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Spectrofluorimetric Method of Analysis for Gabapentin in Spiked Human Plasma and Formulations

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Abstract: Gabapentin is an anticonvulsant widely used in the treatment of epilepsy.A simple, rapid, accurate, precise and economic spectrofluorimetric methods have been developed for the estimation of gabapentin in pharmaceutical formulations and in spiked human plasma. The analysis and validation dine by using SHIMADZU RF – 5301 Spectofluorimeter, equipped with Xenon arc lamp, using quartz cell (1×1×4.5) cm, with R-450-01photomultiplier detector.
The absorbance was found to increase linearly with increasing concentration of gabapentin over the concentration range of 0.2 – 2.0 µg/ml for Gabapentin with a mean r^2 value 0.9989. Recovery studies gave satisfactory results indicating that none of common additives and excipients interfere the assay method. Various analytical parameters were evaluated and the results were validated by statistical data. The proposed method was found to be simple, sensitive, accurate, precise and economic and can be successfully applied for the analysis and validation of gabapentin in both pharmaceutical dosage form and Human plasma.
Key words: Gabapentin, method development, validation.

Introduction

Gabapentin (GBP), 1-(amino methyl) cyclohexane acetic acid, is a structural analogue of the inhibitory neurotransmitter *g*-amino butyric acid as shown in Fig 1. GBP is also used in the treatment of neuropathic pain [1, 2]. In recent years, gabapentin has been used widely as an adjunct for the treatment of acute postsurgical pain. Several methods for determination of GBP in pharmaceutical dosage forms as well as in biological fluids were found in literature surveys. Literature suggested methods such as LC/MS/MS[4-7], GC/MS[8-9], HPTLC[10], HPLC[11-13], GLC[20], capillary electrophoresis, spectrofluorimetry and colorimetry[14]. In the present study, a spectroflourimeteric method had been developed for the analysis and validation of gabapentin in human plasma and pharmaceutical formulation.

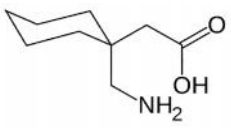

Fig 1: Chemical structure of Gabapentin

Experimental

Chemicals and Reagents Used:

All chemicals used were of analytical grade and solvents were of spectroscopic grade. Gabapentin was kindly supplied by Zydus Cadila, Ahmedabad, India. All reagents were of analytical grade purity or high grade purity. Ethyl acetoacetate (Merck Hohenbrunn Germany), formaldehyde 37% (Merck KGaA 64271 Darmstadt, Germany), glacial acetic acid (Merck KGaA 64271 Darmstadt, Germany), sodium acetate trihydrate (Merck KGaA 64271 Darmstadt, Germany), were used in this work. Blank Human Serum samples were kindly supplied form Prathama Blood Centre, Research Foundation, Vasna, Ahmedabad, India.

Instrumentation:

SHIMADZU RF – 5301 Spectofluorimeter, equipped with Xenon arc lamp, using quartz cell (1×1×4.5) cm, with R-450-01photomultiplier detector was used for the measurements.

Preparation Of Standard Calibration Curves

Preparation of Reagent Solutions:

Ethyl acetoacetate solution (0.8 M) was prepared by diluting 10 mL of the reagent to 100 mL with ethanol. Formaldehyde solution (25%) was prepared by diluting 67.7 mL of 37% to 100 mL with distilled water. Acetic acid-sodium acetate buffer (pH = 4.0) was prepared by mixing 0.2 M sodium acetate solution with 0.2 M acetic acid solution and adjusting the pH to 4.0 using pH meter.

Preparation of standard stock solution (50 µg / ml):

Standard stock solution of Gabapentin (50 µg/ml) was prepared on daily basis by dissolving 5mg of the authentic standard in distilled water and diluting to 100 ml with distilled water. Working standards were prepared by diluting appropriate volume of the stock solution.

On scanning the working standard solution of gabapentin, 398 nm and 315 nm were selected as emission and excitation wavelengths respectively for measuring fluroscence intensity.

Preparation of calibration curve for Gabapentin:

Appropriate volumes of the stock solution of Gabapentin, to give final concentration of 0.2-2 µg/ml, were taken in 20 mL test tubes. 1 mL of 25% formaldehyde and 2 mL of pH 4.0 buffer was added to each test tube. The mixture was gently shaken followed by the addition of 1 mL of ethyl acetoacetate. The components of mixture were, again, gently shaken and heated on a water bath adjusted at 70 °C for 30 min. The contents of the test tubes were cooled by immersing in tap water and then transferred to 25 mL volumetric flasks and diluted to the mark with distilled water. The fluorescent intensity was measured at λmax 398 nm using λmax315 nm. A blank experiment was carried out simultaneously. The corrected fluorescence intensity was plotted against the final drug concentration (µg/ml) to obtain the calibration curve. Alternatively, the corresponding regression equation was derived. The regression equation and correlation coefficient was determined for Gabapentin.

