

ANALYSIS OF ELECTRONIC CIGARETTE CARTRIDGES, REFILL SOLUTIONS, AND SMOKE FOR NICOTINE AND NICOTINE RELATED IMPURITIES

Michael L. Trehy, Wei Ye, Michael E. Hadwiger, Terry W. Moore, James F. Allgire, Jeffrey T. Woodruff, Shafiq S. Ahadi, John C. Black, and Benjamin J. Westenberger

Food and Drug Administration, Division of Pharmaceutical Analysis, Saint Louis, Missouri, USA

□ *The objective of this study was to determine nicotine and the nicotine related impurities, that is, cotinine, myosmine, anatabine, anabasine, and β -nicotyrine, in electronic cigarette cartridges, the liquid used to fill the cartridges, and from smoke generated using the electronic cigarette devices. An HPLC method was validated for the determination. Samples of nicotine containing products were purchased via the internet from NJOY, Smoking Everywhere, CIXI, and Johnson Creek. Electronic cigarette devices were purchased from NJOY, Smoking Everywhere, and CIXI. The results from the testing found that (1) the nicotine content labeling was not accurate with some manufacturers, (2) nicotine is present in the “smoke” from electronic cigarettes, and (3) nicotine related impurities contents in cartridges and refills were found to vary by electronic cigarette manufacturer.*

Keywords anatabine, cigarette, cotinine, e-cigarette, nicotine, smokeless

INTRODUCTION

“Electronic cigarettes” are a recent entry into the market place.^[1] The marketing of electronic cigarettes as a healthier alternative to smoking traditional cigarettes has raised some concerns.^[2–6] In addition to the claim to deliver nicotine, the electronic cigarette manufacturers also claim electronic cigarettes are able to deliver other products including pharmaceuticals.^[7–9] Electronic cigarettes have been reported to have different smoking characteristics from conventional cigarettes^[10] and to have

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Address correspondence to Michael L. Trehy, Food and Drug Administration, Division of Pharmaceutical Analysis, 1114 Market St, Saint Louis, MO 63101, USA. E-mail: Michael.Trehy@fda.hhs.gov

ineffective nicotine delivery.^[11] Samples of nicotine containing products were purchased via the internet from NJOY, Smoking Everywhere, CIXI, and Johnson Creek. Electronic cigarette devices were purchased from NJOY, Smoking Everywhere, and CIXI. As part of an assessment of electronic cigarettes, the nicotine and nicotine related impurities, that is, cotinine, myosmine, anatabine, anabasine, and β -nicotyrine, were determined in selected products and in the "smoke" that would be inhaled by a consumer. Sample extracts of the products were analyzed using a validated gradient HPLC method described in this paper. The "smoke" of the cigarettes was analyzed following a "puff" procedure developed to simulate the use of the electronic cigarettes. The results obtained when testing the "smoke" from electronic cigarettes was compared with results obtained using the same methodology with a popular brand of cigarette.

Gas chromatographic,^[12] capillary electrophoresis,^[13] and liquid chromatographic^[14] methods have been used for the determination of nicotine. The United States Pharmacopeia (USP): Nicotine Transdermal System assay method was modified and initially used for the determination of nicotine and nicotine related impurities.^[14] However, due to poor retention of cotinine and poor resolution between nicotine and anabasine by the USP method, an improved HPLC gradient method was developed. In this paper, we present the improved HPLC conditions and results obtained analyzing several brands of electronic cigarettes.

EXPERIMENTAL

Chemicals

Nicotine bitartrate dihydrate lot G1C070 was purchased from USP and found to contain 7.59% water by Karl Fischer titration. Nicotine content was calculated based on the chemical formula and corrected for water content. Cotinine catalog lot #2-XAL-53-1, myosmine lot #1-JLI-26-1, anabasine lot #2-BHW-20-1, β -nicotyrine lot #1-WHH-126-2, and anatabine tartrate (2:3) lot #9-BHW-79-2 were purchased from Toronto Research Chemicals, Inc. All were listed at 98% purity. Anatabine content was calculated based on the chemical formula and corrected for purity. OmniSolv acetonitrile high purity solvent and ammonium hydroxide from EM Science were purchased from Fisher Scientific. Ammonium formate was purchased from Sigma-Aldrich. Figure 1 provides the structures for the analytes of interest in this study.

Electronic Cigarette Devices and Cartridges

Samples of electronic cigarette devices and cartridges were purchased via the internet. Numerous suppliers of these devices exist and can be

