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Q-Absorbance Ratio Spectrophotometric Determination of Hydrochlorothiazide and Telmisartan in Combined Dosage Form

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ABSTRACT:

In Q absorbance ratio method, Hydrochlorothiazide and Telmisartan were quantified using their absorptivity values of at selected wavelengths, viz., 270 nm and 295 nm respectively. And the isoabsorptive point was found to be 284nm.

Objective- The objective of the present investigation was to developed the simple, prscise,and accurate method with validation.

Methods- The Q absorbance ratio method permits simple, rapid and direct determination of Hydrochlorothiazide and Telmisartan in commercially available tablet dosage form without previous separations and can therefore be used for routine analysis of both drugs in quality control laboratories.The concentrations in the range of 5-25µg/mL of mixed working standard and two sampling wavelengths of 270 nm (λmax of Hydrochlorothiazide), and 284 nm (isoabsorptive point) gave optimum accuracy, precision, time, economy, and sensitivity for this method.

Result - The percentage recoveries for Hydrochlorothiazide and Telmisartan were found to be 99.73±0.66 and 99.98±0.87for this method respectively. The contents estimated using the proposed method was found in agreement with the labelled amount Table 1.The relative standard deviations was found to be within the limit, indicating good accuracy, precision, and repeatability of the proposed method.

Conclusion- The results of analysis of two drugs from tablet formulation using method was found close to 100%, Standard deviation was satisfactorily low indicating accuracy and reproducibility of the method. Recovery studies was satisfactory which showed that there is no interference of excipients.

KEYWORDS:

Hydrochlorthizide, telmisartan, Q-absorbance ratio method, UV spectrophotometry

INTRODUCTION

Telmisartan (TELM) is chemically known as 4'-[(1,4'-dimethyl-2-propyl [2,6'-bi-1 H-benzimidazol]-1'-yl) methyl] [1,1'-biphenyl]-2-carboxylic acid.[1–3] TELM is an anti hypertensive drug (angiotensin-II receptor antagonist) and prevent binding of Angiotensin-II to the AT1 receptor. This is used for treatment of hypertension and diabetic nephropathy with an elevated serum creatinine and proteinuria (>300 mg/day) in patients with type-2 diabetes and hypertension.[4,5]The UV spectrophotometric method was developed and validated as per International Conference on Harmonization(ICH) guidelines.[6] Spectrophotometry is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (200–380 nm).TELM is official in British Pharmacopoeia (2009) and Indian Pharmacopoeia (2010), which recommends UV spectrophotometry for its analysis.

Q-ABSORPTION RATIO METHOD

Let it be one drug X and Y

According to Q-Absorption ratio method, use the ratio of absorption at two selected wavelengths. One is at iso-absorptive point and other being the λmax of one of the two components. Two equations were constructed as described below, using the relationship ax1=ay1 at λ1 and L=1. Equations are;

At λ1 A1 = ax1Cx + ax1Cy (because ax1=ay1) ……. (1)

At λ2 A2 = ax2Cx + ay2Cy ……. (2)

Dividing equation (2) by (1), we get

A2/A1 = (ax2Cx + ay2Cy)/(ax1Cx + ax1Cy) …… (3)

Let Cx/(Cx+Cy)=Fx & Cy/(Cx+Cy)=Fy

Dividing Equation (3) by Cx+Cy, we get

A2/A1 = (ax2Fx+ay2Fy)/(ax1Fx+ax1Fy)

But Fy =1 - Fx

A2/A1 = (ax2Fx+ay2-ay2Fx)/ax1 …… (4)

A2/A1 = (ax2Fx/ax1) – (ay2Fx/ ay1) + (ay2/ay1) (because ax1=ay1)

Let ax2/ax1 = Qx, ay2/ay1 = Qy & A2/A1 = QM

So, QM = FxQx – FyQy + Qy

Fx = (QM-Qy)/(Qx-Qy) …… (5)

This equation gives the fraction of mixture that determine the absolute concentration of X and Y.

