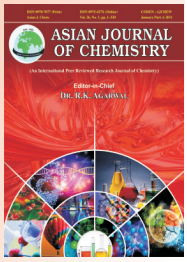
Asian Journal of Chemistry; Vol. 26, No. 21 (2014), 7105-7108

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.15885



Evaluation of an HPLC Method for Quality of Fructus of *Ligustrum lucidum* Ait Pieces

LAN LUAN, YONG-QING XIAO*, LI LI and CUN ZHANG

Institute of Chinese Materia Madica, China Academy of Chinese Medical Sciences, Beijing, P.R. China

*Corresponding author: E-mail: x.heqi@163.com

Received: 8 June 2013; *Accepted*: 4 Septermber 2014; *Published online*: 30 September 2014; AJC-16080

A facile and reproducible HPLC method was established for quality evaluation of fructus of *Ligustrum lucidum* Ait pieces, Both typical chromatograms analysis and determination analysis for main active ingredient were employed to evaluate the quality of fructus of *Ligustrum lucidum* Ait pieces. It is successfully applied to distinguish fructus of *Ligustrum lucidum* Ait from the processed products by typical chromatograms analysis and reveal the main active ingredient change rules by determination analysis. The method for typical chromatograms analysis was validated for precision, repeatability and stability, The method for determination was validated through the following performance criteria *i.e.*, linearity, precision, repeatability, stability, accuracy, limit of detection and limit of quantification. This method is suitable for routine quantitative analysis of fructus of *Ligustrum lucidum* Ait before and after roasted.

Keywords: HPLC, Quality evaluation, Fructus of *Ligustrum lucidum* Ait pieces, Typical chromatograms analysis, Determination analysis.

INTRODUCTION

Chinese medicines (CMs) are being used more and more widely throughout the word, are comprised of a complex multi-component nature, processing of Chinese Material Medica (CMM) is a pharmaceutical technique to fulfill the different requirements of therapy, dispensing and making preparations according to traditional Chinese medicine theory. Those processed products are named as decoction pieces, which are used in clinics. There is a close relationship between processing, safety and efficacy of Chinese medicines. Therefore, quality evaluation for decoction pieces is important to maintain their quality and ensure their safe use. After processing, the material's composition will change and the quality evaluation standard should be change too. However, little efforts have been paid in interpreting or understanding of the differences in Chinese Material Medica and the produce products. Thus, the quality evaluation method is required for quality control of decoction pieces.

Fructus ligustri lucidi (Nü Zhen-zi in chinese), derived from the fructus of *Ligustrum lucidum* Ait[1], is one of the traditional Chinese medicine which has long been used to treat hepatitis, endocrine and metabolic diseases, recurrent respiratory tract infections and other diseases[2-5]. Triterpenoids, iridoids, phenylethanoidal glycosides in fructus of *Ligustrum lucidum* Ait has been reported to contribute to the biological activities of these pieces, which have demonstrated significant pharmacological activities such as antitumor, hepato protection, regulating immune function, antisenile effect, antiinfammation, reducing hypercholesterolemia, *etc*[6-12]. The wine stew products is be widely used in clinical. In terms of quantitative evaluation analysis of fructus of *Ligustrum lucidum* Ait and the processed products, several analytical methods have been reported for quality evaluation, including MECC, X-ray diffraction Fourier typical chromatograms spectra[13,14]. However, the preparation of samples solutions was laborious and time-consuming. The HPLC method, which has good sensitivity, less interference and lower limits of detection, is very convenient and sensitive for quality evaluation of fructus of *Ligustrum lucidum* Ait pieces.

The aims of this study is to establish a facile and reproducible HPLC method for quality evaluation of fructus of *Ligustrum lucidum* Ait pieces. Optimization of the extraction conditions and HPLC method were followed.

