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Cost-Effective HPLC Method for Identification of Rosmarinic Acid in Methanol Extract of *Ocimum Basilicum*

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Abstract: Prescribing herbal remedies is now becoming common by many practitioners, who propose herbal treatments along with conventional medicine systems to treat various ailments considering their synergistic effects. Rosmarinic acid is a potent phenolic compound and caffeic acid derivative. It has many miraculous biological activities. Anti-allergic, anti-angiogenic, anti-depressant, anti-inflammatory, antitumor, anti-microbial activity, and antiviral effect of rosmarinic acid has already been documented in previous literature. It is widely present in many formulations because of its hepatoprotective and antioxidant effect. This study aimed to develop and validate a RP-HPLC method for the standardization of *O. basilicum* L. raw material and extracts by using rosmarinic acid as a marker. The study also aimed to quantify the rosmarinic acid content in the methanol extract of *O. basilicum* leaves. A simple and cost-effective High Pressure Liquid Chromatography (HPLC) method for determining rosmarinic acid in *Ocimum basilicum* plant extract has been developed and validated. Methanol-water-orthophosphoric acid in the ratio of 95:5:1 was used as the mobile phase using a C18 reversed-phase column for the HPLC method. Signals were detected at 254 nm. The rosmarinic acid content was found to be 4.2 mg/10 mg of the plant extract. The method is sensitive and reproducible and ideal for quick routine analysis and can be employed for detecting rosmarinic acid in other herbs. Despite the availability of literature on the medicinal properties of this plant and its chemical constituents, only a limited number of papers have been published on the determination of the rosmarinic acid in *Ocimum basilicum* plant using RP-HPLC. Moreover, this is the first report that quantifies rosmarinic acid in the plant extract by HPLC without using expensive columns and complex buffer systems that deteriorate the chromatographic columns and in turn, enhance the cost of the used process.

Keywords: rosmarinic acid, high pressure liquid chromatography, *Ocimum basilicum* extract, linearity, accuracy, precision.

罗勒甲醇提取物中迷迭香酸的高性价比高效液相色谱法鉴定

摘要： 处方草药疗法现在已成为许多从业者的普遍做法，考虑到它们的协同作用，他们建议将草药疗法与传统医学系统一起治疗各种疾病。迷迭香酸是一种有效的酚类化合物和咖啡酸衍生物。它具有许多神奇的生物活性，迷迭香酸的抗过敏,抗血管生成、抗抑郁、抗炎、

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抗肿瘤、抗微生物活性和抗病毒作用已在以前的文献中有所记载。它还显示出良好的抗氧化活性。已开发并验证了一种简单且经济高效的高压液相色谱高效液相色谱方法，用于测定罗勒植物提取物中迷迭香的含量。甲醇-水-正磷酸以 95:5:1 的比例作为流动相，采用 C18 反相柱进行高效液相色谱法。在 254 纳米处检测到信号。发现迷迭香的含量为 4.2 毫克/10 毫克的植物提取物。该方法灵敏且重现性好，是快速常规分析的理想选择，可用于检测其他草药中的迷迭香酸。

关键词：迷迭香酸，高压液相色谱，罗勒提取物，线性，准确度，精密度。

1. Introduction

Ocimum basilicum L (sweet basil) is widely spread over Asian and African countries. Genus *Ocimum basilicum* is the most abundantly found among the entire Ocimum genus. It is an annual plant also known as cooking herb (1). Rosmarinic acid (RA) is a caffeic acid derivative commonly found in many plants of the *Lamiaceae* family [2], the structure is given in Figure 1.

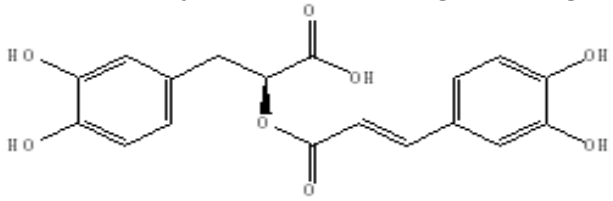

Fig. 1 Structure of rosmarinic acid

It is a potent antioxidant phenolic compound [3]. Anti-inflammatory and anti-viral effects of rosmarinic acid have also been reported [4]. It has also been reported in Ociumum basilicum plant, commonly known as sweet basil [5-7]. The plant is also rich in alkaloids, tannins, flavonoids and oligosaccharides [8-10]. *Ocimum basilicum* has been used for decades to treat gastrointestinal disorders, headache, anti-inflammatory neurodegenerative and cardiovascular disorders, and diabetes [11]. The cardioprotective properties can be concluded by modulation of various antioxidants parameters thus improving the overall antioxidant protection of myocardial tissue [12]. Many studies reported chemical constituents obtained from the plant, but only limited data is available on the determination of these constituents by HPLC [13-14]. This study aims to develop a simple and precise and validated method for the detection and quantification of rosmarinic acid in *Ocimum basilicum* extract.

