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Information theory applied to chromatographic fingerprint of herbal medicine for quality control

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Abstract

At present, the construction of chromatographic fingerprints plays an important role in the quality control of complex herbal medicines. In this work, information theory was applied to obtain chromatographic fingerprints with good performance. Moreover, according to the characteristics of the chromatographic fingerprints obtained, some modifications of the calculation of the information content were conducted. In comparison with the information content from several chromatographic fingerprints obtained, reliable chromatographic fingerprints with a high separation degree and uniform concentration distribution of chemical components could be determined. The successful application of information theory with modification to simulated chromatographic fingerprints together with real herbal medicines such as *Rhizoma chuanxiong* and *Ginkgo biloba* from different sources demonstrated clearly that the proposed method to determine chromatographic fingerprints was reasonable and reliable and it was user-friendly. Chromatographic fingerprints determined with high separation degrees and uniform concentration distribution of chemical ingredients might also chemically represent characteristic components of herbal medicines for quality control.

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Keywords: Pharmaceutical analysis; Information theory; *Rhizoma chuanxiong*; *Ginkgo biloba*

1. Introduction

Herbal medicine (HM) has a long therapeutic history over thousands of years and is currently still serving many of the health needs of a large population of the world. However, as pointed in Ref. [1]

Despite its existence and continued use over many centuries, and its popularity and extensive use during the last decade, traditional medicine has not been officially recognized in most countries. Consequently, education, training and research in this area have not been accorded due attention and support. The quantity and quality of the safety and efficacy data on traditional medicine are far from sufficient to meet the criteria needed to support its use worldwide. The reasons for the lack of research data are due to not only to health care policies, but also to a lack of adequate or accepted research methodology for evaluating traditional medicine.

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currently existing approaches for quality assessment can not fulfill the practical requirements of the safety and efficacy of HMs. One of these reasons might be that, unlike a chemically synthetic drug of high purity, a HM and/or a HM formula may consist of hundreds of complex phytochemicals. As a result, it becomes very difficult or impossible in most cases to identify most of these components by means of common approaches [2–4]. In general, only a few marker or pharmacologically active components are employed for evaluating the quality and authenticity of a HM, identifying the presence of a HM in a HM preparation, and finding out the quantitative herbal composition of a HM product. Yet, it is a common consent that this approach is barely satisfactory for quality control of a HM and HM products with multiple chemical components [4]. Many other problems specific to the quality assessment of HM have been also investigated in Ref. [4].

In 2004, the Chinese State Drug Administration (SDA) will regulate the composition of liquid injection with HM ingredients for assuring stringent quality by chemical assay and standardization. Fingerprints of HMs and HM liquid injections are recommended for this purpose. In addition, among the various experimental techniques, chromatographic methods are highly recommended for determining the fingerprints of these products [5–11].

According to the definition of chromatographic fingerprints of a HM, a chromatographic fingerprint is in practice a chromatographic pattern of some common kinds of pharmacologically active and chemically characteristic components in the HM under study [5,6,10]. This chromatographic profile should feature the fundamental attributions of ‘‘integrity’’ and ‘‘fuzziness’’ or ‘‘sameness’’ and ‘‘differences’’ so as to chemically represent the HM investigated [10,12]. It suggests that with the help of the chromatographic fingerprints obtained, the authentication and identification of a HM can be accurately conducted (‘‘integrity’’) even if the number and/or concentration of chemically characteristic constituents are not very similar in different samples of this HM (‘‘fuzziness’’) or, chromatographic fingerprints could successfully demonstrate both the ‘‘sameness’’ and ‘‘differences’’ between various samples [10,13]. Thus, we should globally (considering multiple constituents) not locally (considering only few marker components) evaluate the quality of HMs. However, as for a HM, there are always hundreds of components and many of them are in too low amounts. On the other hand, there usually exists variability within the different and even the same herbal materials [4,14]. As a result, to obtain reliable chromatographic fingerprints chemically representing pharmacologically active and characteristic components is not a trivial work. The performance of a chromatographic fingerprint obtained is closely dependent on the chromatographic separation degrees and concentration distribution of all chemical components in the HM investigated.

It is well known that chromatography has a very powerful separation ability, suggesting the separation of complex systems like HMs into many relatively simple sub-systems. Furthermore, hyphenated chromatographic and spectrometric approaches such as high-performance liquid chormatography–diode array detection (HPLC–DAD), gas chromatography–mass spectrometry (GC–MS), capillary electrophoresis (CE)–DAD and HPLC–MS, could show greatly improved performances in terms of the elimination of instrumental interference, retention time shift correction, selectivity, chromatographic separation abilities and measurement precision [15–17]. If hyphenated chromatography is further combined with chemometric approaches, clear pictures might be developed for the chromatographic fingerprints obtained. These excellent properties are the so-called dimension advantages proposed by Booksh and Kowalski [18]. Hyphenated chromatography will become the primary tool for obtaining chromatographic fingerprints of HMs for quality control [12–14,19–36].

In this study, the construction of chromatographic fingerprints of *Rhizoma chuanxiong* [37–41] and *Ginkgo biloba* [16,17,42–45] was investigated as a method for their quality control. In order to select chromatographic fingerprints with a high separation degree and uniform concentration distribution of chemical components, information theory was applied to evaluate the performance of the obtained chromatographic fingerprints. In addition, according to the features, ‘‘integrity’’ and ‘‘fuzziness’’ or ‘‘sameness’’ and ‘‘differences’’ of chromatographic fingerprints obtained for quality control of HMs, some modification of the calculation of the infor-

mation content was carried out. On the other hand, in order to determine whether the chromatographic fingerprints selected can chemically present pharmacologically active and marker components from *R. chuanxiong* [37–41] and *G. biloba*, some chemometric approaches and GC–MS analysis are employed. Chromatographic fingerprints simulated also demonstrate the reliability and applicability of information theory with some modification in this work.

2. Theory

From the above, the performance of a chromatographic fingerprint is closely dependent on its separation degree and concentration distribution of each chemical component. This section will employ the information theory to evaluate the performance of a chromatographic fingerprint obtained. The basic principles on information theory have been described in detail in Refs. [46–51]. Hence, only a brief introduction and some reasonable improvements on the calculation of the information content from the chromatographic fingerprints obtained are given here.

According to Refs. [46–50], the information content of a chromatogram with lots of peaks might be calculated by means of various approaches. As for these methods, only the signal intensity, retention time, peak area and/or peak height of each independent peak without overlapping, not the whole chromatographic curve, are taken into consideration [46–50]. So, these methods might be termed local, not global, approaches. On the other hand, in order to calculate the information content of a chromatogram correctly, it is absolutely necessary for these approaches to identify the peaks without overlapping and estimate the noise and/or error level of a chromatographic fingerprint investigated [46–50]. However, the determination of chromatographic peaks along with the estimation of noise and/or error is not a trivial task in practice. Furthermore, if a chromatographic peak is overlapped with its adjacent peak(s), the calculation of the information content will become complex. Vertical splitting is conventionally used for this situation and both peaks on two sides of an overlapping peak cluster are taken as pure ones. This approximate treatment on a chromatogram with some overlapping peak clusters will cause some errors in the calculation of information content.

