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"Dilute & Shoot" approach for rapid determination of trace amounts of nicotine in zero-level e-liquids by reversed phase liquid chromatography and hydrophilic interactions liquid chromatography coupled with tandem mass spectrometry – electrospray ionization.

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Abstract

Two analytical procedures are proposed where HILIC and RPLC techniques are coupled with tandem mass spectrometry detection for rapid determination of trace amounts of nicotine in zero-level liquids for electronic cigarettes. Samples are prepared on the basis of the approach "dilute & shoot" which makes this important step quick and not complicated. The chromatographic separation was carried out on a Zorbax XDB column (RPLC method) and Ascentis Si column (HILIC mode). Within-run precisions (CVs) measured at three concentration levels were as follows: 0.73%, 0.98% and 1.44% for RPLC method and 1.39%, 1.44% and 0.57% (HILIC mode). Between-run CVs were as follows: 1.94%, 1.02% and 1.22% for RPLC mode and 1.49%, 1.20% and 1.22% for HILIC mode. The detection limits of RPLC and HILIC modes were 4.08 ng/mL and 3.90 ng/mL respectively. The proposed procedures are rapid, not complicated, sensitive and are suitable for fast determination of trace amounts of nicotine in zero-level liquids for electronic cigarettes.

Keywords: nicotine; electronic cigarettes; RPLC-MS/MS; HILIC-MS/MS;

1. Introduction

Tobacco leaves are rich with closely related alkaloids like: nicotine, anabasine, anatabine, nornicotine, nicotyrine, myosmine, 2,3'-dipyridyl and cotinine [1]. The most popular and well known alkaloid is nicotine due to its potential as one of the most addictive substances. From the pharmaceutical point of view nicotine plays an important role as the agent responsible for numerous behavioural and physiological effects [2-5]. There are many ways to consume the tobacco and receive nicotine. Nicotine products can be divided into those that produce smoke like cigarettes, pipes or cigars and to those that do not produce smoke for instance gums and inhalers [3].

Recently, manufacturers mainly located in China have been producing electronic cigarettes and equipment for them. Such devices are powered by batteries and produce vapour from liquid containing nicotine and mixture of glycols (mainly polypropylene glycol as solvent) [6]. The cartridges are filled with liquids that contain different amount of nicotine and flavours. Sometimes colorants are used to encourage potential customers. The content of specific flavours (fruits, mint, branded cigarettes taste) can simulate the real sensations of cigarette smoking [6, 7]. Some cartridges and liquids may contain nicotine at trace amount level [8].

There are some known analytical procedures for the determination of nicotine and its derivatives in various types of samples. Up to now UV detection has been frequently applied for the determination of nicotine [9-15]. Information found in recent publications indicate that the most popular ones are based on the application of high and ultra performance liquid chromatography (HPLC and UPLC), coupled with mass spectrometry (MS) and tandem mass spectrometry (MS/MS) [4, 16-25] due to sensitivity, confidence and versatility. Gas chromatography coupled with flame ionization detection [1], MS and MS/MS [24, 26-32], time-of-flight MS [33, 34], electron capture detector (ECD) [35], nitrogen chemiluminescence detection [36] or nitrogen-phosphorous detection (NPD) [37] is used as well for determination of nicotine concentration. Moreover, developed methods with the use of capillary electrophoresis coupled with UV detection [38, 39], MS [40] and electrochemiluminescence detector [41] have been reported for the determination of nicotine. Detection by UV is not as sensitive as MS/MS detection and further analysis and evaluation of nicotine content in zero-level liquids have to be done.

The aim of the project was to develop a rapid, simple and sensitive methods for the determination and quantification of nicotine in zero-level liquids for electronic cigarettes by reversed phase liquid chromatography (RPLC) and by hydrophilic interactions liquid

chromatography (HILIC) coupled with tandem mass spectrometry-electrospray ionization in multiple reaction monitoring (MRM) mode. Sample preparation is based on the approach 'dilute & shoot' due to simple and stable composition of the matrix. Two proposed analytical methods allow determining the concentration of nicotine at trace amount in zero-level liquids in less than 4 minutes per single analysis run.

2. Materials and methods

2.1 Chemicals

Standards of racemic nicotine, acetaminophen (internal standard for the RPLC mode of separation), pyridoxine hydrochloride (vitamin B6; internal standard for the HILIC mode of separation) and ammonium formate were purchased from Sigma Aldrich (St. Louis, USA). Acetonitrile HPLC gradient (ACN) and methanol HPLC gradient (MeOH) were purchased from Merck KGaA (Darmstadt, Germany). Formic acid (FA) and ethanol were purchased from POCH (Gliwice, Poland). Propylene glycol and glycerol were purchased from EasyChem (Szamotuły, Poland). Deionized water (H_2O) was prepared with the use of the HLP5 system from Hydrolab (Wiślina, Poland).

2.2 Samples

Forty one liquids from seven different producers marked with zero-level of nicotine were purchased from stores of popular distributorsof electronic cigarettes on the Polish market. Four producers placed information on the liquids' bottles that product may contain nicotine. Two producers did not include any information about nicotine content. One of the producers gave information about possible trace levels of nicotine.

2.3 Preparation of standards and calibration solutions

Stock solutions of nicotine, acetaminophen and pyridoxine were prepared by dissolving the weighted amount of standards in the following solutions: in a mixture of H_2O and MeOH (75:25) for the RPLC mode of separation, in a mixture of H_2O and ACN (25:75) for the HILIC mode of separation. The final concentration of nicotine and acetaminophen was 10 µg/mL and pyridoxine was 40 µg/mL. Calibration solutions were made by dilution of stock solutions in the mobile phase (separately for the RPLC and HILIC) to obtain the following concentrations: 5, 10, 50, 100, 150, 200 and 400 ng/mL. In each calibration solution, the IS concentration was 100 ng/mL (RPLC mode) and 200 ng/mL (HILIC mode). Standards, stock

solutions and calibration solutions were stored in refrigerator at 4˚C. Every two weeks new stock solutions and calibration solutions were prepared.

