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3
4 **“Dilute & Shoot” approach for rapid determination of trace amounts of nicotine in zero-**
5 **level e-liquids by reversed phase liquid chromatography and hydrophilic interactions**
6 **liquid chromatography coupled with tandem mass spectrometry – electrospray**
7 **ionization.**

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15 **Abstract**

16 Two analytical procedures are proposed where HILIC and RPLC techniques are coupled with
17 tandem mass spectrometry detection for rapid determination of trace amounts of nicotine in
18 zero-level liquids for electronic cigarettes. Samples are prepared on the basis of the approach
19 “dilute & shoot” which makes this important step quick and not complicated. The
20 chromatographic separation was carried out on a Zorbax XDB column (RPLC method) and
21 Ascentis Si column (HILIC mode). Within-run precisions (CVs) measured at three
22 concentration levels were as follows: 0.73%, 0.98% and 1.44% for RPLC method and 1.39%,
23 1.44% and 0.57% (HILIC mode). Between-run CVs were as follows: 1.94%, 1.02% and
24 1.22% for RPLC mode and 1.49%, 1.20% and 1.22% for HILIC mode. The detection limits of
25 RPLC and HILIC modes were 4.08 ng/mL and 3.90 ng/mL respectively. The proposed
26 procedures are rapid, not complicated, sensitive and are suitable for fast determination of trace
27 amounts of nicotine in zero-level liquids for electronic cigarettes.

28
29 **Keywords:** nicotine; electronic cigarettes; RPLC-MS/MS; HILIC-MS/MS;

30

31 1. Introduction

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

40 Recently, manufacturers mainly located in China have been producing electronic cigarettes
41 and equipment for them. Such devices are powered by batteries and produce vapour from
42 liquid containing nicotine and mixture of glycols (mainly polypropylene glycol as solvent)
43 [6]. The cartridges are filled with liquids that contain different amount of nicotine and
44 flavours. Sometimes colorants are used to encourage potential customers. The content of
45 specific flavours (fruits, mint, branded cigarettes taste) can simulate the real sensations of
46 cigarette smoking [6, 7]. Some cartridges and liquids may contain nicotine at trace amount
47 level [8].

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

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

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

70

71 2. Materials and methods

72 2.1 Chemicals

73 Standards of racemic nicotine, acetaminophen (internal standard for the RPLC mode of
74 separation), pyridoxine hydrochloride (vitamin B6; internal standard for the HILIC mode of
75 separation) and ammonium formate were purchased from Sigma Aldrich (St. Louis, USA).
76 Acetonitrile HPLC gradient (ACN) and methanol HPLC gradient (MeOH) were purchased
77 from Merck KGaA (Darmstadt, Germany). Formic acid (FA) and ethanol were purchased
78 from POCH (Gliwice, Poland). Propylene glycol and glycerol were purchased from
79 EasyChem (Szamotuły, Poland). Deionized water (H₂O) was prepared with the use of the
80 HLP5 system from Hydrolab (Wiślina, Poland).

81

82 2.2 Samples

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

88

89 2.3 Preparation of standards and calibration solutions

90 Stock solutions of nicotine, acetaminophen and pyridoxine were prepared by dissolving the
91 weighted amount of standards in the following solutions: in a mixture of H₂O and MeOH
92 (75:25) for the RPLC mode of separation, in a mixture of H₂O and ACN (25:75) for the
93 HILIC mode of separation. The final concentration of nicotine and acetaminophen was 10
94 µg/mL and pyridoxine was 40 µg/mL. Calibration solutions were made by dilution of stock
95 solutions in the mobile phase (separately for the RPLC and HILIC) to obtain the following
96 concentrations: 5, 10, 50, 100, 150, 200 and 400 ng/mL. In each calibration solution, the IS
97 concentration was 100 ng/mL (RPLC mode) and 200 ng/mL (HILIC mode). Standards, stock

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

100

101 2.4 Sample preparation

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

105

106 2.5 Preparation of fortified samples

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

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

118

119 2.6 MS/MS conditions

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

132

133 2.7 HPLC conditions

134 Separation was carried out with the use of HPLC-MS/MS system with the Agilent 1200 series
135 containing a pump coupled with photodiode array detector (DAD), degasser, column oven
136 and autosampler. The RPLC mode was performed on analytical column Zorbax XDB-C8
137 (150x4.6 mm, 5 μm with pore size 100Å). The column temperature was set to 35°C. Mobile
138 phase consisted of H₂O with 0.05% of FA (A) and MeOH with 0.05% of FA (B), while flow
139 rate was set to 0.7 mL/min. Injection volume was set to 5 μL . Isocratic flow conditions were
140 chosen for this method: 75% of A and 25% of B. Total time of analysis was 4 minutes. In case
141 of RPLC mode the acetaminophen was chosen as internal standard.

