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RESEARCH ARTICLE

FORMULATION OPTIMIZATION AND CHARACTERIZATION OF NEWSTABLE READY TO USE (RTU) INJECTABLE FORMULATION OF ROCURONIUM BROMIDE

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Abstract

Commercially available injectable Rocuronium Bromide, a non-depolarizing neuromuscular blocker intended to store in a refrigerator at 2-8°C, requires an additional storage facility. The primary objectives of the present research were to design a stable, Ready to use (RTU) formulation of Rocuronium Bromide (RCB) Injection for Intravenous (IV) administration for storage at room temperature. Beta cyclodextrin (BCD) forms inclusion complexes with RCB. Edetate Disodium (EDTA) helps in achieving within the specification limit of Impurity C (Imp. C), a major metabolite of rocuronium bromide and Assay. Other excipients were selected to achieve a stable injectable formulation and cause less pain at the injection site. The formulation was optimized by Design of experiments (DoE) applying Central Composite Design (CCD) using Design Expert software and established design space where the formulation meets the desired acceptance criteria. The obtained optimized formulation can be terminally sterilized by moist heat and evaluated for thermal stability and robustness. The robustness of the formulation was established through Temperature excursion studies, pH sensitivity, and Oxygen sensitivity studies. Shelf life was estimated for 19 months when stored at 22°C which is an improvement to the currently available brands with 2-8°C storage. The formulation does not contain acetate buffer, which was identified as the root cause of the pain at the vascular site and may cause less vascular pain at the injection site. The current research work is encouraging in a positive way to achieve the objective of stable Rocuronium bromide injection at ambient conditions to enhance patient compliance.

Introduction:-

Rocuronium Bromide (RCB) is a nondepolarizing neuromuscular blocker commonly used in perioperative care. It is indicated as a supplementary to general anaesthetic medications for the muscle relaxation during surgeries or while using mechanical ventilators.RCB causes muscle relaxation and, depending on the dose and duration between doses the onset of action may be rapid to intermediate. It is available for intravenous (IV) administration either as bolus injection or continuous infusion. RCB Injections are commercially available at concentration of 10 mg/mL in a 5 ml or 10 ml containers for multiple doses. The well-known brands are Zemuron®, Esmeron® andEslax® approved in US, Europe, and Japan respectively.

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The commercially available RCB injections are intended to store at refrigerated condition i.e., 2-8°C. Any products that are intended to store in 2-8°C need an additional storage device such as refrigerator for storage and also required a cold chain supply which is expensive and not feasible all the times. While drug products intended to store at room temperature does not require any additional equipment for storage or transportation purposes, this makes it the first choice for any formulators developing drug products. The properties of drug product which affect the stability are the deciding factors for the intended storage condition selection. If the drug product is stable enough then the room temperature is the most preferred storage condition. If stable RCB Injections are formulated, can be stored at ambient conditions and it will help to avoid additional storage equipment in hospitals.

Also, the products intended for IV administration of RCBare associated with vascular pain at along with withdrawal reflexes of hand.Even, when the patient is non conscious, there is a movement of arm during the injection and recall a pain after coming to consciousness, some patients reported. The reflex pain is associated with the ,use of high concentrations of acetate buffer used in the brand formulations of RCB.(1)Numerous studies were performed on reduction of withdrawal reflexes and pain associated with RCB injection.(2,3)The possible options are to develop new formulations, combined medication are possible ways to clinically benefit the patients from the pain and withdrawal reflexes.

The combined medication method i.e., use of additional drugs along with RCB are studied by various researchers. The use of Ketamine, Ondansetron, Lidocaine, Tramadol, Fentanyl, Remifentanil, Alfentanil, Magnesium Sulphate, Thiopental, Sevoflurane inhalation, Paracetamol, Nitrous oxide, Propofol, Alfaxalone, Sufentanil, Tramadol, Meperidine etc before injecting RCBshown to reduce the vascular pain or simultaneous movement at various levels.(3) However, diluting the RCB Injection also shown to reduce the vascular pain to some extent.(4)

The new formulation strategies includein the patent EP3162370A, where, formulating with Glycine/Hydrochloric acid buffer system at pH 2.8-3.2 claimed to reduce the pain and it is approved for commercial use in Japan. They claimed that the formulation is stable at 40°C and at 6 Month, the Impurity C (Imp. C) levels found to be less than 5.0%. Also, the said formulation is less painful than the Brand formulation ESLAX®and identified that use of higher acid concentration is the cause for the pain during injection. But, the Impurity C levels are very higher than the Chinese Pharmacopoeia limit of Not more than (NMT) 2.0%w/v.

There are other publications onRCB injection with stability enhancement. A Chinese patentCN101653412B claims that, RCB injection is stable with EDTA and Calcium EDTA at concentrations of 1 mg/mL to 10 mg/mL, pH was maintained by Acetate buffer system. The solutions were able to be sterilized by terminal sterilization at 115°C for 15 minutes. The formulation was shown to be stable upto 6 months at 30°C.(5)However, the sterilization at 115°C is not the industry standard and shelf life of the formulation was not revealed.

EP2712611A1, revealed a formulation with D-gluconic acid, and preferred buffer system is Acetate/citrate buffer and adjusted the pH to 3.8-4.0. The solutions were able to sterilized by terminal sterilization at 121°C for 15 minutes. The formulation was evaluated in 40°C and 55°C for 48 days and made a shelf-life estimation of about 120 months as per Zero order degradation kinetics.(6)The research work documented a very long predicted shelf life but there is no consideration for the risk of vascular pain.

