Int. J. Chem. Sci.: 14(4), 2016, 2461-2466
ISSN 0972-768X
www.sadgurupublications.com

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF ANTIRETROVIRAL DRUGS IN TABLET DOSAGE FORMS

K. PARAMESWARA RAO*

Department of Chemistry, Andhra Loyola College, VIJAYAWADA – 520008 (A.P.) INDIA

ABSTRACT

Reversed phase liquid chromatographic method has been developed and subsequently validated for the determination of drugs in tablet formulations. Standard stock solutions of different drugs were prepared by transferring accurately weighed drug and dissolving in a solution suitably. This solution was sonicated for 20 min to achieve complete dissolution and made up to the mark with mobile phase. From the standard stock solution different concentrations of working standard solutions of drugs were prepared with the same mobile phase. The developed method offers several advantages in terms of simplicity in mobile phase, mode of elution, easy sample preparation steps and comparative short run time which makes the method specific and reliable for its intended use in routine analysis determination of drug in tablet dosage forms.

Key words: RP-HPLC, Drugs, Validation and tablet formulations.

INTRODUCTION

Reversed phase liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the determination of some drugs in tablet forms. The drugs Darunavir, Atazanavir, Indinavir, Zolmitriptan and Glibenclamide are developed in tablet form. Darunavir is an antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS. Molecular formula is $C_{27}H_{37}N_3O_7S$. Molecular weight is 547.66. Melting point of drug is 74^oC. 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. 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]. Atazanavir Sulfate (ATV)[1] methyl N′-[(1S)-1-{N-[(2S,3S)-2-hydroxyl-

*Author for correspondence; E-mail: kp.rao1982@gmail.com

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

Indinavir sulfate, chemically known as [1(1*S*, 2*R*), 5(*S*)]-2, 3, 5-trideoxy-N-2, 3-dihydro-2-hydroxy-1H-inden-1-yl)-5-[2-[[(1, 1-dimethylethyl)amino]carbonyl]-4-(3 pyridinyl-methyl)-1-piperazinyl]-2-phenylmethyl)-D-erythro-pentonamide sulfate (1:1) salt[2], is a potent protease inhibitor of Human Immunodeficiency Virus (HIV) widely used in the treatment against the acquired immune deficiency syndrome (AIDS) and is prescribed in combination with other protease inhibitors, nucleoside analogues or reverse transcriptase inhibitors. Zolmitriptan (S)-4-{[3-(2-dimethylaminoethyl)-1H-indol-5-yl] methyl}-1, 3-oxazolidin-2-one is an oral, selective serotonin receptor agonist used for the treatment of acute migraine attacks. Glibenclamide is chemically known as 5-chloro-N-[2-[4 [(cyclohexylamino) carbonyl] amino] sulfonyl] phenyl] ethyl]-2-methoxy benzamide is second generation sulphonyl ureas drug widely used in treatment of type 2 diabetic patients. It acts by inhibiting ATP-sensitive potassium channels in pancreatic beta cells causing cell membrane depolarization (increasing intracellular calcium in the beta cell) which stimulates the insulin release. A detailed literature survey for the above mentioned drugs revealed that several analytical methods such as spectrophotometric and HPLC were reported for the quantification of the drugs that are laborious and time consuming. Rao et al. have published their results on different oxide materials, luminescent materials, polymers, glasses and on different drugs in their earlier studies[3-12].

EXPERIMENTAL

Preparation of standard 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). Standard stock solution of Atazanavir was prepared by accurately weighing and transferring 50 mg of Atazanavir (99.9% pure) into a 100 mL volumetric flask. To the above flask, add 60 mL 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. Accurately weighed about 50.0 mg of Indinavir and transferred into a 100 mL volumetric flask then, add

60 mL of methanol and sonicated to dissolve. Cool the solution to room temperature and diluted to mark with methanol [stock solution]. Daily working standard solutions of Indinavir were prepared with mobile phase containing Indinavir at a concentration of 5.0-15.0 µg/mL and each of the these dilutions (20 µL) was injected six times in to the column, with flow rate of 1.0 mL/min and peak area of each of the drug concentrations, retention times were recorded.

