Journal of Chromatography A, 1220 (2012) 50–56

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Separation and identification of flavonoids from complex samples using off-line two-dimensional liquid chromatography tandem mass spectrometry

Jing Zeng[a,b], Xiuli Zhang[a], Zhimou Guo[a], Jiatao Feng[a], Jianguo Zeng[b], Xingya Xue[a,*], Xinmiao Liang[a,*]

[a] Key Lab of Separation Science for Analytical Chemistry, Key Lab of Natural Medicine, Liaoning Province, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China

[b] National Research Center of Engineering Technology for Utilization of Functional Ingredients from Botanicals, Hunan Agriculture University, Changsha 410128, China

ARTICLE INFO

Article history:
Received 2 August 2011
Received in revised form
14 November 2011
Accepted 22 November 2011
Available online 28 November 2011

Keywords:
Off-line two-dimensional liquid chromatography
Identification
Flavonoids
Library searching
Traditional Chinese Medicines

ABSTRACT

In this study, a LC-based identification strategy was proposed and off-line two-dimensional liquid chromatography was developed for the separation and identification of flavonoids from complex samples. The highly orthogonal separation system was composed of a CD column, an OEG column and an XTerra C_{18} column. The CD column was employed for the first dimensional separation and the OEG column and XTerra C_{18} column were used for the second dimensional separation in parallel. A mixture of six traditional Chinese medicines was used as a complex sample in testing this method. Unknown peaks were identified by matching their retention times and accurate mass molecular weights with those of references. The identification efficacy of parallel unidimensional liquid chromatography and off-line two-dimensional liquid chromatography was compared, and the latter was demonstrated to be more efficient for the identification of target compounds—especially co-eluted compounds or minor compounds from complex samples.

1. Introduction

Separation and identification of target compounds from complex samples are important in many fields, such as scientific research, international trade, food safety and sports. Conventional methods for the chemical identification, i.e., infrared spectrometry and ultraviolet spectrometry have questionable specificity for distinguishing structurally similar compounds [1,2]. In addition, gas chromatography tandem electron impact ionization mass spectrometry, though it is a powerful identification method in general, can only be used to detect polar, thermo-labile and high-mass compounds [3,4]. Liquid chromatography tandem mass spectrometry (LC–MS), however, seems to be applicable for the analysis of natural products, foods, drugs and other samples without additional sample derivatization. Moreover, LC–MS provides suitable separation modes (RPLC, HILIC, IEC, etc.) that can be used for many different types of samples.

As far as we know, identifying co-eluted or structurally similar compounds with similar fragments using conventional unidimensional liquid chromatography tandem mass spectrometry (1D-LC–MS) remains a great challenge. Due to the limited peak capacity of 1D-LC, analytes often elute simultaneously during LC separation, preventing their differentiation by retention time. In addition, co-eluted compounds with similar structures may produce highly similar fragments, making it difficult to differentiate them according to their product ions [5]. Furthermore, a universal MS/MS library that can be searched to rapidly identify analytes from complex samples is still lacking. Therefore, retention times and mass spectral matching provided by 1D-LC–MS are still insufficient for the unambiguous identification of complex samples. Two-dimensional liquid chromatography coupled with mass spectrometry, however, could provide better results.

Recent advances in the theory and application of orthogonal liquid chromatography afford researchers new strategies to separate and identify co-eluted or structurally similar compounds from complex samples [6]. Co-eluted or structurally similar compounds in one chromatogram would likely be separated in the second chromatogram, since 2D-LC provides greatly enhanced peak capacity and selectivity [7]. Deng and co-workers developed an off-line 2D-RP/RP method for the separation of *Dracaena Cochinchinensis* (*lour.*). Single peaks from the first dimension were resolved into at least eight peaks on the second chromatogram, demonstrating good separation efficiency [8]. Chen et al. developed an on-line 2D-SAX/RP

* Corresponding authors. Tel.: +86 411 84379519; fax: +86 411 84379539.
E-mail addresses: xuexy@dicp.ac.cn (X. Xue), liangxm@dicp.ac.cn (X. Liang).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.11.043

method for the analysis and identification of *Flos Lonicera*, revealing the potential of 2D-LC for complex sample analysis [9].

In a previous study [10], we separated and identified flavonoids from two relatively simple samples (fractions of *Dalbergia odorifera* T. Chen and *Scutellaria baicalensis* Georgi) using a parallel 1D-LC method, though several review papers discussed the recent trends in application of chromatographic method in research of flavonoids [11–13]. It is important to note that parallel 1D-LC approaches such as that used in our previous study can only be applied to relatively simple samples. For complex samples, 1D-LC is not competent due to limited peak capacity.

Two-dimensional liquid chromatography associated with enlarged peak capacity and enhanced selectivity seemed to be a potentially suitable approach for the separation and identification of these complex samples. Thus, in this study, an off-line 2D-LC approach was developed for the separation and identification of complex samples, using a previously established orthogonal separation system and library of 82 flavonoids. The efficacy of a parallel 1D-LC approach and the off-line 2D-LC approach was compared with respect to the identification of complex samples.

