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Research Article

Development and Validation of a Stability Indicating RP-HPLC Method for Determination of Eperisone Hydrochloride in Bulk Drug.

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ABSTRACT
A simple, new, specific and correct stability showing RP-HPLC method development and validation for the assessment of Eperisone hydrochloride in the bulk drug and dosage form. Waters C18, 5um (4.6×250mm) column were used as stationary phase among mobile phase comprise of mixture of Acetonitrile: 0.01M Ammonium acetate buffer (30:70 v/v), (pH -3.5). The flow rate at 1.0 mL/min and λ max at 256 nm. Retention time was 7.7 min. The linearity of method was inspect in the concentration scale of 40-90 µg/ml with correlation coefficient (r^2) 0.999. The develop method was validated for the linearity, precision, accuracy, SST and FD studies like acidic, alkaline, oxidative and thermal stress conditions were accomplish as such ICH guidelines. The results observed in the study were among the acceptable limits and hence this method used for the estimation of Eperisone HCl in dosage form.

KEYWORDS
Eperisone, HPLC, validation, forced degradation, dosage form.

1. INTRODUCTION

Eperisone HCl (Figure 1) acts by vascular smooth muscles relaxing and skeletal muscles, also demonstrates an action such as decrease of myotonia, elevation of circulation and inhibit the pain reflex. Chemically it is the 4-ethyl-2-methyl-piperdino prophenone HCl. Eperisone HCl also promote voluntary movement of the up and low extremities without decreasing muscle power, it is therefore useful between the starting stage of rehabilitation and as a supporting drug in between second rehabilitative therapy. [1,2,3]

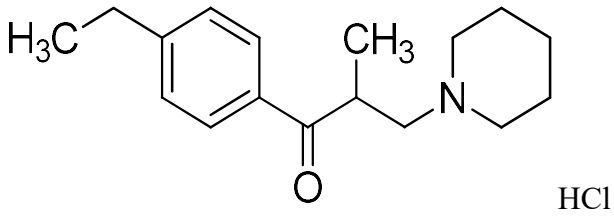

Fig. 1. Chemical Structure of Eperisone.

2. MATERIALS AND METHODS

2.1. Instrumentation

HPLC system consists of Shimadzu, Kyoto Japan having model LC-2010CHT and other instrument used such as Electronic balance made by Shimadzu, Japan. pH meter by Equiptronics and ultra Sonicator consist of Spectra Lab containing model USB 40 and Hot air oven made by Bio Technics India.

2.2. Chemicals and Reagent

HPLC grade methanol and acetonitrile was given from Loba chemical pvt ltd., Mumbai and Merck Ltd., Mumbai, India resp., and all other chemicals which are analytical grade such as ammonium acetate buffer, sodium acetate buffer, sodium hydroxide and hydrogen peroxide was purchased from Loba chemical pvt ltd and Hydrochloric acid was given from S. D. Fine-Chem Ltd. Mumbai, India.

2.3. Selection of Detection wavelength

From UV Spectrophotometric studies, λ max of Eperisone hydrochloride obtained was 256 nm.

2.4. Chromatographic conditions

A mixture of Acetonitrile: 0.01M Ammonium acetate buffer (30:70 v/v), (pH -3.5) was found to be the most suitable mobile phase for ideal chromatographic separation of Eperisone hydrochloride. The solvent mixture was filtered through 0.45 µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 20 µL, columns temperature 40˚C and the column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 256 nm. The run time was set at 10 min.

2.5. Preparation of 0.01 M Ammonium Acetate buffer

0.77 gm of Ammonium Acetate was dissolved in 1000 ml water, to prepare 0.01M Buffer solution. (pH 3.5 adjusted using *O*-phosphoric acid)

2.6. Preparation of mobile phase

Mobile phase was prepared by mixing 30 ml of HPLC grade Acetonitrile with 70ml of 0.01M Ammonium acetate buffer solution. Mobile phase was filtered through 0.45µ membrane filter (Nylon filter) and sonicated in an ultrasonicator before use for 15 min.

2.7. Preparation of diluents

Mixture of Acetonitrile: 0.01 M Ammonium Acetate Buffer solutions (30:70 v/v) were used as diluents.

2.8. Preparation of Standard Stock Solutions and Calibration Curves

The standard stock solution was prepared by weighing 100 mg of Eperisone hydrochloride. The weighed drug was transferred to 100 ml volumetric flask and volume was made up to the mark with methanol to yield a solution containing 1000 µg/ml of Eperisone hydrochloride. Appropriate aliquots from stock solution were transferred in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations 40-90 µg/ml of Eperisone hydrochloride. Calibration curves were constructed by plotting peak area versus concentrations and regression equations were computed for Eperisone hydrochloride.

