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VALIDATED SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF FENOFIBRATE AND ATORVASTATIN IN SYNTHETIC MIXTURE AND IN BULK TABLET DOSAGE FORM

***[1]Godge Ganesh Raosaheb, [2]Garje Mahesh Arjun, [3]Dode Aniket Balaprasad, [4]Dhaygude Anil Machindra, [5]Wagh Divya Vinayak and [6]Fartade Sachin Jalindar**

[1,2]Department of Pharmaceutics, Dr. Vithalrao Vikhe Patil Foundation's College of Pharmacy, Viladghat, Ahmednagar, Maharashtra, India.

[3]Department of Quality Assurance, Dr. Vithalrao Vikhe Patil Foundation's College of Pharmacy, Viladghat, Ahmednagar, Maharashtra, India.

[4]Department of Pharmaceutical Chemistry, Dr. Vithalrao Vikhe Patil Foundation's College of Pharmacy, Viladghat, Ahmednagar, Maharashtra, India..

[5]Department of Pharmacology, Dr. Vithalrao Vikhe Patil Foundation's College of Pharmacy, Viladghat, Ahmednagar, Maharashtra, India.

[6]Department of Pharmaceutics, School of Pharmacy, Swami Ramanand Tirth Marathwada University, Nanded, Maharashtra, India.

***Corresponding Author: Godge Ganesh Raosaheb**

Department of Pharmaceutics, Dr. Vithalrao Vikhe Patil Foundation's College of Pharmacy, Viladghat, Ahmednagar, Maharashtra, India.

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ABSTRACT

A simple, accurate, sensitive, precise and rapid UV- Spectrophotometric simultaneous equation method have been developed for the simultaneous analysis and estimation of Fenofibrate and atorvastatin in bulk and combined tablet dosage form formulation. To determine the absorption maximum, each drug Fenofibrate and atorvastatin were scanned in wavelength range of 200-400 nm in spectra measurement mode using the double beam UV-Spectrophotometer (Jasco). 287 nm (λmax for fenofibrate) and 249 nm (λmax for atorvastatin) respectively were selected as a sampling wavelengths in methanol as solvent. Beer's limit were obeyed in the range of 5-30 µg/ml for both Fenofibrate and atorvastatin. The correlation Coefficients for both drugs found to be satisfactory. Validation parameter such as its accuracy, linearity, precision, limit of detection (LOD), limit of quantitation (LOQ) were studied for proposed method according to the ICH guidelines. Results of all parameters were found to be satisfactory. The proposed method can be used effectively for routine analysis and estimation of fenofibrate and atorvastatin in combined synthetic mixture and bulk dosage form.

KEYWORDS: Fenofibrate, Atorvastatin, UV- Spectroscopy, Simultaneous estimation method.

1. INTRODUCTION

Atorvastatin is mainly used as an antihyperlipidemic agent in cardiovascular risk conditions. Atorvastatin belongs to the class of antihyperlipidemic agents known as statins. It is intended for lowering cholesterol level in the body. It acts by enzyme inhibition mechanism. Atorvastatin act by competitively inhibiting the 3-hydroxy-3-methyl glutaryl Co-enzyme-A (HMG-CoA) reductase. HMG-CoA reductase is a rate determining enzyme in in the biosynthesis of cholesterol via mevalonate pathway. This enzyme catalyzes the HMG-CoA conversion into mevalonate. Atorvastatin primarily shows its action in liver. It c auses the decrease in hepatic cholesterol level, hence hepatic uptake of cholesterol increases and it results in lowering of plasma cholesterol level.[1]

Statins can reduce mortality and morbidity associated with coronary heart disorder. Atorvastatin appears as white crystalline powder. It is practically in soluble in water, slightly soluble in methylene chloride and soluble in methanol. Atorvastatin calcium is chemically {[R-(R, R*)]-2-(4-flurophenyl)-ß, □-dihydroxy-5-(1-methylethyl) -3-phenyl-4-[phenylamino) carbonyl]-14-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate}. It is available in commercial pharmaceutical formulations for the treatment of hypercholesterolemia. it is effective to reduce both cholesterol and triglycerides.[2,3]

Dose:[4] 10-40 mg per day (80 mg max.)

