Optimized and Validated Spectrophotometric Methods for the Determination of Pregabalin in Pharmaceutical Formulation Using Ascorbic Acid and Salicylaldehyde

Sherin F. Hammad[1] and Ola M. Abdallah[2]*

[1]Analytical Chemistry Department, Faculty of pharmacy, Tanta University, Tanta, Egypt
[2]Analytical Chemistry Department, Faculty of pharmacy (girls), Al-Azhar University Cairo, Egypt
*olamody @yahoo.com

Abstract: Two simple and selective spectrophotometric methods have been developed for the determination of the γamino-*n*-butyric acid derivative pregabalin (PGB). The first method is based on the reaction of pregabalin, as a primary amine compound, with ascorbic acid in presence of dimethylformamide to give a purple colored product measured at 530 nm. The second method is based on the derivatization of PGB with salicylaldehyde (SA) at neutral pH, the reaction conditions were optimized and the derivative absorbed maximally at 410 nm. The methods showed linearity in wide ranges of 5.0–50 μg mL^{-1} for the first method and 5–60 μg mL^{-1} for the second one. The proposed methods were extensively validated and the results obtained by adopting the two methods were statistically analyzed and compared with those obtained from a reported method. The two proposed methods were applied successfully to the determination of pregabalin in pharmaceutical dosage form. The mean recovery from commercial capsules was 102.86% ± 0.67 and 100.41% ± 1.31 for the first and second method respectively.
[Sherin F. Hammad and Ola M. Abdallah. **Optimized and Validated Spectrophotometric Methods for the Determination of Pregabalin in Pharmaceutical Formulation Using Ascorbic Acid and Salicylaldehyde.** *J Am Sci* 2012;8(12):118-124]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 18

Keywords: Pregabalin; ascorbic acid; salicylaldehyde; spectrophotomety

1. Introduction

Pregabalin (PGB), (S)-3-(aminomethyl)-5-methylhexanoic acid, Fig. 1 is a structurally related to the inhibitory neurotransmitter γ aminobutyric acid (GABA). PGB is an antiepileptic drug used for neuropathic pain, as an adjunct therapy for partial seizures, and in generalized anxiety disorder and diabetic neuropathy **[1, 2].**

As to our best knowledge, there is no official analytical method for analyzing of PGB in any pharmacopeia. Certain successful attempts have been made for the determination of pregabalin using different analytical techniques. The generally used analytical techniques are gas chromatography-mass spectrophotometry (GC-MS) **[3],** HPLC methods coupled with varying detection techniques like tandem mass spectrometry **[4, 5]** and photodiode array detector **[6].** These methods may involve procedural variations including pre- and post- column derivatization **[7-9].** Of course, the above mentioned techniques are sensitive but expensive. Spectrophotometry is the technique of choice even today due to its inherent simplicity. It is frequently used in the laboratories of the developing countries to overcome versatile analytical problems. PGB, as such, has a poor UV/ visible absorbance profile. In the literature, few spectrophotometric methods have been reported for its determination using different chromogenic & fluorogenic agents **[10-15]**, these spectrophotometric methods are still preferable especially in laboratories where modern and expensive apparatus is not available.

We found it is very pertinent to investigate and develop novel spectrophotometric methods for determination of PGB in bulk powder and pharmaceutical preparations. In the present study, two sensitive spectrophotometric methods were applied; the first method is based on the reaction of PGB with ascorbic acid in presence of dimethyl formamide to give purple colored product [method I]. The second method depends on the reaction of PGB with salicylaldehyde at pH 7.0 to form yellow colored product [method II]. Both methods are direct simple using cheap reagents and do not involve any extraction step which make them suitable for routine analysis of the drug in bulk or in pharmaceutical formulations.

2. Experimental

Apparatus

Spectrophotometric measurements were carried out using a Shimadzu (Japan) UV-1800 PC double beam spectrophotometer with 1 cm glass cells. Absorbance values were measured at 530 nm for method I and 410 nm for method II.

pH measurements were made with HANNA pH 211 Microprocessor pH Meter with double junction glass electrode.

Reagents and solutions

Pregabalin pure sample was kindly provided by Pfizer Egypt S.A.E under the authority of Pfizer

Inc.USA and its subsidiary in UK with a purity of 100.16% as determined by the reference method [14].

