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Characterization of methyl orange and its photocatalytic degradation products by HPLC/UV–VIS diode array and atmospheric pressure ionization quadrupole ion trap mass spectrometry

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Abstract

HPLC/UV–VIS diode array and HPLC/MS techniques were successfully applied to the analysis of sulfonated molecules present in samples coming from the photocatalytic degradation of the azo dye indicator, methyl orange. The substrate was chosen as a simple model for the study of reactions involving the more complex commercial products used for the dyeing of textile fibers. Unexpected MS fragmentation path was observed, due to the very stable methyl orange molecular structure. The chromatographic information were combined with the obtained MS, MS/MS data and the UV–VIS diode array spectra and allowed to rationalize the molecular structures attributable to the various degradation products. (Int J Mass Spectrom 214 (2002) 247–256) © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Azo dyes represent about one-half of the dyes actually used in the textile industry and, as a consequence, a relevant problem is related to the release of these products in the environmental phases [1]. In fact, the effluent streams coming from textile plants must be treated in order to remove the toxic or carcinogenic dye residues and their by-products, whereas an effective effluent decolorization is usually required by most government regulations.

Since processes based on biodegradation are usually scarcely applicable to textile wastewaters due to the simultaneous presence of toxic residual dyestuffs, surfactants and other harmful additives, the use of advanced oxidation techniques has been proposed. In particular, the possible application of heterogeneous photocatalysis [2–4] for the treatment has been investigated as an alternative to conventional methods.

The fundamentals of this approach, abundantly described in the literature cited, can be summarized as follows: irradiation with light of proper wavelength causes electron excitation from the semiconductor valence band to the conduction band, generating

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electron holes pairs. Both electron and holes migrate to the surface of the semiconductor particles, where they can react with water and dissolved oxygen to form various oxidizing species, including the highly reactive hydroxyl radicals, whereas superoxide and perhydroxyl radicals are formed from the reaction of excited electrons with oxygen. The highly reactive oxygen-containing species formed are able to attack and oxidize the organic molecules, generally leading to a complete mineralization of the substrate. When photocatalysis is applied to degrade dyes, in addition to the above mentioned processes, photosensitization [5] of the dye occurs upon excitation by visible light. After the electron transfer from the excited dye to the semiconductor particle, reduction of adsorbed oxygen to form superoxide anion radicals and reaction of the cationic dye radical with other active oxygen species occur.

Several recent studies concerned, in particular, the photocatalytic treatment of aqueous wastes containing single or mixed azo dyes [5–10] and showed very encouraging results. Most of the reported studies were mainly focused on the examination of the primary process, whereas less attention has been paid to a detailed analysis of the reaction mechanisms. More recently [11,12] the use of GC-MS analysis to identify intermediates coming from the photocatalytic degradation of azo dyes were reported. Nevertheless, a lack of information exists about the possible presence of hydrophilic reaction intermediates still containing the chromophoric groups, which cannot be extracted efficiently in the solvents usually used in GC-MS analysis.

In the present work, we investigate the photocatalytic degradation, in the presence of TiO_2 (anatase) dispersions, of methyl orange, a well known sulfonated azo dye indicator. This molecule was chosen as a simple model for the study of reactions involving the more complex commercial products commonly used for the dyeing of textile fibers. Other investigations on the photobleaching of methyl orange in the presence of the same semiconductor have been previously reported [13,14], but these studies were silent about the mechanism of the decolorization process and about the nature of the reaction intermediates formed. Sulfonated azo dyes have poor thermal stability and low volatility and we thus considered the application of HPLC-MS as a suitable analytical approach for the determination of methyl orange and its sulfonated degradation products. The HPLC-MS technique has been already proposed [15,16] for the analysis of mixtures of sulfonated azo dyes in water and wastewater, but not yet applied to the separation and identification of their degradation products.

2. Experimental

2.1. Chemicals

The following analytical-grade reagents (all from Merck) were used as received: methyl orange, ammonium acetate, acetonitrile (Lichrosolv). Ultra pure water was provided by a Milli-Q system (Millipore). TiO_2 P25 from Degussa having a surface area of ca. $55\,m^2\,g^{-1}$ and a measured size of the primary particles around 20–30 nm (20) was used in all the photocatalytic experiments.

2.2. Degradation experiments

Most degradation experiments were performed under aerobic conditions in stirred cylindrical closed cells (40 mm i.d. × 25 mm high) made of Pyrex glass, on 5 ml of aqueous TiO_2 dispersions containing $20\,mg\,L^{-1}$ of methyl orange and $600\,mg\,L^{-1}$ of semiconductor. The initial pH of these solutions was 5.2. A 1500 W xenon lamp, equipped with a 340 nm cut-off filter was used (Solarbox, from Cofomegra, Milan) to produce a simulated sunlight to irradiate the stirred dispersions. The temperature within the cell was ca. 55 °C.

