ISSN : 0974-7419 Volume 10 Issue 7

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 10(7) 2011 [430-439]

Spectrophotometric and conductometric determination of gabapentin in pure form and pharmaceutical preparations

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Received: 21st October, 2010 ; Accepted: 31st October, 2010

ABSTRACT

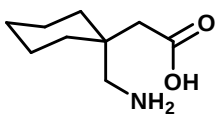
Four simple, sensitive and reproducible methods were developed for the determination of gabapentin (GPT) in pure form and in pharmaceutical preparations. Methods A and B are based on the reaction of cupric chloride with gabapentin to form stable complex, which could be measured spectrophotometrically at λ_{max} 246 nm (method A) or by using conductometric technique (method B). While method C and D depends on the formation of ion pair complex between the studied drug and bromothymol blue, bromocresol green respectively which was extractable with methylene chloride. The concentration ranges were 40-95 μg ml^{-1}, 1-15 mg, 100-800 and 10-150 μg ml^{-1} for methods A, B, C and D respectively.The optimization of various experimental conditions were described.The results obtained showed good recoveries, Ringbom optimum concentration ranges were calculated, in addition to molar absorpivity, Sandell's sensitivity, detection and quantification limits. The methods were successfully applied to the determination of GPT in bulk and pharmaceutical preparations.The results were favorably comparable with the official method. The molar combining ratio for methods (A-B) was found to be (2:1) (drug: reagent) while for method (C-D) it was found to be (1:1).

KEYWORDS

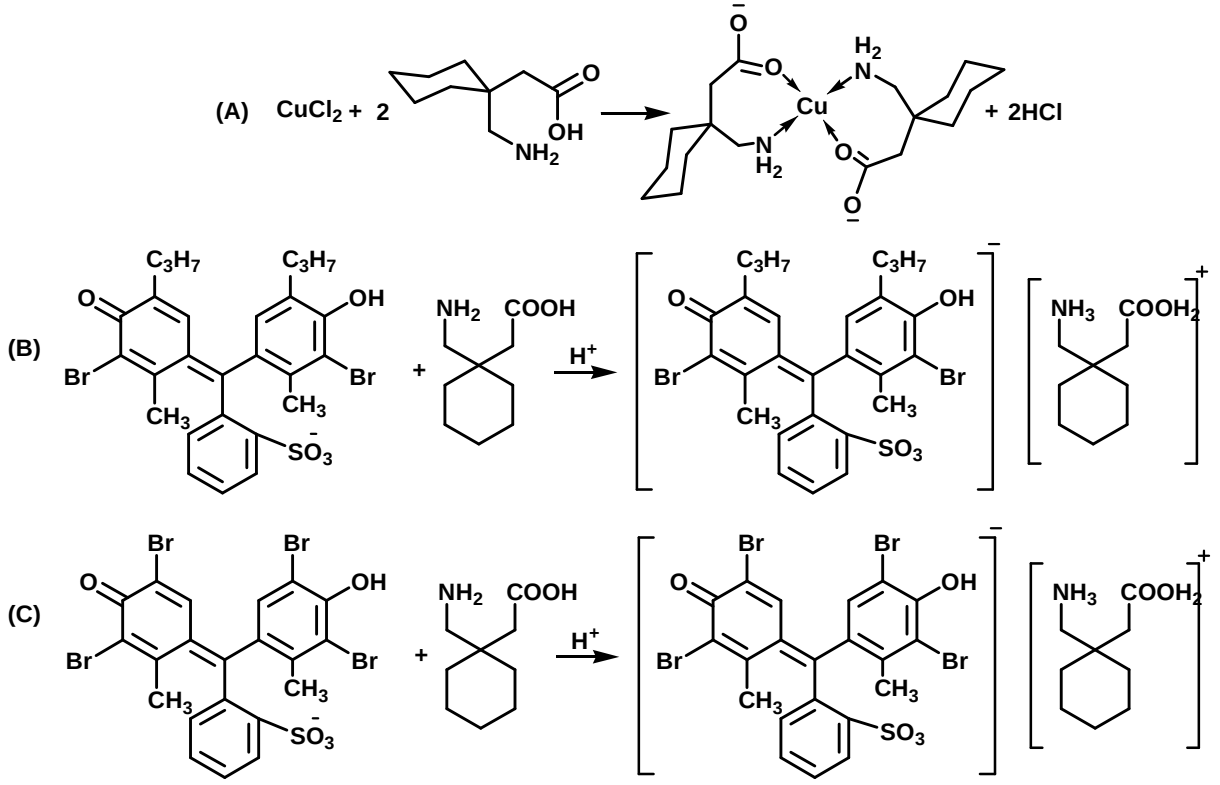
Gabapentin;
Cupric chloride;
Bromothymol blue;
Bromocresol green;
Spectrophotometrically;
Conductometric technique.

