Characterization of Sulfonamides by Flow Injection and Liquid Chromatography–Electrospray Ionization-Mass Spectrometry after Online Photoderivatization

Abdulqawi Numan and **Neil D. Danielson***

Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056

Abstract

The online photochemical identification of six sulfa compounds, sulfadiazine, sulfamerazine, sulfamethoxazole, sulfaisoxazole (SIX), sulfamoxole (SMX), and sulfamethizole, are investigated using flow injection and liquid chromatography (LC)–electrospray ionization-mass spectrometry (MS). Although the identification of some of the mentioned sulfonamides can be performed by recognizing their respected protonated molecules, more positive MS identification of many of these compounds is possible because they undergo various phototransformation processes to produce different product profiles. The LC separation and online photolysis of a mixture containing the geometric isomers SIX and SMX is such an example. With no photolysis, the MS spectra for SIX and SMX are virtually identical, showing primarily the sodiated molecule at *m/z* 290 with a relative abundance of 100% in addition to a few small peaks caused by fragments. With photolysis, SMX is found to form multiple major ions from 100 to 241 amu. However, SIX follows a similar fragmentation pathway either with or without photolysis. Online photochemistry should be a viable approach to extend the capabilities of LC instruments interfaced to a single quadrupole MS detector.

Introduction

In general, the literature has revealed that electrochemical (EC), UV–vis, or fluorescence (FL) are the most frequent detection techniques applied with online photochemistry for a variety of aromatic amines such as amino acids and pharmaceuticals. Liquid chromatography (LC)–EC with photochemical derivatization permitted detection of pharmaceuticals such as phenobarbital, cocaine, methylphenidate, and several benzodiazepines below 1 mg/L (1). With online photolysis, several sulfonamides as well as other classes of nitrogenous pharmaceuticals generated primary amine photoproducts that could be reacted with *o*-phthalaldehyde and mercaptoethanol to form fluorescent isoindole derivatives (2). Flow injection (FI) of sulfamethazine using sulfite as a sensitizing agent with online photochemical-induced FL detection has been reported from 0.02 to 2 mg/L, and pharmaceutical formulations were analyzed (3). We have studied the photolysis behavior of several sulfa drugs via off-line and online approaches under different solvent and pH conditions. UV absorbance at a longer wavelength (330 nm), particularly for photolyzed sulfamethoxazole (SMO), permitted its FI determination in the presence of trimethoprim (4). The determination of photolyzed SMO by FL has also been recently reported (5). Although these detectors provide good quantitation, qualitative information about sample components is lacking. One of the primary advantages of mass spectrometry (MS) is definite identification of many organic compounds eliminating the uncertainty of matching chromatographic retention factors of standards with sample peaks.

The application of MS to the determination of antibiotic sulfa drugs is quite common in the literature. One review article (6) on the determination of sulfa drugs and other antibiotics using LC–MS and another review article (7) covering the analysis of sulfa drugs and antibiotics in meat and milk are available. Direct liquid introduction LC–triple quadrupole MS for the analysis of several sulfa drugs in biological samples was described in an early report (8). Although 18 sulfonamides were separated by LC with ion-spray tandem MS detection, special emphasis was directed toward sulfadimethoxine, which is used in the aquaculture industry (9). The separation and determination of 21 sulfa drugs in milk samples (10), 5 selected sufonamides including the banned compound dapsone in milk (11), 10 sulfonamides in honey (12), and 14 sulfa compounds in waste water (13) using gradient elution LC and electrospray ionization (ESI) MS–MS have been published. The mass spectra of sulfonamides tend to be dominated by the (*p*-aminophenyl)sulfonyl *m/z* 156 peak with small protonated molecule [MH^+] peaks. Even though identification was possible by using the $[MH]^+$ peak and subsequent fragmentation utilizing MS–MS, the approach was most successful for nonisomeric compounds. Sulfa isomeric pairs sulfamethazine–sulfisomidine and sulfamethoxypyridazine–sulfameter

* Author to whom correspondence should be addressed: email danielnd@muohio.edu.

could be slightly distinguished by relative intensities of the same fragment peaks, primarily that of *m/z* 156 (9). However, the isomers sulfaisoxazole (SIX) and sulfamoxole (SMX) basically followed the same fragmentation pattern with similar peak intensities (9). The differentiation of sulfa isomers SMX and SIX, both of which showed the same protonated molecule at *m/z* 268 by standard MS, has been reported by Bateman et al. who applied quasi MS–MS–MS in conjunction with capillary electrophoresis (14). Using in-source collision-induced dissociation (CID), the *m/z* 113 fragment common to both isomers was generated. MS–MS permits isolation of this specific ion and fragmentation to form characteristic isomeric ions. Recently, the fragmentation pattern of a wide range of compounds including amino-, acetylated-, methyl-, and unsubstituted-aromatic sulfonamides has been investigated using electrospray MS–MS with either ion trap or Fourier transform ion cyclotron resonance MS (15). However, a method using the lower cost single quadrupole MS detector designed for LC could potentially be useful for quality control analysis of sulfonamide samples.

