Analytical Methods

Simultaneous determination of iridoid glycosides, phenethylalcohol glycosides and furfural derivatives in Rehmanniae Radix by high performance liquid chromatography coupled with triple-quadrupole mass spectrometry

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Abstract

In this study, a sensitive and selective method for simultaneously quantifying eight major components (four iridoid glycosides, three phenethylalcohol glycosides and one furfural derivative) of Rehmanniae Radix by high performance liquid chromatography coupled with triple-quadrupole mass spectrometry (HPLC–TQ-MS) was developed. The sample preparation was executed using an optimised ultrasonic method with complete extraction efficiencies of eight analytes. For mass spectrometry, selected ion recording (SIR) scan mode was used to improve the sensitivity and selectivity. The established method was validated in terms of linearity, sensitivity, precision, accuracy and stability, and successfully applied to determine the contents of the eight analytes in different batches of raw and processed Rehmanniae Radix, which confirmed that the established method was reliable and useful for “holistic” quality evaluation of Rehmanniae Radix. The quantitative results indicated that the quality of commercial raw or processed Rehmanniae Radix was remarkably inconsistent.

Keywords:
Rehmanniae Radix
Iridoid glycosides
Phenethylalcohol glycosides
Furfural derivatives
HPLC–TQ-MS
SIR scan mode

1. Introduction

Rehmanniae Radix (Dihuang in Chinese), which is derived from the root of Rehmannia glutinosa Libosch., is a commonly used dietary supplement and medicinal herb in China or other eastern countries. In traditional Chinese medicine, there are two types of Rehmanniae Radix frequently used in clinical prescription in terms of the processing method, i.e., raw Rehmanniae Radix (Sheng Dihuang) which is obtained by drying the fresh root of R. glutinosa and used for removing pathogenic heat from blood, nourishing Yin and promoting the production of body fluids, and processed Rehmanniae Radix (Shu Dihuang) which is gained by braising raw Rehmanniae Radix with rice wine and used for nourishing Yin, supplementing the blood and benefitting the marrow (Pharmacopoeia of the People’s Republic of China, 2010). Besides used for medicinal purpose, Rehmannia Radix has also been regarded as one of the most popular dietary herbal supplements for daily health care in China (Bian, 2010), in particular as the major formula herbal material used in some traditional tonic ointments, such as Jade ointment (Zhou, Liu, & Zhong, 1995).

Pharmacological studies showed that Rehmanniae Radix possess wide actions on the blood system, immune system, endocrine system, cardiovascular system and the nervous system (Zhang, Li, & Jia, 2008). Phytochemical investigations revealed that iridoid glycosides, e.g., catalpol, aucubin, leonuride and melittoside (Nishimura, et al., 1989; Oshio & Inouye, 1981; Shoyama, Matsumoto, & Nishioka, 1986), phenethylalcohol glycosides, e.g., acteoside, echnacoside and isoacteoside (Shoyama, Matsumoto, & Nishioka, 1987; Shoyama et al., 1986) and furfural derivatives, e.g., 5-hydroxymethyl–2-furfural (Anh, Sung, Franke, & Wessjohann, 2003; Li, Chen, & Zhu, 2005) were the major components that might be responsible for the overall bioactivities of Rehmanniae Radix. For example, catalpol has been proved to possess lowering blood-glucose and neuroprotective effects (Guo, Liu, Wang, & Yu, 2009). Acteoside was reported to present liver protecting action (Lee et al., 2004), and 5-hydroxymethyl-2-furfural (5-HMF) was confirmed to have activity of anti-anaemia (Lin et al., 2008). Thus, these three kinds of components should be employed as chemical markers for “holistic” quality control of Rehmanniae Radix.

HPLC-UV method was developed to quantify catalpol (Luo, Zhang, Sun, & Cui, 1994), or separately determine catalpol, acteoside and 5-HMF (Wang, Jin, Li, & Bian, 2008). However, it is understandable that iridoid glycosides, which only present weak absorptions in the end of ultraviolet region, may not be detected...
Table 1

