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Extractive Spectrophotometric Methods for the Determination of Gabapentin in Pharmaceutical Dosage Forms

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ABSTRACT

Two simple, rapid, sensitive, accurate, precise and economic spectrophotometric methods have been developed for the estimation of Gabapentin (GBP) in pharmaceutical formulations. During the course of study, it was observed that acidic solution of the drug formed colored ion-association complexes with Bromocresol Green (BCG) (Method I) and Bromothymol Blue (BTB) (Method II) which were soluble in chloroform. This property of the drug was followed for the development of colorimetric methods for analysis of drug. The complex of GBP with BCG and BTB showed λ_{max} at 416 nm and 421 nm, respectively. The linearity range for GBP was 10-120 µg ml^{-1} and 40-90 µg ml^{-1} for BCG and BTB, respectively. Recovery studies gave satisfactory results indicating that none of common additives and excipients interfere the assay method. The proposed methods are found to be simple, accurate and reproducible those were successfully applied for the analysis of pharmaceutical dosage forms.

Keywords: Gabapentin (GBP), Colorimetric analysis, Bromocresol Green (BCG), Bromothymol Blue (BTB), Extractive spectrophotometry.

INTRODUCTION

The new anti-convulsion drug Gabapentin (1-(aminomethyl) cyclohexaneacetic acid) is a structural analogue of aminobutyric acid (GABA) and its action is attributed to the irreversible inhibition of the enzyme GABA-transaminase, thus preventing the physiological degradation of GABA in the brain. [1]

Currently, GBP and its pharmaceutical dosage forms are official in USP and also different analytical methods are reported for its determination. These include high-performance liquid chromatography [2], liquid chromatography-mass spectrometry [3], gas chromatography-mass spectrometry [4], capillary electrophoresis [5], potentiometry [6], spectrofluorimetry [7], and colorimetry. [8] Literature survey does not reveal any simple extractive spectroscopic method for determination of GBP. The present manuscript describes simple and sensitive extractive spectroscopic procedures for the determination of GBP in pharmaceutical dosage forms.

MATERIALS AND METHODS

Apparatus

A Shimadzu 1700 UV Visible spectrophotometer with 1 cm quartz cells was used for all absorbance measurements. Spectra were automatically obtained by UV-Probe (Version 2.10) system software. A calibrated digital pH meter was used for pH measurements.

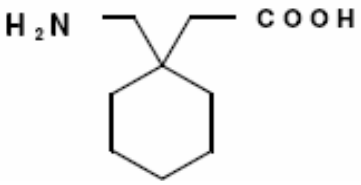

Fig. 1: Structure of Gabapentin

Reagents

All chemicals used were of analytical reagent grade and distilled water was used throughout the study. BCG and BTB reagents were supplied by Merck Chemicals Limited, India. Solution of BCG (0.04% w/v), solution of BTB (0.04 % w/v) and buffer solutions [9] (pH 2.0 to 5.5) were prepared. GBP pure drug was kindly supplied by Torrent Research Centre, Gandhinagar, India as a gift sample. The commercially available capsules and tablets of GBP were procured from local market labeled to contain 300 mg GBP in both the formulations.

Preparation of standard solutions

A standard stock solution of 1 mg ml^{-1} was prepared by dissolving GBP in distilled water. Working standard solution (200µg ml^{-1}) was then prepared by suitable dilution of the stock with distilled water.

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Optimization of conditions

Conditions under which reaction of GBP with dyes fulfill the essential requirements were investigated. All conditions studied were optimized at room temperature (25±2°C).

Selection of suitable pH buffer solution

Buffer solutions of different pH (2.0 to 5.5) were prepared. Working standard solution (5.0 ml) was pipette out and added to separating funnels. BCG solution (2.0 ml) and buffer solutions (2.0 ml) of different pH were added to each. Shaken well and extracted with 10 ml of chloroform. Later the extracts were taken into 10 ml volumetric flasks, treated with anhydrous sodium sulphate and volume was made up with chloroform, and then absorbances were measured at 416 nm. Same procedure was applied for the BTB then absorbances were measured at 421 nm. It was found that drug with BCG and BTB gave maximum absorbance at pH 4.0 (Fig. 2 and 3).