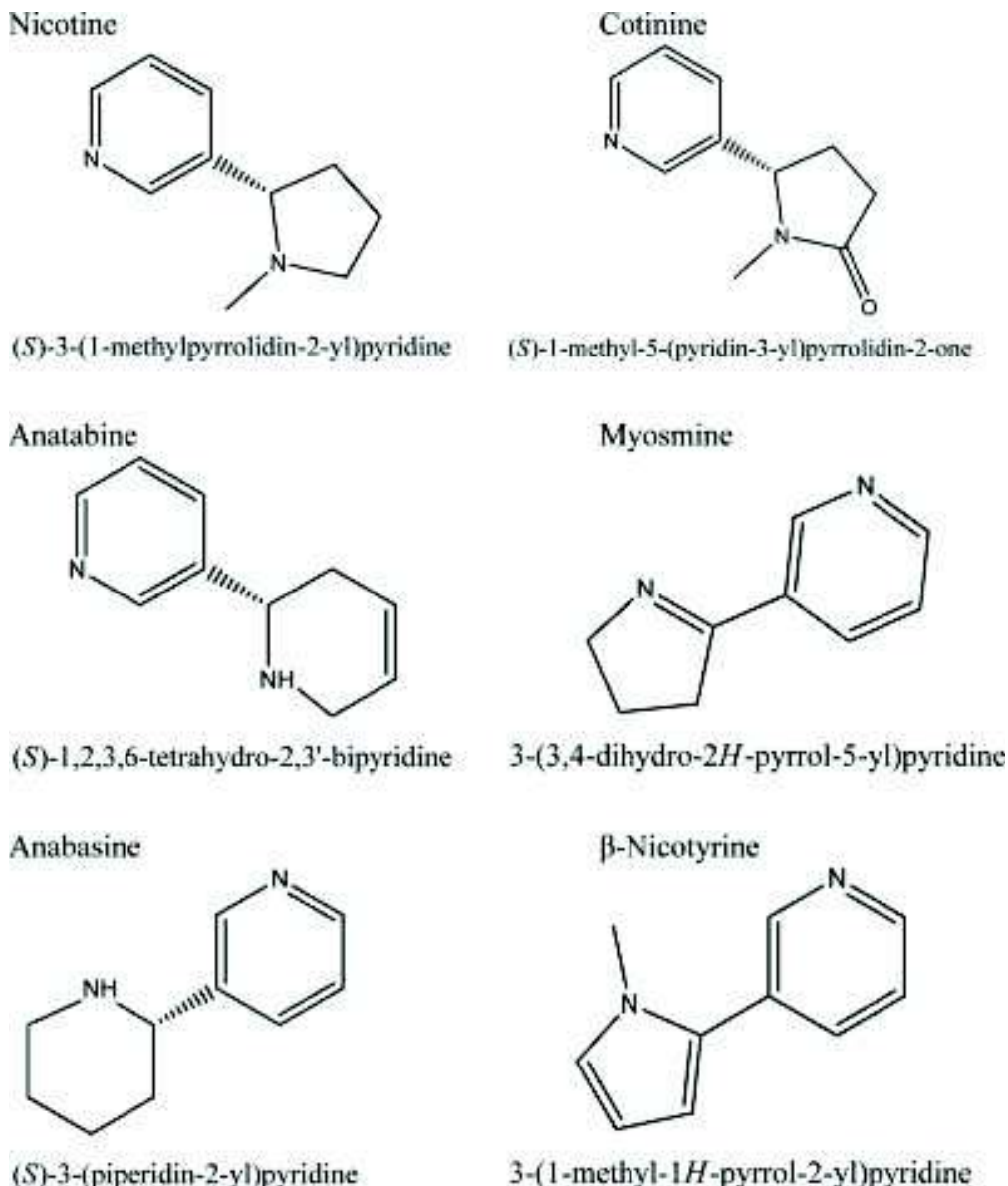


FIGURE 1 Structure for targeted analytes in this study are provided below.

found in many booths in malls in the United States. This study represents a random sampling of four of these suppliers of cartridges, refills, and electronic cigarette devices.

Equipment

Two LC systems were used for analysis of samples. A Shimadzu HPLC with SPD-M10Avp diode array detector, DGU-14A Degasser, LC-10ADvp Pump, CTO-10ASvp Column Oven, SIL-10ADvp Auto injector, FCV-10ALvp Flow Controller, and SCL-10Avp System Controller was used for method development and for gradient analysis. The data were collected and processed employing Shimadzu CLASS-VP software version 5.032.

An Agilent-1100-1 HPLC system with a variable wavelength detector, degasser, quaternary pump, column thermostat, and auto sampler was used for isocratic analysis.

Chromatographic Conditions

A Phenomenex Gemini-NX 5 μ C18 110A 15 cm \times 4.6 mm column was used with the column temperature at 35°C and a flow rate of 1.0 ml/min. The injection volume was 10 μ L, and quantification was carried out at 260 nm. UV spectra were collected over the wavelength range 200 nm to 350 nm. Eluent A was prepared to contain 10% acetonitrile in 20 mM ammonium formate adjusted to pH 8.7 by addition of approximately 1 mL of concentrated aqueous ammonia per 2 liters of eluent A. Eluent B consisted of 100% acetonitrile. The gradient program was a series of linear gradients from initial conditions of 100% A to 80% A and 20% B at 10 min, then to 10% A and 90% B at 20 min then to 100% A at 21 min with a total run time of 30 min.

The Agilent-1100-1 HPLC system was used with a Supelco LC-18-DB 25 cm \times 4.6 mm 5-micron column. The injection volume was 10 μ L and quantification was carried out at 260 nm. Flow rate was set to 1.2 mL/min with a run time of 15 min using isocratic elution. The mobile phase was 23% acetonitrile with 0.1% triethylamine in water. The pH was adjusted to 9.1 with phosphoric acid.

Preparation of Standard Solutions

Stock solutions of cotinine, anabasine, anatabine, myosmine, nicotine, and β -nicotyrine were prepared in 50% methanol in water. Through serial dilutions of the stock standard with 10% acetonitrile in water, working standards were prepared from approximately 0.001 mg/mL to approximately 0.05 mg/mL. These standards were then analyzed by the HPLC procedure to determine the limit of detection (LOD) and limit of quantification (LOQ) following ICH guidelines.^[15]

Preparation of Cartridge Samples and Refill Solutions for Content Analysis

NJOY and Smoking Everywhere cartridges contained a plug at one end of the cartridge. The plug was intended to be removed when the electronic cigarette device containing a heating element was inserted into the cartridge. Removal of the plug allowed access to the cartridge's contents of a propylene glycol solution of nicotine adhering to a loose fibrous pad.

The pad was easily removed with tweezers, unwound, and transferred to an Erlenmeyer flask along with the plug and cartridge itself. Fifty mL of methanol was added to the flask, and the flask was stoppered.

The CIXI cartridge contained a plug with an opening that allowed air to be drawn through the cartridge and was not intended for removal. In this style of cartridge, there was a heating element embedded in the fibrous pad. Removal of the plug allowed access to the cartridge's propylene glycol solution of nicotine adhering to the tightly wound fibrous pad. The pad could only be removed by breaking the wires running to the heating element. It could then be removed with tweezers, unwound, and transferred to an Erlenmeyer flask along with the plug and cartridge itself. Fifty mL of methanol was added to the flask and the flask was stoppered.

The flasks were placed on a platform shaker and shaken for 90 min. The extract was transferred to 50 mL Erlenmeyer flasks for analysis. Extracts were clear and did not require filtration prior to analysis.

Refill solutions from Johnson Creek were in individual vials. Analysis of refill solutions was made after dilution with mobile phase or 10% acetonitrile and ran by the HPLC procedure.

Puff Analysis

Fifty mL of extraction solution (10% acetonitrile in water) and a magnetic stirrer was added to a tall-form gas washing fritted cylinder bottle similar to Fisher catalog #03-040A with 150 mL cylinder and coarse frit. The trap was placed on top of a magnetic stirrer and mixed slowly throughout the trapping process. The electronic cigarette or standard cigarette was attached to the sparger via tubing in such a manner as to minimize the amount of tubing exposed to the air passing through the electronic cigarette to the sparger. The outlet from the gas washing bottle was connected via tubing to a Dräger manual air pump with a capacity of 100 mL. At one minute intervals, a puff of 100 mL of air was drawn through the electronic cigarette through the sintered glass frit into the gas washing bottle containing the extraction solution. A total of 30 puffs were collected in this manner. The electronic cigarette was observed to stay lit for approximately 2 sec after the 100 mL puff of air was initially actuated, and the air flow into the washing bottle stopped approximately 4 sec after the 100 mL puff of air was initiated. The electronic cigarette device contains an air flow sensor which is actuated by the puff of air. When actuated, the electronic cigarette LED light at the tip of the cigarette lights and the heating element is heated. The electronic cigarette was observed to light each time the air was drawn through the tube. An experiment set up to trap the "smoke"

from an electronic cigarette is shown in Figure 2a and is configured with two traps to confirm that tobacco related impurities were being confined to the first trap. Normal operation involved using only a single trap. An experiment to trap smoke from a traditional cigarette is shown for comparison in Figure 2b.

