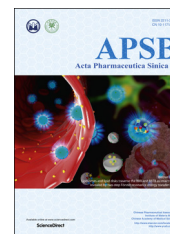




Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

Rapid and sensitive liquid chromatography–tandem mass spectrometric method for the quantitative determination of potentially harmful substance 5,5'-oxydimethylenebis (2-furfural) in traditional Chinese medicine injections



Qingce Zang^a, Yang Gao^a, LuoJiao Huang^a, Jiuming He^a, Sheng Lin^a,
Hongtao Jin^b, Ruiping Zhang^a, Zeper Abliz^{a,c,*}

^aState Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100050, China

^bNew Drug Safety Evaluation Center, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

^cCentre for Bioimaging & Systems Biology, Minzu University of China, Beijing 100081, China

Received 27 July 2017; received in revised form 8 October 2017; accepted 12 October 2017

KEY WORDS

5,5'-Oxydimethylenebis
(2-furfural);
LC–MS/MS;
Quantitative analytical
method;
Traditional Chinese
medicine injection;
Quality control

Abstract With the rapid development and wide application of traditional Chinese medicine injection (TCMI), a number of adverse events of some TCMI have incessantly been reported and have drawn broad attention in recent years. Establishing effective and practical analytical methods for safety evaluation and quality control of TCMI can help to improve the safety of TCMI in clinical applications. In this study, a sensitive and rapid high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) method has been developed and validated for the quantitative determination of potentially harmful substance 5,5'-oxydimethylenebis (2-furfural, OMBF) in TCMI samples. Chromatographic separation was performed on a C18 reversed-phase column (150 mm × 2.1 mm, 5 μm) by gradient elution, using methanol–water containing 0.1% formic acid as mobile phase at the flow rate of 0.3 mL/min. MS/MS detection was performed on a triple quadrupole mass spectrometer with positive electrospray ionization in the multiple reaction-monitoring mode. The method was sensitive with a limit of quantification of 0.3 ng/mL and linear over the range of 0.3–30 ng/mL ($r=0.9998$). Intra- and inter-day precision for analyte was <9.52% RSD with

*Corresponding author at: State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100050, China. Tel.: +86 10 63165218.

E-mail address: zeper@imm.ac.cn (Zeper Abliz).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

<https://doi.org/10.1016/j.apsb.2017.11.002>

2211-3835 © 2018 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

recoveries in the range 88.0–109.67% at three concentration levels. The validated method was successfully applied to quantitatively determine the compound OMBF in TCMI and glucose injections. Our study indicates that this method is simple, sensitive, practicable and reliable, and could be applied for safety evaluation and quality control of TCMI and glucose injections.

© 2018 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

As a new dosage form of traditional Chinese medicine (TCM), traditional Chinese medicine injection (TCMI) is considered to be a great achievement of modernization of TCM. TCMI has been extensively used in China to treat a variety of diseases, including bacterial and viral infections, musculoskeletal disorders, cancer, cardiovascular and cerebrovascular dysfunction^{1–3}. However, many serious adverse drug reactions (ADRs) of TCMI in some patients, including anaphylactic shock and fatal anaphylaxis, have been reported in recent years^{4–6}. Because TCMI is a complex concoction made from extracts derived from a single herb or a group of herbs in a composite formula, and chemical ingredients in herb medicine, which vary greatly with the geographical origin of the species, time of harvest, cultivation practice, methods of processing, and storage condition, further contribute to the complexity and instability of TCMI^{7–11}. Moreover, the quality control of TCMI is still unresolved for its complex composition. Particularly, current methods for monitoring the potentially harmful components in TCMI produced in the procedure of preparation, transportation and storage, are inadequate. Thus, it is imperative and urgent to develop practicable and reliable analytical methods for the purposes of improving the safety and quality of TCMI.

5-Hydroxymethyl furfural (5-HMF, C₆H₆O₃, Fig. 1A), a common product of the Maillard reaction, is generated by acid-catalysed thermal dehydration from fructose, saccharose and to a lesser degree from glucose^{12–14}. Thus, it can be easily found in many processed sugar- or starch-rich foods and heat-sterilised glucose/fructose solutions for pharmaceutical preparations^{15–17}. Excessive 5-HMF can cause skin irritation, damage to striated muscles¹⁸ liver cancer¹⁹, or induce aberrant crypt foci in the colon²⁰. Therefore, the content of 5-HMF in dextrose injection was strictly limited in *Chinese Pharmacopoeia* and *United States Pharmacopoeia*^{21,22}. In our previous study, we have found that there were significant differences in the content of 5-HMF in TCMI samples produced by different manufacturers or even different batches from the same manufacturer²³.

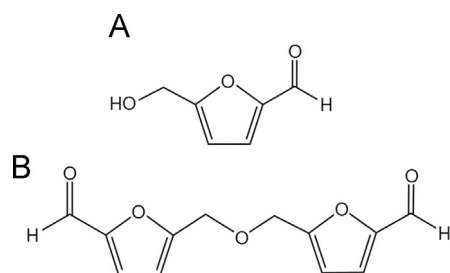


Figure 1 The chemical structures of (A) 5-hydroxymethyl furfural and (B) 5,5'-oxydimethylenebis (2-furfural).

