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Liquid chromatographic–electrospray mass spectrometric analysis of Coptidis Rhizoma

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Abstract

Seven protoberberine alkaloids from Coptidis Rhizoma were separated on a Cosmosil 5C18-MS column (5 μm, 250×4.6 mm I.D.) using ammonium acetate buffer (isocratic) or ammonium acetate–sodium dodecyl sulfate buffer (gradient) with UV and electrospray ionization mass spectrometric (ESI-MS) detection. Selected-ion monitoring was used to resolve the compounds in an overlapped peak and collision-induced dissociation reaction was used to obtain structural infomation of individual components. By use of this LC–UV–ESI-MS method, these protoberberine alkaloids (berberine, palmatine, coptisine, epiberberine, jatrorrhizine, columbamine and berberastine) in a crude extract of Coptidis Rhizoma can be easily separated and identified within 50 min.

Keywords: Coptids Rhizoma; Protoberberine alkaloids; Alkaloids

1. Introduction

Coptidis Rhizoma (*huang-lien*) is a commonly used Chinese herb drug with the effects of clearing heat, drying up dampness, purging toxicosis and detoxicification [1]. It is derived from the dried rhizome of ranunculaceous plant such as *Coptis chinensis* Franch, *C. deltoidea* C.Y. Cheng et Hsiao, *C. japonica* Makino or *C. japonica* Makino var. *dissecta* Nakai, and is known to contain berberine (1), palmatine (2), coptisine (3), epiberberine (4), jatrorrhizine (5), columbamine (6) and berberastine (7) (see Figs. 1–3) as its major bioactive components [2–7]. Several methods have been reported for the determination of some of these seven protoberberine alkaloids, including thin-layer chromatography [5,8–12], micellar chromatography [13], electron microscopic analysis [14–16], high-performance liquid chromatography (HPLC) [17–23], capillary electrophoresis (CE) [24] and capillary electrophoresis–ion spray mass spectrometry (CE–MS) [25].

Electrospray mass spectrometric detection (ESI-MS) is a soft ionization technique that forms mainly M^+ peaks when no additional collison induced dissociation (CID) voltage was applied. These M^+ peaks allow a rapid determination of the molecular mass of a component directly after its elution from the LC column. The use of LC–ESI-MS has already provided on-line molecular mass information in the analysis of some drugs and natural products [26,27]. Separation of the coptis protoberberines directly from a 70% methanol crude extract has been achieved by reversed-phase HPLC using a metha-

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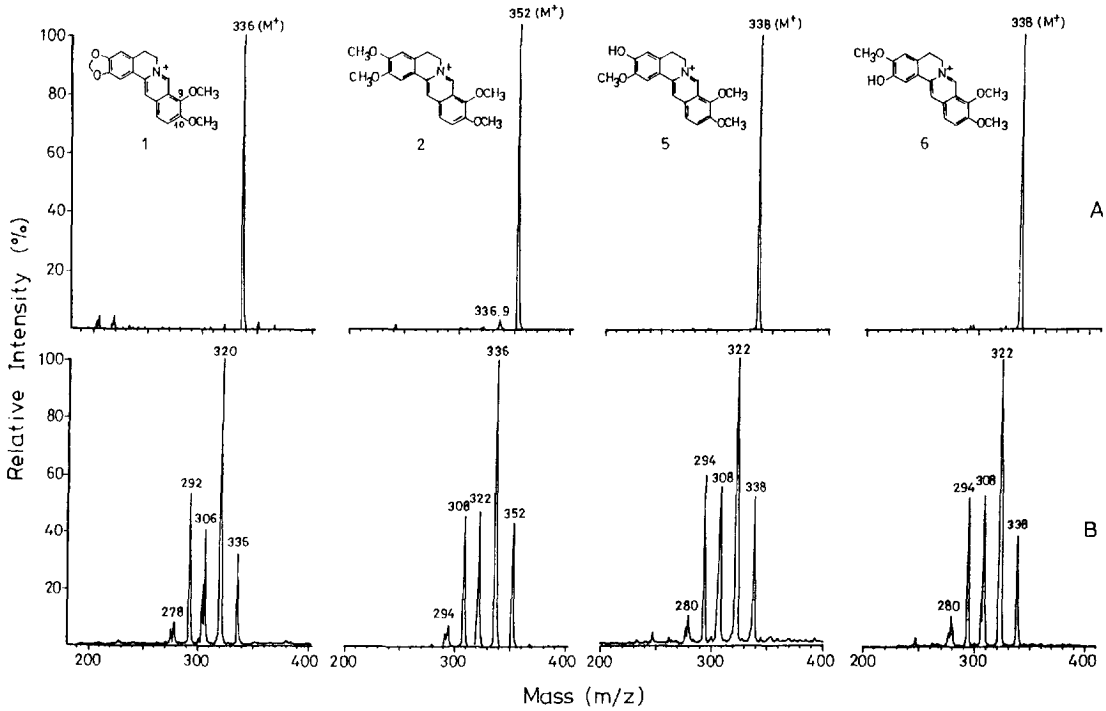

