International Standard Serial Number (ISSN): 2249-6807

*International Journal of Institutional Pharmacy and Life Sciences 2(5): September-October 2012*

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

**Pharmaceutical Sciences** **Research Article……!!!**

Received: 15-08-2012; Revised; Accepted: 29-09-2012

# FORMULATION AND EVALUATION OF PARENTERAL DOSAGE FORM OF ANTI-HIV DRUG USING HYDROTROPS

Ravi B*[1], Nagaraja YS[2], Mahantesha MK[2]

1. Department of QA, Apotex Research private limited, Bangalore. Karnataka -560099, India.
2. Department of Pharmaceutics, S.J.M College of Pharmacy, Chitradurga-577502, Karnataka, India

**Keywords:**

Ritonavir, Human Immunodeficiency Virus, Sodium-p-hydroxy benzoate, Antioxidant, Sodium glycinate

**For Correspondence:**

**Ravi B**

Department of QA, Apotex Research private limited, Bangalore. Karnataka - 560099, India.

**E-mail:**

nagraj.ys@gmail.com

## ABSTRACT

Ritonavir is an antiretroviral drug with activity against Human Immunodeficiency Virus (HIV) type 1. However, only temporary and limited benefits are observed in HIV infected patients treated with Ritonavir alone or in combination to decrease viral burden, the rapid development of resistance have limited their long term efficacy. Hence in the present work an attempt is being made to provide for parenteral drug delivery with having improved therapeutic index for Ritonavir and an intention to develop a stable and effective parenteral formulation, containing the drug Ritonavir. Ritonavir is practically insoluble in water and unstable at higher temperature. The effects of various co solvents in the solubility of Ritonavir have been evaluated. Ritonavir was tried with co solvents such as Sodium-p-hydroxy benzoate, Sodium glycinate and Sodium thiocyanate. The drug was made into injection formulation for administered as a SVP. Various batches of Ritonavir injection formulation were prepared in order to assess the influence of heat, light, atmospheric oxygen and antioxidant on the stability of the drug and the formulations were also subjected to accelerated stability test. Out of all trials, formulation containing Sodium thiocyanate was found to be more soluble, stable and passed all tests satisfactorily.

## INTRODUCTION

Injections include a wide variety of therapeutic agents, e.g., for the treatment of cancer, infections, cardiovascular diseases, arthritis, inflammatory diseases, diabetes, hormonal deficiencies and many other disease states including life threatening emergency conditions. There are more than 400 injections products listed in the USP and, because of the huge number of biotechnology molecules in clinical study, this number will continue to grow rapidly over the next several years. About 80% or greater of all SVPs commercially available are prepared by aseptic processing. LVPs usually involve intravenous infusion, dialysis, or irrigation fluids containing electrolytes, sugar, amino acids, blood, blood products, and fatty lipid emulsions. SVP formulations are simple formulations compared with other pharmaceutical dosage forms, composed of active ingredients, solvent system (preferably aqueous), minimal number of excipients, in the appropriate container and closure packaging system. Formulation scientists have severe restrictions in number and choice of added substances because of safety considerations[1].

Ritonavir, the first anti-HIV compound approved for clinical use is widely used for treatment of AIDS either alone or in combination with other antiviral agents. However, the main limitation to therapeutic effectiveness of Ritonavir is its dose-dependent haematological toxicity, low therapeutic index, short biological half-life, and poor bioavailability. After oral administration, it is rapidly absorbed from the gastrointestinal tract (GIT) exhibiting a peak plasma concentration of 1.2 µg/mL at 0.8 hours. In the systemic circulation, it is first to converted to Ritonavir triphosphate, which is pharmacologically active and prevents the replication of the HIV virus. The biological half-life of Ritonavir triphosphate is 5 hours, thus necessitating frequent administration (3 to 4 times a day) to maintain constant therapeutic drug levels. Since Ritonavir acts as a metabolic antagonist of thymidine and its antiviral effect is time dependent, an adequate parenteral drug delivery of Ritonavir is desired for maintaining anti-AIDS effect and avoiding the strong side effects. Ritonavir is mainly available in dosage forms of tablets solutions and capsules. For an oral dose Ritonavir is extensively metabolized (less than 1% Ritonavir is excreted unchanged in urine) in the liver and half life is 5 hrs. The objective of the present study is to formulate stable Ritonavir injectables to have a rapid therapeutic action. The main problem is involved on the formulation of Ritonavir injection is the extremely the hydrophobic nature of the drug. The aim of the present study is to formulate and evaluate the Parenteral dosage form containing Ritonavir. The objectives of the study are, to study the

