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Study of the Hydrolysis of a Sulfonylurea Herbicide Using Liquid Chromatography with Diode Array Detection and Mass Spectrometry by Three-Way Multivariate Curve Resolution−Alternating Least Squares

Ernst Bezemer and Sarah Rutan*

Department of Chemistry, Virginia Commonwealth University, P.O. Box 842006, Richmond, Virginia 23284-2006

This research is focused on the development of a novel, automated chemometric method for obtaining relevant chemical information from time-course measurements of an evolving chemical system. This paper describes an investigation of the hydrolysis of Ally, which is a sulfonylurea herbicide. The hydrolysis of this compound is observed at different pHs and temperatures by reversed-phase liquid chromatography using a diode array detector. The data are analyzed using a three-way, multivariate curve resolution technique. Of special interest was the application of a closure constraint in the kinetic dimension followed by the determination of the rate constants for each step of the pathway by using a differential equation solver and nonlinear fitting of the data.

Sulfonylurea-based (SU) herbicides are widely used for weed control for grain crops such as corn, wheat, and rice.[1] Their high herbicidal activity and low application rate (10−15 g/ha of the active ingredient) have made these herbicides very popular agents (43 million tons in 1995).[1,2] Not all crops are resistant to these agents, which results in damage when they are sprayed onto neighboring crops or when crops are affected by residual quantities on the field from previous applications. Thus, a better understanding of the degradation process would help agricultural scientists learn how to minimize crop damage.

Various research groups have investigated the biodegradation and hydrolysis of these herbicides, but the complete mechanism has not been determined. The early stages in the metabolic pathway of these compounds have been elucidated by the use of radiolabeled sulfonylureas;[3] however, the in situ degradation was not investigated by these workers. The half-lives of the parent compounds have been reported at various temperatures and pHs, but most of the degradation products were not identified or quantified.[4] It has been shown that capillary electrophoresis can be used to separate the degradation products, but that research group did not identify the individual species.[5] Some researchers have characterized some of the products by using microcolumn liquid chromatography (LC) and fast-atom-bombardment mass spectrometry (MS) but did not undertake time-dependent experiments.[6] Others have used LC with UV−vis detection, but again, these researchers did not obtain kinetic information.[7,8] A study in this research group of one specific SU (Glean) using LC with diode array detection (DAD) showed the applicability of the three-way chemometric methods for characterizing this system.[9]

The problem addressed in this research is the extraction of quantitative kinetic information from poorly resolved chromatograms. The quantitative information is necessary to determine the reaction pathways and rate constants. Chemometric techniques are used to extract this information from experimental data with signal overlap. The data from the hydrolysis of Ally, which is a sulfonylurea herbicide, is used to show the applicability of these methods.

THEORY

The literature on multiway analysis is not consistent in the notation of variables of different orders. The notation used in this paper is as follows: Bold outlined letters ($\mathbb{D}$) are used for three-dimensional tensors. Bold capital letters (**C**) are used for matrices. Small bold letters (**p**) are used for vectors. Capital italic letters (K) are used for the size of a dimension and the total number of measurements. Small italic letters (k) are used for scalars. Small italic letters with subscripts (d_{rsk}) are used to indicate matrix or tensor elements.

The two-way data obtained from a hyphenated instrument such as LC-DAD or LC/MS are well suited for chemometric analysis.[10,11] The UV−vis or mass spectrum in the second dimension contains

* Corresponding author. E-mail: srutan@saturn.vcu.edu.

(1) Beyer, E. M.; Duffy, M. J.; Hay, J. V.; Schlueter, D. D. *Herbicides: Chemistry Degradation and Mode of Action*; Marcel Dekker: New York, 1988.
(2) Short, P.; Colborn, T. *Tox. Indust. Health* **1999**, *15*, 240−275.
(3) Sweetser, P. B.; Schow, G. S.; Hutchison, J. M. *Pestic. Biochem. Physiol.* **1982**, *17*, 18−47.
(4) Berger, B. M.; Wolfe, N. L. *Environ. Toxicol. Chem.* **1996**, *15*, 1500−1507.
(5) Dinelli, G.; Vicari, A.; Brandolini, V. *J. Chromatogr.* **1995**, *700*, 201−207.
(6) Reiser, R. W.; Barefoot, A. C.; Dietrich, R. F.; Fogiel, A. J.; Johnson, W. R.; Scott, M. T. *J. Chromatogr.* **1991**, 91−101.
(7) Braschi, I.; Calamai, L.; Cremonini, M. A.; Fusi, P.; Gessa, C.; Pantani, O.; Pusino, A. *J. Agric. Food Chem.* **1997**, *45*, 4495−4499.
(8) Bossi, R.; Vejrup, K.; Jacobson, C. S. *J. Chromatogr. A* **1999**, *855*, 575−582.
(9) Johnson, K.; de Juan, A.; Rutan, S. C. *J. Chemom.* **1999**, *13*, 1−11.
(10) de Braekeleer, K.; de Juan, A.; Massart, D. L. *J. Chromatogr.* **1999**, *832*, 67−86.
(11) Salau, J. S.; Honing, M.; Tauler, R.; Barcelo, D. *J. Chromatogr.* **1998**, *795*, 3−12.

