Analysis of Acrylonitrile and α -Methacrylonitrile in Vapor Phase of Mainstream Cigarette Smoke Using a Charcoal Trap for Collection*

by

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SUMMARY

A simple procedure for the collection of vapor phase (VP) of mainstream cigarette smoke for analysis has been developed. This procedure consists of collecting the VP on a commercial charcoal trap (ORBO™-32) followed by dissolution in acetone. The acetone extract can be analyzed by a gas chromatography/mass spectrometry (GC/MS) technique. A qualitative analysis of the collected VP has been performed for 3R4F Kentucky reference cigarette, allowing the identification of 138 compounds, some compounds being present in both VP and in particulate phase (PP) of cigarette smoke. A quantitative analysis method for acrylonitrile and α -methacrylonitrile (2-methyl-2-propenenitrile) was also developed, and the level of these compounds in 15 different cigarette brands was measured. Acrylonitrile quantitation was selected since this compound in smoke poses significant health related issues. α -Methacrylonitrile quantitation was selected due to the similar structure of this compound with acrylonitrile. The analyzed cigarettes were several Kentucky reference cigarettes including 1R5F, 2R4F, 3R4F, 2R1F, and 1R3F, several King Size (KS) commercial cigarettes from the US market including Basic Non Filter (NF), Basic Ultra Lights (UL), Newport, Marlboro (Red), Marlboro Menthol, Camel Filter, Camel Lights, Camel Ultra Lights, and two herbal cigarettes, Ecstasy and Dreams. The results for acrylonitrile were in very good agreement with data reported in the literature for 2R4F and 1R5F cigarettes. The levels of α -methacrylonitrile were not previously reported. The correlation between the levels of acrylonitrile and of α -methacrylonitrile with the (wet) total particulate matter (TPM) was evaluated. Although the levels of acrylonitrile and of α -methacrylonitrile in mainstream smoke depend on the TPM values, the correlation is not very strong, indicating that the nature of the cigarette blend and possible other

factors in cigarette construction also influence their levels in smoke. The collection method used in this study allows the subsequent dissolution of VP in a much smaller volume of solvent compared to other methods that use impingers, allows the use of standard GC/MS autosamplers for liquid injection and simple addition of internal standards compared to the methods that use gas bags, and allows a simple and immediate collection of VP as it leaves the Cambridge filter pad. These characteristics represent significant advantages versus other methods commonly used for VP analysis. [Beitr. Tabakforsch. Int. 24 (2010) 145–156]

Keywords: Acrylonitrile, α -methacrylonitrile, vapor phase of cigarette smoke, GC/MS analysis.

ZUSAMMENFASSUNG

Es wurde ein einfaches Verfahren zum Auffangen der Gasphase (GP) des Hauptstromrauchs von Zigaretten zu Analysezwecken entwickelt. Dieses Verfahren besteht im Auffangen der GP in einer handelsüblichen Aktivkohlekartusche (ORBO™-32) und der anschließenden Extraktion mit Aceton. Der Acetonextrakt kann mit Hilfe von Gaschromatographie/Massenspektrometrie (GC/MS) analysiert werden. Eine qualitative Analyse der aufgefangenen GP wurde für die Kentucky-Referenzzigarette 3R4F durchgeführt. Dabei konnten 138 Substanzen identifiziert werden, von denen einige sowohl in der GP als auch in der Partikelphase (PP) des Zigarettenrauchs vorlagen. Eine quantitative Analyse für Acrylnitril und α -Methacrylnitril (2-Methyl-2-propennitril) wurde ebenfalls entwickelt und die Konzentration dieser Verbindungen in 15 verschiedenen Zigaretten sorten bestimmt. Die Quantifizierung von Acrylnitril wurde gewählt, da dieser Rauchbestandteil bedeutende Gesundheitsprobleme verursachen

kann. α -Methacrylnitril wurde aufgrund der dem Acrylnitril ähnlichen Struktur gewählt. Zu den analysierten Zigaretten zählten diverse Kentucky-Referenzzigaretten, darunter 1R5F, 2R4F, 3R4F, 2R1F und 1R3F, diverse auf dem US-Markt erhältliche King-Size-Zigaretten, darunter Basic Non Filter (NF), Basic Ultra Lights (UL), Newport, Marlboro (Red), Marlboro Menthol, Camel Filter, Camel Lights, Camel Ultra Lights sowie zwei Kräuterzigaretten, Ecstasy und Dreams. Die Ergebnisse bezüglich Acrylnitril stimmten sehr gut mit den in der Literatur für 2R4F- und 1R5F-Zigaretten berichteten Daten überein. Über Konzentrationen von α -Methacrylnitril wurden bisher noch nichts publiziert. Die Korrelation zwischen den Konzentrationen von Acrylnitril und α -Methacrylnitril und dem (feuchten) Gesamtkondensat (TPM) wurde untersucht. Obwohl die Konzentrationen von Acrylnitril und α -Methacrylnitril im Hauptstromrauch von den TPM-Werten abhängen, ist die Korrelation nicht sehr stark und deutet darauf hin, dass die Art der Tabakmischung und mögliche andere Designmerkmale der Zigarette ihre Konzentrationen im Rauch beeinflussen. Die in dieser Untersuchung verwendete Sammelmethode ermöglicht die anschließende Lösung der GP in einem viel geringeren Volumen von Lösungsmittel verglichen mit anderen Methoden, bei denen Impinger zum Einsatz kommen. Sie ermöglicht die Verwendung von Standard-Autosamplern für GC/MS zur Flüssigkeitszugabe und zum einfachen Hinzufügen von internen Standards verglichen mit Gasbeutel-Methoden, und sie ermöglicht das einfache und sofortige Sammeln der GP bei Austritt aus dem Cambridge-Filter. Diese Eigenschaften bieten bedeutende Vorteile gegenüber anderen Methoden, die häufig für die Gasphasenanalyse verwendet werden. [Beitr. Tabakforsch. Int. 24 (2010) 145–156]

