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Determination of Pregabalin in Bulk Drug and Pharmaceutical Formulations using Validated Stability-Indicating Spectrophotometric Methods

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Abstract- The present study describes the development and subsequent validation of stability-indicating, accurate, reliable, and sensitive spectro- photometric methods for the determination of Pregabalin in presence of its degradation products, including Dual wavelength and Ratio derivative after derivatization with vanillin reagent. With the Dual wavelength technique, Pregabalin could be determined in the range of 40-160 µg/mL at 390nm and 395.8nm. With the Ratio derivative technique, it could be determined in the above ranges at 401.6nm. All the methods were validated according to the International Conference on Harmonization guidelines and successfully applied to determine Pregabalin in pure form, laboratory-prepared mixtures, and pharmaceutical formulation. The obtained results were statistically compared with reported methods of analysis and there were no significant differences with respect to accuracy and precision of the adopted techniques.

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Determination of Pregabalin in Bulk Drug and Pharmaceutical Formulations using Validated Stability-Indicating Spectrophotometric Methods

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Abstract- The present study describes the development and subsequent validation of stability-indicating, accurate, reliable, and sensitive spectro- photometric methods for the determination of Pregabalin in presence of its degradation products, including Dual wavelength and Ratio derivative after derivatization with vanillin reagent. With the Dual wavelength technique, Pregabalin could be determined in the range of 40-160 µg/mL at 390nm and 395.8nm. With the Ratio derivative technique, it could be determined in the above ranges at 401.6nm. All the methods were validated according to the International Conference on Harmonization guidelines and successfully applied to determine Pregabalin in pure form, laboratory-prepared mixtures, and pharmaceutical formulation. The obtained results were statistically compared with reported methods of analysis and there were no significant differences with respect to accuracy and precision of the adopted techniques.

Keywords: fibromyalgia; pregabalin; spectro-photometric methods; dual wavelength; ratio derivative; derivatization; vanillin reagent.

I. Introduction

Pregabalin (Figure 1) is an anticonvulsant drug used for neuropathic pain and as an adjunct therapy for partial seizures. It has also been found effective for generalized anxiety disorder.

Recent studies have shown that pregabalin is effective at treating chronic pain in disorders such as fibromyalgia and spinal cord injury.

No spectrophotometric methods were reported in major pharmacopeias like USP, EP and BP for determination of Pregabalin. Literature survey revealed many analytical methods for estimation of Pregabalin [1 - 13], However, no stability-indicating spectrophotometric method has been developed for bulk and pharmaceutical formulations.

The proposed methods were found to be easier than published HPLC methods for the determination of Pregabalin, for there is no need to use an internal standard, gradient elution, and time programming to adjust wavelengths. Moreover, the proposed methods are the first spectrophotometric methods for the determination of these drugs in presence of their degradation products. The scientific novelty of the present work is that the methods used are simple, rapid, sensitive, less expensive, and less time-consuming than other published LC methods.

a) *Theoritical Background*

i. *Dual wavelength* [14]

This technique is used for binary mixtures for determination of one component without interference from the other. Two wavelengths are selected where the difference in absorbance of one component at these selected wavelengths is found to be zero, so, the difference in absorbance reflects only the concentration of one of the two components in the mixture.

ii. *Ratio Derivative Spectrophotometric method* [15, 16]

Salinas et al. proposed the spectrophotometric method termed ratio-derivative spectrophotometry, for the simultaneous determination of two compounds in binary mixtures. Their method is based on the derivative of the ratio spectra for a binary mixture. The absorption spectrum of the mixture is divided by the absorption spectrum of a standard solution of one of the compounds and the first derivative of the ratio spectrum is obtained. The concentration of active compounds are then determined from the calibration graphs obtained by measuring the amplitudes at points corresponding to the minimum or maximum wavelengths.

II. Materials and Methods

a) *Chemicals, Pharmaceutical Formulations and Reagents*

(a) *Pregabalin.:* Obtained from Optimus Drugs Ltd (Hyderabad, India).