Weigh accurately about 25 mg of Zolmitriptan into 100 mL volumetric flask. Add 70 mL of diluent and swirl gently to dissolve and make-up the volume with diluent. The calibration curve was plotted over the concentration range 5.104 to 30.626 µg/mL prepared by dissolving aliquots of standard working solution of zolmitriptan with mobile phase. Aliquots (20 µL) of each solution were injected under the operating chromatographic condition described above. Standard stock solution (1.0 $mg.mL^{-1}$) of Glibenclamide was prepared by transferring accurately weighed 50.0 mg of Glibenclamide and dissolving in a 50 mL volumetric flask containing 10 mL of methanol. This solution was sonicated for 20 min to achieve complete dissolution and made up to the mark with mobile phase. From the standard stock solution different concentrations of working standard solutions of Glibenclamide were prepared with the same mobile phase ranging from 2.0-10.0 $\mu g.mL^{-1}$. The solutions were filtered through a 0.45 µm membrane filter before injection.

RESULTS AND DISCUSSION

Analysis of pharmaceutical 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 10 mins 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 1. Analysis of marketed formulations (AZATROR-300 mg) of Atazanavir was carried out by using the proposed method under the above described optimized HPLC conditions. 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. Analysis of marketed formulations (INDINAVIN) of Indinavir was carried out by using the proposed method under the above described optimized HPLC conditions. The % drug content of tablets obtained by the proposed method for Indinavir was found to be 99.99%, respectively. The results are given in Table 3.

Table 1: 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

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

Table 3: Results of analysis of tablet containing Indinavir

Pharmaceutical formulation	Amount of Indinavir*		% Recovery
	Labelled	Found	
Indivan	400 mg	399.96	99.99%

*Average of three determinations

Pharmaceutical formulations of Zolmitriptan were analyzed by the developed method. The assay of the drug present in the each tablet was calculated by comparing the area of the peak of test with the standard. The assay of Zolmitriptan in tablets was found to be 99.49% and the results were presented in Table 4.

Table 4: Analysis of marketed tablets by the proposed method

Drug	Label claim	Quantity found*	Reference method	Statistical results	% Assay
Zolmitriptan (Zomig)	**5.0**	4.92 ± 0.04	4.97 ± 0.08	F = 4.00 t = 1.44	99.49

*Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.262

Pharmaceutical formulations of Glibenclamide were analyzed by the developed method. The assay of the drug present in the each tablet was calculated by comparing the area of the peak of test with the standard. The assay of Glibenclamide in tablets was found to be 99.60% and the results were presented in Table 5.

Table 5: Results of analysis of tablet containing Glibenclamide

Pharmaceutical formulation	Amount of Glibenclamide*		% Recovery
	Labelled	Found	
Micronase (5.0 mg)	5.0 mg	4.98	99. 60%

*Average of three determinations

CONCLUSION

The reported RP-HPLC method developed by the author for the analysis of the different drugs was proved to be simple, rapid and reproducible. The validation data indicate good precision, accuracy and reliability of the developed RP-HPLC method. The developed method offers several advantages in terms of simplicity in mobile phase, mode of elution, easy sample preparation steps and comparative short run time which makes the method specific and reliable for its intended use in routine analysis determination of drugs in tablet dosage forms.

REFERENCES

1. A. K. Ghosh, Z. L. Dawson and H. Mitsuya, Bioorg. Med. Chem., **15(24)**, 7576 (2007).
2. A. K. Patrick and K. E. Potts, Clin. Microbiol. Rev., **11(4)**, 614 (1998).
3. M. C. Rao, Int. J. Chem. Sci., **10(2)**, 1111 (2012).
4. M. C. Rao and K. Ramachandra Rao, Int. J. ChemTech Res., **6(7)**, 3931 (2014).
5. Sk. Muntaz Begum, M. C. Rao and R. V. S. S. N. Ravikumar, J. Inorg. Organometa. Poly. Mater., **23(2)**, 350 (2013).
6. M. C. Rao, J. Crys. Growth, **312(19)**, 2799 (2010).
7. M. C. Rao, Optoelect. Adv. Mater., (Rapid Commu.), **5**, 85 (2011).
8. Sk. Muntaz Begum, M. C. Rao and R. V. S. S. N. Ravikumar, J. Mol. Struct., **1006(1)**, 344 (2011).

9. M. C. Rao, Optoelect. Adv. Mater., (Rapid Commu.), **5(5-6)**, 651 (2011).

10. Sk. Muntaz Begum, M. C. Rao and R. V. S. S. N. Ravikumar, Spectrochim. Acta Part A: Mol. Biomol. Spec., **98**, 100 (2012).

11. M. C. Rao, J. Optoelect. Adv. Mater., **13**, 428 (2011).

12. M. C. Rao and O. M. Hussain, Optoelect. Adv. Mater., **13(2-4)**, 1109 (2011).

Revised : 22.09.2016 *Accepted : 23.09.2016*