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unique species-dependent information and can be used to resolve the overlapping chromatogram. In a simple example with two species, the overall wavelength-dependent absorbance (d_j) is given by eq 1

$$d_j = c_a \times s_{aj} + c_b \times s_{bj} \quad (1)$$

where c_a and c_b are the concentration of species a and b, respectively, and s_{aj}, and s_{bj}, are the molar absorptivities of the species a and b at a specific wavelength, j.

In a more general case with N number of species, the overall wavelength-dependent absorbance is given by eq 2.[12]

$$d_j = \sum_{i=1}^{N} c_i \times s_{ij} \quad (2)$$

The same equation can be written in matrix notation as eq 3

$$\mathbf{D} = \mathbf{C}\cdot\mathbf{S}^T \quad (3)$$

where **C** is a 2-dimensional array of the concentration profiles of each component, $\mathbf{S}^T$ is the transpose of a matrix containing the spectra of the pure components, and **D** is the original data matrix.[13]

Multivariate curve resolution−alternating least squares (MCR/ALS) algorithms sequentially solve for **C** and **S**, as shown in eqs 4 and 5.[14]

$$\mathbf{S}^T = (\mathbf{C}^T\cdot\mathbf{C})^{-1}\cdot\mathbf{C}^T\cdot\mathbf{D} \quad (4)$$

$$\mathbf{C} = \mathbf{D}\cdot(\mathbf{S}^T\cdot\mathbf{S})^{-1}\cdot\mathbf{S} \quad (5)$$

The concentration profiles and pure component spectra are determined by alternating between eqs 4 and 5 until a minimal difference between **D** and ($\mathbf{C}\cdot\mathbf{S}^T$) is obtained.[15] Algorithms for MCR/ALS have been described by Tauler et al. and Bezemer et al.[16,17] The exact algorithm used in this paper has been described in the latter.[17]

To use MCR/ALS algorithms, an initial estimate for either the **C** or the **S** matrix must be obtained. Evolving factor analysis (EFA) is the approach used here for the generation of initial estimates for the concentration profiles. This method is implemented by using singular value decomposition (SVD) on an increasing number of columns of the data set.[17,18] The resulting singular values are combined using the chemical assumption that the first component to start eluting is also the first component to finish eluting.

The ALS calculation needs to be constrained to restrict the range of possible solutions and to result in a chemically reasonable solution. Several types of constraints can be applied to this model, such as unimodality, selectivity, nonnegativity, and closure, which is the application of a mass balance requirement.[17]

If an extra order is present, which in this case is the kinetic information, additional constraints can be applied on the three-way data set. A three-way kinetic trilinear data-set, $\mathbb{D}$ (T × S × K), can be described as the inner product of the retention profiles **R** (T × N), the pure component spectra **S** (S × N), and the kinetic profiles **K** (K × N), according to eq 6,[19]

$$\mathbb{D} = \mathbf{R} \otimes \mathbf{S} \otimes \mathbf{K} + \mathbb{E} \quad (6)$$

where $\mathbb{E}$ is the error tensor, K is defined as the number of kinetic time points, and N is the number of components.

In the ideal case, the data tensor can be unambiguously decomposed into the corresponding matrices using a direct trilinear decomposition (DTD) or the generalized rank annihilation method (GRAM).[20,21] However for real data, in which there can be retention time shifts, peak shape changes, intermolecular interactions that cause spectral changes, and other effects that cause the data set to be nontrilinear, these direct approaches cannot be used, and an iterative method like MCR/ALS has to be employed.

The MCR/ALS algorithm by Tauler and de Juan models only the predetermined number of components. It uses a principal component analysis (PCA) compression to represent the data ($\mathbb{D}$) by using the most significant orthogonal components describing the data set.[22] This ignores information that might be contained in the smaller principal components and can be a potential limitation when analyzing nontrilinear data. In that case, the rank of the data tensor can be higher than the number of real components. The generalized MCR/ALS program developed in our research group does not use this PCA compression.[17] It also allows for a more flexible application of the closure constraint in all dimensions. A thorough comparison between these two programs is given in a previous paper.[17]

One of the multiway constraints, trilinearity, should only be applied to data having ideal behavior where the three-way data tensor can be written as the product of three matrices.[19] The LC-DAD kinetic data described in this paper were found to be very close to trilinear when great care was taken to control the experimental conditions, such as column and solvent temperature; however, the generalized ALS algorithm is able to manage nontrilinearities by relaxing the trilinear constraint for individual species, thus allowing for some components to be trilinear, while others are bilinear.

