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Simultaneous determination of organic acids and iridoid glycosides in traditional Chinese medicine (TCM) preparation reduning injection by rapid resolution liquid chromatography (RRLC) with ultraviolet (UV) detection under segmental monitoring mode

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A sensitive method of the simultaneous determination of organic acids and iridoid glycoside in reduning injection was described by rapid resolution liquid chromatography (RRLC) with ultraviolet (UV) detection under segmental monitoring mode. RRLC separation was achieved by use of a C₁₈ analytical column packed with sorbent of particle size 1.8 μm while the injected sample volume was set to 2 µl. A binary gradient elution program of aqueous phosphoric acid (0.1%, v/v) versus acetonitrile was selected for the quantiative analysis of nine major components. The RRLC-UV method under segmental monitoring mode showed satisfactory linearity, the limit of detection and the limit of quantitation. The method had been successfully applied to the determination of nine major components content in reduning injection samples. The analytical time of the proposed protocol is less than that of conventional HPLC-UV analytical methods, thus RRLC-UV method under segmental monitoring mode could function as an attractive alternative method to be used to evaluate the quality of reduning injection in the process of production.

Key words: Reduning injection, organic acids, iridoid glycosides, rapid resolution liquid chromatography-ultraviolet (RRLC–UV).

INTRODUCTION

Reduning injection made from the aqueous extracts of Flos Lonicerae, Herba Artemisiae Annuae and Gardenia jasminoides was one of most widely used traditional Chinese medicines preparations to treat upper respiratory tract infection in china. It also has good clinical efficacy on herpangina, hand-foot-mouth disease, bronchiolitis and aphthous stomatitis (Guo, 2010; Heng et al., 2010; Shao, 2010; Luo et al., 2009). The chemical constituents of reduning injection mainly included three chemical types: essential oil, organic acids and iridoid glycoside.

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The organic acids of reduning injection which are mainly derived from Flos Lonicerae and Herba A. Annuae, include chlorogenic, 4-caffeoylquinic, 5-dicaffeoylquinic, caffeic and isochlorogenic acids A, B, C. It was reported that these organic acids have been associated with several biological effects, including antioxidant, antibacterial, antiviral and antipyretic properties (Chen et al., 2008; Hung et al., 2006; Mishima et al., 2005). Iridoid glycosides in reduning injection included secoxyloganin and geniposide. The pharmacological studies showed that geniposide had certain analgesic effect and anti-inflammatory effect (Koo et al., 2006). But in the quality standard of reduning injection, only chlorogenic acids and geniposide were quantified and used as chemical markers for the quality control of the preparation, owing to their antipyretic and antibiotic activities as well as their high content in this injection. Therefore, this existed quality evaluation method could not reflect the real and comprehensive active constituents of reduning injection, and is inadequate to control the quality of reduning injection. Consequently, determinations of all types of components such as chlorogenic acid and its analogues and iridoid glycosides in reduning injection could be a better strategy for the comprehensive quality control of reduning injection.

Recently, the rapid-resolution and ultra-performance liquid chromatography (RRLC/UPLC) methods have been established in many laboratories for qualitative and quantitative determinations of active components in TCM materials and preparations (Liu et al., 2010). In contrast to the conventional high performance liquid chromatography (HPLC), the proposed method is an attractive method for analysis, because the improved resolution, shorter retention times, low consumption of harmful organic solvent, higher sensitivity and better performance could be achieved for certain herbal medicines by using RRLC or UPLC (Liang et al., 2010; Qi et al., 2008).

To the best of our knowledge, there were no methods reporting the RRLC determination of organic acids and iridoid glycosides in reduning coupled to UV detection in literatures. In order to comprehensively control the quality of reduning injection, a RRLC-UV method as reported under segmental monitoring mode for simultaneous determination of two category’s compounds in the traditional Chinese medicine preparation reduning injection.

