Original Article

Chemometric Assisted Development and Validation of a Stability-indicating LC Method for Determination of Related Substances in Haloperidol Decanoate Injection

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ABSTRACT

Aim: Haloperidol decanoate injection is a phenyl butyl piperadine derivative with antipsychotic, neuroleptic, antiemetic effects and has multiple related substances as process and degradant impurities. **Objectives:** This study focuses on chemometric assisted liquid chromatographic approach to develop a stability indicating impurity profile of Haloperidol decanoate injection. **Methodology:** Dual experimental designs (combined mixture I-optimal design and response surface historical data design) were employed to resolve all thirteen known impurities of Haloperidol decanoate. Chromatographic separation was achieved on a Hypersil BDS C_{18} (100 x 4.0 mm) 3-μm column to attain separation of related compounds. **Results:** The optimum conditions for the chromatographic system resulted in a mobile phase consisting of tetra butyl ammonium hydrogen sulphate/ 1-decane Sulphonate sodium buffer solution and acetonitrile with linear gradient elution at a flow rate of 1.4 mL/min. Selectivity, forced degradation, linearity, accuracy and precision were demonstrated in a range of 0.75-30.0 μg/mL. **Conclusion:** The optimized method was able to resolve the ghost peak observed because of gradient change of mobile phase and is able to separate both polar and non-polar impurities within 60 min, in single method, with resolution of more than 2.0 between adjacent impurities. The inter-day precision for all impurities and haloperidol decanoate were evaluated and found to have a % RSD of less than 10.

Key words: Design of Experiments, Haloperidol decanoate Injection, Stability indicating method, DoE aided chromatographic method development, Artifact optimization.

INTRODUCTION

Haloperidol Decanoate (HPD), (4-(4-Chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl) piperidin-4-yl decanoate) is the decanoate ester of haloperidol, a phenyl butyl piperadine derivative with anti-psychotic, neuroleptic and antiemetic effects. Haloperidol competitively blocks postsynaptic dopamine (D2) receptors in the mesolimbic system of the brain leading to anti-delusionary and anti-hallucinogenic effects. The antagonistic activity mediated through D2 dopamine receptors in the chemoreceptive trigger zone (CTZ) account for its antiemetic activity. Haloperidol decanoate has a markedly extended duration of effect. It is available in sterile form for intramuscular (IM) injection. The composition of injection dosage form is, each mL of haloperidol decanoate injection, contains 100 mg haloperidol (present as haloperidol decanoate, USP, 141.04 mg) in a sesame oil vehicle, with 1.2% (w/v) benzyl alcohol as

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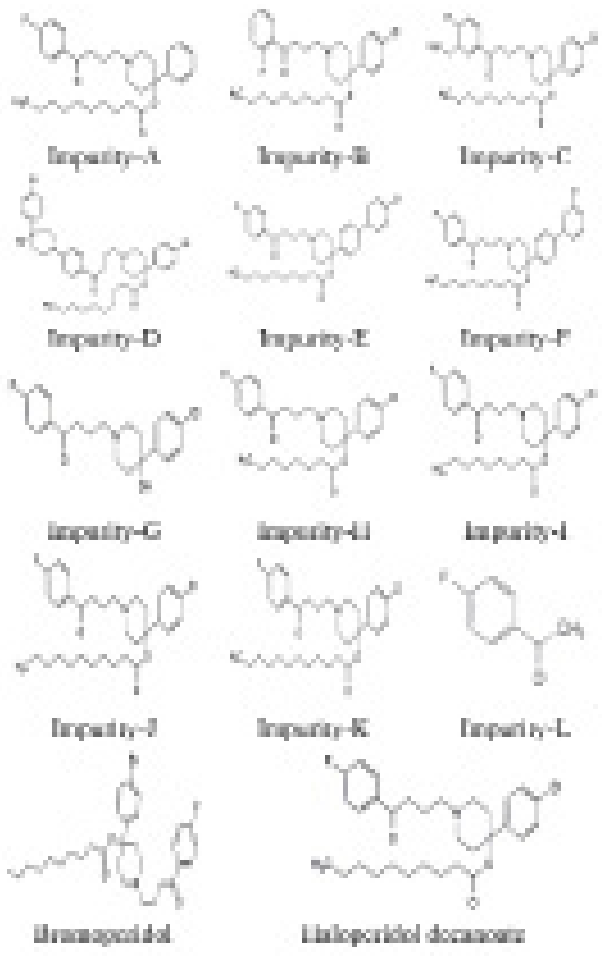

Figure 1: Chemical structures of impurities and Haloperidol decanoate.

a preservative. Similar formulation samples are used for entire study of method development and validation including forced degradation studies. Haloperidol decanoate injection 100 mg/mL is indicated for the treatment of schizophrenic patients who require prolonged parenteral antipsychotic therapy.[1]

Haloperidol decanoate has thirteen known impurities and one unknown degradation product (Figure 1) namely; impurity-A ((1-[4-(4-Fluorophenyl)-4-oxobutyl]-4-phenylpiperidin-4-yl decanoate), impurity-B ((4-(4-Chlorophenyl)-1-(4-(2-fluorophenyl)-4-oxobutyl) piperidin-4-yldecanoate),impurity-C((4-(4-Chlorophenyl)-1-[4-(3-ethyl-4-fluorophenyl)-4-oxobutyl]piperidin-4-yl decanoate), impurity-D ((4-(4-Chlorophenyl)-1-(4-{4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]phenyl}-4-oxobutyl)piperidin-4-yl decanoate), impurity-E ((4-(4'-Chlorobiphenyl-4-yl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl decanoate), impurity-F ((4-(3'-Chlorobiphenyl-4-yl)-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl decanoate), impurity-H(4-(4-Chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl) piperidin-4-yloctanoate),impurity-I(4-(4-Chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl)piperidin-4-yl nonanoate), impurity-J (4-(4-Chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl)piperidin-4-yl undecanoate), impurity-K (4-(4-Chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl)piperidin-4-yl dodecanoate) as process impurities and impurity-G/Haloperidol base (4-[4-(4-Chlorophenyl)-4-hydroxypiperidino]-4-fluorobutyrophenone), Impurity-L (1- (4-Fluro phenyl) ethanone) and a unknown degradant (DP-1) as degradation products.[2] Unknown degradation product is formed by oxidation stress, whereas impurity-G and impurity-L are formed by acid degradation.

