Anal Bioanal Chem (2015) 407:4841–4848
DOI 10.1007/s00216-015-8668-4

NOTE

High-speed homogenization coupled with microwave-assisted extraction followed by liquid chromatography–tandem mass spectrometry for the direct determination of alkaloids and flavonoids in fresh *Isatis tinctoria* L. hairy root cultures

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Received: 6 January 2015 / Revised: 12 March 2015 / Accepted: 27 March 2015 / Published online: 17 April 2015

Abstract A new, simple and efficient analysis method for fresh plant in vitro cultures—namely, high-speed homogenization coupled with microwave-assisted extraction (HSH–MAE) followed by liquid chromatography–tandem mass spectrometry (LC–MS/MS)—was developed for simultaneous determination of six alkaloids and eight flavonoids in *Isatis tinctoria* hairy root cultures (ITHRCs). Compared with traditional methods, the proposed HSH–MAE offers the advantages of easy manipulation, higher efficiency, energy saving, and reduced waste. Cytohistological studies were conducted to clarify the mechanism of HSH–MAE at cellular/tissue levels. Moreover, the established LC–MS/MS method showed excellent linearity, precision, repeatability, and reproducibility. The HSH–MAE–LC–MS/MS method was also successfully applied for screening high-productivity ITHRCs. Overall, this study opened up a new avenue for the direct determination of secondary metabolic profiles from fresh plant in vitro cultures, which is valuable for improving quality control of plant cell/organ cultures and sheds light on the metabolomic analysis of biological samples.

Keywords *Isatis tinctoria* hairy root cultures · High-speed homogenization · Microwave-assisted extraction · Alkaloids · Flavonoids · Liquid chromatography–tandem mass spectrometry

Introduction

Isatis tinctoria L. (Cruciferae) is a biennial herbaceous plant that is widely distributed in China and other Asian countries. *I. tinctoria* root (*Radix isatidis*) is used in traditional Chinese medicine for the treatment of inflammatory diseases [1]. Over the past decades, numerous compounds belonging to various structural classes such as alkaloids, flavonoids, fatty acids, porphyrins, lignans, carotenoids, glucosinolates, cyclohexenones, amino acids, and isoprenoids have been identified in *I. tinctoria* [1–3]. Moreover, extraction methods such as supercritical fluid extraction and pressurized liquid extraction have been applied to obtain extracts from *I. tinctoria* leaves for the analysis of chemical compositions [2–5].

I. tinctoria hairy root cultures (ITHRCs) generated from the genetic transformation of *Agrobacterium rhizogenes* have become feasible to produce active compounds effectively, economically, and in an environmentally friendly way [6]. Therefore, the establishment of a valid analytical method to determine bioactive components and control the quality of ITHRCs is extremely important. Recently, liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) in selected reaction monitoring (SRM) mode has excellent sensitivity and selectivity for elucidating or confirming the chemical structures of target constituents in complex

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Electronic supplementary material The online version of this article (doi:10.1007/s00216-015-8668-4) contains supplementary material, which is available to authorized users.

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biological samples [7]. However, sample preparation is still the most tedious and time-consuming step before LC–MS/MS analysis, which is recognized as the main bottleneck of the analytical process. The development of analytical systems that couple sample pretreatment methods with LC–MS/MS is one of the predominant trends in modern bioanalytical chemistry [8].

Since plant cell/organ cultures contain high water contents, the development of a novel strategy that eliminates biomass drying and enhances extraction efficiency can lead to significant energy and cost savings. High-speed homogenization (HSH) is an effective sample pretreatment technique, which can directly facilitate the destruction of fresh materials for better access to intracellular substances [9]. Microwave-assisted extraction (MAE) is a simple and efficient extraction technique that can decrease the solvent volume, shorten the process time, and improve the extraction efficiency [10]. Moreover, the abundant in situ water in fresh plant cell/organ cultures is caused to rotate under microwave irradiation, and thus the immediate internal change results in a subsequent pressure increase inside plant cells/organs, which leads to breakdown of cell walls and exhaustive release of intracellular target molecules. However, MAE has not been reported being used for the direct extraction of phytochemicals from fresh plant in vitro cultures.

