Jamadar MJ. et al. / Vol 4 / Issue 2 / 2014 / 114-121.

International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF MULTICOMPONENTS IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, precise, and sensitive and a highly selective UV-spectrophotometric method were developed for the simultaneous estimation of atorvastatin calcium and pyridoxine hydrochloride at a maximum wavelength of 259 nm and 288 nm respectively. The method was found to be linear in the range of 2 – 10 µg/ml with mean recovery in the range of 98% - 102% for atorvastatin calcium & pyridoxine hydrochloride. The developed method was validated according to ICH guidelines and it was found to be accurate and precise, thus the proposed method can be successfully applied for simultaneous determination of atorvastatin calcium & pyridoxine hydrochloride in routine analysis work.

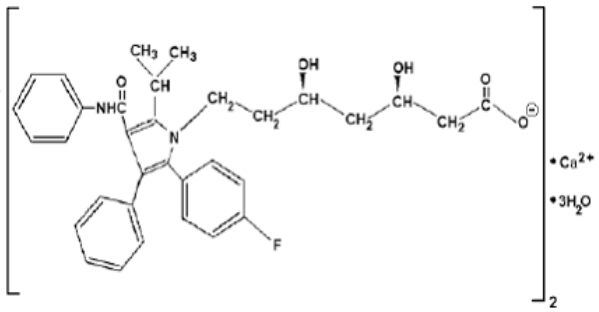
Keywords: Atorvastatin calcium (ATR), Pyridoxine hydrochloride (PYR), UV-Spectrophotometric method.

INTRODUCTION

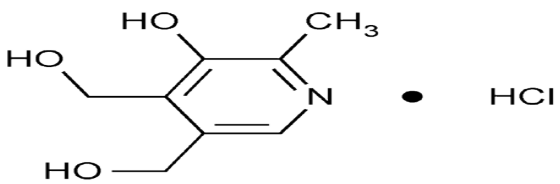
Atorvastatin calcium is (βR,δR)-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methyl ethyl)-3-phenyl-4-((phenylamino) carbonyl)-1H-Pyrrole-1-hepatonoic acid calcium salt (2:1) trihydrate is a therapeutically beneficial drug that works by HMG CoA reductase inhibitor. HMG-CoA reductase catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate, which is the rate-limiting step in hepatic cholesterol biosynthesis. This increases LDL uptake by the hepatocytes, decreasing the amount of LDL-cholesterol in the blood. Like other statins, Atorvastatin also reduces blood levels of triglycerides and slightly increases levels of cholesterol[1-6].

Pyridoxine Hydrochloride is 5-hydroxy-6-methylpyridine- 3, 4-dimethanol hydrochloride. It is B group vitamin which is used in sideroblastic anaemia therapy and anti-isoniazid neuropathy. Pyridoxine assists in the balancing of sodium and potassium as well as promoting red blood cell production. It is linked to cardiovascular health by decreasing the formation of homocysteine [7-9].

Chemical Structure



Atorvastatin calcium



Pyridoxine hydrochloride

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Literature survey revealed that there is no UV method has been reported yet for the analysis of these two drugs in combination without preliminary separation that makes it worthwhile to pursue the present work.

MATERIALS AND METHOD

Atorvastatin calcium and Pyridoxine hydrochloride were obtained as gift sample from Indoco Remedies Ltd, Santacruz Mumbai & Symbiosis Co-Operative Pharmaceutical Ltd Sangli respectively. All solvents were of AR grade obtained from Appasaheb Birnale College of Pharmacy, Sangli Maharashtra.

The present work was carried out on JASCO spectrophotometer, model no. V-530 with 1 cm matched quartz cells was used for experiments. The absorption spectra of reference and test solution were carried out in a 1 cm quartz cell over the range of 200-400nm.

Experimental Condition

According to the solubility characteristics of drug methanol was selected as solvent for analysis. Wavelength of maximum absorption was selected for atorvastatin calcium (ATR) and Pyridoxine hydrochloride (PYR) at 259 nm and 288 nm respectively. From scanning of drugs in UV range.

