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Analytical Method Validation of Related Substance Test Parameter of Fosaprepitant in Fosaprepitant Formulation

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ABSTRACT

Analytical results of pH, light transmission and water content test parameters were found satisfactory. pH of the formulations is on alkaline side as the drug is stable in alkaline compared to acidic environment. Fosaprepitant dimeglumine has four functional groups which have pKa values of 3.05 ± 0.03, 4.92 ± 0.02, 9.67 ± 0.01 and 10.59 ± 0.03. The pka value of 3.05 corresponds to the morpholinium group, the pka of 4.92 corresponds to the monophosphate group, the pka of 9.67 corresponds to the meglumine counter ion, and the pka of 10.59 corresponds to the triazolinone NH group. Water content of the formulation is found around 0.5% level. Chemical evaluation such as assay test parameter result was observed satisfactory wherein the level of assay in all three formulations is around 98%. However, with respect to impurities formation, all the known impurities such as Aprpitant, Impurity A, B, C and D impurity levels were found satisfactory levels in nonaqueous formulations indicating less degradation when compared to degradation in the aqueous environment. It was also to be noted that % content of unknown impurity is satisfactory levels in all the three formulations. From the above experiment, it was concluded that further fine tuning to arrest the degradation impurities in the formulation needs to be worked out and also various other formulation experiments need to be worked out.

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INTRODUCTION:

Fosaprepitant injection is used along with other medications to prevent nausea and vomiting in adults that may occur within 24 hours or several days after receiving certain cancer chemotherapy treatments. Fosaprepitant injection can also be used in children 6 months of age and older[1]. Fosaprepitant injection is in a class of medications called antiemetics[1]. It works by blocking the action of neurokinin, a natural substance in the brain that causes nausea and vomiting. Fosaprepitant injection is *not* used to treat nausea and vomiting that you already have. Fosaprepitant is a substance P/neurokinin-1 (NK1) receptor antagonist, indicated in adults and pediatric patients 6 months of age and older, in combination with other antiemetic agents, for the prevention of: Acute and delayed nausea and vomiting associated with initial and repeat courses of highly emetogenic cancer chemotherapy (HEC) including high-dose cisplatin. Delayed nausea and vomiting are associated with initial and repeat courses of moderately emetogenic cancer chemotherapy (MEC). EMEND for injection.

Fosaprepitant dimeglumine, a prodrug of aprepitant, a substance P/neurokinin-1 (NK1) receptor antagonist, an antiemetic agent, chemically described as 1-Deoxy-1-(methylamino)-D-glucitol[3-[[(2R,3S)-2-[(1R)-1-[3,5- bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-2,5-dihydro-5-oxo-1H-1,2,4- triazol-1-yl]phosphonate (2:1) (salt). Its empirical formula is C23H22F7N4O6P · 2(C7H17NO5) and its structural formula is:

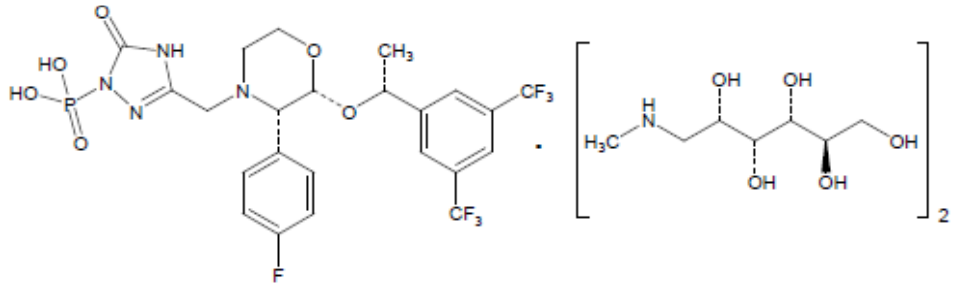

Figure No. 1: Molecular Structure of Fosaprepitant Dimeglumine

Fosaprepitant dimeglumine is a white to off-white amorphous powder with a molecular weight of 1004.83. It is freely soluble in water.

Fosaprepitant is a prodrug of aprepitant and accordingly, its antiemetic effects are attributable to aprepitant. Aprepitant is a selective high-affinity antagonist of human substance

P/neurokinin 1 (NK1) receptors. Aprepitant has little or no affinity for serotonin (5-HT3), dopamine, and corticosteroid receptors, the targets of existing therapies for chemotherapy-induced nausea and vomiting (CINV). Aprepitant has been shown in animal models to inhibit emesis induced by cytotoxic chemotherapeutic agents, such as cisplatin, via central actions. Animal and human Positron Emission Tomography (PET) studies with aprepitant have shown that it crosses the blood-brain barrier and occupies brain NK1 receptors. Animal and human studies have shown that aprepitant augments the antiemetic activity of the 5-HT3-receptor antagonist ondansetron and the corticosteroid dexamethasone and inhibits both the acute and delayed phases of cisplatin-induced emesis.

Antiemetic drugs help to block specific neurotransmitters in the body. These neurotransmitters trigger impulses such as nausea and vomiting, so blocking the impulses will help shut them down.Fosaprepitant dimeglumine is a new drug indicated to prevent nausea and vomiting associated with highly emetogenic cisplatin-based and moderately emetogenic cancer chemotherapy in adults. Due to its complexity in managing, since it requires reconstitution and dilution before intravenous administration. It is a phosphorylated prodrug that is rapidly converted to aprepitant, an oral selective neurokinnin-I receptor antagonist approved[2-5].