INTRODUCTION

Gabapentin (1- (aminomethyl)cyclo-hexaneacetic acid) is a structural analogue of g-aminobutyric acid (GABA) scheme 1 and its action is attributed to the irreversible inhibition of the enzyme GABA-transaminase, thus preventing the physiological degradation of GABA in the brain; a secondary mechanism of a blockade for GABA uptake is also suggested[1], it is an antiepileptic effective in the treatment of partial seizures with or without secondary generalisation and is used as adjunctive therapy in patients unresponsive to or intolerant of standard antiepileptic drugs[2]. A survey of the literature reveals that there were few reported methods for the determination of gabapentin using spectrophotometric technique[3,4], spectrofluorimetry[5,6], capillary electrophoresis[7], LC-MS[8] and HPLC[9].



Scheme 1 : Chemical structure of gabapentin



Scheme 2 : Proposed reactions of gabapentin with: (A) Cupric chloride, (B) Bromothymol blue, (C) Bromocresol green

An inspection of both available methods for the cited drug reveals that only few spectrophotometric work were done, although conductometry is a rapid method and requires simple procedures, it has not been yet applied to the determination of gabapentin. The USP 30 described a liquid chromatographic method for its determination[10].

The aim of this study was to apply simple, accurate, sensitive and reproducible reactions to analyse GPT in pure form and in pharmaceutical preparations, this study described methods that can be used in laboratories where modern and expensive equipment, such as that required for GC or HPLC is not available.

EXPERIMENTAL

Apparatus

Absorption spectra for all measurements were carried out using Shimadzu UV-260 double beam recording spectrometer with a 1 cm cell holder. All conductometric measurements were recorded using Conductometer model CM-1K, Tokyo TOA electronics ltd Japan. The pH values of solutions were measured using a Chemocadet pH meter.

Reagents

Analytical grade reagents and double distilled water were used to prepare all solutions.

GPT pure drug was obtained from Godecke AG, Germany under license of Park-Davis,

Aqueous solutions of 1 and 4 mg ml^{-1} of GPT was prepared by dissolving 100 and 400 mg of pure drug in 100 ml bidistilled water respectively.

Stock solution of cupric chloride (Aldrich Chemical Co. Ltd) was prepared as 0.1% solution in bidistilled water also 10^{-2} M solution was prepared by dissolving 0.171 gm in 100 ml bidistilled water.

Bromothymol blue sodium salt (BDH Chemicals Ltd., Poole, England) was prepared as 0.05% solution in bidistilled water.

Bromocresol green (Aldrich Chemical Co. Ltd) 0.025% solution was prepared by dissolving the weighed amount in 2.5 ml 0.1M NaOH then completed to 100 ml using bidistilled water[11].

Borate buffer pH 7.5was used by dissolving 2.5 g of sodium chloride, 2.85 g of sodium tetraborate and 10.5 g of boric acid in sufficient water to produce 1000

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ml. Adjust the pH if necessary[12].

Also acetate buffer of pH 3.7 was used by dissolving 10 g of anhydrous sodium acetate in 300 ml water, adjust to pH 3.7 with glacial acetic acid and dilute to 1000 ml with water. If necessary, readjust to pH 3.7 with glacial acetic acid or anhydrous sodium acetate as required, before use[12].

Formulations

Gaptin capsules (labeled to contain 100 mg gabapentin per capsule) were obtained from Delta Pharma, Egypt.

Working solution

The contents of ten capsules were emptied, pulverized. An accurately weighed amount equivalent to 100 and 400 mg GPT were extracted by shaking with 50 ml distilled water, filtered, transferred to a 100 ml volumetric flask, completed to the mark using bistilled water. The general procedures were then followed using standard addition method.

General procedures

Spectrophotometric procedure using cupric chloride (method A)

Transfer aliquots containing 40-95 μg $ml^{-1}$of GPT drug solution into 10 ml volumetric flasks, add 1 ml of borate buffer pH 7.5, mix then add 2 ml cupric chloride 0.1 % solution, complete to 10 ml using bidistilled water, measure the absorbance at 246 nm, against a reagent blank solution prepared in the same way without drug.

