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RP-HPLC Method Development Validation and Forced Degradation Studies for Simultaneous Estimation of Torsemide and Spironolactone in Tablet Dosage Form

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ABSTRACT

A simple, accurate and stability indicating high performance liquid chromatographic (HPLC) method was developed for the simultaneous estimation of Torsemide and Spironolactone in combined dosage form. Isocratic RP-HPLC separation was achieved on Kromasil RP- C18 column (250mm×4.6mm; 5µm) using methanol: acetonitrile: water in the ratio of 50:30:20 (v/v), pH6.8, at flow rate of1.0ml/min at ambient temperature. Quantization was achieved by UV detection at 235nm over the concentration range of 10-60µg/ml for torsemide and 25-150µg/ml for spironolactone with percentage recoveries of range 99.688-101.792 and 98.282-101.811for torsemide and spironolactone respectively. Different stress degradation studies like acidic, alkaline, peroxide, thermal etc were measured for both standard drugs and results found that the stress degradation conditions doesn't affect the elution of the both the drugs and hence the developed method was found to be stability indicating method.

Keywords: Torsemide, Spironolactone, RP-HPLC estimation, forced degradation studies.

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INTRODUCTION

Torsemide is sulfonylurea derivative and chemically known as 3-[4-[(3-methylphenyl) amino] pyridin-3-yl] sulfonyl-1-propan-2-ylurea. It acts as diuretic. Spironolactone (SPI) is steroidal derivative and chemically known as 7α-Acetylthio-3-oxo-17α-pregn-4-ene-21, 17- carbolactone. It acts as potassium-sparing diuretics. Diuretics increase the rate of urine flow and sodium excretion and are used to adjust the volume and / or composition of body fluids in a variety of clinical situations including hyper tension, heart failure, renal failure and cirrhosis. Structures of torsemide[1-3] and spironolactone[4] are given in Figures 1 and 2.

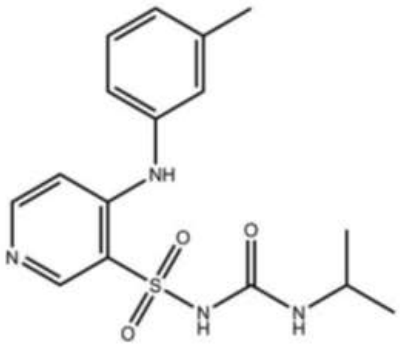

Figure 1: Structure of Torsemide

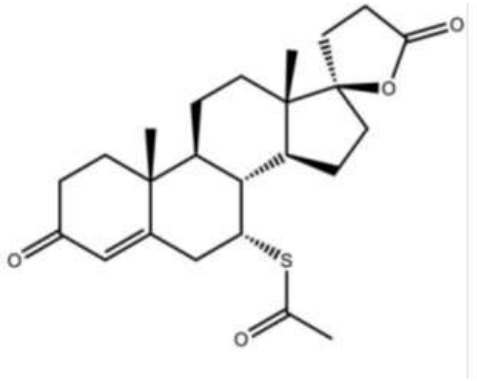

Figure 2: Structure of Spironolactone

The HPLC method is widely employed in quality control assessment of drugs because of its sensitivity, repeatability and specificity. Literature survey revealed that Chromatographic and HPLC methods[5-9] are available for estimation of torsemide and spironolactone individually and in combination with other diuretics in different formulation. Hence simple and specific RP-HPLC procedure has been developed to determine torsemide and spironolactone simultaneously in pharmaceutical dosage forms.

MATERIALS AND METHOD

Apparatus

The HPLC method was performed on PEAK chromatographic system equipped with LC- P7000 torsemide and spironolactone pump UV detector, and Rheodyne injector system fitted with 20µl loop. The HPLC analysis was performed on reversed phase high-performance liquid chromatographic system with isocartic elution mode using a mobile phase of methanol: acetonitrile: water (50:30:20 v/v), pH 6.8, at flow rate of 1.0 ml/min at 30^{o}C temperature.

Reagents and material

The drug samples, torsemide and spironolactone working standard were obtained as gift sample by Macleods Pharmaceuticals Pvt. Ltd Mumbai. The pharmaceutical formulation was procured from

local market. Methanol, acetonitrile and water used were HPLC grade and were purchased from Merck Specialties Private Limited, Mumbai, India. Perchloric acid and remaining buffer solutions used were AR Grade and purchased from Merck Specialties Private Limited, Mumbai, India.

Preparation of standard stock solution

Standard stock solutions were prepared by the transfer of accurately weighed 10 mg of torsemide and 25 mg of spironolactone into 10 ml volumetric flasks separately and dissolved in 10ml methanol. Respective stock solutions were diluted with solvent to obtain the working standard solutions of these drugs.

