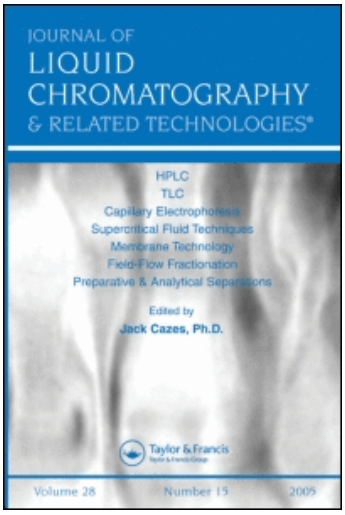

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DEVELOPMENT OF PRACTICAL CHROMATOGRAPHIC METHODS FOR THE ANALYSIS OF ACTIVE ESTERS

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ABSTRACT

Two differing approaches to the liquid chromatographic analysis of active esters used in pharmaceutical processing were developed. Selection of methodology was dictated by the intrinsic chemistries involved and the processing requirements.

The first approach (chromatographic systems A and B) described was employed to monitor a *mesylation reaction* with a relatively simple HPLC impurity profile to a minimum of 99.0% conversion. Chromatographic conditions, designed as a compromise to achieve adequate analyte solubility, specificity, and minimized on-column decomposition within a short analysis time cycle, included an aprotic mobile phase used with a Zorbax SB-CN column at ambient temperature.

The second approach (chromatographic system C) described was employed to monitor a *triflation reaction* to a minimum 97.0% conversion. The impurity profile of this reaction was far more complex than the former reaction, and as a result a more

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sophisticated analysis method was necessary. A chemical derivatization using tetrabutylammonium bromide in sieve-dried acetonitrile was developed to stabilize the reaction product as its bromo analog, permitting longer column residence times to achieve the necessary method specificity.

INTRODUCTION

The separation and identification of trace components contained within complicated matrices is a cornerstone to the control of pharmaceutical processing. The high selectivity and sensitivity of chromatography have positioned separations science as one of the most powerful tools available to the pharmaceutical analytical chemist to control manufacturing quality. However, significant additional challenges to chromatographic methods development are presented for systems in which the analytes of interest are unstable to the analysis conditions employed.

One class of compounds which is often unstable to chromatographic analysis are active esters such as alkylsulfonates. These compounds are weak bases which contain good leaving groups for subsequent chemical reactions.[1] As a result, these compounds are common intermediates used to insert a variety of functionalities into molecules of pharmaceutical interest.

In general, many of the molecules of interest within the pharmaceutical industry are organic bases and therefore the chromatography is dramatically influenced by mobile phase pH, ionic strength, and use of ion-pairing reagents such as ammonium salts.[2] For these analytes, the mobile phase selected for reversed-phase liquid chromatographic methods is often sufficiently acidic to fully protonate the amine functionality and minimize unwanted electrostatic interactions. Unfortunately, an acidic chromatographic environment often hastens the kinetics of on-column hydrolysis of labile analytes, such as alkylsulfonates. A detailed review has been published describing the kinetics of such chemical reactions / equilibria during the liquid chromatographic experiment.[3] Such effects often give rise to artifacts such as broad and asymmetric chromatographic peaks or multiple zones for a single component, which may obscure trace analytes of interest and confound the interpretation of results. However, of equally significant concern within the pharmaceutical industry is the possibility that chromatographic artifacts may lead to incorrect manufacturing conclusions regarding analyte quality.

As a result of this behavior, the chromatographic analysis of acid-labile analytes routinely employs neutral pH in reversed-phase or aprotic solvents in normal phase, if analyte solubility permits. Advances made in the synthesis of silica gel used for both normal and reversed-phase liquid chromatography (type

B silica) have produced a less acidic support due to greater surface hydroxylation and lower metal ion content.[4,5] Additionally, reversed-phase columns are commonly-available which contain extensively-endcapped surface silanols, or were prepared with silanizing agents with bulky organic groups to sterically mask the surface.[6-7] Such efforts are made to deactivate the silanol surface because electrostatic interactions between it and charged analytes serve to decrease desorption kinetics, which often generates asymmetrical chromatographic peak profiles.[8,9] While these stationary phase advances have generally improved the quality of separations under neutral pH conditions, peak tailing is still pervasive due to residual surface activity and may prevent use of these mobile phases for some applications.

