Quantification of Selected Vapour-Phase Compounds using Thermal Desorption-Gas Chromatography*

by

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SUMMARY

A robust method for the analysis of selected vapour phase (VP) compounds in mainstream smoke (MSS) is described. Cigarettes are smoked on a rotary smoking machine and the VP that passes through the Cambridge filter pad collected in a Tedlar® bag. On completion of smoking, the bag contents are sampled onto an adsorption tube containing a mixed carbon bed. The tube is subsequently analysed on an automated thermal desorption (TD) system coupled to a gas chromatography-flame ionization detector (GC-FID) using a PoraPLOT-Q column. Quantification of 14 volatile compounds including the major carbonyls is achieved. Details of the method validation data are included in this paper. This method has been used to analyse the VP of cigarette MSS over a wide range of 'tar' deliveries and configurations with excellent repeatability. Results for the University of Kentucky reference cigarette 1R4F are in good agreement with reported values. [Beitr. Tabakforsch. Int. 21 (2004) 210–215]

ZUSAMMENFASSUNG

Es wird eine robuste Methode zur Analyse ausgewählter Verbindungen in der Gasphase des Hauptstromrauchs von Zigaretten beschrieben. Die Zigaretten wurden mit einer Rotations-Rauchmaschine abgeraucht und die Gasphase, die den Cambridgefilter passiert, wurde in einem Tedlar®-Beutel gesammelt. Nach Beendigung des Abrauchens erfolgte eine Probenentnahme aus dem Beutel auf ein Adsorptionsröhrchen mit einem gemischten Kohlebett. Das Röhrchen wurde dann mit einem automatisierten thermischen Desorptionssystem, gekoppelt mit einem Gaschromatographen und Flammenionisationsdetektor (GC-FID), unter Verwendung einer PoraPLOT-Q-Säule analysiert. Es erfolgte die Quantifizierung von 14 volatilen Verbindungen, einschließlich der wichtigsten Carbonylverbindungen. Es werden detaillierte Daten zur Validierung der Methode mitgeteilt. Die Methode wurde dazu eingesetzt, um die Gasphase des Hauptstromrauchs von Zigaretten mit unterschiedlichen Kondensatgehalten und Produkteigenschaften zu analysieren. Die Wiederholbarkeit der Methode ist sehr gut. Die erzielten Resultate für die Referenzzigarette 1R4F der University of Kentucky sind in guter Übereinstimmung mit publizierten Werten. [Beitr. Tabakforsch. Int. 21 (2004) 210–215]

RESUME

Une méthode robuste pour analyser certains composés de la phase gazeuse de la fumée du courant principal est présentée. Les cigarettes ont été fumées sur une machine à fumer rotative, et la phase gazeuse passée à travers le filtre Cambridge a été recueillie dans des sacs d'échantillonnage Tedlar®. Après fumage, les échantillons ont été injectés sur un tube d'adsorption contenant une couche de charbon mélangée. Ensuite le tube a été analysé sur un système de désorption thermique automatisée couplé à un dispositif de chromatographie en phase gazeuse-détection par ionization de flamme (GC-FID) à l'aide d'une colonne PoraPLOT-Q. Le dosage de 14 composés volatils y compris les carbonyles principaux a été réalisé. Dans cette étude des données détaillées de validation de la méthode sont présentées. Cette méthode a été employée pour analyser la phase gazeuse de la fumée principale de cigarettes d'un large éventail de rendements en goudron et différenciations des produits et ceci avec une répétabilité excellente. Les résultats obtenus avec la cigarette de référence Kentucky 1R4F sont en bon accord avec les valeurs rapportées. [Beitr. Tabakforsch. Int. 21 (2004) 210–215]

Table 1. Amount of compounds in 1 μL of standard

Compound	Amount (μg)
Acetaldehyde (Acetal)	2772
Acetonitrile	1187
Acetone (Aceton)	262
Acrolein (Acrol)	210
Acrylonitrile	81
Benzene	87
2-Butanone (MEK)	268
Butyraldehyde (Butal)	267
Crotonaldehyde (Crotal)	85
Furan	94
Isoprene	1362
Methanol	791
Propionaldehyde (Propal)	201
Toluene	87

INTRODUCTION

High-temperature pyrolysis of tobacco at the burning cone produces a vapour containing volatile compounds, many of which are not present in tobacco (1). The heat of combustion also vapourises many compounds from the tobacco near the burning cone. Whilst many of these compounds condense, some pass through the tobacco rod and filter into the mainstream smoke (MSS) (1). The Cambridge filter pad fractionates the MSS into the particulate phase (having a nominal size greater than 0.3 microns) and the vapour phase (VP) (2). Many of the VP components are of biological interest, e.g. benzene is a known human carcinogen (3). Consequently, several of these chemicals have come under scrutiny by regulators, e.g. in British Columbia and Massachusetts. Additionally many volatile compounds are believed to add to product taste (4).