142 The HILIC mode was performed on analytical column Ascentis Si from Supelco (150x2.1
143 mm, 5 μm with pore size 100 Å). The column temperature was set to 25°C. Mobile phase
144 consisted of ACN with 0.01% of FA (A) and H₂O with 10mM of ammonium formate (B),
145 while flow rate was set to 0.8 mL/min. Injection volume was set to 5 μL . Again, isocratic
146 flow conditions were chosen for this method: 75% of A and 25% of B. Total time of analysis
147 was 4 minutes. In case of HILIC mode the pyridoxine was selected as internal standard.

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

150

151 <insert Figure 1>

152

153 3 Results and discussion

154

155 3.1 Inter-laboratory validation

156

157 3.1.1 Linearity, LOD, LOQ and matrix influence

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

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

168

169 <insert Table 1>

170

171 The obtained values of LOD are proof that with presented methods it is possible to determine
172 the trace amount of nicotine in zero-level liquids for electronic cigarettes. In all cases LOD
173 values are lower than the lowest concentration of calibration solution. High values of
174 coefficient of determination demonstrate an appropriate and acceptable matching of the
175 corresponding points to the calibration curve equation. The influence of matrix components to
176 the calibration curve trends is insignificant and were not observed. Such finding is based due
177 to the similarities and the compatibility of the obtained values of LODs, LOQs and another
178 from the calibration curves obtained without adding the real sample and calibration curves
179 with real sample content. The composition of samples is relatively simple and the influence of
180 alcohols, diols, colorants or flavour components to the nicotine ions is minimal.

181 In order to exclude other effects of sample components and coelution with analyte or IS the
182 randomly selected sample was prepared according to 2.4 (in this case without adding the IS)
183 section and analysis were performed with the usage of DAD detector at 254 nm.

184 Chromatograms of real sample in HILIC and RPLC mode recorded at 254 nm are presented in
185 the Figure 2.

186

187 <insert Figure 2>

188

189 3.1.2 Trueness, intermediate precision and repeatability of the developed methods

190 The developed methods were tested in view of trueness, intermediate precision and
191 repeatability. Fortified liquids were prepared according to the protocol described in section
192 2.5. The fortified samples were prepared according to the protocol described in section 2.4.
193 Three levels of concentrations were prepared to obtain separately 300, 150 and 50 $\mu\text{g/g}$ of
194 nicotine in liquid. After sample preparation step the concentration levels were 300, 150 and
195 50 ng/mL . At the same time unfortified samples were prepared to exclude the influence of
196 ingredients of liquids to the signal coming from nicotine. Six repeats were made for a given
197 level of fortified sample for each of the developed methods. Results are presented in $\mu\text{g/g}$ of
198 liquid and the weight of the sample was included in the calculations. To compare the obtained

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

203

204 <insert Table 2>

205

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

215

216 Repeatability test was done by the analysis of fortified sample at chosen initial concentration
217 150 $\mu\text{g/g}$ of nicotine. The sample was prepared according to the protocol described in section
218 2.4. All analyses were done by HPLC-MS/MS with six repeats during the next three days. No
219 significant difference between recoveries, SDs and CVs values were observed. Results are
220 presented in Table S2 (supplementary material)

221

222

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

227

228 3.1.3 Analysis of real samples

229 Forty one samples of the zero-level content nicotine liquids were analyzed with two
230 presented methods in case of determination of trace amount of nicotine. All samples were
231 prepared according to the presented protocol in 2.4 section. The presented results are in
232 $\mu\text{g/mg}$ not in $\mu\text{g/mL}$. The reason why the results are shown in this way is due to the difference

233 in the density of analyzed samples. Each producer has its own recipe for liquids and the
234 content of propylene glycol, glycerol and ethanol differ amongst the products. Moreover,
235 some producers do not use glycerol or ethanol during preparation of liquids.
236 Results are presented in Table 3 and concentration below LOD and below the calibration
237 curve range were omitted. Examples of chromatograms of real samples are presented in the
238 Figure 1. The distribution of nicotine among the samples of liquids under study for HILIC and
239 RPLC methods is presented in the Figure S2 (supplementary material).

240

241 <insert Table 3>

242

243 The results were calculated as follows: concentrations resulting from the equation of
244 calibration curves (ng/mL) were multiplied by 10 (sample diluted in 10 mL) and divided by
245 the weight of the sample. The final results are presented in $\mu\text{g}_{\text{nicotine}}/\text{g}_{\text{liquid}}$ which is equal to
246 $\text{ng}_{\text{nicotine}}/\text{mg}_{\text{liquid}}$. Among the samples with detected nicotine more than 17 samples contain
247 nicotine at a level below 100 $\mu\text{g}/\text{g}$. However 8 samples contain nicotine at a higher amount.

