Quality Analysis of Chlorogenic Acid and Hyperoside in *Crataegi fructus*

Jin Bae Weon¹, Youn Sik Jung¹, Choong Je Ma¹,²

¹Department of Medical Biomaterials Engineering, College of Biomedical Science, Kangwon National University, ²Department of Biomaterials Engineering, Kangwon National University, Chuncheon 200-701, Korea

Submitted: 21-05-2015 Revised: 01-07-2015 Published: 02-03-2016

**ABSTRACT**

**Background:** *Crataegi fructus* is a herbal medicine for strong stomach, sterilization, and alcohol detoxification. Chlorogenic acid and hyperoside are the major compounds in *Crataegi fructus*. **Objective:** In this study, we established novel high-performance liquid chromatography (HPLC)-diode array detection analysis method of chlorogenic acid and hyperoside for quality control of *Crataegi fructus*. **Materials and Methods:** HPLC analysis was achieved on a reverse-phase C₅µ column (5 µm, 4.6 mm x 250 mm) using water and acetonitrile as mobile phase with gradient system. The method was validated for linearity, precision, and accuracy. About 31 batches of *Crataegi fructus* samples collected from Korea and China were analyzed by using HPLC fingerprint of developed HPLC method. Then, the contents of chlorogenic acid and hyperoside were compared for quality evaluation of *Crataegi fructus*. **Results:** The results have shown that the average contents (w/w %) of chlorogenic acid and hyperoside in *Crataegi fructus* collected from Korea were 0.0438% and 0.0416%, respectively, and the average contents (w/w %) of 0.0399% and 0.0325%, respectively. **Conclusion:** In conclusion, established HPLC analysis method was stable and could provide efficient quality evaluation for monitoring of commercial *Crataegi fructus*.

**Key words:** *Crataegi fructus*, chlorogenic acid, diode array detection, high performance liquid chromatography, hyperoside, quality evaluation

**SUMMARY**

- Quantitative analysis method of chlorogenic acid and hyperoside in *Crataegi fructus* is developed by high-performance liquid chromatography (HPLC)-diode array detection
- Established HPLC analysis method is validated with linearity, precision, and accuracy
- The developed method was successfully applied for quantitative analysis of *Crataegi fructus* sample collected from Korea and China.

**INTRODUCTION**

Herbal medicines have been widely used for the prevention and treatment of many diseases for 1000 of years. Herbal medicines had fewer side effects and were suitable for body. Various pharmacological effects of herbal medicines have been attributed to many bioactive compounds.[1,2] Because of complex composition and variation by culture environment of the compounds in herbal medicines, quality consistency control of herbal medicines is difficult. Thus, accurate and stable analysis method of herbal medicine is required for quality evaluation. Analysis systems, such as high-performance liquid chromatography (HPLC), gas chromatography, and mass spectrometer have been widely used for qualitative and quantitative analysis of compounds in herbal medicines. Among systems, HPLC is the most common system for quality control of herbal medicines using characteristic fingerprint analysis.[3,4] HPLC chromatographic fingerprint technology is the comprehensive peak identification method and is applied to reveal bioactivity and chemical information of herbal medicines.[5] HPLC fingerprint is additionally used to approach a quality analysis of compounds in herbal medicine.[6] *Crataegi fructus* is the fruit part of *Crataegus pinnatifida* Bunge var. typica Schneider (*Rosaceae* family) and is a well-known herbal medicine for strong stomach, analgesic effect, sterilization, and alcohol detoxification. Recent studies showed that *Crataegi fructus* prevents hyperlipidemia induced by alcohol and ameliorate arterial contraction via antioxidant effect.[7] *Crataegi fructus* also showed inhibition effect of sodium nitroprusside-induced cell lipid peroxidation and anti-inflammatory effect.[8]

**Abbreviations used:** HPLC: High-performance liquid chromatography, GC: Gas chromatography, MS: Mass spectrometer, LOD: Limits of detection, LOQ: Limits of quantification, RSD: Relative standard deviation, RRT: Relative retention time, RPA: Relation peak area.