An **antiemetic** is a drug that is effective against vomiting and nausea. Antiemetics are typically used to treat motion sickness and the side effects of opioid analgesics, anesthetics, and chemotherapy directed against cancer. They may be used for severe cases of gastroenteritis, especially if the patient is dehydrated.

Some antiemetics previously thought to cause birth defects appear safe for use by pregnant women in the treatment of morning sickness and the more serious hyperemesis gravidarum[6&7].

Neurokinin-1 (NK-1) receptor antagonists are a new class of antiemetic drugs that possess unique anxiolytic, antidepressant, and antiemetic properties. The discovery of neurokinin-1 (NK-1) receptor blockers was a crucial point in the prevention of emesis associated with cancer chemotherapy[8-9].

The following parameters were considered for the analytical method validation for the assay test parameter of Fosaprepitant in Fosaprepitant Injection.

The following parameters are evaluated during method development.

- Precision
- Accuracy
- Linearity
- Solution Stability
- Specificity (Forced Degradation)

Table No.:1. Summary of the Assay Test Method Validation Results.

Validation Parameter	**Acceptance criteria**	**Results**
System Suitability	% RSD of Fosaprepitant peak from six replicate injections standard preparation should be NMT5.0.	3.2
	Resolution between Fosaprepitant and N-Oxidc impurity should be not less than 1.5 obtained from system suitability solution-I	4.6
	Resolution between Acid impurity and unknown impurity at RRT 0.28 should be NLT obtained from System suitability solution-2.	4.5
	USP plate count/Theoretical plates of Fosaprepitant peak from first injection of standard should not be less than 2000.	72081
	USP tailing factor/Asymmetry of Fosaprepitant peak from first injection of standard should not be more than 2.0.	1.1
Specificity	Diluent and placebo peaks should not interfere with Fosaprepitant and impurities.	There is no interference of diluent, placebo peaks with Fosaprepitant peak and impurity peaks
	The peaks of impurities and Fosaprepitant peak should not interfere with each other.	There is no interference of peaks of impurities and

<table>
<tr><td></td><td></td><td colspan="2">Fosaprepitant with each other.</td></tr>
<tr><td colspan="4">Precision</td></tr>
<tr><td rowspan="2">System precision</td><td>The % RSD of the Retention time for the Fosaprepitant peak obtained from 6 injections of standard preparation should be NMT 1.0</td><td colspan="2">0.0</td></tr>
<tr><td>The % RSD of the Area response for the Fosaprepitant peak obtained from 6 injections of standard preparation should be NMT 5.0</td><td colspan="2">3.2</td></tr>
<tr><td rowspan="10">Method Precision</td><td>For the spiked method precision</td><td>Impurities</td><td>% RSD</td></tr>
<tr><td rowspan="4">%RSD for known impurities (≤0.5%)results from six determinations should be NMT15.0</td><td>Acid Impurity</td><td>3.3</td></tr>
<tr><td>Chloro Impurity</td><td>3.2</td></tr>
<tr><td>Diol Impurity</td><td>3.0</td></tr>
<tr><td>N-Oxide impurity</td><td>6.5</td></tr>
<tr><td rowspan="5">%RSD for % unknownimpuritiesresultsfromsixdeterminations shouldbeNMT10.0</td><td>Specified unidentified impurity at RRT 0.28</td><td>ND</td></tr>
<tr><td>Specified unidentified impurity at RRT 0.44</td><td>4.9</td></tr>
<tr><td>Specified unidentified impurity at RRT 0.65</td><td>7.4</td></tr>
<tr><td>Specified unidentified impurity at RRT 1.14</td><td>ND</td></tr>
<tr><td>Specified</td><td>ND</td></tr>
</table>

		unidentified impurity at RRT 1.16	
	% RSD for single maximum unknown impurity (>05%) results from six determinations should be NMT 15.0.	NA	
	%RSD of total impurities for 6 determinations should be NMT 10.0	3.2	
Intermediate Precision	% RSD for % known impurities from SIX determinationsshouldbeNMT10.0	**Impurities**	**% RSD**
		Acid Impurity	0.8
		Chloro Impurity	2.5
		Diol Impurity	2.7
		N-Oxide impurity	7.8
	%RSD for% known impurities (≤0.5%) results from six determinations should be NMT 15.0	Specified unidentified impurity at RRT 0.28	ND
		Specified unidentified impurity at RRT 0.44	4.4
		Specified unidentified impurity at RRT 0.65	6.8
		Specified unidentified impurity at RRT 1.14	ND
		Specified unidentified	ND

		impurity at RRT 1.16	
	%RSD for single maximum unknown impurity (>05%) results from six determinations Should be NMT 15.0.	4.7	
	%RSD of total impurities for 6 determinations should be NMT 10.0	1.7	
	%RSD of% known impuritiesfor12determinations (method precision & Intermediate precision) should be NMT10.0	**Impurities**	**% RSD**
		Acid Impurity	3.2
		Chloro Impurity	3.0
		Diol Impurity	4.2
		N-Oxide impurity	8.0
	%RSD for known impurities (0.5%) results for 12 determinations (Method precision and Intermediate precision)shouldbeNMT15.0	Specified unidentified impurity at RRT 0.28	ND
		Specified unidentified impurity at RRT 0.44	5.5
		Specified unidentified impurity at RRT 0.65	11.1
		Specified unidentified impurity at RRT 1.14	ND
		Specified unidentified impurity at	ND

