Reconstitution Stability Evaluation Study of Market Brands of Cefixime and Potassium Clavulanate Oral Suspension

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ABSTRACT

The objective of the present study was to evaluate the stability of reconstituted oral suspension of Cefixime and Potassium clavulanate market sample. Cefixime/Clavulanate is a combination of β- lactam and β- lactamase inhibitor which restores the potency of cefixime against the strains produce β- lactamase. Stability evaluation studies of Cefixime and potassium clavulanate oral suspension was performed as per the ICH guidelines Q1A (R2). The various parameters analysed include Description, pH, weight/ml and assay to determination shelf life of reconstituted suspension. Those parameters were evaluated at Initial zero day, 3rd day and 7th day intervals.The storage conditions of sample were cool and dry place (Refrigerator) and normal room temperature. The assay of cefixime and clavulanic acid was carried out using HPLC method. The results of assay indicate that the suspension was within the allowable limits (90 - 120 %). The reconstituted oral suspensions were found to be stable over its intended shelf life of 7 days on the basis of the stability data generated. In view of the results obtained that Cefixime and clavulanate reconstituted oral suspension are recommended to be store at cool (Temperature between 8°C to 15 °C) and the stability of the oral suspension confirmed the ICH & USFDA guidelines.

Keywords
Cefixime, HPLC, potassium clavulanate, pH, Stability evaluation study

INTRODUCTION

Although conventional oral suspension can be administered immediately, there is an important category of suspension that requires mixing prior to administration.These are dry mixtures that require the addition of water at the time of dispencing.The reconstituted system is the formulation of choice when the drug stability is a major concern.After reconstitution, these system have a short but acceptable life if stored at refrigerator temperatures.Reconstitutable oral system show adequate chemical stability of the drug during shelf life.

Stability testing of pharmaceutical products is a complex set of procedures involving considerable cost, time consumption and scientific expertise in order to build in quality, efficacy and safety in a drug formulation. The most important steps during the developmental stages include pharmaceutical analysis and stability studies that are required to determine and assure the identity, potency and purity of ingredients, as well as those of the formulated products (Singh *et al.*, 2000). In other words, it is the extent to which a product retains, within the specified limits, throughout its period of storage and use, the same properties and characteristics possessed at the time of its packaging. Stability testing thus evaluates the effect of environmental factors on the quality of the a drug substance or a formulated product which is utilized for prediction of its shelf life, determine proper storage conditions and suggest labelling instructions. Moreover, the data generated during the stability testing is an important requirement for regulatory approval of any drug or formulation (Singh *et al.*, 2000). In addition, degradation reactions like oxidation, reduction, hydrolysis or racemization, which can play vital role in stability of a pharmaceutical product, also depend on such conditions like concentration of reactants, pH, radiation, catalysts etc., as well as the raw materials used and the length of time between manufacture and usage of the product. A pharmaceutical product may undergo change in appearance, consistency, content uniformity, clarity (solution), moisture contents, particle size and shape, pH, package integrity thereby affecting its stability.To best of our knowledge, there is no published study for this combination.

Table1: Storage Conditions:

Stability study	Storage conditions	Frequency of testing
Room Temperature	25°C ± 2°C/ 60 ± 5% RH	Initial, 1, 3 & 7 days
Refrigerator	5°C ± 3°C	Initial, 1, 3 & 7 days

Experimental

Material and Methods

Cefixime and Potassium Clavulanate oral suspension, the working standard: working standard for cefixime and Potassium Clavulanate, Acetonitrile and Tetrabutyl ammonium Hydroxie solution (10 % aqueous solution),

Orthophosphoric acid, Instruments Applied for Analytical Purpose: pH Meter, KF titrator, HPLC, Refrigerator were obtained from A.L. Ltd. Sikkim, India.

Estimation of Cefixime and Potassium Clavulanate oral suspension by HPLC method: The assay of Cefixime and Clavulanic acid was carried out using HPLC method, which is designated as stability indicating method because of its capability to distinguish the degraded product with parent one. The following table shows the chromatographic conditions.

