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HPLC-DAD-MSⁿ to investigate the photodegradation pathway of nicosulfuron in aqueous solution

M. Benzi · E. Robotti · V. Gianotti

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Abstract The environmental interest of sulfonylurea herbicides was derived from the possibility of diffusion and penetration of these herbicides in the deepest layers of the ground, in particular in sandy or clay-poor soils, up to the ground waters; another interest of the study is their natural degradation pathway which leads to the formation of new species that are potentially more toxic and stable than the precursor herbicides. In this case, a lower persistence in the environment unfortunately does not correspond to a lower toxicity: hence, the importance of the identification of the species can be potentially formed. Here, nicosulfuron, a typical sulfonylurea herbicide, is considered in order to outline the environmental fate of the molecules generating from the simulation of one of the natural processes that can occur, i.e. photoinduced degradation. Aqueous nicosulfuron solutions underwent a simulated sun irradiation: the new species formed during the degradation process were identified by HPLC-DAD-MS/MS and a degradation pathway was proposed. The effect of temperature and the contribution of the hydrolysis were also evaluated. The use of ESI in both positive ion (PI) and negative ion (NI) mode and APCI in PI mode permits to obtain integrated information about the transformation products that can form; moreover, a study of the total ion chromatogram followed by the extraction of the SIM chromatograms of the most intense *m/z* signals made possible the identification of five possible photodegradation transformation products.

Keywords Photoinduced degradation · Sulfonylurea herbicides · Degradation pathway · ESI-MS/MS · APCI-MS/MS

Introduction

Sulfonylurea herbicides are used to control a variety of broad-leaved weeds and grasses in cereals and other row crops and for industrial weed control. They can be considered as “new formulation pesticides” because of their high selectivity and low persistence in the environment [1]. Among this class of pesticides, nicosulfuron, thifensulfuron-methyl, amidosulfuron, azimsulfuron, tribenuron-methyl and rimsulfuron are the most employed formulations.

The environmental interest in sulfonylurea herbicides was derived mainly from the possibility of herbicide diffusion and penetration in the deepest layers of the ground, in particular in sandy or clay-poor soils, up to the ground waters (their sorption depends on different factors, among which are soil pH, soil texture, temperature, etc.). However, another aspect has to be carefully considered: their natural degradation that can bring about the formation of new species potentially more toxic and stable than the precursor herbicides. In this last case, a lower persistence in the environment unfortunately does not correspond to a lower risk [2–6]. Hence, the importance of the identification of the species can be derived from natural degradation.

The natural degradation of sulfonylurea herbicides is already widely being studied especially from the points of view of the hydrolysis [1, 7, 8] and microbial processes that mainly occurs in soil [9–11]; fewer studies regard the photodegradation reaction, both natural [2–4, 6, 12–16] and catalysed by TiO₂ [17–20]. The understanding of the way

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M. Benzi · E. Robotti · V. Gianotti (✉)
DISAV Dipartimento di Scienze dell'Ambiente e della Vita,
University of Piemonte Orientale “Amedeo Avogadro”,
Viale T. Michel 11,
15121 Alessandria, Italy
e-mail: gianotti@mfn.unipmn.it

degradation occurs by solar irradiation is important since the reaction involved can give, if compared with hydrolysis, more complicated degradation pathways: this is due in particular to the more energetic reactions involved that can promote bond breakings.

In this study, nicosulfuron (NICO) is considered (Fig. 1a); it represents a typical sulfonylurea herbicide and one of the most employed new generation herbicides in crop protection. The final aim is to outline the fate of nicosulfuron molecules in the environment simulating one of the natural processes that may occur, i.e. photoinduced degradation. According to the classification by Sarmah et al. [1], NICO is one of the members of *class c* herbicides characterised by the presence in the molecule of both the pyridinic and pyrimidinic rings and by a pK_a value of 4.6.

Aqueous solutions of this herbicide underwent a simulated solar irradiation; high-performance liquid chromatography with photodiode array detection mass spectrometry

(HPLC-DAD-MS)/MS was used to identify the new species formed during the degradation process and to propose a degradation pathway. The temperature effect, the contribution of the hydrolysis and the toxicity were evaluated as well.

Experimental

Reagents

Nicosulfuron 95% was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Standard stock solutions were prepared dissolving 5.00 mg of nico in 1.00 L of ultrapure water and 100.00 mg in 1.00 L of acetonitrile. All solutions were prepared by class A glassware and smaller volumes were picked up by 500 μ l micropipettes and were kept at dark at a temperature of -20 °C.

