RESEARCH ARTICLE

A Particular Separation Method Development and Validation of Nadolol and Bendroflumethiazide by using RP-HPLC

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ABSTRACT

We have developed a simple, general, and reliable HPLC Method and validation for the simultaneous estimation of nadolol and bendroflumethiazide drugs according to (ICH) guidelines. Nadolol and bendroflumethiazide peaks have been observed at a retention time of 1.757, and 3.208 minutes, respectively, and they have kept 5 minutes as a total run time. As per linearity results, the average correlation coefficient of nadolol and bendroflumethiazide is 0.999 which indicates they have good linearity, robustness, and stability.

Keywords: LC-MS/MS, RP-HPLC, Nadolol, Bendroflumethiazide.

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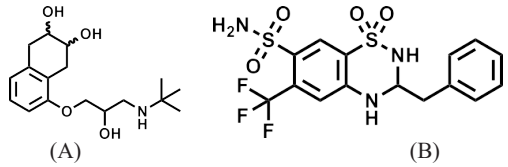
INTRODUCTION

Nadolol (Figure 1) generally can be used to reduce the high BP[1,2] and hear pain[3,4] It is called Corgard in the commercial market. Furthermore, it can also be used for the prevention of migraine[5] headaches and complications of cirrhosis.[6,7] However, it may produce some common side effects such as tiredness, dizziness,[8] reduction of heartbeat.[9] On the other hand, hypertension can be cured by the usage of bendroflumethiazide (Figure 1), which is generally called bendrofluazide in society. It works by preventing sodium reabsorption at the start of[10,11] the distal convoluted tubule. In addition, it plays an important role in the curing of mild heart problems, although loop diuretics are better suited to reducing overload.[12]

RESULTS AND DISCUSSION

Method Development and Optimization

After conducting optimization for specificity, resolution, and retention time, the most appropriate isocratic condition for the separation of Nadolol and Bendroflumethiazide using an inertsil ODS column was determined. 0.1% orthophosphoric acid (OPA) and acetonitrile in a 20: 80 ratio was identified as the mobile phase for this separation. Upon growing concentration of the mobile phase, the resulting chromatogram exhibited an elevation in either background noise or peak broadening, indicating the presence of tailing.



(A) (B)

Figure 1: Chemical compounds of Nadolol (**A**) and Bendroflumethiazide (**B**)

Hence, considering the above-mentioned parameters, Nadolol and Bendroflumethiazide were removed at retaining times of 1.757 and 3.208 minutes, respectively (Figure 2). For additional information, please refer to Table 1, which provides details on the chromatographic parameters implemented in this analytical method.

Validation of Method

Having established guidelines of ICH analytical labs, we have performed the suitability of the HPLC system using standard conditions. Its chromatogram is depicted in Figure 3 and all the results are tabulated in Table 2.

Specificity

Upon completion of the system suitability study, our attention turned to the specificity analysis of the samples (Table 3). In this regard, an appropriate amount of active pharmaceutical ingredient (API) was dissolved in a proper solvent and, then it was subsequently introduced into the high-performance liquid chromatography (HPLC) system. Solution and impurities

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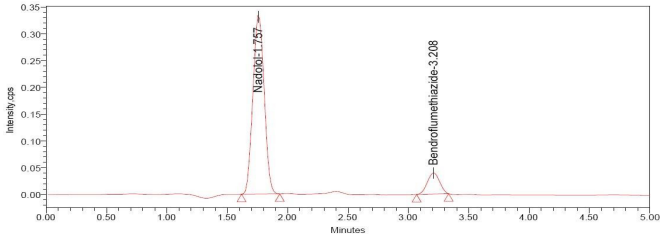


Figure 2: Nadolol and bendroflumethiazide chromatogram peaks

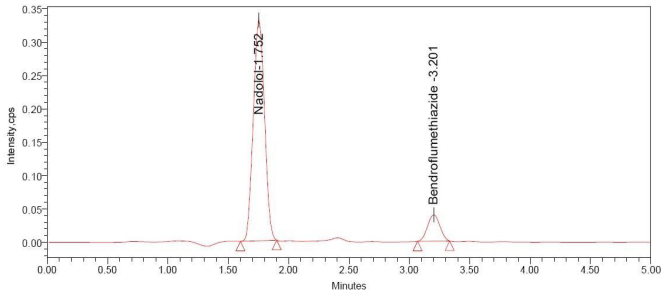


Figure 3: Chromatogram of sample

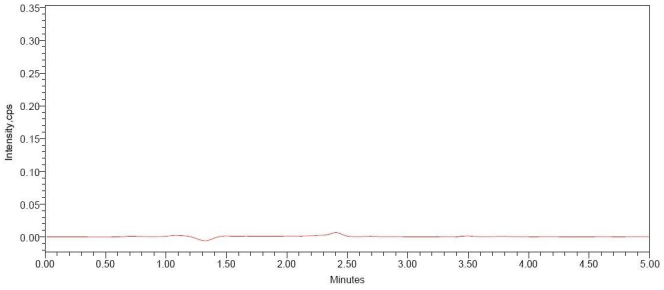
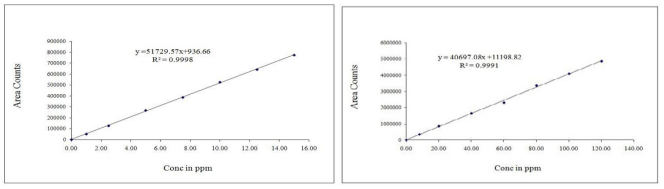


