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QUANTITATIVE ESTIMATION OF ATORVASTATIN-GEMFIBROZIL DRUG COMBINATIONS USING VARIOUS UV SPECTROPHOTOMETRIC TECHNIQUE

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ABSTRACT

Aim: To simultaneously estimate Atorvastatin and Gemfibrozil using UV-Spectroscopic method. **Methodology:** Different simultaneous estimation techniques were used for estimation of Atorvastatin and Gemfibrozil using UV spectroscopy. The methods used were simultaneous equation method, area under curve (AUC) method, Q-analysis or absorbance ratio method. The developed method was validated by following ICH guidelines. **Results:** The absorption spectra was recorded in wavelength region of 200-400nm. Linearity of ATR and GEM was found 2-14µg/ml and 30-90µg/ml respectively. In this work the ATR and GEM were quantified by using three different quantitative methods of UV spectroscopy. The proposed methods confirm the suitability for the estimation of physical mixture of pure drugs as well as for pharmaceutical formulation in combination. The coefficient of correlation for ATR at 223 nm and GEM at 272 nm by all methods was found to be within the range of 0.9738-0.9995 and 0.9798-0.9995 respectively. Both drugs showed good regression values at their respective wavelengths in the all methods. The suggested method's accuracy was tested by estimating pure drug and pharmaceutical formulation, and the results were computed, with no interference from other typical excipients found in pharmaceutical formulations. The presented approach was found to be simple, sensitive, accurate, precise, and cost-effective, and it may be used to determine ATR and GEM individually or as a physical mixture for regular analysis.

KEYWORDS: UV Spectroscopy, Atorvastatin, Gemfibrozil, Simultaneous estimation.

INTRODUCTION

Hyperlipidemia is a important risk factor for cardiovascular disease. It refers to elevated levels of LDL cholesterol and triglycerides in the bloodstream. Atorvastatin is a synthetic lipid-reducing drug that obstructs 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and is the most effective HMG-CoA reductase inhibitor on the market in terms of lowering plasma cholesterol levels.[1]

Gemfibrozil has been shown to be helpful in lowering serum cholesterol, triglyceride, and LDL levels while simultaneously boosting serum HDL. The combination of an HMG-CoA reductase and Gemfibrozil improves function, and new clinical studies indicate the beneficial effects of this combination. Controlled experiments have revealed that this combination not only reduces atherosclerotic plaques, but also increases the risk of myopathy. Experts believe Gemfibrozil diminishes the risk of myopathy when compared to Fenofibrate.

When used with Gemfibrozil, the maximum permitted daily doses of statins are reduced to 10 mg. Gemfibrozil combined with an HMG-CoA leads in better long-term management of lipid idiosyncrasies in mixed lipid diseases than either medication alone. Due to the less incidence of toxicity, combination therapy can be used in patients at high risk of atherosclerotic problems.

A thorough study of the literature revealed that there is no way for assessing Atorvastatin and Gemfibrozil at the same time. In the current study, settings were improved to isolate and properly quantify both medicines at the same time.[1,2]

Multiple medication therapy or the number of pharmaceuticals prescribed to a patient is increasingly prevalent these days. Only a few medications can be mixed; others must be administered individually. When both medicines are present in plasma simultaneously, we must analyze the sample without separating them. As a

result, procedures for simultaneous estimation are increasingly being used for drug estimation in multi-component pharmaceutical formulations due to their intrinsic advantages, which include the avoidance of time-consuming extraction and separation procedures, the minimization of expensive reagents, and the fact that these methods are equally accurate and precise.

According to a literature review, UV techniques for Atorvastatin and Gemfibrozil, have been developed independently. However, there is no mechanism for simultaneously estimating Atorvastatin and Gemfibrozil in combination. As a result, there is a need to create newer, faster, more precise, and reproducible methods for simultaneously estimating Atorvastatin and Gemfibrozil in pharmaceutical dosage form.

MATERIALS AND METHODS

Both the drugs were estimated by UV-spectroscopy by three different methods.

Table No 01: Instrumental Specifications.

UV/Visible Spectrophotometer	SHIMADZU 1800
Software	UV Probe Version 2.43
Balance	Sartorius
pH meter	Elico

CHEMICALS AND REAGENTS

Methanol (AR Grade)-HIMEDIA
Water - Millipore water.

Table No 02: Working standards/ reference standards/ active pharmaceutical ingredients.

Working standard	Source	Potency
Atorvastatin Calcium (ATR)	Medelis Health care	99.8%
Gemfirozil (GMF)	Simson pharma	99.5%
Atorvastatin Calcium (Tablet)	Anax Pharma	10,20mg
Gemfirozil (Tablet)	Actiza Pharma	600mg

Selected spectrophotometric methods to analyse the atorvastatin-gemfibrozil combination:

SPECTROPHOTOMETRIC METHODS

Method A: SIMULTANEOUS EQUATION METHOD

Method B: AREA UNDER CURVE (AUC) METHOD

Method C: Q-ANALYSIS OR ABSORBANCE RATIO METHOD.

Experimental Procedures

a. Selection of Solvent for analysis: The selection of solvents for analysis was carried out by the effect of different solvents on the pure drug and tablet powder. In methanol and ethanol the drugs were soluble. Both drugs are soluble in methanol and stable on long storage.

b. Selection of analytical wavelengths: Standard stock solutions having concentration 10µg/ml of each drug was prepared separately and they were scanned in the wavelength range of 200-400nm and the maximum (λmax) absorbance of both the drugs were found to be 275nm for GEM and 223nm for ATR.

Standard stock solutions having concentration 10µg/ml of each drug was prepared separately and they were scanned in the wavelength range of 200-400nm and the maximum (λmax) absorbance of these two drugs were found to be λ 233nm and 272nm for AML and RAN respectively, 223.8 and 275nm for ATR and GEM respectively. The area under curve was measure at ±5 nm of both λmax. So area measured at λ 218.8-228.8nm and 270-280nm for ATR and GEM respectively. For Q-absorbance method the overlain spectrum was used to determine isoabsorptive point which was found to be 250.9 for ATR-GEM.