**Correspondence:** Prof. Choong Je Ma, Department of Medical Biomaterials Engineering, College of Biomedical Science, Kangwon National University, Hyoja-2 Dong, Chuncheon 200-701, Korea. E-mail: cjma@kangwon.ac.kr

DOI: 10.4103/0973-1296.177904

**Cite this article as:** Weon JB, Jung YS, Ma CJ. Quality analysis of chlorogenic acid and hyperoside in *Crataegi fructus*. Phcog Mag 2016;12:98-103.
Chlorogenic acid and hyperoside are major bioactive compounds in *Crataegus fructus*. Chlorogenic acid, a related family of hydroxycinnamic acid, showed anti-diabetic and anti-lipidemic effects.\[^9\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\]

Previous study reported simultaneous determination of eight polyphenols in the leaves of *Crataegus pinnatifida* by HPLC and HPLC analysis method of chlorogenic acid, hyperoside, and rutin for quality control of *Crataegus fructus*.\[^13,14\]

We determine HPLC analysis method and improve the resolution of hyperoside using modified gradient system of mobile phase based on previous studies. In this study, quantitative determination of chlorogenic acid and hyperoside in *Crataegus fructus* was developed and analyzed in 31 *Crataegi fructus* samples collected from Korea and China.

**MATERIALS AND METHODS**

**Chemicals and plant materials**

Water and acetonitrile as HPLC grade were purchased from J.T Baker. Standards of chlorogenic acid and hyperoside are both obtained from the Ministry of Food and Drug Safety (Korea) [Figure 1]. The 4 batches of *Crataegi fructus* samples (collected from Korea, 11D2001, 2002, 2024, 2027) were obtained from the Ministry of Food and Drug Safety (Korea); 11 batches of *Crataegi fructus* samples (collected from Korea, 12D3017-3027) and 16 batches of *Crataegi fructus* samples (collected from China, 12D3001-3016) were purchased from Kyungdong mart (Seoul, Korea).

**Preparation of samples and standard solution**

Samples of *Crataegi fructus* powder were accurately weighed and extracted by sonication with 70% methanol for 60 min. Before HPLC analysis, extract solutions were filtered with 0.45 μm membrane filter. Stock standard solution of chlorogenic acid and hyperoside were prepared in methanol at a concentration of 1000 μg/mL. Stock standard solutions appropriately diluted to obtain work standard solutions by methanol.

**High-performance liquid chromatography-diode array detection condition**

HPLC was performed on Dionex Ultimate 3000 HPLC system (Dionex, Germany) equipped with a pump (LPG 3X00), an auto sampler (ACC-3000), a column oven (TCC-3000SD), and diode array UV/VIS detector (DAD-3000(RS)). HPLC chromatogram data were processed using Dionex Chromeleon™ Chromatography Data System. Chromatographic separation was conducted on Agilent eclipse XDB-C18 column (150 mm × 4.6 mm, 5 μm) at column temperature 30°C. The mobile phase consisted of aqueous with 0.1% trifluoroacetic acid solution (A) acetonitrile (B) using gradient elution system of 5% (B) at 0–5 min and 5–50% (B) at 5–40 min and flow rate was 1.0 mL/min. The ultraviolet (UV) wavelength was selected and monitored at 254 nm according to the wavelength of chlorogenic acid and hyperoside.

**RESULTS AND DISCUSSION**

**Optimization of chromatographic conditions**

In the previous study, resolution of hyperoside and rutin is low. Therefore, we evaluated HPLC condition for improvement of resolution of hyperoside. Mobile phase system (acetonitrile-water, methanol-water, and acetonitrile and aqueous with 0.1% trifluoroacetic acid), gradient elution system, and column temperature (25°C, 30°C and 35°C) were tested to obtain good resolution.\[^15,16\]

Acetonitrile and water were found to be effective for chromatographic separation, and trifluoroacetic acid was used for the inhibition of compounds and improvement of peak shape. Gradient elution system was proposed to achieve good separation. Overall, gradient elution system was varied as follows: 0–5 min, 5% acetonitrile; and 5–40 min, 5–50% acetonitrile based on previous study. Flow rate of 1.0 ml/min and column temperature of 30°C were selected. The detection UV wavelength of 254 nm was selected according to the wavelength of two compounds. We evaluated resolution value (R) of hyperoside using the retention time (t<sub>R1</sub> and t<sub>R2</sub>) and the widths (W<sub>R1</sub> and W<sub>R2</sub>) of two peaks. R ≥1.5 represents good peak resolution in many cases. Resolution value of hyperoside was 1.76.

