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Validated Isocratic Reversed Phase Liquid Chromatographic Method for the Determination of Darunavir in Pure and Formulations

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ABSTRACT

Reversed phase liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the determination of Darunavir in pure and formulations. Separation was achieved with symmetry C_{18}-Column using the mobile phase of Phosphate Buffer (pH adjusted to 2.5) and Acetonitrile in the ratio 70:30 v/v, at a flow rate of 1.5 mL/min and UV detection was performed at 255 nm. The developed method was linear over a range of 5.0-25 µg/mL for Darunavir. The accuracy of the developed method was demonstrated at three concentration levels in the range of 50–150 % and the recovery of Darunavir was found to be in the range of 99.22–99.83 %. The developed method was said to be simple, selective and accurate and is useful for the assay of Darunavir in dosage forms and can be further employed in the quality control analysis of bulk manufacturing and formulations units.

Keywords: Darunavir, RP-HPLC, Validation, Formulations and uses.

INTRODUCTION

Darunavir is an antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS. Molecular formula is C27H37N3O7S. Molecular weight is 547.66. Melting point of drug is 74 °C. It is an amorphous white, solid, freely soluble in methanol, acetonitrile and soluble in ethanol. Darunavir contains a bis-tetrahydro-furnanyl (bis-THF) moiety and sulfonamide isostere; the drug is administered as its ethanolate salt. The chemical structure of Darunavir is shown in Figure 1. Darunavir [(1S, 2R)-3-[[(4-aminophenyl) sulfonyl] (2-methylpropyl) amino]-2-hydroxy-1-(phenylmethyl) propyl]-carbamic acid hexahydrofuro [2, 3-b] furan-3-yl ester monoethanolate is an antiretroviral agents [1].