Due to the unstable character of 5-HMF, it is readily hydrolyzes to levulinic acid and formic acid under acidic aqueous conditions^{24,25}. In addition, 5-HMF can also participate in hydrogenation, esterification and polymerization reactions²⁶. 5,5'-Oxydimethylenebis (2-furfural, OMBF, C₁₂H₁₀O₅, Fig. 1B), a coloured polymer of 5-HMF, is a by-product of the Maillard reaction²⁷, and is generally produced from acid-catalyzed dehydration reaction of 5-HMF^{28,29}. We occasionally found this compound in TCMI samples through imitating the high-temperature/high-pressure sterilization procedures of glucose injection production. Our further study revealed that OMBF has immunosensitizing potential by acting as a neo-antigen or neo-epitope to elicit a mixed type-1 and type-2 immune response, and exposure to OMBF may represent a safety concern for humans³⁰. However, no studies have been reported on screening this potentially harmful substance in TCMI and glucose injections. We herein developed a rapid, simple and sensitive HPLC–MS/MS method for quantitative determination of OMBF in TCMI and glucose injections. The developed method in this study could rapidly and sensitively determine OMBF with short analysis time, low limits of detection and quantification, and could also contribute to improve the safety and quality of TCMI and glucose injections.

2. Material and methods

2.1. Chemicals and reagents

OMBF was prepared using the procedure outlined by Larousse et al.³¹ A purity of >98% was detected by HPLC. Formic acid was purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade methanol was purchased from Merck (Muskegon, MI, USA). Pure water was obtained from the Wahaha Group Co., Ltd. (Hangzhou, China). Other chemicals were of analytical grade.

TCMI samples (Shuxuening Injection, Qingkailing Injection, Chaihu Injection, Huangqi Injection, Xuesaitong Injection, Shuanghuanglian Injection, Shenmai Injection, Mailuoning Injection, Zhiyinhuang Injection, Tianmasu Injection, Gegensu Injection, Chuanhuning Injection, Guanxinning Injection, Dengzhanxixin Injection, Shengmai Injection, Ciwujia Injection, Chuanxinlian Injection, Danxiangguanxin Injection, Xiyanning Injection) and glucose injection samples produced by different Chinese pharmaceutical factories were purchased at local pharmacies.

2.2. Sample preparation

Accurately weighed OMBF was dissolved in methanol to prepare a 0.335 mg/mL stock solution. Standard working solutions of OMBF at concentrations of 0.3, 0.6, 1.2, 2.4, 5, 15 and 30 ng/mL were prepared by serial dilutions of the stock solution with methanol. TCMI samples and glucose injection samples were

filtered through a 0.22 µm Nylon membrane and kept at 4 °C before use.

2.3. HPLC–MS/MS conditions

Samples analyses were performed on an Agilent 1200 series rapid resolution liquid chromatography system (Agilent technologies, Waldbronn, Germany) equipped with a binary gradient pump, autosampler, column oven and diode array detector. A Zorbax SB C18 column (150 mm × 2.1 mm, 5 µm; Agilent, USA) was used for separation. The mobile phase consisted of water with 0.1% (v/v) formic acid (A) and methanol (B) with a gradient elution. The gradient conditions were as follows: initial 5% B maintained for 10 min to balance the column, 0–5 min, maintained at 5% B; 5–18 min, increased to 100% B in 18 min; and 18–30 min, 100% solvent B. The flow rate was 0.3 mL/min, and the column temperature was kept at 35 °C throughout the analysis. The injected sample volume was 10 µL.

Mass spectrometry experiments were analyzed on an API 4000 triple quadrupole mass spectrometer (Applied Biosystems Sciex, Ontario, Canada), with an electrospray ion (ESI) source in the positive ion mode. The optimized mass spectrometric conditions for the compound OMBF included the following conditions: source temperature, 450 °C; ESI source voltage, 5 kV; nebulizer gas (Gas1), 60 psi; turbo gas (Gas2), 55 psi; curtain gas (CUR), 30 psi; entrance potential (EP), 10 V; declustering potential (DP), 55 V; and collision energy (CE), 18 eV. Nitrogen gas was used for both nebulizing and drying. The dwell time was 50 ms, with a 5 ms pause between scans. The MS/MS detection was operated at unit resolution in the multiple reaction monitoring (MRM) mode. The mass transitions of the protonated precursor/product ion pairs that were used to record the selected ion mass chromatograms of OMBF were m/z 235.1→109.0. Data acquisition and processing were performed using Analyst 1.5.1 supplied by AB SCIEX (Foster City, CA, USA).

2.4. Method validation procedure

The quantitative HPLC–MS/MS method was validated by assessing linearity, limit of detection (LOD) and quantification (LOQ), precision, accuracy, stability and recovery, according to the US Food and Drug Administration (FDA)³² and China Food and Drug Administration (CFDA) guidelines³³ for the validation of analytical methods.

