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Evaluation of gas chromatography–Fourier transform infrared spectroscopy–mass spectrometry for analysis of phenolic compounds

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ABSTRACT

A gas chromatography (GC)–infrared (IR) spectroscopy–mass spectrometric (MS) system was evaluated for the identification and quantitation of 50 target phenolic compounds. Six columns of varying polarities were tested to achieve optimum chromatographic resolution of the phenolic compounds. The low polarity columns (HP-1, DB-5) gave resolution of 41 of the 50 phenolics; higher polarity columns (DB-17, DB-1701, DB-210, Nukol) were not as effective. Standard solutions containing 50 target phenolic compounds with concentrations in the range of 10 to 600 ng/μl were prepared. The solutions were injected into the selected column (HP-1) using cool on-column injection with a retention gap and with the IR detector and mass spectrometer run in the full scan mode. Fully resolved peaks could be easily identified and quantitated by either IR spectroscopy or MS. For co-eluting compounds spectral subtraction (IR) or selected ion (MS) techniques were employed as appropriate to identify the individual phenolics.

INTRODUCTION

A recent report [1] detailed a method for the determination of alkylated phenolics in air. Analysis of the final extract was performed using fused-silica capillary column gas chromatography (GC) with flame ionisation detection (FID) or mass spectrometric (MS) detection. The method was deemed adequate but the results indicated a clear need for increased specificity in the identification and quantitation of phenolic compounds. Although GC–MS is a powerful technique the mass spectra for positional isomers are usually virtually identical and hence, without good chromatographic separation, compound identification cannot be definitive. In contrast, the infrared (IR) spectra of such isomers can be very different, allowing unambiguous compound identification [2,3], and overlapping chromatographic peaks can be re-

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TABLE I

CAPILLARY COLUMNS, SUPPLIERS AND OPTIMUM TEMPERATURE PROGRAMS

Column	Description	Supplier	Ramp 1					Ramp 2			Ramp 3		
			Initial T (°C)	Hold Time (min)	Rate (°C/min)	Final T (°C)	Hold Time (min)	Rate (°C/min)	Final T (°C)	Hold Time (min)	Rate (°C/min)	Final T (°C)	Hold Time (min)
HP-1	25 m × 0.20 mm I.D., 0.5-μm film	Hewlett-Packard	55	1	2	124	0	5	170	0	10	280	1
DB-5	30 m × 0.25 mm I.D., 0.25-μm film	J & W Scientific	50	1	4	164	0	40	280	3	NA[a]	NA	NA
DB-17	30 m × 0.25 mm I.D., 0.25-μm film	J & W Scientific	50	4	4	178	0	25	280	2	NA	NA	NA
DB-1701	30 m × 0.25 mm I.D., 0.25-μm film	J & W Scientific	50	4	5	180	0	15	280	1	NA	NA	NA
DB-210	30 m × 0.25 mm I.D., 0.25-μm film	J & W Scientific	60	1	4	140	0	10	250	0	NA	NA	NA
Nukol	20 m × 0.25 mm I.D., 0.25-μm film	Supelco	50	1	20	150	14	3	200	0	10	230	22

[a] NA = Not applicable.

solved into the two components using spectral subtraction techniques [4]. The combination of the two techniques (GC–IR–MS) is especially powerful [5,6].

Direct gas chromatography of parent phenols is now feasible with the advent of fused-silica capillary columns and avoids the problems encountered in derivatisation of phenolic compounds. The present study evaluates the use of cool on-column sample injection–fused-silica capillary GC (employing a variety of stationary phases) coupled with tandem IR spectroscopy and MS for the identification and quantitation of 50 target phenolic compounds.

EXPERIMENTAL

Materials and instrumentation

Pure phenolic compounds. The phenolic kit supplied by Supelco Canada contained 49 compounds. 2,3,5,6-tetramethylphenol was provided by Varian Canada. Methanol (99.7%) supplied by Fisher Scientific and chloroform (99.7%) supplied by Caledon Labs. were used as solvents for the phenolic compound standards.

Fused silica capillary columns. Table I presents information on the six fused-silica capillary columns and the suppliers.

Gas chromatography–flame ionisation detection. An HP-5890 gas chromatograph equipped with a flame ionisation detector was used for initial investigation of phenolics separation by six capillary columns of varying stationary phase polarity. A cool on-column injection port with a 26 gauge steel needle guide (HP P/No. 19245-20540) was used for sample introduction, which allowed the application of an auto sampler/auto injecter system (HP 7673A). The column was connected to the injector via a fused-silica retention gap (1.0 m × 0.53 μm) coated with 0.1 μm of methyl silicone (HP P/No. 19095-10050). The connection of the retention gap and the column was made possible by using an appropriate press-fit glass connector (HP P/No. 4041-2173). The GC oven conditions were varied with each type of column. Other parameters were: helium flow-rate, 1.5 ml/min (column) and 35 ml/min (make up gas); hydrogen flow-rate, 30 ml/min; air flow-rate, 320 ml/min; injector, cold on-column; detector, 280°C; injection; 1 μl solution for each GC run.

