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Development and Validation of New Stability Indicating RP-HPLC Method for the Assay of Atazanavir in Pure and Dosage Forms

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ABSTRACT

Reversed phase liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the determination of Atazanavir in pure and formulations. The studies on selection of mobile phase and flow rate basing on peak parameters were extensively carried for the development of the proposed method. The mobile phase composition of buffer (pH-4.5) and acetonitrile in the ratio of 750:250 v/v Atazanavir peak was eluted at void volume with long retention time. The regression value for Atazanavir was found to be 0.9964 exhibiting the response, in the linear from range 2.0-10.0 µg/mL respectively. The % RSD for precision is < 2.0 confirming that the developed RP-HPLC method is sufficiently precise and the total run time required for the method is only 4.007 min. for eluting Atazanavir. The accuracy of the developed method was demonstrated at three concentration levels in the range of 50–150 %. The developed method was said to be simple, selective and accurate and is useful for the assay of Atazanavir in dosage forms and can be further employed in the quality control analysis of bulk manufacturing and formulations units.

Keywords: Atazanavir, RP-HPLC, Validation, Recovery Studies, Precision, Ruggedness,

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INTRODUCTION

Atazanavir Sulfate (ATV) [1] methyl N′-[(1S)-1-{N-[(2S, 3S)-2-hydroxyl-3-[(2S)-2-[(methoxycarbonyl) amino]-3, 3-dimethylbutanamido]-4-phenylbutyl]-N′-{[4-(Pyridin-2-yl) phenyl] methyl} hydrazine carbonyl}-2, 2-dimethyl propyl] carbamate (Figure-1), is an azapeptide HIV-1 protease inhibitor (PI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1).

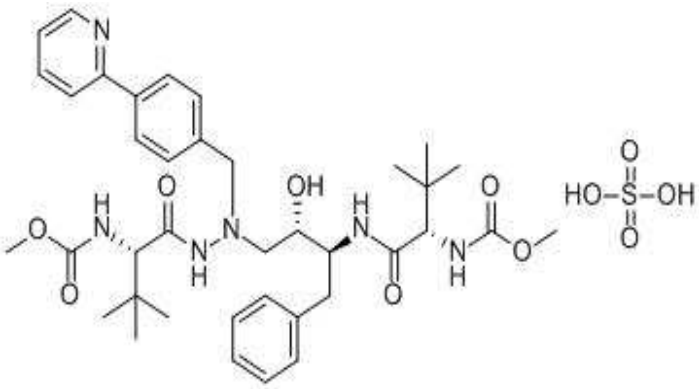

Figure-1: Molecular structure of Atazanavir Sulfate

Atazanavir selectively inhibits the virus-specific processing of viral Gag and Gag-poly proteins in HIV-1- infected cells, thus preventing the formation of mature virions. Atazanavir is also sometimes used to prevent infection in healthcare workers or other people who were accidentally exposed to HIV. Keeping in view of the above discussion, in the present study the authors have attempted in developing of few analytical methods for Atazanavir in pure and dosage forms using visible spectrophotometry and High performance liquid chromatography as analytical tools. Basing on the analytical survey till date few HPLC methods [2-10] and no stability-indicating HPLC method was reported for the assay of Atazanavir in pure and dosage forms. The present study is aimed in development and validation of stability indicating method assay of Atazanavir in pure and dosage forms by RP-HPLC as per ICH guidelines. 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 [11-34]. Rao et al. have reported their work on different oxide materials in their earlier studies [35-65]. The RP-HPLC method developed by the authors in this present investigation facilitated in studying the stability the Atazanavir related drugs when exposed to various degradation conditions (acidic, basic, heat and oxidative) and would be of great applicable in the analysis of purity and pharmacokinetic studies for quality control and clinical monitoring laboratories.

MATERIALS AND METHODS

***Instrumentation*:** The analysis of the Atazanavir was carried out on HPLC Waters system equipped with Waters 2695 separation module having maximum pressure of 5000 psi, Waters 2996 photodiode array and a reverse phase HPLC column X-terra RP- C_8 [150 mm x 4.6 mm I.D; particle size 5 µm]. The output of signal was monitored and integrated using Waters Empower 2 software. Electronic analytical balance (DONA) and Micro pipette (In labs, 10-100 µl) were employed in the present analysis. All the glassware employed in the present analysis were cleaned with hot water and dried in hot air oven whenever required.