Analysis of tablet dosage form:

Contents of twenty capsules were accurately weighed and average weight per capsule was determined, contents were grounded to fine powder. An accurately weighed quantity of the pulverized powder equivalent to 5 mg Gabapentin was transferred into 100 ml volumetric flask, dissolved in sufficient amount of distilled water and diluting to 100 ml with distilled water to obtain a standard stock solution'A' of 50 µg/ml. From the above stock 'A' solution, appropriate aliquot (1ml) was pipetted out and was transferred to a 25 ml volumetric flask. To the above solution, 1 ml of 25% formaldehyde and 2 ml of pH 4.0 buffer was added. The mixture was gently shaken followed by the addition of 1 ml of ethyl acetoacetate. The components of mixture were, again, gently shaken and heated on a water bath adjusted at 70 °C for 30 min. The contents of the flask were cooled by immersing in

tap water and then diluted to the mark with distilled water to obtain final concentration of 2 µg/ml. The fluorescent intensity of the prepared solution was measured at λmax 398 nm using λmax 315 nm against a reagent blank. Six different solutions containing 2µg/ml of gabapentin were prepared and analyzed in same manner as mentioned under the general procedure. The nominal content of the capsule was determined by using the calibration curve.

Procedure for Spiked Human Plasma:

Control samples of human plasma 2.0 ml were spiked with different concentration levels of Gabapentin using above prepared stock solution and transferred into centrifugation tubes, then completed with acetonitrile to final volume of 4 ml, and centrifuged for 15 min at a rate of 3000 rpm. The clear supernatant fluid was transferred to a 25 ml volumetric flask and to this solution, 1 ml of 25% formaldehyde and 2 ml of pH 4.0 buffer was added. The mixture was gently shaken followed by the addition of 1 ml of ethyl acetoacetate. The components of mixture were, again, gently shaken and heated on a water bath adjusted at 70 °C for 30 min. The contents of the flask were cooled by immersing in tap water and then diluted to the mark with distilled water to obtain final drug concentration range of 0.2-2 µg/ml. The fluorescent intensity of above prepared solution was measured at λmax 398 nm using λmax315 nm and simultaneously a blank experiment was also carried out. The nominal content of the drug in plasma was determined by using the corresponding calibration curve.

Method Validation:

The developed analytical method was subjected to validation with respect to various parameters such as linearity, limit of quantification (LOQ), limit of detection (LOD), accuracy, precision, recovery studies, specificity and reproducibility as per the ICH guidelines.

Linearity and range:

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity. By using the above mentioned procedure a linear regression equation was obtained. The regression plots showed that there was a linear dependence of fluorescence intensity on the concentration of the drug over the ranges cited in Table 1, 2.

Accuracy:

It is closeness of the test results obtained to the true value. It is often expressed as % recovery which is performed by analyzing known added amounts of analyte. Also it can be determined by applying the procedure to quantitatively prepared samples.

Procedure for determination of Accuracy:

Recovery studies were carried out by applying the method to drug sample present in capsule dosage form to which known amount of gabapentin corresponding to 50%, 100% and 150% of label claim was added (standard addition method). An accurately weighed quantity of the pulverized capsule powder equivalent to 5 mg Gabapentin was transferred into 100 ml volumetric flask, dissolved in sufficient amount of distilled water and diluting to 100 ml with distilled water to obtain a standard stock solution'A' of 50 µg/ml. From the stock 'A' solution containing 50 µg/ml, appropriate aliquot (1ml) was pipetted out and was transferred to three different 25 ml volumetric flask. To the above solutions appropriate aliquot corresponding to 80%, 100% and 120% of the label claim was added respectively. The above prepared solution was added with 1 ml of 25% formaldehyde and 2 ml of pH 4.0 buffer. The mixture was gently shaken followed by the addition of 1 ml of ethyl acetoacetate. The components of mixture were, again mixed gently and heated on a water bath adjusted at 70 °C for 30 min. The contents of the flask were cooled by immersing in tap water and then diluted to the mark with distilled water and filtered through Whatmann filter paper No.41. The fluorescent intensity of the prepared solutions was measured at λmax 398 nm using λmax 315 nm against a reagent blank. At each level of recovery

studies, three determinations were performed. The results obtained were compared with expected results and were statistically validated.

