Beiträge zur Tabakforschung International/*Contributions to Tobacco Research* Volume 24 · No. 2 · Juli 2010

DOI: 10.2478/cttr-2013-0881

HPLC-MS Determination of Acrolein and Acetone Generated from $^{13}C_3$ -Labeled Glycerol Added to Cigarette Tobacco Using Two Machine-Smoking Regimes*

by

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SUMMARY

The extent of blend glycerol degradation in a burning cigarette to form acrolein and acetone has been quantitatively determined by the addition of glycerol-$^{13}C_3$ to three styles of a leading commercial cigarette brand. Multiple Cambridge pads soaked with a solution of 2,4-dinitrophenylhydrazine (DNPH) were employed to trap hydrazone derivatives of low molecular weight carbonyl compounds in both mainstream and sidestream smoke. High performance liquid chromatography coupled with negative ion mass spectrometry was used to isolate DNPH derivatives of the volatile carbonyl products of combustion and to ascertain their concentration. Acrolein, acetone, and propionaldehyde were the principal compounds of interest. The DNPH derivatives of acrolein-$^{13}C_3$ and acetone-$^{13}C_3$ were independently synthesized, and they served as external standards for absolute quantitation. The cost of fully labeled propionaldehyde precluded its use in this study. The brand styles selected for study represent the cigarette design features that are most prevalent in the U.S. market today and afford a representative range of standardized "tar" yields (14, 10, and 5 mg/cig, respectively by the Cambridge Filter Method). The brand styles studied are part of a commercial cigarette brand family that does not contain additives to the tobacco blend, including glycerol. Mainstream smoke was generated by an automated smoking machine employing the standard Cambridge Filter Smoking Regime and a more intense regime requiring larger, more frequent puffs and 100% vent blocking that is specified for regulatory purposes by the Canadian federal government. The research indicated that only a small fraction of added glycerol (~0.25%–0.30%, w/w) was converted to the two compounds of interest, with the larger portion generally observed in sidestream smoke. Less than 0.1% of the added glycerol was converted to acrolein in mainstream smoke for all cigarette designs and smoking regimes studied.[Beitr. Tabakforsch. Int. 24 (2010) 48–57]

ZUSAMMENFASSUNG

Durch die Zugabe von Glycerin-$^{13}C_3$ zu drei verschiedenen Sorten einer führenden kommerziellen Zigarettenmarke wurden quantitativ Acrolein und Aceton bestimmt, die bei der Zersetzung von Glycerin im Tabak einer brennenden Zigarette gebildet werden. Mehrlagige Cambridge-Filter wurden mit einer Lösung von 2,4-Dinitrophenylhydrazine (DNPH) getränkt, um die Hydrazinderivate der niedermolekularen Carbonylverbindungen im Hauptstromrauch und im Seitenstromrauch aufzufangen. Die bei der Verbrennung entstandenen DNPH-Derivate der flüchtigen Carbonylprodukte wurden mit Hilfe von HPLC (High performance liquid chromatography), gekoppelt mit negativer Ionen-Massenspektrometrie, isoliert und ihre Konzentration bestimmt. In dieser Arbeit lag das hauptsächliche Interesse bei den Komponenten Acrolein, Aceton und Propionaldehyd. Zur absoluten Quantifizierung dienten die DNPH-Derivate von unabhängig synthetisiertem Acrolein-$^{13}C_3$ und Aceton-$^{13}C_3$ als externe Standards. Die Kosten von vollständig markiertem Propionaldehyd überstiegen den Nutzen in dieser Arbeit. Die Zigarettenmarken, die in dieser Arbeit ausgewählt wurden, stellen einen repräsentativen Querschnitt von standardisierten Kondensatwerten (14, 10 und 5 mg/zig bestimmt mit der Cambridge-Filter-Methode) und den Merkmalen des

*Received: 6th August 2009 – accepted: 25th February 2010

Zigarettendesigns, die im heutigen U.S. Markt vorherrschend sind, dar. Die hier untersuchten Markenformate sind Teil einer kommerziellen Zigarettenmarkenfamilie, die keine Additive, also auch kein Glycerin, in der Tabakmischung enthält. Der Hauptstromrauch wurde mit einer automatisierten Rauchmaschine nach der standardisierten Cambridge-Filter-Methode und einer intensiveren Methode produziert. Bei der intensiveren Methode, die für regulatorische Zwecke der kanadischen Regierung spezifiziert ist, werden längere und häufigere Züge genommen und die Filterventilation wird zu 100% blockiert. Die Ergebnisse deuten darauf hin, dass nur ein kleiner Anteil des zugesetzten Glycerins (~0,25%–0,30%, w/w) zu den beiden untersuchten Substanzen umgesetzt wird. Dabei konnte grundsätzlich eine größere Portion im Seitenstromrauch beobachtet werden. Bei allen untersuchten Zigarettendesigns und Abrauchverfahren wurde weniger als 0,1% des zugesetzten Glycerins zu Acrolein im Hauptstromrauch umgesetzt. [Beitr. Tabakforsch. Int. 24 (2010) 48–57]