Cx/(Cx=Cy) = (A2/A1)-(ay2/ay1)/(ax2/ax1)-(ay2/ay1) ……. (6)

Both equation (5) & (6) gives the fraction, rather than the concentration of X and consequently of Y in the mixture in the term of absolute ratio. As, these are independent of concentration only approximate rather than accurate. If the absolute concentration of X & Y than rearrange equation (1), we get

Cx+Cy = A1/ax1 .. …… (7)

From equation (6) & (7), we get

Cx/(A1/ax1) = (QM-Qy)/(Qx-Qy)

Cx= {(QM-Qy)/(Qx-Qy)}* (A1/ax1) …… (8)

& Cy= {(QM-Qx)/(Qy-Qx)}* (A1/ay1) ……..(9)

Finally equation (8 & 9) gives the absolute concentration value of drug X & Y (Beckett and Stenlake, 2005).

CHEMICAL STRUCTURE OF HYDROCHLOROTHIZIDE

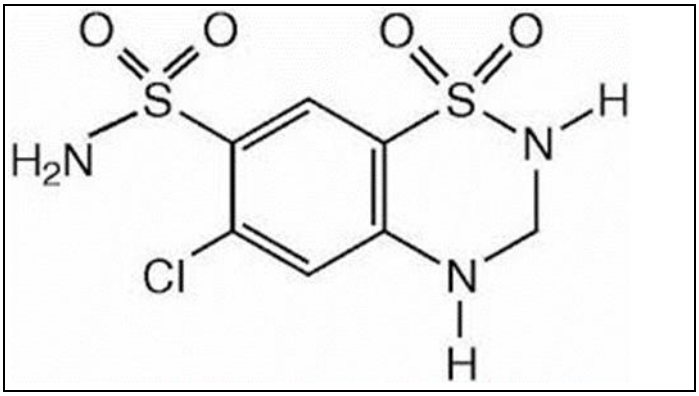


Figure 1.structure of hydrochlorthizide

CHEMICAL STRUCTURE OF TELMISARTAN

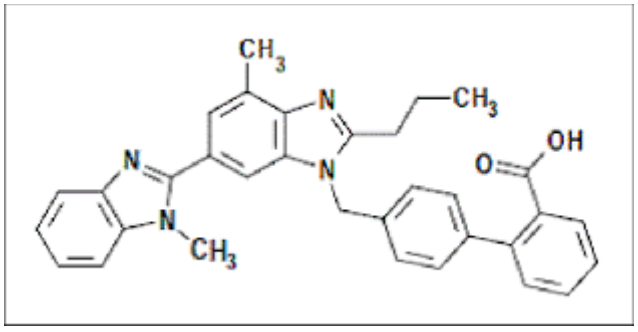


Figure 2.structure of telmisartan

MATERIAL AND METHODS

CHEMICALS AND REAGENTS

Hydrochlorthizide and telmisartan was procured from glenmark pharmaceuticals ,nashik .reagents were used NaOH(Sodium Hydroxide) Modern Industries supplied by Research Chem Industries,Mumbai.(2016)

INSTRUMENTATION

UV-Visible spectrophotometer-Agilent cary 60 with cuvette cells of 1 cm light path were used for the measurement of absorbance.electronic balance schimadzu BL-220H was used for weighing of samples.clas 'A'volumetric glassware were used.ultrasonicator WUC-2.0Lwensar was used for sonication purpose.

PROCEDURE:

Preparation of 0.1 N Sodium hydroxide

0.4 gm of sodium hydroxide was accurately weighed and transferred in100ml volumetric flask,to it approximately 30 ml of distilled water was added to dissolve the NaOH and then final volume was then made upto the mark with water.