In patent WO 2008/065142 A1, the inventors formulated RCB injection using Sulfoalkylethane-beta cyclodextrin (SBECD) at 8 mg/mL using Phosphate buffer system and pH adjustment to 7.1 using ammonium hydroxide solution. However, the formulations were analysed after autoclaving for 1 hr at 121°C and after storing for 44 hours in 22-24°Cwhich may be insufficient to estimate the shelf life.(7)

Thus, earlier research performed on RCB was not able to address the objective of present research work using quality by design approach. The use of statistical designstransformed the optimization of formulation in a more understandable way than the conventional one factor at a time method. Another advantage is the statistically planned design of experiments leads to defining design space for the desired responses in acceptable range. The formulation changes within the design space would not require a further regulatory review and helps to reduce the post approval submission processes.(8)

The present research envisaged to prepareReady to use, Stable formulation of RCB Injectionthat can be stored in room temperature that may causes reduction in pain at the injection site Optimized by Response Surface study applying CCD.

BCD which is known to impart stability to drug molecules by entrapping the drug in the cavity of BCD.(9,10)EDTAhelps to improve the stability by sequestering the metal ions in aqueous solutions. In the composition, rationale for selection of Mannitol as tonicity agent is to make a more stable formulation of RCB. As the EP patent EP2712611A1(7), Formulation with mannitol is showing more stability than that of Sodium chloride. The concentration of Mannitol was varied to adjust the osmolality in the range of 270-310 mOsmol/Kg. The Glycine/Hydrochloric Acid buffer was chosen with an objective to prepare a formulation that helps to reduce the pain at the injection site when given as IV bolus. EP3162370A(5), shown an advantage and compatibility of Glycine/Hydrochloric acid buffer for RCB and its advantage in pain reduction at injection site. The pH range of 3.0±0.2 was chosen as the Degradation studies of RCB suggest that it is less hydrolysed in presence of acid and suitable pH range as per literature is 2.5 to 3.5 and many commercial formulations are made at pH 4.0 including the Branded products. So, the pH of 3.0±0.2 was chosen which is within the range.

The response surface designs are most widely used for the optimization of responses. The objective is to optimize the best formulation accurately without going for a tedious full factorial design followed by construction of design space. Response surface study was suggested by the Design expert software considering the number of factors or independent variables (Two) and Central composite design was applied. The Central composite design enables us to optimize the formulation factors and evaluate the responses, interaction between formulation factors and quadratic effects of the formulation factors on the responses.

The box-wilson central composite design is a factorial design with 2k or 2k-p additional points which are also known as axis points or star points. These are based on Two level factorial designs, augmented with centre and axial points to fit quadratic models.(11–13)

As per the preliminary laboratory research, Impurity C is the metabolite of RCB. The major degradation pathway of RCB is through hydrolysis under higher pH, resulting in Impurity C as major degradant. Under higher pH there is an increase in levels of Impurity C with temperature. BCDs form an inclusion complex by entrapment of drug molecule in the cavity of BCD and impart stability to the drug product.

Materials and Methods:-

Materials:-

Rocuronium Bromide was procured from Aspen Oss B.V, Netherlands. Beta cyclodextrin was obtained as a gift sample from Shanghai T&W pharmaceuticals. Edetate disodium, Glycine, Hydrochloric acid were procured from Merck, China. All remaining materials and reagents were of analytical reagent grade and HPLC grade wherever necessary and were used without further purification. Milli-Q-water prepared inhouse was used for all experiments after passing through 0.2µm membrane filter.

The formulation was optimized by using the Response surface method (RSM) that provides how formulation factors can influence the desired responses and statistically establish the design space, where the obtained formulation displays the desired acceptance criteria. When the Optimal combined design was applied for the given formulation factors, it has resulted in application of Response Surface study throughBox Wilson Central composite design (CCD).

Preparation of Injectable Solution:

The formulations were prepared in laboratory using magnetic stirrer. Required amount of Milli Q water was taken in a clean dried beaker and bubbled with nitrogen till dissolved oxygen reaches below 2 ppm. In the 80% batch size of Milli Q water, added and dissolved the required quantity of BCDunder continuous stirring followed by Glycine, EDTA, RCB. Upon formation of clear solution, pH was made up to 3.0 ±0.2 using 1 M Hydrochloric acid solution and final volume is made with Milli Q water, stirred to form a homogenous solution. The obtained solution was filtered through 0.22µ Polyether sulfone membrane filter. The filtered solution is filled into 5 mL clear, type I glass ampoules and sealed with nitrogen headspace. The sealed ampoules were terminally sterilized with Moist heat using Autoclave at 121°C for 15 minutes.

Optimization by Box Wilson central composite design:

The design of experiments was planned tounderstand the impact of BCD, Edetate disodium on the stability of the formulation. The stability is depending on the Impurity C and Assay of RCB and are chosen as the responses for the design of experiments to optimize the formulation. The Input factors and responses for the design of experiments were given in Table 1.

Table 1:- Input factors and responses for Design of experiments.

Independent Formulation Factors				
Factor Identification	Formulation Factors	Units	Levels	
			Low	High
X1	Concentration of BCD	Molar Ratio (or) mg/mL	0.5 (or) 9.3	2 (or) 37
X2	Concentration of EDTA	mg/mL	0.2 mg/mL	2 mg/mL

Dependent Responses			
Response Identification	Response Name	Units	Target or Range
R1	Assay of RCB	%w/v	95-105
R2	EP Impurity C	%w/v	Less than 0.5

Upon the input of the formulation factor and responses in the Design expert software for optimization purpose, an optimal combined design has recommended CCDand the obtained design matrixpresented in Table 2. A total of 13 formulations were prepared according to the design matrix and evaluated.

Table 2:- Design matrix obtained for the formulation factors.

