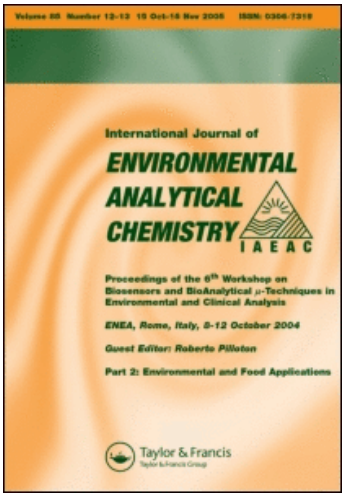

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Application of coupled liquid chromatography-mass spectrometry in hydrolysis studies of the herbicide ethametsulfuron-methyl

Youbin Si[a]; Ligan Zhang[a]; Kazuhiro Takagi[b]
[a] College of Resource and Environmental Science, Anhui Agricultural University, Hefei 230036, China [b] Department of Environmental Chemistry, National Institute for Agro-Environmental Sciences, Ibaraki 305-8604, Japan

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Application of coupled liquid chromatography–mass spectrometry in hydrolysis studies of the herbicide ethametsulfuron-methyl

YOUBIN SI[†]*, LIGAN ZHANG[†] and KAZUHIRO TAKAGI[‡]

[†]College of Resource and Environmental Science, Anhui Agricultural University, Hefei 230036, China
[‡]Department of Environmental Chemistry, National Institute for Agro-Environmental Sciences, Ibaraki 305-8604, Japan

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The hydrolysis of the sulfonylurea herbicide ethametsulfuron-methyl [methyl 2-[[[[(4-ethoxy-6-methylamino-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate] was studied in aqueous buffers of different pH values. The reaction was first-order and pH-dependent. Ethametsulfuron-methyl was more persistent in neutral or weakly basic than in acidic solution. Eleven degradation products were detected and tentatively identified by LC/MS/MS analysis. At all pH values studied, the primary pathway of degradation was the cleavage of the sulfonylurea bridge. However, minor degradation pathways have also been observed, such as *O*-de-ethylation, *N*-demethylation, and opening of the triazine ring.

Keywords: Ethametsulfuron-methyl; Hydrolysis; Metabolites; LC/MS/MS

1. Introduction

Recently, an increasing number of pesticides entering the environment have become a major concern. In fact, any decision concerning environmental protection and pollution control is dependent on our ability to identify and measure the xenobiotic materials in the ecosystem. One of the most important aspects in our understanding of the fate of pesticides in the environment is the knowledge of their degradation mechanism(s). Such degradation processes may lead to the formation of new chemicals with reduced toxicity or, in some cases, increased toxicity, to aquatic biota. For example, several metabolites of the organophosphate and carbamate insecticides are more toxic than the parent compound, especially to fish [1,2]. Knowledge of hydrolytic degradation pathways and kinetics in the normal pH

*Corresponding author. Fax: + 86-551-5120833. E-mail: ybsi2002@yahoo.com.cn

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range of the aquatic environment (5.5–8.0) is crucial in the prediction of the fate and transport behavior of chemicals [3].

Sulfonylurea herbicides are a relatively new class of herbicides, which are characterized by high selectivity and herbicidal activity at very low application rates (2−75 g of active ingredient ha^{-1}) [4]. They can undergo chemical and/or biological degradation. The main mechanisms of transformation are considered to be chemical hydrolysis and microbial degradation, the former being of utmost important for the environmental dissipation of these herbicides [5].

The low use rate and extensive breakdown of the sulfonylurea herbicides requires very sensitive analytical techniques for the identification of their environmental metabolites, since only very small quantities of metabolites are isolated [6]. Direct determination of sulfonylurea herbicides by gas chromatography (GC)/mass spectrometry (MS) has not yet been possible due to their polarity and thermal instability. Derivatization prior to GC/MS has been proven to be both unfeasible and time-consuming for all sulfonylurea herbicides. The method of choice for identification of sulfonylurea herbicide metabolites is liquid chromatography (LC)/MS [7−10]. Since these compounds are temperature-sensitive, their mass spectra typically show very weak or no molecular ions with electron ionization, chemical ionization, or thermospray ionization when the thermospray vaporizer temperature is set for optimum sensitivity [11,12]. A low-temperature mass spectral ionization technique, such as fast-atom bombardment (FAB) or electrospray ionization (ESI), is needed to obtain prominent molecular ions for unequivocal molecular-weight assignments. Reiser *et al.* have shown LC/FAB-MS to be a very powerful analytical method for the identification of unknown sulfonylurea herbicide metabolites [13].

Ethametsulfuron-methyl [methyl 2-[[[[(4-ethoxy-6-methylamino-1,3,5-triazin-2-yl)-amino]carbonyl]amino]sulfonyl]benzoate] is a widely applied sulfonylurea herbicide for post-emergence weed control in oilseed rape (*Brassica napus* L.) [14,15]. Like other sulfonylurea herbicides, it inhibits the enzyme acetolactate synthase (ALS) [E.C. 4.1.3.18], which stops plant cell division by inhibiting biosynthesis of the essential amino acids valine and isoleucine. It is applied once per growing season and is highly active at low application levels (5−15 g ha^{-1}) [14]. No study concerning the degradation mechanism of ethametsulfuron-methyl and the identity of its by-products is available.

In this paper we describe the use of LC/MS/MS analysis as a rapid and selective method to evaluate the degradation kinetics and determine the degradation products of ethametsulfuron-methyl. On the basis of the results obtained, degradation pathways were proposed.

2. Experimental

2.1 *Chemicals*

Technical ethametsulfuron-methyl (97.5%) was obtained from Du Pont Inc., Wilmington, USA. Before use, ethametsulfuron-methyl was further purified to analytical grade by column chromatography. The purity was determined by HPLC and found to be greater than 99%. All solvents used were pure analytical HPLC grade solvents (Aldrich Chemical Co., Milwaukee, WI, USA).

Table 1. Buffer solution prepared for the aqueous hydrolysis of ethametsulfuron-methyl.

