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Research Article

DEVELOPMENT AND VALIDATION OF A REVERSE-PHASE LIQUID CHROMATOGRAPHIC METHOD FOR ASSAY AND RELATED SUBSTANCES OF HALOPERIDOL FOR 50MG/ML AND 100MG/ML

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ABSTRACT

Objective: The main objective of current study is to develop and validate RP-HPLC, simple, precise, accurate and specific chromatographic method for the determination of Related Impurities of Haloperidol in pharmaceutical formulations.

Methods: A High Performance Liquid Chromatograph instrument and BEH YMC -pack ODS-A 150 X 4.6 mm, 3µ were used for determination of Haloperidol and its related impurities. Mobile phase-A-2.5% Tetrabutylammonium hydrogen sulphate, Mobile phase-B Acetonitrile and Mobile phase-C Isopropyl alcohol .The flow rate of 1.0 mL/min was set with gradient program, the temperature of column compartment maintained at 40°C and Ultra violet detection done at 230nm wavelength. The Haloperidol and its related impurities(IMP-L,H,I,B and D) peaks eluted at 37.049, 5.320, 14.820, 23.008, 34.052 and 72.471 minutes and then run time was set as about 92minutes.

Results: The correlation coefficient (≥ 0.999) shows the linearity of response against concentration over the range of LOQ to 300%. The observed result shows that the method is rapid, precise, accurate and simple. The method was validated as per ICH guidelines.

Conclusion: The developed and validated High performance liquid chromatographic method is suitable for determination of Haloperidol and its related impurities in pharmaceutical formulations which is more useful with respect to regular Laboratory analysis.

Keywords: Haloperidol, Related substances, RP-HPLC, Validation

INTRODUCTION

Haloperidol (HAR) is a tertiary amine that occurs as a white or almost white powder, is practically insoluble in water, and is slightly soluble in alcohol, methanol and methylene chloride [1].

The IUPAC name of Haloperidol is 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-4 piperidinyl decanoate with empirical formula $C_{31}H_{41}ClFNO_3$.The Chemical structure of Haloperidol shown in Figure-1. It is a dopamine inverse agonist of the typical antipsychotic class of medications. It is a butyrophenone derivative and has pharmacological effects similar to the phenothiazines. The dissolving nature of Haloperidol tablets increase using Camphor as a subliming agent [2]. Haloperidol shown that neuroleptic drugs tend to decrease the permeability of a variety of biological membranes for various organic and inorganic molecules, including water, and that they exert this effect in minute concentration[3].Literature survey revealed that there was HPLC methods have been reported for the estimation of haloperidol and trihexyphenidyl individually and simultaneously in pharmaceutical dosage forms[4,5,6,7] The present work describes a simple, gradient RP-HPLC method for the determination of Haloperidol and its related substances in injection form as per ICH guidelines[8,9,10].

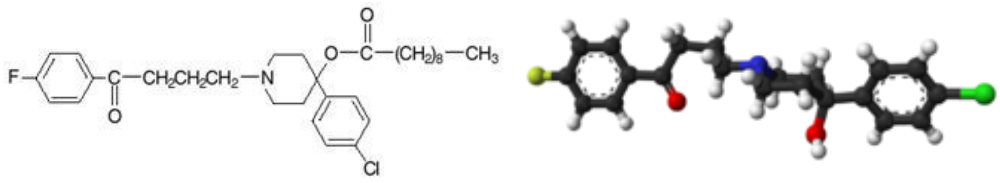

Fig. 1: Structure of Haloperidol

MATERIALS AND METHODS

Chemicals

Qualified standards for drug substance and impurities were obtained from Bio Leo Analytical Laboratory and were used without any further purification. The chemicals like Tetrabutylammonium hydrogen sulphate, Acetonitrile (ACN, Isopropyl alcohol were purchased from Merck, Mumbai. Millipore water generated from Pall system. The analytical column used was YMC -pack ODS-A 150 X 4.6 mm, 3µ.

Instruments

A Waters prominence HPLC system equipped with a quaternary UFLC LC-20AD pump, a DGU-20A$_5$ degasser, a SPD-M20A diode array detector, a SIL-20AC auto sampler, a CTO-20AC column oven and CBM-20A communications bus module was used for method development and validation studies. Second HPLC system Agilent 1200 series with high pressure liquid chromatographic instrument provided with Auto sampler and VWD UV detector, thermostatted column compartment connected with EZ Chrome software.

Standard Stock preparation

Weigh and transfer about 20.0mg of Haloperidol Decanoate standard in to 50 mL volumetric flask, dissolve and make up the volume with Isopropyl alcohol.and mix well. Further Isopropyl alcohol.1.0mL of above solution in 100mL volumetric flask, make up the volume with Isopropyl alcohol and mix well. (Equivalent to 0.004 mg/Ml

Placebo preparation:

Dilute 2mL of Placebo solution into 50 mL volumetric flask with Isopropyl alcohol.