Preparation of Standard stock solution of ATR and GEM

Stock solution was prepared by dissolving 100mg of accurately weighed ATR and GEM in to 100ml volumetric flask, dissolved with 10ml of methanol and the final volume was adjusted to 100ml with methanol to give the stock solution 1000µg/ml concentration. From the resulting solution 1ml of ATR and 1ml of GEM were placed in 100ml volumetric flask and volume adjusted with methanol to give solution of 100µg/ml of ATR solution and 100µg/ml of GEM (stock B). From stock solution B 0.2-1.0ml of ATR and 3-9 ml of GEM were pipetted in to 10ml volumetric flasks and the volume was made up with methanol to get concentration of 2-14µg/ml of ATR and 30-90µg/ml of GEM. The absorbance of resulting solution was measured against 223nm and 275nm. For AUC resulting solution was measured at λ 218.8-228.8nm and 270-280nm. For Q-Analysis method absorbance was measured against 223nm, 275nm, and 250.9nm.

Assay of ATR and GEM in dosage form Tablet

Various aliquots were prepared and suitably diluted with methanol to give final concentration of 2, 4, 6, 8, 10, 12, 14µg/ml for ATR and 30, 40, 50, 60, 70, 80, 90µg/ml for GEM in different volumetric flasks of 10 ml capacity. The absorbance of prepared aliquots mixture of ATR and

GEM was measured against 223nm and 275nm. By substituting the values of A1 and A2 the values of Cx and Cy can be calculated by solving the two equations simultaneously.

RESULT AND DISCUSSION

Method Development

Table No 3: Calibration data of ATR (2-16 µg/ml) and GEM (30-90 µg/ml).

SL.NO	ATR		GEM	
	Conc.	Absorbance	Conc.	Absorbance
01.	2	0.08	30	0.24
02.	4	0.14	40	0.32
03.	6	0.21	50	0.40
04.	8	0.26	60	0.48
05.	10	0.34	70	0.55
06.	12	0.43	80	0.63
07.	14	0.48	90	0.70

Table No 4: Absorbance of ATR at 223.8nm and 275nm for simultaneous estimation method.

SL.NO	Concentration of ATR (µg/ml)	Absorbance		E1%1cm	
		223.8	275	233.8	275
01.	2	0.08	0.010	350	50.0
02.	4	0.14	0.020	370	50.0
03.	6	0.21	0.032	333	56.0
04.	8	0.29	0.042	325	52.0
05.	10	0.35	0.051	340	51.0
06.	12	0.42	0.056	325	50.0
07.	14	0.49	0.062	350	52.0
			Average	341.4	51.5

Here ax1= 341.4, ax2= 51.5.

Table No 5: Absorbance of GEM at 223.8nm and 275nm for simultaneous estimation method.

SL.NO	Concentration of GEM (µg/ml)	Absorbance		E1%1cm	
		223.8	275	223.8	275
01.	30	0.812	0.240	270	80.0
02.	40	1.086	0.320	270	80.0
03.	50	1.354	0.400	270	80.0
04.	60	1.567	0.480	260	80.0
05.	70	1.747	0.550	240	81.0
06.	80	1.901	0.630	237	78.0
07.	90	2.120	0.698	230	80.0
			Average	257	79.3

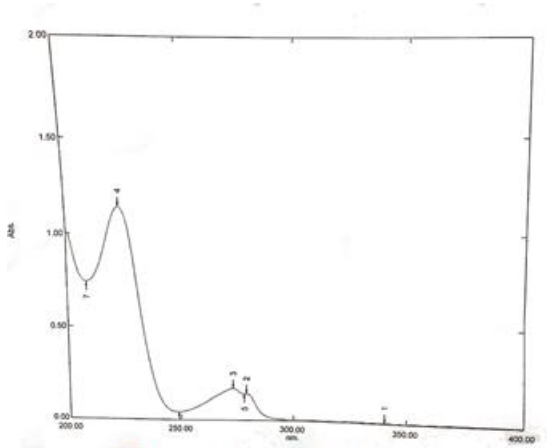
Here ay1= 79.3, ay2= 257.

Table No 6: Absorbance of mix (ATR and GEM) by simultaneous estimation method.

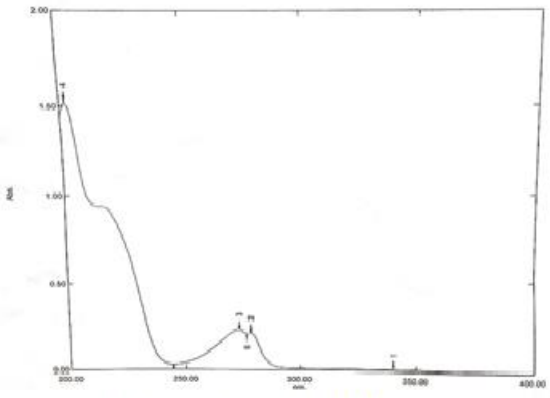
SL.NO	Concentration of ATR and GEM (mix in µg/ml)		ABSORBANCE		Concentration obtained		% ERROR	
	AML	RAN	223.8nm	275nm	AML	RAN	AML	RAN
01.	2	30	0.427	0.092	2.00	31.2	0.000	-4.00
02.	4	40	0.590	0.130	3.80	40.0	5.000	0.00
03.	6	50	0.763	0.166	5.90	50.0	1.660	0.00
04.	8	60	0.937	0.207	8.00	59.8	0.000	0.33
05.	10	70	0.980	0.315	9.80	70.0	2.000	0.00
06.	12	80	1.151	0.399	11.9	79.0	0.833	1.25
07.	14	90	1.218	0.412	14.0	90.0	0.000	0.00

Table No 7: Absorbance of assay mixtures in tablet dosage form.

