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# Capillary electrophoresis as an analytical tool for monitoring nicotine in ATF regulated tobacco products

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**Abstract**

Tobacco products are classified at different excise tax rates according to the Code of Federal Regulations. These include cigars, cigarettes, pipe tobacco, roll-your-own tobacco, chewing tobacco and snuff. Nicotine is the primary determinant of what constitutes a tobacco product from a regulatory standpoint. Determination of nicotine, therefore, is of primary importance and interest to ATF. Since nicotine is also the most abundant alkaloid found in tobacco, comprising about 98% of the total alkaloid content, a rapid method for the determination of nicotine in ATF regulated products is desirable. Capillary electrophoresis (CE), as an analytical technique, is rapidly gaining importance capturing the interest of analysts in several areas. The unique and powerful capabilities of CE including high resolution and short analysis times, make it a powerful analytical tool in the regulatory area as well. Preliminary studies using a 25 m*M* sodium phosphate buffer, pH 2.5 at 260 nm have yielded promising results for the analysis of nicotine in tobacco products. Application of an analytical method for the determination of nicotine by CE to ATF regulated tobacco products will be presented.

*Keywords:* Tobacco products; Nicotine

## 1. Introduction

Commercial tobacco products can be classified into two groups: smoking tobacco products and smokeless tobacco products. Examples of smoking tobacco products include cigars, cigarettes, pipe tobacco and roll-your-own (RYO) tobacco. Smokeless tobacco products include chewing tobacco and snuff. Nicotine is the active principal component in all tobacco products. It is also the most abundant alkaloid found in tobacco, occurring in relatively large concentrations (0.5–8.0%) [1], as their salts in tobacco leaves [2], and comprising about 98% of the total alkaloid content [3].

The Bureau of Alcohol, Tobacco and Firearms (ATF), an independent agency within the US Department of the Treasury, is responsible for revenue compliance on tobacco products. A primary mission of ATF is regulation of the tobacco industry. Tobacco products are classified at different tax rates according the Code of Federal Regulations (Title 27 of the CFR PART 270.11). The comparative basis of this tax classification is as follows: cigars vs. cigarettes; pipe tobacco vs. roll-your-own; chewing tobacco vs. snuff. For example, the tax on cigarettes is approximately ten-fold greater than the tax on cigars. The current tax rate is $1.125 per thousand for small cigars compared to $12.00 per thousand for small cigarettes (Table 1).

Identification of tobacco products relies on a variety of tests including a determination of nicotine. The rationale for analyzing nicotine in ATF regulated products, regardless of the type of tobacco product, is two-fold: (a) regulatory: the presence, not

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Table 1
Excise tax rates effective January 1, 1993

| Tobacco product | Tax rate |
|---|---|
| Small cigars | $1.125 per thousand |
| Large cigars | 12.75% of price not to exceed $30 per thousand |
| Small cigarettes | $12.00 per thousand |
| Large cigarettes | $25.20 per thousand |
| Cigarette papers | 0.75 cents per 50 papers |
| Cigarette tubes | 1.5 cents per 50 tubes |
| Smokeless snuff | 36 cents per pound |
| Chewing tobacco | 12 cents per pound |
| Pipe tobacco | 67.5 cents per pound |

the amount, of nicotine is the primary determinant of what constitutes a tobacco product. (b) Abundance: nicotine is the most abundant tobacco alkaloid, comprising about 98% of the total alkaloid content.

Nicotine is strongly chromophoric in the UV region and is directly detected at about 260 nm [4]. Nicotine has been analyzed using spectrophotometry [5], gas chromatography [6], high-performance liquid chromatography [7,8] and more recently capillary electrophoresis [9].

In the past at the ATF National Laboratory Center, nicotine in tobacco products was determined using spectrophotometry and gas chromatography. Unfortunately, there are inherent disadvantages associated with both these methods. The disadvantage of spectrophotometry, despite the simplicity of technique and instrumentation, is that structurally related tobacco alkaloids (for example, nornicotine) and other UV absorbing compounds would absorb in the 260 nm region thus interfering with that of nicotine. Nevertheless, prior analysis of nicotine in ATF regulated tobacco products reported total alkaloids as nicotine.

Nicotine was then determined using gas chromatography as described by Gottscho et al. [6]. This technique worked rather well especially since the method was originally applied to the analysis of nicotine in commercial tobacco products. Despite the short run times, gas chromatography also suffers in that the extraction step employs organic solvents (chloroform, hexane) in relatively large volumes, is tedious and time consuming and its impact on the environment.

Capillary electrophoresis (CE) as an analytical technique is rapidly gaining importance capturing the interest of analysts in several areas [10]. The unique and powerful capabilities of CE including high efficiency, high resolution, short analysis times, low sample consumption and selectivity make it a powerful analytical tool that is complementary to other analytical techniques such as HPLC.

To my knowledge, this is the first such report on the application of CE to the analysis of nicotine in tobacco products in the regulatory area. Preliminary results using a sodium phosphate buffer, pH 2.5 for extraction of nicotine from tobacco products followed by UV absorbance detection at 260 nm have yielded promising results [11]. In this paper, nicotine obtained in aqueous extracts from tobacco products is determined using CE followed by UV aborbance detection at 260 nm, making it a powerful analytical tool in the regulatory area as well. The focus of this paper will be directed mainly toward the development of a CE method for the separation and identification, with a minor emphasis on its future use as a quantitative tool, for the analysis of nicotine in ATF-regulated tobacco products.

## 2. Experimental

### 2.1. Reagents

All solutions were prepared from reagent grade chemicals. The water used was purified using the NANOpure water purification system (SYBRO/Barnstead, Boston, MA, USA). Sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$), sodium phosphate dibasic ($Na_2HPO_4$), sodium hydroxide (NaOH) and phosphoric acid, 85% ($H_3PO_4$) were obtained from

Fisher Scientific (Fair Lawn, NJ, USA). (−) Nicotine was obtained from Sigma (St. Louis, MO, USA).