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Sample type</th>
<th>Locality/pharmacy shop/hospital</th>
<th>5-HMF</th>
<th>Catalpol</th>
<th>Leonuride</th>
<th>Aucubin</th>
<th>Melittoside</th>
<th>Acteoside</th>
<th>Isoacteoside</th>
<th>Echinacoside</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>JPACM-02-01</td>
<td>Raw materials</td>
<td>Wanxi region, Henan province</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>JPACM-02-02</td>
<td>JPHICWM c, Nanjing</td>
<td>1289.69 (2.88)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>JPHICWM, Nanjing</td>
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<td>-</td>
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<tr>
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<td>JPHICWM, Nanjing</td>
<td>5677.33 (5.84)</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>JPACM-02-05</td>
<td>Yifeng Pharmacy, Nanjing</td>
<td>11617.37 (6.61)</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>JPACM-02-06</td>
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<td>5677.33 (5.84)</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td>JPACM-02-09</td>
<td>Processed materials</td>
<td>5980.98 (0.45)</td>
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<td>-</td>
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<td>6201.02 (1.42)</td>
<td>-</td>
<td>-</td>
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<tr>
<td>JPACM-02-13</td>
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<td>1947.85 (0.66)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>JPACM-02-12</td>
<td>Baiyun Pharmacy, Guangzhou</td>
<td>1980.39 (4.09)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The data were presented as average of triplicates.

Not detected.

c JPHICWM: Jiangsu Province Hospital on Integration of Chinese and Western Medicine.

d Trace amount (Under the limit of quantification).

The data was present as average of triplicates.

MS, 1H-NMR and 13C-NMR), and their purities were determined these compounds were elucidated by their spectral data (UV, IR, University of Denmark, Copenhagen, Denmark. The structures of from Rehmanniae Radix in Department of Chemistry, Technical Sichuan Victory Co. Ltd. (Chengdu, China). Melittoside was isolated and processed Rehmanniae Radix. Quantitative evaluating the ‘holistic’ quality of commercial raw and processed Rehmanniae Radix.

High performance liquid chromatography triple-quadrupole tandem mass spectrometry (HPLC–TQ-MS) technique has advantages of mass fragmentation and many scan modes which is helpful for not only identity assignment through rationalisation of fragments, but also sensitivity improvement and selectivity enhancement through multiple scan modes (Robert, 2003). Selected ion recording (SIR), one of the scan modes, has been widely used in pharmaceutical and herbal product analysis since this approach can ensure higher sensitivity and selectivity with unnecessary baseline chromatographic separation of the target analytes by selectively detecting characteristic ions of the analytes. So it greatly facilitates the determination of marker compounds in the complicated matrixes for comprehensive quality evaluation of medicinal herbs (Luo, Chen, & Yao, 2005).

In the present study, an HPLC–TQ-MS method with SIR scan mode was firstly developed and validated for simultaneous quantification of eight major bioactive components (four iridoid glycosides, three phenethylalcohol glycosides and one furfural derivative) in Rehmanniae Radix, and was successfully used for quantitatively evaluating the “holistic” quality of commercial raw and processed Rehmanniae Radix.

2. Experimental

2.1. Chemicals, reagents and herb materials

Acetonitrile (HPLC grade) from Tedia Co., INC. (Fairfield, USA) and formic acid (analytical grade) from Nanjing Chemical Reagent Co. (Nanjing, China) were purchased. Ultra-pure water was produced by a Milli-Q water purification system (Milford, MA, USA).

The reference compounds, 5-HMF, acteoside, isoacteoside, echinacoside, leonuride, catalpol and aucubin were purchased from Sichuan Victory Co. Ltd. (Chengdu, China). Melittoside was isolated from Rehmanniae Radix in Department of Chemistry, Technical University of Denmark, Copenhagen, Denmark. The structures of these compounds were elucidated by their spectral data (UV, IR, MS, 1H-NMR and 13C-NMR), and their purities were determined to be higher than 95.0% by HPLC–UV analysis.

One batch of Rehmanniae Radix crude drug (JPACM-03-01) was collected from Henan province, the genuine cultivating region of R. glutinosa in China. The commercial samples (Yinpin in Chinese) of raw and processed Rehmanniae Radix (JPACM-03-02 to JPACM-03-13) were purchased from different pharmacy shops and hospitals in Nanjing and Guangzhou, China (Table 1). All the samples were authenticated by Dr. S. L. Li morphologically according to the standard of China Pharmacopoeia (Part I, 2010 Version). The voucher specimens were deposited at Department of Pharmaceutical Analysis and Metabolomics, Jiangsu Province Academy of Traditional Chinese Medicine, Nanjing, China.

