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Colorimetric determination of gabapentin in pharmaceutical formulation

Hisham E. Abdellatef *, Hawa M. Khalil

Faculty of Pharmacy, Analytical Chemistry Department, Zagazig University, Zagazig, Egypt

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Abstract

Three accurate, simple and precise colorimetric methods for the determination of gabapentin in capsules are developed. The first method is based on the reaction of gabapentin with vanillin (Duquenois reagent) in the presence of McIlvain buffer pH 7.5 and the color developed was measured at 376 nm. The linearity range was found to be 80–360 μg ml^{-1}. The second is based on the reaction of the primary amino group of gabapentin with ninhydrin reagent in *N*,*N*′-dimethylformamide (DMF) medium producing a colored product which absorbs maximally at 569 nm. Beer's law is obeyed in the concentration range 40–280 μg ml^{-1} of gabapentin. The third method is based on the reaction of gabapentin with *p*-benzoquinone (PBQ) to form a colored product with λ_{max} at 369 nm. The products of the reaction were stable for 2 h at 30 °C, shifts of the wavelength of maximum absorbance were not observed for up to 24 h after starting the reaction. The absorbance is proportional to gabapentin concentration in the range 80–320 μg ml^{-1}. The optimum experimental parameters for the reactions have been studied. The validity of the described procedures was assessed. 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 capsules. The procedures were rapid, simple and suitable for quality control application.

Keywords: Colorimetric determination; Gabapentin; Duquenois reagent; Ninhydrin; *p*-Benzoquinone

1. Introduction

The new anti-convulsant drug gabapentin (1-(aminomethyl)cyclo-hexaneacetic acid) is a structural analogue of γ-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; a secondary mechanism of a blockade for GABA uptake is also suggested [1]. Currently, gabapentin and its pharmaceutical dosage forms are not found in any pharmacopoeia and different analytical methods are reported for its determination. These include high-performance liquid chromatography (HPLC) [2–7], spectrofluorimetry [8,9], gas chromatography–mass spectrometry (GC–MS) [10,11], capillary electro-

* Corresponding author. Tel.: +20-258-75401; fax: +20-055-2303266
E-mail address: ezzat_hisham@hotmail.com (H.E. Abdellatef).

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phoresis [12] and spectrophotometry applying Hantzsch reaction [13]. To my best knowledge, no more attempts have been made to determine gabapentin by colorimetric method and the literature are still poor in such analytical procedures.

This paper suggests simple and sensitive colorimetric procedures for the determination of gabapentin in capsules. The methods are based on the reaction of primary amino group of gabapentin with many color reagents as vanillin, ninhydrin and *p*-benzoquinone (PBQ).

2. Experimental

2.1. Apparatus

Shimadzu 1601 UV recording spectrophotometer with 1 cm.

2.2. Materials and reagents

All reagents were of analytical grade. Double distilled water was used.

1) Gabapentin pure drug and Neurontin® capsules (labeled to contain 400 mg gabapentin per capsule) were obtained from Godecke AG/Germany under license of Park-Davis.
2) McIlvain buffer was prepared by mixing 35.5 ml of 0.2 M disodium hydrogen phosphate with 64.5 ml of 0.1 M citric acid and pH was adjusted to 7.5 with 0.1 N sodium hydroxide.
3) Duquenois reagent was prepared by mixing 2 g of vanillin with 0.3 ml of acetaldehyde and the volume was completed to 50 ml with ethyl alcohol. The reagent is stored in dark.
4) Ninhydrin reagent: about 0.2 g% (w/v) of ninhydrin in *N*,*N*′-dimethylformamide (DMF) and should be freshly prepared.
5) 0.1 M ethanolic solution of PBQ (Riedel-de Haën).
6) 0.1 M phosphate (Na_2HPO_4–NaH_2PO_4) solution adjusted by NaOH to pH 7.5.

2.3. Standard solutions

Solution of 4.0 mg ml^{-1} was prepared by dissolving 400 mg gabapentin in 100 ml distilled water. The solution was stored in a cool (<25 °C) and dark place.

