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Original Article

Development of a UV Spectroscopy Method to Validate p-Coumaric Acid and Assess the Antioxidant Activity of a p-Coumaric Acid Gel Product

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ABSTRACT

Ultraviolet spectroscopy was one of the simple, rapid, cost-effective, and precise instrumental methods. The objective was the development of the U.V spectroscopy method for the validation of p-coumaric acid according to the International Council for Harmonization [Q2 (R1)] guidelines reported in this work. Antioxidant activity was performed by the DPPH (2,2-diphenyl-1-picrylhydrazyl). The U.V method still not developed for the above drug was the research gap so validation has been studied using ethanol and phosphate buffer 6.8pH. Method A entailed the preparation of the sample and standard solutions in ethanol, whereas method B entailed the preparation of the solution in a mixture of phosphate buffer pH 6.8. Two methods were employed to select unique wavelengths for drug analysis. Method A selected 229 nm, whereas method B selected 284 nm. The drug's linearity of method A and method B was found in the concentration range of 2–10 µg/mL (R^2=0.98) and 5–25 µg/mL (R^2= 0.99), subsequently the accuracy of both methods by computing recovery percentages at 80, 100, and 120 percent. It was found that the recovery percentage using both approaches was between 80 and 90 percent. The lower percentage relative standard deviation (RSD) readings showed, how accurate were both approaches. The precision of the procedure was intended to be repeatable both within and between days. The fact that the % RSD value is less than 2 suggests that both approaches are accurate. With the use of ± wavelength, the robustness of both techniques was investigated. The active ingredient p-coumaric acid was included in the aqueous gel of HPMC K100M (Hydroxy methyl cellulose) and Carbopol 971p polymer and performed antioxidant activity using DDPH free radical scavenger test (IC50). The result was found to be 0.833 mg/mL. *Conclusion*: p-coumaric acid in pharmacologic dosage form was successfully analyzed by the U.V method and proven for significantly good anticipated antioxidant activity.

KEYWORDS: Validation, p-coumaric acid, International Council for Harmonization (ICH), topical gel, spectroscopy, Antioxidant.

1. Introduction

p-coumaric acid is an organic compound that is a hydroxy derivative of cinnamic acid, i.e., 4- 4-hydroxycinnamic acid and a phenolic acid having therapeutic effects against various diseases.[1] p-coumaric acid is a (Biopharmaceutical classification system) BCS class II active drug a small amount is soluble in ethanol, (Dimethyl sulfoxide) DMSO and (Dimethyl formamide) DMF. P-coumaric acid is available in two forms *trans*-p-coumaric acid and *cis*-coumaric acid.[2-6] It is obtained from natural and synthetic derivatives of p-coumaric acid and extraction methods, i.e., classical methods like solvent extraction, acidification, alkaline extraction, etc., and newer approaches techniques like clip-off method, sugaring-out method, soft microwave extraction, etc., have been reported in the literature for its qualitative and quantitative estimation in different plant materials. [7]

Numerous researchers have described the multi-faceted medicinal properties of p-coumaric acid, primarily it possesses antioxidant activity that is responsible for neutralizing free radicals in the body. Due to this, it is a good candidate for safeguarding cells against oxidative stress and inflammation. The skin benefits of p-coumaric acid are especially remarkable, as it can be capable of guarding against (Ultraviolet) UV-induced damage and