Conductometric procedure (method B)

Transfer suitable aliquot of sample solution containing 1-15 mg of drug to a 50 ml calibrated flask and make up to the mark with bidistilled water. Transfer the contents of the calibrated flask to a beaker and immerse the conductivity cell, titrate sample solution using 10^{-2} M cupric chloride. Measure the conductance subsequent to each addition of reagent solution and after thorough stirring for two minutes, correct it for dilution effect[13] by means of the following equation, assuming that conductivity is a linear function of dilution.

$$\Omega^{-1}_{correct} = \Omega^{-1}_{obs} [\nu_1+\nu_2/\nu_1]$$

where Ω^{-1}_{obs} is the observed electrolytic conductivity, v_1 is the initial volume and v_2 is the volume of reagent added. Construct a graph of corrected conductivity *versus* the volume of added titrant and determine the end-point.

Ion pair procedure using bromothymol blue (method C)

Into 125 ml separating funnels, transfer aliquots containing 100 – 800 μg$ml^{-1}$of GPT drug solution, treat the drug solution with about 1.5 ml of acetate buffer pH 3.7, add 1.5 ml of 0.05 % bromothymol blue mix then extract the aqueous solution with an equal volume of methylene chloride, shake for 30 seconds, allow the mixture to separate into two phases. Collect the organic layer and dry with anhydrous sodium sulfate, complete to 10 ml with methylene chloride, measure the absorbance of the extracts at 411 nm, against a reagent blank prepared according to the same treatment without drug.

Ion pair procedures using bromocresol green (method D)

Into 125 ml separating funnels, transfer aliquots containing10–150μgml^{-1} of GPT drug solution, add bout 1.5 ml of acetate buffer pH 3.7, add 2.5 ml of 0.025 % bromocresol green mix then extract the aqueous solution with an equal volume of methylene chloride and shake for 30 seconds, allow the mixture to separate into two phases. Collect the organic layer and dry with anhydrous sodium sulfate, complete to 10 ml with methylene chloride, measure the absorbance of the extracts at 411 nm, against a reagent blank prepared according to the same treatment.

Determination of the stoichiometry of the reaction

In order to ascertain the stoichiometry of reaction Job's method of continuous variation[14] was carried out using the same molarity of drug and reagent.

RESULTS AND DISCUSSION

Spectrophotometric procedures using cupric chloride (method A)

Binary complexes were widely used in spectrophotometric analysis of many pharmaceutical compounds[15-

[17]. In this paper, the formed binary complex consists of the studied drug GPT and the metal ions, copper (II). This complex is water soluble with absorption maximum at 246 nm, figure 1. The effects of the reagent concentrations, pH, temperature and dilution with respect to maximum sensitivity, adherence to Beer's law and stability, was studied through control experiments, the experimental conditions were established by varying each parameter individually and observed the effect on the absorbance of the colored species[18], as well as measured or calculated factors and parameters TABLE 1.

Effect of cupric chloride concentration

The addition of 2 ml of 0.1% cupric chloride solution was sufficient to obtain the maximum absorbance for the recorded samples. Smaller amounts resulted in incomplete complex formation while increased concentrations had no effect on complex formation (Figure 2).

Effect of buffer pH and volume

Different buffer solutions were used to increase the color intensity of the formed ion-pair. Variation of the pH in the range from (2-11), resulted in the conclusion that 1 ml of borate buffer pH 7.5 was enough to give the maximum absorbance (Figure 3).

Effect of temperature

It was found that the reaction proceeds maximally at room temperature, no heating was required.

Effect of diluting solvent

Different solvents were tried including bidistilled water, methanol, ethanol and acetone, to achieve maximum absorbance water was found to be the most convenient solvent.

Effect of reaction time

Maximum absorbance was obtained through 5 minute.

Conductometric procedures using cupric chloride (method B)

Conductometric analysis can be used in many titration procedures when ionic solutions are involved. As the conductance of a solution is related to the total ionic content, it can be applied to follow reactions that results in a change in this quantity. Conductance measurements are used successfully in quantitative titration of systems in which the conductance of the solution varies before and after the equivalence point. In these cases, the titration curve can be represented by two lines intersecting at the end point[19,20].

Investigations were carried out to establish the most favorable conditions for the ion associates formation of gabapentin with cupric chloride to achieve sharp end point.

The optimum conditions for performing the titration in a quantitative manner were elucidated as described below.

Effect of solvent

Seven different titrations were attempted:

1. Aqueous drug solution with aqueous reagent solution
2. Ethanol drug solution with ethanol reagent solution
3. Drug solution with reagent solution, both in ethanol-water (50%, v/v) mixture
4. Methanol drug solution with methanol reagent solution
5. Drug solution with reagent solution, both in methanol-water (50 v/v) mixture
6. Acetone drug solution with acetone reagent solution
7. Drug solution with reagent solution, both in acetone-water (50% v/v) mixture

Preliminary experiments showed that procedure in aqueous media was the most suitable for successful results, because in other procedures turbid solution was formed which caused some errors.

