Volume 9, Issue 7, July – 2024 International Journal of Innovative Science and Research Technology
ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL952

Development and Validation of a UV- Visible Method for Determining Piperine in Bulk and Formulation

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Abstract:- Standardization of herbal preparations is crucial because the growing use of herbal medicines and the globalization of the herbal medicinal industries have raised serious concerns about safety among the public and health authorities. However, the majority of the time, it was discovered that the standardization procedure was challenging and time-consuming. Thus, piperine from *Piper nigrum* in the commercial formulation could be quantitatively determined and standardized using an easy-to-use and trustworthy UV-Visible Spectrophotometric technique. In accordance with WHO guidelines, UV-visible spectrophotometric investigations of several parameters are part of the study. This work presents a spectrophotometric technique in the UV area at 342 nm that is easy to use, quick, sensitive and accurate. It was established how much piperine was present in the sample and validated. Therefore, piperine in the commercial formulations may be reliably quantified using the suggested method.

Keywords:- *Standardization, UV-Visible Spectroscopy, Piperine, Piper Nigrum.*

I. INTRODUCTION

Quality control of herbals is of greater importance for preservation of quality of the natural herbs and products. When the quality control aspect has identification of substance, adulterants, and substitutes; purity of material; and assay of active chemical constituent of greater importance of the particular herb, then they are called as pharmacopoeial aspects of quality control. The process where the qualitative and quantitative values of herbs are measured against the prescribed or set standards and parameters is standardization. [1, 2]

Based on the different important evaluation parameters like organoleptic properties, ash values, moisture content, microbial contamination, and chromatographic and spectroscopic evaluations, the WHO for the standardization of herbal drugs with current and future trends has set guidelines for standardization methods and procedures. [3, 4]

In the study piperine is considered as the marker compound, which is an alkaloid present in black pepper (*Piper nigrum*), one of the most widely used spices. It is also present in long pepper (*Piper longum*), and other Piper species fruits belonging to the family of Piperaceae. Piperine is responsible for the black pepper distinct biting quality. Piperine has many pharmacological effects and several health benefits, especially against chronic diseases, such as reduction of insulin-resistance, anti-inflammatory effects, and improvement of hepatic steatosis. [5, 6]

The formulation of piperine is well- known and traditionally used for digestive health and as a dietary supplement. Standardization of piperine has been done by using sophisticated techniques like HPTLC, HPLC etc. which is complex and time consuming. Thus we have developed a method of standardization of piperine and its formulation using UV – Visible spectrophotometry, which is reliable, reproducible and simple.

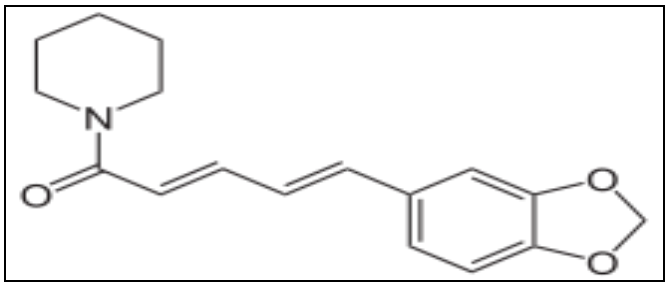

Fig 1 Structure of Piperine

The raw material *(Piper nigrum*) was collected from RK Sweets and Super market at Chelakara, Thrissur District, Kerala.

The *Piper nigrum* that we have purchased is of good quality.


Fig 2 Piper Nigrum

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➢ *Formulation Profile*

Fig 3 Commercial Formulation

➢ *Purchase of Formulation*

The formulation was procured from ARKURE HEALTH CARE, Haryana, India through the online shopping site AMAZON.

➢ *Formulation Specifications*

- It is a dietary supplement of powdered piperine also a nutraceutical.
- It contains ZERO artificial ingredients, antibiotics, binders, hormones, fillers, soya yeast, starch, additives and excepients.
- It is FREE of additives, allergens, fillers, dairy, gluten and GMO.
- It is claimed to be a 100% pure and potent supplement.

