Determination of 5-Hydroxymethylfurfural, N\textsubscript{(Carboxymethyl)Lysine and N\textsubscript{(Carboxyethyl)Lysine in 16 Traditional Chinese Medicine Injections Based on HPLC and UPLC-MS/MS

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Abstract

**Background:** Traditional Chinese medicine injections (TCMIs) are widely applied to treat many chronic diseases. However, product quality problems occur occasionally due to unknown constituents in TCMIs. 5-hydroxymethylfurfural (5-HMF), N-\((\text{carboxymethyl})\)lysine (CML) and N-\((\text{carboxyethyl})\)lysine (CEL) are three compounds generated during food and Chinese medicinal herb processing and may be harmful to human health.

**Methods:** In this study, the contents of 5-HMF, CML and CEL were determined by high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC). For 5-HMF, the separation was performed on a Hypersil ODS2 column (250 mm×4.6 mm, 5 µm), and the column temperature was set at 30°C. The mobile phase was composed of water-methanol (95:5) at a flow rate of 1.0 mL/min. For CML and CEL, separation was performed on a CORTECS HILIC UPLC column (2.1 mm×50 mm, 1.6 µm), and the column temperature was set at 40°C. The mobile phase was composed of acetonitrile-water (3:7) at a flow rate of 0.3 mL/min. Multiple-reaction monitoring mode was employed for analyte determination with positive ionization.

**Results:** The contents of 5-HMF in 16 TCMIs varied from 0.19 to 74.98 µg/mL, with a larger variation than the contents of CML and CEL. The Ciwujia injection had the highest content of 5-HMF, and the Qingkailing injection had the lowest 5-HMF content. The contents of CML and CEL among these TCMIs were 0.51-7.32 ng·mL\(^{-1}\) and 0.38-5.49 ng·mL\(^{-1}\), respectively. The contents of CML and CEL in the Shuxuetong injection were much higher than in the others.

**Conclusions:** The methods established in this study were simple, rapid and accurate and could provide a theoretical basis for the quality evaluation of TCMIs.

**Background**

Traditional Chinese medicine (TCM) is a diagnostic system of ancient medical practice that has evolved over thousands of years to help prevent and cure diseases [1]. As the most effective and popular administration form of TCM [2], traditional Chinese medicine injections (TCMIs) are widely applied to treat acute upper respiratory tract infections [3], angina pectoris [4], heart failure [5] and other chronic diseases. Owing to the uncertainty of the active constituents and the complexity of TCM prescriptions, adverse drug reactions (ADRs) induced by the toxicity of TCMIs have occurred occasionally [6]. In recent years, the potential toxicity of TCMIs has seriously affected its clinical application [7].

5-Hydroxymethylfurfural (5-HMF, Fig. 1A) is a furanic compound that is produced by the Maillard reaction and caramelization during food processing and Chinese medicinal herb preparation [8]. 5-HMF formation in TCMs is mainly derived from the heating decomposition of sugars under acidic conditions [9]. The intake of 5-HMF is harmful to human health because it stimulates the upper respiratory tract, skin and mucous membranes [10–12]. In Chinese Pharmacopeia, the content of 5-HMF in glucose injections has been regulated since 1985 [13].
Advanced glycation end-products (AGEs) are stable oxidant compounds produced by nonenzymatic reactions between nucleic acids, amino acids, proteins and reducing sugars [14–16]. AGEs have been shown to accumulate in tissues and are related to atherosclerosis [17], Alzheimer’s disease [18], diabetes [19] and other chronic diseases. More than 20 AGEs have been identified in both intra- and extracellular tissue proteins [20]. As two typical AGEs, N^\(\text{II}\)-(carboxymethyl)lysine (CML, Fig. 1B) and N^\(\text{II}\)-(carboxyethyl)lysine (CEL, Fig. 1C) are mainly generated by the reactions between lysine, methylglyoxal (MGO) and glyoxal (GO) [21, 22] and are often used as biomarkers to evaluate the contents of AGEs in foods [23, 24].

To analyse the contents of 5-HMF, CML and CEL in food matrices, many techniques, including high-performance liquid chromatography (HPLC) [25], reversed-phase high-performance liquid chromatography (RP-HPLC) [26], liquid chromatography-mass spectrometry (LC-MS) [27], high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) [11], ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) [28] and enzyme linked immunosorbent assays (ELISA) [29], have been employed to develop analytical methods. Because of the lack of specific antibodies and matrix effects, the ELISA method has not been widely applied as a detection method. With the advantage of repeatability and stability, HPLC, HPLC-MS/MS and UPLC-MS have been commonly used to identify and determine the contents of AGEs in various matrices [25, 28]. To date, 5-HMF has been analysed in shengmaiyn [30], Zuo Gui Wan [31] and other TCMIs [32]. However, studies on the determination of CML and CEL in TCMIs are lacking.

