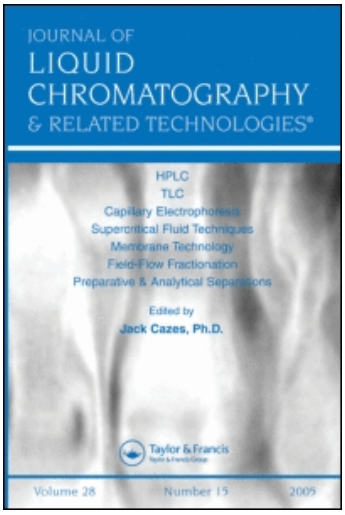

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

QUANTITATIVE DETERMINATION OF PHENOLIC ACIDS IN *LONICERA JAPONICA* THUNB. USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

Chidananda Swamy Rumalla[a]; Bharathi Avula[a]; Jianping Zhao[a]; Troy J. Smillie[a]; Ikhlas A. Khan[ab]
[a] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, Mississippi, USA [b] Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, Mississippi, USA

Online publication date: 03 January 2011

To cite this Article Rumalla, Chidananda Swamy , Avula, Bharathi , Zhao, Jianping , Smillie, Troy J. and Khan, Ikhlas A.(2011) 'QUANTITATIVE DETERMINATION OF PHENOLIC ACIDS IN *LONICERA JAPONICA* THUNB. USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY', Journal of Liquid Chromatography & Related Technologies, 34: 1, 38 — 47
To link to this Article: DOI: 10.1080/10826076.2010.534399
URL: http://dx.doi.org/10.1080/10826076.2010.534399

Journal of Liquid Chromatography & Related Technologies, 34:38–47, 2011

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826076.2010.534399

Taylor & Francis
Taylor & Francis Group

QUANTITATIVE DETERMINATION OF PHENOLIC ACIDS IN *LONICERA JAPONICA* THUNB. USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

Chidananda Swamy Rumalla,[1] Bharathi Avula,[1] Jianping Zhao,[1] Troy J. Smillie,[1] and Ikhlas A. Khan[1,2]

[1]*National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, Mississippi, USA*
[2]*Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, Mississippi, USA*

□ *A new, simple, precise, and rapid high performance thin layer chromatography (HPTLC) method has been developed and validated for the simultaneous quantitative determination of three phenolic acids, namely, 3,5-O-dicaffeoylquinic acid, 3,4-O-dicaffeoylquinic acid and chlorogenic acid from flower buds of* Lonicera japonica. *The separation of these compounds was carried out on silica gel* $^{60}F_{254}$ *eluted with ethyl acetate:chloroform:methanol:formic acid:water (6:2.5:1.5:0.6:0.1 v/v/v/v) and a detection wavelength at 330 nm. The developed method gave a good linear regression relationship between peak area and an observed concentration range of 200–1200 ng spot*$^{-1}$ *for the three aforementioned marker compounds. Spike recoveries were within 97.81–101.59% and the RSD values of precision were in the range of 0.65–3.21%. The method presented good results for the following parameters: selectivity, linearity, accuracy, precision, robustness, as well as limit of detection and limit of quantification. Therefore, this method could provide a simple analytical tool for the quality control of* Lonicera japonica *raw material and its products.*

Keywords *Lonicera japonica* Thunb, caprifoliaceae, high performance thin layer chromatography, phenolic acids

INTRODUCTION

The flower buds of *Lonicera japonica* Thunb. (family: Caprifoliaceae) is one of the most commonly used traditional Chinese medicinal plants, and it is used as an anti-inflammatory herbal medicine in China. It also used for the treatment of a variety of diseases including arthritis, diabetes mellitus, fever, infections, sores, and swelling.[1,2] Pharmacological studies report

Address correspondence to Ikhlas A. Khan, Asst. Director, National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA. E-mail: ikhan@olemiss.edu

that the extract of the flower buds of *L. japonica* have many biological activities including, hepatoprotective, cytoprotective, antimicrobial, antibiotic, antioxidative, antiviral, and anti-inflammatory.[3–8] Different classes of compounds with diverse structures including cerebrosides, flavonoids, iridoids, polyphenols, alkaloids, and saponins have been reported from various parts of the plant.[2,9–13] Recent studies indicated that flowers of *L. japonica* also exhibited good antioxidant activities,[14] which may be partly responsible for some of its medicinal functions. Phenolic acids were believed to be one of the beneficial components for antioxidant activity and were chosen as marker compounds for this study. Several analytical methods including mainly HPLC-UV and HPLC-MS have been reported from literature for qualitative and quantitative analysis of *L. japonica*.[1,14–18] HPTLC is a widely accepted technique for its high accuracy, precision, reproducibility of results in addition to its low per sample operating cost, easy sample preparation, and short analysis time. To the best of our knowledge, this is the first time report of a quantitative determination method for phenolic acids in *L. japonica* using HPTLC.

