VOLUME-8, ISSUE-1, JANUARY-2019 • PRINT ISSN No 2277 - 8160

Original Research Paper

Pharmaceutical

# A VALIDATED UV SPECTROSCOPIC METHOD DEVELOPMENTFOR ESTIMATION OF INDOMETHACIN IN BULK AND ITS FORMULATION

| | |
|---|---|
| **Dr.K.Thejomoorthy** | Associate Professor,Malineni Lakshmaiah College Of Pharmacy, Kanumalla Road, Singaryakonda,Prakasam Dt-523101. |
| **A.Padma*** | Assistant professor, Sankar reddy institute of pharmaceutical sciences, Bestavaripeta, prakasm Dt-523101. *Corresponding author |
| **B. Snehith** | Department of Pharmaceutical Analysis, JNTUA- Oil Technological and Pharmaceutical Research Institute, Ananthapuramu-515001, A. P, India. |
| **S.Raghu** | Department of Pharmaceutical Analysis, JNTUA- Oil Technological and Pharmaceutical Research Institute, Ananthapuramu-515001, A. P, India. |
| **Karedla.Sai Siddhartha** | Department of Pharmaceutical Analysis, JNTUA- Oil Technological and Pharmaceutical Research Institute, Ananthapuramu-515001, A. P, India. |

**ABSTRACT** A novel UV Spectroscopic method was developed for the determination of purity of Indomethacin. The method was developed using Shimadzu 1800 UV-Visible double beam spectrophotometer with uv probe software with Ethanol used as solvent. The compound is monitored at 266 nm. The developed method was validated as per ICH guidelines with respect to specificity, linearity, limit of detection, limit of quantification, accuracy, precision and robustness. This method was also suitable for the assay determination of Indomethacin in pharmaceutical dosage forms.

**KEYWORDS** : Indomethacin, ethanol, ICH guidelines, Specificity, Linearity

## INTRODUCTION:

Indomethacin is a nonsteroidal anti-inflammatory drug(NSAID) commonly used as a prescription medication to reduce fever, pain, stiffness, and swelling from inflammation. It works by inhibiting the production of prostaglandins, molecules known to cause these symptoms[1-2]. Indomethacin is a non-steroidal antiinflammatory agent (NSAIA) with antiinflammatory, analgesic and antipyretic activity[3]. Its pharmacological effect is thought to be mediated through inhibition of the enzyme cyclooxygenase (COX), the enzyme responsible for catalyzes the rate-limiting step in prostaglandin synthesis via the arachidonic acid pathway[4]. Generally Chemically it is2-[1-(4-chlorobenzoyl)-5- methoxy-2-methylindol-3-yl]acetic acid.

From extensive survey of literature on Indomethacinreveals that few reported works UV spectroscopyfor the determination of Indomethacin pure drug and its pharmaceutical dosage form to the best of our knowledge. So the present emphasis was given to develop simple,selectivce, precise and accurate UV method for determination of Indomethacin in bulk and its tablet dosage form. The developed method to be validated in accordance to ICH Q2 (R1) guidelines[11-12].

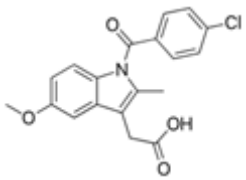


**Figure 1:Chemical structure of Indomethacin**

## MATERIALS AND METHODS

### Chemicals:

An analytically pure sample of Indomethacin was procured as gift sample from Jagsonpal laboratories (Hyderabad, India). Capsule formulation [Indocap],Pro laboratories Formulation Pvt. Ltd. India] was procured from a local pharmacy with labelled amount75mg per capsule.

Distilled water and ethanol was used as solvent for dilution of Indomethacin. Distilled water was prepared by distillation unit in Lab.

### Instrument used:

For the current study UV/VIS double beam spectrophotometer Shimadzu 1800 incorporated with UV probe software,having deuterium lamp.

## METHODOLOGY

### Selection of solvent:

The selection of solvent was done based upon the drug solubility, stability and absorbance maxima of the compound in the particular solvent. 10 mg of Indomethacin was weighed and solubility of this sample was checked in the 0.1N HCL, 0.01N NaoH, Methanol, Ethanol, Phosphate buffer pH6.8 and distilled water.From the reported studies as ethanol 50% was not used for the determination of Indomethacin. Hence the current method was developed in 50%v/v ethanol.

### Preparation of standard stock solution:

Indomethacin pure 100 mg was weighed and transferred to a 100 ml volumetric flask and dissolved in ethanol. It was dissolved properly and diluted up to the mark with diluent to obtain final concentration of 1000 µg/ml. 5µg/ml solution was prepared from the stock solution was prepared using distilled water, which was used as working standard.