### 2.2. Apparatus

CE was performed on a SpectraPHORESIS 500 System (Thermo Separation Products, Fremont, CA, USA) equipped with an autosampler, UV absorbance detector and a data system using SpectraPHORESIS software version 1.04.

The capillary was preconditioned prior to its first use and thereafter at the beginning of each run as follows: 10 min wash with 1.0 *M* NaOH at 60°C, 5 min wash with 0.1 *M* NaOH at 60°C, 5 min wash with distilled deionized water at 60°C, 5 min wash with run buffer at 20°C. Electrolyte solutions were degassed using vacuum for 2 min prior to each run. Two buffer blank runs and two sample runs were performed prior to starting the actual analysis to allow the system to stabilize, and for the electrolyte solutions, standards and samples to equilibrate to the run conditions. The capillary was rinsed with 0.1 *M* NaOH and then with distilled, deionized water each for 1 min between runs in a postrun capillary wash. The capillary was rinsed and filled with the run buffer in a prerun capillary wash for 1 min. CE analysis of nicotine was performed using a 44 cm× 100 μm fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) in 25 m*M* sodium phosphate buffer, pH 2.5 at 20°C. Samples were injected hydrodynamically for 2 s and separation was performed at 10 kV with run times usually around 10 min. Nicotine was detected by monitoring the absorbance at 260 nm.

### 2.3. Samples

Tobacco products were the routine samples provided by the Wine, Beer and Spirits Regulation Branch of ATF and received by the Tobacco Laboratory at the National Laboratory Center. These samples were stored at 4°C until analysis. Filler tobacco was separated from the other components in the case of both cigar and cigarette samples. In addition, cigars were analyzed for their wrappers as well in keeping with the ATF regulatory requirements. Snuff, in its moist or dry form was used as is. Chewing tobacco, as a plug or loose leaf in a pouch, and pipe tobacco required some comminution in order to obtain a working sample size.

### 2.4. Sample preparation

Samples were used as is or ground depending on the type of tobacco product. Cigar filler tobacco, cigarette filler tobacco and cigar wrapper were ground in Wiley Mill equipped with a 20-mesh screen (Arthur H. Thomas, Philadelphia, PA, USA). Chewing tobacco, as a plug or in a pouch, was cut into small pieces using a pair of scissors or a razor blade and used as is. Pipe tobacco (large pieces were usually cut into small pieces) was used as is.

A sample of tobacco, as is or ground (100 mg), was extracted with occasional shaking in 10 ml of distilled, deionized water at room temperature for 1 h. Tobacco samples were used fresh, usually in less than 4 h. The aqueous tobacco extracts were diluted 1:10 with water, an aliquot (2 ml) filtered through a 0.2 μm disposable filter directly into an autosampler vial prior to analysis by CE.

## 3. Results and discussion

Nicotine being a basic molecule, could be separated in CE using a low pH buffer system such as phosphate or citrate [12]. A CE method suggested by Dionex (Sunnyvale, CA, USA) for the separation of nicotine was initially used [13]. This included a 75 cm×75 μm capillary column that was run at 25 kV at 25°C with UV absorbance detection at 265 nm. A 25 m*M* sodium phosphate buffer, pH 2.5 with 100 m*M* hexanesulfonic acid as an ion-pairing reagent was adopted. Nicotine under these conditions eluted around 15.5 min.

On this basis, I modified the method with the ultimate goal of shortening the run times, while maintaining the quality of resolution. The modifications were made following a careful examination of both the structure and chemistry of nicotine.

### 3.1. Nicotine as a function of pH

When dissolved in water, nicotine can exist in one of the three forms depending on the pH of the solution: unprotonated (free base), monoprotonated

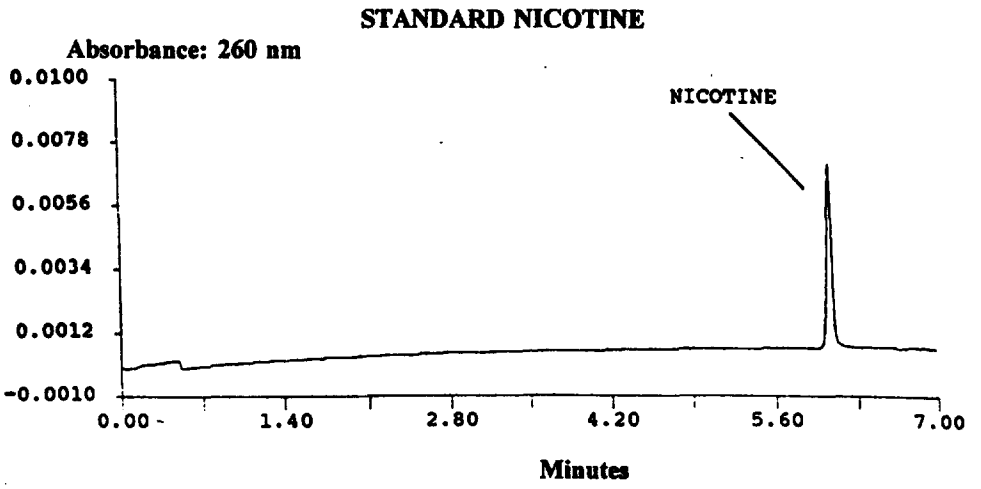


Fig. 1. Electropherogram of standard nicotine at a concentration of 17.24 μg/ml. Separation conditions as described in Section 2.2.

and diprotonated [9,14]. This pH dependence has a definite impact on the CE performance of nicotine as shown below. Nevertheless, the modifications of the CE method for the analysis of nicotine involved the use of a 100 μm capillary column, a lower voltage (10 kV) applied across a shorter column (44 cm) in the presence of a dilute electrolyte buffer (25 m*M* sodium phosphate buffer) with UV absorbance detection at 260 nm. The CE performance of this modified method was evaluated using nicotine at pH 6.9 and pH 2.5 to determine the best response.

