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The Simultaneous Determination of Phenolic Compounds by GC and GC/MS

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Abstract

To develop a simple, rapid and simultaneous analytical method of phenolic compounds using gas chromatography (GC) and gas chromatography/mass spectrophotometer (GC/MS), this experiment was carried out to search the retention times of capillary columns and the characteristics of fragment ions in electron impact mass spectra. Most of trimethylsilyl derivatives and underivatized phenolic compounds were separated very well on three kinds of capillary columns (HP-1, Ultra-2 and HP-35). Quantitative determination of phenolic compounds was achieved by internal standards (*p*-hydroxybenzoic acid isopropyl ester, *p*-hydroxybenzoic acid ethyl ester). Calibration plots were linear in the investigated range, and the limits of detection were about 5 ng at split mode method. When analyzed by three columns, the separation times were fairly constant on two nonpolar columns, but a few compounds showed slightly different separation order by the intermediate polar HP-35 column. The important characteristic patterns of TMS derivatives of phenolic compounds on the EI/MS spectra appeared at the base peak of $[M-15]^+$ ion and presented at high abundance in most TMS derivatives of phenolic compounds. $[M]^+$, $[M-CH_3-COO]^+$, $[M-Si(CH_3)_4]^+$ and $[M-Si(CH_3)_4-CH_3]^+$ ions were also observed in mass spectra of these compounds. Although several compounds have the same retention times on GC column, it might be possible to identify these compounds by the different patterns of mass fragment ions. The TMS derivatives, thus, provide additional information for identification of phenolic compounds in biological systems.

Key words: phenolic compounds, GC/MS, GC

INTRODUCTION

Phenolic compounds are commonly occurring constituents in the plant world. The interest of phenolic compounds in food have been increased scientifically and commercially in recent years. Phenolic compounds aid in the maintenance of tissue, fresh flavor, taste, color and prevention of oxidation deterioration. Recent finding suggest that these compounds might be useful bioactive components due to antioxidative (1-3), antinflammatory (4,5), antimutagenic (6,7), and other biological active properities (8).

The phenolic compounds which occur in food material may be classified into eight groups, namely, simple phenols, benzoic acids, cinnamic acids, coumarins, lignans, flavonoids, tannins and lignins (9). These compounds are distributed widely in plants and are also found in processed foods of plant and medicinal plants.

Qualitative and quantative analysis of phenolic compounds mainly depend upon thin-layer chromatography (TLC) (10), column chromatography (CC) (11), high performance liquid chromatography (HPLC) (10,12-17) and gas chromatography (GC) (10, 18-21). TLC gives difficulties due to its poor separation and quantification. CC requires highly trained techniques and limitation for common uses because of time consumption and economic reason. HPLC is widely used for the determination of phenolic compounds but it is difficult to identify the compounds with similar retention time and their isomers.

This study was attempted to establish a rapid, accurate and simultaneous analytical method for the phenolic compounds widely distributed in food by GC and GC/MS.

MATERIALS AND METHODS

Reagents and standards

Phenolic acids tested in the experiment were three

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simple phenols, twelve benzoic acids, nine coumarins and fourteen other phenolics. Three simple phenols were of catechol, resorcinol and pyrogallol. Twelve benzoic acids were of benzoic acid, salicylic acid, *m*-hydroxy benzoic acid, *p*-hydroxy benzoic acid, gentisic acid, α-resorcylic acid, β-resorcylic acid, protocatechuic acid, gallic acid, 2,3,4-hydroxy benzoic acid, vanillic acid and syringic acid. Six cinnamic acids were of *trans*-cinnamic acid, *o*-coumaric acid, *p*-coumaric aid, caffeic acid, ferulic acid and sinapinic acid. Nine coumarins were of umbelliferon, 4-hydroxycoumarin, scopoletin, esculetin, 4-methylesculetin, fraxetin, esculin, dicoumaol, psoralen and methoxalen. Fourteen other phenolics were of cinnanic alcohol, cinnamic aldehyde, thymol, chlorogenic acid, vanillin, *o*-hydroxyacetophenone, *p*-hydroxyacetophenone, acetosyringone, homovanillic acid, homovanillic alcohol, homoveratric acid, quinic acid. isosafrol and eugenol. The standards of phenolic compounds were purchased from Sigma and Aldrich. Internal standards were *p*-hydroxy benzoic acid isopropyl ester and dehydroacetic acid (Tokyo Chemicals Co.) and 1,1,1,3,3,3-hexamethylsilane (HMDS) and trifluoroacetic acid (TFA) for derivatization were obtained Jansen Co.

GC analysis

A gas chromatography (Hewlett Packard, model 5890 series II) equipped with a flame ionization detector (FID) and three different capillary columns, i.e. HP-1 (0.20 μm × 0.33 μm × 25 mm, nonpolar), Ultra-2 (0.20 mm × 0.33 μm × 25 mm, nonpolar) and HP-35 (0.32 μm × 0.25 μm × 25 mm, intermediate) were used. The injector and detector temperature was 250°C and 300°C respectively. Split mode (1:30) was applied to sample injection. The carrier gas was N_2 and head pressure was 10 psi for HP-1 and ultra-2 and 5 psi for HP-35. Oven temperature profile was 100°C initial temp, 5min. initial holding temp., 7°C/min. increasing rate 320°C final temp., and 20min. final holding time. Data system recorded by HP 3390 integrator.

