http://dx.doi.org/10.5012/bkcs.2013.34.9.2691

Measurement of Aldehydes in Replacement Liquids of Electronic Cigarettes by Headspace Gas Chromatography-mass Spectrometry

Hyun-Hee Lim and Ho-Sang Shin[†,*]

Department of Environmental Science, [†]Department of Environmental Education, Kongju National University, Kongju 314-701, Korea. [*]E-mail: hshin@kongju.ac.kr
Received May 12, 2013, Accepted June 19, 2013

The electronic cigarette (E-cigarette) is a battery-powered device that aerosolizes nicotine so that it is readily delivered into the respiratory tract. The analytical data regarding the substances present in E-cigarettes are very limited. The aim of this study was to measure the concentration of aldehydes-formaldehyde (FA), acetaldehyde (AA) and, acrolein (AL)-in 225 replacement liquid brands from 17 E-cigarette shops sold in the Republic of Korea by headspace solid-phase micro extraction and gas chromatography-mass spectrometry (HS-SPME GC-MS). The concentration range of FA and AA was 0.02-10.09 mg/L (mean = 2.16 mg/L, detected in 207 of 225 samples) and 0.10-15.63 mg/L (mean = 4.98 mg/L, detected in all samples), respectively. AL was not detected in any of 225 replacement liquids. FA and AA were originally present in almost all replacement liquids of electronic cigarettes.

Key Words : Aldehydes, Electronic cigarette replacement liquid, Headspace solid-phase micro extraction, Gas chromatography-mass spectrometry

Introduction

The electronic cigarette (E-cigarette) is a battery powered device that aerosolizes nicotine so that it is readily delivered into the respiratory tract. E-cigarettes consist of a plastic or stainless steel tube, an electronic heating coil, a liquid cartridge, a lithium battery, and an atomization chamber.[1] The temperature at the center of the heating coil was > 350 °C.[2] The device is designed to be refilled with replacement liquids containing propylene glycol (or glycerol), nicotine and the desired flavor blend, which produce the aromas and flavors of tobacco, chocolate, mint, fruit, and coffee.[1,3,4] E-cigarette manufacturers have claimed that their products are safe alternatives to tobacco and contain little more than water vapor, nicotine and propylene glycol, which is used to create artificial smoke in theatrical productions. However, many consumers doubt the safety of the products. Moreover, the analytical data regarding the substances present in E-cigarettes are very limited at this point.

Several studies reported the presence of hazardous compounds in the liquids or in the vapors of E-cigarettes.[5-9] Laugesen reported that acetaldehyde, acetone, ethanol, formaldehyde (FA), cresol, xylene, propylene and styrene were present in the vapors of E-cigarettes,[5] and Hadwiger *et al.* detected the presence of amino-tadalafil and rimonabant in the liquids of E-cigarettes.[6] US FDA detected diethylene glycol in 1 of 18 cartridges tested.[7] NJOY E-cigarette company identified propylene glycol, glycerin, nicotine, acetaldehyde (AA), 1-methoxy-2-propanol, 1-hydroxy-2-propanone, acetic acid, 1-menthone, 2,3-butanediol, menthol, carvone, maple lactone, benzyl alcohol, 2-methyl-2-pentenoic acid, ethyl mantel, ethyl cinnamate, myosamine, benzoic acid, 2,3-bipyridine, cotinine, hexadecanoic acid, and 1,1-oxybis-2-propanol in the vapors of E-cigarettes.[8] Kazushi *et al.* reported a study on the identification of carbonyl compounds in the vapors generated from E-cigarettes.[9] We have also reported the concentrations of tobacco-specific nitrosamines in replacement liquids of E-cigarettes.[10]

