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ORIGINAL ARTICLE

# Quantification of erythromycin in pharmaceutical formulation by transmission Fourier transform infrared spectroscopy

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**Abstract** A simple, cost-effective and environmental friendly analytical method was developed for the quantification of erythromycin in tablet formulation using transmission Fourier Transform Infrared (FT-IR) spectroscopy for routine quality control analysis. There is no need of sample preparation except pellet formation for FT-IR analysis. Use of solvent was totally avoided in this method. Calibration was carried out by using simple Beer's law in the FT-IR region between 1743 and 1697 $cm^{-1}$. The excellent coefficient of determination ($R^2$ = 0.998) was achieved with 0.0247 and 1.14 root mean square error of prediction (RMSEP) and root mean square error of cross validation (RMSECV), respectively. The results of the study revealed that the transmission FT-IR spectroscopy could be effectively used for rapid determination of active ingredients like erythromycin in pharmaceutical formulations to control the quality of finished products.



## 1. Introduction

Erythromycin is a broad-spectrum macrolide antibiotic produced by a strain of *Streptomyces erythreus* (Amin and Issa, 1996). Structurally, erythromycin is a 14-membered lactone ring (Fig. 1) with ten asymmetric centers and two sugar molecules (L-cladinose and D-desoamine). It seems to be very difficult to produce erythromycin synthetically. Patients allergic to Penicillin are often treated with erythromycin due to its antimicrobial activity almost similar or slightly wider than Penicillin (Norouzi et al., 2009). It is also a very effective antibacterial drug often used for the treatment of pneumonia, throat, bronchitis and ear infections, as well as respiratory and urinary tract infections (Avramov Ivic et al., 2008).

A number of analytical techniques such as ultraviolet (UV), high performance liquid chromatography (HPLC), capillary electrophoresis, various electrochemical detections, near infrared (NIR) and liquid chromatography/mass spectrometry (LC/MS) have been applied for the determination and qualitative analysis of erythromycin in raw materials, dosage forms and biological samples. It is difficult to achieve high sensitivity with UV detection because erythromycin lacks a UV chromophore (Deubel et al., 2006). UV method has been developed for the determination of erythromycin by formation of a blue-colored complex with gentian violet in alkaline medium (Amin and

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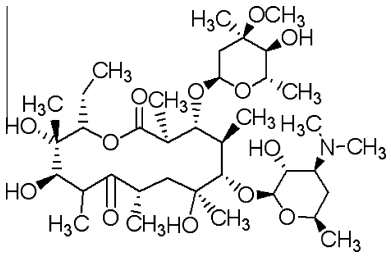


**Figure 1** Chemical structure of erythromycin.

lssa, 1996). The chromatographic methods need a large amount of solvents and costly reagents for derivatization to achieve better sensitivity as a few methods are reported (Torano and Guchelaar, 1998; Weitao et al., 2005). Then, capillary electrophoresis (CE) coupled with end-column electro chemiluminescence (ECL) detector for determination of erythromycin has also been used (Biyang et al., 2007) but CE has not been used widely in the pharmaceutical laboratories. Comparatively, being cheaper and faster than chromatography, electrochemical methods using the oxidation behavior for the determination of erythromycin with various types of electrodes have been reported (Norouzi et al., 2009; Avramov Ivic et al., 2008; Nagwa et al., 2004; Huaisheng et al., 2000; Yudi et al., 1993). The use of NIR spectroscopy for the determination of erythromycin by dissolving in the suitable solvent and also in solid state has been also described (Qu et al., 2007; Yan and Chang, 2006). Almost all of these established analytical methods are complicated, laborious and time consuming (Ventura et al., 2006). Furthermore, these require dissolution of the samples in the proper solvents and then often extraction is performed with organic solvents which are toxic to human health and environment. The pharmaceutical regulatory authorities for good manufacturing practices (GMP) always entail accurate and rapid analysis of finished pharmaceutical products, such as tablets and capsules, to quantify the active ingredient (Moros et al., 2007). However, with the FT-IR spectroscopy, the spectra could be recorded without any appreciable pretreatment. The FT-IR group of National Centre of Excellence in Analytical Chemistry (NCEAC) has already developed the methods using FT-IR spectroscopy for the determination of different quality parameters of oils and fat (Sherazi et al., 2007, 2009, 2011; Van.De voort et al., 2008). The main objective of the present study was the development of a rapid, cheap and environment friendly analytical method for the determination of Erythromycin in tablet formulations for routine quality control analysis, based on transmission FT-IR spectroscopy without using any solvent.