### *3.2. Capillary electrophoresis of nicotine in sodium phosphate buffer as a function of pH*

CE analysis of nicotine was performed in sodium phosphate buffer as a function of pH. Table 2 shows the CE precision data for ten replicate injections of standard nicotine at pH 6.9 and pH 2.5. Two important experimental observations can be made from this study. First, its impact on the net mobility of nicotine: At pH 6.9, nicotine exists as the monoprotonated species and is highly mobile eluting at 2.99 min. Lowering the pH to 2.5, nicotine as the diprotonated species is less mobile and elutes at 6.63 min. Second, the peak area counts of 7464 for nicotine at pH 6.9 was obtained at a concentration 100-fold greater than the peak area counts of 4422 obtained for nicotine at pH 2.5. As a diprotonated species (pH 2.5), the UV absorbance of nicotine at 260 nm is observed to be greater than that observed for nicotine as a monoprotonated species (pH 6.9). This is agreement with similar observations reported by others [5,9]. Based on these results, a 2.5 pH sodium phosphate buffer was subsequently used in all studies for the CE analysis of nicotine.

### *3.3. Capillary electrophoresis of nicotine in sodium phosphate buffer at pH 2.5*

Once the decision was made to use a 2.5 pH sodium phosphate buffer, all studies were performed using the CE separation conditions as described in Section 2.2. Fig. 1 shows the electropherogram of an aqueous solution of standard nicotine at a concentration of 17.24 μg/ml.

Table 2
CE analysis of nicotine in sodium phosphate buffer as a funtion of pH

| Buffer pH | Nicotine | | |
|---|---|---|---|
| | Concentration (μg/ml) | Migration time (min) | Area counts |
| 6.9 | 172.4 | 2.99 | 7464 |
| 2.5 | 1.724 | 6.63 | 4422 |

Table 3
Precision data for nine replicate injections of standard nicotine at two different concentrations in phosphate buffer, pH 2.5

| Concentration (μg/ml) | Migration time (C.V.%) | Area counts (C.V.%) |
|---|---|---|
| 1.724 | 0.69 | 9.04 |
| 17.24 | 1.09 | 4.29 |

The performance criteria of this CE method were assessed by evaluating the precision of the analysis at two different concentrations of nicotine. Table 3 shows the intra-day precision data for nine replicate injections of standard nicotine at two different concentrations (1.724 and 17.24 μg/ml, respectively). Results from these studies show that the precision of sample injection is a function of the sample concentration. A greater precision for migration times is seen at the lower nicotine concentration (1.724 μg/ml), while a greater precision for area counts is seen at the higher nicotine concentration (17.24 μg/ml) [12].

### 3.4. Capillary electrophoresis of nicotine in tobacco products

#### 3.4.1. Beedies

The very first tobacco product to be analyzed for nicotine by CE at the National Laboratory Center was known as beedi. According to the Dictionary of Tobacco Terminology, a beedi (also called bidi, biri)

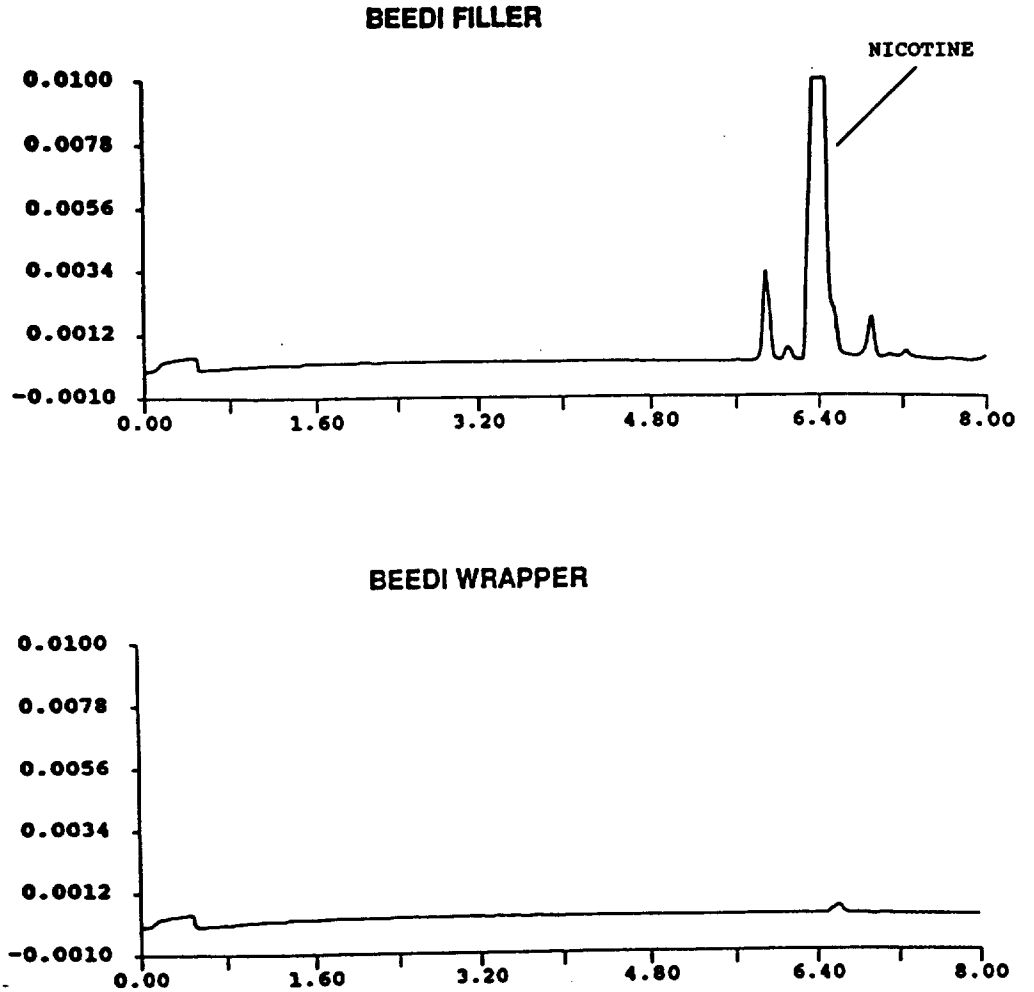


Fig. 2. CE analysis comparing the wrapper and filler extracts from a beedi. Separation conditions as described in Section 2.2.