PREPARATION OF SOLUTION

a) Preparation of standard solution

Standard stock solution of Telmisartan and Hydrochlorothiazide was prepared by dissolving 10 mg of each drug separately in 10mL volumetric flask using 0.1N sodium hydroxide as solvent. Stock solutions of 1000 µg/mL were obtained in this manner. From these stock solutions, working standard solutions of concentration 100 µg/mL each were preparedby appropriate dilutions. Working standard solutions were scanned in the entire UV range to determine the λmax. The λmax of Hydrochlorothiazide and Telmisartan were found to be 270 nm and295 nm respectively. The maximum absorption (λmax) of hydrochlorothizide was found at 270 nm and iso-absorptive point at 284 nm. Absorption and absorptivity for a series of standard solutions were recorded at selected wavelengths

Calibration curves

Seven standard dilutions of each drug were prepared separately having concentrations of 5-25 µg/mL. The absorbances of these standard solutions were measured at 270 nm and 295 nm and calibration curve was plotted. The absorptivity coefficients of the two drugs were determined using calibration curve.

Preparation of sample solution

Sample solution containing both the drugs was prepared by dissolving 10 mg of each drug in 10mL volumetric flask using 0.1N sodium hydroxide to give stock solutions of 1000 µg/mL. From this stock solution, working standard solution of 100 µg/mL concentration was prepared by appropriate dilution. Seven standard dilutions of concentrations of 5,10,15,20, and 25 µg/mL was prepared from working standard solution. The absorbance of this sample solution was measured at 270 nm and 284 nm and their concentrations were determined using proposed analytical methods.

Methodology

Absorption ratio method uses the ratio of absorptions of two selected wavelength, one of which is iso-absorptive point and other being the λmax of one of the two components. From the overlain spectra of two drugs (as shown in figure 5), it shows that hydrochlorothizide and telmisartan having iso-absorptive point at 284 nm. The second wavelength used is 270 nm, which is the λmax of hydrochlorothizide. Working standard solutions having concentration 5,10,15,20,and 25µg/ml for hydrochlorothizide and telmisartan were prepared 0.1 N NaOH and the absorbance at 284 nm (iso-absorptive point) and 270 nm (λmax of hydrochlorothizide) were measured and absorptivity coefficient were calculated using calibrations curve. The concentration of two drugs in the mixture can be calculating by using the equation (3 & 4), we get

Cx= {(QM-Qy)/(Qx-Qy)}* (A1/ax1)

Cy= {(QM-Qx)/(Qy-Qx)}* (A1/ay1)

where, A1 and A2 are the absorbance of mixture at 284 nm and 270 nm; ax1 and ay1 are absorptivities of Hydrochlorothiazide and Telmisartan at 284 nm; ax2 and ay2 are absorptivities of Hydrochlorothiazide and Telmisartan at 270 nm; QM = A2/A1, Qx = ax2/ ax1, Qy = ay2/ay1.

CH=0.7953-1.3056/0.3595-1.3056×A/459.136------(3)

CT=0.7953-0.3595/1.3056-0.3595×A/251.996------(4)

Figure-3 Overlain spectra of hydrochlorothizide and telmisartan at 284nm

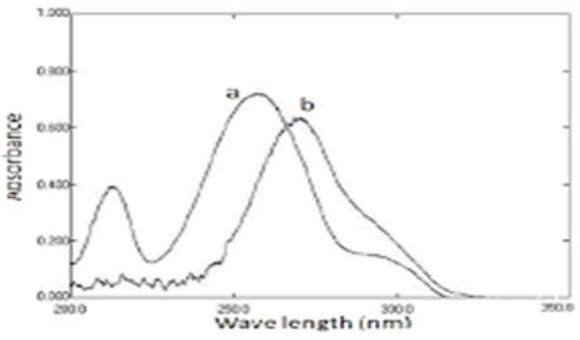


Table-1.Q-Method calculation for Hydrochlorothizide-Hydrochlorothizide-

concentration (µg/mL)	absorbance		absorptivity	
	270nm	284nm	Ax1	Ax2
5	0.1664	0.0385	332.8	77
10	0.3376	0.0622	337.6	62.2
15	0.4430	0.0814	295.3	54.26
20	1.6940	0.9334	847	466.7
25	1.2082	0.4129	483.28	165.16
mean			459.136	165.064