Reagent's concentration

Different concentrations of cupric chloride solution were tried ranging from 2.5×10^{-3} to 2×10^{-2} Molar solution. The optimum concentration of the reagent was 10^{-2} M in titration of the studied drug to achieve a constant and highly stable conductance reading within 1-2 min of mixing. Concentrations less or more than these limits showed only poor inflection at the end point.

Representative titration curve is shown in figure 4. Two straight lines are obtained, intersecting at the endpoint, the first branch ascending the second one, conductance values slightly increase after the equivalence point.

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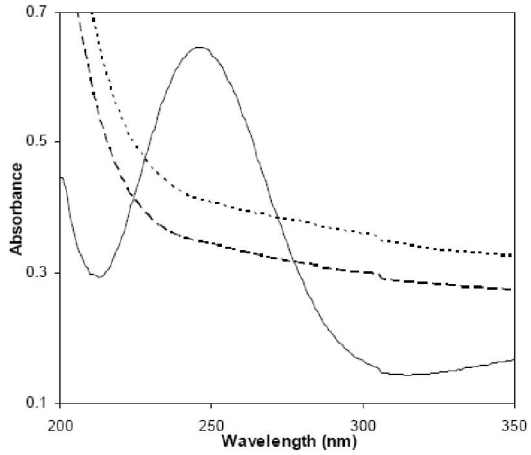

Figure 1 : Absorption spectra of the complex formed through reaction of: 75 µg ml^{-1} gabapentin with cupric chloride in presence of borate buffer pH 7.5 (——), 75 µg ml^{-1} gabapentin with borate buffer pH 7.5 (·····), cupric chloride with borate buffer pH 7.5 (— —)

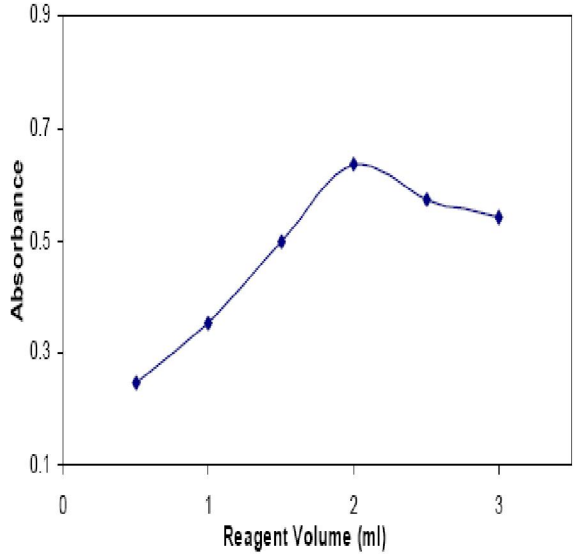

Figure 2 : Effect of 0.1 % cupric chloride volume on the reaction between cupric chloride and 75 µg ml^{-1} gabapentin

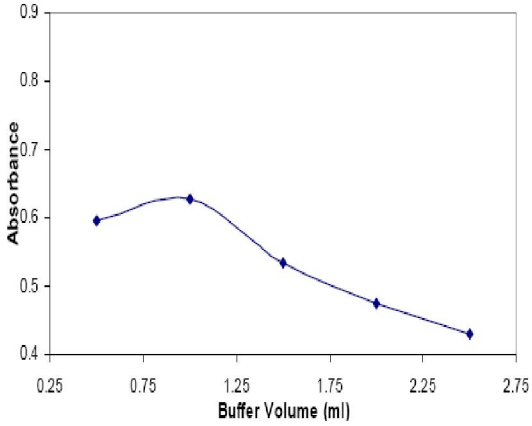

Figure 3 : Effect of borate buffer pH 7.5 volume on the reaction between cupric chloride and 75 µg ml^{-1} gabapentin

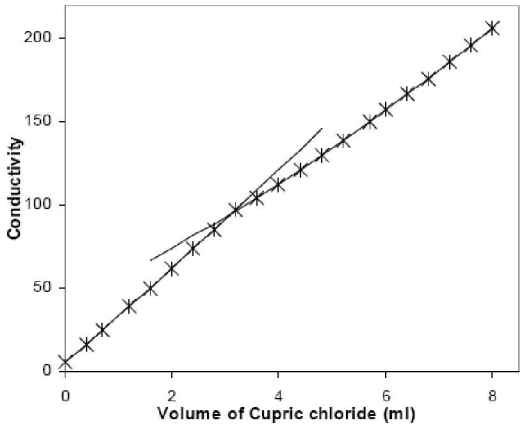

Figure 4 : Conductometric titration curve of 11 mg gabapentin *vs* 10^{-2} M cupric chloride