2.4 Sample preparation

Approximately 10 mg of each sample was weighted into a 10 mL flask and 100 µL (RPLC mode) or 50 µL (HILIC mode) of IS was added, depending on the used method. Finally, the flask was filled up to 10 mL with the mobile phase for the chosen mode of separation.

2.5 Preparation of fortified samples

The main ingredients of liquids for electronic cigarettes are: propylene glycol (>70%), glycerol (>15%) and ethanol (>10%). The rest of the components are complex alcohols, diols, flavours and colorants. The liquid for fortification with nicotine was prepared by mixing 75% of propylene glycol, 15% of glycerol and 10% of ethanol. To such liquid nicotine was added to obtain 50, 150 and 300 µg/g of analyte per gram of liquid. Fortified samples and unfortified laboratory made samples of liquid were prepared according to the protocol described in section 2.4.

To examine the influence of the sample matrix components another calibration solutions were prepared in the same range and in the same way as described in section 2.3. Furthermore, for every 10 mL of each calibration solution 10 mg of randomly selected real sample was added. The nicotine content in chosen real sample was below LOD.

2.6 MS/MS conditions

Analyses were done using a Q-Trap 4000 triple quadrupole mass spectrometer from Applied Biosystems (Foster City, USA) with electrospray ionization in positive ion mode. For the setting the parameters of MRM mode, the infusion analyses were performed with solutions containing 100 ng/mL of nicotine, pyridoxine and acetaminophen. The positive ion mode tandem mass spectra of nicotine, acetaminophen and pyridoxine and their structures are presented in Figure S1 (supplementary material). In order to evaluate optimal parameters for MS/MS ion source for RPLC and HILIC modes flow injection analyses (FIA) of a standard solution of nicotine (100 ng/mL) were done. Operational parameters of ion source were optimized in order to obtain the highest intensity for nicotine. Parameters of the MRM mode for the analyte and internal standards as well as ion source parameters are presented in Table S1 (supplementary material). All data were collected and processed using Analyst 1.5.2 Software and ChemStation B.04.02 SP1.

2.7 HPLC conditions

Separation was carried out with the use of HPLC-MS/MS system with the Agilent 1200 series containing a pump coupled with photodiode array detector (DAD), degasser, column oven and autosampler. The RPLC mode was performed on analytical column Zorbax XDB-C8 (150x4.6 mm, 5 µm with pore size 100Å). The column temperature was set to 35˚C. Mobile phase consisted of H_2O with 0.05% of FA (A) and MeOH with 0.05% of FA (B), while flow rate was set to 0.7 mL/min. Injection volume was set to 5 µL. Isocratic flow conditions were chosen for this method: 75% of A and 25% of B. Total time of analysis was 4 minutes. In case of RPLC mode the acetaminophen was chosen as internal standard.

The HILIC mode was performed on analytical column Ascentis Si from Supelco (150x2.1 mm, 5 µm with pore size 100 Å). The column temperature was set to 25˚C. Mobile phase consisted of ACN with 0.01% of FA (A) and H_2O with 10mM of ammonium formate (B), while flow rate was set to 0.8 mL/min. Injection volume was set to 5 µL. Again, isocratic flow conditions were chosen for this method: 75% of A and 25% of B. Total time of analysis was 4 minutes. In case of HILIC mode the pyridoxine was selected as internal standard.

Chromatograms of mixtures of standard of racemic nicotine and chosen IS for each mode and examples of chromatograms of real samples are presented in the Figure 1.

<insert Figure 1>

3 Results and discussion

3.1 Inter-laboratory validation

3.1.1 Linearity, LOD, LOQ and matrix influence

Calibration curves were constructed using the internal standard method. Seven calibration solutions were made from standard solutions of nicotine as described in section 2.3. Each calibration solution contained a specific amount of IS (100 ng/mL of acetaminophen for RPLC mode and 200 ng/mL of pyridoxine for HILIC mode). Each solution was analyzed three times. The values of limits of detection (LODs) were calculated by multiplying the constant term in the equation of the calibration curve by 3.3 and dividing by the slope of the calibration curve. The values of the limits of quantitation (LOQs) were calculated by

multiplying LODs by 3. Equations of calibration curves, values of LODs, LOQs, coefficients of determination (R^2), standard deviations of slope (S_a) and standard deviations of constant term (S_b) are summarized in Table 1.

<insert Table 1>

The obtained values of LOD are proof that with presented methods it is possible to determine the trace amount of nicotine in zero-level liquids for electronic cigarettes. In all cases LOD values are lower than the lowest concentration of calibration solution. High values of coefficient of determination demonstrate an appropriate and acceptable matching of the corresponding points to the calibration curve equation. The influence of matrix components to the calibration curve trends is insignificant and were not observed. Such finding is based due to the similarities and the compatibility of the obtained values of LODs, LOQs and another from the calibration curves obtained without adding the real sample and calibration curves with real sample content. The composition of samples is relatively simple and the influence of alcohols, diols, colorants or flavour components to the nicotine ions is minimal.
In order to exclude other effects of sample components and coelution with analyte or IS the randomly selected sample was prepared according to 2.4 (in this case without adding the IS) section and analysis were performed with the usage of DAD detector at 254 nm. Chromatograms of real sample in HILIC and RPLC mode recorded at 254 nm are presented in the Figure 2.

