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Full Length Research Paper

A novel method for the determination of pregabalin in bulk pharmaceutical formulations and human urine samples

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A simple isocratic reversed phase HPLC method was developed and validated for the analysis of pregabalin in bulk, pharmaceutical formulations and human urine samples, the separation was accomplished on a C_{18} 5 µm ODS hypersil column (250 mm × 4.6 mm) using a methanol acetonitrile - 0.02 M di - potassium hydrogen orthophosphate (K_2HPO_4) (pH - 7.00) (3: 1: 16, v/v/v) mobile phase. The compound eluted isocratically at a flow rate of 1.0 ml /min. The UV detector was set at 210 nm for the detection of pregabalin. The method was linear over the range of 0.75 - 6.00 µg/ml. The method was validated with respect to accuracy, precision, linearity, ruggedness, limit of detection and limit of quantitation. Robustness testing was also conducted to evaluate the effect of minor changes to the chromatographic system and to establish appropriate system suitability parameters. This method was used successfully for the quality assessment of 5 pregabalin drug products and human urine samples with good precision and accuracy.

Key words: Pregabalin, isocratic system, validation, high performance liquid chromatography, pharmaceutical formulations**,** human urine samples.

INTRODUCTION

Pregabalin (PGB), (S) - 3 - amino methyl hexanoic acid, is a structural analogues of - amino butyric acid (GABA) as shown in (Figure1) . It is a white crystalline solid. It is soluble in water and in both basic and acidic aqueous solutions. It is a new anticonvulsant and anal-gesic medication that was recently approved for adjunctive treatment of partial seizures in adults in both the United States and Europe and for the treatment of neuropathic pain from postherpetic neuralgia and diabetic neuropathy. It is both structurally and pharma-cologically related to the anticonvulsant and analgesic medication gabapentin and both compounds were originally synthesized with the hope of modulating brain GABA receptors and GABA synthetic enzymes. These compounds are inactive at $GABA_A$ and $GABA_B$ recap-

tors (Piechan, 2004). The mechanism of action of pregabalin has been characterized only partially and in particular, the cellular and molecular details of its action to reduce neurotransmitter release are incompletely known. The primary high - affinity binding site for pre-gabalin in forebrain tissues is the $_2$ – type 1 auxiliary subunit of voltage - gated calcium channels (Gee, 1996) and this interaction seems to the required for the pharmacological actions of the medications (Taylor, 2004; Belliotti, 2005). The identification of the $_2$ - binding sites has lead to the speculation that pregabalin act pharmacologically specifically in neurons by modu-lating the action of synaptic calcium channels. This hypothesis is supported by several findings that prega-balin reduce calcium influx into synaptosomes prepared from human brain (Fink, 2000; Hoff, 2002) and it subtly reduce calcium dependent overflow of neurotransmit-ters from several different neuronal tissues and reduce synaptic responses. PGB is thought to be useful for treating any other conditions, pain, physiological conditions associa-

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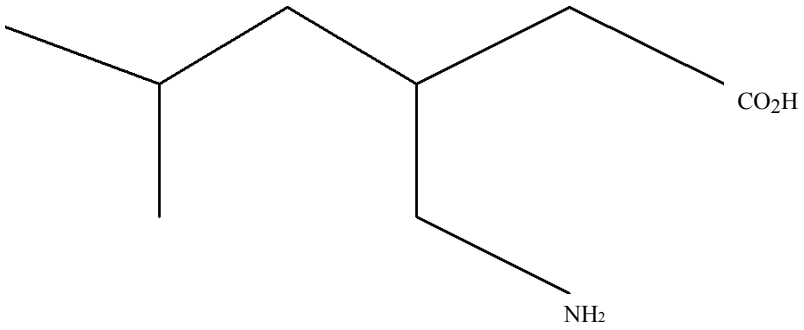

Figure 1. Structure of pregabalin.

