

**APPLICATION OF UV SPECTROPHOTOMETRIC METHOD  
DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS  
ESTIMATION OF GABAPENTIN AND MECOBALAMINE IN  
PHARMACEUTICAL DOSAGE FORM**

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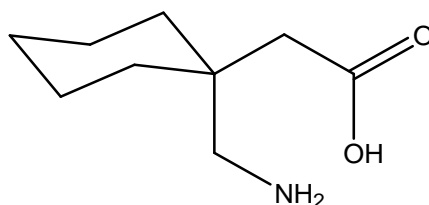
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**ABSTRACT**

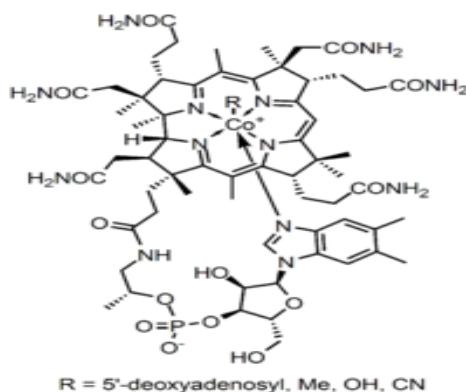
Two precise, simple, accurate, reproducible, rapid and economical UV spectrophotometric methods have been developed for simultaneous estimation of gabapentine and mecobalamine in tablet dosage form by using 0.1N HCL as solvent. Method I is based on formation and solving of simultaneous equation method, known as Vierordt's method. Gabapentine and mecobalamine show absorbance maxima at 283 nm and 274 nm respectively, so absorbance was measured at the same wavelength for the estimation of gabapentine and mecobalamine in tablet combination. Method II is based on principle of Q-analysis, known as absorbance ratio method. Absorbance was measured at 285 nm (isobestic point) and 274 nm ( $\lambda_{max}$  of Methylcobalamin.).

gabapentine and mecobalamine obeys Beer's law in the concentration range of 5 to 30  $\mu\text{g/ml}$ . Percentage estimation of gabapentine and mecobalamine in tablet dosage form by method I is 99 and 99.85 and by method II is 99 and 99.89 respectively with standard deviation  $\leq 2$ . Methods are validated according to ICH guidelines and can be applied for routine analysis of drugs in tablet dosage form.

**KEYWORDS:** Gabapentine and mecobalamine.

**INTRODUCTION****Fig. 1: Gabapentin.**

Gabapentin (GBP) (1-(amino methyl) cyclohexaneacetic acid), is an antiepileptic drug which is a structural analogue of neurotransmitter  $\gamma$ -amino butyric acid (GABA). Methylcobalamin (MC) is a coenzyme form of Vitamin B<sub>12</sub> which is biologically active.<sup>[1]</sup> Several methods are cited in literature for determination of GBP & MC individually by UV-Vis spectroscopy<sup>1</sup>, HPLC<sup>2</sup>, LC-MS<sup>3,5</sup>, GC-MS<sup>4</sup> & HPTLC<sup>6</sup> for individual drug but for combination only RPHPLC<sup>7</sup> method was reported. Hence the objective of work is to develop a simple, economic & precise UV is spectrophotometric method for this combination in commercial dosage form like tablet.<sup>[2]</sup>

**Fig. 2: Methylcobalamin.**

Vitamin B<sub>12</sub>, also called cobalamin, is a water-soluble vitamin that has a key role in the normal functioning of the nervous system via the synthesis of myelin.<sup>[3]</sup> Methylcobalamin is equivalent physiologically to vitamin B<sub>12</sub> and can be used to prevent or treat pathology arising from a lack of vitamin B<sub>12</sub> intake (vitamin B<sub>12</sub> deficiency). Methylcobalamin is also used in the treatment of peripheral neuropathy, diabetic neuropathy, and as a preliminary treatment for amyotrophic lateral sclerosis.<sup>[4,5]</sup>

The review of literature revealed that several methods are available for the determination of gabapentin and vitamin B<sub>12</sub> individually. Reported methods for estimation of gabapentin were Spectrophotometric<sup>[6]</sup>, HPLC<sup>[7,8]</sup>, HPTLC<sup>[9]</sup>, and LC-MS<sup>[10]</sup> and for vitamin B<sub>12</sub> were

Spectrophotometric<sup>[11]</sup>, HPLC<sup>[11]</sup> and HPTLC.<sup>[11]</sup> But there is no any analytical method yet reported for simultaneous estimation of these drugs in combination.