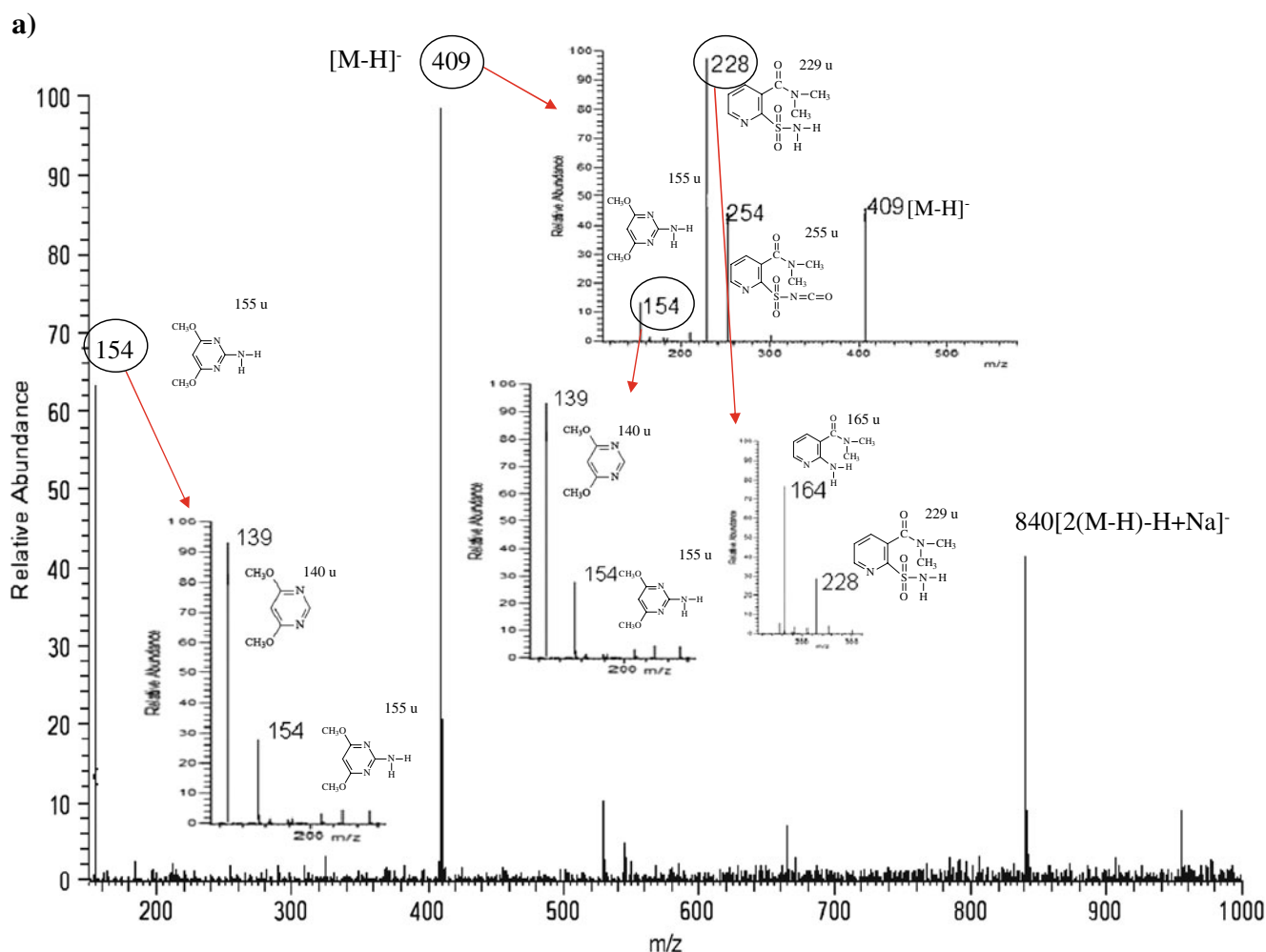


Fig. 1 MS spectra obtained in the characterisation study. **a** ESI-negative ion mode. **b** ESI-positive ion mode. **c** APCI-negative ion mode

