Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Validation of High-Performance Liquid Chromatography Method for Quantification of Costunolide Content in *Laurus nobilis*

Puttaswamy Naveen, Harakanahalli Basavegowda Lingaraju[1], Siddappa Chandrappa[1], Koretekera Shekharappa Priya, Kodimule Shyam Prasad[1]

Analytical Research and Development, [1]Phytochemistry Lab, Vidya Herbs Pvt. Ltd, Bangalore, Karnataka, India

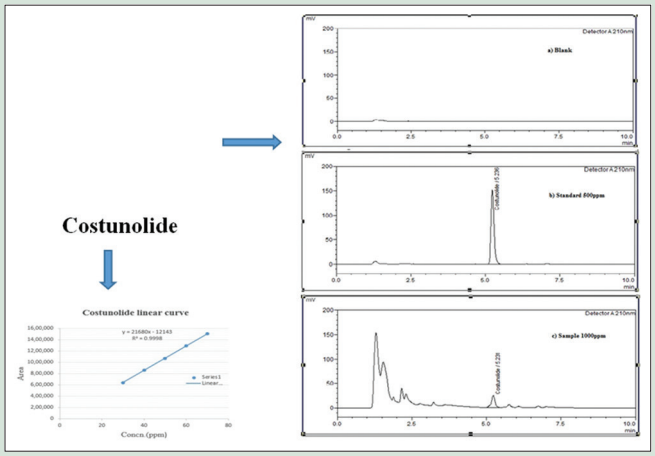
ABSTRACT

Objective: To establish a method to determine the content of costunolide in *Laurus nobilis* leaves by high-performance liquid chromatography (HPLC). **Materials and Methods:** The separation was performed on a reversed-phase C18 column (100 Å, 150 mm × 4.6 mm, 5 µm pore size) using a mobile phase composed of water:acetonitrile (40:60) at a flow rate of 1.0 ml/min. The detection was carried out on a ultraviolet detector at 210 nm. The developed method was validated according to the requirements for International Conference on Harmonisation guidelines. **Results:** The proposed method for costunolide was validated for linearity with excellent correlation coefficient ($r^2 > 0.999$). The relative standard deviation (RSD) is less than 1% in precision (i.e repeatability and intermediate) of the method. The recovery rate for costunolide was within 100.54%–102.62%. The limit of detection and limit of quantitation were 2.29 and 6.64 parts per million, respectively. **Conclusion:** The developed HPLC method is simple, rapid, precisely, accurately, and widely accepted and it is recommended for efficient assays in routine work.

Key words: Costunolide, *Laurus nobilis*, linearity, precision, recovery, validation

SUMMARY

- The present study was projected to develop and validate a reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantification of costunolide from the leaf extract of *Laurus nobilis*. The developed method was validated according to the requirements for International Conference on Harmonisation guidelines. A simple, rapid, precise, and specific RP-HPLC method was developed and validated for the quantification of costunolide in *L. nobilis*.



Abbreviation Used: HPLC: High-performance liquid chromatography; UV: Ultraviolet; ICH: International Conference on Harmonisation; r^2: Correlation coefficient; WHO: World Health Organization; ppm: Parts per million; % RSD: Percentage relative standard deviation

Correspondence:
Dr. Puttaswamy Naveen,
Analytical Research and Development,
Vidya Herbs Pvt. Ltd, Jigani, Anekal Taluk,
Bengaluru, Karnataka, India.
E-mail: analytical@vidyaherbs.com

DOI: 10.4103/pr.pr_31_18

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Over the centuries, the use of medicinal herbs has become an important part of daily life despite the progress in modern medicine and pharmaceuticals research. Approximately 3000 plants species are known to have medicinal properties in India.[1] According to the World Health Organization, more than one million people trust on herbal medicines around the world to some extent. India has a wide array of medicinal plants comprising about 25,000 species, of which 150 species are commercially used for extracting medicines or drug formulation.[2]

Laurus nobilis L. is the member of family *Lauraceae* which comprises 32 genera and represented about 2000–2500 species and it is also called Bay Laurel, Sweet Bay, Grecian Laurel, True Bay, and Bay Tree.[3] The *Lauraceae* family distributed in the subtropics and tropics of Eastern Asia, South and North America, Mediterranean area, and Europe. The dried leaves are used extensively in cooking, and the essential oil is generally used in the flavoring industry.[4]

