International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.5, pp 1490-1500, Sept-Oct 2014

Force Degradation Study of Berberine Chloride by Using Stability Indicating HPLC Method

P S Tarte*, G R Shedharkar

Nanded Pharmacy College, Shyam Nagar, Kasturba Matru Svea Sangh, Shyam Nagar, Nanded-431605, Maharashtra, India

***Corres.author: pawantarte@rediffmail.com**
Contact No. 91-9373429270

Abstract: Berberine chloride was subjected to degradation under different stress conditions prescribed by International Conference on Harmonization. The samples so obtained were subjected to develop a stability-indicating high performance liquid chromatographic (HPLC) method for the berberine chloride. The drug was well separated from degradation products using a reversed-phase (Hypersil C18 column) column and a mobile phase comprising of acetronitrile: phosphate buffer 0.05 M (pH3) in ratio 25:75 v/v. The Peak purity index is greater than the single point threshold in all the control and degradation samples to the peak of BER.
The detection limit and quantification limit were found to be 0.212 ppm and 0.642 ppm, respectively. The method was validated for precision, robustness, linearity and range. Results obtained after validation study, indicating that the proposed single method allowed analysis of berberine chloride, in the presence of their degradation products formed under a variety of stress conditions. This method is helpful to industry for stability study.
Keywords: Berberine hydrochloride, HPLC, Validation, Stability, Degradation.

Introduction

Berberine chloride (BER) is a type of isoquinoline alkaloid, with a variety of biological and pharmacological actions, for example antidiabetic activity [1], antitumor properties [2], bacteriocidal property [3], as well as antiatherosclerotic activity [4] and anti-inflammatory effects. Berberine hydrochloride is a therapy for many diseases either alone or in combination with other phytoconstituents. So far significant methods have been available for the analysis of BER, such as HPLC [5-6], capillary electrophoresis [7], resonance Rayleigh scattering, spectrofluorimetry, electrochemical analysis, and chemiluminescence. [8]

Though the different analytical methods available for the analysis of BER, the very few methods are stability indicating methods were documented for its determination. Considering the need for simple, accurate, economical and precise stability indicating analytical method for BER, the reverse phase HPLC method was developed for the simultaneous determination of BER and its degradation product.

Materials and methods

Chemicals and reagents

Berberine hydrochloride was supplied by J. L. Chaturvedi College of Pharmacy, Nagpur. All the chemicals used for the study were of AR and HPLC grade. Millipore membrane filters (0.45µ) were used for filtration of mobile phase and working solutions. Double distilled and membrane filtered water was used throughout the experimental work.

HPLC instrumentation and conditions

HPLC Shimadzu (LC-2010) equipped with pump (LC-30AD) and PDA detector (SPD-M20A). Each chromatogram was analyzed with LC Solutions software. Separations were achieved by using Hypersil C18 column (150 mm × 4.6 mm, 5 µm). The mobile phase consisted of acetronitrile: phosphate buffer 0.05 M (pH3) in ratio 25:75 v/v was filtered through a 0.45 µm membrane filter to degas and pumped from the respective solvent reservoirs to the column at a flow rate of 1 ml/min.

Forced Degradation of BER [9-16]

Acid degradation:

Accurately weigh quantity of BER was transferred to 100 ml of volumetric flask to it 20 ml of 1 M hydrochloric acid solution was added. The resulting solution was refluxed for 5 h at 80°C. After cooling at room temperature, acid was neutralized with 1M sodium hydroxide solution then diluted with mobile phase to obtain a final concentration of 100µg/ml.

Base degradation

Condition 1: Accurately weigh quantity of BER was dissolved in 5 ml of diluent, to it 20 ml of 1 M sodium hydroxide solution was added and mixture was refluxed for 30 min.

Condition 2: Accurately weigh quantity of BER was dissolved in 5 ml of diluent to it 20 ml of 1 M sodium hydroxide solution was added and resulting solution was refluxed for 3 h.

Allow the solutions to cool at room temperature and neutralized them with 1M hydrochloric acid and then diluted with mobile phase to obtain a final concentration of 100µg/ml.

Hydrogen peroxide degradation:

Accurately weigh quantity of BER was subjected to peroxide degradation (10 ml, 30%) for 1 h at 80°C. After cooling at room temperature, mixture was diluted with mobile phase to obtain a concentration of 100µg/ml.

Dry heat degradation:

To execute dry heat degradation, 10 mg of drug was kept in oven at 105ºC for 12 h, allowed it to cool at room temperature and diluted with mobile phase to obtained concentration of 100µg/ml.

Photolytic degradation:

Photolytic degradation was performed under two different conditions 1) at 254 nm and 2) at 365 nm. For both the conditions 10 mg of BER was transfer to 100 ml of volumetric flask and diluted with mobile phase to a mark. Both the solutions were then exposed to above mentioned conditions for 24 h.

Water hydrolysis

Accurately weigh 10 mg of BER was refluxed with 20 ml of water at 80ºC for 4 h. Resulting solution was cool and diluted with mobile phase to obtain a concentration of 100µg/ml.