Gas chromatography–infrared–mass spectrometry. The Hewlett-Packard GC–IR–MS system consisted of an HP 5890 GC connected to an HP 5965A Fourier transform (FT) IR detector and to an HP 5970B MS detector. The central control of the IR detection was the HP 59970C Chemstation which was installed with the HP 59965 IR operating software and equipped with an HP 7957 Winchester drive (80 Mb) and an HP 9144 tape drive (80 Mb). The MS detector was controlled by an HP 59970 MS Chemstation with an HP 9133 drive. The column connections to the GC were similar to those used in the GC–FID system. The flow from the GC capillary column entered the IR detector through a heated interface, passed through the light pipe and returned to the GC oven through a second interface. The IR detector exit interface was lined with a fused-silica capillary transfer line (0.1 mm I.D.) which extended through the heated interface of the MS detector to the ion source. Operational conditions for the GC–IR–MS were: IR light pipe, IR and MS interface temperatures, 290°C; ion source temperature, 230°C; electron impact, 70 eV; electron multiplier, 1800 V; scan-rate MSD (40–300 a.m.u./s); FT-IR scan-rate, 3.3 scans/s (4000–750 wave numbers); optical resolution, 8 wave numbers. Helium sweep gas (0.5

ml/min) was introduced at the inlet and outlet of the IR light pipe interface to minimize peak tailing resulting from dead volume effects in the sample path. The total gas flow-rate through the light pipe was approximately 2 ml/min and the total flow-rate at the second interface was therefore 2.5 ml/min. The flow-rate allowed to enter the MS detector was approximately 0.5 ml/min, the remaining 2.0 ml/min flow was vented to a charcoal collector tube.

Procedures

This section describes the protocols used for different tests using the analytical systems. The objective was to evaluate available analytical components and optimise conditions for resolution and detection of phenolic compounds.

Purity tests for 50 phenolic compounds

The individual standards of phenolics were prepared in chloroform (about 200 ng/μl) and stored in the dark at 4°C. All standards were analysed on HP-1, DB-5, DB-17, DB-1701, DB-210 or Nukol columns using GC–IR–MS and GC–FID. Only one peak was detected for each compound injected into the system. The MS and IR spectra of each phenolic compound were compared to the spectra of the National Bureau of Standards (MS) and the US Environmental Protection Agency (IR) libraries to assess compound purity. The results indicated no evidence of contamination in the standard solutions of the 50 phenolic compounds.

MS and IR library synthesis. To minimise search time MS and IR libraries for the 50 phenolics were prepared using the individual phenolic standard solutions (about 200 ng/μl) and the DB-5 column.

Preparation of quantitation mixtures of phenolics. The 50 target phenolics were divided into five groups each containing ten completely resolved phenolics as determined by GC–FID fitted with the DB-5 column. For each group twelve quantitative mixtures were prepared initially in methanol but later in chloroform at concentrations from 5 to 1000 ng/μl per component. Ten composite quantitative solutions of all 50 phenolics were also prepared at concentrations from 10 to 600 ng/μl. For the dinitrophenol and 3,4,5-trichlorophenol compounds the concentrations were up to 1500 ng/μl due to their low detectabilities by the GC–IR–MS system.

Optimisation of GC temperature program. For each column, at least six temperature programs were evaluated to determine the best conditions for separation of the target phenolics. The composite quantitative mixture of all phenolics with concentration of 100 ng/μl each was used. The identification of peaks in each chromatogram was performed using both IR and MS library searches. To verify peak identity the five solutions containing ten phenolic compounds were injected into the system using the same temperature program. Table I presents the optimum temperature program for each column.

Calibration tests. The HP1 column was selected to investigate the linear range and the detection and quantitation limits of the MS and IR detectors for each phenol. Since the presence of possible interferences of overloading effects were unknown, the calibrations for individual phenolic compounds were performed using the ten compound mixture standards. These results were compared to the results from the mixtures containing all 50 target phenolics. The calibration was performed using the full scan mode for the IR and MS detectors. Three methods of quantitation were used: (i)

for fully resolved peaks calibration curves were established based upon the total responses of the IR and MS detectors, (ii) for peaks containing co-eluting isomers (*e.g.* 3- and 4-methylphenol, 2,4- and 3,5-dimethylphenol and 3- and 4-ethylphenol), the IR data were selected since spectral subtraction could be reliably performed. The IR spectra for each phenolic compound at different concentrations were abstracted and used for measuring the adsorption at different wavenumbers. The calibration curves of these phenolics were established based upon the relationship between absorption and concentration for each phenol at each selected wavelength according to the Lambert–Beer law. If the unknown chromatographic peak was a mixture of two isomers (*e.g.*, 3- and 4-methylphenol), the 4-methylphenol contribution to absorption intensity was subtracted from the mixture's intensity to give the intensity that was attributable to the pure spectrum of 3-methylphenol. From this pure spectrum, the absorption at a suitable wavelength was measured and the concentration of 3-methylphenol was calculated based upon the previous calibration, (iii) for peaks of co-eluting phenolics for which the mass spectra are different, selected MS ion chromatograms of characteristic mass ranges of each compound were used to establish the calibration curves.