Preparation of sample solution

Sample solution was prepared from finely ground uniform size powder of 20 tablets (Tide Plus-torsemide 10mg and spironolactone 25mg). Initially, 10mg of torsemide was accurately weighed and transferred quantitatively into a 10ml volumetric flask. The solution was sonicated for about 15 min after adding 5ml of methanol. Then the solution was mixed with mobile phase and made up to the mark of volumetric flask. After filtration, a concentration of 40µg/ml was obtained by the dilution with mobile phase. As on the label claim of the combined tablet 100µg/ml of spironolactone was obtained. Simultaneous estimation of torsemide and spironolactone in combined fixed dosage form was done by the use of combined solution.

Method Development

The developed stability indicating RP-HPLC method was aimed to resolve torsemide and spironolactone under system suitability test and stability test in all forced degradation studies. During the method development, solubility, partition coefficient and polarity of the drugs were considered. Different mobile phases were used to optimize the desired HPLC method. Individual standard solutions of 10µg /ml for both drugs were prepared and scanned by the UV-Vis spectrophotometer separately to select the suitable monitoring wavelength. Wavelength of 235 nm was selected for the entire study as the two active ingredients showed optimum response near 235 nm in ultraviolet absorption spectra (Figure 3). These mobile phases differ in the percentages, additives, pH, the organic solvents type, strength, and temperature. The best conditions selected were based on minimizing peak tailing, improving peak symmetry, column efficiency, resolution, and total analysis time. At a mobile phase ratio of methanol: acetonitrile: water in 50:30:20 (v/v) gave better separation compared to all the conditions. But in these conditions, theoretical plates were found to be less. To improve the theoretical plates, pH of mobile phase was changed by using orthophosphoric acid for acidic nature and sodium perchlorate for basic nature. Finally at pH of 6.8 was found to be suitable for analysis of torsemide and spironolactone. It was found that the

temperature had a negligible influence on resolution and tailing factors, therefore room temperature was selected for the entire analysis. In these optimized conditions, well resolved, retained and accepted system suitability was observed for both the drugs. The optimized conditions were given in Table 1 and the system suitability conditions were tabulated in Table 2. The retention times for torsemide and spironolactone were 4.55 and 5.95 min respectively.

Table 1: Optimized Chromatographic Conditions

S. No	Parameter	Results
1	MP	Methanol: Acetonitrile: Water in 50:30:20 (v/v)
2	Wavelength	235nm
3	Stationary Phase	Kromasil RP- C18
4	pH of MP	6.8 with 0.1M Sodium Perchlorate
5	Flow Rate	1.0ml/min
6	Pump Mode	Isocratic
7	Pump Pressure	11.2±7MPa

Table 2: Validation results for System suitability

S. No	Parameter	Result
1	API Concentration	Torsemide – 40µg/ml Spironolactone - 100µg/ml
2	RT	Torsemide – 4.55min Spironolactone -5.95min
3	Resolution	Torsemide – Spironolactone – 4.95
4	Area	Torsemide – 451609 Spironolactone – 956848
5	Theoretical Plates	Torsemide – 4311 Spironolactone – 6789
6	Tailing Factor	Torsemide – 0.68 Spironolactone -0.94