HPLC chromatograms of standard compounds and *Crataegi fructus* sample were obtained without interfering peaks on optimized separation condition [Figure 2].

**Method validation**

**Linearity, limits of detection, and limits of quantification**

The calibration curves of chlorogenic acid and hyperoside were constructed by plotting the peak area versus six different concentrations of standard solutions. Limits of detection (LOD) and limits of quantification (LOQ) of two compounds were investigated at noise ratio of 3 and 10, respectively. The linear regression equations were present as Y = ax + b (a: The slope of the calibration curve, b: The intercept of calibration curve, x: Concentration and Y: Peak area of compound). Regression equations and correlation coefficients of chlorogenic acid, showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.\[^10-12\] Hyperoside is one of the phenolic compounds and showed antiviral activity, anti-inflammatory effect, and protective effect on PC12 cells against cytotoxicity.
acid and hyperoside were  \( Y = 0.0857x + 0.1784 \) (\( R^2 = 0.9995 \)) and  \( Y = 0.1669x + 0.349 \) (\( R^2 = 0.9986 \)), respectively. The LOD of chlorogenic acid and hyperoside were 0.26 µg/ml and 0.04 µg/ml, respectively, and the LOQ were found to be 0.78 µg/ml and 0.12 µg/ml for chlorogenic acid and hyperoside, respectively [Table 1].

Results showed that this HPLC method has good linearity and high detection sensitivity for chlorogenic acid and hyperoside.

**Precise and accuracy**

The inter- and intra-day test with chlorogenic acid and hyperoside at three different concentrations were performed to investigate the precision of this HPLC method. Previously repeatability of this method was indicated and determined as relative standard deviation (RSD) values of inter- and intra-day. The intra-day test was continuously analyzed by injection standard solution 5 times on the same day and the inter-day test was analyzed for 3 consecutive days (1, 3, and 5 days).

The intra- and inter-day RSD values of chlorogenic acid and hyperoside were in the range from 0.05–1.02% to 0.62–2.49%, respectively [Table 2].

Accuracy of analysis method was determined as recovery test. Recovery was investigated by spike test. Three different concentrations of chlorogenic acid and hyperoside were added into the previously analyzed *Crataegi fructus* sample, and then the sample was analyzed in triplicate. Accuracy (%) was calculated by difference of spiked and unspiked sample.

The recovery value of two compounds ranged from 97.02% to 109.39% with RSD <1.17% [Table 3].

**Quantitative analysis of chlorogenic acid and hyperoside in Crataegi fructus samples**

To obtain the HPLC fingerprint of *Crataegi fructus*, 31 batches of *Crataegi fructus* from different areas were analyzed by optimized HPLC method. Peaks of chlorogenic acid and hyperoside are identified in all the samples. Among different peaks, chlorogenic acid and hyperoside have relatively high intensity peak in *Crataegi fructus* [Figure 3]. Relative retention time and relation peak area of chlorogenic acid and hyperoside peak were calculated [Table 4].

This HPLC method was applied for the quantitative determination of chlorogenic acid and hyperoside in 31 *Crataegi fructus* samples using calibration curve of two compounds. Among *Crataegi fructus* sample, 15 species were collected from Korea and 16 species were collected from China. The content results of chlorogenic acid and hyperoside were shown in Table 5.