EXPERIMENTAL SECTION

Chemicals and Materials. In this research, one specific member of the SU family is investigated; methyl-2-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]aminosulfonyl]-benzoate.[23] This herbicide is known under the trade name Metsulfuron methyl or Ally and will be called Ally for the

(12) Harris, D. C. *Quantitative Chemical Analysis*, 3rd ed.; W. H. Freeman and Company: 1991.
(13) Blyth, T. S.; Robertson, E. F. *Matrices and Vector Spaces*; Chapman & Hall: 1986.
(14) Mallat, E.; Barcelo, D.; Tauler, E. *Chromatographia* **1997**, *47*, 342−350.
(15) Tauler, R.; Smilde, A.; Kowalski, B. *J. Chemom.* **1994**, *9*, 31−58.
(16) Tauler, R. B. *D TRAC* **1993**, 319−327.
(17) Bezemer, E. C.; Rutan, S. C. *Chemom. Intell. Lab. Sys.* **2001**, in press.
(18) Meader, M.; Zilian, A. *Chemom. Intell. Lab. Syst.* **1988**, *3*, 205−213.
(19) Sanchez, E.; Kowalski, B. R. *J. Chemom.* **1990**, *4*, 29−45.
(20) Booksh, K. S.; Lin, Z.; Wang, Z.; Kowalski, B. R. *Anal. Chem.* **1994**, *66*, 2561−2569.
(21) Wilson, B. E.; Sanchez, E.; Kowalski, B. R. *J. Chemom.* **1989**, *3*, 493−498.
(22) Gargallo, R.; Tauler, R.; Cuesta Sanchez, F.; Massart, D. L. *Trends Anal. Chem.* **1996**, *15*, 279−286.
(23) Rodriguez, M.; Orescan, D. B. *Anal. Chem.* **1998**, 2710−2717.

remainder of the paper. This compound was generously donated to us by DuPont de Nemours. All other chemicals were purchased from Aldrich and used without further purification unless otherwise stated. The water was purified using a Nanopure ultrapure water system from Barnstead.

Sample Preparation. The pH 2.00 and pH 4.00 buffers were made using aspartic acid ($pK_{a_1} = 2.0$ and $pK_{a_2} = 3.9$).[24] The pH 3.00 and pH 5.00 buffers were made using citric acid ($pK_{a_1} = 3.1$ and $pK_{a_2} = 4.8$).[24] These buffer components were chosen for their low UV−vis absorption and pK_a values close to the desired pH's. All buffers had a concentration of 0.0050 M, which is 2 orders of magnitude larger than the concentration of the analyte, thereby providing adequate buffer capacity. The pHs of these buffers were adjusted to the indicated pH using concentrated hydrochloric acid or solid sodium hydroxide. Particulates in all liquid phases were removed by 0.45-μm membrane vacuum filtration, and solvents were degassed by a helium purge.

For all of the kinetic experiments, 3 mg of Ally was dissolved in 0.5 mL of acetonitrile. This solution was diluted to 100 mL with the corresponding buffer solution (pH 2, 3, 4, or 5). Aliquots of the Ally solution were stored in closed vials and kept in a waterbath held at 25, 35, 45, or 53 °C, depending on the experiment. The temperature of the waterbath up to its highest setting was verified by a calibrated thermometer. At each time point, a sample was taken, after which the vial was discarded.

Procedure. The LC-DAD instrument was a Hewlett-Packard 1090 system. The eluate spectra (1 scan/sec) were observed from 212 to 330 nm at 2 nm intervals. The mobile phase for the LC-DAD experiments consisted of 50% acetonitrile and 50% of the pH 2 aqueous buffer mixed by the instrument. The flow rate was 0.4 mL/min, and the column temperature was controlled at 38 °C.

The ESI-MS experiments without prior chromatographic separation were carried out on a Nermag 3010 MS in positive ion mode using a 2800 V electrospray potential and a 0.5 μL/min flow-rate. The internal standard was 2-chlorobenzenesulfonamide. The hydrolysis was observed in a 0.01 M HCl solution, as opposed to the pH 2 aspartate buffer.

The LC/MS experiments were performed using a Hewlett-Packard 1050 LC pump coupled to a Micromass Quattro LC mass detector via an electrospray interface (ESI) in positive ion mode. The mobile phase for the LC/MS experiments was 50% acetonitrile and 50% purified water. Both phases contained 0.1% formic acid. The flow rate for these experiments was 0.2 mL/min. Both the LC-DAD and LC/MS separations were performed on a Phenomenex LUNA C_{18} column (150 × 4.6 mm) with 5-μm particles. Samples were injected using a 20-μL sample loop. Both of the MS experiments used alternate aqueous acids to avoid interference from the aspartate buffer ions.

Data Analysis Methods. The ESI-MS data were binned from −0.50 to +0.49 for each nominal mass-to-charge ratio. Furthermore, intensities were summed for components that were also present as solvent complexes, sodiated ions, or dimers (i.e., M^+, $M(s)^+$, $M(Na)^+$, $(M)_2^+$). Last, the intensity ratio between this total component intensity and the internal standard was calculated. The same procedure was used to treat the LC/MS data.