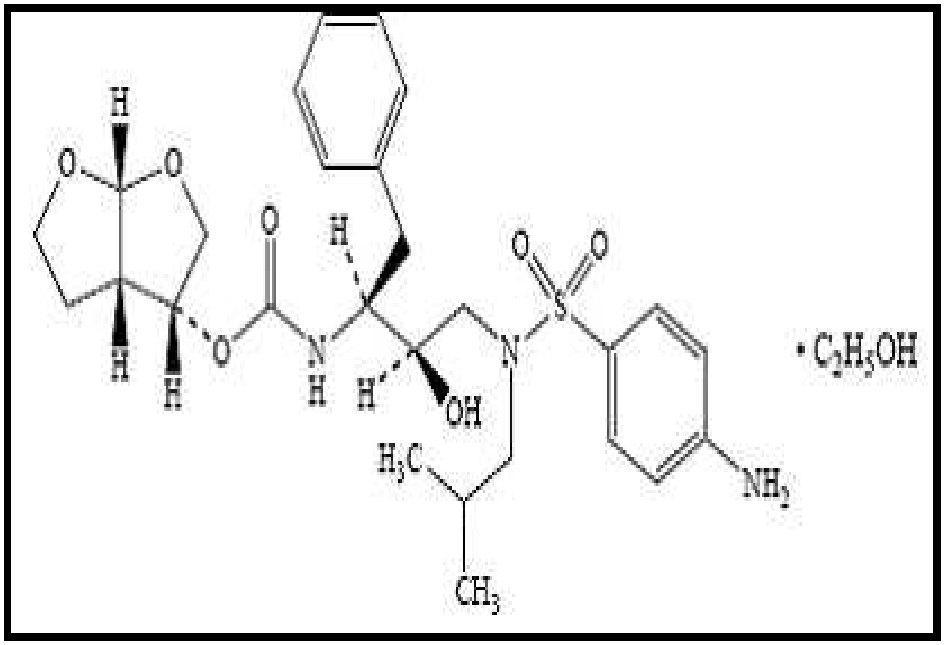

Figure 1: Chemical Structure of Darunavir

Several analytical methods have been reported for the determination of Darunavir in pure drug, pharmaceutical dosage forms and in biological samples using spcetrophotometry liquid chromatography, electro kinetic chromatography high performance thin layer chromatography either in single or in combined forms. Darunavir was designed to form robust interactions with the protease enzyme from many strains of HIV, including strains from treatment-experienced patients with multiple resistance mutations to PIs. A few analytical methods were reported for the determination of Darunavir in plasma and pharmaceutical dosage forms that include LC-MS [2-4], electrophoresis [5] and HPLC [6-8] methods. Darunavir selectively inhibits the cleavage of HIV-1 encoded Gag-Pol polyproteins in infected cells, thereby preventing the formation of mature virus particles [9]. Darunavir is co-administered with ritonavir and with other antiretroviral agents, is indicated for the treatment of human immunodeficiency virus (HIV-1) infection [10]. Metal oxides play a very important role in many areas of chemistry, physics and materials science. The metal elements are able to form a large diversity of oxide compounds. In technological applications, oxides are used in the fabrication of microelectronic circuits, sensors, piezoelectric devices and fuel cells, coatings for the passivation of surfaces against corrosion and as catalysts [11-19]. We have presented our results on different oxide materials in our earlier studies [20-89]. In the present study we reported a sensitive, accurate and precise RP-HPLC method for the estimation of Darunavir in bulk and in tablet dosage forms have developed and validated.

MATERIALS AND METHODS

Chemicals and Reagents: Darunavir was obtained from Hetero Drugs, Hyderabad as gift sample and Prezista (Darunavir) Tablets (300 mg) orange colored film coated tablets available in the local pharamacy were used in the present study. Acetonitrile (HPLC grade), Potassium dihydrogen orthophosphate (AR-grade, Merck), Trimetyl amine (AR-grade, Merck) Orthophosphoric acid (AR-grade, Merck) and HPLC grade water were used in the preparation of different solutions (Mobile phase, Stock and working) in the present work.

Instrumentation: Quantitative HPLC was performed on a Waters 2695 HPLC system equipped with quaternary, low-pressure mixing pump and Waters 2996 Photodiode Array Detector (wavelength range of 190-800 nm). The output signal was monitored and integrated using Waters Empower software. A Hypersil ODS C_{18} column (250 mm × 4.6 mm, 5 µm) was used for separation. Shimadzu analytical electronic balance was used in the present study.

Preparation of Phosphate Buffer: The preparation of Phosphate Buffer solution was made by dissolving about accurately weighed 3.3954 gm of Potassium dihydrogen phosphate into a 1000 ml volumetric flask containing 1000 ml of HPLC grade water. 1000 ml with HPLC water and to the above solution add 1.5 ml of trimethyl amine and mix well. Adjust the pH to 2.5 with dilute orthophosphoric acid. This solution was mixed well, filtered and used for the mobile phase preparation.

Preparation of Standard Darunavir Stock Solution: About 50 mg of Darunavir reference standard was exactly weighed and dissolved in a 50 mL volumetric flask with the mobile phase to prepare the stock solution and was this stock solution was further diluted with the mobile phase according to the requirement (concentration within the linearity limits i.e., 5.0 – 25 µg/mL).

RESULTS AND DISCUSSION

A) Method Development: In the present study method development was started with the selection of appropriate mobile phase. For these initial trials were exclusively carried out by the author using the following mobile phases of varying compositions Phosphate Buffer (pH-2.5) and Acetonitrile in the ratios 50:50, 60:40 and 70:30 v/v respectively. Mobile phase of composition 50:50 has been rejected due to a lack of Darunavir signal on chromatogram.