The content (w/w %) of chlorogenic acid in *Crataegi fructus* ranges were as follows: 0.0129–0.1065% (Korea) and 0.0121–0.1125% (China). The contents of hyperoside ranged from 0.0150–0.0660% (Korea) to 0.0204–0.0560% (China). The average content of chlorogenic acid in

### Table 1: Regression equation, correlation coefficient (R²), limits of detection, and limits of quantitation

| Compound   | Linear range (µg/ml) | Regression Equation | \( R^2 \) (n=6) | LOD (µg/ml) | LOQ (µg/ml) |
|------------|----------------------|---------------------|-----------------|-------------|-------------|
| Chlorogenic acid | 4.38–70.00            | \( Y = 0.0857x + 0.1784 \) | 0.9995          | 0.26        | 0.78        |
| Hyperoside  | 4.06–65.00           | \( Y = 0.1669x + 0.349 \) | 0.9986          | 0.04        | 0.12        |

LOD: Limits of detection; LOQ: Limits of quantitation

### Table 2: Analytical results of intra- and inter-day variability

| Compound   | Concentration (µg/ml) | Intra-day (n = 5) | Inter-day (n = 5) |
|------------|-----------------------|-------------------|-------------------|
|            |                       | Mean ± SD (µg/ml) | RSD (%) | Accuracy (%) | Mean ± SD (µg/ml) | RSD (%) | Accuracy (%) |
| Chlorogenic acid | 35.00                | 32.92 ± 0.22      | 0.66    | 94.06        | 33.72 ± 0.79      | 2.35    | 96.35        |
|             | 17.50                 | 16.05 ± 0.16      | 1.02    | 91.70        | 15.85 ± 0.16      | 1.00    | 90.58        |
|             | 8.75                  | 7.89 ± 0.04       | 0.56    | 90.13        | 8.01 ± 0.05       | 0.67    | 91.56        |
| Hyperoside  | 32.50                 | 31.53 ± 0.05      | 0.56    | 97.02        | 32.23 ± 0.80      | 2.49    | 99.18        |
|             | 16.25                 | 14.93 ± 0.02      | 0.15    | 91.86        | 16.00 ± 0.35      | 2.19    | 98.49        |
|             | 8.13                  | 7.41 ± 0.01       | 0.10    | 91.21        | 7.31 ± 0.05       | 0.62    | 90.02        |

SD: Standard deviation; RSD: Relative standard deviation
Crataegi fructus collected from Korea (0.0477%) higher than that in China sample (0.0399%). Moreover, the average content of hyperoside in Crataegi fructus collected from Korea (0.0450%) was higher than that in China sample (0.0325%).

Table 3: Results of recovery test

| Compound      | Spiked amount (μg/ml) | Measured amount (μg/ml) | RSD (%) | Recovery (%) |
|---------------|-----------------------|-------------------------|---------|--------------|
| Chlorogenic acid | 35.00               | 35.53±0.19              | 0.53    | 101.52       |
|               | 17.50                | 17.04±0.14              | 0.83    | 97.37        |
|               | 8.75                 | 8.86±0.05               | 0.51    | 101.25       |
| Hyperoside    | 21.67                | 23.69±0.15              | 0.64    | 97.02        |
|               | 10.83                | 11.85±0.14              | 1.17    | 109.39       |
|               | 5.42                 | 5.87±0.06               | 0.95    | 108.33       |

RSD: Relative standard deviation

Table 4: Relative retention time and relation peak area of the chlorogenic acid and hyperoside in Crataegi Fructus

| Compound      | Retention time (min) | RRT     | RPA |
|---------------|----------------------|---------|-----|
| Chlorogenic acid | 12.36               | 0.967   | 0.997 |
| Hyperoside    | 18.86               | 0.913   | 0.910 |

RRT: Relative retention time; RPA: Relation peak area

Table 5: Contents (μg/mg) of the chlorogenic acid and hyperoside in the 30 Crataegi Fructus

