

表5 国産たばこ8銘柄の葉中グリセロール類濃度 (n=5)

Sample	Cigarette brand	Concentration (mg/g, CTF)			
			Propylene glycol	Glycerol	Triethylene glycol
MEVIUS 1	MEVIUS ONE 100's	AVG	2.50	15.03	< LOQ
		SD	0.03	0.47	
		CV	1.36	3.15	
MEVIUS 3	MEVIUS EXTRA LIGHTS	AVG	2.82	13.44	< LOQ
		SD	0.10	0.29	
		CV	3.59	2.14	
MEVIUS 6	MEVIUS SUPER LIGHTS	AVG	2.78	16.39	< LOQ
		SD	0.27	1.05	
		CV	9.54	6.41	
MEVIUS 8	MEVIUS LIGHTS	AVG	2.61	13.48	< LOQ
		SD	0.08	0.51	
		CV	2.97	3.81	
MEVIUS 10	MEVIUS ORIGINAL	AVG	2.67	16.05	< LOQ
		SD	0.09	0.41	
		CV	3.42	2.55	
SS 14	SEVEN STARS	AVG	2.76	18.51	< LOQ
		SD	0.06	0.75	
		CV	2.12	4.04	
ECHO 15	ECHO	AVG	2.93	18.39	< LOQ
		SD	0.04	0.78	
		CV	1.24	4.22	
WKB 19	WAKABA	AVG	3.00	18.81	< LOQ
		SD	0.11	0.47	
		CV	3.76	2.50	

< LOQ, less than limit of quantitation

AVG, average; SD, standard deviation, CV, coefficient variation

表6 メンソールたばこ11銘柄の葉中グリセロール類濃度 (n=5)

Sample	Cigarette brand	Concentration (mg/g, CTF)			
		Propylene glycol	Glycerol	Triethylene glycol	
BVL1	BEVEL FALIR MENTHOL	AVG	5.08	11.24	<LOQ
		SD	0.19	0.43	
		CV	3.75	3.86	
PIA1	PIANISSIMO petil menthol one	AVG	3.23	10.39	<LOQ
		SD	0.10	0.19	
		CV	2.95	1.81	
VS1	VIRSINIA SLIMS One	AVG	4.36	13.44	<LOQ
		SD	0.13	0.44	
		CV	2.95	3.25	
PIA3	PIANISSIMO ultra lights	AVG	2.96	10.77	<LOQ
		SD	0.06	0.22	
		CV	1.98	2.03	
VS3	VIRSINIA SLIMS ULTRA Lights	AVG	8.41	16.32	<LOQ
		SD	0.23	0.45	
		CV	2.76	2.76	
MB4	Marlboro ULTRA LIGHTS	AVG	8.31	14.71	<LOQ
		SD	0.31	0.38	
		CV	3.72	2.55	
PIA5	PIANISSIMO icene menthol	AVG	4.68	12.37	<LOQ
		SD	0.04	0.25	
		CV	0.77	2.03	
BVL6	BEVEL LIGHTS	AVG	1.42	<LOQ	<LOQ
		SD	0.02		
		CV	1.15		
VS6	VIRSINIA SLIMS Lights	AVG	8.98	17.33	<LOQ
		SD	0.18	0.31	
		CV	2.01	1.80	
MB8	Marbolo LIGHTS 100's	AVG	7.76	11.98	<LOQ
		SD	0.03	0.10	
		CV	0.42	0.80	
KOOL	KOOL Boost 8	AVG	6.74	33.20	<LOQ
		SD	0.18	0.79	
		CV	2.66	2.37	

< LOQ, less than limit of quantitation

AVG, average; SD, standard deviation, CV, coefficient variation

表7 メンソールたばこ11銘柄及び国産たばこ8銘柄の葉中グリセロール類濃度

Cigarette brand	Concentration (mg/g, CTF)			
	Propylene glycol	Glycerol	Triethylene glycol	
11 domestic menthol cigarette	AVG	5.63	15.17	< LOQ
	SD	2.56	6.75	
	CV	45.43	44.49	
8 domestic cigarette	AVG	2.76	16.26	< LOQ
	SD	0.16	2.18	
	CV	5.90	13.42	

< LOQ, less than limit of quantitation

AVG, average; SD, standard deviation, CV, coefficient variation

表8 標準たばこ葉中のポロニウムの分析結果

Standard Cigarette		Polonium-210				
Brand	No.	(m Bq/g)	(m Bq/本)	Mean	*SD	**CV (%)
3R4F	1	13.7	10.1	11.4 ± 1.50	1.50	13.2
	2	14.4	10.7			
	3	14.9	11.0			
	4	18.9	14.0			
	5	15.1	11.2			
1R5F	1	10.5	5.8	5.6 ± 0.39	0.39	6.7
	2	9.6	5.3			
	3	11.2	6.2			
	4	9.6	5.2			
	5	10.4	5.7			
CM6	1	23.3	17.5	17.0 ± 1.05	1.05	6.2
	2	23.2	17.5			
	3	21.9	16.5			
	4	23.9	18.0			
	5	20.4	15.4			

*SD : standard deviation, **CV : coefficient of variation

表9 国産たばこ8銘柄のたばこ葉中ポロニウムの分析結果

Standard Cigarette		Polonium-210				
Brand	No.	(m Bq/g)	(m Bq/本)	Mean	SD	CV (%)
MEVIUS ONE	1	29.4	17.2	16.8 ± 0.34	0.34	1.9
	2	28.3	16.5			
	3	28.1	16.5			
	4	28.7	16.8			
	5	29.2	17.1			
MEVIUS Extra Lights	1	31.7	18.0	18.5 ± 1.45	1.45	7.6
	2	36.8	20.8			
	3	33.2	18.8			
	4	31.1	17.6			
	5	30.7	17.4			
MEVIUS Super Lights	1	28.7	15.9	15.5 ± 0.39	0.39	2.4
	2	27.5	15.3			
	3	27.0	15.0			
	4	28.0	15.5			
	5	28.4	15.8			
MEVIUS Lights	1	31.9	19.3	18.9 ± 1.12	1.12	5.7
	2	29.1	17.6			
	3	32.7	19.8			
	4	29.9	18.0			
	5	33.2	20.1			
MEVIUS Original	1	28.5	16.1	16.8 ± 0.45	0.45	2.7
	2	29.9	16.9			
	3	30.6	17.3			
	4	30.1	17.0			
	5	29.8	16.8			
Seven Stars	1	35.9	24.1	25.0 ± 0.73	0.73	2.8
	2	37.2	24.9			
	3	36.9	24.7			
	4	38.8	26.0			
	5	37.7	25.3			
echo	1	33.5	17.9	17.4 ± 0.63	0.63	3.5
	2	33.9	18.1			
	3	32.4	17.3			
	4	31.9	17.1			
	5	31.1	16.6			
わかば	1	36.0	24.3	25.7 ± 1.72	1.72	6.5
	2	39.8	26.9			
	3	41.3	27.9			
	4	35.6	24.1			
	5	37.8	25.5			

表10 国産たばこ3銘柄の主流煙中ポロニウム-210の分析結果

たばこ銘柄	mBq/本					たばこ葉から 主流煙への 移行率 (%)
	たばこ葉		フィルター		ガス	
	Mean	喫煙法	Mean	SD	Mean	
Mevius One	16.8	ISO	0.1<		<0.76	7.6
		HCI	1.3	± 0.26	<0.76	
Mevius Original	16.8	ISO	1.1	± 0.05	<0.76	6.8
		HCI	2.6	± 0.53	<0.76	15.8
Sevnen Stars	25.0	ISO	1.7	± 0.06	<0.76	6.9
		HCI	4.0	± 0.16	<0.76	15.9

表11 国産たばこ3銘柄の主流煙中ポロニウム-210の分析結果

	mBq/本					たばこ葉から 主流煙への 移行率 (%)
	たばこ葉		フィルター		ガス	
	Mean	喫煙法	Mean	SD	Mean	
Mevius One	16.8	ISO	8.5	± 0.75	<0.7	50.8
		HCI	7.1	± 0.84	<0.7	42.5
Mevius Super Light	15.5	ISO	5.5		<0.7	35.5
		HCI	5.9		<0.7	38.1
Sevnen Stars	25.0	ISO	9.3	± 0.85	<0.7	37.1
		HCI	8.7	± 1.77	<0.7	34.8