<insert Figure 2>

3.1.2 Trueness, intermediate precision and repeatability of the developed methods
The developed methods were tested in view of trueness, intermediate precision and repeatability. Fortified liquids were prepared according to the protocol described in section 2.5. The fortified samples were prepared according to the protocol described in section 2.4. Three levels of concentrations were prepared to obtain separately 300, 150 and 50 µg/g of nicotine in liquid. After sample preparation step the concentration levels were 300, 150 and 50 ng/mL. At the same time unfortified samples were prepared to exclude the influence of ingredients of liquids to the signal coming from nicotine. Six repeats were made for a given level of fortified sample for each of the developed methods. Results are presented in µg/g of liquid and the weight of the sample was included in the calculations. To compare the obtained

mean recoveries an ANOVA test was conducted. The null hypothesis is that means of recovery resulting from both methods are equal, due to the similarity in SD and CV. The objective of the test was to accept or reject such hypothesis. The confidence level was 95% and α=0.05 Data gathered from trueness test and ANOVA test are presented in Table 2.

<insert Table 2>

Calculated F values are greater than $F_{critical}$ and p-values are smaller than α. The obtained results from the ANOVA test indicate a rejection of the hypothesis that the means are equal. The conclusion is that the effectiveness of the two presented methods is different for recovery of nicotine. Furthermore $F_{calculated}$ (2.32) < $F_{critical}$ (4.17), hence there is no significant difference between the two methods at 0.05 confidence level. The analysis of variance for each spiking level demonstrated that RPLC method is more suitable than HILIC method for lower levels of concentration. However, the analysis of variance of HILIC method (more than six times smaller than for RPLC method) is a proof for adjustment of this method to higher concentration levels.

Repeatability test was done by the analysis of fortified sample at chosen initial concentration 150 µg/g of nicotine. The sample was prepared according to the protocol described in section 2.4. All analyses were done by HPLC-MS/MS with six repeats during the next three days. No significant difference between recoveries, SDs and CVs values were observed. Results are presented in Table S2 (supplementary material)

The results are satisfactory and it was proved and concluded that it is possible to analyze liquids for electronic cigarettes in case of determination of trace amount of nicotine. The recovery values are at acceptable levels and after sample preparation HPLC-MS/MS analysis with both or one of the presented methods is possible.

3.1.3 Analysis of real samples

Forty one samples of the zero-level content nicotine liquids were analyzed with two presented methods in case of determination of trace amount of nicotine. All samples were prepared according to the presented protocol in 2.4 section. The presented results are in µg/mg not in µg/mL. The reason why the results are shown in this way is due to the difference

in the density of analyzed samples. Each producer has its own recipe for liquids and the content of propylene glycol, glycerol and ethanol differ amongst the products. Moreover, some producers do not use glycerol or ethanol during preparation of liquids.

Results are presented in Table 3 and concentration below LOD and below the calibration curve range were omitted. Examples of chromatograms of real samples are presented in the Figure 1. The distribution of nicotine among the samples of liquids under study for HILIC and RPLC methods is presented in the Figure S2 (supplementary material).

<insert Table 3>

The results were calculated as follows: concentrations resulting from the equation of calibration curves (ng/mL) were multiplied by 10 (sample diluted in 10 mL) and divided by the weight of the sample. The final results are presented in $\mu g_{nicotine}/g_{liquid}$ which is equal to $ng_{nicotine}/mg_{liquid}$. Among the samples with detected nicotine more than 17 samples contain nicotine at a level below 100 µg/g. However 8 samples contain nicotine at a higher amount.

4. Conclusions

Current trends allow smokers to use tobacco substitutes containing nicotine in various forms including the latest fashion: electronic cigarettes. There is a lot of controversy about the use and safety of electronic cigarettes and some countries (Australia, Hong Kong, Brazil) prohibit their sale. Other countries such as Poland, Belgium, and Germany have not introduced so far legal restrictions on the e-cigarettes. This means that the nicotine content in liquids for filing e-cigarettes is not controlled. Particularly noteworthy are liquids that do not contain nicotine and are intended as help in quitting smoking.

Developed methods may be used independently or simultaneously to verify the concentration of nicotine in the liquids identified as zero-level. Presented methods are rapid, reproducible and do not require complex equipment. Moreover, with the HPLC it is possible to perform the analysis in a similar time to that of a UPLC. The LOD and LOQ values obtained for the two methods are at satisfactory level. Selected compounds as internal standards are easy available, cheap, stable and the probability that they are present in the liquids for e-cigarettes is very low. Furthermore, the sample preparation step is fast and simple. Additionally, presented methods may be used as a part of quality control for e-liquids, only the dilution of the samples should be compatible in such cases.

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Figures

Figure 1. Left panel. Multiple-reaction monitoring chromatograms obtained with column Zorbax XDB-C8 (150x4.6mm): A) mixture of racemic nicotine (100 ng/mL) and IS (acetaminophen 100 ng/mL), B) sample of Producer C – taste "Chocolate" ($C_{Nicotine}$=320.95 ± 2.02 µg/g), C) sample of Producer G – taste "Vanilla" ($C_{Nicotine}$=88.48 ± 0.95 µg/g), D) sample of Producer D – taste "Desert Ship ($C_{Nicotine}$=10.05 ± 0.15 µg/g). Right Panel. Multiple reaction monitoring obtained with column Ascentis Si (150x2.1): E) mixture of racemic nicotine (100 ng/mL) and IS (pyridoxine 200 ng/mL), F) sample of Producer C – taste "Chocolate" ($C_{Nicotine}$=312.32 ± 1.51 µg/g), G) sample of Producer G – taste "Vanilla" ($C_{Nicotine}$=84.19 ± 1.55 µg/g), H) sample of Producer D – taste "Desert Ship ($C_{Nicotine}$=9.74 ± 0.16 µg/g).