(b) *75 and 150 mg capsules of Pregabalin (Irenypathic®).:* Produced by Amoun Pharmaceuticals Inc. (Cairo, Egypt).

(c) *Methanol, hydrochloric acid, sodium hydroxide, potassium permanganate, sulphuric acid, sodium sulphite, disodium hydrogen phosphate, citric acid anhydrous, ethyl alcohol (96%) and vanillin.*— Analytical reagent grade were purchased from Scharlau (Scharlab S.L, Sentmenat, Spain)

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(d) *Double distilled water.:* Prepared by using Millipore Milli-Q plus water purification system (Millipore Corp., Billerica, MA).

b) Equipments

(a) *Double-beam UV-Vis spectrophotometer (Shimadzu 1650 PC)* connected to a computer fitted with UVPC personal spectroscopy software version 2.42 (Shimadzu) was used to process absorption and derivative spectra.: (Shimadzu Corp., Kyoto, Japan).

(b) *Hotplate (WiseStir) with temperature controller*.: Used to carry out degradation studies for all solutions (Daihan Scientific Co. Ltd, Korea).

(c) *pH-meter (Orion).:* Equipped with combined glass electrode for pH adjustment (Thermo Scientific).

(d) *Ultrasonic bath.:* Elma (Danbury, CT).

c) Preparation of solutions

ICH guidelines Q1A_R2 (2.1.2) and Q2_R1(part II - 1.2) don't mention specified conditions or reagents for stress testing of drug substance. So, mild conditions (0.1N HCl or 0.1N NaOH) were used at first but didn't give complete degradation. So, drastic conditions were used to achieve complete degradation. $KMnO_4$ was used instead of hydrogen peroxide because it gave better results and the degradation reaction could be controlled or stopped on the contrary with hydrogen peroxide where the reaction could not be stopped.

1) *Stock standard solution.:* Stock standard solution of Pregabalin (5 mg/mL) was prepared by dissolving 500 mg of the drug in water, sonicated and completed to volume with the same solvent in a 100 mL volumetric flask. Then, 5 mL were further diluted to 50 mL with water. The required concentrations were prepared by serial dilutions.

2) *Oxidative-induced forced degradation of Pregabalin (1000 µg/mL). :* In a conical flask, 10 mL 0.1N $KMnO_4$ were added on 10 mL from stock standard solution (5mg/mL), the conical flask was covered with a funnel, heated on a hot plate - adjusted at 140 ^{0}C - for 60 min, cooled, then 0.5 mL of 4.5M H_2SO_4 and 1M sodium sulphite solution were added till discoloration, excess sulphuric acid was neutralized with 1M NaOH using a calibrated pH meter, and completed to 50 mL with water. Complete degradation was checked using HPLC.

Pregabalin is only sensitive to oxidative degradation. Acid, base, dry heat degradation and photo-degradation were tried but no significant change in the peak area appeared, indicating stability of Pregabalin to acid, alkaline, thermal and Photo-degradation.

3) *Sample preparation.:* Separately, the contents of 75 mg and 150 mg capsules were mixed, an amount equivalent to 500 mg of Pregabalin was accurately weighed, volume was completed to 100 mL with water then sonicated for 15 minutes and filtered. Then, 1 mL was further diluted to 50 mL with water.

4) *Vanillin reagent.:* Two grams of vanillin were weighed, volume was completed to 50 mL with ethyl alcohol (96%)

5) *Buffer pH 7.5.:* Prepared by mixing 35.5 mL of 0.2M disodium hydrogen phosphate anhydrous with 64.5 mL of 0.1M citric acid anhydrous, pH adjusted to 7.5 with 1M NaOH.

d) Procedures

The absorption spectra of the intact drug and its degradates are strongly overlapped, so application of the traditional spectral techniques failed to resolve this problem (Figure 2). On the other hand, this spectral overlapping was sufficient to demonstrate the resolving power of the proposed methods.