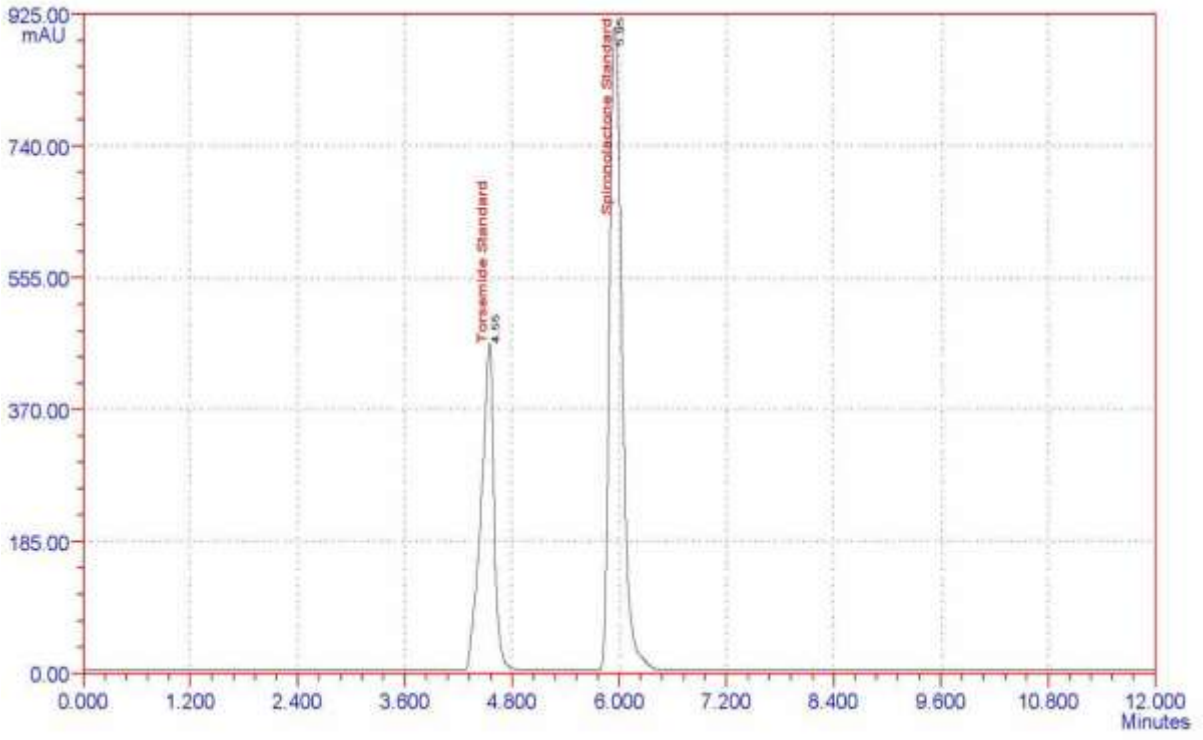

Figure 3: Standard graph of Torsemide and Spironolactone

Method validation

Calibration graph (linearity)

Torsemide and spironolactone standard stock solution was used for the preparation of subsequent aliquots. By serial dilution various aliquots were prepared. Calibration graphs were constructed by plotting peak area Vs concentration of torsemide and spironolactone. The calibration graphs were plotted over 6 different concentrations in the range of 10-60µg/ml for torsemide and 25-150µg/ml for spironolactone. Aliquots (20µl) of each solution were injected under the operating chromatographic conditions described above [Number of replicates (n=6)]. The precision of the instrument was checked by injecting mixed standard solution of torsemide and spironolactone repeatedly (n = 6). The intraday and inter day precision of the proposed method was determined by analyzing mixed standard solution of torsemide and spironolactone at concentration of 40µg/ml and 100µg/ml respectively for six times on the same day and on 3 different days. The results are reported in terms of relative standard deviation. The LOD and LOQ were calculated for both drugs using the following equations according to International Conference on Harmonization guidelines[10-12].

DL = $3.3\sigma/S$

QL = $10\sigma/S$

Where σ = the standard deviation of the response

S = the slope of the calibration curve.

The standard solution of torsemide (40 µg/ml for HPLC method) and spironolactone (100 µg/ml for HPLC method) and sample solution of torsemide (40 µg/ml for HPLC method) and spironolactone (100 µg/ml for HPLC method) were prepared and analyzed after 24 hrs by storing the solutions at room temperature. Accuracy of method was observed from recovery results. The two placebos accurately spiked with different concentrations of the active ingredient. Recovery assessment was obtained by using standard addition technique which was done by adding known quantities of pure standards at three different levels in 50%, 100% and 150% to the pre-analyzed sample formulation. From the recovery measurements, the amount of drug recovered and percentage recovery were calculated. Robustness of the proposed new method included six deliberate variations to same chromatographic parameters. The modifications included different mobile phase ratios, different detector wavelengths and percentage variations in the mobile phase (in the range of ± 5 of the nominal value and the normal %). The percentage change in each of the changed condition was calculated.

Analysis of torsemide and spironolactone in tablet dosage form

The response of sample solutions were measured at 235 nm for quantization of torsemide and spironolactone by the method described above. The amount of torsemide and spironolactone present in the sample solution were determined by applying values of peak area to regression equation of the calibration graph.

FORCED DEGRADATION STUDIES

Degradation studies were measured under acidic, aqueous, basic, peroxide, thermal, Sun light and UV light conditions.

Light (Normal and UV light)

In this type of degradation, sample was taken in open petri dish and exposed to normal light and UV light for about 48 hours. Sample solutions were prepared and injected into HPLC under the chromatographic conditions. The degradants were evaluated from the chromatogram and compared to initial values (Figure 4 and 5).