Table 2: Chromatographic Condition

Column	250 mm x 4.6 mm x 5 µm, ODS(preferably Supelco 516 C-18 DB)
Flow rate	1.5 ml/min
Detector	UV-VIS
Wavelength	220 nm
Injection Volume	20 µl.
Column Temperature	30ºC

Reconstitution Stability Study

Stability study as per the ICH guidelines was carried out for the Cefixime and Potassium clavulanate oral suspension.

Table 3: Stability protocol and product details

Product Name	Generic Name	Label Claim
Cefixime and Clavulanate Potassium for oral suspension (50 mg/5 ml+31.25 mg/5 ml)	Cefixime and Clavulanate Potassium for oral suspension (50 mg/5 ml+31.25 mg/5 ml)	Assay (% label claim) Each 5 ml contains: Cefixime 50 mg Clavulanic acid 31.25 mg Freely reconstituted

Table 4: API's details

Name of the API	Cefixime IP as Trihydrate equivalent to Anhydrous Cefixime
	Potassium Clavulanate Diluted IP equivalent to Clavulanic Acid
Primary Pack	30 ml Amber coloured Bottle with Aluminium cap.

Table 5: Packing components details

Primary Pack	30 ml Amber coloured Bottle with Aluminium cap.
	PL. inner plug 20 mm
	Aluminium screw cap 20 mm
Secondary Pack	PP cap, P.L.M cup 10 ml

Table 6: Stability study plan

Sr. No.	Test interval	Parameters to be analyzed
1	0 day , Initial	I,II,III,IV and V
2	3 days	I,II ,III and V
3	7 days	I,II ,III and V

Table 7: Parameters to be analyzed

Sr. No	Parameter	Category
1.	Description	I
2.	pH	II
3.	Wt/ml	III
4.	Water Content (By KF)	IV
5.	Assay (By HPLC)	V

Table 8: Acceptance criteria for parameters tested

Sr. No.	Tests	Specification
1.	Description (As is) Reconstituted (5.0 g /30 ml)	White to off-white granular free flowing powder filled in 30 ml amber coloured glass bottle. White coloured homogenous suspension with flavour.
2.	pH	Between 3.5 and 4.5
3.	Wt/ml	1.05 ± 0.05 g/ml
4.	Water Content (By KF)	NMT 7.5% w/w
5.	Assay (% label claim) Each 5 ml contains: Cefixime 50 mg Clavulanic acid 31.25 mg Freely reconstituted	NLT 90.0% and NMT 120.0% of the labeled amount of Cefixime. NLT 90.0% and NMT 120.0% of the labeled amount of Clavulanic acid.
	Reconstitution Limit	Not less than 80 % of Labelled amount of Cefixime and Clavulanic acid.

RESULT AND DISCUSSION

Cefixime and Potassium Clavulanate oral suspension in the final package were tested for the analytical parameters such as description, pH, Moisture content and assay the results given in tables (9 and10). The remaining sample containers were divided to get twice the number required to carry out the analysis at prescribed sampling intervals, to compensate any problems during the analysis.

After giving the first injection of standard solution, the chromatogram was processed and system suitability report was generated, in each case starting from zero to 7th day. It was observed that the plate count for Cefixime and clavulanic acid was found to be 23213425 and 13178785 respectively. The column efficiency was found to be more than 2500 theoretical plates for both the peaks; therefore the system was suitable and appropriate for further study, for the rest of the period. The % RSD for the peaks was found to be 0.06% and 0.07% which is with in the allowable limit of 2.0 %. The initial % potency was found to be 108.6 % and 112.5 % for cefixime and clavulanic acid respectively. It was observed that the plate count for cefixime and clavulanic acid in 0 day was found to be 23201239 and 13167858 respectively. For the 3 rd day, it was observed that the plate count for cefixime and Clavulanic acid was found to be 24233726 and 13485418 respectively. For the 7 thdays it was observed that the plate count for cefixime and clavulanic acid was found to be 22533728 and 13869458 respectively. The % potency and result for both the conditions are given in table 9 & 10 respectively.

