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Original Article

UV-spectrophotometric Method Development and Validation for Piperine Estimation in Black Pepper, Ayurvedic Formulation and Novel Nano Formulation: A Perfect Quality Assessment Tool

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ABSTRACT

Aim: The current study aims to establish and validate a UV-spectrophotometric approach for estimating piperine in black pepper, commercial Ayurvedic preparation, and novel piperine-loaded nanoformulation. **Materials and Methods:** The UV-spectrophotometric technique was developed using methanol as a solvent. Piperine showed the absorbance at 342 nm. In accordance with ICH Q2 (R1) guidelines, the developed method was extensively validated in terms of selectivity, linear range, precision, accuracy, robustness, ruggedness, and reproducibility. The new method was also effectively used for precise estimation of piperine in black pepper, commercial Ayurvedic preparation and novel piperine loaded nanoformulation. The stability indicating study of the established UV-spectroscopic approach was carried as per as per ICH Q1A (R^2) guidelines. **Results:** The optimal parameters for piperine analysis were established using methanol as solvent. With the line equation y = 0.067x + 0.005, the maximum absorption wavelength was found to be 342 nm. Between concentration ranges of 0.5–32µg/mL, it showed a linear response. The linear regression coefficient was found to be 0.999. The method's linearity, accuracy, ruggedness, specificity, and sensitivity were all validated, and all validation results were confirmed to be in acceptable ranges. Piperine was found to be stable and less prone to degradation under acidic, basic, thermal and photolytic stress conditions whereas oxidation degradation showed extremely degradable characteristics. **Conclusion:** The established approach will serve as standard quality control tool for precise estimation of piperine in herbal formulations and can be used for quality analysis.

Keywords: Piperine, Ayurvedic Preparation, UV-spectroscopic, Black pepper, Nanoformulation, ICH.

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INTRODUCTION

Piperine (Figure 1) is an alkaloid derived from the fruits of the black pepper plant (*Piper nigrum* Linn). Because of its distinct pungency and flavour, black pepper ranks first among spices and kitchen uses, earning it the moniker "the king of spices". Traditional Indian, Chinese, and Arabic medicine has used black pepper for numerous ailments for a very long time.[1] Piperine has numerous pharmacological activities, including antioxidant, anti-arthritic, anti-inflammatory, and anti-depressant properties. Many studies on piperine have been conducted in association with other phytochemicals, which has resulted in enhanced bioavailability, preventive and therapeutic responses.[2,3] The therapeutic characteristics of piperine make it an excellent candidate for the treatment of a variety of disorders. The main challenge in developing piperine-based formulations for therapeutic usage is their limited water solubility due to their hydrophobic nature, which lowers their bioavailability.[4] Certain drawbacks were reported from traditional dosage forms such as poor solubility and permeability, poor bioavailability, degradation by gastrointestinal enzyme, food interactions, and toxicity. Novel lipid-based nanoformulation has received considerable interest in drug delivery methods to circumvent these limitations. Liposomes, nanoemulsions, niosomes, ethosomes, solid lipid nanoparticles, and transferosomes are nano-sized carriers that have been designed to overlook such barriers arising from traditional dosage forms. These drug delivery systems are non-toxic, have high drug entrapment efficiency, and provide long-term drug release.[5,6] In other hand,

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Quality control in any herbal based formulation become more imperative task while assessing the quality.[7] Quality control tools are crucial for determining predictability and consistency of the pharmaceutical dosage forms and for confirming the applicability of pharmaceutical dosage forms.[8] The researchers face the problem of developing a suitable and valid method for quality assessment while developing novel drug delivery systems. This necessitates the development of a simple, quick, accurate, and efficient analytical method.[9] Modern analytical instruments are extremely important in the quality monitoring and standardisation of herbal medicines. The spectroscopic and chromatographic methods, in particular, play an important role in the quality control and analytical validation of herbal preparations.[10] Spectrophotometry remains a preferred approach among the different methods available due to its simplicity, specificity, and low cost.[11] From the literature review, it is observed that very few spectroscopic methods for estimation of piperine have been reported.[12-18] These reported methods have their own limitations so there is need to design UV-spectroscopic method for piperine estimation. Therefore, the objective of the current work was to develop and validate a simple, quick, sensitive, exact, and accurate UV-spectrophotometric method for piperine estimation and its application for estimation of piperine in black pepper, commercial Ayurvedic preparation, and novel piperine-loaded nanoformulation.

MATERIALS AND METHODS

Materials

Piperine (Analytical grade) received as a gift sample from Himalaya Drug Company, Bengaluru, India and Biomed Ingredients Pvt. Ltd., Goa, India. A gift sample of phospholipid (LIPOID 90G) was obtained from Lipoid GmbH, Germany. Commercial Ayurvedic preparation (Trikatu Churna) purchased from local pharmacies. Other chemicals and reagents used in the research work were of analytical grade.

Instrument

Shimadzu UV-spectrophotometer model 1800 with UV probe software along with 1 cm quartz cells was used for analysis. The glassware's were thoroughly cleaned using methanol prior to use.