This study aimed to develop an efficient and sensitive analytical method based on LC–MS/MS for the simultaneous determination of six alkaloids and eight flavonoids in ITHRCs. HSH coupled with MAE was used for the sample preparation, and its superiority compared with conventional methods was evaluated in terms of extraction efficiency and green aspects. A sensitive and accurate LC–MS/MS method was established and validated for simultaneous qualitative and quantitative analysis of 14 target compounds. The applicability of the HSH–MAE–LC–MS/MS method developed was checked by screening high-productivity ITHRCs among eight candidates.

Materials and methods

Materials and reagents

Eight *I. tinctoria* hairy root lines (I–VIII) were successfully induced via the genetic transformation of *A. rhizogenes* LBA9402 strain in our laboratory [6]. Eight ITHRCs (I–VIII) originating from different hairy root lines were harvested by filtration after 4 weeks of cultivation, and their moisture contents were predetermined for the further quantitative analysis. The standard compounds epigoitrin (EPI), isatin (ISA), indole-3-carboxaldehyde (INC), tryptanthrin (TRY), indigo (ING), indirubin (INR), rutin (RUT), neohesperidin (NEO), buddleoside (BUD), liquiritigenin (LIQ), quercetin (QUE), isorhamnetin (ISR), kaempferol (KAE), and isoliquiritigenin (ISL) were purchased from Weikeqi Biological Technology (Sichuan province, China). Other reagents of either analytical grade or optical grade were obtained from Beijing Chemical Reagents (Beijing, China). Ultrapure water with a resistivity of 18.3 MΩ cm was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

HSH–MAE procedure

HSH pretreatment was achieved with a T 18 digital Ultra-Turrax system (IKA, Staufen Germany) at a homogenization speed of 8,000 rpm of. MAE was performed with a MAS-II microwave-accelerated reactor (Sineo Chemical Equipment, Shanghai, China). The extraction temperature was monitored by an infrared temperature sensor and controlled by feedback to the microwave power regulator. The results of our preliminary experiments showed that ITHRCs III, V, and VII exhibited better capabilities of biomass production as compared with the other candidates. For the purpose of obtaining a more abundant source of raw materials and more quickly, ITHRC III was selected as the representative for sample extraction by HSH–MAE and conventional procedures in this study.

Fresh ITHRC III (5.0 g) was directly added to the Ultra-Turrax device for the HSH pretreatment. Subsequently, the homogenates and 80 % ethanol solution (30 mL) were introduced into the reaction flask, which was placed symmetrically in the microwave resonance cavity. After the extraction, the solution obtained was centrifuged and filtered through a 0.22-μm nylon membrane for the LC–MS/MS analysis. To achieve the optimum efficiency by HSH–MAE, a Box–Behnken design was applied to survey the effects of homogenization time, extraction temperature, microwave power, and extraction time on the sum yield of target analytes. The actual and coded levels of independent variables used in the experimental design are summarized in Table S1. The experimental data were analyzed statistically with Design-Expert 7.0 (Stat-Ease, Minneapolis MN, USA). Analysis of variance (ANOVA) was performed to calculate and simulate the optimal values of the parameters tested.

Conventional procedures

The washed ITHRC III was dried in a vacuum drier at 60 °C for 36 h. The materials obtained were ground to fine powders and extracted by reported Soxhlet extraction (SE) and ultrasound-assisted extraction (UAE) methods with slight modifications [11, 12]. For SE, root powders (0.5 g) were placed in a Soxhlet apparatus and extracted with 80 % ethanol solution (30 mL) at 90 °C for 4 h. For UAE, root powders (0.5 g) were extracted with 80 % ethanol solution (30 mL) in a KQ-250DB ultrasonic bath (Kun-shan Ultrasonic Instrument,

China) for 120 min. After the extraction, the subsequent analysis was the same as for HSH–MAE.

LC–MS/MS analysis

An Agilent 1100 series high-performance LC system (Agilent Technologies, San Jose, CA, USA) coupled to an API 3000 triple-quadrupole mass spectrometer (Applied Biosystems, Concord, ON, Canada) equipped with a Phenomenex Gemini C_{18} 110 Å reversed-phase column (250 mm×4.6-mm inner diameter, 5 μm) was applied for the analysis of target compounds in ITHRCs. The binary mobile phase consisted of acetonitrile (solvent A) and 0.002 % formic acid aqueous solution (solvent B) using the following gradient program: 0–3 min, 35 % solvent A; 3–5 min, 35–40 % solvent A; 5–15 min, 40–50 % solvent A; 15–20 min, 50–60 % solvent A; 20–25 min, 60–80 % solvent A; 25–28 min, 80 % solvent A; and 28–30 min, 80–35 % solvent A. The column temperature was maintained at 30 °C. The flow rate was 1.0 mL/min, and the injection volume was 10 μL.