MATERIALS AND METHODS

Reference substances, reagents and chemicals

Three reference compounds including chlorogenic acid, caffeic acid and geniposide were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, P.R. China). Six reference standards including secoxyloganin, isochlorogenic acids A, B, C, 4-caffeoylquinic acid and 5-dicaffeoylquinic acid were offered by Jiangsu Kanion Pharmaceutical Co., Ltd (Lianyungang, China). Their structures were elucidated by their spectra data (MS, 1H NMR and 13C NMR). The purities of nine reference standards were analyzed on Eclipse plus C18 column (4.6 × 50 mm, 1.8 μm, Agilent Technologies, USA). The purity of each compound was determined to be higher than 98% by normalization of the peak areas as detected by HPLC-UV. All of the standard substances were stored in the refrigerator at 4°C. Acetonitrile was chromatographic pure and was purchased from Dima Technology Inc. (USA). De-ionized water was purified with a Milli-Q Academic ultra-pure water system (Billerica, MA, USA) prior to usage as RRLC mobile phase. Phosphoric acid was purchased from the First Chemical Company of Tianjin (Tianjin, China); chromatographic grade methanol was purchased from Hanbang Science & Technology Company (Nanjing, P.R. China). All samples were kindly offered by Jiangsu Kanion Pharmaceutical Co., Ltd, produced in the year 2009 to 2010.

Preparation of standard solutions

Nine standard stock solutions were prepared by accurately weighed amounts of the each reference compounds and dissolving them in 50% methanol (v/v). Working standard solutions were prepared by diluting the mixed standard solution to give six different concentrations for the calibration curves. The standard stock and working solutions were all stored in dark brown calibrated flasks at 4°C.

Preparation of sample solutions

Reduning injection was diluted with 50% methanol at the ratio of 1:100 and filtrated through a membrane filter (0.45 μm), and an aliquot of 2 μl of the filtrate was injected for RRLC-UV analysis.

Apparatus and chromatographic conditions

RRLC analyses were performed on an Agilent 1200 SL HPLC (Agilent Technologies, USA) equipped with a binary pump and a micro-vacuum degasser, a multi-wavelength (MW) detector, an autosampler, a column temperature controller. The column configuration composed of an Agilent Zorbax Extend-reversed-phase C18 column (100 × 3.0 mm I.D., 1.8 μm particle size) and an Agilent Zorbax C18 guard column. The UV detection wavelength at 0 to 5 min was set at 225 nm for 4-cafeoylquinic acid, 5-dicaffeoylquinic acid, chlorogenic acid, caffeic acid and geniposide; at 237 nm from 5 to 8 min for secoxyloganin; from 8 to 10 at 324 nm for isochlorogenic acid A, isochlorogenic acid B and isochlorogenic acid C. A constant flow rate of 0.7 ml/min was employed throughout the analysis. All analyses were performed at 30°C and volume of solution injected into the column was 2 μl. The mobile phase comprised of (A) aqueous phosphoric acid (0.1%, v/v) and (B) acetonitrile using a gradient elution of 9.5% B at 0 to 4 min, 9.5 to 19% B at 4 to 6 min, 19% B at 6 to 10 min and the re-equilibration time of gradient elution was 5 min.

Validation procedure

Calibration curves, limits of detection (LOD) and limits of quantification (LOQ)

The linear dynamic range, LOD, recovery and precision were evaluated for the method developed. 50% methanol stock solution containing the nine reference components were prepared and diluted to an appropriate concentration for the construction of calibration curves. Six concentrations of the mixed standard solution were injected in triplicate, and their regression equations were calculated in the form of Y = A × X + B. The dilute solution was further diluted to a series of concentrations with 50% methanol.
to obtain the LOD and LOQ. The LOD and LOQ under the present chromatographic conditions were determined at a signal-to-noise (S/N) ratio of 3 and 10, respectively.

**Precision, repeatability, stability and recoveries**

The precision was determined by analyzing the six replicates on the same day and inter-day variation was determined in three consecutive days. The relative standard deviation (RSD) of retention time and peak areas was taken as a measure of precision.

Stability was tested with an extract solution of the same sample at 4°C and analyzed at 0, 4, 8, 12, 24, 48, and 72 h within 3 days. To confirm the repeatability, five different working solutions prepared from the same batch of reduning injection were analyzed. The RSD was also taken as the measures of stability and repeatability.