Structure[20]

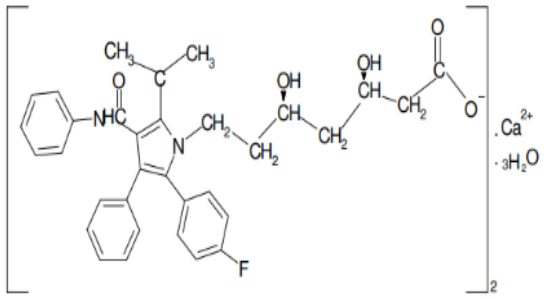

Figure 1: Structure of atorvastain calcium.

Fenofibrate is official drug Indian pharmacopoeia. It is a member of fibrate class. Fenofibrate has IUPAC name as propane-2-yl-2{4-[4-chlorophenyl)-carbonyl] phenoxy}-2-methyl propanoate.[5,6] It is used as a lipid regulating agent in the patients which are at the risk of cardiovascular disorders.[7] Fenofibrate act by increasing lipolysis and triglycerides rich particles are eliminated from plasma by activating lipoprotein lipase and by reducing apoprotein C-III production. Apoprotein C-III is an inhibitor of lipoprotein lipase activity.[8] It causes reduction in in both LDL (low density lipoprotein) and VLDL (Very low density lipoprotein) levels. Fenofibrate also increases the level of high density lipoprotein (HDL).[9]

Structure[8]

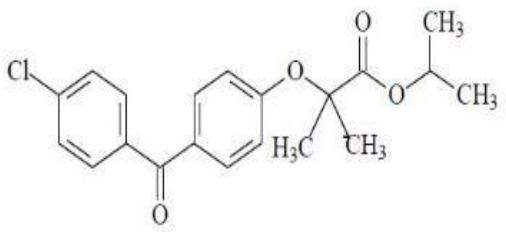

Figure 2: Structure of fenofibrate.

In this work, an attempt is made to provide simple and convenient validated analytical UV- spectrophotometric method for simultaneous estimation of fenofibrate and atorvastatin in pure and bulk tablet dosage form.

2. MATERIALS AND METHODS

2.1 Instruments

A double beam UV- visible spectrophotometer (model-Jasco -V630) is used with a spectral band width of 1.5 nm and automatic wavelength corrections. Pair of 10 mm matched quartz cell was used for experimental work.

2.2 Reagents and Chemicals

All chemicals and reagents used were of analytical reagent grade. Fenofibrate was obtained from Arti Drugs, Mumbai and atorvastatin was obtained fromCadila Healthcare, Ahmedabad, as a gift sample. Analytical HPLC grade methanol was procured from Rajesh Chemicals, Mumbai. The commercially available tablet formulation with brand name Atorec^{TM} - F (Emcure pharmaceuticals LTD) containing atorvastatin 10 mg and fenofibrate 160 mg was purchased from local market.

2.3 Procedure

2.3.1. Preparation of Standard Stock Solution

10 mg quantity of fenofibrate weighed accurately and transferred in in 100 ml volumetric flask. Sufficient quantity of methanol[8] is added to the flask to dissolve the drug and solution is sonicated for 15 min and then diluted up to 100 ml with same solvent, so as to obtain concentration of 100 µg/ml. Similarly, an accurately weighed quantity of about 10 mg of atorvastatin calcium was taken in 100 ml of volumetric flask and dissolve in sufficient quantity of methanol. Solution is sonicated for 15 min and diluted up to 100 ml with same solvent methanol so as to get the concentration of 100 µg/ml. These stock solutions are used for preparation of subsequent dilutions for calibration curve.

2.3.2. Selection of Wavelength / Determination of λmax

The solutions of fenofibrate and atorvastatin were scanned in UV range 400 to 200 nm in 10 mm quartz cell against blank solution separately. The overlain spectra (fig.4) shows λmax for fenofibrate at 287 nm and for atorvastatin λmax was found to be at 249 nm. By using this two λmax, estimation of drug were carried out by simultaneous equation method.