表12 現存するたばこ製品の規制戦略

規制の種類	規制を実施している国	規制の例	参照
害と毒性の規制			
排出物制限	EU諸国、オーストラリア、シンガポールなど	EU諸国：2004年1月以降、加盟国内で無料配布用にはリリース、マーケティング、または製造された紙巻きたばこのイールドは、ISO喫煙法のもとでタールについては紙巻きたばこ1本あたり10mg、ニコチンについては紙巻きたばこ1本あたり1mg、一酸化炭素については紙巻きたばこ1本あたり10mgを超えないものとする。これらのタール制限は、1992年に実施された紙巻きたばこ1本あたり15mgおよび1997年に実施された紙巻きたばこ1本あたり12mgをさらに低減したものである。	2001年6月5日の欧州議会および評議会の2001/37/EC指令 http://europa.eu/legislation_summaries/public_health/health_determinants_lifestyle/c11567_en.htm (2011年4月23日アクセス)
低延焼性	カナダ、米国、オーストラリアなど	オーストラリア：2010年3月23日以降、オーストラリアで製造されるかオーストラリアに輸入されるすべての紙巻きたばこに強制基準が適用される。2010年9月23日以降、いつでもどこで製造されたかに関わらず、またいつオーストラリアに輸入されたかに関わらず、オーストラリアに供給されるすべての紙巻きたばこに強制基準が適用される。	オーストラリア競争・消費者委員会 (ACCC)。Product Safety Australia：強制基準：火災危険低減紙巻きたばこ： http://www.productsafety.gov.au/content/index.phtml/itemId/974720/fromItemId/974709 (2011年4月23日アクセス)
製品禁止	オーストラリア、ニュージーランド、EU	ニュージーランド：噛むか、または他の（喫煙以外の）経口使用に適するというラベルが付けられているか、または他に記載されている販売用に輸入された噛みたばこ	1990年喫煙対策環境法 http://www.legislation.govt.nz/act/public/1990/0108/latest/DLM223191.html (2011年4月23日にアクセス)
製品毒素規制	スウェーデンなど	スウェーデン：たばこ会社であるSwedish Matchがたばこ製品内の一定の毒素の制限を開発し、Gothiatek規格と呼ばれるようになった。	Gothiatek規格。 http://www.swedishmatch.com/en/Snus-and-health/Our-quality-standard-GothiaTek/GothiaTek-standards/ (2011年4月23日にアクセス)
誘引性の規制			
添加物と成分の制限/禁止	カナダ、米国、オーストラリア、タイ、フランス、シンガポール、リトアニアなど	カナダ：2010年7月現在、カナダは、メントールを除き香味特性があったり、香味を高めたりする添加物を禁止した。カフェインやビタミンなどの添加物も禁止した。	カナダ司法省。青少年向けたばこ販売に関する取締法 (Cracking Down on Tobacco Marketing Aimed at Youth Act) http://laws-lois.justice.gc.ca/PDF/2009_27.pdf
中毒性の規制	この分野で法律を実施している国はまだな		
試験と開示	カナダ、ブラジル、オーストラリア、タイ、米国、ベネズエラ、ニュージーランドなど	カナダ：カナダで販売されており、市場占有率が1%を超えるすべての銘柄について、ISOおよびカナダ保健省の喫煙法を使用した26個のたばこ全体の化学成分、たばこ重量、pH、41個の排出物の開示。製造業者と輸入業者はまた、3つの毒性試験を実施して報告しなくてはならない。また、すべての消費者たばこ製品製造業者は、その消費者たばこ製品に関して、製造業者により、または製造業者の代理として、1年間に実施し、継続し、完了した各研究活動を毎年報告するものとする。これには、(a) 毒性、(b) 健康への影響、(c) 成分、(d) 味と香味、(e) 改良、(f) マーケティング、(g) 消費者の使用方法に関する研究が含まれるが、これらに限定されるものではない。	カナダ保健省。たばこ報告規制。 http://www.hc-sc.gc.ca/heps/tobac-tabac/legislation/reg/indust/index-eng.php (2010年4月18日にアクセス)

文献[7]のTable 1を日本語に翻訳

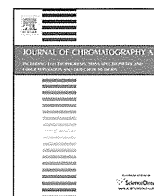
Ⅱ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
稲葉 洋平	タバコ煙に含まれる化学物質	G.I.Research	20	277-286	2012
Uchiyama S, Inaba Y, Kunugita N	Ozone removal in the collection of carbonyl compounds in air	J Chromatogr A	1229	293-297	2012
Uchiyama S, Sakamoto H, Ohno A, Inaba Y, Nakagome H, Kunugita N	Reductive amination of glutaraldehyde 2,4-dinitrophenylhydrazone using 2-picoline borane and high-performance liquid chromatographic analysis	Analyst	137	4274-4279	2012
杉山晃一, 稲葉洋平, 大久保忠利, 内山茂久, 高木敬彦, 樺田尚樹	国産たばこ主流煙中たばこ特異的ニトロソアミン類の異なる捕集法を用いた測定	日本衛生学雑誌	67	423-430	2012
Ino T, Shibuya T, Saito K, Inaba Y	Relationship between body mass index of offspring and maternal smoking during pregnancy	Int J Obes (Lond)	36	554-558	2012
稲葉洋平, 大久保忠利, 内山茂久, 樺田尚樹	国産たばこ銘柄のたばこ葉に含有されるニコチン, たばこ特異的ニトロソアミンと変異原性測定	日本衛生学雑誌	68	46-52	2013
Matsumoto M, Inaba Y, Yamaguchi I, Endo O, Hammond D, Uchiyama S, Suzuki G	Smoking topography and biomarkers of exposure among Japanese smokers: associations with cigarette emissions obtained using machine smoking protocols	Environ Health Prev Med	18	95-103	2013
杉田和俊, 松本真理子, 稲葉洋平, 遠藤治, 内山茂久, 樺田尚樹	GC/MS によるニコチン分析の検討と公定法との比較	分析化学	62	253-257	2013

稲葉洋平, 大久保忠利, 杉田和俊, 内山茂久, 緒方裕光, 櫻田尚樹	薬用吸煙剤ネオシーダーの葉中及び主流煙中の有害化学成分と変異原活性の測定	日本衛生学雑誌	69	31-38	2013
Uchiyama S, Ohta K, Inaba Y, Kunugita N	Determination of Carbonyl Compounds Generated from the E-cigarette Using Coupled Silica Cartridges Impregnated with Hydroquinone and 2,4-Dinitrophenylhydrazine, Followed by High-Performance Liquid Chromatography	Anal Sci.	29	1219-1222	2013
Uchiyama S, Tomizawa T, Inaba Y, Kunugita N	Simultaneous determination of volatile organic compounds and carbonyls in mainstream cigarette smoke using a sorbent cartridge followed by two-step elution	J Chromatogr A.	1314	31-37	2013
稲葉洋平, 内山茂久, 櫻田尚樹	国産たばこ製品の有害性の評価	日本小児禁煙研究会雑誌	3	31-39	2013



Simultaneous determination of volatile organic compounds and carbonyls in mainstream cigarette smoke using a sorbent cartridge followed by two-step elution



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ABSTRACT

We developed a simple method for the simultaneous determination of volatile organic compounds (VOCs) and carbonyls in the mainstream cigarette smoke using a sorbent cartridge at ambient temperature without the traditional cryogenic impinger. A sorbent cartridge is installed between intake filter and the pump of the smoking machine. Collection of cigarette mainstream smoke is performed according to the Canadian Intense regime or the ISO regime. As adsorbent, Carboxen 572 (CX-572) is the most suitable for collection of VOCs and carbonyls in the mainstream cigarette smoke. Elution of VOCs and carbonyls from CX-572 is performed by the two-step elution with carbon disulfide and methanol. VOCs are eluted by first elution with carbon disulfide and carbonyls are eluted by second elution with methanol. For VOCs, a portion of eluate is analyzed by gas chromatography–mass spectrometry. For carbonyls, a portion of eluate is derivatized with enriched 2,4-dinitrophenylhydrazine solution and analyzed by high-performance liquid chromatography. Measurement values by CX-572 cartridge method are very close to those obtained by traditional impinger method except for 2-butanone. Impinger methods use 2,4-dinitrophenylhydrazine solution containing 50% water and 2-butanone–DNPhydrazone may be hydrolyzed with water. In the CX-572 method, the hydrolysis of 2-butanone is prevented because the eluate solution contains no water. CX-572 method can measure cigarette smoke resulting from not only one whole cigarette but also from one puff volume because of its high sensitivity and simple operation.

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1. Introduction

Cigarette smoking is associated with a variety of pulmonary and cardiovascular disorders including emphysema, atherosclerosis and cancer [1–5], and causes 30% of all cancer deaths. Cigarette smoke, which can be divided into gas phase and particulate matter, is a complex mixture consisting of more than 5000 chemicals and at least 50 of these are carcinogenic [6,7]. Volatile organic compounds (VOCs) in gas phase cigarette smoke include hazardous substances, specifically, benzene, 1,3-butadiene, isoprene and acrylonitrile that are carcinogenic and prevalent toxins [8,9]. Benzene induces leukemia both in humans with occupational exposures [10,11] and in experimental animals [12,13]. In International Agency for Research on Cancer (IARC) monographs [14], benzene was classified as a Group-1 compound (*carcinogenic to humans*), citing additional evidence of an increased incidence of acute nonlymphocytic leukemia in workers exposed to benzene.

