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Research Article

Development and validation of a stability indicating RP-HPLC method for simultaneous estimation of Pantoprazole sodium sesquihydrate and Levosulpiride in a combined dosage form

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ABSTRACT

A simple, precise, rapid and accurate stability indicating RP-HPLC method has been developed for the simultaneous estimation of Pantoprazole sodium sesquihydrate (PNT) and Levosulpiride (LSP) in combined capsule dosage form. Formulation containing PNT with LSP are used as proton pump inhibitor (antacid) and prokinetic agent respectively. The chromatographic separation was achieved on Thermo BDS C18 column (250 mm x 4.6 mm, 5.0 µ particle size) using 0.02 M Potassium dihydrogen o- phosphate solution (pH-4 adjusted with o-phosphoric acid): Acetonitrile (60:40 v/v), flow rate was 1.0 mL m^{-1} . Quantification and linearity was achieved at 238 nm over the concentration range of 8 - 48 µg ml^{-1} for pantoprazole sodium and 7.5-45 µg ml^{-1} for levosulpiride. The method was validated for specificity, linearity, accuracy, precision, LOD, LOQ and robustness. The proposed method was optimized and validated as per the ICH guidelines.

Key words: Pantoprazole sodium sesquihydrate, Levosulpiride, stability indicating RP-HPLC; Method Validation

INTRODUCTION

Pantoprazole sodium (PNT) is a sodium 5-(difluoromethoxy)-2-[[(3,4-dimethoxy]-2-pyridinyl) meth -yl]sulfinyl]-1H-benzimidazole ; it is a proton pump inhibitor used for short-term treatment of erosion and ulceration of the oesophagus caused by gastroesophageal reflux disease(GERD). It has molecular formula $C_{16}H_{14}F_2N_3NaO_4S$ with molar mass 405.36 g/mol. A few UV spectrophotometric [Kalure SU. et al., 2012; Patel K. et al., 2013], HPLC [Kampati AK. et al., 2013; Rami Reddy YV. et al., 2012; Saini V. et al.,2009] and HPTLC [Patel S. et al., 2003] methods have been reported individually or in combination with other drugs for estimation of pantoprazole sodium sesquihydrate. Levosulpiride (LSP) is an N-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxy-5-sulfamoylbenzamide ; it is a new orally effective prokinetic agent reported to be a selective antagonist of dopamine D2 receptor activity on both central and peripheral levels. It is an atypical neuroleptic (S)-enantiomer of sulpiride having molecular formula $C_{15}H_{23}N_3O_4S$ with molar mass 341.43 g/mol. Levosulpiride is also claimed to have mood elevating property and used in the treatment of psychoses, particularly negative symptoms of schizophrenia, anxiety disorders, dysthymia, vertigo, dyspepsia, irritable bowel syndrome, and premature ejaculation .Various UV and HPLC methods for LSP have been reported for its estimation individually or in combination with other drugs [Chouvan V. et al., 2011; Jagadeesh B. et al., 2012; Brahmbhatt DD. et al., 2012] Based on the literature available, need was felt to develop stability indicating HPLC method for the estimation of PNT and LSP in combined pharmaceutical dosage forms. The present work describes a simple stability indicating RP-HPLC method for the determination of PNT and LSP in capsule dosage form. The method was validated according to ICH guidelines and was found to be reproducible with good resolution between PNT and LSP.

MATERIALS AND METHODS

MATERIALS AND REAGENTS

Capsules used for analysis were manufactured by Hetero Labs Limited, (Kalyanpur, H.P., India) contained PNT 40 mg and LSP 75 mg per capsule. Pure drug sample of PNT (98.8%) and LSP (99.8%) were obtained as a gift sample from Medley Pharmaceuticals Ltd., Mumbai and Torrent Pharmaceuticals Ltd., Ahmedabad respectively. All solvents and chemicals used in the study were of analytical grade manufactured by Merck specialities Pvt. Ltd (Mumbai, India) and Hexon laboratories Pvt. Ltd, Pune. Double distilled water and capsule placebo was made at lab scale only.

INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS

The HPLC system consisted of a quaternary pump (model Lachrome 1200, double reciprocating HPLC pump, column and UV detector. Data collection and analysis was done using Winchrome software. Separation was achieved on Thermo BDS Hypersil C18 column (250 mm × 4.6 mm, 5.0 µ) columns maintained at ambient temperature. Isocratic elution with 0.02 M Potassium dihydrogen phosphate solution (pH 4 - adjusted with o-phosphoric acid): acetonitrile (60 : 40 v/v) mobile phase at the flow rate of 1.0 ml m^{-1} was carried out. The detection was monitored at 238 nm and injection volume was 20 µl. The peak purity was checked with the UV detector.

