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Sunlight induced degradation of E133 in a commercial beverage

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Abstract

The degradation pathway of E133 dye was investigated, when a commercial beverage containing the dye was subjected to summer sunlight and temperature. The study employed HPLC-DAD and HPLC−MS^n analyses, conducted on both the beverage and model solutions subjected to sunlight irradiation.

The results indicate that E133 was not present in the beverage after 16 days of irradiation and that the degradation products are formed only after long exposure times. Possible structures are proposed but their identification was made difficult by the formation of adducts with other ingredients present. Solid Phase Extraction (SPE) was developed to lower the matrix interferences.

Keywords: E133 (CI 42090); Degradation; HPLC−MS; Non-alcoholic beverage; SPE

1. Introduction

Many dyes are employed in the textile and food industries. The coloured wastes produced are decolourised, before disposal, using different treatment processes [1−8]. Even if public is no longer alarmed by unnatural colours that, in the past, characterized the waters of rivers and lakes, chemists not only know that the absence of colour does not mean the absence of toxicity but also that the uncoloured species formed could be even more toxic than the parent dye. Dye decolourisation can take place naturally under strong sun irradiation and high temperatures and experimental evidence shows that undesired decolourisation can also occur with food dyes allowed by European legislation and widely used in commercial food and beverages. Since many food dyes are aromatic amino-sulfonates, alarm concerns the possible formation of toxic aromatic amines [9−14].

Relatively few degradation studies of aromatic sulfonates are present in the literature [15−17]. The photodegradation of dyes has been studied under anaerobic reductive condition [18] as well as in oxidative condition [19−21], catalysed by peroxidase [22,23] or by TiO_2 [3,24−28].

Moreover, the results obtained have been interpreted differently for different dyes and it is not possible to predict a general behaviour for the degradation pathway of aromatic sulfonate dyes.

To check the possible degradation of food dyes induced by the action of sun and temperature, some commercial beverages were exposed, in sealed bottles, to sun irradiation and high summer temperatures in the town of Alessandria (Piedmont Region, north-west Italy). These experiments showed that some beverages lose their colour within 2 weeks, and in particular those containing CI 42090 (Brilliant Blue FCF E133) and CI 15985 (Sunset Yellow FCF E110) [29,30]. The degradation pathway of E110 in an orange coloured drink has been studied using HPLC−MS methodologies and some degradation intermediates have been identified [29]. This study was first performed on model solutions and then applied to the beverage. The major problems in the identification of the products came from the other ingredients present in the beverage that were shown to participate in the degradation process with the formation of adducts.

As concerns E133 (Fig. 1), a previous paper [30] reported a study performed on model solutions of the dye, different

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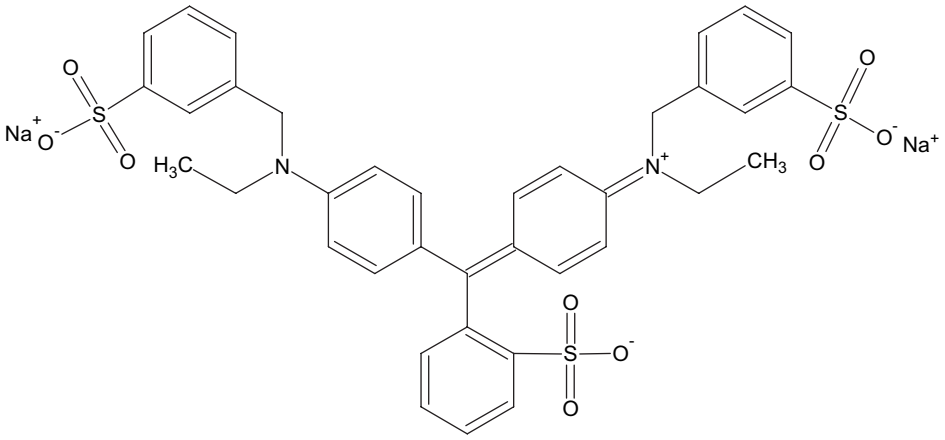

Fig. 1. Molecular structure of the dye Brilliant Blue FCF, E133.

treatments based on oxidative conditions under natural sunlight irradiation, using potassium persulfate at different persulfate:dye molar ratios were employed.

The aim of the present study was to identify the degradation intermediates that form in a beverage as a result of the action of uncontrolled sunlight and temperature so as to mimic the conditions encountered during transport, distribution and storage. The final aim was to evaluate the possible toxicity caused by the degradation process. For this purpose HPLC coupled with mass spectrometry seemed the most suitable analytical method. As reported on the label, the beverage contains, besides the dye, inorganic (sodium chloride, potassium *o*-hydrogen phosphate, magnesium carbonate) and organic species (glucose, fructose, saccharose, citric acid, aroms, E445 emulsion agent). Particular attention is devoted to the extraction procedures, generally based for complex food samples on liquid–liquid extraction with tetrabutylammonium salt [30] or Solid Phase Extraction (SPE) [31–34].

2. Experimental

2.1. Instrumentation

The chromatographic analyses were carried out by (i) a Finnigan Mat Spectra System equipped with a Degasser SCM1000, a gradient pump Spectra System P4000, a Spectra System AS3000 autosampler, interfaced to an SN4000 module and a diode array detector (Spectra System UV6000LP), ESI-MS Finnigan LCQ Duo ion trap detector; (ii) a Merck–Hitachi L-6200 Lichrograph chromatograph equipped with a two-channel Merck–Hitachi D-2500 chromato-integrator and interfaced with a Merck–Hitachi L-4250 UV/vis detector.

A microprocessor pH meter equipped with a combined glass–calomel electrode was employed for pH measurement.

