**DEVELOP A NEW ANALYTICAL METHOD FOR CURCUMINE AND PIPERINE DRUGS BY UV SPECROPHOOMETRY**

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**ABSTRACT**

The development and validation of analytical methods are crucial in the discovery, development, and production of pharmaceuticals. Quality control laboratories employ the official testing methods derived from these processes to verify the identity, purity, potency, and efficacy of drug products..UV Visible spectrophotometric method is that quick ,easy , selective and accurate has been developed for the determination of curcumin and piperine formulations. The spectrophotometric detection was performed using Chloroform as the solvent an absorption maxima 418nm & 239nm . The method was validated for number of validation parameters , and the results shewed that the method can be routine, quality control analysis for curcumine and piperine in herbal formulations. The Linearity range was 2-10μg/ml for Curcumin and 1 -5 μg/ml with a correlation coefficient 0.999 & 0.998

**Keywords:**Curcumin,Piperine,UV-Spectrophotometer

1. **INTRODUCTION**

 Natural plant products are capable of addressing a variety of infections and health issues. A notable example is turmeric, belonging to the Zingiberaceae family, which has been utilized by ancient civilizations for centuries.Turmeric (Curcuma longa Linn) is a plant that thrives in tropical and subtropical regions across the globe. Its primary native habitats include India, Southeast Asia, and Indonesia. Among these countries, India is the leading producer of Curcumin (CUR), accounting for 93.7% of the total global output. Curcuma longa encompasses a variety of economically significant taxa utilized in medicine, culinary applications, coloring agents, and ornamental purposes. Due to India's diverse geographical landscape, the concentration of curcuminoids in turmeric varies significantly across different locations.C. longa extract contains three key compounds: curcumin (Cur), demethoxycurcumin, and bisdemethoxycurcumin, which are found in approximate concentrations of 70–77%, 18–20%, and 5–10%, respectively. Curcuminoids consist of a blend of curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC). Curcumin is chemically characterized as (1E, 6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione.

Piperine (PIP), an alkaloid, is recognized as the primary bioactive component responsible for the characteristic pungent flavor of the plant. Piper nigrum, a flowering vine within the Piperaceae family, has been documented to contain a diverse array of naturally occurring phytochemicals, with PIP being the principal bioactive element contributing to its pungency. PIP is identified as a yellow crystalline compound, with the IUPAC designation of 1-(5-[1,3-benzodioxol-5-yl]-1-oxo-2,4-pentadienyl) piperidine. This plant-derived alkaloid is renowned for its extensive range of biological activities. Research has highlighted PIP's notable properties, including antidepressant, antipyretic, analgesic, anti-inflammatory, antioxidant, hepatoprotective, and anti-diabetic effects, as evidenced by various clinical and pharmacological studies.

CUR and PIP are two dietary polyphenols that have been studied for their potential anti-cancer properties, particularly in relation to colorectal cancer (CRC). The efficacy of curcumin is enhanced when combined with PIP. Both CUR and PIP exhibit poor stability when exposed to light; therefore, all solutions were prepared using amber glassware. While numerous analytical techniques have been established for the quantification of CUR and PIP, the majority of these methods address the compounds separately. Only a limited number of methods have been devised to measure CUR and PIP concurrently in a combined dosage form. This paper examines various analytical approaches developed for the individual and simultaneous determination of CUR and PIP in their combined dosage form.

1. **DRUG PROFILE**

**CURCUMIN**



**CHEMICAL FORMULA** : C21H20O6

**IUPAC NAME**  : 1,7-bis (4-hydroxy- 3-methoxyphenyl) -1,6- heptadiene-3,5-dione

**MOLECULAR WEIGHT** : 368.4

**MELTING POINT** : 1830C

**DRUG CATEGORY**  : Anti-inflammatory, Antioxidant

**PIPERINE**



**CHEMICAL FORMULA** : C17H19 NO3

**IUPAC NAME** : 1,3-Benzodioxol-5-yl)-1-(piperidin-1-yl) penta-2,4-dien-1-one

**MOLECULAR WEIGHT** : 285.3

**MELTING POINT** : 1300C

**DRUG CATEGORY** : Anti-inflammatory

1. **METHODOLOGY**

**Instrument**

The technique was developed utilizing the LASANY Double beam UV-Spectrophotmeter,Model.No.LI2802, MI Series.