Run	BCD (mg/mL)	EDTA (mg/mL)
1	9.3	0.02
2	23.15	0.11
3	37	0.2
4	23.15	0.237279
5	37	0.02
6	9.3	0.2
7	42.7369	0.11
8	23.15	-0.0172792
9	3.56314	0.11
10	23.15	0.11
11	23.15	0.11
12	23.15	0.11
13	23.15	0.11

Evaluation of Optimization trials:

All the formulations as per design matrix were evaluated for critical quality attributes for an injection formulation i.e., Description, Assay of RCB by HPLC, Impurity C by HPLC, pH, Osmolality, Visible Particles. All the experimental results of design matrix were provided in Table 3.

Table 3:- Experimental results of the design matrix.

S. No	**Material name (Units)**	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**
1	RCB (mg/mL)	10	10	10	10	10	10	10	10	10	10	10	10	10
2	BCD (mg/mL) (X1)	9.3	23.25	37	23.25	37	9.3	43	23.25	3.6	23.25	23.25	23.25	23.25
3	Mannitol (mg/mL) (X2)	15	11.5	8.2	11.5	8.2	15	6.8	11.5	16.3	11.5	11.5	11.5	11.5

4	EDTA (mg/mL)	0.02	0.11	0.2	0.24	0.02	0.2	0.11	0	0.11	0.11	0.11	0.11	0.11
5	Glycine (mg/mL)	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
6	Hydrochloric Acid	Qs to pH 3.0 ±0.2	Qs to pH 3.0 ±0.2	Qs to pH 3.0 ±0.2	Qs to pH 3.0 ±0.2	Qs to pH 3.0 ±0.2	Qs to pH 3.0 ±0.2	Qs to pH 3.0 ±0.2	Qs to pH 3.0 ±0.2	Qs to pH 3.0 ±0.2	Qs to pH 3.0 ±0.2	Qs to pH 3.0 ±0.2	Qs to pH 3.0 ±0.2	Qs to pH 3.0 ±0.2
(Description Clear, Colourless solution)		Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
Assay (% w/v) (R1)		97.3	99.1	99	99.2	98.4	98.6	99	97.1	98.4	99.3	99.2	99.3	99.5
Impurity C (% w/v) (R2)		0.81	0.24	0.66	0.52	0.64	0.6	0.72	0.79	0.74	0.23	0.19	0.22	0.24
Osmolality (mOsmol/Kg)		284	296	288	290	300	282	306	294	279	297	299	299	295
Visible Particles		Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
pH		2.9	3.0	2.9	2.9	2.9	3.0	2.9	2.9	3.0	3.0	3.0	3.0	3.0

From the statistical analysis of the obtained results, optimization was performed by ANOVA as per Quadratic model and the Contour response surface plot and 3D response surface plot for the Impurity C and Assay of RCB were provided as Fig. 1,2,3,4.

Figure 1:-Contour response surface plot for Impurity C.

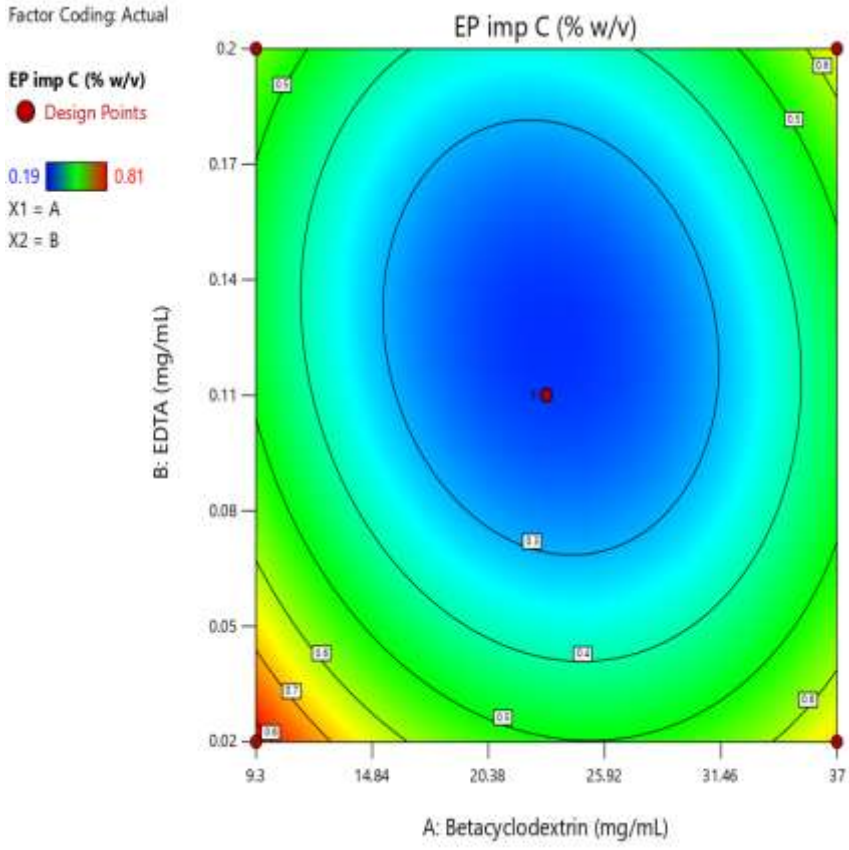


Figure 2:-3D response surface plot for Impurity C.

