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Simultaneous Determination of Furan and Vinyl Acetate in Vapor Phase of Mainstream Cigarette Smoke by GC-MS

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ABSTRACT

A simple and sensitive method for simultaneous determination of furan and vinyl acetate (VA) in vapor phase of mainstream cigarette smoke with cold trap and gas chromatography-mass spectrometry (GC-MS) was developed. A Cambridge filter pad (CFP) was placed in front of the impingers of smoking machine to remove the particle phase from cigarette smoke. Furan and VA in vapor phase of mainstream cigarette smoke were collected in two impingers connected in series by filled with methanol at –78°C. The solutions were added with deuterium-labeled furan-d_4 and VA-d_6 as internal standards and analyzed by GC-MS. The results showed that the calibration curves for furan and VA were linear ($r^2 > 0.9995$) over the studied concentration range. The intra- and inter-day precision values for furan and VA were <7.07% and <9.62%, respectively. The extraction recoveries of furan and VA were in the range of 94.5-97.7% and 92.3-94.9%, respectively. Moreover, the limits of detection for furan and VA were 0.028 µg mL^{-1} and 1.3 ng mL^{-1}, respectively. The validated method has been successfully applied to determine the emissions of furan and VA in the vapor phase of mainstream cigarette smoke under International Organization for Standardization (ISO) and Canadian Intense (CI) smoking regimen.

Key words: vapor phase, mainstream cigarette smoke, furan, vinyl acetate, cold trap, gas chromatography-mass spectrometry.

INTRODUCTION

Both furan and vinyl acetate (VA) were classified as possibly carcinogenic to the human (group 2B) by the International Agency for Research on Cancer (IARC) (Smith et al. 2000). They were also considered as harmful or potentially harmful compounds (HPHCs) in tobacco products and tobacco smoke by US Food and Drug Administration (FDA) (Oldham et al. 2014). Specially, furan was included in Hoffmann List of tobacco carcinogens (Hoffmann et al. 2001). The formation of furan in cigarette smoke was degradation of amino acids and/or reducing sugars, and oxidation of ascorbic acid and poly-unsaturated acids, and recombination

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by Maillard reaction during combustion (Baltes and Bochmann 1987, Becalski and Seaman 2005, Locas and Yaylayan 2004, Yaylayan 2006). The VA monomer was reported to be primarily used in the production of polyvinyl acetate (PVA), which was a thermoplastic polymer commonly used as the sideseam adhesive in cigarette paper in the tobacco industry (Levy and Hinojosa 1992). Hence, the formation of VA in cigarette smoke was probably from PVA adhesives, and some sugars in the tobacco were also involved (Coggins et al. 2013).

Up to now, the major trapping methods of furan in vapor phase of mainstream cigarette smoke were direct injection method (Hatzinikolaou et al. 2006, Grob et al. 1965, Vickroy et al. 1976) and gas bag trapping method (Sampson et al. 2014). Grob et al. (1965) reported on determination of furan in cigarette smoke directly through a gas sampling valve by gas chromatography (GC) coupled with flame ionization detector (FID) without sample collection. Vickroy et al. (1976) presented the gas bag trapping method, that is, fresh smoke was collected with a gas bag and the furan was determined by GC-FID. Hatzinikolaou et al. (2006) developed a method for the analysis of furan in the vapor phase of cigarette smoke by a GC coupling with diode array photometric spectral detection in the ultraviolet region (UV-DAD). However, all of above three reports showed poor separation results and had adverse effect on the accuracy of quantification furan. Adam et al. (2006) developed a novel method for the direct determination of furan in fresh vapor phase of mainstream cigarette smoke by a GC with single photon ionisation time-of-flight mass spectrometry (GC-SPI-TOFMS) without sample collection prior to analysis. Eschner et al. (2011) developed a technique that furan in the vapor phase of mainstream cigarette smoke was analyzed by fast GC coupled to single photon ionisation time-of-flight mass spectrometry. Furthermore, quantification of furan was actualized for two different smoking regimes (International Organization for Standardization) ISO and (Canadian Intense) CI machine smoking regimens). However, soft photoionization and puff-by-puff analysis were adopted in their reports, which made these methods complicated and difficult to operate. Sampson et al. (2014) reported a method that the vapor phase of mainstream cigarette smoke was collected by a gas bag, and furan was detected by solidphase microextraction gas chromatography - mass spectrometry (SPME-GC-MS). And the emissions of furan in the research cigarettes (1R5F and 3R4F) under ISO and CI smoking regimens were reported. However, the SPME technique made the method difficult to operate and time-consuming (Sampson et al. 2014).