Precision:

The precision of an analytical method is the degree of agreement among the individual test results obtained when the method is applied repeatability to multiple sampling of the same homogenous sample under prescribed conditions.

- **Intraday Precision**

  It is the degree of agreement among the individual test results obtained when the method is applied repeatability to multiple sampling of the same homogenous sample within the same day (intra-day).

- **Interaday Precision**

  It is the degree of agreement among the individual test results obtained when the method is applied repeatability to multiple sampling of the same homogenous sample on different days (inter-day).

Procedure for determination of Intra-day Precision:

The intraday precision for Gabapentin in pure form and in spiked human plasma was evaluated by assaying two different concentrations of Gabapentin within the calibration curve range on three successive times in same day in same manner as mentioned under the general procedure.

Procedure for determination of Inter-day Precision:

The interday precision was determined by assaying two freshly prepared concentrations of Gabapentin solution for three successive days in same manner as mentioned under the general procedure.

Limit of detection and limit of quantification:

The lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated as an exact value is termed as limit of detection. The lowest amount of the analyte in the sample that can be quantitatively determined with defined precision under the stated experimental conditions is termed as limit of quantitation.

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH Q2B.

The limit of detection (LOD) was determined by establishing the minimum level at which the analyte can be reliably detected.

LOQ and LOD were calculated according to the following equation:

$$LOQ = 10\ \sigma \backslash S$$

Where; σ = Standard deviation of Response
S = Slope of calibration curve

$$LOD = 3.3\ \sigma \backslash S$$

Where; σ = Standard deviation of Response
S = Slope of calibration curve

Specificity:

Specificity is a procedure to detect quantitatively the analyte in presence of components that may be expected to be present in the sample matrix.

The specificity of the method was investigated by observing any interference encountered from the common tablet excipients such as talc, lactose, starch, and magnesium stearate. These excipients did not interfere with the proposed method.

Robustness:

The robustness of the method adopted is demonstrated by the constancy of the fluorescence intensity with the deliberated minor change in the experimental parameters such as the change in the concentration of ethyl acetoacetate (0.8 M), 0.8 ± 0.1 M, volume of ethyl acetoacetate (1ml) 1ml ± 0.1ml, change in reaction time 30 ± 5 min, concentration of formaldehyde (25%), 25 ± 1%, pH of buffer (4.0), 4.0 ± 0.1 and reaction temperature (70^0C) 70 ± 2 ^{0}C. These minor changes that may take place did not affect the fluorescence intensity of the reaction product.

Ruggedness:

Three sets of experiments for this drug were carried out using two different laboratories and different analysts; no significant difference was obtained between the results in this study.

Optimization Of Experimental Parameters

The spectrofluorimetric properties of the colored product, as well as the different experimental parameters affecting the development of the reaction product and its stability were carefully studied and optimized. Such factors were changed individually while the others were kept constant. The factors include pH, concentration of the reagent, temperature, reaction time and dilution.

Effect of pH:

The influence of pH on the fluorescence intensity of the reaction product was studied. The maximum fluorescence intensity was obtained upon using Acetic acid sodium acetate buffer of pH 4. This value was found to be the optimum pH for Hantzsch's condensation reaction mechanism because condensation of most of the amines with β keto ester has been found to be optimal at pH 3.8 - 4.5. The effect of volume of the buffer was also investigated and it was found that 2 mL of pH 4.0 buffer produced maximum fluorescence signal.

Effect of Reagent Concentration:

The concentration and volume of various reagents affecting the formation of the fluorescent product were carefully studied. The effect of the concentration of ethyl acetoacetate on the condensation reaction was studied in the range of 0.2–1.4 M. It was found that fluorescence intensity increased with increase in concentration up to 0.8 M beyond which the fluorescence intensity decreased due to the formation of water insoluble products. Then the volume of ethyl acetoacetate was varied keeping the concentration constant.

The concentration of formaldehyde was varied from 15–35% and its effect on the fluorescent product formation was monitored. An increase in fluorescence intensity was observed up to 25% of formaldehyde concentration after which decrease occurred in the fluorescence intensity mainly due to formation of water insoluble byproducts. The volume of the formaldehyde was also optimized and 1 ml of the reagent was found sufficient for maximum fluorophore formation

Effect of Temperature:

The reaction temperature was varied from 60–100 °C and its effect on the fluorescent product formation was monitored. It was found that maximum fluorophore formation occurred when the reaction mixture was heated at 70 °C. Beyond this temperature the stability of the fluorophore reduces and fluorescence intensity decreases.