GC/MS analysis

GC/MS analysis was performed with a Fison Instrument MD 800 system equipped with a ultra-2 (0.20 mm × 0.33 μm × 25 mm, nonpolar) capillary column. The carrier gas of He and inlet pressure on the column at 10 psi and split mode (1:30) was applied for sample injection at 250°C. Oven temperature profile was as the same as the GC-FID analysis. The column was connected through the interface to the mass spectrophotometer at 270°C. Mass spectra was obtained at 70 eV over 40~800 amu at a mass filter. Source temperature was 270°C and injection volume was 1 μl. The data system employed the program was developed by Fison Co. The libraries developed by National Institute of Standards and Technology (NIST) and Willey were used for the identification of mass spectra.

Aanalysis of trimethylsilyl derivatives

The derivatization method using pyridine, HMDS and TFA was used (21). 1~2 mg of each phenolic compound was dissolved in 0.5 ml of anhydrous pyridine and 0.45 ml of HMDS in a screw-cap vial and 50 μl TFA was added. The mixture was shaken vigorously for 10 sec and maintained at 70°C for 5 min in water bath until the solution became clear. GC/MS analysis was conducted on the solution that was diluted 50 times. For GC analysis, 10 mg of each phenolic acid was dissolved in 20 ml of methanol, and 1, 3, 5 and 10 ml of this solution was taken and dried at 40°C in vacuum by using rotary evoporator. Each of the residue was derivatized by the above method using pyridine with internal standard (para oxybenzoic acid isopropylester) and the volume of test solutions was finally adjusted to 1 ml.

Analysis of underivatized phenolic compounds

Ten mg of each phenolic acid (psoralen, methoxalen, isosafrol, eugenol and cinnamic aldehyde) was dissolved in 50 ml of methanol, and was analyzed by GC/MS. For GC analysis, 10 mg amount of each phenolic compounds was dissolved in 20 ml of methanol, and then, 1, 3, 5 and 10 ml of the solution were evaporated and dried at 40°C under vacuum by rotary evaporator. Each residue was dissolved in 10 ml of methanol and mixed with internal standard (dehydroacetic acid).

RESULTS

TMS derivatives of simple phenols

Fig. 1 and Table 1 show a typical total ion chromatogram (TIC) and EI/MS spectral data of three TMS derivatives of simple phenols separated on ultra-2 capillary column. As the results show, Peak No. 1 and 2,

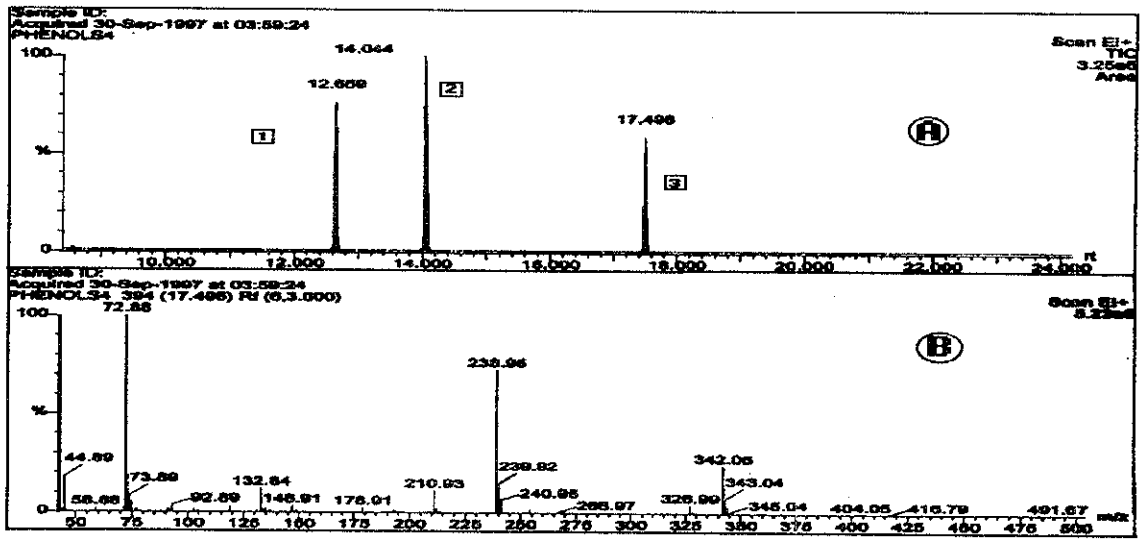

Fig. 1. Total ion chromatogram of TMS derivatives of simple phenols (A) and mass spectra of pyrogallol (B).