248

249 4. Conclusions

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

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

266

267 References

- 268 [1] J. Cai, B. Liu, P. Lin, Q. Su, *J. Chromatogr. A*, 1017 (2003).
 269 [2] D.M. Atrens, *J. Drug Issues*, 31 (2001) 325-394.
 270 [3] F. Marclay, M. Saugy, *J. Chromatogr. A*, 1217 (2010) 7528-7538.
 271 [4] D.M. Shakleya, M.A. Huestis, *J. Chromatogr. B*, 877 (2009) 3537-3542.
 272 [5] Y. Xue, E.F. Domino, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 32 (2008) 1131-
 273 1138.
 274 [6] J.-F. Etter, *BMC Public Health*, 10 (2010).
 275 [7] L. Dawkins, J. Turner, S. Hasna, K. Soar, *Addict. Behav.*, 37 (2012) 970-973.
 276 [8] B.J. Westenberger, Department of Health and Human Service. Food and Drug
 277 Administration, (2009).
 278 [9] J.E. Jablonski, J.E. Schlessler, P. Mariappagoudar, *J. Agr. Food. Chem.*, 54 (2006) 7460-
 279 7465.
 280 [10] A. Aresta, F. Palmisano, C.G. Zambonin, *Food. Chem.*, 93 (2005) 177-181.
 281 [11] C. Oddoze, A.M. Pauli, J. Pastor, *J. Chromatogr. B*, 708 (1998) 95-101.
 282 [12] Y.-L. Chang, P.-L. Tsai, Y.-C. Chou, J.-H. Tien, T.-H. Tsai, *J. Chromatogr. A*, 1088
 283 (2005) 152-157.
 284 [13] B. Sellergrena, A. Zander, T. Renner, A. Swietlow, *J. Chromatogr. A*, 829 (1998) 143-
 285 152.
 286 [14] M. Page-Sharp, T.W. Hale, L.P. Hackett, J.H. Kristensen, K.F. Ilett, *J. Chromatogr. B*,
 287 796 (2003) 173-180.
 288 [15] A.W. Abu-Qare, M.B. Abou-Donia, *J. Chromatogr. B*, 757 (2001) 295-300.
 289 [16] F. Baumann, R. Regenthal, I.L. Burgos-Guerrero, U. Hegerl, R. Preiss, *J. Chromatogr. B*,
 290 878 (2010) 107-111.
 291 [17] M. Concheiro, T.R. Gray, D.M. Shakleya, M.A. Huestis, *Anal. Bioanal. Chem.*, 398
 292 (2010).
 293 [18] H. Kataoka, R. Inoue, K. Yagi, K. Saito, *J. Pharmaceut. Biomed.*, 49 (2009) 108-114.
 294 [19] J. Kuhn, T. Vollmer, C. Martin, D. Hendig, C. Knabbe, *J. Chromatogr. A*, 1217 (2010)
 295 7528-7538.
 296 [20] E.I. Miller, H.-R.K. Norris, D.E. Rollins, S.T. Tiffany, D.G. Wilkins, *J. Chromatogr. B*,
 297 878 (2010) 725-737.
 298 [21] S. Onoue, N. Yamamoto, Y. Seto, S. Yamada, *Eur. J. Drug. Metab. Ph.*, 26 (2011) 416-
 299 422.
 300 [22] K.B. Scheidweiler, D.M. Shakley, M.A. Huestis, *Clin. Chim. Acta*, 413 (2012) 978-984.
 301 [23] P.L. Vieira-Brock, E.I. Miller, S.M. Nielsen, A.E. Fleckenstein, D.G. Wilkins, *J.*
 302 *Chromatogr. B*, 879 (2011) 3465-3474.
 303 [24] D.V. Zagorevski, J.A. Loughmiller-Newman, *Rapid Commun. Mass. Sp.*, 26 (2012) 403-
 304 411.
 305 [25] P. Jacob, L. Yu, M. Duan, L. Ramos, O. Yturralde, N.L. Benowitz, *J. Chromatogr. B*,
 306 879 (2011) 267-276.
 307 [26] M.-J.e. Binette, P. Lafontaine, M. Vanier, L.-K. Ng, *J. Agr. Food. Chem.*, 57 (2009)
 308 1151-1155.
 309 [27] B.M.d. Fonseca, I.E.D. Moreno, A.R. Magalhães, M. Barroso, J.A. Queiroz, S. Ravara, J.
 310 Calheiros, E. Gallardo, *J. Chromatogr. B*, 889-890 (2012) 116-122.
 311 [28] A.M. Hossain, S.M. Salehuddin, *Arab. J. Chem.*, article in press
 312 doi:10.1016/j.arabjc.2010.10.006 (2011).
 313 [29] X. Joya, M. Pujadas, M. Falcón, E. Civit, O. Garcia-Algar, O. Vall, S. Pichini, A. Luna,
 314 R.d.l. Torre, *Forensic Sci. Int.*, 196 (2010) 34-42.
 315 [30] C.N. Man, S. Ismail, G.L. Harn, R. Lajjis, R. Awang, *J. Chromatogr. B*, 877 (2009) 339-
 316 342.