In the present study, we developed HPLC and UPLC-MS/MS techniques to determine the levels of 5-HMF, CML and CEL in TCMIs. Validated methods were used to measure the contents of 5-HMF, CML and CEL in different TCMIs, as they may contribute to AGE formation. Preliminary analysis and evaluation were performed according to the results, which may provide a theoretical basis for standard quality evaluation of TCMIs.

**Methods**

**Materials and reagents**

5-Hydroxymethylfurfural (5-HMF, CAS: 67-47-0, purity > 99%), N^\(\text{II}\)-(carboxymethyl)lysine (CML, CAS: 5746-04-3, purity > 99%) and N^\(\text{II}\)-(carboxyethyl)lysine (CEL, CAS: 5746-03-2, purity > 99%) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and methanol of HPLC grade were obtained from Fisher-Scientific (Waltham, MA, USA). Ultrapure water was obtained from a Millipore Milli-Q Advantage A10 system (Millipore, Bedford, USA). Chinese medicine injections were purchased from an ordinary pharmacy.

**Determination of 5-HMF**

A stock solution of 5-HMF was prepared at a concentration of 0.15 mg/mL, which was further diluted with 10% methanol to a series of calibration standards with concentrations of 0.15, 2.34, 9.38, 37.5, and
75 µg/mL. Quality control (QC) samples were also diluted to concentrations of 0.15, 9.38, and 75 µg/mL. A total of 200 µL of each Chinese medicine injection was diluted 10-fold with 10% methanol prior to HPLC analysis.

HPLC was conducted to determine the content of 5-HMF in different Chinese medicine injections. The separation was performed on a Hypersil ODS2 column (250 mm×4.6 mm, 5 µm, Waters Corp., Milford, MA, USA), and the column temperature was set at 30°C. The mobile phase was composed of water (solvent A) and methanol (solvent B). Gradient elution began with 5% solvent B for 5 min, followed by a linear gradient of 5-15% solvent B from 5 min to 15 min, and then returning to a linear gradient of 15%-5% solvent B from 15 min to 20 min. The flow rate was 1.0 mL/min, and the injection volume was 20 µL.

Method validation was conducted according to the US Food and Drug Administration guidelines [33], and method validation included selectivity, linearity, accuracy, precision, recovery and stability determination.

The selectivity of the method was determined by evaluating 6 blank reagent injections (10% methanol). The linearity was determined by using calibration standard samples at concentrations of 0.15, 2.34, 9.38, 37.5, and 75 µg/mL, and each level was independently injected 3 times. Calibration curves were constructed by plotting the average peak areas (y) of each sample against the calibration concentrations (x). The limits of detection (LODs) and quantification (LOQs) were evaluated by calculating the concentrations corresponding to signal-to-noise (S/N) ratios of 3 and 10, respectively [34]. The intra- and inter-day precision and accuracy were determined by measuring the concentrations of QC samples and were evaluated by the relative standard deviation (RSD). The stability of 5-HMF was determined by evaluating QC samples under three conditions, including three freeze-thaw cycles (-80°C), storage at room temperature for 4 h, storage at 4°C for 1 d, and storage at -20°C for 21 d. The recovery was assessed by calculating the ratios of concentrations between analytes in the QC samples and those in samples with known concentrations.

**Determination of CML and CEL**

A stock solution of CML and CEL was prepared with each at a concentration of 1 mg/mL, which was diluted to working solutions at a series of concentrations of 0.078, 0.313, 1.25, 5, 20, and 640 ng/mL. Two hundred microlitres of a Chinese medicine injection was measured and diluted 10-fold with ultrapure water, and 2 µL was injected into the UPLC system for analysis.