The objective of the present study is to develop a rapid and simple quantification of three phenolic compounds namely 3,5-*O*-dicaffeoylquinic acid (**1**), 3,4-*O*-dicaffeoylquinic acid (**2**), and chlorogenic acid (**3**) in flower buds of *L. japonica* using HPTLC. The method developed is also used for chemical fingerprint analysis.

EXPERIMENTAL

Plant Material

Plant materials were studied including: *Lonicera japonica* Thunb. (voucher no. 727) (LJ-1) obtained from Bejing Yuke Botanical garden; China, *Lonicera japonica* Thunb. (voucher no. 5272) obtained from the Missouri Botanical Garden (LJ-2), and *Lonicera japonica* Thunb. commercial sample (sample no. 5208) (LJ-3) procured from Kalyx.com; Beijing, China. All samples are deposited at the National Center for Natural Products Research (NCNPR), University of Mississippi, USA.

Chemicals

The standard compounds (**1–3**; Fig. 1) were isolated at NCNPR, the identity and purity was confirmed by chromatographic (TLC, HPLC) methods, by the analysis of the spectral data (IR, 1D- and 2D-NMR, HR-ESI-MS) and comparison with published spectral data.[15] Ethyl acetate, chloroform, methanol, formic acid, and water were ACS certified grade purchased from Fisher Scientific (Fair Lawn, NJ, USA).

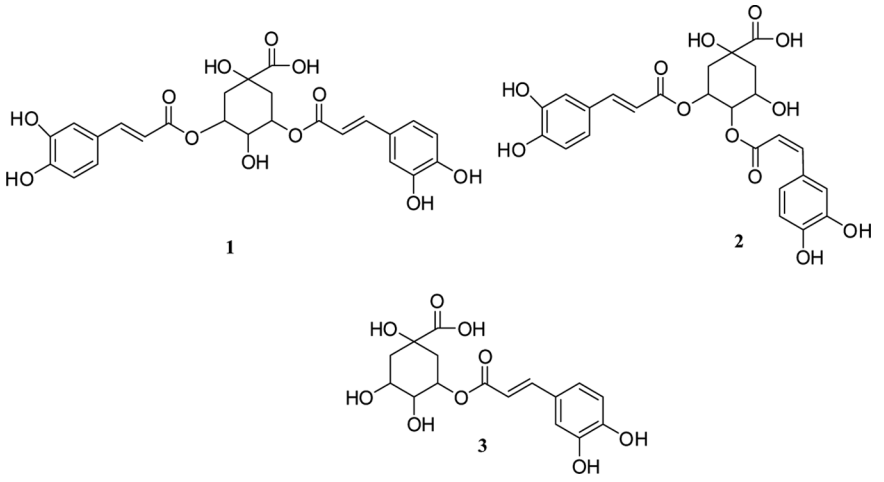

FIGURE 1 Structure of compounds **1–3**.

Analytical Procedure

Chromatography was performed on glass plates (Merck, Darmstadt, Germany) with silica gel $^{60}F_{254}$ (20×10 cm and 10×10 cm). Plates were prewashed with methanol and dried in an oven for 5 min at temperature 105°C. Samples and standards were applied with Linomat 5 semi-automatic applicator with 100 µl syringe as bands (8 mm wide), distance between tracks was 9.7 mm; the first application position was 12.0 mm from the left edge of the plate, and application position from the lower edge of the TLC plate is 8.0 mm. The delivery speed of the syringe was 90 nl/sec. The application parameters were identical for all the analyses performed. Chamber saturation was done using 20×10 cm Whatman filter paper for 20 min (Twin trough chamber 20×10 cm; Analtech, USA). The plates were dried at room temperature, and the layers developed to a height of 8 cm with a mobile phase of ethyl acetate:chloroform:methanol: formic acid:water (6:2.5:1.5:0.6:0.1 v/v/v/v) at a temperature of 21–24°C and a relative humidity of 40–45%. For quantitative determination, the analyte spots corresponding to compounds **1–3** were scanned using a CAMAG TLC scanner 3 under reflectance/absorbance mode at 330 nm with a 6×0.45 mm slit width, a scanning speed of 20 mm/sec and a data resolution of 100 µm/step. Spectral scanning was done over a range of 280–400 nm with 20 mm/sec and data resolution 1 nm/step. Images were taken under 366 nm by using reprostar 3 along with DigiStore 2 Digital Documentation System, winCATS 4 software ver. 1.4.3 (CAMAG, Switzerland).