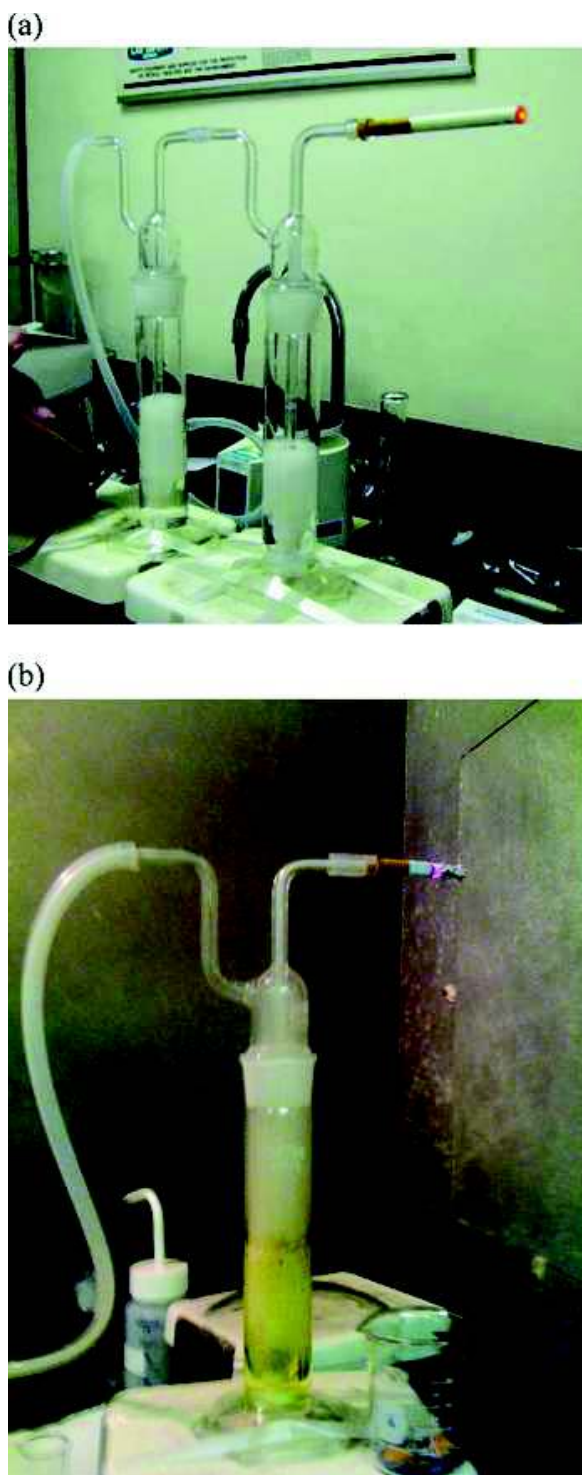


FIGURE 2 (a) Photograph of experimental set up for electronic cigarette testing. (b) Photograph of experimental set up for traditional cigarette testing. (Color figure available online.)

Method Validation

Precision of the HPLC determinative step was evaluated by seven replicate injections of a standard and calculation of the average, standard deviation, and percent relative standard deviation for nicotine and each of the five nicotine related impurities selected for analysis. Limit of detection (LOD) and limit of quantitation (LOQ) were determined following ICH guidelines based on the standard deviation of the response and the slope.^[15] Linearity was determined using serial dilutions of a mixed standard containing all six components. Percent recovery was determined by spiking diluted sample with the analytes and calculating the percent recovery after subtracting the areas due to the sample matrix.

Sample Collection

Samples of electronic cigarettes and devices were purchased over an approximately one year period via internet purchases. The initial survey obtained electronic cigarette devices, refill solutions, and cartridges from NJOY and Smoking Everywhere. The devices, refill solutions, and cartridges were similar in design and properties. A third supplier, Johnson Creek was sampled for cartridges and refill solutions. The Johnson Creek cartridges were similar in design to the first two sets of samples. The final set of samples including cartridges, refill solutions, and devices was collected from the CIXI and the design of the cartridges and electronic cigarette was different.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

The objective of the method was to measure nicotine, cotinine, myosmine, anatabine, anabasine, and β -nicotyrine in electronic cigarette cartridges and from “smoke” generated using the electronic cigarette devices. Information from the internet provided an estimate for the concentration of nicotine likely to be present. No information was available regarding the likely concentrations of nicotine related impurities associated with the nicotine or in the electronic cigarette “smoke.”

Initial analysis followed a procedure similar to the USP method^[14] employing a Supelco NO. 5-875 LC-18-DB 25 cm \times 4.6 mm 5-micron column with isocratic elution using 23% acetonitrile containing 0.1% triethylamine, a flow rate of 1.2 mL per minute, and a column temperature of 35°C. Using these conditions, cotinine was poorly retained, and anabasine

was not completely resolved from nicotine due to the broad peak observed for nicotine at the sample concentrations used for analysis.

An alternative method was developed using gradient elution and a Phenomenex[®] Gemini-NX 5 μ m C18 110A 15 cm \times 4.6 mm column to improve resolution. The LC conditions are listed in the Chromatographic Conditions section. Under these conditions all the components are resolved as shown in Figure 3 with a resolution greater than 4.0 and with peak asymmetry ranging from 1.12 to 1.32 for all the components. The pH of the eluent greatly impacted resolution. When the pH of eluent A is 8.7 all components listed were resolved while at a pH of 8.9 all the peaks were not resolved. This method was used for analysis of electronic cigarette cartridges and for the trapping solution obtained from the electronic cigarette smoke for CIXI and for the analysis of the smoke from a popular cigarette brand.

