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Short communication

## Analysis of trifluoroacetic acid in lyophilized natural products by capillary electrophoresis

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### Abstract

A rapid and simple method for the capillary electrophoretic determination of residual trifluoroacetic acid in lyophilized natural products is described. The technique utilizes 2,6-naphthalenedicarboxylic acid as a separation buffer additive providing indirect ultraviolet absorption detection. Using this method, acceptable precision, accuracy, selectivity, range and linearity were achieved. ©1997 Elsevier Science B.V.

*Keywords:* Trifluoroacetic acid

### 1. Introduction

Trifluoroacetic acid (TFA) is commonly utilized as both a reversed-phase chromatography mobile phase additive and as an agent to facilitate the removal of residual protecting groups (such as *tert.*-butoxycarbonyl) during the purification and downstream organic chemistry of natural products such as glyco- and lipopeptides [1,2]. Although TFA is volatile, upon lyophilization peptide salts can be formed with the TFA anion. To reduce toxicity which might be caused by TFA remaining in the final product, it is desirable to determine residual TFA and monitor its removal.

Capillary electrophoresis (CE) has recently been suggested as an attractive alternative to other methods for the determination of TFA, such as ion chromatography, by Werner et al. [3] who employed proprietary reagents for both capillary wall treatment and analyte detection. In the study described below, 2,6-naphthalenedicarboxylic acid (NDC), a UV-ab-

sorbing carrier ion previously used to obtain successful CE analyses of low-mobility organic anions [4], facilitated both acceptable resolution and excellent sensitivity for the determination of residual TFA in lyophilized natural products.

### 2. Experimental

The chemicals used in this study were obtained from Aldrich (Milwaukee, WI, USA). Separations were performed with a commercial instrument (P/ACE System 2100, Beckman, Palo Alto, CA, USA) equipped with 57 cm (50 cm inlet-to-window) 75  $\mu\text{m}$  I.D. capillaries. The capillaries were thermostated at 25°C. Sample injections were carried out hydrodynamically via application of a low positive pressure (3.45 kPa) for 10 s. The separations were performed by the application of 20 kV potential (outlet=anode), and indirect detection at 280 nm UV was employed. The detector signal was electronically inverted to facilitate peak integration and quantitation. The 2 mM NDC, 5 mM NaOH and 0.5 mM

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cetyltrimethylammonium chloride (CTAC) separation electrolyte was made fresh daily and was utilized non-filtered. To facilitate the solubilization of the NDC, 20-ml volumes of the electrolyte suspension were sonicated using a Vibra Cell sonicator (Sonics and Materials, Danbury, CT, USA). Samples of a lyophilized lipopeptide natural product were obtained from Eli Lilly and were prepared for analysis by dissolving in 0.01 M HCl at a concentration of approximately 0.2 mg/ml in a 5.0-ml volume. To prevent the precipitation of the NDC buffer electrolyte which can occur under acidic conditions, the pH of the samples and standards were adjusted to pH 10–12 by the addition of 10  $\mu$ l concentrated NaOH, and a 20-fold dilution in deionized water was then performed prior to injection. All TFA standards were prepared directly in 1.0 mM NaOH at concentrations of 15.0 and 30.0 ppm (w/v), unless otherwise indicated.

### 3. Results and discussion

The preparation procedure utilized in this study was designed to dissociate the TFA anions from the lyophilized sample prior to CE analysis via displacement by chloride. The use of solubilization in dilute HCl has been demonstrated to be effective for this purpose [3].

Displayed in Fig. 1a,b are typical electropherograms

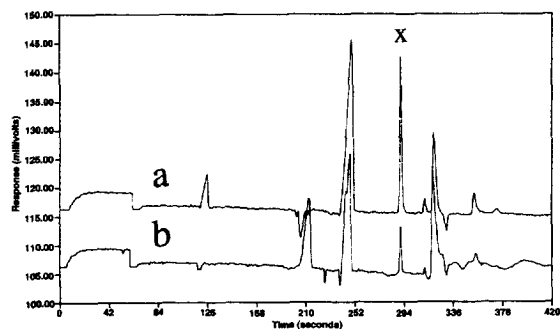


Fig. 1. CE separations of 10-s low pressure injections of (a) a 15 ppm TFA standard, and (b) a solubilized lipopeptide sample obtained in a 57 cm silica capillary using 20 kV applied potential (outlet=+), 280 nm indirect UV detection, and a 2.0 mM NDC, 5 mM NaOH, 0.5 mM CTAC background electrolyte. The peak corresponding to TFA in the profile is marked with an 'x'.