When the sample of Darunavir was analyzed using mobile phase of composition 60:40 peak shape was not good and retention time was ~6 min. and further subsequent attempts were made by lowering the pH of the mobile phase with phosphate buffer and change in organic modifier concentration marked improvement was observed. Eventually, a mobile phase composed of Phosphate Buffer (pH - 2.5) and Acetonitrile in the ratio 70:30 v/v gave the best results. During the course of these studies the injection volume and the mobile phase flow rate was kept constant (20 µL and 0.8 mL min^{-1} respectively). The analytical wavelength was 255 nm. The validated chromatogram for Darunavir so obtained after optimizing various experimental conditions was depicted in Figure 2 respectively.

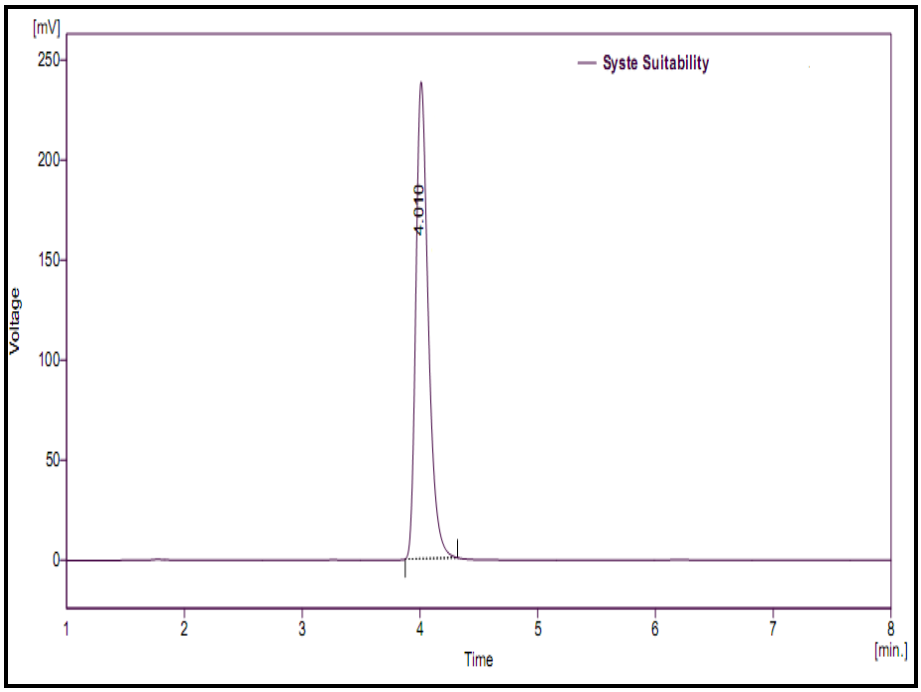

Figure 2: Validation Chromatogram for Darunavir

B) Validation of the Proposed Procedure: The selectivity**,** linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed RP-HPLC method as per the ICH guidelines for the estimation of Darunavir.

Selectivity: This method was selective for the Darunavir (Retention time about 3.308 min). The analysis of the chromatogram of Darunavir (Table 1) revealed the following efficiencies of the column: for Darunavir N = 5574 (where N represents theoretical plate number) and asymmetry 1.24 with the retention time of 4.010 min. The typical excipients included in the drug formulation do not interfere with selectivity of the method.

Table: 1 A system suitability parameters for Darunavir

Name of the Compound	Theoretical Plates	Tailing Factor
Darunavir	5574	1.24

Linearity: Linearity of the present method was evaluated at five concentration levels by diluting the standard Darunavir solutions to give solutions over the range 5.0 – 25 µg mL^{-1} (Table 1). 20 µl of these solutions were injected in triplicate in to HPLC system and the peak areas were recorded. A linearity plot (represented in Figure 3**)** was drawn by plotting obtained peak area verses the concentration data was calibrated by least-squares linear regression analysis and the results of calibration data was given in Table 2. The linearity range was between 5.0 – 25 µg mL^{-1} presented with the equation of 39332 x + 23819 with correlation coefficient (r^2 = 0.9995) closed to unity revealing the good linearity of the present method.

