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VALIDATION PROCESS OF EDTA FOR INFUSION/ INJECTION WITH CEFTRIAXONE AND SULBACTAM

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ABSTRACT
The validation of the manufacturing process to produce ceftriaxone/ sulbactam with EDTA1.5 g/ vial powder for solution for injection/ infusion. Ceftriaxone works by inhibiting the mucopeptide synthesis in the bacterial cell wall. The beta-lactam moiety of Ceftriaxone binds to carboxypeptidases, endopeptidases, and transpeptidases in the bacterial cytoplasmic membrane. These enzymes are involved in cell- wall synthesis and cell division. By binding to these enzymes, Ceftriaxone results in the formation of defective cell walls and cell death. Sulbactam is an irreversible inhibitor of beta-lactamase; it binds the enzyme and does not allow it to interact with the antibiotic. The validation confirms that each stage of the manufacturing process is in control and will consistently produce a product of acceptable quality, as defined by the specifications of product. It is planned that operating variables and control parameters of processes shall be studied and documented. The associated critical product attributes and characteristics shall also be studied. Process validation of ceftriaxone/ sulbactam 1.5g/ vial powder for solution for injection. Process for manufacture of ceftriaxone/ sulbactam 1.5g/ vials powder for solution for injection/ infusion is said to be in state of control. Hence this product can be manufactured by using this process without modifying any parameters

INTRODUCTION

FDA issued a notice announcing the availability of entitled guideline on general principle of process validation (the 1987 guidance in the federal register of may11, 1987(52 FR 17638). The revised guidance conveys FDA's current thinking on process validation and is consistent with basic principles first introduced in 1987 guidance [1,2]. The process validation is standardization of the validation documents that must be submitted in the application for marketing authorization, demonstrating the validity of a given process [3,4]. Components should be treated in a grade D setting at least after washing. Handling of sterile starting materials and components, unless

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subjected to sterilization or filtration through micro-organism-retaining filter later in the process, should be done in a grade A environment with grade B background [5]. Ceftriaxone sodium and sulbactam injection play a therapeutic role. Ceftriaxone sodium acts by inhibiting bacterial cell wall synthesis and sulbactam make irreversible competitive inhibition of β-lactamase. The antimicrobial effect of Ceftriaxone can be enhanced by the two combined. The compound specifically aims to the mechanism of bacterial resistance, extending the life of Ceftriaxone in the treatment-resistant pathogen infections. The aim of this research work process is to validation process & manufacturing of ceftriaxone and sulbactam

MATERIALS AND METHODS

Materials

Ceftriaxone sodium, Sulbactum sodium, and Ethylene diamine tetra acetic acid was procured from Venus Remedies Limited, Baddi, Himachal Pradesh. All other ingredients were of analytical grades

Methods

Three consecutive batches of same size of ceftriaxone/ sulbactam1.5 G/ Vial Powder for solution for injection/ infusion was manufactured as per Batch Manufacturing record. Different samples were collected at different stages of processing, as mentioned in the sampling plan. The collected samples were sent to QC laboratory for the analysis as per testing plan. The results of critical control variables were recorded and monitored. During the processing of the current Good Manufacturing Practice was followed. In case of any deviation observed during the process, they were noted down in the deviation report immediately. The deviation was noted in succession throughout the process, along with corrective action. A validation report was prepared upon the execution of the protocol and testing. Composition of ingredients is shown in table 1.

Table 1. Composition of Ingredients.

S. No.	Name of Ingredient	Function	Reference Standard	Quantity (mg)
1	Ceftriaxone sodium eq.to Ceftriaxone	Active Ingredient	EP	1000
2	Sulbactum sodium eq. to sulbactam	Active Ingredient	EP	500
3	Ethylene diamine tetra acetic Acid	Excipient	EP	37

Dosage Form- Dry powder for injection

Storage Condition- store in a cool and dry place. Protect from light.

The ingredients were weighed according to batch size and the ingredients were blended. The blend was then sent to QC for analysis. The variables during blending was recorded.

Washing and sterilization record of machine parts and garments

The machine parts, garments, rubber plugs and cone blender was sterilized in the autoclave at 121°C and 15 PSI for 30 minutes as per the current validated load pattern.

