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Research Article

STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTIFICATION OF ISOPROTERENOL HCL IN BULK AND ITS FORMULATION BY RP-HPLC USING PDA DETECTION

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ABSTRACT

The objective of the present study was to develop an accurate, precise stability indicating reverse phase liquid chromatographic method to quantify Isoproterenol HCl in bulk and its formulation using PDA detection. The chromatographic separation of the analyte from the degradants was achieved on Phenomenex Luna column with mobile phase composition of Methanol and 0.1% Triethyl amine [pH 7.0], [20:80% v/v] at flow rate of 1.0 ml/min. The analyte separation was monitored using PDA detection at 279nm. The method was linear in the concentration range of 10-60 µg/ml. The method shown acceptable percent relative deviations for the Inter-day (0.58%) and Intra-day (0.65%) precisions. The mean percent recovery for accuracy study was within the limit. From the stability studies method has proven specificity to quantify Isoproterenol HCl in presence of its degradation products. The method shown acceptance as according to ICH guidelines.

Keywords: Stability indicating, Isoproterenol HCl, RP-HPLC, ICH guidelines.

INTRODUCTION

Isoproterenol HCl (IPN)[1] chemically named as 3,4-dihydroxy-α-[(isopropylamino)methyl] benzyl alcohol hydrochloride (Figure 1). It is non-selective β-adrenergic agonist and trace-amine associated receptor 1 (TAAR1) agonist.[2] It is mostly used to treat bradycardia, heart block, chronic obstructive pulmonary diseases and rare in asthma, and as an active bronchodilator.[3-5]

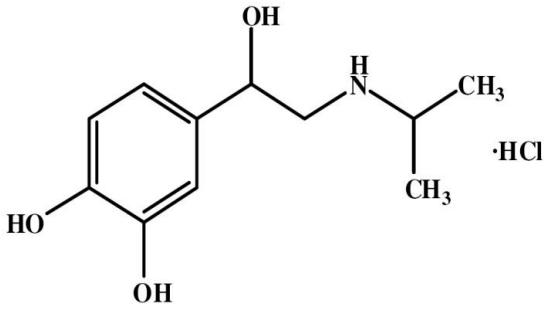

Fig 1: Chemical structure of Isoproterenol HCl (IPN)

The literature review on availability of analytical methods to identify isoproterenol hydrochloride and its metabolites in biological samples such as high performance liquid chromatography, liquid chromatography–mass spectrometry (LC–MS)[6,7] gas-liquid chromatography[8] and poly vinylchloride membrane selective electrode[9], selective separation using bismuth silicate ion-exchanger[10], chemiluminescence determination using luminol-diperiodatoargentate(III)[11] and characterization of new process related impurity using HPLC, LC/ESI-MS, NMR.[12] A sensitive spectrophotometric method recently has been developed for the determination of isopropyl amine, a core moiety of isoproterenol hydrochloride, at the trace level in pharmaceutical drug substances.[13]

The reported methods so far was done in biological samples, there is no information on method development of IPN in bulk and for its formulation, and also no degradation data such as % degradation. In the purview of above discussions and based on non-availability of reliable and sensitive stability indicating RP-HPLC method for IPN the present attempt was made to develop stability indicating assay method by reversed phase HPLC method using PDA detection and to validate as required by International council on harmonization ICH guidelines[14-16]. Thus the developed method could be more appropriate in the quantification of IPN in presence of all possible degradation products during stability testing protocol.