1,3-Butadiene is known to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in humans, including epidemiological and mechanistic studies [15]. Similarly to benzene, 1,3-butadiene was currently classified as a Group-1 carcinogen by IARC [16]. Isoprene has been identified as a carcinogen in humans and experimental animals [17] and was classified as a Group 2B carcinogen (*possibly carcinogenic to humans*) on the basis of sufficient evidence for carcinogenicity at multiple organ sites in both mice and rats, especially male mice, exposed by inhalation [18]. Acrylonitrile was found to be carcinogenic to rats with tumors reported in the central nervous system, mammary gland, and a few other rare tumor sites. IARC classified acrylonitrile in 1999 as Group 2B, based on evidence from experimental animals [19]. Similarly to VOCs, carbonyls such as aldehydes and ketones in cigarette smoke, have received much attention as hazardous substances in studies of environmental and biological chemistry. Long-term exposure to relatively high levels of formaldehyde is known to increase the risk to humans [20–23]. In 2004, IARC reclassified formaldehyde as a Group 1 human carcinogen that causes nasopharyngeal cancer and also concluded that there is a “strong but not sufficient evidence for a causal association between leukemia and occupational exposure to formaldehyde” [24]. Acetaldehyde may be responsible

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for increased risk of head and neck cancer and esophageal cancer and was classified as a Group 2B substance [25–27]. Acrolein is not currently a suspected human carcinogen as, to date, no studies have been conducted to observe its carcinogenic effects on human cells. However, studies in rats have shown an increase in cancerous tumors from ingestion but not inhalation, and Feng et al. [28] have recently suggested a connection between acrolein in cigarette smoke and an increased risk of lung cancer.

Therefore, it is very important to measure VOCs and carbonyls in cigarette smoke and evaluate the effect of smoking on human health. At the present time, measurement of VOCs and carbonyls in the mainstream cigarette smoke is performed by two different collection methods and two analytical instruments. In the VOCs analysis, generally, measurement is performed under Health Canada Intense Regime (HCI) T-116 [29] or CORESTA Recommended Method (CRM) No. 74 [30]. In these regulations, VOCs are collected by passing the mainstream smoke of two or five cigarettes through a glass fiber filter disk and into the cryogenic impinger containing 10 mL methanol cooled to below -70°C by using dry-ice/isopropanol bath. Then, the impinger solutions are injected onto a gas chromatograph/mass spectrometer (GC/MS) for quantitation. In the carbonyl analysis, generally, measurements are performed under HCI T-104 [31] or CRM No. 70 [32]. In these regulations, carbonyls are collected by passing the mainstream smoke of two or five cigarettes into the impinger containing 80 mL or 35 mL DNPH solution. Then, the impinger solutions are injected onto a high performance liquid chromatography (HPLC) for quantitation.

Thus, these traditional impinger methods need large sampling devices, cryogenic bath cooled to below -70°C for VOCs, and very complicated operation. Therefore, we developed the simple method for the measurement of VOCs in the mainstream cigarette smoke using a sorbent cartridge at ambient temperature without the cryogenic impinger.

2. Experimental

2.1. Apparatus and reagents

The GC/MS system (QP 2010 Ultra, Shimadzu, Kyoto, Japan) was used with a fused-silica column (InertCap AQUATIC-2 60 m \times 0.25 mm i.d., $d = 1.4 \mu\text{m}$, GL Sciences, Tokyo, Japan) and operated with temperature programming from 40°C (held for 6 min) to 250°C at $6^{\circ}\text{C}/\text{min}$, with He as carrier gas at 0.61 mL/min and 70 eV EIMS detection operated in full-scan mode from m/z 40–500. The injection volume is 1 μL (split injection, split ratio 10:1; septum purge 1 mL/min; injector temperature 240°C). The HPLC system (Prominence LC-20, Shimadzu, Kyoto, Japan) was used with two LC-20AD pumps, an SIL-20AC autosampler and an SPD M20A photo-diode array detector. The analytical column was an Ascentis RP-Amide, 3 μm particle size, 150 mm \times 3 mm i.d. column (Supelco Inc., Bellefonte, PA, USA). Solution A of the mobile phase mixture was acetonitrile/water (50/50, v/v) containing 10 mmol/L ammonium acetate and solution B was acetonitrile/water (80/20, v/v). HPLC elution was carried out with 100% A for 5 min, followed by a linear gradient from 100% A to 100% B in 50 min and then held for 10 min. The flow rate of the mobile phase was 0.7 mL/min. The column temperature was 30°C and the injection volume was 10 μL .

The cigarette smoke was generated on a single-port piston-type smoking machine Model LM1/PLUS (Heinrich Borgwaldt Hamburg, Germany). Thermal mass flow meter (TSI 4100 Series, TSI Inc.) was used for measuring the smoking machine puff profiles.

Standard 1,3-butadiene solution (2.0 mg/mL in methanol) was purchased from AccuStandard Inc. (New Haven, CT, USA). Benzene- d_6 (99.95%), Isoprene (95.0%), acrylonitrile (97%) benzene (99.7%),

toluene (99.7%), carbon disulfide (99.0%) and methanol (99.8%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Carboxen 563 (CX-563, 20/45 mesh), Carboxen 564 (CX-564, 20/45 mesh), Carboxen 569 (CX-569, 20/45 mesh), Carboxen 572 (CX-572, 20/45 mesh) and activated carbon (AC, 20/45 mesh) were purchased from Sigma–Aldrich Inc. (MO, USA). Anasorb 747 (AS-747, 20/40 mesh) was purchased from SKC Inc. (PA, USA). The water used for HPLC and sample preparation was deionized and purified using a Milli-Q Water System equipped with a UV lamp (Millipore, Bedford, MA, USA). 2,4-Dinitrophenylhydrazine hydrochloride (>98%) was obtained from Tokyo Kasei Co., Ltd. (Tokyo, Japan). The acetonitrile (HPLC grade, >99.9%), ethanol (>99.5), phosphoric acid (85% solution in water), and ammonium acetate (99.999%) were from Sigma–Aldrich Inc. (MO, USA).

Sample cigarettes used in this study were 3R4F, 1R5F from University of Kentucky (Lexington, KY, USA) and CM6 from Cooperation Center for Scientific Research Relative to Tobacco (CORESTA, Paris, France).

2.2. Preparation of the sorbent cartridge (CX-572 cartridge)

CX-572 particles were weighed into a glass tube and conditioned at 380°C for 5 h under a flow of purified nitrogen at 50 mL/min using a tube conditioner (TC-20, Markes Int. Ltd., Mid-Glamorgan, UK). After cooling to room temperature, carbon adsorbents were packed into the polyethylene cartridges (Rezorian tube, 1 mL, Supelco Inc., Bellefonte, PA).

2.3. Preparation of the enriched DNPH-solution

Phosphoric acid (25 mL) and DNPH hydrochloride (1 g) are added in a 50 mL volumetric flask, and then this solution is diluted to 50 mL with acetonitrile. This mixture solution is continuously stirred with a magnetic stirrer until a clear solution is obtained and stored in a refrigerator at 4°C .

2.4. Collection cigarette smoke using a CX-572 cartridge

Test cigarettes are placed at 22°C temperature and 60% humidity. CX-572 cartridge is installed between intake filter and pump of the smoking machine. Collection of cigarette main-stream smoke is performed according to the HCI regime [33] or the ISO regime [34]. In the HCI regime, mainstream smoke constituents are collected under the conditions of 55 mL puff volume, 2 s puff duration, 30 s puff interval, and 100% blocking of the filter ventilation holes with Mylar adhesive tape. In the ISO regime, mainstream smoke constituents are collected under the conditions of 35 mL puff volume, 2 s puff duration, 60 s puff interval, and no blocking of the filter ventilation holes.

2.5. Elution of CX-572 cartridge by two-step elution and analysis

After collection, CX-572 particles together with the frits are removed from cartridge and deposited into the 15 mL septum-sealed vial. Then, 1 mL of carbon disulfide is slowly added into the vial through the septum using the syringe with needle. After letting the sample stand for 10 min, 4 mL of methanol is added and stirred for 10 s. In the case of VOC analysis, a 1 mL portion of eluate solution is transferred to a 1.5 mL autosampler vial, internal standard (10 mg/mL benzene- d_6 , 8 μL) is added, and then, this solution is analyzed by GC/MS under the conditions described in Section 2.1. In the case of carbonyl analysis, a 0.5 mL of portion of eluate solution is transferred to a 5 mL volumetric flask. Then, 0.1 mL of the enriched DNPH solution is added, and after ten minutes, this solution is diluted to 5 mL with ethanol and analyzed by HPLC.

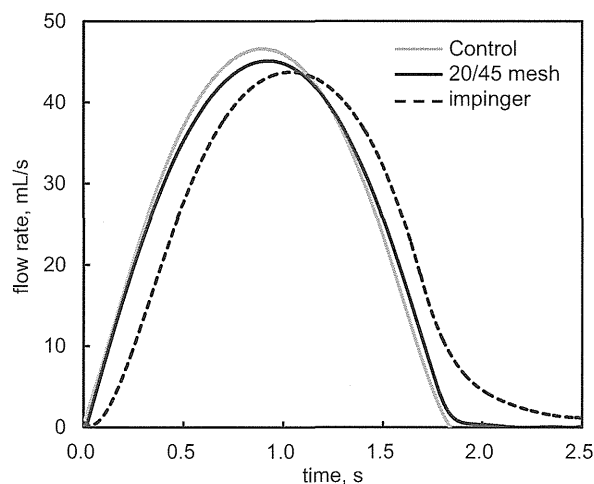


Fig. 1. The changes in flow rates with time during one puff cycle.