***Chemicals & Solvents*:** Ultra-pure water (Milli-Q) used for this assay was obtained from a Millipore system (Bedford, MA, USA). Acetonitrile (HPLC grade) Orthophosphoric acid (GR Grade), Sodium dihydrogen phosphate monohydrate (GR Grade) were purchased from Qualigens Ltd., Mumbai., India. Atazanavir (99.99 % pure) was obtained as gift sample form Emcure pharmaceuticals Ltd and ATAZOR Capsules are available for oral administration in strengths of 300 mg of Atazanavir procured from local pharmacy.

***Preparation of Buffer*:** Accurately weigh and transfer about 2.72 grams of Sodium dihydrogen phosphate monohydrate in 1000 mL of purified water and mix. Adjust pH to 4.5 (± 0.05) with dilute orthophosphoric acid. Before use filter this solution through 0.45 µm membrane filter.

***Preparation of Mobile Phase*:** The mobile phase used in the present assay is prepared by mixing Sodium dihydrogen phosphate buffer (pH-4.5) and acetonitrile in the ratio of 700:300 v/v respectively. Before use the mobile phase is filtered and degassed.

***Diluent Preparation*:** Mobile phase is used as diluent in the present assay.

Preparation of Standard Drug Solution (stock & working): Standard stock solution of Atazanavir was prepared by accurately weighing and transferring 50 mg of Atazanavir (99.9 % pure) into a 100mL volumetric flask. To the above flask, add 60mL of diluent and sonicated to dissolve. Cool the solution to room temperature and diluted to volume with diluent. This stock solution was further diluted by transferring suitable aliquots into a separate 100 mL volumetric flask and diluted up to the mark with diluent to obtain final working concentrations (2.0-10.0 µg/mL) of linearity range, respectively.

***Sample Preparation*:** For analysis of the formulations, twenty tablets (ATAZOR-300 mg) were weighed individually and their average weight is determined. These tablets were then crushed to a fine powder. Powder equivalent to the weight of 50 mg [ATAZANAVIR] was transferred to a 100 mL volumetric flask and dissolved in about 70 mL of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature and finally diluted to the mark with diluent and mixed thoroughly. Later this solution was filtered through 0.45 µm membrane. Transfer a series of aliquots of this above solution into a 100 mL volumetric flask and dilute to volume with the same diluent and proceeded with the above described procedure of the proposed method.

RESULTS AND DISCUSSION

Method Development: To develop a suitable and robust RP-HPLC method for the assay of Atazanavir the chromatographic conditions were optimized by different means (i.e, using different column, different buffer and different mode of HPLC run). Primarily the method development was started with two different columns C8 and C18. Of the two columns [C_8 and C_{18}], X-terra RP-C_8 (150 mm x 4.6 mm I.D; particle size 5 µm) gave satisfactory resolution at 5mins time with the mobile phase. Secondly studies on selection of mobile phase and flow rate basing on peak parameters (height, area, tailing, theoretical plates, capacity factor and resolution) were extensively carried for the development of the proposed method.

The mobile phase composition of buffer (pH-4.5) and acetonitrile in the ratio of 750:250 v/v Atazanavir peak was eluted at void volume with long retention time. The best results was obtained by the when the mobile phase composition of buffer and acetonitrile was slightly changed to the ratio of 700:300 v/v at a flow rate 1.0 mL/min. With this mobile phase Atazanavir eluted at a retention time ~ 4.0 minutes (Figure-2). Finally, a flow rate of 1.0 mL/min with an injection volume of 20 µL and UV detection at 230 nm was found to be best for analysis of Atazanavir. The chromatogram of Atazanavir standard using the proposed method is shown in (Figure-2). System suitability results of the proposed method are presented in Table-1.

Table-1: System Suitability Parameters

Parameters	Atazanavir
Retention time	4.007
Peak area	1571.182
USP Tailing	1.6

***Chromatographic Conditions*:** The chromatographic separation was performed using X-terra RP-C_8 [150 mm x 4.6 mm I.D; particle size 5 µm] column, at ambient temperature, eluted at the flow rate of 1.0 mL/min using gradient run. The mobile phase consisted of sodium di-hydrogen phosphate buffer [pH adjusted to 4.5 ± 0.1 with 10 % phosphoric acid] and acetonitrile in the ratio of 700:300 v/v. This mobile phase was filtered through 0.45 mm membrane filter and degassed prior to use. The wavelength in the present assay was selected by scanning standard solution of drug over 200 to 400 nm using Waters 2996 photodiode array detector. Analysis of Atazanavir was made by injecting sample volume of 10 µL and detection at 230 nm. The complete analysis of Atazanavir was performed with in the chromatographic runtime of 5 minutes respectively.