solubility behavior of the drug in different solvents, to develop an analytical method for assay of, to design and formulate a stable parenteral formulation of Ritonavir, to evaluate prepared parenteral formulations of Ritonavir.

## MATERIAL AND METHODS

Ritonavir is obtained from **Apotex research pvt**, Bangalore.

## PREFORMULATION STUDIES [2,3,4,5,6]

**Solubility studies of Ritonavir in different solvents:** Excess of drug was added to different solvents in 10 ml stoppered volumetric flasks. Then Drug was made to dissolve in the solvent by placing the volumetric flask in the shaker bath at 25° C for 6 hours. The volumetric flasks were then placed at room temperature for 24 hours. The solutions were filtered and appropriate dilutions were made to measure absorbance using UV spectrophotometer, and water as blank. The data are given in Table 2.

**Effect of Temperature on Stability of Drug:** Ritonavir solution in Sodium thiocyanate is filled into vials. The vials were sealed and placed at refrigeration, room-temperature, 50°C, 75°C and 95°C for 1 week and observed for color change and crystal growth. The samples placed at refrigeration and room temperature served as controls. The data are given in Table 3.

**Light Stability of Drug:** Ritonavir solution in Sodium thiocyanate is filled in to 10ml glass vials (amber and clear).Also samples of drug substance are placed in an open Petridish to expose a large surface. Drug and dilutions placed in a light-resistant amber colored glass vials, foil wrapped and in a cardboard box as controls. This is carried out for 4 weeks with weekly examinations for visible color change or precipitation in solution in clear vials, the compound can be considered as potentially light sensitive and should be handled accordingly. The data are given in Table 4.

**Effect of Oxygen on Drug**: Ritonavir in Sodium thiocyanate is filled into vials and placed at 30°C and 40°C. One group is purged and another group is sealed with air. Solutions are observed for color change and drug content. The data are given in Table 5 and 6.

## FORMULATION DEVELOPMENT

Attempts were made to develop a stable parenteral formulation using co solvent/s along with other excipients. The dose selected for formulation was 50 mg of Ritonavir in 1ml solvent. The prepared formulations contain the following ingredients along with their concentrations are given in Table 1.

**Table 1: Concentration of different ingredients used in various trial formulations**

| Ingredients | Formulation (%) | | | | | |
|---|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | **E** | **F** |
| Ritonavir (gm) | 20 | 20 | 20 | 20 | 20 | 20 |
| Sodium-p-hydroxybenzoate (gm) | - | 25 | - | - | - | 25 |
| Sodium glycinate(gm) | - | 24 | - | 24 | - | 24 |
| Sodium thiocyanate (gm) | - | 10 | 15 | - | 22 | 22 |
| Propyl Paraben (gm) | 0.022 | 0.022 | 0.022 | 0.022 | 0.022 | 0.022 |
| Sodiummetabisulphite (gm) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Water for Injection (ml) | q s | q s | q s | q s | q s | q s |

Thus prepared formulations were assayed for drug content respectively and 10ml of these were placed at 5°C, room temperature (RT), 37°C, 40°C and 45°C for six weeks and observed for crystal growth, clarity, pH change, and drug content.

**POST FORMULATION EVALUATIONS** [7,8,9,10]

**Assay of Formulations**:

**Reference Solution Preparation-**100ml of stock reference solutions for each formulation was prepared. The composition of the reference stock solution was similar to that of the respective formulations excluding the drug and also they were diluted similarly as the formulations were diluted using water. This resulting solution is used as reference solution (blank) in comparison with the prepared formulations are measured.

**Sterilization Studies:** The injection samples were taken and conducted LAL test.

**Stability Studies-**For any pharmaceutical dosage form stability of the prepared formulation is a very basic and important factor, from point of view of safety of the patient being treated with and to get a safe and maximum therapeutic response of the drug.