2.2. Reagents

Ultra pure water from a Millipor Milli-Q system was used for the preparation of all solutions. Acetonitrile (HPLC grade) and dichloromethane were purchased from Merck, sodium hydroxide 1.0 N standard solution and ammonium hydroxide 30% from Carlo Erba, sulfuric acid 95–97% from Fluka, methanol 99.9% HPLC grade, C.I. 42090 (Brilliant Blue FCF, E133), tetrabutylammonium bromide (TBA) 99%, citric acid 99% and ammonium acetate 99.999% were all purchased from Sigma–Aldrich. On line nitrogen gas had a purity of 99.9990%.

A stock dye solution was prepared in a dark glass flask at a concentration of 1000.0 mg/L in ultra pure water and was preserved at 4 °C. Dilutions using ultra pure water were performed to obtain the desired concentrations.

2.3. Extraction of the dye from the beverage

Two kinds of solid phase were tested, namely Strata C18-E and Strata-X33 Polymeric from Phenomenex, Waters.

The optimised method makes use of a Waters cartridge (Strata C18-E). The cartridge was conditioned with the reagents added in the order and at the concentrations as follows: 2.0 mL of acetonitrile, 2.0 mL of methanol and 2.0 mL of water at pH 2.0 for sulfuric acid. The cartridge was then charged with 2.0 mL of the sample and washed with 4.0 mL of water to remove possible interfering species. The elution of the dye is then performed with 2.0 mL of a 50/50 v/v acetonitrile/water mixture brought to pH 2.0 for sulfuric acid.

The liquid–liquid extraction of the dye is performed with 3.0 mL of dichloromethane, after the addition of 0.40 mL of TBA (200.0 mM) to 2.0 mL of the sample.

2.4. Chromatographic conditions

When using the UV/vis detector (set at 626 nm, λ_{max} of the dye) the stationary phase was a C18 LiChroCART, HPLC-Cartridge RP-18e (5 μm) 250 × 4.6 mm from Merck and the mobile phase a 60/40 v/v mixture of 20.0 mM ammonium acetate aqueous solution/methanol. The injection volume was 100.0 μL and the flow rate was 1.0 mL/min.

When using the mass spectrometry detector, the stationary phase was a Polaris C18-ether (5 μm) 150 × 4.0 mm from Varian and the mobile phase, flowing under isocratic conditions at

flow rate of 0.2 mL/min, was a 60/40 v/v mixture of 20.0 mM ammonium acetate aqueous solution/methanol; the injection volume was 20.0 μL.

2.5. Mass spectrometry conditions

High purity nitrogen was used as nebulizer gas (operating pressure at 80 of the arbitrary scale 0–100 of the instrument for the sheath gas and 20 for the auxiliary gas); helium (>99.999%) was the quenching agent. The ESI probe tip and capillary potentials were set, respectively, at 4.50 kV and ±46.00 V (depending on the ionisation mode). The heated capillary was set at 200 °C and ion optics parameters were optimised to the following values: tube lens offset, 30.00 V; first octapole voltage, 8.00 V; inter octapole lens voltage, 14.00 V; and second octapole voltage, 13.00 V.

The mass-to-charge range was 50–900 *m/z*. The mass spectrometer was operated both in positive (PI) and negative (NI) ions' full-scan mode (3 micro scans, 50 ms inlet time) and in MS^n mode. PI and NI modes can be usefully used in the identification of unknown species as the degradation intermediates. Tuning was optimised for the mass signal 749 *m/z* (PI) and 747 *m/z* (NI) that respectively, correspond to $[M - 2Na + 3H]^+$ and $[M - 2Na + H]^-$, where M is the mass of the E133 dye.

3. Results

3.1. Construction and validation of the calibration plot

As already observed by different authors [30,36,37], the dye E133, both in a reference standard solution and in a beverage at its λ_{max} of 626 nm, shows three typical peaks of different intensities. The peak of greatest intensity was used in this work. A calibration plot of peak area (y) versus concentration (x) was constructed, in the concentration range between 0.1 and 5.0 mg/L; the concentration levels were 0.1, 0.8, 1.5 and 5.0 mg/L. For each concentration level, three independent replicates, analysed by the HPLC-DAD–MS method, gave a standard deviation below 5%.

In order to validate the calibration plots and in particular to test the statistical significance of the intercept, calculated by the regression analysis, a statistical treatment was performed, based on the calculation of the casual deviation $s_{y/x}$ along the axis y [35]. The regression equation obtained showed a deviation from the intercept value so as to evaluate the significance of the mean value found for the intercept, a t-test was performed to estimate the error associated with the value of the intercept. Since the value of $|t|$ ($t = 0.03$) was lower than the t value tabulated for 95% significance level and $(n - 1)$ freedom degrees ($t = 2.35$), the intercept of the calibration plot was assumed as statistically non-significant. The regression equation was therefore:

$$y = (4389 \pm 101)x, \quad R^2 = 0.9989$$

To evaluate the level of detection (LOD), in the chromatogram an area which corresponds to a signal-to-noise ratio of around 3 is identified and used to proportionally transform sensitivity (given as the peak area for 1.0 mg/L concentration as obtained by the slope of the calibration plot) into concentration units (mg/L). The LOD value was 0.03 mg/L, whereas the quantitation level (0.10 mg/L) was evaluated from the calibration plot as the lowest concentration that could be quantified.

3.2. Extraction

Taking into account the high number and different chemical properties of the components present in the matrix (salts, sugars, organic acids, …) which could interfere with the signal of the dye in the HPLC–MS analysis of the beverage, extraction of the dye from the matrix was performed. At this stage liquid–liquid extraction and Solid Phase Extraction methods were developed and compared.

3.2.1. Liquid–liquid extraction

To extract the polar anionic form of the dye in dichloromethane, an ion-pair with TBA was previously formed; to maximize the extraction recovery, experiments were undertaken at concentrations of TBA ranging between 100.0 mM and 300.0 mM. The procedure in which 2.0 mL of the beverage was added with 0.40 mL of 200.0 mM TBA and extracted with 3.0 mL of dichloromethane gave an extraction recovery of around 97% (Table 1).

