ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Preparation, Characterization and Stability Study of Oral Reconstitutable Suspension of Amoxicillin and Potassium Clavulanate

Pranit P Atpadikar[1], Amol S Sherikar[*1], Mahanthesh M C[2], Disouza J I[1]

[1]Department of Quality Assurance, Tatyasaheb Kore College of Pharmacy, Warananagar – 416 113, Maharashtra, India
[2]Department of Pharmacognosy, Tatyasaheb Kore College of Pharmacy, Warananagar – 416 113, Maharashtra, India

Article History:

Received on: 25 Dec 2021
Revised on: 26 Jan 2022
Accepted on: 28 Jan 2022

Keywords:

Reconstitutable,
Micromeritic,
Carr's Index,
HPLC Assay,
Accelerated Stability

ABSTRACT

To overcome the problem of bitter taste, degradation, and sedimentation, reconstitutable suspension of Amoxicillin and Potassium Clavulanate was prepared. The suspension was prepared by using two different formulations. The suspensions were characterized for micromeritic studies such as bulk density, tap density, Car's index, angle of repose, pH, and viscosity. The HPLC assay was performed for the quantitative determination of Amoxicillin and Clavulanate potassium from suspension. The accelerated stability study was carried out to predict the stability profile of a drug product. Formulated reconstitutable suspension of Amoxicillin and Potassium Clavulanate has IPQC properties. From the results, it is concluded that reconstitutable suspension was successfully prepared and evaluated.

*Corresponding Author

Name: Amol S Sherikar
Phone: 091-9881901262
Email: assherikar.tkcp@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v13i2.452

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

Chemically, Amoxicillin sodium is sodium (2S,5R,6R)- 6-{[(2R)-2-amino- 2-(4-hydroxyphenyl)- acetyl]amino}- 3,3-dimethyl-7-oxo- 4-thia- 1-azabicyclo[3.2.0]heptane-24-carboxylic acid and Clavulanate potassium is potassium (2)-(2R,5R)-3-(2-hydro-xyethylidene)-7-oxo-4-1-azabicyclo[3.2.0]-heptane-2-carboxylate. Both drugs are officially present in USP, BP, and other pharmacopeias. Several Amoxicillin and Clavulanic acid combinations are available on market in the form of tablets and suspension. These products are used for the short-term treatment of different bacterial infections in the upper and lower respiratory tracts, genito-urinary tract, abdominal infections, skin, and soft tissues. The ability of Clavulanate to inhibit β-lactamase extends the broad-spectrum activity of amoxicillin. Amoxicillin is semi-synthetic penicillin with a broad spectrum anti-bactericidal activity, good oral absorption acts through the inhibition of biosynthesis of the cell wall. It is having high efficacy against S. pneumonia and H. influenza.4-6 Clavulanic acid is a potent inhibitor of β-lactamases of both Gram +ve and Gram -ve pathogenic bacteria. Its bioavailability is 60% [1, 2] and has a protective effect on Amoxicillin [3, 4].

The combination of Clavulanic acid with Amoxicillin extends the anti-bacterial spectrum and it is potent against resistant bacteria. Thus this combination is very important for the inhibition of a wide range of bacteria and treatment of a variety of bacterial infections. This combination is active against S. pneumonia, beta-lactamase-producing H. influenza, and M. catarrhalis. It is well known as one of the most active oral agents against S. pneumonia.

Suspension is an intimate mixture of dry, finely divided drugs with excipients, which, upon the addition of a suitable vehicle, yields a suspension [5]. To avoid swallowing problems reconstitutable suspension is preferred which are reconstituted at the time of use and thus can be used as a liquid formulation. The stability of the product is a big concern for tropical countries where products are exposed to elevated temperatures (up to 40°C) and relative humidity (up to 90%), especially during transport and storage. The reconstituted system is the formulation of choice when drug stability is a major concern. After reconstitution, these systems have a short but acceptable life if stored at refrigerator temperatures [6]. Reconstitutable oral systems show adequate chemical stability of the drug during shelf life, avoid the physical stability problems related to solubility, pH, and incompatibilities with other ingredients and also reduce the weight of the final product because the aqueous vehicle is absent and consequently the transportation expenses may be reduced [7].

Stability testing is carried out to check how the product behaves under the influence of a variety of environmental conditions such as temperature, humidity, and light. The purpose of stability studies is to establish a shelf-life to determine the storage conditions. To know about the length of the time and conditions where efficacy, safety, and quality of the product are maintained [8].