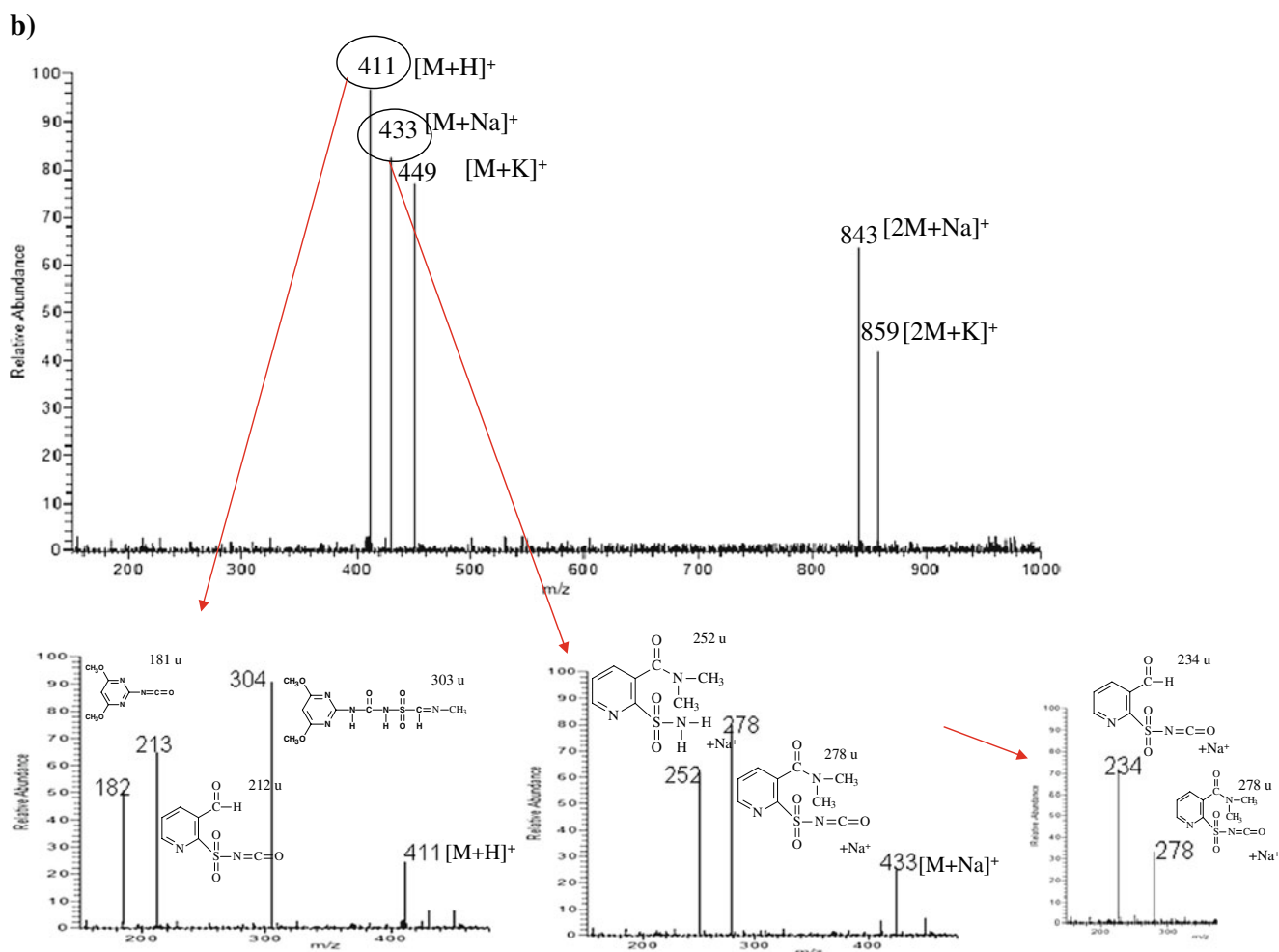


Fig. 1 (continued)

HPLC grade acetonitrile (99.8%) and glacial acetic acid (99.8%) were purchased from Merck (Darmstadt, Germany); ultrapure water was produced by a Millipore Milli-Q system (Milford, MA).

Apparatus

HPLC-DAD-MS/MS analyses were carried out by a Thermo Finnigan Mat Spectra System (Finnigan, San Jose, CA) equipped with a Degasser SCM1000, a gradient pump Spectra System P4000, an Autosampler Spectra System AS3000, interfaced by the module SN4000 to a Spectra System UV6000LP diode array detector (190–700 nm) and to a mass spectrometer detector Finnigan LCQ Duo, equipped with electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) ion sources and with ion trap analyser.

A CoFoMeGra Solar box 3000e (Milan, Italy) was used to simulate sunlight irradiation.

UV–vis analyses were performed by a Jasco V-550 spectrophotometer (Tokyo, Japan) equipped with Spectra

Manager for Windows 95/NT Version 1.53.00 by Jasco Corporation.

HPLC-DAD-MS/MS conditions

The chromatographic conditions derive from a method previously developed in our laboratories for the separation of a mixture of sulfonylurea herbicides [21].

The stationary phase was an endcapped Lichrospher column RP-18 (5 μ m, 250 \times 4 mm i.d.) with a 15 \times 4 mm Lichrospher RP-18 (5 μ m) guard precolumn (Merck, Darmstadt, Germany). The mobile phase was a mixture 55/45% v/v of acetonitrile and acidified water (0.05% v/v of acetic acid), at a flow rate of 0.5 mL min⁻¹. DAD acquisitions were performed in the range 200–600 nm.

Mass spectrometry conditions for both ESI and APCI analyses are reported in the Electronic supplementary material (Table S1), and the full scan data acquisitions were made in the m/z range of 50–1,000 with three microscans and an inlet time of 50 ms. High purity nitrogen

c)