Pregabalin exhibits a very low UV absorption and as a consequence, poor sensitivity will be achieved by conventional UV spectrophotometric methods. Pregabalin contains a primary aliphatic amino group, which is known to react with many color reagents as vanillin. Literature [34] shows that maximum absorbance intensities were achieved using 2 mL of buffer at pH 7.5. It was also found that 2 mL of Vanillin reagent was sufficient for production of maximum and reproducible color intensity. Time required for complete reaction at room temperature was 40 min. Heating leads to decrease in absorbance so reaction was done at room temperature.

This was further applied to perform the below mentioned methods under [*(2.4.1)* and *(2.4.)*] to determine pregabalin in presence of its degradation products.

i. *Dual wavelength (DWL)*

Laboratory prepared mixtures of different concentrations of the intact drug and its degradation product were recorded against blank in the range from 390nm to 430nm for Pregabalin (Figure 3). Determine the absorbance at 390nm and 395.8nm.

The concentrations of Pregabalin in each mixture was determined by calculating the difference in absorbance measured at these wavelengths.

ii. *Ratio Derivative Spectrophotometric method (RDer)*

The absorption spectra recorded in the previously mentioned method *(2.4.1)* was divided by its divisor of oxidative induced degradate spectrum and the first derivative of the absorption spectra obtained was computed. Pregabalin concentrations were determined in each mixture from the absorbance at the amplitudes 401.6nm (Figure 4).

e) Method validation

1. *Linearity.:* Accurately measured aliquots of stock standard solution *(2.3.1)* were separately transferred

into a series of 25 mL volumetric flasks, to produce 40 to 160 µg/mL, on each flask, 2.5 mL of oxidative-induced degradate *(2.3.2)* were added to produce 100 µg/mL, 2 mL of Vanillin reagent *(2.3.4)*, 2 mL of buffer pH 7.5 *(2.3.5)*, flasks were left in dark at room temperature for 40 minutes and then completed to volume with water. Each of these solutions was measured in triplicate as mentioned under *(2.4.1)* and *(2.4.2)*.

2. *Accuracy.: Assay of drug in bulk powder.*— The mentioned procedures under *(2.4.1)* and *(2.4.2)* were repeated by measuring 80, 100, 120 µg/mL Pregabalin standard solutions, prepared from stock standard solution *(2.3.1)*, in triplicate after reaction with Vanillin reagent *(2.3.4)* in presence of buffer pH 7.5 *(2.3.5)* and the concentrations of Pregabalin were calculated by the corresponding regression equation.
3. *Specificity.:* In three separate flasks, accurately measured aliquots of standard stock solution *(2.3.1)* were transferred into a series of 25 mL volumetric flasks to produce 100 µg/mL each, accurately measured aliquots of oxidative-induced degradates *(2.3.2)* were added to produce 80, 100, 120 µg/mL each on a flask, and treated as mentioned under *(2.5.1)*. Each of these solutions was measured in triplicate as mentioned under *(2.4.1)* and *(2.4.2)*.
4. *Precision.:* Six replicates of same concentration (100 µg/mL) were checked for repeatability. The intraday and interday variation for the determination of Pregabalin was carried out at three different concentration levels of 80, 100, 120 µg/mL as mentioned under *(2.4.1)* and *(2.4.2)* after treatment as mentioned under *(2.5.1)*.
5. *Assay of pharmaceutical dosage forms.:* For determination of Pregabalin in 75 mg or 150 mg capsules, from the sample solutions *(2.3.3),* aliquots were transferred to 25 mL volumetric flasks, treated as mentioned under *(2.5.1)* to produce 100 µg/mL and then measured in triplicate as mentioned under *(2.4.1)* and *(2.4.2)*].

