International Jour. Chem.
Vol. 20, No. 1, (2010) 53-60

SPECTROPHOTOMETRIC DETERMINATION OF GABAPENTIN IN PHARMACEUTICAL FORMULATION USING NINHYDRIN AND 1,2-NAPHTHO UINONE-4-SODIUM SULPHONATE

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Abstract : Two simple, rapid and accurate Spectrophotometric methods are presented for the determination of gabapentin. The first method is based on the reaction of the primary amino group of gabapentin with ninhydrin reagent in a water bath in the presence of sodium hydroxide to yield a bluish violet product which absorbs maximally at 566 nm. Beer's law is obeyed in the concentration range 8-50 μgml^{-1} of gabapentin. The second method depends on the reaction of gabapentin with I, 2-naphthoquinone-4-sodium sulphonate in boiling water bath in presence of sodium hydroxide to form a brown colored product measurable at 540 nm. The absorbance is proportional to gabapentin concentration in the range 5-25 μgml^{-1}. The optimum experimental parameters for both reactions have been studied. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The suggested procedures could be used for the determination of gabapentin in pharmaceutical capsules.

1. Introduction

Gabapentin [l-(aminomethyl) cyclohexaneacetic acid] is a new generation antiepileptic drug used for the treatment of partial seizures with or without secondary generalized tonic-clonic convulsions. Gabapentin is a structural analogue of γ-aminobutyric acid (GABA), an inhibitory neurotransmitter and its action is attributed to the irreversible inhibition of the enzyme GABA-transaminase, thus preventing the physiological degradation of GABA in the brain (Parfitt and Martindale, 1999). Gabapentin and its pharmaceutical dosage forms are not found in any pharmacopoeia and different analytical methods are reported for their determination. These include gas chromatography-mass spectrometry (Borrey et al., 2005; Kushnir et al., 1999; Van Lente and Gatautis, 1998), liquid chromatography-mass spectrometry (Ifa et al., 2001; Oertel et al., 2009; Carlsson and Reubsaet, 2004), ultra performance liquid chromatography-mass spectrometry (Kasprzyk-Horden et al., 2008), high performance liquid chromatography (Ciavarella et al., 2007; Sagirli et al., 2006; Bahrami and Kiani, 2006; Zhu and Neirinck, 2002; Vermeij and Edelbroek, 2004; Tang et al, 1999; Jiang and Li, 1999; Wad and Kramer, 1998),

Keywords : Spectrophotometric determination; Gabapentin, Ninhydrin; 1,2-Naphthoquinone-4-sodium sulphonate; Pharmaceutical analysis.

liquid chromatography (Chung et al., 2006), capillary electrophoresis (Sekar and Azhaguvel, 2004; Rada et al., 1998), electro-oxidation (Hedge et al., 2009), spectrofluorimetry (Belal et al, 2002; Hassan et al., 2001), spectrophotometry applying Hantzsch reaction (Al-Zehouri et al., 2001), flow analysis spectrophotometry applying piezoelectric pumping (Ribeiro et al.,2007).

Visible spectrophotometric methods are commonly used in industrial laboratories because of their simplicity, selectivity and sensitivity.

Gabapentin was determined in pharmaceutical preparations by one spectrophotometric method (Abdellatef and Khalil, 2003). Therefore, the need for a fast, low cost, accurate, precise and sensitive method is obvious, especially for a routine quality control analysis of pharmaceutical products containing gabapentin.

The aim of the present work is to develop simple, sensitive, and selective spectrophometric procedures for the determination of gabapentin in pure and in pharmaceutical capsules. The proposed methods are based on the reaction of primary amino group of gabapentin with ninhydrin and with l,2-naphthoquinone-4-sodium sulphonate reagents in alkaline medium.

2. Experimental

2.1. Apparatus

All the absorption spectral measurements were made using Ultrospec 2100 pro-88683 Biochrom UV-Visible Spectrometer (Cambridge, UK) with 1 cm matched quartz cells.

2.2. Materials and reagents

All reagents were of analytical grade. Double distilled water was used. Gabapentin pure drug was obtained from EVA Pharma Co. (Batch No. 20060404) and Neurontin capsules (Labeled to contain 300 mg gabapentin) were obtained from Godecke AG/Germany under license of Park-Davis, Pfizer (Batch No. 0021026).

Stock gabapentin solution of 100 μgml^{-1} was prepared by dissolving 0.01 g in distilled water and adjusted to 100 ml with distilled water in 100 ml measuring flask. Working solutions of lower concentration were prepared by serial dilutions.

