



# Ft-IR Spectroscopic Approach for the Quantitative Analysis of few Commercial Drugs in Bulk and Pharmaceutical Formulations

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## Abstract

A simple, rapid, environmentally benign, and cost-effective Fourier Transform Infrared (FT-IR) spectroscopic method was developed and validated for the quantitative determination of Nateglinide (NTG), Tolcapone (TCP), and Topiramate (TPR) in bulk drug substances and pharmaceutical formulations. The proposed analytical approach is based on the relationship between drug concentration and the intensity of characteristic infrared absorption bands, where variations in concentration produce corresponding changes in absorbance, enabling quantitative estimation through calibration models.

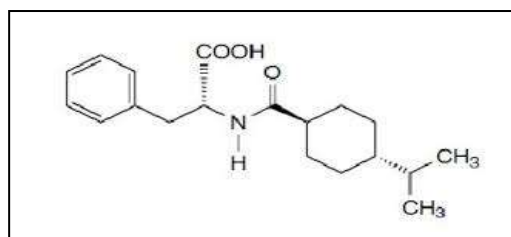
The method was optimized by selecting suitable absorption bands with high sensitivity and reproducibility for each drug. Quantitative analysis of the selected pharmaceuticals in commercial tablet formulations was successfully performed without interference from formulation excipients, demonstrating the method's selectivity. Validation studies were carried out following accepted analytical guidelines, and the method was evaluated for linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, and ruggedness. The developed procedure exhibited satisfactory analytical performance, with excellent linear correlations, low relative standard deviations, and acceptable recovery.

The calibration curves established for the selected drugs were found to be reliable for routine quantitative analysis of both pure drug samples and pharmaceutical dosage forms. The absence of extensive sample preparation, reduced solvent consumption, and shorter analysis time make the proposed FT-IR method a practical and sustainable alternative to conventional analytical techniques. Therefore, the method can be effectively employed for quality control and routine assay applications in pharmaceutical industries and analytical laboratories.

**Keywords:** Fourier Transform Infrared Spectroscopy (FT-IR), Nateglinide, Tolcapone, Topiramate, Quantitative Analysis, Pharmaceutical Formulations, Method Validation.

## 1. INTRODUCTION

### 1.1. Nateglinide (NTG)

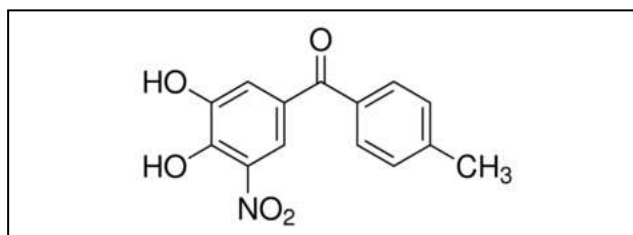


#### Structure of Nateglinide (fig.1)

Nateglinide (NTG), chemically known as *N-(trans-4-isopropylcyclohexanecarbonyl)-D-phenylalanine*, is an orally active antidiabetic drug used in the management of Type 2 Diabetes Mellitus [1,2]. It stimulates insulin secretion from pancreatic  $\beta$ -cells by blocking ATP-sensitive potassium channels, thereby helping to control postprandial blood glucose levels.

Several analytical methods, including spectrophotometry [3], RP-HPLC [4], and HPTLC [5], have been employed for the determination of Nateglinide in pharmaceutical formulations and biological samples. Despite their reliability, these techniques may involve complex procedures, costly instrumentation, and substantial solvent usage. Consequently, there is a need for simpler, rapid, and environmentally friendly analytical methods for routine quality control.

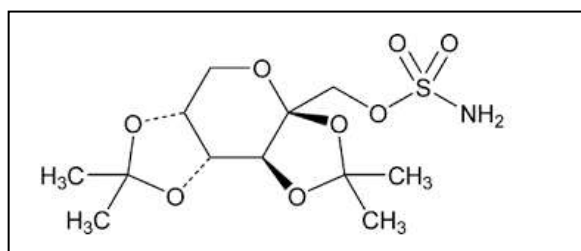
### 1.2Tolcapone: (TCP):



### Structure of Tolcapone (fig.2)

Tolcapone (TCP) is a reversible COMT inhibitor widely used in combination with levodopa/carbidopa for the treatment of Parkinson's disease. It increases dopamine availability by reducing its metabolic degradation, thereby enhancing therapeutic efficacy [6,7]. Quantitative determination of TCP has been reported using techniques such as RP-HPLC [8], HPLC–MS/MS [9], and Geomatrix technology [10].

### 1.3 Topiramate (TPR):



### Structure of Topiramate (fig.3)

Topiramate (TPR) is a broad-spectrum anticonvulsant drug, chemically designated as 2,3:4,5-bis-O-(1-methylethylidene)-β-D-fructopyranose sulfamate. It is widely used in the treatment of epilepsy, migraine prophylaxis, Lennox–Gastaut syndrome, and other neurological disorders [11,12]. Quantitative analysis of TPR has been reported using UV spectrophotometry [13], HPLC [14 344], HPTLC [15], colorimetric methods [16], and LC–MS/MS [17].

### 2. ABOUT THE METHOD

The quantitative application of FT-IR spectroscopy is based on the proportional relationship between drug concentration and the intensity of characteristic vibrational absorption bands. This relationship enables the construction of calibration models for accurate drug quantification.