Further, standard addition technique was followed:

In three separate flasks, accurately measured aliquots of a previously analyzed sample solution of 75 mg capsules *(2.3.3)* were transferred into a series of 25 mL volumetric flasks to produce 100 µg/mL each, accurately measured aliquots of standard stock solution *(2.3.1)* were added to produce 20, 40, 60 µg/mL each on a flask, treated as mentioned under *(2.5.1)* and then measured in triplicate as mentioned under *(2.4.1)* and *(2.4.2)*.

The same steps were repeated with sample solution 150 mg capsules *(2.3.3)*.

III. Results and Discussion

a) Method validation

Method validation was performed according to the ICH guidelines for the suggested spectrophotometric methods.

Linearity.: was evaluated by analyzing different concentrations of Pregabalin in the ranges of 40 to 160 µg/mL. The analysis was performed according to the experimental conditions previously mentioned in *(2.4.1) and (2.4.2)*. Results are summarized in (Table 1).

Accuracy.: The accuracy of the results was checked by applying the proposed methods for the determination of % recoveries of 3 different concentrations of the drug in bulk powder. The concentrations were obtained from the corresponding regression equation, and the recoveries were calculated (Table 4).

Precision.: Precision of the obtained results of three concentrations of Pregabalin (80, 100, 120 µg/mL) were evaluated by three replicate determinations to estimate the intraday and interday variations. Then, the RSD% was calculated (Table 1).

LOD and LOQ.: Approaches based on the SD of the intercept and the slope were used for determining the LOD and LOQ, where

LOD = 3.3 x SD/slope and LOQ = 10 x SD/slope

These were determined experimentally for the proposed methods and are presented in Table 1.

Specificity.: The developed methods were found to be specific and selective. The intact drug can be detected without interference from its degradation products and formulation excipients. Recovery and relative standard deviations were calculated. Results are summarized in Table 2.

b) Analytical applications

The proposed methods were successfully applied to commercial preparations, and the standard addition technique was performed. The concentrations were calculated using the corresponding regression equation. Results are summarized in Table 3.

c) Statistical analysis

A statistical comparison of the results obtained by the proposed methods and the reported method [3] for determination of Pregabalin was done. The significant difference between groups was tested by *t*-test as shown in Table 5. The test ascertained that there was no significant difference with respect to accuracy and precision between the proposed methods and the reported method [3].

IV. Conclusion

The paper describes simple, inexpensive, precise, accurate, and sensitive methods for determination of Pregabalin in bulk drug as well as in

pharmaceutical dosage forms, also, can separate the drug from its degradation products, so, can be described as stability-indicating assay methods. The minimum sample preparation and the speed of analysis are the main advantages of these methods over other analytical procedures, unlike the HPLC procedures, the instrument is simple and inexpensive using a small quantity of reagents, thus, cost and time saving.

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References Références Referencias