(24) Harris, D. C. *Quantitative Chemical Analysis*, 2nd ed.; W. H. Freeman and Company: New York, 1982.

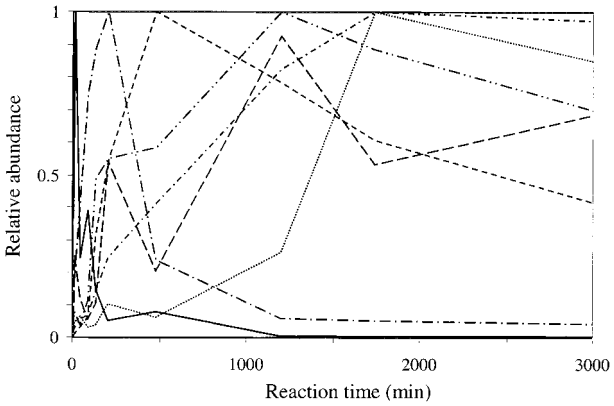


Figure 1. Time dependence of MS positive ions for Ally (—), OH−Ally (—•—•—), ring-opened Ally (----), benzenesulfonamide (-••-••-), triazine (—••—••—), OH-triazine (— — —), and ring-opened triazine (•••••••).

The LC-DAD data were converted to ASCII by a macro supplied by Hewlett-Packard and transferred to an off-line PC. The HPLC-DAD data were analyzed using the generalized MCR/ALS routine, as described in a previous paper, using MATLAB 5.3 from MathWorks on a Dell OptiPlex GX1 PC.[17]

The kinetic profiles resolved by the ALS algorithm were further analyzed by a nonlinear fitting program called Gepasi.[25−28] This program requires the input of a chemical model, which it uses to build and solve the differential equations corresponding to the model. Subsequently, the program fits the kinetic profiles to obtain rate constants for each of the steps in the degradation pathway. The built-in genetic algorithm (500 generations; population, 20) was used to find the optimal rate constants for describing the kinetic profiles. The precision of the rate constants was determined by changing the individual rate constants until the sum of squares error of the fit to the data was 10% above the optimum fit.

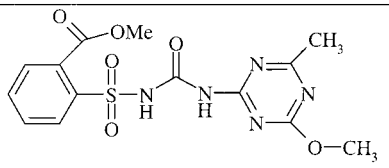
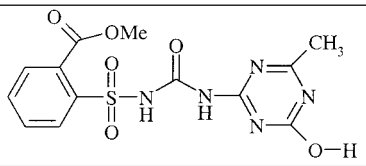
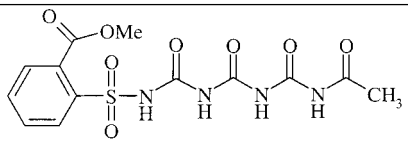
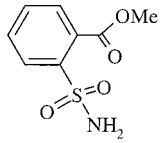
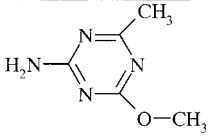
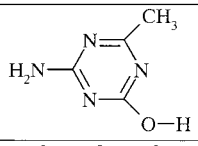
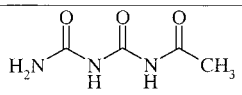
RESULTS AND DISCUSSION

The determination of the kinetic parameters was accomplished by the chemometric analysis of the LC-DAD data. However, before the degradation pathways and reaction rates were determined using the LC-DAD data, the chemical species involved were identified. To do this, two MS experiments were performed. In the first experiment, aliquots of the reaction mixture were directly injected into the electrospray source, whereas in the second, several representative aliquots of the reaction mixture were separated chromatographically before being analyzed by MS using the LC/MS setup.

ESI-MS Analysis. Figure 1 shows the relative abundance ratios of the degradation products relative to the internal standard for this direct MS experiment for the first 50 h of the experiment. The reaction was observed for a total of 4 days, but no additional information was revealed at longer reaction times. Although the signal-to-noise ratio is low, the profiles clearly showed three intermediates under these conditions. The first intermediate had a maximum abundance at ∼4 h, the second had its highest

(25) Mendes, P. Gepasi, version 3.22, http://gepasi.dbs.aber.ac.uk/softw/gepasi.html, 2000.
(26) Mendes, P. *Comput. Appl. Biosci.* **1993**, *9*, 563−571.
(27) Mendes, P. *Trends Biochem. Sci.* **1997**, *22*, 361−363.
(28) Mendes, P.; Kell, D. B. *Bioinformatics* **1998**, *14*, 869−883.