All mass spectra of target analyses were acquired in SRM mode with an electrospray ionization (ESI) source operating in negative ion mode. The universal operational parameters were set as follows: ion source temperature 300 °C; ion spray voltage −4,500 V; nebulizing gas 12 a.u.; curtain gas 10 a.u.; collision gas 6 a.u.; focusing potential −75 V; and entrance potential −10 V. To obtain the highest signal response for each analyte, the specific parameters for acquiring the optimal precursor/product ion combinations were optimized–that is, declustering potential, collision energy, and collision cell exit potential (Table 1). The contents of the target compounds were calculated from corresponding calibration curves based on the dry weight of roots. For fresh ITHRCs, dry weights were obtained by converting fresh weights by the aid of the moisture contents.

Statistical analysis

The results are expressed as the mean±the standard deviation. The data were statistically analyzed using SPSS, version 17.0 (SPSS, Chicago, IL, USA). Differences between means were determined by ANOVA with Duncan's test on the level of significance declared at $P<0.05$.

Results and discussion

Optimization of HSH–MAE conditions

HSH can handle fresh plant materials in a continuous stream with turbulence, shear stress, and friction, which can induce the rupture of plant matrices for enhancement of the following extraction process [9]. MAE has attracted significant attention in the extraction of active constituents from plant materials, owing to its special heating mechanism, moderate capital cost, and outstanding performance under atmospheric conditions. Microwave radiation can penetrate into plant materials, provide localized heating in samples, and act as a driving force to destroy plant matrices so that analytes can diffuse out and dissolve in extraction solvents [10].

Table 1 Mass spectrometry parameters for the target analytes

Analyte	DP (V)	CE (V)	CXP (V)	Ion combinations (amu)
EPI	−19	−20	−8	127.8→58.0
ISA	−48	−20	−5	146.0→118.0
INC	−40	−37	−9	143.9→115.1
TRY	−52	−33	−12	247.2→218.9
ING	−40	−35	−11	261.0→217.0
INR	−93	−37	−5	261.2→157.0
RUT and NEO	−52	−42	−14	609.3→301.4
BUD	−55	−18	−8	591.5→283.1
LIQ and ISL	−40	−29	−5	255.4→119.0
QUE	−48	−32	−8	301.0→151.0
ISR	−43	−34	−5	315.0→300.1
KAE	−31	−50	−4	285.3→183.1

The nebulizing gas, curtain gas, and collision gas were set at 12, 10, and 6 a.u., respectively, the ion source temperature was 300 °C, the ion spray voltage was −4500 V, and the focusing potential and entrance potential were −75 and −10 V, respectively

BUD buddleoside, *CE* collision energy, *CXP* cell exit potential, *DP* declustering potential, *EPI* epigoitrin, *INC* indole-3-carboxaldehyde, *ING* indigo, *INR* indirubin, *ISA* isatin, *ISL* isoliquiritigenin, *ISR* isorhamnetin, *KAE* kaempferol, *LIQ* liquiritigenin, *NEO* neohesperidin, *QUE* quercetin, *RUT* rutin, *TRY* tryptanthrin

Considering that the numbers of experiments necessary for optimizing the extraction conditions can be reduced by a statistical optimization design, the homogenization time, extraction temperature, microwave power, and extraction time were optimized with respect to the yield of total target analytes by the Box–Behnken design. On the basis of the ANOVA results of the quadratic model constructed (see Table S2), the optimal operational parameters were obtained as follows: extraction temperature 58 °C, homogenization time 52 s, microwave power 505 W, and extraction time 8.2 min. Under the optimal conditions, the yield of total target analytes was 959.01± 8.63 μg/g from the actual experiments, which was a good fit for the value (965.97 μg/g) forecasted by the regression model. Therefore, the optimal extraction conditions obtained were reliable and practical.