2.9. Preparation of solutions for forced degradation studies

A) 0.1M Hydrochloric acid: 0.85 ml of HCL was diluted in 100ml of HPLC grade water to obtain 0.1 M HCL.

B) 0.1M Sodium hydroxide: 0.4 gm of NaOH was dissolved in 100 ml of HPLC grade water to obtain 0.1M NaOH.

C) 3% Hydrogen peroxide solution: 6% H_2O_2 was diluted to 10 ml using HPLC grade water to obtain 3% H_2O_2 solution.

***2.10. Validation of the Analytical Methods*[4,5,6]**

Validation of optimized HPLC method was done according to ICH guideline Q2 (R1) for following parameters

2.11. Linearity and range

The linearity of analytical process is its caliber (within a given range) to observed test results which are directly proportional to the concentration (Quantity) of analyte in the sample. Linearity was settled by three injections of solutions containing drug in the concentration scale 40.00-90.00 µg/ml for Eperisone hydrochloride. The peak areas vs. concentration of drug were assessing by linear regression analysis.

2.12. Precision

The precision of analytical method given as the closeness of the results between sequences of measurements observed from many sampling of same sample under prescribed conditions. Repeatability was observed by using six replicates of working standard (60 µg/ml). Intra-day variation was evaluated out by injecting 3 different concentrations (40, 60, 80 µg/ml) for3 times in a day, and inter-day precision was assessed by injecting these three different concentrations on

three different days over a time of one week.

2.13. Accuracy (Recovery studies)

The accuracy of a method is the closeness of the test results of the method to the true value. Accuracy may be often declared as % of recovery by assay of known mixed amounts of analyte to blank matrix. Accuracy is a calculate of turenss of the analytical method. The analytical recovery is used as a control step for the analytical method. Recovery studies were compass at 75.0%, 100.0% and 125.0% levels using standard addition method. Standard drug solution of Eperisone HCl was spiked at 75.0%, 100.0% and 125.0% of the degraded sample solution (500µg/ml.) For each level three replicates were make and injected onto column under optimized HPLC conditions. The calculation for total recovery of Eperisone HCl was performed and results are expressed in terms of % recovery ± S.D and % R.S.D values.

2.14. Robustness

Robustness of the method was performed by purposefully different some chromatographic parameters. These parameters contain change in flow rate (0.9ml and 1.1ml), temperature (390c and 410c) and wavelength (254nm and 256nm) etc. The results observed by changing all these conditions are indicated in terms of % RSD values. These studies were performed on concentration of 50µg/ml.

2.15. Limit of detection (LOD) and Limit of quantification (LOQ)

Limit of detection of analytical method is given as the less Quantity of analyte in a sample can be estimated but not quantitated as exact value. Limit of quantification of analytical method it is defined less Quantity of an analyte can be quantitatively estimated among accuracy and precision and used for the identification of the degraded products.

LOD and LOQ of the method determined by using following formula,

$$LOD = 3.3 \times N/B \text{ ---Formula 1}$$

$$LOQ = 10 \times N/B \text{ ---Formula 2}$$

2.16. Analysis of Marketed Formulation

Ten tablets of Eprisan® (Eisai Pharmaceuticals India Pvt. Ltd) 50 mg per tablet were weighed and ground into a fine powder using a glass mortar and pestle. A portion equivalent to about 10 mg of Eperisone hydrochloride was accurately weighed and transferred to a 100 ml volumetric flask. Approximately 70 ml of methanol was added to the flask and the contents were sonicated for 20 min, the solution was cooled and volume was adjusted to the mark with proper shaking. The resulting solution was filtered through Whatman filter paper no.41. This sample stock solution was further diluted with the same diluents to obtain 50 µg/ml of Eperisone hydrochloride of solution was injected twice into HPLC under the optimized chromatographic conditions. The concentration of the drug in sample was calculated by measuring their peak areas and comparing with peak areas of pure drug solutions of respective concentrations. The results are discussed under result and Discussion section.

***2.17. Forced degradation studies*[15,16,17,18]**

Forced degradation studies were performed to determine stability indicating properties of the drug and specificity of the method.

2.18. Acid Degradation sample

20 mg of pure drug was transferred to round bottom flask. To this, 40 ml of 0.1M HCL was added and this reaction mixture was refluxed at 80^0 C for about 3 hrs. After every 30 min, 5 ml of refluxed sample was removed. Further, 1 ml of the above solution was neutralized using 1ml of 0.1M NaOH solution and then diluted to 10 ml with diluents to obtain concentration of 50 µg/ml of Eperisone hydrochloride. This solution was mixed well and was filtered through 0.45µm filter (Nylon filter) and injected onto column under optimized RP-HPLC conditions.

