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Short communication

Liquid chromatography–electrospray mass spectrometric analysis of curcuminoids and sesquiterpenoids in turmeric (*Curcuma longa*)

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Abstract

On-line high-performance liquid chromatography–UV diode-array and electrospray mass spectrometry have been used simultaneously to analyze curcuminoids and sesquiterpenoids in a fresh turmeric (*Curcuma longa*) extract. Five major components: curcumin (**1**), demethoxycurcumin (**2**), bisdemethoxycurcumin (**3**), ar-turmerone (**5**) and curlone (**6**) have been unambiguously identified, based on their UV spectra, mass spectra and retention times in comparison with the data of standard compounds. This method provides a reliable fingerprint for turmeric, distinguishing it from other Curcuma species.

Keywords: *Curcuma longa*; Turmeric; Curcuminoids; Sesquiterpenoids

1. Introduction

Turmeric (from the rhizome of *Curcuma longa* L., family *Zingiberaceae*) is a main ingredient of curry powders. It is well-known for its coloring, flavoring and digestive properties.

Traditional doctors in India and China have long used turmeric as a remedy for hepatitis, sepsis, dyspepsia and a number of other disorders. Recent studies have not only confirmed many of these older applications but have found several new and useful properties as well, such as anticancer and antiviral. Turmeric has also been found to increase bile output and to scavenge free radicals [1–4].

Three major yellow pigments (3–5% of raw plant) have been isolated from turmeric [5]. These diarylheptane derivatives are curcumin (**1**), demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**). An asymmetrical derivative, dihydrocurcumin (**4**), was also isolated. Generally called curcuminoids, these compounds give turmeric its distinctive color.

The major constituents of turmeric's volatile oil (2–7% of raw plant) are bisabolane sesquiterpene [6,7]. They are ar-turmerone (**5**), curlone (**6**), α-turmerone (**7**), β-turmerone (**8**) and bisacumol (**9**). Other sesquiterpenes include zingiberene (**10**), curcumenone (**11**), curcumenol (**12**), procurcumenol (**13**), dehydrocurdione (**14**) and germacrone-13-al (**15**). The chemical structures of these compounds are shown in Fig. 1.

Curcuminoids and essential oils are the major active constituents of turmeric. The analysis of curcuminoids in turmeric is crucial to determine the quality of plant material or its processed products.

Two HPLC methods have been reported which analyze the individual curcuminoids [8,9]. The sepa-

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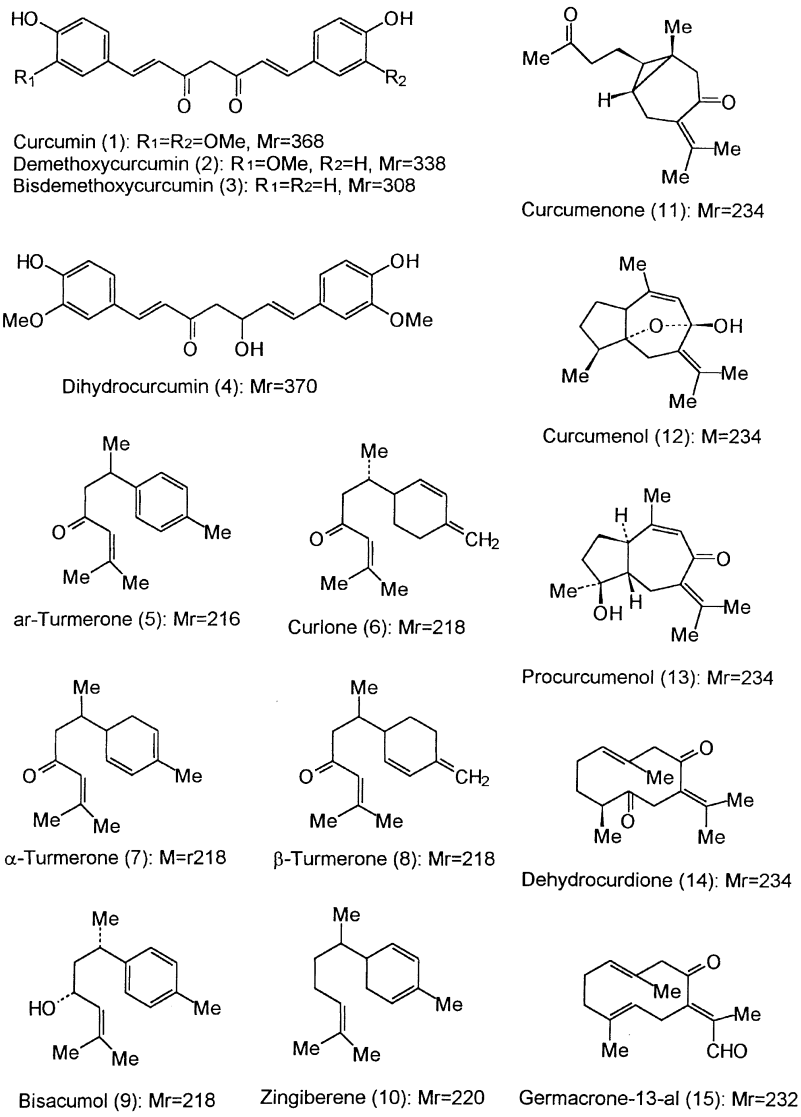