Development of HPLC method:

Each and every preparation was analysed by HPLC method. The separations were achieved by using, acetronitrile: phosphate buffer 0.05 M (pH 3 with ortho- phosphoric acid) in ratio 25:75 v/v as the mobile phase. It was filtered through 0.45 µ membrane filter and degassed before use. The working concentration 100 µg/ml was used for all degradation samples. The elution was carried out at the flow rate of 1ml /min. Detection was carried out at 271 nm at ambient temperature. A typical chromatogram of Berberine chloride was shown in (Fig. 1).

System suitability was determined by injecting the standard solution five times. System suitability parameters i.e. peak area, tailing factor, retention time (Rt) and theoretical plate fall within 2% RSD during routine performance of the method. The data obtained is summarized in Table 1.

Table 1: System suitability parameters of BER

Injection	Retention time (min)	Tailing	No. of theoretical plate	Peak area
1	10.504	1.62	6746	2007044
2	10.499	1.63	6842	1996623
3	10.488	1.63	6889	2011375
4	10.488	1.63	6905	2012644
5	10.488	1.63	7008	2008144
Mean	10.493	1.627	6878.00	2007166
Std. Dev.	0.01	0.0045	95.4856	6321.72
%RSD	0.07	0.2747	1.3883	0.315

Validation Parameters [17-18]

Linearity

The linearity of analytical method was established by injecting 20 to 140 µg/mL of BER and peaks were recorded and plotted graph, concentration versus area response of BER. The range of analytical method was established with a suitable level of precision, accuracy and linearity. Results are shown in Table 2.

Table 2: Linearity study of BER

Sr. No.	Concentration (µg/ml)	Peak area*	Slop (m)	'r'
1	20	305108	15427.3	0.9994
2	40	621307		
3	60	916621		
4	80	1200762		
5	100	1492508		
6	120	1840895		
7	140	2179853		

Precision

Precision of analytical method was ascertained by replicate estimation of same concentrations of drug for five times. The results are expressed as SD and % RSD of series of measurements. Results of precision study are shown in Table 3.

Table 3: Results of precision study of BER

Injection	Retention time (min)	Peak area	Tailing	Plates count
1	10.504	2007044	1.62	6746

2	10.499	1996623	1.63	6842
3	10.488	2011375	1.63	6889
4	10.488	2012644	1.63	6905
5	10.488	2008144	1.62	7008
Mean	10.494	2007166	1.63	6878.00
SD±	0.01	6321.72	0.01	95.49
%RSD	0.07	0.315	0.34	1.39

LOQ and LOD

LOQ and LOD values were determined by preparing the BER solutions of different concentrations (i.e. 0.3, 0.6, 0.9 1.2 and 1.5 ppm) and peak response of the prepared solutions was determined by applying same chromatographic conditions as mention above. Graph was plotted concentration versus area response of BER solutions. Results of LOD and LOQ analysis are shown in Table 4.

Table 4: LOD and LOQ study results of BER

Sr. No.	ppm	Area* response	Slop	r ‘	Styex	LOD	LOQ
1	0.3	3391					
2	0.6	6838					
3	0.9	13381	17241	0.993	1106.36	0.212	0.642
4	1.2	17014					
5	1.5	24165					

*results are the mean of 3 replicates

Robustness

The robustness of the method is determined by changing the flow rate (1.0 mL/min ± 0.2 ml/min) of the method. Robustness of the method was studied using five replicates at a concentration level of 100 µg /ml and RSD value at different flow rate was determined. Results are shown in Table 5.

Table 5: Results of robustness study

Sr. No.	Flow 0.8 ml/min		Flow 1 ml/min		Flow 1.2 ml/min	
	RT	Area	RT	Area	RT	Area
1	8.340	1309780	11.869	1599865	14.313	1873978
2	8.485	1306223	11.872	1525697	14.289	1837106
3	8.524	1315603	11.843	1535555	14.342	1841179
4	8.569	1310948	11.866	1538139	14.297	1828819
5	8.539	1316098	11.896	1531689	14.316	1838394
Mean	8.532	1311731	11.869	1546189	14.311	1843895
SD	0.03	4147.88	0.02	30369	0.02	17435.46
% RSD	0.29	0.316	0.16	1.964	0.14	0.946

Result and Discussion

Most of the methods available for determination of BER are not stability indicating. The present proposed method was used for the complete separation of the analyte in presence of its impurity and the method can be successfully applicable to perform long-term and accelerate stability studies of Berberine chloride. The representative chromatogram obtained for Berberine chloride is shown in Fig. 1. The theoretical plates were 6878 ±1.39 (more than 2000) and tailing was1.63 ±0.34 (less than 2) for the Berberine chloride peak. The method permits detection and quantitative determination of BER in the presence of its degradation products with higher accuracy and precision.