Table-2.Q- method calculation for telmisartan

Telmisartan-

concentration (µg/mL)	absorbance		absorptivity	
	270nm	284nm	Ax1	Ax2
5	0.1265	0.1626	253	325.2
10	0.2644	0.3372	264.4	337.2
15	0.3743	0.4984	249.53	332.26
20	0.4985	0.6500	249.25	325
25	0.6095	0.8137	243.8	325.48
mean			251.996	329.028

Estimation in the marketed solution

Twenty tablets were weighed and crushed to a fine powder. An accurately weighed powder sample equivalent to 10 mg of Telmisartan was transferred to a 10 ml volumetric flask, dissolved in 10 ml 0.1N sodium hydroxide, shaken for 10 min and the volume was made up to the mark with 0.1N sodium hydroxide. The solution was then filtered through Whatman filter paper no. 41. The solution was further diluted to get different concentrations in the range of 5-25 µg/mL of both the drugs. For this method the absorbances of the sample solution, i.e., A 1 and A 2 , were recorded at 284 nm and 270 nm respectively, and concentration of two drugs in the sample were determined using the equations(1) and (2). The analysis procedure was repeated three times with the formulation. The result of analysis of the formulation is shown in Table 1 and 2.

METHOD VALIDATION

The method validation parameters like linearity, range,precision, accuracy, repeatability, limit of detection and limit of quantitation were checked as per ICH guidelines.

Linearity and range

The linearity for Telmisartan and Hydrochlorothiazide were determined at seven concentration levels, ranging from 5-25µg/mL using working standard.the linearity shown in figure 5 and 6.

Linearity of HCT

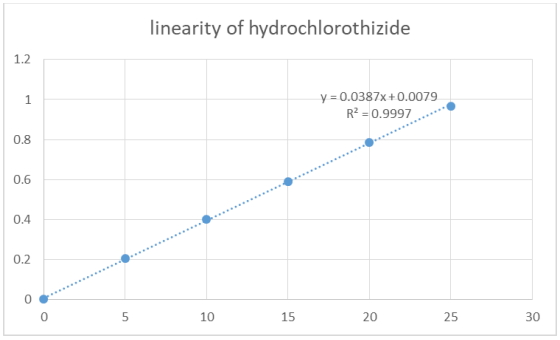
Table 3.Calibration of hydrochlorothizide

Sr.no	concentration	absorbance
1	5	0.2041
2	10	0.4002
3	15	0.5911
4	20	0.7845
5	25	0.9666

F(x)=0.0387171429x+0.019

R2=0.9997

Figure 5.calibration curve of linearity of hydrochlorothizide



Linearity of TLM

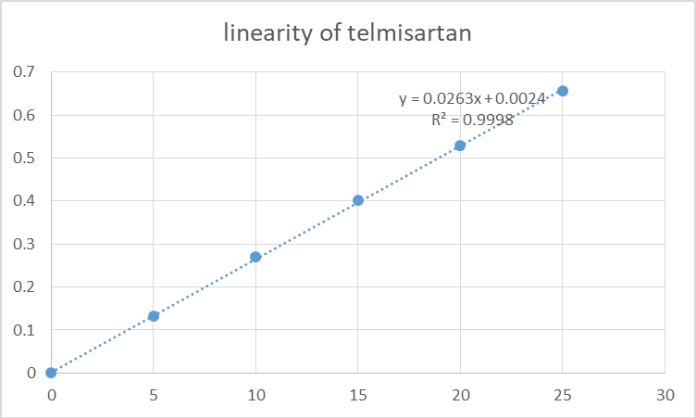
Table 4. Calibration of telmisartan

Sr.no	concentration	absorbance
1	5	0.1313
2	10	0.2701
3	15	0.4012
4	20	0.5301
5	25	0.6558