2.3.3. Construction of Standard Calibration Curve

For each drug, six working standard solutions are prepared by pipette out the aliquots from standard stock solutions of fenofibrate and atorvastatin in 10 ml volumetric flask and diluted up to 10 ml by solvent methanol to get working solutions of concentration 5 to 30 µg/ml. These series of different concentrations of fenofibrate and atorvastatin were scanned at 287 nm and 249 nm respectively and absorbance were recorded. The standard calibration curve was constructed by plotting concentrations versus absorbance over the range of 5 to 30 µg/ml. The correlation coefficient was found to be as 0.998 and 0.997 for obtained calibration curve of fenofibrate and atorvastatin respectively.

2.3.4. Preparation of Mixed Standard Solution

Accurately weighed 160 mg of fenofibrate and 160 mg of atorvastatin were weighed accurately into 100 ml clean and dry volumetric flask, methanol was added to dissolve the drugs. The mixed standard solution was sonicated for 15 minutes and then final volume up to 100 ml mark is made with methanol. The prepared mixed standard solution were subjected to UV spectrophotometric analysis upon suitable solution.

2.3.5. Preparation of Stock Solution of Marketed Formulation

Twenty tablets of Atorec-F containing 160 mg of fenofibrate and 10 mg of atorvastatin calcium were

weighed and finely powdered. Average weight per tablet is calculated. A quantity of tablet powder equivalent to 16 mg fenofibrate was weighed accurately and transferred to a 100 ml of volumetric flask. Powder is dissolved in methanol with vigorous shaking and solution is sonicated for 20 minutes. This solution contain 16 mg fenofibrate and 1 mg atorvastatin. Now add 15 mg of atorvastatin to the above solution and the final volume up to 100 ml mark was made with same solvent. Now, this stock solution contain 16 mg of fenofibrate and 16 mg of atorvastatin. The prepared test stock solution was filtered through the whatman filter paper. From this stock solution further dilutions were made. The resulting solution was analyzedat λmax of both the drugs against solvent blank.[21-32]

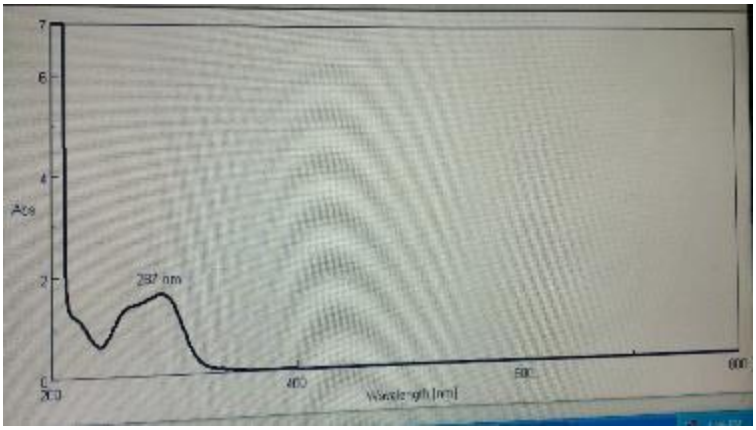

Figure 3: UV spectrum of Fenofibrate.

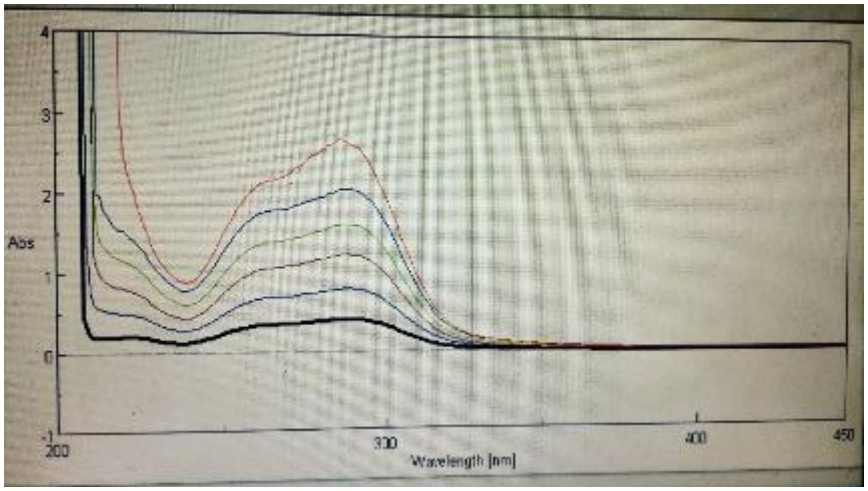

Figure 4: Overlay spectrum of fenofibrate (5-30 µg/ml).