Table 1. Structures and Capacity Factors

structure	compound name	capacity factors	
		LC-DAD[a]	LC-MS[b]
	Ally	1.75 (0.01)	1.9 (0.1)
	OH-Ally	0.53 (0.01)	0.55 (0.06)
	ring-opened Ally	1.47 (0.02)	1.43 (0.08)
	benzenesulfonamide	0.93 (0.01)	0.97 (0.03)
	triazine	0.19 (0.04)	0.16 (0.01)
	OH-triazine	0.77 (0.02)	-0.08 (0.09)
	ring-opened triazine	0.72 (0.03)	-0.18 (0.08)

[a] 50% ACN and 50% pH 2.00 0.0050M aspartate mobile phase [b] 50% ACN and 50% aqueous mobile phase with 0.1% formic acid.

abundance at ~8 h, and the third, at ~24 h. These experiments were also performed in parallel using the LC-DAD in order to determine the influence of the change in buffer for the reaction. Changing the reaction buffer from pH 2.00 aspartate to pH 2.00 HCl had no effect on the reaction kinetics.

LC/MS Analysis. In the LC/MS experiments, the aliquots were taken at time points when the reactant, intermediates, and products were predicted to be at their peak concentrations. The separation conditions used for the LC-DAD experiments could not be used because of interference from the buffer ions. Furthermore, this MS detector required a lower flow rate than the 0.4 mL/min used for the other experiments. This instrument did not have a thermostated column compartment, nor did it have an absorption detector; therefore, all samples were analyzed in parallel using the LC-DAD instrument using the same mobile phase as used for the LC/MS instrumental setup. Constraints on instrumental availability did not allow for complete kinetic studies to be obtained using this technique.

Table 1 shows the capacity factors under both sets of LC conditions for the important species in the degradation pathway. The values were determined by calculating the average and standard deviation of a minimum of 5 runs. The peaks from the LC/MS experiments and the LC-DAD experiments using the 50% ACN and 50% pH 2.00 aspartate mobile phase were matched on the basis of the capacity factor and according to similarities in the corresponding kinetic and spectral profiles. All of the low-molecular-weight components had small capacity factors. These components were presumed to be charged under these acidic mobile phase conditions. The large difference in the capacity factor of Ally versus OH−Ally was also due to the use of an acidic mobile phase. Ally is uncharged under these mobile phase conditions, whereas OH−Ally is ionized, and thus, is far less retained by the C_{18} stationary phase.

In comparing the capacity factors between the formic acid and aspartate mobile phases, some changes in the retention order occurred. As pointed out by Bosch et al., even though the pH aspartate aqueous phase was lower than that of the formic acid aqueous phase, in the presence of 50% ACN, the trend in acidities may be reversed.[29,30]

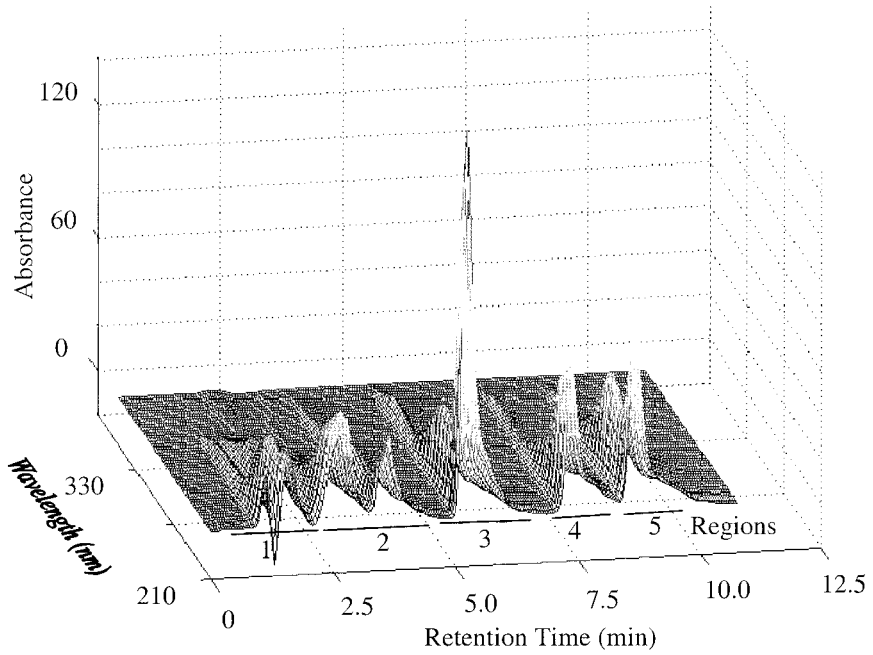


Figure 2. Typical LC-DAD chromatogram. Region 1 contains the system peak and is not analyzed. Region 2 contains four components. Regions 3, 4, and 5 contain one component each.

