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Research article

Validation of spectrophotometric dissolution method for modified release Trimetazidine pharmaceutical dosage form

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Abstract

A spectrophotometric method was validated for the determination of trimetazidine (modified released) rate of release. The dissolution conditions comprised of USP II (Paddle), with a Dissolution medium (Phosphate Buffer pH 6.8.) with Revolution 75 rpm. Detection was carried out at 231± 1 nm. The linear regression analysis data for the calibration plots showed good linear relationship within the concentration range 0.00800 mg/ml-0.03200 mg/ml. The value of correlation coefficient was found to be 1.00. The recovery of trimetazidine hydrochloride was about 95–105%. Based on the test results of linearity, accuracy and precision the range of method is established 80%-120%. The method was validated as per ICH guidelines.

Introduction

Trimetazidine (TRMZ); 1-[(2,3,4-trimethoxyphenyl)methyl] piperazine dihydrochloride (Figure 1) is a clinically effective antianginal agent that has been used in the prophylaxis and management of anginapectoris, and in ischemia of neurosensorial tissues as in Meniere's disease[1]. The anti-anginal efficacy of TRMZ is comparable to propranolol but it does not reduce cardiac rate–pressure product or coronary blood flow [2]. Trimetazidine exhibits some cytoprotective effects on myocardial energy metabolism and exerts an antianginal effect in the absence of significant hemo-dynamic effects [3]. For these clinical successes, TRMZ has become unique among the antianginal agents, and it has been clinically used throughout many countries worldwide [4, 5].

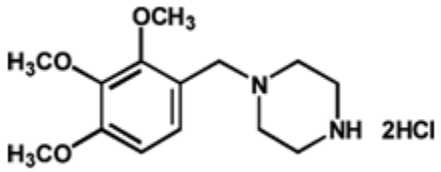

Figure 1. Structure of Trimetazidine

Trimetazidine dihydrochloride have been determined in pharmaceutical formulations and/or biological fluids by high- performance thin-layer chromatography [6], liquid chromatography [7–9], gas chromatography–mass spectrometry [10], adsorptive stripping voltammetry [11], and chemi-luminescence [12]. Spectrophotometry, because of its inherent simplicity, is considered a more convenient alternative technique. Since TRMZ contains weakly absorbing chromophores in its molecule, only few spectrophotometric methods have been reported for its determination [13–16]. These methods include direct UV measurements [13], formation of ion-pair associates with bromophenol blue [14], methyl orange and tropaelin [15], formation of complex with Fe(III) chloride[16], and formation of enamine derivative with acetaldehyde–chloranil combination [14]. These methods are somewhat insensitive, time consuming and/or not simple to perform. Therefore, the development of simple and sensitive methods for the determination of release of TRMZ was necessary.

Experimental

Materials used

Subject: In-house developed tablets
Working standard: In-house standard (Complies with EP)

Dissolution Condition:

Apparatus	:	USP II (Paddle)
Dissolution medium	:	Phosphate Buffer pH 6.8.
Volume	:	900 ml
Revolution	:	75 rpm
Temperature	:	37°C ± 0.5°C
Dissolution Time	:	1.0 hour, 2 hours,4 hours,8 hours

Procedure for the Method of Dissolution

Preparation of Phosphate Buffer pH 6.8
Dissolve 47.6 gm potassium dihydrogen phosphate (monobasic potassium phosphate in a 7.0 liter purified water, adjust to pH 6.8 with 0.1 M Sodium hydroxide solution.

Standard solution
Weigh accurately about 50.0 mg working standard (Reference Standard) of Trimetazidine hydrochloride in 50 ml volumetric flask. Add 30 ml phosphate buffer solution pH 6.8 and sonicate for 5.0 minutes and shake to dissolve with phosphate buffer and then make volume up to the mark with the same solvent. Dilute 2 ml of the above solution to 100 ml with the same solvent. Finally filter the solution through 0.45 µ membrane filter.

Sample solution
Put 900 ml dissolution medium in all vessels. Switch on the heater of dissolution Apparatus (with set temperature 37°C± 0.5°C). When the temperature is achieved at 37°C, place one tablet in each vessel. After completion of every segment as 1 hour, 2 hours, 4 hours & 8 hours keeping rpm 75, withdraw samples of 10 ml and filter through whatman filter paper (size # 40). Take 5 ml of filter solution into 10 ml volumetric flask and volume up to the mark with the phosphate buffer solution pH 6.8.(Note: After removing the 10 ml of sample from disso apparatus, 10 ml of phosphate buffer should be added to maintain the sink condition)

Measurement
Measure the absorbance of standard solution and sample solution in a suitable spectrophotometer in 1 cm cell at 231 ± 1nm. Blank with phosphate buffer pH 6.8