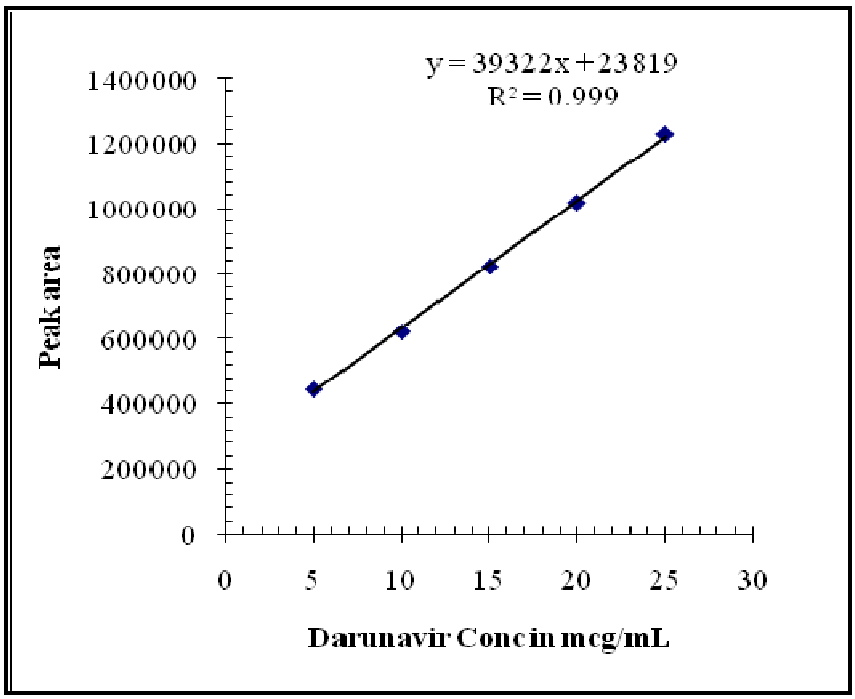

Figure 3: Linearity Graph of Valsartan

Table 2: Calibration Results Data

Regression Parameters	Results
Regression equation; Slope (b)	39321.78
Intercept (a)	238196.7
Correlation coefficient	0.9995
Standard deviation on intercept(S_a)	379.429
Standard deviation on slope (S_b)	657.717
Standard error on estimation(S_e)	10399.42
Limits of Detection (LOD)[µg/ml]	0.0289
Limits of Quantification [LOQ)[µg/ml]	0.022

Precision: The precision of the present analytical procedure was done by injecting six repeated injections of standard and sample solutions and recording the response factor of drug peaks. The mean, SD and % RSD were calculated and are presented in Table 3. These results revealed that the developed method was found to be precise.

Table 3: Results of Precision by the Proposed Method

S. No	Retention time	Peak area
1	4.012	806270
2	4.049	805846
3	4.051	807713
4	4.054	808291
5	4.052	808342
6	4.053	806951
Avg*	4.045	807235
Std Dev	0.0163	1049.78
% RSD	0.403	0.130

Average of six determinations

Accuracy: To assess accuracy of the present method, freshly prepared placebo of the Darunavir pharmaceutical formulations were spiked with various amounts of pure Darunavir at 50, 100 and 150 % respectively. Each solution

was injected in triplicate and the peak areas were used to calculate mean, SD and RSD % and the values (Table 4) were found to be less than 2 % indicating the good accuracy of the proposed method.

Table 4: Results of Accuracy by the Proposed Method

Spike Level	Amount (µg /mL) added	Amount (µg/mL) found	% Recovery	Mean % Recovery
50 %	20	19.84	99.20	99.22
50 %	20	19.85	99.28	
50 %	20	19.84	99.20	
100 %	60	59.92	99.80	99.83
100 %	60	59.92	99.80	
100 %	60	59.96	99.90	
150 %	100	99.83	99.73	99.62
150 %	100	99.87	99.69	
150 %	100	99.67	99.46	

*****Average of three determinations*

Ruggedness: Ruggedness is expressed in terms of percentage relative standard deviation and statistical analysis showed no significant difference between results (Table 5**)** obtained employing different analyst.