EXPERIMENTAL

HPLC was performed on a LC-20A series HPLC system (Shimadzu Corporation, Japan) consisting of a 2-liquid gradient system, high speed auto-sampler, column oven and UV-visible detector. An Agilent Luna C_{18} (250 × 4.6 mm, 5 µm) was maintained at 35 °C. Detection wavelength was set at 224 nm. The mobile phase for typical chromatograms analysis consisted of methanol (A) and 0.05 % phosphoric acid (B) at a flow-rate of 1 mL min^{-1}, A gradient program was as follows: 0-10 min, 5 B %; 10-40 min, 5-25 B %; 40-50 min, 25 B %; 50-90 min, 25-35B %; 90-120 min, 35B %; injection volume:10 µL. The

mobile phase for determination analysis consisted of acetonitrile (A) and 0.1 % phosphoric acid (B) at a flow-rate of 1 mL min^{-1}, A gradient program was as follows: 0-15 min, 15-35 B %; 15-25 min, 35-50 B %; injection volume: 5 µL.

A versatile plant pulveriser (Tianjin, China) was used to power the medicines into powder. An KH3200SPV ultrasonic generator (50Hz, 1200W) from KunShan, HeChuang Ultrasonics Co, Ltd. (Jiangsu, China) and An DZKW-D electric-heated thermostatic water bath from HuangYe aerospace instrument factory (Hebei, China) were used to extract components from samples.

Methanol and acetonitrile (HPLC grade) were purchased from Fisher Scientific (New Jersey, USA), All other chemicals were of analytical grade and used without further purification and the water used as pure water (Wahaha Group Co., Ltd., China) for sample preparation and preparation of mobile phases for HPLC analysis. The herb were purchased from Ji-Ren Pharmaceutical and Pieces of Chinese medicine in An, hui Hu-Qiao plant. Standards of salidroside, *p*-tyrosol and specnuezhenide were purchased from National Institutes for Food and Drug Control (Beijing, China), the structures were shown in Fig. 1.

Preparation of standard and samples solutions: Methanol containing standard compounds were prepared and diluted to appropriate concentrations for the construction of calibration curves for the quantitative analysis.

The dried fructus of *Ligustrum lucidum* Ait pieces were milled to powder, sieved through a No. 60 mesh, 0.5 g powder samples were accurately weighed and extracted ultrasonically by 50 mL 50 % (v/v) methanol-water solution for 45 min for the quantitative analysis; and 0.5 g powder samples were accurately weighed and extracted, with 50 mL 50 % (v/v) methanol-water solution for 1 h, cool and filter, use the filter as the test solution of fingerprint analysis, all above the sample solutions were then made up the loss weight with 50 % (v/v) methanol-water solution after extraction and filtered through 0.45 µm nylon filters into amber sample vials for HPLC analysis.

RESULTS AND DISCUSSION

Typical chromatograms analysis for fructus of *Ligustrum lucidum* Ait pieces: The analytical method for typical chromatograms analysis was validated for precision, repeatability and stability by measurement of relative peak height and retention time for the wine stew product.

Method precision was based on analysis of sample solution for five times. The relative standard deviation (RSD) values of 18 common peaks (Fig. 2) height and retention time were lower than 5 and 1, respectively.

The repeatability was assessed by analyzing five independently samples. The RSD values of 18 common peaks height and retention time were lower than 4.6 and 1 %, respectively.

The stability test was performed with a sample solution over 24 h in room temperature. The corresponding RSD values of 18 common peaks height and retention time were less than 4.8 and 1 %, respectively.

The results indicated that the developed method was validated and applicable for sample analsis. Typical chromatograms of fructus of *Ligustrum lucidum* Ait and the produced product were shown in Fig. 2.

It is obvious that the major chemical constituents of fructus of *Ligustrum lucidum* Ait had qualitative and quantitative changes after processing, the height of peaks 1, 5, 6, 7, 8 and 9 were found significantly higher in the wine stew fructus of *Ligustrum lucidum* Ait and the height of peaks 14 and c was lower after processing, The peaks a and b disappear after processing and three new peaks 12, 17 and 18 appear at wine stew products, It is successful to distinguish raw herb from the processed products by typical chromatograms analysis.