2.3. Chromatographic conditions

Samples were taken from the reaction vessels and filtered through a 0.45 μm cellulose membranes (Millex, Millipore). After a defined irradiation time, the residual dye and its degradation products were determined according to the below described conditions.

HPLC analyses were carried out with a pump Thermoquest Model TP 4000, a Thermoquest AS300 auto sampler and a Thermoquest UV 6000 diode array detector.

Separations were obtained under isocratic conditions using a RP-C18 column (Lichrospher RP-18, 250 mm × 4.6 mm; 5 mm particles, Merck, Darmstadt, Germany) and a mobile phase composed of acetonitrile–ammonium acetate 10 mM pH 6.8 (24/76 (v/v)); flow rate 0.8 mL min^{-1}. The eluent from the chromatographic column successively enter the UV–VIS diode array detector, the ESI interface and the quadruple ion trap mass analyzer.

2.4. Mass spectrometry

MS and MS^n analyses in the negative ions mode were performed on a Thermoquest LCQ ion trap mass spectrometer equipped with an atmospheric pressure ionization (API) interface and an ESI ion source. The mass range was 100–350 amu.

High purity nitrogen was used as nebulizer and auxiliary gas (operating pressure, respectively, at 60 and 10 units of the arbitrary scale 0–100 of the instrument).

The ESI probe tip and capillary potentials were set at 4.5 kV and –8 V, respectively.

The heated capillary was set to 220 °C and the ion optics parameters were optimized to the following values:

tube lens voltage, 5.00 V;
first octapole voltage, 2.25 V;
inter octapole lens voltage, 20.0 V;
second octapole voltage, 7.00 V.

3. Results and discussion

The degradation of methyl orange during the irradiation was monitored together with the formation of the inorganic final products (SO_4^{2-}, NO_3^-, NH_4^+) and the TOC evolution; the complete substrate disappearance was observed after ca. 35 min. Nevertheless at this time the complete mineralization has not yet occurred; in particular the released sulfate did not yet reach the sulfur stoichiometric amount [17]. In order to assess the nature of the organic sulfonated intermediates present in the final degradation stages, the chosen irradiation time (5, 10, 15, 22, 27, 37 min) correspond to substrate degradation percentage falling in the range 75–100%. With the aim to achieve preliminary information about the mass spectrometric behavior of methyl orange, ESI mass spectra profiles (MS, MS^2, MS^3) were obtained by direct infusion in ESI ion source at 10 μL min^{-1} of a 10 ppm methanolic solution of the pure standard. The parent molecule provides a signal corresponding to a negative ion at *m*/*z* 304. The MS/MS analysis of this ion gives three significant *m*/*z* values: 289.2, 240.3, 156.2. The MS/MS/MS analysis of the ion 289.2 provides the *m*/*z* values 260.2, 224.2 and again 156.2, whereas the analysis in the same conditions of the ion 240 gives only a fragment of *m*/*z* 225. A visualization of the results is shown in Fig. 1 from which it is possible to draw some conclusions about the bonds more susceptible to breakdown as a consequence of collisions of suitable energy. The most striking feature of the proposed fragmentation scheme is the unavoidable presence of odd electrons species. As it is known high energy homolitic rupture of bonds is an event generally excluded in electrospray ionization owing to the fact that only even electrons ions ($[M + H^+]$ or $[M - H^+]$) are formed. Such ions normally fragment giving neutral molecules by concerted rupture of multiple bonds. However, the structure of methyl orange molecule (Fig. 1) does not allow easy formation of neutral fragments if one excludes SO_2 or SO_3 molecules. Homolytic bond breaking becomes thus competitive in spite of the absence of any stabilization of the odd electron by the conjugated π electron system due to the difference in the orbital symmetry. After this preliminary characterization of the fragmentation behavior of methyl orange molecule we analyzed the constituents of its solutions submitted to degradation conditions for different times.

