Journal of the Maharaja Sayajirao University of Baroda ISSN : 0025-0422

SIMULTANEOUS ESTIMATION AND VALIDATION OF METOPROLOL TARTRATE & HYDROCHLOROTHIAZIDE IN COMBINED DOSAGE FORM BY RP HPLC METHOD

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ABSTRACT:
A simple and rapid reversed phase high performance liquid chromatographic (HPLC) stability indicating method was developed and validated for the Metoprolol Tartrate (MET) & Hydrochlorothiazide(HTZ) in combined Dosage form . The column used was Inertsil ODS 3V C_{18}, 150 x 4.6mm, i.d. 5µm and a mobile phase composed of Acetonitrile : buffer (Sodium acetate trihydrate) (20:80), pH 5.0 adjusted with acetic acid. The flow time was set at 1.0mL/min. Analysis was performed using UV detection at 275nm.. The retention times of MET and HTZ were found to be 5.72 mins and 3.46 mins respectively. The developed method was validated for accuracy, linearity and robustness as per the ICH guidelines. The stability of the method was tested by subjecting the drugs to acidic, basic, oxidative and photolytic degradation studies.
Keywords: Metoprolol Tartrate (MET) ; Hydrochlorothiazide (HTZ) , RP-HPLC; Validation

1. Introduction:

Metoprolol Tartrate (MET) is the Tartrate salt of Metoprolol, a cardio selective competitive beta-1adrenergic receptor antagonist with antihypertensive properties and devoid of intrinsic sympathomimetic activity. Hydrochlorothiazide(HTZ) is the most commonly prescribed thiazide diuretic. It is indicated to treat edema and hypertension. Metoprolol and Hydrochlorothiazide are widely used in the treatment of hypertension, cardiac and renal diseases. Chemically, Metoprolol is 1-[4-(2-methoxyethyl) phenoxy]-3-[(1-methylethyl)amino]-2-propanol and Hydrochlorothiazide is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide.

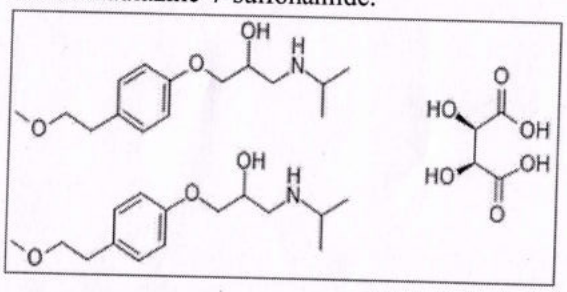


Fig 1. Chemical structure of Metoprolol Tartrate

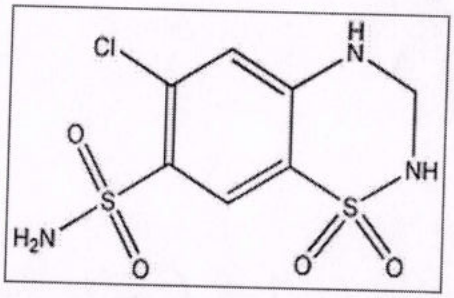


Fig 2. Chemical structure of Hydrochlorothiazide

Literature survey reveals that various analytical techniques viz, UV spectrophotometry, [11-13] high performance liquid chromatography (HPLC) [4-10] and were reported for the analysis of MET and HTZ in pharmaceuticals. Few HPLC methods have been reported for the simultaneous determination of MET and HTZ [4-10]. Differential pulse voltametric method has been developed and reported for the determination of MET & HTZ . Our aim was to develop proper method which estimates both the analytes in a shorter time and to develop low cost method. The present study describes an isocratic, reversed-phase HPLC method using ultraviolet detection for the determination of Metoprolol Tartrate and Hydrochlorothiazide from tablet dosage form.

2. Experimental:

2.1 Chemicals and reagents:

Methanol HPLC grade was procured from Merck India Limited, Mumbai. Water HPLC grade was obtained from Merck Specialties Private Limited, Mumbai. Reference standards of MET and HTZ were gifted sample . Sodium acetate trihydrate buffer and glacial acetic acid was purchased from Merck India.