Buffer solution	pH	Preparation for 100 mL of buffer solution
A	4.0 ± 0.1	18.0 mL of 0.2 M NaOAc + 82.0 mL of 0.2 M HOAc
B	5.0 ± 0.1	70.5 mL of 0.2 M NaOAc + 29.5 mL of 0.2 M HOAc
C	6.0 ± 0.1	87.7 mL of 0.1 M KH_2PO_4 + 12.3 mL of 0.05 M $Na_2B_4O_7$
D	7.0 ± 0.1	62.3 mL of 0.1 M KH_2PO_4 + 37.7 mL of 0.05 M $Na_2B_4O_7$
E	8.0 ± 0.1	46.5 mL of 0.1 M KH_2PO_4 + 53.5 mL of 0.05 M $Na_2B_4O_7$

Five buffer solutions were used to study the aqueous hydrolysis of ethametsulfuron-methyl (table 1).

2.2 *Hydrolysis study*

Hydrolysis of ethametsulfuron-methyl was carried out in aqueous buffer solutions within the pH range 4.0–8.0 (Table 1). To avoid microbial degradation, buffer solutions were sterilized by filtration (Millex GS/AP20 0.22 μm, Millipore, Bedford, MA), and glass equipment was autoclaved for 20 min at 16 psi and 121°C. Aseptic techniques were adopted throughout the study to maintain sterility.

A stock solution containing 100 mg L^{-1} of ethametsulfuron-methyl in acetonitrile was prepared. Triplicate 40.0 mL samples, each containing 5.0 mg L^{-1} of herbicide, were obtained by diluting the stock solution with the appropriate buffer solution. The treated buffer solutions were stored in the dark at ambient temperature (25 ± 1°C) in 50 mL centrifuge glass tubes.

An aliquot of 1 mL from each tube was aseptically removed periodically, starting on 0 day to 28 days, and analyzed by LC/MS/MS.

2.3 *Analytical procedure*

The LC was performed using a C_{18} Hypersil ODS column (200×4.6 mm i.d., with a 5 μm particle size). The composition of the mobile phase was 30% water and 70% methanol. The eluent was passed through the sample injection valve to an electrospray ion source at a flow rate of 0.6 mL min^{-1}. A sample volume of 20 μL was injected by an autosampler.

MS detection was achieved using a Finnigan LCQ advantage LC/MS^n triple-quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) equipped with an electrospray (ES) ion source.

Liquid N_2 was used as both the drying and ES nebulizing gas at flow rates of 500 and 20 L h^{-1}, respectively. The instrument was operated in +ve or −ve ion mode with an ion source temperature of 95°C, a capillary needle potential of +3.75 KV, and a cone voltage of 15 V.

The total ion chromatogram (TIC) of LC/MS was obtained by scanning the first quadrupole from m/z 80 to 500 at a rate of 400 mass units (amu) s^{-1} in full scan mode with an interscan delay of 0.10 s. Data were acquired in continuum mode.

3. Results and discussion

3.1 *Hydrolysis rates*

The hydrolysis of ethametsulfuron-methyl, monitored at different pH values, followed first-order kinetics, with significant determination coefficient ($P < 0.05$), thus indicating that the half-life was independent of initial herbicide concentration; this is in agreement with results of preliminary studies at different concentrations (unreported data) and with the data presented by Dinelli *et al.* on other sulfonylureas [16].

Hydrolysis rates of ethametsulfuron-methyl showed variation with pH as do those of other sulfonylureas (figure 1). The observed rate constant, k, and calculated half-lives ($t_{1/2} = \ln 2/k$) are given in table 2. Ethametsulfuron-methyl was relatively stable to hydrolytic degradation in neutral (pH 7.0) and alkaline (pH 8.0) buffer solution at 25 °C with less than 30% degradation after 28 days. Hydrolysis was more rapid under acidic pH conditions. The degradation rate of ethametsulfuron-methyl at pH 4.0 was more than 10-fold faster than at pH 7.0 (half-lives were 8.9 and 90.0 days, respectively). Higher pH values seem to strongly reduce the hydrolysis rate of ethametsulfuron-methyl. The hydrolysis rate of ethametsulfuron-methyl at pH 8.0 is a little higher than that at pH 7.0 (table 2, figure 1). This implied an increase in degradation rate when pH values were increased in alkaline buffers. Similar results have been observed for other sulfonylurea herbicides [17–19]. The significantly larger rate of alkaline hydrolysis

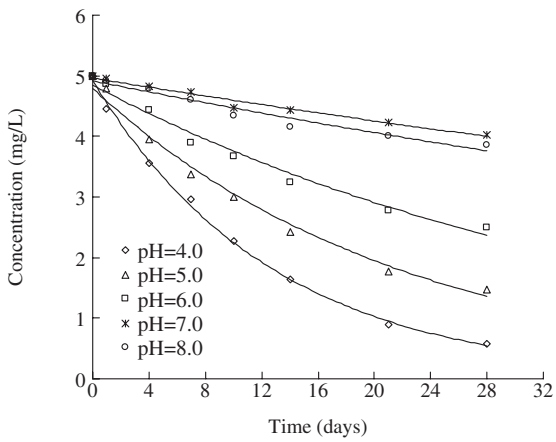


Figure 1. Disappearance of ethametsulfuron-methyl at different pH values.