UPLC-MS/MS system was employed to analyse the concentrations of CML and CEL in Chinese medicine injections. The separation was achieved with a CORTECS HILIC UPLC column (2.1 mm×50 mm, 1.6 µm, Waters Corp., Milford, MA, USA) maintained at 40°C. Gradient elution was performed with water (solvent A) and acetonitrile (solvent B) with the following programme: 0-1 min (100% B), 1-2.5 min (100-60% B), 2.5-4 min (60% B), and 4-6 min (100% B). The flow rate was 0.3 mL/min, and the injection volume was 2 µL. Quantification of CML and CEL were performed by using standards and by reference to an external standard calibration curve. Data are reported as means±SD of triplicate experiments.
Mass spectrometric detection was achieved on a Waters Xevo TQ mass spectrometer (Waters Corp., Milford, MA, USA) with electrospray ionization (ESI) in multiple reaction monitoring (MRM) mode. MS detection was performed in positive ionization mode. The parent ions for CML and CEL were observed at m/z 205 and 219, respectively. CML and CEL were fragmented to produce ion signals at m/z 130.14→84.10 and m/z 130.15→84.10. Data acquisition was accomplished with Masslynx software version 4.1 (Waters Corp., Milford, MA, USA).

The method validation conducted as outlined in the description of “Determination of 5-HMF”. In brief, 6 blank reagent samples (ultrapure water) were used to measure the selectivity. The intra-/inter-day precision and accuracy were calculated using calibration standards at 0.078, 1.25 and 20 ng/mL. The precision was determined by the RSD, and the accuracy was expressed as RE. Recovery was determined by comparing the peak area ratios of samples spiked with calibration standards and the original amount. The stability of the working solution for CML and CEL was evaluated under three freeze-thaw cycles (-80°C), at room temperature for 4 h, at 4°C for 1 d, and at -20°C for 21 d.

**Results And Discussion**

**Quantification of 5-HMF**

**Chromatogram of 5-HMF**

The chromatograms of 5-HMF in the reference solution and that in the Honghua injection are presented in Fig. 2. The retention time of 5-HMF was 9 min, and no interference peaks were observed.

**Linearity**

In the range of 0.15-75 µg/mL, the linear regression curve of 5-HMF was expressed as follows: \( Y=1.159 \times 69 \times 108X+68 \times 068.1, R^2=0.999 \). Excellent linearity indicated that the peak areas responded proportionally to the concentrations of 5-HMF. The LOD and LOQ of 5-HMF were 2.45 ng/mL and 7.59 ng/mL, respectively.

**Precision, accuracy and recovery**

The intra-day precision and inter-day precision are listed in Table 1, and the inter-day precision (RSD) and accuracy (RE) ranged from 2.15 to 5.35% and 2.27 to 4.79%, respectively; the intra-day precision and accuracy ranged from 1.53 to 3.97% and 1.70 to 3.88%, respectively. The recoveries of the three tested concentrations were more than 80.2%.

**Stability**

The stability of 5-HMF was verified under various storage conditions (Table 2). The levels of 5-HMF did not change significantly, with an RE ranging from -0.31 to 6.16. The results showed that the analyte exhibited excellent stability under the four storage conditions.
Quantification of 5-HMF in TCMIs by HPLC

The contents of 5-HMF in 16 TCMIs are listed in Table 3, and the results showed that the contents of 5-HMF were notably high in the Ciwujia (74.98 µg/mL) and Shengmai (65.18 µg/mL) injections and were low in the Qingkailing, Kudiezi and Tianmasu injections, which were even under 1 µg/mL. 5-HMF could not be detected in the Qukeluding, Xingnaojing, and Yinxing leaf extracts or in the Shuxuening, Salvianolate and Xueshuantong injections. According to the literature, the contents of 5-HMF in different TCMIs varied from 0.009 to 4100 mg/L [35], and the results in our research were also within that range. Although the content of 5-HMF in glucose injections is limited (11.8 µg/mL) in the Chinese Pharmacopeia [13], we found that the contents of 5-HMF varied with different injection types, manufacturers and batches; thus, we suggested formulating unified content standards for 5-HMF in different TCMIs.

Quantification of CML and CEL

Mass spectra and chromatography

The UPLC-MS chromatograms of CML and CEL standards and in the Ciwujia injection are shown in Fig. 3. The chromatographic separation was optimized, and the retention times of CML and CEL were 0.6 min. The precursor ions for CML and CEL were observed at m/z 130.14 and 130.15, respectively. CML and CEL were quantified by the major ion at m/z 84.10 (Fig. 4).

Method validation

As shown in Table 4, within the range of 0.078-640 ng/mL, the regression equations of CML and CEL presented great linearity with correlation coefficients ($R^2$) >0.99. The LODs and LOQs were 0.15 and 0.48 for CML and 0.13 and 0.41 for CEL, respectively.