Fig. 1. ESI mass spectra for 9,10-dimethoxyl protoberberine alkaloids: (A) no CID, (B) CID= −30 V. 1, berberine; 2, palmatine; 5, jatrorrhizine; 6, columbamine.

nol–acetonitrile–buffer gradient system [23]. Coupling of LC–UV with MS should be able to offer added capability by providing important information necessary for the identification of these compounds in a complex mixture.

This paper presents the results obtained by LC–UV–ESI-MS in analysis of the crude extract of a Coptidis Rhizoma sample.

2. Experimental

2.1. Reagents and materials

Berberine chloride, palmatine chloride and sodium dodecyl sulfate (SDS) were purchased from Sigma (St. Louis, MO, USA), coptisine chloride and ammonium acetate were obtained from Nacalai Tesque (Kyoto, Japan). Berberastine, columbamine, jatrorrhizine and epiberberine were isolated from coptis rhizome [7,19]. Deionized water from a Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare all buffer and sample solutions. Acetonitrile and methanol were of LC grade (Fisons, Loughborough, UK). Coptidis Rhizoma was purchased from the Chinese herbal market in Taipei (Taiwan).

2.2. Preparation of Coptidis Rhizoma extracts

A 0.2-g sample of pulverized Coptidis Rhizoma was extracted with 70% methanol (7 ml) by stirring at room temperature for 30 min, then centrifuged at 1500 *g* (Universal, Hettich Zentrifugen) for 10 min. The extraction was repeated three times. After combining them together and filtering through a No.1 filter-paper, the Coptidis Rhizoma extract was diluted to 50 ml with 70% methanol. This solution was passed through a 0.45-μm filter and 2 μl of the filtrate was then injected into the LC–MS system directly.

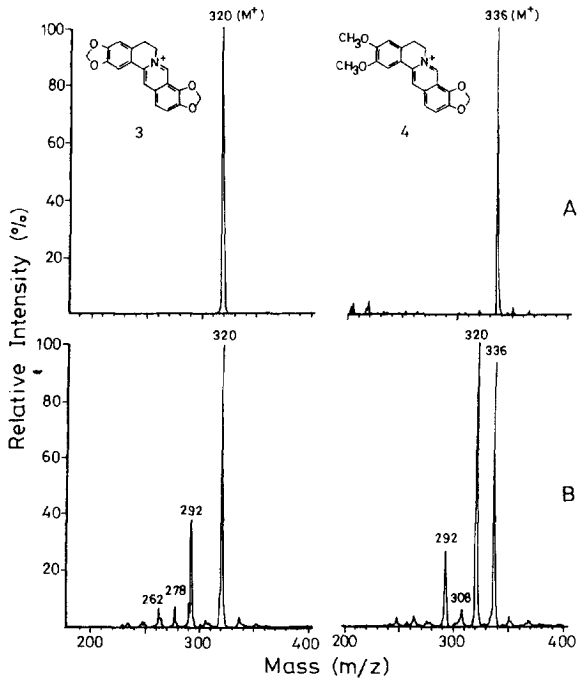

Fig. 2. ESI mass spectra for 9,10-methlenedioxyl protoberberine alkaloids: (A) no CID, (B) CID= −30 V. 3, coptisine; 4, epiberberine.

