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High-performance liquid chromatography–electrospray mass spectrometric analysis of pungent constituents of ginger

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Abstract

A gradient elution reversed-phase HPLC separation of ginger extract has been developed, which gives better separation than previous isocratic separations. HPLC–UV–electrospray MS has been successfully utilized to identify the individual pungent constituents in the chromatogram of ginger extract. Seven compounds were positively identified as the major pungent constituents of ginger based on their UV spectra, $[M+H]^+$, $[M+Na]^+$ and characteristic sodiated dimer $[2M+Na]^+$ ions, and comparison to data for the purified standards: [6]-gingerol and [6]-shogaol. The pungent compounds were assigned as [6]-gingerol, [8]-gingerol, [10]-gingerol, [6]-shogaol, [8]-shogaol, [10]-shogaol and [6]-gingediol. Another eight minor compounds were tentatively identified as gingerol analogues. © 1998 Elsevier Science B.V.

Keywords: Zingiber officinale; Ginger; Gingerol; Shogaol

1. Introduction

Ginger is the rhizome of *Zingiber officinale* Roscoe (Zingiberaceae), a plant cultivated in many tropical and subtropical countries. The part used is the pungent rhizome, commonly called 'root' both in fresh and dried forms. Oleoresin has been obtained by solvent extraction of dried unpeeled ginger, as peeled ginger loses much of its essential oil content. Major exporting countries are India, Australia, Fiji, Nigeria, and China. As a spice, it is widely consumed in Asian cooking. It is used in Chinese traditional medicine as a stomachic, antiemetic, antidiarrheal and cardiotonic, for the treatment of several gastrointestinal and respiratory diseases. Powdered ginger is also used for treatment of motion sickness [1]. The constituents responsible for the pungent taste of ginger are a homologous series of phenolic ketones, known as [4]-, [6]-, [8]-, [10]- and [12]-gingerols [2]. The shogaol series of compounds, even more pungent than the gingerols, is virtually absent from fresh ginger, and is derived from the corresponding gingerols during thermal processing or long-term storage [3]. Shogaols are gingerol analogues with a 4,5 double bond, resulting from elimination of the 5-hydroxy group. The chemical structures of gingerol, shogaol and related compounds are depicted in Fig. 1.

GC–MS has been used to analyze gingerols in ginger extract [4,5]. Because the main pungent principles, [6]-, [8]- and [10]-gingerol, are thermally unstable and decompose under high temperature during GC analysis, there were some artifacts detected. HPLC has been used for ginger analysis [6–9]; however, quantification may require authentic gingerols and shogaols as retention time standards.

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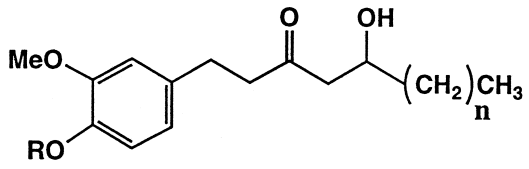
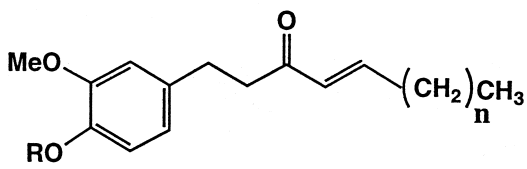
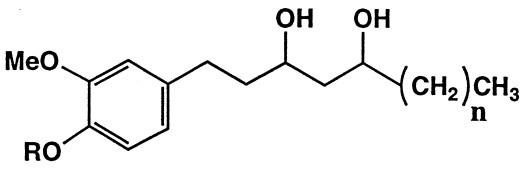


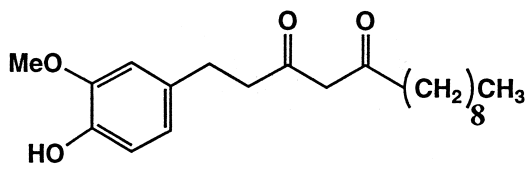
Compound	n	R	Mr
[4]-gingerol (**1**)	2	H	266
[6]-gingerol (**2**)	4	H	294
[8]-gingerol (**3**)	6	H	322
[10]-gingerol (**4**)	8	H	350
Me-[6]-gingerol (**5**)	4	Me	308



Compound	n	R	Mr
[6]-shogaol (**6**)	4	H	276
[8]-shogaol (**7**)	6	H	304
[10]-shogaol (**8**)	8	H	332
Me-[6]-shogaol (**9**)	4	Me	290



Compound	n	R	Mr
[6]-gingediol (**10**)	4	H	296
[8]-gingediol (**11**)	6	H	324
[12]-gingediol (**12**)	10	H	380
Me-[12]-gingediol (**13**)	10	Me	394



[10]-gingerdione (**14**) Mr = 348

Fig. 1. Ginger pungent constituents discussed in this paper.