3.2.2. Solid Phase Extraction (SPE)

The extraction process was optimised for the sorbent, washing agents and eluents by performing each series of experiments on the extraction of a standard solution of E133 (3.0 mg/L) with a C18 and a polymeric phase.

As regards the washing solutions used to remove water soluble interfering species prior to elution, the use of different volumes of ultra pure water and NH_4OH solution (at pH = 9.0) revealed that a volume of 4.0 mL of ultra pure water offered a good compromise of rapidity and efficiency.

In the case of the eluting reagents, when using the polymeric sorbent, best results (recovery of around 80%) were obtained using 2.0 mL methanol with respect to mixtures v/v of acetonitrile/water at different pH values. With C18 sorbent, it was found that the recovery increased as the polarity of the elution system increased and was near to 100% when a mixture of 50/50 v/v acetonitrile/acidic water (pH = 2.0 for sulfuric acid) was used. On the basis of these results, the extraction procedure used C18 sorbent, 4.0 mL of ultra pure water as

Table 1
Optimization of recovery for the liquid–liquid extraction of dye as a function of TBA concentration

TBA (mM)	Recovery (%)
100.0	80.83 ± 1.74
200.0	97.50 ± 0.50
300.0	73.33 ± 3.48

washing solution and 2.0 mL of a 50/50 v/v mixture acetonitrile/water at pH 2.0 with sulfuric acid as eluent.

3.3. Analysis of the beverage

3.3.1. Mass spectrometry characterisation of the dye

The mass spectrometric characterisation of the dye was performed through the identification of the major product-ions that formed in the collisional sequential fragmentations of MS^n experiments. ESI mass spectra profiles (MS, MS^2, MS^3, MS^4, MS^5) were obtained by the direct infusion (flow rate, 20.0 μL min^{-1}) into the ESI ion source of a 10.0 mg/L methanol solution of the standard dye.

The molecular structure of the dye shows three negative sulfonated groups saturated with Na^+ and one protonable aminic functionality (Fig. 1). To collect the maximum information that could be useful to identify the degradation products of E133 in the beverage, mass characterisation was performed both in positive (PI) and negative (NI) ionisation modes.

The molecular mass of the dye was 792 amu; the molecule provides well recognizable signals corresponding to two positive pseudo-molecular ions, respectively, at 749 *m/z* and 771 *m/z*. The peak at 749 *m/z* is due to the $[M - 2Na + 3H]^+$, whereas the peak at 771 *m/z* corresponds to $[M - Na + 2H]^+$. The characterisation in NI mode showed three negative pseudo-molecular ions, respectively, at 373 *m/z*, 747 *m/z* and 769 *m/z*. The peak at 373 *m/z* is due to the $[M - 2Na]^{2-}$, whereas the peaks at 747 *m/z* and 769 *m/z* represent $[M - 2Na + H]^-$ and $[M - Na]^-$, respectively. Each precursor ion was then fragmented with MS^n analysis and the characteristic product-ions are summarized in Tables 2 and 3.

3.3.2. Degradation pathway in the commercial beverage

Five bottles of the commercial, blue-coloured beverage were bought from a local food store. One bottle (here indicated as t_0) was used as reference and stored inside, protected from sunlight and high temperature. The other four bottles were exposed in their sealed original bottles to the sun irradiation during July–September 2004 in the town of Alessandria (north-west Italy). The progressive reduction of the blue colour could be observed by the naked eye. When the variation of colour became significant, one of the bottles was removed from sun exposure, 30.0 mL of the beverage were collected from the bottle using a syringe, avoiding as possible the contact with atmosphere and was immediately analysed. Simultaneously, a control sample (30.0 mL) was collected and stored at −20 °C.

The first degraded sample (indicated as t_1) was removed after 10 days of sun exposition, when the colour had changed from Brilliant Blue to pale blue (Fig. 2). Following the colour variation, a second bottle (t_2) was removed after 11 days and a third bottle (t_3) after 16 days, when the beverage was completely uncoloured. The fourth bottle (t_4) was kept under the sun for a further 10 days to determine if the degradation process further proceeds in the absence of colour. Fig. 2 shows the progressive changes in colour of the drink, after, respectively, 0, 10, 11, 16 and 26 days of exposure to sunlight.

All the samples (t_0, t_1, t_2, t_3 and t_4) were subjected to the SPE C18 extraction process and the solutions were submitted to HPLC-DAD–MS analysis.

As expected, the relative intensities of the dye peaks at 749 *m/z* (PI) and 747 *m/z* (NI) decrease according to the extent of decolourisation of the beverage. In particular, the HPLC-DAD and MS chromatograms of sample t_1 (Fig. 3) showed the presence of a new peak with an R_t of 8.35 min (Fig. 3b). HPLC-DAD analysis showed for this species two λ_{max} at 229 nm and 316 nm (Fig. 3c) and HPLC–MS analysis showed a signal for *m/z* = 166 (Fig. 3d).

Table 2
MS^n characterisation of E133 in PI mode

m/z	MS	Fragment
749	MS	$[M - 2Na + 3H]^+$
458	MS^2	$[M - 2Na + H–SO_3–⬡ –CH_2NCH_2CH_3–⬡]^+$
171	MS^3	$[CH_2–⬡–SO_3H]^+$
579	MS^2	$[M - 2Na + 3H–SO_3–⬡–CH_2]^+$
458	MS^3	$[M - 2Na + H–SO_3–⬡ –CH_2NCH_2CH_3–⬡]^+$
771	MS	$[M - Na + 2H]^+$
601	MS^2	$[M - Na + 2H–SO_3CH_2–⬡]^+$
691	MS^2	$[M - Na + 2H–SO_3]^+$