As we know, a chromatographic fingerprint, which is in fact a concentration distribution curve of several chromatographic peaks, might be regarded as a continuous signal determined by its chromatographic shape. According to Ref. [51], the information content of a continuous signal might be simply expressed as the following:

$$\Phi = -\int p_x \log p_x \mathrm{d}x \qquad (1)$$

where p_x is the probability or concentration of chemical components distribution function. In theory, if and only if p_x with unchangeable variance is characterized by normal distribution can its information content Φ reach its maximum [51].

Under an ideal situation, all the chromatographic peaks from a chromatogram can be separated completely and each peak confined to a narrow zone might correspond to a normal distribution profile [52]. A chromatographic fingerprint with all of peaks just completely separated should be featured by maximal information content. Further separation cannot provide any more information and is unnecessary. On the contrary, if any of chromatographic peaks is overlapped with its adjacent one(s), this peak will surely show non-Gaussian normal distribution and therefore undoubtedly cause a loss of the information content. Thus, the chromatographic shape is closely dependent on the separation degrees of chemical components. On the other hand, it is known that the concentration distribution of each chemical component can affect the chromatographic shape.

In this study, the construction of chromatographic fingerprints aims at evaluating the quality of HMs. This evaluation is based on the similarities and/or differences of the chromatographic shapes, or the separation degree and concentration distribution of each chemical component from chromatographic fingerprints obtained between various HMs investigated. As a result, both the separation degrees and concentration distributions of components involved in a chromatographic fingerprint should be taken into consideration for this evaluation. To achieve this

goal, some reasonable modification on the calculation of the information content (Φ) based on Eq. (1) is employed here. A chromatographic fingerprint is first normalized with its overall peak area equal to one and then its information content is calculated based on Eq. (2):

$$\Phi = -\int p_x/[\mathrm{sum}(p_x)]\log p_x/[\mathrm{sum}(p_x)]\mathrm{d}x \qquad (2)$$

where p_x is the real chromatographic response of all chemical components involved in the chromatographic fingerprint under study and sum(p_x) is the sum of p_x. In this paper, Φ obtained on the basis of Eq. (2) is taken as the real information content from a chromatographic fingerprint investigated.

From the above, there might be, at least, two advantages of the calculation of the information content based on Eq. (2) over the other approaches in Refs. [46–50]. As for Eq. (2), the whole chromatogram (global) not the retention time, peak intensity, peak width, peak area and/or peak height from each peak identified (local) are taken into consideration. Moreover, it is unnecessary for Eq. (2) to identify the chromatographic peaks at first and the noise might have a small influence on the calculation of the information content.

Table 1
Information content of simulated chromatographic fingerprints with different separation degrees in Fig. 1

	Data 1	Data 2	Data 3	Data 4
R_s	1.50	0.63	0.31	2.00
Φ	6.04	5.50	4.83	6.04

Fig. 1 shows four simulated chromatographic fingerprints with different separation degrees (data 1, data 2, data 3 and data 4). Here, the concentration distributions of the four peaks are the same. The values of the chromatographic resolution (R_s) are displayed in Table 1. Fig. 1a with R_s equal to 1.50 is a chromatogram just separated completely. In Fig. 1b and c, the peaks are seriously overlapped with each

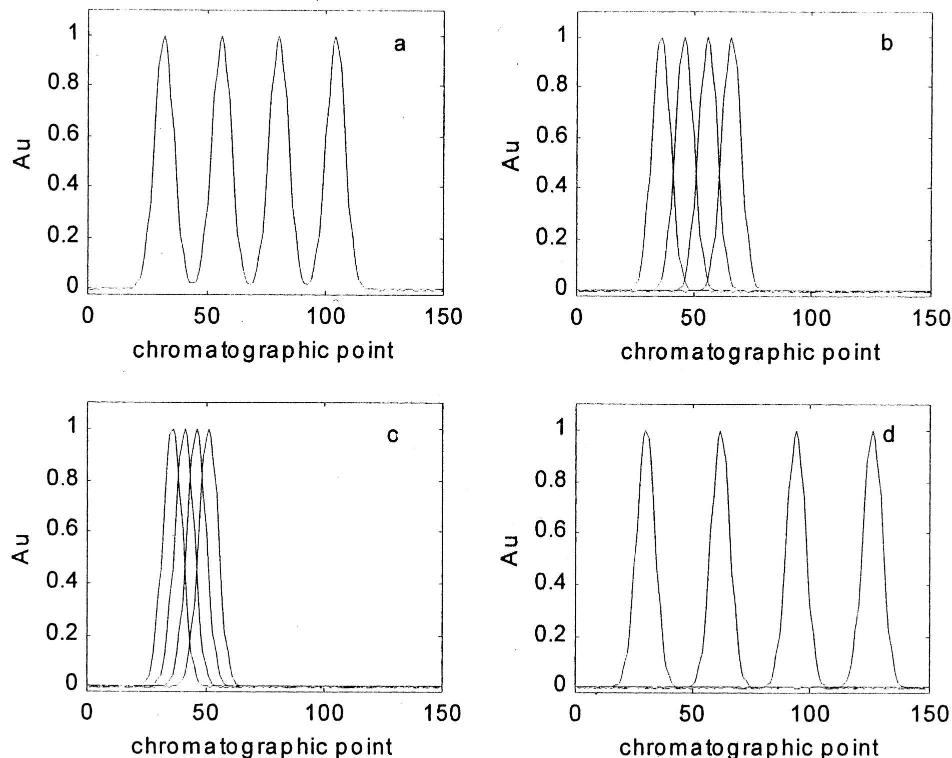


Fig. 1. Chromatographic fingerprints simulated with different separation degrees.