Obtaining these unstable compounds requires great expenditure of time or money. Additionally, some previous isocratic HPLC separations were less than optimal [3,7,9].

The combination of HPLC with UV photodiode-array detection and mass spectrometry offers on-line UV and mass spectra for each peak. It therefore supplies more data for precise assignment of known compounds in a chromatogram. There are many different LC–MS interfaces, each with a certain selectivity and its own restrictions [10]. In an electrospray (ES) interface, the ions are formed from the liquid phase by ejection from shrinking charged droplets. This is more suitable for the analysis of thermally labile compounds such as the gingerols. ES is a soft ionization and usually gives a protonated molecule $[M+H]^+$ without fragmentation. In this paper, we will report an improved HPLC separation of ginger constituents and the direct identification of the peaks in HPLC chromatograms from fresh ginger and ginger oleoresin by HPLC–UV–ES-MS analysis. Pure [6]-gingerol (**2**) and [6]-shogaol (**6**) were isolated by preparative TLC as our own standard compounds for definite confirmation of peak identification. Their structures were confirmed by ^{1}H-NMR analysis [11,12].

2. Experimental

2.1. Instrumentation

A HPLC 1090 Series II instrument (Hewlett–Packard, Palo Alto, CA, USA) with a photodiode-array detector set at 280 nm (for signal A) and 230 nm (for signal B) was coupled with a HP 5989 B quadrupole mass spectrometer. UV spectra were taken in the region of 200–500 nm. Chromatographic conditions were: column, Waters Symmetry C_{18}, 5 μm, 150×2.1 mm (Waters, Milford, MA, USA); eluent (A) water and (B) acetonitrile. The gradient elution had the following profile: 0–8 min, 45–50% B; 8–17 min, 50–65% B; 17–32 min, 65–100% B;32–38 min, 100% B; 38–40 min, 100–45% B.

Flow-rate, 0.2 ml/min; temperature, 48°C. Mass

range measured: 200–800 μ, quadrupole temperature, 150°C; EM volts 2173. The spectra were acquired in the positive mode. ES interface was HP 59987 A; drying N_2 temperature, 350°C, 40 ml/min; nebulizing N_2, $5.5 \cdot 10^5$ Pa (80 p.s.i.). The HPLC was directly connected to the mass spectrometer without stream splitting.

2.2. Plant material and sample preparation

Fresh ginger *(Zingiber officinale* Rosc.) was purchased from a local supermarket. One gram of fresh ginger was ground in a mill. Samples were refluxed with 20 ml of methanol for 1 h. The methanol solution was filtered through a 0.45-μm nylon Acrodisk 13-mm filter (Gelman, USA). An 8-μl volume of the sample solution was injected into the HPLC column. Ginger oleoresin, from an ethanolic extract, was purchased from China (Wallshine, Hunan, China). A 50-mg sample was dissolved in 10 ml methanol. The methanol solution was filtered as above. An 8-μl volume of the sample solution was injected into the HPLC column.

2.3. Isolation of [6]-gingerol (*2*) and [6]-shogaol (*6*)

The 1 g of ginger oleoresin was partitioned between water and ethyl acetate. The ethyl acetate layer was concentrated to dryness in vacuo. The residue was dissolved in methanol and extracted with *n*-hexane. The methanol part was evaporated in vacuo. The residue (100 mg) was dissolved in ethanol for TLC separation. TLC was carried out on a silica gel 60 F_{254} plate, 20×20 cm, 0.5 mm (E. Merck, Darmstadt, Germany) with a mixture of *n*-hexane–acetone (8:2) as developing solvent. The bands of [6]-gingerol (R_F 0.25) and [6]-shogaol (R_F 0.52) were scraped from the plate and extracted with methanol. Sample purities were checked by HPLC. ^{1}H-NMR was measured on a Bruker AM400 spectrometer in $CDCl_3$, tetramethylsilane as internal standard.