Although no universal approach is available, the literature guidelines for confirmation criteria in gas chromatography–MS, LC–MS, and MS–MS require at least three specific ions (16,17) with a reproducibility of ± 5%. A reproducibility of 20% of the intensity ratio of diagnostic ions has been considered an acceptable criterion for confirmation purposes as well. Such a requirement was not easily attained for the determination of sulfamethazine using various instruments (6). More recently, a new approach defining a certain number (usually 3 or 4, depending on the hazardous nature of the compound) of identification points (IPs) was reported (18). Different analytical techniques such as LC with photodiode array detection and LC with fluorescence detection, as well as LC–MS, can each provide one IP. LC–MS–MS can potentially provide multiple IPs; ion recognition of four diagnostic ions with a relative intensity of $\geq$ 10% of the base peak is recommended for one IP for each type of MS scan. Logically, LC–MS with and without photochemistry could provide two or perhaps more IPs if experimental conditions are varied.

Applications in which MS is coupled with online photochemistry are not wide-spread. Some studies of ruthenium metal complexes with bipyridine, bipyrazine, and other similar ligands (19–21) showed ligand release from bidentate but not tridentate complexes. Photolysis products of aryl methyl ethers dissolved in methanol–water or water was studied quantitatively using membrane introduction MS (22). The rate constants for the generation of five products upon photolysis of the substituted triphenylethylene compound idoxifene were determined by online electrospray MS (23). Characterization of β-lactams in a quadrupole ion trap has been compared using photodissociation and collision-activated dissociation (24).

Applications involving LC with online photolysis and MS detection are also not common. Drugs such as barbiturates, O3-monoacetylmorphine–O6-monoacetylmorphine, and cannabidol–Δ^9-tetrahydrocannabinol could be distinguished by thermospray MS (25). In the same study, the online photolysis LC–MS analysis of psilocyabin in mushrooms was performed. *N*-nitrosodialkylamines in beer samples (26); nitrosation products in cosmetic material (27); and pesticides such as *N*-heterocyclic compounds, phenylureas, and carbamates (28) have all been characterized by online photolysis LC–MS. In the pesticide study, photosensitizers such as acetone and benzophenone caused additional photodegradation products to be formed upon photolysis of triazine compounds. We have recently reported that indole compounds such as 5-hydroxytryptophol and serotonin, which fragment similarly, can be easily distinguished by FI or LC with online photochemistry and MS detection (29).

This report describes the combination of online photochemistry with FI and LC–MS for the identification of closely related, pharmaceutically relevant sulfonamides such as sulfadiazine (SDZ), sulfamerazine (SMR), SMO, sulfamethizole (SMZ), SIX, and SMX. Upon photolysis, more extensive fragmentation with loss of the parent ion peak was observed for most of the compounds. To the best of our knowledge, this is the first application of online photoderivatization with MS to differentiate between geometric isomers such as SMX and SIX without the use of the MS–MS option. The long-term objective is to show that online photochemistry can expand the capability of a low-cost LC equipped with a single quadrupole MS detector.

Experimental

Reagents

All of the reagents used in this project were of analytical grade. The sulfa compounds (SDZ, SMR, SMO, SMZ, SIX, and SMX) were purchased from Sigma (St. Louis, MO). Ammonium formate (97%), formic acid (88%), and HCl (49%) were obtained from Fisher Scientific (Foster City, CA). The high-performance-LC-grade methanol (MeOH) and acetonitrile (ACN) were purchased from Burdick and Jackson (Muskegon, MI). Distilled, deionized water was prepared in our laboratory with a Millipore Milli-Q system (Billerica, MA). All mobile phases were filtered through 0.45-µm nylon membrane filters from Whatman (Middlesex, U.K.). The pH measurements were done using Corning model 443i pH meter (Corning, NY).