HSH–MAE superiority

Conventional SE and UAE methods were used for the extraction of phytochemicals from dried plant hairy root cultures [11, 12]. Accordingly, the HSH–MAE approach was

evaluated for its superiority against these traditional methods. The obvious advantages of HSH–MAE were mainly reflected as follows: simplicity in operation (a successive procedure without biomass drying and grinding), highest efficiency (9.1 min as against 240 min for SE and 120 min for UAE), improved yield (959.01 μg/g as against 887.62 μg/g for SE and 832.73 μg/g for SE), lowest energy cost (0.079 kWh/mg as against 48.31 kWh/mg for SE and 47.33 kWh/mg for UAE), and minimal CO_2 generation (0.063 kg/mg as against 38.65 kg/mg for SE and 37.86 kg/mg for UAE). The energy consumption was determined by a wattmeter and was based on the extraction of 1 mg total target analytes. The calculation of the amount of CO_2 ejected was done in accordance with a previous report [13].

Use of conventional mechanical grinding technology for dried biomass can result in the local overheating of materials, thus leading to the thermal degradation of susceptible compounds [9]. HSH can rapidly pulverize fresh plant materials in a continuous slurry stream and avoid the localized increased temperatures. Moreover, conventional extraction techniques require a long extraction time at high temperature, which will increase the risk of degradation of thermolabile compounds. However, the operational conditions of MAE are quite moderate, which can effectively avoid the risk of thermal degradation of sensitive analytes. Additionally, microwave irradiation can cause instantaneous localized heating inside plant cells/organs, which will effectively deactivate enzymes and thus reduce or prevent the enzymatic hydrolysis of analytes during extraction. These beneficial properties contributed to an increase in the yield of target compounds during HSH–MAE and helped to produce high-quality extracts for the accurate determination of secondary metabolic profiles. Overall, the proposed HSH–MAE is a simple, low-cost, green, and effective method for the direct extraction of alkaloids and flavonoids from fresh ITHRCs.

HSH–MAE mechanism

Since plant cell walls and membranes present formidable barriers to permeation by extraction solvents, cells have to be disrupted in the extraction process. Therefore, ITHRC samples before and after extraction were examined by cytohistological observations to clarify the extraction mechanism. Fig. S1A in the ESM shows a typical micrograph of untreated ITHRCs. After HSH (see ESM Fig. S1B), ITHRCs were obviously dispersed from an intact organ into numerous cells (arrows). After HSH–MAE (see ESM Fig. S1C), ITHRC cells exhibited an evident rupture of cellular matrices (arrows). During the extraction process, microwave irradiation led to internal thermal stresses in the plant cells caused by the localized heating effect, which resulted in pressure buildup within cells exceeding their capacity for expansion and rupture, and a movement of intracellular substances into solvent could thus be envisaged. These phenomena in this study suggested that HSH–MAE is a feasible sample preparation technique for the extraction of intracellular phytochemicals from fresh plant in vitro cultures.

Fig. 1 Liquid chromatography–tandem mass spectrometry (LC–MS/MS) total ion chromatogram obtained in selected reaction monitoring (SRM) mode of a standard mixture (**a**), and product ion mass spectra of **b** rutin (RUT), **c** epigoitrin (EPI), **d** neohesperidin (NEO), **e** isatin (ISA), **f** buddleoside (BUD), **g** indole-3-carboxaldehyde (INC), **h** liquiritigenin (LIQ), **i** quercetin (QUE), **j** isorhamnetin (ISR), **k** kaempferol (KAE), **l** isoliquiritigenin (ISL), **m** tryptanthrin (TRY), **n** indigo (ING), and **o** indirubin (INR). The elution order of the target compounds was as follows: RUT (*1*), EPI (*2*), NEO (*3*), ISA (*4*), BUD (*5*), INC (*6*), LIQ (*7*), QUE (*8*), ISR (*9*), KAE (*10*), ISL (*11*), TRY (*12*), ING (*13*), INR (*14*)