Standard Stock and Sub Stock Solution

UV analysis was done by using the standard stock solution of 100 µg/ml of each ATR and PYR by dissolving 10 mg of each standard drug separately in methanol. For calibration curve aliquots of 2, 4, 6, 8, 10 µg/ml was prepared by using stock solution.

Sample Preparation

Ten tablets of Atherochek-5 containing 5 mg of ATR and 10 mg of PYR were weighed and finely powdered separately. Powder equivalent to 5 mg of ATR and 10 mg of PYR was weighed and transferred to a volumetric glass and drug was sonicated with 15 ml quantities of methanol, and then final volume of the solution was made up to 25ml with methanol to get a stock solution containing 200 µg/ml of ATR and 400 µg/ml of PYR, and further dilutions were made to get a concentration of 5 µg/ml of ATR and 10 µg/ml of PYR. The contents were mixed and filtered through a Whatmann filter paper.

Wavelength Selection

The standard solution of ATR and PYR were separately scanned at different concentration in the range of 200-400 nm and the λ max was determined shown in fig no.1.

Linearity

The linearity for spectrophotometric method was established in the concentration of 2, 4, 6, 8, and 10 µg/ml for both the drugs. Shown in fig no.2 to 5.

Method Validation

Accuracy was determined by recovery study. The recovery experiment was carried out by spiking the preanalysed sample of the multicomponent tablets with their different known concentration of standard i.e. ATR and PYR. Precision for the method were determined by repeatability, intraday & interday precision for both drugs.

Recovery

To evaluate the accuracy, precision and reproducibility of the method, known amount of pure drug was added to the preanalyzed sample of tablet powder and the mixture was analyzed for the drug content using the proposed method. The percentage recovery was calculated. The recovery experiments indicated the absence of interference from the commonly encountered Pharmaceutical additives and recipients.

Procedure for Recovery Studies

Recovery studies were carried out by standard addition method at three levels, 80%, 100%, and 120%. In this method a known amount of standard drug solution were added to preanalysed tablet solution and absorbance were measured at 259.0nm and 288.0nm (λ_{max} of ATR and PYR respectively) and the concentration of both drugs were determined using simultaneous equation. At each level three determinations were performed and results were obtained.

RESULTS AND DISCUSSION

Determination of Absorption maxima

By appropriate dilution of two standard drug solutions with methanol, solutions containing 10 µg/ml of atorvastatin calcium and 10 µg/ml of pyridoxine hydrochloride were scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption for both the drugs. Atorvastatin calcium and pyridoxine hydrochloride showed absorbance maxima at 259 nm (λ_1) and 288 nm (λ_2) respectively. The overlain spectra showed λ max of both drugs and also iso-absorptive points at 281 nm. (Fig.no.3.)

Linearity

The appropriate dilutions are prepared as per dilution which are scanned and the absorbance of above solutions were measured at the selected wavelengths and the calibration curves were constructed by plotting the absorbance against the concentration for all the drugs the concentration in sample solution was calculated by using formula Abs = A + B * C, The correlation coefficient for ATR and PYR was found to be 0.999, and 0.999 respectively. Calibration curves are shown in Fig.2 to.5 while absorbance is shown in Table no.1 and Table no.2.

Simultaneous Equations

$$C_{PYR} = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \quad \text{----------} \quad (1)$$

$$C_{ATR} = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \quad \text{----------} \quad (2)$$

Precision

Repeatability of method was established by analyzing various replicates of sample. All the solutions were analyzed thrice, in order to record intra-day & inter-day variation in the result. The result obtained for intra-day and inter-day variations of both the drugs are shown in table no.4 to 7.

Result of Tablet formulation Analysis

A tablet dosage form of Atorvastatin calcium and Pyridoxine hydrochloride was analyzed by simultaneous equation, the percentage in dosage form were determined and presented in table no.8. Assay results obtained are within limit, referred in table no.10.