RESUME

L'ampleur de la dégradation du mélange de glycérol dans une cigarette en train de se consumer pour former de l'acroléine et de l'acétone a été quantifiée par l'addition de glycérol-$^{13}C_3$ aux trois sortes de cigarettes d'une marque leader sur le marché. Plusieurs tampons Cambridge imbibés d'une solution de 2,4-dinitrophénylhydrazine (DNPH) ont été employés pour piéger des dérivés d'hydrazone de composés de carbonyle de faible poids moléculaire dans la fumée principale et la fumée latérale. La chromatographie liquide à haute performance en tandem avec la spectrométrie de masse des ions négatifs a été employée pour isoler les dérivés DNPH des produits carbonyles volatiles de combustion et pour établir leur concentration. Acroléine, acétone et propionaldéhyde ont été les principaux composés étudiés. Les dérivés DNPH de l'acroléine-$^{13}C_3$ et de l'acétone-$^{13}C_3$ ont été synthétisés indépendamment et ils ont servi de normes externes pour la quantification absolue. Le coût du propionaldéhyde entièrement marqué exclut son utilisation dans cette étude. Les sortes de cigarettes de marque sélectionnées pour l'étude présentent les caractéristiques de conception des cigarettes actuellement les plus courantes sur le marché américain et offrent une gamme de taux de «goudron » standardisée représentative (14, 10 et 5 mg/cig respectivement selon la méthode de filtre Cambridge). Les sortes de cigarettes de la marque étudiées ici font partie d'une famille de cigarettes de marque commercialisées qui ne contiennent pas d'additifs au mélange de tabac, y compris le glycérol. La fumée principale a été générée par une machine à fumer automatique utilisant un régime de fumage standard pour le filtre Cambridge et un régime plus intense exigeant des bouffées plus grandes et plus fréquentes et obstruction des orifices à 100%, ce qui est spécifié par le gouvernement fédéral canadien à des fins de régulation. La recherche a indiqué que seule une faible fraction du glycérol ajouté (~0,25% à 0,30%, p/p) a été convertie dans les deux composés étudiés, la majeur partie ayant généralement été observée dans la fumée latérale. Moins de 0,1% du glycérol ajouté a été converti en acroléine dans la fumée principale pour toutes les conceptions de cigarettes et les régimes de fumage étudiés. [Beitr. Tabakforsch. Int. 24 (2010) 48–57]

INTRODUCTION

Glycerol (1,2,3-trihydroxypropane) is a naturally occurring component of tobacco and is also among the major tobacco additives in U.S. domestic cigarettes. Approximately 1–3% glycerol is routinely used as a humectant to aid tobacco processing, to improve product shelf life, and to enhance cigarette smoke quality. While glycerol is not a cigarette smoke toxicant, it is considered to be a precursor source from which acrolein is formed via dehydration under some pyrolytic conditions (1). Acrolein, an irritant and lacrimator, is a compound of regulatory interest, with annual reporting of smoke yields required in some jurisdictions (2, 3). Acrolein is present in both mainstream and sidestream cigarette smoke at concentrations typically between ~2–150 µg/cig, depending upon the cigarette design studied and the machine smoking regime applied for testing (4). Generally, mainstream smoke (MS) yields increase with more intense machine smoking, suggesting the possibility that a greater rate of conversion from a precursor molecule such as glycerol may occur with more intense smoking. Thus, establishing a better understanding of the precursor/fate relationship by which acrolein may be formed from glycerol during cigarette smoking provides a potential opportunity to design and develop consumer-acceptable cigarettes with reduced levels of this toxicant in MS.

The extent of glycerol decomposition to form acrolein when a cigarette is smoked continues to be an open area of scientific discussion and debate. Pyrolysis of neat glycerol in air at 700 °C produces extensive decomposition of glycerol with acrolein as a principal product (5). However, under pyrolysis conditions deemed more relevant to the complex and dynamic environment in a burning cigarette, glycerol remains 99.8% intact (6). Several reports that have considered the potential conversion of added glycerol to acrolein when a cigarette is smoked provide disparate results (7–9). Findings for glycerol conversion to MS acrolein have ranged from 0% contribution to MS (8) to an increase of ~26% in MS acrolein (9). Glycerol conversion rates as great as 1% have been reported; however, the only associated acrolein increase was found in sidestream smoke (SS, 8). Findings for glycerol conversion to MS acetone have ranged from 0% contribution to MS (8) to an increase of ~7% in MS acetone (9).

Research reports that address the glycerol/acrolein precursor/fate relationship have examined a limited number of cigarette designs to date and all work has been conducted with a single smoking regime (7–9). The purpose of the study reported here is to quantitatively measure the extent of tobacco blend glycerol degradation to two low molecular weight carbonyl compounds, acrolein and acetone, as a function of a broader range of cigarette designs and smoking regimes. The brand styles selected for study are part of a commercial cigarette brand family that does not contain additives to the tobacco blend, including glycerol. However, the brand styles studied represent the principal cigarette design features (tobacco weights, expanded tobacco content, filter ventilation, etc.) that are most prevalent in the U.S. market today and afford a represen-

Table 1. Mobile phase gradient schedule for separation of dnph carbonyl derivatives via reversed phase liquid chromatography [a]

Time (min)	A (%) [b]	B (%) [c]
0	100	0
12	80	20
13	65	35
13.5	100	0
18	100	0

[a] Flow rate: 0.3 mL/ min; column: Agilent Zorbax Eclipse XDB-C18 150 × 2.1 mm, 5 µm

[b] Solvent A: water-acetonitrile-THF-*iso*-propanol : 59:30:10:1 (*v/v*)

[c] Solvent B: acetonitrile-water : 65:35 (*v/v*)

tative range of standardized "tar" yields (14, 10, and 5 mg/cig, respectively, by the Cambridge Filter Method, 10). Generation of mainstream smoke employing both the standard Cambridge Filter Smoking Regime and a more intense regime (one that requires larger, more frequent puffs and 100% filter vent blocking) provides the opportunity to examine the robustness of results when generated with multiple smoking regimes. Also, data generated with multiple smoking regimes may be more relevant to the range of ways in which smokers smoke cigarettes.

EXPERIMENTAL

Reagents

High performance liquid chromatography grade acetonitrile, water, tetrahydrofuran, and isopropanol were purchased from EMD Chemicals Inc. (Gibbstown, NJ USA). Acrolein-$^{13}C_3$, acetone-$^{13}C_3$ and glycerol-$^{13}C_3$ were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA USA). The cigarette brand styles for study, which contained no additives to the tobacco blend, were Winston Full Flavor King Soft Pack, Winston Light King Soft Pack, and Winston Ultralight King Hard Pack. Standard Cambridge Filter Method Smoking Regime "tar" yields for the three brands were 14, 10, and 5 mg/cig respectively.

