Method Development and Validation for the Simultaneous Estimation of Pregabalin, Methylcobalamin and Nortriptyline in Sustained Release Tablet Dosage Form Using UV-Spectrophotometry

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

To method develop and validate simple, linear, rapid, cost effective, precise, accurate and economical for the simultaneous estimation of Pregabalin, Methylcobalamin and Nortriptyline in sustained release tablet dosage form using UV-Spectrophotometry. The drug obeyed the Beer's law

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and showed good correlation of concentration with absorption which reflect in linearity. The UV-Spectroscopic method was developed for estimation of Pregabalin, Methylcobalamin and Nortriptyline in sustained release tablet dosage form and also validated as per ICH guidelines. The method is based on measurement of absorbance at three wavelengths 219 nm, 222 nm, and 239 nm in Ethanol and distilled water. Linearity graph of Pregabalin, Methylcobalamin and Nortriptyline were found to be linear in the concentration ranges of 30-150 µg/ml, 0.6-3.0 µg/ml and 2-10 µg/ml. The correlation coefficient (R^2) values at three wavelengths 219 nm, 222 nm, and 239 nm for Pregabalin are 0.9996, 0.9929, and 0.9996; for Methylcobalamin (R^2) values are 0.9998, 0.9991, and 0.9997; for Nortriptyline (R^2) values are 1.0, 0.9992 and 0.9997. The low % RSD values indicate method to be accurate and precise. The % recoveries of Pregabalin, Methylcobalamin and Nortriptyline were in the range of (99.30%-1-1.61%), (98.89%-100.02%) and (99.69%-100.23%) which was within standard acceptance limits. All other validation parameters were performed by following respective ICH guidelines.

1. INTRODUCTION

Pregabalin is a derivative of [gamma-amino butyric acid - GABA] with anti-convulsant, analgesic and anti-epileptic activities. Pregabalin neither bind directly to GABA-A or GABA-B receptors and nor alter GABA degradation. Binding of pregabalin to [Voltage Dependent Calcium Channels - VDCC] subunits stop the influx of calcium and also the following neurotransmitters like dopamine, serotonin and nor epinephrine are prevented [1]. Even the MOA is not-known, but pregabalin specifically attaches to [alpha2delta - A2D] subunits of (VDCC) situated in the [Central nervous system – CNS].

IUPAC NAME of Pregabalin: (3S)-3-(amino methyl)-5-methylhexanoic acid.
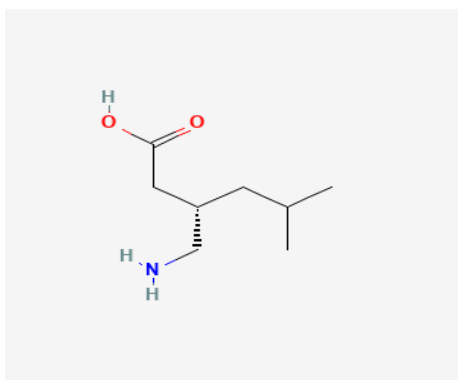

Fig. 1. Structure of pregabalin

Methylcobalamin is an active and synthetic form obtained from Vit. B12 which are able to pass through [Blood Brain Barrier – BBB], that can be administered in treating or preventing Vit.B12 deficiency and its allied complications.

Methylcobalamin is supposed to replace endogenous vitamin B12 [2]. Vitamin B12 is required for haematopoiesis, RNA and DNA production, haematopoiesis and fat, carbohydrate and protein metabolism. It helps in improving iron associated functions in body's metabolic cycle and it helps folic acid in choline synthesis.

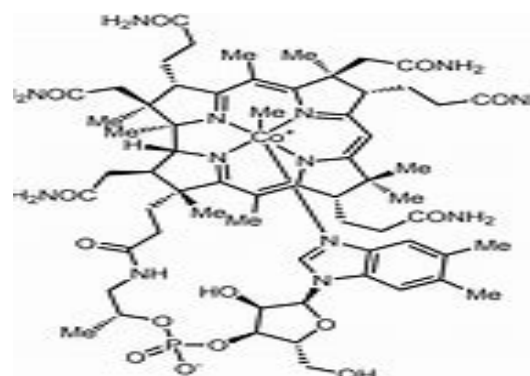IUPAC NAME of Methylcobalamin: Carbanide.

Fig. 2. Structure of methylcobalamin

Nortriptyline is an antidepressant agent which is prescribed for short-term treatment of disease depression and mental illness [3]. Nortriptyline agent inhibits the nor-epinephrine presynaptic receptors, and it works by restricting the uptake and reuptake of neurotransmitter and in increasing the concentration in the synaptic cleft of CNS. Nortriptyline binds to histaminergic, cholinergic and α-adrenergic receptors. For long-term treatment with nortriptyline, it shows a decrease of adrenergic receptors which leads to increased stimulation of adrenergic receptors.

IUPAC NAME of Nortriptyline: 3-(10, 11-Dihydro-5H-dibenzo [a, d] cyclohepten-5-ylidene)-N-methyl-1-propanamine.
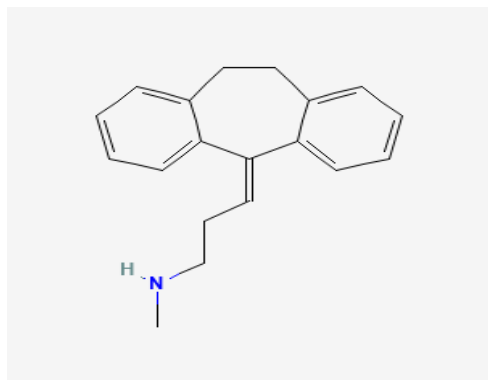

Fig. 3. Structure of nortriptyline

Pregabalin, Methylcobalamin and Nortriptyline are a three-drug combination medicine which is having its potential in treating neuropathic pain. It functions by lowering pain by monitoring calcium channel activity of the nerve cells. It helps in regulating mood and increases the chemical passengers' levels in brain that supports in protection of nerve fibers [4].

An attempt to make method rapid, easy, simple, accurate, cost-effective and economical, simultaneous estimation method for Pregabalin, Methylcobalamin and Nortriptyline in a sustained release form is developed and validated as per ICH guidelines.

2. MATERIALS AND METHODS

Instrumentation: UV Spectrophotometer
Double beam UV-visible spectrophotometer with two same cuvettes of 1cm light path made up of quartz was used for taking spectral readings. We observed spectral readings at four different wavelengths 219 nm, 222 nm and 239 nm. UV analyst was the software used to load readings on to UV-visible spectrophotometer.