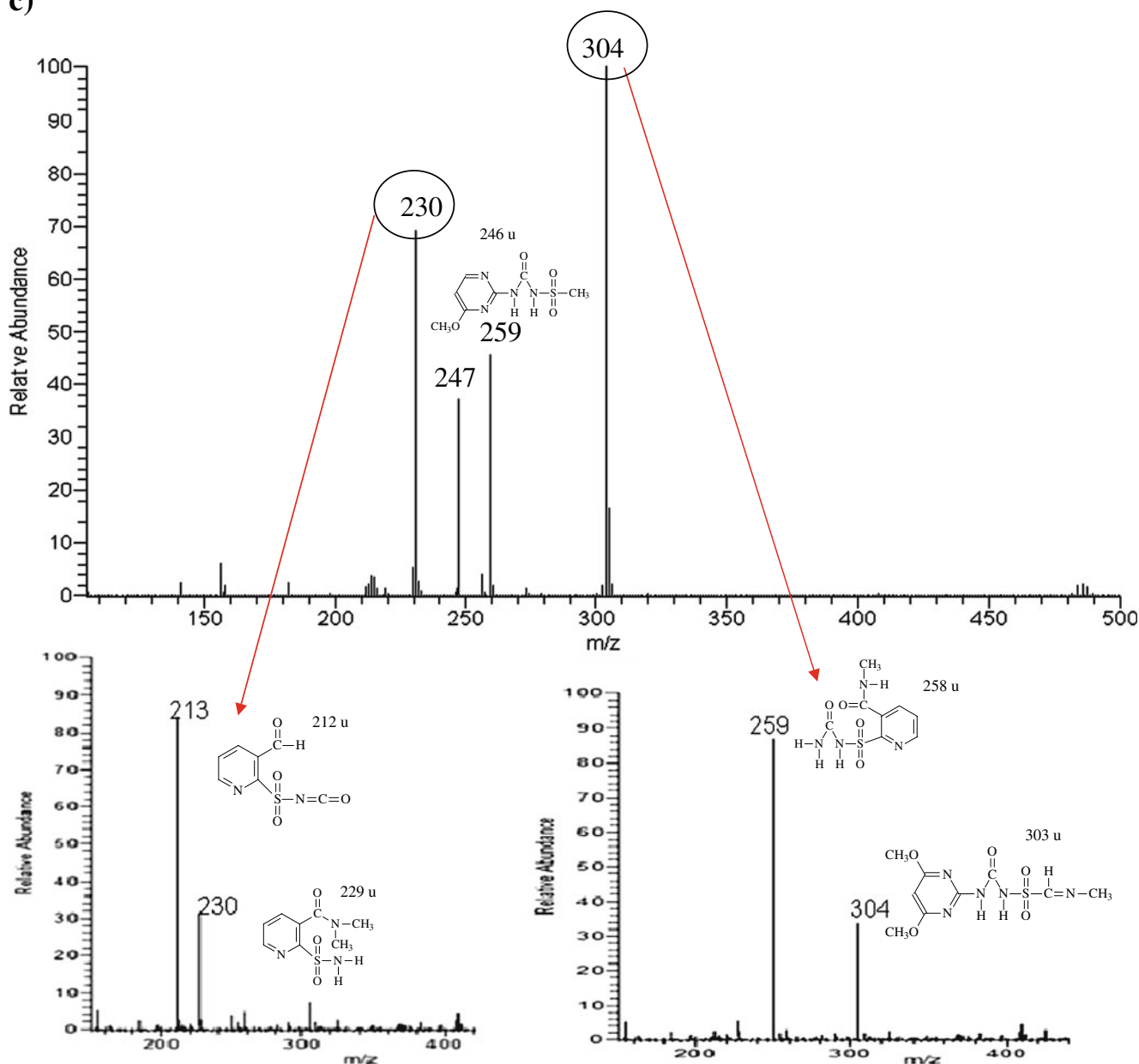


Fig. 1 (continued)

gas was used as nebuliser and helium (>99.999%) was used as quenching agent.

UV-vis spectroscopy conditions

Spectroscopic analyses were performed in the range 190–800 nm using Milli Q water as reference solution.

Solar box irradiation conditions

A 10.00 mL of 5.00 mg L⁻¹ herbicide solution in ultrapure water were introduced in quartz cells and exposed to solar

box irradiation for fixed exposure times (0, 24, 48, 120, 168, 240, 288, 360, 432, 480, 600, 672 and 816 h). The instrumental conditions were chosen simulating the average sun irradiation and temperature in the period from May to September, as monitored by the meteorological station of the DISTA (Department of Advanced Sciences and Technologies of the University of Eastern Piedmont). The density of radiation of the xenon lamp was set at 260 W m⁻² and temperature at 30 °C. The filter for light radiation employed was an outdoor UV filter. These conditions were the result of a study, carried out in our laboratory, that compared experiments performed on solutions irradiated

with sunlight and with the solar box. The results are similar for sunlight irradiation and irradiation by the solar box, but the last one is better since it is characterised by a constant intensity and is continuously available.

Hydrolytic degradation and temperature effect study: experimental conditions

A 10.00 mL of 5.00 mg L⁻¹ herbicide solution in ultrapure water were introduced in quartz cells protected by an aluminium foil and put: (1) at dark and at a temperature of 21±1 °C for the evaluation of the hydrolysis contribution to the degradation; (2) at dark and at a temperature of 35±1 and 70±1 °C to evaluate the effect of temperature. The temperature control was obtained by a thermostatic bath.

Chemical oxygen demand evaluation

The chemical oxygen demand (COD) values were evaluated by the ISO6060:1989 regulation [22] on the aqueous NICO solution at an initial concentration of 5.00 mg L⁻¹ both photoirradiated and preserved at dark at different increasing times (from 0 to 34 days).

Ecotoxicological tests

The acute toxicity test by *Vibrio fischeri* has been performed on NICO solutions dissolved in ultrapure water at an initial concentration of 5.00 mg L⁻¹ both not irradiated and irradiated by the solar box for 600 min (a time greater than $t_{1/2}$); each experiment was repeated three times.

V. fischeri is a bioluminescent gram-negative bacterium living in sea waters: the monitored effect consists in the decrease of bioluminescence due to the inhibition of *luciferase*. The luminescence is recorded after 15 min of incubation at 15 °C [23]. This test ensures repeatability, accuracy and sensitivity [24]. The toxic effect depends on the pollutant concentration and is due to the interaction between the molecular structure of the pollutant and the bioindicator or specific parts of its organism, when they are put in contact for prefixed times. Since bacterial bioluminescence is directly related to cellular respiration, a decrease in luminescence indicates a cellular toxicity.

Results and discussion

Mass characterisation of the analytes

In order to optimise instrumental sensitivity in mass spectrometric analyses and correctly identify and characterise the possibly formed photodegradation products, frag-

mentation patterns were built by direct infusion in the mass spectrometer of aqueous solutions of the analyte at 5.00 mg L⁻¹. Fragmentation patterns were built using both the ESI and the APCI ion sources.

Electronic supplementary material (Table S2) and Fig. 1a–c report the results of ESI and APCI characterization for NICO; infusion experiments were performed using both positive (PI) and negative ion (NI) mode both for ESI and APCI characterisation. Since the APCI in negative ion mode resulted inapplicable due to a too low sensitivity, the results obtained by this characterisation will not be presented.

The ESI analysis of NICO shows, both in PI and in NI mode (Fig. 1 a,b), the presence of the pseudo-molecular ion at m/z 411 ($[M+H]^+$) and 409 ($[M-H]^-$), respectively. Moreover, in PI mode, the signals at m/z 433 and 449 can be found, attributed to the sodium and potassium adducts ($[M+Na]^+$ and $[M+K]^+$, respectively) of the pseudo-molecular ions, as well as the signals at m/z 843 and 859, attributed to the sodium and potassium adducts of the dimeric form of the pseudo-molecular ion ($[2M+Na]^+$ and $[2M+K]^+$). In NI mode, the signal at 840 m/z was found, attributed again to the sodium adduct of the dimeric form of the pseudo-molecular ion.

In positive ion mode the MS/MS experiment on the pseudo-molecular ion (m/z 411) gives the fragments at m/z 304, 213 and 182 (Fig. 1a). The m/z 182 fragment derives from the cleavage of the C–N bond of the sulfonylureic bridge; the fragment at m/z 304 derives from the opening of the pyridinic ring and the m/z 213 fragment derives from the cleavage of the C–N bond of the sulfonylureic bridge and from the loss of the N–CH₂–CH₃ group.