2. Experimental

2.1. Materials and reagents

The three columns used in this study were of an XTerra 5 μm MS C_{18} column (150 mm × 2.1 mm, Waters), which will subsequently be referred to as a C_{18} column, a homemade 5 μm Click OEG column (150 mm × 4.6 mm) [14], and a homemade 5 μm Click CD column (150 mm × 4.6 mm) [15], which will subsequently be referred to as an OEG column and CD column, respectively. The C18 SPE cartridge was purchased from Sipore Co. LTD. (Dalian, China).

HPLC-grade acetonitrile was purchased from TEDIA (Fairfield, USA), formic acid and ammonium formate were purchased from Acros (Cambridge, USA), and water was prepared using a Milli-Q system (MA, USA).

Six Traditional Chinese Medicines (TCMs): *Dalbergia odorifera* T. Chen, *Scutellaria baicalensis* Georgi, *Scutellaria barbata* D. Don, *Citrus aurantium* L., *Glycyrrhiza glabra* L., and kudzu were purchased from the Dalian BaiYun pharmacy (Dalian, China).

2.2. LC–MS conditions

Determination of retention times of unknowns was carried out on a Waters Alliance HPLC system, consisting of a 2695 HPLC pump and a 2996 photodiode array detection system. Data acquisition and processing were conducted using Waters Empower software. Chromatographic conditions are shown in Table 1.

MS experiments were performed on a Waters Micromass Q/TOF system (Milford, MA, USA) operated in positive ion mode. The operation parameters used are listed as follows: capillary voltage: 2 kV; cone voltages: 20 V; desolvation gas temperature: 300 °C; source temperature: 100 °C; cone gas: 50 L/h; desolvation gas: 1000 L/h. The mass scan range was set to 50–850 m/z with a scan time of 0.3 s and an interscan delay of 0.1 s. Masslynx was employed as the chemstation for data acquisition.

Table 1
Separation conditions for the three columns in this study.

Columns	Mobile phases	Gradient	Flow rate (ml/min)
C_{18}	A: 0.2% Formic acid–H_2O; B: 0.2% Formic acid–ACN	(95:5) → (30:70) (v:v) (A:B) (15 min)	0.3
OEG	A: 0.2% Formic acid–H_2O; B: 0.2% Formic acid–ACN	(95:5) → (40:60) (v:v) (A:B) (30 min)	1
CD	A: H_2O; B: ACN; C: 100 mM ammonium formate	(65:5:30) → (30:40:30) (v:v:v) (A:B:C) (30 min)	1

2.3. Selection of complex samples

In a previous study, hundreds of natural compounds from TCMs were purified and characterized in our laboratory, from which 82 flavonoids were selected as references for the construction of a library [10]. These flavonoids partly came from several TCMs, including *Dalbergia odorifera* T. Chen, *Scutellaria baicalensis* Georgi, *Scutellaria barbata* D. Don, *Citrus aurantium* L., *Glycyrrhiza glabra* L., kudzu. Therefore, a mixture of these six TCMs was selected for method optimalization, compounds from these TCMs would likely be identified in a library search.

2.4. Sample preparation

Six TCMs (5 g of *Dalbergia odorifera* T. Chen, 5 g of *Scutellaria baicalensis* Georgi, 5 g of *Scutellaria barbata* D. Don, 5 g of *Citrus aurantium* L., 5 g of *Glycyrrhiza glabra* L., and 5 g of kudzu) were mixed together and extracted using ultrasound with 200 mL methanol for 2 h. The supernatant was concentrated with a spray dryer. The resulting extract was dissolved in 10 mL 50% methanol–water (v:v), and then filtered through a 0.22 μm membrane. The sample was further cleaned by solid phase extraction (SPE). A 1 g C_{18} SPE cartridge was pre-conditioned with 5 mL methanol and 5 mL water, then 2 mL extract was loaded onto the cartridge, followed by 5 mL water and 5 mL methanol. The acquired fraction was concentrated to about 30 mg mL^{-1} of methanol and stored at 4 °C. The injection volume for the first dimension on the CD column was 70 μL. The injection volumes for the OEG and C_{18} columns were 30 μL and 10 μL, respectively.

2.5. Software and interval settings

Following a previous study [10], retention time thresholds for the C_{18} column and OEG column were set to 0.10 min and 0.3 min, respectively. The accurate molecular weight interval was set to 20 ppm.