Fig. 2 reports the chromatograms monitored in full scan MS, corresponding to solutions of methyl orange degraded at 0, 5, 10, 15, 22 and 27 min, respectively. As it can be seen there is a number of degradation products variably present at the various times; the

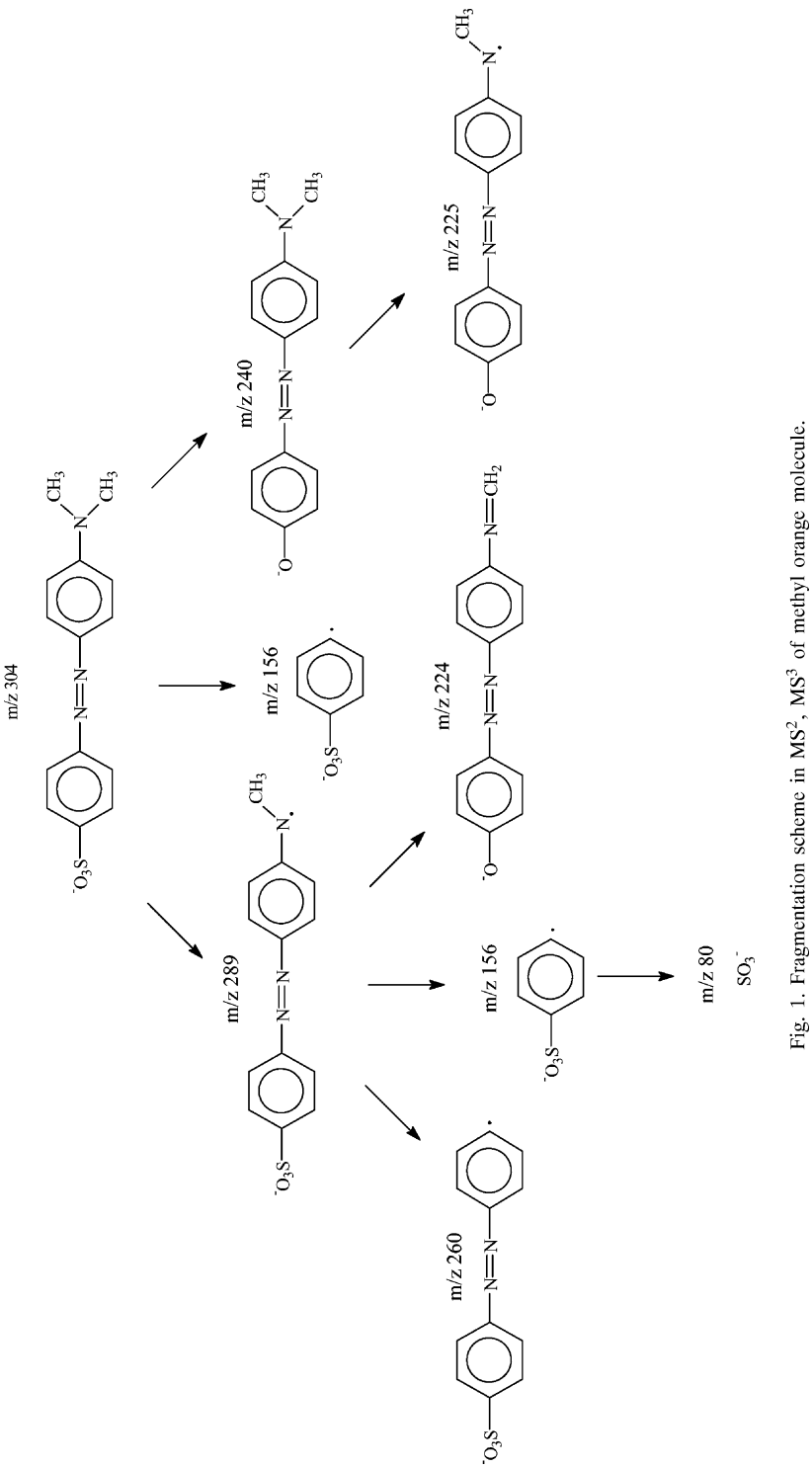

Fig. 1. Fragmentation scheme in MS^2, MS^3 of methyl orange molecule.