Instrumentation

All FI and LC experiments were conducted on a Agilent (Hewlett-Packard, Palo Alto, CA) series 1100 instrument equipped with a binary mobile phase delivery system, an autosampler with a vacuum degasser, and a variable wavelength UV–vis detector. The entire system was controlled with Agilent Chemstation software. For the high-performance liquid chromatographic (HPLC) separation, a Supelco C_{18} DB column with the dimensions of 250- × 2.1-mm i.d. (5-µm particle size and 150-Å pore size) was used. The photochemistry took place in a home-made knitted ethylenetrifluoroethylene (ETFE) tube reactor (2.5 m × 0.5 mm with a volume of 0.5 mL) that was knitted on a 1- × 1-cm plastic grid mesh. The fluoropolymer ETFE has a higher transparency than polytetrafluoroethylene (Teflon) (4). The plastic grid mesh was placed inside a metal shield that housed a low pressure 8-W Hg lamp (Aura Industries, Staten Islands, NY). The reactor was inserted between the autosampler and the UV–vis detector when FI was employed and between the LC column and the UV–vis detector when HPLC was used. A small fan was placed

directly over the reactor to ensure a consistent ambient temperature during the photolysis.

The LC system was interfaced to a Bruker Ion Trap Esquire-LC MS (Billerica, MA). A positive atmospheric pressure ESI source was used in all experiments. Nitrogen nebulization gas with a pressure of 38 psi, flow rate of 8 L/min, and drying temperature of 360°C was used. The capillary voltage was set to –4000 V and the end plate offset was –500. Other parameters that included skim 1 voltage (28.9 V), octopole (2.45 V), trap drive level (50%), trap drive (40.7), target (30,000), maximum accumulation time (50.00 ms), and spectra average (15 scans) were used for all analysis.

Procedure

In general, the standard sulfonamides were dissolved in a 10% MeOH or ACN–90% aqueous mobile phase and the solutions sonicated for 5 min or until no visible particles were seen. In some cases, the organic solvent composition was increased to 25%. This was done to avoid any solubility problem as observed with SDZ when a 2mM ammonium formate (pH = 3)–ACN (90:10 ratio) mobile phase was used. Mobile phase A was either water or buffer and mobile phase B was ACN or MeOH, depending on the experimental conditions. After warming up the photoreactor for at least 15 min, triplicate injections (30 µL) of each sample were made. The UV–vis signal was monitored at 330 nm, as previously described (4). For comparison purposes, triplicate injections for each sample were made when the photoreactor lamp was off. The scan range, between 50 and 350 amu, was used after checking one full scan trace for each sample in the range of 50 to 600 amu. In the case of FI, the flow rate and the photolysis time were 0.2 mL/min and 2.5 min, respectively.

The flow rate for all LC work was set for 0.3 mL/min, which gave a photolysis time of 1.7 min. A 30-µL sample of 150 mg/L SDZ and SMR was injected and separated using H_2O–ACN–formic acid. In this case, mobile phase A was 10:90% ACN–H_2O with 0.1% formic acid, and mobile phase B contained 100% ACN with 0.1% formic acid. The gradient started with 0% B and increased to 100% in 10 min. It was held constant for 2 min and then decreased sharply to 0% B and held there for 8 min. The isomers SMX and SIX with a concentration of 150 mg/L were separated using an ACN–ammonium formate gradient. The mobile phase A contained 2mM ammonium formate (pH = 3) and mobile phase B consisted of 100% ACN. The same gradient as described previously was used.

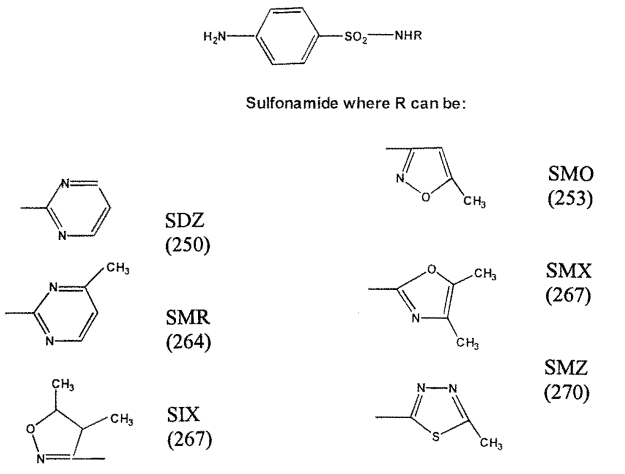

Figure 1. Chemical structures of selected sulfa compounds.