The method was linear over the range of standards prepared and had a percent relative standard deviation of less than 1% for seven replicate injections of the highest standard tested for each of the analytes. Linearity as measured by the correlation coefficient was 1.000 for all the analytes tested. A comparison of the LOD and LOQ values using isocratic elution and the Supelco LC-18-DB column compared to the results obtained using gradient elution and the Phenomenex Gemini-NX 5 μ m C18 110A 15 cm \times 4.6 mm column are shown in Table 1. The limit of detection (LOD) and limit of quantitation (LOQ) were determined by calculating the standard error for the

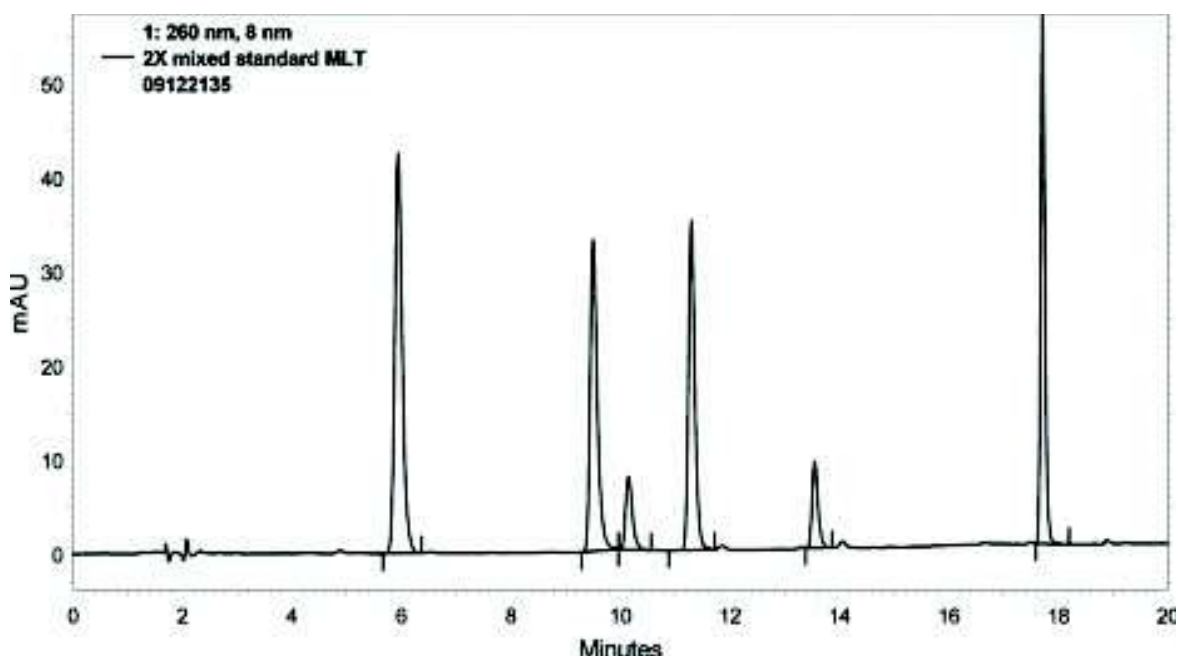


FIGURE 3 Chromatogram for nicotine and nicotine related impurities. Retention times; cotinine (5.94 min), anabasine (9.515 min), anatabine (10.155 min), myosmine (11.307 min), nicotine (13.557 min), and β -nicotyryne (17.717 min).

TABLE 1 Comparison of LOD and LOQ Values for Isocratic Elution with a Supelco LC-18-DB 250 mm Column versus a Phenomenex Gemini-NX 5 μ C18 110A 15 cm \times 4.6 mm Column

LOD, LOQ, Linearity, and %RSD for Isocratic HPLC Method w Supelco

	Range $\mu\text{g/mL}$ Std	LOD – LOQ $\mu\text{g/mL}$ Tobacco Soln	Recovery (%) at Max Range	RSD (%) 7 inj at Max Range
Cotinine	0.02–24.2	0.57–1.70	81.8	0.62
Anabasine	0.02–22.8	6.10–18.50	67.8	3.37
Anatabine	0.03–34.6	1.10–3.40	93.4	2.97
Myosmine	0.01–10.3	1.10–3.30	95.9	1.23
Nicotine	0.06–72.4	34.00–104.00		0.77
β -Nicotyrine	0.01–9.3	0.30–0.96	100.8	1.70

LOD, LOQ, Linearity, and RSD (%) for Gradient HPLC Method Phenomenex

	Range $\mu\text{g/ml}$ Std	LOD – LOQ $\mu\text{g/mL}$ Tobacco Soln	Recovery (%) at Max Range	RSD (%) 7 inj at Max Range
Cotinine	1.4–87.6	0.2–0.70	99.4	0.3
Anabasine	1.2–76.5	0.4–1.30	100.2	0.3
Anatabine	0.3–15.9	0.1–0.30	100.1	0.5
Myosmine	0.6–35.4	0.1–0.30	99.4	0.3
Nicotine	0.2–15.3	0.1–0.25	99.6	0.4
β -Nicotyrine	0.6–36.3	0.1–0.40	100.4	0.3

intercept and dividing by the slope of the line of best fit and then multiply by 3.3 for the LOD and by 10 for the LOQ.^[15] The percent recovery was determined by spiking a diluted sample at the high end of the concentration range used for the linearity determination.

Design Differences in Electronic Cigarette Devices

Samples of electronic cigarette devices and cartridges were obtained from NJOY, Smoking Everywhere, and CIXI. NJOY and Smoking Everywhere were indistinguishable in design. NJOY and Smoking Everywhere had used a rubber plug possibly made of silicone to seal the end of the cartridge prior to connecting to the electronic cigarette device. The plug absorbed a portion of the nicotine which likely was partially responsible for lower nicotine values than indicated on the internet. CIXI had a different cartridge design and electronic cigarette device from the other two manufacturers.

All three electronic cigarette devices contain a rechargeable battery, a light emitting diode (LED), and an air flow sensor that is activated when the flow of air through the device exceeds a predetermined level. On activation, the LED light is turned on along with the heating element as shown in Figure 2a. The consumer can visually observe whether sufficient air is

being drawn through the device to activate the heater. In the case of manufacturers NJOY and Smoking Everywhere, the heating element was part of the cigarette device and when the cartridge plug was removed and the cartridge attached to the electronic cigarette device, the heating element was placed in contact with the nicotine containing solution on a fiber mat. CIXI had placed the heating element inside the cartridge and electrical contact was made when the cartridge was connected to the device.