HPLC-MS/MS analysis

All HPLC separations were obtained using an Agilent (Wilmington, DE USA) HPLC 1100 series equipped with diode array detector, column heater, and Thermo Survey (San Jose, CA USA) Auto-Sampler. A methanol solution (10 µL) of each smoke trap extract was injected onto the chromatographic column via the auto-sampler. The column for all HPLC separations was an Agilent Zorbax Eclipse XDB-C_{18} (150 mm x 2.1 mm, 5 µm). The gradient delivery and binary mobile phase are noted in Table 1 and the accompanying footnote respectively. The mobile phase was delivered to the HPLC column at a flow rate of 0.3 mL/min.

For mass spectral analysis, the HPLC column effluent (10 µL injection volume) was pumped directly (without split) into the spray chamber of a Thermo Instrument TSQ triple quadrupole mass spectrometer (Thermo Finnigan, San Jose, CA USA) equipped with an atmospheric pressure chemical ionization (APCI) source. The instrument was calibrated with a solution of polytyrosine according to the manufacturer's recommendation. Tuning parameters were established and optimized via trial and error in order to achieve reasonable MS sensitivity and analyte confirmation. MS parameters for separation and detection of the derivatized carbonyl compounds in the negative ion mode are listed below:

Spray voltage:	3200 V
Discharge current:	4.0 µA
Sheath gas pressure:	60 (arbitrary value)
Auxiliary gas pressure:	20 (arbitrary value)
Capillary temperature:	350 °C
APCI vaporization temperature:	400 °C

Cigarette core injection

Glycerol-$^{13}C_3$ was added to each cigarette by injection of 40 µL of a methanol solution (0.45 mg/µL). The loaded syringe was inserted through the center of the tobacco rod to a depth of 3 mm from the tipping paper. The syringe was then backed out of the cigarette; while the plunger was depressed over a period of five minutes to uniformly inject the glycerol solution without producing damp spots on the wrapping paper. The core injected cigarettes were then placed in a sealed container at ambient temperature/ humidity for three weeks prior to machine smoking. Depending upon the brand style, 2.5–3.0% (w/w) labeled glycerol was achieved in the tobacco blend based upon the amount of injected glycerol and the weight of the tobacco in the particular brand style.

Preparation of Cambridge filter pads for carbonyl compound collection

Multiple Cambridge filter pads were employed for trapping mainstream and sidestream smoke. Each 44 mm Cambridge glass fiber filter pad was pre-treated with a solution of recrystallized DNPH in acetonitrile (749.8 mg of recrystallized DNPH/50 mL acetonitrile) that also contained 100 mL of perchloric acid. Fifty milliliters of the DNPH solution was sufficient to saturate 23 pads (i.e. yielding approximately 33 mg of DNPH/pad). After treatment, the filter pads were later placed in a ventilated hood at room temperature for 30 min. in order to remove residual solvent prior to smoking. At this point, the treated Cambridge pads were ready for smoke collection. Given the DNPH loading per pad, the use of three treated Cambridge pads provided approximately 7,300 µg/cig of MS carbonyl compound collection capacity, based on acetaldehyde reaction stoichiometry. Similarly, the use of five treated Cambridge pads provided approximately 12,000 µg/cig of sidestream smoke (SS) carbonyl compound collection capacity, based on acetaldehyde reaction stoichiometry.

Smoke generation and collection

Two sets of smoking conditions were employed: (a) 35 cm^3 puff volume/60 sec puff frequency/2 sec puff duration with no vent-blocking and (b) 55 cm^3 puff volume/30 sec puff frequency/2 sec puff duration with 100% vent-blocking.

These smoking regimes are typically referred to as the Cambridge Filter Method Smoking Regime and the Canadian Intense Smoking Regime, respectively. With the latter, filter vent-blocking was achieved with "Scotch™ Transparent Tape 600" applied to 100% of the filter circumference from the mouth-end of the filter to the end of the tipping. Cigarettes were smoked to the length of the filter over-wrap plus 3 mm, using a Borgwaldt Smoking Machine pneumatic panel (Heinr. Borgwaldt GmbH, Hamburg, Germany) and a "Total Smoke Collection Chamber." The smoke generation and collection protocol was based on a previously published method reported by STEVENS and BORGERDING (11). MS was collected using three 44 mm Cambridge DNPH-impregnated glass fiber filter pads in series. Cigarettes were lit with an electric lighter. SS was collected using five 44 mm Cambridge DNPH-impregnated filter pads in series. During lighting and smoking of each cigarette, dry air flowed through the chamber at a rate of 1.25 L/min. Cigarettes were extinguished by flooding the chamber with helium. Each smoke session consisted of three cigarettes per replicate and a total of three replicates. After each smoking, the filter pads remained in the holder for approximately 3 min to ensure complete reaction. Each pad extract was then individually extracted and filtered before injection into the LC-MS/MS system.

Preparation of standard acrolein/acetone-DNPH derivatives

To approximately 17.4 mL of acetonitrile was added 4.5 mg of acrolein-$^{13}C_3$ (or 4.65 mg of acetone-$^{13}C_3$), 260.8 mg of recrystallized DNPH (excess) and 34.8 µL of perchloric acid. The solution was mixed gently and allowed to stand for 10 min after which 0.1 mL of pyridine was added. The resulting mixture was diluted to 10 mL with acetonitrile. The concentration of standard DNPH derivative ($^{13}C_3$) was calculated to be 1.82 mg/mL in the case of acrolein and 1.88 mg/mL in the case of acetone.

RESULTS AND DISCUSSION

Cigarette smoke is a complex mixture consisting of more than 5300 constituents (12, 13). MS (smoke drawn from the mouth-end of the cigarette during smoking) and sidestream smoke (SS, smoke from the lit end) are formed from complex overlapping mechanisms, including pyrolysis, pyrosynthesis, distillation, sublimation, condensation and combustion (14). Individual smoke constituents may be directly transferred from the tobacco column or formed from one, or more, precursor molecules. Thus, accurately discerning the fate of a particular cigarette constituent is a formidable analysis challenge.

