Reversed Phase Ion-Pair Liquid Chromatographic Determination of Nicotine in Commercial Tobacco Products. 2. Cigarettes

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A reversed phase ion-pair liquid chromatographic method for the determination of nicotine in commercial tobacco products was previously developed and optimized (Ciolino, L. A.; Turner, J. A.; McCauley, H. A.; Smallwood, A. W.; Yi, T. Y. *J. Chromatogr.* **1999a**, *852* (2), 451−463) and provided reliable results for the determination of nicotine in commercial moist snuff (Ciolino, L. A.; McCauley, H. A.; Fraser, D. B.; Barnett, D. Y.; Yi, T. Y.; Turner, J. A. *J. Agric. Food Chem.* **1999b**, *47*, 3706−3712). The method uses an aqueous-based sample extraction and provides rapid separation of nicotine from the minor tobacco alkaloids and other commercial tobacco components. In the present work, the method is evaluated for the determination of nicotine in commercial cigarettes and compared to both an official AOAC method for total alkaloids in tobacco (AOAC, *AOAC Official Methods of Analysis of AOAC International*, 16th ed.; AOAC International: Gaithersburg, MD, 1995; pp 30−31), and a published GC method (Lyerly, L. A.; Greene, G. H. *Beitr. Tabakforsch.* **1976**, *8* (6), 359−361). Good agreement was obtained between the ion-pair LC method and the GC method with relative differences in determined nicotine contents of 0.6 to 5% for a series of commercial and reference cigarettes.

Keywords: *Nicotine; cigarettes; tobacco; alkaloids; reversed phase ion-pair liquid chromatography*

INTRODUCTION

In Part 1 (Ciolino et al., 1999b), we reported on the evaluation of a reversed phase ion-pair liquid chromatographic method for the determination of nicotine in commercial moist snuff. This method was based on conditions that were optimized in separate experiments (Ciolino et al., 1999a). We report here on the evaluation of this approach for the determination of nicotine in commercial cigarettes.

Validation data were generated for two reference cigarette composite samples (University of Kentucky Reference 1R4F and University of Kentucky Reference 2R1F) and eight commercial cigarette composite samples. The nicotine contents of the 10 composite samples were also determined using two other methods for comparison: the AOAC steam distillation method for the determination of total alkaloids in tobacco (AOAC, 1995) and a GC method based on the determination of the nicotine in commercial tobacco (Gottscho et al., 1988; Lyerly and Greene, 1976). This latter method was reported to provide reproducible and accurate results for a variety of commercial tobaccos with nicotine contents ranging from 0.05 to 3.0 wt % (Lyerly and Greene, 1976).

MATERIALS AND METHODS

Composite Preparation. Eight different brands of commercial cigarettes were purchased from stores in the Greater Cincinnati area. Reference Cigarettes 1R4F and Reference Cigarettes 2R1F were obtained from the University of Kentucky Tobacco Health Research Institute (THRI). Each composite was prepared in a 1 gal glass jar using the contents of 20 packs (20 cigarettes/pack) of cigarettes. To remove the tobacco filler from the cigarette, the cigarette wrapper was sliced along the length of the cigarette using a razor blade. Two cigarettes from every fifth pack were sampled for individual nonvolatile determination immediately after the wrapper had been sliced open. The tobacco filler from these cigarettes was not added into the composite. After all of the tobacco filler was placed in the jar, the contents were thoroughly mixed and additional samples were taken for a second nonvolatile determination. When not in use, the composite samples were sealed in the glass jars and stored in a refrigerator. Additional nonvolatile determinations were conducted on the composites at 2−3 week intervals throughout the study. Total composite weights ranged from 300 to 340 g except for composite 7, which weighed 200 g. The lower weight for composite 7 was due to the lower weight of tobacco filler contained in each cigarette for this product (average of 510 mg of tobacco filler/cigarette) versus all of the other products (averages ranging from 760 to 870 mg of tobacco filler/cigarette).