2.1 Chemicals and Reagents

Standard reagents of Pregabalin and Nortriptyline were gifted by Medley Pharmaceuticals Ltd. as standard gift samples, Methylcobalamin were gifted by Flagship Biotech International Pvt. Ltd. Ethanol was made available from D Y Patil University School of Pharmacy, Nerul. All other reagents used were specifically of analytical grade [5]. All the lab apparatus and glassware's used for this study were made up of Borosilicate glass. The sustained release tablet formulations used which included all three drugs was manufactured by Medley Pharmaceuticals Ltd. Under the brand name Nortipan –M.

2.2 Preparation of Solutions [6]

1) **Preparation of Stock solution:**10 mg of Methylcobalamin, 10 mg of Nortriptyline and 10 mg of Pregabalin working standard was weighed with the help of analytical balance and transferred into 10 ml individual volumetric flasks appropriately and the volume of volumetric flask was made up to the 10 ml mark with the help of Ethanol as reagent. The mixture was homogenously dissolved with the help of Sonicator for 15 min's. At last final working concentration of Pregabalin, Methylcobalamin and Nortriptyline were 1000 µg/ml.

2) **Preparation of Calibration curve:** While preparing aliquots' of Pregabalin, Methylcobalamin and Nortriptyline 1 ml was withdrawn from stock solution and diluted with ethanol to prepare dilution. Later, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml and 1.2 ml of aliquots were taken from stock solution to prepare calibration curve by observing spectral measurements at all three wavelengths 219 nm, 222 nm, 225 nm and 239 nm. We get a series of concentration ranges for each drug such as 30-150 µg/ml for Pregabalin, 0.6-3.0 µg/ml for Methylcobalamin and 2 – 10 µg/ml for Nortriptyline.

3) **Preparation of Sample solution:** Weigh 20 tablets of Nortipan-M Sustained release dosage form. All tablets were added to mortar and pestle and were triturated till we get smooth powder. Then, powder quantity was weighed which was equivalent to the 75 mg of Pregabalin, 1.5 mg of Methylcobalamin and 10 mg of Nortriptyline and transferred to the volumetric flask. Later was diluted with ethanol. The entire solution was sonicated for 15 min's to get uniform homogenous solution, it was filtered with the help of Whatman paper and sample solution was collected for the study.

3. RESULTS AND DISCUSSION

The main ultimate goal of this study was to develop spectrophotometric study for Nortipan-M

Sustained release tablet by simultaneous equation method using Pregabalin, Methylcobalamin and Nortriptyline standards and sample solution [7].

3.1 Simultaneous Equation Method

This method is based upon Beer-Lambert's Law to estimate amount of absorption of drug at specified wavelength. In this method determination we have analyzed over wavelength ranges 219 nm, 222 nm and 239 nm. The absorbance of Pregabalin, Methylcobalamin and Nortriptyline were determined and studied with the help of formula [8].

Formula: The total absorbance of solution is the total sum of absorbance of individual components.

$$Cx = A_2*ay_1 – A_1*ay_2 / ax_2*ay_1 – (ax_2*ay_2)$$
$$Cy = A_1*ax_2 – A_2*ax_1 / ax_2*ay_1 – (ax_2*ay_2)$$

Where,

Cx and Cy = Concentration of drug X and Y.
A_1 and A_2 = Absorbance at $λ_1$ and $λ_2$.
ax_1 and ay_1 = Abosrptivity of drugs X and Y at $λ_1$.

ax_2 and ay_2 = Abosrptivity of drugs X and Y at $λ_2$.

3.2 Determination of absorptivity value

The absorptivity values for Pregabalin, Methylcobalamin and Nortriptyline were calculated using below mentioned formula and the results were presented in Table 1, Table 2 and Table 3.

Formula: Absorptivity = Absorbance/ Concentration.

3.3 Method Validation

3.3.1 Linearity

The Linearity of Pregabalin, Methylcobalamin and Nortriptyline was analyzed at different concentration regions and at different wavelengths [9]. The studied data showed linear in the ranges of 30-150 µg/ml, 0.6-3.0 µg/ml and 2-10 µg/ml. Evaluation based on visual characteristics was done by analyzing standard deviation, slope of calibration, regression factor and intercept. The values are thoroughly estimated and summarized in Table 1, Table 2 and Table 3.
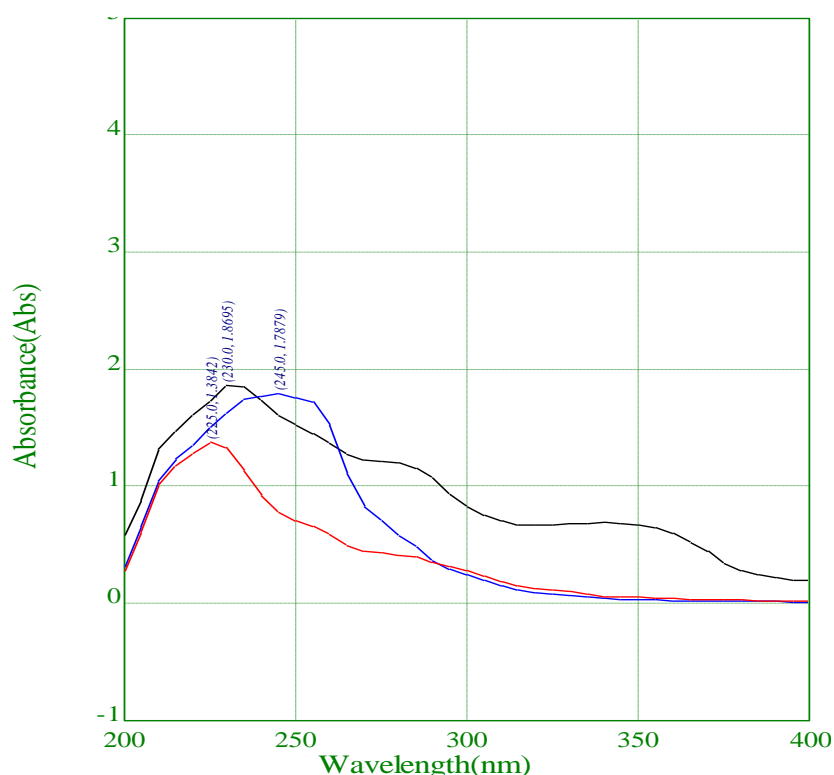

Fig. 4. Overlain spectra of standard Solution of Pregabalin, Methylcobalamin and Nortriptyline

