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Gas chromatography–mass spectrometry of carbonyl compounds in cigarette mainstream smoke after derivatization with 2,4-dinitrophenylhydrazine

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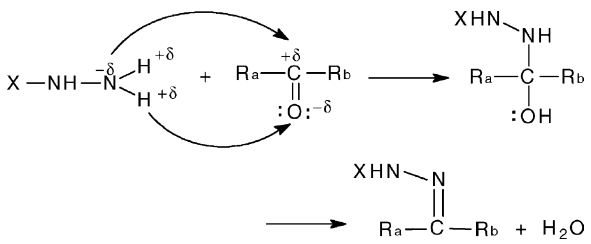
Abstract

An improved gas chromatography–mass spectrometry (GC–MS) method was described for the analysis of carbonyl compounds in cigarette mainstream smoke (CMS) after 2,4-dinitrophenylhydrazine (DNPH) derivatization. Besides formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, methyl ethyl ketone, butyraldehyde, and crotonaldehyde that are routinely analyzed in cigarette smoke, this technique separates and allows the analysis of several C_4, C_5 and C_6 isomeric carbonyl compounds. Differentiation could be made between the linear and branched carbon chain components. In cigarette smoke, the branched chain carbonyls are found at higher level than the linear chain carbonyls. Also, several trace carbonyl compounds such as methoxyacetaldehyde were found for the first time in cigarette smoke. For the analysis, cigarette smoke was collected using DNPH-treated pads, which is a simpler procedure compared to conventional impinger collection. Thermal decomposition of DNPH–carbonyl compounds was minimized by the optimization of the GC conditions. The linear range of the method was significantly improved by using a standard mixture of DNPH–carbonyl compounds instead of individual compounds for calibration. The minimum detectable quantity for the carbonyls ranged from 1.4 to 5.6 μg/cigarette.

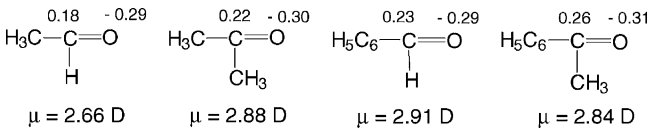
Keywords: Cigarette smoke; Derivatization, GC; Carbonyl compounds; Dinitrophenylhydrazine

1. Introduction

The high reactivity and selectivity of 2,4-dinitrophenylhydrazine (DNPH) serves well as a derivatization reagent to determine carbonyl compounds in complex sample matrices [1–33]. The reaction is assumed to start either as a nucleophile attack to the carbon or an electrophile attack of a proton to the oxygen of the carbonyl group and is followed by an elimination reaction as shown below:



The efficiency of this reaction depends on charge distribution on the carbonyl group (and the dipole moment μ of the molecule), which is a function of the nature of substituents R_a and R_b. The values of $+\delta$, $-\delta$ and the dipole moment μ for acetaldehyde, acetone, benzaldehyde, and acetophenone are given below as examples showing the negative partial charge on the oxygen and the positive one on the carbon involved in the carbonyl double bond:



The values for the charges and for μ were calculated using a MOPAC molecular orbital package [16]. For the reagent, the localized charges depend on the nature of the substituting group of hydrazine. Examples for some substituted hydrazines used as reagents are shown below:

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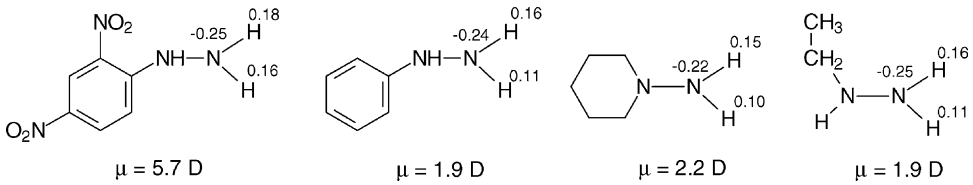
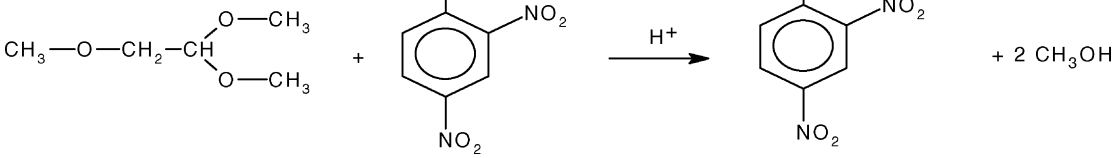


The formal charges on the active hydrogens and on the nitrogen atom indicate that 2,4-dinitrophenylhydrazine should have a higher reactivity in the reaction with the carbonyl compounds compared to the other three reagents. This reactivity explains the common use of DNPH.

The charges on the reacting hydrogen atoms are increased if the substituted hydrazine is protonated. Typically, strong acids such as perchloric acid are added to promote such protonation. From this point, the reaction mechanism is very likely an electrophile attack of a proton to the oxygen of the carbonyl group. Since the presence of strong acids is undesirable for chromatographic separations, pyridine is typically added after the derivatization takes place. Pyridine addition also stabilizes the DNPH derivatives.



The DNPH–carbonyl compounds can be analyzed by HPLC or gas chromatography–mass spectrometry (GC–MS). The HPLC procedure has been typically preferred to the GC–MS due to its robustness and good repeatability. However, in very complex mixtures such as cigarette smoke condensate, the application of the HPLC separation has the potential of interferences, and the determination of certain carbonyl compounds present at trace levels is difficult. For this reason, a GC–MS procedure was needed to separate a number of these carbonyl compounds [3,11,24–27,32]. The GC coupled with MS detection, compared to HPLC, has the advantages that positive identification of each DNPH–carbonyl compound can be achieved, as well as potentially better separation. These qualities are particularly important in the measurement of carbonyl compounds in cigarette smoke condensate, since there were over 4000 compounds reported in cigarette smoke [34] and there is a high potential for interferences. The present study describes an optimized GC–MS procedure for the analysis of several carbonyl compounds in the cigarette mainstream smoke (CMS) using DNPH derivatization. Improvements have been made for smoke collection using DNPH-treated pads and for the limited linearity range using standard mixtures of DNPH–carbonyl compounds instead of individual compounds.

