World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 7.409

SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF FENOFIBRATE AND ATORVASTATIN USING HYDROTROPY PHENOMENA

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Article Received on 21//04/2025 **Article Revised on 11/05/2025** **Article Accepted on 01/06/2025**

ABSTRACT

Aim: The aim of this study is to develop and validate a spectrophotometric method for the simultaneous estimation of fenofibrate and atorvastatin using the hydrotropy phenomena. The objective is to establish a reliable, cost-effective, and environmentally friendly analytical technique for the simultaneous quantification of these two pharmaceutical compounds in a single assay. **Objectives:** Investigate and identify a suitable hydrotropic agent that enhances the solubility of both fenofibrate and atorvastatin, facilitating their simultaneous analysis. Determine the optimal experimental conditions, including the concentration of the selected hydrotropic agent and the wavelength of maximum absorbance for both fenofibrate and atorvastatin. **Method Development:** Develop a spectrophotometric method for the simultaneous estimation of fenofibrate and atorvastatin based on the hydrotropy phenomena. Construct calibration curves for fenofibrate and atorvastatin to establish the relationship between concentration and absorbance under the optimized conditions and Validate the developed method according to International Conference on Harmonisation (ICH) guidelines. **Conclusion:** The developed spectrophotometric method for the simultaneous estimation of fenofibrate and atorvastatin using hydrotropy phenomena is a reliable and accurate analytical tool. The method is simple, cost-effective, and environmentally friendly, making it suitable for routine analysis in pharmaceutical laboratories. The use of hydrotropy not only enhances the solubility of the drugs but also simplifies the analytical procedure. The validation results demonstrate the reliability of the method for quantifying fenofibrate and atorvastatin over a specified concentration range. The method shows good linearity, precision, accuracy, and robustness, meeting the criteria set by regulatory guidelines. This suggests that the developed method can be applied for routine quality control analysis of pharmaceutical formulations containing fenofibrate and atorvastatin.

KEYWORDS: Fenofibrate, Atorvastatin, Hydrotropy, Spectrophotometric method.

1. INTRODUCTION

1.1 Hydrotropes

Since about 70% of recently discovered drug candidates have poor aqueous solubility, the primary issue facing the pharmaceutical industry at the moment is connected to tactics that increase drug solubility. One of the key characteristics to achieve desired pharmacological response is solubility. The bioavailability of a medicine determines its therapeutic effectiveness, which is ultimately determined by the drug's moiety's solubilityCarl A. Neuberg first used the term "hydrotropy" in 1916. The solubility of weakly and sparingly soluble medicines in water can be improved using hydrotropes. It is a chemical phenomenon that makes a medication that dissolves poorly in water more soluble by adding a second solute (hydrotrope). When one solute is present in excess, it makes another solute more soluble (Navale *et al.,* 2010; Mitra *et al.,* 2011).

Through "salt in" or "salt out" effects, hydrotropic agents are ionic organic salts with the ability to alter the solubility of a solute in a particular solvent. The term "hydrotropism" refers to the phenomena of salts that exhibit the "salt in effect" of non-electrolytes. Although they lack colloidal characteristics, they increase solubility by generating weak interactions with the molecules of the solution. A hydrotropic molecule interacts with a less water-soluble molecule through dipole-dipole or weak van der Waals interactions (Pandey *et al.,* 2022; Tripathi *et al.,* 2022).

1.1 Mechanism of hydrotropic solubalization

The enhancement of solubility by hydrotrope is based on the self-association of hydrotrope and the association of hydrotropes with solute. Various hypothetical and investigational efforts are being made to clarifying the mechanisms of hydrotrope.

The available proposed mechanisms can be abridged according to four designs

a) Hydrotropes self-associate to form aggregates. Another name for it is stacking. Additionally, it attracts the molecules of the solute in the aqueous phase. As a result, solubility increases as hydrotropic agent concentration does.

b) The interaction between the hydrotropes and the solute results in a complex with increased aqueous solubility.

c) Intermolecular hydrogen bonding modifies the solvent's structure. The solute's solubility is altered as a result of hydrogen bonding.

d) Hydrotropes build up surrounding the hydrophobic solute, acting as a bridge to promote solubility, but there is no contact. Reduced Gibbs energy results in higher solubility (Srinivas and Balasubramanian, 1998).

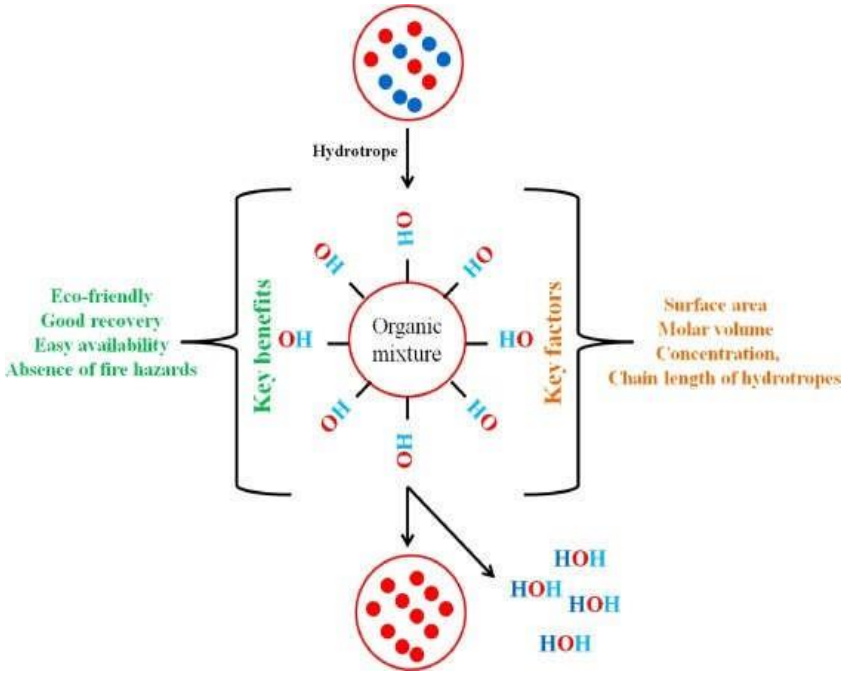

Figure 1.1: Hydrotropes.