Figure 2. Chromatograms of real sample recorded at 254 nm: A) HILIC mode, B) RPLC mode.

Tables

Table 1. Data gathered from equations of calibration curves for two presented methods.

Table 2. Recovery, standard deviations (SD), coefficients of variation (CV) and variance analysis (ANOVA) taken from HPLC–MS/MS analysis of spiked samples at three levels.

Table 3. Concentration of nicotine in zero-level liquids for electronic cigarettes.

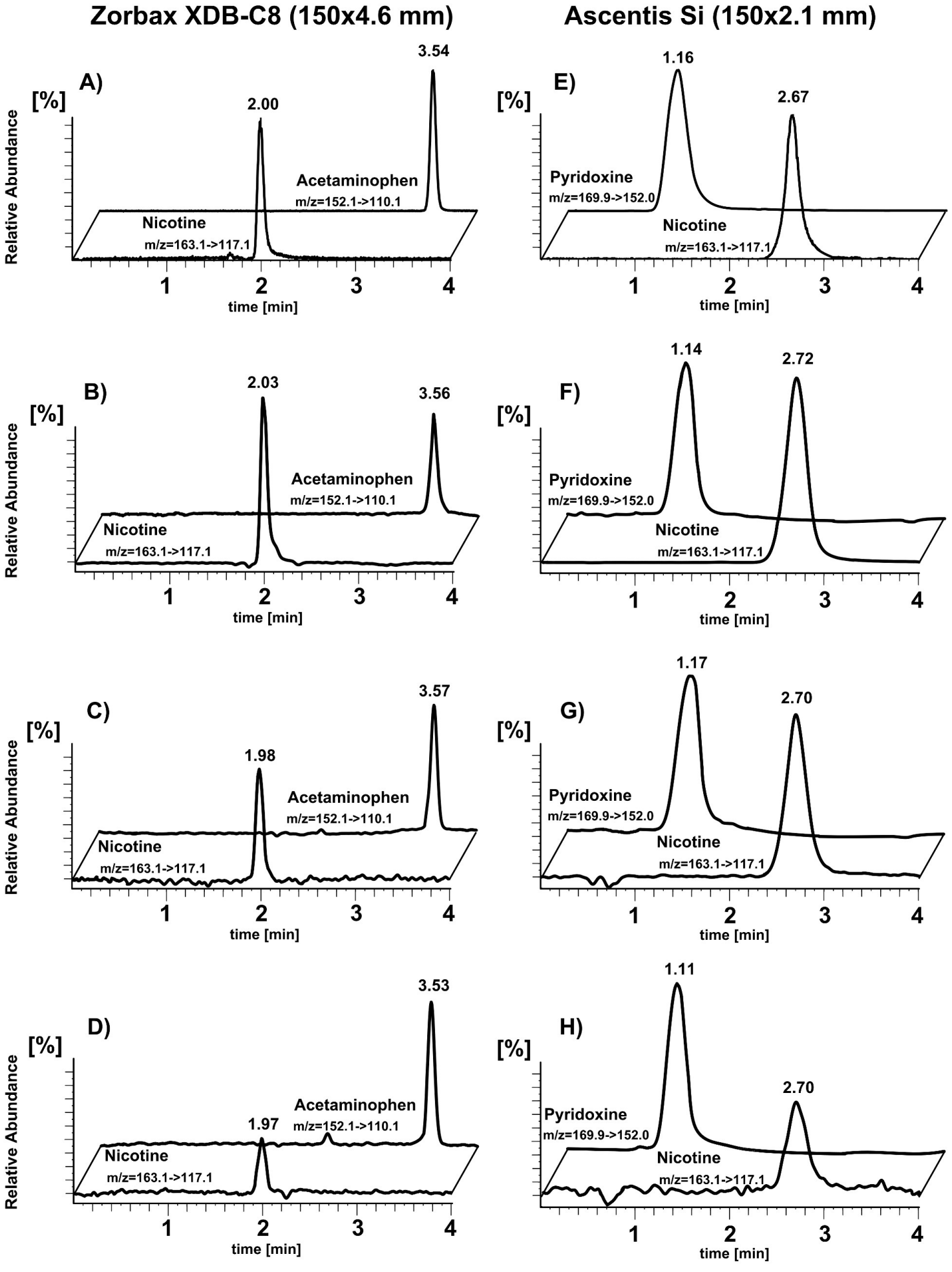
Supplementary material

Figure S1. The positive ion mode tandem mass spectra of standards of nicotine, acetaminophen and pyridoxine each at a concentration of 100 ng/mL, molecular weights and structures.

Figure S2. Distribution of nicotine among the samples of liquids for electronic cigarettes for HILIC and RPLC methods

Table S1. Optimal parameters for the monitored ion transitions (MRM) and chosen operational parameters of ion source.

Table S2. Recovery, standard deviations and coefficients of variations taken from HPLC-MS/MS analysis of one fortified sample at initial concentration 150 µg/g.