- 317 [31] K. Shrivastava, D.K. Patel, Food Chemistry, 1222 (2010) 314-318.
 318 [32] M. Sleiman, R.L. Maddalena, L.A. Gundel, H. Destailhats, J. Chromatogr. A, 1216
 319 (2009) 7899-7905.
 320 [33] P. Begley, S. Francis-McIntyre, W.B. Dunn, D.I. Broadhurst, A. Halsall, A. Tseng, J.
 321 Knowles, R. Goodacre, D.B. Kell, Anal. Chem., 81 (2009) 7038-7046.
 322 [34] V. Lopez-Avila, J. Cooley, R. Urdahl, M. Thevis, Rapid Commun. Mass. Sp., 26 (2012)
 323 2714-2724.
 324 [35] J.M. Moore, D.M. Cooper, T.C. Kram, R.F.C. Klein, J. Chromatogr. A, 645 (1993) 273-
 325 281.
 326 [36] N. Ramírez, M.Z. Özel, A.C. Lewis, R.M. Marcé, F. Borrull, J.F. Hamilton, J.
 327 Chromatogr. A, 1219 (2012) 180-187.
 328 [37] L. Malafatti, P.P. Maia, M.C.G. Martins, M.E.P.B.d. Siqueira, I. Martins, Braz. J. Pharm.
 329 Sci., 46 (2010) 769-776.
 330 [38] A.A. Dahab, N.W. Smith, J. Sep. Sci., 35 (2012) 66-72.
 331 [39] S. Kodama, A. Morikawa, K. Nakagomi, A. Yamamoto, A. Sato, K. Suzuki, T.
 332 Yamashita, T. Kemmei, A. Taga, Electrophoresis, 30 (2009) 349-356.
 333 [40] C.-W. Chiu, H.-H. Liang, H.-Y. Huang, Electrophoresis, 28 (2007) 4220-4226.
 334 [41] J. Sun, H. Du, T. You, Electrophoresis, 32 (2011) 2148-2154.

335
 336
 337 **Figures**

338
 339 **Figure 1.** Left panel. Multiple-reaction monitoring chromatograms obtained with column
 340 Zorbax XDB-C8 (150x4.6mm): A) mixture of racemic nicotine (100 ng/mL) and IS
 341 (acetaminophen 100 ng/mL), B) sample of Producer C – taste “Chocolate” ($C_{\text{Nicotine}}=320.95 \pm$
 342 $2.02 \mu\text{g/g}$), C) sample of Producer G – taste “Vanilla” ($C_{\text{Nicotine}}=88.48 \pm 0.95 \mu\text{g/g}$), D)
 343 sample of Producer D – taste “Desert Ship” ($C_{\text{Nicotine}}=10.05 \pm 0.15 \mu\text{g/g}$). Right Panel. Multiple
 344 reaction monitoring obtained with column Ascentis Si (150x2.1): E) mixture of racemic
 345 nicotine (100 ng/mL) and IS (pyridoxine 200 ng/mL), F) sample of Producer C – taste
 346 “Chocolate” ($C_{\text{Nicotine}}=312.32 \pm 1.51 \mu\text{g/g}$), G) sample of Producer G – taste “Vanilla”
 347 ($C_{\text{Nicotine}}=84.19 \pm 1.55 \mu\text{g/g}$), H) sample of Producer D – taste “Desert Ship” ($C_{\text{Nicotine}}=9.74 \pm$
 348 $0.16 \mu\text{g/g}$).

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

351
 352
 353
 354 **Tables**

355
 356 **Table 1.** Data gathered from equations of calibration curves for two presented methods.