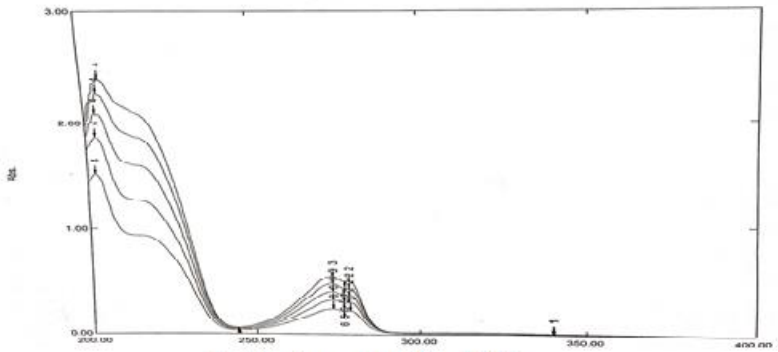
SL.NO	ATR (µg/ml)		GEM (µg/ml)		Absorbance		% Error	
	Conc. Tkn.	Conc. Obt	Conc. Tkn.	Conc. Obt.	223.8nm (A1)	275nm (A2)	ATR	GEM
01.	2.0	1.80	30	29.6	0.418	0.102	10	1.33
02.	4.0	4.00	40	39.0	0.541	0.126	0.000	2.50
03.	6.0	5.60	50	49.7	0.620	0.150	6.66	0.60
04.	8.0	8.00	60	59.0	0.850	0.180	0.000	1.60
05.	10	9.80	70	69.5	0.900	0.215	0.00	0.71
06.	12	12.0	80	79.0	0.926	0.285	2.000	1.25
07.	14	13.9	90	89.8	0.970	0.310	0.714	-0.22



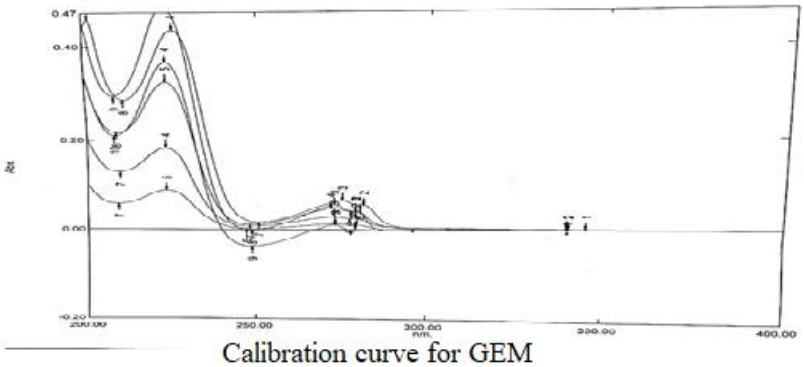
UV spectrum for ATR



UV spectrum for GEM

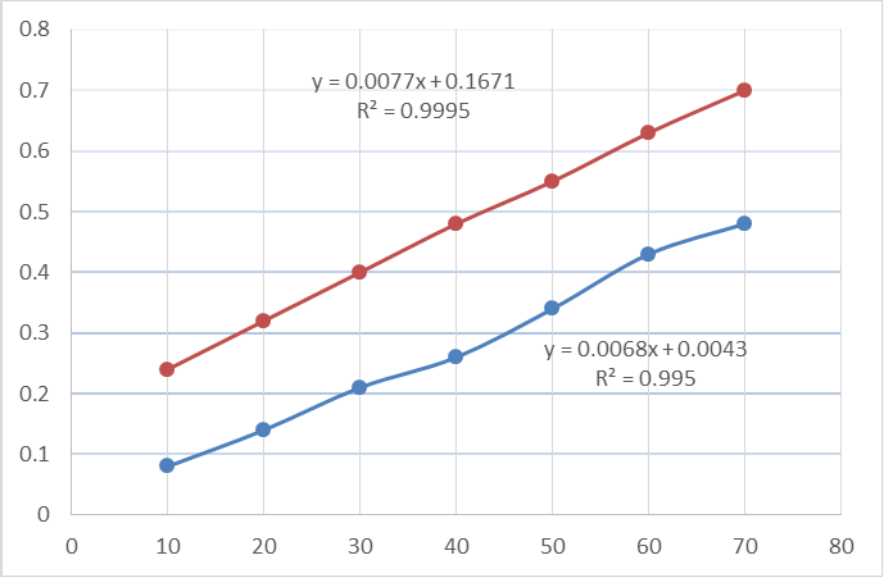


Calibration curve for ATR



Calibration curve for GEM

Fig. No. 1: Uv spectrum and calibration curve for ATR and GEM.



Graph 1: Calibration graph for ATR (2-14 µg/ml) and GEM (30-90 µg/ml).

Table No. 8: %Recovery study data for ATR and GEM by simultaneous estimation method.

Level	ATR		GEM		Total conc. taken (µg/ml)		Abs at λmax		Amt. of std. recovered (µg/ml		% Recovery	
	Std. soln	Sample mix soln	Std. soln	Sample mix soln	ATR	GEM	ATR	GEM	ATR	GEM	ATR	GEM
80%	10	4	10	40	14	50	0.49	0.43	4.2	502	101.40	100.50
80%	10	4	10	40	14	50	0.48	0.44	4.1	50.3	102.50	100.75
80%	10	4	10	40	14	50	0.49	0.43	4.2	50.3	101.40	100.33
100%	10	6	10	60	16	70	0.60	0.49	6.3	60.2	100.30	100.33
100%	10	6	10	60	16	70	0.61	0.50	6.4	60.1	100.10	100.16
100%	10	6	10	60	16	70	0.60	0.50	6.2	60.1	100.10	100.16
120%	10	8	10	80	18	90	0.68	0.69	8.2	80.3	100.40	100.37
120%	10	8	10	80	18	90	0.68	0.68	8.3	80.2	100.37	100.25
120%	10	8	10	80	18	90	0.68	0.69	8.2	80.3	100.25	100.37

Table No. 9: Calibration data of AUC of ATR (2-14µg/ml) and GEM (30-90µg/ml).