FT-IR spectroscopy offers several advantages, including rapid analysis, high accuracy, minimal sample preparation, and low sample consumption. Pharmaceutical samples in various physical forms can be analysed within a short time while maintaining excellent wavelength precision and spectral resolution. Furthermore, quantitative evaluation can be performed using spectral parameters such as peak area, peak height, or peak ratio, allowing reliable determination of analytes in pharmaceutical formulations [19–23]

## EXPERIMENT

**3.1 Instrumentation:** Infrared spectra were recorded using a **SHIMADZU Prestige-21 Fourier Transform Infrared (FT-IR) spectrophotometer** equipped with NaCl cells of 0.1 mm path length. Sample weighing was performed using a **Dhona 200 single-pan electronic balance**.

**3.2 Materials and Reagents:** Spectroscopic-grade chloroform was used throughout the study as the solvent. All chemicals and reagents employed were of analytical-reagent grade and used without further purification.

**3.3 Preparation of Standard Drug Solutions:** Standard stock solutions of Nateglinide (NTG), Tolcapone (TCP), and Topiramate (TPR) were prepared by accurately weighing 2.0 g of each drug and transferring it separately into 100 mL volumetric flasks. The contents were dissolved and diluted to volume with chloroform to obtain the stock solutions. Appropriate aliquots of these solutions were further diluted with the same solvent to prepare working standard solutions of the desired concentrations.

## 4. PROCEDURE

Aliquots of the standard working solutions equivalent to 1–10 mL were transferred into a series of 10 mL volumetric flasks and diluted to volume with chloroform. Each prepared solution was introduced into a 0.1 mm NaCl cell using a syringe, and the FT-IR spectrum was recorded against a reagent blank after allowing the solution to equilibrate for approximately 2 min. Spectra were obtained at different concentration levels to evaluate the analytical response.

Calibration curves were constructed by correlating drug concentration with the corresponding spectral response (absorbance) at the selected characteristic absorption bands. Quantitative analysis was performed using the regression equations derived from the calibration plots, following the Beer–Lambert relationship.

## 5. ASSAY OF PURE DRUG SAMPLE

To evaluate the accuracy and precision of the proposed FT-IR method, standard solutions containing Nateglinide (NTG), Tolcapone (TCP), and Topiramate (TPR) within their respective Beer–Lambert concentration ranges were

analyzed. The concentration ranges investigated were 1.6–16.0 mg mL<sup>-1</sup> for NTG, 2.8–28.0 mg mL<sup>-1</sup> for TCP, and 1.7–17.0 mg mL<sup>-1</sup> for TPR.

Calibration curves were established by plotting drug concentration against the corresponding absorbance values at the selected analytical bands. Each concentration level was analyzed in triplicate, and the average absorbance was used for data processing. Relative response values were calculated from the absorbance-to-concentration ratio, and calibration data exhibiting responses within 95–105% of the mean value were considered for regression analysis. The resulting calibration plots were subsequently employed for quantitative determination of the drugs.

### 5.1 Procedure For Assay Of Pure Drug

The accuracy and precision of the proposed FT-IR method were evaluated through recovery studies performed within the Beer–Lambert concentration range of each drug. Standard solutions at selected concentration levels were analyzed in triplicate, and the percentage recovery was calculated. The results are presented in Table 4.

Method precision was assessed by calculating the relative standard deviation (%RSD), while accuracy was determined from the recovery values obtained. The low %RSD values (< 2%) and satisfactory recoveries demonstrate the good precision, accuracy, and reliability of the proposed method for the quantitative determination of the studied drugs.

## 6. IR band assignment and construction of calibration curves

### 6.1 Nateglinide:

**6.1.1 Band assignment:** The IR spectrum of Nateglinide is shown in (Fig.5). From this spectrum it is clear that significant peaks are exhibited at 1236.37 cm<sup>-1</sup> (-C-O stretch (carboxylic acid)), 1386.82 cm<sup>-1</sup> (-C-H bending (alkane)), 1450.47 cm<sup>-1</sup> (-C-H bending (alkane)), 1498.69 cm<sup>-1</sup> (-C=C stretch (aromatic)), 1668.43 cm<sup>-1</sup> (-C=O stretch (amide)), 1718.58 cm<sup>-1</sup> (-C=O stretch (acid)), 1751.36 cm<sup>-1</sup> (-C=O stretch), 2860.43 cm<sup>-1</sup> (-C-H stretch (alkane)), 2931.80 cm<sup>-1</sup> (-C-H stretch (alkane)), 3010.88 cm<sup>-1</sup>, 3024.38 cm<sup>-1</sup> (-O-H stretch (carboxylic acid)), 3035.96 cm<sup>-1</sup> (-C-H stretch (aromatic)) and 3433.29 cm<sup>-1</sup> (-N-H stretch) and these frequencies may be assigned as follows:

### 6.1.2. Linearity observed frequencies and Calibration

The data related to frequency Vs Optical density at 5 mL concentration is tabulated below.

| Frequency (cm <sup>-1</sup> ) | 1386.82 | 1498.68 | 1718.58 | 3433.29 |
|-------------------------------|---------|---------|---------|---------|
| Optical density               | 0.231   | 0.261   | 0.319   | 0.263   |

From the spectral data analysis of 1-10 mL of drug solution, it was observed that optical densities of a few frequencies (4) exhibited linearity against drug concentration (Fig. 4)