Unknown tests. Sixteen samples containing mixtures of phenolic compounds which were unknown to the analyst were prepared. The GC–IR–MS, equipped with an HP-1 column, was used for the identification and quantitation of the 16 samples. Samples 1 and 10 were analyzed in triplicate. The identification of unknown compounds was based on retention time and the analysis of IR and MS spectra. For quantitation of unknowns one of the above three quantitation methods was used; in all cases quantitation was carried out relative to known standards analysed under similar conditions.

RESULTS AND DISCUSSION

Solvent effect

Initial use of methanol as solvent resulted in peak splitting which was not resolved by use of a retention gap. Grob [7] has indicated that the retention gap technique fails if the injected liquids do not wet the surface of the column inlet. Chloroform was tested as a substitute for methanol and was found to be suitable for all target phenolic compounds. Testing on the GC–IR–MS with six different columns confirmed the elimination of peak splitting phenomena by using chloroform as the solvent.

Column performance

The column performance was evaluated based on the number of phenolics that could be eluted and the number of well resolved peaks from a chromatogram of the mixtures of 50 phenolic compounds. IR and MS identifications are greatly simplified if components of complex mixtures can be resolved by use of an appropriate chromatographic column. Six capillary columns of varying polarity were evaluated for the separation of the 50 component phenolic mixture. Six or more different temperature programs were tested for each column. Table II indicates the retention times using the optimum temperature program for each column. It is apparent that the HP-1 and DB-5 columns gave the greatest number of resolved peaks (resolution defined as 20%

TABLE II

RETENTION TIMES OF 50 PHENOLIC COMPOUNDS ON SIX DIFFERENT CAPILLARY COLUMNS WITH OPTIMUM TEMPERATURE PROGRAMS

No.	Compound	Retention time (min)					
		HP-1	DB-5	DB-17	DB-1701	DB-210	Nukol
1	Phenol	18.364	9.436	16.05	19.148	8.173	27.471
2	2-Methylphenol	23.286	11.951	18.978	20.637	9.885	27.226
3	3-Methylphenol	24.840	12.721	19.794	21.722	10.845	31.159
4	4-Methylphenol	24.761	12.691	19.824	21.722	10.810	30.807
5	2,3-Dimethylphenol	32.186	16.458	24.191	24.180	13.095	33.462
6	2,4-Dimethylphenol	30.040	15.346	22.601	23.117	12.503	30.616
7	2,5-Dimethylphenol	30.218	15.405	23.150	23.131	12.590	30.474
8	2,6-Dimethylphenol	26.952	13.827	21.148	21.086	11.600	22.484
9	3,4-Dimethylphenol	33.262	17.014	24.698	25.056	14.797	36.443
10	3,5-Dimethylphenol	31.610	16.129	23.329	24.222	13.791	34.585
11	2,3,5-Trimethylphenol	38.643	19.852	27.343	26.473	16.261	36.365
12	2,3,6-Trimethylphenol	36.044	18.400	26.045	24.616	15.257	28.915
13	2,4,6-Trimethylphenol	34.110	17.390	24.631	23.702	14.050	26.859
14	2-Ethylphenol	29.288	14.957	22.247	22.943	11.654	30.139
15	3-Ethylphenol	31.600	16.142	23.533	24.393	13.329	34.856
16	4-Ethylphenol	31.328	16.028	23.447	24.333	13.186	34.594
17	2-Chlorophenol	19.060	9.699	15.907	16.880	8.255	19.775
18	3-Chlorophenol	33.630	17.134	24.690	26.951	13.956	42.581
19	4-Chlorophenol	33.477	17.155	25.180	26.968	14.442	42.409
20	2,3-Dichlorophenol	32.016	16.341	24.135	23.025	13.388	35.606
21	2,4-Dichlorophenol	31.454	16.013	23.213	23.488	12.922	35.041
22	2,5-Dichlorophenol	31.702	16.102	23.150	23.586	12.895	35.678
23	2,6-Dichlorophenol	33.804	17.300	25.278	23.787	14.990	32.314
24	3,4-Dichlorophenol	44.917	24.761	33.405	33.096	20.706	59.649
25	3,5-Dichlorophenol	44.188	23.955	31.333	32.581	18.925	55.805
26	2,3,4-Trichlorophenol	43.151	23.153	31.676	29.804	19.236	45.678
27	2,3,5-Trichlorophenol	41.822	21.972	29.428	28.821	17.389	44.114
28	2,3,6-Trichlorophenol	43.786	23.723	32.273	29.446	20.328	43.851
29	2,4,5-Trichlorophenol	42.890	22.822	30.511	29.803	18.741	45.768
30	2,4,6-Trichlorophenol	42.543	22.644	30.396	28.454	18.885	41.614
31	3,4,5-Trichlorophenol	50.289	30.741	38.772	36.306	24.893	ND
32	2,3,4,5-Tetrachlorophenol	48.995	29.128	37.342	ND[a]	23.541	ND
33	2,3,5,6-Tetrachlorophenol	48.862	29.004	37.213	33.073	23.744	ND
34	Pentachlorophenol	52.859	32.240	40.532	ND	26.775	ND
35	2-Chloro-5-methylphenol	26.418	13.377	19.816	19.987	11.072	23.765
36	4-Chloro-2-methylphenol	38.294	19.787	27.940	28.230	16.787	42.034
37	4-Chloro-3-methylphenol	39.404	20.458	28.605	29.024	17.307	44.250
38	2-Bromophenol	24.221	12.370	19.805	19.414	10.120	25.913
39	3-Bromophenol	39.173	20.292	28.812	29.611	16.583	47.789
40	4-Bromophenol	39.027	20.287	29.200	29.635	17.060	47.049
41	2,4-Dibromophenol	42.017	22.297	31.272	28.619	18.030	43.629
42	2,6-Dibromophenol	43.319	23.394	32.971	28.618	19.942	40.808
43	2-Nitrophenol	28.255	14.726	22.758	20.735	15.873	19.143
44	3-Nitrophenol	46.620	26.812	36.638	35.011	25.475	ND
45	4-Nitrophenol	48.010	28.569	38.277	36.123	26.775	57.577
46	2,4-Dinitrophenol	46.934	27.628	37.575	ND	27.810	ND
47	2,5-Dinitrophenol	45.079	26.312	36.547	ND	26.984	ND
48	2,6-Dinitrophenol	48.214	29.506	39.281	34.621	28.927	ND
49	3,4-Dinitrophenol	55.344	33.839	ND	ND	ND	ND
50	2,3,5,6-Tetramethylphenol	42.884	22.877	30.663	27.940	18.680	34.747