Calculation:

$$\text{Factor (F)} = \frac{W_{Std.} \times 2 \times 900 \times 10 \times P_{Std.}}{A_{Std.} \times 50 \times 100 \times 35 \times 5}$$

$$\% \text{ of Dissolution} = F \times Ab_S$$

Where,
Ab_S =Absorbance of sample solution
$W_{Std.}$ =Weight taken of Working Standard in mg
$P_{Std.}$ =Potency of Working Standard in percentage
$A_{Std.}$ =Absorbance of standard solution

Methods Validation & Observations

Specificity
The specificity of the method for dissolution is tested by reading following solutions into the Absorbance system:

- Blank
- Dissolution media
- Standard solution
- Placebo Preparation

Blank: Dissolution Media

Dissolution Media
Dissolve 47.6 gm potassium dihydrogen phosphate (monobasic potassium phosphate in a 7.0 liter purified water, adjust to pH 6.8 with 0.1 M Sodium hydroxide solution.

Standard solution
Weigh accurately about 50.0 mg working standard (Reference Standard) of Trimetazidine hydrochloride in 50 ml volumetric flask. Add 30 ml phosphate buffer solution pH 6.8 and sonicate for 5.0 minutes and shake to dissolve with phosphate buffer and then make volume up to the mark with the same solvent. Dilute 2 ml of the above solution to 100 ml with the same solvent. Finally filter the solution through 0.45 µ membrane filter.

Sample solution
Put 900 ml dissolution medium in all vessels. Switch on the heater of dissolution Apparatus (with set temperature 37°C± 0.5°C). When the temperature is achieved at 37°C, take a quantity of powder about 277.0 mg of formulation placebo in each vessel. After completion of every segment as 1 hour, 2 hours, 4 hours & 8 hours keeping rpm 75, withdraw samples of 10 ml and filter through whatman filter paper (size # 40). Take 5 ml of filter solution into 10 ml volumetric flask and volume up to the mark with the phosphate buffer solution pH 6.8.

Calculation:

$$\text{Factor (F)} = \frac{WStd. \times 2 \times 900 \times 10 \times P_{Std.}}{AStd. \times 50 \times 100 \times 35 \times 5}$$

$$\% \text{ of Dissolution} = F \times Ab_P$$

Where,
Ab_S =Absorbance of Placebo solution
WStd. =Weight taken of Working Standard in mg
PStd. =Potency of Working Standard in percentage
AStd. =Absorbance of standard solution

Observations results and acceptance criteria are shown in table 1 and 2.

Table 1. Observation for Specificity

Raw Data Analysis					
Sl. No.	Sample ID	Interference (%)	Mini.	Max.	Mean
1.0	Vessel_01	1.99%			
2.0	Vessel _02	1.55%	1.55%	1.99%	1.74 %
3.0	Vessel _03	1.99%			
4.0	Vessel _04	1.55%			
5.0	Vessel _05	1.78%			
6.0	Vessel _06	1.55%			

Table 2. Acceptance Criteria & Results

Specification	Result
Placebo interference should not exceed 2%	1.74%

Linearity

To check the linearity prepare a dilution series of standard solution from 60 to 120% of the nominal concentration. Take absorbance separately for each concentration & calculate correlation coefficient, r² from the calibration curve from Absorbance.

Trimetazidine Hydrochloride Stock solution

Dissolve 20.00 mg Trimetazidine Hydrochloride API into 100 ml volumetric flask with Dissolution media. (0.200 mg/ml of Trimetazidine Hydrochloride).

Table 3. Linearity concentration

Concentration level in (%) of the active ingredients concentration	Volume of stock solution added (ml) in 50 ml volumetric flask with Dissolution media	Approx. final concentration in (mg/ml)
40	2	0.00800
60	3	0.01200
80	4	0.01600
100	5	0.02000
120	6	0.02400
160	8	0.03200

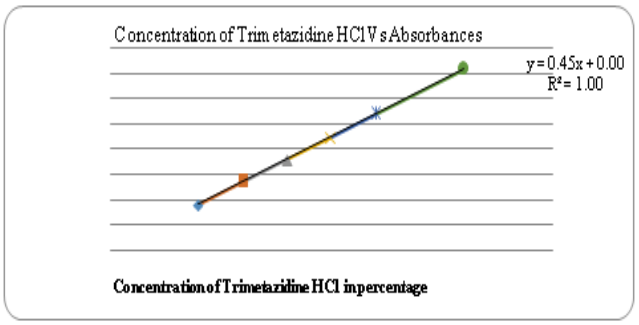

Figure 2. Different concentration of Trimetazidine Hydrochloride VS Average absorbance

Table 4. Different concentration of Trimetazidine Hydrochloride and respective absorbance

