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Design And Development Of Generic Formulation By Applying Reverse Engineering Approach

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Abstract

Introduction: The innovator formulation is meat for its high cost as Innovator Company used to invest millions of dollars. The generic formulation is an alternative to beat the cost. The criteria of generic formulation is should have the bioequivalence as that of RLD (Reference Listed Drug). Reverse engineering is decoding of the RLD to find out qualitative and quantitative composition. Rationale behind reverse engineering is if qualitative and quantitative composition of the formulations is same then performance of the formulation will also be same. That helps to speed up the process of generic formulation development and reduces cost and time. The formulation used in study is anti-opioid uni-dose nasal spray 4mg/0.1ml. The formulation contains of anti-opioid drug, benzoalkonium chloride(BKC), EDTA, sodium chloride (NaCl) and pH adjusters like hydrochloric acid, sodium hydroxide.
Objectives: The main objective of current research is to perform reverse engineering of RLD to find out the qualitative and quantitative composition by using different analytical methods and validate the same as per ICH guideline. To develop generic formulation by using data obtained from reverse engineering. To perform similarity matrixing for in-house and RLD formulation.
Methods: Different analytical methods were used to perform the reverse engineering of the RLD to find out the qualitative and quantitative composition like HPLC for benzoalkonium chloride and EDTA. Flame photometry for the NaCl, and the Nano-drop UV for the Anti-opioid drug. The Assay, pH, osmolarity and viscosity were performed for complete characterization of the RLD while for in-house to show the similarity matrixing.
Results: The de-formulation was performed for all the components and the methods were validated as per ICH guideline. The correlation coefficient was >0.99 for all components in all the methods. The recovery results ranged from 99.4 to 99.6% for anti-opioid drug, 99.83−100.33% for BKC, 100.02−100.23% for NaCl and 100.3−100.7% for EDTA. The obtained % RSD for precision study were 0.87% (intraday) and 0.76% (interday) for anti-opioid drug, 0.01% (intraday) and 0.1 (interday) for BKC, 0.62% (intraday) and 0.54 (interday) for NaCl and 0.62%(intraday) and 0.54 (interday) for EDTA. There were no interference of other components and methods are specific.
Conclusions: The reverse engineering gave critical information about the formulation apart from qualitative and quantitative composition like process used by innovator to stabilize the formulation, viscosity of formulation and mechanism of uni-dose nasal spray to provide the better efficacy. That saves time, cost and manpower incurred for the trial batches. The validation of methods gave an assurance for the qualitative and quantitative composition helped for getting affordable bioequivalent generic formulation.

Keywords: De-formulation, Reverse Engineering, ant-opioid nasal spray.

1. Introduction

Reverse engineering/de-formulation implemented for faster development of generic formulation development. During reverse engineering the Reference listed drug (RLD) formulation is decoded to get the qualitative and quantitative composition. Reverse engineering process aims to recreate the generic formulation which can show similar performance as that of RLD.(1) The main rational behind this is when the qualitative and quantitative composition of formulation is known then the performance of generic formulation will also be same.

So to achieve the similarity matrixing between the RLD and generic, one has to keep the qualitative and quantitative composition same as that of RLD. (2)
Parenteral drug product injections, topical products, ophthalmic, and otic solutions can get biowaiver by proving the similarity in qualitative and quantitative aspects with RLD products. Generic formulation can be submitted by Abbreviated New Drug Application (ANDA) to get biowaiver it is necessary to show similarity matrixing with respect to physicochemical properties with RLD. Moreover, Q1 (Qualitative composition) and Q2 (Quantitative Composition) should be identical for Parenteral drug products. For the generic formulation to get similarity in performance with respect to RLD,(3) It is necessary to know the qualitative and quantitative composition of RLD this can be possible by decoding the formula of RLD.(4-7)
Rising deaths due to prescription and illicit opioid overdose was an alarming situation in USA. Although opioid reversal injection is also available which safe and efficacious treatment for opioid overdose is, it is sometimes unavailable on

time due to legal and practical restrictions for layman. Now a day opioid reversal nasal spray is available as over the counter product in US market to overcome this issue, but the product is not affordable economically for everyone. The administration of nasal spray doesn't require trained person or physician like injection layman can also administer the formulation at home. [8]