2.19. Alkali Degradation sample

20 mg of pure drug was transferred to round bottom flask. To this, 40 ml of 0.1M NaOH was added and this reaction mixture was refluxed at 80^0c for about 3 hrs. After every 30 min, 5 ml of refluxed sample was removed. Further, 1 ml of the above solution was neutralized using 1ml of 0.1M HCL solution and then diluted to 10 ml with diluents to obtain concentration of 50 µg/ml of Eperisone hydrochloride. This solution was mixed well and was filtered through 0.45µm filter (Nylon filter) and injected onto column under optimized RP-HPLC conditions.

2.20. Hydrogen Peroxide Degradation sample

20 mg of pure drug was transferred to round bottom flask. To this 40 ml of 3% H_2O_2 was added and this reaction mixture was kept at room temperature for about 4 hrs. After every 1 hr, 5 ml of sample was removed. Further 1 ml of the above solution was diluted to 10ml with diluent to obtain concentration of 50µg/ml. Mixed well and filter through 0.45µm filter and injected onto column under optimized RP-HPLC conditions.

2.21. Neutral Degradation sample

20 mg of pure drug was transferred to round bottom flask. To this 40 ml of HPLC grade water was added and this reaction mixture was refluxed at 80^0c for about 3 hrs. After every 30 min, 5ml of sample was removed. Further 1 ml of the above solution was diluted to 10ml with diluent to obtain concentration of 50µg/ml. Mixed well and filter through 0.45µm filter and injected onto column under optimized RP-HPLC conditions.

2.22. Photo Degradation (Sun light) sample

The photochemical stability of the drug was studied by exposing the stock solution of 1000 µg/ml Eperisone hydrochloride to direct sunlight for 8 hrs on a wooden plank and kept on the terrace in the month of February. Samples were withdrawn at every 2 hrs. The resultant solution was injected into the system under optimized conditions

2.23. Dry Heat degradation sample

Solid Eperisone hydrochloride packed in the vial was exposed to dry heat in an oven at 80^0 C for 8 hrs. After 8 hrs, 10 mg of the drug was dissolved in 10 ml of methanol and sonicated for 20 min. Final dilution was made in the mobile phase to obtain a predetermined concentration of 50 µg/ml solution to study the effect of dry heat degradation.

3. RESULTS AND DISCUSSION

In this work a simple, novel, accurate and precise stability indicating RP-HPLC method have been optimized, developed and validated for determination of Eperisone hydrochloride. In the formulations by using Waters, C-18 (250.0 mm X 4.6 mm, 5 µm) column, in isocratic mode, at 40° C temperature using a MP containing mixture of Acetonitrile : Buffer(0.01M ammonium acetate) (30:70 v/v) (pH-3.5). Resulted chromatographic obtained peak was free from tailing in good shape and better resolved FR was 1.0 ml/min and Detection was carried at 256 nm. The result of optimized condition where shown in table 1.

Table 1.Optimized Chromatographic Conditions.

Parameters	Condition
Column	Waters C18, 5um (4.6×250mm) column
Column Temperature	40˚C
Mobile Phase	Acetonitrile : 0.01M Ammonium acetate buffer (30:70 v/v),(pH -3.5)
Buffer:	0.01M Ammonium acetate buffer solution.
Detection	256 nm
Injection Volume	20 µl
Run Time	10 minutes
Retention time	7.7 minutes
Flow rate	1 ml/min

3.1. Linearity and Range

EPE showed good correlation coefficient in concentration range of 40-90 µg/ml (r2=0.999). Linearity was evaluated for a set of six standard working solutions containing 40-90 µg/ml for EPE. The calibration graphs of linearity and acceptance of Beer's law were validated by evaluating correlation coefficient and S.D. values given within accepted limit.

Table 2. Linearity results of EPE.

Concentration (µg/ml)	Area
40	2950615
50	3561556
60	4178110
70	4792339
80	5412129
90	6115635

n=6

Table 3. Linear regression data for calibration curves.

Parameters	EPE
Linearity range µg/ml	40-90 µg/ml
r^2 ± SD*	0.999±0.0018
Slope ± SD*	70606.67± 5689.73
Intercept ± SD*	74800.67± 24036.49
Y= mx + c	y = 62832x + 41767

n=6

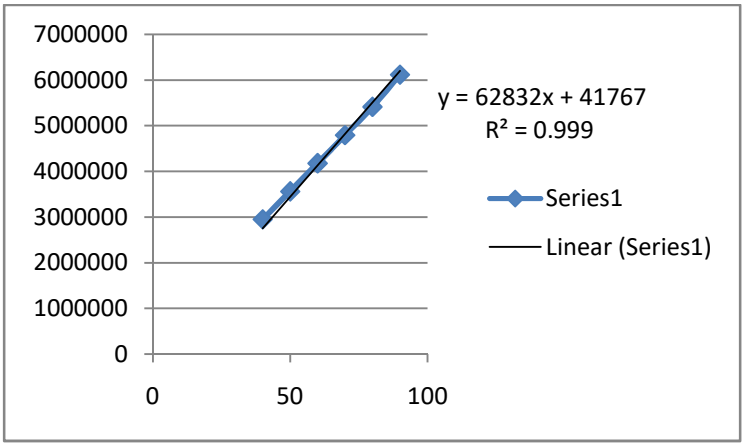

Fig. 2. Linearity curve of Eperisone Hydrochloride.