with psychomotor stimulants, inflame-mation, gasintestinal damage, alcoholism, insomnia, and various psychiatric disorders, including mania and bipolar disorder. There is no official method developed for the analysis of pregabalin till now and therapeutic importance of the drug has engendered development of assays for the quantification of PGB. A through literature search has revealed that only a few analytical methods are available for determination of pregabalin in bulk drugs and pharmaceutical formulations (Onal, 2009) . Liquid chromatography - mass spectrophotometry (LC - MS), LC with fluorescence detection were used to determine pregabalin in human plasma and serum (Mandal, 2008; Oertel, 2008; Vermeij, 2004). All of these methods are very expensive because these methods require long and tedious pretreatment of the samples, laborious clean up procedures (including extraction with solvent) and derivatization for the analysis of PGB. There is no HPLC method without derivatization for the analysis of PGB. So there is need for the development of a HPLC method for the analysis of PGB. Hence, an attempt has been made to develop a simple, efficient and selective method for the analysis of PGB in bulk, pharmaceutical formulations and human urine samples. The method requires no derivatization steps. HPLC instrumentation with UV detection, which is readily available in most analytical and pharmaceutical laboratories was used. A total analysis run time of less than 10 min was achieved. The method was used successfully to evaluate 5 marketed PGB drug products and human urine samples.

EXPERIMENTAL

Materials

i.) Pregabalin (Vardhman Chemtech Ltd, Punjab, India) used as a standard. ii.) Pharmaceutical formulations of pregabalin such as Gabanext 75 (Nicholas Piramal India Ltd., Mumbai, India), Pregalin 75 (Torrent Pharmaceutical Ltd., Baddi, India), Neugaba 75 (Sun Pharmaceutical Industries, Jammu, India), Mahagaba 75 (Mankind Pharma Ltd., New Delhi, India) and Maxgalin 75 (Sun Pharmaceutical Industries, Jammu, India) were purchased from local markets. iii.) Di - potassium hydrogen orthophosphate (K_2HPO_4) was purchased from Qualigens fine chemicals, Mumbai, India. iv.) Acetonitrile was HPLC grade purchased from Qualigens fine chemicals, Mumbai, India. v.) Orthophosphoric acid was LR grade purchased from Qualigens fine chemicals, Mumbai, India. vi.) All other chemicals were of analytical grade and used without any further purification.

Determination of appropriate UV wavelength

A suitable wavelength was required for the determination of pregabalin. The appropriate wavelength for the detection of drug in mobile phase was determined by wavelength scanning over the range of 200 - 400 nm with a UV 3000$^+$ (LABINDIA®, India) UV/VIS spectrophotometer.

Instrumentation

i.) The HPLC used was model LC – 2010 CHT, Shimadzu, Kyoto, Japan with Pump model 2 LC - 10 ADvp, Autosampler model SIL - 10 ADvp, column oven model CTO - 10 A (C) vp. The detector was a UV detector model SPD - 10 A (V) vp. The system was driven by a HP - 5502. ii.) The 2487 Waters HPLC (Singapore) with 515 pumps was also used. The system was driven by Sync Master 794 MG, Samsung. iii.) UV/VIS Spectrophotometer, Model No UV 3000$^+$, LABINDIA® (Mumbai, India)iv.) Digital pH - meter, Sr No 9492, Toshniwal instrument manufacture Pvt Ltd (Mumbai, India). vi.) The data processing system were run with Breeze softwere for 2487 HPLC and LC solution for LC – 2010 CHT (Shimadzu).

vii.) The HPLC column used was a C_{18} 5 µm ODS hypersil column (250 mm × 4.6 mm) (Thermo Scientific,

UK). The mobile phase filtration unit was Ultipor ® N_{66} ® Nylon 6, 6 membrane (Pall Life Sciences, Mumbai, India), Lot No 06 - 07 ID000784.

Chromatographic system and conditions

The proposed method was performed using a liquid chromatography of model LC - 2010 CHT (Shimadzu, Kyoto, Japan). Separation was operated on C_{18} 5 µm ODS hypersil column (250 mm × 4.6 mm) using methanol - acetonitrile - 0.02 M di – potassium hydrogen orthophosphate (K_2HPO_4) (pH - 7.00) (3: 1: 16, v/v/v) mobile phase at a flow rate of 1.0 ml/min. di – potassium hydrogen orthophosphate solution was prepared by dissolving 3.5 g K_2HPO_4 in 1000 ml double distilled water. Final pH of the mobile phase was adjusted to 7.00 with 0.01 M orthophosphoric acid, prepared daily and degassed by passing through a 0.45 µm Ultipor filter and ultrasonication for 10 min. All separations were performed at room temperature with detection at 210 nm.

