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# DEVELOPMENT AND VALIDATION OF A DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF IMEGLIMIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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## ABSTRACT

**Objective:** To develop a novel, simple and economical derivative spectrophotometric method as the literature survey indicated that no method had been reported till date for the estimation of Imeglimin a new tetrahydrotriazine-containing class of oral antidiabetic agents, the "glimins" and also there is a need for a validated UV spectrophotometric method to estimate the drug in bulk and pharmaceutical dosage forms. **Materials and Methods:** A UV spectrophotometric method was developed on Shimadzu UV-1800 double beam spectrophotometer using water as solvent for all measurements. The maximum absorption of Imeglimin was found to be at 237 nm for zero order and the absorbance's at its first and second derivatives were measured at 236 nm and 240 nm, respectively. **Results:** The developed derivative method proved to be linear in the concentration range of 2-12 µg/ml for Imeglimin and shows a good correlation coefficient. The precision of the developed method was less than the maximum permissible limit (% RSD < 2) set by the ICH guidelines. The limit of detection (LOD) and limit of quantification (LOQ) were 0.306 µg/ml and 0.9299 µg/ml, respectively. Excellent % recovery (98% - 101%) with less than 2% RSD indicates that the method was accurate, also found to be robust and rugged for the intended use. **Conclusion:** The developed UV method was simple, eco-friendly, precise and accurate as per ICH guidelines.

The proposed method can be used in quality control for routine analysis of Imeglimin in bulk and pharmaceutical dosage form.

**Keywords: derivation method, Imeglimin, first derivative, uv spectrophotometric method, validation**

## INTRODUCTION:

Imeglimin **[1]** is the first of the "glimins," **[2]** a new class of glucose-lowering **[3]** drug developed for the treatment of type 2 diabetes mellitus (T2DM) **[2]**. Imeglimin, a dihydro-1, 3, 5-triazine that has been studied as a potential novel anti-diabetic medication is a cyclic Metformin derivative **[4]**. Correcting abnormalities in both insulin secretion and insulin sensitivity **[5]** is a requirement for achieving optimal glucose management in type 2 diabetes **[6]**. Imeglimin has a special method of action that specifically targets the three pathophysiologic elements of type 2 diabetes: decreased muscle glucose uptake, excessive hepatic gluconeogenesis, and increased apoptosis in beta cells. It is an oxidative phosphorylation inhibitor that reduces hepatic gluconeogenesis, boosts muscular glucose uptake, and returns insulin levels to normal. Recent phase II and phase III studies have demonstrated the efficacy of Imeglimin alone and in combination with other hypoglycemic agents, in improving glycated hemoglobin (HbA1c) and fasting plasma glucose (FPG) levels **[7]**. Therefore, it may be suitable for safe and effective combination with other drugs commonly used to treat type 2 diabetes and its common complications.

This is a crystalline white powder, very soluble in water **[8]**, methanol, and ethanol. Chemically, it is (6R)-(+)-4-dimethylamino-2-imino-6-methyl-1,2,5,6-tetrahydro-1,3,5-triazine**[9]** **Figure 1,** with a molecular weight of 191.6 and a molecular formula of $C_6H_{14}ClN_5$.

## OBJECTIVE:

After reviewing literature, no derivative spectrophotometric method **[10, 11]** has been reported till date for determination of Imeglimin. As Imeglimin is a recently launched antidiabetic drug, a validated UV spectrophotometric method for determining Imeglimin in pharmaceutical preparations is needed. In this study, we developed and validated a new derivative spectroscopy method for the evaluation of Imeglimin in formulations according to ICH recommendations.

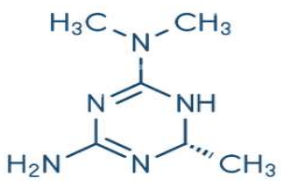

**Figure 1: Structure of Imeglimin**

## MATERIALS AND METHODS

### Equipment:

The proposed work was carried out on a UV-1800 make is **SHIMADZU** and UV-3200 make is **LAB INDIA** UV- Visible spectrophotometer, with a 1 cm quartz matched cells, weighing was done on electronic balance **(Shimadzu-BL220H**), sonicated with **Sonica**, Ultrasonic cleaner, Spincotech PVT LTD.