2.4. Solvent and chemicals

Methanol, acetonitrile and water were HPLC grade (VWR, Seattle, WA, USA). Ethyl acetate, *n*-hexane, acetone were reagent grade (Sigma, St. Louis, MO, USA).

3. Results and discussion

3.1. HPLC–MS of fresh ginger extract

Both the HPLC–UV and HPLC–ES-MS chromatograms of a fresh ginger extract are shown in Fig. 2. Peaks 2, 3, 6 and 10 all showed similar UV spectra characteristic of gingerol compounds, having a UV absorption maximum at 282 nm and a shoulder at 230 nm. Peak 2 has a protonated molecule $[M+H]^+$ at m/z 297, an adduct ion $[M+Na]^+$ at m/z 319 and a sodiated dimer ion $[2M+Na]^+$ at m/z 615. It is identified as [6]-gingediol (**10**). Peaks 3, 6 and 10 showed protonated molecules $[M+H]^+$ at m/z 295, 323 and 351, adduct ions $[M+Na]^+$ at m/z 317, 345 and 373, and sodiated dimer ions $[2M+Na]^+$ at m/z 611, 667 and 723, respectively. The molecular mass of the compound for peak 6 was 28 μ more than the compound for peak 3. In the biosynthetic point of view, this should indicate the difference of two methylene units. The same homologous relationship was observed between peaks 10 and 6. Therefore, they are assigned as [6]-gingerol (**2**), [8]-gingerol (**3**), and [10]-gingerol (**4**), respectively, in order of decreasing polarity. Moreover, our purified [6]-gingerol (**2**) had the same retention time and MS spectrum as peak 3. These compounds in the chromatogram appeared in the same consecutive order as in the previous work by Chen et al. [5].

A small peak, 7, was assigned as [6]-shogaol (**6**), based on a protonated molecule $[M+H]^+$ at m/z 277 and an adduct ion $[M+Na]^+$ at m/z 299. Peaks 4, 8, 9, 11, 12 and 14 are tentatively assigned, as methyl [6]-gingerol (**5**), [12]-gingediol (**12**), methyl-[6]-shogaol (**9**), methyl-[12]-gingediol (**13**), [10]-gingerdione (**14**), and [8]-gingediol (**11**), based on their UV spectra, protonated molecules and adduct ions listed in Table 1. Peaks 5, 13 and 15 remain unidentified, because there are no dominant ions or ions cannot be matched with known ginger compounds.