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

359

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

361

362 Supplementary material

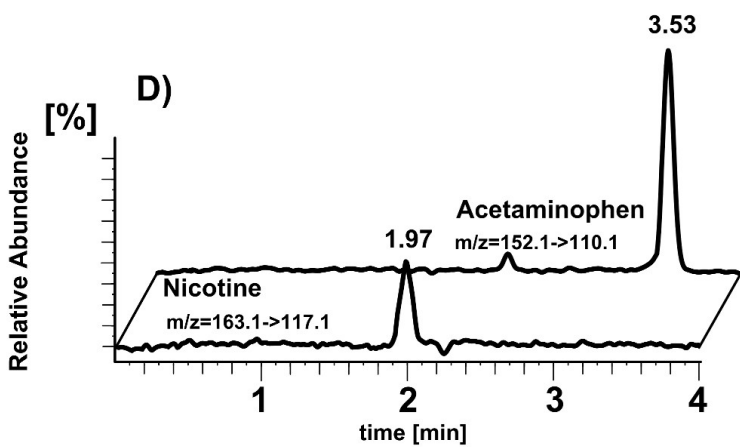
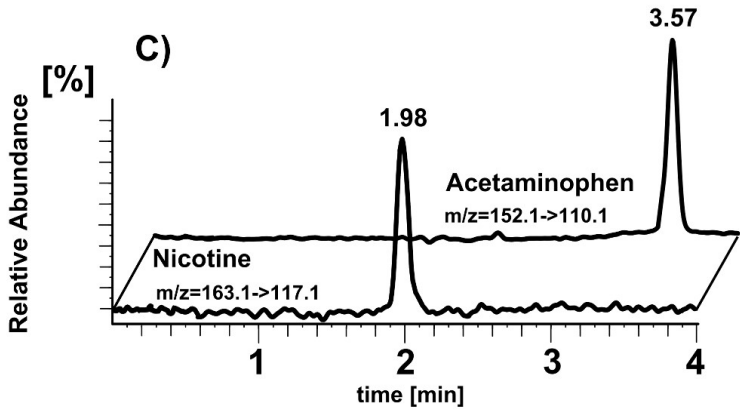
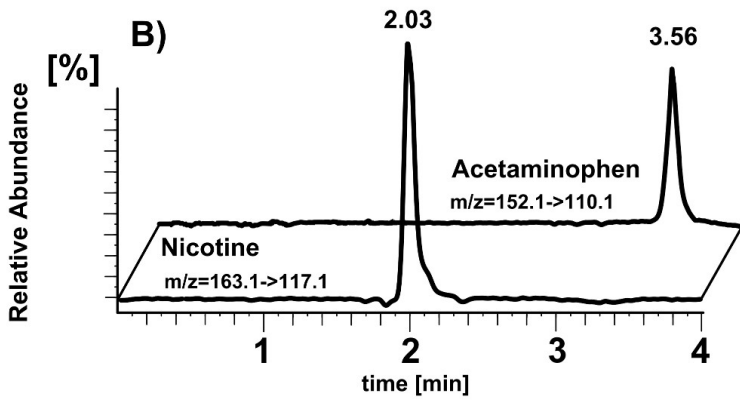
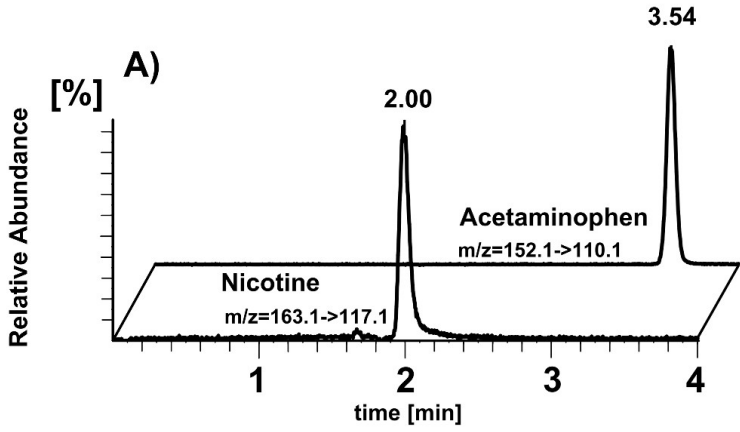
363 Figure S1. The positive ion mode tandem mass spectra of standards of nicotine,
364 acetaminophen and pyridoxine each at a concentration of 100 ng/mL, molecular weights and
365 structures.