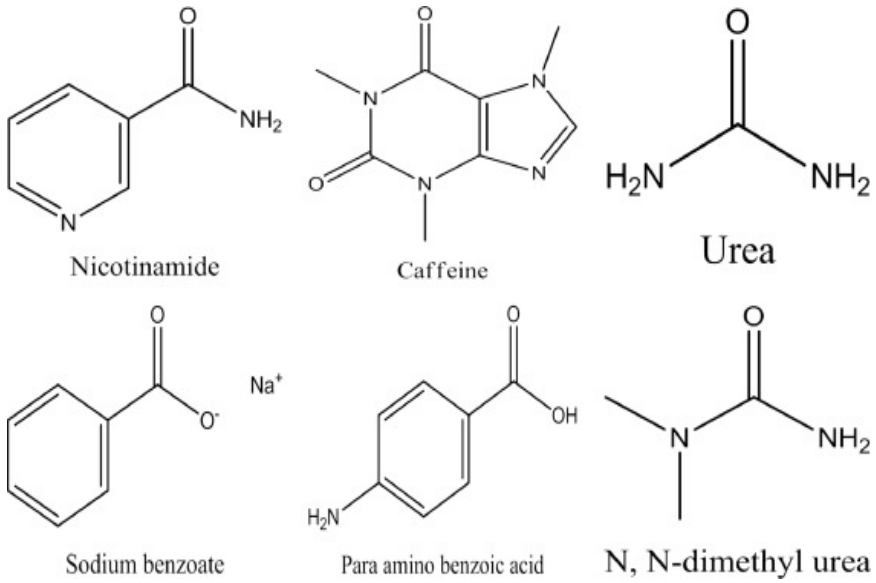

Figure 1.2: Some common hydrotropic agents.

1.2 Environmental aspects

Given that the octanol-water partition coefficient of hydrotropes is 1, they have a poor potential for bioaccumulation. According to research, hydrotopes

have very low vapour pressures (2.0x10-5 Pa), making them barely volatile. They can degrade aerobically. More than 94% of the activated sludge is removed during the secondary wastewater treatment process. Studies on fish's acute toxicity have revealed an LC50 > 400 mg active ingredient (a.i.)/L. The EC50 for Daphnia is >318 mg a.i./L. Green algae are the most vulnerable species, with EC50 values between 230 and 236 mg a.i./L and No Observed Effect Concentrations (NOEC) between 31 and 75 mg a.i./L. It was determined that the aquatic Predicted No Effect Concentration (PNEC) was 0.23 mg a.i./L. Since the ratio of Predicted Environmental Concentration (PEC) to Predicted Natural Environmental Concentration (PNEC) has been shown to be 1, hydrotropes have does not have any hazard to environment (Stanton *et al.,* 2010; Maheshwari *et al.,* 2009).

1.2.1 Necessity of method development

Drug evaluation exhibits the identity characterization and resolution of the drugs in combination like dosage forms and organic fluids. At some point of producing technique and development of drug the principal purpose of analytical strategies is to generate data regarding efficiency (which might be directly connected with the need of a identified dose), impurity (related to safety of the medication), bioavailability (consists of key drug traits like crystal kind, uniformity of drug and release of drug), stability(that shows the degradation product), and effect of manufacturing parameters to verify that the production of drug product is steady.

1.3 Analytical method

Analytical method includes use of a specified technique and detailed-stepwise instructions which are used in qualitative, quantitative or structural analysis of a sample for one or more analytes (Kissinger PT, 2002).

Analytical methods are mainly classified into two types: Classical methods and Instrumental methods. A method in which the signal is proportional to the absolute amount of analyte is called classical method. A method in which the signal is proportional to the analytes concentration is called instrumental method (Harvey, 2000).

Classical methods are divided into 3 main types are: a) Separation of analyte, b) Qualitative analysis and c) Quantitative analysis. Separation of analyte includes extraction, distillation, precipitation and filtration. Qualitative analysis includes boiling point, freezing point, colour, odour, density, reactivity and refractive index. Quantitative analysis includes gravimetric analysis and volumetric analysis.

2. DRUG PROFILE

2.1 Fenofibrate

Brand name: Antara, Cholib, Fenoglide, Fenomax, Lipidil Supra, Lipofen, Tricor, Triglide
Background: Fenofibrate is a fibric acid derivative like clofibrate and gemfibrozil. Fenofibrate is used to treat primary hypercholesterolemia, mixed dyslipidemia, severe hypertriglyceridemia.
Mol. weight: 360.83
IUPAC Name: propan-2-yl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoate
Chemical formula: $C_{20}H_{21}ClO_4$

Structure of drug

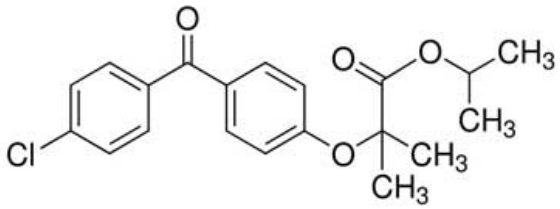

Figure 2.1: Structure of Fenofibrate.

2.2 Atorvastatin

Brand Names: Atorvaliq, Caduet, Lipitor, Lypqozet
Background: Atorvastatin (Lipitor®), is a lipid-lowering drug included in the statin class of medications. By inhibiting the endogenous production of cholesterol in the liver, statins lower abnormal cholesterol and lipid levels, and ultimately reduce the risk of cardiovascular disease. (https://go.drugbank.com/drugs/DB01076).
Mol. weight: 558.63
IUPAC Name: (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2- yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid
Chemical formula: $C_{33}H_{35}FN_2O_5$

Structure of drug

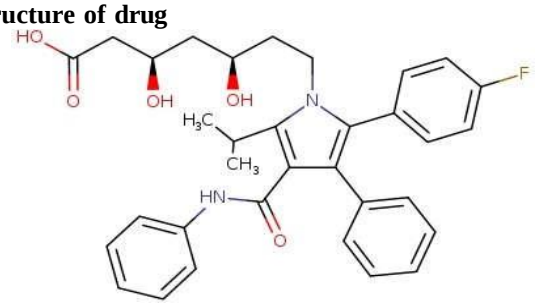

Figure 2.2: Structure of Atorvastatin.