Determination of Limit of Detection

The Limit of Detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD was calculated using the following

Formula:

$$LOD = \frac{3.3 \times \text{Standard deviation of y intercept}}{\text{Slope of calibration curve}}$$

Atorvastatin Calcium**:** 0.101µg/ml

Pyridoxine Hydrochloride: 0.1175µg/ml.

Determination of Limit of Quantification

The Limit of Quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. LOD was calculated using the following formula:

$$LOQ = \frac{10 \times \text{Standard deviation of y intercept}}{\text{Slope of calibration curve}}$$

Atorvastatin Calcium: 0.308µg/ml

Pyridoxine Hydrochloride: 0.356µg/ml

Recovery Studies

Accuracy and sensitivity of analysis was determined by performing recovery studies by spiking different concentrations of pure drug in the preanalyzed sample. Results of recovery studies are shown in Table. No.11 to 14.

Table 1. Calibration of Atorvastatin Calcium at 259 nm & 288 nm.

Sr. No.	Concentration (µg/ml)	Absorbance (259 nm)	Absorbance (288 nm)
1	2	0.2014	0.1124
2	4	0.4123	0.2103
3	6	0.6152	0.3125
4	8	0.8120	0.4257
5	10	0.9855	0.5321

Correlation coefficient of Atorvastatin Calcium 259.0nm was found to be 0.999.

Table 2. Calibration of Pyridoxine Hydrochloride 288.0nm

Sr. No.	Concentration (µg/ml)	Absorbance (288.0 nm)	Absorbance (259.0 nm)
1	2	0.1228	0.0165
2	4	0.2410	0.0324
3	6	0.3634	0.0487
4	8	0.4857	0.0652
5	10	0.6129	0.0836

Correlation coefficient for Pyridoxine Hydrochloride 288.0nm was found to be 0.999

Table 3. Absorptivity Values for Atorvastatin Calcium and Pyridoxine Hydrochloride.

Components	Absorptivity at 259.0nm	Absorptivity at 288..0nm
Atorvastatin Calcium (x)	1050.7 (ay_1) ± 0.6293	555.35(ay_2) ±0.6218
Pyridoxine Hydrochloride (y)	83(ax_1) ± 0.1736	691.9 (ax_2) ±0.5057

Table 4. a) Intraday precision for Atorvastatin Calcium at 259.0nm

Conc. (µg/ml)	Absorbance (259.0nm)			Mean	±SD	% RSD
	Trial 1	Trial 2	Trial 3			
4	0.4123	0.4113	0.4126	0.4120	0.00068	0.1651
6	0.6152	0.6150	0.6173	0.6158	0.0012	0.2068
8	0.8120	0.8118	0.8115	0.8117	0.00025	0.0310

Table 4. b) Intraday precision for Atorvastatin Calcium at 288.0nm

Conc. (µg/ml)	Absorbance (288.0nm)			Mean	±SD	% RSD
	Trial 1	Trial 2	Trial 3			
4	0.2103	0.2113	0.2123	0.2113	0.001	0.4732
6	0.3125	0.3188	0.3157	0.3155	0.0031	0.9984
8	0.4257	0.4277	0.4281	0.42717	0.0012	0.3010

Table 5. a) Intraday precision for Pyridoxine Hydrochloride at 288.0nm

Conc. (µg/ml)	Absorbance (288.0nm)			Mean	±SD	% RSD
	Trial 1	Trial 2	Trial 3			
4	0.2410	0.2415	0.2420	0.2415	0.0002	0.4942
6	0.3634	0.3648	0.3642	0.3641	0.0007	0.1928
8	0.4857	0.4856	0.4860	0.4857	0.0002	0.0428

Table 5. b) Intraday precision for Pyridoxine Hydrochloride 259.0nm

Conc. (µg/ml)	Absorbance (259.0nm)			Mean	±SD	% RSD
	Trial 1	Trial 2	Trial 3			
4	0.0324	0.0312	0.0326	0.03207	0.0004	1.4255
6	0.0487	0.0477	0.0481	0.04817	0.0005	1.0448
8	0.0652	0.0642	0.0658	0.06586	0.0010	1.5573

