ABSTRACT
Oral suspensions of antibiotics are mainly available as dry powders for reconstitution; many reconstituted antibiotic suspensions are to be kept refrigerated in order to get the optimal benefit from the drug. However, many patients do not keep to the specified storage conditions for different reasons like no refrigerator and irregular power supply that may result in various degrees of degradation of the product. The aim of the study was to determine the stability of cefuroxime axetil (Brand name) oral suspension (125mg/5ml) at different temperature storage conditions (stored at room temperature/25°C and refrigerated 2-8°C conditions). This study investigated stability of cefuroxime axetil oral suspension at different temperature storage conditions of erratic power supply and no refrigeration. Three different batches from cefuroxime axetil 125mg/5ml oral suspensions (Zinnat) were reconstituted and stored in refrigerator (2-8°C) and the same batches are stored at room temperature 25°C over a period of 14 days. Samples from the suspension were assayed using a validated HPLC method and the concentration and percentage dissolution of cefuroxime axetil were measured using HPLC and UV spectrophotometer. Reconstituted cefuroxime axetil stored at refrigerator (2-8°C) is stable for 10 days over 90%, after that start to degradation and also the percentage dissolution over 60% for the first 10 days after that less than 60% outside the acceptance limits of USP. While concentration percentage of cefuroxime axetil stored at room temperature were over 90% up to 5th day, degradation was extensive by 7th day with cefuroxime axetil concentration falling to 80% outside the acceptance limits and percentage dissolution over 60% for the first 3 days after that the amount of dissolved drug was less than 60% and reach to 33% outside the acceptance limits of USP. Cefuroxime axetil suspension after reconstitution was found stable for 10 days, if stored at refrigerated condition but 3 days only at room temperature.

Keywords: Stability; Cefuroxime axetil; Reconstitution; oral suspension; Degradation.

INTRODUCTION
Cefuroxime axetil (acetoxyethyl ester of cefuroxime) is orally active prodrug of cefuroxime, second generation semi-synthetic cephalosporin antibiotic. Upon oral administration, cefuroxime axetil is absorbed from gastrointestinal tract and rapidly hydrolyzed by nonspecific esterase in the intestinal mucosa and blood releasing microbiologically active form (free acid), cefuroxime. Cefuroxime exerts its bactericidal effect against a range of gram-positive and gram-negative bacteria by inhibiting the synthesis of bacterial cell wall.1-3 Cefuroxime axetil (Figure 1) has a carbamoyl group in position 3, which accounts for considerable metabolic stability, and a methoxymino group in position 7, which provides resistance to β-lactamase attack and, together with a furyl ring, contributes to the antibacterial properties of the molecule by enhancing its activity against Gram-negative bacteria. A 1-acetoxyethyl ester group in the position 4 of cefuroxime axetil ensures its lipophilicity and promotes the intestinal absorption of cefuroxime.

For the preparation of pharmaceutical formulations only the amorphous form is used. It has better physicochemical and biological properties than the crystalline form, e.g. significantly higher solubility and bulk density as well as higher degree of absorption after oral administration.4,5 Due to their possible composition, pharmaceuticals are especially sensitive to environmental factors. Strict storage conditions are necessary for the maintenance of integrity and product activity. Stability is defined as the capacity of a drug substance or drug product to remain within the established specifications to maintain its identity, strength, quality, and purity throughout the retest or until expiry date period.6

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Stability testing of an active substance or finished product provides information on how the quality of drug substance or drug product varies with time influenced by a variety of environmental factors such as temperature, humidity and light. Knowledge from stability studies enables understanding of the long-term effects of the environment on drugs. Stability testing also provides information about the degradation mechanisms, potential degradation products, possible degradation pathways of drug as well as interaction between the drug and the excipients in drug product. The gained information is applied in the development of manufacturing processes, selection of proper packaging and storage conditions, and determination of product’s shelf life and expiration date.

Instructing patients on storage of reconstituted antibiotics at home is a challenge for pharmacists in this situation. This study is important to ascertain effect of in-home storage conditions on stability of reconstituted suspensions of cefuroxime axetil and give insight on appropriate pharmacists’ instructions when adequate refrigeration is unachievable.

Suspensions of cefuroxime axetil are available for use in children and must be refrigerated (2-8°C) to maintain effectiveness once reconstituted. Liquid formulations generally tend to have much shorter shelf-lives than solid formulations and once opened should be used within 2 weeks to avoid any microbial contamination or reduction in activity.

The nature of syrup formulations in terms of added adjuncts such as sweetening, flavoring, suspending, stabilizing and preserving agents makes the liquid formula a complex one that is very prone to physical, chemical and microbiological instability. Stability is defined as the capacity of a drug substance or drug product to remain within the established specifications, to maintain its identity, strength, quality, and purity throughout the retest or until expiry date period.

