Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2023, 15(3): 1-4
(http://www.derpharmachemica.com/archive.html)

UV Spectrophotometric method development and Validation for the Quantitative measurement of Rosmarinic Acid

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Received: 04-Mar-2023, Manuscript no: dpc-23-92246, **Editor assigned**: 06-Mar-2023, PreQC No: dpc-23-92246, **Reviewed**: 20-Mar-2023, QC No: dpc-23-92246, **Revised**: 22-Mar-2023, Manuscript No: dpc-23-92246, **Published**: 29-Mar-2023, **DOI**: 10.4172/0975-413X.15.3.1-4

ABSTRACT

The main aim of the current research was development and validation of simple, accurate UV spectrophometric method for measurement of Rosmarinic acid (RA). Method validation parameters including linearity, precision, specificity, accuracy, and limit of detection (LOD), limit of quantification (LOQ) were established. 327 nm was found to be the maximum wavelength of RA in solution. Calibration curve illustrated a linear correlation between the absorbance and concentration in the range of 1-14 µg/ml with a correlation coefficient (r^2) of 0.9987. ICH guideline was studied for the linearity, accuracy, precision, LOD and LOQ. The method may be efficient for the determination of RA in liposomes and requires inexpensive instrument.

Keywords: Rosmarinic acid; Method validation; UV spectrophotometry; ICH guidelines

INTRODUCTION

RA is composed of 3,4-dihydroxyphenyllactic acid and ester of caffeic acid (Figure 1)and derived from *Lamiaceae* family [1]. Several biological effects were established for RA, such as anti-inflammatory, antioxidant, anticataract and neuroprotective. The antioxidant activity of RA depends on polyphenolic groups that are free radical scavengers. Kuo and colleagues investigate the antioxidant and anti-apoptotic activity of RA by decreasing the level of ROS and malondial-dehydrate (MDA) as well. They illustrated that the potential glioprotective effect of RA on H_2O_2 induced oxidative stress in astrocytes [2]. The beneficial effect of RA on Alzheimer's disease was investigated by Baluchnejadmojarad et al., suggesting that RA decreased the accumulation of Aβ deposits leading to malondialdehyde and nitrite production [3]. RA is considered highly lipophilic and slightly soluble in water. This molecule is shown good solubility in most organic solvents. The powder of RA is in the color red-orange. The molecular weight of RA is 360.32 g/mol, and the melting point is 171-175°C [4].

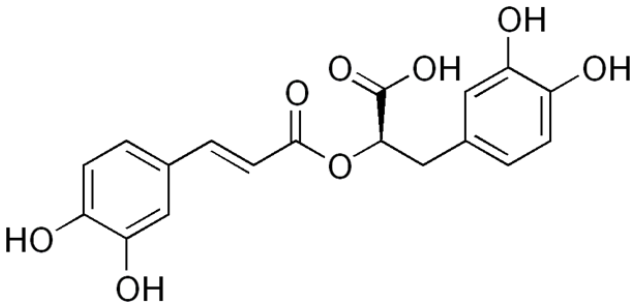

Figure 1: Chemical structure of Rosmarinic acid

In this work, we find out a simple, accurate, sensitive, and validated spectrophotometric method for determination of RA that was found in many natural products.

Materials and methods

Equipment

UV-visible spectrophotometer (UV-vis), model SPECORD S600 by Analytic Jena.

Materials

RA was purchased from Sigma-Aldrich Company. Sodium deoxycholate and soy phosphatidylcholine was used in this study were obtained from Sigma-Aldrich company. All other chemical were in pharmaceutical grade.

Method validation of RA

The method was validated in terms of linearity, accuracy, and precision according to ICH Q2 Analytical Validation [5].

Selection of wavelength for analysis of RA

An appropriate volume of standard stock solution of RA (0.5 ml) was transferred into a 10 ml volumetric flask, diluted to a mark with ethanol: distilled water (1:9) to give a 5 µg/ml concentration. The resulting solution was scanned in the UV range (250–400 nm). In spectrum, RA showed absorbance maximum at 327 nm.

Preparation of standard stock solution

Accurately weighed 5 mg of RA was transferred to a 100 ml volumetric flask and dissolved in 10 ml methanol by shaking manually for 2 min. The volume was adjusted with the distilled water up to the mark to give the final strength, 50 µg/ml. the method described by Yücel et al. with some modifications [6].

Preparation of working standard solutions

Different working solutions were prepared from the stock solution in the range of 1-14 µg/ml. The calibration cure plot is illustrated as absorbance vs. concentration.