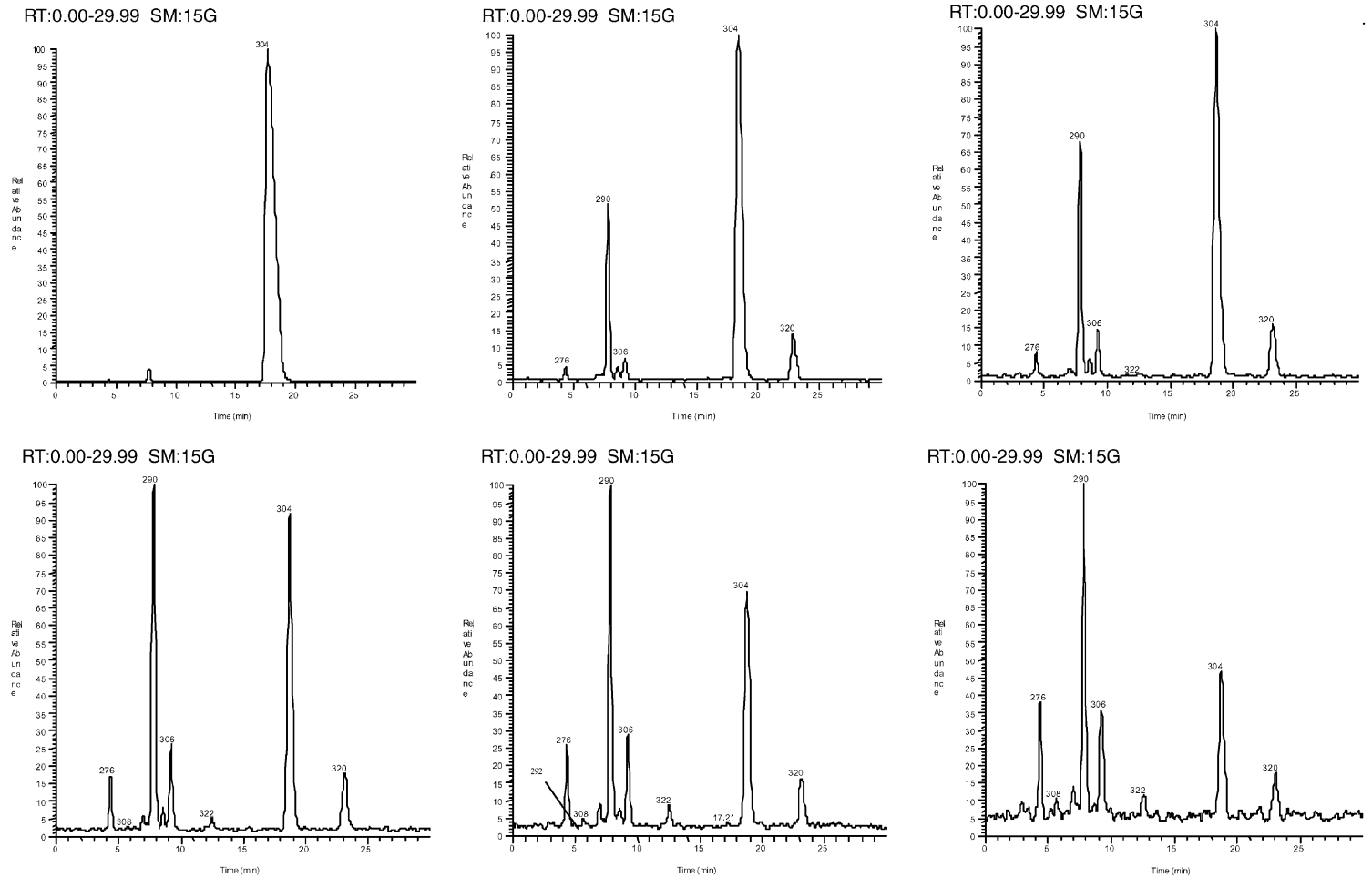

Fig. 2. Chromatograms monitored in full scan MS corresponding to solutions of methyl orange degraded at 0, 5, 10, 15, 22 and 27 min; each peak is characterized by its m/z value.

chromatogram corresponding at 37 min irradiation shows only very low signals and it is not reported. The significant peaks present at the various degradation times are labeled with the corresponding *m*/*z* values. It is worth noticing that there are four species having *m*/*z* values higher than that of the parent molecule but only one of them (*m*/*z* 320) exhibits a longer retention time. The remaining three species correspond to lower *m*/*z*