is "a form of cigarette found in India; it consists of granulated tobacco rolled in a section of Indian ebony leaf and tied with thread" [15]. Prior records have shown that neither was this product analyzed for nicotine, nor a determination made to properly classify it for tax purposes according to the Code of Federal Regulations (27 CFR PART 270.11). According to the Dictionary of Economic Plants, the species of Indian ebony leaf used for wrapping beedies in India is *Diospyros melanoxylon Rox* [16,17], and, according to Hegnauer's Chemotaxonomie der Pflanzen, this and the other *Diospyros* species contain no alkaloids [18,19]. Based on this information, beedies, therefore, provided a tobacco product that was an ideal and challenging substrate ready to be analyzed for nicotine, and its subsequent proper classification from a regulatory standpoint. This beedi product was prepared for analysis by first

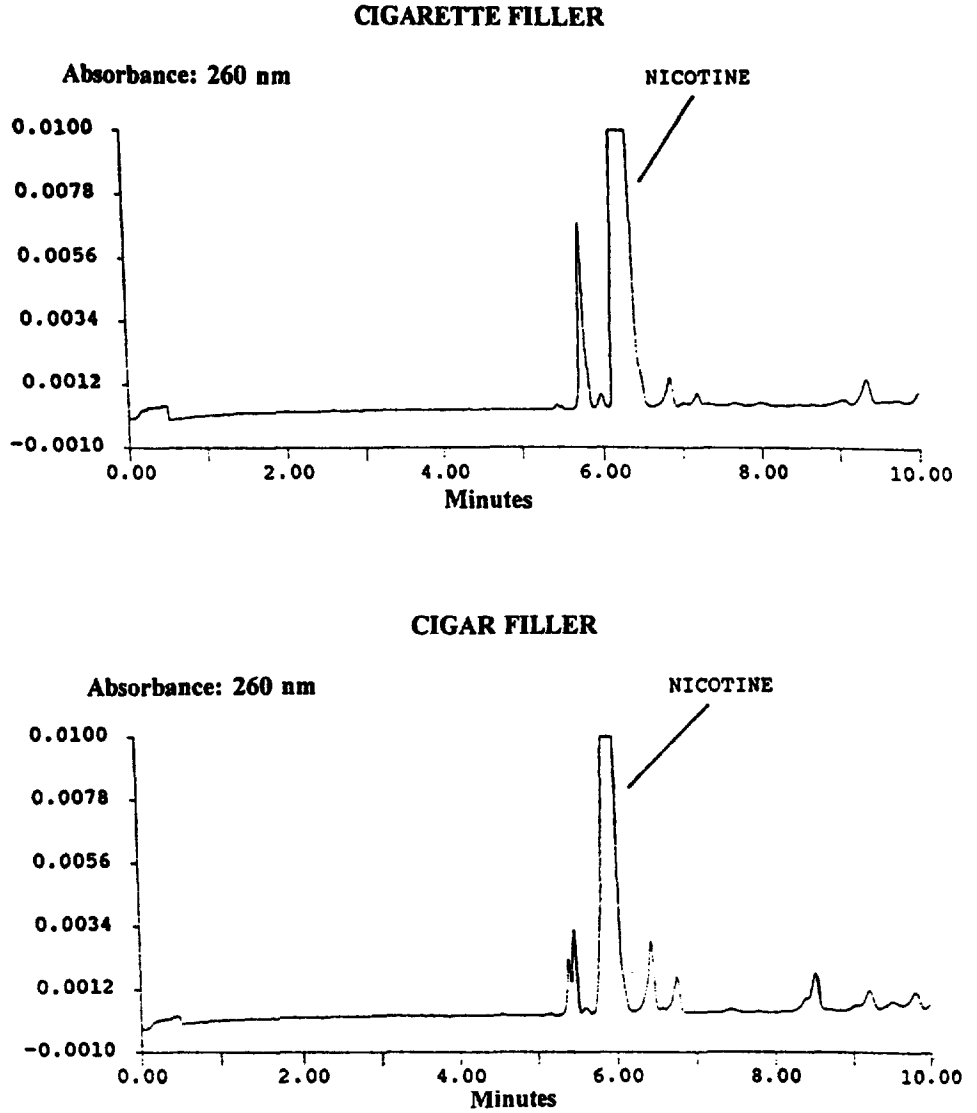


Fig. 3. CE analysis of aqueous extracts from cigarette and cigar fillers. Separation conditions as described in Section 2.2.

separating the filler from its wrapper. The filler from one beedi (0.1625 g) was extracted with 10 ml of deionized water with occasional shaking at room temperature for 1 h. No attempt was made to basify the water to improve the extraction efficiency [9]. Nicotine obtained from the aqueous beedi filler extract was filtered directly into the autosampler vial for CE analysis. The beedi wrapper was similarly extracted and analyzed by CE. Results of this CE analysis showed the presence of nicotine in the filler, indicating that the beedi is consistent with a tobacco product. CE analysis of the wrapper showed an absence of nicotine, indicating that the beedi is consistent with a cigarette and would be properly classified as such. Based on these CE results, we were able to show that the beedi was indeed a tobacco product, and would be properly classified as a cigarette. The CE conditions included a 75 cm×75