Standard stock preparation:

100 mg of Metoprolol Tartrate and 12.5 mg of Hydrochlorothiazide working standard were accurately weighed and transferred to a $100cm^3$ volumetric flask and $10cm^3$ of dimethyl formamide was added and sonicated to dissolve. The solution was cooled to room temperature diluted with diluents. 5 cm^3 of this solution was diluted to $25cm^3$ with diluents. The concentration of the solution was found to be 100ppm of MET and 12.5 ppm of HTZ.

Preparation of sample solution:

Ten tablets containing the drugs Metoprolol Tartrate and Hydrochlorothiazide were weighed and their average weight was calculated. These tablets were powdered and weight equivalent to one tablet containing 100mg of MET and 12.5 mg of HET was taken in a 100mL dilution flask. Then 50mL of Dimethyl formamide was added to it and sonicated for 20-25 mins at an ambient temperature with intermittent swirling, cooled to room temperature and diluted upto the mark with diluent. Then solution from the flask was filtered through syringe filter.

2.2 Chromatographic Conditions:

The chromatographic system consist of a Waters HPLC system having Waters 501 isocratic pump equipped with Waters ™ 717plus autosampler and a Waters 486 tunable absorbance UV-detector. The data was recorded using Millenium^{32} chromatographic software. Separation was performed on a 150 mm × 4.6 mm i.d., 5 µ particle size Inertsil ODS 3V C_{18} column. Mobile phase consisted of a mixture of Acetonitrile : buffer (Sodium acetate trihydrate) (20:80), pH 5.0 adjusted with acetic acid. The wavelength was set at 275nm.

2.3 Method Validation:

The method was validated as per ICH guidelines [3] for specificity, linearity, quantification limit, precision, accuracy, recovery and stability [1]. Specificity was investigated by analyzing the blank diluents and samples of 100% level for any interference of the endogenous material at the retention times of MET and HTZ. No interference was observed from the peaks due to mobile phase and placebo at the retention time of MET and HTZ.

Metoprolol Tartrate and Hydrochlorothiazide tablets were subjected to acidic, basic, oxidative, thermal and photolytic degradation about 10% - 30% for forced degradation conditions.

The linearity of the method was tested by taking several aliquots of standard solutions of MET and HTZ in 100 cm^3 volumetric flask. The linearity range was 50% - 100%.

The accuracy of the method was determined by recovery experiments. A standard addition method was employed for this experiment. A known quantity of each drug substance (MET and HTZ) corresponding to three different levels i.e 50%, 100% and 150 % in triplicate and analyzed as per the sample preparation. Each set of addition was repeated three times. The accuracy was expressed as a percentage of analytes recovered by the assay.

The system precision & method precision of the method was demonstrated by five replicate injections of standard and sample were made and percentage RSD was calculated.

The robustness of the method was checked by changing the chromatographic conditions. The organic phase of the mobile phase was varied by ±5% while pH of the buffer was varied by ±0.2 units. The three different sample solutions were injected in each varied condition and the assay was checked.

3. Results and Discussions

3.1 Optimization of the chromatographic conditions

In order to develop an isocratic reverse phase HPLC method for the simultaneous determination of MET and HTZ in combined dosage form, the chromatographic conditions were optimized. For better separation and resolution the different buffers were tried. It has been found that sodium acetate trihydrate buffer with pH 5.0 adjusted with glacial acetic acid gave better peak shape than other buffers. The different compositions of mobile phase were changed for getting better separation of these analytes. Thus the mobile phase composed of the mixture of acetonitrile and buffer (sodium acetate trihydrate) in the ratio of (20:80v/v) was finalized. The better separation, peak symmetry and reproducibility were obtained with Inertsil C18, 150mm x 4.6mm, 5µm column compared to Thermo BDS Hypersil C8, 150 mm x 4.6mm, 5µm column. Both these analytes gave better response at 275 nm wavelength using UV detector. The flow rate kept was 1.5mL/min. There was no peak tailing observed under these optimized chromatographic conditions. The retention times of MET and HTZ were found to be 5.72 mins and 3.46 mins respectively.