Table 1. Absorptivity Value for Pregabalin (30-150 µg/ml)

Sr. No	Concentration (µg/ml)	Wavelength = 219 nm		Wavelength = 222 nm		Wavelength = 239 nm	
		Absorbance	Absorptivity	Absorbance	Absorptivity	Absorbance	Absorptivity
1	30 µg/ml	0.142	0.0047	0.147	0.0049	0.153	0.0051
2	60 µg/ml	0.288	0.0048	0.296	0.0049	0.312	0.0052
3	90 µg/ml	0.423	0.0047	0.443	0.0049	0.462	0.0051
4	120 µg/ml	0.575	0.0047	0.609	0.0050	0.615	0.0051
5	150 µg/ml	0.708	0.0047	0.699	0.0046	0.783	0.0052
		Mean:	0.0047	Mean:	0.0049	Mean:	0.0051
	S.D (Standard deviation)		0.000045	S.D	0.00015	S.D	0.000054
	% R.S.D (% Relative standard deviation)		0.947%	% R.S.D	3.121%	% R.S.D	1.066%

Table 2. Absorptivity Value for Methylcobalamin (0.6-3.0 µg/ml)

Sr. No	Concentration (µg/ml)	Wavelength = 219 nm		Wavelength = 222 nm		Wavelength = 239 nm	
		Absorbance	Absorptivity	Absorbance	Absorptivity	Absorbance	Absorptivity
1	0.6 µg/ml	0.025	0.041	0.021	0.035	0.032	0.053
2	1.2 µg/ml	0.051	0.042	0.044	0.036	0.063	0.052
3	1.8 µg/ml	0.077	0.042	0.064	0.036	0.094	0.052
4	2.4 µg/ml	0.101	0.042	0.085	0.035	0.128	0.053
5	3.0 µg/ml	0.127	0.042	0.109	0.036	0.158	0.052
		Mean:	0.042	Mean:	0.036	Mean:	0.052
		S.D	0.00044	S.D	0.00054	S.D	0.00054
		% R.S.D	1.070%	% R.S.D	1.539%	% R.S.D	1.045%

Table 3. Absorptivity value for Nortriptyline (2-10 µg/ml)

Sr. No	Concentration (µg/ml)	Wavelength = 219 nm		Wavelength = 222 nm		Wavelength = 239 nm	
		Absorbance	Absorptivity	Absorbance	Absorptivity	Absorbance	Absorptivity
1	2 µg/ml	0.096	0.048	0.077	0.038	0.126	0.063
2	4 µg/ml	0.191	0.047	0.156	0.039	0.252	0.063
3	6 µg/ml	0.286	0.047	0.234	0.039	0.372	0.062
4	8 µg/ml	0.384	0.048	0.312	0.039	0.504	0.063
5	10 µg/ml	0.480	0.048	0.380	0.038	0.620	0.062
		Mean:	0.048	Mean:	0.039	Mean:	0.063
		S.D	0.00054	S.D	0.00054	S.D	0.00054
		% R.S.D	1.151%	% R.S.D	1.419%	% R.S.D	0.875%

Table 4. Accuracy studies of Pregabalin, Methylcobalamin and Nortriptyline

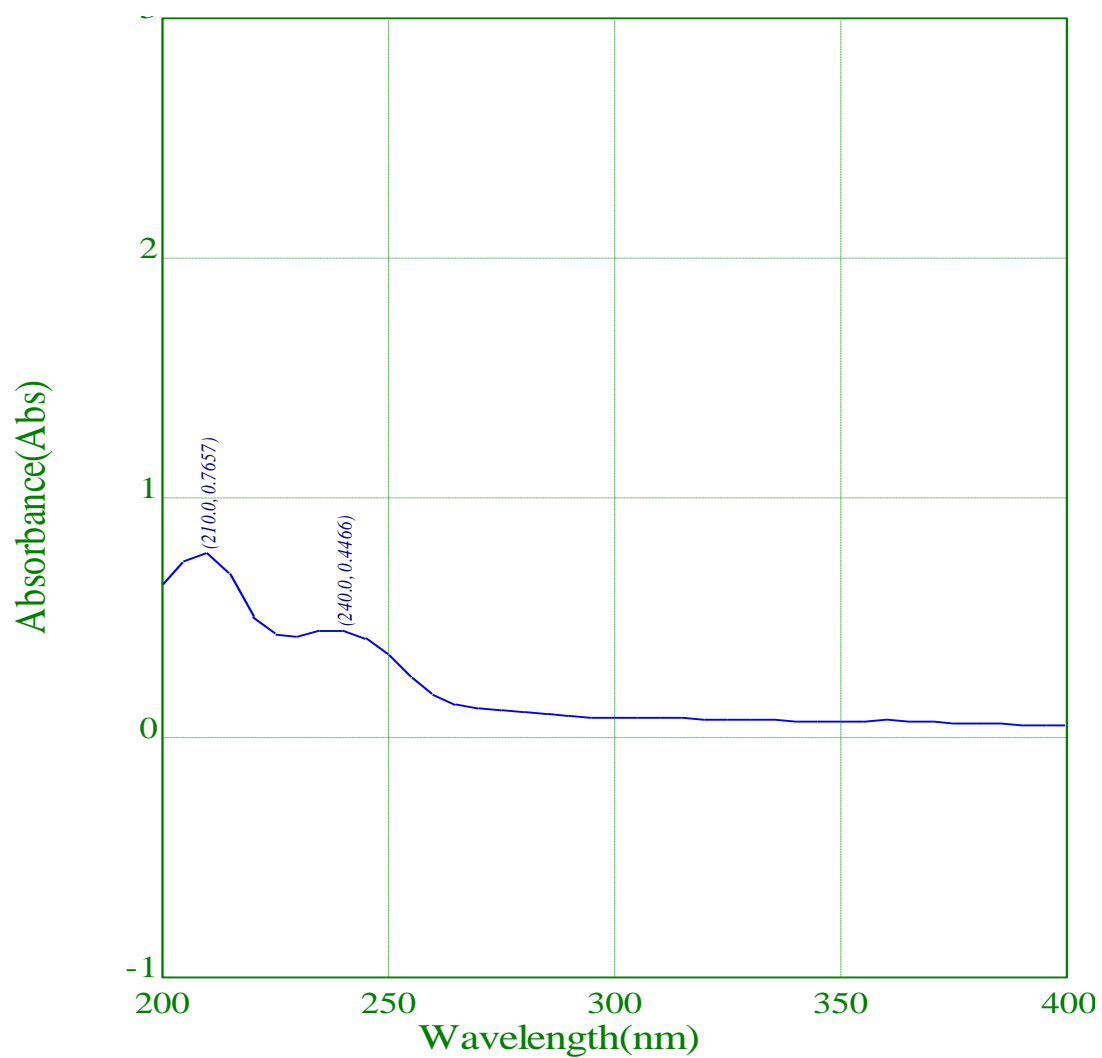| Drug	Spiked level (%)	Amount taken (µg/ml)	Amount added (µg/ml)	% Recovery
Pregabalin	80	50	50	99.41%
	100	50	70	99.30%
	120	50	90	101.61%
Methylcobalamin	80	1	1	99.48%
	100	1	1.2	100.02%
	120	1	1.4	98.89%
Nortriptyline	80	4	3.2	100.64%
	100	4	4	99.69%
	120	4	4.8	100.23%