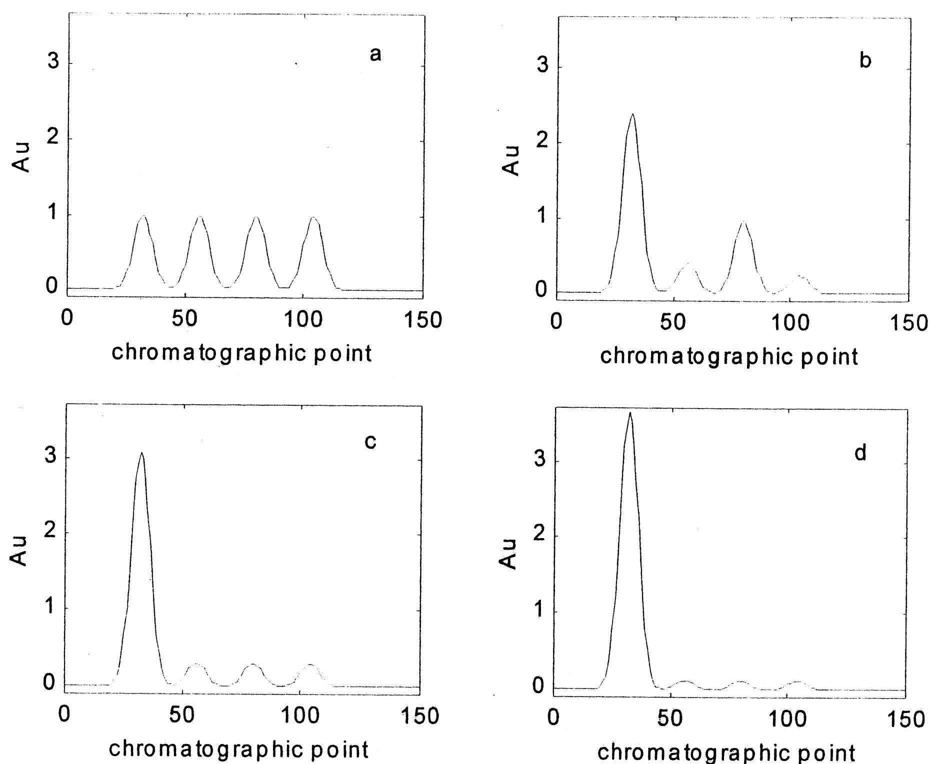


Fig. 2. Chromatographic fingerprints simulated with different concentration distributions.

other as R_s values are 0.63 and 0.31, respectively. The peaks are further separated as R_s is 2.00 in Fig. 1d. Table 1 also displays the values of Φ from these above-simulated chromatographic fingerprints. As seen from Table 1, Φ in both Fig. 1a and d reach the maximal values. It suggests that the further chromatographic separation from Fig. 1a–d (R_s from 1.50 to 2.00), which can not cause any addition to the information content Φ, is unnecessary. However, the serious overlapping situation in Fig. 1b and c (R_s = 0.63, 0.31) surely causes a loss of the information content.

Fig. 2 represents four simulated chromatographic fingerprints with different concentration distributions of each chemical component (fingerprint 1, fingerprint 2, fingerprint 3 and fingerprint 4). Here, the separation degrees of the four peaks are the same. In fact, the chromatographic fingerprint in Fig. 2a is the same as in Fig. 1a. The relative percent of the areas of the first, second, third and fourth peaks to the overall peak area is displayed in Table 2. Fig. 2a, which is a chromatographic fingerprint with a uniform concentration distribution of each peak, has the maximal information content (6.04). In Fig. 2b–d,

Table 2
Information content of simulated chromatographic fingerprints with different concentration distribution in Fig. 2

	First (%)*	Second (%)*	Third (%)*	Fourth (%)*	Φ
Fingerprint 1	25.00	25.00	25.00	25.00	6.04
Fingerprint 2	60.00	10.00	24.00	6.00	5.56
Fingerprint 3	76.93	7.69	7.69	7.69	5.18
Fingerprint 4	91.00	3.00	3.00	3.00	4.62

Note: * relative percent of the areas of the first, second, third and fourth peaks to the overall peak area.

the relative percent of the first peak becomes high, suggesting the non-uniformity of the concentration distribution of components. The information content values are 5.56, 5.18 and 4.62, respectively.

3. Experimental

3.1. Simulated chromatographic fingerprints

For the simulated data, all chromatographic fingerprints contain several peaks with different separation degrees and concentration distribution of peaks. Each chromatographic peak is constructed with one Gauss peak.

3.2. Materials

R. chuanxiong and *G. biloba* samples were purchased from several pharmaceutical stores, companies and collected from different producing areas in the mainland and in Hong Kong. All of these samples were identified by one of authors (advanced pharmaceutist) from Guangzhou Institute of Drug Control, and an advanced analytical researcher in the Hong Kong Governmental Laboratory.

CH_3OH, H_3PO_4, K_2HPO_4, CH_3CN, isopropanol (C_3H_8O) and citric acid ($C_6H_8O_7$) were of analytical grade. Double distilled water was used.

3.3. Instruments

An RT-80 pulverizer made in Taiwan, a 5810 centrifuge from Eppendorf (Germany), an Ultra Turrax T25 basic stirrer from IKA (Malaysia), a CQ250 ultrasonic cleaner made in Shanghai, a Hewlett-Packard HP-1100 HPLC system coupled with a G1315A diode array detector and a Hewlett-Packard 5890 Series II GC system coupled with a 5972 Series mass-selective detector were employed in this study.

3.4. Extraction

3.4.1. Extraction of raw materials of R. chuanxiong

All of the raw materials of *R. chuanxiong* were dried for about 60 min at 30 °C at first. Then, about 0.5 g of dried and pre-pulverized herbal materials were extracted using an Ultra Turrax T25 basic stirrer (11 000 rpm) with 30 ml of CH_3OH for 2 min. After centrifugation for about 20 min, the upper solution was filtered through a glass filter covered with a filter paper. Next, the solution was evaporated under reduced pressure to about 1 ml and then diluted with methanol to 5 ml in a volumetric flask. A 1-ml volume of this solution was then filtered through a Millipore filtration unit type HV, 0.45 μm. Volumes of 20 μl and 2.5 μl of this solution were injected into the HPLC and GC–MS systems, respectively.

3.4.2. Extraction of extracts and products of G. biloba

Pre-weighed extracts of *G. biloba* were dissolved with 5 ml methanol. A 1-ml volume of this solution was filtered through a Millipore filtration unit type HV 0.45 μm. A 10-μl volume of this solution was injected into the HPLC system.

Pre-weighed *G. biloba* products were extracted using the CQ250 ultrasonic cleaner with 20 ml methanol for 15 min. The extract was left to stand for a moment at room temperature. The solution was then filtered through a glass filter covered with a filter paper. Next, the solution was evaporated under vacuum to about 1 ml and then diluted with methanol to 5 ml in a volumetric flask. A 1-ml volume of this solution was then filtered through a Millipore filtration unit type HV 0.45 μm. A 10-μl volume of this solution was injected into the HPLC system.

3.5. Chromatographic procedure

3.5.1. Determination of raw materials of R. chuanxiong with HPLC–DAD

The column was a LiChrosorb RP18 (Hewlett-Packard, 200×4.6 mm I.D.) with the following mobile phases: (i) at the starting time, the mobile phase was composed of A (CH_3OH)–B (water–K_2HPO_4–H_3PO_4, pH 3) (40:60, v/v). Then, this mobile phase was changed to A–B (80:20, v/v) after 50 min; (ii) A and B were mixed at 10:90 (v/v) at first and then linearly changed to A–B (100:0, v/v) after 60 min. The flow rates were (i) 0.7 ml/min; and (ii) 1.0 ml/min. The column temperature was 25 °C. The wavelength scanning range was 190–400 nm at 2 nm/step.