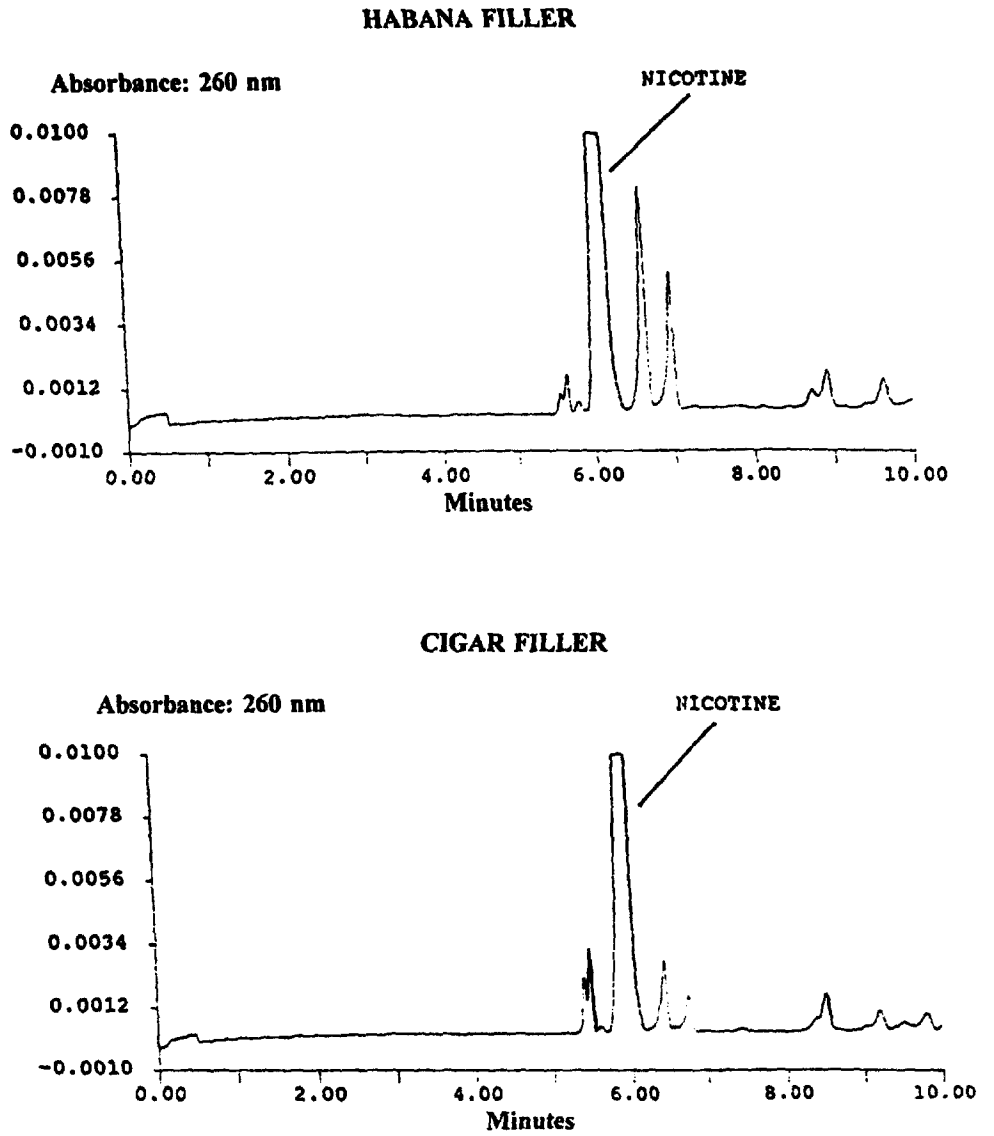


Fig. 4. CE analysis of aqueous extracts from habana and cigar fillers. Separation conditions as described in Section 2.2.

μm capillary column that was run at 25 kV at 25°C with UV absorbance detection at 265 nm, and 25 m*M* sodium phosphate, pH 2.5 with 100 m*M* hexanesulfonic acid, as the running buffer [13].

Because of the long migration times observed with CE under conditions using an ion-pairing reagent as mentioned above with beedies, subsequent analysis of nicotine in tobacco products was performed using the described CZE separation conditions. Results are shown in Fig. 2. In case of the beedi sample, the wrapper did not analyze positive for nicotine in agreement with published reports ( [18,19]; Fig. 2).

### 3.5. Other applications

The CZE method was applied to the analysis of nicotine in ATF regulated tobacco products other than the beedies. These included cigars, cigarettes,