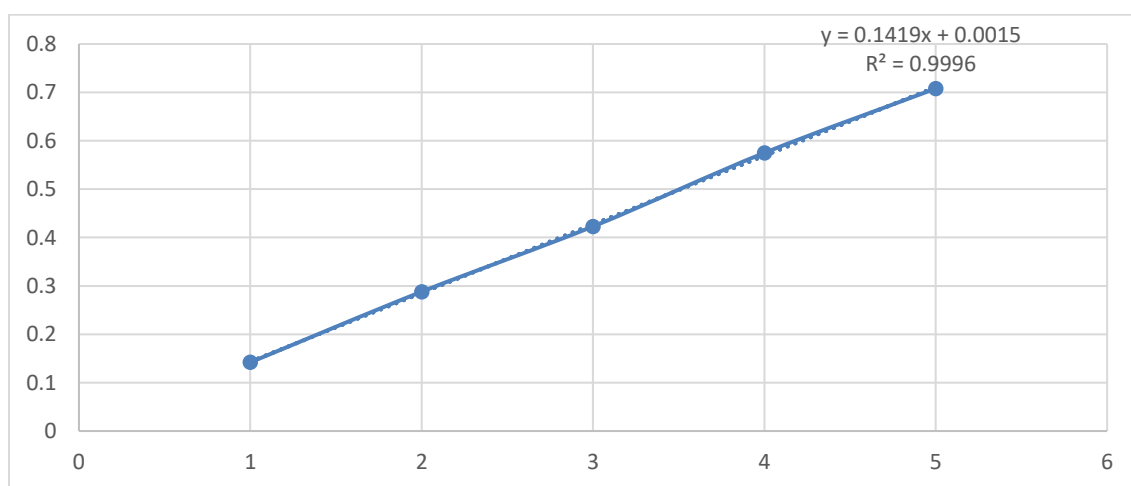**Fig. 5. Overlain spectra of formulation**

Fig. 6. Linearity graph of pregabalin at 219 nm
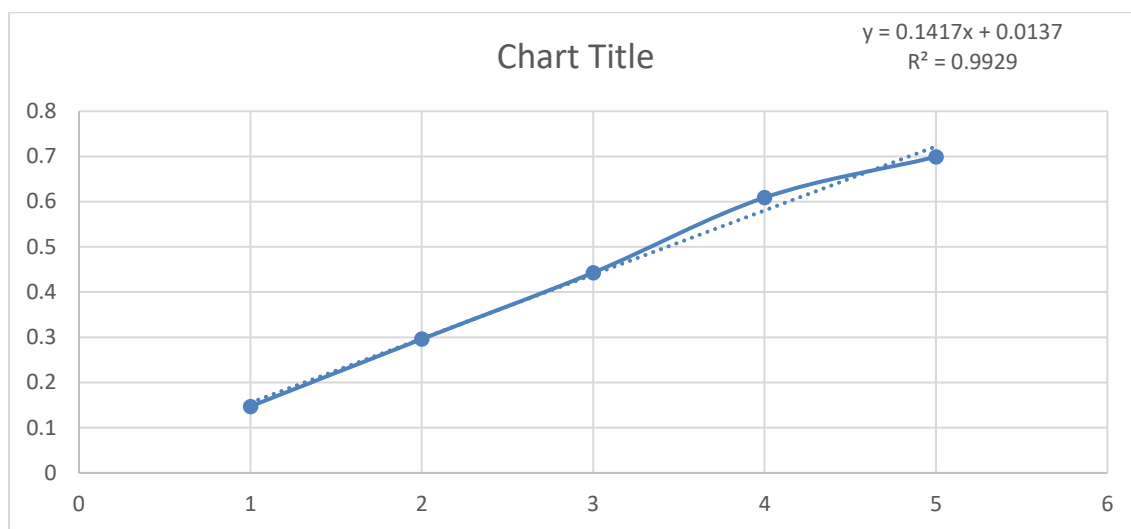
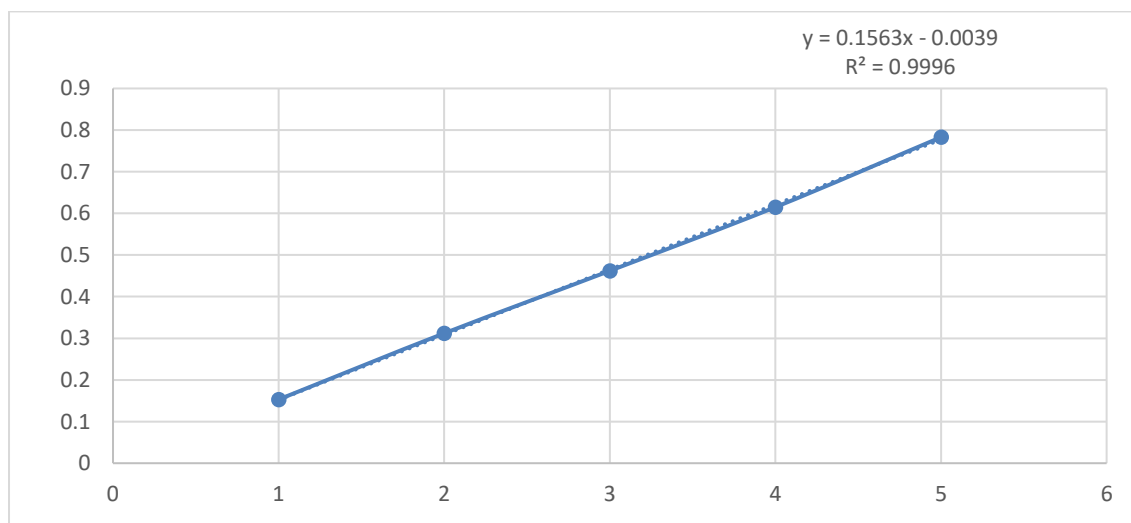**FIG. 7. Linearity graph of pregabalin at 222 nm**
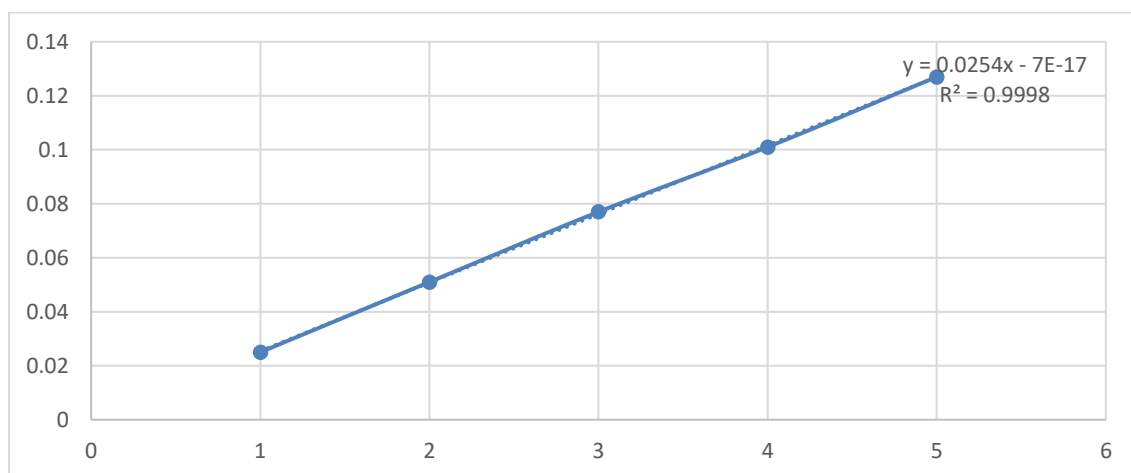

