Journal of Chromatography, 505 (1990) 365–374
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 296

Separation and quantitation of phenolic compounds in mainstream cigarette smoke by capillary gas chromatography with mass spectrometry in the selected-ion mode

E. J. NANNI*, M. E. LOVETTE, R. D. HICKS, K. W. FOWLER and M. F. BORGERDING
R. J. Reynolds Tobacco Company, Bowman Gray Technical Center, Winston-Salem, NC 27102 (U.S.A.)
(First received April 14th, 1989; revised manuscript received January 9th, 1990)

SUMMARY

Cigarette smoke condensate is a complex chemical matrix and determination of phenolic compounds in it frequently requires extensive and laborious sample preparation. By utilizing derivatization techniques and capillary column gas chromatography with mass spectrometry in the selected-ion mode, separation and quantitation of selected phenolic compounds found in mainstream cigarette smoke can be accomplished with minimal sample preparation.

This method has been used to determine concentrations of phenol, *o*-cresol, *m*-cresol, *p*-cresol, catechol, resorcinol and hydroquinone in cigarette smoke condensate from a number of commercially available cigarettes and a new cigarette which heats, but does not burn, tobacco. Unlike tobacco-burning cigarettes, levels of the phenolic compounds in the new cigarette smoke are at or below the detection limits for most of the compounds. This result is attributed to the unique design of the new cigarette.

INTRODUCTION

Phenol, cresols and dihydroxybenzenes are well known components of cigarette smoke condensate (CSC). They are formed from pyrolysis of tobacco during the smoking process and contribute to the flavor and aroma of tobacco smoke[1].

Phenolic compounds in tobacco smoke have been determined by a number of techniques[2–15]; however, quantitation of these compounds is often difficult because of the complex chemical matrix in which they occur and the very low concentrations at which they are present. Gas chromatography (GC) has been widely applied to the determination of phenolic compounds but even capillary column GC separations often require preliminary ancillary chromatographic separations or purifications such as solvent partitioning, column chromatography, and/or acid–base extraction. These ancillary purification steps often lead to losses of phenolic constituents, artifactual

0021-9673/90/$03.50

formation of phenols, and the inevitable increase in analytical imprecision. Therefore, an ideal analytical method for these compounds should minimize sample handling and preparation.

A simple, reliable, and accurate method has been developed to determine seven phenolic compounds in CSC with minimal sample preparation. The seven compounds that can be easily separated and quantified by the method are phenol, *o*-cresol, *m*-cresol, *p*-cresol, catechol, resorcinol and hydroquinone.

In short, sample preparation involves collection of CSC by electrostatic precipitation followed by derivatization of the CSC with neat bis-N,O-trimethylsilyl-trifluoroacetamide (BSTFA), which reacts with the phenolic compounds to form their respective mono- and bis-trimethylsilyl (TMS) ethers. The reaction mixture is separated by GC and the TMS ethers are detected using selected-ion mass spectrometry (SIMS). With at least ten-fold excess of BSTFA, silylation (reaction 1) reaches completion within 15 min.

$$\begin{matrix} C_6H_5OH \\ \text{and} \\ C_6H_4(OH)_2 \end{matrix} + \begin{matrix} \text{OTMS} \\ | \\ CF_3C{=}NTMS \end{matrix} \xrightarrow{80°C} \begin{matrix} C_6H_5(OTMS) \\ \text{and} \\ C_6H_4(OTMS)_2 \end{matrix} + \begin{matrix} \text{O} \\ \| \\ CF_3CNH_2 \end{matrix} \qquad (1)$$

Moreover, the method cleanly separates *m*- and *p*-cresols, a separation which, heretofore, has not been easily achieved and thus allows for their accurate quantitation during a single analysis.

The method has been applied to determination of seven phenolic compounds in CSC from various commercial cigarettes, 1R4F Kentucky Reference cigarettes, and several prototypes of a new cigarette that heats, but does not burn, tobacco. Unlike the tobacco-burning cigarettes, levels of the phenolic compounds in the CSC from the new cigarette are at or below the detection limits for most of the compounds.