Concentration level in (%) of the active ingredients concentration	Approx. final concentration in (mg/ml)	Absorbance for Trimetazidine Hydrochloride Individual	Average
40	0.00800	0.180 0.180 0.181	0.180
60	0.01200	0.273 0.273 0.272	0.273
80	0.01600	0.359 0.360 0.359	0.359
100	0.02000	0.447 0.447 0.447	0.447
120	0.02400	0.538 0.539 0.537	0.538
160	0.03200	0.716 0.716 0.716	0.716

Table 5. Linearity regression

From Figure 2: Regression equation , y = y = 0.45x + 0.00, $R^2 = 1.00$

Correlation coefficient R^2	1.00
Intercept	0.45
Slope of regression line	0.00

Table 6. Acceptance Criteria & Results

Sl. No.	Acceptance Criteria	Results
01.	Correlation coefficient : ≥ 0.990	1.00
02.	Intercept	0.45
03.	Slope regression line	0.00

Range

Data taken from linearity studies to establish range.

Remarks: Based on the test results of linearity, accuracy and precision the range of method is established as 80 – 120% of the target concentration.

Table 7. Acceptance Criteria & Results for Range

Acceptance Criteria	Results
80 – 120 % of the limit concentration of active ingredient.	Complies

Precision

System precision (System suitability)

To check the repeatability of the system, reading the standard solution 10 times, immediate one after another,

under conditions as similar as possible. Calculate the coefficient of Variation.

Standard solution

Weigh accurately about 50.0 mg working standard of Trimetazidine hydrochloride in 50 ml volumetric flask.Add 30 ml phosphate buffer solution pH 6.8 and sonicate for 5.0 minutes and shake to dissolve with phosphate buffer and then make volume up to the mark with the same solvent. Dilute 2 ml of the above solution to 100 ml with the same solvent. Finally filter the solution through 0.45 µ membrane filter.

Measurement

Measure the absorbance of standard solution and sample solution in a suitable spectrophotometer in 1 cm cell at 231 ± 1nm. Blank with phosphate buffer pH 6.8.Observations are shown in table 8 and 9.

Table 8: Ten replicates reading of standard solution

No. of Sample	Absorbance for Trimetazidine Hydrochloride	Average Absorbance	Coefficient of variation (%)
01.	0.453		
02.	0.452		
03.	0.453		
04.	0.451		
05.	0.452	0.452	0.174
06.	0.453		
07	0.451		
08	0.452		
09	0.452		
10	0.453		

Table 9. Acceptance Criteria & Results for Precision

Sl. No.	Acceptance Criteria	Results
01.	Coefficient of variation is less then 2.0%.	0.174%

Accuracy or Recovery

Prepare standard solution of 80%, 100%, &120% in respect to the nominal concentration level. In parallel to the standard solution, mix the quantity of excipients and also prepare 80%, 100%, & 120% of the declared amount of active ingredient at each planned time point, individual samples for each level and analyse according to the test procedure. Calculate the % recovery and conclude whether the method accuracy meets the requirement of current approach for the analysis of the method.

Trimetazidine Dihydrochloride Stock solution

Dissolve 20.00 mg Trimetazidine Dihydrochloride API into 100 ml volumetric flask with Dissolution media. (0.2000 mg/ml of Trimetazidine Dihydrochloride)

Table 10. Accuracy / Recovery standard concentration

Concentration level in (%) of the active ingredients concentration	Volume in 50 ml volumetric flask with Dissolution Media	Standard concentration in (mg/ml)
80	4	0.01600
100	5	0.02000
120	6	0.02400

Preparation of Trimetazidine Dihydrochloride MR tablet 35 mg accuracy test solutions

Take three 250 ml volumetric flask and labeled it as 80%, 100% & 120%. Weigh and transfer placebo equivalent to 3 tablets into the marked volumetric flask each. Weigh 80.0 mg, 100.0 mg and 120.0 mg of Trimetazidine Dihydrochloride API. And add it into the 80%, 100%, 120% marked volumetric flask respectively. Add 250 ml of dissolution media each volumetric flask respectively and sonicate for 10 minutes. Transfer 5 ml of this each solution into 100 ml volumetric flask and volume upto the mark with the mobile phase Prepare at least 3 samples at each level.
Table 11 describes the concentration of sample at different level.

Table 11. concentration of sample at different level.

Concentration level in (%) of the active ingredients concentration	Approx. final concentration in (mg/ml)
80 x 3 sample	0.01600
100 x 3 sample	0.02000
120 x 3 sample	0.02400

Observation

The sample solution for evaluating the Accuracy / Recovery was prepared as 80% – 120% of nominal analyte of Trimetazidine Hydrochloride.

