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UV-VIS SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF GABAPENTIN AND METHYLCOBALAMIN IN BULK AND TABLET

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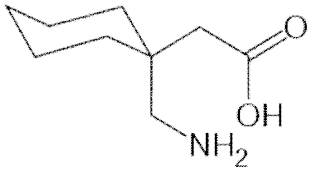
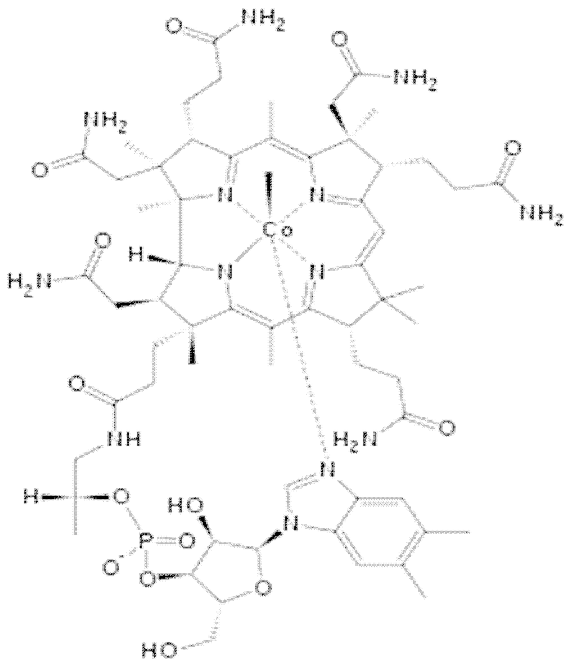
ABSTRACT: Fixed dose combination tablet containing Gabapentin & Methylcobalamin is widely used for peripheral neuropathy. A simple, economic & precise UV-Vis spectrophotometric method has been developed for the estimation of Gabapentin & Methylcobalamin in tablet dosage form. The estimation was based upon measurement of absorbance of tablet in distilled water at λmax 351nm for Methylcobalamin and 405nm for Gabapentin after reacting with 0.2% ninhydrine in N, N'- dimethylformamide. Beer Lambert's law obeyed over a concentration range of 50-300µg/ml for Gabapentin (r^2 = 0.9949) & 1-7µg/ml for Methylcobalamin (r^2 = 0.9993). The mean results of estimation in tablet were 100.43±0.15% & 100.48±0.18% by standard curve method and 100.58±0.17% and 102.31±1.25% by double point standardization of the label claim for Gabapentin & Methylcobalamin respectively. The method has been validated with respect to linearity, range, accuracy & precision.
KEY WORDS: UV-Vis Spectrophotometry, Gabapentin, Methylcobalamin.

INTRODUCTION

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



Gabapentin



Methylcobalamin

EXPERIMENTAL

INSTRUMENTS & REAGENTS

Uv-Vis spectrophotometer, make- JASCO, model- UV V-630 with 1.0 cm matched quartz cells was used. Chemicals of S. D. fine chemicals were used for analysis like Ninhydrine AR, N, N'-dimethylformamide AR. Reference samples of GBP & MC were procured as gift samples from Wockhardt Ltd. & Merck Pharmaceuticals Ltd. India. Tablet dosage form Gabaneuron (Gabapentin-300mg + Methylcobalamin-0.5mg) of Aristo pharmaceuticals was procured from market**.** Distilled water was used for the preparation of all solutions.

GENERAL PROCEDURE

PREPARATION OF WORKING STANDARD SOLUTIONS

Gabapentin 50mg weighed accurately and transferred to a 50ml volumetric flask, dissolved & diluted to volume with distilled water to get stock solution of 1000µg/ml. From this various dilutions of 50, 100, 150, 200, 250 and 300 µg/ml were made by reacting each dilution with 2ml of 0.2% ninhydrin in N,N'-dimethylformamide and volume was made up to mark with distilled water. After complete dilution each flask was heated on water bath at 85±5ºC for 5 min & cooled to room temperature. In another 50ml amber colored volumetric flask 50mg of methylcobalamin was accurately weighed & diluted with distilled water to get stock solution of 1000µg/ml. The further dilutions are made to get the final concentration of 1-7µg/ml of MC**.**

PREPARATION OF SAMPLE SOLUTION

Twenty tablets were accurately weighed & powdered. The quantity equivalent to 300mg of GBP & 0.5mg of MC were transferred to 100ml amber colored volumetric flask and to this 60ml distilled water was added & sonicated for 15 min at room temperature & then diluted to the mark with distilled water. The sample solution was filtered through whatmann filter paper prior to use.