2. Experimental

2.1. Reagents and materials

DNPH, pyridine, anthracene, and perchloric acid (70% solution) were obtained from Aldrich (Milwaukee, WI, USA), acetonitrile with 99.99% purity was obtained from EM Science (Gibbstown, NJ, USA), and 2,4-dinitrophenylhydrazones of formaldehyde, acetaldehyde, acetone, propionaldehyde, acrolein, methyl ethyl ketone, *n*-butyraldehyde and crotonaldehyde were obtained from TCI America (Portland, OR, USA). Methoxyacetaldehyde–DNPH and 3-hydroxy-2-butanone–DNPH were not commercially available. Methoxyacetaldehyde–DNPH has been synthesized for the purpose of quantitation. The synthesis using methoxyacetaldehyde dimethyl acetal (98% purity, Aldrich) as a starting compound is shown below:

For this synthesis, 2 g of recrystallyzed DNPH was dissolved in 80 ml CH_3CN using sonication to speed the dissolution. After complete dissolution, 1.3 ml methoxyacetaldehyde dimethyl acetal and then 6 ml of 0.5 M H_2SO_4 were added and mixed at room temperature. The reaction mixture was kept for 1 h in an ultra sonic bath. An orange precipitate formed when part of the CH_3CN was evaporated. This precipitate was filtered and discarded. The remaining solution was concentrated, and the methoxyacetaldehyde–DNPH was crystallized. The material was then recrystallized from CH_3CN. The GC–MS trace of the pure material obtained by this procedure shows only one peak. The mass spectrum of this compound is given in Fig. 1.

This spectrum shows the molecular ion *m*/*z* 254 corresponding to the expected compound, and the fragmentation pattern is similar to that for other derivatives of carbonyl compounds with DNPH (the mass spectrum of methoxyacetaldehyde–DNPH derivative is not available in the Wiley or US National Institute of Standards and Technology (NIST) mass spectra libraries). The purity of methoxyacetaldehyde–DNPH was also verified using a LC–MS technique [35].

For the positive identification of 3-hydroxy-2-butanone, the DNPH derivative of this compound was also synthesized,

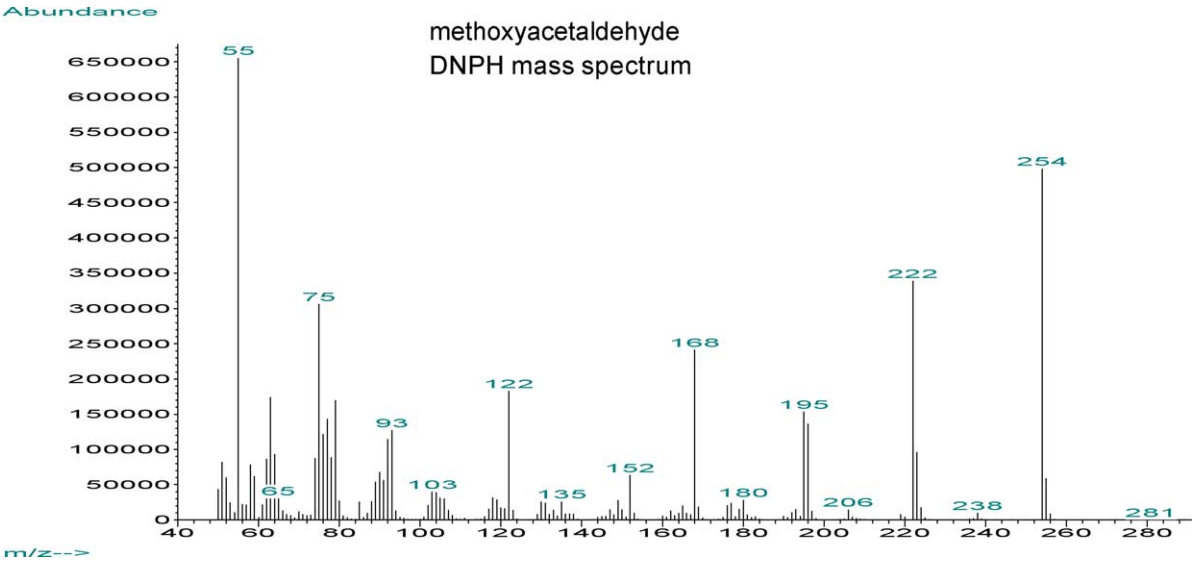


Fig. 1. Positive ion electron impact ionization mass spectrum (70 eV) of methoxyacetaldehyde–DNPH derivative.

but it was used only for qualitative purposes. The 3-hydroxy-2-butanone–DNPH compound was obtained by mixing stoichiometric amounts of DNPH and 3-hydroxy-2-butanone in a dilute solution (20 μg/ml) in acetonitrile, followed by the addition of a drop of perchloric acid (70%). The resulting solution was injected in the GC–MS instrument for the determination of the retention time of the DNPH derivative. The compound showed a major ion at *m/z* 266, although the M_r for the compound is 268.

Two types of cigarettes were used as samples in the study. The cigarettes were 1R4F and 2R4F Kentucky reference cigarettes available from University of Kentucky, Kentucky Tobacco Research and Development Center (KTRDC).

2.2. Sample collection and derivatization

The first step in the analysis of carbonyl compounds in cigarette smoke was to treat the Cambridge filters (pads) commonly used for smoke collection with a DNPH solution. For this purpose, a solution of DNPH was made by using 1500 mg recrystallized DNPH (7.58 mmol), 100 ml acetonitrile, and 200 μl 70% perchloric acid (2.2 mmol). This solution had a 0.3 molar ratio of the acid to DNPH. Then, ten 92 mm Cambridge pads or forty 44 mm pads were saturated with the solution; the pads were dried in a vacuum oven at 35 °C for about 1 h and kept in a closed container (desiccator without drying material). The mass for DNPH should be 150 mg/pad for 92 mm, and 35 mg/pad for 44 mm. The pads should maintain a certain level of solvent, since the completely dried pads have reduced reactivity with the carbonyl compounds from smoke and the recovery was not complete. For this reason, for a 92 mm pad weighing initially about 1.5 g, the final mass after impregnation with DNPH solution and drying should be about 4.5–5.0 g.