1.2% (w/v) aqueous solutions of ninhydrin reagent (E. Merck, DARMSTADT) and 0.2% (w/v) l,2-naphthoquinone-4-sodium sulphonate reagent, NQS (BDH chemical Ltd.) were freshly prepared. Aqueous solutions of 0.03M and 0.005 M sodium hydroxide (BDH Laboratory Supplies) were prepared by dissolving an appropriate weight in 100 ml distilled water.

2.3. General procedures

2.3.1. Method 1 (Using ninhydrin reagent)

Into 10 ml measuring flasks, different aliquots of stock drug solution were transferred to provide final concentration range 8-50 μgml^{-1}. To each flask, 0.3 ml of 0.03 M NaOH and 0.8 ml of 1.2% ninhydrin reagent were successively added. Each flask was heated in a water bath of 90 ± 2°C for 15 min. After the flask had been cooled to room temperature, the solution was made up to the mark with distilled water. The absorbance of each colored solution was measured against a reagent blank at 566 nm. The calibration graph was prepared by plotting absorbance versus concentration of gabapentin. Alternatively, the corresponding regression equation was derived.

2.3.2. Method 2 (Using NQS reagent)

Into 10 ml measuring flasks, different aliquots of standard drug solution were transferred to provide final concentration range 5-25 μgml^{-1}. To each flask, 0.5 ml of 0.005 M NaOH and 0.4 ml of 0.2% NQS reagent were successively added. Each flask was heated on a water bath at 80 ± 2°C for 10 min. After the flasks had been cooled to room temperature, each solution was made up to the mark with distilled water. The absorbances of the colored solutions were measured against a reagent blank at 540 nm. The calibration graph was prepared by plotting absorbance versus concentration of gabapentin. Alternatively, the corresponding regression equation was derived.

2.4. Procedure for capsules

The content of four capsules was emptied out as completely as possible. An accurately weighed amount of the powder equivalent to 0.0Ig of the drug was dissolved in 100 ml of distilled water. The procedure was continued as described under general procedures. Nominal content of capsules was calculated either from the previous plotted calibration graph or by using the regression equation.

3. Results and discussion

Gabapentin contains a primary aliphatic amino group which is known to undergo condensation reaction in alkaline medium with ninhydrin reagent or substitution reaction with NQS reagent (Pesez and Bartos, 1974).

3.1. Optimization of the reactions conditions

3.1.1. Method 1 (using ninhydrin reagent)

Ninhydrin reagent is used for the determination of an aliphatic primary amine or an amino acid group (Rahman and Azmi, 2001; Nobrega Jde et al., 1994). The presence of an aromatic ring inhibits the response; the inhibition increasing the nearer amine group is to the ring. The reaction is usually carried out by heating for a short time in an alkaline medium of sodium hydroxide. Gabapentin reacts with ninhydrin reagent in NaOH medium via oxidative deamination of the primary amino group followed by the condensation of the reduced ninhydrin to form the colored reaction product-Ruhemenn's purple-with λ_{max} at 566 nm (Fig. 1).

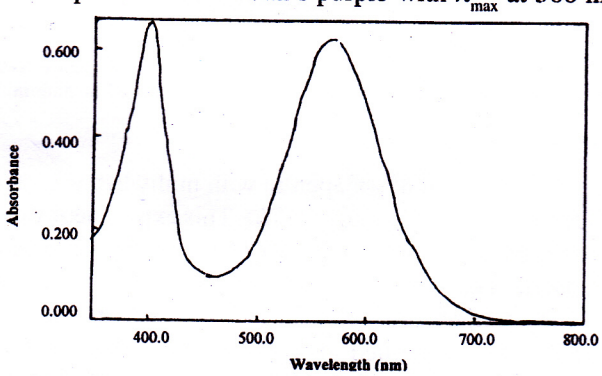


Figure-1 : Absorption spectrum of Gabapentin/ninhydrin product in presence of NaOH.

To optimize the conditions, a number of parameters such as reagent concentration, temperature and time were investigated. The optimum conditions were established by varying one variable and observing the effect on the absorbance of the colored product.

3.1.1.1. Effect of concentration of NaOH

In alkaline medium ninhydrin condenses with gabapentin to give the bluish-violet colored species.

It is noteworthy to mention that NaOH is essential for the production and stability of the color. The role of NaOH may be to stabilize the electron deficient carbon atom in the colored product formed which is measurable at 566 nm. Various volumes of NaOH were tested and found to be 0.3 ml of 0.03 M.

3.1.1.2. Effect of concentration of ninhydrin reagent.

0.8 milliliters of 1.2g (w/v) of ninhydrin reagent was found optimum to maximize the color intensity.

3.1.1.3. Effect of temperature and time

Gabapentin was capable of reaction with ninhydrin only at higher temperatures. Maximum color was obtained by heating on a water bath at 90 ± 2°C for 15 min. The developed color was stable for 2h.

