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Separation and detection of compounds in *Honeysuckle* by integration of ion-exchange chromatography fractionation with reversed-phase liquid chromatography-atmospheric pressure chemical ionization mass spectrometer and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis

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Abstract

A hyphenated method for the isolation and identification of components in a traditional Chinese medicine of *Honeysuckle* was developed. Ion-exchange chromatography (IEC) was chosen for the fractionation of *Honeysuckle* extract, and then followed by concentration of all the fractions with rotary vacuum evaporator. Each of the enriched fractions was then further analyzed by reversed-phase liquid chromatography-atmospheric pressure chemical ionization mass spectrometer (RPLC-APCI/MS) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) with matrix of oxidized carbon nanotubes, respectively. It can be noted totally more than 117 components were detected by UV detector, APCI/MS and MALDI-TOF/MS in *Honeysuckle* extract except the 145 components identified by MALDI-TOF/MS alone with this integrated approach, and 7 of them were preliminary identified according to their UV spectra and mass spectra performed by APCI/MS and MALDI-TOF/MS, respectively. The obtained analytical results not only indicated the approach of integration IEC fractionation with RPLC-APCI/MS and MALDI-TOF/MS is capable of analyzing complex samples, but also exhibited the potential power of the mass spectrometer in detection of low-mass compounds, such as traditional Chinese medicines (TCMs) and complex biological samples.

Keywords: *Honeysuckle*; Ion-exchange chromatography fractionation; LC-APCI/MS; MALDI-TOF/MS; Integrated approach

1. Introduction

As a mainstream separation tool, multi-dimensional separation system in conjunction with modern identified technologies has shown the powerful separation ability, high peak capability and intensive detectability in the analysis of complex samples. Various multi-dimensional chromatography techniques, such as gas chromatography (GC) coupled with GC [1] and liquid chromatography (LC) coupled to LC [2], have been described and applied in different research areas. As a typical format of multi-dimensional separation system, comprehensive two-dimensional liquid chromatography has been commonly used in the research of proteomics [3], metabolomics [4], and in the characterizing of other complex samples [5,6] due to the high degree of automation and versatility, since it appeared in 1978 [7]. As another attractive approach, off-line two-dimensional system has also been established and widely applied in the resolution and identification of peptides [8], proteins [9] and other complex mixtures [10], because it not only can offer easy operation, free choice of solvents to be used and less overlap between the individual separations in the second dimension, also permit varied concentration of volumes in instances of trace analysis.

Because traditional Chinese medicines (TCMs) provide almost infinite resources for drug development, they are gaining more and more attention in modern pharmaceutical institution. Analysis of the components in TCMs is an important subject in order to ensure the reliability and repeatability of pharmacological and clinical research, to understand their bioactivities and possible side effects of active compounds and to enhance product quality control. However, the compositions of TCMs

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are very complicated and usually contain hundreds of chemical constituents, but only a few of them may have pharmaceutical and/or toxic activity. Therefore, efficient and selective methods are required for the analysis and identification of these ingredients from TCMs. Different analytical techniques including thin-layer chromatography (TLC) [11], GC [12], LC [13], biochromatography [14,15] and micellar electrokinetic chromatography (MEKC) [16] have been applied for this purpose. However, with respect to multi-dimensional techniques, up to now, there are only a few reports on the application of multi-dimensional separation system coupled with mass spectrometry for the analysis of TCMs. Zhang et al. [17] applied comprehensive two-dimensional capillary LC with MEKC for the resolution of neutral components in *liquorice*, a typical ingredient in TCMs. Hundreds of components in *liquorice* were separated and identified using such a comprehensive two-dimensional system and the total peak capacity of it was as high as 2000. Yang et al. [18] developed a multi-dimensional counter-current chromatographic system and applied for the preparative separation of isorhamnetin, kaempferol and quercetin from crude flavone aglycones of *Ginkgo biloba* L. and *Hippophae rhamnoides* L. We [19,20] have also constructed a comprehensive two-dimensional liquid chromatography coupled to mass spectrometry system and successfully applied it in the separation and identification of compounds in *Rhizoma chuanxiong* and *G. biloba*.

In this work, an integration approach of ion-exchange chromatography (IEC) fractionation with reversed-phase liquid chromatography-atmospheric pressure chemical ionization mass spectrometer (RPLC-APCI/MS) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) analysis was established and applied for the resolution and determination of components in *Honeysuckle* extract, another most commonly used drug in the prescriptions of TCMs [21]. In this method, APCI/MS and MALDI-TOF/MS were employed to detect the corresponding molecular weight information of components. Totally, more than 117 components in the extract of *Honeysuckle* were well resolved and detected by UV detector, APCI/MS and MALDI-TOF/MS in terms of this hyphenated approach excluding the 145 components identified by MALDI-TOF/MS alone, and 7 of them were preliminary identified according to their UV spectra, mass spectra obtained with APCI/MS and MALDI-TOF/MS, respectively.

2. Experimental

2.1. Instrumentation

The IEC column was prepared by packing Kromasil-SCX (5 μm, 120 Å, Sweden) into a stainless steel column (150 mm × 4.6 mm i.d.), and a linear gradient from 0.5% acetic acid buffer to methanol/0.5% acetic acid buffer (66/34, v/v) in 65 min was used for the fractionation of *Honeysuckle* extract, the flow rate was set as 0.8 mL/min. Kromasil-ODS (5 μm, 120 Å, Sweden) was packed into a column (150 mm × 4.6 mm i.d.) in-house and used to couple with APCI/MS. Separation of IEC fractions were performed on RPLC with a multi-stepwise gradient elution at flow rate of 0.7 mL/min, the mobile phase was adopted with acetonitrile in 0.1% (v/v) acetic acid buffer (6/94, v/v) in the first 23 min, then changed to acetonitrile/0.1% (v/v) acetic acid buffer (14/86, v/v) for the next 17 min, and finally changed to acetonitrile/0.1% (v/v) acetic acid buffer (27/73, v/v) in the rest time. The analytes eluted off the ODS column were detected with an SPD-10Avp diode array detector (Shimadzu, Kyoto, Japan) and an APCI/MS (Shimadzu) simultaneously. The APCI probe voltage was set at 1800 V, the nebulizing gas flow was at 2.5 L/min, the APCI, CDL and block temperature was set at 400 °C, 250 °C and 200 °C, respectively. The mass range [*m*/*z*] was set from 0 to 1000 and the scan speed was set at 0.5 scan/s.