In this study, the inter-/intra-day precision of CML was less than 3.52% and 2.96%, while the inter-/intra-day accuracy of CML was below 2.68% and 3.48%, respectively. For CEL, the inter-/intra-day precision ranged from 0.89% to 3.31% and 1.90% to 3.77%, respectively, and the inter-/intra-day accuracy ranged from 0.94% to 3.20% and -0.29% to 4.15%, respectively. The mean recoveries of CML and CEL were 81.4% and 79.6%, respectively. All results were investigated and are listed in Table 4 and Table 5. These results indicated that the method was feasible and efficient for the detection and quantification of CML and CEL.

Stability

The stability of CML and CEL was investigated under four storage conditions (Table 6). The results indicated that the analytes exhibited great stability, as the concentrations were not significantly changed under the different conditions.

Quantification of CML and CEL in TCMIs by UPLC-MS/MS
The contents of CML and CEL in 16 TCMIs are listed in Table 7, and the results indicated that CML and CEL were detected in 5 and 9 TCMIs, respectively. The contents of CML and CEL in these injections were 0.51-7.32 ng·mL-1 and 0.38-5.49 ng·mL-1, respectively. The contents of CML (7.32 ng/mL) and CEL (5.49 ng/mL) in the Shuxuetong injection were much higher than in the other injections. The CEL level in the Shuanghuanglian powder injection was the lowest (0.38 ng/mL), while the CML level in the Ciwujia injection was the lowest (0.51 ng/mL). We deduced that high protein and fat levels raised the CML and CEL contents in the Shuxuetong injection, as its components were Hirudo and Lumbricus; likewise, high levels of CML and CEL were frequently generated during milk processing [24]. Moreover, the Ciwujia injection is rich in flavones, anthraquinones and other secondary metabolites that could stabilize MGO and GO to limit the generation of CML and CEL during the Maillard reaction [36].

Conclusions

A validated, accurate analytical method using HPLC was developed for the determination of 5-HMF in TCMIs. Simultaneously, we developed and validated a rapid and sensitive method using UPLC-MS/MS for the determination of CML and CEL in TCMIs for the first time. Based on the methods, the contents of 5-HMF, CML and CEL in 16 TCMIs were successfully quantified. The methods exhibited excellent recoveries, inter-/intra-day precision and low LODs and LOQs. Among the results, the Ciwujia injection had the highest content of 5-HMF, and the Qingkailing injection had the lowest 5-HMF content. On the other hand, the contents of CML and CEL in the Shuxuetong injection were much higher than in the others. The methods exhibited simple, rapid and high accuracy that could provide a theoretical basis for the quality evaluation of TCMIs.

Abbreviations

TCM: traditional Chinese medicine; TCMIs: traditional Chinese medicine injections; ADRs: adverse drug reactions; 5-HMF: 5-Hydroxymethylfurfural; AGEs: Advanced glycation end-products; CML: N\(^\text{ε}\)-(carboxymethyl)lysine; CEL: N\(^\text{ε}\)-(carboxyethyl)lysine; MGO: methylglyoxal; GO: glyoxal; HPLC: high-performance liquid chromatography; RP-HPLC: reversed-phase high-performance liquid chromatography; LC-MS: liquid chromatography-mass spectrometry; HPLC-MS/MS: high-performance liquid chromatography coupled to tandem mass spectrometry; UPLC-MS: ultra-performance liquid chromatography-mass spectrometry; ELISA: enzyme linked immunosorbent assays; ESI: electrospray ionization; MRM: multiple reaction monitoring; LODs: limits of detection; LOQs: limits quantification.

Declarations

Ethics approval and consent to participate

This study was approved by the Committee of Institute of 4 Special Animal and Plant Sciences, Chinese Academy of Agricultural Science.
Consent for publication

Not applicable.

Consent for publication

Not applicable.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable

Authors’ contributions

LZ, YS conceived and designed the study; LZ analysed the data and wrote the paper; RG conducted parts of experiment methodology; CL performed the study; All authors read and approved the final manuscript.

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Availability of data and materials

All data used to support the findings of this study are available from the corresponding author upon request.