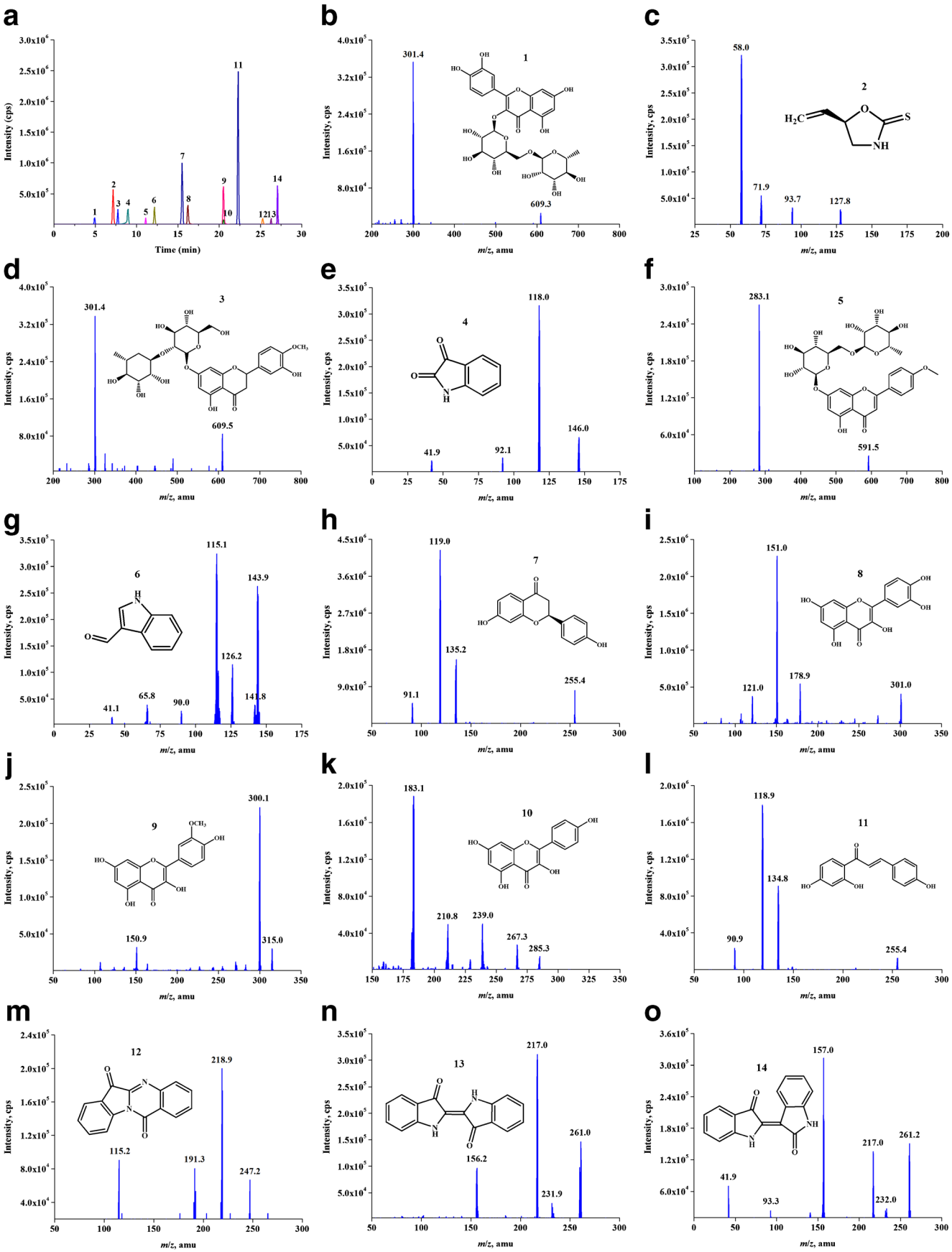
Establishment of the LC–MS/MS method

To obtain the efficient separation of analytes by LC, the mobile phase and elution program should be optimized. The best results were obtained using an acetonitrile–water mixture with 0.002 % formic acid, which provided satisfactory peak shape, baseline stability, and ionization efficiency. Moreover, the gradient elution program developed as described in "LC–MS/MS analysis" offered a short run time (30 min) and sufficient resolution of 14 target analytes, which provided a benefit with respect to the matrix effect in the following ESI-MS/MS analysis.