Optimization of volume of buffer

Volume of buffer was optimized by changing volume of buffer and other parameters were kept constant. Buffer solution of pH 4.0 was prepared. Working standard solution (5.0 ml) was transferred in seven separating funnels, different volume of buffer solution was added in the different separating funnels and BCG solution (2.0 ml) or BTB solution (2.0 ml) was added in each. Shaken well and extracted with 10 ml of chloroform. Later the extracts were taken into 10 ml volumetric flasks, treated with anhydrous sodium sulphate and volume was made up with chloroform, and then absorbances were measured at 416 nm and 421 nm for BCG and BTB, respectively. It was found that after addition of 2.0 ml of buffer in BCG and 2.5ml of buffer in BTB, absorbance became constant. Hence 2.0 ml and 2.5 ml of buffer solution were optimized for BCG method and BTB method, respectively (Fig. 4 and 5).

Optimization of volume of dye

Volume of dye was optimized by changing volume of dye and other parameters were kept constant. Working standard solution (5.0 ml) was transferred in six separating funnels; buffer solution (2.5 ml) was added in each separating funnel. Different volume of BCG solution was added in each. Shaken well and extracted with 10 ml of chloroform. Later the extracts were taken into 10 ml volumetric flasks, treated with anhydrous sodium sulphate and volume was made up with chloroform, and then absorbances were measured at 416 nm for BCG. Same procedure was performed for BTB using buffer (2.0 ml) and different volume of dye solution, and then absorbances were measured at 421 nm for BTB. It was found that after addition 2.0 ml of BCG solution and 1.5 ml of BTB solution, absorbance became constant. Hence 2.0 ml of BCG solution and 1.5 ml of BTB solution were optimized for the proposed methods (Fig. 6 and 7).

Stability study of drug dye complexes

The stability of the drug dye complexes was determined individually for both the dyes (BCG and BTB). Working standard solution (5.0 ml) was pipette out and added to a series of separating funnels. Buffer solution (2.0 ml) and BCG solution (2.0 ml) were added to each. Shaken well and extracted with 10 ml of chloroform. Later the extracts were taken into 10 ml volumetric flasks, treated with anhydrous sodium sulphate and made up the volume with chloroform. The absorbances were measured periodically at an interval of 30, 60, 90, 120, 180, 240, 300 and 360 minutes at 416 nm. Same procedure was applied for the BTB using 2.5 ml of Buffer solution and 1.5 ml of BTB solution, and then absorbances were measured at 421 nm. Finally it was found that BCG-GBP complex was stable at least for 6 h, whereas BTB-GBP complex was stable at least for 4 h.

Validation of the proposed methods

Linearity

Method I

From the working standard solution, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml were transferred to a series of separating funnels and buffer solution (2.0 ml) was added to each separating funnels, then BCG solution (2.0 ml) was added and shaken well, and then 10 ml of chloroform was added to each and shaken well and kept for few minutes. Later the extracts were taken into 10 ml volumetric flasks, treated with anhydrous sodium sulphate and made up the volume with chloroform, and then absorbance of the solution was measured at 416 nm against reagent blank. Final concentrations of analyzed solutions were 10µg ml^{-1} to 120µg ml^{-1}. The standard calibration plot (Fig. 8) was prepared to calculate the amount of the analyte drug in unknown samples.

Method II

From the working standard solution, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 ml was transferred to a series of separating funnels and buffer solution (2.5 ml) was added to each separating funnels, then BTB solution (1.5 ml) was added and shaken well, and then 10 ml of chloroform was added to each and shaken well and kept for few minutes. Later the extracts were taken into 10 ml volumetric flasks, treated with anhydrous sodium sulphate and made up the volume with chloroform, and then absorbance of the solution was measured at 421 nm against reagent blank. Final concentrations of analyzed solutions were 40µg ml^{-1} to 90µg ml^{-1}. The standard calibration plot (Fig. 9) was prepared to calculate the amount of the analyte drug in unknown samples.

Accuracy (% Recovery)

The accuracy of the proposed methods was performed by calculating recovery of GBP by the standard addition method. Known amounts of standard solutions of GBP were added at 50, 100 and 150% levels to prequantified sample solutions of 20µg ml^{-1} GBP and 40µg ml^{-1} GBP for BCG and BTB, respectively. At each level of the amount 3 determinations were performed. The amount of GBP was estimated by applying obtained values to regression equation. (Table 1 and 2)

Method precision (% Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of 50µg ml^{-1} GBP and 70µg ml^{-1} GBP for BCG and BTB, respectively without changing the parameters for the method. The repeatability was expressed in terms of relative standard deviation (RSD). (Table 3)

Intermediate precision (Reproducibility)

The intraday and interday precision of the proposed methods were performed by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 6 different concentrations of standard solutions of GBP (20, 40, 60, 80, 100 and 120µg ml^{-1}) for BCG and GBP (40, 50, 60, 70, 80 and 90µg ml^{-1}) for BTB. The results were reported in terms of relative standard deviation (RSD).