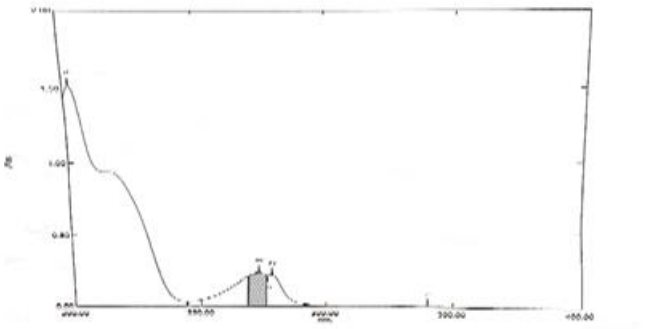
Sl.No	ATR		GEM	
	Conc (µg/ml)	AUC at 218-228nm	Conc (µg/ml)	AUC at 267-277nm
01.	2	0.056	30	0.213
02.	4	0.130	40	0.290
03.	6	0.170	50	0.360
04.	8	0.225	60	0.429
05.	10	0.291	70	0.494
06.	12	0.335	80	0.560
07.	14	0.383	90	0.591

Table No 10: Area under Curve of mix (ATR and GEM) by AUC method.

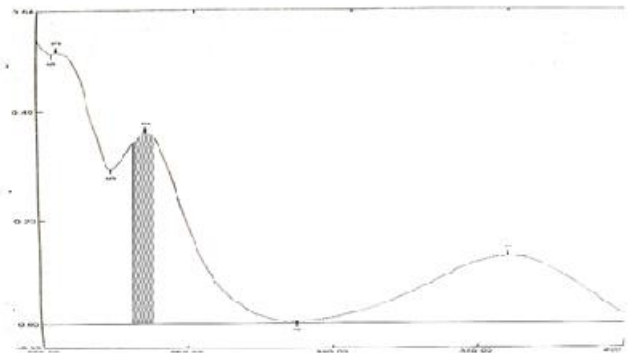
Sl. No	Concentration of ATR and GEM (mix in µg/ml)		AUC		Concentration obtained		% ERROR	
	ATR	GEM	218-228	270-280	AML	RAN	AML	RAN
01.	2	30	0.0860	0.352	1.9	29.0	5.0	3.33
02.	4	40	0.120	0.549	4.1	40.0	-2.5	0.00
03.	6	50	0.155	0.707	5.9	49.2	1.66	1.66
04.	8	60	0.195	0.865	8.0	60.0	0.00	0.00
05.	10	70	0.296	0.930	10.0	69.3	0.00	1.00
06.	12	80	0.392	1.120	11.9	80.0	0.833	0.00
07.	14	90	0.456	1.260	14.0	88.9	0.00	1.22

Table No. 11: Absorbance of assay mixtures in tablet dosage form ATR and GEM.

Sl. No	Concentration of ATR and GEM (mix in µg/ml)		AUC		Concentration obtained		% ERROR	
	ATR	GEM	218-228	270-280	AML	RAN	AML	RAN
01.	2	30	0.091	0.295	1.90	29.0	5.00	3.33
02.	4	40	0.110	0.459	4.00	38.9	0.00	2.75
03.	6	50	0.145	0.695	5.80	49.5	3.33	1.00
04.	8	60	0.170	0.710	7.86	58.2	1.75	3.00
05.	10	70	0.262	0.910	9.89	69.0	1.10	1.42
06.	12	80	0.310	1.105	11.8	79.8	1.16	0.25
07.	14	90	0.411	1.310	13.9	88.7	0.71	1.44

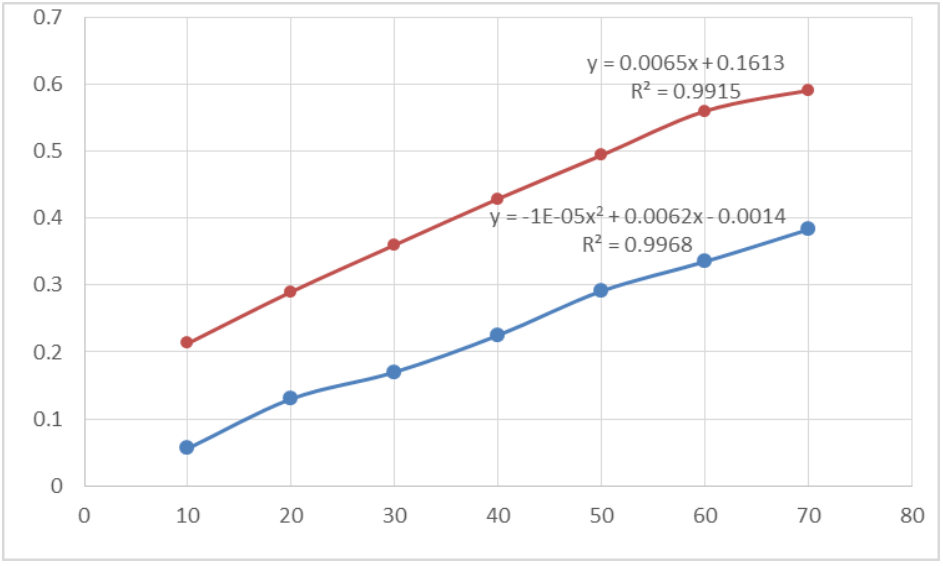


Area under Curve of GEM in 218-228nm



Area under Curve of AML in 228-238nm

Fig. No. 2: UV spectra for Area Under Curve for ATR and GEM.