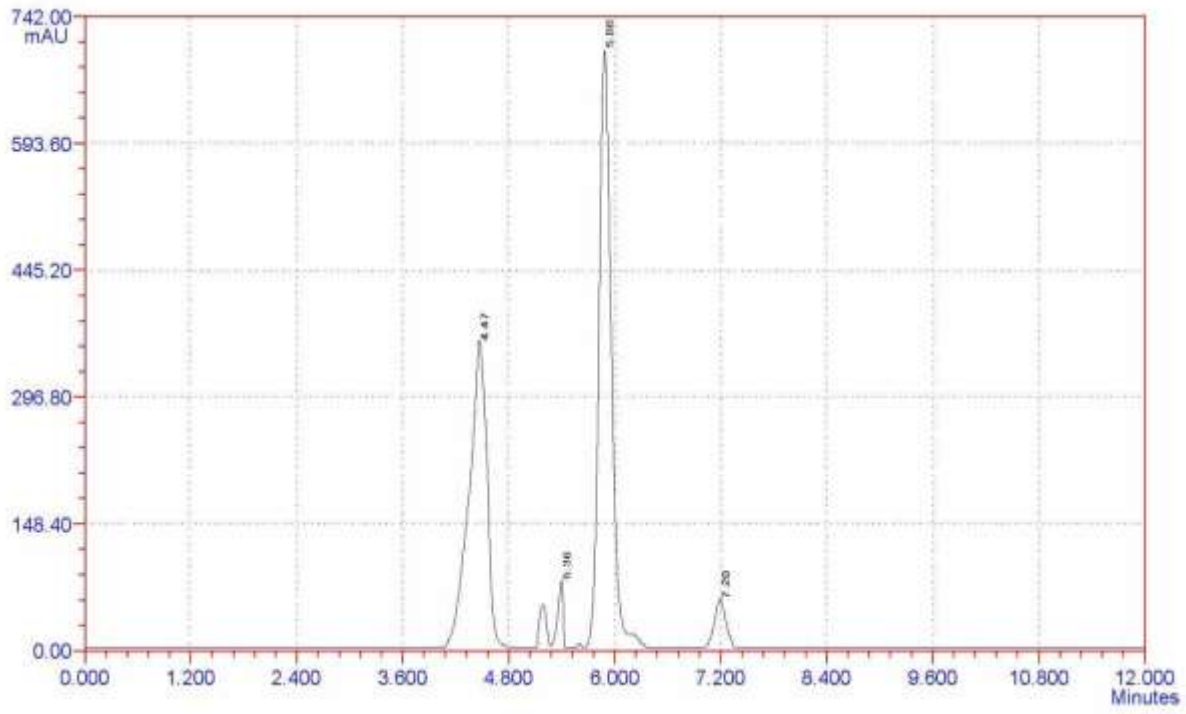

Figure 4: Normal light degradation graph of Torsemide and Spironolactone

Thermal

In thermal degradation, sample was taken in a Petri dish and kept in oven at 40°C and 80°C up to 48 hours. After thermal exposal, the prepared sample solution was injected into HPLC under the chromatographic conditions. The evaluated degradants from the chromatogram were compared with basic values (Figure 6).

