Stability Indicating Validated RP-HPLC Method Development for Simultaneous Estimation of Benidipine Hydrochloride and Telmisartan from Pharmaceutical Dosage Form

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Abstract: *Stability indicating validated RP-HPLC method was developed for simultaneous estimation of Benidipine Hydrochloride (BPH) and Telmisartan (TEL) from combined dosage form. The separation of drugs was achieved on a Phenomenax C_{18} Column (250×4.6 mm, 5µm particle size) with UV detection 237 nm using a mobile phase consisting of Methanol: Acetonitrile: water in the ratio of 70:20:10 v/v at a flow rate 0.8ml/min. Retention time was found as 2.51 min for BPH and 3.227 min for TEL. Both drugs obey Beers law in the range of 2-10 µg/ml for BPH and 5-25 µg /ml for TEL. The proposed method was validated as per ICH guidelines for linearity, range, precision, accuracy, robustness and LOD, LOQ and stress degradation studies were carried out under acidic, alkaline, photolytic, thermal degradation condition as per SIAM as described by ICH guidelines. The method is accurate, precise and rapid for routine analysis of BPH and TEL from dosage form. Stress degradation studies proved methods rigidity.*

Keywords: Benidipine Hydrochloride (BPH), TEL (TEL), RP-HPLC, Validation, Stability studies

1. Introduction

BPH [1-2] is a salt form of synthetic dihydropyridine derivative that has anti-hypertensive action. And this dihydropyridine calcium channel blocker is used for the treatment of hypertension. It is reno-cardioprotective and chemically it is 3-(3R)-1 benzylpiperidin-3-yl5-methyl (4R) -2, 6 dimethyl-4-(3-nitrophenyl) - 1, 4-dihydropyridine-3, 5-dicarboxylate hydrochloride. TEL [1-5] is an angiotensin II receptor antagonist (ARB) used in management of hypertension. Generally angiotensin II receptor blockers (ARB) such as TEL bind to angiotensin II type 1 (AT1) receptor with high affinity causing inhibition of the action of angiotensin II on vascular smooth muscle, ultimately to a reduction in arterial blood pressure and chemically it is 2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl] methyl} phenyl) benzoic acid

BPH, TELis drugs used for the treatment of hypertension. Methods such as UV spectroscopic [6] RP-HPLC [7] were reported for estimation of BPHlonelyor along with other antihypertensive agent. Methods such as RP-HPLC [8-14], UV spectroscopic [15] were reported for determination of TEL alone or along with other antihypertensive agents. After literature survey it was found that there is no stability indicating or stress degradation studied method reported for estimation of both drugs in combined dosage form.TEL is official in BP [16] and in IP [17] whereas BPH was not yet included.

2. Materials and Methods

The Pure sample of BPH was procured from Niksan Pharmaceutical Ankleshwar, and TEL was procured from Cadila Healthcare Ahmadabad, India. HPLC grade Water, Acetonitrile, Methanol, Glacial Acetic Acid, NAOH was procured from Merck chemicals, Mumbai.

2.1 Instrumentation

A RP-HPLC system with Rheodyne injector, dual wavelength UV- VIS absorbance detector was used throughout the study. Phenomenax C_{18} (250×4.6.5 µm) Column was used for separation of drugs. A Shimadzu model UV-VIS 1700 a double beam UV-VIS spectrophotometer with spectral bandwidth of 2 nm and wavelength accuracy of ±1nm with 10 mm matched Quartz cells was used for. Electronic balance Afcoset FX 300 was used for weighing of samples.