maintaining healthy skin overall. It was also endowed with cardioprotective, anti-melanogenic, antimutagenic, antiplatelet and immunomodulatory activities.[8-11] p-coumaric acid was devoid of appreciable cytotoxicity at the range of effective concentration.[13] Researchers have cited that analytical methods for the quantification of p-coumaric acid via the utilization of Infrared Spectroscopy (IR), High-performance liquid Chromatography (HPLC), and titrimetric methods usually take a considerable amount of time and effort. This work emphasized the establishment of a less complicated UV spectroscopy technique for the confirmation of p-coumaric acid. However, the p-coumaric inclusion into aqueous gel composed of HPMC K4M and carbopol 971p gel and assessed its antioxidant potential for dermal use. The principle behind DPPH is the donation of a single electron (Dini & Laneri, 2021; Pyrzyńska & Pękal, 2013). Spectrophotometric estimation of the disappearance or disappearance rates of DPPH due to reaction with antioxidants provides the quantitative basis for this assay. The DPPH radical, whose unique purple color and high absorbance at 517 nm have made it widely used, reduces upon reaction with an antioxidant molecule to produce a drop in absorbance that was measurable (Gülçın & Alwasel, 2023; Shargi et al., 2020)

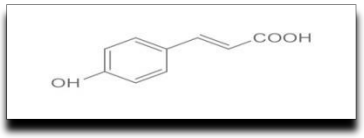

Fig.1. Structure of p-coumaric acid [(E)-3-(4 hydroxyphenyl)-2-propionic acid]

The literature review reveals comparatively few simple UV spectrophotometric methods for the determination of p-coumaric acid in pharmaceutical dosage form. To identify the light absorption by chemical compound solutions, a UV spectrophotometric method must be developed.

2. Materials and Methods

A pure drug powder sample of p-coumaric acid procured from Yucca Enterprises A-246, Antop Hill ware Housing Co, Wadala (E), Mumbai, disodium hydrogen phosphate and potassium dihydrogen phosphate (6.8 pH Buffer chemicals) procured from Loba Chemie Pvt. Ltd.(Mumbai, India), HPMC K100M was procured from Analab Fine Chemicals (Mumbai), carbopol 971p procured from Loba Chemie Pvt. Ltd.(Mumbai, India) ethanol solvent from S.D. Fine Chemicals Ltd. Mumbai. UV spectrophotometer Jasco V730 for UV validation.

3. Methods A

4.1.a.Preparation of standard stock solution (ethanol)

10 mg of p-coumaric acid drug was weighed and transferred into 10mL ethanol then obtained stock solution having a concentration of 1000µg/mL. Working solutions were prepared by suitable dilution with ethanol. 1mL of the stock solution was diluted up to 10mL ethanol to prepare a concentration of 100µg/ml solution, then 1mL of this solution was diluted up to 10mL to prepare a 10µg/mL concentration solution, further dilutions were obtained by the above method.

4.1.b.Selection of wavelength(ethanol)

2mL of p-coumaric acid of 100µg/mL solution was taken and diluted up to 10mL by ethanol obtained from 20µg/mL solution, It was scanned under a UV range of 200-400nm, with maximum absorbance observed at 300nm.

4. Method B

4.2.a. Preparation of standard stock solution (buffer)

10mg of p-coumaric acid drug was weighed and dissolved in 10ml of prepared phosphate buffer (6.8 pH) 1mg/mL stock solution was obtained. Further dilution with buffer till 10mL gets working solutions such as 100µg/mL, 10µg/mL, etc.

4.2.b. Selection of wavelength(buffer)

2ml of p-coumaric acid of 100µg solution was taken and diluted up to 10mL by 6.8 pH buffer solution to prepare 20µg/mL solution. It is scanned under the ultraviolet range 200-400nm. Maximum absorbance observed at 284nm.[12]

4.3.1**Analytical methods of validation**

The UV method for quantifying p-coumaric acid was validated based on the guideline. Different variables like linearity, accuracy, precision, robustness, the limit of detection (LOD), and the limit of quantification (LOQ) were evaluated.[14-17]

4.3.1.1. Linearity

The linearity of an analytical method produces test results that are directly proportional to the concentration of analyte within the sample. For the formation of linearity make five sets of each concentration and take the absorbance of each concentration. By using the obtained data plot the linearity calibration graph.

4.3.1.2. Accuracy

Accuracy was confirmed for methods A and methods B by percentage recovery of the best-known concentration of p-coumaric acid. The method was replicated three times for each concentration.