Literature reference methods for determination of impurities of HPD active substance (API) are listed in European pharmacopoeia and United States pharmacopoeia.[2,3] However these methods are not suitable for injection dosage form, as benzyl alcohol (preservative) and benzaldehyde (impurity of benzyl alcohol), present in injection, will interfere with early eluting impurities (impurity-G and impurity-L). All other literature reported methods are either for Haloperidol base or only address assay of Haloperidol decanoate.[4-8] These literature reported methods are not suitable Haloperidol decanoate as the impurities of haloperidol and haloperidol decanoate are different. An artefact (ghost peak) was observed close to the retention time of haloperidol decanoate peak in pharmacopeial methods, which also interferes with impurity-A and impurity-B. Major reason for artefacts are potential impurities of mobile phase that elute as common band, when elutropic strength of mobile phase increases gradually in a gradient run. Other reasons include biofilm formation in HPLC instrument, contamination of water purification system with bacteria, possible contamination from additives of mobile phase. Additional sources for artefact can be plastic leachable, airborne absorption of phthalates, detergent residues on glass and ion pair reagents present in mobile phase.[9] Another type of artefacts are called as 'vacancy peaks' which are caused when UV absorbing impurities present in mobile phase equilibrate the column and form background. These can be resolved by increasing mobile phase B concentration.[10] Stabilizers, additives used in the solvents and reagents may also interact with mobile phase solvents to form byproducts to contribute artefacts.[11] Cleaning water with milli-Q ion exchange system can remove artefacts contributed from water source.[12-14]

Because of above listed discrepancies, none of the literature reported methods were found suitable for injection dosage form. Moreover the diluent used in literature based methods is a combination of aqueous

buffer and organic phase. As injection dosage form has sesame oil as vehicle, aqueous diluents are not suitable and pure organic solvent as diluent is to be investigated for suitability. Target for impurity elution is set as resolution of not less than 1.5 between all closely eluting impurities with non-interfering peaks arising from blank and placebo components of injection dosage form. A method for estimation of impurities of haloperidol decanoate was developed and validated by resolving ghost peak (artefact) of gradient elution with a diluent suitable for the evaluation of related substances of haloperidol decanoate injection.

MATERIALS AND METHODS

Chemicals and Reagents

Haloperidol decanoate injection (each mL contains 141.04 mg of Haloperidol decanoate, 1.2% of Benzyl alcohol as preservative and sesame oil as vehicle) and Placebo solution (each mL contains 1.2% of Benzyl alcohol as preservative and sesame oil as vehicle) was supplied by GVK BIO, Formulations (Hyderabad, India). Haloperidol decanoate API was supplied by RPG life sciences (Mumbai, India). Haloperidol decanoate reference standard and bromoperidol decanoate impurity were procured from USP. Impurity-L and impurity-G were sourced from Sigma Aldrich, USA. Rest of all impurities were purchased from Analytica chemie Inc., (Bangalore, India). Acetonitrile (ACN), Isopropyl alcohol (IPA) and methanol were procured from Merck Life Sciences Pvt. Ltd (Mumbai, India) and are of HPLC grade. Tetra butyl ammonium hydrogen sulphate (Analytical grade) was procured from SRL laboratories (Bangalore, India). 1-Decane sulphonic acid sodium (AR grade) was procured from Finar chemicals (Bangalore, India). All other chemicals were of Merck-Emplura grade. Ultra-pure water was obtained from Millipore® water purification system (Merck Millipore, Hyderabad, India).

Apparatus and Equipment

HPLC studies were carried out on Waters Alliance 2698 liquid chromatograph (Waters corporation, USA), which was equipped with a Photo diode array detector. Hypersil BDS, C_{18} Column (100 mm x 4.0 mm, 3µm particle size, Thermo Fisher Scientific India Pvt. Ltd, Mumbai, India) was utilized in the study. pH was observed by using pH/Ion analyzer (LP139SA, Polmon, Bangalore, India). Entire method development studies were performed with linear gradient programme with buffer as mobile phase-A and acetonitrile (ACN) as mobile phase-B. Other equipment used were micro balance (ME 5, Sartorius, Switzerland), analytical balance (XB220A, Precisa Gravimetric AG, Dietikon, Switzerland). Pipettes and remaining glassware were of Borosil make. 0.45µm Polyvinylidene fluoride (PVDF) filters (Merck Millipore, Bangalore, India) were used for the filtration of sample solutions. Stat-Ease Design-Expert, version-10 software was used during DoE studies so as to generate experimental designs and to analyze the obtained responses.

Procedure and Details of Test product

Haloperidol decanoate (HPD) sample solution at 2968 µg mL^{-1} was prepared by dissolving about 1.978 g of haloperidol decanoate injection in 100.0 mL of isopropyl alcohol. Each g of haloperidol decanoate injection contains 150.04 mg of haloperidol decanoate and density of haloperidol decanoate injection is 0.94 g mL^{-1}. All calculations for recovery were made against HPD diluted standard, prepared by serially diluting HPD stock solution in IPA to achieve a solution of 15 µg mL^{-1}. Linearity solutions for all impurities and HPD were prepared by diluting stock solutions to a concentration of around 0.75, 1.50, 3.75, 7.50, 12.00, 15.00 and 30.00 µg mL^{-1}. Accuracy was studied at four different levels including LOQ and in triplicate preparation at each level. Accuracy solution were prepared by spiking impurities to HPD injection in IPA as diluent at a concentration of 1.50, 7.50, 15.00 and 22.50 µg mL^{-1}.

