Quantitative Determination of Phenol in Cigarette Smoke

A. W. SPEARS

Research Division, P. Lorillard Co., Inc., Greensboro, N. C.

▶ Phenol is quantitatively determined in cigarette smoke by a method involving solvent partition, steam distillation, and gas chromatography. Purity of the isolated phenol and cresols has been demonstrated by spectrophotometry and the accuracy of the method has been established by use of the isotope dilution technique. Semiquantitatively, the method can be extended to the cresols and other volatile phenols. A new gas chromatographic column has been developed which gives improved resolution of phenolic compounds. Results of phenol analyses on the smoke of commercial cigarettes have been obtained.

ALTHOUGH several investigators (*2–4, 6*) report the determination of phenolic compounds in cigarette smoke, only Hoffmann and Wynder's method was considered an analytical method, since all others are indirect and circuitous and not applicable to routine determinations. The Hoffmann and Wynder method is similar to that developed in this laboratory, with the exception of the gas chromatographic resolution of components. The column which they employ is similar to that reported by Payn (*5*). Several of the components of cigarette smoke are unresolved by this column and resolution is lost with increasing age of the packing.

Carelli *et al.* (*1*) reported the separation of a cresol mixture on a column packed with finely divided polyhexamethylene adipamide, where cyclohexane was the mobile phase. This separation resulted from differences in the stability of the association complexes formed between phenolic compounds and the polyamide. Direct application of the polyamide to gas chromatography gave separations of phenolic compounds, but the peaks were asymmetric. However, on addition of a small amount of liquid phase the asymmetry was negligible and the resolution between some compounds was increased.

To quantitate the method, C^{14} phenol was used as a tracer and then an internal standard (*o*-hydroxyacetophenone) was adopted after correlation with the tracer. The relative standard deviation of the method was 2%.

EXPERIMENTAL PROCEDURE

One hundred nonfilter or 200 filter cigarettes were smoked on a commercial Phipps and Bird smoking machine to a butt length of 23 or 25 mm., respectively. The machine was adjusted so that a 35-cc. puff, over a 2-second duration, was taken once a minute. The collection vessels were two traps of 100-cc. volume each, immersed in dry ice–acetone slurry, followed by a drying tube containing loosely packed glass wool wet with 2*N* sodium hydroxide. The condensate was removed from the collection vessels and the glass wool by washing with 75 to 100 ml. of 2*N* sodium hydroxide.

The appropriate amount of C^{14} phenol and/or *o*-hydroxyacetophenone in cyclohexane was added to the sodium hydroxide solution of smoke condensate. (The added concentration of *o*-hydroxyacetophenone should approximate the anticipated concentration of phenol, 9 and 3 mg. per 100 cigarettes for nonfilter and filter cigarettes, respectively.) The alkaline mixture was cooled to 0° C., acidified with 20% sulfuric acid, and extracted with three 50-ml. portions of ether. The combined ether extracts were washed with three 50-ml. portions of a saturated aqueous solution of sodium bicarbonate and then extracted with five 40-ml. portions of 2*N* sodium hydroxide. The combined aqueous sodium hydroxide extracts were cooled to 0° C., acidified with 20% sulfuric acid, and distilled with steam at a rate of 2 ml. per minute until 240 to 250 ml. of distillate had been collected in a distillation receiver containing 0.5 ml. of 2*N* sodium hydroxide. The distillate was cooled to 0° C., acidified with 20% sulfuric acid, and extracted with three 20-ml. portions of ether and the combined extract was dried over 10 grams of anhydrous sodium sulfate for about 10 minutes. The ether solution was decanted from the desiccant and evaporated with agitation in a bath at about 55° C., without the aid of an inert carrier gas. The mixture was evaporated near dryness, as indicated by an increase in viscosity of the mixture. Although about 10% of the phenol is lost in the evaporation, a corresponding amount of *o*-hydroxyacetophenone is evaporated and elaborate precautions need not be taken.

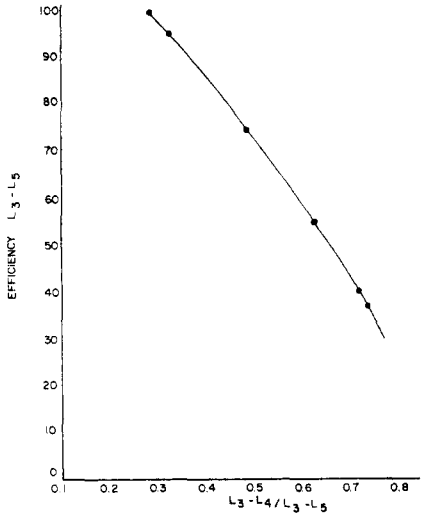

Figure 1. Quench correction of C^{14} phenol

Three or four drops of dry methanol were added and 0.02 to 0.03 ml. of the supernatant liquid was subjected to gas chromatography. (In the case of the C^{14} phenol the sample was diluted to 1.0 ml. with toluene and a larger aliquot was used for gas chromatography.)