➢ *Supplement Facts*

- Serving size: 10mg
- Amount per serving
- Piperine black pepper seed extract powder (*Piper nigrum*) * 10mg (standardized to contain minimum 95% piperine)
- Daily value not established
- Made from black pepper seed

➢ *Directions*

Take 10 mg twice a day; preferably with meals or as directed by your physician.

➢ *Suggested use*

- Add one serving to your favorite dish or drink for a nutritious boost.
- Can be mixed with warm milk, honey, juices & smoothies.
- Discontinue use if side effects appear.

➢ *Benefits of Commercial Formulation*

- Enhances absorption of whole food and supplements.
- Enhance thermogenic functions.
- Supports digestive health.
- Increases the bioavailability of water-soluble nutritional compounds.

➢ *Warning*

The statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any diseases. If you are currently taking any medication, consult with a physician prior to use. Don't exceed suggested use. This product shouldn't be used as a substitute for a varied diet. Keep out of reach of children.

II. MATERIALS AND METHODS

A. Preparation of Ethanolic Extract

➢ *Collection of Raw Material*

The raw material (*Piper nigrum*) was collected from RK Sweets and Super market at Chelakkara, Thrissur District, Kerala.

➢ *Procurement of chemicals*

All the chemicals and solvents used were procured from NICE CHEMICALS PVT LTD. KOCHI, KERALA.

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➢ *Materials used*

Black pepper, heating mantle, soxhlet apparatus, cotton, 95% ethanol.

➢ *Preparation of Ethanolic Extract*

100g of black pepper was weighed and powdered. Then extracted with 150 ml 95% ethanol using a Soxhlet apparatus at 60 °C. The extraction procedure was carried out for 3 hours. Then the obtained extract was concentrated on heating mantle by adjusting the temperature to 60^0c. [7, 8]

B. Isolation of Piperine from Raw Material

➢ *Materials used*

China dish, extract, 15ml 10% KOH, 95% ethanol.

➢ *Procedure of Isolation*

- The ethanolic extract of piperine was taken and acetone was added in which piperine is not soluble.
- It was kept overnight in the refrigerator.
- The solution was taken out and the upper layer was decanted.
- Yellow colored needle shaped crystals of piperine was obtained.

Fig 4 Crystals of Piperine

C. Development of TLC Profile for Piper Nigrum

➢ *Materials used*

Wagner's reagent, standard piperine, aluminium-backed silica gel TLC plate, acetone and n-hexane (3:2), filter paper and commercial formulation are among the items were used.

➢ *Procedure*

The mobile phase used is acetone and n- hexane in the ratio of 3:2. After saturation precoated aluminium TLC plate spotted with isolated piperine, and commercial formulation and kept in the TLC chamber for development. After the development of chromatogram it was air dried. Then the separated samples were detected by spraying the Wagner's reagent. Then the R_f values were calculated and compared. [9, 10]

D. Developing and Validating UV-Visible Spectrophotometric Standardisation Method of Piperine

A simple and reliable UV- Visible spectophotometric method was developed and validated for estimation of piperine in raw material and formulation. The λ_{max} of piperine was obtained at 342nm. Shimadzu-1800 series model was used for the study. It was scanned at 200- 800 nm UV range with working standard solutions with a concentration of 10µg/ml were scanned against ethanol as a blank.

➢ *Preparation of Standard Solution (1000µg/ml)*

100 mg of piperine was transferred into 100ml standard flask. 30ml ethanol was added to it and shaken for 5 min. The final volume was made up to 100ml with ethanol.

➢ *Preparation of Working Standard Solution*

Aliquots about 10ml was taken in 100ml standard flask and volume was made up to 100ml with ethanol to obtain concentration of 100µg/ml. Appropriate aliquots of the standard solution were taken and diluted with ethanol in separate 10ml standard flask to prepare standard solutions of piperine having concentrations ranging from 10-100µg/ml. The absorbance of each standard solutions was measured at 342nm using ethanol as blank.

➢ *Preparation of Solution of Formulation*

100mg of the sample was transferred to 100ml standard flask.10ml of ethanol was added to it and shaken for 5 min. It was made up to 100ml with ethanol to obtain concentration of 1000µg/ml.