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

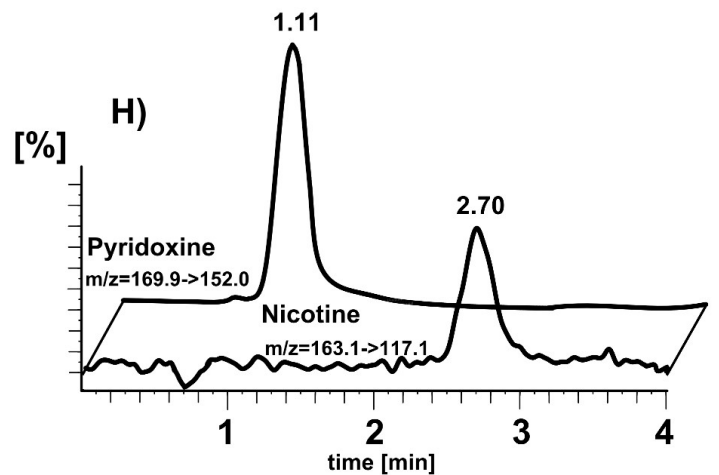
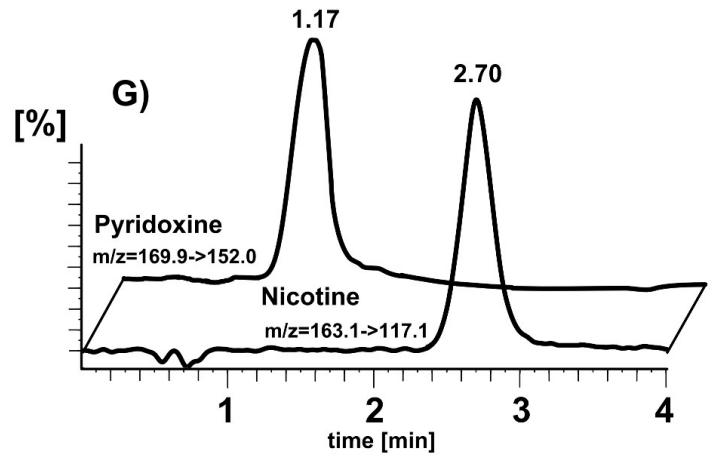
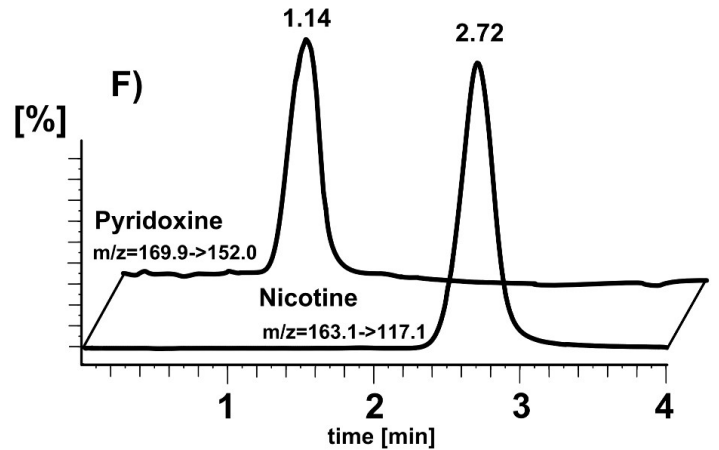
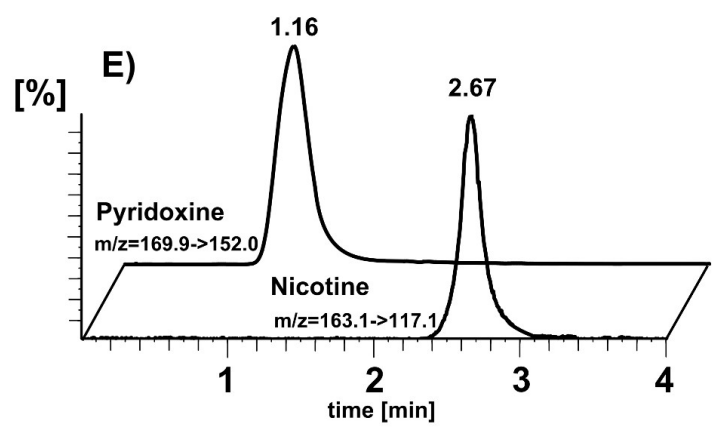
368 Table S1. Optimal parameters for the monitored ion transitions (MRM) and chosen
369 operational parameters of ion source.

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

Zorbax XDB-C8 (150x4.6 mm)



Ascentis Si (150x2.1 mm)