EXPERIMENTAL

Instrumentation and apparatus

A Heinreich Borgwaldt smoking machine (RM 20/CS), central electrostatic smoke trap, and high-tension generator (Model 251) were used to generate and trap CSC for analysis. A Hewlett-Packard (HP) 5890 GC system and HP 5970 mass spectrometry (MS) system with a direct interface were employed to separate and detect components with samples being introduced to the gas chromatograph by an HP 7673 automatic sampler. Control of the gas chromatograph, mass selective detector, and automatic sampler was accomplished using an HP 59770 MS ChemStation. The compounds of interest were separated on a 30 m, narrow bore (0.23 mm I.D.), 5% phenylmethylsilicone capillary column (DB5-30N) available from J & W Scientific. Conditions employed to carry out the GC–MS analyses are found in Table I. The mass spectral ions (*m*/*z*) used for quantitation were 151 and 166 for phenol; 91, 165 and 180 for *o*-, *m*- and *p*-cresol; 149, 185 and 200 for *o*-chlorophenol (internal standard); and 73, 239 and 254 for catechol, resorcinol and hydroquinone.

TABLE I

GC–MS CONDITIONS

GC capillary column:	DB5-30N (30 m × 0.23 mm I.D., 0.25-μm film)
GC oven program:	
Initial temperature:	50°C
Initial time:	3 m
Program rate:	5°C/min
Final temperature:	230°C
Total run time:	55 min
Injector temperature:	225°C
Transfer line temperature:	250°C
Injection mode:	Splitless, 1 μl, 30 s purge
Mass spectra acquisition:	Selected-ion monitoring, 4 groups
Column head pressure:	*Ca.* 15–20 p.s.i.g. (to maintain a flow of *ca.* 30–35 ml/min through the split vent)

Reagents and chemicals

All chemicals were reagent grade quality or better and were used as received without further purification. Phenol (99 + %), *o*-chlorophenol (98 + %) (CAS registry No. 106-48-9), *o*-cresol (99 + %) (95-48-7), *m*-cresol (99%) (108-39-4), *p*-cresol (99 + %) (106-44-5), catechol (99 + %) (120-80-9), resorcinol (98 + %) (106-46-3) and hydroquinone (99 + %) (123-31-9) were all obtained from Aldrich; methyl *tert.*-butyl ether and methylene chloride were purchased from Burdick & Jackson Labs.; and BSTFA (74785-85-6) with 1% trimethylchlorosilane (TMCS) was purchased from either Pierce or Regis.

Preparation of stock solutions and standards

A stock solution (*ca.* 1000 μg/ml) of the phenolic analytes was prepared by accurately weighing approximately 100 mg each of phenol, *o*-cresol, *m*-cresol, *p*-cresol, catechol, resorcinol, and hydroquinone into a 100-ml volumetric flask. The mixture was diluted to the mark with methyl *tert.*-butyl ether. Approximately 100 mg of *o*-chlorophenol were accurately weighed into another 100-ml volumetric flask and diluted to the mark with methyl *tert.*-butyl ether; this solution was the internal standard stock solution. Utilizing both stock solutions calibration standards were prepared as follows. To each of six 5-ml reaction vials 2.5 ml of BSTFA was added. Each vial was sealed with a septum cap and 12 μl of *o*-chlorophenol internal standard stock solution were added by syringe. Appropriate amounts of the analyte stock standard solutions were added by syringe such that concentrations in the reaction vials were 0.1, 0.5, 1.0, 5.0 and 10.0 μg/ml, respectively. The vials were heated overnight at 80°C in a LabLine multi-heating block to ensure completion of the silylation reaction; although as will be discussed in the Results and Discussion section, the reaction is complete within 15 min. After cooling, aliquots from each reaction vial were transferred by syringe to autosampler vials and were used to calibrate the GC–MS system.

Sample preparation

For each cigarette sample, CSC was obtained by smoking twenty cigarettes on

a Heinreich Borgwaldt (HB) 20-port smoking machine. The mainstream smoke particulate phase was condensed by electrostatic precipitation with a HB high-tension generator, a HB electrostatic precipitation (EP) trap, and glass EP tubes. For all samples the Federal Trade Commission (FTC) puffing regimen, *i.e.*, a 35-ml puff of 2 s duration every 60 s, was employed. EP tubes and endcaps were tared prior to smoking and weighed after smoking in order to determine the amount of CSC or wet total particulate matter yielded by the twenty cigarettes. Samples for GC–MS were then otained by accurately weighing *ca.* 25 mg CSC into 5-ml reaction vials, adding 2.5 ml BSTFA and 12 μl *o*-chlorophenol internal standard stock solution to the reaction vials, and heating the reaction vials at 80°C on a LabLine multi-heating block. The reaction vials were heated at least 15 min to ensure completion of the reaction. Aliquots from the reaction vials were then transferred to autosampler GC vials for analysis.