Results and Discussion

Mass spectra with no photolysis

The chemical structures and the molecular weights of the sulfonamides that were studied in this project are shown in Figure 1. The only difference exhibited by these compounds is the identity of the R-group. This closed resemblance leads to a common fragmentation pathway, which results in the formation of what is known as generic specific ions, as reported previously (6,7,13). These ions are formed when the bond between the sulfur and the nitrogen atoms is cleaved and the charge is associated with the $[H_2NPhSO_2]^+$ (*m/z* 156) fragment (12,14). Subsequent loss of SO_2 to give the aniline carbocation $+C_6H_5NH_2$ (*m/z* 92) is also possible. A rearrangement process can give the $[H_2NPhO]^+$ *m/z* 108 ion (15). Compound-specific ions are also possible to form when the charge is retained by the heteroaromatic amine containing group (R). For example, $[R\text{-}NH_3]^+$ (loss of $HNPhSO_2$) and $[R\text{-}NHSO_3]^+$ (loss of aniline) are possible. Loss of H_2SO_2 to form unknown fragments has also been recently reported (15). ESI mass spectra previously reported for sulfonamides generally show the base peak as *m/z* 156 with smaller $[MH]^+$, *m/z* 108, and *m/z* 92 peaks (9,12). Other more identifying peaks for a particular sulfonamide are quite low in intensity. A peak summary with intensities in parentheses for the sulfonamide isomer SIX is 156(100), 113(59), 108(19), 92(18), 139(1), and 175(1), and for SMX is 156(100), 113(36), 108(21), and 92(12) (9). The similarity of these peak summaries, with no $[MH]^+$ peaks noted, is striking.

Figure 2 represents the background corrected mass spectra of the sulfa compounds obtained with FI–ESI-MS, when the photoreactor lamp is off, using a MeOH–water (90:10) mobile phase. For SDZ (Figure 2A), the sodiated molecular ion $[MNa]^+$ is the base peak, typical for all the spectra in Figure 2. One reason for the presence of sodiated ions in these mass spectra is that some of the sulfa compounds were actually sodium salts, and no separation of sodium from the sulfonamide is possible by FI. Another possibility is instrument contamination caused by the ubiquitous nature of sodium. The molecular ion $[MH]^+$ and the 156 *m/z* peak are also evident for SDZ, again true for all the spectra. The compound-specific ion for SMR (Figure 2B) at *m/z* 110 is present but with a very low relative abundance. The sulfa SMO (Figure 2C) with a $[MH]^+$ at *m/z* 254 does not show the compound specific ion at *m/z* 99. The mass spectrum is dominated by the sodiated molecule at *m/z* 276. The isomers SIX (Figure 2D) and SMX (Figure 2E) exhibit the same fragmentation pathway with the exception that *m/z* 92 is not present in the mass spectrum of SMX. The *m/z* 113 peak that is considered compound specific ion for SMX and SIX is present in both mass spectra. The SMZ mass spectrum (Figure 2F) shows the sodium ion adduct as the base peak (100%) at *m/z* 293 in addition to the protonated molecule and the group specific ions with low intensity. The compound specific ion with *m/z* 116 is not formed under this experimental condition. The 108 *m/z* peak is evident for SDZ, SMO, and SMZ.

The mass spectral information in Figure 2 may be enough to distinguish some of the sulfonamides, however, unequivocal differentiation between the isomers SIX and SMX is not evident.

The effect of other solvents on the mass spectra of sulfa compounds when the photoreactor lamp is off is also studied. Solvent combinations of MeOH–H_2O (90:10, v/v) and ACN–H_2O (90:10, v/v) are considered in two separate experiments. Comparable results to the data shown in Figure 2 are obtained (data not shown). The mass spectra of the sulfonamides are also obtained with no photolysis employing an acidic mobile phase at pH = 3. Most of the compounds are characterized by the high abundance of the protonated molecules and the group specific ions (data not shown).

Photochemistry of sulfa compounds

The photochemistry of sulfonamides has been well documented. The formation of free radicals such as R'XS$^{\bullet}O_2$ and RS$^{\bullet}O_2$X and the elimination of SO_2 was an initial outcome upon the photo-irradiation of the sulfa compounds (30). The rupture of the N–S bond, loss of SO_2, photodeprotection to form free amine, C–S bond fission, and the formation of azobenzene and aniline could then occur. A bimolecular redox reaction to form RSO_3 and R–S from $ArSO_2^{\bullet}$ was also reported (31,32).