The provision of rapid means of quality control, which ensures that no unexpected changes in the stored product are occurred like: Crystal growth, pH changes, Clarity and % Drug content. The data are given in Table 9 to12. **Crystal Growth-**10 ml of the each prepared formulations B, F were placed at refrigeration, room temperature, 37°C, 40°C and 45°C respectively for six weeks and observed for crystal growth. The data are given in Table 15.

**pH Changes-**10ml of the each prepared formulations B, F were kept at different temperatures/conditions such as refrigeration, room temperature, 37°C, 40°C, 45°C and under light. At regular time intervals the samples were examined for pH changes for six weeks using a digital pH meter. The data are given in Table 14.

**Clarity**-10ml of the formulations were placed at refrigeration, room temperature, 37°C, 40°C and 45°C for 6 weeks and observed for color change or turbidity. The data are given in Table 16.

**% Drug Content**-The drug content of the formulations B, F were determined by following the same procedures as mentioned in assay. The estimates were done at intervals of one week up to six weeks. The data are given in Table 17 and 18.

**RESULTS AND DISCUSSION**

**Figure 1: F.T.I.R. image of Ritonavir. Ritonavir showed a characteristic starching at 3134 $cm^{-1}$, which corresponds to its N-H group.**

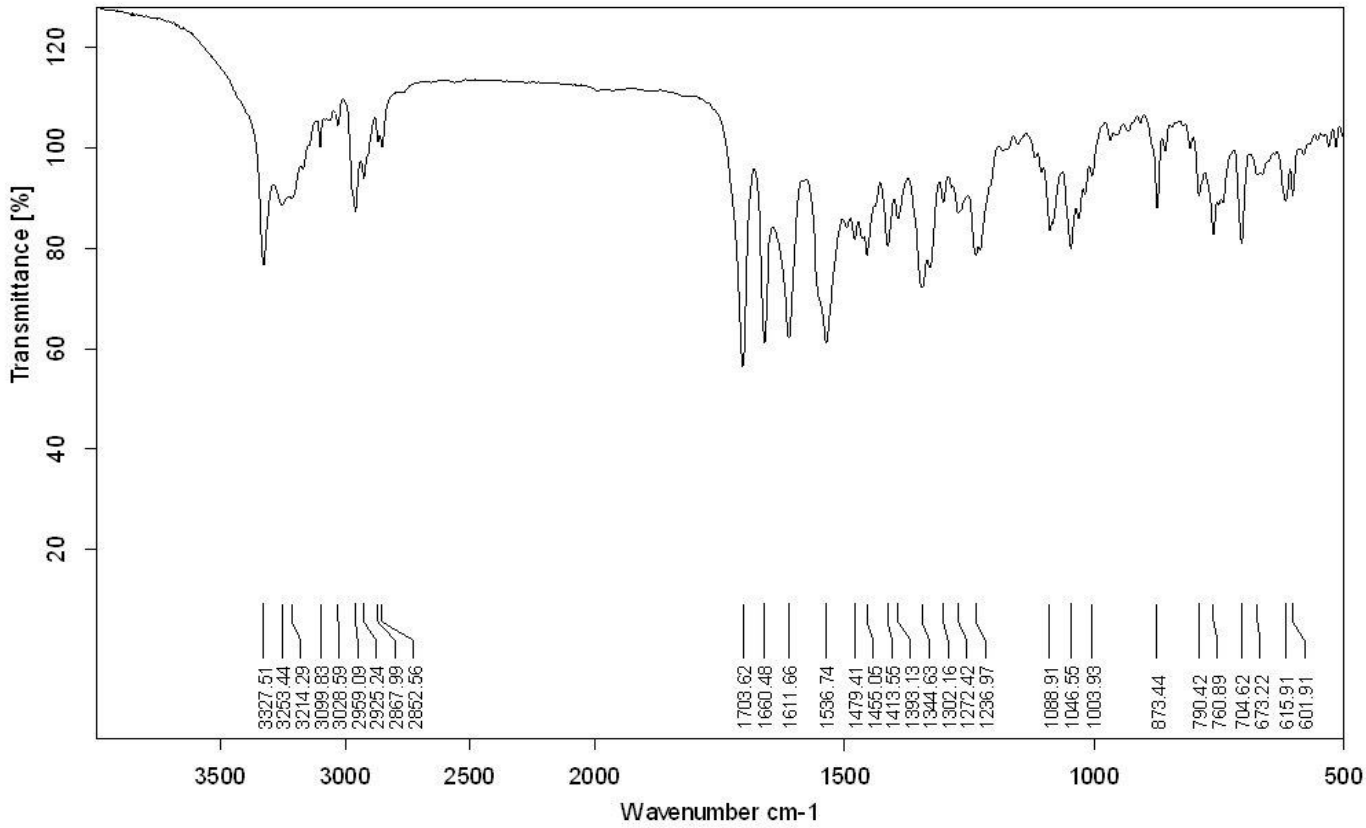

**Figure 2: D.S.C. image of Ritonavir. Ritonavir showed a characteristic exothermic peak at 232.82$^0$C, which corresponds to its melting point.**