The purpose of accelerated stability testing is to increase the rate of chemical degradation and physical change of a drug by using exaggerated storage conditions as part of the formal stability testing program. The data thus obtained, in addition to those derived from real-time stability studies, may be used to assess longer-term chemical effects under non-accelerated conditions and to evaluate the impact of short-term excursions outside the label storage conditions, as might occur during shipping or transportation. The results of accelerated testing studies are not always predictive of physical changes. Real-time (Long-Term) stability studies experiments on the physical, chemical, biological, biopharmaceutical, and microbiological characteristics of a drug, during and beyond the expected shelf-life and storage periods of samples under the storage conditions expected in the intended market. The results are used to establish the shelf-life, to confirm the projected shelf-life, and to recommend storage conditions [9].

The main objective of the present work was to prepare, characterize, and carry out stability studies of reconstitutable suspension of Amoxicillin and Potassium Clavulanate.

MATERIALS AND METHODS

Amoxicillin and Potassium Clavulanate were collected from Maneesh Pharmaceutical's ltd, Mumbai as a gift sample. Analytical grade chemicals and reagents were used throughout the experimental work.

Preparation of suspensions

Two different trials were planned to develop the formulations. This suspending agent (xanthan gum) was used in the first formulation whereas it was omitted in the second one as shown in Table 1.

The procedure involves the following steps:

1. Dispensed API and all Raw materials.
2. Sifted Potassium Clavulanate, Aspartame, Aerosil, Sodium Benzoate, Sodium Citrate, Orange Flavor, Citric Acid, Talcum, and Mannitol.
3. Xanthan Gum, Sodium Benzoate, and Sodium Citrate dried in tray dryer at 60°C.
4. Blended all RM without lubricant for 10 Min.
5. Lubricated all RM with lubricant for 5 Min [10–12].
6. Then finally formulation was ready for filling [13, 14].

Characterization of Suspension

Flow Properties/Micromeritic

The prepared granules of Amoxicillin and Potassium Clavulanate oral suspension were characterized by their micromeritic properties such as bulk density, tapped density, Carr's compressibility index [15, 16], and angle of repose [17, 18].

pH

The digital pH meter was used to measure the pH of the prepared formulation.

Differential Scanning Calorimetry (DSC)

About 3-5 mg of sample in 100 μL aluminium pan was measured with a DSC 1 (Mettler-Toledo, DSC 1 star system, Mumbai, India). A scan rate of 10^0/min and a nitrogen gas flow of 40 ml/min was applied. The measurement was conducted in a temperature range of 40^0C to 150°C.

X-Ray Diffraction (XRD)

The crystallinity in the prepared formulation was investigated by a transmission diffractometer

Table 1: List of Product Information

S. No.	Name of Ingredient	Formulation 1	Formulation 2
		Quantity (mg)	
1.	Amoxicillin	200	200
2.	Potassium Clavulanate	28.5	28.5
3.	Aspartame	4.0	4.0
4.	Aerosil	10.0	10.0
5.	Xanthan Gum	3.1	-
6.	Sodium Benzoate	4.25	4.25
7.	Sodium Citrate	1.00	1.00
8.	Orange Flavor	4.00	4.00
9.	Citric Acid	3.50	3.50
10.	Talcum	3.5	3.5
11.	Mannitol	161.34	164.44

Table 2: HPLC Assay Parameter

S. No.	Parameter	System Specification
1.	Mobile Phase (Gredient)	Buffer: Methanol 95:5
2.	Column	30 cm $\times$ 4.0 mm id (3 to 10 microns) L1. Or equivalent.
3.	Wave length	220 nm
4.	Flow Rate	2.0 ml/min
5.	Injection volume	20μl
6.	Column Temperature	Ambient temperature
7.	Detector	UV detector

Table 3: Physical/flow properties/micromeritics of Amoxicillin and Potassium Clavulanate Oral Suspension (Formulation 2)

S. No.	Test Parameter	Specifications	Results
1.	Description	Off white colored granular powder	Complies
2.	Bulk density	-	0.5
3.	Tap Density	-	0.7
4.	Angle of Repose	-	15
5.	Carr's Index	-	9
6.	pH	3.82 - 6.6	4.73

Table 4: Viscosity and Thixotropy of Amoxicillin and Potassium Clavulanate Oral Suspension

Batch. No.	Viscosity	Thixotropy
TR01	1.0030 pa/s	58.4639 pa/s
TR02	1.2894 pa/s	56.754 pa/s
TR03	1.2572 pa/s	57.532 pa/s