The air flow necessary to actuate the flow sensor was determined by connecting the device to a vacuum and measuring the air flow with a Gilmont air flow meter. The minimum air flow to actuate the heating element and LED was determined to be 52 mL per second. In the puff analysis testing used in this study, a 100 mL puff was drawn through the device at 1 min intervals. The LED light was observed to stay lit for approximately 2 sec and air flow had completely stopped after 4 sec. Typical conditions for testing cigarettes under FTC^[12,16,17] conditions is 1 min puff interval, 2 sec puff duration, and 35 mL total puff volume. The flow rate for these conditions would not actuate the electronic cigarette device.

In order to evaluate the nicotine delivery under greatly varying conditions, the amount of nicotine delivered was determined with no pause between puffs with and without battery attached to simulate performance with a dead battery and compared to the standard procedure used in this study which employed a 30 sec pause between puffs. The electronic cigarette's nicotine delivery was greatly enhanced by reducing the pause between puffs and removing the battery greatly reduced nicotine delivery Figure 4. Nicotine delivery appeared to be greatly impacted by the temperature of the nicotine solution so that repeated heating in a short time

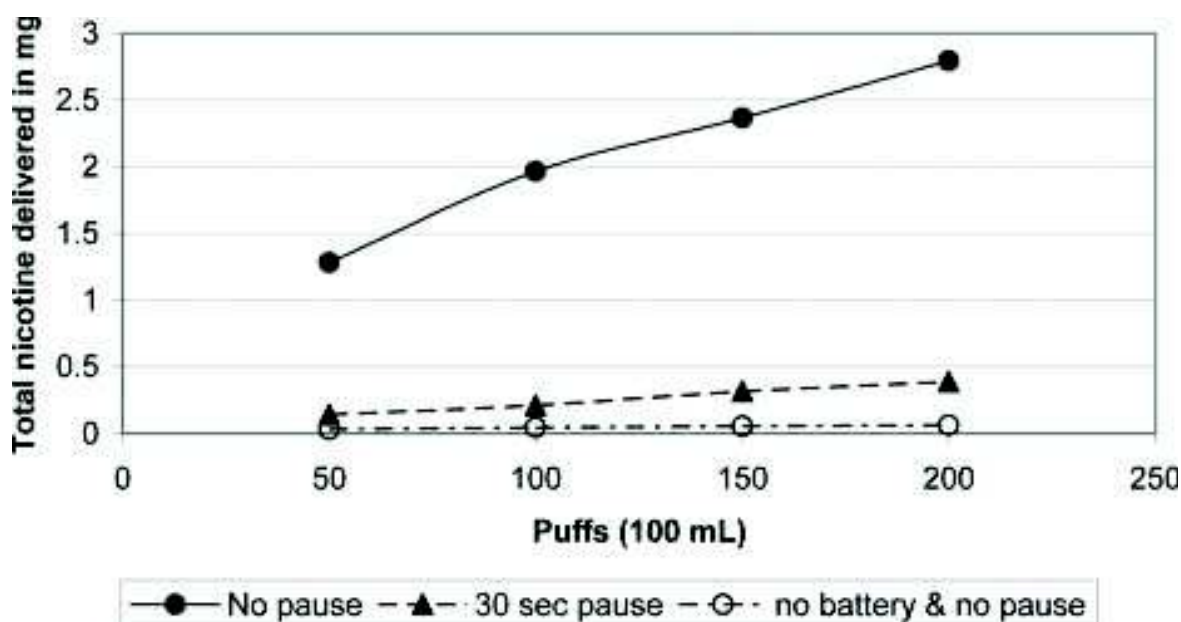


FIGURE 4 Nicotine delivery is greatly impacted by time between puffs.

interval enhanced nicotine release. Since most people who smoke take a pause of variable length, the actual amount of nicotine delivered is likely to be highly variable.

Nicotine Content of Electronic Cigarette Cartridges

Nicotine content labeling issues existed with all of the manufacturers tested. Results are summarized in Table 2. Methanol extracts of the cartridges were diluted with Milli-Q water and analyzed by HPLC following the modified USP procedure^[14] and compared to the results employing the gradient procedure. NJOY and Smoking Everywhere have results which overlap dosage levels so that little differentiation exists. CIXI is completely inconsistent. Some cartridges labeled as containing nicotine, did not contain nicotine and some cartridges labeled as not containing nicotine, did contain nicotine. Further, when a particular flavor type from CIXI labeled as containing nicotine was analyzed, only one of the seven cartridges analyzed was found to contain nicotine.

Nicotine Related Impurities in Cartridge Extracts

The nicotine content and nicotine related impurities were determined in the cartridge extracts using the gradient elution method. Results are

TABLE 2 Nicotine Label Content versus Nicotine Content Found for Cartridges from Three Different Manufacturers

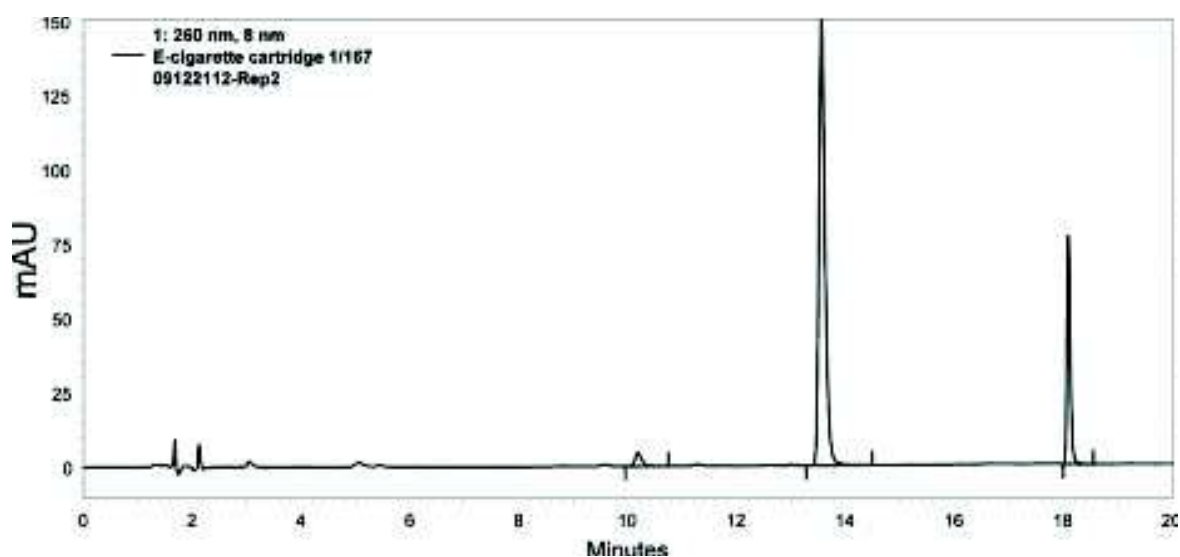
Smoking Everywhere		NJOY		CIXI	
Label mg Nicotine per Cartridge	Actual mg Nicotine per Cartridge	Label mg Nicotine per Cartridge	Actual mg Nicotine per Cartridge	Label mg Nicotine per Cartridge	Actual mg Nicotine per Cartridge
0	0.1	6	5.2	0	0.07
0	0.0	12	5.3	0	12.90
0	0.0	12	4.3	0	21.80
0	0.1	12	4.8	16	0.09
0	0.0	18	6.8	16	21.00
6	1.0			16	13.00
6	1.6			24	0.09
6	2.7			24	13.20
6	1.8			24	20.60
11	5.2			24	15.10
11	2.7				
11	4.4				
16	6.0				
16	4.2				
16	5.5				