2. Methods and Materials

Plants were collected from the Karachi University and authenticated by a taxonomist with voucher number GH NO 942382. The leaves were washed and dried under shade and was extracted with methanol for 48 hours. The extract was then filtered and evaporated using a rotary evaporator. All the chemicals used were of AR grade and were purchased from Merck. The reference standard compound of rosmarinic acid was purchased from Sigma-Aldrich.

2.1. Preparation of Reference Standard and Sample Solutions

The stock solution 100 µg/ml of Rosmarinic acid is prepared by reconstituting 10 mg of Rosmarinic acid in 100 ml of HPLC grade methanol. Similarly, 100 µg/ml of extract solution was prepared by using dried extract. The solutions were sonicated for 15 minutes using an ultrasonic sonicator. Dilutions were made from the rosmarinic acid stock solution in concentrations of 80 µg/ml, 60 µg/ml, 40 µg/ml, 20 µg/ml by diluting each of these solutions with the mobile phase followed by filtration using 0.45 micron membrane filter. These solutions were then injected in the HPLC column to be analyzed.

2.2. Chromatographic Conditions for HPLC

Shimadzu model High Pressure Liquid Chromatography-LC-20 AT, with UV-visible detector SPD-10A (V), Class LC-20 Version 1:62 was used in the study. C18 column with dimensions 150 mm x 4.6 mm, with 5 µm internal diameter at ambient temperature was used for the separation, pump is as in isocratic approach.

2.3. Selection of Wavelength and Mobile Phase

The methanolic *Ocimum basilicum* extract and rosmarinic solution (100 mg/ml) were scanned at a wide range of spectrum. The *Ocimum* extract and standard solutions measured with Uv detector showed a good result at 254 nm for the RP-HPLC.

Mobile phase was composed of 95:5:1 ratio of methanol-water-orthophosphoric acid. The pH was maintained at 3-4. Then, 20 µL of the sample was injected at a flow rate of 1ml/min. Wavelength used was 254 nm for detection.

3. Results and Discussion

In this study, the method development for Rosmarinic acid as marker was based on RP-HPLC and

RP-UHPLC. Different types of columns and mobile phase were employed to get better separation and a resolved peak. The column was selected on the basis of good resolutions of peaks. Wavelength was selected using UV spectra of standard and extract samples overlaid. The best response was found at 254 nm. Chromatograms are shown in Figures 2–3, which revealed good separation.

