International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

VALIDATED SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF PREGABALIN IN PHARMACEUTICALS BASED ON CHARGE TRANSFER REACTION

K.O. Merin[*1],
S.E. Cicy[1], V.Sheeja[1]

[1]Grace College of Pharmcy, kodunthirapully P.O, Palakkad-678004, Kerala.

ABSTRACT

A simple, rapid, sensitive & specific spectrophotometric method has been developed for the estimation of Pregabalin in bulk and capsule dosage form. The estimation was based on the charge transfer complexation reaction of pregabalin as n-electron donor with π-acceptor picric acid to give yellow colored radical anion species which can be measured at 364nm in acetonitrile. The color was found to be stable for more than 4 hours. Beer Lambert's law was obeyed over a concentration range of 15-30µg/ml (r^2 = 0.998). The effect of reaction time, reagent concentration on sensitivity and stability of the complexes formed has been examined. The results of estimation in capsules were 99.37% & 100.13% by standard curve method and single point standardization of the label claim for Pregabalin. The method has been validated with respect to linearity, range, accuracy, precision, robustness and ruggedness. Association constants and standard free energy changes(k_f) has aiso been calculated.The excipients present in the formulations do not interfere with the assay procedure.

Correspondence to Author

K.O. MERIN

Grace College of Pharmcy, kodunthirapully P.O, Palakkad-678004, Kerala.

Email: merinoommen@gmail.com

KEYWORDS : *Spectrophotometry, Pregabalin, picric acid, charge-transfer complex,formulation.*

INTRODUCTION

Pregabalin (PGB), (S)-3-(aminomethyl)-5-methylhexanoic acid is an anticonvulsant drug which has demonstrated clinical benefit for neuropathic pain and as an adjunct therapy for partial seizures with or without secondary generalization in adults. It has also been found effective for generalized anxiety disorder. Its chemical structure is demonstrated in Figure. 1

No official (pharmacopoeia) method has been found for the assay of Pregabalin and in its formulations. However, many studies have been reported for the determination of PGB in pharmaceuticals and biological fluids[1] including visible spectrophotometry[2,3], RP-HPLC[4,5],LC-MS[6]. Charge transfer complexation reactions [7-11] are popular for their sensitivity in the assay of drugs and, therefore, they have received considerable

attention for the quantitative determination of many pharmaceutical compounds. The aim of this study is to develop simple, rapid, sensitive & specific spectrophotometric method for the determination of PGB in bulk and capsule dosage form.

MATERIALS AND METHODS

Instrument used: A SHIMADZU model PHARMASPEC-1800 UV-Visible double beam spectrophotometer with 1 cm matched quartz cell was used for recording spectra and absorbance measurements.

Reagents: All reagents used were of analytical grade and were obtained from s.d. fine chemicals, Mumbai.

Picric acid (PA): 0.1% of picric acid was prepared by accurately weighing 50mg and dissolving it in 50ml dichloromethane. Pregabalin was kindly supplied by Influx Pharmaceuticals Mumbai (India). Capsule dosage form Nuramed,Zydus Cadilla Healthcare LTD was procured from market**.**

Experimental

Preparation of working standard solutions: 5mg of pregabalin was accurately weighed and transferred to 10ml volumetric flask, dissolved in 1ml water and diluted to mark with acetonitrile to give a stock solution of 500µg/ml.

Method

Aliquot of the working standard solution of Pregabalin x ml, (x=0.3, 0.35, 0.4, 0.45, 0, 5, 0.55, 0.6) (15-30µg/ml) were added into a series of 10ml volumetric flasks. To each of the above aliquots of pregabalin, 0.5ml 0.1% of picric acid solution in dichloromethane was added and mixed thoroughly. The volume was brought up to mark with acetonitrile, mixed thoroughly and absorbance of each species was measured at 364nm against reagent blank. Calibration graphs were constructed by plotting the absorbance against the concentration of the drug as shown in Figure. 2. Overlay spectra of PGB is shown in figure 3.