Lyrica® capsules, Batch no. 1102 (labeled to contain 50 mg PGB each), product of Pfizer company, obtained from a local pharmacy.

L-ascorbic acid, Salicylaldehyde (SA) and 1,2-Naphthoquinone-4-sulphonate sodium (NQS) solution (Sigma-Aldrich) .

Ascorbic acid solution (0.2%) was prepared by dissolving 100 mg in 0.5 mL of distilled water, volume was adjusted to 50 mL with dimethyl formamide (DMF).

Salicylaldehyde solution (0.3% v/v) was prepared by diluting 0.15 ml of stock salicylaldehyde (SA) to 50 ml with methanol.

1,2-Naphthoquinone-4-sulphonate sodium solution was freshly prepared as 0.5% (w/v) aqueous solution, for reference method.

Acetate buffer solution (pH 7.0) was prepared by adjusting the solution of 1.0 M anhydrous sodium acetate with diluted acetic acid.

Borate buffer solutions (0.2 M) were prepared by mixing appropriate volumes of 0. 2 M boric acid with 0.2 M borax and adjusting the pH to 10.5 for reference method.

Drug stock solution was prepared by dissolving 25 mg PGB in 25 ml of distilled water, then a working solution of a concentration of 100 µg mL^{-1} was prepared in DMF for method I and in methanol for method II.

All the reagents used were of analytical grade

General procedures

Calibration:

Method I

Different aliquots of 100 µg mL^{-1} PGB standard solution in DMF were quantitatively transferred into a series of screw capped glass tubes, so as to contain the drug within the concentration range of 5-50 µg mL^{-1}. To each tube, 2 mL of ascorbic acid solution (0.2%) was added and volumes were adjusted to 5 mL with DMF. Solutions were heated on a water bath with a temperature maintained at 100 ±5°C for 45 minutes. After the mentioned time, tubes were cooled to room temperature. The content of tubes was transferred to a series of 10 mL standard flasks and completed to volume with DMF. Absorbance of the solution in each flask was measured at 530 nm against the reagent blank. Calibration graph was constructed by plotting the absorbance versus final concentration of the PGB. Regression equation was computed.

Method II:

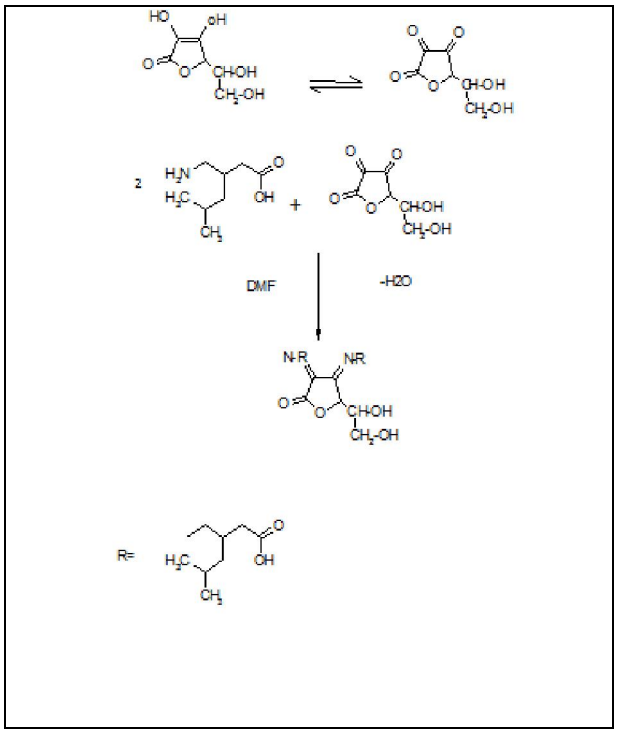
Different aliquots of 100 µg mL^{-1} PGB standard solution in methanol were quantitatively transferred into a series of screw capped glass tubes, so as to contain the drug within the concentration range of 5-50 µg mL^{-1}. 2.5 mL of (SA) was added followed by 1.5 mL of acetate buffer (pH 7); contents of each tube were mixed well using a vortex mixer then heated on water bath with a temperature maintained at 70°C for 15 minutes. After the mentioned time, tubes were cooled to room temperature. The content of tubes was transferred to a series of 10 mL standard flasks and adjusted to volume with methanol. Absorbance of the solution in each flask was measured at 410 nm against the reagent blank. Calibration graph was constructed by plotting the absorbance versus final concentration of the PGB. Regression equation was computed.