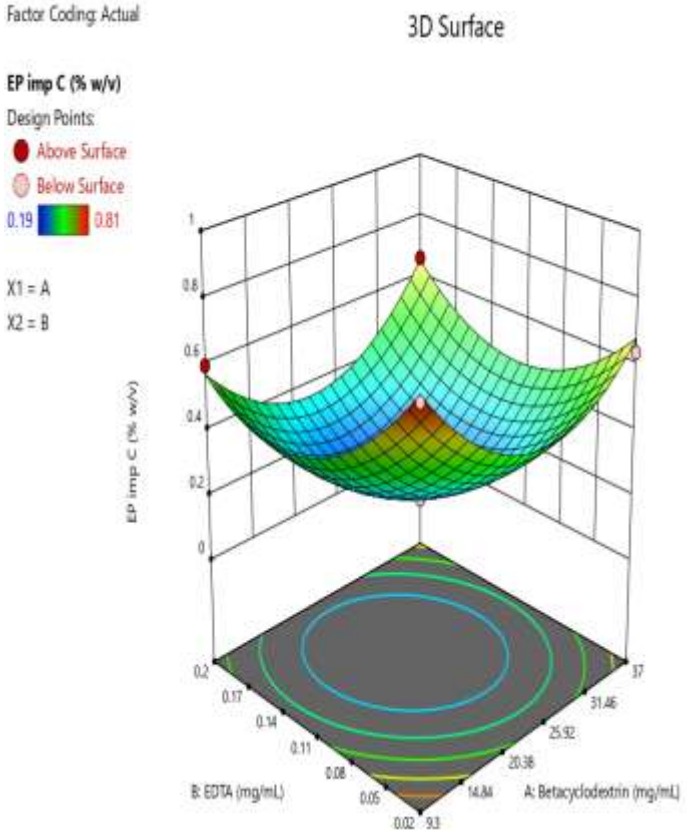


Figure 3:-Contour response surface plot for Assay of RCB.