RESULTS AND DISCUSSION

Method validation studies

A number of method validation experiments were conducted prior to the determination of the selected phenolic compounds in CSC. The dynamic range of the method, the ability to separate and detect the compounds of interest, the method precision, and the method accuracy were each assessed to validate the procedure for application to the analysis of CSC. As with any procedure involving reaction chemistry, an accurate measurement of each analyte is possible only if the derivatization process yields quantitative results. Therefore, studies were also conducted to determine the optimum reaction time and the optimum CSC sample size. These studies were conducted both with CSC from a cigarette which burns tobacco and with CSC from a cigarette which only heats tobacco because of the potential for different types of sample matrix effects.

Evaluation of dynamic range and chromatographic resolution of all analytes. Dynamic range of the method was investigated using thirteen cigarettes which yield a wide range of wet total particulate matter. The cigarettes also yield a wide concentration range of phenolic compounds. When samples representative of this range were prepared and analyzed by GC–SIMS, two different MS electron multiplier dynode voltages were employed in order to obtain maximum sensitivity and linear response for the entire range of interest. Hence, two sets of standards were required to construct calibration curves. All calibration curves were linear with r^2 values of 0.998 or better for the seven phenolic compounds in the concentration ranges of 0.1 to 5.0 μg/ml and 1.0 to 50 μg/ml, respectively. The dynode voltage was lowered 400 V relative to the autotune set voltage to record chromatograms of standards in the higher concentration range as compared to those in the lower concentration range. The voltage was set appropriately depending upon the type of sample to be analyzed. Fig. 1 illustrates a typical GC–MS chromatogram of a *ca.* 5 μg/ml standard solution using the higher concentration range settings.

Tables II and III list results for at least six replicate determinations of the seven phenolic compounds in the CSC from thirteen different cigarettes. For all cigarettes studied, except an ultra-low-"tar" tobacco-burning cigarette and the new cigarette that heats, but does not burn, tobacco, calibration curves constructed from

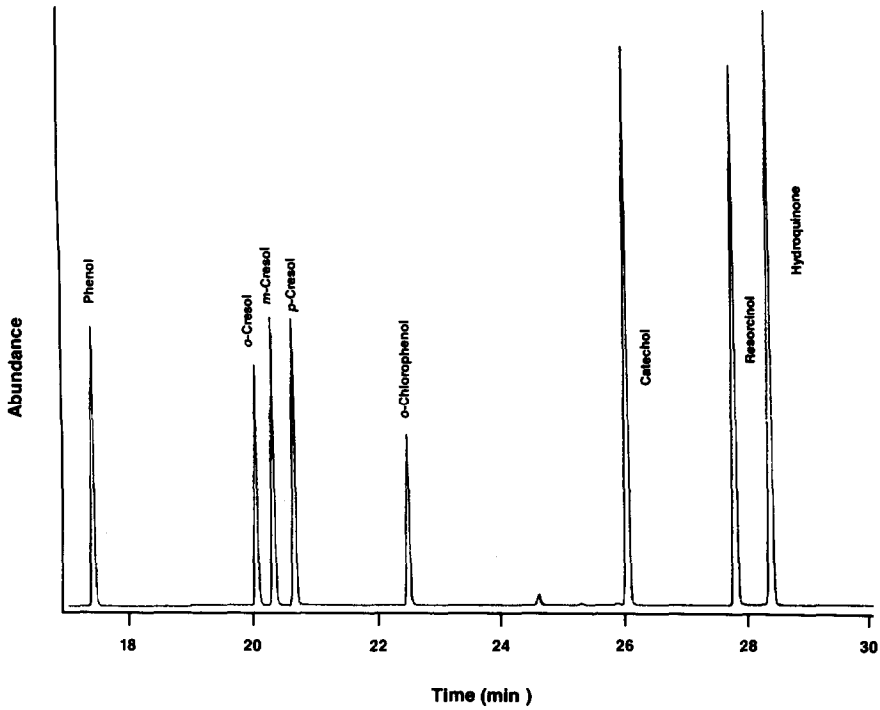

Fig. 1. Chromatogram of set No. 2 standards. Concentration of all standards is 5 μg/ml.