2.2. Sample preparation

Rehmanniae Radix materials were cut into pieces (about 0.5 × 0.5 × 0.2 cm). The sliced samples were then accurately weighed (approximately 0.5000 g) and ultrasonic-extracted with 25 mL.
methanol for 30 min. The extracted solutions were filtered through a 0.22 μm PTFE syringe filter for HPLC–TQ-MS analysis.

2.3. Liquid chromatography

The HPLC analysis was performed on a Waters Alliance HPLC 2695 system (Waters Corp., MA, USA), equipped with a binary solvent delivery system, auto-sampler, and a photo-diode array (PDA) detector. The separation was achieved on an Alltech Altima C18 column (250 mm × 4.6 mm, 5 μm) with a Phenomenex C18 guard column. The mobile phase is consisted of (A) 0.2% formic acid aqueous solution and (B) acetonitrile containing 0.2% formic acid. The gradient elution was optimised as follows: 1% B (0–16 min), 1–40% B (16–35 min). The flow rate was 1.0 mL/min and split 0.3 mL/min for mass spectrometry analysis. The column and auto-sampler temperature were maintained at 35 °C and 10 °C, respectively. The injection volume was 20 μL.

2.4. Mass spectrometry

Mass spectrometry was performed on a Micromass Quattro-Micro™ triple-quadrupole mass spectrometer (Waters Corp., MA, USA) with electrospray ionisation (ESI) interface in positive mode. The capillary voltage was 3500 V, desolvation gas and temperature were set to 450 L/h and at 400 °C, the cone gas was 50 L/h, and source temperature was set at 110 °C. The cone voltages were separately set from 25 to 80 V for different analytes.

2.5. Method validation

The method for quantitative analysis was validated in terms of linearity, sensitivity, precision, accuracy and stability.

Methanol stock solutions of eight reference compounds were diluted to appropriate concentrations for the construction of calibration curves. Six concentrations of the solution were analysed in triplicates, and the calibration curves were constructed by plotting the peak areas versus the concentrations of analytes.

The stock solutions were diluted to a series of appropriate concentrations with methanol, and an aliquot of the diluted solutions were injected into HPLC–TQ-MS for analysis. The limits of detection (LODs) and limits of quantification (LOQs) under the present conditions were determined at an S/N (signal to noise) of about 3 and 10, respectively.

Intra- and inter-day variations were chosen to determine the precision of the developed assay. For intra-day variability test, the Rehmannia Radix sample (JPACM-02-08) was extracted and analysed for six replicates within one day, while for inter-day variability tests, the same sample was examined in duplicates for consecutive three days. Variations were expressed by the RSDs of the data.

The spike recovery test was used to evaluate the accuracy of the method. About 0.2500 g of Rehmannia Radix sample (JPACM-02-03) with known contents of the target analytes was weighed, and different amounts (high, middle and low level) of reference standards (5-HMF: 12.50, 10.42 and 8.34 μg; catalpol: 633.98, 528.32 and 422.66 μg; leonuride: 72.78, 60.65 and 48.52 μg; aucubin: 60.00, 50.00 and 40.00 μg; melittoside: 14.93, 12.44 and 9.95 μg; acteoside: 31.43, 26.19 and 20.96 μg; isoeucoside: 49.21, 41.01 and 32.81 μg; echinacoside: 6.64, 5.53 and 3.67 μg) were spiked, then extracted and analysed in triplicates. The spike recoveries were calculated by following equation: Spike recovery (%) = (total amount detected-amount original)/amount spiked × 100%

The stability test was performed by analysing the sample extract over period of 2 h, 4 h, 6 h, 8 h, 12 h, 24 h, the RSDs of the peak areas of each analyte were taken as the measures of stability.

3. Results and discussion

3.1. Optimization of sample preparation procedure

Generally, in sample preparation procedures, herbal materials are usually dried and pulverized into well-distributed powders for facility of extraction and ensuring high repeatability of the quantitative results (Sun & Zhuang, 1999). However, Rehmanniae Radix is always very sticky under room temperature owing to its high contents of polysaccharides (Liu et al., 2009). Moreover, according to the literatures, some bioactive components in Rehmanniae Radix, such as 5-HMF and catalpol are thermal labile (Yang & Cao, 2009; Li, Zhang, Liu, Zhang, & Liu, 2005). Hence, in this study, Rehmanniae Radix was directly cut into homogeneous thin slices for further extraction.