Table 3
MS^n characterisation of E133 in NI mode

m/z	MS	Fragment
373	MS	$[M - 2Na]^{2-}$
769	MS	$[M - Na]^-$
689	MS^2	$[M - NaSO_3]^-$
495	MS^3	$[M - 2\ NaSO_3–CH_3–⬡]^-$
453	MS^4	$[M - 2\ NaSO_3–CH_3–⬡ –CH_3NCH_3]^-$
424	MS^5	$[M - 2\ NaSO_3–CH_3–⬡ –CH_3NCH_3–CH_2CH_3]^-$
747	MS	$[M - 2Na + H]^-$
667	MS^2	$[M - 2NaSO_3 + H]^-$
587	MS^3	$[M - 2NaSO_3]^-$
558	MS^4	$[M - 2NaSO_3 + H–CH_2CH_3]^-$
529	MS^5	$[M - 2NaSO_3 + H–2CH_2CH_3]^-$

Fig. 2. Five bottles of commercial beverage containing E133 are subjected to sun exposure degradation and are indicated as t_0–t_4. The variation of intensity of blue colour can be observed by naked eye.

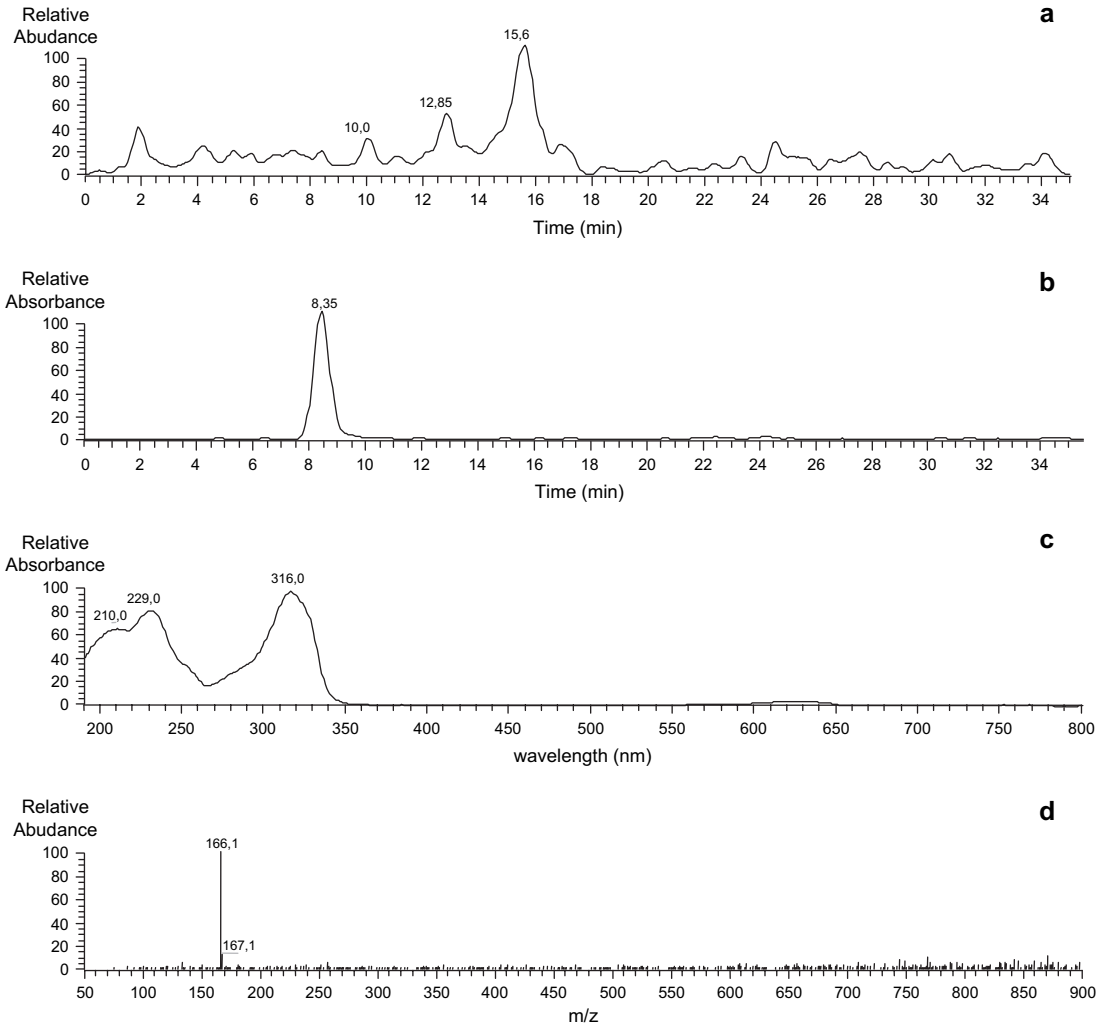

Fig. 3. (a) HPLC-DAD–MS chromatogram of the sample t_0 reporting the three peaks typical to E133 after extraction of 749 *m/z* signal; (b) HPLC-DAD–MS chromatogram of sample t_1 where a new signal is present (8.35 min); (c) DAD spectrum of the new signal; (d) mass spectrum of the new signal. The conditions of HPLC-DAD–MS analysis are reported in Section 2.4.

The sample collected after 11 days of exposure to sunlight, (t_2), discoloured a paler blue colour. As expected, the relative intensities of the peaks at 749 *m/z* (PI) and 747 *m/z* (NI) had further decreased. The HPLC-DAD–MS analyses did not show new UV absorbing species while the mass analysis showed in NI a mass signal at 295 *m/z* with an R_t of about 18 min.

The sample t_3, collected after 16 days of sun exposure, was uncoloured. HPLC-DAD analysis confirmed the absence of dye and also showed no evidence of the presence of other species that absorbed in the UV/vis region. In turn, mass analysis in NI confirmed the absence of the *m/z* signal of the dye and showed, besides the signal at 295 *m/z* already observed for the sample t_2, the presence of a new peak eluting at 2.80 min and with an *m/z* ratio of 181.