2.4. General procedure

2.4.1. Method 1 (using Duquenois reagent)

Into 10 ml measuring flasks, different aliquots of drug solution (0.2–0.9 ml) were transferred to provide final concentration range 80–360 μg ml^{-1}. To each flask, 1 ml of Duquenois reagent and 1 ml of McIlvain buffer of pH 7.5 were successively added and set aside at room temperature for 30 min. The volume was made up to the mark with distilled water and the absorbance was measured against a reagent blank at 376 nm. The calibration graph was prepared by plotting absorbance vs. concentration of gabapentin.

2.4.2. Method 2 (using ninhydrin reagent)

Into 10 ml measuring flasks, different aliquots of drug solution (0.1–0.7 ml) were transferred to provide final concentration range 40–280 μg ml^{-1}. To each flask, 2 ml of ninhydrin reagent in *N*,*N*′-DMF was added. The volume was made up to the mark with distilled water and the flask was heated on a waterbath at 90 ± 5 °C for 5 min. After the flask had been cooled to room temperature and the solution was made up to the mark with water. The absorbance of the solution was measured against a reagent blank at 569 nm. The calibration graph was prepared by plotting absorbance vs. concentration of gabapentin.

2.4.3. Method 3 (PBQ reagent)

Into 10 ml measuring flasks, different aliquots of drug solution (0.2–0.8 ml) were transferred to provide final concentration range 80–320 μg ml^{-1}. To each flask, 0.5 ml of 0.1 M phosphate solution and 0.2 ml of 0.1 M ethanolic solution of PBQ were successively added. The volume was made up to the mark with distilled water and the flask was heated on a waterbath at 90 ± 5 °C for 5 min. After the flask had been cooled to room temperature and the solution was made up to the

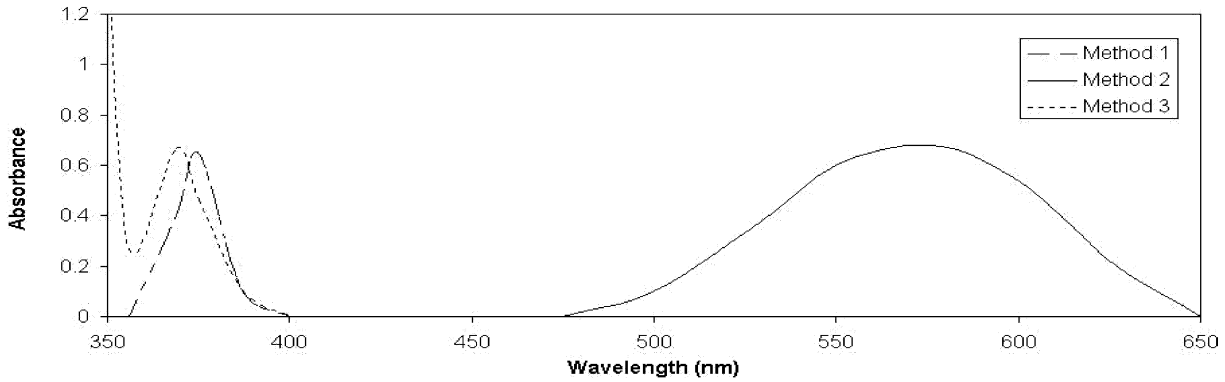


Fig. 1. Absorption spectra of reaction product between gabapentin (240 μg ml^{-1}) and Duquenois reagent (method 1), ninhydrin (method 2) or PBQ (method 3).

mark with distilled water. The absorbance of the solution was measured against a reagent blank at 369 nm. The calibration graph was prepared by plotting absorbance vs. concentration of gabapentin.

2.5. Procedures for capsule

The content of five capsules was emptied out as completely as possible. An accurately weighed amount of the powder equivalent to 400 mg of the drug was dissolved in 100 ml of water. The procedure was continued as described under general procedures.