3.2 Method Validation

The proposed method was shows short elution time and good separation between MET and HTZ. The proposed method was validated as per the ICH guidelines with respect to specificity, linearity, precision and accuracy.

The method was linear over the range 50-150 µg/mL and 6.25-18.75 µg/mL for MET and HTZ respectively. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was

$y = 897633.7931x - 2604.7931+004$ ($r^2= 1.0000$) for MET and $y = 400644.1379x + 685.2621$ ($r^2= 1.0000$) for HTZ. The results show that an excellent correlation between response factor and concentration of drugs.

The developed method was validated for system precision (repeatability) and method precision. Six injections of mixed standards of 100 µg/mL of MET and 12.5 µg/mL of HTZ were injected and %RSD calculated for injection repeatability. Similarly Six replicate injections of sample of 100% level were injected and assayed according to the procedure. The relative standard deviation of 0.07% and 0.04% respectively. The result shows that the method was precise.

The accuracy of the method was determined by the standard addition method at three different levels. Placebo spiked recovery for MET and HTZ was carried out in the standard addition method. Each determination was performed in triplicates. The accuracy was then calculated as the percentage of the standard drug recovered by the recovery study. The amount of MET and HTZ added and recovered was calculated for all the nine determination. The results are well within the acceptance limit and hence the method is accurate.(Table 1 & 2)

Table 1: Placebo Spiked Recovery of Metoprolol Tartrate

Recovery Level	Amount added (µg)	Amount recovered (µg)	% Recovery	Mean	%RSD
50%	50.45 50.32 50.65	50.20 50.25 50.98	99.50 99.86 100.65	100.00%	0.587%
100%	100.34 100.56 100.10	100.78 99.97 99.34	100.43 99.41 99.24	99.69%	0.649%
150%	150.46 150.39 150.89	149.44 150.32 150.23	99.32 99.95 99.41	99.56%	0.34%

Table 2: Placebo Spiked Recovery of Hydrochlorothiazide

Recovery Level	Amount added (µg)	Amount recovered (µg)	% Recovery	Mean	%RSD
50%	6.35 6.32 6.65	6.25 6.28 6.50	98.42 99.36 99.74	98.51%	0.82%
100%	12.34 12.56 12.35	12.40 12.50 12.34	100.48 99.52 99.91	99.97%	0.484%
150%	18.46 18.75 18.89	18.44 18.76 18.70	99.89 100.05 98.99	99.64%	0.572%

The robustness of the method was performed by deliberately changing the chromatographic conditions. The organic strength of the mobile phase was varied by ±5% while pH of the buffer was varied by ±0.2 units. The change in wavelength of the detector was ±2nm. The standard solution and three different sample preparations were injected in each varied condition and the assay was checked. Under all varied conditions, it has been found that the %RSD for the assay values for MET and HTZ were found to be well within the acceptance limit of 2%(Table 3)

Table 3: Robustness experiment for Metoprolol Tartrate

Name of the sample	Change in wavelength 273nm(-2)	Change in wavelength 277nm(+2)	Change in pH of Mobile phase 4.8 (-0.2)	Change in pH of Mobile phase 5.2 (+0.2)	Change in organic phase composition -5%	Change in organic phase composition +5%