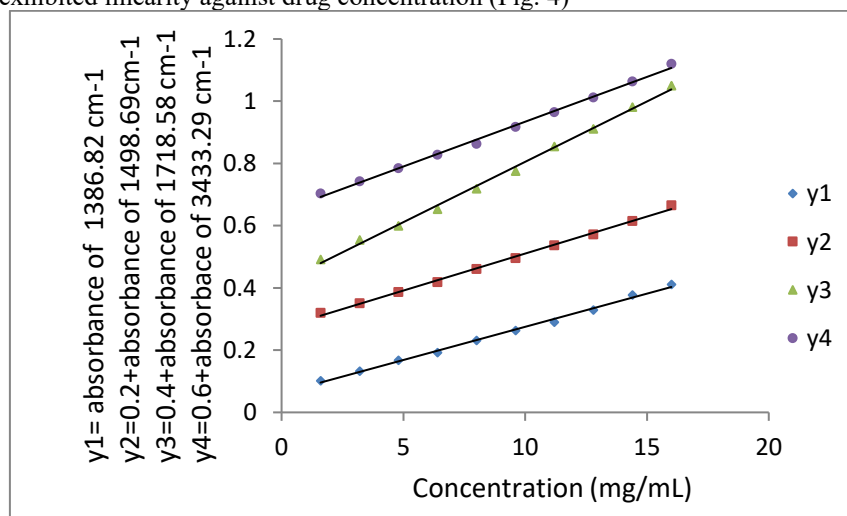


Fig.4. Calibration curves of Nateglinide

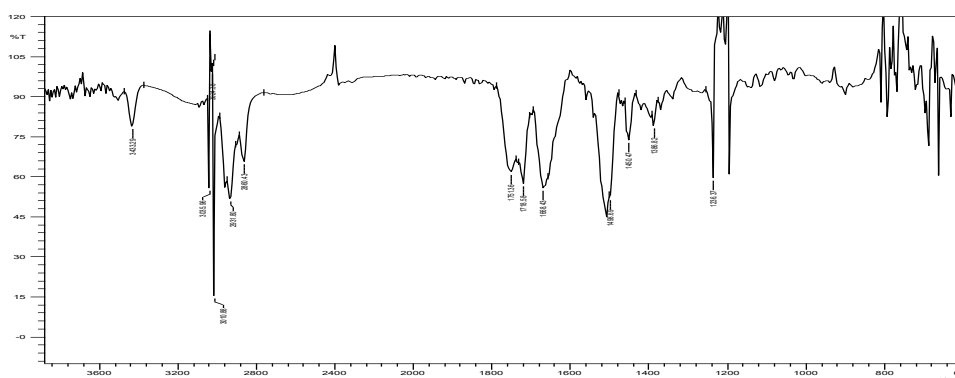


Fig.5. IR Spectrum of Nateglinide

## 6.2 Tolcapone

**Band assignment** The IR spectrum of Tolcapone is shown in (Fig:59). From this spectrum it is clear that significant peaks are exhibited at  $1109.07\text{cm}^{-1}$  (-C-O stretch (alcohol)),  $1182.36\text{cm}^{-1}$  (-C-O stretching),  $1298.09\text{cm}^{-1}$  (-C-O stretch(alcohol)),  $1342.46\text{cm}^{-1}$  (-N-O-stretch(nitro))  $1394.53\text{cm}^{-1}$  (-O-H bending (alcohol)),  $1450.47\text{cm}^{-1}$  (-C-H bending (alkane-methyl group)),  $1548.84\text{cm}^{-1}$  (-N-O stretching (nitro compound)),  $1625.99\text{cm}^{-1}$  (-C=C bending (aromatic)),  $1658.78\text{cm}^{-1}$  (-C=O stretch) and  $3545.16\text{cm}^{-1}$  (-O-H stretching (alcohol)) as marked by the instrument and these frequencies may be assigned as follows,

### a) b) Linearity observed frequencies and Calibration

The data related to frequency Vs Optical density at 5mL concentration is tabulated below

| Frequency( $\text{cm}^{-1}$ ) | 1109.07 | 1298.09 | 1342.46 | 1625.99 | 1658.78 | 3545.16 |
|-------------------------------|---------|---------|---------|---------|---------|---------|
| Optical density               | 0.2171  | 0.6427  | 0.4546  | 0.2262  | 0.2324  | 0.2149  |

From the spectral data analysis of 1-10 mL of drug solution, it was observed that optical densities of a few frequencies (6) exhibited linearity against drug concentration (fig.6)