Effect of the Reaction Time and stability of the product:

Different time intervals were tested to ascertain the time after which the solution attains its highest fluorescence intensity. It was found that after 30 min of heating the reaction product reaches the highest fluorescence

intensity and remains stable at room temperature for 2 hr. The stability of the fluorescent reaction product was determined by measuring the fluorescence intensity at regular interval up to 120 min. It was found the no significant change occurred in the fluorescence intensity of the reaction product.

Thus the fluorescent reaction product is stable and will not affect the result of analysis even if the fluorescence intensity is measured after 2 hr of the dilution.

Results And Discussion

The ethyl acetoacetate and formaldehyde reacts with amino group of the Gabapentin in slightly acidic media following the Hantzsch's condensation reaction mechanism. In the first step the ethyl acetoacetate reacts with formaldehyde producing diethyl-2, 4-diacetyl pentanedioate. In the second step the diethyl-2, 4-diacetyl pentanedioate undergo condensation with amino group of the Gabapentin producing fluorescent product.The fluorescent reaction product showed maximum fluorescence intensity at λmax 398 nm when excited at λmax315 nm (Fig 2, 3).

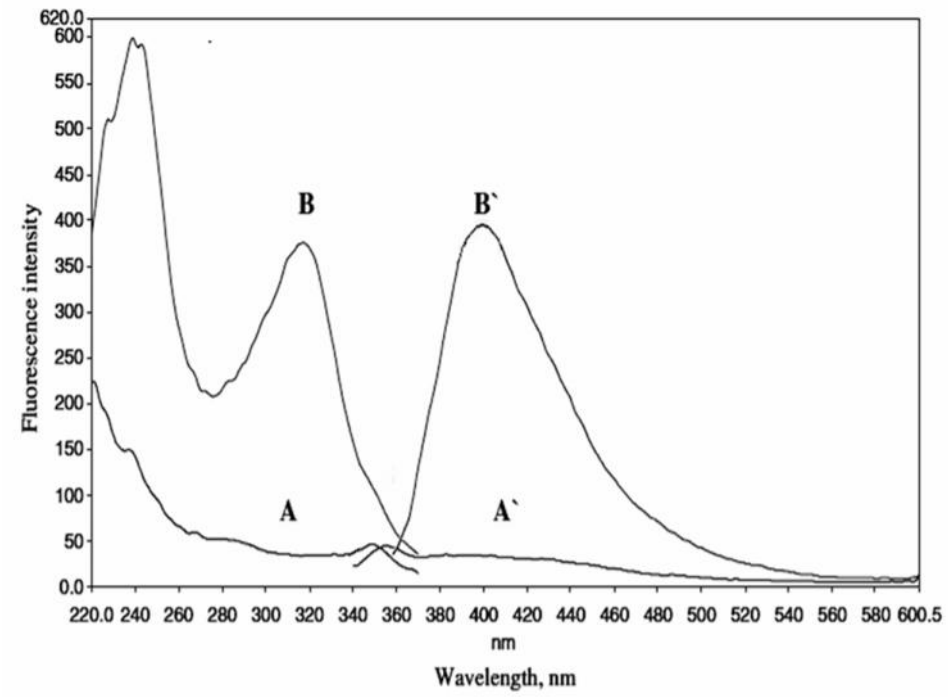

Fig 2: Fluorescence spectra of A and B excitation and emission spectra of blank solution and Fluorescence spectra of A' and B' excitation and emission spectra of Gabapentin (0.2 µg/ml) with dansyl chloride at pH 9.8

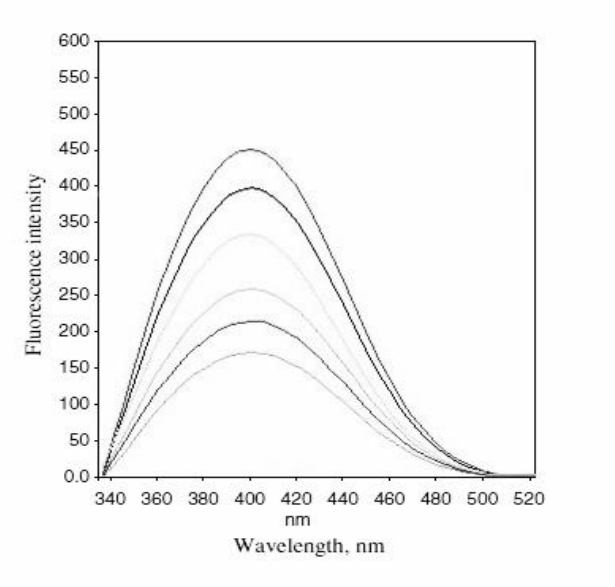

Fig 3: Overlain Fluorescence emission Spectra of Gabapentin (0.04-0.24 µg/ml) at 398nm