Selection of Detection Wavelength

After chromatographic development, bands were scanned over the range 200–400 nm (spectrum scan speed 20 nm/sec) and the spectra were overlaid. All three compounds showed maximum absorbance at 330 nm, and it was selected as the wavelength for determination.

Standard Solution

Individual stock solutions of standard compounds were prepared at a concentration of 1.0 mg/mL in methanol. The calibration curve was prepared at five different concentration levels. The range of the calibration curve was 200–1200 ng/spot. Table 1 shows the calibration data and the calculated limit of detection and limit of quantification for the HPTLC method. Further dilution was made in methanol to provide a working standard solution of 100 μg/mL. For quantification, all working samples were applied in triplicate.

Sample Preparation

Dry ground flower buds (100 mg) of *L. japonica* were sonicated in 2.5 mL of methanol:water (1:1) for 30 min followed by centrifugation for 15 min at 3300 rpm. The supernatant was transferred to a 10 mL volumetric flask. The procedure was repeated four times and respective supernatants combined. The final volume was adjusted to 10.0 mL with methanol:water (1:1) and mixed thoroughly. Prior to injection, an adequate volume (ca. 2 mL) was passed through a 0.45 μm nylon membrane filter. Each sample solution was injected in triplicate.

System Validation

Scanner validation was performed by scanning the same spot five times. Applicator validation was performed by spotting 3 μl (100 μg/mL) of working standard five times and peak areas were obtained by scanning. Precision of the system was expressed as RSD (%).

TABLE 1 Regression Equation, Correlation Coefficient (r^2), LOD, and LOQ for Three Phenolic Acids from *L. japonica* (LJ-1) Using the HPTLC Method

Compounds	Regression Equation	r^2	LOD ng/spot	LOQ ng/spot	R_f
1	$Y = -2121 + 16.84X - 0.00485X^2$	0.9994	100	200	0.66
2	$Y = 2519.836 + 25.401X - 0.008X^2$	0.9989	120	200	0.56
3	$Y = -1823 + 18.23X - 0.00376X^2$	0.9998	120	200	0.37

Y = area; X = amount (ng).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were determined by spotting a series of diluted solutions with known concentrations. Limit of detection (LOD) and limit of quantification (LOQ) were defined as the signal-to-noise ratio equal to 2 or 3 and 10, respectively.

Repeatability

The sample of *L. japonica* was used to achieve repeatability testing for intra-day and inter-day. The data used to calculate RSD (%) of intraday repeatability was the mean value for a total of nine applications.

Recovery

Plant sample (LJ-1) after exhaustive extraction of five times and drying was spiked with known amounts of the standard compounds at two different concentrations (300 and 500 ng), extracted again, and analyzed under optimized conditions.

Specificity

The specificity of the method was ascertained by analyzing standard compounds and samples. The bands for compounds **1–3** in sample solutions were confirmed by comparing the R_f and spectra of the bands with that of standards.

Robustness

Robustness is a measure of the methods ability to remain unaffected by small, but deliberate, variations in the method conditions and is an indication of the reliability of the method. For the robustness measurement, two parameters, mobile phase composition and developing TLC distance, were checked. The three different mobile phases with slight differences in their composition were used and developing distance was checked, varying between 70, 85, and 90 cm. No considerable effect on the analysis was observed.

RESULTS AND DISCUSSION

Method Development

The composition of the mobile phase for TLC was optimized by testing different solvent mixtures of varying polarity. The best results were

obtained using ethyl acetate:chloroform:methanol:formic acid:water (6:2.5:1.5:0.6:0.1 v/v/v/v). The selected mobile phase produced highly symmetrical peaks showing good resolution (Fig. 2) for compounds **1–3** in the