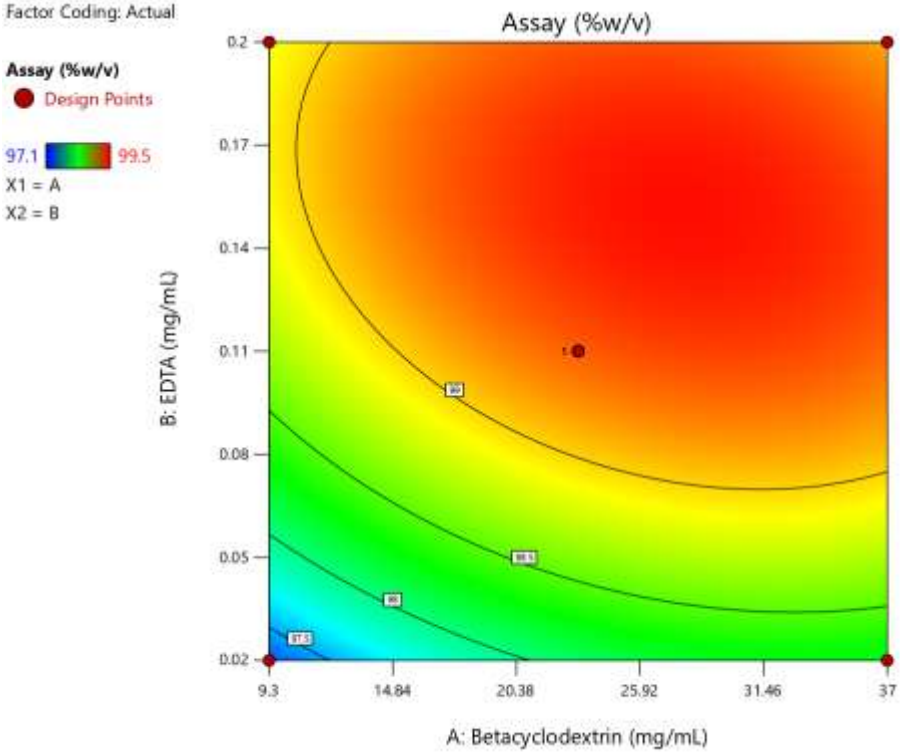
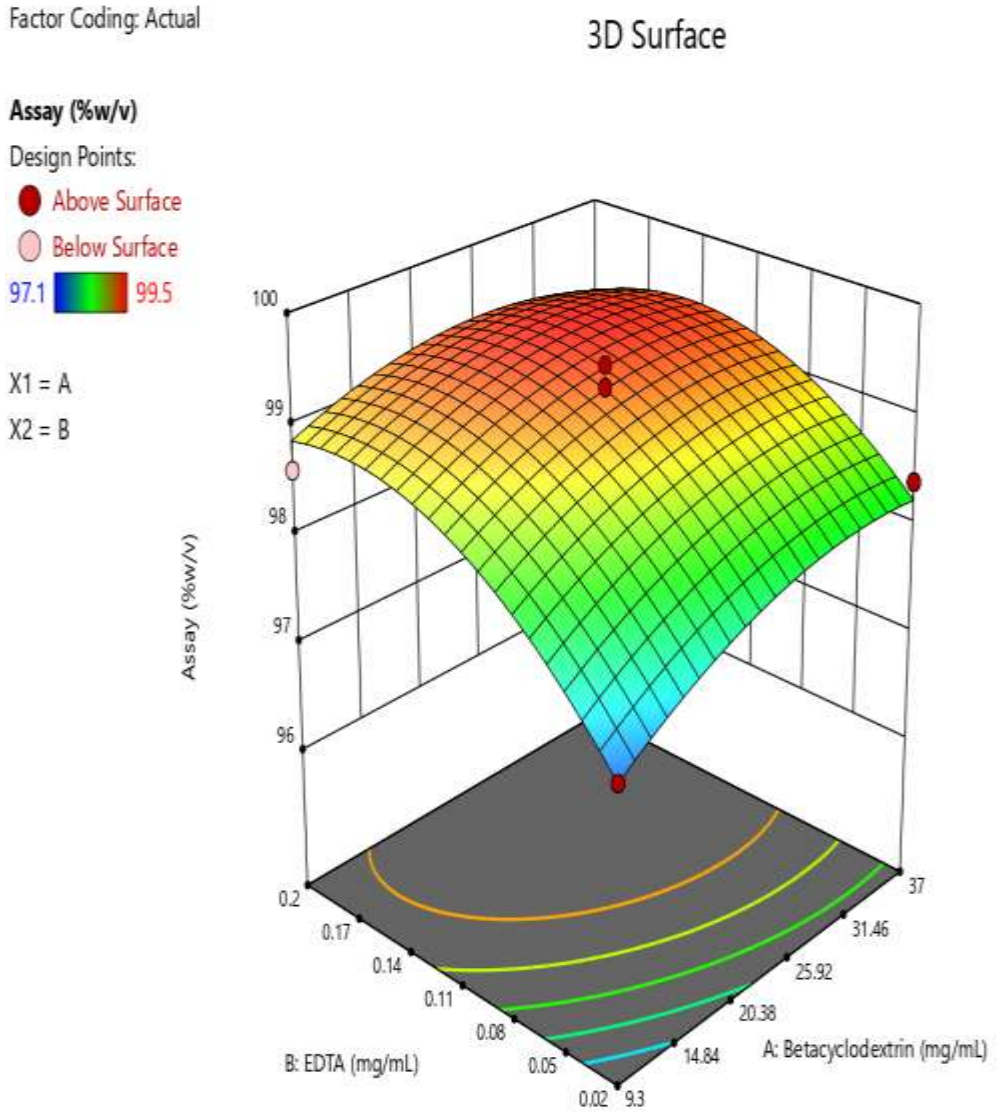
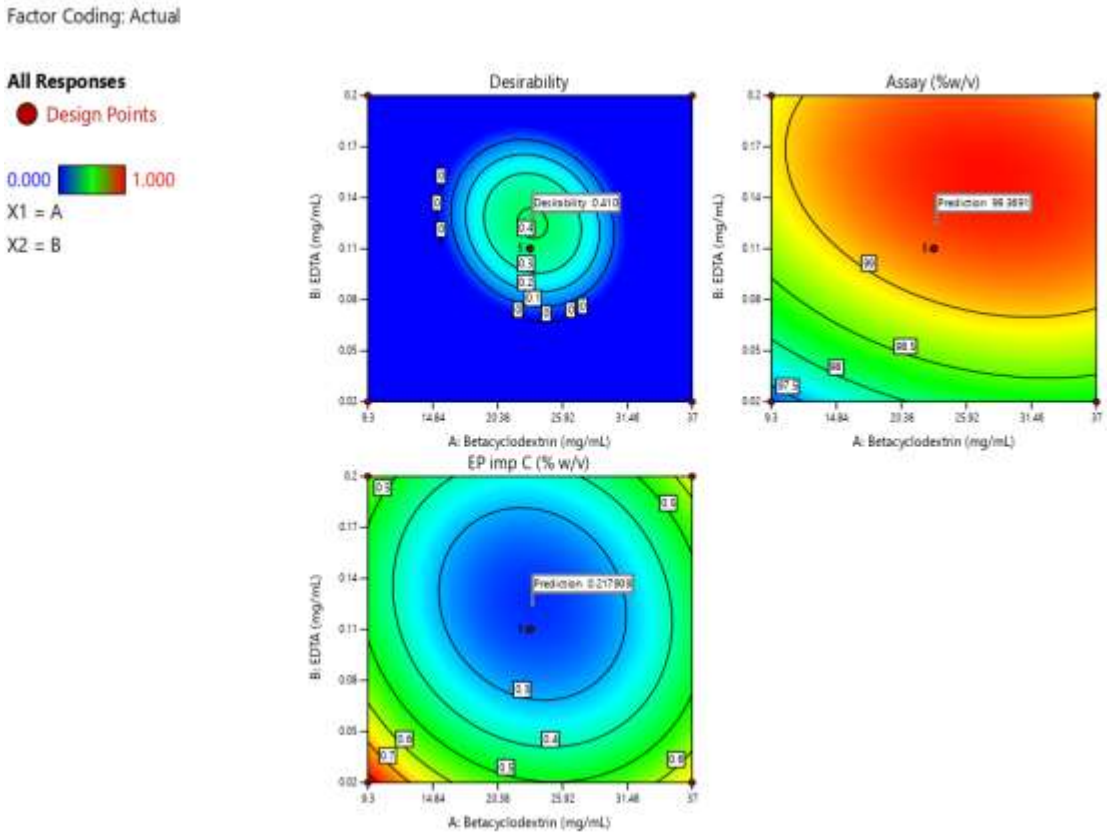


Figure 4:-3D response surface plot for Assay of RCB.



