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Research Article

A SENSITIVE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF PREGABALIN IN PURE DRUG AND PHARMACEUTICAL FORMULATIONS THROUGH BENZOYLATION

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ABSTRACT

Pregabalin is chemically related to an antiepileptic drug *gabapentin*. Chemical name of pregabalin is; (S)-3-isobutyl *GABA*, (S)-(+)-3-(aminomethyl)-5-methylhexanoic acid. It is an alpha2delta-ligand that has been found effective in a variety of animal models of neuropathic and nociceptive pain. It is found from IUPAC name that pregabalin does not have chromophoric group. To be UV sensitive, it is compulsory to have a chromophoric group in the structure. Thus, major aim of present research paper was to introduce a chromophoric group in pregabalin structure and make it UV sensitive. This was achieved by converting primary amine group of pregabalin in UV sensitive product through reaction with benzoyl chloride. A new sensitive and selective spectrophotometric method has been developed and validated according to ICH guidelines for determination of UV sensitive product of pregabalin in pure drug and pharmaceutical formulation. UV sensitive reaction product is determined at 223 nm using UV-VIS spectrophotometer. Developed method shows best results in terms of linearity, accuracy, precision, LOD and LOQ. It obeys Lambert-Beer's law in the range of 2.5-12.5 µg mL^{-1}, shows good linearity [r^2 = 0.9997] and accuracy [99.71 % w/w]. The suggested procedure can be used for determination of pregabalin for pharmaceutical formulation. Thus, present research paper will be useful for routine analysis of pregabalin without need of sophisticated techniques.

KEYWORDS: Pregabalin; gabapentin; benzoyl chloride; benzoyl derivative; ICH guidelines; UV-VIS Spectrophotometer.

INTRODUCTION

The structure of Pregabalin (PGL) is shown in Figure 1, (S) - 3 - amino methyl hexanoic acid. It is a white crystalline solid with molecular formula $C_8H_{17}NO_2$, molecular mass 159.23 g/mol and melting point 190-192 ºC[1]. It is an anticonvulsant and analgesic medication that is both structurally and pharmacologically related to gabapentin. PGL is recently approved for adjunctive treatment of partial seizures in adults in United States and Europe for the treatment of neuropathic pain from postherpetic neuralgia and diabetic neuropathy[1-3]. PGL is less metabolised in human and unchanged parent compound represents majority (≥ 90 %) of drug-derived material[4].

A through literature search has revealed that very few analytical methods are available for determination of PGL in pure drug and pharmaceutical formulations. Few methods such as HPLC with fluorescence detection[5], LC-MS-MS from human plasma[6], Spectrophotometric and spectrofluorimetric methods[7] are available in literature. I.P. 2010 has monograph of PGL, but analytical procedure is HPLC method[8]. Thus, all the reported methods are quite complicated and can't be utilized for routine analysis of PGL. UV-VIS spectrophotometric technique is most preferable technique for routine analysis, but it cannot be utilized for estimation of PGL. Since, PGL is less UV sensitive due to lack of strong chromophoric group, which is the basic condition to be UV-VIS sensitive.

The present method is based on UV-VIS spectrophotometry. In this proposed research paper chromophoric group is attached to PGL moiety through benzoylation[9-10]. PGL is a primary amine compound which is allowed to react with benzoyl chloride to form benzoylated derivative of PGL. This benzoylated reaction product is UV sensitive and shows maximum absorption at 223 nm.

The method was developed and validated according to ICH guidelines[11] for the estimation of UV sensitive product of PGL in pure form and this validated method was applied for determination of PGL in pharmaceutical formulation. Hence, in the present investigation we proposed to develop and validate a simple, sensitive, accurate, and cost effective spectrophotometric method for determination of PGL in pure and pharmaceutical formulation.

MATERIALS AND METHODS

Instrumentation

Spectrophotometric measurements were made on a PerkinElmer Lambda 35 double beam UV Visible spectrophotometer with a fix slit width of 1 cm coupled with computer loaded with PerkinElmer Win lab Pro Software.

Reagents

Pure PGL was procured as a gift sample from Alembic Pharmaceuticals Ltd. Vadodara, India. Formulation (capsules) of PGL (Microlabs Ltd., PREGATOR Batch no PGRY0018, Manufacturing Date: Jun 2009, Expiry date: Nov 2010) containing 75 mg of PGL per capsule was obtained from local drug store, Moga, India. Other reagents such as benzoyl chloride, sodium hydroxide pellets, hydrochloric acid and methanol were obtained from CDH Labs, New Delhi, India. All other reagents and chemicals were of analytical grade (AR). 1 N sodium hydroxide & 5 % hydrochloric acid were prepared in distilled water[8].