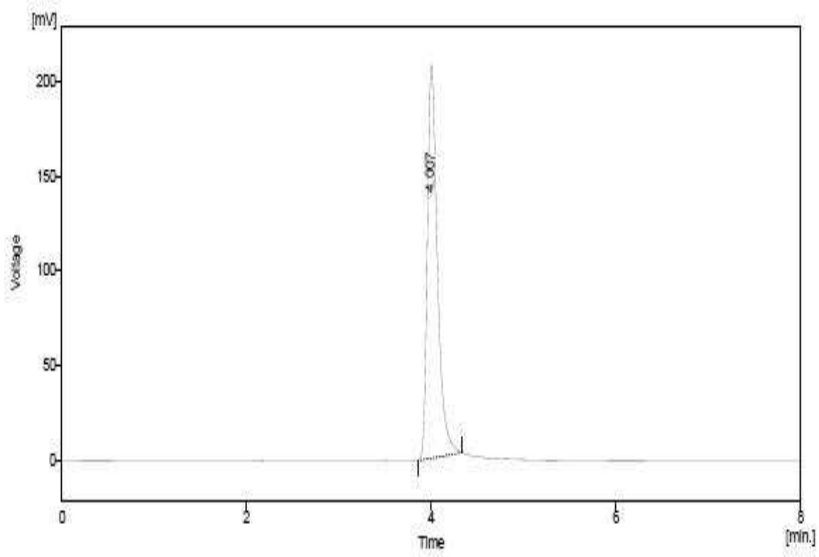

Figure-2: Validative Chromatogram of Atazanavir

***Method Validation*:** The developed RP-HPLC method was validated for system suitability, specificity linearity, accuracy, precision, robustness and ruggedness studies as per IC norms. All the validation studies were carried out by injecting replicate injections of the standard and sample solutions of Atazanavir into the HPLC column under the above said optimized chromatographic conditions.

System Suitability: System suitability tests were performed as per the ICH Guidelines to confirm the suitability and reproducibility of the system. The test was carried out by repetitively injecting the standard solution of Atazanavir at the concentration level 10 µg/mL. The results of system parameters [Theoretical plates, retention time, tailing factor] were determined and are presented in Table-1 respectively.

Specificity: Specificity is the ability of the method to unequivocally assess the analyte in the presence of components, which may be expected to be present. The specificity of the developed HPLC method for Atazanavir was carried out in the presence of its degradation products in blank (stress studies) and bulk drug separately.

Blank Interference: A study to establish the interference of blank was conducted. Diluent was injected into the chromatograph in defined above chromatographic conditions and the blank chromatogram was recorded. Chromatogram of blank solutions showed no peaks at the retention time of atazanavir peak. This indicates that the diluent solution used in sample preparation do not interfere in the assay of Atazanavir in Atazanavir tablets.

***Forced Degradation Studies*:** In order to establish the stability indicating nature of the developed method, force degradation of Atazanavir was performed under various stress conditions. Intentional degradation was attempted to stress conditions exposing it to acid (5N hydrochloric acid), alkali (5N NaOH) and hydrogen peroxide (30 %) to evaluate the ability of the proposed method to separate Atazanavir from its degradation products.

From the results of degradation studies it was observed that upon treatment of Atazanavir with different strengths of base (5N NaOH), acid (5N HCl) and hydrogen peroxide (30 %) no degradation was observed and the results, chromatograms of various degradation studies of Atazanavir indicating the suitability of the developed method to study stability of Atazanavir under various forced degradation conditions viz. acid, base and oxidative degradation.

