Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1090 (2005) 107–115
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

HPLC-MS degradation study of E110 Sunset Yellow FCF in a commercial beverage

Fabio Gosetti, Valentina Gianotti, Stefano Polati, Maria Carla Gennaro *

Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale "Amedeo Avogadro", Spalto Marengo 33, 15100 Alessandria, Italy

Received 16 March 2005; received in revised form 29 June 2005; accepted 4 July 2005
Available online 1 August 2005

Abstract

Experimental evidence has shown that a beverage containing Sunset Yellow FCF (labelled as E110 in the European Union), when exposed to natural conditions of summer temperature and sunlight, losses its colour. To possibly identify the degradation pathway and collect information on the potential toxicity of the uncoloured species formed, different degradation conditions, under both oxidising and reducing environments, were simulated in laboratory. Experiments were carried out under the following conditions: (i) thermally induced degradation, (ii) visible photo induced degradation, (iii) UV-photo induced conditions in oxidising environment (addition of hydrogen peroxide, Fenton reaction) and (iv) UV-photo induced conditions in reducing environment (addition of sulphide and ascorbic acid, addition of ascorbic acid in the absence and in the presence of saccharose). Decolourisation process was observed in oxidant conditions when applying the Fenton reaction but the reaction was too quick to be progressively followed. On the other hand, it was also possible to study the degradation reaction observed in reducing conditions in the presence of ascorbic acid. The HPLC-MS results gave evidence for the cleavage of the double bond and the protonation of the azo groups. The loss of colour is therefore not due to a mineralization process but to the formation of a dimeric form of 5-amino-6-hydroxy-2-naphthalene sulfonate and, likely, of *p*-amino-benzensulfonate.

Keywords: Dye; Degradation; HPLC-MS; Aromatic amines

1. Introduction

This study was initiated by the information reported in literature according which some allowed food azo-dyes can degrade in the food itself during the phases of storage and transport, before and during the commercialisation [1]. Degradation often results in a loss of colour.

Literature studies underline that decolourisation of azo-dyes does not necessarily mean mineralization and report examples in which the decolourisation corresponds to the formation of toxic species. The use of bioluminescent bacterium *Vibrio fischeri* showed that the uncoloured products formed in the destruction of Reactive Black have an EC50value around 100 times lower than the original dye and that toxicity and genotoxicity of decolourised Acid Orange 7 largely increase due to the production of 1-amino-2-naphthol [2].

Aromatic amines are reported to form in the degradation process of azo-dyes, but in this case it is necessary to distinguish the possible content of aromatic amines originally present in the commercial dyes (as impurities or process side products) from aromatic amines that can be produced by degradation processes [3–7].

The formation of aromatic amines for degradation is generally explained as the result of a reduction process, which leads to the cleavage of the azo double bond. So, reduction of azo-dyes used in toys and assumed with saliva can be induced in vivo by the action of intestinal anaerobic bacteria or of hepatic azo reductase with the possible formation of aromatic amines [8–10]. A model study was performed in which nine azo-dyes generally employed to colour toys were undergone to reduction with a 10/1 excess of sodium dithionite: the uncoloured products, obtained at 65 °C in only five

* Corresponding author. Fax: +39 0131 287416.
E-mail address: gennaro@mfn.unipmn.it (M.C. Gennaro).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.07.024

minutes, contained aniline, *o*-toluidine, 1,4-diaminobenzene and 2,4-dimethylaniline [8]. In the reduction of other dyes, performed with dithionite under similar conditions and in the presence of citrate buffer, other authors provided evidence of the formation of benzidine, *p*-phenylenediamine and aniline [11]. In acetate buffer containing EDTA, the reduction of synthetic food azo-dyes by ascorbic acid, studied by differential-pulse polarographic technique, evidenced the formation of aniline, sulphonic acid, naphthionic acid and sulphanilic acid [1].

The degradation of azo-dye (amaranth E123) in soft drinks was evidenced already in 1987, when no restriction on the admitted quantity was yet present [12]. It was shown that amaranth was reduced to naphthionic acid and amino-*R*-salt, which is unstable and is readily oxidised to naphthoquinone. The reducing effect was ascribed to L-ascorbic acid, that is often added to drinks as nutritional or antioxidant supplement. In addition, a protective effect of the sugars present in the beverage towards the reductive potentialities of ascorbic acid was ascribed: the effect was explained by competitive chelation reactions taking place between sugars and the metal ions present, which, if uncomplexed, could catalyse the oxidation of ascorbic acid.

Very few are the studies of degradation of sulfonated azo-dyes reported in literature. They concern some alkylbenzene sulfonates [13,14], aromatic sulfonates [15], dyes [16,17], and drugs [18]. The degradation processes are induced by catalysts and UV-irradiation, and the degradation is studied by liquid [13–15] and gas chromatography [19–21] with mass spectrometric detection. Literature results concerning the degradation reaction of aromatic sulfonates are too few to be correlated and do not indicate a common pathway that could be applied to make hypothesis about the structure of the intermediates and about the degradation pathway. This is likely also due to the fact that the matrix seems often to play an important role.

On these bases, a series of experiments was carried out in our laboratories, in which commercial beverages were undergone to natural conditions of high temperatures and sunlight. Two samples of the most common commercial beverages were bought in the local stores: one sample, in its sealed bottle, was exposed to summer light and temperature while the other was stored indoor. The beverages that showed the more relevant variation or loss of colour were those containing Sunset Yellow FCF and Brilliant Blue FCF, labelled, respectively, E110 and E133 in the European Union. The variations could be observed by naked eye and became evident after only 5 days of exposure.