The sample t_4 collected after about 26 days of sun exposure was uncoloured and HPLC-DAD chromatograms did not show the presence of any peak. HPLC–MS analysis, with respect to the analysis of the sample t_3, revealed that the signal at 295 *m/z* was lower by about 86%, while the signal at 181 *m/z* was no longer present.

As a conclusion of this study in which degradation was followed as a function of exposure to sun the sequence of the chromatograms recorded at the different times of exposure shows that the intensity of the signal of the dye at 747 *m/z* (NI) progressively decreased in correspondence to the progressive decolourisation of the beverage. The same results were obtained in PI following the signal with *m/z* = 749 (Fig. 4).

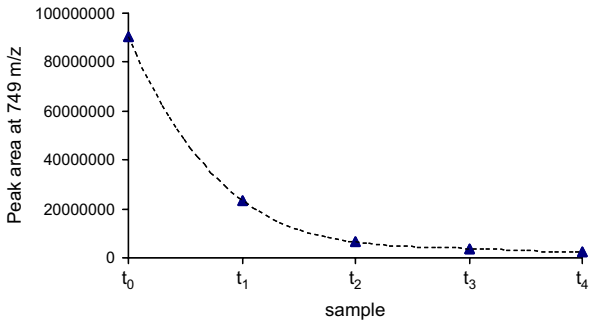

Fig. 4. The decrease of peak area (relative units) at 749 *m/z* (PI) as a function of irradiation time. HPLC-DAD–MS conditions are reported in Section 2.4.

A species with 295 *m/z* that was not present in the original sample t_0 was formed; this species had a maximum value for the sample t_1 and then progressively decreased to 14% for sample t_4. The species at 181 *m/z* formed in t_3 but was no longer present in t_4. The identification of the species at *m/z* 295 and 181 was not possible, possibly due to the matrix effect that is particularly relevant notwithstanding the extraction process to which the beverage has been subjected.

3.3.3. Model solutions

In order to collect information that might identify the intermediates that are formed during the degradation process and whose *m/z* signals have been found in mass analysis, four model solutions of the dye were prepared in the absence of the other components in the beverage and in two different redox environments. Two aqueous solutions of dye at 2.50 mg/L (the same concentration present as that in the beverage) were prepared: one was fluxed with ultra pure nitrogen gas and the other was kept under atmospheric oxygen. Both the solutions were exposed, in Pyrex-glass bottles, to sun irradiation. Since after three months of sun exposure no colour variation could be observed, it was concluded that the decolourisation process observed in the beverage does not involve the dye alone but is due to possible interactions between the dye and the other components present in the beverage. Since the component present at highest concentration was citric acid, two model solutions were prepared containing the dye and citric acid in the same molar ratio (1:100) as in the beverage and the extent of degradation was followed during exposure to solar irradiation, in both oxidant and reductive conditions. At this stage, before irradiation, one solution was fluxed with ultra pure nitrogen gas and both the bottles were sealed.

During irradiation, the sample containing the dye and citric acid in the presence of atmospheric oxygen changed colour from blue to dark-blue, while the same solution in the presence of nitrogen was completely decolourised only after 7 days.

HPLC-DAD–MS analysis of the uncoloured solution showed the absence of signals of the dye at 749 *m/z* (PI) and 747 *m/z* (NI) and the presence of a new peak, eluting at about 1.7 min. Fig. 5 shows the chromatogram in diode

