Research Paper

New Validated Diffuse Reflectance Infrared Fourier Transform Spectroscopic Method for the Quantification of Levofloxacin in Pharmaceutical Dosage Form

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Levofloxacin is a synthetic fluoroquinolone antibiotic available in different dosage forms for the treatment of various bacterial infections. Here in, we report the validated diffuse reflectance infrared Fourier transform spectroscopic method for quantitative analysis of levofloxacin in the solid dosage forms. This method offers the advantage of being eco-friendly that is applied for solid state samples. Appropriate quantities of levofloxacin and dry potassium bromide were mixed to get the desired concentration of samples for Fourier transform infrared spectroscopic analysis using a diffuse reflectance sampling interface. The peak in the Fourier transform infrared spectrum around 1724 cm^{-1} was observed for the carbonyl group of levofloxacin. The calibration curve was plotted for the area under the curve of carbonyl peak against the concentration of calibration standards. A linear calibration curve with a correlation coefficient of 0.999 was obtained in the range of 0.3-1.8 % w/w of levofloxacin. The calibration curve method was linear, precise and accurate as per guidelines. The method employed for quantification of levofloxacin in three marketed tablet dosage forms and laboratory physical mixture yielded a mean recovery of 101.32 and 98.11 percent by weight, respectively. Overall, the precision, accuracy and reproducibility of the proposed diffuse reflectance infrared Fourier transform spectroscopy method make it appropriate for quantitative analysis of levofloxacin in solid dosage forms.

Key words: Levofloxacin, fourier transform infrared spectroscopy, diffuse reflectance infrared fourier transform spectroscopy, validation, quantification, analysis

The role of pharmaceutical analysis in the drug developmentand manufacturing is mainly focused on the identification and quantification of drugs and organic impurities. The most important aspect of quality control of pharmaceuticals includes the availability of robust, time and cost effective established analytical methods. Infrared (IR) spectroscopy is a widely recommended official method for the identification of pharmaceuticals. The use of IR and near infrared (NIR) spectroscopy for the identification of pharmaceuticals almost eliminated the use of classical identification techniques such as color and chemical tests[1,2]. Many chromatographic methods are used for the quantification of bulk drugs and pharmaceuticals of which high performance liquid chromatography (HPLC) is a widely recommended official methods[3]. In about 45 % of the monographs in the twenty-ninth United States Pharmacopoeia (USP), HPLC is recommended for the analysis of small organic molecules in bulk materials. The other official methods recommended for the analysis of pharmaceuticals include gas chromatography, thin layer chromatography, ultraviolet spectrophotometry and electroanalytical techniques[4].

Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy is a relatively newer IR technique that has shown potential for the quantification of solid samples[5,6]. DRIFT spectroscopic methodology has the advantages of being the least complex procedures

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involving simple sample preparation, eco-friendly solid state analysis and do not need the drug extraction procedure. Analysis of various drug samples using the DRIFT spectroscopic method is reported in the literature[7-15]. The USP describes the IR method for quantification of simethicone in bulk and pharmaceutical formulations[16].

Chemically, levofloxacin is the S enantiomer (*L isomer*) of ofloxacin and has approximately twice the potency and substantially less toxicity than ofloxacin (fig. 1)[17]. Levofloxacin acts as an antibacterial against a wide range of bacterial pathogens. It inhibits deoxyribonucleic acid (DNA) gyrase in susceptible organisms resulting in the degradation of chromosomal DNA and interference with cell division and gene expressions[18]. Various analytical methods are reported for the estimation of levofloxacin in pharmaceuticals such as UV spectrophotometric[19-22], spectrofluorometric[23], HPLC[24-26], reverse phase (RP) HPLC[27], Reverse Phase ultra-performance liquid chromatography (RP-UPLC)[28], high-performance thin layer chromatography (HPTLC)[29] and voltammetry[30]. For the quantification of levofloxacin in bulk and pharmaceutical dosage forms, the liquid chromatography method is described in USP[31]. To the best of our knowledge, the Fourier transform infrared spectroscopy (FTIR) method using diffuse reflectance sampling technique for the quantitative analysis of levofloxacin in bulk or tablet dosage form was not available in the literature. Therefore, the objective of the present study was to develop and validate the solid state quantification method for levofloxacin in tablet dosage forms employing the DRIFT spectroscopic method.

MATERIALS AND METHODS

Chemicals and reagents:

Julphar Gulf Pharmaceutical Industries, Ras Al Khaimah provided the gift sample of levofloxacin and analytical grade potassium bromide (KBr) procured from the HiMedia, India.

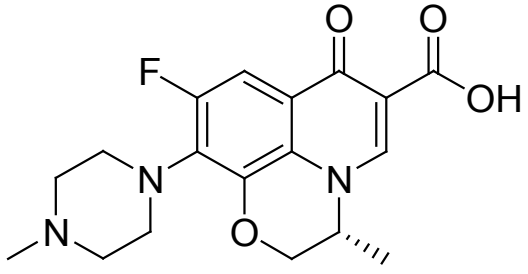

Fig. 1: Chemical structure of levofloxacin

FTIR Instrumentation:

All the diffuse reflectance spectra were collected using a Cary 630 FTIR spectrophotometer (Agilent Technologies, USA) equipped with a diffuse reflectance sampling interface (650-4000 cm^{-1}, 32 scans, resolution of 8 cm^{-1} and background spectrum of gold). Data collection and analysis were carried out using Cary 630 MicroLab PC and Agilent Resolution Pro software, respectively.