Carbonyls are among the compounds present at high levels in the E-cigarettes. Long-term exposure to carbonyl compounds, such as FA and AA, increases the cancer risk.[11-13] Both the International Agency for Research on Cancer[11] and the US Environmental Protection Agency (US EPA)[12] classified FA as "carcinogenic to humans" in Group 1. US EPA set the acceptable daily intake (ADI) of FA as 0.2 mg/kg body weight and warned of the potential adverse health effects in the cases where the intake level FA exceeded the ADI. AA is also toxic, an irritant, and a probable carcinogen.[13] Acrolein (AL) is very toxic through all routes of administration and may cause respiratory and ocular irritation. On the basis of the findings of *in vitro* and animal experiments, the Deutsche Forschungsgemeinschaft (DFG) classified AL as a group 3B carcinogen.[14] Moreover, Feng *et al.* have reported a relationship between AL in cigarette smoke and an increased risk of lung cancer.[15]

Therefore, accurate measurements of the concentration of carbonyl compounds in E-cigarettes are important both for determining the formation mechanism of carbonyls and for evaluating their effect on human health. Moreover, because of the emerging evidence of cancer risk from carbonyls, an efficient technique for measurement of their concentration is needed.

Recently, we developed and validated an analytical method for detecting the carbonyl compounds in water by headspace solid-phase micro extraction and gas chromatography-mass

spectrometry (HS-SPME GC–MS) after derivatization with 2,2,2-trifluoroethylhydrazine (2,2,2-TFEH).[16,17] Although the HS-SPME GC-MS method was successful in detecting the carbonyl compounds in water or foods, was not effective in the case of replacement liquids of E-cigarettes that have propylene glycol as a major constituent. Because the analytes are well dissolved in the glycol, their extraction from the matrix and their detection need to be studied.

In this study, we developed a simultaneous analytical method of AL, FA and AA in the replacement liquids of the E-cigarettes by headspace solid-phase micro extraction and gas chromatography-mass spectrometry (HS-SPME GC-MS) based on the modification of the method developed in our previous study,[16,17] and for the quantification of AL, FA and AA in the 225 replacement liquid brands from 17 E-cigarette manufacturers in the Republic of Korea.

Experimental

Materials. All organic solvents used were of HPLC grade. Sodium chloride, 2,2,2-TFEH (70 wt % solution in water), AL, FA, AA and acetone-d_6 (AC-d_6) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Commercially available SPME fibers, 65 μm-polydimethylsiloxane-divinylbenzene (PDMS-DVB), were purchased from Supelco (Bellefonte, PA, USA). According to the manufacturer' instructions, the fibers were conditioned in an extra split/splitless port with helium carrier gas prior to each adsorption.

Replacement Liquids for Electronic Cigarettes. A total of 225 replacement liquids were purchased from 17 E-cigarette shops of various regions of the Republic of Korea during the period July, 2011 to June 2012. These shops directly imported the replacement liquids China or USA. All samples were analyzed within 2 months of purchase after storage in a refrigerator at 4 °C.

Headspace Solid Phase Microextraction Procedures. The sample (extraction and derivatization) was prepared in 10-mL headspace vials with carried-lined screw caps. To 0.5 mL of the E-cigarette liquid sample or sample-spiked propylene glycol, 50 μL of AC-d_6 (1.0 mg/L), 4.5 mL Milli-Q water, 1.6 g of NaCl and 2.0 mL of TFEH solution (2.0%) were added. The pH was adjusted to 9.0 with the buffer (sodium bicarbonate:potassium carbonate = 3:1, *w/w*). The derivatization/adsorption was carried out in a headspace vial with continuous shaking, and the derivatives were desorbed in the injection port for successive analysis and then passed onto the column for analysis. Derivatization was performed for an adsorption time of 40 min at a temperature of 50 °C. Calibration curves for aldehydes were obtained by derivatization after adding 0.005, 0.01, 0.05, 0.25, 0.5, 1.25, 2.5, 5.0, 12.5 and 25.0 μg of the analyte standard solutions (0.1-100.0 mg/L) and 50 μL of AC-d_6 (1.0 mg/L; internal standard) in 0.5 mL of propylene glycol. The corresponding concentrations of the standards were 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, and 50.0 mg/L. The ions selected by SIM were *m/z* 69, 83 and 152 for AL-TFEH; *m/z* 43, 57, and 126 for FA-TFEH; *m/z* 42, 71, and 140 for AA-TFEH and *m/z* 62, 91, and 160 for AC-d_6-TFEH (internal standard). The ratio of the peak area of the standard to that of the internal standard was used to quantify the analytes.