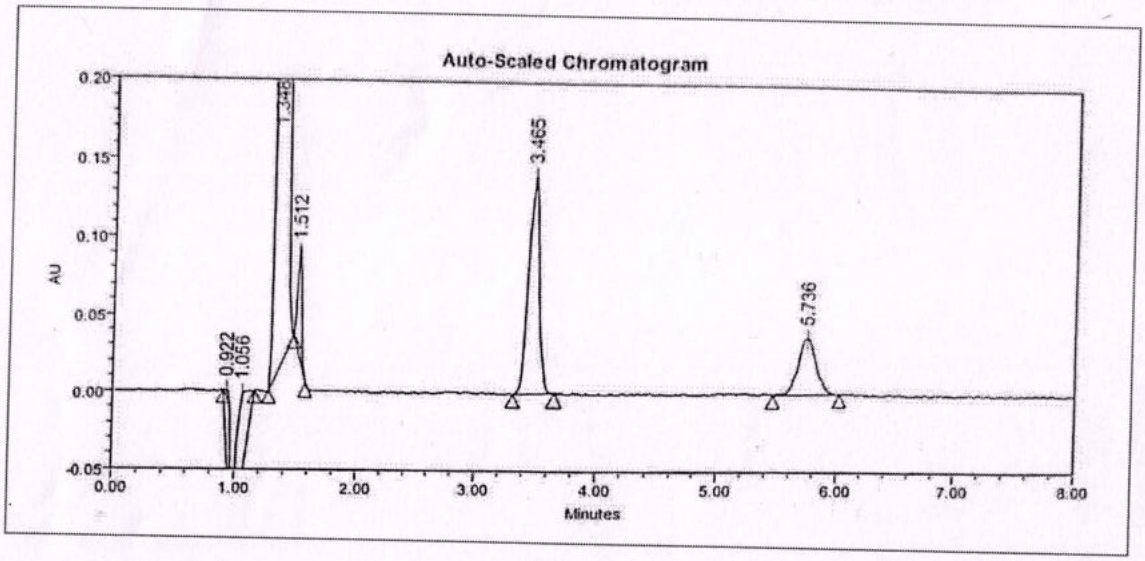
Sample 1	99.45	96.80	99.22	98.80	99.21	99.34
Sample 2	98.23	97.45	98.65	99.12	99.67	98.96
Sample 3	99.34	97.65	99.06	99.44	98.56	98.42
Mean	99.00	97.30	98.98	99.12	99.15	98.91
SD	0.67	0.44	0.29	0.32	0.56	0.46
%RSD	0.68	0.45	0.30	0.32	0.56	0.47

Table 4 : Robustness experiment for Hydrochlorothiazide

Name of the sample	Change in wavelength 273nm(-2)	Change in wavelength 277nm(+2)	Change in pH of Mobile phase 4.8 (-0.2)	Change in pH of Mobile phase 5.2 (+0.2)	Change in organic phase composition -5%	Change in organic phase composition +5%
Sample 1	97.54	96.78	97.76	97.30	97.21	96.45
Sample 2	98.10	97.87	97.35	97.69	97.67	97.95
Sample 3	97.67	97.65	97.87	97.94	97.56	97.99
Mean	97.77	97.43	97.66	97.64	97.48	97.46
SD	0.29	0.58	0.27	0.32	0.24	0.88
%RSD	0.30	0.59	0.28	0.33	0.25	0.90

The specificity of the method was checked by injecting a sample solution. No chromatographic interference was observed from endogenous material. The chromatogram of 100% sample solution of MET and HTZ is shown in fig 3.

Fig 3 Chromatogram of 100% Sample Solution of MET & HTZ



The Specificity of the method is also checked by exposing 100% sample solution of MET and HTZ to stress conditions i.e. 5M sodium hydroxide, 0.1M hydrochloric acid, 10% hydrogen peroxide ,thermal & photolytic degradation. The degradation products were separated from their parent compounds. The results of the forced degradation studies indicated a high degree of selectivity and specificity of this method for MET and HTZ. The details are given in Table 4.

Table 5 : Forced degradation study of MET and HTZ

Conditions	Temp	Time	% degradation	
			MET	HTZ
1N HCl	70^0C	15min	1.55%	9.69%
5 N NaOH	70^0C	15min	3.28%	6.74%
10% H_2O_2	70^0C	15min	12.82%	13.26%
Thermal	105^0C	24hrs	1.99	3.34
Photolytic		24hrs	2.08	4.22

4. Applications:

The validated stability indicating HPLC method was applied to the simultaneous determination of MET and HTZ in tablet dosage form. The samples were analysed and the assay results are as per the label claim shown in Table 6.