RESUME

Une procédure simple de collecte de la phase vapeur (VP) de la fumée principale de cigarette à des fins d'analyse a été développée. Cette procédure consiste à collecter la VP dans un piège à charbon commercial (ORBO™-32) puis à la dissoudre dans l'acétone. L'extrait d'acétone peut être analysé par une technique de chromatographie gaz/spectrométrie de masse (GC/MS). Une analyse qualitative de la VP collectée a été réalisée avec une cigarette de référence Kentucky 3R4F, permettant l'identification de 138 composants, dont certains étaient présents à la fois en VP et en phase particulaire (PP) de la fumée de cigarette. Une méthode d'analyse quantitative de l'acrylonitrile et du α -méthacrylonitrile (2-méthyl-2-propénitrile) a également été développée et le taux de ces composants a été mesuré pour 15 marques différentes de cigarettes. La quantification d'acrylonitrile a été choisie car ce composant de la fumée pose des problèmes de santé significatifs. La quantification du composant α -méthacrylonitrile a été sélectionnée pour sa structure similaire à celle de l'acrylonitrile. Plusieurs cigarettes de référence Kentucky ont été analysées dont 1R5F, 2R4F, 3R4F, 2R1F et 1R3F, ainsi que plusieurs cigarettes commerciales King Size (KS) du marché américain dont Basic Non Filter (NF), Basic Ultra Lights (UL), Newport, Marlboro (Red), Marlboro Menthol, Camel Filter, Camel Lights, Camel Ultra Lights et deux cigarettes aux

plantes Ecstasy et Dreams. Les résultats relatifs à l'acrylonitrile étaient très similaires aux données rapportées dans la littérature pour les cigarettes 2R4F et 1R5F. Les niveaux de α -méthacrylonitrile n'avaient pas fait l'objet de rapports au préalable. La corrélation entre les niveaux d'acrylonitrile et de α -méthacrylonitrile et le total des particules (humides) (TPM) a été évaluée. Bien que les niveaux d'acrylonitrile et de α -méthacrylonitrile dans la fumée principale de cigarette dépendent des valeurs TPM, la corrélation n'est pas très forte, ce qui indique que la nature du mélange de cigarette et d'autres facteurs potentiels dans la fabrication de la cigarette influencent également leurs niveaux dans la fumée. La méthode de collecte utilisée dans cette étude permet la dissolution ultérieure de la VP dans un volume nettement inférieur de solvant comparé à celui d'autres méthodes utilisant des impacteurs. Elle permet d'utiliser des auto-échantillonneurs GC/MS standard pour l'injection de liquide ainsi qu'un simple ajout d'étalons internes comparé aux méthodes utilisant des ballonnets à gaz. Elle permet enfin une collecte simple et immédiate de la VP lorsqu'elle s'échappe du tampon-filtre Cambridge. Ces caractéristiques représentent des avantages significatifs par rapport aux autres méthodes généralement employées pour l'analyse de la VP. [Beitr. Tabakforsch. Int. 24 (2010) 145–156]

INTRODUCTION

Vapor phase (VP) of mainstream cigarette smoke is a complex mixture of gases and volatile liquids that are not retained when the smoke passes a Cambridge pad. Analysis of VP of cigarette smoke is an important subject since a number of toxicants such as HCN, acetaldehyde, ethylene oxide, and acrylonitrile are VP constituents. Most techniques for the analysis of VP of cigarette smoke depend on the collection in impingers with a solvent (typically also cooled; 1–5) or in a Tedlar gas bag (6, 7). Other analytical techniques for VP analysis involve cryogenic trapping (with no solvent), trapping on Tenax® (8), direct transfer of VP smoke to an analytical instrument (9–12) or even solid phase microextraction (13). Further separation and identification is typically done using GC or GC/MS analysis. Each collection procedure has advantages and disadvantages, related to a number of parameters such as collection efficiency, pressure drop through the impinger(s) when they are installed between the cigarette and the pneumatic panel of the smoking machine, stability of VP smoke components in the gas phase, adsorption on the wall of the Tedlar bag (in case of this type of collection), ease of addition of an internal standard to the sample, degassing in the GC syringe (in case of solvent collection), etc. An alternative procedure for the collection of the VP of cigarette smoke can utilize a charcoal trap (14, 15), which allows the quantitative collection of a large number of volatile compounds (16), and has considerable advantages over other collection techniques, as further described. Qualitative analysis of the charcoal trap extract leads to the identification of a considerable number of other components of the VP, with potential for their quantitation. The application of a charcoal trap for sample collection for quantitative purposes is described in this study only for the analysis of

acrylonitrile and α -methacrylonitrile. Acrylonitrile quantitation was selected since this compound in smoke poses significant health related issues. An IARC working group classified acrylonitrile as possibly carcinogenic to humans (Group 2B) based on sufficient evidence in experimental animals (17–19), while the US-EPA classified acrylonitrile as probable human carcinogen based on limited evidence of carcinogenicity in humans (17). For these reasons, the compound is considered a highly undesirable toxicant in smoke (20). On the other hand, α -methacrylonitrile is not considered a carcinogen, although it is a known toxicant (21). α -Methacrylonitrile quantitation was selected due to the similar structure of this compound with acrylonitrile.