Stability testing provides information about the degradation mechanisms, potential degradation products, possible degradation pathways of drug as well as interaction between the drug and the excipients in drug product.

Therefore medicines are stored at room temperature or kept in fridge’s that has no power supply for several hours in a day thereby exposing these drugs to excessive temperatures far more than the room temperatures which ultimately may cause decomposition of both the excipients and active ingredient(s). Yousif [14], in a Sudanese study reported that the rate of unsuitable storage conditions of drugs was 26.0%, compared to 31.8% in the Papua New Guinea study and that there was a higher rate of inappropriate storage in rural areas due to the lack of refrigeration. Drugs are chemicals that react to external stimuli such as, heat, humidity, light, microbial agents and dust. In many cases, such reactions can only lead to physical changes such as discoloration of the drug product. In many other cases, the reaction may affect the drug more seriously leading to the reduction or elimination of its effectiveness and/or potency. There are cases of drugs that, when affected, not only the failure of the drug to exert a therapeutic effect, but also cause adverse effects on the patient’s health. Therefore storage conditions must not be taken lightly; studies have shown different drug in-home storage practices, some store or keep their drugs on the dining table, top of the refrigerator, first aid boxes, in their bags, in the car, closed cupboard or drawer, suit case, in the kitchen and even the bathroom and these practices may result to degradation.

A study carried out to determine the chemical stability of amoxicillin and potassium clavulanate (250/62 Co-amoxiclav) oral suspension stored at room temperature 20°C and 8°C over a period of 11 days showed amoxicillin is stable for 7 days at both temperatures. Potassium clavulanate maintains at least 90% of its initial concentration for 7 days at 8°C but shows more than 40% degradation in the same time period at room temperature of 20°C. The time taken for the original concentration of potassium clavulanate to drop to 90% of its value at room temperature of 20°C is 2 days.

The aim of present study was to determine the stability of cefuroxime axetil oral suspension at different temperature storage conditions (stored at room 25°C and refrigerated 5°C conditions). Determination of cefuroxime (as cefuroxime axetil) was performed by dissolution testing and by content uniformity.

MATERIALS AND METHODS

Reagents

All reagents such as sodium dihydrogen orthophosphate anhydrous, sodium hydroxide and ortho-phosphoric acid were of analytical grades while Methanol was of HPLC grade. Sodium dihydrogen phosphate and disodium hydrogen phosphate were provided by Across Co. (Germany). Cefuroxime axetil working standard was provided by Dobfar Company (Italy).

Sample collection

Three different batches of cefuroxime axetil (Zinnat®) were used for this study. It is available as dry powder containing 125 mg as cefuroxime axetil per/5 ml for reconstitution in water for oral use. The samples were purchased from a reputable and registered pharmacy and were within the stated expiry date on pack are summary in table 1.

Table 1. Illustrate the number of batches of sample collection and its expiry date

| Table 1. Illustrate the number of batches of sample collection and its expiry date |
|---|---|---|---|
| Name of drug | Batch N.O. | Sample N.O. | Manufacturing date | Expiry date |
| Zinnat (original brand) | 444433 | (6 bottle) | 10/2009 | 10/2011 |
| | 423233 | (6 bottle) | 06/2009 | 06/2011 |

Instrumentation and chromatographic conditions

Cefuroxime axetil were assayed using High Performance Liquid Chromatography (HPLC) (Shimadzu; Japan). The analysis was carried out using HPLC system; the column is C25cm.

Mobile phase: 0.2M monobasic phosphate: methanol (620: 380) was prepared by dissolving of ammonium phosphate in distilled water (DW) to 1000 ml. The flow rate was 1.5ml/min. The UV detection was set at wave length 278 nm for cefuroxime axetil. 10 µL of the sample was injected into the HPLC and the system was maintained at ambient temperature.

Preparation of stock and calibration solutions

120 mg of cefuroxime axetil was transferred to a 50ml volumetric flask, dissolved and diluted with methanol to a 50ml then standard solutions 50%, 75%, 100% and 150% of the working concentration standard were prepared and diluted to the final volume with 0.2M monobasic
ammonium phosphate after that three replicate measurements of each solution by HPLC and the reading obtained for each solution is plotted against its corresponding theoretical concentration and linear regression analysis was performed. The process was replicated more than one in different time.