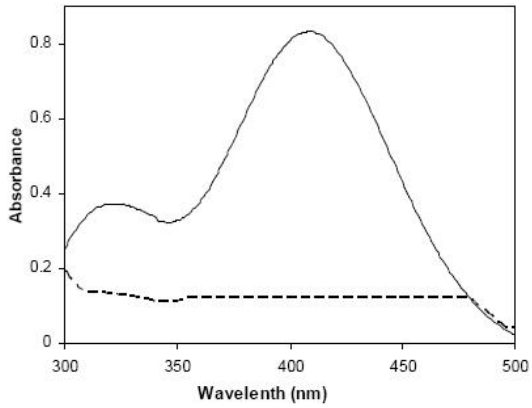

Figure 5 : Absorption spectra of the ion pair formed through reaction of: 800 µg ml^{-1} gabapentin with 0.05 % bromothymol blue (——), blank solution (———)

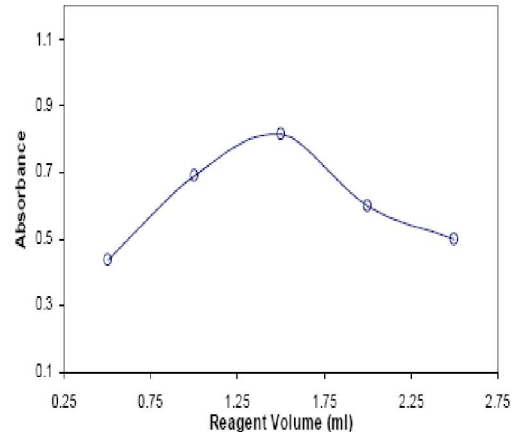

Figure 6 : Effect of volume of 0.05 % bromothymol blue on the reaction of bromothymol blue with 800 µg ml^{-1} gabapentin

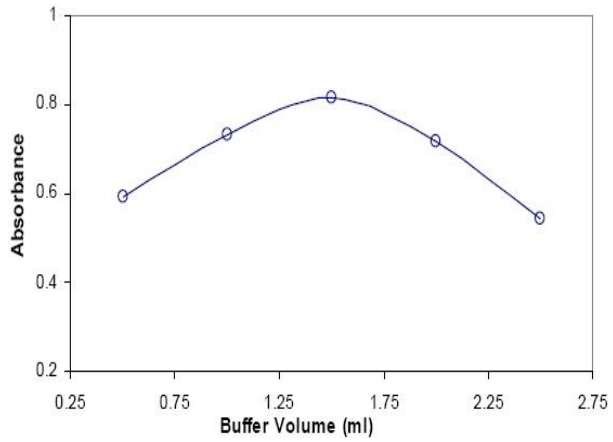

Figure 7 : Effect of volume of acetate buffer pH 3.7 on the reaction of bromothymol blue with 800 µg ml^{-1} gabapentin

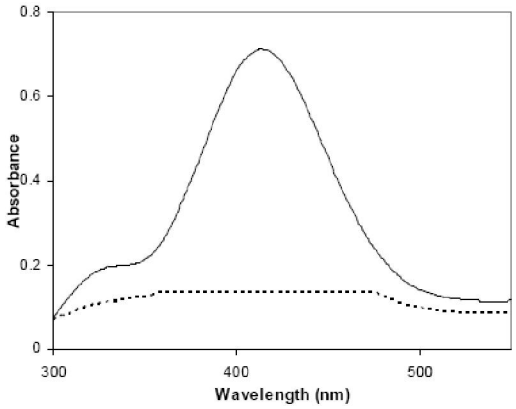

Figure 8 : Absorption spectra of the ion pair formed through reaction of: 100 µg ml^{-1} gabapentin with 0.025% bromocresol green (——), blank solution (……)

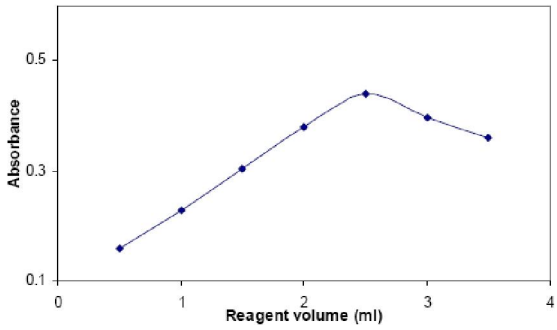

Figure 9 : Effect of volume of 0.025 % bromocresol green on the reaction of bromocresol green with 50 µg ml^{-1} gabapentin