A manuscript was already published reporting an HPLC-MS study of degradation of E133 [17]. In this paper, the degradation pathway observed for a beverage containing E110 is presented (Fig. 1).

Already in 1983 the possible degradation of E110 and other food dyes was evidenced, as that induced by strong sunlight when the bottled drinks are displayed in bright sunlight in shop windows or garage forecourt. The result of the study, performed by differential-pulse polarography, evidenced the complete loss of colour, that was ascribed to the photo-induced reductive action of ascorbic acid and to the formation of sulphanilic acid [1].

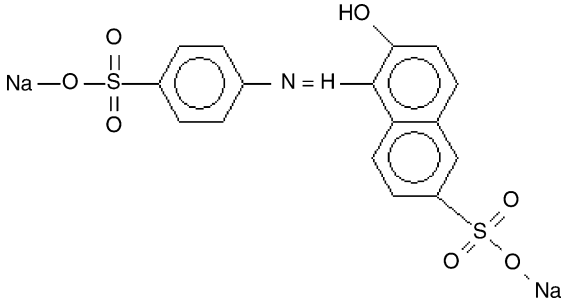

Fig. 1. Sunset Yellow FCF (E110) chemical structure.

The photodegradation of dyes has been studied in anaerobic reductive environment [22], as well as in oxidant conditions through Fenton reaction in the presence [23] and in the absence of EDTA [24,25], catalysed by peroxidase [26,27] or by addition of TiO_2 [28–32]. In particular for E110 a degradation mechanism has been proposed, that leads to the formation of benzene sulfonate, induced by Fe(III) and H_2O_2 [23].

With the aim to identify the products that E110 naturally forms in the bottled drink for exposure to light and high summer temperatures, model solutions were undergone in laboratory to simulated degradation conditions. Thermal, UV–vis irradiation as well as reductive and oxidant conditions were compared. Strong oxidative conditions associated to UV light were employed. The use of hydrogen peroxide was experimented in which the hydroxyl radicals formed in the photolysis of hydrogen peroxide oxidise organic material RH leading to the formation of organic radicals $R^{\bullet}$, that in turn with molecular oxygen produce super oxide radicals. Other oxidative conditions were simulated, according to the Fenton reaction, that is very efficacious for the degradation of different organic species [25] and is utilised in the destruction of toxic chemicals [27,33,34]. The oxidising agents are hydroxyl radicals that due to their high oxidation potential, of the order of 2.80 V, are able to oxidise all the organic substances leading to the formation of CO_2 and H_2O. Hydroxyl radicals are produced through the reaction of hydrogen peroxide with Fe^{2+} and Fe^{3+} ions, in acidic medium, according to the reactions:

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + {}^{\bullet}OH + H_2O$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + {}^{\bullet}OOH + H^+$$

$$Fe^{3+} + {}^{\bullet}OOH \rightarrow Fe^{2+} + O_2 + H^+$$

The oxidising power of Fenton reaction can be enhanced by UV irradiation, likely due to the direct formation of the

hydroxyl radical and to regeneration of Fe^{2+} ions through the following photolytic reaction:

$$[Fe^{III}(OH)]^{2+} \xrightarrow{h\nu} {}^{\bullet}OH + Fe^{2+}$$

To propose a degradation pathway, the use of HPLC methods interfaced to mass spectrometry permits to discuss the structure of the intermediates formed. In particular, the characterisation of the dye through MS^n fragmentations gives information about the bonds that more easily can break for collision of suitable energy.

2. Experimental

2.1. Reagents

Ultra pure water from a Millipor Milli-Q system (Milford, MA, USA) was used for the preparation of all the solutions. HPLC-grade methanol from Merck (Darmstadt, Germany) was filtered before use through a 0.45 μm membrane (Millex, Millipore). Sunset Yellow FCF (C.I. 15985-E110), hydrogen peroxide 35%, L-ascorbic acid 99%, D-(+)-saccharose 98%, ammonium acetate 99.999% were purchased from Aldrich (Milwaukee, WI, USA). Iron (II) sulfate hexahydrate was purchased from Riedel-de Haen (Seelze, Germany). Acetic acid and triethylamine (TEA) were Merck (Darmstadt, Germany) analytical grade chemicals. Sulphuric acid 97% was acquired from Fluka (Milwaukee, WI, USA). On line nitrogen gas had a purity of 99.9990%.

2.2. Instrumentation

The HPLC analysis of the dye was performed with a Finnigan Mat Spectra System equipped with a Degaser SCM1000, a gradient pump Spectra System P4000, an Autosampler Spectra System AS3000, interfaced by the module SN4000 to a diode array detector Spectra System UV6000LP and to ESI-MS ion trap detector Finnigan LCQ Duo.

A Microprocessor pH meter (Hanna Instrument, Portugal), equipped with a combined glass-calomel electrode, was employed for pH measurements. The spectrophotometric analyses were carried out with a spectrophotometer Jasco V-550.

2.3. Samples preparation

The 1000.0 mg/L standard solution of food-dye Sunset Yellow FCF was prepared in ultrapure water and kept in dark glass bottle at 4 °C. The solution is stable for about 6 months.

2.4. Chromatographic conditions

The stationary phase was a Polaris C18-Ether column (150 mm × 4.6 mm, 5 μm) (Varian, CA, USA). The mobile phase was a mixture of 63% aqueous solution 20.0 mM of ammonium acetate and 37% CH_3OH, at flow rate 0.20 mL/min. The injection volume was 20.0 μL.