Preparation of sample for 50 mg/mL

Transfer 2ml of sample into 50mL volumetric flask dissolve and dilute to volume with Isopropyl alcohol.

Preparation of sample 100mg/mL

Transfer 1ml of sample into 50mL volumetric flask dissolve and dilute to volume with Isopropyl alcohol

Table 1: Gradient Program

Time in (min)	%Mobile phase-A	%Mobile phase-B	%Mobile phase-C	Flow rate (mL/min)
Initial	55.0	45.0	0.0	1.0
45	55.0	45.0	0.0	1.0
80	40.0	60.0	0.0	1.0
80.01	0.0	0.0	100.0	0.8
84.0	0.0	0.0	100.0	0.8
84.01	55.0	45.0	0.0	1.0
92	55.0	45.0	0.0	1.0

Chromatographic conditions

The chromatographic column used was YMC -pack ODS-A column with dimensions of 150 mm X 4.6 mm with 3 µm particle size. The gradient method was employed, with the mobile phase-A is 25% Tetrabutylammonium hydrogen sulphate, Mobile phase –B is Acetonitrile and mobile phase –C is Isopropyl alcohol. The column temperature was maintained at 45.0 ^{o}C and detection was monitored at a wavelength of 230 nm. Injection volume was 10 µl and the mobile phase flow was set at 1.0 mL/min. The Isopropyl alcohol was used as diluents for preparation of solutions. The gradient program was given in Table 1.

Method Validation

The developed method for determination of Haloperidol and its related substances was validated for system suitability along with method selectivity, specificity, linearity, range, precision (Repeatability and Intermediate precision),accuracy, limits of detection and Limit of quantification according to the ICH guidelines.

System suitability

The system suitability was conducted using diluted standard preparation and evaluated by injecting six replicate injections.

Specificity

Specificity is the ability of analytical method to assess un equivocally the analyte in the presence of component that may be expected to be present, such as impurities, degradation products and matrix components. Performed the specificity parameter of the method by injecting Diluent, standard preparation, and sample preparation spiked with impurities into the chromatographic system and evaluated by making three replicate injections.

Linearity

Performed the linearity with Haloperidol standard and impurities in the range of LOQ to 300% of specification limit. Recorded the area response for each level and calculated slope, intercept & correlation coefficient. Also performed precision at higher level by injecting six times into the chromatographic system.

Precision and Accuracy

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (Coefficient of variation) of series of measurements. The system precision was conducted using all the impurities spiked to Haloperidol and evaluated by making six replicate injections. The Accuracy of the method by recoveries of all the impurities was determined by analyzing Haloperidol sample solutions spiked with each impurity at three different concentration levels ranging from LOQ to 300%.

LOD and LOQ

The LOD and LOQ were determined for Haloperidol and each of the impurities based on the standard deviation of (SD) of the response and slope (S) of the regression line as per ICH guidelines.

Forced degradation studies

Forced degradation of Haloperidol was carried out according to ICH guidelines Q1A (R2). About 5.0 ml of HAR was subjected to forced degradation under acidic, basic and neutral conditions by refluxing with each 10.0 mL of 0.1N HCl, 0.1N NaOH and water at 80°C for 4 Hours. Oxidation of HAR (5.0 mL) was carried out using 3% H_2O_2 for 7 days. Thermal Stressed of HAR vial was exposed in Hot air oven at 80°C for 4 hours. Photolytic of HAR vial is exposed to an illumination of 1.2 million Lux hours of cool fluorescent light and an integrated near UV energy exposure of 200 watt hours / m^2 simultaneously in a photo stability chamber maintained at 25°C. After the prescribed time, samples were collected and stored in a refrigerator at 5 °C.

Robustness

The method robustness was studied by deliberately changing the percentage of organic modifier, flow rate, and column temperature.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Method development includes selection of appropriate chromatographic conditions/factors like detection wave length, selection and optimization of stationary and mobile phases. The longer wavelength of 230 nm was selected since it produces less noise, which minimizes problems that may exhibit around the active ingredient when attempting to quantify Haloperidol and its related substances-B, D, H, I and L. Moreover, all related impurities are also detected satisfactorily at the same wavelength and hence it is selected as detection wavelength. Preliminary development trials were performed with various ODS columns of different types and dimensions from different manufacturers were tested for the peak shape and the number of theoretical plates for specification concentrations. Finally by switching to Zorbax YMC -pack ODS-A 150 X 4.6 mm, 3µ column there was a substantial increase in the theoretical plates(17623) with a significant improvement in the peak shapes with 1.1 tailing factor. It also produced adequate resolution between Haloperidol and its related substances.