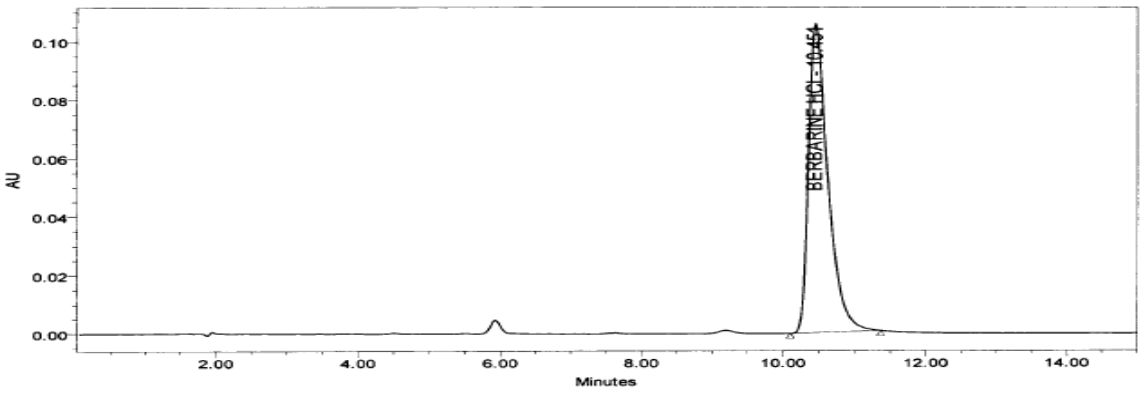


Fig 1. Chromatogram of BER

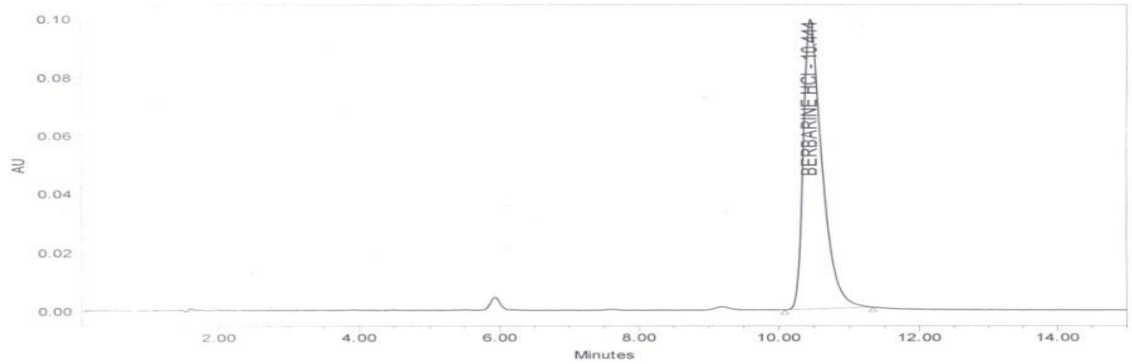


Fig 2. Chromatogram of 1 M HCl degradation at 80°C for 5 h

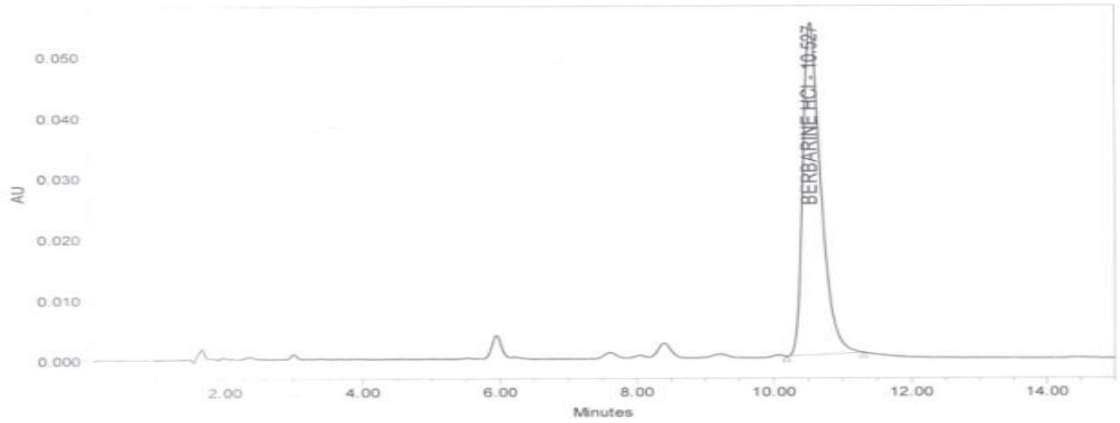


Fig 3. Chromatogram of 1 M NaOH degradation at 80°C for 30 min

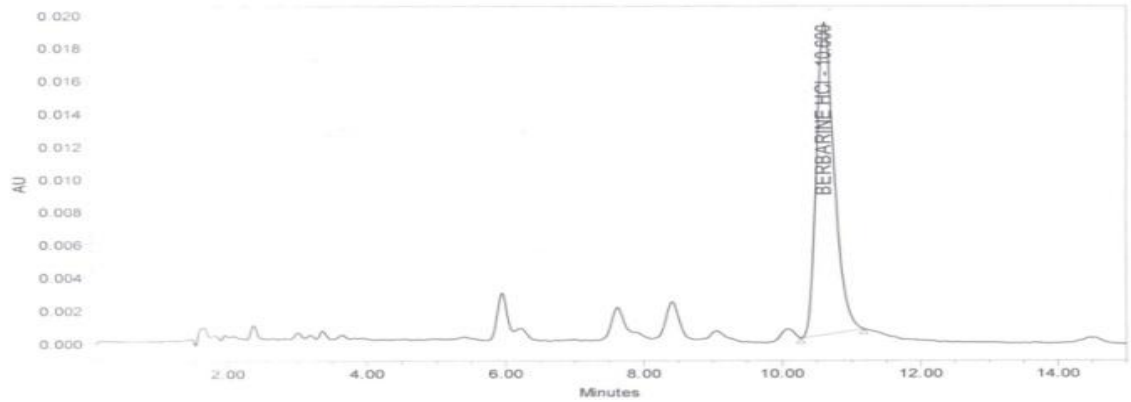


Fig 4. Chromatogram of 1 M NaOH degradation at 80°C for 3 h

As per ICH guidelines it was subjected to different stress conditions to obtained degradation in the range of 5-30%. The chromatograph of BER degradation in acidic, peroxide and basic condition showed change in peak area, so it was found to be unstable in acidic, peroxide and basic (Fig. 5-7) conditions. BER showed 