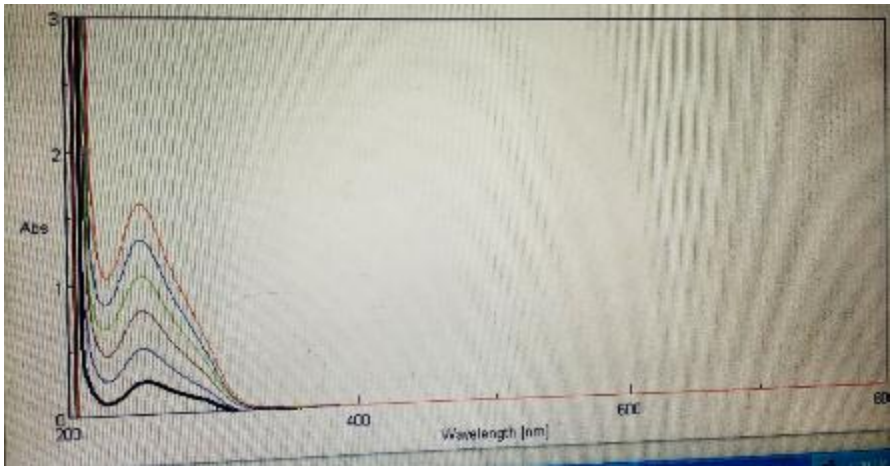

Figure 5: Overlay spectra of atorvastatin (5-30 µg/ml).

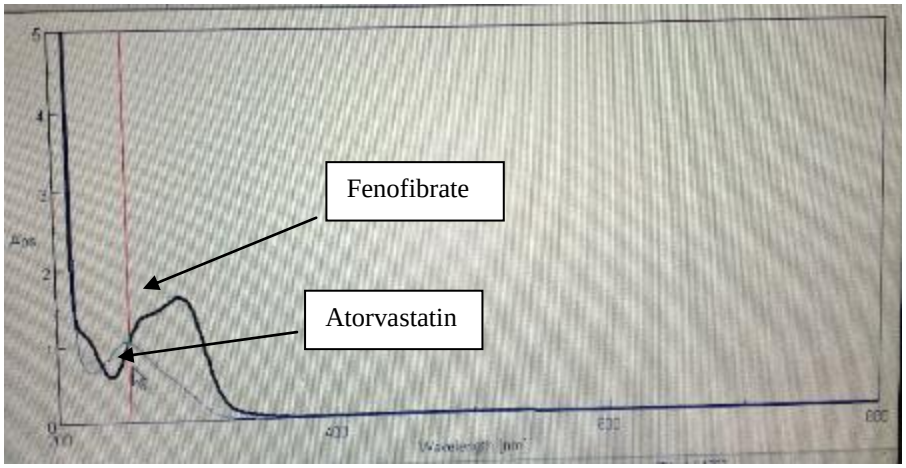

Figure 6: Overlay spectra of fenofibrate and atorvastatin.