MATERIALS AND METHODS

Standard drug of IPN (99.99 %) was kindly provided by Mylan laboratories, Bangalore, Karnataka, India. HPLC grade solvents were procured from Merck Pvt Ltd, India, and Triethyl amine, Orthophosphoric acid was obtained from Qualigens, Mumbai, India. All glass wares used were calibrated for class A type. Shimadzu LC 20 AD system equipped with photodiode array detector (PDA), LC solutions software, 20µL rheodyne manual injector was used in this study. Chemicals were weighed using calibrated Axis LC GC balance. pH measurements were performed on Elico Digital pH meter LI 120.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

The optimum condition for chromatographic analysis of IPN and its degradation products were performed on a reversed-phase LC Phenomenex Luna C18 (250 ×4.6 mm, 5µ particle size) column. The mobile phase consisting methanol and 0.1% triethyl amine buffer (pH adjusted to 7.0 with OPA) at the ratio of 20:80 % v/v was pumped at 1.0 ml/min flow rate. The contents of the mobile phase and all samples were filtered through 0.45 µ membrane filter and degassed before analysis. The eluents were monitored at 279 nm.

PREPARATION OF CALIBRATION STANDARDS OF IPN

10 mg of IPN was accurately weighed and dissolved in 10 ml of HPLC grade methanol to get 1000 µg/ml. From this stock, stock solution working standards ranging from 10 – 60 µg/ml were prepared in the mobile phase and injected in triplicate. The concentration (x-axis) versus mean peak area (Y-axis) served as a calibration curve for quantification of IPN.

PREPARATION OF SAMPLE SOLUTION (ASSAY)

A quantity equivalent to 10 mg of IPN was drawn into a 10 ml volumetric flask. A small quantity of methanol was added to dissolve and sonicate for 5 min. The final volume was made up to the mark with the same solvent and filtered through 0.45 µ filter to give 1000 µg/ml of IPN. From the above solution various aliquots were further prepared with mobile phase and analyzed. The results are shown in Table 3.

PREPARATION OF STOCK SOLUTION FOR STRESS STUDIES

Weighed a quantity of 10 mg of IPN was carefully transferred into a 10 ml volumetric flask, dissolved completely in water (for neutral degradation studies) and the volume was made up to mark to get 1000 µg/ml. The same procedure was used to prepare stock solutions for stress studies viz for acid hydrolysis, base hydrolysis, and oxidation respectively with HCl (0.1M), NaOH (0.1M) and hydrogen peroxide (3% v/v). Thermal degradation was carried out for solid State IPN by heating the samples over a period in a hot air oven, at 70 ^{0}C. Photo-degradation was carried on solid Sample by exposing to natural sunlight. Blank was analyzed under the same condition to assess the method specificity.

RESULTS AND DISCUSSION

In order to achieve good separation various experimental trials were carried out for the optimization of chromatographic conditions for the analysis of IPN. Different buffer pH conditions and different proportions of HPLC grade solvents like methanol, acetonitrile and water were tried. However, methanol and 0.1% triethyl amine buffer (pH adjusted to 7.0 with OPA) at the ratio of 20:80 % v/v achieved good satisfactory results at a flow rate of 1.0 ml/min measured at 279 nm as detection wavelength. The optimized chromatogram was depicted in fig 2. The suitability of the method was proven from six replicate injections of IPN. The percent RSD for all the parameters were within 2% and shown in table 1. The result shows the applicability of this method for the routine analysis.

Table 1: System suitability parameters for the developed method (IPN)

Parameters	Results
Chromatographic Column	Phenomenex Luna C18 (250 x 4.6 mm i.d, 5µm)
Mobile phase	Methanol and water (0.1% TEA) (20:80% v/v)
Flow rate	1.0 ml/min
Detection wavelength	279 nm
Retention time (Rt)*	3.3 ± 0.1 min
Tailing factor*	1.62 ± 0.01
Theoretical plates*	> 4000
Repeatability (% RSD)*	0.61

* Number of Injections (n=6)

Linearity was performed over the concentration range of 10 – 60 µg/ml (25 to 150 % of range of analyte). The regression line was obtained from the calibration curve using least squares procedure. The correlation coefficient (r^2) was 0.999. The results were presented in table 4.

Precision studies were assessed from triplicate standard samples in terms of repeatability, reproducibility and intermediate precision. The percent RSD values were shown in table 2.

