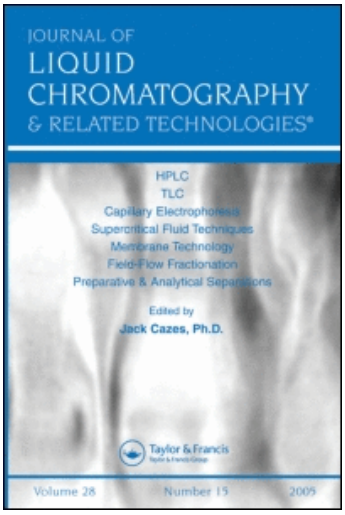

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Wang, Tao , Chen, Yadan W. , Zheng, Hong and Novak, Thomas J.(1998) 'Subambient Temperature Reversed-Phase High-Performance Liquid Chromatography and Hydrolysis Kinetics of a Para-Substituted Benzenesulfonyl Chloride', Journal of Liquid Chromatography & Related Technologies, 21: 9, 1359 — 1377
To link to this Article: DOI: 10.1080/10826079808005883
URL: http://dx.doi.org/10.1080/10826079808005883

J. LIQ. CHROM. & REL. TECHNOL., 21(9), 1359–1377 (1998)

SUBAMBIENT TEMPERATURE REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND HYDROLYSIS KINETICS OF A PARA-SUBSTITUTED BENZENESULFONYL CHLORIDE

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ABSTRACT

It was demonstrated that a para-substituted benzenesulfonyl chloride could be chromatographed in reversed-phase HPLC system using a C_{18} column which was cooled to 6°C with acetonitrile/0.1% H_3PO_4 (pH = 2.6) as mobile phase. The study of the hydrolysis kinetics of this sulfonyl chloride in a batch reactor, under different conditions, provided useful information on the influence of temperature and water concentration on the pseudo-first-order hydrolysis rate constant. The determination of apparent hydrolysis rate constant in a chromatographic reactor enabled the assessment of the suitability of a reversed-phase HPLC method for the determination of impurity profile of this sulfonyl chloride material. It was shown that the method was suitable for its purpose. It was also determined that, in the chromatographic reactor, the hydrolysis rate constant in the stationary phase was approximately an order of magnitude smaller than that in the mobile phase.

INTRODUCTION

In the synthesis of a pharmaceutical compound, a para-substituted benzenesulfonyl chloride $RC_6H_4SO_2Cl$ (named RP-sulfonyl chloride in this paper) is used as a key raw material, a material whose chemical structure is incorporated into the structure of the final drug substance. It is, therefore, important to have an analytical method for the determination of the impurity profile of this material in order to ensure its quality which, in turn, affects the quality of the final drug substance. It is known that sulfonyl chlorides are reactive with water, forming the corresponding sulfonic acids as the hydrolysis products.[1] Therefore, in addition to the determination of other impurities present in the bulk RP-sulfonyl chloride, it is also necessary to determine the level of RP-sulfonic acid ($RC_6H_4SO_2OH$) which may exist in the RP-sulfonyl chloride powder as a hydrolysis product caused by either the adsorbed moisture in the RP-sulfonyl chloride powder or trace amount of water present during the preparation of the RP-sulfonyl chloride material.

High-performance liquid chromatography (HPLC) has been widely used in the pharmaceutical industry as a routine analytical technique for the determination of impurity profiles of bulk pharmaceutical chemicals and their synthetic intermediates. The high efficiency, speed, and ease of operation offered by HPLC are advantageous over other techniques for impurity profile determination. It was, therefore, desirable to develop a HPLC method for the determination of the impurity profile of the RP-sulfonyl chloride material.

Sulfonyl chloride compounds have been extensively used as derivatizing reagents in the HPLC separations of a variety of compounds.[2-10] Examples of the sulfonyl chloride compounds used in derivatization include 5-dimethylaminonaphthalene-1-sulfonyl chloride,[2-4] 4-dimethylamino-azobenzene-4′-sulfonyl chloride,[4-6] and acenaphthene-5-sulfonyl chloride[7] for derivatizing amino acids, p-toluenesulfonyl chloride[8] and (1S)-(+)-camphor-10-sulfonyl chloride[9] for derivatizing amines, and 2-fluorene-sulfonyl chloride[10] for sensitive UV-fluorescent detection of phenols. However, a recent literature survey did not reveal any reports on the direct chromatography of sulfonyl chloride compounds. Therefore, it was of interest to develop a direct HPLC method for RP-sulfonyl chloride analysis.

Due to the reactivity of sulfonyl chloride compounds with water and alcohols,[1] normal phase chromatography, using aprotic solvents and a cyano (CN) column (rather than silica or diol columns which have excessive -OH groups on the stationary phase), would be preferred to avoid on-column degradation. However, our experiments showed that the RP-sulfonic acid interacted with the CN column so strongly that it could not be eluted using a

mobile phase as strong as 100% tetrahydrofuran,[11] making the monitoring of this hydrolysis product difficult. Therefore, a reversed-phase method which could elute both RP-sulfonyl chloride and RP-sulfonic acid was desirable, provided that a method of controlling the on-column hydrolysis of RP-sulfonyl chloride was available.