ASSAY PROCEDURE FOR CAPSULE DOSAGE FORMULATION

Twenty tablets were finely powdered. An accurately weighed amount of the powder equivalent to 5 mg of pregabalin was transferred to a 10 ml standard volumetric flask, dissolved in 1ml of water, sonicated for 5 minutes and solution was made up to the mark with acetonitrile. The solution was then filtered through whatmann filter paper.The first 10ml portion of the filtrate was discarded. 0.45ml of remaining sample stock solution was transferred to 10ml volumetric flask and 0.5ml of picric acid solution was added and made up to 10ml with acetonitrile and absorbance was noted at 364nm. The concentrations of the drugs were calculated using equation of standard curve method & double point standardization.

A) Standard Curve method:

For Pregabalin y = 0.053x - 0.023

B) Single point standardization:

$$C_{test} = \frac{(A_{test} \times C_{std})}{A_{std}}$$

Where

A_{test} = Absorbance of test solution

C_{std} = Concentration of standard

A_{std} = Absorbance of standard

C_{test} = concentration of test solution

RESULTS

An attempt was made to develop a simple, rapid, sensitive and specific analytical method for analysis of PGB in bulk and capsule dosage form.

Absorption spectra

The reaction of Pregabalin base (PGB) as n-electron donor and the π-acceptor , picric acid(PA) results in the formation of charge-transfer (C-T) complex.Absorption spectra of the yellow color PGB- PA charge transfer complex is shown in Figure. 3, quantified spectrophotometricaly, with a maximum absorbance (λ_{max}) at 364 nm. The results of analysis of marketed formulation are given in the Table 1.

Figure 1: Structure of Pregabalin

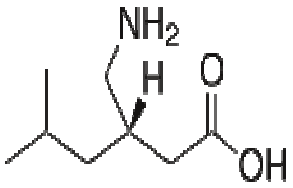

Figure 2: Calibration curve of Pregabalin

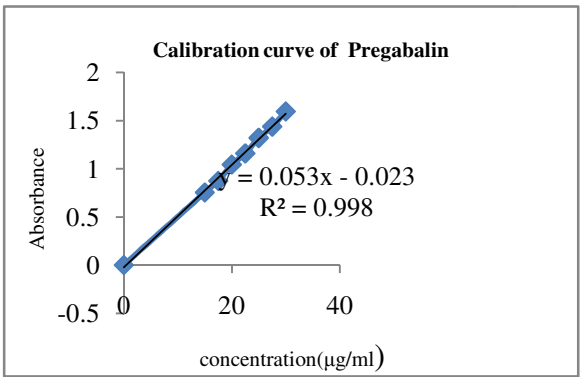

Figure 3: Overlay spectrum of Pregabalin

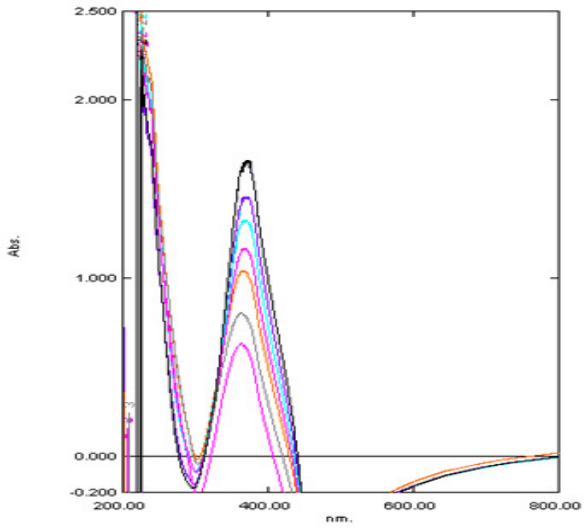

Table 1: Results of marketed formulation analysis

Marketed formulation	Method	Drug	Label claim	Estimated amount	%Label claim
Nuramed(PGB 75 mg,Zydus Cadilla,Health Care LTD)	standard curve method	Pregabalin	75mg	74.53mg	99.37
	Single point standardisation	Pregabalin	75mg	75.10mg	100.13