The MS/MS experiment performed on the sodium adduct (m/z 433) gives the fragments at m/z 278 and 252. The signal at m/z 278 corresponds to an adduct with Na⁺ (Fig. 1a) and deriving from the cleavage of the C–N bond of the sulfonylureic bridge that after a further fragmentation (MS³) leads to the loss of the group N–CH₂–CH₃ with the formation of the fragment at m/z 234.

The potassium adduct (m/z 449) in the MS/MS experiment gives the fragment at m/z 268 that can be ascribed to the same molecules corresponding at the signal at m/z 252 already described, with the substitution of the sodium by the potassium.

The MS/MS experiments in NI mode (Fig. 1b) performed on the pseudo-molecular ion (m/z 409) give three fragments at m/z 228, 254 and 154; the signal m/z 254 can be attributed to the structure reported in Fig. 1b that, by the further loss of –C=O group, gives the signal at m/z 228; both the fragments are confirmed by the MS³ experiment, giving a fragment at m/z 164.

The fragment at m/z 154 can be assigned to structure reported in Fig. 1b, confirmed by MS³ experiment giving the signal at m/z 139 for a loss of a –NH₂ group.

The analysis performed with the APCI interface shows that in PI mode the most intense signals were found at m/z 304, 259, 247 and 230. In Fig. 1c the structural assignments are reported. In particular, the signals at m/z 304 and at m/z 230 correspond to the molecules already found in ESI characterisation; the signal at m/z 259 derives from the loss of a $-CH_3$ group on the pyridinic ring and the loss of the pyrimidinic portion of the molecule. The signal at m/z 247 derives from the loss of the pyridinic ring and of the groups $-NCH_3$ and $-OCH_3$.

Solubility and calibration curves

Literature reports that solubility for NICO in water at 25.0 °C is 0.07 g L⁻¹ [25]; however, the standard solution could not be prepared at this concentration since an evident turbidity of the solution was observed. The maximum solubility of the analyte in ultrapure water was evaluated by comparison of the chromatographic peak of the aqueous solution with the calibration curve of the analyte built on the solutions prepared in acetonitrile (in the range of 0.50–10.00 mg L⁻¹) in which NICO is completely soluble: the analyte proved to be completely soluble in ultrapure water up to a concentration level of 5.00 mg L⁻¹. The calibration curve of NICO in ultrapure water was therefore built in the concentration range 0.05–5.00 mg L⁻¹ using the peak area of the extracted chromatogram at 240 nm from DAD detector data. The calibration curve ($y = 7.6010^4 + 3.1910^5x$) results were satisfactory since the R^2 value obtained was 0.999. The model resulted significant also by ANOVA: the sum of squares (SS) for the residuals equals 1.65 10⁹ while the SS of the regression equals 3.90E-12 (F -Fisher_{calculated}=3.1E-4 while $F_{tab, 1, 13, 95\%}$ =4.67). No lack of fit was detected. This calibration curve was used to quantify NICO during degradation, i.e. the extent of the degradation process.

Preliminary analyses by UV–vis spectrophotometry

To obtain a first evidence of the degradation of the analyte at the experimental conditions considered, UV–vis absorbance spectra at different exposure times (from 0 to 34 days) were measured in the range from 190 to 600 nm. The UV–vis spectra of NICO are reported in Fig. 2a: the spectrum before the irradiation (0 min) shows the presence of one maximum of absorbance at 240 nm that disappears after 14 days of photoirradiation and a band maximum at 255 nm that shifts at 267 nm during the same irradiation time. After 27 days of irradiation, the intensity of the new band increases and a new band appears at 215 nm, continuously increasing in intensity up to 34 days of degradation. The results point out that degradation occurs and degradation products are formed.

Preliminary UV spectroscopy analyses performed also on the solution exposed at temperatures of 35 and 70 °C;

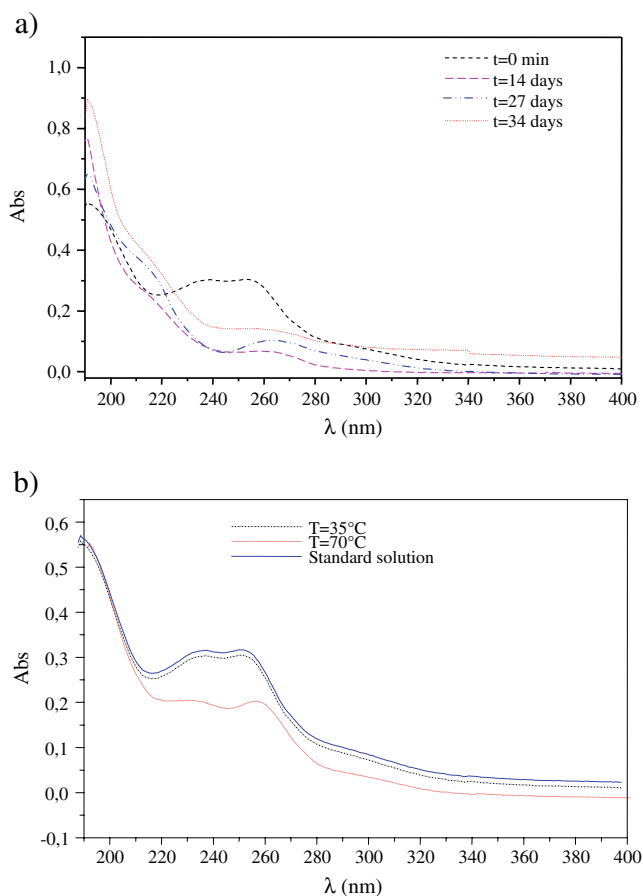


Fig. 2 UV–vis spectra of a nicosulfuron aqueous solution (5.0 mg L⁻¹): **a** after 0, 14, 27 and 34 days of photoirradiation and **b** after 15 days at dark and at different temperature

after 15 days (360 h) the spectra shown in Fig. 2b indicate that an appreciable effect of the temperature is registered at 70.0 °C. These results suggest the importance of a strict control of the temperature during the irradiation experiments performed in the solar-box to avoid the synergistic effect of temperature and photoirradiation.