The use of reduced and cryogenic column temperatures is a useful technique to allow high-performance liquid chromatographic separation of unstable compounds.[12] Applications of this technique include separation of thermally labile metal diketonate complexes[12-14] and chromatography of an active mesylate.[15] In our case, the use of subambient column temperature appeared to be an attractive method of controlling the on-column hydrolysis of RP-sulfonyl chloride.

This present paper describes a HPLC method that involves the direct chromatography of RP-sulfonyl chloride in the reversed-phase mode using subambient column temperature for the determination of the impurity profile of RP-sulfonyl chloride material. Hydrolysis kinetics of RP-sulfonyl chloride in a batch reactor and a chromatographic reactor was investigated in order to evaluate the suitability of the impurity profile method. The pseudo-first-order hydrolysis reaction rate constants under different conditions (temperature, water concentration in the reaction medium, and reactor type) were determined and compared to gain information about the hydrolysis reaction.

EXPERIMENTAL

Instrumentation

The chromatographic system consisted of a SpectraSystem P4000 HPLC pump, an AS3000 autosampler equipped with a 20 μL sample loop and a sample tray whose temperature could be controlled, and a UV2000 variable wavelength UV detector (Thermo Separation Products, Piscataway, NJ). The column used was an Inertsil 5 ODS-2 column (5 μm, 250 x 4.6 mm, MetaChem Technologies Inc, Torrance, CA). A Model 7955 column temperature controller (Jones Chromatography, Lakewood, CO) was used to control the column temperature. The temperatures of the autosampler tray and the column housing in the column temperature controller were monitored using a thermometer. Chromatograms were acquired and processed by a PE Nelson data system equipped with Access*Chrom software (version 1.9.5; PE Nelson, Cupertino, CA). Mass spectra from LC/MS were acquired using a Finnigan

Model 7000 TSQ mass spectrometer (San Jose, CA). Atmospheric pressure ionization was used, incorporating an electrospray interface operated in the negative ion mode. In the LC/MS experiments, the acidic mobile phase modifier was trifluoroacetic acid.

Materials

The HPLC grade acetonitrile was purchased from Fisher Scientific (Fair Lawn, NJ). The acetonitrile used for sample preparation was dried over 4 Å molecular sieves. The phosphoric acid (85%) was purchased from Fisher Scientific. The RP-sulfonyl chloride was provided by the Process Research and Development Department of Merck Research Laboratories (Rahway, NJ). The RP-sulfonic acid solution was prepared by mixing 3 mL water with 7 mL RP-sulfonyl chloride solution in acetonitrile (0.1 mg/mL) and leaving the solution at room temperature for 24 hours to convert the RP-sulfonyl chloride to RP-sulfonic acid. The chromatographic peak of the hydrolysis product was confirmed by LC/MS to be due to the RP-sulfonic acid as the deprotonated molecular ion and the trifluoroacetate ion adduct.

Chromatographic Conditions for Determination of Impurity Profile of RP-Sulfonyl Chloride Material

The column temperature was maintained at 6°C. The flow rate was 1.0 mL/min. The mobile phase components were: 0.1% phosphoric acid in water with pH adjusted to 2.6 with 5 N NaOH (A) and acetonitrile (B). A mobile phase gradient program was used: 30%A/70%B to 10%A/90%B in 20 min, followed by a 10 min hold. The UV detection was performed at 220 nm.

Determination of Hydrolysis Rate Constants of RP-Sulfonyl Chloride in Batch Reactor

For the determination of the hydrolysis rate constants of RP-sulfonyl chloride in a batch reactor at 25°C, approximately 7.5 mg of RP-sulfonyl chloride was weighed into a 50 mL volumetric flask and dissolved in 35 mL or 45 mL dry acetonitrile. An aqueous solution of 0.1% H_3PO_4 with pH adjusted to 2.6 was then added to the flask to bring the volume to the mark so that the reaction medium had 30:70 or 10:90 (v/v) water:acetonitrile. After mixing, the solution was transferred into a HPLC autosampler vial and injected into the HPLC system described in the instrumentation section using acetonitrile as

mobile phase with a flow rate of 0.5 mL/min. The column temperature was 25°C. Detection was at 220 nm. The sample vial was maintained at 25°C in the autosampler and subsequent injections of the solution from the same vial were made at 44 min intervals. The area count of the RP-sulfonyl chloride peak was recorded for each reaction time point.

For the determination of rate constants at 6°C, the sample preparation procedure was the same except that the acetonitrile solution of RP-sulfonyl chloride and the aqueous solution of 0.1% H_3PO_4 (pH = 2.6) were chilled to 5°C before mixing. After mixing, the solution was immediately transferred into the HPLC autosampler vial which was maintained at 6°C in the autosampler throughout the experiment. Injections of the solution from the same vial were made at 44 min intervals. The HPLC conditions were the same.