3.5.2. Determination of raw materials of R. chuanxiong with GC–MS

The column was a HP-5MS column (crosslinked 5% PH MS siloxane, 30 m×0.25 mm I.D., 0.25 μm film thickness); the column temperature was maintained at 80 °C for 2 min at first, and then programmed from 80 °C to 230 °C at 5 °C/min; the inlet temperature was 230 °C; the carrier gas helium at a flow-rate of 1.0 ml/min; the positive ion electron impact (EI^+) mode was used at an electron energy of 70 eV; the scan range was 30–400 u at 2.025 scan/s and the ionization source temperature was 280 °C.

3.5.3. Determination of extracts and products of G. biloba with HPLC–DAD

The column was a Spherisorb ODS2 C_{18} (250×4 mm I.D.) with the following mobile phase: at the beginning, the mobile phase consisted of water–CH_3CN–isopropanol–citric acid (1000:200:30:4.92, w/w). Then, it was linearly changed to water–CH_3CN–isopropanol–citric acid (1000:470:50:6.08, w/w) after 25 min. The flow-rate was 1.0 ml/min. The column temperature was 25 °C and the wavelength scanning range was 200–400 nm at 1 nm/step.

3.6. Data analysis

Data analyses were performed on a Pentium-based IBM-compatible personal computer. All programs were coded in MATLAB 5.1 for windows.

4. Results and discussion

4.1. Determination of the chromatographic fingerprint simulated with the most information content

In order to further interpret the reliability and applicability of the calculation of information content with modification proposed in this paper, we simulated six other chromatographic fingerprints with much more complexity. Fig. 3 expresses these

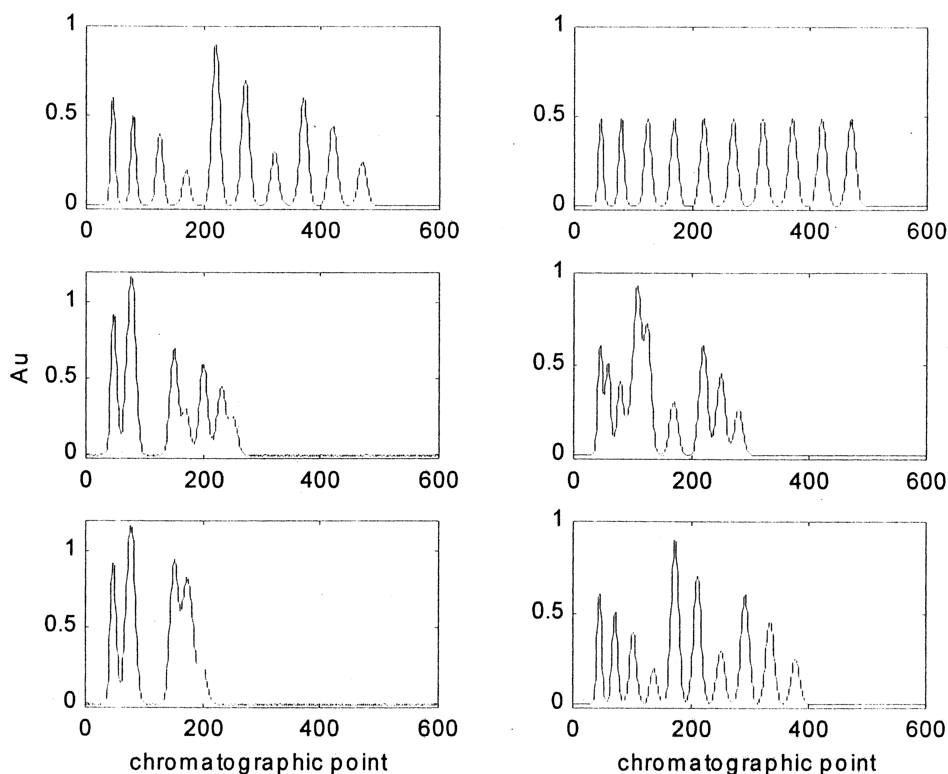


Fig. 3. Chromatographic fingerprints simulated with different separation degrees and concentration distributions.

Table 3
Information content and number of peaks identified for simulated chromatographic fingerprints with different separation degrees and concentration distribution in Fig. 3

	Simu 1	Simu 2	Simu 3	Simu 4	Simu 5	Simu 6
Φ	7.82	7.95	7.25	7.53	6.85	7.82
No. peaks	10	10	7	9	5	10

chromatographic fingerprints simulated (simu 1, simu 2, simu 3, simu 4, simu 5 and simu 6) containing 10 peaks with different separation degrees and concentration distribution. Among these simulated data, all 10 peaks in Fig. 3a, b and f exhibit themselves as normal distribution curves on account of their complete separation. In addition, the concentration distribution of chromatographic peaks in Fig. 3b is uniform. However, the chromatographic peaks in Fig. 3c–e are slightly or even seriously overlapped and non-uniformly distributed. Thus, all their concentration curves of chemical components must deviate from normal distribution profiles with non-uniform distribution. Visually detected from Fig. 3a to Fig. 3f, their performances might be: Fig. 3b>Fig. 3a>Fig. 3f>Fig. 3d>Fig. 3c>Fig. 3e.

Table 3 shows their information content and the number of the peaks identified from these simulted chromatographic fingerprints. As seen from Table 3, the information content in Fig. 3b reaches the maximal value. Φ in both Fig. 3a and Fig. 3f are equal to each other. It suggests that the performance of Fig. 3f should be, in practice, the same as that of Fig. 3a. Further separation from Fig. 3f to Fig. 3a is unnecessary. The comparison of the information content and the number of peaks identified from chromatographic fingerprints in Fig. 3c to Fig. 3e, it is in much accordance with their performances. The