DISCUSSION

Reaction scheme

A charge-transfer complex [12] (CT complex) or electron-donor-acceptor complex is a complex formed as a result of association of two or more molecules, in which a fraction of electronic charge is transferred between the molecular entities. The resulting electrostatic attraction provides a stabilizing force for the molecular complex. The source molecule from which the charge is transferred is called the electron donor and the receiving species is called the electron acceptor. The attraction is created by an electronic transition into an excited electronic state, and is best characterized as a weak electron resonance. The excitation energy of this resonance occurs very frequently in the region between (360-800nm) of the electro-magnetic spectrum, which produces usually intense colour characteristic for these complexes. Therefore, pregabalin an n-electron donor has allowed to react with a pi-electron-acceptor, picric acid, to produce yellow colored charge transfer complex peaking at 364nm. Charge transfer complexation reactions have been extensively utilized for the determination of electron-donating basic nitrogenous compounds using π-acceptor (picric acid). The application of picric acid for the quantitative estimation of orphendrine citrate and phentolamine mesylate injections listed in the USP.picric acid reacts with electron donor molecule to form charge transfer molecules.

Reaction with π-acceptor

Amines are organic compounds and functional groups that contain a basic nitrogen atom with a lone pair.When an amine is combined with a plolynitrophenol,a donor-acceptor interaction[13] takesplace. According to lewis-acid base theory, A Lewis base, is any species that donates a pair of electrons to a Lewis acid to form a Lewis adduct, so pregabalin which is having a methylamino group donates electrones to picric acid(lewis acid-acceptor),which results in the formation of complexes between the pair of molecule PGB-PA . The nature of the attraction in a charge-transfer complex is not a stable chemical bond, and is thus much weaker than covalent forces. Quantum mechanically, this is described as a resonance between the non-bonded state |D, A> and the dative state $|D^{+}...A^{-}>$ [12]. The formation of the dative state is an electronic transition giving rise to the colorful absorption bands.

The color of CT complexes is reflective of the relative energy balance resulting from the transfer of electronic charge from donor to acceptor. These colors are caused by electronic transitions by the absorption of light. A charge transfer band entails promotion of electron from a metal-based orbital into an empty ligand based orbital (Metal to Ligand Charge Transfer or MLCT). The converse also occurs: excitation of an electron in a ligand-based orbital into an empty metal-based orbital (Ligand to Metal Charge Transfer or LMCT). This phenomenon can be observed with the aid of electronic spectroscopy.

Reaction Mechanism

Higuchi and Brochmann-Hanssen[14] have reported that the basic aliphatic amines form salts with picric acid in organic solvents which are much more intensely colored than picric acid itself, and this is due to the fact that the negatively charged picrate ion (phenolate ion) is intensely colored (yellow), where as the undissociated form, as it exists in neutral or acidic solvents is very lightly colored. Similarly, Saito and Matsunaga[15] reported that when the aliphatic amine is combined with a polynitrophenol, the fore field produces an acid-base interaction which leads to the formation of true phenolate by proton transfer.Suggested reaction pathway is given in figure .4 and shown as follows:

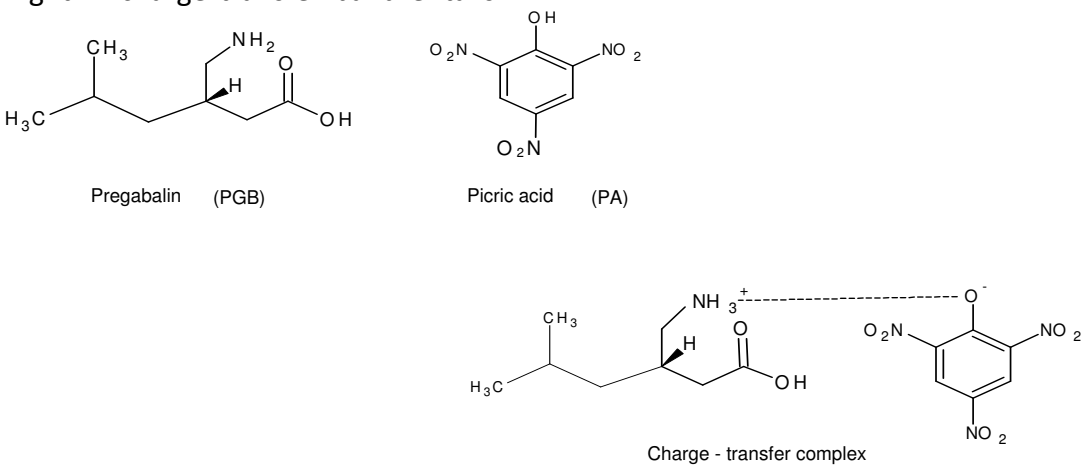

Figure : 4 Suggested reaction pathway

OPTIMIZATION OF EXPERIMENTAL VARIABLES

Some experimental variables which were found to affect the color intensity and stability of the resulting charge-transfer complexes were optimized to achieve maximum analytical sensitivity and adherence to Beer's law.

Effect of reagent concentration

The optimum reagent concentration required to achieve maximum sensitivity of the developed radical anion species was ascertained by adding different volumes (0.2–2 ml) of 0.1-0.5% solution of PA to a constant concentration of PGB (20 µg/ml). Maximum absorbance of PGB-PA charge-transfer complexes was found at 0.5 mL of 0.1 % solution of PA, beyond this value the absorbance decreases.

Hence, 0.5 mL of 0.1% PA solution was used throughout this study.

Effect of solvent

The possibility to prepare PGB solution in different solvents was restricted since its solubility is limited to a few solvents such as water,methanol and ethanol . Even though PGB is freely soluble in water and insoluble in acetonitrile, PGB can't be prepared in water because water gave intense yellow color with PA. So, to prepare the stock solution, PGB was first dissolved in a minimum amount of water and subsequently diluted with acetonitrile. The results showed that the effect of amount of water used to prepare the stock solution of PGB was negligible.In order to select a suitable solvent to prepare the reagent solution used in the study, the reagent was prepared separately in different solvents such as 1,4-dioxane, chloroform,acetonitrile, acetone, t-butanol,2-propanol and dichloromethane.The reaction of PGB with PA was carried according to the procedure mentioned above,dichloromethane was best suited for the preparation of PA solution. Similarly, the effect of the diluting solvent was studied for all the methods and the results showed that the ideal diluting solvent to achieve maximum sensitivity and stability of the colored species was acetonitrle.

Effect of reaction time and stability of the C-T complexes

The optimum reaction times were determined by measuring the absorbance of the complex formed upon the addition of reagent solution to PGB solution at room temperature. The reaction of PGB with PA was instantaneous and complete color developed immediately after the addition of reagent solution. The absorbance of the resulting CT complexes remained stable for at least more than 4 h .

Association constants and standard free energy changes(k_f)

The association constants was determined for Donor-Acceptor association (pregabalin-picric acid) using Benesie

Hildebrand equation[16,17] shown in Table. 2

$[D]o/Abs = (1/[A]o)\ (1/K_f\ \varepsilon) + 1/\varepsilon$

[D]o = concentration of donor (pregabalin)

Abs = Absorbance of CT complex at wavelength λ

[A]o = concentration of acceptor (picric acid)

K_f = equilibrium constant for DA complex formation

ε = molar absorptivity of DA complex at λ

From the above mentioned equation, on plotting the values of D_O vs A, straight lines were obtained.

The standard free energy changes(ΔG^0)

The standard free enegy changes of complexation[19-21] were calculated from the association constants by the following equation and shown in table. 2

$\Delta G^0 = -2.303RT\log K_f$

Where ΔG^0 = standard change of reaction in Gibbs free energy (KJ mol^{-1})

R = the gas constant (0.001987 K cal $mol^{-1}deg^{-1}$) [18, 22, 23]

T = the temperature in kelvin(273+ ^{0}C)

K_f = the association constant of CT complex

METHOD VALIDATION

Precision

In order to determine the precision of the proposed methods, pure drug solutions (PGB) at a particular concentration level (within the working range) were prepared and analyzed in three replicates during the same day (intra-day precision) and on three consecutive days (inter-day precision) and the results are presented in Table. 3. Percentage relative standard deviation (RSD %) for intra-day was ≤ 0.149 and for inter-day it was ≤ 0.086 indicating repeatability and usefulness of the proposed methods in the routine analysis.