Vial washing and sterilization record

The vial was washed with 5µ and 2µ filtered HPW water using vial washing machine. Clarity of washed vials was checked every 2 hours.

Tunnel sterilization record

The washed vials was sterilized online using dehydrogenated tunnel as per latest validated parameter.

Homogeneity

In the test of homogeneity, the null hypothesis says that the distribution of a categorical response variable is the same in each population. The data were Collected and analyzed.

Filling and stoppering records

The sterilized powder is stored in powder hopper is agitated by pair of mechanical agitators for maintaining consistency and uniform bulk density. The powder wheel having eight ports rotates at the pre-determined speed below the powder hopper with practically no clearance. Powder wheel consist of Piston in each port and behind the powder wheel vacuum plate is provided there is no clearance between powder wheel and vacuum plate due to back spring pressure.

Precise volume of powder is sucked in to the port of powder wheel during vacuum according to the piston length different fill size can be achieved. The excess powder is doctored off by a doctor blade. Doctor blades can be adjusted from outside also without removing powder hopper. When powder wheel indexes further and remain in the port due to the vacuum till it reaches just vertically above the container. The time dose of Compressed air (Nitrogen Gas), sterilized low pressure air sequentially

flushes out powder from the port of powder wheel in to the container one by one. The filled containers are immediately separated on the conveyor by vial separator and moves further for stoppering operation. (Different grooves separator can be used which depends on the container diameter or multiple dosing systems).

The sterilized, siliconized, rubber stoppers stored in the vibrator bowl moves to vibratory bowl and stacked vertically in the rubber stopper chute. The container is held firmly between pair or timing belt to pick up rubber stopper from exit end of the chute. Further the container is passed between two pressing roller for tight fit fixing of rubber stopper.

Procedure of assay using HPLC

HPLC apparatus manufactured by Shimadzu, HPLC model: LC-2050C was used for detection of drugs. It comprised of Parallel-type double plunger pump, variable wavelength UV detector, Auto-sampler, Column oven. Chromatographic separation was done isocratically at ambient temperature. C8 column was used (250 mm × 4.6 mm, 5 µm particle size). Thermo Scientific mobile phase composition of 10 ml of 40% Tetra Butyl Ammonium Hydroxide (TBAH) in 1000 ml of water (pH 5.5, maintained by dil Phosphoric acid) and acetonitrile (70:30, v/v) at a flow rate of 2.0 ml min-1 was used. Detector was set at 227 nm with a total run time of 10 mins, and sample injection of 20 µL was injected at 37 ºC. The eluent was monitored with a UV detector set at 227 mm.

86.1 mg of sample diluted to 50.0 ml with mobile phase and mixed properly. Samples were further diluted by mobile phase, which has a final concentration of 100.12µg ml-1 of CEF and 50.06 mg ml-1 of SUL and then injected into the HPLC system.

RESULT & DISCUSSION

The objective of this work is to confirm that the process validation of ceftriaxone/ sulbactam 1.5 g/ vial powder for solution for injection.

It includes manufacturing, and filling process, sterilization of equipments and components, environmental control and monitoring program and aseptic practices of manufacturing personnel are adequate to qualify for manufacture the sterile product and to maintain the stability and sterility of the product during filling and final capping in vial section. This process validation report applies to the manufacturing process of ceftriaxone/ sulbactam 1.5 g/ vial powder for solution for injection for one batches. The following batch are taken for process validation.

Table 2. Record for blending activity

Time	Temperature °C	RH%	Blending speed in RPM
	X Batch	X Batch	
60 Minutes	23.2	26	20
120 Minutes	22.0	27	20
180 Minutes	22.9	27	20
240 Minutes	23.1	26	20