A Model 702-A Nuclear-Chicago liquid scintillation counter was used to determine the concentrations of C^{14} phenol. Suitable standards were prepared and a quenching curve was obtained by using the isolated phenols as the quencher (Figure 1). The instrumental parameters were as follows: discriminator settings, L_3 1.5, L_4 2.9, and L_5 9.9; data and gate voltage 950 and 1476 volts, respectively; sample temperature 0° C. A quenching factor of only about 0.5 was obtained with the phenol mixture when the solvent was toluene and the scintillation solution was 0.5% 2,5-diphenyloxazole and 0.03% 2-*p*-phenylenebis-(5-phenyloxazole) in toluene. Hence, one half the sample isolated for gas chromatography, 0.5 ml. in toluene, was added to 15 ml. of the scintillator solution and both the counts per minute and the quenching factor were determined. The counting efficiency for an unquenched sample was about 70% and about 12,000 c.p.m. was a convenient amount of C^{14} phenol for a single experiment.

The gas chromatography was carried out on a Perkin-Elmer Model 154-D Vapor Fractometer equipped with a

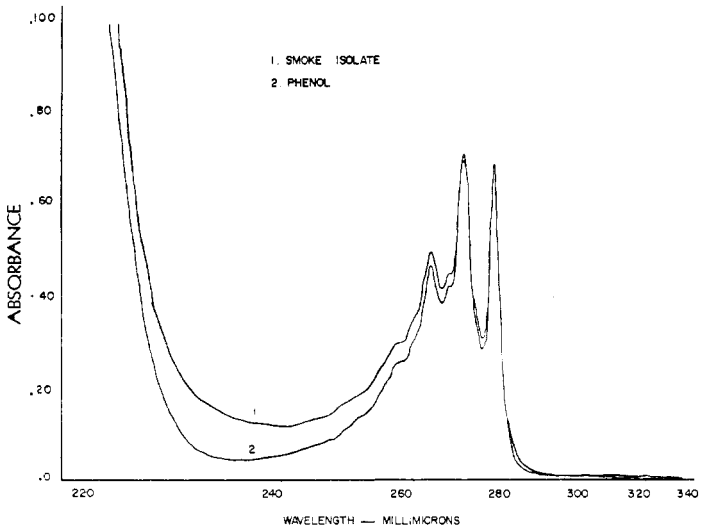

Figure 2. Ultraviolet spectra

1-mv. recorder and approximately 8000-ohm thermistors. The detector voltage was 8 volts. Peak areas were determined by a Disc integrator.

The gas chromatography column was prepared in the following manner: A solution of commercial nylon 66 in 98% formic acid was filtered through a borosilicate glass plug and added to 42- to 60-mesh firebrick to give 25% nylon by weight. The formic acid was removed by flash evaporation and the firebrick shaken with water. The mixture was placed in a blender for a few minutes to break up any lumps and the brick was washed several times with water, methanol, and cyclohexane, respectively. The dry weight of the coated brick was recorded and 8% Ucon oil 50 HB 2000, based on the weight of uncoated brick, was added. The oil was added in cyclohexane solution and the solvent was removed by flash evaporation. Finally, the material was passed through screens to give 40- to 60-mesh particles. A 6-foot, $^1/_4$-inch stainless steel column was prepared with this packing and conditioned at 168° C. for 24 hours.

The column used for this work gave excellent results at a temperature of 168° C. and a helium flow of 78 ml. per minute with a sample which did not contain more than 0.5 mg. of phenol. However, other columns, prepared in the same way, gave optimum results at a slightly higher or lower temperature. At the optimum operational temperature for any particular column the 2,4-dimethylphenol peak (No. 14 in Figure 5) is centered between the phenol and 3- and 4-methylphenol peak. Too high a concentration of phenols gave rise to asymmetric peaks. The same column has been in use for 17 months and has allowed approximately 200 chromatograms with little or no loss in resolution of the individual phenols.