Strategically placed, isotopically labeled compounds are an ideal means to follow the fate of an individual tobacco blend constituent when a cigarette is smoked, since the fate of labeled compounds can be followed directly based on the isotopic enrichment. Practical limitations to the approach include limited availability of labeled compounds (i.e., labeled analogs of only a small percentage of the materials present in tobacco are available) and the fact that most compounds in the tobacco blend are present at trace levels. Glycerol, however, is well suited to such an approach as it is both readily available with isotopic labeling and is typically present in the tobacco blend at percentage levels. Therefore, the extent of blend glycerol degradation in a burning cigarette to form acrolein and acetone is quantitatively assessed in this work based upon monitoring the fate of glycerol-$^{13}C_3$ added to commercial cigarette brand styles.

Table 2. Molecular masses of DNPH derivatives of selected volatile carbonyl compounds and their negative ion analogs. Negative ion value in parenthesis.

Derivative	Molecular Mass
Acrolein-$^{12}C_3$	236 (235)
Acetone-$^{12}C_3$	238 (237)
Propionaldehyde-$^{12}C_3$	238 (237)
Acrolein-$^{13}C_3$	239 (238)
Acetone-$^{13}C_3$	241 (240)
Propionaldehyde-$^{13}C_3$	241 (240)

Chromatographic separation and peak assignment

The analysis of low molecular weight carbonyl compounds such as formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, and butyraldehyde in cigarette smoke can be difficult because of their volatility and reactivity. Similar molecular weights for these small molecules also provide additional challenge. The analytical approach followed in this study is based on the derivatization procedure reported by DONG and MOLDOVEANU (15), an adaptation of the liquid chromatography reported by WANG *et al.* (16) and selective mass spectrometric detection in the negative ion mode. Formation of DNPH derivatives and separation via liquid chromatography address the volatility and reactivity associated with the compounds of interest. Mass spectrometry provides the ability to identify and selectively quantitate both natural abundance and isotopically labeled compounds.

Mainstream and sidestream smoke samples generated with either smoking regime yielded qualitatively similar chromatographic traces. Chromatographic peak assignments were established from standard solutions and from mass spectral data. Figure 1, a typical sidestream smoke separation from glycerol-$^{13}C_3$ fortified cigarettes, summarizes chromatographic peak assignments for the compounds of interest. The molecular mass of the pertinent carbonyl products expected to possibly arise from the smoking process are listed in Table 2. All mass spectral data were obtained in the negative ion mode; therefore the observed masses are one unit less than the actual molecular mass of the respective derivative.

Figure 1A shows the SIM 237.8–238.2 mode response. Inspection of Table 2 suggests that acrolein is the only isotopically labeled compound expected to produce a response for this ion mass range. However, three peaks were found at approximately 11.87, 12.92, and 14.24 min. The peak at 12.92 corresponds to the acrolein-$^{13}C_3$ molecular ion as detected in the negative ion mode. The peaks at 11.87 and 14.24 min correspond to the negative ion analogs of the acetone-$^{12}C_3$ and propionaldehyde-$^{12}C_3$ M+1 ions, respectively (Figure 2).