The major phytoconstituents present in the *L. nobilis* leaves and fruits contain sesquiterpene lactones,[5] alkaloids,[6] glycosylated flavonoids,[7] monoterpene, and germacrane alcohols.[8,9] *L. nobilis* has been reported for its antioxidant,[10] wound healing,[11] neuroprotective,[12] antiulcerogenic,[13] anticonvulsant,[14] analgesic,[15] anti-inflammatory,[16] antimutagenic,[17] immunostimulant,[18] antiviral,[19] antibacterial,[20] and antifungal activities.[21]

In the present study, we have developed a simple, optimized, and validated reversed-phase high-performance liquid chromatography (RP-HPLC) method for quantitative determinations of costunolide in the leaves of *L. nobilis*. The developed method was validated as per the International Conference on Harmonisation (ICH) guidelines.[22]

Cite this article as: Naveen P, Lingaraju HB, Chandrappa S, Priya KS, Prasad KS. Validation of high-performance liquid chromatography method for quantification of costunolide content in *Laurus nobilis*. Phcog Res 2018;10:422-5.

MATERIALS AND METHODS

Materials

Costunolide (HPLC grade ≥97%) was used as standard and was procured from Sigma-Aldrich (India). All reagents and solvents were of analytical and HPLC grade. The powdered leaf material of *Laurus nobilis* was procured from Europe.

Preparation of the extract

Laurel leaf extract of *L. nobilis* leaves was prepared by Soxhlet extraction of 100 g of powdered floral material using 70% alcohol at 80°C for about 5 h in three successive batch extractions. The first batch was extracted by adding 500 ml solvent for about 3 h, and further two successive extractions were done by adding 300 ml/batch for 1 h. After completion of extraction, the cooled liquid was concentrated by evaporating its liquid contents in a rotary evaporator till dryness. The extract powder was used for further experiments.

Instrumentation and chromatographic conditions

HPLC was performed on a Shimadzu LC2030 C Prominence-i (Japan) system equipped with a quaternary low-pressure gradient solvent delivery LC2030 pump with high-pressure switching valves, online LC2030 degasser unit, a high sensitivity LC2030 ultraviolet (UV) detector, and high-speed drive LC2030 autosampler with a 100 µl loop, and it accommodates 216 samples at a time with direct access rack system and large capacity column oven. The system was controlled and data were analyzed by LabSolutions software (Shimadzu LC2030 C Prominence). A separation was carried out in Kinetex C18 column (100 Å, 150 mm × 4.6 mm, 5 µm pore size). The mobile phase consists of isocratic elution with a low-pressure gradient using water: acetonitrile (40:60) with a flow rate of 1.0 ml/min and the injection volume of 5 µl. All solutions were degassed and filtered through a 0.45-µm pore size filter. The column was maintained at 26°C throughout the analysis, and the UV detector was set at 210 nm. 100% methanol was used as a diluent for assay by HPLC analysis, and the total LC run time was 10 min. The instrument was calibrated and qualified before the analysis. Using these chromatographic conditions, it was possible to confirm the retention time (RT) of costunolide by injection of corresponding standard separately.

Preparation of standard solution

Accurately weighed appropriate amount of costunolide standard was mixed and dissolved in 100% methanol in a 10 ml volumetric flask to obtain a stock solution 500 parts per million (ppm). Before analysis, the solution was filtered through 0.20 µm nylon membrane filters.

Preparation of sample solution

The appropriate amount of dried laurel leaf extract was mixed and dissolved in methanol in a 50 ml volumetric flask to obtain a stock solution of 1000 ppm. Further, the sample solution was obtained by diluting the 5.0 ml stock solution to 10 ml volumetric flask and made up with methanol to get a final concentration of 1000 ppm. Before analysis, the solution was filtered through 0.20 µm nylon membrane filters.

Preparation of spiked sample

Three different volumes (0.8, 1, and 1.2 ml) of standard solution and 5 ml of sample (1000 ppm) solution are added into 10 ml of volumetric flask separately. The standard stock solution was spiked into the sample to determine recovery. Before analysis, the solutions were filtered through 0.20 µm nylon membrane filters.

Validation of the method

In the present study, a simple isocratic RP-HPLC method was developed according to the ICH guidelines. The method is validated for linearity, precision, repeatability, accuracy, limit of detection (LOD), limit of quantification (LOQ), and robustness.

Specificity

Specificity of the HPLC method is demonstrated by the separations of the analytes from other potential components such as impurities, degradants, or excipients. In this study, the specificity was demonstrated by running a blank, standard, and sample solution. In addition, the resolution between the peaks, tailing factor, and theoretical plates is determined.

Linearity

Linearity was determined by different known concentrations of costunolide standard solution in triplicate by diluting the standard stock solution. The standard solutions were injected, and the peak area was measured. For linearity study, five aliquots in the range of 0.6–1.4 ml of standard stock solution (i.e., 100 ppm) were taken and diluted to 10 ml to obtain different concentrations in the range of 30–70 ppm.