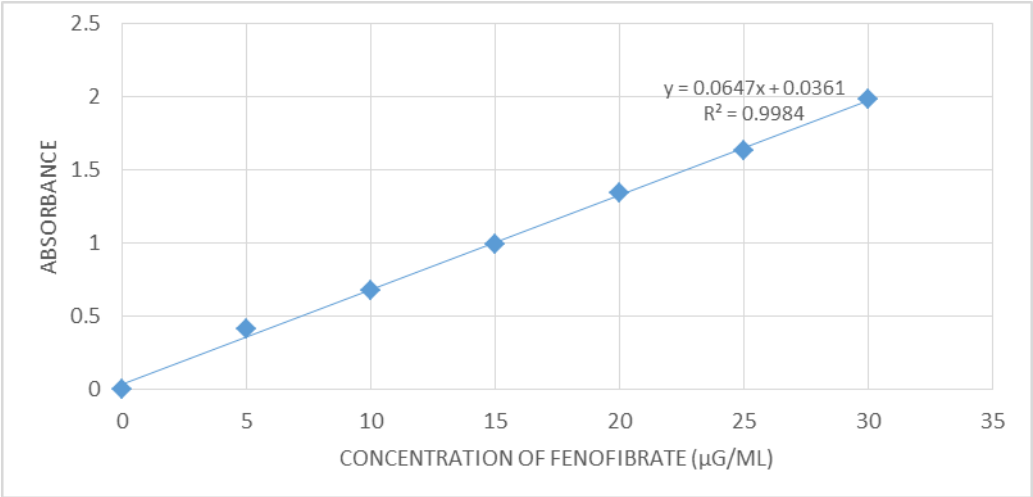

Figure 7: Standard calibration curve for fenofibrate.

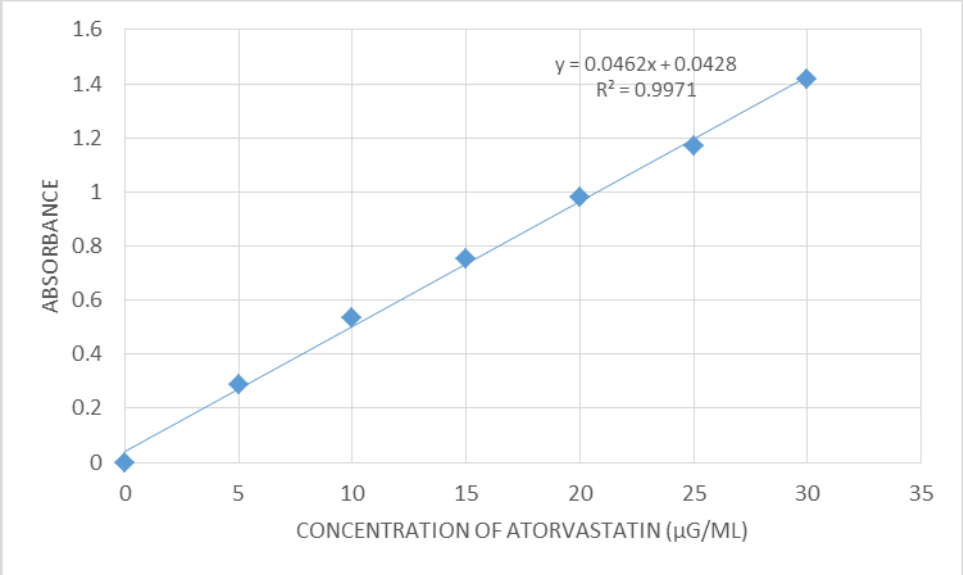

Figure 8: Standard calibration curve for atorvastatin.

2.4. Simultaneous Equation Method[11,12]

The simultaneous equation technique is based on the absorption of the fenofibrate and atorvastatin calcium at their respective λmax, 287nm and 249nm were two wavelengths which was selected for the development of the simultaneous equations. The drug content in tablet formulation was calculated by following equations using the mean absorptivity.

$$Cx = \left| \frac{A2.ay1 - A1.ay2}{ax2.ay1 - ax1.ay2} \right|$$

$$Cy = \left| \frac{A2.ax1 - A1.ax2}{ax1.ay2 - ax2.ay1} \right|$$

Where, A1 and A2 are absorbance values of test solutions at 287 nm and 249 nm respectively. Cx and Cy are concentrations of fenofibrate and atorvastatin respectively in µg/mlin test solution. ax1 and ax2 are the mean absorptivity coefficient of atorvastatin calcium at 287 nm and 249 nm respectively; whereas, ay1 and ay2 are the mean absorptivity coefficient of atorvastatin calcium at 287 nm and 249 nm respectively. By substituting the above values in equation, Cx and Cy can be calculated.[33-42]

2.5. Method Validation

2.5.1.Linearity

Appropriate dilutions of prepared stock solutions of fenofibrate and atorvastatin were analyzed at their respective wavelength maxims. During the analysis, Beers limit was obeyed by both fenofibrate and atorvastatin for simultaneous equation method and absorbance ratio method. The Beer-Lambert's concentration range is 0 to 30 ug/mL for both the drug. Data of linearity for both the drugs is given in table.

