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Analyses of Phenolics in Cigarette Smoke by GC-MS with the Multiple Ion Selection Technique

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Abstract □ Improvements in the analytical methodology used in the gas chromatographic/mass spectral analysis of phenolic compounds from cigarette smoke are described. For the direct analysis of crude samples, pyridine extraction and the glass capillary column GC was used for the separation of phenolics as trimethylsilyl derivativatives. The separations of cigarette smoke on Carbowax 20M and SE-54 wall coated open tubular columns are given. Improved methodology for the routine quantitation of the identified components using the computer-controlled multiple ion selection technique of MS presented. Considerations pertaining to routine analyses of a multitude of complex smoke samples are also discussed.

Keywords □ Phenolics in cigarette smoke condensate, Pyridine extraction, Capillary column GC-MS, Computer-controlled multiple ion selection technique.

Phenolic compounds are components of tobacco and its smoke that are pyiosyntheized during the smoking process. These phenols are important contributors to the flavor and aroma of cigarette smoke.[1] Studies have been shown that some dihydroxybenzenes, such as catechols, are important co-carcinogens of tobacco smoke.[2,3] The weak acid fraction of cigarette smoke condensate (CSC) has been to contain mono-and dihydroxybenzenes and their alkylated derivatives and to possess high tumor-promoting activity in mouse-back painting bioassay.[4,5]

Our environment contains numerous mono-and dihydroxybenzenes derived from both natural and man-made sources.[6] Due to their importance, phenolic constituents have been determined by a variety of methods.[7,10] Howerever, presence within a complex matrix. CSC is a good example of a most perplexing mixture that contains many thousands of compounds. Of the developed methods, gas chromatography (GC) of the mono-and dihydroxybenzene compounds[11], or of their derivatives[12] has been the most widely used analytical technique. Most GC procedures require some type of prior chromatographic separation or purification to produce a purified phenolic fraction. Solvent partitioning, column chromatography, and acid-base extractions have been employed without the realization that they often lead to severe losses of many phenols, especilly the biologically important dihydroxybenzenes (catechol, hydroquinone, and their alkyl derivatives). Consequently, such procedures should be avoided in devising quantitative determinination of these compounds. The best analytical technique for the phenolic compounds should rely on the direct analysis of crude samples, without elaborate sample purification precedures.

It also became apparent that conventional packed column GC was unaceptable, due to a lack of compound resolution. Even after isolation procedure[13] produced pure phenolic isolates (fractions), identification and quantitation of

many phenolic components was impossible with normal packed column GC. This difficulty was resolved by the glass capillary column GC and the computer-controlled multiple ion selection (MIS) technique of MS, which significantly increased the resolutien and improved the quantitation of compounds in complex CSC mixture.

MIS based on channel switching between several masses, turns the mass spectrometer into an ideal detectors: sensitive, specific, versatile. MIS can be realized by the following three possibilities: 1) computer-aided adjustment of the MS accelerating voltage, *i.e.*, electrical peak jumping, 2) computer-aided adjustment of the Ms magnetic field, *i.e.*, magnetic peak jumping, 3) combined jumping, *i.e.*, 1) and 2) Electrical peak jumping is very fast, jump time of approximately 10-20 milliseconds irrespective of mass, as compared to magnetic jumping, jump time of approximately 300-500ms, but has the disadvantage of restricted jump range and loss of sensitivity with increasing jump distance. The disadvantages of possibilities 1) and 2) are practically compensated by using possibility 3).

In the present paper, the methodology developed for the direct glass capillary GC and computer-controlled MIS analyses of the phenolic compounds from CSC is described.

EXPERIMENTAL METHODS

Materials

Dry pyridine and the silylating reagent, N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) plus 1% trimethylchlorosilane (TMCS), were obtained from Pierce Chemical (Rockford, Illinois). All phenolic standards were purchased from Aldrich and Sigma Co. (St. Louis, U.S. A.).

Sample Preparation

Cigarettes were conditioned by keeping them in a room with 22±3°C and 65% relative humidity for 48 hours before use. For analysis, five cigarettes selected by weight and resistance to draw were smoked through Cambridge filter pad using the Phillip Morris twenty channel smoking machine. Standard puff parameters (35ml volume/puff, 2 sec duration/puff, and 1 puff/min) were used to smoked the cigarettes to a 30mm butt. The filter pad was transferred to a screw capped vial. Ten ml portion of dry pyridine and excess amount of anhydrous sodium sulfate were added. And then the sample was allowed to stand overnight.

Silylation Procedure

Aliquots (100μl) of standard mixtures and sample were mixed with 100 μl of BSTFA(+1 % TMCS) in a screw-capped vial. The mixture was heated at 60°C for 30min prior to GC-MS analyses.