Zorbax XDB-C8 (150x4.6 mm)
Ascentis Si (150x2.1 mm)
A)
B)
C)
D)
E)
F)
G)
H)
[%]
Relative Abundance
time [min]
Acetaminophen
m/z=152.1->110.1
Nicotine
m/z=163.1->117.1
Pyridoxine
m/z=169.9->152.0
2.00
3.54
2.03
3.56
1.98
3.57
1.97
3.53
1.16
2.67
1.14
2.72
1.17
2.70
1.11
2.70

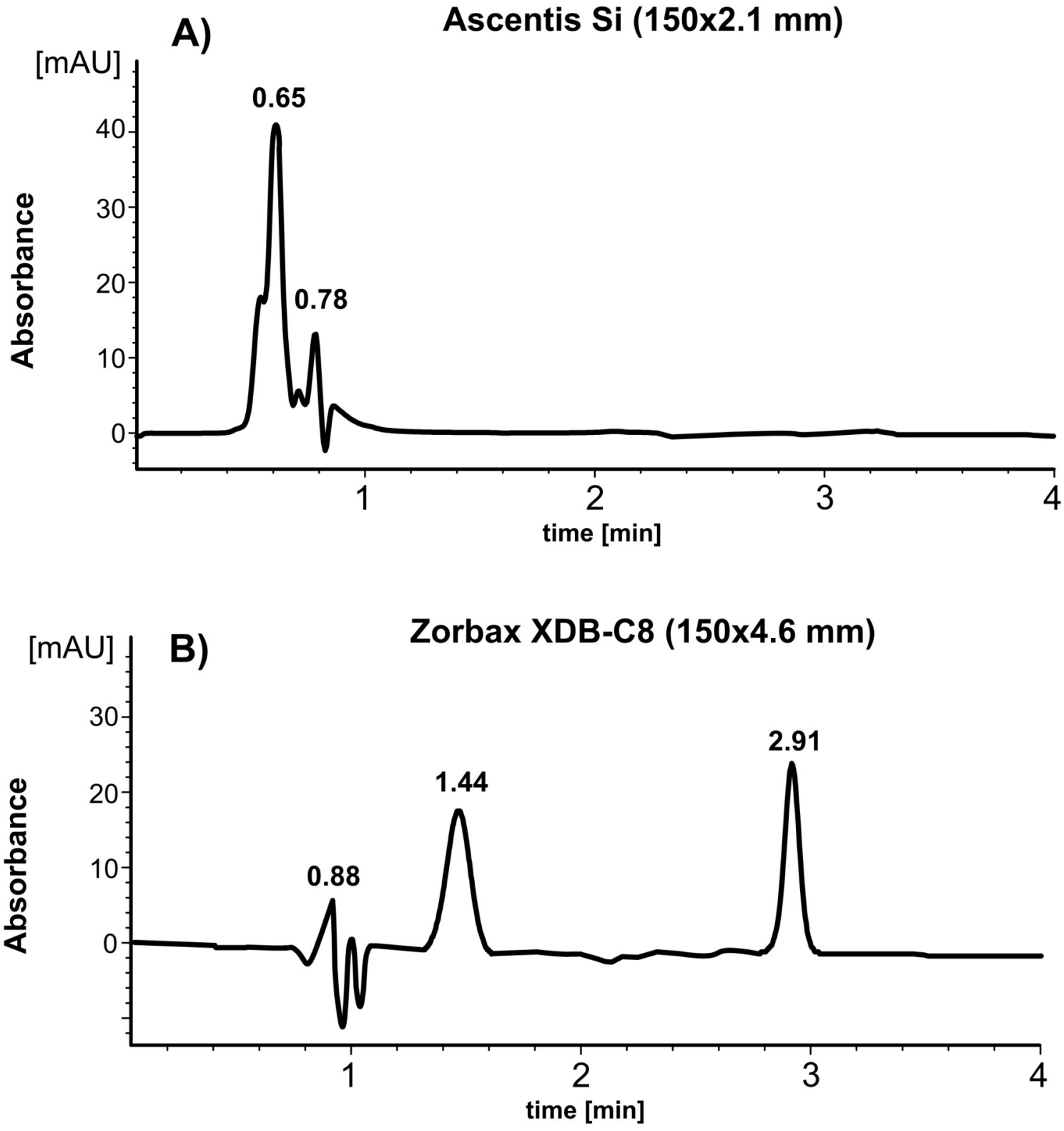

A)
Ascentis Si (150x2.1 mm)
[mAU]
Absorbance
0.65
0.78
40
30
20
10
0
1
2
3
4
time [min]
B)
Zorbax XDB-C8 (150x4.6 mm)
[mAU]
Absorbance
0.88
1.44
2.91
30
20
10
0
1
2
3
4
time [min]

Table 1. Data gathered from the equations of calibration curves for two presented methods.

Analyte	Calibration curve equation (5-400 ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	S_a	S_b	R^2
RPLC mode (Zorbax XDB-C8 150 x 4.6 mm)						
Nicotine	y = 0.0142243x + 0.1720	4.08	12.24	0.000096	0.018	0.9991
Nicotine (matrix influence)	y = 0.0141687x + 0.278	4.19	12.58	0.000074	0.018	0.9997
HILIC mode (Ascentis Si 150 x 2.1 mm)						
Nicotine	y = 0.0006367x + 0.00331	3.90	11.70	0.0000041	0.00075	0.9992
Nicotine (matrix influence)	y = 0.0006254x + 0.00365	4.43	13.30	0.0000068	0.00084	0.9993

Table 2. Recovery, standard deviations (SD), coefficients of variation (CV) and variance analysis (ANOVA) taken from HPLC–MS/MS analysis of spiked samples at three levels.

Analyte	Spiking level (ug/g)	Mean recovery (µg/g) (%) (n=6)	SD	CV (%)
RPLC mode (Zorbax XDB-C8 150 x 4.6 mm)				
Nicotine	50	51.20 (102.4)	0.37	0.73
	150	148.22 (98.8)	1.45	0.98
	300	296.08 (98.36)	2.92	1.94
HILIC mode (Ascentis Si 150 x 2.1 mm)				
Nicotine	50	49.37 (98.7)	0.69	1.39
	150	151.34 (100.9)	2.18	1.44
	300	296.45 (98.8)	1.68	0.57
Analysis of variance (two way) ANOVA				
Source of variation	F value	F critical test	p-value	α
Sample	96819.26	3.32	$7.09*10^{-58}$	
Columns	2.32	4.17	0.14	0.05
Interaction	9.67	3.32	0.00057	
Spiking level (µg/g)	RPLC variance		HILIC variance	
50	0.055		0.19	
150	0.84		1.90	
300	7.24		1.14	

Table 3. Concentration of nicotine in zero-level liquids for electronic cigarettes.