Standard solutions

Stock standard solution of pregabalin was prepared by dissolving an appropriate amount of the compound in mobile phase to give a final concentration of 0.75 mg/ml. Standard solutions of pregabalin (2.0, 3.5 and 5.0 µg/ml) were prepared by subsequent dilution. A phosphate buffer containing 0.02 M K_2HPO_4 (3.50 gm in 1000 ml distilled water) and adjusting the pH to 7.0 by adding with 0.01 M orthophosphoric acid.

METHODS

System Suitability

System suitability tests is an integral part of liquid chromatogramphic method. It is used to verify that the resolution of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations and sample to be analyzed constitute an integral system that can be evaluated as such.

Procedure for determination of pregabalin

Aliquots of stock solution (0.75 mg/ml) were transferred in to a 50 ml volumetric flask and volumes were completed to the mark with the mobile phase to produce solutions in the concentration range 0.75 - 6.0 µg/ml. 20 µl of the solution was injected into the HPLC system. The eluents were detected by the UV detector with the wavelength of 210 nm. The signals emerging from the detector were integrated as peak area and a calibration graph of peak area against the concentration of pregabalin was plotted. Alternatively, the regression equation was derived.

Procedure for pharmaceutical formulations

1 capsule (claiming 75 mg of pregabalin) was accurately weighed and finely powdered. A quantity of the powder equivalent to 75 mg of PGB was extracted by shaking with 20 ml of the mobile phase, followed by another 2 extractions each with 10 ml mobile. After passing through a 0.45 µm Millipore filter, the solution was diluted with mobile phase to obtain a concentration of about 0.75 mg/ml. It was further diluted according to the need and then analyzed following the proposed procedures. The nominal content of the capsule was calculated either from the previously plotted calibration graphs or using regression equation.

Procedure for the determination of pregabalin in human urine samples

Aliquot volumes of human urine samples were transferred into small separating funnel. 5 ml of carbonate buffer pH - 9.4 (prepared by dissolving 26.5 g sodium carbonate and 21.0 g sodium bicarbonate in 500 ml distilled water) was added and solution was mixed well. The solution was then extracted with 3 × 5 ml of diethyl ether. The ether extract was collected and evaporated. The residue was dissolved in 5 ml of mobile phase and above general procedure was then followed. The nominal content of PGB was determined from the corresponding regression equation.

METHOD VALIDATION

Solution stability

The stability of the reference pregabalin sample solutions at room temperature was evaluated with the help of HPLC systems.

Specificity and selectivity

The specificity and selectivity of the proposed method was evaluated by estimating the amount of pregabalin in the presence of common excipients such as sodium stearyl fumarate, magnesium stearate, starch, lactose, glucose, fructose, talc and methyl cobalamin. The ability to separate all the compounds (excipients and substance) from PGB in the sample was demonstrated by assessing the resolution between the peaks corresponding to various substances.

Linearity

The linearity of the method was constructed for pregabalin reference standard solutions by plotting the concentrations of the compound versus peak area response. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method (Daraghmeh, 2001). The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation.

Accuracy and precision

The accuracy and precision of the method was evaluated within the linear range based on the analysis of pregabalin reference standard samples and pharmaceutical products at 2.0, 3.5 and 5.0 µg/ml. 5 independent analysis were performed at each con-