2.5. Mass spectrometry conditions

The MS experiments were conducted by means of Thermoquest LCQ Duo ion-trap mass spectrometer from Finnigan (San Jose, CA, USA) equipped with an atmospheric pressure ionization (API) interface and an electrospray (ESI) ion source. High purity nitrogen was used as nebulizer (operating pressure at 60 of the arbitrary scale 0–100 of the instrument), and helium (>99.999%) served as quenching agent. The ESI probe tip and capillary potential were set at 4.50 kV and −30.00 V, respectively. The heated capillary was set at 300 °C and ion optics parameters were optimized to the following values: tube lens offset, 10.00 V; first octapole voltage, 4.75 V; inter octapole lens voltage, 30.00 V; second octapole voltage, 6.00 V.

The mass to charge range was 50–500 *m*/*z*. The mass spectrometer was operated in negative ion full-scan mode (3 micro scans, 50 ms inlet time) and in MS^n mode. The method conditions employed in the MS analysis make use of the tuning method optimised for the peak at 203 amu.

3. Results

To reproduce in laboratory the loss of colour of the beverages and to make hypothesis about the possible reactions and intermediates, some strategies were employed to simulate the decolouration of E110 dye.

3.1. Thermally induced degradation

A first attempt to simulate the loss of colour naturally undergone by the beverage when exposed to natural conditions of summer temperature and solar UV–vis irradiation was performed, by exposing to temperatures up to 50 °C a standard solution of the dye at the same concentration as reported on the label of the beverage (0.11 mM). The experiment was carried on for about 30 days, following the signal of the dye in the solution through an HPLC method with UV detection at λ = 480 nm (2000 nm/min). No variation in the retention time and no decrease in the signal could be observed.

This result suggested that the decolourisation observed has likely to be ascribed to the effect of UV–vis irradiation more than to high temperatures.

3.2. Visible region photo induced degradation

It is worth to underline that the soft drink containing the E110 dye is commercialised in poly-ethylene-terephthalate (PET) bottles. Since this material absorbs the UV light at wavelengths lower than 300 nm, we supposed that the UV–vis radiations at higher wavelengths could be responsible for the degradation of the dye. 200.0 mL of a standard 0.11 mM solution of the dye was therefore placed in a glass cell and

irradiated with a 40 W tungsten-wire lamp. HPLC analysis showed that also in these conditions no variation occurred in 2 months of experiment.

3.3. Oxidative condition according to the Fenton reaction

According to literature data, visible light seems not to be efficacious in increasing the reaction velocity. It must be always observed that the most of these data concern the oxidation of organic species which do not absorb visible light, while, when the Fenton reaction was applied to an UV-absorbing species [25] the reaction was greatly accelerated by visible light ($\lambda > 470$ nm), very likely through a mechanism in which the excited dye reacts with Fe(III) ions to regenerate Fe(II) which preferentially reacts with hydrogen peroxide to give hydroxyl radicals.

A similar behaviour could occur for E110 dye, when undergone to Fenton reaction. To 100.0 mL of 0.30 mM aqueous solution of Sunset Yellow E110 dye were simultaneously added 10.0 mL of a solution 0.30 mM of $FeSO_4 \cdot 7H_2O$ (brought at pH 2.0 for sulphuric acid to prevent precipitation of $Fe(OH)_3$) and 10.0 mL of a solution 35.0 mM of H_2O_2.

In these conditions, the colour of the solution changes quickly, from yellow-orange to dark brown and then to yellow-brown, due to the formation of Fe(III) aquoions. The sequence of the colours is the same both in the absence and in the presence of sulphuric acid. The loss of the characteristic yellow-orange colour of the dye is already observed in the first 3–4 min and is therefore impossible to study the sequential degradation steps. To lower the velocity of the degradation reaction and to be able to follow spectrophotometrically the variations of colour, a new mixture was prepared directly in the spectrophotometric cell, using lower concentrations of the reagents. To 3.00 mL of a 0.06 mM solution of the dye contained in the cell were added 10 μL of the solution 30 mM of Fe(II) brought at pH 2.0 for H_2SO_4 and 10 μL of H_2O_2 35.0 mM. The cell was capped and shaken. Every 15 s successive absorbance scans were recorded in the wavelength range 200–700 nm (scanning velocity 2000 nm/min). The spectra, obtained at successive times, and compared with a spectrum recorded at $t = 0$, show that the absorbance at 480 nm (at which correspond the maximum of absorbance of the dye) is progressively decreasing as a function of time (Fig. 2).

The loss of colour is likely due to the cleavage of the azo-bond but no confirm could be obtained by mass spectrometry on the degraded solution, due to the too high noise and to the too high number of *m*/*z* signals present: the strong excess of $HO^{\bullet}$ radicals and the high concentration of metal ions (Fe^{2+}, Fe^{3+}) suppress and perturb the mass signal and decrease the sensitivity.

To identify the intermediates, another degradation technique must be employed. Methods based on reduction effects were tried.

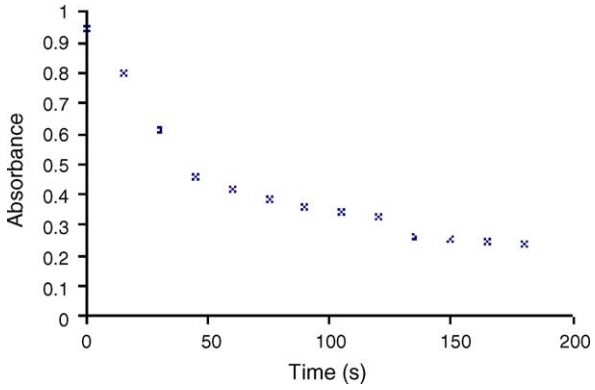

Fig. 2. Behaviour of the absorbance measured at 480 nm (at which E110 shows its maximum of absorbance) as a function of degradation time.