6% degradation in 1M HCl for 5 h at 80℃ with change in peak area (Fig. 2). In basic solution it's found to be highly unstable with 48% degradation in 1M NaOH for 30 min at 80℃ (Fig. 3) and 83% degradation in 1M NaOH solution for 3h at 80℃ (Fig. 4). BER found to be 19 % degraded in peroxide condition (30% H2O2, at 80℃ for 1 h) (Fig. 5).

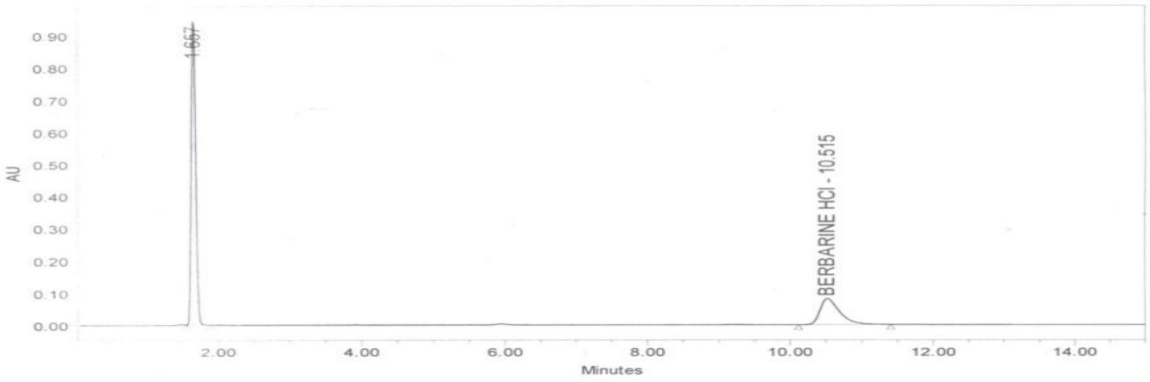


Fig 5. Chromatogram of 30% H_2O_2 degradation at 80°C for 1H

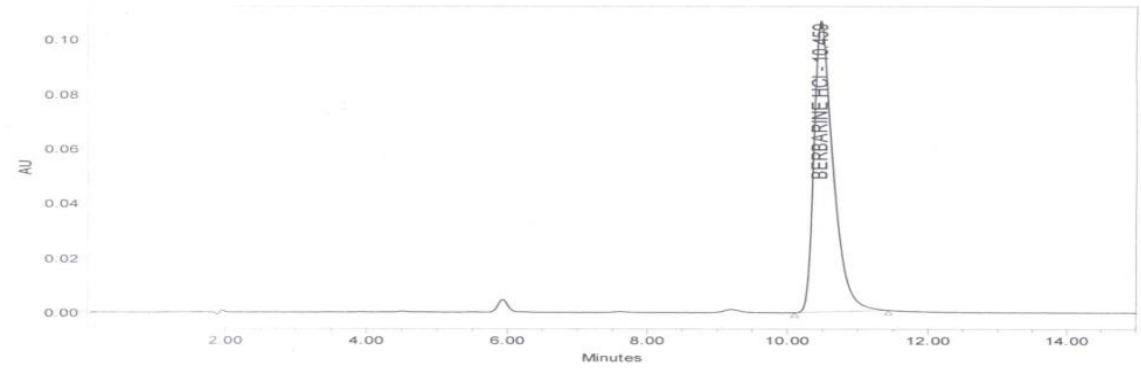


Fig 6. Chromatogram of dry heat degradation

It was found to be stable to dry heat (Fig. 6), photolytic degradation (Fig. 7-8) and water hydrolysis (Fig. 9) as there no additional peak and change in peak area.