		RRT 1.16	
	% RSD for single maximum unknown impurity (>05%)for 12 determinations (method precision & Intermediate precision) should be NMT 15.0	NA	
	% RSD for the total impurities results from 12 determinations (method precisions & Intermediate precision) should be NMT 10.0.	2.5	

SYSTEM SUITABILITY:

To verify that the analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set. Injected Diluent (Blank) (one injection), Standard Preparation (6injections), recorded chromatograms and checked the system suitability.

Table No.:2. Results of System Suitability

Acceptance Criteria	**Results**
%RSD of Fosaprepitant peak from six replicate injections standard preparation should be NMT 5.0.	3.2
Resolution between Fosaprepitant and N-Oxide impurity should be not less than 1.5 obtained from system suitability solution-I	4.6
Resolution between Acid impurity and unknown impurity at RRT 0.28 should be NLT 1.5 obtained from System suitability solution-2	4.5
USP plate count/Theoretical plates of Fosaprepitant peak from first injection of standard should not be less than 2000.	72081
USP tailing factor/Asymmetry of Fosaprepitant peak from first injection of standard should not be more than2.0.	I.I

Data Interpretation:

From the above results, it was concluded that the system is suitable for Analytical Method Validation.

SPECIFICITY:

Specificity is the ability of analytical method to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components.

Performed the specificity parameter of the method by injecting Diluent (Blank), Placebo Solution, System Suitability Solution-1, System Suitability Solution-2, Standard Preparation, Placebo Preparation, Sample Preparation, Acid Impurity, Diol Impurity, Chloro Impurity, N-Oxide Impurity and Sample spiked with impurities into the Chromatographic System and recorded the Retention Times.

Acceptance Criteria:

Diluent and placebo peaks should not interfere with Fosaprepitant and impurities.

The peaks of Impurities and Fosaprepitant peak should not interfere with each other

Table No.:3. Results of Specificity

Solutions		**Retention time*(in* min.)**
Blank		-
Placebo Solution		-
System Suitability Solution-I	Acid Impurity	8.546
	Diol Impurity	13.831
	Chloro Impurity	23.705
	Fosaprepitant	26.335
	N-Oxide Impurity	29.181
SystemSuitabilitySolution-2	UnknownatRRT0.28	7.503
	Acid impurity	8.548
	Fosaprepitant	26.355
Standard Solution		26.912
Sample Solution	Acid Impurity	ND
	Diol Impurity	13.853
	Chloro Impurity	ND
	N-Oxide Impurity	ND

	Fosaprepitant	26.382
Individual Impurities	Acid Impurity	8.619
	Diol Impurity	13.866
	Chloro Impurity	23.759
	N-Oxide Impurity	29.238
Sample Spiked with Impurities	Acid Impurity	8.585
	Diol Impurity	13.859
	Chloro Impurity	23.739
	N-Oxide Impurity	29.201
	Fosaprepitant	26.370

Data Interpretation:

From the above results, it was concluded that there was no interference of peaks of Diluent, Impurities and Fosaprepitant with each other.

PRECISION:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous samples. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (Coefficient of variation) of series of measurements.

SYSTEM PRECISION:

The system precision is checked by using standard chemical substances to ensure that the analytical system is working properly. The retention time and area response of six determinations should be measured and calculated% relative standard deviation.

Injected Diluent (Blank) (one injection), and Standard preparation (6Injections) and checked the system suitability parameter.

Acceptance criteria:

- The% RSD of the Retention time for the Fosaprepitant peak obtained from 6 injections of standard preparation should be NMT 1.0

➢ The %RSD of the Area response for the Fosaprepitant peak obtained from 6 injections of standardpreparationshouldbeNMT5.0

Table No.:4. Results of System Precision

Injection No.	**Fosaprepitant**	
	Retention Time (in minutes)	**Area Response**
I	26.767	40348
2	26.753	37264
3	26.756	38173
4	26.754	40121
5	26.749	37979
6	26.740	39164
Mean	26.753	38842
% RSD	0.0	3.2

Data Interpretation:

From the above results, it was concluded that Retention time & Area responses were consistent as evidenced by relative standard deviation. Hence, it was concluded that the system precision parameters meet the requirement of method validation.

METHOD PRECISION:

In method precision a homogeneous sample of a single batch should be analyzed six times. This indicates whether a method is giving consistent results of a single batch. Analyzed the six sample preparations of Fosaprepitant for Injection 30mg/vial of the same batch as per analytical procedure. Calculated the % of impurities.

For Spiked Method Precision

➢ % RSD for% known impurities results from six determinations should be NMT 10.0

➢ % RSD for known impurities(≤ 0.5%) results from six determinations should be NMT 15.0

➢ % RSD for single maximum unknown impurity (>05%) results from six determinations

Should be NMT 15.0.