3. Results and discussion

3.1. Selection of particle size of adsorbent

Changes in flow rate for puff duration (puff profile) will affect the generation of chemical substances from cigarette. Puff profile of smoking machine may vary depending on particle size of adsorbent in the sorbent cartridge. Therefore, the changes in flow rate and pressure drop at puff duration were measured using a thermal mass flow meter. The test particle sizes of adsorbent were 20/45, 45/60, 60/80, 80/120 mesh. 300 mg of each adsorbent was packed into the polyethylene cartridge. For reference, impinger filled with 35 mL DNPH solution [32] was tested. Thermal mass flow meter (TSI 4100) was set in the smoking machine between cigarette and sorbent cartridge. Then, the smoking machine was run under the conditions of 55 mL puff volume, 2 s puff duration based on the HCI regime [33]. Fig. 1 shows the changes in flow rates with time during

one puff cycle. Maximum flow rate was decreased when the particle size was decreased down to 80/120 mesh. The puff duration was longer when particle size decreased. Moreover, pressure drops were observed in cases of smaller particle sizes (data not shown). Puff profile of impinger was shifted toward longer puff duration and not completed within 2 s. Puff profile using 20/45 mesh size is very similar to that with no cartridge (control) and pressure drop is lowest in comparison to that for other mesh sizes. From these results, 20/45 mesh size was selected for the sorbent cartridge.

3.2. Selection of adsorbents for the sorbent cartridge

CX-563, CX-564, CX-569, CX-572, AC, and AS-747 were packed in a two-bed sorbent tube separated with frits (1 mm thickness). Both beds contained the same sorbent. Adsorbent contents of front (F) and rear (R) beds was as follows: F/R; 50 mg/450 mg, 100 mg/400 mg, 200 mg/300 mg, 300 mg/200 mg, 400 mg/100 mg, 500 mg/0 mg. Mainstream cigarette smoke from reference cigarette CM6 was collected with each sorbent tube using smoking machine according to the HCI regime [33]. CM6 generates the highest concentrations of VOCs and carbonyls in mainstream cigarette smoke among the reference cigarettes. After collection, in each case adsorbent particles of front bed and rear bed were removed from sorbent tube and analyzed by the procedure described above. Fig. 2 shows changes in collection efficiency of propene, 1,3-butadiene, isoprene, benzene and toluene with amount of adsorbent. The collection efficiency was calculated from the ratio of the peak area of adsorbates of front (F) and rear (R) beds; $F/(F+R) \times 100$. 500 mg of adsorbents can collect all target compounds except for propene. Propene in cigarette smoke is not a target compound but can become an index component for ability of adsorbents because of its low boiling point (-47.6°C) and its difficulty to adsorption. Overall adsorption abilities decrease in the following order: CX-572 > AC > AS-747 > CX-564, CX-563, CX-569 and are about the same as the following order of surface area: CX-572 (1100 mg/m^2) > AC (1070 mg/m^2) > AS-747 (980 mg/m^2) > CX-564 (400 mg/m^2), CX-563 (510 mg/m^2), CX-569 (485 mg/m^2).

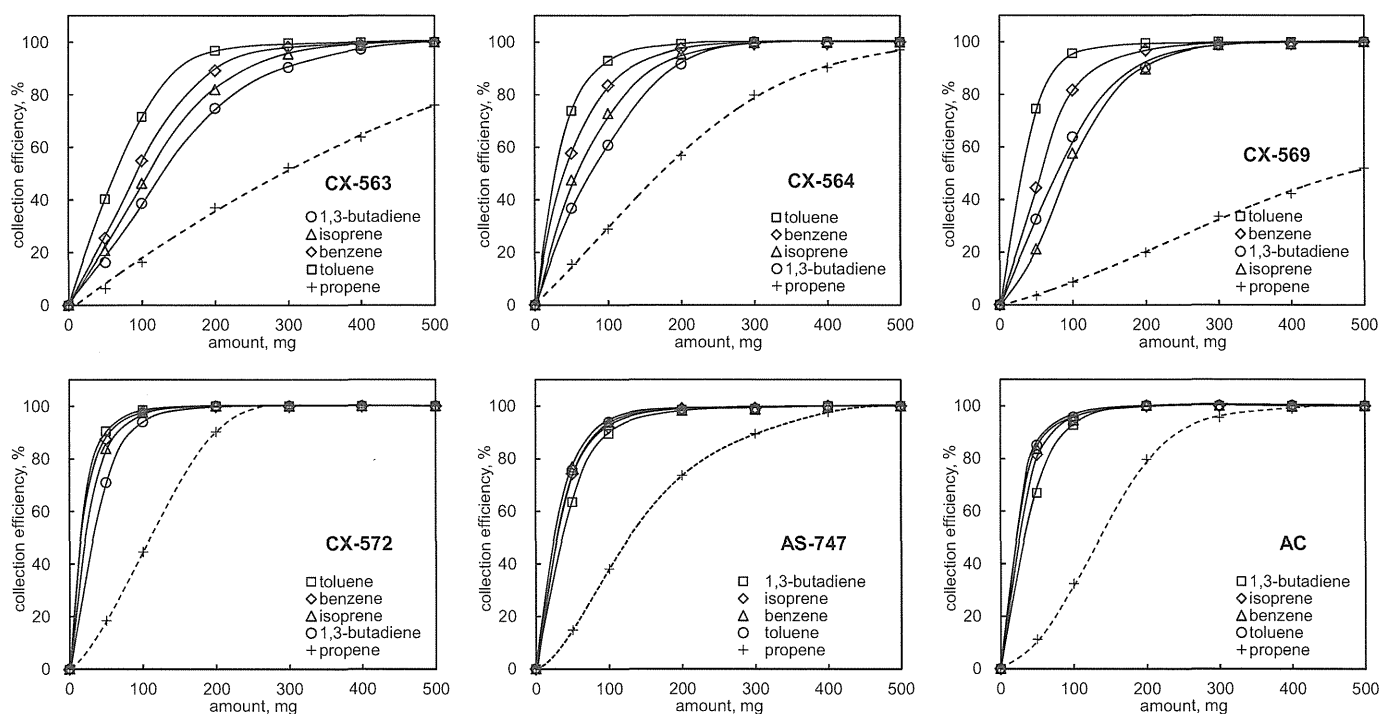


Fig. 2. Changes in collection efficiency with amount of sorbent.

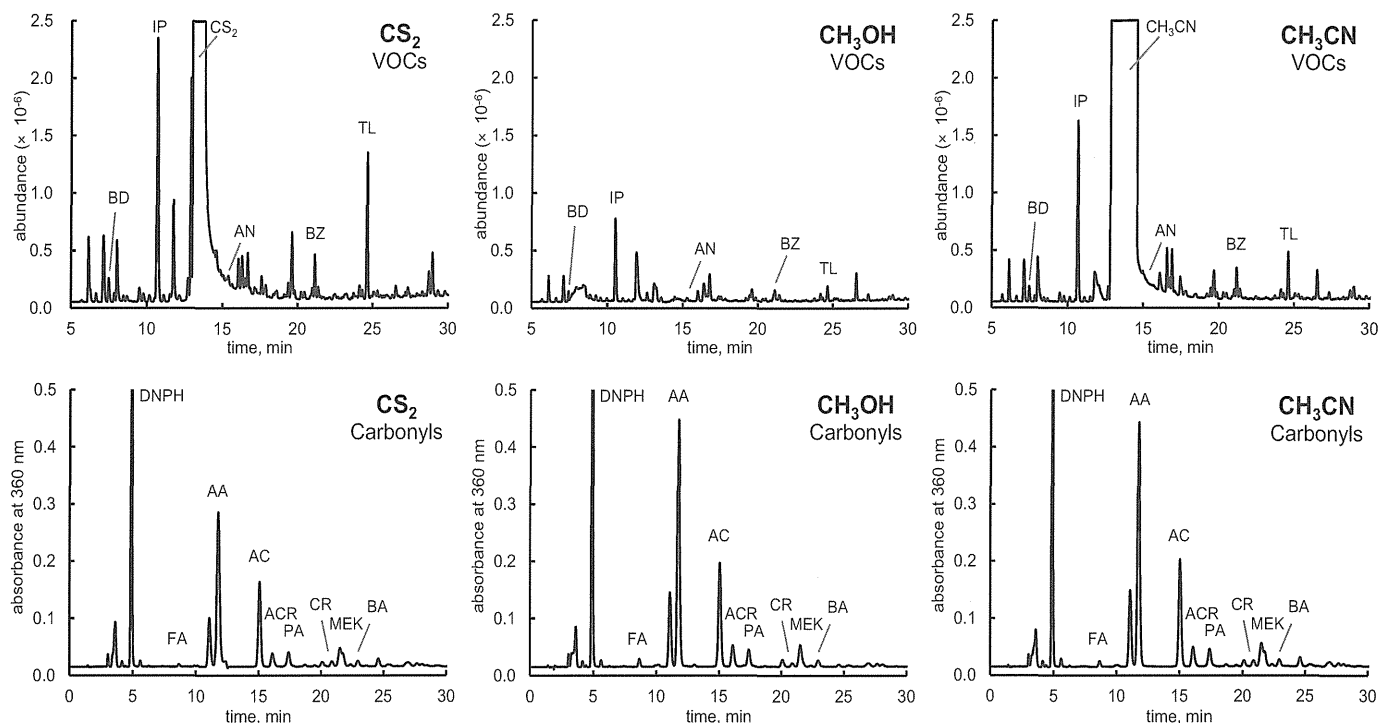


Fig. 3. Chromatographic profiles of VOCs (upper panel) and carbonyls (lower panel) eluted with the various eluents from CX-572 cartridge collected with mainstream cigarette smoke. BD: 1,3-butadiene; IP: isoprene; AN: acrylonitrile; BZ: benzene; TL: toluene; FA: formaldehyde-DNPhydrazone; AA: acetaldehyde-DNPhydrazone; AC: acetone-DNPhydrazone; ACR: acrolein-DNPhydrazone; PA: propanal-DNPhydrazone; CR: crotonaldehyde-DNPhydrazone; MEK: methyl ethyl ketone (2-butanone)-DNPhydrazone; BA: butanal-DNPhydrazone.