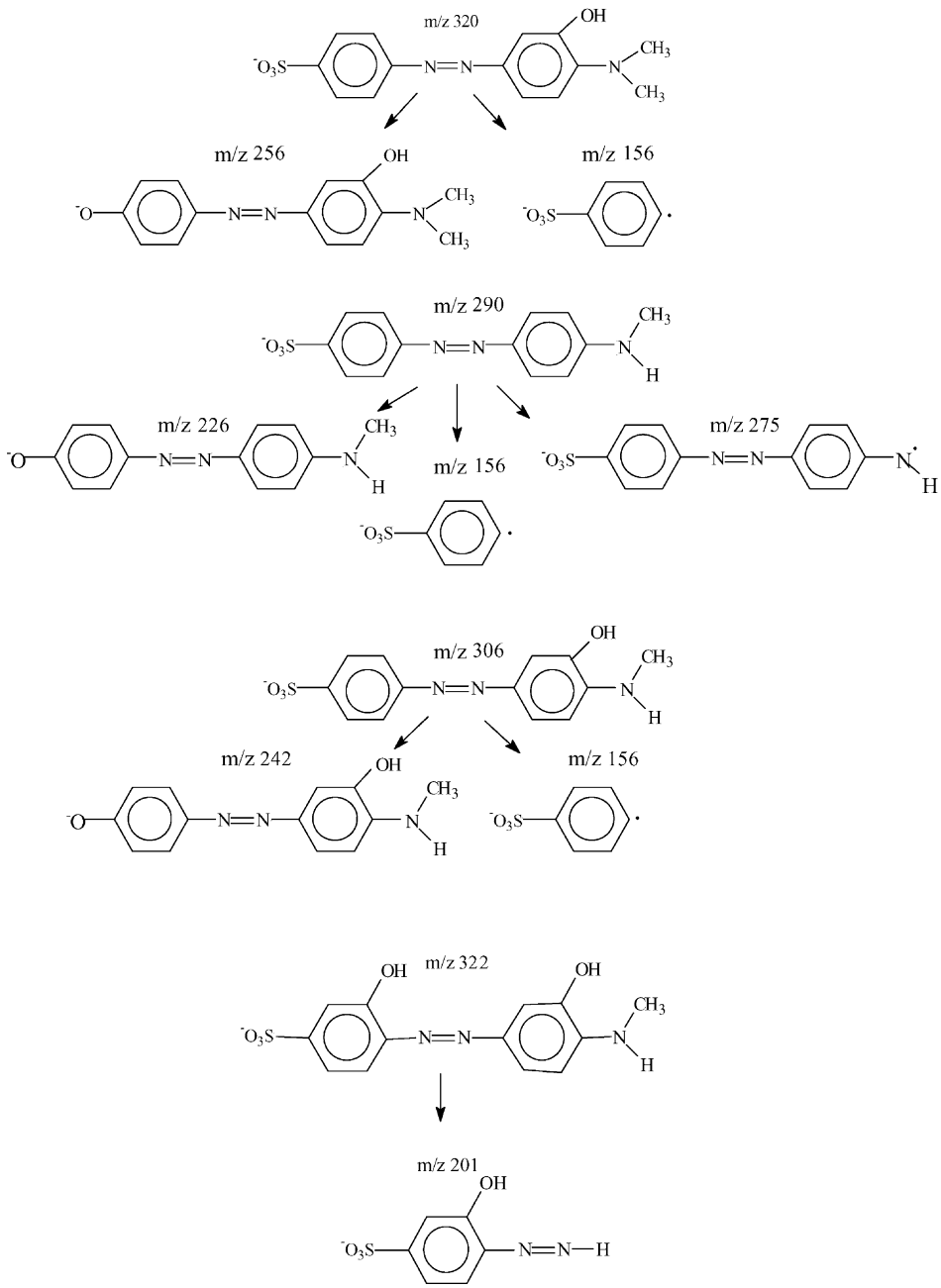

Fig. 3. Fragmentation scheme of methyl orange degradation products.

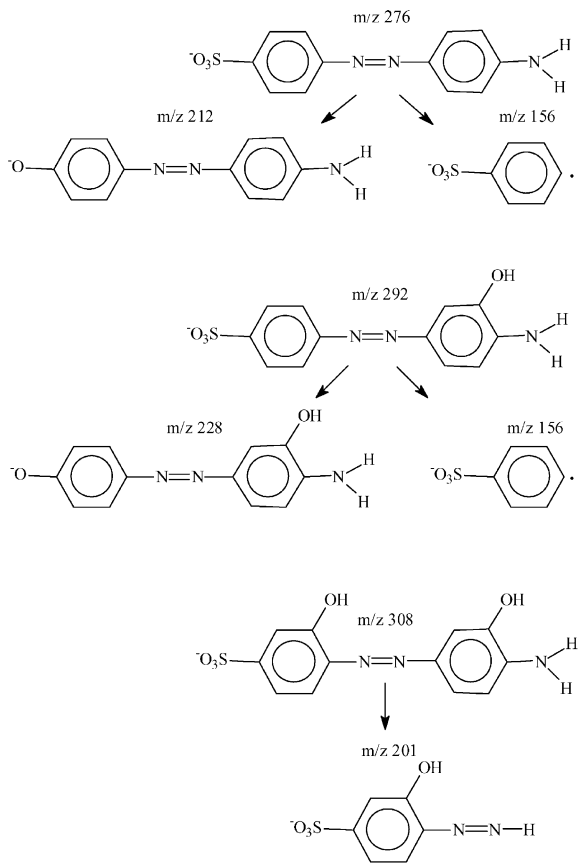

Fig. 3. (*Continued*).