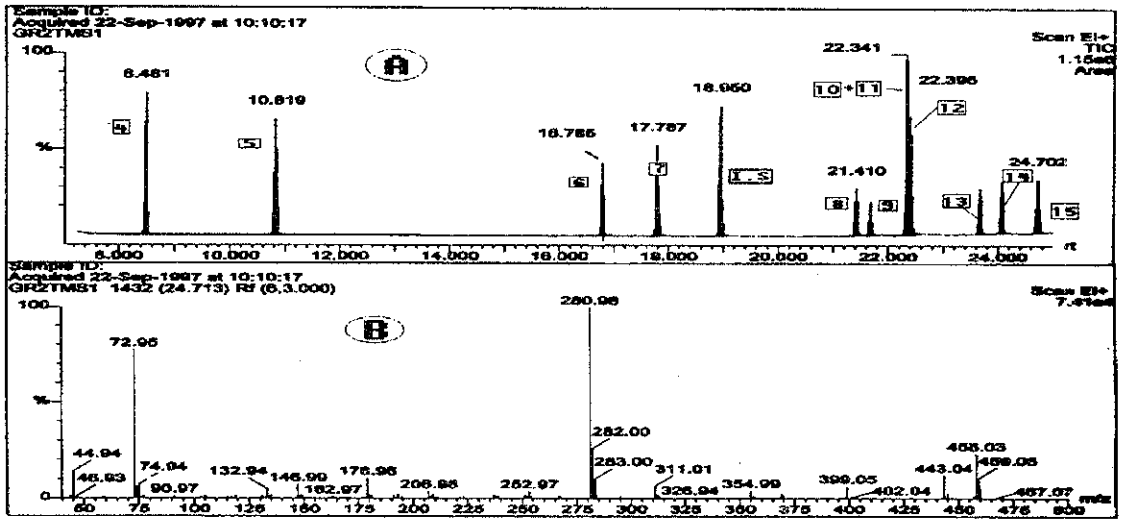

Fig. 2. Total ion chromatogram of TMS derivatives of benzoic acids (A) and mass spectra of gallic acid (B).

isomeric phenols with two hydroxyl groups, are separated very well and their spectra also appeared a distinct difference. In the case of resorcinol, an $[M-15]^+$ ion (*m/z* 239) is a base peak and molecular ion, $[M]^+$ ion were present at high abundance, but $[M]^+$, $[M-15]^+$ ($[M-CH_3]^+$), $[M-88]^+$ ($[M-Si(CH_3)_4]^+$) and $[M-103]^+$ ($[M-Si(CH_3)_4-CH_3]^+$) fragment ions were relatively weak in catechol. The pyrogallol with 3-hydroxyl groups show its base peak at *m/z* 73 ion. While $[M]^+$ and $[M-CH_3]^-$ ions are weak, The *m/z* 239 ion suspected as $[M-Si(CH_3)_4-CH_3]^+$ are resulted in a high abundance (71%).

TMS derivatives of benzoic acids

Benzoic acids are widely used as a synthetic preservative in various foods and six kinds including para-oxy

Table 1. Principal ions observed in the EI mass spectra of TMS derivatives of simple phenols

Peak No.	Systematic name	Common name	M.W.	M.W. (TMS)	Fragment ions *m/z*, (intensity as % of base peak)
1	1,2-benzenediol	catechol	110	254	254 (12), 239 (6), 166 (4), 151 (6), 136 (4), 73(100)
2	2,4-benzenediol	resorcinol	110	254	254 (65), 239(100), 147 (13), 133 (22), 91 (17), 73 (69)
3	2,3,4-benzenetriol	pyrogallol	126	342	342 (23), 327 (5), 239 (71), 210 (11), 133 (13), 73(100)

Table 2. Principal ions observed in the EI mass spectra of TMS derivatives of benzoic acids

Peak No.	Systematic name	Common name	M.W.	M.W. (TMS)	Fragment ions *m/z*, (intensity as % of base peak)
4	benzene carboxylic acid	benzoic acid	122	194	194 (7), 179(100), 135 (53), 105 (60), 77 (39)
5	2-hydroxy benzoic acid	salicylic acid	138	282	282 (2), 267(100), 209 (19), 135 (18), 73 (92)
6	3-hydroxy benzoic acid	m-hydroxy benzoic acid	138	282	282 (29), 267(100), 223 (63), 193 (54), 73 (59)
7	4-hydroxy benzoic acid	p-hydroxy benzoic acid	138	282	282 (17), 267(100), 223 (97), 193 (66), 73 (80)
8	4-hydroxy,3-methoxy benzoic acid	vanillic acid	168	312	312 (40), 297(100), 267 (80), 253 (60), 73 (70)
9	2,5-dihydroxy benzoic acid	gentisic acid	154	370	370 (2), 355(100), 297 (10), 223 (11), 73(100)
10	3,5-dihydroxy benzoic acid	α-resocylic acid	154	370	370(100), 355 (82), 311 (29), 70 (28), 73(100)
11	2,4-dihydroxy benzoic acid	β-resocylic acid	154	370	370 (1), 355(100), 281 (18), 223 (7), 73 (58)
12	3,4-dihydroxy benzoic acid	protocatechuic acid	154	370	370 (59), 355 (43), 311 (42), 193(100), 73 (48)
13	4-hydroxy,3,5-dimethoxy benzoic acid	syringic acid	198	342	342 (56), 327(100), 312 (69), 297 (79), 73 (52)
14	2,3,4-trihydroxy benzoic acid		170	458	458 (0), 443(100), 281 (48), 147 (16), 73 (95)
15	3,4,5-trihydroxy benzoic acid	gallic acid	170	458	458 (20), 443 (12), 281(100), 179 (11), 73 (81)