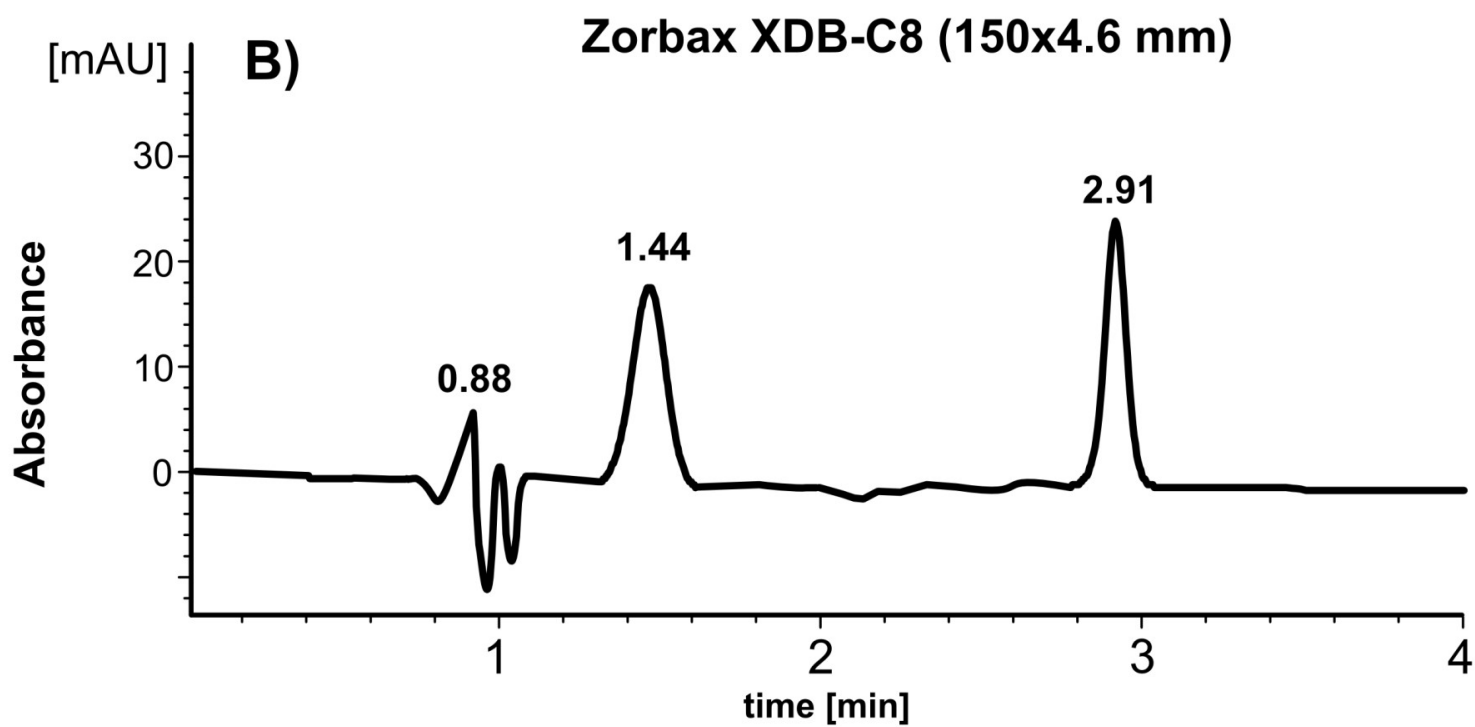
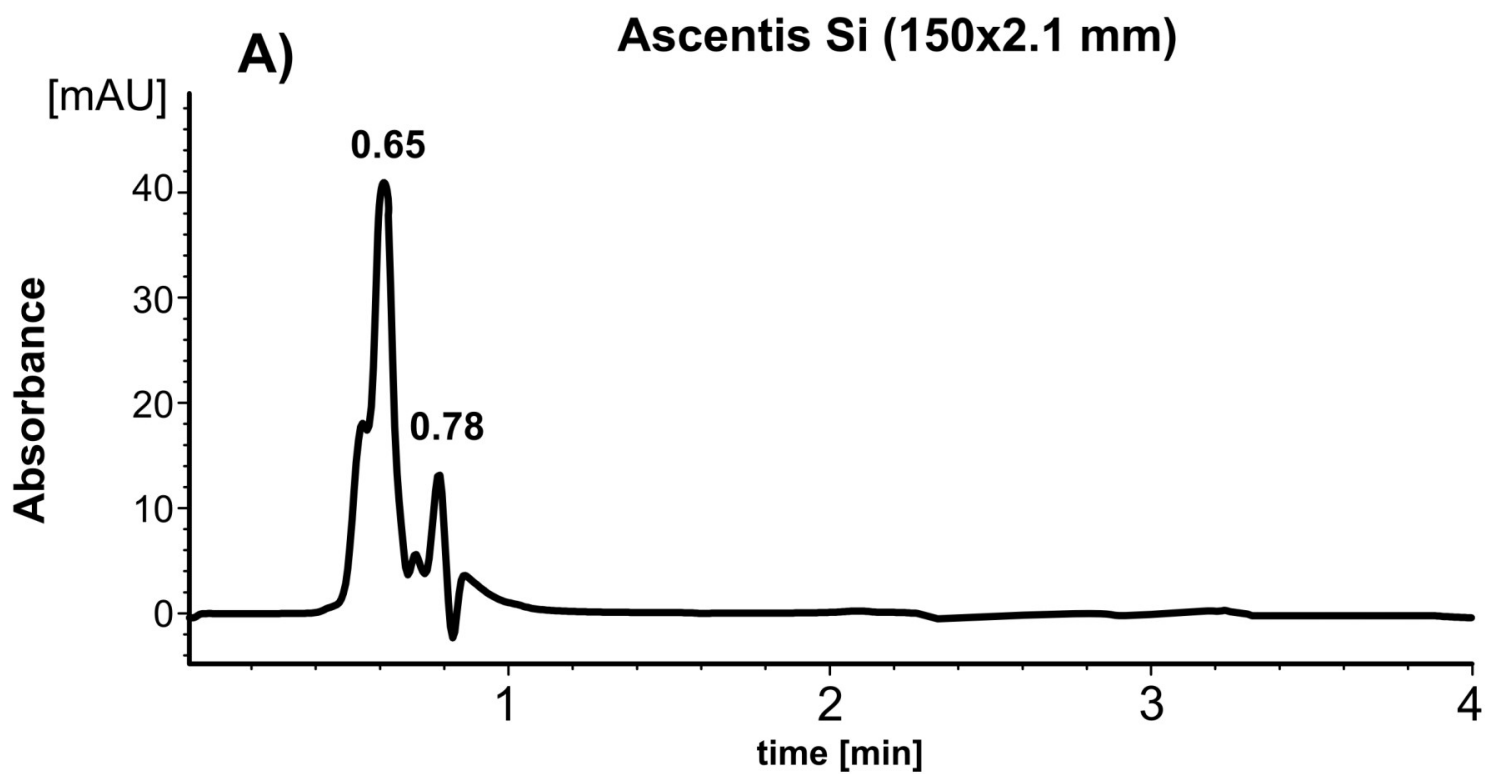