values so their structure may be considered as a moiety of the original molecule. There is a numerical agreement between some molecular weights and the hypothesis of the formation of monohydroxylated (*m*/*z* 320, 306, 292) and dihydroxylated (*m*/*z* 322, 308) products. All the species present were reanalyzed in MS/MS conditions in order to achieve further details mainly about the substitution position of OH groups. Combining the information coming from the chromatographic runs with the obtained MS and MS/MS data we rationalized the molecular structures attributable to the various degradation products. The structural details of the following discussion are reported in Fig. 3. From the chromatographic point of view the more important factor determining the retention time seems to be the presence or the absence of the methyl groups. The species of *m*/*z* 276 (no more methyl groups bonded to nitrogen atom of amino group, Fig. 3) has the lower retention time, followed by the species of *m*/*z* 290 (one methyl group left) and the parent molecule *m*/*z* 304 (both methyl groups present). The insertion of one hydroxyl group in the aromatic rings of each of the above cited molecules induced by the operating degradation mechanism gives more than one species present in different amounts and at different retention times. In Fig. 4 are reported as an example the comparison of the chromatographic behavior of monohydroxylated species with respect to the non-hydroxylated ones at *m*/*z* 304 and 290. The hydroxylated species exhibiting retention times faster than that of the respective non-hydroxylated molecule are in lower concentration whereas the monohydroxylated species present in greater amount shows a chromatographic behavior opposite than expected, that is a slowing down of the elution time in respect to the non-hydroxylated homologous. The same is true for the dihydroxylated species (not reported in the Fig. 4) which further delayed with respect to monohydroxylated species. Such chromatographic behavior is at first glance surprising and we can only try an explanation hypothesizing the insertion of hydroxyl groups mainly in *ortho* positions to azo and $N(R_1R_2)$ groups. As a matter of fact the formation of an internal hydrogen bond favors such type of substitution and at the same time partially engages the lone electronic pairs on the $N(R_1R_2)$ group and on the nitrogen atom of the azo group reducing their important role in retention mechanism. The MS/MS results visualized in Fig. 3 may help to support this hypothesis. As it can be seen the monohydroxylated species (*m*/*z* 320 and 306) fragment loosing SO_2 molecule and alternatively giving as typical product the odd electron ion at *m*/*z* 156 corresponding to the phenylsulfonic radical. On the contrary the species of *m*/*z* 322 and 308, homologous dihydroxylated of the species at *m*/*z* 290 and 276, respectively, loose no more SO_2 molecule neither forms the ion at *m*/*z* 156 but give both the MS/MS fragment at *m*/*z* 201.

Then we can deduce that the first hydroxylation occurs at the ring containing the $N(R_1R_2)$ group; as this substituent is an electron donor and thus *ortho*