3. Experimental Work and Results

3.1 Standards and Reagents

Standards and reagents play a crucial role in the experimental procedures conducted for the analysis of Fenofibrate and Atorvastatin. The reference standards for Fenofibrate (FND) and Atorvastatin (ATV) were generously provided by a pharmaceutical company, while various hydrotropic agents, such as Urea, Sod. Alginate, N, N dimethyl urea, and Sod. Benzoate, were procured from Merck Chemical Division, Mumbai. Additionally, commercial tablets of Fenofibrate and Atorvastatin were sourced from the local drug market for experimental use.

The reagents employed in the experiments were of high quality and sourced from Merck Specialties Pvt. Ltd.,

Mumbai. These included Methanol and Acetonitrile, both of HPLC grade, as well as Potassium Dihydrogen ortho Phosphate and Water.

The experiments utilized a range of apparatus and instrumentation to ensure precision and accuracy in the analyses. Various glassware, such as volumetric flasks, pipettes, measuring cylinders, and beakers, made of Borosilicate glass type I, were employed for volumetric measurements and sample preparations. Whatmann Filter Paper No.41 was used for filtration purposes.

In terms of instrumentation, the experiments employed the following devices:

3.2 Identification and characterization of drugs

3.2.1 Physical characterization of drugs

The drugs Fenofibrate and Atorvastatin were physically characterized on the beginning of appearance, color and odor. All these parameter were recorded and compared with the literature.

- **Melting point determination**

The melting point determined used for the strength of mind of melting point of Fenofibrate and Atorvastatin by the open capillary methods. The melting point of drug was recorded and compared with literature values. The Melting point of Fenofibrate and Atorvastatin was found to be 79-82°C and 155-187°C respectively.

3.2.3 FT-IR study carried out by KBr press pellet technique

The concentration of the sample in KBr should be in the range of 0.2% to 1%. The pellet is a lot thicker than a liquid film, consequently a decrease concentration in the sample is required (Beer's Law). For the die set that you'll be the usage of, about 80 mg of the mixture is wanted. Too excessive of an attention causes typically difficulties to obtain clean pellets. This pellet keeps into the sample cell and scanned between 4000-400 $c.m^{-1}$ and IR spectra are obtained.

3.3 Method: Simultaneous estimation of Fenofibrate and Atorvastatin using mixed hydrotropic solubilizing gents. Fenofibrate and Atorvastatin combination recently launched in the market for the treatment of diabetes in the strength of 2:30 mg. till date there is no method for the spectrophotometric estimation of Fenofibrate and Atorvastatin in combination by using hydrotropic agent. Following are the marketed formulation to be estimated by using hydrotrotropic phenomemon.

Commercial Formulation
Name of Tablet: Tonact Tg-20

Strength
- Fenofibrate: 20mg
- Atorvastatin: 160mg

3.4 Theme of work

Solubility

Solubility of Fenofibrate and Atorvastatin was determined at 25±1°C. Accurately weighed 10mg Fenofibrate and Atorvastatin was added in different 10 ml volumetric flask containing different solvent and placed at mechanical shaker for 8 hrs. After 8 hrs filter both solution were filtered through whatman filter paper No. 41. The filtrates were diluted suitably and analyzed visually.

Table 3.1: Solubility of drug in different solvents.

S. No.	Solvents	Solubility	
		FND	ATV
1	Water	-+	-+
2	Hot water	-+	-+
3	Cold water	-+	-+
4	2M Sodium acetate	+	+
5	8M Urea	+	+
6	2M Sodium Citrate	+	+
7	2M Sodium Benzoate	+	+
8	2M Ammonium Acetate	++	++
9	2M Sod. Citrate	++	++
10	2M Sodium acetate: 2M Sodium Benzoate (1:1)	+	+
11	2M Urea:2M Sodium acetate (1:1)	+	+
12	2M Sodium citrate:8M Urea (1:1)	+	+
13	2M Sodium citrate:8M Urea (1:1)	+	+
14	2M Ammonium Acetate: 2M Sod. Citrate (1:1)	+++	+++

- **Determination of Solubility Enhancement by UV VIS. Spectroscopy**

Solubility studies were performed in distilled water 2M Sodium acetate, 8M Urea, 2M Sodium Citrate, 2M Sodium Benzoate, 2M Ammonium Acetate, 2M Sod. Citrate, 2M Sodium acetate: 2M Sodium Benzoate, 2M Urea: 2M Sodium acetate, 2M Sodium citrate: 8M Urea, 2M Sodium citrate: 8M Urea, 2M Ammonium Acetate: 2M Sod. Citrate at room temperature (25 ± 2^0 C). An excess amount of drug was added to 100ml of

solvent in screw- capped glass vials; these were mechanically shaken for 48 hours at 25°C until equilibrium was achieved. Aliquots were withdrawn, filtered through a membrane filter (0.45□) and spectrophotometrically analyzed for solubility.

Table 3.2: Results of solubility enhancement by UV VIS. Spectroscopy.

S. No.	Solvents	Solubility Enhancement (folds)	
		FND	ATV
1	2M Sodium acetate	4	7
2	8M Urea	5	8
3	2M Sodium Citrate	6	8
4	2M Sodium Benzoate	4	6
5	2M Ammonium Acetate	7	8
6	2M Sod. Citrate	6	8
7	2M Sodium acetate: 2M Sodium Benzoate (1:1)	7	5
8	2M Urea:2M Sodium acetate (1:1)	6	4
9	2M Sodium citrate:8M Urea (1:1)	7	9
10	2M Sodium citrate:8M Urea (1:1)	8	7
11	2M Ammonium Acetate: 2M Sod. Citrate (1:1)	16	18

- **Selection of solvent system**

FND and ATV were scanned in various hydrotropic agent in the spectrum mode over the UV range (200-400) and 2M Ammonium Acetate: 2M Sod. Citrate (1:1) was found to be most appropriate because:

- Both drugs are soluble in it.
- Both drugs are stable in it.
- Both drugs exhibit good spectral characteristics in it.
- 2M Ammonium Acetate: 2M Sod. Citrate solutions have no interference with the λ_{max} of both drugs.
- More than 16 folds solubility enhancement forFND and more than 18 folds solubility enhancement for ATV.