The comparison between the absorbance of the sulfa compounds with and without photolysis at 330 nm is depicted in Figure 3 using a 10:90 MeOH–H_2O mobile phase. These UV absorption data indicate the formation of the photodegradation products that have an absorbance at a longer wavelength compared with the starting materials. The absorbance of photolysis at

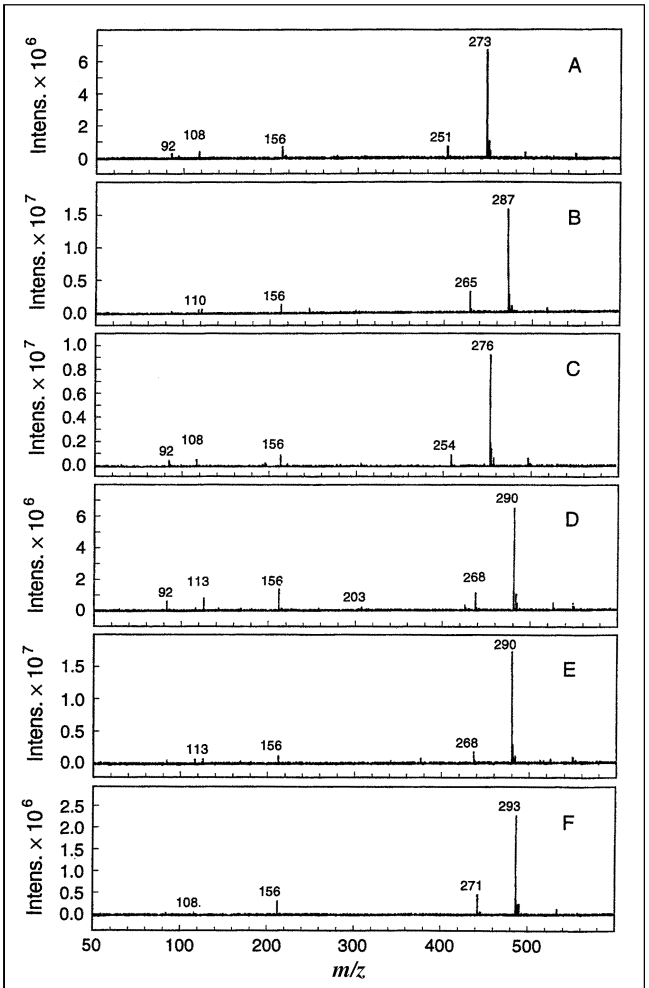

Figure 2. Representative mass spectra of selected sulfonamides without photolysis. For the figure, 100 mg/L of (A) SDZ, (B) SMR, (C) SMO, (D) SIX, (E) SMX, and (F) SMZ. Injection volume, 30 µL; flow rate, 0.2 mL/min; solvent, MeOH–H_2O (10:90). Other parameters as in the Experimental section.

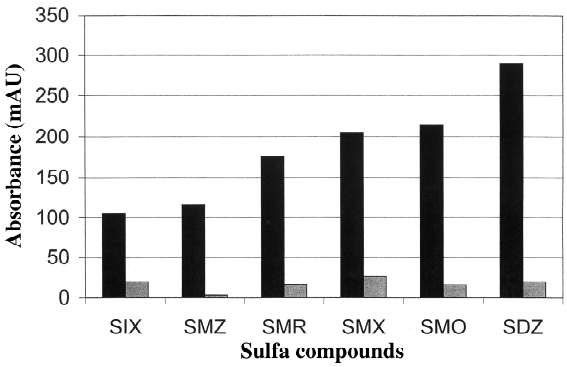

Figure 3. Average absorbance in milliabsorbance units (mAU) of the sulfonamides at 330 nm when photoreactor lamp was on (black scale) and when lamp was off (gray scale). Analyte concentration, 100 mg/L (n = 3); flow rate, 0.2 mL/min; and solvent, MeOH–H_2O (10:90).