MALDI-TOF/MS analysis were performed on the Bruker AutoFlex™ instruments (Bruker Co., Bremen, Germany) by using matrix of the oxidized carbon nanotubes [22,23], thus the interferences of abundant matrix ion signals in the low mass range with conventionally used organic matrices for the analysis of low-mass compounds were eliminated. The instrument was equipped with a nitrogen laser (λ = 337 nm) to desorb and ionize the samples and its available accelerating potential is in the range of +20 kV/−20 kV. As reported method in our previous works [22,23], the MALDI uses a ground-steel sample target, on which 1 μL of the oxidized carbon nanotubes solution was deposited and dried to form a thin layer of matrix. Then 1 μL of concentrated fraction was deposited and dried on the layer of matrix for MALDI analysis. The analytical range of laser energy was adjusted to slightly above the threshold to obtain good resolution and signal-to-noise ratios. All mass spectra shown were obtained in delayed extraction reflection under pressure less than 1×10^{-4} Pa with delayed time of 40 ns; each spectrum was typically added by 30 laser shots. External mass calibration was obtained by using two points that bracketed the mass range of interest.

The enrichment and concentration of samples were accomplished with RE-85A rotary vacuum evaporator (Shanghai, China) at 45 °C.

2.2. Chemical and reagents

Methanol and acetonitrile were chromatographic grade; acetic acid was analytical grade; distilled water used in all experiments was purified by a Milli-Q system (Milford, MA, USA). Caffeic acid and chlorogenic acid standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). *Honeysuckle* was ordered from a local Chinese medicine store.

2.3. Extraction of Honeysuckle

Two grams of *Honeysuckle* was first crushed with a grinder and immersed in 100 mL of 40% ethanol for 24 h, and then heated to boiling for 45 min. The extract was filtered through a 0.45 μm membrane and stored at 4 °C in the absence of light for subsequent experiments.

3. Results and discussion

As a typical kind of TCMs, *Honeysuckle* has been widely used for the treatment of various kinds of chronic diseases, such as cancer, heart disease and aging [24]. The effective components of this herb are flavonoids, triterpenoid saponins and polyphenolics [25]. The chemical and physical properties of these compounds, such as hydrophobicity, are significantly different. In order to choose the suitable LC separation modes in the integration approach, the experiments of *Honeysuckle* extract on SCX and ODS columns were performed, and the obtained separation chromatograms are shown in Fig. 1. Due to different separation mechanisms on these two columns, different pattern of chromatograms were observed, the separation of a solute on IEC and RPLC was mainly determined by the electrostatic and hydrophobic interaction, respectively. Comparing with the experimental results given in Fig. 1, the ODS column shows better resolution and higher efficiency compared with the SCX column, also the mobile phases used in RPLC are much compatible with APCI/MS detector. Therefore, RPLC with ODS column was chosen for further separation and identification of IEC fractions with detection by UV and APCI/MS.

The mobile phase compositions for fractionation of *Honeysuckle* extract on IEC were optimized with the optimization strategy based on uniform design and genetic algorithm previously reported [26]. The extract of *Honeysuckle* was separated on the SCX column under the linear gradient elution with the optimal conditions, and the corresponding chromatogram is illustrated in Fig. 2. It can be seen all the components in *Honeysuckle* extract were eluted completely from the column in 55 min. So, the eluate from the SCX column was collected every 5 min manually, and a

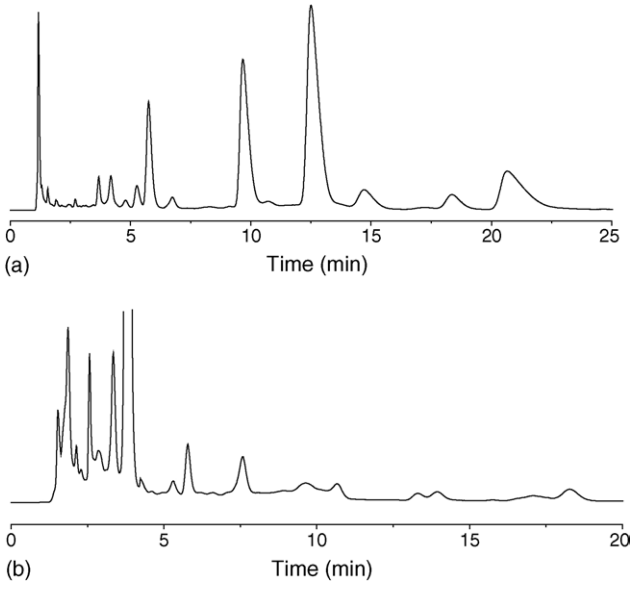

Fig. 1. Chromatograms for *Honeysuckle* extract on SCX and ODS columns. *Experimental conditions*: (a) mobile phase, methanol/0.5% acetic acid buffer (16/84, v/v); column, 150 mm × 4.6 mm i.d. packed with 5 μm Kromasil SCX; flow rate, 0.8 mL/min; injection volume, 10 μL; detection wavelength, 238 nm and (b) mobile phase, acetonitrile/0.1% acetic acid buffer (13/87, v/v); column, 150 mm × 4.6 mm i.d. packed with 5 μm Kromasil ODS. Other conditions are the same as in (a).

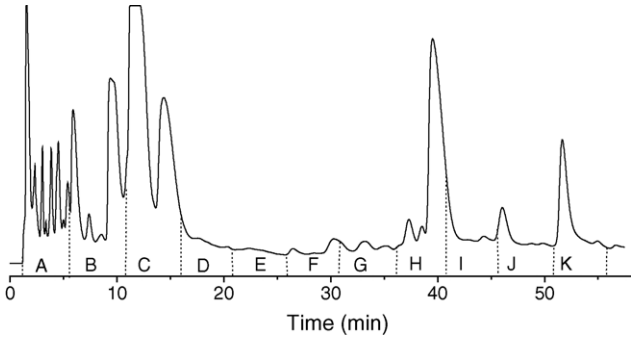

Fig. 2. Chromatogram for fractionation of *Honeysuckle* extract on SCX column. *Experimental conditions*: mobile phase with linear gradient elution from 0.5% acetic acid buffer to methanol/0.5% acetic acid buffer (66/34, v/v) in 65 min; injection volume, 20 μL. Other conditions are the same as in Fig. 1a.

total number of 11 fractions were obtained for further treatment and analysis as shown in Fig. 2.