Table 2. Determination of rate constant (k) and half-life ($t_{1/2}$) for the hydrolysis of ethametsulfuron-methyl at different pH values.

pH	$k \times 10^{-3}$ (days^{-1})	r	$t_{1/2}$ (days)
4.0 ± 0.1	78.3	0.99	8.9
5.0 ± 0.1	44.9	0.99	15.4
6.0 ± 0.1	25.5	0.99	27.2
7.0 ± 0.1	7.7	0.98	90.0
8.0 ± 0.1	9.6	0.97	72.2

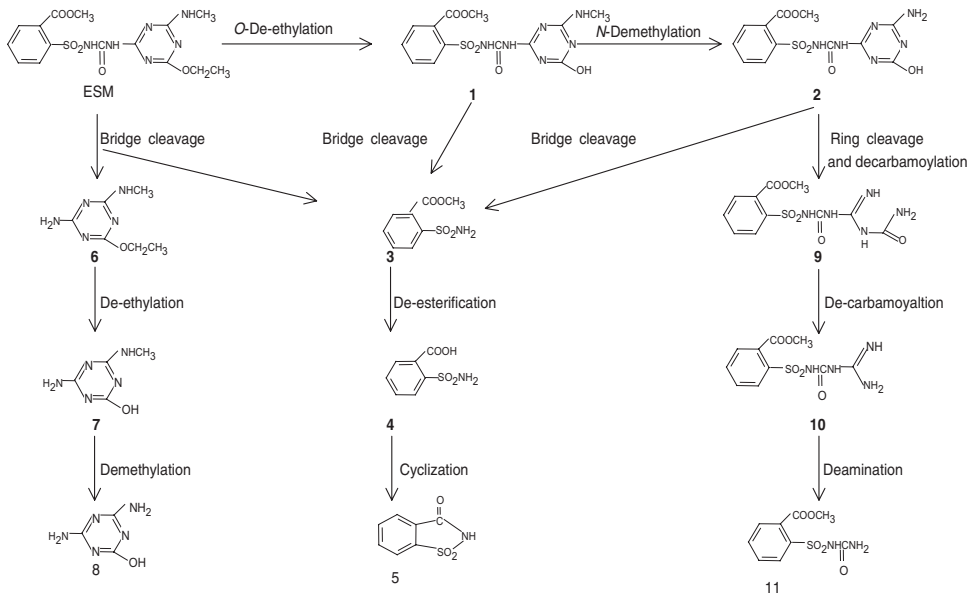


Figure 2. Products and proposed pathways of chemical hydrolysis of ethametsulfuron-methyl (ESM).

of ethametsulfuron-methyl than that in neutral water may be attributed to the base-catalyzed reaction involving hydroxyl ion attack on the bridge carbonyl.

3.2 *Hydrolysis metabolites*

In aqueous hydrolysis studies at different pH values with ethametsulfuron-methyl, eleven degradation products were formed, which were later tentatively identified using mass spectra obtained for each product (figure 2). Tentative assignment of the by-products was made on the basis of molecular ion peaks and fragment ions as obtained from LC/MS/MS studies (table 3, figure 3).

A characteristic ion for an authentic standard of ethametsulfuron-methyl (m/z 411) was obtained using positive-ion electrospray ionization mass spectrometry. The spectrum of ions obtained following fragmentation of ethametsulfuron-methyl (411 $[MH]^+$) included the acidic sulfonamide portion of the herbicide (m/z 217) and the triazine portion of the parent molecule (m/z 196, 170 and 133). The negative-ion electrospray ionization mass spectrum of the parent herbicide has a characteristic parent ion (m/z 409) and expected daughter ions of ethametsulfuron-methyl (m/z 200, 183, and 168).

Hydrolysis product **1** had an m/z value of 381 (17, $[M-H]^-$) that corresponds to the loss of an ethyl group from ethametsulfuron-methyl and was identified as methyl 2-[[[[[4-hydroxy-6-(methylamino)-1,3,5-triazin-2-yl]amino]carbonyl]amino]-sulfonyl]benzoate. The mass spectrum of the daughter ions of product **1** showed many characteristic ions of *O*-deethyl-ethametsulfuron-methyl including fragment ion peaks at 289 (41, 381–$COOCH_3$–CH_3–OH – H), 213 (5, 381–$C_3N_3(NHCH_3)$(OH)–CONH), 182 (100, 213–OCH_3), 156 (5, 182–CO + 2H), 137 (5, 182–CONH–2H), and 97 (12).

Table 3. Mass spectral data of ethametsulfuron-methyl (ESM) and its hydrolysis products.

Product	Ionization mode	Retention time (t/min)	Relative molecular mass	Mass found (m/z, %)	Proposed structure
ESM	Positive	5.89	410	411 (100, $[MH]^+$) 217 (1) 196 (8) 170 (2) 133 (2)	
	Negative	5.86	410	409 (100, $[M-H]^-$) 200 (3) 183 (3) 168 (12)	
1	Negative	2.75	382	381 (17, $[M-H]^-$) 289 (41, $381-COOCH_3-CH_3-OH-H$) 213 [5, $381-C_3N_3(NHCH_3)(OH)-CONH$] 182 (100, $213-OCH_3$) 156 (5, $182-CO+2H$) 137 (5, $182-CONH-2H$) 97 (12)	methyl 2-[[[[[4-hydroxy-6-(methylamino)-1,3,5-triazin-2-yl]amino]carbonyl]amino]sulfonyl]benzoate
2	Negative	4.81	368	367 (8, $[M-H]^-$) 289 (9, $367-COOCH_3-OH-2H$) 214 [5, $367-C_3N_3(NH_2)(OH)-CONH+H$] 182 (2, $214-OCH_3-H$) 137 (4, $182-CONH-2H$) 119 (3, $182-SO_2+H$) 97 (3)	methyl 2-[[[[[4-hydroxy-6-(amino)-1,3,5-triazin-2-yl]amino]carbonyl]amino]sulfonyl]benzoate
3	Negative	5.01	215	214 (58, $[M-H]^-$) 197 (8, $214-NH_3$) 183 (100, $214-OCH_3$) 151 (16, $214-SO_2+H$) 135 (14, $151-NH_2$) 121 (13, $135-CH_2$) 91 (56, $214-COOCH_3-SO_2$)	methyl 2-sulfonamide-methylbenzoate