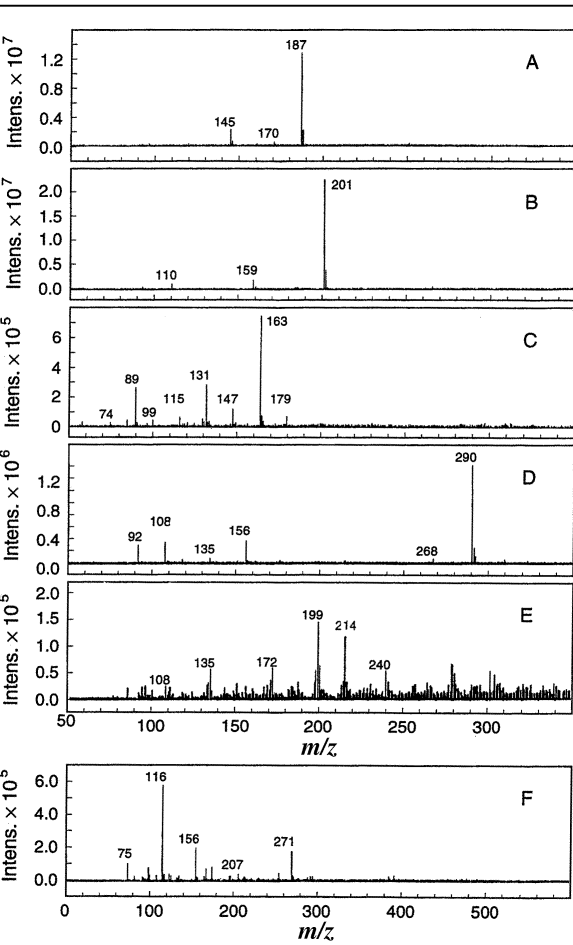

Figure 4. Representative mass spectra of photolyzed sulfa compounds in MeOH–H_2O (10:90). Abbreviations and other parameters as in Figure 2.

330 nm ranged from 5 to 13 times. The enhancement of the UV absorbance at 330 nm of the sulfa drugs upon photolysis using ACN–H_2O (90:10) ranged from 22 times for SIX to 59 times for SMO (data not shown). Using a 10:90% ACN–ammonium formate (pH = 3.0) mobile phase, the enhancement in the UV absorbance was comparable with those obtained in MeOH–H_2O (10:90). Possibly some of these products could aid in the differentiation of sulfa compounds by MS.

Mass spectra with online photolysis using a MeOH–H_2O mobile phase

Figure 4 represents the mass spectra of the sulfonamides with online photolysis using a MeOH–water (10:90) mobile phase. The relative standard deviation (n = 3) for the peak intensities for eleven major ions from 89 to 215 amu ranged from 0% to 13% with an average of 5.7%. The mass spectra of SDZ and SMR that differ only in one methyl group on the heterocyclic rings are characterized by the formation of two base peaks, *m/z* 187 (Figure 4A) and *m/z* 201 (Figure 4B), respectively. These ions may be formed by losing SO_2 from the protonated molecules $[MH\text{-}SO_2]^+$ of the corresponding compound. This has been observed previously (33). This photodegradation process is unique for these two compounds. The spectrum of SDZ shows an unidentified smaller peak with *m/z* 145. In Figure 4C, the disappearance of the protonated molecule (*m/z* 254) and the group-specific ions from the mass spectrum of SMO with online photolysis is noted. This phototransformation results in the formation of new ions *m/z* 163, 131, and 89 in addition to other ions with lower relative abundance including the compound specific ion at *m/z* 99. The base peak with *m/z* 163 might be formed by C–S bond fission, which is possible upon the photolysis of sulfa compounds. However, this ion has an extra proton that could be acquired from the solvent. When the preparation and the photolysis of SMO is conducted in 100% ACN, in which the chance of acquiring a proton from the solvent is minimal, the mass spectrum is dominated by the compound specific ion with *m/z* 99 (data not shown). This ion has a relative abundance of 100% and is formed by the cleavage of the S–N bond.