Aliquots about 10ml was taken in 100ml standard flask and volume was made up to 100ml with ethanol to obtain concentration of 100 µg/ml. From that 10ml solution was transferred to 10ml standard flask and volume was made upto 10ml with ethanol to obtain concentration of 10µg/ml. Prepared sample solution was analysed and the content of piperine in sample was found out.

➢ *Estimation of Piperine in Samples*

Absorbance was measured at 342nm for the sample solutions and concentration of piperine in was calculated.

➢ *Method Validation*

ICH guidelines were followed for the validation of analytical methods developed for precision, repeatability, accuracy, LOD, LOQ.

➢ *Linearity*

The solutions were prepared by pipetting 1,2,3,4,5 and 6ml from working stock solution into 10ml standard flask and the volume was adjusted to mark with ethanol to produce 10 to 60 µg/ml respectively. The absorbance of solutions was measured at 342nm. Calibration curve was generated by taking the absorbance verses concentration.

➢ *Precision*

The precision of method was determined by repeatability, interday, and intraday precision.

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➢ *Repeatability*

Aliquots of 1ml of working standard solutions were transferred to 10ml standard flask and volume was adjusted with ethanol to get concentration of 10 µg/ml. The absorbance of solutions was measured six times and %RSD was calculated.

➢ *Inter Day Precision*

Aliquots of 2,3 and 4ml of working standard solutions were transferred to 10 ml standard flask and volume was adjusted with ethanol to get concentration of 2,3 and 4 µg/ml. The absorbance of solution was measured 3 times and %RSD was calculated. For interday the analysis was carried on different 3 days in a week.

➢ *Intraday Precision*

Aliquots of 2,3 and 4ml of working standard solution were transferred to 10ml standard flask and volume was adjusted with ethanol to get concentration of 2,3 and 4 µg/ml. The absorbance of solution was measured 3 times and % RSD was calculated. For intraday the analysis was carried out at different intervals on the same day.

➢ *Accuracy*

For the accuracy of proposed method, recovery studies were performed by standard addition method at 3 different levels. A known amount of standard pure drug was added to pre- anlyzed powder and the sample was then analyzed by proposed method.

➢ *Limit of Detection*

The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected.

LOD=3.3*SD/slope (1)

➢ *Limit of Quantification*

The quantification limit is generally determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision. [11, 12,13,14,15,16,17,18]

LOQ= 10*SD/slope (2)

III. RESULT

➢ *Development of TLC Profile for Piper Nigrum*

According to the literature review the R_f value of standard piperine was found to be 0.48. When we did the experiment R_f value of isolated piperine was found to be 0.48. So it is similar to that of the value of R_f that we observed in the literature review. So we can consider it as standard piperine.

When we did the experiment the R_f value of the standard piperine was 0.48 and commercial formulation was 0.48.

So we can conclude that the commercial formulation contains piperine because it has similar R_f value as that of standard.

➢ *Developing and Validating UV-Visible Spectrophotometric Standardisation Method of Piperine*

- *Method Development*

Analytical method was developed and validated according to ICH Q2 guidelines. The proposed method was found to be simple, sensitive, accurate, precise, economical and rapid for the qualitative and quantitative estimation of piperine in sample solutions.