Linearity & Detector Response (lod & loq): The Peak-area ratios of Atazanavir obtained from selected chromatograms were utilized for construction of calibration curve. The linearity of the calibration curve was constructed by plotting the peak- area ratios versus the concentrations of atazanavir. A standard curve was obtained in the concentration range of 2.0-10.0 µg/mL. The linear regression data, slope, intercept and correlation coefficient [r^2] of standard curve were calculated. The result showed that with-in the concentration range mentioned above,

there was an excellent correlation between peak area ratio and concentration of Atazanavir. LOD (defined at s/n = 3:1) for Atazanavir were found to be 0.0601 respectively (Figure-3).

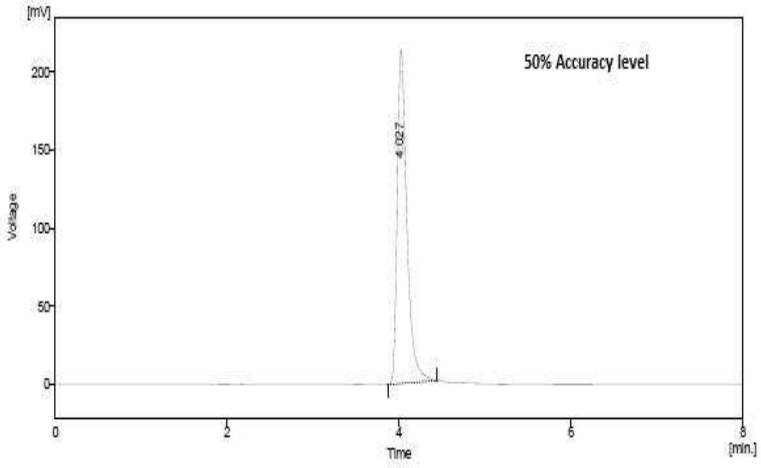

Figure-3: Linearity Curve of Atazanavir

***Method Precision*:** Repeatability of the developed method was studied by carrying out method precision. Method precision was determined from results of six independent determinations at 100 % of the standard concentrations of Atazanavir. The relative standard deviation was found to be 1.701. The results of method precision gave high values of mean assay and low values of standard deviation and % RSD studies revealing that the proposed RP-HPLC method is precise.

Accuracy [Recovery Studies]: To study accuracy of the method, recovery experiment was carried out by applying the standard addition method and is expressed as the percentage of analytes recovered by the assay. A known quantity of drug substance corresponding to 50 %, 100 % and 150 % of the label claim of drug were added, to determine if there are positive or negative interferences form excipients present in the formulations. Each set of addition were repeated three times. The percentage recoveries of Atazanavir were found in the range of 99.92 to 100 % from a series of spiked concentrations and the results and their respective chromatograms are reported. These results indicated that the proposed RP-HPLC method is highly accurate for atazanavir in pure and it's dosage forms (Figure-4).

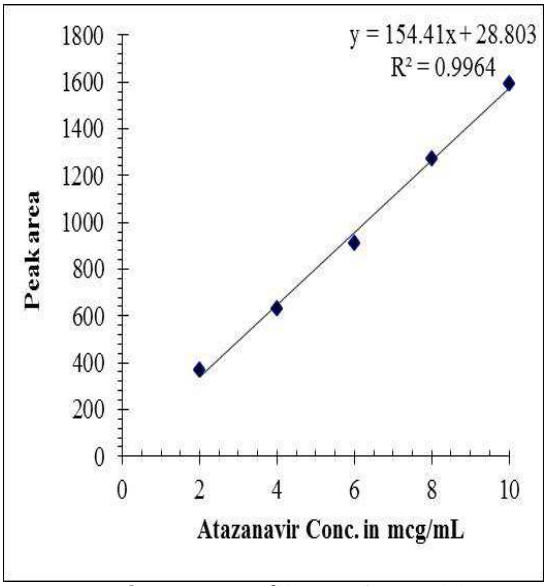

Figure - 4: HPLC Chromatogram of Atazanavir at 50% Accuracy Level

Robustness: In evaluating the robustness of developed RP-HPLC method a few parameters like flow rate and temperature deliberately changed. Each of the above mentioned factor mentioned above was changed at three levels (-1, 0, +1) at one time with respect to optimized parameters. The results of robustness studies for Atazanavir evaluated that there is no insignificant differences in peak areas and less variability in retention times with the standard revealing that the developed RP-HPLC method is robust for Atazanavir assay.