Collection efficiencies for carbonyls were similar to VOCs and decrease in the following order: acrolein > acetone > formaldehyde > acetaldehyde. 200 mg of CX-572 completely adsorbed all carbonyls.

Based on these results, 300 mg of CX-572 particles was selected as the sorbent cartridge.

3.3. Selection of eluents for the sorbent cartridge

Mainstream cigarette smoke from reference cigarette CM6 was collected with CX-572 cartridge using a smoking machine according to the HCl regime [33]. After collection, CX-572 particles were removed from cartridge and deposited into the 15 mL septum-sealed vial. Then, 5 mL of methanol, isopropanol, ethyl acetate, acetonitrile, or carbon disulfide was added to the vial using the syringe with needle and the vial was left standing for 30 min. In the case of VOC analysis, a portion of eluate solution was transferred into a 1.5 mL autosampler vial and analyzed by GC/MS under the conditions described above. In the case of carbonyl analysis, a 0.5 mL of portion of eluate solution was transferred to a 5 mL volumetric flask. Then, 0.1 mL of the enriched DNPH solution was added, and after ten minutes, this solution was diluted to 5 mL with ethanol and analyzed by HPLC. Fig. 3 shows chromatograms of VOCs and carbonyls eluted with carbon disulfide, methanol and acetonitrile.

Regarding VOCs, among all the solvents tested, carbon disulfide exhibits excellent elution ability to VOCs, however, cannot completely elute acrylonitrile. Carbon disulfide has weak polarity and not suitable for polar substances such as acrylonitrile and carbonyls. Regarding carbonyls, polar solvents such as acetonitrile, methanol, 2-propanol, ethyl acetate and dimethyl sulfoxide exhibit excellent elution ability and can completely elute the target compounds, however, carbon disulfide is not completely to

elute carbonyls. Therefore, there were no one solvent acceptable as eluent for both VOCs and carbonyls. Accordingly, the two-step elution with polar and nonpolar solvents was examined. VOCs are eluted by first elution with carbon disulfide and carbonyls are eluted by second elution with methanol. Mainstream cigarette smoke of CM6 was collected with CX-572 cartridge and VOCs and carbonyls were analyzed by the two-step elution method described in Section 2.5. Fig. 4 shows chromatograms of VOCs (upper panel) and carbonyls (lower panel) eluted by the two-step elution method using carbon disulfide and methanol. All target compounds, 1,3-butadiene, isoprene, acrylonitrile, benzene, toluene, acetaldehyde, acetone, acrolein, propanal, 2-butanone and butanal, were completely eluted by the two-step elution method. Moreover, VOCs and carbonyls in eluate solution never reabsorb into CX-572 particles in the carbon disulfide/methanol mixture solution.

In the analysis of acrolein using a traditional DNPH-cartridge, acrolein DNPhydrazone is decomposed rapidly in the DNPH-cartridge and forms DNPH and acrolein DNPhydrazone adducts [35–42]. However, CX-572 method uses no DNPH-cartridge and derivatization with DNPH is performed in the eluate solution. When derivatization of carbonyls in the eluate is performed using enriched DNPH solution, acrolein DNPhydrazone decompose in acidified aprotic solvent solution such as acetonitrile, and this decomposition can be inhibited by the addition of a protic solvent such as ethanol [42]. Fig. 5 shows the changes in acrolein DNPhydrazone with time in ethanol, methanol and acetonitrile dilution to the 0.5 mL carbon disulfide – methanol eluate from CX-572 cartridge collected with mainstream cigarette smoke. The decomposition of acrolein DNPhydrazone was depressed by ethanol.

Based on these results, carbon disulfide and methanol were selected as eluents for two-step elution to CX-572 method and ethanol was selected as dilution solvent for carbonyl analysis.

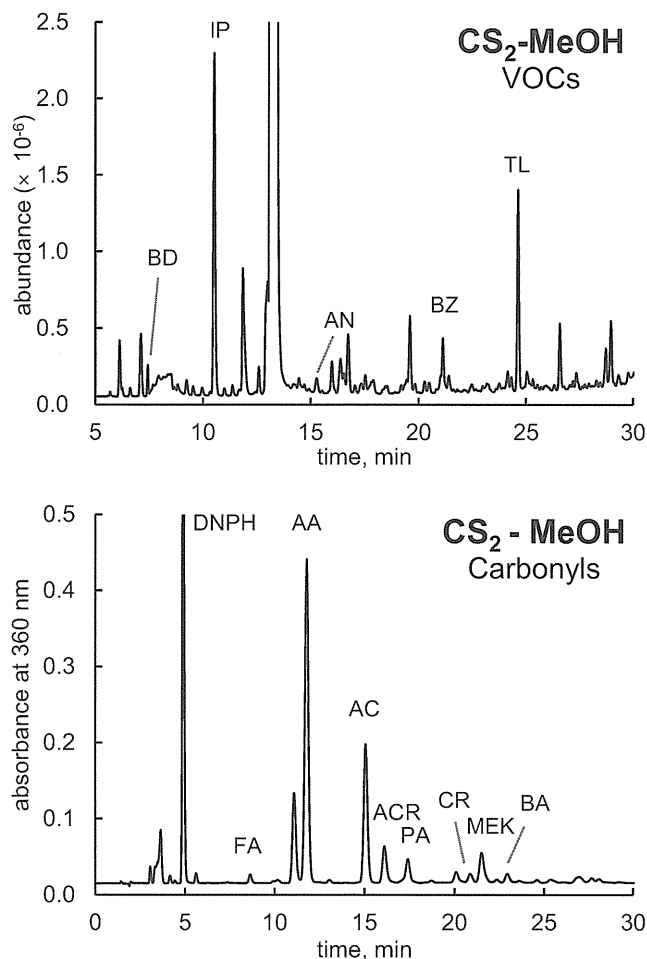


Fig. 4. Chromatographic profiles of VOCs (upper panel) and carbonyls (lower panel) eluted by the two-step elution method using carbon disulfide and methanol. BD: 1,3-butadiene; IP: isoprene; AN: acrylonitrile; BZ: benzene; TL: toluene; FA: formaldehyde-DNPhydrazone; AA: acetaldehyde-DNPhydrazone; AC: acetone-DNPhydrazone; ACR: acrolein-DNPhydrazone; PA: propanal-DNPhydrazone; CR: crotonaldehyde-DNPhydrazone; MEK: methyl ethyl ketone (2-butanone)-DNPhydrazone; BA: butanal-DNPhydrazone.

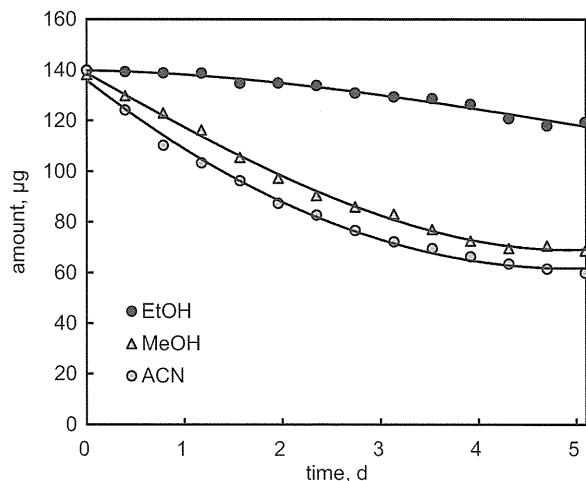


Fig. 5. Decrease of acrolein DNPhydrazone with time in ethanol, methanol and acetonitrile to the eluate from Carboxen 572 cartridge collected with main stream cigarette smoke.

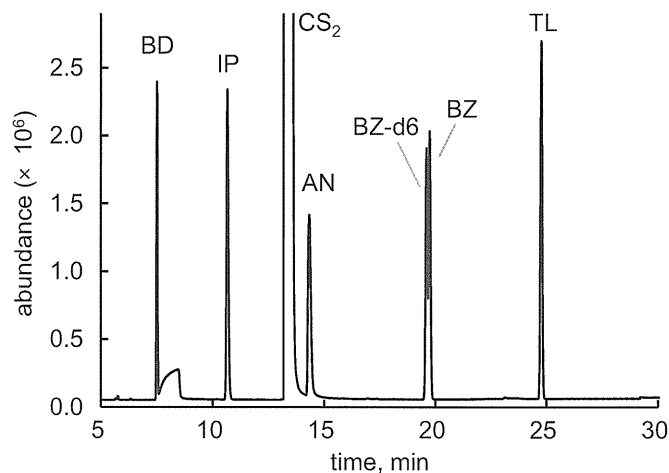


Fig. 6. Total ion chromatogram of a mixed standard solution. BD: 1,3-butadiene; IP: isoprene; AN: acrylonitrile; BZ-d6: benzene-d6; BZ: benzene; TL: toluene.