In virtue of the spectral patterns of RUT, EPI, NEO, ISA, BUD, INC, LIQ, QUE, ISR, KAE, ISL, TRY, ING, and INR obtained by ESI-MS/MS in SRM mode (Fig. 1b–o), the precursor ion–product ion combinations as well as the declustering potential, collision energy and collision cell exit potential were optimized, and the results are summarized in Table 1. Additionally, these ion combinations were defined as quantifiers and qualifiers for the 14 target analytes in LC–MS/MS analysis. A representative LC–MS/MS total ion chromatogram of standard mixtures is presented in Fig. 1a.

Method validation

As the results in Table 2 show, all calibration curves exhibited excellent linearity ($R^2 \geq 0.9862$) within the range of concentrations tested. The limits of detection and the limits of quantification for all target analytes were less than 2.14 and 7.05 ng/mL, respectively. Typical SRM chromatograms at the limits of detection for the target compounds are shown in ESM Fig. S2. Moreover, the relative standard deviations of intraday and interday measurements for the retention time of all target analytes were less than 0.55 % and 0.92 %, respectively, and for the peak area they were less than 5.78 % and 7.19 %,


a
Intensity (cps)
Time (min)
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3
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5
6
7
8
9
10
11
12
13
14
b
301.4
609.3
1
Intensity, cps
m/z, amu
c
58.0
71.9
93.7
127.8
2
Intensity, cps
m/z, amu
d
301.4
609.5
3
Intensity, cps
m/z, amu
e
118.0
146.0
92.1
41.9
4
Intensity, cps
m/z, amu
f
283.1
591.5
5
Intensity, cps
m/z, amu
g
115.1
143.9
126.2
65.8
90.0
41.1
6
Intensity, cps
m/z, amu
h
119.0
135.2
91.1
255.4
7
Intensity, cps
m/z, amu
i
151.0
178.9
121.0
301.0
8
Intensity, cps
m/z, amu
j
300.1
315.0
150.9
9
Intensity, cps
m/z, amu
k
183.1
210.8
239.0
267.3
285.3
10
Intensity, cps
m/z, amu
l
118.9
134.8
90.9
255.4
11
Intensity, cps
m/z, amu
m
218.9
115.2
191.3
247.2
12
Intensity, cps
m/z, amu
n
217.0
261.0
156.2
231.9
13
Intensity, cps
m/z, amu
o
157.0
217.0
261.2
41.9
93.3
232.0
14
Intensity, cps
m/z, amu

Table 2 Calibration curves, limit of detection (*LOD*), limit of quantification (*LOQ*), precision, and recovery for the 14 target compounds as determined by the high-seed homogenization–microwave-assisted extraction–liquid chromatography–tandem mass spectrometry method developed

Analytes	Calibration curves[a]	R^2	Linear range (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	Intraday RSD[b] (%)		Interday RSD[b] (%)		Standard addition recovery[c] (%) (mean±RSD; n=3)
						RT	PA	RT	PA	
EPI	$y=173x+4.67\times10^3$	0.9999	7.85–7850	0.99	3.27	0.33	4.05	0.74	4.85	96.92±2.87
ISA	$y=4430x+3.41\times10^5$	0.9994	5.92–5920	0.07	0.23	0.41	1.89	0.65	6.37	101.34±1.68
INC	$y=1710x+5.06\times10^5$	0.9961	7.92–7920	0.09	0.29	0.20	2.61	0.41	4.97	95.82±3.40
TRY	$y=151x+829$	0.9992	8.04–8040	2.14	7.05	0.39	5.78	0.57	5.38	96.28±2.51
ING	$y=182x+1.30\times10^4$	0.9930	1.54–1540	0.38	1.25	0.14	4.17	0.38	3.02	98.83±1.05
INR	$y=1200x+4.76\times10^5$	0.9992	2.46–2460	0.15	0.49	0.28	2.93	0.29	6.52	100.64±2.88
RUT	$y=317x+1.86\times10^4$	0.9995	3.38–3380	0.33	1.10	0.55	4.08	0.53	4.83	97.25±1.64
NEO	$y=563x+3.05\times10^4$	0.9993	3.46–3460	0.23	0.77	0.46	3.26	0.77	7.19	94.93±3.05
BUD	$y=419x+3.15\times10^5$	0.9986	3.69–3690	0.28	0.93	0.37	1.76	0.40	4.88	102.56±0.94
LIQ	$y=12300x+2.08\times10^6$	0.9957	4.54–4540	0.01	0.03	0.27	5.88	0.92	6.31	99.27±2.01
QUE	$y=7470x+2.63\times10^5$	0.9997	3.85–3850	0.08	0.27	0.22	2.64	0.35	3.71	95.01±1.29
ISR	$y=8030x+1.36\times10^6$	0.9953	3.54–3540	0.02	0.04	0.43	3.90	0.63	6.73	105.77±4.83
KAE	$y=542x+1.35\times10^5$	0.9977	7.69–7690	0.12	0.41	0.50	5.26	0.58	2.88	96.38±4.57
ISL	$y=15700x+4.64\times10^6$	0.9862	4.23–4230	0.01	0.03	0.37	2.44	0.46	5.97	103.94±2.88