3. Results and discussion

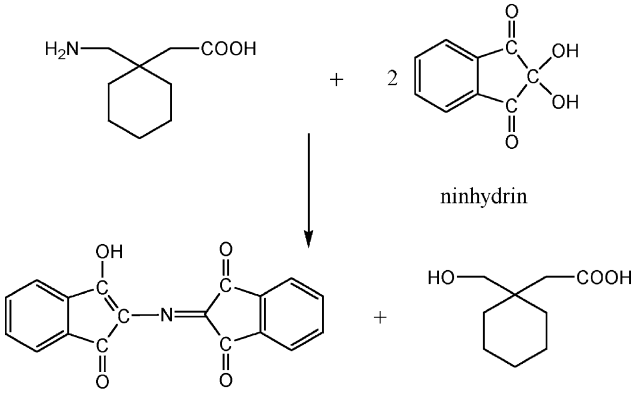
Gabapentin exhibits a very low UV absorption, with $A^{1\%}_{1\ cm}$ at 276 nm = 6.5 and as a consequence, poor sensitivity will be achieved by conventional UV spectrophotometric methods. Gabapentin contains a primary aliphatic amino group, which is known to react with many color reagents as vanillin, ninhydrin and PBQ.

3.1. Optimization of the reactions conditions

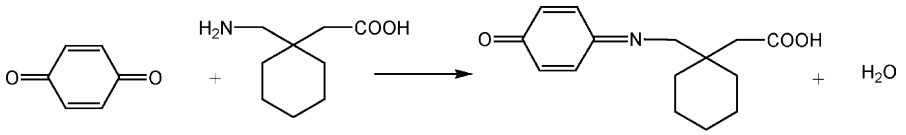
3.1.1. Method 1 (using Duquenois reagent)

Duquenois reagent is used for the determination of amino group containing compounds [14,15]. The reagent consists mainly of vanillin, which contains an aldehyde group that could be react with the primary amino group of gabapentin via condensation mechanism to give colored product.

The McIlvain buffer of pH 7.5 was necessary to achieve the reaction and to obtain the color. Trials are carried out to use buffers with different pH values. However, the pH value 7.5 was found to be the best to obtain the color in a specified time 30 min at room temperature and maximum wavelength at 376 nm. Fig. 1 shows the absorption spectrum of the reaction colored product. The change in buffer concentration and pH led to change in λ_{max}.



Scheme 1. The suggested reaction pathway between gabapentin and ninhydrin in DMF.



Scheme 2. The suggested reaction pathway between gabapentin and *p*-PBQ.

Table 1
Optical characteristics and statistical data of the regression equations for the determination of gabapentin using the proposed methods

Parameters	Method 1 (using Duquenois reagent)	Method 2 (using ninhydrin reagent)	Method 3 (using PBQ reagent)
Linearity range (μg ml^{-1})	80–360	40–280	80–320
Molar absorptivity (mol^{-1} l cm^{-1})	4.57×10^2	5.16×10^2	4.63×10^2
Sandell's sensitivity (μg cm^{-1})	0.374	0.331	0.369
Regression equation			
Intercept, *a*	0.0096	0.011	0.004
Slope, *b*	0.00263	0.00297	0.00269
Correlation coefficient, *r*	0.9993	0.9992	0.9991

3.1.2. Method 2 (using ninhydrin reagent)

Ninhydrin reagent is used for the determination of an aliphatic primary amine or an amino acid group [16–18]. The presence of an aromatic ring inhibits the response; the inhibition increasing the nearer amino group is to the ring. The reaction is usually carried out by heating for a short time in a mixture of water and an organic solvent (2-propranol, butanol, DMF). The reaction product is measured between 550 and 580 nm depending on the reaction condition [19].

Gabapentin reacts with ninhydrin reagent in DMF 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 569 nm (Fig. 1 and Scheme 1).