Table 5: Quantitative HPLC Assay of Amoxicillin and Potassium Clavulanate Oral Suspension

Name of Drug	Specification	% found
Amoxicillin	90%-120% of Labeled amount	103.97
Potassium Clavulanate	90%-120% of Labeled amount	117.76

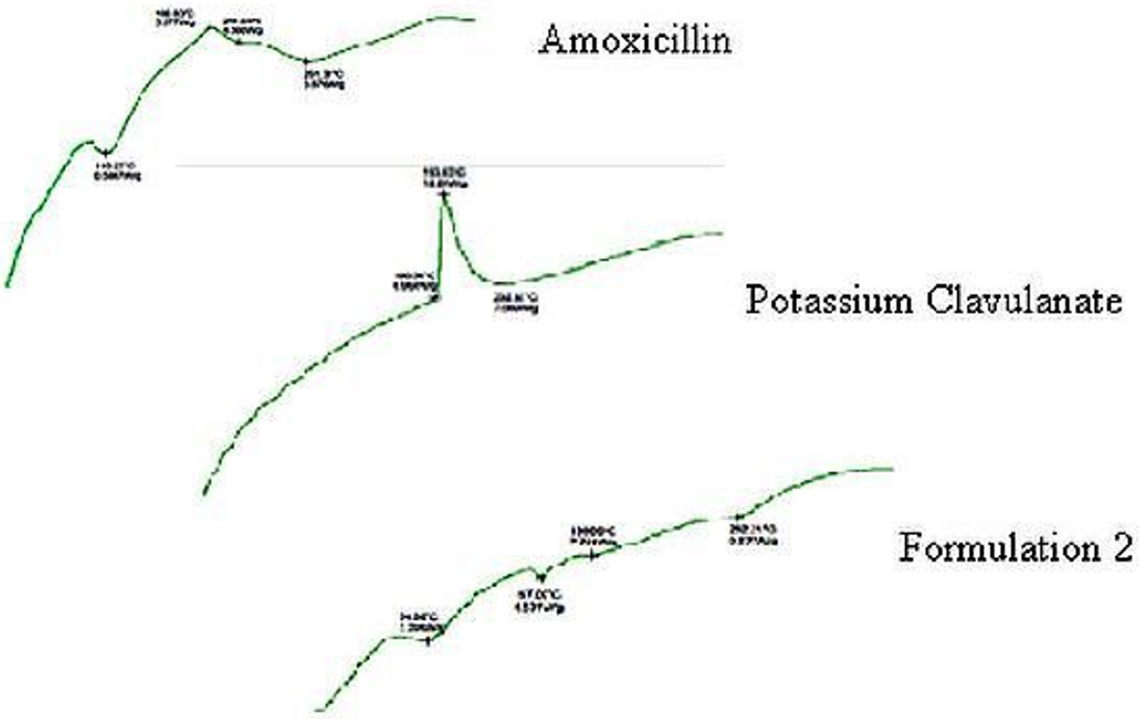

Figure 1: DSC of Amoxicillin, Potassium Clavulanate and Formulation 2 X-Ray Diffraction (XRD)

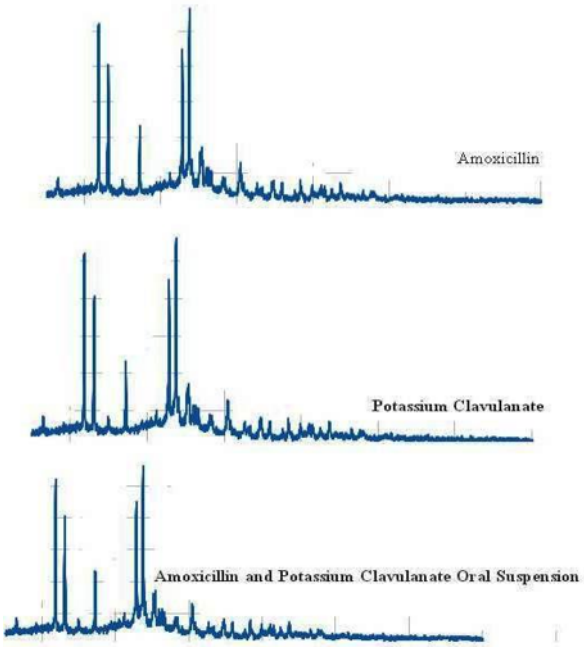

Figure 2: XRD of Amoxicillin, Potassium Clavulanate and Formulation 2

Table 6: Result of Accelerated Stability Studies

Test Parameter	0 Month	3 Month	6 Months
Bulk density	0.5	0.6	0.5
Tap Density	0.7	0.8	0.7
pH	4.80	4.75	4.81
Amoxicillin	103.97 %	102.45 %	101.68 %
Potassium Clavulanate	117.76 %	116.28 %	115.41 %