PA peak area, *RSD* relative standard deviation, *RT* retention time

[a] The calibration curves were constructed by plotting the peak areas versus the concentration (ng/mL) of each analyte, and each regression equation included eight data points

[b] RSD (%)=(standard deviation/mean)×100

[c] The data are presented as the average of three determinations, where standard addition recovery (%)=(amount found – original amount)/spiking amount×100

respectively. Furthermore, the recoveries of all target analytes obtained using spiked samples ranged between 94.93 % and 105.77 %, with relative standard deviations ranging from 0.94 % to 4.83 %. Overall, the aforementioned data indicate that the present analytical method possesses good accuracy, sensitivity, and stability for the quantification of target alkaloids and flavonoids in ITHRCs.

Application of the HSH–MAE–LC–MS/MS method

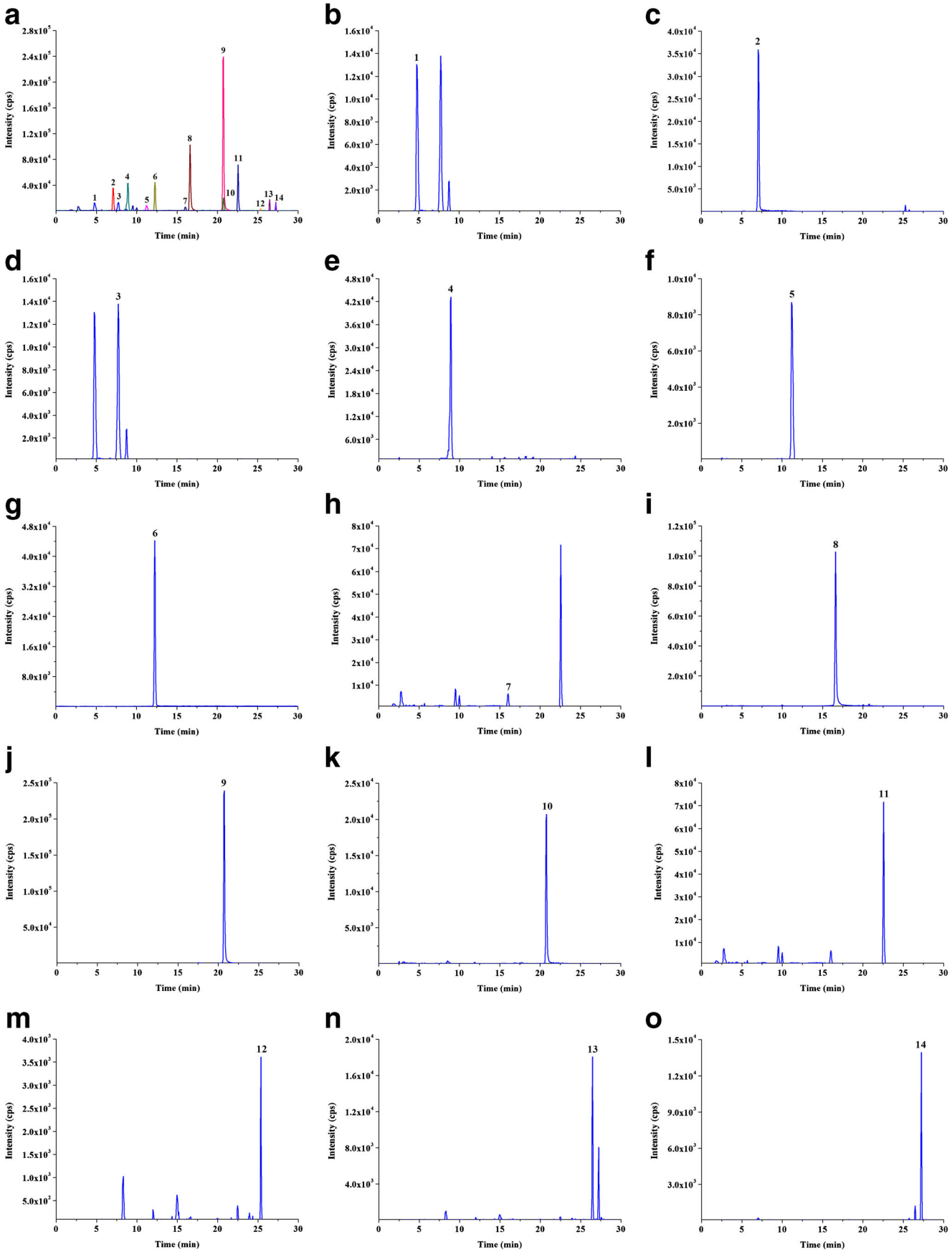
Owing to the uncertainty of *A. rhizogenes* T-DNA integration into the host plant genome, different derived *I. tinctoria* hairy root lines often show considerable diverse patterns of biosynthesis of secondary metabolites [14]. Therefore, the proposed HSH–MAE–LC–MS/MS method under optimal conditions was applied for the determination of target alkaloids and flavonoids in eight candidate ITHRCs (I–VIII) originating from distinct *I. tinctoria* hairy root lines. Quantitative results for 14 target analytes in different ITHRCs are shown in Table S3.