The major trapping methods of VA in vapor phase of mainstream cigarette smoke are direct injection (Norman et al. 1968), cold trap (Diekmann et al. 2002) and gas bag trapping combining with solid-phase microextraction (SPME) (Sampson et al. 2014). The VA content in cigarette smoke could be determined by GC-MS. Norman et al. (1968) reported a method that the VA form mainstream cigarette smoke was quantitatively analyzed by GC-FID, but no VA was detected in the vapor phase of mainstream cigarette smoke under ISO smoking conditions because of its poor sensitivity and selectivity. Diekmann et al. (2002) reported a method that the VA in vapor phase of mainstream cigarette smoke was trapped in three impings connected in series at -78 °C filled with acetone, the impinger solutions were fortified with VA-d_6 as internal standard and analyzed by GC-MS. This method was proved to have a good linearity, excellent quantitative limit and perfect precision. However, three impinges connected in series and acetone were used in their reports, which made the method tedious and environmentally unfriendly. Sampson et al. (2014) reported a method that the VA of the vapor phase from cigarette smoke was detected by GC-MS combining with solidphase microextraction technique. Similarly, the application of solidphase microextraction made the method difficult to operate and time-consuming.

In this study, A Cambridge filter pad (CFP) was placed in front of the impingers to remove the particle phase from the smoke. Then, furan and VA in vapor phase of mainstream cigarette smoke were collected in 2 impingers connected in series at –78°C filled with methanol. The impinger solutions were spiked with deuterium-labeled internal standards (i.e. furan-d_4 and VA-d_6) and analyzed by GC-MS. Moreover, furan and VA in vapor phase of mainstream cigarette smoke under ISO and CI machine smoking regimens were detected in this study.

MATERIALS AND METHODS

CHEMICALS AND CIGARETTES

Furan and VA were purchased from Ultra Scientific Inc. (North Kingstow, RI). Furan-d_4 and VA-d_6 were purchased from Cambridge Isotope Laboratoris, Inc. (Andover, MA), and methanol (HPLC grade) was purchased from Dikama Corporation (Richmond Hill, NY). The reference cigarettes 1R5F and 3R4F were purchased from the University of Kentucky (Lexington, KY) and 15 commercial cigarettes were purchased from Chinese market with packet labeled tar ranged from 3 to 11 mg cig^{-1}.

PREPAREATION OF STOCK SOLUTIONS

The stock solutions of furan and VA were prepared in methanol at the concentrations of 1000 and 10 µg mL^{-1}, respectively. The stock solutions of furan-d_4 and VA-d_6 were prepared in methanol at concentrations of 2000 and 20 µg mL^{-1}, respectively. The standard solutions of furan and VA were prepared by diluting with methanol at the concentration range of 0.2-50.0 µg mL^{-1}and 0.02-0.5µg mL^{-1}, respectively. The concentrations of furan-d_4 and VA-d_6 in the standard solutions were 10 mL^{-1}and 0.1µg mL^{-1}, respectively.

SAMPLES COLLECTION AND GC-MS ANALYSIS

Before smoking, the cigarettes were conditioned with ISO 3402:1999 recommended conditions of 22 ± 1 ºC and 60 ± 2% relative humidity for 48 hours. The mainstream cigarette smoke was generated on an automatic, 20-port RM 20H rotary smoke machine (H. Borgwaldt, Hamburg, Germany). Ten cigarettes were smoked following the ISO 3308 smoking regimen (35mL puff volumn with 2 s durations every 60 s) and five cigarettes were smoked following the Canadian intense T-115 smoking regimen (55mL puff volumn with 2 s durations every 30 s, 100% ventilation blocked), respectively. The cigarette mainstream smoke collection was adapted from a CORESTA recommended method (CORESTA 2013). A Cambridge filter pad (CFP) was placed in front of the impingers to remove the particle phase from cigarette smoke. Furan and VA in vapor phase of mainstream cigarette smoke were collected in two impingers connected in series filled with 15 mL methanol per impinger. The impingers were cooled to -78 ºC with a mixture isopropanol and dry ice prior to smoking. After smoking, each impinger solution was equivalently spiked with 75 µL furan-d_4 and 75 µL VA-d_6 stock solution and stirred vigorously. Then two impinger solutions were combined and kept in the Dewar flasks for further use.