Table 3. Vial washing record

Pressure								
HPW 1± 0.2 kg/ cm2			**Filtered HPW 1± 0.2 kg/ cm^2**			**Compressed air 1± 0.5 kg/ cm^2**		
X	Y	Z	X	Y	Z	X	Y	Z
1.2	1.0	1.2	1.2	1.2	1.2	1.2	1.4	1.4.
1.2	1.2	1.2	1.1	1.2	1.1	1.4	1.3	1.4
1.2	1.2	1.1	1.1	1.0	1.2	1.4	1.2	1.4
1.1	1.2	1.2	1.2	1.0	1.2	1.3	1.2	1.4
1.1	1.1	1.1	1.2	1.1	1.2	1.3	1.4	1.4
1.2	1.1	1.2	1.2	1.1	1.1	1.4	1.4	1.2
1.2	1.2	1.2	1.1	1.0	1.2	1.4	1.2	1.2

Table 4. Line clearance check list for tunnel sterilization

Observation			**Remarks**
Check and ensure cleanliness of sterilization tunnel			Cleaned
Check and ensure the pressure differential between filling room and washing area is minimum of 1.5 mm of water.			Filling room 7.0mm Washing room 3.6 mm
Check and ensure PLC set parameter for the vial type to be sterilized are as per required size of vial.			Yes
Check and ensure that the vial washing machine is running and washed vials are released clarity.			Released
Identify the line with status label.			Identified
Conveyor speed	**Conveyor start temperature**	**Set temperature**	**Pressure zone**
160	300 °C	300 °C- 330 °C	18 mm

Batch No. X

Table 5. Homogeneity data

Limit: 95-105% and RSD< 5% for Ceftriaxone; 95-105% and RSD< 5% for Sulbactam; 95-105% and RSD< 5% for EDTA

Homogeneity	60 minutes			120 minutes			180 minutes			240 minutes		
	Cef	Sul	EDTA	Cef	sul	EDTA	Cef	Sul	EDTA	Cef	Sul	EDTA
S1	93.2	108.3	130.8	95.0	95.7	189.5	99.7	99.7	101.6	100.4	94.3	102.4
S2	92.7	93.5	320.0	98.3	100.5	176.2	97.3	99.1	102.6	94.6	100.3	103.1
S3	78.6	124.0	261.2	106.7	81.6	123.7	99.6	99.6	101.9	100.2	100.7	102.8
S4	83.9	108.6	340.4	94.6	95.1	110.8	99.8	99.8	102.7	94.7	99.9	103.6
S5	90.3	94.3	125.4	96.8	98.9	87.0	99.1	99.0	101.6	99.30	100.3	103.9
S6	100.5	88.0	245.8	107.0	81.5	255.4	99.7	99.5	102.4	100.6	100.5	102.5
Average	89.8	102.6	237.2	99.7	92.2	157.1	99.5	99.5	102.1	99.9	100.3	103.1
% RSD	8.5	13.1	38.60	5.70	9.2	39.50	0.27	0.33	0.47	0.51	0.34	0.58

Table 6 Observation

S. No.	Observation	Remarks
1	Check for the removal of previous product left over if any	Removed
2	Check and ensure that filling area is cleaned, disinfected and fumigated as per schedule.	Yes
3	Check and ensure that positive pressure, RH, and temperature are in standard level.	Temp- 24°C RH -27%
4	Check and ensure that container gross weight of raw materials.	Matches
5	Check and ensure that filling machine part and accessories are sterilized and available in sterile area.	Sterilized
6	Ensure that sterilizing tunnel is started and required vials sterilization is in progress.	Yes.
7	Identifying the filling line with status label.	Identified
8	Check and ensure that sterile rubber stoppers are available.	Available
9	Check and ensure that availability of hand disinfectant for hand disinfection.	Available
10	Ensure that target fill weight of raw material/ blend material as per QC report and calculation sheet.	Yes
11	If there is any product change over, check for the compliance of Trace water sample.	Yes
12	Ensure that the particle counts are in limit.	Yes

Table 7. Filling data

Test	Acceptance criteria	Method	Result		
Appearance before reconstitution	White to pale yellow or cream color crystalline powder, free from extraneous particles	Visual inspection	Complies		
Appearance after reconstitution	When powder constituted with water for injection, should be form clear and particle free solution	Visual inspection	Complies		
Mean weight	Standard weight± 2.0%	Current Ph Eur.	**Begin**	**Middle**	**End**
S1			1.736	1.738	1.766
S2			1.745	1.764	1.748
S3			1.765	1.748	1.758
S4			1.738	1.768	1.749
S5			1.758	1.762	1.776
S6			1.755	1.771	1.771
S7			1.749	1.748	1.759
S8			1.768	1.738	1.763
S9			1.773	1.749	1.744
S10			1.754	1.772	1.761
Average			1.754	1.756	1.760
RSD			0.71	0.75	0.58