3.4. Preparation of standard solution

The standard stock solutions (2 mg/mL) of isoprene, acrylonitrile, benzene and toluene were prepared by dissolving their reagents in absolute methanol. 1,3-Butadiene is inconvenient for laboratory use because it has very low melting point (-108.9°C) and boiling point (-4.4°C) and exist as gas. Therefore, commercially available 1,3-butadiene standard solution (2 mg/mL in methanol) was used as the standard stock solution. Portions (1 mL) of 1,3-butadiene, isoprene, acrylonitrile stock solutions and portions (0.4 mL) of benzene and toluene stock solution were added into the 10 mL volumetric flask. After addition of 2 mL of carbon disulfide, the solution was diluted to 10 mL with methanol. Solvent of this standard mixture is the same as sample eluate and consists of 20% carbon disulfide and 80% methanol. This standard mixture solution contains 1,3-butadiene (0.2 mg/mL), isoprene (0.2 mg/mL), acrylonitrile (0.2 mg/mL), benzene (0.08 mg/mL) and toluene (0.08 mg/mL). After placing 0, 0.2, 0.4, 0.6, 0.8, 1.0 mL of standard mix solution in autosampler vials and diluting with 20% carbon disulfide methanol solution, 8 μL of benzene-d6 (10 mg/mL; internal standard) is added and analyzed by GC/MS under the conditions described in Section 2.1. Fig. 6 shows the total ion chromatograms of a mixed standard solution. All target compounds are completely separated under the analytical conditions of this study. Benzene-d6 (Internal standard) and benzene are partially overlapped but can be separated with mass chromatograms of m/z 84 and 78 fragments.

Preparation of standard solution for carbonyls was according to Health Canada method T-104 [31].

3.5. Limit of detection, limit of quantitation, reproducibility and recovery

The limit of detection (LOD) and limit of quantitation (LOQ) of a CX-572 cartridge method was calculated using linear regression theory [43]. 10 μL of VOC mixture standard solution (0.05 mg/mL each) and 10 μL of carbonyl mixture standard solution (0.5 mg/mL each) were introduced into the 10 CX-572 cartridges and analyzed using the analytical conditions described above. The LOD and LOQ were calculated as being three times the standard deviation obtained from the data of 10 samples (Table 1). The reproducibility of CX-572 cartridge method was estimated from data of 10 cartridges spiked with 10 μL of VOC mixture standard solution (5 mg/mL) and carbonyl mixture standard solution (50 mg/mL).

Table 1
LOD, LOQ, reproducibility and recovery of CX-572 cartridge method.

Compound	LOD (μg)	LOQ (μg)	RSD (%)	Recovery (%)
1,3-Butadiene	0.07	0.24	5.9	106
Isoprene	0.04	0.15	3.7	106
Acrylonitrile	0.07	0.22	5.6	98.1
Benzene	0.03	0.10	2.4	103
Toluene	0.03	0.09	2.1	99.3
Acetaldehyde	0.26	0.88	1.2	99.2
Acetone	0.15	0.49	1.5	103
Acrolein	0.13	0.45	2.2	102
Propanal	0.24	0.80	3.3	96.3
Crotonaldehyde	0.07	0.25	1.3	102
2-Butanone	0.14	0.46	1.7	102
Butanal	0.29	0.97	2.3	99.0
i-Valeraldehyde	0.31	1.1	3.2	95.9

The relative standard deviation (RSD) and recovery are shown in Table 1.

3.6. Measurement of real sample cigarettes

VOCs and carbonyls in mainstream cigarette smoke from reference cigarettes (CM6, 3R4F, 1R5F) were analyzed by CX-572 method. Table 2 shows the amounts of VOCs and carbonyls in mainstream cigarette smoke measured by CX-572 method. For reference, CORESTA data published at March 2013 [30,32] are also shown in parenthesis in Table 1. Formaldehyde was removed from the target compounds because it was trapped in the Cambridge filter pad before the CX-572 cartridge. We have confirmed that 63% of formaldehyde in mainstream cigarette smoke was trapped into the Cambridge filter pad. The Cambridge filter pad collects the particle fraction containing water. Formaldehyde is highly hydrophilic

Table 2
VOCs and carbonyl in mainstream cigarette smoke measured by CX-572 method. $\mu\text{g}/\text{cigarette}$. Data indicates the mean value ($n=5$) of the CX-572 method and the parenthesis indicates the mean value from CORESTA [30,32].

Compound	CM6		3R4F		1R5F	
	ISO	HCI	ISO	HCI	ISO	HCI
Puff number	8.6	12	8.0	9.8	6.5	6.4
1,3-Butadiene	65 (61)	110 (110)	37 (40)	94 (100)	12 (12)	88 (91)
Isoprene	590 (560)	940 (1000)	310 (350)	750 (910)	110 (120)	750 (890)
Acrylonitrile	12 (12)	24 (25)	8.2 (8.5)	27 (27)	2.0 (2.1)	25 (28)
Benzene	70 (60)	120 (110)	47 (42)	110 (97)	15 (14)	88 (79)
Toluene	120 (87)	220 (170)	84 (67)	220 (170)	21 (19)	160 (140)
Acetaldehyde	670 (690)	1100 (1300)	570 (550)	1400 (1600)	200 (140)	1200 (1400)
Acetone	320 (270)	580 (520)	250 (210)	600 (600)	87 (63)	540 (490)
Acrolein	72 (69)	120 (130)	56 (48)	140 (160)	15 (9.3)	110 (120)
Propanal	62 (53)	120 (100)	49 (42)	130 (120)	16 (12)	100 (99)
Crotonaldehyde	25 (21)	53 (48)	15 (11)	51 (50)	2.0 (2.4)	42 (36)
2-Butanone	130 (62)	240 (130)	97 (52)	220 (150)	34 (14)	190 (110)
Butanal	37 (37)	68 (70)	26 (25)	65 (72)	10 (7.7)	57 (59)
i-Valeraldehyde	22 (n.a.)	49 (n.a.)	16 (n.a.)	48 (n.a.)	5.1 (n.a.)	40 (n.a.)

n.a.: not available.

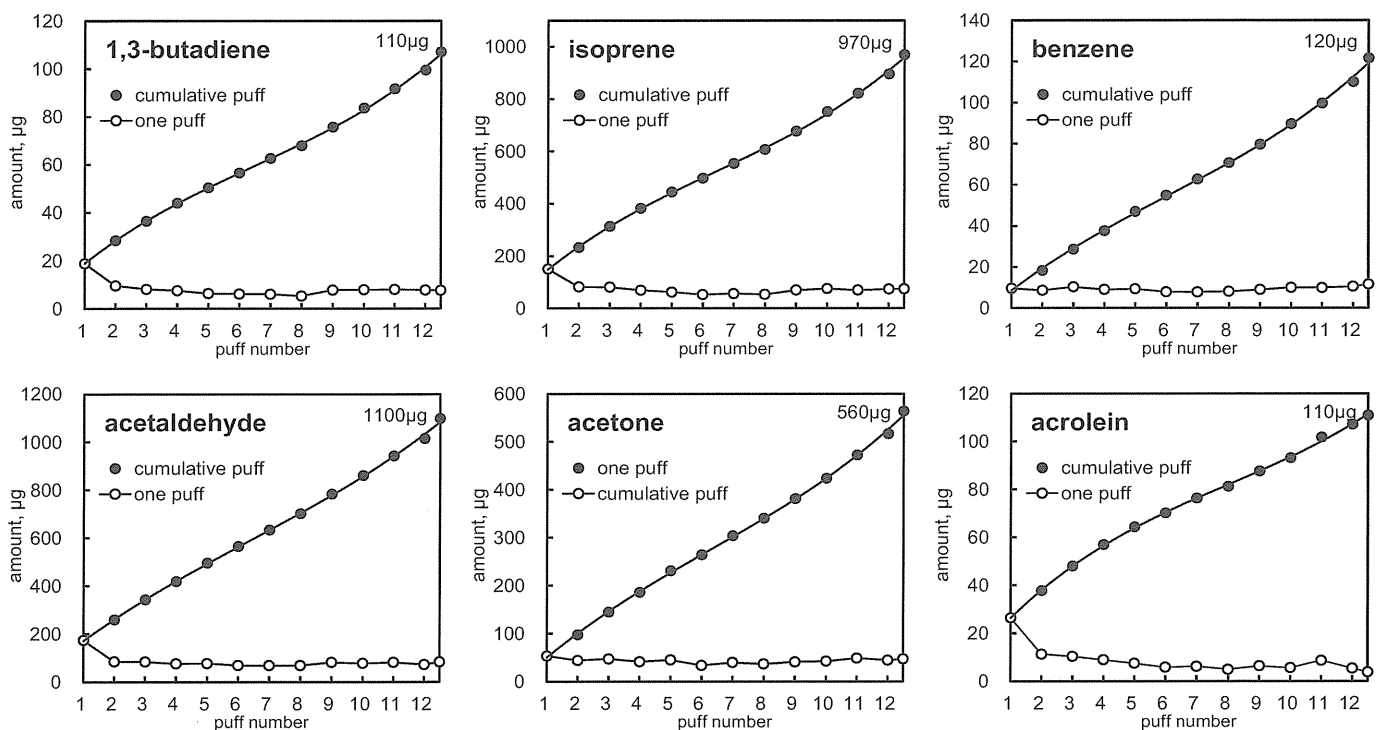


Fig. 7. Changes in amounts of VOCs and carbonyls in mainstream cigarette smoke with puff number. Open circles indicate every one puff data and closed circles indicate cumulative data.

and easy to solve into the Cambridge filter containing tar with water. It was suggested that DNPH-silica cartridge method without Cambridge filter pad is more suitable for collection of formaldehyde in mainstream cigarette smoke. All target compounds except for formaldehyde passed through the Cambridge filter pad.