3.2. Precision studies

Repeatability studies were studied by giving 6 replicates of working standard (60 µg/ml). The results for repeatability studies of HPLC. *(as shown in Table 4)*

Table 4. Repeatability studies.

Precision	Amount (µg/ml)	Area	Mean Area ± SD*	%RSD*
Repeatability (n=3)	60	4727958	4615536 ± 79580.86	1.724
	60	4554807		
	60	4727958		
	60	4554807		
	60	4563250		
	60	4564434		

Intraday precision was performed by repeated injections of pure drug solutions at three concentrations (40, 60 and 80 µg/ml). Interday precision studies were performed as same

concentrations at three different days over a time of 1 week. Precision was expressed as % Relative Standard Deviation values *(as shown in Table 5, 6)*

Table 5. Intraday precision studies.

Precision	Amount (µg/ml)	Area	Mean Area ± SD	%RSD*
	40	3145580		0.394
Intra-day	40	3125539	3142139 ± 12390.32	
(n=3)	40	3155298		
	60	4527958	4549066 ± 15434.62	0.339
	60	4554807		
	60	4564434		
	80	5546208	5533552 ± 15231.13	0.275
	80	5542318		
	80	5512129		

Table 6. Inter-day precision studies.

Precision	Amount (µg/ml)	Area	Mean Area ± SD	%RSD*
	40	3145580		0.394
Inter-	40	3125539	3142139 ±	
day	40	3155298	12390.32	
(n=3)	60	4527958	4549066 ±	0.339
	60	4554807	15434.62	
	60	4564434		
	80	5546208	5533552 ±	0.275
	80	5542318	15231.13	
	80	5512129		

3.3. Accuracy (Recovery studies)

Standard drug sample when spiked with the degraded sample at three given concentration levels (75%, 100%, 125%) showed satisfactory recovery of EPE. Using the developed method, the average mean recovery ± S.D. of the added drug was 100.09 ± 0.3743. *(as shown in Table 7)*

Table 7. Results for accuracy studies.

Spike level	Amount added (µg/ml)	Area	Amount recovered (µg/ml)	% Recovery	Mean % recovery ± S.D.	% R.S.D.*
		5496133	81.55	99.60		
75%	81.5	5492562	81.51	100.01	99.84 $\pm$ 0.165	0.16
		5496133	81.51	99.60		

		6304990	94.62	100.66		
100%	94	6350125	94.59	100.63	100.63±0.015	0.015
		6304990	94.62	100.66		
		6768602	102	99.51		
125%	102.5	6817453	103	100.98	100.24 ±0.73	0.014
		6768602	102	99.51		

n=3

The RT of Eperisone HCl was 7.7min,cuts down on all time of sample analysis and the method was cost effective and also it required very less amount of mobile phase the theoretical plate was 5107 observed .Tailing factor was 1.6.which indicate better performance of column .typical chromatogram of drug shown in fig. 3.