Selection of Wavelength

Different solvents were used to assess the solubility of the piperine. Based on solubility pattern of piperine, methanol was found to be suitable solvent for analysis. The UV-spectrophotometer was used to scan piperine (10 µg/mL) between 800 nm to 400 nm, piperine showed highest absorption at 342 nm (Figure 1).

Preparation of stock solution

Accurately weighed quantity of piperine (10 mg) was dissolved in methanol in 10 mL amber coloured volumetric flasks. Further the working dilutions of piperine such as 0.5, 1, 2, 4, 8, 16 and 32 µg/mL were prepared by using above stalk solution.

Preparation of calibration curve

A linear regression equation was derived using a standardised curve with concentration on the X-axis and absorbance on the Y-axis. The absorbance was measured at 342 nm.

Method Validation

The method was developed and validated in compliance with to ICH Q2 (R1) guidelines to assess Specificity and Selectivity, Linearity, Accuracy, System Precision, Sensitivity, Ruggedness, Robustness, and Stability.[19-21] Specificity and selectivity parameters were evaluated in order to verify that none of the component interfered during analysis of piperine. The absence of absorbance at 342 nm served as evidence of the method's specificity and selectivity. Linearity range was determined at 342 nm by analysing 0.5-32 µg/mL of piperine. By plotting the area versus concentration, calibration curve was generated and correlation value R^2 was estimated. The intraday and interday precision tests were used to investigate precision. The % RSD was computed after recording the absorbance on the same day at three different intervals and on three different days. Recovery studies were conducted to verify accuracy, and % mean recovery of the sample was calculated at three distinct levels i.e., 50%, 100%, and 150% of the sample solutions. The sensitivity of proposed method was estimated using the Limit of Quantification (LOQ) and Limit of Detection (LOD). The signal-to-noise ratios for LOD and LOQ, which are the lowest detectable and measurable concentrations of analytes, are 3:1 and 10:1, respectively. Ruggedness was analysed by repeating the recommended approach on two distinct instruments and having independent analysts examine the repeatability and percent RSD was calculated. The robustness was carried out to evaluate the influence of a small but deliberate variation in the spectrometric condition for determination of piperine. The analysis of the developed method at different wavelengths and solvent compositions was used to assess its robustness. The stability of the solution was examined by exposing it to unstable environments for 72 hr.

Preparation of piperine loaded lipid based nanoformulation[22-24]

The novel lipid-based nanoformulation (transfersomes) prepared by thin film hydration method. In this approach, accurately weighed quantity of phospholipon 90G (phospholipids) and tween 80 (an age activator) were dissolved in a beaker containing chloroform and methanol (1:1 V/V) while piperine was dissolved in methanol in another beaker. Solutions from both beakers were combined and placed in a round bottom flask. The organic solvent was then evaporated using a rotary evaporator at (60°C) and at 60 rpm while operating under reduced pressure. After the solvent had completely evaporated, a thin layer has been

observed on the inner wall of the flask. The lipid film was then rehydrated with phosphate buffer (pH 7.4) until the entire thin layer scraped off. Furthermore, hydrated transfersomes subjected for ultra-sonication for 5 min to reduce particle size. The resultant piperine loaded nanoformulation stored in amber coloured container until further use.

Preparation of Black pepper and commercial Ayurvedic preparation samples for analysis of piperine[19]

The established UV spectrophotometric method was used to determine the quantity of piperine in black pepper (*Piper nigrum* Linn) as well as in Trikatu churna, a commercial Ayurvedic preparation. The black pepper fruits were converted into powder form. Accurately weighed quantity of black pepper powder was dissolved in methanol and it was subjected for sonication for 15 min. The resultant solution was filtered using 0.45 µm syringe filter. Further, dilutions were prepared and piperine content was estimated. The same method was used for estimation of piperine in *Trikatu churna*. The method for estimating piperine content using UV-spectrophotometric method was similar to that described above.

Stability indicating parameters[25-28]

Samples were subjected to acid, base, oxidation, and photolytic degradation in order to evaluate the stability of the established UV-Spectroscopic approach as per ICH Q1A (R2) guidelines. The percentage degradation was estimated. For acid degradation study, freshly prepared dilution of piperine (10 µg/mL) was stressed for two hours in 0.1 N HCl on a water bath at 80°C. The samples were scanned between 200-400 nm to get spectra. Piperine solution (10 µg/mL) was made using primary and secondary stock solutions and diluted with 0.1 NaOH. In both cases, the spectra were scanned after being stressed for two hours at 80°C for base degradation study of piperine. Oxidative degradation study of piperine was carried out by hydrogen peroxide method. Piperine (10 µg/mL) prepared from primary and secondary stock solutions and diluted with 30% H_2O_2. After stressing for 2 hr at 80°C, the samples were scanned between 200-400 nm to get spectra. Piperine (10 mg) was exposed to UV light to determine photo degradation for 2 hr. Piperine (10 µg/mL) was prepared in methanol and scanned to obtain the spectra.

