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Quantification of Vigabatrin Using Visible Spectrophotometry and HPTLC by Derivatization Method

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Abstract: Two analytical methods were developed for the determination of vigabatrin in formulation. The first method is visible spectrophotometry based on vigabatrin reaction with ninhydrin reagent in phosphate buffer (pH 7.4) to form a purple-colored chromogen (Ruhemann's purple) exhibiting maximum absorbance at 572 nm. Derivatization of Vigabatrin was carried out by treating the drug solution with phosphate buffer and ninhydrin reagent and heating for 15 min. The resulting solutions were analysed at 572 nm. The second method is HPTLC of Vigabatrin was achieved using Merck TLC aluminium plates precoated with silica gel G 60 F_{254}.The derivatized samples were applied on the TLC plate, and chromatographic separation was achieved using acetone: chloroform (3:7, v/v) as the mobile phase. The developed chromatogram was scanned densitometrically at 572 nm. The developed methods were validated for linearity, precision, accuracy, LOD, LOQ, and robustness as per ICH Q2(R2) guidelines. The calibration curve was linear over the concentration range of 15–48 ng/band in HPTLC method and 10–50 µg/mL in visible spectroscopic method, with correlation coefficient (r^2) > 0.99. The proposed methods were found to be specific, precise, accurate, and robust. Both the methods are economical and successfully applied for the analysis of vigabatrin in tablet dosage form. The amount obtained is as per with label claim of the product. Two methods are first of their kind and shall be applied to the routine quality control analysis of Vigabatrin in formulation.

Keywords: *Vigabatrin, Derivatization, Visible, HPLTC, Formulation.*

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I. INTRODUCTION

Vigabatrin is an antiepileptic agent used in the treatment of epilepsy. The IUPAC name of vigabatrin is 4-amino-5-hexenoic acid. It has the molecular formula $C_6H_{11}NO_2$. The drug is marketed in tablet and oral powder dosage forms, with film-coated tablets available in a strength of 500 mg.

A literature survey (1-13) revealed that only a few HPLC methods based on derivatization detection have been reported for vigabatrin. However, there are no visible spectrophotometric and HPTLC methods available for its analysis in pharmaceutical formulations are limited. Hence, the present work describes two simple, sensitive, accurate, precise, and cost-effective UV-visible spectrophotometric and HPTLC methods for the determination of vigabatrin in pharmaceutical formulations. The structure of vigabatrin (Figure 1) shows that it has very less chromophore, which is prompting for derivatization in order improve its detection before quantification.

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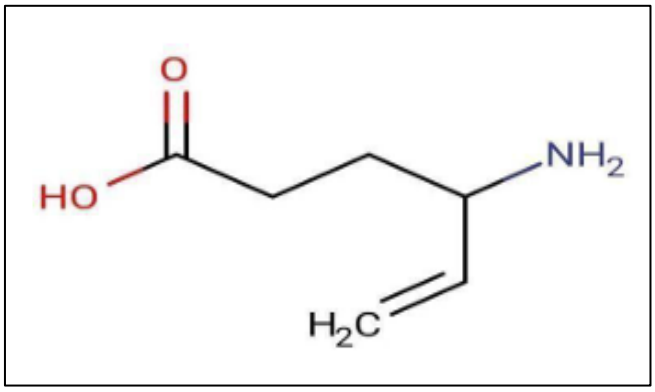

Fig 1. Structure of vigabatrin

II. MATERIALS AND METHODS

➢ *Chemicals and reagents*

Vigabatrin working standard (Viganext) was obtained from MSN Laboratories Private Limited, Telangana, India. The marketed formulation used in the study was procured from a local pharmacy, coimbatore. Methanol (AR grade) was procured from Loba Chemie Pvt. Ltd., Mumbai, India; ethanol (AR grade) from Krishna Pharma, Hyderabad, India; acetone (AR grade) and chloroform (AR grade) from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

➢ *Equipment*

Visible spectrophotometric analysis was performed using a Jasco V-630 UV/Visible spectrophotometer (Jasco Corporation, Tokyo, Japan). HPTLC analysis was carried out using a CAMAG HPTLC system (CAMAG, Switzerland) equipped with a Linomat 5 applicator, TLC Scanner 4, and Vision CATS software version 3.1. Pre-coated aluminium-backed TLC plates with silica gel 60 F_{254} (Merck Specialties Pvt. Ltd., Mumbai, India) were used as the stationary phase for HPTLC analysis.