Linearity:

The linearity of the proposed method was established by least square regression analysis of the calibration curve. The constructed calibration curve was linear over the concentration range of 0.2 – 2.0 µg/ml for Gabapentin with a mean r^2 value 0.9989 respectively (Table 1, 2 and Fig 4).

Table: 1 Result of calibration curve for Gabapentin

Concentration (µg/ml)	Relative Fluorescence Intensity Mean ± Std. Deviation (n=6)	% CV
0	0.0000 ± 0.0000	0.0000
0.2	128.5 ± 1.8708	1.4558
0.4	168.33 ± 1.6329	0.9700
0.8	221.83 ± 1.7224	0.7764
1.2	290.66 ± 1.9663	0.6765
1.6	360 ± 1.7888	0.4969
2	418.83 ± 1.8348	0.4380

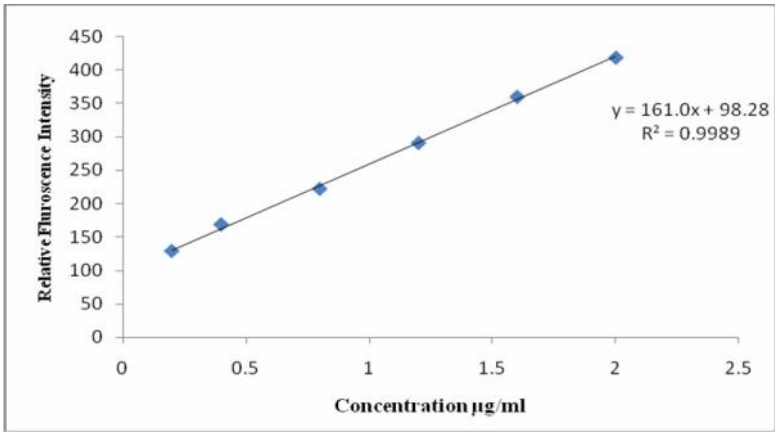

Fig: 4 Calibration curve for Gabapentin by Spectrofluorimetric method

Table: 2 Statistical analysis of the calibration curve of Pregabalin

Parameter	Gabapentin
Linear Range (µg/ml)*	0.2-2.0
Slope*	160.9833 ± 1.3585
Intercept*	98.28 ± 1.1972
Correlation coefficient*	0.9989
Limit of Detection (µg/ml)*	0.02
Limit of Quantitation (µg/ml)*	0.08

Assay:

Six different solutions containing 2µg/ml of gabapentin were prepared and analyzed in same manner as mentioned under the general procedure. The nominal content of the capsule was determined by using the calibration curve. All the results were depicted in table 3, 4.

Table: 3 Assay results of capsule dosage form

Sr. No.	Amount present in (µg/ml)	Amount obtained in (µg/ml)	Amount obtained in %
1	2	1.98	99.00
2	2	1.97	98.50
3	2	1.96	98.00
4	2	1.96	98.00
5	2	1.97	98.50
6	2	1.97	98.50

Table: 4 Statistical Validation Data for Tablet Formulation

Drug	Mean Assay*	Standard Deviation*	Co-efficient of Variation*
Gabapentin	98.41	0.3763	0.3823

*n = 6

Accuracy:

A recovery study was also performed to determine the accuracy and precision of the proposed method. Recovery experiments were performed at three levels, 80%, 100% and 120% of the labeled amount of the drug in capsule formulation. Three replicate samples of each concentration levels were prepared and the percentage recovery at each level (n = 3), and mean % recovery (n = 9) were determined and summarized in Table 5, 6. **The** mean (%) recovery was found to be 99.00% with % RSD value > 2% respectively.