Formula to calculate % degradation

$$\% \text{ Degradation} = \frac{(\text{Initial degradation} - \text{Final degradation})}{\text{Initial degradation}} * 100$$

Statistical analysis

Each validation and stress degradation parameters were performed in triplicates, and the data were reported as mean ± SD. The mean, standard deviation, % relative standard deviation (%RSD), slope, and correlation coefficient of the experimental data were determined using Microsoft Excel. The calibration curve of piperine was subjected to an ANOVA analysis using the Graph Pad Prism software (GraphPad Software Inc., CA, USA).

RESULTS AND DISCUSSION

A UV-spectrophotometric approach was developed using Shimadzu UV-1800 system and solvent as methanol for estimation of piperine. Table 1 shows the specifics of the established approach.

Method validation

According to ICH Q2 (R1) guidelines, the proposed method was extensively validated for specificity and selectivity, linearity, accuracy, system precision, sensitivity, ruggedness, robustness, and stability.

Specificity and Selectivity

The specificity and selectivity of the approach were underscored by the solvent spectrum, which revealed that there was no interference from absorbance at 342 nm. Figure 2 illustrates the UV spectra of solvent (methanol), Figure 3 depicts the spectrum of piperine at 342 nm.

Linearity range

By analysing 0.5-32 µg/mL of piperine, linearity range was determined at 342 nm. The linearity of the calibration curve was validated by the value of correlation coefficients. The correlation value R2 was found to be 0.999 which indicates a good correlation between absorbance and concentration. The linearity and range are shown in Table 2, and the calibration curve is shown in Figure 4.

System precision

The results of precision study were represented in % RSD and it was found to be less than 2% (Table 3). It clearly indicates precision of the method as it complies with acceptable limits of <2%. Table 4 and Table 5 represents intraday and interday precision respectively.

Table 1: Parameters of method development.

Parameters	Specifications
Instrument	UV-spectrophotometer
Make	Shimadzu
Model	1800
Software	UV Probe
Analyte	Piperine
Solvent	Methanol
λ_{max} Piperine	342 nm

Accuracy

Accuracy data was obtained at three distinct levels, such as 50%, 100%, and 150%. The % mean recovery of the drug is within the acceptance criteria of 99.0% - 102.0% suggesting good recovery value and accuracy. Table 6 represents accuracy of method.

Table 2: Linearity and range data of Piperine.

Sl. No.	Concentration (µg/mL)	Absorbance of Piperine at 342 nm
1	0.5	0.036
2	1	0.075
3	2	0.151
4	4	0.271
5	8	0.542
6	16	1.09
7	32	2.16
R^2		0.999
LOD		0.052 µg/mL
LOQ		0.159 µg/mL

Sensitivity

The signal-to-noise ratios for LOD and LOQ, which are the lowest detectable and measurable concentrations of analytes, are 3:1 and 10:1, respectively. The developed UV spectrometric technique was sensitive for estimation of piperine in black pepper, commercial Ayurvedic preparation and in nanoformulation with detection and quantification limits for piperine found to be 0.052 and 0.159 µg/mL at 342 nm, respectively.

Ruggedness

In order to assess ruggedness, independent analysts repeated the suggested procedure on two different instruments and results showed percent RSD of less than 2%, indicating that the developed method is rugged. Table 7 depicts the ruggedness of method.

Table 3: System Precision of Piperine.

Sl. No	Concentration (µg/mL)	Piperine	% RSD
1	2	0.152	0.380
2	2	0.151	
3	2	0.152	
4	6	0.415	0.139
5	6	0.414	
6	6	0.414	
7	10	0.699	0.143
8	10	0.697	
9	10	0.698	

Table 4: Data for Intraday Precision of Piperine.

Sl. No	Concentration (µg/mL)	Piperine (Morning)	Piperine (Afternoon)	Piperine (Evening)
1	2	0.151	0.152	0.152
2	2	0.156	0.155	0.154
3	2	0.152	0.151	0.151
%RSD		1.7292	1.3635	1.0027
4	4	0.271	0.27	0.271
5	4	0.274	0.277	0.274
6	4	0.276	0.273	0.275
%RSD		0.9195	1.2848	0.7615
7	6	0.416	0.414	0.415
8	6	0.421	0.412	0.423
9	6	0.427	0.421	0.411
%RSD		1.3071	1.1369	1.4675

Table 5: Interday Precision data for Piperine.

Sl. No	Concentration (µg/mL)	Piperine (DAY 1)	Piperine (DAY 2)	Piperine (DAY 3)
1	2	0.153	0.152	0.151
2	2	0.155	0.153	0.152
3	2	0.157	0.156	0.153
%RSD		1.2903	1.3546	0.6578
4	4	0.272	0.271	0.27
5	4	0.274	0.272	0.271
6	4	0.276	0.274	0.272
%RSD		0.7299	0.5609	0.3690
7	6	0.414	0.412	0.41
8	6	0.42	0.415	0.414
9	6	0.423	0.419	0.417
%RSD		1.0936	0.8455	0.8489

Table 6: Accuracy data for Piperine.