3.4. Photo induced degradation in reductive environment

3.4.1. Addition of sulphide and ascorbic acid

According to literature results, a photo-induced degradation can be achieved both by addition of sulphide [35] and of a mixture of ascorbic acid and sulphide [12,35]. Since the beverage contains ascorbic acid, it is of interest to study its role in the degradation process; two mixtures (100.0 mL) 0.30 mM of the dye and 3.0 mM of sodium sulphide are then prepared and to one of them 100.0 mL of ascorbic acid (200.0 mg/L) are also added. The solutions contained in glass vials, are sealed and put under stirring at 30 °C for 3 weeks. Every 2 days, on aliquots of the solutions A/λ spectra are recorded in the wavelength region 200–700 nm.

The solution that contains the dye and sulphide does not show any variation of absorbance and in particular at 480 nm, that corresponds to the maximum of absorbance of E110. The spectrum of the solution that also contains ascorbic acid also shows no decrease of absorbance at 480 nm, but only a decrease around 262 nm, typical of ascorbic acid, which naturally decrease since it is being oxidized to dehydroascorbic acid.

3.4.2. Addition of ascorbic acid and saccharose in atmosphere of nitrogen and oxygen

Even if the previous results suggest that ascorbic acid does not play a role in inducing degradation of the dye, we wished to take into account a possible synergic action between ascorbic acid and saccharose: according to some authors in fact saccharose protects ascorbic acid from oxidation [12].

To check the influence of reductive environment in the degradation process and taking into account that the beverage is commercialised in sealed PET bottles in which the atmospheric oxygen has been removed, some samples were saturated with nitrogen gas to avoid the oxidation of ascorbic acid by the atmospheric oxygen.

New degradation experiments are then carried out for the mixture of the dye and ascorbic acid, with and without

saccharose and in the presence of atmosphere of respectively oxygen and nitrogen gas. The molar concentration and the molar ratios were chosen in agreement with the composition of the beverage. Two sets of four solutions are prepared, respectively containing: (a) E110 dye at concentration 0.11 mM; (b) E110 dye at concentration 0.11 mM and ascorbic acid 11.00 mM; (c) E110 dye at concentration 0.11 mM and saccharose 11.00 mM and (d) E110 dye at concentration 0.11 mM, ascorbic acid 11.00 mM and saccharose 11.00 mM. In particular the model solution (d) contains the three components (dye, ascorbic acid and saccharose) at the same concentrations reported on the label of the drink. One set of solutions is exposed to atmosphere and the other is fluxed with ultrapure nitrogen gas and sealed. All the solutions are then exposed to sun irradiation.

After 5 days, while no loss of colour is observed for the solutions (a and c), in both oxidative and inert environments, a complete decolourisation is observed for the solutions (b and d) prepared in inert N_2 gas and containing E110 and ascorbic acid, respectively, in the absence and in the presence of saccharose. The degradation process therefore seems not to involve only the dye, but to be due to the interaction of the dye with ascorbic acid, in the absence of atmospheric oxygen. To study therefore the degradation reaction of solution (b) in atmosphere of N_2, an HPLC method interfaced with mass spectrometry is developed. The HPLC-MS analysis is preceded by a study of mass characterization of the dye, which could be helpful in the identification of the intermediates.

3.5. Mass characterisation of E110 dye

ESI mass spectra profiles (MS, MS^2, MS^3, MS^4) are obtained by direct infusion (flow rate 20.0 μL min^{-1}) in ESI ion source of a 10.0 mg/L methanolic solution of the standard. Due to two negative sulphonated groups present in the structure of the dye (Fig. 1), the characterization study was carried out in negative ion-mode. The parent molecule (molecular mass 452 amu) provides well recognizable signals corresponding to three negative pseudo-molecular ions at 203, 407 and 429 *m/z*. The signal at 203 *m/z* is ascribed to $[M - 2Na]^{2-}$, whereas the signals at 407 and 429 *m/z* are ascribed, respectively to $[M - 2Na + H]^-$ and $[M - Na]^-$. Each peak relative to these pseudo-molecular ions is then fragmented with MS^n analysis and the characteristic product ions are summarized in Table 1.

3.6. HPLC-MS investigation of the E110 degradation pathway induced by ascorbic acid in N_2 environment

Fig. 3 shows the HPLC chromatogram of a mixture of the dye and ascorbic acid (each at concentration of 2.0 mg/L)