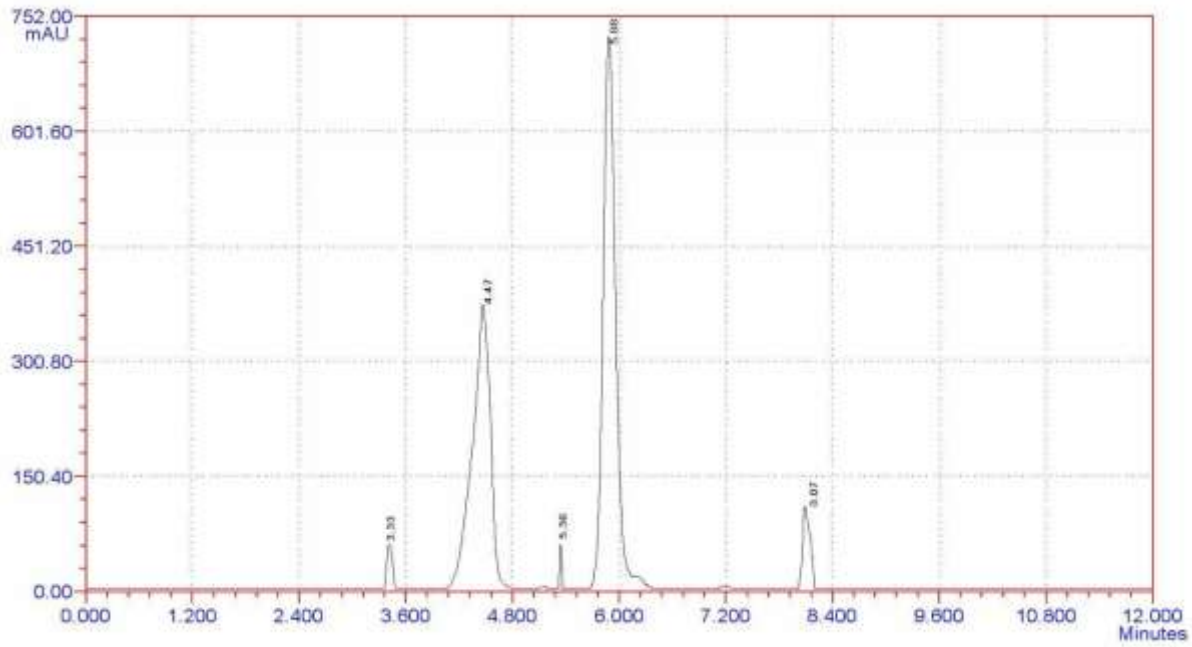

Figure 5: UV light degradation graph of Torsemide and Spironolactone

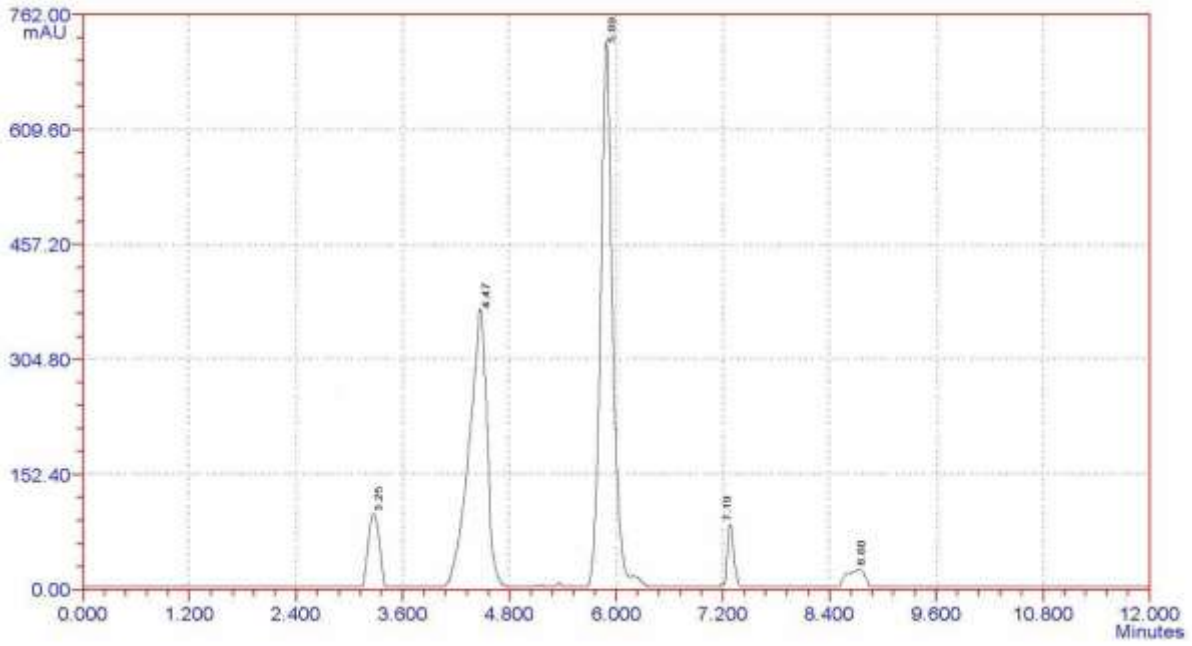

Figure 6: Thermal degradation graph of Torsemide and Spironolactone

Acid

Acid hydrolyzed sample solution was prepared by taking 300 mg of sample in 20 ml of 0.1 N hydrochloric acid. After 48 hours, 5 ml of acid hydrolyzed sample solution was taken into 25 ml volumetric neutralized with 5 ml of 0.1 N sodium hydroxide solution. Later it was made up the volume with diluent. The above solution was injected into HPLC under chromatographic

conditions and degradants were evaluated from the chromatogram and compared with standard values (Figure 7).