SELECTION OF ANALYTICAL WAVELENGTH

Selected dilutions were scanned and absorbance maxima 405 & 351nm were selected for analysis of GBP & MC respectively (Graph 1).

CALIBRATION CURVE FOR WORKING STANDARDS

Absorbance of prepared dilutions was reported at 405 & 351nm for GBP & MC respectively & graph was plotted. The coefficient of correlation (r^2) of 0.9949 for GBP & 0.9993 for MC was obtained (Graph 2 & 3).

ASSAY PROCEDURE FOR TABLET FORMULATION

The absorbance of prepared sample solution was determined at 351nm for the estimation of Methylcobalamin. The 5ml of remaining sample stock solution was diluted to 10ml to get 1500µg/ml of GBP. From the above solution 1ml is transferred to 10ml volumetric flask & treated in same manner as given for working standard of GBP & absorbance was noted at 405nm. The concentrations of the drugs were calculated by equation of standard curve method & double point standardization[8].

A) Standard Curve method:

For Gabapentin $y = 0.001x - 0.013$

For Methylcobalamin $y = 0.027x + 0.001$

B) Double point standardization:

$$C_{test} = \frac{(A_{test} - A_{std1})(C_{std1} - C_{std2}) + C_{std1}(A_{std1} - A_{std2})}{A_{std1} - A_{std2}}$$

Where,

A_{test} - Absorbance of test solution

A_{std1} - Absorbance of std_1

A_{std2} - Absorbance of std_2

C_{std1} - Highest concentration than test solution

C_{std2} - Lowest concentration than test solution

METHOD VALIDATION[9]

The method was validated as per ICH guidelines.

SPECIFICITY

The specificity of the method was investigated by observing any interference encountered from the excipients of the tablet. It was shown that these excipients do not interfere with the proposed method.

PRECISION

The precision was determined at two levels, i.e. system repeatability & method repeatability. System repeatability determined by measurement of six replicates of bulk. Method repeatability determined by measurement of six replicates of sample.

LINEARITY AND RANGE

The analytical concentration ranges over which the drugs obeyed Beer Lambert's law were found to be 50–300µg/ml for GBP (r^2 = 0.9949) & 1-7µg/ml for MC (r^2= 0.9993).The standard calibration curve is given in graph 2 & 3. The data of absorbance Vs drug concentration were treated by linear least square regression analysis (Table no.1).

RESULTS AND DISCUSSION

An attempt was made to develop a simple accurate and precise analytical method for analysis of GP and MC in combined tablet dosage form. The simultaneous equation method, Q-analysis and area under curve (AUC) method were tried for this combination but not successful due to poor absorption of Gabapentin. Hence an indirect method was developed. The authenticity and purity of bulk drug was confirmed by m. p., IR spectroscopy & TLC. The method is validated with respect to linearity, range & precision. The results of marketed formulation analysis of both methods were given in table no.2.

CONCLUSION

The proposed method was validated as per ICH guidelines. The standard deviation and standard error mean calculated for the method are low, indicating high degree of precision of the method. Hence, it can be concluded that the developed UV-Vis spectrophotometric method is accurate, precise and selective and can be employed successfully for the estimation of Gabapentin & Methylcobalamin in tablet formulation.