TABLE 3 Results from CIXI for Nicotine and Nicotine Related Impurities in Cartridges Expressed as mg/Cartridge

Nicotine, Flavor	Cotinine	Myosmine	Anatabine	Anabasine	Nicotine	β -Nicotyryne
None, regular	ND	<LOQ	ND	ND	0.08	ND
24 mg, regular	ND	<LOQ	ND	ND	0.09	<LOQ
16 mg, regular	ND	<LOQ	ND	ND	0.09	ND
24 mg, E-cialis	ND	0.05	0.49	<LOQ	13.24	ND
0 mg, E-cialis	ND	0.05	0.49	<LOQ	12.92	ND
0 mg, E-rimonabant	ND	0.08	0.82	<LOQ	21.82	ND
24 mg, E-rimonabant	ND	0.08	0.77	<LOQ	20.57	ND
16 mg, E-rimonabant	ND	0.08	0.79	<LOQ	21.04	ND
16 mg, E-cialis	ND	0.05	0.50	<LOQ	13.01	ND
24 mg, Marlboro Taste	ND	0.08	0.57	<LOQ	15.11	ND
LOD	0.04	0.02	0.02	0.07	0.01	0.02
LOQ	0.12	0.05	0.05	0.21	0.04	0.06

ND = not detected.

tabulated in Table 3 for CIXI. All nicotine containing products contained nicotine related substances. Results shown are representative of impurity levels observed for other manufacturers except for anatabine levels. Nicotine used by CIXI for preparation of nicotine solutions contained anatabine at 3.77% of the nicotine concentration while other manufacturers had anatabine levels at 0.2% or less of the nicotine concentration. Myosmine was found at approximately 0.4% of the nicotine concentration while cotinine, anabasine and β -nicotyryne were not detected. Figure 5 shows a chromatogram for a cartridge from CIXI labeled High (nicotine content of 16 mg). Nicotine content of the cartridge was determined to be 21.0 mg with 0.79 mg of anatabine.

**FIGURE 5** Cartridge extract from electronic cigarette labeled High E-Rim. Anatabine peak at 10.15 min, nicotine peak at 13.6 min, and Rimonabant peak at 18 min.

Nicotine Content of Refill Solutions

Table 4 summarizes the results for nicotine content found on analyzing two manufacturers of electronic cigarette refill solutions. Johnson Creek consistently matched the label specification with the range 100.2% to 110.8% of label value. However, CIXI did not consistently match the label claim. The nicotine content followed the flavor labeling so that all “regular flavor” refill solutions did not contain nicotine even though they were labeled to contain variable concentrations of nicotine. Of greatest concern was the high nicotine content in refill solutions labeled to contain no nicotine.

Nicotine and Nicotine Related Impurities in Puff Trapping Solutions

Electronic cigarettes were found to deliver nicotine at a consistent rate for a given cartridge and electronic cigarette device as shown in Figure 6 when a constant puff pause interval of 1 min was used. However, cartridge contents vary significantly from one cartridge to another so that actual performance from one electronic cigarette cartridge combination to another is highly variable. This is especially true from one manufacturer to another. The variability within a single manufacturer and cartridge is similar to that observed when a popular cigarette was tested under the same conditions as shown in Table 5.

Although, nicotine related impurities were not detected in the puffs above the LOQ for the electronic cigarettes, this is attributable to the lower nicotine delivery observed and not the absence of nicotine impurities in the

TABLE 4 Nicotine Results for e-Cigarette Refills from Two Manufacturers

CIXI Label mg Nicotine/mL (flavor)	mg Nicotine/mL	% of Label	Johnson Creek Label mg Nicotine/mL (flavor)	mg Nicotine/mL	% of Label
0 (Regular Flavor)	0	–	24 (Original Smoke RPG)	25.6	106.5
24 (Regular Flavor)	0	0	24 (Tennessee Cured RPG)	25.4	106.0
16 (Regular Flavor)	0	0	24 (Tennessee Cured PG)	25.4	105.9
24 (Cialis)	13	54	18 (Tennessee Cured PG)	19.9	110.8
0 (Cialis)	12	–	0 (Tennessee Cured PG)	0.0	–
16 (Cialis)	13	81	24 (Simply Strawberry PG)	24.8	103.2
24 (Marlboro Flavor)	14	58	18 (Simply Strawberry PG)	18.0	100.2
0 (Rimonabant)	21	–	24 (Espresso PG)	24.9	103.6
24 (Rimonabant)	20	83	18 (Espresso PG)	18.8	104.4
16 (Rimonabant)	20	125	0 (Espresso PG)	0.0	–
			24 (Original PG)	24.6	102.5
			18 (Original PG)	18.9	105.2