Table 5: Accuracy data of Gabapentin sample in spiked standard drug solution

Level of % recovery	Amount present (mg)	Amount of standard drug added (mg)	Total amount recovered (mg)	% Recovery
80%	5	4	8.93	99.22
	5	4	8.90	98.88
	5	4	8.92	99.11
100%	5	5	9.91	99.10
	5	5	9.90	99.00
	5	5	9.89	98.90
120%	5	6	10.87	98.81
	5	6	10.92	99.27
	5	6	10.91	99.18

Table: 6 Statistical Validation Data for Accuracy determination

Level of % recovery	Mean*	Standard Deviation*	Co-efficient of Variation*
80%	99.07	0.1734	0.1750
100%	99.00	0.1100	0.1111
120%	99.08	0.2437	0.2459

Precision:

Both intra-day and inter-day precision were determined using ICH guidelines and the statistical validation data for both intra-day and inter-day precision are summarized in Table 7-10 respectively. The variation was found to be showing least % RSD value indicating high grade of precision of the method.

Table: 7 Determination of Intra-day Precision of Gabapentin

Sr. No.	Amount present in (µg/ml)		Relative Fluroscence Intensity		Amount found in (µg/ml)	
	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
1	0.4	0.8	167	219	99.22	98.73
2	0.4	0.8	169	223	100.41	100.54
3	0.4	0.8	165	221	98.03	99.63

Table: 8 Statistical Validation Data for Intra-day Precision

Components	Mean*	Standard Deviation*	Co-efficient of Variation*
Sample A	99.22	1.19	1.1993
Sample B	99.63	0.905	0.9083

*n = 3

Table: 9 Determination of Inter-day Precision of Gabapentin

Days.	Amount present in (µg/ml)		Relative Fluroscence Intensity		Amount found in (µg/ml)	
	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
1	0.4	0.8	164	217	97.44	97.83
2	0.4	0.8	169	218	100.21	98.28
3	0.4	0.8	166	220	98.61	99.18

Table: 10 Statistical Validation Data for Inter-day Precision

Components	Mean*	Standard Deviation*	Co-efficient of Variation*
Sample A	98.91	1.6755	1.6939
Sample B	98.44	1.7012	1.7281

The proposed method was also successfully applied for the determination of Gabapentin in spiked human plasma. The % mean recovery was found to be 99.03 % with a least % RSD value 0.1111 indicating high sensitivity and accuracy of the proposed method for the determination of Gabapentin in biological fluids as shown in table 11.

Table: 11 Determination of Gabapentin sample in spiked human plasma

Blank Plasma Samples (ml)	Amount added (µg/ml)	Amount found (µg)	Recovery* (%)
2	0.2	0.198	99.00
2	0.4	0.394	98.50
2	0.8	0.789	98.63
2	1.2	1.187	98.92
2	1.6	1.592	99.50
2	2	1.98	99.00
Mean (%) recovery**			98.93
± SD**			0.3491
RSD (%) **			0.3528

where, *n = 3 and **n = 6

Robustness:

The robustness of the method adopted is demonstrated by the constancy of the fluorescence intensity with the deliberated minor change in the experimental parameters. Results were as shown in table 12-18.

Table: 12 Robustness Results for variations in concentration of ethyl acetoacetate on 2.0 µg/ml of gabapentin

Method Parameter concentration of ethyl acetoacetate (0.8 M)	Relative Fluorescence Intensity*	Amount found (µg/ml)*	Amount obtained %*
0.7	416	1.98	99.00
0.8	417	1.99	99.5
0.9	421	2.01	100.5

*n=3

Table: 13 Robustness Results for variations in volume of ethyl acetoacetate on 2.0 µg/ml of gabapentin

Method Parameter Volume of ethyl acetoacetate (1ml)	Relative Fluorescence Intensity*	Amount found (µg/ml)*	Amount obtained %*
0.9	417	1.99	99.5
1.0	418	1.99	99.5
1.1	421	2.01	100.5

*n=3

Table: 14 Robustness Results for variations in Reaction Time on 2.0 µg /ml of gabapentin

Method Parameter Reaction Time (30 min)	Relative Fluorescence Intensity*	Amount found (µg/ml)*	Amount obtained %
25	416	1.98	99.0
30	417	1.99	99.5
35	417	1.99	99.5

*n=3

Table: 15 Robustness Results for variations in Reaction Temperature on 2.0 µg /ml of gabapentin

Method Parameter Reaction Temperature (70^{o}C)	Relative Fluorescence Intensity*	Amount found (µg/ml)*	Amount obtained %
68	415	1.97	98.5
70	415	1.97	98.5
72	416	1.98	99.0

*n=3

Table: 16 Robustness Results for variations in concentration of Formaldehyde on 2.0 µg/ml of gabapentin