Table 5: Results of Ruggedness by the Proposed Method

Ruggedness	Analyst -1		Analyst-2	
No of Injections	Retention Time	Area	Retention Time	Area
Injection-1	4.012	806270	4.010	805863
Injection-2	4.049	805846	4.049	805846
Injection-3	4.051	807713	4.043	806513
Injection-4	4.054	808291	4.032	805391
Injection-5	4.052	808342	4.052	808342
Injection-6	4.053	806951	4.051	807951
Avg*	4.045	807235	4.039	806651
Std Dev	0.0163	1049.78	0.016	1218.61
% RSD	0.403	0.130	0.401	0.151

*****Average of six determinations*

Robustness*:* The robustness of the present RP-HPLC method was ascertained by varying two parameters (flow rate, composition of mobile phase) from the optimized chromatographic conditions and the statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of parameters were introduced indicating the robust of the developed method

Assay of Commercial Formulations: 10 tablets **(**Prezista -300 mg) were purchased from the local pharmacy was weighed and finely powdered. An accurately weighed quantity of tablet powder equivalent to 50 mg was transferred into 50 ml volumetric flask add 20 ml of diluents, sonic ate to dissolve for 10mins and dilute to volume with diluents (mobile phase). The solution was then filtered through 0.45 µ filter. From this aliquots of this solution were transferred and diluted to a series of 100 ml volumetric flasks and the volume in each flask was made up to the mark with distilled water to give concentrations (5.0 – 25 µg/ml) that obey within the linearity. The results are given in Table 6.

Table 6: Results of Analysis of Darunavir Formulations

Pharmaceutical Formulation	Amount of Darunavir		% of Recovery
	Labeled	Found*	
Alkeran	300 mg	299.98	99.99 %

**All the values are the averages of three determinations*

CONCLUSION

In the present study, a new RP-HPLC assay method for Darunavir was developed by following the ICH guidelines. Greater reproducibility was obtained for calibration plots and it was determined by calculating the slope, intercept and % RSD for each standard plot. The method was found to be robust as there was no significant change in the peak area and retention time. This chromatographic assay fulfilled all the requirements for being a reliable and feasible method with respect to precision, accuracy, linearity and recovery and therefore, this developed HPLC method can be reliably adopted for routine quality control analysis of darunavir in its tablets form.