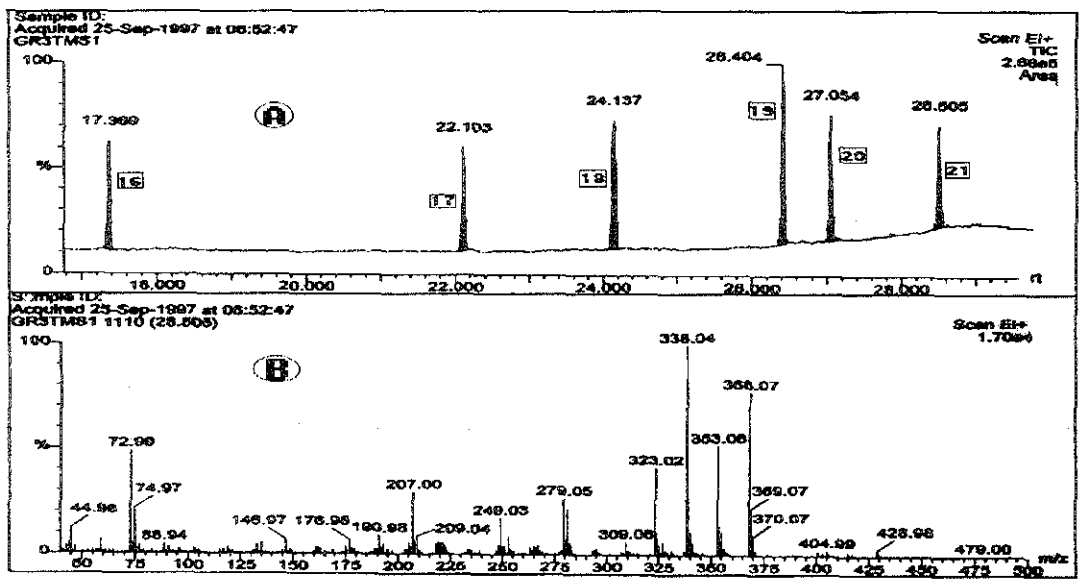

Fig. 3. TIC chromatogram of TMS derivatives of cinnamic acids (A) and mass spectra of sinapinic acid (B).

family are permitted to use in food in Korea.

Fig. 2 shows the typical TIC of TMS derivatives of benzoic acids and Table 2 is the mass spectral data of these compounds on EI-MS. It is possible to identify the isomeric compounds (Peak No. 5, 6 and 7) by their mass spectra. These isomers showed the base peak at $[M-15]^+$ ion but revealed different abundance at *m/z* 282 and *m/z* 223 ions that were suspected to be $[M]^+$ ion and $[M-CH_3-COO]^+$ ion, respectively. Four isomeric compounds with two hydroxyl groups (Peak No. 9, 10, 11 and 12) are appeared to have similar retention time on GC except No. 9, but revealed different mass spectra. The base peak of Peak No. 10, 11 and 12 compounds appeared at *m/z* 370, *m/z* 355 and *m/z* 193 ion, respectively. and this observation made it possible to identify the four isomers.

TMS derivatives of cinnamic acids

Cinnamic acid is one of phenolic compounds having a C_6-C_3 structure and is synthesized from phenylalanine *via* shikimic acid pathway. It exists as free form, *glycosides, sugar ester or esters with cell wall com*-pounds. Cinnamic acids are known to be effective in growth regulation and resistance to diseases. Fig. 3 shows the TIC for the separation of six TMS derivatitives of cinnamic acid. Since the compounds were separated very well, it was possible to identify the cinnamic acids by retention time. As noted in Table 3, $[M]^+$ and $[M-15]^+$ ions were appeared as an important ions.

TMS derivatives of coumarins

The natural coumarins in plant have its basic skeleton

Table 3. Principal ions observed in the EI mass spectra of TMS derivatives of cinnamic acids

Peak No.	Systematic name	Common name	M.W.	M.W. (TMS)	Fragment ions *m/z*, (intensity as % of base peak)
16	3-phenylpropanoic acid	*trans*-cinnamic acid	148	220	220 (37), 205(100), 161 (55), 131 (63), 103 (55)
17	2-hydroxy cinnamic acid	o-coumaric acid	164	308	308 (42), 293 (70), 161 (25), 147 (90), 73(100)
18	4-hydroxy cinnamic acid	p-coumaric acid	164	308	308 (55), 293(100), 249 (59), 219 (70), 73 (65)
19	4-hydroxy,3-methoxy cinnamic acid	ferulic acid	180	338	338(100), 323 (88), 308 (73), 293 (65), 249 (58)
20	3,4-hydroxy cinnamic acid	caffeic acid	194	396	396 (72), 381 (22), 307 (20), 219(100), 73(65)
21	4-hydroxy,3,5-dimethoxy cinnamic acid	sinapinic acid	224	368	368 (85), 353 (52), 338(100), 323 (40), 73 (40)

Table 4. Principal ions observed in the EI mass spectra of TMS derivatives of coumarins

Peak No.	Systematic name	Common name	M.W.	M.W. (TMS)	Fragment ions *m/z*, (intensity as % of base peak)
22	4-hydroxy coumarin		162	234	234 (41), 219(100), 206 (55), 175 (82), 73 (48)
23	7-hydroxy coumarin	umbelliferon	162	234	234 (78), 219(100), 163 (59), 89 (15), 73 (30)
24	7-hydroxy 6-methoxy coumarin	scopoletin	192	264	264 (45), 234(100), 206 (50), 103 (12), 73 (60)
25	6,7-dihydroxycoumarin	esculetin	178	322	322 (40), 307 (40), 206 (10), 175 (7), 73(100)
26	7,8-dihydroxy,6-methoxy coumarin	fraxetin	208	352	352 (28), 337 (71), 307 (5), 263 (5), 73(100)
27	6,7-dihydroxy,4-methyl coumarin	4-methylesculetin	192	352	352 (28), 337(100), 307 (5), 263 (5), 73 (80)
28	6,7-dihydroxy 6-glucoside coumarin	esculin	340	700	363(100), 331 (60), 323 (40), 272 (45), 234 (62)
29	3,3'-methylene-bis(4-OH-coumarin)	dicoumaol	336	700.	480 (5), 465 (17), 305 (20), 233 (30), 73(100)