1. Shah, D.A, Patelia, E.M., & Mori, A. (2013). Simultaneous Estimation of Pregabalin and Methylcobalamine in Pharmaceutical Formulation by HPTLC-Densitometry Method. *J. Chromat. Separation Techniq*., 4(2), 169-172.
2. Shep, S.G., & Lahoti, S.R. (2013) Development and Validation of UV Spectrophotometric Method of Pregabalin In Bulk And Pharmaceutical Formulation. *Int. J. ChemTech Res.,* 5(5), 1264-1270.
3. Kasawar, G.B., & Farooqui, M.N. (2010) Development and Validation of HPLC Method for the Determination of Pregabalin in Capsules. *Indian J. Pharm. Sci.,* 72(4), 517-519.
4. Vukkum, P., Babu, J.M., & Muralikrishna, R. (2015) Stress degradation behavior of Pregabalin, identification of degradation impurities and development of stability indicating UPLC method. *International journal of pharmaceutical sciences and research,* 6(6), 2241-2257.
5. Reddy, M.N.C., & Sekhar KB, C. (2012) RP-HPLC Determination of Related substances of Pregabalin in bulk and pharmaceutical dosage form. *International Journal of Chemical and Pharmaceutical Sciences,* 3(2), 40-46.
6. Gujral, R.S., Haque, S.K.M., & Kumar, S. (2009) A novel method for the determination of pregabalin in bulk pharmaceutical formulations and human urine samples. *African Journal of Pharmacy and Pharmacology,* 3(6), 327-334.
7. Bali, A., & Gaur, P. (2011) A novel method for spectrophotometric determination of pregabalin in pure form and in capsules. *Chemistry Central Journal,* 5(59), 1-7.
8. Onal, A., & Sagirli, O. (2009) Spectrophotometric and spectrofluorimetric methods for the determination of pregabalin in bulk and pharmaceutical preparation. *Spectrochimica Acta Part A, molecular and biomolecular spectroscopy*, 72, 68-71.
9. Mishra, S.K., Gurupadhyya, B.M., & Verma, S. (2012) Stability indicating RP-HPLC method for determination of Pregabalin using ICH guidelines. *International Journal of Natural Product Science,* 1, 115-116.
10. Walash, M.I., Belal, F.F., El-Enany, N.M., & El-Maghrabey, M.H. (2011) Utility of certain nucleophilic aromatic substitution reactions for the assay of Pregabalin in capsules. *Luminescence,* 26, 342–348.
11. Prasad, M.K.C., Sagar, G.V., & Sudhakar, P. (2013) Simultaneous HPTLC method for estimation of Gabapentin and Pregabalin. *Int. J. Pharm. Pharm. Sci.*, 5(4), 326-333.
12. Kumar, B.S.J., Kumar, R.V., & Sinha, V.R. Stability Indicating HPTLC Method For The Determination of Pregabalin In Bulk And Pharmaceutical Dosage Forms. *Analytical Chemistry: An Indian Journal,* 12(10), 377-383.
13. Saleh, H.M., EL-Henawee, M.M., Ragab, G.H., & Mohamed, O.F. (2014) Spectrophotometric and spectrofluorimetric determination of Pregabalin via condensation reactions in pure form and in capsules. *International Journal of Pharmaceutical, Chemical and Biological Sciences*, 4(3), 738-747.
14. Oza, C.K., Nijhawan, R., Pandya, M.K., Vyas, A.J., & Patel, A.I. (2012) Dual Wavelength Spectro-photometric method for the simultaneous determination of Paracetamol and Nabumetone in API and in tablet dosage form. *Asian J. Pharm. Ana.*, 2(4). 122-127.
15. Dinc, E., Baydan, E., Kanbur, M., & Onur, F. (2002) Spectrophotometric multicomponent determination of sunset yellow, tartrazine and allura red in soft drink powder by double divisor-ratio spectra derivative, inverse least-squares and principal component regression methods. *Talanta,* 58, 579-594.
16. Abdel-Ghany, M.F., Abdel-Aziz, O., Ayad, M.F., & Mikawy, N.N. (2015) Simultaneous Determination of Octinoxate, Oxybenzone, and Octocrylene in a Sunscreen Formulation Using Validated Spectrophotometric and Chemometric Methods. *Journal of AOAC International,* 98(5), 1215-1225.

Table 1: Calibration data for the determination of Pregabalin by the proposed methods

Method	Range µg/mL	Regression equation (Y = bC + a)[a]	r^2	LOD, µg/mL	LOQ, µg/mL	Repeatability RSD%	Intraday RSD%	Interday RSD%
DWL	40 - 160	Y = 0.0 C - 0.021	0.9998	3.092	9.369	0.573	0.862	0.803
RDer	60 - 140	Y = 0.012 C + 0.069	0.9992	6.022	18.25	0.139	0.117	0.206

aa = Intercept, b = slope, and C = concentration of drug in µg/mL
P+OD= Pregabalin and oxidative degradates
DWL= Dual wavelength, RDiff= Ratio difference, RDer= Ratio Derivative

