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Assessment of Azithromycin in Pharmaceutical Formulation by Fourier-transform Infrared (FT-IR) Transmission Spectroscopy

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Abstract

A simple, rapid and economical method for azithromycin quantification in solid tablet and capsule formulations has been developed by applying Fourier-transform Infrared (FT-IR) transmission spectroscopy for regular quality monitoring. The newly developed method avoids the sample preparation, except grinding for pellet formation and does not involve consumption of any solvent as it absolutely eliminates the need of extraction. KBr pellets were employed for the appraisal of azithromycin while acquiring spectra of standards as well as samples on FT-IR. By selecting the FT-IR carbonyl band (C=O) in the region 1,744–1,709 cm^{-1} the calibration model was developed based on simple Beer's law. The excellent regression coefficient (R^2) 0.999 was accomplished for calibration set having standard error of calibration equal to 0.01 mg. The current work exposes that transmission FT-IR spectroscopy can definitely be applied to determine the exact amount of azithromycin to control the processing and quality of solid formulations with reduced cost and short analysis time.

Keywords: FT-IR transmission spectroscopy; Azithromycin; Quantitative analysis; Pharmaceutical formulation; Tablets

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Introduction

Azithromycin is 15-membered semi synthetic antibiotic (Fig. 1) belonging to macrolide family with azalactone ring which is structurally different from erythromycin, as at position C-9 it does not contain keto oxygen instead at position C-10 it contains methyl-substituted nitrogen and thus activity of azithromycin is enhanced 15 times as compared to erythromycin [1, 2]. It is commonly prescribed for the treatment of some bacteria causing infections, like upper and lower respiratory tract infections, throat, laryngitis and bronchitis infections, middle ear infections and pneumonia [3]. Furthermore, it also works as an efficient therapeutic agent for the treatment of diseases that are sexually transmitted, tonsillitis, and infections of skin [4].

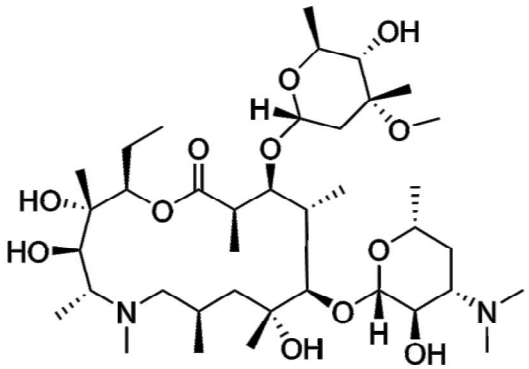

Figure 1. **Chemical structure of azithromycin.**

Numerous liquid chromatographic (LC) methods are reported for determination of azithromycin in human plasma and nearly all involve electrochemical detection to attain sufficient sensitivity as UV detection shows little

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bit less sensitivity for macrolides analysed in body fluids. Barrett et al. has used Tandem LC for quantification of azithromycin in plasma samples of human. Torano and Guchelaar have worked on a strategy using fluorescence detection for quantification of macrolide antibiotics in serum. Nirogi used solid-phase extraction then quantitative analysis was done on LC/MS/MS. The chromatographic techniques require huge amount of solvents, lengthy experimental procedures for sample clean-up, and also demand expensive equipment that might not be available in many laboratories [5-12]. The survey of published literature reveals that quantitative methods are meant for the measurement of azithromycin in bodily fluids like plasma, tissues etc and waste water. Detection is generally electrochemical, MS, UV and fluorescence after derivatization [13, 14].
Another popular method for quantitative analysis is FT-IR spectroscopy, which has proved to be a promising tool for the quantification of variety of samples. The NCEAC FT-IR group has credibly worked for development of new methods by applying FT-IR transmission spectroscopy to analyse various quality factors of oils and fat [15-19]. FT-IR spectroscopy has previously been applied for quantification of azithromycin by dissolving it in toluene [20]. Electroanalytical techniques offer another possibility for estimating this compound due to a suitable electroactive site in its structure hence DPV method has also been applied [21]. The quality monitoring authorities in pharmaceutical industries need exact analysis of formulations produced so as to confirm that these contain the necessary quantity of the active component in order to follow good manufacturing practice (GMP) rules [22].

