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Research Article

Absorbance Ratio Spectrophotometric Method for the Estimation of Nebivolol Hcl and Hydrochlorthiazide in their Combined Dosage Form

RK. Patel* and JB. Patel

Department of Pharmaceutical Chemistry, K. B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India.

ABSTRACT
A simple, rapid, accurate, precise and sensitive absorbance ratio spectrophotometric method was developed for the simultaneous estimation of Nebivolol HCl (NEB-H) and Hydrochlorothiazide (HCTZ) in bulk as well as in the pharmaceutical formulation. Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. Nebivolol HCl and Hydrochlorothiazide have shown an isoabsorptive point at 287 nm in methanol. The second wavelength used is 271 nm, which is λ-max of Hydrochlorthiazide in methanol. Calibration curves were linear in range of 10-**80** µg/mL **(**r^2=0.999) and 2 -**16** µg/mL **(**r^2=0.999) for Nebivolol HCl and Hydrochlorothiazide respectively. The method was validated statistically.

Keywords: Hydrochlorothiazide, Nebivolol Hcl, Absorbance ratio method.

INTRODUCTION

Chemically Nebivolol is α, α'–[iminobis (methylene)] bis [6-flouro-3,4,-dihydro-2H-1-benzopyran-2- methanol hydrochloride with competitive & selective B1-receptor antagonist[1]. Chemically Hydrochlorothiazide is 6-Chloro-3,4-dihydro-2H- 1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide with diuretic activity[1]. Both of the drugs are used for the treatment of hypertension. A literature survey regarding quantitative analysis of these drugs revealed that attempts were made to develop analytical methods for NEB-H using RP-HPLC[2-4], HPTLC[5] and Spectrophotometry[6-7]. As far as HCTZ is concerned, some reports are available for its estimation in dosage form using spectrophotometry[8-15], RP-HPLC[10,13,16-34], HPTLC[15,35-38]. This paper describes spectrophotometric method for the determination of NEB-H and HCTZ in mixtures without prior separation. Also the proposed method is shown to be useful in determination of both drugs in combined tablet formulation.

MATERIALS & METHODS
MATERIALS
Spectrophotometric analysis was carried out on a SHIMADZU 2400 series double beam spectrophotometer with a fixed slit width (1nm) attached to the computer with UV-Probe, version 2.21 software for obtaining the spectra. Pure drug samples of Nebivolol Hcl and Hydrochlorothiazide were kindly gifted by Torrent Pharmaceutical Ltd., Ahmedabad-Mehsana Highway, Gujarat-382721, Unichem Laboratories Ltd., Raigad. and Micro Laboratories Ltd., Banglore. The gift samples were used as standard without further purification. Methanol (AR Grade, CDH® (P) LTD., Sarigam, Gujarat) was used as solvent in this work. The commercial pharmaceutical preparations were procured from the local market.

METHODS
Preparation of Standard Solutions
A 10 mg of standard NEB-H and HCTZ were weighed and transferred to 100 mL separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100 µg/mL each of NEB-H and HCTZ.

METHODOLOGY
Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max

of one of the two components. From the overlay spectra of two drugs, it is evident that NEB-H and HCTZ show an isoabsorptive point at 287 nm. The second wavelength used is 271 nm, which is the λ-max of HCTZ. Working standard solutions having concentration range 10-80 µg/ml for NEB-H and 2-16 µg/ml for HCTZ were prepared in methanol and the absorbance at 271 nm and 287 nm were measured and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations.

$$CX = [(QM – QY) / (QX -QY)] \times A1/ax1 .... (1)$$
$$CY = [(QM – QX) / (QY -QX)] \times A2/ay1 … (2)$$

Where, A1 and A2 are absorbance of mixture at 271 nm and 287 nm; ax1 and ay1 are absorptivities of HCTZ and NEB-H at 271 nm; ax2 and ay2 are absorptivities of HCTZ and NEB-H respectively at 287 nm; QM = A2 / A1, QX = ax2 / ax1 and QY = ay2 / ay1.

Analysis of Tablet Sample
A total of 20 tablets were accurately weighed and powdered. Tablet powder equivalent 12.5 mg of HCTZ and 5 mg NEB-H was taken for the analysis and dissolved in 100 mL methanol and were further diluted with methanol to get the solution containing 12.5 µg/mL of HCTZ and 5 µg/mL of NEB-H. The absorbance of solution was measured at 271 nm and 287 nm. Absorbances at two wavelengths were substituted in absorbance ratio equation to calculate the amount of drugs present in tablet.