| Number  | Species     | Collection Place | Mean ± SD | RSD (%) | Mean ± SD | RSD (%) |
|---------|-------------|------------------|-----------|---------|-----------|---------|
|         | Chlorogenic acid | Hyperoside      |           |         |           |         |
| 11D2001 | C. pinnatifida | Korea            | 0.0575 ± 0.0001 | 0.1509  | 0.0660 ± 0.0002 | 0.3374  |
| 11D2002 | C. pinnatifida | Korea            | 0.0129 ± 0.0002 | 1.3330  | 0.0212 ± 0.0001 | 0.3349  |
| 11D2024 | C. pinnatifida | Korea            | 0.0407 ± 0.0001 | 0.2565  | 0.0447 ± 0.0001 | 0.0752  |
| 11D2027 | C. pinnatifida | Korea            | 0.0008 ± 0.0001 | 12.8378 | 0.0036 ± 0.0001 | 0.8172  |
| 12D3017 | C. pinnatifida | Korea            | 0.0339 ± 0.0008 | 2.2902  | 0.0432 ± 0.0002 | 0.0405  |
| 12D3018 | C. pinnatifida | Korea            | 0.0177 ± 0.0008 | 4.3347  | 0.0150 ± 0.0004 | 2.3626  |
| 12D3019 | C. pinnatifida | Korea            | 0.0365 ± 0.0016 | 4.4143  | 0.0381 ± 0.0032 | 8.3023  |
| 12D3020 | C. pinnatifida | Korea            | 0.0955 ± 0.0034 | 3.5614  | 0.0586 ± 0.0025 | 4.2608  |
| 12D3021 | C. pinnatifida | Korea            | 0.0170 ± 0.0003 | 2.0472  | 0.0513 ± 0.0002 | 0.3165  |
| 12D3022 | C. pinnatifida | Korea            | 0.1065 ± 0.0002 | 0.1930  | 0.0655 ± 0.0001 | 0.0958  |
| 12D3023 | C. pinnatifida | Korea            | 0.0725 ± 0.0008 | 1.1144  | 0.0402 ± 0.0000 | 0.0837  |
| 12D3024 | C. pinnatifida | Korea            | 0.0674 ± 0.0080 | 11.8288 | 0.0620 ± 0.0082 | 13.2434 |
| 12D3025 | C. pinnatifida | Korea            | 0.0383 ± 0.0079 | 20.5923 | 0.0600 ± 0.0114 | 18.9926 |
| 12D3026 | C. pinnatifida | Korea            | 0.0314 ± 0.0019 | 6.0345  | 0.0368 ± 0.0006 | 1.6618  |
| 12D3027 | C. pinnatifida | Korea            | 0.0405 ± 0.0004 | 1.0013  | 0.0394 ± 0.0001 | 0.2681  |

Mean 0.0477 ± 0.0017 4.8436 0.0450 ± 0.0012 2.3878

Mean 0.0753 ± 0.0009 1.2617 0.0341 ± 0.0002 0.6131

Mean 0.0121 ± 0.0012 10.1521 0.0283 ± 0.0021 7.4884

Mean 0.0139 ± 0.0006 4.3536 0.0373 ± 0.0002 0.5682

Mean 0.0276 ± 0.0007 2.6444 0.0290 ± 0.0004 1.3112

Mean 0.0258 ± 0.0016 6.2941 0.0325 ± 0.0012 3.8327

Mean 0.0162 ± 0.0014 8.5103 0.0204 ± 0.0021 10.4409

Mean 0.0167 ± 0.0003 1.9890 0.0369 ± 0.0008 2.1406

Mean 0.0801 ± 0.0036 4.5450 0.0497 ± 0.0028 5.5294

Mean 0.1125 ± 0.0004 0.3935 0.0312 ± 0.0001 0.4089

Mean 0.0360 ± 0.0017 4.8581 0.0349 ± 0.0019 5.3955

Mean 0.0254 ± 0.0004 1.4704 0.0560 ± 0.0002 0.2687

Mean 0.0174 ± 0.0006 3.4547 0.0263 ± 0.0002 0.8685

Mean 0.0620 ± 0.0004 0.7211 0.0315 ± 0.0002 0.5474

Mean 0.0179 ± 0.0002 0.9555 0.0234 ± 0.0007 2.8732

Mean 0.0421 ± 0.0005 1.0901 0.0221 ± 0.0010 4.5483

Mean 0.0580 ± 0.0009 1.6060 0.0258 ± 0.0003 1.0983

Mean 0.0399 ± 0.0010 3.3937 0.0325 ± 0.0009 2.9992

SD: Standard deviation; RSD: Relative standard deviation; C. pinnatifida: Crataegus pinnatifida

The difference of contents in Crataegi fructus was due to cultivation environment including the year of the plant cultivation, harvest time, plant origins, climate, and cultivated location.

Principal components analysis (PCA) was conducted by scatterplot for quantitative evaluation. Results of PCA showed that the contents of Crataegi fructus collected from Korea and China were different by two developed clusters [Figure 4].