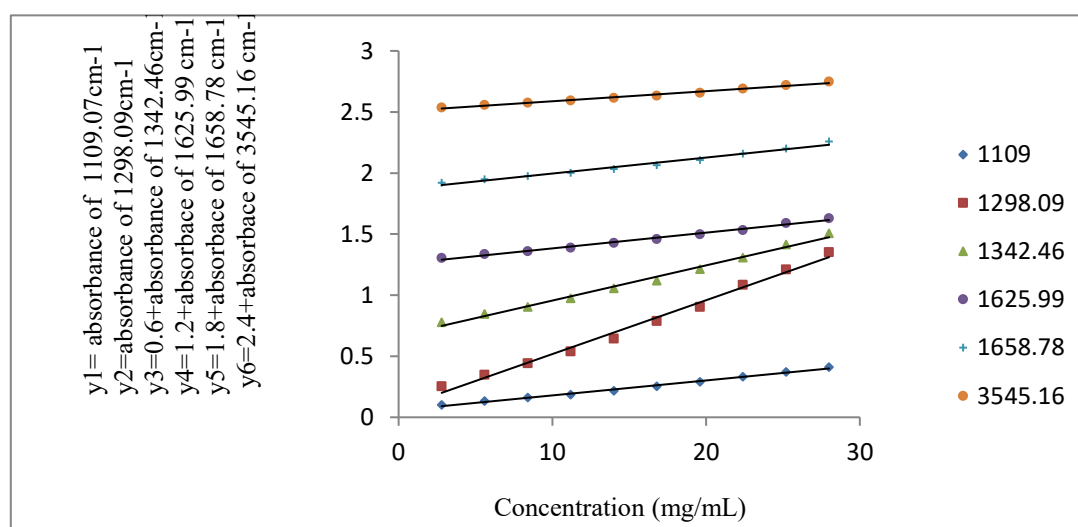


Fig.6. Calibration Curves of Tolcapone

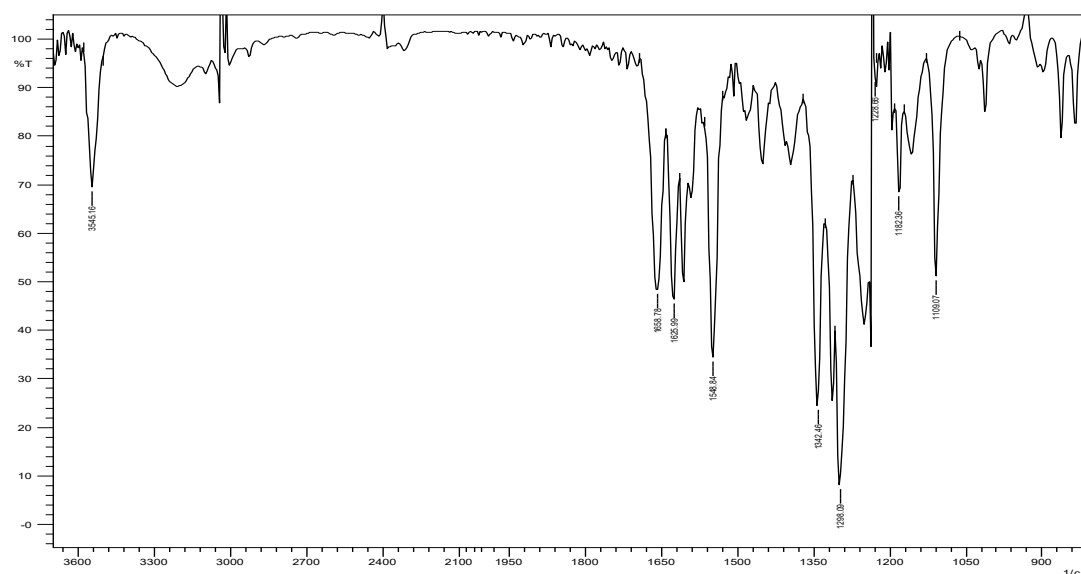


Fig.7. IR Spectrum of Tolcapone

## 6.3 Topiramate:

### Band assignment

The IR spectrum of Topiramate is shown in (Fig. 3). From this spectrum it is clear that significant peaks are exhibited at  $887.26\text{cm}^{-1}$  (-C-H bending),  $1074.35\text{cm}^{-1}$  (-S=O stretching),  $1180.44\text{cm}^{-1}$  (-C-O stretch),  $1384.89\text{cm}^{-1}$

<sup>1</sup> (-C-H bending (alkane) gem dimethyl), 2939.52cm<sup>-1</sup> (-C-H stretching (alkane)), 2995.45cm<sup>-1</sup> (-C-H stretching (alkane)), 3354.21cm<sup>-1</sup> (-N-H stretching (primary amine)) and 3446.79cm<sup>-1</sup> (-N-H stretching (primary aliphatic amine)) as marked by the instrument and these frequencies may be assigned as follows,

### 6.3.2 Linearity observed frequencies and Calibration

The data related to frequency Vs Optical density at 5mL concentration is tabulated below:

|                              |         |         |         |         |
|------------------------------|---------|---------|---------|---------|
| Frequency(cm <sup>-1</sup> ) | 1074.35 | 1384.89 | 2939.52 | 3354.21 |
| Optical density              | 0.862   | 0.851   | 0.241   | 0.764   |

From the spectral data analysis of 1-10 mL of drug solution, it was observed that optical densities of a few frequencies (5) exhibited linearity against drug concentration (Fig.8).