Published approaches to the chromatographic analysis of active alkylsulfonates vary with the chemical nature of the analyte, its intended use, and the requirements for quantitation of the chemical system. Normal phase liquid chromatography at subambient temperature was used to avoid on-column hydrolysis of an active methanesulfonyl ester intermediate of a pharmaceutical compound.[10] A reversed-phase liquid chromatographic separation of p-benzenesulfonyl chloride at subambient temperature was reported to minimize on-column hydrolysis of the parent compound to a manageable 0.3%.[11] Yet another approach reported the indirect determination of an N-hydroxysuccinimidyl-activated polyethylene glycol ester by on-line hydrolysis after aprotic solvent gel permeation chromatography.[12]

This paper will discuss the chromatographic analysis of two active ester reaction systems used to support real time processing of intermediates to drug candidates, hence minimized analysis time cycle with minimum acceptable quantitative ability for key analytes is of major concern. The first example described is formation of the methanesulfonyl ester **II** of tryptophol intermediate **I** (Figure 1). This transformation is employed in the manufacture of rizatriptan benzoate, a 5-HT_1D receptor agonist approved for the treatment of migraine, and will be referred to as the *mesylation reaction* in this manuscript.[13-15] The second example

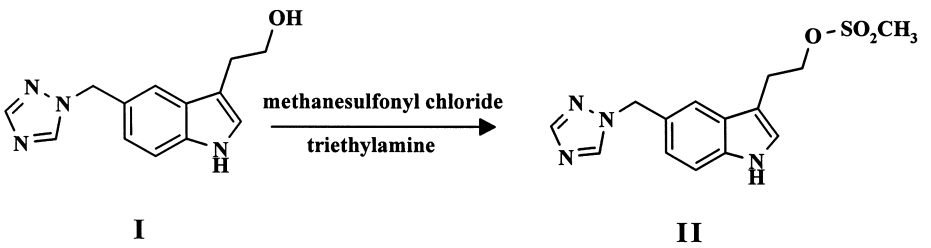
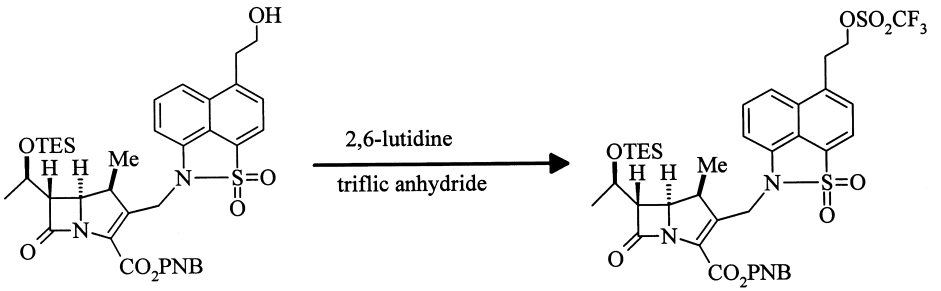


Figure 1. Mesylation reaction scheme.



III **IV**

Figure 2. Triflation reaction scheme.

is the formation of the trifluoromethanesulfonyl ester **IV** of alcohol intermediate **III** (Figure 2), which will be referred to as the *triflation reaction* in this manuscript. Compound **V** is the penultimate intermediate to a compound which was evaluated as an anti-MRSA antibiotic.[16-20]

Normal phase chromatographic methods with a silica or diol stationary phase and hexane / isopropanol mobile phases at reduced analysis temperature were evaluated for both reactions in consideration in this work. However, due to poor peak shape, component selectivity, and limited solubility in the case of the *mesylation reaction*, this approach was determined to be unsuccessful for both of the reactions in question. The focus of this paper will therefore be investigations into the reversed-phase liquid chromatographic analysis of these two reaction systems, and the differing approaches required to meet the analysis needs of these two chemical systems.

EXPERIMENTAL

Materials

Compounds **I**, **II**, **III**, and **IV** were synthesized in the process chemistry department of Merck Research Laboratories. HPLC grade acetonitrile was purchased from Fisher Scientific (Fairlawn, NJ) and dried over molecular sieves where indicated. HPLC grade monobasic potassium phosphate buffer (KH_2PO_4) was obtained from JT Baker (Phillipsburg, NJ) and benzyltriethylammonium chloride and tetrabutylammonium bromide were purchased from Aldrich (Milwaukee, WI). Zorbax RX-C8, Zorbax SB-CN, and Zorbax SB-Phenyl

columns, all 25 cm x 4.6 mm i.d. with 5 μm packing, were obtained from MAC-MOD Analytical (Chadds Ford, PA).