2.2 Optimization of mobile phase

The main objective of the experiment was to optimize the assay method for simultaneous estimation of BPH and TEL. Different composition of mobile phase was prepared and utilized. The different composition of mobile phase used includes Methanol: Water in the ratio 70:30(pH 4.0), Methanol: Water in the ratio 50:50(pH 3.8) and Acetonitrile: Water in the ratio 90:10(pH 4.8). The optimized mobile phase Methanol: Acetonitrile: Water in the ratio 70:20:10(pH 4.4) adjusted with HPLC grade Glacial acetic acid shown good resolution of BPH and TEL with t_R 2.51 and t_R 3.2227 respectively and suits chromatographic conditions.. System suitability parameters are within the acceptable range with this mobile phase and other chromatographic conditions. The Mobile Phase was prepared by mixing HPLC grade solvent in proportion 70% v/v Methanol, 20% v/v Acetonitrile and 10% v/v Water in a clean & dry flask (Methanol: Acetonitrile: Water 70:20:10). The mobile phase was filtered through membrane filter under vacuum and pH was adjusted using HPLC grade Glacial Acetic Acid and Sonicated in Ultrasonicator to remove any air or gases.

2.3 Optimization of the wavelength selection

In present study 10µg/ml of BPH and 10 µg/ml of TEL were prepared in methanol. After observing UV spectrum of both drugs wavelength of 237 nm was selected for further study and in the HPLC method development. Chromatogram was recorded by positioning UV detector at 237 nm wavelength.

2.4 Preparation of Standard Solutions

Standard stock solution was prepared by accurately weighing pure drug powder equivalent to 10 mg of BPH and 10 mg of TEL separately and transferred into 50 ml clean & dry volumetric flask separately containing mobile phase. The solution was sonicated for 10 min after making volume up to the mark with mobile phase to get standard stock solution. Standard solutions were prepared from stock solution and filtered through syringe filter of pore size 0.45 µ and injected 20 µl filtered solution with Hamilton syringe. The chromatograph of both drug were recorded. The peak area recorded for each conc. and calibration graph was plotted. A series of working standard solution with concentration range in 2-10 µg/ml for BPH and 5-25 µg/ml of TEL were prepared and injected in column.

2.5 Assay of tablet formulation

The twenty tablets of formulation of BPH and TEL were weighed and average weight of each tablet was found. Tablets of formulation were triturated into a fine powder form with a glass mortar pestle. Tablet powder equivalent to 2 mg BPH and 20 mg of TEL was quantitatively transferred into a 50 ml volumetric flask and dissolved in mobile phase and volume was made up to 50 ml with mobile phase. The solution was sonicated for 15 min, to ensure the uniform and homogenous solution of the drug. Further solution was centrifuged at 500 rpm for 10 min and the clear supernatant was collected and filtered through membrane syringe filter (pore size 0.45 µm). The aliquots of clear solution were further diluted to 10 ml with mobile phase to obtain 2ug/ml of BPH and 20 µg/ml of TEL. Each sample solution was injected and the peak areas were measured for the determination of BPH and TEL in tablet formulation.

2.6 Validation of the method

The method was validated as per ICH guidelines. The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, selectivity and specificity. Accuracy was assessed by measuring recovery at three different levels. Precision assessed by measurement of intra and inter day precision.

3. Results and Discussion

3.1 HPLC method development and optimization

The optimized chromatograph of both drugs in mobile phase was shown in Figure 1 and 2. The peak area was recorded for respective conc. and calibration graph was plot and shown in Figure 6. A series of working standard solution with concentration range found linear in 2-10 µg/ml of BPH and 5-25 µg/ml of TEL. The system suitability parameters for this chromatographic condition were summarized in (Table 1)

Table 1: Results of system suitability parameters of BPH and TEL

Parameters	BPH	TEL	Acceptable range
Retention time*	2.412	5.021	NA
Tailing factor*	1.13	1.30	NMT 2
Asymmetrical factor*	1.167	1.020	1
Number of Theoretical plates	2879	15854	MT 2000
Resolution	2.398	9.323	Within 2 to 10
Flow rate ml/min	0.8 ml/min	0.8 ml/min	NA
Run time	8 min	8 min	NA
Column temp	40 deg c	40 deg c	NA
Injection volume	20 µl	20 µl	Na

3.2 Linearity

A series of 2-20 µg/ml of BPH and 5-25 µg/ml of TEL were prepared and injected at 332 nm detection wavelengths. Regression equation was summarized in table 3.

3.3 Specificity

The specificity of the method was confirmed by comparing retention time of pure drug with retention time of separated drug from mixed standard solution by injecting mixed standard solution in the column (Figure 4).