System suitability

The RSD from six replicate injections of diluted standard preparation was 1.0 %. Theoretical plates and Tailing factor for Haloperidol Decanoate peak 17623 and 1.1

Selectivity

Performed the specificity parameter of the method by injecting Diluent, Standard preparation, and Sample preparation, Placebo preparation, Impurity-B, Impurity-D, Impurity-H, Impurity-I, Impurity-L and sample spiked with impurities into the chromatographic system and recorded the retention times. Specificity study of the method proved the separation of impurity peaks from Haloperidol .peak Specificity results of Haloperidol given in the below table 2.The selectivity Chromatograms Shown in the Fig.2,3 and 4.

Linearity

To demonstrate the linearity with Haloperidol Decanoate standard, Impurity-B, Impurity-D, Impurity-H, Impurity-I and Impurity-L in

the range of LOQ to 300% of specification limit.

Correlation coefficient of haloperidol and its related compounds was 1.000. Plotted a graph of Haloperidol standard and Impurities concentration (ppm) on X-axis and Area responses on Y-axis. Moreover, the value of intercept is within ± 5% of the area response at 100% level. Precision at higher level RSD was NMT 5.0%. Linearity data of Haloperidol and its relative impurities given in the below table 3.The linearity curves of Haloperidol and its related substances Shown inthe Fig.5,6,7,8,9 and 10.

Table 2: Selectivity results of Haloperidol

Solution	Retention Time in (min)
Diluent	-
Placebo	-
Standard preparation	37.049
Sample as such	37.956
Impurity-L	5.32
Impurity-H	14.820
Impurity-I	23.008
Impurity-B	34.052
Impurity-D	72.471