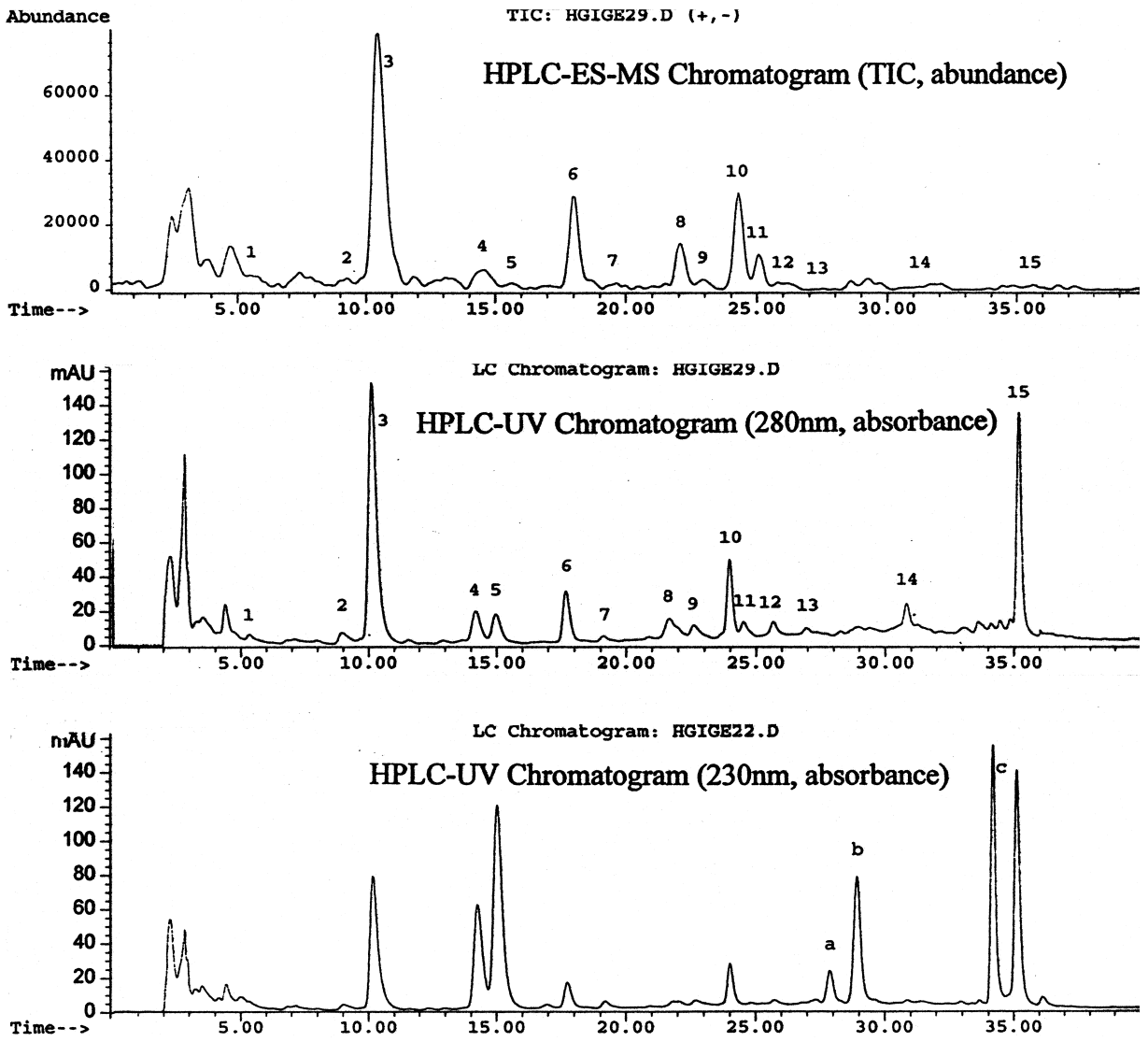


Fig. 2. Simultaneous HPLC–UV and HPLC–ES-MS chromatogram of a fresh ginger methanol extract, without post-column stream splitting. Chromatographic conditions were as described in Section 2. Peak assignments are shown in Table 1.

3.2. HPLC–MS of ginger oleoresin extract

Both the HPLC–UV and HPLC–ES-MS chromatograms of a ginger oleoresin extract are shown in Fig. 3. The UV spectra of peaks 4, 8 and 11 showed spectra characteristic of shogaol. An α, β-unsaturated ketone moiety as an additional chromophore in shogaol gives rise to a much stronger UV absorption than gingerol in the lower wavelength region at 228 nm. The UV spectra of [6]-gingerol (**2**) and [6]-shogaol (**6**) are shown in Fig. 4. Peaks 4, 8 and 11 showed protonated molecules $[M+H]^+$ at m/z 277, 305 and 333; adduct ions $[M+Na]^+$ at m/z 299, 327 and 355; sodiated dimer ions $[2M+Na]^+$ at m/z 575 and 631 respectively (peak 11 did not show a sodiated dimer ion). They are assigned as [6]-shogaol (**6**), [8]-shogaol (**7**), and [10]-shogaol (**8**). Moreover, our purified [6]-shogaol (**6**) had the same retention

Table 1
Peak assignments for analysis of a fresh ginger methanol extract

Peak number	Retention time (min)	$[M+H]^+$ (m/z)	$[M+Na]^+$ (m/z)	$[2M+Na]^+$ (m/z)	Other ions (m/z)	UV λ_{max} (nm)	Identification
1	5.2	267	289	–	–	–	[4]-gingerol[a]
2	9.0	297	319	615	–	230 sh, 282	[6]-gingediol
3	10.2	295	317	611	–	230 sh, 282	[6]-gingerol
4	14.1	–	331	–	–	268, 330	methyl-[6]-gingerol[b]
5	15.0	–	–	–	315, 375	270, 330	n.d.
6	17.8	323	345	667	–	230 sh, 282	[8]-gingerol
7	19.1	277	299	–	–	230, 282	[6]-shogaol
8	21.8	403	–	–	–	230 sh, 282	[12]-gingediol[a]
9	22.7	291	313	–	–	230, 282	methyl-[6]-shogaol[a]
10	24.0	351	373	723	–	230, 282	[10]-gingerol
11	24.6	–	417	–	–	230, 280	methyl-[12]-gingediol[a]
12	25.7	–	371	–	–	230, 280	[10]-gingerdione[a]
13	27.0	425	447	–	371, 447	–	n.d.
14	30.9	–	347	–	–	230, 280	[8]-gingediol
15	35.1	–	–	–	–	230, 300	n.d.