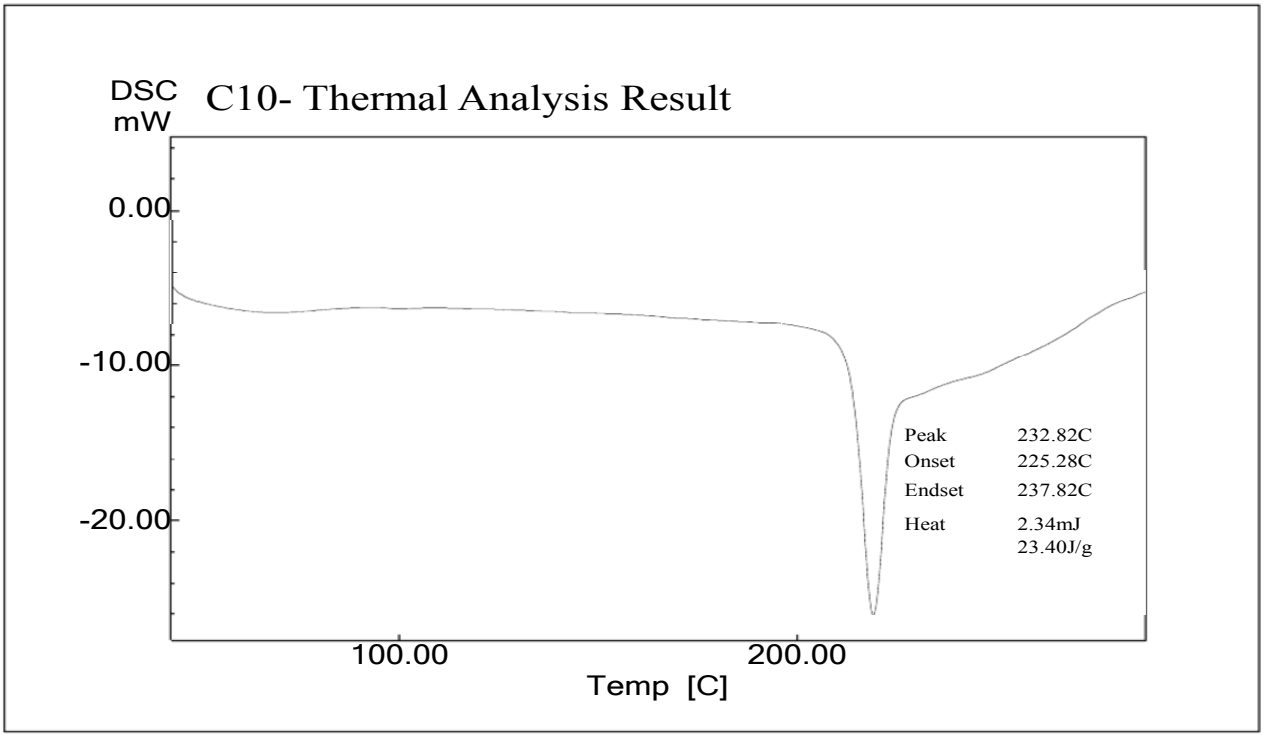


**Table 2: Concentration of different ingredients used in various trial formulations**

| Sl. No | Solvents | Concentration mg/ml | Standard deviation |
|---|---|---|---|
| 1 | DM Water | 2.1439 | ± 0.118 |
| 2 | Sodium-p-hydroxy benzoate | 30.5571 | ± 0.103 |
| 3 | Sodium-m-hydroxy benzoate | 9.9295 | ± 0.016 |
| 4 | Xylenesulphonic acid | 7.2613 | ± 0.109 |
| 5 | Citric acid | 14.9327 | ± 0.104 |
| 5 | Cremophor EL | 10.3129 | ± 0.106 |
| 6 | Proline | 17.0579 | ± 0.108 |
| 7 | Span 20 | 13.0516 | ± 0.106 |
| 8 | Lecithin | 08.20031 | ± 0.107 |
| 9 | Sodium glycinate | 24.6870 | ± 0.103 |
| 10 | Sodium thiocyanate | 51.1455 | ± 0.103 |
| 11 | Sosium naphenate | 12.747 | ± 0.103 |

## STABILITY EVALUATION

Various stress tests are performed on solid and solution samples to establish the effect of heat, light and oxygen on the drug substance stability.

### 1. Heat stability

**Table 3: Heat stability profile of Ritonavir**

| Temperature (°C) | Duration (weeks) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Refrigeration | - | - | - | - |
| Room temperature | - | - | - | - |
| 40 | - | - | - | + |
| 50 | + | + | + | + |
| 75 | + | + | + | + |

+ Colour change, - No colour change

### 2. Light stability

**Table 4: Light stability study of Ritonavir**

| Withdrawal week | Observations | |
|---|---|---|
| | Clear | Amber |
| 1 | - | - |
| 2 | - | - |
| 3 | + | - |
| 4 | + | - |

- Clear, + Turbidity

### 3. Effect of oxygen

**Table 5: Test for color change after a week**

| Temperature(°C) | Air sealed vials | Perged vials |
|---|---|---|
| 25 | - | - |
| 30 | - | - |

+ colour change, - no colour change

### Estimation of drug content:

**Table 6: Drug content in freshly prepared drug solution**

| Absorbance at 252nm | Concentration in µg/ml | Concentration in mg/ml |
|---|---|---|
| 0.814 | 9940.53 | 49.9405 |