Linearity study

The calibration curve was obtained at seven concentration levels of RA solutions (1-14 µg/ mL). The linearity was evaluated with triplicate determinations at each concentration level by the least square regression method. The spectrum was recorded at 327 nm. The calibration plot was constructed as concentration *vs*. absorbance. Figure 2 is depicted the absorbance vs. concentration of RA at 327 nm.

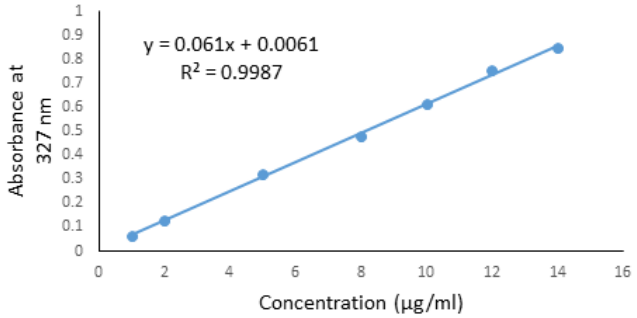

Figure 2: Calibration curve of RA at 327 nm

Accuracy

To the preanalyssed sample solutions, a known amount of standard stock solution was added at different levels, i.e., 80%, 100%, and 120%. The solutions were analyzed by the proposed method.

Precision

The precision of the method was studied as intraday and interday variations. Intraday precision was determined by analyzing the 8, 10, and 12 µg/ml of RA solutions three times in the same day. Interday precision was determined by analyzing the 8, 10, and 12 µg/ml of RA solutions daily for 3 days over the period of a week.

Sensitivity

The sensitivity of measurements of RA by the use of the proposed method was estimated in terms of the limit of detection (LOD) and limit of Quantification (LOQ). The LOD and LOQ were calculated using equation LOD = 3.3 × σ/S and LOQ = 10 × σ/S, respectively where σ is the standard deviation of the response (n = 3), and S is the slope of the corresponding calibration curve.

Repeatability

Repeatability was determined by analyzing 10 µg/ml concentration of RA solution for six times.

RESULTS

Linearity study

The linear regression data for the calibration curves showed a good linear relationship over the concentration range 1-14 µg/ml for RA. Linear regression equation was found to be $Y = 0.061\ X + 0.0061$ ($r^2 = 0.9987$). The result is expressed in table 1.

Table 1: Linearity studies

Concentration (µg/ml)	Absorbance in 327 nm Mean ± SD (n=3)	% RSD
1	0.063 ± 0.001	1.59
2	0.128 ± 0.002	1.96
5	0.322 ± 0.003	1.17
8	0.483 ± 0.003	0.63
10	0.612 ± 0.004	0.68
12	0.765 ± 0.003	0.40
14	0.85 ± 0.004	0.53

Accuracy

The solutions were reanalyzed by the proposed method; results of recovery studies are reported in Table 2, which showed that the % amount found was between 97.70 and 102.38 with % RSD < 2.

Table 2: Accuracy studies

Pre-analyzed sample solution (µg/ml)	Amount of drug added (µg/ml) (n=3)	Amount recovered (µg/ml) (n=3)	% Recovery	% RSD
10	8	7.81 ± 0.07	97.70 ± 0.93	0.96
	10	9.93 ± 0.05	99.3 ± 0.52	0.53
	12	12.28 ± 0.06	102.38 ± 0.50	0.49

Precision

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These results show the reproducibility of the assay. The % RSD values found to be less than 2 indicate this method precise for the determination of RA in the formulation, as illustrated in table 3.

Table 3: Precision studies

Component	Concentration (µg/ml)	Intraday precision (n=3)		Interday precision (n=3)	
		Conc. Found	%RSD	Conc. Found	%RSD
Rosmarinic acid	10	9.82 ± 0.17	1.78	9.93 ± 0.07	0.79
	12	11.70 ± 0.05	1.3	11.82 ± 0.05	0.45
	14	13.80 ± 0.15	1.1	13.93 ± 0.09	0.71

Sensitivity

The linearity equation was found to be Y = 0.061X + 0.0061. The LOD and LOQ for RA were found to be 0.36 µg/ml and 1.11 µg/ml, respectively.

Repeatability

Repeatability was determined by analyzing 10 µg/ml concentration of RA solution for six times, and the % amount found was between 96% and 101% with % RSD < 2. The result of repeatability studies is depicted in table 4.

Table 4: Repeatability studies

Component	Amount taken (µg/ml) (n=6)	Amount recovered (µg/ml) (n=6)	Amount found (%)	% RSD
Rosmarinic acid	10	9.78 ± 0.19	97.83 ± 1.94	1.98

CONCLUSION

The UV-spectrophotometry for determination of RA in natural products was developed and validated. The proposed method displayed to be linear, precise, repeatable, and accurate. Finally, it can't be used for quantification of RA loaded into liposomes.

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