RESULTS

Utilization of this method has led to the identification of the phenolic compounds listed in Table I. The last two compounds, 3- and 4-methoxyphenol, have not been previously reported, but their identification is tentative at this time. The purity of the

Table I. Phenolic Compounds of Cigarette Smoke

Component	Retention time, min.	Major ultraviolet absorption[a] maxima, mμ
2-Methoxyphenol	9.5	281.5, 275.5
2,6-Dimethylphenol	13.5	277.5, 272
2,4,6-Trimethylphenol	18.2	284, 279, 277
2-Methylphenol	21.2	278.5, 272
Phenol	23.8	278, 271.5, 265
2,4-Dimethylphenol	27.2	285, 279
2,5-Dimethylphenol	27.2	281, 275.5
4-Methylphenol	30.0	286, 279.5, 276.5, 273
3-Methylphenol	31.2	280, 273
3-Ethylphenol	42.0	279, 272
4-Ethylphenol	42.0	285, 278.5, 275, 272
4-Methoxyphenol	90	301, 294, 290
3-Methoxyphenol	108	280.5, 273.5
Internal standard (2-hydroxyacetophenone)	7.8	

[a] Solvent for all ultraviolet spectra was cyclohexane.

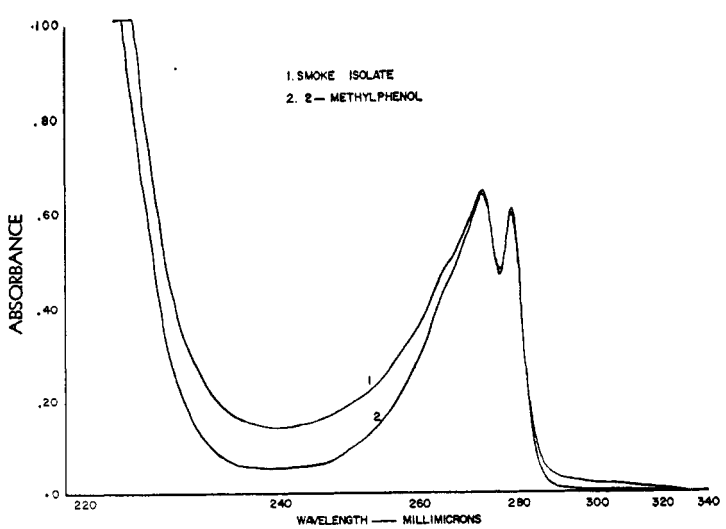

Figure 3. Ultraviolet spectra

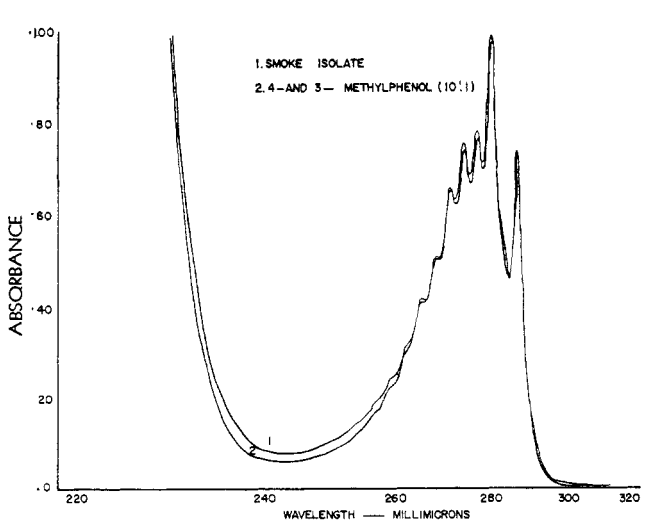

Figure 4. Ultraviolet spectra

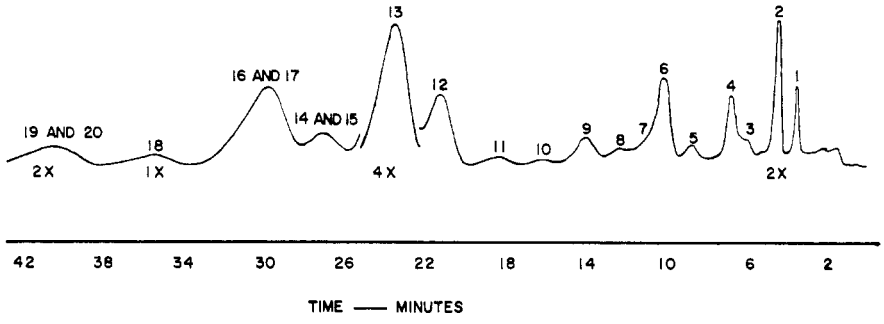

Figure 5. Gas chromatogram

Table II. Comparison of Methods

Sample	Tracer	I. S.
I	13.6 μg./cig.	13.9 μg./cig.
II	73.1 μg./cig.	78.0 μg./cig.
III	2.15 mg./g.	2.10 mg./g.
IV	...	3.08 mg./g.
V (IV + 2.50 mg. phenol)	...	5.56 mg./g.