**FORMULATION DEVELOPMENT**

A stable parenteral formulation of water soluble drug Ritonavir was formulated after performing trials with various solvents. Thus prepared formulations were subjected for various tests and results are discussed in the following section.

**Table 7: % Drug content of various formulation trials containing Ritonavir**

| Formulation | Drug content (mg/ml) | % Drug content |
|---|---|---|
| A | 50.590 | 101.156 |
| B | 50.858 | 104.663 |
| C | 50.290 | 100.156 |
| D | 50.311 | 102.660 |
| E | 50.008 | 102.159 |
| F | 50.310 | 100.156 |

* Each value is an average of three determinations

**Table 8: LAL test.**

| Test | Observation | Report |
|---|---|---|
| LAL | No gel formation | Passed |

**Post Formulation Studies**

**Effect of different temperature on crystal growth:**

**Table 9: Effect of different temperature on crystal growth**

| Formulation | RT | 40°C | Light |
|---|---|---|---|
| **A** | - | - | - |
| **B** | - | - | - |
| **C** | + | + | + |
| **D** | - | - | - |
| **E** | - | + | + |
| **F** | - | - | - |

+ Crystal growth, - No crystal growth

In the formulations A, E, D and F no crystals were developed after two weeks. So A, E, D and F are stable at temperatures studied

**Effect of different temperature on clarity:**

**Table 10: Effect of different temperature on clarity**

| Formulation | RT | 40°C | Light |
|---|---|---|---|
| **A** | - | - | - |
| **B** | - | - | - |
| **C** | + | + | - |
| **D** | - | - | - |
| **E** | - | + | - |
| **F** | - | - | - |

+ Turbid, - Clear

A, B, D and F are clear after two weeks. So A, B, D and E are stable at temperatures studied

**Effect of different temperature on colour change:**

**Table 11: Effect of different temperature on colour change**

| Formulation | 5°C | RT | 40°C | Light |
|---|---|---|---|---|
| **A** | - | - | - | - |
| **B** | - | - | - | - |
| **C** | + | + | + | + |
| **D** | - | - | - | - |
| **E** | - | + | + | + |
| **F** | - | - | - | - |

+colour change, - no colour change.