The LOD and LOQ of the analyte were calculated by analyzing a series of dilute standard solutions of known concentration at signal-to-noise ratios (S/N) of 3:1 and 10:1, respectively. The LOQ served as the lowest standard on the calibration curve in this analytical method. Standard seven point calibration curves, covering the range 0.3–30 ng/mL, were used for determination of linearity. A weighted ($1/x$) least squares regression analysis was used to determine the intercepts, slopes and correlation coefficients (r). Linearity was considered to be acceptable when correlation coefficients were higher than 0.99 and calibrators had accuracies of 85%–115% and precisions within ±15% RSD.

The intra-day precision and accuracy of the analytical method were assessed by processing and analyzing five replicates of the OMBF standard solutions at three concentration levels. The inter-day precision and accuracy were evaluated over 3 days by analyzing 15 samples ($n=5$ for each concentration level) each day. Precision of determination was expressed as the percentage

relative standard deviation (% RSD) and accuracy was expressed as the percentage of nominal values. The acceptance limits were <15% RSD for precision and 85%–115% for accuracy.

The recovery was also evaluated by adding OMBF standard solution to a TCMI sample. In this experiment, Mailuoning injection (Lot No. 20140544) containing 75.0 ng/mL of OMBF was used. The Mailuoning Injection was diluted 10 times with water. 1 mL of diluted Mailuoning Injection was added to a 2 mL volumetric flask. Then 0.4, 0.5 and 0.6 mL of OMBF standard solution (15 ng/mL) were added to the flasks, respectively, and the volumes were adjusted to 2 mL by adding water. To determine recovery at LOQ, the Mailuoning Injection was diluted to 0.6 ng/mL. A 0.2 mL of OMBF standard solution (15 ng/mL) was spiked into 1 mL of diluted Mailuoning Injection, and then the volumes were adjusted to 2 mL by adding water. These samples were prepared in triplicate for each concentration level and were disposed as described above, and analyzed with the procedure. The average recovery was estimated by the Eq. (1):

$$\text{Recovery (\%)} = \frac{(\text{Amount determined} - \text{Original amount})}{\text{Amount spiked}} \times 100 \quad (1)$$

Stability studies were carried out as part of the method validation. OMBF stability in terms of short-term stability and long-term stability were assessed by analyzing replicates ($n=3$) of standard solution samples at concentrations of 0.6, 12 and 24 ng/mL, and a TCMI sample (Mailuoning Injection, Lot No. 20140544). The standard solutions of OMBF were prepared in methanol. The short-term stability was evaluated after exposure of the standard solution samples of OMBF and the TCMI sample Mailuoning Injection to room temperature for 0, 2, 4, 8, 12 and 24 h, and the long-term stability was examined at room temperature over 0, 2, 3, 7 and 15 days.

The absolute matrix effect was also determined by comparing the chromatographic peak areas of OMBF spiked into blank solvent with peak areas obtained from the same concentration of OMBF in the glucose injection. The matrix effect was calculated using the Eq. (2):

$$\text{Matrix effect (\%)} = \frac{(\text{Peak area of analyte spiked in blank solvent})}{(\text{Peak area of analyte in glucose injection})} \times 100 \quad (2)$$

The experiment was also evaluated at three concentrations of the analyzed compound ($n=6$ for each concentration level).

Residual action was also evaluated in this experiment by analyzing blank samples after injection high concentration of OMBF (30 ng/mL, $n=6$). The acceptance limit was <20% LOQ.

3. Results and discussion

3.1. Optimization of HPLC–MS/MS conditions

To optimize the chromatographic separation, a serial of preliminary experiments were performed, testing different mobile phases consisting of methanol, acetonitrile or mixture of acetonitrile and methanol as an organic phase and water with different mobile phase additives, such as formic acid and acetic acid at various concentrations. The addition of aqueous formic acid and acetic acid reduced peak tailing and improved the response of OMBF in positive ESI mode. Finally, methanol/0.1% aqueous formic acid (v/v) was selected as the best mobile phase for the chromatographic separation.

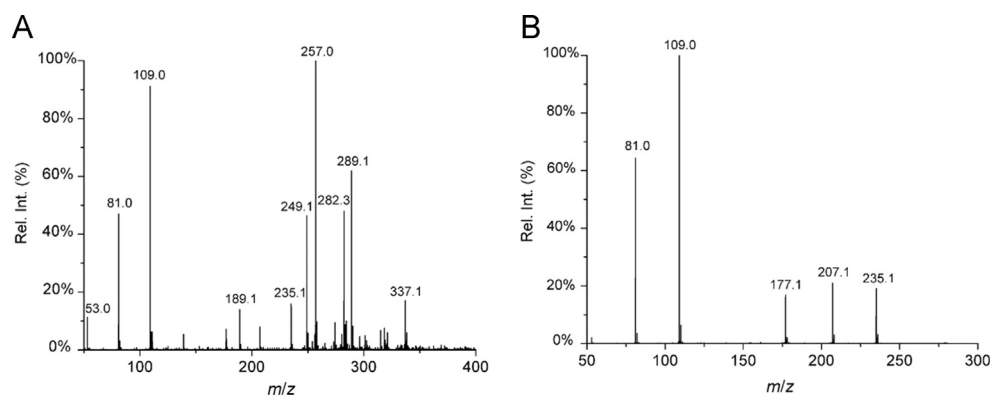


Figure 2 ESI-MS spectrum (A) of 5,5'-oxydimethylenebis(2-furfural), and product ion scan (MS/MS) spectrum (B) of the precursor ion $[M+H]^+$ in the positive ion mode.