2.5.2. Precision (Repeatability)

Inter-day precision and intra-day precision was evaluated by using marketed tablet powder equivalent to 100 % of label claim amount of fenofibrate and atorvastatin. It is expressed in terms of SD and % RSD. SD and % RSD was obtained by repeating assay of four replicates of single sample concentration three times on the same day and on different days.

2.5.2. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ for fenofibrate and atorvastatin calcium was calculated on the basis of standard deviation of slope and y- intercept. It calculated as follows.

$$LOD = \frac{3.3 \times \sigma}{S}$$

$$LOQ = \frac{10 \times \sigma}{S}$$

Where, σ – The standard deviation of response
S- The slope of calibration curve

2.5.4. Accuracy

Accuracy of the methods is determined by recovery studies. It is performed by standard addition method. Known amount of standard fenofibrate and atorvastatin was added in the preanalyzed tablet powder, in 80%, 100% and 120% of label claim and concentration was determined.

3. RESULT AND DISCUSSION

The Beer-Lambert's concentration range is 5- 30 µg/mL for fenofibrate and atorvastatin calcium at 287 nm and 249 nm respectively. The coefficient of correlation for Fenofibrate at 287 nm and atorvastatin calcium at 249 nm is 0.9984 and 0.9971, respectively. Both Fenofibrate and atorvastatin shows good regression values at their respective wavelength (λmax). From recovery study it is revealed that any small change in the concentration of Fenofibrate or atorvastatin in the solution can be detected accurately by this method. Percentage estimation of fenofibrate and atorvastatin calcium in selected tablet dosage form was found to be 98.85% and 97.20% respectively, with standard deviation less than 2.

The reliability and validity of the proposed method is assessed by recovery studies. Result of recovery study was found to be within the prescribed limit and results of recovery study are shown in table. No interference in result observed in the result due to tablet excipients.

The limit of detection (LOD) and limit of quantitation (LOQ) values for fenofibrateare 0.3017 and 0.9144 and for atorvastatin calcium are 0.2912 and 0.9009, respectively. Low value of LOD and LOQ indicate that the method has good sensitivity.

Precision of method was determined by repeatability and intermediate precision study by intra-day and inter-day precision. The % RSD was calculated for fenofibrate and atorvastatin calcium. % RSD value not more than 2.0% indicate good repeatability and intermediate precision. An intermediate precision is a study of variation within laboratory in different days.

Table 1: Optical Characteristics.

Parameters	Name of componant	
	Fenofibrate	**Atorvastatin**
Wavelength λmax (nm)	287 nm	247 nm
Beer's law limit (µg/ml)	5-30 µg/ml	5-30 µg/ml
Correlation coefficient R^2	0.9984	0.9971
Slope	0.0647	0.0462
Intercept	0.0361	0.0427
Intra- day (% RSD)	0.6802	0.5351
Inter-day (% RSD)	0.9835	0.9912
LOD (µg/ml)	0.3017	0.2912
LOQ (µg/ml)	0.9144	0.9009

Table 2: Results of recovery study.

Drug	Level of Recovery (%)	Recovery (%)	SD	% R.S.D.
Fenofibrate	80	97.23	0.0585	0.0602
	100	98.71	0.0450	0.0456
	120	98.97	0.1107	0.1112
Atorvastatin	80	98.53	0.1721	0.1749
	100	98.67	0.1652	0.1675
	120	99.03	0.2722	0.2743

*SD= Standard Deviation, RSD= Relative Standard Deviation

Table 3: Results of analysis of tablet.

Formulation	Label claim (mg/ Tab)	Amount found (mg)	% of drug content	SD	% RSD	SE
Synthetic Mixture	Fenofibrate (160 mg)	159.07	99.41	0.1414	0.1405	0.0816
	Atorvastatin (160 mg)	157.96	98.72	0.3151	0.3191	0.1819
Tablet	Fenofibrate (160 mg)	158.16	98.85 %	0.3253	0.3291	0.1878
	Atorvastatin (10 mg)	9.72	97.20 %	0.9451	0.9690	0.5456

*SD= Standard Deviation, RSD= Relative Standard Deviation, SE= Standard Error

4. CONCLUSION

The proposed UV spectrophotometric methods are simple, useful, rapid reliable and provide acceptable accuracy, linearity, Precision and reproducibility. Methods can be adopted for routine simultaneous analysis of fenofibrate and atorvastatin calcium in pure form and pharmaceutical tablet dosage form.