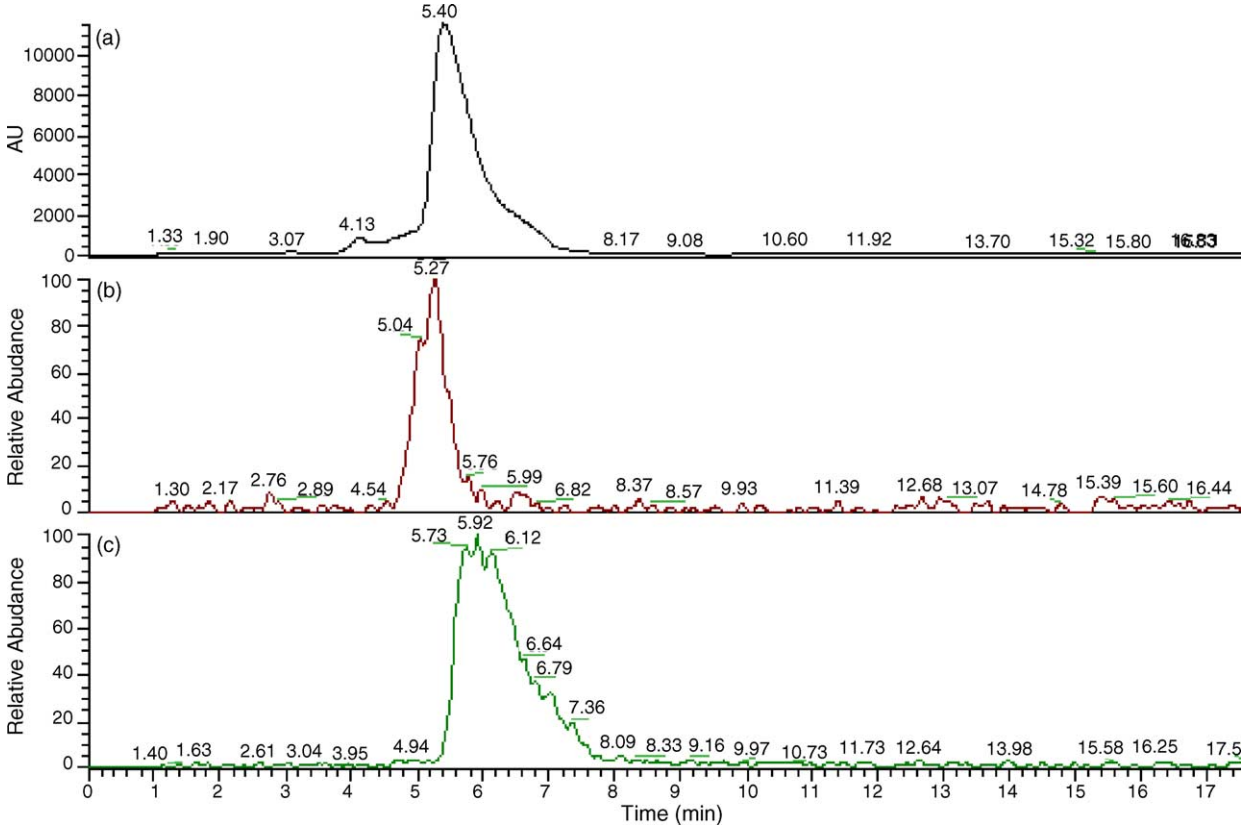

Fig. 3. Chromatogram of a mixture of dye and ascorbic acid, each at concentration of 2.0 mg/L. Stationary phase: Polaris C18-Ether column (150 mm × 4.6 mm, 5 μm). Mobile phase: 63% aqueous solution 20.0 mM of ammonium acetate and 37% CH_3OH mixture; flow rate 0.20 mL min^{-1}, injection volume 20 μL. Peak detection: photodiode array (a), mass spectrometry (b and c), extracting the signal of ascorbic acid at 175 *m/z* (b) and of E110 dye at 203 *m/z* (c).

with detection in photodiode array and mass spectrometry (ESI negative mode). By extracting from total ion current (TIC) the signals at 175 and at 203 *m*/*z*, respectively for the ascorbic acid $[M-H]^-$ and for the dye $[M-2Na]^{2-}$, it was possible to separate the two components of the drink.

In turn, Fig. 4 reports the LC–MS chromatogram recorded on the degraded solution. It can be observed that the peak of the dye (eluting at 5.92 min) is no more present while a new peak is present (at 6.76 min). This result, that suggests that a degradation of E110 has taken place with the formation of a new species, is investigated by mass analysis. The mass spectrum that corresponds to the new peak formed shows two mass signals respectively at 237 *m*/*z* (more intense) and at 239 *m*/*z*. The signal at 237 *m*/*z* can be ascribed to a dimeric