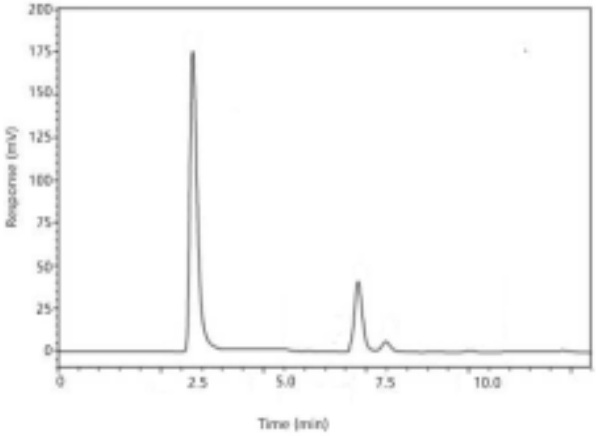

Fig. 2 RP-HPLC chromatogram of rosmarinic acid

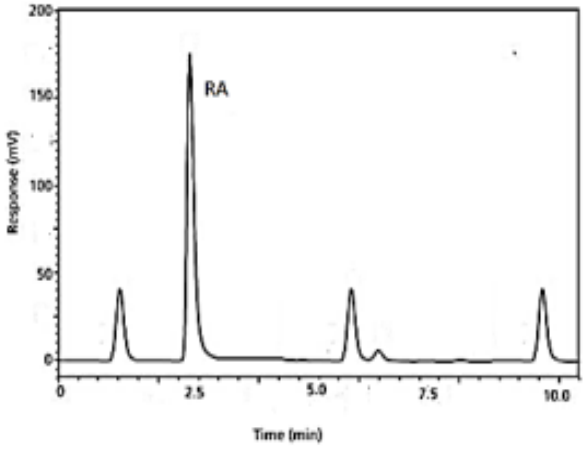

Fig. 3 RP-HPLC chromatogram of rosmarinic acid in *Ocimum basilicum* extract

The aim of this study is to establish an accurate, precise and reproducible method with sensitivity and cost effectiveness to quantify Rosmarinic acid in a plant extract.

3.1. Method Validation

The developed method was validated under the guidelines provided by ICH.

3.2. Linearity

Linearity of the developed method was assessed using the regression equation with r2 = 0.989 (Figure 4). Calibration curve was established by plotting between the peak height and concentration of test solution to obtain the regression equation.

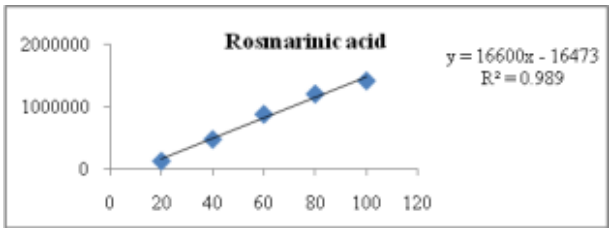

Fig. 4 Linearity of the prescribed method

3.3. Precision

A precise method should be repeatable and reproducible. In the current study the inter-day and intraday variations were evaluated on different concentrations in Table 1. The %RSD was within the limit and no significant variation was found among %Recovery at these concentrations that confirms the precision of the developed method.

Table 1 Interday and intraday precision

Methods	Conc. Injected	Inter-day		Intra-day	
	mg mL^{-1}	%RSD	%Recovery	%RSD	%Recovery
HPLC	20	0.12	99.93	0.08	99.9
	40	0.1	100.2	0.09	100.3
	60	0.23	100.06	0.07	100.1
	80	0.52	100.17	0.062	100.2
	100	0.26	100.02	0.059	100.5

3.4. Accuracy

Three consecutive concentrations 100±20% was prepared; %RSD and Recovery was evaluated. %RSD was found in the range of 0.00021 to 0.00029 while percentage Recovery was obtained between 99 and 101 that indicate the method accuracy as shown in Table 2.

Table 2 Accuracy of the prescribed method

Methods	Conc. µgmL^{-1}	%RSD	% Recovery
HPLC	80%	0.00026	99.99
	100%	0.00029	99.99
	120%	0.00021	100.00

3.5. Limit of Detection and Limit of Quantification

Dilutions of the rosmarinic acid solution were prepared and the limits of detection and quantification were calculated (Table 3).

Table 3 Limit of detection and limit of quantification

HPLC	LOD =	3.3*SD/Slope	0.00073 µg/ml
	LOQ =	10*SD/Slope	0.0022 µg/ml

3.6. Specificity and Selectivity

Obvious and sharp peaks were observed that indicated the specificity and selectivity of the prescribed method.

3.7. Quantification of Rosmarinic Acid in *Ocimum Basilicum* Plant Extracts

Thus, 100 µg/ml of *Ocimum basilicum* plant extract was prepared and analyzed under the same conditions

as used for rosmarinic acid detection. The RA peak was observed around the same retention time as it was observed in the standard RA determination. RA content was determined using the linearity data obtained for RA determination. The RA content was found to be 4.2 mg/10 mg of *Ocimum basilicum* extract.

The proposed method was developed and validated for the analysis of rosmarinic acid. This can be used for the analysis of RA in other herbal extracts. A literature survey reported many other methods for their determination with the limitation of complex instrumentation and usage of expensive columns and solvents. The proposed method is the simplest method for the analysis using a simple C18 column and cost-effective solvents. The method was validated according to the ICH guidelines. The method has high selectivity; it can detect RA without interference with other plant constituents. The method was précised and has a high level of accuracy.