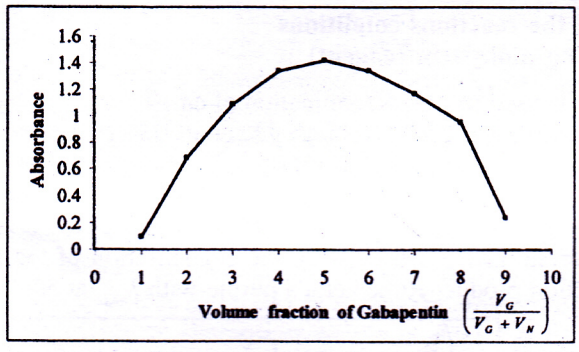


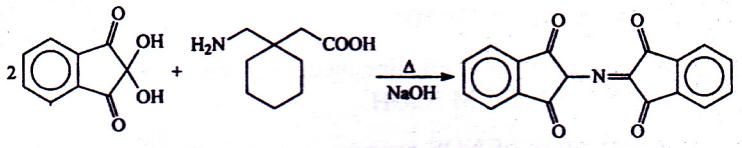
Figure-2 : Determination of Stoichemistry of the reaction of gabapentin (0.001M) and ninhydrin (0.001 M) by continuous variation method at 566nm.

3.1.1.4. Stoichiometry of the reaction

The Stoichiometry of the reaction of gabapentin with ninhydrin was studied by the continuous variation method (Job's method) (Inczedy, 1976). This experiment was conducted by using aqueous solutions of 0.001 M gabapentin and 0.001 M ninhydrin. Nine mixtures of gabapentin and ninhydrin wee prepared. The volumes of gabapentin solution used varied from 9.0 to 1.0 ml, and those of ninhydrin solution from 1.0 to 9.0 ml; total volume was always 10.0 ml, then the procedure was continued as described under general procedures. The Job's plot (Fig-2) reached a maximum value at a mole fraction of 5.0, which confirmed that molar ratio between

gabapentin and ninhydrin in the reaction product is 1:2. Hence, the reaction pathway in Scheme 1 was proposed.

3.1.2. Method 2 (using NQS reagent)



Ninhydrin Gabapentin Violet-product

Scheme (1) : Proposed mechanism of the reaction between gabapentin and ninhydrin in presence of NaOH.

1,2-Naphthoquinone-4-sodium sulphonate reagent (NQS) is used for the determination of aliphatic primary and secondary amines. Gabapentin reacts with NQS reagent in NaOH medium with heating in a water bath where replacement of the sulphonate group of the naphthoquinone sulphonic acid by the amino group on gabapentin takes place to give the color product, N-alkylaminonaphthoquinone which absorbs maximally at 540 nm (Fig. 3). Scheme 2 shows the possible reaction pathway predicted from literature (Pesez and Bartos, 1974) and from results of the present work.

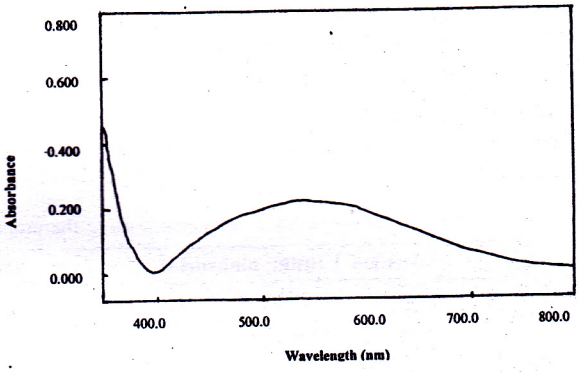
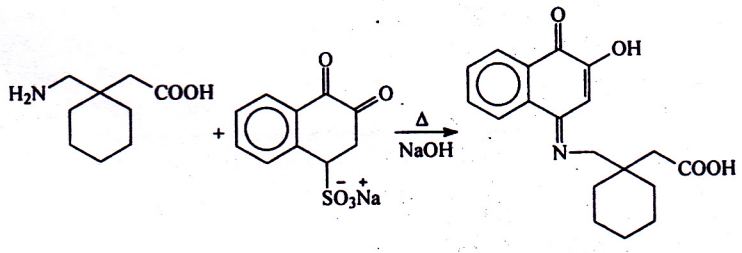


Figure-3 : Absorption spectrum of Gabapentin/NQS product in presence of NaOH.



Gabapentin 1,2-naphthoquinone-4-sodium sulphonate (brown product)

Scheme (2) : Proposed mechanism of the reaction between gabapentin and NQS in presence of NaOH.