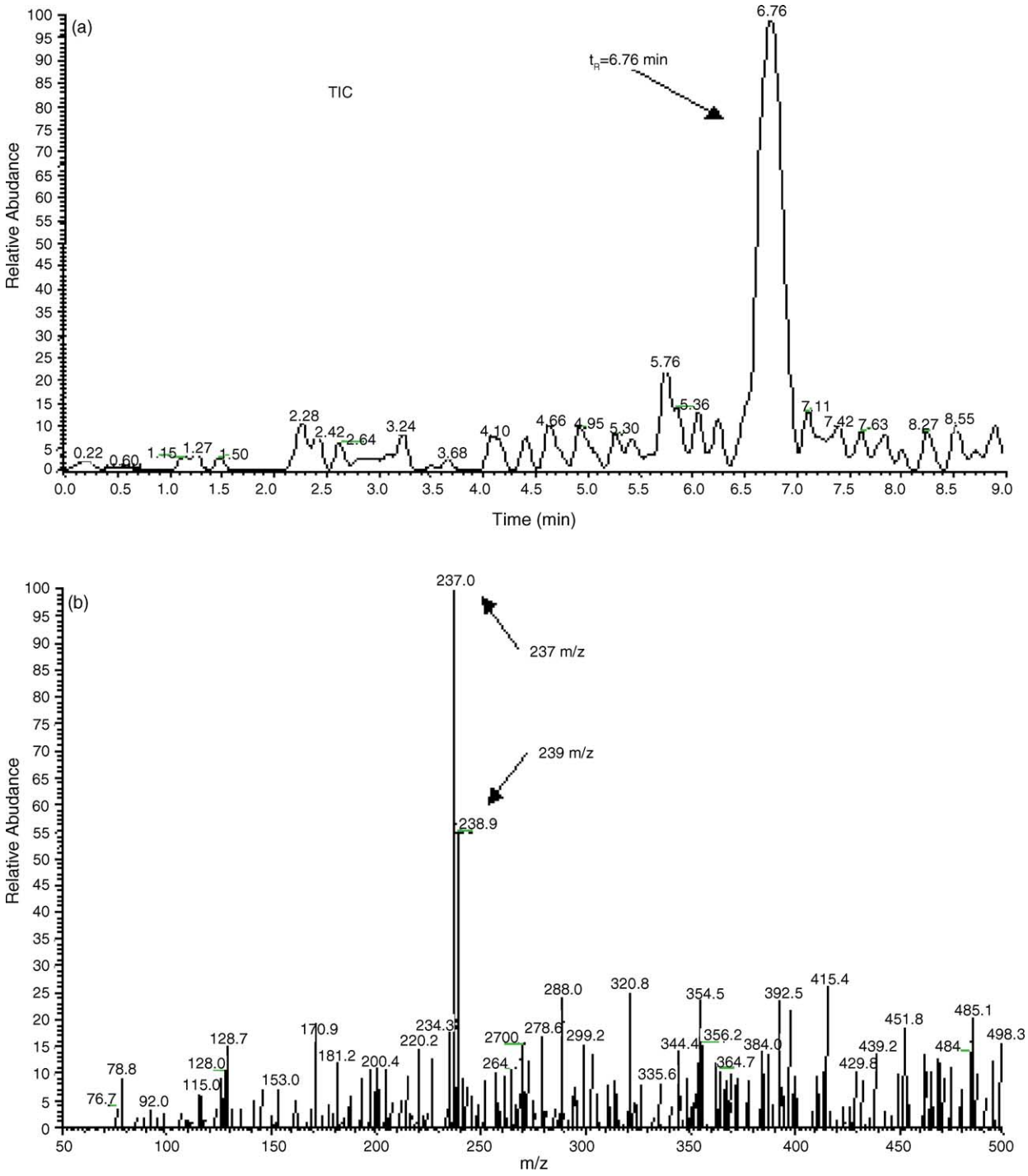

Fig. 4. (a) TIC chromatogram of the solution containing a 1/100 dye/ascorbic acid molar ratio solution recorded after 5 days. Chromatographic conditions as in the figure. (b) Mass spectrum for the peak at 6.67 min.

Table 1
MS^n characterization of Sunset Yellow FCF (E110). The pseudo-molecular ions are reported in bold

m/z	MS	Fragment
203	–	$[M - 2Na]^{2-}$
429	–	$[M - Na]^-$
365	MS^2	$[M - Na - SO_2]^-$
285	MS^3	$[M - Na - SO_2 - SO_3]^-$
170	MS^4	$[M - 2NaSO_3 - \text{C}_6\text{H}_6]^-$
349	MS^2	$[M - Na - SO_3]^-$
407	–	$[M - 2Na - H]^-$

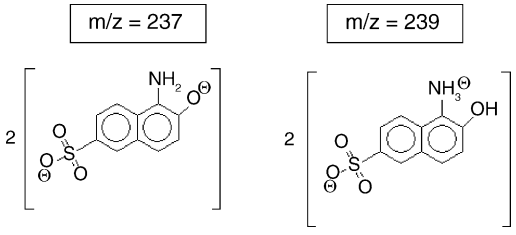

Fig. 5. Chemical structures ascribed to the mass signals at 237 and 239 *m/z*.

form of 5-amino-6-hydroxy-2-naphthalensulfonate while the signal at 239 *m/z* likely corresponds to the same species in which the two aminic groups are protonated (Fig. 5). This pattern confirms the hypothesis that E110 in the degradation process could behave as other azo-dyes for which has been reported the cleavage of the azo bond with the formation of aromatic amines [3–10]. To confirm the identification of the signal at 237 *m/z* a MS–MS spectrometric analysis is performed: the signal at 173 *m/z* (Fig. 6) can be ascribed to

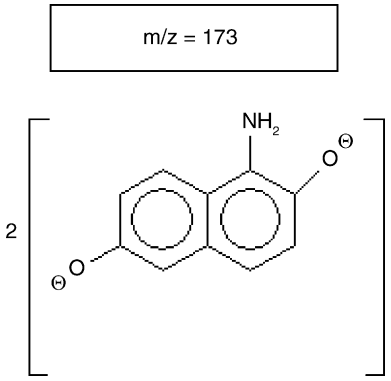

Fig. 6. Chemical structure ascribed to the mass signal at 173 *m/z*.

the loss of SO_2 from the sulphonic group of the 5-amino-6-hydroxy-2-naphthalensulfonate previously identified (237 *m/z*).

In order to collect more information on the intermediates, a decrease in the advancing of the degradation reaction is experimented through the addition of a lower concentration of ascorbic acid. A model solution containing the dye and ascorbic acid, each at concentration 0.11 mM, is prepared and undergone to the same degradation conditions as solution (b). After 5 days the solution is coloured, but the colour has varied from orange to dark-orange; then the colour does not change for further (up to 2 months) exposure to summer light.