Fig. 8. Linearity graph of pregabalin at 239 nm

Fig. 9. Linearity graph of Methylcobalamin at 219 nm

Table 5. Intraday precision data for analysis of Pregabalin at 219 nm, 222 nm and 239 nm

Lvl No.	Concentration (µg/ml)	Absorbance			Mean			Standard Deviation (S.D)			% Relative Standard Deviation (R.S.D)		
		219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm
1	60 µg/ml	0.288 0.285 0.284	0.423 0.421 0.425	0.575 0.576 0.576	0.285	0.423	0.575	0.0020	0.0020	0.0005	0.729%	0.423%	0.100%
2	90 µg/ml	0.423 0.425 0.422	0.443 0.443 0.445	0.462 0.463 0.466	0.423	0.443	0.463	0.0015	0.0011	0.0020	0.361%	0.260%	0.449%
3	120 µg/ml	0.575 0.577 0.573	0.609 0.608 0.603	0.615 0.615 0.617	0.575	0.606	0.615	0.0020	0.0032	0.0011	0.348%	0.530%	0.188%

Table 6. Intraday precision data for analysis of Methylcobalamin at 219 nm, 222 nm and 239 nm

Lvl No.	Concentration (µg/ml)	Absorbance			Mean			Standard Deviation (S.D)			% Relative Standard Deviation (R.S.D)		
		219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm
1	1.2 µg/ml	0.051 0.050 0.051	0.044 0.046 0.043	0.063 0.061 0.061	0.050	0.044	0.061	0.0005	0.0015	0.0011	1.140%	3.446%	1.872%
2	1.8 µg/ml	0.077 0.075 0.074	0.064 0.063 0.067	0.094 0.093 0.095	0.075	0.064	0.094	0.0015	0.0020	0.0010	2.028%	3.219%	1.064%
3	2.4 µg/ml	0.101 0.103 0.104	0.085 0.088 0.089	0.128 0.125 0.130	0.102	0.087	0.127	0.0015	0.0020	0.0025	1.488%	2.384%	1.971%

Table 7. Intraday precision data for analysis of Nortriptyline at 219 nm, 222 nm and 239 nm

Lvl No.	Concentration (µg/ml)	Absorbance			Mean			Standard Deviation (S.D)			% Relative Standard Deviation (R.S.D)		
		219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm
1	4 µg/ml	0.191 0.193 0.194	0.156 0.157 0.153	0.252 0.251 0.255	0.192	0.155	0.252	0.0015	0.0020	0.0020	0.793%	1.340%	0.824%
2	6 µg/ml	0.286 0.286 0.285	0.234 0.236 0.239	0.372 0.370 0.374	0.285	0.236	0.372	0.0005	0.0025	0.0020	0.202%	1.065%	0.538%
3	8 µg/ml	0.384 0.385 0.385	0.312 0.315 0.316	0.504 0.505 0.507	0.384	0.314	0.505	0.0005	0.0020	0.0015	0.150%	0.662%	0.302%

Table 8. intraday precision data for analysis of nortipan-m Tablet formulation at 219 nm, 222 nm and 239 nm

Lvl No.	Days	Absorbance			Mean			Standard Deviation (S.D)			% Relative Standard Deviation (R.S.D)		
		219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm
1	Day 1	0.643 0.641 0.650	0.548 0.550 0.547	0.525 0.523 0.528	0.644	0.548	0.525	0.0047	0.0015	0.0025	0.733%	0.279%	0.479%
2	Day 3	0.517 0.514 0.514	0.430 0.428 0.431	0.436 0.437 0.434	0.515	0.429	0.435	0.0017	0.0015	0.0015	0.356%	0.356%	0.351%
3	Day 7	0.608 0.609 0.605	0.514 0.514 0.517	0.503 0.505 0.501	0.607	0.515	0.503	0.0020	0.0017	0.0020	0.343%	0.336%	0.398%
4	Day 10	0.590 0.588 0.591	0.431 0.431 0.434	0.403 0.407 0.408	0.589	0.432	0.406	0.0015	0.0017	0.0026	0.259%	0.401%	0.652%

Table 9. Interday precision data for analysis of pregabalin at 219 nm, 222 nm and 239 nm