3. Results and discussion

3.1. Selection of separation approach

In our previous work [10], three columns (the C_{18} column, OEG column and CD column) with different selectivities operated in a parallel 1D-LC approach were used for the separation and identification of two relatively simple fractions of TCMs. As mentioned, both the parallel 1D-LC approach and 2D-LC approach can be used for separation, depending on sample complexity. As shown in Fig. 1, general identification schemes of parallel 1D-LC and 2D-LC are proposed. For relatively simple samples, the compounds can be separated on two or three columns in parallel. Retention times and accurate molecular weights of detected peaks can be matched with those of references. The parallel 1D-LC approach is easy and flexible, and is widely used for impurity evaluation in pharmaceutical industry [16–19]. First of all, selectivity of the parallel 1D-LC system is primarily considered because the impurities involved in active pharmaceutical ingredients are possibly structurally similar compounds. In addition, limited peak capacity typically associated with parallel 1D-LC is still applicable because pharmaceuticals are relatively simple samples. Finally, orthogonality of parallel 1D-LC system is easy to change by means of changing column [20], mobile phase pH [21], additives [22], and temperature [23].

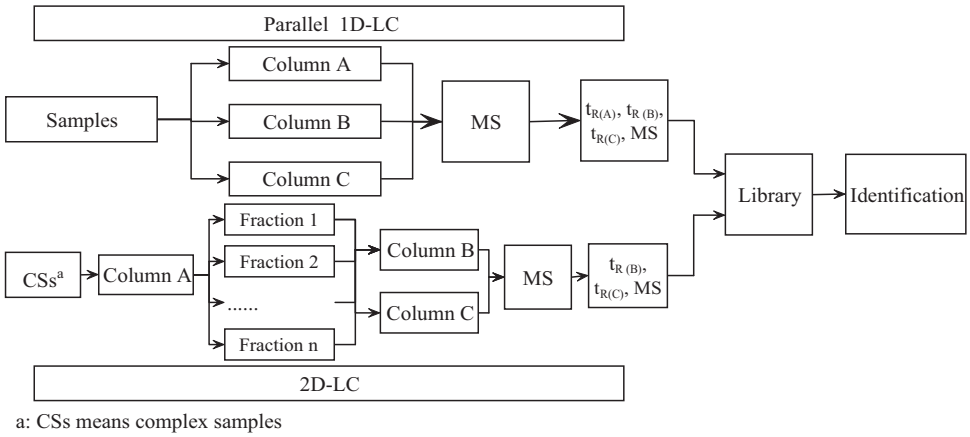

Fig. 1. Flow chart of general chemical identification.

For more complex compounds, the parallel 1D-LC approach is obviously not competent due to limited peak capacity and selectivity. Two-dimensional liquid chromatography, associated with significantly improved peak capacity and enhanced selectivity, is a promising technique for the separation of different types of complex samples [24–27]. As shown in Fig. 1, one of the three columns can be used for first dimensional separation, and then eluted fractions can be separated on the two remaining columns in the parallel second dimensional separation. While the first dimensional separation of complex fractions would yield numerous fractions, more peaks would be revealed by the second dimensional separation, because the second dimension would provide different separation selectivity.

3.2. Construction of an off-line 2D-LC system

For the separation and identification of complex samples, both on-line 2D-LC and off-line 2D-LC are applicable, but the former is always limited by equipment and interface. In this study, off-line 2D-LC provided easy operation, and free concentrated fractions was used for the separation and identification of the mixture of six TCMs. In our recent study [10], a highly orthogonal separation system, consisting of a C_{18} column, OEG column and CD column was established. When the three columns are used in off-line 2D-LC approach, any one of the three columns can be used for first dimensional separation, and the remaining columns can be used for second dimensional separation in parallel.