Method Validation[39-40]
The method was validated for linear range, accuracy, precision, Limit of detection (LOD) and Limit of quantification (LOQ).
  (a) Linearity and range
       solutions of different concentrations were prepared and checked for the linearity.
  (b) Precision
       Precision of the method was measured in terms of the interday and intraday precision. The experiment was repeated for three times in a day using three different concentrations (3×3) for intraday and three different concentrations on three consecutive days for interday precision.
  (c) Accuracy
       Accuracy of the method was determined by performing recovery study from previously analyzed tablet powder by standard addition method at three different levels.

  (d) LOD and LOQ
       LOD and LOQ were found out by the following equations.

LOD = 3.3 Sa / b and LOQ = 10 Sa / b, where Sa = standard deviation of the intercept and b = slope of calibration curve.

RESULTS AND DISCUSSION
Methanol was a solvent of choice owing to high solubility of NEB-H and HCTZ and also there was no shift in the absorbance maxima of NEB-H and HCTZ in methanol. The overlain UV absorption spectrum of HCTZ and NEB-H showing isoabsorptive point (287 nm) in methanol is shown in [Figure 1]. The validation parameters were studied at all the wavelengths for the proposed method. The calibration curves for NEB– H and HCTZ at both the selected wavelengths were obtained by plotting the absorbance Vs the concentration. NEB – H and HCTZ were found to be linear in the range of 10-18 µg/mL and 2-16 µg/mL with r^2 = 0.999 and 0.999 at both the wavelengths respectively [Figure 2,3,4,5]. The regression analysis of the calibration curves is shown in [table 1].The recoveries of NEB – H and HCTZ were found to be 100.67 - 102.25 and 98.67 -101.87 respectively, which were satisfactory [Table 2]. The validation parameters are summarized in [Table 1]. The proposed spectroscopic method was applied to combined dosage form (Tablet). The results obtained for NEB-H and HCTZ were comparable with the corresponding label claim percentage [Table 4].

CONCLUSION
The proposed Absorbance spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of NEB-H and HCTZ in synthetic mixture. The method utilizes easily available and cheap solvent for analysis of both drugs hence the method was also economic for estimation of NEB-H and HCTZ from synthetic mixture and may be successfully applied in laboratories for the determination of NEB-H and HCTZ in combined dosage form. High recoveries show that the method is free from the interference from excipients used in the commercial pharmaceutical preparations.

**Table 1: Regression analysis and summary
of validation parameters for HCTZ and NEB-H**

| Parameters	HCTZ	NEB-H
Wavelength	271	287
Linear range (µg/ml) 2-16		2-16
Correlation coefficient (r^2)	0.999	0.999
Slope	0.065	0.0095
Intercept	0.0021	0.0051
Intraday Precision	0.782	0.888
Interday Precision	1.526	1.255
LOD	0.143	0.650
LOQ	0.432	1.969
% Recovery	98.67-101.87	100.67-102.25

Table 2: Result of recovery study

Sample Concentration (µg/mL)		Amt. of Drug Added (µg/mL)		Amt. recovered (µg/mL)		% Recovery*	
HCTZ	NEB-H	HCTZ	NEB-H	HCTZ	NEB-H	HCTZ	NEB-H
12.5	5.0	10.00	4.00	10.18	4.09	101.80	102.25
12.5	5.0	12.00	5.00	12.38	5.06	99.04	101.20
12.5	5.0	15.00	6.00	14.80	6.04	98.67	100.67

* Mean of three determinations

Table 3: Assay result

Formulations	Label claim (mg)		% Assay* ± SD	
	HCTZ	NEB-H	HCTZ	NEB-H
Brand A	12.5	5.00	99.04 ±0.158	100.70 ±0.090

* Average of three determination
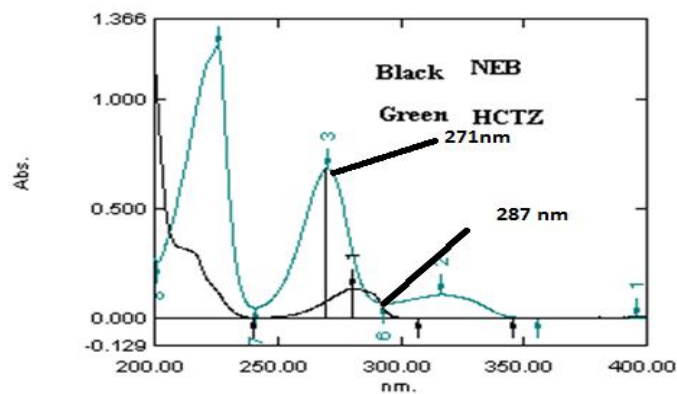
**Fig. 1: Overlain spectra of HCTZ
(10 µg/mL) and NEB-H (10µg/mL)**