3.4 Assay

Assay of formulation was performed and chromatograph of formulation shown in (Figure 5). The percentage purity was found out and results were statistically obtained and tabulated in (Table2) each sample solution was injected and the peak areas were measured for the determination of BPH and TEL in tablet formulation.

Table 2: Assay results of Tablet formulation

Formulation	Drug	Label claim (mg/ tablet)	Amount found (mg) n = 6	Drug Content (%)	Std Deviation	% Relative Std deviation
Benitowa-TM	BPH	4	4.0124	100.31	1.5857	1.580
	TEL	40	39.908	99.77	0.87505	0.8770

Precision
The precision study was carried out by injecting working sample solution five times also the intermediate precision was carried out by interday and intraday precision. SD and % RSD shows method precise and summarized in Table 3.

Accuracy
Analysis of marketed formulation was carried by injecting sample corresponding to equivalent weight into HPLC system and recovery studies were carried out. The results are summarized in Table 3.

Robustness
Robustness was performed by injecting sample solution of BPH and TEL and chromatograph was monitored at ± 0.8 ml/min flow rate and at ± 237 nm detection wavelengths. SD and % RSD tabulated in Table 3.

LOD/LOQ
LOD and LOQ of BPH by the proposed method were determined using calibration graph method (Figure 6) and calculated as 3.3σ/s and 10 σ/s respectively. The results are summarized in Table 3.

Table 3: Summarized results of validation parameters

Parameters		Benidipine HCL	TEL
Beer's lawrangeμg/ml		2-10μg/ml	5-25μg/ml
Regression equation		Y=69.494x-20.497	Y=81.409x-34.841
Specificity		Specific	Specific
Repeatability % RSD		0.63	1.57
Intermediate precision (SD and % RSD)		1.5873 0.6355	2.0459 0.1108
Accuracy		100.31 %	99.77 %
Robustness	(± 0.2 ml flow rate) SD and % RSD	0.3327 0.0838	0.1489 0.1445
	(± 2 nm in wavelength) SD and % RSD	0.476 0.474	0.558 0.556
LOD		2.94	0.19
LOQ		1.19	2.57

*LOD= Limit of detection, LOQ = Limit of quantification
SD = standard deviation RSD = Relative standard deviation

Stress Degradation Study

Preparation of standard solution
Tablet formulation weighted equivalent to 4 mg BPH and 40 mg TEL and transfer into 10 ml volumetric flask containing mobile phase. The solution was sonicated for 10 min and then adjusts the volume up to the mark with same mobile phase to get standard stock solution.

Acid degradation study
To 0.05 ml of stock solution of BPH and TEL, 0.05 ml of 1N HCL was added separately and solution was kept for 30 min at 60°C. resultant solution was diluted to obtain BPH and TEL 10μg/ml. These solutions were injected and chromatograms were recorded to assess the stability of drug under the influence of acid (Figure 7).

Base degradation study
To 0.05 ml of stock solution of BPH and TEL, 0.05 ml of 1N NAOH was added separately solution was kept for 30 min at 60°C. resultant solution was diluted to obtain Benidipine HCL and TEL 10μg/ml. These solutions were injected and chromatograms were recorded to assess the stability of drug under influence of base (Figure 8).

Photolytic degradation study
To 0.05 ml of stock solution of BPH and TEL, 0.05 ml of water was added separately and solution was kept in sunlight for 6 Hrs. The resultant solution was diluted to obtain BPH and TEL 10μg/ml. These solutions were injected and chromatograms were recorded to assess the stability of drug under photolytic condition (Figure 9).