[a] Peaks were tentatively identified.
n.d., peak identification not determined.
[b] UV spectrum does not match gingerol analogue.

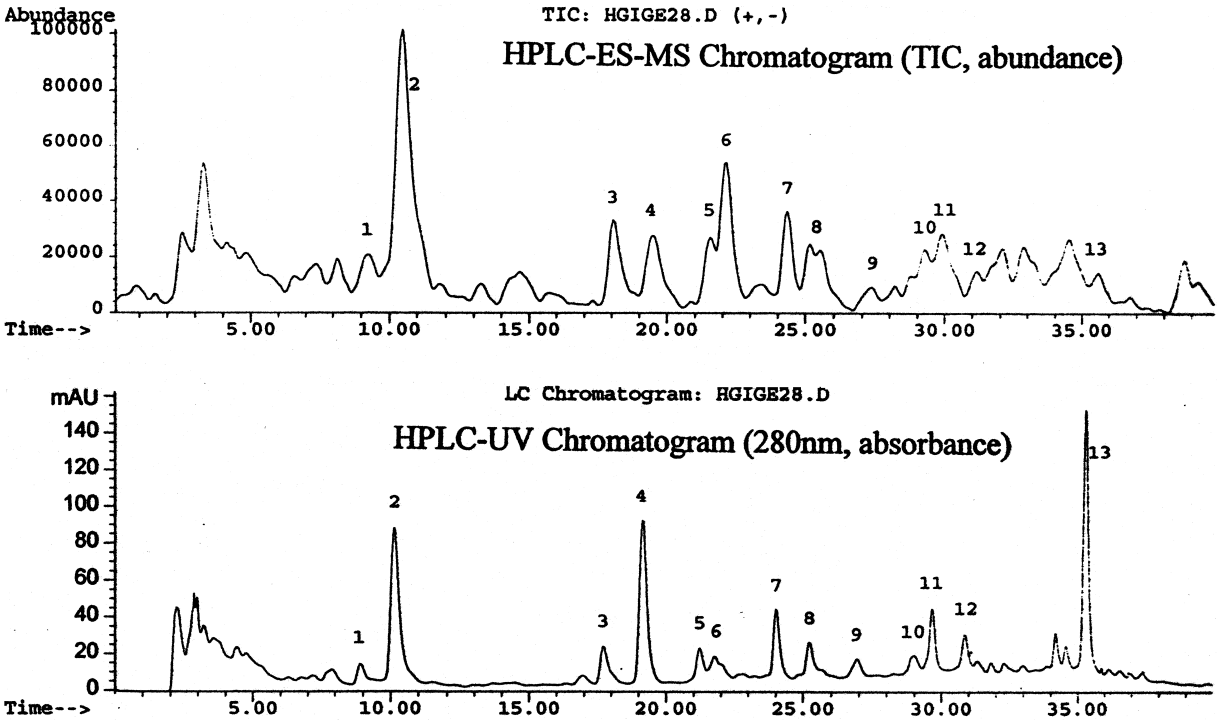


Fig. 3. Simultaneous HPLC–UV and HPLC–ES-MS chromatogram of a ginger oleoresin extract, without post-column stream splitting. Chromatographic conditions were as described in Section 2. Peak assignments are shown in Table 2.