Ruggedness: The ruggedness of the proposed RP-HPLC method was evaluated by a different analyst and different analyst and different instrument in the same laboratory. The % RSD for peak areas of Atazanavir was calculated. These results revealed that the % RSD was within the limits indicating that the developed RP-HPLC method was found to be rugged.

***Analysis of Marketed Formulation*:** Analysis of marketed formulations (**AZATROR-300 mg**) of Atazanavir was carried out by using the proposed method under the above described optimized HPLC conditions (Figure-5). The % drug content of tablets obtained by the proposed method for Atazanavir was found to be 99.98 %, respectively. The results are given in Table-2.

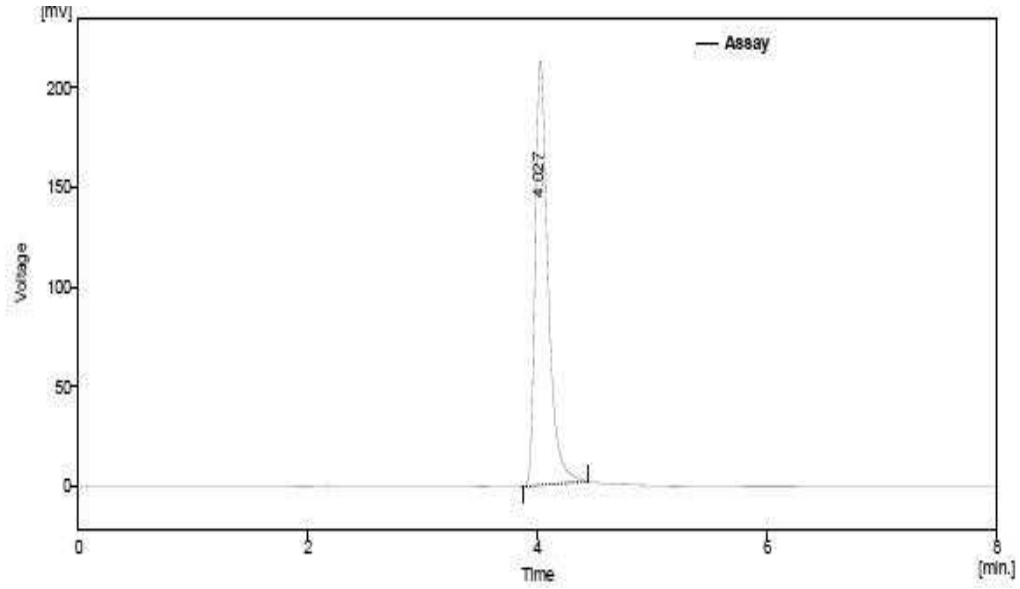

Figure-5: Validative Chromatogram of Atazanavir in Formulation

Table 2: Results of Analysis of Tablet Containing Atazanavir

Pharmaceutical Formulation	Amount of ATAZANAVIR*		% of Recovery
	Labeled	Found*	
AZATOR	300 mg	299.96	99.98 %

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

CONCLUSION

In the present study a simple, feasible, stability indicating reversed-phase HPLC method has been successfully developed and validated for the assay of Atazanavir in pure and its pharmaceutical dosage forms. Based on peak purity results, obtained from the analysis of force degraded samples using described method, it can be concluded that that the developed method is specific for the estimation of Atazanavir in presence of degradation products. The regression value for Atazanavir was found to be 0.9964 exhibiting the response, in the linear from range 2.0-10.0 µg/mL respectively. The % RSD for precision is < 2.0 confirming that the developed RP-HPLC method is sufficiently precise and the total run time required for the method is only 4.007 min. for eluting Atazanavir. Moreover, it is concluded that the results of specificity, linearity, precision, accuracy and method robustness established not only the validation of the proposed RP-HPLC method but also exhibiting its reliability in assaying Atazanavir in pure and its pharmaceutical dosage forms