4	Negative	3.21	201	200 (100, $[M-H]^-$) 182 (15, $200-H_2O$) 156 (9, $200-CO_2$) 137 (4, $200-SO_2+H$) 121 (3, $137-NH_2$)	2-sulfonamide-benzoic acid
5	Negative	2.70	183	182 (100, $[M-H]^-$) 169 (1, $182-NH+2H$) 155 (2, $182-CO+H$) 137 (2, $182-CONH-2H$) 119 (1, $182-SO_2+H$)	1-dioxy-3-keto-1,2-benzisothiazole
6	Positive	12.13	169	170 (100, $[MH]^+$) 142 (3, $170-NHCH_3+2H$) 113 (2, $142-CH_2CH_3$) 97 (1, $113-NH_2$)	4-ethoxy-6-(methylamino)-1,3,5-triazin-2-amine
7	Positive	11.67	141	142 (100, $[MH]^+$) 113 (72, $142-NHCH_3+H$) 97 (20, $113-NH_2$)	4-amino-6-(methylamino)-1,3,5-triazin-2-ol
8	Negative	2.64	127	127 (100, $[M]^-$) 113 (14, $127-NH_2+2H$) 97 (4, $113-NH_2$)	4,6-diamino-1,3,5-triazin-2-ol
9	Positive	3.18	343	344 (1, $[MH]^+$) 333 (17, $344-CH_3+4H$) 318 (3, $333-NH$) 296 (100, $344-OCH_3-NH_3$) 274 (7, $318-CONH_2$)	methyl 2-[[[[[[[amino(carbonyl)amino](imino)methyl]amino]carbonyl]amino]sulfonyl]benzoate
10	Negative	2.97	300	299 (1, $[M-H]^-$) 272 (100, $299-CH_3-NH+3H$) 243 (1, $272-CH_2NH_2+H$) 228 (1, $243-NH$)	methyl 2-[[[[[amino(imino)methyl]amino]carbonyl]amino]sulfonyl]benzoate
11	Positive	4.77	258	258 (100, $[M]^+$) 228 (5, $258-OCH_3+H$) 213 (7, $228-NH$) 139 (10, $258-SO_2-NH-CONH+2H$) 105 (9, $213-SO_2-CONH_2$)	methyl 2-carbamoylsulfamoylbenzoate

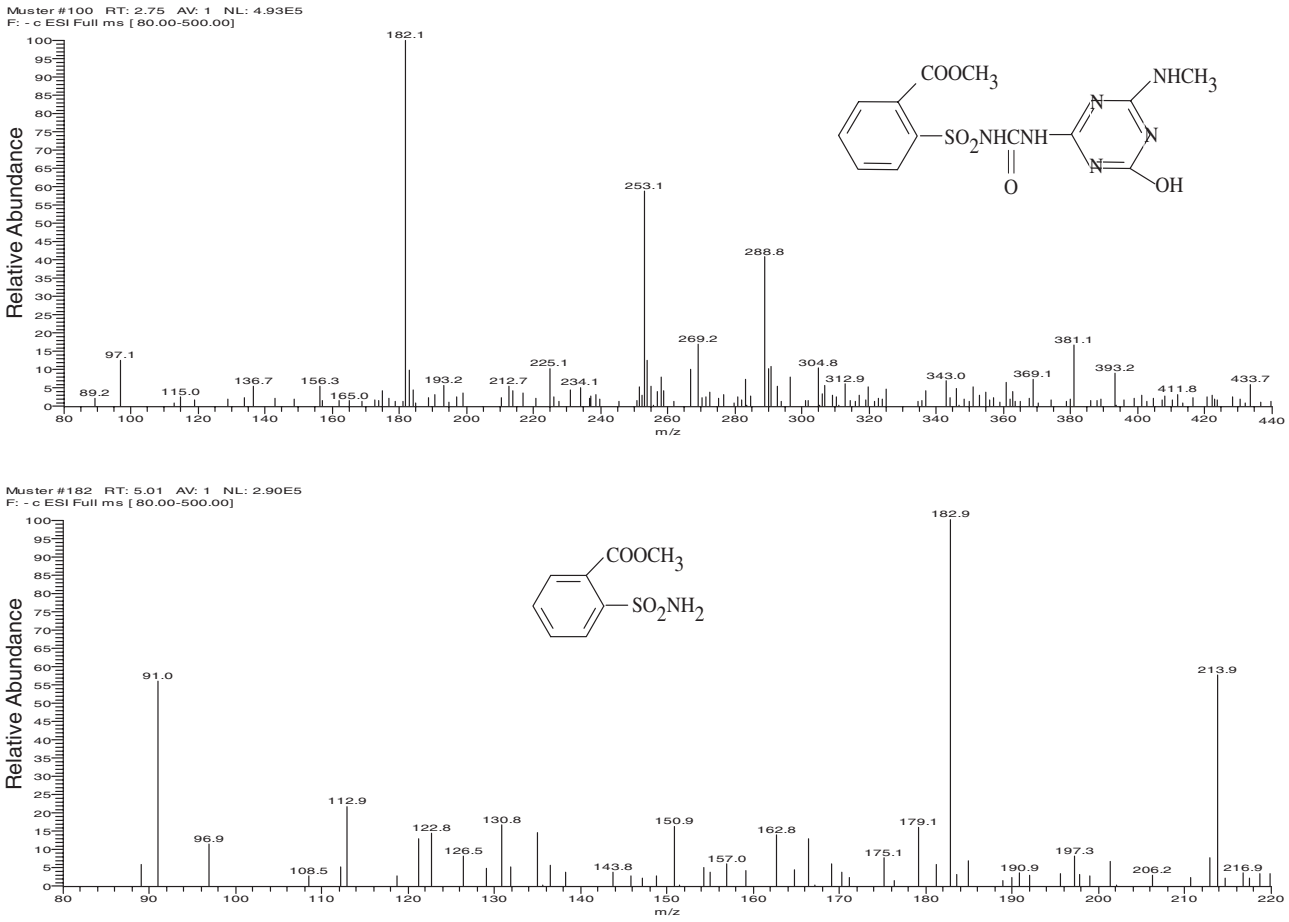


Figure 3. Electrospray ionization mass spectrum of degradation products of ethametsulfhuron-methyl.