Table 6. a) Interday precision for Atorvastatin Calcium at 259.0nm

Conc. (µg/ml)	Absorbance (259.0nm)			Mean	±SD	% RSD
	Day 1	Day 2	Day 3			
4	0.4160	0.4140	0.4150	0.415	0.001	0.2409
6	0.6082	0.6190	0.6140	0.6137	0.0054	0.8806
8	0.8120	0.8090	0.8110	0.744	0.0015	0.2053

Table 6. b) Interday precision for Atorvastatin Calcium 288.0nm

Conc. (µg/ml)	Absorbance (288.0nm)			Mean	±SD	% RSD
	Day 1	Day 2	Day 3			
4	0.2160	0.2155	0.2182	0.2165	0.0014	0.6632
6	0.3190	0.3135	0.3175	0.3166	0.0028	0.8978
8	0.4281	0.4271	0.4290	0.4280	0.00095	0.2220

Table 7. a) Interday precision for Pyridoxine Hydrochloride at 288.0nm

Conc. (µg/ml)	Absorbance (288.0nm)			Mean	±SD	% RSD
	Day 1	Day 2	Day 3			
4	0.2485	0.2422	0.2474	0.2460	0.0033	1.367
6	0.3688	0.3635	0.3651	0.3658	0.0027	0.7431
8	0.4802	0.4855	0.4858	0.4851	0.0012	0.2543

Table 7. b) Interday variation for Pyridoxine Hydrochloride at 259.0nm

Conc.(µg/ml)	Absorbance (259.0nm)			Mean	±SD	% RSD
	Day 1	Day 2	Day 3			
4	0.0324	0.0340	0.0334	0.03327	0.00010	0.3180
6	0.0487	0.0497	0.0481	0.04883	0.00080	1.6553
8	0.0652	0.0660	0.0657	0.06563	0.00040	0.6157

Table 8. Analysis of Tablet formulation.

Sr. No.	Label Claim (mg)		Amount found (mg)		% of Label Claim	
	ATR	PTR	ATR	PTR	ATR	PTR
1.	5	10	5.00	10.18	100.01	101.80
2.	5	10	5.09	10.10	101.80	101.00
3	5	10	5.03	9.98	100.70	99.80

Table 9. Absorbance values for the Tablet

Sr. .No.	A_1 (259. nm)	A_2 (288.nm)
1.	0.6102	0.9824
2.	0.6183	0.9812
3.	0.6125	0.9705

Table 10. Statistical Validation of Tablet formulation

Component	Mean	Standard Deviation	% RSD	Standard Error
ATR	100.83	0.9028	0.89537	0.5212
PYR	100.86	1.007	0.99841	0.5812

Table 11. Recovery studies for ATR

Level of % Recovery	Amount present (mg/tab)		Amount added (mg)		Amount Found ATR	% Recovery
	ATR	PYR	ATR	PYR	259.0nm	ATR
80	5	10	4	0	8.85	98.33
	5	10	4	0	9.15	101.66
	5	10	4	0	8.95	99.44
100	5	10	5	0	10.05	100.5
	5	10	5	0	9.95	99.5
	5	10	5	0	9.93	99.3
120	5	10	6	0	11.12	101.09
	5	10	6	0	11.06	100.54
	5	10	6	0	10.96	99.63

Table 12. Statistical Validation for recovery studies of ATR

Level of % Recovery	% Mean Recovery	Standard Deviation	% RSD	Standard error
	ATR	ATR	ATR	ATR
80	99.81	1 .696	1.6992	0.9789
100	99.76	0.6429	0.6445	0.3712
120	100.42	0.7374	0.7343	0.4257

Table 13. Recovery studies for PYR

Level of % Recovery	Amount present(mg/tab)		Amount added(mg)		Amount FoundPYR	% Recovery
	ATR	PYR	ATR	PYR	288nm	PYR
80	5	10	0	8	18.10	100.55
	5	10	0	8	17.90	99.44
	5	10	0	8	18.15	100.83
100	5	10	0	10	20.05	100.25
	5	10	0	10	20.10	100.5
	5	10	0	10	19.95	99.75
120	5	10	0	12	21.95	99.75
	5	10	0	12	22.10	100.45
	5	10	0	12	21.97	99.86