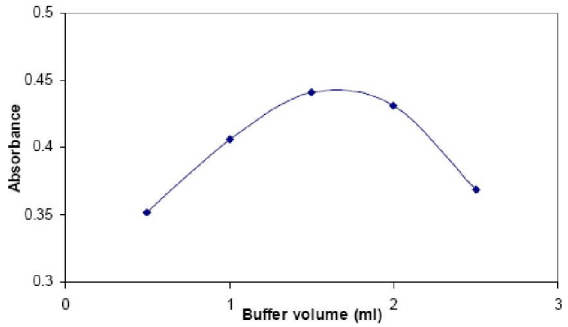

Figure 10 : Effect of acetate buffer pH 3.7 volume on the reaction of bromocresol green with 50 µg ml^{-1} gabapentin

The conductances measured before the addition of the titrant (volume of Cu^{+2}þ equal zero) is related to the dissociation of the proton of the carboxylic group present in the gabapentin molecule. Up to the equivalence point, the titration involves the gradual substitution of the protons of the gabapentin molecule by cations of Cu^{+2}. This increase of the conductance is because the mobility of the ion H^+ is larger than that of ion Cu^{+2}þ, causing an increase in the slope of the conductometric curve (first branch of the curve). After the equivalence point, the measured conductance is the sum of the Cu^{+2}þ and Cl^- present in the solution. As the sum of the mobilities of those ions is smaller than that of the H^+ cation, there is a decrease in the slope of the second section of the titration curve. The equivalence point is defined as the point of intersection of the two straight segments.

The conductometric titrations of different volumes of 10^{-2} M cupric chloride solution were performed. The results show an obvious maximum in the conductance curve at drug-reagent molar ratio of (2:1). The optimum concentration ranges for determination of gabapentin was in the range of 1-15 mg. At such range, distinct inflections and stable conductance reading were obtained.

Ion pair procedures using bromothymol blue (method C)

The utility of bromothymol blue as ion-pairing reagent in assay of gabapentin is investigated here. The spectra of the reaction products show characteristic λ_{max} at 411 nm (Figure 5).

The experimental conditions were established by varying one variable and observing its effect on the absorbance of the colored species as discussed below:

Effect of bromothymol blue concentration

1.5 ml of 0.05 % bromothymol blue was found to be sufficient for giving best results, (Figure 6).

Effect of buffer pH and volume

Using different buffers of different pH in the range from (2-11), it was found that the intensity of the color of the formed complex increased when 1.5 ml of ac-

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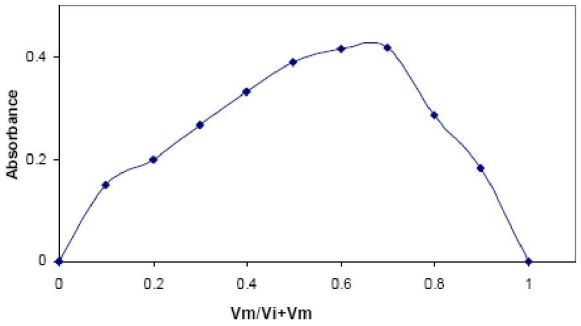

Figure 11 : Determination of the stoichiometry of the reaction of: Gabapentin(5×10^{-3}M)and cupric chloride (5×10^{-3} M)

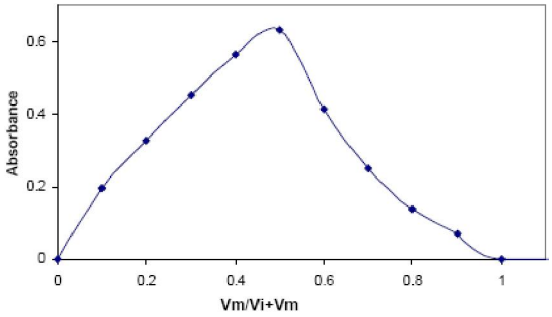

Figure 12 : Determination of the stoichiometry of the reaction of: Gabapentin (1.25×10^{-3}M) and bromothymol blue (1.25×10^{-3} M)

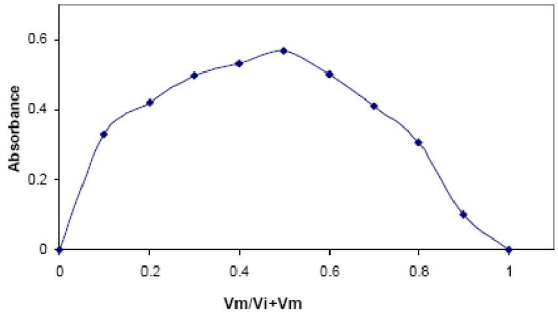

Figure 13 : Determination of the stoichiometry of the reaction of: Gabapentin (1.25×10^{-3}M) and bromocresol green (1.25×10^{-3} M)

etate buffer of pH 3.7 was used (Figure 7).