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REFERENCES

[1] A.K. Ghosh, Z.L. Dawson, H. Mitsuya, *Bioorg. Med. Chem.*, **2007,** 15(24), 7576–7580.
[2] L. Goldwirt, S. Chhun, E. Rey, O. Launay, V. Jean Paul, G. Pons and V. Jullien, *J. Chromatogr. B,* **2007**, 857, 327-331.
[3] M.S. Avolio, M. Sciandra, L. Baietto, *J. Chromatogr. B,* **2007**, 859, 234-240.
[4] M. Takahashi, Y. Kudaka, N. Okumura, A. Hirano, K. Banno, *Biol .Pharm. Bull.*, **2007**, 30, 1947-1949.
[5] S. Leonard, S. Schepdae, T. Ivanyi, I. Lazar, J. Rosier M. Vanstockem, *J. Chromatogr. B,* **2005**, 26, 627-632.
[6] B. Ramprasad, A. Lanka, P. Srinivasu, R.P. Jayachandra, J.V.L.N.S. Rao, *Asian J. Pharm. Res.*, **2011**, 1(1), 10-14.
[7] L. Satyanarayana, S.V. Naidu, M. Narasimha Rao, S. Alok Kumar K. Suresh, *Asian J.Res. Pharm. Sci.*, **2011**, 1(3), 74-76.
[8] N. Bhavini, N. Bhanubhai, N. Chaganbhai, *Int. J. Pharm. Sci.*, **2012**, 4(3), 270-273.
[9] K. McKeage, C.M. Perry, S.J. Keam, *Darunavir Drugs*, **2009**, 69(4), 477- 503.
[10] A. K. Ghosh, Z. L. Dawson, H. Mitsuya, Darunavir, Bioorg. Med. Chem., **2007**, 15(24), 7576-7580.
[11] M.C. Rao, O.M. Hussain, *Res. J. Chem. Sci*, **2011**, 1, 92-95.
[12] M.C. Rao, *J. Non-Oxide Glasses*, **2013**, 5, 1-8.
[13] M.C. Rao, O.M. Hussain, *Res. J. Chem. Sci.*, **2011**, 1 (7), 76-80.
[14] M.C. Rao**,** *Res. J. Recent. Sci*, **2013**, 2(4), 1-8.
[15] M.C. Rao, *Int. J. Adv. Phar. Bio. Chem*, **2013**, 2 (3), 498-500.
[16] M.C. Rao, *Int. J. Mod. Phys., Conf. Series*, **2013**, 22, 576-582.
[17] M.C. Rao, *Int. J. Chem. Sci*, **2012**, 10(2), 1111-1116.
[18] M.C. Rao, K. Ramachandra Rao, *Int. J. Chem Tech Res*., **2014**, 6(7), 3931-3934.
[19] S.M. Begum, M.C. Rao, R.V.S.S.N. Ravikumar, *J. Inorg. Organometa. Poly. Mater*, **2013**, 23(2), 350-356.
[20] S.M. Begum, M.C. Rao, R.V.S.S.N. Ravikumar, *J. Mol. Struct*., **2011**, 1006(1), 344- 347.
[21] S.M. Begum, M.C. Rao, R.V.S.S.N. Ravikumar, *Spectrochim. Acta Part A: Mol. & Biomol. Spec.*, **2012**, 98, 100-104.
[22] K. Ravindranadh, RVSSN Ravikumar, M.C. Rao, *Int. J. Mod. Phys., Conf. Series*, **2013**, 22, 346-350.
[23] K. Ravindranadh, M.C. Rao, R.V.S.S.N. Ravikumar, *J. Mater. Sci: Mater. Elect*., **2015,** 26, 6667-6675.