It was observed that the loss in potency of Clavulanic acid is more compared to potency of cefixime simultaneously significant changes in description. Though there is increased loss in potency of Clavulanic acid in room storage condition at 7 th day and out of specification result observed. This is considred to be significant, since the loss is > 5 % (as per Q1A (R2) and USFDA) in both the Cefixime and Clavulanic acid at room condition. i.e from 108.6 % to 103.0% and 112.5% to 100.0 % respectively.

Similarly the potency of Cefixime and Clavulanic acid in Refrigerated condition found insignificant at 3rd day study, but significant at 7th day study. The loss of potency within the limit of reconstituted powder and description also complying.

Table 9: % Assay potency result of Cefixime and Clavulanic acid in Room Temperature

Sr. No.	Tests	Specifications	Reconstitution study result		
			Initial 0 day	**After 3rd day**	**After 7th day**
1	Description	@	Complies	Complies	SC
2	%Water content (By KF)	Not more than 7.5 % w/w	2.15 %	NA	NA
3	pH (By pH Meter)	Between 3.5 to 4.5	3.77	3.89	4.10
4	Wt/ml	1.05 ± 0.05 g/ml	1.04 g/ml	1.03 g/ml	1.02 g/ml
5	% Assay (By HPLC) Reconstitution Limit Not less than 80 % of Labelled amount of Cefixime and Clavulanic acid.	**Cefixime** Not less than 90.0% and Not more than 120.0% of the labeled amount of Cefixime.	108.6 %	103.0 %	98.0 %
		Clavulanic acid Not less than 90.0% and Not more than 120.0% of the labeled amount of Clavulanic acid.	112.5 %	100.0 %	78.0 %

@ White to off-white granular free flowing powder filled in 30 ml amber coloured glass bottle. After reconstitution give white coloured homogenous suspension with characteristic flavour.

SC: Significant changes in decription as discoloration of colour and texture observed.

Table 10: % Assay potency result of Cefixime and Clavulanic acid in Refrigerated condition

Sr. No.	Tests	Specifications	Reconstitution study result		
			Initial 0 day	**After 3rd day**	**After 7th day**
1	Description	@	Complies	Complies	Complies
2	%Water content (By KF)	Not more than 7.5 % w/w	2.15 %	NA	NA
3	pH (By pH Meter)	Between 3.5 to 4.5	3.77	3.82	3.92
4	Wt/ml	1.05 ± 0.05 g/ml	1.04 g/ml	1.03 g/ml	1.05 g/ml
5	% Assay (By HPLC) Reconstitution Limit Not less than 80 % of Labelled amount of Cefixime and Clavulanic acid.	**Cefixime** Not less than 90.0% and Not more than 120.0% of the labeled amount of Cefixime.	108.6 %	106.0 %	105.0 %
		Clavulanic acid Not less than 90.0% and Not more than 120.0% of the labeled amount of Clavulanic acid.	112.5 %	108.0 %	88.5 %

CONCLUSION

"Reconstitution Stability Evaluation Study of Market Brands of Cefixime and Potassium Clavulanate Oral Suspension" was performed as per ICH guidelines Q1A (R2) by analyzing various parameters such as description, pH, water content and assay at initial, 3 rd day and 7 th day interval at room temperature (25±2°C/60±5%RH) and Refrigerated conditions (5°C ± 3°C). All the parameters do not found to be changed significantly over a study period. The shelf life of the reconstituted oral suspension was found to be stable up to 7 days in refrigerated condition and 3 days in normal room temperature. In view of the results obtained that cefixime and Clavulanic acid oral suspension tested are recommended to store at refrigerated condition without compromising the potency and significant changes of drug products.The stability of oral suspension confirmed the ICH & USFDA guidelines.