Table 2: Results of Precision studies

Parameter	Repeatability		Reproducibility		Intermediate Precision	
	Retention time (%RSD)	Peak Area	Retention time (%RSD)	Peak Area	Retention time (%RSD)	Peak Area
Result	0.61	0.68	0.83	0.65	0.74	0.58

Accuracy studies were carried in triplicate at three concentration levels of LOQ (50%, 100% and 150%). The percent mean recovery was summarized in table 3.

Table 3: Accuracy results

Spiked Concentration level (%)	Amount added (µg/ml)	Amount Found (µg/ml)	Recovery (%)	Mean Recovery (%)
50	20.01	19.98	99.87	100.92
		20.38	101.86	
		20.22	101.03	
100	40.02	40.60	101.44	100.02
		39.98	99.90	
		39.51	98.73	
150	60.03	60.08	100.07	99.26
		59.21	98.63	
		59.48	99.08	

The limits of detection (LOD) and quantitation (LOQ) was determined with respect to 1 mg/ml of IPN. The LOD and LOQ values were found to be 1.97 and 5.99 µg/ml respectively.

Robustness of the method was evaluated by analyzing the sample for a slight variation in optimized parameters. The method was found to be robust from the results obtained as in table 4. The summary of all validation parameters was shown in the below table 4.

Table 4: Validation parameters for the developed method (IPN)

Parameters	Results
Specificity	3.3 ± 0.1 min
Linearity	10 -60 µg/ml (r^2 : 0.999)
Repeatability (% RSD)	0.68
Precision	
a. Intraday	0.65
b. Inter-day	0.58
Accuracy by % Recovery	
50 % level	100.92 (% RSD: 0.45)
100 % level	100.02 (% RSD: 0.75)
150 % level	99.26 (% RSD: 0.59)
LOD	1.97
LOQ	5.99
Assay	100.84
Robustness	
Flow rate (± 0.1 ml/min)	0.79 (% RSD)
Temperature (ºC)	1.52 (% RSD)
Wavelength (nm)	1.28 (% RSD)

The ability to measure the analyte in presence of degradants was well determined from the specificity studies. The specificity of the developed liquid chromatographic method was carried at concentration of 100% by subjecting IPN to acid (0.1M HCl), alkaline (0.1M NaOH), oxidative (3.0% v/v peroxide), thermal (70ºC) and Photo light. Peak purity was assessed for IPN by PDA detection for all stress samples. All the chromatograms were shown in fig 2, 3 and assay for the stress samples was performed and compared with reference standard and results were shown in table 5.