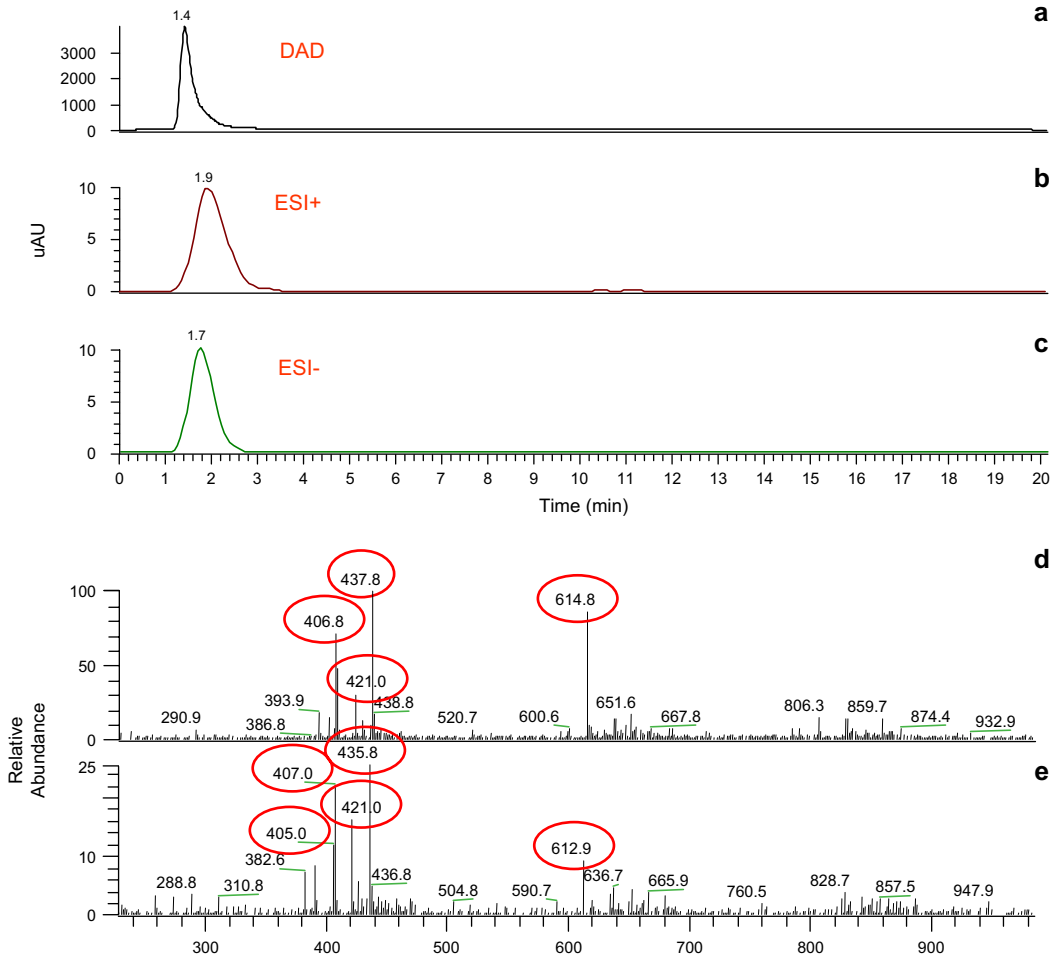

Fig. 5. HPLC-DAD–MS analysis of uncoloured solution of E133:citric acid in molar ratio 1:100 in diode array detection (a), TIC (total ion current) in PI mode (b), TIC (total ion current) in NI mode (c), mass spectrum in PI mode (d) and NI mode (e). Analysis conditions are reported in Section 2.4.

array detection (a), mass detection in PI mode (b), NI mode (c) and the mass spectrum in PI (d) and NI (e) mode. The mass signals do not correspond to those found in the beverage.

For the most intense mass signals reported in Fig. 5d and e, through the following fragmentations by MS^n, possible chemical structures were conjectured (Figs. 6 and 7). It can be seen that in all the proposed structures, a hydrogen atom is always bound to the central carbon atom of a triarylic dye structure, which precludes electronic delocalisation in the aromatic structure of the dye and inhibits the chromophoric properties in the visible region, so justifying the absence of colour.

The same analysis was then carried out for the degradation of the model dye/citric acid solution in which atmospheric oxygen was not removed and which was of a dark-blue colour. HPLC-DAD–MS analysis showed that three peaks, as those shown by the dye are present, but the UV/vis spectrum recorded for the second peak showed a λ_{max} at 601 nm, which was significantly different from that of E133 (626 nm). Mass detection of this peak in NI corresponded to an *m/z* signal of 823 amu; moreover, mass analysis revealed two signals of, respectively, 869 *m/z* and 915 *m/z*.

Taking into account that the signals observed were all correlated by a difference of 46 amu, which corresponds to the molecular mass of formic acid, the formation of adducts with formic acid, which could be derived from the cleavage of citric acid present in the solution seems probable.

4. Conclusions

The irradiation of a commercial beverage containing the blue dye E133 showed that the beverage gradually discolors, and completely loses its colour after 16 days. Degradation of dyes in sealed beverages can therefore occur in every day life under uncontrolled conditions that can be encountered during transport, distribution and storage. Laboratory experiments also showed that solutions of the standard dye alone do not undergo any degradation under the same exposure time, but that degradation is assisted by other components present in the beverage. It was clearly shown that citric acid, which is the component present in the beverage at the highest concentration (100-fold that of the concentration of the dye), may result in the formation of metabolites.

On the other hand, the degradation products formed when irradiating the model solutions containing the dye and citric acid in the same concentration as in the beverage, revealed different structures to those formed from the degradation of the beverage.

On the basis of the results obtained using model solutions, it is opinion of the authors that the different ingredients of the drink can give rise to different interactions in the degradation pathway. Due to the large number of components present in the beverage, their reactions on one hand make the identification of the degradation species formed difficult and, on the other hand, preclude the identification of a general pathway that could be applied to coloured beverages to evaluate the

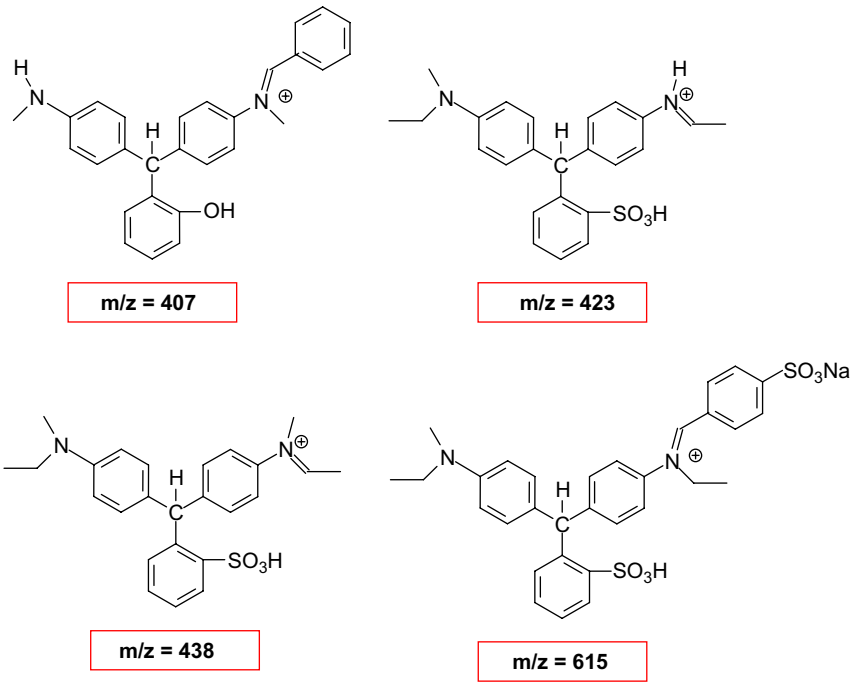

Fig. 6. Proposed chemical structures for the intermediates originated in the model solution containing E133 (3.16 mM) and citric acid in molar ratio 1:100; the mass signals are obtained by ion trap working in PI mode. Conditions are reported in Section 2.5.