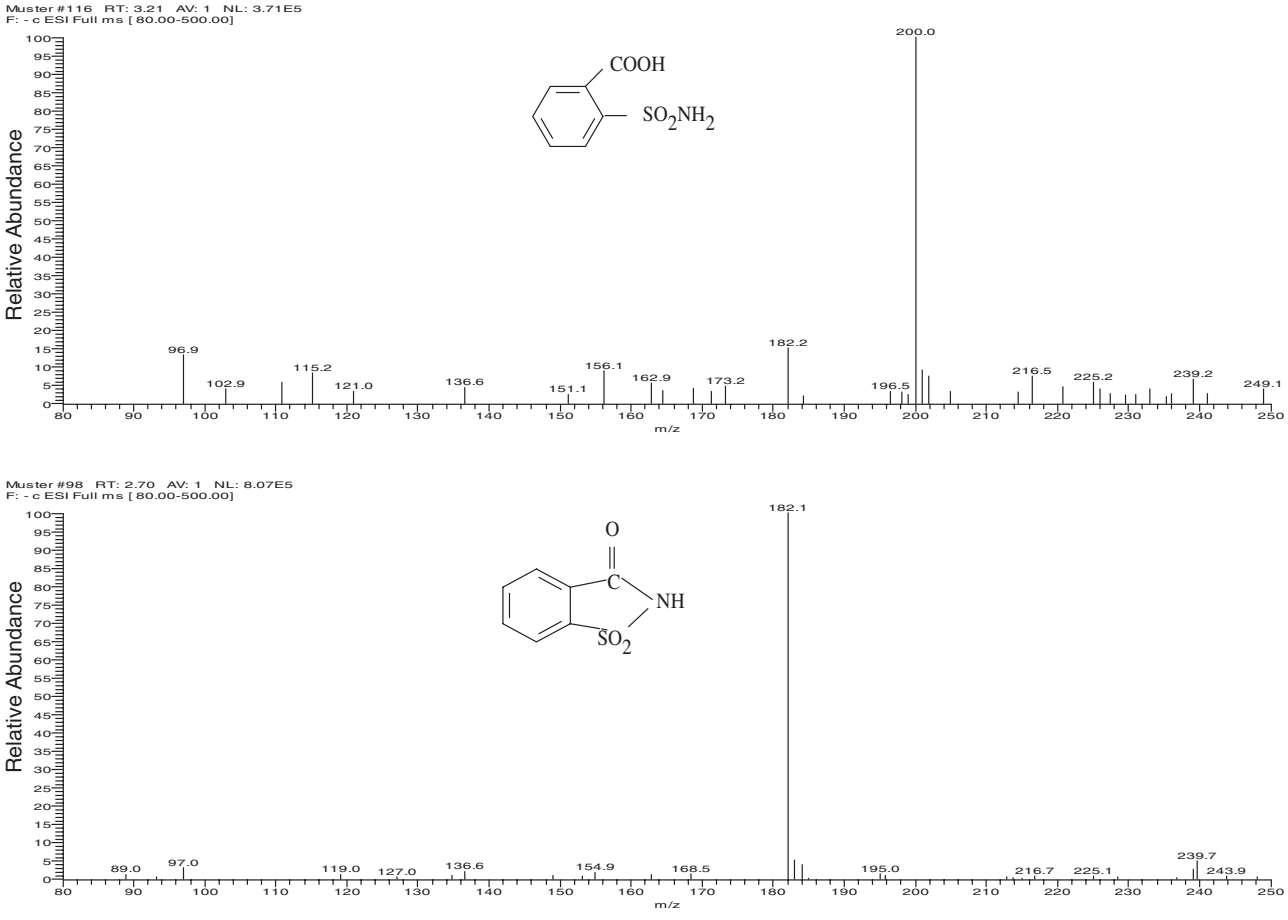
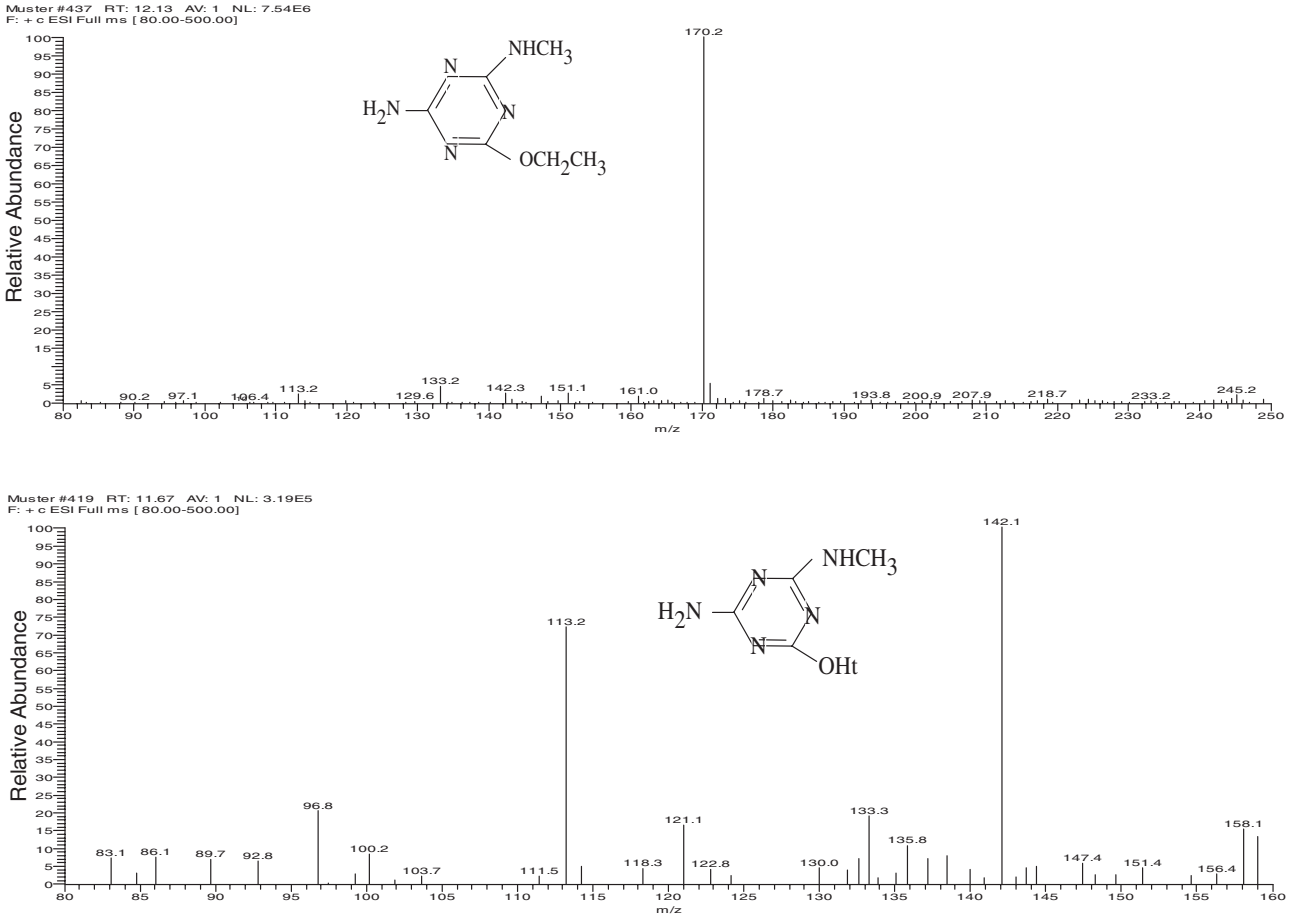