Table 2: Specificity results of Pregabalin using the proposed methods

Method	Claimed conc. (µg/mL)	Imp. conc. added (µg/mL)	Recovery %	Av. recovery ±RSD%	Method	Recovery %	Av. recovery ±RSD%
DWL	100	80	100.96	100.32 ±0.735	RDer	101.66	101.23 ±1.024
		100	99.52			100.05	
		120	100.48			101.98	

Table 3: Application of standard addition technique for the determination of Pregabalin in formulations using the proposed methods

Method	Claimed conc. (µg/mL)	Std conc. added (µg/mL)	Recovery % (dosage form 1)	Recovery% (dosage form 2)	Method	Recovery % (dosage form 1)	Recovery % (dosage form 2)
DWL	100	20	100.00	100.00	RDer	98.14	98.14
		40	98.84	100.00		98.45	98.39
		60	101.55	99.22		98.18	98.43
		Av. recovery ±RSD%	100.13 ±1.359	99.74 ±0.448	Av. recovery ±RSD%	98.25 ±0.171	98.32 ±0.159

Table 4: Results of recovery studies of Pregabalin

DWL		
Theoritical conc. (µg/mL)	Actual conc found (µg/mL)	Recovery%
80	78.97	98.71
100	97.28	97.28
120	100.76	120.91
RDer		
Theoritical conc. (µg/mL)	Actual conc found (µg/mL)	Recovery%
80	79.85	99.81
100	100.37	100.37
120	120.38	100.32

Table 5: Statistical comparison between the results of the proposed spectrophotometric methods and the reported method for determination of Pregabalin

Statistical term	Reported method	DWL (P+OD)	RDer (P+OD)
Accuracy			
mean recovery	100.258	100.13	98.25
Variance	0	1.852	0.028
t-test	2.776	0.164	-20.58
	(t-tabulated)	(t-calculated)	(t-calculated)
Precision - intraday			
mean recovery	100.059	100.36	99.25
Variance	0.241	0.918	0.986
RSD% mean	0.723	0.862	0.117
t-test	2.776	0.487	1.258
Precision - interday			
mean recovery	100.02	100.14	99.09
Variance	0.207	1.089	0.845
RSD% mean	0.762	0.803	0.206
t-test	2.776	0.18	-1.56

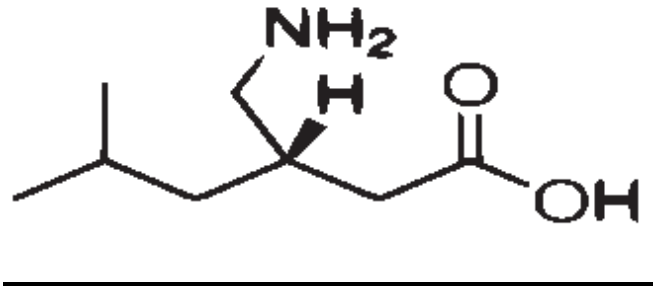

Figure 1: Pregabalin [(*S*)-3-(aminomethyl)-5-methylhexanoic acid] intact drug

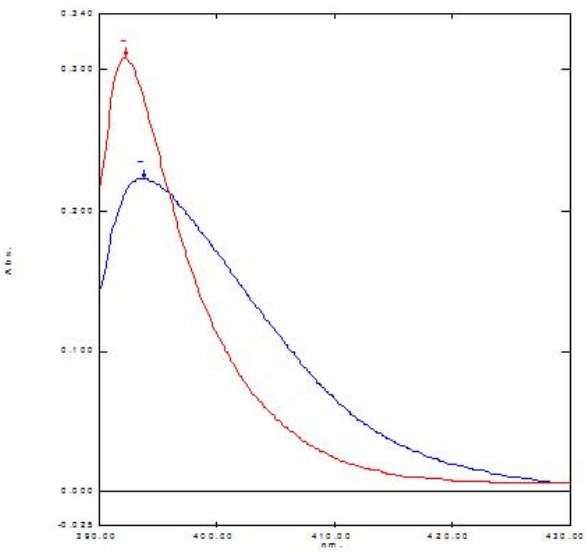

Figure 2: Pregabalin 0.1mg/ml (blue) + oxidative degradation 0.1mg/ml (red)