Drug	Level	Absorbance	Recovery Amount (µg/ml)	%Mean Recovery ± SD (*n*=3)
Piperine	50%	0.131	1.012	101.2±0.2
		0.129	0.977	
		0.127	0.975	
	100%	0.268	3.029	102±0.4
		0.275	3.016	
		0.251	3.042	
	150%	0.407	4.94	99.7±0.3
		0.409	4.91	
		0.402	4.97	

Robustness

The robustness of the developed method shows insignificant impact on the absorption level through the analysis by altering the λ_{max} and solvent composition. Robustness data for piperine is depicted in Table 8. The % RSD was found to be within limits suggesting that developed method is robust.

Stability

The solution was exposed to unstable surroundings for 72 hr in order to assess its stability. Table 9 displays stability of method.

Application of the validated method for estimation of Piperine in Black pepper, Commercial Ayurvedic preparation and Nano formulation

The established analytical approach was utilized to estimate the piperine content in black pepper, commercial Ayurvedic preparation and Nano formulation. The concentration of piperine in black pepper, commercial Ayurvedic preparation and Nano formulation was revealed to be 99.27, 98.40 and 98.51%

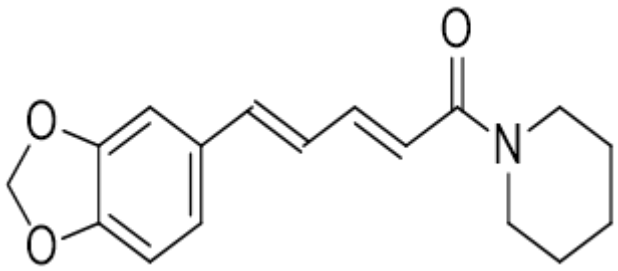

Figure 1: Structure of Piperine.

respectively. These values were all within the range specified on the label. This well-established analytical approach can be utilised in industries for routine quality control analysis of piperine as there were no additional peaks observed for the UV-spectrum of black pepper, commercial Ayurvedic preparation and Nano formulation, indicating that other ingredients used in those formulations cannot interfere with the parenting spectrum of piperine. Figure 5 summarizes the piperine estimation. Figure 5A represents UV spectrum for Normal (A), Figure 5B represents

Table 7: Data for Ruggedness of Piperine.

Sl. No	Concentration (µg/mL)	Change in analyst	Change in instrument
		Piperine	Piperine
1	2	0.151	0.153
2	2	0.152	0.155
3	2	0.153	0.157
% RSD		0.6578	1.2903
4	4	0.273	0.276
5	4	0.274	0.271
6	4	0.271	0.274
% RSD		0.5602	0.9195
7	6	0.416	0.413
8	6	0.42	0.415
9	6	0.418	0.416
% RSD		0.4784	0.3683

Table 8: Robustness data for Piperine.

Sl. No	Concentration (µg/ml)	Change in Wavelength		Change in solvent make (methanol: distilled water)
		Piperine		Piperine
		340nm	344nm	(8:2)
1	2	0.151	0.15	0.157
2	2	0.154	0.155	0.156
3	2	0.152	0.152	0.152
%RSD		1.0027	1.6520	1.7069
4	4	0.274	0.277	0.275
5	4	0.271	0.276	0.277
6	4	0.273	0.27	0.275
%RSD		0.5602	0.2743	0.4188
7	6	0.416	0.414	0.416
8	6	0.412	0.41	0.411
9	6	0.416	0.413	0.412
%RSD		0.5569	0.5048	0.6406

Table 9: Solution stability data of Piperine.

Solution stability		Fresh stock dilutions	Old stock dilutions
Replicates	Concentration	Piperine	Piperine
1	2µg/ml	0.151	0.149
2	2µg/ml	0.152	0.15
3	2µg/ml	0.15	0.149
4	2µg/ml	0.154	0.152
5	2µg/ml	0.155	0.154
6	2µg/ml	0.152	0.1515
RSD		1.2222	1.2987