Determination of Hydrolysis Rate Constant of RP-Sulfonyl Chloride in Chromatographic Reactor

For the determination of the hydrolysis rate constant in the chromatographic reactor, the same HPLC system was used with 30:70 (v/v) 0.1% H_3PO_4 (pH = 2.6):acetonitrile as mobile phase. The column temperature was maintained at 6°C. The RP-sulfonyl chloride sample solution was made in dry acetonitrile at a concentration of 0.1 mg/mL. p-Terphenyl was added in the sample solution as an inert internal standard at a concentration of 0.04 mg/mL. The sample was injected into the HPLC system with constant mobile phase flow rates at 0.15, 0.3, 0.4 and 0.7 mL/min, respectively. The peak areas of RP-sulfonyl chloride and p-terphenyl, along with their retention times obtained at each flow rate, were recorded. The retention time of non-retained compound (t_0) was measured with uracil.

RESULTS AND DISCUSSION

The Reversed-Phase HPLC Method for Impurity Profile Determination

A number of publications reported the dependence of the hydrolysis rate constants of various sulfonyl chloride compounds, including benzenesulfonyl chloride, on the pH of the reaction solutions.[16-18] Basically, the rate constants remained unchanged at the pH range below 7.0 and increased with pH at the pH range above 7.0. The pH of the mobile phase used in our HPLC method was chosen to be 2.6, based on the considerations of minimizing on-column

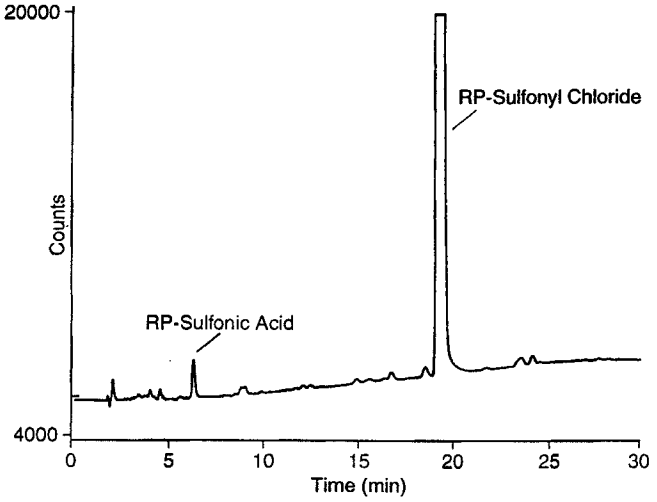

Figure 1. Impurity profile chromatogram of a typical RP-sulfonyl chloride sample. Column: Inertsil 5 ODS-2 (5 μm, 250 x 4.6 mm); column temperature: 6°C; mobile phase gradient: 30%A/70%B to 10%A/90%B in 20 min followed by a 10 min hold, where A is 0.1% phosphoric acid in water with pH adjusted to 2.6 and B is acetonitrile; flow rate: 1.0 mL/min; UV detection: 220 nm.

hydrolysis of RP-sulfonyl chloride and providing appropriate separation. Subambient column temperature (6°C) was also used to reduce the on-column hydrolysis. Figure 1 shows the chromatogram of a typical RP-sulfonyl chloride sample obtained using the impurity profile method. The method provided adequate separation of the RP-sulfonic acid and other impurities from the RP-sulfonyl chloride, which eluted at a reasonable retention time (19.3 min). Both RP-sulfonic acid and RP-sulfonyl chloride showed excellent peak shapes.

The major concern in using the reversed-phase method was that the on-column hydrolysis of RP-sulfonyl chloride might occur to such an extent that the levels of RP-sulfonic acid and RP-sulfonyl chloride determined by the method would deviate from their actual levels in the sample.

Therefore, it was necessary to study the kinetics of the hydrolysis and determine the hydrolysis reaction rate constant of RP-sulfonyl chloride in order to evaluate the extent of on-column hydrolysis that could occur during the 19.3 min retention time.