Method Parameter Concentration of formaldehyde (25%)	Relative Fluorescence Intensity*	Amount found (µg/ml)*	Amount obtained %
24	416	1.98	99.0
25	416	1.98	99.0
26	417	1.99	99.5

*n=3

Table: 17 Robustness Results for variations in pH of buffer on 2.0 µg/ml of gabapentin

Method Parameter Buffer pH (4.0)	Relative Fluorescence Intensity*	Amount found (µg/ml)*	Amount obtained %
3.9	415	1.97	98.5
4.0	416	1.98	99.0
4.1	415	1.97	98.5

*n=3

Table: 18 Statistical validation of Robustness Results for variations in Method Parameters

Method Parameters	Mean*	Standard Deviation*	Co-efficient of Variation*
Concentration of ethyl acetoacetate (0.8 M)	99.67	0.7637	0.7662
Volume of ethyl acetoacetate (1ml)	99.83	0.5773	0.5782
Reaction Time (30 min)	99.33	0.2887	0.2906
Reation Temp (70^0C)	98.67	0.2887	0.2925
Concentration of formaldehyde (25%)	99.17	0.2887	0.2911
Buffer pH (4.0)	98.67	0.2887	0.2925

*n=3

Optimization Of Experimental Parameters

Effect of pH:

The influence of pH on the fluorescence intensity of the reaction product was studied. The fluorescence intensity increased with increase in pH up to 4.0 after which slight decrease was observed in the signal (Fig 5).

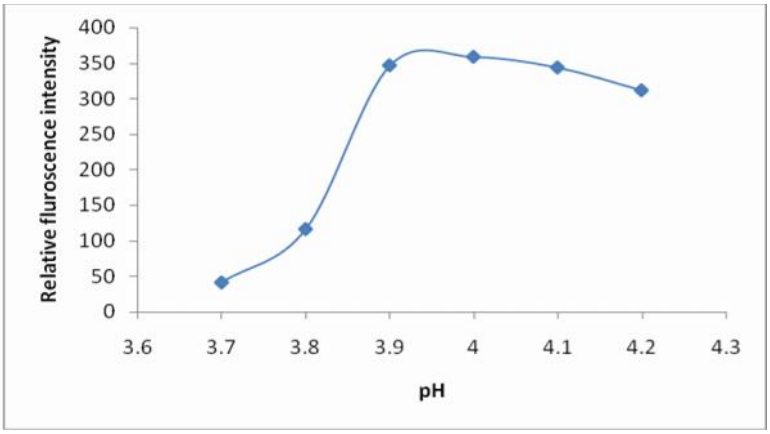

Fig: 5 Effect of pH on Fluorescence Intensity

Effect of Temperature:

The reaction temperature was varied from 60–100 °C and its effect on the fluorescent product formation was monitored. It was found that maximum fluorophore formation occurred when the reaction mixture was heated at 70 °C. Beyond this temperature the stability of the fluorophore reduces and fluorescence intensity decreases. (Fig. 6 - 8).