EXPERIMENTAL

Sampling using a charcoal trap

For each sample, three cigarettes were smoked using a Borgwaldt rotary machine RM20/CSR (Borgwaldt, 22525 Hamburg, Germany). The particulate phase (PP) of mainstream cigarette smoke was collected on one 92 mm Cambridge pad. The pad holder was connected to a charcoal trap for the collection of components from the VP, and further to the pneumatic panel of the smoking machine. The trap was an ORBO™-32 Small trap (Supelco, Bellefonte, PA 16823–0048 USA) containing charcoal in two sections indicated as A and B. Section A of the tube contains 100 mg adsorbent charcoal and is designed to retain the analytes. Section B of the tube contains an additional 50 mg adsorbent charcoal and it is used as backup in case of breakthrough of the analyte. Smoking was done for all samples using 35 mL puff volume, 2 sec puff duration and 60 sec puff interval (indicated as 35/2/60), similar to Federal Trade Commission (FTC) smoking recommendations for a linear machine (22) and using the measurement of the exhaust flow of the Borgwaldt smoking machine between 4.17 and 4.36 m/s. Three of the cigarette brands were smoked in more intensive conditions using 60 mL puff volume, with a puff duration of 2 sec each puff taken at 30 sec interval (indicated as 60/2/30 conditions). The cigarette vent was not blocked in the experiments. After the cigarettes were smoked, one clearing puff was taken.

For each sample, the charcoal from section A of the ORBO™-32 trap was transferred into a 1.5 mL GC vial (Agilent, Wilmington, DE 19808, USA), and 1 mL acetone containing 20 $\mu\text{g/mL}$ $^2\text{H}_3$ -acrylonitrile was added. The $^2\text{H}_3$ -acrylonitrile was obtained from CDN Isotopes (Pointe-Claire, Quebec, H9R 1H1, Canada) and the acrylonitrile (further used for calibration) was obtained from Sigma/Aldrich (St. Louis, MO, 63168, USA). For several samples, the charcoal from section B of the trap was processed similarly. The acetone dissolved immediately the compounds adsorbed in the charcoal. The solution from the vials containing the VP components (without separating the extracted charcoal) was further analyzed by a GC/MS procedure.

The charcoal trap collection procedure is very simple. The ORBO™-32 trap can be connected immediately after the Cambridge pad holder and before the pneumatic panel of

the smoking machine, avoiding further aging of the VP of cigarette smoke in gas phase. The pressure drop across the ORBO™-32 trap is very low, with an average for three measurements of 460 mm H_2O (4.51 kPa). The use of a solvent for the dissolution of the compounds retained in the trap, allows the addition of internal standards at the desired concentration. However, the volume of the solvent used for the dissolution can be as low as 0.5 mL (in this study 1.0 mL solvent was used). This low volume of solvent is much smaller than the volume of solvent typically needed for collection in impingers, such that a more concentrated solution of VP constituents can be obtained compared to impinger collection technique. The use of a liquid injection (as opposed to a gas phase injection) allows the use of standard GC/MS equipment with an autosampler. The procedure does not require special thermal desorption equipment as in the case of adsorption on Tenax®.

Sample analysis

The analysis was performed on a 6890/5973 GC/MS system (Agilent, Wilmington, DE 19808, USA). The separation was performed on a DB-1701 column 60 m, 0.25 mm internal diameter, and 1 μm film thickness (J&W Scientific from Agilent). The liner used for the injection port of the instrument was single tapered with deactivated glass wool. The operating parameters for the GC/MS instrument are given in Table 1.

A typical chromatogram for a 3R4F cigarette smoked under 35/2/60 regimen is shown in Figure 1. The figure also shows an expanded window containing the extracted ions $m/z = 56$ corresponding to $^2\text{H}_3$ -acrylonitrile (retention time 16.33 min) and $m/z = 53$ corresponding to acrylonitrile (retention time 16.45 min) and 2-methylfuran (retention time 16.63 min).

The identification of the peaks in the chromatograms was performed using the data processing capability of the mass spectrometer, and mass spectral libraries Wiley7n and NIST02. Also AMDIS_32 (Version 2.1) program with NIST2 mass spectral library were utilized for the identification of compounds in some coeluting peaks. Except for $^2\text{H}_3$ -acrylonitrile, acrylonitrile, and α -methacrylonitrile, no other peak in the chromatogram was identified using standards.

Acrylonitrile and α -methacrylonitrile quantitation

The quantitation of acrylonitrile was performed utilizing the ratio of peak area of the ion $m/z = 53$ of the analyte vs. that of the ion $m/z = 56$ of the internal standard (extracted ions) using the following formula:

$$C_{\text{acrylonitrile}} (\mu\text{g/mL}) = \frac{\text{Peak_area_analyte}}{\text{Peak_area_I.S.}} \cdot C_{\text{I.S.}} \cdot F \quad [1]$$

Where C indicates the concentration (of the sample or of the standard), the concentration of the internal standard being 20 $\mu\text{g/mL}$. F is a response factor for the ratio nondeuterated/deuterated acrylonitrile. This factor was determined by generating calibration curves (compound level) vs. (measured area counts) in the range 0.5 $\mu\text{g/mL}$ to 40 $\mu\text{g/mL}$ of standard solutions for both acrylonitrile and $^2\text{H}_3$ -acrylonitrile using five concentration levels and

triplicate injections. The dependence equation concentration vs. area for acrylonitrile was:

$$C(\mu\text{g/mL}) = 7.34697 \text{ E-}7 \cdot \text{Peak_Area with } R^2 = 0.99883 [2]$$

and the dependence equation for $^2\text{H}_3$ -acrylonitrile was:

$$C(\mu\text{g/mL}) = 6.77603 \text{ E-}7 \cdot \text{Peak_Area with } R^2 = 0.99856 [3]$$

The slopes ratio of expressions [2] and [3] gave the response factor nondeuterated/deuterated acrylonitrile, with $F = 1.0843$.