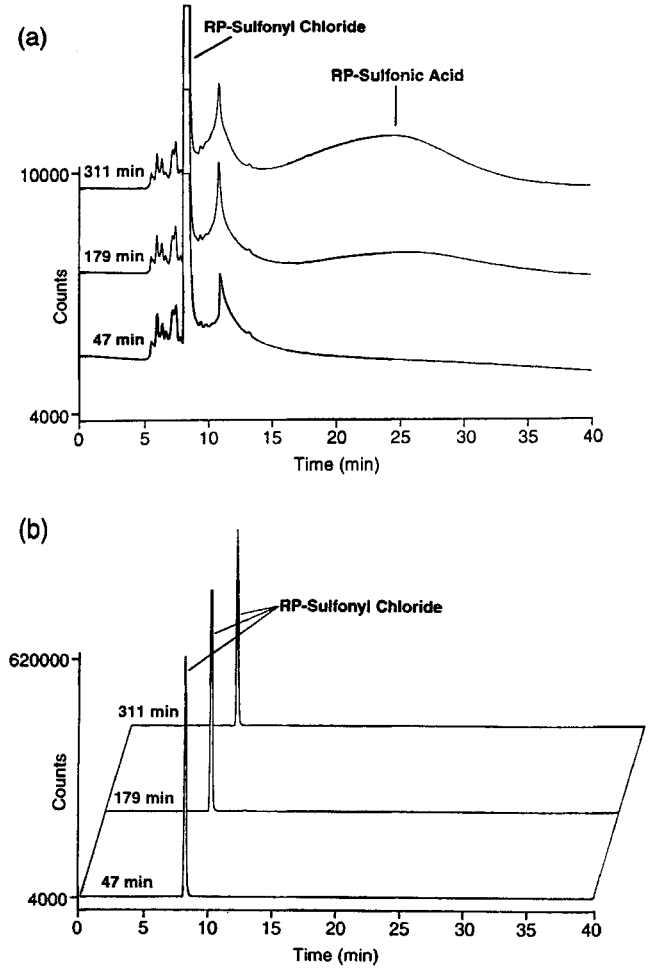

Figure 2. Chromatograms of batch reactor samples at different reaction times as shown in (a) detailed scale and (b) full scale. Reaction medium: 0.1% phosphoric acid (pH = 2.6)/acetonitrile (10/90, v/v); reaction temperature: 25°C. HPLC conditions: Inertsil 5 ODS-2 column (5 μm, 250 x 4.6 mm) with acetonitrile as mobile phase at a flow rate of 0.5 mL/min; column temperature: 25 °C; UV detection: 220 nm.

Determination of Hydrolysis Rate Constants in a Batch Reactor

Before determining the hydrolysis rate constant of RP-sulfonyl chloride in the chromatographic system, it was of interest to first determine the rate

constants in a batch reactor under different conditions in order to gain information about the hydrolysis reaction itself without involving the chromatographic process.

Robertson and Rossall[19,20] reported that the hydrolysis of a series of para-substituted benzenesulfonyl chloride compounds followed pseudo-first-order reaction kinetics. In our batch reactor study, the hydrolysis occurred when RP-sulfonyl chloride in acetonitrile solution was mixed with the aqueous solution of 0.1% H_3PO_4. The molar concentration of water in the mixture (5.6 M or 16.7 M) was so much larger than the mole concentration of RP-sulfonyl chloride (approximately 4 x 10^{-4} M) that the water concentration remained virtually unchanged; only the concentration of RP-sulfonyl chloride decreased noticeably as the hydrolysis proceeded. When the hydrolysis is a pseudo-first-order reaction, the following equation exists:

$$\ln[C] = \ln[C_o] - kt \qquad (1)$$

where C is the concentration of RP-sulfonyl chloride at time t, C_o is the concentration of RP-sulfonyl chloride at the beginning of the reaction, and k is the pseudo-first-order reaction rate constant. If samples from the reactor are taken and analyzed for RP-sulfonyl chloride concentration at certain reaction time points, the hydrolysis rate constant can be determined from the slope of the plot of ln[C] versus t.

Among the different techniques for analyzing the concentrations of components of interest in reaction mixtures, HPLC is a very useful tool because of its ability to simultaneously separate and quantitate the different components with high speed, reliability, and relatively simple operation. Applications of HPLC to reaction studies have been reported.[21-23] In our present study, HPLC was used to measure the peak area of RP-sulfonyl chloride in the reaction mixture at different time points of the hydrolysis reaction. Acetonitrile was used as mobile phase to avoid on-column hydrolysis of RP-sulfonyl chloride. A number of chromatograms of the reaction samples taken at various reaction time points under one set of reaction conditions are shown in Figure 2. The traces in Figures 2(a) and 2(b) represent the same chromatograms, respectively, but they are shown in different scales. Figure 2(a) demonstrates the separation of RP-sulfonyl chloride from the impurities and shows the growth of the RP-sulfonic acid peak with reaction time; whereas, Figure 2(b) depicts the decrease of the size of the RP-sulfonyl chloride peak with reaction time.

By comparing the chromatograms in Figures 1 and 2(a), one can notice the major difference caused by changing the mobile phase from acetonitrile-water mixture to acetonitrile — the elution order of RP-sulfonyl chloride and

RP-sulfonic acid was reversed. Horvath's research group reported dual retention mechanisms involving both solvophobic and silanolphilic interactions for silica-bonded hydrocarbonaceous stationary phases in reversed-phase systems.[24] The authors observed that the retention behaviors of crown ethers and amino peptides on silica-bonded hydrocarbonaceous stationary phases with binary hydro-organic eluents were not regular as expected on the basis of the solvophobic effect. When retention factors of crown ethers and amino peptides were plotted against the volume fraction of water in the eluent, the authors observed minima. The irregular retention behavior was attributed by the authors to silanolphilic effect (solute interactions with residual silanol groups on the surface of the stationary phase) which became dominant when the eluent was rich in organic solvent. The reversed elution order of RP-sulfonyl chloride and RP-sulfonic acid observed in our case was probably also due to the silanolphilic effect which became dominant when the eluent was rich in acetonitrile. Compared with RP-sulfonyl chloride, RP-sulfonic acid is expected to have stronger hydrogen bondings with the silanol groups.