Nonvolatile Determination. Percent nonvolatiles was determined by drying a weighed portion of tobacco (sample weight = 1−2 g) in a laboratory oven for 3 h at 105 °C, cooling the samples in a desiccator, and reweighing. The percent by weight volatile material (100% − % nonvolatiles) was used as an estimate of percent by weight moisture.

Ion-Pair LC Method. *(a) Instruments and Conditions.* Each of the four analysts used one of the following liquid chromatograph systems or component combinations: Hewlett-Packard Series II 1090 with diode array detector; Hewlett-Packard 1050 with multiwavelength detector; Hewlett-Packard 1050 with diode array detector; Waters 600 E pump, Waters 712 WISP autosampler, and Waters 996 diode array detector. Zorbax Rx C_{18} (analysts 1, 3, and 4) or Supelco PKB-100 C_{18} (analyst 2) columns, both 5 μm, 15 cm × 4.6 mm i.d.,

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were used for all determinations. The injection volume was 10 μL. Detection was at 259 nm.

The mobile phase buffer was 50 mM citric acid and 10 mM sodium octanesulfonate for all determinations. Analyst 2 used a buffer pH of 3.0 with 65:35 buffer/methanol and a flow rate of 1.0 mL/min. Analysts 1, 3, and 4 used a buffer pH of 3.20−3.25 with 70:30 buffer/methanol and a flow rate of 1.0 mL/min.

(b) Reagents and Standards. Citric acid monohydrate (ACS reagent), sodium octanesulfonate (98% or Ultra grade), and nicotine hydrogen tartrate salt (catalog no. N-5260) were obtained from Sigma (St. Louis, MO). The nicotine content of the nicotine salt was determined at 32.6% at the beginning of the study and at 32.6% after the study using CORESTA Recommended Method 39 (CORESTA, 1994). This corresponds to a purity of 100% (100.3%) as the salt. Nicotine standards were prepared in the mobile phase buffer to cover the calibration range 0−135 μg/mL.

(c) Sample Preparation. Approximately 0.1−0.2 g of composited cigarette tobacco filler was accurately weighed into a 20 mL glass scintillation vial. Mobile phase buffer (10 mL) was added using a pipet, and the vial was sonicated for 30 min. The liquid extract was subsequently drawn off using a fine-tip disposable pipet and set aside. Second and third extractions of the original sample were conducted using 10 mL volumes of mobile phase buffer, sonicating, and drawing off the liquid extract after each extraction. The three extraction volumes were combined and filtered through a 0.45 μm nylon filter into an LC vial for analysis. Eight replicate samples were prepared for each composite. All values for nicotine content are based on peak area quantitation and are reported on an "as is" basis (not corrected for moisture content).

(d) Sample and Standard Preparation, Single Extraction Experiments. Approximately 0.1−0.2 g of composited cigarette tobacco filler was accurately weighed into a 20 mL glass scintillation vial. Mobile phase buffer (10 mL) was added using a pipet, and the vial was sonicated for 30 min. The liquid extract was filtered through a 0.45 μm nylon filter into an LC vial for analysis. Eight replicate samples were prepared for each composite. Nicotine standards were prepared in the mobile phase buffer to cover the calibration range 0−500 μg/mL. All values for nicotine content are based on peak area quantitation and are reported on an "as is" basis (not corrected for moisture content).

(e) Spike/Recovery Experiments Using a Single Extraction. For spike recovery experiments, an appropriate volume of a nicotine hydrogen tartrate solution prepared in mobile phase buffer was spiked onto 1 g of composited cigarette tobacco filler to provide an additional nicotine content equal to the previously determined nicotine content of the tobacco sample. The sample was then analyzed according to section d above (single extraction), increasing the extracting solvent volume to 100 mL. Because of the increased sample weight used in the spike/recovery experiments, unspiked 1 g samples were also analyzed for nicotine content. All spiking experiments were conducted in duplicate.

(f) Homogeneity Experiment. Analyst 2 repeated the nicotine determinations for all of the assigned cigarette composites according to section d above (single extraction) using an increased sample weight of 1.5−2.0 g and an extraction solvent volume of 100 mL. Five replicate samples were prepared for each composite.