Assay of dosage form:

Powder content of ten capsules labeled to contain 50 mg PGB each, were mixed. Amount of the powder equivalent to one capsule content was accurately weighed, transferred into 50 mL volumetric flask then dissolved in 20 ml water and sonicated for 10 minutes. The volume was brought to 50 mL with water and final solution was filtered. 5.0 mL of this filtrate was transferred to 50 mL volumetric flask and diluted to volume with DMF for method I, with methanol for method II and with water for the reference method. Thereby a working solution of 100 µg mL^{-1} was obtained .Complete as under the procedure "calibration". The nominal contents of the capsules were calculated using the corresponding regression equation and compared to reference method.

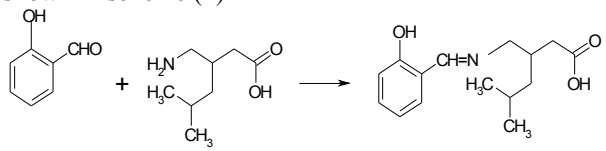
3. Results and discussion

Pregabalin has no specific absorbance since it is an aliphatic compound and lacks any chromophores which are essential for light absorption. This renders its spectrophotometric determination as a challenging problem. Such problem is highly exaggerated when it is necessary to determine the drug especially in pharmaceutical preparations. However, aliphatic nature of PGB and presence of a primary amino group makes it susceptible to derivatization. PGB as a primary amine reacts with ascorbic acid in DMF medium to produce colored product which absorbed maximally at 530 nm under the optimized conditions, ascorbic acid undergoes oxidation resulting in the formation of dehydroascorbic acid, Fig. 2 **[16].** The carbonyl group of dehydroascorbate reacts with primary amino group of PGB to form a colored condensation product. By analogy to the previous report **[17]**, the reaction is proposed to proceed as shown in scheme (1).



Scheme (1): The proposed mechanism for the reaction of PGB with ascorbic acid

Since pregabalin has no absorbance beyond 210 nm. Therefore, salicylaldehyde was used in this study to react with the primary aliphatic amine in PGB to form a schiff's base which is measured at 410 nm, Fig. 3. Derivatization with salicylaldehyde was carried out to increase the spectrophotometric sensitivity with bathochromic shift to visible region. Derivatization reaction is proposed to proceed as shown in scheme (2)



Scheme (2): The proposed mechanism for the reaction of PGB with salicylaldehyde

Optimization of the reaction variables

Method I

Effect of volume of ascorbic acid:

The influence of the concentration of the reagent was studied using different volumes of 0.2% ascorbic acid. 2 mL of 0.2% ascorbic acid was chosen as the optimum volume of the reagent.

Further increase of the reagent volume produced a gradual decrease in the absorption intensity.

Effect of temperature and heating time:

The effect of temperature and heating time on the formation of the colored product were also optimized. At room temperature, the addition of ascorbic acid did not lead to the formation of any colored product and higher temperatures were required to accelerate the reaction. The color intensity was found to be increased with increasing temperature and maximum absorbance was obtained following heating on a water bath at a temperature of 100 ± 5°C for 45 minutes.

Method II

Many variables affected the reaction of PGB with salicylaldehyde which include volume of the reagent, volume of buffer, temperature and pH. These variables were studied by varying each in turn while keeping all others constant (one factor at a time) and in order to study the interaction of the important factors; temperature, volume of buffer and volume of reagent a factorial design for these factors was made to obtain the optimum results.

Validation of the proposed methods

The validity of the proposed methods was tested regarding linearity, specificity, accuracy, repeatability and intermediate precision according to ICH Q2 (R1) recommendations **[18]**.