Table 5. Principal ions observed in the EI mass spectra of TMS derivatives of other related phenolics

Peak No.	Systematic name	Common name	M.W.	M.W. (TMS)	Fragment ions *m/z*, (intensity as % of base peak)
30	5-methyl-2-isopropylphenol	thymol	150	222	222 (25), 207(100), 165 (7), 91 (10), 73 (87)
31	2-hydroxyacetophenone	0-hydroxyacetophenone	136	208	208 (0), 193(100), 175 (26), 151 (20), 73 (12)
32	3-phenyl-2-propen-1-ol	cinnamyl alcohol	134	206	206 (42), 191 (42), 117(100), 115 (50), 73 (70)
33	4-hydroxyacetophenone	p-hydroxyacetophenone	136	208	208 (23), 193(100), 151 (17), 91 (10), 73 (25)
34	4-hydroxy,3-methoxy benzyl alcohol	homovanillic alcohol	168	312	312 (20), 297 (10), 209(100), 179 (20), 73 (67)
35	3,4-methyl phenyl acetic acid	homoveratric acid	196	268	268 (40), 253 (20), 209(100), 151 (40), 73(100)
36	4-hydroxy,3'5'-methoxy acetophenone	acetosyringone	196	268	268 (40), 253 (65), 238 (84), 223(100), 73 (40)
37	4-hydroxy,3-methoxy phenyl acetic acid	homovanillic acid	182	326	326 (30), 267 (26), 209 (42), 179 (32), 73(100)
38	1,3,4,5-tetrahydroxyhexanecarboxylic acid	quinic acid	192	556	556 (0), 345 (67), 255 (32), 147 (40), 73(100)
39	3-(3,4-dihydroxycinnamoyl) quinic aicd	chlorogenic acid	354	786	786 (0), 345 (67), 307 (30), 255 (42), 73(100)

of 2H-1-benzopyran-2-one. Most coumarins are synthesized from *trans* p-coumaric acid and bind hydroxyl group at C_7 position. Coumarins are classified into simple coumarin and furanocoumarin. These compounds possess a lot of physiological active functions.

The Peak No. 23 compound, umbelliferon containing one hydroxyl group is present in plant with its isomer, 4-hydroxycoumarin. From the analysis of mass fragment ions(Table 4), these two compounds showed their base peak at $[M-15]^+$ ion, but gave different abundance at $[M]^+$ ion. It could be possible to identify these two isomers on the basis of different fragment ion patterns, i.e, *m/z* 206 and 175 ions for Prak No. 22, *m/z* 191 and 163 ions for Peak No. 23, respectively.

TMS derivatives of other related phenolics

The other related phenolic compounds listed in Table 5 are presented as free form or intermediate metabolite or can be produced from hydrolysis of phenolic compounds in plants. $[M]^+$ and $[M-15]^+$ ions were not detected on quinic acid, an intermediate metabolites of shikimic acid pathway and on chlorogenic acid. Both compounds showed their base peak at *m/z* 73 .

Underivatized phenolics

The separation of phenolic compounds containing aldehyde, methyl and methoxyl groups can be done by nonpolar column without derivatization. As shown in Table 6, their mass spectra were different from TMS derivatives of phenolic acids. Psoralen, a typical linear furanocoumarin, showed the base peak at *m/z* 158 but other compounds were appeared the base peak at $[M]^+$ ions. It might be possible to identify these compounds by the different patterns of mass fragment ions and retention time.

Table 6. Principal ions observed in the EI mass spectra of underivatized phenolics

Peak No.	Systematic name	Common name	M.W.	Fragment ions *m/z*, (intensity as % of base peak)
40	3-phenyl,2-propenol	cinnamic aldehyde	131	131(100), 103 (65) 77 (63), 63 (13), 51 (41)
41	4-allyl,1,2-*methylene dioxybenzene*	isosafrole	162	162(100), 135 (27), 131 (72), 104 (72), 77 (59), 63 (62)
42	2-methoxy,4-[2-prophenyl]phenol	eugenol	164	164(100), 149 (65), 131 (55), 103 (60), 91 (60), 77 (72)
43	8-methoxy[Furano-3',2':6,7-coumarin]	methoxalen	216	216(100), 201 (30), 173 (55), 145 (25), 89 (32), 63 (25)
44	Furo[3,2-8] coumarin	psoralen	187	187 (14), 158(100), 130 (27), 102 (49), 76 (20), 51 (28)

Table 7. Retention Time of TMS derivatives and underivatized phenolics on three different capillary columns