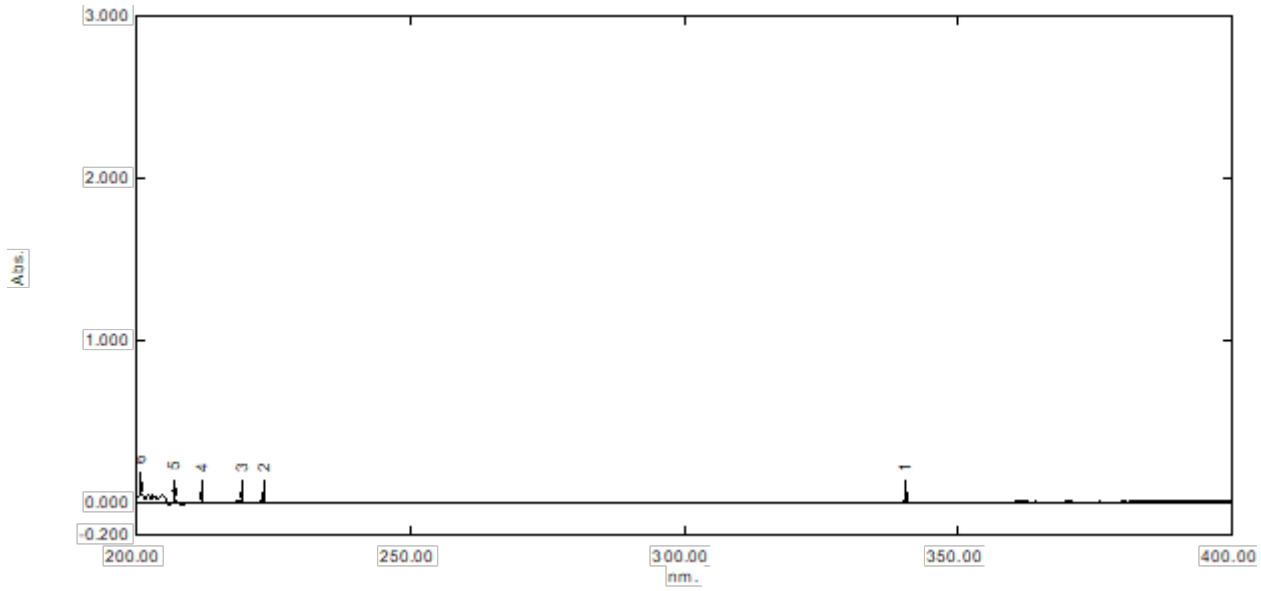

Figure 2: UV-spectrum of methanol.

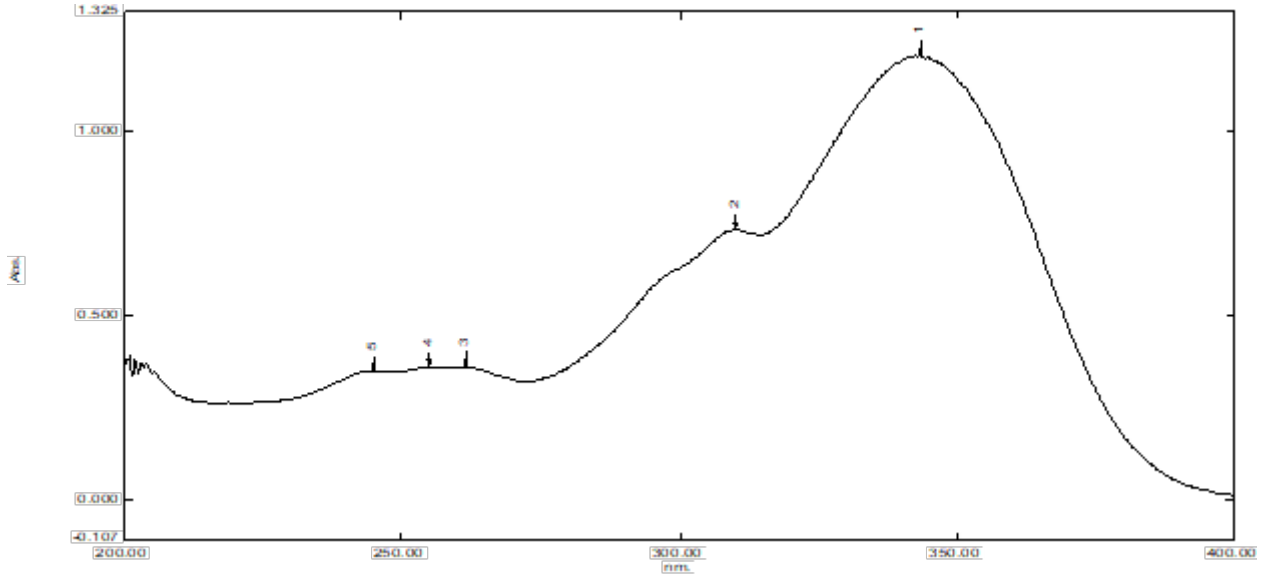

Figure 3: UV-spectrum of Piperine.

UV spectrum of Black pepper (B), Figure 5C represents UV spectrum for Commercial Ayurvedic preparation (C), Figure 5D represents UV spectrum for Lipid based nanoformulation (D).

Stability indicating parameters

Samples were subjected forced degradation analysis to evaluate the stability of the established UV-spectroscopic technique. The percentage degradation was computed and it was found as acceptable and in within limits. Figure 6 represents results of forced degradation study. Piperine was slightly degraded in 0.1 N HCl acid degradation study. After heating 0.1 N HCl for two hours at 80°C, the spectra was visible. The acid degradation findings confirmed that piperine is stable and less prone to degradation under acidic conditions. Figure 6 A represents acid degradation study of piperine. There are no degradation peaks

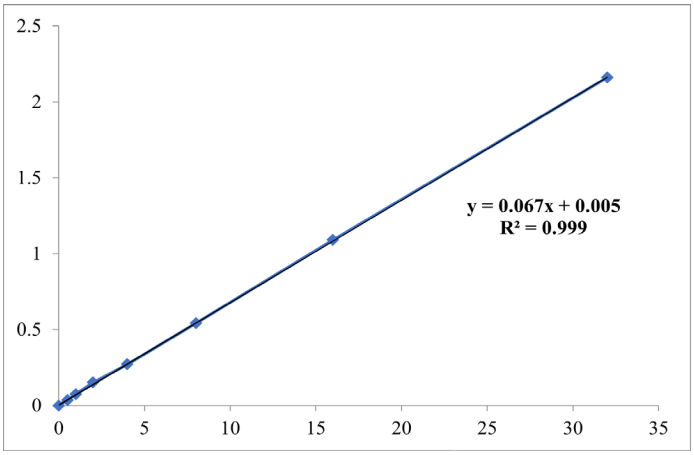

Figure 4: Linearity curve for Piperine.