Ultrasonic-assisted extraction and hot reflux are usually used for sample preparation in the analysis of medicinal herbs (Yang & Kong, 2010). However, as mentioned above, extraction with high temperature may cause degradation of some components in Rehmanniae Radix. So these two sample preparation methods were compared in this study, and the hot-sensitive compounds 5-HMF and catalpol were selected as chemical markers to evaluate the method. It was found that the contents of 5-HMF and catalpol in samples extracted by hot reflux (peak area: 55043 and 259779, respectively) are less than those by ultrasonic-assisted extraction (peak area: 65721 and 271503, respectively). Ultrasonic-assisted extraction was therefore adopted in this study for sample preparation. In addition, to avoid possible glucosidase-evolved hydrolysis of iridoid glycosides and phenethylalcohol glycosides in Rehmanniae Radix during extraction (Li et al., 2010), the herbal material

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**Fig. 1.** Extraction yields of eight analytes with different extraction durations (n = 3) ***: P < 0.01; **: P < 0.05; *: P < 0.1.
was sonicated with 100% methanol, and the temperature of the ultrasonic bath was maintained at 30 ± 1 °C with running water.

Our previous study has confirmed that extraction efficiencies of analytes should always be tested in analytical method development, so that spike recovery can really represent the accuracy of analytical methods for medicinal herbs (Xu et al., 2012). Thus, extraction efficiency in sample preparation was investigated in this study. The extraction yields of seven analytes (aucubin was not included) were measured and compared using the LC-MS method.
detectable in the tested sample) with five extraction durations (10, 20, 30, 45 and 60 min) were studied. All samples were tested in triplicates. The extraction yields (peak areas) of each analyte with different extraction durations were shown in Fig. 1, and the total yields of seven analytes were compared statistically by one-way ANOVA. From Fig. 1, it was found that the total extraction yields of seven analytes increased (very) significantly ($P < 0.05$ or $P < 0.01$) with the extension of the extraction duration from 10 to 30 min, and presented a platform ($P > 0.1$) till 45 min, then declined very remarkably ($P < 0.01$) till 60 min. This trend was obviously contributed by 5-HMF whose extraction yield decreased since 30 min (Fig. 1), possibly owing to the degradation of 5-HMF during long time extraction (Li et al., 2005). Thus, 30 min was selected as the optimal extraction time for sample preparation.

3.2. Optimization of the MS conditions

The mass spectrometric conditions, such as ion mode, source temperature, desolvation gas flow and dry temperature, were optimised manually for high sensitivity by injecting the standard solutions and comparing the ratio of signal/noise. It was found that in positive ion mode, when capillary voltage was 3500 V, desolvation gas flow and temperature were 450 L/h and at 400 °C, the cone gas flow was 50 L/h, the source temperature was at 110 °C, the cone voltages were separately set from 25 to 80 V for different analytes, all analytes showed abundant molecular ion [M + H]$^+$ or adduct ion [M + Na]$^+$ (Fig. 2). Therefore, the molecular ion or adduct ion of each analyte were selected for SIR scan mode. The optimised cone voltages and the molecular ion or adduct ion of each analyte were summarized in Table 2.

3.3. Method validation

The linearity, regression and linear ranges of eight analytes were summarized in Table 3. The data indicated good relationship between concentrations and peak areas of the analytes within the test ranges ($R^2 \geq 0.9990$). The LOQs and LODs of all analytes were less than 6.2 and 2.6 ng on column, respectively (Table 3). The overall RSDs of intra- and inter-day variations for eight analytes were not more than 6.70% and 7.62% respectively. The established method also had acceptable accuracy with spike recovery of 90.96–108.66% for all analytes. As to stability test, the RSDs of the peak areas for eight analytes detected within 24 h were lower than 3.65% (Table 3). All these results demonstrated that the established HPLC–TQ-MS method was linear, sensitive, precise, accurate, and stable enough for simultaneous quantification of eight major components in Rehmanniae Radix.