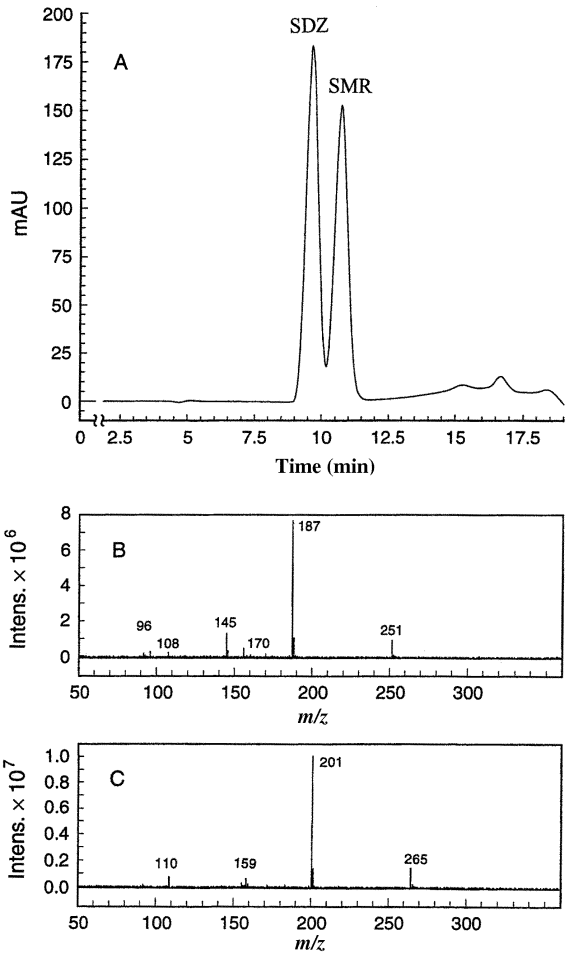

Figure 5. LC–UV–MS of a mixture containing 150 mg/L SDZ and SMR with photolysis. For the figure, (A) UV trace at 330 nm, SDZ (t_R = 9.6 min), and SMR (t_R = 10.7 min); (B) background corrected mass spectrum of SDZ; and (C) background corrected mass spectrum of SMR. Flow rate, 0.3 mL/min; photolysis time, 2 min; and gradient elution of mobile phase A with B as explained in the Procedure section.

Online photolysis does not seem to make a major change in the mass spectrum of SIX (Figure 4D). The sodiated molecule, in addition to a few minor generic ions, still dominates. The detection limit based on a clearly defined 290 *m/z* peak as compared with other peaks was 0.5 mg/L. The sulfa SMX, on the other hand, is found to follow a unique phototransformation pathway leading to the formation of major ions such as *m/z* 240, 214, and 199, as shown in Figure 4E. The 108 *m/z* peak not seen in the unphotolyzed spectra is now evident in both the SIX and SMX photolyzed spectra. These data for SIX and SMX were still reproducible even when taken 6 months apart. The detection limit for SMX was estimated to be 10 mg/L; below this value, the mass spectra showed considerable variation. Between 10 and 50 mg/L, the dominant peak was *m/z* 164, which was different than that at higher concentrations. Qualitative reproducibility of SMX photolysis may be an issue if the concentration of SMX is quite varied.

The online photolysis of SMZ (Figure 4F) results in the formation of a distinguishable mass spectrum characterized by the base peak at *m/z* 116. This ion is specific for SMZ, which is formed by the cleavage of the S–N bond. The cleavage of the S–N bond, leading to the formation of compound specific ion with a 100% relative abundance in aqueous organic solvent, was observed only for SMZ.

Mass spectra with online photolysis using an ACN–aqueous mobile phase

Generally, the presence of 10% ACN instead of MeOH in the aqueous mobile phase is found to produce similar results as those reported in Figure 4 for most of the sulfa compounds. The only difference observed in the mass spectrum for the photolysis of SMX is the presence of a major ion at *m/z* 151 and the base peak now at *m/z* 134.

The phototransformation of the sulfa compounds is investigated using a 10:90 ACN–ammonium formate (pH = 3.0) mobile phase. This apparent pH is well below the pK_a values of the secondary amine (range 5.5–7.0) and slightly higher than the pK_a of the primary amine attached to the aromatic ring (~ 2 for all sulfonamides) (17). The mass spectra of SDZ, SMR, and SMX are similar to those presented in Figure 4. The mass spectrum of

SMO shows a dominant peak (100% relative intensity) with *m/z* 204. The only difference observed in the mass spectrum of SIX is the increase in the relative abundance of the generic ions *m/z* 156 [61.4%, relative standard deviation (RSD) = 0.3%], *m/z* 108 (62.0, RSD = 2.3%), and *m/z* 92 (51.5%, RSD = 9.6%) for triplicates (data not shown).

LC–MS with and without online photolysis

The applicability of the developed method for the separation and identification of two pairs of sulfonamides in two different mixtures is tested using online photolysis and LC–UV–MS. Representative LC–UV and MS outputs with online photolysis for the separation of SDZ and SMR are shown in Figure 5. The photoreactor does introduce some band broadening, making the use of gradient elution as well as a faster flow rate (0.3 mL/min) a desirable alternative even though complete baseline resolution of the peaks is not necessary for MS characterization. Without the photoreactor, the analyte has a peak width of less than 0.5 min at the baseline (data not shown). The increase in the flow rate to 0.3 mL/min decreases the photolysis time to 1.7 min compared with 2.5 min as in FI. This change does not affect the efficiency of online photolysis substantially. SDZ and SMR undergo phototransformation in a similar way to form the base peaks *m/z* 187 and 201, respectively, as described in the FI experiment. The MS results of the same separation without photolysis are also comparable with the data shown in Figures 2A and 2B.