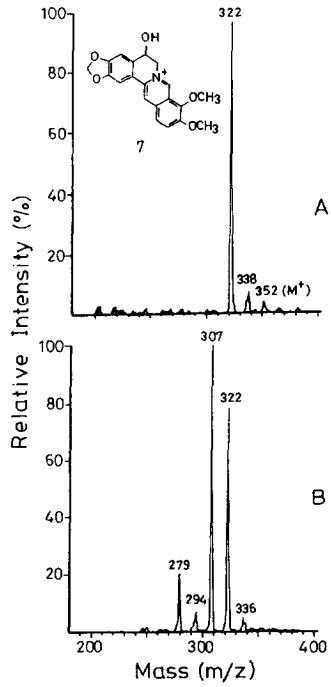

Fig. 3. ESI mass spectra for 7, berberastine: (A) no CID, (B) CID= −30 V.

2.3. ESI-MS analyses

A Finnigan MAT TSQ-700 (San Jose, CA, USA) triple quadrupole mass spectrometer equipped with an electrospray ionization source was used. Nitrogen was used as the nebulizing gas at a backing pressure of $5\sim6\cdot10^{-6}$ Torr (1 Torr=133.322 Pa). The electrospray needle was maintained at 4.5 kV and heated-capillary temperature was set at 200°C.

The individual alkaloid samples were delivered by a syringe pump (Harvard Apparatus, Cambridge, MA, USA) at a rate of 5 μl/min; and the samples of synthetic mixture and coptis crude extract were delivered by a HPLC system at a rate of 0.85 ml/min. The HPLC system consisted of a Consta Metric 4100 MS pump (Tsp, Riviera Beach, FL, USA), a gradient controller, a UV detector (λ=270 nm) and a Rheodyne 7725 injector (5-μl loop). Separations were performed on a Cosmosil 5C18-MS column (5 μm, 250×4.6 mm I.D., Nacalai Tesque) with a μ-Bondapak C_{18} precolumn (Millipore, Milford, MA, USA). Two eluent systems were used in this separations. Solvent system I was an isocratic elution of acetonitrile–acetate buffer (30:70) (acetate buffer consisted of 50 m*M* ammonium acetate and 2% acetic acid). Solvent system II followed a linear gradient elution, using the eluents A and B [A: acetonitrile–buffer (40:60) (the buffer consisted of 50 m*M* ammonium acetate, 2% acetic acid and 5 m*M* SDS); B: water–acetonitrile–methanol (10:45:45)], according to the following profile: 0–15 min, 100–65% A, 0–35% B; 15–30 min, 65% A, 35% B.

To obtain fragmentation information for each component, an additional offset voltage of −30 V was applied to the octapole to get desired fragments (CID). The lens and quardrupole voltages were optimized to maximum ion current for electrospray. Ions were detected by scanning the first quadrupole, and the scans were monitored in the range of m/z 50–500 in 0.5 s.

3. Results and discussion

3.1. Electrospray

Figs. 1–3 show the ESI mass spectra for individual alkaloids dissolved in 70% methanol, recorded at 0 and −30 V CID offset. With exception of berberastine, the mass spectrum of each alkaloid tested consisted exclusively of M^+ ion as the base peak when no additional voltage of CID. Fragment peaks from CID reactions appear at −30 V CID offset and provide structural information about molecules. After confirming with the results obtained from an ESI-MS–MS experiment (including daughter ions scan, parent ions scan and neutral loss ions scan) [28], the fragment peaks shown in Figs. 1–3 were interpreted as listed in Table 1. Data in Table 1 showed that the structures of these protoberberine alkaloids can be easily elucidated by the CID reactions, and all had $[M-CH_4]^+$ ions as their base peaks except 3 and 7. Compound 3 with no methoxyl group consised M^+ as base peak and compound 7 with a secondary hydroxyl group exhibited $[M-HCHO-CH_3]^+$ as the base peak. As a general rule, compounds with two methoxyl groups at C9 and C10 of the isoquinoline ring (1, 2, 5, 6 and 7), exhibit a fragment ion at *m/z* $[M-HCHO]^+$, and with methylenedioxyl group (3 and 4), however, give a fragment ion at *m/z* $[M-C_2H_4]^+$ when a high octapole voltage (CID) is used.

3.2. Composition of LC buffer

A rapid method for simultaneous separation of the coptis protoberberine alkaloids had been developed in our laboratory [23] by using a mixture of acetonitrile and acetate–SDS–diethylamine buffer solution (0.87 *M* acetic acid, 0.12 *M* sodium acetate, 11.56 m*M* SDS and 16.12 m*M* diethylamine) as eluent. However, this solvent system was not suitable for the LC–MS analysis owing to the presence of sodium ion and high-concentration salts. Eliminating SDS and diethylamine, using ammonium acetate instead of sodium acetate and reducing the concentration of both acetate and acetic acid, a buffer solution consisted of 50 m*M* ammonium acetate and 2% acetic acid was developed. In this condition (solvent system I), all the alkaloids could be well separated except compounds 4 and 5, which overlapped completely.