Therefore, it is reasonable to expect RP-sulfonic acid to have longer retention time when the silanolphilic effect becomes dominant. The broad peak shape of RP-sulfonic acid in Figure 2(a) could also be due to strong hydrogen bondings between the RP-sulfonic acid and the residual silanol groups. In any case, the separation in Figure 2 allowed quantitation of RP-sulfonyl chloride.

Since the concentration C of RP-sulfonyl chloride is proportional to the area count A of the chromatographic peak of RP-sulfonyl chloride, i.e., $C = mA$, where m is a constant, equation (1) can be expressed as:

$$\ln[A] = \ln[A_o] - kt \qquad (2)$$

where A_o is the area count of RP-sulfonyl chloride peak at the beginning of the reaction. Therefore, in our study, ln[A] values were plotted versus t values and the pseudo-first-order reaction rate constant was obtained from the slope of the plot.

In our study, reaction rate constants at four different sets of conditions were determined (see Table 1) by using the procedure described in the experimental section and plotting ln[A] versus reaction time t (Figure 3). The plots obtained under the different conditions were all linear. Since a plot that is really curved may appear to be straight within experimental error over a small range, reaction data should be taken to at least 75% completion of the reaction.[25] In our study, the data for plot (a) in Figure 3 were taken to over 80% completion of the reaction. The excellent linearity ($r^2 = 1.000$)

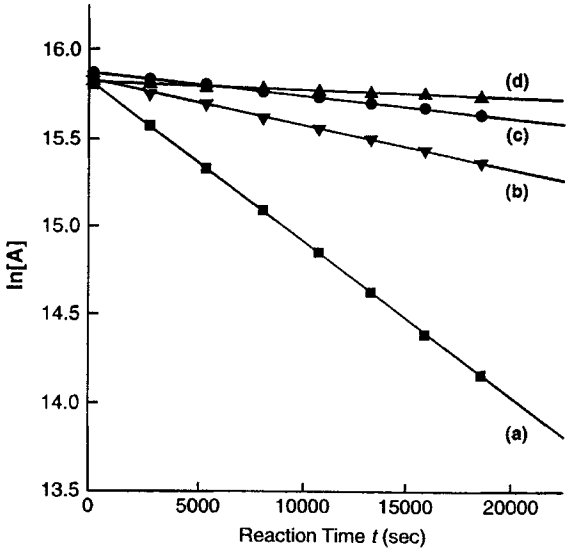

Figure 3. Plots of logarithm of peak area A of RP-sulfonyl chloride versus reaction time t obtained from a batch reactor under different conditions. (a) Water/acetonitrile ratio = 30/70 (v/v), 25°C, r^2 = 1.000; (b) water/acetonitrile ratio = 10/90 (v/v), 25 °C, r^2 = 0.999; (c) water/acetonitrile ratio = 30/70 (v/v), 6 °C, r^2 = 0.998; (d) water/acetonitrile ratio = 10/90 (v/v), 6 °C, r^2 = 0.989.

Table 1

Pseudo-First-Order Hydrolysis Reaction Rate Constants Under Different Conditions in a Batch Reactor

Temperature (°C)	Reaction Medium (A) aq[a]/ACN[b] = 30/70 (v/v)	Reaction Medium (B) aq[a]/ACN[b] = 10/90 (v/v)
6	$k = 1.3 \times 10^{-5}$ (sec^{-1}) SD[c] $= 2.0 \times 10^{-7}$ (sec^{-1})	$k = 4.5 \times 10^{-6}$ (sec^{-1}) SD[c] $= 2.0 \times 10^{-7}$ (sec^{-1})
25	$k = 8.9 \times 10^{-5}$ (sec^{-1}) SD[c] $= 2.3 \times 10^{-7}$ (sec^{-1})	$k = 2.5 \times 10^{-5}$ (sec^{-1}) SD[c] $= 2.7 \times 10^{-7}$ (sec^{-1})

[a] aq. = 0.1% phosphoric acid in water with pH adjusted to 2.6; [b] ACN = acetonitrile; [c] SD = standard deviation.

demonstrates that the reaction is indeed pseudo-first-order. Swain and Scott reported that the hydrolysis rate constant of benzenesulfonyl chloride in 50%/50% water-acetone mixture at 25 °C was 2.4 x 10^{-4} sec^{-1}.[26] According to the results of Robertson and Rossall,[19] the variation of hydrolysis rate constant of benzenesulfonyl chloride caused by different para-substituents was within an order of magnitude. Considering these, the hydrolysis rate constant of RP-sulfonyl chloride (8.9 x 10^{-5} sec^{-1}), determined in 30%/70% water-acetonitrile mixture at 25°C, is reasonable.