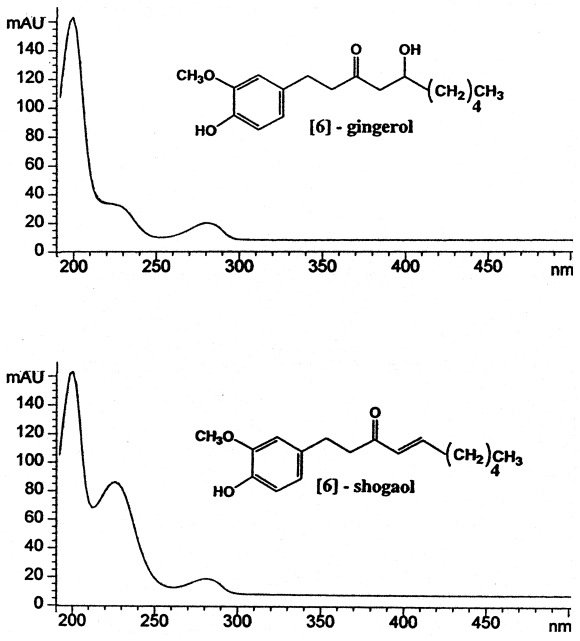


Fig. 4. The UV spectra of [6]-gingerol and [6]-shogaol.

time and MS spectrum as peak 4. Peaks 1, 2, 3, and 7 are assigned as [6]-gingediol (**10**), [6]-gingerol (**2**), [8]-gingerol (**3**), and [10]-gingerol (**4**), respectively. Peak 5 is probably an isomer of [8]-gingerol (**3**). Peak 6 is tentatively identified as [12]-gingediol (**12**). Peaks 9, 10, 12, and 13 were not identified (Table 2).

Our results show that in the mass spectra of [6]-, [8]- and [10]-gingerol, the highest ion signal intensity was observed for the $[M+Na]^+$ ions, yet in [6]-, [8]- and [10]-shogaol, the highest ion signal intensity was seen for $[M+H]^+$ ions. The [6]-, [8]- and [10]-gingerols, [6]- and [8]-shogaols, and [6]-gingediol formed the sodiated dimer ions in electrospray ionization. Sodiated dimer ions provide additional data for definite identification of these compounds. The mass spectra of these compounds are shown in Fig. 5. Zhou and Hamburger noticed that capsaicin, which is structurally related to gingerol, formed the protonated dimer ions $[2M+H]^+$ in ES-MS [13], but gave no detailed descriptions. Possessing the same chromophore and approximate molecular mass as the gingerols, as well as ready commercial availability in pure form, capsaicin may represent an external standard for the direct quantification of pungent compounds in ginger oleoresin [14].

The addition of acetic acid or trifluoroacetic acid to the mobile phase will enhance the intensity of $[M+H]^+$ or $[M+Na]^+$ ions in most LC–MS applications. In the case of ginger, however, it produced many other ions in the chromatogram, obscuring the interpretation of the mass spectra. We therefore did not add any acid to the mobile phase in this analysis.

Table 2
Peak assignments for analysis of a ginger oleoresin extract

Peak number	Retention time (min)	$[M+H]^+$ (m/z)	$[M+Na]^+$ (m/z)	$[2M+Na]^+$ (m/z)	Other ions (m/z)	UV λ_{max} (nm)	Identification
1	9.0	297	319	615	–	228 sh, 282	[6]-gingediol
2	10.2	295	317	611	–	228 sh, 282	[6]-gingerol
3	17.8	323	345	667	–	228 sh, 282	[8]-gingerol
4	19.2	277	299	575	–	228, 282	[6]-shogaol
5	21.2	323	345	667	–	228 sh, 282	[8]-gingerol isomer[a]
6	21.8	–	403	–	–	228 sh, 282	[12]-gingediol[a]
7	24.0	351	373	723	–	230, 282	[10]-gingerol
8	25.2	305	327	631	550[b]	228, 282	[8]-shogaol
9	27.0	–	–	–	548	280	n.d.
10	29.0	–	–	–	427, 449	230, 282	n.d.
11	29.6	333	355	–	427, 449[b]	230, 282	[10]-shogaol
12	30.9	–	–	–	–	280	n.d.
13	35.2	–	–	–	–	230, 300	n.d.

[a] Peaks were tentatively identified.
n.d., peak identification not determined.
[b] Impurity ion.