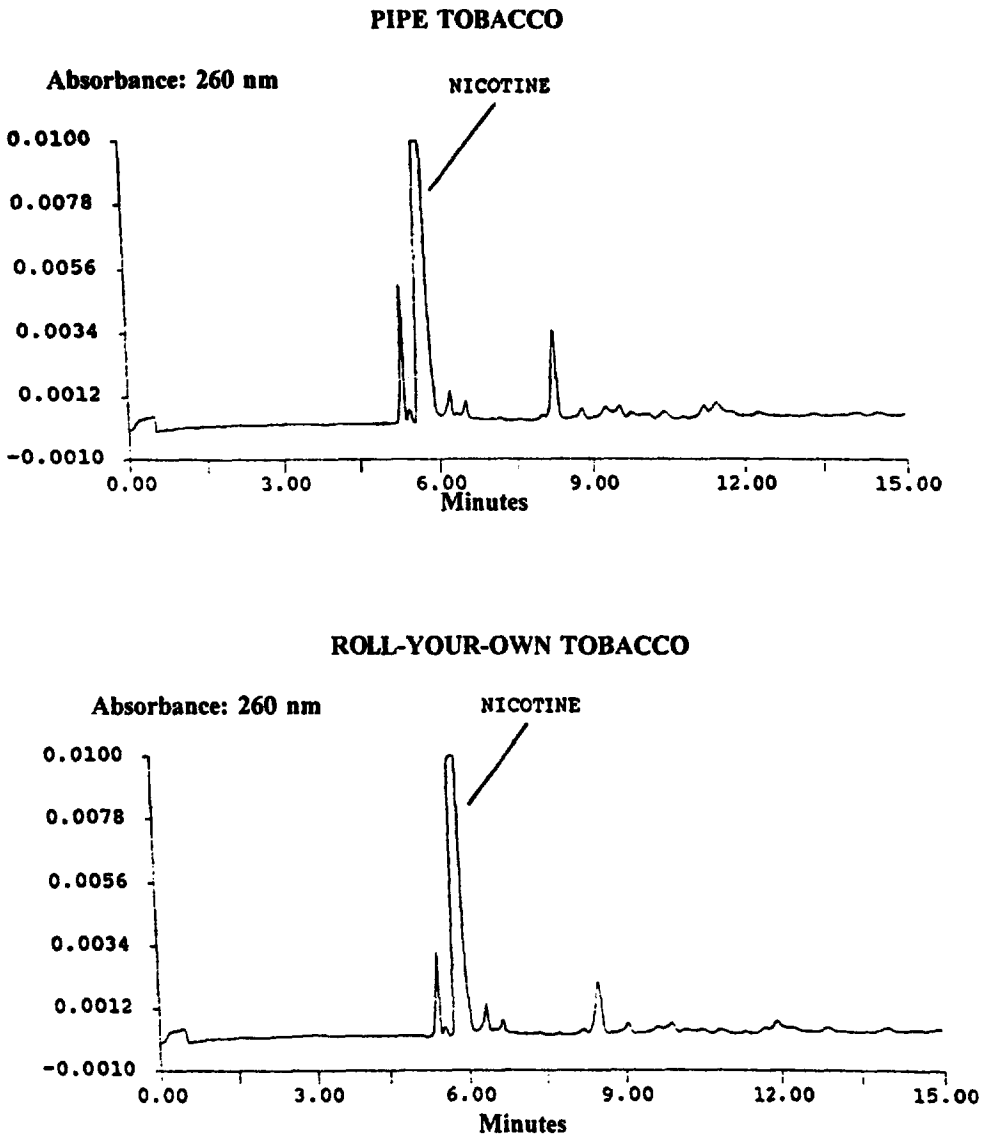


Fig. 5. CE analysis of aqueous extracts from pipe tobacco and roll-your-own (RYO) tobacco. Separation conditions as described in Section 2.2.

pipe tobacco, roll-your-own (RYO) tobacco, chewing tobacco and snuff. In all cases, about 100 mg of the tobacco product was extracted with 10 ml of deionized water with shaking for 1 h. Again, no attempt was made to basify the deionized water for improved extraction efficiency. The aqueous extract was filtered through a 0.2 μm filter directly into the autosampler vial and analyzed by CE. Figs. 3–6 show sample electropherograms of tobacco extracts prepared from a cigarette, cigar (filler), habana cigar (filler), pipe tobacco, roll-your-own, chewing tobacco and snuff. In case of the cigar samples, the wrappers were also analyzed in addition to the fillers. The cigar wrapper analyzed positive for nicotine in keeping with the regulatory definition of a cigar according to the Code of Federal Regulations (Title 27 of the CFR PART 270.11) (Fig. 7). A single distinct peak for nicotine was detected in all cases. Under these conditions, the capillary buffer seems to provide just the exact buffering capacity required for

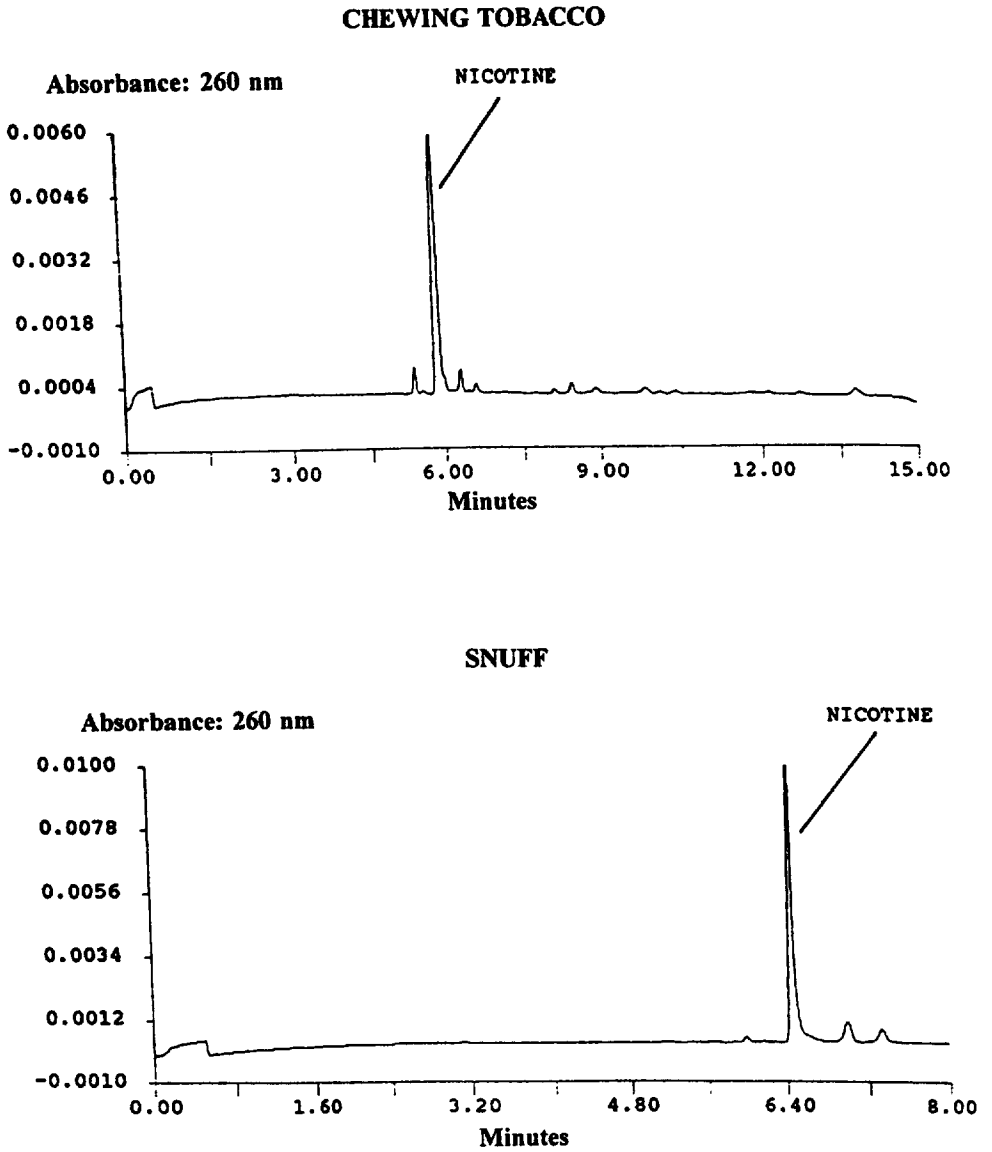


Fig. 6. CE analysis of aqueous extracts from chewing tobacco and snuff. Separation conditions as described in Section 2.2.