PREPRATION OF STANDARD SOLUTIONS AND CALIBRATION CURVE

Mixture of Standard stock solution of PNT and LSP (in ratio 1:1.875 mg ml^{-1}) was prepared in mobile phase. To study the linearity range of each component, serial dilutions of PNT and LSP were made from 8 to 48 µg ml^{-1} and 7.5 to 45 µg ml^{-1}, respectively in mobile phase and injected on to column. Calibration curves were plotted as concentration of drugs versus peak area response. From the standard stock solutions, a mixed standard solution was prepared containing the analytes in the given ratio and injected on to column. The system suitability test was performed from six replicate injections of mixed standard solution.

ANALYSIS OF CAPSULE FORMULATIONS

Twenty capsules were weighed accurately and a quantity of capsule powder equivalent to 40 mg of PNT (75 mg of LSP) was weighed and dissolved in the 50 ml of mobile phase with the aid of ultrasonication for 20 min and solution was filtered through Whatman paper No.41 and then membrane filtered through 0.2µ filter paper and transferred into a 50 ml volumetric flask. Volume was made up to mark. The solution was suitably diluted with mobile phase to get 16 µg ml^{-1} of PNT & 30 µg ml^{-1} of LSP. The structure of analytes and a typical chromatogram obtained from a sample solution is shown in Figure No. 1 and 2 respectively.

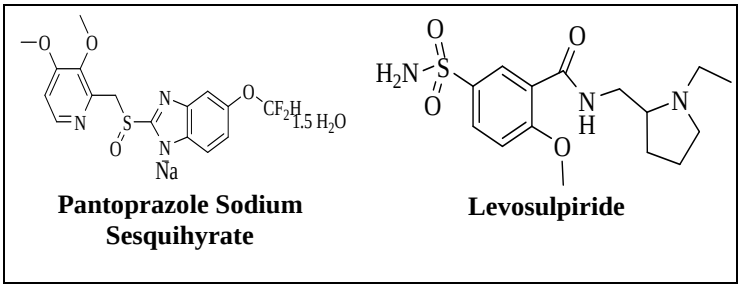

Figure No. 1: Structures of Analytes

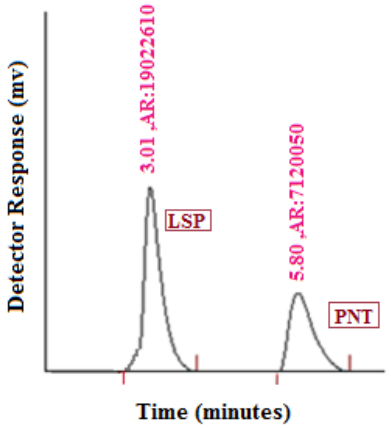

Figure No. 2: Chromatogram of Working Standard Mixture of PNT (40 µg ml^{-1}) and LSP (75 µg ml^{-1})

METHOD DEVELOPMENT

The HPLC method was validated in terms of precision, accuracy and linearity according to ICH guidelines. Method precision was determined using nine independent test solutions. The intermediate precision of the assay method was also evaluated as inter-day and intra-day precision. The accuracy of the assay method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high) of the authentic standards were added to the placebo. The mixtures were extracted and analyzed using the developed HPLC method. The Limit of Detection (LOD) and Limit of Quantification (LOQ) for analytes were determined based on the calibration curves at a signal to noise ratio of 3:1 and 10:1 respectively. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The degradation studies were done by subjecting the drugs to various stress conditions like acid, alkali, hydrogen peroxide. Photo stability was determined by storing the samples for 24 h under UV light in photo stability chamber. The thermal stability was also determined by keeping the drug in hot air oven at 80 °c temperature for 24 h.

METHOD OPTIMIZATION

Well defined symmetrical peaks were obtained upon measuring the response of eluent under the optimized conditions after thorough experimental trials. The UV

detector response of PNT and LSP was studied at 238 nm (λmax.of LSP) which gave highest sensitivity.

MOBILE PHASE COMPOSITION

Several modifications in the mobile phase composition were carried out in order to study the possibilities of changing the selectivity of the chromatographic system. These modifications included the change of the type and ratio of the organic modifier, the pH, the concentration of 0.02M potassium dihydrogen phosphate solution, the flow rate, the temperature and the stability of PNT and LSP was also studied for the optimization of the mobile phase. The system suitability parameters were as shown in Table No.1.