In order to increase the concentrations of components in the collected fractions and enhance their detection sensitivity in the following analysis by RPLC-APCI/MS and MALDI-TOF/MS, each of the 11 fractions was enriched and concentrated to 1/20th-volume ratio of original with rotary vacuum evaporator.

Subsequently, all the collected and concentrated fractions were injected onto the ODS column, allowing further separation of components in IEC fractions under multi-stepwise gradient elution. Typical chromatograms for separation of the 11 fractions with detection of UV and APCI/MS are shown in Fig. 3. As it can be seen, the coeluting peaks in the fractions from the SCX column are more resolved with RPLC due to the improved resolving power and different separation selectivity of the ODS column. Simultaneously, all the 11 fractions were further analyzed by MALDI-TOF/MS in both negative and positive ionization modes with the matrix of oxidized carbon nanotubes. It was found that a large number of individual species of low-mass compounds were particularly detected in the fractions with the high detection accuracy and sensitivity, and the obtained mass spectra are illustrated in Fig. 4.

It should be pointed out that some analytes separated on SCX column were possibly presented in more than one fraction, so chromatograms of different fractions on RPLC show some peaks with the same UV and MS spectra, which should be identified as the same components, even they were detected in different fractions, and also the mass spectra analyzed by MALDI-TOF/MS supported this evidence. For example, more than 32 peaks were separated on the ODS column and identified with their UV and MS spectra in fraction A as shown in Fig. 3a, and 5 of them were also observed in fraction B as shown in Fig. 3b, so these five peaks are regarded as the same components in faction A and not marked again in the chromatogram of fraction B in Fig. 3b. All the well-separated components can be possibly identified by comparing their retention times on the ODS column, UV spectra, APCI/MS spectra and corresponding MALDI-TOF/MS spectra, respectively. By summarizing the experimental results as shown in Figs. 3 and 4, totally more than 117 components from the extract of *Honeysuckle* were detected by UV detector, APCI/MS and MALDI-TOF/MS and 145 components were detected by

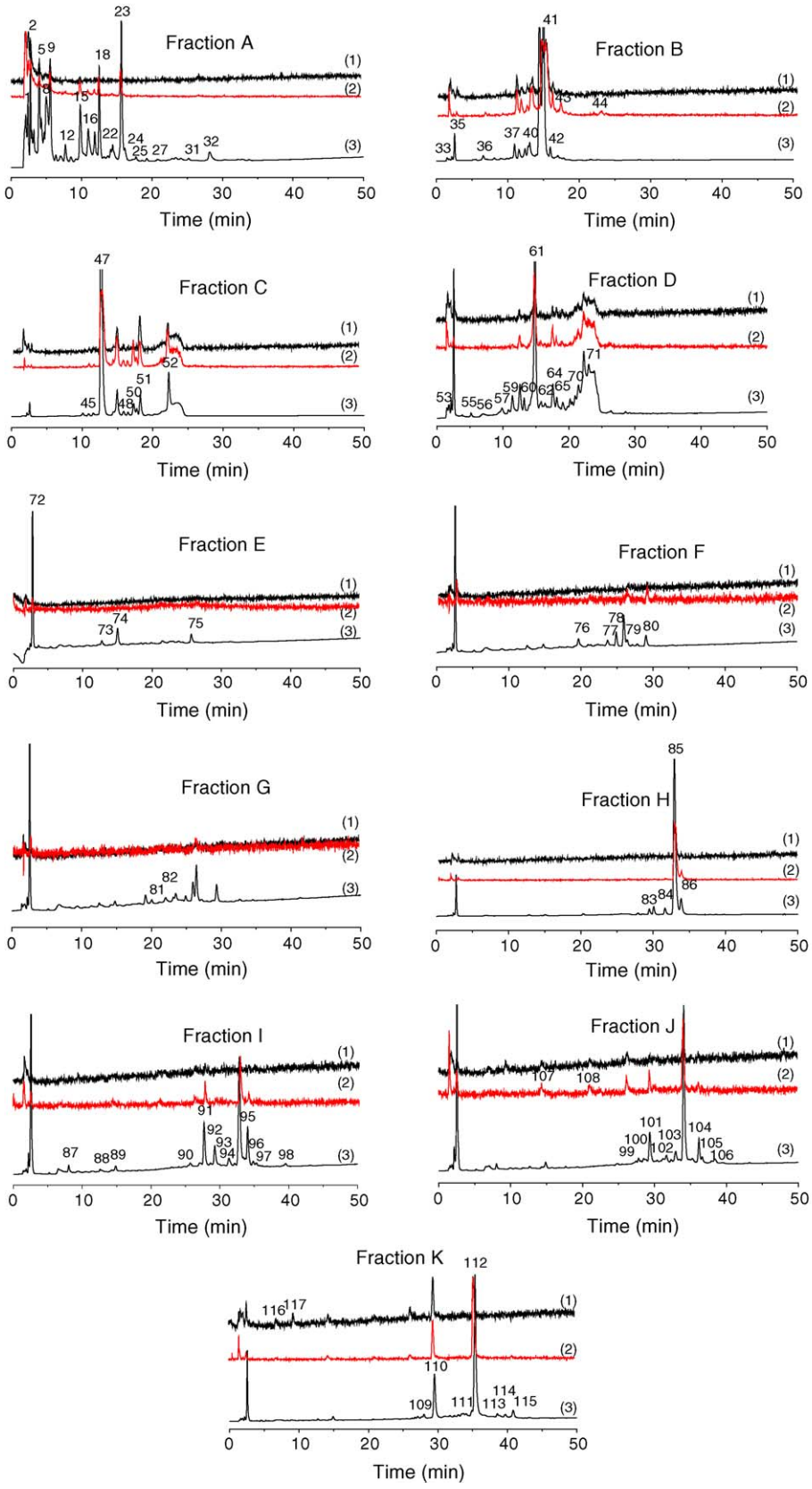

Fig. 3. (A–K) Chromatograms for reversed-phase liquid chromatography separation of the fractions A–K from SCX column of *Honeysuckle* extract with detection by UV and APCI/MS. *Experimental conditions*: mobile phase with acetonitrile/0.1% acetic acid buffer (6/94, v/v) from 0 min to 23 min, and with 14% acetonitrile (v/v) from 23 min to 40 min, then with 27% acetonitrile (v/v) from 40 min to 50 min; flow rate, 0.7 mL/min; injection volume, 20 μL. Other conditions are the same as in Fig. 1(b). The height of all peaks determined by (1) relative intensity using APCI positive ion mode; (2) APCI negative ion mode and (3) the relative UV absorbance.