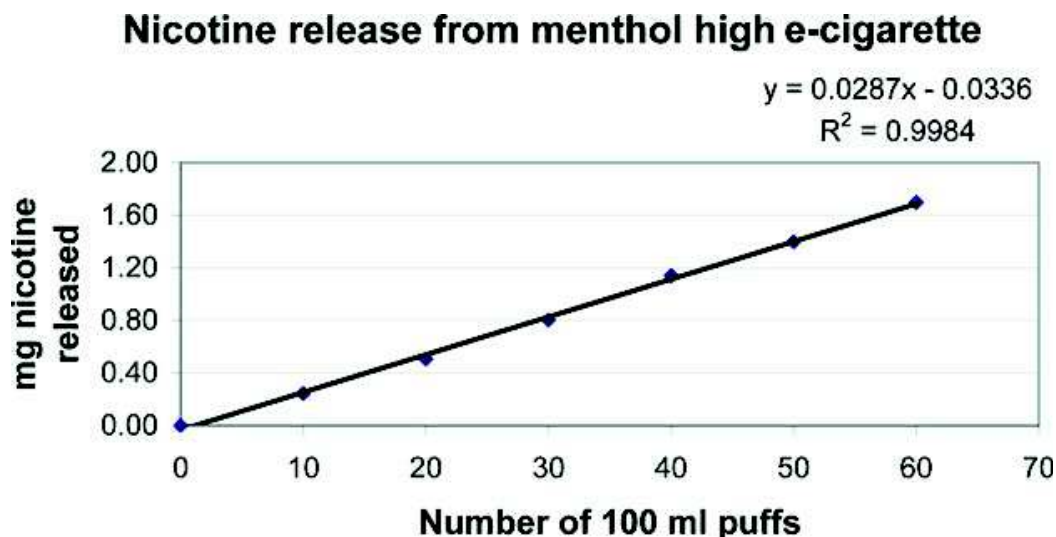


FIGURE 6 Nicotine release from an electronic cigarette versus the number of 100 mL puffs. (Color figure available online.)

smoke. Note that at the nicotine levels observed, the corresponding amount of anatabine that would be present is below the method LOD. In the trapping solution for smoke from a traditional cigarette, anabasine was the nicotine related impurity present at the highest relative concentration at 12% of the nicotine content. Results for nicotine related impurities are shown in Table 6. In Figure 7, chromatograms from the trapping solutions after thirty 100 mL puffs from (a) a popular traditional cigarette is compared to (b) an electronic cigarette using a cartridge labeled to contain 24 mg of nicotine/cartridge. The impurity level as a percentage of the area for nicotine appears to be lower in the trapping solution from the electronic cigarette than in the trapping solution from a traditional cigarette.

TABLE 5 Simulated Smoking Evaluation Results for Nicotine Delivery

	μg Nicotine/100 mL Puff Measured	mg Nicotine/Cartridge Measured
E-cigarette label value Smoking Everywhere		
11 mg/cartridge	10.6	4.77
16 mg/cartridge	26.8, 34.9, 43.2	6.76
E-cigarette NJOY		
6 mg/cartridge	9.9	1.57
12 mg/cartridge	15.7	5.15
18 mg/cartridge	31.5	5.98
E-cigarette CIXI		
16 mg/cartridge	14.4, 4.4, 6.6	21.0
24 mg/cartridge	0	0
24 mg/cartridge	2.1, 2.4, 0.6	13.2
Traditional Cigarette		
Marlboro	193, 154, 152	

TABLE 6 Simulated Smoking Evaluation for Nicotine Related Impurities

Nicotine, Flavor	Cotinine	Myosmine	Anatabine	Anabasine	Nicotine	β -Nicotyrine
E-cigarette CIXI						
High	ND	ND	ND	ND	254	ND
E-High*	ND	ND	<LOQ	ND	292	ND
E-High (duplicate cartridge)	ND	ND	<LOQ	ND	50	ND
Traditional cigarette						
Traditional Cigarette (equivalent to 3.75 cigarettes)	313	220	117	540	4,558	<LOQ
LOD $\mu\text{g}/30$ 100 mL puffs	10	5	4.7	20	10	20
LOQ $\mu\text{g}/30$ 100 mL puffs	35	16	14	60	40	60

Results in $\mu\text{g}/\text{thirty}$ 100 mL puffs which is equal to a total volume of 3,000 mL of smoke. Under these conditions, eight puffs were possible per traditional cigarette.

*Only one out of 7 cartridges tested from this lot contained nicotine at level close to label claim. Result is for cartridge close to label value.