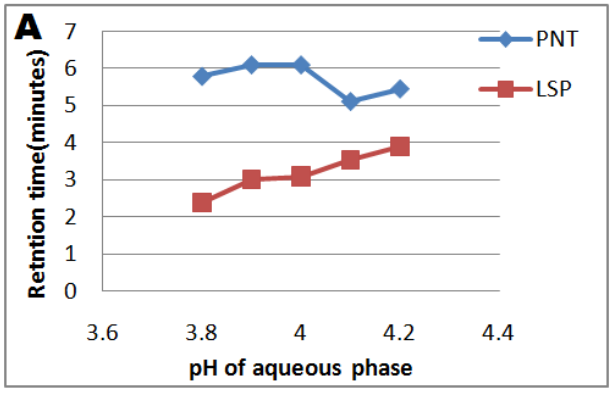

Figure No 3: Effect of pH (A), Proportion of 0.02 M phosphate solution in mobile phase (B) and effect of flow rate (C) on retention times of PNT and LSP

Table No.1: System suitability parameters at optimized chromatographic conditions

Name of analytes	System suitability	
	Parameters	Results
PNT	Theoretical plates	53824
	Peak Tailing	1.25
	% R.S.D.	0.07520
LSP	Theoretical plates	22650
	USP resolution	3.75
	Peak Tailing	1
	% R.S.D	0.10147

TYPE OF ORGANIC MODIFIER

Initially methanol and water in different ratios were tried which showed peak broadening. Further various combinations of acetonitirile and water. Then acetonitrile and methanol were tried but the resolution was very less. So methanol was replaced by 0.02 M potassium dihydrogen phosphate solution with different pH and concentration. Hence potassium dihydrogen phosphate solution (pH-4): acetonitrile (60:40 v/v) was suitable to get resolved and sharp peak. Acetonitrile was the organic modifier of choice giving symmetrical narrow peaks and good resolution reported in Table No.1

RATIO OF ORGANIC MODIFIER

The effect of changing the ratio of organic modifier on the selectivity and retention times of the test solutes was investigated using mobile phases containing concentrations of 60-40% Acetonitrile. Table No. 1 shows that 40% acetonitrile was the best one giving well resolved peaks and higher no. of theoretical plates. Ratios less than 40% resulted in peak broadening, where as ratios higher than 40% resulted in peaks with very less unacceptable retention times.

EFFECT OF pH

The effect of changing the pH of aqueous phase in the mobile phase on the selectivity and retention times of the test solutes was investigated using 0.02 M potassium dihydogen phosphate solution of pH ranging from 3.0-6.0. pKa of LSP is 11, so in the acidic pH probability of drug being ionised form is more, which is in turn has an effect on peak shape and retention time. In order to ensure complete separation and high resolution, the chosen pH of the aqueous phase is 4 (Figure No. 3(A)) There were asymmetric and broad peaks of both the analytes at pH values < or > 4.0

PROPORTION OF 0.02 M POTASSIUM DIHYDROGEN PHOSPHATE SOLUTION IN THE MOBILE PHASE

The effect of changing the proportion of 0.02 M potassium dihydrogen phosphate solution on the selectivity and retention times of the test solutes was investigated using mobile phases containing proportion of 40, 50, 60, 70 and 80% v/v of potassium dihydrogen phosphate solution. Figure No. 3(B) shows that 60% v/v phosphate solution

was found to be the most suitable giving best resolution and highest theoretical plate count, while lower concentrations showed asymmetric and tailing problem.

EFFECT OF FLOW RATE

The effect of flow rate on the formation and separation of peaks was studied by varying the flow rate from 0.8 - 1.2 ml m^{-1}; Figure No.3(C) shows that flow rate of 1 ml m^{-1} was optional for good separation and resolution of peaks in a reasonable time.

RESULTS AND DISCUSSION

METHOD VALIDATION

The method was validated, in accordance with ICH guidelines, for linearity, range, accuracy, precision, LOD and LOQ, specificity, ruggedness, and robustness [ICH-Q2B, 2006]

LINEARITY AND RANGE

For the construction of calibration curves, six calibration standard solutions were prepared over the concentration range. Linearity was determined for PNT in the range of 8-48 µg ml^{-1} and for LSP 7.5-45 µg ml^{-1}. The correlation coefficient ('r^2') values were > 0.999 (n = 6). Typically, the regression equations for the calibration curve was found to be y =44953x-11795 for PNT, y=6367x−34596 for LSP.