Optimization of chromatographic conditions: The instrument and separation condition for preparation of peaks in quantitative analysis were described, scanning from 190 to 400 and 224 nm was selected as detection wavelength for acquiring chromatograms. the gradient program described above was chosen as it allowed the three major peaks to be clearly separated. Typical chromatograms of standards and samples were shown in Fig. 3.

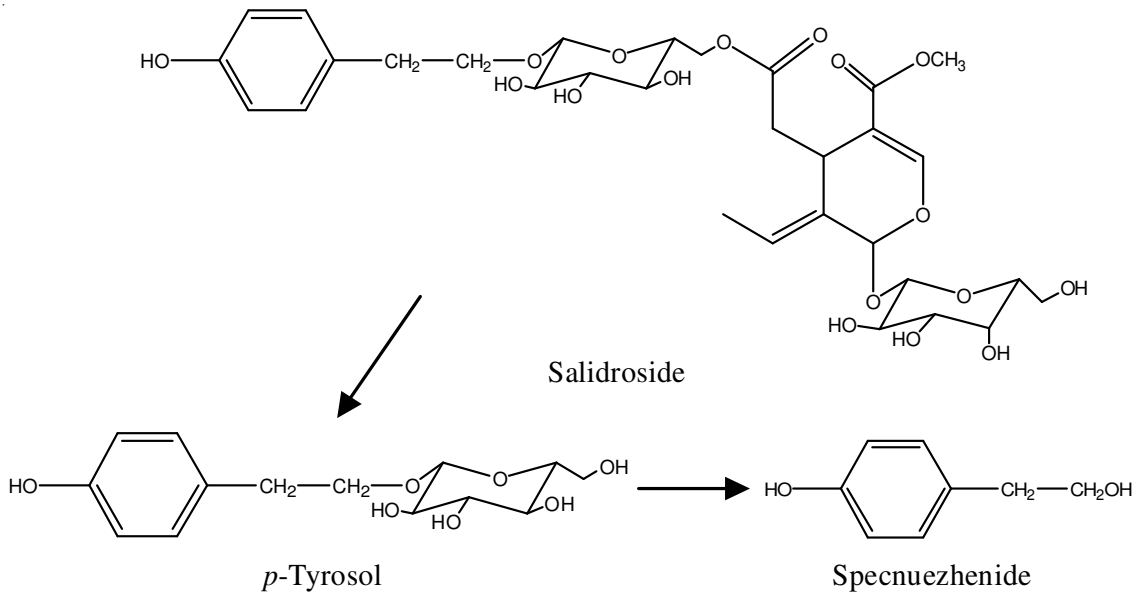


Fig. 1. Molecular structures of compounds in this study

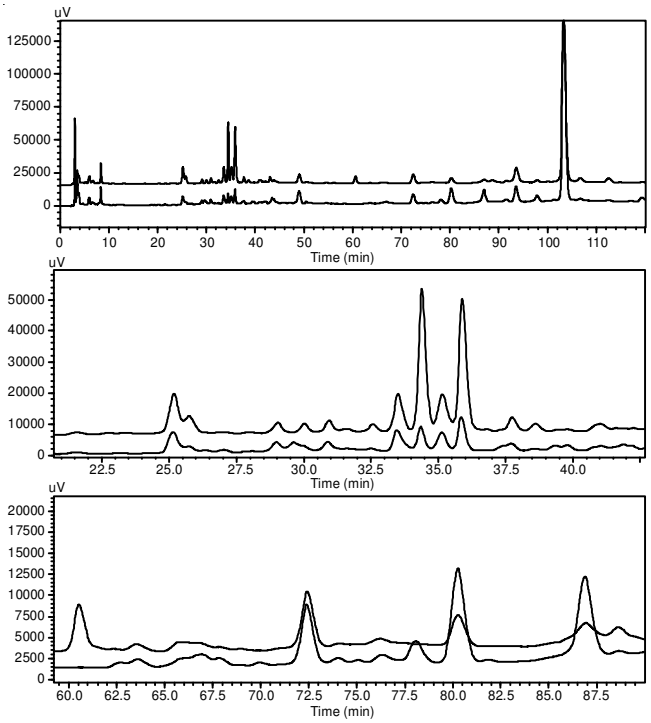


Fig. 2. Chromatograms of fructus of *Ligustrum lucidum* Ait (b) and the wine stew product (a) 6 salidroside; 7 *p*-tyrosol; 16 specnuezhenide