Almost all measurement values by CX-572 method are very close to CORESTA data [30,32] except for 2-butanone. Measurement values of 2-butanone by CX-572 method are twofold higher than CORESTA data. In acidic aqueous solutions, 2-butanone-DNPhydrazone is hydrolyzed back to the 2-butanone and DNPH with water [44]. Impinger methods use DNPH solution containing 50% water (28 mol/L) for impinger and 2-butanone-DNPhydrazone may be hydrolyzed with water. In the CX-572 method, the hydrolysis of 2-butanone may be prevented because the eluate solution contains no water.

CX-572 method can measure VOCs and carbonyls in mainstream cigarette smoke from not only one whole cigarette but also from one puff volume because of its high sensitivity and simple operation. CX-572 cartridge installed in the smoking machine can be exchanged with new cartridge at 5 s within puff interval of HCl and ISO regime (30 s, 60 s). Fig. 7 shows the changes in amounts of VOCs and carbonyls in mainstream cigarette smoke with puff number while one cigarette had been smoked according to the HCl regime. Sample cigarette is CM6. Amounts of 1,3-butadiene, isoprene, acetaldehyde and acrolein at the first puff were twofold higher than second puff and after that, however, benzene, toluene, acetone and 2-butanone were not so varied at every puff. Cumulative data show cubic curve in Fig. 7 and the data of last puff correlates with the data from one cigarette. Cumulative values at last puff in Fig. 7 are very close to the one cigarette data described in Table 1.

4. Conclusions

A sorbent cartridge method (CX-572 method) for measurement of VOCs and carbonyls in cigarette mainstream cigarette smoke developed in this study has the advantages of high efficiency, high sensitivity, and very simple operation. CX-572 cartridge was shown to be capable of collection of VOCs and carbonyls in cigarette smoke at room temperature. Moreover, it can measure cigarette smoke not only from one whole cigarette but also from one puff volume because of its high sensitivity and simple operation.

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Determination of Carbonyl Compounds Generated from the E-cigarette Using Coupled Silica Cartridges Impregnated with Hydroquinone and 2,4-Dinitrophenylhydrazine, Followed by High-Performance Liquid Chromatography

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Carbonyl compounds in E-cigarette smoke mist were measured using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine, followed by high-performance liquid chromatography. A total of 363 E-cigarettes (13 brands) were examined. Four of the 13 E-cigarette brands did not generate any carbonyl compounds, while the other nine E-cigarette brands generated various carbonyl compounds. However, the carbonyl concentrations of the E-cigarette products did not show typical distributions, and the mean values were largely different from the median values. It was elucidated that E-cigarettes incidentally generate high concentrations of carbonyl compounds.

Keywords E-cigarette, carbonyl compounds, acrolein, glyoxal, methylglyoxal, glycerol, propylene glycol

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Introduction

E-cigarettes (electronic cigarettes or e-cigs) are battery-powered devices designed to deliver nicotine to a smoker in the form of a vapor, and were first introduced into the Chinese market in 2004. Currently, they are widely used around the world. In the United States, as of 2011, approximately 21% of adults who smoked traditional cigarettes had used electronic cigarettes, which was an increase from 10% in 2010, according to a study released by the Centers for Disease Control and Prevention.¹ Overall, approximately 6% of all adults have tried E-cigarettes, and these estimates are nearly double those from 2010.¹ It was reported in the news media in 2013 that electronic cigarettes were beginning to gain cultural acceptance, and sales were growing rapidly.²

An electronic cigarette contains three essential components: a plastic cartridge that serves as a mouthpiece and a reservoir for a liquid, an "atomizer" that vaporizes the liquid, and a battery. The liquid used to produce the vapor in electronic cigarettes is a solution of propylene glycol and/or glycerin and/or polyethylene glycol mixed with concentrated flavors and, optionally, a variable percentage of liquid nicotine concentrate. These base liquids have been widely used as food additives, as base solutions in personal care products, such as toothpaste, and in medical devices, such as asthma inhalers. However, there are few reports on chemical compounds in E-cigarette smoke mist; moreover, the health effects of inhaling nicotine vapor into the lungs are uncertain.

We have developed a new method (the HQ-DNPH method)

for the determination of acrolein and other carbonyl compounds in cigarette smoke using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine³ (DNPH), and we reported that E-cigarettes sometimes accidentally generate various carbonyl compounds, such as formaldehyde, acetaldehyde, acrolein, glyoxal, and methyl glyoxal.^{3,4} In these previous studies, we concluded that ethylene glycol was oxidized to formaldehyde and glyoxal; propylene glycol was oxidized to formaldehyde, acetaldehyde, and methylglyoxal; and glycerol was oxidized to formaldehyde, acrolein, glyoxal, and methylglyoxal.⁴ In this study, we determined the concentration of various carbonyl compounds generated from a total of 363 E-cigarettes (13 brands). The results are presented herein.

Experimental

Apparatus and reagents

An HPLC system (Shimadzu, Kyoto, Japan) with two LC-20AD pumps, an SIL-20AC autosampler and an SPD M20A photodiode array detector, was used. The analytical column was an Ascentis Express RP-Amide (2.7 μ m particle size, 150 mm \times 4.6 mm i.d., Supelco Inc., Bellefonte, PA). The column temperature was 40°C, and the injection volume was 10 μ L. Solution A of the mobile phase mixture was composed of acetonitrile/water (40/60 v/v) containing 5 mmol/L ammonium acetate; solution B was composed of acetonitrile/water (75/25 v/v). HPLC elution was carried out with 100% A for 8 min, followed by a linear gradient from 100% A to 100% B in 37 min, and then maintained constant for 15 min using 100% B. The flow rate of the mobile phase was 0.7 mL/min.

An LM1/PLUS (Borgwaldt Technik GmbH, Hamburg,

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Germany) smoking machine was used for the collection of cigarette smoke.

The water used for the HPLC analysis and sample preparation was deionized and purified using a Milli-Q Water System equipped with a UV lamp (Millipore, Bedford, MA). 2,4-Dinitrophenylhydrazine hydrochloride (>98%) was obtained from Tokyo Kasei Co., Ltd. (Tokyo, Japan). Acetonitrile (HPLC grade, >99.9%), ethanol (>99.5%), hydroquinone (>99%), phosphoric acid (85% solution in water), and ammonium acetate (99.999%) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). The silica gel (spherical, 60/80 mesh, 120 Å mean pore size) was acquired from AGC Si-Tech. Co., Ltd. (Fukuoka, Japan).

The DNPH-impregnated silica cartridge (DNPH-cartridge) and the hydroquinone-impregnated silica cartridge (HQ-cartridge) were prepared according to previous reports.^{3,4}

Collection and analysis of E-cigarette smoke

Before collecting smoke from the E-cigarettes, an HQ-cartridge and a DNPH-cartridge were connected. The coupled cartridges were then connected between the mouthpiece of the E-cigarette and the smoking machine, and the smoke from the E-cigarette was drawn into the coupled cartridges from the HQ-cartridge to the DNPH-cartridge according to the Canadian intense regimen,⁵ (55 mL puff volume, 2-s puff duration, 30-s puff interval, and 10 puffs). After collection, the coupled cartridges were extracted using acetonitrile containing 1% phosphoric acid in a direction opposite to the air sampling direction until the total volume of the solution was 4.5 mL. After 10 min, ethanol (0.5 mL) was added to the eluate, and the solution was analyzed by HPLC. If the extraction was not performed immediately, the HQ-DNPH cartridge set was decoupled, and the individual cartridges were capped with stoppers.

Results and Discussion

Analysis of E-cigarette smoke by the HQ-DNPH method

Various types of carbonyl compounds were detected in the E-cigarette smoke. Figure 1 shows a representative chromatogram of a sample eluate by HPLC analysis with UV (360 nm) detection. In the HQ-DNPH method, it is possible to analyze C1 - C10 carbonyl compounds, and C1 - C3 carbonyl compounds, such as formaldehyde, acetaldehyde, acetone, acrolein, propanal, glyoxal, and methylglyoxal, were detected.

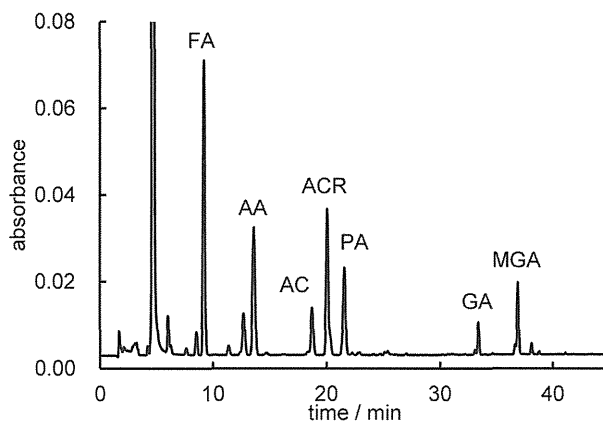


Fig. 1 Representative chromatogram of carbonyl DNPhydrazones derivatized from DNPH with carbonyls found in E-cigarette smoke. FA, formaldehyde; AA, acetaldehyde; AC, acetone; ACR, acrolein; PA, propanal; GA, glyoxal; MGA, methylglyoxal.