The objective was to develop and validate a robust method for the analysis of selected VP compounds, with a good sample turnaround time and minimal requirements on resources (personnel and instrumentation). Methods for the quantification of volatile compounds in MSS can be divided into four categories depending on their trapping approach: direct injection (5–8), cryogenic trapping (9–12), adsorbent trapping (13,14) and sample bag (15,16). Of these methodologies, adsorbent trapping was chosen as it produces a homogenous sample, requires no modification to the smoking machine and permits analysis "off-line" due to the stability of the adsorbed sample.

EXPERIMENTAL

Smoking

Commercially available cigarettes from the United Kingdom (UK) market, with deliveries ranging from 1 to 12 mg 'tar', were conditioned according to International Standard (ISO) 3402 (17) and used without further selection. Twenty cigarettes per sample were smoked on a Borgwaldt RM20 smoking machine in accordance with ISO 4387 (18). The

MSS was separated using a 92 mm Cambridge filter pad and the VP collected in an opaque Tedlar® bag (SKC Ltd, Dorset, UK). Immediately on completion of smoking, the VP was sampled onto prepacked thermal desorption (TD) tubes (Markes Ltd, Pontyclun, UK) using an Alpha-1 personal air sampler (Shawcity, Faringdon, UK) operating at a flow rate of 100 mL/min. The TD tubes contained two adsorbent beds in series, Carbotrap™ and Carboxen™. Three samples were smoked per product with two analyses from each collection bag, giving a total of six measurements per product.

Calibration

A mixed calibration standard was prepared to mimic the relative concentrations of the 14 analytes typically found in the MSS of UK products. The concentrations of the working standard are given in Table 1. All chemicals used were supplied by Sigma-Aldrich, were of analytical grade and used as received without further purification. Other standards were prepared by serial dilution in heptane. One microlitre of the standard solution was volatilised in a modified gas chromatograph (GC) injection port at 150 ˚C and carried onto a TD tube using a stream of inert gas (19).

Analysis

The sample was introduced using a Perkin Elmer Automated Thermal Desorber (ATD400) coupled to a Perkin Elmer Autosys XL gas chromatograph with flame ionisation detector (GC-FID). Separation of the components was achieved using a PoraPLOT-Q column (50 m × 0.32 mm i.d. × 1 μm film thickness, Varian-Chrompack) with a flow of Helium at 2 mL/min. A temperature program of T_{int} 90 ˚C, 2˚/min to 120 ˚C (hold 10 min) 4˚/min to 220 ˚C (hold 10 min) was used to optimise separation giving a total run time of 60 min. Typical chromatograms for a standard and a MSS sample are given in Figures 1 and 2, respectively.

Calculation

The amount of each VP component in the sample was determined using the following equation:

$$\text{Analyte (μg/cig)} = A_s \times RF \times N \times c$$

where A_s = average peak response for the sample, RF = response factor for standard, N = puff number, c = conversion factor (accounts for puff volume [35 mL], number of cigarettes [20] and amount sampled [200 mL]).

RESULTS

Validation

The instrument precision, range, linearity and limit of quantitation (LOQ) of the method were assessed in accordance with International Conference on Harmonisation guidelines (20). Recovery was determined using the method of standard addition by introducing a known amount of standard onto a TD tube containing VP matrix. Peak purity
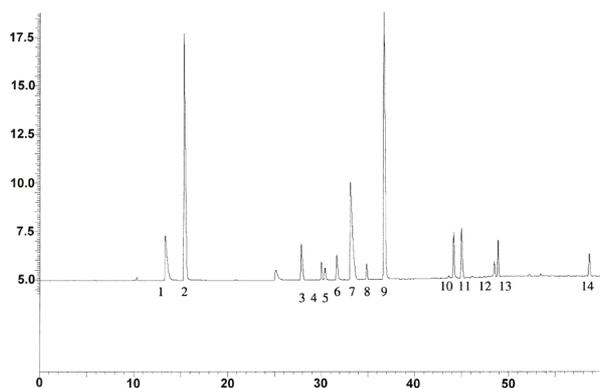
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Figure 1. Typical chromatogram of standard injection (1 = methanol, 2 = acetaldehyde, 3 = acetonitrile, 4 = acrolein, 5 = furan, 6 = propionaldehyde, 7 = acetone, 8 = acrylonitrile, 9 = isoprene, 10 = butyraldehyde, 11 = 2-butanone, 12 = crotonaldehyde, 13 = benzene, 14 = toluene)

Figure 2. Typical chromatogram of MSS sample

was confirmed by mass spectrometry (MS) using a Perkin Elmer Turbomass system in conjunction with the ATD-GC. The validation results are summarised in Table 2.