The data in Table 1 indicate that, when the reaction temperature was lowered from 25°C to 6°C, the reaction rate constant decreased approximately 6-fold, indicating the benefit of reduced temperature in reducing the reaction rate. The reaction media in columns A and B in Table 1 were chosen to simulate the mobile phase composition at the beginning and the end of the solvent gradient program in the HPLC impurity profile method. The k values in column A are approximately three times those in column B under the same temperatures. This is attributed to the fact that the concentration of water in reaction medium A was three times that in reaction medium B. Since the hydrolysis reaction is pseudo-first-order, the water concentration in the reaction medium is expected to affect the pseudo-first-order rate constant k. Therefore, the approximately three-fold ratio between the k values in column A and column B was reasonable.

From the two k values obtained at 6°C, it was determined that 1.5% and 0.5% hydrolysis would occur within 19.3 min in the batch reactor with water:acetonitrile ratios in the reactor being 30:70 and 10:90 (v/v), respectively. These values were calculated using equation (1), with t = 1158 sec, k = 1.3 x 10^{-5} sec^{-1} and 4.5 x 10^{-6} sec^{-1}, respectively. In these two situations in the batch reactor, the water:acetonitrile ratio was maintained constant in each case. If the percentage of water in the reaction medium is changed from 30% to 10% in 20 min, as in the situation of the mobile phase in the gradient program of the impurity profile method, the overall hydrolysis rate constant in the reactor in this 20 min time interval would be between 1.3 x 10^{-5} and 4.5 x 10^{-6} sec^{-1}. If the average of the two k values is used to estimate the overall hydrolysis rate constant in such a reactor with changing water concentration, the extent of hydrolysis occurring in the reactor within 19.3 min would be 1.0%.

In the HPLC impurity profile method, the situation was different from that in a batch reactor with changing water concentration in the reaction medium. In addition to the hydrolysis reaction in the mobile phase, the situation involved a chromatographic process in which the RP-sulfonyl chloride molecules spent a significant amount of time in the C_{18} stationary phase while traveling through

the column. The hydrolysis rate constant in the stationary phase was expected to be smaller than that in the mobile phase, due to restricted access of water molecules to the RP-sulfonyl chloride molecules in the stationary phase. Therefore, the overall apparent hydrolysis rate constant in the chromatographic system was expected to be smaller than that in the batch reactor and the extent of on-column hydrolysis in 19.3 min was expected to be less than 1.0%. To account for the effect of the chromatographic process involved, the apparent hydrolysis rate constant in a chromatographic reactor was determined.

Determination of Hydrolysis Rate Constant in a Chromatographic Reactor

A chromatographic reactor is a chromatographic system in which both reaction and separation take place. In a liquid chromatographic reactor, after a sample of a reactant is injected, the reaction takes place in the column as the reactant travels through the column and reacts with other species that are present in the column. A reactor chromatogram can then be obtained as the species involved in the reaction are separated by the column and eluted through the detector. The use of a liquid chromatographic column as a reactor to study the reaction kinetics and kinetic processes has been reviewed.[27] Applications of liquid chromatographic reactors to study reaction kinetics,[28] as well as to characterize a chemically bonded stationary phase using kinetics in the LC reactor,[29] have been reported.

The commonly used method for determination of reaction rate constants in chromatographic reactors is the "inert standard" method.[27] For a pseudo-first-order reaction, if a known amount of inert standard material (I) is added to the reactant solution before injection, then the area of the reactant peak A_R on the reactor chromatogram is related to the apparent (or composite) pseudo-first-order rate constant k_{app} by

$$\ln[A_R/A_I] = - k_{app}\, t_R + \ln[A_R/A_I]_{t=0} \qquad (3)$$

where t_R is the retention time of the reactant and A_I is the peak area of the inert standard.[27,28] The slope of a plot of $-\ln[A_R/A_I]$ versus t_R gives the value of k_{app} where

$$k_{app} = k_m(t_m/ t_R) + k_s(t_s/ t_R) \qquad (4)$$

and where k_m and k_s are the rate constants in the mobile and stationary phases, respectively, and t_m and t_s are the residence times in the mobile and stationary phases, respectively.[27,28] With A_R/A_I and t_R measured experimentally from a series of reactor chromatograms obtained using various mobile phase flow