CONFLICT OF INTEREST

There is no conflict of interest in this review.

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REFERENCES

1. Virani P, Sojitra R, et.al. Atorvastatin: A Review on Analytical Methods and its Determination in Pharmaceuticals and Biological Matrix. Inventi Rapid: Pharma Analysis & Quality Assurance, 2014; 4: 1-11.
2. Ashour S. New Kinetic Spectrophotometric Method for determination of Atorvastatin in Pure and Pharmaceutical Dosage Forms. Pharm Anal Acta, 2013; 4(5): 1-7.
3. Indian Pharmacopoeia. Monograph of atorvastatin calcium, 2007; 2: 225-226.
4. Chawla PA, Pandey S, et.al. Various Analytical Methods for Analysis of Atorvastatin: A Review. J Drug Deliv Ther, 2019; 9(3-s): 885-899.
5. Indian Pharmacopoeia, The Indian Pharmacopoeia Commission, 2007; 3: 1676-1678.
6. Kondawar MS, Kamble KG, et.al. UV spectrophotometric estimation of Ezetimibe and Fenofibrate in Bulk Drug and Dosage Form Using simultaneous equation Method. Int J ChemTech Res, 2011; 3(2): 749-754.
7. Thriveni J, Rambabu R et.al. Development and Validation of RP- HPLC Method for Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Bulk and Pharmaceutical Dosage Forms. Int J Res Pharm Chem, 2013; 3(2): 208-212.
8. Karunakaran A, Subhash V, et.al. Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Bulk and in Tablet Dosage Form by Uv-Spectrophotometry and RP- HPLC. S J Pharm Sci, 2011; 4(1): 58-63.
9. Sevda RR, Ravetkar AS, et.al. UV Spectrophotometric Estimation of Rosuvastatin Calcium and Fenofibrate in Bulk and in Tablet Dosage Form Using Simultaneous Equation Method. Int J ChemTech Res, 2011; 3(2): 629-635.
10. Patel B, Jadav A, et.al. Development and Validation of Derivative spectroscopic Method for the Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Tablet. Int J Pharm Res Rev, 2013; 2(7): 1-6.
11. Sawant RL, Joshi RS. Handbook of Experimental Ultraviolet Absorption Spectroscopy. 1st edition, Career publications, 23-26.
12. Sawant RL, Hadawale SD, et.al. Spectrophotometric Method for Simulataneous Estimation of Rabeprazole Sodium and Aceclofenac from the