### Chemicals and reagents:

Imeglimin standard was obtained as gift sample from Dr.Reddy's Laboratories, Hyderabad, **(Imextor)** tablets with label claim 500 mg manufactured by Exmed Pharmaceuticals were purchased from local market.

### Preparation of Stock and Working Solution:

As the drugs was found to be soluble in water. The standard stock solution was prepared by dissolving 25 mg of Imeglimin with little amount of water and then the volume was made upto 25 ml with distilled water to acquire a concentration of 1000 µg/ml and sonicated for 10 minutes to ensure complete solubilization. A working standard solution of 10 µg/ml was prepared by appropriately diluting the stock solution with distilled water.

### Appropriate wavelength selection for Imeglimin analysis

**Method I** (Zero order) preparation of 10 µg/ml working standard and scanning at 200–400 nm UV; Imeglimin has an absorbance maximum at 237 nm.

**Method II** (First derivative UV-Spectrophotometry using amplitude), generated an absorption spectrum of Imeglimin and recorded the amplitude at 236 nm.

**Method III** (Second derivative UV-Spectrophotometry using amplitude); these derived spectra have a narrow spectral bandwidth. This result in better resolution and the appearance of overlapping bands those are lost in the original spectrum. Therefore, it is advantageous to select the correct wavelength. Additionally, measurements of analyte concentrations with interference or methods in which two or more analyte in a mixture are derived can be performed more simply and accurately.

The absorption spectrum of Imeglimin was converted to second order and the amplitude was recorded at 240 nm. The selection of the wavelength in all the methods is shown in **Figure 2**.

**Calibration curve preparation**

Standard stock solution was appropriately diluted to obtain final concentrations in the range of 2-12 µg/ml. For each prepared solution, absorbance was measured at the wavelengths selected above. A calibration curve was drawn between concentration and absorbance with a correlation coefficient of 0.999, 0.998 and 0.998 for zero order, first derivative and second derivative respectively.