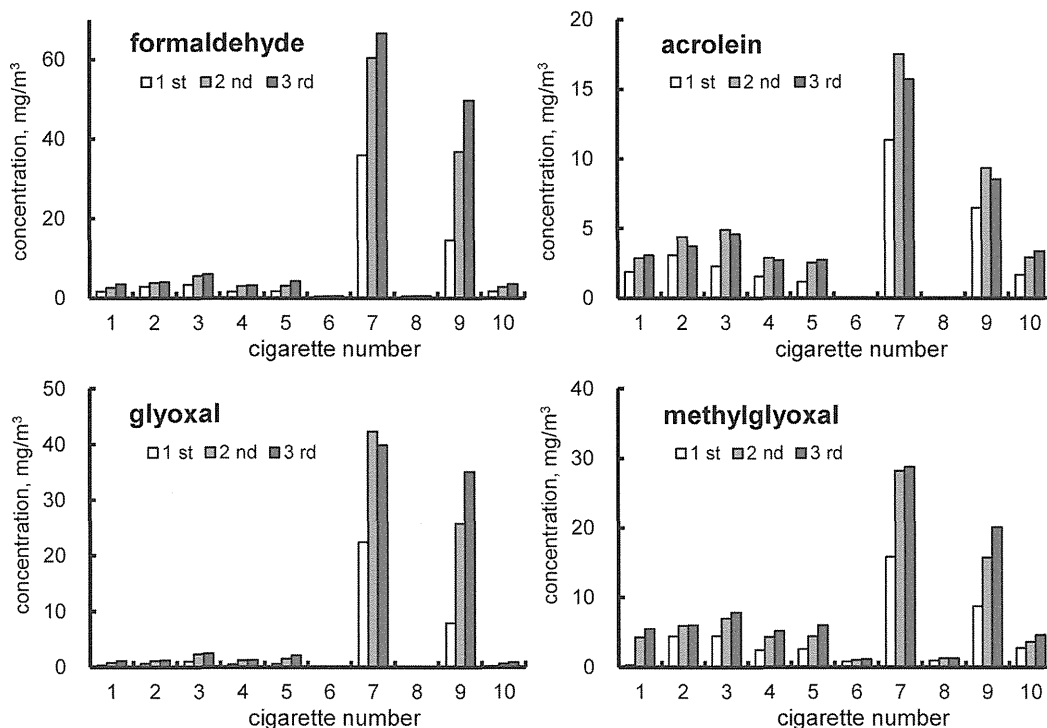


Fig. 2 Concentrations of formaldehyde, acrolein, glyoxal, and methylglyoxal generated from 10 different E-cigarettes of the same brand. Reproduced with permission from Fig. 3 in Ref. 4.

Table 1 Concentrations (mg/m³) of major carbonyl compounds generated from 13 brands of E-cigarettes

Product	N_{high} N_{low}	FR	Formaldehyde	Acetaldehyde	Acrolein	Propanal	Glyoxal	Methylglyoxal
A	16	31	61 ± 64	48 ± 51	7.5 ± 6.9	16 ± 19	4.6 ± 6.5	5.3 ± 5.7
	35		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B	6	20	44 ± 19	0.3 ± 0.1	12 ± 4.3	2.0 ± 1.2	29 ± 12	20 ± 7.8
	24		2.6 ± 1.6	n.d.	2.2 ± 1.6	n.d.	n.d.	3.7 ± 2.2
C	8	27	40 ± 28	1.7 ± 2.5	9.7 ± 10	6.1 ± 6.3	18 ± 9.5	22 ± 10
	22		3.1 ± 2.6	n.d.	1.1 ± 1.1	n.d.	1.3 ± 1.4	2.1 ± 1.9
D	12	24	28 ± 12	25 ± 12	36 ± 18	24 ± 19	7.7 ± 4.1	11 ± 7.5
	37		1.5 ± 1.8	n.d.	n.d.	n.d.	n.d.	n.d.
E	14	40	31 ± 14	27 ± 11	34 ± 12	27 ± 15	8.2 ± 4.4	8.6 ± 7.9
	21		1.3 ± 1.5	n.d.	1.2 ± 1.7	n.d.	n.d.	n.d.
F	2	40	12 ± 1.7	2.8 ± 0.2	2.0 ± 0.1	0.7 ± 0.1	2.8 ± 0.7	5.8 ± 0.9
	3		3.6 ± 3.1	1.6 ± 0.4	1.2 ± 0.5	n.d.	n.d.	1.6 ± 1.5
G	1	4	53	19	19	6.3	17	37
	25		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H	5	17	19 ± 8.9	8.3 ± 4.3	8.1 ± 4.0	n.d.	4.6 ± 0.9	8.4 ± 5.7
	25		1.7 ± 2.6	n.d.	n.d.	n.d.	n.d.	n.d.
I	6	20	5.8 ± 1.9	11 ± 5.9	11 ± 4.2	14 ± 4.1	n.d.	n.d.
	24		2.8 ± 2.6	4.8 ± 5.2	5.0 ± 4.9	6.0 ± 6.2	n.d.	n.d.
J	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	4		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
K	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	30		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
L	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	30		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	13		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

The upper line indicates the mean value for the high-concentration group, and the lower line indicates the mean value for the low-concentration group. Indices N_{high} and N_{low} indicate the number of E-cigarettes that generated high and low concentrations of carbonyl compounds, respectively. FR indicates the failure rate, which was calculated using the following equation: $\text{FR} = N_{\text{high}}/(N_{\text{high}} + N_{\text{low}}) \times 100$. Values are mean \pm SD. n.a., not available; n.d., not detected.

Concentration of carbonyl compounds in E-cigarette smoke

The concentration of carbonyl compounds in the smoke mist from 13 brands of E-cigarettes sold in Japan was determined by the HQ-DNPH method. The analysis of these actual brands of E-cigarettes revealed very large variations in the carbonyl concentrations among not only different brands, but also different examples of the same products. Typical distributions of the carbonyl concentrations were not observed for any of the E-cigarettes tested, and the mean values were largely different from the median values. These concentration variations were not caused by the analytical method, because the HQ-DNPH method has good reproducibility (RSD less than 2.1%).³ We previously reported that the smoke mist generated from E-cigarettes unexpectedly contains carbonyl compounds.⁴ This conclusion is based on the fact that for the same E-cigarette products, it was found that some E-cigarettes generated high concentrations of carbonyl compounds, while others did not. Figure 2 shows the concentrations of formaldehyde, acrolein, glyoxal, and methylglyoxal generated from 10 electronic cigarettes of the same brand. These results represent triplicate measurements for 10 samples. As can be seen in the figure, the number 7 and 9 E-cigarettes generated peculiarly high concentrations of carbonyl compounds. Therefore, the resulting data were divided into two groups based on the formaldehyde concentration (10 mg/m³): a high concentration group and a low concentration group. Table 1 shows the concentrations of the major carbonyl compounds generated from 13 brands of E-cigarettes. In the table, the top entry in each cell indicates the mean value for the high-concentration group, and the lower

entry indicates the mean value for the low-concentration group. The indices N_{high} and N_{low} indicate the number of E-cigarettes that generated high and low concentrations of carbonyl compounds, respectively. FR indicates the failure rate, which was calculated by the following equation: $\text{FR} = N_{\text{high}}/(N_{\text{high}} + N_{\text{low}}) \times 100$.

Four (J, K, L, M) out of the 13 E-cigarette brands did not generate any carbonyl compounds. The other nine E-cigarette brands (A, B, C, D, E, F, G, H, I) generated various carbonyl compounds. The concentrations of carbonyl compounds obtained for the high concentration group were significantly higher than that determined for the low concentration group. The maximum concentrations of formaldehyde, acetaldehyde, acrolein, propanal, glyoxal, and methylglyoxal were 260, 210, 73, 83, 42, and 38 mg/m³, respectively. For a typical cigarette smoking experience of 10 puffs, these values translate to maximum concentrations of 140 μg formaldehyde/cigarette, 120 μg acetaldehyde/cigarette, 33 μg acrolein/cigarette, 46 μg propanal/cigarette, 23 μg glyoxal/cigarette, and 21 μg methylglyoxal/cigarette. Most notably, very high concentrations of formaldehyde were measured in the smoke from the E-cigarettes. Glyoxal and methylglyoxal are peculiar to E-cigarette smoke, and have not been detected in the mainstream smoke from normal cigarettes. Glyoxal is known to be mutagenic to *Salmonella typhimurium* strains TA100, TA102, and TA104.^{6,7} It has been shown that glyoxal reacts with guanine residues in DNA.⁸ Its tumor promoting activity has also been reported.^{9,10} Methylglyoxal, the most mutagenic of all aldehydes, is known to inhibit formaldehyde metabolism, thus