- % RSD of total impurities for 6 determinations should be NMT 10.0

Table No.:5. Results of Method Precision

Sample Preparation	Spiked Precision (%)					
	Acid Impurity	Diol Impurity	Chloro Impurity	N-Oxide Impurity	Spec unid imp at RRT 0.28	Spec unid imp at RRT 0.44
1	0.221	0.215	0.198	0.183	ND	0.215
2	0.203	0.220	0.207	0.182	ND	0.232
3	0.204	0.203	0.217	0.212	ND	0.246
4	0.208	0.216	0.201	0.181	ND	0.227
5	0.210	0.221	0.209	0.192	ND	0.238
6	0.204	0.217	0.204	0.181	ND	0.221
Mean	**0.208**	**0.215**	**0.206**	**0.189**	**NA**	**0.230**
%RSD	**3.3**	**3.0**	**3.2**	**6.5**	**NA**	**4.9**

Sample Preparation	Spec unid imp at RRT 0.65	Spec unid imp at RRT 1.14	Spec unid imp at RRT 1.16	Single maximum	Total Impurities
1	0.142	ND	ND	NA	1.252
2	0.138	ND	ND	NA	1.281
3	0.155	ND	ND	NA	1.347
4	0.134	ND	ND	NA	1.234
5	0.143	ND	ND	NA	1.291
6	0.124	ND	ND	NA	1.249
Mean	**0.139**	**NA**	**NA**	**NA**	**1.276**
% RSD	**7.4**	**NA**	**NA**	**NA**	**3.2**

Data Interpretation:

From the above results, it was concluded that the method was precise.

INTERMEDIATE PRECISION:

The intermediate precision ensures that the analytical results will remain unaffected with change in analyst and day.

Repeated the method precision for spiked sample set by other analyst using different column, different instrument on different day.

Precision Matrix:

The precision activity [Method Precision & Intermediate Precision] was carried using 2 different scientist and found compliance to the requirement.

Calculated the % of impurities. Compared the results obtained in method precision and intermediate precision.

Acceptance Criteria

- % RSD for % known impurities from six determinations should be NMT10.0
- % RSD for % known impurities (≤0.5%) results from six determinations should be NMT 15.0
- % RSD for single maximum unknown impurity (≥05%) results from six determinations should be NMT 15.0.
- % RSD of total impurities for 6 determinations should be NMT I0.0
- % RSD of % known impurities for 12 determinations (method precision & Intermediate precision) should be ≤ NMT10.0
- % RSD for known impurities (≤0.5%) results for 12 determinations (Method precision and Intermediate precision) should be NMT 15.0
- % RSD for single maximum unknown impurity(≥ 05%)resultsfor12determinations(method precision & Intermediate precision) should be NMT 15.0
- % RSD for the total impurities results for 12 determinations (method precision & Intermediate precision) should be NMT10.0.

Table No.:6. *Results of Intermediate Precision*

Sample Preparation	Spiked Precision (%)					
	Acid Impurity	Diol Impurity	Chloro Impurity	N-Oxide Impurity	Spec unid imp at RRT 0.28	Spec unid imp at RRT 0.44
1	0.200	0.211	0.207	0.172	ND	0.227
2	0.198	0.200	0.210	0.157	ND	0.228
3	0.201	0.202	0.208	0.170	ND	0.208
4	0.199	0.196	0.215	0.184	ND	0.206
5	0.198	0.207	0.205	0.166	ND	0.211
6	0.202	0.199	0.219	0.195	ND	0.217
Mean	**0.200**	**0.203**	**0.211**	**0.174**	**NA**	**0.216**
%RSD	**0.8**	**2.7**	**2.5**	**7.8**	**NA**	**4.4**

Sample Preparation	Spec unid imp at RRT 0.65	Spec unid imp at RRT 1.14	Spec unid imp at RRT 1.16	Single maximum	Total Impurities
1	0.126	ND	ND	0.048	1.276
2	0.127	ND	ND	0.050	1.259
3	0.115	ND	ND	0.051	1.245
4	0.108	ND	ND	0.051	1.250
5	0.110	ND	ND	0.049	1.238
6	0.121	ND	ND	0.055	1.297
Mean	**0.118**	**NA**	**NA**	**0.051**	**1.261**
% RSD	**6.8**	**NA**	**NA**	**4.7**	**1.7**

Table No.: 7

Comparison of the results obtained in Method precision and Intermediate Precision

	Sample Preparation	Acid impurity (%)	Diol impurity (%)	Chloro impurity (%)	N-Oxide impurity (%)	Spec unid imp at RRT 0.28 (%)
Method precision	1	0.221	0.215	0.198	0.183	ND
	2	0.203	0.220	0.207	0.182	ND
	3	0.204	0.203	0.217	0.212	ND
	4	0.208	0.216	0.201	0.181	ND
	5	0.210	0.221	0.209	0.192	ND
	6	0.204	0.217	0.204	0.181	ND
Intermediate precision	7	0.200	0.211	0.207	0.172	ND
	8	0.198	0.200	0.210	0.157	ND
	9	0.201	0.202	0.208	0.170	ND
	10	0.199	0.196	0.215	0.184	ND
	11	0.198	0.207	0.205	0.166	ND
	12	0.202	0.199	0.219	0.195	ND
Mean		**0.204**	**0.209**	**0.208**	**0.181**	**NA**
% RSD		**3.2**	**4.2**	**3.0**	**8.0**	**NA**