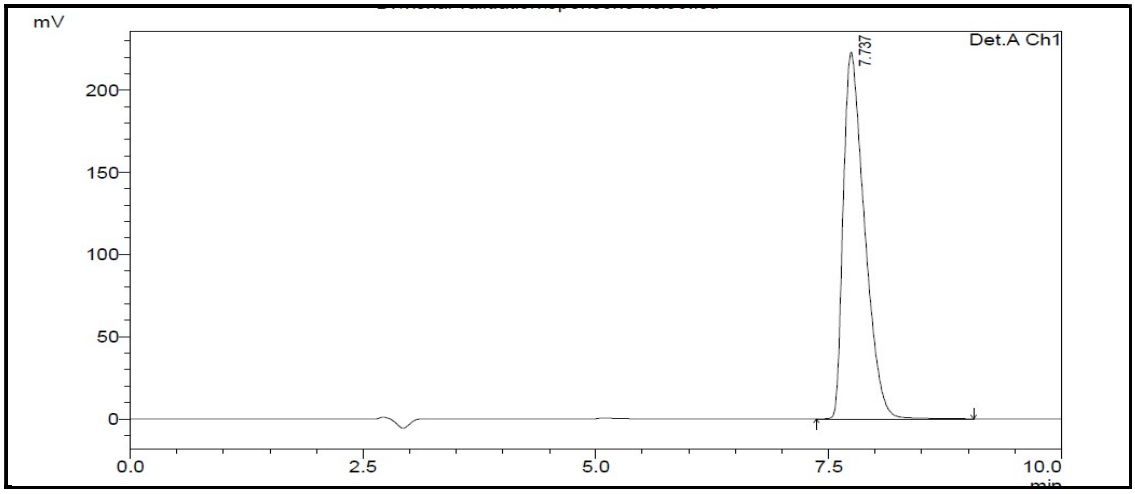

Fig. 3. Typical Chromatogram of Eperison hdychloride.

3.4. Analysis of Marketed Formulation by RP-HPLC method

The peak at tR 7.7 ± 0.02 min was observed for EPE in chromatogram of drug sample extracted from tablet. Practical results of EPE quantity in tablet shows as percentage of label claim were in better agreement with the label claim there by advising that there is no interference from different excipients, which are present in tablet. The mean drug content and S.D. was 99.80 ± 0.254 for EPE. Which contents no interfering spectra of the marketed preparation within run time indicate that excipient used in formulation can't interfere with estimation of drug by proposed RP-HPLC method.

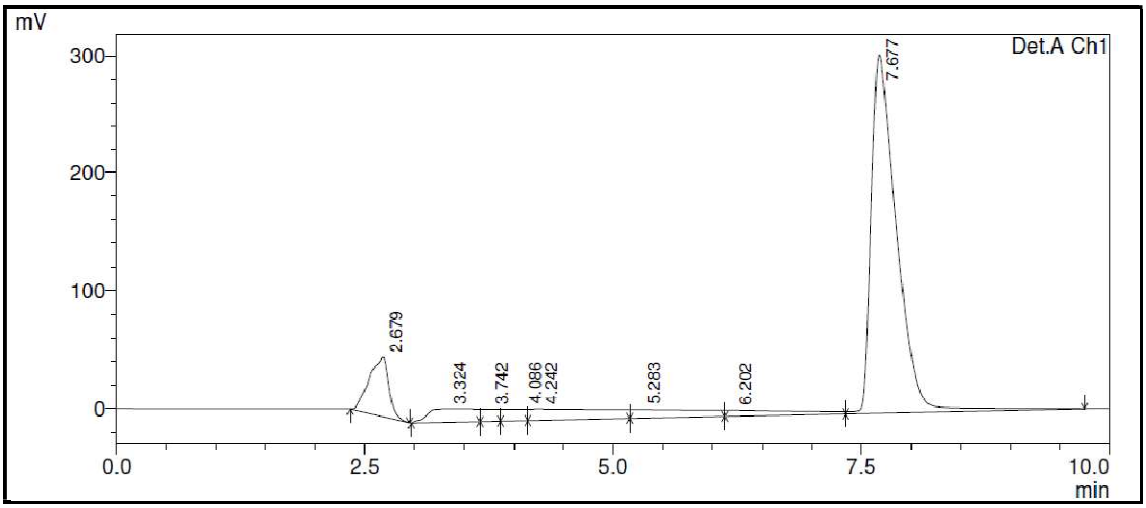

Fig. 4. Chromatogram of marketed formulation of EPE t_R=7.6±0.02min Mobile phase- ACN: 0.01M ammonium acetate buffer (30:70v/v), 1 ml/min, Waters C18, 5um (4.6×250mm) column.

Table 8. Assay of EPE in commercial tablet. (Eprisan®).

Parameters	EPE
Label Claim (mg)	50
Actual content found (mg)	49.546
Drug content (%) ± SD*	98.9% ± 0.254
% RSD*	0.257

n=3

The selectivity of method was stated by absence of any peaks without drug on retention time of drug. The limit of detection found to be 1.12 µg/ml and LOQ was 3.40 µg/ml which indicate sensitivity of method. System suitability verifies the reproducibility and resolution of the method. SST parameters for the method shows within the acceptable limit. (*as given in Table 9)*

Table 9. System suitability parameters.

Sr. No	Parameters	Obtained value	USP specifications
1	Retention time *(t_R)*	7.7 ± 0.02 min.	-
2	Theoretical plates	5107.229	Greater than 2000.
3	Linearity Range	40-90µg/ml	
4	Correlation Coefficient	0.999	≥0.999
5	LOD (µg/ml)	1.12	
6	LOQ (µg/ml)	3.40	
7	Tailing factor	1.6	Less than 2.

Robustness was observed that there were no marked changes in chromatogram. Each factor described under table 9 was changed at 3 levels (-1, 0, 1). One factor was changed at single time to find out effect. Robustness is evaluated at a concentration level 50µg/ml for EPE. Insignificant variation in chromatograms areas and little variability in RT were observed.