Peak No.	Systematic name (Common name)	*Retention Time(min)*		
		Ultra-2	HP-1	HP-35
1	1,2-benzenediol (catechol)	12.66	14.61	12.63
2	2,4-benzenediol (resorcinol)	16.20	15.93	14.22
3	2,3,4-benzenetriol (*pyrogallol*)	19.70	19.50	17.16
4	benzene carboxylic acid (benzoic acid)	13.08	12.58	12.02
5	2-hydroxy benzoic acid (salicylic acid)	18.99	18.56	17.51
6	3-hydroxy benzoic acid (m-hydroxy benzoic acid)	19.96	19.57	18.40
7	4-hydroxy benzoic acid (p-hydroxy benzoic acid)	21.13	20.77	19.64
8	4-hydroxy,3-methoxy benzoic acid (vanillic acid)	23.58	23.06	22.41
9	2,5-dihydroxy benzoic acid (gentisic acid)	23.74	23.45	21.94
10	3,5-dihydroxy benzoic acid (α-resocylic acid)	24.44	24.09	22.38
11	2,4-dihydroxy benzoic acid (β-resocylic acid)	24.51	24.11	22.49
12	3,4-dihydroxy benzoic acid (protocatechuric acid)	24.58	24.22	22.53
13	4-hydroxy,3,5-dimethoxy benzoic acid (syringic acid)	25.79	25.14	24.97
14	2,3,4-trihydroxy benzoic acid	26.18	25.86	23.76
15	3,4,5-trihydroxy benzoic acid (gallic acid)	26.75	26.43	24.47
16	3-phenylpropanoic acid (*trans*-cinnamic acid)	19.68	18.95	19.08
17	2-hydroxy cinnamic acid (o-coumaric acid)	24.40	23.87	23.33
18	4-hydroxy *cinnamic acid (p-coumaric acid)*	26.45	25.93	25.27
19	4-hydroxy, 3-methoxy cinnamic acid (ferulic acid)	28.74	28.13	27.85
20	3,4-hydroxy cinnamic acid (caffeic acid)	29.34	28.97	27.50
21	4-hydroxy, 3,5-methoxy cinnamic acid (sinapinic acid)	30.82	30.14	30.13
22	4-hydroxy coumarin	25.12	24.00	23.56
23	7-hydroxy coumarin (umbelliferon)	25.13	24.17	25.53
24	7-hydroxy 6-methoxy coumarin (scopoletin)	28.27	27.10	28.91
25	6,7-dihydroxycoumarin (esculetin)	29.19	28.37	28.89
26	7,8-dihydroxy,6-methoxy coumarin (fraxetin)	30.19	29.24	30.06
27	6,7-dihydroxy,4-methyl coumarin (4-methylesculetin)	31.00	30.14	37.61
28	6,7-dhydroxy 6-glucoside coumarin (esculin)	43.31	42.14	40.41
29	3,3'-methylene-bis[4-OH-coumarin] (dicoumaol)	74.41	73.98	51.75
30	5-methyl-2-isopropylphenol (thymol)	14.67	14.41	12.51
31	2-hydroxyacetophenone (*0-hydroxyacetophenone*)	16.29	15.50	15.55
32	3-phenyl-2-propen-1-ol (cinnamyl alcohol)	17.18	16.68	16.01
33	4-hydroxyacetophenone (p-hydroxyacetophenone)	18.18	17.48	17.73
34	4-hydroxy,3-methoxy benzyl alcohol (homovanillic alcohol)	22.64	21.86	21.17
35	3,4-methyl phenyl acetic acid (homoveratric acid)	22.76	22.21	23.04
36	4-hydroxy,3'5'-methoxy acetophenone (acetosyringone)	23.85	22.93	24.05
37	4-hydroxy,3-methoxy phenyl acetic acid (homovanillic acid)	23.79	23.12	22.73
38	1,3,4,5-tetrahydroxyhexanecarboxylic acid (quinic acid)	25.41	25.45	21.55
39	3-(3,4-dihydroxycinnamoyl) quinic aicd (chlorogenic acid)	42.72	42.07	38.32
40	3-phenyl,2-propenol (cinnamic aldehyde)	13.85	12.68	14.83
41	4-allyl,1,2-methylene dioxybenzene (isosafrole)	15.78	14.93	15.80
42	2-methoxy,4-[2-prophenyl]phenol (eugenol)	16.29	15.45	16.61
43	8-methoxy[Furano-3',2':6,7-coumarin] (methoxalen)	26.15	25.06	30.61
44	*Furo[3,2-8] coumarin (psoralen)*	29.82	28.43	26.99

GC analysis

Table 7 shows the retention time of 39 TMS derivatives and 5 underivatives separated by HP-1 (nonpolar), Ultra-2 (relatively weak nonpolar) and HP-35 (intermediate) columns. The separation time was fairly constant on two nonpolar columns, but a few compounds showed slightly different separation order on the HP-35 column.

The head pressure of HP-35 column (0.32 mm inner size) was reduced to 5 psi to maintain similar retention time of nonpolar columns. Figs. 4 and 5 are the GC chromatogram and five underivatized phenolic compounds.

Calibration plots of each compounds were linear at 0.1 ~10 μg (r^2=0.998) and detection limits were about 5 ng at split mode method. In the case of using 0.53 mm capillary column in the splitless mode, the value of correlation coefficients and detection limit of compounds could be much more improved.