		% Impurity					
	Sample Preparation	Spec unid imp at RRT 0.44	Spec unid imp at RRT 0.65	Spec unid imp at RRT 1.14	Spec unid imp at RRT 1.16	Single Maximum	Total Impurities
Method precision	1	0.215	0.142	ND	ND	NA	1.252
	2	0.232	0.138	ND	ND	NA	1.281
	3	0.246	0.155	ND	ND	NA	1.347
	4	0.227	0.154	ND	ND	NA	1.234
	5	0.238	0.134	ND	ND	NA	1.291
	6	0.221	0.143	ND	ND	NA	1.249
Intermediate precision	7	0.227	0.126	ND	ND	NA	1.276
	8	0.228	0.127	ND	ND	NA	1.259
	9	0.208	0.115	ND	ND	NA	1.245
	10	0.206	0.108	ND	ND	NA	1.250
	11	0.211	0.110	ND	ND	NA	1.238
	12	0.217	0.121	ND	ND	NA	1.297
Mean		**0.223**	**0.129**	**NA**	**NA**	**NA**	**1.268**
% RSD		**5.5**	**11.1**	**NA**	**NA**	**NA**	**2.5**

Data Interpretation:

From the above results, it was concluded that the method was rugged.

FORMULAE FOR CALCULATION:

a) Correlation Coefficient (r): $r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{n(\sum x^2) - (\sum x)^2}\sqrt{n(\sum y^2) - (\sum y)^2}}$

∑ = Sum of, x =Conc. Of the Component, y =Av. Area response ratio of component,
n = number of observations

b) Slope(a)= $\frac{(n\sum XY - \sum X \sum Y)}{(n \sum X2 - (\sum X)2)}$

c) The equation of straight line: Y=aX+b

d) Tailing factor (T): T= (a+b)/2a

e) Theoretical plates (N): $N=16(V_e/W_b)^2$

f) Resolution (Rs) $=2(t_2-t_1)/ w_1+w_2$

t_2: Retention time of peak (2)
t_1: Retention time of peak (1)
w_2: Peak width at the base line of peak (2)
w_1: Peak width at the base line of peak (1)

g) Intercept on the Y axis (b) = $\overline{Y} - a\ \overline{X}$, ($\overline{X}$ =mean values of X), ($\overline{Y}$ =mean values of Y)

h) Calculate the content of any Known/Unknown impurity with respect to the Carfilzomil label claim as per below formula:

$$\text{Content of Impurity in \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{N} \times \frac{P}{100} \times \frac{100}{LC} \times RF$$

AT	:	Area of peak response of Known/Unknown Impurity from test preparation.
AS	:	Average area of Carfilzomib peak from the Standard preparation
WS	:	Weight of Carfilzomib standard taken in mg
DS	:	Dilution of standard Preparation in mL
DT	:	Dilution of sample preparation in mL
N	:	No.of vials reconstituted for test preparation
P	:	Potency of Carfilzomib standard (on as is basis)
LC	:	Label claim of Carfilzomib in (30 mg/vial)
RF	:	Response factor for Known impurities

For unknown impurities the RF is calculated as 1.0
Total Impurities = Sum of Known and Unknown impurities

i) **RF Calculation:**

$$RRF = \frac{\text{Slope of Impurity from linearity}}{\text{Slope of Carfilzomib from linearity}}$$

$$RF = \frac{1}{RRF}$$

	RF	RRT(about)	% LOD	% LOQ
Carfilzomib			0.014	0.042
Acid Impurity	0.86	0.33	0.013	0.039
Chloro Impurity	1.02	0.90	0.012	0.036
Diol Impurity	1.02	0.53	0.015	0.044
N-Oxide Impurity	1.11	1.12	0.015	0.044

Table No.:8. Details of RF, RRT, LOD & LOQ of Impurities.

Various chromatograms which are part of assay test parameter analytical method validation is presented below