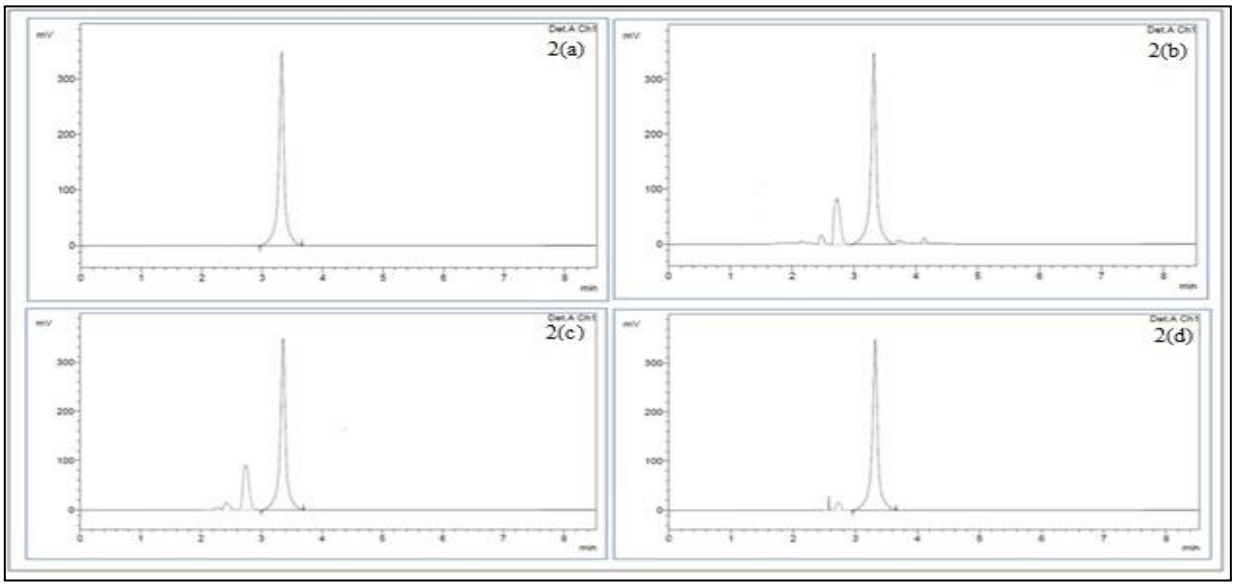

Figure 2(a): Optimized chromatogram of Isoproterenol HCl on C18 column (IPN)
Figure 2(b): Chromatogram of acid degradation in 0.1M HCl
Figure 2(c): Chromatogram of alkaline degradation in 0.1M NaOH
Figure 2(d): Chromatogram of oxidative degradation in 3% v/v H_2O_2

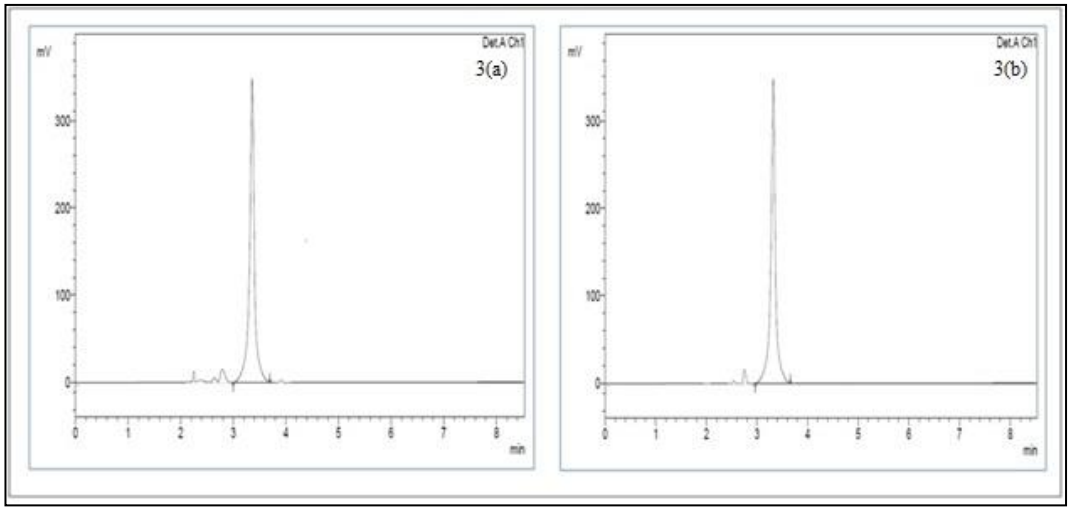

Figure 3(a): Chromatogram of photolytic degradation
Figure 3(b): Chromatogram of thermal degradation

Table 5: Stress degradation Profile of IPN

Stress Conditions	Duration	% Assay	% Degradation	Mass balance
0.1M HCl	5 days	95.12	4.88	99.82
0.1M NaOH	30 h	96.09	3.91	98.95
3.0% H_2O_2	2 h	97.12	3.88	99.45
Photolytic	30 min	94.48	5.52	99.78
Thermal (70 ºC)	6 h	95.45	4.55	99.86

CONCLUSION

In this study, a validated simple, specific and reliable isocratic RP-HPLC method with PDA detection was developed for the estimation of Isoproterenol HCl in bulk and its pharmaceutical formulation. The compound was subjected to forced degradation applying several stress conditions. The proposed method successfully separated the degradants, estimate the active content. The proposed method is specific and stability-indicating power. The developed method herein was found to be in good agreement and can be adapted to regular quality control analysis.

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