Method A

The accuracy of this method was determined by preparing solutions in ethanol, different concentrations of 80%, 100%, and 120% (because when we determine accuracy will take 20 lower and above concentrations) in which the amount of drug was kept constant that is 10ug and the amount of pure drug was varied that is 8ug, 10ug, 12ug for 80%, 100%, 120% respectively. The solutions were prepared in triplicate in ethanol and absorbance was recorded.

Method B

The accuracy of this method was determined by preparing (6.8 pH phosphate buffer), in different concentrations of 80%,100%, and 120% in which the amount of drug was kept constant that is 10ug and the amount of pure drug was varied that is 8ug, 10ug, 12ug for 80%, 100%, 120% respectively. The solutions were prepared in triplicate in a buffer and absorbance was recorded. Accuracy was indicated by % recovery.[12]

4.3.1.3.Precision

Precision-measured closeness among the information obtained in entire experiments under the same conditions.

Method A

Intraday precision

Six solutions of ethanol of the same concentration were prepared and analyzed thrice a day that was morning,

afternoon and evening. The result was indicated by % RSD.

Interday precision

Six solutions of ethanol of the same concentration were prepared and analyzed thrice for three consecutive days and absorbance was recorded. The result obtained in % RSD.

Method B

Intraday precision

Six solutions of phosphate buffer (6.8 pH) of the same concentration were prepared and analyzed thrice a day that is morning, afternoon and evening. The result was indicated by %RSD.

Interday precision

Six solutions of 6.8 pH phosphate Buffer of the same concentration were prepared and analyzed thrice for three consecutive days and absorbance was recorded. The result obtained in %RSD.

4.3.1.4 Robustness

The robustness of an analytical method is a measurement of its capacity to remain not affected by a small variation in method parameters. Variation of wavelength evaluates robustness.

4.3.1.5 Limit of detection (LOD)

The detection limit of an individual analytical procedure was the lowest amount of analyte in a sample that can be detected not quantified. LOD was detected by the following formula:

$$LOD = 3.3 (SD/S)$$

Where, SD = the standard deviation of response, S = the slope of the calibration curve.

4.3.1.6 **Limit of quantification (LOQ)**

The quantification limit of an individual analytical procedure was the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

$$LOQ = 10 (SD/S)$$

Where, SD = standard deviation of response, S = slope of calibration curve.

4.4. Preparation of gel:

The gel preparation was prepared using a cold mechanical process according to the composition presented in Table 1. 1 g of p-coumaric acid was dissolved using 15 ml of glycerin with the assistance of mild heat. Weighted amounts of each 1g HPMC K100M and Carbopol 971P were dispersed in 75 ml of distilled water under constant stirring using a magnetic stirrer so that no lump in the dispersion was present. 10 % sodium hydroxide solution dropwise was added to it to achieve gel formation. At room temperature, lastly, the required amount of sodium benzoate was added and the formulation was checked. The formulated product carried out antioxidant activity.

Table 1. Composition of p-coumaric acid gel

Sr no	Ingredients	Quantity	Cateogory
1	p-coumaric acid	1g	Active ingredient
2	HPMC K100M	1g	Gelling polymer
3	carbopol 971p	1g	Gelling polymer
4	glycerin	15mL	Humectant
5	sodium benzoate	0.1g	Preservative
6	10 % NaOH	Quantity sufficient	pH Adjuster
7	Distilled Water	100mL	Vehicle

4.5.**Antioxidant activity**

Antioxidant activity tests were conducted by DPPH radical scavenging assay. 10mg of formulation dissolved in 10 mL of methanol. From the stock solution taken 1000µL diluted solutions (100uL, 200uL, 300uL, 400uL, 500uL, 600uL) respectively add 3ml methanol in every test tube similarly, taken readings of one blank solution of methanol, 5 Stock solutions (test tube no. 1, 2, 3, 4, 5) and 1 Control (test tube no. 6). The mixture then mixed vigorously using vortex for 1 minute. The mixture solution was incubated for 30 minutes at room temperature. The absorbance was determined at 517 nm. Percent inhibition expressed in terms of inhibitory concentration 50(IC50) based on a regression equation by (Boulanouar et al.,2017)[18-20]

