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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHOD FOR ESTIMATION OF PARGEVERINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, sensitive and specific stability indicating high performance liquid chromatographic (HPLC) method for the estimation of Pargeverine Hydrochloride was developed and validated. Pargeverine Hydrochloride was separated and quantified using stainless steel column (25 cm × 4.0 mm) packed with end-capped octadecylsilyl silica gel for chromatography (5 µm) (Nucleosil C18 is suitable) OR Equivalent, using a mixture of Acetonitrile: Methanol: 1% Ammonium Acetate pH 4.5 (40:40:20v/v/v) and at a flow rate of 1.0 ml/min. Quantification was achieved with an UV detector at 252 nm over the concentration range of 7.5–45 µg/ml. The applied HPLC method allowed the separation and quantification of Pargeverine Hydrochloride with good linearity (r2=0.999) in the studied concentration range. The method was validated as per the International Conference on Harmonization (ICH) guidelines. Pargeverine Hydrochloride stock solution was subjected to different stress conditions. The degraded product peaks were well resolved from the pure drug peak with significant difference in their retention time values. Stressed samples were assayed using developed HPLC method. Statistical analysis of the data showed that the method is precise, accurate, reproducible, and selective for the analysis of Pargeverine Hydrochloride. The method was successfully applied to the estimation of Pargeverine Hydrochloride in Oral Solution dosage form.

KEYWORDS: Pargeverine Hydrochloride**,** HPLC, Stability indicating method.

INTRODUCTION

Pargeverine Hydrochloride is solid white powder with molecular weight of 373.87g/mol and molecular formula $C_{21}H_{24}ClNO_3$. Chemically Pargeverine hydrochloride is 2-(dimethyl amino) ethyl 2, 2-diphenyl-2-prop-2 ynoxyacetate hydrochloride. Pargeverine hydrochloride is soluble in water, methanol and chloroform and having melting point of 170-175˚C. Therapeutic category of Pargeverine hydrochloride is antispasmodic mainly used Pargeverine hydrochloride used in the treatment of gastrointestinal and smooth muscle spasm. Pargeverine hydrochloride, also known as Propinox hydrochloride, is an antispasmodic that presents a dual mechanism of pharmacologic action: musculotropic and anticholinergic. It functions as a musculotropic agent, acting directly on the visceral smooth muscle cells by blocking L-type calcium channel and

conferring its antispasmodic activity. The anticholinergic activity of propinox is derived from a moderate and non-selective blockade of muscarinic M2 and M3 receptors of cholinergic fibers. Pargeverine hydrochloride generally used in dose of 5-10 mg orally and 5- 10 mg/ml parenterally. Propinox Hydrochloride rapidly and completely absorbed after oral administration, reaching peak plasma level in about 60 min of the oral dose. Pargeverine hydrochloride highly 91% bound to plasma protein and volume of distribution 2 L/kg. Pargeverine hydrochloride metabolizes by liver with half life of 4 hours and elimination by hepatic biotransformation eliminate through fecal and renal.

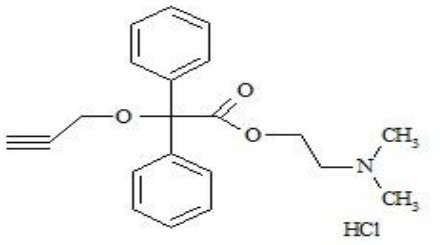

Figure 1: Structure of Pargeverine Hydrochloride

Literature survey reveals that there is no stability indicating analytical method reported for estimation of Pargeverine Hydrochloride. Hence it was thought worthwhile to develop stability indicating method for estimation of Pargeverine Hydrochloride in Pharmaceutical dosage form.

MATERIAL AND METHOD

Material and Reagents

Pure Pargeverine Hydrochloride was obtained as gratis sample from Aum Research Laboratories Ahmadabad, India. Acetonitrile, water, methanol of HPLC grade were Purchased from RANKEM (India) Mumbai. Ammonium acetate, Ortho phosphoric acid, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide were purchased from Finar chemicals (India) Private Limited.