Table 2. Rate Constants and Fitting Errors for Ally Degradation Pathway

pH	temp, °C	k_1, 10^{-4} min^{-1}	k_2, 10^{-4} min^{-1}	k_3, 10^{-4} min^{-1}	k_4, 10^{-4} min^{-1}	k_5, 10^{-4} min^{-1}	k_6, 10^{-4} min^{-1}	k_7, 10^{-4} min^{-1}	fit error ALS, %	fit error GEPASI, %
2	45	61 ± 19	33 ± 13	43 ± 11	5.7	4.9	1.3	0.13	24	4.0
3	45	23 ± 6	19 ± 8	15 ± 4	0.2	0.26	0.05	3.3	27	0.12
4	45	15 ± 3	1.9 ± 0.9	11 ± 3	0.13	0.34	0.33	0.36	22	1.5
5	45	2.9 ± 0.4	1.1 ± 0.2	1.2 ± 0.2	0.054	2.1	1.0	2.8	34	0.18
2	25	9 ± 3	1.0 ± 0.7	10 ± 3	<0.01	0.26	<0.01	<0.01	25	11
2	35	44 ± 18	20 ± 9	14 ± 5	0.02	7.6	<0.01	0.03	32	2.1
2	45	61 ± 19	33 ± 13	43 ± 11	5.7	4.9	1.3	0.13	24	4.0
2	53	120 ± 40	70 ± 30	110 ± 30	22	19	1.9	0.01	32	3.2

Chemometric Analysis. Figure 2 shows a typical DAD chromatogram of an aliquot of the Ally solution at pH 2 after 4 h of reaction at 45 °C. Region 1 contains the system peak. Region 2 contains four components. Regions 3, 4, and 5 contain only a single component. To apply three-way chemometric methods to these data, a set of initial estimates had to be generated for the ALS algorithm. There were three prominent peaks that are baseline-separated in regions 3, 4, and 5, respectively. Initial estimates for these three components were generated by performing EFA on each peak individually, followed by two-way alternating least squares. Nonnegativity on both the spectral and concentration profiles were the only constraints applied. Subsequent two-way ALS analysis resulted in a closer approximation to the true spectral and chromatographic responses than the initial EFA result.

The earlier eluting components in region 2 were treated in a different manner. The envelope was divided in two smaller envelopes, each containing the significant part of two of the four overlapped components, and the following procedure was applied to both envelopes. EFA was performed on each of the smaller envelopes for the experiment when both components are present. Once again, two-way ALS was performed to refine the coarse initial estimates. Three-way ALS was carried out on this part of the data set using this matrix with two components as an initial estimate for each of the experiments. The two components were constrained to nonnegativity and vertical unimodality.[31] The vertical unimodality constraint was necessary to resolve these severely overlapped peaks with very similar spectral profiles. This procedure was repeated for the second peak envelope.

The two chemometrically resolved profiles for each of the smaller peak envelopes in region 2 were combined to give four profiles. The retention profiles of these four peaks as resolved by the ALS algorithm are shown in Figure 3A, and the corresponding spectral profiles are shown in Figure 3B. These four profiles were subsequently merged with the profiles of the three baseline-separated components that were isolated earlier to form a three-way initial guess tensor consisting of seven components. No extra components were used to account for background effects, because an eighth component did not improve the fit.

Nonnegativity constraints were applied to the retention, spectral, and kinetic profiles of all the components. Vertical

(29) Bosch, E.; Bou, P.; Allemann, H.; Roses, M. *Anal. Chem* **1996**, *68*, 3651−3657.
(30) Bosch, E.; Espinosa, S.; Roses, M. *J. Chromatogr.* **1998**, *824*, 137−146.
(31) de Juan, A. Ph.D. dissertation, Universitat de Barcelona, Barcelona, Spain, 1997.

