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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TRIMETAZIDINE BY EXTRACTIVE SPECTROPHOTOMETRY

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ABSTRACT: Rapid, simple, sensitive and accurate spectrophotometric methods have been developed for the determination of Trimetazidine in pure and pharmaceutical formulations. Various methods were developed for estimation of trimetazidine .but a simple extractive photometric method using has not been developed .This method is based on the formation of chloroform soluble ion pair complex of Trimetazidine with bromothymol blue (BTB) in buffer of pH 5.0 with absorption maximum at 409nm. Reaction conditions were optimised to obtain the maximum colour intensity. The absorbance was found to increase linearly with increase in concentration of Trimetazidine. Calibration graph was plotted and the correlation coefficient was found to be 0.998.Beer's law was obeyed in the concentration range of 1- 7 µg/ml with molar absorptivity of 30*10^3 l /mol /cm .Various analytical parameters have been evaluated and the results have been validated by statistical data. This method has been successfully applied for the assay of drug in pharmaceutical formulations. No interference was observed from common pharmaceutical adjuvants.The proposed method is simple, accurate and suitable for quality control applications.

INTRODUCTION: Trimetazidine chemically 1-[(2,3,4-trimethoxyphenyl)methyl]piperazine, (**figure 1**) is a cellular acting anti ischemic agent [5]. It acts by inhibiting mitochondrial long chain 3-KAT thus favouring 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, HPLC methods 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 accurate spectrophotometric methods for determination of Trimetazidine by formation of an ion pair complex with bromothymol blue at pH 5.

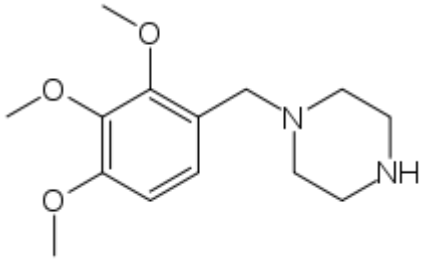

FIG. 1: STRUCTURE OF TRIMETAZIDINE [6]

EXPERIMENTAL:

Instruments: Spectral and absorbance measurements were made on an ELICO SL 210 UV –Vis spectrometer by using 1cm quartz cells. Shimadzu BL 2208 electronic balance was used for weighing the sample.

Reagents and standards: Bromothymol blue solution was prepared by dissolving 0.1g of bromothymol blue in 100ml of 50%v/v ethanol. pH 5 buffer was prepared by dissolving 0.68 g of potassium dihydrogen phosphate in 100ml of water and adjust the pH to 5 with 10M potassium hydroxide as per I.P.

Trimetazidine pure drug was obtained as a gift sample from Orchid health care. Formulation used for studies was developed by Cipla with brand name trivedon -20.

All chemicals and reagents used were of analytical grade.

Preparation of stock solution of drug: 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.

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

Choice of organic solvent: A number of organic solvents such as chloroform, carbon tetrachloride, dichloromethane, benzene and toluene were examined for the extraction of ion pair complex in order to provide an applicable extraction procedure. Chloroform was preferred for its selective extraction of ion pair complex from the aqueous solution. Shaking time of 0.5-4min provided a constant absorbance and hence 2 min was used as an optimum shaking time throughout the experiment.

Selection of pH of buffer: The drug reagent complex extracted into the organic solvent should be stable. In order to determine the pH in which the complex formed is stable, various trials were carried out using phosphate buffers of pH 2,4,5,7. It was observed that maximum stability was observed with pH 5 buffer as evidenced by its maximum absorbance. Hence pH 5 buffer was selected to develop the method and a volume of 1ml was used for the ion pair formation.

Effect of dye concentration: The effect of dye concentration on the intensity of the color developed at the selected wavelength and constant trimetazidine concentration was critically examined using different milliliters of reagent . The results indicated that the maximum absorbance was found with 1ml of reagent and beyond which the absorbance became constant. Therefore 1ml of dye stuff was used for ion pair formation through out the experiment.