Instrument

HPLC analysis was performed on Agillent 1100 and stainless steel column (25 cm × 4.0 mm) packed with end-capped octadecylsilyl silica gel for chromatography (5 µm) (Nucleosil C18 is suitable) used. The HPLC system was equipped with “EZChrom Elite” software. A double beam UV-visible spectrophotometer (Shimadzu, model UV-1800) having two matched quartz cells

with 1 cm light path and loaded with UV probe software was used for recording of spectra and measuring absorbance. An electronic analytical weighing balance (0.1 mg sensitivity, Mettler Toledo ME 204), digital pH meter (Hanna model HI 2215), a sonicator (Digital Ultra Sonicator Cleaner, LMUC Series-LABMAN)

Method

Selection of wavelength

Suitable wavelength for the HPLC analysis was determined by recording UV spectrums in the range of 200-400 nm. Here 10 µg/ml of solution of Pargeverine Hydrochloride in methanol are to be prepared and measured. Suitable wavelength selected for estimation of Pargeverine Hydrochloride is 252nm. (Fig. 2)

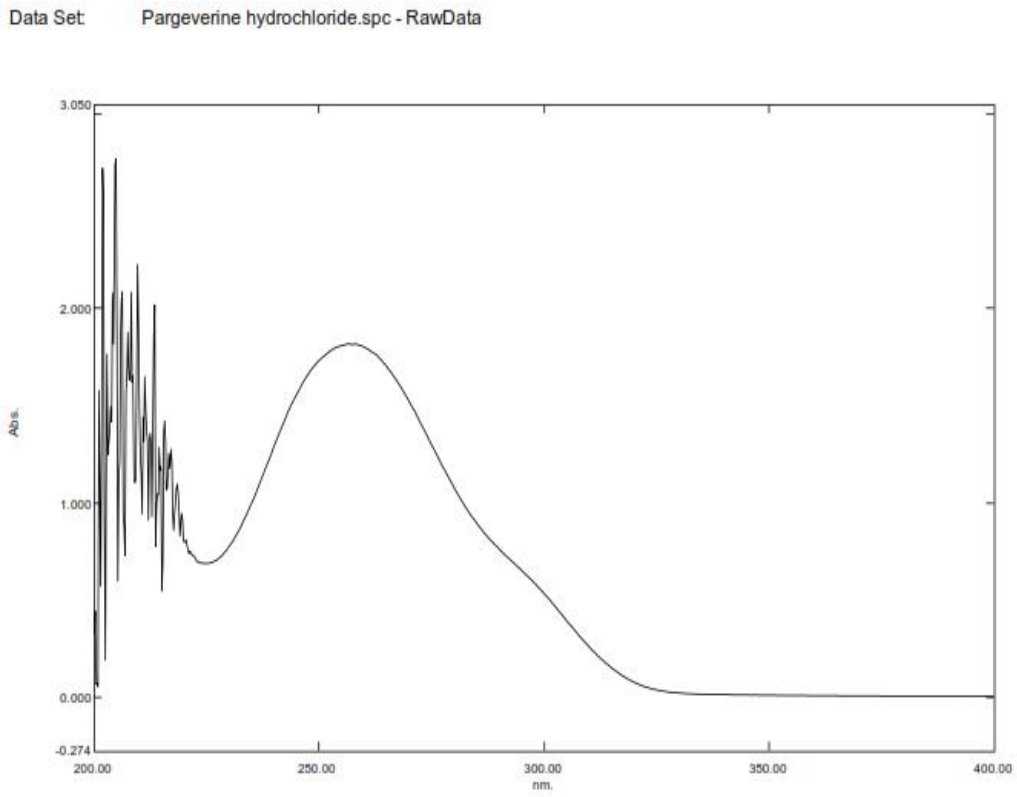

Figure 2: UV Spectra of Pargeverine Hydrochloride

Chromatographic conditions

The developed method uses a stainless steel column (25 cm × 4.0 mm) packed with end-capped octadecylsilyl silica gel for chromatography (5 µm) (Nucleosil C18 is suitable) OR Equivalent, mobile phase of Acetonitrile: Methanol: 1% Ammonium Acetate (pH 4.5) in the proportion of 40:40:20 v/v/v. The mobile phase was set at a flow rate of 1.0 ml/min and the volume injected was 20µl for every injection. The detection wavelength was set at 252 nm.

Preparation of 1% Ammonium acetate (pH 4.5): Accurately weigh 1g of ammonium acetate and add 80ml of HPLC grade water and adjust the pH 4.5 with Ortho Phosphoric acid and make up volume up to 100ml with HPLC grade water.

Mobile Phase Preparation: The mobile phase was prepared by mixing Acetonitrile, Methanol and 1% Ammonium acetate (4.5pH) in the ratio of 40:40:20 v/v/v and later it was sonicated for 10 min for the removal of air bubbles.

