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Development and Validation of RP-HPLC method for Simultaneous estimation of combined drug in Pharmaceutical formulation

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Abstract

Analytical techniques hold the key to the design, development, standardization and quality control of medical products. In the present research work a modest attempt has been made to develop validated analytical methods for the determination of single or combined dosage form. Research had done to developed simple, rapid and sensitive, stable and highly effective simple RP-HPLC method for determination of Nebivolol HCL and Telmisartan, to validate methods as per ICH Guidelines, to analysed Nebivolol HCL and Telmisartan using validated methods quantitatively and qualitatively. Solubility,wavelength, Optimization of chromatographic condition, Linearity, System Suitability test, proposed method for estimation of both drugs,validation studies performed in this article. Method successfully quantified the selected analytes from tablet formulation.No interference of additives etc.is encountered in this method further studies on other pharmaceuticals formulation would throw more light on those study.

Keywords: RP-HPLC, Nebivolol HCL, Telmisartan, Validation

Introduction

Analytical techniques hold the key to the design, development, standardization and quality control of medical products. In the present research work a modest attempt has been made to develop validated analytical methods for the determination of single or combined dosage form. Estimation of degradants generated during formulation and storage of finished products using techniques like UV-Visible Spectrophotometer, HPLC, HPTLC and UPLC (1-3). The solvent composition may be attended gradually to give gradient elution. The rate of distribution between stationary and mobile phase is controlled by diffusion process. In diffusion minimized a faster and effective separation can be achieved. These properties may include solubility, shifting of max, overlapping of the absorbance, etc. But the various sophisticated analytical instruments are now overcoming these problems. (2,4)

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The various methods of analysis can be broadly classified into two categories; Classical methods and Instrumental methods, UV-Visible spectrophotometry, High performance liquid chromatography (HPLC) and High performance thin layer chromatography (HPTLC) are the most widely used techniques (2,4,5). Chromatography may be defined as a method of separating a mixture of components into individual components through equilibrium distribution between two phases that is mobile phase and stationary phase(6). HPLC is the method of choice for checking peak purity of new chemical entities, monitoring reaction changes is in synthetic procedures or scale up, evaluating new formulations and carrying out quality control / assurance of the final drug products(7).The essential parts of the High Performance Liquid Chromatography are, Solvent reservoir and Treatment system, Mobile phase, Pump system, Sample Injection System, Column, Detector, Recording and interpretation unit. The most widely used detector in HPLC is the UV-absorption or spectrophotometer. In this detector the changes in UV-absorption when the solution passes through a flow cell is measured. UV detectors are concentration sensitive and have the advantage that they don't destroy the solute. UV detection can utilizes the fixed emission line of a mercury line (254 nm) to allow the detection of molecules with some absorption at this wavelength. The continuous emission of energy by deuterium lamp can be utilized in conjunction with a monochromator to provide a variable wavelength detector. Double beam UV detectors are available which can record the spectrum if the flow is stopped; while the solute passes through cell. Not all molecules possess sufficiently strong UV chromophore for satisfactory UV absorption. Bile acids, lipids, sugars etc., are examples of such compounds (4,5).

Validation of an analytical method is the "A documented programme, which provides a high degree of assurance that a specific process will consistently produce, a product meeting its pre-determined specifications and quality attributes" (4,5).The following are typical analytical performance characteristics which may be tested during methods validation, Accuracy, Precision, Repeatability, Intermediate precision, Linearity, Specificity, Range, Robustness System suitability determination (2,3,4,6).