### UV Spectrophotometric method

#### Materials and Methods

UV-Visible double beam spectrophotometer, (Jasco model 2201) with spectral bandwidth of 1 nm, wavelength accuracy of  $\pm 0.3$  nm and a pair of 1mm matched quartz cell was used. The commercially available Gabapentine and Methylcobalamin was procured from local market.

#### Preparation of standard stock solution and calibration curve

The standard stock solution of Gabapentine and Methylcobalamin were prepared by dissolving 10 mg of Gabapentine and Methylcobalamin in 0.1 N HCl in a separate 100 ml volumetric flask and final volume was adjusted with the same solvent in 100 ml of volumetric flask to get a solution containing 100  $\mu\text{g/ml}$  of Gabapentine and 100  $\mu\text{g/ml}$  of Methylcobalamin respectively.

Working standard solutions of 10 $\mu\text{g/ml}$  for Gabapentine and Methylcobalamin were scanned in the entire UV range of 200-400 nm to determine their  $\lambda_{\text{max}}$ . The  $\lambda_{\text{max}}$  of Gabapentine and Methylcobalamin is found to be 283 nm and 274 nm respectively and isobestic point is at 285 nm from overlain spectra as shown in **Fig.3**. Six working standard solutions with concentration 5, 10, 15, 20, 25, 30 $\mu\text{g/ml}$  for Gabapentine and Methylcobalamin were prepared in 0.1N HCl from stock solution. The absorbance of resulting solutions were measured at their respective  $\lambda_{\text{max}}$  and isobestic point and plotted a calibration curve to get the linearity and regression equation as shown in **Fig. 4 and 5**.

#### Method I (Simultaneous equation method)

Simultaneous equation method of analysis is based on the absorption of both drugs at their wavelength maximum. Two wavelengths selected for the development of the simultaneous equation are 283 nm and 274 nm. The absorptivity values were determined for Gabapentine are 0.14621( $a_{x1}$ ), 0.08121 ( $a_{x2}$ ) and for Methylcobalamin are 0.07191 ( $a_{y1}$ ), 0.1771 ( $a_{y2}$ ) at 283nm and 274 nm respectively. These values are means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in equation 1 and 2 to obtain the concentration of these drugs.

$$C_{\text{Gabapentine}} = \frac{(A_2 \times a_{y1}) - (A_1 \times a_{y2})}{a_{x2} \times a_{y1} - a_{x1} \times a_{y2}} \dots\dots\dots \text{Eqn. 1}$$

$$C_{\text{Methylcobalamin}} = \frac{(A_1 \times ax_2) - (A_2 \times ax_1)}{ax_2 \times ay_1 - ax_1 \times ay_2} \dots\dots\dots \text{Eqn.2}$$

Where C<sub>FLX</sub> and C<sub>QTF</sub> are concentration of Gabapentine and Methylcobalamin respectively in µg/ml. A<sub>1</sub> and A<sub>2</sub> were the absorbance of the sample at 283 nm and 274 nm respectively.

**Method II (Absorbance ratio method)**

Absorbance ratio method of analysis is based on the absorbance at two selected wavelengths, one of which is an isobestic point and the other being the absorption maximum of one of the two drugs. From overlain spectra (**Fig.3**) 285 nm (isobestic point) and 274 nm (λ<sub>max</sub> of Methylcobalamin) are selected for the formation of Q absorbance equation (Eqn.3 and 4). The absorptivity values determined for Gabapentine are 0.08121 (ax<sub>1</sub>), 0.04958 (ax<sub>2</sub>) and for Methylcobalamin are 0.17710 (ay<sub>1</sub>), 0.17794 (ay<sub>2</sub>) at 274 nm and 285 nm respectively. These values are means of six estimations. The absorbance and absorptivities at these wavelengths were substituted in equation 3 and 4 to obtain the concentration of these drugs.