Thermal degradation study
Standard solution of BPH and TEL was placed in oven at 105°C for 6 hrs. Resultant solution was diluted to obtain BPH and TEL10μg/ml. These solutions were injected and chromatograms were recorded to assess the stability of drug under the influence of heat (Figure 10)**.**

Table 4: Results of Stress Degradation Studies

Sr. No	Type of Degradation	Benidipine HCL		TEL	
		% obtained	% degraded	% obtained	% degraded
1	Acid	94.25 %	5.75 %	95.75 %	4.29 %
2	Alkali	93.19 %	7.12 %	95.320 %	4.68 %
4	Thermal	91.018 %	8.97 %	92.57 %	7.43 %
5	Photo light	97.68 %	2.32 %	98.15 %	1.85 %

4. Conclusion

Developed chromatographic method is scientifically sound and capable of giving reproducible, reliable and precise results. The stability indicating and stress degradation studied RP HPLC chromatographic method for simultaneous estimation of BPH and TEL is rigid and stable suitable for analysis.

5. Acknowledgement

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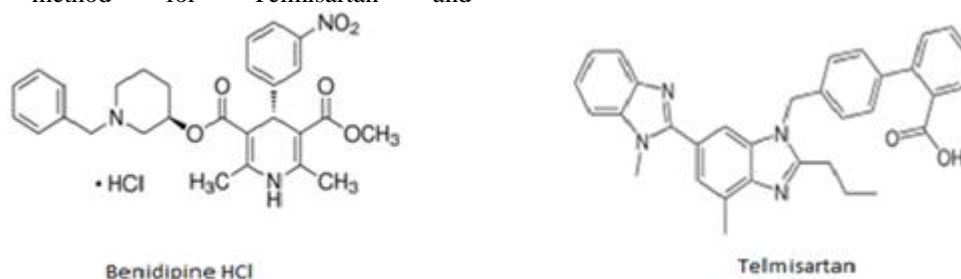
Figure 1 Chemical structure of Benidipine Hydrochloride and Telmisartan

Figure 1: Chemical structure of Benidipine HCL and Telmisartan
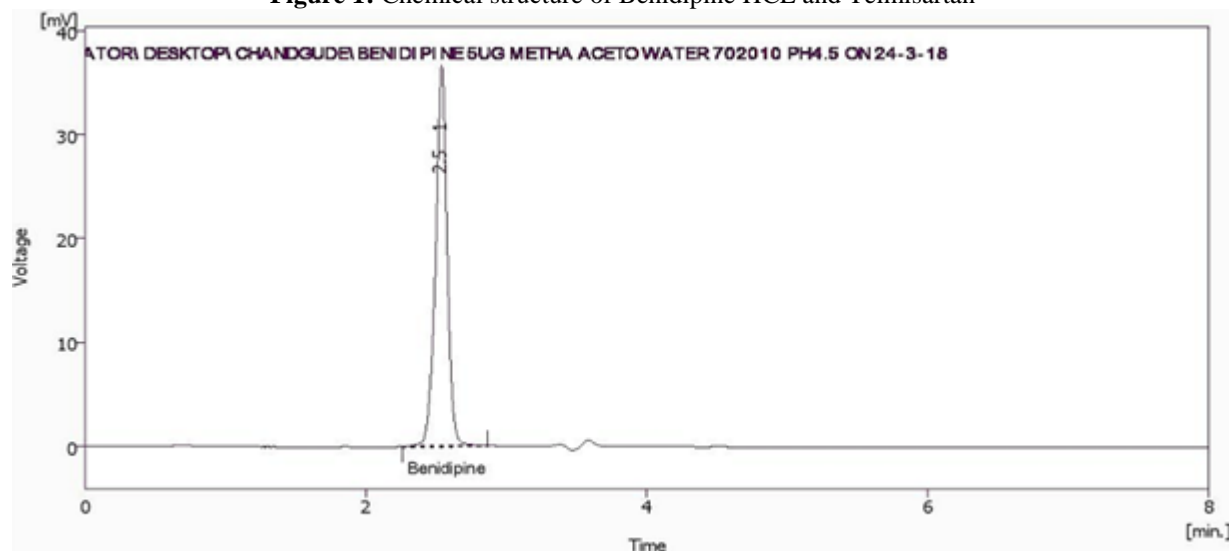