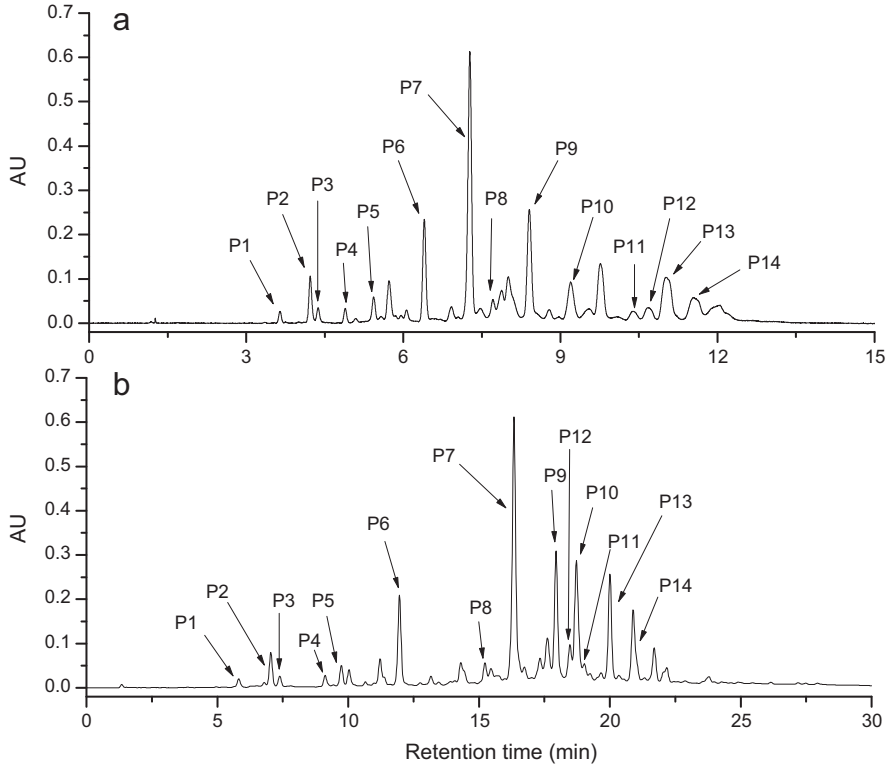

Fig. 2. Separation of the SPE extract of six TCMs on C18 column (a) and OEG column (b). Chromatographic conditions are detailed in Section 2.2.

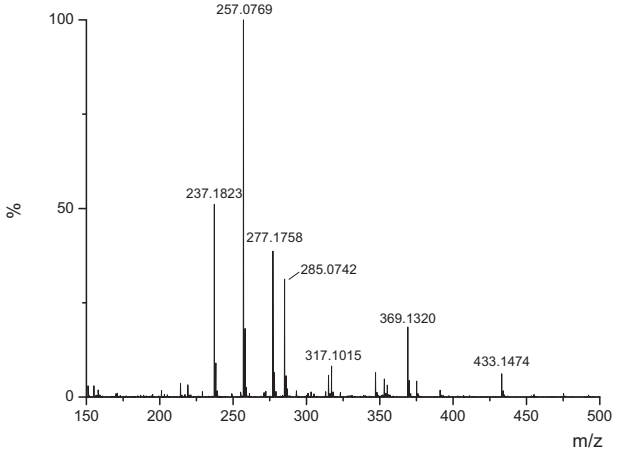

Fig. 3. Mass spectrum of broader Peak 14.

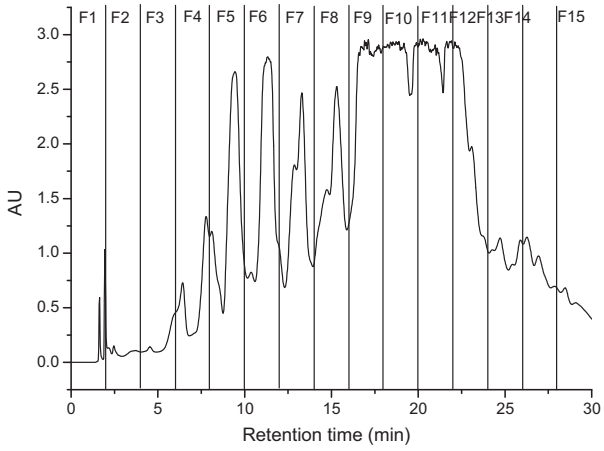

Fig. 4. Fractionation of the extract of six TCMs on CD column. Inject volume: 70 μL, other chromatographic conditions are detailed in Section 2.2.

For the fractionation of the extract containing six TCMs, the CD column was selected for the first dimensional separation. CD is a type of novel stationary phase, demonstrating HILIC/RPLC mixed-mode retention behavior [15]. Cyclodextrins (CDs) are a series of cyclic oligosaccharides with a hydrophobic cavity and hydrophilic exterior faces, which can provide mixed interactions with analytes, such as hydrophobic interaction, hydrophilic interaction, and stereo interaction. In addition, a 2D-RP/HILIC-LC system, coupled with C_{18} and CD column was successfully established for the separation of *Carthamus tinstorius Linn* [28].

For the second dimensional separation, the C_{18} column and OEG column were employed. C_{18} is one of the most popular stationary phases for the separation of medium to weak polar compounds due to its high efficiency, stability and reproducibility. OEG is also a novel stationary phase, which showed good performance for the separation of *Lamiophlomis rotate* [14]. Oligo (ethylene glycol) (OEG) is flexible chain-molecules with four ether bonds and a hydroxyl group. Besides hydrophobic interaction afforded by conventional C_{18} column, the unique structure can provide additional hydrogen bonding and dipole–dipole interaction. Additionally, a 2D-RP/RP-LC system, coupled with OEG and C_{18}, was established and demonstrated higher orthogonality than C_{18}/CN and C_{18}/C_{18}.

The three columns with different functional groups afford fairly different selectivities, demonstrating high orthogonality, even though it is operated under RP mode. While conventional RP columns would not afford such high orthogonality. The established off-line 2D-LC system seems to be applicable for the separation and identification of the extract containing six TCMs.