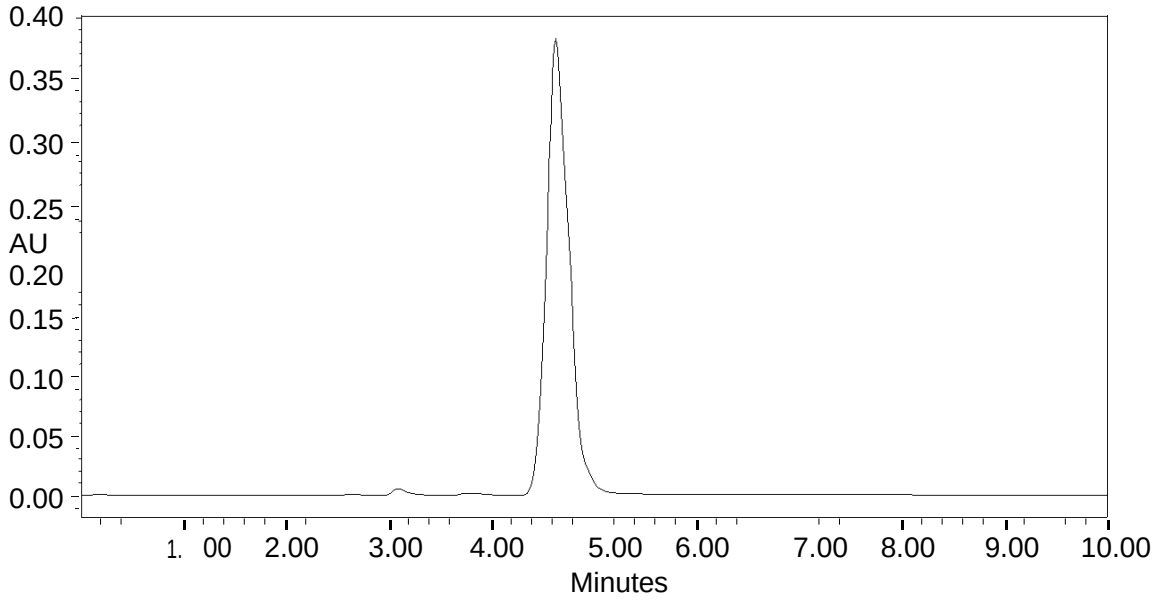

Figure 2. HPLC Chromatograms of Pregabalin (5 µg/ml).

centration level within 1 day (intra day precision) as well as for five consecutive days (inter day precision).

Recovery experiments were carried out by standard addition method. For this, 1.0 (or 2.5 and 4.0 µg/ml) of reference pregabalin solution (0.75 mg/ml) was transferred into a 50 ml volumetric flask followed by 1.5 µg/ml of sample solution (0.75 mg/ml) and the volume was completed up to the mark with the mobile phase. The total amount was determined from the previously plotted calibration graphs or using regression equation.

RESULTS AND DISCUSSIONS

Optimization of chromatographic conditions was achieved by monitoring varying columns and mobile systems. Silica columns such as a µ Bondapak column with different mobile phases did not give a suitable peak shape for analysis. On the other hand, C_{18} 5 µm ODS hypersil column (250 mm × 4.6 mm) gave better results. After trying different ratios of mixtures of methanol - acetonitrile - 0.02 M di-potassium hydrogen orthophosphate (K_2HPO_4), the best results were achieved by using a mixture of methanol - acetonitrile - 0.02 M di-potassium hydrogen orthophosphate (pH - 7.00) (3 : 1 : 16, v/v/v) as mobile phase. Excellent chromatogramphic specificity with no interference from dosage form excipients was observed. Moreover, a suitable retention time for PGB was achieved. Typical chromatograms obtained from the standard solution of PGB, assay preparation of capsules. Under the chromatographic conditions described, PGB was well resolved and eluted at about 4.632 min (Figure 2), the total run time was within 10 min. Good baseline resolution and peak shape can be observed.

System suitability

The system suitability of the proposed method was evaluated after spiking PGB and closely eluted compounds such as 3-isobutyl glutaric acid and (R) - (-) - 3 - (carbamoylmethyl) -5- methyl hexanoic acid. The resolution of PGB with 3 - isobutyl glutaric acid and (R) - (-) - 3 - (carbamoylmethyl) - 5 - methyl hexanoic acid is more than 2.

Determination of suitable UV wavelength

In order to investigate the appropriate wavelength for the determination of PGB, solution of PGB in mobile phase was scanned by UV spectroscopy in the range 200 - 400 nm. The maximum absorbance was observed at 210 nm. Alternatively, solution of PGB in the same mobile phase was also injected to HPLC directly at different wavelength. But the maximum peak area was observed at 210 nm. Therefore, it was concluded that 210 nm is the most appropriate wavelength for the analysis of PGB with suitable sensitivity.

Specificity

The specificity and selectivity of the proposed method was evaluated by estimating the amount of pregabalin in the presence of common excipients such as sodium stearyl fumarate, magnesium stearate, starch, lactose, glucose, talc and methyl cobalamin. The HPLC chromatograms recorded for the mixture of the drug excipients revealed almost no peaks within a retention time

Table 1. Summary of optical and regression characteristics of the proposed method.