- **Establishment of stability profile**

Stability of both drugs was observed by dissolving FND and ATV in 2M Ammonium Acetate: 2M Sod. Citrate (1:1) solution used as solvent. Solution of FND and ATV was prepared in the conc. of 2 □g/ml and 20 □g/ml respectively and scanned under time scan for 30 min. Spectra of both drugs under time scan shows that of both drugs are stable in mixed hydrotropic solution.

- **Linearity range and calibration graph**

Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80`mL mixed hydrotropic solution containing 2M Ammonium Acetate: 2M Sod. Citrate (1:1) and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to 100ml with mixed hydrotropic agent to get a concentration of 1000 µg/ml (Stock-A) for both drugs.

Preparation *of Sub Stock Solution (Stock-B)*

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of FND and ATV and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with RO Water that gave concentration of 100 µg/ml (Stock-B).

Preparation of Working Standard Solution

1) Aliquots of 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0ml withdrawn with help of pipette from standard stock solution (Stock-B) separately in 10 ml volumetric flask and volume was made up to 10 ml with RO Water. This gave the solutions of 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml respectively for FND.
2) 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with RO Water. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for ATV.

- **Selection of wavelength for linearity**

Solutions of 2□g/ml of FND and 20□g/ml ATV were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of FND and ATV was observed at 282.0 nm and 244.0 nm, respectively. FND and ATV showed linearity in the concentration range of 2-10 □g/ml and 10-50□g/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration.

To study the linearity of FND and ATV the selected wavelength are:

1. λ_{max} of FND	282.0 nm
2. λ_{max} of ATV	244.0 nm

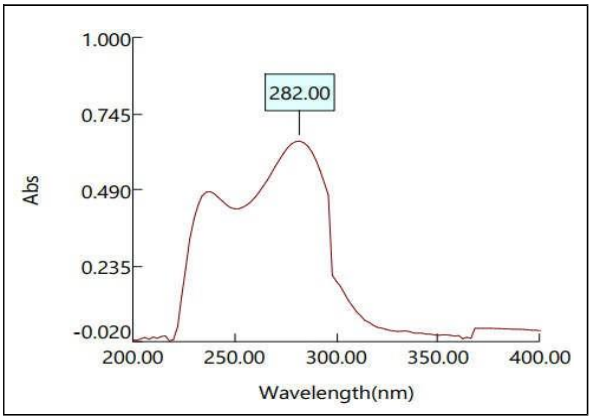

Figure 3.1: Determination of λ_{max} of FND.

Table 3.3: Linearity of FND At λ_{max} = 282.0 nm.

Standard Conc. (□g/ml)	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Mean	S.D.	% RSD
2	0.198	0.197	0.196	0.198	0.197	0.1972	0.001	0.424
4	0.385	0.384	0.385	0.386	0.384	0.3848	0.001	0.217
6	0.599	0.598	0.597	0.597	0.598	0.5978	0.001	0.140
8	0.795	0.794	0.796	0.797	0.798	0.796	0.002	0.199
10	0.995	0.994	0.996	0.997	0.997	0.9958	0.001	0.131

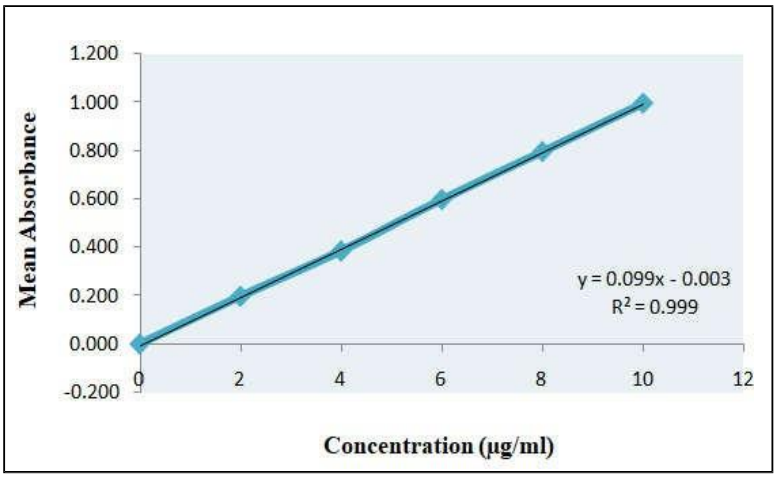

Figure 3.2: Calibration Curve of FND.

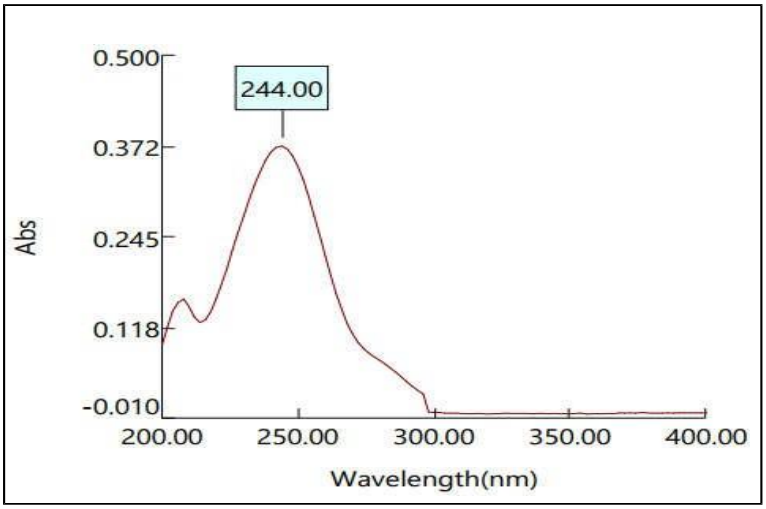

Figure 3.3: Linearity of ATV.

Table 3.4: Linearity of ATV At λ_{max} = 244.0 nm.