[24] K. Ravindranadh, M.C. Rao, R.V.S.S.N. Ravikumar, *Appl. Mag. Reson*., **2015**, 46(1), 1-15.
[25] K. Ravindranadh, M.C. Rao, R.V.S.S.N. Ravikumar, *J. Luminesce*., **2015**, 159, 119-127.
[26] M.C. Rao, *J. Crys. Growth*, **2010**, 312(19), 2799–2803.
[27] M.C. Rao, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2011**, 5, 85-88.
[28] M.C. Rao**,** O.M. Hussain, *IOP Conf. Series: Mater. Sci. Eng.*, **2009**, 2, 012037 (p.1-4).
[29] M.C. Rao, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2011**, 5(5-6), 651-654.
[30] M.C. Rao, *J. Optoelect. & Adv. Mater*., **2011,** 13, 428-431.
[31] M.C. Rao, O.M. Hussain, *Optoelect. & Adv. Mater*., **2011,** 13(2-4), 1109-1113.
[32] M.C. Rao, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2012**, 6, 511-515.
[33] M.C. Rao, O.M. Hussain, *Eur. Phys. J. Appl. Phys.*, **2009**, 48(2), 20503 (p.1-6).
[34] M.C. Rao**,** O. M. Hussain, *Ind. J. Eng. Mater. Sci.*, **2009**, 16, 335-340.
[35] M.C. Rao, Inter. *J. Pure & Appl. Sci.*, **2010**, 3, 365–370.
[36] M.C. Rao, *J. Optoelect. & Adv. Mater.*, **2010**, 12, 2433-2436.
[37] M.C. Rao, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2010**, 4, 2088-2091.
[38] M.C. Rao, *J. Optoelect. & Adv. Mater.*, **2011**, 13, 78-81.
[39] M.C. Rao, K. Ravindranadh, Sk. Muntaz Begum, G. Nirmala, *AIP Conf. Proc.*, **2011**, 349, 641-642.
[40] M.C. Rao, O. M. Hussain, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2012**, 6, 263- 266.
[41] M.C. Rao, *Res. J. Chem. Sci.*, **2012**, 2(3), 74-79.
[42] M.C. Rao, Sk. Muntaz Begum, E.Sivanagi Reddy, O.M.Hussain , *AIP Conf. Proc.*, **2012**, 1447, 613-614.
[43] M.C. Rao, *Int. J. Chem. Sci.*, **2012**, 10(2), 1111-1116.
[44] M.C. Rao, *J. Intense Pulsed Lasers & Appl. Adv. Phys.*, **2012**, 2, 45-47.
[45] M.C. Rao, *J. Optoelect. & Biomedical Mater*., **2013**, 5, 9-16.
[46] M.C. Rao, *J. Chem. Bio. Phy. Sci. Sec. C*, **2013**, 3(2), 1412-1424.
[47] M.C. Rao, *Int. J. Mod. Phys., Conf. Series*, **2013**, 22, 355-360.
[48] M.C. Rao, *J. Chem. Bio. Phy. Sci. Sec. C*, **2013**, 4(1), 496-500.
[49] M.C. Rao, *J. Chem. Bio. Phy. Sci. Sec. C*, **2014**, 4(2), 1502-1505.