## 2. Experimental

### 2.1. Reagents and samples

Analytical grade Erythromycin (98%) used for calibration in the present study was obtained from Sigma Aldrich. Spectroscopic grade KBr was used for the preparation of sample pellets. The different commercially available tablet samples containing Erythromycin as an active ingredient were purchased from medical stores of local markets.

### 2.2. FT-IR spectral measurements

For the acquisition of infrared spectra of standards as well as samples in the tablet form, Thermo Nicolet 5700 FTIR spectrometer equipped with removable KBr optics and deuterated triglycine sulfate (DTGS) detector was used. The instrument was controlled by commercially available IR spectra analysis software package OMNIC (Thermo Nicolet Analytical Instruments, Madison, WI). All spectra were recorded averaging 32 scans in the range of 4000–400 $cm^{-1}$ at a resolution of 4 $cm^{-1}$. The spectrum of each standard as well as sample was ratioed against a fresh background spectrum recorded from KBr pellet.

### 2.3. FT-IR calibrations

A set of 16 standards of Erythromycin containing the range between 0.005 and 1.0 mg in KBr was prepared to formulate

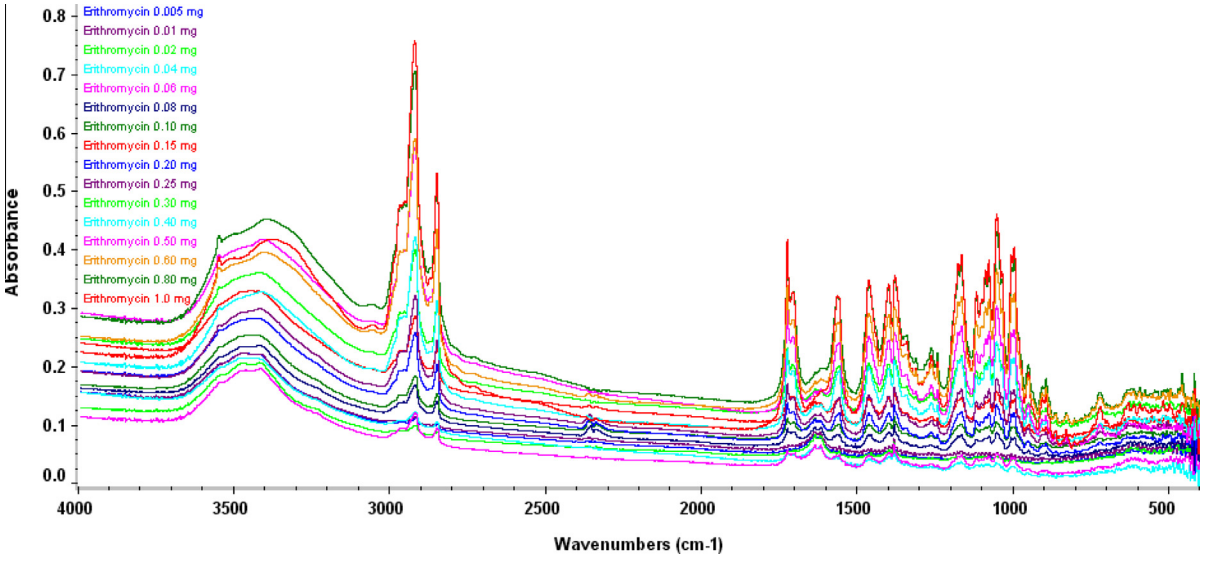



**Figure 2** FTIR spectra of erythromycin standards in mid infrared region (4000–400 $cm^{-1}$).

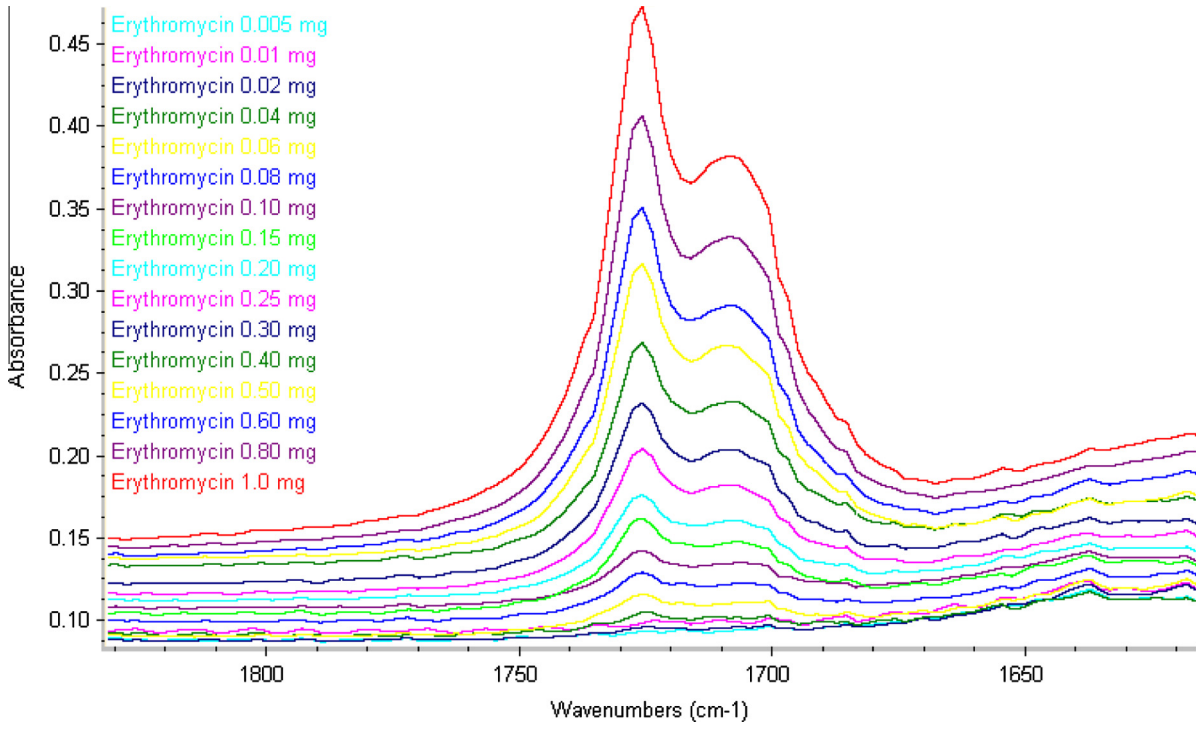



**Figure 3** The absorbance of carbonyl band of erythromycin standards in increasing concentrations.

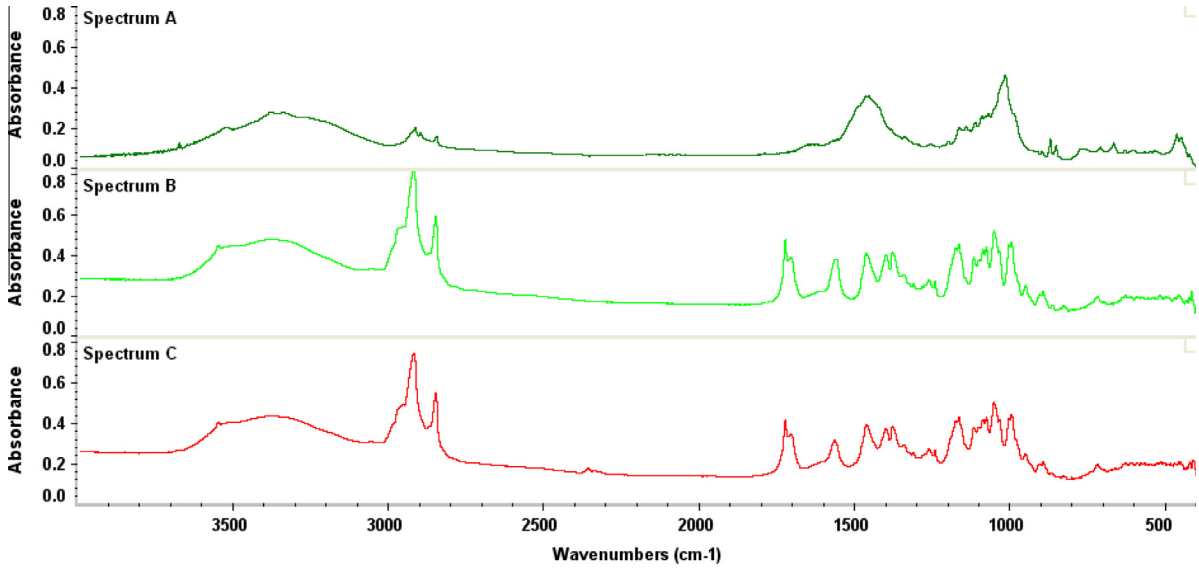
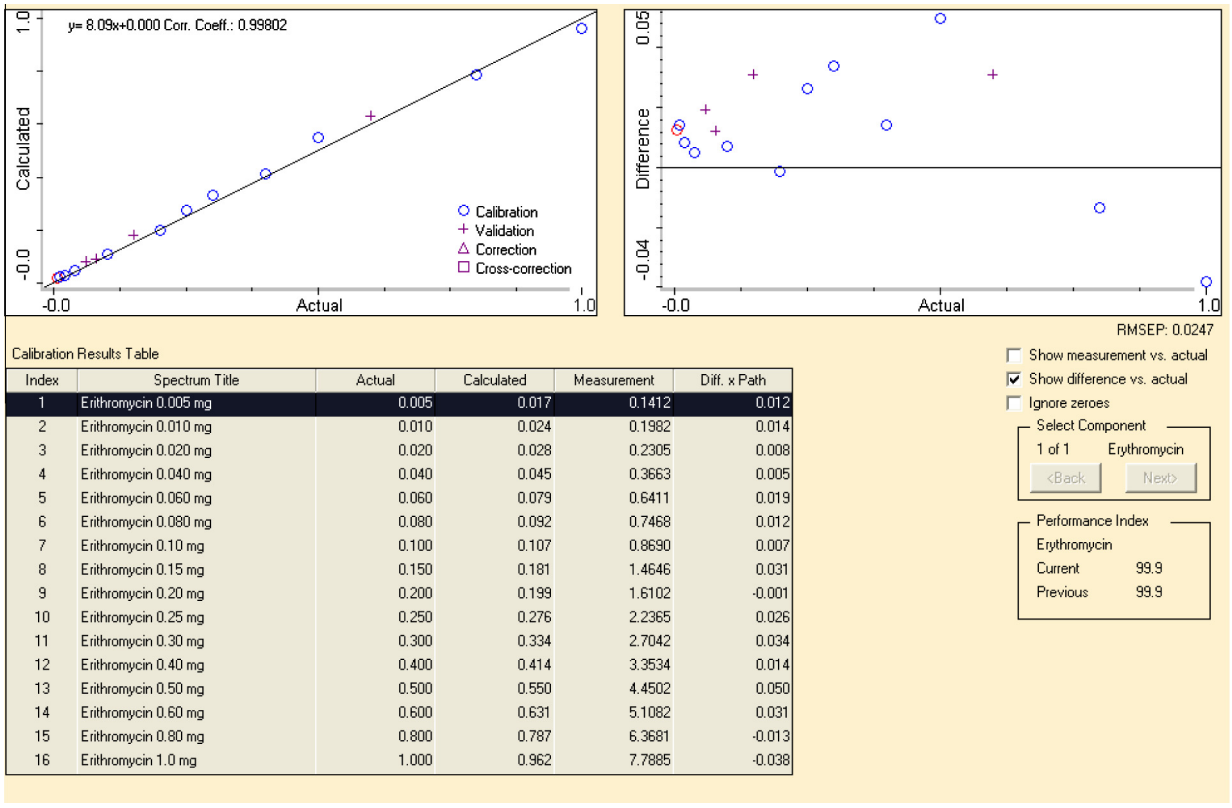



**Figure 4** Spectrum A represents the mixture of excipients (i.e. avecil, magnesium stearate, starch and talcum powder) used in solid erythromycin formulation, spectrum B is erythromycin standard and spectrum C represents Erythromycin tablet sample.

the 100 mg pellets of total weight. By the use of Omnic software and TQ Analyst program, simple Beer's law method was developed for the quantitative determination of Erythromycin in tablet formulations. In the TQ Analyst program Erythromycin standards spectra ranging from 0.005 mg to 1.0 mg were selected, that were already recorded. Specific region of ketone band i.e. 1743–1697 $cm^{-1}$ was specified in the TQ Analyst program, 2004 (Thermo Electron Corporation, Madison WI, USA) for best results. Area under the band for all Erythromycin standards was calculated by the software package itself which saved plenty of time and labor to compute the band area of each standard. A very good calibration was also generated with excellent regression using the TQ Analyst software.