rams of a 15.0 ppm TFA standard and a solubilized lipopeptide sample, respectively. The extraneous peaks in the profiles were determined to be generated by contaminants present in the NaOH used for sample preparation, as demonstrated by the overlay of a standard with a matrix blank (5.0 ml 0.01 M HCl+10  $\mu$ l concentrated NaOH) in Fig. 2. Under the conditions described, the values of peak asymmetry and theoretical plate count were 1.25 and  $1.7 \times 10^5$ , respectively. A test of precision using a lyophilized sample found to contain  $2.7 \times 10^5$  ppm TFA or approximately 2 ppm TFA in the injected sample (seven injections analyzed on three different days) generated a relative standard deviation (R.S.D.) of 14.5% (within-day %R.S.D. ranged from 1.8 to 2.6). The method was found to be linear over the domain of 1.0–100.0 ppm (slope=82.42 peak area units/ppm, Y intercept=12.19 peak area units, standard fit error=2.08 %R.S.D., correlation coefficient=0.999) using peak area for quantitation.

To evaluate recovery and the possibility of matrix effects on the assay, a series of standards were prepared in triplicate at each of four levels (2.0, 5.0, 10.0 and 20.0 ppm TFA) both in the absence and presence of a lipopeptide sample. The recovery of TFA was determined by direct comparison of the integrated peak areas of the spikes versus the standards at each concentration. Recoveries at the 2.0, 5.0, 10.0 and 20.0 ppm TFA levels averaged 135.8, 95.6, 103.6 and 97.9%, respectively, indicating com-

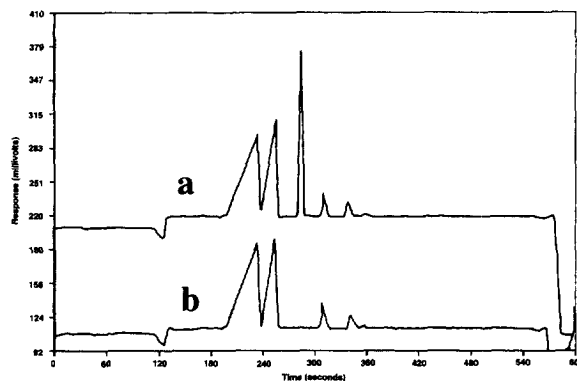


Fig. 2. CE separations of 10-s low pressure injections of (a) a 100 ppm TFA standard, and (b) a matrix blank obtained in a 57 cm silica capillary using 20 kV applied potential (outlet=+), 280 nm indirect UV detection, and a 2.0 mM NDC, 5 mM NaOH, 0.5 mM CTAC background electrolyte.

plete extraction of the TFA from the lyophilized sample. The slope of a plot of peak area versus concentration for this standard series was 95.2 arbitrary units. The slope of a calibration plot of standards prepared in 1.0 mM NaOH was 92.3 units, only a 3% difference. In addition, an overlay of the separation of a TFA standard with a matrix blank provided further evidence for the absence of matrix interferences (see Fig. 2).

The durability of the method was assessed by repeatedly injecting a solubilized lipopeptide sample. To minimize the effects of buffer depletion [5], four sets of 4.0 ml buffer vials were utilized for this study (i.e. 25 injections per set). After 100 injections, the theoretical plate count and migration time remained constant, indicating the lack of accumulation of matrix components on the capillary walls. The %R.S.D. of the peak area from this set of analyses was 2.3.

#### 4. Conclusions

A rapid and simple assay for TFA in lyophilized natural products has been described. The linear range of the method extends over at least two orders of magnitude, and the day-to-day precision is comparable to that which has been reported for the CE analysis of a variety of inorganic anions [6]. On the basis of a signal-to-noise ratio of 3, the detection limit is about 0.5 ppm, which at a 1.0 mg/ml

concentration of dissolved natural product sample corresponds to levels of impurity below 0.05%. This sensitivity is superior to that which had been achieved through the use of other methods such as headspace capillary gas chromatography for the determination of residual TFA in bulk drug substances [7].

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