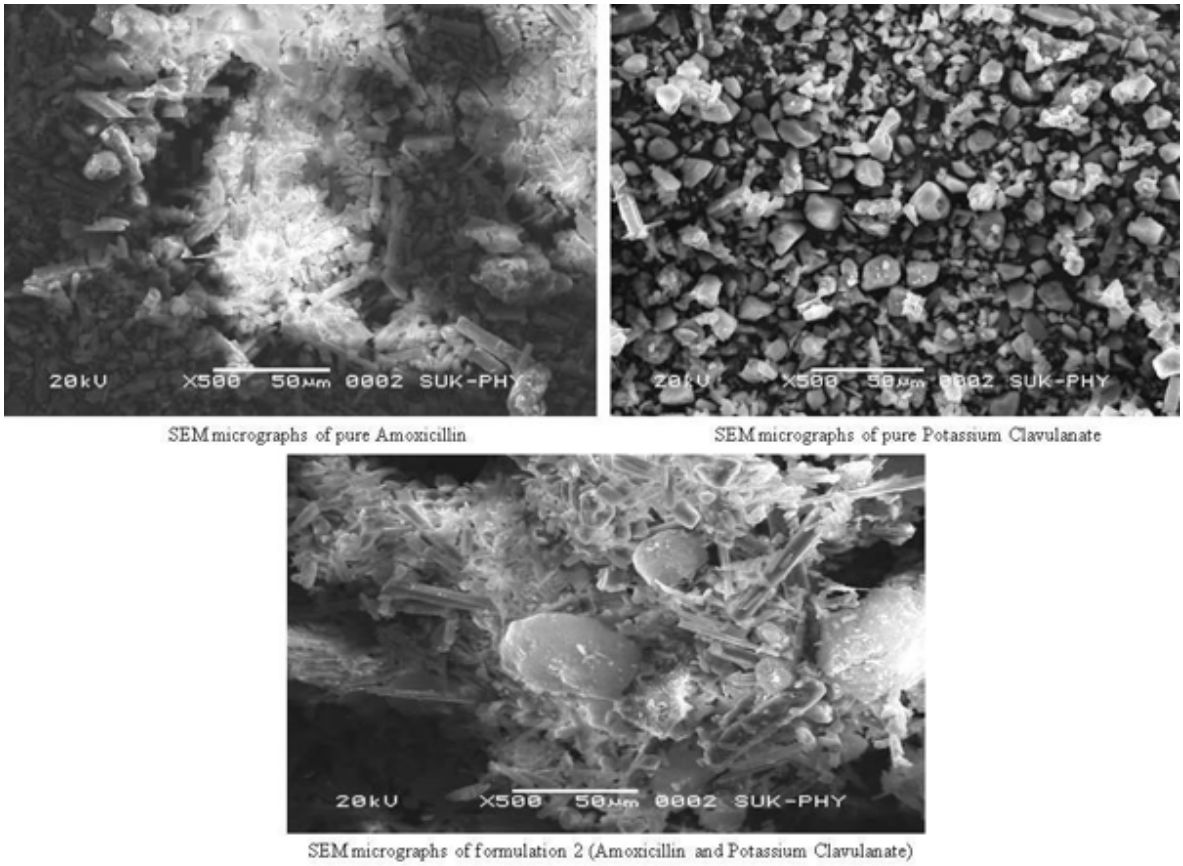

Figure 3: SEM Micropraphs of Amoxicillin, Potassium Clavulanate and Formulation 2

(rigaku miniflex, Mumbai, India). Diffraction patterns were obtained at a voltage of 45 kV and a current of 40 mA. Samples were scanned in a 2 θ range from $5^0$to 70^0 with a scanning speed of 2^0/min and an intensity of 1000 cps.

Scanning Electron Microscopy (SEM)

The surface morphology of formulation was analyzed by a scanning electron microscope model JEOL, JSM-5400 (Japan) coupled with energy dispersive X-ray analysis (EDAX).

Viscosity and Thixotropy

The viscosity of the samples was determined using a Brookfield digital viscometer. The sample temperature was controlled at 25^0 C before each measurement.