Accuracy

The accuracy of the method was determined by recovery experiments. A known quantity of the pure drug was added to the pre-analyzed sample formulation at 60%, 80% and 100%levels. The percentage recovery and standard deviations were calculated. The recovery studies were carried out 6 times and the percentage recovery and percentage relative standard deviation of the percentage recovery were calculated and given in Table. 4

Analytical data parameters

The linear regression equations were obtained by the method of least squares and the Beer's law

range, detection wavelength, correlation coefficient, slope and intercept for both methods are summarized in Table. 5.

Selectivity

Upon analysis of placebo blank solution as described under "assay procedure for capsule dosage formulation", the resulting absorbance readings for both the methods were same as reagent blank, inferring no interference from the placebo. The percent recoveries of drugs were 100.15 for PGB. This confirms the selectivity of methods in the presence of the commonly employed capsule excipients.

Robustness and ruggedness

To evaluate the robustness of the methods, the temperature of sample solutions was changed and the effect of this change on the absorbance of the sample solutions was studied. The results of this study are presented in Table. 6 and indicated that the proposed methods are robust. Method ruggedness was evaluated by performing the analysis following the recommended procedures by two different analysts. From the %RSD values presented in Table. 6, one can conclude that the proposed methods are rugged.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ values were calculated to check the detection limit and quantitation limit of the method by using following equations;

$$LOD = \frac{3.3\sigma}{S} \qquad LOQ = \frac{10\sigma}{S}$$

Where σ the standard deviation and S is is the slope of the curve.

The values are concluded in the table .7.

Table 2 : (molar absorptivity,standard free energy and association constant)

Molar absorptivity(ε)	Standard free energy changes(ΔG^0)	Association constant(k_f 10^3)
51768.499	-4.09	1.00002

Table 3: Precision

Drug	Amount(µg/ml)	Intraday		Interday	
		%Content	%RSD	%Content	%RSD
		99.37		99.11	
		99.11		99.2	
Pregabalin	7.5	99.28	0.149	99.28	0.086

Table 4 Accuracy

Drug	Theoretical % target level	Amount added(µg/ml)	Amount recovered(µg/ml)	%Recovery	%RSD
	60	12	11.95	99.58	0.23
	80	16	15.98	99.87	0.275
Pregabalin	100	20	20.03	100.15	0.35

Table. 5 Analytical data parameters

Parameters	Pregabalin
Beer's law limit	15-30µg/ml
Detection limit	364nm
Regression equation	y=0.053x-0.023
Correlation coefficient	0.998
Slope	0.053x
Intercept	0.023

Table. 6 Ruggedness and Robustness

Drug	Amount taken(µg/ml)	Method Robustness			Method Ruggedness		
		Amount found(µg/ml)			Amount found(µg/ml)		
		Parameter altered(temperature)			Parameter altered(analysts)		
		25 ^{0}C	35 ^{0}C	%RSD	25 ^{0}C	35^{0}C	%RSD
pregabalin	20	19.93	19.95	0.252	19.95	19.98	0.351

Table. 7 LOD & LOQ

Pregabalin	
LOD(µg/ml)	LOQ(µg/ml)
3.99	12.09

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 spectrophotometric method is simple, rapid, sensitive and specific and can be employed successfully for the estimation of Pregabalin in bulk and capsule formulation.

ACKNOWLEDGMENT

We wish to acknowledge, Infux Pharmaceuticals, Mumbai, India, for providing the gift sample of Pregabalin. We also thank Principal, staff and faculties of Grace College of Pharmacy, Palakkad, Kerala, for permission and facilities to carry out the research.