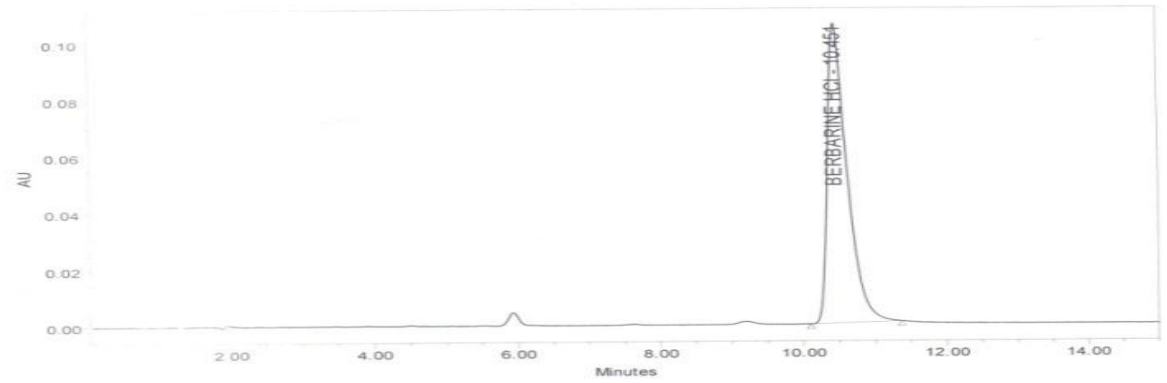


Fig 7. Chromatogram of photolytic degradation at 254nm

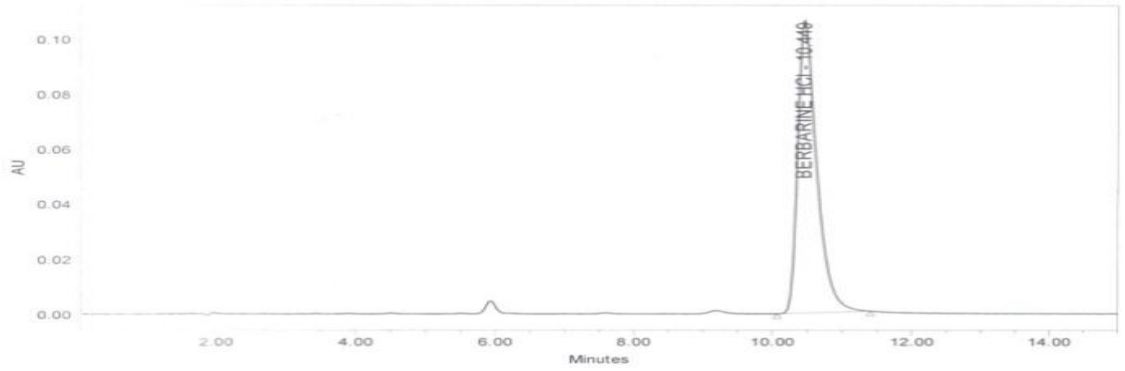


Fig 8.Chromatogram of photolytic degradation at 365nm

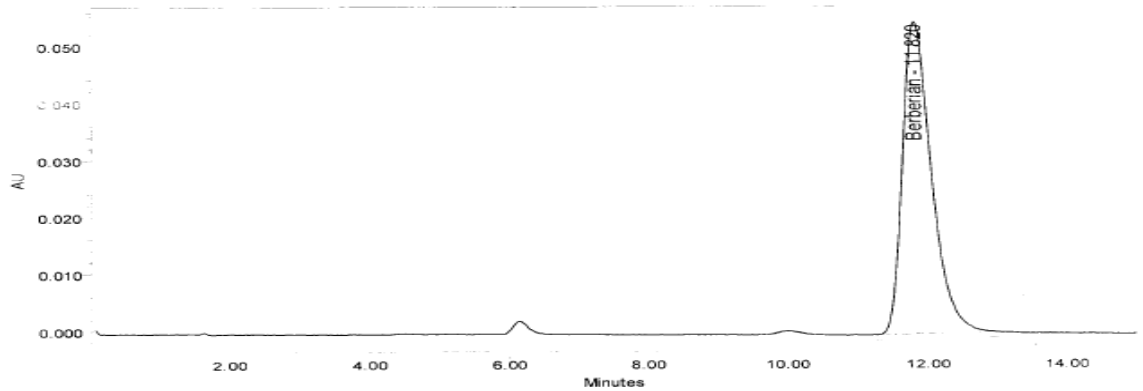
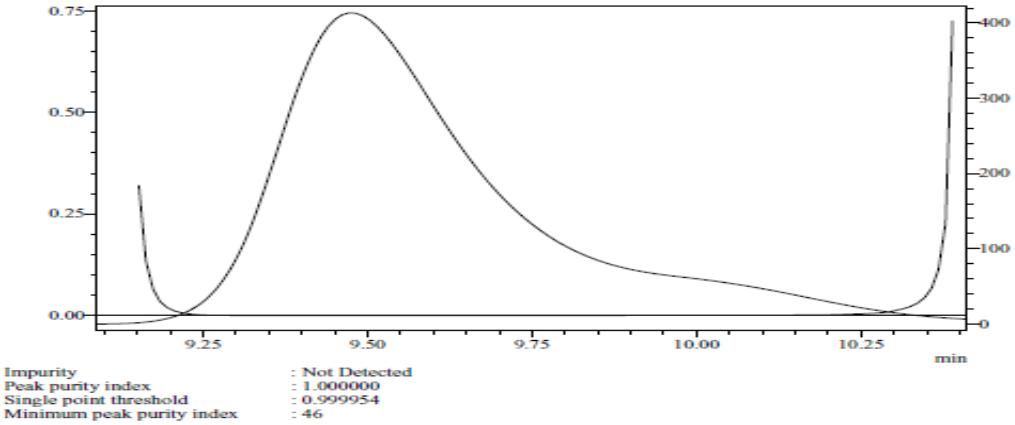


Fig 9. Chromatogram of water hydrolysis

The drug can be analyzed in the presence of different degradation products by using isocratic liquid chromatographic conditions and mobile phase containing acetronitrile: phosphate buffer 0.05 M (pH 3 with ortho- phosphoric acid) in a ratio 25:75 v/v.