Artefact Optimization

Literature reference method from USP monograph was implemented and observed that multiple artefacts were generated, at 20 to 30 min retention time, because of steep rise in gradient change (Figure 2). Hence to resolve artefacts, different gradient programmes were experimented with the help of DoE software as chemometric tool. In the first place response to be optimized was selected as artefact intensity, which is peak height of the artefact in milli-Absorbance units (mAU) multiplied by a factor of 10, for ease of representation in DoE. IPA as diluent blank and HPD diluted standard solution were injected to confirm artefact. The analyzed factors i.e. concentration of buffer (A) and acetonitrile (B) composition as mixture variables at start of gradient programme and gradient ramp change (C) as numeric variable were selected based on literature reference, as they have more influence on the response under study. All the selected variables for this design were pertaining to critical process parameters (CPP). The combined mixture I-optimal design (CMD) consisted of twelve experiments including combination of factors at different levels. The ranges studied for the factors were 20.00%-40.00% of ACN in mobile phase at

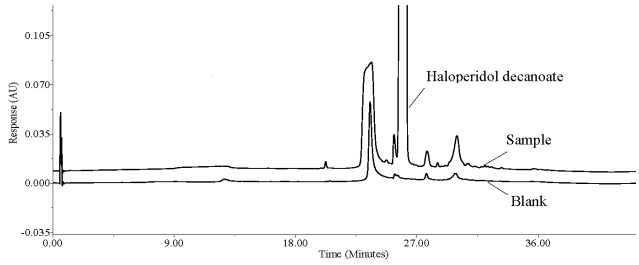

Figure 2: Representative chromatogram with artefact in literature method.

initial gradient and 0.50%-2.00%/ min of gradient ramp change at 6.0 min (Table 1). The study ranges were consciously selected around the existing method values for the selected variables. Existing method has 20.00% of ACN as initial gradient and the range for study is extended up to 40.00% as higher organic ratio at initial stage of gradient programme will reduce the intensity of artifact. Gradient ramp of literature method is 1.3%/ min and both slow and fast gradient ramp change were studied to see impact on artifact.

Table 1: Combined mixture design for ghost peak optimisation.

Run	Buffer %	ACN %	Gradient ramp % /min	Blank peak intensity (mAU)[a]
1	70	30	1.25	1
2	70	30	1.25	1
3	80	20	2.00	5
4	60	40	0.50	0
5	71	29	1.82	2
6	80	20	0.50	3.5
7	60	40	2.00	0.5
8	60	40	1.43	0
9	70	30	1.25	1
10	80	20	0.50	3.5
11	80	20	2.00	5
12	72	28	0.76	1

[a] Height of peak is multiplied by a factor of 10 for ease of interpretation

Application of DoE for Chromatographic Separation

Further to resolve closely eluting impurities, different experiments were conducted by varying mobile phase pH and also introducing a secondary ion pair reagent. Ion pair reagent concentration was selected as critical variable to separate polar impurities (impurity-G and L) and gradient programme ramp change over time was experimented to separate impurity-A and I, while studying the impact of change in pH of mobile phase on both critical separation goals.

Ion pair chromatography usually employs reagents such as alkyl sulfonates or alkyl ammonium salts, added to the mobile phase, which bind with opposite charged ions of analytes to increase the retention time. The retention for non-charged analytes is usually not affected with ion pair reagents. By varying type and concentration of ion pair reagent in sensible ranges the separation is attained.[15] Various methods were developed with ion pair reagent for complex separations involving structurally similar analytes with similar polarity.[16] Quaternary ammonium salts were used for acidic compounds separation and alkyl sulfonates for basic compounds separation.[17] When multiple analytes are to be separated in single chromatographic run, DoE tool was experimented for better evaluation and understanding of relation between variables of method on responses.[18]

As optimized conditions with encouraging resolution between all closely eluting impurities was not achieved with OFAT experiments, response surface methodology with historical data design (RSH) was implemented by combining all the fourteen OFAT experiments to DoE (Table 2). The study ranges were 0-5 g/L of 1-decane sulfonic acid as ion pair reagent in mobile phase, 1.87-4.30 range for pH of mobile phase and 0.2-1.5 %/ min of gradient change for ACN in mobile phase at 6 min. Ion pair reagent is studied from no reagent in mobile phase to 5 g/L of reagent, based on literature references of most commonly used ion pair concentrations. Existing mobile phase pH was observed around 1.87 and hence sligh variation in pH of mobile phase was brought in as critical variable as difference in pH of mobile phase will influence ionization of analytes and thus retention properties. Further as it was observed from CMD design that slow gradient ramp is helping to retain and resolve impurities a wide range of slowest gradient to fast change over time were selected. The studied responses were resolution between impurity-G and L (R1), resolution between impurity-A and I (R2) and retention time of impurity-G (R3). It is worthwhile to note that impurity-G being highly polar among all the impurities elutes close to void peak and hence was of importance during method optimization. Ion pair reagent concentration and pH of the mobile phase were pertaining to CMA of the method and gradient ramp was pertaining to CPP of the method. Final method with optimized conditions was validated to prove reliability, stability indicating nature of the method and applicability to HPD injection dosage form.

Table 2: Response surface historical data design for chromatographic separation optimisation.