Table 14. Statistical validation for PYR

Level of % Recovery	% Mean Recovery	Standard Deviation	% RSD	Standard Error
	PYR	PYR	PYR	PYR
80	100.27	0.7351	0.73312	0.4244
100	100.16	0.3819	0.38129	0.2205
120	100.00	0.3912	0.39118	0.2259

Table 15. Summary of the present study Result (UV –method)

Parameters	Atorvastatin Calcium	Pyridoxine Hydrochloride
Detection Wavelength	259.0nm	288.0nm
Beers Law Limit	2-10µg/ml	2-10µg/ml
Absorptivity	1050.7gm/100ml	691.9gm/100ml
Regression Equation	y = mx + c	y = mx + c
Slope	0.099	0.016
Intercept	+0.007	-0.001
Correlation Coefficient	0.999	0.999
Limit of Detection(LOD)	0.101µg/ml	0.1175µg/ml
Limit of Quantification (LOQ)	0.308µg/ml	0.356µg/ml

Fig 1.Individual spectra of Atorvastatin calcium.

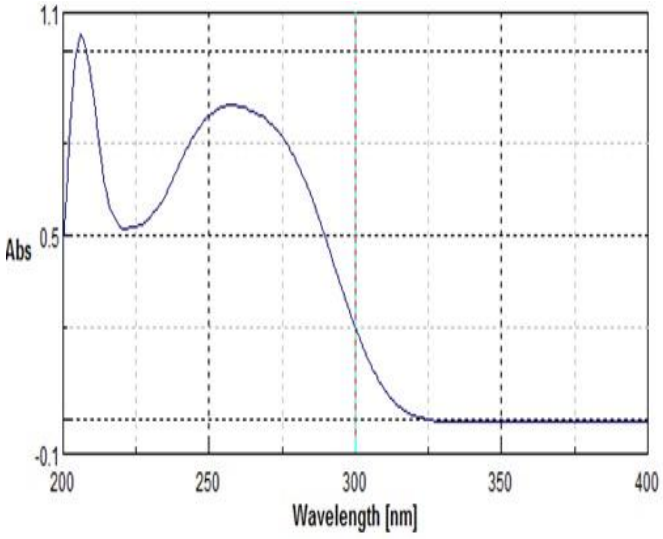


Fig 2. Individual absorption spectra of pyridoxine hydrochloride.

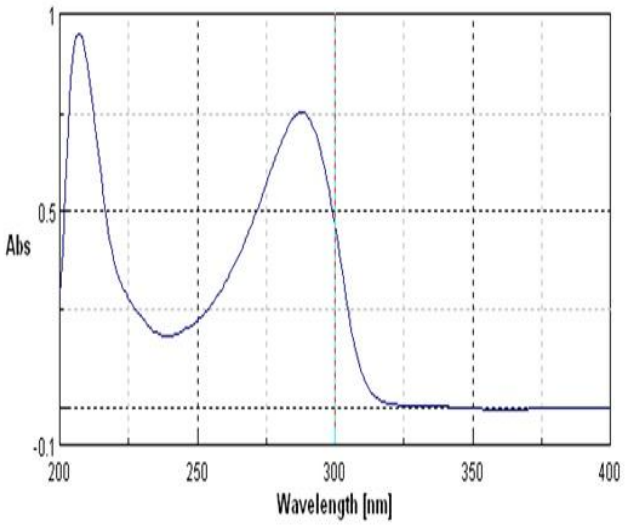


Fig 3. Overlain of both the drugs (iso-absorptive point at 281 nm)