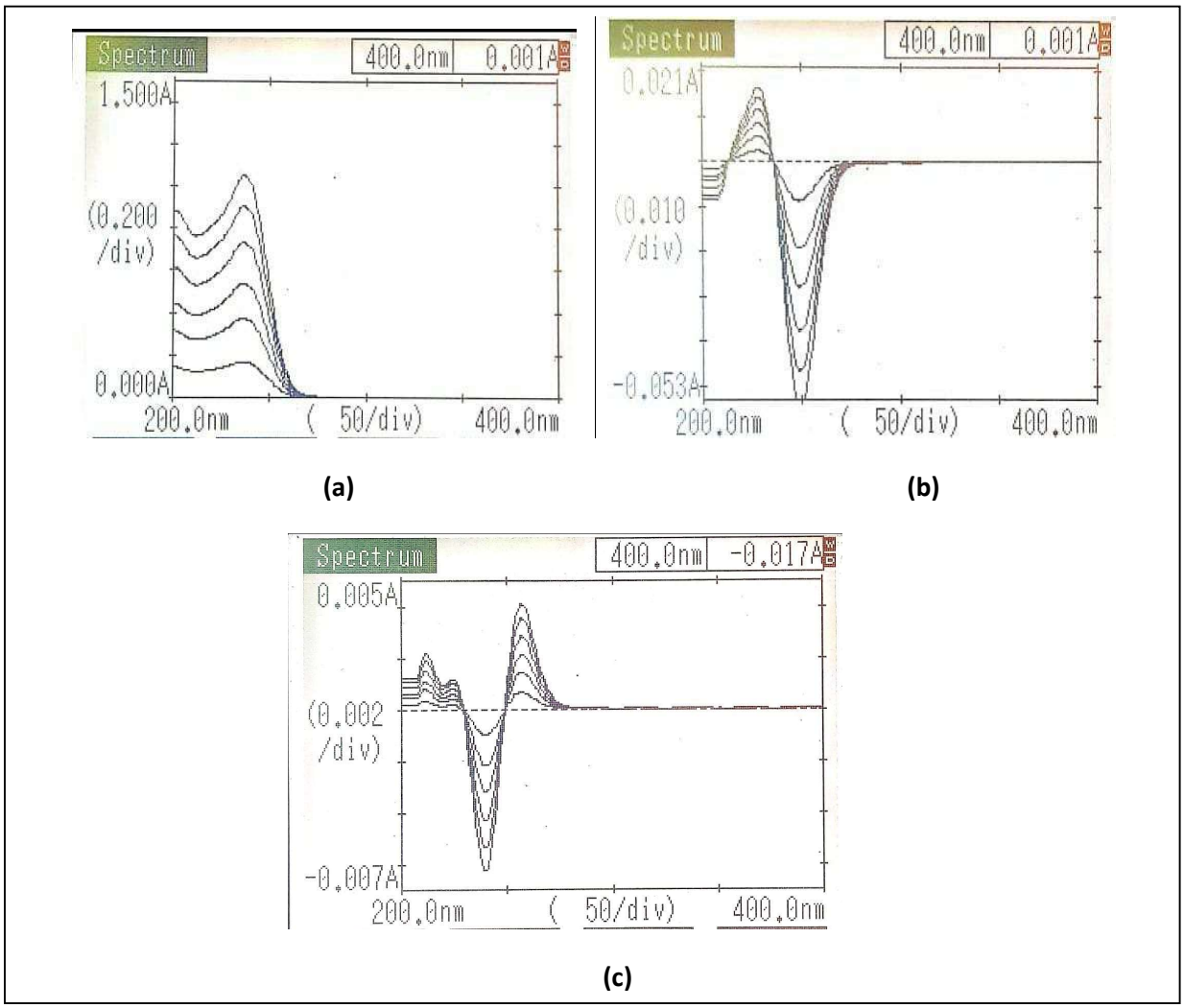


**Figure 2: (a) Zero order (b) First derivative (c) Second derivative spectrum of Imeglimin**

**ANALYSIS OF THE MARKETED FORMULATION:**

10 tablets were weighed and powdered. A powdered tablet equivalent to 100 mg of Imeglimin was precisely weighed, transported to a 100 ml volumetric flask, dissolved in water using a sonicator, and then the remaining capacity was filled with water. The resultant solution was filtered using Whatman filter paper, and 1 ml of the filtrate was added

to a 10 ml volumetric flask to make the required amount of solution. The volume was adjusted with water up to 10 ml to obtain a solution with a concentration of 100 µg/ml, which was then further diluted to create a 10 µg/ml concentration. Tablet excipients that are typically present did not interfere. In the % Analysis, the zero order percentages were 98.71%, the first derivative was 98.70%, and the second derivative was 99.21%.

Weight of 10 Tablets =5620 mg

10 Tablets average weight = 5620/10 =562 mg

$$\text{Weight to be taken} = \frac{\text{Average weight X Equivalent Weight}}{\text{Label claim}}$$

$$= \frac{562 \text{ mg X } 100 \text{ mg}}{500 \text{ mg}} = 112 \text{ mg}$$

$$\text{Assay} = 100 * \frac{\text{Absorbance Sample}}{\text{Absorbance Standard}} * \frac{\text{Concentration of Standard}}{\text{Concentration of Sample}}$$

**Table 1: Analysis of Market Formulation of Imeglimin**

| S. No | METHOD | LABEL CLAIM | Standard Absorbance (10 µg/ml) | Sample Absorbance (10 µg/ml) | % ASSAY |
|---|---|---|---|---|---|
| 1 | Zero order | 500mg | 0.921 | 0.933 | 98.71 |
| 2 | First derivative | 500mg | 0.914 | 0.926 | 98.70 |
| 3 | Second derivative | 500mg | 0.904 | 0.912 | 99.12 |

## METHOD VALIDATION

Methodology validation is the process of building narrative evidence that a system, procedure, or movement has been implemented or tested and maintained a desired level of consistency through all phases. Approved scientific strategies are essential for improving diagnostic techniques and have been time and again tested for specificity, linearity, accuracy, precision, range, limits of detection, and quantization cutoffs. In summary, the development and approval of a systematic strategy confirms that accurate and reliable potency estimation of medicinal products has been performed.