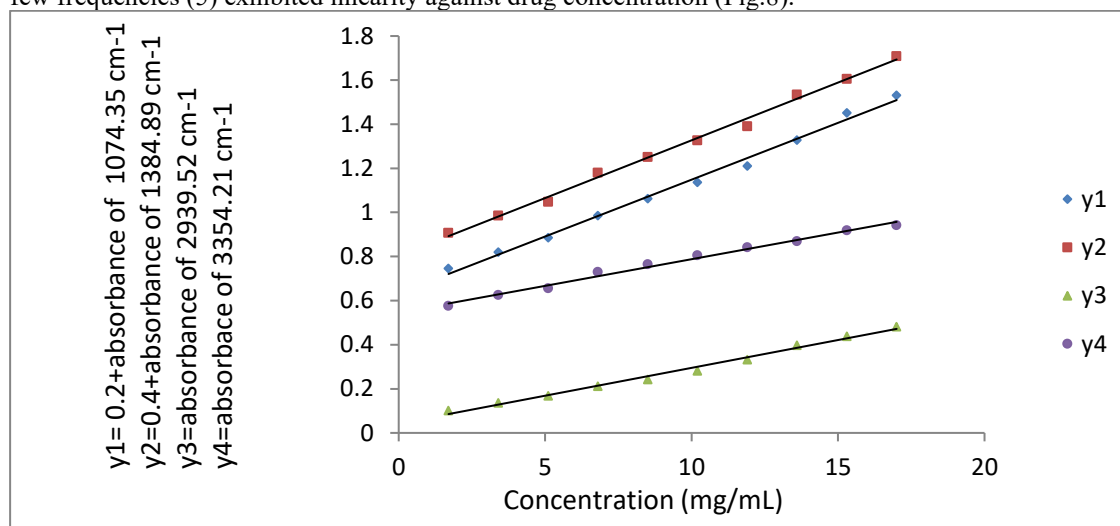


Fig.8. Calibration Curves of Topiramate

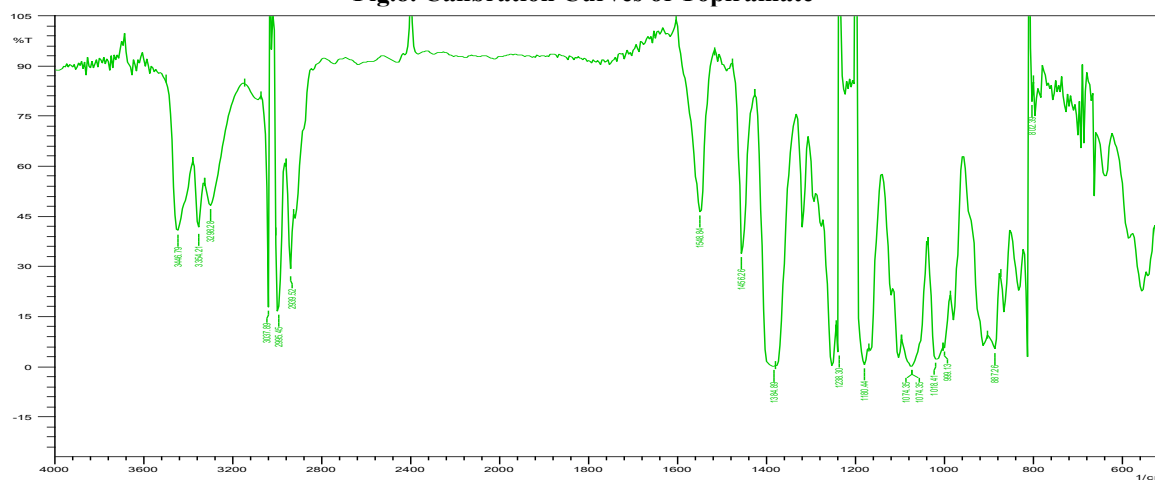


Fig. 9. IR Spectrum of Topiramate

## 7. PROCEDURE FOR ANALYSIS OF TABLETS

**7.1 Nateglinide:** For the assay of tablet formulations, three Glinat® tablets (120 mg NTG per tablet) were powdered and mixed thoroughly. An accurately weighed portion of the powder equivalent to 300 mg of Nateglinide was transferred to a 100 mL volumetric flask, extracted with chloroform, filtered, and diluted to volume with the same solvent to obtain the stock sample solution.

**7.2 Tolcapone:** For the analysis of Tolcapone tablet formulations, three tablets containing 100 mg of Tolcapone each were finely powdered and mixed thoroughly. An accurately weighed portion of the powder equivalent to 250 mg of Tolcapone was transferred into a 100 mL volumetric flask and extracted with chloroform. The resulting solution was filtered, and the filtrate was diluted to volume with the same solvent to obtain the stock sample solution for subsequent analysis.

### 7.3 Topiramate:

For the analysis of Topiramate tablet formulations, three Topamate® tablets containing 100 mg of Topiramate each were finely powdered and mixed thoroughly. An accurately weighed portion of the powder equivalent to 250 mg of Topiramate was transferred into a 50 mL volumetric flask and extracted with chloroform. The resulting

solution was filtered, and the filtrate was diluted to volume with the same solvent to obtain the stock sample solution for subsequent analysis.

## 8. Method Of Validation

The proposed FT-IR methods were validated with respect to linearity, accuracy, precision, selectivity, limit of detection (LOD), limit of quantification (LOQ), and ruggedness. Calibration curves were constructed by plotting absorbance against concentration within the respective Beer–Lambert ranges of the drugs.

Method precision was evaluated by performing six replicate analyses at selected concentration levels, while accuracy was assessed through recovery studies. The percentage recovery and relative standard deviation (%RSD) values obtained demonstrated satisfactory accuracy and precision, with %RSD values below 2% for all the investigated drugs (Table 2).

The limits of detection and quantification were calculated based on the standard deviation of the intercepts and the slope of the corresponding calibration curves using the following equations:

$$\text{LOD} = 3.3\sigma/S$$

$$\text{LOQ} = 10\sigma/S$$

where  $\sigma$  represents the standard deviation of the intercept ( $n = 6$ ) and  $S$  is the slope of the calibration plot.

The selectivity of the method was evaluated by analyzing pure drug samples in the presence of commonly used pharmaceutical excipients. Recovery studies indicated that the excipients did not interfere with the determination of the drugs, confirming the selectivity of the proposed methods.

Ruggedness was assessed by performing the analysis using different analysts and instruments. The results showed no significant variation in absorbance values, indicating that the developed methods are reliable and reproducible under normal laboratory conditions.