The calibration curve for α -methacrylonitrile was obtained

in the range 0.25 $\mu\text{g/mL}$ to 5 $\mu\text{g/mL}$ using four different levels. The quantitation was done using the ion $m/z = 67$. The dependence equation for α -methacrylonitrile was:

$$C(\mu\text{g/mL}) = 1.20490 \text{ E-}6 \cdot \text{Peak_Area with } R^2 = 0.99859 [4]$$

The quantitation for α -methacrylonitrile was done using formula [1] also taking as an internal standard $^2\text{H}_3$ -acrylonitrile, and a response factor $F = 1.6399$. The response factor (nondeuterated α -methacrylonitrile)/(deuterated acrylonitrile) is not very close to 1.0, the two compounds having structural differences besides the replacement of hydrogen with deuterium atoms.

Figure 1. Typical chromatogram for VP from a 3R4F cigarette smoked in 35/2/60 regimen. The expanded window shows the extracted ions $m/z = 56$ corresponding to $^2\text{H}_3$ -acrylonitrile (retention time 16.33 min) and $m/z = 53$ corresponding to acrylonitrile (retention time 16.45 min) and 2-methylfuran (retention time 16.63 min).

Validation of acrylonitrile quantitation method

The method for acrylonitrile analysis was validated for several requirements. The selectivity for the analysis was very good for acrylonitrile, the peak eluting with no interference, although the separation of the extracted ion $m/z = 53$ for the acrylonitrile peak and for 3-methylfuran was not at the baseline (see Figure 1). The peaks were nevertheless separated enough to not affect the peak area integration by the data processing software of the mass spectrometer. The relative standard deviation of acrylonitrile measurements for a number of 18 samples

analyzed in triplicate varied between 0.77% and 4% indicating very good precision of the measurement. Repeatability for acrylonitrile analysis could not be evaluated in different matrices since only analysis of smoke was performed. However, the results obtained on 3R4F cigarette by five replicate measurements within a three week period showed a relative standard deviation (RSD%) of 3.15%.

The accuracy of the procedure was verified for 2R4F cigarettes and 1R5F cigarette smoked under 35/2/60 regimen, by comparing the results with the results reported in the literature for a Coresta 2006 Joint Experiment (23).

Figure 2. The results for acrylonitrile levels in the smoke of 2R4F and 1R5F cigarettes (35/2/60 smoking), as reported in the literature (23) and as obtained in the present study.

Figure 2 displays the results reported by different laboratories (23) and the results obtained in this study (as average of triplicate measurement). As seen from Figure 2 the results from the present study are within the range reported by many other laboratories. Also, the results are in good agreement with other reported levels of acrylonitrile in 2R4F cigarette (24).

The linearity of the acrylonitrile measurement was verified in the range 0.5 µg/mL to 40 µg/mL, with the trendline showing a $R^2 = 0.99883$ (see relation [2]). This proves a very good linearity within the specified range.

For the determination of the limit of detection, a standard containing 0.1 µg/mL acrylonitrile was analyzed five times. The results generated a standard deviation $SD = 0.00462$ mg/mL. This result would lead to an estimated $LOD = 0.014$ µg/mL and an estimated $LOQ = 0.046$ µg/mL for the method.

The recovery of the acrylonitrile analysis in VP of cigarette smoke using charcoal trap collection is dependent on two criteria. The first is the retention efficiency of VP in the ORBO™-32 trap. For the proof of complete retention of VP components in Section A of the ORBO™-32 trap, three 3R4F cigarettes were smoked using 60/2/30 regimen. After smoking, the analysis of the active charcoal from Section B of the trap was performed in identical conditions as for Section A. The analysis indicated no acrylonitrile present, as well as no other compounds from the VP. The second criteria is related to the completeness of the extraction in acetone of the acrylonitrile from the charcoal. The complete extraction was verified by repeated extraction with three portions of 1 mL acetone of the same charcoal from Section A of a trap where VP smoke from three 3R4F cigarettes was collected. The solutions were mixed and analyzed. Taking into account the three fold dilution, the

difference between single extraction and three repeated extractions (duplicate samples) was within 5% RSD. The maximum retention capacity of the ORBO™-32 trap was not measured, and the completion of the VP retention in extreme conditions such as for a 15 mg 'tar' cigarette smoked under intense regimen was also not verified, since such conditions were not applicable for the cigarettes evaluated in the present study. However, the loading capacity for acetaldehyde is at least 40–50 µg for 1 mg charcoal (in the presence of other vapor phase components). This indicated that the ORBO™-32 small trap can retain without losses the vapor phase smoke from three cigarettes. The retention of VP from ten 3R4F cigarettes smoked under 60/2/30 regimen was verified on a ORBO™-32 Large trap (400 mg charcoal in Section A). The charcoal from Section B or the Large trap did not contain any VP components. The charcoal from Section A was transferred into a 4 mL vial and extracted with 3 mL acetone. The results of acrylonitrile quantitation (duplicate samples) were close to the standard procedure for three cigarettes, with less than 10% deviation. A full validation of the collection of VP smoke in an ORBO™-32 Large trap was beyond the scope of the present study.

