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Non-target screening of Allura Red AC photodegradation products in a beverage through ultra high performance liquid chromatography coupled with hybrid triple quadrupole/linear ion trap mass spectrometry

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ABSTRACT

The study deals with the identification of the degradation products formed by simulated sunlight photoirradiation in a commercial beverage that contains Allura Red AC dye. An UHPLC–MS/MS method, that makes use of hybrid triple quadrupole/linear ion trap, was developed. In the identification step the software tool information dependent acquisition (IDA) was used to automatically obtain information about the species present and to build a multiple reaction monitoring (MRM) method with the MS/MS fragmentation pattern of the species considered.

The results indicate that the identified degradation products are formed from side-reactions and/or interactions among the dye and other ingredients present in the beverage (ascorbic acid, citric acid, sucrose, aromas, strawberry juice, and extract of chamomile flowers).

The presence of aromatic amine or amide functionalities in the chemical structures proposed for the degradation products might suggest potential hazards to consumer health.

1. Introduction

The potential impact on human health of food additives is increasingly at the centre of the attention of public opinion, scientists and legislators. In particular, the use in food of many dyes has been banned and positive lists containing the permitted colours together with the maxima amounts allowed in the different kinds of food and beverages have been prepared (European Directive 94/36/EC, 1994; http://www.fda.gov/ForIndustry/ColorAdditives/ColorAdditiveInventories).

Food dyes are generally characterized by large molecular masses containing strong anionic or cationic charges that prevent important adsorptions in the gastro intestinal tract. Anyway, some amounts of dyes (up to about 2 mg/day) are adsorbed: dyes can undergo extensive metabolic reduction by intestinal micro flora and the aromatic amines formed can be rapidly adsorbed. Then, aromatic amines can be oxidized and acetylated by P450 and acetyl transferase and become genotoxic (Shimada, Kano, Sasaki, Sato, & Tsudua, 2010).

Results published by Tsuda et al. (2001) and based on Comet assay tests evidenced effects of Allura Red AC (an azoic food dye) on nuclear DNA migration in mouse in vivo. Possible risks to health induced by dyes including Allura Red AC are denounced in other studies performed both on humans and animals (Mizutani, 2009; Shrestha, Bhattarai, Lee, & Cho, 2006).

Studies have been conducted in UK (Tennant, 2008), Ireland (Connolly et al., 2010), and Italy (Fallico, Chiappara, Arena, & Ballistreri, 2011) to evaluate the total average intake of dyes from non-alcoholic beverages. Different situations have been envisaged and: it is difficult to obtain a definite answer also because in many commercial products the dye amount is not specified but often indicated as "quantum satis". In particular, the Regulation EC No. 1333/2008 of the European Parliament and of The Council of 16 December 2008 on food additives requires that the labelling of food commercial products that contain Sunset Yellow (E110), Quinoline Yellow (E104), Carmoisine (E122), Allura Red (E129), Tartrazine (E102) and Ponceau 4R (E124) must include, besides the qualitative and quantitative composition, the following additional information: "name or E number of the colour(s): may have an adverse effect on activity and attention in children" (Regulation EC 1333/2008, 2008).

It is worthwhile to remind here that in particular Allura Red AC has been undergone by the European Food Safety Authority (EFSA) to a re-evaluation by the Panel on Food Additives and Nutrient Sources (EFSA Panel), after the results of some studies (Bateman

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et al., 2004; Schab & Trinh, 2004; McCann et al., 2007 denouncing that the exposure to Allura Red AC leads to increased hyperactivity in children.

However, the final EFSA verdict was "In the context of the overall weight of evidence and in view of the considerable uncertainties, such as the lack of consistency and relative weakness of the effect and the absence of information on the clinical significance of the behavioural changes observed, the Panel concludes that the findings of the study cannot be used as a basis for altering the ADI of the respective food colours or sodium benzoate" (European Food Safety Authority, 2008). The EFSA Panel concluded that a causal relationship between exposure to colour additives and hyperactivity in children in the general population has not been established, no change in the acceptable daily intake (ADI) was warranted and these dyes were considered safe to use under the previously established limits.

Safety tests on food dyes and studies about dye toxicity are extensively present in literature and all summarize that food dyes can be considered safe to consume at the approved ADIs (Gloria, 2004; Marmion, 1991; Watson, 2001; WHO Food Additives Series 65, 2012).

But a risk not enough known and evidenced in recent studies is the possibility that safe food dyes undergo degradation in beverages for the action of sun light irradiation, to which the commercial beverages are often exposed during the steps of transport and storage (Gianotti, Angioi, Gosetti, Marengo, & Gennaro, 2005; Gosetti et al., 2004, 2008; Gosetti, Gianotti, Mazzucco, Polati, & Gennaro, 2007; Gosetti, Gianotti, Polati, & Gennaro, 2005). Often, but not always, the degradation is clearly evidenced by naked eye through decolorization or colour variations of the beverage. The colour variation or decolorisation are due to side-reactions, assisted by light irradiation, that take place among the dye and other ingredients of the beverages.