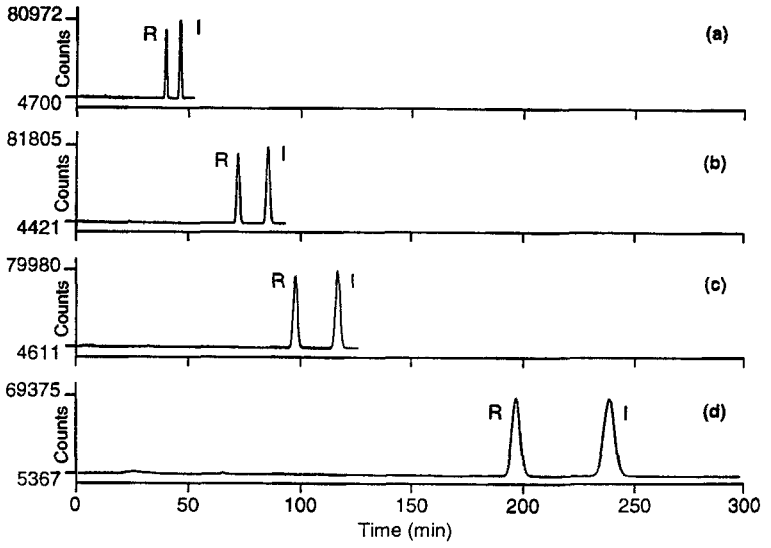

Figure 4. Series of reactor chromatograms for RP-sulfonyl chloride hydrolysis reaction in the chromatographic reactor at 6°C. Column: Inertsil 5 ODS-2 (5 μm, 250 x 4.6 mm); mobile phase: 30/70 (v/v) 0.1% H_3PO_4 (pH = 2.6)/acetonitrile. Mobile phase flow rates: (a) 0.7 mL/min; (b) 0.4 mL/min; (c) 0.3 mL/min; (d) 0.15 mL/min. R: reactant (RP-sulfonyl chloride); I: inert standard (p-terphenyl).

rates, a plot of $-\ln[A_R/A_I]$ versus t_R can be constructed. In our experiment, the RP-sulfonyl chloride sample was prepared in dry acetonitrile to prevent hydrolysis of the sample before injection. p-Terphenyl was chosen as the inert standard because its retention time was close to that of RP-sulfonyl chloride. Figure 4 shows a series of chromatograms obtained at various mobile phase flow rates, using 30:70 (v/v) 0.1% H_3PO_4 (pH = 2.6):acetonitrile as mobile phase and maintaining the column temperature at 6°C. Due to the slow hydrolysis under these conditions, the decrease of A_R/A_I ratio with longer retention time t_R is not obvious by visual observation of the reaction chromatograms. However, the plot of data obtained from the electronically integrated peak areas on the chromatograms indicates the decrease of $\ln[A_R/A_I]$ with longer t_R (Figure 5). The linear relationship between $\ln[A_R/A_I]$ and t_R indicates that the on-column hydrolysis is pseudo-first-order. The k_{app} determined from the slope of the plot was 2.5×10^{-6} sec^{-1} with a standard deviation of 1.0×10^{-7} sec^{-1}. Compared with the k value (1.3×10^{-5} sec^{-1}) from the batch reactor at 6°C with the reaction medium being the same as the mobile phase used in the chromatographic reactor, the k_{app} value was five-fold smaller,

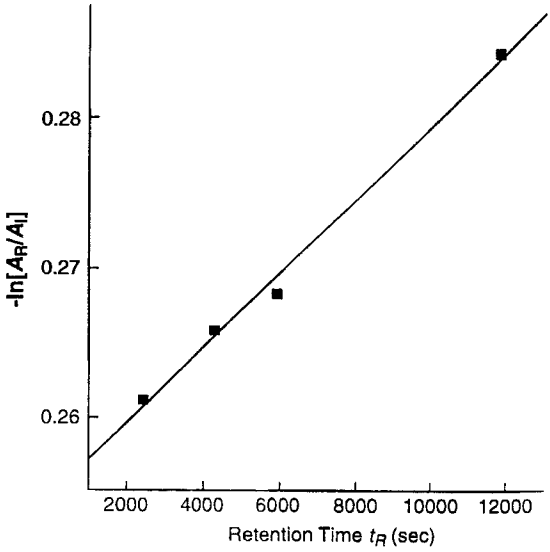

Figure 5. Plot of negative logarithm of A_R/A_I versus retention time of RP-sulfonyl chloride obtained from the chromatographic reactor. $r^2 = 0.996$. A_R: peak area of RP-sulfonyl chloride; A_I: peak area of inert standard (p-terphenyl).

as expected. From equation (4), one could calculate k_s (the hydrolysis rate constant in the stationary phase) using the apparent rate constant k_{app} and the rate constant in the mobile phase k_m which was obtained from the batch reactor. The value of t_m was obtained from the retention time of non-retained uracil; t_s was obtained from the retention time t_R of RP-sulfonyl chloride, subtracting the value of t_m. When the mobile phase was 30:70 (v/v) 0.1% H_3PO_4 (pH = 2.6):acetonitrile and the column temperature was 6°C, the k_s was calculated to be 1.6×10^{-6} sec^{-1}. As expected, the hydrolysis rate constant in the stationary phase k_s was much smaller (about 10-fold) than that in the mobile phase k_m (1.3×10^{-5} sec^{-1}).