Table 12. Accuracy / Recovery observation

Concentration of Trimetazidine Hydrochloride (mg/ml)	% of nominal concentration	Average Absorbance (Standard)	Average Absorbance (Sample)	Recovery from sample	% Recovered
0.01612	80	0.359	0.367	0.01648	102.23
0.02015	100	0.445	0.452	0.02047	101.57
0.02418	120	0.535	0.543	0.02454	101.49
				Average	101.76
				Max.	102.23%
				Min.	101.49%

Remarks: Individual recovery for Trimetazidine Hydrochloride is from 101.49 – 102.23% and mean recovery is 101.76%.

Table 13. Acceptance Criteria & Results for Recovery

S. No.	Acceptance Criteria	Results
01.	Individual recovery % must be between 85 - 115 %	101.49 – 102.23%
02.	Mean recovery % must be between 95 - 105 %	101.76%

Robustness

Stability of the analytical solutions (Dissolution Study only for after one hour)

The stability of analytical solution is demonstrated by carrying out the analysis on the Reference and Test solution immediately after they are prepared and then at suitable intervals at room temperature.

The test solution to be kept on bench top under normal laboratory conditions and to be analyzed at suitable time intervals to establish bench top solution stability up to 8 hrs.

In a table 14 summarize the % change between the initial results and the results at each time point calculated with respect to the fresh standard where appropriate.

Standard and sample solutions are prepared as per test method and analyzed initially and at different time intervals by keeping the solution at room temperature (about 25°C).

Table 14. Robustness (Standard sample solution)

	Standard Solution			Sample solution		
Time in Hours	Absorbance	Results	% Change	Absorbance	Results	% Change
Initial	0.451	-	-	0.168	37.93%	-
4	0.449	100.32%	-	0.169	38.21%	0.28%
8	0.453	99.56%	0.76%	0.170	38.10%	0.11%

Remarks: From the above study, there is no significant change in % result of standard & sample solution a suitable interval after 4 hours & 8 hours.

Table 15. Robustness (Degradation for time interval)

Sl. No.	Acceptance Criteria	% Change regard to initial	
01.	Standard solution: ± 2.0% with regard to initial	8 Hr	0.76%
02.	Sample solution: ± 2.0% with regard to initial	4 Hr	0.28%
		8 Hr	0.11%

Influence of the variation in test parameters

The evaluation of robustness, which assesses the effect of making small, deliberate changes to the dissolution conditions. Parameters to be varied are dependent on the dissolution procedure and analysis type. They may include

a) pH
b) Volume
c) Agitation rate

pH change of dissolution media : 6.66 and 6.94(required 6.8)
Volume : 500 ml and 1000 ml (required 900 ml)
Agitation rate : 50 rpm & 100 rpm (required 75 rpm)

a) pH: Dissolution is done by changing pH keeping other parameters unchanged. The results are shown in table 16.

b) Dissolution is done by changing Dissolution medium volume keeping other parameters unchanged. The results are shown in table 17.

c) Agitation rate: Dissolution is done by changing agitation rate (rpm) keeping other parameters unchanged. The results are shown in table 18.

Table 16. Robustness (pH change)

Raw data analysis			
	Dissolution medium pH		
Time	Mean dissolution results at pH-6.66	Mean dissolution results at (Standard pH) pH-6.80	Mean dissolution results at pH-6.94
1st hour	36.61%	36.94%	36.96%
2nd hours	55.85%	55.84%	56.17%
4th hours	72.51%	72.79%	72.84%
8th hours	96.16%	95.77%	96.48%

Table 17. Robustness (Volume change)

Raw data analysis			
	Dissolution medium pH		
Time	Mean dissolution results in 500 ml medium	Mean dissolution results in 900 ml medium(Standard medium)	Mean dissolution results in 1000 ml medium
1st hour	37.83%	36.94%	36.09%
2nd hours	57.49%	55.84%	56.36%
4th hours	74.80%	72.79%	75.66%
8th hours	97.08%	95.77%	96.43%

Table 18.Robustness (Dissolution RPM change)

Raw data analysis			
	Dissolution medium pH		
Time	Mean dissolution results at 50 rpm	Mean dissolution results at 75 rpm (Standard agitation)	Mean dissolution results at 100 rpm
1st hour	36.29%	36.94%	38.09%
2nd hours	55.36%	55.84%	57.32%
4th hours	72.01%	72.79%	74.31%
8th hours	93.94%	95.77%	95.79%

Table 19. Acceptance Criteria & Result

Acceptance Criteria	Result
Must be Robust	Complies

Conclusion

A simple, sensitive, specific, accurate and precise UV spectrophotometric method was validated for the routine analysis of modified release tablet dosage form of trimetazidine. The method is sensitive enough for the detection of analyte in pharmaceutical formulation when compared to the research works found in the literature. The method can be employed for the routine analysis of dissolution of trimetazidine modified release tablet dosage form.

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