Opioid reversal nasal spray is safe and effective during opioid overdose treatment and can be administered by layman during emergency, can shorten the time of reversal of opioid toxicity and reduces chances of opioid-related deaths. Drug in formulation is an opioid antagonist it rapidly reverses an opioid overdose. The mode of action is it attaches to opioid receptor and displaces the opioid and quickly restores normal breathing if breathing slowed down or stopped due to opioid overdose.[9]

The drug in formulation does not have any effect on those who do not have opioid in their system. Opioid reversal nasal spray works only within 30 to 90 minutes by reversing opioid by drug in overdose. But many opioid remain in the body beyond 30-90min. Due to this there may be possibility that person can experience the effects of an opioid overdose after effect of drug wears off. Few opioid are potent and may require multiple doses. [10]

The current research targets the de-formulation of marketed opioid reversal nasal spray formulations. The study on marketed RLD aims generic formulation development to match the performance of generic formulation with the RLD. Opioid reversal nasal spray consist of the following Ingredients: anti-opiod drug (4mg/0.1ml) as an active pharmaceutical ingredient, Benzoalkonium chloride as preservative, ethylenediaminetetraacetic acid (EDTA) as a chelating agent, and NaCl as a tonicity agent. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were used for adjustment of pH.

Fig 1 Process of generic development by Reverse engineering

The anti-opioid drug concentration was known still as part of confirmation the drug concentration was analyzed by using the nano-drop UV, this gave an idea about the interference of other component in formulation and helped a lot to take decision regarding orthogonal tools to find out the other components. Nano-drop UV is highly sensitive UV spectrophotometer requires a single drop to analyze the formulation.

EDTA used with Benzoalkonium chloride to enhance the antimicrobial activity of formulation, acts as a chelating agent in the formulation is not a UV active, derivatization technique with an ion-interaction chromatographic technique were used to quantify EDTA on high performance liquid chromatography (HPLC) with a UV detector to get qualitative and quantitative composition. The isocratic method was applied for the analysis of EDTA. The $CuSO_4$ was used to derivetised EDTA by forming EDTA-Cu complex.

$$Cu^{2+} + (EDTA)^{2} \rightarrow Cu(EDTA)^{2-} + 2H^{+}$$

Benzoalkonium chloride (BAC) is used as a bacteriostatic agent in the nasal spray formulation, as a preservative. BAC, is a mixture of alkyldimethylbenzylammonium chloride, a quaternary ammonium salt, The alkyl substituent's C12 and C14 with the quaternary ammonium salt. The homologues are having different physical, chemical and microbiological properties. The efficacy of a BAC depends on the content of appropriate homologues in the mixture. Therefore it is necessary to know benzoalkonium chloride identity and content in nasal spray formulation. So it is necessary that method should resolve both the homologous peak to get proper qualitative and quantitative composition.

Sodium chloride is used as osmotic agent. The osmolarity of dosage affect nasal the nasal absorption of drug. The higher concentration of drug can cause higher bioavailability, also leads to toxicity to nasal epithelial tissue. Also act as moisturizer to nasal passage.
The flame photometry was used to quantify the NaCl. The flame photometer was used to determine the concentration of NaCl in a formulation. The flame photometer works on principle an alkali metal salt drawn into a non-luminous flame get ionize, absorbs energy from the flame and emits light of characteristic wavelength as the excited atom comes to ground state. The intensity of emission is proportional to concentration of Sodium chloride in the solution Sodium chloride consist of sodium and chloride. [11-12]

2. Objectives

Anti-opioid nasal spray consists of 4 major components an anti-opioid drug, Benzoalkonium chloride EDTA solubilised in NaCl saline solution. The anti-opiod drug concentration is lable claim which is known and need to cross verify for the efficacy of formulation.
To find out specific analytical method for the quantification of various component present in the formulation validate the same as per ICH guideline
To develop the generic formulation by using data obtained from the reverse engineering of RLD.
To perform the similarity matrixing for RLD and In-house batch.