Linearity:

The calibration graphs obtained by plotting the values of the absorbance versus the final concentration of the drug (100 µg mL^{-1}) were found to be rectilinear over the concentration ranges cited in Table 1. The proposed methods were evaluated for the accuracy as percent recovery and the precision as percent relative standard deviation (% RSD), Tables 1 and 2.The validity of the methods was proved by statistical evaluation of the regression data, regarding the standard

Deviation of the residuals (Sxy), the standard deviation of the intercept (Sa) and standard deviation of the slope (Sb). The results are shown in Table 1.

The small values of the figures indicate low scattering of the points around the calibration line and good linearity over the working concentration ranges.

Limit of quantitation and limit of detection:

The limits of quantitation (LOQ) were determined by establishing the lowest concentration that can be measured according to ICH Q2 (R1) recommendation below which the calibration graph is non linear.

The limits of detection (LOD) were determined by evaluating the lowest concentration of the analyte that can be readily detected. The results are summarized in Table 1.

LOQ and LOD were calculated according to the following equations **[18]**:

$LOQ = 10\ \sigma/S$

$LOD = 3.3\ \sigma/S$

Where σ is the standard deviation of the intercept, and b is the slope of the calibration curve.

Accuracy:

To test the validity of the proposed methods they were applied to the determination of pure sample of PGB over the chosen concentration ranges. The accuracy was determined by calculating % recovery ± SD, Table 2.

Precision:

Repeatability (intra-day) was performed over a specified concentration range through replicate analysis of three concentrations of PGB in pure form on three successive occasions. The results are presented in Table 3.

Intermediate precision (inter-day) was tested by repeated analysis of PGB in pure form using selected concentrations for a period of 3 successive days. The results are shown in Table 3.

High % recovery, low SD and low % RSD indicate high accuracy and precision of the proposed method, respectively.

Robustness:

The robustness of the procedures adopted in the two proposed method was demonstrated by the constancy of the absorbance intensity with the deliberated minor changes in the experimental parameters. For Method I; changes included the heating temperature (100 ±5°C) and heating time (45± 5 minutes). For method II; changes included the volume of the buffer solution (1.5 ± 0.2 mL), volume of salicylaldehyde (2.5 ± 0.3 mL), heating temperature (70 ± 5°C) . These minor changes that may take place during the experimental operation did not affect the absorbance of the reactions products.

Selectivity:

The Selectivity of the methods was investigated by observing any interference encountered from the common capsule excipients, such as lactose monohydrate, corn starch and talc. These excipients did not interfere with the proposed methods of analysis.

Stability:

The stability of final measured sample solutions was examined and responses were found to be stable for at least 1 hour at room temperature. This allows the processing of large batches of samples and their comfortable measurements with convenience.

Stoichiometry of the reactions:

Stoichiometry in the two methods was studied by adopting the limiting logarithmic method [19] for method I. The two straight lines were obtained using increasing concentrations of the reagent while keeping the concentration of the drug constant and using increasing concentrations of the drug while keeping the concentrations of the reagent constant. Plots of log absorbance versus log ascorbic and log PGB gave two straight lines, slopes of which were 0.448 and 0.955 for ascorbic acid and PGB, respectively Fig. 4. Hence, it is concluded that the two molecules of the drug condenses with one molecule of ascorbic acid.

For method II, the determination of the stoichiometry of the reaction of PGB and salicylaldehyde was performed using Job's variation method [20] where the same concentration of PGB and salicylaldehyde (1.0 mM) was used. The obtained results indicate that the ratio of the reaction is 1:1 which means that one molecule of PGB condenses with one molecule of salicylaldehyde, Fig. 5.

Analysis of the pharmaceutical dosage form

Table 4. shows the results of the assay for pregabalin carried out on marketed formulation by the two proposed methods and reference method. The results obtained were compared statistically by the student's t-test and variance ratio F-test. The experimental t- and F- values did not exceed the theoretical one at 95% confidence level, indicating the absence of any significant difference between the compared methods.