During chromatographic determination blank solution (Mobile phase) should not show any interference at the retention time corresponding to the peak BER and its impurities. The blank solution of acid, base and peroxide degradation should not show any interference at the retention time corresponding to the peak of BER and its impurities. The Peak purity index is greater than the single point threshold in all the control and degradation samples to the peak of BER. (Fig. 10-16)



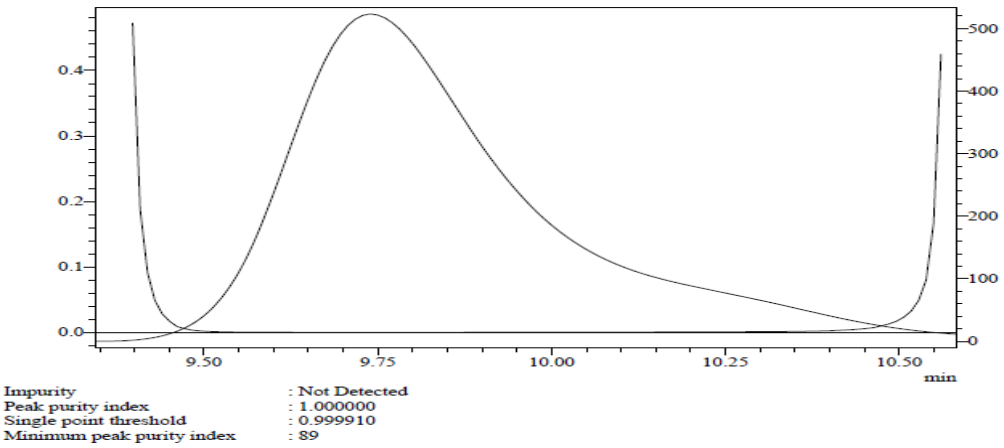


Fig 10. Peak purity chromatogram of acid degradation

Fig 11. Peak purity chromatogram of base degradation

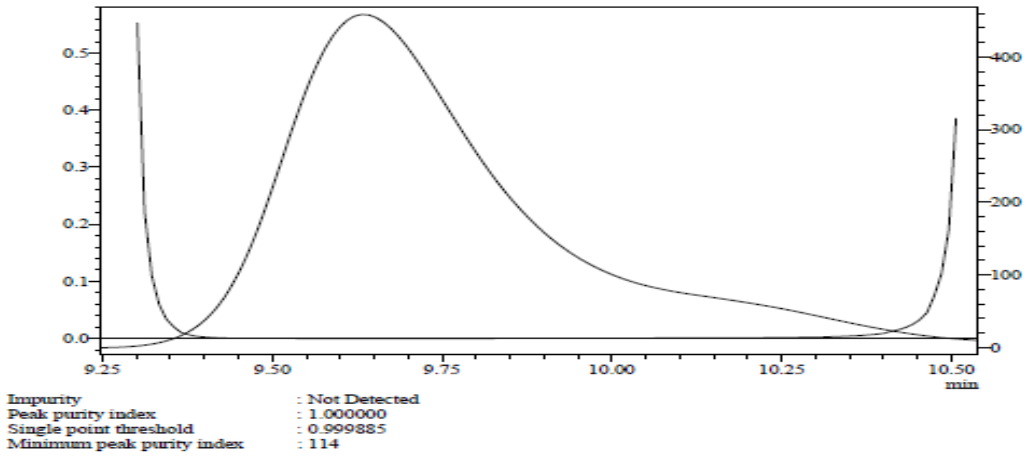