### *2.4. Sample preparation procedure*

In this method no prior sample preparation is required for FTIR run except grinding. The tablet samples were weighed




Calibration Results Table

| Index | Spectrum Title | Actual | Calculated | Measurement | Diff. x Path |
|---|---|---|---|---|---|
| 1 | Erithromycin 0.005 mg | 0.005 | 0.017 | 0.1412 | 0.012 |
| 2 | Erithromycin 0.010 mg | 0.010 | 0.024 | 0.1982 | 0.014 |
| 3 | Erithromycin 0.020 mg | 0.020 | 0.028 | 0.2305 | 0.008 |
| 4 | Erithromycin 0.040 mg | 0.040 | 0.045 | 0.3663 | 0.005 |
| 5 | Erithromycin 0.060 mg | 0.060 | 0.079 | 0.6411 | 0.019 |
| 6 | Erithromycin 0.080 mg | 0.080 | 0.092 | 0.7468 | 0.012 |
| 7 | Erithromycin 0.10 mg | 0.100 | 0.107 | 0.8690 | 0.007 |
| 8 | Erithromycin 0.15 mg | 0.150 | 0.181 | 1.4646 | 0.031 |
| 9 | Erithromycin 0.20 mg | 0.200 | 0.199 | 1.6102 | -0.001 |
| 10 | Erithromycin 0.25 mg | 0.250 | 0.276 | 2.2365 | 0.026 |
| 11 | Erithromycin 0.30 mg | 0.300 | 0.334 | 2.7042 | 0.034 |
| 12 | Erithromycin 0.40 mg | 0.400 | 0.414 | 3.3534 | 0.014 |
| 13 | Erithromycin 0.50 mg | 0.500 | 0.550 | 4.4502 | 0.050 |
| 14 | Erithromycin 0.60 mg | 0.600 | 0.631 | 5.1082 | 0.031 |
| 15 | Erithromycin 0.80 mg | 0.800 | 0.787 | 6.3681 | -0.013 |
| 16 | Erithromycin 1.0 mg | 1.000 | 0.962 | 7.7885 | -0.038 |

**Figure 5** TQ analyst calibration of erythromycin standards.

and ground to fine powder in mortar to reduce the particle size. The KBr pellets were prepared by mixing 1 mg samples with 99 mg of dried, finely powdered potassium bromide then condensed in the 13-mm die at a pressure of 6 tons for 5 min. The pellets were scanned from 4000 to 400 $cm^{-1}$ on Thermo Nicolet 5700-FTIR spectrometer.

### 2.5. Limit of detection and quantification

For the determination of limit of detection (LOD) and limit of quantification (LOQ) of the proposed method, the selected band area was measured at low concentrations of standards, until the erythromycin related signal disappeared. The analysis at the lowest amount which produced substantial signal was repeated eleven times and calculated by the following formula (Memon et al., 2010):

$$\mathrm{LOD} = 3 \times \mathrm{SD} \times C/M$$

where: SD is the standard deviation; $C$ is the concentration of analyte and $M$ is the mean band area.

While LOQ was determined by the same way with the following equation:

$$\mathrm{LOQ} = 10 \times \mathrm{SD} \times C/M$$

### 2.6. Recovery efficiency

The recovery efficiency (RE) was determined by the ratio of erythromycin recovered (%) to the erythromycin content (mg) added. The calculation was carried out by the following equation:

$$R\ (\%) = (C - B/A) \times 100$$

where, $R$ is the erythromycin recovered (%), (B) is actual concentration present in the sample before addition of erythromycin (C) is the concentration of erythromycin after addition and (A) added level of erythromycin.

The CV (%) of the data set was calculated and used as relative standard deviation (RSD, %) as in the AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals (AOAC International, 2002).

## 3. Results and discussion

The results of this study accomplish significant benefits in terms of rapidity, accuracy, simplicity and green method by the use of FTIR spectroscopy for measuring quantity of the desired active species while performing quality control of pharmaceuticals. Fig. 2 represents the transmission FTIR spectrum

**Table 1** Results for the erythromycin in the tablet formulations.

| Sample | Erythromycin labeled mg/Tablet | Erythromycin found mg/Tablet |
|---|---|---|
| Sample 01 | 500 | 489.81 ± 0.642 |
| Sample 02 | 500 | 492.54 ± 0.394 |
| Sample 03 | 500 | 497.66 ± 0.056 |
| Sample 04 | 250 | 253.17 ± 0.472 |
| Sample 05 | 250 | 246.5 ± 0.085 |