Besides the calibration and verification of linearity in the range 0.25 µg/mL to 5 µg/mL no other validation steps for the α -methacrylonitrile quantitation were performed.

RESULTS AND DISCUSSION

A qualitative analysis of the VP components was performed for 3R4F cigarette. A full list of compounds identified by MS library search is given in Appendix 1. This Appendix

Table 1. GC/MS operating parameters.

Parameter	Description	Parameter	Description
Initial oven temperature	30 °C	injection volume	1.0 µL
Initial time	11.0 min	carrier gas	helium
Oven temperature rate first ramp	1.5 °C/min	purge time	1.0 min
Final temperature first ramp	60 °C	purge flow	1.1 mL/min
Final time	0 min	flow mode	constant flow
Oven temperature rate second ramp	5.0 °C/min	flow rate	1.1 mL/min
Final temperature second ramp	100 °C	average gas velocity	27 cm/s
Final time	1.0 min	nominal initial pressure	16.91 psi
Oven temperature rate third ramp	10 °C/min	MSD transfer line	280 °C
Final temperature	260 °C	ion source temperature	230 °C
Final time	4.0 min	MSD EM offset	250 V
Total run time	60 min	MSD solvent delay	4.0 min
Inlet temperature	26 °C	MS operating mode	scan
Inlet mode	splitless	mass range a.m.u.	23–550

Table 2. Selected compounds detected in both VP and PP of mainstream smoke of 3R4F cigarette, and their estimated proportion in PP.

No.	Compound	% PP
1	1,3-Butadiene	0.21
2	Acetaldehyde	0.41
3	Furan	0.82
4	Acetonitrile	2.84
5	2,3-Butanedione	8.95
6	Benzene	1.43
7	3-Penten -2-one	12.14
8	2,3-Pentandione	12.57
9	Toluene	3.54
10	Pyridine	43.23
11	<i>o</i> -Xylene	9.05
12	<i>p</i> -Xylene	11.72
13	3-Methylpyridine	61.07
14	Styrene	22.09
15	2,6-Dimethyl-2,6-octadiene	33.54
16	2-Methyl-2-cyclopenten-1-one	48.67
17	Ethyl-3-methylbenzene	52.80
18	Limonene	41.62
19	Hydroxyacetonitrile	69.37
20	Dihydro -2-methyl-3(2H)-furanone	95.31
21	Acetic acid	96.39
22	Propionic acid	99.52
23	1,2-Propandiol	99.92
24	Nicotine	99.92

also indicates the relative content of a specific compound in vapor phase. This content was calculated exclusively based on the area counts of the peaks in the chromatogram, and cannot be considered quantitative. It gives only an estimate of the peak intensities corresponding to each listed compound. Also, the complexity of the VP of cigarette smoke is considerably higher than indicated by the list of identified compounds. Numerous compounds present at low levels were not considered for identification, although their spectra were frequently very well defined.

Some of the compounds present in VP of cigarette smoke are also found in PP. Table 2 gives a list of selected compounds analyzed in both VP and PP of 3R4F cigarette, and the estimated proportion of each compound present in PP. The calculation was done using the ratios of the peak areas of each individual compound in the chromatogram for the VP and for the PP, the chromatograms being generated using identical conditions as described in Table 1. Many compounds from mainstream cigarette smoke are not included in the list since their detection either in VP or in PP was not feasible. When a compound is below the detection limit for the analytical measurement, the calculation of the true contribution to PP or VP cannot be determined. As seen from Table 2, analysis of some compounds can be done exclusively in VP (or exclusively in PP) since more than 99% of that compound is present in only one phase, but many compounds are present in both VP and PP.

Further quantitative analysis was performed for acrylonitrile and α -methacrylonitrile. These compounds were not detected in the PP of mainstream cigarette smoke. The analysis was performed on several Kentucky reference cigarettes including 1R5F, 2R4F, 3R4F, 2R1F, and 1R3F, several King Size (KS) commercial cigarettes from the US market including Basic Non Filter (NF), Basic Ultra Lights (UL), Newport, Marlboro (Red), Marlboro Menthol, Camel Filter, Camel Lights, Camel Ultra Lights, and two herbal cigarettes, Ecstasy and Dreams. The results were obtained from triplicate sample analyses and are given in Table 3.

The levels of acrylonitrile and α -methacrylonitrile were further compared to the level of total particulate matter (TPM) from the analyzed cigarettes. Figure 3 show the dependence of the levels of acrylonitrile on TPM and Figure 4 shows the dependence for α -methacrylonitrile.

Although both acrylonitrile and α -methacrylonitrile levels in smoke depend on the TPM of the cigarette, the correlation is not very strong, indicating that the nature of the cigarette blend and possible other factors in cigarette construction also influence the levels of these compounds in smoke.

Table 3. The levels of acrylonitrile and α -methacrylonitrile (in $\mu\text{g}/\text{cig}$) in various cigarettes (triplicate samples).