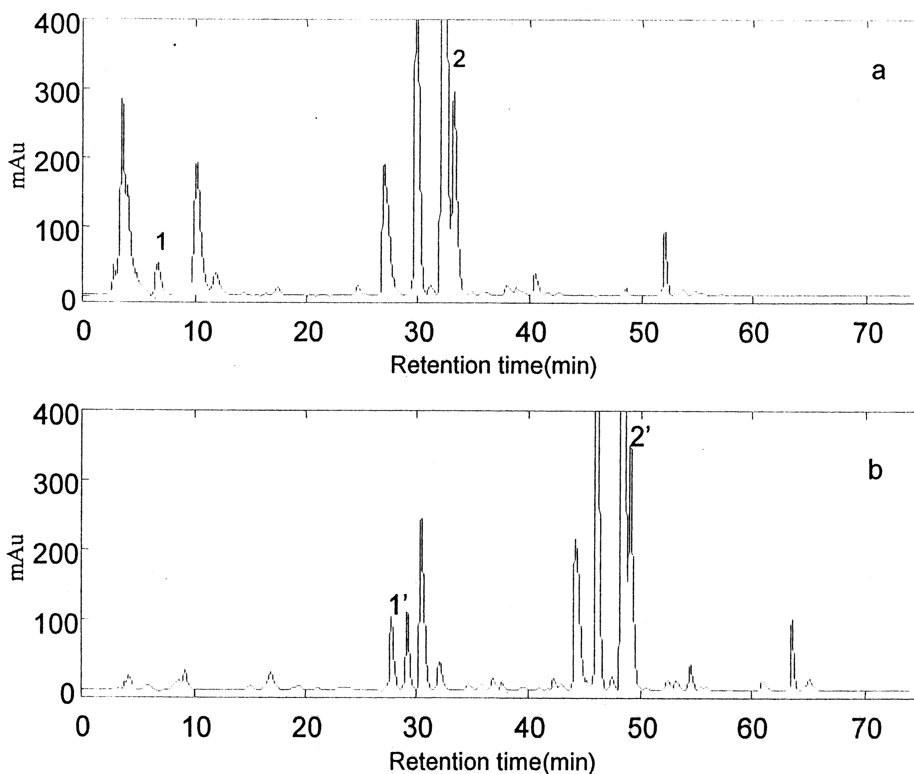


Fig. 4. Chromatographic fingerprints from *Rhizoma chuanxiong* under conditions i (a) and ii (b).

Table 4
Information content and number of peaks identified of chromatographic fingerprints from a *Rhizoma chuanxiong* sample detected under two conditions in Fig. 4

	i	ii
Φ	7.08	7.74
No. peaks	47	60

See Section 3.5.1 for conditions i and ii.

information content calculated on the basis of the modification here could be used to evaluate the performances of these simulated chromatographic fingerprints.

4.2. Selection of a chromatographic fingerprint of R. chuanxiong with high quality

Fig. 4 shows the chromatographic fingerprints of the same raw material of *R. chuanxiong* detected at 254 nm under conditions i (Fig. 4a) and ii (Fig. 4b), respectively (see Section 3.5.1). As can be seen from these profiles, only 47 chromatographic peaks appear in Fig. 4a while there are about 60 chromaographic peaks identified in Fig. 4b (see Table 4). Fig. 4b shows greatly improved performance in terms of the chromatographic separation degree. On the other hand, the concentration distribution of each peak in Fig. 4b is also more uniform than in Fig. 4a. From it, detection conditions ii should be better than i.

Table 4 also displays the information content Φ from the two chromatographic fingerprints obtained (Fig. 4a and Fig. 4b). As seen from Table 4, the information content in Fig. 4b is much more than in Fig. 4a. Thus, the chromatogram in Fig. 4b could be selected as the chromatographic fingerprint of *R. chuanxiong* with high quality.

In order to further determine whether the chromatographic fingerprints obtained can chemically represent some pharmacologically active and marker compounds existing in *R. chuanxiong*, several multiresolution approaches in chemometric and GC–MS determination are employed here [15,16]. Take the

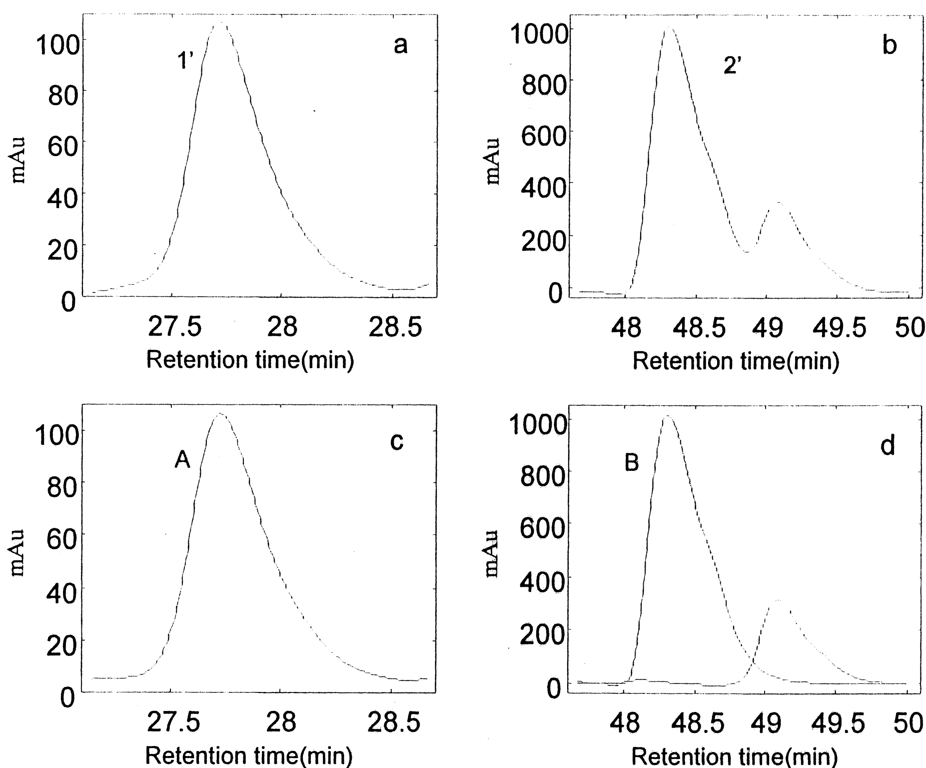


Fig. 5. Peak clusters 1′ (a), 2′ (b) and their resolved results (c, d).