- *UV Spectra of Piperine*

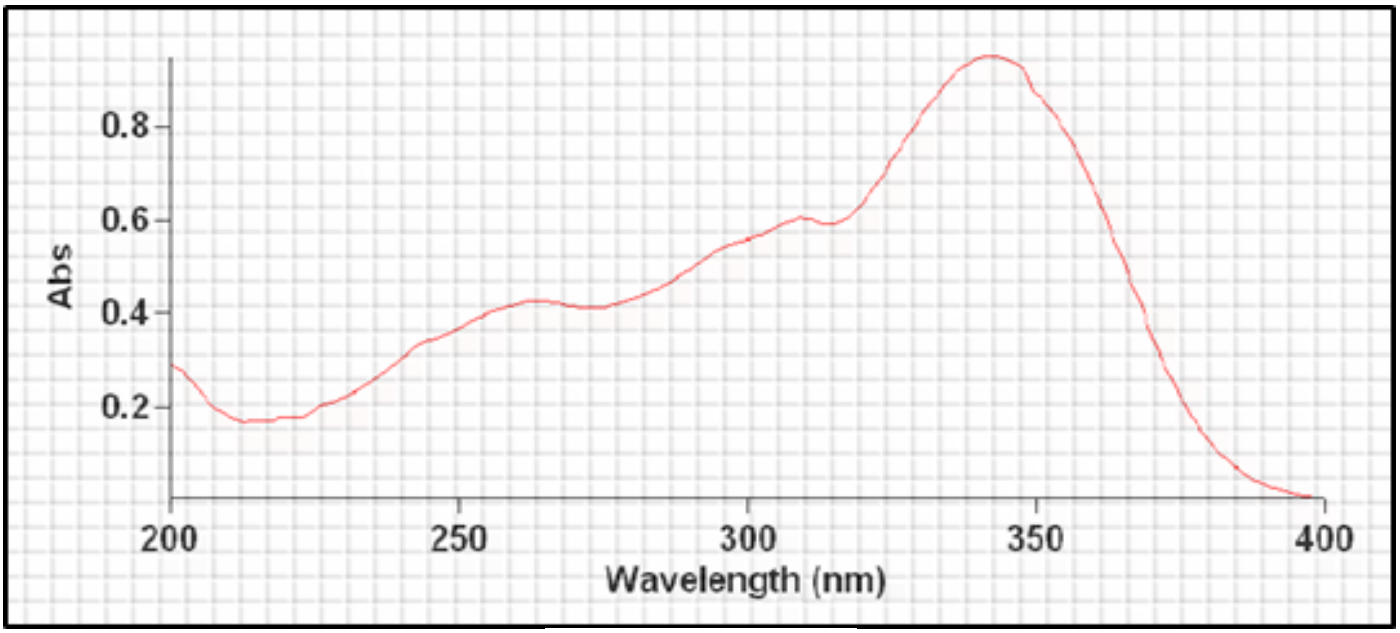

Fig 5 UV Spectra of Piperine

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- *Calibration Curve of Standard Piperine (Isolated)*