Parameters	Pregabalin
Linear dynamic range (g/ml)	0.75 – 6.00
Regression equation[a]	Y = 1836.2 + 467.24 *X*
S_at S_a[b]	10.0622.41
S_bt S_b[b]	2.716.04
Correlation coefficient (r)	0.9999
LOD (g/ml)	0.669
LOQ (g/ml)	0.221
Variance (S_o^2) of calibration line	9.78×10^{-2}

[a]With respect to Y = a + b *X*, where X is the concentration in g/ml, Y is peak area; [b]Confidence interval of the intercept and slope at 95 % confidence level and ten degrees of freedom (t = 2.228)

Table 2. Summary of accuracy and precision results of the proposed method in pure form.

Proposed methods	Amount (g/ml)		RSD (%)	REC.	SAE[b]	C.L.[c]
	Taken	Found ± SD[a]				
Intra day assay	2.00	1.999 ± 0.002	0.122	99.990	2.4×10^{-3}	6.7×10^{-3}
	3.50	3.502 ± 0.006	0.167	100.056	2.6×10^{-3}	7.2×10^{-3}
	5.00	5.001 ± 0.002	0.036	100.017	8.0×10^{-4}	2.2×10^{-3}
Inter day assay	2.00	1.999 ± 0.003	0.162	99.990	1.5×10^{-3}	4.2×10^{-3}
	3.50	3.502 ± 0.006	0.117	99.980	1.8×10^{-3}	5.0×10^{-3}
	5.00	4.999 ± 0.003	0.068	99.980	1.5×10^{-3}	4.2×10^{-3}

[a]Mean for 5 independent analyses. [b]SAE, standard analytical error. [c]C.L., confidence limit at 95% confidence level and 4 degrees of freedom (t = 2.776).

range of 10 min. The study of the absence of excipients showed that none of the peaks appears at the retention time of PGB and it was concluded that the developed method is selective in relation to the excipients of the final preparation.

Solution stability

The solution stability was ascertained from HPLC peak area of reference standard samples. The peak area was obtained at 4.632 min retention time with a UV detector of wavelength of 210 nm (2.000 AUFS). The standard sample solutions were kept at room temperature for 15 days, it was observed that there was no change in peak area of these solutions.

Accuracy and precision

Under the optimum experimental conditions, the peak area - concentration plot for the proposed method was found to be rectilinear over the range of 0.75 - 6.0 µg/ml. Linear regression analysis of calibration data gave the regression equation cited in Table 1 with correlation coefficient close to unity. Statistical analysis of regression line was made regarding the standard deviation of residuals (S_o), standard deviation of slope (S_b) and standard deviation of intercept (S_a) and the values are summarized in Table 1.The days of precision assays were carried out through replicate analysis (n = 5) of PGB corresponding to 2.0, 3.5 and 5.0 µg/ml for the proposed method in pure form and pharmaceutical formulations (Table 2 and 3). The interday precision was also evaluated through replicate analysis of the pure drug and pharmaceutical formulations samples for 5 consecutive days at the same concentration levels as used in the within day precision (Table 2 and 3). As can be seen from the Table 2 that the recovery and relative standard deviation (RSD) by intraday and interday precision were in the ranges 99.990 - 100.056%, 0.036 - 0.167% and 99.98 - 99.99%, 0.068 - 0.162% respectively. As can be seen from Table 3 that that the recovery and RSD by intraday and interday precision were in the ranges 99.940 - 99.983%; 0.174 - 0.785% and 99.927 - 99.970%, 0.203 -0.851% respecttively. The precision results are satisfactory. The proposed method was used for estimation of PGB from capsules after spiking with 1.0, 2.5 and 4.0 µg/ml of additional pure drug. The results are reported in Table 4. As can be seen from the Table 4 that recoveries ranged from 99.906 to 100.088% with relative standard deviation between 0.119 to 0.465%. The proposed method was further extended to the *in vitro* determination of PGB in human urine samples. The results are sum-

Table 3. Summary of accuracy and precision results of the proposed method in pharmaceutical formulations.