3.3. Separation and identification of the extract

For testing the separation efficiency of 1D-LC approach, the SPE extract of the six TCMs was separated on a C_{18} column and OEG column in parallel (see Fig. 2). The retention times and accurate molecular weights of peaks were imported into the library and searched automatically. As a result, fourteen compounds, P1–P14, were identified with the C_{18} column (see Fig. 2a) and OEG column (see Fig. 2b), and the identification results are shown in Table 2. Most visual peaks were identified by matching retention times on the two columns and accurate molecular weights. Fig. 2a shows that peaks eluted from 10 to 12 min were broader than normal chromatographic peaks, suggesting that these peaks were composed of more than one compound. We analyzed the mass data of peaks eluted at about 12 min (see Fig. 3), and we found that in addition to the high-abundance molecular ion peak $[M+H]^+$ (m/z = 257.0769), numerous low-abundance ion peaks were also present (m/z 237.1823, m/z 277.1758, m/z 285.0742, m/z 369.1320, m/z 433.1474). This result provided further evidence that multiple compounds elute simultaneously due to the limited peak capacity of a unidimensional approach.

In contrast to the parallel 1D-LC approach, the mixture of six TCMs was separated and identified using off-line 2D-LC approach. As shown in Fig. 4, the extract of the six TCMs was fractionated on the CD column. In order to simplify the extract, the eluted compounds were manually collected at 2 min intervals from 0 to 30 min (totally 15 fractions were collected). The resulting fractions were concentrated under nitrogen, re-dissolved into 0.2 mL of methanol, and then separated on C_{18} column and OEG column, respectively. The representive chromatograms are shown in Fig. 5. In total, 25 compounds (P1–P25) were clearly identified and are listed in Table 3. It is clear that an off-line 2D-LC approach provides more efficient identification than a parallel 1D-LC approach.

For off-line 2D-LC, peak capacity is increased by one order of magnitude, allowing compounds to be well-separated. Co-eluted (unresolved) peaks in the first dimension would likely be separated in the second dimension using orthogonal columns. But parallel 1D-LC cannot separate hundreds of compounds from complex samples due to limited peak capacity, and thus many of these compounds eluted simultaneously. For example, Peak 14 (P14) (identified as wogonin using 1D-LC) in Fig. 2a seems broader than other peaks, suggesting that it is composed of several co-eluted compounds. Using 2D-LC approach, we found that P24 (identified as pinocembrin and shown in Fig. 5g1) was shielded by P14 (shown in Fig. 5f1). Structures of wogonin and pinocembrin are very similar, which was shown in Fig. 6. Pinocembrin is a dihydro-flavonoid, while wogonin is a flavonoid with additional 8-methoxy group. The two compounds have fairly similar retention time on C_{18} column. However, the two compounds can be separated into different fractions on CD column due to its unique mixed interactions afforded by cyclodextrin bonded phase. As mentioned in Section 3.2, oligo (ethylene glycol) can provide hydrogen bonding and dipole–dipole interaction. Therefore, the two compounds can also be differentiated on OEG column with special separation selectivity.

P22 (identified as liquoritigenin and shown in Fig. 5f1) and P26 (not identified and shown in Fig. 5e1) eluted at 8.0 min and 8.1 min with off-line 2D-LC, respectively. Therefore, the two peaks inevitably overlapped on the 1D-LC separation chromatogram, but could be successfully resolved into different fractions on the CD column during first dimensional separation. In addition, large differences in the abundance of compounds in samples make it difficult to identify minor compounds with a 1D-LC approach. As shown in Fig. 2, the 14 compounds identified with 1D-LC were almost major peaks. Using 2D-LC, minor compounds can be potentially identified. For example, when 1D-LC was used, the

