic dyes Tartrazine; Indigo carmine; Sunset yellow; Brilliant blue; Fast green and semi synthetic dye Na-Cu-Chlorophyllin separated on UPLC column Aquatic UPLC HSS T3 100 A, 1.8 $\mu$ m, 2.1mm\*100mm and gradient elution using solvent A as 0.1% formic acid buffer (pH 6.8) and solvent B 0.1% Formic acid buffer in ACN (pH 6.8) and the flow-rate of 1.0 mL/min, injection volume 5 $\mu$ L, equipped with mass detector.



**Figure 2.** Mass Spectra of All separated Synthetic and semi-synthetic food dyes



**Figure 3.** Structure and Molecular masses of synthetic and semi-synthetic dyes

## 5. Conclusions

The versatility of UPLC as an analytical tool is used as an ideal technique for analytical quality control of research and development laboratories in the food and beverage industry, especially when minimal clean-up needs to be performed. We have proposed a rapid and interference-free UPLC method developed for the quality control department of candies producers using synthetic food colors. The determination of the synthetic color additives in beverages using mass detector is performed in a short time. The results confirm that the proposed method works well and is useful for a quality control or screening of the synthetic colors added in soft drinks and foods.

## Acknowledgments

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## Reference

- [1] de Andrade F I, Guedes M I F, Vieira Í G P, Mendes F N P, Rodrigues P A S, Maia C S C, Ávila M M M and de Matos Ribeiro L 2014 Determination of synthetic food dyes in commercial soft drinks by TLC and ion-pair HPLC *Food chemistry* **157** 193-8

- [2] Poul M, Jarry G, Elhkim M O and Poul J-M 2009 Lack of genotoxic effect of food dyes amaranth, sunset yellow and tartrazine and their metabolites in the gut micronucleus assay in mice *Food and chemical toxicology* **47** 443-8
- [3] Arnold L E, Lofthouse N and Hurt E 2012 Artificial food colors and attention-deficit/hyperactivity symptoms: conclusions to dye for *Neurotherapeutics* **9** 599-609
- [4] Joseph S Analysis of color additives in sweets
- [5] Qi P, Lin Z-h, Chen G-y, Xiao J, Liang Z-a, Luo L-n, Zhou J and Zhang X-w 2015 Fast and simultaneous determination of eleven synthetic color additives in flour and meat products by liquid chromatography coupled with diode-array detector and tandem mass spectrometry *Food chemistry* **181** 101-10
- [6] Tsai C-F, Kuo C-H and Shih D Y-C 2015 Determination of 20 synthetic dyes in chili powders and syrup-preserved fruits by liquid chromatography/tandem mass spectrometry *journal of food and drug analysis* **23** 453-62
- [7] Yoshioka N and Ichihashi K 2008 Determination of 40 synthetic food colors in drinks and candies by high-performance liquid chromatography using a short column with photodiode array detection *Talanta* **74** 1408-13
- [8] Garcia-Falcón M and Simal-Gándara J 2005 Determination of food dyes in soft drinks containing natural pigments by liquid chromatography with minimal clean-up *Food Control* **16** 293-7
- [9] Heidarizadi E and Tabaraki R 2016 Simultaneous spectrophotometric determination of synthetic dyes in food samples after cloud point extraction using multiple response optimizations *Talanta* **148** 237-46
- [10] Yamjala K, Nainar M S and Ramiseti N R 2016 Methods for the analysis of azo dyes employed in food industry—a review *Food chemistry* **192** 813-24
- [11] Rovina K, Prabakaran P P, Siddiquee S and Shaarani S M 2016 Methods for the analysis of Sunset Yellow FCF (E110) in food and beverage products—a review *TrAC Trends in Analytical Chemistry* **85** 47-56
- [12] Dixit S, Purshottam S, Khanna S and Das M 2011 Usage pattern of synthetic food colours in different states of India and exposure assessment through commodities preferentially consumed by children *Food Additives & Contaminants: Part A* **28** 996-1005
- [13] Gosetti F, Gianotti V, Angioi S, Polati S, Marengo E and Gennaro M 2004 Oxidative degradation of food dye E133 Brilliant Blue FCF: liquid chromatography–electrospray mass spectrometry identification of the degradation pathway *Journal of Chromatography A* **1054** 379-87
- [14] Scotter M J, Castle L and Roberts D 2005 Method development and HPLC analysis of retail foods and beverages for copper chlorophyll (E141 [i]) and chlorophyllin (E141 [ii]) food colouring materials *Food additives and contaminants* **22** 1163-75
- [15] Gosetti F, Gianotti V, Polati S and Gennaro M C 2005 HPLC-MS degradation study of E110 Sunset Yellow FCF in a commercial beverage *Journal of Chromatography A* **1090** 107-15
- [16] Vachirapatama N, Mahajaroensiri J and Visessanguan W 2008 Identification and Determination of Seven Synthetic Dyes in Foodstuffs and Soft Drinks on Monolithic C18 Column by High Performance Liquid Chromatography *Journal of Food & Drug Analysis* **16**
- [17] Yang W, Wang J, Li X and Du Z 2011 A new method research for determination of natural pigment crocin yellow in foods by solid-phase extraction ultrahigh pressure liquid chromatography *Journal of Chromatography A* **1218** 1423-8