TABLE II

CONCENTRATIONS OF PHENOL AND CRESOLS IN VARIOUS PRODUCTS BY GC–MS

n.d. = None detected. Values in parentheses are the detection limits for the respective compound.

Product	*Concentration (μg/cigarette) ± S.D. (n ⩾ 6)*			
	Phenol	*o-Cresol*	*m-Cresol*	*p-Cresol*
1R4F	7 ±1	1.8 ± 0.4	1.6 ± 0.4	4.1 ± 0.6
Brand A	12.0 ± 0.7	4.1 ± 0.1	3.92 ± 0.07	7.4 ± 0.3
Brand B	6.1 ± 0.2	1.8 ± 0.3	1.9 ± 0.2	4.0 ± 0.1
Brand C	7.7 ± 0.7	0.33 ± 0.07	1.8 ± 0.1	3.5 ± 0.2
Brand D	17 ± 2	2.0 ± 0.5	3.2 ± 0.3	8 ± 1
Brand E	8.7 ± 0.5	0.9 ± 0.2	1.9 ± 0.1	4.3 ± 0.3
Brand F	7 ± 1	1.8 ± 0.2	2.1 ± 0.1	4.8 ± 0.2
Brand G	3.6 ± 0.1	1.70 ± 0.04	2.01 ± 0.05	3.3 ± 0.1
Brand H[a]	0.26 ± 0.02	n.d. (0.2)	n.d. (0.2)	0.24 ± 0.03
NC-1	0.29 ± 0.05	n.d. (0.2)	n.d. (0.2)	n.d. (0.2)
NC-2	0.25 ± 0.01	n.d. (0.2)	n.d. (0.2)	n.d. (0.2)
NC-3	n.d. (0.1)	n.d. (0.2)	n.d. (0.2)	n.d. (0.2)
NC-4	n.d. (0.1)	n.d. (0.2)	n.d. (0.2)	n.d. (0.2)

[a] Brand H is an ultra-low-"tar" cigarette.

TABLE III

CONCENTRATIONS OF DIHYDROXYBENZENES IN VARIOUS PRODUCTS BY GC–MS

n.d. = None detected. Values in parentheses are the detection limits for the respective compound.

Product	*Concentration (μg/cigarette) ± S.D. (n ⩾ 6)*		
	Catechol	*Resorcinol*	*Hydroquinone*
1R4F	38 ± 5	3.0 ± 0.7	37 ± 5
Brand A	50 ± 1	7.7 ± 0.3	56 ± 2
Brand B	37 ± 2	4,7 ± 0.3	41 ± 1
Brand C	42 ± 4	1.2 ± 0.1	48 ± 7
Brand D	58 ± 3	1.8 ± 0.2	50 ± 4
Brand E	38 ± 1	1.3 ± 0.2	40 ± 1
Brand F	43 ± 2	6 ± 1	40 ± 5
Brand G	45 ± 2	6.3 ± 0.4	46 ± 2
Brand H[a]	3.4 ± 1.7	0.58 ± 0.08	n.d. (0.4)
NC-1	2.1 ± 0.9	0.8 ± 0.6	1.3 ± 0.6
NC-2	1.2 ± 0.4	n.d. (0.4)	n.d. (0.4)
NC-3	0.42 ± 0.06	n.d. (0.4)	n.d. (0.4)
NC-4	0.5 ± 0.1	n.d. (0.4)	n.d. (0.4)

[a] Brand H is an ultra-low-"tar" cigarette.

high-concentration-range standards were used to calculate the amounts of the seven phenolic compounds in the particulate phases. Results for the ultra-low-"tar" and the new cigarette prototypes were determined using the lower-concentration-range standards. The new cigarette prototypes yielded substantially lower amounts of all seven analytes when compared to most tobacco-burning cigarettes. Some new cigarette prototypes, although generally lower, yielded amounts similar to those from the ultra-low-"tar" cigarette. However, the NC-4 new cigarette prototype, which is the culmination of product development efforts to date, yielded substantially less of each analyte detected than did the ultra-low-"tar" cigarette.