3. Methods

EDTA:[13-14]
Preparation of 2M Ammonium Acetate solution: 3.08g of ammonium acetate in 20ml of volumetric flask 10ml of water was added to it and sonicated for 5min, volume was made upto the mark.

Buffer Preparation: 50mM tetrabutylammonium hydrogen sulfate buffer prepared by adding 17g of tetrabutylammonium hydrogen sulfate in 1000ml of MilliQ water sonicated to dissolve mixed well and pH was adjusted to 4.40 using 2M ammonium acetate solution.

Mobile Phase: Mobile phase was prepared by mixing 300ml of methanol, 170ml of buffer and water 530ml in mobile phase reservoir bottle and degassed using sonicator for 20mins. (Methanol:Buffer:Water) (300:170:530)

Diluent: Diluent was prepared by dissolving 0.24g of ammonium acetate in 1000ml of water and pH was adjusted to 4.4 with glacial acetic acid; to it 0.5g of copper sulfate pentahydrate was added.
Calibration curve was plotted using different calibration standards prepared by using derivatization of EDTA by diluent 5ug/ml, 10ug/ml, 15ug/ml, 20ug/ml, 25ug/ml.

Analyzed unknown concentration of sample against calibration curve and concentration found by using formula.

$$x = \frac{\mathrm{y} - \mathrm{C}}{\mathrm{m}}$$

Where, y-Area obtained of sample,
C- intercept.
m-Slope.

The method was validated using ICH guideline by using following chromatographic condition:

Chromatographic condition:

Chromatographic Condition	
Column	Agilent C8 4.6 X 150mm X 5u,
Flow Rate	0.6ml/min,
Wavelength	254nm
Run time	12.8min,
Injection Volume	20ul,
Column Temp	25°C.
Retention time	7.7min

Benzoalkonium chloride: [15]
Buffer: Ammonium phosphate buffer was prepared by dissolving 11.5g of Ammonium di-hydrogen phosphate in 1litre HPLC reservoir bottle containing 800ml of water, and volume was made upto 1000ml by milliQ Water.

Mobile Phase: was prepared by mixing 400ml of ammonium phosphate buffer with 600ml of Acetonitrile and 5ml of triethalamine, degassed using sonicator for 20 min and filtered using 0.45u filter paper. Buffer:Water (400:600).

Diluent: water used as diluent analysed as blank solution. The calibration curve was plotted using different calibration standards 5ug/ml, 10ug/ml, 15ug/ml, 20ug/ml, 25ug/ml by dissolving solution of BAC STD in diluents. Calculated unknown concentration of sample against calibration curve by using above mentioned formula.
The method was validated using ICH guideline by using following chromatographic condition:

Chromatographic condition:

Chromatographic Condition	
Column	Agilent C8 4.6 X 150mm X 5u,
Flow Rate	0.9ml/min,
Wavelength	215nm
Run time	7.9min,
Injection Volume	20ul,
Column Temp	25°C.
Retention time	7.9min

Sodium chloride: [16]
NaCl quantification was performed using flame photometer The flame photometer consist of detector which detect the flame colour based on presence of element in solution. The intensity of flame is directly proportional to the amount of Sodium in the formulation. The flame and the pump pressure was adjusted and cone type flame was adjusted. The normal gas flame is blue which turns yellow when the sample contains sodium metal. The amount of Na is equivalent to the amount of NaCl present in the sample. The calibration curve was plotted using the different concentration of NaCl standards solution and the sample was analyzed. Directly the instruments shows result in ppm.

