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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF TRIMETAZIDINE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT: A simple and sensitive UV spectrophotometric method has been developed for the quantitative estimation of trimetazidine in bulk drug and pharmaceutical dosage forms (tablets). Trimetazidine exhibited absorption maximum at 269 nm in 0.1 N HCl and obeyed Beer's law in concentration range 5-25 µg/ml. The result of analysis in this method has been validated statistically and by recovery studies. This method is extended for the analysis of drug in pharmaceutical formulations.

INTRODUCTION: Trimetazidine [1],chemically 1-[(2,3,4-trimethoxyphenyl)methyl] piperazine, (figure 1)is a cellular acting anti ischemic agent. It acts by inhibiting mitochondrial long chain 3-KAT, thus favoring glucose oxidation at the expense of fatty acid oxidation. This drug is official in IP and BP. From literature review it is known that several spectrophotometric [2, 3], HPLC [4, 5], HPTLC methods [6] have been developed for the determination of trimetazidine.

Though modern methods of analysis for purity, assay of any drug afford simplicity, speed, good specificity and excellent precision and accuracy, they involve sophisticated instruments which are not in the reach of most laboratories and small scale industries. So an attempt was made to develop simple, sensitive UV spectrophotometric method for the quantitative estimation of trimetazidine in bulk drug and pharmaceutical dosage forms. In this method, trimetazidine exhibits absorption maximum at 269 nm in 0.1 N HCl and obeyed Beer's law in concentration range 5-25 µg/ml. The result of analysis in this method has been validated statistically and by recovery studies. The method is extended for the analysis of drug in pharmaceutical formulations.

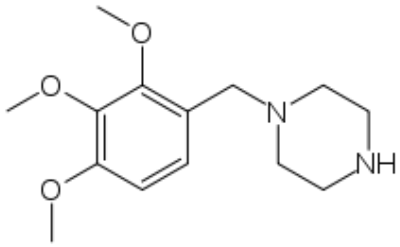

FIG. 1: STRUCTURE OF TRIMETAZIDINE [7]

EXPERIMENTAL

Instruments used: All spectral measurements were done on Elico double beam UV-Vis spectrophotometer Model SL 210.

Reagents: Analytical grade reagents were used.

(i) Hydrochloric acid (0.1 N, Qualigens).
(ii) Double distilled water

Working standard of Drug Solution: A 1mg/ml solution of trimetazidine was prepared by dissolving 100mg of Trimetazidine in 100 ml of distilled water. From this solution 1ml was taken into a 10ml volumetric flask and diluted to the volume with distilled water to produce a concentration of 100µg/ml.

Sample preparation: Ten tablets of commercial samples of Trimetazidine were accurately weighed and powdered. An amount of powder equivalent to 50mg was weighed separately and add 10ml of 0.1N HCl. shake vigorously and dilute to 50 ml with 0.1N HCl. The solution was filtered.

Assay: Prepare 20µg/ml solution from previously prepared 1mg/ml solution of sample by using 0.1N HCl. The absorbance was measured and it was compared with that of the standard. Total content present and the % purity were calculated.

Optimization of Reaction conditions: The optimum conditions for quantitative estimation of the drug were established via a number of preliminary experiments.

Selection of Solvent: The solubility of Trimetazidine was determined in a variety of solvents as per Pharmacopoeial standards. Solubility tests for Trimetazidine were performed using various solvents. As per solubility, HCl was selected as a solvent to develop the method.

Selection of detection Wavelength: Sensitivity of the method depends upon the proper selection of wave length. An ideal wave length is one that gives good response for all the components to be detected.

Trimetazidine solution of concentration 15µg/ml was prepared from standard stock solution using 0.1N HCl and it was scanned between 200 to 400 nm. The λ max of Trimetazidine was found to be 269 nm. The spectrum was shown below in **fig. 2**.