Extraction procedure: In to a series of 50 ml separating funnel appropriate volume of Trimetazidine solution was placed followed by 1.0 ml of buffer and 1.0 ml of BTB reagent and shake well . Then 10 ml of chloroform was added to each funnel. The contents were shaken for 2 min and the two layers were allowed to separate.Extract was scanned between 400 to 800 nm. The λ max of Trimetazidine was found to be 409 nm. The spectrum was shown in **figure 2**.

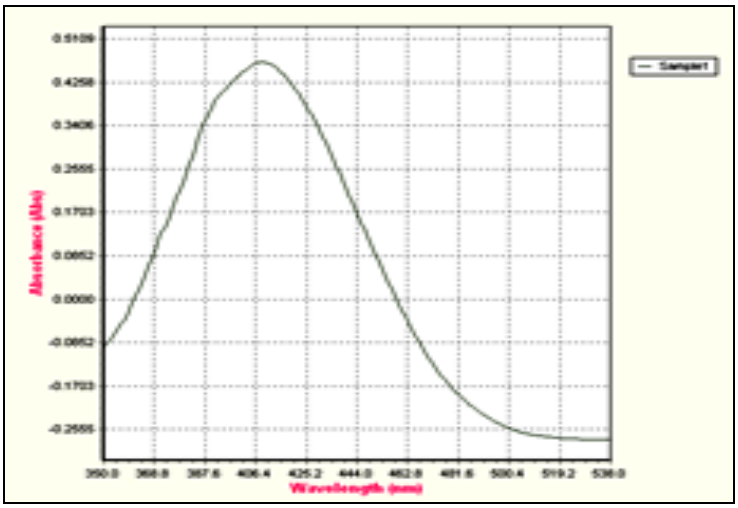

FIGURE 2: SPECTRUM OF TRIMETAZIDINE

Ruggedness: Analyst variation and instrument variation were observed by taking 5µg/ml and their %RSD was calculated. results are tabulated in **table 5 and 6**.

Robustness: Nm variation was checked by taking 6µg/ml solution at 3 different wavelengths such as 408,409 and 410nm and their %RSD was calculated. Results are tabulated in **table 7** .

Procedure for assay of drugs in dosage forms: Ten tablets of commercial samples of Trimetazidine were accurately weighed and powdered. An amount of powder equivalent to 50mg was weighed separately and made upto 50ml with distilled water. The solution was filtered and subjected to recommended procedure for the determination. The results were displayed in **table 8**.

Analytical data: Calibration graph was constructed by measuring the absorbance at seven concentration levels, which showed linear response of absorbance in relation to concentration of trimetazidine over the range of 1-7µg/ml. Regression analysis of calibration graphs indicated linear relationship. Table summarises the analytical parameters, molar absorptivity and the results of statistical analysis of the experimental data. The detection limits were found to be 0.216 µg/ml respectively. The repeatability of the proposed procedure was checked by performing six replicate determinations of trimetazidine. The percent relative standard deviation (%RSD) and recoveries were found to vary over the range of 0.523%and 98.035-99.99% respectively.

The accuracy of the proposed method was demonstrated by recovery experiments, which were carried out by taking a fixed amount of pure drug to the sample matrix .the analytical results are summarised in **table 9**.

TABLE 1: LINEARITY READINGS

Concentration(µg/ml)	Absorbance
1	0.241
2	0.315
3	0.3876
4	0.451
5	0.521
6	0.582
7	0.6735