Figure 4: Chromatogram of blank



(A) (B)

Figure 5: Linearity plots of (A) Nadolol and (B) Bendroflumethiazide

chromatograms were obtained (Figure 4) and, we have not observed any indications of interference of the chromatogram.

Linearity

To evaluate linearity, the sample concentrations were meticulously prepared using a rigorous procedure. For nadolol, concentrations ranging from 8 to 120 µg/mL were selected. The resulting regression equation was determined as Y = 40697.08x + 11198.82, with a high correlation coefficient of 0.9993, indicating a robust linear relationship between concentration and response (Figure 5).

Similarly, for bendroflumethiazide, concentrations ranging from 1 to 15 µg/mL were employed, leading to a regression equation of Y = 51729.57x + 936.66 and an impressive correlation coefficient of 0.9998, affirming an excellent linear correlation between concentration and response (Figure 5).

Table 1: HPLC results of Nadolol and Bendroflumethiazide

S.No.	Variable	Conditions
(i)	Column	Intertsil ODS 150x4.6mm, 3.5 µ
(ii)	Rate of flow	1 ml/min
(iii)	Sample running time	5 min
(iv)	Volume of injection	10 µl
(v)	Wavelength of light source	270 nm
(vi)	Mobile Phase	0.1% OPA: CAN 80:20

Table 2: HPLC system precision results

S. No.	*Suitability variable of system*	*Acceptance Criteria*	*Rug Name*	
			Nadolol	*Bendroflumethiazide*
(i)	RSD (%)	NMT 2.0	0.61	1.41
(ii)	USP Tailing	NMT 2.0	1.02	1.11
(iii)	USP Plate Count	NLT 3000	2563	4256

Table 3: Linearity information of sample

S.No.	*Nadolol*		*Bendroflumethiazide*	
	Conc (µg/mL)	*Area*	*Conc (µg/mL)*	*Area*
Linearity-1	8	345962	1	50621
Linearity-2	20	856254	2.5	126254
Linearity-3	40	1652478	5	267845
Linearity-4	60	2315796	7.5	386521
Linearity-5	80	3365241	10	526415
Linearity-6	100	4102635	12.5	642417
Linearity-7	120	4869575	15	774952
Slope	40697.08		51729.57	
Intercept	11198.82		936.66	
CC	0.9993		0.9998	

Table 4: Robustness of sample

S. No.	Name of Variable	%RSD for purity	
		Nadolol	Bendroflumethiazide
(i)	Rate of Flow: 0.8 mL/min	1.38	0.94
(ii)	Rate of Flow: 1.2 mL/ min	0.56	0.33
(iii)	Organic solvent (+ 10%) (88:12)	0.83	0.76
(iv)	Organic solvent (- 10%) (72:28)	1.27	1.52

Robustness

Robustness results are inserted in Table 4. Here it is noted that variables like flow rate (± 0.2 mL) and organic solvent composition (± 10%) were altered to investigate robustness. Notably, these adjustments resulted in negligible changes in the percentage relative standard deviation.

Stability

The stability of nadolol and bendroflumethiazide was studied at room temperature and 2 to 8°C with intervals of every 6 hours. The results are shown in Table 5. The final results clearly recommended that there is no significant deviation of purity at the above-said temperatures.

Table 5: Results of stability

S. No.	*Monitoring of time*	*Quality of Nadolol at rt*	*Quality of Nadolol at 2–8oC*	*Quality of Bendroflumethiazide at rt*	*Quality of Bendroflumethiazide at 2–8oC*
(i)	Initial	99.98	99.96	99.99	99.96
(ii)	6 hours	99.95	99.94	99.95	99.92
(iii)	12 hours	99.92	99.89	99.92	99.87
(iv)	18 hours	99.91	99.85	99.84	99.85
(v)	24 hours	99.87	99.80	99.79	99.82