MALDI-TOF/MS alone in the integration of IEC fractionation with RPLC-APCI/MS and MALDI-TOF/MS analysis system, and the distribution of them is shown in Fig. 5. It can be seen from Figs. 3–5, among the 117 components, totally 11 components could be simultaneously detected by UV detector, APCI/MS and MALDI-TOF/MS as summarized in Table 1, and 42 components were detected by both UV detector and APCI/MS as listed in Table 2. Moreover, 58 components could be only detected by UV detector and 6 were detected only by APCI/MS with positive or negative ion detection modes as listed in Table 3, respectively. Besides these, the 145 dominant molecular ion peaks were detected by MALDI-TOF/MS alone as listed in Table 4. Those results strongly indicated that LC-APCI/MS and MALDI-TOF/MS with matrix of oxidized carbon nanotubes are very complimentary for detection and analysis of small molecules in complex samples.

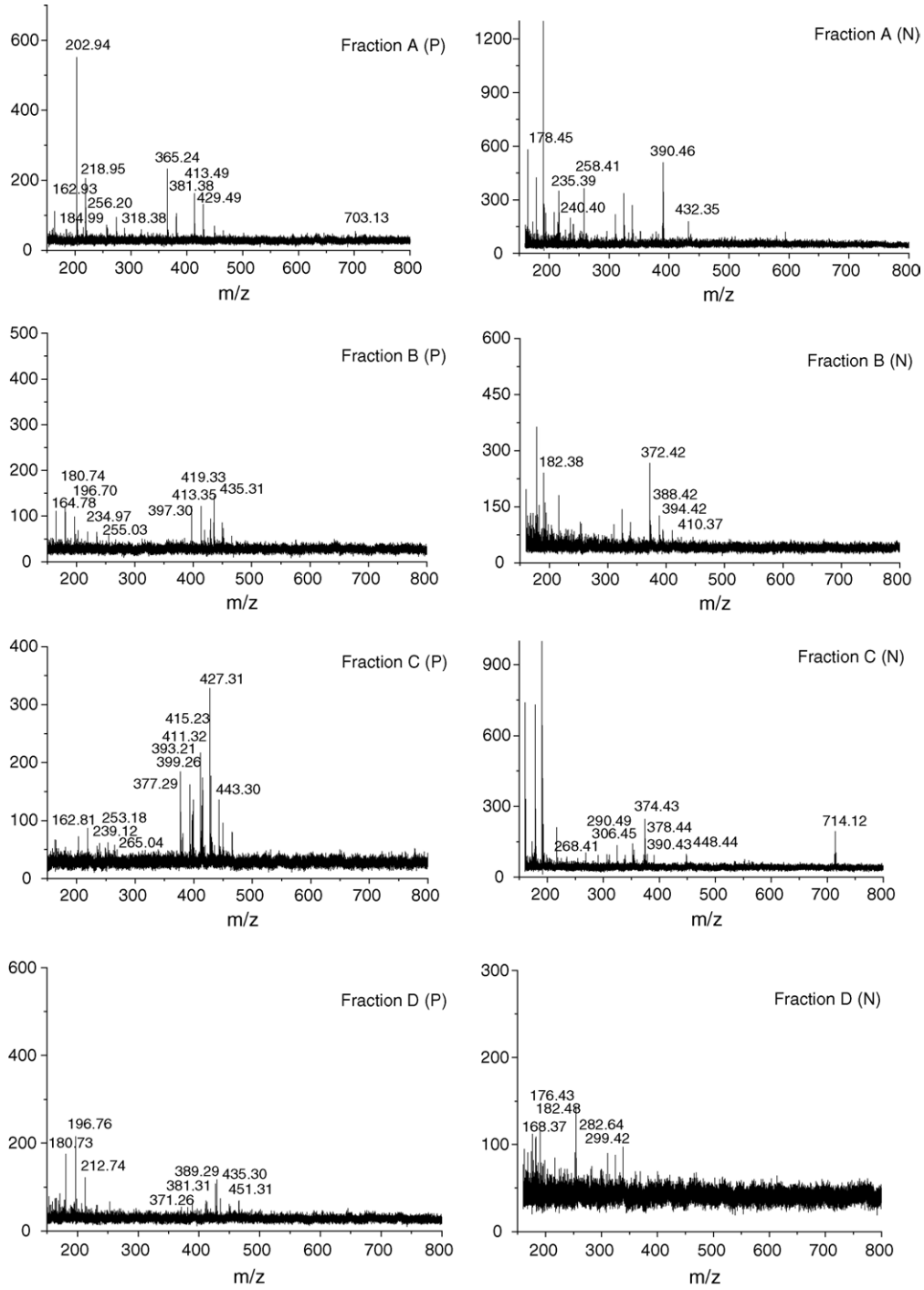

Fig. 4. (A–K) Mass spectra for the fractions A–K from SCX column of *Honeysuckle* extract analyzed with MALDI-TOF/MS. The mass spectra of all fractions were obtained with the laser power adjusted to slightly above the threshold energy for all of the components with the matrix of oxidized carbon nanotubes and detected in (P) positive ion mode and (N) negative ion mode.