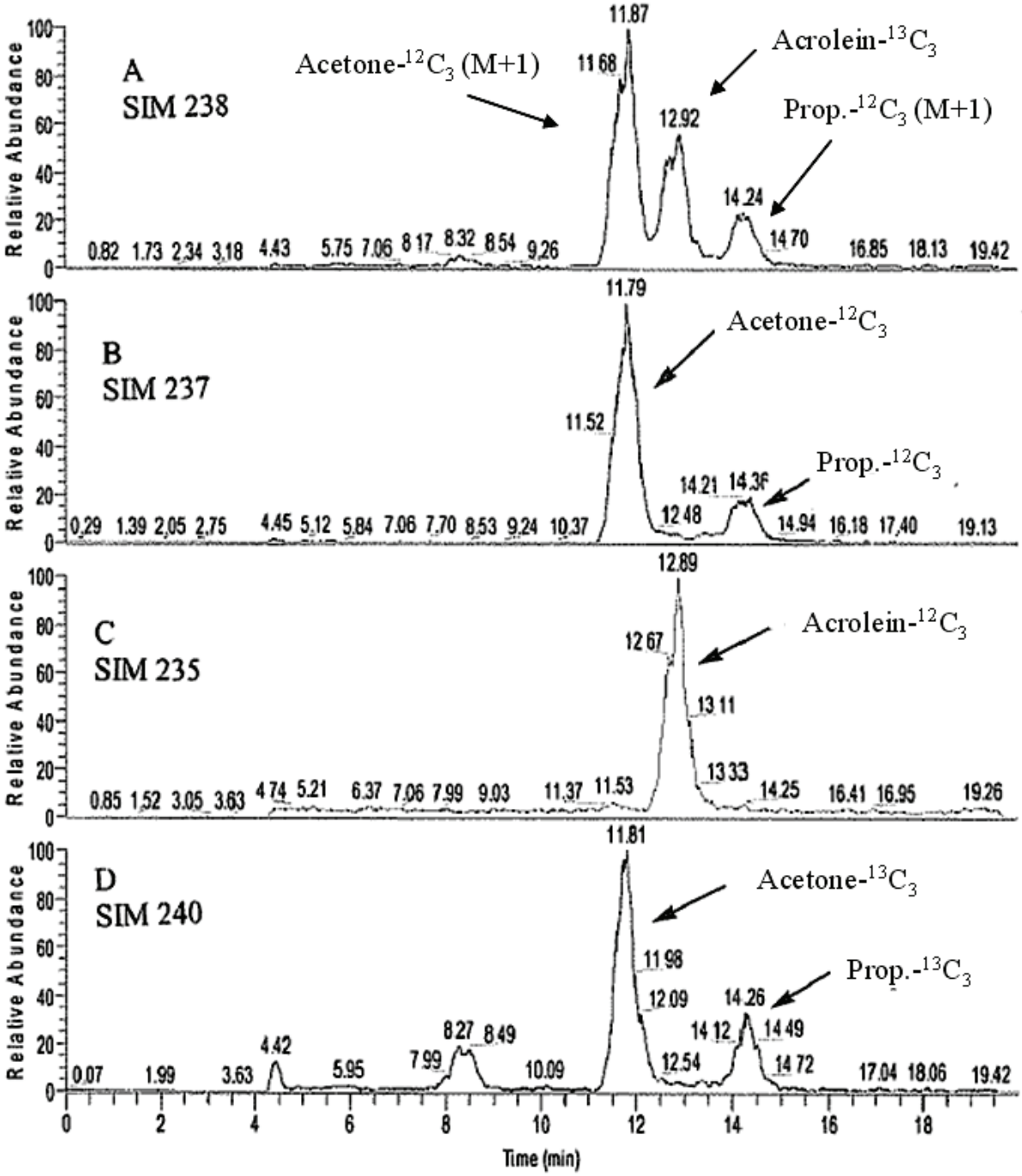

Figure 1. HPLC-MS separation of a sidestream smoke extract from a cigarette following a labeled glycerol spike, a 35/60/2 smoking regime, and derivatization via DNPH (A) SIM, m/z 237.8–238.2; (B) SIM, m/z 236.8–237.2; (C) SIM, m/z 234.8–235.2; and (D) SIM, m/z 239.8–240.2.

Response from non-isotopically labeled carbonyl compounds is expected. The cigarette brand styles studied in this work are part of a commercial cigarette brand family that does not contain additives to the tobacco blend, including glycerol. These cigarettes do, however, produce acrolein, acetone and propionaldehyde concentrations in mainstream and sidestream smoke that are typical of other cigarette brands styles that include glycerol as a tobacco additive. Response from non-isotopically labeled carbonyl compounds therefore arises from incomplete tobacco combustion during smoking, rather than from glycerol. It is important to note that since the M+1 ion is a minor ion within the acetone and propionaldehyde spectra, Figure 1A is a first indication that very little conversion of labeled glycerol to acrolein-$^{13}C_3$ occurs since the molecular ion response observed from acrolein-$^{13}C_3$ is similar to, or smaller, than the M+1 response observed for the other non-labeled compounds.

Figure 1B, the chromatographic trace for SIM 236.8–237.2, and Figure 1C, the chromatographic trace for SIM

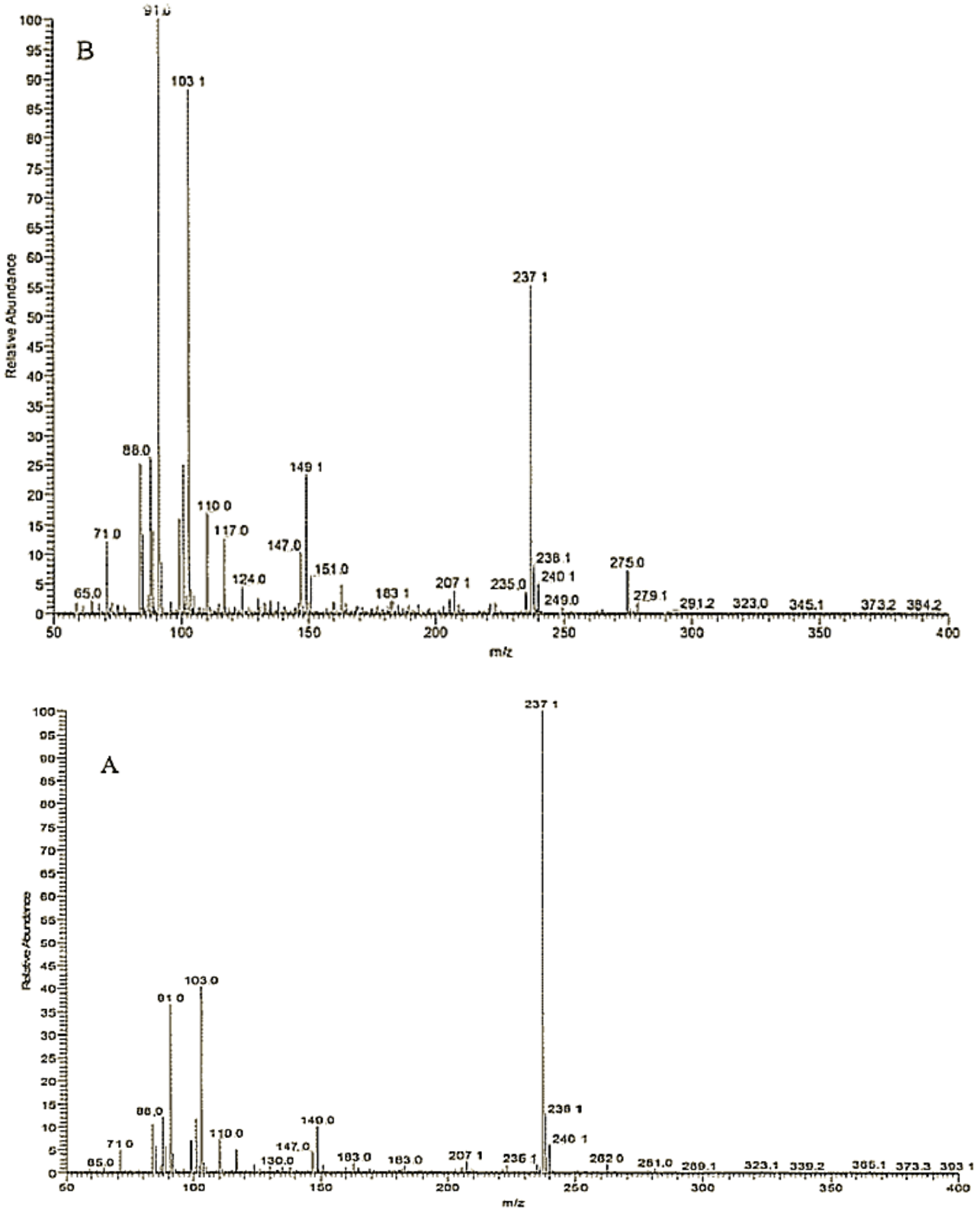

Figure 2. The mass spectra of components eluting at (A) 11.8 min (acetone) and (B) 14.2 min (propionaldehyde) in Figure 1.