III. EXPERIMENTAL METHODS

➢ *Method Development*

- *Preparation Of Standard Stock Solution*

The drug Vigabatrin was weighed (10 mg) into a 10 ml standard flask. It was dissolved and made up to volume with methanol (1000 µg/ml).

- *Preparation Of Working Solution*

From the stock solution 1ml was pipetted out and transferred to 10 ml standard flask and made up to the volume with methanol to obtain the final concentration of 100µg/ml. This is used for treating with ninhydrin in order to develop two methods and quantify the drug.

- *Preparation of Ninhydrin Solution.*

The 0.2%w/v Solution of ninhydrin was prepared by dissolving 0.02g of ninhydrin in 10 ml of ethanol.

- *HPTLC Method*

The mobile phase, stationary phase was selected by trial and error method for derivatized vigabatrin, after scanning method was validated.

- *Derivatization Procedure*

To the suitable volumes of vigabatrin working standards, addition of optimized volumes of phosphate buffer and ninhydrin reagent were done. After heating, cooling, and dilution to volume, 1 µL of the solution (30 ng/band) was applied to a TLC plate and developed under optimized conditions. Also analysed by spectrophotometric method. The responses (absorbance/chromatogram) was recorded, and the vigabatrin content was calculated.

IV. METHOD VALIDATION

After the development of two methods the suitability of them was confirmed by studying.

A. Analytical method validation parameters, as described below:

➢ *Linearity*

Under optimized experimental conditions, linearity was established between absorbance and corresponding concentrations of the coloured product. The study was performed at five concentration levels (10–50 µg/mL). Calibration curves were constructed, and regression equations and correlation coefficients were calculated.

In HPTLC, Linearity was evaluated by applying aliquots of 0.5 µL,0.6 µL,0.8 µL,1.0 µL,1.2 µL,1.4 µL and1.6µL of vigabatrin stock solution (100 µg/mL) onto precoated TLC plates to obtain concentration levels of 15 ng/band,18 ng/band,24 ng/band,30 ng/band,36 ng/band,42 ng/band, and 48ng/band. Plates were developed under optimized chromatographic conditions and scanned to construct the calibration curve.

➢ *Precision*

Precision of two methods was studied by Intra-day, Inter-day and Repeatability.

- Intra-day precision was evaluated by analyzing standard solutions at two concentration levels three times on the same day, and %RSD was calculated.

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- Inter-day precision was assessed by analyzing the same concentrations three times on two different days, and %RSD was calculated.
- Repeatability was determined by analyzing a single concentration six times on the same day, and %RSD was calculated.

➢ *Accuracy*

Accuracy was evaluated by recovery studies at the 50% level by spiking a known amount of pure vigabatrin into the pre-analysed formulation. Percent recovery was calculated from the difference between the amount of drug recovered after standard addition and the amount originally present, relative to the amount of vigabatrin added.

$$\% \text{ Recovery} = \frac{\text{Amount of drug found after addition} - \text{Amount found before addition of the standard drug}}{\text{Amount of standard drug added}}$$

➢ *Limit of Detection (LOD) and Limit of Quantification (LOQ)*

The LOD and LOQ were calculated using the following equations:

$LOD = 3.3\sigma/S$

$LOQ = 10\sigma/S$

where σ is the standard deviation of the intercept and S is the slope of the calibration curve.

➢ *Stability Studies*

The stability of the developed colored solution was evaluated by storing solutions in the concentration range of 10–50 µg/mL at room temperature for 24 hours. Absorbance was monitored to assess colour stability over time.

Stability of vigabatrin on the chromatographic plate was evaluated by storing the developed plate at room temperature for 2 hours. The plate was rescanned, and peak areas were compared with those of freshly scanned plates.

➢ *Robustness*

Robustness was assessed by introducing small, deliberate variations in the detection wavelength and evaluating their effect on analytical performance.

Robustness for HPTLC method was examined by introducing small, deliberate variations in mobile phase composition, chamber saturation time, and development distance, and evaluating their effect on chromatographic performance.