## 9. Factors Effecting Absorbance

### 9.1 Effect of concentration of Drug

The influence of drug concentration on the FT-IR response was investigated by preparing a series of solutions of varying concentrations in chloroform. The corresponding spectra were recorded over the range of 600–4000  $\text{cm}^{-1}$ , and calibration plots were constructed by relating absorbance to concentration. A linear relationship was observed within a specific concentration range, beyond which deviations from Beer–Lambert behavior occurred. The concentration ranges exhibiting satisfactory linearity were selected for subsequent analysis and used for the preparation of standard stock solutions of Nateglinide (Table 1), Tolcapone (Table 2), and Topiramate (Table 3).

## 10. ANALYSIS OF PHARMACEUTICALS

The applicability of the proposed FT-IR methods was evaluated by analyzing commercial tablet formulations containing the selected drugs within their respective Beer–Lambert concentration ranges. Each assay was performed in six replicates to assess method precision. Accuracy was determined through recovery studies, and the results were expressed as percentage recovery and relative standard deviation (%RSD). The satisfactory recovery values and low %RSD (<2%) obtained for all formulations confirmed the accuracy, precision, and suitability of the proposed methods for routine pharmaceutical analysis (Table 5). The results further demonstrated the absence of interference from formulation excipients, highlighting the reliability of the developed methods for quality control applications.

## 11. Results And Discussion

Among the IR absorption bands investigated for NTG, TCP, and TPR, only selected frequencies exhibited satisfactory linearity and were utilized for quantitative determination. The remaining bands showed scattered responses, possibly due to intermolecular interactions, overlapping bands, and differences in vibrational modes, including bending and combination vibrations, which may influence the spectral response.

## 12. ANALYTICAL DATA

A good linear relationship between absorbance and concentration was observed within the selected Beer–Lambert ranges for all the investigated drugs. Analytical performance characteristics, including Sandell's sensitivity, limit of detection (LOD), and limit of quantification (LOQ), were calculated in accordance with ICH guidelines [24] and are summarized in Tables 1–3. The low LOD and LOQ values obtained indicate the high sensitivity of the proposed FT-IR methods. Furthermore, regression analysis of the calibration data was performed using the least-squares method, and the corresponding regression parameters, namely slope ( $b$ ), intercept ( $a$ ), and correlation coefficient ( $r$ ), are also presented in Tables 1–3.

**Table 1: Analytical and Validation Parameters for the Quantitative Determination of Nateglinide by FT-IR Spectroscopy at Selected Absorption Bands**

| Parameters<br>/ $\nu$ ( $\text{cm}^{-1}$ )  | 1386.82                 | 1498.69                | 1718.58                 | 3433.29                 |
|---------------------------------------------|-------------------------|------------------------|-------------------------|-------------------------|
| Beer's Law Limits (mg<br>$\text{mL}^{-1}$ ) | 1.6-16                  | 1.6-16                 | 1.6-16                  | 1.6-16                  |
| Molar Absorptivity                          | $0.2023553 \times 10^2$ | $0.238065 \times 10^2$ | $0.2003714 \times 10^2$ | $0.2043391 \times 10^2$ |

|                                                   |                    |                    |                    |                    |
|---------------------------------------------------|--------------------|--------------------|--------------------|--------------------|
| (L mol <sup>-1</sup> cm <sup>-1</sup> )           |                    |                    |                    |                    |
| Sandell's sensitivity (mg cm <sup>-2</sup> )      | 0.047619           | 0.043478           | 0.026316           | 0.034483           |
| LOD (mg mL <sup>-1</sup> )                        | 0.222234           | 0.101454           | 0.18422            | 0.173822           |
| LOQ (mg mL <sup>-1</sup> )                        | 0.673435           | 0.307438           | 0.558242           | 0.526733           |
| Slope,b                                           | 0.012              | 0.023              | 0.038              | 0.029              |
| Intercept,a                                       | 0.061              | 0.072              | 0.021              | 0.042              |
| Correlation Coefficient (R)                       | 0.997998           | 0.998499           | 0.997998           | 0.996995           |
| Standard Deviation of Intercept (S <sub>a</sub> ) | 0.001414           | 0.000707           | 0.002121           | 0.001528           |
| Standard Deviation of Slope (S <sub>b</sub> )     | 0.140714           | 0.001414           | 0.000707           | 0.002082           |
| Regression Equation<br>Y=a+bx                     | y = 0.021x + 0.061 | y = 0.023x + 0.072 | y = 0.038x + 0.021 | y = 0.029x + 0.042 |

**Table 2: Analytical and Validation Parameters for the Quantitative Determination of Tolcapone by FT-IR Spectroscopy at Selected Absorption Bands**