Cigarettes

The concentration of the 14 VP components were measured in nine commercially available brands (A–I) and three reference cigarettes: the CORESTA monitor cigarette (CM3), University of Kentucky reference cigarette 1R4F and our in-house monitor product (Mon). The configuration details of the cigarettes and the variability of the measurement of the VP components (expressed as coefficients of variation, CoV) are given in Tables 3 and 4, respectively. Figures 3 and 4, respectively, illustrate the results obtained for the analysis of carbonyls and volatile organic components in the MSS of the Kentucky 1R4F reference cigarette using this method compared to results from other laboratories.

DISCUSSION

Methods to measure volatile components of MSS all have their limitations. The use of serial impingers often requires
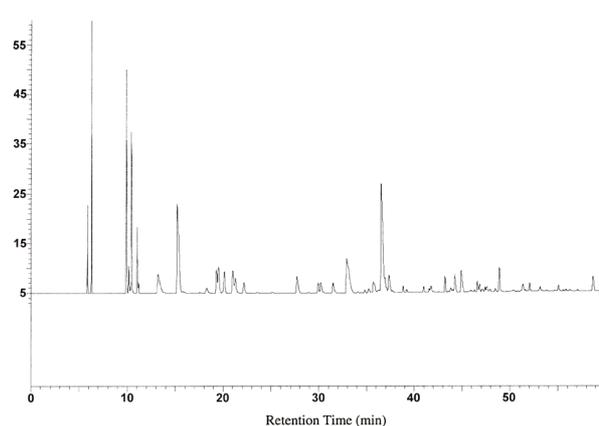
modification of the smoking machine, which can cause difficulties maintaining ISO smoking conditions (10). As the volatile carbonyls are generally trapped separately from other volatile compounds, two separate smoke runs and analyses are required per sample (9). Whilst direct injection can analyse all the VP components using only one instrument, sample turnaround is limited as samples can only be introduced during smoking (5). Due to the limited sample volume that can be introduced into a GC, it is difficult to get a homogenous sample from a cigarette with direct injection. Transfer lines are also required to be kept as short as possible. A gas-sampling valve can be used to obtain an aliquot from the collection bag that will be relatively homogeneous (15). To minimise the ageing of the VP it is imperative that the bag is sampled as soon as possible, hence analysis must occur immediately on completion of the smoking run.

To overcome the problems of homogeneity and turnaround time, the VP can be sampled from the collection bag onto adsorbent tubes. Once adsorbed, all the volatile components remain stable for at least 24 h (25–27). This allows the smoking to continue while the operator is present, with automated after-hours analysis for improved efficiency. Careful selection of the adsorbents in the TD tube allows complete adsorption of all volatile chemicals of interest. The TD tubes used in this study have two carbon beds. The

Table 2. Summary of method validation results

Compound	Instrument precision (%CoV)	LOQ (µg/cig)	Range (µg/cig)	Linearity	Mean recovery (%)
Acetaldehyde	3	1.89	LOQ-1256	0.995	99
Acetonitrile	2	1.88	LOQ-1130	0.999	109
Acetone	1	1.88	LOQ-1122	0.999	103
Acrolein	2	2.00	LOQ-1198	0.998	111
Acrylonitrile	2	1.92	LOQ-1152	0.998	114
Benzene	2	2.08	LOQ-1248	0.999	109
2-Butanone	2	1.92	LOQ-1150	0.999	111
Butyraldehyde	3	1.91	LOQ-1142	0.999	107
Crotonaldehyde	3	2.01	LOQ-1208	0.999	115
Furan	2	2.23	LOQ-1338	0.997	111
Isoprene	2	1.62	LOQ-972	0.998	99
Methanol	2	1.88	LOQ-1130	0.998	112
Propionaldehyde	2	1.92	LOQ-1150	0.999	109
Toluene	1	2.06	LOQ-1236	0.999	111