### Validation Parameters

Validation parameters are used to provide documented evidence that the performance of a method meets the requirements of the intended analytical application. The purpose of the verification is to demonstrate that the analytical results obtained using a particular method are suitable for that purpose and are as specified below.

**Method Validation:**

This study was conducted to develop a new inexpensive and convenient method for the spectroscopic determination of Imeglimin. The method was tested for linearity, accuracy, precision and reliability according to the ICH guidelines.

**Linearity**

The Linear relationships must be evaluated using a variety of analysis methods. A minimum of 5 concentrations is recommended to establish linearity. It can be directly detected with API by diluting the standard stock solution with the proposed method. The correlation coefficient, the point of intersection with the ordinate, and the slope of the regression line should be provided.

**Accuracy**

To ensure the reliability of the above method, recovery studies were performed by mixing known amounts of the standard drug with formulation samples. Recovery studies were conducted at three different levels of 50%, 100% and 150% levels. The contents were analyzed using the proposed zero order, first derivative and second derivative spectroscopy.

**Precision**

The precision of the method is the degree of agreement between individual test results when the method is repeated on multiple samples of a homogeneous sample. Validation of analytical methods requires not only the performance of feature parameters, but also statistical processing of analytical data for both Intraday and Interday. These treatments determine the tolerance for variation in analytical data. The precision of analytical procedure is usually expressed as the variance, standard deviation, or coefficient of variation of a set of measurements.

**Reproducibility**

Reproducibility is also referred to as intra-assay precision. Reproducibility is defined as short-term accuracy under identical conditions of use.**Robustness**

Robustness evaluation should be considered during the design phase and depends on the type of method being studied. The reliability of the assay with respect to intentional changes to method parameters should be demonstrated.

**LOQ (Limit of Detection) and LOQ (Limit of Quantification)**Response standard deviation and determined by the linearity slope.

The detection limit (DL) can be expressed as: DL = 3.3 σ/ S where σ = standard deviation of the response, S = slope of the calibration curve. The limit of quantification (LOQ) can be expressed as LOQ = 10 σ/S. where σ = standard deviation of the response S = slope of the calibration curve. The slope can be estimated from the calibration curve of the analyte.

**RESULTS AND DISCUSSION:**

**Linearity**

The standard stock solution of the drug was prepared by dissolving 10 mg of the drug in 10ml of water (because the drug is water-soluble) and then adjusted to 10ml with water to obtain a concentration of 1000 µg/ml. Further dilution of this solution with water yields a range of solutions containing various concentrations from 2-12 µg/ml. Absorbance was recorded at λ max 237 nm. The equation of the calibration curve using UV spectroscopy was found to be y = 0.096 x - 0.019 for method I with $R^2$ = 0.998 and y = 0.099 x -0.011 for method II with $R^2$ = 0.999 and y=0.094x-0.012 for Method-II with $R^2$=0.998. The absorbance was plotted for each concentration to obtain a calibration curve shown in **Figure 3** and data can be found in (**Table 2)**.