Kinetics of the photodegradation experiments

The photodegradation of NICO was studied by HPLC-DAD-MS/MS analyses on the ultrapure water herbicide solutions photoirradiated in a solar box for a maximum of 34 days (816 h).

At regular time intervals the remaining concentration of the analyte in the photoirradiated solution was evaluated by its chromatographic peak area. After about 8 days, the concentration of the analyte was considerably reduced.

The concentration C of the analyte was calculated by the calibration curve after a photo irradiation of: 0, 24, 48, 120, 168, 240, 288, 360, 432, 480, 600, 672 and 816 h.

In order to calculate the kinetic order of the photodegradation reaction, two different functions of the concen-

tration of the analyte were plotted as a function of time: $\ln C$ (natural logarithm of the concentration) and $1/C$ (inverse of the concentration). The best linear interpolation was obtained using $\ln C$ (Fig. 3) indicating that the degradation process follows a first order reaction with a resulting $t_{1/2}$ value of 115 h and a kinetic constant of 0.006 h^{-1} .

Photodegradation study

Aliquots of 1.00 mL of NICO solutions used for the photodegradation study were submitted to HPLC-DAD-MS/MS analyses.

The use of ESI and APCI in both PI and NI mode permits to obtain integrated information about the transformation products that can form; moreover, a study of the chromatogram acquired in total ion current (TIC) followed by the extraction of the selected ion monitoring chromatograms (Fig. 4) of the most intense m/z signals have made it possible the identification of five possible photodegradation transformation products (afterwards indicated as *M1*, *M2*, *M3*, *M4* and *M5*). In order to sum up all the information collected, Fig. 5 shows the entire pathway of NICO photodegradation, proposed together with the structures associated to the fragments obtained in the MSⁿ experiments.

The proposed structures were obtained using the molecular masses identified in the mass spectra by the pseudo-molecular ions according to the Nitrogen rule, the UV-vis data collected by the DAD detector and the spectrophotometric analyses; when possible, the structures proposed were confirmed by the MSⁿ experiments performed by the direct infusion of the degraded solutions in the mass analyser.

The transformation product *M1* can be detected in HPLC-NI ESI chromatograms (Fig. 4a) as a peak with retention time of 4.8 min by the extraction of the m/z 313; after 6 days of irradiation the peak is well defined and its area increases with the irradiation time. The proposed structure derives from the opening of the pyrimidinic ring.

M2 transformation product corresponds to m/z 369 and is detected by the extraction of this signal in HPLC-PI ESI chromatograms as a peak at retention time of 5.3 min (Fig. 4b): this peak is already present in the NICO solution not irradiated but its area increases along with the

degradation time. This transformation product seems to derive from an impurity already present in the standard powder at a low concentration and increases during photodegradation. The proposed structure that derives from the loss of the two methyl groups on the substituents of the pyridinic ring and the methyl group on the pyrimidinic ring was confirmed by the fragment obtained in the MS/MS experiment characterised by the m/z 325 due to the further loss of $-\text{NH}_2$ and $-\text{OCH}_3$ units.

Another possible transformation product (*M3*), co-eluting with *M2*, is evidenced both in PI/NI ESI (Fig. 4c) and in PI APCI (Fig. 4d) chromatograms by the extraction, from the TIC, of the m/z values corresponding to 229 u. The corresponding peak forms starting from 24 h of irradiation and increases its intensity along with degradation. It can be pointed out that the proposed structure is the same as the signal corresponding to 229 u already found in the APCI characterisation study: the MS/MS experiments performed on both the initial solution and on the photo-irradiated one show the same fragment at 212 u.

A signal at m/z 259 in PI APCI chromatograms (Fig. 4e) was also found only in the samples collected after 24 and 48 h of irradiation and corresponds to the fragment already found in the characterisation study (molecule F in Fig. 1b), ascribed to an intermediate (*I1*). This molecule can be likely the precursor of the transformation product *M3* previously described since the disappearance of *I1* coincides with the formation of *M3* and the conversion implies only the loss of $-\text{NH}_2$ and $-\text{CH}_3$ groups.

Another possible transformation product (*M4*) was found both in NI/PI ESI and in PI APCI chromatograms (Fig. 4f) by the extraction of the m/z values corresponding to 155 u. A peak at retention time of 11.3 min that is visible after 24 h of irradiation and increases the intensity along with the photoirradiation time is obtained. The structure already proposed in the characterisation (Fig. 2c) is also confirmed by the MS/MS experiment that shows the formation of the fragment at 99 m/z compatible with the mentioned assignment (Fig. 5).

Finally, a peak at retention time of 24.7 min was observed in the NI ESI chromatograms (Fig. 4g) starting from about 18 days of photoirradiation; the area of this peak increases for about 10 days and then remains constant. The peak is characterised by an m/z value of 293 that in the MS/MS fragmentation experiment leads to the formation of two fragments at 222 and 237 u. The hypothesised structure for the corresponding transformation product (*M5*) derives from the loss of the pyridinic ring and the addition of a $-\text{OCH}_3$ group.