F(x)=0.0257605714x+0.019

R2=0.9998

Figure 6. calibration curve of linearity of telmisartan



Precision

The precision of the method was evaluated by inter day and intra day variation studies. In intra day studies, working solutions of standard and sample were analysed thrice in a day and percentage relative standard deviation (% RSD) was calculated. In the inter day variation studies, working solution of standard and sample were analysed on three consecutive days and percentage relative standard deviation (% RSD) was calculated. The data is shown in table6.

Accuracy

The accuracy of the method was determined by recovery studies. The recovery studies were performed by the standard addition method at 80%, 100% and 120% level and the percentage recoveries were calculated and are shown in Table 6.

LOD

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula and shown in Table 6.

LOD = 3.3 (σ / S)

Where, S = slope of calibration curve, σ = standard deviation of the response.

LOQ

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in Table 6.

LOQ = 10 (σ / S)

Where, S = slope of calibration curve, σ = standard deviation of the response.

RESULT AND DISCUSSION

In the present work, new method, namely, Q-absorption ratio method was used for the Q-method estimation of Hydrochlorothiazide and Telmisartan in commercially available tablet dosage form. The concentrations in the range of 5-25µg/mL of mixed working standard and two sampling wavelengths of 270 nm (λmax of Hydrochlorothiazide), and 295 nm (λmax of Telmisartan) gave optimum accuracy, precision, time, economy, and sensitivity for this method. The proposed procedure was successfully applied to the determination of Hydrochlorothiazide and Telmisartan in the commercially available tablets dosage form, and the results are shown in Table 5. The recovery studies were carried out at different concentrations by spiking a known concentration of standard drug to the pre analysed sample and contents were reanalysed by proposed methods. The results of marketed formulation analysis and recovery studies are depicted in Table 5. The method was validated statistically for range, linearity, precision, accuracy, repeatability, LOD, and LOQ Table 8. Accuracy was ascertained on the basis of recovery studies. Precision was calculated as inter and intra day variation for both the drugs. The percentage recoveries for Hydrochlorothiazide and Telmisartan were found to be 99.73±0.66 and 99.98±0.87for this method respectively. The contents estimated using the proposed method was found in agreement with the labelled amount Table 6.The relative standard deviations was found to be within the limit, indicating good accuracy, precision, and repeatability of the proposed method.

Table5: Result of tablet analysis

sr.no.	drug	Label claim	Q- method	
		Mg/tab	%found±RSD	%recovery±RSD
1	TLM	40	99.87±0.32	99.98±0.87

2	HCT	12.5	97.63±0.55	99.73±0.66

Mean±RSD of observations, n=no of determinations

Table 6: Summary of validation parameters for Q-method

Sr.no	parameter	HCT	TMS
1	Linearity range(µg/mL)	5-25(µg/mL)	5-25(µg/mL)
2	Correlation coefficient(r2)	0.9997	0.9998
3	Precision(RSD)	97.63±0.55	99.87±0.32
4	Accuracy (%)	99.73±0.66	99.98±0.87
5	LOD	0.031(µg/mL)	0.054(µg/mL)
6	LOQ	0.130(µg/mL)	0.236(µg/mL)

RSD=Relative standard deviation
n=Number of determination
LOD=Limit of detection
LOQ= Limit of quantitation

CONCLUSION

The Q- method permits simple, rapid and direct determination of Hydrochlorothiazide and Telmisartan in commercially available tablet dosage form without previous separation. The results of analysis of two drugs from tablet formulation using method was found close to 100%, Standard deviation was satisfactorily low indicating accuracy and reproducibility of the method. Recovery studies was satisfactory which showed that there is no interference of excipients.

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