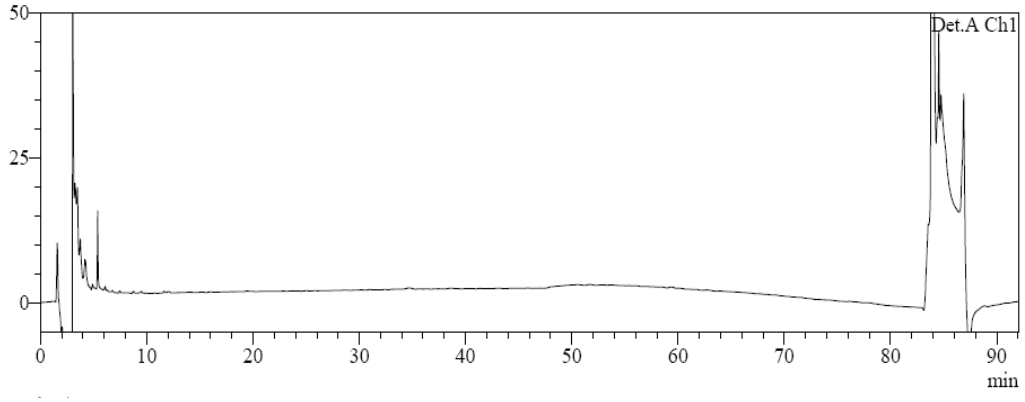

Fig. 2: Chromatogram of Blank

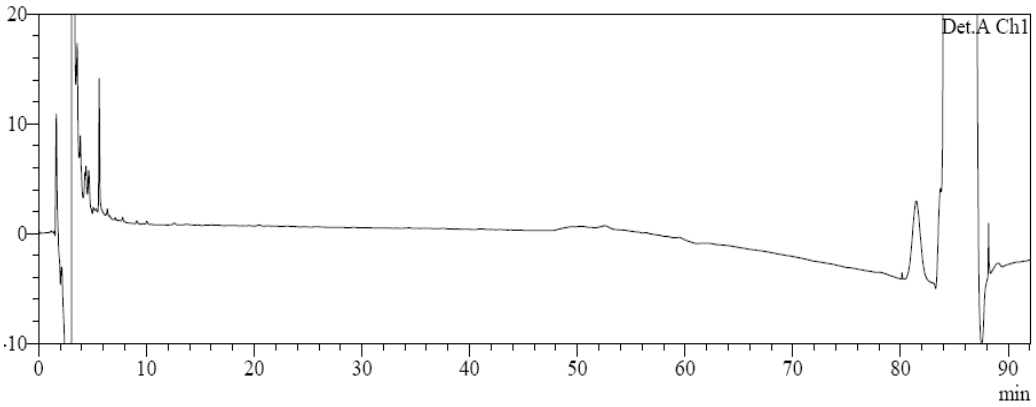

Fig. 3: Chromatogram of Placebo

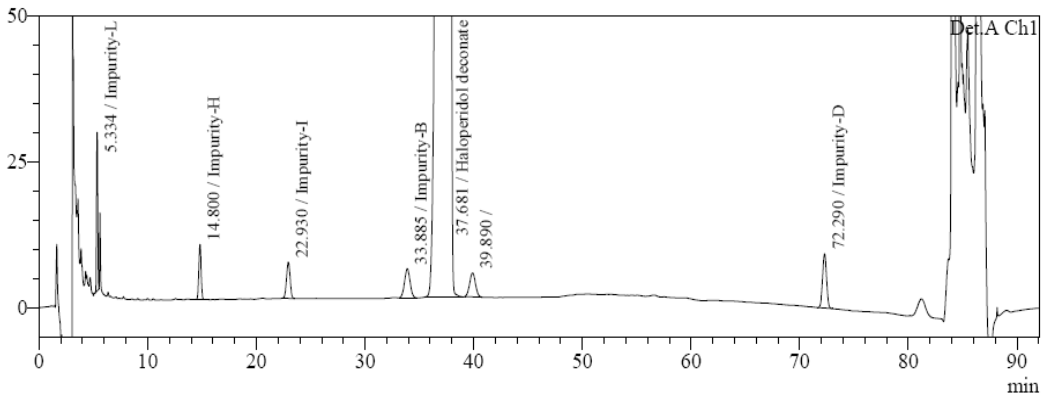

Fig. 4: Chromatogram of Haloperidol and its impurities

Table 3: Linearity results of Haloperidol.

S. No.	Concentration in ppm	Area Response
1.	0.2475	3435
2	3.7132	67306
3.	11.7585	207614
4.	35.8942	646110
5.	89.7356	1621121