The mass analysis of this solution shows a signal at 409 *m/z* (Fig. 7) that can be ascribed to the protonation of the azo group. The degradation pathway in the presence of ascorbic acid (Fig. 8) shows the formation of 5-amino-6-hydroxy-

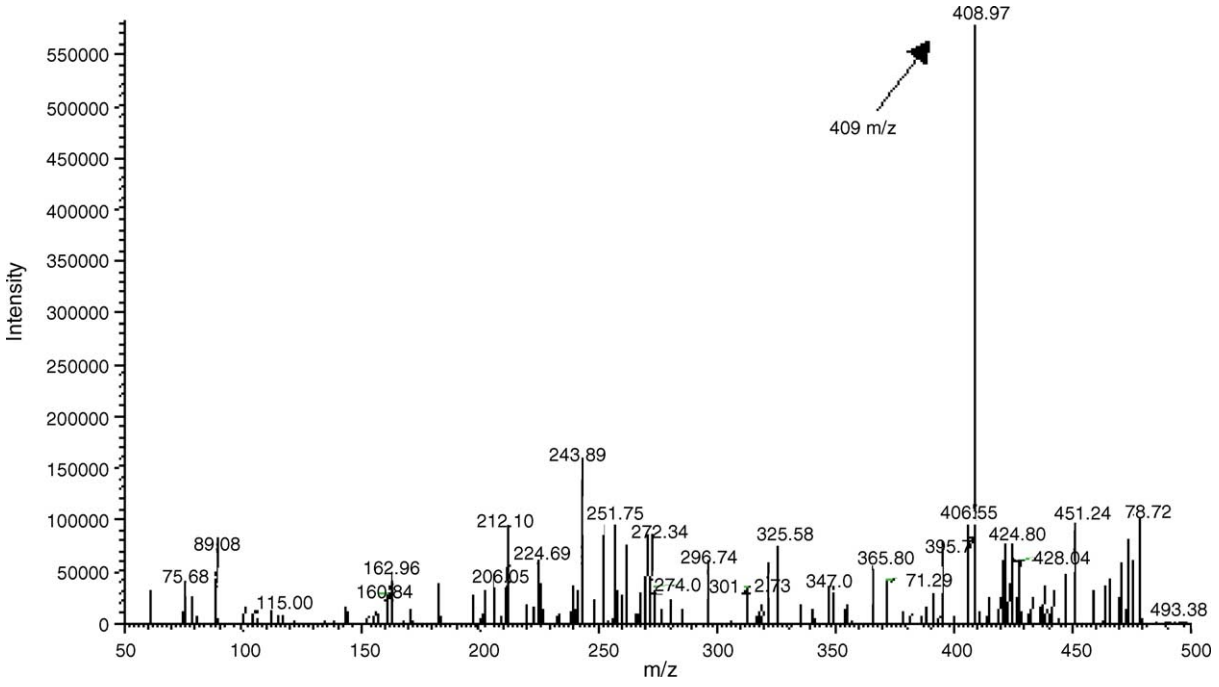

Fig. 7. Mass spectrum for the degraded solution containing a dye/ascorbic acid 1/1 molar ratio.

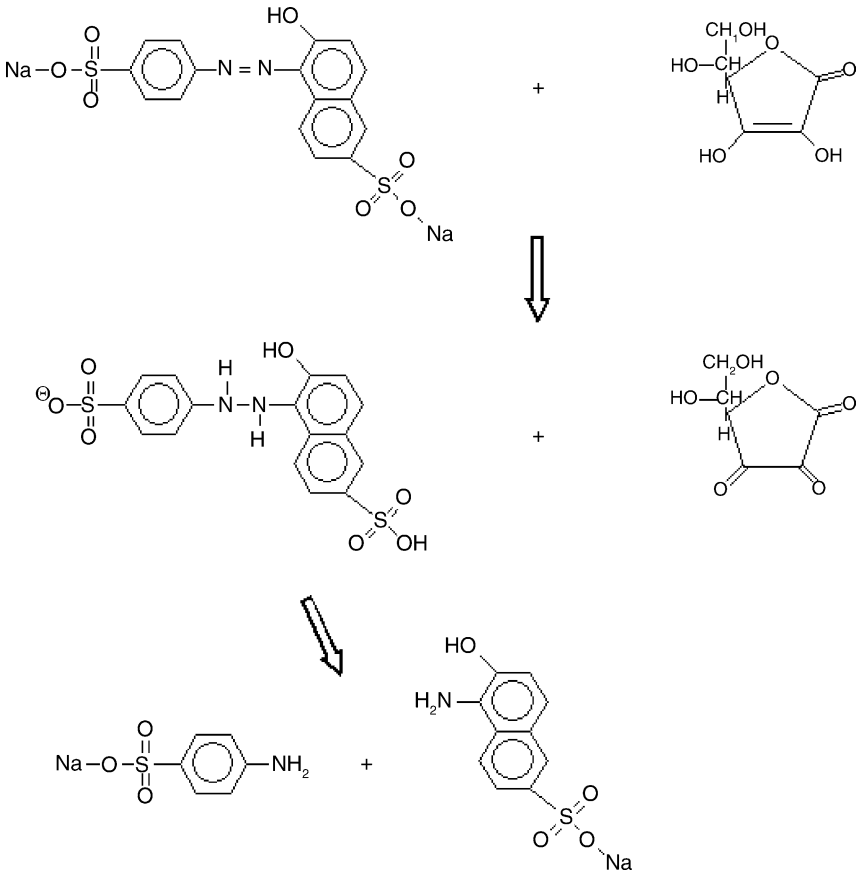

Fig. 8. Degradation pathway of the dye E110 in the presence of ascorbic acid.

2-naphthalensulfonate, whose dimeric form is identified by mass analysis. The simultaneous formation of *p*-amino benzensulfonate is also suggested, in agreement with the results obtained by other authors in TiO_2 solar photocatalytic degradation of indigoid dye [36], and that showed that naphthalene ring is more stable to the degradation than benzene ring.