4. Conclusion

Rosmarinic acid has many beneficial effects on the human system. It has a remarkable antioxidant activity; and is widely used in the food and cosmetic industries. This therapeutic compound is widely spread in the nature.

Industries focus on optimum methods to obtain a good yield of this compound within a short time and minimal use of expensive equipment and solvents. To identify and quantify rosmarinic acid is the crucial step. Many reported methods use expensive and high purity solvents, long detection times that increase the cost of the implied method when implemented on an industrial scale.

For this reason, this study reports a simple method, that is sensitive and less time consuming. Method was validated according to the ICH guidelines. Limits of detection and quantification are sufficient to analyze rosmarinic acid in *Ocimum basilicum*. This method is ideal for the quick routine analysis.

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References

[1] BILAL A, JAHAN N, AHMED A, et al. Phytochemical and pharmacological studies on *Ocimum basilicum* Linn-A review. *International Journal of Current Research and Review*, 2012, 23: 73-83. https://doi.org/10.1016/s1001-7844(13)60020-6

[2] PETERSEN M, & SIMMONDS MS. Rosmarinic acid. *Phytochemistry*, 2003, 62: 121-125. https://doi.org/10.1016/s0031-9422(02)00513-7.

[3] ERKAN N, AYRANCI G, AYRANCI E. Antioxidant activities of rosemary (*Rosmarinus Officinalis L.*) extract, blackseed (Nigella sativa L.) essential oil, carnosic acid, rosmarinic acid and sesame oil. *Food Chemistry*, 2008, 110: 76-82. https://doi.org/10.1016/j.foodchem.2008.01.058

[4] SWARUP V, GHOSH J, GHOSH S, SAXENA A, BASU A. Antiviral and anti-inflammatory effects of rosmarinic acid in an experimental murine model of Japanese encephalitis. *Antimicrobial Agents and Chemotherapy*, 2007, 51: 3367-3370. https://doi.org/10.1128/aac.00041-07

[5] BAIS HP, WALKER TS, SCHWEIZER HP, VIVANCO JM. Root-specific elicitation and antimicrobial activity of rosmarinic acid in hairy root cultures of *Ocimum basilicum* Plant. *Physiology and Biochemistry*, 2002, 40: 983-995. https://doi.org/10.1016/s0981-9428(02)01460-2

[6] TADA H, MURAKAMI Y, OMOTO T, SHIMOMURA K, ISHIMARU K. Rosmarinic acid and related phenolics in hairy root cultures of *Ocimum basilicum. Phytochemistry*, 1996, 42: 431-434. https://doi.org/10.1016/0031-9422(96)00005-2

[7] KINTZIOS S, MAKRI O, PANAGIOTOPOULOS E, SCAPETI M. In vitro rosmarinic acid accumulation in sweet basil (*Ocimum basilicum L.*). *Biotechnology Letters*, 2003, 25: 405-408.

[8] GOVINDARAJAN M, SIVAKUMAR R, RAJESWARY M, YOGALAKSHMI K. Chemical composition and larvicidal activity of essential oil from *Ocimum basilicum* (L.) against *Culextritaeniorhynchus, Aedesalbopictus* and *Anopheles subpictus* (Diptera: Culicidae). *Experimental Parasitology*, 2013, 134: 7-11. https://doi.org/10.1016/j.exppara.2013.01.018

[9] HUSSAIN AI, ANWAR F, SHERAZIST, PRZYBYLSKI R. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry*, 2008, 108: 986-995. https://doi.org/10.1016/j.foodchem.2007.12.010

[10] CHIANG LC, NG LT, CHENG PW, CHIANG W, LIN CC. Antiviral activities of extracts and selected pure constituents of *Ocimum basilicum*. *Clinical and Experimental Pharmacology and Physiology*, 2005, 32: 811-816. https://doi.org/10.1111/j.1440-1681.2005.04270.x

[11] MIRAJ S, & KIANI S. Study of pharmacological effect of *Ocimum basilicum*: A review. *Der Pharmacia Lettre*, 2016, 8: 276-280.

[12] SHARMA M, KISHORE K, GUPTA SK, JOSHI S, ARYA DS. Cardioprotective potential of *Ocimum sanctum* in isoproterenol induced myocardial infarction in rats. *Molecular and Cellular Biochemistry*, 2001, 225: 75-83.