Reaction with benzoyl chloride (benzoylation)

Stock solution of 1 mg mL^{-1} of PGL was prepared in methanol. Aliquot of 1 part of PGL was allowed to react with 1 part of benzoyl chloride in the presence of sodium hydroxide solution and was kept on stirring for about four hours. Few drops of hydrochloric acid solution were added to precipitate out final product. The product was filtered and vacuum dried. Optimum time of reaction completion was determined with TLC. The aliquots of reaction products were prepared in methanol (2.5-12.5 µg/ml). Absorbance of final dilutions was recorded at 223 nm against methanol. Zero order spectra of UV sensitive PGL product (2.5-12.5 µg/ml) are shown in Figure 2.

Test (pharmaceutical formulation) solution of PGL

PGL from formulation was extracted with water and treated for benzoylation. The test dilutions were prepared of benzoylated product using methanol.

RESULTS AND DISCUSSION

In order to have a good UV sensitivity, amino group of PGL is allowed to react with benzoyl chloride in alkaline condition (NaOH 1N), & stirred for about four hours. The benzoylated product is precipitated out with HCl (few drops) in form of white product. The reaction mechanism is describes in Figure 3. The optimum time of reaction completion is determined with TLC. As with time spot of reactant is disappeared from the product indicates formation of benzoyl derivative. The TLC images at different interval of time which indicates completion of reaction after 4 hours is explained in Figure 4. The reaction stoichiometry was found to be a good approximate 1:1 (drug: reagent), confirming that one molecule of PGL reacts with one molecule of benzoyl chloride[9]. The structure of benzoylated product of PGL is shown in Figure 5. Benzoyl chloride is chromogenic reagent used for many reaction processes. It is very commonly available reagent in each of laboratory compare to the other reagents used for PGL estimation[7]. The proposed procedure is quite simple and covers a wide range of PGL concentration compare to previous reported methods[12].

The Benzoylated product was analyzed for stability and it is found to be stable for 48 hours. Stability of PGL is explained in Figure 6. Developed method was validated for determination of benzoylated product. Validation parameters of developed method are shown in (**Table 1**). Linear relationship found

between absorbance and concentration of benzoylated product (at λ_{max}) in the range of 2.5-12.5 µg/ml. Correlation coefficient (r^2) was found to be 0.9997. It is clearly observed from (**Table 1**) that all the validation parameters are acceptable and within range of ICH guidelines.

To determine accuracy of the method, standard spiking technique was used. Different amount of pure benzoylated product was added to benzoylated drug product of the pharmaceutical formulation and assayed. Percentage recovery of added standards to assay sample was calculated and summarized in (**Table 2**). It is found that results obtained from proposed method are accurate and accuracy found to be 99.71 % w/w.

The applicability of method was checked by % purity (assay) of PGL in capsules. Capsules were converted in benzoylated product and % purity was determined. Results were satisfactory and accurate as shown in (**Table 3**). It is found that there was no interference from various excipients such as lactose, starch etc. Thus, method is found to be specific and selective.

In conclusion, we can say that proposed method gives UV sensitive derivative of PGL through benzoylation. This benzoylated product can be easily quantified using UV-VIS spectrophotometer as compared to parent moiety. Newly developed spectrophotometric method for estimation of PGL is sensitive, specific, accurate, precise, rapid, and cost effective according to ICH guidelines. Method is applicable and suitable for routine quality control of PGL and pharmaceutical formulation without any interference of excipients.

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Table 1: Validation Parameters Results Obtained by the Method

Validation Parameters	Results
Absorption maxima, λ_{max} (nm)	223
Beer's law Limit (µg/ml)	2.5-12.5
Regression equation (Y^a) Slope (b) Intercept (a)	Y=0.0472X+0.0812 0.0472 0.0812
Coefficient of determination (r^2)	0.9997
Limit of detection LOD, (µg/ml)	0.31
Limit of quantitation LOQ (µg/ml)	0.87
Accuracy (% w/w)	99.71±1.23
Precision (% R. S. D)*	Intra-day = 0.52 Inter-day = 1.24
Robustness (% R. S. D)	Less than 1 %
% Purity (w/w)	99.46

* R.S.D. = Relative standard deviation

Table 2: Results of recovery studies by standard addition method

Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	% Recovery
8	7.93	99.24
10	9.87	98.79
12	12.13	101.12
Mean		99.71
Std. Dev.		1.23