Assessment of the Impurity Profile Method

When the k_{app} value (2.5×10^{-6} sec^{-1}) obtained from the chromatographic reactor with isocratic mobile phase composition of 30:70 (v/v) 0.1% H_3PO_4 (pH = 2.6): acetonitrile is substituted into equation (1) for k, the ratio C/C_o for RP-sulfonyl chloride at 1158 sec (19.3 min) is calculated to be 0.997, which means the on-column hydrolysis of RP-sulfonyl chloride has occurred 0.3% in 19.3

min. Since the water concentration in the mobile phase used in the impurity profile method changes from 30% to 10% during the gradient program, the k_{app} value in such a chromatographic reactor should be smaller than 2.5×10^{-6} sec^{-1}, due to the gradually lowered water content in the mobile phase. Therefore, when the impurity profile method is used, the extent of on-column hydrolysis of RP-sulfonyl chloride is less than 0.3%.

In a chromatographic system, the process of separating the sample components begins immediately after the sample is injected. In the chromatographic system described in the impurity profile method, the RP-sulfonic acid originally present in the sample began to be separated immediately after the injection and eluted at 6.2 min (Figure 1). The RP-sulfonyl chloride had higher affinity to the stationary phase than the RP-sulfonic acid and eluted at 19.3 min.

During the course of elution, less than 0.3% RP-sulfonyl chloride was hydrolyzed causing a less than 0.3% reduction of the peak area of RP-sulfonyl chloride. The trace amount of RP-sulfonic acid generated from the hydrolysis is expected to spread into the baseline between the RP-sulfonic acid and RP-sulfonyl chloride peaks.

In Figure 1, the RP-sulfonic acid peak was well separated from the RP-sulfonyl chloride peak and showed excellent peak shape suggesting that the area of the RP-sulfonic acid peak represented only the amount of RP-sulfonic acid present in the original sample.

The RP-sulfonyl chloride also showed excellent peak shape indicating that the extent of on-column hydrolysis was minimal.

In typical RP-sulfonyl chloride samples, the level of each individual impurity was below 0.5%. Since the percentage of each component was calculated based on the total area counts of the peaks due to the impurities as well as RP-sulfonyl chloride, the less than 0.3% reduction of the main peak area due to RP-sulfonyl chloride would not cause noticeable change of the percentage of each component. Therefore the accuracy of the results obtained from the impurity profile method was satisfactory.

The precision of the impurity profile method was also evaluated by making four consecutive injections of an RP-sulfonyl chloride sample solution. The area counts and their standard deviations of the RP-sulfonic acid and RP-sulfonyl chloride peaks are listed in Table 2. The excellent precision of the method is demonstrated by the small %RSD values in the table.

Table 2

Precision of the Impurity Profile Method

Injection Number	Area Counts of RP-Sulfonic Acid	Area Counts of RP-Sulfonyl Chloride
1	15360	4843532
2	15365	4821716
3	15474	4831124
4	15424	4818699
%RSD:	0.4	0.2

Table 3

Influence of Column Temperature on Column Performance

Column Temp. (°C)	No. of Theoretical Plates of RP-Sulfonyl Chloride Peak	Tailing Factor of RP-Sulfonyl Chloride Peak
6	18810	1.07
11	21830	1.04
17	25560	1.03
25	32910	1.02

The effect of column temperature on column performance was also investigated based on the RP-sulfonyl chloride peak (see Table 3). The lowering of column temperature from 25°C to 6°C did not significantly change the peak symmetry. Although the column efficiency at 6°C was reduced compared to that at 25 °C, the separation achieved at 6 °C (Figure 1) was satisfactory.

CONCLUSION

The RP-sulfonyl chloride was successfully chromatographed in the reversed-phase HPLC system at a column temperature of 6°C. The reversed-phase HPLC impurity profile method provided satisfactory separation of the impurities, including RP-sulfonic acid, present in the bulk RP-sulfonyl chloride material. The kinetic results of RP-sulfonyl chloride in the batch reactor

provided useful information about the hydrolysis. The hydrolysis reaction was determined to be a pseudo-first-order reaction. The hydrolysis rate constant was reduced by approximately 6-fold when the reaction temperature was lowered from 25°C to 6°C. Based on the apparent hydrolysis rate constant in the chromatographic reactor, it was shown that only less than 0.3% RP-sulfonyl chloride would hydrolyze on the column when the impurity profile method was used, and the method was suitable for its purpose. Finally, it was demonstrated that, in the chromatographic reactor, the hydrolysis rate constant in the stationary phase k_s was approximately 10-fold smaller than that in the mobile phase k_m.

ACKNOWLEDGMENTS

The authors wish to thank Drs. R. S. Egan, N. Grinberg, and V. Antonucci for reviewing the manuscript and providing fruitful discussions. The authors also thank Drs. G.-J. Ho, J. Chung, and N. Yasuda for providing the samples of RP-sulfonyl chloride.

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Received August 25, 1997
Accepted September 17, 1997
Manuscript 4607