Standard Conc. (□g/ml)	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Mean	S.D.	% RSD
10	0.253	0.254	0.253	0.254	0.255	0.2538	0.001	0.330
20	0.512	0.513	0.512	0.513	0.512	0.511	0.001	0.107
30	0.759	0.758	0.757	0.759	0.758	0.7582	0.001	0.110
40	1.015	1.014	1.015	1.014	1.013	1.0142	0.001	0.082
50	1.229	1.228	1.227	1.228	1.227	1.2278	0.001	0.068

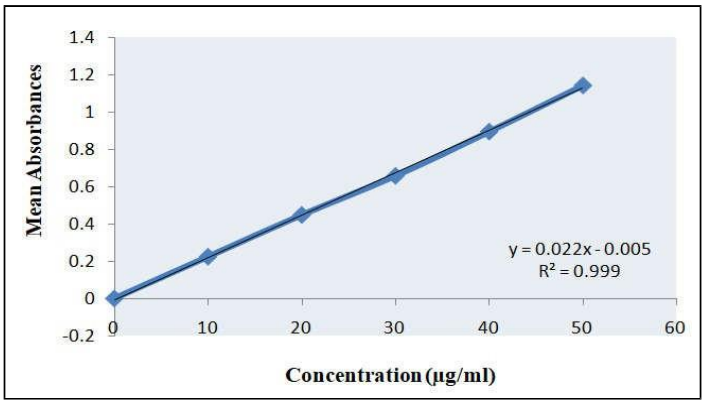

Figure 3.4: Calibration Curve of ATV.

3.5 Method (Simultaneous equation method)

3.5.1 Study of overlay spectra

Working standard solution from the standard stock solution prepared in concentration 2µg/ml of FND and 20µg/ml of ATV were scanned in the spectrum mode over the range of 200-400 nm against RO Water as blank and the overlain spectra of the two were recorded. FND showed an absorbance peak at 282.0 nm, whereas ATV at 244.0 nm. The overlain spectra also showed isoabsorptive points at 225.0 nm. Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method.

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 216.0 nm and 232.0 nm that are λ_{max} of FND and ATV respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%,\ 1cm}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

$$C\ GLP = \frac{A1ay2 - A2ay2}{ax1ay2 - ax2ay1} \quad \text{.....Eq. (1)}$$

$$C\ PGZ = \frac{A1ax2 - A2ax2}{ax1ay2 - ax2ay1} \quad \text{.....Eq. (2)}$$

Where, A_1 and A_2 are absorbances of mixture at 282.0 nm and 244.0 nm respectively, ax_1 and ax_2 are absorptivities of FND at λ_1 (282.0 i.e. λ_{max} of FND) and λ_2 (244.0 i.e. λ_{max} of ATV) respectively and ay_1 and ay_2 are absorptivities of ATV at λ_1 and λ_2 respectively. C_{ATV} and C_{FND} are concentrations of FND and ATV respectively. Both the drugs in 20:160 ratio and the criteria for obtaining maximum precision [i.e. absorbance ratio $(A_2/A_1)/ax_2/ax_1$ and ay_2/ay_1] by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the FND and ATV.

3.6 Validation of simultaneous equation method A_1: Linearity

Linearity of both drugs was established by response ratios of drugs. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio.

Table 3.5: Response Ratio of FND and ATV.

S. No.	FND			ATV		
	Conc. (µg/ml)	ABS	Response Ratio	Conc. (µg/ml)	ABS	Response Ratio
1	2	0.1972	0.079	10	0.2538	0.022
2	4	0.3848	0.077	20	0.511	0.022
3	6	0.5978	0.076	30	0.7582	0.022
4	8	0.796	0.076	40	1.0142	0.022
5	10	0.9958	0.075	50	1.2278	0.023

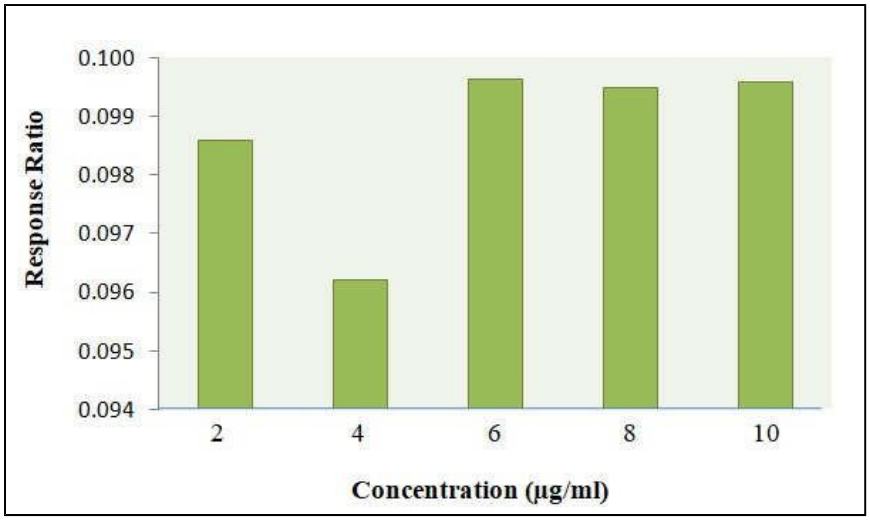

Figure 3.5: Graph of Response ratio graph for linearity for FND

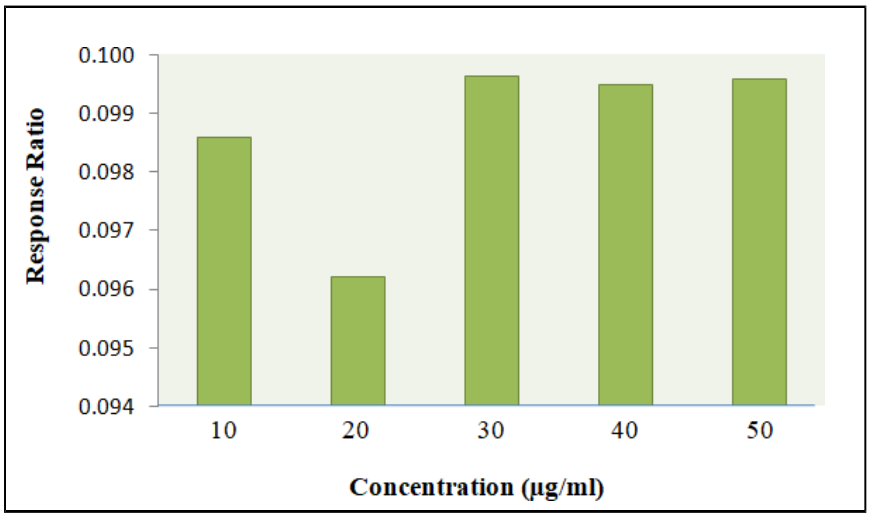

Figure 3.6: Graph of Response ratio graph for linearity for ATV B_1: Accuracy.