Precision

Precision was determined by studying the repeatability and intermediate precision. The repeatability was determined at a minimum of three different concentration levels of costunolide standard. The intermediate precision was carried for another day by different analyst. It is not necessary to study these effects individually. The precision was expressed as percentage relative standard deviation (% RSD).

Accuracy

The accuracy of the method was tested by adding standard solutions with three known concentrations of costunolide in the sample. The standard stock solution was spiked into the samples to determine recovery. Three different volumes (0.8, 1, and 1.2 ml) of standard stock solution were added to the sample solution (1000 ppm). Triplicate injections were made with all the spiked samples.

$$\% \text{ of recovery} = (b - a)/c \times 100$$

where "*a*" is the amount of drug found in the sample before addition of standard drug, "*b*" is the amount of drug found after addition of standard drug, and "*c*" is the amount of standard drug added.

Limit of detection

Detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated under the stated experimental conditions. The quantitation limit (QL) may be expressed as: 10 × standard deviation of lowest concentration/slope of the calibration line. Calculated the standard deviation of the response and the slope of costunolide.

Limit of quantification

QL of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with accuracy. The QL may be expressed as: 10 × standard deviation of lowest concentration/slope of the calibration line. Calculated the standard deviation of the response and the slope of costunolide.

Robustness

The robustness of an analytical procedure is its ability to remain unaffected by small variations in the analytical parameters. The

robustness is evaluated by varying the analytical parameters such as changing the mobile-phase concentration, temperature, and many other simple parameters. In this validation, we change the temperature 26°C to 30°C. The robustness of the method was done at three different standard concentration levels of 40, 50, and 60 ppm and sample concentration of 1000 ppm (n = 6). The corresponding peak areas were recorded and expressed in terms of % RSD.

Statistical analysis

Results were expressed as mean ± standard deviation. The data were submitted to statistical analysis using Excel software.

RESULTS AND DISCUSSION

An RP-HPLC method was developed and validated for the determination of costunolide in *L. nobilis*. Several mobile-phase compositions were tried, and following chromatographic parameters provided the best separation for the analysis of costunolide. Water: acetonitrile (40:60 v/v) was used as mobile phase.

In specificity, an optimized chromatogram of blank, standard, and sample is shown in Figure 1. The RT of costunolide reference standard was found to be 5.236. The 100% test (sample) chromatograms confirm the presence of costunolide RT at 5.231 without any interference. The sample solution is overlay with the standard solution, so the method was specific. The chromatographic parameters such as column efficiency and peak symmetry were done to the standard solution according to the ICH guidelines. The theoretical plates 4604 and the tailing factor 1.30 were observed, which indicated that column efficiency is satisfactory.

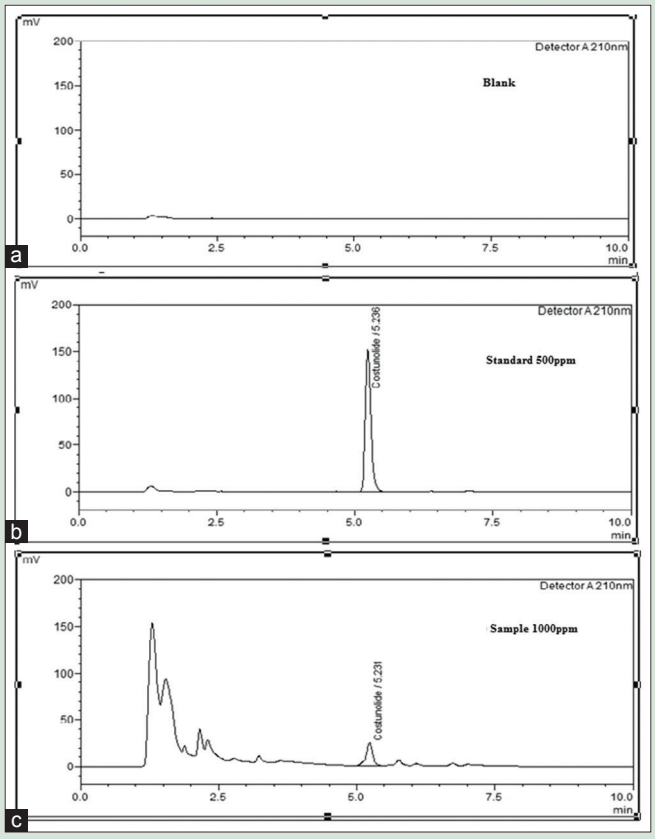


Figure 1: Optimized HPLC chromatogram of costunolide (a) Blank (b) Standard (c) Sample

The calibration plots were linear in the range 30, 40, 50, 60, and 70 ppm and the correlation coefficient (r^2) of costunolide was 0.999 as shown in Figure 2. The minimum acceptable correlation coefficient is 0.990.[22,23] The r^2 value is >0.990. This indicates that the costunolide obeys Beer–Lambert law and good fitting of the curve. The method shows good linearity parameter in the concentrations in the range of 30–70 ppm.