Recovery test was used to evaluate the accuracy of this method. Each sample was analyzed in triplicate. The total amount of each component was calculated from the corresponding calibration curve. The average recoveries were counted by the formula:

\[
\text{Recovery} (\%) = \frac{\text{amount found} - \text{original amount}}{\text{amount spiked}} \times 100\%, \quad \text{and} \quad \text{RSD} (\%) = \frac{\text{SD/mean}}{100}\%.
\]

The percentage recovery of the samples was also calculated.

**RESULTS AND DISCUSSION**

**Optimization of chromatographic separation of RRLC**

Method for rapid, highly resolving and efficient determination of reduning injections was of great interest. RRLC methods have been accepted to offer greater resolution, good sensitivity and high speed of analysis for complex system, thus RRLC-UV method was investigated to control the quality of reduning injections.

In order to obtain good chromatographic separation and ideal peak distribution of the sample, the chromatographic conditions including mobile phase, flow rates, column temperature and detection wavelength, were investigated, respectively. Firstly, the analytical performance of the compositions of mobile phases (methanol-water, methanol-0.1% formic acid, 0.1% (v/v) phosphoric acid and acetonitrile, 0.1% formic acid and acetonitrile) were compared in terms of separation and ability to suppress tailing peaks for each compound. Thus, 0.1% (v/v) phosphoric acid and acetonitrile were finally preferred as the optimized composition of mobile phases. It was well known that the flow rate was a crucial factor for shortening analytical time in RRLC (Nakajima et al., 2007), therefore the effect of flow rates was studied in the range of 0.3 to 1.0 ml/min in 0.1 units, and finally 0.7 ml/min was selected. The effects of column temperature on the separation were investigated in the range 20 to 50°C. The result showed that the running time decreased when the column temperature increased, but the resolution of isochlorogenic acid A and isochlorogenic acid B become worse. To obtain the best separation, it was fixed at 30°C throughout the analysis. The detection wavelength of these two category’s compounds was then investigated. It was reported that the segmental monitoring strategy based on variable wavelength detection (VWD) was very useful for comprehensive quality control of herbal medicines (Ma et al., 2012). Based on the retention time and the maximal UV absorption of each target compound, segmental monitoring was designed as in apparatus and chromatographic conditions. The choice of detection with segmental monitoring in a run could provide an optimum S/N for simultaneous analysis of nine target components.

Based on chromatographic conditions optimized, nine main active components could be eluted with baseline separation in 10 min. The typical chromatographic profiles of the standard solution and the real sample solution are shown in Figure 1. Almost no interference was presented in the chromatographic separation, and each target peak in reduning injection had a good resolution. The target components in the chromatographic profile of the sample solution were identified by comparing the retention times and the characteristic of the UV spectra of these peaks with those presented in the chromatogram of the mixed standard solution.

**Comparison of LC-UV and RRLC-UV method**

The analytical performance of newly established RRLC-UV and conventional LC-UV methods were compared in terms of analytical time and solvent saving. The result demonstrated that the chromatographic analysis time for RRLC on columns packed with 1.8-μm particles was less than 10 min without a loss in resolution. It was found that a great improvement in analytical time was obtained comparing with up to 60 min when LC-UV method was used in our previous research. The reduced solvent consumption was also friendly to environment and financial expense. Thus RRLC method had its advantages over LC-UV in terms of time saving and solvent saving, and subsequently was a powerful tool for the analysis of complex system such as Chinese herbal prescription reduning injection.

**Validation of the method**

All detailed descriptions of the regression curves are listed in Table 1. The good linearity (Coefficient of determination r^2>0.999) were achieved for nine active components. Meanwhile, as clearly shown in Table 1, the LOD and LOQ for seven phenolic acids and two iridoid glycosides range from 0.0003 to 0.005 μg/ml and from 0.001 to 0.016 μg/ml, respectively. The RSD values of peak areas were lower than 3.0% for both precision and stability tests. Moreover, all the RSD values of the analysis’ repeatability for the solution of real sample were
also lower than 3.0% for peak area. The recoveries of all investigated components ranged from 96.1 to 103%, and their RSD values were all less than 3.0%. It was concluded that the developed method possessed the reliability and accuracy for the measurement of these nine major components in reduning injection.