Apparatus. Under the following instrumental condition, AL, FA and AA were identified and to quantified. All mass spectra were obtained with an Agilent 7890/5975B instrument. The ion source was operated in the electron ionization (EI; 70 eV) mode. Full-scan mass spectra (*m/z* 40-800) were recorded for analyte identification. A HP-INNOWax capillary column (30 m × 0.25 mm I.D. × 0.25 μm film thickness) was used for the separation of the aldehyde derivatives. The samples were injected in the splitless mode. The flow rate of helium as carrier gas was 1.0 mL/min, and the injector temperature was 240 °C. The oven temperature programs were as follows: initial temperature of 40 °C (held for 5 min), and then increased to the final temperature of 205 °C at 15 °C/min.

Results and Discussion

Chromatography and Validation. According to the manufacturer, propylene glycol makes up 89-90% of the liquid in the nicotine cartridge that generates the mist and vapor in the E-cigarette smoke.[5] Propylene glycol is a colorless, nearly odorless, clear, viscous liquid and miscible with water, acetone, diethyl ether, and chloroform. Therefore, selecting a liquid-liquid extraction method as a pretreatment method for the analytes from the replacement liquids of the E-cigarettes is problematic.

We recently reported that carbonyl compounds reacted with TFEH to form volatile hydrazones, which could be used for the determination of carbonyl compounds in water or fermented foods by HS SPME GC-MS.[16,17] In the first reported study on the detection of FA in fermented foods,[16] the optimum derivatization conditions were as follows: 4 mg TFEH, the reaction solution pH 4.0, reaction temperature 40 °C, and reaction time 30 min. On the other hand, in the second reported study on the detection of AL in water,[17] the optimum derivatization conditions were: 20 mg TFEH, the reaction solution pH 10, reaction temperature 60 °C, and reaction time 50 min. CAR–PDMS was used as the optimum fiber in both methods. AA was not identified using the above mentioned method. Therefore, an HS-SPME GC–MS method based on the derivatization using TFEH to detect simultaneously AL, FA, and AA present in the liquids of the E-cigarettes (propylene glycol base) is plausible. The optimum derivatization conditions were analyzed using the sample-spiked propylene glycol or the liquid of the E-cigarette. There were no significant differences in the optimum fiber, extraction/derivatization temperature, heating time, and pH when compared to our previous study[17] on AL detection in water. However, the amount of TFEH amount used was increased amount by 40 mg, which may be due to its increasing consumption in the real sample. The optimum derivatization conditions to detect simultaneously AL, FA, and AA in the liquids of the E-cigarettes were as follows:

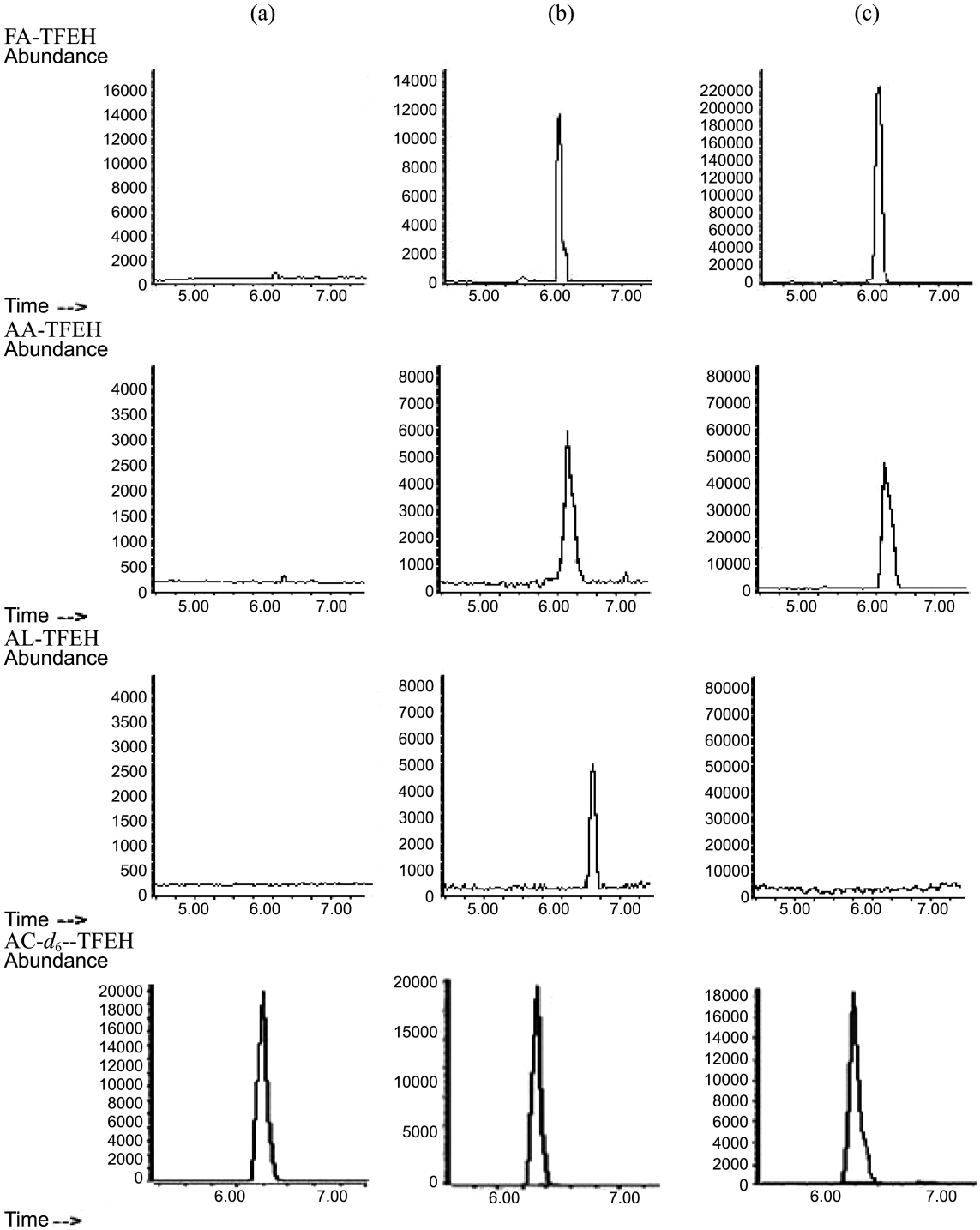

Figure 1. HS-SPME GC-MS extracted ion chromatogram of (a) the blank sample (propylene glycol), (b) standard sample spiked in concentration of standards 0.050 mg/L, (c) the replacement liquid sample of E-cigarette with FA 1.0 mg/L and AA 0.6 mg/L. AC-d_6 as internal standard was spiked at 100 μg/L in each sample. (FA-TFEH = 6.006 min; AA-TFEH = 6.245 min; AL-TFEH = 6.598 min; AC-d_6-TFEH = 6.205 min).

TFEH 40 mg, the reaction solution pH 10.0, reaction temperature 60 °C, and reaction time 50 min. These conditions were successfully applied to the analysis of analytes in replacement liquids of E-cigarettes.

Figure 1 shows a HS-SPME/GC–MS chromatogram after the derivatization of analytes. A semipolar stationary phase (INNOWax) was used for the GC separation of the derivatives. Each derivative showed a sharp peak, and the compound was quantified as the integration of the peak area.

The analytes were confirmed by the comparison of EI mass spectra of the extract obtained from the samples and the authentic standards after the derivatization. The analytes were identified based on the retention time and MS spectrum.