Limit of detection and Limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by calculating the signal-to-

noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by International Conference on Harmonization (ICH) guideline:

$$LOD = 3.3 \times \sigma/S \text{ and } LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response

S = slope of the calibration curve

Table 1: Drug recovery study in capsule dosage form

Drug	Level	Amount of sample taken (µg ml^{-1})	Amount of standard spiked (%)	Mean % Recovery ± SD*
GBP-BCG	I	20	50 %	99.23 ± 0.77
	II	40	100 %	100.4 ± 0.77
	III	60	150 %	100.3 ± 1.60
GBP-BTB	I	30	50 %	98.85 ± 0.23
	II	60	100 %	100.5 ± 0.29
	III	90	150 %	101.5 ± 1.17

* Mean % Recovery ± SD of six observations

Table 2: Drug recovery study in tablet dosage form

Drug	Level	Amount of sample taken (µg ml^{-1})	Amount of standard spiked (%)	Mean % Recovery ± SD*
GBP-BCG	I	20	50 %	99.23 ± 0.77
	II	40	100 %	99.36 ± 1.18
	III	60	150 %	99.91 ± 1.07
GBP-BTB	I	30	50 %	99.16 ± 0.48
	II	60	100 %	100.9 ± 0.11
	III	90	150 %	99.34 ± 0.80

* Mean % Recovery ± SD of six observations

Table 3: Precision data for GBP

S. No.	Absorbance of GBP	
	BCG (GBP: 50µg ml^{-1})	BTB (GBP: 70µg ml^{-1})
1	0.412	0.521
2	0.412	0.52
3	0.414	0.522
4	0.413	0.524
5	0.411	0.521
6	0.415	0.519
Mean	0.413	0.521
SD	0.001	0.002
RSD	0.357	0.330

Table 4: Analysis of marketed formulation (capsule dosage form) of GBP by proposed methods (n = 6)

Sample No.	Label Claim	Amount Found		% Label Claim	
	GBP (mg/cap)	GBP (mg/cap)		GBP (mg/cap)	
		BCG	BTB	BCG	BTB
1	300	301.1	298.6	100.4	99.52
2	300	304.8	297.7	101.6	99.22
3	300	302.0	297.0	100.7	99.01
4	300	301.1	297.9	100.4	99.32
5	300	297.3	296.7	99.11	98.91
6	300	304.8	298.8	101.6	99.62
Mean				100.6	99.27
SD				0.93	0.28

Table 5: Analysis of marketed formulation (tablet dosage form) of GBP by proposed methods (n = 6)

Sample No.	Label Claim	Amount Found		% Label Claim	
	GBP (mg/tab)	GBP (mg/tab)		GBP (mg/tab)	
		BCG	BTB	BCG	BTB
1	300	300.5	295.1	100.2	98.36
2	300	294.9	294.2	98.31	98.07
3	300	303.2	293.6	101.1	97.87
4	300	296.8	295.4	98.92	98.46
5	300	294.0	295.4	98.00	98.46
6	300	299.5	294.8	99.85	98.27
Mean				99.38	98.25
SD				1.18	0.24

Determination of GBP in pharmaceutical formulations

Capsule

Powder from 10 capsules was collected and weighed. The capsule powder equivalent to 100 mg of GBP was dissolved in distilled water in 100 ml volumetric flask. Finally the volume was made up to 100 ml with distilled water then preceded as described above for pure drug. The nominal content of the capsules was determined either from the calibration curve or using the regression equation. (Table 4)

Tablet

Twenty tablets were accurately weighed and powdered. The tablet powder equivalent to 100 mg of GBP was weighed and dissolved in distilled water in 100 ml volumetric flask. Finally the volume was made up to 100 ml with distilled water then preceded as described above for pure drug. The nominal content of the tablets was determined either from the calibration curve or using the regression equation. (Table 5)