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of Fenofibrate and Atorvastatin to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels.

Table 3.6: Recovery study of FND (80% level).

FND tablet (mg)	Std.FND Added (mg)	Rep-1		Rep-2		Rep-3		FND % Mean
		FND Found	% Found	FND Found	% Found	FND Found	% Found	
2	**1.6**	1.58	98.75	1.59	99.38	1.55	96.88	98.33
4	**3.2**	3.19	99.69	3.18	99.38	3.16	98.75	99.27
6	**4.8**	4.79	99.79	4.75	98.96	4.69	97.71	98.82
8	**6.4**	6.38	99.69	6.35	99.22	6.38	99.69	99.53
10	**8**	7.89	98.63	7.95	99.38	7.89	98.63	98.88
							MEAN*	98.97
							SD*	0.459
							% RSD*	0.464

* Mean of 3 replicate and 5 concentrations

Table 3.7: Recovery study of FND (100% level).

FND tablet (mg)	Std.FND Added (mg)	Rep-1		Rep-2		Rep-3		FND % Mean
		FND Found	% Found	FND Found	% Found	FND Found	% Found	
2	**2**	1.98	99.00	1.85	92.50	1.89	94.50	95.33
4	**4**	3.65	91.25	3.92	98.00	3.85	96.25	95.17
6	**6**	5.98	99.67	5.78	96.33	5.69	94.83	96.94
8	**8**	7.92	99.00	7.82	97.75	7.84	98.00	98.25
10	**10**	9.98	99.80	9.96	99.60	9.88	98.80	99.40
							MEAN*	97.02
							SD*	1.835
							% RSD*	1.891

* Mean of 3 replicate and 5 concentrations

Table 3.8: Recovery study of FND (120% level).

FND tablet (mg)	Std.FND Added (mg)	Rep-1		Rep-2		Rep-3		FND % Mean
		FND Found	% Found	FND Found	% Found	FND Found	% Found	
2	**2.4**	2.38	99.17	2.39	99.58	2.33	97.08	98.61
4	**4.8**	4.75	98.96	4.75	98.96	4.78	99.58	99.17
6	**7.2**	7.19	99.86	7.19	99.86	7.16	99.44	99.72
8	**9.6**	9.58	99.79	9.58	99.79	9.58	99.79	99.79
10	**12**	11.69	97.42	11.85	98.75	11.95	99.58	98.58
							MEAN*	99.18
							SD*	0.580
							% RSD*	0.585

* Mean of 3 replicate and 5 concentrations

Table 3.9: Recovery study of ATV (80% level).

ATV Tablet (mg)	Std. ATV Added (mg)	Rep-1		Rep-2		Rep-3		ATV % Mean
		ATV Found	% Found	ATV Found	% Found	ATV Found	% Found	
10	**8**	7.95	99.38	7.82	97.75	7.75	96.88	98.00
20	**16**	15.69	98.06	15.68	98.00	15.69	98.06	98.04
30	**24**	23.85	99.38	23.96	99.83	23.85	99.38	99.53
40	**32**	31.74	99.19	31.58	98.69	31.74	99.19	99.02
50	**40**	39.65	99.13	39.56	98.90	39.95	99.88	99.30
							MEAN*	98.78
							SD*	0.714
							% RSD*	0.723

* Mean of 3 replicate and 5 concentrations

Table 3.10: Recovery study of ATV (100% level).

ATV Tablet (mg)	Std. ATV Added (mg)	Rep-1		Rep-2		Rep-3		ATV % Mean
		ATV Found	% Found	ATV Found	% Found	ATV Found	% Found	
10	**10**	9.98	99.80	9.95	99.50	9.74	97.40	98.90
20	**20**	19.85	99.25	19.74	98.70	19.78	98.90	98.95
30	**30**	29.96	99.87	29.65	98.83	29.74	99.13	99.28
40	**40**	39.45	98.63	39.74	99.35	39.65	99.13	99.03
50	**50**	49.85	99.70	49.87	99.74	49.85	99.70	99.71
							MEAN*	99.17
							SD*	0.334
							% RSD*	0.337

* Mean of 3 replicate and 5 concentrations

Table 3.11: Recovery study of ATV (120% level).

ATV Tablet (mg)	Std. ATV Added (mg)	Rep-1		Rep-2		Rep-3		ATV % Mean
		ATV Found	% Found	ATV Found	% Found	ATV Found	% Found	
10	**12**	11.85	98.75	11.78	98.17	11.85	98.75	98.56
20	**24**	23.65	98.54	23.85	99.38	23.74	98.92	98.94
30	**36**	35.85	99.58	35.96	99.89	35.44	98.44	99.31
40	**48**	47.85	99.69	47.45	98.85	47.82	99.63	99.39
50	**60**	59.89	99.82	59.12	98.53	59.65	99.42	99.26
							MEAN*	99.09
							SD*	0.343
							% RSD*	0.346

* Mean of 3 replicate and 5 concentrations

C_1: Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week. The results are shown in tables 6.12-6.13.

C_1-1: *Repeatability*

Table 3.12: Repeatability of FND.

Replicate	Concentration Found					
	2	**4**	**6**	**8**	**10**	
Replicate-1	1.98	3.95	5.85	7.83	9.85	
Replicate-2	1.85	3.85	5.89	7.98	9.74	
Replicate-3	1.92	3.65	5.65	7.83	9.65	
Replicate-4	1.94	3.96	5.74	7.63	9.83	
Replicate-5	1.86	3.74	5.96	7.85	9.95	
Mean	1.91	3.83	5.818	7.824	9.804	
% Mean	95.5	95.75	96.966667	97.8	98.04	**96.811**
S.D.	0.055	0.134	0.123	0.125	0.114	**0.110**
% R.S.D.	0.057	0.140	0.127	0.128	0.116	**0.114**

Table 3.13: Repeatability of ATV.