We evaluated reproducibility of this HPLC method by cross-monitoring with other researchers. Cross-monitor was performed using some Crataegi fructus samples and compared to the content results of chlorogenic acid and hyperoside of other researchers. Reproducibility is determined from the difference between two test results obtained from same HPLC conditions. However, production of HPLC system and column is different. The results are listed in Table 6 and there was no difference between the contents of chlorogenic acid and hyperoside in other researchers [Figure 5].

The results of cross-monitor indicated that our HPLC method had a good reproducibility.

CONCLUSION

In this study, we developed quantitative analysis method of chlorogenic acid and hyperoside in Crataegi fructus and validated with linearity, precision, and accuracy. Results of validation indicated that this HPLC method was accurate and stable for the analysis of Crataegi fructus.
Moreover, the developed method was successfully applied for quantitative analysis of Crataegi fructus sample collected from Korea and China. The results of validation and Crataegi fructus sample analysis could be used as a reference for monitoring and quality control of Crataegi fructus.

Acknowledgment
This work was carried out with the support of the Ministry of Food and Drug Safety.

Financial support and sponsorship
Nil.

Conflicts of Interest
The authors declare no conflicts of interest.

REFERENCES

1. Lu AP, Jia HW, Xiao C, Lu QP. Theory of traditional Chinese medicine and therapeutic method of diseases. World J Gastroenterol 2004;10:1854-6.
2. Jiang WY. Therapeutic wisdom in traditional Chinese medicine: A perspective from modern science. Trends Pharmacol Sci 2005;26:558-63.
3. Steinmann D, Ganzera M. Recent advances on HPLC/MS in medicinal plant analysis. J Pharm Biomed Anal 2011;55:744-57.
4. Drasar P, Moravcova J. Recent advances in analysis of Chinese medical plants and traditional medicines. J Chromatogr B Analyt Technol Biomed Life Sci 2004;812:3-21.
5. Gong F, Liang YZ, Xie PS, Chau FT. Information theory applied to chromatographic fingerprint of herbal medicine for quality control. J Chromatogr A 2003;1002:25-40.
6. Gu M, Ouyang F, Su Z. Comparison of high-speed counter-current chromatography and high-performance liquid chromatography on fingerprinting of Chinese traditional medicine. J Chromatogr A 2004;1022:139-44.
7. Kao ES, Wang CJ, Lin WL, Yin YF, Wang CP, Tseng TH. Anti-inflammatory potential of flavonoid contents from dried fruit of Crataegus pinnatifida in vitro and in vivo. J Agric Food Chem 2005;53:430-6.
8. Chu CY, Lee MJ, Liu WL, Yin YE, Tseng TH. Anti-inflammatory activity of hyperoside through the suppression of nuclear factor-κB activation in mouse peritoneal macrophages. Am J Chin Med 2011;39:171-81.
9. Wu LL, Yang XB, Huang ZM, Liu HZ, Wu GX. In vivo and in vitro antiviral activity of hyperoside
extracted from Abelmoschus manihot (L) medik. Acta Pharmacol Sin 2007;28:404-9.

13. Bae YH, Cuong TD, Lee JH, Woo MH, Choi JS, Min BS. Quantitative analysis of bioactive compounds in the Fruits of Crataegus pinnatifida by high-performance liquid chromatography. Nat Prod Sci 2012;18:83-8.

14. Ying X, Wang R, Xu J, Zhang W, Li H, Zhang C, et al. HPLC determination of eight polyphenols in the leaves of Crataegus pinnatifida Bge. var. major. J Chromatogr Sci 2009;47:201-6.

15. Heyman AN, Henry RA. Importance of controlling mobile phase pH in reversed phase HPLC. Keystone Tech Bull 1999;99-06:1-7.

16. Dolan JW. The Importance of Temperature. Walnut Creek, California, USA: LC Resources Inc.; 2002.

ABOUT AUTHOR

Choong Je Ma, has completed his PhD at the age of 32 years from Seoul National University and postdoctoral studies from the University of Michigan. He is the professor of Department of Medical Biomaterials Engineering, College of Biomedical science, Kangwon National University, Korea. He has published more than 20 papers in reputed journals.