No.	Cigarette	Smoking	Acrylonitrile		α -Methacrylonitrile	
			Average	RSD%	Average	RSD%
1	1R5F	35/2/60	1.93	3.61	0.52	2.74
2	1R5F	60/2/30	9.85	4.01	2.48	0.88
3	2R4F	35/2/60	7.44	2.66	1.84	0.30
4	2R4F	60/2/30	15.27	3.54	4.92	4.61
5	3R4F	35/2/60	7.31	3.70	1.95	0.04
6	3R4F	60/2/30	15.47	1.48	4.74	1.12
7	2R1F	35/2/60	11.54	1.98	3.74	3.53
8	1R3F	35/2/60	10.83	1.97	2.79	0.99
9	Basic NF	35/2/60	8.82	0.77	2.23	2.18
10	Basic UL	35/2/60	5.10	1.50	1.30	0.46
11	Newport	35/2/60	13.87	0.83	3.13	2.10
12	Marlboro	35/2/60	10.83	2.64	2.26	2.56
13	Marlboro Menthol	35/2/60	10.77	3.06	2.23	0.86
14	Camel Filter	35/2/60	11.49	2.58	2.38	2.01
15	Camel Lights	35/2/60	9.54	3.09	1.95	2.63
16	Camel UL	35/2/60	4.15	1.07	0.95	0.25
17	Ecstasy	35/2/60	15.82	0.83	3.72	2.66
18	Dreams	35/2/60	6.63	3.25	1.21	0.67

Figure 3. Variation of acrylonitrile in vapor phase cigarette smoke as a function of TPM of the cigarette.

CONCLUSIONS

A simple procedure for the collection of VP of mainstream cigarette smoke for analysis has been evaluated. This procedure consists of collecting the VP on a commercial charcoal trap (ORBO™-32) followed by dissolution in

acetone and GC/MS analysis. The ORBO™-32 trap can be connected immediately after the Cambridge pad holder and before the pneumatic panel of the smoking machine, avoiding further aging of the VP of cigarette smoke in gas phase. The pressure drop across the ORBO™-32 trap is very low. The use of a solvent for the dissolution of the

compounds retained in the trap, allows the addition of internal standards at the desired concentration. However, the volume of the solvent used for the dissolution can be as low as 0.5 mL (in this study 1.0 mL solvent was used). This low volume of solvent is much smaller than the volume of solvent typically needed for collection in impingers, such that a more concentrated solution of VP constituents can be obtained compared to impinger collection. The use of a liquid injection (as opposed to a gas phase injection) allows the use of standard GC/MS equipment with an autosampler. A qualitative analysis of the collected VP has been performed for 3R4F cigarette smoked under 35/2/60 regimen, allowing the identification of 138 compounds,

some present also in PP. A quantitative analysis for acrylonitrile and α -methacrylonitrile was also developed, and the level of these compounds in smoke from 15 different cigarette brands was measured. The results for acrylonitrile were in very good agreement with data reported in the literature for 2R4F and 1R5F cigarettes. The levels of α -methacrylonitrile were not previously reported. Although both acrylonitrile and α -methacrylonitrile levels in smoke depend on the TPM of the cigarette, the correlation is not very strong, indicating that the nature of the cigarette blend and possible other factors in cigarette construction also influence the levels of these compounds in smoke.

Figure 4. Variation of α -methacrylonitrile levels in vapor phase cigarette smoke as a function of TPM of the cigarette.

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APPENDIX

Appendix 1. Identification of VP components in the mainstream smoke of 3R4F cigarette. Tentative identifications are indicated by ‘?’