Preparation of standard solution: Weigh accurately about 30 mg of Pargeverine hydrochloride working standard in 100 ml volumetric flask, add about 50 ml of Diluent to dissolve with the aid of ultrasound for about 5 minutes with occasional shaking and make volume with Diluent. Dilute 1ml of this solution in 10ml of volumetric flask and make up to mark with Diluent.

Preparation of sample solution: Transfer 5 ml of sample solution (Equivalent to 30 mg of Pargeverine hydrochloride) in 100 ml of volumetric flask, add about 50 ml of mobile phase to dissolve with the aid of ultrasound for about 10 minutes with occasional shaking and make volume with mobile phase. Filter the solution through 0.22µm. Discard first 5 ml of the filtrate. Dilute 1ml of this filtrate solution in 10ml of volumetric flask and make up to mark with mobile phase.

Method validation

Validation of the developed HPLC method was carried out as per the International Conference on Harmonization (ICH) guidelines Q2 (R1).

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products.

Specificity Part-A

In specificity Part-A spiked solution of Pargeverine Hydrochloride was used and separation and resolution were observed between Pargeverine Hydrochloride standard solution, placebo solution and its formulation.

Specificity Part-B

In specificity Part-B, forced degradation study carried out in which sample was subjected to various stress conditions like acidic hydrolysis, basic hydrolysis, oxidative degradation, thermal degradation, photolytic degradation. For this purpose injection formulation and control placebo sample were used.

Acid Hydrolysis

Take accurately and transfer about 1ml of sample solution in 10ml of volumetric flask. Add

1ml of 1N HCl in above flask, than put it at put it at room temperature for 24 hr. Neutralize it with 1ml of 1N NaOH respectively and make up with diluent.

Base Hydrolysis

Take accurately and transfer about 1ml of sample in 10ml of volumetric flask. Add 1ml of 1N NaOH in above flask, than put it at room temperature for 24 hr. Neutralize it with 1ml of 1N HCl respectively and make up with diluent.

Oxidative Hydrolysis

Take accurately and transfer about 1ml of sample in 10ml of volumetric flask. Add 1ml of 3 % H_2O_2 than put it at 60º C temperature in heat chamber for 24hr.After that make up to 10ml with diluent.

Thermal Degradation: Take 5ml of sample in vial and keep at 80º C temperature in heat chamber for 48hr.Than take 1ml in volumetric flask of 10ml and make up 10ml with diluent.

Photolytic Degradation

Take accurately and transfer about 1 ml of sample in 10 ml of volumetric flask. In photolytic degradation sample was put for 1 cycle in photolytic chamber (U.V/Not less than 200 watt hour's square meter^{-1}, VIS/ not less than 1.2 million Lux hr). After completion of 1 cycle dilute it up to 10 ml with diluent.

Calibration curve (linearity of the HPLC method)

Calibration curves were constructed by plotting peak area vs. concentrations of Pargeverine Hydrochloride, and the regression equations were calculated. The calibration curves were plotted over the six different concentration range 7.5–45 µg/ml of Pargeverine Hydrochloride. Aliquots of standard working solution were transferred to a series of 10 mL volumetric flasks and diluted to the mark with the mobile phase. Aliquots (20 µL) of each solution were injected under the operating chromatographic condition described above (n = 6).

Precision

Repeatability

The repeatability studies were carried out by estimating six replicates of same concentration of Pargeverine hydrochloride (30µg/ml) and result reported in terms of relative standard deviation.

Intermediate precision

The intermediate precision studies were carried out by estimating six replicates of same concentration of Pargeverine hydrochloride (30µg/ml) with different equipment and different analyst and result reported in terms of relative standard deviation.

Accuracy (% recovery)

The accuracy of the method was determined by calculating the recovery of Pargeverine Hydrochloride by the standard addition method. Known amounts of standard drug of Pargeverine Hydrochloride is added in 5ml of placebo solutions of oral syrup dosage form and make dilution with mobile phase to make 50%, 100% & 150% (15, 30 and 45 µg/ml). The amount of Pargeverine Hydrochloride was estimated by comparing with average area of 6 standards.

Robustness

Robustness of the method was studied by changing the flow rate (±10%) and the pH (±0.5%) of mobile phase.

