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Short Communication

Ion chromatographic method for the simultaneous determination of trifluoromethanesulfonic acid and trifluoroacetic acid

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ABSTRACT

Mixtures of trifluoromethanesulfonic acid (TFMSA) and trifluoroacetic acid (TFA) are typically gaining acceptance as reagent mixtures used to cleave peptides from solid-phase resins as well as removing residual protecting groups, e.g., benzyl, *tert*.-butoxycarbonyl, tosyl, etc., from peptides. Due to the toxic nature of these strong acids, their quantitative determination in these materials is essential. In this laboratory a suppressed ion chromatographic method with conductivity detection has been used to quantitate trace amounts of TFMSA and TFA that may remain after deprotection. A Dionex anion column (AS4A) was used for all separations with 1.5 m*M* Na_2CO_3–2.5 m*M* $NaHCO_3$–4% (v/v) acetonitrile as the eluent. Method validation included: standard linearity plots and standard addition-recovery data. It was shown that various inorganic trace impurities occasionally present in the peptide samples did not interfere with the determination of TFMSA and TFA.

INTRODUCTION

Trifluoromethanesulfonic acid (TFMSA) in combination with trifluoroacetic acid (TFA) is used with increasing frequency to remove peptides from solid-phase resins as well as residual protecting groups [e.g., tosylate, benzyl, *tert*.-butoxycarbonyl (Boc)] from peptides [1]. Subsequent to the deprotection step, various wash stages are used to remove the residual TFMSA and TFA. It is critical to monitor their concentration levels during these wash stages to ensure their removal.

Various methods for the assay of TFMSA and TFA have been devised. These include for TFA: ion-selective electrodes [2], GC [3–7], IR spectrometry [8], isotachophoresis [9–12], reversed-phase HPLC [13], non-aqueous acid-base titrations [14], spectrophotometry [15] and ion-exchange chromatography [16–18]. Of the above-mentioned techniques ion chromatography is considered to be a relatively convenient and sensitive technique for the quantitative determination of TFA [16], and easily lends itself as an automated technique for multi-sample analysis. The available literature on quantitative determination of TFMSA is sparse, a noteworthy technique being the use of a trifluoromethanesulfonate-selective liquid membrane electrode [19].

The work described below presents an alternative way to quantitate TFMSA and TFA simultaneously, using ion chromatography with simple

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isocratic elution. The method has been applied to the determination of TFMSA and TFA in water-soluble peptides used in our pharmaceutical research.

EXPERIMENTAL

Reagents

All reagents used were of analytical grade. Sodium carbonate and sodium bicarbonate were obtained from Baker. Sulfuric acid, potassium chloride, sodium nitrate and sodium sulfate were obtained from Fisher. TFMSA and sodium fluoride were obtained from Aldrich. Sodium dihydrogenphosphate and ammonium acetate were purchased from Mallinckrodt. TFA was obtained from EM Science. The various water-soluble peptide salts used in this work were obtained in house. Deionized water ($18 \cdot 10^4\ \Omega$ m) was used to prepare the stock and the working standard solutions of various salts. The eluents used in this work were prepared by dissolving appropriate quantities of salts in deionized water followed by addition of the organic modifier, filtration through 0.45-μm membrane filters (obtained from Alltech Assoc.), and degassing for 5 min with helium prior to use.

Instrumentation

A Dionex Series 45OOi ion chromatograph equipped with a conductivity detector and gradient pump capabilities and an anion micro membrane suppressor was used for all experiments. The integrator used was a Spectra-Physics Model 4100. A Dionex Ion Pac AS4A analytical column (250 mm x 4 mm I.D.) composed of 15-μm polystyrene-divinylbenzene substrate agglomerated with anion-exchange latex beads was used for all runs. The latex beads (0.05 μm diameter) were completely aminated thus carrying the ion-exchange sites. A Dionex AG4A guard column was used in combination with the analytical column.

Sample preparation

The various peptide salts (e.g., acetate or phosphate form) were dissolved in deionized water to yield sample solutions at approximately 1 mg/ml. In some cases, 1 or 2 min of agitation by vortex-mixing was used to ensure complete dissolution of the peptide salt.

RESULTS AND DISCUSSION

Optimization of the mobile phase

Initial attempts to separate TFA and TFMSA involved the use of a borate eluent prepared as 1 m*M* sodium tetraborate. With this eluent TFMSA was not eluted from the column presumably due to its relatively strong interaction with the stationary phase. TFA, as a result of its weaker affinity for the resin, was eluted, though it was retained more strongly than other singly charged anions, e.g., fluoride, acetate and chloride. It was decided to use a stronger eluent to separate and quantitate TFMSA and TFA in a single run, and, as such, a carbonate-bicarbonate eluent was selected as the eluent of choice. The HCO_3^{-1}/CO_3^{-2} ratio was varied to yield an eluent suitable for the simultaneous determination of TFMSA and TFA. In the absence of an organic modifier in the mobile phase TFMSA eluted as a tailing peak. The addition of acetonitrile was found to reduce the extent of tailing with somewhat shorter retention time for TFMSA. It had no apparent effect on the TFA peak.

Fig. 1 shows a typical chromatogram of standard solution of TFMSA and TFA in water. The additional peaks seen in the chromatogram are due to trace amounts of inorganic impurities, *e.g.*, chloride, nitrate, phosphate and sulfate present in the TFMSA and TFA reagents. Reagents obtained from other manufacturers showed the presence of the same impurities. No interference is observed from these impurities in the determination of TFMSA and TFA (see also Fig. 3). Fig. 2 shows a chromatogram of an investigational peptide (acetate salt) in water at *ca.* 1 mg/ml, showing the presence of a trace amount of TFA with no TFMSA detected.