Table 10. Robustness data in terms of retention time for EPE (change in flow rate, temperature and Wavelength).

Factor	Level	EPE (t_R in min)	Peak area
A: Change in Flow Rate (1 ml/min)			
0.9	-1	7.75	4422289
1	0	7.61	4450308
1.1	+1	7.41	4465412
	% RSD*	**0.401**	
B: Change in temperature (30^0 C)			
39^0 C	-1	7.75	4422289
40^0 C	0	7.61	4450308
41^0 C	+1	7.41	4465412
	% RSD*	**0.1943**	
C: Change in Wavelength			
255 nm	-1	7.63	4584512
256 nm	0	7.62	4520389
257 nm	+1	7.63	4535140
	% RSD*	**0.603**	

****n=3***

Table 11. Summary of the Validation Parameter.

Sr. No.	**Parameters**	**Results**	**Standard values**
1	Linearity Range	40-90µg/ml	-
2	Straight line equation	y = 62832x + 41767	-
3	Correlation Coefficient	0.999	≥0.999
	Precision (%RSD)		
	Repeatability* (n=6)	1.72	≤2.0%R.S.D.
4	Intra-day* (n=3)	0.394	≤2.0%R.S.D.
	Inter-day* (n=3)	0.345	≤2.0%R.S.D.
5	Average % Recovery ± S. D.* (n=3)	100.23 ± 0.063	≤2.0%R.S.D.

6	LOD (µg/ml)	1.12	-
7	LOQ (µg/ml)	3.40	-
8	Robustness:		
	changing in flow rate	Complies	≤2.0%RSD
	changing in Wavelength	Complies	≤2.0%RSD
	changing in temperature	Complies	≤2.0%RSD

3.5. Stress Degradation Studies

Stress studies performed on the drug sample according to ICH Q1A (R2) guideline. The EPE was subjected to different stress testing as hydrolysis, oxidation, thermal stress, photo and UV light. The different stress studies were optimized in such way that the drug will degrade at least 20-30%.

Table 12. Summary of degradation studies.

Deg. Conditions	**Time(hrs)**	**% degradation of EPE**	**t_R of degradation products(min)**
Acid (0.1 M HCL)	1	27.44	Deg. A = 3.2, Deg. B = 5.4
Alkali (0.1M NaOH)	1	11.9	Deg. C= 3.6
Neutral Degradation (HPLC water)	1	15	No extra peak
H_2O_2 (3 %) RT	1	24	Deg. D=4 min
Dry heat	8	12	3.2, 4.0, 5.4
Sunlight light	8	6	None

Acidic hydrolysis

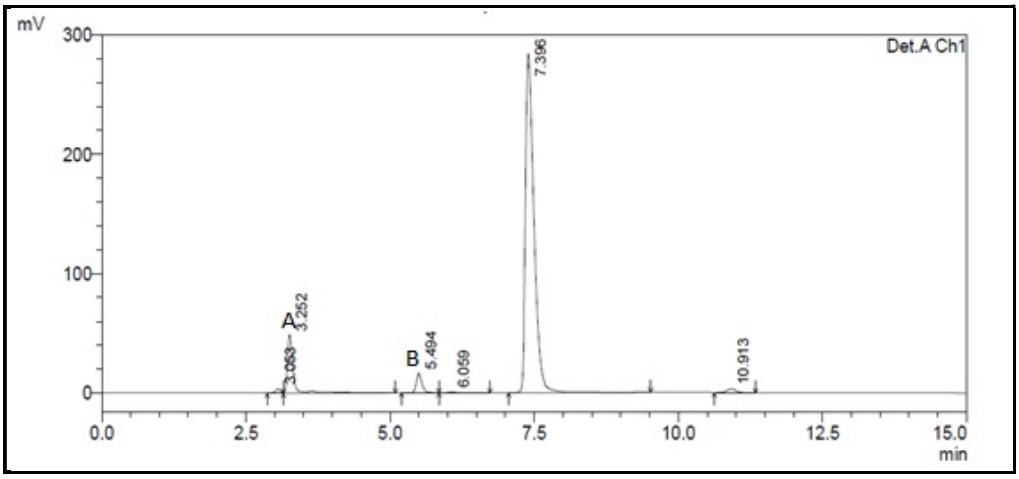

Fig. 5. Chromatogram of Acid degradation condition.