Precision was evaluated based on the % RSD value. The data pertaining to repeatability (intra) and intermediate precision are summarized in Table 1. In repeatability, the % RSD of peak area of costunolide standard concentration of 40, 50, and 60 ppm was found to be 0.103%, 0.120%, and 0.117%, respectively. In intermediate precision, the % RSD of peak area of costunolide standard concentration of 40, 50, and 60 ppm was found to be 0.203%, 0.074%, and 0.126%, respectively. These precision presented % RSD values are <1.0%, so the method was found to be highly precise and reproducible.

Percentage recovery was calculated from differences between the peak areas obtained for spiked and standard solutions as shown from the data in Table 2. The accuracy of the method was evaluated by adding the standard solution of 40, 50, and 60 ppm (i.e., 0.8, 1.0, and 1.2 ml) to known sample solutions and was found to be 101.44%, 102.62%, and 100.54%, respectively. The average % RSD at three different levels spiked sample of costunolide was found to be 0.23%, 0.15%, and 0.11%, respectively. Therefore, this HPLC method can be regarded as selective, accurate, and precise.

LOD was found to be 2.29 ppm and LOQ was 6.64 ppm. Low LOD and LOQ of costunolide enable the detection and quantitation of this costunolide in *L. nobilis* at low concentrations.

Table 1: Results of precision (n=6)

Costunolide standard conc	Repeatability			Intermediate precision		
	Mean area	SD	% RSD	Mean area	SD	% RSD
40 ppm	859536	888	0.103	860302	1748	0.203
50 ppm	1065608	1275	0.120	1064565	786	0.074
60 ppm	1288769	1513	0.117	1286975	1624	0.126

Table 2: Results of accuracy (n=3)

Recovery study of Costunolide						
Amount added (ppm)	Mean area (b)	SD	% RSD	a	c	% recovery
40	1065325	2404	0.226	192362	860989	101.34%
50	1287534	1944	0.151	192362	1067169	102.62%
60	1489861	1685	0.113	192362	1290498	100.54%

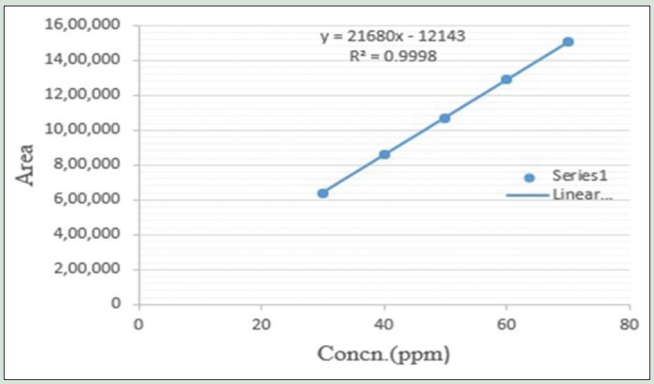


Figure 2: Linear curve of costunolide

The robustness of the method was done at three different concentrations of standards 40, 50, 60, and sample of 1000 ppm at six injections. The % RSD was found to be ≤1%, which shows ability to remain unaffected by small variation in the analytical parameters. This indicates that the proposed method was stable, precise, and reproducible.

CONCLUSION

The developed method is simple, accurate, precise, specific, selective, and robust. Therefore, the method was proved to be suitable for costunolide determination in *L. nobilis*. Further explorations are needed to investigate the standardization of individual phytoconstituents of *L. nobilis*.