[a] ND = Not detected.

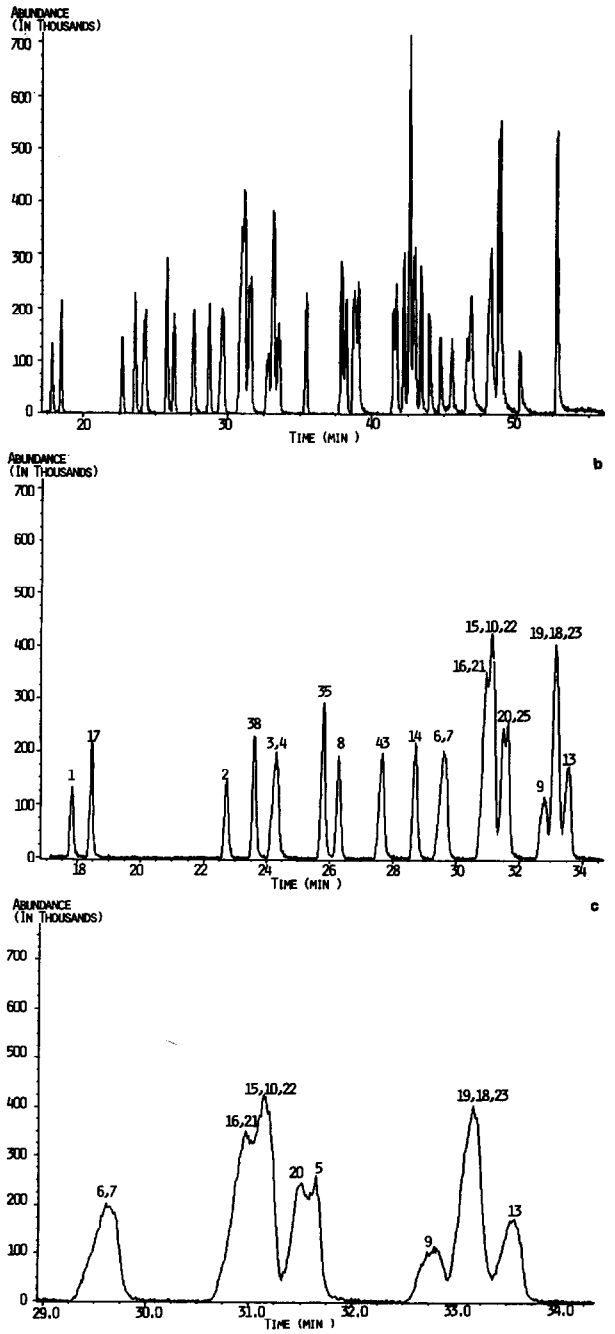

Fig. 1.

(*Continued on p. 304*)