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

1) Two milliliters of 0.2 g% of ninhydrin reagent was found optimum to maximize the color intensity.
2) Gabapentin was capable of reaction with ninhydrin only at higher temperatures. Maximum color was obtained by heating on a waterbath at 90 ± 5 °C for 5 min prolonged heating decreased the color intensity, and so the reaction time should be controlled. The developed color was stable for 2 h.
3) Different dilution solvents such as water, ethanol, methanol, isopropanol, acetone, dioxan and acetonitrile have been tried, but water gave the best results.

3.1.3. Method 3 (PBQ reagent)

PBQ reagent is used for the determination of an aliphatic primary amine or an amino acid group. Scheme 2 shows the possible reaction pathway predicted from literature [20–22] and from results of the present work, where the free primary amine moiety of gabapentin condenses with carbonyl group of PBQ to form the condensation product.

Under the reaction condition used, which include heating to 90 °C, it was observed that the product of the reaction of PBQ and gabapentin at about 369 nm; for most pharmaceutical compounds [20–22], the products of the reaction absorb in the range 390–670 nm, but most of them at about 490–500 nm. On changing the pH

Table 2
Evaluation of the accuracy and precision of the proposed methods

	Added	Found ± S.D.[a]	RSD (%)	SAE	Confidence limits[b]
Method 1 (using Duquenois reagent)	100	101 ± 1.05	1.040	0.470	101 ± 1.304
	200	203 ± 1.12	0.552	0.501	203 ± 1.391
	300	298 ± 1.31	0.440	0.586	298 ± 1.627
Mean			0.677	0.519	
Method 2 (using ninhydrin reagent)	80	79 ± 1.11	1.423	0.496	78 ± 1.379
	160	156 ± 1.36	0.872	0.601	156 ± 1.688
	240	235 ± 1.52	0.647	0.680	235 ± 1.888
Mean			0.981	0.595	
Method 3 (using PBQ reagent)	100	99 ± 0.96	0.970	0.429	99 ± 1.192
	200	193 ± 0.83	0.430	0.371	193 ± 1.031
	300	293 ± 1.01	0.345	0.452	293 ± 1.355
Mean			0.582	0.417	

[a] Mean ± standard deviation for five determinations.
[b] Confidence limits at $P = 0.95$ and four degrees of freedom.

Table 3
Determination of gabapentin in Neurontin® using the proposed methods compared with reference method [13]

Pharmaceutical preparation	% Recovery ± S.D.[a]			Reference method[b] [13]
	Method 1 (using Duquenois reagent)	Method 2 (using ninhydrin reagent)	Method 3 (using PBQ reagent)	
Neurontin® capsules (400 mg gabapentin/capsule)	99.35 ± 0.62; $t = 0.28$[c]; $F = 5.37$[d]	99.65 ± 0.98; $t = 0.13$; $F = 2.19$	99.59 ± 0.65; $t = 0.16$; $F = 4.98$	99.55 ± 1.45

[a] Spectrophotometric method.
[b] Mean ± standard deviation for five determinations.
[c] Tabulated t-value for $P = 0.05$ and eight degrees of freedom is 2.306.
[d] Tabulated F-value for $P = 0.05$ and $f_1 = f_2 = 4$ is 6.39.

to 0.5, 6.0, 7.0 or 0.8, a shift of the maximum absorbance to shorter wavelengths was observed. Zaia et al. [23] observed a similar shift to shorter wavelengths upon reaction of PBQ with proteins and amino acid.

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 volume of the reagent, selectivity of the solvent, reaction time and stability. The optimum conditions were incorporated into general procedures.

3.2. Linearity, accuracy and precision

The methods were tested for linearity, accuracy and precision. By using the above colorimetric procedures, linear regression equations were obtained. The regression plots showed a linear dependence of the 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, the correlation coefficients obtained by the linear least-squares treatment of the results.

In order to determine the accuracy and precision of the methods, solution containing three different

concentrations of gabapentin were prepared and analyzed in five replicates. The analytical results obtained from this investigation are summarized in Table 2. The mean relative standard deviation (RSD) and the mean standard analytical error (SAE) can be considered to be very satisfactory.