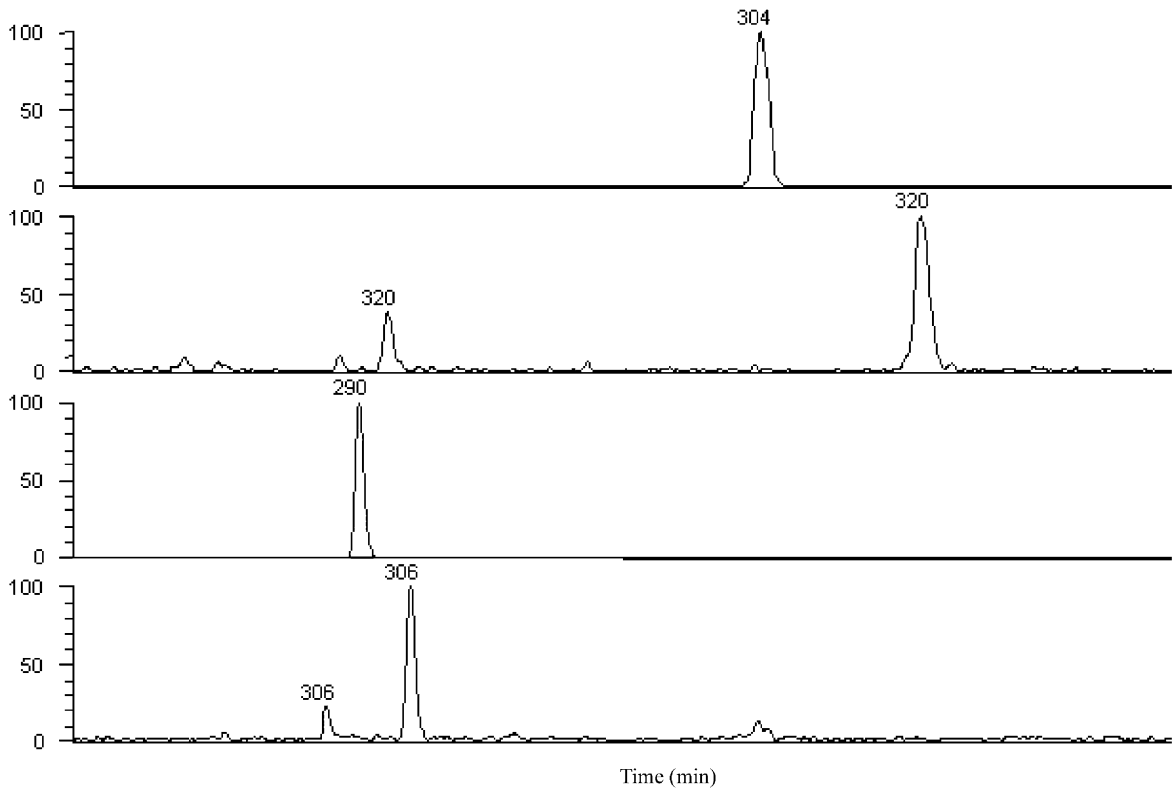

Fig. 4. Chromatographic run of the sample at 22 min of degradation displayed at the different *m*/*z* values.

directing, and is the part of the molecule containing the negative charge which is mainly involved in the fragmentation process.

The introduction of the second hydroxyl group in the ring containing the SO_3^- group is supported by the absence of the fragmentation pathway observed for the monohydroxylated species. As a matter of fact a different fragmentation product is formed whose proposed structure presented in Fig. 3 is in accordance with the electron withdrawing and *meta* orientating properties of the SO_3^- group.

In conclusion such a very peculiar chromatographic behavior seems to depend on the structure of the species involved and is controlled by the more or less availability of the interactions between the mobile phase and the nitrogen-containing moieties of the molecules which, together with the SO_3^- group, are mainly responsible of the chromatographic behavior.

As a further support of the formation of hydroxylated species we can bring forward the experimental evidence provided by the profile of the UV spectrum obtained for each peak by diode array detection. In Fig. 5 are reported, in the sake of example, the UV spectra for the species of *m*/*z* 290, 306 and 322. As it can be seen there is a spectrophotometric evidence we can consider a confirmation of the formation of hydroxylated degradation products, that is a bathochromic shift of the main absorption maximum of these species in respect to the non-hydroxylated homologous.

As regards the degradation mechanisms, it is confirmed that till to 27 min there is a substantial presence of species retaining the chromophore group. At 37 min there is little evidence by UV detection of the presence of such species and the chromatographic run shows the presence of early eluting species having spectral evidences completely different. However, the