% Radical scavenging activity or % Inhibition =
(1 - Absorbance of sample/Absorbance of control) x100%

5. Results

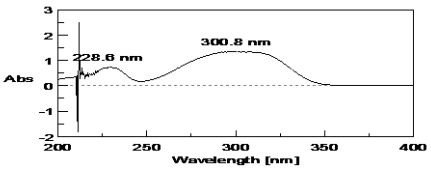

Fig.2.Absorbance spectra of p-coumaric acid in ethanol.

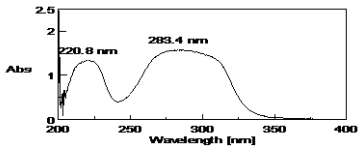

Fig.3.Absorbance spectra of p-coumaric acid in 6.8 pH Buffer

The validation process of a method comes up whenever a novel protocol is proposed or when an amendment has been made to an already approved protocol. A method is validated if and only if it is suitable for its required purpose and yields sound output, which can be properly interpreted by the user. Results have been presented separately in the section for method A, and method B.

5.1 **For method A**

Table 2. Linearity of p-coumaric acid in ethanol

Sr.no.	Concentration (µg/mL)	Absorbance
1.	5	0.151
2.	10	0.479
3.	15	0.600
4.	20	0.928
5.	25	1.216

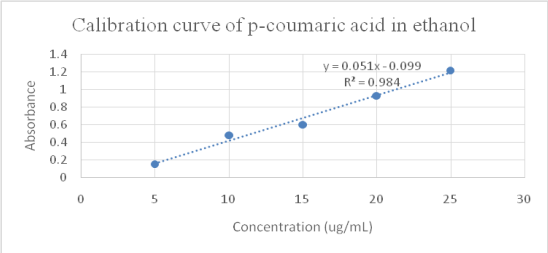

Fig.4.Calibration curve of p-coumaric acid in ethnol

5.1 For Method B

Table 3. Linearity of p-coumaric acid in pH 6,8 buffer

Sr.no.	Concentration (µg/mL)	Absorbance
1.	2	0.364
2.	4	0.465
3.	6	0.726
4.	8	0.888
5.	19	1.223

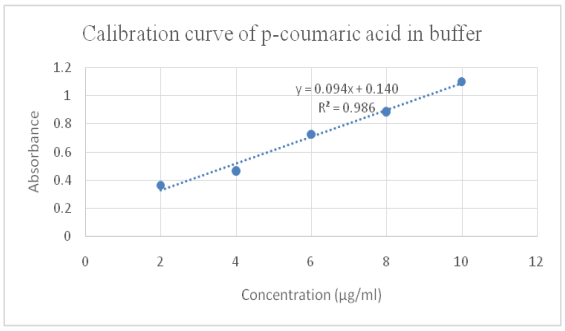

Fig.5.Calibration curve of p-coumaric acid in ethnol

5.2. Accuracy

Table.4.Accuracy determination of p-coumaric acid (method A)

Sr.no.	% recovery level	Mean of absorbance	Standard deviation	% Relative standard deviation
1.	80%	0.458	0.012	0.026
2.	100%	0.668	0.076	0.113
3.	120%	0.022	0.024	1.082

Table.5.Accuracy determination of p-coumaric acid (method B)

Sr.no.	% recovery level	Mean of Absorbance	Standard deviation	% Relative standard deviation
1.	80%	0.872	0.026	0.0229
2.	100%	1.086	0.1011	0.0925
3.	120%	1.425	0.077	0.0492

5.3. Precision

Table 6.Intraday precision study of p-coumaric acid (method A)