AOAC Method 967.02 (AOAC, 1995). *(a) Samples.* Three replicate analyses were conducted for each cigarette composite using sample weights of 5 g. The reported nicotine contents were not corrected for the purity of the nicotine free base standard. All values are reported on an "as is" basis (not corrected for moisture content).

(b) Instrument, Apparatus, and Standards. See Part 1 (Ciolino et al., 1999b).

GC Method (Gottscho et al., 1988; Lyerly and Greene, 1976). *(a) Instrument and Conditions.* All GC determinations were made using a Hewlett-Packard 5890 Series II gas chromatograph with an FID detector. The GC column and conditions were the same as for the moist snuff validation study (Ciolino et al., 1999b), which are based on the work of Gottscho et al. (1988): Alltech catalog no. C6089 glass column (2% KOH and 10% Carbowax 20 M, support 80−100 mesh Chromosorb WHP, 6 ft length, 4 mm i.d., 0.25 in. o.d.). The column was preconditioned by the manufacturer. The column temperature (isothermal) was 190 °C; injector and detector temperatures were both 230 °C. The carrier gas flow rate (helium) was 30 mL/min. The injection volume was 5 μL.

Note: To the best of our knowledge, the column specified by Lyerly and Greene (1976) is not commercially available (stainless steel packed with 10% Castorwax and 3% KOH on 60/80 mesh Chromosorb W, 3 ft length, $^1/_8$ in. i.d.).

(b) Standards. The internal standard *n*-hexadecane (99+%) was obtained from Sigma. See the section c under AOAC Method 967.02 of Part 1 (Ciolino et al., 1999b) for a description of the nicotine free base standard.

(c) Samples. Five replicate analyses were conducted for the cigarette composites using 1 g samples. Sample extractions were conducted as specified by Lyerly and Greene (1976). The reported nicotine contents were not corrected for the purity of the nicotine free base standard and are reported on an "as is" basis (not corrected for moisture content).

Experimental Design for Validation Studies. Four analysts participated in the ion-pair LC portion of the validation study. To compare results between and among analysts in an efficient design, the analysts were assigned to the composites as shown in Table 2. This design provided for two separate determinations (two different analysts) for each of the commercial composites (composites 1−8) and for a comparison of results from all four analysts based on both of the reference composites (composites 9 and 10). This design also allowed results from each analyst to be compared to results from every other analyst at least once. The analyst assignments were the same for the single extraction experiments. The AOAC method and the GC method were conducted by two additional analysts (one per method) who did not participate in the LC portion of the study.

Table 1. Nonvolatile Determinations for Cigarettes: Individual Packs and Composited Product

composite no.	individual packs		composited product	
	% nonvolatiles	%RSD	% nonvolatiles	%RSD
1	85.3	2.3	85.1	1.5
2	84.2	0.5	84.7	0.4
3	85.4	0.3	85.3	0.2
4	84.2	0.7	84.5	0.4
5	84.8	0.6	85.1	0.4
6	85.1	0.3	85.8	0.4
7	85.9	0.6	86.2	0.3
8	85.2	0.7	84.8	0.6
9	86.5	0.6	86.3	0.4
10	83.8	2.0	84.0	1.8

RESULTS AND DISCUSSION

Nonvolatile Determination and Composite Homogeneity. Commercial cigarettes are typically packaged in paper or foil packs. Once a pack is opened, moisture may be lost due to evaporation. Nonvolatile determinations were conducted on the products immediately after the packs were opened to determine the approximate moisture content of the fresh product. Nonvolatile determinations were also conducted immediately after the products had been composited.