Optimum separation conditions for all the seven coptis alkaloids were carried out using eluents of different organic content (acetonitrile 30–40%) and SDS concentration (0–10 m*M*) in a linear gradient system. The presence of SDS was found to be necessary. A well-separated chromatogram for the seven protoberberine alkaloids could be achieved only if a 5 m*M* SDS was added. At SDS concentrations lower than 5 m*M*, 4 and 5 were not separated well; at concentrations higher than 5 m*M*, the heated-capillary of the MS system was found to be contaminated seriously. The optimum separation was therefore obtained with a buffer solution of 40% acetonitrile, 50 m*M* ammonium acetate, 2% acetic acid and 5 m*M* SDS in pump A, and a mixture of 10% water, 45% acetonitrile and 45% methanol in pump B (solvent system II).

3.3. Analysis of a synthetic mixture

An on-line LC–UV–ESI-MS method was established by injecting the berberine standard into LC

Table 1
Fragment peaks of the coptis alkaloids (*m/z*)

Compound	CID=0V						CID=−30 V				
	M^+	$[M-HCHO]^+$	M^+	$[M-CH_4]^+$	$[M-HCHO]^+$	$[M-HCHO-H_2]^+$	$[M-CH_4-C_2H_4]^+$	$[M-HCHO-C_2H_4]^+$	$[M-C_2H_4]^+$	$[M-HCHO-CH_3]^+$	$[M-HCHO-CH_3CO]^+$
1	336[a]		336	320[a]	306	304	292	278			
2	352[a]		352	336[a]	322		308	294			
3	320[a]		320[a]						292		
4	336[a]		336	320[a]			292		308		
5	338[a]		338	322[a]	308		294	280			
6	338[a]		338	322[a]	308		294	280			
7	352	322[a]			322					307[a]	279

[a] Base peak.

system, by using solvent systems I and II as eluent and by tuning the sheath and auxiliary gases to an optimal condition. Under such conditions, the m/z value of the mobile phase cluster was kept below 150 and the molecular ion peak for berberine was obviously present at 336. After applying the background-substracted technique and optimizing the CID offset, the ESI mass spectra of berberine obtained were the same as that of the flow injection shown in Fig. 1. The detection limit ($S/N=3$) was about 120 fmol.

A mixture of seven authentic alkaloids was then injected into the HPLC system directly and was found to separated successfully by optimizing the concentrations of ammonium acetate, SDS and organic solvents; the alkaloid structures were identified by comparing the retention time, M^+ and CID fragments with those obtained from the individual authentic alkaloids.

As solvent system I was used, a well-separated chromatogram for the synthetic mixture was obtained except for 4 and 5. Compounds 4 and 5 completely overlapped either in the total ion current chromatogram or in the UV chromatogram, and had to be resolved by using the selected-ion monitoring (SIM) method with the selection of m/z 336 and 338 from the total ion current chromatogram. When solvent system II was applied, all seven compounds could be separated completely and the ESI mass spectra were found to be as good as those of solvent system I.

According to the results obtained, both solvent system I (isocratic phase system) using SIM technique and solvent system II (gradient phase system) using cleansing of heated-capillary frequently (once every 2 h) can be used in the analysis of the synthetic mixture.