Instrumentation

Two Shimadzu HPLC systems, which included a SIL-10A autoinjector, SCL-10A system controller, LC-10AS gradient pumps, and SPD-10AV UV/VIS detector, were used for this work. A model 7955 HPLC column heater/chiller (Jones Chromatography, Lakewood, CO) was used for subambient chromatographic investigations.

Chromatographic Methods and Sample Preparation

Chromatographic system A was developed to monitor the *mesylation reaction* used to form **II**. It used a 10 mM aqueous KH_2PO_4 / acetonitrile linear mobile phase gradient from 20 to 60 % (v/v) organic over 10 minutes with a Zorbax RX-C8 column. The mobile phase flow rate was 1.5 mL/min, injection volume was 20 μL, and column temperature was ambient unless otherwise noted, and a UV detection wavelength of 226 nm was selected. Reaction samples were diluted 100x in acetonitrile, followed by a 5x dilution in anhydrous ethanol, for a final diluent composition of 80% ethanol / 20% acetonitrile (v/v). These solutions were immediately analyzed to avoid excessive sample degradation.

Using chromatographic system A, the kinetics of decomposition of **II** in mixtures of potential mobile phase components were evaluated. Solutions of **II** were prepared in 100% acetonitrile, 80% methanol / 20% acetonitrile (v/v), 50% ethanol / 50% acetonitrile (v/v), and 80% ethanol / 20% acetonitrile (v/v) diluents from a single stock solution of **II** in acetonitrile. Five successive injections of each fresh solution were made with chromatographic system A over the course of approximately 65 minutes, and the response of **II** with respect to time was used to calculate the rate constant for decomposition at ambient temperature in each solvent system.

Chromatographic system B was also developed to monitor the *mesylation reaction* used to form **II**. It used an isocratic mobile phase of 95% acetonitrile / 5% methanol (v/v) with a Zorbax SB-CN column. Mobile phase flow rate was kept constant at 0.5 mL/min and injection volume was held constant at 20 μL. Column temperature was held at ambient unless otherwise noted and a UV detection wavelength of 226 nm was selected. Typical *mesylation reaction* samples were diluted 1000x in acetonitrile to achieve a 0.07 mg/mL sample concentration of **II** and were immediately analyzed, although the stability of **II** in dry acetonitrile was experimentally confirmed for a minimum of an hour.

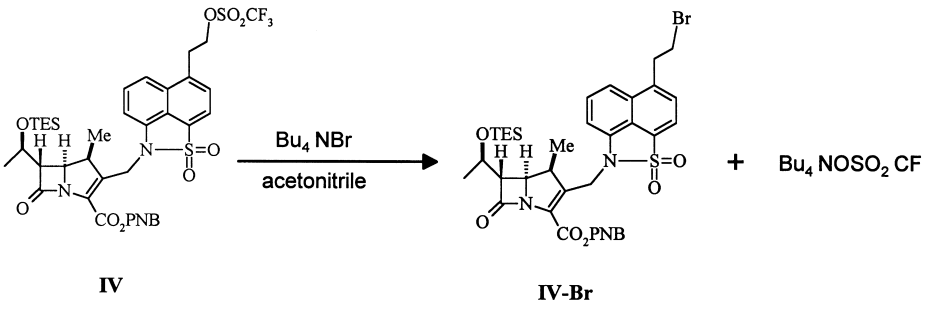
Chromatographic system C was developed to monitor the *triflation reaction* used to form **IV**. It used a 5 mM aqueous KH_2PO_4 (pH 4.5) / acetonitrile linear mobile phase gradient from 60 to 100 % (v/v) organic over 30 minutes with a Zorbax SB-Phenyl column. Mobile phase flow rate was kept constant at 1.0 mL/min and injection volume was held constant at 20 μL. Column temperature was held at ambient unless otherwise noted and a UV detection wavelength of 245 nm was selected.