Run	Variables			Responses		
	A g/L	B	C % min^{-1}	R_1	R_2	R_3
1	0	1.87	1.5	2.8	-1.45	0.72
2	0	4.3	1.5	2.8	-1.45	0.72
3	2	1.87	1.5	1.6	0.30	1.01
4	4	1.87	1.5	1.6	0.31	1.01
5	0	6.00	1.5	2.8	-1.45	0.76
6	3	4.00	1.5	0.2	0.30	1.31
7	3	3.00	1.5	0.4	0.30	1.36
8	3	1.87	0.8	1.5	0.70	1.81
9	4	1.87	0.8	1.1	0.76	1.62
10	5	1.87	1.5	2.1	0.96	1.90
11	5	1.87	0.9	1.8	4.50	1.72
12	5	1.87	0.2	2.3	6.16	2.03
13	5	1.87	1.9	2.5	0.95	2.10
14	5	1.87	0.4	2.2	8.90	1.91

A, ion pair reagent concentration; B, pH of mobile phase; C, gradient ramp at 6 min; R_1, resolution between impurity-G and L; R_2, resolution between impurity-A and I; R_3, impurity-G retention time.

RESULTS AND DISCUSSION

Resolving Artefact with Combined Mixture I-optimal Design

Artefact evaluation with combined mixture I-optimal design (CMD) discovered that the initial mobile phase composition in combination with gradient ramp were having significant impact on intensity of artefact. Response evaluation was performed with 3D surface plots and observed that highest response for artefact was observed at initial mobile phase composition of buffer and acetonitrile in the ratio of 80:20 v/v, with a gradient change of mobile phase-B at 2%/ min. Whereas no artefact was observed at an initial mobile phase composition of 60:40 v/v and gradient ramp of below 1% / min (Figure 3). Further, method optimization was performed with DoE software using 'constraints' option to achieve desired artefact intensity as '0' and criteria for method optimization are presented in Table 3. CMD design was able to predict several solutions and most promising results are with buffer and acetonitrile at 60:40 v/v composition and 0.78%/ min of mobile phase-B change, as depicted in desirability graph (Figure 4). It can be noticed that the optimized method has low resolution for impurity-G and L as well impurity-A and I which were closely eluting.

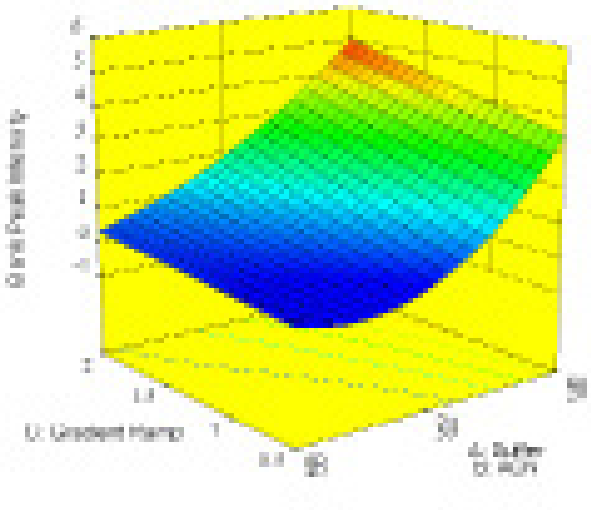

Figure 3: Response surface plot for artefact intensity as a function of mobile phase composition and gradient ramp.

Chromatographic Optimization with Response Surface Historical Data Design

Based on the outcome of CMD design, linear gradient programme was set with 35 min runtime, which was devoid of artefacts. As the gradient programme of optimized method was with higher concentration of organic phase, compared to literature reference method, the resolution between closely eluting impurities (impurity-G and L, impurity- A and I) was compromised and hence these were identified as critical separation goals for further experiments. In-order to achieve this separation goal, different alkyl sufonates as additional ion pair reagent were experimented with OFAT approach and observed that 1-Decane sulfonic acid is able to retain impurity-G and L with apposite resolution. Effect of ion pair reagent was evaluated and observed that with increase in ion pair reagent concentration, retention of impurity-G was increasing also with improvement in resolution between impurities-A and I. Impurity-G was eluting in void with no alkyl sulfonate as ion pair in mobile phase. It was noticed that there is elution pattern change for impurity-A and I. At low or no ion pair reagent (alkyl sulfonate), impurity-I was eluting first followed by impurity-A (Figure 5a). At mid-range of additional ion pair reagent concentration, both impurities were merging and at high concentration of additional ion pair, at about 5 g, there was good resolution between impurities and elution order is impurity-A followed by impurity-I (Figure 5b). Further evaluation of responses was made with 3D surface plots. Figure 6a indicates that ion pair concentration has impact on elution pattern for impurity-A and I, indicated by both negative and positive values for resolution. Whereas low or optimum

Table 3: Criteria for optimisation of factors and responses.						
Optimization	**Factors**	**Response**	**Goal**	**Lower Limit**	**Upper Limit**	**Importance**
CMD	Buffer		is in range	60	80	3
	ACN		is in range	20	40	3
	Gradient ramp		is in range	0.5	2	3
		Intensity of artifact	is target = 0	0	2	5
RSH	A			0	7	3
	B			1.87	6	3
	C			0.2	1.875	3
		R_1		1.5	2.82	3
		R_2		1.5	8.9	5
		R_3		1	2.101	3

CMD: Combined Mixture I-Optimal Design; RSH: Response Surface Historical data design; Int: blank peak intensity; A, ion pair reagent concentration; B, pH of mobile phase; C, gradient ramp at 6 min; R_1, resolution between impurity-G and L; R_2, resolution between impurity-A and I; R_3, impurity-G retention time.

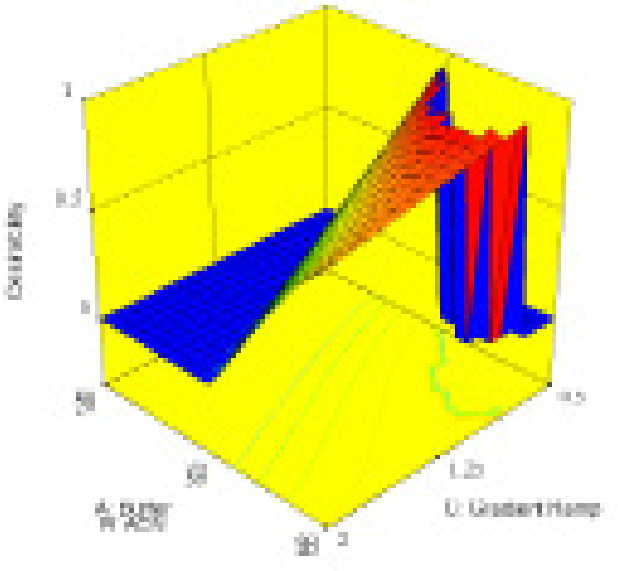

Figure 4: Response surface of global desirability from CMD design for artefact intensity as a function of gradient ramp and mobile phase composition.