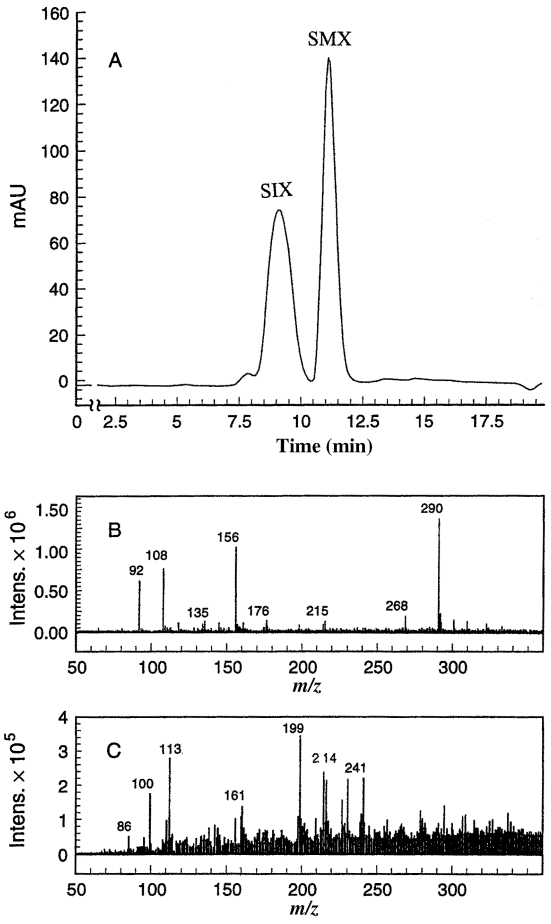

Figure 6. LC–UV–MS of 150 mg/L mixture of SIX and SMX with photolysis. For the figure, (A) UV trace at 330 nm, SIX (t_R = 9.1 min), and SMX (t_R = 11.1 min); (B) background subtracted mass spectrum of SIX; and (C) background subtracted mass spectrum of SMX. Other parameters as in Figure 5.

The baseline separation of a mixture containing the isomers SMX and SIX is shown in Figure 6A. As expected, the mass spectrum of SIX with online photolysis (Figure 6B) exhibits the presence of the $[MNa]^+$ peak at *m/z* 290 with a relative abundance of 100% in addition to the generic ions *m/z* 156 (71.6%, RSD = 4.0), *m/z* 108 (56.9%, RSD = 1.8), and *m/z* 92 (45.1%, RSD = 3.0%) for *n* = 3. Similar results were obtained with no photolysis (data not shown). The background correction of the SMX mass spectrum (Figure 6C) with photolysis revealed the formation of few major ions such as *m/z* 241, 214, 199, and 113, with relative abundance RSD values ranging from 0% to 7%. The *m/z* 113 peak, quite intense using this mobile phase as compared with Figure 4E, is considered by the literature as a compound characteristic ion for SMX and SIX (8,14). However, this ion is only present in the mass spectrum of SMX. The noise level in the SMX spectrum seems to have increased by a factor of 2–3 using this mobile phase as compared with MeOH–H_2O. The issue of detection limit is important if biological samples containing sulfa drug residues are of interest; however, this method should work for serum samples in which levels of SMX and SIX are in the 50–100-mg/L range (34). Using FI–MS with or without photolysis, mass spectra of samples of SMX (50 mg/L) and SIX (50 mg/L) mixed with bovine serum albumin (400 mg/L) showed no significant changes as compared with corresponding mass spectra without protein.

Conclusion

In this work, we have demonstrated the potential use of online photoderivatization in conjunction with FI–ESI-MS and LC–ESI-MS for the identification of closely related aromatic sulfa compounds. Without photolysis, sulfonamides were found to follow basically a common fragmentation pathway way leading to the formation of group specific ions. Thus, the clear distinction between isomers was not possible using conventional MS. Upon photolysis, however, the sulfa compounds underwent different phototransformation pathways that made the differentiation between the isomers and other similarly structured compounds possible. Because loss of the protonated molecular ion peak caused by photolysis was common for these sulfonamides, mass spectra taken both with and without photolysis is recommended for identification. Other sulfonamide isomer pairs such as sulfamethazine–sulfisomidine (9), sulfamethoxypyridazine–sulfameter (9), and sulfadimethoxine–sulfadoxine (12) also have very similar ESI mass spectra and could potentially be more easily distinguished using online photochemistry. Online photochemistry should in general be a viable option for extending the capability of any commercially available electrospray single quadrupole MS designed specifically for LC detection.

Acknowledgments

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