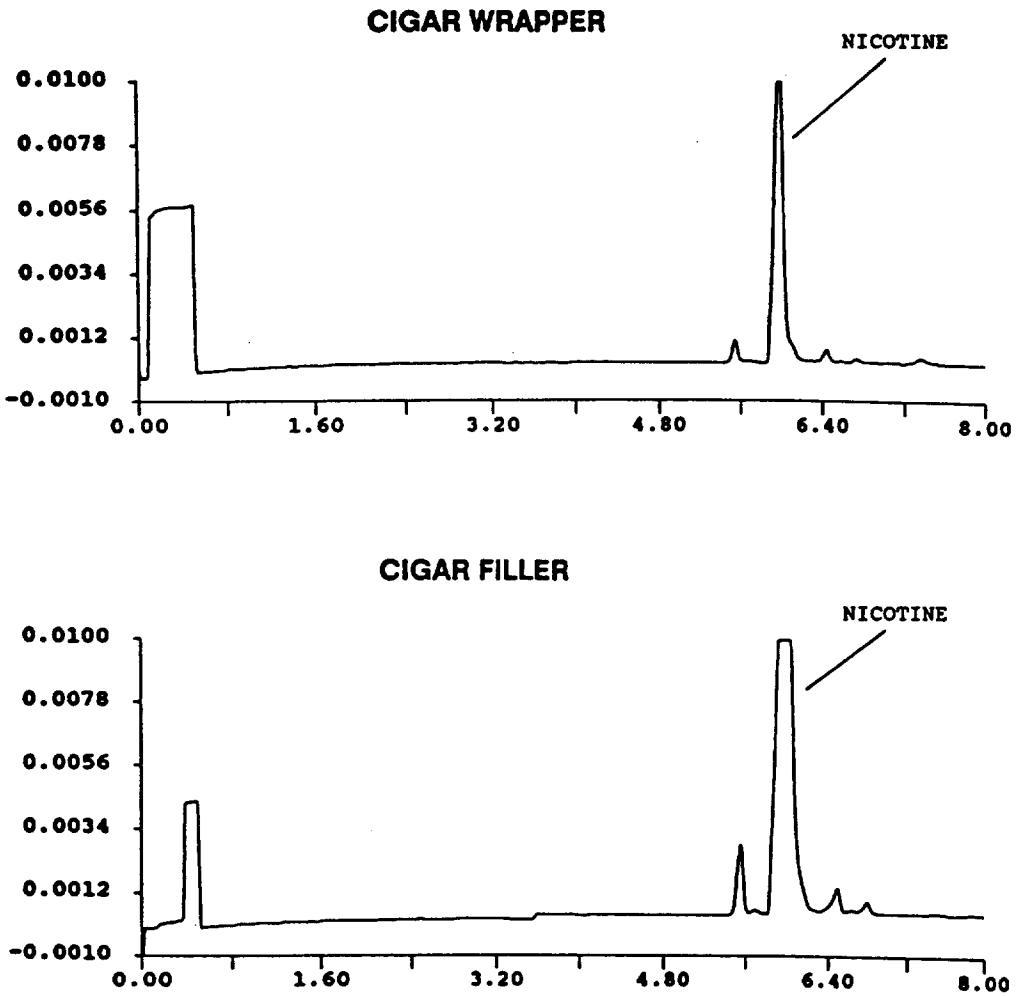


Fig. 7. CE analysis comparing the wrapper and filler extracts from a cigar. Separation conditions as described in Section 2.2.

the CE analysis of ATF regulated tobacco products. The pH of 2.5 is acidic enough for nicotine to exist as a diprotonated species [14], thereby providing a maximum UV absorbance at 260 nm. The buffer concentration of 25 m*M* sodium phosphate seems to provide the ionic strength for maintaining just the right electroosmotic flow observed in this study. Under these conditions, nicotine appeared as a single predominant peak around 6.0 min, with a fairly high UV response at 260 nm, and very well separated from the other peaks. The original 25 m*M* solution of capillary buffer once prepared was used repeatedly, after storing at 4°C, for over a year with virtually no loss in its buffering capacity.

From the electropherograms of the different tobacco products analyzed, minor peaks, in addition to the major nicotine peak, were also detected with virtually no interference. The electropherograms were generally clean and straightforward. Efforts have not been made to identify the minor peaks at the present time. This is because (a) nicotine is the most abundant tobacco alkaloid and (b) the presence, not the amount, of nicotine is the primary determinant of what constitutes a tobacco product from a regulatory standpoint. Nevertheless, minor alkaloids such as nornicotine, anabasine and anatabine have been reported to occur in tobacco and tobacco products [1]. Two of the minor alkaloids, nornicotine and

anatabine, have been identified in a CE analysis of a Kentucky Reference tobacco sample [9]. Table 4 shows the application of the CE method to the analysis of nicotine in a wide variety of ATF regulated tobacco products.