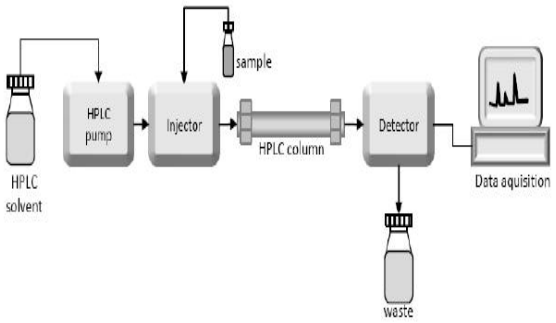

Fig. 1: Instrumentation of HPLC

Material and Methods

Nebivolol HCL, Telmisartan from Lupin Research park, Methanol (HPLC grade), Distilled Water (HPLCgrade), Ortho Phosphoric Acid (HPLCgrade) from Merck Ltd.India,UV-Visible Spectrophotometer model 2080, HPLC Agilent 1100, pH MeterSystonic Sy-614A, Balance CY 104 (Micro Analytical Balance), Ultrasonicator Meta-lab1.5 L 50 were used.

Optimization of chromatographic condition

The mobile phase was prepared by methanol & 0.1% OPA acid having pH-7 (80:20% v/v). To take 400ml of methanol and 100ml of 0.1% OPA acid having pH-7. Figure 3,4,5. To take10mg of Nebivolol HCL and 80mg Telmisartan dissolved in 10ml methanol. Add 1 drops of tri-ethylamine and Sonicated for 10mins. And take 0.2ml from stock solution and dissolved in 10ml mobile phase. This solution holds 20ppm. Of Nebivolol HCL & 80ppm Telmisartan. Table 1,2,3,4. (13,14)

Linearity

Accurately weighed Nebivolol HCL 5mg and Telmisartan 40mg dissolved in 10 ml Methanol. (15,16) And add 1drop tri-ethylamine. And sonicate for 10min. This solution holds 500µg/ml of Nebivolol HCL and 4000µg/ml of Telmisartan. Take 0.1ml from above solution dissolved in 10ml mobile phase. For linearity study to take 10µg/ml, 20µg/ml, 30µg/ml,40µg/ml& 50µg/ml sample are prepared. And inject to record the chromatogram of linearity. (17,18,19,20)

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre analysed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analysed. Statistical validation of recovery studies shown in Table no.7. (21,22)

Specificity

Specificity was measured as ability of the proposed method to obtained well separated peaks for Nebivolol HCL and Telmisartan without any interference from component of matrix. Shown in Table no. 12,13, Figure 7,8. (23,24,25)

Robustness

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. (26) To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate on retention time and tailing factor of drug peak was studied. The mobile phase composition was changed in ± 1 ml proportion and the flow rate was varied by ± 0.1 ml min^{-1}, of optimized chromatographic condition. (27,28) The results of robustness studies are shown in Table No.14 &15. System suitability parameters were also found satisfactory; hence the analytical method would be concluded.

Results and Discussion

Selection of wavelength

This solution is scan by Uv-visible spectrophotometer under a scanning wavelength 200nm-400nm. The wavelength of Nebivolol HCL & Telmisartan was found to be 286nm. And hence this wavelength is used for method development purpose. As show in Fig.2

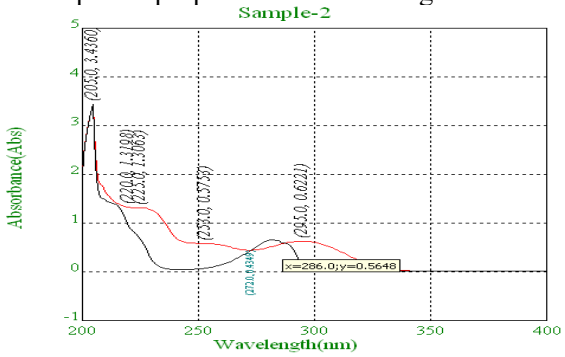

Fig. 2: UV-visible spectra. of Nebivolol HCL & Telmisartan

Selection of mobile phase

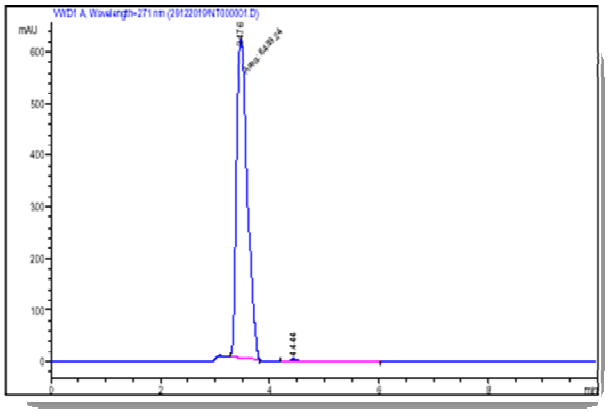

Fig. 3: chromatogram of Trail-1

Table 1: Detail of chromatogram of Trail-1

Sr.No	Drug	Rt time	Area	Plates	Symmetry
1.	TEL	3.476	8439.23	1611	1.10
2.	NEB	4.444	62.32	687	0.47