An aliquot of the impinger solutions was used to analyze the contents of furan and VA in cigarette smoke by GC-MS (7890/5975C, Agilent Technologies, U.S.) fitted with a Supelco VOCOL (60 m × 0.32 mm i.d., and 1.8µm film thickness) capillary column. The split mode was a ratio of 10:1 and each injection volume was 2 µL. The temperature and pressure of the inlet were 180°C and 8.7 psi, respectively. Helium carrier gas was maintained at 1.5 mL min^{-1}, which corresponded to a linear velocity of about 31.4 cm s^{-1}. The GC oven was started at 40°C and held for 6 min, then heated to 220°C at 20°C min^{-1} and held for 6 min. The MS was operated in EI (70eV) mode. The MS transfer line temperature was set at 230°C, and an ion source temperature and an MS quadrupole were maintained at 230°C and 150°C, respectively. Enhanced ChemStation (MSD ChemStation. F.01.01.2317) of Agilent Technologies was applied to determine chromatogram peak areas automatically (Choe et al. 2012).

RESULTS AND DISCUSSION

COLUMN SELECTION AND TRAPING PREPARATION

Initially, three different GC columns were compared, including Agilent J&W DB-624 (60 m × 0.25 mm i.d., and 1.4 µm film thickness) column, Agilent J&W DB-624 (60 m × 0.32 mm i.d., and 1.8 µm film thickness) and Supelco VOCOL (60 m × 0.32 mm i.d., and 1.8µm film thickness). The peaks of furan and VA were overlapped by other peaks when both Agilent J&W columns were employed. However, when Supelco VOCOL column was used, furan and VA could be separated sufficiently, as shown in Fig. 1.

The trapping efficiency of furan and VA in vapor phase of mainstream cigarette smoke was evaluated under ISO and CI smoking regimens. Three impingers containing 15 mL methanol per impinger were connected in series after CFP. And the vapor phase of mainstream cigarette smoke was collected by the three impingers. Two types of cigarettes (Virginia type cigarette and blended type cigarette) were examined and the results were shown in Table I. It showed that the furan and VA were found in both the first and second impinger solution, while they were not observed in the third impinger solution. Therefore only two impingers were enough to trap furan and VA in vapor phase of mainstream cigarette smoke completely.

METHOD VALIDATION

Linearity, limit of detection (LOD) and limit of quantitation (LOQ)

The calibration curves were produced for furan and VA with a concentration range of 0.2-50.0 µg ml^{-1} and 0.02- 0.5µg ml^{-1}, respectively. The linear regression equation of furan was Y=0.993X + 0.030 and that of VA was Y= 1.178X + 0.049. The correlation coefficient (r^2) for furan was 0.9997 and that for VA was 0.9993. The method exhibited excellent linear response in the selected concentration range.