Effect of extraction time and times of extractions

It was found that a single extraction of the ternary complex for 30 seconds was sufficient for complete extraction.

Effect of organic solvent type

Chloroform, methylene chloride, diethyl ether and benzene were all tried.

Methylene chloride was found to be the most convenient solvent for the studied drug.

TABLE 1 : Spectral characteristics and statistical data of the regression equations for the product formed through reactions (A, C and D)

Items	Method A	Method C	Method D
Linearity range (μg ml^{-1})	40-95	100-800	10-150
Ringbom range (μg ml^{-1})	56.2-85.11	199.5-707.9	19.95-85.11
Molar absorptivity* (mol^{-1} cm^{-1})	1.33×10^{3}	1.99×10^{2}	1.54×10^{3}
Sandell's sensitivity (μg ml^{-1} per 0.001 A)	7.76×10^{-4}	1.17×10^{-4}	8.98×10^{-4}
Regression equation			
Intercept (a)	- 0.549	0.076	0.175
Slope (b)	0.016	0.00092	0.0053
Correlation coefficient(r)	0.9995	0.9998	0.9999
Variance	1.09	1.38	1.71
Limit of detection LOD(μg ml^{-1})	1.179	1.44	1.61
Limit of quantitation LOQ(μg ml^{-1})	3.89	4.75	5.30

***calculated on the basis of the molecular weight of the drug**

Effect of aqueous to organic phase ratio

Varying the ratio of aqueous phase to organic phase didn't cause any reasonable change in the results so a (1:1) ratio was rather used.

Ion pair procedure using bromocresol green (method D)

The utility of bromocresol green as ion-pairing reagent in assay of gabapentin is investigated here. The spectra of the reaction products show characteristic λ_{max} at 411 nm (Figure 8).

The experimental conditions were established by varying one variable and observing its effect on the absorbance of the colored species as discussed below:

Effect of bromocresol green concentration

Experiments was carried out in which the volume was kept constant at 10 ml while the concentration of reagent was increased; revealed that 2.5 ml of 0.025 % is the optimum concentration (Figure 9).

Effect of buffer pH and volume

Using different buffers of different pH in the range from (2-11), 1.5 ml of acetate buffer of pH 3.7 was found to be the most convenient buffer (Figure 10).

Effect of extraction time and times of extractions

It was found that single extraction of the ternary complex for 30 seconds was sufficient for complete extraction.

TABLE 2 : Determination of gabapentin by using methods (A-D)

Method A			Method B			Method C			Method D		
Taken (μg ml^{-1})	Found (μg ml^{-1})	Recovery %	Taken (mg)	Found (mg)	Recovery %	Taken (μg ml^{-1})	Found (μg ml^{-1})	Recovery %	Taken (μg ml^{-1})	Found (μg ml^{-1})	Recovery %
40	40.25	100.63	1	0.99	99.31	100	98.91	98.91	10	9.81	98.11
50	49.00	98.00	3	3.01	100.47	200	201.09	100.54	25	24.91	99.62
60	58.88	98.13	5	5.00	100.00	300	303.26	101.09	50	50.00	100.00
75	74.69	99.58	7	7.02	100.29	400	405.43	101.36	75	76.42	101.89
85	83.13	97.79	9	8.99	99.89	500	492.39	98.48	100	101.13	101.13
90	89.25	99.17	11	10.96	99.63	800	803.26	100.41	150	149.62	99.75
95	94.56	99.54	13	13.01	100.08						
			15	15.07	100.47						
Mean*±S.D. 98.98± 1.04			100.02 ± 0.405			100.13 ± 1.173			100.08 ± 1.309		
(p = 0.05)											
N 7			8			6			6		
S.D. 1.04			0.405			1.173			1.309		
R.S.D. 1.05			0.404			1.172			1.308		
V 1.09			0.164			1.38			1.71		
S.E. 0.394			0.143			0.479			0.534		

***Mean of three different experiments**

TABLE 3 : Determination of gaptin capsule by using methods (A-D)