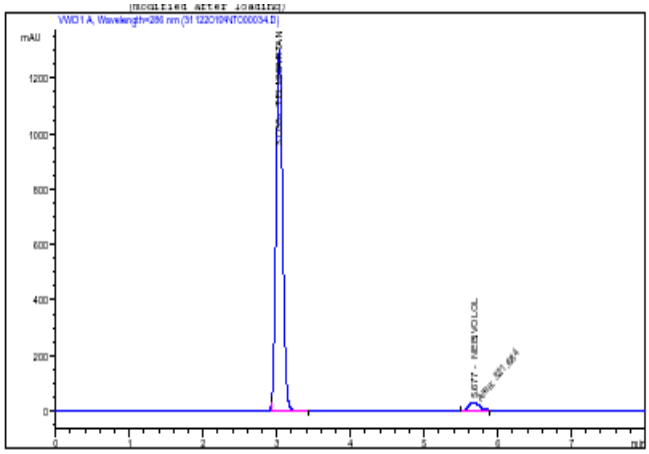

Fig.4: Chromatogram of Trail-2

Table 2: Detail of chromatogram of Trail-2

Sr.No	Drug	Rt time	Area	Plates	Symmetry
1.	TEL	3.609	8166.29	7911	1.22
2.	NEB	4.035	325.57	11967	0.48

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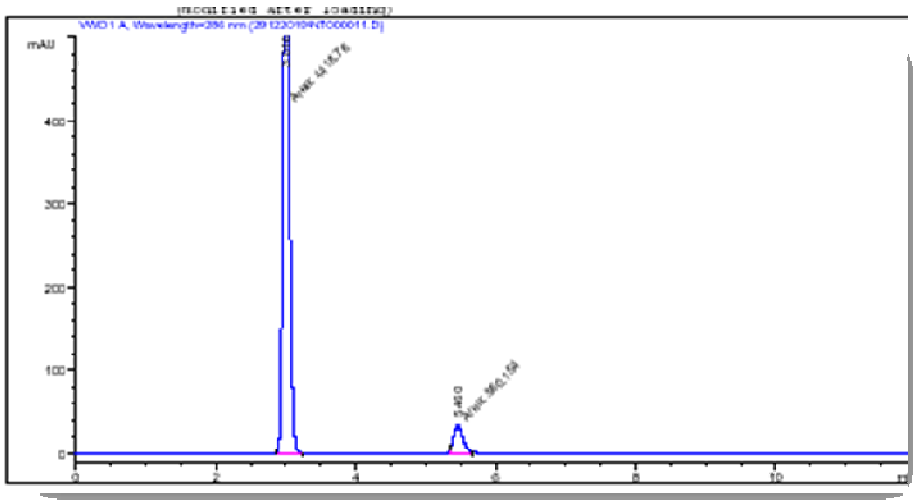

Fig. 5: Chromatogram of Trail-3

Table 3: Detail of chromatogram of Trail-3

Sr.No	Drug	Rt time	Area	Plates	Symmetry
1.	TEL	3.004	4418.68	4812	0.99
2.	NEB	5.469	361.86	6460	0.80