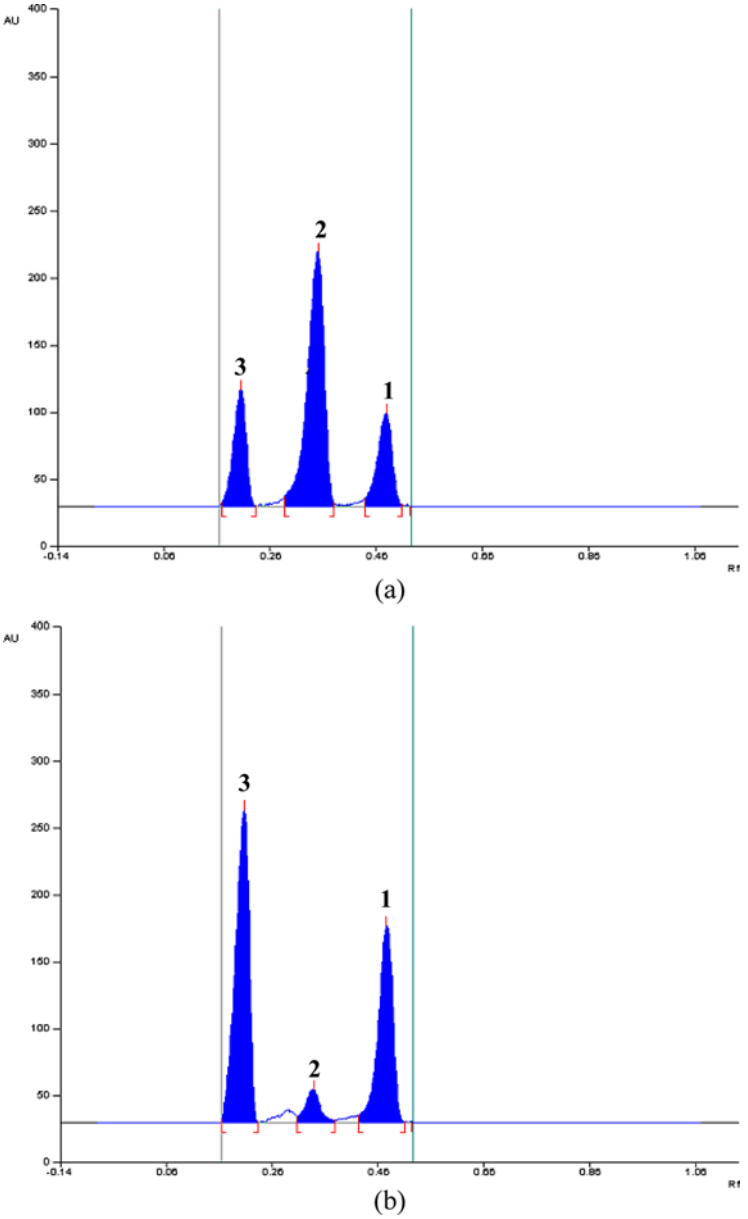

FIGURE 2 (a) The HPTLC separation of standard compounds mixture (b) extract of L. *japonica*; detection under reflectance/absorbance mode at 330 nm. (Figure available in color online.)

sample extract and the R_f values of compounds **1–3**. Peak purity tests were performed on all three compounds satisfactorily by comparing the spectra of the standard and sample tracks. Full UV spectra scans were acquired from 280–400 nm. The spectral scans for compounds **1–3** indicated the maxima at 330 nm and, hence, this wavelength was used for quantification.

Validation

The calibration plots were found to be linear over the ranges 200–1200 ng/spot for standard compounds **1–3** with correlation coefficients of ranging from 0.9994 to 0.9999. Limits of detection obtained for compounds **1–3** were 100, 120, and 120 ng/spot, respectively, and the limit of quantification was 200 ng $spot^{-1}$, for all the compounds. Intra-day variation, as RSD (%), was found to be 0.65 to 2.43% and inter-day variation, as RSD (%), was found to be 1.54 to 3.21 for compounds **1–3** (Table 2). The recovery percentage of compounds **1–3** were in the range of 97.81–101.59% (Table 3).

Plant Sample Analysis

For positive identification, a sample must exhibit bands with chromatographic characteristics, including color and R_f values (0.66 for **1**, 0.56 for **2**,

TABLE 2 Intra- and Inter-day Precision of Plant Sample LJ-1 Assayed Under Optimized Conditions for Compounds **1–3** Using HPTLC Method

	Intra-day (n = 3)			
Compounds	Day-1	Day-2	Day-3	Inter-day (n = 9)
1	1.99 (2.40)	2.10 (0.75)	2.08 (0.65)	2.07 (1.54)
2	0.58 (1.56)	0.63 (2.43)	0.62 (1.28)	0.61 (2.47)
3	3.52 (1.32)	3.65 (1.10)	3.74 (1.01)	3.67 (3.21)

Values in mg/100 mg of sample; relative standard deviation are given in parentheses.