Combined Capsule Dosage Form. Pharm Methods, 2011; 2(3): 193-197.
13. Rath SK, Samantaray SV, et.al. Development and Validation of New Analytical Method for the Estimation of Atorvastatin Calcium Hydrate Residue by Using UV Spectrophotometer. Int J PharmSci Res, 2013; 4(9): 3416-3425.
14. Bhinge SD, Malipatil SM, et.al. A New Approach to the RP-HPLC Method for Simultaneous Estimation of Atorvastatin Calcium and Fenofibrate in Pharmaceutical Dosage Forms. E-J Chem, 2012; 9(3): 1223-1229.
15. Ilango K, Shiji PS Kumar. Validated Spectrophotometric Methods for the Simuoltaneous Determination of Telmisartan and Atorvastatin in Bulk and Tablets. Pharm Methods, 2012; 3(2): 112-116.
16. Sharma JRK, Chippa RC, et.al. Quantitative Estimation of Fenofibrate in Bulk Drug and Tablet by UV Visible Spectroscopy. J Drug Deliv Ther, 2012; (3): 129-131.
17. Jain N, Jain D, et.al. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Atorvastatin Calcium and Fenofibrate in Tablet Dosage Form. Indian J Pharm Sci, 2008; 70(2): 263-265.
18. Pawar PY, Bhagat AR, et.al. Simultaneous Estimation of Atorvastatin Calcium and Aspirin in Pure and Capsule Dosage Form by Using U.V. Spectrophotometric Method. Der Pharma Chemica, 2013; 5(3): 98-103.
19. Gupta KR, Askarkar SS, et.al. Validated Spectrophotometric determination of Fenofibrate in Formulation. Der Pharmacia Sinica, 2010; 1(1): 173-178.
20. Zaheer Z, Farooqui MN, Mangle AA, Nikalje AG. Stability- Indicating High Performance Liquid Chromatographic Determination of Atorvastatin Calcium in Pharmaceutical Dosage Form. Afr J Pharm Pharmacol, 2008; 2(10): 204-210.
21. Shaikh S., Merekar AN, Godge GR and Gaikwad MR. Formulation And In-Vitro Evaluation Of Buccal Mucoadhesive Tablets Of Catopril By Using Natural And Synthetic Polymers, World Journal of Pharmaceutical Research, 2016; 5(7): 1296-1315.
22. Kute VC and Godge G. Preparation & In-Vitro Evaluation Of Inclusion Complexes Of Simvastatin Tablet With Cyclodextrins. World Journal of Pharmaceutical Research, 2016; 5(2): 1022-1041.
23. Godge GR. et.al. Formulation development and in-vitro evaluation of sustained release tablets of telmisartan by solid dispersion technology. Asian Journal of Pharmaceutical Technology & Innovation, 2016; 04(17): 131-139.
24. Chemate SZ., Godge GR., Pawa KK. and Rupnar KA. Preparation and evaluation of hollow calcium pectinate beads for floating-pulsatile drug delivery. Turk J Pharm Sci, 2016; 13(1): 91-102.
25. Raskar MA, Godge GR, Chitale AB and Giri PD. Validated simultaneous spectrophotometric estimation of telmisartan, hydrochlorthiazide and amlodipine besylate in combined tablet dosage form. Der Pharmacia Lettre, 2012; 7(11): 120-124.
26. Godge GR Misal AV and Pawar PY. Formulation and Evaluation Of Mouth Dissolving Tablet With Taste Masking Resin. International Journal of Life Sciences and Review, 2015; 1(7): 253-263.
27. Godge G, Labade S and Misal A. Oral Bioavailability Improvement Using Solid Dispersion Techniques: A Review. International Journal of Life Sciences and Review, 2015; 1(7): 243-252.
28. Hiremath S, Godge G, Sonawale B and Shirsath R. Pharmaceutical Advances In Cyclodextrin Inclusion Complexes For Improved Bioavailability Of Poorly-Soluble Drugs International Journal of Pharmaceutical Sciences and Nanotechnology, 2015; 8(3): 2894-2905.
29. Godge GR and Labade SP. Preparation of Solid Dispersion of Poorly Water Soluble Drug Formulation And Consideration. International Journal of Pharma Sciences and Research, 2015; 6(5): 897-903.
30. Godge GR, and Hiremath SN. An Investigation into the Characteristics of Natural Polysaccharide: Polymer Metoprolol Succinate Tablets for Colonic Drug Delivery. Mahidol University Journal of Pharmaceutical Sciences, 2014; 41(2): 7-21.
31. Godge GR and Hiremath SN. Colon Targeted Drug Delivery System: A Review. Int. J. Pharm.Drug Ana, 2013; 2(1): 35- 48.
32. Godge GR and Hiremath SN. Development and Evaluation of Colon Targeted Drug Delivery System by Using Natural Polysaccharides/Polymers. Dhaka Univ. J. Pharm. Sci, 2014; 13(1): 105-113.
33. Hiremath SN, Godge GR. Preparation and in vitro Evaluation of Inclusion Complexes of Nelfinavir with Chemically Modified β-cyclodextrins. Dhaka Univ. J. Pharm. Sci, 2012; 11(2): 107-116.
34. Sanap DS, Garje MA, Godge GR. Probiotics, their Health Benefits and Applications for Development of Human Health: A Review, Journal of Drug Delivery and Therapeutics, 2019; 9(4-s): 631-640.
35. Sanap DS, Godge GR. Recent Trends in Medicated Chewing Gum Technology: A Review on History, Formulation Strategies and Characterization. Int. J. Pharm. Sci. Rev. Res., 2019; 59(2): 72-85.