PRECISION AND ACCURACY

The precision of repeatability was studied by replicate (n=6) analysis of capsule solutions. The precision was also studied in terms of intra-day changes in peak area of drug solution on the same day and on three different days over a period of one week. The intra-day and inter-day variation was calculated in terms of percentage relative standard deviation and the results are given in Table No.2. Accuracy of the method was calculated by recovery studies at three levels by standard addition method. The mean percentage recoveries obtained for PNT and LSP were 99.93% and 100.21%, respectively, reported in Table No.3.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ)

The LOD and LOQ values were found to be 0.0321 and 0.0974 µg ml^{-1} and 0.004172 and 0.012643 µg ml^{-1} for PNT and LSP, respectively.

Table No. 2: Results of precision

Name of analyte	Precision of the method (n=6)		
	Actual Conc.(µg/ml)	Measured conc. (µg/ml), %RSD	
		Intra-day	Inter-day
PNT	40	39.6, 0.3303	39.7, 0.5434
LSP	75	74.8, 0.2642	74.4, 0.6157

Table No.3: Results of Capsule Analysis and Accuracy studies

Compound (Label Claim)	Formulation Study (n=6)			Recovery (accuracy) Study		
	Batch Capsule	% Assay Found	%RSD	Recovery Level	% Recovery	%RSD (n=3)
PNT (40 mg)	Batch I	99.06	0.2765	80	99.63	0.2167
				100	99.78	0.6299
	Batch II	99.55	0.3303	120	100.4	0.6333
LSP (75 mg)	Batch I	100.16	0.1865	80	100.71	1.020
				100	100.03	0.3469
	Batch II	99.88	0.2642	120	99.9	1.443

ROBUSTNESS

Robustness of the method was investigated under a variety of conditions including changes of flow rate, % of buffer and pH of buffer. The dilutions were injected in triplicate for each condition and effect on the retention time, tailing factor and % assay was calculated. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust as shown in Table No. 4

Table 4: Results of robustness study

Chromatographic changes (n=3)						
Parameter	Retention time		Tailing factor		% Drug found	
	PNT	LSP	PNT	LSP	PNT	LSP
Flow Rate (± 0.1 ml/m)						
0.9	6.83	3.41	1.25	1.1	100.51	100.48
1.0	6.10	3.10	1.2	1	100.7	99.0
1.1	5.64	2.86	1.18	0.9	100.8	99.83
Mean± S.D.	6.18 ±0.5841	3.12 ±0.2757	1.21 ±0.036	1 ±0.1	100.67 ±0.1473	99.77 ±0.7418
Mobile phase composition (± 1 v/v)						
61:39	5.80	2.96	1.3	1.2	100.1	100.97
60:40	6.10	3.10	1.2	1	99.59	100.5
59:41	6.47	3.11	1.12	1.1	99.98	99.45
Mean± S.D.	6.12 ±0.3356	2.98 ±0.0251	1.24 ±0.0529	1.1 ±0.1	99.89 ±0.2666	100.31 ±0.7721
pH of Buffer						
3.9	6.10	3.01	1.22	1.11	100.0	100.4
4	6.10	3.10	1.2	1	99.98	100.3
4.1	6.11	3.11	1.2	1.13	99.96	100.03
Mean± S.D.	6.10 ±0.0057	3.07 ±0.0519	1.2 ±0.0115	1.08 ±0.07	99.98 ±0.02	100.25 ±0.1913

SPECIFICITY

The specificity of the HPLC method is illustrated in Figure No. 4, where complete separation of PNT and LSP was noticed in presence of capsule placebo. In addition there was no any interference at the retention time of PNT and LSP in the chromatogram of capsule solution. This shows

that the peak of analytes was pure and excipients in the formulation did not interfere the analytes.

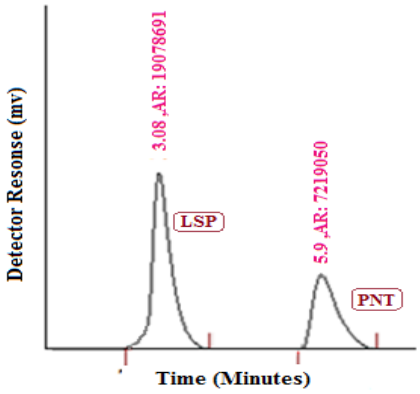

Figure No. 4: HPLC chromatogram of PNT & LSP in Capsule formulation

FORCED DEGRADATION STUDIES:

For the forced degradation studies, the drugs were subjected to acidic, alkaline, oxidative, thermal & photolytic degradation. Accurately about 10 mg of PNT & LSP weighed & added separately in a series of 25 ml volumetric flask.