TABLE 3 Accuracy for Three Phenolic Acids from *L. japonica* (LJ-1)

Compounds	Added Amount (ng)	Recovered Amount (ng)	Recovery[a] (%)	RSD (%)
1	300	301.32	100.44	1.10
	500	503.06	100.61	0.81
2	300	294.62	97.81	1.39
	500	504.77	99.96	1.13
3	300	304.78	101.59	2.32
	500	498.32	99.66	1.33

[a]Each value is the mean of triplicate analyses.

TABLE 4 Percentage Content of Three Compounds (**1–3**) from Samples of *L. japonica*

Samples	**1**	**2**	**3**
LJ-1	2.070 ± 1.55	0.610 ± 2.73	3.668 ± 3.21
LJ-2	1.685 ± 0.52	0.886 ± 1.46	3.041 ± 0.12
LJ-3	1.693 ± 1.66	0.545 ± 1.41	2.620 ± 0.21

Mean (n = 3) ± %RSD.

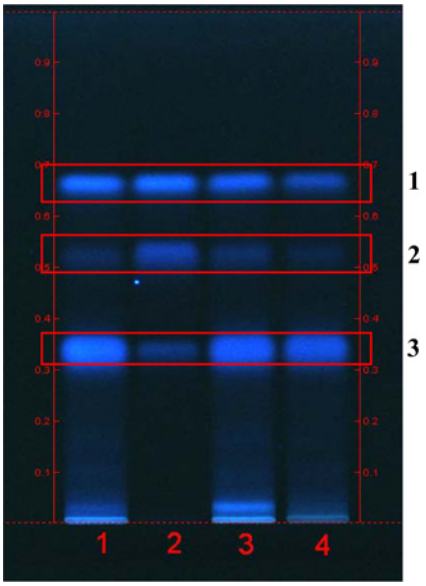

FIGURE 3 HPTLC chromatogram: Track-1; LJ-1, Track-2; Std mix-3 [3,5-*O*-dicaffeoylquinic acid (1), 3,4-*O*-dicaffeoylquinic acid (2) and chlorogenic acid (3)], Track-3; LJ-2, Track-4; LJ-3 at 366 nm. (Figure available in color online.)

and 0.37 for **3**), similar to those of standard compounds (Fig. 3 and Table 4). The percentage content of compounds **1–3** were 2.07, 0.6, and 3.7% for sample LJ-1; 1.69, 0.89, and 3.0% for sample LJ-2; and 1.69, 0.55, and 2.62% for sample LJ-3, respectively. The mean percent content of compounds **1–3** are shown in Table 4.

CONCLUSION

The developed HPTLC method is very simple, and suitable for the rapid screening of flower buds of *L. japonica* for its phenolic profile. The method can be applied without any special pretreatment of the sample. All three compounds can be assayed on a single plate with a single solvent system and a single scanning wavelength (330 nm). A large number of

samples can be analyzed at the same time without compromising accuracy. This is the first HPTLC densitometric method for the estimation of compounds **1–3** from flower buds of *L. japonica*.

ACKNOWLEDGMENTS

This research is funded in part by The United States Department of Agriculture Agricultural Research Service, Specific Cooperative Agreement No. 58-6408-2-0009 and is supported in part by Science Based Authentication of Dietary Supplements and Botanical Dietary Supplement Research funded by the Food and Drug Administration grant Nos. 5U01FD002071-09 and 1U01FD003871-01, respectively.