Application

Traditionally Chinese Medicines (TCM) could be selected for search and development of new antiviral TCM preparation on the basis of their medicinal use, e.g. against infections. Reduning injection was made from the aqueous extracts of *Flos Lonicerae*, *Herba A. annuae*, and *G. jasminoides* which were widely used as drug against infections. To our knowledge, the investigated analytes in reduning injection covered two chemical types, namely, seven caffeoylquinic acid derivatives (1, 2, 3, 4, 7, 8 and 9) and two iridoid glycosides (5 and 6). Ingredients 1, 2, 3, 4, 7, 8 and 9 were identified to be 5-dicaffeoylquinic acid, chlorogenic acid, 4-caffeoylquinic acid, caffeic acid, isochlorogenic acid B, isochlorogenic acid A, and isochlorogenic acid C. Caffeoylquinic acid derivatives are natural functional ingredients isolated from many herbal medicines and possess a broad range of pharmacological properties, including antioxidant, antibacterial, antihistaminic, anticancer, and other biological effects (Nakajima et al., 2007; Kwon et al., 2003). Recently, it has been demonstrated that isochlorogenic acid A and isochlorogenic acid C showed significant analgesic activity in the acetic acid-induced mouse writhing test (Dos Santos et al., 2005). Moreover, chlorogenic acid, isochlorogenic acid B and isochlorogenic acid A possessed potent anti-respiratory syncytial virus (RSV) activity against RSV (Li et al., 2005). Ingredients 5 and 6 were identified to be geniposide and secoxyloganin. It was also reported that geniposide had certain analgesic effect and anti-inflammatory effect (Koo et al., 2006). Thus, it would be useful for quality control of reduning injections to develop a method for comprehensive and accurate identification and determination organic acids and iridoid glycosides in this TCM preparation.

Thirteen batches of commercially available reduning injections were analyzed to assess the feasibility of the proposed method for the quality control of reduning injection. The typical RRLC chromatograms of mixed standard substances and reduning injection samples are shown in Figure 1. The contents of the nine active constituents in reduning injection samples from different batches are listed in Table 2. These results showed that the total content of nine major compounds varied from 26.51 to 41.29 mg/ml. Meanwhile, there are four more abundant components, namely 4-caffeoylquinic acid, 5-dicaffeoylquinic acid, chlorogenic acid and geniposide in reduning injection samples, whose contents were higher than 1.0 mg/ml and accounted for almost 85 to 95% of total contents acquired. Their contributions were more than 10% of total content. But another five components including caffeic acid, secoxyloganin, isochlorogenic acid...
A, B and C which were less than 1 mg/ml could be the relatively minor components. Most of their contribution ranged from 1.0 to 10.0% of total content except for caffeic acid and isochlorogenic acid A. Considering the aforementioned pharmacological properties, it was indicated that 4-caffeoylquinic acid, 5- dicafeoylquinic acid, chlorogenic acid, geniposide caffeic acid, secoxyloganin, isochlorogenic acid A, isochlorogenic acid B and isochlorogenic acid C should be selected as marker components for quality control of reduning injection. It was also

Table 1. The calibration curves, LODs and LOQs. Precision, repeatability, stability and recovery of the developed method for the nine active components of the assay.