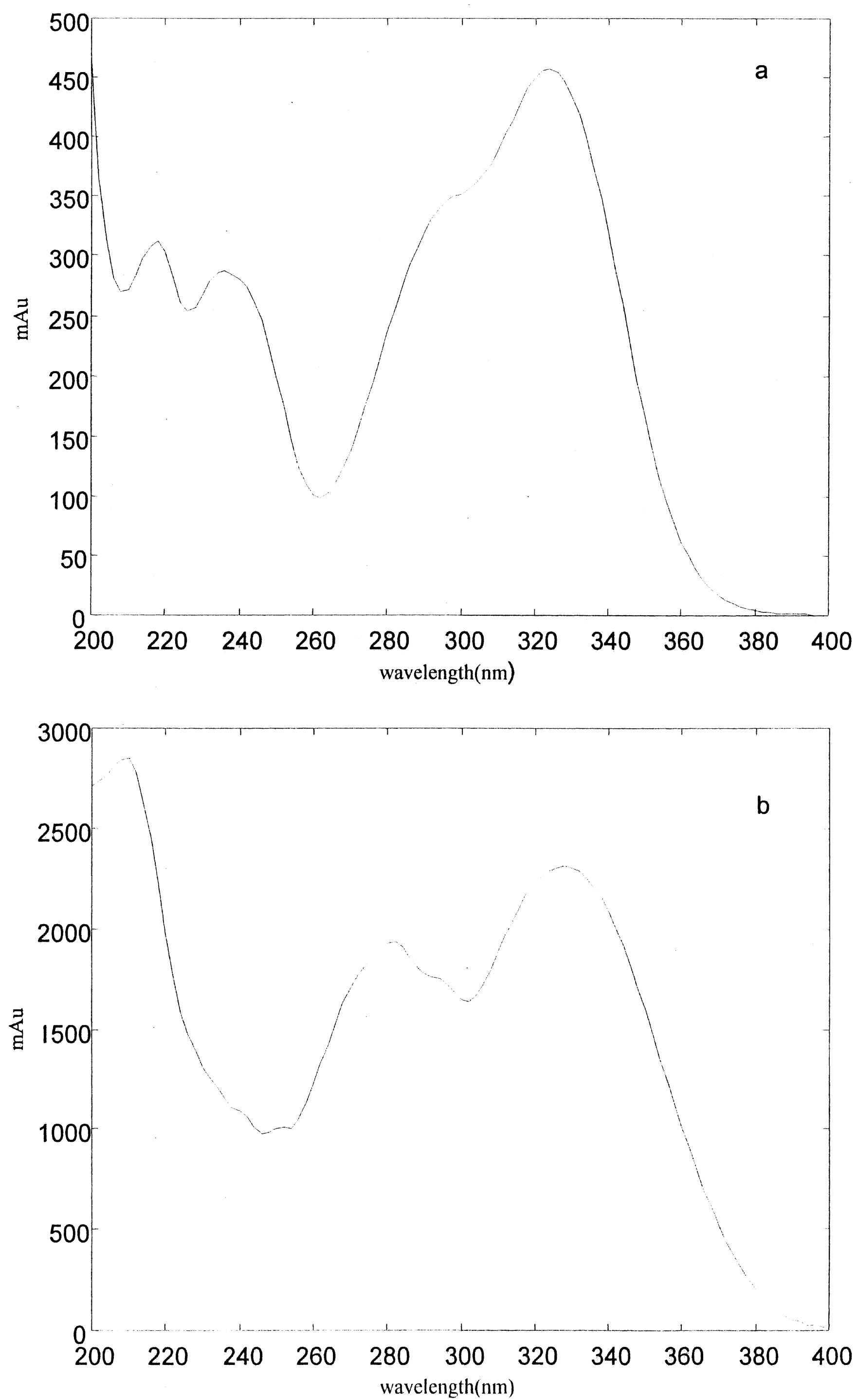
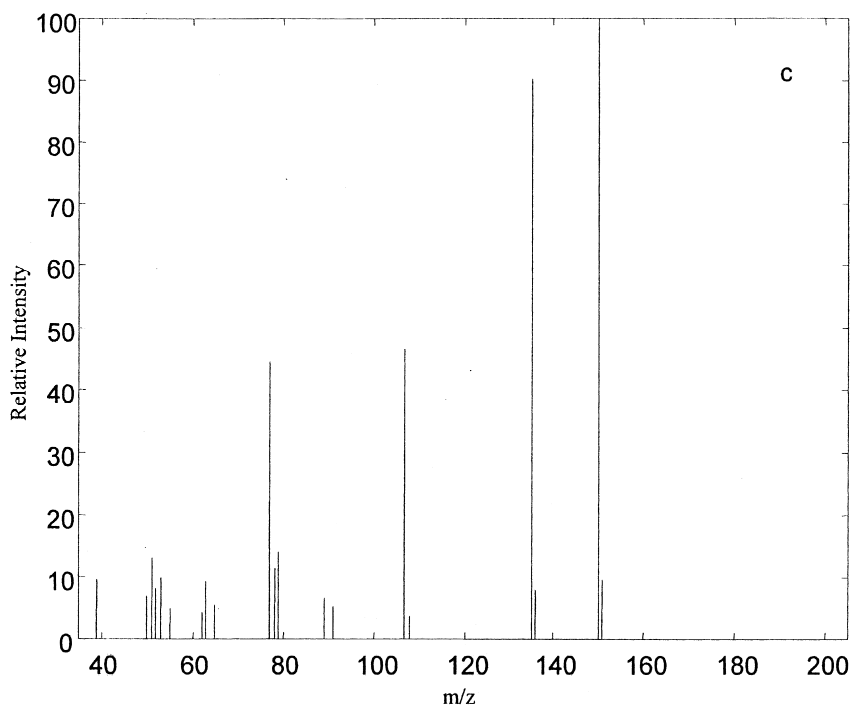


Fig. 6. UV and mass spectra of ferulic acid (a and c) and bultylidene dihydrophthalide (b and d).



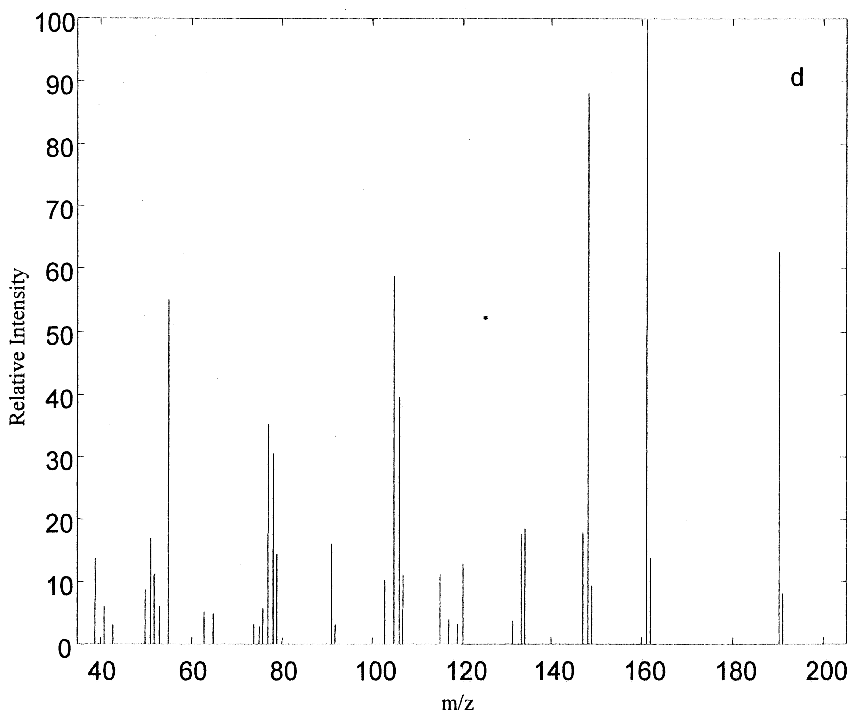


Fig. 6. (*continued*)