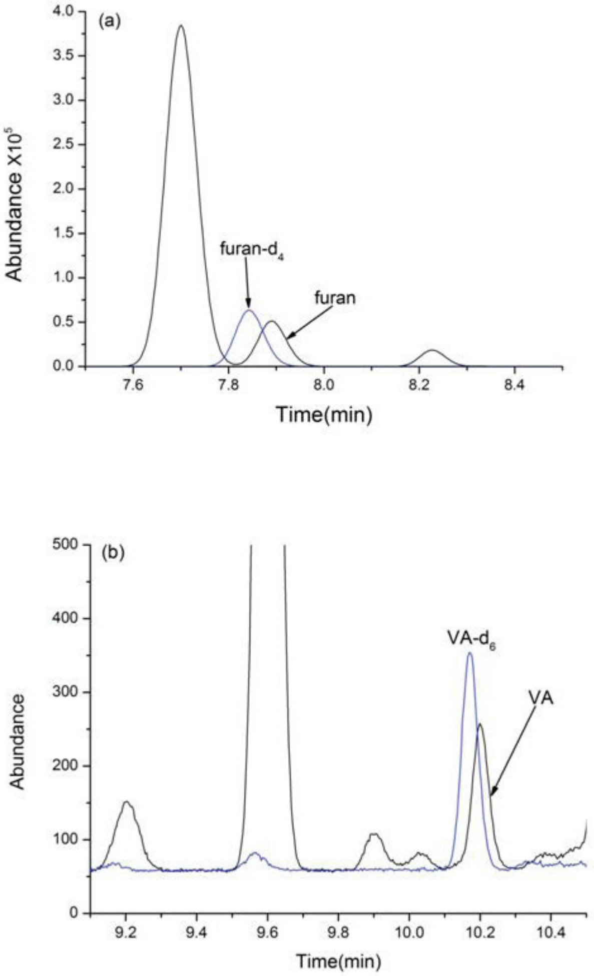

Figure 1- GC-MS chromatograms of furan (m/z = 68), furan-d_4 (m/z = 72) (a) and VA(m/z = 86), VA-d_6 (m/z = 92) (b) in vapor phase of mainstream cigarette smoke sample.

TABLE I
The trapping efficiency of furan and VA in vapor phase of mainstream cigarette smoke under ISO and CI smoking regimens.

cigarette	furan						VA					
	ISO smoking regimen			CI smoking regimen			ISO smoking regimen			CI smoking regimen		
	1st (%)	2nd (%)	3rd (%)	1st (%)	2nd (%)	3rd (%)	1st (%)	2nd (%)	3rd (%)	1st (%)	2nd (%)	3rd (%)
Virginia type	90.6	9.4	0.0	87.1	12.9	0.0	95.0	5.0	0.0	93.4	6.6	0.0
Blended type	92.7	7.3	0.0	90.2	9.8	0.0	96.9	3.1	0.0	94.9	5.1	0.0

TABLE II
Intra-day, inter-day precision, and recovery of furan and VA.

Analyte	Intra-day precision		Inter-day precision		Recovery	
	Measurement ± SD (μg mL^{-1})	RSD(%) (n=6)	Measurement ± SD (μg mL^{-1})	RSD(%) (n=6)	Tested concentration (μg mL^{-1})	Recovery (%) (±SD, n=3)
furan	7.94±0.39	4.96	7.81±0.55	7.07	5.0	94.5±4.7
					10.0	96.2±4.3
					20.0	97.7±2.9
VA	0.0978±0.0067	6.86	0.1008±0.0097	9.62	0.05	92.3±6.5
					0.10	93.8±5.0
					0.20	94.9±3.5

The limit of detection (LOD) and limit of quantitation (LOQ) are terms used to describe the smallest concentration of a measure and that can be reliably measured by an analytical procedure. The concentration of 0.028 μg mL^{-1} was found to be the LOD of furan, and LOQ of furan was 0.092 μg mL^{-1}. While the LOD and LOQ of VA were 1.3 ng mL^{-1} and 4.5 ng mL^{-1}, respectively. Under the ISO and CI smoking regimens, the LOQ of furan was observed as 0.28μg cig^{-1} and 0.56 μg cig^{-1}, respectively, while LOQ of VA was 13.5ng cig^{-1} and 27.0 ng cig^{-1}, respectively.

Precision

Intra-day and inter-day precisions were determined using the reference cigarette 3R4F (the names of these cigarettes provided by University of Kentucky for research purposes) which were analyzed six times on the same day and six successive days. As shown in Table II, the intra-day precisions of furan and VA were determined as 4.96% and 7.07%, respectively, and the inter-day precisions of furan and VA were 6.86% and 9.62%, respectively. These results, which fulfilled the acceptable criteria for accuracy and precision, indicate that the method is reliable and reproducible for the quantitative analysis of the analytes in furan and VA contents in vapor phase of mainstream cigarette smoke (Wang et al. 2013).