Table 1. Calibration curve, detection limit, precision and accuracy of analytes in a control sample

Analytes	Calibration curve (y = ax + b)				Detection limit (mg/L)		Precision and Accuracy (%)		
	Linear range (mg/L)	a	b	r^2	LOD	LOQ	Spiked conc. (mg/L)	Accuracy	Precision
FA	0.01-50.0	1.786	0.087	0.9995	0.0031	0.0098	1.0	94.6	5.41
							5.0	96.0	1.36
							10.0	96.8	3.76
AA	0.01-50.0	0.5038	0.027	0.9998	0.0035	0.0113	1.0	101.5	6.51
							5.0	100.4	4.94
							10.0	95.2	1.55
AL	0.01-50.0	0.0555	-0.053	0.9998	0.0063	0.0190	1.0	99.7	6.24
							5.0	98.0	5.21
							10.0	100.0	4.73

Validation of the method was performed. Chromatograms of the propylene glycol samples spiked with AL, FA, and AA standards and the original sample are shown in Figure 1. The peaks of AL–TFEH, FA–TFEH, and AA–TFEH were symmetrical. The retention times of FA–TFEH, AA–TFEH, AL–TFEH, and AC-d_6–TFEH were 6.006, 6.245, 6.598, and 6.205 min, respectively. In original samples, no interfering peak was observed in the chromatograms near the retention times of analytes due to discriminatory nature of SPME.

A typical standard curve was obtained by computing a regression line of the ratios of the peak area of AL–TFEH, FA–TFEH, and AA–TFEH to that of AC-d_6–TFEH as a function of concentration using a least-squares fit. An investigation of this curve demonstrated a linear relationship with correlation coefficients that were consistently higher than 0.998 (see Table 1).

The limit of detection (LOD) and limit of quantitation (LOQ) of the analytes in the coupled derivatization and extraction method were calculated to be 3.14 and 10 times the standard deviation obtained from the data of 7 replicate measurements.[17] The test was performed using the control propylene glycol spiked at a concentration of 5.0 μg/L, in which analytes were not detected.[18] The LOD and LOQ were 6.3 and 19.0 μg/L for AL, 3.1 and 9.8 μg/L for FA, and 3.5 and 11.3 μg L for AA, respectively. The precision and accuracy of the assay were acceptable (Table 1). The relative standard deviation was less than 6% for five independent determinations at 1, 5 and 10 mg/L (Table 1).

The developed method was used to identify and quantify AL, FA, and AA in the 225 replacement liquids of E-cigarettes. FA and AA were detected in the concentration range of 0.02-10.09 mg/L (mean concentration = 2.16 mg/L, detected in 207 of 225 samples) and 0.10-15.63 mg/L (mean concentration = 4.98 mg/L, detected in all samples), respectively, as shown in Table 2 and Figure 2. However, AL was not detected in any of the samples tested.

Table 2. Analytical results of aldehydes in the liquid samples of E-cigarettes purchased from Korea shop (mg/L)

Blend	Sample Nr	FA			AA		
		Detected Conc Range	Mean	Detection Samp Nr	Detected Conc Range	Mean	Detection Samp Nr
A	5	0.31-3.77	1.89	5	3.66-11.81	8.06	5
B	15	0.03-4.41	0.96	13	0.25-6.42	1.87	15
C	5	0.28-2.17	0.69	4	0.53-1.31	0.98	5
D	11	0.02-2.12	0.48	10	0.36-4.74	1.41	11
E	3	0.49	0.16	1	0.31-1.16	0.68	3
F	25	0.04-2.37	0.84	23	0.29-8.30	2.92	25
G	7	0.38-1.05	0.58	7	0.42-2.81	2.03	7
H	20	0.06-2.66	0.53	16	0.10-3.80	1.51	20
I	32	0.10-6.56	0.91	32	0.12-9.93	2.08	32
J	21	0.20-9.67	2.32	21	0.16-8.21	2.24	21
K	19	0.02-2.19	0.58	19	0.69-5.82	2.21	19
L	19	0.05-1.11	0.41	14	0.19-1.75	0.88	19
M	4	0.46-10.09	3.68	4	0.95-11.98	4.42	4
N	19	0.19-4.98	1.07	19	0.92-6.47	3.51	19
O	6	0.36-7.13	2.88	6	1.30-15.63	8.05	6
P	5	0.24-7.14	1.96	5	1.33-13.31	4.26	5
Q	2	0.58-7.82	4.20	2	0.34-7.26	3.80	2
R	7	0.02-0.31	0.15	6	0.64-3.08	1.33	7