Figure 3. Continued.



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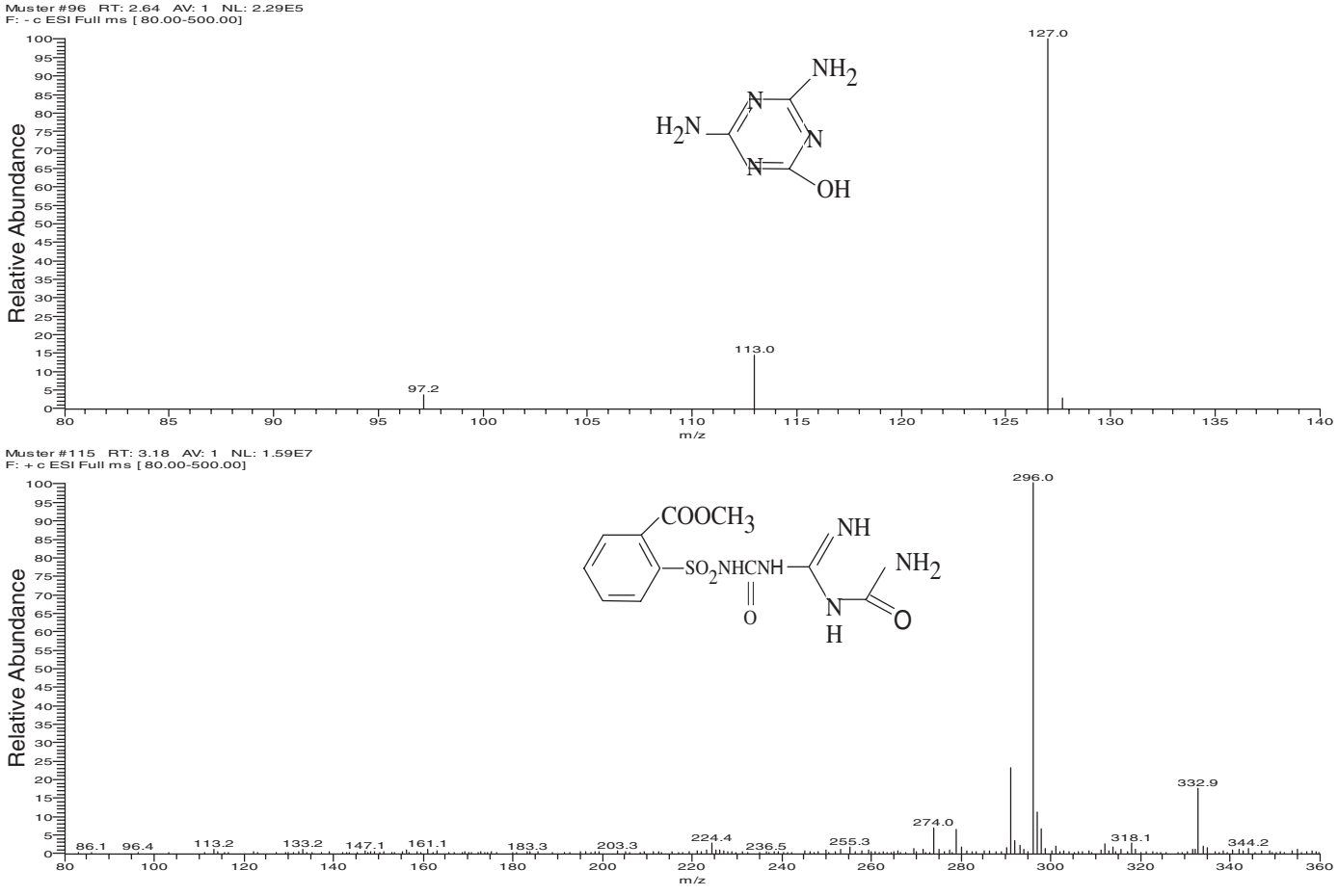