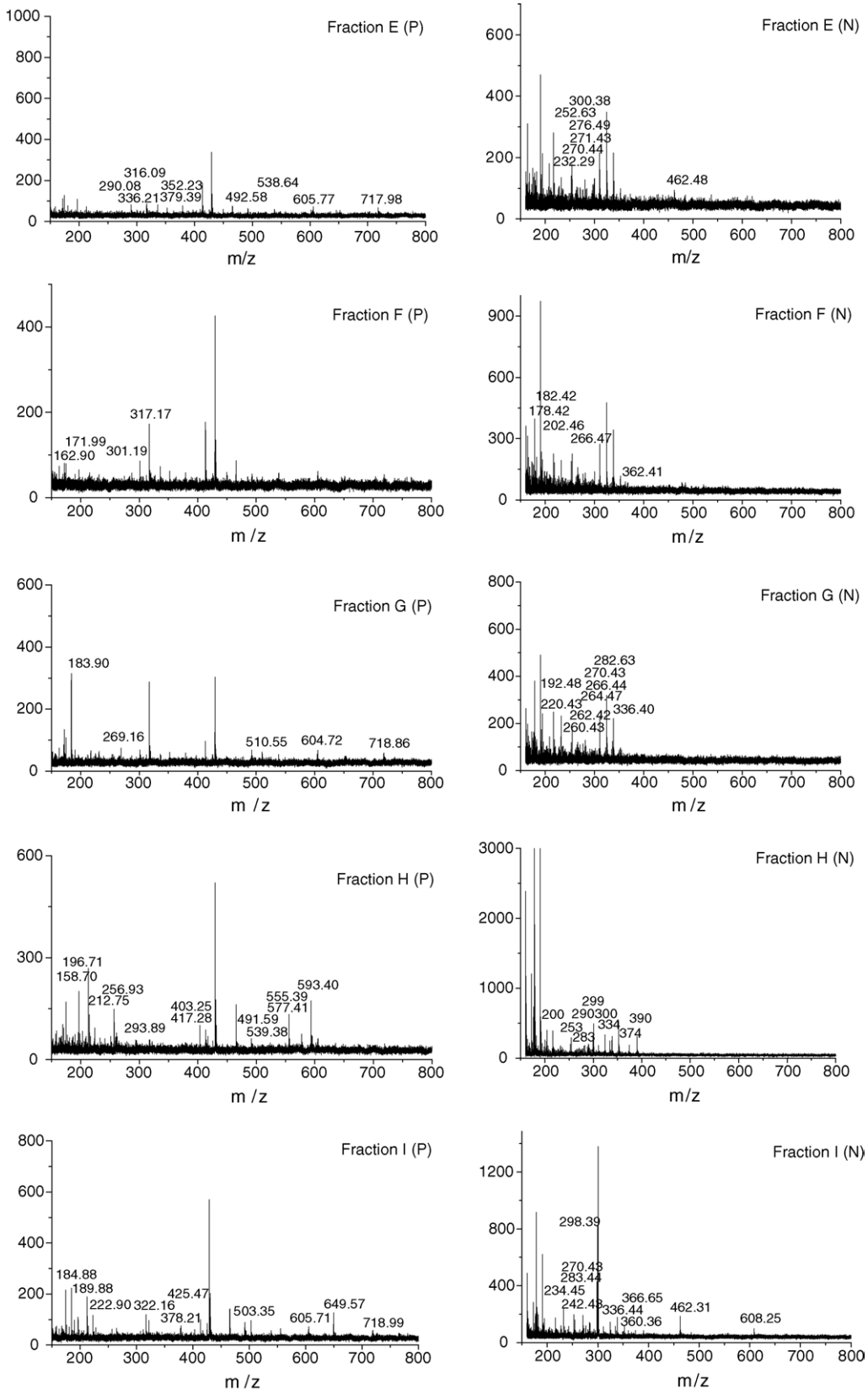

Fig. 4. (*Continued*)

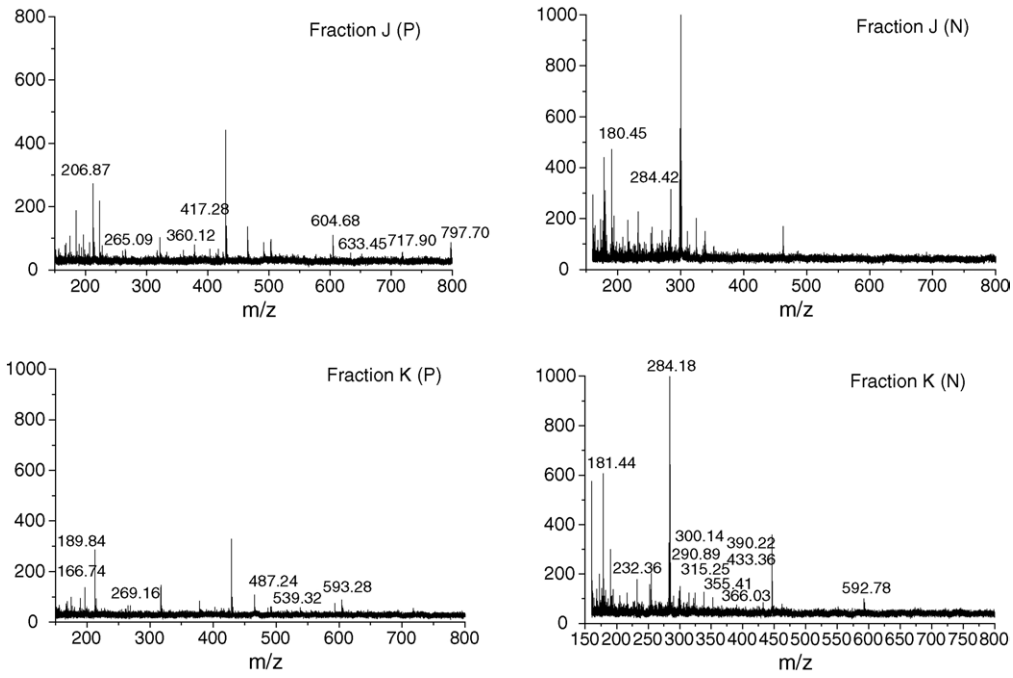

Fig. 4. (*Continued*).

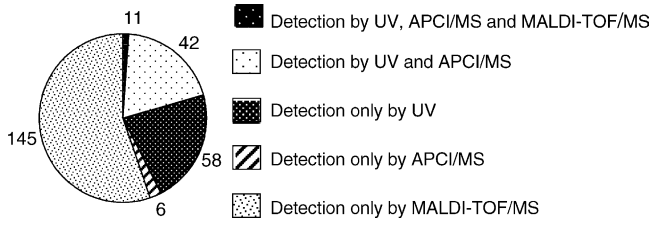

Fig. 5. Distribution of all the 262 components in *Honeysuckle* extract detected by UV detector, APCI/MS and MALDI-TOF/MS, respectively.