Time/ Duration	Conc. (µg/mL)	Mean of absorbance	Standard deviation	% Relative standard deviation (%RSD)
Morning	10	0.788	0.0627	0.079
Afternoon	10	0.869	0.075	0.086
Evening	10	0.817	0.118	0.145

Table.7.Interday precision study of p-coumaric acid (method A)

Time/ Duration	Conc. (µg/mL)	Mean absorbance	Standard deviation	% Relative standard deviation
Morning	10	0.788	0.063	0.074
Afternoon	10	0.851	0.076	0.088
Evening	10	1.433	0.082	0.057

Table.8.Intraday precision study of p-coumaric acid (method B)

Day	Conc. (µg/mL)	Mean absorbance	Standard deviation	% Relative standard deviation
First	10	1.230	0.084	0.068
Second	10	1.141	0.042	0.037
Third	10	1.141	0.047	0.042

Table.9.Interday precision study of p-coumaric acid (method B)

Day	Conc. (µg/mL)	Mean absorbance	Standard deviation	% Relative standard deviation
First	10	1.230	0.085	0.069
Second	10	1.091	0.132	0.121
Third	10	1.172	0.233	0.199

5.4.Robustness

Table.10.Evaluation data of robustness study for p-coumaric acid using 300nm ± 2nm (method A)

Sr.No.	Wavelength 302nm	Wavelength 298nm
1.	0.090	0.248
2.	0.128	0.396
3.	0.583	0.538
4.	0.633	0.840
5.	0.111	0.928
Mean	0.309	0.590
SD	0.245	0.258
%RSD	0.800	0.431

Table.11.Evaluation data of robustness study for p-coumaric acid using 284nm ± 2nm

Sr.No.	Wavelength 282nm	Wavelength 286nm
1.	0.120	0.117
2.	0.341	0.340
3.	0.427	0.425

4.	0.799	0.801
5.	0.801	0.800
Mean	0.498	0.497
SD	0.266	0.268
%RSD	0.543	0.531

5.5. Limit of detection and Limit of quantification

Table No.12.Evaluation data of LOD and LOQ for p-coumaric acid (method A)

Sr.No.	Drug	Limit of detection (µg/mL)	Limit of quantification (µg/mL)
1.	p-coumaric acid	1.792	5.420

Table No.13.Evaluation data of LOD and LOQ for p-coumaric acid (method B)

Sr.No.	Drug	Limit of detection (µg/mL)	Limit of quantification (µg/mL)
1.	p-coumaric acid	5.449	16.512

5.6. Table No.14 Antioxidant activity using the DPPH method

Formulation	Activity	IC_{50} (mg/mL)
p-coumaric acid prepared gel	DPPH antioxidant free radical scavenging test	0.833

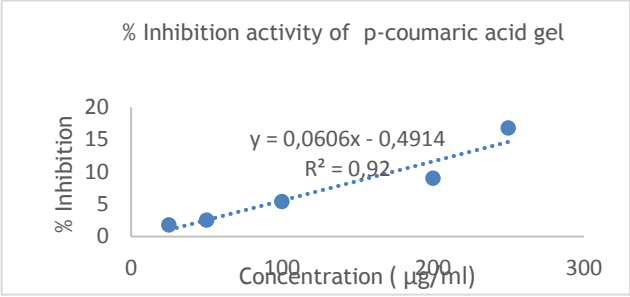

Fig.6.percent inhibition of p-coumaric acid gel

6. Discussion

6.1. Linearity: For method A and method B, The absorbance of p-coumaric acid was measured at 229nm and 283nm. The calibration curves in ethanol and phosphate buffer pH 6.8 were plotted in a concentration range of 5-25µg/mL and 2-10ug/mL versus absorbance. The calibration curve for p-coumaric acid was linear over the concentration range of 5-25µg/mL and 2-10ug/mL, as shown in Table 2 and Table 3. The equation of the regression line was found to be 0.0516x+0.00992 and 0.1071x+0.0905. The correlation coefficient (R^2) was found to be 0.98 and 0.97 as shown in Figure 4 and Figure 5

6.2.**Accuracy**: method A and method B, the value of SD and %RSD were found to be < 2%, these result confirms that the current method is accurate as shown in Table 3 and Table 4.