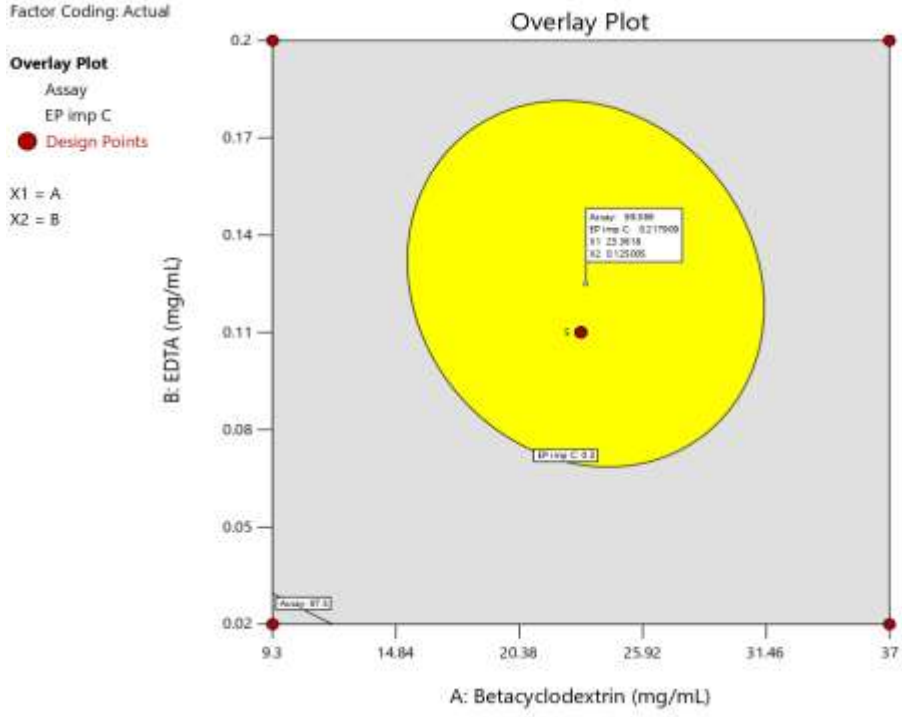
Upon the analysis of responses for the chosen formulation factors, there are no interactions. Hence the design moved to further for Optimization. The Numerical optimization analysis was performed with the criteria of assay between 97.5% w/v to 102.5 % w/v. The Criteria for Impurity C is desired to be less than 0.3%w/v, the analysis was provided in the Figure 5.

Figure 5:- Numerical optimization analysis.



Further upon graphical optimization analysis, design space wasestablished and was provided in Figure 6.

Figure 6:- Design space for the optimized formulation.



From the above optimization graph, the design space for the Formulation factors is established and are tabulated in Table 4.

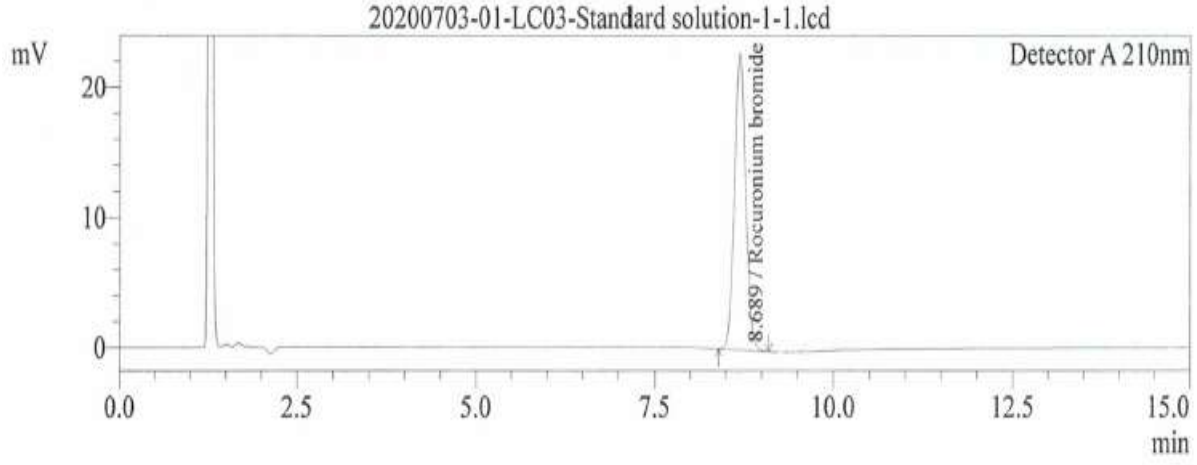
Table 4:- Design space.

Factor	Knowledge space	Design space	Control space
BCD Concentration (mg/mL)	18.0-27.0	15.0-30.0	20.0 -25.0
EDTA Concentration (mg/mL)	0.08-0.16	0.07-0.18	0.09-0.13

Characterization of Formulations:

Assay by HPLC: RCBAnalysis were performed by using Shimadzu HPLC (model Prominence-I LC2030C 3D plus series) with Photodiode array detectors with a run time of 15 minutes. The method employed a Hypersil silica (250x4.6)mm,5µm column, injection volume of 5µL, and mobile phase composed of acetonitrile: phosphate buffer (pH 7.4) (90:10, %v/v) under an isocratic flow of 2.0 mL/min. This method was previously validated according to ICH Guidelines (ICH-Q2 (R1), 2005).(14) A typical chromatogram was presented as Figure 7.