No.	Compound	Retention time	MW	Formula	% in VP
1	Propane	4.26	44	C ₃ H ₈	0.36
2	Isobutane (2-methylpropane)	4.59	58	C ₄ H ₁₀	0.17
3	2-Butene (E)	4.93	56	C ₄ H ₈	2.82
4	2-Butene (Z)	5.18	56	C ₄ H ₈	1.08
5	1,3-Butadiene	5.25	54	C ₄ H ₆	0.44
6	1-Pentene	5.94	70	C ₅ H ₁₀	1.31
7	Acetaldehyde	6.08	44	C ₂ H ₄ O	2.83
8	2-Methyl-2-butene	6.72	70	C ₅ H ₁₀	1.56
9	3-Methyl-1-butene	6.96	70	C ₅ H ₁₀	0.87
10	2-Pentene	7.23	70	C ₅ H ₁₀	0.27
11	2-Methyl-1,3-butadiene	7.87	68	C ₅ H ₈	22.33
12	Furan	8.53	68	C ₄ H ₄ O	0.10
13	1,4-Pentadiene	8.92	68	C ₅ H ₈	0.11
14	1,3-Cyclopentadiene	9.77	66	C ₅ H ₆	0.19
15	Acetone (Solvent)	11.7–13.7	58	C ₃ H ₆ O	—
16	Acetic acid methyl ester	13.82	74	C ₃ H ₆ O ₂	7.95
17	Acetonitrile	15.03	41	C ₂ H ₃ N	2.18
18	2-Methylpropanal	15.83	72	C ₄ H ₈ O	0.63
19	<i>o</i> 3-Acrylonitrile	16.33	56	C ₃ D ₃ N	0.41
20	Acrylonitrile	16.45	53	C ₃ H ₃ N	0.39
21	2-Methylfuran	16.63	82	C ₅ H ₆ O	1.77
22	2-Methylpropenal	16.76	70	C ₄ H ₆ O	0.31
23	3-Methylfuran	17.97	82	C ₅ H ₆ O	0.35
24	2,4-Hexadiene (Z,Z ?)	18.79	82	C ₆ H ₁₀	0.67
25	Butanal	19.42	72	C ₄ H ₈ O	0.25
26	1-Methyl-1,3-cyclopentadiene	19.60	80	C ₆ H ₈	0.15
27	Methyl vinyl ketone	20.11	70	C ₄ H ₆ O	0.62
28	2-Butanone	20.80	72	C ₄ H ₈ O	4.87
29	2,3-Butanedione	20.89	86	C ₄ H ₆ O ₂	1.07
30	1-Heptene	22.08	98	C ₇ H ₁₄	0.36
31	Methacrylonitrile	22.82	67	C ₄ H ₅ N	0.12
32	Benzene	23.37	78	C ₆ H ₆	4..86
33	Thiophene	25.26	84	C ₄ H ₄ S	0.01
34	3-Methylbutanal	26.52	86	C ₅ H ₁₀ O	0.71
35	2,4-Hexadienal (E,E ?)	27.02	96	C ₆ H ₈ O	0.15
36	2-Methylbutanal	27.19	86	C ₅ H ₁₀ O	0.45
37	2,5-Dimethylfuran	27.57	96	C ₆ H ₈ O	2.38
38	Isobutyronitrile (1-cyano-1-methylethane)	27.86	69	C ₄ H ₇ N	0.42
39	3-Penten-2-one	28.78	84	C ₅ H ₈ O	0.42
40	2-Butenal	29.09	70	C ₄ H ₆ O	0.69
41	2-Butenenitrile	30.27	67	C ₄ H ₅ N	0.35
42	1-Pentene-3-one	31.23	84	C ₅ H ₈ O	0.05
43	2-Pentanone	31.39	86	C ₅ H ₁₀ O	0.88
44	Vinylfuran	31.97	94	C ₆ H ₆ O	0.12
45	3-Pentanone	32.18	86	C ₅ H ₁₀ O	0.35
46	2-Methyl-1,5-hexadiene	32.51	96	C ₇ H ₁₂	0.05
47	2,3-Pentanedione	33.00	100	C ₆ H ₈ O ₂	0.11
48	2-Methyl-1-heptene	33.48	112	C ₈ H ₁₆	0.11
49	Octane	33.79	114	C ₈ H ₁₈	0.21
50	1-Octene	33.92	112	C ₈ H ₁₆	0.21
51	Butanenitrile	34.33	69	C ₄ H ₇ N	0.29
52	2-Methyl-1,6-heptadiene	34.53	110	C ₈ H ₁₄	0.09
53	3-Methylcyclohexanone	34.73	112	C ₇ H ₁₂ O	0.05
54	Dimethyldisulfide	34.89	94	C ₂ H ₆ S ₂	0.59
55	2-Butenenitrile (Isomer?)	35.46	67	C ₄ H ₅ N	0.09
56	Toluene	35.58	92	C ₇ H ₈	7.21

Appendix 1. (cont.)

No.	Compound	Retention time	MW	Formula	% in VP
57	Methyl isobutyl ketone	36.26	100	C ₆ H ₁₂ O	0.11
58	1-Methyl-1 <i>H</i> -pyrrole	36.54	81	C ₅ H ₇ N	0.50
59	Pyridine	36.86	79	C ₅ H ₅ N	0.26
60	2-Ethyl-5-methylfuran	37.09	110	C ₇ H ₁₀ O	0.25
61	2-Methyl-2-butenal mix	37.22	84	C ₅ H ₈ O	0.12
62	Octanal	37.22	128	C ₈ H ₁₆ O	0.10
63	2-Methylbutanenitrile mix	37.81	109	C ₆ H ₉ N	0.17
64	1-Cyclopropyl ethanone mix	37.81	84	C ₅ H ₈ O	0.43
65	2,3,5-Trimethylfuran	38.40	110	C ₇ H ₁₀ O	0.11
66	Methyl thiocyanate	38.63	73	C ₂ H ₃ NS	0.29
67	3-Methylbutanenitrile	38.85	109	C ₇ H ₁₁ N	0.51
68	?-Methyl-2-pentalenal	39.05	98	C ₅ H ₁₀ O	0.09
69	3-Hexanone	39.44	100	C ₆ H ₁₂ O	0.30
70	3,3,5-Trimethylcyclohexene	39.53	124	C ₉ H ₁₆	0.09
71	2,3-Hexanedione	39.89	114	C ₆ H ₁₀ O ₂	0.05
72	2-Hexanone	40.20	100	C ₆ H ₁₂ O	0.04
73	4-Methyl-3-penten-2-one	40.54	98	C ₆ H ₁₀ O	1.59
74	3-(1-Methylethyl)cyclopentene	40.76	110	C ₈ H ₁₄	0.03
75	3-(1-Methylethyl)cyclohexene	41.16	124	C ₉ H ₁₆	0.17
76	Nonane	41.30	128	C ₉ H ₂₀	00.23
77	1-Nonene	41.38	126	C ₉ H ₁₈	0.19
78	2-Ethyl-1 <i>H</i> -pyrrole	41.52	95	C ₆ H ₉ N	0.11
79	Cyclopentanone	41.75	84	C ₅ H ₈ O	0.34
80	4-Nonene	42.00	126	C ₉ H ₁₈	0.18
81	1,6-Dimethyl-1,3,5-heptatriene	42.06	122	C ₉ H ₁₄	0.22
82	Ethylbenzene	42.16	106	C ₈ H ₁₀	0.93
83	1,6-Dimethyl-1,3,5-heptatriene isomer	42.24	122	C ₉ H ₁₄	0.17
84	1-(2-Furanyl)-1-propanone	42.47	124	C ₇ H ₈ O ₂	0.11
85	<i>o</i> -Xylene	42.61	106	C ₈ H ₁₀	1.23
86	2-Methyl-2-pentalenal	43.18	98	C ₆ H ₁₀ O	0.09
87	2,4-Dimethyl-2,4-heptadiene	43.33	124	C ₉ H ₁₆	0.04
88	2-Methylcyclopentanone	43.77	98	C ₆ H ₁₀ O	0.23
89	<i>p</i> -Xylene	43.90	106	C ₈ H ₁₀	0.19
90	3,7-Dimethyl-1,6-octadiene	44.05	138	C ₁₀ H ₁₈	0.03
91	3-Methylpyridine	44.23	93	C ₆ H ₇ N	0.04
92	Styrene	44.29	104	C ₈ H ₈	0.12
93	3-Methylcyclopentanone	44.39	98	C ₆ H ₁₀ O	0.12
94	3-Methylcyclopentanone (Isomer?)	44.72	98	C ₆ H ₁₀ O	0.23
95	Furfural	44.74	96	C ₅ H ₄ O ₂	0.04
96	(1-Methylethyl)benzene	44.78	120	C ₉ H ₁₂	0.03
97	4-Methylpentanenitrile	44.97	97	C ₆ H ₁₁ N	0.18
98	4-Ethenyl-1,4-dimethylcyclohexene	45.06	136	C ₁₀ H ₁₆	0.34
99	4-Hydroxy-4-methyl-2-pentanone	45.20	116	C ₆ H ₁₂ O ₂	0.11
100	Decane	45.49	142	C ₁₀ H ₂₂	0.12
101	4,5-Dimethyl-2,6-octadiene	45.56	138	C ₁₀ H ₁₈	0.54
102	2,7-Dimethyl-1,6-octadiene	45.69	138	C ₁₀ H ₁₈	0.21
103	4-Methyl-3-penten-2-ol	45.81	100	C ₆ H ₁₂ O	0.07
104	Indane	45.88	118	C ₉ H ₁₀	0.10
105	Propylbenzene	45.89	120	C ₉ H ₁₂	0.03
106	2,6-Dimethyl-2,6-octadiene	45.96	138	C ₁₀ H ₁₈	0.33
107	1-(Methylethyl)benzene (Isomer?)	46.11	120	C ₉ H ₁₂	0.21
108	Ethyl-3-methylbenzene	46.21	120	C ₉ H ₁₂	0.15
109	2-Methyl-2-cyclopentene-1-one	46.60	96	C ₆ H ₈ O	0.09