For the analysis, 10 conditioned cigarettes were smoked through two pads, the first 92 mm diameter and the second 44 mm diameter containing DNPH (total of 185 mg), using a Borgwaldt RM 20/CSR rotary machine. The smoking was performed using conditions as recommended by the International Standard Organization (ISO) [36–38]. This requires a puff volume of 35 ml, puff interval of 60 s, and a puff duration of 2 s. The air flow at the cigarette burning zone was typically 200 ± 30 mm/s. Conditions as recommended by the US Federal Trade Commission (FTC) [39] can also be utilized.

After smoking, the pads remained in the holder for 3 min for the completion of the reaction. The two pads were then combined and extracted with 50 ml extracting solution prepared from acetonitrile, which contains 0.5 μg/ml anthracene (used as an internal standard) and 2% pyridine. The pads were mechanically shaken for 5 min. Then, 1 ml extract was added to 5 ml of the same extracting solution.

The trapping efficiency using two Cambridge filters treated with DNPH solution was evaluated by analyzing the gas after the two pads using a vapor phase technique reported in the literature [40]. Only traces of carbonyl compounds were detected in the gas phase. The quantitative results indicated that the reaction efficiency is better than 96% for all major carbonyl compounds analyzed.

2.3. Chromatographic separation and detection

For the chromatographic analysis, an Agilent 6890/5973 GC–MS system equipped with a Supelco Equity-5 (30 m × 0.32 mm i.d., 0.25 μm) column was used. The injector temperature was 230 °C, and an Agilent Marlin microseal septa was used to avoid leaks caused by the relatively high column head pressure. The injection was done

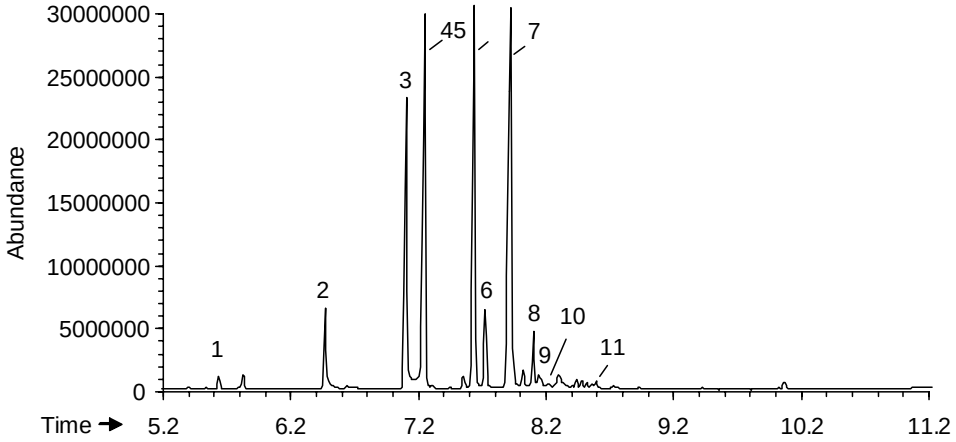


Fig. 2. Typical chromatogram for the GC–MS analysis of aldehydes and ketones using DNPH derivatization of the mainstream smoke of a 1R4F cigarette.

in the splitless mode with a splitless injection glass liner that was deactivated and contained no glass wool. The separation was done under a constant flow of 3.8 ml/min. The GC oven was programmed with the initial temperature of 100 °C for 0.1 min, followed by a ramp of 18 °C/min, up to 330°. A solvent delay of 4.5 min preceded the MS spectra acquisition which covered a mass range of 50–450 amu. The total run time was 12.88 min. A typical chromatogram obtained from the smoke of 1R4F Kentucky reference cigarette is shown in Fig. 2. The peak identifications and the molecular masses of the analyzed compounds are given in Table 1.

The solutions of derivatized carbonyl compounds in cigarette smoke were stable and can be analyzed with good results within 3 days after the preparation. However, due to the common possibilities of contamination, the background of a blank sample should be evaluated, preferably with each batch of samples. If present, this background should be subtracted from the result of the analysis.

Table 1
Carbonyl compounds determined in the smoke of a 1R4F cigarette

Peak no.	Component	Molecular mass	Quantitation
1	Anthracene (internal standard)	178	
2	Formaldehyde–DNPH	210	Yes
	2,4-Dinitrobenzenamine	183	
3	*syn*-Acetaldehyde–DNPH	224	Yes
4	*anti*-Acetaldehyde–DNPH	224	Yes
5	Acetone–DNPH	238	Yes
6	Propionaldehyde–DNPH	238	Yes
	Acrolein–DNPH	236	Yes
7	2,4-Dinitrophenyl hydrazine	198	
8	Methyl ethyl ketone–DNPH	252	Yes
9	Methacrolein–DNPH	250	
10	*n*-Butyraldehyde–DNPH	252	Yes
11	Crotonaldehyde–DNPH	250	Yes

3. Results and discussion

As indicated in Fig. 2, the chromatogram for the GC–MS analysis of aldehydes and ketones using DNPH derivatization from the CMS is rather simpler. The 11 peaks labeled corresponding to typical aldehyde–DNPH and ketone–DNPH from the smoke make up more than 98% of the peak area of the whole chromatogram. Although there are more than 4000 compounds from CMS [34], the majority of the vapor phase compounds such as benzene, toluene and isoprene, etc. are not trapped during the smoke collection. Other non-carbonyl compounds that are trapped on the pads, such as nicotine, eluted much quicker from the column than the DNPH–carbonyl compounds. This was not shown in the chromatogram in Fig. 2. Of the eleven analytes, acetaldehyde and acetone are the two most abundant compounds accounting for more than 80% of all carbonyls. Therefore, it is also a challenge in optimizing the chromatographic conditions to analyze some trace level carbonyl compounds in the smoke.