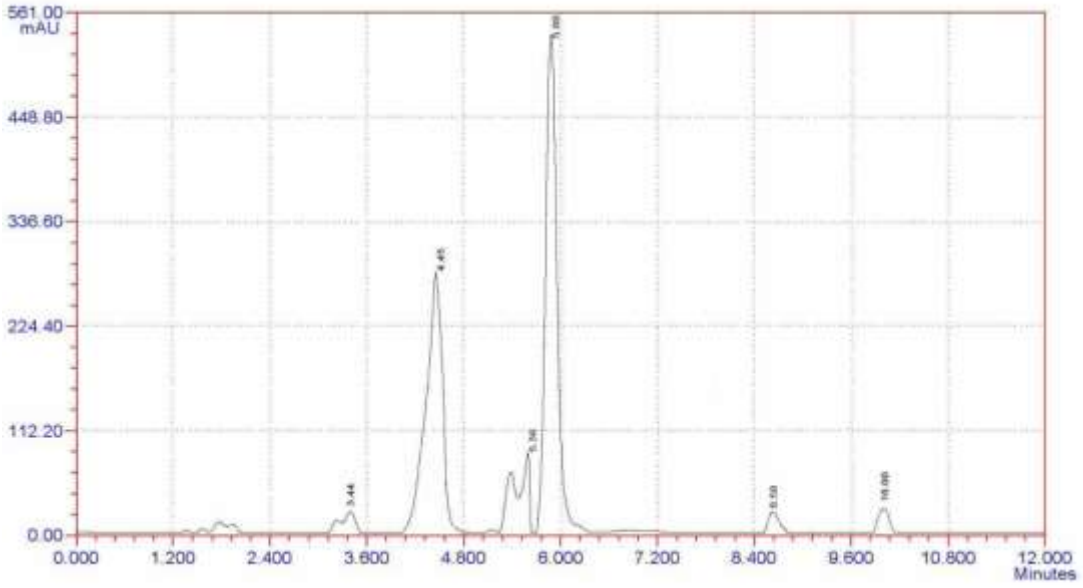

Figure 7: Acid degradation graph of Torsemide and Spironolactone

Base

In order to prepare base hydrolyzed sample solution, 300 mg of sample was dissolved in 20 ml of 0.1 N sodium hydroxide solution. After 48 hours, 5 ml of base hydrolyzed sample solution was neutralized with 5 ml of 0.1 N hydrochloric acid solution in 25 ml volumetric flask and and made up the mark with diluent. Degradants were evaluated by injecting sample solution into HPLC and were compared with original values (Figure 8).

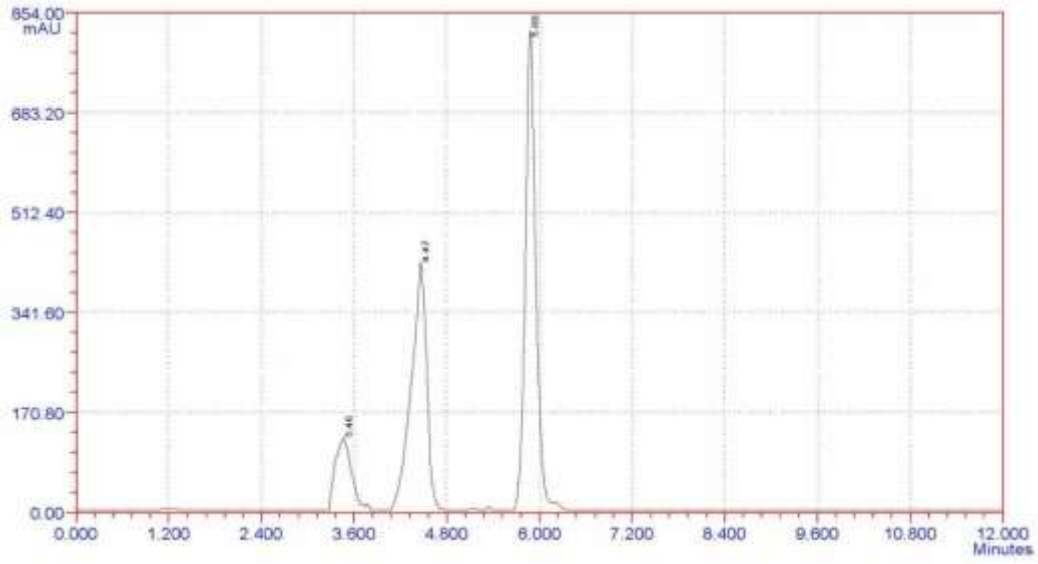

Figure 8: Base degradation graph of Torsemide and Spironolactone

Hydrogen Peroxide

For the preparation of oxidized sample solution, 300 mg of sample was dissolved in 20 ml of 3% hydrogen peroxide. After 48 hours, 5 ml of oxidized sample solution was transferred into25 ml volumetric flask and made up the volume with diluent. By injecting sample solution into HPLC, the degradents were evaluated and compared with initial values (Figure 9).

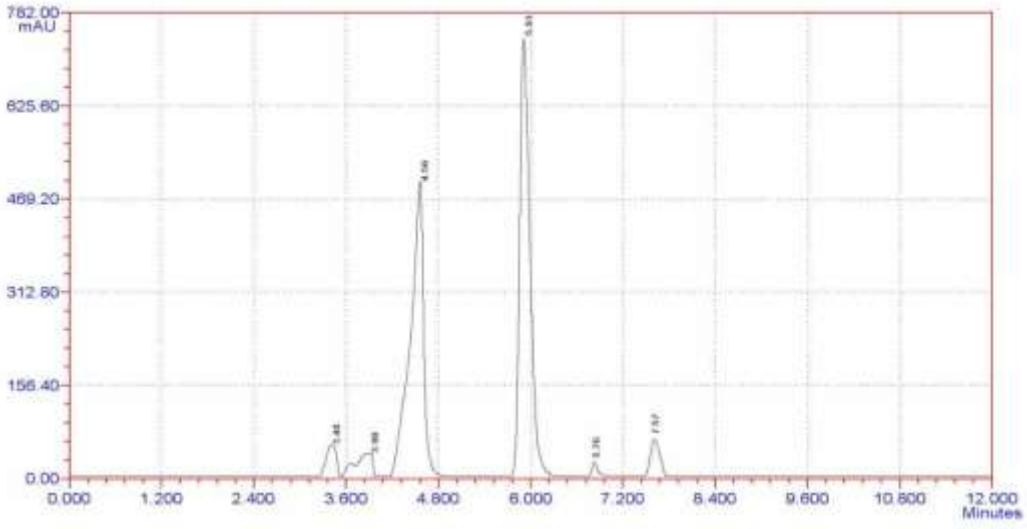

Figure 9: Peroxide degradation graph of Torsemide and Spironolactone

Aqueous

In the preparation of aqueous sample solution, 300 mg of sample was dissolved in double distilled water. The prepared solution was allowed to rest for 48 hours and then 5ml of sample solution was taken into 25 ml volumetric flask and made up to the volume with diluents. The degradants were evaluated from the chromatogram and compared with standard values (Figure 10).