REFERENCES

1. Gujral.R.S, Manirul. Haque.S.K and Kumar.S. A novel method for the determination of pregabalin in bulk pharmaceutical formulations and human urine Samples. African Journal. Pharmacy and Pharmacology.3(6);2009: 327-334.
2. Alka B,Prateek.G. A novel method for spectrophotometric determination of pregabalin in pure form and in capsules. Chemistry Central Journal. 5(59);2011: 1-10.
3. Walash.M.I, Belal.F.F,El-Enany.N.M and El-Maghrabey.M.H. Utility of certain nucleophilic aromatic substitution reactions for the assay of pregabalin in capsules.Chemistry Central Journal.5(36);2011.
4. Ashu M, Parmar, Nagarajan K and Vijendra Singh. Development and validation of rapid HPLC method for determination of Pregabalin in bulk drug and capsule dosage forms. Scholars Research Library.3(1);2011:482-489.
5. Kannapan. N, Nayak. S.P, Venkatachalam. T, Prabhakaran.V. Analytical RP-HPLC Method for Development and Validation of Pregabalin and Methylcobalamine in Combined Capsule Formulation. Journal of Applied Chemicl. Research.13;2010:85-89.
6. Uma.G, Manimala.M,Vasudevan.M,Karpagam.S,Deecarman.Lc-ms-ms method for the determination of pregabalin in human plasma. International. journal of Pharmacy and Pharmaceutical Sciences. 4(3);2012:108-112.
7. Siddiqui. F.A et al. Spectrophotometric determination of gabapentin in pharmaceutical formulations using ninhydrin and p-acceptors.European Journal of Medicinal Chemistry. 45; 2010: 2761-2767.
8. Sameer A. M, Abdulrahman , Basavaiah.k. Sensitive and Selective Spectrophotometric Determination of Gabapentin in Capsules Using Two Nitrophenols as Chromogenic Agents.

International Journal of Analytical. Chemistry;2011.
9. Neeti.S, Ishaat M. K, Afaq A . Spectrophotometric and spectroscopic studies of charge transfer complexes of p-toluidine as an electron donor with picric acid as an electron acceptor in different solvents. Spectrochimica Acta Part A. 75;2010:1347-1353.
10. Madihalli S. R, Kanakapura .B .Optimized and validated spectrophotometric methods for the determination of levocetirizine in pharmaceuticals based on charge transfer reaction. Journal of the Association of Arab.Universities for Basic and Applied Sciences.12;2012: 33-41.
11. Neeti .S, Afaq.A ,Spectrophotometric and spectroscopic studies of charge transfer complex of 1-Naphthylamine as an electron donor with picric acid as an electron acceptor in different polar solvents. Journal of. Molecular Structure.977;2010:197-202.
12. http://en.wikipedia.org/wiki/Charge-transfer_complex
13. http://en.wikipedia.org/wiki/Pi_interaction.
14. Higuchi.T, Brochmann- Hanssen.E, Pharmaceutical Analysis, CBS Publishers, New Delhi, India:1997.
15. Saito.G , Matsunaga.Y. Charge-transfer and proton transfer in the formation of molecular complexes. I. The complex isomerization of some anilinium picrates by melting, Bulletin of the Chemical Society. of Japan. 44;1971: 3328–3335.
16. Benesi, H. A, Hildebrand, J. H. J. Am. Chem. Soc.,71; 1949: 71: 2703.
17. Mohr, Peter J, Taylor, Barry N, Newell, David B. (2008).
18. "CODATA Recommended Values of the Fundamental Physical Constants: 2006". Rev. Mod. Phys. 80 (2);2008: 633–730.
19. Greiner, Walter, Neise, Ludwig, Stöcker, Horst. Thermodynamics and statistical mechanics. Springer-Verlag;1995: 101.
20. Perrot, Pierre (1998). A to Z of Thermodynamics. Oxford University Press.
21. http://en.wikipedia.org/wiki/Gibbs_free_energy.
22. http://en.wikipedia.org/wiki/Gas_constant.
23. Bibcode2008RvMP...80..633M. doi:10.1103/RevModPhys.80.633. http://physics.nist.gov/cuu/Constants/index.html.

*****