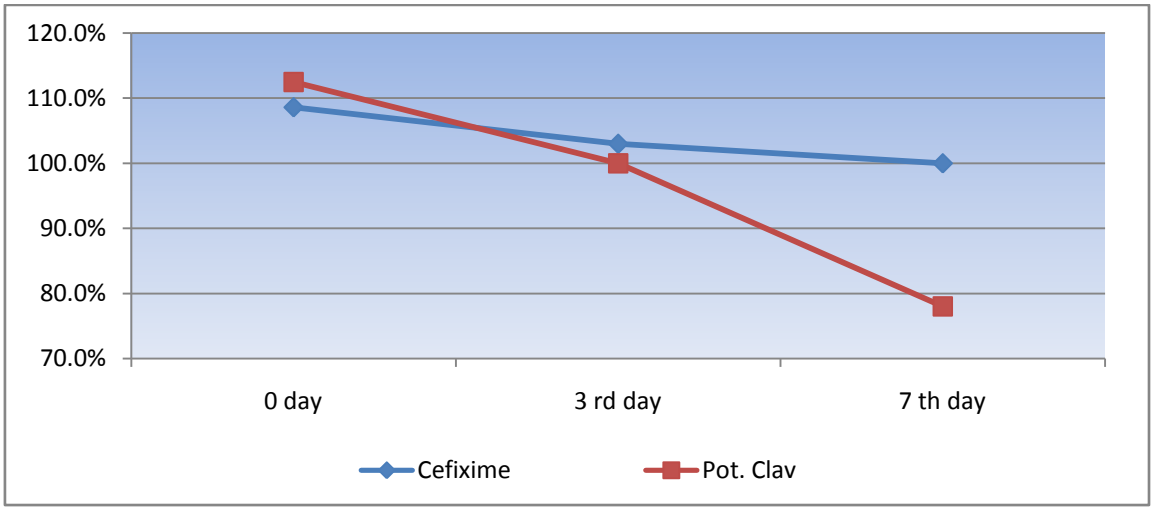

Fig 1: % assay results of Cefixime and potassium clavulanate at room condition

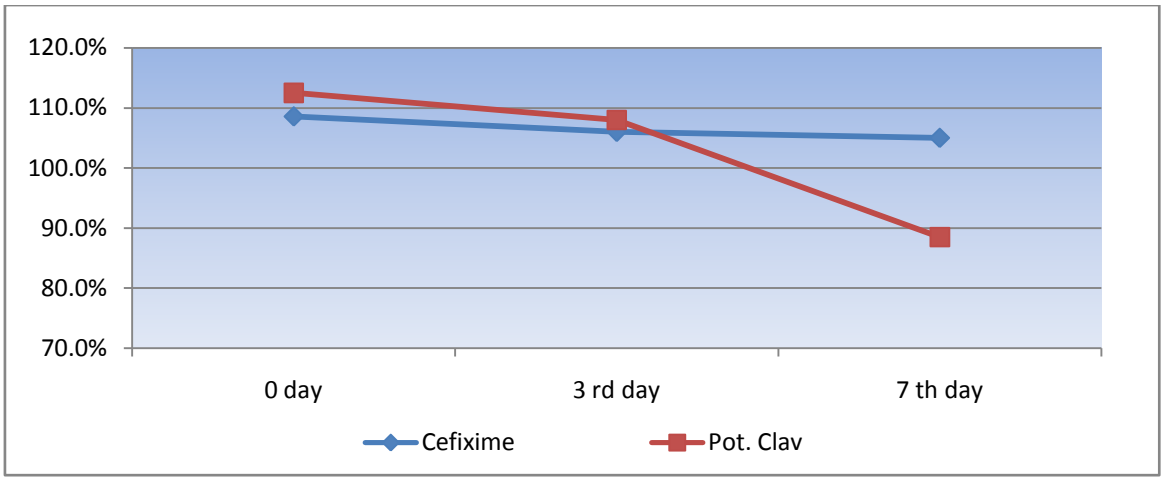

Fig 2: % assay results of Cefixime and potassium clavulanate at Refrigerated condition

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