Table 1 Concentration Vs Absorbance of Standard Piperine

S. no.	Concentration (µgm/ml)	Absorbance
01	10	0.11
02	20	0.23
03	30	0.35
04	40	0.44
05	50	0.52
06	60	0.73

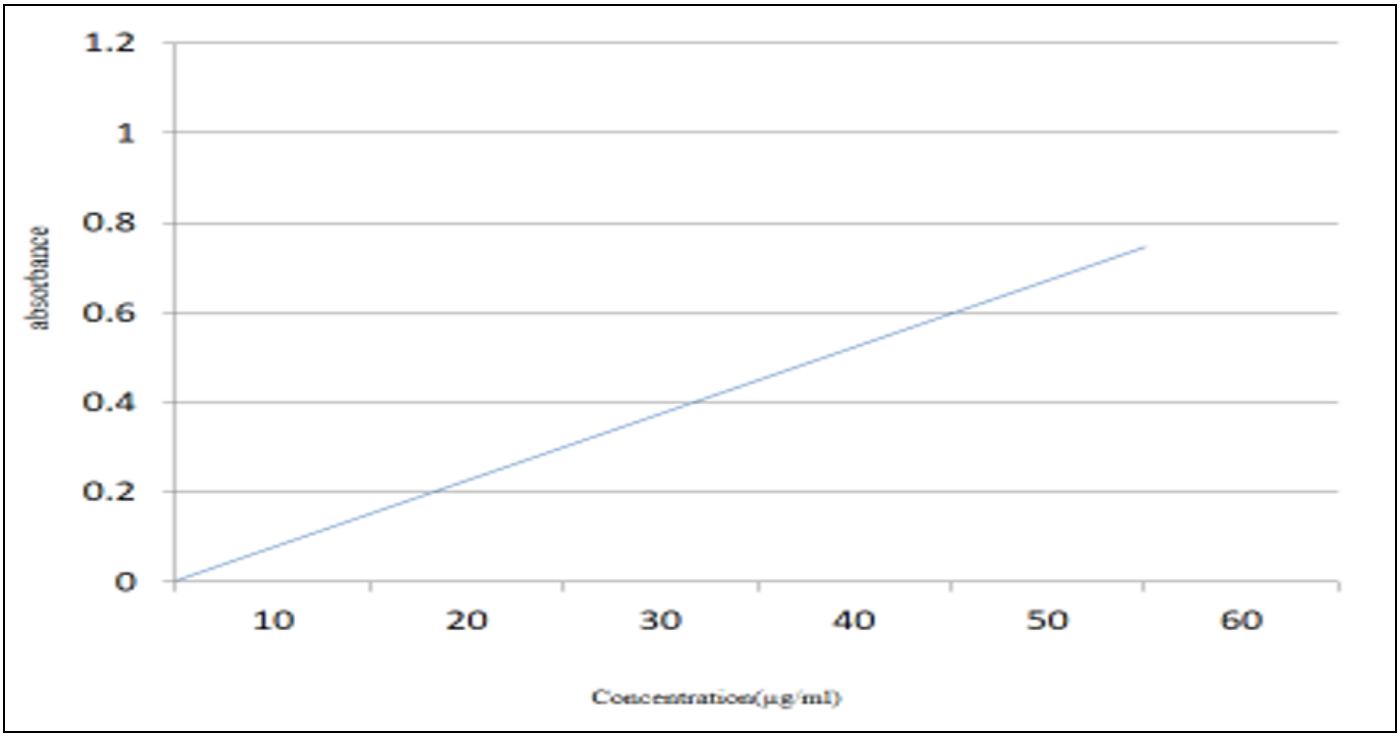

Fig 6 Calibration Curve of Standard Piperine

Slope (y) = 0.0131

Since the calibration curve and the slope obtained for the standard piperine (isolated) matches with that of the literature reference, so it can be confirmed to be the standard.

- *Estimation of Percentage Purity*

Table 2 Determination of Absorbance and Standard Deviation

Concentration[µg/ml]	Absorbance of standard piperine (a)	Absorbance of commercial formulation (b)	S.D (x) of commercial formulation x = b- a
40	0.110	0.109	0.001
	0.235	0.210	0.025
	0.356	0.354	0.027
	0.441	0.440	0.001
	0.526	0.523	0.003
	0.736	0.735	0.026

RSD = 0.101

Percentage purity = (100-RSD)