B. Application of the Developed Methods to Formulation

The developed method was applied to the analysis of a marketed tablet formulation containing 10 mg of vigabatrin. Ten tablets were weighed, finely powdered, and an amount equivalent to 10 mg of vigabatrin was transferred to a 10 mL volumetric flask, diluted with methanol, and sonicated for 30 minutes. The solution was filtered, and suitable dilutions were prepared. Optimized volumes of phosphate buffer and ninhydrin reagent were added, followed by heating, cooling, and dilution to volume with methanol. The visible spectrum was recorded, and the vigabatrin content was determined using the calibration curve.

In HPTLC, after heating, cooling, and dilution to volume, 1 µL of the solution (30 ng/band) was applied to a TLC plate and developed under optimized conditions. The chromatogram was recorded, and the vigabatrin content was calculated.

V. RESULTS AND DISSCUSSION

A. Method Development

The following sections present the method development of visible spectrophotometric, colorimetric, and HPTLC techniques for vigabatrin analysis.

➢ *Visible Spectroscopic Method*

A visible spectrophotometric method for the determination of vigabatrin was developed based on derivatization with ninhydrin. Preliminary solubility studies were carried out using different solvents, and methanol was selected as the solvent due to its good solubilizing capacity and stability for the drug. The derivatization conditions were systematically optimized to achieve maximum color intensity and stability of the colored complex. Phosphate buffer concentration, ninhydrin reagent volume, heating temperature, and reaction time were evaluated, and optimum conditions were selected to ensure reproducible and sensitive measurements.

The spectrophotometric detection wavelength was selected by scanning the derivatized vigabatrin solution in the visible region. The overlain spectrum exhibited maximum absorbance at 572 nm, which was chosen as the analytical wavelength for further studies. Under the optimized conditions, the derivatized drug showed consistent absorbance, confirming the suitability of the developed colorimetric method for quantitative analysis.

➢ *Colorimetric Reaction Mechanism*

Vigabatrin contains a primary aliphatic amine and a carboxylic acid group but lacks an inherent chromophoric system. Therefore, the proposed method is based on derivatization through the reaction of the primary amine group of vigabatrin with ninhydrin, a reagent widely used for the detection and quantification of amino acids, peptides, and proteins.

Upon treatment with ninhydrin, vigabatrin undergoes a series of characteristic reactions. Initially, the primary α-amino group of vigabatrin undergoes oxidative deamination and decarboxylation in the presence of ninhydrin, resulting in

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the release of ammonia (NH_3), carbon dioxide (CO_2), and the corresponding aldehyde, pent-4-enal. During this process, ninhydrin is reduced to hydrindantin. Subsequently, the liberated ammonia reacts with another molecule of ninhydrin and hydrindantin to form a highly conjugated purple-colored chromophore known as Ruhemann's purple (diketohydrindylidene-diketohydrindamine). The proposed reaction mechanism is illustrated in Figure 2.