The proposed methods for the determination of gabapentin were applied to commercial capsules together with the reference method [13]. These determinations were carried out on the same batch of samples. The results obtained showed that the calculated t- and F-values did not exceed the theoretical values (95% confidence limits for the five degrees of freedom, Table 3) from which we can conclude that the proposed methods do not differ significantly from reference method.

4. Conclusion

The data given above reveal that the proposed methods are simple, accurate and sensitive with good precision and accuracy. With these methods, one can do the analysis with speed at low cost without losing accuracy. 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 these drugs in quality control laboratories.

References

[1] D. Ouellet, H.N. Bockbrader, D.L. Wesche, D.Y. Shapiro, E. Garofalo, Epilepsy Res. 47 (3) (2001) 229–241.
[2] Q. Jiang, S. Li, J. Chromatogr. B 727 (1–2) (1999) 119–123.
[3] P.H. Tang, M.V. Miles, T.A. Glauser, T. DeGrauw, J. Chromatogr. B 727 (1–2) (1999) 125–129.
[4] D.F. Chollet, L. Goumaz, C. Juliano, G. Anderegg, J. Chromatogr. B 746 (2) (2000) 311–314.
[5] D.R. Ifa, M. Falci, M.E. Moraes, F.A. Bezerra, M.O. Moraes, G. de Nucci, J. Mass Spectrom. 36 (2) (2001) 188–194.
[6] N. Ratnaraj, P.N. Patsalos, Ther. Drug Monit. 20 (4) (1998) 430–434.
[7] N. Wad, G. Kramer, J. Chromatogr. B 705 (1) (1998) 154–158.
[8] F. Belal, H. Abdine, A. Al-Majed, N.Y. Khalil, J. Pharm. Biomed. Anal. 27 (1–2) (2002) 253–260.
[9] E.M. Hassan, F. Belal, O.A. Al-Deeb, N.Y. Khalil, J. AOAC Int. 84 (4) (2001) 1017–1024.
[10] M.M. Kushnir, J. Crossett, P.I. Brown, F.M. Urry, J. Anal. Toxicol. 23 (1) (1999) 1–6.
[11] F. Van Lente, V. Gatautis, Clin. Chem. 44 (9) (1998) 2044–2045.
[12] P. Rada, S. Tucci, J. Perez, L. Teneud, S. Chuecos, L. Hernandez, Electrophoresis 19 (1998) 2976–2980.
[13] J. al-Zehouri, S. al-Madi, F. Belal, Arzneimittelforschung 51 (2) (2001) 97–103.
[14] M.A. Shehata, S.M. Tawakkol, L.E. Abdel Fattah, J. Pharm. Biomed. Anal. 27 (2002) 729–735.
[15] C.L. O'Neal, D.J. Crouch, A.A. Fatah, Forensic Sci. Int. 109 (3) (2000) 189–201.
[16] N. Rahman, S.N. Azmi, Farmacokinetics 56 (10) (2001) 731–735.
[17] N. Nobrega Jde, O. Fatibello-Filho, C. Vieira Ida, Analyst 119 (9) (1994) 2101–2104.
[18] I. Molnar-Perl, M. Pinter-Szakacs, Anal. Biochem. 177 (1) (1989) 16–19.
[19] S. Görög, Ultraviolet–Visible Spectrophotometry in Pharmaceutical Analysis, CRC Press, New York, 1995, p. 318.
[20] L. el-Sayed, Z.H. Mohamed, A.A. Wahbi, Analyst 111 (8) (1986) 915–917.
[21] A.M. Mohamed, H.F. Askal, G.A. Sale, J. Pharm. Biomed. Anal. 9 (7) (1991) 531–538.
[22] A. Numan, B. Musial, N. Anielson, J. Pharm. Biomed. Anal. 30 (3) (2002) 761.
[23] D.A.M. Zaia, W.J. Barreto, N.J. Santos, A.S. Endo, Anal. Chim. Acta 277 (1993) 89–95.