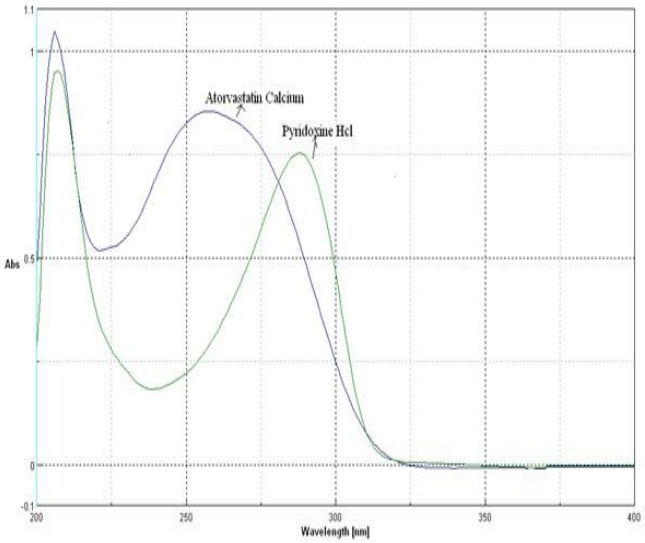


Fig 4. Calibration curve for Atorvastatin Calcium 259 nm

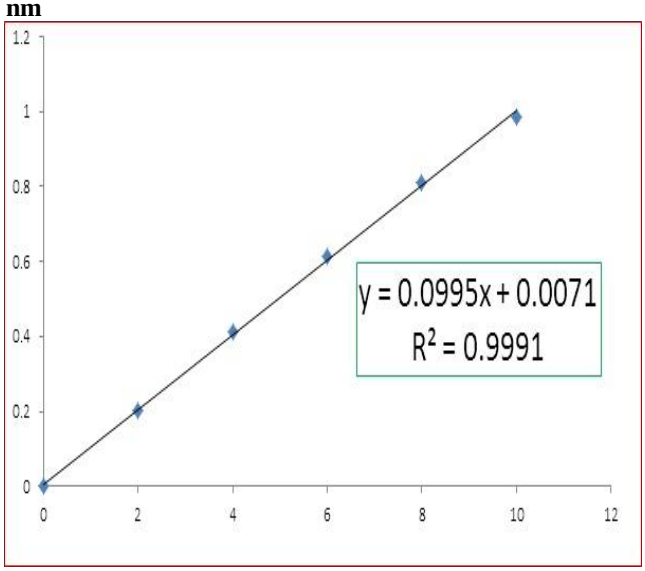


Fig 5. Calibration curve for Atorvastatin Calcium 288.0 nm

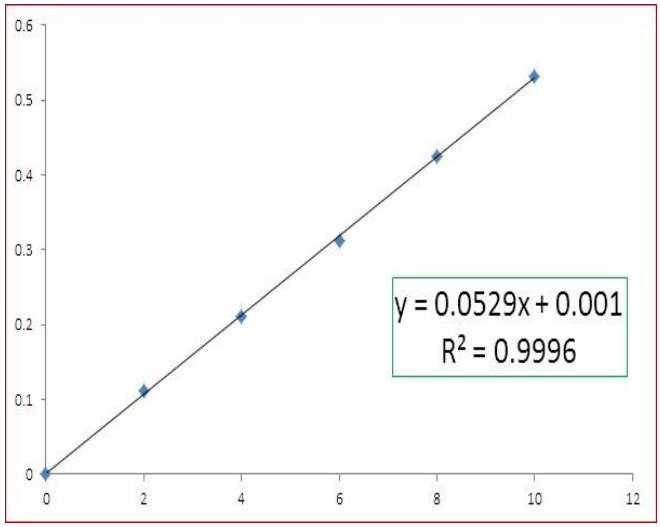


Fig 6. Calibration curve for Pyridoxine Hydrochloride 288.0nm

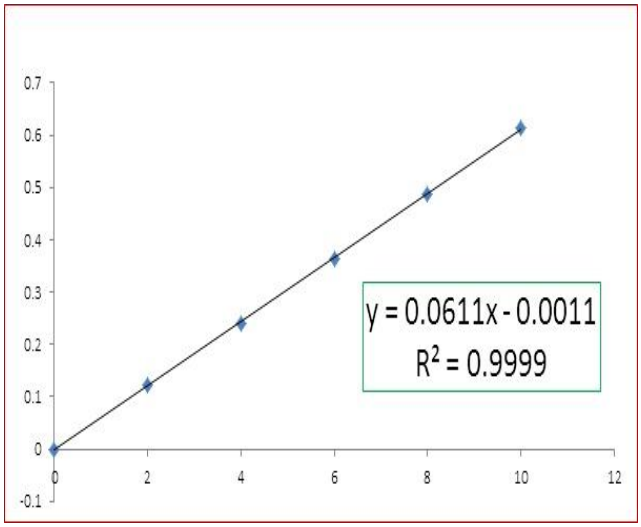
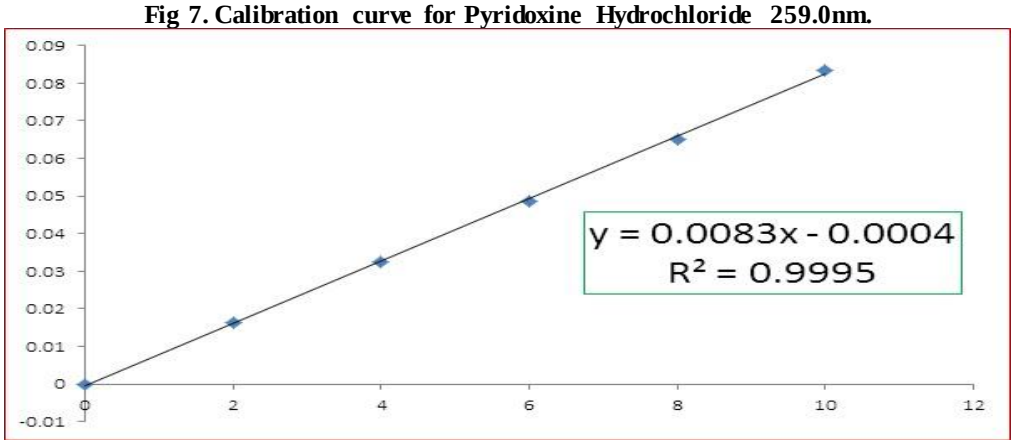


Fig 7. Calibration curve for Pyridoxine Hydrochloride 259.0nm.



DISCUSSION

The proposed method for determination of atorvastatin calcium and pyridoxine hydrochloride show for UV spectrophotometric method, the Linearity was determined at different concentration ATR and PYR were showed linearity in the concentration range of 2-10 µg/ml, 2-10 µg/ml with correlation coefficient of 0.999 & 0.999 respectively. Limit of detection (LOD) and Limit of quantitation (LOQ) were determined by standard deviation of response and slope of calibration curve. LOD and LOQ were found to be 0.101µg/ml, 0.308µg/ml for ATR & 0.1175µg/ml, 0.356µg/ml for PYR respectively. Marketed formulation was analyzed and amount of drug determined by proposed method was in good agreement with the labeled claim. The results of the Marketed formulation were found to be 100.83 ±0.9028, 100.86±1.007 for ATR and PYR respectively. Result of analysis of marketed formulation showed % relative standard deviation values of 0.89537and 0.99841for ATR and PYR which indicates repeatability of the method. The reproducibility of sample was expressed in terms of SD and % RSD. There was no interference from the common excipients present in tablets. The results i.e. % RSD < 2 % signifies the precision of the method. The proposed method was validated as per the ICH guideline.

CONCLUSION

The proposed spectrophotometric method is accurate, precise and reliable for the simultaneous measurement of ATR and PYR in combined dosage form. The developed spectrophotometric method was validated for simultaneous estimation of ATR and PYR using linearity, range, accuracy and precision. The %RSD for all parameters was found to be less than 2. This indicates the validity of method and assay results obtained by this method, are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of ATR and PYR in multi-component pharmaceutical preparation.

ACKNOWLEDGEMENTS

We are sincerely thankful to Indoco Remedies Ltd, Santacruz Mumbai & Symbiosis Co-Operative Pharmaceutical Ltd Sangli for allowing us to work at their esteemed premises and also oblige for providing working standards of atorvastatin calcium and pyridoxine hydrochloride as a gift sample.

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