Table 1 provides the average and percent relative standard deviations (%RSDs) for both the individual packs and the composited product nonvolatile determinations. On the basis of these results, all of the commercial cigarette brands had moisture contents ranging from 14 to 16 wt %. For all of the cigarettes tested, the %RSD based on the individual packs was <2.3%, showing a high degree of pack to pack consistency. In

Table 2. Results of Nicotine Determinations Using Ion-Pair LC Method (Three Extractions): Percent by Weight, As Is Basis

composite no.	analyst no. 1 av	1 SD	1 %RSD	2 av	2 SD	2 %RSD	3 av	3 SD	3 %RSD	4 av	4 SD	4 %RSD	analyst to analyst precision[a] (%)
1				1.65	0.06	3.4	1.56	0.08	5.4				5.6
2	1.65	0.11	6.4				1.60	0.06	4.0				3.1
3							1.67	0.08	4.9	1.66	0.07	4.4	0.6
4	1.35	0.13	9.7	1.49	0.09	5.7							9.9
5							1.58	0.06	4.1	1.63	0.09	5.5	3.1
6	1.61	0.10	6.4	1.58	0.07	4.2							1.9
7				2.02	0.07	3.7				2.00	0.08	4.2	1.0
8	1.49	0.11	7.2							1.54	0.08	5.4	3.3
9	1.82	0.05	2.7	1.80	0.06	3.5	1.75	0.08	4.8	1.80	0.10	5.5	3.9
10	1.55	0.10	6.6	1.59	0.11	7.0	1.59	0.05	3.3	1.66	0.08	5.1	6.9

[a] See text for details of calculation of analyst to analyst precision.

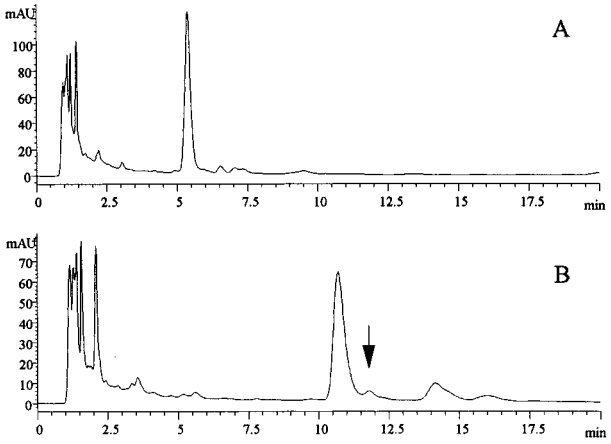

Figure 1. Chromatograms of a commercial cigarette product obtained using optimized reversed phase ion-pairing conditions and a variation: (A) buffer at pH 3.0, 65:35 buffer/methanol; (B) buffer at pH 3.2, 70:30 buffer/methanol. The variation increased the resolution between nicotine and a minor component, indicated by the arrow in (B).

all cases, the %RSD after compositing was <2% and, in most cases, was also less than the RSD based on the individual packs, showing that the composited samples were well mixed and homogeneous with respect to moisture.

Nonvolatile determinations were also made at 2−3 week intervals throughout the study so that if any moisture changes occurred, the nicotine content could be related to that of the fresh product. The difference in moisture content as measured on the fresh composites and throughout the study was <3% on a relative basis for all of the commercial cigarette brands and <5% relative for the reference cigarette composites. No trends were observed in moisture gain or loss. Therefore, no corrections were made to any of the nicotine determinations based on moisture content.

Ion-Pair LC Method, Chromatographic Performance, Figures of Merit, and Nicotine Content Results. Typical chromatograms obtained using the reversed phase ion-pair conditions are shown in parts A and B of Figure 1. In the present work, two variations of a mobile phase based on the 50 mM citric acid, 10 mM sodium octanesulfonate buffer were used. The first variation comprised 65:35 buffer/methanol with a buffer pH of 3.0 (Figure 1A). This mobile phase is identical to the mobile phase used for the determination of nicotine in moist snuff (Ciolino et al., 1999b). The second variation comprised 70:30 buffer/methanol with a buffer pH of 3.20−3.25 (Figure 1B). The second mobile phase was used to increase the resolution between nicotine and an unidentified, partially coeluting component, which was detected in several of the commercial cigarette brands (Figure 1B, component indicated by arrow). Both mobile phase variations use an optimal buffer (citrate) and are in the optimum region for pH and ion-pairing agent concentration as determined in separate experiments (Ciolino et al., 1999a).