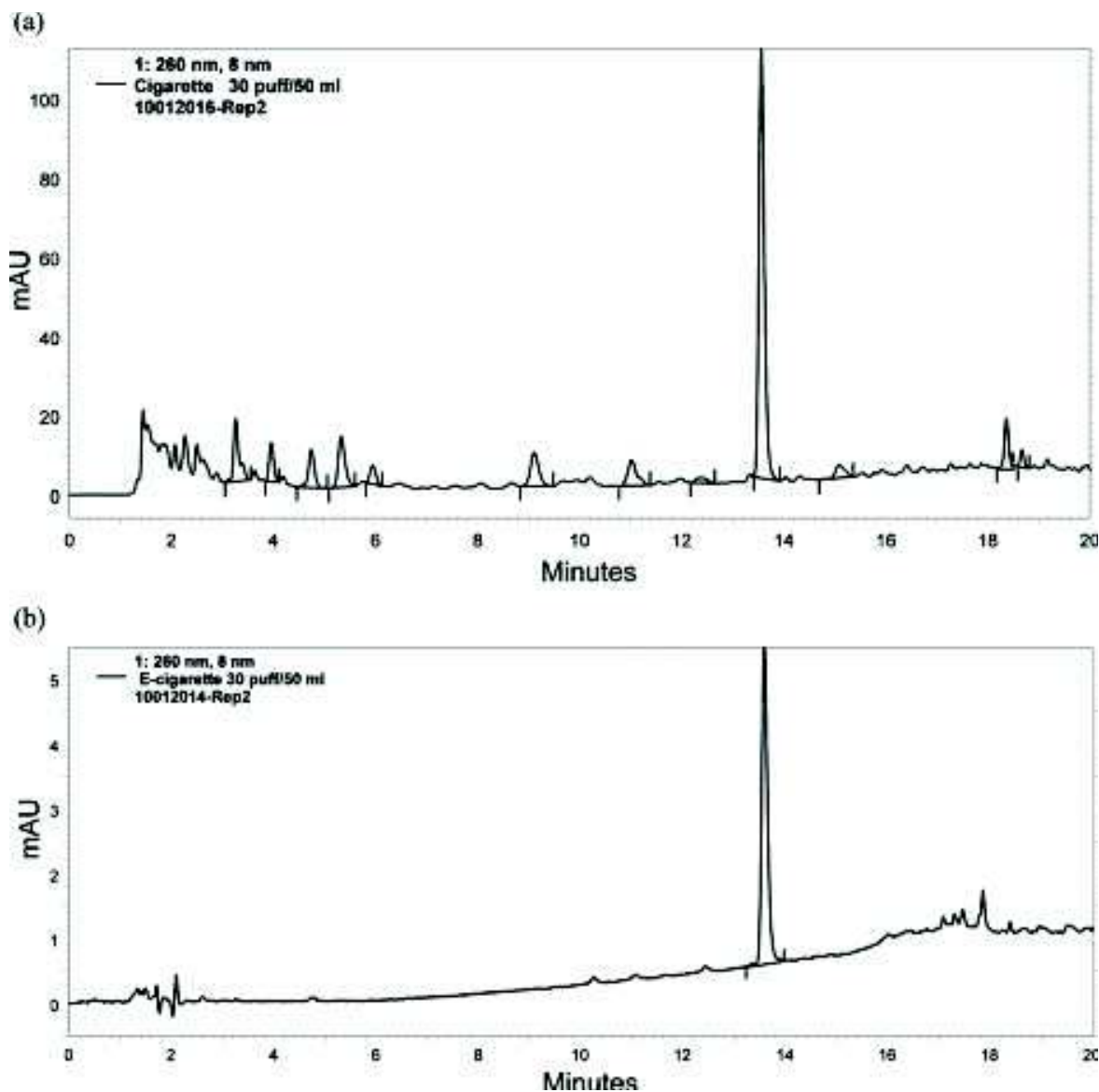


FIGURE 7 (a) Trapping solution after collecting thirty 100 mL puffs from a popular brand of cigarette. (b) Trapping solution after collecting thirty 100 mL puffs from E-High electronic cigarette.

Rimonabant and Amino-Tadalafil

As previously reported,^[9] CIXI markets several products containing additional active ingredients including rimonabant and amino-tadalafil. The Cialis E-Cartridges and E-Liquids were mislabeled as they contained amino-tadalafil not tadalafil, the reference listed drug in Cialis. The E-Cartridges contained 12 mg amino-tadalafil/cartridge; there was no label declaration. The E-Liquid contained 17 mg/mL in one sample and 5 mg/mL in another; there were no label declarations. The manufacturer's website indicated there was 16 mg of tadalafil in the cartridge and 20 mg/mL tadalafil in the E-Liquid.

The Rimonabant E-Cartridges contained 3 mg rimonabant per cartridge, and two E-Liquids samples contained 3 mg/mL and 4 mg/mL rimonabant; there were no label declarations. The manufacturer's website indicated there was 20 mg/mL rimonabant in the E-Liquid and 16 mg/cartridge in the E-Cartridges.

Analysis of the puff trapping solutions from electronic cigarettes using cartridges containing rimonabant and amino-tadalafil did not detect either rimonabant or amino-tadalafil in the trapping solution. The results suggest that under our testing conditions transfer of rimonabant and amino-tadalafil to the vapor phase is low.

CONCLUSIONS

The gradient elution method was developed to allow for separation of the nicotine related impurities and for their determination in cartridge extracts of the electronic cigarettes. Nicotine was shown to be delivered using electronic cigarette devices although the amount of nicotine delivered will be greatly impacted by the "smoking" habits of the consumer. Significant labeling issues were found to exist with products in the market place with respect to product labeling accuracy. Some products were found to contain high concentrations of nicotine when labeled not to contain nicotine.

REFERENCES

1. Lik, H. PCT Int. Appl. (2004), WO 2004080216 A1 20040923.
2. Pauly, J.; Quiang, L.; Barry, M. B. Tobacco-Free Electronic Cigarettes and Cigars Deliver Nicotine and Generate Concern. *Tob. Control* **2007**, *16*, 357.
3. Kuehn, B. M. FDA: Electronic Cigarettes May Be Risky. *JAMA* **2009**, *302*, 937.
4. Wollscheid, K. A.; Kremzner, M. E. Electronic Cigarettes: Safety Concerns and Regulatory Issues. *Am. J. Health-Syst. Pharm.* **2009**, *66*, 1740–1742.
5. Flouris, A. D.; Oikonomou, D. N. Electronic Cigarettes: Miracle or Menace? *BMJ* (Clinical Research ed.) **2010**, *340*, c311.

6. Trtchounian, A.; Talbot, P. Electronic Nicotine Delivery Systems: Is There a Need for Regulation? *Tob. Control* **2011**, *20*, 47–52.
7. Jian, H. Electronic Cigarette Atomizing Solution Containing Tobacco Leaf Extract, Propanediol, Tobacco Essence and Nicotine. *Faming Zhuanli Shenqing Gongkai Shuomingshu*, CN 101461566 A 20090624, 2009.
8. Jian, H. Preparation of a Spray Solution Containing Chinese Medicinal Extracts for Electronic Cigarettes. *Faming Zhuanli Shenqing Gongkai Shuomingshu*, CN 101461565 A 20090624, 2009.
9. Hadwiger, M. E.; Trehy, M. L.; Ye, W.; Moore, T.; Allgire, J.; Westenberger, B. J. Identification of Amino-Tadalafil and Rimonabant in Electronic Cigarette Products Using HPLC with Diode Array and Tandem Mass Spectrometric Detection. *J. Chromatogr. A* **2010**, *1217*, 7547–7555.
10. Trtchounian, A.; Williams, M.; Talbot, P. Conventional and Electronic Cigarettes (E-Cigarettes) Have Different Smoking Properties. *Nicotine Tob. Res.* **2010**, *12*, 905–912.
11. Eissenberg, T. Electronic Nicotine Delivery Devices: Ineffective Nicotine Delivery and Craving Suppression After Acute Administration. *Tob. Control* **2010**, *19*, 87–88.
12. Watson, C. H.; Trommel, J. S.; Ashley, D. L. Solid-Phase Microextraction-Based Approach to Determine Free-Base Nicotine in Trapped Mainstream Cigarette Smoke. *J. Agric. Food Chem.* **2004**, *52*, 7240–7245.
13. Yang, S. S.; Smetena, I.; Goldsmith, A. I. Evaluation of Micellar Electrokinetic Capillary Chromatography for the Analysis of Selected Tobacco Alkaloids. *J. Chromatogr. A* **1996**, *746*, 131–136.
14. United States Pharmacopeia. USP32/NF27 Vol. 3, 2009; pp 3079–3081.
15. ICH Q2B, Validation of Analytical Procedures: Methodology, 1996.
16. Pankow, J. F.; Tavakoli, A. D.; Luo, W.; Isabelle, L. M. Percent Free-Base Nicotine in the Tobacco Smoke Particulate Matter of Selected Commercial and Reference Cigarettes. *Chem. Res. Toxicol.* **2003**, *16*, 1014–1018.
17. Rustemeier, K.; Piade, J. J. Determination of Nicotine in Mainstream and Sidestream Cigarette Smoke. In *Analytical Determination of Nicotine and Related Compounds and Their Metabolites*; Gorrod, J. W., Peyton, J. III, Eds.; Elsevier: Amsterdam, 1999, pp. 489–529.