Graph 02: Calibration graph for ATR (2-14µg/ml) and GEM (30-90µg/ml).

Table No. 12: %Recovery study data for ATR and GEM by Area under Curve method.

Level	ATR		GEM		Total conc. taken (µg/ml)		Absorbance		Amt. of std. recovered (µg/ml		% Recovery	
	Std. soln	Sample mix soln	Std. soln	Sample mix soln	ATR	GEM	ATR	GEM	ATR	GEM	ATR	GEM
80%	10	4	10	40	14	50	0.410	0.368	14.23	50.30	100.16	100.60
80%	10	4	10	40	14	50	0.411	0.367	14.36	50.40	102.57	100.80
80%	10	4	10	40	14	50	0.410	0.368	14.20	50.30	101.40	100.60
100%	10	6	10	60	16	70	0.460	0.452	15.80	70.50	100.70	100.71
100%	10	6	10	60	16	70	0.459	0.450	16.30	70.40	100.50	100.50
100%	10	6	10	60	16	70	0.458	0.452	15.81	70.50	100.70	100.71
120%	10	8	10	80	18	90	0.522	0.572	18.36	90.10	100.10	100.10
120%	10	8	10	80	18	90	0.522	0.570	18.36	90.23	102.00	100.25
120%	10	8	10	80	18	90	0.521	0.572	18.50	90.10	100.27	100.10

Table No. 13: Absorbance of ATR and GEM at 275nm, 223nm and 250.90nm.

Sl. No	Conc (µg/ml)	ATR						Conc. (µg/ml)	GEM					
		Absorbance			$E^{1\%}$ 1CM				Absorbance			$E^{1\%}$ 1CM		
		223	275	250.9	223	275	250.9		223	275	250.9	223	275	250.9
01.	2	0.07	0.010	0.003	350	50	15	30	0.812	0.24	0.055	270	80	18
02.	4	0.15	0.020	0.014	375	50	35	40	1.086	0.32	0.098	270	80	24
03.	6	0.20	0.036	0.023	333	56	38	50	1.354	0.40	0.145	270	80	29
04.	8	0.26	0.042	0.026	325	52	32	60	1.567	0.48	0.168	260	80	28
05.	10	0.34	0.048	0.035	340	51	35	70	1.747	0.55	0.187	240	78	26
06.	12	0.39	0.056	0.039	325	50	32	80	1.901	0.63	0.218	230	80	27
07.	14	0.48	0.061	0.042	335	53	33	90	2.100	0.72	0.246	250	78	26
		Average			341	51.5	33.7		Average			257	79.3	25.3

ax1=33.7, ax2=341 and ay1=25.3, ay2=257.

Table No. 14: Absorbance of mixture (ATR and GEM) in std. drugs by Q-absorbance equation method.

Sl.No	Concentration of ATR and GEM (mix in µg/ml)		Absorbance (nm)			Concentration By Method I (in µg/ml)		Concentration By Method II		% Error Method-I		% Error Method-II	
	ATR	GEM	223	275	250.9	ATR	GEM	ATR	GEM	ATR	GEM	ATR	GEM
01.	2	30	0.427	0.092	0.083	1.70	29.60	1.90	28.8	15.0	1.33	10.0	4.00
02.	4	40	0.590	0.130	0.097	4.03	38.20	3.60	39.6	-0.75	1.00	5.30	1.00
03.	6	50	0.763	0.166	0.115	5.68	49.10	6.02	48.8	5.30	1.80	-0.30	2.40
04.	8	60	0.937	0.207	0.181	8.00	58.8	7.90	59.3	0.00	2.00	1.25	1.16
05.	10	70	0.980	0.315	0.197	9.81	69.3	9.8	70.2	1.90	1.00	2.00	-0.02
06.	12	80	1.151	0.399	0.218	11.96	79.2	11.8	79.6	0.33	1.00	1.60	0.50
07.	14	90	1.290	0.406	0.235	13.20	88.6	13.9	88.3	3.50	1.50	3.50	0.77