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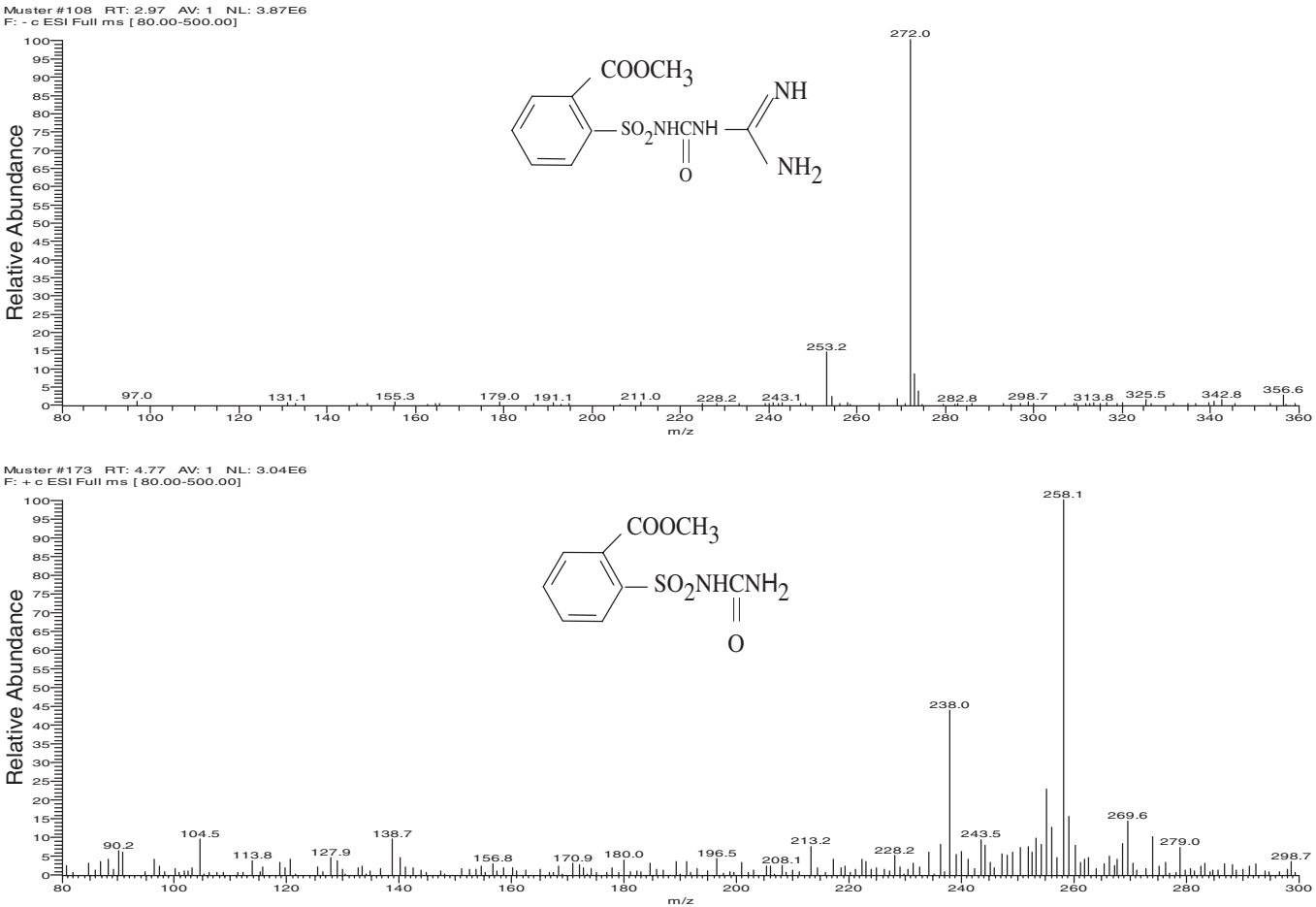

Figure 3. Continued.

The negative-ion electrospray ionization mass spectrum of product **2** showed a molecular ion with an m/z value of 367 (8, $[M–H]^-$), which corresponds to the loss of methyl and ethyl constituents from ethametsulfuron-methyl. Therefore, product **2** was identified as methyl 2-[[[[(4-hydroxy-6-(amino)-1,3,5-triazin-2-yl] amino] carbonyl]amino] sulfonyl]benzoate. Further examination of the daughter ions of product **2** revealed characteristic ions of *N*-demethyl-*O*-de-ethyl-ethametsulfuron-methyl, including fragment-ion peaks at 289 (9, 367–$COOCH_3 - OH$–2H), 214 (5, 367–$C_3N_3(NH_2)(OH)$–CONH + H), 182 (2, 214–OCH_3–H), 137 (4, 182–CONH–2H), 119 (3, 182–SO_2 + H), and 97 (3). Products **1** and **2** have been detected in plant metabolites of ethametsulfuron-methyl [15,20,21].

The mass spectrum of product **3** showed a quasi-molecular-ion peak at 214 (58, $[M–H]^-$) and fragment-ion peaks at 197 (8, 214–NH_3), 183 (100, 214–OCH_3), 151 (16, 214–SO_2 + H), 135 (14, 151–NH_2), 121 (13, 135–CH_2) and 91 (56, 214–$COOCH_3$–SO_2). It was tentatively identified as methyl 2-sulfonamido-methyl-benzoate. Compound **3** has been reported as a degradation product of ethametsulfuron-methyl in water [22] and of metsulfuron-methyl in soil [23].

A quasi-molecular-ion peak at m/z 200 (100, $[M–H]^-$) in the mass spectrum of product **4** and ion fragment peaks at m/z 182 (15, $200 - H_2O$), 156 (9, $200 - CO_2$), 137 (4, $200 - SO_2 + H$) and 121 (3, $137 - NH_2$), helped to identify the compound as 2-sulfamoylbenzoic acid. Compound **4** was also reported as a soil metabolite of sulfonamido-benzoic acid [24].

Product **5** was tentatively identified as 1-dioxy-3-keto-1,2-benzisothiazole according to its mass spectrum, which showed a quasi-molecular-ion peak at m/z 182 (100, $[M–H]^-$) and ion peaks at m/z 169 (1, $182 - NH + 2H$), 155 (2, $182 - CO + H$), 137 (2, $182 - CONH - 2H$) and 119 (1, $182 - SO_2 + H$). A compound similar to this product was identified as a hydrolysis product of chlorimuron and nicosulfuron in aqueous solution [25,26].

The mass spectrum of product **6**, which was detected in positive mode, showed a protonated molecular-ion peak at m/z 170 (100, $[MH]^+$) and characteristic ion-fragment peaks at m/z 142 (3, $170 - NHCH_3 + 2H$), 113 (2, $142 - CH_2CH_3$), and 97 (1, $113 - NH_2$). On the basis of this result, this compound is assigned the structure 4-ethoxy-6-(methylamino)-1,3,5-triazin-2-amine. Compound **6** has been reported as a hydrolysis product of ethametsulfuron-methyl [22]. An analogue of **6** was observed during the hydrolysis of triflusulfuron-methyl in aqueous buffer [27].