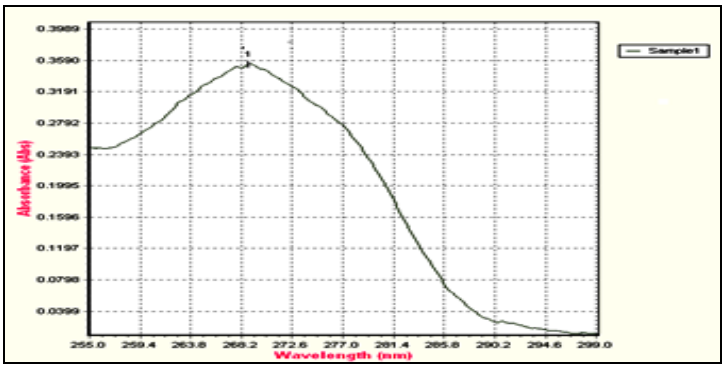

FIG. 2: SPECTRUM OF TRIMETAZIDINE

Validation: Validation is one of the most important steps in method development for analytical determinations. The main validation parameters such as linearity and range, accuracy and precision, LOD, LOQ, ruggedness were evaluated for the developed method.

1. **Linearity:** The calibration curve was constructed by considering the absorbance measured at five concentration levels of Trimetazidine i.e.(5-25µg/ml) and was shown in fig 3. Using the method of least squares, a line of best fit was taken and the correlation coefficient, slope, and y-intercept were calculated. The amount of drug was computed either from calibration curve or from regression equation. Linearity readings were given in **table 1**.

2. **Precision:** Six individual preparations of Trimetazidine drug substance were prepared with a target concentration of about 15µg/ml and their absorbances were measured and their %RSD was calculated. And the results were tabulated in **table 2.**

3. **Specificity:** The specificity of the method was demonstrated by establishing a lack of interference from the diluent blank. Blank solution was prepared and its absorbance was measured. No interference was measured with the blank.

4. **Accuracy:** Accuracy was studied through recovery experiments at the level of 3 concentrations 15ug/ml, 20ug/ml, and 25ug/ml. All the 3 concentrations were prepared and their absorbance was measured. % recovery was calculated for the 3 concentrations. And were tabulated in **table 3**.

5. **Ruggedness:** Analyst variation and instrument variation were observed by taking 15µg/ml and their %RSD was calculated. And the results were tabulated in **table 4 and 5**.

6. **Robustness:** Wavelength variation was checked at the level of 3 concentrations 15ug/ml,20ug/ml, and 25ug/ml concentration . All the 3 concentrations were prepared and their absorbance was measured at 3 different wavelengths such as 268 ,269 and 270nm and their %RSD was calculated. And the results were tabulated in **table 6**.

7. **LOD & LOQ:** Limit of detection was calculated using the formula;

$$3.3\sigma/s$$

Limit of quantification was calculated using the formula;

$$10\sigma/s$$

Where σ is standard deviation and s is the slope.

Assay: The method was applied to marketed formulation and the amount found in the sample solution was found. Results were given in **table 7.**

RESULTS AND DISCUSSION: The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity are presented in Table 8.The regression analysis using the method of least squares was made for the slope (b), intercept (a) and correlation(r) from different concentrations and results are summarized in **Table 8.** The results showed that this method has reasonable precision.

1. **Linearity**:

TABLE 1: LINEARITY READINGS

Concentration(µg/ml)	Absorbance
5	0.175
10	0.276
15	0.377
20	0.479
25	0.562

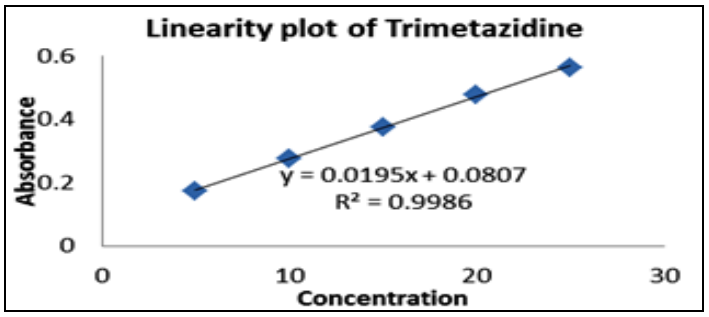

FIGURE 3: LINEARITY PLOT

2. **Precision:**

TABLE 2: REPEATABILITY READINGS

Concentration (µg/ml)	Absorbance
15	0.3334
15	0.3329
15	0.3332
15	0.3331
15	0.3336
15	0.3328
Average	0.333167
Standard deviation	0.000301
% RSD	0.090345

%RSD was found to be within the limits. So the method was found to be precise.