Table No 4: Showing result of Experimental Trials

Trial	Column used	Mobilephase, Flow Rate and Wavelength	Inj. Vol.	Observation	Conclusion
1	C_{18} (AGILENT) (250×4.6m)	Methanol & 0.1% OPA acid (90:10% v/v). Flow Rate=0.7ML, Wavelength-286nm(pH-3)	20 µl	Well resolved peaks were not obtained	Hence rejected
2	C_{18} (AGILENT) (250 ×4.6mm)	80% Methanol: 20% Water (0.1 % OPA) Flow rate 0.7 ml. Wavelength-286nm(pH-3)	20 µl	Well resolved peaks were not obtained	Hence rejected
3	C_{18} (AGILENT) (250 ×4.6mm)	80% Methanol: 20% Water (0.1 % OPA) Flow rate 0.7 ml. Wavelength-286NM (pH-7)	20 µl	Well resolved peaks were obtained	Hence selected

Linearity study

Linearity of Nebivolol was observed in the range of 10-50 µg/ml and Telmisartan was observed in the range of 10-50µg/ml Detection of wavelength used was 286 nm. The calibration curve yielded correlation coefficient (r^2) 0.9999 &

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0.9996 for Nebivolol and Telmisartan respectively. As per Table no.5 and Figure no. 6.

Table 5: Result of standard calibration curve for Nebivolol HCL

Sr No.	Conc	Area I	Area II	Mean	SD	%RSD
1	40	79.71	80.23	79.97	0.37	0.46
2	80	162.3	161.21	161.76	0.77	0.48
3	120	245.13	248.61	246.87	2.46	1.00
4	160	325.89	328.86	327.38	2.10	0.64
5	200	407.99	406.93	407.46	0.75	0.18
		R^2=0.9999	M=16.40	C=1.284	Avg=1.29	Avg=0.55

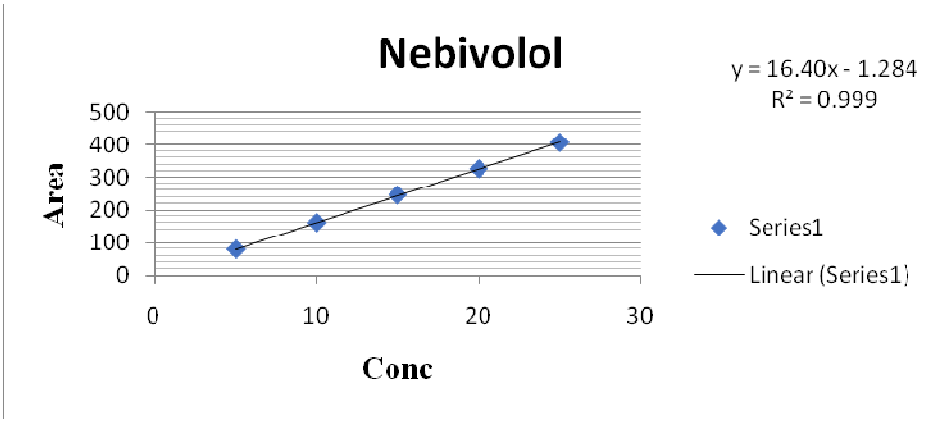

Fig. 6: Standard Calibration Curves for Nebivolol HCL

System suitability test

System suitability was performed to verify, whether the resolution and reproducibility of the chromatographic system are adequate.

Table 6: Result of system suitability for Nebivolol HCL and Telmisartan

Sr. No.	Peak area		Retention Time		Asymmetry		Theoretical plates	
	NEB	TEL	NEB	TEL	NEB	TEL	NEB	TEL
1	244.21	6280.86	5.69	3.04	0.75	0.91	7135	5587
2	243.32	6295.31	5.67	3.03	0.46	0.91	7090	5425
3	244.10	6270.15	5.68	3.05	0.55	0.92	7145	5547
4	242.15	6285.25	5.66	3.06	0.67	0.94	7065	5326

5	245.17	6291.26	5.70	3.07	0.71	0.93	7180	5598
Mean	243.79	6284.56	5.68	3.05	0.62	0.92	7123	5497
S. D	1.12	9.77	0.01	0.01	0.01	0.01	45.63	117.50
C.V	0.45	0.15	0.17	0.32	1.61	1.08	0.64	2.13

Accuracy

Accuracy of method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 99.52-100.99 %.As per table no.7

Table 7: Statistical Validation of Recovery Studies.

Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation	% RSD
80	**Nebivolol HCL**	100.54	0.65	0.44
	Telmisartan	99.70	6.33	0.17
100	**Nebivolol HCL**	100.99	0.58	0.35
	Telmisartan	99.52	7.06	0.17
120	**Nebivolol HCL**	100.1	0.49	0.27
	Telmisartan	99.54	0.96	0.23

Precision

Precision studies were carried out using parameter like intra-day and inter-dayprecision, the study showed that the results were within acceptance limit. i.e.%RSD below 2.0 indicating reproducibility of method. Results as shown in following table no.8,9,10,11.

Result of Intraday Telmisartan

Table 8: Intra-day Precision study Telmisartan

Sr No.	Conc	Area-I	Area-II	Mean	Amt Found	% Amt Found	SD	%RSD
1	80	4165.59	4168.69	4167.14	79.30	99.13	0.96	0.02
2	120	6290.41	6292.61	6291.51	120.79	100.65	1.56	0.02
3	160	8391.47	8386.03	8388.75	161.74	101.09	3.85	0.04

Result of Intraday Nebivolol HCL

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Table 9: Intra-day Precision study Nebivolol HCL

Sr No.	Conc.	Area-I	Area-II	Mean	Amt Found	% Amt Found	SD	%RSD
1	10	161.55	168.69	165.12	10.14	101.46	0.96	0.58
2	15	239.03	234.09	236.56	14.82	98.85	3.49	1.47
3	20	328.8	322.19	325.50	19.92	99.60	4.67	1.43

Interday Telmisartan

Table 10: Interday Telmisartan study

Sr No.	Conc.	Area-I	Area-II	Mean	Amt Found	% Amt Found	SD	%RSD
1	80	4166.32	4168.87	4167.60	79.31	99.14	0.96	0.02
2	120	6287.74	6291.11	6289.43	120.75	100.62	2.38	0.03
/3	160	8389.15	8392.55	8390.78	161.78	101.11	2.51	0.02

Interday Nebivolol HCL

Table 11: Interday Nebivolol HCL study

Sr No.	Conc.	Area-I	Area-II	Mean	Amt Found	% Amt Found	SD	%RSD
1	10	161.23	165.78	163.51	10.04	100.40	0.96	0.58
2	15	241.52	242.91	242.22	14.84	98.93	0.98	0.40
3	20	330.09	326.5	328.30	20.09	100.45	2.54	0.77

Specificity

API and the Tablet sample solution prepared as per the proposed method to check for the interference if any peak at the retention time of Nebivolol and Telmisartan. Thus, no interference was found at the Retention time of Nebivolol and Telmisartanwhich is 3.00 & 5.46min respectively, Shown in Figure no. 7 and 8, Table no.12,13.