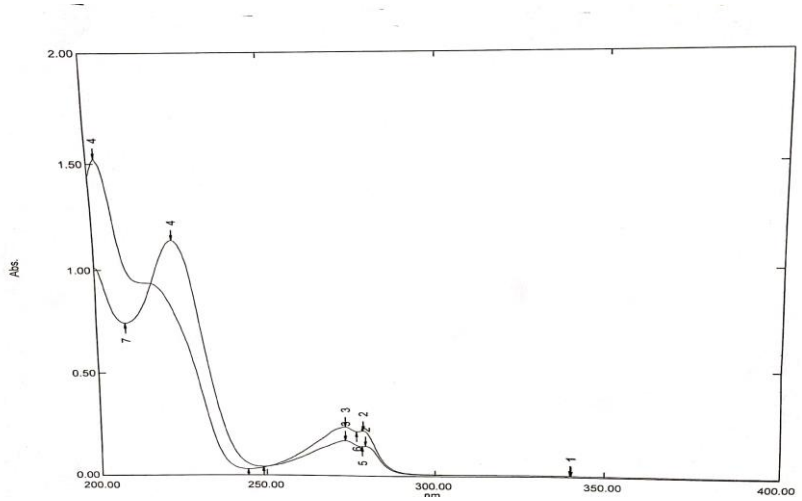
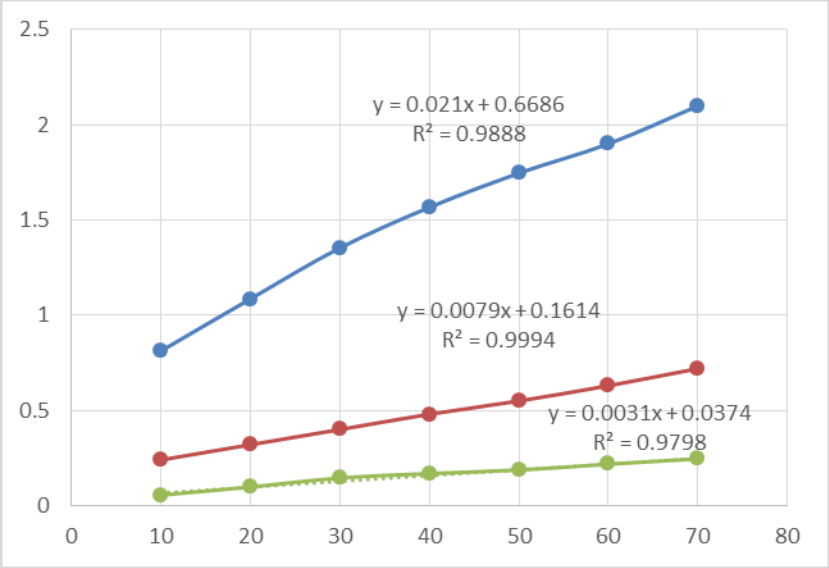
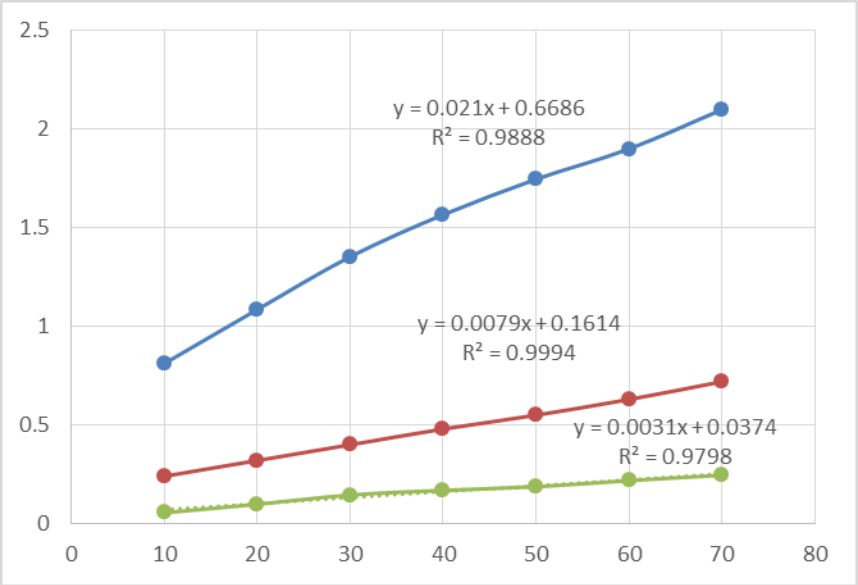


Fig. No. 3: Overlain UV spectrum of ATR (10µg/ml) and GEM (10µg/ml).



Graph 3: Calibration graph for ATR at all three wavelengths.



Graph 4: Calibration graph for GEM at all three wavelengths.

Table No 15: % Recovery study data for ATR and GEM by Graphical absorbance method.

Level	ATR		GEM		Total conc. taken (µg/ml)		Absorbance		Amt. of std. recovered (µg/ml		% Recovery	
	Std. soln	Sample mix soln	Std. soln	Sample mix soln	ATR	GEM	ATR	GEM	ATR	GEM	ATR	GEM
80%	4	1.6	10	48	5.6	58	0.019	0.152	5.82	50.30	100.16	100.60
80%	4	1.6	10	48	5.6	58	0.019	0.150	5.65	50.40	102.57	100.80
80%	4	1.6	10	48	5.6	58	0.019	0.148	5.51	50.30	101.40	100.60
100%	4	2.0	10	50	6.0	60	0.026	0.169	6.50	60.50	100.70	100.71
100%	4	2.00	10	50	6.0	60	0.030	0.166	6.30	60.40	100.50	100.50
100%	4	2.00	10	50	6.0	60	0.031	0.168	6.23	60.28	100.70	100.71
120%	4	2.4	10	72	6.4	82	0.049	0.186	6.90	81.30	100.10	100.10
120%	4	2.4	10	72	6.4	82	0.053	0.185	6.80	82.60	102.00	100.25
120%	4	2.4	10	72	6.4	82	0.051	0.190	6.60	81.50	100.27	100.10

Method Validation

Linearity

In this method the absorption spectra was recorded in wavelength region of 200-400nm. Linearity of AML and RAN was found 2-14µg/ml and 10-80µg/ml respectively, ATR and GEM was found 2-14µg/ml and 30-90µg/ml respectively. In this work the AML and RAN, ATR and GEM were quantified by using five different quantitative methods of UV spectroscopy. The three methods followed are simultaneous equation method, Area under curve method and Q-analysis (Graphical ratio absorbance spectroscopy).

Table No 16: Linearity of ATR-GEM in Methanol.

Sl. No	ATORVASTATIN			GEMFIBROZIL		
	Conc	Abs	E1% 1cm	Conc	Abs	E1% 1cm
1.	2	0.07	350	30	0.24	80.00
2.	4	0.14	375	40	0.32	80.00
3.	6	0.23	333	50	0.40	80.00
4.	8	0.29	325	60	0.48	78.00
5.	10	0.36	340	70	0.55	78.75
6.	12	0.42	325	80	0.63	79.00
7.	14	0.53	330	90	0.72	80.10

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed methods. Repeatability was determined by preparing six replicates of same concentration of the sample and the absorbance was measured. Intraday and interday precision studies were done. The % RSD was calculated for ATR and GEM.

Table No. 17: Intraday Precision data for ATR.