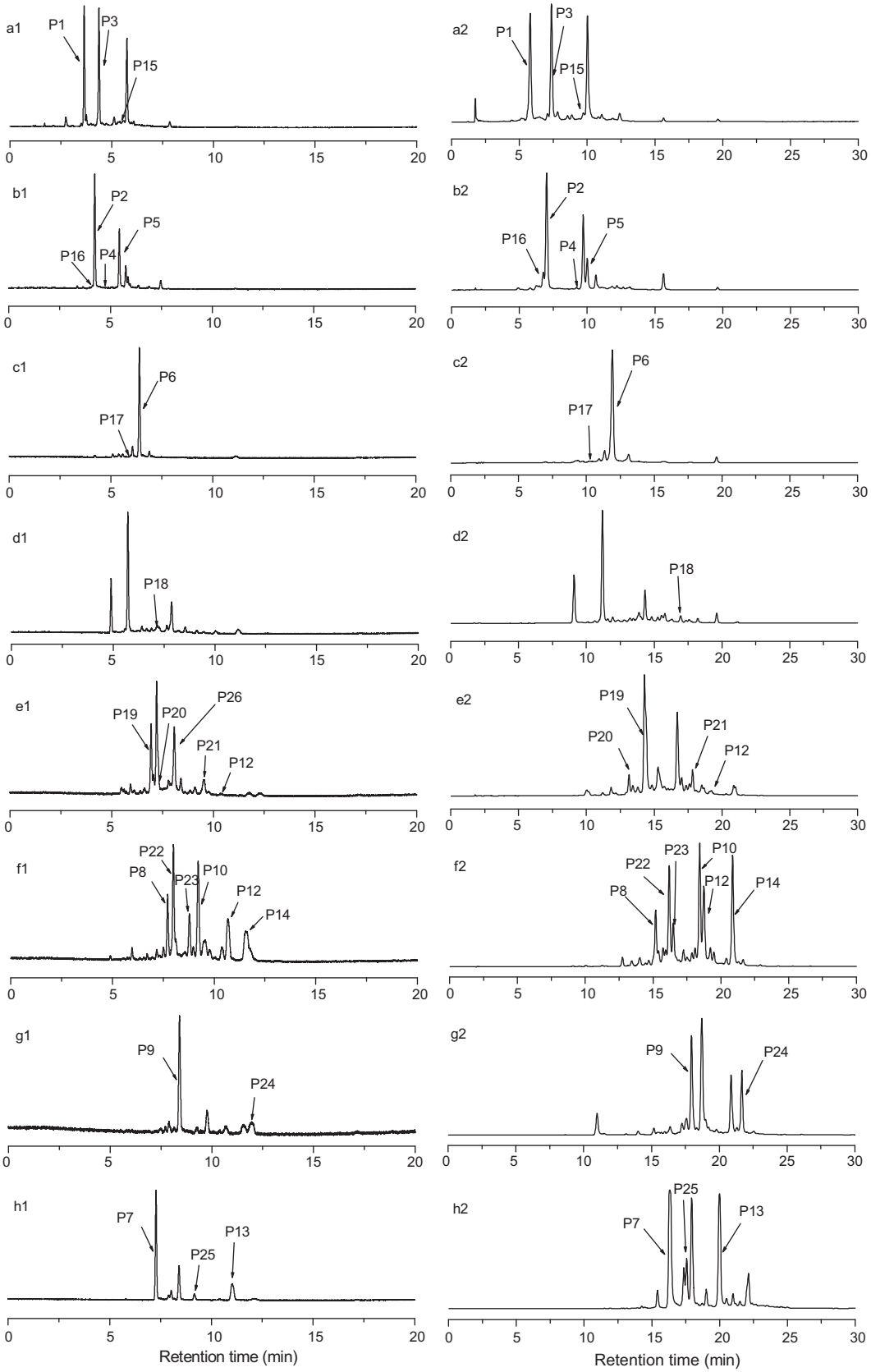

Fig. 5. Separation of Fr4 (a1), Fr5 (b1), Fr6 (c1), Fr7 (d1), Fr8 (e1), Fr9 (f1), Fr10 (g1), Fr11 (h1) on C18 column and Fr4 (a2), Fr5 (b2), Fr6 (c2), Fr7 (d2), Fr8 (e2), Fr9 (f2), Fr10 (g2), Fr11 (h2) on OEG column. Chromatographic conditions are detailed in Section 2.2.

Table 2
Identification of the peaks using parallel 1D-LC method.

Peak no.	$t_{R\ (C18)}$[a]	$t_{R\ (OEG)}$[b]	$\Delta t_{R\ (C18)}$[c]	$\Delta t_{R\ (OEG)}$[d]	Deviation[e]	Identification
P1	3.65	5.87	0.04	0.13	0.5	5-Hydroxypuerarin
P2	4.22	7.01	0.04	0.07	7.2	Puerarin
P3	4.38	7.44	0.05	0.14	2.7	3′-Methoxypuerarin
P4	4.89	9.18	0.05	0.04	5.3	Daidzin
P5	5.43	9.8	0.04	0.09	2.6	Chrysin-6-C-α-L-arabinopyranosyl-8-C-β-D-glucopyranoside
P6	6.4	12.02	0.04	0.07	1.3	Hesperidin
P7	7.27	16.35	0.02	0.21	4.7	Baicalin
P8	7.71	15.28	0.06	0.02	9.4	Daidzein
P9	8.41	17.96	0.03	0.23	10.2	Wogonoside
P10	9.2	18.76	0.03	0.27	8.8	Naringenin
P11	10.39	19.08	0.04	0.05	9.3	Formononetin
P12	10.69	18.53	0.04	0.22	8.8	3′-O-methylviolanone
P13	11.02	20.06	0.03	0.08	2.7	Sativanone
P14	11.54	20.94	0.03	0.02	8.4	Wogonin

[a] $t_{R\ (C18)}$ means determined retention time of unknown peaks on C_{18} column (min).
[b] $t_{R\ (OEG)}$ means determined retention time of unknown peaks on OEG column (min).
[c] $\Delta t_{R\ (C18)}$ means absolute difference of retention times between determined retention time of unknown peaks and references on C_{18} column (min).
[d] $\Delta t_{R\ (OEG)}$ means absolute difference of retention times between determined retention time of unknown peaks and references on OEG column (min).
[e] Deviation means accurate mass weight deviation between theoretical mass weight and measured mass weight (ppm).

Table 3
Identification results by using off-line 2D-LC method, for other details see Table 2.