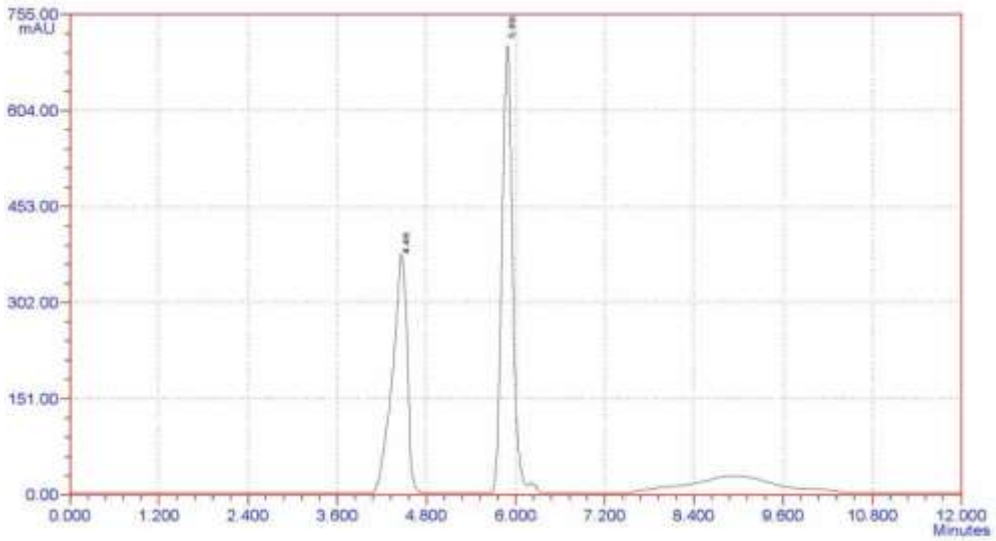

Figure 10: Aqueous degradation graph of Torsemide and Spironolactone

RESULTS AND DISCUSSION

High performance liquid chromatography (HPLC) is one of the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product and used for determining drug product stability. Stability indicating analytical method is defined as a validated analytical procedure that accurately and precisely measure active ingredients (drug substance or drug product) free from process impurities, excipients and degradation products. The literature survey revealed that no stability indicating HPLC method was detected for the simultaneous analysis of torsemide and spironolactone. Hence a validated, simple and accurate stability indicating HPLC method was developed for the simultaneous estimation of torsemide and spironolactone in pharmaceutical formulations. Linearity was observed in the concentration range of 10-60µg/ml for torsemide and 25-150µg/ml for spiranolactone. Linear regression equation was found to be y = 8486.x + 10922 (R^2 = 0.999) for torsemide and y = 9793.x - 11767 (R^2 = 0.999) spiranolactone. Linearity results were given in Table 3 and calibration curves were given in Figures 11 and 12 for torsemide and spiranolactone respectively. In precision experiment % RSD was found to be (1.221, 0.595 for intraday, 1.186, 0.174 for interday precision and 1.96, 0.60 for ruggedness respectively for torsemide and spiranolactone) very less. This confirms that, the method is having high repeatability, reproducibility. A very less % change was observed when small changes were made in the optimized conditions; hence the method is applicable for the routine analysis. A very high % recovery, more than 98% was observed for recovery study. Hence the method is found to be accurate. Limit of detection was found to be 0.05µg/ml and 0.08µg/ml for torsemide and spiranolactone respectively. Hence the method has a very high sensitivity. Summary of validation results were shown in Table-4.