peak clusters 1, 2, 1′ and 2′ in Fig. 4 as examples. Both the peak clusters 1 and 1′ are pure peaks representing ferulic acid—a pharmacologically active and marker compound. However, the peak clusters 2 and 2′ are overlapping peaks containing two components, one of which is another pharmacologically active and marker compound of butyllidene dihydrophthalide. Fig. 5 shows the peak clusters 1′ and 2′ (Fig. 5a and Fig. 5b) and their resolved results (Fig. 5c and Fig. 5d). In Fig. 5, peak A represents ferulic acid while peak B denotes bultylidene dihydrophthalide. GC–MS determination can further determine these two components (see Section 3.5.2). Their UV obtained directly by HPLC–DAD and mass spectra from GC–MS are shown in Fig. 6. As a result, the chromatographic fingerprint obtained in Fig. 4b with much information content could also surely chemically represent *R. chuanxiong*. Thus, Fig. 4b can be selected as the chromatographic fingerprint of *R. chuanxiong* for quality control.

4.3. Determination of chromatographic fingerprints of G. biloba

Flavonoids are one kind of pharmacologically active and chemically characteristic ingredient of *G. biloba*. Nowadays, a standard extract denoted EGB 761, which is a well-defined and beneficial extract from *G. biloba*, is recognized worldwide. This standard extract of EGB 761 is mainly composed of 33 flavonoids [19,20,42–45]. As a result, the chromatographic fingerprint of *G. biloba* obtained for quality control should be very similar to that of EGB 761.

Fig. 7a–c show three chromatographic fingerprints (360 nm) of EGB 761, one extract and one product of *G. biloba* provided by a pharmaceutical company, respectively. These chromatographic fingerprints are also obtained by use of HPLC–DAD in this study. In these figures, the peaks denoted by 1 represent one of the pharmacologically active and marker com-

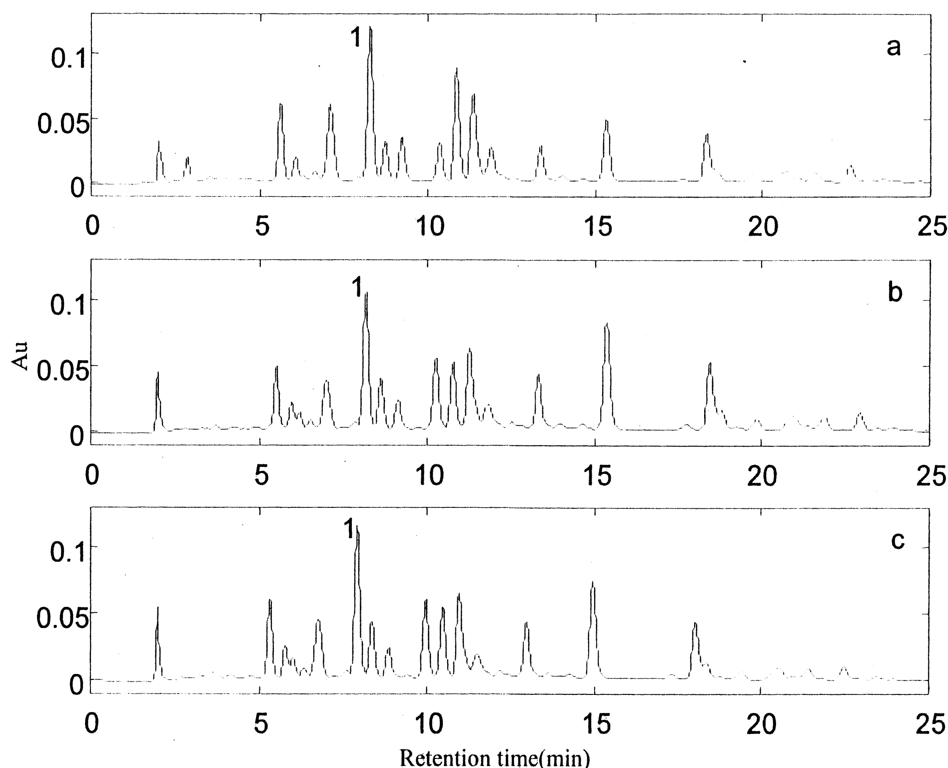


Fig. 7. Chromatographic fingerprints of EGB 761 (a), one extract (b) and one product (c) of *Ginkgo biloba* at 360 nm. Peak 1 represents luteolin ($C_{15}H_{10}O_6$).

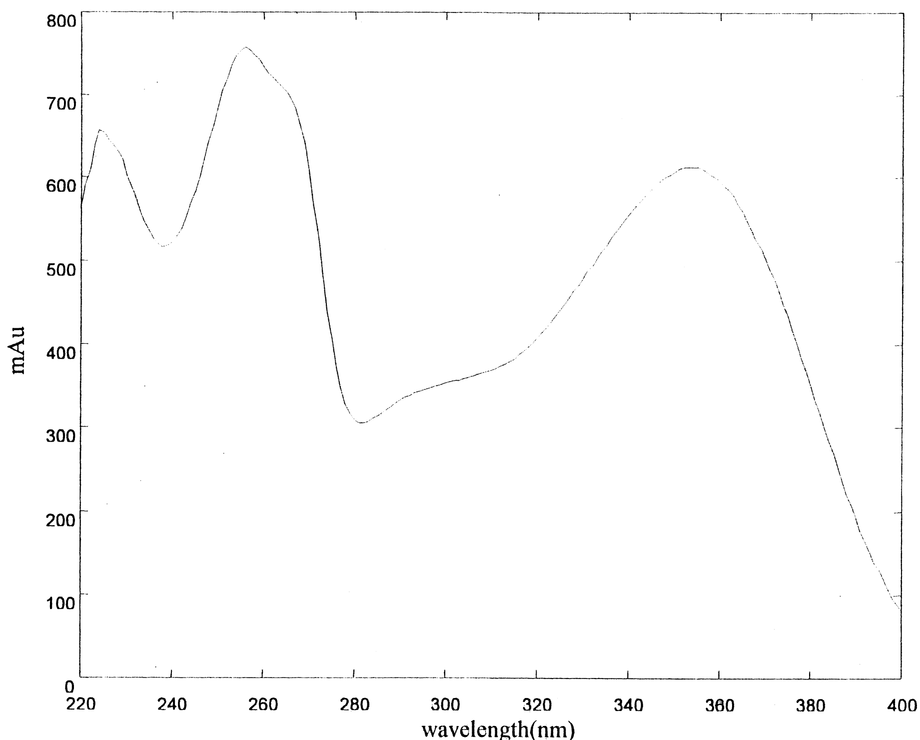


Fig. 8. UV spectrum of luteolin.

ponents named luteolin ($C_{15}H_{10}O_6$) whose UV spectrum is shown in Fig. 8.