RESULT AND DISCUSSION

Method development and Optimization

Validation of a stability-indicating analytical method should demonstrate the capability of the method for the Quantitation of the active pharmaceutical ingredient and the determination of possible degradation products without any interference. To obtain the best chromatographic conditions, the mobile phase was optimized to provide sufficient selectivity and sensitivity in a short separation time. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (RT), number of theoretical plates (N) and Peak Asymmetry (A) etc are considered. A satisfactory separation obtain with stainless steel column (25 cm × 4.0 mm) packed with end-capped octadecylsilyl silica gel for chromatography (5 µm) (Nucleosil C18 is suitable) OR Equivalent, mobile phase composition is Acetonitrile: methanol: 1% ammonium acetate pH 4.5 (40:40:20v/v/v), flow rate was 1.0 ml/min as it provided the best chromatographic performance and acceptable peak characteristics, including tailing factor and the number of theoretical plates at the wave length of 252nm.The retention time of Pargeverine hydrochloride is 5.0min and run time is 10 min. The degradation products was obtained, confirming the stability-indicating capability of the proposed method. The optimized conditions of the HPLC method were validated for the analysis of Pargeverine Hydrochloride in Oral syrup formulation Esandil Compuesto syrup. The sample peak was identified by comparing the retention times with the standard solutions. System suitability parameters were within the acceptance limits, ideal for the injected sample. Integration peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step.

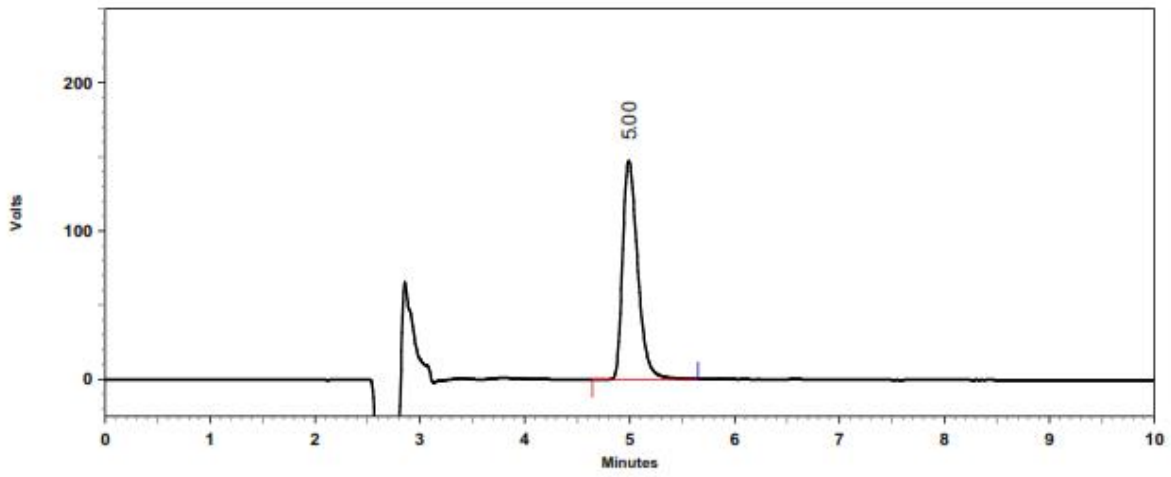

Figure 3: Chromatogram of Standard drug optimize condition

Table 1: System suitability Parameter for standard					
Name	**Retention Time**	**Area**	**%Area**	**Theoretical Plates**	**Asymmetry**
Pargeverine Hydrochloride	5.0 min.	24815686	100%	5826	1.47