Alkaline hydrolysis

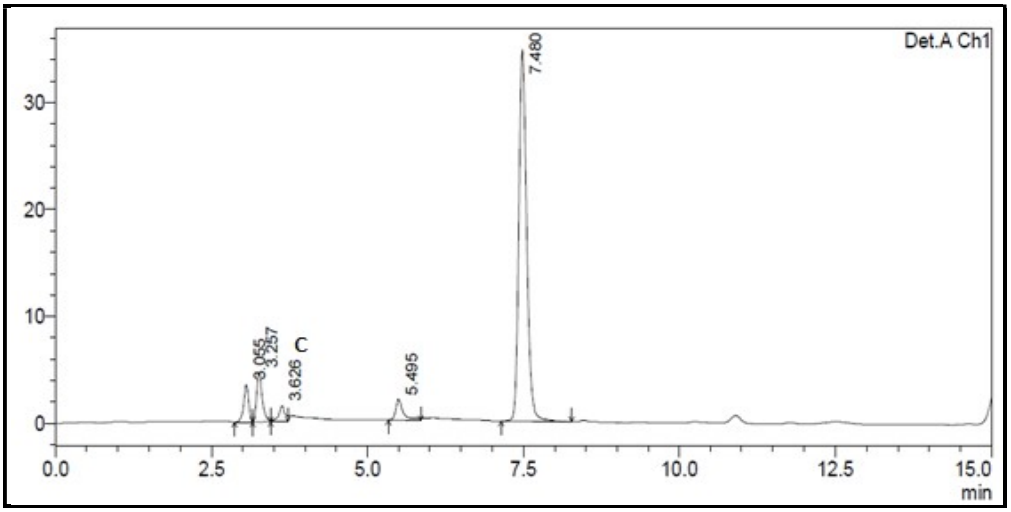

Fig. 6. Chromatogram of Alkaline degradation.

Neutral hydrolysis

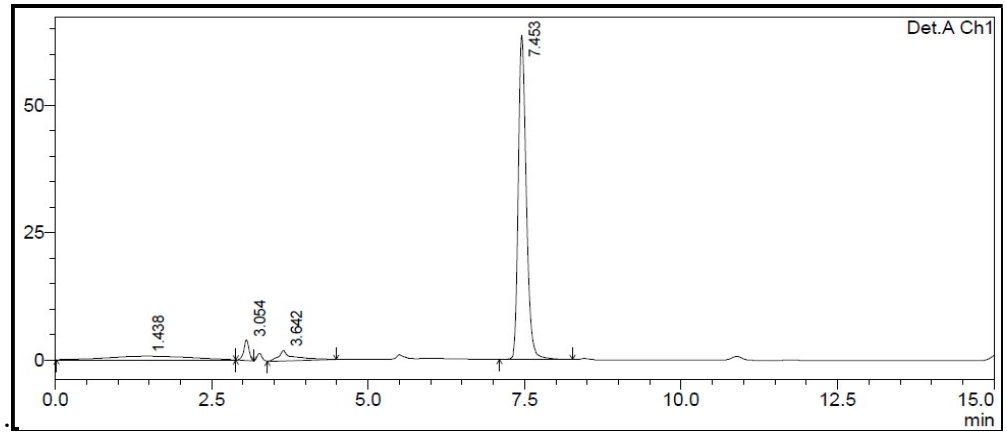

Fig. 7. Chromatogram of Neutral Degradation.

Oxidative degradation

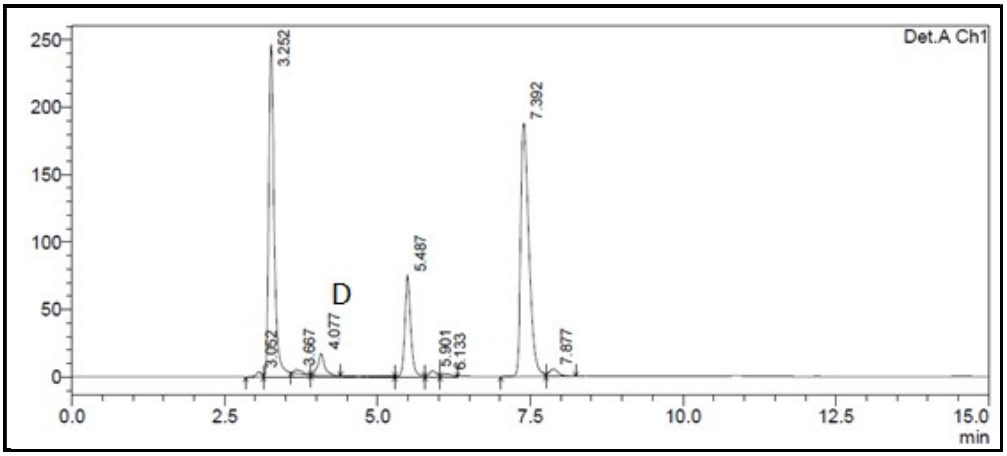

Fig. 8. Chromatogram of Oxidative Degradation.