Table (1): Performance data and statistical parameters

Parameter	Method I	Method II
Concentration range (μg g mL^{-1})	5-50	5-60
Limit of detection (LOD)	0.5	3
Limit of Quantitation (LOQ) (μg mL^{-1})	1.0	5.0
Regression Parameters		
Slope ± SD (Sb)	0.0144 ± 1.91E-0.3	0.0130 ± 2.85E-0.3
Intercept ± SD (Sa)	0.0080 ±0.0049	-0.0150 ± 0.0102
SD of residual (Sxy)	0.0082	0.0130
Correlation Coefficient (r)	0.9996	0.9996
ε (l/mol./cm)	2229.22	2069.99
Sandell´s sensitivity	0.0714	0.0769

Table (2): Results of recovery studies of PGB in pure form

Parameter	Method I			Method II		
	Conc. taken (μg mL^{-1})	Conc. found (μg mL^{-1})	% found	Conc. taken (μg mL^{-1})	Conc. found (μg mL^{-1})	% found
	15	15.14	100.95	20	20.07	100.38
	25	25.10	100.57	30	30.30	101.03
	35	34.92	99.79	40	40.30	100.96
Mean ± S.D			100.43 ± 0.59			100.79 ± 0.36

Table (3): Results of precision study of the proposed methods for the analysis of PGB

Parameter	Method I			Method II		
Conc. of PGB (μg mL^{-1})	15	25	35	20	30	40
Interday						
% found	100.9	100.57	100.41	96.15	98.71	100.96
	98.0	96.80	102.48	96.15	101.02	101.00
	100.46	100.42	99.77	99.38	98.00	99.03
Mean	99.78	99.26	100.89	97.23	99.24	97.56
± S.D.	1.56	1.9	1.15	1.86	1.57	1.13
% RSD	1.56	1.9	1.13	1.91	1.58	1.12
Intra-day						
% found	101.9	100.57	101.6	97.23	98.71	100.96
	98.75	102.0	99.77	96.15	98	101.02
	100.95	101.43	100.41	96.5	99.5	101.38
Mean	100.53	101.33	100.59	96.63	98.74	101.12
S.D.	1.62	0.72	0.93	0.55	0.75	0.23
% RSD	1.61	0.71	0.92		0.76	0.23

Table (4): Statistical analysis of the results obtained by the two proposed and reference methods for the determination of pregabalin in capsules

Pharmaceutical preparation	Conc. Taken (μg mL^{-1})	Recovery %		
		Method I	Method II	Reference method***
Lyrica® Capsules B.N.	5.0	104.00	103	104.12
	10.0	102.38	101.33	103.33
	20.0	103.33	101.2	103.44
Mean		103.23	101.84	103.63
SD		0.81	1.00	0.42
Variance		0.65	1.00	0.17
t		0.82 (2.78)*	1.86 (2.78)*	
F		3.72 (6.39)**	5.72 (6.39)**	

*, ** The theoretical values of t and F, respectively at (p=0.05)

*** PGB was determined using 1,2-naphthoquinone-4-sulphonate sodium (NQS), yielding an orange colored product that was measured at 473 nm.