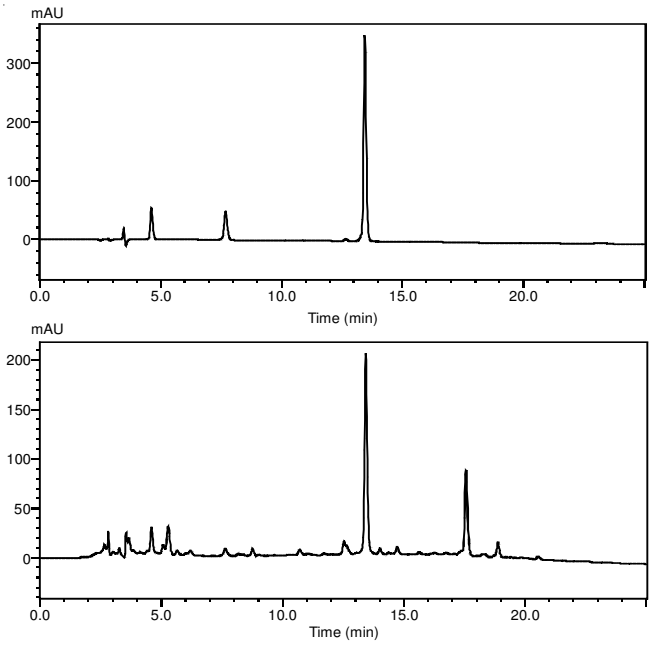


Fig. 3. Chromatograms of standards solutions (a) and sample solutions (b) 1 salidroside; 2 *p*-tyrosol; 3 specnuezhenide

Optimization of sample preparation: In order to achieve the optimal extraction conditions, variables involved in the extraction procedure such as reflux and ultrasonic extraction, extraction solvents, extraction time, solvent volume were investigated.

In this experiment, reflux and ultrasonic extraction methods were employed to extract samples, the reflux extraction was higher efficiency for the samples, four solvents were investigated to optimize the optimal solvent for extraction of samples. The solvents used were 50 % (v/v) ethanol-water, 30 % (v/v) methanol-water, 50 % (v/v) methanol-water, 70 % (v/v) methanol-water, whilst total peak areas of the analytes of interest reached the highest values when 50 % (v/v) methanol-water was employed as extraction solvent. Thus, 50 % (v/v) methanol-water was the most efficient solvent for the extraction of the samples. In the assay, extraction efficiency in samples was compared by reflux extraction with 50 mL 50 % (v/v) methanol-water for 15, 30, 45 and 60 min, respectively. The results indicated that the highest extraction efficiency was obtained by reflux extraction for 60 min in 50 % (v/v) methanol-water. the results were shown in Table-1.

Method validation: The calibration curves of the individual standards was constructed using seven concentrations, by plotting peak areas against the concentration of analytes. Good linearity (r = 1) was observed in calibration curves over the concentration ranges investigated. The limit of detection and limit of quantification were determined at a signal-to-noise ratio (S/N) of 3 and 10, respectively. the results were shown in Table-2.

TABLE-2
REGRESSION EQUATIONS, LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION FOR THREE STANDARDS

No	Linear regression	Linear range (μg mL^{-1})	r	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
1	$y = 3 \times 10^6$-806.23	1.37-27.38	1	0.04	0.135
2	$y = 3 \times 10^6$-803.47	1.65-32.90	1	0.06	0.17
3	$y = 1 \times 10^6 + 4871.5$	20.60-412.00	1	0.021	0.10

1. Salidroside; 2. *p*-Tyrosol; 3. Specnuezhenide

The precision of the method was assessed by measurement of repeatability of peak area in five analyses of the same stew fructus of *Ligustrum lucidum* Ait. The RSD were all less 3 % for three components.