| Component | Linear regression | Linear range (μg/ml) | R² | LOD (μg/ml) | LOQ (μg/ml) | Precision (RSD%) | Stability (RSD%) | Repeatability (RSD%) | Recovery (%) |
|-----------|-------------------|----------------------|----|-------------|-------------|-----------------|-----------------|--------------------|--------------|
| 1         | Y = 5.658X + 5.7979 | 0.037-74.52 | 0.9997 | 0.003 | 0.010 | 1.26 | 0.80 | 0.20 | 100 | 1.89 |
| 2         | Y = 4.6757X + 9.1186 | 0.052-209.10 | 0.9997 | 0.001 | 0.003 | 1.49 | 1.27 | 0.19 | 100 | 2.27 |
| 3         | Y = 4.2217X + 3.7556 | 0.051-219.9 | 0.9998 | 0.002 | 0.005 | 1.96 | 0.90 | 2.28 | 103 | 1.95 |
| 4         | Y = 9.0125X + 2.2496 | 0.013-26.95 | 0.9994 | 0.002 | 0.008 | 1.58 | 0.50 | 0.78 | 103 | 0.67 |
| 5         | Y = 2.7618X + 7.3283 | 0.063-253.8 | 0.9991 | 0.002 | 0.006 | 1.10 | 1.50 | 0.24 | 104 | 0.69 |
| 6         | Y = 5.1673X + 1.7106 | 0.059-59.85 | 0.999 | 0.005 | 0.016 | 0.61 | 1.51 | 0.43 | 97.4 | 1.68 |
| 7         | Y = 9.4589X + 4.8758 | 0.037-74.00 | 0.9998 | 0.002 | 0.007 | 0.25 | 1.45 | 0.48 | 96.1 | 0.59 |
| 8         | Y = 26.796X + 5.5663 | 0.083-16.76 | 0.9996 | 0.003 | 0.008 | 1.26 | 1.72 | 1.39 | 96.6 | 0.81 |
| 9         | Y = 8.5638X + 6.3869 | 0.076-76.00 | 0.9996 | 0.002 | 0.007 | 0.68 | 1.89 | 0.64 | 102.3 | 0.69 |

*In the regression equation, the X value is the concentration of Compounds (μg/ml), the Y value is the peak area. LOD (Limit of detection), LOQ (Limit of quantification). The relative standard deviation (%). Recovery (%) = (Amount determined – Amount original)/ Amount spiked x 100%.

Table 2. Contents of nine components at samples from different batches (mg/ml).

| Sample batch | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | Total content |
|--------------|----|----|----|----|----|----|----|----|----|---------------|
| S1080707     | 3.31 | 8.05 | 3.61 | 0.15 | 11.95 | 0.97 | 0.41 | 0.08 | 0.50 | 29.02 |
| S2080309     | 3.27 | 8.32 | 4.88 | 0.11 | 12.79 | 0.62 | 0.39 | 0.07 | 0.39 | 30.85 |
| S3091012     | 2.89 | 7.99 | 3.56 | 0.18 | 11.41 | 0.95 | 0.30 | 0.05 | 0.35 | 27.68 |
| S4090907     | 3.60 | 10.3 | 4.37 | 0.24 | 13.77 | 0.34 | 0.41 | 0.18 | 0.38 | 33.56 |
| S5091013     | 2.86 | 8.07 | 3.42 | 0.13 | 10.55 | 0.63 | 0.34 | 0.07 | 0.44 | 26.51 |
| S6091105     | 3.80 | 10.5 | 3.69 | 0.22 | 13.89 | 1.91 | 0.70 | 0.15 | 0.78 | 35.61 |
| S7091115     | 2.97 | 8.09 | 3.63 | 0.16 | 10.64 | 0.96 | 0.54 | 0.12 | 0.67 | 27.76 |
| S8091206     | 3.78 | 8.66 | 4.45 | 0.19 | 11.15 | 1.40 | 0.81 | 0.18 | 0.91 | 31.52 |
| S9091218     | 4.50 | 11.0 | 5.44 | 0.21 | 13.91 | 2.16 | 1.21 | 0.28 | 1.45 | 40.15 |
| S10100201    | 3.07 | 8.00 | 3.68 | 0.13 | 10.48 | 0.27 | 0.77 | 0.17 | 0.32 | 26.88 |
| S11100223    | 4.35 | 11.0 | 5.04 | 0.29 | 14.56 | 2.90 | 1.13 | 0.29 | 1.39 | 40.98 |
| S12100206    | 3.89 | 10.9 | 4.7 | 0.29 | 14.81 | 2.72 | 0.90 | 0.24 | 1.09 | 39.53 |
| S13100216    | 4.02 | 11.5 | 4.94 | 0.27 | 15.37 | 2.72 | 1.00 | 0.26 | 1.25 | 41.29 |
demonstrated that the established RRLC-UV method could be applied to evaluate the quality of reduning injection.