Proposed methods	Amount (g/ml)		RSD (%)	REC.	SAE[b]	C.L.[c]
	Taken	Found ± SD[a]				
Intra day assay						
Gabanext - 75	2.00	1.999 ± 0.016	0.785	99.949	0.0070	0.0194
Neugaba - 75	2.00	1.999 ± 0.012	0.584	99.949	0.0052	0.0144
Maxgalin - 75	2.00	1.999 ± 0.008	0.404	99.949	0.0036	0.0100
Pregalin - 75	2.00	1.999 ± 0.010	0.512	99.946	0.0046	0.0128
Mahagaba - 75	2.00	1.999 ± 0.014	0.699	99.949	0.0063	0.0175
Gabanext - 75	3.50	3.498 ± 0.007	0.191	99.955	0.0030	0.0083
Neugaba - 75	3.50	3.499 ± 0.010	0.274	99.967	0.0043	0.0119
Maxgalin - 75	3.50	3.499 ± 0.010	0.297	99.967	0.0046	0.0128
Pregalin - 75	3.50	3.497 ± 0.011	0.310	99.918	0.0049	0.0136
Mahagaba - 75	3.50	3.498 ± 0.011	0.295	99.955	0.0048	0.0133
Gabanext - 75	5.00	4.997 ± 0.009	0.174	99.940	0.0039	0.0108
Neugaba - 75	5.00	4.997 ± 0.010	0.203	99.940	0.0045	0.0125
Maxgalin - 75	5.00	4.999 ± 0.010	0.190	99.974	0.0043	0.0119
Pregalin - 75	5.00	4.997 ± 0.009	0.180	99.949	0.0040	0.0111
Mahagaba - 75	5.00	4.999 ± 0.010	0.194	99.983	0.0043	0.0119
Inter day assay						
Gabanext - 75	2.00	1.999 ± 0.017	0.851	99.949	0.0076	0.0211
Neugaba - 75	2.00	1.999 ± 0.012	0.601	99.927	0.0054	0.0150
Maxgalin - 75	2.00	1.999 ± 0.009	0.464	99.970	0.0042	0.0117
Pregalin - 75	2.00	1.999 ± 0.011	0.569	99.970	0.0051	0.0142
Mahagaba - 75	2.00	1.999 ± 0.014	0.691	99.949	0.0062	0.0172
Gabanext - 75	3.50	3.499 ± 0.012	0.341	99.967	0.0053	0.0147
Neugaba - 75	3.50	3.498 ± 0.012	0.348	99.943	0.0054	0.0150
Maxgalin - 75	3.50	3.498 ± 0.012	0.331	99.943	0.0052	0.0144
Pregalin - 75	3.50	3.498 ± 0.011	0.323	99.943	0.0051	0.0142
Mahagaba - 75	3.50	3.498 ± 0.013	0.384	99.943	0.0060	0.0167
Gabanext - 75	5.00	4.997 ± 0.011	0.217	99.932	0.0049	0.0136
Neugaba - 75	5.00	4.997 ± 0.013	0.264	99.940	0.0059	0.0164
Maxgalin - 75	5.00	4.998 ± 0.010	0.207	99.957	0.0046	0.0128
Pregalin - 75	5.00	4.997 ± 0.011	0.227	99.940	0.0051	0.0142
Mahagaba - 75	5.00	4.997 ± 0.010	0.203	99.940	0.0045	0.0125

[a]Mean for 5 independent analyses. [b]SAE, standard analytical error. [c]C.L., confidence limit at 95% confidence level and 4 degrees of freedom (t = 2.776).

marized in Table 5. These results are satisfactorily accurate and precise. The performance of the proposed method was studied with other existing reference method (Onal, 2009) and (Mandal, 2008). In case proposed method and reported (Onal, 2009) me-thod, the proposed method do not need any derivati-zation with higher recovery in pharmaceutical formula-tions but the reported method requires derivatization for the analysis. On the other hand, the RSD value of the reported (Mandal, 2008) method is relatively higher than the proposed method.

Robustness

The robustness of the method relative to each operational parameter was checked and investigated. The influences of small changes in the mobile phase composition and buffer pH were studied to determine the

Table 4. Summary of data for the determination of pregabalin in pharmaceutical preparations by standard addition method.

Formulations	Amount g/ml)			Recovery (%)	RSD (%)	SAE[b]
	Taken	Added	Found ± SD[a]			
Gabanext-75	1.50	1.00	2.499 ± .011	99.974	0.465	0.0052
	1.50	2.50	3.999 ± .012	99.981	0.311	0.0056
	1.50	4.00	5.499 ± .011	99.984	0.199	0.0049
Neugaba-75	1.50	1.00	2.502 ± .009	100.060	0.363	0.0041
	1.50	2.50	3.997 ± .012	99.917	0.314	0.0056
	1.50	4.00	5.499 ± .013	99.979	0.242	0.0059
Maxgalin-75	1.50	1.00	2.499 ± .011	99.991	0.438	0.0049
	1.50	2.50	4.004 ± .009	100.088	0.212	0.0038
	1.50	4.00	5.501 ± .008	100.023	0.142	0.0035
Pregalin- 75	1.50	1.00	2.502 ± .011	100.060	0.419	0.0047
	1.50	2.50	3.997 ± .007	99.917	0.168	0.0030
	1.50	4.00	5.499 ± .007	99.976	0.131	0.0032
Mahagaba- 75	1.50	1.00	2.498 ± .008	99.906	0.329	0.0037
	1.50	2.50	3.999 ± .009	99.981	0.231	0.0041
	1.50	4.00	5.499 ± .007	99.984	0.119	0.0029