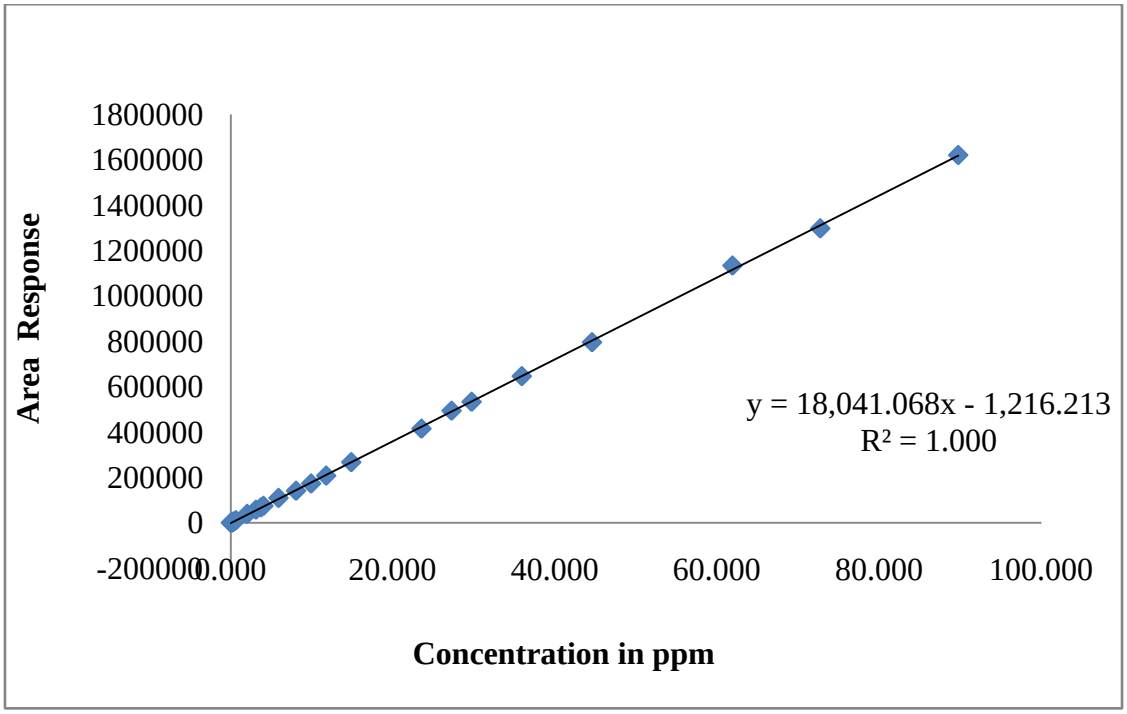

Fig. 5: Linearity of Haloperidol

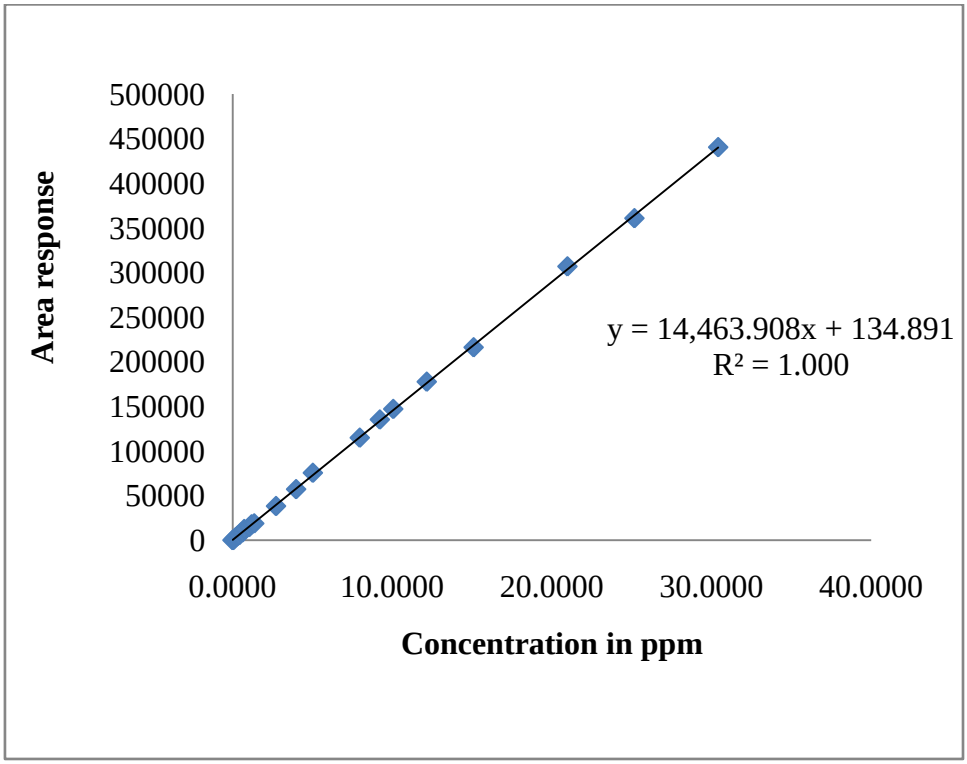

Fig. 6: Linearity of IMP –B

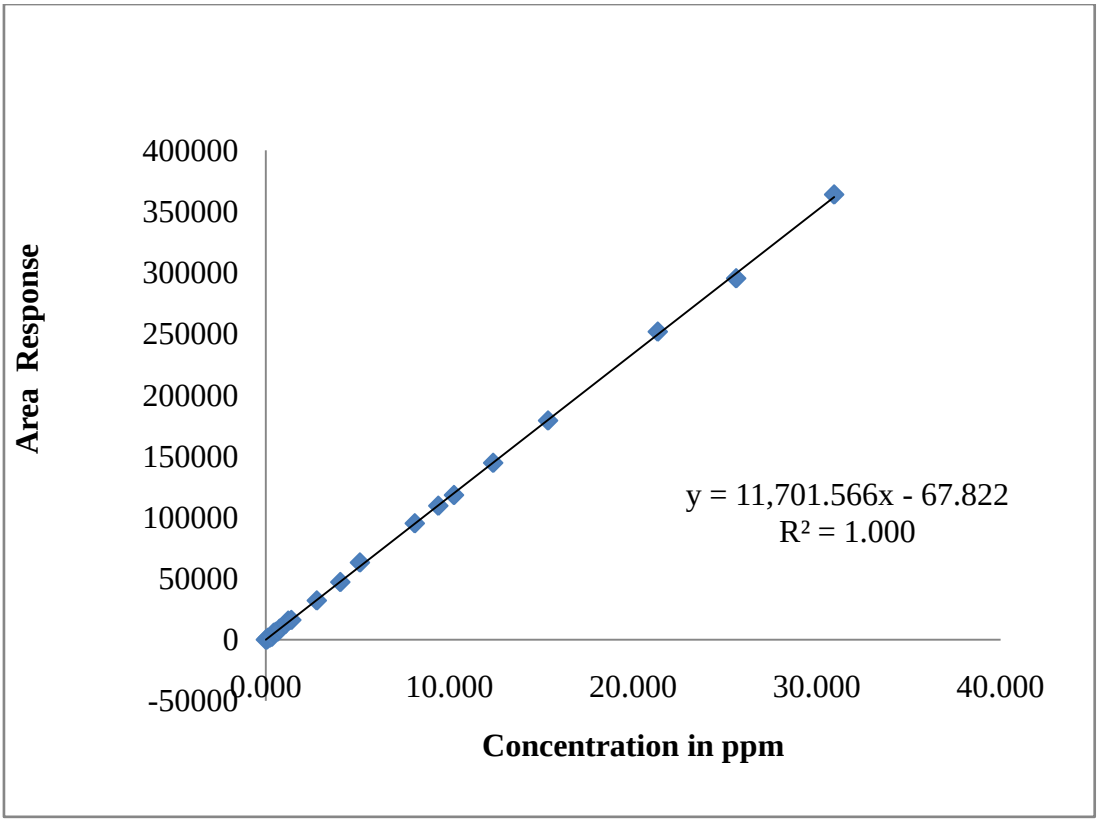

Fig. 7: Linearity graph of IMP-D