Table I lists the optimum chromatographic conditions used for all subsequent experiments.

Interference study

The common anionic impurities expected to be present in our peptide samples include chloride, fluoride, sulfate, nitrate, TFMSA and TFA.

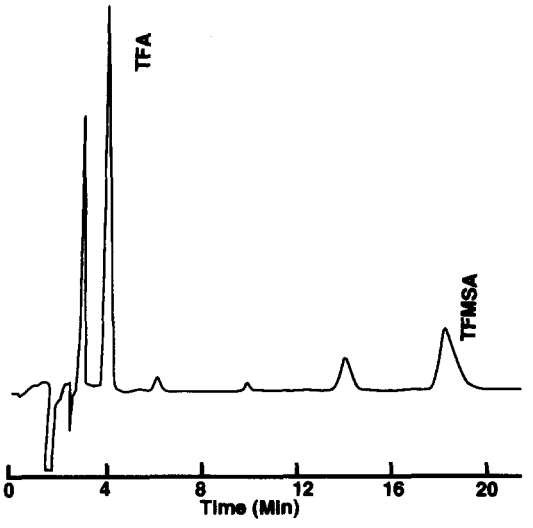

Fig. 1. Typical chromatogram of a standard solution of TFMSA and TFA in water, each at 1 μg/ml; see Table I for chromatographic conditions.

TABLE I

CHROMATOGRAPHIC PARAMETERS

Column	Dionex Ion Pac AS4A (15 μm, 250 mm × 4 mm I.D.)
Eluent	2.5 m*M* $NaHCO_3$–1.5 m*M* Na_2CO_3–4% (v/v) acetonitrile
Regenerant	27 m*M* H_2SO_4
Eluent flow-rate	1 ml/min
Regenerant flow-rate	4-5 ml/min
Detector output range	1 μS
Injection volume	25 μl
Chart speed	0.5 cm/min

Aside from these, phosphate and acetate, as the counter ions in the peptide salts, also need to be considered as potential interfering anions when analyzing for TFMSA and TFA. Fig. 3 shows the chromatogram of a standard mixture of the various anions mentioned above at the 1 μg/ml

Fig. 2. Typical chromatogram of a solution of peptide (acetate salt) in water, ca. 1 mg/ml, showing the presence of a residual amount of TFA; see Table I for chromatographic conditions.

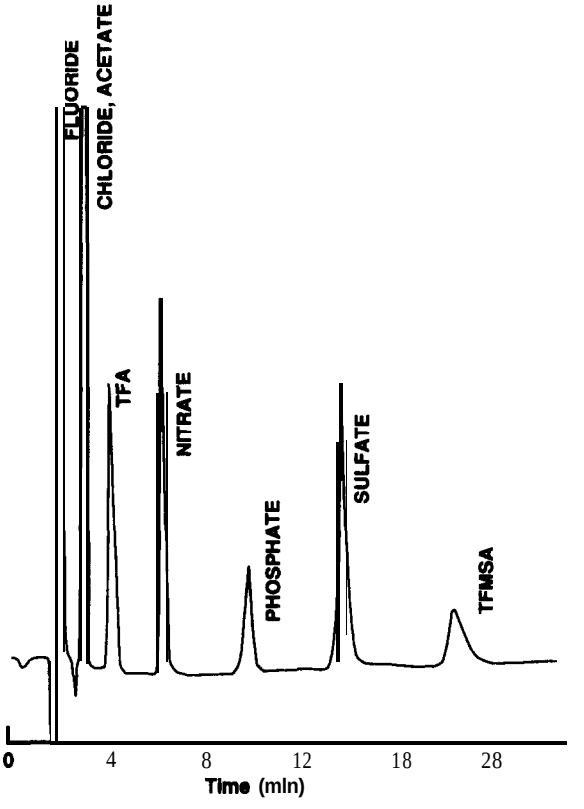

Fig. 3. Typical chromatogram of a standard mixture of anions in water (1 μg/ml each); see Table I for chromatographic conditions.

TABLE II

RECOVERY OF TFMSA AND TFA FROM PEPTIDE ACETATE

Amount added (μg)	Amount found (μg)	Recovery (%)
TFMSA		
2.68	***2.64***	***98.5***
5.36	***5.40***	101
8.04	8.06	100
TFA		
2.96	***2.84***	***95.9***
5.92	***5.68***	***95.9***
8.88	***8.64***	***97.3***

level. Fluoride, chloride and acetate are seen to elute early in the run and no interference is observed from the other anions in the mixture.

TFMSA and TFA standard linearity

At a detector setting of 1 μS the peak areas varied linearly with concentration over the 0.3-3 μg/ml range for TFA and 1-15 μg/ml range for TFMSA. The linearity range for both TFA and TFMSA was determined by applying the method of "Linearity plots" [20] to the calibration curve data.

Standard addition-recovery study

Known amounts of TFMSA and TFA were added to flasks containing a constant and known amount of the peptide solution (*ca.* 1 mg/ml), and resulting solutions were chromatographed. Recovery values are listed in Table II.

CONCLUSION

An ion chromatographic method is described for the determination of TFMSA and TFA using isocratic elution with conductivity detection. The method has been applied to water-soluble pharmaceutical preparations.

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