Method A			Method B			Method C			Method D		
Taken added (μg ml^{-1})	Found (μg ml^{-1})	Recovery %	Taken (mg)	Found (mg)	Recovery %	Taken added (μg ml^{-1})	Found (μg ml^{-1})	Recovery %	Taken added (μg ml^{-1})	Found (μg ml^{-1})	Recovery %
40 -	40.56	101.41	2	1.99	99.30	100 -	101.09	101.09	10 -	9.62	96.23
40	40.69	101.72	4	4.01	100.18	400	395.65	98.91	10	10.19	101.89
60	59.44	99.06	8	8.01	100.13	500	493.48	98.70	20	20.38	101.89
65	66.19	101.83	10	10.10	101.03	600	590.22	98.37	25	25.28	101.13
70	70.56	100.80				700	680.43	97.20	30	30.38	101.26
80	79.94	99.92				800	798.91	99.86	100	100.75	100.75
Mean*±S.D. 100.67 ± 1.18			100.16 ± 0.706			98.61 ± 0.962			101.38 ± 0.495		
(p = 0.05)											
N 5			4			5			5		
S.D. 1.18			0.706			0.962			0.495		
V 1.39			0.499			0.926			0.245		
S.E. 0.529			0.353			0.430			0.221		

***Mean of three different experiments**

Effect of organic solvent type

Chloroform, methylene chloride, diethyl ether and benzene were all tried.

Methylene chloride was found to be the most convenient solvent for the studied drug.

Effect of aqueous to organic phase ratio

Varying the ratio didn't cause any reasonable change in the results so a (1:1) ratio was rather used.

Stoichiometric relationship

Using Job's method of continuous variation, the molar ratio of gabapentin to cupric chloride was found to be (2:1), while for bromothymol blue and bromocresol green it was found to be (1:1) (Figure11-13). The mechanisms of the methods were suggested in Scheme 2.

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TABLE 4 : Statistical data for the determination gabapentin using method (A-D) compared with reference method[4]

Item	Reference method	Method A	Method B	Method C	Method D
Mean±S.D.	99.59±0.650	98.98±1.04	100.02±0.405	100.13±1.173	100.08±1.309
(p=0.05)					
N	5	7	8	6	6
S.D.	0.650	1.04	0.405	1.173	1.309
R.S.D	0.653	1.05	0.404	1.172	1.308
V	0.423	1.09	0.164	1.38	1.71
t	-	1.15(2.228)*	1.48 (2.201)*	0.913(2.262)*	0.758(2.262)*
F	-	2.58 (4.53)*	2.58 (4.12)*	3.26 (5.19)*	4.04 (5.19)*

***Theoretical values of t and F at p = 0.05**

METHODS OF VALIDATION

The developed analytical methods were validated as per ICH guideline and USP requirement[21,22], applying a pharmaceutical preparation analysis. Under the described experimental conditions, calibration graphs were constructed for all of the methods, TABLE 1 ans 2 summarized the values of Beer's Law limits, Ringbom concentrations ranges, regression equations, correlation coefficients, molar absorptivity, Sandell's sensitivety, limit of detection (LOD) and limit of quantification (LOQ) for each method. As can be seen from TABLE 1, linear relationship was found between the absorbance at λ_{max} and the concentration of the drug in the ranges of 40-95 μg ml^{-1}, 100-800 and 10-150 μg ml^{-1} for methods A, C and D respectively. It was observed that method D is the most sensitive one. The optimum concentration ranges of gabapentin that can be measured accurately as evaluated from the Ringbom plot were 56.2-85.11, 199.5-707.9, 19.95-85.11 μg ml^{-1} for methods A, C and D respectively. The limit of detection (LOD) and limit of quantification (LOQ) were also determined.

To check accuracy of the proposed methods, the standard addition technique was applied; the results of analysis of the commercial dosage forms and the recovery study are shown in TABLE 3.

The average percent recoveries indicating good accuracy of the methods. Experiments showed that there was no interference from the additives e.g. lactose, fructose, magnesium stearate and starch.

The methods performance was assessed using the t-test (for accuracy)and a variance ratio F-values did not exceed the theoretical values (95% confidence limit), so we concluded that the proposed methods does not differ significantly from the official one, (TABLE 4).

CONCLUSION

The data given above reveals that the proposed methods introduce new techniques for the determination of gabapentin. The studied methods showed the advantage of being simple, accurate and sensitive with good precision and accuracy. Using of bromocresol green allowed the detection of gabapentin in small amount of 10 μg ml^{-1}. With these methods, one can do the analysis in a short time at low cost without losing accuracy. The proposed methods can be used as alternative methods to reported ones for the routine determination of gabapentin in the pure form and in pharmaceutical formulations.

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