Replicates	SIM		AUC		QAM	
	Abs	Conc	Abs	Conc	Abs	Conc
1.	0.200	59.9	0.170	60.0	0.023	59.9
2.	0.201	60.01	0.170	60.0	0.022	59.8
3.	0.200	59.9	0.171	60.1	0.022	59.8
Mean	0.2003	59.93	0.1703	60.03	0.0223	59.83
Std Deviation	0.00047	0.0518	0.00047	0.0471	0.00047	0.0471
%RSD	0.28819	0.1059	0.3389	0.0961	2.8515	0.0964

Table No. 18: Intraday Precision data for GEM.

Replicates	SIM		AUC		QAM	
	Abs	Conc	Abs	Conc	Abs	Conc
1.	0.48	5.8	0.431	6.0	0.168	5.8
2.	0.48	5.8	0.432	6.1	0.170	6.0
3.	0.48	5.8	0.432	6.1	0.169	5.9
Mean	0.48	5.8	0.4316	6.066	0.169	5.9
Std Deviation	0.00	0.00	0.00047	0.0471	0.0008	0.0814
%RSD	0.00	0.00	0.1337	0.9514	0.5917	1.6914

Table No. 19: Interday Precision data for ATR.

Replicates	Days	SIM		AUC		QAM	
		Abs	Conc	Abs	Conc	Abs	Conc
1.	Day 1	0.219	59.8	0.170	60.0	0.020	59.5
2.	Day 2	0.218	60.1	0.169	60.1	0.026	60.1
3.	Day 3	0.221	59.3	0.168	60.3	0.024	59.8
Mean		0.2198	59.733	0.1703	60.13	0.0233	59.83
Std Deviation		0.001528	0.0518	0.00047	0.1521	13.093	0.0471
%RSD		0.69644	0.4041	0.3389	0.2504	0.0035	0.664

Table No. 20: Interday Precision data for GEM.

Replicates	Days	SIM		AUC		QAM	
		Abs	Conc	Abs	Conc	Abs	Conc
1.	Day 1	0.45	5.9	0.431	5.92	0.163	5.6
2.	Day 2	0.42	6.1	0.435	5.86	0.172	6.2

3.	Day 3	0.43	6.0	0.438	5.83	0.171	6.1
Mean		0.433	6.0	0.434	5.87	0.168	5.96
Std Deviation		0.0152	0.2521	0.0035	0.0485	0.0049	0.0614
%RSD		3.521	0.6250	0.8079	0.7806	2.975	1.714

Ruggedness

Ruggedness was performed for AML and RAN, ATR and GEM by different analyst on different day. The results obtained are within the limits. The % RSD calculated for all the methods were found to be less than 3%.

Table No. 21: Ruggedness data for ATR and GEM.

ANALYST-01.

Concentration		SIM		AUC		QAM	
ATR	**GEM**	**ATR**	**GEM**	**ATR**	**GEM**	**ATR**	**GEM**
6	60	0.20	0.48	0.171	0.420	0.23	0.168
6	60	0.19	0.48	0.170	0.421	0.22	0.167
6	60	0.20	0.47	0.171	0.420	0.23	0.167
6	60	0.20	0.48	0.170	0.420	0.23	0.167
6	60	0.21	0.47	0.171	0.420	0.23	0.167
6	60	0.20	0.47	0.171	0.420	0.23	0.168
Mean		0.20	0.476	0.1706	0.4202	0.228	0.1676
Std. Deviation		0.0062	0.00489	0.0004	0.0004	0.004	0.0048
%RSD		3.1622	1.1531	0.3025	0.0971	1.7879	0.3086

ANALYST-02.

Concentration		SIM		AUC		QAM	
ATR	**GEM**	**ATR**	**GEM**	**ATR**	**GEM**	**ATR**	**GEM**
6	60	0.20	0.48	0.171	0.420	0.23	0.168
6	60	0.19	0.48	0.170	0.421	0.22	0.167
6	60	0.20	0.47	0.171	0.420	0.23	0.167
6	60	0.20	0.48	0.170	0.420	0.23	0.167
6	60	0.21	0.47	0.171	0.420	0.23	0.167
6	60	0.20	0.47	0.171	0.420	0.23	0.168
Mean		0.20	0.476	0.1706	0.4202	0.228	0.1672
Std. Deviation		0.0063	0.00489	0.00048	0.0004	0.004	0.0004
%RSD		3.1622	1.1531	0.30257	0.09713	1.7879	0.3086

Robustness

Robustness of the proposed method was determined by variance in method parameter like temperature. The %RSD calculated for all the methods were found to be less than 3%.

Table No. 22: Robustness data for ATR and GEM.

AT 18°C.

SIM		AUC		QAM	
ATR	**GEM**	**ATR**	**GEM**	**ATR**	**GEM**
0.19	0.47	0.171	0.420	0.23	0.168
0.19	0.47	0.170	0.421	0.22	0.167
0.20	0.47	0.172	0.420	0.21	0.168
0.21	0.48	0.170	0.421	0.23	0.167
0.21	0.47	0.170	0.421	0.23	0.167
0.20	0.47	0.171	0.420	0.23	0.168
0.20	0.472	0.1706	0.4206	0.224	0.1674
0.0089	0.004	0.008	0.00048	0.008	0.00048
5.000	0.8665	0.4784	0.13025	3.174	0.3269

AT ROOM TEMPERATURE

Concentration		SIM		AUC		QAM	
ATR	**GEM**	**ATR**	**GEM**	**ATR**	**GEM**	**ATR**	**GEM**
6	60	0.19	0.47	0.171	0.420	0.23	0.168

6	60	0.19	0.47	0.170	0.421	0.22	0.167
6	60	0.20	0.47	0.172	0.420	0.21	0.168
6	60	0.21	0.48	0.170	0.421	0.23	0.167
6	60	0.21	0.47	0.170	0.421	0.23	0.167
6	60	0.20	0.47	0.171	0.420	0.23	0.168
Mean		0.20	0.472	0.1706	0.4206	0.224	0.1674
Std. Deviation		0.0089	0.004	0.008	0.00048	0.008	0.00048
%RSD		5.000	0.8665	0.4784	0.13025	3.174	0.3269

Sensitivity

The LOD and LOQ values from Method A-C for for ATR 1.25,1.25,1.00µg/ml and 0.5µg/ml respectively, for GEM 5 and 10µg/ml respectively. Low values of LOD and LOQ indicates good sensitivity of proposed methods shown in Table No: 23.