Table 8. Reconciliation of finished product

		Batch X
A	Total quantity to be produced	50000
B	In process check	128
C	Rejects during filling, plugging and sealing	38
D	Optical rejects	29
E	Spot check rejects	Nil
F	QC samples	129
G	Good quantity	49679
H	Actual quantity produced(G+F)	49808
	Production yield (NLT=97%)	99.61
	Total quantity transfer for quarantine area	49679

HPLC Graph for assay of Finished Product

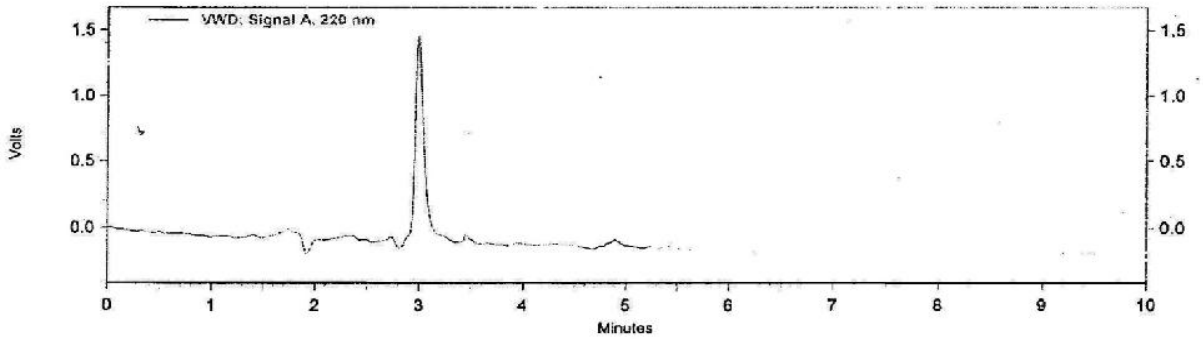

Fig. 1 : Chromatogram of blank

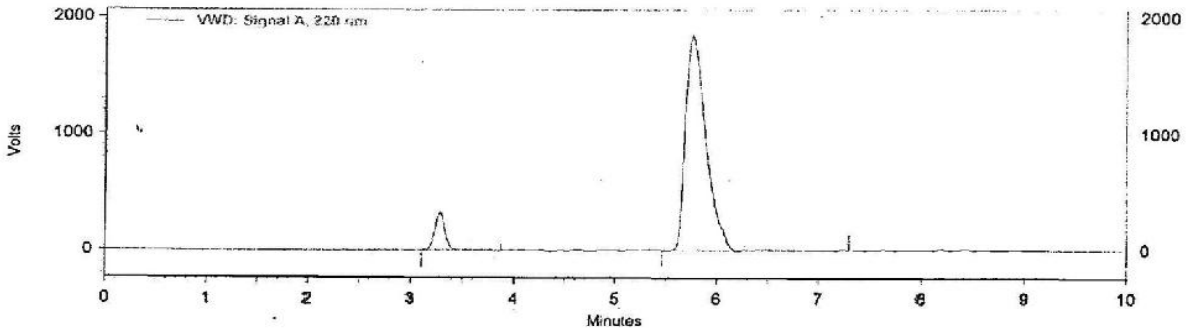

Fig. 2: Chromatogram of cetriaxone and sulbactum standard