234.8–235.2, confirm the retention times for unlabeled acetone, propionaldehyde and acrolein. Figure 1D, the chromatographic trace for SIM 239.8–240.2, confirms the retention time for isotopically labeled acetone. As expected, labeled and natural abundance analogs for each carbonyl compound co-elute at a single retention time.

Acrolein and acetone trapping efficiency

Prior to quantification, trapping efficiency experiments were first conducted with the Canadian Intense Smoking Regime, the more intense of the two smoking regimes studied, to ensure quantitative trapping of acrolein and

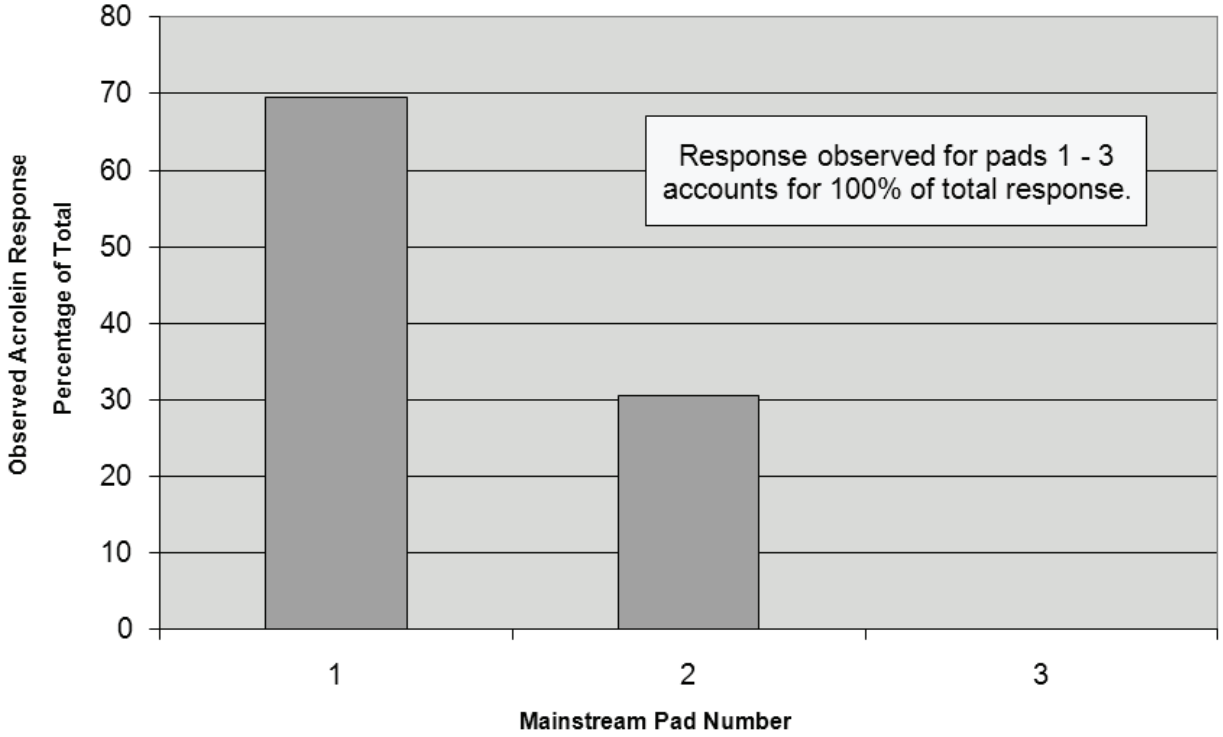

Figure 3. Mainstream acrolein trapping efficiency via pad number: Winston Light, Canadian Intense Regime

acetone. Mainstream smoke and sidestream smoke collection were each accomplished in initial experiments with three treated Cambridge pads per smoke stream. Results from these experiments suggested that mainstream smoke acrolein and acetone could be quantitatively trapped with three Cambridge pads with no detectable response observed for the last pad in the series (Figure 3). However, sidestream smoke acrolein and acetone were not efficiently trapped with three Cambridge pads as indicated by a substantial response for each compound on the third collection pad. Sidestream smoke collection and quantification was ultimately achieved with a series of five treated Cambridge pads (Figure 4). While acrolein and acetone response was observed for each Cambridge pad, an approximately exponential decay in observed response for the final four pads in the five pad series indicates that about 85% of expected acrolein or acetone response is accounted for with five Cambridge pads.

Table 3. Added glycerol converted to acrolein %, (CV). Results based on 3 MS Cambridge pads and 5 SS Cambridge pads.

	Mainstream smoke		Sidestream smoke	
Winston cigarette brand style	Cambridge Filter Regime [a]	Canadian Intense Regime [b]	Cambridge Filter Regime	Canadian Intense Regime
Full flavor	0.030 (12.0)	0.047 (12.6)	0.109 (2.9)	0.106 (11.2)
Light	0.032 (4.4)	0.062 (6.8)	0.113 (7.4)	0.097 (3.2)
Ultralight	0.020 (10.0)	0.077 (9.6)	0.132 (2.1)	0.077 (2.1)

[a] 35 cm^3 puff volume/60 sec puff frequency/2 sec puff duration with no vent-blocking.

[b] 55 cm^3 puff volume/30 sec puff frequency/2 sec puff duration with 100% vent-blocking.