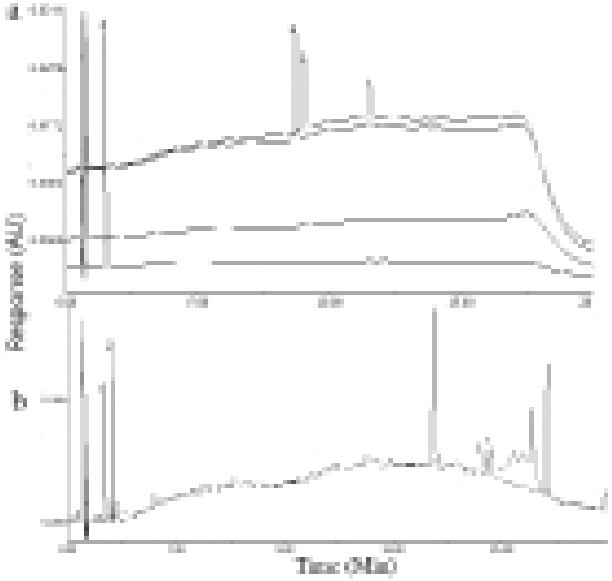

Figure 5: Chromatograms depicting effect on ion pair concentration on impurity separation and elution pattern; (a) No ion pair reagent, (b) 3 g of ion pair reagent, 1 impurity-G, 2 impurity-L, 3 impurity-A, 4 impurity-I.

gradient ramp is favorable for better resolution. Figure 6b explain relation of ion pair reagent concentration in combination to mobile phase pH on resolution between impurity-G and L. Improved resolution was observed at mid to low pH and higher concentration of ion pair reagent. Figure 6c depicts impact of ion pair reagent on retention time of impurity-G which was positive with increase in concentration, while gradient ramp does not have any impact.

Method Optimization, Desirability Graphs and Overlay Plots

Final chromatographic conditions were predicted with 'constraints' option for RSH design with given parameters in Table 3. Desirability graph from RSH design indicates,

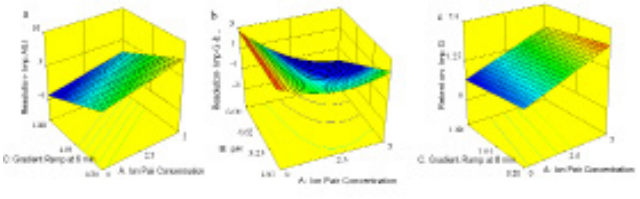

Figure 6: Response surface plot for chromatographic separation optimization; (a) Resolution between impurity-A and I as a function of ion pair reagent concentration and gradient ramp, (b) Resolution between impurity-G and L as a function of ion pair reagent concentration and pH of mobile phase, (c) Retention time of impurity-G as a function of ion pair reagent concentration and gradient ramp.

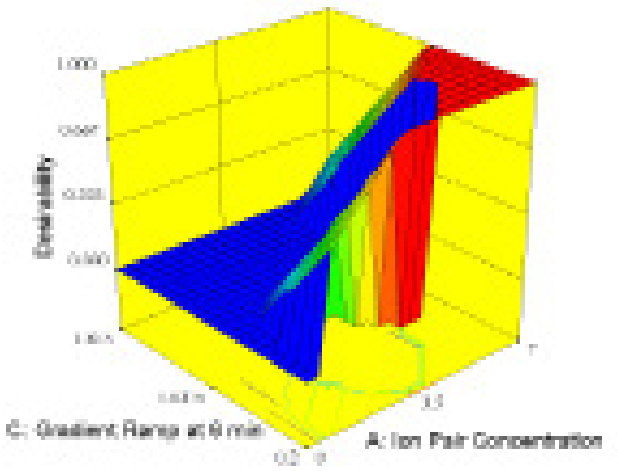

Figure 7: Response surface of global desirability from RSH design for chromatographic separation optimisation as a function of ion pair reagent concentration and gradient ramp.

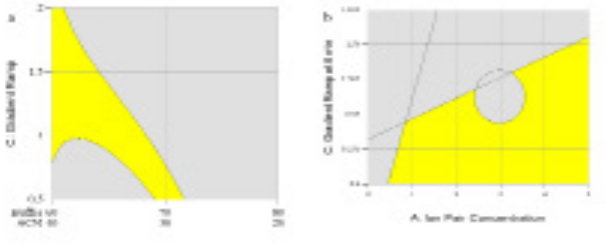

Figure 8: Overlay plots depicting MODR; (a) CMD design as a function of mobile phase composition and gradient ramp, (b) RSH design as a function of ion pair concentration and gradient ramp at 6 min.