Table 6: Optical characteristics

Parameters	BCG	BTB
λ_{max}, nm	416	421
Beer Lambert's law limits, µg ml^{-1}	10-120	40-90
Molar absorptivity*	1627.70	1123.72
Sandell's sensitivity, µg cm^{-2} /0.001 Absorbance unit*	0.1138	0.1750
Regression equation y=mx+c	y = 0.0065x + 0.0895	y = 0.0145x - 0.4744
Slope (m)	0.0065	0.0145
Intercept (c)	0.0895	-0.4744
Correlation coefficient (r^2)	0.9966	0.9954
Limit of detection (LOD)	2.90	10.86
Limit of quantification (LOQ)	8.77	32.91
Repeatability (%RSD, n=6)	0.36	0.33
Precision (%RSD)		
Interday (n = 3)	0.14-1.08	0.31-1.63
Intraday (n = 3)	0.77-1.90	087-1.77

** Average of six determinations*

RESULTS AND DISCUSSION

During the course of study, it was observed that acidic solution of the drug formed colored ion-association complexes with BCG and BTB which were soluble in chloroform. This property of the drug was followed for the development of colorimetric methods for analysis of drug. The complex of GBP with BCG and BTB showed λ_{max} at 416 nm and 421 nm, respectively. These developed methods were used for the estimation of GBP from two formulations (GABAPIN Capsule and Tablet). Both methods involve formation of ion-associated complex with BCG and BTB at pH 4.0 exhibiting λ_{max} at 416 nm and 421 nm, respectively (Fig. 10 and 11). The method commonly used in the determination of certain amines and quaternary ammonium compounds that do not absorb or absorb weakly in the ultraviolet region. [10] The proposed methods were based on addition of an amine in its ionized form to an ionized dye, yield a salt (ion-pair) that was extracted into an organic solvent such as chloroform or dichloromethane. The indicator dye was added in excess and the pH of the aqueous solution was adjusted to a value where both the amine and dye were in the ionized forms. The ion pair was separated from the excess indicator by extraction into the organic solvent. In these methods Beer's law was obeyed with BCG and BTB in the concentration range of 10-120µg ml^{-1} and 40-90µg ml^{-1},

respectively. Optical characteristics of GBP were given in the Table 6.