**Sample preparation and estimation of cefuroxime axetil**

**Preparation of standard:** Quantity equivalent to about 125 mg of cefuroxime, were weighed and transferred to 100 ml volumetric flask then dissolved with methanol and completed to volume with methanol then 5ml was transferred to 50ml volumetric flask and 13.8 ml of methanol was added and completed to the volume with 0.2 M of monobasic ammonium phosphate to get the concentration of 0.0125 mg/ml.16

**Preparation of sample:** Eighteen samples of cefuroxime axetil (125mg/5ml) oral suspension were freshly reconstituted with distilled water (DW). The reconstituted preparations were distributed into groups (n=9) and were subjected to two different simulated conditions that represent the different storage conditions.

Samples stored under condition (A) were refrigerated with fluctuating temperatures between 2-8°C. Samples in condition (B) were stored at room temperatures of 25°C for a period of 14th days.

An accurately 5ml of cefuroxime axetil oral suspension was measured and transferred to a 100-ml volumetric flask, freshly mixed and free from air bubbles, then 50 mL of methanol was added and shake by mechanical means for about 10 minutes then diluted with methanol to volume, and mixed. Finally a portion of this stock solution was filtered, and 5.0 mL of the filtrate was transferred to a 50-ml volumetric flask then 13.8 mL of methanol was added and diluted with 0.2 M Monobasic ammonium phosphate to get the concentration of 0.0125 mg/ml.

**Dissolution test for cefuroxime axetil**

Dissolution tester (Pharmatest PT-DT 7) Apparatus II, Time: 30min, Speed: 50rpm, Volume: 900 mL (blank 0.07 M pH 7.0 phosphate buffer with D.W) and Medium: 0.07 M pH 7.0 phosphate buffer was prepared by dissolving 3.7 g of monobasic sodium phosphate and 5.7 g of anhydrous dibasic sodium phosphate in 1000 mL of water, then the pH was adjusted to 7.16

**Preparation of standard:** Equivalent to 125mg of cefuroxime axetil was added to 100ml volumetric flask and dissolved by dissolution medium then 1ml from the solution was taken to 100ml volumetric flask and the volume was completed by dissolution medium. The final concentration is 0.0125 mg/ml.

**Preparation of sample:** Dissolution tester (Pharmatest PT-DT 7) was used and at the end of dissolution time (after 30 min), 1ml was taken after filtration by suitable filter into 50ml volumetric flask and the volume was completed by dissolution medium finally the concentrations of sample and standard were measured at wave length: 280nm, using UV/ Visible spectrophotometer (Shimadzu; Japan).

**RESULTS AND DISCUSSION**

**Linearity**

For cefuroxime axetil, a good linear response was found between the peak area and the concentration, with a correlation coefficient 0.997. (Table 2, Figure 2)

**Table 2. Calibration concentration of cefuroxime axetil (CFA)**

<table>
<thead>
<tr>
<th>S No</th>
<th>%</th>
<th>Concentration (µg/ml)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>120</td>
<td>2734007</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>180</td>
<td>4149126</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>240</td>
<td>5336057</td>
</tr>
<tr>
<td>4</td>
<td>125</td>
<td>300</td>
<td>6441395</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>360</td>
<td>7886758</td>
</tr>
</tbody>
</table>

Figure 2. Linearity of area of CFA against concentration by µg/ml

The results of the study are summarized in tables 3 and 4 which show the concentrations of cefuroxime axetil and the dissolution rate respectively as percentage of assay and dissolution rate during 14th day. Also figures 3, 4 and 5 illustrated the percentage of cefuroxime which degraded in different batches during this period of study at storage condition.

**Table 3. Percentage Concentration of Cefuroxime Axetil ± SD. (Storage Conditions 2-8°C and at 25°C)**

<table>
<thead>
<tr>
<th>B No</th>
<th>444433</th>
<th>451273</th>
<th>423233</th>
<th>444433</th>
<th>451273</th>
<th>423233</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>Assay % at refrigerator temp. (2-8°C)</td>
<td>Assay test % at room temp. (25°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0day</td>
<td>104±0.55</td>
<td>101±0.66</td>
<td>102±0.75</td>
<td>103±0.69</td>
<td>101±0.67</td>
<td>101±0.85</td>
</tr>
<tr>
<td>3rdday</td>
<td>99±0.58</td>
<td>96±0.68</td>
<td>95±0.76</td>
<td>93±0.54</td>
<td>98±0.27</td>
<td>93±0.93</td>
</tr>
<tr>
<td>7thday</td>
<td>98±0.72</td>
<td>93±0.74</td>
<td>92±0.64</td>
<td>93±0.77</td>
<td>86±0.93</td>
<td>88±0.69</td>
</tr>
<tr>
<td>9thday</td>
<td>94±0.86</td>
<td>92±0.48</td>
<td>92±0.97</td>
<td>92±0.43</td>
<td>87±0.71</td>
<td>88±0.51</td>
</tr>
<tr>
<td>14day</td>
<td>92±0.91</td>
<td>91±0.87</td>
<td>89±0.34</td>
<td>89±0.98</td>
<td>86±0.80</td>
<td>85±0.84</td>
</tr>
</tbody>
</table>