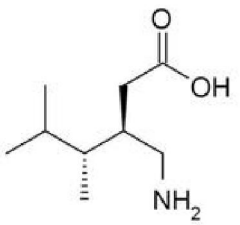


Fig. 1: Chemical structure of pregabalin

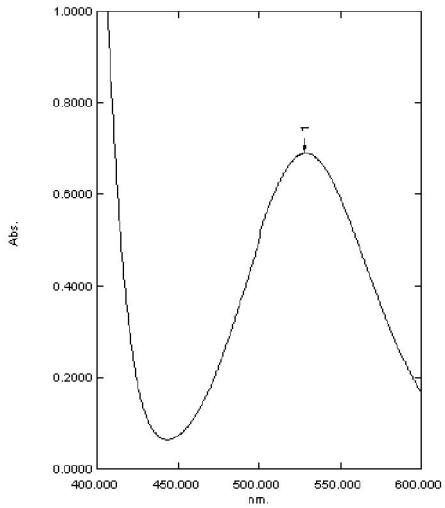


Fig. 2: Absorption spectrum of PGB- ascorbic acid derivative

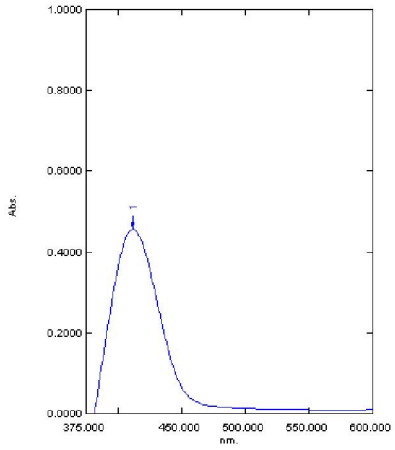


Fig. 3: Absorption spectrum of PGB – salycilaldehyde derivative

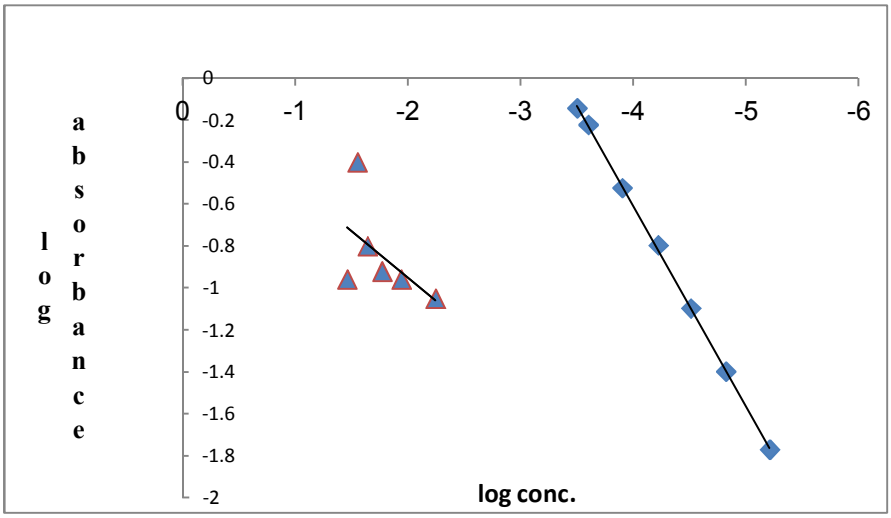


Fig. 4: Limiting logarithmic plot for molar reactivity of PGB with ascorbic acid;for ▲: log A vs log [reagent] with [PGB] kept constant; ■ log A vs log [PGB] with [reagent] kept constant.

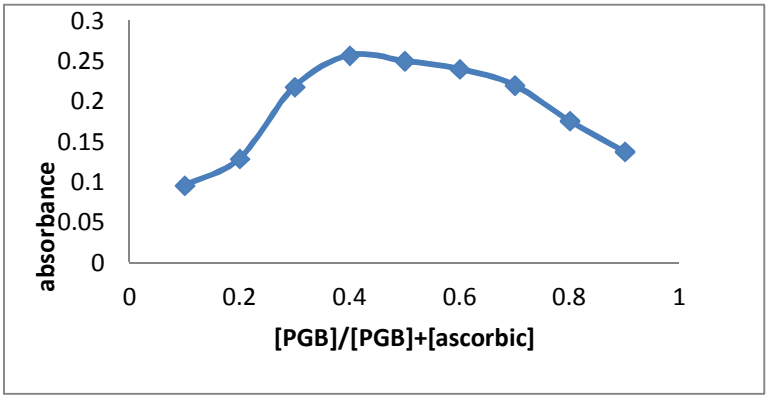


Fig. 5: Job's plot for the determination of stoichiometry of the reaction between PGB and salicylaldehyde

4. Conclusion

The present study was devoted to explore ascorbic acid and salicylaldehyde as derivatizing reagents for the development of spectrophotometric methods for the determination of PGB in its marketed form (capsules). The proposed methods are simple, inexpensive and sensitive for the determination of pregabalin in bulk as well as in capsules. There is no requirement of any sophisticated apparatus as in chromatographic methods. Omission of extraction step with organic solvent is an added advantage.