Assay by (HPLC)

The HPLC analysis was carried out on Agilent HPLC (Compact LC) 1120 with parameters given in Table 2. The following procedure was employed for the HPLC assay.

1. Weighed accurately about 125.0 mg of Amoxicillin Trihydrate working standard in 50 ml volumetric flask. Added 30ml water shake well and stirred for 45 min and diluted up to mark with water (Solution B).
2. Weighed accurately about 65.0 mg of Potassium Clavulanate diluted with Microcrystalline Cellulose 1.1 working standard in 50.0 ml volumetric flask, added 30 ml water, shake and stirred for 45 minutes and dilute up to the mark with water

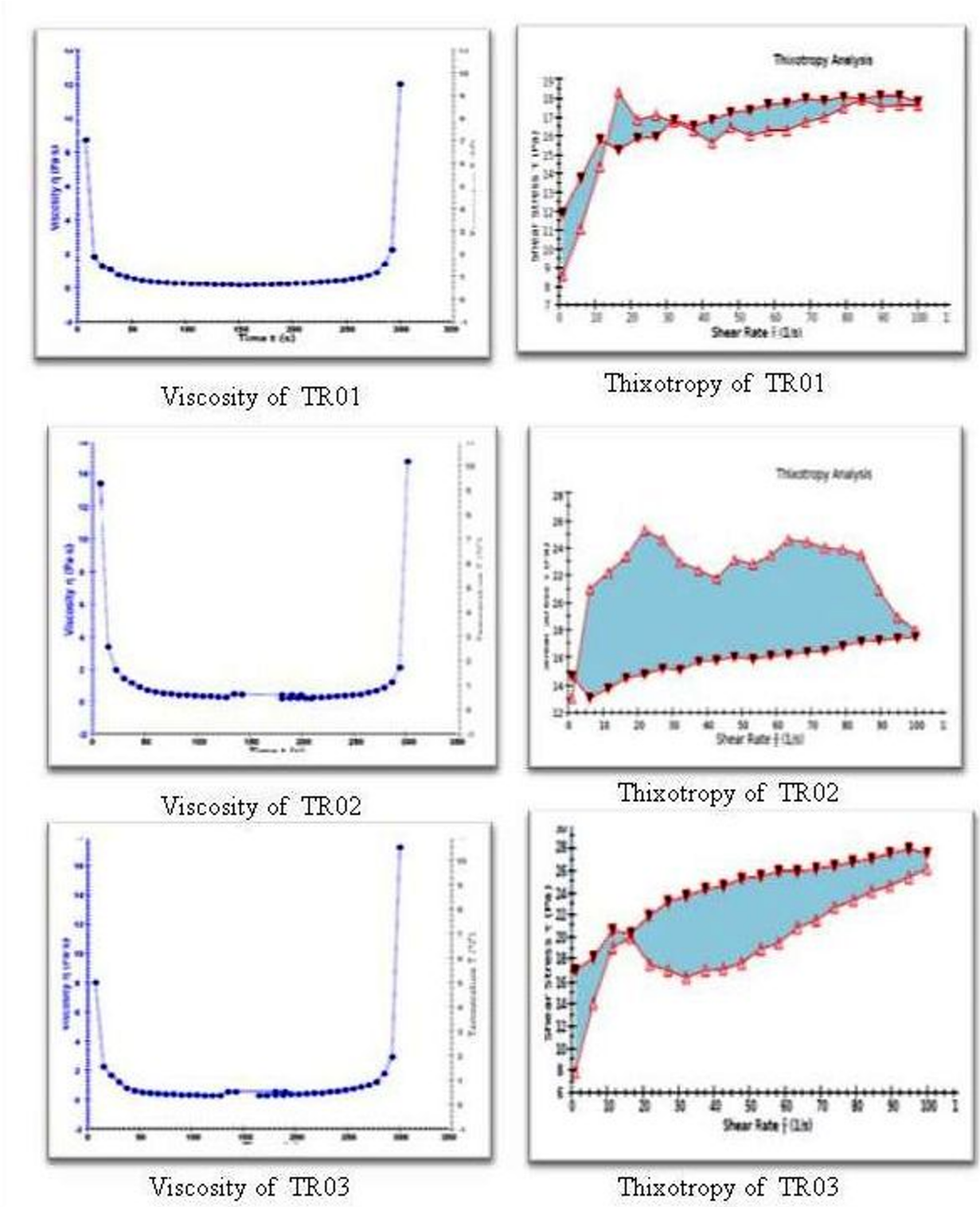

Figure 4: Viscosity and Thixotropy of Batch TR01, TR02 and TR03

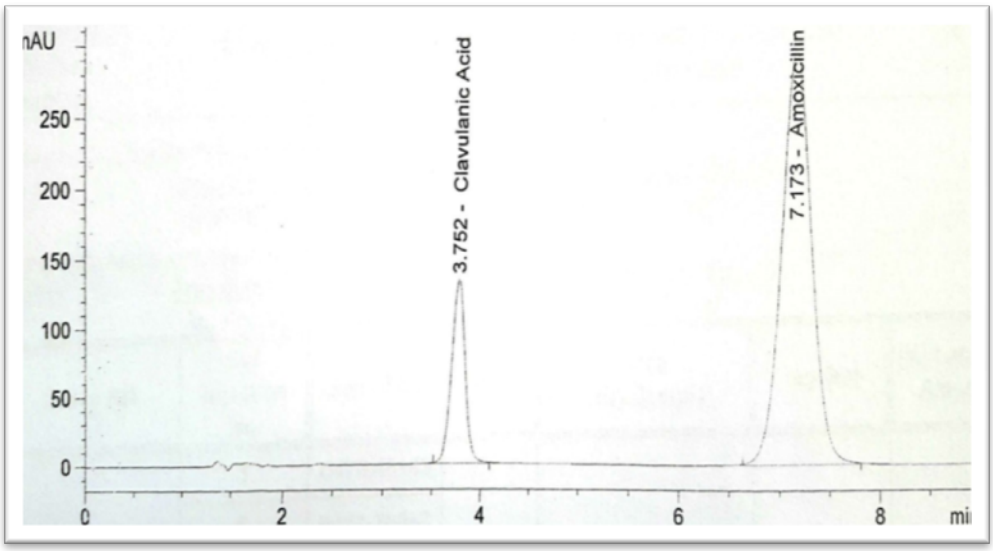

Figure 5: HPLC Chromatogram of Formulation 2

(Solution A).

3. Mix Standard Preparation: Pipette out 10 ml of solution A in a 50 ml volumetric flask. Added 10 ml of solution B and make up the volume up to mark with water.
4. Sample Preparation: Reconstituted the bottle of suspension with water till volume up to the mark and shaken vigorously as directed in the labeling. 5 ml suspension was diluted with water up to 500 ml to obtain about 0.5 mg of Amoxicillin per ml. Filtered off the assay preparations. Injected in duplicate & recorded the area [19–21], calculated mean area of the sample [22].

Calculation

Calculation for – Amoxicillin Trihydrate IP equivalent to Amoxicillin (L.A. 200 mg/5ml) Mg/5ml =