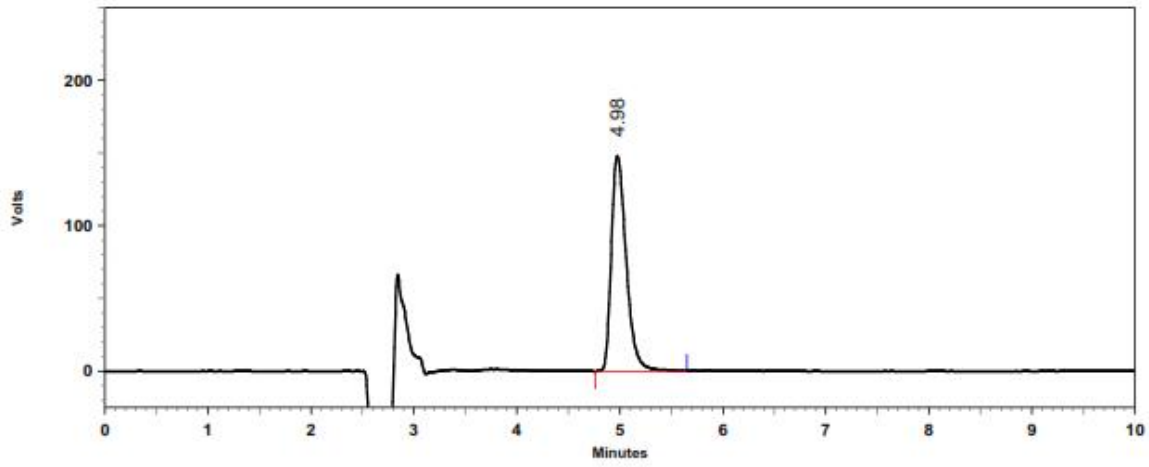

Figure 4: Chromatogram of sample

Table 2: Assay of test sample and system suitability parameter					
Retention Time	**Area**	**%Area**	**Theoretical Plates**	**Asymmetry**	**% Assay**
4.98	24939380	100	5807	1.48	99.79

Method validation

Forced degradations are performed to provide indications of the stability-indicating properties of an analytical method, particularly when there is no information available about the potential degradation products. The results from the stress testing studies indicated that the method was specific for Pargeverine hydrochloride. The drug was found to be unstable in acidic medium. The degradation products were completely distinguishable from the parent compound. Acidic stress lead to 14.26 % of degradation with two unknown degradation peaks at 3.94 and 7.18 min, respectively, whereas a prominent peak of Pargeverine Hydrochloride was stable at 4.98 min (Fig. 5a) and recovery was 84.42%. Alkali stress lead to 11.72 % of degradation with two unknown degradation peaks at 3.82 and 7.67 min, respectively, whereas a prominent peak of Pargeverine Hydrochloride was stable at 4.98 min (Fig. 5b) and recovery was 86.49%. Peroxide stress lead to 3.87% degradation unknown degradation peak at 8.24 min, whereas a prominent peak of Pargeverine hydrochloride was stable at 5.12 min (Fig. 5c) and recovery was 93.26%. In thermal and UV degradation conditions Pargeverine hydrochloride is stable with recovery of 99.26% & 98.46% respectively (Fig. 2d & e). Table 3 outlines the results of degradation study of Pargeverine hydrochloride at each stress condition.