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### Tables

**Table 1 Precision, accuracy and recovery of 5-HMF in QC samples (n=5)**

| 5-HMF concentration (µg/mL) | Inter-day | Intra-day | Recovery (%) |
|-----------------------------|-----------|-----------|--------------|
|                             | RSD (%)   | RE (%)    | RSD (%)      | RE (%)    |          |
| 0.15                        | 5.35      | 4.79      | 3.97         | 3.88      | 80.2±1.2 |
| 9.38                        | 2.55      | 2.41      | 1.53         | 1.70      | 81.3±1.8 |
| 75                          | 2.15      | 2.27      | 2.80         | 2.71      | 82.5±2.4 |

**Table 2 Stability of 5-HMF under different conditions (n=5)**

| Conditions                  | Concentration (µg/mL) | RSD (%) | RE (%) |
|-----------------------------|-----------------------|---------|--------|
| Freeze-thaw (−80℃, 3 cycles) | 0.15                  | 5.80    | 6.16   |
|                             | 9.38                  | 7.18    | 1.13   |
|                             | 75                    | 2.30    | 1.73   |
| RT, 4 h                     | 0.15                  | 5.27    | 5.02   |
|                             | 9.38                  | 3.64    | 1.96   |
|                             | 75                    | 3.42    | -0.31  |
| 4℃, 1 day                   | 0.15                  | 5.51    | 6.16   |
|                             | 9.38                  | 2.38    | 2.60   |
|                             | 75                    | 4.81    | 2.58   |
| -20℃, 21 days               | 0.15                  | 5.94    | 3.19   |
|                             | 9.38                  | 3.29    | 1.28   |
|                             | 75                    | 3.67    | 0.57   |

**Table 3 Contents of 5-HMF in 16 traditional Chinese medicine injections**
| No. | Sample                      | Lot number | 5-HMF/(µg·mL⁻¹) |
|-----|-----------------------------|------------|-----------------|
| 1   | Honghua injection           | 170406     | 2.83±0.16       |
| 2   | Qingkailing injection 1     | 15112463   | 0.45±0.07       |
| 3   | Kudiezi injection           | 171106     | 0.50±0.12       |
| 4   | Ciwujia injection           | 20151003   | 74.98±0.62      |
| 5   | Qingkailing injection 2     | 180104A2   | 0.19±0.05       |
| 6   | Danshenchuanxiongqin injection | 20170626 | 7.79±0.15       |
| 7   | Qukeuling injection         | 170301010  | —               |
| 8   | Xingnaojing injection       | 1707042    | —               |
| 9   | Yinxing leaf extract        | 19870304   | —               |
| 10  | Shuxuening injection        | 170605     | —               |
| 11  | Tianmasu injection          | 20170305-2 | 0.48±0.02      |
| 12  | Shuxuetong injection        | 17041622   | 2.41±0.32       |
| 13  | Shuanghuanglian powder      | 1611627    | 2.27±0.25       |
| 14  | Salvianolate for injection  | 17040624   | —               |
| 15  | Xueshuantong for injection  | 17070113   | —               |
| 16  | Danshen for injection       | 1706730    | 3.64±0.20       |
| 17  | Shengmai injection          | 1702241    | 65.18±0.46      |

‘—’ not detected

Table 4: Linearity of CML and CEL response

| Analytes  | Regression equation | R²   | Linear range (ng/mL) | LOD (ng/mL) | LOQ (ng/mL) |
|-----------|---------------------|------|----------------------|-------------|-------------|
| CML       | $Y=7.74562\times10^5X$ -685.514 | 0.995 | 0.078-640            | 0.15        | 0.48        |
| CEL       | $Y=1.08648\times10^6X$ -1106.17  | 0.996 | 0.078-640            | 0.13        | 0.41        |

Table 5: Precision, accuracy and recovery of CML and CEL in QC samples (n=5)

| Analytes  | Concentration (ng/mL) | Inter-day RSD (%) | Inter-day RE (%) | Intra-day RSD (%) | Intra-day RE (%) | Recovery (%)  |
|-----------|-----------------------|-------------------|-----------------|------------------|------------------|--------------|
| CML       | 0.078                 | 1.78              | -1.96           | 0.96             | 1.06             | 80.2±1.5     |
|           | 1.25                  | 1.97              | 0.34            | 0.93             | 0.37             | 78.5±2.2     |
|           | 20                    | 3.52              | 2.68            | 2.96             | 3.48             | 85.5±1.4     |
| CEL       | 0.078                 | 0.89              | 0.94            | 2.27             | 1.92             | 82.3±2.1     |
|           | 1.25                  | 1.64              | 0.61            | 1.90             | -0.29            | 76.9±3.2     |
|           | 20                    | 3.31              | 3.20            | 3.77             | 4.15             | 79.6±1.2     |