**Table 4. Percentage of Dissolution rate ± SD. (Storage conditions 2-8°C and at 25°C)**

<table>
<thead>
<tr>
<th>B No</th>
<th>444433</th>
<th>451273</th>
<th>423233</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>Dissolution rate % at refrigerator temp. (2-8°C)</td>
<td>Dissolution rate % at room temp. (25°C)</td>
<td></td>
</tr>
<tr>
<td>0day</td>
<td>83±0.89</td>
<td>70±0.71</td>
<td>74±0.67</td>
</tr>
<tr>
<td>3rdday</td>
<td>80±0.35</td>
<td>68±0.52</td>
<td>71±0.95</td>
</tr>
<tr>
<td>7thday</td>
<td>79±0.48</td>
<td>63±0.82</td>
<td>70±0.83</td>
</tr>
<tr>
<td>9thday</td>
<td>60±0.57</td>
<td>59±0.63</td>
<td>64±0.34</td>
</tr>
<tr>
<td>14day</td>
<td>58±0.65</td>
<td>56±0.72</td>
<td>55±0.81</td>
</tr>
</tbody>
</table>

The procedure separately inject equal volume (about 10µl) of the standard preparation and the assay preparation into the chromatography, record the chromatograms and measure the response for the major peaks and calculate the percentage of the cefuroxime for oral suspension in the quantity of the sample taken by the formula:

\[
\text{Assay}\% = \frac{r_n}{r_s} \times \frac{\text{conc. of standard}}{\text{conc. of sample}} \times 100
\]

In which \( r_n \) and \( r_s \) are the sums of the peak responses of the cefuroxime axetil diastereoisomers A and B obtained from the assay preparation, respectively.
The suspensions were judged to be stable if the components maintained at least 90% of the label concentrations and the amount of drug release not less than 60% (USP).

During the test period, the concentration of cefuroxime axetil was found to be stable for 10th days under the refrigerator storage condition (A) and after that the concentration of cefuroxime axetil less than 90%. While the concentration of cefuroxime axetil at 5th days found to be stable under room temperature storage condition (B) and after this period the concentration of cefuroxime axetil less than 90% outside the acceptance limits of USP (Table 3 and figure 3, 4 and 5).

The suspensions were judged to be stable if the dissolution rate maintained at least 60% of the label concentrations (USP).

During the tests period, dissolution rate of cefuroxime axetil was found to be stable for 10th days under condition (A) for two batches and after that the dissolution of cefuroxime axetil less than 60%. While the dissolution of cefuroxime axetil at first day found to be stable under condition (B) till 3rd day only one batch are stable but after this period the dissolution rate of cefuroxime axetil less than 60% reach to 33% out the limit of USP (Table 4 and figure 6, 7 and 8).

The rate of degradation in suspensions are zero-order kinetic, in which the concentration in solution depends on the drug's solubility as the drug decomposes in solution, more drug is released from the suspended particles so that the concentration remains constant. This concentration is the drug's equilibrium solubility in a particular solvent at a particular temperature.17

Cefuroxime axetil of innovator brand is formulated from the amorphous materials and during storage transform to crystalline form which is less soluble than amorphous form. The batches which storage at room temperature gave value lower than 60% on receipt and throughout the storage period that may be due to the cefuroxime axetil may be change to the crystalline form, which is less soluble than amorphous form.

The result of this study is similar to the work done by Naidoo, (2006), which showed that only amoxicillin suspension stored between 2°C and 8°C for 7 days showed the lowest level of degradation.18

Dan Diaconu, (2006) study the stability of the
reconstituted suspension (at 5°C and, respectively, at 25°C) has been studied and the kinetic equations that describe the degradation of the active substances have been established; the administration period for the reconstituted suspension has been shown as being of 6 days at room temperature and, respectively, 8 days if the product is kept in the refrigerator.\textsuperscript{19}

Florey et al reported that 200-220 mg/ml of CF are stable for 24 hours at room temperature or 48 hours at 5°C.\textsuperscript{20}

**CONCLUSION**

Reconstituted of brand name of cefuroxime axetil oral suspension is stable for at least 10th days when stored at refrigerator at 2-8°C. When stored at room temperature cefuroxime axetil constituent was stable for first 3 days at room temperature. Cefuroxime axetil oral suspension must be stored after reconstitution at refrigerator to be stable during its shelf life.

**ACKNOWLEDGEMENT**

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**REFERENCES**