**Table 2** Recovery test of erythromycin from tablet samples after exogenous addition of known amount of standards.

| | | | | By FT-IR | | Acceptable recovery(%) |
|---|---|---|---|---|---|---|
| | (A) | (B) | (C) | Recovery[a](%) | CV[b](%) | (AOAC, 2002) |
| 1 | 25 | 246.5 | 270.81 ± 0.21 | 97.24 | 0.83 | 90–108 |
| 2 | 50 | 246.5 | 296.43 ± 0.16 | 99.86 | 0.75 | |
| 3 | 100 | 246.5 | 348.26 ± 0.28 | 101.76 | 1.03 | |

(A) Exogenous addition.
(B) Before addition.
(C) After addition.
[a] Recovery (%) = (C − B)/A × 100.
[b] Coefficient of variation was obtained from the mean of triplicate tests.

of the erythromycin in the mid infrared region recorded in the form of KBr pellets. The homogenous composition was achieved by carefully measuring specific amounts of the erythromycin standard and KBr ratio by applying equal pressure for all pellets.

Fig. 3 specifically shows the absorbance of the carbonyl (C═O) band of erythromycin standard (0.005 to 1.0 mg) in the FTIR region between 1743 and 1697 $cm^{-1}$. The carbonyl band was selected for the quantification of erythromycin due to the well established fact that no excipients exhibit absorption in this region.

Other bands appearing at 2980 to 2900 $cm^{-1}$, 2860 to 2840 $cm^{-1}$ and 1480 to 1440 $cm^{-1}$ have some interfering bands appearing in the same region due to the mixture of the excipients present in the formulation. The excipients commonly used in the solid commercial formulation of erythromycin are starch, magnesium stearate, lactose, avecil and talcum powder. FT-IR spectra of mixed excipients are shown in Fig. 4A. The main idea of the achieving green method was fully achieved in the sense that strategy of avoiding extraction brought overwhelming success as clearly shown in the figure that two spectra i.e. sample and the erythromycin standard both spread over the surface of each other and there is no significant interference bands hence proving the method to be practical.

A simple Beer's law quantitative model based upon the measurement of the band area of the selected region was applied for the quantitative determination of erythromycin in the pharmaceutical formulations using a specified region and a good calibration curve with a linear regression of 0.998 as quoted in Fig. 5 was achieved. The slope and intercept were determined corrected with one point baseline, and then the obtained regression equation was used for the calculation of erythromycin concentration in the tablets. The % difference plot between the actual and calculated values for the calibration standards is also shown in the figure. The calibration clearly shows the tremendous sensitivity of transmission FT-IR spectroscopy for the determination of erythromycin in the pharmaceutical formulation in the form of tablets.

From the calibration, the assessment of the errors was carried out by calculating the residual mean standard error of calibration (RMSEC) after comparing the actual concentration with the computed one for each component with the standard deviation of 0.158. The root mean square error of prediction (RMSEP) was 0.0247. The quantitative model was also verified through root mean square error of cross validation (RMSECV) which was found to be 1.14.

$$Y = 8.09x + 0.00$$

The regression equation obtained from the calibration of standards was afterwards utilized for the calculation of the concentration of erythromycin active in the real samples. The summary of the results obtained by the proposed method for erythromycin tablets is presented in Table 1. The concentration of erythromycin determined was found to be in the range of 97.96–101.26% with labeled contents in analyzed tablet samples and is within the permissible limits of the pharmacopoeia.

The statistics of erythromycin recovery tests (Table 2) on the one selected tablet sample revealed high recovery performance (97.24, 99.86 and 101.76 %) with high precision (%CV = 0.83, 0.75, and 1.03) of the proposed method. Satisfactory recovery is 90–108% (AOAC International, 2002). The % recovery results revealed that there is no significant interference effect from any other constituent present in the matrix and hence prove that this method is feasible without any solvent extraction. LOD and LOQ calculated for this method were found to be 0.006 mg $g^{-1}$ and 0.018 mg $g^{-1}$ respectively. These results clearly prove the validity of the proposed method using transmission FT-IR spectroscopy for quantitative analysis.

## 4. Conclusion

The proposed method for the determination of erythromycin in tablets using FTIR spectroscopy for quality control analysis is a straightforward analytical method which is very simple as it does not require any complex procedure. This is an economical and environmentally friendly method avoiding the use of hazardous chemicals or solvents and could be effectively used in the pharmaceutical industry for GMP as a rapid and green method. This may be applied as a capable method for the speedy quality assessment/quality control (QA/QC) of the active ingredients like erythromycin in the pharmaceutical preparations.

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