Table 10: List of previously reported publications.

Title	Solvent Wavelength (nm)	Limitations	Applications	References
UV-spectrophotometric method development for estimation of Piperine in Chitrakadi Vati.	Methanol 342 nm	Validation part is not fully explored, Limited applications.	It can be applied for routine analysis of piperine in polyherbal formulations containing piperine.	14
Quantitative analysis of piperine in Ayurvedic formulation by UV Spectrophotometry.	Methanol 342.5 nm	Lack of validation and stability study.	Can be used for quantification of Piperine in Ayurvedic formulation.	15
Spectrophotometric determination of piperine in Trikatu Churna: An Ayurvedic Formulation.	Ethanol 342.5 nm	Limited applications, Validation part is not explored.	Method can be used for quality control of Trikatu churna.	16
UV Spectrophotometric determination of piperine in NavasayaChurna: A Polyherbal Formulation.	Ethanol 342.7nm	Limited applicability, Validation required.	Suitable for routine analysis of piperine in Navasaya churna.	17
Development of fingerprints for an Ayurvedic formulation Ajmodadi churna, via piperine estimation by UV-Spectrophotometry.	Methanol 342.5 nm	Lack of validation and stability.	Method can be used for the routine analysis of piperine in Ajmodadi churna and its crude drugs.	18
Simultaneous estimation of Piperine with other drug				
Development and validation of bivariate UV-visible spectroscopic method for simultaneous estimation of curcumin and piperine in their combined nanoparticulate system.	Methanol 342 nm	Lack of forced degradation behaviour of curcumin and piperine.	The method can be applied for estimation of curcumin and piperine from nanoparticulate system.	13
UV-Vis Spectroscopy to enable determination of the dissolution behavior of solid dispersions containing curcumin and piperine.	Methanol 335.5 nm	Limited applications,Lack of stability result.	Method can be used for the determination of curcumin or piperine during dissolution studies.	29
Development and validation of simultaneous UV-spectrophotometric method for the determination of Resveratrol and Piperine in pharmaceutical dosage form.	Methanol 340 nm	Not applicable for low concentration samples due to linearity concentration is high.	Method can be used for simultaneous analysis of Resveratrol and Piperine.	30

visible when piperine is heated at 80°C with 0.1 N NaOH. The acid and base degradation analyses revealed that piperine was more stable under basic stress conditions than acidic stress conditions, suggesting good stability of the developed method. Figure 6 B represents base degradation study. When compared to other methods of degradation, 30% H_2O_2 oxidation degradation showed extremely oxidative degradable characteristics. Oxidative degradation involves an electron transfer mechanism. Piperine was found to be unstable in an oxidative environment. This is due to the amine present in piperine as amines are susceptible