Decolorization or fading of coloured syrups and beverages in the presence of L-ascorbic acid, as for example in sport-type drinks, was also observed by other authors, who also showed that addition of vitamin B2 or other reducing species can prevent decolorization (Chang, 1994). The decolorization is generally explained with the reduction of nitrogen–nitrogen double bond and the formation of different photoproducts, often characterized by structures of aromatic amines potentially toxic (Chang, 1994; Gosetti et al., 2005, 2007, 2008).

On the basis of these considerations this work is devoted to study the photodegradation of Allura Red AC, disodium 6-hydroxy-5-((2-methoxy-5-methyl-4-sulphophenyl)azo)-2-naphthalenesulphonate, in a commercial preparation expressly commercialized for children.

To check the content of synthetic dyes in food, suitable analytical methods are required. The analytical methods present in literature for Allura Red AC determination in food are based on spectrophotometry (Kucharska & Grabka, 2010; Pourreza, Rastegarzadeh, & Larki, 2011; Soylak, Unsal, & Tuzen, 2011), spectrophotometry and high performance liquid chromatography (HPLC) correlated with chemometrics (Dinc, Aktas, Baleanu, & Ustundag, 2006; Kucharska et al., 2010; Pourreza et al., 2011; Soylak et al., 2011), voltammetry and differential pulse polarography (Kucharska et al., 2010; Zhang, Zhang, Lu, Yang, & Wu, 2010), capillary electrophoresis (Kucharska et al., 2010), HPLC-diode array detection (HPLC-DAD) (Kirschbaum, Krause, Pfalzgraf, & Bruckner, 2003; Yang, Yin, & Shao, 2011), and HPLC-DAD–tandem mass spectrometry (HPLC-DAD–MS/MS) (Ji, Feng, Chen, & Chu, 2011).

To identify the main degradation products formed by photoirradiation of the drink containing Allura Red AC dye, the LC–MS/MS is a powerful tool since it is very suitable for the identification and determination of a wide molecular weight range of polar, semivolatile and thermally labile compounds. The ability to perform non-target screening on a routine basis is made possible through advancements in LC–MS/MS technology, including hybrid system like the triple quadrupole/ion trap (QQQ/IT). When it is not used a target analyte list, the detection step is not based on any *a priori* knowledge, such as retention times and previous information on precursor and product ions. Therefore, it becomes very important to concatenate more MS experiments, such as full scan, enhanced resolution and MS/MS, to obtain the widest information for the identification of unknown chromatographic peaks.

2. Materials and methods

2.1. Reagents

HPLC methanol (Chromasolv, >99.9%), Allura Red AC (>80%), and ammonium acetate (99%) were purchased from Sigma–Aldrich (Milwaukee, USA). Ultrapure water was obtained through a Millipore Milli-Q system (Milford, MA).

The stock water standard solution of Allura Red at 1000.000 mg L^{-1} was prepared, properly diluted in ultrapure water and preserved at 4 °C in dark glass vials.

The beverage has been bought at a supermarket. It is contained in a 250 mL polyethylene terephthalate bottle and its declared ingredients are water, ascorbic acid, citric acid, sucrose, aromas, strawberry juice, and extract of chamomile flowers without addition of carbon dioxide.

The beverage was filtered before UHPLC–MS/MS analysis on 0.45 μm nylon filter (VWR International, Darmstadt, Germany).

2.2. Apparatus

To simulate sunlight irradiation, a CoFoMeGra Solar box 3000e (Milan, Italy) was used, that assures, with respect to natural sunlight, continuous availability and constant intensity. The irradiation density of the xenon lamp was settled at 600 Wm^{-2} and the temperature at 35 °C. An outdoor UV glass filter was employed.

The LC/MS analysis was performed by UFLC XR Shimadzu (Kyoto, Japan) system equipped by a DGU-20A3 Degasser, two LC-20ADXR Pumps, a SIL-20AXR Autosampler, a CTO-20A column compartment and a CMB-20A system controller. The system was interfaced with a 3200 QTrap™ LC–MS/MS system (AB Sciex, Concord, Canada) by a Turbo V™ interface equipped with an ESI probe. The 3200 QTrap™ data were processed by Analyst 1.5.1 software (Toronto, Canada).

Dissolved oxygen determination was performed by a WTW-Multi 3420 meter (WTW, Milan, Italy).

The microbiological experiments were performed by incubators and heating ovens Genesis series Top (Fratelli Galli, Milan, Italy), WTB Binder APT line BED series, WTB Binder KB 53 model (WTB Blinder, Tuttlingen, Germany) and by autoclave Stematic III from PBI International (Milan, Italy).

The high nutrient agar medium was "Yeast extract agar" from Lab M (Heywood, Lancashire, UK) containing 6.0 g of tryptone, 3.0 g of yeast extract, 15.0 g of agar in 1000 mL of distiled water.

For the cultivation of anaerobiotic and microaerophilic microorganisms "Anaerocult mini" systems were purchased from Merck (Darmstadt, Germany).

2.3. UHPLC–MS/MS conditions

The stationary phase was an Acquity HSS-T3 column (2.1 mm × 100 mm, 1.8 μm) (Waters, Milford, USA). The mobile phase was a mixture of ammonium acetate 1.0 mM in ultrapure water (A) and ammonium acetate 1.0 mM in methanol (B), eluting at flow rate 0.400 mL min^{-1} in the gradient conditions reported in