Corresponding author
Ola M. Abdallah
Analytical Chemistry Department, Faculty of pharmacy (girls), Al-Azhar University Cairo, Egypt
olamody @yahoo.com

References

1. Tassone DM, Boyce E, Guyer J, Nuzum, D. Pregabalin: a novel gamma amino butyric acid analogue in the treatment of neuropathic pain, partial-onset seizures, and anxiety disorders. Clin. Ther. 2007; 29(1): 26-48.
2. Hamandi K, Sander JW. Pregabalin: a new antiepileptic drug for refractory epilepsy. Seizure 2006; 15(2): 73-78.
3. Mudiam MKR, Chauhan A, Jain R, Fatima, G, Malhotra E, Murthy, RC. Development, validation and comparison of two microextraction techniques for the rapid and sensitive determination of pregabalin in urine and pharmaceutical formulations after ethyl chloroformate derivatization followed by gas chromatography-mass spectrometric analysis. J. Pharm. and Biomed. Anal. 2012; 70: 310-319.
4. Vaidya V, Yetal S, Roy S, Gomes N, Joshi S. LC-MS-MS Determination of Pregabalin in Human Plasma. Chromatographia 2007; 66 (11-12): 925-928.
5 .Mandal U, Sarkar A, Gowda K, Agarwal S, Bose A, Bhaumik U, Ghosh D, Pal T. Determination of Pregabalin in Human Plasma Using LC-MS-MS. Chromatographia 2008; 67 (3-4): 237-243.
6. Kasawar G, Farooqui M. Development and validation of HPLC method for the determination of pregabalin in capsules. Indian J. Pharm. Sci. 2010; 72(4): 517-519.
7. Vermeij T, Edelbroek P. Simultaneous high-performance liquid chromatographic analysis of pregabalin, gabapentin and vigabatrin in human serum by precolumn derivatization with o-phtaldialdehyde and fluorescence detection. J Chromatogr B Analyt. Technol. Biomed. Life Sci. 2004; 810 (2): 297-303.
8. David B, Millington C. Analysis of Pregabalin at Therapeutic Concentrations in Human Plasma/Serum by Reversed-Phase HPLC. Ther. Drug Monit. 2005; 27(4): 451-456.
9. Jadhav A, Pathare D, Shingare M. Validated enantioselective LC method, with precolumn derivatization with Marfey's reagent, for analysis of the antiepileptic drug pregabalin in bulk drug samples. Chromatographia 2007; 65(3-4): 253-256.
10. Salem H. Analytical Study for the Charge-Transfer Complexes of Pregabalin. E-J Chem. 2009; 6: 332-340.
11. Onal A, Olcay S. Spectrophotometric and spectrofluorimetric methods for the determination of pregabalin in bulk and pharmaceutical preparation. Spectrochim Acta A 2009; 72(1): 68-71.
12. Gujral R, Manirul S, Kumar S. A. novel method for the determination of pregabalin in bulk pharmaceutical formulations and human urine samples. J. Pharm. Pharmacol. 2009; 3(6): 327-334.
13. Rasha A. Spectrofluorimetric and Spectrophotometric Determination of Pregabalin in Capsules and Urine Samples. Int. J. Biomed. Sci. 2010; 6(3): 260- 267.
14. Walash MI, Belal F, El-Enany N, El-Maghrabey MH. Utility of certain nucleophilic aromatic substitution reactions for the assay of pregabalin in capsules. Chem Cent. J. 2011; 5 (36): 10 pages.
15. Sowjanya K, Thejaswini JC, Gurupadayya BM, Indupriya M. Spectrophotometric Determination of Pregabalin Using Gibb's and MBTH reagent in Pharmaceutical Dosage Form. Der Pharma Chemica, 2011; 3(1): 112-122.
16. Sandulescu R, Mirel S, Oprean R. The development of spectrophotometric and electroanalytical methods for ascorbic acid and acetaminophen and their applications in the analysis of effervescent dosage forms. J. Pharm.Biomed. Anal. 2000; 23(1): 77-87.
17.Rahman N, Hoda MN. Validated spectrophotometric methods for the determination of amlodipine besylate in drug formulations using 2,3-dichloro 5,6-dicyano 1,4-benzoquinone and ascorbic acid. J. Pharm. Biomed. Anal. 2003; 31(2): 381-392.
18. ICH Harmonized Tripartite Guideline: Validation of Analytical Procedures. Text and Methodology, Q2 (R1), Current Step 4 Version. Parent Guidelines on Methodology 2005.
19. Rose J. Advanced Physicochemical experiments. Pitman: London, England; 1964.
20. Werner L, Boltz D. Theory of continuous variations plots and a new method for spectrophotometric determination of extraction and formation constants. Anal. Chem.,1971; 43 (10): 1265–1272.

10/12/2012