$$C_{\text{Gabapentine}} = \frac{Q_M - Q_Y}{Q_Y - Q_X} \times \frac{A_1}{ax_1} \dots\dots\dots \text{Eqn.3}$$

$$C_{\text{Methylcobalamin}} = \frac{Q_M - Q_X}{Q_X - Q_Y} \times \frac{A_1}{ay_1} \dots\dots\dots \text{Eqn.4}$$

Q<sub>M</sub>, Q<sub>X</sub> and Q<sub>Y</sub> were obtained from Eqn.no.5, 6, 7 respectively.

$$Q_M = \frac{A_2}{A_1} \dots\dots\dots \text{Eqn.5}$$

$$Q_X = \frac{ax_2}{ax_1} \dots\dots\dots \text{Eqn.6}$$

$$Q_Y = \frac{ay_2}{ay_1} \dots\dots\dots \text{Eqn.7}$$

Where C<sub>FLX</sub> and C<sub>QTF</sub> are concentration of Gabapentine and Methylcobalamin respectively in µg/ml. A<sub>1</sub> and A<sub>2</sub> were the absorbance of the sample at 285 nm and 274 nm respectively.

**Validation of developed methods<sup>[13]</sup>**

**Linearity**

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed method. For method I and II, the Beer-Lambert’s concentration range was found to

be 5-30  $\mu\text{g/ml}$  for both Gabapentine and Methylcobalamin. The linearity data of both methods are presented in **Table.1**.

### *Accuracy*

To check the accuracy of the proposed methods, recovery studies were carried out at 80, 100 and 120% of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The results of the recovery studies are shown in **Table.2**.

### *Repeatability*

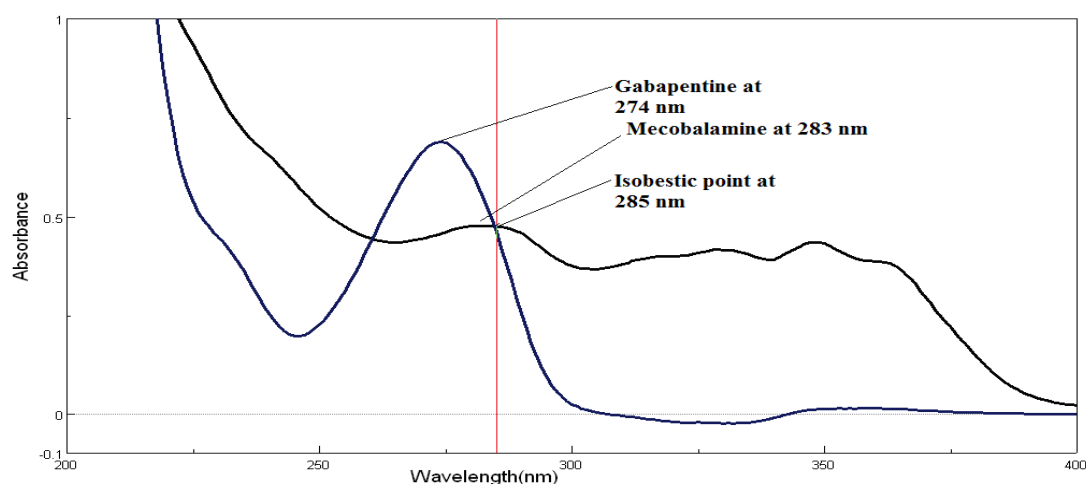
To check the degree of repeatability of these methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with synthetic mixture. The standard deviation and coefficient of variation were calculated. The results of statistical evaluation are given in **Table.2**.

### *Intermediate Precision (Interday and Intraday precision)*

The Interday and intraday precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively. The results of the same are presented in **Table.3**.

### *Limit of Detection (LOD) and Limit of Quantitation (LOQ)*

The LOD and LOQ of Gabapentine and Methylcobalamin by proposed methods were determined using calibration standards. LOD and LOQ were calculated as  $3.3 \sigma/S$  and  $10 \sigma/S$  respectively. Where S is the slope of the calibration curve and  $\sigma$  is the standard deviation of response. The results of the same are quoted in **Table.3**.