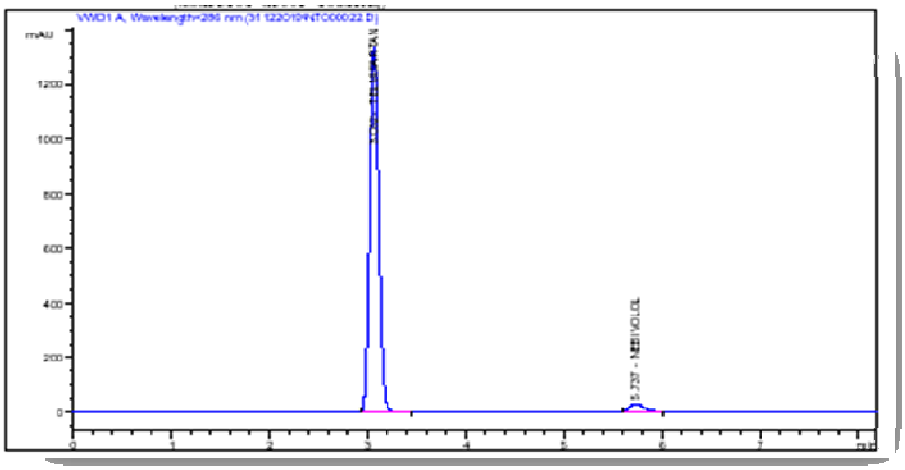

Fig. 7: A chromatogram of API

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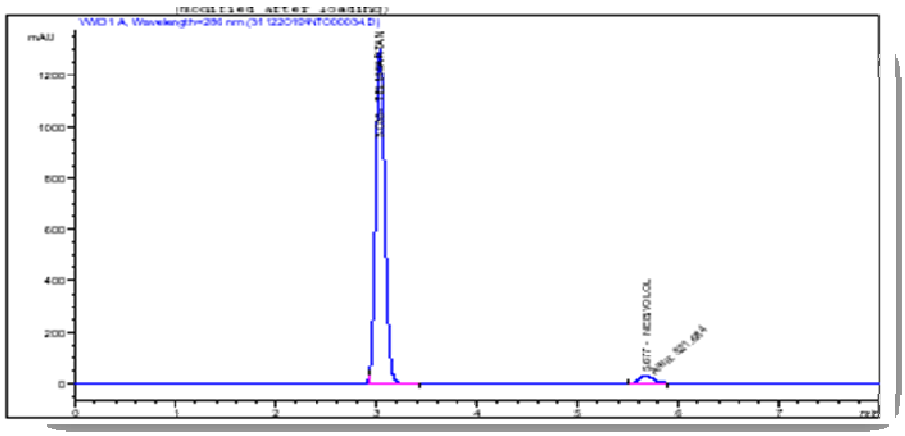

Fig. 8: A chromatogram of Tablets

Table 12: Detail of chromatogram of API

Sr.No	Drug	Rt time	Area	Plates	Symmetry
1.	TEL	3.062	8395.49	5247	0.96
2.	NEB	5.737	325.86	7255	0.75

Table 13: Detail of chromatogram of Tablets

Sr.No	Drug	Rt time	Area	Plates	Symmetry
1.	TEL	3.036	8380.08	5287	0.90
2.	NEB	5.677	326.68	7250	0.53

Robustness

To evaluate the robustness of the method, the parameters selected were varied at three levels. The results indicate that less variability in retention time and tailing factor were observed in following table no. 14,15.

Table 14: Result of Robustness Study of Nebivolol HCL

Parameters	Conc. (µg/ml)	MEAN	SD	%RSD
Mobile phase composition-(81+19)	40	323.16	6.13	1.90
Mobile phase composition-(79+21)	40	304.20	2.72	0.90
Wavelength change285nm	40	335.00	2.11	0.63
Wavelength Change 287nm	40	326.46	1.64	0.50
Flow rate change(0.9ml)	40	396.73	3.94	0.99
Flow rate change(1.1ml)	40	320.46	5.27	1.64

Table 15: Result of Robustness Study of Telmisartan

Parameters	Conc. (µg/ml)	MEAN	SD	%RSD
Mobile phase composition-(81+19)	40	8122.17	15.98	0.20
Mobile phase composition-(79+21)	40	8372.90	24.72	0.73
Wavelength change285nm	40	8111.70	21.96	0.27
Wavelength Change 287nm	40	8044.71	70.59	0.88
Flow rate change(0.9ml)	40	9134.71	17.41	0.19
Flow rate change(1.1ml)	40	7531.67	111.45	1.48

The analysis of tablet formulation was done and the results obtained within the limits. The results obtained for validation study were within the limit specified by the ICH guidelines and hence the method was found to be linear, precise. The results of recovery study were within ICH limits, thus indicating the accuracy of method. The present work involved the development of accurate, precise, and simple suitable RP-HPLC method for estimation of the drugs in multicomponent tablet formulation. Method successfully quantified the selected analytes from tablet formulation. No interference of additives etc.is encountered in this method further studies on other pharmaceuticals formulation would throw more light on those study.

Conclusion

The method was completely validated showing satisfactory data for all the method validation parameters tested. Hence this method can be introduced into routine use for determination of Telmisartan and Nebivolol HCL.

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