4. Conclusions

The study, addressed to the identification of degradation intermediates of Sunset Yellow FCF (E110) in a commercial beverage, identifies the conditions under which the degradation proceeds with the decolourization of the dye. Different strategies are developed to simulate the decolourisation of E110 dye and to investigate the degradation intermediates. A thermally induced degradation does not show any variation of colour. In a similar way, a visible light region photo-induced degradation does not involve any colour variation. Strongly oxidative conditions according to the Fenton reaction provoke a loss of colour but the variation is too quick to be studied, also because the strong excess of hydroxylic radicals and the high concentration of iron ions suppress and perturb the spectrometer mass signal. Methods based on reduction effects are then experimented. The first, that is based on the addition of sulphide and ascorbic acid, does not give any variation of absorbance for the dye. The second method is based on the addition of ascorbic acid and saccharose in atmosphere of nitrogen and oxygen. A complete decolourisation are obtained for the degradation induced by ascorbic acid in atmosphere of nitrogen and exposure to sunlight, while the role of saccharose seems to have no effect. The results obtained by HPLC-MS indicate that the loss of colour is due to the cleavage of the azo bond and not to a complete mineralization of the dye. A degradation pathway is presented in which a dimeric form of 5-amino-6-hydroxy-2-naphthalensulfonate has been identified and the simultaneous formation of *p*-amino benzensulfonate is proposed.

References

[1] A.G. Fogg, A.M. Summan, Analyst 108 (1983) 691.
[2] A. Gottlieb, C. Shaw, A. Smith, A. Wheatley, S. Forsythe, J. Biotech. 101 (2003) 49.
[3] J.F. Lawrence, J. Chromatogr. Sci. 25 (1987) 444.
[4] F.E. Lancaster, J.F. Lawrence, J. Assoc. Off. Anal. Chem. 66 (1983) 1424.
[5] F.E. Lancaster, J.F. Lawrence, Food Add. Contam. 8 (1991) 249.
[6] F.E. Lancaster, J.F. Lawrence, Food Add. Contam. 6 (1989) 415.
[7] C.-S. Lu, S.-D. Huang, J. Chromatogr. A 696 (1995) 201.
[8] M.C. Garrigos, F. Reche, M.L. Marin, A. Jimenez, J. Chromatogr. A 976 (2002) 309.

[9] S.W. Collier, J.E. Storm, R.L. Bronaugh, Toxicol. Appl. Pharmacol. 118 (1993) 73.
[10] S. Borros, G. Barbera, J. Biada, N. Agullo, Dyes Pigm. 43 (1999) 189.
[11] A. Pielesz, I. Baronowska, A. Rybak, A. Wlochowicz, Ecotoxicol. Environ. Saf. 53 (2002) 42.
[12] L. Marovatsanga, R. Macrae, Food Chem. 24 (1987) 83.
[13] J. Fernández, J. Riu, E. Garciá-Calvo, A. Rodríguez, A.R. Fernández-Alba, D. Barceló, Talanta 64 (2004) 69.
[14] S. González, M. Petrovic, D. Barceló, J. Chromatogr. A 1052 (2004) 111.
[15] T. Storm, T. Reemtsma, M. Jekel, J. Chromatogr. A 854 (1999) 175.
[16] C. Baiocchi, M.C. Brussino, E. Pramauro, A. Bianco Prevot, L. Palmisano, G. Marcì, Int. J. Mass Spectrom. 214 (2002) 247.
[17] F. Gosetti, V. Gianotti, S. Angioi, S. Polati, E. Marengo, M.C. Gennaro, J. Chromatogr. A 1054 (2004) 379.
[18] P. Calza, E. Pelizzetti, M.C. Brussino, C. Baiocchi, J. Am. Soc. Mass Spectrom. 12 (2001) 1286.
[19] W.-H. Ding, S.-H. Tzing, J.-H. Lo, Chemosphere 38 (1999) 2597.
[20] T.P. Knepper, J. Chromatogr. A 974 (2002) 111.
[21] T. Reemtsma, J. Chromatogr. A 733 (1996) 473.
[22] A. Plum, G. Braun, A. Rehorek, J. Chromatogr. A 987 (2003) 395.
[23] S. Nam, V. Renganathan, P.G. Tratnyek, Chemosphere 45 (2001) 59.
[24] F. Chen, Y. Xie, J. He, J. Zhao, J. Photochem. Photobiol. A: Chem. 138 (2001) 139.
[25] K. Wu, Y. Xie, J. Zhao, H. Hidaka, J. Mol. Catal. A: Chem. 144 (1999) 77.
[26] J.T. Spadaro, V. Renganathan, Arch. Biochem. Biophys. 312 (1994) 301.
[27] J.T. Spadaro, L. Isabelle, V. Renganathan, Environ. Sci. Technol. 28 (1994) 1389.
[28] V. Augugliaro, C. Baiocchi, A. Bianco Prevot, E. Garcia-Lopez, V. Loddo, S. Malato, G. Marcì, L. Palmisano, M. Pazzi, E. Pramauro, Chemosphere 49 (2002) 1223.
[29] R.J. Davis, J.L. Gainer, G. O'Neal, I.-W. Wu, Water Environ. Res. 66 (1994) 50.
[30] Y. Wang, Water Res. 34 (2000) 990.
[31] M. Holčapek, P. Jandera, P. Zderadička, J. Chromatogr. A 926 (2001) 175.
[32] M. Holčapek, P. Jandera, J. Přikryl, Dyes Pigm. 43 (1999) 127.
[33] W.G. Kuo, Water Res. 26 (1992) 881.
[34] J.H. Carey, E.G. Cosgrove, B.G. Oliver, Can. J. Chem. 55 (1977) 2373.
[35] F.P. van der Zee, G. Lettinga, J.A. Field, Chemosphere 44 (2001) 1169.
[36] C. Galindo, P. Jacques, A. Kalt, J. Photochem. Photobiol. A: Chem. 141 (2001) 47.