TABLE 1: Linear regression data for calibration curves

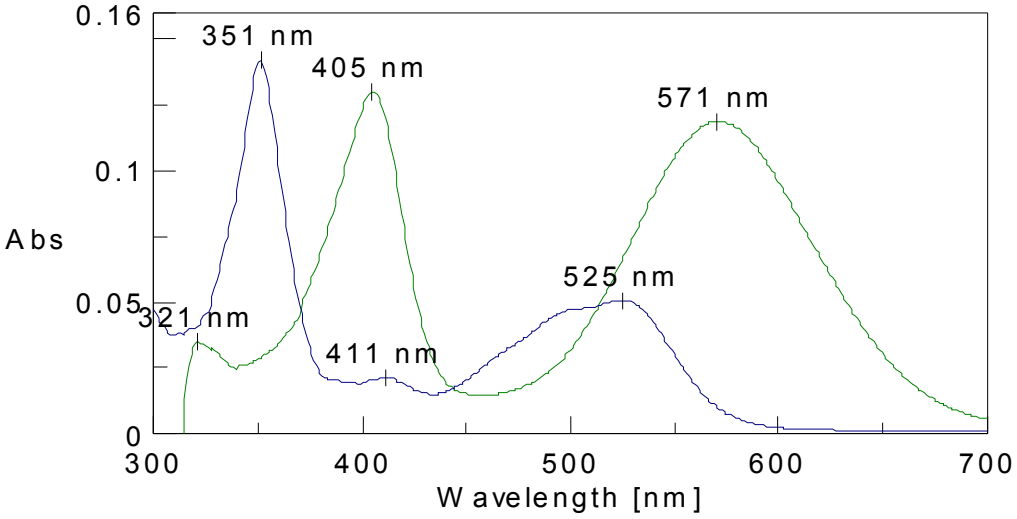
Parameters	GBP	MC
Detection Wavelength(nm)	405	351
Beer's Law Limit (µg/ml)	50-300	1-7
Regression equation	y = 0.001x - 0.013	y = 0.027x +0.001
Correlation Coefficient (r)	0.9949	0.9993
Intercept (c)	-0.013	0.001
Slope (m)	0.027	0.001

TABLE 2: Results of marketed formulation analysis

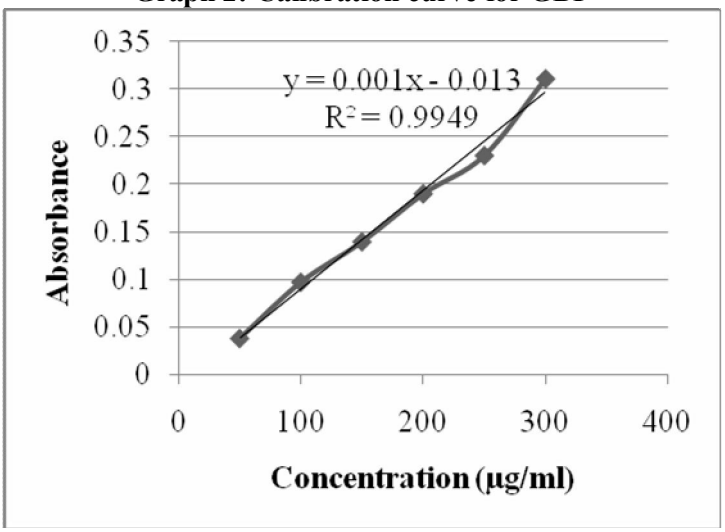
Marketed Formulation	Label claim (mg/tab)		Amount found (mg/tab) ± SEM*		Amount found in % ± SEM*	
Gabaneuron (GBP+MC Aristo Pharmaceuticals)	GBP	MC	GBP	MC	GBP	MC
Standard Curve Method	300.0	0.500	301.9 ±0.7234	0.502 ±0.0013	100.43 ±0.1534	100.48 ±0.1892
Double Point Standardization	300.0	0.500	301.7 ±0.5217	0.511 ±0.0062	100.58 ±0.1759	102.31 ±1.256

*Average of six determinations

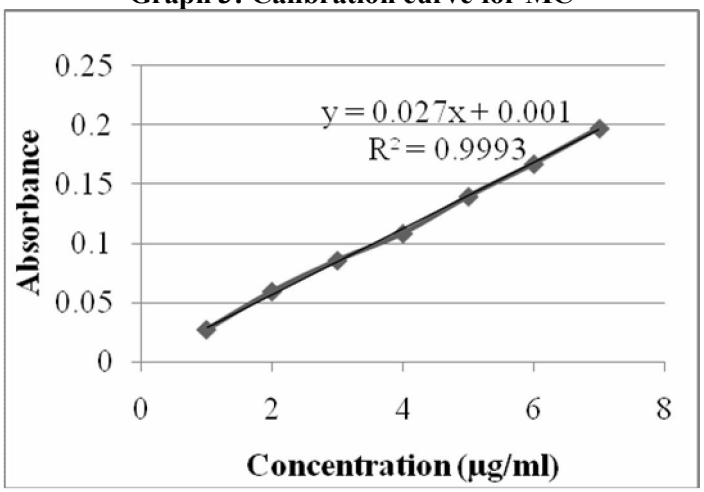
Graph 1: Overlay Spectra of GBP & MC



Graph 2: Calibration curve for GBP



Graph 3: Calibration curve for MC



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