Fr. no.[a]	Peak no.	$t_{R\ (C18)}$	$t_{R\ (OEG)}$	$\Delta t_{R\ (C18)}$	$\Delta t_{R\ (OEG)}$	Deviation	Identification
Fr4	P1	3.65	5.80	0.04	0.06	6.7	5-Hydroxypuerarin
	P3	4.38	7.37	0.05	0.07	7.6	3′-Methoxypuerarin
	P15	5.40	9.73	0.01	0.02	2.0	Chrysin-8-C-α-L-arabinopyranosyl-6-C-β-D-glucopyranoside
Fr5	P16	4.12	6.77	0.09	0.09	2.4	Puerarin-6″-apioside
	P2	4.22	7.01	0.04	0.07	2.6	Puerarin
	P4	4.77	9.21	0.07	0.07	2.6	Daidzin
	P5	5.44	10.01	0.02	0.03	5.3	Chrysin-6-C-α-L-arabinopyranosyl-8-C-β-D-glucopyranoside
Fr6	P17	5.97	10.22	0.04	0.02	2.7	Formononetin-8-C-apiosyl (1→6)-glucoside
	P6	6.40	11.92	0.04	0.03	3.3	Hesperidin
Fr7	P18	7.18	16.31	0.04	0.26	5.2	Scutellarin
Fr8	P19	6.94	14.29	0.04	0.10	17.3	3′,4′,7-Trihydroxyflavanone
	P20	7.30	13.15	0.05	0.09	9.1	4′-Methoxydadzin
	P21	9.52	17.83	0.03	0.04	8.9	Alpinetin
	P12	10.42	19.22	0.07	0.19	2.6	Formononetin
Fr9	P8	7.71	15.20	0.06	0.10	11.4	Daidzein
	P22	8.00	16.20	0.05	2.99	8.6	Liquoritigenin
	P23	8.79	16.50	0.05	0.04	19.3	Violanone
	P10	9.22	18.44	0.05	0.05	18.0	Naringenin
	P12	10.69	18.75	0.04	0	8.5	3′-O-methylviolanone
	P14	11.55	20.87	0.04	0.09	16.5	Wogonin
Fr10	P9	8.41	17.95	0.03	0.24	4.1	Wogonoside
	P24	11.96	21.66	0.03	0.30	9.4	Pinocembrin
Fr11	P7	7.27	16.31	0.02	0.25	2.7	Baicalin
	P25	9.17	17.57	0.04	0	9.1	Vestitone
	P13	11.02	20.00	0.03	0.02	14.7	Sativanone

[a] Fr. no. means fraction number fractionated on CD column.

minor compound P25 (see Fig. 5h1) was shielded by major compound P10 (see Fig. 5f1), since they eluted at almost the same time. However, the two peaks were clearly identified as vestitone and naringenin using 2D-LC.

As far as we know, compounds with similar chemical and physical properties are common in complex samples [26,29]. A 2D-LC approach for separation not only enhanced peak capacity and selectivity, but also reduced sample complexity. Minor peaks covered by major peaks were exposed and identified. Therefore, we recommend 2D-LC for the separation and identification of complex samples. With the development of increasingly rapid chromatography and new equipment, comprehensive on-line 2D-LC will be an excellent technique that does not require the investment of huge amounts of time or money for the identification.

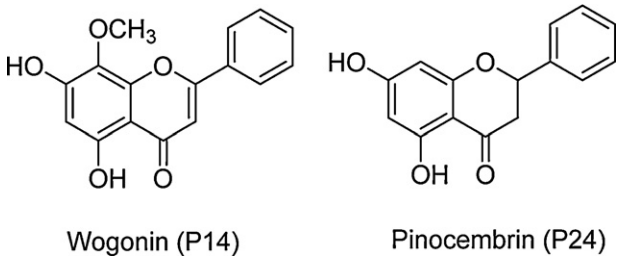

Fig. 6. Structures of wogonin (P14) and pinocembrin (P24).

4. Conclusion

In this study, an off-line 2D-LC identification method, based on a previous established orthogonal separation system, was developed to test identification efficiency for complex samples. Two-dimensional liquid chromatography not only simplified complex samples, but also enlarged peak capacity and enhanced selectivity. This allowed for the successful separation of co-eluted and minor compounds. With this approach, unresolved or minor compounds in one chromatogram would likely be separated and revealed in the second chromatogram. In total, 25 compounds, including minor compounds and compounds unresolved with1D-LC, were unambiguously identified with off-line 2D-LC. In contrast only 14 compounds, all of which were highly abundant in their samples, could be identified using parallel 1D-LC. Therefore, we propose that an off-line 2D-LC approach, such as the method established in this study, is more efficient for the identification of complex samples.

Additionally, the two columns used for the second dimensional separation in this study can be replaced with one column to improve throughput if necessary. Ultra-performance liquid chromatography is a good technique for second dimensional separation due to its enhanced resolution and shortened time of analysis. Although this type of off-line 2D-LC method would be relatively time consuming and labor-intensive, it is important to recognize that identification based on orthogonal separation and library searching can be transferred to on-line 2D-LC, which could greatly speed up the identification process.