Product **7** was tentatively identified as 4-amino-6-(methylamino)-1,3,5-triazin-2-ol according to its mass spectrum, which showed a quasi-molecular-ion peak at m/z 142 (100, $[MH]^+$) and ion peaks at m/z 113 (72, $142 - NHCH_3 + H$) and 97 (20, $113 - NH_2$). A product analogous to **7** was found in soil metabolites of chlorsulfuron under laboratory and field conditions [28,29].

The mass spectrum of product **8** showed a pseudo-molecular ion peak at m/z 127 (100, $[M]^-$) and fragment-ion peaks at m/z 113 (14, $127 - NH_2 + 2H$) and 97 (4, $113 - NH_2$). It was tentatively identified as 4,6-diamino-1,3,5-triazin-2-ol.

When hydrolysis experiments were conducted with acidic aqueous solutions of ethametsulfuron-methyl (pH 4 and 5), some additional metabolites were generated. They were mainly composed of product **9**, as confirmed by LC/MS analysis. Two other products, **10** and **11**, were also detected (table 3, figure 3).

Product **9** was tentatively identified as methyl 2-[[[[[[amino(carbonyl)-amino]-(imino)methyl]amino]carbonyl]amino]sulfonyl]benzoate; its mass spectrum showed

a quasi-molecular-ion peak at m/z 344 (1, $[MH]^+$) and fragment-ion peaks at m/z 333 (17, $344-CH_3+4H$), 318 (3, $333-NH$), 296 (100, $344-OCH_3-NH_3$), and 274 (7, $318-CONH_2$). Compound **9** has been reported as a soil metabolite of metsulfuron-methyl [30]. Analogous compounds have been described in the hydrolysis of thifensulfuron and thifensulfuron-methyl [31].

The mass spectrum of product **10** showed a quasi-molecular-ion peak at m/z 299 (1, $[M-H]^-$), with fragment-ion peaks at m/z 272 (100, $299-CH_3-NH+3H$), 243 (1, $272-CH_2NH_2+H$), and 228 (1, $243-NH$). It was tentatively identified as methyl 2-[[[[[amino(imino)methyl]amino]carbonyl]amino]sulfonyl]benzoate. A compound analogous to **10** was identified in poultry metabolites of bensulfuron-methyl [13].

The mass spectrum of product **11** contained a quasi-molecular-ion peak at 258 (100, $[M]^+$) and fragment peaks at m/z 228 (5, $258-OCH_3+H$), 213 (7, $228-NH$), 139 (10, $258-SO_2-NH-CONH+2H$) and 105 (9, $213-SO_2-CONH_2$). It was tentatively identified as methyl 2 carbamoylsulfamoylbenzoate. An analogous metabolite has been observed in a soil-dissipation study of chlorsulfuron [28].

3.3 *Hydrolysis pathways*

After identification of the various degradation products, tentative pathways for the hydrolytic degradation of ethametsulfuron-methyl in water are proposed (figure 2). The hydrolysis process of ethametsulfuron-methyl involves three degradation pathways.

One route includes the cleavage of the sulfonylurea bridge yielding products **3** and **6**. De-esterification of product **3** yielded **4**. Product **4** underwent cyclization/dehydration reactions to yield saccharin, product **5**. Product **7** appears to be formed by de-ethylation of product **6**. Product **7** undergoes a demethylation to give product **8**. Hydrolytic cleavage of the sulfonylurea bridge yielding the corresponding sulfonamide and heterocyclic amine is very common during the degradation of sulfonylurea herbicides in water [32–34].

The second route, involving *O*-deethylation and *N*-demethylation reactions at the substituted triazine ring of ethametsulfuron-methyl, led to products **1** and **2**. Dealkylation and hydroxylation of the substituted triazine ring have been observed during the degradation of some sulfonylurea herbicides [35].

The third route implies the opening of the triazine ring of ethametsulfuron-methyl. The *O*-deethylation of the ethoxy group on the triazine ring affords hydroxyethametsulfuron. The latter metabolite is unstable under acidic aqueous conditions (pH 4–5) and degrades further through two different pathways: cleavage of the sulfonylurea bridge to give sulfonamide and hydroxytriazine, and opening of the triazine ring to yield the carbamoyl guamidine product **9**. Product **10** appears to be formed by cleavage of the urea N–C bond and protonation of the amino group of **9**. Product **10** undergoes deamination to give product **11**. Products **9**, **10** and **11** were unstable in water, and finally probably resulted in the formation of compound **3**.

The triazine ring-opening phenomenon was first described for metsulfuron-methyl degradation, which involved substitution of the methoxy group of the triazine ring by OH, followed by hydrolytic cleavage of the triazine ring [36]. Studies carried out on triasulfuron [17], thifensulfuron-methyl [37], and chlorsulfuron [28,29] (three sulfonylureas sharing a methoxy substitution in the triazine ring with chlorosulfuron) showed hydrolysis pathways similar to those of metsulfuron-methyl. For all three

sulfonylureas, in addition to their respective sulfonamide and aminotriazine derivatives, products both of *O*-demethylation and hydrolytic breakdown of the triazine ring were isolated and identified. Therefore, this kind of reaction seems common to methoxytriazine-substituted sulfonylurea herbicides. Sarmah and Sabadie suggested that the cleavage of the triazine ring was the most important degradation pathway for these herbicides under acidic conditions [33]. Similar degradation routes have also been proposed for prosulfuron hydrolysis in aqueous solution [38].

4. Conclusions

The hydrolysis rate of ethametsulfuron-methyl is pH-dependent. On the basis of the results obtained and in accord with degradation studies of other sulfonylurea herbicides, the cleavage of the sulfonylurea linkage must be considered as the predominant pathway, whereas *O*- and *N*-dealkylation reactions at the substituted triazine ring, and hydrolytic cleavage of the triazine ring, are the second and third major metabolic pathways.

We have shown that the use of LC/MS/MS as an useful analytical technique for identification of the unknown metabolites generated in this study.

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