- [18] de Andrade F I, Guedes M I F, Vieira Í G P, Mendes F N P, Rodrigues P A S, Maia C S C, Ávila M M M and de Matos Ribeiro L 2014 Determination of synthetic food dyes in commercial soft drinks by TLC and ion-pair HPLC *Food chemistry* **157** 193-8
- [19] Tateo F and Bononi M 2004 Fast determination of Sudan I by HPLC/APCI-MS in hot chilli, spices, and oven-baked foods *Journal of Agricultural and Food Chemistry* **52** 655-8
- [20] González M, Gallego M and Valcárcel M 2003 Liquid chromatographic determination of natural and synthetic colorants in lyophilized foods using an automatic solid-phase extraction system *Journal of agricultural and food chemistry* **51** 2121-9
- [21] Dixit S, Purshottam S, Khanna S and Das M 2011 Usage pattern of synthetic food colours in different states of India and exposure assessment through commodities preferentially consumed by children *Food Additives & Contaminants: Part A* **28** 996-1005
- [22] Ma K, Yang Y N, Jiang X X, Zhao M and Cai Y Q 2012 Simultaneous determination of 20 food additives by high performance liquid chromatography with photo-diode array detector *Chinese Chemical Letters* **23** 492-5
- [23] Chen Q-c, Mou S-f, Hou X-p, Riviello J and Ni Z-m 1998 Determination of eight synthetic food colorants in drinks by high-performance ion chromatography *Journal of Chromatography A* **827** 73-81
- [24] Huang H-Y, Shih Y-C and Chen Y-C 2002 Determining eight colorants in milk beverages by capillary electrophoresis *Journal of chromatography A* **959** 317-25
- [25] Ahlström L-H, Eskilsson C S and Björklund E 2005 Determination of banned azo dyes in consumer goods *TrAC Trends in Analytical Chemistry* **24** 49-56
- [26] Jonnalagadda P R, Rao P, Bhat R V and Nadamuni Naidu A 2004 Type, extent and use of colours in ready-to-eat (RTE) foods prepared in the non-industrial sector—a case study from Hyderabad, India *International journal of food science & technology* **39** 125-31
- [27] Breithaupt D E 2004 Simultaneous HPLC determination of carotenoids used as food coloring additives: applicability of accelerated solvent extraction *Food chemistry* **86** 449-56
- [28] Chanlon S, Joly-Pottuz L, Chatelut M, Vittori O and Cretier J 2005 Determination of Carmoisine, Allura red and Ponceau 4R in sweets and soft drinks by Differential Pulse Polarography *Journal of Food Composition and Analysis* **18** 503-15
- [29] Garcia-Falcón M and Simal-Gándara J 2005 Determination of food dyes in soft drinks containing natural pigments by liquid chromatography with minimal clean-up *Food Control* **16** 293-7

# Corrigendum: Simultaneous identification of synthetic and natural dyes in different food samples by UPLC-MS

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Description of corrigendum e.g,

## **Page 6:**

In the Acknowledgements section, the following text appears:

*“The authors acknowledge the research grant obtained under Indo-Taiwan S&T Cooperation Programme (Project reference no. GITA/DST/TWN/P-72/2015) for this study. Also, the authors acknowledge VIT University, Vellore 632014 for the characterization facility used in this study.”*

This should read:

*“The authors highly acknowledge VIT University, Vellore-India and National Tsing Hua University, Hsinchu-Taiwan for the instrumental facilities given to analyse the samples and the financial support obtained from Indo-Taiwan Collaborative project (Ref. No: GITA/DST/TWN/P-72/2015 and MOST 104-2923-M-007-MY3).”*