6.3. Precision: In the proposed method A and Method B, precision was studied as the repeatability of each concentration (%RSD<2) for intraday and interday precision. The %RSD of method A for intraday analysis of p-coumaric acid was found in the range of 0.079-0.145%, the observed result confirms that the intraday precision method was accurate and for interday analysis of p-coumaric acid was found in the range of 0.057-0.088%, the observed result confirms that the interday precision method is accurate. Similarly, method B for intraday analysis of p-coumaric acid was found in the range of 0.037-0.068 %, the observed result confirms that the intraday precision method was accurate. Interday analysis of p-coumaric acid was found in the range of 0.068-0.198%, the observed result confirmed that the interday precision method was accurate as shown in Table 6, Table 7, Table 8 and Table 9.

6.4. **Robustness:** The proposed method A and method B were found to be robust as there is no interference from changes in wavelength as shown in Table 10 and Table 11.

6.5.Limit of detection and Limit of quantification: LOD and LOQ of the proposed UV spectrophotometric method were found to be 5.449 and 16.512 for method A and 1.792 and 5.420 for method B. The lower LOQ value indicated that the proposed method would be suitable for analyzing samples containing even small quantities of p-coumaric acid as shown in Table 12 and Table 13.

6.6.Antioxidant Activity: the method development, further the drug was incorporated in an aqueous gel and antioxidant activity was analyzed using a DPPH radical scavenging assay. DPPH is known as stable free radical α-diphenyl-ß-picrylhydrazyl. The assay used the measurement of the scavenging ability of the antioxidants against DPPH. An odd electron of a nitrogen atom in DPPH was reduced by accepting a hydrogen atom from antioxidants. The reduction of color intensity (violet) of DPPH indicates the inhibition of free radicals. Correlation in Figure 2. In this research, the formulation contains p-coumaric acid composed polyphenol content, which has high antioxidant activity shown in (Table 14). the proposed research revealed higher antioxidant activities of the prepared formulation IC50 0.833mg/ml which inhibited 50% of free radicals produced by DPPH.

B1	0.272	5.122	25
B2	0.270	8.236	50
B3	0.262	10.445	100

7. Conclusion

UV spectrometry's validation of p-coumaric acid was developed using various ICH guidelines. This method resulted to be noncomplicated, precise, accurate irrespectively robust can be applied for determining P-coumaric acid in pharmaceutical dosage forms. In the validation parameters of both methods A and B, the % RSD was less than 2%. The accuracy of current methods was confirmed by carrying out accuracy parameters that showed the results within the range. The UV spectrometric method has been developed to quantify p-coumaric acid in topical gel formulation. The validation method proves that this was an accurate method for quantifying p-coumaric

acid in the formulation and showed potential antioxidant activity. The relevance of the test of the formulation was the authentication of p-coumaric acid when blended into a formulation to determine its claimed antioxidant efficacy and confirm its effectiveness in several applications. This test involves a controlled medium to measure its radical-scavenging potential and reducing capacity. In addition, the behavior of p-coumaric acid in association with other compounds in the formulation should be precisely assessed to avoid compromising its antioxidant activity.

Appendix

Author Contributions: Dr. Ashwini R Madgulkar contributes to imparting knowledge necessities of validation, methodology and interpretation of results. Ms.Minakshi Shinde was a contributor for searching and reading comprehensive research articles through avail subscription access of our college as per Journal guidelines. All authors read and approved the final manuscript acknowledgment. As an author I have written and technology study done of validation and formulation by performing activity.

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Conflict of Interest: The authors declare no conflict of interest, financial or otherwise.

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