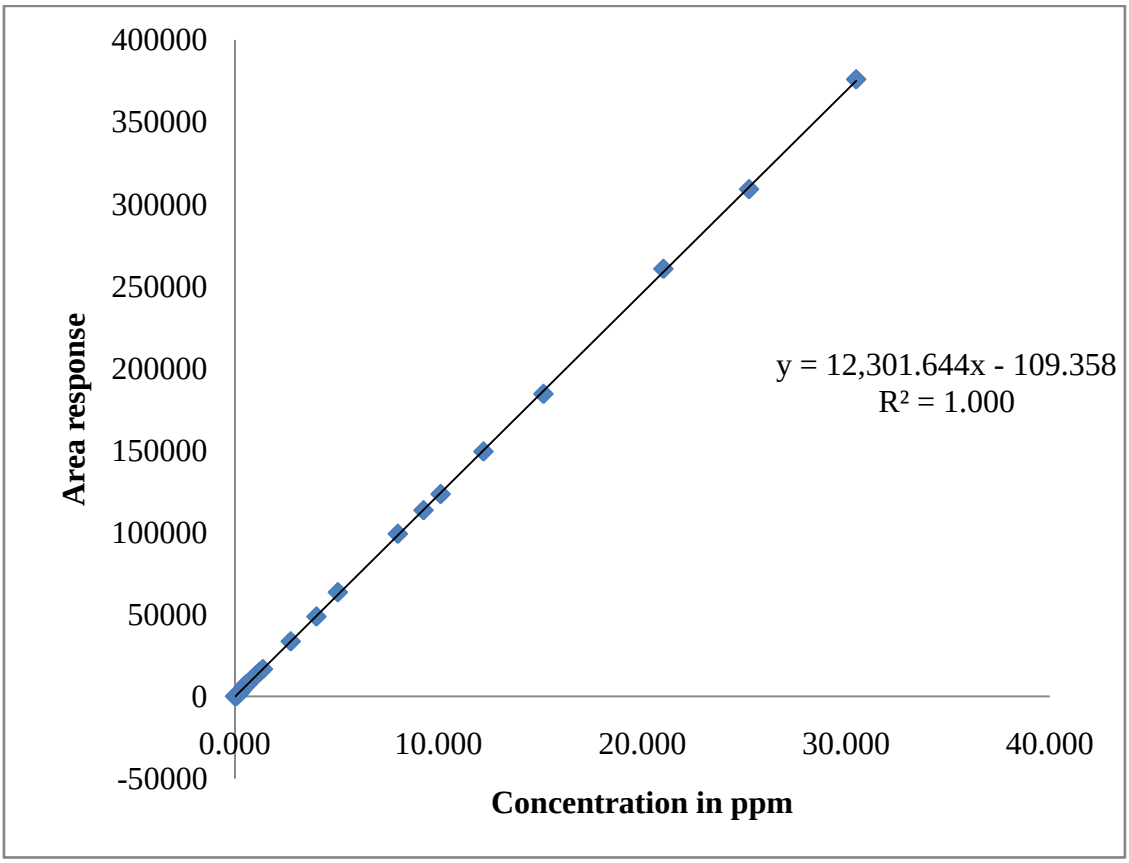

Fig. 8: Linearity graph of IMP-H

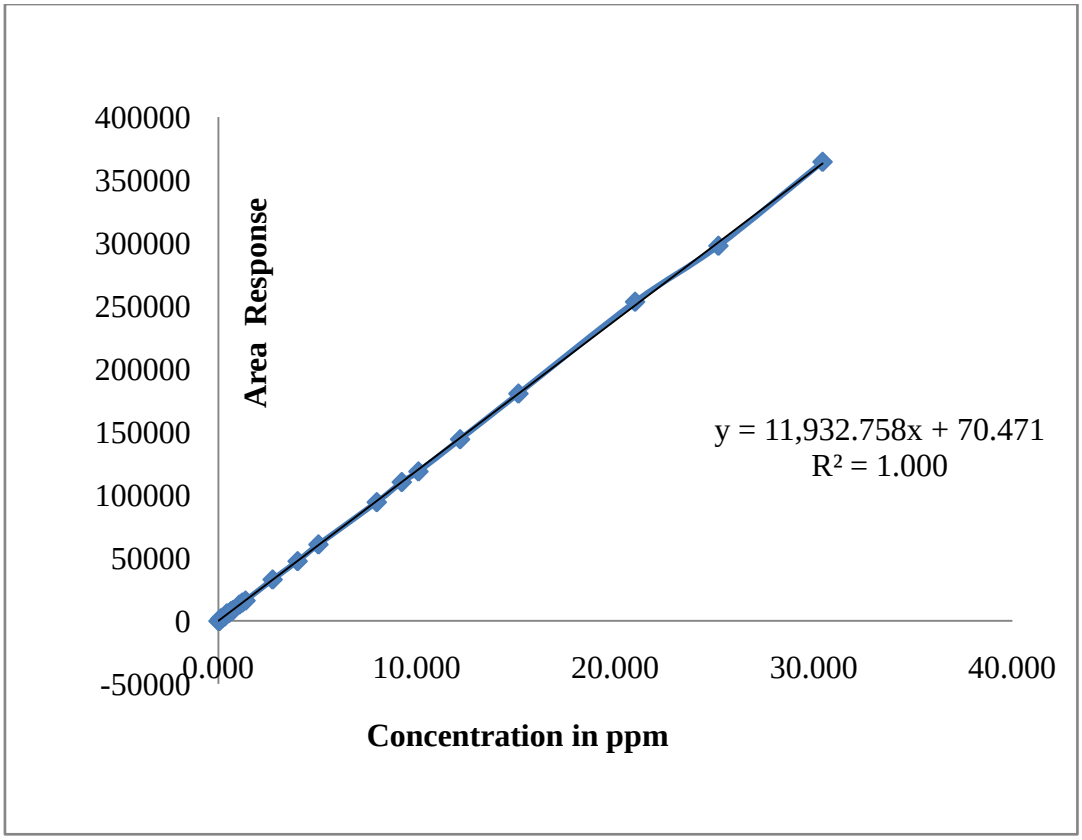

Fig. 9: Linearity graph of IMP-I

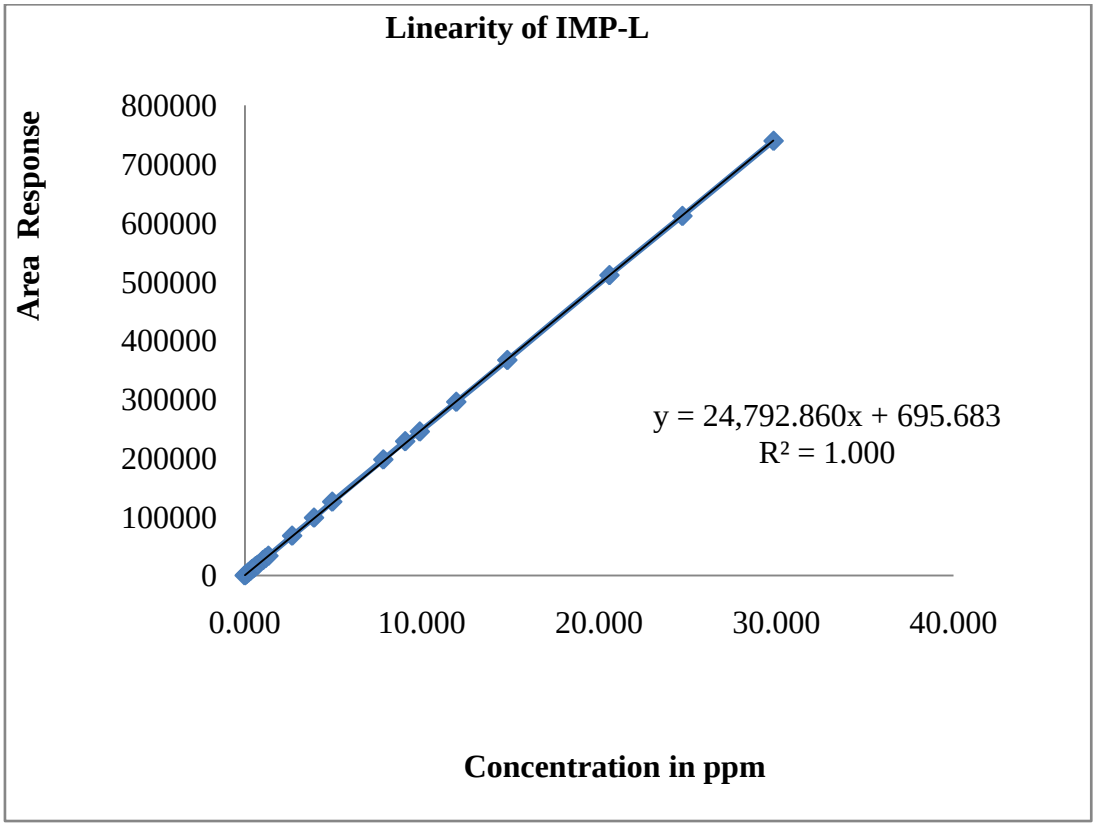

Fig. 10: Linearity graph of IMP-L

Accuracy

Accuracy study found that the mean % of recovery was more than 85% and less than 115% at each level LOQ to 300% of concentration levels, hence method is accurate. The accuracy results are given table 4:

Precision

The % RSD of the Retention time for peaks obtained from 6 injections of standard preparation 0.3.Method precision and intermediate precision results found that the % RSD of the impurities 0.05 % and total impurities for 6 determinations is less than 15.0 and 10.0 The % RSD of the impurities 0.05 % and total impurities for 12 determinations (Method precision & Intermediate precision) is 15.0 and 10.0.Precision results are given in the table 5.

LOD and LOQ

The distinct visible peak observed at LOD level concentration. The concentrations of LODs and LOQs were verified for precision by the analysis of solutions having Haloperidol and its impurities at these levels in six replicates and found that % RSD below 10. The LOD and LOQ data given table 6.Chromatogram of LOQ preparation Shown in the Fig.11

Table4: Accuracy results

S. No.	Level in %	Compound	% Mean Recovery
	LOQ	Haloperidol	100.5
2.	50%	Impurity-B	99.7
3.	100%	Impurity-D	102.0
4.	150%	Impurity-H	105.0
5.	200%	Impurity-I	108.8
6.	300%	Impurity-L	03.3

Table 5: Precision results

S. No.	Retention Time(in min)	Area response
1.	33.902	55155
2	33.741	55626
3.	33.907	55050
4.	33.980	55164
5.	33.707	55102
6.	33.916	56432
Mean	33.859	55422
% RSD	0.3	1.0

Table6: LOD and LOQ establishment data

LOD & LOQ	Haloperidol	IMP-B	IMP-D	IMP-H	IMP-I	IMP-L
LOD (ppm)	0.137	0.139	0.165	0.140	0.141	0.119
LOD (% w.r.t sample conc.)	0.007	0.007	0.008	0.007	0.007	0.006
LOQ (ppm)	0.458	0.463	0.551	0.467	0.469	0.395
LOQ (% w.r.t sample conc.)	0.023	0.023	0.028	0.023	0.023	0.020

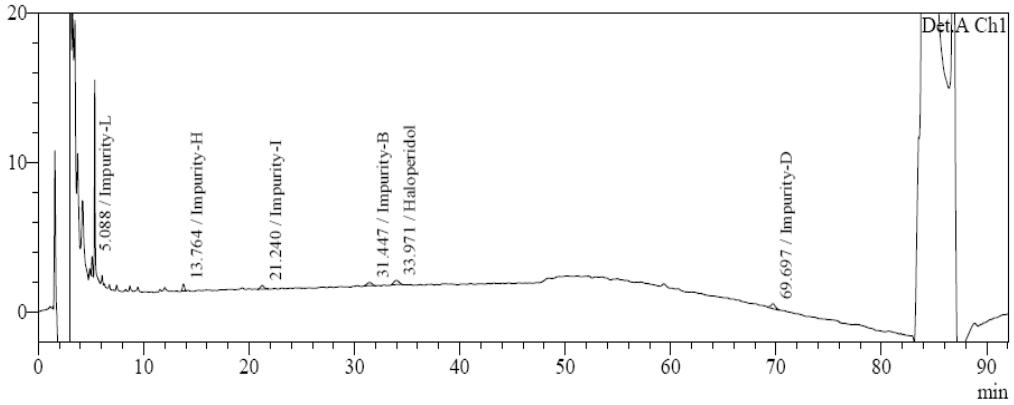

Fig. 11: Chromatogram of LOQ preparation

Force degradation study

The degradation study of all exposed samples were found spectrally pure as evidenced that the purity factor of haloperidol in all stress conditions was 1.000 and all known and unknown impurities are well separated from Haloperidol peak.

Stability in analytical solutions

The standard solution is stable for 63 hours at 2-8°C (%Difference for Haloperidol Decanoate peak is 3.8)and the Sample solution is stable for 37 hours at 2-8°C (%Difference for Haloperidol Decanoate peak is 4.1)

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CONCLUSIONS

A simple gradient RP-LC method has been developed and validated for the determination of related substances of Haloperidol drug substance. The developed method has been found to selective, sensitive, precise, robust, and stability indicating. The method can be directly adopted in quality control laboratories for routine analysis

with respect to determination of related substances and assay and also for the analysis of stability samples.

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