Replicate	Concentration Found					
	10	**20**	**30**	**40**	**50**	
Replicate-1	9.98	19.98	29.74	39.92	49.95	
Replicate-2	9.85	19.95	29.96	39.98	49.85	
Replicate-3	9.78	19.85	29.85	39.87	49.78	
Replicate-4	9.65	19.78	29.65	39.78	49.85	
Replicate-5	9.84	19.65	29.82	39.78	49.78	
Mean	9.82	19.84	29.80	39.86	49.84	
% Mean	98.2	99.21	99.34	99.66	99.68	**98.2**
S.D.	0.120	0.134	0.117	0.088	0.070	**0.120**
% R.S.D.	0.122	0.135	0.118	0.088	0.070	**0.122**

C_1-2: *Intermediate Precision*

C_1-2.1: Day-to-Day Variation

Table 3.14: Day-to-Day Variation of FND.

Replicate	Concentration Found					
	2	**4**	**6**	**8**	**10**	
Day – 1	1.93	3.85	5.78	8.01	9.98	
Day – 2	1.98	3.79	5.69	7.95	9.78	
Day – 3	1.96	3.93	5.85	7.96	9.92	
Mean	1.957	3.857	5.773	7.973	9.893	
% Mean	97.833	96.417	96.222	99.667	98.933	**97.814**
S.D.	0.025	0.070	0.080	0.032	0.103	**0.062**
% R.S.D.	0.026	0.073	0.083	0.032	0.104	**0.064**

Table 3.15: Day-to-Day Variation of ATV.

Replicate	Concentration Found					
	10	20	30	40	50	
Day – 1	9.98	19.95	29.78	39.98	49.85	
Day – 2	9.95	19.89	29.85	39.65	49.87	
Day – 3	9.96	19.87	29.69	39.78	49.69	
Mean	9.963	19.903	29.867	39.807	49.868	
% Mean	99.633	99.515	99.557	99.518	99.736	**99.592**
S.D.	0.015	0.015	0.015	0.015	0.015	**0.015**
% R.S.D.	0.150	0.075	0.050	0.038	0.030	**0.069**

C_1-2.2: Analyst to analyst variation

Table 3.16: Analyst-to-Analyst Variation of FND.

Replicate	Concentration Found					
	10	20	30	40	50	
Analyst -1	9.95	19.89	29.98	39.96	49.98	
Analyst -2	9.65	19.85	29.65	39.89	49.65	
Mean	9.8	19.87	29.815	39.925	49.815	
% Mean	98.00	99.35	99.38	99.81	99.63	**99.235**
S.D.	0.212	0.028	0.233	0.049	0.233	**0.151**
% R.S.D.	0.216	0.028	0.235	0.050	0.234	**0.153**

Table 3.17: Analyst-to-Analyst Variation of ATV.

Replicate	Concentration Found					
	10	20	30	40	50	
Analyst -1	1.99	3.95	5.98	7.95	9.95	
Analyst -2	2.01	3.92	5.83	7.96	9.87	
Mean	2.000	3.935	5.905	7.955	9.910	
% Mean	100.000	98.375	98.417	99.438	99.100	**99.066**
S.D.	0.014	0.021	0.106	0.007	0.057	**0.041**
% R.S.D.	0.014	0.022	0.108	0.007	0.057	**0.042**

C_1-3: *Reproducibility*

Table 3.18: Reproducibility of FND.

Replicate	Concentration Found					
	2	4	6	8	10	
Replicate-1	1.95	3.96	5.98	7.98	9.98	
Replicate-2	1.85	3.85	5.85	7.85	9.85	
Replicate-3	1.96	3.93	5.96	7.96	9.65	
Replicate-4	1.98	3.94	5.78	7.88	9.78	
Replicate-5	1.83	3.88	5.82	7.93	9.63	
Mean	1.914	3.912	5.878	7.92	9.778	
% Mean	95.7	97.8	97.96	99	97.78	**97.649**
S.D.	0.069	0.045	0.088	0.054	0.145	**0.080**
% R.S.D.	0.072	0.047	0.090	0.055	0.148	**0.082**

Table 3.19: Reproducibility of ATV.

Replicate	Concentration Found					
	10	20	30	40	50	
Replicate-1	9.96	19.85	29.98	39.68	49.98	
Replicate-2	9.98	19.78	29.98	39.96	49.89	
Replicate-3	9.87	19.69	29.65	39.78	49.78	
Replicate-4	9.87	19.85	29.89	39.65	49.69	
Replicate-5	9.89	19.99	29.78	39.98	49.85	
Mean	9.928	19.860	29.880	39.842	49.865	
% Mean	99.283	99.300	99.600	99.604	99.730	**99.504**
S.D.	0.052	0.110	0.142	0.154	0.110	**0.114**
% R.S.D.	0.053	0.111	0.142	0.155	0.110	**0.114**

3.6.1 Analysis of tablet sample

Twenty marketed tablets of Fenofibrate and Atorvastatin were weighed and ground to a fine powder; amount equal to 2mg of FND was taken in 10 ml volumetric flask. The ATV present in this amount of tablet powder was 16mg. Then 8 ml of 2M Ammonium Acetate: 2M Sod. Citrate (1:1) solution was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with RO Water to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from simultaneous equation method. The procedure was repeated for five times.

Table 3.20: Analysis of tablet formulation of Fenofibrate and Atorvastatin.

Drug	Label claim (mg)	Amount found (mg)	Label claim (%)	S.D.	% RSD
FND	20	19.85	99.25	0.145	0.225
ATV	160	158.65	99.16	0.215	0.284

4. DISCUSSION

Simultaneous estimation of Fenofibrate (FND) and Atorvastatin (ATV) using mixed hydrotropic solubilizing agents. The developed methods were found to be linear. The linearity study results for Glimepiride (FND) and Atorvastatin (ATV) using the designated analytical method are presented in Table 3.2. The working λmax values, representing the wavelengths at which maximum absorbance occurs, were found to be 282 nm for FND and 244 nm for ATV.

The Beer's law limits, which define the linear concentration range, were determined as 2-10 µg/ml for FND and 10-50 µg/ml for ATV. The high correlation coefficients (r2) of 0.999 for both FND and ATV indicate a robust linear relationship between concentration and response. Additionally, the calculated slopes (m) and intercepts (c) further confirm the method's sensitivity and accuracy.