GC-MS Analyses

GC-MS analyses for trimethylsilyl (TMS) derivatives of crude CSC were performed on a Varian 3700 gas chromatograph linked with a Varian MAT212 double focusing mass spectrometer. GC condrtions for GC-MS analyses were as follows: column, wall coated open tubural (WCOT) glass (borosilicate) column (20m×0.25mm i.d.) coated with SE-54; helium flow rate, 1.0ml/min; column temperature, isothermally 60°C for 2 min and programmed at 10°C/min to 240°C; splitless injection mode; 30 sec injector purge delay time. And MS conditions were as follows; ion source temperature, 250°C; ionization voltage,70 eV; emission current, 1mA. Samples were spiked with the internal standard, p-sec-butylphenol. GC peak identifications were achieved by comparison of the gas chromatographic retention times with those of authentic compounds and confirmed by their mass spectra.

Quantitation

Quantitations of the identified components were performed by using the computer-controlled MIS technique. A Varian Model SS MAT 188 data system (computer, PDP 11/34) was used for this purpose. To determine the concentration of compounds in a real sample, standard mixtures and samples were injected into the GC-MS. During data measurement and acquisition, MIS traces of monitored masses were displayed selectively. The areas of selective traces were quantificated. The result shows the intensity ratios between the peaks of standard and measured component. The corresponding component concentration in the original sample was directly read from the calibration list.

RESULTS AND DISCUSSION

Some problems were observed intially in attempting to isolate quantitively the weak acids from the condensate by using typical fraction procedure. Losses of phenolics from the aqueous basic solution during the solvent washing step to remove interfering nonaciding nonacidic contaminants, and considerable losses of the free phenolics during concentration of the final extract containing the weakly acidic substances. And studies have shown that phenolics, especially biologically important dihydroxybenzenes, catechol and hydroquinone, are readily oxidized upon standing for even short periods of time. Thus, rapid analysis of samples was essential and also, direct analysis of phenolic samples would require the the shortest storage time.

Pyridine (pH=8) was chosen for the direct extraction of phenolics from CSC in order to avoid the defects of fractionation procedure. And the high resolution glass capillary columns were used for the direct analyses the complex CSC extract. WCOT fused silica capillary column coated with Carbowax 20M was chosen for the initial attempts at separating the underivatized compounds. The separation of phenols in underivatized crude CSC on a Carbowax 20M column proved that this column

Table I: Identified phenolic compounds and their amounts in commercial cigarette smoke condensate.

Peak No. (Figure. 2)	Compound (TMS derivative)	Molecular ion (TMS derivative)	Amount (μg/cigarette)
1	Phenol	166	119.9
2	*o*-Cresol	180	10.5
3	*m*-Cresol	180	13.7
4	*p*-Cresol	180	32.4
5	2,5-Xylenol	194	1.4
6	2,4-Xylenol	194	1.1
7	*o*-Methoxyphenol	196	14.2
8	*o*-Ethylphenol	194	6.4
9	*m*-Ethylphenol	194	2.3
10	*o*-Vinylphenol	192	3.8
11	2,3-Xylenol	194	0.9
12	*p*-Ethylphenol	194	4.2
13	3,5-Xylenol	194	0.8
14	3,4-Xylenol	194	0.7
15	2,3,5-Trimethylphenol	208	9.3
16	3,4,5-Trimethylphenol	208	5.4
17	*p*-Vinylphenol	192	31.3
18	Catechol	254	300.6
19	Resorcinol	254	84.2
20	4-Methylcatechol	268	48.1
21	3-Methylcatechol	268	32.3
22	Hydroquinone	254	172.3
23	Methylhydroquinone	268	160.3
24	4-Vinylguaiacol	222	14.2
25	4-Ethylcatechol	282	72.4
26	4-Vinylcatechol	280	145.2
27	α-Naphthol	218	2.3
28	β-Naphthol	218	1.1
29	Pyrogallol	342	38.4