3.4. Quantification of eight analytes in raw and processed Rehmanniae Radix

The developed HPLC–TQ-MS method was applied to quantify eight analytes in 13 Rehmanniae Radix samples. Typical

<table>
<thead>
<tr>
<th>Compound</th>
<th>Selected ion (m/z)</th>
<th>Cone voltage (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HMF</td>
<td>127.20 [M + H]$^+$</td>
<td>25.0</td>
</tr>
<tr>
<td>Catalpol</td>
<td>385.40 [M + Na]$^+$</td>
<td>50.0</td>
</tr>
<tr>
<td>Leonuride</td>
<td>371.50 [M + Na]$^+$</td>
<td>50.0</td>
</tr>
<tr>
<td>Aucubin</td>
<td>369.50 [M + Na]$^+$</td>
<td>50.0</td>
</tr>
<tr>
<td>Melittoside</td>
<td>547.40 [M + Na]$^+$</td>
<td>50.0</td>
</tr>
<tr>
<td>Acteoside</td>
<td>647.50 [M + Na]$^+$</td>
<td>70.0</td>
</tr>
<tr>
<td>Isoacteoside</td>
<td>647.50 [M + Na]$^+$</td>
<td>70.0</td>
</tr>
<tr>
<td>Echinacoside</td>
<td>809.50 [M + Na]$^+$</td>
<td>80.0</td>
</tr>
</tbody>
</table>

Table 2

Selected ions and optimal cone voltages of eight compounds for SIR scan.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linearity</th>
<th>LOQ (ng)</th>
<th>LOD (ng)</th>
<th>RSD, Intra-day</th>
<th>RSD, Inter-day</th>
<th>RSD, Spike Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HMF</td>
<td>25.0</td>
<td>1.4</td>
<td>4.2</td>
<td>4.82</td>
<td>6.59</td>
<td>99.999 (3.24)</td>
</tr>
<tr>
<td>Catalpol</td>
<td>50.0</td>
<td>2.4</td>
<td>4.5</td>
<td>4.51</td>
<td>6.61</td>
<td>99.999 (3.41)</td>
</tr>
<tr>
<td>Leonuride</td>
<td>50.0</td>
<td>1.6</td>
<td>2.1</td>
<td>2.18</td>
<td>4.41</td>
<td>99.999 (3.41)</td>
</tr>
<tr>
<td>Aucubin</td>
<td>50.0</td>
<td>0.6</td>
<td>0.6</td>
<td>0.55</td>
<td>1.14</td>
<td>99.999 (3.41)</td>
</tr>
<tr>
<td>Melittoside</td>
<td>50.0</td>
<td>0.6</td>
<td>0.6</td>
<td>0.55</td>
<td>1.14</td>
<td>99.999 (3.41)</td>
</tr>
<tr>
<td>Acteoside</td>
<td>70.0</td>
<td>0.6</td>
<td>0.6</td>
<td>0.55</td>
<td>1.14</td>
<td>99.999 (3.41)</td>
</tr>
<tr>
<td>Isoacteoside</td>
<td>70.0</td>
<td>2.6</td>
<td>2.6</td>
<td>2.65</td>
<td>5.45</td>
<td>99.999 (3.41)</td>
</tr>
<tr>
<td>Echinacoside</td>
<td>80.0</td>
<td>2.6</td>
<td>2.6</td>
<td>2.65</td>
<td>5.45</td>
<td>99.999 (3.41)</td>
</tr>
</tbody>
</table>

Table 3

Calibration curves, LODs, LOQs, repeatability, accuracy and stability of the assay.

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2281
Fig. 3. Representative chromatograms of reference compounds and Rehmanniae Radix samples. A1–A8: Chromatograms of eight reference compounds in SIR scan mode; B1–B7: Representative chromatograms of raw Rehmanniae Radix sample (JPACM-03-04) in SIR scan mode; C1–C7: Representative chromatograms of processed Rehmanniae Radix sample (JPACM-03-10) in SIR scan mode 1: 5-HMF; 2: Catalpol; 3: Leonuride; 4: Aucubin; 5: Melittoside; 6: Acteoside; 7: Isoacteoside; 8: Echinacoside.
Shengdihuang SIR of 7 Channels ES+

Time

-0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50 35.00

%

-10 90

B1

Shengdihuang SIR of 7 Channels ES+

Time

-0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50 35.00

%

-10 90

B2

Shengdihuang SIR of 7 Channels ES+

Time

-0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50 35.00

%

-10 90

B3

Shengdihuang SIR of 7 Channels ES+

Time

-0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50 35.00

%

-10 90

B4

Shengdihuang SIR of 7 Channels ES+

Time

-0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50 35.00

%

-10 90

B5

Shengdihuang SIR of 7 Channels ES+

Time

-0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50 35.00

%

-10 90

B6

Shengdihuang SIR of 7 Channels ES+

Time

-0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50 35.00

%

-10 90

B7

Fig. 3 (continued)
Fig. 3 (continued)
chromatograms of reference compounds, raw and processed Rehmanniae Radix samples were shown in Fig. 3A1–A8, B1–B7, C1–C7. It could be seen that all investigated analytes were clearly detected with baseline separation through SIR scan mode.