Producer	Taste/Flavour	Detected concentration of nicotine in zero-level liquids (μg/g) ± SD (n=3)		Absolute difference in concentration among methods (μg/g)
		HILIC mode	RPLC mode	
A	Menthol	-	-	-
	Cherry	160.22 ± 1.81	166.35 ± 1.17	6.13
	Marlboro	-	-	-
B	Strawberry	-	-	-
	Chocolate	-	-	-
	Orange	-	-	-
	Camel	-	-	-
	Watermelon	-	-	-
	Grape	-	-	-
C	Chocolate	312.32 ± 1.51	320.95 ± 2.02	8.63
	Coffee	125.93 ± 0.92	127.76 ± 1.14	1.83
	RedBull	41.30 ± 0.33	39.07 ± 0.35	2.23
	L&M	-	-	-
	Marlboro	-	-	-
	Camel	-	-	-
	Strawberry	-	-	-
	Cherry	205.42 ± 1.03	207.33 ± 1.24	1.91
	Apple	74.63 ± 0.72	71.76 ± 0.54	2.87
D	Desert Ship	9.74 ± 0.16	10.05 ± 0.15	0.31
	Cherry	338.46 ± 1.96	332.49 ± 1.92	5.97
	USA Mix	30.97 ± 0.40	29.32 ± 0.52	1.64
	Menthol	5.82 ± 0.12	5.30 ± 0.07	0.52
	Fruit Mix	-	-	-
E	Cuban Tobacco	26.94 ± 0.78	28.56 ± 0.16	1.62
	Café Latte	14.90 ± 0.20	14.01 ± 0.07	0.90
	English Black Tea	-	-	-
	Energy Drink	-	-	-
	Strong Mint	-	-	-
F	Tiramisu	19.90 ± 0.35	18.32 ± 0.37	1.57
	Cherry	6.15 ± 0.14	6.21 ± 0.20	0.06
	Coffee	5.11 ± 0.08	5.55 ± 0.73	0.44
G	Watermelon	318.28 ± 0.97	315.58 ± 1.55	2.70
	Banana	151.33 ± 1.66	148.89 ± 1.16	2.44
	Vanilla	84.19 ± 1.55	88.48 ± 0.95	4.28
	Camel	23.26 ± 0.33	22.03 ± 0.22	1.23
	Marlboro	20.37 ± 0.29	22.56 ± 1.04	2.19
	RedBull	53.47 ± 0.17	47.15 ± 0.97	6.32

	Blackberry	22.82 ± 0.13	23.36 ± 0.95	0.54
	Cherry	280.75 ± 2.59	283.53 ± 1.58	2.78
	Menthol	72.75 ± 0.55	69.06 ± 0.36	3.69
	Fruit Mix	34.40 ± 0.19	31.18 ± 0.31	3.22

Table S1. Optimal parameters for the monitored ion transitions (MRM) and chosen operational parameters of ion source

Parameters for the monitored ion transitions

Name	Transition[a]	Declustering Potential (V)	Entrance Potential (V)	Collision Cell Exit Potential (V)	Collision Energy (V)
Nicotine	<u>163.1→130.1</u>	56	10	8	29
	163.1→117.1			20	37
Acetaminophen	<u>152.1→110.1</u>	61		18	23
	152.1→93.1			16	31
Pyridoxine	<u>169.9→152.0</u>	91		12	19
	169.9→134.0			10	27