Appendix 1. (cont.)

No.	Compound	Retention time	MW	Formula	% in VP
110	2,5,6-Trimethyl-1,3,6-heptatriene	46.80	136	C ₁₀ H ₁₆	0.33
111	Acetone cyanhydrin (Artifact ?)	46.86	85	C ₄ H ₇ NO	1.35
112	1-Methyl-4-(1-methylethyl)cyclohexene	47.04	138	C ₁₀ H ₁₈	0.23
113	3-(Methylthio)propanal	47.16	104	C ₄ H ₈ OS	0.54
114	1,2,3-Trimethylbenzene	47.25	120	C ₉ H ₁₂	0.09
115	Limonene	47.40	136	C ₁₀ H ₁₆	1.52
116	Dimethyltrisulfide	47.47	126	C ₂ H ₆ S ₃	0.11
117	3,4-Dimethyl-2-cyclopenten -1-one	47.55	110	C ₇ H ₁₀ O	0.02
118	Formic acid ethenyl ester	47.85	72	C ₃ H ₄ O ₂	0.75
119	2-Hydroxypropanenitrile	47.90	71	C ₃ H ₅ NO	0.85
120	1,3,5-Trimethylbenzene	48.21	120	C ₉ H ₁₂	0.03
121	Undecane	48.22	156	C ₁₁ H ₂₄	0.02
122	1-Undecene	48.26	154	C ₁₁ H ₂₂	0.03
123	5-Ethylbicyclo[2.2.1]-2-heptene	48.58	122	C ₉ H ₁₄	0.09
124	Hydroxyacetonitrile	48.64	57	C ₂ H ₃ NO	0.14
125	Acetolacetate ?	48.68	116	C ₆ H ₈ O ₃	0.20
126	Butanethioic acid S-methyl ester	49.33	118	C ₅ H ₁₀ OS	0.43
127	3-Methylbicyclo[3.3.0]oct-2-en-8-one	49.59	136	C ₉ H ₁₂ O	0.25
128	1,3-Propanediol	49.73	76	C ₃ H ₈ O ₂	0.67
129	5-Methyl-1,5-hexadien-3-ol	49.80	112	C ₇ H ₁₂ O	0.12
130	6-Methylbicyclo[3.3.0]-2-en-7-one	50.28	136	C ₉ H ₁₂ O	0.14
131	3-Methylbicyclo[3.3.0]-2-en-6-one	50.45	136	C ₉ H ₁₂ O	0.24
132	Isobutylene epoxide ?	50.72	72	C ₄ H ₈ O	0.49
133	Acetyl-5-norbornene ?	51.10	136	C ₉ H ₁₂ O	0.26
134	Dihydro-2-methyl-3(2H)-furanone ?	51.65	100	C ₅ H ₈ O ₂	0.21
135	1,3,5-Undecatriene	51.73	150	C ₁₁ H ₁₈	0.24
136	1,6-Heptadien-4-ol ?	52.43	112	C ₇ H ₁₂ O	0.57
137	Butanoic acid phenyl ester ?	53.53	164	C ₁₀ H ₁₂ O ₂	0.11
138	Nicotine	55.22	162	C ₁₀ H ₁₄ N ₂	0.02