In order to test the repeatability, six sample solutions of wine stew fructus of *Ligustrum lucidum* Ait in Anhui Province were prepared. The contents of three components were 1.43, 0.63 and 35.36 mg/g and the RSDs were 0.66, 0.93 and 1.05 %, respectively. Thus repeatability was very good.

TABLE-1
EFFECTS OF REFLUX AND ULTRASONIC EXTRACTION, EXTRACTION SOLVENTS, EXTRACTION TIME, SOLVENT VOLUME ON THE EXTRACTION YIELD OF COMPONENTS

No	Extraction method (mg/g)		Extraction solvent				Extraction time (min)				Solvent volume (mL)		
	Ultrasonic extraction	Reflux extraction	50 % Ethanol-water	30 % Methanol-water	50 % Methanol-water	70 % Methanol-water	15	30	45	60	15	25	50
1	1.46	1.61	1.61	1.6	1.61	1.61	1.56	1.47	1.63	1.54	1.57	1.55	1.61
2	0.60	0.62	0.61	0.6	0.62	0.6	0.62	0.62	0.66	0.65	0.69	0.66	0.65
3	35.04	47.65	38.07	36.6	47.76	37.74	36.06	37.76	39.81	39.43	38.61	37.65	47.76

1. Salidroside; 2. *p*-Tyrosol; 3. Specnuezhenide

For stability test, the same sample solution was analyzed for 0, 4, 8, 12 and 24 h at the room temperature. The RSDs of contents of the three components in the same sample were 2.18, 1.62 and 1.44 %, respectively, which indicated that the sample was stable over 24 h under the experimental conditions.

In order to evaluate the accuracy of this method, the recovery was performed by adding standard solutions with known content of three components (that same as repeatability). The samples were then extracted according to the procedure described above and analyzed. The recovery of each component was calculated as the percentage of the net amount of each compound obtained after extraction from that had been added prior to the extraction. The recoveries were 97-101 %, 100-103 and 102-105 % and the RSDs were 1.70, 1.30 and 1.20 %, respectively. It was indicated that the extraction method was efficient enough for determination of the three components in fructus of *Ligustrum lucidum* Ait and the processed products.

Sample analysis: The established HPLC method was applied to determination in fructus of *Ligustrum lucidum* Ait and the processed products. The contents of the three components in different samples are listed in Fig. 4.

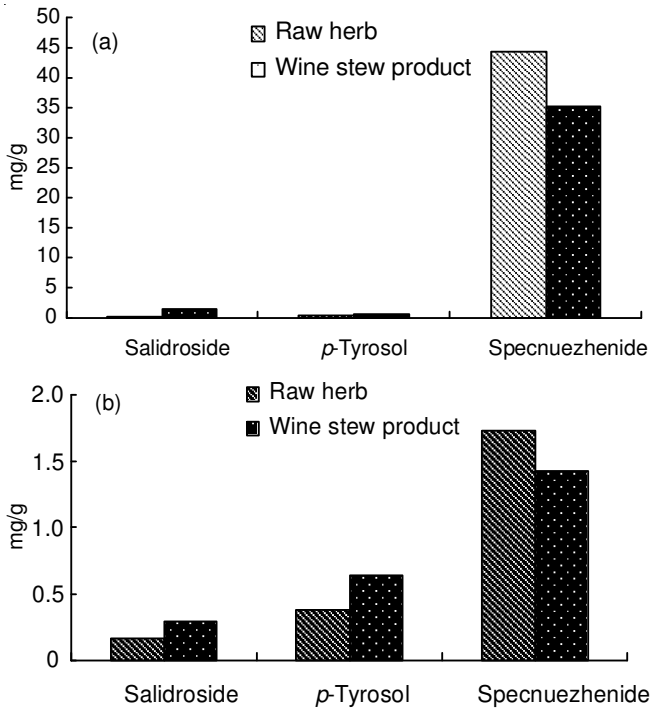


Fig. 4. Measurement results of components in fructus of *Ligustrum lucidum* Ait pieces (a) Ji-Ren pharmaceutical; (b) Pieces of Chinese medicine in An, hui Hu-Qiao plant