So, infront of these commonly employed methodologies the significant target of current approach was to develop a straightforward method omitting the otherwise imperative sample pre-treatment, less time consuming, sensitive and alternative method capable of estimation of azithromycin in tablet formulations using FT-IR spectroscopy for routine quantitative monitoring to counter the existing laborious methods. As FT-IR is analytical technique which allows rapid quantitative measurement as it is fast and non-destructive in nature, which is an extremely useful means for analyzing solid as well as liquid pharmaceuticals without requiring any solvent. The rise in industrial significance for FT-IR during current decade is result of remarkable advancements in the technique along with more accessibility of fast-scan instruments which facilitate smooth measurements using potential chemometric techniques with less or without sample pre-treatment.

Experimental

Reagents and samples

Standard azithromycin (98%) of analytical grade for the calibration in the current study was obtained from Sigma Aldrich (CAS No. 83905-01-5). Potassium bromide (KBr) used for the sample as well as pellets formation was spectroscopic grade. Various tablet and capsule formulation samples with azithromycin as an active component were obtained from different pharmaceutical manufacturing companies and drug stores of local market (Manufacturing. Date: November/ December 2010).

FT-IR analysis

FT-IR spectra of all standards as well as samples in the pellet form were acquired with 5700 FT-IR spectrometer of Thermo Nicolet equipped with KBr optics and deuterated triglycine sulfate (DTGS) detector. The FT-IR data analysis was conducted using infrared spectra analysis software package OMNIC. All recorded spectra were average of 32 scans in mid Infrared (IR) region of 4000–400 cm^{-1} by optimizing resolution of the instrument at 4 cm^{-1}. Each time before obtaining spectrum of sample or standard a fresh background spectrum from KBr pellet was recorded. All the spectra were recorded in triplicate to obtain reproducible results.

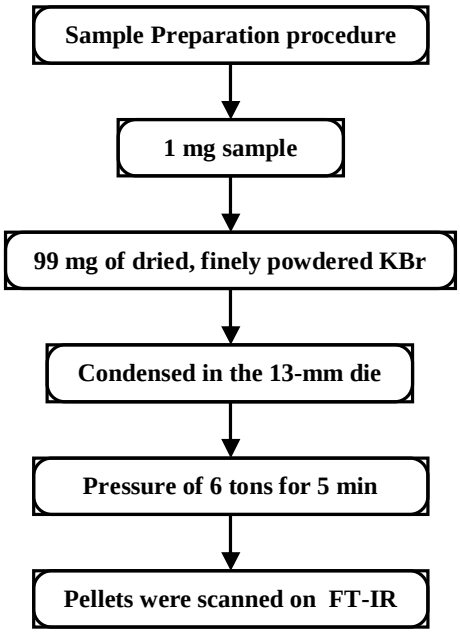
Calibrations and statistical analysis

A set consisting 14 various concentration of azithromycin standards ranging from 0.005 mg to 1 mg were mixed in KBr to make each time the pellete of 100 mg by total weight to ensure homogeneity and required ratio. Simple Beer's law model was developed from the calibration data set for the appraisal of azithromycin in real pharmaceutical samples using chemometric

software package Turbo Quant (TQ) Analyst. The already recorded spectra of azithromycin standards were used in the TQ analyst software which specified the prominent region of carbonyl band i.e. 1,744–1,709 cm^{-1} so as to obtain better results. The peak area of all azithromycin standards was computed by the software automatically hence saved much time and hard work to work out the peak area for all standards. An excellent calibration was also achieved with an excellent regression with the help of software.

Procedure for sample preparation

For FT-IR run except grinding no prior sample treatment is needed in this method. The tablet samples were weighed and grinded to fine powder in mortar to decrease the particle size. The KBr pellets were prepared by mixing 1 mg samples with 99 mg of dried, finely powdered KBr then condensed in the 13-mm die at a pressure of 6 tons for 5 min. The pellets were scanned in mid IR region on Thermo Nicolet 5700-FT-IR spectrometer.



Recovery efficiency

The recovery efficiency (RE) was checked by the ratio of azithromycin recovered to the azithromycin content (mg) added. The recovery was calculated using the following equation:

$$R\ (\%) = (C–B/A)\ x100$$

Where, R shows the azithromycin recovered (%), (B) indicates the actual concentration that was present in the selected sample before addition of azithromycin (C) is the final concentration of azithromycin after addition of standard and (A) gives the value of azithromycin amount added.

The CV (%) of the data set was determined and used as relative standard deviation (RSD, %) as in the AOAC guidelines [23].

Results and Discussion

The outcome of the current work brings about considerable benefits achieved like speed, accuracy, ease and expediency by the use of FT-IR spectroscopy for calculating exact amount of the desired ingredient during analysis of pharmaceuticals to control the quality and quantity of product. The generation of the calibration curve was done using the spectra of the standards as given in (Fig. 2). which incorporates the region of aliphatic carbonyl (C=O) group at 1,744–1,709 cm^{-1}.