These experimental results indicated that the method being described here could provide several potential advantages in the analysis of complex samples. Firstly, the integration of IEC fractionation with RPLC system has shown the high peak capacity and powerful resolution ability in isolation of complex samples owing to the free choice of columns and separation modes. Secondly, the LC coupling APCI/MS makes the system a stronger capacity for quickly screening the mass of major components, and also it can increase the separation capacity because of the high sensitivity and selectivity with low chemical noise. Thirdly, as a popular and versatile analysis method, MALDI-TOF/MS provides a completely different principle from LC/MS in determination of components. The mass spectrum of a sample by

Table 1
Eleven components separated from the extract of *Honeysuckle* with detection by UV detector, APCI/MS and MALDI-TOF/MS

Fraction	Peak	*m/z*		
		APCI/MS		MALDI-TOF/MS
		(P)	(N)	(P)
A	9	391.05	389.05	413.49 $(M+Na)^+$, 429.49 $(M+K)^+$
	12	391.05	389.10	413.49 $(M+Na)^+$, 429.49 $(M+K)^+$
	23	–	389.05	413.49 $(M+Na)^+$, 429.49 $(M+K)^+$
B	41	375.05	373.05	397.30 $(M+Na)^+$, 413.34 $(M+K)^+$
C	46	355.05	353.05	377.24 $(M+Na)^+$, 393.21 $(M+K)^+$
	52	–	403.05	427.31 $(M+Na)^+$, 443.30 $(M+K)^+$
D	65	359.05	357.10	381.31 $(M+Na)^+$
H	85	–	515.05	539.36 $(M+Na)^+$, 555.39 $(M+K)^+$
	86	–	515.05	539.36 $(M+Na)^+$, 555.39 $(M+K)^+$
I	89	340.20	–	378.41 $(M+K)^+$
K	112	–	515.05	538.56 $(M+Na)^+$

Notes: (–) denotes the peak not detected.

Table 2
Forty two components separated from the extract of *Honeysuckle* with detection by UV detector and APCI/MS

Fraction	Peak	*m*/*z* APCI/MS	
		(P)	(N)
A	1	–	290.00
	2	–	190.90
	5	375.05	373.05
	15	355.05	353.05
	16	–	375.05
	17	–	373.05
	18	–	375.05
B	33	–	305.00
	34	–	121.95
	35	–	118.90
	36	–	134.90
	37	465.10	463.10
	38	–	401.05
	39	471.15	469.05
	40	–	375.05
	42	509.15	507.15
C	45	–	465.15
	47	–	401.05
	48	–	449.10
	49	355.00	353.00
	50	–	449.10
	51	419.05	417.05
D	53	–	191.0
	60	–	222.95
	61	–	373.00
	62	342.00	340.00
	63	357.00	355.00
	64	339.00	337.00
	66	–	445.10
	67	301.10	299.10
	68	–	447.10
	69	–	462.95
	70	389.10	387.10
	71	–	447.10
E	75	566.35	564.25
I	87	–	227.05
	90	566.35	564.30
	91	302.95	301.00
	95	–	291.00
J	104	–	291.05
K	110	–	134.90
	111	227.05	225.05

Notes: (–) denotes the peak not detected.

MALDI-TOF/MS not only support the identification of individual analyte by UV and APCI/MS, but also particularly provide a tool for resolution of analytes completely different from LC/MS.

Comparing with the structural data reported in literatures of the components in *Honeysuckle* [27–31], 7 of the 262 components were identified as quercetin-3-*O*-β-D-glucoside (peak 37), luteolin-7-*O*-α-D-glucoside (peak 44), chlorogenic acid 4 (peak 46), quercetin (peak 91), 3,5-*O*-dicaffeoylquinic acid (peak 112), luteolin (peak 116) and caffeic acid, respectively. The analysis of structural information for them obtained from

Table 3
Components separated from the extract of *Honeysuckle* with detection only by UV detector and APCI/MS, respectively

Fraction	Peak	UV	APCI/MS	
			m/*z* (P)	*m*/*z* (N)
A	3	+	–	–
	4	+	–	–
	6	+	–	–
	7	+	–	–
	8	+	–	–
	10	+	–	–
	11	+	–	–
	13	+	–	–
	14	+	–	–
	19	+	–	–
	20	+	–	–
	21	+	–	–
	22	+	–	–
	24	+	–	–
	25	+	–	–
	26	+	–	–
	27	+	–	–
	28	+	–	–
	29	+	–	–
	30	+	–	–
	31	+	–	–
	32	+	–	–
B	43	–	–	461.15
	44	–	–	447.10
D	54	+	–	–
	55	+	–	–
	56	+	–	–
	57	+	–	–
	58	+	–	–
	59	+	–	–
E	72	+	–	–
	73	+	–	–
	74	+	–	–
F	76	+	–	–
	77	+	–	–
	78	+	–	–
	79	+	–	–
	80	+	–	–
G	81	+	–	–
	82	+	–	–
H	83	+	–	–
	84	+	–	–
I	88	+	–	–
	92	+	–	–
	93	+	–	–
	94	+	–	–
	96	+	–	–
	97	+	–	–
	98	+	–	–
J	99	+	–	–
	100	+	–	–
	101	+	–	–
	102	+	–	–
	103	+	–	–
	105	+	–	–
	106	+	–	–
	107	–	340.10	338.10

Table 3 (*Continued*)

Fraction	Peak	UV	APCI/MS	
			m/*z* (P)	*m*/*z* (N)
	108	–	453.30	451.30
K	109	+	–	–
	113	+	–	–
	114	+	–	–
	115	+	–	–
	116	–	286.95	284.95
	117	–	162.95	160.95

Notes: (+) denotes the peak detected and (–) denotes the peak not detected.

UV spectra, mass spectra of APCI/MS and MALDI-TOF/MS is described as follows.