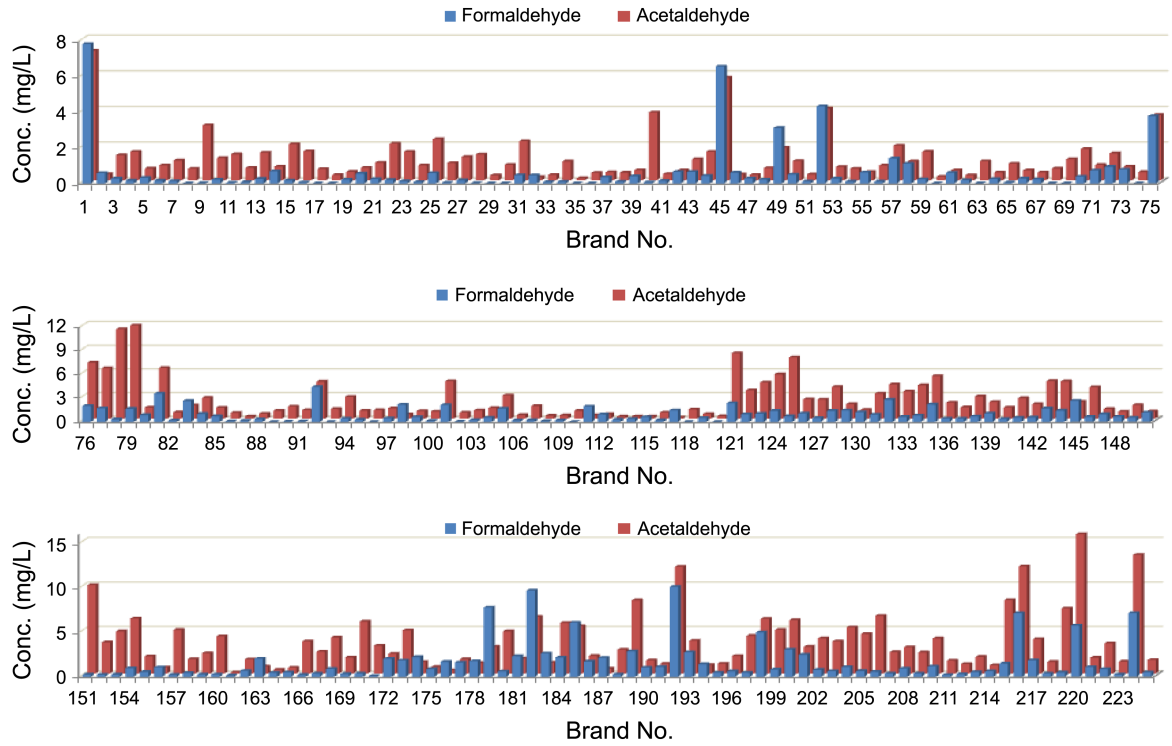

Figure 2. Analytical results of aldehydes in the liquid samples of E-cigarettes purchased from 17 E-cigarette shops of various regions of Republic of Korea (mg/L).

The correlation between FA and AA in the same sample was analyzed. FA and AA correlated relatively well with one another ($r^2 = 0.351$, $P = 0.001$), suggesting their similarity in properties. These compounds can exist in raw materials or can be produced naturally during preparation procedures. They can be controlled to the minimum concentration, if a check is undertaken in many raw materials and processing procedure

Conclusion

A study on detecting the presence of AL, FA, and AA in the replacement liquids of E-cigarettes is necessary for evaluating their effect on human health. FA and AA were identified and quantified in 225 replacement liquids obtained from 17 shops, by GC–MS. FA and AA were detected in the concentration range of 0.02-10.09 mg/L (mean concentration = 2.16 mg/L, detected in 205 of 225 samples) and 0.10-15.63 mg/L (mean concentration = 4.98 mg/L, detected in all samples). US EPA set the ADI of FA as 0.2 mg/kg body weight and warned of potential adverse effects on health in the cases where the intake level FA exceeded the ADI. This amount corresponds to 10.09 mg of FA per 1 L of the replacement liquid, which cannot be achieved by normal consumption E-cigarettes

Although the amount detected in replacement liquids of E-cigarettes is relative low, they should be controlled to the lowest possible concentrations in raw materials because of the absence of a system to verify their formation in a heating coil at > 350 °C.