Linear calibration curves were obtained by all four analysts over the specified concentration range (0−135 μg/mL) with correlation coefficients (r^2) all >0.9999 for the 11 calibration curves generated during this portion of the study. The average nicotine contents, standard deviations, and %RSDs obtained by the four analysts for the 10 cigarette composites are given in Table 2. %RSDs ranged from 3 to 10% across analysts and composites. The overall method precision defined as the overall average %RSD was 5%. Each of the eight commercial composites (composites 1−8) was analyzed by two analysts, and the reference composites (composites 9 and 10) were analyzed by all four analysts. Analyst to analyst precision as defined in Part 1 (Ciolino et al., 1999a) ranged from ∼1 to 10% (Table 2, last column).

Both the %RSDs and the analyst to analyst precision results obtained for cigarette tobacco filler were higher than the corresponding results obtained in Part 1 for moist snuff (all <5%). It was suspected that the higher variability obtained for cigarette tobacco filler relative to moist snuff was due to the relative homogeneity of the two tobacco matrices and the relatively small sample size used in the analysis. Many commercial brands of moist snuff appear to be more homogeneous than the tobacco filler used in commercial cigarettes; this may be due to the extensive processing of the tobacco used in moist snuff including its fermentation and cutting. The higher moisture content of moist snuff (up to 55 wt %) relative to that of cigarette tobacco filler (14−16 wt %) may also contribute to a more homogeneous distribution of nicotine within the moist snuff matrix. The suspicion that the higher variability for cigarette tobacco filler was due to the matrix, and not the analytical method, was confirmed in subsequent experiments in which larger samples were taken for analysis and the extraction volume was increased proportionately (see Homogeneity Experiment).

Efficiency of a Single Extraction, Ion-Pair LC Method. The efficiency of a single extraction for extracting nicotine from cigarette tobacco filler was measured in an analagous manner to measurements made for moist snuff as described in Part 1 (Ciolino et al.,

Table 3. Results of Nicotine Determinations Using Ion-Pair LC Method (Single Extraction): Percent by Weight, As Is Basis

composite no.	analyst no. 1 av	1 SD	1 %RSD	2 av	2 SD	2 %RSD	3 av	3 SD	3 %RSD	4 av	4 SD	4 %RSD	analyst to analyst precision[a] (%)
1				1.50	0.06	4.2	1.52	0.10	6.9				1.3
2	1.50	0.10	6.4				1.47	0.06	4.3				2.0
3							1.60	0.03	1.9	1.65	0.07	4.0	3.1
4	1.34	0.09	6.9	1.36	0.07	4.9							1.5
5							1.46	0.07	4.6	1.59	0.05	3.4	8.5
6	1.47	0.09	6.4	1.47	0.11	7.4							0.0
7				1.90	0.08	4.2				1.89	0.09	4.8	0.5
8	1.46	0.05	3.1							1.48	0.05	3.6	1.4
9	1.70	0.08	4.6	1.58	0.05	3.2	1.59	0.09	5.9	1.70	0.10	6.2	7.3
10	1.50	0.08	5.5	1.43	0.09	6.1	1.45	0.11	7.5	1.58	0.12	7.9	10

[a] See text for details of calculation of analyst to analyst precision.