The method demonstrates its suitability for the quantitative analysis of Glimepiride and Atorvastatin, providing essential information for their concentration determination in pharmaceutical formulations. The specified wavelengths contribute to precise measurements, enhancing the reliability of the method in pharmaceutical quality control and research applications.

Table 4.1: Results of Linearity of Fenofibrate and Atorvastatin.

	Method	
Parameter	**FND**	**ATV**
Working λ_{max}	282 nm	244 nm
Beer's law limit (µg/ml)	2-10	10-50
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	0.099	0.024
Intercept (c)*	-0.003	0.008

The recovery studies conducted on the marketed formulations of Glimepiride (FND) and Atorvastatin (ATV) are summarized in Table 4.2. At various recovery levels, namely 80%, 100%, and 120%, the mean percentages of recovery along with standard deviations were calculated. For FND, the recovery percentages were 98.97%±0.459 at 80%, 97.02%±1.835 at 100%, and 99.18%±0.580 at 120%. Similarly, for ATV, the recovery percentages were 98.78%±0.714 at 80%, 99.17%±0.334 at 100%, and 99.09%±0.343 at 120%.

These results indicate a high level of accuracy and precision in the recovery of FND and ATV from the marketed formulations. The close agreement between the observed and expected concentrations at different recovery levels demonstrates the reliability and effectiveness of the developed analytical method. The low standard deviations suggest good precision and reproducibility of the method, making it suitable for the quantification of FND and ATV in commercial formulations. These findings contribute to the robustness and reliability of the proposed analytical method for quality control purposes in the pharmaceutical industry.

Table 4.2: Results of Recovery Studies on Marketed Formulations.

Recovery Level %	% Recovery (Mean±SD)*	
	FND	**ATV**
80	98.97±0.459	98.78±0.714
100	97.02±1.835	99.17±0.334
120	99.18±0.580	99.09±0.343

*Average of three determination

Table 4.3 presents the results of the validation parameters, including precision, for the developed method for Glimepiride (FND) and Atorvastatin (ATV). The precision was evaluated through various aspects, namely repeatability, day-to-day precision, analyst-to-analyst variation, and reproducibility.

For repeatability, the mean precision for FND was found to be 96.811%±0.110, and for ATV, it was 98.2%±0.120. In the context of day-to-day precision, the mean values were 97.814%±0.062 for FND and 99.592%±0.015 for ATV. Analyst-to-analyst variation showed mean precision values of 99.235%±0.151 for FND and 99.066%±0.041 for ATV. Lastly, the reproducibility of the method resulted in mean precision values of 97.649%±0.080 for FND and 99.504%±0.114 for ATV.

The obtained results demonstrate the robustness and reliability of the developed analytical method for the quantification of FND and ATV. The low standard deviations across various precision parameters indicate consistent and accurate performance, making the method suitable for routine analysis and quality control in pharmaceutical applications.

Table 4.3: Results of validation (Mean±SD)*

Parameter		Method	
		FND	ATV
Precision*	Repeatability	96.811±0.110	98.2±0.120
	Day-to-Day	97.814±0.062	99.592±0.015
	Analyst-to-Analyst	99.235±0.151	99.066±0.041
	Reproducibility	97.649±0.080	99.504±0.114

*Average of five determination

The analysis of the tablet formulation of Glimepiride (FND) and Atorvastatin (ATV) is presented in Table 4.4, providing information on the label claim, the amount found, and the percentage of label claim, along with standard deviations and the percentage relative standard deviation (% RSD) for each parameter.

For Glimepiride, the label claim was 20 mg, and the amount found was 1.98 mg, resulting in a percentage label claim of 99.00%. The standard deviation (S.D.) was recorded as 0.225, with a % RSD of 0.235. These values indicate a close agreement between the labeled and found amounts of Glimepiride, suggesting the accuracy and precision of the analytical method in quantifying this component in the tablet formulation.

These results of percentage assay indicate a high level of accuracy and precision in determining the Atorvastatin content in the tablet formulation. The low standard deviations and % RSD values in both cases suggest that the developed method is reliable and reproducible for the quantitative analysis of FND and ATV in tablet formulations.

Table 4.4: Analysis of Tablet Formulation of FND and ATV.

Drug	Label claim (mg)	Amount found (mg)	Label claim (%)	S.D.	% RSD
FND	20	19.85	99.25	0.145	0.225
ATV	160	158.65	99.16	0.215	0.284

5. SUMMARY AND CONCLUSION

Summary

The development and validation of a spectrophotometric method for the simultaneous estimation of fenofibrate and atorvastatin using the hydrotropy phenomena have been successfully achieved. The method offers a cost-effective and efficient way to analyze these two important pharmaceutical compounds in a single assay.

The hydrotropy phenomena, which involve the use of a large amount of aqueous solution of hydrotropic agents, have been exploited to enhance the solubility of the poorly water- soluble drugs. In this study, a suitable hydrotropic agent was identified and utilized to improve the solubility of both fenofibrate and atorvastatin, enabling their simultaneous estimation in a single spectrophotometric analysis.

The spectrophotometric method was developed and optimized by studying the absorption spectra of fenofibrate and atorvastatin in the presence of the hydrotropic agent. The wavelength of maximum absorbance for both drugs was determined, and calibration curves were constructed. The method was validated according to International Conference on Harmonisation (ICH) guidelines for parameters such as linearity, precision, accuracy, robustness, and specificity.

CONCLUSION

The developed spectrophotometric method for the simultaneous estimation of fenofibrate and atorvastatin using hydrotropy phenomena is a reliable and accurate analytical tool. The method is simple, cost-effective, and environmentally friendly, making it suitable for routine analysis in pharmaceutical laboratories. The use of hydrotropy not only enhances the solubility of the drugs but also simplifies the analytical procedure.

The validation results demonstrate the reliability of the method for quantifying fenofibrate and atorvastatin over a specified concentration range. The method shows good linearity, precision, accuracy, and robustness, meeting the criteria set by regulatory guidelines. This suggests that the developed method can be applied for routine quality control analysis of pharmaceutical formulations containing fenofibrate and atorvastatin.

In conclusion, the spectrophotometric method developed for the simultaneous estimation of fenofibrate and atorvastatin using hydrotropy phenomena is a valuable addition to the arsenal of analytical techniques for pharmaceutical analysis, providing an efficient and sustainable solution for the quantification of these two important drugs in combination.

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