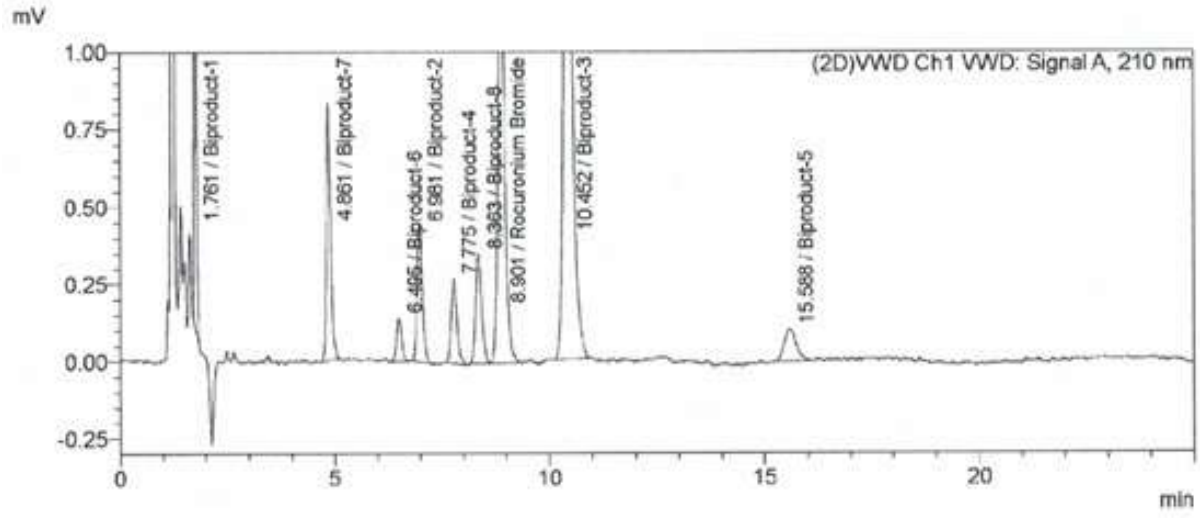
Figure 7:- Typical Chromatogram from Assay of RCB by HPLC.



Related substances by HPLC:

RCBAnalysis were performed by using Shimadzu HPLC (model Prominence-I LC2030C 3D plus series) with Photodiode array detectors with a run time of 25 minutes. The method employed a Hypersil silica (250x4.6)mm,5µm column, injection volume of 5µL, and mobile phase composed of acetonitrile: phosphate buffer (pH 7.4) (90:10, %v/v) under an isocratic flow of 2.0 mL/min. This method was previously validated according to ICH Guidelines (ICH-Q2 (R1), 2005).(14) A typical chromatogram was presented as Figure 8.

Figure 8:- Typical Chromatogram from Related substances of RCB by HPLC.



Osmolality:
Osmolality of formulations were determined by Gonotec® Osmomat 3000 Freezing point osmometer. pH was measured by using Mettler Toledo pH meter for all formulations.

Evaluation of Optimized Formulation:
From the experimental Design data, the optimized formula is containing BCD at23.25 mg/mL and EDTA at 0.11 mg/mL.The formulation was studied for below studies for characterization and robustness.

1. Thermal stability study up to 3 months as per ICH Q1A (R2)
2. Photostability study as per ICH Q1B
3. Freeze thaw study (Three cycles)
4. pH sensitivity at lower and higher pH
5. Oxygen Sensitivity study
6. Short term Excursion study at 50°C

Thermal Stability study:
The prepared samples of optimized formulation were loaded into stability chambers in stability conditions as per guideline ICH Q1A (R2)(15), and additionally loaded in intended storage condition.After holding the samples in storage conditions mentioned above for the scheduled time, samples were withdrawn and analysed for critical parameters of Assay of RCB and Imp. C, the data is provided in Table 5.

Photostability study (PS):
RCB Injection samples were exposed to light intensity of not less than 1.2 million lux hours and near ultraviolet energy of not less than 200 watt hours/square meter. All samples are exposed by placing horizontally in photostability chamber as per guideline ICH Q1B.(16)The samples are exposed in ampoules i.e., primary pack, Ampoules packed in secondary pack. Also, ampoules wrapped in aluminium foil are used as control. The exposed samples were evaluated for Critical parameters (Table 6).

Freeze thaw study (FZ):
RCB Injection samples were subjected to freezing at -20°C for two days followed by thawed in room temperature and the same samples held in 40°C±2°C/75% RH±5% RH for two days. This constitutes one freeze thaw cycle. The same process is repeated for another two times to complete three successive freeze thaw cycles. The exposed samples were evaluated for Critical parameters (Table 6).

Short term Excursion study (ST):
RCB Injection samples were held at 50°C for 6 weeks and tested for critical parameters at Initial, 2 Weeks, 4 Weeks, and 6 Weeks (Table 6).

pH sensitivity Study (PH):
RCB Injection formulations were prepared at lower pH of 2.8 and higher pH of 3.2. Samples were loaded into stability chambers at Accelerated stability condition. The samples were tested at Initial, 1M and 3 M time points for critical parameters (Table 7).

Oxygen Sensitivity study (OS):
RCB Injection formulations were prepared at without using Nitrogen during preparation and filling, samples were loaded into stability chambers at Accelerated stability condition. The samples were tested at Initial, 1M and 3 M time points for critical parameters (Table 7).

Results and Discussion:-

Table 5:- Thermal stability data.

Storage Condition	Time Point	Assay of RCB (%w/v)	Impurity C (%w/v)
Long term Stability (25°C±2°C / 60% RH±5% RH)	Initial	100.1	0.22
	15 Days	99.8	0.30
	1 Month	99.5	0.37
	2 Month	99.1	0.45

	3 Month	98.7	0.55
Intermediate Stability (30°C±2°C / 65% RH±5% RH)	Initial	100.1	0.22
	15 Days	99.6	0.38
	1 Month	99.2	0.49
	2 Month	98.8	0.66
	3 Month	97.9	0.88
Accelerated Stability (40°C±2°C / 75% RH±5% RH)	Initial	100.1	0.22
	15 Days	99.2	0.34
	1 Month	98.3	0.56
	2 Month	97.5	0.78
	3 Month	96.9	0.97
Intended Storage condition(22°C±2°C/ 60% RH±5% RH)	Initial	100.1	0.22
	15 Days	99.9	0.26
	1 Month	99.7	0.31
	2 Month	99.4	0.38
	3 Month	99.1	0.47

Table 6:- Photo stability, Freeze thaw and short-term excursion data.