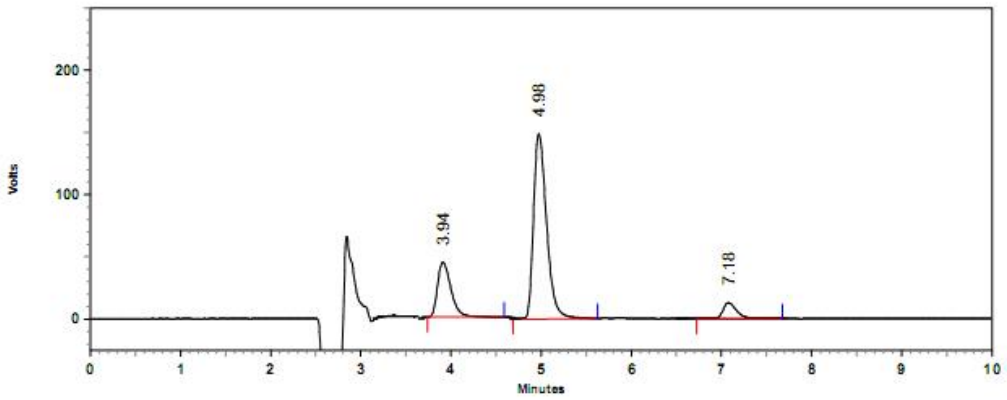

Figure 5 (a): Chromatogram of Acid Hydrolysis

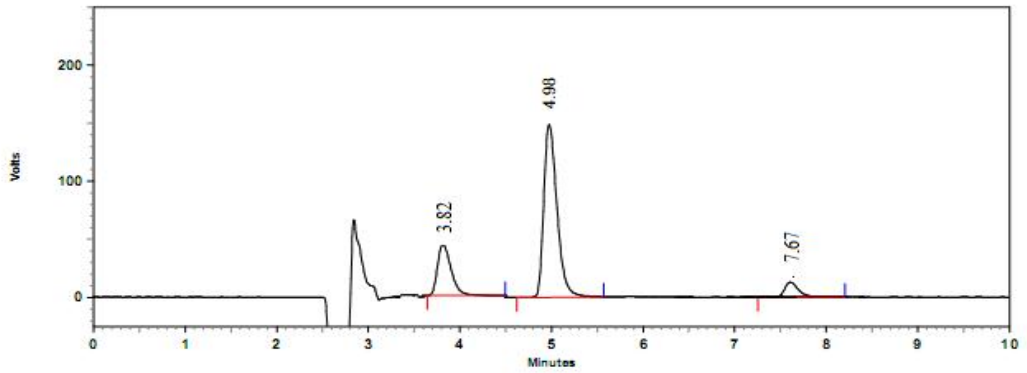

Figure 5(b): Chromatogram of Basic Hydrolysis

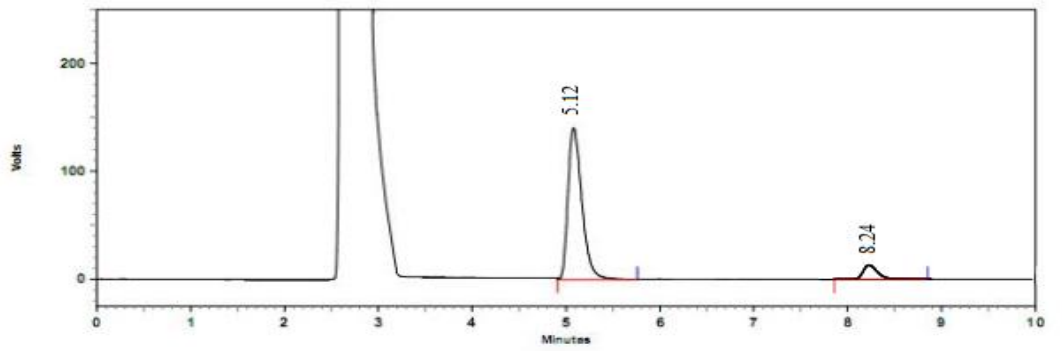

Figure 5 (c): Chromatogram of Oxidative Hydrolysis

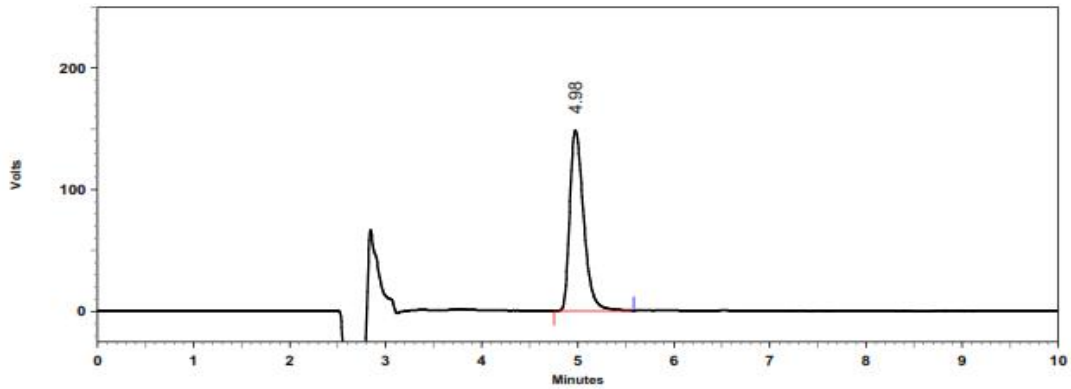

Figure 5(d): Chromatogram of Thermal Degradation

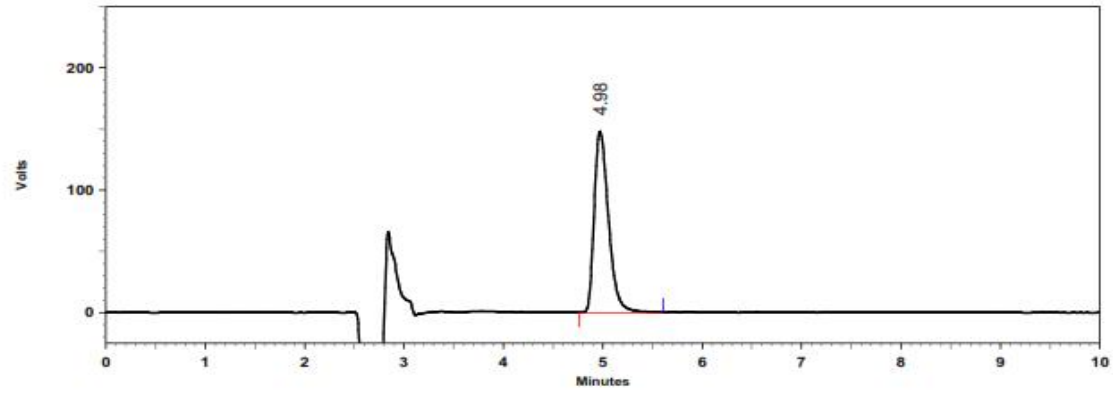

Figure 5(e): Chromatogram of Photolytic Degradation

Specificity is the ability to accurately and specifically measure the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. It is a measure of the degree of interference from other active ingredients, excipients, impurities, and degradation products. Specificity in a method ensures that a peak response is due to a single component only. In the present study, the ability of the method to separate the drug from its degradation products and the non-interference of the excipients indicate the Specificity of the method. Values of peak purity index were higher than 0.9999. These results indicated that the proposed method is specific and stability-indicating, and can be applied for stability studies and QC analysis of Pargeverine Hydrochloride in pharmaceutical products.

Table 3: Summary of Forced Degradation

Sr. no.	Condition	% Assay	% Assay after Degradation	% Degradation observed	% Mass Balance
1	1N HCl_24hr	99.79	84.42	14.26	98.89
2	1N NaOH_24hr	99.79	86.49	11.72	98.42
3	3% H_2O_2_24hr_60º C	99.79	93.26	3.87	97.33
4	80º C_24hr	99.79	99.26	-	99.26
5	Photolytic	99.79	98.46	-	98.46

The linearity of a method is defined as its ability to provide measurement results that are directly proportional to the concentration of the analyte. The linearity of the detector was obtained by diluting the analyte stock solution and measuring the associated responses, whiles the linearity of the analytical method was determined by making a series of concentrations of the analyte from independent sample preparations (weighing and spiking). The linearity data described in the