Acknowledgements

This work was supported by The Creative Research Group Project by NSFC (No. 21021004) and Project of National Science Foundation of China (30801513). Thanks to Lillian Nordahl for the manuscript revision.

References

[1] B.L. Milman, Trends Anal. Chem. 24 (2005) 493.
[2] M. Valcarcel, S. Cardenas, B.M. Simonet, C. Carrillo-Carrion, J. Chromatogr. A 1158 (2007) 234.
[3] P. Marquet, Ther. Drug Monit. 24 (2002) 125.
[4] L. Rivier, Anal. Chim. Acta 492 (2003) 69.
[5] C. Wagner, M. Sefkow, J. Kopka, Phytochemistry 62 (2003) 887.
[6] G. Guiochon, N. Marchetti, K. Mriziq, R.A. Shalliker, J. Chromatogr. A 1189 (2008) 109.
[7] M. Dumarey, Y.V. Heyden, S.C. Rutan, Anal. Chem. 82 (2010) 6056.
[8] Z.Q. Teng, R.J. Dai, W.W. Meng, Y. Chen, Y.L. Deng, Chromatographia 74 (2011) 313.
[9] X.G. Chen, Z.L. Jiang, Y. Zhu, J.Y. Tan, Chromatographia 65 (2007) 141.
[10] J. Zeng, X. Zhang, Z. Guo, J. Feng, X. Xue, X. Liang, J. Chromatogr. A 1218 (2011) 1749.
[11] C.D. Stalikas, J. Sep. Sci. 30 (2007) 3268.
[12] J. Vacek, B. Klejdus, L. Lojkova, V. Kuban, J. Sep. Sci. 31 (2008) 2054.
[13] E. de Rijke, P. Out, W.M.A. Niessen, F. Ariese, C. Gooijer, U.A.T. Brinkman, J. Chromatogr. A 1112 (2006) 31.
[14] Z.M. Guo, Y.F. Liu, J.Y. Xu, Q. Xu, X.Y. Xue, F.F. Zhang, Y.X. Ke, X.M. Liang, A.W. Lei, J. Chromatogr. A 1191 (2008) 78.
[15] Z.M. Guo, Y. Jin, T. Liang, Y.F. Liu, Q. Xu, X.M. Liang, A.W. Lei, J. Chromatogr. A 1216 (2009) 257.
[16] G. Xue, A.D. Bendick, R. Chen, S.S. Sekulic, J. Chromatogr. A 1050 (2004) 159.
[17] J. Pellett, P. Lukulay, Y. Mao, W. Bowen, R. Reed, M. Ma, R.C. Munger, J.W. Dolan, L. Wrisley, K. Medwid, N.P. Toltl, C.C. Chan, M. Skibic, K. Biswas, K.A. Wells, L.R. Snyder, J. Chromatogr. A 1101 (2006) 122.
[18] E. Van Gyseghem, M. Jimidar, R. Sneyers, D. Redlich, E. Verhoeven, D.L. Massart, Y. Vander Heyden, J. Chromatogr. A 1074 (2005) 117.
[19] E. Van Gyseghem, S. Van Hemelryck, M. Daszykowski, F. Questier, D.L. Massart, Y. Vander Heyden, J. Chromatogr. A 988 (2003) 77.
[20] Y.M. Liu, Z.M. Guo, Y. Jin, X.Y. Xue, Q. Xu, F.F. Zhang, X.M. Liang, J. Chromatogr. A 1206 (2008) 153.
[21] J. Zhang, Y. Jin, Y.F. Liu, Y.S. Xiao, J.T. Feng, X.Y. Xue, X.L. Zhang, X.M. Liang, J. Sep. Sci. 32 (2009) 2084.
[22] K. Valko, S. Espinosa, C.M. Du, E. Bosch, M. Roses, C. Bevan, M.H. Abraham, J. Chromatogr. A 933 (2001) 73.
[23] Y. Mao, P.W. Carr, Anal. Chem. 73 (2001) 4478.
[24] H. Malerod, E. Lundanes, T. Greibrokk, Anal. Methods 2 (2010) 110.
[25] I. Francois, K. Sandra, P. Sandra, Anal. Chim. Acta 641 (2009) 14.
[26] P. Dugo, F. Cacciola, T. Kumm, G. Dugo, L. Mondello, J. Chromatogr. A 1184 (2008) 353.
[27] P. Jandera, J. Sep. Sci. 29 (2006) 1763.
[28] Y.M. Liu, X.Y. Xue, Z.M. Guo, Q. Xu, F.F. Zhang, X.M. Liang, J. Chromatogr. A 1208 (2008) 133.
[29] M.X. Gao, C.H. Deng, S. Lin, F.L. Hu, J. Tang, N. Yao, X.M. Zhang, J. Sep. Sci. 30 (2007) 785.