Recovery

A recovery study was performed by spiking three different concentration levels of standard solutions of furan and VA as shown in Table II. The

TABLE III
Furan and VA yields in vapor phase of mainstream cigarette smoke samples under ISO and CI smoking regimens (n=3).

samples	packet labeled tar (mg)	furan		VA	
		ISO smoking regimen (μg cig^{-1})	CI smoking regimen (μg cig^{-1})	ISO smoking regimen (μg cig^{-1})	CI smoking regimen (μg cig^{-1})
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
1R5F	1.8	7.1±0.3	38.3±1.3	0.096±0.008	0.658±0.042
3R4F	7.9	23.7±1.3	53.5±2.6	0.294±0.020	0.829±0.051
1#	3	11.5±0.6	50.7±2.6	0.156±0.012	0.631±0.039
2#	5	16.3±0.9	48.9±2.3	0.221±0.017	0.642±0.041
3#	6	19.4±1.1	57.3±3.1	0.237±0.014	0.703±0.047
4#	8	20.9±1.0	43.6±1.8	0.312±0.023	0.657±0.037
5#	8	22.8±1.5	47.1±2.0	0.308±0.021	0.624±0.034
6#	10	26.3±1.4	57.6±2.7	0.366±0.027	0.810±0.056
7#	11	27.7±1.7	60.2±3.1	0.412±0.029	0.938±0.076
8#	11	30.3±1.8	70.3±4.9	0.449±0.032	1.014±0.085
9#	5	16.0±0.7	46.8±2.5	0.212±0.013	0.617±0.042
10#	6	17.6±0.7	42.3±2.4	0.247±0.016	0.689±0.044
11#	6	21.5±1.3	54.7±2.9	0.239±0.017	0.654±0.036
12#	7	18.2±0.9	43.6±2.2	0.274±0.023	0.643±0.040
13#	8	21.1±1.6	45.9±1.8	0.286±0.026	0.740±0.054
14#	8	20.3±1.4	42.6±1.5	0.332±0.024	0.796±0.063
15#	11	29.6±2.1	66.5±3.4	0.397±0.031	0.958±0.071

recovery of furan was between 94.5% and 97.7% of the expected value. The recovery of VA was between 92.3% and 94.9%, respectively. These results indicated that the extracts had little or no detectable coeluting endogenous substances that could influence the extraction of the analytes.

Stability

Stability tests were performed by analyzing furan and VA solutions under two different storage conditions (room temperature and -20 °C) for 32 h. The difference was less than 4.9%, indicated that the sample of furan and VA was quantitatively stable under two different storage conditions.

APPLICATION UNDER ISO AND CI MACHINE SMOKING REGIMENTS

To Apply this optimal method, two reference cigarettes (1R5F and 3R4F) and 9 commercial cigarette purchased from China market were used and furan and VA in vapor phase of mainstream cigarette smoke under ISO and CI machine smoking regimens were determined. As shown in Table III, the packet labeled tar yields of these commercial cigarettes ranged from 3 mg cig^{-1} to 11 mg cig^{-1}, and sample 1-7# were Virginia type cigarettes, sample 8-15# were blended type cigarettes. Furan and VA in vapor phase of mainstream cigarette smoke were quantitatively analyzed under ISO and CI smoking regimens. The results found that the emissions of furan and VA in vapor phase of mainstream cigarette smoke ranged of 7.1-30.3 µg cig^{-1} and 0.096- 0.449 µg cig^{-1}, respectively, under ISO smoking regimen. However, the furan and VA in the vapor phase of mainstream cigarette smoke yields ranged of 38.3-70.3 µg cig^{-1} and 0.624-1.014 µg cig^{-1} respectively, under CI smoking condition. It indicated that the furan and VA yields in the vapor of the mainstream cigarette smoke under CI smoking condition were much higher than those under ISO smoking regimen. This trend was more significant in the cigarettes with the packet labeled tar below 6 mg cig^{-1}. The reason might be due to the different smoke parameters (e.g., larger puff volume, shorter duration and 100% vent blocking under CI smoking regimen than those under ISO smoking regimen) and different cigarette design parameters (e.g., cigarette paper and filter ventilation) (Roemer and Carchman 2011). This method was proved to be easy to operate, more sensitive and selective, and could be successfully applied to detect the emissions of furan and VA for the reference cigarettes (1R5F and 3R4F) and commercial cigarettes purchased from China market.

CONCLUSIONS

A simple and sensitive method for simultaneous determination of furan and vinyl acetate (VA) in vapor phase of mainstream cigarette smoke with cold trap and gas chromatography-mass spectrometry (GC-MS) was developed. This method had a good linearity, quantitation limit, precision and perfect recovery. The furan and VA yields in the vapor phase of the mainstream cigarette smoke under CI smoking condition are much higher than those under ISO smoking regimen, This method is applicable to the determination of fuan and VA in the vapor phase of mainstream cigarette smoke under ISO and CI smoking rengimen.

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