Estimation of detection limits. Using the low-range standards and the procedure outlined above, detection limits for the seven phenolic compounds by GC–SIMS were calculated and are listed in Table IV. The detection limit for a particular compound

TABLE IV

DETECTION LIMITS FOR ANALYSIS OF PHENOLIC COMPOUNDS BY GC–MS

Compound	*Amounts (μg/cigarette)*
Phenol	0.1
o-Cresol	0.2
m-Cresol	0.2
p-Cresol	0.2
Catechol	0.3
Resorcinol	0.4
Hydroquinone	0.4

according to the procedure of Miller and Miller[16] is defined as the analyte concentration giving a signal equal to the blank signal plus three standard deviations of the blank. The *y*-intercept and associated standard deviation calculated from regression analysis of the standard calibration curve are used as an estimate of the blank signal and the standard deviation of the blank. Note that many compounds in the CSC from the new cigarette prototypes were not detected according to these parameters (see Table II).

Method precision. The precision of the method was estimated using CSC from 1R4F Kentucky Reference cigarettes. These cigarettes are standard reference cigarettes which can be purchased from the University of Kentucky. In this study, ten aliquots of 1R4F CSC were derivatized and analyzed for the seven compounds. Results are shown in Table V. For all compounds good precision was obtained with relative standard deviation ranging from *ca.* 1 to 5%.

CSC sample size and optimum reaction time. The amount of BSTFA employed to derivatize CSC was chosen after first considering the expected hydroxyl content of CSC and a need to minimize sample dilution which in turn maximizes sensitivity. In preparation of CSC samples 2.5 ml of neat BSTFA was employed. This amount of BSTFA was chosen based on estimates of the number of hydroxyl substituents expected in new-cigarette CSC. Because of the unique cigarette design, the new-cigarette CSC is very different from that of other cigarettes. New-cigarette CSC is composed of *ca.* 80–90% water and glycerol[17,20] and, as such, is much richer in hydroxyl functionality than is CSC from cigarettes which burn tobacco. Thus, for a *ca.* 25 mg sample of new-cigarette CSC it was calculated that 2.5 ml BSTFA should provide at least 10 × as much reagent as would be required to react with all hydroxyl substituents in the samples. In order to maximize sensitivity, the reaction is performed in neat BSTFA without addition of any other solvent.

To corroborate these estimates and to ensure that sufficient BSTFA was utilized in the method, a series of samples containing *ca.* 25, 50, 75, and 100 mg of new cigarette and 1R4F CSC, respectively, were reacted with 2.5 ml BSTFA. Figs. 2 and 3 illustrate, on a per-cigarette basis, the effects of increasing the amounts of either new cigarette or 1R4F CSC on the determination of the phenolic compounds. As the figures show, no or very little effect was found; however, we did discover that with 1R4F smoke aerosols 2.5 ml BSTFA was insufficient to derivatize all of the catechol and hydroquinone in samples greater than 25 mg. The reason for this is that aliquots of 1R4F aerosol greater

TABLE V

PRECISION STUDY

Compound	*Relative standard deviation (%)*
Phenol	3.8
o-Cresol	4.9
m-Cresol	2.4
p-Cresol	3.8
Catechol	4.6
Resorcinol	5.4
Hydroquinone	1.4

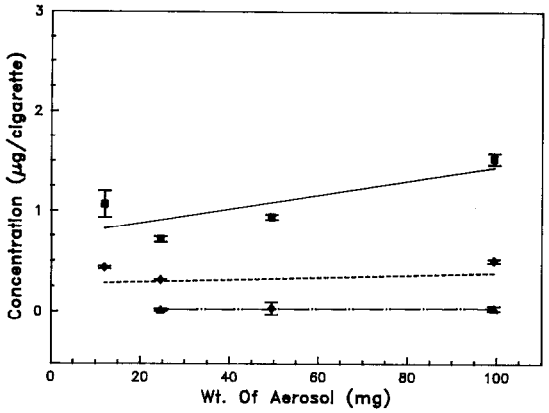

Fig. 2. Effect of varying new-cigarette smoke condensate upon yield of resorcinol (▲), hydroquinone (◆) and catechol (■). Error bars depict 95% confidence intervals.

than 25 mg are not soluble in 2.5 ml BSTFA and catechol and hydroquinone are not extracted sufficiently into this solvent. With a 25-mg aerosol sample 2.5 ml BSTFA are sufficient to consume all the phenolic hydroxyl functions and to drive the reaction to completion.