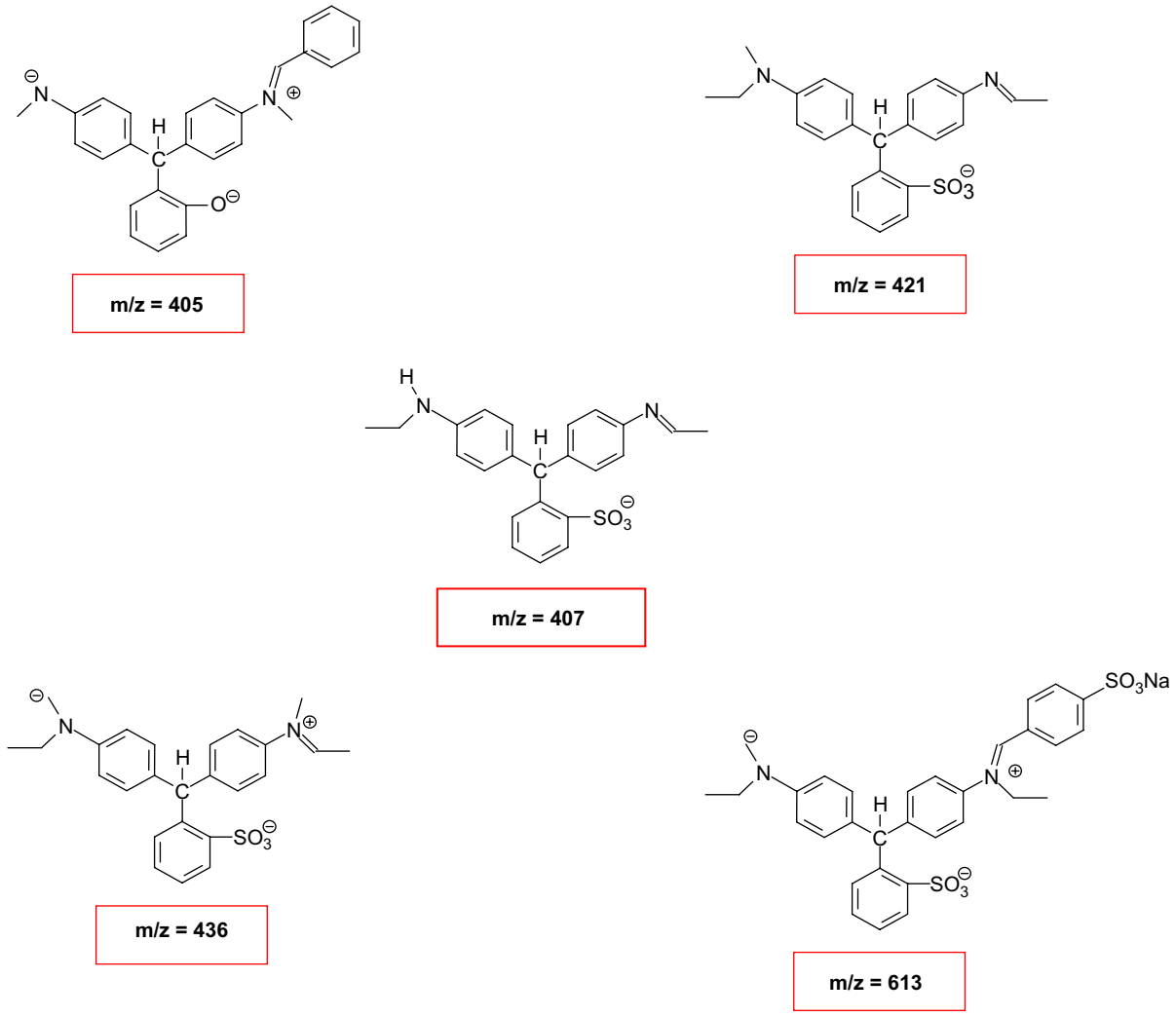

Fig. 7. Proposed chemical structures for the intermediates originated in the model solution containing E133 (3.16 mM) and citric acid in molar ratio 1:100; the mass signals are obtained by ion trap working in NI mode. Conditions are reported in Section 2.5.

possible toxicity of the species formed as a result of sunlight irradiation.

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References

[1] Azmi W, Sani RK, Banerjee UC. Biodegradation of triphenylmethane dyes. Enzyme Microb Technol 1998;22:185–91.
[2] Davila-Jimenez MM, Elizalde-Gonzalez MP, Gutierrez-Gonzalez A, Pelaez-Cid AA. Electrochemical treatment of textile dyes and their analysis by high-performance liquid chromatography with diode array detection. J Chromatogr A 2000;889:253–9.
[3] Davis RJ, Gainer JL, O'Neal G, Wu I-W. Photocatalytic decolorization of wastewater dyes. Water Environ Res 1994;66:50–3.
[4] Nam S, Tratnyek PG. Reduction of azo dyes with zero-valent iron. Water Res 2000;34:1837–45.
[5] Pielesz A. The process of the reduction of azo dyes used in dyeing textiles on the basis of infrared spectroscopy analysis. J Mol Struct 1999;511:337–44.
[6] Seshadri S, Bishop P. Anaerobic/aerobic treatment of selected azo dyes in wastewater. Waste Manag 1994;14:127–37.
[7] Sponza DT, Isik M. Decolorization and azo dye degradation by anaerobic/aerobic sequential process. Enzyme Microb Technol 2002;31:102–10.
[8] van der Zee FP, Lettinga G, Field JA. Azo dye decolourization by anaerobic granular sludge. Chemosphere 2001;44:1169–76.
[9] Eskilsson CS, Davidsson R, Mathiasson L. Harmful azo colorants in leather determination based on their cleavage and extraction of corresponding carcinogenic aromatic amines using modern extraction techniques. J Chromatogr A 2002;955:215–27.
[10] Fogg AG, Summan AM. Differential pulse polarographic monitoring of permitted food colouring matters and ascorbic acid in accelerated light degradation studies and the spectrophotometric determination of the ammonia and simpler amines formed. Analyst 1983;108:691.
[11] Garrigós MC, Reche F, Marín ML, Jiménez A. Determination of aromatic amines formed from azo colorants in toy products. J Chromatogr A 2002;976:309–17.
[12] Gottlieb A, Shaw C, Smith A, Wheatley A, Forsythe S. The toxicity of textile reactive azo dyes after hydrolysis and decolourisation. J Biotechnol 2003;101:49–56.
[13] Lu C-S, Huang S-D. Trace determination of aromatic amines or phenolic compounds in dyestuffs by high-performance liquid chromatography with on-line preconcentration. J Chromatogr A 1995;696:201–8.