Table III. Precision of Method

Sample	Run	Phenol [I. S.] μg./cig.
I	1	13.7
	2	13.4
II	1	16.7
	2	16.8
III	1	23.4
	2	25.5
IV	1	21.0
	2	19.3
V	1	0.763 mg./g.
	2	0.706 mg./g.
VI	1	74.6
	2	73.1
	3	72.8
VII	1	67.2
	2	66.0
		Rel. std. dev. 2%

components which were determined as phenol, 2-methylphenol, and 3- and 4-methylphenol was indicated by the ultraviolet spectra (Figures 2, 3, and 4, respectively). The latter two components were partially resolved by the gas chromatographic column and because of slight overlap with the preceding 2,4-dimethylphenol peak a representative sample of the methylphenols could not be collected (see Table I and Figure 5). Consequently, the ratio of 4- to 3-methylphenol indicated by Figure 4 is not quantitative. Figure 5 shows a typical chromatogram of the phenolic fraction of cigarette smoke. Peaks 1, 2, 3, 4, 5, 7, 8, 10, and 18 have not been identified; peaks 6, 9, 11, 12, 13, 14, 15, 16, 17, 19, 20, and 21 have been identified as 2-methoxyphenol, 2,6-dimethylphenol, 2,4,6-trimethylphenol, 2-methylphenol, phenol, 2,4-dimethylphenol, 2,5-dimethylphenol, 4 - methylphenol, 3 - methylphenol, 3-ethylphenol, 4-ethylphenol, and 3,4-dimethylphenol (tentative), respectively. Figure 6 shows a typical chromatogram where the internal standard has been added.

Table II gives the results obtained on samples containing both C^{14} phenol and the internal standard. Table III shows the results of duplicate determinations on a number of samples where the internal standard was used. A relative standard deviation of 2% is estimated, and assuming that isotope dilution studies can be made with the same precision, the relative error is estimated as 2%. Table IV presents a spectrum of phenol values obtained on commercial cigarettes. The values of other phenolic constituents have not been reported, since they are isolated with less precision than reported for phenol. However, the ratios of phenol constituents were found to be semiquantitatively constant for most samples studied. The following ratios have been calculated: phenol: 3- and 4-methylphenol: 2-methylphenol: 2,4- and 2,5-dimethylphenol: 2-methoxyphenol, 10: 5.6: 2.3: 1.9: 1.3.

Table IV. Phenol Values of Commercial Cigarettes

Sample brand[a]	Phenol/ cig., μg.	Filter efficiency[b]
1 (85 mm.) NF	108	...
2 (85 mm.) NF	104	...
3 (85 mm.) NF	76	...
4 (70 mm.) NF	81	...
5 (70 mm.) NF	59	...
6 (85 mm.) F	35	41
7 (85 mm.) F	34	44
8 (85 mm.) F	33	42
9 (85 mm.) F	31	39
10 (85 mm.) F	28	69
11 (85 mm.) F	24	44
12 (85 mm.) F	24	41
13 (85 mm.) F	23	51
14 (85 mm.) F	21	65
15 (85 mm.) F	21	43
16 (85 mm.) F	14	54
17 (85 mm.) F	14	42
18 (85 mm.) F	12	45
19 (85 mm.) F	9	60

[a] NF nonfilter.
F filter.
[b] % condensable smoke removed by filter. Condensable smoke determined by collection in solvent of toluene-ethanol–aqueous HCl and evaporation to constant weight in vacuum oven.

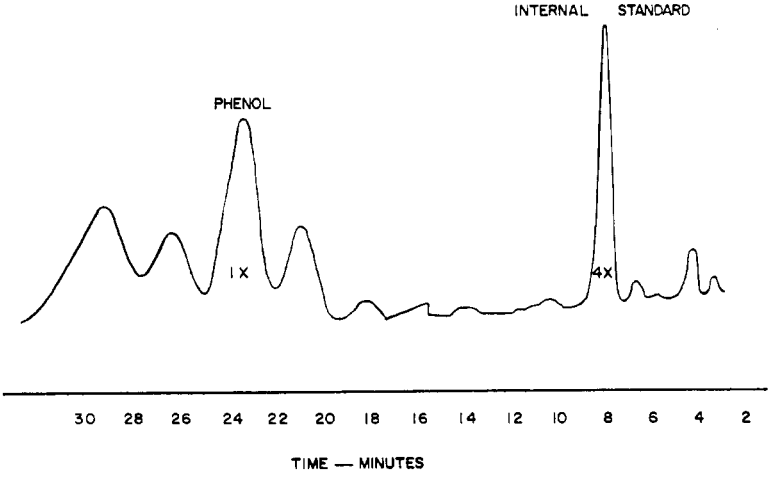

Figure 6. Gas chromatogram

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