In order to evaluate if and how degradation occurs in absence of photoirradiation, the study was conducted also on NICO solutions at 5.00 mg L^{-1} , obtained starting from

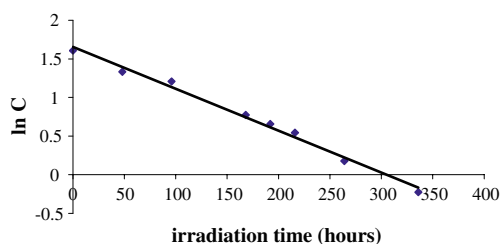


Fig. 3 Logarithmic decay for the photodegradation of nicosulfuron

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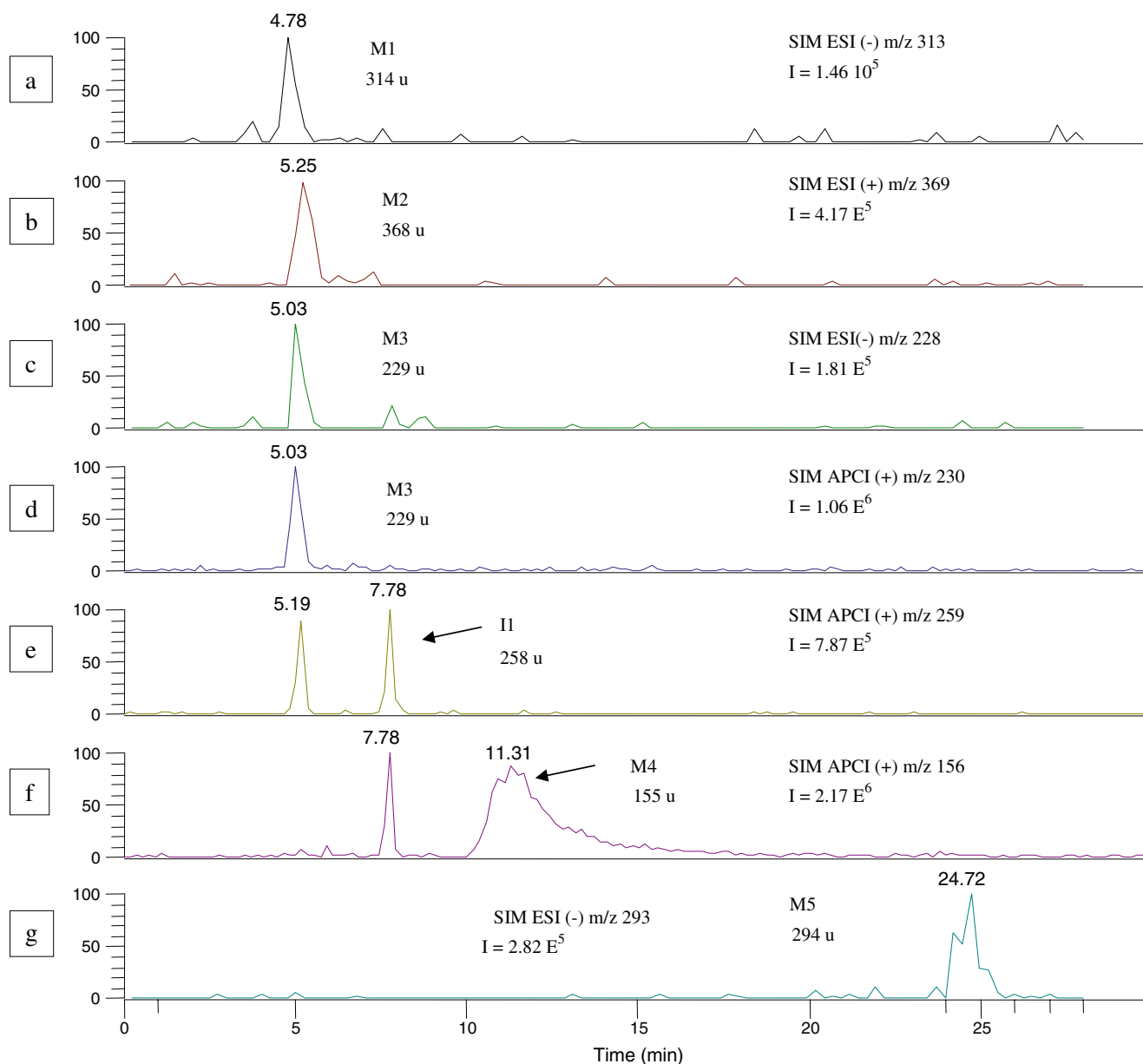


Fig. 4 HPLC-MS chromatograms extracted from the TIC in SIM mode of the transformation products (*M1–M5*) and the impurity identified (*I1*)

the same mother solution, preserved at dark at room temperature.

The HPLC-DAD-MS/MS analysis performed on the aliquots collected in the same time intervals of the photodegradation experiment shows that the hydrolysis does not occur significantly; only after 4 months of dark preservation a 10% of the analyte degrades and the formation of the hydrolysis transformation product *M4* (Figs. 4f and 5) already found in the photodegradation experiments is observed; the hypothesized structure was found also in literature [16, 26] where it is confirmed as a hydrolysis product.

Moreover, the COD values evaluated on the NICO solution both underwent to photoirradiation and preserved in the dark confirmed that a complete mineralisation is not achieved: about 20% and 90%, respectively, of the initial oxidable species remain after 34 days.