The contents of eight analytes in 13 Rehmanniae Radix samples were summarized in Table 1. It is well accepted that 5-HMF is an artefact produced during hot-processing of Rehmanniae Radix (Cao, Liang, Yang, Liu, & Duan, 2010). So 5-HMF was regarded as the characteristic marker for identification of processed Rehmanniae Radix (Wang, Jia, Li, & Ji, 2009). In fact, 5-HMF has been selected as the exclusive marker for identification of processed Rehmanniae Radix in Chinese pharmacopoeia (Vol. 1, 2005 version). However, in this study, 5-HMF was unexpectedly detected with considerable contents in all commercial raw Rehmanniae Radix samples purchased from pharmacy shops and hospitals, whereas this compound was not detectable in the crude drug of Rehmanniae Radix (JPACM-02-01) which was collected from genuine cultivating region of R. glutinosa (Wanxi, Henan province, China), suggesting that these commercial raw Rehmanniae Radix might be inappropriately hot-treated during preparation of commercial raw Rehmanniae Radix.

From Table 1, it was also found that catalpol was detected in both raw and processed Rehmanniae Radix samples, but the contents in raw Rehmanniae Radix samples were far higher than those in processed Rehmanniae Radix samples, which is in agreement with previous findings that catalpol was abundantly present in raw Rehmanniae Radix but decreased sharply during preparation of processed Rehmanniae Radix (Guo et al., 2009). It should be noted that aucubin could not be detected in all five processed Rehmanniae Radix and three of eight raw Rehmanniae Radix samples, and leonuride could not be detected in processed Rehmanniae Radix samples, or the content in processed Rehmanniae Radix was much lower than that in raw Rehmanniae Radix, indicating that these two iridoid glycosides may be also hot-sensitive components of Rehmanniae Radix.

It was astonished to find that there were significant difference among raw Rehmanniae Radix or processed Rehmanniae Radix samples in terms of total or individual contents of the investigated bioactive components. For example, in three batches of raw Rehmanniae Radix samples (JPACM-02-02, JPACM-02-03 and JPACM-02-04) collected from Jiangsu Province Hospital on Integration of Chinese and Western Medicine, the content of catalpol was from 5204 to 10566 μg/g, the content of melittoside was from 88 to 249 μg/g, and the total contents of eight investigated components were from 7547 to 13672 μg/g, suggesting that the “holistic” quality of the commercial Rehmanniae Radix samples were remarkably inconsistent. Therefore, standardization of both post-harvest handling and processing of Rehmanniae Radix is strongly recommended to assure the quality consistency of this commonly used medicinal herb.

4. Concluding remarks

In this study, an HPLC–TQ-MS with SIR scan mode method for simultaneous quantification of eight major bioactive components (four iridoid glycosides, three phenethylalcohol glycosides and one furfural derivative) in Rehmanniae Radix was established and validated. Compared with previously reported HPLC-UV method, the established method was more sensitive and selective for the determination of three types of components in Rehmanniae Radix. The newly established method was successfully used to quantify these eight bioactive components in commercial raw and processed Rehmanniae Radix samples, the results indicated that the quality of commercial raw or processed Rehmanniae Radix was remarkably inconsistent. Standardization of both post-harvest handling and processing procedures of Rehmanniae Radix is recommended to assure the quality consistency of this commonly used medicinal herb.

Acknowledgements

This study was financially supported by research starting fund from Jiangsu Province Academy of Traditional Chinese Medicine (RC1101), the Administration of Traditional Chinese Medicine of Jiangsu Province (LZ11066), the open fund from Key Laboratory of New Drug Delivery System of Chinese Materia Medica in Jiangsu Province Academy of Traditional Chinese Medicine (No. 2011NDDCM02003) and the Grants from Macao Science and Technology Development Fund (013/2008/A1).

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