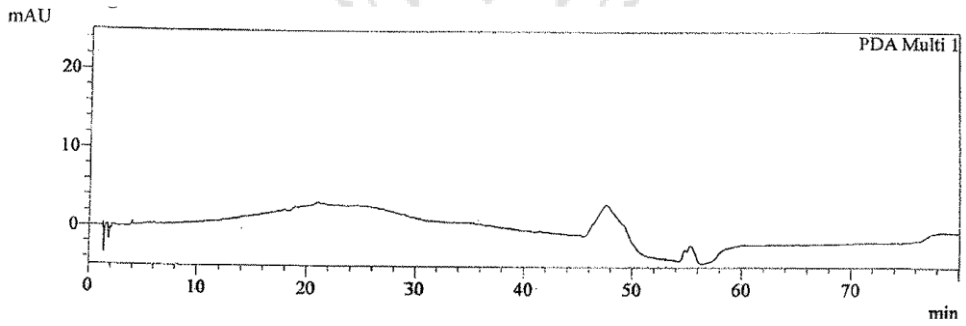

Figure No.:2. Chromatogram of Blank

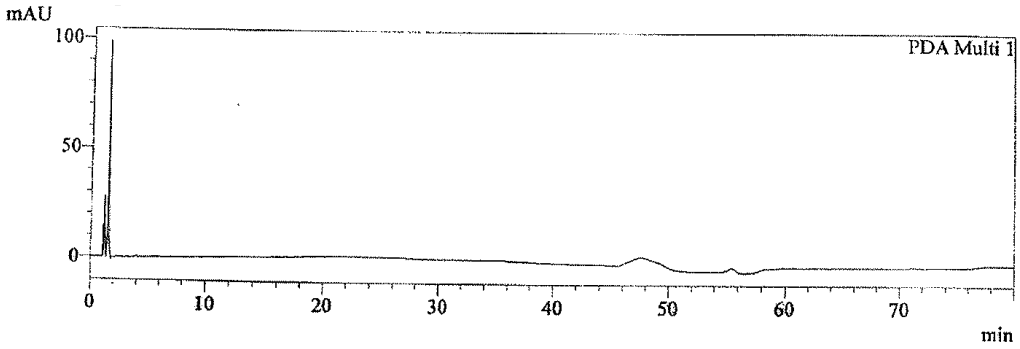

Figure No.:3. Chromatogram of Placebo Solution

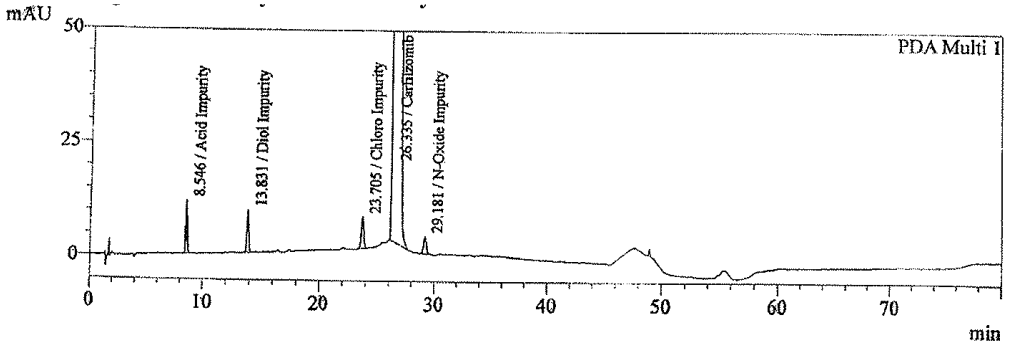

Figure No.:4. Chromatogram of System Suitability Solution-I

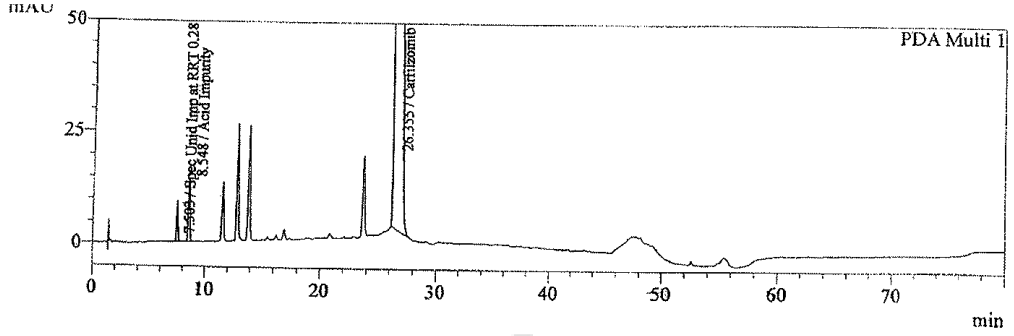

Figure No.:5. Chromatogram of System Suitability Solution-II

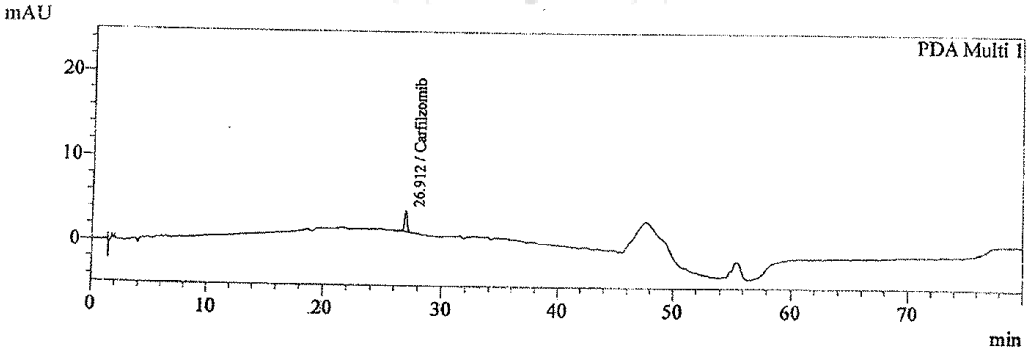

Figure No.:6. Chromatogram of Standard Solution

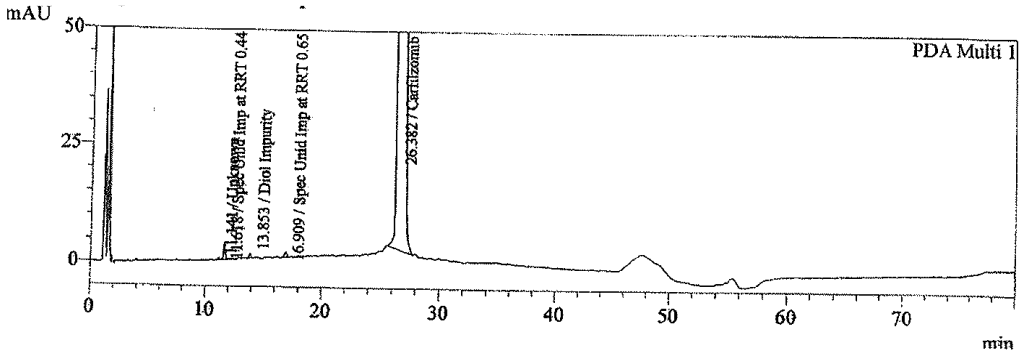

Figure No.:7. Chromatogram of Sample Solution

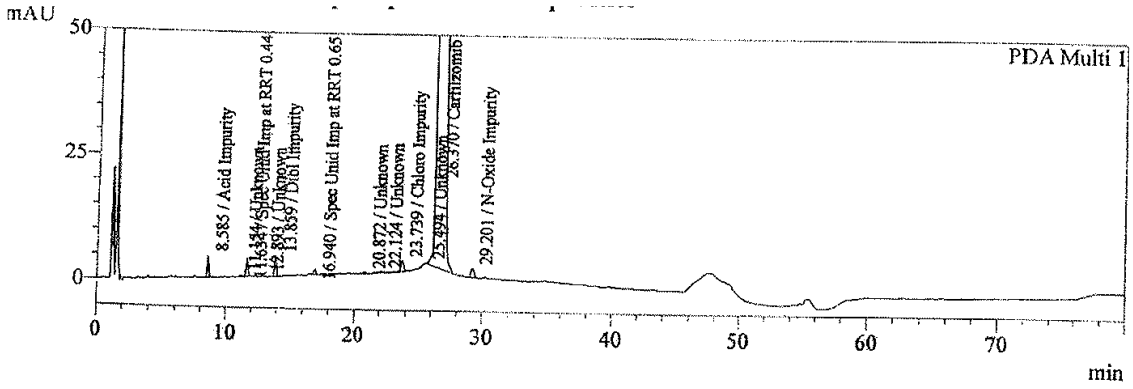

Figure No.:8. Chromatogram of Sample with Spiked Impurities

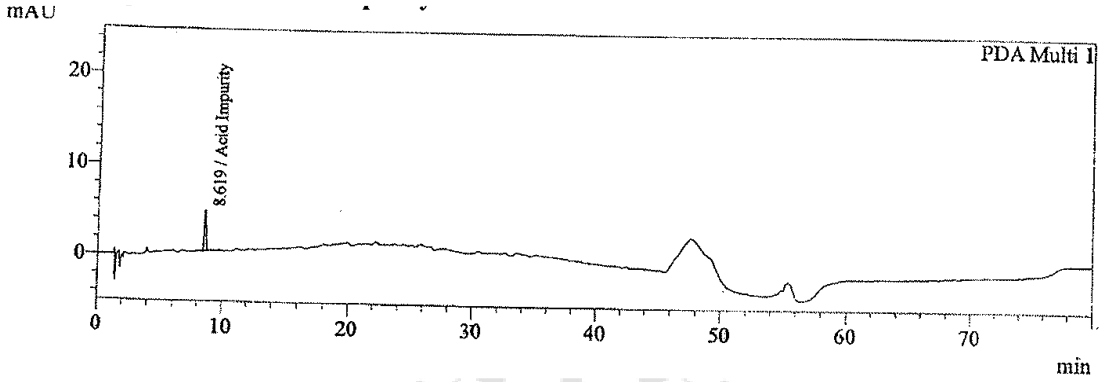

Figure No.:9. Chromatogram of Acid Impurity

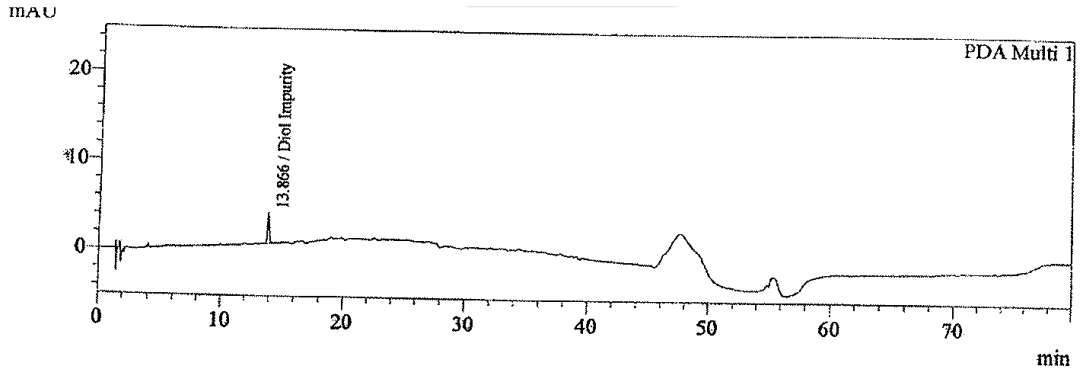

Figure No.:10. Chromatogram of Diol Impurity

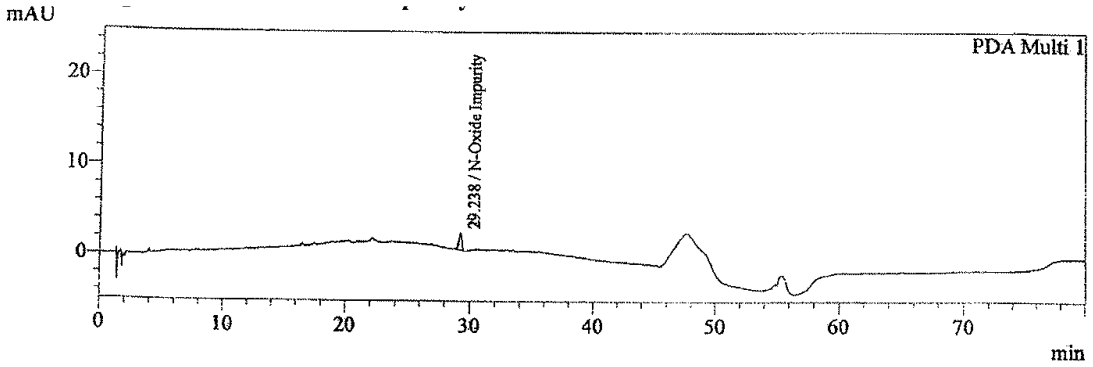

Figure No.:11. Chromatogram of Chloro Impurity

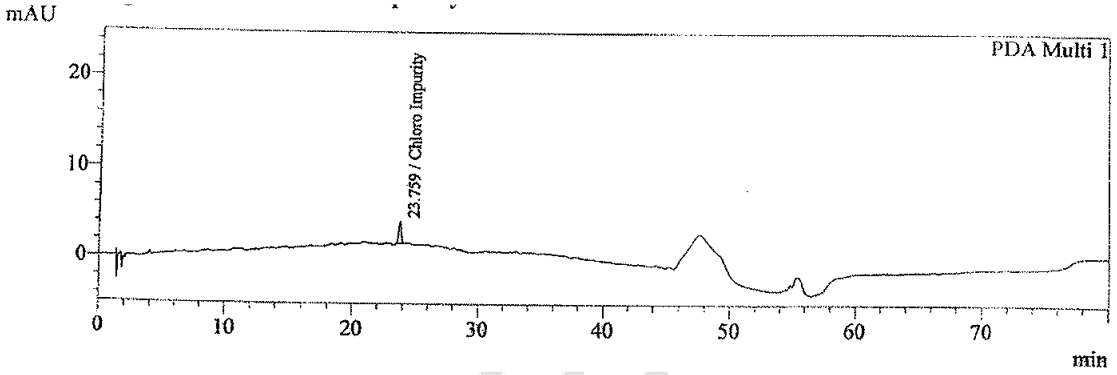

Figure No.:12. Chromatogram of N-Oxide Impurity

Analytical results of pH, light transmission and water content test parameters were found satisfactory. pH of the formulations is on alkaline side as the drug is stable towards alkaline compared to acidic environment. Fosaprepitant dimeglumine has four functional groups which have pKa