**Fig. 3: Overlain spectra of Gabapentine and Methylcobalamin.**

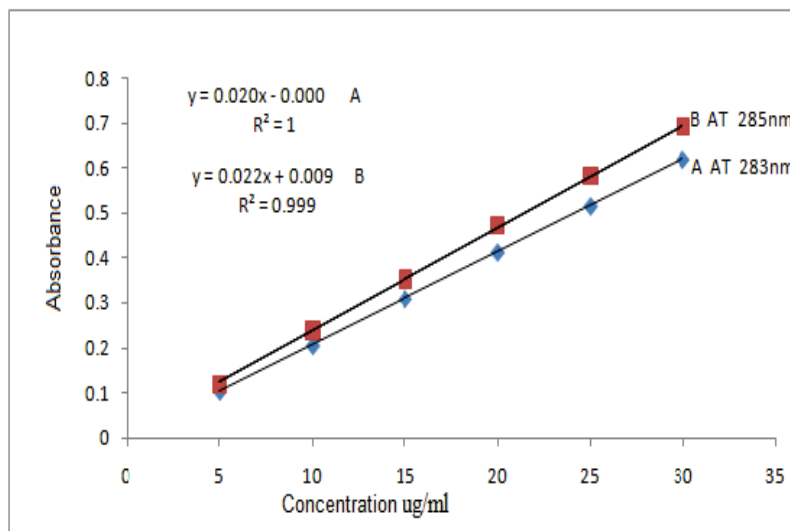


Fig. 4: Calibration curve and regression equation of Gabapentine in 0.1N HCl.

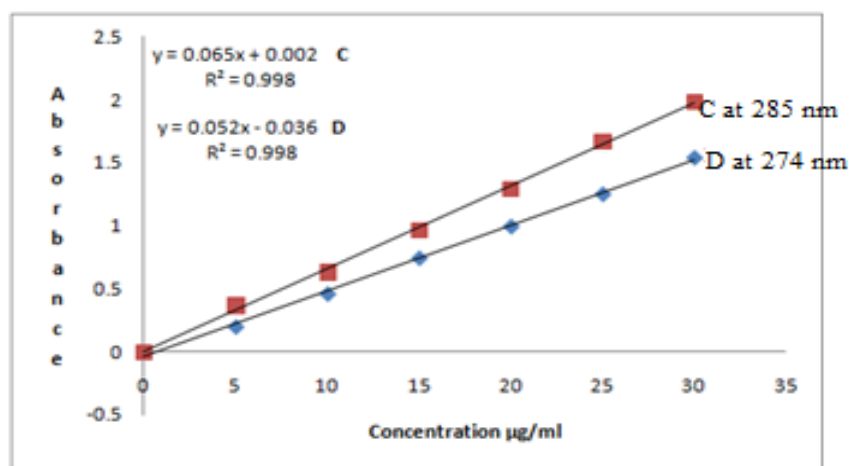


Fig. 5: Calibration curve and regression equation of Methylcobalamin in 0.1N HCl.

Table 1: Optical Characteristics for Gabapentine and Methylcobalamin.

Parameters	Gabapentine		Methylcobalamin	
	283nm	285nm	274nm	285nm
Working wavelength	283nm	285nm	274nm	285nm
Bear's law limit(µg/ml)	5-30	5-30	5-30	5-30
Absorptivity*	0.1462	0.0495	0.1771	0.1779
Correlation coefficient*	0.999	0.997	0.998	0.998
Intercept*	0.008	0.026	0.036	0.002
Slope*	0.026	0.047	0.052	0.065

\*Average of six estimations.

**Table 2: Recovery studies.**

Method	Level of recovery (%)	% Recovery $\pm$ S.D.#	
		Gabapentine	Methylcobalamin.
I	80	98.5 $\pm$ 0.45	99.45 $\pm$ 0.82
	100	99.42 $\pm$ 0.60	100.5 $\pm$ 0.45
	120	99.60 $\pm$ 0.97	101.2 $\pm$ 0.54
II	80	98.56 $\pm$ 0.54	100.54 $\pm$ 0.23
	100	99.45 $\pm$ 0.65	101.2 $\pm$ 0.12
	120	100.56 $\pm$ 0.78	99.60 $\pm$ 0.45

#mean of three determinations, SD: Standard Deviation

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