Table 6: Precision results

Analyte	*Standard Conc.*	*RSD (%)*
Nadolol	80	0.71
Bendroflumethiazide	10	0.55

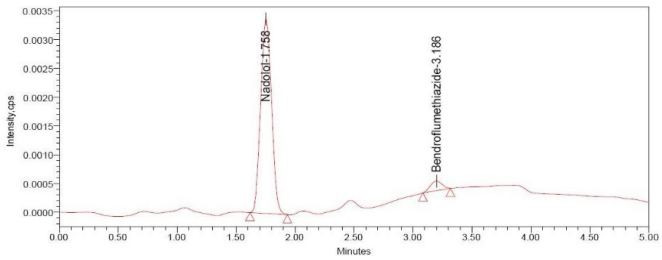


Figure 6: COD chromatogram

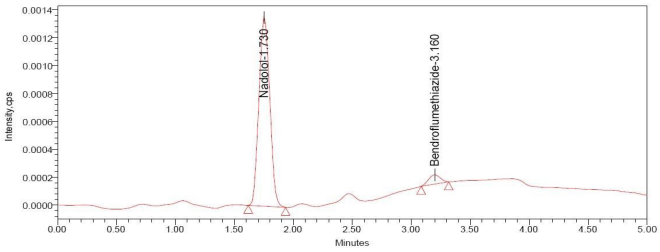


Figure 7: COQ chromatogram

Precision

To determine the precision of the analytical method, a comprehensive approach was incorporated in accordance with the established guidelines set forth by the International Council for Harmonisation (ICH). The sample underwent a meticulous analytical procedure, strictly adhering to the ICH instructions, encompassing every step from sample preparation to the conclusive determination of results (Table 6).

Cap of detection and quantification

As per the below-shown chromatogram nadolol and bendroflumethiazide, CoD concentrations were observed at 0.1 and 0.013 µg/mL, respectively. Whereas CoQ concentrations of nadolol and bendroflumethiazide were identified at 0.33 and 0.041 µg/mL, respectively (Figure 6). The results indicate the LoQ concentrations (Figure 7) are higher than the concentrations of LoD.

Accuracy

The accuracy of the sample was conducted using three compositions (50, 100, and 150%). APIs of nadolol having concentrations 40, 80, and 120 µg/mL and bendroflumethiazide bearing concentrations 5, 10, and 15 µg/mL were prepared, and their accuracy was tested (Table 7 and 8). Their accuracy was identified at the level of 99.4–100.1%.

Table 7: Accuracy of nadolol

S.No.	*Level (%)*	*Accuracy (%)*	*Average accuracy (%)*
(i)	50	99.6	99.5
(ii)		99.3	
(iii)		99.7	
(iv)	100	99.8	99.7
(v)		99.8	
(vi)		99.6	
(vii)	150	99.5	99.4
(viii)		99.2	
(ix)		99.4	

Table 8: Accuracy of bendroflumethiazide

S.No.	*Level (%)*	*Accuracy (%)*	*Average accuracy (%)*
(i)	50	100.2	100.1
(ii)		100.1	
(iii)		99.9	
(iv)	100	99.8	100.0
(v)		99.9	
(vi)		100.4	
(vii)	150	99.7	99.5
(viii)		99.5	
(ix)		99.3	

Table 9: Nadolol and bendroflumethiazide degradation studies

Degradation condition	*%Degradation of Nadolol*	*%Degradation of Bendroflumethiazide*
Unstressed degradation	0.1	0.1
Acid degradation	16.5	15.7
Alkali degradation	16.2	15.1
Peroxide degradation	14.3	14.3
Reduction degradation	13.8	12.7
Thermal degradation	11.9	13.4
Hydrolysis degradation	12.4	11.6

Degradation studies

The present study involved conducting forced degradation experiments to evaluate the activity of nadolol and bendroflumethiazide samples. The results demonstrated the efficacy of the employed analytical method in detecting and characterizing degraded products. Furthermore, these investigations provided valuable insights into the drug substances' susceptibility to instability under various conditions, thereby emphasizing the importance of implementing appropriate measures during formulation to mitigate potential instabilities. A comprehensive compilation of the degradation results can be found in Table 9. Notably, the findings indicated that the percentage of acid-induced degradation was notably higher for both nadolol and bendroflumethiazide compared to other degradation pathways.

DISCUSSION

In this investigation, RPHPLC method was employed using ODS column. The combination of 0.1% OPA and acetonitrile (20:80) was taken as mobile phase. This chromatographic system was utilized to effectively separate nadolol and bendroflumethiazide, enabling their accurate quantification and analysis. Analytical samples were prepared as per the guidelines of ICH and the FDA to check the reliability, accuracy, and precision of nadolol and bendroflumethiazide. All the activities have shown moderate to good results. Furthermore, a comparison was made between the analysis of the market preparation and our validated assay method. The results showed that our method successfully detected the drug contents without any overlapping peaks observed in the chromatograms of marketed products. Notably, the robustness of our method remained unaffected even by varying parameters also, demonstrating the reliability and consistency of the method across different conditions.

CONCLUSION

RP-HPLC validation has been accomplished for the selective determination of nadolol and bendroflumethiazide. The degradation behavior of the drugs indicated a higher susceptibility under acidic conditions. The regression line equation derived from the calibration curve showed excellent predictability for quantifying drug concentrations within the range of 8 to 120 µg/mL for nadolol and 1 to 15 µg/mL for bendroflumethiazide, utilizing the corresponding peak areas. Furthermore, the method was subjected to rigorous validation demonstrating its reliability, sensitivity, robustness, and specificity. It successfully enabled the accurate and sensitive detection of nadolol and bendroflumethiazide.

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