The TQ analyst generates the calibration in an efficient way. The computed peak area was used to develop simple Beer's law model which was then put into practice for the quantitative analysis of azithromycin in solid formulations such as capsules and tablets using particular region and a fine calibration was also accomplished with excellent regression as shown in Fig. 3. From the calibration set slope as well as intercept was computed and corrected with baseline of two points.

$$Y = 4.42\ x + 0.00$$

The regression equation given above was subsequently utilized for the calculation of concentration of azithromycin in the samples. The computed calibration curve alongwith % difference plot for theoretical and computed values in the range of 0.005–1.0 mg azithromycin standards are displayed in (Fig. 3). From the calibration remarkable sensitivity of transmission FT-IR spectroscopy is clearly evident for the quantification of azithromycin in the pharmaceutical samples as shown in (Table 1).

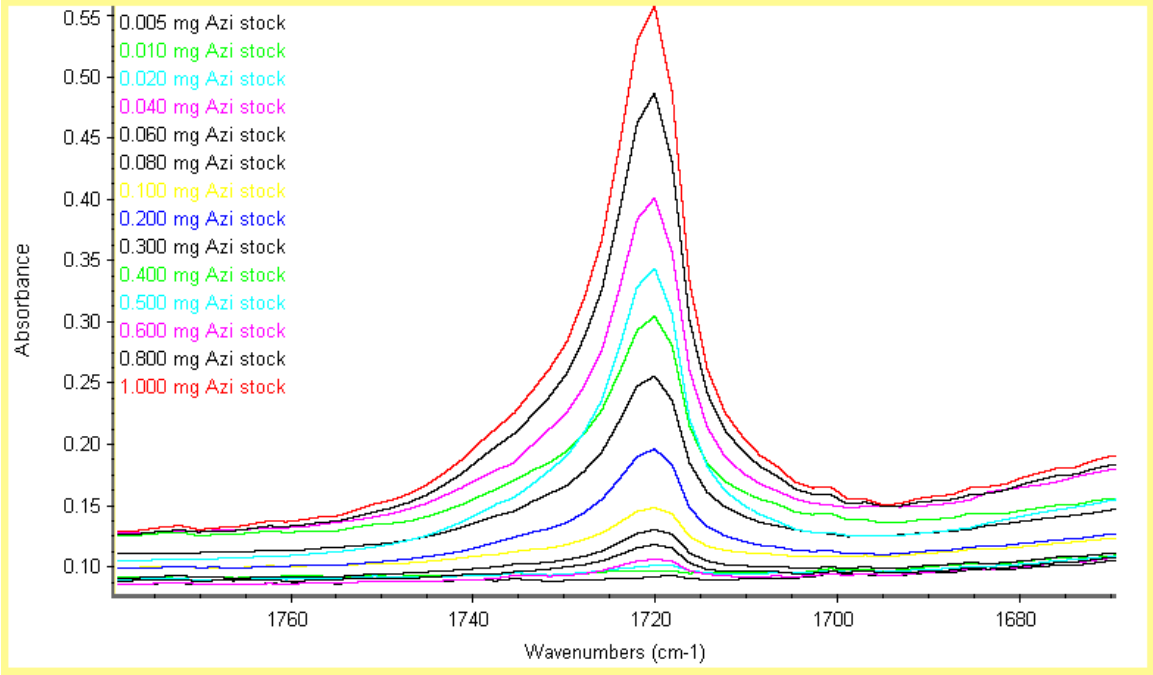
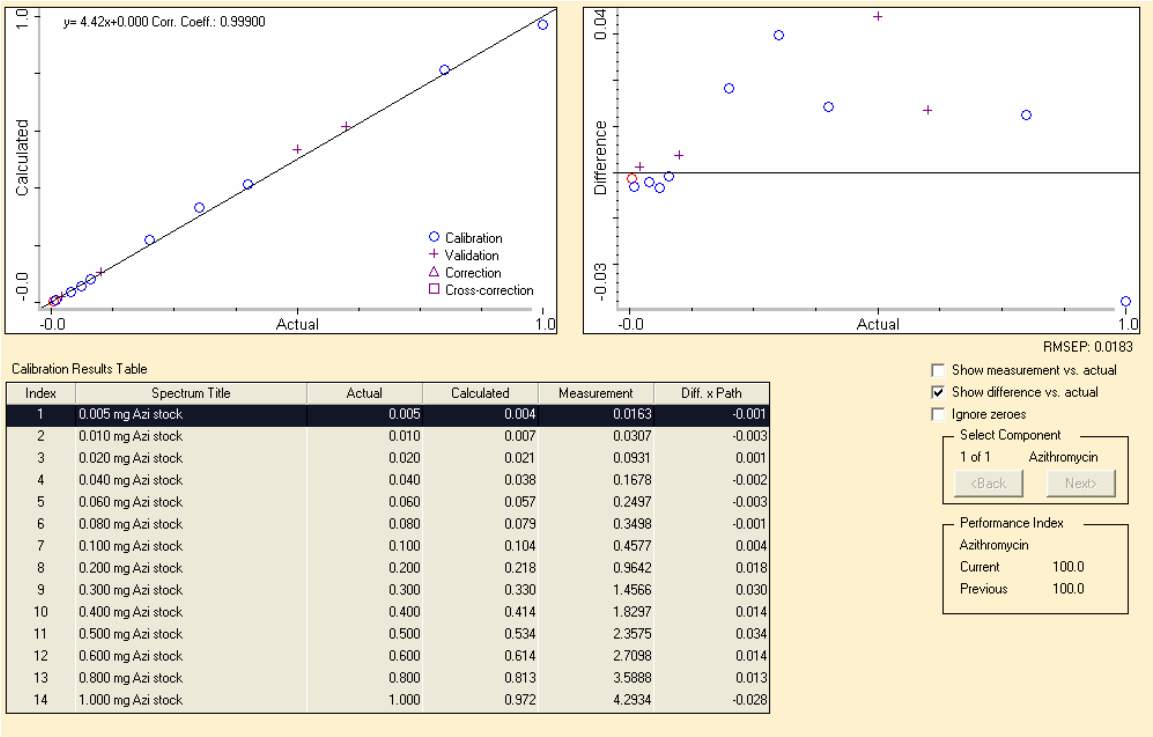