Table 4. Nicotine Recovery from Cigarette Tobacco in a Single Extraction Using Citric Acid/IPR Buffer

composite no.	single extrn % nicotine, overall lab av	three extrn % nicotine, overall lab av	% recovery (single extraction) ratio one/three extrn	% recovery (single extraction) spike/recovery
1	1.51	1.61	94	not tested
2	1.49	1.63	91	93
3	1.63	1.67	98	90
4	1.35	1.42	95	92
5	1.53	1.61	95	not tested
6	1.47	1.60	92	94
7	1.90	2.01	94	not tested
8	1.47	1.52	97	not tested
9	1.64	1.79	92	91
10	1.49	1.60	93	not tested

Table 5. Effect of Sample Weight on Analysis Precision for Cigarette Composites (Single Extraction)

composite no.	0.1−0.2 g sample % nicotine	0.1−0.2 g sample %RSD	1.5−2.0 g sample % nicotine	1.5−2.0 g sample %RSD
1	1.50	4.2	1.49	2.5
4	1.36	4.9	1.34	0.8
6	1.47	7.4	1.44	1.3
7	1.90	4.2	1.85	0.5
9	1.58	3.2	1.59	1.9
10	1.43	6.1	1.46	1.5
av		5.0		1.4

1999b). The entire ion-pair LC portion of the study was repeated by modifying the method to use only a single 10 mL extraction. The nicotine standard calibration curve was extended to cover the concentration range 0−500 μg/mL to reflect the higher concentration of nicotine obtained in a single extraction. Again, linear calibration curves were obtained by all four analysts with correlation coefficients (r^2) >0.9999 for the 10 calibration curves generated during this portion of the study.

The average nicotine contents, standard deviations, and %RSDs obtained by the four analysts for the 10 cigarette composites using a single 10 mL extraction are given in Table 3. The higher variability associated with the cigarette tobacco filler matrix was again evident in the precision data. %RSDs obtained by the four analysts were similar to the results obtained with three extractions, ranging from 2 to 8%. The overall method precision was 5%. Analyst to analyst precision (Table 3, last column) ranged from 0 to 10%.

Table 4 lists the average nicotine contents (average across analysts) obtained for the 10 cigarette composites using one vs three extractions. As was reported for moist snuff, the citric acid/sodium octanesulfonate buffer extracts most of the nicotine content of the tobacco filler in a single extraction. The percentage of the nicotine removed in the first extraction ranged from 91 to 98% across composites, with an average of 94%.

The efficiency of a single extraction was also measured in spike/recovery experiments. Nicotine was spiked onto the tobacco filler at a level equal to its previously determined nicotine content so that the total nicotine content after spiking was twice the amount prior to spiking. To minimize the variability associated with the matrix, larger samples were used (1 g) with the extraction volume increased to 100 mL. Results are given in Table 4 (last column) and show an average of 92% recovery across composites.

As with moist snuff, all of the results for cigarettes that are based on a single extraction represent a reasonable estimate (within 10%) of the actual nicotine content as determined from exhaustive extractions. For cigarettes, it was *not* necessary to include a centrifugation step between extractions, as was done with moist snuff (Ciolino et al., 1999b), because the liquid extract could be readily drawn off between extractions using a disposable pipet. This was due to the larger size of the cigarette tobacco filler pieces. Even so, the use of a single extraction simplifies the sample preparation and may be a suitable approach for monitoring changes in nicotine content for a given product or for making product comparisons when one is working with large numbers of samples.

Homogeneity Experiments. It was suspected that the higher variation in nicotine determinations observed for cigarette tobacco filler (method precision = 5% based on overall average RSD) versus moist snuff (method precision = 1.3%) was due to the higher variability of the cigarette tobacco filler matrix relative to the moist snuff matrix. To test this supposition, analyst 2 repeated the analysis of six of the cigarette composites using a higher sample weight, in the range of 1.5−2.0 g. Note that the previous sample weight range was 0.1−0.2 g. Results are given in Table 5 and indicate that the precision was significantly improved by using a larger sample weight. The average %RSDs were 5.0% using a 0.1−0.2 g sample and 1.4% using a 1.5−2.0 g sample. Although the precision was poorer using the lower sample weight range, the nicotine determination (average nicotine content) was not significantly affected. All of the nicotine contents determined using the lower sample weight range differed by <3% on a relative basis from the nicotine contents determined using the higher sample weight range.