Reaction samples of **IV** from the *triflation reaction* were chemically derivatized to the corresponding bromo analog with tetrabutylammonium bromide in acetonitrile to stabilize the analyte with respect to hydrolysis and permit longer column residence time to achieve component separations (Figure 3). A 50 μL aliquot of the *triflation reaction* was added to 10 mL of a solution of 6 mM tetrabutylammonium bromide in sieve-dried acetonitrile. The reaction was vigorously shaken for approximately one minute prior to HPLC analysis of the solution without further dilution.

RESULTS AND DISCUSSION

Development of Mesylation Reaction Methods

A reversed-phase HPLC method (system A) was evaluated to monitor the conversion of **I** to **II**, with an minimum analysis goal of rapidly and accurately quantitating unreacted **I**. However, the composition of water needed for retention in system A led to significant on-column degradation of **II** → **I** as demonstrated in Figure 4, which compromised quantitation of residual **I**. By visual inspection, the use of subambient column temperature (5°C) served only to further broaden



IV **IV-Br**

Figure 3. Tetrabutylammonium bromide derivatization of compound **IV**.

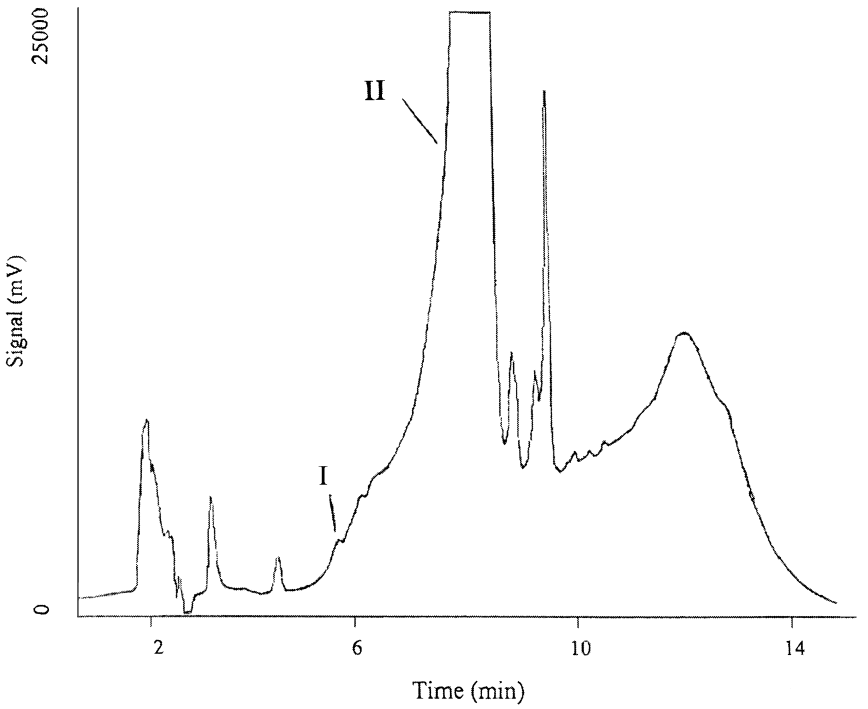


Figure 4. Typical mesylation reaction chromatogram obtained with chromatographic system A.

chromatographic peaks due to reduced mass transfer rates, without any obvious reduction in on-column degradation.

Despite these limitations, system A was a useful tool to determine the stability of **II** prepared in different chromatographic diluents, which served as a screening process for potential mobile phases. Assuming that the on-column decomposition of **II** is constant for a given set of chromatographic parameters, such as chromatographic system A, the level of **II** detected as a function of residence time in the diluent is related to the stability of **II** in the sample diluent via the first order rate law:

$$\ln[\textbf{II}\text{ response}] = -kt + \ln[\textbf{II}\text{ response at } t=0] \quad (1)$$

The first order rate constants (k) for decomposition of **II** at ambient temperature were calculated for solutions of compound **II** prepared in the alcohol / acetonitrile diluents described in the Experimental Section for chromatographic system A. The results are summarized in Table 1. Good linear fits were obtained

Table 1. First Order Rate Constants (k) for the Decomposition of Compound II in Alcohol/Acetonitrile Mixtures at Ambient Temperature

Diluent Composition (v/v)	k (min^{-1})	R (Linear Fit)
100% Acetonitrile	8.12E-5	0.982
80% Anhydrous ethanol / 20% acetonitrile	6.69E-4	0.992
50% Anhydrous ethanol / 50% acetonitrile	3.38E-4	0.998
80% Methanol / 20% acetonitrile	1.06E-3	0.996

(R >0.98) for all four diluent experiments. Decomposition occurred at the slowest rate in 100% acetonitrile, as anticipated, however void volume elution was observed for numerous bonded phases with acetonitrile as the mobile phase. Therefore, addition of a polar mobile phase modifier to the acetonitrile mobile phase was needed to achieve minimum selectivity requirements at the expense of some added on-column decomposition.