Figure 2. **The calibration of carbonyl band of azithromycin standards in increasing concentration by FTIR transmission spectroscopy.**



Calibration Results Table

Index	Spectrum Title	Actual	Calculated	Measurement	Diff. x Path
1	0.005 mg Azi stock	0.005	0.004	0.0163	-0.001
2	0.010 mg Azi stock	0.010	0.007	0.0307	-0.003
3	0.020 mg Azi stock	0.020	0.021	0.0931	0.001
4	0.040 mg Azi stock	0.040	0.038	0.1678	-0.002
5	0.060 mg Azi stock	0.060	0.057	0.2497	-0.003
6	0.080 mg Azi stock	0.080	0.079	0.3498	-0.001
7	0.100 mg Azi stock	0.100	0.104	0.4577	0.004
8	0.200 mg Azi stock	0.200	0.218	0.9642	0.018
9	0.300 mg Azi stock	0.300	0.330	1.4566	0.030
10	0.400 mg Azi stock	0.400	0.414	1.8297	0.014
11	0.500 mg Azi stock	0.500	0.534	2.3575	0.034
12	0.600 mg Azi stock	0.600	0.614	2.7098	0.014
13	0.800 mg Azi stock	0.800	0.813	3.5888	0.013
14	1.000 mg Azi stock	1.000	0.972	4.2934	-0.028

Figure 3. **The band area of corbonyl group (1744-1709cm^{-1}) of the Azithromycin standards.**

Table 1. **Regression results of the calibration of carbonyl band area (1,744-1,709 cm^{-1}) versus various concentrations of the azithromycin standards.**

Y = A + B * X

Parameter	Value	Error
A	0.02841	0.02641
B	4.41492	0.06173
R	0.999	
SD	0.07179	
N	14	
P	<0.0001	

The evaluation of errors was performed on the basis of calibration to compare actual concentrations with the calculated for each component with the standard deviation of 0.0163. The residual mean standard error of prediction (RMSEP) was also found to be 0.0183.

(Fig. 4) shows transmission FT-IR spectra of standards of azithromycin and tablet samples containing azithromycin as an active constituent. A prominent band between 1,744–1,709 cm^{-1} was selected for the quantification. The purpose of newly developed method was completely accomplished as there seems no major interference by the excipients present in tablet formulation as the spectra of tablet samples stretch over the surface of azithromycin standard spectra indicating the very negligible interference of other sample matrix.