Table 1. Data gathered from the equations of calibration curves for two presented methods.

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 ($\mu\text{g/g}$) \pm SD (n=3)		Absolute difference in concentration among methods ($\mu\text{g/g}$)
		HILIC mode	RPLC mode	
A	Menthol	-	-	-
	Cherry	160.22 \pm 1.81	166.35 \pm 1.17	6.13
	Marlboro	-	-	-
B	Strawberry	-	-	-
	Chocolate	-	-	-
	Orange	-	-	-
	Camel	-	-	-
	Watermelon	-	-	-
	Grape	-	-	-
C	Chocolate	312.32 \pm 1.51	320.95 \pm 2.02	8.63
	Coffee	125.93 \pm 0.92	127.76 \pm 1.14	1.83
	RedBull	41.30 \pm 0.33	39.07 \pm 0.35	2.23
	L&M	-	-	-
	Marlboro	-	-	-
	Camel	-	-	-
	Strawberry	-	-	-
	Cherry	205.42 \pm 1.03	207.33 \pm 1.24	1.91
Apple	74.63 \pm 0.72	71.76 \pm 0.54	2.87	
D	Desert Ship	9.74 \pm 0.16	10.05 \pm 0.15	0.31
	Cherry	338.46 \pm 1.96	332.49 \pm 1.92	5.97
	USA Mix	30.97 \pm 0.40	29.32 \pm 0.52	1.64
	Menthol	5.82 \pm 0.12	5.30 \pm 0.07	0.52
	Fruit Mix	-	-	-
E	Cuban Tobacco	26.94 \pm 0.78	28.56 \pm 0.16	1.62
	Café Latte	14.90 \pm 0.20	14.01 \pm 0.07	0.90
	English Black Tea	-	-	-
	Energy Drink	-	-	-
F	Strong Mint	-	-	-
	Tiramisu	19.90 \pm 0.35	18.32 \pm 0.37	1.57
	Cherry	6.15 \pm 0.14	6.21 \pm 0.20	0.06
G	Coffee	5.11 \pm 0.08	5.55 \pm 0.73	0.44
	Watermelon	318.28 \pm 0.97	315.58 \pm 1.55	2.70
	Banana	151.33 \pm 1.66	148.89 \pm 1.16	2.44
	Vanilla	84.19 \pm 1.55	88.48 \pm 0.95	4.28
	Camel	23.26 \pm 0.33	22.03 \pm 0.22	1.23
	Marlboro	20.37 \pm 0.29	22.56 \pm 1.04	2.19
	RedBull	53.47 \pm 0.17	47.15 \pm 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		8	29
	163.1→117.1			20	37
Acetaminophen	<u>152.1→110.1</u>	61	10	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