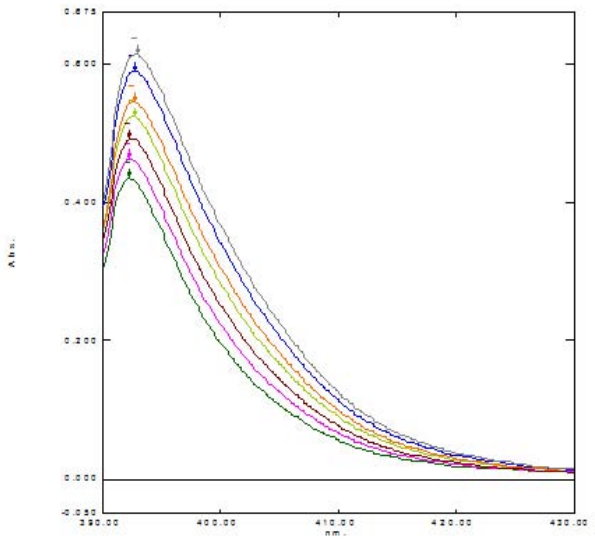

Figure 3: Pregabalin (40 - 160μg/ml) + oxidative degradation (100μg/ml) by Dual wavelength

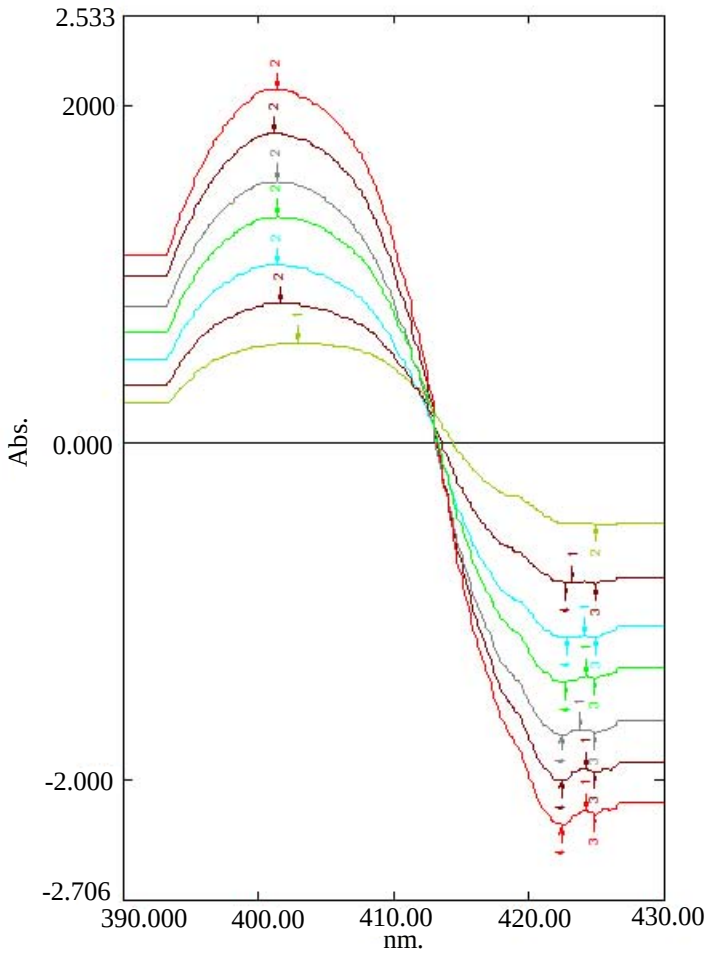

Figure 4: Pregabalin (40 - 160μg/ml) + oxidative degradation (100μg/ml) by Ratio derivative

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