Lvl No.	Hours	Absorbance			Mean			Standard Deviation (S.D)			% Relative Standard Deviation (R.S.D)		
		219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm
1	0 hr	0.282 0.280 0.279	0.419 0.421 0.421	0.570 0.571 0.576	0.280	0.420	0.572	0.0015	0.0011	0.0032	0.545%	0.275%	0.562%
2	3 hr	0.269 0.271 0.274	0.417 0.410 0.401	0.565 0.569 0.571	0.271	0.409	0.568	0.0025	0.0080	0.0030	0.927%	1.959%	0.538%
3	6 hr	0.273 0.275 0.671	0.419 0.428 0.428	0.569 0.571 0.573	0.273	0.425	0.571	0.0020	0.0051	0.0020	0.733%	1.223%	0.350%

Table 10. Interday precision data for analysis of Methylcobalamin at 219 nm, 222 nm and 239 nm

Lvl No.	Hours	Absorbance			Mean			Standard Deviation (S.D)			% Relative Standard Deviation (R.S.D)		
		219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm
1	0 hr	0.051 0.052 0.050	0.046 0.044 0.043	0.063 0.062 0.066	0.051	0.044	0.063	0.0010	0.0015	0.0020	1.961%	3.446%	3.270%
2	3 hr	0.053 0.058 0.059	0.045 0.046 0.048	0.064 0.068 0.060	0.056	0.046	0.064	0.0032	0.0015	0.0040	5.673%	3.297%	6.250%
3	6 hr	0.051 0.050 0.057	0.043 0.041 0.046	0.067 0.069 0.061	0.052	0.043	0.065	0.0037	0.0025	0.0041	7.188%	5.808%	6.340%

Table 11. Interday Precision data for analysis of Nortriptyline at 219 nm, 222 nm and 239 nm

Lvl No.	Hours	Absorbance			Mean			Standard Deviation (S.D)			% Relative Standard Deviation (R.S.D)		
		219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm
1	0 hr	0.191 0.190 0.194	0.155 0.151 0.153	0.250 0.251 0.250	0.191	0.153	0.250	0.0020	0.0020	0.0005	1.086%	1.307%	0.231%
2	3 hr	0.197 0.196 0.190	0.152 0.157 0.156	0.250 0.251 0.257	0.194	0.155	0.254	0.0037	0.0026	0.0032	1.948%	1.707%	1.262%
3	6 hr	0.196 0.194 0.193	0.150 0.151 0.153	0.251 0.258 0.256	0.194	0.151	0.255	0.0015	0.0015	0.0030	0.786%	1.009%	1.914%

Table 12. Interday precision data for analysis of Nortipan-m Tablet formulation at 219 nm, 222 nm and 239 nm

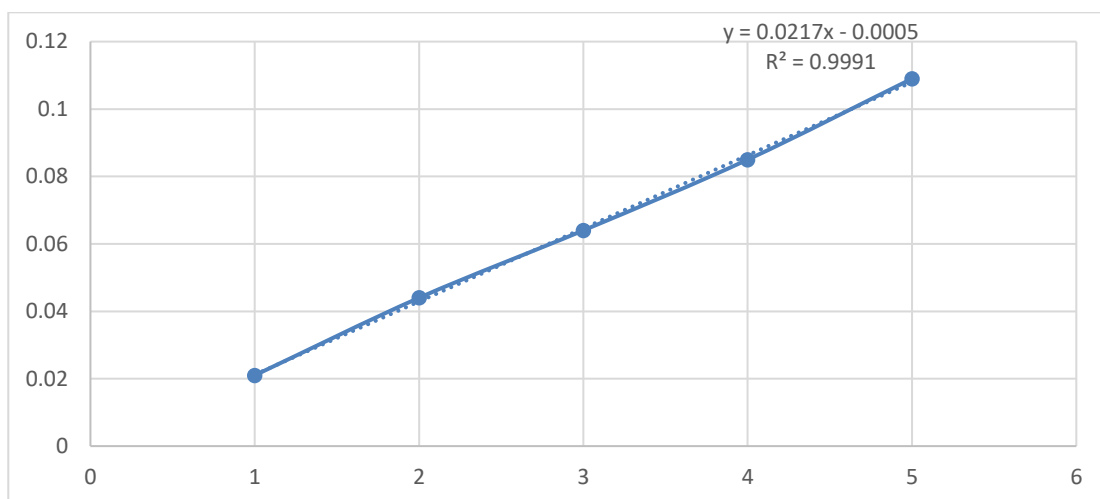| Lvl No.	Hours	Absorbance			Mean			Standard Deviation (S.D)			% Relative Standard Deviation (R.S.D)		
		219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm
1	0 hr	0.621 0.616 0.619	0.437 0.435 0.433	0.426 0.421 0.427	0.618	0.435	0.424	0.0025	0.0020	0.0032	0.407%	0.460%	0.757%
2	3 hr	0.510 0.508 0.513	0.414 0.411 0.412	0.418 0.417 0.419	0.510	0.412	0.418	0.0025	0.0015	0.0010	0.493%	0.370%	0.239%
3	6 hr	0.678 0.675 0.675	0.575 0.577 0.573	0.550 0.551 0.555	0.676	0.575	0.552	0.0017	0.0020	0.0026	0.256%	0.348%	0.479%

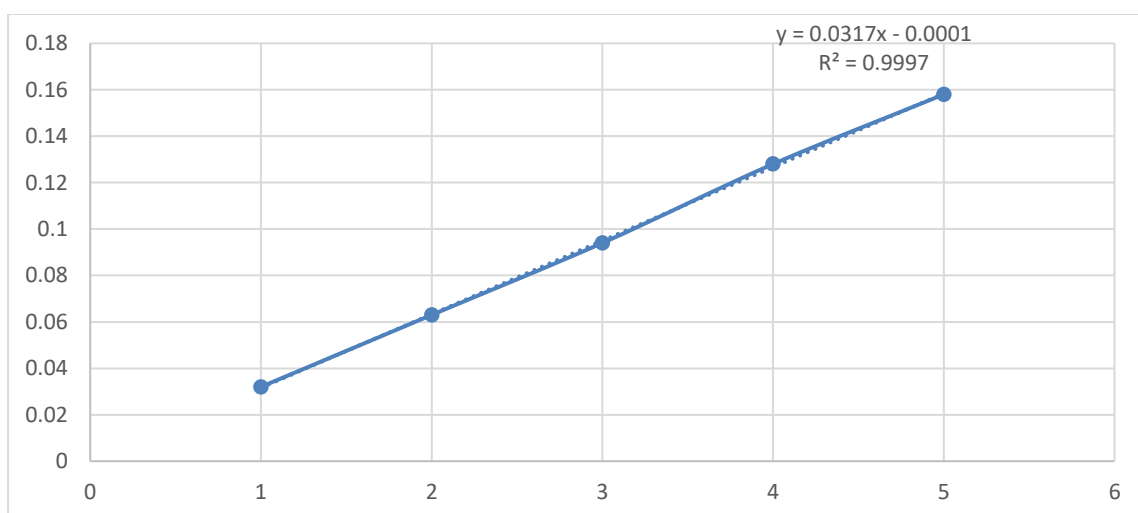**Fig. 10. Linearity graph of Methylcobalamin at 222 nm**