ACIDIC DEGRADATION:

Acid induced degradation was performed by adding 1 ml of 0.05 M and 0.1M Hydrochloric acid (HCl) separately to each volumetric flask containing PNT & LSP respectively. The volume was made up to 10 ml with distilled water & refluxed for 30 minutes at 40 ^{o}c. The solution was cooled and neutralized with Sod. Hydroxide solution. This solution was sufficiently diluted to 100 ml with distilled water and filtered through whatmann filter paper 41. 1 ml of this solution was pipetted out and diluted to 10 ml with mobile phase.

ALKALINE DEGRADATION:

Base induced degradation was performed by adding 1 ml of 0.1 M Sodium hydroxide (NaOH) to each volumetric flask containing PNT & LSP. The solutions were processed in the same way as acid degradation and neutralized with hydrochloric acid.

OXIDATIVE DEGRADATION:

Hydrogen peroxide (H_2O_2, 3% and 6%) were prepared & 1 ml was added to each volumetric flask containing PNT & LSP . The flasks were kept at room temperature for 20 minutes. The solution was diluted to 100 ml with mobile phase. The sample solution was filtered through whatmann filter paper 41. From this solution, 1ml pipetted out and diluted to 10 ml with mobile phase.

DEGRADATION UNDER DRY HEAT (THERMAL DEGRADATION):

For dry heat degradation, the standard drugs were placed in an oven at 80°C for 24 h. Appropriate dilutions of 10 µg were prepared in mobile phase and then analyzed under the optimized chromatographic conditions.

PHOTO-STABILITY STUDIES:

For photo stability study, the standard drugs were exposed to UV light in photo-stability chamber for 24 h (lux illumination). Appropriate dilutions 10 µg were prepared in mobile phase and then analyzed under the optimized chromatographic conditions.

RESULTS OF FORCED DEGRADATION STUDIES:

Table 5: Results of forced degradation studies

S. N.	Stress Condition	Temperature and Time	% assay of active substance		% Degradation of active substance	
			PNT	LSP	PNT	LSP
1.	Acid (0.05 M HCl)	At Room Temperature For 20 m	78 %	--	22 %	Stable
	0.1 M HCl		--	82.45 %	NA	17.55 %
2	Alkali (0.1 M NaOH)		88.39 %	85.28 %	11.61 %	14.72 %
3	Oxide (3 % H_2O_2)		93 %	--	7 %	Stable
	6 % H_2O_2		--	86.23 %	NA	13.77 %
4	Thermal degradation	80^{0}c for 24 h	92.86 %	91.49 %	7.14 %	8.51 %
5	Photo degradation	24 h	86.46 %	86.97 %	13.54 %	13.03 %

Result of forced degradation studies are given in Table No. 5. According to the forced degradation studies carried out for PNT and LSP, it was found that PNT undergoes 22 % degradation when exposed to 0.05 M HCl while LSP was found to be stable. Further both the drugs were subjected to higher stress condition with 0.1 M HCl, wherein 17.55% degradation was recorded for LSP. The degradation peak of LSP was not resolved. PNT underwent complete degradation when exposed to 0.1 M HCl. The chromatogram of acidic degradation for PNT and LSP in 0.05 M and 0.1 M HCl are as shown in Figure No.5 & 6 respectively.

Under alkaline conditions PNT showed 11.61 % degradation while LSP showed 14.72 % degradation but no separate degradation peak was obtained for LSP. The chromatogram of alkaline degradation for PNT and LSP in 0.1 M NaOH is as shown in Figure No. 7 & 8 respectively.

PNT showed 7%, 7.14%, 13.54% degradation under oxidative, photo & thermal degradation conditions respectively. LSP showed 13.77 %, 8.51%, 13.03% degradation under oxidative, photo & thermal degradation conditions respectively. The degradation peaks were well resolved from the main peak.

The chromatogram of oxidative, photo & thermal degradation for PNT are as shown in Figure No. 9, 11, 13 respectively & for LSP in Figure No.10, 12 and 14 respectively.