calibration curve of HCTZ at 271.0 nm
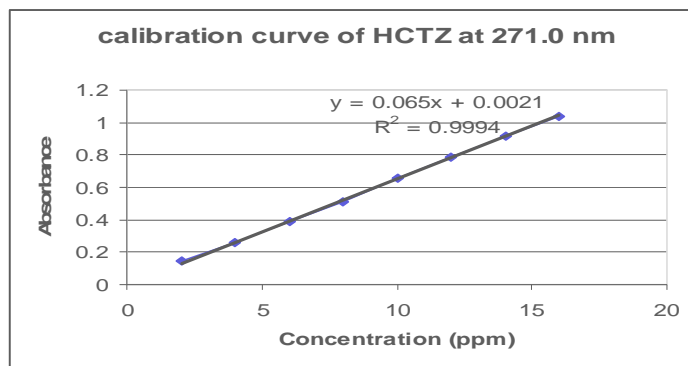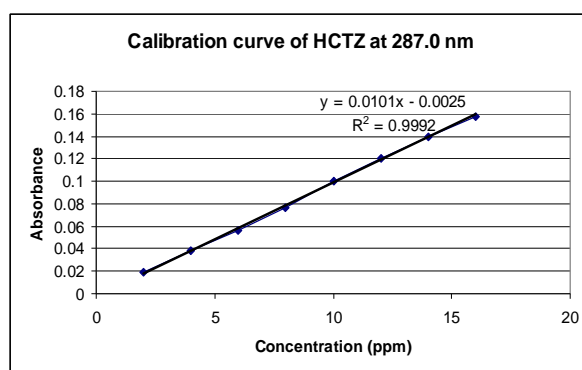
$y = 0.065x + 0.0021$
$R^2 = 0.9994$

Fig. 2: Calibration Curve of HCTZ at 271.0 nm

Calibration curve of HCTZ at 287.0 nm

$y = 0.0101x - 0.0025$
$R^2 = 0.9992$

Fig. 3: Calibration Curve of HCTZ at 287.0 nm

Calibration curve for NEB at 271.0 nm
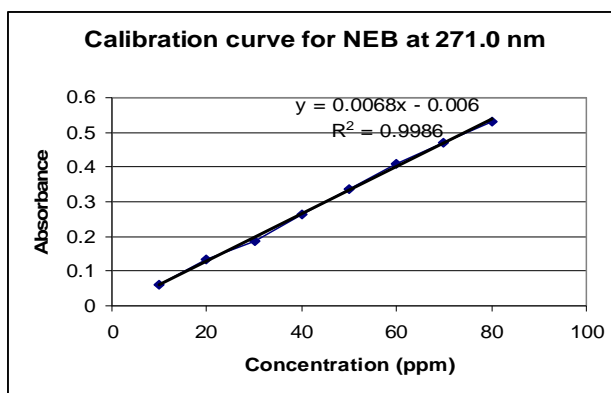
$y = 0.0068x - 0.006$
$R^2 = 0.9986$

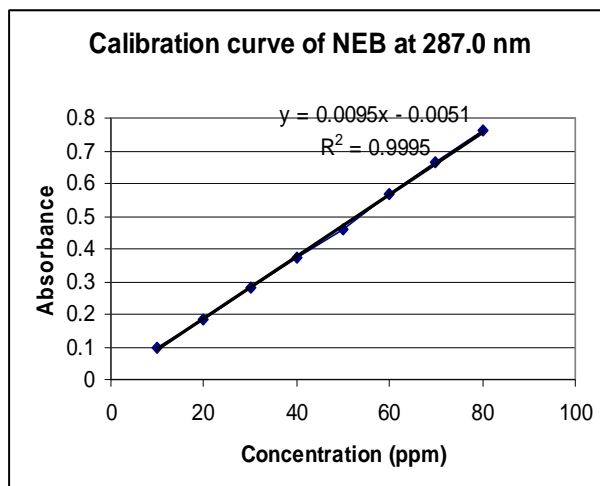**Fig. 4: Calibration Curve of NEB-H at 271.0 nm**

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Fig. 5 Calibration Curve of NEB-H at 287.0 nm