Conversion of glycerol to acrolein and acetone during smoking

The average percent conversions of glycerol-$^{13}C_3$ to acrolein-$^{13}C_3$ and acetone-$^{13}C_3$ observed in this study together with associated analytical precision data are summarized in Tables 3 and 4. In general, precision of the replicate determinations was ~10%, or less. From the data, it is clear that that a small fraction (~0.25–0.30%) of added glycerol was converted to acrolein and acetone upon smoking; with contributions to both mainstream and sidestream smoke found. Glycerol conversion to mainstream acrolein (Table 3) was less than ~0.08% for all cigarette brand styles when cigarettes were smoked with either the standard Cambridge Filter Method Regime or the Canadian Intense Regime. Slightly greater acrolein conversion rates were consistently observed for the more intense of the two smoking regimes. Conversion of glycerol to sidestream smoke acrolein was greater than that observed for mainstream smoke, even though the extent of conversion was also quite small (less than ~0.15%). Unlike mainstream smoke, nominally greater rates of conversion to sidestream smoke acrolein were found for the standard Cambridge Filter Smoking Regime. Data trends for the conversion of glycerol to acetone are similar to trends observed for acrolein (Table 4), although conversion to sidestream acetone is less than ~0.08%.

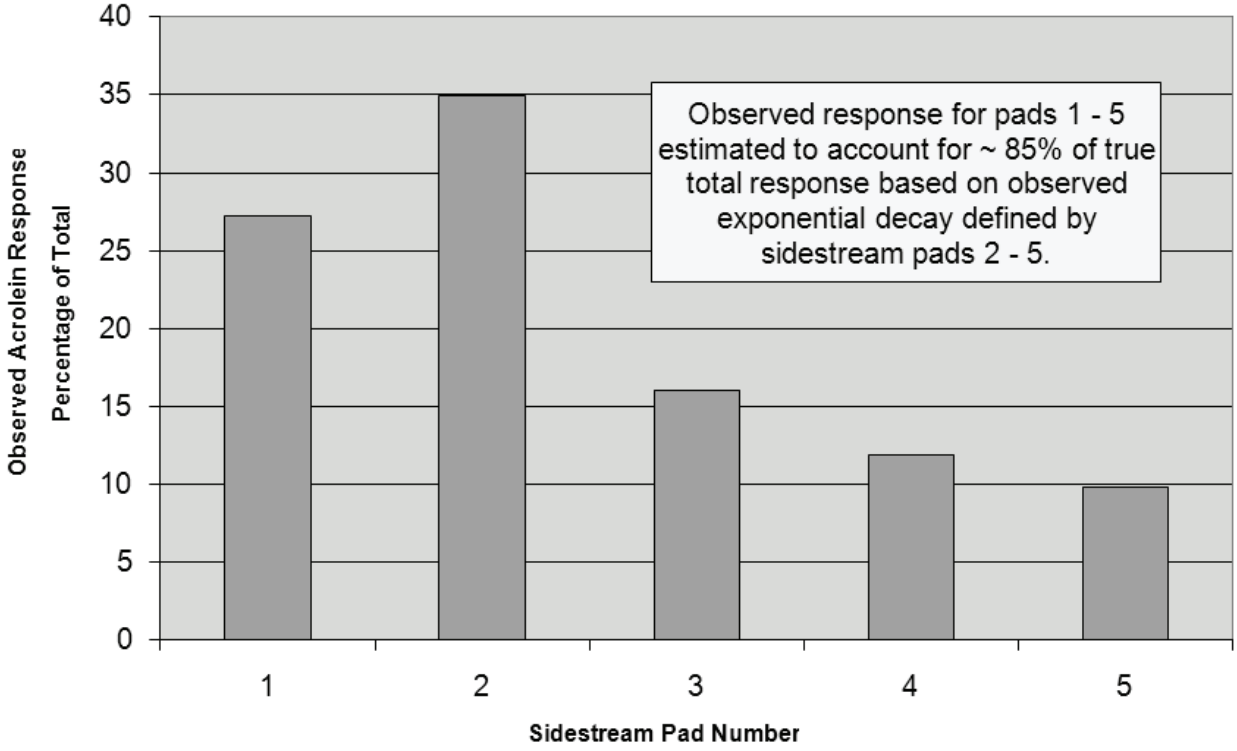

Figure 4. Sidestream acrolein trapping efficiency via pad number: Winston Light, Canadian Intense Regime

Tables 5 and 6 express the results obtained for acrolein and acetone on a mass basis (µg/cig). For mainstream smoke, the decomposition of glycerol during smoking yielded ~2–9 µg/cig acrolein and ~2–7 µg/cig acetone, depending upon the cigarette configuration and the smoking regime applied. For sidestream smoke, the decomposition of glycerol during smoking yielded ~8–15 µg/cig acrolein and ~6–9 µg/cig acetone, depending upon the cigarette configuration and the smoking regime applied. Although a small absolute difference, conversion of glycerol to SS acrolein and acetone was greater for the Cambridge Filter Regime than for the Canadian Intense Regime. Mainstream results did not follow this trend, with greater results observed for the Canadian Intense Regime.

It is useful to consider the extent of glycerol conversion to mainstream smoke acrolein and acetone in the context of typically observed mainstream smoke values. Comparison of results in Tables 5 and 6 to MS values reported by COUNTS *et al.* (4) for U.S. cigarettes suggests that added glycerol could be the precursor source for approximately 6–11% of the mainstream smoke acrolein and approximately 1–2% of the mainstream smoke acetone when cigarettes are smoked with the standard Cambridge Filter Method Regime. These estimates are based on at least three assumptions: (1) the Cambridge Filter Smoking Regime applied in this work and the ISO smoking regime applied in the COUNTS *et al.* study yield comparable smoke values, (2) the amount of glycerol added to the Marlboro cigarettes in the COUNTS *et al.* study is comparable to the amount of isotopically labeled glycerol added to the Winston cigarettes in this study and (3) minor differences in product design and tobacco blend between the products in the COUNTS study and the current work do not substantially affect glycerol decomposition upon smoking. Smaller conversion values are found for the more intense smoking regime. Canadian Intense Regime data from the two studies considered in a like manner suggest that glycerol could be the precursor source for approximately 4–7% of the mainstream smoke acrolein and approximately 1% of the mainstream smoke acetone. Clearly, such results indicate that glycerol is not the principal precursor material for acrolein formation in cigarette smoke.