### Preparation of sample solution:

Weight equivalent to 25 mg of drug Indomethacin tablet dosage form and transfer into 25 ml volumetric flask and dissolve in 50%v/v ethanol, the contents were sonicated for 5 min to enhance solubility of the drug and then finally made up to the volume. From this aliquot of 20 µg mL-1 was prepared and used.

## METHOD VALIDATION:

### Linearity and Range:

Calibration standards of Indomethacin covering the range 1-6µg/ml, were prepared with the suitable dilution made from stock solution. The calibration curves were obtained by plotting the intensity of absorbance against of concentration. The slope and

intercept of the calibration line were determined by linear regression using the least squares method.

**Precision:**

The precision of an analytical method is the degree of agreement among individual test results, when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as %RSD.

The intra & inter-day precision was evaluated by analyzing six sample solutions (n = 6), at the final concentration of analyses (5µg/ml) of Indomethacin. The Indomethacin concentrations were determined and the relative standard deviations (%RSD) were calculated.

**Accuracy:**

Accuracy is the closeness of the test results obtained by the method to the true value. Indomethacin reference standards were accurately weighed and added to a mixture of the tablets excipients, recovery studies were carried out by adding three different concentration levels 80%, 100% and 120% respectively. At each level, samples were prepared in triplicate and the recovery percentage was determined. The % recovery was calculated.

**Detection and quantification limits:**

Limit of detection LOD and limit of quantification LOQ were calculated by using the standard deviation from the precision and the slope of linearity.

**Limit of detection (LOD):**

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. From the standard stock solution 0.5 ml was pipette out into 10 ml volumetric flask and the volume was made up to the mark with distilled water. The Limit of detection was found to be 0.17273µg/ml.

**Limit of quantification(LOQ):**

Based on the LOD strength (0.5 mcg / ml, standard solution), the LOQ values were calculated by multiplication with three times. From the standard stock solution 0.5 ml was pipette out and transferred into 10 ml volumetric flask and volume was made up to the mark with distilled water. The Limit of Quantification was found to be 0.52343µg/ml.

**Robustness:**

The robustness of the method was determined to assess the effect of small but deliberate changes of the conditions on the determination of Indomethacin. The different variations are change of wave length by ± 2 nm from developed spectroscopic conditions. The concentration of the solution analyzed was 5µg/ml.

**RESULTS AND DISCUSSION**

Indomethacin exhibits maximum absorbanceat 266 nm. So determination of Indomethacin by UV spectrophotometric method was thus attempted. Beer's law was obeyed in the concentration range of 1 to 6µg/ml. Interday and intraday studies showed high degree of repeatability of an analytical method under normal operating conditions. The accuracy of the method was determined by investigating the recovery of the drugs using spiked concentrations of the standard drug. Precision was determined by analysis of capsule containing Indomethacin. The results were tabulated in the following tables.

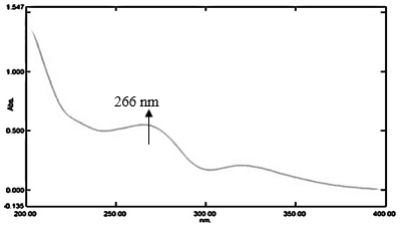


**Figure 2: UV spectra of Indomethacin showing absorbance at 266 nm**

**Table -1 Results Of Calibration Curve At 266 Nm For Indomethacin**

| S.No | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1 | 1 | 0.165 |
| 2 | 2 | 0.256 |
| 3 | 3 | 0.368 |
| 4 | 4 | 0.461 |
| 5 | 5 | 0.552 |
| 6 | 6 | 0.647 |

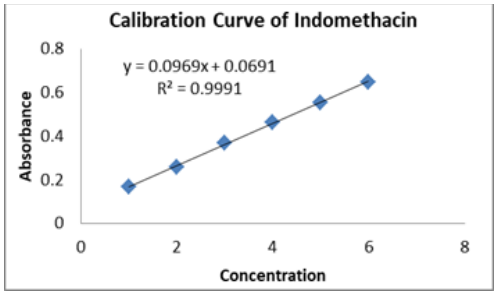


**Figure 3:Calibration curve for Indomethacin**

**Table-2 Precision Results Of Indomethacin**

| S.No | Precision | |
|---|---|---|
| | Intra day | Inter day |
| 1 | 0.561 | 0.548 |
| 2 | 0.549 | 0.551 |
| 3 | 0.545 | 0.563 |
| 4 | 0.551 | 0.546 |
| 5 | 0.554 | 0.543 |
| 6 | 0.549 | 0.542 |
| Avg | 0.5515 | 0.54883 |
| STD | 0.00502 | 0.00701 |
| %RSD | 0.91114 | 1.27724 |