Determination of toxicity

Ecotoxicological tests were performed by *V. fischeri* on both not irradiated and photoirradiated solutions (at concentration of 5.00 mg/L) to obtain I% values that indicate the percentage of inhibition induced by the

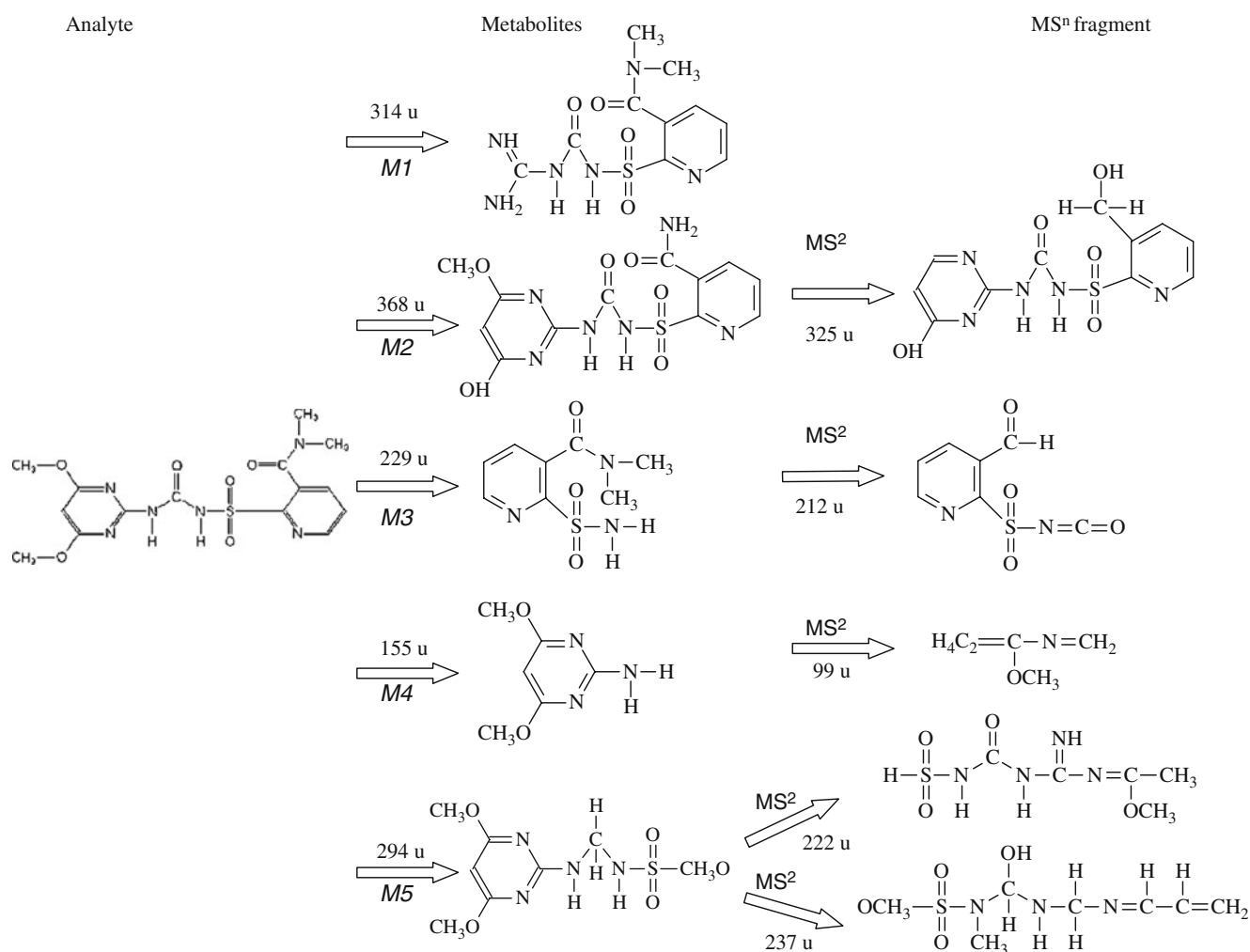


Fig. 5 Proposed structures and MS/MS characterisation of the transformation products of the nicosulfuron photodegradation

chemicals on the activity of the organisms: I% ranging between 0 and 20 corresponds to a not toxic sample; the sample is weakly toxic for values between 20 and 50 and toxic for values greater than 50.

In the case of a previous study on a sulfonylureic pesticide [15] all the tests performed indicated that the degradation products were not more toxic than the herbicide itself; the photoirradiated solution of chloroaniline instead resulted more toxic than the non-photoirradiated one [27].

In our study, the average values of I% obtained are 18.4 ± 0.6 and 43.2 ± 0.8 for the non-photoirradiated solution and the photoirradiated one, respectively. The results indicate that the pesticide solution at the initial concentration seems not particularly toxic, but toxicity increase when it is irradiated.

Conclusions

Aqueous nicosulfuron solutions underwent simulated sun irradiation: the new species formed during the degradation

process were identified by HPLC-DAD-MS/MS and the integrated use of ESI and APCI both in PI and in NI mode and the MS/MS fragmentation study has made it possible to find the presence of five transformation products.

The structures of the transformation products can only be hypothesised since their confirmation can be obtained only by a NMR study or by the synthesis of the intermediates. The structures presented derive substantially from the loss of the rings substituents, bond cleavages in the sulfonylureic bridge and opening of the pyridinic and pyrimidinic rings itself, as generally reported in literature about photodegradation studies performed on other sulfonylureic pesticides [15–18]. In particular, only the work by Halle et al. [16] is performed on nicosulfuron directly photodegraded on a simulated cuticular wax film: some transformation products found by Halle et al. correspond to those identified in the present study while the others present similar structural modifications.

Only in the case of the transformation product M5, the addition of a radical is hypothesised (radical·OCH₃)

whereas many examples are reported in literature about radical ($\cdot\text{OCH}_3$, $\cdot\text{OH}$, ...) addition to organic molecules undergone photoirradiation in aqueous solution [27–29]. This fact was also reflected in the values obtained from the toxicological test which indicated an increase of the toxicity in the photoirradiated solution but do not reach the I%, >50 as in the case of more substituted photoproducts [27]. Moreover, the contribution of the hydrolysis during the photodegradation was evaluated and resulted very small since only after 4 months of dark preservation a 10% of the analyte degraded. The hydrolysis transformation product was in any case identified as the *M4* transformation product.

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