Fig. 6 shows UV and APCI mass spectra of peaks 37, 44, 46, 91, 112 and 116, respectively. The APCI/MS spectrum of peak 37 as shown in Fig. 6a exhibits mass ion signals at *m*/*z* 465.10 (M^+) and *m*/*z* 463.10 (M^-). By comparing the mass spectrum with literature data [27], peak 37 was preliminary identified as quercetin-3-*O*-β-D-glucoside. Gao et al. [27] separated and identified luteolin-7-*O*-α-D-glucoside from *Honeysuckle*, the molecular weight was measured as 448.38 and the UV spectra with maximal absorption at 256 nm, these information are quite similar with peak 44 shown in Fig. 6b, so the compound associated with peak 44 could be identified as luteolin-7-*O*-α-D-glucoside. Chlorogenic acid was reported as an active compound in *Honeysuckle* [28], the UV spectrum, APCI/MS and MALDI-TOF/MS

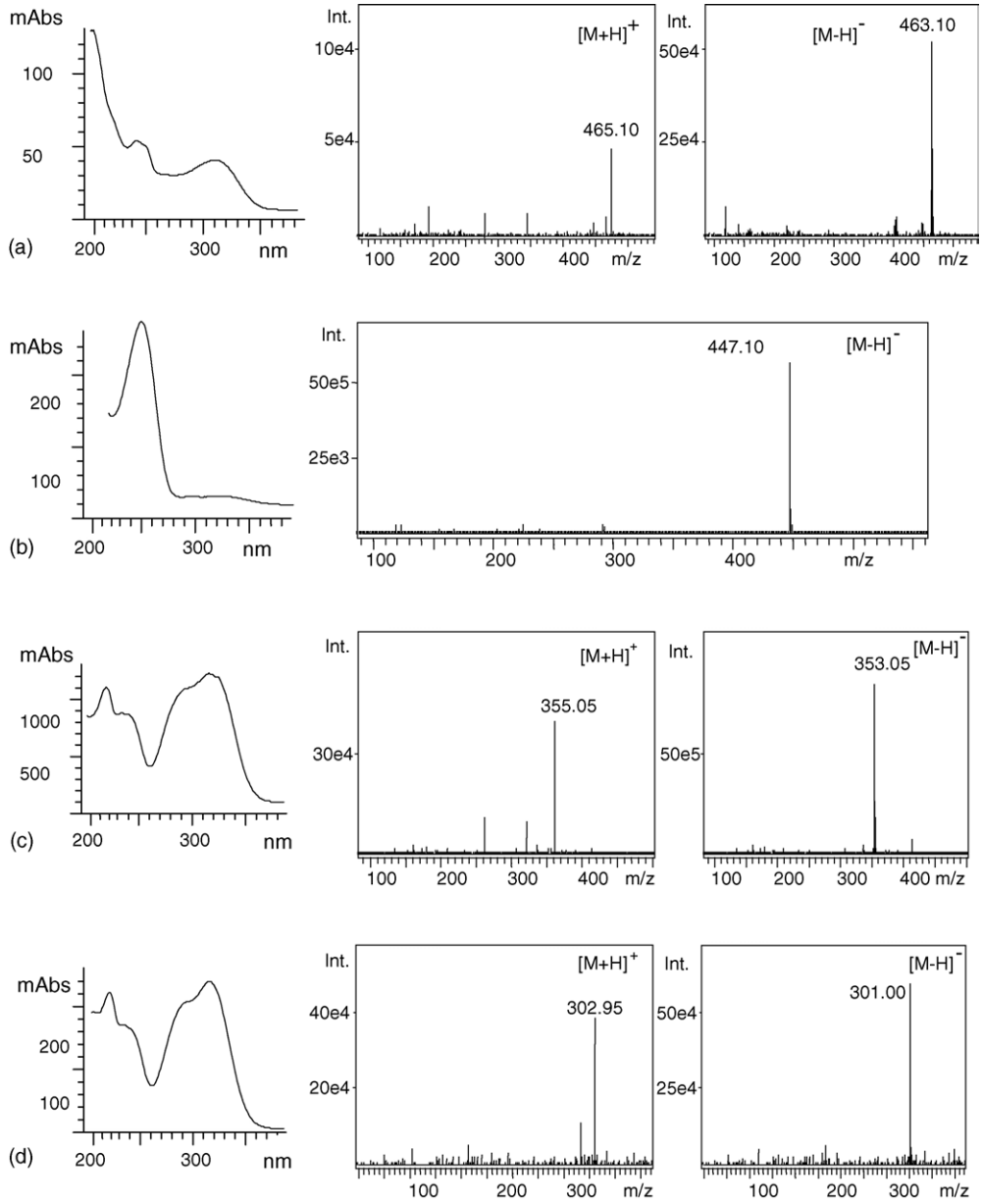

Fig. 6. UV spectra and APCI mass spectrum for detection of components in *Honeysuckle* extract. *Solutes*: (a) peak 37; (b) peak 44; (c) peak 46; (d) peak 91; (e) peak 112; (f) peak 116 in Fig. 3.

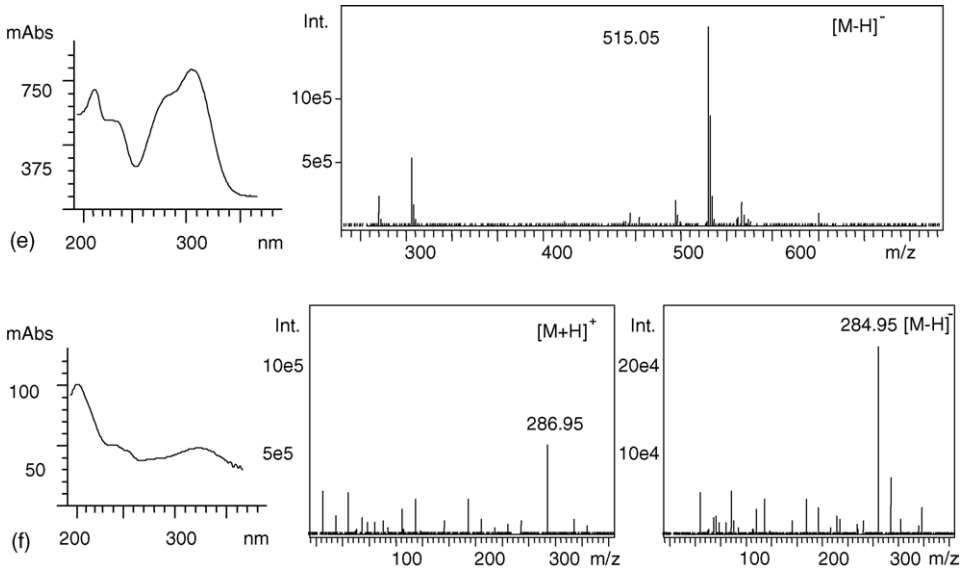

Fig. 6. (*Continued*).