Since this method involves reaction chemistry, experiments were performed in order to understand the time required for the reaction to reach completion. For these experiments the new cigarette and 1R4F cigarettes were employed and *ca.* 25 mg of mainstream smoke aerosols from the cigarettes were reacted with 2.5 ml BSTFA at 80°C for various lengths of time. Fig. 4 is a representative plot of the amounts of dihydroxybenzenes found in 1R4F aerosols *versus* reaction time. The figure reveals that the reaction is complete within 15 min and that carrying out the derivatization for as long as 18 h has no ill-effects on the analyses. As one might suspect, the TMS ethers of the phenolic compounds are stable, even at temperatures as high as 80°C for 24 to 48 h.

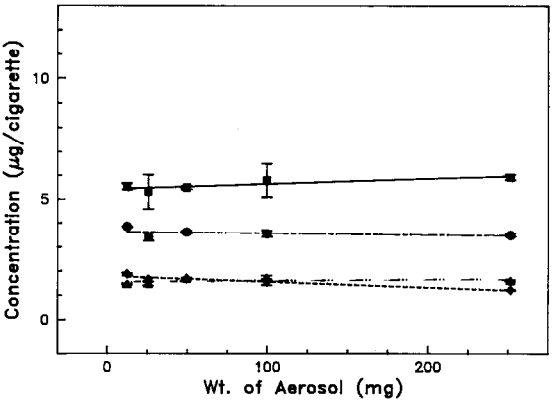

Fig. 3. Effect of varying 1R4F Kentucky Reference cigarette smoke condensate upon yield of *m*-cresol (●), *p* -cresol (▲), *o*-cresol (◆) and phenol (■). Error bars depict 95% confidence intervals.

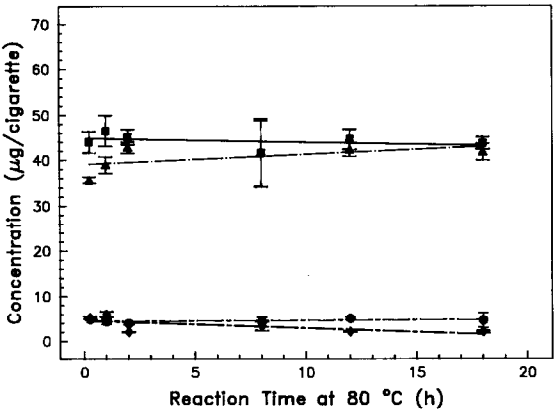

Fig. 4. Effect of varying reaction time of 1R4F Kentucky Reference cigarette smoke condensate with excess BSTFA upon yield of hydroquinone (▲), resorcinol (◆), phenol (●) and catechol (■). Error bars depict 95% confidence intervals.

Comparison of results using different methods

In order to probe the accuracy of the present GC–MS method, results were compared with those obtained by two other methods used at the R. J. Reynolds Tobacco Co.: a liquid chromatography–fluorescence (LC–F) method[18] and a GC method[19]. Fig. 5 compares results obtained by the GC–MS method with the LC–F method. Unlike the GC–MS method the LC–F method cannot separate *m*-cresol from *p*-cresol. Because the GC–MS and LC–F methods yield comparable results, we believe that the GC–MS method yields an accurate measure of phenolic compound concentrations in the mainstream particulate matter. Note there are small differences in amounts of the seven compounds found by each technique: the LC–F method results in slightly higher concentrations of phenolic compounds in the CSCs as compared to the GC–MS method. For the determination of phenol only, both the

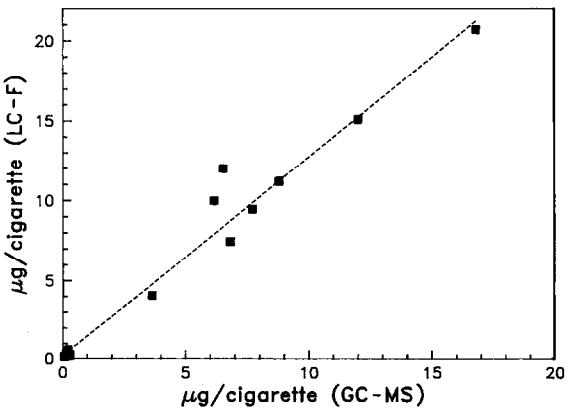

Fig. 5. Comparison of yields of phenol from thirteen cigarette samples by a liquid chromatography–fluorescence detection method (LC–F) with the method that uses BSTFA derivatization followed by gas chromatography–mass spectrometry (GC–MS).