a gradient ramp of 0.2 to 1.0 %/ min with 1-decane sulfonic acid sodium concentration, above 3 g/ L of mobile phase (Figure 7) was favorable for good resolution. Selected solutions, out of the DoE predictions, were methodically evaluated with numerical optimization criteria and overlay graph, to understand the method operable design region (MODR) of experimental design.[19-21] Final gradient ramp selection from suggested solution of RSH was made by thorough evaluation of MODR with evaluation of overlay plots of both the designs. Overlay plots of CMD (Figure 8a) and RSH design (Figure 8b) indicate a gradient ramp of 0.4%/ min is suitable, as common factor for both designs, to achieve goal of no artefact and desirable separation between all impurities. The finalized chromatography was performed with hypersil BDS C_{18}, (100 x 4.0 mm, 3µ particle size) HPLC column with a flow rate of 1.4 mL/ min, column temperature operated at 30°C and 10 µL of analytical solutions injected to chromatograph. Mobile phase-A is 27g/ 5g per liter of tetra butyl ammonium hydrogen sulphate / 1-decane sulphonic acid sodium salt respectively in water with pH adjusted to 2.1 with dilute sulfuric acid and acetonitrile used as mobile phase-B. The elution was continued with gradient change of mobile phase-B starting at 40% volume and linearly increased to 45% volume over 6 min. Further gradient ramp for mobile phase-B was increased to 55% until 30 min, which was kept on hold for five minutes. The mobile phase gradient is then slowly re-equilibrate initial composition to prepare the instrument for next run. Detection is made at 230 nm with UV-Vis detector and total run time was kept at 60 min.

Statistical Significance of terms

The statistical significance of main effects and interaction effects for both the designs was evaluated with coefficients table. Each of the effects were monitored for *p*-value for significance. From the CMD design for artifact optimization, it was observed that buffer and ACN concentration in mobile phase as main effects, buffer concentration in combination with gradient ramp and buffer concentration in combination with ACN as interaction effects were found to be very significant with *p*-value less than 0.01. Whereas interaction effects for combination all three factors was found to be significant with moderate impact on selected with p-value between 0.01-0.05. All other effects for CMD design were not of significance. Statistical significance for RSH design revealed that response-1 (resolution between impurity-G and L) is influenced by Ion pair reagent concentration, pH of mobile phase and combination of both as main effects with p-value, very significant (less than 0.01). Whereas gradient change over time was having moderate significance on response-1 and there was no significant interaction effect. For response-2 (resolution between impurity-A and I) gradient ramp was only main effect which is very significant, while all other main and interaction effects did not have any significance. Response-3 (retention of impurity-G) is very significant for changes in ion pair reagent concentration alone while any other main and interaction effect does not have any significance.

Method Validation

The method was validated following ICH Q2 (R1) guidelines on validation of analytical procedures.[22-24] The following parameters were evaluated: specificity, linearity, Limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy and range. Thorough forced degradation studies were performed in hydrolytic, oxidation and photolytic stress conditions and mass balance with peak purity criteria were established to prove stability indicating nature of the method.

Table 4: Accuracy and precision.

Impurity Name	Level	Concentration (%)	Nominal (µg mL^{-1})	Predicted (µg mL^{-1})	Average Area	% Recovery	% CV
Impurity-A	LOQ	0.02	1.514	1.426	5476	93.5	7.8
	50%	0.25	7.568	7.200	27647	94.3	11.5
	100%	0.50	15.135	13.546	52019	88.8	9.5
	150%	0.75	22.703	22.703	75934	86.4	4.2
Impurity-B	LOQ	0.02	1.515	1.37	13093	89.8	1.6
	50%	0.25	7.575	7.506	71722	98.4	3.9
	100%	0.50	15.15	13.66	130524	89.5	8.5
	150%	0.75	22.725	25.713	245692	112.3	2.0
Impurity-C	LOQ	0.02	1.505	1.277	8834	83.7	8.9
	50%	0.25	7.523	6.999	50004	91.7	4.2
	100%	0.50	15.045	14.458	103294	94.7	8.8
	150%	0.75	22.568	23.269	166235	101.7	3.7
Impurity-D	LOQ	0.02	1.506	1.35	11696	88.5	5.0
	50%	0.25	7.53	7.789	67473	102.1	3.8
	100%	0.50	15.06	13.57	117544	88.9	5.5
	150%	0.75	22.59	20.536	177887	89.7	7.4
Impurity-E	LOQ	0.02	1.503	1.326	7577	86.9	4.8
	50%	0.25	7.515	7.351	42015	96.3	8.6
	100%	0.50	15.03	14.434	82494	94.5	3.1
	150%	0.75	22.545	23.272	133005	101.6	1.1
Impurity-F	LOQ	0.02	1.543	1.5	11515	98.3	1.6
	50%	0.25	7.714	7.948	61042	104.2	1.8
	100%	0.50	15.428	13.924	106931	91.2	9.8
	150%	0.75	23.141	23.717	182147	103.6	4.9
Impurity-G	LOQ	0.02	1.506	1.322	10742	86.6	8.6
	50%	0.25	7.53	7.215	58635	94.6	5.7
	100%	0.50	15.06	13.722	111509	89.9	6.0
	150%	0.75	22.59	21.297	173071	93	4.2
Impurity-H	LOQ	0.02	1.514	1.545	12601	101.2	4.9
	50%	0.25	7.571	7.497	60924	98.3	3.1
	100%	0.50	15.143	15.183	123380	99.5	6.6
	150%	0.75	22.714	21.948	178362	95.8	11.3
Impurity-I	LOQ	0.02	1.620	1.655	13595	108.4	6.2
	50%	0.25	8.100	7.447	61183	97.6	4.8
	100%	0.50	16.200	14.556	119587	95.3	6.5
	150%	0.75	24.300	24.506	201337	107	5.3
Impurity-J	LOQ	0.02	1.531	1.687	12055	110.6	1.6
	50%	0.25	7.654	6.666	47618	87.3	3.7
	100%	0.50	15.308	14.874	106257	97.5	5.6
	150%	0.75	22.961	23.353	166835	102	7.0
Impurity-K	LOQ	0.02	1.853	1.673	5078	109.6	6.4
	50%	0.25	9.263	7.952	24144	104.2	7.3
	100%	0.50	18.525	16.491	50069	108	8.7
	150%	0.75	27.788	23.804	72277	104	2.8
Impurity-L	LOQ	0.02	1.526	1.394	19049	91.3	3.8
	50%	0.25	7.631	6.835	93390	89.6	4.3
	100%	0.50	15.263	13.655	186571	89.5	5.5
	150%	0.75	22.894	20.403	278774	89.1	6.4
Bromoperidol	LOQ	0.02	1.725	1.524	16334	99.9	4.4
	50%	0.25	8.625	8.444	90487	110.6	6.0
	100%	0.50	17.25	16.012	171593	104.9	8.5
	150%	0.75	25.875	22.538	241525	98.4	5.9

Table 5: Linearity, range and relative response factor.