Table 4
145 components in 11 fractions from SCX column identified with MALDI-TOF/MS with matrix of oxidized carbon nanotubes alone

Fraction	No.	MALDI-TOF/MS		No.	MALDI-TOF/MS	
		m/z (P)	*m/z* (N)		*m/z* (P)	*m/z* (N)
A	1	162.93		8		178.45
	2	184.99		9		235.39
	3	202.94 $(M+Na)^+$, 218.95 $(M+K)^+$		10		240.40
	4	256.20		11		258.41
	5	318.38		12		390.46
	6	365.24 $(M+Na)^+$, 381.30 $(M+K)^+$		13		432.35
	7	703.13				
B	14	164.78		19		182.38
	15	180.74 $(M+Na)^+$, 196.70 $(M+K)^+$		20		372.42
	16	234.97		21		388.42
	17	255.03		22		394.42
	18	419.33 $(M+Na)^+$, 435.31 $(M+K)^+$		23		410.37
C	24	162.81		32		290.49
	25	239.12		33		306.45
	26	253.18		34		374.43
	27	265.04		35		378.44
	28	399.26		36		390.43
	29	411.32 $(M+Na)^+$, 427.31$(M+K)^+$		37		448.44
	30	415.23		38		714.12
	31		268.41			
D	39	180.73		44		168.37
	40	196.76 $(M+Na)^+$, 212.74 $(M+K)^+$		45		176.43
	41	371.26		46		182.48
	42	389.29		47		282.64
	43	435.30 $(M+Na)^+$, 451.31 $(M+K)^+$		48		299.42
E	49	290.08 $(M+Na)^+$, 316.09 $(M+K)^+$		56		232.29
	50	336.21 $(M+Na)^+$, 352.23 $(M+K)^+$		57		252.63
	51	379.39		58		270.44
	52	492.58		59		271.43
	53	538.64		60		276.49
	54	605.77		61		300.38
	55	717.98		62		462.48

Table 4 (*Continued*)

Fraction	No.	MALDI-TOF/MS		No.	MALDI-TOF/MS	
		m/z (P)	*m/z* (N)		*m/z* (P)	*m/z* (N)
F	63	162.90		67		182.42
	64	171.99		68		202.46
	65	301.19 $(M+Na)^+$, 317.17 $(M+K)^+$		69		266.47
	66		178.42	70		362.41
G	71	183.90		78		260.43
	72	269.16		79		262.42
	73	510.55		80		264.47
	74	604.72		81		266.44
	75	718.86		82		270.43
	76		192.48	83		282.63
	77		220.43	84		336.40
H	85	158.70		94		252.58
	86	196.71 $(M+Na)^+$, 212.75 $(M+K)^+$		95		282.62
	87	256.93		96		290.42
	88	293.89		97		299.37
	89	403.25		98		300.38
	90	417.28		99		334.39
	91	491.59		100		374.38
	92	577.41 $(M+Na)^+$, 593.40 $(M+K)^+$		101		390.33
	93		200.41			
I	102	184.88		111		234.45
	103	189.88		112		242.43
	104	222.90		113		270.43
	105	322.16 $(M+Na)^+$	298.39	114		283.44
	106	425.47		115		336.44
	107	503.35		116		360.36
	108	605.71		117		366.65
	109	649.57		118		462.31
	110	718.99		119		608.25
J	120	206.87		125	633.45	
	121	265.09		126	717.90	
	122	360.12		127	797.70	
	123	417.28		128		180.45
	124	604.68		129		284.42
K	130	166.74		138		290.89
	131	199.84		139		300.14
	132	269.16		140		315.25
	133	487.24		141		355.41
	134	593.28		142		366.03
	135		181.44	143		390.22
	136		232.36	144		433.36
	137		284.18	145		592.78

Notes: P denotes positive detection mode and N denotes negative detection mode.

spectra of standard of chlorogenic acid is quite accordant to peak 46, which are revealed in Figs. 6c and 4c, respectively, so peak 46 was preliminary identified as chlorogenic acid. It can be seen in Fig. 6d that peak 91 shows molecular ion signals at *m/z* 302.95 (M^+) and *m/z* 301.00 (M^-), which were similar to those of quercetin, so peak 91 was identified as quercetin according to the literature data [29]. As it can be seen in Fig. 6e that peak 112 shows mass ion signals at *m/z* 515.05 (M^-) and the corresponding Na^+ adduct ion peaks at *m/z* 539.32 $(M+Na)^+$on MALDI-TOF/MS spectrum were also observed as indicated in Fig. 4k, which were similar to those of 3,5-*O*-dicaffeoylquinic acid reported in literature [30], so peak 112 was identified as 3, 5-*O*-dicaffeoylquinic acid. By comparing the mass spectrum of peak 116 and that of luteolin [29], peak 116 was preliminary identified as luteolin, whose APCI/MS spectrum exhibits mass ion signals at *m/z* 286.95 (M^+) and *m/z* 284.95 (M^-) as shown in Fig. 6f. As reported by Zhao et al. [31], caffeic acid was also an active compound in *Honeysuckle* and the molecular weight was calculated as 180.16, it was not detected neither by UV detector nor with APCI/MS, but the corresponding Na^+/K^+ adduct ion peak at *m/z* 202.94 $(M+Na)^+$ and *m/z* 218.95 $(M+K)^+$ were characterized by MALDI-TOF/MS analysis as shown in Fig. 4a.

4. Conclusions

An integration of IEC fractionation with RPLC-APCI/MS and MALDI-TOF/MS system is particularly constructed and successfully employed in the isolation and detection of com-

ponents in *Honeysuckle* extract, a commonly used traditional Chinese medicine. The collected fractions from SCX column were further enriched, and then separated on an ODS column and identified by UV detector and APCI/MS as well as MALDI-TOF/MS with matrix of oxidized carbon nanotubes, the LC combined APCI/MS further reduces the chance of undetected components in coeluting peaks by UV detector and MALDI-TOF/MS also shows resolution ability completely different from LC/MS with the accuracy and sensitivity for determination of low-mass compounds. With this integrated approach more components were well resolved and detected from the extract of *Honeysuckle*, totally more than 117 components were detected by UV detector, APCI/MS and MALDI-TOF/MS, and also 145 components could be detected with MALDI-TOF/MS alone, among them 7 components were preliminary identified according to their UV spectra and mass spectra performed by APCI/MS and MALDI-TOF/MS, respectively. The obtained results not only indicated the approach of integration IEC fractionation with RPLC-APCI/MS and MALDI-TOF/MS is capable of analyzing complex samples, but also exhibited its potential in identification of low-mass compounds, such as TCMs and complex biological samples.

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