From the rate constant data for alcohol / acetonitrile solutions of **II** found in Table 1, addition of 5% alcohol to acetonitrile mixtures increases k (min^{-1}) by an acceptable 10% for ethanol and 15% for methanol, while experimentally (chromatographic system B) providing adequate chromatographic properties to monitor the conversion of **I** → **II** (Figure 5).

The method precision for quantitation of residual **I** in two *mesylation reaction* samples with chromatographic system B was evaluated. Samples from one reaction at 90% completion and another at >99% completion (estimated from the charge of methanesulfonylchloride) were prepared for analysis in acetonitrile diluent using clear glass volumetric flasks. The amount of residual **I** determined was 10.9 weight % with 0.5% RSD for the former sample and 0.5 weight% with 1.4% RSD for the latter sample, with no observation of peak distortion. Detector response at 226 nm was linear (R>0.999) for both **I** and **II** over the concentration ranges of 9.80E-2 to 7.84E-5 mg/mL and 5.89E-5 to 8.18E-2 mg/mL, respectively.

The limit of detection (LOD) and limit of quantitiation (LOQ) for **I** were calculated as 5.89E-5 mg/mL and 1.47E-4 mg/mL, respectively, which correspond to 0.5 and 0.2 weight% of the target concentration. Typical chromatographic resolution of 4.5 between **I** and **II** was achieved on multiple Zorbax SB-CN columns used during development.

Development of Triflation Reaction Methods

Chromatographic system C was developed to monitor the conversion of **III** → **IV** during the *triflation reaction*, once again with a minimum goal of quanti-

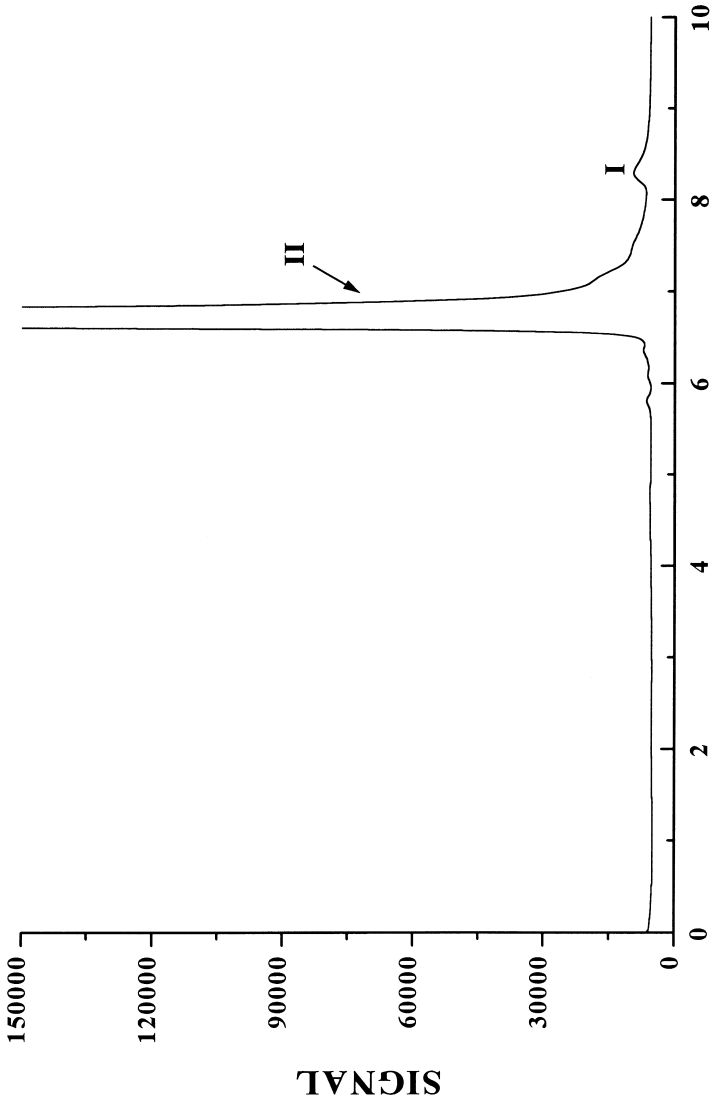


Figure 5. Typical mesylation reaction chromatogram obtained with chromatographic system B.