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Table 3. Details of cigarettes smoked

Cigarette	Configuration rod/filter (mm) [a]	Filter	Ventilation	'Tar' (mg/cig)	Nicotine (mg/cig)	Blend style [b]
A	KS (64/20)	Y	Y	12	1.0	Am
B	KS (64/20)	Y	Y	11	0.9	V
C	KS (64/20)	Y	Y	5	0.5	V
D	KS (64/20)	Y	N	12	0.8	V
E	KS (59/25)	Y	Y	1	0.1	V
F	LL (74/25)	Y	Y	10	0.9	V
G	LL (74/25)	Y	Y	8	0.7	V
H	LL Slim (72/27)	Y	Y	5	0.5	V
I	Untipped (59)	N	N/A	12	1.0	V
Monitor	KS (64/20)	Y	Y	11	0.9	V
CM3	KS (64/20)	Y	N	15	1.5	V
1R4F	KS (57/27)	Y	Y	10	0.8	Am

[a] KS, king size; LL, longer length; Slim, circumference ≈20 mm.
[b] Am, American blend; V, Virginia.

Table 4. Variability of vapour phase measurement (%CoV)

Compound	A	B	C	D	E	F	G	H	I	Mon	CM3	1R4F
Acetaldehyde	5	6	7	5	5	11	10	7	6	12	9	7
Acetonitrile	4	3	6	5	8	7	7	8	8	12	8	7
Acetone	6	3	7	5	4	9	6	8	6	9	7	10
Acrolein	4	3	5	5	4	9	6	7	5	9	6	6
Acrylonitrile	5	4	10	11	10	7	8	10	7	10	12	9
Benzene	4	3	6	5	4	9	5	8	5	8	7	5
2-Butanone	5	3	7	6	5	10	7	7	7	9	6	6
Butyraldehyde	3	2	10	4	9	10	8	7	7	14	7	6
Crotonaldehyde	7	6	8	8	5	11	6	8	11	11	10	13
Furan	5	4	9	8	6	9	9	9	10	14	9	8
Isoprene	5	5	4	11	4	3	8	7	5	8	9	6
Methanol	9	7	9	5	5	9	11	7	10	12	8	11
Propionaldehyde	6	8	7	8	4	11	11	10	6	12	9	6
Toluene	8	6	12	8	11	9	7	8	10	9	10	10

first carbon bed traps compounds in the boiling range n-C_5 to n-C_{14} whilst the second bed, a carbonised molecular sieve, traps those with a boiling point <80 ˚C.

Initial experiments were performed to optimise bed density, particle size and sampling air-flow as well as the choice of adsorbent to ensure that all the required volatile species were adequately trapped and retained by the adsorbents. Further work on aged samples was undertaken to confirm the stability of the adsorbed species over the duration of entrapment, i.e. the maximum time between sampling and analysis. There was no loss of volatiles from the sealed TD tubes for a period of up to 48 h however, it is recommended that all analysis be carried out within 24 h of sampling. Loss of sample after this period can be further prevented by the use of Swagelok™ type end-caps, which have demonstrated no loss of a range of compounds for a period of 21 days at room temperature (27). These compounds covered a wide range of volatility from freon 12 to hexachlorobutadiene on mixed adsorbent bed TD tubes similar in composition to those used in the present study. Further gas-phase reactions of trapped species are largely prevented as the individual molecules are kept apart as they are retained in separate pores within the adsorbents (26).

Matching an unknown peaks retention time with known standards does not provide sufficient evidence of a peaks identity or purity. To clarify this MS can be used either in parallel or as an alternate detector to the FID. Using the above matrix of products we found sufficient peak purity to rely upon the peak area counts derived from the FID for calculations. However, is it recommended that peak purity be checked for each sample type using GC-MS. The choice of FID for this work was based largely upon its widespread availability in laboratories throughout the world and its robustness. Appendix 1 details suitable MS conditions and ions for detection of the VP components listed.