[50] M.C. Rao**,** *J. Chem. Bio. Phy. Sci. Sec. C*, **2014**, 4(3), 2555-2559.
[51] M.C. Rao, S.M. Begum, *Optoelect. & Adv. Mater., (Rapid Commu.,)*, **2012**, 6, 508-510.
[52] M.C. Rao, O.M. Hussain, *J. Alloys Compd.*, **2010**, 491(1), 503-506.
[53] M.C. Rao, K. Ravindranadh, *Der Pharma Chemica*, **2016**, 8, 243-250.
[54] M.C. Rao, K. Ravindranadh, M.S. Shekhawat, *AIP Conf. Proc.*, **2013**, 1536, 215-216.
[55] K. Ravindranadh, M.S. Shekhawat, M.C. Rao, *AIP Conf. Proc.*, **2013**, 1536, 219-220.
[56] K. Ravindranadh, R.V.S.S.N. Ravikumar, M.C. Rao, *J. Non Oxide Glasses*, **2013**, 5, 39-45.
[57] G. Nirmala, R.V.S.S.N. Ravikumar, M.C. Rao, *J. Optoelect. & Biomedical Mater.*, **2013**, 5, 57-62.
[58] M.C. Rao, K. Ravindranadh, M.S. Shekhawat, *AIP Conf. Proc.*, **2016,** 1728, 020077 (1-4).
[59] K. Ravindranadh, R.V.S.S.N. Ravikumar, M.C. Rao, *AIP Conf. Proc.*, **2016**, 1728, 020079 (1-4).
[60] T. Samuel, K. Sujatha, K. Ramachandra Rao, M.C. Rao, *AIP Conf. Proc.*, **2016**, 1728, 020080 (1-4).
[61] M.C. Rao, K. Ravindranadh, *J. Chem. Bio. Phy. Sci. Sec. C*, **2016**, 6(3), 944-950.
[62] K. Ravindranadh, D. Sridhar Kumar, K. Durga Venkata Prasad, M.C. Rao, *Int. J. ChemTech Res.*, **2016**, 9(4) 598-603.
[63] Ch. Srinivasa Rao, M.C. Rao**,** T. Srikumar, *Int. J. Chem Tech Res*., **2014**, 6(7), 3935- 3938.
[64] T. Srikumar**,** Ch. Srinivasa Rao, M.C. Rao, *Int. J. Chem Tech Res.*, **2014**, 6(11), 4697-4701.
[65] Ch. Srinivasa Rao, T. Srikumar, M.C. Rao, *Int. J. Chem Tech Res.*, **2014**, 7(1), 420-425.
[66] Ch. Srinivasa Rao, T. Srikumar, S. Shantkriti, M.C. Rao, *Int. J. Tech Chem Res.*, **2015**,1(1), 66-69.
[67] Ch. Srinivasa Rao, M.C. Rao, *Int. J. Chem Tech Res.*, **2015**, 8(2), 524-527.
[68] M.C. Rao, K. Ravindranadh, *Der. Pharm. Che.*, **2016**, 8(7), 74-79.
[69] K. Ravindranadh, K. Koteswara Rao, M.C. Rao, *J. Chem. Pharm. Res.*, **2016**, 8(5), 310-313.
[70] M.C. Rao, *J. Chem. Pharm. Res.*, **2016**, 8(5), 450-456.
[71] M.C. Rao, K. Ravindranadh Rao, *J. Chem. Pharm. Res.*, **2016**, 8(5), 677- 684.
[72] K. Ravindranadh, M.C. Rao, *J. Chem. Pharm. Res*., **2016,** 8(5), 721-724.
[73] M.C. Rao, *Int. J. Mod. Phys., Conf. Series*, **2013**, 22, 11-17
[74] M.C. Rao, *Int. J. Mod. Phys., Conf. Series*, **2013,** 22, 355-360.
[75] M.C. Rao, *Int. J. Mod. Phys., Conf. Series*, **2013**, 22, 385-390.
[76] M.C. Rao, *Res. J. Recent Sci.*, **2013**, 2(3), 67-73.
[77] M.C. Rao, K. Ravindranadh, T. Rosemary, *J. Chem. Bio. Phy. Sci. Sec. C*, **2013**, 4(1), 469-473
[78] K. Ravindranadh, M.C. Rao, R.V.S.S.N. Ravikumar, *J. Non Oxide Glasses*, **2013**, 5,39-45
[79] M.C. Rao, *J. Optoele. Biomed. Mater.*, **2013**, 5(1), 9-16.
[80] G. Nirmala, R.V.S.S.N. Ravikumar, M.C. Rao, *J. Optoelect. & Biomedical Mater.*, **2013**, 5, 57-62.
[81] M.C. Rao, K. Ravindranadh, *Int. J. Adv. Phar. Bio. Chem.*, **2013**, 2(2), 368-371.
[82] K. Ravindranadh, M.C. Rao, *Int. J. Adv. Phar. Bio. Chem.*, **2013**, 2(1), 190-200.
[83] K. ParameswaraRao, B. V. Ramesh, Ch. Siva Prasad, M. C. Rao, *Der Pharm. Lett.* **,2016**, 8(9), 341-348.
[84] Sk. Muntaz Begum, K. Ravindranadh, M.C. Rao, R.V.S.S.N. Ravikumar, *AIP Conf.Proc.*, **2013**, 1536, 27-28.
[85] P.V. Prasad, K. Ramachandra Rao, M.C. Rao, *Int. J. Chem Tech Res.*, **2014**, 7(1), 269-274.
[86] P.V. Prasad, K. Ramachandra Rao, M.C. Rao**,** *J. Mol. Struc.*, **2015**, 1085, 115-120.
[87] S. Rajyalakshmi, B. Brahmaji, K. Samatha, K. Ramachandra Rao, M.C. Rao, *Int. J. ChemTech Res.*, **2016**, 9(1), 7-14.
[88] M.C. Rao, *Int. J. Chem Tech Res.*, **2014**, 6(3), 1904-1906.
[89] M.C. Rao**,** K. Ramachandra Rao, *Int. J. Chem Tech Res.*, **2014,** 6(7), 3931-3934.