Acknowledgment

We are grateful to the all members of Analytical Research and Development at Vidya Herbs Pvt. Ltd., Bengaluru, for their cooperation and technical help.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Prakasha HM, Krishnappa M, Krishnamurthy YL, Poornima SV. Folk medicine of NR Pura Taluk in Chikamaglur district of Karnataka. Indian J Trad Knowl 2010;9:55-60.
2. Majumder P, Paridhavi M. An ethno-phytochemical and pharmacological review on novel Indian medicinal plants used in herbal formulations. Int J Pharm Sci 2013;5:74-83.
3. Garg SN, Siddiqui MS, Agarwal SK. New fatty acid esters and hydroxyl ketones from fruits of *Laurus nobilis*. J Nat Prod 1992;55:315-9.
4. Bauer, K, Garbe D. Common Fragrance and Flavor Materials, Preparation, Properties and Uses. Weinheim, Germany: Vancouver Coastal Health; 1985.
5. Harborne JB, Baxter H, Moss GP, editors. Phytochemical Dictionary. London: Taylor and Francis; 1999. p. 2521-98.
6. Pech B, Bruneton J. Alkaloids of *Laurus nobilis*. J Nat Prod 1982;45:560-3.
7. Fiorini C, David B, Fouraste I, Vercavteren J. Acylated kaempferol glycosides from *Laurus nobilis* leaves. Phytochemistry 1998;47:821-4.
8. Novak M. A monoterpene alcohol from *Laurus nobilis*. Phytochemistry 1985;24:858.
9. Appendino G, Taglipietra S, Nano GM, Cisero M. A sesquiterpene alcohol from the fruits of *Laurus nobilis*. Phytochemistry 1992;31:2537-38.
10. Elmastas M, Gulcin I, Isildak O, Kufrevioglu OI, Ibaoglu K, Aboul-Enein HY. Radical scavenging activity and antioxidant capacity of bay leaf extracts. J Iran Chem Soc 2006;3:258-66.
11. Nayak S, Nalabothu P, Sandiford S, Bhogadi V, Adogwa A. Evaluation of wound healing activity of *Allamanda cathartica*. L. And *Laurus nobilis*. L. Extracts on rats. BMC Complement Altern Med 2006;6:12.
12. Ham A, Shin J, Oh K, Lee S, Nam K, Koo U, *et al*. Neuroprotective effect of the n-hexane extracts of *Laurus nobilis* L. In models of Parkinson's disease. Biomol Ther 2011;19:118-25.
13. Afifi FU, Khalil E, Tamimi SO, Disi A. Evaluation of the gastroprotective effect of *Laurus nobilis* seeds on ethanol induced gastric ulcer in rats. J Ethnopharmacol 1997;58:9-14.
14. Sayyah M, Valizadeh J, Kamalinejad M. Anticonvulsant activity of the leaf essential oil of *Laurus nobilis* against pentylenetetrazole- and maximal electroshock-induced seizures. Phytomedicine 2002;9:212-6.
15. Sayyah M, Saroukhani G, Peirovi A, Kamalinejad M. Analgesic and anti-inflammatory activity of the leaf essential oil of *Laurus nobilis* Linn. Phytother Res 2003;17:733-6.
16. Esra K, Ilkay O, Erdem Y. Evaluation of some plants used in Turkish folk medicine for their anti-inflammatory and antinociceptive activities. Pharm Biol 2007;45:547-55.
17. Samejima K, Kanazawa K, Ashida H, Danno G. Bay laurel contains antimutagenic kaempferol coumarate acting against the dietary carcinogen 3-amino-1-methyl-5H-pyrido [4, 3-b] indole. J Agric Food Chem 1998;46:4864-8.
18. Bilen S, Bulut M. Effect of laurel (*Laurus nobilis*) on the non-specific immune responses of rainbow trout (*Oncorhynchus mykiss*, Walbaum). J Anim Vet Adv 2010;9:1275-7.
19. Loizzo MR, Saab AM, Tundis R, Statti GA, Menichini F, Lampronti I, *et al*. Phytochemical analysis and *in vitro* antiviral activities of the essential oils of seven Lebanon Species. Chem Biodivers 2008;5:461-70.
20. Ozcan B, Esen M, Sangun MK, Coleri A, Caliskan M. Effective antibacterial and antioxidant properties of methanolic extract of *Laurus nobilis* seed oil. J Environ Biol 2010;31:637-41.
21. Corato DU, Trupo M, Leone GP, Sanzo DG, Zingarelli G, Adami M. Antifungal activity of the leaf extracts of laurel (*Laurus nobilis* L.), orange (*Citrus sinensis* Osbeck) and olive (*Olea europaea* L.) obtained by means of supercritical carbon dioxide technique. J Plant Pathol 2007;89:83-91.
22. International Conference on Harmonization (Q2B) Note for Guidance on Validation of Analytical Procedures: Methodology, Geneva, Switzerland: IFPMA, International Conference on Harmonization; 1996.
23. International Conference on Harmonization (Q2A) Validation of Analytical Procedures: Text and Methodology. Geneva, Switzerland: International Conference on Harmonization; 2005.