3.1. Optimization of chromatographic conditions

Previous studies have shown that there were several obstacles encountered in the GC separation of DNPH–carbonyl compounds such as thermal stability of the analytes, ruggedness of the GC inlet and column, and the formation of *syn*- and *anti*-isomers [3,24,26,27]. The thermal stability is not only related to the chromatographic conditions of injector temperature and oven programming, but also to the acidity of the sample solution. In fact, the acidity of the sample solution has far more effects than the chromatographic conditions. As stated in Section 2, rather high column temperature (330 °C) and injection temperature (230 °C) were used in this study, and no measurable effects were seen. Up to 280 °C for injection was tested, and no significant change in the responses was noticed as compared to 230 °C. As discussed below, many previous investigators used high acid/DNPH

molar ratios which are not favorable to the GC separation. Even with the addition of a high amount of pyridine, the chromatography eventually deteriorated for the samples with high acid/DNPH molar ratio. In some extreme cases, the chromatogram only gives peaks corresponding to the degradation of column stationary phase.

Chromatography can be improved by applying a higher flow rate of the carrier gas, which means reduced retention times during the chromatographic separation [32]. In this study, a constant flow rate was optimized at 3.8 ml/min for the 30 m column with 320 μm i.d. Flow rates higher than 2 ml/min may sacrifice some efficiency of the column, but it is necessary in this case. It is also vital to keep the glass liner cleaned. Only the deactivated ones contained no glass wool should be used. The glass wool can affect the thermal stability of the analytes as a catalyst and also affect the delectability of the analytes at low concentration due to the absorption by the glass wool as discussed below. Excessive DNPH left in the reaction solution can also affect the performance of chromatography since DNPH undergoes thermal degradation giving unwanted peaks in the chromatogram. One such peak is 2,4-dinitrobenzenamine which coeluted with formaldehyde as indicated in Fig. 2 and Table 1.

3.2. Advantages using DNPH treated pads in smoke collection

Previous studies on the analysis of carbonyl compounds from the CMS were conducted by purging the smoke through one or two impingers containing DNPH solution [13–15,29,31], or using a silica gel cartridge coated with acidified DNPH [33]. In some cases, an ice bath was used to increase the reaction efficiency [15], and in others, the DNPH solution was made of aqueous and chloroform layers in order to concentrate the DNPH derivatives [13]. Using impingers increases the pressure drop across the collection system during the smoking which is a critical point for the standardized smoking conditions by FTC or ISO, since the pressure drop may affect the smoke puff profile and the smoke collection accuracy.

In regular smoking, the standard conditions recommended of using 44 or 92 mm Cambridge pads for both the FTC or ISO conditions [36–39]. Therefore, minimum alteration of smoking conditions is essential for method development. In this particulate case, the DNPH-treated pads were made according to the procedure as described in the Section 2. Only little amount of solvent is needed when compared to the conventional impinger collection. Also, flexibility is added for more cigarettes being smoked (up to 10 per analysis), other than just one or two as used by the previous ones. Table 2 showed such comparisons between the conventional method and the new method.

3.3. Assessment of the acidity required for reactions between DNPH and carbonyl compounds

As discussed above, addition of a small amount of acid is needed in order to promote the reactions between the carbonyls and DNPH. However, excessive acid will yield adverse results but generates significantly higher amount of formaldehyde from the reagents [17,18–21,23]. Also excessive acid in the solution will damage the column stationary phase.

Table 2 gives the summary from previous and current studies for carbonyl analysis using DNPH. As shown in Table 2, the molar ratio for the acid to DNPH in this study was about 0.3 which was about 10 times smaller than others who used the same perchloric acid [14,29,31]. In the case of using hydrochloric acid, the molar ratio of acid to DNPH was as high as 99 [13,15]. This is a critical point leading the successful GC–MS separation of the DNPH–carbonyl compounds from the smoke in this study. Since the reaction occurs directly from fresh smoke on the DNPH-treated pads with little amount of acetonitrile, a very high efficiency of the reaction is expected. This is because of the high concentration of the DNPH reagent precipitated on the pads, compared to the conventional impingers at 0.6–4.0 mg/ml. An aliquot (1 ml) of the reaction solution that was diluted with 5 ml extraction solution for GC–MS analysis is just for the consideration of saving solvent (see Section 2).

Table 2
Optimization of the reaction and the molar ratio for acid/DNPH

	Ref. [13]	Ref. [15]	Ref. [14]	Ref. [29]	Ref. [31]	This study
HPLC or GC	HPLC	HPLC	HPLC	HPLC	HPLC	GC
Impingers or pads	1 impinger	2 impingers	1 impinger	2 impingers	1 impinger	2 pads
Cooled bath used	No	Yes	No	No	No	No
Reaction solution (ml)	40	400	50		80	3[b]
DNPH concentration (mg/ml)	4.0	0.6	1.3	2.2	2.4	60[c]
Cigarettes smoked	1	2	2		1	10
DNPH (mg/cigarette)	160	120	31.0		192	18.5
Acid/DNPH (mmol/mmol)	99.0[a]	66.0[a]	7.3	2.6	2.4	0.3

[a] Hydrochloric acid was used in this case.
[b] The DNPH-treated pads contained little solvent (approximately 2.5–3 ml).
[c] Since there was little solvent on the treated pads, the extraction solution was saturated with DNPH and the DNPH may exist in a precipitated form on the pads. Therefore, the concentration of 60 mg/ml is only estimation for comparison purpose.

Also, much less amount of DNPH per cigarette was used than the conventional impinger collection. As indicated in Fig. 2, too high level of DNPH interferes the chromatographic separation, since the excessive DNPH coeluted with *i*-butyraldehyde.