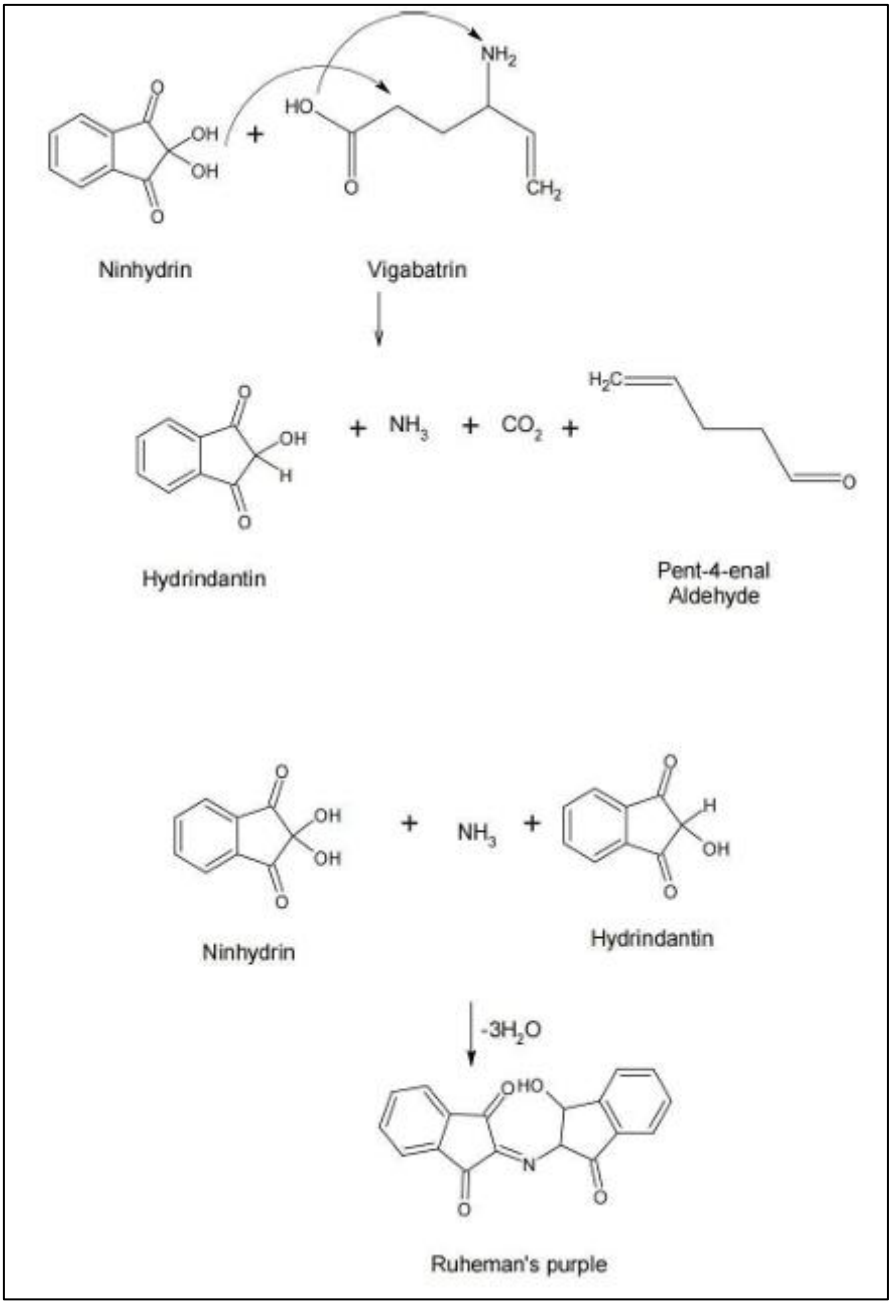

Fig 2. Scheme of Vigabatrin, Ninhydrin Complex Formation

➢ *HPTLC Method*

In HPTLC method for vigabatrin, a pre-coated silica gel $60F_{254}$ plate was used as the stationary phase, and methanol was selected as the solvent for sample preparation. Several mobile phase systems were investigated to achieve optimal separation, and an acetone–chloroform system was finalized based on spot compactness and resolution. The optimized chromatographic conditions produced well-defined and reproducible spots, and densitometric detection was carried out at 572 nm after derivatization, demonstrating the applicability of the developed HPTLC method for routine analysis. A typical densitogram of vigabatrin retained in TLC plate shown in Fig 3.

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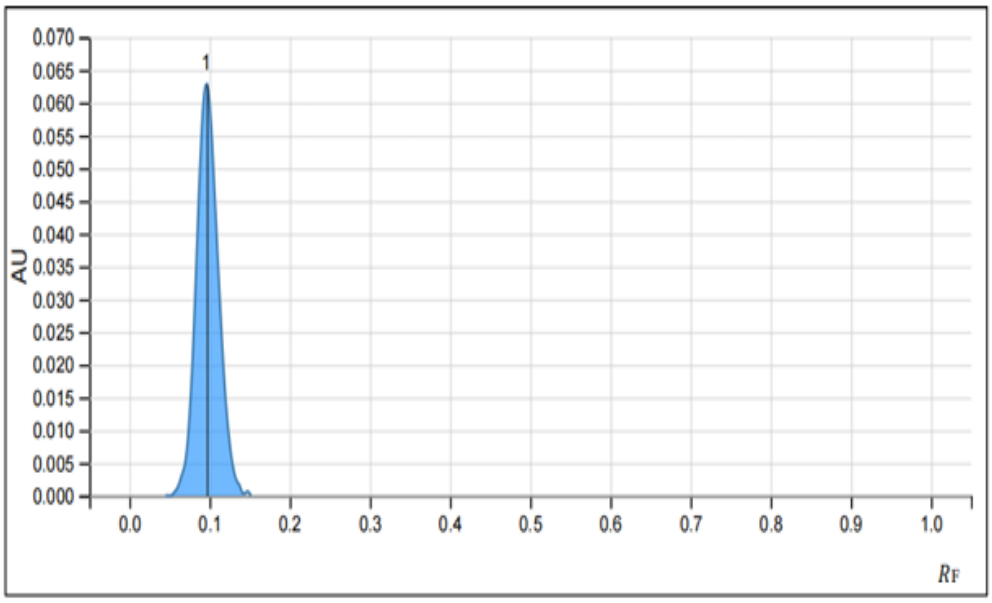

Fig 3. HPTLC Standard Chromatogram of Vigabatrin (48 ng/band) Recorded Under Optimized Chromatographic Conditions.