A, B and F show no colour change up to $40^0$C after two weeks. So A, B and F are stable at temperatures studied.

**SCALE UP STUDIES** [11,12,13,14]

**ASSAY OF THE FORMULATIONS**

**Table 12: Drug content of B, F**

| Formulation | Drug content (mg/ml) * | % Drug content |
|---|---|---|
| B | 50.27 | 101.156 |
| F | 50.678 | 104.663 |

* Each value is an average of three determinations

**ACCELERATED STABILITY STUDIES**

**pH CHANGES**

**Table 14: pH changes of formulation B, F at different temperatures/conditions on ageing**

| Formulation | Withdrawal Week | 37°C | 40°C | Light |
|---|---|---|---|---|
| B | 0 | 6.18 | 6.28 | 6.28 |
| | 1 | 6.25 | 6.25 | 6.28 |
| | 2 | 6.25 | 6.27 | 6.20 |
| | 3 | 6.29 | 6.29 | 6.20 |
| | 4 | 6.22 | 6.23 | 6.25 |
| | 5 | 5.24 | 6.22 | 6.24 |
| | 6 | 6.20 | 6.27 | 6.29 |
| F | 0 | 6.26 | 6.26 | 6.26 |
| | 1 | 6.23 | 6.22 | 6.15 |
| | 2 | 6.29 | 6.27 | 6.19 |
| | 3 | 6.25 | 6.24 | 6.17 |
| | 4 | 6.25 | 6.21 | 6.14 |
| | 5 | 6.23 | 6.21 | 6.10 |
| | 6 | 6.25 | 6.29 | 6.17 |

**CRYSTAL GROWTH**

**TABLE 15: Crystal growth of formulation B, F at different temperatures/conditions on ageing**

| Formulation | Withdrawal Week | 37°C | 40°C | 45°C | Light |
|---|---|---|---|---|---|
| B | 0 | - | - | - | - |
| | 1 | - | - | - | - |
| | 2 | - | - | - | - |
| | 3 | - | - | - | - |
| | 4 | - | - | - | - |
| | 5 | - | - | - | - |
| | 6 | - | - | - | - |
| F | 0 | - | - | - | - |
| | 1 | - | - | - | - |
| | 2 | - | - | - | - |
| | 3 | - | - | - | - |
| | 4 | - | - | - | - |
| | 5 | - | - | - | - |
| | 6 | - | - | - | - |

+crystal growth, - no crystal growth

No crystal growth was observed in the formulations at different temperatures/conditions.

## CLARITY STUDIES

**Table 16: Clarity of formulation B, F at different temperatures/conditions on ageing**

| Formulation | Withdrawal Week | 37°C | 40°C | 45°C | Light |
|---|---|---|---|---|---|
| B | 0 | - | - | - | - |
| | 1 | - | - | - | - |
| | 2 | - | - | - | - |
| | 3 | - | - | - | - |
| | 4 | - | - | - | - |
| | 5 | - | - | - | - |
| | 6 | - | - | - | - |
| F | 0 | - | - | - | - |
| | 1 | - | - | - | - |
| | 2 | - | - | - | - |
| | 3 | - | - | - | - |
| | 4 | - | - | - | - |
| | 5 | - | - | - | - |
| | 6 | - | - | - | - |

+ turbid, - clear

All the formulations were clear at different temperatures/ conditions

## DRUG CONTENT

**Table 17: Percent drug content of formulation B at different temperatures/conditions on ageing.**

| Sample withdrawal (week) | % Drug Content | | |
|---|---|---|---|
| | 37°C | 40°C | Light |
| **0** | 101.116 | 101.156 | 101.156 |
| **1** | 101.015 | 100.973 | 101.000 |
| **2** | 100.873 | 100.761 | 100.728 |
| **3** | 100.758 | 100.537 | 100.569 |
| **4** | 100.569 | 100.365 | 100.296 |
| **5** | 100.470 | 100.107 | 100.100 |
| **6** | 100.017 | 99.897 | 99.901 |