3.4. Discussion on quantitation

The quantitation of carbonyl compounds using the GC–MS analysis raised some problems. Calibration curves for each carbonyl compound were generated in two ways. One was to use the standards of DNPH–carbonyl compounds individually to obtain the calibration. Another was to use the standard mixture of all major DNPH–carbonyl compounds. It was noted that the slope of the calibration curves for individual carbonyl compounds was significantly smaller at low concentrations than at high concentrations. As a consequence, non-zero intercepts on the calibration curves were generated, leading to a limited linearity range and relatively high limits of quantitation. Using uncorrected calibration curves would produce much higher values than the true carbonyl concentration.

However, the compounds at the same concentration analyzed in a mixture showed higher areas. This effect is shown in Fig. 3, where the ratios (area as individual)/(area of the individual in a mixture) are plotted for several compounds at several concentrations. The ratio (area as individual)/(area of the individual in a mixture) would be expected equal to one, but it is below one at lower concentrations. For example, formaldehyde–DNPH response was 30% lower at 10 ppm using the individual standard than using the standard mixture. At the same level, acrolein–DNPH was about 60% lower than the mixture. This difference, however, diminishes when the concentration increases. This suggests that the decomposition or adsorption of the analytes occurred on the active sites in the injection port or during the GC analysis with little and constant rates.

As the carbonyl compounds were always present as a mixture in cigarette smoke, this problem as indicated Fig. 3, was overcome by performing the calibration of each individual carbonyl compound using a standard mixture and not solutions of individual ones. The calibration mixtures were made such that each individual carbonyl compound was in concentrations to cover the range typically found for the solution after the collection of smoke. The upper concentration standard contained: formaldehyde 10 μg/ml, acetaldehyde 120 μg/ml, acetone 40 μg/ml, propionaldehyde 10 μg/ml, acrolein 10 μg/ml, methyl ethyl ketone 10 μg/ml, *n*-butyraldehyde 5 μg/ml, and crotonaldehyde 5 μg/ml. This mixture of solution was further diluted into different levels of concentrations to generate the calibration curves. These curves showed a linear dependence with R^2 values between 0.998 and 1.0.

Another problem encountered during GC–MS quantitation of DNPH derivatives was related to the formation of two peaks for individual compounds, due to the generation of *syn* and *anti* forms. Typically, the *anti* form is present as the main component with the *syn* form below 4%. Acrolein–DNPH and crotonaldehyde–DNPH showed only about 0.5% *syn* form. However, the ratio may vary depending on the acidity of the sample. For compounds such as acetaldehyde, methyl ethyl ketone, propionaldehyde or butyraldehyde, the quantitation should be done based on calibrations for both forms followed by the sum of the results.

3.5. Precision of the method

The results for the analysis of eight common carbonyl compounds found in mainstream smoke of 1R4F and 2R4F Kentucky reference cigarettes are shown in Table 3. The table gives the averages in μg/cigarette and the relative standard deviations (R.S.D.) for five replicates. The smoking was done using ISO conditions.

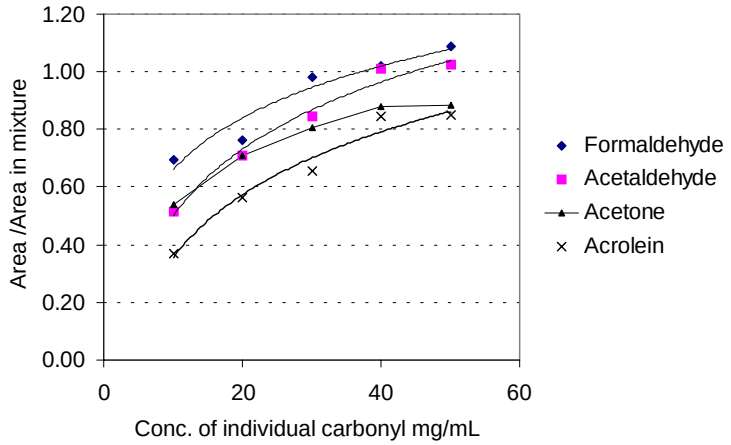


Fig. 3. The ratio (area as individual)/(area of the individual in a mixture) for different DNPH derivatives of carbonyl compounds at concentrations between 10 and 60 μg/ml.

Table 3
The levels in μg/cigarette of eight common carbonyl compounds in mainstream smoke of 1R4F and 2R4F reference cigarettes

	Average (μg/cigarette)		R.S.D. (%)	
	1R4F	2R4F	1R4F	2R4F
Acetaldehyde	619.4	583.7	2.1	5.8
Acetone	233.9	261.6	3.1	16.2
Acrolein	47.1	50.3	3.4	6.4
Butyraldehyde	12.6	12.6	1.6	2.4
Crotonaldehyde	18.5	20.1	4.9	4.5
Formaldehyde	22.9	23.2	9.2	17.7
Methyl ethyl ketone	69.9	73.5	2.0	8.4
Propionaldehyde	46.5	44.0	2.2	5.9
Total	1070	1069.0	2.0	7.1

As seen in Table 3, the R.S.D. for these analyses is about 5% for most analytes, except for acetone and formaldehyde for 2R4F cigarettes where higher R.S.D. (%) were observed. These results were compared with those obtained in a collaborative study for the same cigarettes [41]. The collaborative study included six laboratories, all using impingers with DNPH solution to trap smoke and HPLC analysis for the analysis of 2,4-dinitrophenylhydrazones. The results from the collaborative study are shown in Table 4, where the repeatability R.S.D.$_{-r}$ and the reproducibility R.S.D.$_{-R}$ are also shown. These were calculated based on the usual expressions [42]:

$$\text{R.S.D.}_{-r} = \frac{S_{-r}}{\bar{X}} \times 100\% \quad \text{and}$$
$$\text{R.S.D.}_{-R} = \frac{S_{-R}}{\bar{X}} \times 100\% \qquad (1)$$

where $\bar{X}$ is the average among the laboratories; $S_{-r} = \sqrt{(1/p)\sum_{i=1}^{p} S_i^2}$, p the total number of labs, S_i standard deviations (S.D.) within the lab; $S_{-R} = \sqrt{S_{\bar{X}}^2 + ((n-1)/n)S_{-r}^2}$, n the number of replicates in each laboratory, and $S_{\bar{X}}$ is the standard deviation between the averages for each laboratory.