Dry heat degradation

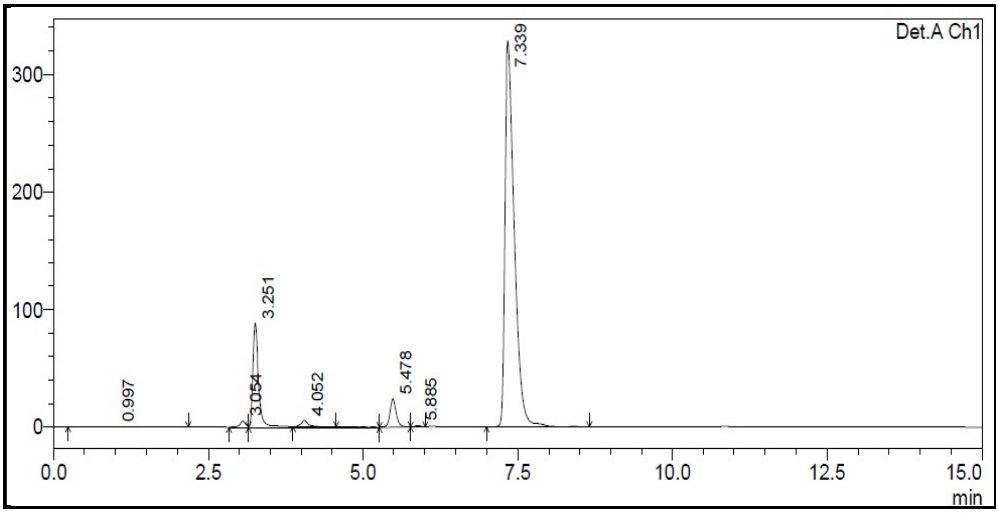

Fig. 9. Chromatogram of Dry heat Degradation.

Photo degradation studies

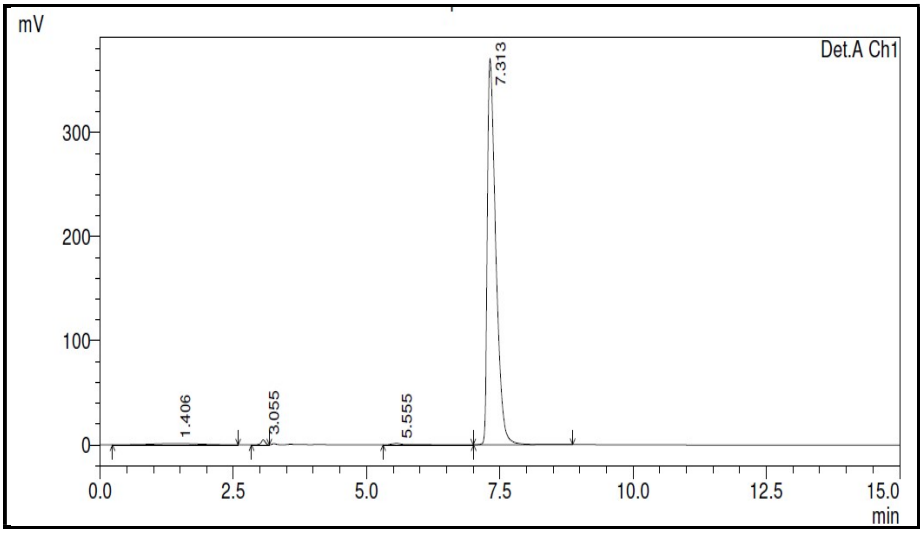

Fig. 10. Chromatogram of EPE under Photo degradation studies.

4. CONCLUSION

The present study represents a simple, reproducible and specific stability indicating RP-HPLC method for Eperisone hydrochloride in API. RT of Eperisone hydrochloride 7.7 min ± 0.2 min. drug content of marketed tablet formulation was done and percentage purity found to be 98.9% ± 0.01.The developed method was validated for linearity, accuracy, precision, LOD, LOQ and specificity and robustness. Drug Linearity was obtained in range of 40-90µg/ml (r2=0.9995). LOD and LOQ 1.12µg/ml and 3.40µg/ml respectively. % RSD values of Reproducibility, Precision was < 2 and it showed that method was precise. Percentage recovery was ob 100.63% obtained, thus method was accurate. There was not much change in tR and area of Eperisone hydrochloride by change in flow rate and oven temperature, thus developed method was robust.

The proposed method was employed for FDS of Eperisone hydrochloride API. The stress condition of degradation study showed that EPE was susceptible to Acidic, Basic, Oxidative and thermal degradation and different degradations studies were at 27.40, 11.9, 24 and 12% respectively. The degradation study indicates that Eperisone hydrochloride was stable to Neutral and Photolytic condition without giving any interference degradation peaks.

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