present study demonstrate an acceptable linearity for Pargeverine hydrochloride over the range of 25–150% of the target concentration. Linear correlation was obtained between peak areas and concentrations of Pargeverine hydrochloride in the range of 7.5–50 µg/ml. The following regression equation was found by plotting the peak area (y) versus the Pargeverine hydrochloride concentration (x) expressed in µg/ml y = 83904x – 60920. The correlation coefficient R^2 = 0.999 obtained for the regression line demonstrates the excellent relationship between peak area and concentration of Pargeverine hydrochloride. Data of regression analysis are summarized in Table 4.

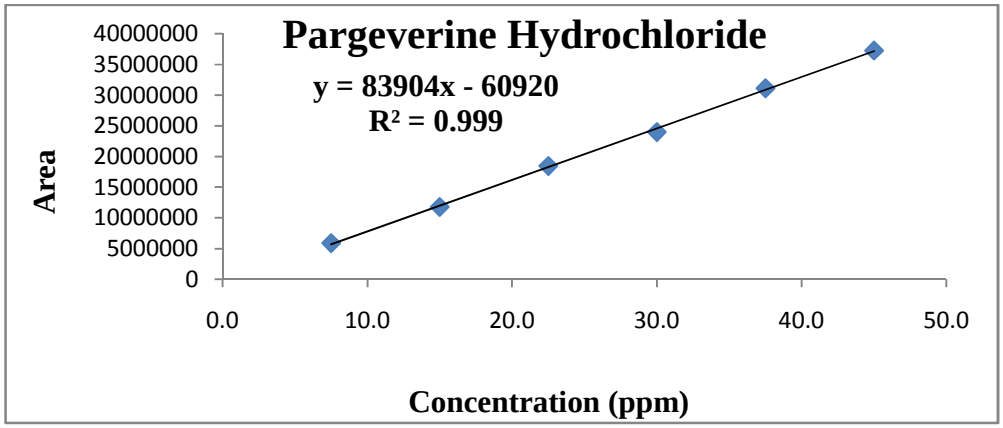

Figure 6: Calibration curve of Pargeverine Hydrochloride

Table 4: Regression analysis of calibration curve for Pargeverine Hydrochloride for the proposed HPLC

Parameter	Pargeverine Hydrochloride
Concentration range (µg/ml)	7.5-45 µg/ml
Slope	83904
Intercept	60920
Correlation coefficient R^2	0.999

The precision, evaluated as the repeatability of the method, was studied by calculating the RSD for six determinations of the 30 µg/ml sample of Pargeverine Hydrochloride performed on the same day and under the same experimental conditions. The obtained RSD value was 0.27%. The RSD values for repeatability study was found to be <1%, which indicate that the proposed method is repeatable. The intermediate precision was assessed by RSD for six determination of 30 µg/ml analyze by different analyst in different equipment. The obtained RSD was 0.82 and

0.97 for area and RT respectively. The RSD values for intermediate precision was found to be <2%, which indicate that the proposed methods are reproducible.