REFERENCES

1. Qian, Z. M.; Li, H. J.; Li, P.; Ren, M. T.; Tang, D. Simultaneous Qualitation and Quantification of Thirteen Bioactive Compounds in Flos *Lonicerae* by High-Performance Liquid Chromatography with Diode Array Detector and Mass Spectrometry. *Chem. Pharam. Bull.* **2007**, *55*, 1073–1076.
2. Song, W.; Li, S.; Wang, S.; Wu, Y.; Zi, J.; Gan, M.; Zhang, Y.; Liu, M.; Lin, S.; Yang, Y.; Shi, J. Pyridinium Alkaloid-Coupled Secoiridoids from the Flower Buds of *Lonicera japonica*. *J. Nat. Prod.* **2008**, *71*, 922–925.
3. Chang, W. C.; Hsu, F. L. Prostag. Inhibition of Platelet Activation and Endothelial Cell Injury by Polyphenolic Compounds Isolated from *Lonicera japonica*. *Leukotr. Ess.* **1992**, *45*, 307–312.
4. Chang, C. W.; Lin, M. T.; Lee, S. S.; Liu, K. C.; Hsu, F. L.; Lin, J. Y. Differential Inhibition of Reverse Transcriptase and Cellular DNA Polymerase-Alpha Activities by Lignans Isolated from Chinese Herbs, *Phyllanthus Myrtifolius* Moon, and Tannins from *Lonicera japonica* Thunb and *Castanopsis Hystrix*. *Antivir. Res.* **1995**, *27*, 367–374.
5. Lee, S. J.; Choi, J. H.; Chang, H. W.; Kang, S. S.; Kim, H. P. Suppression of Mouse Lymphocyte Proliferation *In Vitro* by Naturally-Occurring Biflavonoids. *Life Sci.* **1995**, *57*, 551–558.
6. Shi, Y.; Shi, R. B.; Lu, W. R. Chemical and Pharmacological Studies on *Lonicera japonica* Planted in China. *Chin. Pharm. J.* **1999**, *34*, 724–727.
7. Xiang, T.; Xiong, Q. B.; Ketut, A. I.; Tezuka, Y.; Nagaoka, T.; Wu, L. J.; Kadota, S. Studies on the Hepatocyte Protective Activity and the Structure-Activity Relationships of Quinic Acid and Caffeic Acid Derivatives from the Flower Buds of *Lonicera Bournei*. *Planta Med.* **2001**, *67*, 323–325.
8. Leung, H. W. C.; Kuo, C. L.; Yang, W. H.; Lin, C. H.; Lee, H. Z. Antioxidant Enzymes Activity Involvement in Luteolin-Induced Human Lung Squamous Carcinoma CH27 Cell Apoptosis. *Eur. J. Pharmacol.* **2006**, *534*, 12–18.
9. Kakuda, R.; Imai, M.; Yaoita, Y.; Machida, K.; Kikuchi, M. Secoiridoid Glycosides from the Flower Buds of *Lonicera japonica*. *Phytochemistry.* **2000**, *55*, 879–881.
10. Teng, R. W.; Wang, D. Z.; Chen, C. X. Two Triterpenoid Saponins from *Lonicera japonica*. *Chin.Chem. Lett.* **2000**, *11*, 337–340.
11. Kumar, N.; Singh, B.; Bhandari, P.; Gupta, A. P.; Uniyal, S. K.; Kaul, V. K. Biflavonoids and Volatile Oil Constituents from *Lonicera japonica*. *Phytochemistry.* **2005**, *66*, 2740–2744.
12. Kumar, N.; Singh, B.; Gupta, A. P.; Kaul, V. K. Lonijaposides, Novel Cerebrosides from *Lonicera japonica*. *Tetrahedron.* **2006**, *62*, 4317–4322.
13. Bi, Y. F.; Tian, Y.; Pei, S. S.; Liu, H. M. Secoiridoid Glycosides from Flos *Lonicerae*. *Chin.Tradit. Herbal Drugs.* **2008**, *39*, 18–21.
14. Tang, D.; Li, H. J.; Chen, J.; Guo, C. W. Li, P. Rapid and Simple Method for Screening of Natural Antioxidants from Chinese Herb Flos *Lonicerae japonicae* by DPPH-HPLC-DAD-TOF/MS. *J. Sep. Sci.* **2008**, *31*, 3519–3526.

15. Xing, J. B.; Li, H. J.; Li, P.; Liu, Y. Chin. Studies on Chemical Constituents in Dried Buds of *Lonicera japonica* Thunb. *J. New Drugs.* **2002**, *11*, 856–896.
16. Xiong, Y.; Gao, H.; Wang, Z.; Zhu, J. Comparison on HPLC Fingerprint Profiles of *Lonicera japonica* Bud Obtained by Various Dryness Techniques. *Zhongguo Zhong Yao Za Zhi.* **2009**, *34*, 1015–1017.
17. Xiang, Z. X.; Gao, S. L. Application and Study of HPLC Fingerprint of *Lonicera japonica* for Identification of Geo-Authenticity. *Zhongguo Zhong Yao Za Zhi.* **2008**, *33*, 996–998.
18. Bhandari, P., Kumar, N.; Gupta, A. P.; Singh, B.; Kaul, V. K. A Rapid RP-HPTLC Densitometry Method for Simultaneous Determination of Major Flavonoids in Important Medicinal Plants. *J. Sep. Sci.* **2007**, *30*, 2092–2096.