Impurity Name	LOD[a]	Concentration Range (µg mL^{-1})	Area		Slope (m)	Intercept (c)	Correlation Coefficient (r)	RRF[b]
			Minimum	Maximum				
Impurity-A	0.025	0.767-30.27	3391	98091	3242205	2792	0.9972	0.43
Impurity-B	0.025	0.768-30.03	6598	239707	8011949	6725	0.9948	1.07
Impurity-C	0.025	0.762-30.09	4286	160510	5415521	4192	0.9948	0.73
Impurity-D	0.025	0.763-30.12	4606	214447	7241077	6265	0.9934	0.97
Impurity-E	0.025	0.762-30.06	2782	140807	4774685	3511	0.9939	0.64
Impurity-F	0.025	0.782-30.86	3749	193398	6384095	4951	0.9941	0.86
Impurity-G	0.025	0.763-30.12	5941	202483	6786109	5419	0.0060	0.91
Impurity-H	0.025	0.767-30.29	5368	206208	6892768	3953	0.9969	0.92
Impurity-I	0.025	0.821-32.4	6740	218550	6838863	5529	0.9953	0.92
Impurity-J	0.025	0.776-30.62	5613	179965	5937469	5524	0.9949	0.80
Impurity-K	0.025	0.939-37.05	1770	91854	2525744	2137	0.9946	0.34
Impurity-L	0.025	0.773-30.53	8460	342665	11395626	7666	0.9957	1.53
Bromoperidol	0.025	0.882-34.8	9491	307303	8931744	10765	0.9933	1.20
Haloperidol Decanoate	0.025	0.774-30.56	7771	226917	7453624	10204	0.9925	1.00

[a] % with respect to sample concentration (3000 µg mL^{-1} as Haloperidol decanoate)[b] Relative response factor of impurity with respect to Haloperidol decanoate

Linearity, Range and RRF Establishment

The studied range of the method was 5 to 200 % of the specification concentration. Specification for impurities was set as 0.5% (15 µg mL^{-1}) of HPD concentration in sample solution (3000 µg mL^{-1}). Linearity was evaluated at 7 levels for all known impurities and HPD peak and all the solutions were prepared in IPA as diluent. Observed correlation coefficient values indicate linear response for all impurities across range. Relative response factor (RRF) values for each impurity were calculated with ratio of impurity slope to that of HPD slope, as observed from linearity curve (Table 5). LOD of the method is observed around 0.75 µg mL^{-1} and LOQ around 1.5 µg mL^{-1}. Established correlation co-efficient values for all impurities and HPD from linearity curve met the acceptance criteria of not less than 0.99.

Accuracy and Precision

Accuracy of the method was performed at four different levels by spiking all known impurities at pre-determined concentration ranges to HPD injection. Accuracy at 100% of target impurities specification (0.5% of sample concentration; 15 µg mL^{-1}) was performed in six replicates to evaluate method precision. Accuracy at LOQ level was also performed in six replicates to prove method range at low level. For all accuracy level, % recovery of impurities and HDP were calculated along with % CV for replicate preparation at each level. All recoveries were calculated by applying relative response factor (RRF) for impurities against an external standard prepared with HPD at concentration of 0.5% with respect to sample preparation (15 µg mL^{-1}). All the tested levels were able to meet the acceptance criteria for recovery (85-115 %) and precision (% CV < 15%), indicating method suitability for routine use. Accuracy and precision results from the study are presented in Table 4.

Specificity

Specificity of the method was carried out by evaluating different kinds of interferences, i.e. those produced by blank, placebo, known impurities and degradation products. For the first case, diluent as blank solution is tested. For placebo and known impurity interference, sesame oil with benzyl alcohol as placebo and all known impurities spiked to sample were evaluated. Benzyl alcohol is present as preservative in injection and hence interference of benzyl alcohol and related impurities was also verified. Benzyl alcohol was not detected at method detection wavelength of 230 nm while benzaldehyde, impurity of benzyl alcohol, is separated from impurity-G. Overlaid chromatograms of specificity study from final method chromatographic conditions along with LOQ solution are presented in Figure 9.

Forced Degradation

Stability indicating nature of the method for degradation products was proven with forced degradation studies at different hydrolytic, oxidation, thermal and photolytic stress conditions. Several permutation and combinations

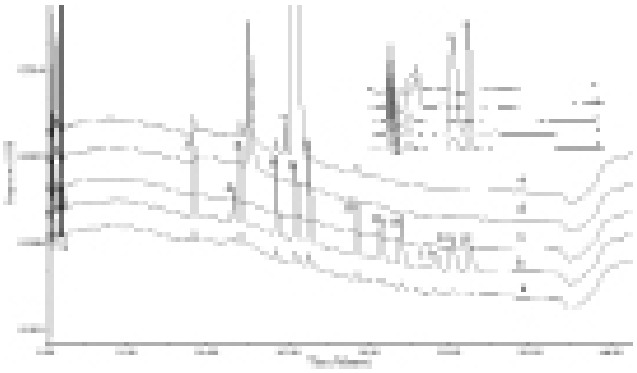

Figure 9: Chromatograms obtained with optimized conditions; a All impurities at LOQ, b All impurities at specification level (0.5%), c Blank., d Placebo, e Sample solution, 1 impurity-G, 2 impurity-L, 3 impurity-H, 4 impurity-A, 5 impurity-I, 6 impurity-B, 7 DP-I, 8 HPD, 9 bromoperidol, 10 impurity-J, 11 impurity-C, 12 impurity-D, 13 impurity-K, 14 impurity-F, 15 impurity-E.