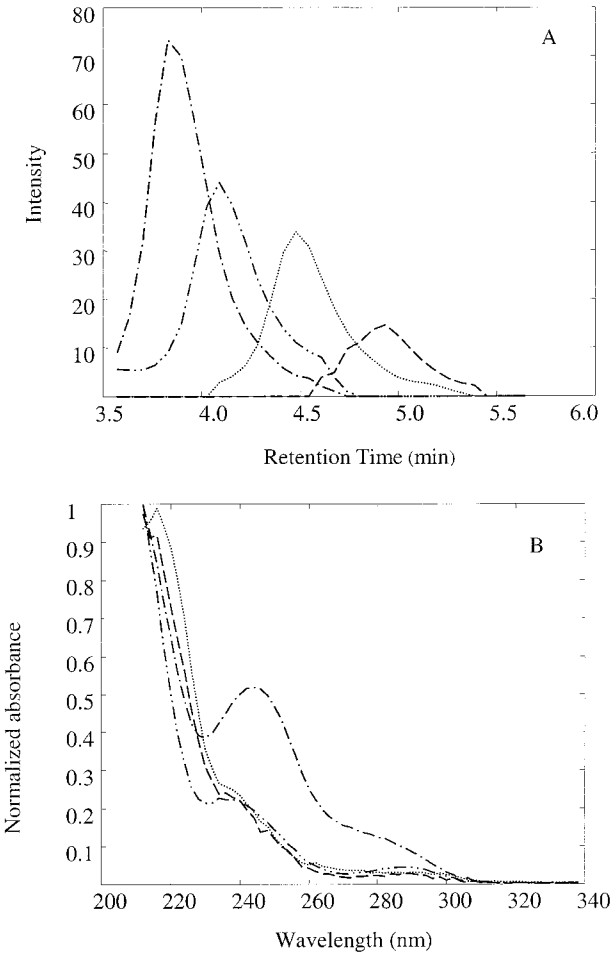


Figure 3. Retention profiles (A) of OH−Ally (−·−·−·−), triazine (−··−··−··), OH-triazine (− − −), and ring-opened triazine (·····). The spectral profiles (B) of OH−Ally (−·−·−·−), triazine (−··−··−··), OH-triazine (− − −) and ring-opened triazine (·····).

unimodality as well as trilinearity was applied to all components.[31] The local rank was also restricted, because each of the latter three peaks was due to a single component. The closure constraint was implemented in the algorithm developed here and resulted in obtaining absolute concentrations rather than relative intensities. It forces the sum of the concentrations of the included components in the mass balance to a set value. The components that included the triazine ring or the ring-opened triazine were included in one closure, and the second closure contained the components that were assumed to include the benzenesulfonamide unit. The application of the trilinearity constraint and the closure constraint resulted in the ALS fit error shown in Table 2. Without these constraints, the fit error was under 10% for most experiments. This indicates that the data are not perfectly trilinear; therefore, the quantification of the peak area was inaccurate, resulting in a less accurate implementation of the closure constraint and, thus, a poorer fit to the data.

Nonlinear Kinetic Fitting of the Rate Constants. From these experiments, a tentative degradation mechanism was determined and is shown in Figure 4. The kinetic profiles found by three-way analysis of the LC-DAD were fit to a model describing this scheme using Gepasi differential equation solver. Figure 5 shows a comparison between the chemical model with the fitted rate constants and the kinetic profiles resolved by ALS. It indicates three intermediates with reaction times for their respective peak concentration that correspond to the maximum peak concentrations seen in the ESI-MS experiments.

The fitting results for all of the reaction conditions are summarized in Table 2. At low pHs and high temperatures, the main pathway for degradation was hydrolysis of the methyl group on the triazine ring followed by ring opening; however, there was a trend that indicated that at higher pHs and lower temperatures, the predominant pathway could become the hydrolysis of the sulfonylurea bridge, as is often reported in the literature.[1]

Other research groups have studied Ally at a range of pHs and temperatures and have reported the half-lives or pseudo-first-order rate constants.[1,4] The half-life of Ally for this study can be calculated by dividing the natural log of two by the sum of k_1 and k_3; however, the only direct comparison that was available was for the hydrolysis at pH 5 and 45 °C.[1,4] Beyer et al. reported a

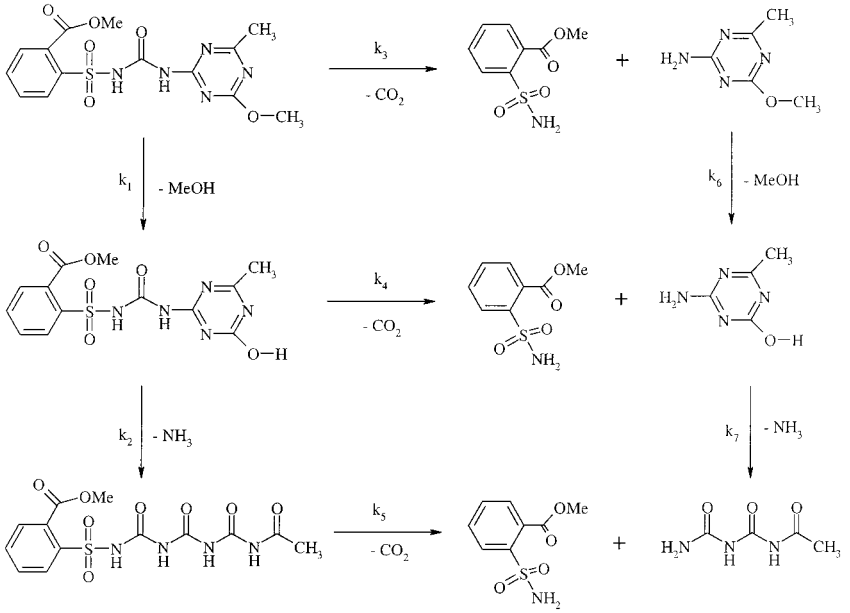


Figure 4. Proposed degradation pathway of Ally.