Study	**Initial**	**Photostability Study**			**Freeze Thaw Study (Three Cycles)**	**Short Term Excursion study at 50°C**		
Test Parameter / Conditions &Time points		**Primary Pack**	**Secondary Pack**	**Aluminium Wrap**		**2 Week**	**4 Week**	**6 Week**
Description (Clear, Colourless solution)	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
Assay (% w/v)	99.9	99.4	99.6	99.4	99.2	99.1	98.5	98.0
Impurity C (% w/v)	0.20	0.23	0.25	0.22	0.32	0.47	0.72	0.95
Osmolality (mOsmol/Kg)	298	296	296	295	298	295	296	296
Visible Particles	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
pH	3.0	2.9	3.0	3.0	2.9	3.0	2.9	2.9

Table 7:- Robustness study data (pH Sensitivity and Oxygen sensitivity study data).

Study	**pH sensitivity study-Lower pH 2.8 @ 40°C±2°C/75% RH±5% RH**			**pH sensitivity study-Higher pH 3.2 @ 40°C±2°C/75% RH±5% RH**			**Oxygen sensitivity study @ 40°C±2°C/75% RH±5% RH**		
Test Parameter & Time points	Initial	1 Month	3 Month	Initial	1 Month	3 Month	Initial	1 Month	3 Month
Description (Clear, Colourless solution)	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies
Assay (% w/v)	100.0	98.4	97.3	99.8	98.6	97.0	98.4	99.3	99.2
Impurity C (% w/v)	0.24	0.38	0.85	0.26	0.54	0.99	0.23	0.56	1.05
Osmolality (mOsmol/Kg)	302	301	301	288	290	294	297	297	299
Visible Particles	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
pH	2.8	2.8	2.8	3.2	3.2	3.2	3.0	3.0	3.0

Optimization trial data reveals that, there is reduction in Impurity C with use of BCD upto certain extent but again impurity raised. The concentration of EDTA has a direct effect in the formulation and the Impurity C levels are

gradually decreasing with increase in the EDTA. However, any one of BCD or EDTA may not be able to stabilize the formulation. Hence, the stable formulation was obtained at the levels of 1.25 molar ratio of BCD and 0.11 mg/mL of EDTA.As without EDTA formulation has reduced assay, it clearly shows EDTA has direct impact on the Assay. The impact of BCD is not clear form the data, but also unable to ignore the factor. Formulations were prepared as per the design matrix generated by Design Expert® software (trial version 13, Stat -ease Inc). All the executed batches were analysed for the critical quality attributes and responses were entered into the software. Based on the regression analysis by Analysis of Variance (ANOVA), recommended the Quadratic model to navigate the design space. The Contour response surface plot and 3D response surface plot for the responses were generated by numerical optimization. Based on the desired criteria, design space was created by graphical optimization. The obtained optimized formula was verified by preparing three consecutive batches and their critical responses were compared against 95% prediction interval.

Thermal stability data reveals the obtained formulation was able to maintain the impurity levels below 1.0% w/v by the end of 3 M in all stability conditions including accelerated stability condition. All other critical quality attributes were meeting the acceptance criteria at the 3 M time point in all stability conditions. The stability results were extrapolated for long-term stability condition and Intended storage condition, using the statistical tool Minitab 19 trial version (Minitab, LLC). The predicted shelf life for Assay is 15.7 months, 20.2 months whereas for Impurity C is 13.6 months and 19.9 months when stored at long-term stability condition and Intended storage conditions respectively.As evidenced in data, Optimized formulation was found to be stable to light in primary pack. Hence, the formulation is photostable and no additional precautions are required while handling.After three consecutive freeze thaw cycles, there are no significant changes in impurity levels, assay and other critical quality attributes. Also, when stored at 50°C, there is an increasing trend of impurity but are within the acceptable range considering the higher hydrolysis rate with temperature. However, the data suggest, the formulation can sustain the temperature variations during transit of drug product.

pH Sensitivitystudy was performed to evaluate the robustness of the formulation. The lower pH formulation has shown lower impurity levels than that of higher pH formulation. This once again confirm that lower pH favours the stability of formulation. The optimized formulation is found to be stable in the proposed pH range.In Oxygen sensitivity, where formulation is prepared under ambient condition without using inert gas at any stage. The Impurity C levels tend to increase slightly and are crossing 1.0%w/v by 3M at accelerated condition. These results are slightly higher than that of formulation prepared using inert gas. Hence, the formulation manufacturing requires inert atmosphere while bulk solution preparation, filtration and filling to get a better stable product.The formulation is not containing the acetate buffer which is found to be the causative for the withdrawal reflexes or burning pain at the injection site with RCB injection brands. However, the pain assessment studies are scheduled after fine tuning the optimized formulation for even better stability results i.e., up to 24 months in long term stability condition.

Conclusion:-

The current work envisaged to address the requirement of special storage equipment such as refrigerators which are required with the existing marketed formulations of RCB injections. The formulation was containing BCD, a complexing agent and EDTA, an antioxidant which are optimized to provide a stable formulation. Other excipients are chosen by keeping a view on stability and pain reduction. The optimization was performed using the CCD and was confirmed with three reproducible batches. A design space was established for the chosen formulation variables to meet the desired criteria. During stability evaluation, found to be stable up to 3months in 40°C. When stored at 22°C, predicted shelf life of 19 months was attained which is sufficient for regulatory filing of the formulation and is capable of commercialization. However, there is a lot of work required be performed in this regard including clinical studies about the withdrawal reflexes. Conclusively, the obtained data is fairly encouraging and gave a hope of a product which is stable at ambient temperature and provide a better patient compliance.

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