$$\frac{Mean\ area\ of\ test}{Mean\ area\ of\ Std} \times \frac{Wt\ of\ Std}{50} \times \frac{10}{5} \times \frac{1000}{5} \times \frac{Std\ purity}{100} \times$$

$$5\%Assay\ of\ Amoxicillin = \frac{\frac{mg}{5ml} \times 100}{200}$$

(Limit: NLT 90%-120% labeled amount)

Calculation for – Potassium Clavulanate diluted IP equivalent to Clavulanic Acid (L.A. 28.5 mg/5 ml) Mg/5ml =

$$\frac{Mean\ area\ of\ test}{Mean\ area\ of\ Std} \times \frac{Wt\ of\ Std}{50} \times \frac{10}{5} \times \frac{1000}{5} \times \frac{Std\ purity}{100} \times$$

$$5\%Assay\ of\ Clavulanic\ acid = \frac{\frac{mg}{5ml} \times 100}{28.5}$$

(Limit: NLT 90%-120% labeled amount)

Stability Study

The optimized batch of Amoxicillin and Potassium Clavulanate oral suspension were stored at 40°C±2°C/75±5° C % RH for 3 months and 25°C±2°C/60±5% RH for 3 months in a stability chamber and the effects of storage condition on the preparation were studied by Physical characterization, HPLC Study. Accelerated stability of sample tested at 0, 3, 6 months.

RESULTS AND DISCUSSION

After the development of formulation, 1 appearance and uniformity of the suspension was checked and it was found improper. Xanthan Gum does not dissolve properly after reconstitution of suspension. Formulation 1 is not palatable for pediatric patients because crystal remains in suspension. Hence formulation 1 study is stopped at this stage no further study was carried out.