[13] WANG H, PROVAN GJ, & HELLIWELL K. Determination of rosmarinic acid and caffeic acid in aromatic herbs by HPLC. *Food Chemistry*, 2004, 87: 307-311. https://doi.org/10.1016/j.foodchem.2003.12.029

[14] ACHAMLALE S, REZZONICO B, & GRIGNON-DUBOIS M. Rosmarinic acid from beach waste: Isolation and HPLC quantification in Zostera detritus from Arcachon lagoon. *Food Chemistry*, 2009, 113: 878-883. https://doi.org/10.1016/j.foodchem.2008.07.040

参考文:

[1] BILAL A、JAHAN N、AHMED A 等。罗勒的植物化学和药理研究-一个综述。国际当前研究与评论杂志，2012，23：73-83。https://doi.org/10.1016/s1001-7844(13)60020-6

[2] PETERSEN M, 和 SIMMONDS MS.。迷迭香酸。植物化学，2003，62：121-125。https://doi.org/10.1016/s0031-

9422(02)00513-7。
[3] ERKAN N, AYRANCI G, AYRANCI E. 迷迭香(迷迭香.)提取物、黑籽(黑种草.)精油、鼠尾草酸、迷迭香酸和芝麻油的抗氧化活性。食品化学，2008，110：76-82。https://doi.org/10.1016/j.foodchem.2008.01.058
[4] SWARUP V, GHOSH J, GHOSH S, SAXENA A, BASU A. 迷迭香酸在日本脑炎实验鼠模型中的抗病毒和抗炎作用。抗菌剂和化疗，2007，51：3367-3370。https://doi.org/10.1128/aac.00041-07
[5] BAIS HP、WALKER TS、SCHWEIZER HP、VIVANCO JM。迷迭香酸在罗勒植物毛状根培养物中的根特异性诱导和抗菌活性。生理学与生物化学, 2002, 40：983-995。https://doi.org/10.1016/s0981-9428(02)01460-2
[6] TADA H, MURAKAMI Y, OMOTO T, SHIMOMURA K, ISHIMARU K. 罗勒毛状根培养物中的迷迭香酸和相关酚类物质。植物化学，1996，42：431-434。https://doi.org/10.1016/0031-9422(96)00005-2
[7] KINTZIOS S, MAKRI O, PANAGIOTOPOULOS E, SCAPETI M. 甜罗勒(罗勒.)中的体外迷迭香酸积累。生物技术快报, 2003, 25：405-408。
[8] GOVINDARAJAN M, SIVAKUMAR R, RAJESWARY M, YOGALAKSHMI K. 罗勒(大号.)精油对 三带喙库蚊、伊蚊 和按蚊(双翅目：鲶科)的化学成分和杀幼虫活性。实验寄生虫学，2013，134：7-11。https://doi.org/10.1016/j.exppara.2013.01.018
[9] HUSSAIN AI, ANWAR F, SHEAZIST, PRZYBYLSKI R. 罗勒(罗勒)精油的化学成分、抗氧化和抗菌活性取决于季节变化。食品化学，2008，108：986-995。https://doi.org/10.1016/j.foodchem.2007.12.010
[10] CHIANG LC, NG LT, CHENG PW, CHIANG W, LIN CC. 罗勒提取物和精选纯成分的抗病毒活性。临床与实验药理学与生理学，2005，32：811-816。https://doi.org/10.1111/j.1440-1681.2005.04270.x
[11] MIRAJ S, 和 KIANI S. 罗勒药理作用研究：综述。药书, 2016, 8：276-280。
[12] SHARMA M, KISHORE K, GUPTA SK, JOSHI S, ARYA DS。罗勒在异丙肾上腺素诱导的大鼠心肌梗死中的心脏保护作用。分子和细胞生物化学, 2001, 225：75-83。
[13] WANG H, PROVAN GJ, & HELLIWELL K. 高效液相色谱法测定香草中的迷迭香酸和咖啡酸。食品化学，2004，87：307-311。https://doi.org/10.1016/j.foodchem.2003.12.029
[14] ACHAMLALE S、REZZONICO B 和 GRIGNON-DUBOIS M. 来自海滩废物的迷迭香酸：来自阿卡雄泻湖的带状疱疹的分离和高效液相色谱定量。食品化学，2009，113：878-883。https://doi.org/10.1016/j.foodchem.2008.07.040