[14] Pielesz A, Baranowska I, Rybak A, Wlochowicz A. Detection and determination of aromatic amines as products of reductive splitting from selected azo dyes. Ecotoxicol Environ Saf 2002;53:42–7.
[15] Fernández J, Riu J, Garciá-Calvo E, Rodríguez A, Fernández-Alba AR, Barceló D. Determination of photodegradation and ozonation by products of linear alkylbenzene sulfonates by liquid chromatography and ion chromatography under controlled laboratory experiments. Talanta 2004;64:69–79.
[16] González S, Petrovic M, Barceló D. Simultaneous extraction and fate of linear alkylbenzene sulfonates, coconut diethanol amides, nonylphenol ethoxylates and their degradation products in wastewater treatment plants, receiving coastal waters and sediments in the Catalonian area (NE Spain). J Chromatogr A 2004;1052:111–20.
[17] Storm T, Reemtsma T, Jekel M. Use of volatile amines as ion-pairing agents for the high-performance liquid chromatographic–tandem mass spectrometric determination of aromatic sulfonates in industrial wastewater. J Chromatogr A 1999;854:175–85.
[18] Plum A, Braun G, Rehorek A. Process monitoring of anaerobic azo dye degradation by high-performance liquid chromatography–diode array detection continuously coupled to membrane filtration sampling modules. J Chromatogr A 2003;987:395–402.
[19] Nam S, Renganathan V, Tratnyek PG. Substituent effects on azo dye oxidation by the Fe-III–EDTA–H_2O_2 system. Chemosphere 2001;45:59–65.
[20] Chen F, Xie Y, He J, Zhao J. Photo-Fenton degradation of dye in methanolic solution under both UV and visible radiation. J Photochem Photobiol A Chem 2001;138:139–46.
[21] Wu K, Xie Y, Zhao J, Hidaka H. Photo-Fenton degradation of dye under visible light radiation. J Mol Catal A Chem 1999;144:77–84.
[22] Spadaro JT, Renganathan V. Peroxidase-catalysed oxidation of azo dyes: mechanism of disperse yellow 3 degradation. Arch Biochem Biophys 1994;312:301–7.
[23] Spadaro JT, Isabelle L, Renganathan V. Hydroxyl radical mediated degradation of azo dyes: evidence for benzene generation. Environ Sci Technol 1994;28:1389–93.
[24] Augugliaro V, Baiocchi C, Bianco Prevot A, Garcia-Lopez E, Loddo V, Malato S, et al. Azo-dyes photocatalytic degradation in aqueous suspension of TiO_2 under solar irradiation. Chemosphere 2002;49:1223–30.
[25] Wang Y. Solar photocatalytic degradation of eight commercial dyes in TiO_2 suspension. Water Res 2000;34:990–4.
[26] Holčapek M, Jandera P, Zderadička P. High performance liquid chromatography–mass spectrometric analysis of sulphonated dyes and intermediates. J Chromatogr A 2001;926:175–86.
[27] Holčapek M, Jandera P, Přikryl J. Analysis of sulphonated dyes and intermediates by electrospray mass spectrometry. Dyes Pigments 1999;43:127–37.
[28] Baiocchi C, Brussino MC, Pramauro E, Bianco Prevot A, Palmisano L, Marcì G. Characterization of methyl orange and its photocatalytic degradation products by HPLC/UV–vis diode array and atmospheric pressure ionization quadruple ion trap mass spectrometry. Int J Mass Spectrom 2002;214:247–56.
[29] Gosetti F, Gianotti V, Polati S, Gennaro MC. HPLC–MS degradation study of E110 Sunset Yellow FCF in a commercial beverage. J Chromatogr A 2005;1090:107–15.
[30] Gosetti F, Gianotti V, Angioi S, Polati S, Marengo E, Gennaro MC. Oxidative degradation of food dye E133 Brilliant Blue FCF. Liquid chromatography–electrospray mass spectrometry identification of the degradation pathway. J Chromatogr A 2004;1054:379–87.
[31] Bovanová L, Brandšteterová E. Direct analysis of food samples by high-performance liquid chromatography. J Chromatogr A 2000;880:149–68.
[32] Loos R, Alonso MC, Barceló D. Solid-phase extraction of polar hydrophilic aromatic sulfonates followed by capillary zone electrophoresis–UV absorbance detection and ion-pair liquid chromatography–diode array UV detection and electrospray mass spectrometry. J Chromatogr A 2000;890:225–37.
[33] de Souza SVC, Junqueira RG, Ginn R. Analysis of semicarbazide in baby food by liquid chromatography tandem mass spectrometry (LC–MS–MS). In-house method validation. J Chromatogr A 2005;1077:151–8.
[34] Shao B, Han H, Hu J, Zhao J, Wu G, Xue Y, et al. Determination of alkylphenol and bisphenol A in beverages using liquid chromatography/electrospray ionization tandem mass spectrometry. Anal Chim Acta 2005;530:245–52.
[35] Mille JN, Miller JC. Statistics and chemometrics for analytical chemistry. 4th ed. England: Prentice Hall; 2000.
[36] Gianotti V, Angioi S, Gosetti F, Marengo E, Gennaro MC. J Liq Chromatogr Relat Technol 2005;28:923–37.
[37] Lawrence JF, Lancaster FE, Conacher HBS. Separation and detection of synthetic food colors by ion-pair high-performance liquid chromatography. J Chromatogr 1981;210:168–73.