Manufacturers of these replacement liquids claimed that it was not known whether AA detected from the mist of E-cigarettes is an artifact, as AA could have been formed due to heating of the ethyl alcohol during GC–MS measurement.[5] Based on this study, we conclude that FA and AA are originally present in almost all replacement liquids of E-cigarettes.

Acknowledgments. This study was funded in part by Ministry of Health and Welfare (research number: 68-C6-0040).

References

1. Etter, J. F.; Bullen, C. *Addiction* **2011**, *106*, 2017.
2. Schripp, T.; Markewitz, D.; Uhde, E.; Salthammer, T. *Indoor Air* **2013**, *23*, 25.
3. American-Legacy-Foundation. Electronic Cigarette ('ecigarette') Fact Sheet. American Legacy Foundation, 2009. Available at: http://www.legacyforhealth.org/PDFPublications/ECIGARETTE_0909_temp.pdf, http://www. webcitation.org/5zUApidHT), Accessed October 10, 2012.
4. Westenberger, B. J. *U.S. Food and Drug Administration evaluation of E-cigarettes.* Center for Drug Evaluation and Research, Division of Pharmaceutical Analysis. Rockville, MD: U.S. Food and Drug Administration, 2009. http://www.fda.gov/downloads/Drugs/ScienceResearch/UCM173250.pdf. Accessed October 10, 2012.
5. Laugesen, M. *Safety Report on the Ruyan E-Cigarette Cartridge and Inhaled Aerosol*. Christchurch, New Zealand: Health New Zealand Ltd; 2008.
6. Hadwiger, M. E.; Trehy, M. L.; Moore, W.; Ye, T.; Algire, J.; Westenberger, B. *J. Chromatogr. A* **2010**, *1217*, 7547.

7. Food and Drug Administration (FDA). Summary of Results: Laboratory Analysis of Electronic Cigarettes Conducted By FDA, US: Food and Drug Administration (FDA), July 2009, http://www.fda.gov/NewsEvents/PublicHealth Focus/ucm173146.htm (Accessed October 10, 2012.; archived at http://www.webcitation.org.5zUAMMHkS).
8. Park, M. *Exponent*. 2009, NJOY E-cigarette Health Risk Assessment. CA: Exponent, http://truthaboutecigs.com/science/5.php, Accessed February 10, 2012.
9. Ohta, K.; Uchiyama, S.; Inaba, Y.; Nakagome, H.; Kunugita, N.; *Bunseki Kagaku* **2011**, *60*, 791.
10. Kim, H. J.; Shin, H. S. *J. Chromatogr. A* **2013**, *1291*, 48.
11. International Agency for Research on Cancer (IARC). Monographs on the evaluation of carcinogenic risks to humans, Vol 88, methanal, 2-butoxyethanol and 1-*tert*-buxy-2-propanol. Lyon, France, 2006.
12. US Environmental Protection Agency, Integrated Risk Information System (IRIS) on Methanal, National Center for Environmental Assessment, Office of Research and Development, Washington, DC. 1999.
13. EPA 749-F-94-003a, Chemical summary for ethanal prepared by office of pollution prevention and toxics. U.S. Environmental Protection Agency, 1994.
14. DFG, List of MAK- and BAT-values. Wiley-VCH, Weinheim, 2006.
15. Feng, Z.; Hu, W.; Hu, Y.; Tang, M. S. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15404.
16. Kim, H. J.; Shin, H. S. *J. Sep. Sci.* **2011**, *34*, 693.
17. Shin, H. S.; Lim, H. H. *Int. J. Food Sci. Tech.* **2012**, *47*, 350.
18. USEPA Method SW 846, Quality Control, 1992; p 26 (Chapter 1).