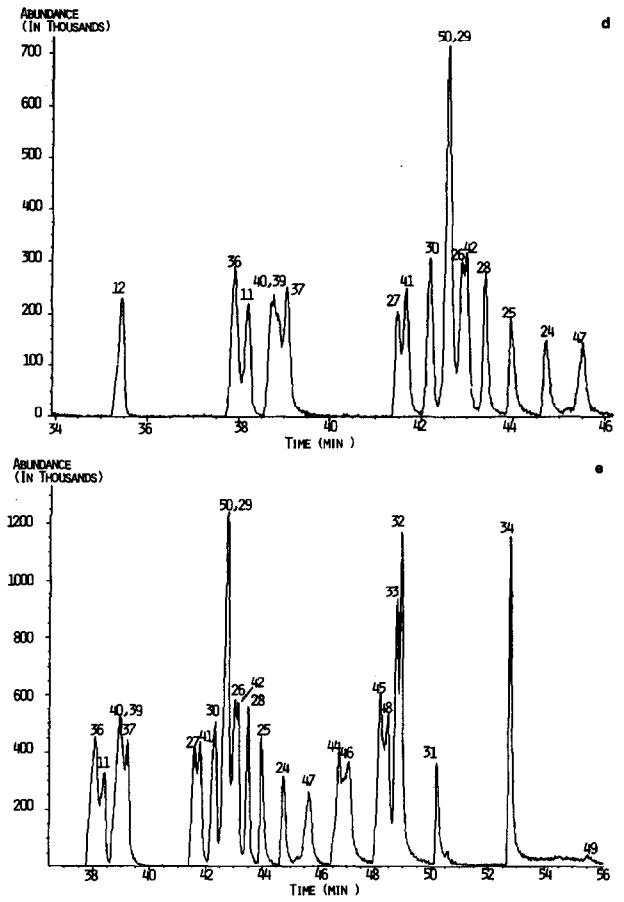

Fig. 1. Standard (a) and expanded (b–e) chromatograms of the 50 phenolics analysed on the HP-1 column using the optimum temperature program. TIC = Total ion chromatogram.

peak to valley); slightly superior peak resolution favoured the use of the HP-1 column. Both columns operate primarily on the principle of dispersive interactions; with each the maximum number of peaks observed at the 100 ng injection level was found to be 41 when 50 compounds were injected. Co-elution was still a problem in that some compounds could not be resolved (*e.g.* 3- and 4-methylphenol, 2,4- and 2,5-dimethylphenol, 2,3,5,6-tetramethylphenol and 2,4,5-trichlorophenol) and some other compounds were only partially resolved. A typical chromatogram for the HP-1 column is shown in Fig. 1. The added selectivity afforded by use of other columns with potential dipole, and acid–base phase interactions proved useful in providing separation of some of the compounds; however, these interactions also resulted in losses of significant numbers of highly polar phenolics. This effect is demonstrated by the results of the high polarity Nukol column tested which gave the worst performance of all the columns in terms of the number of compounds lost during chromatography. These findings are consistent with those of Korhonen [8,9]. He reported on

TABLE III

DETECTION LIMITS (ng/μl) AND LINEAR DYNAMIC RANGE FOR TARGET PHENOLIC COMPOUNDS FROM STANDARDS PREPARED AS TEN COMPOUND MIXTURES

No.	Compound	IR		MS	
		High	Low[a]	High	Low
1	Phenol	200	40	400	20
2	2-Methylphenol	500	50	500	20
3	3-Methylphenol	500	20	500	20
4	4-Methylphenol	450	40	450	40
5	2,3-Dimethylphenol	500	30	300	20
6	2,4-Dimethylphenol	400	20	400	10
7	2,5-Dimethylphenol	200	50	400	10
8	2,6-Dimethylphenol	350	40	350	10
9	3,4-Dimethylphenol	600	50	600	40
10	3,5-Dimethylphenol	400	50	400	10
11	2,3,5-Trimethylphenol	400	50	400	10
12	2,3,6-Trimethylphenol	500	20	500	10
13	2,4,6-Trimethylphenol	600	20	600	10
14	2-Ethylphenol	300	20	300	20
15	3-Ethylphenol	600	20	300	10
16	4-Ethylphenol	500	70	500	20
17	2-Chlorophenol	400	30	400	10
18	3-Chlorophenol	400	50	400	10
19	4-Chlorophenol	300	50	400	40
20	2,3-Dichlorophenol	600	40	700	20
21	2,4-Dichlorophenol	400	40	500	30
22	2,5-Dichlorophenol	400	40	400	20
23	2,6-Dichlorophenol	400	30	400	20
24	3,4-Dichlorophenol	300	70	300	60
25	3,5-Dichlorophenol	400	40	500	20
26	2,3,4-Trichlorophenol	400	50	400	40
27	2,3,5-Trichlorophenol	400	50	400	50
28	2,3,6-Trichlorophenol	250	20	600	20
29	2,4,5-Trichlorophenol	300	70	450	70
30	2,4,6-Trichlorophenol	700	50	700	10
31	3,4,5-Trichlorophenol	1200	30	1000	70
32	2,3,4,5-Tetrachlorophenol	300	70	600	50
33	2,3,5,6-Tetrachlorophenol	200	50	500	50
34	Pentachlorophenol	600	80	600	60
35	2-Chloro-5-Methylphenol	400	30	400	20
36	4-Chloro-2-Methylphenol	250	60	400	20
37	4-Chloro-3-Methylphenol	250	40	500	30
38	2-Bromophenol	300	20	700	20
39	3-Bromophenol	600	50	600	40
40	4-Bromophenol	400	40	400	20
41	2,4-Dibromophenol	600	50	600	40
42	2,6-Dibromophenol	300	40	400	30
43	2-Nitrophenol	400	20	500	40
44	3-Nitrophenol	400	50	400	70
45	4-Nitrophenol	400	70	600	80
46	2,4-Dinitrophenol	800	100	800	120
47	2,5-Dinitrophenol	800	100	800	150
48	2,6-Dinitrophenol	1200	100	1200	170
49	3,4-Dinitrophenol	600	300	600	300
50	2,3,5,6-Tetramethylphenol	150	30	320	20

[a] Signal-to-noise ratio 3.