Figure 2 Chromatograph of Benidipine

Figure 2: Optimized Chromatogram for Benidipine HCL (BEN)
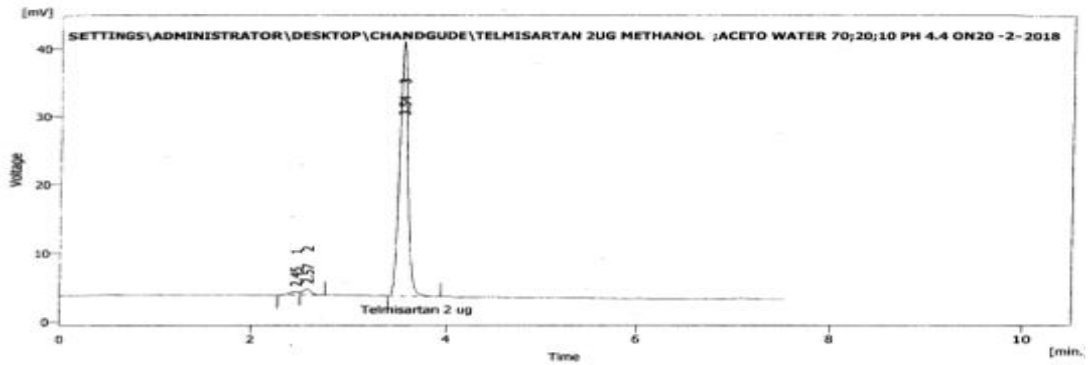Chromatograph of Benidipine HCL with retention time 2.51 min

Figure 3: Optimized Chromatogram for Telmisartan (TEL)
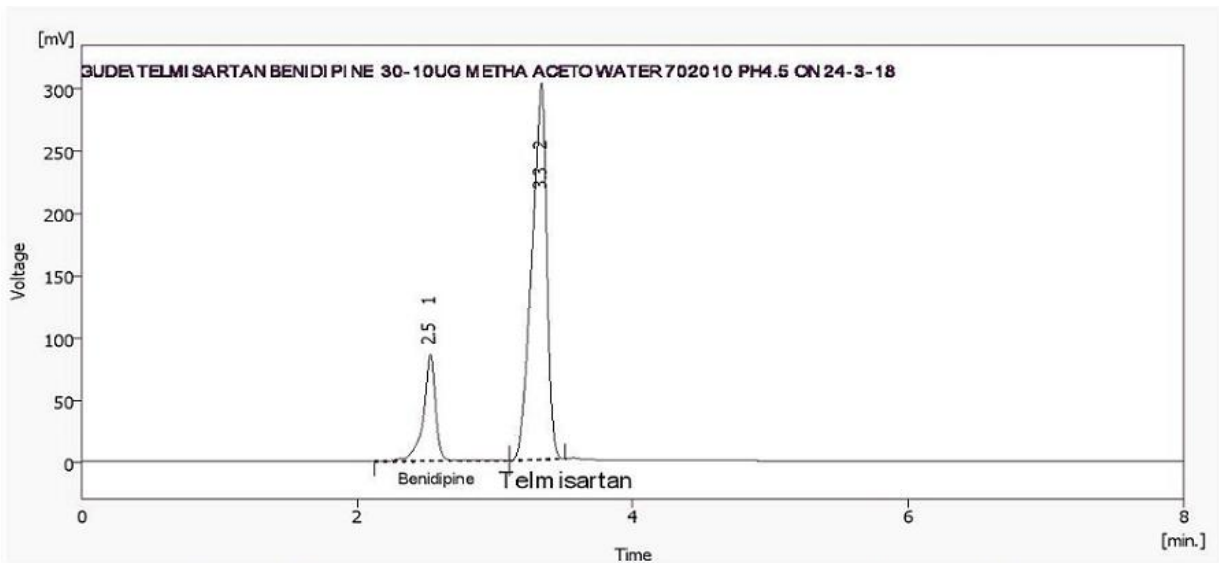Chromatograph of Benidipine HCL (BPH) with retention time 3.32 min

Figure 4 Chromatograph of Benidipine and Telmisartan from mixed standard solution