The results of quantitative analysis clearly indicated that the change of before and after roasted from contents of salidroside, *p*-tyrosol and specnuezhenide (Fig. 4). After processing, specnuezhenide in fructus of *Ligustrum lucidum* Ait was degraded, then the degradation products-secondary glycoside (salidroside) was further degraded to the *p*-tyrosol (Fig. 1). Thus, it appears that the expected decrease in contents of specnuezhenide and contents of salidroside and *p*-tyrosol increased compared to the sample of raw herb.

Conclusion

Quality evaluation for decoction pieces is important to maintain their quality and ensure their safe use. Our study in the paper demonstrated on the typical chromatograms analysis and determination analysis method combine with in quality evaluation of *Ligustrum lucidum* Ait pieces, it is successful to distinguish decoction from the raw herb. And has been applied successfully to evaluate quality of fructus of *Ligustrum lucidum* Ait pieces.

ACKNOWLEDGEMENTS

This research was supported by the Support Program of the Institute of Chinese Materia Madica, China Academy of Chinese Medical Sciences (2011ZDXK-02).

REFERENCES

1. The State Pharmacopoeia Commission of P.R. China, Pharmacopoeia of the People, Republic of China, Chemical Industry Press, China, p. 43 (2010).
2. H.M. Lin, F.L. Yen, L.T. Ng and C.C. Lin, *J. Ethnopharmacol*., **111**, 129 (2007).
3. Z.-D. He, H. Dong, H.-X. Xu, W.-C. Ye, H.-D. Sun and P.P.-H. But, *Phytochemistry*, **56**, 327 (2001).
4. M. Niikawa, H. Hayashi, T. Sato, H. Nagase and H. Kito, *Mutation Res. Genetic Toxicol*., **319**, 1 (1993).
5. G. Chen, L.Y. Zhang, X.L. Wu and J.N. Ye, *Anal. Chim. Acta*, **530**, 15 (2005).
6. E.Q. Xia, Y.Y. Yu, X.R. Xu, G.F. Deng, Y.J. Guo and H.B. Li, *Ultrason. Sonochem*., **19**, 772 (2012).
7. H.-Y. Ju, S.C. Chen, K.-J. Wu, H.-C. Kuo, Y.-C. Hseu, H. Ching and C.-R. Wu, *Food Chem. Toxicol*., **50**, 492 (2012).
8. K.Y.-Z. Zheng, Z.-X. Zhang, A.J.-Y. Guo, C.W.-C. Bi, K.Y. Zhu, S.L. Xu, J.Y.-X. Zhan, D.T.-W. Lau, T.T.-X. Dong, R.C.-Y. Choi and K.W.-K. Tsim, *Eur. J. Pharmacol*., **679**, 34 (2012).
9. Z. Wang, C.C. Hsu, C.N. Huang and M.C. Yin, *Eur. J. Pharmacol*., **628**, 255 (2010).
10. J. Liu, *J. Ethnopharmacol*., **49**, 57 (1995).
11. Z.H. Wang, C.- Hsu and M.- Yin, *Food Chem*., **112**, 914 (2009).
12. S.R. Jensen and H. Franzyk, *Phytochemistry*, **60**, 213 (2002).
13. H.X. Liu, Y.H. Shi, D.X. Wang, G.L. Yang, A.M. Yu and H.Q. Zhang, *J. Pharm. Biomed. Anal*., **32**, 479 (2003).
14. K.B. Shi and Z.H. Sheng, *Zhong Yao Cai Za Zhi*, **30**, 643 (2003).