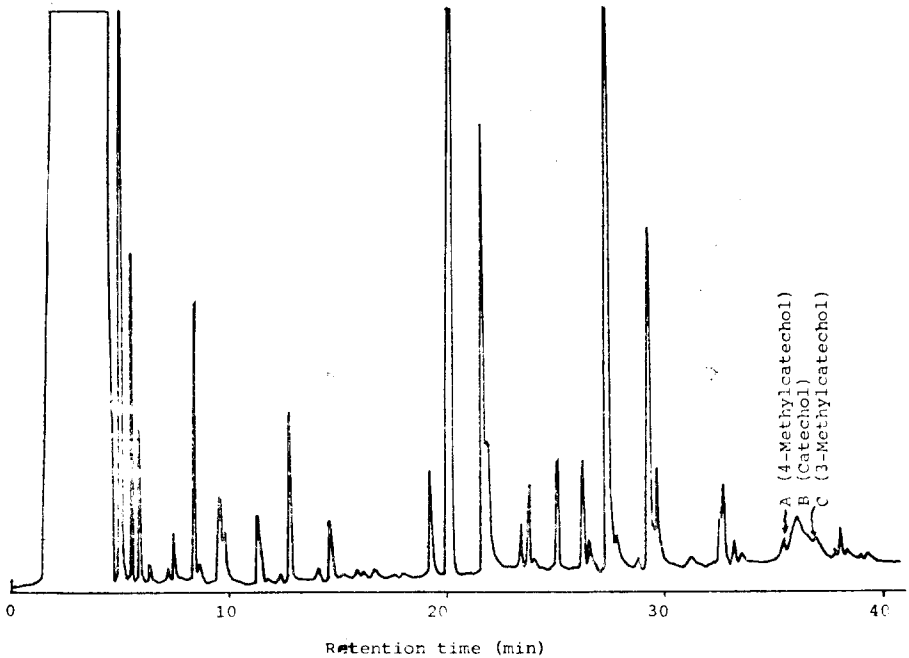

Fig. 1: Chromatogram of the underivatized CSC on a fused silica Carbowax 20M WCOT column. Conditions: 50m×0.20mm i.d.; 50°C for 5 min; temperature programmed from 50 to 200°C/min; splitless injection mode; 30sec injector purge delay time; flame ionization detector. Instrument: Hewlett-Pachard Model 5880A GC.

separated most of these compounds with little tailing or decomposition. Unfortunately, the biologically important catechol and its alkyl derivatives (peak A-C, Figure 1) were strongly adsorbed on the column surface.

Because of the limited success in separating the underivatixed phenols on a Carbowax 20M WCOT column, the decision was made to analyze them as their TMS derivatives on a SE-54 WCOT capillary column. As TMS derivatives, the polar phenols behaved like aliphatic hydrocarbons, which allowed me to take full advantage of the selectivity of the nonpolar SE-54 liquid phase. Figure 2 shows the total ion current (TIC) chromatogram of TMS derivatives of CSC on a SE-54 WCOT column. And results of identification of peaks in Figure listed in Table I.

To consider derivatizing agent, BSTEA was superior to BSA. BSTFA was more volatile than BSA, and the excess reagent eluted well in advance of TMS-phenol. When BSA was used, TMS-phenol coeluted with the reagent peaks. And the TMS derivatives were stable, showing no signs of decomposition even when kept in closed vials for as 2 months.

Quantitative analyses of the phenolic compounds from tobacco smoke were routinely performed by using a computer-controlled MIS technique. The flow chart of practical practical procedure for routine MIS is shown in Figure 3. The MIS acquisition was called, each MIS traces can be monitored up to 8 different masses within specific time periods. When used with a routine MIS procedure, it can process a very large number of samples and accumulate ready

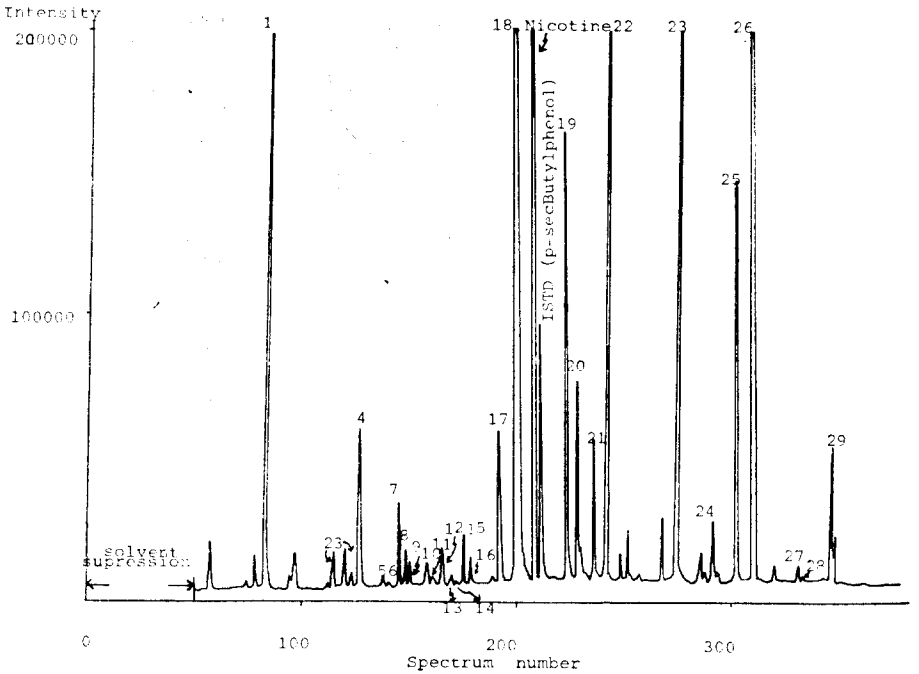

Fig. 2: TIC chromatogram of the TMS derivatives of CSC separated on a borosilicate glass SE-54 WCOT column.