Percentage purity of commercial formulation = 99.99% w/w

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL952

- *Estimation of Piperine in Commercial Formulation*

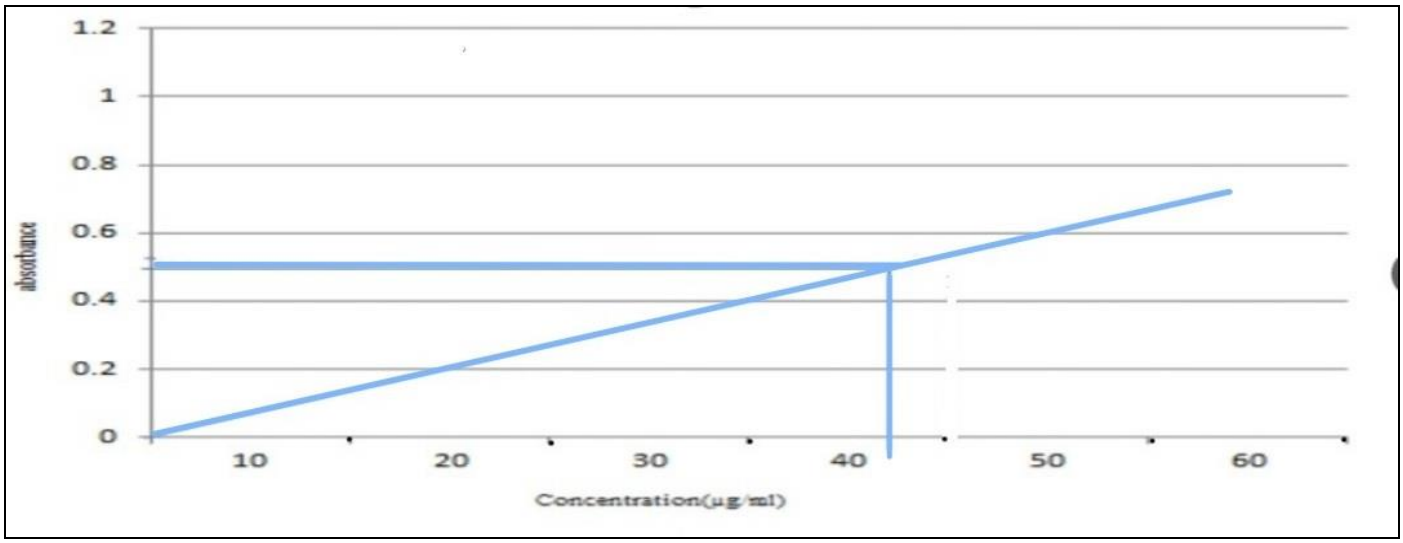

Fig 7 Extrapolated Graph of Piperine

The absorbance values obtained was recorded and piperine content in formulation was found to be 3.8 µg/38 µg.

➢ *Method Validation*

- *Specificity*

The developed method was found to be highly specific as there were no inferences with the excepients in the formulation.

- *Linearity and Range*

Table 3 Concentration Vs Absorbance for Linearity

Sl No	Conc(µg/ml)	Piperine (ml)	Ethanol (ml)	Absorbance						Mean
1	10	1	9	0.11	0.11	0.10	0.12	0.11	0.10	0.108
2	20	2	8	0.23	0.22	0.22	0.23	0.22	0.21	0.221
3	30	3	7	0.35	0.33	0.34	0.35	0.33	0.34	0.340
4	40	4	6	0.44	0.42	0.43	0.43	0.44	0.43	0.431
5	50	5	5	0.52	0.53	0.52	0.52	0.53	0.52	0.523
6	60	6	4	0.73	0.71	0.72	0.73	0.72	0.72	0.721