The precision of the results from the collaborative study were compared to those of the GC–MS technique described in this study. Since the number of replicates for the collaborative study and the GC–MS technique were equal, it was possible to apply the statistical F-ratio for comparing the results obtained using the GC–MS analysis, and the results reported in Table 4. For this purpose, the repeatability standard deviations, S.D. − total, for the whole group of methods including six literatures reported data and the GC–MS data were first calculated. From the S.D. values generated for the GC–MS technique as reported in Table 3, and the S.D. − total, the ratios F = (S.D. for GC–MS)/(S.D. − total) were calculated. The critical value for F at 0.5% significance level for seven laboratories and five replicates of each sample is 1.77. Only the F-value for acetone for 2R4F cigarettes exceeded this number indicating a lower precision for the GC–MS technique, compared to the analytical techniques applied in other laboratories. All other data showed equal precision for the collaborative study and the GC–MS technique. This leads to the conclusion that the precision of the GC–MS technique is similar to that of the HPLC with impinger collection of the sample.

3.6. Accuracy of the method

The results obtained by the GC–MS procedure and the averages obtained for the collaborative study [41] are compared in Table 5. The relative differences (Diff%) for each compound were calculated relative to the collaborative study values.

As seen from Table 5, for most compounds, the results show good agreement between the GC–MS analysis and the average of the collaborative study. The levels for acrolein and butyraldehyde levels are, however, systematically lower for the GC–MS technique than for the collaborative study. The lower levels for acrolein obtained in the GC–MS technique can be explained by the cyclization reaction of the acrolein–DNPH derivative that may take place in the GC injection port or during the chromatographic separation. This reaction can be written as follows:

Table 4
Averages and R.S.D.$_{-r}$ and R.S.D.$_{-R}$ values obtained for carbonyl compounds in the mainstream smoke of 1R4F and 2R4F reference cigarettes in a collaborative study including six laboratories [41]

	Average (μg/cigarette)		R.S.D.$_{-r}$ (%)		R.S.D.$_{-R}$ (%)	
	1R4F	2R4F	1R4F	2R4F	1R4F	2R4F
Acetaldehyde	623.88	560.48	6	5	13	15
Acetone	293.15	264.74	6	5	7	5
Acrolein	60.64	58.77	8	7	16	14
Butyraldehyde	33.93	29.58	9	7	12	9
Crotonaldehyde	15.90	16.18	15	11	43	43
Formaldehyde	22.19	21.61	8	10	9	14
Methyl ethyl ketone	68.08	62.72	7	7	30	25
Propionaldehyde	51.54	43.92	7	5	15	13

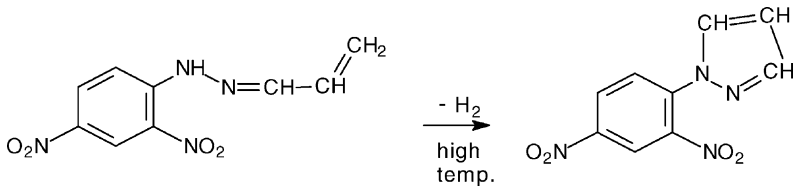


Table 5
Comparison of the results obtained by the GC–MS procedure and the averages obtained for the collaborative study (in μg/cigarette) [41]

	GC–MS 1R4F	Collaborative study 1R4F	Diff%	GC–MS 2R4F	Collaborative study 2R4F	Diff%
Acetaldehyde	619.4	623.88	−0.72	583.7	560.48	4.14
Acetone	233.9	293.15	−20.21	261.6	264.74	−1.19
Acrolein	47.1	60.64	−22.33	50.3	58.77	−14.41
Butyraldehyde	12.6	33.93	−62.86	12.6	29.58	−57.40
Crotonaldehyde	18.5	15.9	16.35	20.1	16.18	24.23
Formaldehyde	22.9	22.19	3.20	23.2	21.61	7.36
Methyl ethyl ketone	69.9	68.08	2.67	73.5	62.72	17.19
Propionaldehyde	46.5	51.54	−9.78	44	43.92	0.18

The resulting 1-(2′,4′-dinitrophenyl)pyrazole has been detected in the GC–MS chromatograms of the standard solutions of acrolein–DNPH, as well as in the smoke samples after derivatization. Also, 1-(4′-nitrophenyl)pyrazole was identified in the chromatograms, possibly resulting from acrolein–DNPH. This compound was suspected as resulting from acrolein–DNPH by Tejada [33] and identified as *x*-acrolein. The levels of the two pyrazole related compounds increase as the acidity of the sample increases. For crotonaldhyde–DNPH, 2-methyl-1-(2′,4′-dinitrophenyl)pyrazole and 2-methyl-1-(4′-nitrophenyl)pyrazole were found in the chromatogram as well, but with very trace level. Similar cyclization reaction was found by Vogel et al. using HPLC [30], who identified 3-methyl-1-(2′,4′-dinitrophenyl)pyrazol from the derivatization of 3-butyn-2-one using DNPH. Catalytic cyclization of 3-butyn-2-one–DNPH may play a major role in this case, since very mild temperature was applied in the use of HPLC.

The explanation for the disagreement in the level of butyraldehyde is that while the GC–MS procedure differentiates between *n*-butyraldahyde and 2-methylpropionaldehyde (*i*-butyraldehyde), the HPLC separation does not. The extracted ion chromatogram for *m*/*z* 252 showing several C_4 carbonyl compounds in the mainstream smoke of a 1R4F cigarette is shown in Fig. 4. In the GC–MS procedure, the levels of 2-methylpropionaldehyde are similar to those obtained for the compound reported as “butyraldehyde” in the collaborative study. By adding the GC–MS results for 2-methylpropionaldehyde to the results for *n*-butyraldehyde, the total is higher than the value reported by the HPLC procedures for butyraldehyde. These results are shown in Table 6 (differences reported to collaborative study value).