tating residual **III**. Significant levels of on-column decomposition of **IV** → **III** were observed, as seen in Figure 6, despite reducing the column temperature to 0°C. The approach used to chromatographically monitor the *mesylation reaction* (system B, i.e., rapid analysis under conditions which maintain acceptable levels of degradation) was not possible for the *triflation reaction* because the impurity profile is far too complex to achieve the required minimum method specificity. As a result, all subsequent investigations were focussed on stabilizing **IV** to hydrolysis to permit longer column residence times and achieve adequate separation, while maintaining the integrity of chromatographic zones.

Addition of quartenary ammonium salts to liquid chromatographic mobile phases is a quite common technique employed to mask strong anionic sites on the stationary phase, which if left available will contribute to peak asymmetry for organic amine analytes.[21] The addition of approximately 10 mM of tetrabutylammonium bromide to the mobile phase of chromatographic system C produced a similar chromatogram as seen in Figure 6 for a *triflation reaction* sample, with a

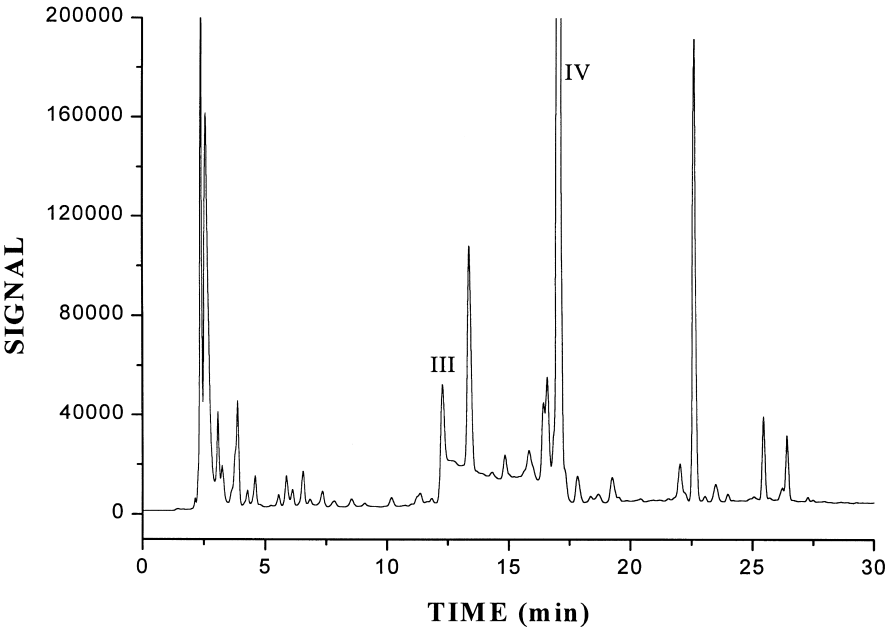


Figure 6. Typical triflation reaction chromatogram obtained with chromatographic system C without derivatization of compound **IV**.

significant baseline rise between the peaks resulting from **III** and **IV**. However, the onset of peak asymmetry in the tail region of **IV** was also observed, suggesting simultaneous on-column production of a more retained species as a result of the ammonium salt additive to the mobile phase, presumably the bromo analog of **IV** (**Br-IV**).