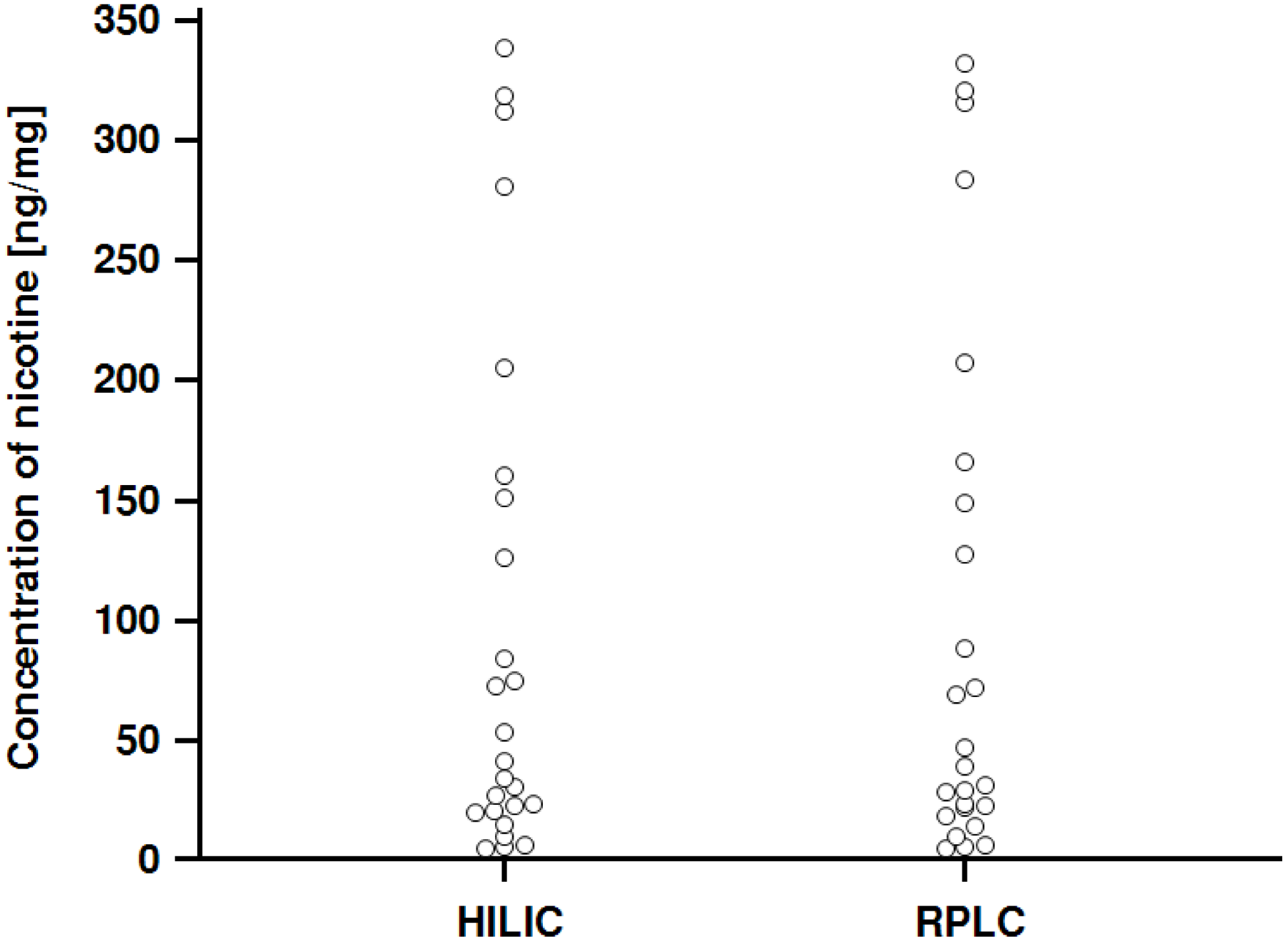
MS/MS operational parameters of the ion source

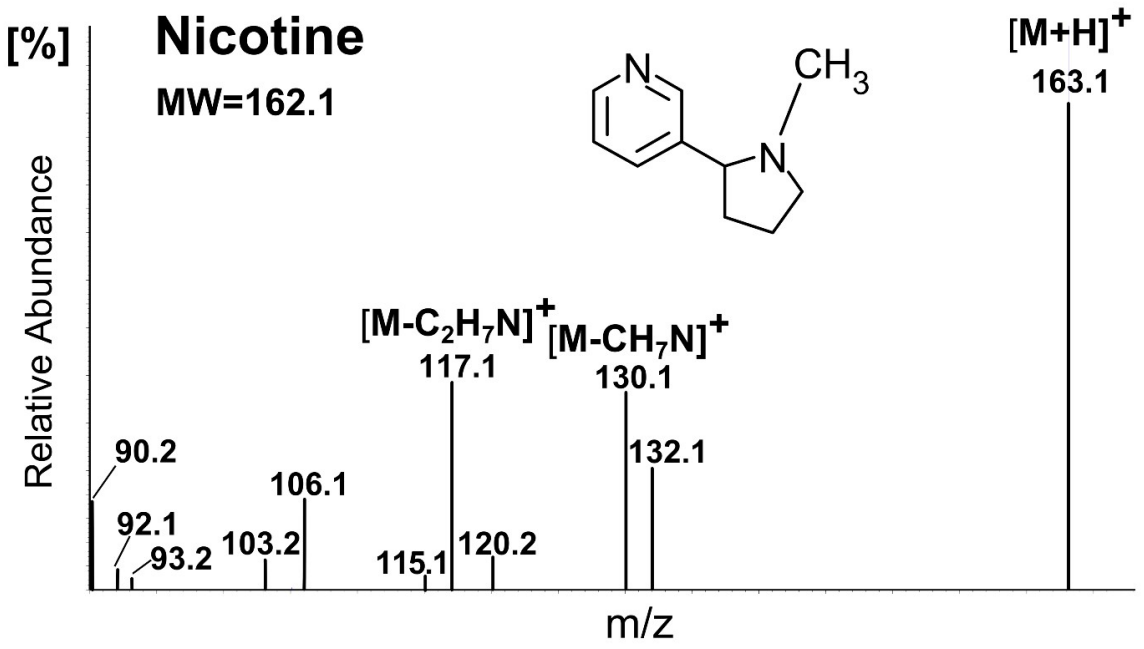
	Curtain Gas (psi)	Temperature (˚C)	Nebulizer Gas (psi)	Turbo Gas (psi)
RPLC mode	15	600	50	60
HILIC mode	50	550		50