Table 6: Stability of CML and CEL in different storage conditions (n=5)
| Conditions | Theoretic CML (ng/mL) | Measure CML (ng/mL) | RSD (%) | RE (%) | Theoretic CEL (ng/mL) | Measure CEL (ng/mL) | RSD (%) | RE (%) |
|------------|----------------------|---------------------|---------|-------|----------------------|---------------------|---------|-------|
| Freeze-thaw (~80°C, 3 cycles) | 0.078 | 0.08±0.0 | 10.23 | -1.45 | 0.078 | 0.08±0.0 | 8.69 | -2.76 |
| | 1 | 1.25 | 1.26±0.0 | 1.23 | 1.14 | | 1.25 | 1.25±0.0 | 2.20 | 0.08 |
| | 2 | 20 | 21.31±1.28 | 6.01 | 6.53 | | 20 | 20.41±0.83 | 4.04 | 2.05 |
| | 3 | 0.078 | 0.08±0.0 | 1 | 8.69 | | 0.078 | 0.08±0.0 | 1 | -1.71 |
| | 4 | 1.25 | 1.29±0.0 | 3.86 | 3.47 | | 1.25 | 1.29±0.0 | 3.86 | 3.47 |
| | 5 | 20 | 20.86±0.83 | 3.97 | 4.3 | | 20 | 19.80±0.96 | 4.84 | -0.98 |
| | 6 | 0.078 | 0.08±0.0 | 1 | 7.99 | | 0.078 | 0.08±0.0 | 6.85 | -2.44 |
| | 7 | 1.25 | 1.25±0.0 | 2.98 | -0.26 | | 1.25 | 1.26±0.0 | 3.85 | 0.53 |
| | 8 | 20 | 21.09±1.18 | 5.61 | 5.47 | | 20 | 21.44±1.22 | 5.71 | 6.95 |
| | 9 | 0.078 | 0.08±0.0 | 1 | 6.94 | | 0.078 | 0.08±0.0 | 8.95 | -1.32 |
| | 10 | 1.25 | 1.26±0.0 | 1.63 | 0.43 | | 1.25 | 1.27±0.0 | 6.16 | 1.62 |
| | 11 | 20 | 21.20±1.18 | 5.55 | 6.00 | | 20 | 20.61±1.58 | 7.68 | 1.84 |

| No. | Sample | Lot number | CML/ ng·mL⁻¹ | CEL/ ng·mL⁻¹ |
|-----|--------|------------|--------------|--------------|
| 1   | Honghua injection | 170406 | - | 0.47±0.02 |
| 2   | Qingkailing injection | 15112463 | 0.95±0.06 | 1.07±0.07 |
| 3   | Kudiezi injection | 171106 | - | - |
| 4   | Ciwujia injection | 20151003 | 0.51±0.01 | 0.61±0.02 |
| 5   | Qingkailing injection | 180104A2 | 1.32±0.11 | 1.31±0.08 |
| 6   | Danshenchuanxiang qin injection | 20170626 | - | - |
| 7   | Qukeluding injection | 170301010 | - | - |
| 8   | Xingnaojing injection | 1707042 | - | - |
| 9   | Yinxiangyin leave extract injection | 19870304 | - | - |
| 10  | Shuxuening injection | 170605 | - | - |
| 11  | Tianmasu injection | 20170305-2 | - | - |
| 12  | Shuxuetong injection | 17041622 | 7.32±0.23 | 5.49±0.15 |
| 13  | Shuanghuanglian powder injection | 1611627 | - | 0.38±0.02 |
| 14  | Salvianolate for injection | 17040624 | - | 0.58±0.06 |
| 15  | Xueshuantong for injection | 17070113 | - | 1.59±0.15 |
| 16  | Danshen for injection | 1706730 | - | - |
| 17  | Shengmai injection | 1702241 | 0.56±0.04 | 0.67±0.03 |

'—' not detected

**Figures**
Figure 1

The chemical structures of 5-HMF (A), CML (B) and CEL (C)
Figure 2

Chromatograms of 5-HMF in a standard solution (A) and in the Honghua injection (B)
Figure 3

Typical MRM chromatograms of CML and CEL in a standard solution (A) and in the Ciwujia injection.
Figure 4

The product mass spectra of the [M+H]+ ions of CML and CEL in a standard solution (A, B) and in the Ciwujia injection (C, D)