Anti-opioid drug: [17-18]
The drug was quantified using Nano-drop UV spectrophotometer. The interference of the Benzoalkonium chloride was seen that's why 283nm wavelength was selected. The diluent for the samples and standard was water. So the water was used as blank and analyzed. 10PPM standard solution was prepared and 10 PPM sample solution was prepared and analyzed using Nano-drop UV spectrophotometer. And % assay was calculated by following formula:

$$\%\text{Assay} = \frac{\text{Abs of sample}}{\text{Abs of std}} \text{ X } \frac{\text{Wt of Std}}{\text{volume of diluent}} \text{ X } \frac{\text{volume of diluent}}{\text{Volume of sample}} \text{ X } \frac{\text{Potency}}{100} \text{ X } \frac{1}{\text{lable claim}} \text{ X } 100$$

The method was validated as per ICH guidelines

Method Validation: [19-20]
Specificity: the specificity was performed for each component by analysing the standard, sample and blank.
Linearity: Calibration curve were plotted for each component to check the lineariy of samples.
LOD and LOQ: lowest limit of detection and highest limit of detection were calculated using the formula.

$$\text{LOD} = \frac{3.3\,\sigma}{S}$$

$$\text{LOQ} = \frac{10\sigma}{S}$$

Where, σ- standard deviation of the response S- Slope of the calibration curve.
Precision: Precision study for analytical methods was performed for all the components to calculate the % assay, mean assay, % Deviation and % relative standard deviation.
Accuracy: Accuracy of the method was performed by standard addition method at 3 different levels. 80%, 100% and 120% was added in sample.
Solution stability study: The solution stability for all components was established for 24 hrs at room temp in the sample solutions.
Robustness: The small deliberate change was performed to check the robustness of study and found the method was robust.

Development of In-house nasal Spray: [21-22]
On decoding the formula of RLD in-house batch was taken and design the formulation by following recipe.

Preparation of 0.74% NaCl solution: 74mg of NaCl (mw 58.44) was dissolved in 100ml of purified water, and used as vehicle for nasal spray.

Preparation of Formulation Placebo:

50mg of EDTA was dissolved in the 50ml of 0.74%NaCl solution to it 10mg of Benzoalkonium chloride 50% was added and mixed well.

Preparation of nasal spray: 2.2g of drug was dissolved in 30ml of placebo and volume was made upto 50ml and ph was adjusted to 4.5 by 0.1N HCl or 0.1N NaOH solution and filled in the glass tube and capped with rubber cork the glass tube was inserted in the nasal spray device needle pointing towards the rubber cork and capped plunger.

Similarity Matrixing: [23]

The similarity matrixing was performed using different performance parameters

Assay of anti-opioid drug:

Assay of drug were perform using nano-drop UV spectrophotometer to check similarity in efficacy.

pH of solution:

1ml of sample was taken in separate test tube of RLD and in-house batch respectively, labelled and pH was reported at 25°C.

Osmolarity:

The performance of osmometer was verified with purified water and 0.9% NaCl solution.

50ul of sample taken in the ependrop tube and sample was fixed to the Osmometer ensured sensor dipped in the ependrop tube, and push the sensor to upward direction.

4 Results

Specificity: the specificity was performed by injecting the standard sample and blank. There were no interference of other component an all components (Figures 2,3,4).

Linearity: Response of the all components was found to be linear in the investigation concentration range and the linear regression equations were reported with correlation coefficient 0.99 for all components. (Table 3,7,11,15).

LOD and LOQ: lowest limit of detection and highest limit of detection were calculated for all components and reported. (Table 4,8,12,16).