TABLE IV

METHODS OF CALIBRATION FOR 50 PHENOLICS COMPOUNDS

No.	Compound	Method
1	Phenol	Total response of IR and MS
2	2-Methylphenol	Total response of IR and MS
3	3-Methylphenol	IR method, absorption *vs.* concentration at a single wavelength (1600 or 1156 cm^{-1})
4	4-Methylphenol	IR method, absorption *vs.* concentration at a single wavelength (1515 or 1174 cm^{-1})
5	2,3-Dimethylphenol	MS: mass range: 106.5–122.5
6	2,4-Dimethylphenol	IR method, absorption *vs.* concentration at a single wavelength (3656 or 2933 cm^{-1})
7	2,5-Dimethylphenol	IR method, absorption *vs.* concentration at a single wavelength (1515 or 1230 cm^{-1})
8	2,6-Dimethylphenol	Total response of IR and MS
9	3,4-Dimethylphenol	MS: mass range: 106.5–122.5
10	3,5-Dimethylphenol	MS: mass range: 120.5–121.5
11	2,3,5-Trimethylphenol	Total response of IR and MS
12	2,3,6-Trimethylphenol	Total response of IR and MS
13	2,4,6-Trimethylphenol	MS: mass range: 106.5–122.5
14	2-Ethylphenol	Total response of IR and MS
15	3-Ethylphenol	IR method, absorption *vs.* concentration at a single wavelength (1600 of 1155 cm^{-1})
16	4-Ethylphenol	IR method, absorption *vs.* concentration at a single wavelength (1514 or 1174 cm^{-1})
17	2-Chlorophenol	Total response of IR and MS
18	3-Chlorophenol	MS: mass range: 127.5:128.5
19	4-Chlorophenol	MS: mass range: 127.5–128.5
20	2,3-Dichlorophenol	MS: mass range: 125.5–164.5
21	2,4-Dichlorophenol	MS: mass range: 125.5–164.5
22	2,5-Dichlorophenol	MS: mass range: 125.5–164.5
23	2,6-Dichlorophenol	MS: mass range: 161.5–164.5
24	3,4-Dichlorophenol	Total response of IR and MS
25	3,5-Dichlorophenol	Total response of IR and MS
26	2,3,4-Trichlorophenol	MS: mass range: 195.5–198.5
27	2,3,5-Trichlorophenol	MS: mass range: 195.5–198.5
28	2,3,6-Trichlorophenol	Total response of IR and MS
29	2,4,5-Trichlorophenol	MS: mass range: 195.5–198.5
30	2,4,6-Trichlorophenol	MS: mass range: 195.5–198.5
31	3,4,5-Trichlorophenol	Total response of IR and MS
32	2,3,4,5-Tetrachlorophenol	Total response of IR and MS
33	2,3,5,6-Tetrachlorophenol	Total response of IR and MS
34	Pentachlorophenol	Total response of IR and MS
35	2-Chloro-5-Methylphenol	Total response of IR and MS
36	4-Chloro-2-Methylphenol	Total response of IR and MS
37	4-Chloro-3-Methylphenol	MS: mass range: 106.5–142.5
38	2-Bromophenol	Total response of IR and MS
39	3-Bromophenol	MS: mass range: 171.5–174.5
40	4-Bromophenol	MS: mass range: 171.5–174.5
41	2,4-Dibromophenol	MS: mass range: 251.5–254.5
42	2,6-Dibromophenol	MS: mass range: 251.5–254.5
43	2-Nitrophenol	Total response of IR and MS
44	3-Nitrophenol	MS: mass range: 138.5–139.5
45	4-Nitrophenol	MS: mass range: 138.5–139.5
46	2,4-Dinitrophenol	MS: mass range: 183.5–184.5
47	2,5-Dinitrophenol	Total response of IR and MS
48	2,6-Dinitrophenol	MS: mass range: 183.5–184.5
49	3,4-Dinitrophenol	Total response of IR and MS
50	2,3,5,6-Tetramethylphenol	MS: mass range: 134.5–150.5

the unsuitability of OV-351 and free fatty acid phase column liquid phases for the analysis of phenolic compounds, particularly the chloro substituted phenolics; these columns are similar to the Nukol column in terms of polarity. The column related losses were highlighted by 3,4-dinitrophenol which proved universally difficult to analyze due to poor peak shape and tailing. The low sensitivity to this compound is likely due to significant losses of the compound occurring in the chromatographic column or on the detector surfaces rather than the inherent detector limitations. Interesting features of the Nukol column were its ability to resolve completely two pairs of isomers, 3- and 4-methylphenol, 3- and 4-ethylphenol and to resolve partly 2,4- and 2,5-dimethylphenol compounds which are not easily resolved with the other columns. This revealed the advantage offered by the more polar Nukol column for separation of certain phenolic isomers. Unfortunately, other groups of phenolics appeared to remain unresolved with this column.