| Parameters / $\nu$ (cm <sup>-1</sup> )                     | 1109.07                     | 1298.09                    | 1342.46                   | 1548.84                 | 1625.99                    | 1658.78                    | 3545.16                   |
|------------------------------------------------------------|-----------------------------|----------------------------|---------------------------|-------------------------|----------------------------|----------------------------|---------------------------|
| Beer's Law Limits (mg mL <sup>-1</sup> )                   | 2.8-28                      | 2.8-28                     | 2.8-28                    | 2.8-28                  | 2.8-28                     | 2.8-28                     | 2.8-28                    |
| Molar Absorptivity (L mol <sup>-1</sup> cm <sup>-1</sup> ) | 0.09856301 X10 <sup>2</sup> | 0.2070799 X10 <sup>2</sup> | 17.42906 X10 <sup>2</sup> | 0.1456 X10 <sup>2</sup> | 0.1012955 X10 <sup>2</sup> | 0.1177877 X10 <sup>2</sup> | 0.132133 X10 <sup>2</sup> |
| Sandell's sensitivity (mg cm <sup>-2</sup> )               | 0.083333                    | 0.021739                   | 0.035714                  | 0.058824                | 0.083333                   | 0.076923                   | 0.125                     |
| LOD (mg mL <sup>-1</sup> )                                 | 0.477107                    | 0.32349                    | 0.623641                  | 0.593039                | 0.615124                   | 0.472409                   | 0.647419                  |
| LOQ (mg mL <sup>-1</sup> )                                 | 1.445779                    | 0.980272                   | 1.889822                  | 1.797089                | 1.864011                   | 1.431541                   | 1.961876                  |
| Slope,b                                                    | 0.012                       | 0.046                      | 0.028                     | 0.017                   | 0.012                      | 0.013                      | 0.008                     |
| Intercept,a                                                | 0.056                       | 0.044                      | 0.067                     | 0.083                   | 0.053                      | 0.065                      | 0.105                     |
| Correlation Coefficient R                                  | 0.996494                    | 0.998999                   | 0.99549                   | 0.996494                | 0.997497                   | 0.990454                   | 0.993479                  |
| Standard Deviation of Intercept (S <sub>a</sub> )          | 0.001735                    | 0.004509                   | 0.005292                  | 0.003055                | 0.002237                   | 0.001861                   | 0.00157                   |
| Standard Deviation of Slope (S <sub>b</sub> )              | 0.056294                    | 0.008737                   | 0.004163                  | 0.004163                | 0.003786                   | 0.002082                   | 0.002082                  |
| Regression Equation<br>Y=a+bx                              | y = 0.012x + 0.056          | y = 0.046x + 0.044         | y = 0.028x + 0.067        | y = 0.017x + 0.083      | y = 0.012x + 0.061         | y = 0.013x + 0.065         | y = 0.008x + 0.105        |

**Table 3: Analytical and Validation Parameters for the Quantitative Determination of Topiramate by FT-IR Spectroscopy at Selected Absorption Bands**

| Parameters / $\nu$ (cm <sup>-1</sup> )                     | 1074.35                  | 1384.89                  | 2939.52                   | 3354.21                  |
|------------------------------------------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
| Beer's Law Limits (mg mL <sup>-1</sup> )                   | 1.7-17                   | 1.7-17                   | 1.7-17                    | 1.7-17                   |
| Molar Absorptivity (L mol <sup>-1</sup> cm <sup>-1</sup> ) | 1.107907X10 <sup>2</sup> | 1.010092X10 <sup>2</sup> | 0.2016192X10 <sup>2</sup> | 1.149828X10 <sup>2</sup> |
| Sandell's sensitivity (mg cm <sup>-2</sup> )               | 0.010989                 | 0.019231                 | 0.04                      | 0.041667                 |
| LOD (mg mL <sup>-1</sup> )                                 | 0.550365                 | 0.479124                 | 0.349239                  | 0.210035                 |
| LOQ (mg mL <sup>-1</sup> )                                 | 1.667773                 | 1.451891                 | 1.058301                  | 0.636469                 |

|                                                        |                    |                    |                    |                    |
|--------------------------------------------------------|--------------------|--------------------|--------------------|--------------------|
| <b>Slope, b</b>                                        | 0.091              | 0.052              | 0.025              | 0.024              |
| <b>Intercept, a</b>                                    | 0.431              | 0.403              | 0.042              | 0.545              |
| <b>Correlation Coefficient (R)</b>                     | 0.993479           | 0.997497           | 0.99549            | 0.994987           |
| <b>Standard Deviation of Intercept (S<sub>a</sub>)</b> | 0.015177           | 0.00755            | 0.002646           | 0.001528           |
| <b>Standard Deviation of Slope (S<sub>b</sub>)</b>     | 0.260001           | 0.003512           | 0.023288           | 0.003055           |
| <b>Regression Equation Y=a+bx</b>                      | y = 0.091x + 0.431 | y = 0.052x + 0.403 | y = 0.025x + 0.042 | y = 0.024x + 0.545 |

\*Limit of determination as the weight in  $\mu\text{g}$  per mL of solution, which corresponds to an absorbance of  $A = 0.001$  measured in a cuvette of cross-sectional area 1 cm<sup>2</sup> and path length of 1 cm.  $Y^{**} = a + bX$ , where Y is the absorbance and X concentration of drugs in  $\mu\text{g}$  per mL.

### 13. ACCURACY AND PRECISION

The accuracy and precision of the methods were evaluated by analyzing the pure drug solution at 6 different levels by choosing them in working limits. The relative error (%) which is a measure of accuracy & RSD (%) a measure of precision are summarized in Table 2 and reveal the high accuracy and precision of the methods.

### 14. ROBUSTNESS AND RUGGEDNESS

Ruggedness of these developed methods were evaluated by performing same analysis with 3 different analysts and also performing analysis on 3 different spectrophotometers by the same analyst.