of heat and additive concentrations were tested to arrive at suitable degradation conditions that will yield degradation in the range of 2 to 20%. Photolytic stress was performed both at visible stress and UV stress as per ICH Q1B conditions and the product is stable to light exposure.[25] While heat stress is not able to generate significant degradants at 80°C for about 2 days. It was observed that acid, base and peroxide stress conditions were able to generate considerable degradation of HPD injection. Acid stress was performed by heating HPD injection in diluent at 80°C for about 1 hr., with addition of about 10 mL 1N alcoholic hydrochloric acid. Base degradation studies were performed by heating HPD Injection in diluent. The presence of any overlapping peak at known impurities along with peak purity of HPD were evaluated to prove stability indicating nature of the method. Peak purity and mass balance studies confirm noninterference of degradation products and the data was presented in Table 6. Peroxide stress and base hydrolysis were found to be highest labile conditions and an unknown degradation product was observed in peroxide stress at high levels (DP-I), which is also seen during routine stability studies of HPD injection (Figure 10). Impurity-G and L were observed to be degradation products in base degradation stress.

Table 6: Forced degradation data.

Impurity Name	Degradation condition			
	Control	Base	Acid	Peroxide
Impurity-A	0.01	ND	ND	ND
Impurity-B	0.05	0.03	ND	ND
Impurity-C	ND	ND	ND	ND
Impurity-D	ND	ND	ND	ND
Impurity-E	ND	ND	ND	ND
Impurity-F	ND	ND	ND	ND
Impurity-G	ND	2.66	1.20	ND
Impurity-H	0.04	0.06	0.02	0.04
Impurity-I	0.04	0.09	ND	ND
Impurity-J	0.04	0.08	ND	0.03
Impurity-K	ND	ND	ND	ND
Impurity-L	ND	0.50	0.06	ND
Bromoperidol	ND	ND	ND	ND
DP-I	0.01	0.01	0.02	4.82
Single maximum unknown	0.01	0.29	1.77	0.09
Total Impurities	0.19	4.78	5.62	5.04
%Assay	99.9	91.4	93.9	92.9
Mass balance	NA	96.1	99.4	97.9
Purity angle	0.402	0.241	0.414	0.980
Purity threshold	0.634	0.331	1.652	1.435

ND: Not detected; RRT: Relative retention time; Base: 0.25 N NaOH at 80°C for 2 hr.; Acid: 1 N HCl at 80°C for 1 hr.; Peroxide: 30% hydrogen peroxide for 1 hr. at room temperature.

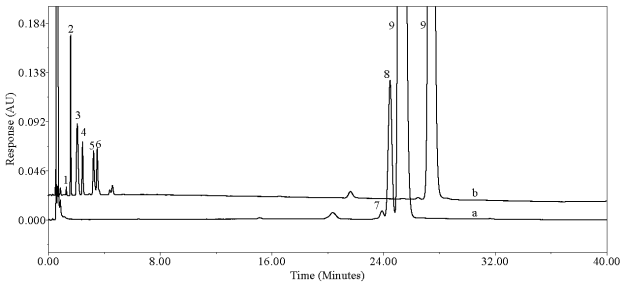

Figure 10: Forced degradation chromatograms; a Peroxide stress, b Base stress, 1 impurity-G, 2 impurity-L, 3 unknown impurity-I, 4 unknown impurity-II, 5 unknown impurity-III, 6 unknown impurity-IV, 7 impurity-B, 8 DP-I, 9 HPD.

CONCLUSION

The developed method over this work for the determination of related substances of haloperidol decanoate in depot injection is specific, linear, precise and accurate and allows to obtain reliable results of impurities without any interference from blank or sample matrix.

The application of design of experiments methodology with dual designs was successfully employed to resolve artefacts and to resolve all known and unknown impurities of HPD. The use of historical data design for separation and method optimization of related substances method is employed successfully as a novel approach. The optimized method is able to estimate all impurities with known precision and accuracy. This method was successfully implemented for routine quantification of impurities in HPD injection for release and shelf life testing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

USP: United States Pharmacopoeia; **DP:** Degradation Product; **API:** Active Pharmaceutical Ingredient; **DOE:** Design of Experiments; **HPLC:** High Performance Liquid Chromatography; **UV:** Ultra Violet light; **IPA:** Isopropyl Alcohol; **OFAT:** One Factor at a Time; **LOQ:** Limit of Quantitation; **LOD:** Limit of Detection; **ANOVA:** Analysis of Variance; **FDS:** Fraction of Design Space; **MODR:** Method Operable Design Region; **HPD:** Haloperidol Decanoate.

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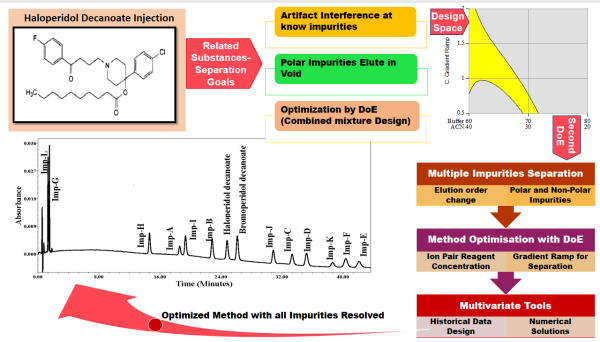
PICTORIAL ABSTRACT



SUMMARY

Related substances method for Haloperidol Decanoate injection is developed with the help of design of experiments. Dual designs are implemented to resolve artifact interference and to separate closely eluting impurities. With the help of historical data design tool, critical resolutions of closely eluting impurities is optimized. Second ion pair reagent is introduced to retain polar impurities. Design space for all the critical variables of the method is established. The final chromatographic conditions are validated to establish accuracy, precision and range of the method.

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