Fig 12. Peak purity chromatogram of peroxide degradation

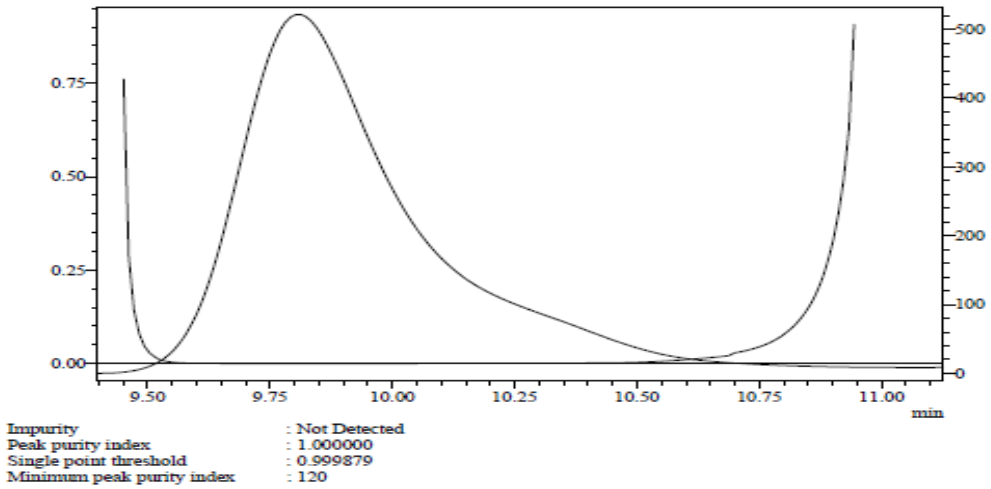


Fig 13. Peak purity chromatogram of heat degradation

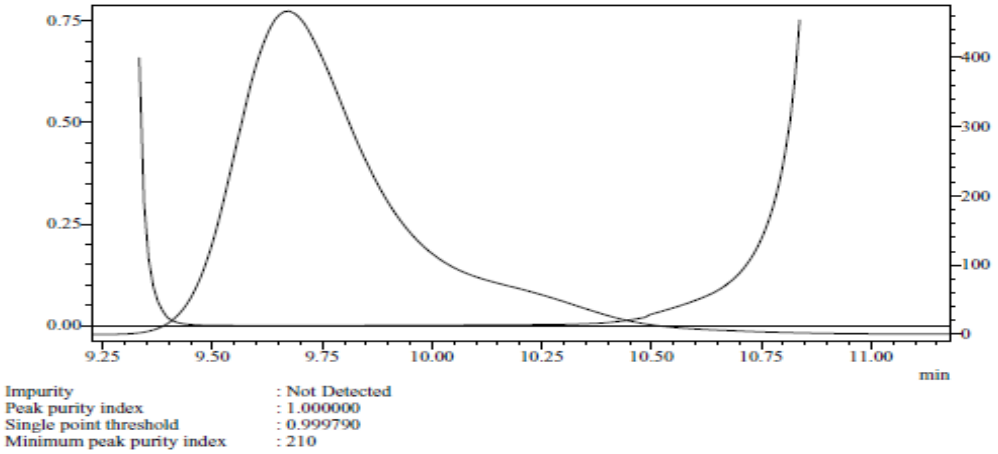


Fig 14. Peak purity chromatogram of water hydrolysis

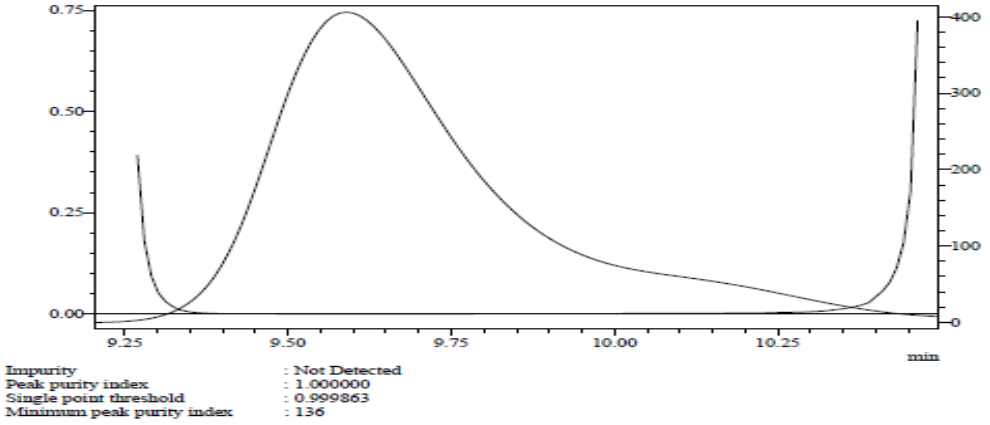


Fig 15. Peak purity chromatogram of photolytic degradation at 254 nm

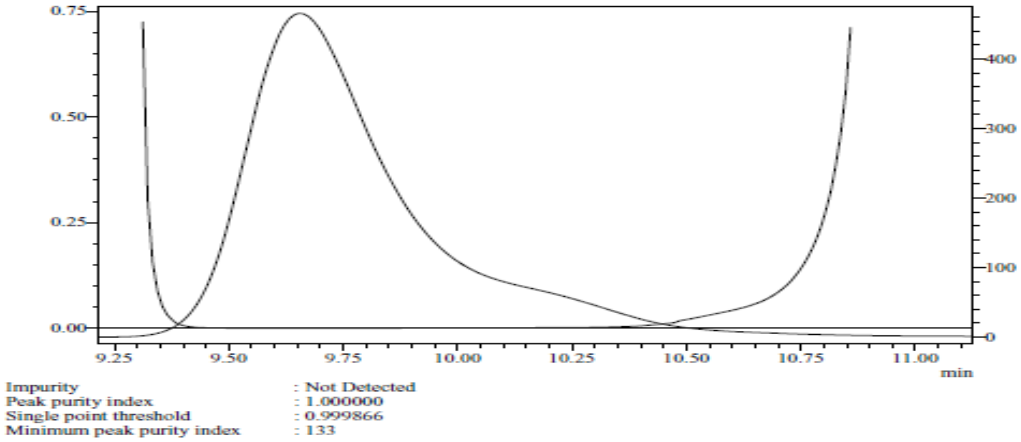


Fig 16. Peak purity chromatogram of photolytic degradation at 365 nm

Thus the developed chromatographic method does not show interference of mobile phase, solvent mixture and degradation product in chromatogram at the λ-max of the analyte. Results of degradation study are shown in Table 6.