Precision: The employed method was found to be precise as the %RSD values for the intermediate precision studies were <1.0% The obtained % RSD for precision study were 0.87% (intraday) and 0.76% (interday) for anti-opioid drug, 0.01% (intraday) and 0.1 (interday) for BKC, 0.62% (intraday) and 0.54 (interday) for NaCl and 0.62%(intraday) and 0.54 (interday) for EDTA. (Table 5,9,13,17).

Accuracy: Accuracy of the method was performed by standard addition method at 3 different levels. 80%, 100% and 120% was added in sample. The recovery results ranged from 99.4 to 99.6% for anti-opioid drug, 99.83−100.33% for BKC, 100.02−100.23% for NaCl and 100.3−100.7% for EDTA. confirms that the method was accurate (Table 6,10,14,18).

Robustness: The small deliberate change was performed to check the robustness of study and found the method was robust.

On reverse engineering the quantity of ingredients used in formulation

Table 1 Qualitative reverse engineering of RLD

Test	Result
Assay	99.76%
pH	4.52
Osmolarity	224

Table 2 Quantitative composition by reverse engineering

Ingredient	Amount observed
BAC	0.01 mg/0.1ml
EDTA	0.1 mg/0.1ml
NaCl	0.74 mg/0.1ml
Anti-opioid drug	4 mg/0.1ml

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Method validation
For Benzoalkonium chloride:

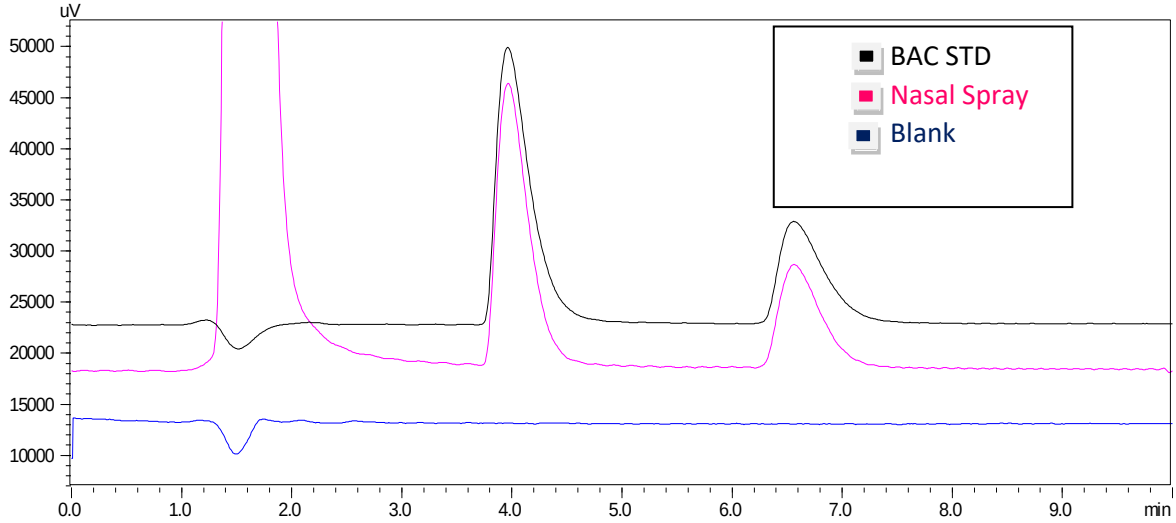

Fig 2 Specificity of BAC

Table 3 Linearity for BAC

Linearity	
CONC (ug/ml)	AREA
5	402221
10	823322
15	1326365
20	1816052
25	2314645

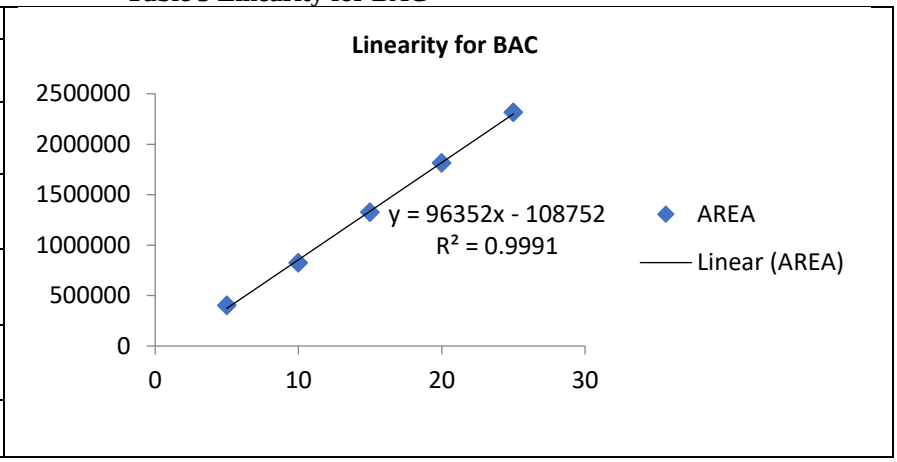

Table 4 LOD and LOQ for BAC

LOD	2.16 ug/ml
LOQ	6.54 ug/ml

Table 5 Precision Study for BAC

Sr. No.	Intraday	Interday
1	99.97	101.35
2	99.97	101.6
3	99.94	101.31
Mean	99.96	101.42
SD	0.017	0.157
%RSD	0.0173	0.155

Table 6 Accuracy Study BAC

Sr. No.	Level	%recovery	mean	SD	%RSD
1	80	99.50	99.83	0.293	0.29
2	80	99.97			
3	80	100.03			
4	100	100.43	100.33	0.083	0.08
5	100	100.27			