GC–MS and LC–F methods were compared with another GC method used at the R. J. Reynolds Tobacco Co.[19] and good agreement was found among the three methods for amounts of phenol found in the CSCs.

CONCLUSIONS

An accurate and reliable method for analysis of seven phenolic compounds in CSC has been developed. This method involves BSTFA derivatization followed by capillary GC and MS of the TMS ethers. Although the method includes derivatizations, sample preparation is minimal. The method has been successfully applied to determination of seven phenolic compounds in CSC from thirteen different cigarettes. Amounts of the seven phenolic compounds in CSC from all of the tobacco-burning cigarettes in this investigation correlated to FTC "tar" values. Although the amounts of CSC from new cigarettes that heat, but do not burn, tobacco are comparable to those of low-"tar" cigarettes, the concentrations of the seven phenolic compounds in the new cigarette smoke aerosols are 10 to 100 × less than that from tobacco-burning cigarettes.

ACKNOWLEDGEMENTS

We would like to thank Dr. R. A. Lloyd and Mr. J. A. Giles for many helpful suggestions in the development of this method and in the preparation of this manuscript. We would also like to thank Mr. C. H. Risner and Analytical Services at the R. J. Reynolds Tobacco Co. for sharing results obtained by their methods, liquid chromatography–fluorescence and gas chromatography for phenol, respectively.

REFERENCES

1 A. G. Kallianos, *Recent Adv. Tob. Sci.*, 2 (1976) 61.
2 M. E. Snook and P. J. Fortson, *Anal. Chem.*, 15 (1979) 1814.
3 M. E. Snook, P. J. Fortson and O. T. Chortyk, *Tob. Sci.*, 24 (1980) 30.
4 W. J. Chamberlain, M. E. Snook and J. L. Baker, *Anal. Chim. Acta*, 111 (1979) 235.
5 W. A. Court, *J. Chromatogr.*, 130 (1977) 287.
6 G. A. Smith and D. A. King, *Chem. Ind.*, (1964) 540.
7 K. D. Brunnemann, H. C. Lee and D. Hoffmann, *Anal. Lett.*, 9 (1976) 939.
8 M. R. Guerin and G. Olerich, *Tob. Sci.*, 20 (1976) 44.
9 S. Ishiguro, S. Cato, S. Sugawara and Y. Kajuraki, *Agr. Biol. Chem.*, 40 (1976) 977.
10 M. Legtonen, *J. Chromatogr.*, 202 (1980) 413.
11 R. W. Souter and R. H. Bishara, *J. Chromatogr.*, 140 (1977) 245.
12 A. DiCorcia, *J. Chromatogr.*, 80 (1973) 69.
13 P. Buryan, J. Macak and V. M. Nabivach, *J. Chromatogr.*, 148 (1978) 203.
14 J. Hrivnak and J. Macak, *Anal. Chem.*, 43 (1971) 1039.
15 J. Hoshika, *J. Chromatogr.*, 157 (1978) 277.
16 J. C. Miller and J. N. Miller, *Statistics for Analytical Chemistry*, Wiley, New York, 1984.
17 M. F. Borgerding, R. D. Hicks, J. E. Bodnar, D. M. Riggs, E. J. Nanni, G. W. Fulp, Jr., W. C. Hamlin and J. A. Giles, *J. Assoc. Off. Anal. Chem.*, submitted for publication.
18 C. H. Risner and S. Cash, R. J. Reynolds Tobacco Company, personal communication.
19 J. M. Martin and H. L. Gilleland, R. J. Reynolds Tobacco Company, personal communication.
20 *Chemical and Biological Studies On New Cigarette Prototypes That Heat Instead Of Burn Tobacco*, R. J. Reynolds Tobacco Company, Winston-Salem, NC, 1988.