Table 6: Stress study results of BER

Stress condition	Duration	Temp rature	Peak area (STD)	Peak area* (SPL)	% content *	±SD	% RSD	Peak purity
1M HCl	5h	80°C	2007166	1835443	94.24	0.88	0.93	Passes
1 M NaOH	30 min	80°C	2007166	988851	51.16	0.49	0.97	Passes
1 M NaOH	3 h	80°C	2007166	339002	16.73	0.14	0.89	Passes
30 % H_2O_2	1h	80°C	2007166	1534079	80.93	0.54	0.67	Passes
Dry heat	12 h	105°C	2007166	2017370	100.51	0.50	0.49	Passes
254nm	24 h	-	2007166	2005715	100.86	0.53	0.52	Passes
365 nm	24 h	-	2007166	2016432	101.40	0.46	0.45	Passes
Water hydrolysis	4 h	80°C	1473717	1561452	101.41	0.80	0.79	Passes

*results are the mean of three replicates

The method was validated for parameters like precision, linearity, LOD, LOQ, and robustness. Linearity was studied over the concentration range 20–140 µg/mL and the regression equation was found to be y= 15427x – 11748 with correlation coefficient of 0.9988, indicating that method is accurate. The LOD was found to be 0. 212 ppm and the LOQ was found to be 0.642 ppm. The % RSD in precision and robustness studies was found to be less than 2.0%, indicating that the method is precise and robust.

Conclusion

The mobile phase, solvent mixture and degradation product under same condition as that of analytical conditions of BER does not show any interference, specificity is satisfactory. Method should be linear, precise

and accurate across the suitable analytical range. The method has been robust towards deliberate minor changes in the method parameters. The method can be used in quality control laboratory for release of production batches and stability study.

References

1. Zhou JY and Zhou SW. Berberine regulates peroxisome proliferator-activated receptors and positive transcription elongation factor b expression in diabetic adipocytes. European Journal of Pharmacolog 2010; 649 (1–3): 390–97.
2. SinghT, Vaid M, Katiyar N, Sharma S, Katiyar SK. Berberine, an isoquinoline alkaloid, inhibits melanoma cancer cell migration by reducing the expressions of cyclooxygenase-2, prostaglandin E and prostaglandin E receptors. Carcinogenesis 2011; 32 (1): 86–92.
3. Lee IA, Hyun YJ, Kim DH. Berberine ameliorates TNBS-induced colitis by inhibiting lipid peroxidation, enterobacterial growth and NF-κB activation. European Journal of Pharmacology 2010; 648 (1–3): 162–70.
4. Wu M, Wang J, Liu LT. Advance of studies on anti-atherosclerosis mechanism of Berberine. Chinese Journal of Integrative Medicine2010; 16 (2): 188–92.
5. Chang YX, Qiu YQ, Du LM, Li CF, Wu H. Simultaneous determination of palmatine and berberine using cucurbit[7]uril as mobile phase additive by HPLC. Journal of Liquid Chromatography & Related Technologies 2011; 34 (20): 2629–39.
6. Wang ZG, Du Q, X. Qiu et al., "Simultaneous determination of six herbal components in intestinal perfusate by high-performance liquid chromatography," Biomedical Chromatography, vol. 23, no. 8, pp. 798–803, 2009.
7. Chen JB, Zhu HM, Chu VM et al. Quality control of a herbal medicinal preparation using high-performance liquid chromatographic and capillary electrophoretic methods. Journal of Pharmaceutical and Biomedical Analysis 2011. 55 (1): 206–10.
8. Wang JC, Zhang Q, Cai DF. Stability-Indicating Validated HPLC Method for Analysis of Berberine Hydrochloride and Trimethoprim in Pharmaceutical Dosage Form. Journal of Chemistry 2013; 2013: 1-9.
9. Bakshi M, Singh S. Development of validated stability-indicating assay methods-critical review. J Pharm Biomed Anal 2002; 28:1011–1040.
10. Xu QA, Trissel LA. Stability indicating HPLC methods for drug analysis. 3rd ed. Londan (UK): Pharmacutical Press; 1979.
11. Singh S, Bakshi M. Guidance on Conduct of Stress Tests to Determine Inherent Stability of Drugs, Pharm Tech On-Line 2000; April: 1-14.
12. Kumarn BSP, Annapurna MM, Pavani S. Development and validation of a stability indicating RP-HPLC method for the determination of Rufinamide. J Pharm Ana 2013; 3(1): 66–70.
13. Ngwa G. Forced Degradation as an Integral Part of HPLC Stability-Indicating Method Development. Drug Delivery Technology. June 2010, 10 (5): XX.
14. Shah DA, Bhatt KK, Mehta RS, Baldania SL, Gandhi TR. Stability indicating RP-HPLC estimation of nebivolol hydrochloride in pharmaceutical formulations Indian J Pharm Sci 2008; 70: 591-595.
15. Damle Dangi M, Chaudhari D, Sinker M, et al. Stability indicating HPTLC method for estimation of nebivolol hydrochloride and amlodipine besylate in combination. Eur asian J Anal Chem 2010; 5(2): 161-169.
16. Sherikar O, Mehta P. Comprehensive assessment of degradation behaviour of aspirin and atorvastatin singly and in combination by using a validated RP-HPLC method. Sci Pharm 2013; 81: 195–210.
17. ICH Q1A (R2). Stability Testing of New Drug Substances and Products. Geneva: International Conference on Harmonization; 2003.
18. ICH Q2 (R1). Validation of Analytical Procedures: Text and Methodology. Geneva: International Conference on Harmonization; 2005.

*****