6	100	100.29			
7	120	100.49	100.24	0.41	0.41
8	120	99.77			
9	120	100.48			

For EDTA

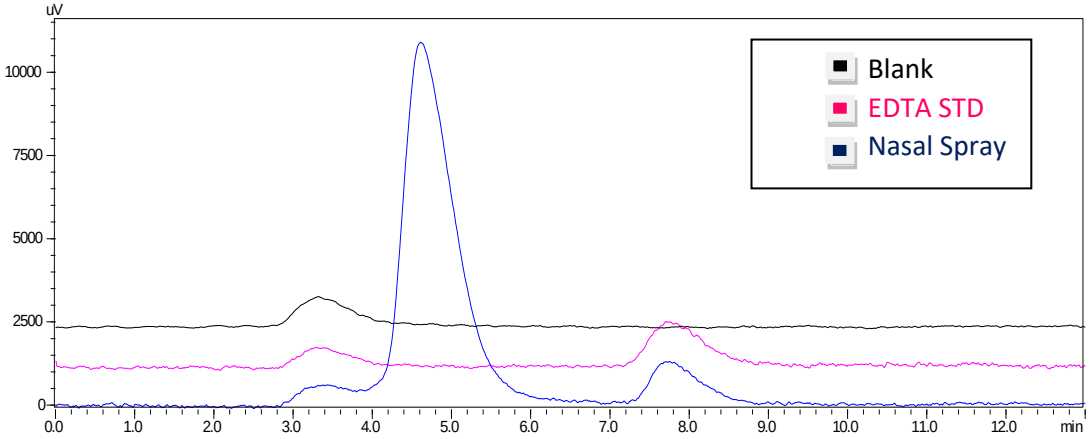

Fig 3 Specificity of EDTA

Table 7 Linearity curve for EDTA

Linearity	
CONCENTRATION	AREA
25	28668
50	56255
75	86230
100	114411
125	140235

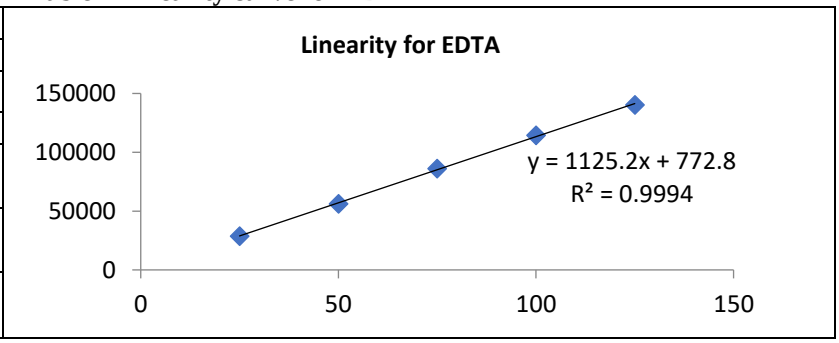

Table 8 LOD and LOQ:

LOD	8.37 ug/ ml
LOQ	25.37 ug/ml

Table 9 Precision Study EDTA

Sr. No.	Intra-day	Interday
1	99.59	100.35
2	99.69	99.94
3	100.82	100.2
Mean	100.03	100.16
SD	0.68	0.20
%RSD	0.68	0.20

Table 10 Accuracy study for EDTA

Sr. No.	Level	%recovery	mean	SD	%RSD
1	80	100.06			
2	80	100.25	100.29	100.30	0.25
3	80	100.57			
4	100	99.51			
5	100	99.3	99.70	99.70	0.44
6	100	100.2			

7	120	100.31	100.33	100.34	0.22
8	120	100.57			
9	120	100.48			

Table 11 Linearity curve for NaCl

Linearity	
Cocn %w/v	PPM
0.16	161
0.45	451
0.65	650
0.75	741
0.9	900
1	1000

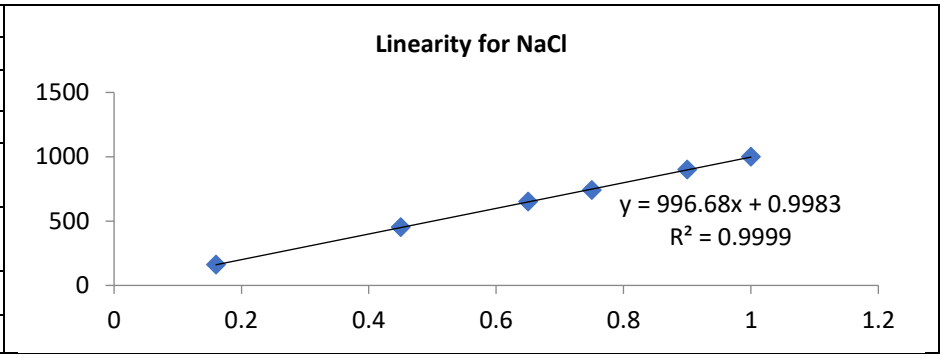

Table 12 LOD and LOQ

LOD	0.03%
LOQ	0.10%

Table 13 Precision Study NaCl

Sr. No.	Intra-day	Interday
1	100.81	100.27
2	99.59	101.35
3	100.4	100.67
Mean	100.27	100.76
SD	0.62	0.55
%RSD	0.62	0.54

Table 14 Accuracy Study NaCl

Sr. No.	Level	%recovery	mean	SD	%RSD
1	80	99.7	100.23	0.46	0.46
2	80	100.52			
3	80	100.45			
4	100	99.53	100.02	0.74	0.74
5	100	99.66			
6	100	100.87			
7	120	100	100.08	0.37	0.3
8	120	100.49			
9	120	99.75			

Anti-opioid drug:

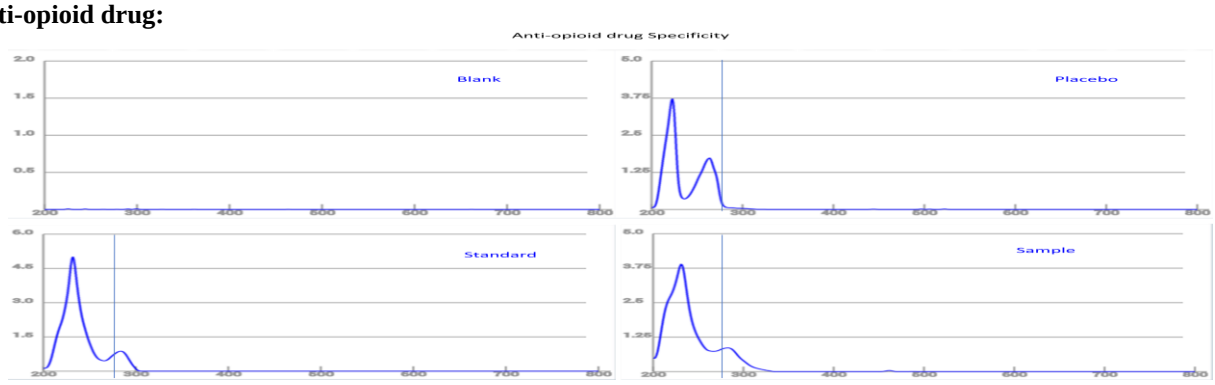

Fig 4 Specificity of anti-opioid drug

Table 15 Linearity curve for anti-opioid drug

Linearity	
CONC (ug/ml)	AREA
100	0.251
200	0.457
300	0.653
400	0.868
500	1.137
600	1.325
700	1.512

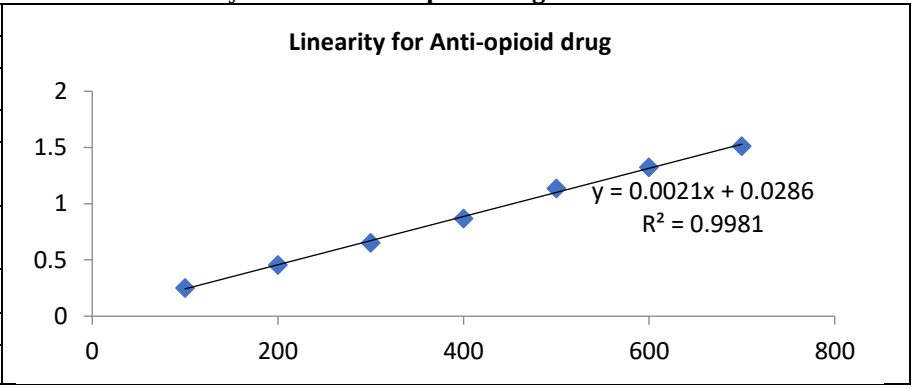

Table 16 LOD and LOQ

LOD	82ug/ml
LOQ	249 ug/ml

Table 17 Precision study

Sr. No.	Intra-day	Interday
1	100.23	99.77
2	99.31	100.7
3	98.5	99.19
Mean	99.35	99.89
SD	0.87	0.76
%RSD	0.87	0.76

Table 18 Accuracy study for anti-opioid drug

Sr. No.	Level	%recovery	mean	SD	%RSD
1	80	99.27	99.59	0.29	0.29
2	80	99.65			
3	80	99.85			
4	100	98.79	99.56	0.68	0.68
5	100	100.05			
6	100	99.82			
7	120	98.24	98.4	0.23	0.23
8	120	98.66			
9	120	98.29			

Similarity matrixing:

Table 19 Similarity Matrixing

Test	RLD	In-house
Assay	99.76%	99.30%
pH	4.52	4.50
Osmolarity	224	221

4. Discussion

The reverse engineering applied to anti-opioid nasal spray to find out the qualitative and quantitative composition of the formulation. This helped to develop the generic formulation within limited time and cost. The validation of methods employed assured for the authenticity of results. This helped to move ahead for the development of the generic formulation by using data obtained by the reverse engineering. On similarity matrixing the rational used for the study was successfully worked out this gave confidence on each level.

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