Table 5 shows the information content and number of peaks identified from these chromatographic fingerprints obtained. As seen from Table 5, the information content values are approximately close to each other, suggesting the same chromatographic shapes or separation degrees and concentration distribution of all chemical components. On the other hand, 34, 30 and 28 chemical components could be identified in Fig. 7a–c, respectively. Clearly, the number of their chemical components is close to that of flavonoids (33). Thus, the chromatographic fingerprints obtained in Fig. 7a–c featured high separation degrees and uniform concentration distribution of chemical components.

In fact, as seen from Fig. 7, these chromatographic patterns are very similar to each other. The similarities between these three chromatographic fingerprints are over 0.95, which might be obtained by calculating the correlative or congruent coefficient of these profiles. Thus, the ‘‘integrity’’ [10] or ‘‘sameness’’ [12] of the chromatographic fingerprints obtained is demonstrated successfully here. The information content Φ, which is similar in the chromatographic fingerprints obtained in Fig. 7, might also explain their similarities.

On the other hand, we can also investigate the

Table 5
Information content and number of peaks identified of chromatographic fingerprints from three *Ginkgo biloba* samples in Fig. 7

	EGB 761	*Ginkgo biloba* extract	*Ginkgo biloba* product
Φ	10.98	10.79	10.67
No. peaks	34	30	28

difference between these chromatographic fingerprints obtained. Seen from the chromatographic pattern of EGB 761, this chromatographic fingerprint might be divided into five sections denoted I, II, III, VI and V (see Fig. 9). There are 21 main peaks existing in these sections. The concentration distribution of each chemical component from EGB 761 can be demonstrated clearly within these five sections. If the similar procedure is employed for Fig. 7b and c, the same fingerprint sub-regions can be also obtained. In comparison with these fingerprint sub-regions, small dissimilarities obviously exist. Thus, in addition to the "integrity" or "sameness", the "fuzziness" [10] or "difference" [19] between chromatographic fingerprints of EGB 761, the extract and product of *Ginkgo biloba* can also be exhibited in Fig. 7 and Fig. 9.

From the above, chromatographic profiles with high separation degrees and uniform concentration distribution of chemical components in Fig. 7 could demonstrate the similarity and difference of chemically characteristic components from *G. biloba*. As a result, Fig. 7 could be certainly selected as the chromatographic fingerprint for the quality control of *G. biloba*.

The chromatographic fingerprints of *R. chuanxiong* and *G. biloba* from different sources, their corresponding stabilities and recoveries and the information content have been also studied. This research covers 50 *R. chuanxiong* samples and 82 *G. biloba* ones. All the results obtained show that, the determination of chromatographic fingerprints with high separation degrees and uniform concentration distribution of chemical components on the basis of the present methodology in this study are very satisfactory to obtain chromatographic fingerprints of HMs for quality control. They are not discussed in detail here.

From the above, chromatographic fingerprints of HMs for quality control are obtained by means of HPLC–DAD in this work. These kinds of hyphenated instruments could take full advantage of the chromatographic separation and spectrometric qualitative determination of some pharmacologically active and marker compounds such as ferulic acid and bultylidene dihydrophthalide existing in *R. chuan-*

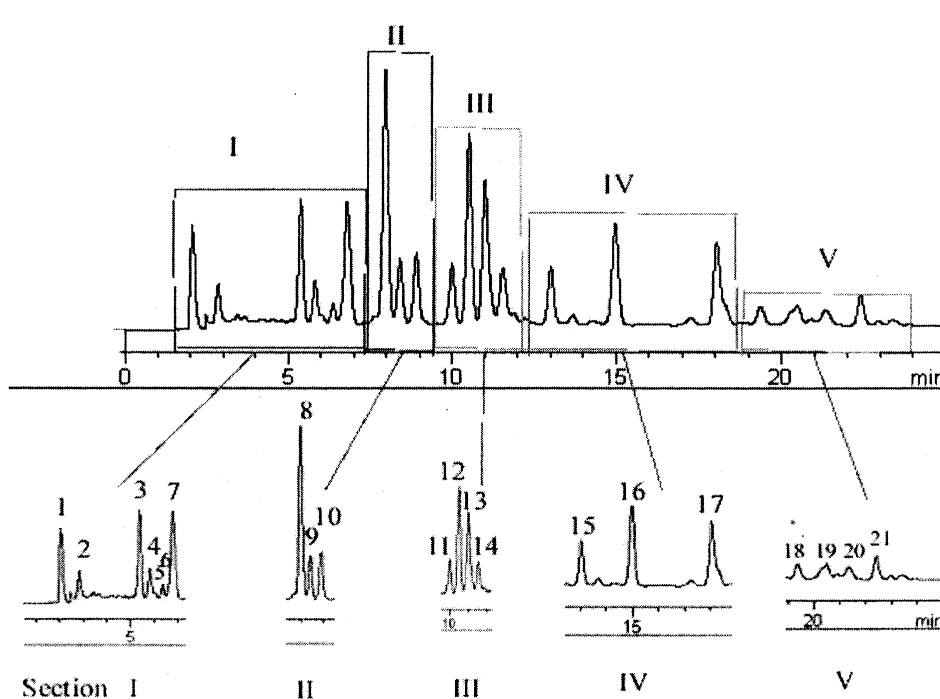


Fig. 9. Five sub-regions of the chromatographic fingerprint of EGB 761 in Fig. 7a.

xiong and luteolin from *G. biloba*. Moreover, hyphenated chromatography could easily conduct the calibration of background and correction of baseline shift, correction of retention time shift, selection of wavelengths, purity identification of chromatographic peaks and resolution of overlapping peaks into pure chromatograms and spectra. Future research will further discuss the advantages of hyphenated chromatography for chromatographic fingerprints of herbal medicines for quality control in detail.

5. Conclusion

Nowadays, the construction of chromatographic fingerprints of complex herbal medicines, especially with hyphenated chromatography, has become one of the most powerful approaches to conduct quality control. However, the performance of a chromatographic fingerprint obtained with several peaks is closely relative to the separation degree and concentration distribution of each chemical component. In this study, the information theory with modification based on the characteristics of chromatographic fingerprints was applied to evaluate chromatographic fingerprints obtained. In comparison to the information content from chromatographic fingerprints investigated, it is easy for us to select a chromatographic fingerprint with a high separation degree and uniform concentration distribution of all chemical components. The application of the information theory with modification of simulated and real herbal medicine chromatographic fingerprints demonstrated clearly that, such the tool like information theory could surely show the prosperous prospect for pharmaceutists and analytical chemists to directly address very difficult problems in chromatographic fingerprints of herbal medicines for quality control.

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