Accuracy

Accuracy of proposed methods was determined using recovery studies. The recovery studies were carried out by adding different amount (80%, 100% and 120%) of pure drug to the pre analysed formulation. The mean recoveries were found in the range of 100.1-105%. The results of a recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed methods. The results are shown in Table no: 8, 12, 15.

Table No. 23: Calibration Data for ATR and GEM.

PARAMETERS	SIM		AUC		QAM	
	ATR	GEM	ATR	GEM	ATR	GEM
λmax (nm)	223.8	275	218-228	270-280	250.9	250.9
E1% 1cm	341	51.5	289	71.0	337	25.3
Slope*	0.0077	0.0068	0.0065	0.0062	0.0031	0.0031
Intercept*	0.1671	0.0043	0.1613	0.0014	0.0375	0.0374
Correlation coefficient	0.9995	0.9950	0.9915	0.9968	0.9938	0.9798
Linearity and range (µg/ml)	2-40	30-90	2-100	30-400	2-40	30-90
LOD(µg/ml)	1.25	5	1.25	5	1	5
LOQ(µg/ml)	0.5	10	0.5	10	0.5	10

CONCLUSION

In UV spectrophotometric technique three methods were used for simultaneous estimation of ATR and GEM. Method-A involves the measurement absorbances at selected wavelengths and calculating the concentration drugs by using simultaneous equation. Method-B involves measurement area under curve at selected wavelengths and calculates the concentration of drugs by using simultaneous equation. Method-C involves Q-analysis (Graphical ratio absorbance method) in which measurement of absorbances at isobestic point and a wavelength maximum of any one drug is used for calculation.

- The simultaneous estimation was carried to estimate the concentration in both individually and in the combination.
- The AUC method is an upgrade method of simultaneous equation method in with the Area under the curve was measured instead of absorbance at wavelength ±5nm of λmax of both the drugs.
- In Q-analysis method the estimation is carried out at isobestic point and the wavelength at which absorbance is close to absorbance at isoabsorptive point was much accurate and precise than the other methods.

The striking advantages of all the spectrophotometric methods developed are economical, accurate and precise. These are generally fast and economical in comparison to the more time consuming chromatographic techniques often used for the assay of formulation.

BIBLIOGRAPHY

1. Murdock DK, Murdock AK, Murdock RW, Olson KJ, Frane AM, Kersten ME, Joyce DM, Gantner SE. Long-term safety and efficacy of combination Gemfibrozil and HMG-CoA reductase inhibitors for the treatment of mixed lipid disorders. American heart journal, 1999 Jul 1; 138(1): 151-5.
2. Islam U, Jillani SM, Ashfaq M. Determination of Atorvastatin and Gemfibrozil in human plasma by reversed-phase liquid chromatography. Lat. Am. J. Pharm., 2010; 29(8): 1383-8.
3. ICH guidelines, validation of analytical procedures; methodology. International Conference on Harmonization, Q2B: Geneva; Nov, 2005.
4. Hansen S, Pedersen-Bjergaard S, Rasmussen K. Introduction to pharmaceutical chemical analysis. Chichester, West Sussex: John Wiley & Sons Inc., 2012.
5. Jain H K, Patel K H. Development and Validation of UV Spectrophotometric Area under Curve Method for Estimation of Loratadine in Bulk and Tablet

Dosage Form. Am. J. Pharma Tech Res, 2013; 3(4): 492-99.
6. Pandey G, Mishra B. A new analytical Q-absorbance ratio method development and validation for simultaneous estimation of Lamivudine and Isoniazid. ISRN Spectroscopy, 2013 Dec 16.
7. Gidwani B, Patel L, Gupta A, Kaur GD. Ultra-violet spectrophotometric method for estimation and validation of Amlodipine in bulk and tablet formulation. J Anal Pharm Res., 2017; 4(6): 00125.
8. Patil PR, Rakesh SU, Dhabale PN, Burade KB. Simultaneous UV-spectrophotometric method for estimation of Losartan Potssium and Amlodipine Besylate in tablet dosage Form. Asian J Res Chem., 2009; 2(1): 183-7.
9. Kasture AV, Ramteke M. Simultaneous UV-spectrophotometric method for the estimation of Atenolol and Amlodipine Besylate in combined dosage form. Ind J Pharm Sci., 2006; 68(3): 394-396.
10. Vanparia DJ, Chodavadia RM, Patel PB, Kalsaria NM, Marolia BP, Shah SA. Spectrophotometric Estimation of Ranolazine in Bulk and Pharmaceutical Dosage Form by UV-Specrtophotometry. International Journal of Chemical and Analytical Science, 2011; 2(9): 1150-2.
11. Sharma A, Prakash D, kumar Singh S. Development and validation of UV spectrophotometric method for the estimation of Ranolazine in bulk drug and pharmaceutical formulation. Int. J. ChemTech Res., 2010 Oct; 2(4): 1945-8.
12. Rao NV, Sowjanya K, Ramachandran D. UV-Spectrophotometric assay method development and validation of Gemfibrozil in bulk and tablet formulation. BOPAMS, 2015 Oct; 3(4).
13. Vikas PC, Karkhanis VV. Spectrophotometric Estimation of Gemfibrozil In bulk and pharmaceutical dosage forms. Int Res J Pharm, 2011.