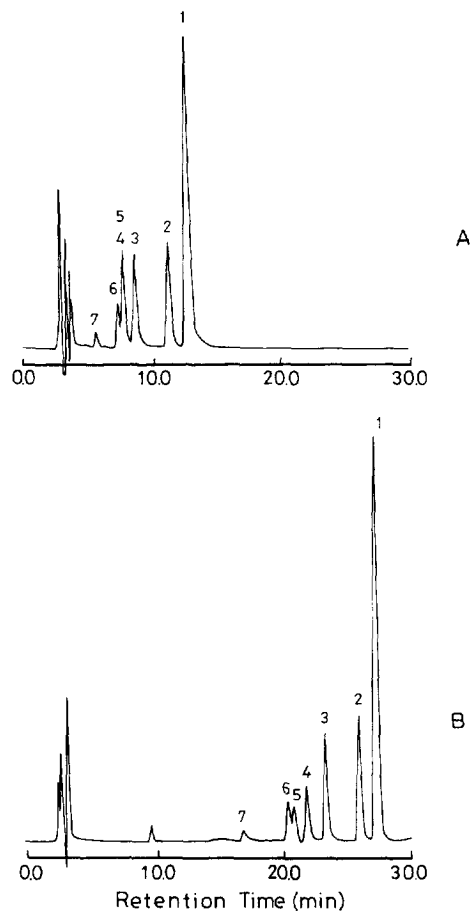

Fig. 4. HPLC chromatograms of Coptidis Rhizoma extract with UV detection at 270 nm using (A) solvent system I, (B) solvent system II as eluent. 1, berberine; 2, palmatine; 3, coptisine; 4, epiberberine; 5, jatrorrhizine; 6, columbamine; 7, berberastine.

3.4. Separation and identification of the alkaloids in Coptidis Rhizoma extracts

Since reproducible and structurally informative LC–MS full-scan background-substracted CID mass spectra were obtained in this work, the components present in the extracts of Coptidis Rhizoma could be identified. The same experimental conditions as described in Section 3.3 for the synthetic mixture were used. Fig. 4A shows the UV chromatogram whereas Fig. 5 shows the corresponding total ion current chromatogram for Coptidis Rhizoma using solvent system I as eluent. The identification of each peak was based on a comparison of the retention time and the background-substracted CID mass spectrum from each component in the extracts with that of standard under the same CID LC–MS conditions. Compounds 4 and 5 were also resolved and identified by SIM method. Fig. 4B and Fig. 6 show the UV and total ion current chromatograms of Coptidis Rhizoma with the use of solvent system II as eluent.

As a result, solvent systems I and II were both suitable for the separation and identification of these seven protoberberine alkaloids in a crude extract of Coptidis Rhizoma. Nevertheless, when quantitation