**Table 2: Standard curve data of Imeglimin**

| S. No. | Concentration in µg/ml | Method- I 237 nm | Method- II 236 nm | Method- III 240 nm |
|---|---|---|---|---|
| 1 | 2 | 0.19 | 0.188 | 0.187 |
| 2 | 4 | 0.369 | 0.366 | 0.363 |
| 3 | 6 | 0.556 | 0.551 | 0.547 |
| 4 | 8 | 0.750 | 0.737 | 0.74 |
| 5 | 10 | 0.94 | 0.946 | 0.904 |
| 6 | 12 | 1.15 | 1.157 | 1.141 |
| Correlation coefficient | | 0.998 | 0.999 | 0.998 |
| Slope | | 0.096 | 0.095 | 0.094 |

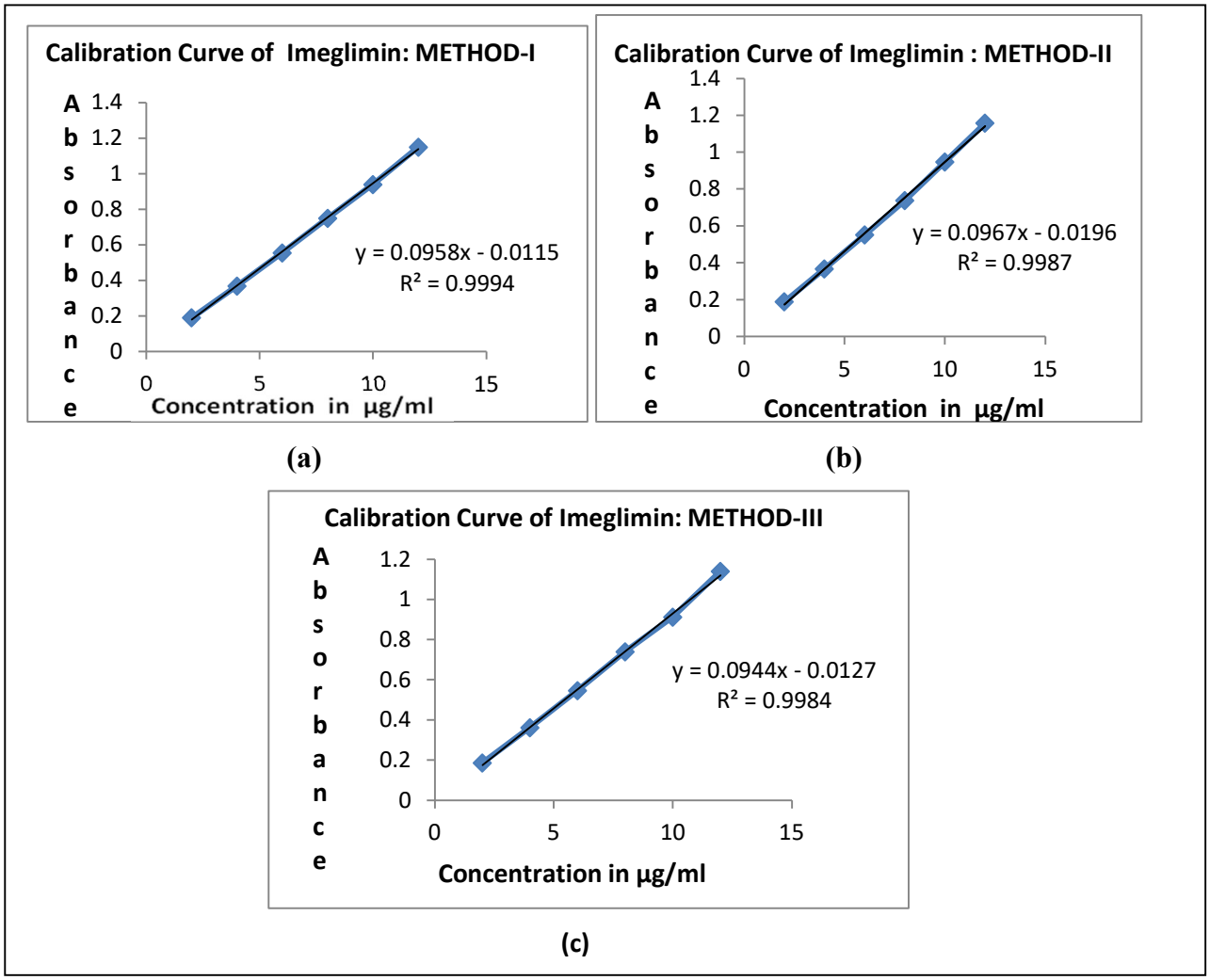


**Figure 3: Plot of Linearity and Range for (a) zero order (b) First derivative (c) Second derivative of Imeglimin**

**Precision:**

By examining the corresponding responses six times on the same day and six other days in the same concentration (10 µg/ml) of Imeglimin standard solution without altering the method parameters, the intra- and inter-day precision of the proposed approach was assessed. Relative standard deviation is used to express results (%RSD). The outcomes from UV-Spectroscopy for intraday and interday variations are displayed in **(Table 3)**.

**Accuracy:**

To test the accuracy of the method, a recovery study was performed by adding the drug standard solution (100 µg/ml) to the pre-analyzed sample solution (100 µg/ml) at three levels: 50%, 100%, and 150%. The drug concentration and recovery (%) were

determined by a linear equation. The results obtained are presented in (**Table 4)**.

**Robustness:**

By doing the study under different detection wavelength (±237 nm) circumstances and observing the impact on absorbance, the robustness of the approach was assessed. This approach has consistently worked. Results for Imeglimin robustness are displayed in **(Table 5)**.

## Ruggedness:

By doing two separate analyses by 2 different analysts 6 times for 10 µg/ml, noting the respective absorbance, and reporting the results as a percentage RSD, ruggedness was established. The outcome of Imeglimin toughness is depicted in **(Table 6)**.