Table 1 Intra- and inter-day assay precisions and accuracies of the developed HPLC-MS/MS method for the determination of OMBF.

| Nominal (ng/mL) | Intra-day ($n=5$) | | | Inter-day ($n=15$) | | |
|-----------------|----------------------------|----------------------------|---------------------------|----------------------------|----------------------------|---------------------------|
| | Determined (mean \pm SD) | Precision ^a (%) | Accuracy ^b (%) | Determined (mean \pm SD) | Precision ^a (%) | Accuracy ^b (%) |
| 0.6 | 0.69 \pm 0.01 | 7.42 | 113.33 | 0.68 \pm 0.07 | 9.52 | 113.33 |
| 12 | 12.64 \pm 0.23 | 1.82 | 105.33 | 12.50 \pm 0.21 | 1.66 | 104.17 |
| 24 | 23.78 \pm 0.36 | 1.50 | 99.08 | 23.95 \pm 0.40 | 1.65 | 99.79 |

^aExpressed as $RSD(\%) = (SD/mean) \times 100$.

^bCalculated as $(\text{mean determined concentration}/\text{nominal concentration}) \times 100$.

Full scan and MS/MS mass spectra were obtained from infusion of 10 ng/mL standard solution of OMBF at a flow rate of 0.1 mL/min. The protonated molecular ion $[M+H]^+$ and the sodium adduct ion $[M+Na]^+$ of OMBF were observed at m/z 235.1 and 257.0, respectively, in the positive ion mode mass spectrum (Fig. 2). The MS/MS spectrum of the precursor ion at m/z 235.1 displayed characteristic product ions at m/z 207.1, 177.1, 109.0 and 81.0. The product ion at m/z 109.0, which had the highest relative intensity, was chosen as the quantitative ion.

The optimization of ESI-MS/MS parameters was performed for analyte in infusion experiments: 2 ng/mL standard solution of OMBF was infused at a constant flow-rate of 5 μ L/min into the mass spectrometer using a syringe pump. The following parameters were tested: nebulizer gas, turbo gas, curtain gas, spray voltage, capillary temperature, entrance potential, declustering potential and collision energy. The optimal conditions are given in the experimental section.

3.2. Method validation

3.2.1. Sensitivity and linearity

The calibration curve for OMBF was $y=62169x+814.94$ (x , concentration of reference substance; y , peak area). Good results were achieved in the range of 0.3–30 ng/mL for OMBF, with an excellent correlation coefficient ($r=0.9998$). The LOD ($S/N=3$) and LOQ ($S/N=10$) for OMBF were 0.1 and 0.3 ng/mL, respectively. The LOQ was determined as the lowest concentration point on the calibration curve that could be quantitated with an accuracy

Table 2 Recoveries of OMBF in traditional Chinese medicine injection.

| No. | Original amount (ng/mL) | Spiked (ng/mL) | Measured (ng/mL) | Recovery (%) |
|-----|-------------------------|----------------|------------------|--------------|
| 1 | 0.3 | 1.5 | 1.62 | 88.0 |
| 2 | 0.3 | 1.5 | 1.58 | 88.33 |
| 3 | 0.3 | 1.5 | 1.65 | 90.0 |
| 4 | 3.75 | 3.0 | 6.76 | 100.33 |
| 5 | 3.75 | 3.0 | 6.74 | 99.67 |
| 6 | 3.75 | 3.0 | 7.04 | 109.67 |
| 7 | 3.75 | 3.75 | 7.81 | 108.27 |
| 8 | 3.75 | 3.75 | 7.44 | 98.40 |
| 9 | 3.75 | 3.75 | 7.36 | 96.27 |
| 10 | 3.75 | 4.5 | 7.89 | 92.00 |
| 11 | 3.75 | 4.5 | 8.02 | 94.89 |
| 12 | 3.75 | 4.5 | 8.21 | 99.11 |

within $\pm 15\%$ bias of nominal concentration and precision not exceeding 15% coefficient of variation.

3.2.2. Precision and accuracy

Precision and accuracy were determined by analyzing high, medium and low standard concentrations of OMBF samples on the same day (intra-day, $n=5$) and continuously for 3 days (inter-day, $n=15$). Intra- and inter-day precision and accuracy, as shown in Table 1, were in the range 1.50%–9.52%, and 99.08%–113.33%, respectively.