Flow Properties/Micromeritic

Amoxicillin and Potassium Clavulanate Oral Suspension (Formulation 2) physical parameters were checked like Bulk density, Tap density, Angle of repose, Carr's Index, pH. Description complies with the specification. Bulk density 0.5, Tap density 0.7, Angle of repose 15, Carr's Index 9, and pH 4.73 these all physical parameter result complies with the predetermined specification found well within the limit and found satisfactory.

After the development of formulation, 2 appearances and uniformity of the suspension were checked and it was found proper.

Formulation 2 was palatable for pediatric patients because the uniformity of suspension was found satisfactory. Also, the taste is pleasant which masks the bitter taste of Amoxicillin. The results are given in Table 3.

Differential Scanning Calorimetry (DSC)

Figure 1 shows the DSC thermogram, the melting point and the melting enthalpy of crystalline Amoxicillin were 110.250 C and 0.5867 w/g respectively and Amorphous Potassium Clavulanate were 186.540C and 6.994w/g has been observed. In DSC of Amoxicillin and Potassium Clavulanate Oral suspension, it clearly indicated that distinct melting point of Amoxicillin and Potassium Clavulanate changes to 94.99^0C. This implies that the crystalline form of drug transfer to amorphous in Amoxicillin and Potassium Clavulanate Oral suspension.

Figure 2 shows the X-ray diffraction pattern of pure Amoxicillin, Potassium Clavulanate, and Amoxicillin and Potassium Clavulanate oral suspension. The diffraction pattern of Amoxicillin suggests that Amoxicillin is purely crystalline and Potassium Clavulanate is purely amorphous. From the X-ray diffraction pattern of Amoxicillin and Potassium Clavulanate Oral suspension is showed that there is the formation of amorphous form because there is a decrease in the intensity of peaks.

Scanning Electron Microscopy (SEM)

Figure 3 depicts SEM images of pure Amoxicillin, Potassium Clavulanate, and Amoxicillin and Potassium Clavulanate oral suspension. The bar on the picture indicates the degree of magnification (50 μm for all images). The SEM micrograph of pure Amoxicillin, Potassium Clavulanate revealed irregular shape crystals. An image of Amoxicillin and Potassium Clavulanate oral suspension indicates that in some extend Amoxicillin and Potassium Clavulanate transfer to amorphous form.

Viscosity and Thixotropy

The viscosity and thixotropy study was carried out for three consecutive batches i.e. TR01, TR02, and TR03. The result is given in Table 4. Figure 4 depicts the viscosity and thixotropic behavior of all the batches.

Assay by (HPLC)

For detection of the sample, HPLC wavelength was selected 220 nm. The HPLC chromatogram is shown in Figure 5. It is observed that for Amoxicillin retention time was 3.871 min, area of peak 1408.65173, the height of peak 141.10555, plates 3747 and for Potassium Clavulanate retention time was 7.179 min, area of peak 6699.26221, the height of peak 328.24149, plates 3006.

Table 5 shows the amount of drug found in the formulation.

Stability Study

The stability study studies of the formulation are performed on stability condition $40 \pm 2^\circ$C/ $75 \pm 5\%$ RH (Accelerated stability study) and $25 \pm 2^\circ$C/ $60 \pm 5\%$ RH (Long term stability studies) revealed that no significant changes in the physical parameter and analytical parameter.

In accelerated stability at time point zero months, physical parameters checked like Bulk density, Tap density, pH. Description complies with the specification. After HPLC analysis, it is observed that the assay of Amoxicillin was 103.97% and Potassium Clavulanate is 117.76% which is well within the standard limit of assay 90%-120% of the labeled amount.

The accelerated stability study at time point three months, physical parameter checked like Bulk density, Tap density, pH. Description complies with the specification. The assay of Amoxicillin by HPLC produced 102.45% and Potassium Clavulanate 116.28% which is well within the standard limit of assay 90%-120% of the labeled amount.

Accelerated stability study at time point six months, physical parameter checked like Bulk density, Tap density, pH. Description complies with the specification. In HPLC assay is observed that Amoxicillin was 101.68% and Potassium Clavulanate is 115.41% which is well within the standard limit of assay 90%-120% of the labeled amount. The results are given in Table 6.