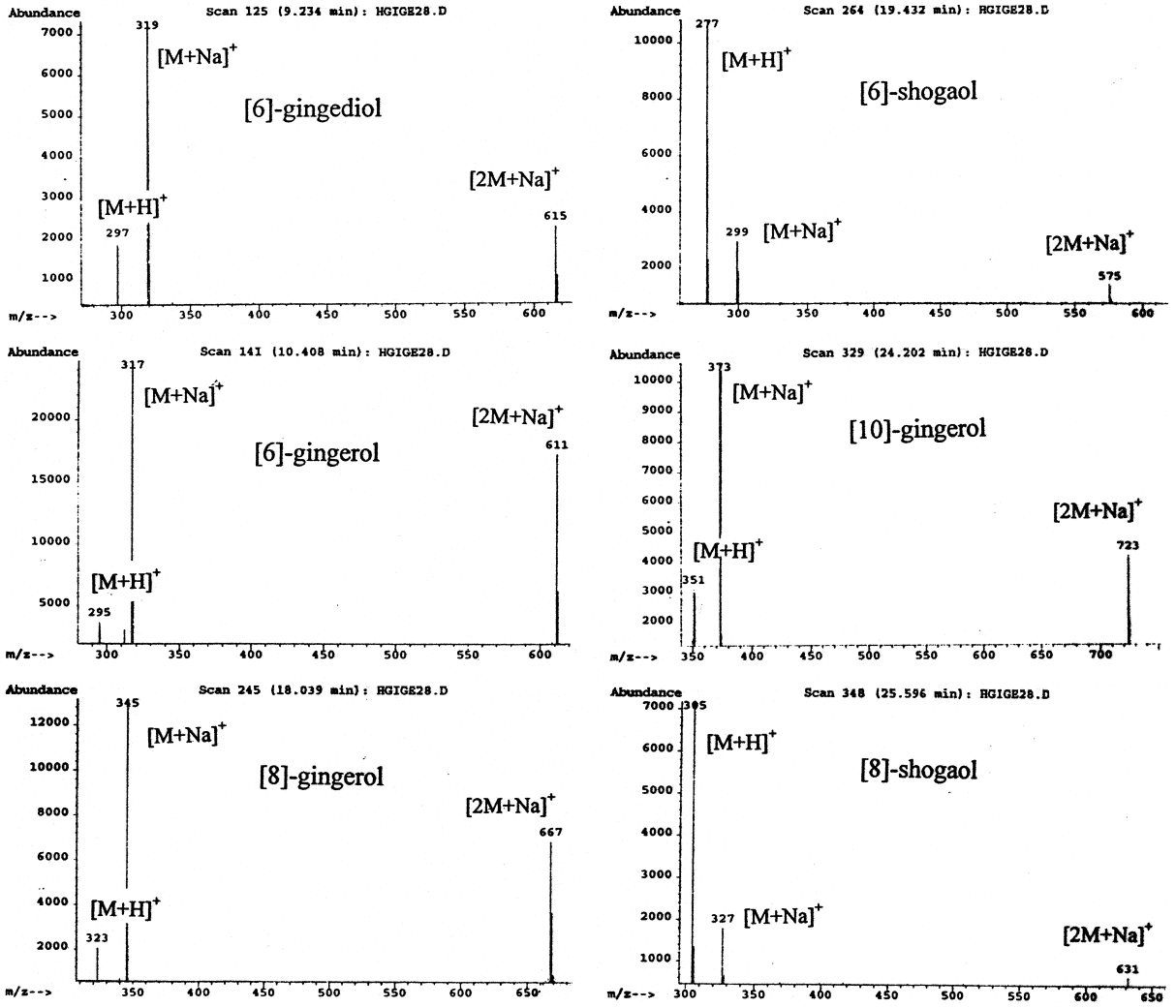


Fig. 5. Mass spectra of [6]-gingediol, [6]-gingerol, [8]-gingerol, [10]-gingerol, [6]-shogaol and [8]-shogaol.

Using UV 230 nm for detection (see Fig. 2, bottom), there are some other large peaks (a, b, c) in the chromatogram, the UV spectra of which are different than that of gingerols and shogaols. These peaks remain unidentified. Our results demonstrate that [6]-shogaol exists in fresh ginger as a minor constituent. However, ginger oleoresin contains [6]-, [8]- and [10]-shogaol as major constituents owing to their conversion from the corresponding gingerols. For processed ginger products, evaluation of pungency is an important quality assessment. Our chromatogram provides a valuable fingerprint for standardization of ginger extract. UV 280 nm, as suggested by Wood [9], rather than 230 nm, is a specific wavelength for detection of gingerols and shogaols.

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