Table 6. Analysis of formulation: Betaloc H (Label Claim: MET 100mg + HTZ 12.5 mg)

Drug	Std wt (mg)	Label Claim (mg)	Mean Std area	Mean Sample area	Amount Present	% Assay
Metoprolol Tartrate	100mg	100mg	400528	384719	99.95 mg	99.97%
Hydrochlorothiazide	12.5mg	12.5mg	897872	868806	12.45 mg	99.30%

5. Conclusion:

The isocratic RP- HPLC method has proved to be simple, specific, precise and accurate and is suitable for simultaneous estimation of Metoprolol Tartrate (MET) and Hydrochlorothiazide (HTZ). The proposed method gives a good resolution among these analytes. High percentage of recovery shows that the method is accurate.

Acknowledgement

The authors are thankful to Department of Chemistry, Mithibai College, Mumbai, India for their support.

References:

1. United States Pharmacopoeia, 28, NF 23, the USP Convention, Rockville, 2007.

2. Carlos Alberto RossiSalamanca-NetoAna Paula PiresEiseleVitória GouveiaResta JessicaScremin Elen "Differential pulse voltammetric method for the individual and simultaneous determination of

antihypertensive drug metoprolol and its association with hydrochlorothiazide in pharmaceutical dosage forms" Sensors and Actuators B: Chemical Volume 230, July 2016, Pages 630-638

3. ICH, Q2 (R1) "Validation of analytical procedure, Test and methodology, International Conference of harmonization, Geneva, 2005

4. N. D. Rawool* and A. Venkatchalam "Analytical Method for the Simultaneous Estimation of Hydrochlorothiazide and Metoprolol Tartrate using RP HPLC" Indian J Pharm Sci. 2011 Mar-Apr; 73(2): 219–223

5. Nesrin K. Ramadan, Heba M. Mohamed & Azza A. Mostafa " Chromatographic Methods for the Simultaneous Determination of Metoprolol Tartrate and Hydrochlorothiazide in the Presence of Hydrochlorothiazide Degradation Product". JPC – Journal of Planar Chromatography – Modern TLC vol 26, pages 510–516(2013)

6. R El-Bagary; E Elkady; A Faqeh. "Simultaneous determination of Atenolol, Amiloride hydrochloride and Hydrochlorothiazide using reversed phase liquid chromatography" J. Chem. Pharm. Res., 2011, 3 (6), 320-329.

7. Jaiswal; Yogini; Talele; Gokul; Surana; Sanjay, "Application of HPLC for the Simultaneous Determination of Ethamsylate and Mefenamic Acid in Bulk Drugs and Tablets" Journal of Liquid Chromatography & Related Technologies (2007), 30(8), 1115-1124.

8. D.K.Mandloi, P.K.Tyagi, V.K.Rai, S. Dey, R.K Ashada and P.Mohanraj "Method development and validation of RP- HPLC in the application of invitro dissolution study of Lamivudine in bulk drug and tablet formulation " J.Chem. Pharma. Res. (2009),1(1), 286-296

9. Boyka G. Tsvetkova* and Lily P. Peikova "Determination of metoprolol tartrate and hydrochlorothiazide in tablet dosage form by high performance liquid chromatography". Journal of Chemical and Pharmaceutical Research, 2013, 5(1):168-171

10. Madhukar A. Badgujar* and Kiran V. Mangaonkar "Simultaneous determination of paracetamol and mefenamic acid in tablet dosage form by high performance liquid chromatography" Journal of Chemical and Pharmaceutical Research 2011, 3(4):893-898

11. G Sowjanya; P Gangadhar; P Ramalingeswara Rao; P Subrahmanyam; P Suresh. "Simultaneous UV Spectrphotometric estimation of enalapril maleate and hydrochlorothiazide in tablets" J. Chem. Pharm. Res., 2012, 4 (7), 3483-3488.

12. K Gupta; M Tajne; S Wadodkar. "New Spectrophotometric Method for Simultaneous determination of Metoprolol Tartarate and Hydrochlorthiazide in Tablets" Ind. J. Pharm. Sci., 2008, 70 (4), 511-513.

13. G Garg; S Saraf; S Saraf. "Spectrophotometric and Column High-Performance Liquid Chromatographic Methods for Simultaneous Estimation of Metoprolol Tartrate and Hydrochlorothiazide in Tablets" J. AOAC Int., 2008, 91 (5), 1045-1050.