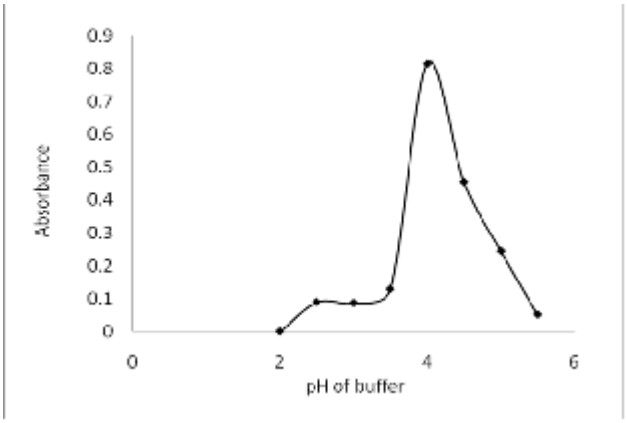

Fig. 2: Optimization of pH of buffer for GBP-BCG complex

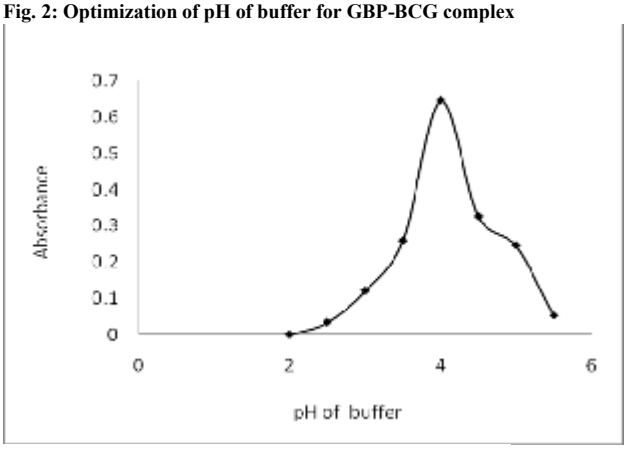

Fig. 3: Optimization of pH of buffer for GBP-BTB complex

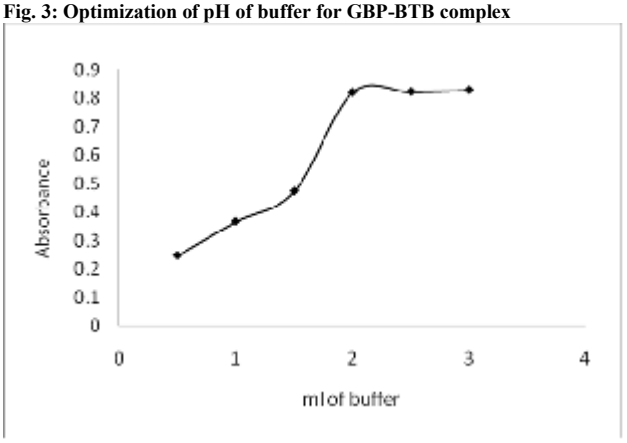

Fig. 4: Optimization of volume of buffer for GBP-BCG complex

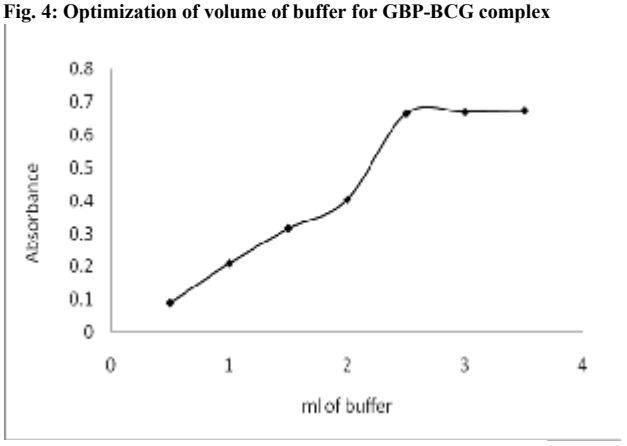

Fig. 5: Optimization of volume of buffer for GBP-BTB complex

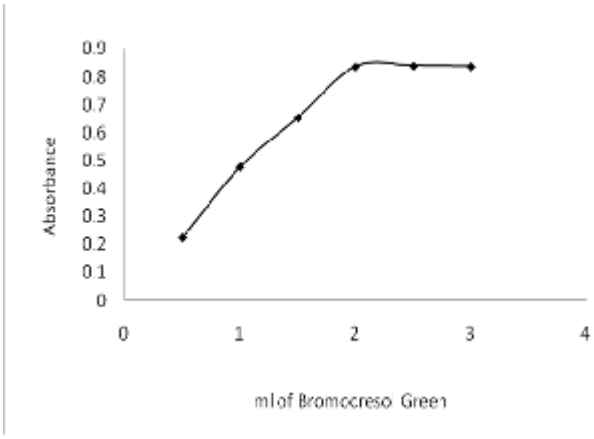

Fig. 6: Optimization of volume of dye for GBP-BCG complex

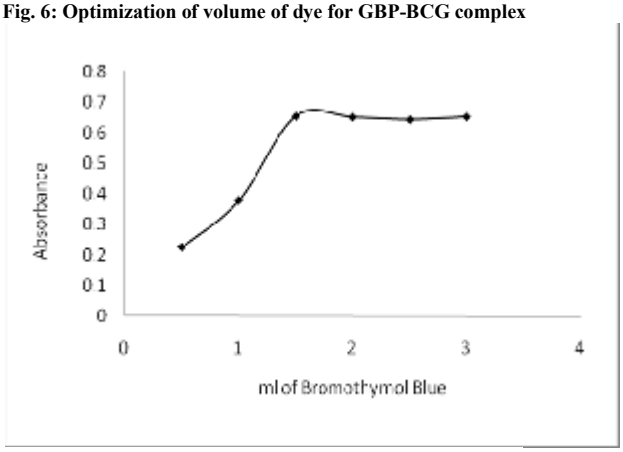

Fig. 7: Optimization of volume of dye for GBP-BTB complex

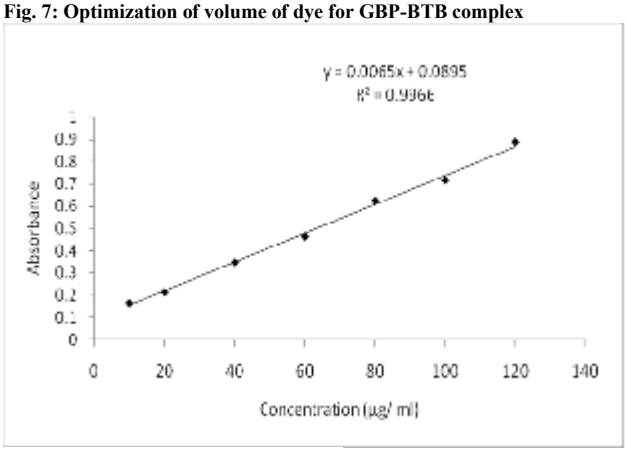

Fig. 8: Calibration curve for GBP-BCG

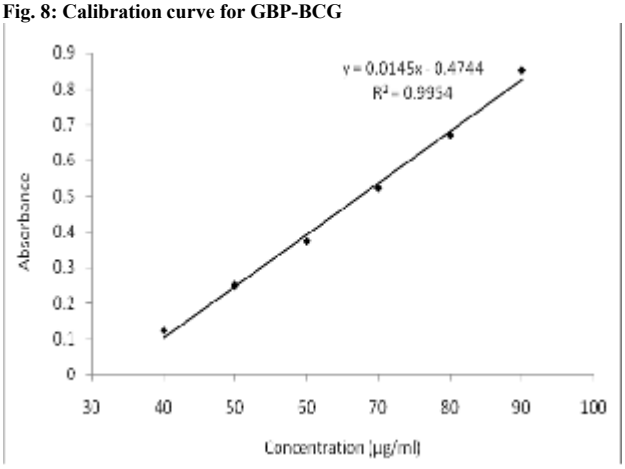

Fig. 9: Calibration curve for GBP-BTB

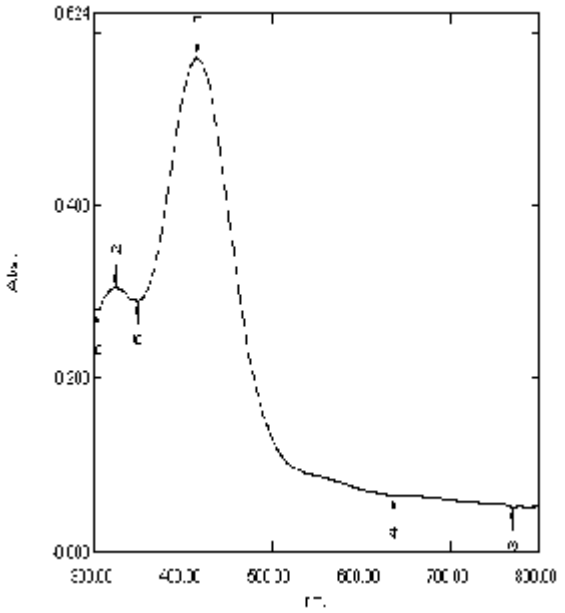

Fig. 10: Representative spectra of GBP-BCG showing λmax at 416 nm

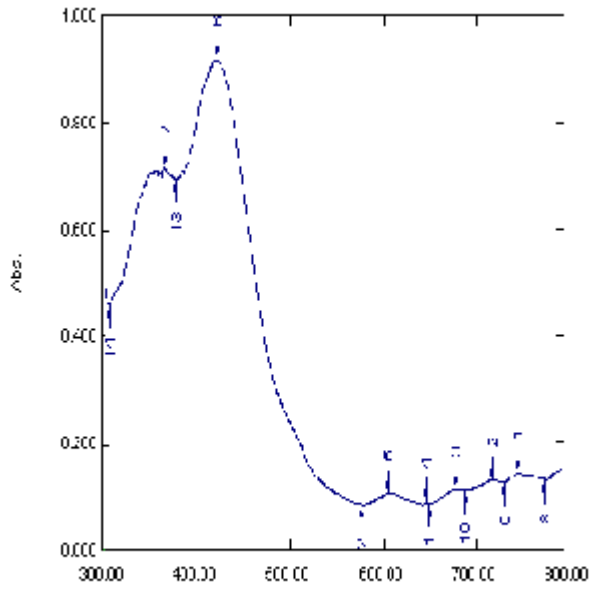

Fig. 11: Representative spectra of GBP-BTB showing λmax at 421 nm

The molar absorptivity and sandell's sensitivity showed that the methods are sensitive. The optimum conditions for color development had been established by varying the different parameters involved. For testing the accuracy and reproducibility of the proposed methods, recovery studies were performed. The data obtained by recovery studies indicate non-interference from the excipients used in the formulations. The percentage recoveries were close to 100%. This study revealed that the common excipients and other additives such as lactose, starch, gelatin, talc and magnesium stearate that are usually present in the tablet dosage forms do not interfere in the analysis. Thus, it can be concluded that the proposed methods are found to be simple, sensitive and accurate that can be used for the determination of GBP in their pharmaceutical dosage forms in a routine manner.

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