Therefore, determined limit of detection (LOD) and limit of quantification (LOQ) values were 0.01 and 0.05, respectively. These markedly demonstrate high sensitivity of transmission FT-IR spectroscopy by applying the proposed method. Furthermore, evaluation of the accuracy of the method was carried out by employing the recovery test.

The results of azithromycin recovery tests are given in (Table 2) on the one selected real sample which indicates very good recovery performance (98.4, 101.2 and 105.2 %) with good precision of (CV = 2.1, 1.4 and 1.7 %) by applying the developed method. As per AOAC [23] the satisfactory recovery is in the range of 90–108%. It is also revealed through the recovery results that the other substances present in the matrix does not show any significant interference effect hence the strategy for the new method becomes successful without carrying out extraction.

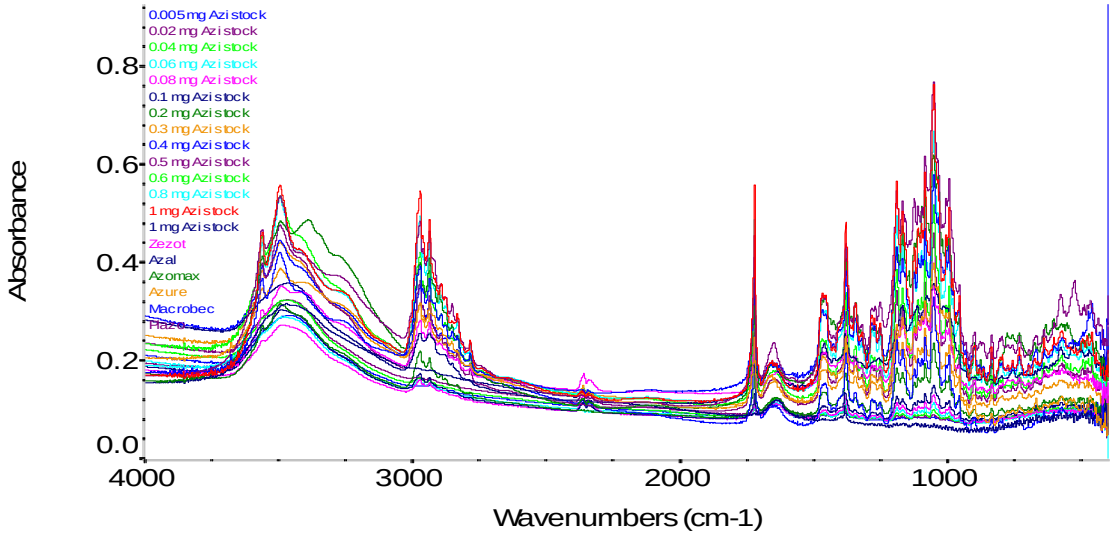


Figure 4. **Transmission FT-IR spectrum of a tablet samples containing azithromycin as active ingredient and standards of azithromycin.**

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

(A)	(B)	By FT-IR (C)	*Recovery*[a] *(%)*	*CV*[b] *(%)*	Acceptable recovery (%) [23]
25	251	275.6 ± 1.3	98.4	2.1	90-108
50	251	301.6 ± 0.8	101.2	1.4	
100	251	356.2 ± 1.4	105.2	1.7	

(A) Exogenous addition
(B) Before addition
(C) After addition
[a]Recovery (%)=(C–B)/A x100.
[b]Coefficient of variation was obtained from the mean of triplicate tests.

(Table 3) shows the results for azithromycin tablets achieved through the proposed method. The quantity of azithromycin active ingredient computed from analyzed tablet samples was in the range of 90-105 % and matched with the labelled data provided by manufacturer. These results are within the permissible limits as mentioned in pharmacopoeia.

Table 3. **Results for the azithromycin in the tablet formulations.**

Sample	Azithromycin labeled	Azithromycin found
Sample 01	250	255.59 ± 1.18
Sample 02	250	251.28 ± 0.83
Sample 03	250	257.61 ± 0.67
Sample 04	250	247.59 ± 1.04
Sample 05	250	254.51 ± 0.58

[a] The amounts are expressed in mg/Tablet on the dry weight basis.

Conclusion

This new assay method aimed for the assessment of azithromycin in tablets by FT-IR transmission spectroscopy is simple, inexpensive and environmental friendly eliminating the consumption of toxic chemicals; it can be widely applied in pharmaceutical industry as it has the potential of a rapid and economical method to fulfil the core requirement of GMP. This may be the potential alternate for rapid quality monitoring of azithromycin in the pharmaceutical formulations.

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