There are several literature reports providing results for analysis of carbonyl compounds in the CMS [14,29,43–48], some including data for 1R4F cigarettes [14,29,45–48]. Among theses, one paper [45] does not collect smoke using a standard protocol such as FTC or ISO. It simply adsorbs continuously the smoke of a burning cigarette into a vacuumed separatory funnel, rendering the reported results unuseful. The results from the other three reports are

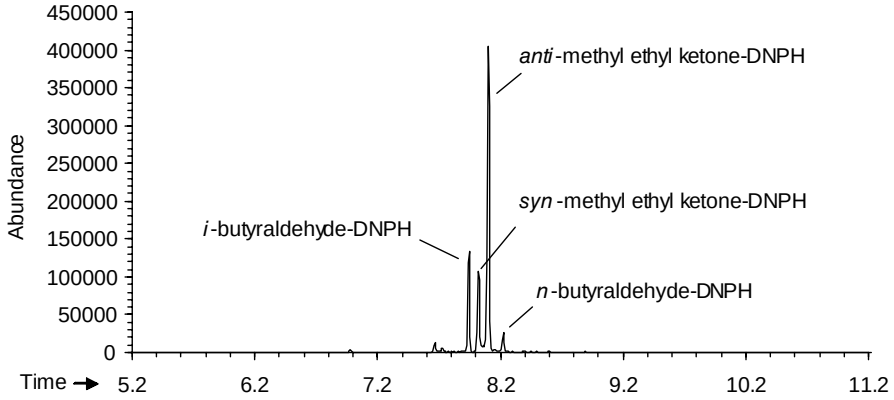


Fig. 4. Extracted ion chromatogram for *m*/*z* 252 showing several C_4 carbonyl compounds in the mainstream smoke of a 1R4F cigarette.

Table 6
Results for *n*-butyraldehyde, 2-methylpropionaldehyde and total butyraldehyde obtained by the GC–MS technique and in the collaborative study for 1R4F and 2R4F cigarettes

	GC–MS 1R4F	Diff% interlaboratory	GC–MS 2R4F	Diff% interlaboratory
Butyraldehyde	12.6	−62.9	12.6	−57.4
2-Methylpropionaldehyde	31.0	−8.6	30.8	4.1
Total	43.6	+28.5	43.4	+46.7

compared in Table 7 with the data from this study. As seen from Table 7, the method using DNPH derivatization with GC–MS analysis performed in this study does not give results significantly different from other published results. However, the agreement is not as good as the one obtained in the comparison with the results from the interlaboratory study [41], where the smoking protocol was very similar to that used in this study.

3.7. Limit of detection (LOD) and limit of quantitation (LOQ)

Table 8 shows the LOD and LOQ values. The lowest level of standard was analyzed as an unknown sample five times. The LOD is calculated as three times the SD and the LOQ as 10 times the SD. Except for acrolein and crotonaldehyde, lower than 3 μg/cigarette LOQ was obtained for all the analytes. As discussed above, acrolein–DNPH and crotonaldehyde–DNPH went through some measurable thermal and catalytic rearrangements for the formation of pyrazols during the chromatographic separation, which might be an explanation for the higher LOD and LOQ values for acrolein and crotoaldehyde.

Table 7
Comparison of the results (in μg/cigarette) for the levels of carbonyl compounds in mainstream smoke of 1R4F from several studies

	Ref. [14]	Ref. [29]	Ref. [46]	This study
Acetaldehyde	709–826	659	682	619.4
Acetone			272	233.9
Acrolein		73	72	47.1
Formaldehyde	23–28	29	11.8	22.9

Table 8
Limits of detection (LOD) and quantification (LOQ) for several carbonyl compounds

	LOD (μg/ml)	LOD (μg/cigarette)	LOQ (μg/cigarette)
Acetaldehyde	0.020	0.60	2.0
Acetone	0.019	0.57	1.9
Acrolein	0.047	1.41	4.7
Butyraldehyde	0.029	0.87	2.9
Crotonaldehyde	0.056	1.68	5.6
Formaldehyde	0.014	0.42	1.4
Methyl ethyl ketone	0.029	0.87	2.9
Propionaldehyde	0.024	0.72	2.4

3.8. Other carbonyl compounds measured in smoke

The application of the GC–MS technique for the analysis of carbonyl compounds in the CMS allowed the detection of several other carbonyl compounds which are at low levels. This advantage opens the possibility for quantitation of a number of compounds that are not typically analyzed in the HPLC technique. Among these are methacrolein, *n*-valeraldehyde, *i*-valeraldehyde, 3-pentanone, 2-pentanone, 1-penten-2-one, 1-peneten-3-one, cyclopentanone, and other carbonyl compounds such as cinnamaldehyde, furfural, 3-hydroxy-2-butanone, hydroxypentanone, and methoxyacetaldehyde.

As an example, Fig. 5 shows the extracted ion *m*/*z* 266 corresponding to several saturated C_5 carbonyl compounds. 3-Hydroxy-2-butanone with M_r 268 is also seen in this figure, since the ion *m*/*z* 266 is a major ion in the spectrum of this compound. Special attention was paid to the quantitation of methoxyacetaldehyde in mainstream smoke, since this compound was not previously reported in smoke [34]. The extracted ion chromatogram for the ion 254 corresponding to DNPH–methoxyacetaldehyde is shown in Fig. 6.

Table 9 shows the quantitation results for several carbonyl compounds that are not typically analyzed in cigarette smoke. These results were obtained from five replicates. As seen from Table 9, similar to butyraldehyde, *i*-valeraldehyde predominates over *n*-valeraldehyde. The same table shows that the content of methoxyacetaldehyde in the CMS of the reference cigarettes was around 1.2 μg/cigarette.