**Limit of detection (LOD) and limit of quantification (LOQ):**

The formulas LOD = 3.3*/S and LOQ = 10*/S are used to determine LOD and LOQ from linearity data, where is the standard deviation of the y intercept of the linearity equation and S is the slope of the analytical calibration curve. Calculations as in found that the LOD and LOQ for UV spectroscopy were 0.3068 µg/ml and 0.9299 µg/ml, respectively **(Table 7)**.

**Specificity:**

The Proposed method was specific as none of the excipients interfered with the studied drug so the method can be applied for assaying the commercial dosage forms.

**Table 3: Intraday and Interday precision of Imeglimin**

| INTRADAY | | | | INTERDAY | | | |
|---|---|---|---|---|---|---|---|
| **Sample No** | **Method- I 237 nm** | **Method- II 236 nm** | **Method- III 240 nm** | **Day** | **Method- I 237 nm** | **Method- II 236 nm** | **Method- III 240 nm** |
| **1** | **0.91** | **0.903** | **0.893** | **Day 1** | **0.906** | **0.903** | **0.899** |
| **2** | **0.915** | **0.91** | **0.91** | **Day 2** | **0.903** | **0.897** | **0.901** |
| **3** | **0.916** | **0.909** | **0.899** | **Day 3** | **0.906** | **0.903** | **0.893** |
| **4** | **0.926** | **0.92** | **0.91** | **Day 4** | **0.93** | **0.925** | **0.922** |
| **5** | **0.931** | **0.924** | **0.915** | **Day5** | **0.9** | **0.891** | **0.882** |
| **6** | **0.937** | **0.93** | **0.921** | | | | |
| **Mean** | **0.9196** | **0.916** | **0.908** | **Mean** | **0.9097** | **0.9038** | **0.8994** |
| **SD** | **0.0104** | **0.0102** | **0.0103** | **SD** | **0.012** | **0.0128** | **0.0146** |
| **%RSD** | **1.1309** | **1.1135** | **1.134** | **%RSD** | **1.3191** | **1.4162** | **1.6276** |