REFERENCES
1. Budavari S. The Merck Index, New Jersey, 18th edition (2006): 827:1112.
2. Ramakrishna NVS, Vishwottam KN, Koteshwara M, Manoj S, Santosh M and Varma DP. Rapid quantification of nebivolol in human plasma by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. J Pharm Biomed Anal. 2005;39(5):1006-1013.
3. Rajeswari K, Sankar GG, Rao AL, Raju DB and Rao JVNL. RP-HPLC estimation of nebivolol in dosage form. Asian J Chem. 2005;17(2):1259-63.
4. Aboul-Enein HY and Ali I. HPLC enatiomeric resolution of nebivolol on normal and reversed amylose based chiral phases. Pharmazie. 2001;56(3):214-13.
5. Reddy TS and Devi PS. Validation of a high performance thin layer chromatography method with densitometric detection for quantitative analysis of nebivolol hydrochloride in tablet formulations. J Chromatography. 2007;20(2):149-152.
6. Kaila MM, Mondal N, Ghosh LK and Gupta BK. A validated UV spetrophotometric method for estimation of nebivolol hydrochloride in bulk and pharmaceutical formulation. Pharmazie. 2007;62(7):486-87.
7. Bhatt LR, Vora AT and Damle MC. Simultaneous estimation of nebivolol hydrochloride and amlodipine besylate in bulk and combined tablets dosage form by U.V. specrophotometry. Indian Drugs. 2007;44(12):915-19.
8. Sahu R and Patel VB. Simultaneous spectrophotometric estimation of hydrochlorothiazide and bisoprolol fumarate in combined dosage forms. Indian J Pharm Sci. 2006;68(6):764-767.
9. Panderi IE. Simultaneous determination of benazepril hydrochloride and hydrochlorothiazide in tablets by second-order derivative spectrophotometry. J Pharm Biomed Anal. 1999; 21(2):257-265.
10. Satana E, Altinay S, Goger NG, Ozkan SA and Senturk Z. Simultaneous determination of valsartan and hydrochlorothiazide in tablets by first derivative ultraviolet spectrophotometry and LC. J Pharm Biomed Anal. 2001;25(5-6):1009-13.
11. Walily AFEl, Belal SF, Heaba EA and Kersh El. A Simultaneous determination of enalapril maleate and hydrochlorothiazide by First derivative ultraviolet spectrophotometry and high performance liquid chromatography. J Pharm Biomed Anal. 1995;13(7):851-6.
12. Erk N. Application of First derivative ultraviolet spectrophotometry and ratio derivative spectrophotometry for the simultaneous determination of candesartan cilexetil and hydrochlorothiazide. Pharmazie. 2003;58(11):796-800.
13. Erturk S, Cetin SM and Atmaca S. Simultaneous determination of moexipril hydrochloride and hydrochlorothiazide in tablets by first derivative spectrophotometry and high performance liquid chromatography method. J Pharma Biomed Anal. 2003; 33(3):505-11.
14. Kowalczuk D and Hopkala H. Application of derivative spectrophotometry for simultaneous determination of quinapril and hydrochlorothiazide in combination tablets. J AOAC Int. 2004;87(4):847-51.

15. Shah SA, Rathod IS, Suhagia BN, Savale SS and Patel JB. Simultaneous determination of losartan and hydrochlorothiazide in combined dosage forms by First derivative spectrophotometry and high performance thin layer chromatography. J AOAC Int. 2001;84 (6):1715-23.

16. Patel LJ, Suhagia BN, Shah PB and Shah RR. Simultaneous estimation bisoprolol fumarate and hydrochlorothiazide in tablets dosage form by RP-HPLC method. Indian J Pharma Sci. 2006;68(5):635-638.

17. Wankhede SB, Tajne MR, Gupta KR and Wadodkar SG. RP-HPLC method for simultaneous estimation of telmisartan and hydrochlorothiazide in tablet dosage form. Indian J Pharma Sci. 2007;69(2):298-300.

18. Pai PNS, Shenoy KRP and Pandey J. Simultaneous RP-HPLC determination of metoprolol tartrate and hydrochlorothiazide in tablets. Indian J Pharma Sci. 2005;67 (5):608-610.

19. Suhagia BN, Shah RR and Patel DM. Development of RP-HPLC method for evaluating losartan potassium and hydrochlorothiazide in tablets. Indian J Pharma Sci. 2005;67(1):37-42.

20. Wei YQ, Zou HB and Yuan JR. Simultaneous LC determination of chlorogenic acid and hydrochlorthiazide in zhenju jiangya tablets. J Chromatographia. 2006;64(11-12): 709-711.