3.9. Factors affecting the accuracy in the measurement of carbonyl compounds in CMS with the DNPH GC–MS method

As discussed above, there were a number of factors to affect the accuracy of the analytical numbers for the carbonyl compounds. Such factors are, but not limited to, the numbers of cigarettes smoked, cigarettes homogeneity, condition controls for the smoking machine such as puff volume and air flow rate, efficiency of the reaction using DNPH, and the treatment of reaction solution with or without purification or concentration of the formed DNPH–carbonyl compounds. As discussed above, poor linear range at low concentration can significantly affect the accuracy of the data. Therefore, optimization of the condition for each step is very critical for the successful application in the quanti-

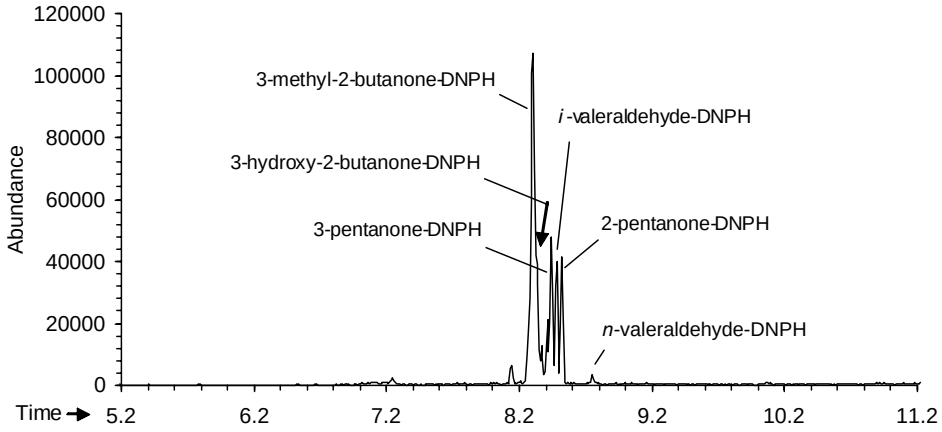


Fig. 5. Extracted ion *m/z* 266 corresponding to several C_5 carbonyl compounds in cigarette mainstream smoke.

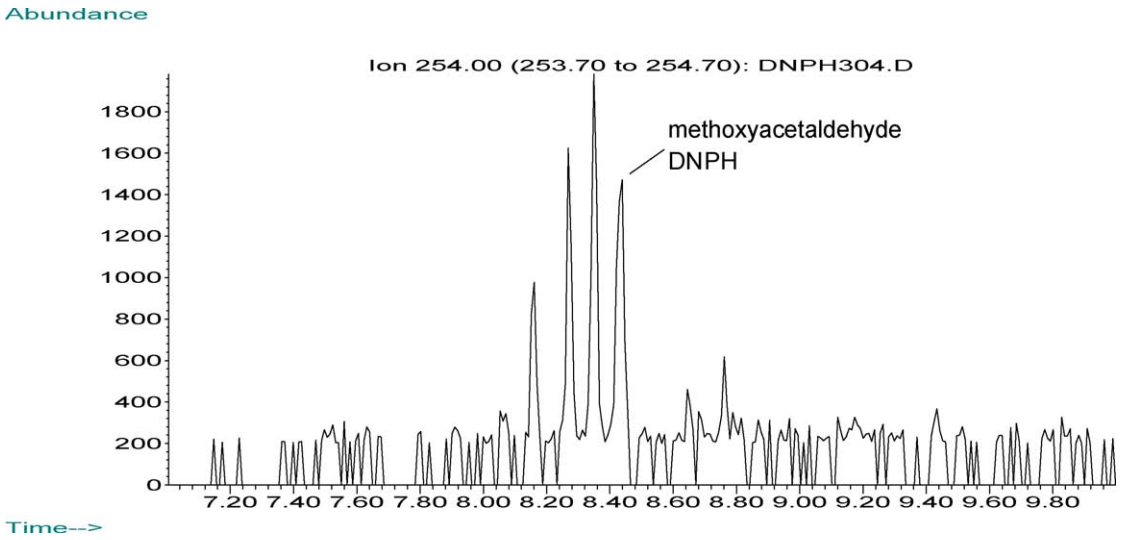


Fig. 6. Extracted ion chromatogram for the ion with *m/z* 254 in the control cigarette showing methoxyacetaldehyde–DNPH peak.

tative analysis of carbonyl compounds in the CMS. Nevertheless, this study has demonstrated that not only for those routinely analyzed but also for some others at trace levels, this GC–MS technique could be utilized for the quantitative and qualitative analysis of the carbonyl compounds in the CMS.

Table 9
Analysis of other carbonyl compounds in cigarette mainstream smoke for 1R4F and 2R4F Kentucky reference cigarette (ISO smoking)

	1R4F		2R4F	
	Average (μg/cigarette)	R.S.D. (%)	Average (μg/cigarette)	R.S.D. (%)
Methacrolein	23.8	2.1	25.5	7.8
n-Valeraldehyde	2.4	7.3	3.0	11.8
i-Valeraldehyde	25.8	1.0	26.1	3.2
3-Pentanone	9.0	12.8	8.4	10.1
2-Pentanone	5.0	13.1	5.6	6.0
Methoxyacetaldehyde	1.1	13.6	1.3	6.1

4. Conclusions

A GC–MS technique has been developed for the analysis of DNPH derivatives of carbonyl compounds in the CMS. The method uses DNPH-treated pads for smoke collection, with several advantages compared with the collection in impingers. The collection on the pad does not affect the puff profile, and is simpler. The GC separation provides a higher resolution of the compounds compared to typical HPLC, and the MS detection leads to positive identification of each compound, which is a major advantage when the analysis is done in a very complex matrix such as CMS. The new GC–MS technique can be used for the quantitation of a number of carbonyl compounds including formaldehyde, acetaldehyde, acrolein, etc. commonly measured in cigarette smoke. The precision and accuracy of these measurements are in the same range with typical HPLC analysis of DNPH–carbonyl derivatives. In addition, the method allows detailed analysis of various isomers of short aliphatic chain carbonyl

compounds. The dominance of *iso* chain versus normal chain for C_4 and C_5 compounds was demonstrated. Methoxyacetaldehyde was quantitated and reported for the first time in cigarette smoke. Also, other carbonyl compounds not analyzed previously in cigarette smoke can be quantitated.

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