**Table 4: Accuracy studies for Method-I, Method-II and Method-III at three concentration level**

| METHOD -I | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **DRUG** | **% LEVEL** | **Volume of sample taken (ml)** | **Volume of standard added (ml)** | **Final Concentration in (µg/ml)** | **Mean of Absorbance (n=3)** | **% Recovery** | **Mean** | **SD** | **%RSD** |
| **IMEGLIMIN** | **50** | **0.4** | **0.2** | **6** | **0.454** | **99.5** | **99.5** | **1.2** | **1.206** |
| | **100** | **0.4** | **0.4** | **8** | **0.6713** | **100.7** | | | |
| | **150** | **0.4** | **0.6** | **10** | **0.8826** | **98.3** | | | |
| **METHOD -II** | | | | | | | | | |
| **DRUG** | **% LEVEL** | **Volume of sample taken** | **Volume of standard added (µg/ml)** | **Final Concentration in (µg/ml)** | **Mean of Absorbance (n=3)** | **% Recovery** | **Mean** | **SD** | **%RSD** |
| **IMEGLIMIN** | **50** | **0.4** | **0.2** | **6** | **0.458** | **99.5** | **99.4** | **1.1** | **1.1** |
| | **100** | **0.4** | **0.4** | **8** | **0.6766** | **100.5** | | | |
| | **150** | **0.4** | **0.6** | **10** | **0.8893** | **98.3** | | | |
| **METHOD -III** | | | | | | | | | |
| **DRUG** | **% LEVEL** | **volume of samples taken** | **Volume of standard added (µg/ml)** | **Final Concentration in (µg/ml)** | **Mean of Absorbance (n=3)** | **% Recovery** | **Mean** | **SD** | **%RSD** |
| **IMEGLIMIN** | **50** | **0.4** | **0.2** | **6** | **0.451** | **99.5** | **99.5** | **1.1** | **1.1** |
| | **100** | **0.4** | **0.4** | **8** | **0.6656** | **100.6** | | | |
| | **150** | **0.4** | **0.6** | **10** | **0.874** | **98.4** | | | |

**Table 5: Robustness of Imeglimin**

| S. No. | Wave length (nm) | Mean of Absorbance (n=6) | Mean % Recovery | Mean | SD | % RSD |
|---|---|---|---|---|---|---|
| **1** | **237** | **0.921** | **100.7** | **0.913** | **0.00854** | **0.9358** |
| **2** | **236** | **0.914** | **100** | | | |
| **3** | **240** | **0.904** | **99** | | | |

**Table 6: Ruggedness of Imeglimin**

| RESULTS OF ANALYST TO ANALYST VARIATION | | | | | |
|---|---|---|---|---|---|
| **S.NO** | **Different analyst** | **Weight of tablet powder taken(mg)** | **Method absorbance (n=6)** | | |
| | | | **Method-I** | **Method-II** | **Method-III** |
| **1** | **Analyst-I** | **112.4** | **0.906** | **0.903** | **0.899** |
| **2** | **Analyst-II** | **112.4** | **0.903** | **0.897** | **0.901** |
| **Mean** | | | **0.9045** | **0.9** | **0.9** |
| **SD** | | | **0.002121** | **0.00424264** | **0.00141421** |
| **%RSD** | | | **0.23453** | **0.47140452** | **0.15713484** |

**Table 7: LOD & LOQ values of Imeglimin**

| Parameter | Method-I | Method-II | Method-III |
|---|---|---|---|
| LOD | 0.3068 | 0.4625 | 0.5979 |
| LOQ | 0.9299 | 1.4016 | 1.812 |

## CONCLUSION:

Based on a calibration curve and a derivative approach, the suggested derivative spectrophotometric method was created for the determination of Imeglimin. This approach has been tested and is simple, sensitive, precise and accurate. The excipients typically contained in tablets were not interfering in any way. This technique can be used successfully for regular analysis of Imeglimin in pharmaceutical formulations because it has been validated in accordance with ICH recommendations.

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**CONFLICTS OF INTEREST:** The authors declare that there is no conflict of interests regarding the publication.

## ABBREVIATION USED:

**API**: Active pharmaceutical ingredient, **RSD**: Relative standard deviation, **SD**: Standard deviations.

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