21. Erram S and Tipnis HP. Simultaneous estimation of penbutolol sulfate and hydrochlorothiazide from tablets by RP-HPLC. Indian J Pharma Sci. 1992;54(6): 245-249.

22. Mashru RC, Sutariya VB and Thakker AJ. High performance liquid chromatographic method for simultaneous determination of fosinopril sodium and hydrochlorothiazide in tablets formulation. Ars Pharmaceutica. 2006;47(4):376-384.

23. Baing MM, Vaidya VV, Sane RT, Menon SN and Dalvi K. Simultaneous RP-LC determination of losartan potassium, ramipril, and hydrochlorothiazide in Pharmaceutical Preparations. J Chromatograpia. 2006;64(5-6):293-296.

24. Huang T, Zhong H, Yang R, Shao L, Zhena X and Duan G. Simultaneous determination of captopril and hydrochlorothiazide in human plasma by RP-LC from linear gradient elution. J Pharm Biomed Anal. 2006;41(2):644-648.

25. Carlucci G, Palumbo G, Mazzeo P and Quaglia MG. Simultaneous determination of losartan and hydrochlorothiazide in tablets by high performance liquid chromatography. J Pharm Biomed Anal. 2000;23(1):185-9.

26. Panderi IE and Parissi- Poulou M. Simultaneous determination of benazepril and hydrochlorothiazide by micro-bore liquid chromatography. J Pharm Biomed Anal. 1999;21(5):1017-24.

27. Belal F, Al-Zaagi IA, Gadkariem EA and Abounassif MA. Stability-indicating LC method for the simultaneous determination of ramipril and hydrochlorothiazide in dosage forms. J Pharm Biomed Anal. 2001;24(3):335-42.

28. Zecevic M, Zivanovic LJ and Agatonovic S. Statistical optimization of a reversed-phase liquid chromatographic method for the analysis of amiloride and Hydrochlorothiazide in tablets. J Pharm Biomed Anal. 2000;22(1):1-6.

29. Zendelovska D and Stafilov T. Development of solid phase extraction method and its application for determination of hydrochlorothiazide in human plasma using HPLC, Biomed Chromatography. 2004;18(2):71-6.

30. Butterfield AG, Lovering EG and Sears RW. Simultaneous determination of reserpine and hydrochlorothiazide in two component tablet formulations by HPLC. Indian J Pharm Sci. 1978;67(5):650-3.

31. Menon GN and White LB. Simultaneous determination of hydrochlorothiazide and triaterene in capsule formulations by HPLC. J Pharm Sci. 1981;70(9):1083-5.

32. Walily AF and Belal SF. Simultaneous estimation of enalapril maleate and hydrochlorothiazide by first derivative ultraviolet and HPLC. J Pharm Biomed Anal. 1995;13(7):851-6.

33. Kartal M and Erk N. Simultaneous determination of hydrochlorothiazide and amiloride hydrochloride by ratio spectra derivative and HPLC, J Pharm Biomed Anal. 1999;19(3-4):477-85.

34. Erk N. Analysis of binary mixture of losartan potassium and hydrochlorothiazide by using HPLC, ratio spectra derivative spectrophotometric and compansation technique. J Pharm Biomed Anal. 2001;24(4):603-11.

35. Shah NJ, Suhagia BN, Shah RR and Shah PB. Development and validation of a HPTLC Method for the simultaneous estimation of telmisartan and hydrochlorothiazide in tablet Dosage Form. Indian J Pharm Sci. 2007;69(2):202-205.

36. Shah NJ, Suhagia BN, Shah RR and Patel NM. Development and validation of a HPTLC method for the simultaneous

estimation of irbesartan and hydrochlorothiazide in tablet dosage form. Indian J Pharm Sci. 2007;69(2):240-243.

37. El-Gindy A, Ashour A, Abdel-Fattah L and Shabana MM. Application of LC and HPTLCdensitometry for the simultaneous determination of benazepril hydrochloride and hydrochlorothiazide. J Pharm Biomed Anal. 2001;25(2):171-9.

38. Walode SG, Charde MS, Tajne MR and Kasture AV. Development of HPTLC method for simultaneous estimation of captopril and hydrochlorothiazide in combined dosage form. Indian Drugs. 2005;42(6):340-344.

39. Anonymous: International Conference on Harmonization, Q2A Text on Validation of Analytical Procedures, International Conference on Harmonization, (CPMP/ICH/381/95), 1995.

40. Anonymous: International Conference on Harmonization, Q2B Validation of Analytical Procedures: Methodology, International Conference on Harmonization, (CPMP/ICH/381/95), 1996.