Fig. 1. Chemical structures of curcuminoids and sesquiterpenes found in *Curcuma longa*.

rations of these compounds were not satisfactory because of tailing peaks and poor resolution. A recent paper has reported the baseline separation of three curcuminoids, using a non-silica polymeric column [10]. However, this study did not identify any of the minor peaks and the volatile oil constituents in the chromatogram.

A combination of high-performance liquid chromatography (HPLC), photodiode-array UV–Vis and mass spectrometry (MS), using an electrospray (ES) interface, has proven to be a useful on-line system for establishing the identity of components in botanical extracts [11–13]. The purpose of this research was to use HPLC–UV–ES-MS for the identification of turmeric constituents. Not only individual curcuminoids but also a number of major essential oil constituents could be identified from a single HPLC run.

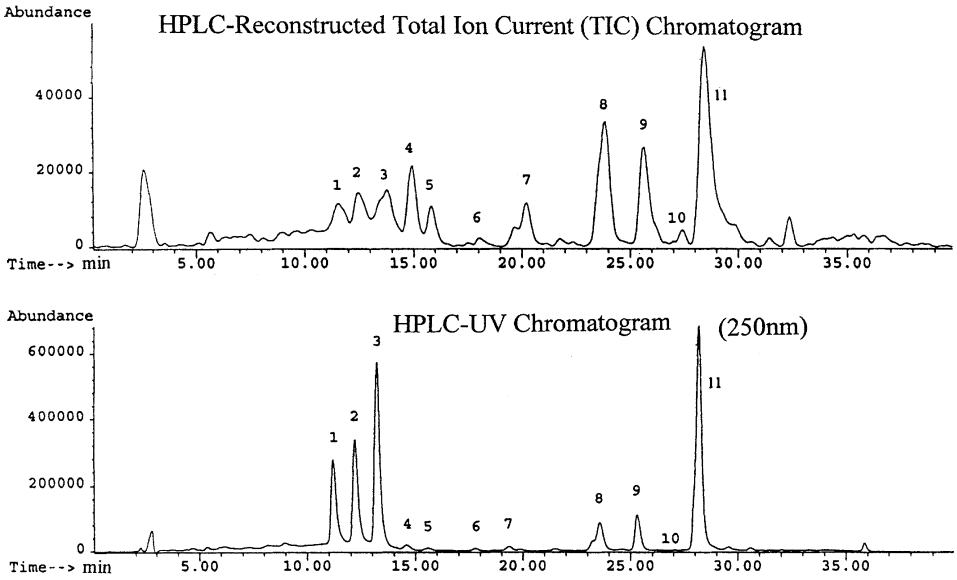

Fig. 2. Simultaneous HPLC-reconstructed total ion current (TIC) chromatogram (top panel) and HPLC–UV (250 nm) chromatogram (bottom panel) of fresh turmeric extract, without postcolumn stream splitting. Chromatographic conditions are as described in Section 2.1. Peak identifications are shown in Table 2.

2. Experimental

2.1. Instrumentation

HPLC separation was performed using a 1090 series II HPLC (Hewlett-Packard, Palo Alto, CA, USA) with a photodiode-array detector set at 425 nm (for signal A) and 250 nm (for signal B). UV spectra were taken in the region of 200–500 nm. A Waters Symmetry C_{18} column (5 μm, 15 cm×2.1 mm) with a Sentry guard column (Symmetry, C_{18}, 5 μm, 20×3.9 mm) (Waters, Milford, MA, USA) were used. The column temperature was set at 48°C. The profile of the gradient elution was: (A) water (0.25% HOAc) and (B) acetonitrile, 0–17 min, 40–60% B; 17–32 min, 60–100% B; 32–38 min, 100% B; 38–40 min, 100–40% B at a flow-rate of 0.2 ml/min.