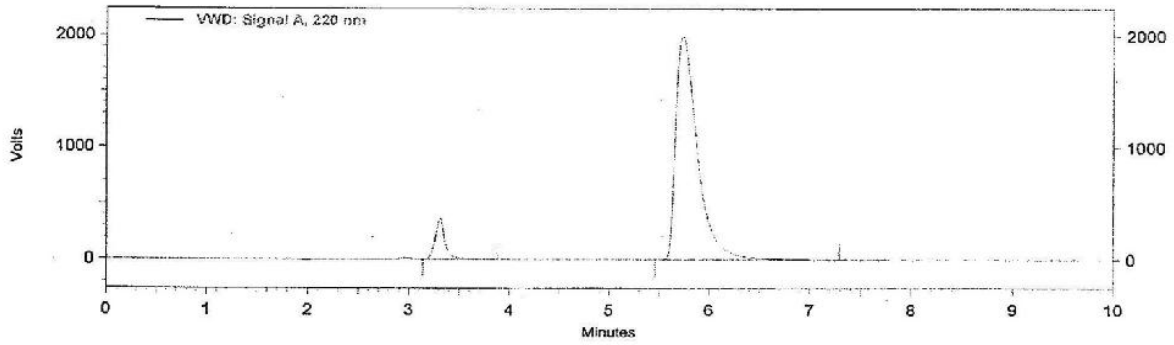

Fig. 3 : Chromatogram of ceftriaxone and sulbactum sample

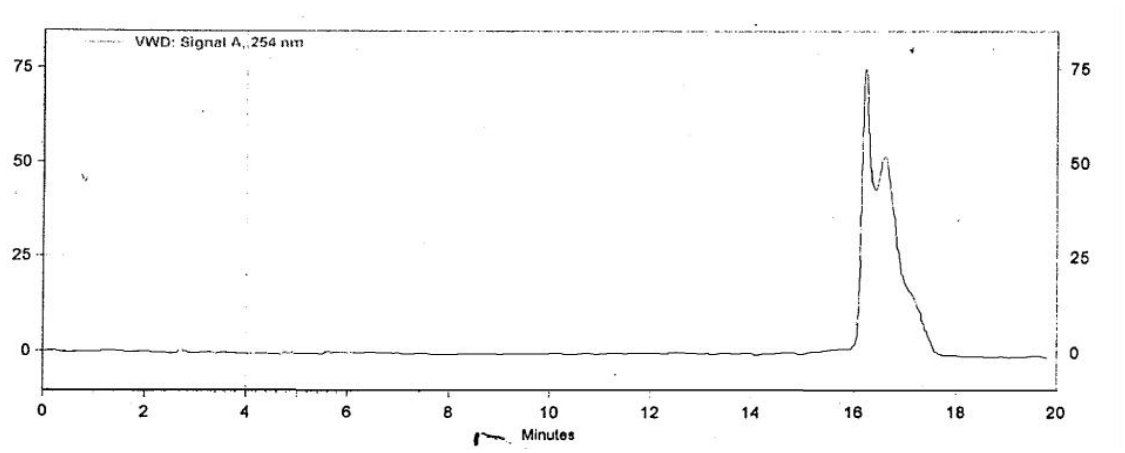

Fig. 4 : Chromatogram of EDTA – Blank

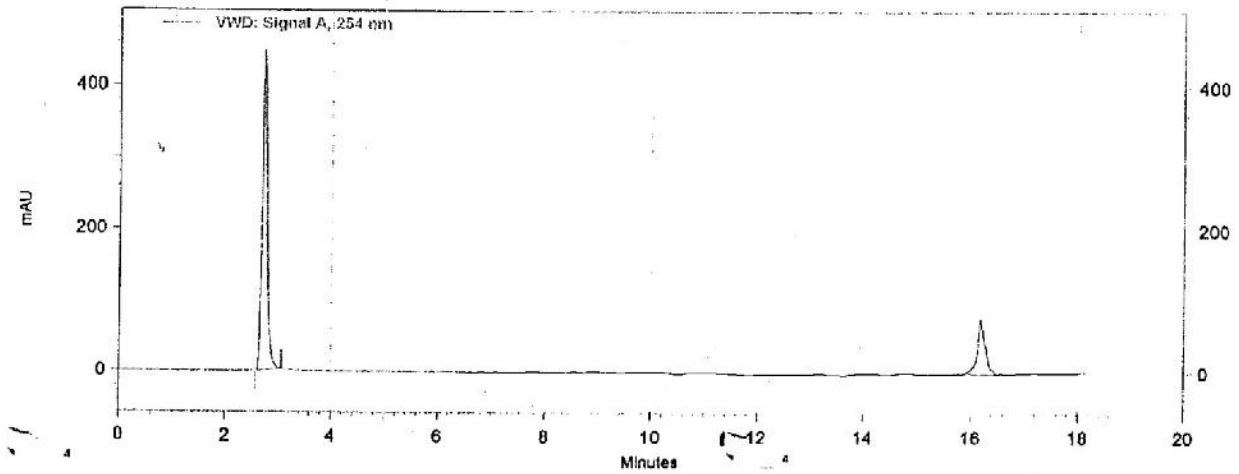

Fig. 5: Chromatogram of EDTA –Standard

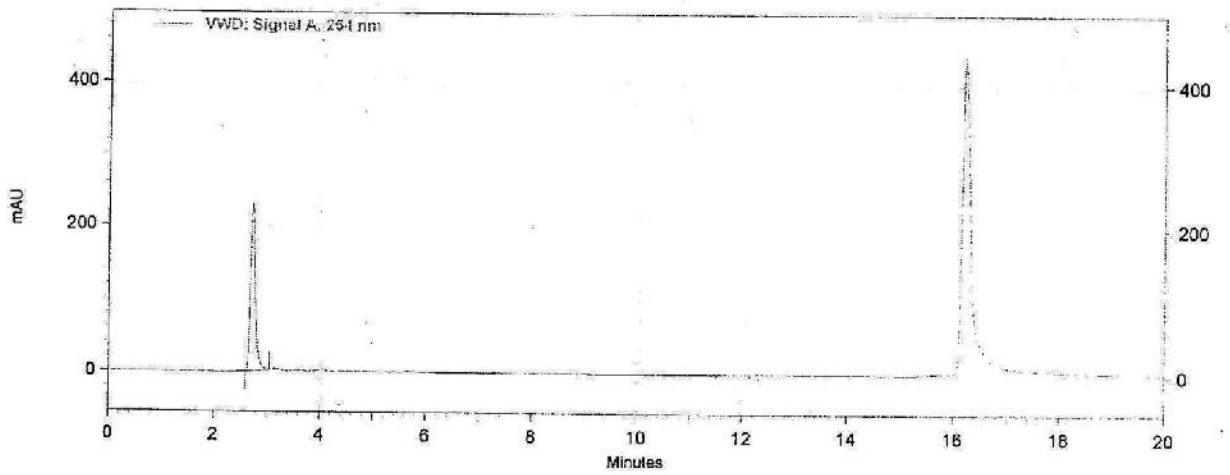

Fig. 6: Chromatogram of EDTA –Sample

Assay: method and observation for batch X

Weight of standard (ceftriaxone): 51.99 mg Dilution: 100ml

Weight of standard (sulbactam): 24.65 mg Dilution: 100 ml

Weight of sample: 85.99 mg Dilution: 100 ml

Table 9. Observation of assay method of batch X

Sr. No.	Area of standard		Area of sample	
	Ceftriaxone	Sulbactam		
1	452180983	37886603	506308698	41263841
2	452393864	38054734	506274055	41316589
3	452728262	38080376	-	-
4	452872980	38047430	-	-
5	452695199	38077553	-	-
Average	452574257.6	38029339.2	506291376.5	41290215
Std. Dev.	280528.97	81048.01		
RSD %	0.06	0.21		

System suitability parameters

Table 10. System Suitability Parameter

.**Parameter**	**Ceftriaxone Sodium**	**Sulbactum sodium**
Tailing factor	1.988	1.223
Theoretical plates	3772	5899
RSD	0,06	0.21
Resolution	9.096	