The short-term variability of this method is given by the CoV of replicate samples of the 9 products over a short period of time (typically over one day). From Table 4 it can be seen that the CoV was ≤12% for all these products regardless of configuration, delivery or ventilation level. The longer-term variability of the method was established over a three-month period for three products, namely CM3, 1R4F and an in-house monitor product. Table 4 shows the longer-term variability was ≤14% indicating consistency of the method under normal operating conditions.
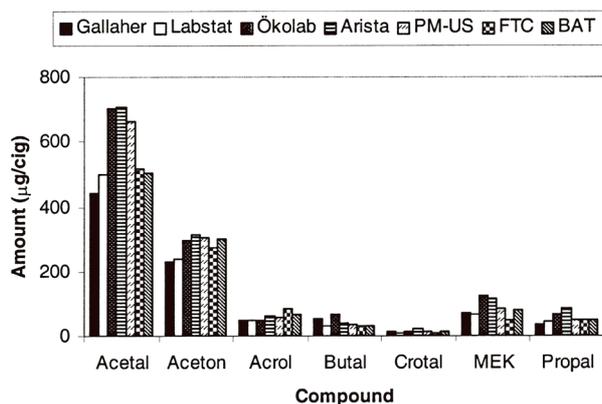
Figure 3. Comparison of 1R4F carbonyl compounds data (References: Labstat (21), Arista (22), PM-US (23), BAT (24). Personal communication from Ökolab & Filtrona Technology Centre (FTC)).
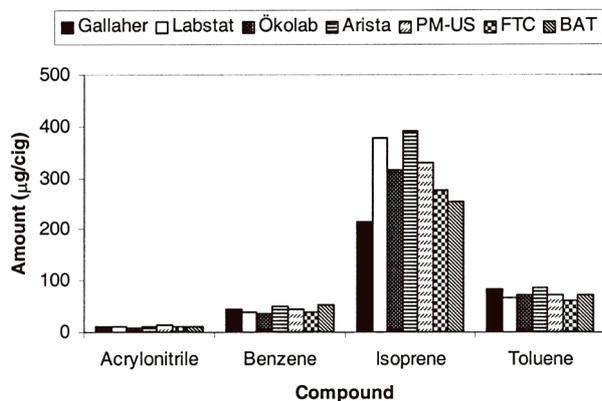

Figure 4. Comparison of 1R4F volatile organic compound data. (References: Labstat (21), Arista (22), PM-US (23), BAT (24). Personal communication from Ökolab & Filtrona Technology Centre (FTC)).

As can be seen from Figures 3 and 4, the VP data for the Kentucky 1R4F reference cigarette obtained by this method are in general agreement with results from six independent laboratories using alternate methodologies. Unfortunately, there was no data available for the comparison of acetonitrile, furan and methanol results.

Although there is no internationally agreed methodology for the determination of vapour-phase chemicals in MSS, there appears to be some consistency between the laboratories detailed above. To trap the carbonyls from MSS, most of the laboratories use a solution of 2,4-dinitrophenyl-hydrazine (DNPH) and analyse the resultant derivatives by high performance liquid chromatography (HPLC) using ultraviolet (UV) detection. A separate smoking is required for the volatile organic compounds (VOCs) as they are trapped using chilled liquid impingers and analysed by GC-MS, normally using selected ion monitoring (SIM). The laboratories do, however, differ in the type of smoking machine (linear or rotary) used, the number of cigarettes (5, 10 or 20) per determination and the number and volume of the liquid impingers.

Despite the similarities of the methodologies used, the results obtained for the 1R4F cigarette differ between laboratories, however, these differences are within the inter-laboratory variability expected for the measurement of "Hoffmann"-type analytes in MSS (28). Furthermore, the

results obtained from this study using a unique methodology are consistent with the other results presented for the 1R4F given the inter-laboratory variability shown above.

CONCLUSION

Thermal desorption-gas chromatography with flame ionisation detection (TD-GC-FID) provides a simple and consistent method for the quantification of VP compounds in MSS from cigarettes of different configurations. A run time of 60 min provides information on eleven of the volatile chemicals currently required by the British Columbia regulations using only one analytical instrument. Smoking is also carried out under ISO conditions with no modifications to the smoking machine and minimal preparation of materials. The method can be applied to a wider range of volatile chemicals by careful selection of the adsorbent materials.

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Appendix. Suitable gas chromatography-mass spectrometry conditions and ions for detection of the vapour phase components

Table A1. Target and qualifier ions for mass spectrometry detection

Compound	Target	Qualifier
Acetaldehyde	29	43
Acetonitrile	41	40
Acetone	43	58
Acrolein	56	55
Acrylonitrile	53	52
Benzene	78	52
2-Butanone	43	72
Butyraldehyde	43	72
Crotonaldehyde	41	70
Furan	39	68
Isoprene	68	53
Methanol	31	29
Propionaldehyde	58	57
Toluene	91	92

Perkin-Elmer TurboMass: transfer line 220 ˚C; ion source 200 ˚C, electron energy 70 eV, emission current 150 µA.