**Chemicals and Reagents**

Curcumin(CUR) and Piperine(PIP) were purchased from surya herbal labs,Hyderabad.Methanol (HPLC grade), acetonitrile (HPLC grade), water (HPLC grade), and chloroform were sourced from Merck Chemicals Private Limited in Mumbai, India.

**Selection of wavelength**

The λmax of the two ingredients i.e., Curcumin and Piperine were found to be 239 nm and 418 nm respectively in mobile phase as solvent system.

**Preparation of standard solution of Curcumin**

10 mg of Curcumin was weighed accurately and transferred into 100 ml volumetric flask. About 10 ml of Chloroform was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 100 μg/ml of Curcumin.

**Preparation of standard solution of Piperine**

10 mg of Piperine was weighed accurately and transferred into 100 ml volumetric flask. About 10 ml of Chloroform was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 10μg/ml of Piperine.

**Preparation of mix. Standard solution of Curcumin & Piperine**

Accurately weighed 10 mg of Curcumin and 10 mg of piperine were transferred to two different10 ml volumetric flask. About 3 ml of mobile phase was added and sonicated to dissolve. The volume was made up to mark with same solvent. Then 1 ml of Curcumin & 0.1 ml of piperine were diluted to 10 ml with the solvent system. The resultant solution was filtered through a 0.45 m membrane filter and degassed under ultrasonic bath prior to use. From the above standard solution several working standard solutions are prepared by serial dilution technique.

 

Fig 01: Standard UV Spectrum of Curcumin Fig 02: Standard UV Spectrum of Piperine



Fig 03: Sample UV Spectrum of Curcumin & Piperine

**Method Validation**

**Linearity**

The linearity of the method developed was confirmed by preparing fresh aliquots from the working standard solution. Subsequent dilutions were performed to create a concentration range of 10-50 µg/ml by transferring aliquots of 1.0, 2.0, 3.0, 4.0, and 5.0 ml into a series of 10 ml volumetric flasks, followed by dilution with chloroform. Absorbance measurements were taken in triplicate for each concentration at wavelengths of 244nm and 418nm, using chloroform as the blank. A calibration curve was constructed with concentration plotted on the X-axis and absorbance on the Y-axis.

|  |  |  |
| --- | --- | --- |
| S.N | Conc (µg/ml) | Absorbance |
| 1 | 10 | 0.091 |
| 2 | 20 | 0.152 |
| 3 | 30 | 0.223 |
| 4 | 40 | 0.286 |
| 5 | 50 | 0.351 |

Table01: Data Showing Linearity fro Curcmin

Figure04:Linearity curve for Curcumin

|  |  |  |
| --- | --- | --- |
| S.N | Conc (µg/ml) | Absorbance |
| 1 | 1 | 0.121 |
| 2 | 2 | 0.145 |
| 3 | 3 | 0.168 |
| 4 | 4 | 0.192 |
| 5 | 5 | 0.214 |

Table02: Linearity curve of Piperine

Figure05:Linearity curve for Piperine

|  |  |  |
| --- | --- | --- |
| **PARAMETERS** | **CURCUMIN** | **PIPERINE** |
| Max(nm) | 418 | 244 |
| Linearity range(µg/ml) | 10-50 | 1-5 |
| Correlation coefficient (r2) | 0.999 | 0.999 |
| Slope (m) | 0.0639 | 0.0233 |
| Intercept (c) | 0.0317 | 0.0981 |
| Regression equation (y=mx+c) | 0.0639x+0.0317 | 0.0233x+0.0981 |
| Correlation Coefficeint r2 | 0.999 | 0.998 |

Table 03 : Data Showing for both the drugs

**PRECISION**

Precision is a measure of the agreement among the values obtained when the same solution is repeatedly assayed. Repeatability also termed as intraday precision was obtained by evaluating the absorbance of six replicates of working standard solution 4µg/ml of curcumin and piperine on a single day. Interday precision was calculated in three different days at different times intervals. The % relative standard deviation (RSD) serves as a measure of precision.

|  |  |
| --- | --- |
| **S. No.** | **Absorbance** |
| 1 | 100.6 |
| 2 | 100.3 |
| 3 | 99.8 |
| 4 | 99.2 |
| 5 | 99.9 |
| 6 | 99.5 |
| Mean | 99.8 |
| SD | 0.4 |
| %RSD | 0.5 |