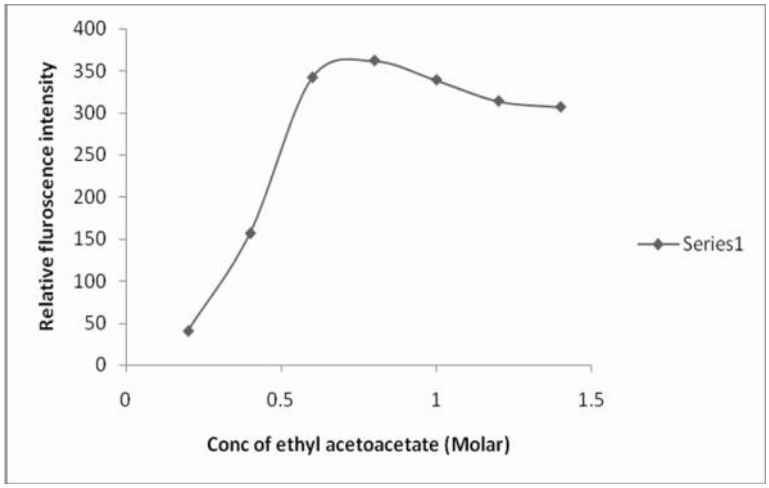

Fig: 6 Effect of Concentration of ethyl acetoacteate on Fluorescence Intensity

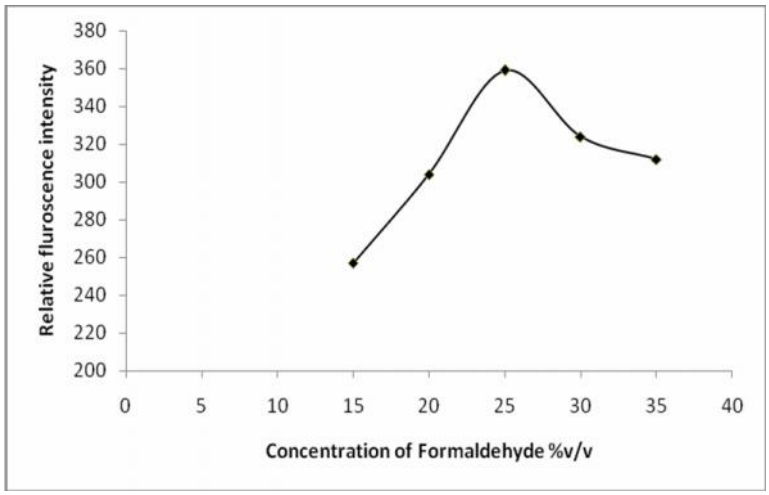

Fig: 7 Effect of Concentration of Formaldehyde %v/v on Fluorescence Intensity

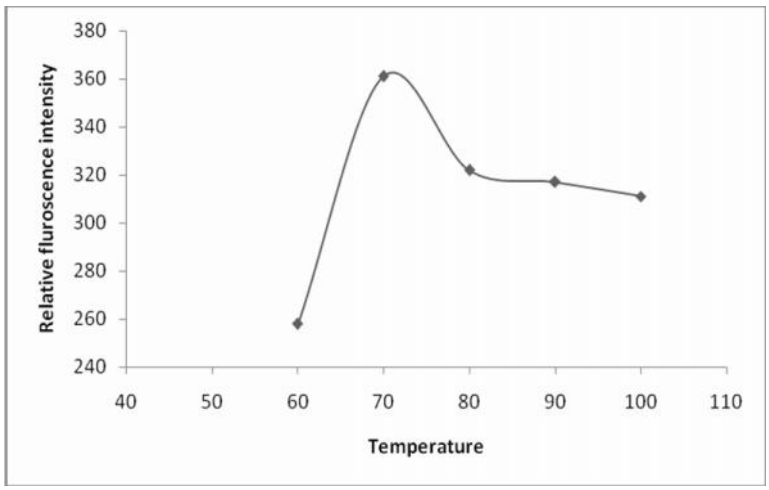

Fig: 8 Effect of reaction temperature on Fluorescence Intensity

Effect of the Reaction Time and stability of the product:

Different time intervals were tested to ascertain the time after which the solution attains its highest fluorescence intensity. It was found that after 30 min of heating the reaction product reaches the highest fluorescence intensity and remains stable at room temperature for 2 hr. The stability of the fluorescent reaction product was determined by measuring the fluorescence intensity at regular interval up to 120 min (Fig 9). It was found there was no significant change occurred in the fluorescence intensity of the reaction product.

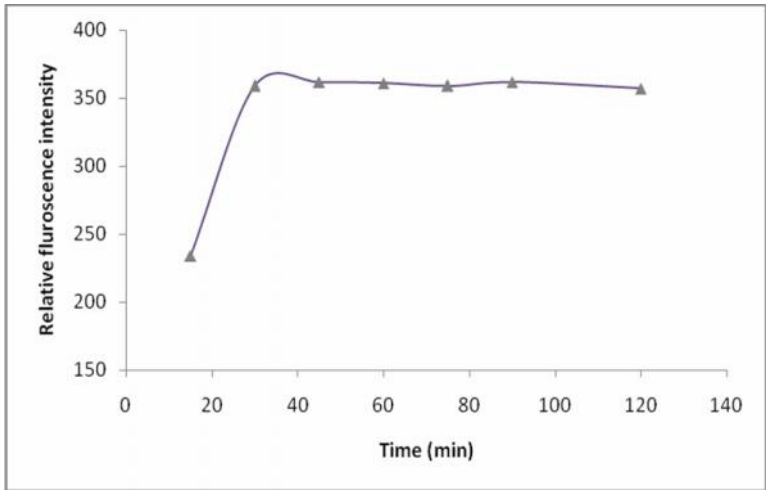

Fig: 9 Effect of reaction time on Fluorescence Intensity

Conclusion

Developed spectrofluorimetric method was found to be simple, accurate, precise, and linear across the analytical range. The method was selective for the determination of gabapentin in pharmaceutical formulations, and plasma.

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