Table 4
CE analysis of nicotine in ATF regulated tobacco products

| Tobacco product | Sample No. | Nicotine | |
|---|---|---|---|
| | | Wrapper | Filler |
| Cigarette | 16648 | NA | + |
| | 16649 | NA | + |
| | 16650 | NA | + |
| | 16651 | NA | + |
| | 16652 | NA | + |
| | 16653 | NA | + |
| | 16654 | NA | + |
| | 16655 | NA | + |
| | 16656 | NA | + |
| | 16657 | NA | + |
| | 16659 | NA | + |
| | 16660 | NA | + |
| | 16661 | NA | + |
| | 16662 | NA | + |
| | Marlboro | NA | + |
| | Newport | NA | + |
| Beedies | 16586 | – | + |
| | 16606 | – | + |
| | 16607 | – | + |
| | 16647 | – | + |
| Cigars | 16574 | + | + |
| | 16582 | + | + |
| | 16664 | + | + |
| | Winchester Little Cigar | + | + |
| | Habana Cigar | + | + |
| Pipe tobacco | 16634 | NA | + |
| | 16635 | NA | + |
| Chewing tobacco | 16645 | NA | + |
| Roll-your-own | 16636 | NA | + |
| | 16637 | NA | + |
| Herbal smoking (non tobacco) | 16608 | – | – |
| | 16658 | – | – |
| Herbal smokeless (non tobacco) | 16665 | NA | – |

NA=Not Applicable.

### 3.6. Quantitative analysis of nicotine by capillary electrophoresis

An experiment to quantitate nicotine in selected tobacco products was attempted. Quantitation was done by performing a calibration run using standard nicotine solutions. Nicotine standard solutions ranging in concentration between 1.724–17.24 μg/ml were prepared and used in a four-level calibration run. An acceptable correlation coefficient of 0.992 was calculated from the calibration plot of peak area response at 260 nm versus nicotine concentration (Fig. 8). Table 5 shows the quantitative analysis of nicotine in selected ATF regulated tobacco products. These results are in general agreement with reported nicotine amounts for such products [1].

### 3.7. Future studies

Future studies at the National Laboratory Center will focus on extending the range and scope of this application to: (a) additional studies on the quantitative analysis of nicotine and the other minor alkaloids in ATF regulated tobacco products; (b) CE profiling the ATF regulated tobacco products and (c) characterizing the ATF regulated tobacco products.

## 4. Conclusions

CE is shown to be a simple, rapid, useful and powerful analytical tool for monitoring nicotine in ATF regulated tobacco products. Nicotine, the predominant tobacco alkaloid and the primary regulatory determinant, can be quantitatively monitored. CE nicotine results obtained here have provided respectable data with respect to system suitability parameters: precision, accuracy, linearity and sensitivity. CE is amenable to ATF regulatory compliance with the added advantage of reduced turnaround time. Since the capillary buffer is aqueous and with very little of it being consumed, CE is both environmentally friendly and economical. Eventually, CE has the potential of "fingerprinting" ATF regulated products for classification purposes.

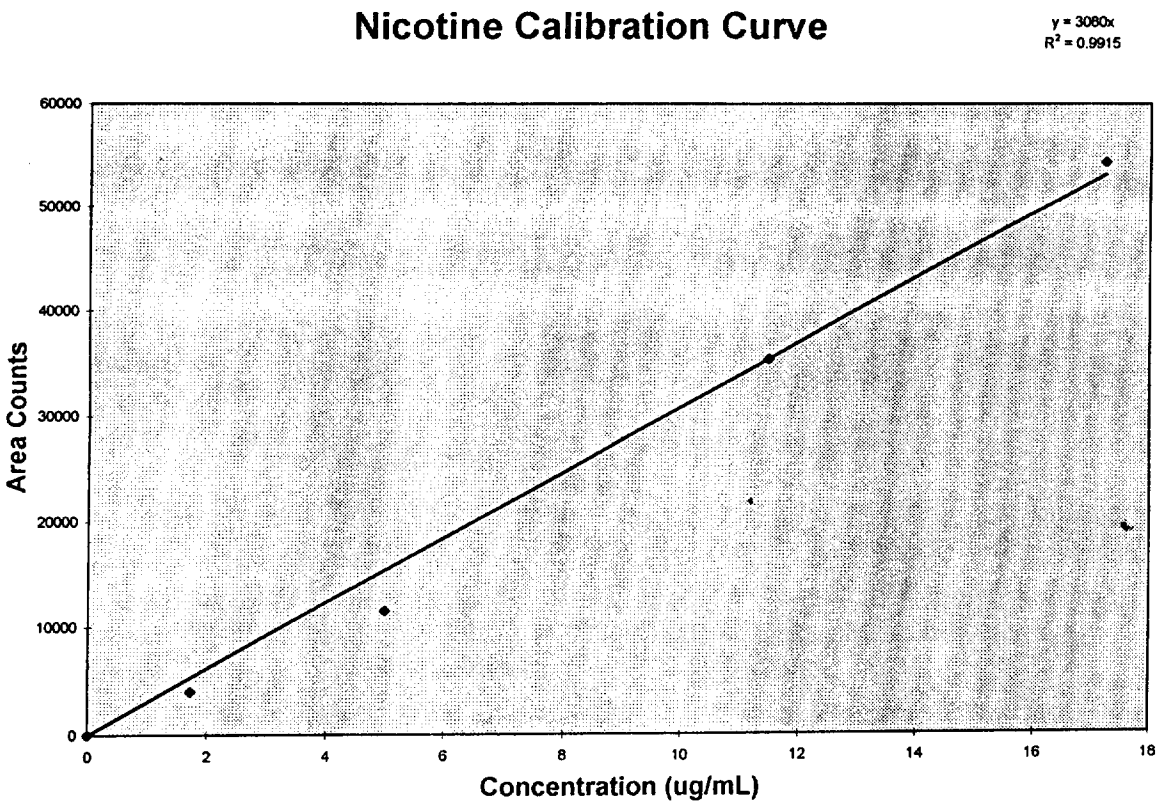


Fig. 8. CE calibration plot of peak area response at 260 nm vs. nicotine concentration.

Table 5
CE quantitative analysis of nicotine in ATF regulated tobacco products

| Tobacco product | Nicotine (%) |
|---|---|
| Cigarette | 1.75 |
| Cigar | 1.39 |
| Habana | 1.70 |
| Beedi | 3.49 |
| Herbal Filter | 0.00 |

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