System calibration

Calibration of the GC–IR–MS system was performed by injection of working standard solution prepared as ten compound mixtures. The total ion chromatogram (MS) and the total response chromatogram (IR) were used to set up the calibration curves. Because of the limitation on carrier gas flow (0.5 ml/min) to the MS only about 20% of the amount injected was transmitted to the MS. The linear range observed for each compound is given in Table III; the detection limits for each phenolic were estimated based on the calibration curves and on a signal-to-noise ratio of three. These values represent the identification and quantitation limits for each phenolic. Essentially all compounds gave linear calibration with MS over the range indicated. Non-zero intercepts were evident indicating that some adsorption of phenolic compounds may have occurred in the chromatographic system. Many of the phenolics showed linear calibration with the IR, but some exhibited complex deviations from linearity. In part, these are explainable by light absorption which deviated from the Lambert–Beer law. Calibration tests with the 50 compound mixtures gave similar results except that the co-elution of certain phenolics complicated the method of quantitation and gave somewhat narrower dynamic ranges and higher detection limits. For co-eluting compounds with similar mass spectra but unique IR features, the method of choice for determination of concentration was based on the IR response. In this method the total IR absorption spectrum is obtained first and the spectral subtraction was performed to obtain the pure IR spectrum. For example, the IR spectrum of coeluted 3,5-dimethylphenol and 3-ethylphenol, which have similar mass spectra, was subtracted by the 3,5-dimethylphenol spectrum to obtain a pure spectrum of 3-ethylphenol. The wavelength unique for the specific compound was selected to generate a response relationship. These are generally characterized by non-zero intercepts, a narrow linear range and a saturation of response at higher concentrations. Where co-elution of non-isomeric phenolic compounds occurred the phenols generally exhibited unique mass spectra. Hence a characteristic mass for the compound could be selected and its response utilized to generate a calibration curve. The calibration curves for these compounds were generally linear. For compounds that were chromatographically resolved, calibration was based on the total ion chromatogram (MS) or the total response chromatogram (IR) depending on which gave optimum response. In general these compounds gave linear relationships. Table IV summarises the selected detector and method of calibration for each phenol.

TABLE V

RESULTS OF UNKNOWN SAMPLE ANALYSES

Sample No.	Compound identification	Quantitation (ng/μl)			
		IR Result	MS Result	Design concentration	Detection limit
1	3-Methylphenol	100/98/106	–[b]	100	60
	2,4-Dimethylphenol	82/90/104	–	100	30
	2,4,6-Trimethylphenol	–	57/58/58	100	30
	3-Chlorophenol	ND/ND/ND[a]	ND/ND/ND	100	30
2	Blank solvent	ND	ND	ND	–
3	2-Methylphenol	538	502	500	20
	2,6-Dimethylphenol	57	40	40	20
	4-Ethylphenol	578	–	500	70
	2,6-Dichlorophenol	–	117	100	20
	3-Bromophenol	–	198	250	30
	2,4,6-Trichlorophenol	–	100	100	30
	3,4-Dichlorophenol	ND	ND	40	50
	2,4-Dinitrophenol	–	105	250	120
4	2-Methylphenol	380	240	250	20
	4-Ethylphenol	72	–	40	70
	3-Bromophenol	–	64	100	30
	3,4-Dichlorophenol	906	375	500	50
5	Phenol	100	98	100	20
	3-Methylphenol	75	–	100	60
	2-Bromophenol	102	110	100	20
	3-Bromophenol	–	58	100	30
	4-Chloro-3-Methylphenol	–	41	100	30
6	2-Methylphenol	ND	ND	10	20
	2,6-Dimethylphenol	ND	ND	0.8	20
	4-Ethylphenol	ND	–	10	70
	2,6-Dichlorophenol	–	ND	2	20
	3-Bromophenol	–	ND	5	30
	2,4,6-Trichlorophenol	–	ND	2	30
	3,4-Dichlorophenol	ND	ND	0.8	50
	2,4-Dinitrophenol	–	ND	5	120
7	2-Methylphenol	470	630	500	20
	2,6-Dimethylphenol	35	53	40	20
	4-Ethylphenol	549	–	500	70
	2,6-Dichlorophenol	–	120	100	20
	3-Bromophenol	–	230	250	30
	2,4,6-Trichlorophenol	–	130	100	30
	3,4-Dichlorophenol	ND	ND	40	50
	2,4-Dinitrophenol		270	250	120
8	2-Methylphenol	96	98	100	20
	2,6-Dimethylphenol	300	260	250	20
	2,6-Dichlorophenol	–	510	500	20
	2,4,6-Trichlorophenol	–	460	500	30
	2,4-Dinitrophenol	–	ND	40	120

TABLE V (*continued*)