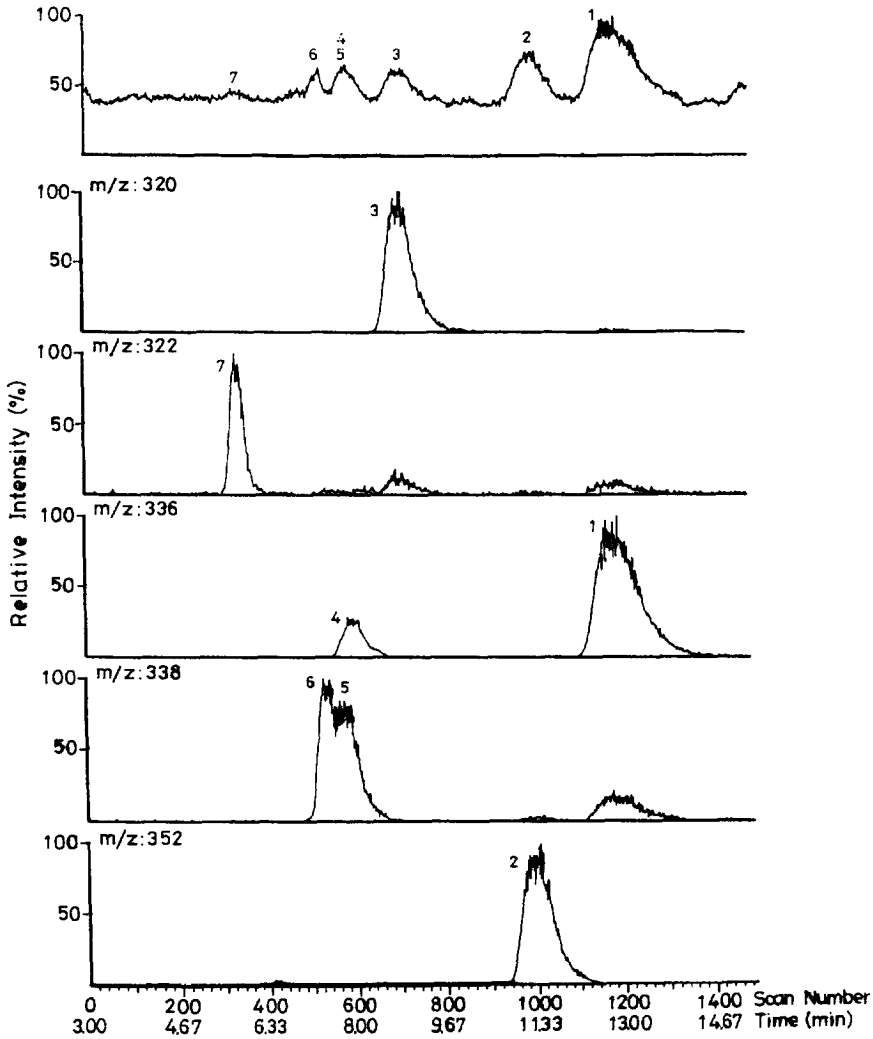

Fig. 5. Total ion current chromatogram of Coptidis Rhizoma extract using SIM and the solvent system I.

is required the current methodology is insufficient, owing to the overlapping peaks of compounds 5 and 6.

4. Conclusion

Combined LC–UV–ESI-MS allows a more efficient separation than HPLC alone. ESI-MS enables one to determine not only the molecular mass of protoberberine alkaloids, but also the presence of various functional groups according to observed losses from the M^+ ion during CID by adjusting the MS parameter. The reported results also demonstrate the feasability of adding SDS to the mobile phase, the suitability of using a conventional LC column in the experiment, and the utility of this coupled technique for the analysis of *Coptis* crude extracts using either isocratic or gradient elution.

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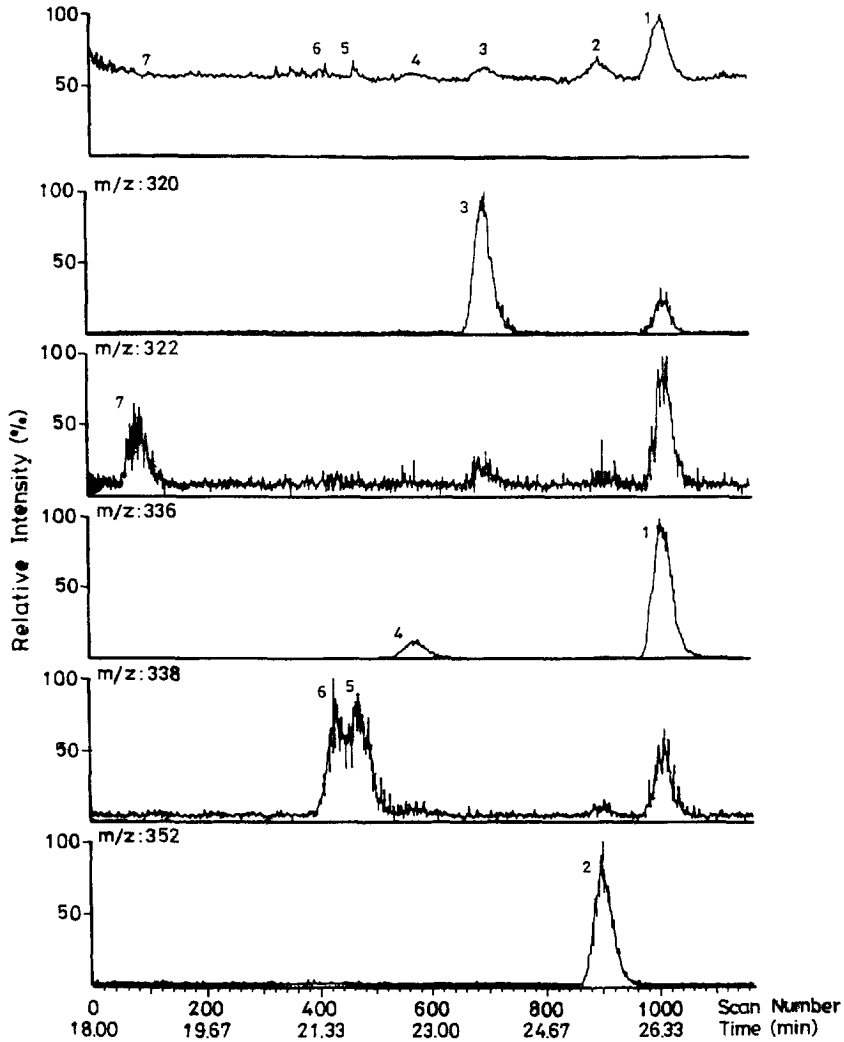

Fig. 6. Total ion current chromatogram of Coptidis Rhizoma extract using SIM and the solvent system II.

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