B. Method validation

➢ *Linearity*

Under optimized conditions, a linear relationship was observed between the absorbance of derivatized vigabatrin and its concentration over the range of 10–50 µg/mL. Linearity was evaluated using five concentration levels, and the regression equation and correlation. The overlay visible spectra is shown in Fig .4.

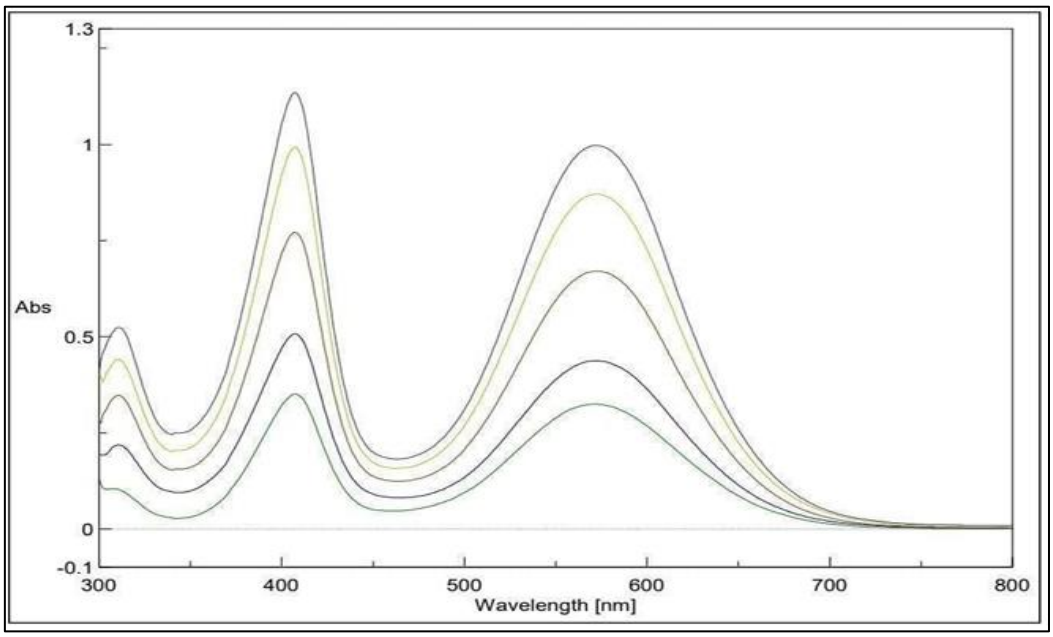

Fig 4. Overlay visible spectra of Vigabatrin standards (10-50µg/ml)

In HPTLC Linearity was assessed by constructing a calibration curve (Fig 5) over the concentration range of 15–48 ng/band. The regression equation obtained was $y = 4.839 \times 10^{-8}x - 3.294 \times 10^{-4}$, with a correlation coefficient (r) of 0.998, indicating good linearity between peak area and concentration of vigabatrin.