Batch Release Sheet of X Batch

Table 11. Batch Release Sheet of X Batch

Batch No. X Batch Size- 50000vials			
TEST	**ACCEPTANCE CRITERIA**	**METHODS**	**Result**
Appearance before reconstitution	White to pale yellow or cream color crystalline powder, homogeneous, free from extraneous particles	Visual inspection	Complies
Identification By UV detector	Positive for ceftriaxone, sulbactum and EDTA	Current Ph Eur.	Complies
Appearance after reconstitution	When powder constituted with water for injection, should be form clear and particle free solution	Visual inspection	Complies
pH(10%w/v solution)	Between 5.0 and 8.0	Current Ph Eur.	6.43
Water content	Not more than 11.0%	Current Ph Eur.	9.44
Particulate matter (visible particles)	free from visible particles	Current Ph Eur.	Complies
Particulate matter (sub visible particles) ≥ 10µm ≥25µm	 Not more than6000/vial. Not more than 600/vial	Current Ph Eur.	 3045 117
Clarity of reconstituted solution	Test solution not more than opalescent of reference solution I	Current Ph Eur.	Complies
Color of reconstituted solution	Test solution not more than opalescent of reference solution Y3	Current Ph Eur.	Complies
Uniformity of dosage units	Between 85.0% to 115.0% of the labeled claim	Current Ph Eur.	Complies
Bacterial endotoxins	not more than 0.20EU/mg	Current Ph Eur.	Complies
Sterility	Should be sterile	Current Ph Eur.	Complies

Batch No. X Batch Size- 50000vials			
TEST	**ACCEPTANCE CRITERIA**	**METHODS**	**Result**
Related substances ceftriaxone sodium			
Impurity A	Not more than 1.0% Not more than 1.0% Not more than 1.0% Not more than 1.0% Not more than 1.0% Not more than 0.2%	In -house	Not detected
Impurity B			0.005%
Impurity C			0.460%
Impurity D			Not detected
Impurity E			0.016%
Any unspecified imp.	Not more than 4.0%	In -house	0.015%
Total Impurities			0.516%
Sulbactam Sodium			
Impurity A			Not detected
Impurity B	Not more than 0.5% Not more than 0.1% Not more than 0.2% Not more than 0.1% Not more than 0.2% Not more than 0.1% Not more than 0.1%	In -house	Not detected
Impurity C			Not detected
Impurity D			Not detected
Impurity E			Not detected
Impurity F			Not detected
Any unspecified imp.	Not more than 1.0%		0.008%
Total impurities			0.008%
Assay by HPLC			
Ceftriaxone EP	Between 95.0 and 105.0%	In -house	99.16%
Sulbactam EP	Between 95.0 and 105.0%		101.02%
EDTA EP	Between 95.0 and 105.0%		104.37%
Dissolution time	not more than 3.0 minute	In -house	Complies

Table 12 .Batch number and batch size detail.

Sr. No.	Batch Number	Batch Size
1	X	50000 vials

The batch of ceftriaxone / sulbactam 1.5 g/ vial powder for solution for injection/infusion were validated for manufacturing and filling process. All the parameters mentioned in the protocol were monitored and found within the specifications. The parameters monitored are temperature, relative humidity, appearance, identification, water, pH, homogeneity and assay. All the parameters were within the set specifications. The parameters include components washing and sterilization, fill weights, in process checking of vials for sealing and visible particulate matters. All the parameters were found satisfactory. Filling process was monitored for viable particles through settling plates and volumetric air samplings. All the parameters were found satisfactory.

Filled vials were randomly sampled and analyzed for parameters, which includes appearance before reconstitution, Identification, Appearance after reconstitution, Water content, pH (10%w/v solution), Particulate matters (visible particle), Particulate matters (sub-visible particle), Clarity of reconstituted solution, Color of reconstituted solution, Weight uniformity, Bacterial endotoxin, Sterility, Related substance, Assay and dissolution time of ceftriaxone/ sulbactam 1.5g/ vials powder for

solution for injection / infusion. All the results found within the set specifications of in house and pharmacopoeia.

CONCLUSION

All the steps carried out as per the batch manufacturing record, Wright from starting to packaging and the parameters were found satisfactory. Process for manufacture of ceftriaxone/ sulbactam 1.5g/ vials powder for solution for injection / infusion is said to be in state of control. Hence this product can be manufactured by using this process without modifying any parameters.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The author declare no conflict of interest.

AUTHOR CONTRIBUTION

Md. Semimul Akhtar collected the contents and performed the literature survey. Akash Babu designed the work, corrected and made necessary revisions in the manuscript. Sudip Kumar Mandal contributed in drafting the Manuscript. All the authors framed the final manuscript.

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