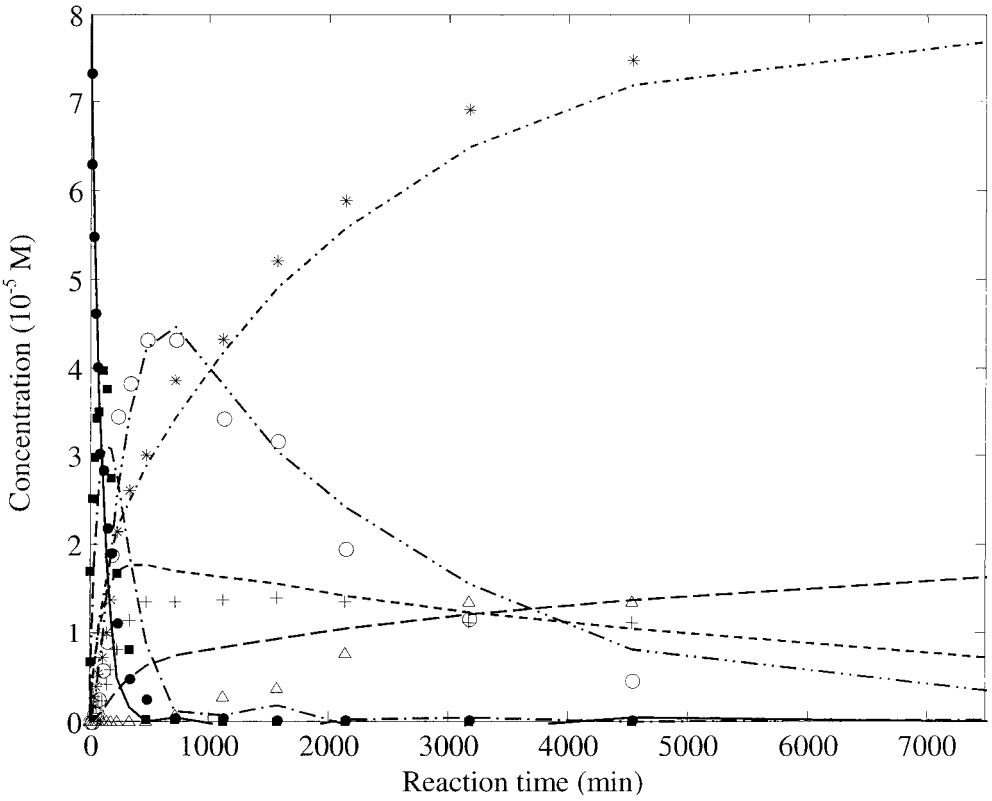


Figure 5. Time dependence of Ally at pH 2 and 45 °C and its degradation products as the ALS kinetic profiles (symbols) and the chemical model with the rate constants fit by Gepasi (lines) for Ally (— ●), OH−Ally (—·—·—·— ■), ring-opened Ally (------ +), benzenesulfonamide (-·-·-·- *), triazine (—··—··—·· ○), OH-triazine (— — — △), and ring-opened triazine (····· ×).

half-life of 2.1 days under these conditions, but our results indicated a half-life of 1.2 ± 0.3 days. Our other measurements were not directly comparable to any literature value because of mismatches in both temperature and pH.

The calculated optimal fit was verified by changing the value of the different rate constants and simulating the kinetic profiles. In all cases, the sum of squares error increased upon both positive and negative changes in the rate constants. The precision of the first three rate constants was determined by changing each rate constant individually and determining when the sum of squares error was 10% worse than the best fit. The last four rate constants showed several local minimums. Those values are much less precise, and it was not possible to determine the precision of their values.

CONCLUSION

This paper has shown the applicability of three-way chemometric methods to kinetic studies performed by LC-DAD and uses a general approach for the generation of the initial estimates. The overlapped components in the chromatogram were resolved, and all species were identified with the aid of MS and LC/MS experiments. The implementation of the closure constraint in the third dimension resulted in the determination of the absolute concentrations rather than relative ones. This was necessary to be able to implement the kinetic modeling procedure. This modeling was implemented using the Gepasi program, which calculated the rate constants that give the best fit to the kinetic profiles determined by ALS. The rates of degradation determined by this method agreed with the half-lives reported in the literature for acidic conditions.[1,4] The overall rates increased with lower pH and higher temperature. In addition, at these conditions, the predominant degradation pathway proceeded via the hydrolysis of the triazine-methoxy group, followed by ring opening, whereas direct hydrolysis of the sulfonylurea bridge was the minor pathway. This was different from what has been demonstrated to happen under ambient conditions, where the predominant pathway is hydrolysis of the sulfonylurea-bridge.[1]

The application of three-way curve-resolution methods to kinetic data is of great advantage when studying reaction pathways of environmental pollutants. The chemometric methods can resolve the kinetic profiles of the reacting species from interfering components, such as those found in soil and water samples. And even though this paper demonstrates the resolution of kinetic profiles from a degradation reaction, it can easily be used to observe the temporal or seasonal profiles of those components in the field. In addition, this approach is easily generalized to any number of kinetic studies in fields beyond environmental science, including pharmaceutical, biomedical, chemical, and materials sciences.

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