values of 3.05 ± 0.03, 4.92 ± 0.02, 9.67 ± 0.01 and 10.59 ± 0.03. The pka value of 3.05 corresponds to the morpholinium group, the pka of 4.92 corresponds to the monophosphate group, the pka of 9.67 corresponds to the meglumine counter ion, and the pka of 10.59 corresponds to the triazolinone NH group. Water content of the formulation is found around 0.5% level. Chemical evaluation such as assay test parameter result was observed satisfactory wherein the level of assay in all three formulations is around 98%. However, with respect to impurities formation, all the known impurities such as Aprpitant, Impurity A, B, C and D impurity levels were found satisfactory levels in nonaqueous formulations indicating less degradation when compared to degradation in the aqueous environment. It was also to be noted that % content of unknown impurity is satisfactory levels in all the three formulations. From the above experiment, it was concluded that further fine tuning to arrest the degradation impurities in the formulation needs to be worked out and also various other formulation experiments needs to be worked out.

Analytical Method Development & Validation:

In order to understand the assay and impurities levels in the present research work, an in-house analytical method development for Assay and Related substances was developed and it is learned that methods were found stability indicating in nature. As a part of analytical method validation, Assay and Related substances test parameter of the finished product validation was carried out. The details of the method validation were captured in the materials and methods. The validation exercise was carried in compliance to the ICH guidelines for MethodValidationQ2 (R1) and USP39<1225>Validation of Compendial Methods. The analytical method validation was carried out satisfactorily with the parameters like precision, accuracy, robustness and linearity. The validated method was applied to analyze initial samples of aqueous trails [FF1 to FF3] and non-aqueous formulations [NFF1 to NFF3] and the stability exposed and photostability exposed samples of optimized formulation [NFF1].

CONCLUSION:

The adopted method of HPLC for an estimation of impurities under related substances test parameter of Fosaprepitant in Fosaprepitant Injection is validated and the method is found specific and precise. A system suitability test was established and related parameters were recorded. Hence this method stands validated and could be used for regular and stable exposed samples analysis.

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