Table 3 Results of stability tests carried out on standard solutions of OMBF at three concentrations, and a TCMI sample (Mailuoning Injection) under different storage conditions.

| Theoretical concentration (ng/mL) | Short-term stability | | | | | | Long-term stability | | | | | | | | |
|-----------------------------------|----------------------------------|------|------|------------------|------|------|----------------------------------|-------|-------|------------------|-------|--------------|------|-------|-------|
| | Determined concentration (ng/mL) | | | Precision (RSD%) | | | Determined concentration (ng/mL) | | | Precision (RSD%) | | | | | |
| | 0 h | 2 h | 4 h | 8 h | 12 h | 24 h | 0 d | 2 d | 3 d | 7 d | 15 d | Accuracy (%) | | | |
| 0.6 | 0.76 | 0.68 | 0.65 | 0.74 | 0.62 | 0.67 | 7.7 | 114.7 | 0.69 | 0.74 | 0.60 | 0.81 | 0.62 | 12.32 | 115.2 |
| 12 | 12.0 | 12.4 | 12.7 | 12.8 | 13.0 | 12.5 | 2.8 | 104.7 | 12.84 | 12.54 | 12.32 | 12.7 | 12.8 | 1.69 | 105.3 |
| 24 | 23.2 | 24.2 | 25.1 | 25.2 | 25.3 | 24.1 | 3.4 | 102.1 | 24.2 | 24.26 | 23.8 | 24.1 | 24.3 | 8.30 | 100.6 |
| 75.0 | 74.8 | 74.6 | 79.9 | 81.2 | 78.9 | 76.2 | 3.6 | 103.5 | 76.2 | 74.8 | 79.2 | 79.2 | 79.4 | 2.73 | 103.7 |

The results revealed that all values were in the acceptable ranges, indicating the method was reliable and reproducible for the determination of OMBF in traditional Chinese medicine injections.

3.2.3. Recovery and stability

For the recovery test, known amounts (low, medium, and high) of the OMBF were spiked into samples and then prepared as test solutions. The determination was performed in triplicate, and the average recoveries and RSD were calculated and summarized in Table 2. The developed method had good accuracy with the overall recovery of 88.0%–109.67%, with the RSD of 7.27%. Thus, the recoveries of OMBF were consistent, precise and reproducible within the acceptance criteria.

The results of short-term stability and long-term stability of OMBF in TCMI are shown in Table 3. All the results indicated that the analyte was stable at room temperature for 24 h, and also unaffected by storage at room temperature for 15 days.

3.2.4. Matrix effect and residual action

The matrix effects of the analysis were within the range of 95%–105%, indicating that no significant ion suppression or enhancement of glucose solution was observed using the current method. After injection high concentration of OMBF sample, the residue in blank sample was 5.8% of LOQ. The residual action in the present method thus meet the acceptance criteria.

Based on all of these validation results, the present method was considered to be suitable for the quantitative analysis of OMBF in TCMI samples.

3.3. Quantitative determination of OMBF in TCMI and glucose injection samples

59 TCMI and glucose injection samples from different batches or different manufacturers were analyzed using the established HPLC–MS/MS method. A representative MRM chromatogram of TCMI samples is shown in Fig. 3A. Peak identity was confirmed by both retention time compared to that of the reference analyte (Fig. 3B) and by the characteristic ion pairs. The results of quantitative determination of OMBF in TCMI and glucose injections are shown in Table 4. OMBF was detected in 6 samples, and the content of OMBF was in the range 0.37–127 ng/mL. Obviously, the concentrations of OMBF were significantly different between different batches from one manufacturer as well as between different manufacturers. Such significant differences in the content of OMBF would likely to be associated with the variability of sugar content in the raw material and the pH value of TCMI. In our previous study, we found Mailuoning Injection (Lot No. 20120451) contained a high concentration of 5-HMF (1420 µg/mL)²¹. In this study, two batches of Mailuoning Injection (Lot Nos. 20140928 and 20140544) produced by the same manufacturer, were also found to contain high concentration of OMBF. These products may increase the risk to cause adverse drug reaction. In addition, it is worth noting that the OMBF was also detected in a glucose injection sample. Therefore, further toxicity studies on compound OMBF should be conducted, and it is strongly recommended that limit criteria for the content of OMBF in TCMI and glucose injection should be established to ensure the safety in clinical application.

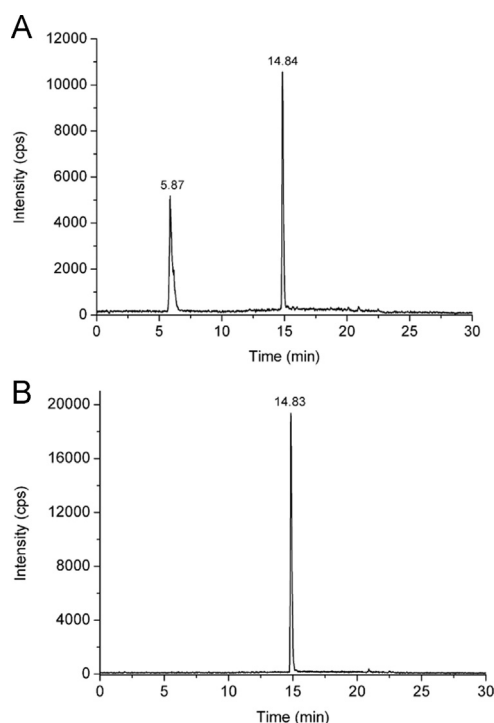


Figure 3 Representative MRM chromatograms of (A) a TCM sample (Mailuoning Injection, Lot No. 20140544), (B) standard solution of OMBF at 2.4 ng/mL analyzed by HPLC–MS/MS method. The retention time for OMBF was 14.8 min.