Table 6. Comparison of Nicotine Content Determination Results for Ion-Pair LC Method vs AOAC and GC Methods (Percent by Weight, As Is Basis)

composite no.[a]	ion-pair LC method (three extrn)	GC method[b]	AOAC 967.02
1	1.61	1.59	1.79
2	1.63	1.60	1.85
3	1.67	1.68	1.94
4	1.42	1.41	1.65
5	1.61	1.62	1.85
6	1.60	1.52	1.75
7	2.01	1.92	2.31
8	1.52	1.53	1.75
9	1.79	1.74	1.96
10	1.60	1.54	1.81

[a] Composites 1−8 were made from eight different brands of commercial cigarettes. Composites 9 and 10 were made from University of Kentucky Tobacco and Health Research Institute 1R4F Cigarettes and 2R1F Cigarettes, respectively. [b] GC Method (Lyerly and Greene, 1976).

Ion-Pair LC Method vs AOAC and GC Methods. The nicotine contents of the 10 cigarette composites were also determined using the AOAC steam distillation method (AOAC, 1995) and a GC method based on the GC conditions of Gottscho et al. (1988) and the sample preparation procedures of Lyerly and Greene (1976). This latter method was reported to provide reproducible and accurate results for a variety of commercial tobaccos with nicotine contents ranging from 0.05 to 3.0% (Lyerly and Greene, 1976).

A comparison of the average nicotine contents of the 10 cigarette composites as determined by the ion-pair LC method versus the AOAC and GC methods is given in Table 6. As with moist snuff, both the ion-pair LC method and the GC method gave lower nicotine content results for cigarette tobacco filler than the AOAC method because the AOAC method is not selective for nicotine and measures total alkaloids. The nicotine content as determined by the GC method averaged 87% of the total alkaloids as determined by the AOAC method.

Comparison of the results between the ion-pair LC method and the GC method shows that relative differences in determined nicotine contents of 0.6−5% were obtained across composites. Thus, the two chromatographic methods provided good agreement for the determination of nicotine in commercial cigarettes. Although two reference cigarettes were included in this study (composites 9 and 10), the nicotine contents for these cigarettes were not reported in the accompanying literature (THRI, 1990). We are also not aware of any other published reports of nicotine determinations for these cigarettes.

Evaluation of the Ion-Pair LC Method for Commercial Cigarettes. The advantages of the ion-pair LC method for the determination of nicotine in commercial moist snuff were cited in Part 1 (Ciolino et al., 1999b) and were also realized for the determination of nicotine in commercial cigarettes. The use of an aqueous-based extraction solvent that also serves as the mobile phase buffer provides for simplified sample preparation, results in minimal noise in the chromatographic baseline, and avoids the generation of halogenated waste. The convenience and reliability of the use of the nicotine salt as the analytical standard were also cited.

Initially, the chromatographic conditions used in Part 1 for moist snuff (pH 3.0, 65:35 buffer/methanol) were applied to the analysis of commercial cigarettes. As these conditions were applied to the various cigarette brands, the spectral purity of the nicotine peak was monitored using a diode array detector. A minor component that partially coeluted with nicotine was detected in several of the cigarette brands (the presence of this component altered the nicotine content determination on the order of 1% on a relative basis). The mobile phase conditions were slightly modified (pH 3.2, 70:30 buffer/methanol) to provide sufficient resolution of nicotine from the interfering component as indicated by the spectral purity of the nicotine peak. These modified conditions have since been shown to be suitable for moist snuff. Both sets of conditions have been demonstrated to provide rapid separation of nicotine from the minor tobacco alkaloids (Ciolino et al., 1999a), as well as separation of nicotine from a host of other unidentified endogenous tobacco components and commercial additives.

We have used the ion-pair LC method for the determination of nicotine in >1000 commercial tobacco samples. We are continuing to investigate minor modifications of the method that will make it more convenient, cost-effective, and reliable. These include the use of column thermostating to increase the precision of the nicotine peak retention times, the elimination of the sodium octanesulfonate in the extraction solvent (probably not necessary and is costly relative to the citric acid), and the use of column wash procedures that will extend the usable lifetime of the column.

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