DISCUSSION

More than 1,000 phenolic compounds are known to be present in nature. Flavonoids, simple monocyclic phenols, phenyl propanoids and phenolic quinone are included in this group and reported to have important physiological

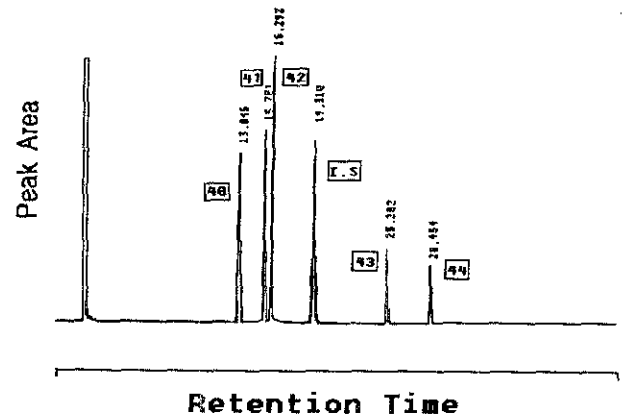

Fig. 5. Capillary gas chromatogram of five phenolic compounds.

active functions. Due to the similar UV spectrum and formation of complexes of these compounds, it is very difficult to identify each compounds.

This study was conducted to obtain more accurate qualitative and quantative method by separating these compouds with GC and identifying them with retention times and the charateristics of mass fragment ions. Generally, it is essential to derivatize the compound by substituting with nonpolar functional group, if it is nonvolitale or contain the active hydrogen, hydroxyl and carboxyl groups (22).

For the rapid and simple trimethylsilyl derivatization

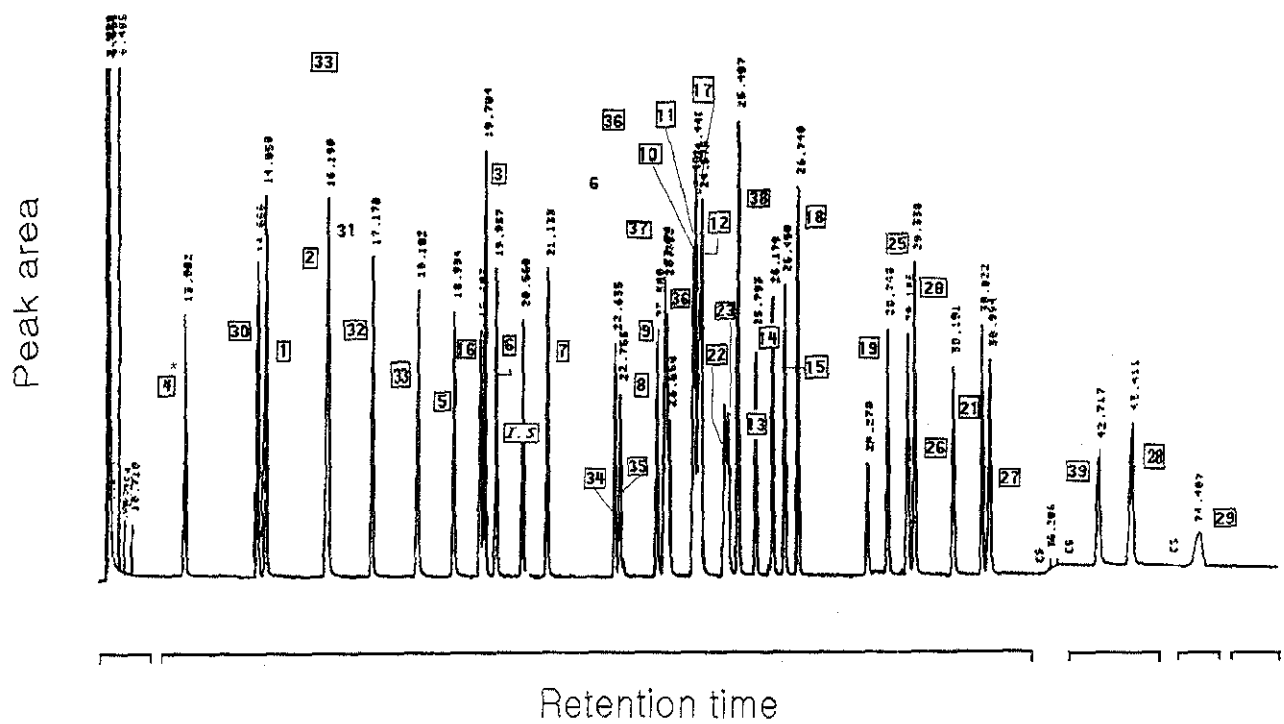

Fig. 4. Capillary gas chromatogram of 39TMS derivatives of phenolic compounds.
*The identified name of Peak No. were listed in Table 7.

of phenolic compounds and its isomers, the reaction using pyridine, HMDS and TFA was successfully achieved at 70°C for 5 min and samples were separated well by GC column. TMS derivatives must be analyzed as soon as possible. TMS derivatives studied in this experiment were not changed when stored below 0°C for 15 days, but mutipeaks were shown when stored at room temperature.

In mass spectral analysis, it was possible to identify the compounds with similar retention time and the pattern of mass fragment ions produced. The presence of strong $[M-15]^+$ ion, the base peak of most TMS derivatives of phenolic compounds used in this experiment, attributed to the loss of a methyl radical and the formation of a bond between the silicon atom of the TMS group and adjacent carbonyl oxygen. The result is agreed with Creaser et al. (20) who found that the $[M-15]^+$ ion was major fragment ion from the study of mass spectrum of flavonoid aglycones. Loh et al. (23) also reported the same result from the trimethylsilylation study of saccharin. This study suggested that nonflavonoid phenolic compounds could be identified by the relative retention time on column and fragment patterns obtained by GC/MS spectra.