The accuracy was assessed by the standard addition method for three replicate determinations of three different solutions containing 50%, 100%, and 150% of target concentration of Pargeverine hydrochloride. The recoveries were obtained in a range of 101.22-103.31% for Pargeverine hydrochloride using the proposed HPLC method. (Table 5) The high values indicate that the proposed HPLC method is accurate.

The results and the experimental range of the selected variables evaluated in the robustness assessment are given in Table 5, together with the optimized values. There were no significant changes in the chromatographic pattern when the modifications were made in the experimental conditions, thus showing the method to be robust.

Table 5: Summary of validation Parameter

SR. NO.	PARAMETERS	RESULT
1	**Linearity & Range (n=6)**	
	Linearity & Range	7.5 – 45µg/ml
	Regression Equation	y = 83904x - 60920
	Regression coefficient	R^2 = 0.999
2	**Accuracy**	**%Recovery**
	50%	101.22
	50%	103.29
	150%	103.31
3	**Precision**	
	Repeatability	%RSD = 0.27
	Intermediate Precision	%RSD of Standard = 0.82
		%RSD of Test = 0.62
4	**Robustness**	
	(A) Change in Flow Rate	
	1. Flow Rate 0.9ml/minute	%RSD of Standard = 0.33
		%RSD of Test = 1.50

	2. Flow Rate 1.1ml/minute	%RSD of Standard = 1.62 %RSD of Test = 0.50
	(B) Change in pH	%RSD of Standard = 0.38
	1. pH 4.1	%RSD of Test = 0.79
	2. pH 5.1	%RSD of Standard = 1.37 %RSD of Test = 1.06

CONCLUSION

All the parameters and results were found within the acceptance limit. Hence it could be conclude that the developed stability indicating RP-HPLC method was simple, selective, rapid, specific, sensitive, linear, accurate, precise and robust. Therefore the method is found to be specific for the quantitative estimation of Pargeverine Hydrochloride. It can be applied for the stability samples. So the proposed method can be used in pharmaceutical analysis for stability monitoring and routine quality control sample.

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REFERENCES

1. ICH, Q2B, Validation of Analytical Procedures: Text and Methodology, International Conference on Harmonization, (2005), IFPMA, Geneva, Switzerland.
2. USP-35/NF28 2015, volume II, Asian Ed., United state Pharmacopoeial Convention, Inc., Rockville, MD, Page no. - 3686, 3671, 4283.
3. Kim Huynh-Ba Handbook of Stability Testing in Pharmaceutical Development, pp: 144.
4. Singh R, Rehman Z. —Current trends in forced degradation study for pharmaceutical product development 2012; 3 (1).
5. Blessy M, Patel R, Prajapati P, Agrawal Y. —Development of forced degradation and stability indicating studies of drugs — A review, J Pharm Anal 2014, 4(3), 159–65.
6. Brümmer AH, Manager CS, Life SGS, Services S. —How to approach a forced degradation study, 2011, (January)

7. George N, —Forced Degradation as an Integral Part of HPLC Stability Indicating Method Development, Drug Delivery Technology, 2010, 10, 1
8. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, —Stability testing of new drug substances and products Q1A (R2) 6 February 2003
9. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, —Stability testing: Photo stability testing of new drug substances and products Q1B 6 November 1996
10. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, — Stability testing for new dosage forms Q1C, November 1996
11. Patel R., Patel P. and Patel N., —Stability indicating HPLC Method – A Review International Research Journal of Pharmacy, 2011, 5, 79-87
12. Pargeverine-Drugs.com, September 2016, https://www.drugs.com/international /pargeverine.html
13. Chemical Book, Pargeverine Hydrochloride, September 2016, http://www.chemicalbook.com/ProductChemicalPropertiesCB7764927_EN.htm
14. Pubchem, Pargeverine Hydrochloride, September 2016, https://pubchem.ncbi.nlm.nih.gov/compound/53398649
15. SAAPedia , Pargeverine Hydrochloride, September 2016, http://www.saapedia.org/en/saa/?type=detail&id=9933
16. Safety Sheet for Pargeverine Hydrochloride, October 2016, http://www.seqchem.com/safetysheet.php?SQIndex=SRP01076p
17. Chembel, Pka pargeveriene, October 2016, https://www.ebi.ac.uk/chembldb/compound/inspect/CHEMBL1872101
18. Step-by-step Analytical Methods Validation and protocol in Quality System Compliance Industry, by G. A. Shabir, J, Chromatogra A., Vol. 987, 2003,57.