Figure 4: Chromatogram of Benidipine HCL (BPH) and Telmisartan (TEL)
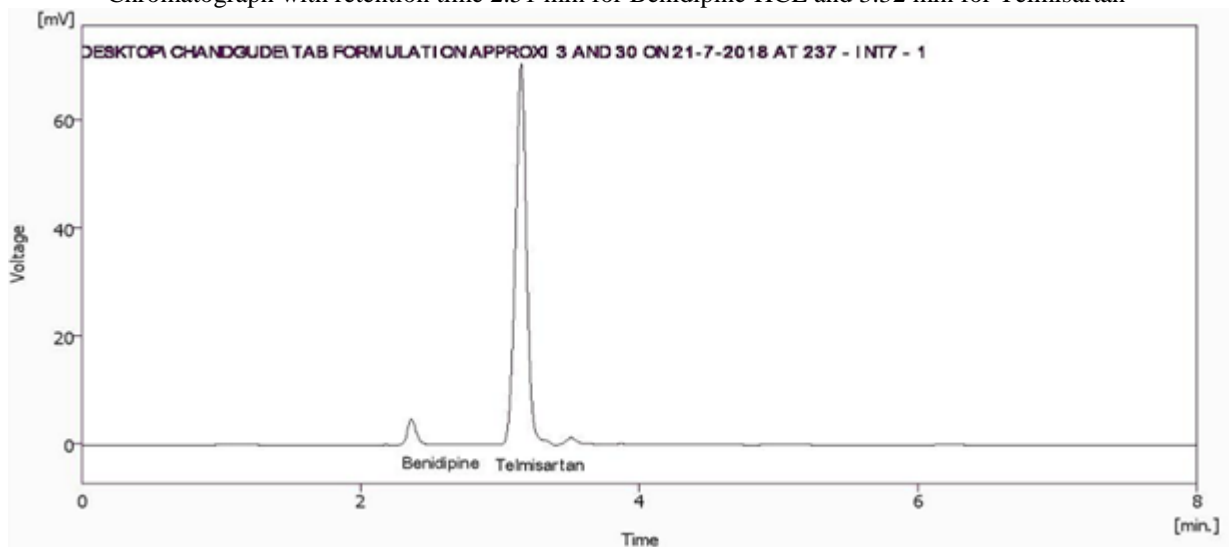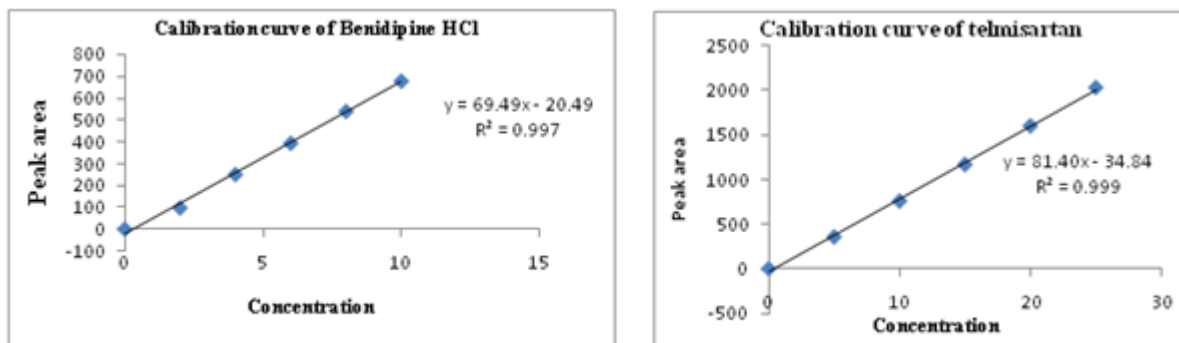Chromatograph with retention time 2.51 min for Benidipine HCL and 3.32 min for Telmisartan

Figure 5 Chromatograph of Benidipine and Telmisartan from Tablet formulation

Figure 5: Chromatogram of Benidipine HCL and Telmisartan from tablet formulation
Chromatograph with retention time 2.53 min for Benidipine and 3.34 min for Telmisartan

Figure 6 Calibration curves of Benidipine HCL and Calibration curves of and Telmisartan