Sample No.	Compound identification	Quantitation (ng/μl)			
		IR Result	MS Result	Design concentration	Detection limit
9	2-Methylphenol	ND	ND	40	20
	3-Bromophenol	–	ND	40	30
	2,4-Dinitrophenol	–	ND	100	120
10	3-Ethylphenol	141/146/145	–	100	90
	2,3,5,6-Tetramethylphenol	–	118/129/123	100	30
	2,4,5-Trichlorophenol	–	93/88/92	100	70
11	2,6-Dimethylphenol	80	94	100	20
	4-Ethylphenol	100	–	100	70
	2,6-Dichlorophenol	–	240	250	20
12	2-Methylphenol	604	400	500	20
	2,6-Dimethylphenol	26	38	40	20
	4-Ethylphenol	530	–	500	70
	2,6-Dichlorophenol	–	82	100	20
	3-Bromophenol	–	120	250	30
	2,4,6-Trichlorophenol	–	86	100	30
	3,4-Dichlorophenol	ND	ND	40	50
	2,4-Dinitrophenol	–	140	250	120
13	Phenol	103	84	100	20
	3-Ethylphenol	94	–	100	90
	3,5-Dimethylphenol	–	88	100	30
	2,3,5,6-Tetramethylphenol	–	69	100	30
14	2,6-Dimethylphenol	600	500	500	20
	2,6-Dichlorophenol	–	40	40	20
	2,4,6-Trichlorophenol	–	46	40	30
	3,4-Dichlorophenol	58	67	100	50
	2,4-Dinitrophenol	–	380	500	120
15	2-Methylphenol	ND	ND	25	20
	2,6-Dimethylphenol	ND	ND	2	20
	4-Ethylphenol	ND	–	25	70
	2,6-Dichlorophenol	–	ND	5	20
	3-Bromophenol	–	ND	12.5	50
	2,4,6-Trichlorophenol	–	ND	5	30
	3,4-Dichlorophenol	ND	ND	2	50
	2,4-Dinitrophenol	–	ND	12.5	120
16	4-Ethylphenol	300	–	250	70
	3-Bromophenol	–	360	500	50
	2,4,6-Trichlorophenol	–	240	250	30
	3,4-Dichlorophenol	210	160	250	50

[a] ND = Not detected in sample.
[b] Not quantitated in sample.

Unknown test results

The ability of the GC–IR–MS system to identify and quantitate co-eluting phenolics was assessed by analysis of sixteen check samples fortified with compounds/

concentrations unknown to the analyst. Optimum HP-1 column conditions were used and quantitation was based on calibration data derived from composite standard solutions containing all 50 target phenolics using the method of quantitation outlined in Table IV. The phenolics present in the check samples were identified by retention time and by use of the spectral libraries. For co-eluting compounds the spectral subtraction technique (IR) or selected ion method (MS) were employed as appropriate to identify the individual phenolics; quantitation of the phenolics was based on the method reported in Table IV.

The composition and concentrations of the check samples are given in Table V together with the results obtained by IR and MS analysis. Sample 2 was a solvent blank and samples 3, 7 and 12 were replicate aliquots of the same solution. In general the phenolic compounds were correctly identified in the check samples when the compounds were present at concentrations substantially above the detection limit. No false positives were reported and no phenolics were reported in the blank solvent (sample 2) or in samples (6 and 15) in which fortification of the phenols was at levels at or below the instrument detection limit. The quality of the quantitative data varied from compound to compound and to some extent depended on the method of quantitation. Good agreement was obtained between sample data and the design concentration for 2,6-dimethylphenol (samples 3, 6–8, 11, 12, 14 and 15) which had good chromatographic resolution and which was quantitated using total response of the two detectors. MS data were somewhat more accurate than IR data in the 40 to 500 ng/μl range and for the replicate samples (3, 7 and 12) fortified at 40 ng/μl the precision of the MS data (43.7 $\pm$ 8.1 ng/μl) was better than the precision of the IR data (39.3 $\pm$ 15.9 ng/μl). 3,4-Dichlorophenol is not reported as present in samples (3, 6, 7, 12 and 15), however, when present at twice the detection level or higher (samples 4, 14 and 16) it is correctly identified. Quantitation, however, indicate a consistently low bias for this compound of approximately 30%. When quantitation was carried out by selected mass MS, data for 2,4,6-trichlorophenol showed good accuracy in the 40 to 500 ng/μl range (samples 3, 6–8,12 and 14–16), and for the replicate samples (3, 7 and 12) fortified at 100 ng/μl the precision was reasonable (105 $\pm$ 23 ng/μl). When quantitation was carried out by single wavelength IR, data for 4-ethylphenol showed good accuracy in the 100 to 500 ng/μl range (samples 3, 4, 6, 7, 11, 12, 15 and 16) and for the replicate samples (3, 7 and 12) fortified at 500 ng/μl the precision was good (552 $\pm$ 24 ng/μl). For replicate samples (3, 7 and 12) accuracy and precision for 2-methylphenol (IR, 537 $\pm$ 67 ng/μl; MS, 511 $\pm$ 115 ng/μl), 3-bromophenol (MS, 183 $\pm$ 57 ng/μl) and 2,4-dinitrophenol (MS, 172 $\pm$ 87 ng/μl) was the least satisfactory. Its poor detection limit (120 ng/μl) and losses in the system are probable reasons for poor results found with 2,4-dinitrophenol. The poor performance with 2-methylphenol and 3-bromophenol is not as easily understood however. The reproducibility of analysis within the same sample is demonstrated by the triplicate analyses performed on samples 1 and 10 (precision was better than $\pm$ 10%).

CONCLUSIONS

The data clearly indicate the utility of a GC–IR–MS system for the identification and quantitation of phenolic compounds although detection limits determined with the system are higher than those found using GC–FID or GC–MS alone. Identi-

fication/quantitation of the phenolic compounds was faster using MS data analyses compared to using IR data analysis but IR detection was superior in identification of unknowns, especially for isomeric compounds. The coupling of the two detectors with the GC is a great advantage for the analysis of phenolic compounds.

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