Unlike the Marlboro cigarettes studied by COUNTS *et al.* which contain typical levels of added glycerol, the Winston brand styles studied in this work are part of a commercial cigarette brand family that does not contain additives to the tobacco blend, including glycerol. However, the yields of mainstream smoke acrolein and acetone observed for Winston cigarettes are comparable to other U.S. cigarettes, providing additional evidence for non-glycerol materials as the primary source of low molecular weight carbonyl compounds in cigarette smoke. Comparison of the amounts

Table 4. Added glycerol converted to acetone %, (CV). Results based on 3 MS Cambridge pads and 5 SS Cambridge pads.

	Mainstream smoke		Sidestream smoke	
Winston cigarette brand style	Cambridge Filter Regime	Canadian Intense Regime	Cambridge Filter Regime	Canadian Intense Regime
Full flavor	0.032 (15.1)	0.040 (17.1)	0.076 (1.5)	0.075 (15.8)
Light	0.031 (8.8)	0.053 (13.2)	0.071 (8.7)	0.066 (4.7)
Ultralight	0.022 (2.8)	0.057 (7.6)	0.077 (0.7)	0.051 (14.5)

Table 5. Added glycerol converted to acrolein-$^{13}C_3$, µg/cig, (CV). Results based on 3 MS Cambridge pads and 5 SS Cambridge pads.

	Mainstream smoke		Sidestream smoke	
Winston cigarette brand style	Cambridge Filter Regime	Canadian Intense Regime	Cambridge Filter Regime	Canadian Intense Regime
Full flavor	3.4 (12.0)	5.2 (12.6)	12.2 (2.9)	11.8 (11.2)
Light	3.6 (4.4)	6.9 (6.8)	12.7 (7.4)	10.8 (3.2)
Ultralight	2.2 (10.0)	8.6 (9.6)	14.7 (2.1)	8.6 (2.1)

Table 6. Added glycerol converted to acetone-$^{13}C_3$, µg/cig, (CV). Results based on 3 MS Cambridge pads and 5 SS Cambridge pads.

	Mainstream smoke		Sidestream smoke	
Winston cigarette brand style	Cambridge Filter Regime	Canadian Intense Regime	Cambridge Filter Regime	Canadian Intense Regime
Full flavor	3.7 (15.1)	4.6 (17.1)	8.7 (1.5)	8.7 (15.8)
Light	3.6 (8.8)	6.1 (13.2)	8.2 (8.7)	7.6 (4.7)
Ultralight	2.5 (2.8)	6.5 (7.6)	8.8 (0.7)	5.9 (14.5)

of acrolein and acetone observed for Winston cigarettes without additives and the amounts of isotopically labeled acrolein and acetone observed for Winston cigarettes containing labeled glycerol suggests that adding typical levels of glycerol to Winston cigarettes would increase acrolein yields by ~5–7% and ~3–7% when smoked with the standard Cambridge Filter Method Regime and Canadian Intense Regime, respectively, depending upon the cigarette configuration. Acetone yields would increase by ~1–2% and 1% when smoked with the standard Cambridge Filter Method Regime and Canadian Intense Regime, respectively, depending upon the cigarette configuration.

Pyrolysis studies as a model for conversion of glycerol to acrolein and acetone during smoking

As discussed by STOTESBURY *et al.* (17), pyrolysis techniques offer several advantages as a model system for studying the fate of tobacco additives during smoking. Advantages include rapid evaluation, minimal sample preparation, use of straightforward instrumental analysis techniques and relatively simple data analysis since samples are free of tobacco combustion materials. STOTESBURY *et al.* also note a significant limitation of pyrolysis as a model for combustion during smoking. That limitation is the need to verify that the experimental conditions chosen for study produce results that are meaningful in terms of actual smoke chemistry. Such verification is ideally accomplished by following the fate of isotopically labeled materials added to a cigarette as has been done in this work for glycerol.

Following the fate of an isotopically labeled compound precludes the limitations that may occur when pyrolysis is not a perfect model for the burning cigarette, which is often the case as many pyrolysis experiments fall short of the ideal scenario described by STOTESBURY *et al.* (17). For example, a wide range of glycerol pyrolysis conditions have been reported that produce varying degrees of glycerol decomposition to form acrolein (1, 5, 6). Results from this study (Tables 3 and 4) suggest that the conditions developed by BAKER and BISHOP (6) to approximate the pyrolysis zone of a burning cigarette are reasonable and appropriate for the intended purpose.

CONCLUSIONS

In conclusion, the extent of blend glycerol degradation in a burning cigarette to form acrolein and acetone has been quantitatively determined by the addition of glycerol-$^{13}C_3$ to three styles of a leading commercial cigarette brand. A small fraction of added glycerol (~0.25–0.30%, w/w) was converted to the two carbonyl compounds of interest, with the largest response observed in sidestream smoke. Less than 0.1% of the added glycerol was converted to acrolein in mainstream smoke for all cigarette designs and smoking regimes studied. Thus, glycerol as typically added to U.S. cigarettes is not the principal source of MS acrolein or acetone, a finding that was consistent for all cigarette designs and smoking regimes studied. Additionally, results from this study suggest that the analytical conditions developed by BAKER and BISHOP to approximate the pyrolysis zone of a burning cigarette are reasonable and appropriate for the intended purpose.

ACKNOWLEDGMENTS

This research was supported by a monetary gift to the Department of Chemistry from R. J. Reynolds Tobacco Company. Drs. Jianxin Yu and Mehdi Ashraf-Khorassani were selected as RJRT-Harold McNair Fellows in 2005.

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