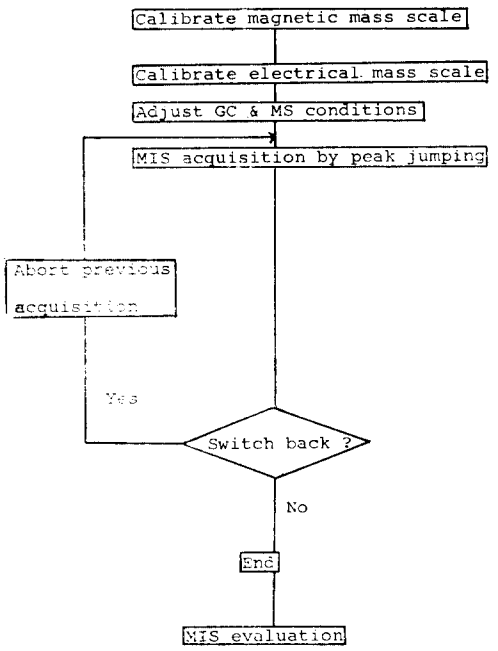

Fig. 3: Flow diagram for the computer-controlled routine MIS procedure.

to use results.

The spectra from GC-MS analysis of the TMS derivatives of the reference phenolic compounds were similar. There were prominent ions at m/e 73, M-15, and M. Most phenolic compounds investigated fairly prominent molecular ions, M, and base peak was generally M-15. Table II represents prominent ions of TMS-cresol. Although nearly all molecular ion peak of phenolic components were not base peak, the intensity of molecular ion of phenolic components were not base peak the intensity of of molecular ion of these compounds was sufficient for quantition. All the compounds were measured best in MIS mode on the molecular ion peak. Figure 4 shows MIS plots of standard solutions 1, 2, 3 ppms of TMS-2, 5-xylenol and of quantitation of identified components using the routine MIS procedure are represented in Table I.

Table II: Prominent ions in mass spectrum of TMS derivative of *o*-cresol.

m/e	Relative intensity	Fragment
180	41	M^+
165	100	$[M-CH_3]^+$
91	33	(tropyllium ion)
73	23	$[Si(CH_3)_3]^+$
65	7	(cyclopentadienyl cation)

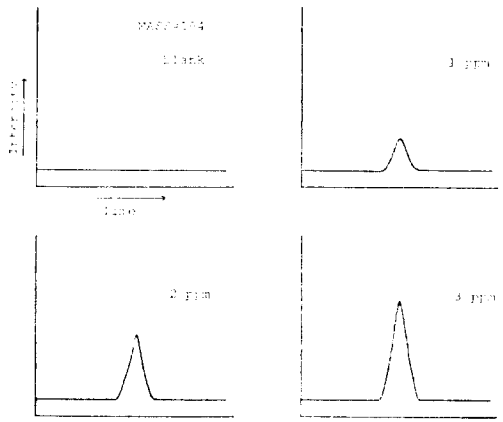

Fig. 4: MIS plot at m/e 194 of blank and standard solutions of TMS-2, 5-xylenol.

The recovery test, known quantities of catechol in methanol were injected to filter pad before extraction with pyridine. The recovery of nearly 100% indicates that pyridine effectively extracts the phenolics and the phenolics and that sufficient BSTFA is present to convert them to the derivatives. The reproducibility of this method was checked at the point of catechol in samples (% standard deviation was below ±2%).

For special applications where an extremely high specificity is needed one has to couple a capillary column to a mass spectrometer working in the computer-controlled MIS mode. The high resolution of the capillary column is ideally completed by the increased selectivity of the computer-controlled MIS program of MS. The specificity during MIS work is improved because the signals from foreign compounds such as column bleeding or biological matrix are well separated from the ions one wishes to detect. Furthermore the advantages of this method for the analysis of phenolics in highly complex CSC include: a relative short analytical time and simple procedure, greater sensitivity than any other GC detectors (ng range detection is readily achieved by using this technique because MIS allows to stay long time in one mass and then, much ions can be assemble), and routine analyses of trace compounds are possible.

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