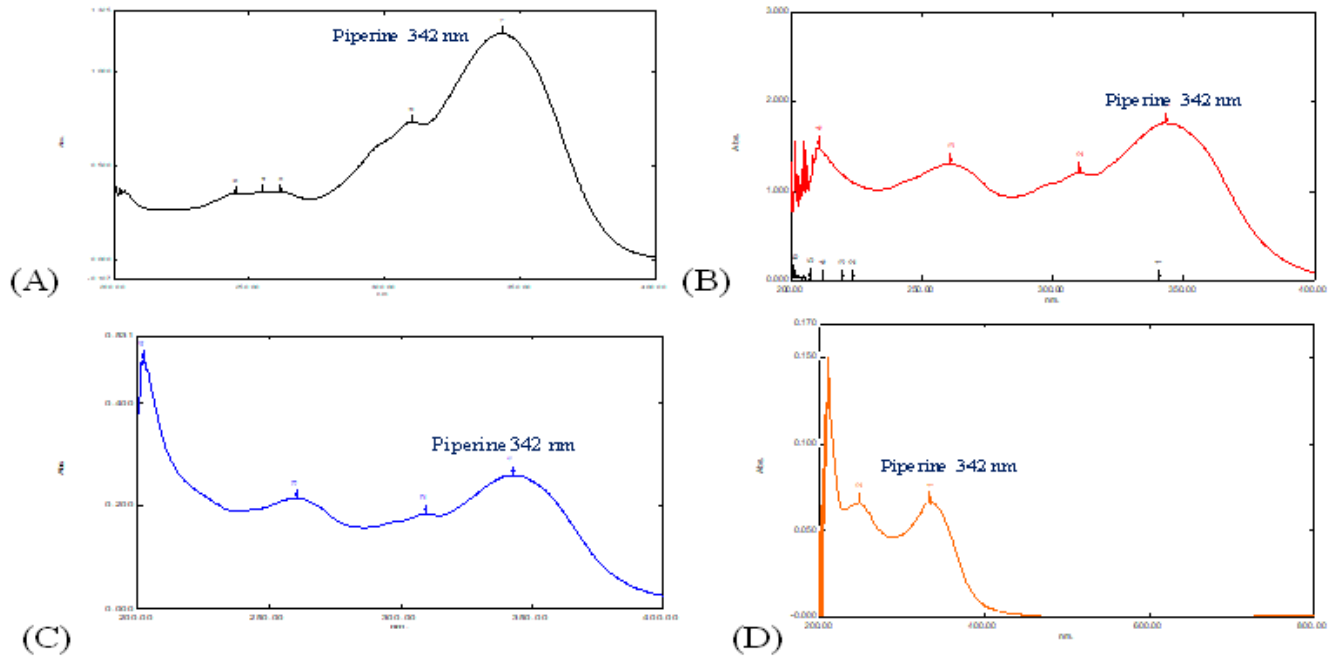

Figure 5: UV-spectrophotometric spectrum for Normal (A), Plant extract Black pepper (*Piper nigrum* L.) (B), Ayurvedic formulation (Trikatu churna) (C), Lipid based nanoformulation (D).

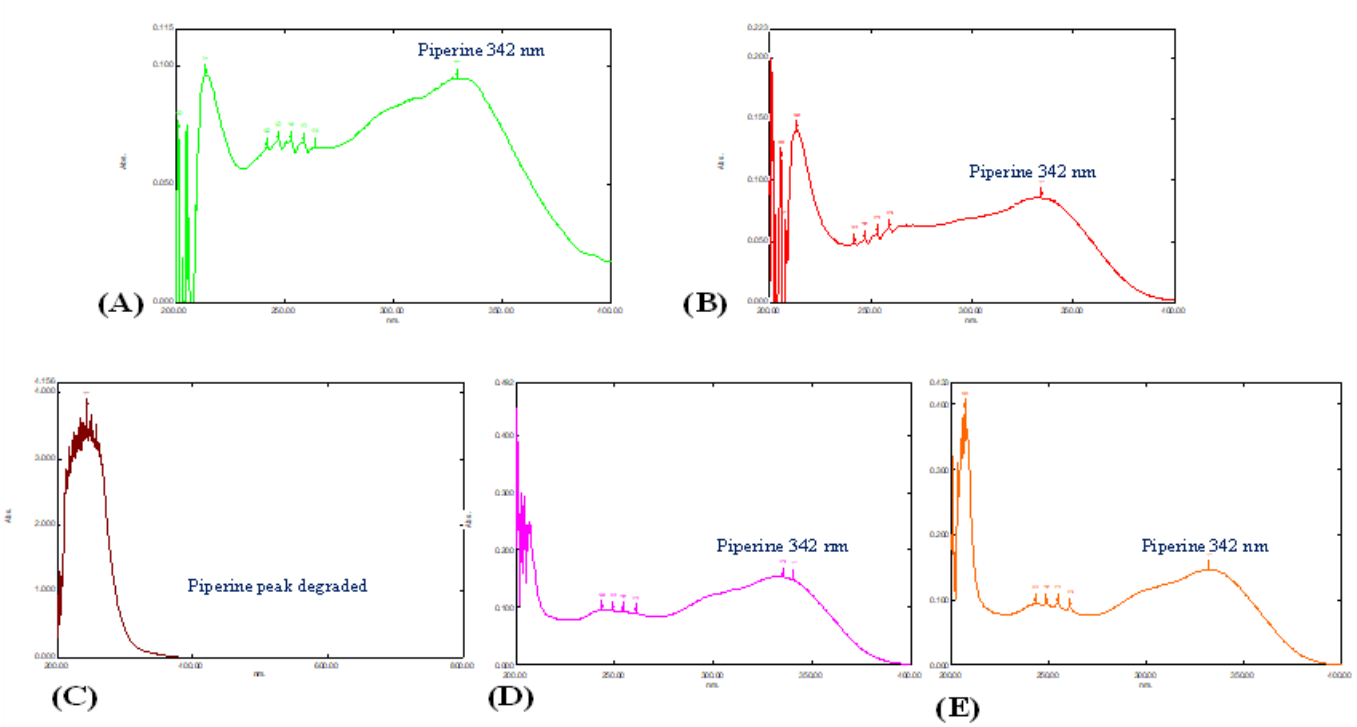

Figure 6: UV-spectra of piperine (10 µg/mL) obtained in the stress degradation assays using Acidic (A), Basic (B), Oxidative (C), Thermal (D), Photolytic (E).

to electron transfer oxidation. Figure 6 C depicts oxidative behaviour of piperine. The results of thermal degradation clearly indicates that heating does not change stability behaviour of piperine because the spectrum remained exactly like normal, with a negligible percent degradation. This could be due to high melting point of piperine, which does not influence its stability. As a result, piperine is found to be stable in thermal degradation analysis. Figure 6 D represents thermal degradation behaviour of piperine. When compared to other degradations, the amount of photolytic degradation was fairly modest and its spectra barely changed. Figure 6 E represents photolytic degradation of piperine.