**Table-3 Determination Of Accuracy Results For Indomethacin**

| S. No | Spike Level | Absorbance | µg/ml Added | µg/ml Found | % Recovery |
|---|---|---|---|---|---|
| 1 | 80 % | 0.461 | 1.98917 | 3.31408 | 166.02 |
| 2 | 100 % | 0.552 | 4.92292 | 4.9639 | 100.81 |
| 3 | 120 % | 0.647 | 8.95726 | 8.93 | 100.33 |

**Table-4 Results For Detection And Quantification Limits**

| S. No | Validation parameter | Result |
|---|---|---|
| 1 | Limit of detection (LOD) | 0.17273 µg/ml |
| 2 | Limit of Quantification (LOQ) | 0.52343µg/ml |

**Table-5 Results Of Robustness Studies**

| S. No | Robust condition | Parameter | Absorbance | % RSD |
|---|---|---|---|---|
| 1 | Wave length ± 2 nm | 264nm | 0.549 | 0.373 |
| 2 | | 266 nm | 0.552 | 0.225 |
| 3 | | 268nm | 0.550 | 0.308 |

**Table -6 The Total Summary Of Method Development And Validation Parameters**

| S No. | Parameters | Results |
|---|---|---|
| 1. | Absorption Maxima (nm) | 266 |
| 2. | Beer's-Lambert's range (µg/ml) | 1-6 |
| 3. | Regression equation (y) | Y = 0.096x + 0.069 |
| 4. | Slope (b) | 0.096 |
| 5. | Intercept (a) | 0.069 |
| 6. | Correlation coefficient (r2) | 0.999 |
| 7. | Intraday precision (% RSD) | 0.91114 |
| 8. | Interday precision (% RSD) | 1.27724 |

| | | |
|---|---|---|
| 9. | Accuracy (% mean recovery) | 99.06-100.56 |
| 10. | Limit of detection (µg / ml) | 0.17273 |
| 11. | Limit of quantification (µg / ml) | 0.52343 |
| 12. | Assay of tablets (%Purity) | 99.45 |

Y = bx + a where x is the concentration of Indomethacin in mcg / ml and Y is the absorbance at the respective λmax.

## CONCLUSION

A novel, simple and cost effective spectrophotometric method for the quantitative estimation of Indomethacin in bulk drug and pharmaceutical formulations have been developed. From the recovery studies noninterference of excipients was observed. Hence the method was found to be precise, robust and accurate. The developed method can be successfully used for routine analysis of Indomethacin in its pure and pharmaceutical formulation.

## REFERENCES


[1] Brayfield, A, ed."Indometacin". Martindale: The Complete Drug Reference. London, UK:Pharmaceutical Press. Retrieved 22 June 2014.

[2] "TGA Approved Terminology for Medicines, Section 1 – Chemical Substances" (PDF). Therapeutic Goods Administration, Department of Health and Ageing, Australian Government. July 1999: 70.

[3] Gouda (2011), "Spectrophotometric and spectrofluorimetric methods for the determination of non steroidal anti-inflammatory drugs, A review". Arabian Journal of Chemistry.

[4] Indomethacin, www.drug bank .in.

[5] Karima fadhil ali,zinah hussein ali (2015)."New assay method UV spectroscopy for determination of Indomethacin in pharmaceutical formulation". Journal of Chemical and Pharmaceutical Research.7(4),1591-1596.

[6] Dhaval D. patel,Vijay joguparthi(2011). "Maintainance of super saturation I: Indomethacin crystal growth kinetic modeling using an online second derivative ultra violet spectroscopic method". Journal of pharmaceutical sciences. 100(7), 2623-2641.

[7] Song- min huo,An – ping deng. "Development and validation of a highly sensitive ELISA for the determination of pharmaceutical Indomethacin in water samples".Science direct. 73(2), 380-386.

[8] Sunil jambhekar,T.maher(2004). "The physico chemical characteristics and bio availability Indomethacin from β- cyclodextrin,hydroxy ethyl-β-cyclodextrin,and hydroxyl propyl-β-cyclodextrin complexes". International journal of pharmaceutics. 270(2), 149-166.

[9] Neelam sawant,P.N.Sanjay pai (2016). "Development and validation of a stability indicating RP- HPLC method for the estimation of Indomethacin in pharmaceutical formulation". Journal of pharmaceutical research. 15(3).

[10] ICH: 12.Q2A, Text on validation of analytical procedure; 1994.

[11] ICH: Q2(R1),Validation of analytical procedures: text and methodology; 2005.