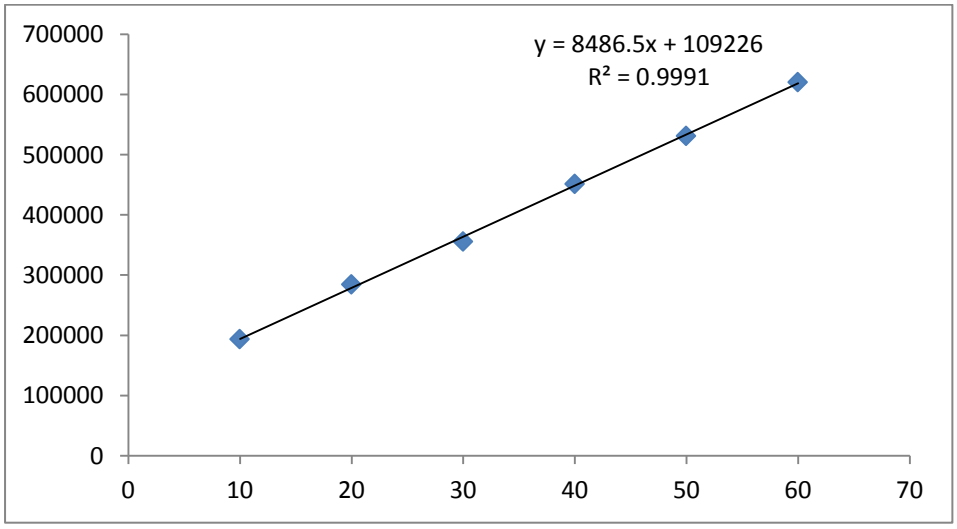

Figure 11: Calibration curve of Torsemide

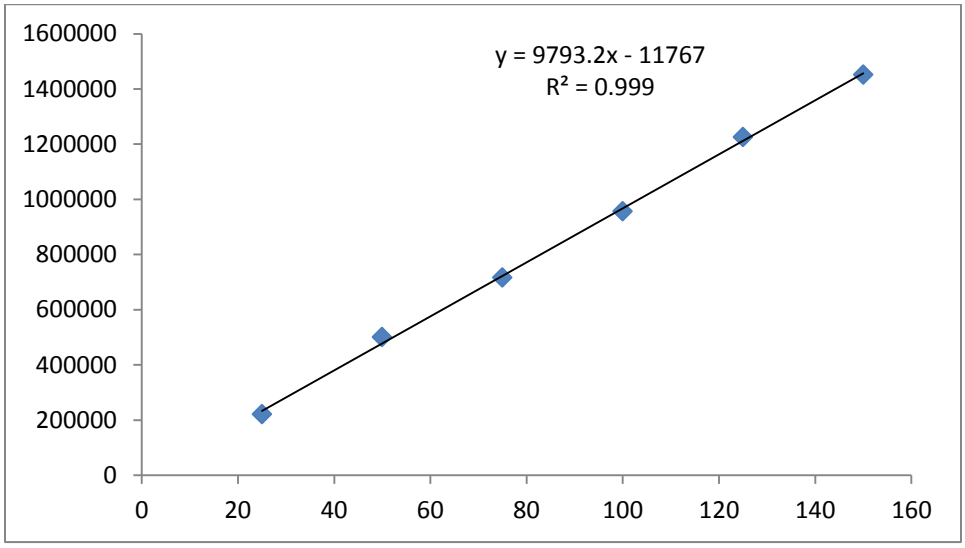

Figure 12: Calibration curve of Spironolactone

Table 4: Summary of validation results

S.No	Parameter	Result		Limit
		Torsemide	Spironolactone	
1	Repeatability in %RSD	1.96	0.60	Below 2
2	Intraday precision	1.221	0.595	Below 2
3	Interday Precision	1.186	0.174	Below 2
4	LOD	0.05µg/ml	0.08µg/ml	---
5	LOQ	0.16µg/ml	0.26µg/ml	---
6	Solution Stability	36 hours	36 hours	---
7	Formulation assay in %	98.247	99.89	98-102%
8	Recovery in %	99.688 -101.792	98.282 - 101.811	98-102%
9	Robustness in % Change	0.06 -1.519	0.556 - 1.239	Below 2

Table 5: Results of Stability Studies

S. No	Condition	No of degradation Peaks Observed
1	Acidic	4
2	Aqueous	0
3	Base	1
4	Peroxide	4
5	Sun Light	2
6	Thermal	3
7	UV light	3

The stability of the molecule was measured by forced degradation studies. Different stress conditions like acidic, aqueous, basic, peroxide, thermal, light and UV light were applied for inducing stress in the molecule. Results of stability studies were shown in Table 5. The samples were analyzed in the optimized conditions. Results confirm that more degradative products were observed in acidic, thermal and peroxide conditions. High stability was observed in all the stress studies except acidic and peroxide conditions. Moreover there was no remarkable change in the

retention time and % assay in all the stress conditions. Hence the method is found to be stability indicating and can be used for separation of degradative products in different stress conditions.

CONCLUSION

The developed stability indicating RP-HPLC method was found to be simple, accurate, and precise and can be used for the routine analysis of torsemide and spironolactone. The method was found to be stable and there was no remarkable change in elutions, separation and analysis of both the drugs under different stress conditions. The simple analytical conditions gave a very less elution time of less than 6min for both the drugs and facilitate the analysis of more number of samples on a less time. Hence the method can be applied for the routines analysis of torsemide and spironolactone.

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