REFERENCES

[1] P.J. Piliero, *Expert Opin. Investig. Drugs*, **2002**, 11, 1295-1301.
[2] D.R. Goldsmith and C.M. Perry, *Atazanavir. Drugs*, **2003**, 63, 1679-1693.
[3] S. Dey, Y.V. Reddy, T. Reddy, S.K. Sahoo, P.N Murthy, S. Mohapatra and S.S. Patro, *Int. J. Pharm. Bio. Sci.*, **2010**, 1, 1-8.
[4] J. Shah, M.R. Jan, M.A. Khan, *J. Chin. Chem. Soc*., **2005**, 52, 347-352.
[5] Anindita Behera, M. Swapan Kumar, Si. Sudam Chandra, D. Gowri Sankar, *Quím. Nova*, **2011**, 8, 34-39.
[6] K. Sathish Kumar, K. Sujana, *Int. J. Pharm. Sci.*, **2011**, 4, 614-618.
[7] D. Cattaneo, F. Maggiolo, D. Ripamonti, N. Perico, *J. Chromatog. Sci.*, **2008**, 46, 485-488.
[8] B.H. Jung, N.L. Rezk, A.S. Bridges, A.H. Corbett, A.D.M. Kashuba, *Biomed. Chromatogr*, **2007**, 21, 1095-1098.
[9] K.M.L. Crommentuyn, A.D.R. Huitema, J.H Beijnen, *J. Pharm. Biomed. Anal*, 2005, 38, 139-143.
[10] V. R. Robledo, W.F. Smyth, *Anal. Chim. Acta*, **2008**, 623, 221-230.
[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. Mod. Phys., Conf. Series*, **2013**, 22, 576-582.
[16] M.C. Rao, *Int. J. Chem. Sci*, **2012**, 10(2), 1111-1116.
[17] M.C. Rao, K. Ramachandra Rao, *Int. J. Chem Tech Res*., **2014**, 6(7), 3931-3934.
[18] S.M. Begum, M.C. Rao, R.V.S.S.N. Ravikumar, *J. Inorg. Organometa. Poly. Mater*, **2013**, 23(2), 350-356.
[19] S.M. Begum, M.C. Rao, R.V.S.S.N. Ravikumar, *J. Mol. Struct*., **2011**, 1006(1), 344- 347.
[20] S.M. Begum, M.C. Rao, R.V.S.S.N. Ravikumar, *Spectrochim. Acta Part A: Mol. & Biomol. Spec*., **2012**, 98, 100-104.
[21] K. Ravindranadh, RVSSN Ravikumar, M.C. Rao, *Int. J. Mod. Phys., Conf. Series*, **2013**, 22, 346-350.
[22] K. Ravindranadh, M.C. Rao, R.V.S.S.N. Ravikumar, *J. Mater. Sci: Mater. Elect*., **2015,** 26, 6667-6675.
[23] K. Ravindranadh, M.C. Rao, R.V.S.S.N. Ravikumar, *Appl. Mag. Reson*., **2015**, 46(1), 1-15.
[24] K. Ravindranadh, M.C. Rao, R.V.S.S.N. Ravikumar, *J. Luminesce*., **2015**, 159, 119-127.
[25] M.C. Rao, *J. Crys. Growth*, **2010**, 312(19), 2799–2803.
[26] M.C. Rao, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2011**, 5, 85-88.
[27] M.C. Rao**,** O.M. Hussain, *IOP Conf. Series: Mater. Sci. Eng.*, **2009**, 2, 012037 (p.1-4).
[28] M.C. Rao, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2011**, 5(5-6), 651-654.
[29] M.C. Rao, *J. Optoelect. & Adv. Mater*., **2011,** 13, 428-431.
[30] M.C. Rao, O.M. Hussain, *Optoelect. & Adv. Mater*., **2011,** 13(2-4), 1109-1113.
[31] M.C. Rao, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2012**, 6, 511-515.
[32] M.C. Rao, O.M. Hussain, *Eur. Phys. J. Appl. Phys*., **2009**, 48(2), 20503 (p.1-6).
[33] M.C. Rao**,** O. M. Hussain, *Ind. J. Eng. Mater. Sci*., **2009**, 16, 335-340.
[34] M.C. Rao, *J. Optoelect. & Adv. Mater*., **2010**, 12, 2433-2436.
[35] M.C. Rao, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2010**, 4, 2088-2091.
[36] M.C. Rao, *J. Optoelect. & Adv. Mater*., **2011**, 13, 78-81.
[37] M.C. Rao, K. Ravindranadh, Sk. Muntaz Begum, G. Nirmala, *AIP Conf. Proc.*, **2011**, 1349, 641-642.
[38] M.C. Rao, O. M. Hussain, *Optoelect. & Adv. Mater., (Rapid Commu.)*, **2012**, 6, 263- 266.
[39] M.C. Rao, *Res. J. Chem. Sci.*, **2012**, 2(3), 74-79.
[40] M.C. Rao, Sk. Muntaz Begum, E.Sivanagi Reddy, O.M.Hussain , *AIP Conf. Proc.*, **2012**, 1447, 613-614.
[41] M.C. Rao, S.M. Begum, *Optoelect. & Adv. Mater., (Rapid Commu.,)*, **2012**, 6, 508-510.
[42] M.C. Rao, O.M. Hussain, *J. Alloys Compd.*, **2010**, 491(1), 503-506.
[43] M.C. Rao, K. Ravindranadh, *Der Pharma Chemica*, **2016**, 8, 243-250.
[44] M.C. Rao, K. Ravindranadh, M.S. Shekhawat, *AIP Conf. Proc.*, **2013**, 1536, 215-216.
[45] K. Ravindranadh, M.S. Shekhawat, M.C. Rao, *AIP Conf. Proc.*, **2013**, 1536, 219-220.
[46] K. Ravindranadh, R.V.S.S.N. Ravikumar, M.C. Rao, *J. Non Oxide Glasses*, **2013**, 5, 39-45.
[47] M.C. Rao, K. Ravindranadh, M.S. Shekhawat, *AIP Conf. Proc.*, **2016,** 1728, 020077 (1-4).
[48] K. Ravindranadh, R.V.S.S.N. Ravikumar, M.C. Rao, *AIP Conf. Proc.*, **2016**, 1728, 020079 (1-4).
[49] T. Samuel, K. Sujatha, K. Ramachandra Rao, M.C. Rao, *AIP Conf. Proc.*, **2016**, 1728, 020080 (1-4).
[50] K. Ravindranadh, D. Sridhar Kumar, K. Durga Venkata Prasad, M.C. Rao, *Int. J. ChemTech Res.*, **2016**, 9(4) 598-603.