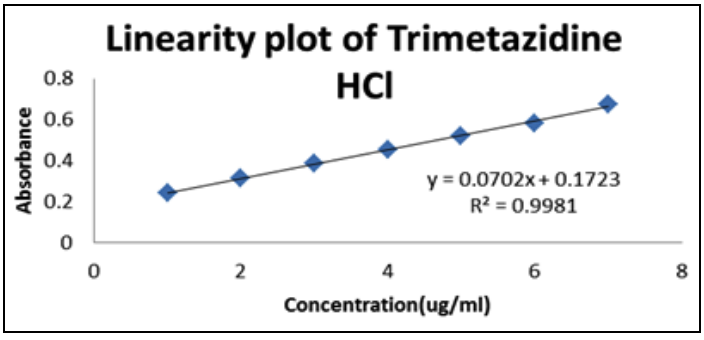

FIGURE 3: STANDARD GRAPH

TABLE 2: STATISTICAL PARAMETERS OF CALIBRATION GRAPH

Slope	**0.070175**
Intercept	0.1723
Regression equation	Y= 0.0702x + 0.1723
Regression coefficient	0.9981
Variance	0.023026
Beers law limit	1-7µg/ml

TABLE 3: REPEATABILITY

Concentration (µg/ml)	Absorbance
4	0.456
4	0.453
4	0.459
4	0.449
4	0.447
4	0.456
Average	0.4533
Standard deviation	0.00459
% RSD	1.01257

TABLE 4: ACCURACY

Concentration (µg/ml)	Absorbance	% Recovery
4	0.4465	98.035%
5	0.518	98.8%
6	0.5916	99.99%

Ruggedness:

TABLE 5: ANALYST VARIATION:

Concentration(µg/ml)	Analyst-1	Analyst-2
5	0.521	0.534
5	0.532	0.536
5	0.528	0.549
5	0.527	0.538
5	0.518	0.54
5	0.524	0.539
Average	0.525	0.539333
Standard deviation	0.00506	0.005203
% RSD	0.963742	0.964629

Instrumental Variation:

TABLE 6: INSTRUMENTAL VARIATION

Concentration(µg/ml)	Instrument-1	Instrument-2
5	0.521	0.531
5	0.532	0.526
5	0.528	0.528
5	0.527	0.539
5	0.518	0.524
5	0.524	0.539
Average	0.525	0.531167
Standard deviation	0.00506	0.006494
% RSD	0.963742	1.222514

Robustness:

TABLE 7: WAVELENGTH VARIATION

Concentration	408nm	409nm	410nm
6	0.6126	0.591	0.6064
6	0.6124	0.5824	0.5923
6	0.6271	0.5874	0.5934
Average	0.617367	0.586933	0.597367
Standard	0.00843	0.004319	0.007842
% RSD	1.365462	0.73585	1.31283

TABLE 8: ASSAY

Label claim	Amount found (mg)	Assay
20mg	20.112	100.56 %±0.64
20mg	20.34	101.7% ±0.93

TABLE 9: SUMMARISED TABLE

Parameter	Value
λ_{max} (nm)	409
Beer's law limits (µg/ml)	1-7
Molar absorptivity ($1\ mol^{-1}\ cm^{-1}$)	30029.384
Linear regression equation	Y=0.0702x + 0.1723
Intercept(a)	0.172
Slope (b)	0.070175
Correlation coefficient(r)	0.9981
Variance (S_o^2)	0.023026
Relative standard deviation (%)	NMT 2%
Recovery (%)	98-102%
LOD	0.216µg/ml
LOQ	0.6 µg/ml

CONCLUSION: The results indicate that the proposed methods are precise, accurate and linear. They comply the method validation in line with ICH guidelines. So they can be transferred from research environment to Quality Control environment to carry out the routine quality control testing of TRIMETAZIDINE as alternative methods in pure drug and in pharmaceutical formulations.

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REFERENCES:

1. 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
2. Ibrahim A. Darwish. Kinetic spectrophotometric methods for determination of Trimetazidine dihydrochloride., Analytica Chimica Acta 551 2005: 222-231
3. 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): 965-970.
4. 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
5. Marzilli M. Trimetazidine: a metabolic agent for the treatment of stable angina,European Heart Journal Supplements ,2001, Supplement O, O12–O15
6. pubchem.ncbi.nlm.nih.gov/summary/ 21109
7. Text on validation of analytical procedures, ICH Hormonised tripartite guidelines, adoption on 27 OCT, 1994.

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