Table 3: Results of analysis of PGL in Capsules

Label Claim (mg)	Amount found (mg)	% Purity
75	74.6	99.46

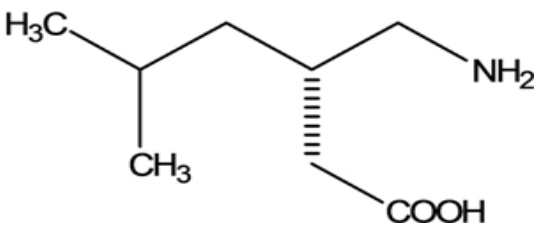

Figure 1: Structure of PGL

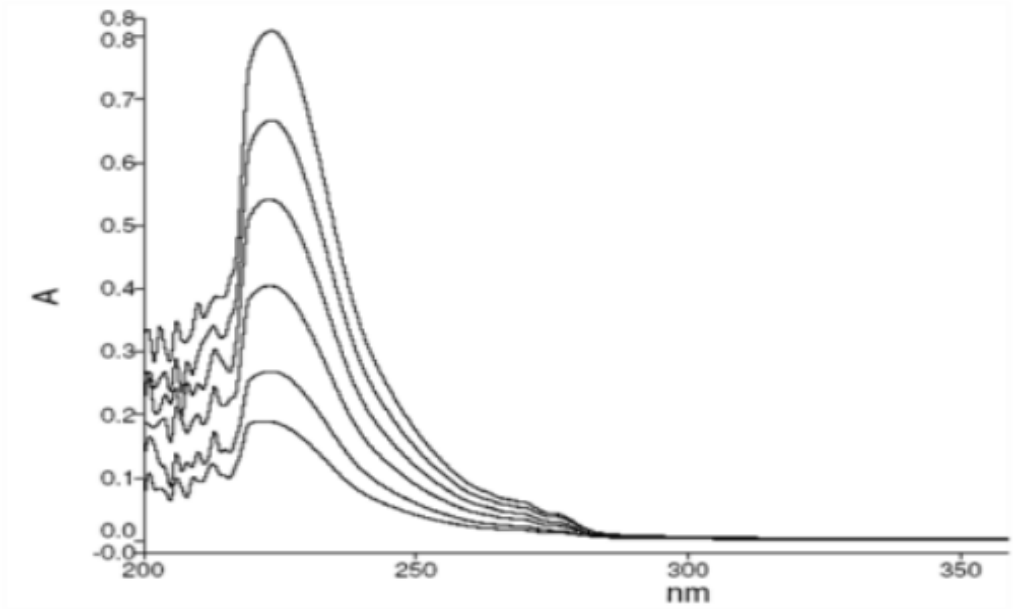

Figure 2: Zero order spectra of benzoylated product of PGL of 2.5-12.5 µg mL^{-1} concentrations