[51] Ch. Srinivasa Rao, M.C. Rao**,** T. Srikumar, *Int. J. Chem Tech Res*., **2014**, 6(7), 3935- 3938.
[52] T. Srikumar**,** Ch. Srinivasa Rao, M.C. Rao, *Int. J. Chem Tech Res.*, **2014**, 6(11), 4697-4701.
[53] Ch. Srinivasa Rao, T. Srikumar, M.C. Rao, *Int. J. Chem Tech Res.*, **2014**, 7(1), 420-425.
[54] Ch. Srinivasa Rao, M.C. Rao, *Int. J. Chem Tech Res.*, **2015**, 8(2), 524-527.
[55] M.C. Rao, K. Ravindranadh, *Der. Pharm. Che.*, **2016**, 8(7), 74-79.
[56] M.C. Rao, *Res. J. Recent Sci.*, **2013**, 2(3), 67-73.
[57] K. Ravindranadh, M.C. Rao, R.V.S.S.N. Ravikumar, *J. Non Oxide Glasses*, **2013**, 5, 39-45
[58] K. ParameswaraRao, B. V. Ramesh, Ch. Siva Prasad, M. C. Rao, *Der Pharm. Lett.* **,** **2016**, 8(9), 341-348.
[59] Sk. Muntaz Begum, K. Ravindranadh, M.C. Rao, R.V.S.S.N. Ravikumar, *AIP Conf. Proc.*, **2013**, 1536, 27-28.
[60] P.V. Prasad, K. Ramachandra Rao, M.C. Rao, *Int. J. Chem Tech Res.*, **2014**, 7(1), 269-274.
[61] P.V. Prasad, K. Ramachandra Rao, M.C. Rao**,** *J. Mol. Struc*., **2015**, 1085, 115-120.
[62] S. Rajyalakshmi, B. Brahmaji, K. Samatha, K. Ramachandra Rao, M.C. Rao, *Int. J. ChemTech Res.*, **2016**, 9(1), 7-14.
[63] M.C. Rao, *Int. J. Chem Tech Res.*, **2014**, 6(3), 1904-1906.
[64] K. Parameswara Rao, B.V. Ramesh, Ch. Siva Prasad, G.V. Ramana, M.C. Rao, *Der Pharm. Lett.*, **2016,** 8(10), 222-228.
[65] K. Ravindranadh, K. Durga Venkata Prasad and M.C. Rao**,** *AIMS Mater. Sci*., **2016**, 3(3) 796-807.