Table 4 Results of OMBF quantification in 59 samples of TCM and glucose injection using the developed HPLC–MS/MS method.

| No. | Sample name | Lot No. | Content (ng/mL) |
|-----|---------------------------------------|------------|-----------------|
| 1 | Shuxuening injection ^{#1} | 1041411041 | n.d. |
| 2 | Shuxuening injection ^{*1#1} | 1041410242 | n.d. |
| 3 | Shuxuening injection ^{#1} | 140726B1 | n.d. |
| 4 | Shuxuening injection ^{*1#1} | 140725B2 | n.d. |
| 5 | Qingkailing injection ^{#2} | 14052902 | n.d. |
| 6 | Qingkailing injection ^{#2} | 14052305 | n.d. |
| 7 | Qingkailing injection ^{*2#2} | 14011208 | n.d. |
| 8 | Qingkailing injection ^{*2} | 5140830B2 | n.d. |
| 9 | Qingkailing injection ^{#2} | 14120951 | n.d. |
| 10 | Qingkailing injection ^{#2} | 14122752 | n.d. |
| 11 | Qingkailing injection ^{#2} | 14121862 | n.d. |
| 12 | Qingkailing injection ^{*2#2} | 14122663 | n.d. |
| 13 | Chaihu injection ^{*3} | 1501092 | n.d. |
| 14 | Chaihu injection ^{*3} | 14121622 | n.d. |
| 15 | Chaihu injection ^{*3} | 311405092 | n.d. |
| 16 | Chaihu injection ^{*3} | 141022D1 | n.d. |
| 17 | Huangqi injection ^{#4} | 141106A3 | n.d. |
| 18 | Huangqi injection ^{#4} | 141126A3 | n.d. |
| 19 | Huangqi injection ^{*4#4} | 141127A2 | n.d. |
| 20 | Huangqi injection ^{*4} | A20141201 | n.d. |
| 21 | Huangqi injection ^{*4} | 1410273 | n.d. |
| 22 | Xuesaitong injection ^{#5} | B20140416 | n.d. |
| 23 | Xuesaitong injection ^{#5} | B20140710 | n.d. |
| 24 | Xuesaitong injection ^{#5} | B20140402 | n.d. |
| 25 | Xuesaitong injection ^{#5} | B20140306 | n.d. |
| 26 | Xuesaitong injection ^{#5} | B20140709 | n.d. |
| 27 | Xuesaitong injection ^{*5#5} | B20140708 | n.d. |

Table 4 (continued)

| No. | Sample name | Lot No. | Content (ng/mL) |
|-----|---|-------------|-----------------|
| 28 | Xuesaitong injection ^{#5} | ZGA1457 | n.d. |
| 29 | Xuesaitong injection ^{*5#5} | ZGA1446 | n.d. |
| 30 | Xuesaitong injection ^{#5} | 14HJ205-11 | n.d. |
| 31 | Xuesaitong injection ^{#5} | 14GJ206-11 | n.d. |
| 32 | Xuesaitong injection ^{#5} | 14GJ205-21 | n.d. |
| 33 | Xuesaitong injection ^{*5#5} | 14GJ205-22 | n.d. |
| 34 | Shuanghuanglian injection ^{#6} | 14094012 | n.d. |
| 35 | Shuanghuanglian injection ^{#6} | 14094013 | n.d. |
| 36 | Shuanghuanglian injection ^{#6} | 14094112 | n.d. |
| 37 | Shuanghuang injection ^{*6#6} | 14094111 | n.d. |
| 38 | Shenmai injection ^{#7} | 13071321 | n.d. |
| 39 | Shenmai injection ^{*7#7} | 140427F1 | n.d. |
| 40 | Mailuoning injection ^{#8} | 20140544 | 75.0 |
| 41 | Mailuoning injection ^{*8#8} | 20140928 | 127 |
| 42 | Zhiyinhuang injection | 140913B1 | n.d. |
| 43 | Tianmasu injection ^{#9} | 14070303-2 | n.d. |
| 44 | Tianmasu injection ^{*9#9} | 1411150111 | n.d. |
| 45 | Gegensu injection ^{#10} | 1411037 | n.d. |
| 46 | Gegensu injection ^{*10#10} | 141004 | n.d. |
| 47 | Chuanhuning injection ^{#11} | 141015 | n.d. |
| 48 | Chuanhuning injection ^{#11} | 140704 | n.d. |
| 49 | Chuanhuning injection ^{*11#11} | 140718 | n.d. |
| 50 | Guanxinning injection | 10214091627 | n.d. |
| 51 | Dengzhanxixin injection | 20150148 | Det |
| 52 | Shengmai injection ^{*12} | 13120711 | 2.44 |
| 53 | Shengmai injection ^{#12} | 14060602 | n.d. |
| 54 | Shengmai injection ^{*12#12} | 14050704 | n.d. |
| 55 | Ciwujia injection | 2014090521 | 0.37 |
| 56 | Chuanxinlian injection | 14062201A | n.d. |
| 57 | Danxianguanxin injection | 251150106 | Det |
| 58 | Xiyanping injection | 2014121403 | 1.18 |
| 59 | Glucose injection | D14070210 | 0.76 |

n.d., not detected—peak not observed or concentration is lower than the LOD.