Comparison with previously published methods

Comparative evaluation of previously reported UV Spectroscopic method for estimation of piperine is represented in Table 10. The comparative evaluation was based on the solvents used, detection wavelength, limitations, and the extent of applicability of the methods. From literature survey, no single UV spectroscopic method available that can be utilised for analysis of piperine from black pepper, marketed Ayurvedic product and in nanoformulation, as well as evaluating the degradation behaviour of piperine using the same parameters of the developed UV spectroscopic method. Extensive literature survey revealed that there is need for development of UV-spectroscopic method for precise estimation of piperine in different formulations. Few literatures were found based on UV-spectroscopy, but they have reported different limitations such as lack of validation as per ICH protocol, higher concentration for linearity range which cannot be applied to smaller concentration samples, limited applications, lack of stability protocol etc. The developed method thus found to be ideal as compared with other previously published works as it can be used for the analysis of piperine in black pepper, commercial preparations and novel lipid based piperine loaded nanoformulations. As the current era is of herbal based new drug delivery systems having various advantages associated with new drug delivery systems. In other hand, herbal medicines mainly lacks in quality analysis. Hence quality analysis of piperine incorporated into nanoformulation could serve as bench marker for establishment of standard quality control tool for precise estimation of piperine. Till date no single literature is available which can be used for analysis of piperine in nanoformulations separately. Hence the developed method is found to be more sensitive, economic and stable when compared to other published literature.

CONCLUSION

The UV-spectroscopic method for estimation of piperine in black pepper, commercial Ayurvedic preparation and Nano formulation was successfully developed and validated in terms of ICH guidelines. This newly developed UV-method, in contrast to earlier approaches, enables simple piperine quantification in herbal formulation. From extensive literature survey, very few UV-spectroscopic methods were reported. These reports have shown various limitations. As quality is a main concern in phyto-pharmaceuticals and these products lacks mainly in quality analysis. Establishment of such quality analysis tools will become a stepping stone in quality assessment of herbal products. The objective of validation of any analytical method is to demonstrate that is suitable for its intended purpose. The developed method was extensively validated as per ICH Q2 (R^1) guidelines and all the validation parameters found in acceptable ranges. The developed method was found to be simple, precise, rugged, and stable for the analysis of piperine. A forced degradation study investigates the stability of a drug under stressful conditions. It is crucial to identify the specific stress factors that cause the deterioration of the drug. The stability of the established UV-spectroscopic approach was carried as per as per ICH Q1A (R^2) guidelines. The results clearly confirmed that piperine is stable and less prone to degradation under acidic, basic, thermal and photolytic stress conditions whereas oxidation degradation showed extremely degradable characteristics. In other hand, analysis of herbal raw material is an imperative task in terms of quality assurance. Herbal phytoconstituents have poor bioavailability due to poor solubility and hence there is an increased attention for development of herbal based Nano formulations. Precise estimation of phytoconstituent loaded in nanoformulations remains a big challenge to the researchers. Thus, the developed UV-spectroscopic method was successfully applied on crude black pepper and piperine based nanoformulation for accurate estimation of piperine. Furthermore, the method was also applied on marketed Ayurvedic preparation known as Trikatu churna for piperine estimation. Based on the results, it can be concluded that the developed method can be used as standard quality analysis tool for piperine containing products.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

The development and validation of the UV Spectroscopic technique for piperine have been collaboratively achieved by all authors. The development and validation of the UV Spectroscopic

technique for piperine was aided by Akshay K. Patil and Dr. Sunil S. Jalalpure provided guidance for the current research project, including advice on reviving the literature and producing the paper work. Supriya S Chimagave and Bhaskar K. Kurangi guided for the writing of manuscript and framing of the research paper.

ABBREVIATIONS

UV: Ultra violet; **µg:** Microgram; **mL:** Millilitre; **µL:** Micro litter; **nm:** Nanometre; **LOD:** Limit of detection; **LOQ:** Limit of quantification; **RSD:** Relative standard deviation; **SD:** Standard deviation; **ICH:** International conference of harmonization; **HCL:** Hydrochloric acid; **NaOH:** Sodium hydroxide; **H_2O_2:** Hydrogen peroxide.

SUMMARY

The UV Spectrophotometric method for estimation of piperine in black pepper, Ayurvedic formulation and novel nanoformulation was successfully established. A very few spectroscopic methods for estimation of piperine have been reported and these reports have their own limitations. During development of new drug delivery systems, researchers particularly face problem in quality analysis. In quality analysis, spectroscopy remains at priority level as analytical tool. In the present work, methanol is used as solvent and detection wavelength was found to be 342 nm. The developed method was validated as per ICH guidelines, all the validation parameters were found to be within limit. The method is found to be simple, robust, accurate, precise and stable. Forced degradation study was carried out in acidic, basic, oxidation, thermal and photolytic condition. The developed method was successfully applied for estimation of piperine in black pepper, Ayurvedic formulation and novel nanoformulation. Therefore, the proposed method can be used as routine quality analysis tool for accurate estimation of piperine in herbal formulation. Researchers facing limitations of quality analysis while developing new drug delivery systems can be overlooked by such analytical methods.

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