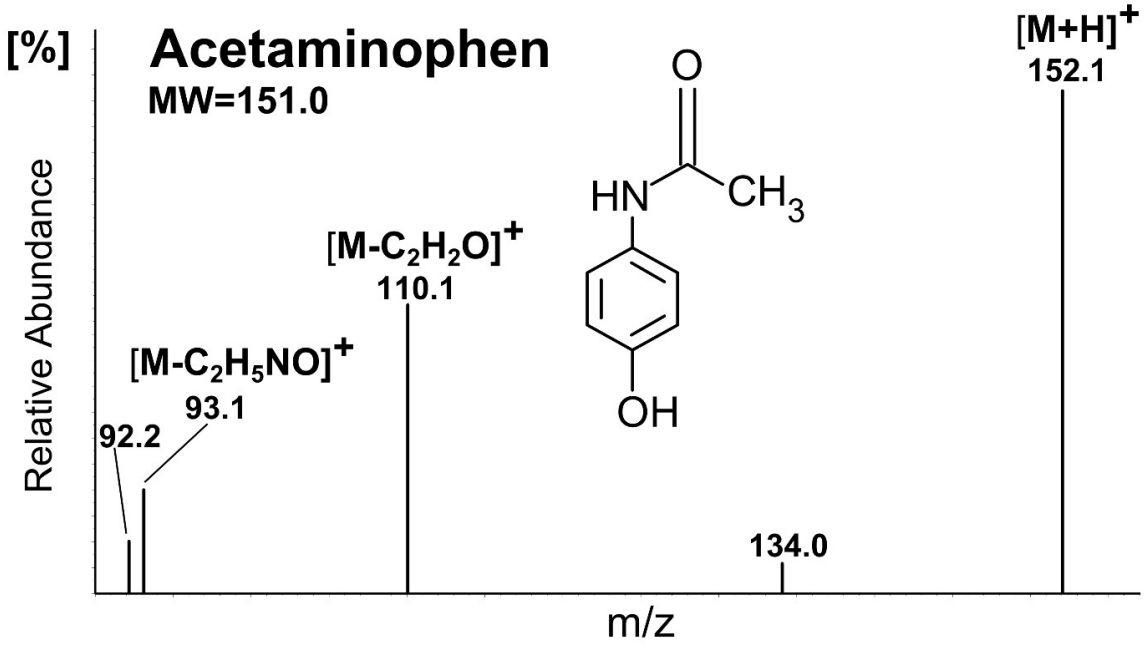
a – quantification ion transitions are underlined

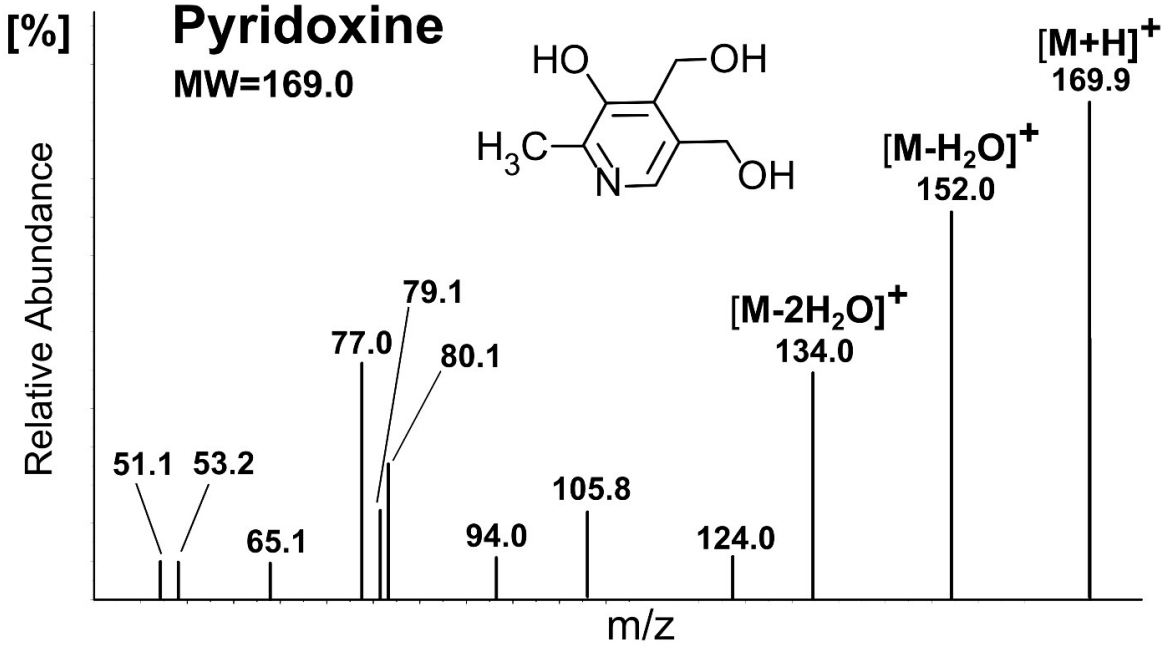
Table S2. Recovery, standard deviations and coefficients of variations taken from HPLC-MS/MS analysis of one fortified sample at initial concentration 150 µg/g.

Analyte	Day	Mean recovery (µg/g) (%) (n=6)	SD	CV (%)
RPLC method (Zorbax XDB-C8 150 x 4.6 mm)				
Nicotine	1	150.39 (100.4)	2.92	1.94
	2	148.19 (98.8)	1.51	1.02
	3	151.21 (100.8)	1.84	1.22
HILIC method (Ascentis Si 150 x 2.1 mm)				
Nicotine	1	153.54 (102.4)	2.29	1.49
	2	154.66 (103.1)	1.85	1.20
	3	153.68 (102.5)	1.87	1.22


Concentration of nicotine [ng/mg]
350
300
250
200
150
100
50
0
HILIC
RPLC


[%]
Nicotine
MW=162.1
[M+H]+
163.1
Relative Abundance
[M-C2H7N]+
117.1
[M-CH7N]+
130.1
90.2
106.1
132.1
92.1
103.2
93.2
115.1
120.2
m/z


[%]
Acetaminophen
MW=151.0
[M+H]+
152.1
Relative Abundance
[M-C2H2O]+
110.1
[M-C2H5NO]+
93.1
92.2
134.0
m/z


[%]
Pyridoxine
MW=169.0
[M+H]+
169.9
Relative Abundance
[M-H2O]+
152.0
[M-2H2O]+
134.0
79.1
77.0
80.1
51.1
53.2
105.8
65.1
94.0
124.0
m/z