Det, detected—peak observed, concentration is lower than the LOQ but higher than the LOD.

*The test samples with the same name from different manufacturers.

#The test samples with the same name but different lot No. from the same manufacturer.

4. Conclusions

In this study, a rapid and sensitive HPLC–MS/MS method has been developed and validated for the quantitative determination of OMBF in TCMs and glucose injections for the first time. It was successfully applied to large-scale screening of OMBF in commercially available TCMs and glucose injections. The developed HPLC–MS/MS method has been elucidated to be a simple, sensitive, practicable and reliable quality control procedure for TCM and glucose injection. In addition, this method can be further adapted for the analysis of OMBF in other herbal medicines or preparations containing this compound.

Acknowledgments

This research was supported by Chinese Academy of Medical Science (CAMS) Innovation Fund for Medical Sciences (Grant Nos. 2016-I2M-1-009 and 2016-I2M-3-010) and National

Scientific and Technological Major Project for New Drugs (Grant No. 2017ZX09101003-002-004).

References

- 1 Wang Z, Wang D, Sui Y, Cui H, Yu Y. Experimental study on anaphylaxis of Qingkailing Injection and its components on Beagle dogs. *J Tradit Chin Med* 2012;**32**:641–5.
- 2 Zhi XW, Su XM, Feng WY, Zhang HM. Effect and mechanism of Danhong Injection on isolated mesenteric arterial rings in rats. *China J Chin Mater Med* 2012;**37**:2607–11.
- 3 Li M, Qiao C, Qin L, Zhang J, Ling C. Application of traditional Chinese medicine injection in treatment of primary liver cancer: a review. *J Tradit Chin Med* 2012;**32**:299–307.
- 4 Wu GL, Zhang L, Yu GY. Analysis on the common causes for traditional Chinese medicinal injection induced adverse reactions and the preventive countermeasures. *Chin J Integr Tradit West Med* 2008;**28**:1137–40.
- 5 Wang L, Yuan Q, Marshall G, Cui X, Cheng L, Li Y, et al. Adverse drug reactions and adverse events of 33 varieties of traditional Chinese medicine injections on National Essential Medicines List (2004 edition) of China: an overview on published literatures. *J Evid Based Med* 2010;**3**:95–104.
- 6 Ji KM, Chen J, Li M, Liu Z, Xia L, Wang C, et al. Comments on serious anaphylaxis caused by nine Chinese herbal injections used to treat common colds and upper respiratory tract infections. *Regul Toxicol Pharmacol* 2009;**55**:134–8.
- 7 Duan B, Hu J, Huang L, Yang X, Chen F. Chemical fingerprint analysis of Gentianae Radix et Rhizoma by high-performance liquid chromatography. *Acta Pharm Sin B* 2012;**2**:46–52.
- 8 Xiao PG, Liu CX. A re-understanding on the safety matters of Chinese herbal medicine. *Chin J Integr Med* 2004;**10**:242–5.
- 9 Fan M, Qin K, Ding F, Huang Y, Wang X, Cai B. Identification and differentiation of major components in three different “Sheng-ma” crude drug species by UPLC/Q–TOF-MS. *Acta Pharm Sin B* 2017;**7**:185–92.
- 10 Liang Q, Ma J, Ma Z, Wang Y, Tan H, Xiao C, et al. Chemical comparison of dried rehmannia root and prepared rehmannia root by UPLC-TOF MS and HPLC-ELSD with multivariate statistical analysis. *Acta Pharm Sin B* 2013;**3**:55–64.
- 11 Li T, Zhuang S, Wang Y, Wang Y, Wang W, Zhang H, et al. Flavonoid profiling of a traditional Chinese medicine formula of Huangqin Tang using high performance liquid chromatography. *Acta Pharm Sin B* 2016;**6**:148–57.
- 12 Mauron J. The Maillard reaction in food: a critical review from the nutritional standpoint. *Prog Food Nutr Sci* 1981;**5**:5–35.
- 13 Antal Jr MJ, Mok WS, Richards GN. Mechanism of formation of 5-(hydroxymethyl)-2-furaldehyde from D-fructose and sucrose. *Carbohydr Res* 1990;**199**:91–109.
- 14 Jain A, Shore AM, Jonnalagadda SC, Ramanujachary KV, Mugweru A. Conversion of fructose, glucose and sucrose to 5-hydroxymethyl-2-furfural over mesoporous zirconium phosphate catalyst. *Appl Catal A-Gen* 2015;**489**:72–6.
- 15 Jalili M, Ansari F. Identification and quantification of 5-hydroxymethyl furfural in food products. *Nutr Food Sci Res* 2015;**2**:47–53.