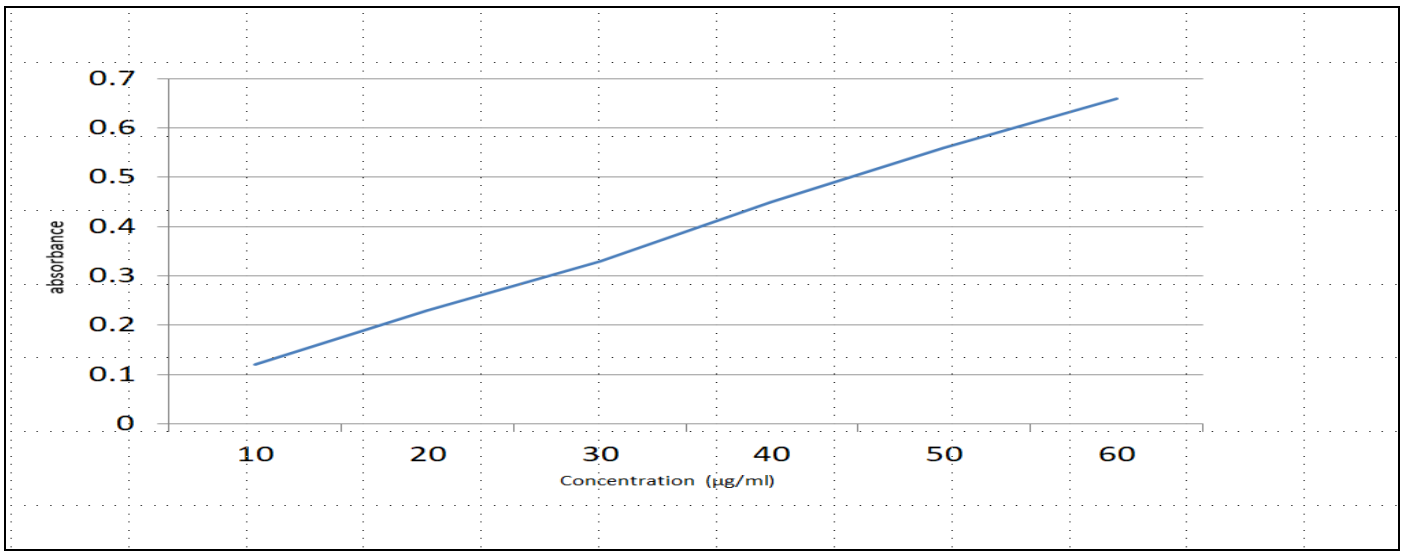

Fig 8 Calibration Curve of Commercial Formulation

Slope (y) = 0.0129

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUL952

- *Accuracy*

Table 4 Determination of Accuracy

Sample	Concentration (µg/ml)		% Recovery
	Standard	Formulation	
S1 50%	10	20	99.73
S2 100%	20	20	99.59
S3 150%	30	30	99.61

➢ *Precision*

- *Intraday*

Table 5 Intraday Determination

Sl no	Concentration	Absorbance
1	40	0.440
2	40	0.441
3	40	0.439
4	40	0.441
5	40	0.440
Average		0.440
RSD		0.440

- *Intraday*

Table 6 Intraday Determination

Conc	D 1	D 2	D 3	D 4	D 5	D 6	Mean ab	SD	RSD
10	0.11	0.11	0.10	0.12	0.11	0.10	0.108	0.007528	6.95%
20	0.23	0.22	0.22	0.23	0.22	0.21	0.221	0.007528	3.4%
30	0.35	0.33	0.34	0.35	0.33	0.34	0.340	0.008944	2.63%
40	0.44	0.42	0.43	0.43	0.44	0.43	0.431	0.007528	1.74%
50	0.52	0.53	0.52	0.52	0.53	0.52	0.523	0.005164	0.987%
									RSD = 3.1414

- *Limit of Detection (LOD)*

The lowest concentration of unknown sample that can be detected, was determined from standard curve was found to be 1.877 µg/ ml.

- *Limit of Quantitation (LOQ)*

The lowest limit of unknown sample at which peak can be quantified was found to be 5.688 µg / ml.

IV. DISCUSSION

The UV-VIS method was found to be simple, accurate, precise, economical and rapid for the estimation of Piperine in samples. The absorption maxima 342 nm was selected for the study and the typical UV spectra of piperine is given in figure 5.

Absorbance of standard solutions in the given concentration range was fund to be linear.

The percentage purity of formulation was found to be 99.99%w/w.

The absorbance values obtained was recorded and piperine content in the formulation was found to be 3. 8 µg/38 µg.

Result for repeatability studies are shown in table 5. Percentage recovery studies was for the 50%, 100% and 150% respectively, percentage recovery of piperine was found to be in between 99.59- 99.73%w/v. The intraday and interday precision studies were performed for three repeated absorbance of same homogenous solution having the concentration of 40 µg/ml and the percentage relative standard deviations are shown in table no: 5, 6.

In this present work an attempt was made to estimate piperine present in formulation. The developed method was found to be simple, precise, economic and can be utilized for routine method for quantification of piperine. The content of piperine in the formulation was quantified.

V. CONCLUSION AND SUMMARY

In this present work an attempt was made to estimate piperine in the formulation. The developed method was found to be simple, rapid, precise and economic and can be utilized for routine method for quantification of piperine. The

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proposed spectrophotometric method is validated in terms of linearity, accuracy, precision and reproducibility. The piperine content in the formulation was quantified. The quantitative comparison of piperine in laboratory and the formulation were comparable.

The presence of piperine could be confirmed by the phytochemical and TLC profile studies.

The concentration of piperine in the formulation was quantified and it was found to be 3. 8 µg/38 µg.

The percentage purity of formulation was found to be 99.99%w/w.

The advantage of this method lies in the simplicity of the sample preparation and less time required. The validated parameters indicate that the developed method is quick, selective and cheap. Hence the developed method is more suitable for the estimation of piperine in products. Piperine quantitation was used to standardize the formulation. formulations of piperine can undergo routine quality control using this UV-visible approach.

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