A HP 5989 B quadrupole mass spectrometer was coupled with the HPLC instrument, using a HP 59987A electrospray interface. The mass range was measured at 200–800 u, and the quadrupole temperature was 150°C; electron multiplier voltage, 2173 V. The spectra were acquired in the positive detection mode. The drying N_2 temperature was 350°C at a flow-rate of 40 ml/min. The nebulizing N_2 pressure was 5.5×10^5 Pa (80 p.s.i.).

Table 1
t_R, UV and MS data of standard compounds

Compound name	t_R (min)	[M+H] m/z	$[M+Na]^+$ m/z	$[2M+H]^+$ m/z	UV λ_{max} (nm)
Curcumin (**1**)	13.3	369	391	759	260 425
ar-Turmelone (**5**)	25.3	217	239	–	238
Curlone (**6**)	28.1	219	241	–	238

2.2. Plant material and sample preparation

Fresh turmeric was purchased from Ginger Ridger Farm (HI, USA) and ground in a mill. One gram was 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). The injection volume was 5 μl.

Curcumin (2.77 mg) was dissolved in 10 ml of methanol in a volumetric flask. The injection volume for the standard solution was 10 μl.

2.3. Isolation of ar-turmerone (*5*) and curlone (*6*)

Crude drug turmeric was purchased from Asia Natural Products (San Francisco, CA, USA). The powdered plant material (214 g) was extracted with acetone (1 l) by percolation. The extract solution was concentrated to dryness in a rotary evaporator. The extract (21 g) was partitioned between aqueous methanol (MeOH–water, 9:1, v/v) and hexane. The hexane portion (9 g) was separated by column chromatography (300 g of silica gel 60, 230 mesh) and eluted by hexane–acetone (97:3, v/v). Fractions of 50 ml were collected and checked by thin-layer chromatography (TLC) analysis (hexane–acetone, 97:3, v/v; silica gel 60, F_{254} plate). Fractions 26–28 offered a viscous oil, curlone (**6**), and fractions 37–40 offered a viscous oil, ar-turmelone (**5**). Their identities were confirmed by ^{1}H-NMR analysis in comparison with literature data [14,15]

2.4. Solvents and chemicals

Methanol, acetonitrile and water were of HPLC grade (VWR, Seattle, WA, USA). Acetic acid, curcumin and hexane were of reagent grade (Sigma, St. Louis, MO, USA). Merck Silica gel 60 and TLC plates were purchased from VWR.

3. Results and discussion

The commercially available standard compound, curcumin (**1**), showed three peaks. The peak area of curcumin was 80% of the total peak area. The other two peaks were identified as compounds **2** and **3**.

Both the HPLC-reconstructed total ion current (TIC) chromatogram and the HPLC–UV (250 nm) chromatogram of a fresh turmeric extract are shown in Fig. 2. Peaks 1, 2 and 3 showed similar UV spectra, which are characteristic of curcuminoids with λ_{max} at 425 nm. They were identified as bisdemethoxycurcumin (**3**), demethoxycurcumin (**2**) and curcumin (**1**), respectively, based on their mass spectra, UV spectra and retention times in comparison with the data of the standard compounds, listed in Table 1.

Compounds **1**, **2** and **3** all showed very intense protonated molecules $[M+H]^+$ at m/z 369, 339 and