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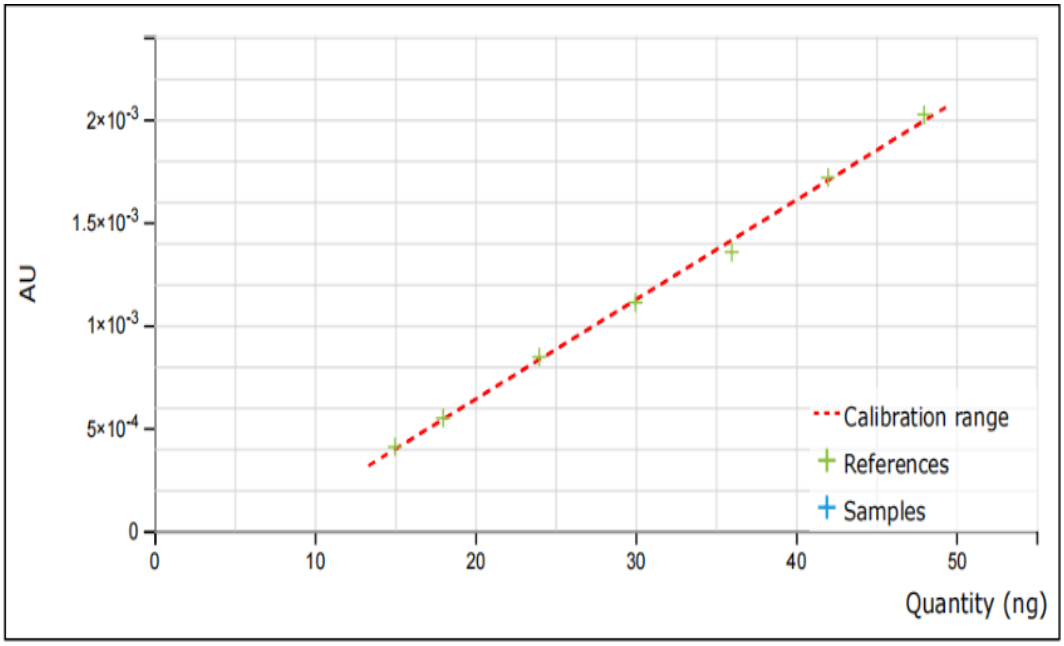

Fig 5. Calibration Curve of Vigabatrin (15-48ng/band)

➢ *Precision*

The %RSD values obtained for intra-day and inter-day precision, as well as repeatability studies of vigabatrin, were within 2%, confirming the precision of the developed method.

➢ *Accuracy*

Recovery studies were performed, and the percentage recovery along with the percentage relative standard deviation (%RSD) of the recovery values were calculated. Accuracy data is presented acceptable %RSD less than 1.5 and value ranged from 95-99%.

➢ *LOD & LOQ*

The sensitivity of the visible method was established by determining the limit of detection (LOD) and limit of quantification (LOQ). For vigabatrin, the LOD and LOQ were found to be 3.16 µg/mL and 9.58 µg/mL, respectively.

The sensitivity of the HPTLC method was established by determining the limit of detection (LOD) and limit of quantification (LOQ) using the regression equation. The LOD and LOQ for vigabatrin were found to be 1.14 ng/band and 8.57 ng/band, respectively.

➢ *Stability*

In visible spectroscopic method, stability of the colour solutions was found to be stable for 22hours.

When the developed chromatographic plate is exposed to the atmosphere, the analyte may undergo decomposition. Therefore, it was necessary to evaluate the stability of the developed plate. The chromatographic plate was found to be stable for up to 2 hours under atmospheric conditions.

➢ *Robustness*

The visible method was demonstrated to be robust, as slight deliberate variations in experimental conditions did not significantly affect the absorbance values or their %RSD. Robustness was evaluated using a vigabatrin concentration of 30 µg/ml.

The HPTLC method was confirmed to be robust, as slight deliberate variations in experimental conditions did not significantly affect the Rf values or the %RSD of peak areas. Robustness was evaluated using 30 ng/band of vigabatrin.

C. Application of the Developed Method to Formulation

The proposed method was successfully applied to formulation. The amount of tablet was calculated by two methods is shown in Table 1.

Table 1. Result of Formulation Analysis

Drug	**Amount Labelled (mg/tab)**	**Amount Estimated (mg/tab)**		**% Label claim**		**%RSD**
		Visible	**HPTLC**	**Visible**	**HPTLC**	
Vigabatrin	10	9.79	9.84	97.90	98.40	<1.30

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VI. CONCLUSION

In this article, simple and reliable visible spectrophotometric and HPTLC methods were described for the estimation of the non-chromophoric drug vigabatrin in pharmaceutical formulations using ninhydrin derivatization. The derivatization reaction produced a stable coloured complex, which enabled effective detection at 572 nm. Both analytical methods showed good linearity within their respective concentration ranges and were validated in accordance with ICH guidelines, demonstrating acceptable accuracy, precision, sensitivity, robustness, and specificity. The proposed methods were successfully applied to the analysis of marketed tablet formulations, and the results obtained were in good agreement with the labelled claim. Hence, these methods can be considered suitable, economical, and convenient for routine quality control analysis of vigabatrin in pharmaceutical dosage forms.

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