The absorptiometric properties of the colored species as well as the influence of different parameters on the color development are extensively studied to determine optimal conditions of the assay procedure. The reaction was studied as a function of the concentration of reagents, heating, time and stability.

3.1.2.1. Effect of concentration of NaOH

This reaction is affected strongly in alkaline medium, where maximum color intensity was obtained upon using 0.5 ml of 0.005 M NaOH.

3.1.2.2. Effect of concentration of NQS reagent

It was found that 0.4 ml of 0.2% (w/v) aqueous NQS solution is optimal for maximum development of the brown color.

3.1.2.3. Effect of temperature and time

Maximum development of the brown color was also attained after heating in water bath at 80 ± 2°C for 10 min. After cooling the color intensity was found to be stable for 2 h.

3.2. Validation

The methods were tested for linearity, accuracy and precision. By using the above spectrophotometric procedures, linear regression equations were obtained. The regression plots showed a linear dependence of absorbance over Beer's law range given in Table-1. The table also shows the results of the statistical analysis of the experimental data, such as the slopes, the intercepts, and the correlation coefficients obtained by the linear least-squares treatment of the results. The limits of detection and quantitation were established according to IUPAC definitions (IUPAC, 1978) and recorded in Table-1.

Table-1 : Optical and regression characteristics of gabapentin using the proposed methods.

Parameters	Method 1 (using ninhydrin)	Method 2 (using NQS)
λ_{max} (nm)	566	540
Linearity ranges (μgml^{-1})	8.0 – 50.0	5.0 – 25.0
Detection limits (μgml^{-1})	2.0	1.0
Quantitation limits (μgml^{-1})	5.0	3.0
* Regression equation:		
Slope (b)	0.0576	0.017
Intercept (a)	–0.110	0.0089
Correlation coefficient (r)	0.9998	0.9998

* With respect to A = a + bC where C is concentration of drug in μgml^{-1} and A is absorbance.

In order to determine the accuracy and precision of the methods, solutions containing different concentrations of pure samples of gabapentin were prepared and analyzed applying the proposed procedures. The analytical results obtained from this investigation are summarized in Table-2.

Table-2 : Determination of gabapentin in pure and dosage forms by the proposed and reference methods.

Drug form	With ninhydrin reagent		With NQS reagent		Reference method[a]
	Taken (μgml^{-1})	Recovery (%)	Taken (μgml^{-1})	Recovery (%)	
Pure gabapentin	8.0	98.8	5.0	100.0	
(B.N. 20060404)	10.0	98.0	8.0	98.8	
	15.0	100.7	10.0	101.0	
	20.0	101.0	15.0	100.7	
	30.0	101.0	20.0	100.0	
	40.0	100.3	25.0	99.6	
	50.0	99.6			
Mean ± S.D.		99.9±1.16		100.0±0.79	99.82±1.37
t-value		0.114(2.201)[c]		0.278(2.228)[c]	(n=6)
F-ratio		1.39(4.39)[c]		3.03(5.05)[c]	
Neurotin 300 mg[b]	8.0	98.8	5.0	98.0	
(B.N. 0021026)	10.0	98.0	8.0	100.0	
	15.0	100.0	10.0	102.0	
	20.0	101.0	15.0	100.7	
	30.0	101.0	20.0	99.5	
	40.0	100.0	25.0	100.0	
	50.0	99.6			
Mean ± S.D.		99.8±1.10		100.0±1.32	99.7±0.98
t-value		0.617(2.228)[c]		0.42(2.262)[c]	(n=5)
F-ratio		1.26(6.16)[c]		1.814(6.26)[c]	

[a] A colorimetric method (Abdellatef et al., 2003)
[b] Parke-Davis, Pfizer
[c] The figures in parenthesis are the tabulated values of t and F at 95% confidence limits.

3.3. Analytical applications

The proposed methods for the determination of gabapentin were successfully applied to commercial capsules together with the reference method (Abdellatef and Khalil, 2003). These determinations were carried out on the same batch of samples. The results obtained showed that the calculated Student's t-test (for accuracy), and variance ratio F-test (for precision) (Miller and Miller, 2000) did not exceed the theoretical values (95% confidence limits for the five degrees of freedom, Table-2), indicating no significance difference between the compared methods regarding accuracy and precision. The proposed methods were more accurate with high recoveries compared to the reference colorimetric method.

4. Conclusion

The data given above reveal that the proposed methods are simple, accurate, sensitive with good precision and accuracy, less time consuming and using simple and inexpensive reagents. The proposed methods can be used as alternative methods to the reported ones for the routine determination of gabapentin capsules. This encourages their successful use in routine analysis of this drug in quality control laboratories.

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