**Table 18: Percent drug content of formulation F at different temperatures/conditions on ageing.**

| Sample withdrawal (week) | % Drug Content | | |
|---|---|---|---|
| | 37°C | 40°C | Light |
| **0** | 104.006 | 104.066 | 104.066 |
| **1** | 103.857 | 103.410 | 103.839 |
| **2** | 103.028 | 103.698 | 103.725 |
| **3** | 103.501 | 103.527 | 103.400 |
| **4** | 103.089 | 103.271 | 103.317 |
| **5** | 103.109 | 103.106 | 103.118 |
| **6** | 102.961 | 102.894 | 102.996 |

## CONCLUSION

The concept of parenteral formulations containing Ritonavir offers a suitable, practical approach to achieve desired stable parenteral preparation with solubility of drug in suitable solvent composition. In present work, parenteral formulation of Ritonavir was prepared successfully by using different concentrations and combinations of Sodium-p-hydroxy benzoate, Sodium glycinate and Sodium thiocyanate in formulation design. These formulations were expected to be stable for sufficiently long time. The conclusions arrived from the above results indicated that the parenteral formulation containing Ritonavir developed was found to be complying satisfactorily with all the evaluation tests performed and was stable for sufficiently longer duration of time.

## REFERENCES

1. Lachmann, Leon., Patrick Deluca, Michael J. Akers,Kinetic Principles and Stability Testing., chapter 26 in the Theory and Practice of Industrial Pharmacy, Bombay: Varghese Publishing House, 1987, page-902.
2. Gopal Krishna, Hodnick WF, Lang W, Lin X, Karra S, Mao J, Almassian B. Pharmaceutical Development and Manufacturing of a Parenteral Formulation of a Novel Antitumor Agent. AAPS PharmSciTech.2001; 2 (3):14.
3. Nahar M, Jain NK. Formulation and evaluation of saquinavir injection. Indian J Pharm Sci 2006; 68:608-14

4. Anupama B. Formulation and evaluation of rofecoxib injection. M.Pharm dissertation: Rajiv Gandhi University of Health Sciences; 2007.
5. Kenneth EA, Leon L, Herbert AL. Pharmaceutical dosage forms: Parenteral medications. Vol 1. Marcel Dekker Inc: New York and Basel; 1989. p. 89-137.
6. Nagaraja YS, Nagaraj TS, Bharati DR, Mahantesha MK. Formulation and Stability studies of Azithromycin Parenteral dosage form. Int Jur Uni Phar lif sci 2012;2(4):270
7. Nahar M, Jain NK. Formulation and evaluation of saquinavir injection. Indian J Pharm Sci 2006; 68:608-14
8. Jusko WJ, Gretch M, Gassett R. J Amer. Precipitation of Diazepam from intravenous preparation. Med Assoc 1973; 225:176.
9. Korttila K, Sothman A, Andersson P. Polyehylene Glycol as a solvent for Diazepam – Bioavailability and clinical effects after intramuscular administration. Acta Pharmacol Toxicol 1976; 139:104.
10. Yalkowsky SH, Valvani SC. Precipitation of solubilized drugs due to Injection or dilution. Int Clin Pharm 1977; 11:417.
11. Swamy PV, Sushma P, Chirag G, Prasad K, Younus AM, Raju SA. Parenteral formulation of zopiclone. Int J Pharm Sci 2008; 70(1):96-9.
12. Subramanyam CVS. Text book of physical pharmaceutics. 2nd ed: Vallabha prakashan ; 200.p. 65-75
13. Nagaraja YS, Nagaraj TS, Bharati DR, Mahantesha MK, Manjutnatha TO. Formulation and evaluation of parenteral dosage form of Lornoxicam using hydrotropic solubilization method. Am. J. Pharm Tech Res 2012; 2(5):573-85
14. Paranjothy KLK, Praveen KA. Stability testing of pharmaceuticals 1979; 17(4): 114-19.