3. **Specificity**: Absorbance of the blank solution was measured and it was found to be very negligible. Absorbance of blank: -0.0729. Hence no interference with blank solution was observed. This indicates the specificity of the method.

4. **Accuracy**:

TABLE 3: ACCURACY READINGS

Concentration (µg/ml)	Absorbance	% Recovery
15	0.3682	98.99%
20	0.469	99.57%
25	0.5746	100.88%

% Recovery was found to be within the limits. This indicates that the method is accurate.

5. **Ruggedness:**

TABLE 4: ANALYST VARIATION READINGS

Concentration (µg/ml)	Analyst-1	Analyst-2
15	0.3334	0.3339
15	0.3329	0.3323
15	0.3332	0.3332
15	0.3331	0.3333
15	0.3336	0.3328
15	0.3328	0.3335
Average	0.333167	0.333167
Standard deviation	0.000301	0.000557
% RSD	0.090345	0.167183

TABLE 5: INSTRUMENTAL VARIATION READINGS

Concentration (µg/ml)	Instrument-1	Instrument-2
15	0.3334	0.3325
15	0.3329	0.3314
15	0.3332	0.3351
15	0.3331	0.3326
15	0.3336	0.3341
15	0.3328	0.3316
Average	0.333167	0.332883
Standard deviation	0.000301	0.001447
% RSD	0.090345	0.43468

% RSD was found to be within the limits. This indicates that the method is rugged.

6. **Robustness**:

TABLE 6: WAVE LENGTH VARIATION READINGS

Concentration (µg/ml)	268nm	269nm	270nm
15	0.365	0.377	0.376
20	0.371	0.375	0.377
25	0.372	0.378	0.377
	0.369333	0.376667	0.376667
	0.003786	0.001528	0.000577
	1.0250	0.40566	0.15318

% RSD was found to be within the limits. This indicates that the method is robust.

RESULT:

TABLE 7: ASSAY

Label claim	Amount found	Assay
20mg	20.296	101.48%
20mg	20.42	102.1%

Summarised Table:

TABLE 8: SUMMARISED TABLE

Parameter	Value
λmax (nm)	**269**
Beer's law limits (µg/ml)	**5-25**
Molar absorptivity (1 mol^{-1} cm^{-1})	**66938.226**
Linear regression equation	**Y=0.0195x + 0.0807**
Intercept (a)	**0.0807**
Slope (b)	**0.01954**
Correlation coefficient(r^2)	**0.9986**
Variance (So^2)	**0.023896**
Relative standard deviation (%)	**NMT 2%**
Recovery (%)	**98-102%**
LOD	**1.2 µg/ml**
LOQ	**3.6 µg/ml**

CONCLUSION: The proposed method is found to be simple, sensitive, selective, economical, accurate and precise and can be used for the determination of trimetazidine in bulk drug and its pharmaceutical dosage forms in a routine manner

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REFERENCES:

1. Marzilli M., Trimetazidine: a metabolic agent for the treatment of stable angina, European Heart Journal Supplements (2001) 3 (Supplement O), O12–O15 .
2. Ganesh M., Jeraldmaria Antony G., Saravankumar A., Rajesh R., Rajasekar K. A New Validated Spectrophotometric Method for determination of Trimetazidine in formulation and comparision with UV method. : Der PharmaChemica, 2009 1(2): 97-104 .
3. Ibrahim A. Darwish., Kinetic spectrophotometric methods for determination of trimetazidinedihydrochloride. Analytica ChimicaActa 551 (2005) 222-231
4. Naushad M. A., , F. J. Ahmad M. Aqil A. Ali, Faisal M. S., Rizwan M. and Faiyaz S. Development and validation of the HPLC method for the analysis of trimetazidine hydrochloride in bulk drug and pharmaceutical dosage forms. , Journal of Analytical Chemistry 2008, 63(10): pg 965-970.
5. Simmy O, Thoppil, P.D. Admin, Trimetazidine: stability indicating RPLC assay method, Journal of Pharmaceutical and Biomedical Analysis 2001, May, Vol. 25, Issue 2, 191-195
6. Simmy O Thoppil, Rita M Cardoza. , Stability indicating HPTLC determination of Trimetazidine as bulk drug and in pharmaceutical formulations. :Journal of Pharmaceutical and Biomedical Analysis 2001, April, Vol. 25, Issue 1, 15-20
7. pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=21109.

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