CONCLUSION

Formulation 2 was developed by the dry granulation method and physical parameters like Bulk density, Tab density, angle of repose, pH, and appearance were checked. Result of physical parameter found well within the limit. After reconstitution, it was observed that the appearance, consistency of suspension were very good and proper. In the HPLC assay study, it was observed that assay of Amoxicillin showed 103.76% to 103.88% of labeled claim and assay of Potassium clavulanate showed 116% to 117% of labeled claim which is well within standard limit 90% to 120% as per IP and USP monograph. From the result of DSC, XRD, and SEM characterization, it was found that Amoxicillin and Potassium clavulanate both has crystalline form. From the result of the stability study, it can be concluded that the prepared formulation is a stable one.

ACKNOWLEDGEMENT

The authors are thankful to Shree Warana Vibhag Shikshan Mandal, Warananagar for providing the necessary facilities required to carry out the above work.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] S P Kaur, R Rao, and S Nanda. Amoxicillin: a broad spectrum antibiotic. *Int J Pharm Pharm Sci*, 3(3):30–37, 2011.

[2] N Peace, O Olubukola, and A Moshood. Stability of reconstituted amoxicillin clavulanate potassium under simulated in-home storage conditions. *J. Appl. Pharm. Sci*, 2(1):28–31, 2012.

[3] J G Avari and M Bhalekar. Cation exchange resins for taste masking and rapid dissolution of sparfloxacin. *Indian Drugs-Bombay*, 41(1):19–23, 2004.

[4] C A Howard. Introduction to pharmaceutical dosage forms. pages 139–166, 1981. Lea and Febiger, Philadelphia, PA.

[5] ICH Q1A (R2). Stability testing of new drug substances and products. 2003. International Conference on Harmonisation.

[6] ICH Guidelines-Q1E. Evaluation of Stability Data. 2003.

[7] J Swarbrick and J C Boylan. Encyclopedia of pharmaceutical technology. page 370, 2002. Marcel Dekker Inc., New York.

[8] S B Bari, B M Mahajan, and S J Surana. Resistance to antibiotics: A challenge in chemotherapy. *Indian Journal of Pharmaceutical Education and Research*, 42(1):3–11, 2008.

[9] L Prabakaran, M Bajpai, and T R Saini. Formulation development and optimization studies of satranidazole oral suspension. *J Pharm Res*, 4:19–23, 2005.

[10] R J Parasimpathomimetics. Martindale-The Extra Pharmacopoeia. 1996. Royal Pharmaceutical Society. London, P. 1413, 1428.

[11] The United State Pharmacopoeia. 1995. Easton: Mack Publishing Company, p.1342.

[12] The Indian Pharmacopoeia. 2007. Ministry of Health and Family Welfare, Government of India.

[13] C R Raymond, J S Paul, and C O Sian. Handbook of pharmaceutical Excipients, The pharmaceutical Press and The American Pharmacist Association. 2006. p. 124-125, 743-747, 821-823.

[14] The British Pharmacopoeia. 1988. The British Pharmacopoeia Commission, p. 105 & 192.

[15] N A Pendharkar and D R Chaple. Simultaneous estimation of azithromycin and ambroxol hydrochloride in combined tablet dosage form by multicomponent mode of analysis. *Asian Journal of Research in Chemistry*, 3(2):3–5, 2010.

[16] J Maurya. Role of process validation in pharmaceutical industry: an overview. *Journal of Harmonized Research in Pharmacy*, 2:151–172, 2013.

[17] S Sharma and G Singh. Process Validation in Pharmaceutical Industry; An Overview. *Journal of Drug Delivery and Therapeutics*, 3(4):184–188, 2013.

[18] M B Wazade, S R Walde, and A M Ittadwar. An Overview of Pharmaceutical Process Validation And Process Control Variables of Tablets Manufacturing Processes In Industry. *International Journal of Pharmaceutical Sciences and Research*, 3(9):3007–3022, 2012.

[19] I R Berry and R A Nash. Pharmaceutical Process Validation. volume 101, pages 25–26, New York, 2013. Marcel Dekker, Inc, New York.

[20] S Alam. An overview Pharmaceutical Process Validation. *J. Adv. Pharm. Edu. and Res*, 2:185–200, 2013.

[21] S S Patil, A S Sherikar, S Patil, and A Patil. Improvement of physicochemical characteristics and dissolution profile of poorly water soluble drug: ketoprofen by solid dispersion technique. *Int. J. Res. Pharm. Sci*, 1:1–4, 2010.

[22] J N Addotey, L Awudzi, and R K Adosraku. Stability studies on reconstituted amoxicillin-clavulanic acid oral powder by HPLC method development and quantification. *International Journal of Pharmaceutical Science and Practice*, 3(1):1–12, 2014.