Conclusion

In this paper, a RRLC-UV method under segmental monitoring mode for rapid separation and quantitative analysis of nine compounds including seven organic acids and two iridoid glycosides, has been developed and successfully applied to determine nine major active components in reduning injection with good precision, accuracy and repeatability. Compared to the reported methods, the established RRLC-UV under segmental monitoring mode was not only suitable for its simplicity and reproducibility, but also specific enough for quality control of reduning injection. It could also be used to guarantee clinical efficacy of reduning injection.

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REFERENCES

Chen Y, Li SG, Lin XH, Luo HB, Li GG, Yao H (2008). On-Line Screening and Identification of Radical Scavenging Compounds Extracted from Flas Lonicerae by LC-DAQ–TOF-MS. Chromatogr 68:327-333.

Dos Santos MD, Gobbo-Neto L, Albarella L, de Souza GE, Lopes NP (2005). Analgesic activity of di-cafeoylquinic acids from roots of Lychnophora ericoides (Arnica daserra). J. Ethnopharmacol. 98:545–549.

Guo QJ (2010). Sixty curative effects of Reduning injection on hand-foot-mouth disease. Modern J. Integr. Tradit. Chin. Western Med. 19:933-934.

Heng YB, Wu LB, Chen GM, Zhu M (2010). Fifty curative effects of Reduning injection on herpangina. Modern. J. Integr. Tradit. Chin. Western. Med. 19:949-950

Hung TM, Na M, Thuong PT, Su ND, Sok D, Song KS, Seong YH, Bae K (2006). Antioxidant activity of cafeoyl quinic acid derivatives from the roots of Dipsacus asper Wall. J. Ethnopharmacol. 108:188-192.

Koo HJ, Lim KH, Jung HJ, Park EH (2006). Anti-inflammatory evaluation of gardenia extract, geniposide and genipin. J. Ethnopharmacol. 103:496–500

Kwon YS, Kim KO, Lee JH (2003). Chemical constituents of Dipsacus asper (II). Kor. J. Pharmacogn. 34:128–131.

Li YL, But PPH, Ooi VEC (2005). Antiviral activity and mode of action of cafeoylquinic acids from Schefflera heptaphylla (L.) Frodin. Antiviral. Res. 8:1-9.

Liang X, Zhang L, Zhang X, Dai W, Li HY, Hu LW, Liu H, Su J, Zhang WD (2010). Qualitative and quantitative analysis of traditional Chinese medicine Niu Huang Jie Du Pill using ultra performance liquid chromatography coupled with tunable UV detector and rapid resolution liquid chromatography coupled with time-of-flight tandem mass spectrometry. J. Pharm. Biomed. Anal. 51:565-571.

Liu J, Chen XY, Hu YH, Cheng G, Zhong DF (2010). Quantification of the major metabolites of bromhexine in human plasma using RRLC-MS/MS and its application to pharmacokinetics. J. Pharm. Biomed. Anal. 51:1134-1141.

Luo YM, Ma ZL, Qiu X (2009). Clinical observation of curative effects of Reduning injection on bronchiolitis. Chin. Modern Med.16:144-146.

Shao X (2010). Clinical observation of curative effects of Reduning injection on aphthous stomatitis. Chin. Pract. Med. 5:144-145.

Ma J, Qi LW, Li HJ, Li P (2012). A segmental monitoring strategy based on variable wavelength detection for quality control of three Polygonaceae herbs. J. Pharm. Biomed. Anal. 62:155–161.

Mishima S, Yoshida C, Akino S, Sakamoto T (2005). Antihypertensive effects of Brazilian propolis: identification of cafeoylquinic acids as constituents involved in the hypotension in spontaneously hypertensive rats. Biol. Pharm. Bull. 28:1909–1914.

Nakajima Y, Shimazawa M, Mishima S, Hara H (2007). Water extract of propolis and its main constituents, cafeoylquinic acid derivatives, exert neuroprotective effects via antioxidant actions. Life Sci. 80:370–377.

Qi LW, Cao J, Li P, Yu QT, Wen XD, Wang YX, Li CY, Bao KD, Ge XX, Cheng XL (2008). Qualitative and quantitative analysis of Radix astragali products by fast high-performance liquid chromatography–diode array detection coupled with time-of-flight mass spectrometry through dynamic adjustment of fragmentor voltage. J. Chromatogr. A 1203:27–35.