Obviously, the levels of alkaloids (EPI, ISA, INC, TRY, ING, and INR) in ITHRC III were significantly higher as compared with the other candidates, whereas ITHRC V was categorized as a high-productivity culture in terms of accumulation of flavonoids (RUT, NEO, BUD, LIQ, QUE, ISR, KAE, and ISL). A representative LC–MS/MS total ion chromatogram of the ITHRC III sample and the corresponding extracted ion chromatograms are shown in Fig. 2. This successful application example indicated that the proposed extraction and analytical method is suitable for quality control of ITHRCs or other plant in vitro cultures.

Conclusions

A rapid, green and effective sample preparation and analytical procedure for fresh plant in vitro cultures—namely, HSH–MAE followed by LC–MS/MS detection—was developed and validated for the simultaneous determination of six alkaloids and eight flavonoids in ITHRCs. In comparison with conventional SE and UAE methods, HSH–MAE under optimal conditions exhibited significant improvements with regard to operation, efficiency, yield, and green aspects in terms

Fig. 2 LC–MS/MS total ion chromatogram obtained in SRM mode of ▶ the *Isatis tinctoria* hairy root culture III sample (**a**), and the corresponding extracted ion chromatograms of **b** RUT, **c** EPI, **d** NEO, **e** ISA, **f** BUD, **g** INC, **h** LIQ, **i** QUE, **j** ISR, **k** KAE, **l** ISL, **m** TRY, **n** ING, and **o** INR. The elution order of the target compounds was as follows: RUT (*1*), EPI (*2*), NEO (*3*), ISA (*4*), BUD (*5*), INC (*6*), LIQ (*7*), QUE (*8*), ISR (*9*), KAE (*10*), ISL (*11*), TRY (*12*), ING (*13*), INR (*14*)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Intensity (cps)
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of saving energy cost and minimization of the generation of waste. Cytohistological investigations also provided evidence of pronounced cellular/tissue damage during HSH–MAE. Moreover, the LC–MS/MS method was proved to have excellent linearity, precision, repeatability, and reproducibility. The feasibility of HSH–MAE–LC–MS/MS was demonstrated by the selection of high-productivity ITHRCs among eight candidates. Overall, the proposed analytical method is valuable for the rapid determination of secondary metabolic profiles in ITHRCs or other plant in vitro cultures.

Acknowledgments The authors gratefully acknowledge financial support by the Application Technology Research and Development Program of Harbin (2013AA3BS014), the Fundamental Research Funds for the Central Universities (2572015AA14, 2572014AA06), and the Special Fund of the National Natural Science Foundation of China (31270618).

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