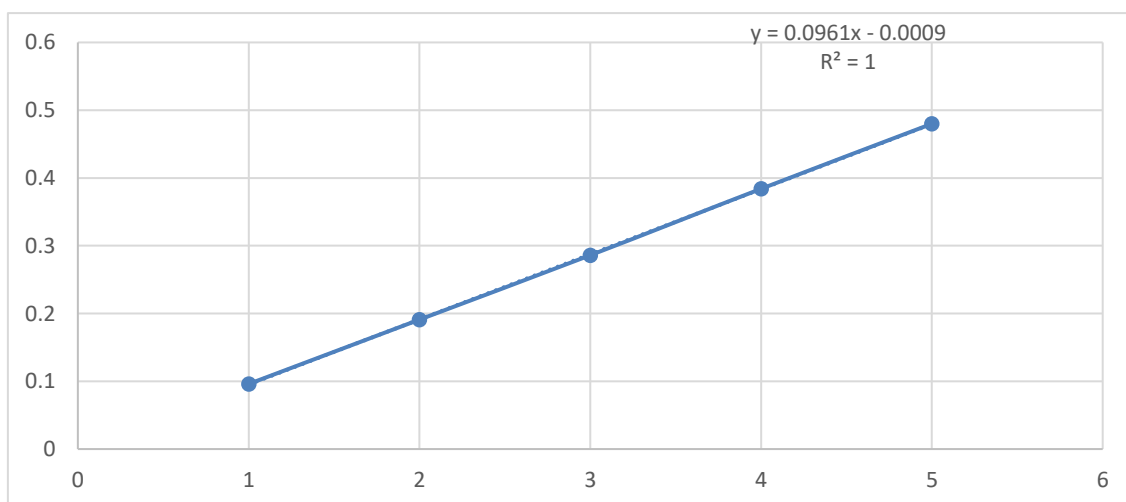**Fig. 11. Linearity graph of Methylcobalamin at 239 nm**
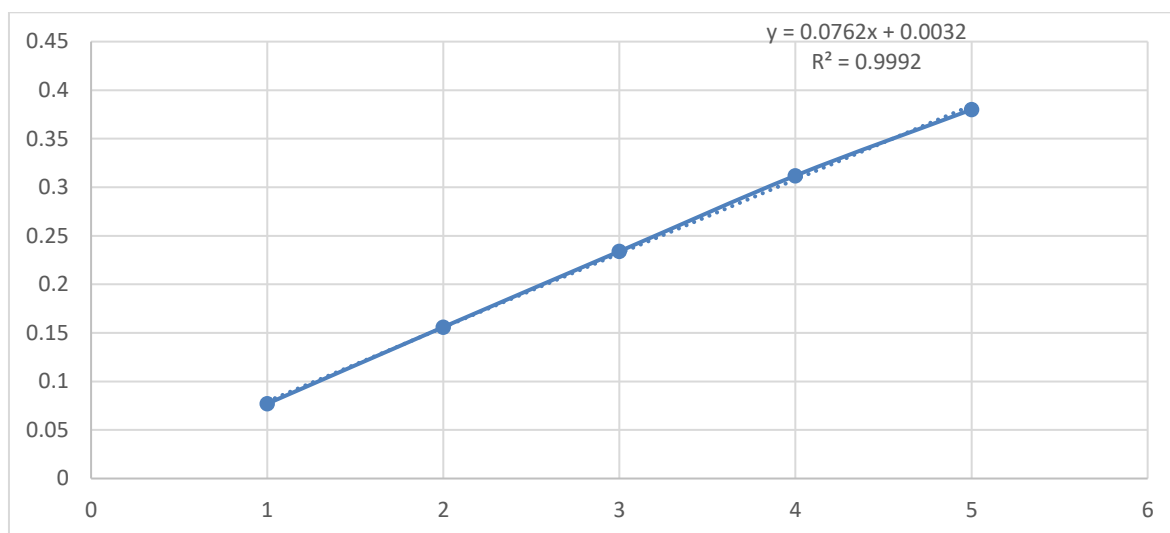

Fig. 12. Linearity graph of nortriptyline at 219 nm

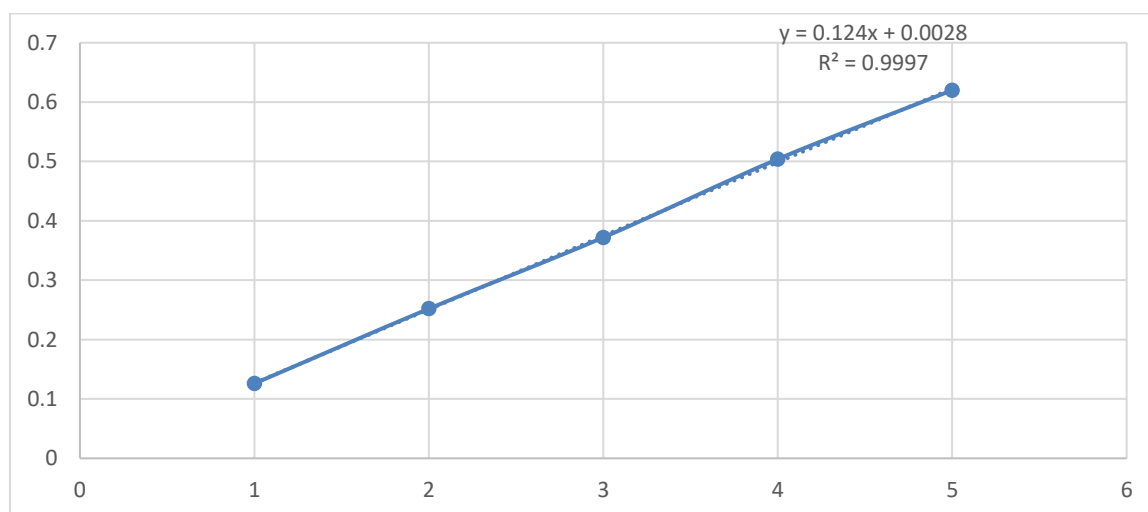**Fig. 13. Linearity graph of Nortriptyline at 222 nm**

Fig. 14. Linearity graph of Nortriptyline at 239 nm

3.3.2 Limit of Detection and Limit of Quantification [12]

The limit of detection (LOD) and limit of quantification (LOQ) for three drugs were calculated practically and theoretically following ICH guidelines. Data mentioned in Table 16 .