[a]Mean for 5 independent analyses. [b]SAE, standard analytical error.

Table 5. Application of the proposed HPLC method to the determination of Pregabalin in human urine samples

Amount added(µg/ml)	Amount found(g/ml)	Recovery (%)
1.0	0.9712	97.12
2.0	1.9686	98.43
3.0	2.9657	98.86
4.0	3.9476	98.69
5.0	4.9093	98.19
6.0	5.8919	98.20
X		98.25
RSD		0.624

robustness of the method, such as the changes in peak area and retention time. The results are summarized in Table 6.The robustness of the method was also assessed by analyzing the active PGB in pharmaceutical formulations. The reference standard sample solution containing 5.0 µg/ml of the drug assayed. The percent recovery ± RSD of the method (99.989 ± 0.150) were found to be appreciable, indicating that the proposed method is robust.

Ruggedness

For the evaluation of ruggedness of the proposed method, the contents of PGB at 5.0 µg/ml were assayed following the recommended procedure using Shimadzu LC 2010 CHT Auto sampler and Waters 2487 HPLC systems. The recoveries ± RSD resulting from the Shimadzu LC 2010 CHT (99.985 ± 0.145) and Waters 2487 (99.981 ± 0.252) were compared.

Conclusion

In conclusion, the proposed HPLC method a simple, accurate and reproducible method for routine *in vitro* tests of PGB in bulk, pharmaceutical formulations and human urine samples. Although several HPLC methods are now available for determination of PGB with UV detection. The major advantages of this method include short retention time, without derivatization with other reagent, stability of the solution, no need for prior separation or purification before analysis, and the applicability of a common HPLC system (isocratic system, UV

Table 6. The influence of Small Changes in pH and Composition of Mobile Phase (Method Robustness)

Mobile Phase Composition	Retension Time (t_R)	Peak area
Methanol – Acetonitrile – Buffer pH = 7.00 (3: 1: 16)	4.632	4170
Methanol – Acetonitrile – Buffer pH = 7.00 (5: 3: 32)	4.636	4088
Methanol – Acetonitrile – Buffer pH = 7.00 (7: 1: 32)	4.555	4055
Methanol – Acetonitrile – Buffer pH = 7.00 (1: 1: 8)	4.761	4069
Methanol – Acetonitrile – Buffer pH = 7.00 (3: 3: 14)	4.339	4060
Methanol – Acetonitrile – Buffer pH = 7.00 (4: 1: 17)	4.327	4057
Methanol – Acetonitrile – Buffer pH = 7.00 (1: 1: 3)	4.780	4085
Methanol – Acetonitrile – Buffer pH = 7.50 (3: 1: 16)	4.754	4008
Methanol – Acetonitrile – Buffer pH = 7.20 (3: 1: 16)	4.701	4019
Methanol – Acetonitrile – Buffer pH = 6.80 (3: 1: 16)	4.543	4043
Methanol – Acetonitrile – Buffer pH = 6.50 (3: 1: 16)	4.504	4047

detector). The short chromatographic time makes this method suitable for the processing of multiple samples in a limited amount of time. In addition, the method has wider linear dynamic range with good accuracy and precision. The method shows no interference from common excipients. Since in human unchanged parent representing 90% of drug is derived in urine (normal capsule is 75 mg strength), this method can be used for estimating unabsorbed PGB in urine samples by very simple, cost effective, fast and efficient method. The statistical parameter and recovery data reveal the good accuracy and precision of the proposed method. Finally, since no pharmacopoeial method for determination of pregabalin in bulk and pharmaceutical formulations have been reported yet, the proposed method could be useful and suitable for the determination of PGB in bulk pharmaceutical formulations and in human urine samples.,

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