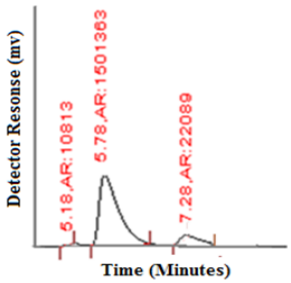

Figure No. 5: Chromatograms of PNT after acidic (0.05M HCl) degradation

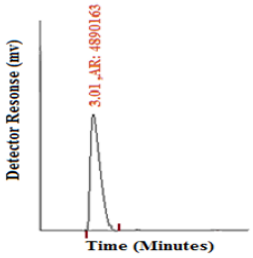

Figure No. 6: Chromatograms of LSP after acidic (0.1M HCl) degradation

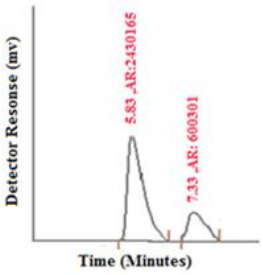

Figure No. 7: Chromatograms of PNT after alkaline (0.1M NaOH) degradation

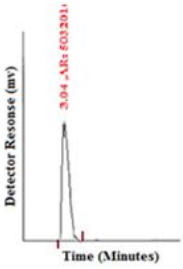

Figure No. 8: Chromatograms of LSP after alkaline(0.1M NaOH) degradation

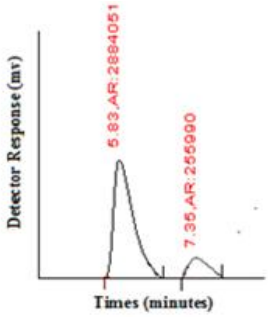

Figure No. 9: Degradation of PNT in 3 % H_2O_2

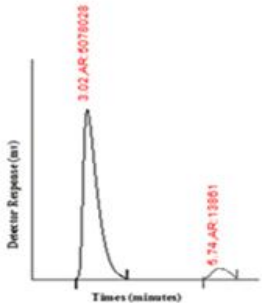

Figure No. 10: Degradation of LSP in 6% H_2O_2

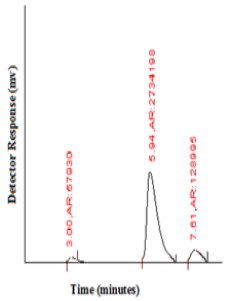

Figure No. 11: Chromatograms of PNT after Photo degradation

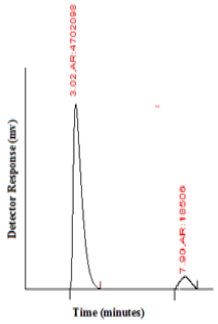

Figure No. 12: Chromatograms of LSP after Photo degradation

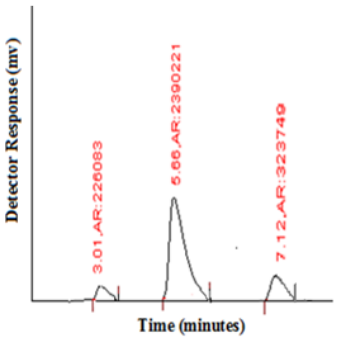

Figure No. 13: Chromatograms of PNT after Thermal degradation

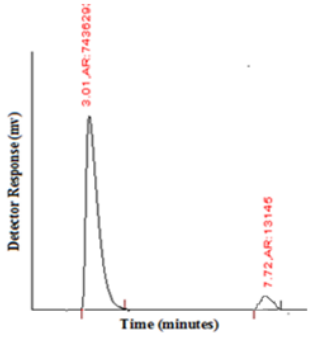

Figure No. 14: Chromatograms of LSP after Thermal degradation

CONCLUSION

Pantoprazole sodium sesquihydrae and levosulpiride are weakly acidic and required the use of reversed phase chromatographic techniques for better resolution and degradation studies. The mobile phase was optimized using different proportions of organic modifier and pH variations. When exposed to the stress conditions, both the drugs were susceptible to oxidative, thermal and photolytic conditions while in the acidic and alkaline conditions PNT underwent degradation with well resolved peaks of the degradant product while LSP underwent degradation but degradant peaks were not resolved. Hence the proposed RP-HPLC method for the simultaneous estimation of Pantoprazole sodium sesquihydrate & Levosulpiride was found to be accurate, precise, reproducible, economic & less time consuming. Therefore the method can be employed for monitoring the stability of both the drugs.

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