**Table 4: Evaluation of Accuracy and Precision of the Proposed FT-IR Method for Pure Drug Samples**

| Drug | Taken (mg/mL) | Found (mg/mL) | Error (%) | Recovery(%) | RSD (%) | Proposed method Mean $\pm$ SD |
|------|---------------|---------------|-----------|-------------|---------|-------------------------------|
| NTG  | 1.6           | 1.59          | 0.62      | 99.37       | 0.1813  | 99.48 $\pm$ 0.1804            |
|      | 3.2           | 3.19          | 0.31      | 99.68       |         |                               |
|      | 4.8           | 4.77          | 0.62      | 99.37       |         |                               |
| TCP  | 2.8           | 2.78          | 0.714     | 99.28       | 0.2739  | 99.58 $\pm$ 0.278             |
|      | 5.6           | 5.59          | 0.178     | 99.82       |         |                               |
|      | 8.4           | 8.37          | 0.357     | 99.64       |         |                               |
| TPR  | 1.7           | 1.69          | 0.588     | 99.41       | 0.1509  | 99.248 $\pm$ 0.149            |
|      | 3.4           | 3.37          | 0.882     | 99.11       |         |                               |
|      | 5.1           | 5.06          | 0.784     | 99.21       |         |                               |

### 15. APPLICATION TO FORMULATIONS

These developed methods were applied for the estimation of drugs in tablets.

The results in Table 5 reveal that the methods are successful for the determination of drugs and that the excipients in the dosage forms do not interfere. The results are compared to the available validated reported [1-18] methods on each drug and the results agree well with the claim and also are in agreement with the results obtained by the literature method.

Statistical evaluation results were used to test the accuracy by Student's t-test and precision by F-test (Table 6) which revealed no significant change was observed between the literature method and proposed method at the 95 % confidence level with reference to accuracy and precision.

Recovery experiments were carried out via standard addition technique to calculate the accuracy and validity of these developed methods. To a fixed and known amount/concentration of drug in tablet powder, pure drug was added at three levels (50, 100 and 150% of the level present in the tablet) and the total amount of drug was found by these developed methods. Each experiment was repeated six times and the percent recovery of pure drugs added (Table 5) was within the permissible limits showing the absence interference by the inactive ingredients in the assay.

**Table 5: Results of assay of tablets (NTG, TCP and TPR) by the proposed methods and statistical evaluation and recovery experiments by standard addition method**

|          | Glinatone (NTG)    | Tolcapone (TCP)    | Topamate (TPR)     |
|----------|--------------------|--------------------|--------------------|
| F-test*  | 0.0469<br>(4.7571) | 0.0341<br>(4.9503) | 0.1298<br>(4.7571) |
| t-test** | 1.6625<br>(3.182)  | 1.6904<br>(2.571)  | 0.2644<br>(3.182)  |



Table 6: F-test and t-test values

| Tablets         | Drug in Table<br>µg/mL | Drug added<br>µg/mL | Total found<br>µg/mL | Error (%) | Recovery (%) | RSD (%) | Reference method<br>Mean±SD | Proposed method<br>Mean±SD |
|-----------------|------------------------|---------------------|----------------------|-----------|--------------|---------|-----------------------------|----------------------------|
| Glinatone (NTG) | 0.50                   | 1.60                | 2.09                 | 0.48      | 99.52        | 0.217   | 99.76<br>±0.74              | 99.68<br>±0.216            |
|                 | 0.50                   | 3.20                | 3.69                 | 0.27      | 99.72        |         |                             |                            |
|                 | 0.50                   | 4.80                | 5.00                 | 0.00      | 100.0        |         |                             |                            |
|                 | 1.60                   | 0.00                | 1.59                 | 0.62      | 99.37        |         |                             |                            |
|                 | 3.20                   | 0.00                | 3.18                 | 0.31      | 99.68        |         |                             |                            |
|                 | 4.80                   | 0.00                | 4.79                 | 0.21      | 99.79        |         |                             |                            |
| Tolcapone (TCP) | 0.50                   | 2.80                | 3.30                 | 0.00      | 100.0        | 0.185   | 99.55<br>±0.59              | 99.75<br>±0.185            |
|                 | 0.50                   | 5.60                | 6.09                 | 0.16      | 99.83        |         |                             |                            |
|                 | 0.50                   | 7.40                | 7.88                 | 0.25      | 99.74        |         |                             |                            |
|                 | 2.80                   | 0.00                | 2.79                 | 0.36      | 99.64        |         |                             |                            |
|                 | 5.60                   | 0.00                | 5.59                 | 0.18      | 99.82        |         |                             |                            |
|                 | 7.40                   | 0.00                | 7.36                 | 0.54      | 99.45        |         |                             |                            |
| Topamate (TPR)  | 0.50                   | 1.70                | 2.18                 | 0.91      | 99.09        | 0.363   | 101.65<br>±0.42             | 99.33<br>±0.360            |
|                 | 0.50                   | 3.40                | 3.87                 | 0.77      | 99.23        |         |                             |                            |
|                 | 0.50                   | 5.10                | 5.59                 | 0.18      | 99.82        |         |                             |                            |
|                 | 1.70                   | 0.00                | 1.69                 | 0.59      | 99.41        |         |                             |                            |
|                 | 3.40                   | 0.00                | 3.36                 | 1.18      | 99.82        |         |                             |                            |
|                 | 5.10                   | 0.00                | 5.08                 | 0.39      | 99.60        |         |                             |                            |

\*t- test and \*\*F-test values from literature.

## 16. Conclusion

The present study described the successful development of new, simple, sensitive, selective, accurate cost-effective and rapid FT-IR spectroscopic method for the accurate determination of the NTG, TCP and TPR drugs in their pharmaceutical form. There is no interference from additives and excipients. The method thus can be used in the quantitative determination of these drugs in pure and pharmaceutical formulations. So, it is the good alternative to the reported methods for the quantitative determination of these drugs.

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