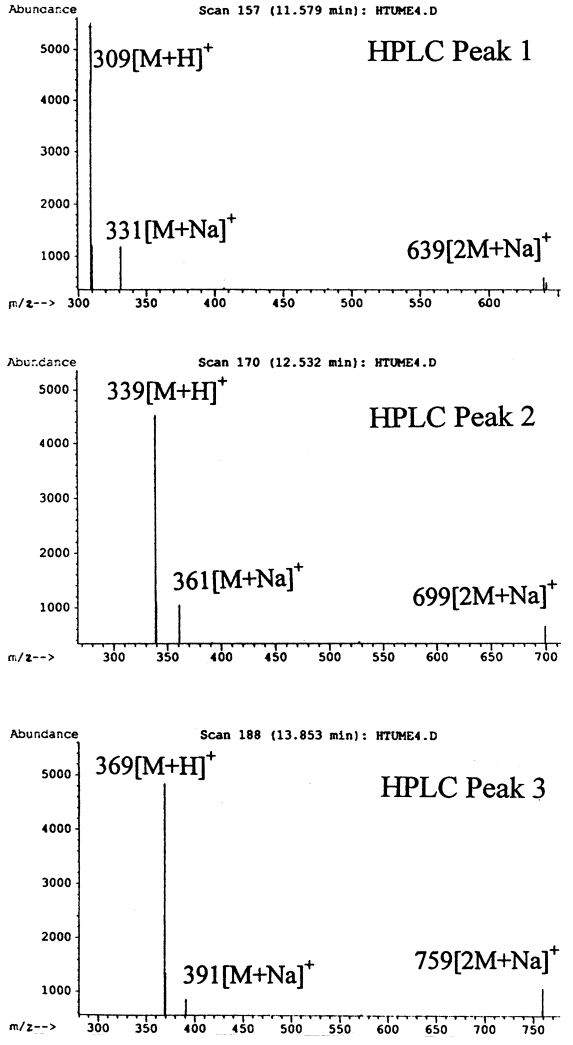

Fig. 3. Mass spectra of HPLC peak 1 (top panel), peak 2 (middle panel) and peak 3 (bottom panel).

Table 2
t_R, UV and MS data and peak assignments for the analysis of a fresh turmeric extract

Peak number	t_R (min)	$[M+H]^+$ m/z	$[M+Na]^+$ m/z	$[2M+H]^+$ m/z	Other ions m/z	UV λ_{max} (nm)	Identification (compound number)
1	11.2	309	331	639	–	250 425	**3**
2	12.2	339	361	699	–	250 425	**2**
3	13.3	369	391	759	–	260 425	**1**
4	14.6	235	257	491	–	n.d.	**11**, **12**, **13** or **14**
5	15.5	259	273	–	523	n.d.	n.i.
6	17.8	233	255	–	–	263	**15**[a]
7	19.4	257	–	–	–	n.d.	n.i.
8	23.6	235	257	–	331 371	238	**11**, **12**, **13** or **14**
9	25.3	217	239	–	–	238	**5**
10	27.1	221	243	–	–	n.d.	**10**[a]
11	28.1	219	241	–	–	238	**6**

n.d., not detectable.
n.i., not identified.
[a] Tentatively identified.

309, less intense adducts ions $[M+Na]^+$ at m/z 391, 361 and 331, and sodiated dimer ions $[2M+Na]^+$ at m/z 759, 699 and 639, respectively. Their mass spectra are shown in Fig. 3.

Peak 9 showed a UV spectrum with λ_{max} at 238 nm, an intense protonated molecule $[M+H]^+$ at m/z 217 and an adduct ion $[M+Na]^+$ at m/z 239. These data correspond to compound ar-turmerone (**5**). Peak 11 showed a UV spectrum with λ_{max} at 238 nm, an intense protonated molecule $[M+H]^+$ at m/z 219 and an adduct ion $[M+Na]^+$ at m/z 241. Compounds **6**, **7** or **8** could be assigned to this peak.

The identities of peaks 9 and 11 as ar-turmerone (**5**) and curlone (**6**) were confirmed by comparing their UV and MS data to the compounds previously isolated and identified by ^{1}H-NMR (see Table 1).

Peaks 4 and 8 were relatively small in the HPLC–UV chromatogram but were large in the HPLC–ES–MS chromatogram. Both exhibited an intense protonated molecule $[M+H]^+$ at m/z 235 and an adduct ion $[M+Na]^+$ at m/z 257. These two peaks can be attributed to the compounds **11**, **12**, **13** or **14** which have the same molecular mass of 234.

Peaks 6 and 10 were tentatively identified as germacrone-13-al (**15**) and zingiberene (**10**), respectively, based on their mass data. Peak assignments, UV and MS data for analysis of a fresh turmeric extract are shown in Table 2.

In addition to turmeric (Jiang Huang), other curcuma plants are officially listed in the Chinese Pharmacopeia: *C. aromatica* (Curcuma tuben, Yu Jin) and *C. zedoaria* or *C. kwangsiensis* (Zedoary, E Zhu). These species also contain curcuminoids. However, only turmeric contains significant amounts of bisabolane-type sesquiterpenes. To distinguish these species by the macroscopic characteristics of their rhizomes alone is problematic [16]. The simultaneous analysis of curcuminoids and essential oils provides a chromatographic profile that is more effective for species distinction.

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