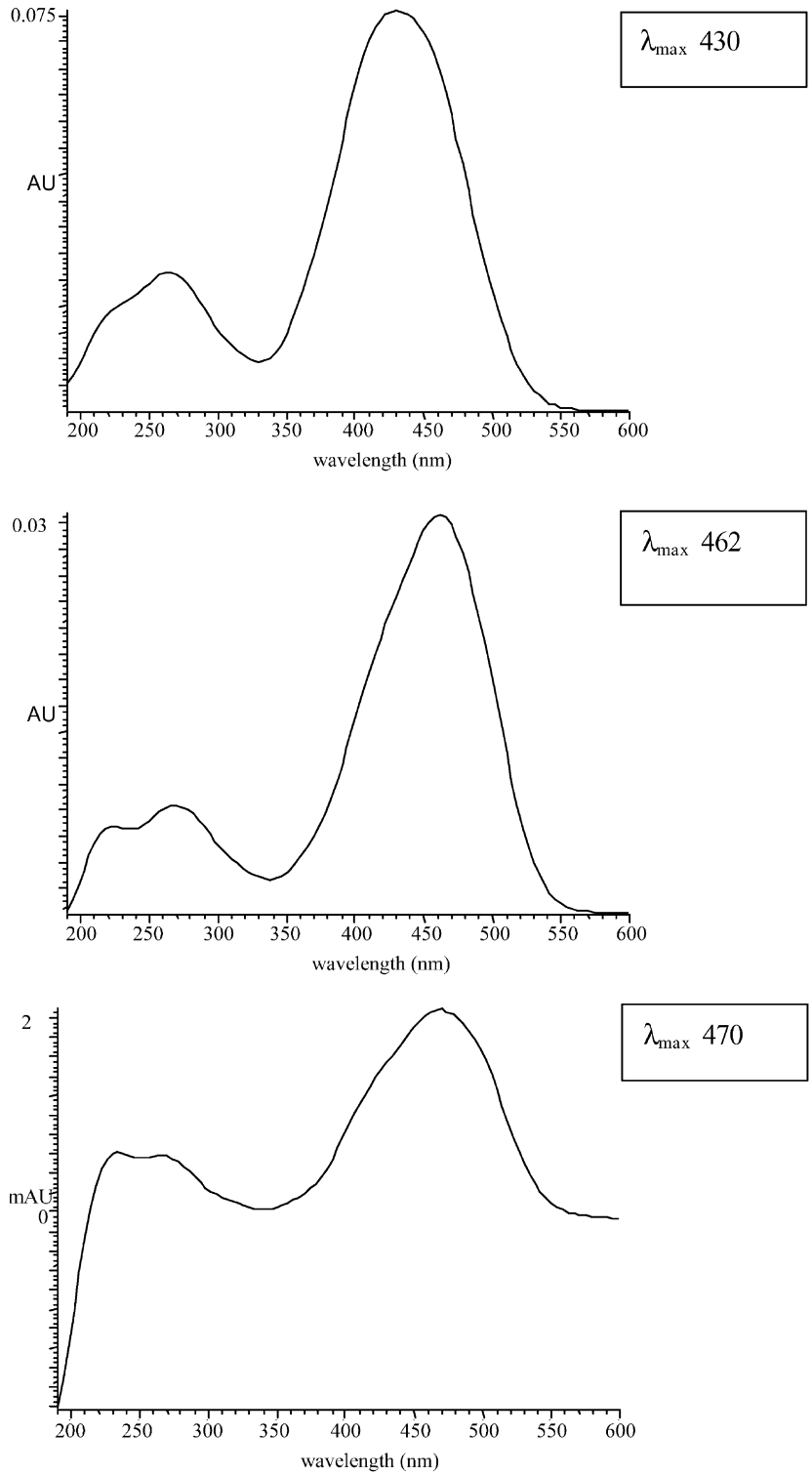

Fig. 5. UV spectra for the species of *m*/*z* 290, 306 and 322.

corresponding analysis by the more sensitive MS detection shows that some species are yet present though at very lower concentrations.

4. Conclusions

The results obtained confirm that HPLC-MS is a suitable technique when hydrophilic molecules present in aqueous matrices have to be separated and characterized. The structural information about the degradation intermediates achieved from MS and MS^2 studies were quite compatible with the photocatalytic degradation steps already reported in literature for other molecules, in particular the introduction of OH groups to aromatic rings. By combining different analytical evidences it was possible to achieve further support to the presence of OH groups inserted in the molecule structure but it is worth noticing that only MS/MS data allowed to make a reasonable proposal of their addition positions.

Acknowledgements

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References

[1] H. Zollinger, Color Chemistry: Synthesis, Properties and Applications of Organic Dyes and Pigments, VCH Publishers, New York, 1987.
[2] D.F. Ollis, E. Pelizzetti, N. Serpone, in: N. Serpone, E. Pelizzetti (Eds.), Photocatalysis Fundamentals and Applications, Wiley, New York, 1989, Chapter 18, p. 603.
[3] D.F. Ollis, H. Al-Ekabi, Photocatalytic Purification and Treatment of Water and Air, Elsevier, Amsterdam, 1993.
[4] M.R. Hoffmann, S.T. Martin, W. Choi, D.W. Bahnemann, Chem. Rev. 95 (1995) 69.
[5] P.V. Kamat, Chem. Rev. 93 (1993) 267.
[6] K. Vinodgopal, I. Bedja, S. Hotchandani, P.V. Kamat, Langmuir 10 (1994) 1767.
[7] C. Nasr, K. Vinodgopal, S. Hotchandani, A.K. Chattopadhyay, P.V. Kamat, Res. Chem. Intermed. 23 (1997) 219.
[8] K. Tanaka, K.K. Padermpole, T. Hisanaga, Water Res. 34 (2000) 327.
[9] H. Chun, W. Yizhong, Chemosphere 39 (1999) 2107.
[10] C. Zhu, L. Wang, L. Kong, X. Yang, L. Wang, S. Zheng, F. Chen, F. Mai Zhi, H. Zong, Chemosphere 41 (2000) 303.
[11] W. Feng, D. Nansheng, H. Helin, Chemosphere 41 (2000) 1233.
[12] F. Herrera, A. Lopez, G. Mascolo, P. Albers, J. Kiwi, Appl. Catal. B: Environ. 29 (2001) 147.
[13] L.C. Chen, T.C. Chou, J. Mol. Catal. 85 (1993) 201.
[14] Y. Wang, Chemosphere 34 (2000) 990.
[15] J. Riu, I. Schönsee, D. Barceló, C. Ràfols, Trends Anal. Chem. 16 (1997) 405, and references therein.
[16] C. Ràfols, D. Barceló, J. Chromatogr. A 777 (1997) 177.
[17] E. Pramauro, et al., unpublished results.