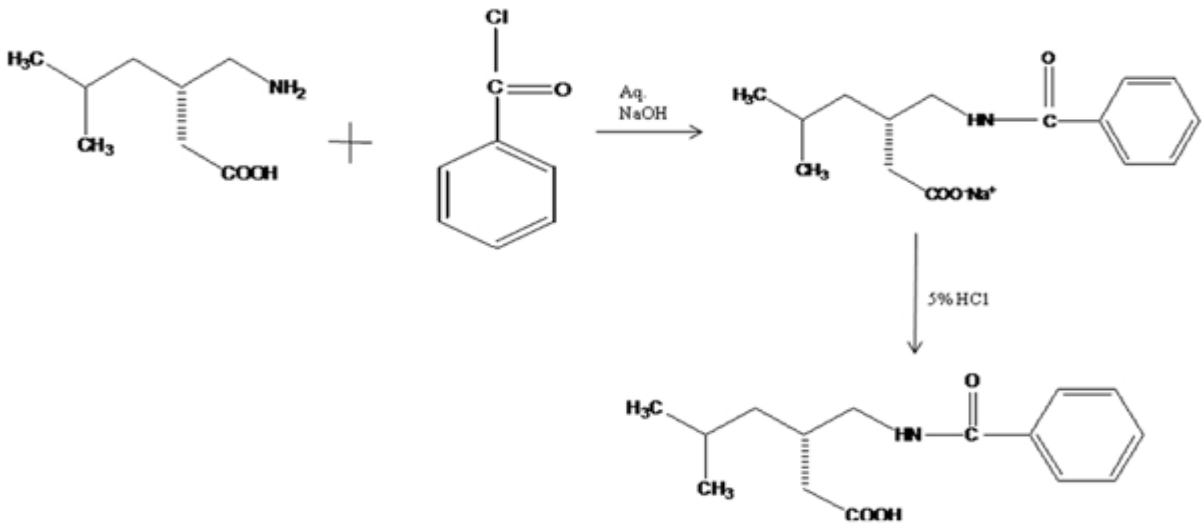

Figure 3: Mechanism of the Reaction

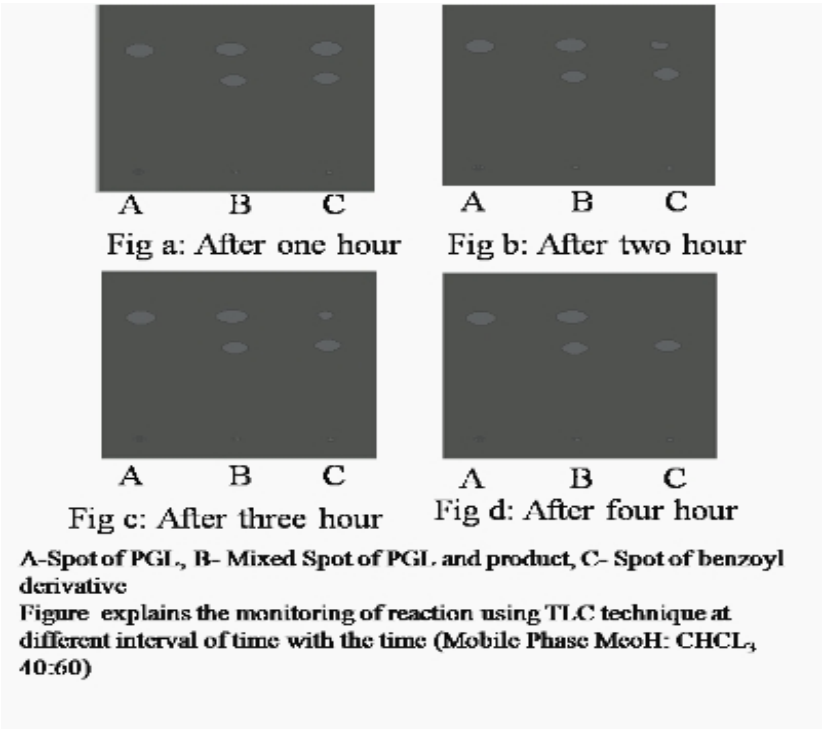

Figure 4: TLC image at different interval of time

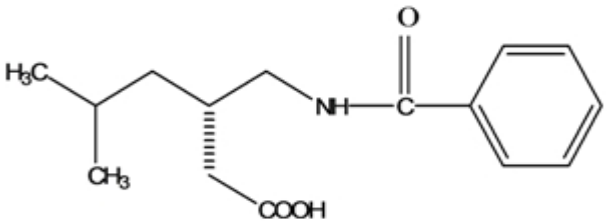

Figure 5: The Structure of the Benzoyl derivative.

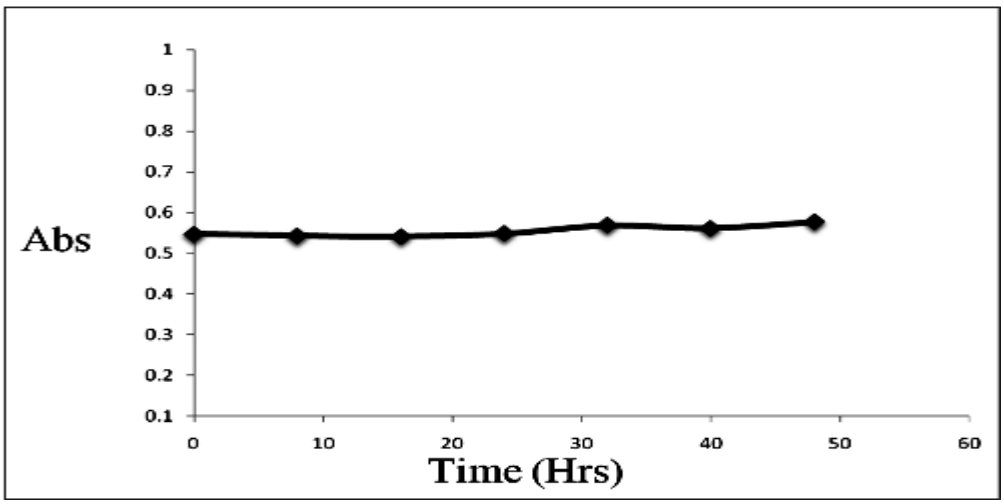

Figure 6: Stability of benzoylated PGL for 48 hours

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