A literature search revealed no references to the direct use of quaternary ammonium salts as derivatization agents. One published report describes the use of tetraalkylammonium salts, with detector-active counterions, as mobile phase additives to indirectly detect amino acids based upon displacement measurements.[22] Other researchers have reported the use of hexdecyltrimethylammonium bromide as a phase transfer agent to facilitate the pentafluorobenzyl derivatization of anions present in aqueous solution for subsequent gas chromatographic analysis.[23]

To further investigate the potential of quaternary ammonium salt as derivatizing agents, benzyltriethylammonium chloride and tetrabutylammonium bro-

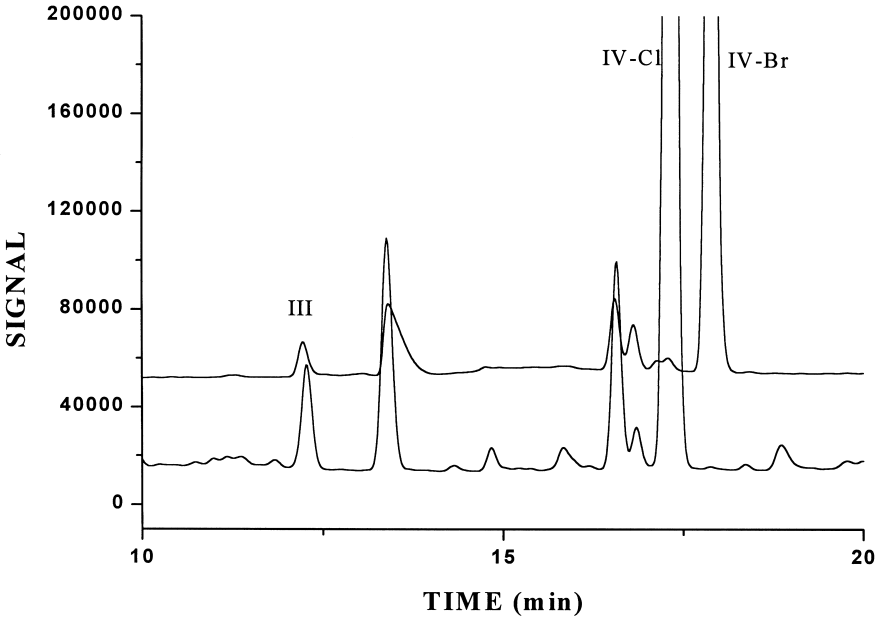


Figure 7. Typical triflation reaction chromatogram obtained with chromatographic system C after tetrabutylammonium bromide derivatization of compound **IV**.

Table 2. Effect of Tetrabutylammonium Bromide on the Derivatization of Compound IV

Concentration (mM) Bu_4NBr	Area% III	Area% IV-Br
2.3	5.3	82.6
4.5	5.3	82.5
6.2	5.0	83.0
8.1	5.3	82.6
%RSD	2.9	0.3

mide were selected to form the corresponding chloro and bromo analogs of **IV** in dry acetonitrile, followed by analysis via chromatographic system C. Figure 7 is an enlargement of the chromatographic region of interest for two derivatized *triflation reaction* samples, in which **IV-Cl** is observed to elute approximately 30 sec after underivatized **IV** (see Figure 6), while **IV-Br** is resolved from underivatized **IV** by over 1 min.

The on-column decomposition observed in Figure 6 was not observed in either chromatogram generated via derivatization and **IV** was not detected above the method LOD of 0.02%, suggesting the derivatization had proceeded to completion. All other chromatographic components were unaffected by the derivatization and the tetrabutylammonium cation introduced no new components into the chromatogram, making it an ideal choice for use with UV detection. As a result, the tetrabutylammonium bromide derivatization was selected for further development.

The concentration of tetrabutylammonium bromide in acetonitrile was varied from 0.75 to 2.60 mg/mL (2.3 to 8.1 mM), which represents up to an order of magnitude molar excess with respect to **IV,** to assess the impact on the derivatization. Additonally, the 6.2 mM Bu_4NBr sample (Table 2) was prepared in acetonitrile which had been sieve-dried for several weeks to evaluate the role of trace water on the derivatization. The HPLC results summarized in Table 2 suggest no bias as a function of Bu_4NBr concentration, but do demonstrate a small advantage in reducing water content. The most significant potential source of water intrusion seems to be from the acetonitrile used and not the ammonium salt, hence sieve-dried solvent is recommended. From these data, the method precision for determination of **III** at 5.3 area% was 2.9% RSD. Analytical samples from two preparative scale *triflation reactions* were monitored in the laboratory via this method (system C) and found to proceed to 1.7 and 2.1 area % residual **III**, respectively, consistent with subsequently-determined batch yields.

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