- 16 de Andrade JK, de Andrade CK, Komatsu E, Perreault H, Torres YR, da Rosa MR, et al. A validated fast difference spectrophotometric method for 5-hydroxymethyl-2-furfural (HMF) determination in corn syrups. *Food Chem* 2017;**228**:197–203.
- 17 Gao W, Qi LW, Liu CC, Wang R, Li P, Yang H. An improved method for the determination of 5-hydroxymethylfurfural in Shenfu Injection by direct analysis in real time-quadrupole time-of-flight mass spectrometry. *Drug Test Anal* 2016;**8**:738–43.
- 18 Bauer-Marinovic M, Taugner F, Florian S, Glatt H. Toxicity studies with 5-hydroxymethylfurfural and its metabolite 5-sulphoxymethylfurfural in wild-type mice and transgenic mice expressing human sulphotransferases 1A1 and 1A2. *Arch Toxicol* 2012;**86**:701–11.
- 19 Monien BH, Engst W, Barknowitz G, Seidel A, Glatt H. Mutagenicity of 5-hydroxymethylfurfural in V79 cells expressing human SULT1A1: identification and mass spectrometric quantification of DNA adducts formed. *Chem Res Toxicol* 2012;**25**:1484–92.
- 20 Zhang XM, Chan CC, Stamp D, Minkin S, Archer MC, Bruce WR. Initiation and promotion of colonic aberrant crypt foci in rats by 5-hydroxymethyl-2-furaldehyde in thermolyzed sucrose. *Carcinogenesis* 1993;**14**:773–5.
- 21 Chinese Pharmacopoeia Commission. Glucose Injection: limit of 5-hydroxymethyl furfural. In: *Pharmacopoeia of the People’s Republic of China*, II. Beijing: China Medical Science Press; 2015. p. 1270.
- 22 United States Pharmacopoeial Convention. Dextrose Injection: limit of 5-hydroxymethylfurfural and related substances. In: *United States Pharmacopoeia and National Formulary* (USP35-NF30). Rockville, MD: United States Pharmacopoeial Convention Inc.; 2012. p. 2862.
- 23 Zang QC, He JJ, Bai JF, Zheng YJ, Zhang RP, Li TG, et al. Rapid screening and quality evaluation for the harmful substance 5-hydroxymethyl furfural in commercially available traditional Chinese medicine injection using LC–MS/MS method. *Acta Pharm Sin* 2013;**48**:1705–9.
- 24 Choudhary V, Mushrif SH, Ho C, Anderko A, Nikolakis V, Markinkovic NS, et al. Insights into the interplay of Lewis and Brønsted acid catalysts in glucose and fructose conversion to 5-(hydroxymethyl) furfural and levulinic acid in aqueous media. *J Am Chem Soc* 2013;**135**:3997–4006.
- 25 Qi L, Mui YF, Lo SW, Lui MY, Akien GR, Horváth IT. Catalytic conversion of fructose, glucose, and sucrose to 5-(hydroxymethyl) furfural and levulinic and formic acids in γ -valerolactone as a green solvent. *ACS Catal* 2014;**4**:1470–7.
- 26 Gilkey MJ, Panagiotopoulou P, Mironenko AV, Jenness GR, Vlachos DG, Xu BJ. Mechanistic insights into metal lewis acid-mediated catalytic transfer hydrogenation of furfural to 2-methylfuran. *ACS Catal* 2015;**5**:3988–94.
- 27 Popoff T, Theander O. Formation of aromatic compounds from carbohydrates. Part III. Reaction of D-glucose and D-fructose in slightly acid, aqueous solution. *Acta Chem Scand B* 1976;**30**:397–402.
- 28 Chundury D, Szmant HH. Preparation of polymeric building blocks from 5-hydroxymethyl and 5-chloromethylfurfuraldehyde. *Ind Eng Chem Prod Res Dev* 1981;**20**:158–63.
- 29 Musau RM, Munavu RM. The preparation of 5-hydroxy-2-furaldehyde (HMF) from D-fructose in the presence of DMSO. *Biomass* 1987;**13**:67–74.
- 30 Lin N, Liu TT, Lin L, Lin S, Zang QC, He JM, et al. Comparison of *in vivo* immunomodulatory effects of 5-hydroxymethylfurfural and 5,5'-oxydimethylenebis (2-furfural). *Regul Toxicol Pharmacol* 2016;**81**:500–11.
- 31 Larousse C, Rigal L, Gaset A. Synthesis of 5,5'-oxydimethylenebis (2-furfural) by thermal dehydration of 5-hydroxymethyl-2-furfural in the presence of dimethylsulfoxide. *J Chem Technol Biotechnol* 1992;**53**:111–6.
- 32 Food and Drug Administration, Guidance for industry: analytical procedures and methods validation, chemistry, manufacturing, and controls documentation (Draft), 2000.
- 33 State food and drug administration, Guidance for validation of analytical methods in the quality control of chemical drugs (No. [H] GPH5-1). 2005. Available from: <http://www.sda.gov.cn/directory/web/WS01/images/u6Rp9KpzuWysGv9jWxrfWzva3vbe0enWpLy8yvXWuLW81K3U8i5wZGY=.pdf>.