REFERENCES

1. Choi, S. W., Kang, W. W., Chung, S. K. and Cheon, S. H. : Antioxidative activity of flavonoids in persimmon leaves. *Food Biotechnol.*, **5**, 119 (1996)
2. Mario, F., Mario, P., Maria, T. B. and Giuseppe, R. : Flavonoids, coumarins, cinnamic acids as antioxidants in a micellar system. structure-activity relationship. *J. Agric. Food Chem.*, **44**, 497 (1996)
3. Joesep, K., Edwin, F., Rina, G., Bruuce, G. and John, E. K. : Natural antioxidants in grapes and wine. *J. Agric. Food. Chem.*, **42**, 64 (1994)
4. Park, J. C., You, Y. B., Lee, J. H. and Kim, N. J. : Anti-inflammatory and anlgesic effects of the components from some edible plants. *J. Korean Soc. Food Nutr.*, **23**, 671 (1994)
5. Kuk, J. H., Ma, S. J. and Pack, K. H. : Isolation and characterization of benzoic acid with antimicrobial activity from needle of *Pinus densiflora*. *Korean J. Food Sci. Technol.*, **29**, 204 (1997)
6. Pack, J. C., Ha, J. O. and Pack, K. Y. : Antimutagenic effect of flavonoids isolated from *Oenanthe javanica*. *J. Korean Soc. Food Sci. Nutr.*, **25**, 588 (1996)
7. Moon, S. H. and Park, K. Y. : Antimutagenic effects of boiled water extract and tannin from persimmon leaves. *J. Korean Soc. Food Sci. Nutr.*, **24**, 880 (1995)
8. Kang, Y. H., Pack, Y. K. and Lee, G. D. : The nitrite scavenging and electron donating ability of phenolic compounds. *Korean J. Food Sci. Technol.*, **28**, 232 (1996)
9. Woo, W. S. : *Research method on the chemistry of natural products*. Mitumsa, Seoul, p.45 (1994)
10. Vanhaelen, M. and Vanhaelen-Fastre, R. : High performance liquid, gas liquid and thin layer chromatography of naturally occurring flavonoids, phenolic and related compounds. *J. Chromatogr.*, **187**, 255 (1980)
11. Han, S. Y., Park, J. C., Park, H. J., Lee. J. H. and Choi, J. S. : Phenolic compounds of the leaves of *Eucommia ulmoides*. *Arch. Pharm. Res.*, **14**, 114 (1991)
12. Leira, E., Botana, A. and Cela, R. : Resolution of complex mixtures of non-flavonoid polyphenol by column-switching high-performance liquid chromatography using octadecyl silica and graphitized carbon column. *J. Chromatogr. A*, **724**, 67 (1996)
13. Koupai-Abyazani, M. R., McCallum, J. and Bojm, B. A., : Identification of constituent flavonoid units in sainfoin proanthocyanidins by reversed-phase high-performance liquid chromatography. *J. Chromatogr.*, **594**, 117 (1992)
14. Guillen, D. A., Barroso, C. G. and Perez-Bustamante, J. A. : Automation of sample preparation as a preliminary stage in the high-performance liquid chromatographic determination of polyphenolic compounds in sherry wines. *J. Chromatogr. A*, **730**, 6 (1996)
15. Pietta, P., Mauri, P. and Bruno, A. : Identification of flavonoids from *Ginkgo* L., *Anthemis nobilis* L and *Equisetum arvense* L. by high-performance liquid chromatography with diode-array UV detection. *J. Chromatogr.*, **553**, 223 (1991)
16. Guillen, D. A., Barroso, C. G. and Perez-Bustamante, J. A. : Selection of column and gradient for the separation of polyphenol in sherry wine by high performance liquid chromatography in cooperating internal standards. *J. Chromatogr. A*, **724**, 117 (1996)
17. Guillen, D. A., Merello, F., Barroso, C. G. and Perez-Bustamante, J. A. : Soild-phase extraction for sample preparation, in the HPLC analysis of polyphenolic compounds in "fino" sherry wine. *J. Agri. Food Chem.*, **45**, 403 (1997)
18. Creaser, C. S., Koupai-Abyazani, M. R. and Stephenson, G. R. : Capillary column gas chromatography of methyl and trimethylsilyl derivatives of some naturally occurring flavonoid aglycones and other phenolics. *J. Chromatogr.*, **478**, 415 (1989)
19. Creaser, C. S., Koupai-Abyazani, M. R. and Stephenson, G. R. : Origin and control of multi-peak formation in the analysis of trimethylsilyl derivatives of flavonone aglycones by capillary column gas chromatography. *J. Chromatogr.*, **586**, 323 (1991)
20. Creaser, C. S., Koupai-Abyazani, M. R. and Stephenson, G. R. : Mass spectra of trimethylsilyl derivatives of naturally occurring flavonoid aglycones and chalcones. *Org. Mass Spectrom.*, **26**, 157 (1991)
21. Landis, W. D., Jonathan, W., White, J. and Jhon, G. P. : Gas-liquid chromatographic test for honey adulteration by high fructose corn syrup. *J. Assoc. Off. Anal. Chem.*, **62**, 186 (1979)
22. McEwen, C. N. and Larsen, B. S. : Mass spectrometry of biological materials 14. Capillary GC/MS for the analysis of biological materials. Marcel Dekker Inc., New York, p.437 (1990)
23. Lho. D. S., Kim, S. K., Lee, J. A., Jeung, H. S., Yoo, B. K. and Park, J. S. : GC/MS analysis of saccharin in foods. *J. Food Hyg. Safety*, **10**, 239 (1995)

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