**INTERDAY AND INTRA DAY PRECISION**

The intraday and inter day precision study of curcumin and piperine was carried out by estimating different concentration of curcumin and piperine six time on same day (Intraday precision) and on different day (Inter day precision) and the results were reported in terms of %RSD. The developed method was found to be precised as a=the average % RSD values for Intraday Inter day precision was found to be 1.6% and 1.5% respectively.

|  |  |  |
| --- | --- | --- |
| **S. No.** | **% Assay Interval-I** | **% Assay Interval-II** |
| 1 | 100.3 | 100.6 |
| 2 | 99.9 | 99.8 |
| 3 | 100.2 | 99.6 |
| 4 | 99.8 | 101.3 |
| 5 | 99.6 | 99.2 |
| 6 | 99.9 | 100.2 |
| **Mean** | **99.9** | **100.1** |
| **SD** | **0.29** | **0.75** |
| **% RSD** | **0.3** | **0.8** |

Table05:Intra day results

|  |  |  |
| --- | --- | --- |
| **S. No.** | **% Assay Interval-I** | **% Assay Interval-II** |
| 1 | 100 | 99.4 |
| 2 | 99.4 | 100.2 |
| 3 | 100.2 | 99.6 |
| 4 | 99.6 | 100.1 |
| 5 | 99.2 | 99.2 |
| 6 | 99.8 | 99 |
| **Mean** | 99.7 | 99.5 |
| **SD** | 0.37 | 0.4 |
| **% RSD** | 0.4 | 0.5 |

#### TABLE 06: Inter Day Precision

**ACCURACY:**

Accuracy was assessed by determination of recovery of method by adding standard drug to the known amount of marketed formulation at three different concentration level 80,100, and 120%taking into consideration %purity of added bulk drug sample. each concentration was analysed three times and percentage recoveries were named (table). Results of recovery study where within the range of 92.62%-100.5% indicating that the develop method is an accurate method for the determination of curcumin and piperine the results are summarized in the table.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Recovery level****(%)** | **Concentration(ug/ml)** | **%Recovery** | **SD** | **%RSD** |
| **Test** | **Standard** |
| 50 | 20 | 19 | 101.02 | 100.12±0.65 | 0.65 |
| 50 | 20 | 20.10 | 99.48 |
| 50 | 20 | 19.98 | 99.86 |
| 100 | 40 | 39.9 | 99.8 | 99.98±0.12 | 0.12 |
| 100 | 40 | 40.01 | 100.09 |
| 100 | 40 | 39.8 | 100.05 |
| 150 | 60 | 59 | 100.25 | 99.95±0.25 | 0.25 |
| 150 | 60 | 59.9 | 99.63 |
| 150 | 60 | 60.02 | 99.99 |

Table 05:Accuracy Studies

### LOD AND LOQ

The LOD and LOQ of the actives were obtained by calculating the signal to noise ratio (S ̸ N) i.e., 3.3 for LOD 10 for LOQ using the following formula

|  |  |  |
| --- | --- | --- |
| **COMPONENTS** | **LOD µg/ml** | **LOQ µg/ml** |
| CUR | 0.0837 | 0.287 |
| PIP | 0.1032 | 0.412 |

# CONCLUSION

The quantitation of Curcumin and Piperine though UV-Spectroscopy accomplishes with the requirements of specificity, precision and accuracy in order to be used as a method for the quality control of Pharmaceuticals. The methods have been evaluated for Linearity, Accuracy, Precision, ruggedness in order to ascertain the suitability of the analytical method. The both methods are very simple as doesn’t include any complicated procedure for preparation of sample etc. rapid and economic nature which makes it especially suitable for routine quality control work.

The above proposed UV Spectroscopic method is based on dissolving the drug in chloroform and further dilutions take measurement of absorbance of the curcumin and piperine at 418nm and 241nm using chloroform as blank. Beer’s law was obeyed in correlation range 1 to 5ug/ml having line equation y = 0.0639x +0.0317 with correlation coefficient value 0.999 and y = 0.0.0233x +0.081 with correlation coefficient value 0.9998.To conclude the developed method can be successfully used for estimation of CUR and PIP Tablet dosage form.

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