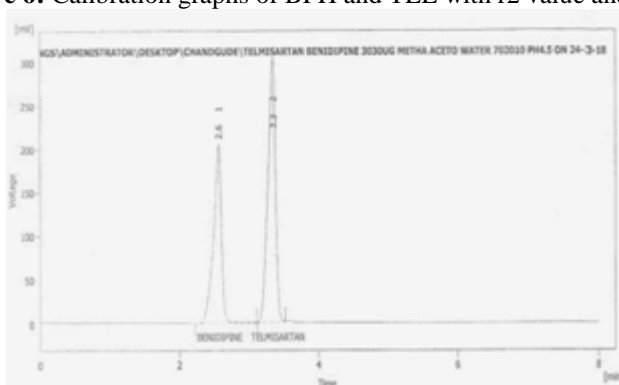**Figure 6:** Calibration graphs of BPH and TEL with r2 value and slope

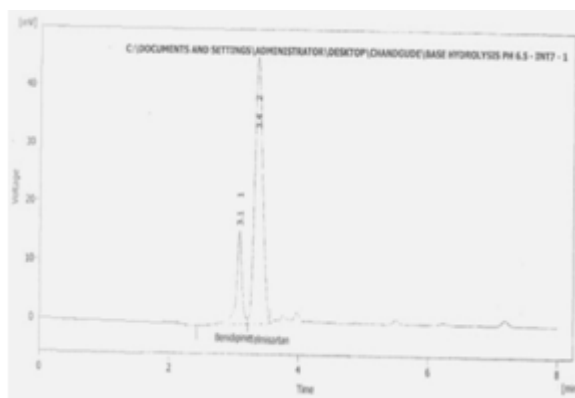**Figure 7:** Acidic Degradation Chromatogram
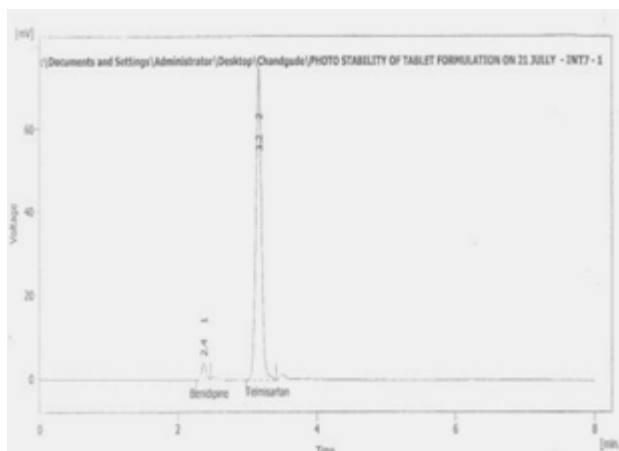

Figure 8: Basic Degradation Chromatogram

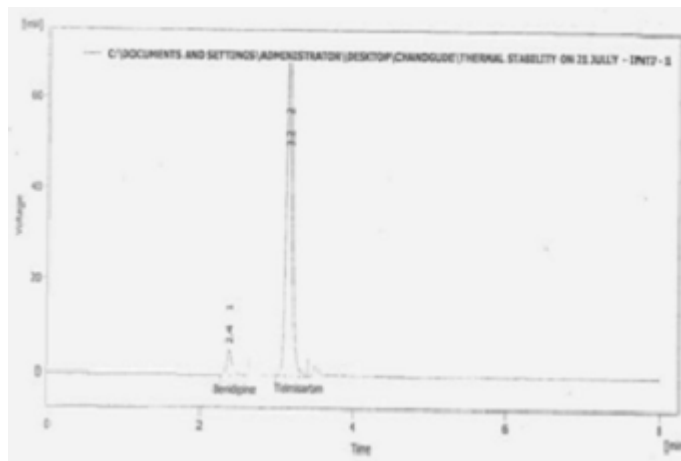**Figure 9:** Chromatograph of photolytuic degradation

Figure 10: Thermal Degradation Chromatogram

Figure 7, 8, 9, 10 Chromatograph of acidic degradation, basic degradation, photolytic degradation and thermal degradation respectively