LOD = 3.3 x standard deviation of response / slope of calibration curve
LOQ = 10 x standard deviation of response / slope of calibration curve

3.3.3 Accuracy (% Recovery) [10]

The accuracy of the drug sample was studied to calculate % recovery. Accuracy was measured for Pregabalin, Methylcobalamin and Nortriptyline at concentration ranges 30-150 μg/ml, 0.6-3.0 μg/ml and 2-10 μg/ml. The % recovery ranges for each drug was observed to be (99.30%-1-1.61%), (98.89%-100.02%) and (99.69%-100.23%). (Table 4).

Precision [11]

Intraday, Interday and Repeatability are the parameters observed for three solutions of three drug concentrations within same day and within 10 days of time-span. Mean, Standard Deviation and % Relative Standard Deviation was calculated respectively. (Table 5 to Table 5).

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Table 13. Repeatability data for Pregabalin at 219 nm, 222 nm and 239 nm

	Absorbance		
	219 nm	**222 nm**	**239 nm**
90 µg/ml	0.423	0.443	0.462
	0.419	0.440	0.463
	0.418	0.439	0.465
	0.421	0.441	0.461
	0.421	0.440	0.466
Mean	0.420	0.440	0.463
Standard Deviation	0.0019	0.0015	0.0020
%Relative Standard Deviation	0.464%	0.344%	0.447%

Table 14. Repeatability data for methylcobalamin at 219 nm, 222 nm and 239 nm

	Absorbance		
	219 nm	**222 nm**	**239 nm**
1.8 µg/ml	0.077	0.064	0.094
	0.080	0.063	0.091
	0.081	0.067	0.093
	0.084	0.069	0.095
	0.076	0.060	0.097
Mean	0.0796	0.0646	0.094
Standard Deviation	0.0032	0.0035	0.0022
%Relative Standard Deviation	4.032%	5.429%	2.379%

Table 15. Repeatability data for nortriptyline at 219 nm, 222 nm AND 239 nm

	Absorbance		
	219 nm	**222 nm**	**239 nm**
6 µg/ml	0.286	0.234	0.372
	0.290	0.237	0.371
	0.285	0.238	0.371
	0.289	0.231	0.368
	0.291	0.233	0.365
Mean	0.288	0.234	0.369
Standard Deviation	0.0025	0.0028	0.0028
%Relative Standard Deviation	0.898%	1.228%	0.780%

Table 16. Iod and Loq of Pregabalin, Methylcobalamin and Nortriptyline

	Parameters					
Drugs	**LOD**			**LOQ**		
	219 nm	**222 nm**	**239 nm**	**219 nm**	**222 nm**	**239 nm**
Pregabalin	0.04	0.04	0.01	0.14	0.14	0.31
Methylcobalamin	0.06	0.22	0.11	0.19	0.69	0.34
Nortriptyline	0.05	0.08	0.05	0.15	0.26	0.16

Table 17. Summary of method validation parameters for pregabalin, Methylcobalamin and Nortriptyline

Parameters	Pregabalin			Methylcobalamin			Nortriptyline		
	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm	219 nm	222 nm	239 nm
Calibration range (µg/ml)	30-150 µg/ml	30-150 µg/ml	30-150 µg/ml	0.6-3.0 µg/ml	0.6-3.0 µg/ml	0.6-3.0 µg/ml	2-10 µg/ml	2-10 µg/ml	2-10 µg/ml
Slope	0.1419	0.1417	0.1563	0.0254	0.0217	0.0317	0.0961	0.0762	0.124
Correlation Coefficient (R^2)	0.9996	0.9929	0.9996	0.9998	0.9991	0.9997	1.00	0.9992	0.9997
Precision (%R.S.D)									
Intraday	0.348% -0.7295	0.260%-0.530%	0.100%- 0.449%	1.140%-2.028%	2.384%-3.446%	1.064%-1.971%	0.150%-0.793%	0.662%-1.340%	0.302%-0.824%
Interday	0.545%-0.927%	0.275%-1.959%	0.350%-0.562%	1.961%-7.188%	3.297%-5.808%	3.270%-5.808%	1.086%-1.948%	1.009%-1.707%	0.231%-1.914%
Repeat-ability	0.464%	0.344%	0.447%	4.032%	5.429%	2.379%	0.898%	1.228%	0.780%
LOD (µg/ml)	0.04	0.04	0.01	0.06	0.22	0.11	0.05	0.08	0.05
LOQ (µg/ml)	0.14	0.14	0.01	0.19	0.69	0.34	0.15	0.26	0.16

Table 18. Analysis of marketed formulation

Sr.No	Drug	Amount labelled	Amount found	% Amount found
1	Pregabalin	75 mg	74.78 mg	99.70%
2	Methylcobalamin	1.5 mg	1.49 mg	99.33%
3	Nortriptyline	10 mg	9.98 mg	99.80%

4. CONCLUSION

The Simultaneous equation method was developed for simultaneous estimation of Pregabalin, Methylcobalamin and Nortriptyline in their sustained release tablet form Nortipan-M. It was validated and estimated by following ICH guidelines. The Linearity was observed in the range of (30-150 μg/ml) for Pregabalin, (0.6-3.0 μg/ml) for Methylcobalamin and (2-10 μg/ml) for Nortriptyline. The correlation coefficient (R^2) values at three wavelengths 219 nm, 222 nm, and 239 nm for Pregabalin are 0.9996, 0.9929, and 0.9996; for Methylcobalamin (R^2) values are 0.9998, 0.9991, and 0.9997; for Nortriptyline (R^2) values are 1.0, 0.9992 and 0.9997. The % recoveries of Pregabalin, Methylcobalamin and Nortriptyline were in the range of (99.30%-1-1.61%), (98.89%-100.02%) and (99.69%-100.23%) which was within standard acceptance limits. By estimating all evaluation and validation parameters (Linearity, LOD, LOQ) we concluded that the simultaneous estimation method for Pregabalin, Methylcobalamin and Nortriptyline was simple, cost effective and economical in its determination in Sustained release tablet. The assay results were achieved and it proved that a successful analytical method can be used for routine analysis of Pregabalin, Methylcobalamin and Nortriptyline in Sustained release tablet by UV Spectrophotometry.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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