Calibration curve:

The calibration curve was plotted using six different solid state calibration standards of levofloxacin with the concentration in the range of 0.3 to 1.8 % w/w. Around 1000 mg calibration standards were prepared by diluting pure levofloxacin with dry KBr, followed by trituration using mortar and pastel. The area under the curve (AUC) for the peak around 1724 cm^{-1} for each standard in the replicate of six was recorded. The calibration curve was prepared by plotting the average AUC of six analyses vs. the concentration of calibration standard. StatFlex version 6.0 software for Windows was used (Artech, Osaka, Japan, http://www.statflex.net).

Method validation:

The proposed diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) method for quantification of levofloxacin was validated for linearity, precision and accuracy[32-34].

Linearity:

Samples of levofloxacin in the range of 0.5-2.0 % w/w in three replicates as described in the calibration curve were analyzed to check the linearity of the calibration curve.

Precision:

Method precision was assessed by intraday and interday repeatability. Three samples of 1.2 % w/w of levofloxacin were analyzed three consecutive times in the same day (d 1). For the same samples, analyses were repeated on d 3 (interday) to check the intermediate precision of the assay method.

Accuracy:

A standard addition method for the recovery of pure drug from the excipients at three different levels (80, 100 and 120 % by weight) was used to check

the accuracy of the assay method. Pure levofloxacin equivalent to 80, 100 and 120 % by weight of label claim were added to pre analyzed tablet powder (Brand 1). Samples appropriately 1.2 % w/w of levofloxacin in six replicates were prepared by dilution with dry KBr and analyzed.

Analysis of physical mixture:

A physical mixture of levofloxacin was prepared in the laboratory using the composition as shown in Table 1. Six samples of a physical mixture containing approximately 1.2 % w/w levofloxacin were analyzed in three replicates.

Analysis of marketed tablet formulations:

For the quantification of levofloxacin in tablet formulation, three brands (Label claim 500 mg) available in the pharmacy were used. After determining the average weight, ten tablets were finely powdered. Six (around 1000 mg) samples containing approximate 1.2 % w/w levofloxacin were prepared by diluting the appropriate quantity of levofloxacin with dry KBr. All the samples were analyzed in three replicates and the percent recovery of levofloxacin was calculated using the following formula.

Percent recovery=(Ru/Rs)×(Cs/Cu)×Avg weight/Label claim×100

Where, Ru=AUC for sample; Rs=AUC for standard; Cu=nominal concentration of sample (% w/w); Cs=concentration of USP levofloxacin (% w/w); Avg weight=Average weight of tablet dosage form.

RESULTS AND DISCUSSION

DRIFT spectroscopy is a well-known technique for the characterization and quantification of solid state samples. Since the organic compounds exhibit intense adsorptions peaks in the 4000-650 cm^{-1} region of the IR spectrum, it is possible to quantitatively analyze them using DRIFT spectroscopy.

The reflectance spectrum for neat sample of levofloxacin exhibited absorbance bands in the range of 3272 (NH_{str}), 3082, 2974, 2936 (CH_{str}), 2888, 2850, 2803, 2262, 2070, 1891, 1735, 1731 ($C{=}O_{str}$), 1696, 1629, 1551, 1544, 1486, 1305, 1288, 1241, 1193, 1007, 953, 903, 873, 799, 779, 695 cm^{-1} (fig. 2a). The most prominent absorbance band corresponding to the carbonyl group in the neat levofloxacin was around 1731 cm^{-1}, while that of diluted samples in dry KBr was within 2.0 absorbance units around 1724 cm^{-1}. The involvement of a range of different path lengths within the sample in reflectance spectroscopy generally results in the enhanced intensities of the weaker bands relative to that of the stronger bands. Dilution with the non-absorbing matrix (such as KBr) ensures all the absorptions are weak, thus minimizes this distortion of band intensities. However, in the spectrum of the neat powder, the stronger bands have approximately the same intensities and their shapes are distorted by the contribution of surface reflection[35], whereas the weaker bands appear similar in both spectra. The representative reflectance spectrum for 10 % w/w levofloxacin in dry KBr is shown in fig. 2b.

TABLE 1: COMPOSITION OF THE PHYSICAL MIXTURE

Composition	Quantity (mg)
Levofloxacin	500
Lactose	150
Microcrystalline cellulose (MCC)	75
Sodium starch glycolate	25
Starch	25
(Hydroxypropyl) methyl cellulose (HPMC)	10

The calibration curve was prepared by plotting the mean AUC obtained for the carbonyl peak versus nominal concentrations (0.3-1.8 % w/w) is shown in fig. 3. The reflectance spectra for the levofloxacin calibration samples diluted with dry KBr are depicted in fig. 4a-f. The mean AUC was calculated for each concentration and the data is shown in Table 2. The calibration curve is described by the equation y=a+bx, where "y" represents peak area and "x" represents the concentration of levofloxacin. The regression equation was found to be "y=4.54367+6.3959x" with a coefficient of correlation of 0.999 for the calibration curve.

The linearity curve was prepared by plotting the mean AUC obtained for the carbonyl peak (around 1724 cm^{-1}) for the diluted levofloxacin samples (0.5 to 2.0 % w/w in dry KBr) verses nominal concentrations (fig. 5). The regression equation for the linearity curve was "y=4.3995+6.3476x" with a coefficient of correlation of 1.0. This supports that in the concentration range of 0.5-2.0 % w/w the proposed method was linear.

The coefficient of variation (percentage relative standard deviation (% RSD)) is used to express the precision, whereas the mean of standard deviation (SD) is used for the expression of the accuracy of an analytical method. The intraday and inter day precision study data for levofloxacin are shown in Table 3. For 1st d precision studies, the RSD (%) values for the three samples were observed in the range of 1.37-1.67 while for 3rd d precision studies, the range was 1.47-1.84. The

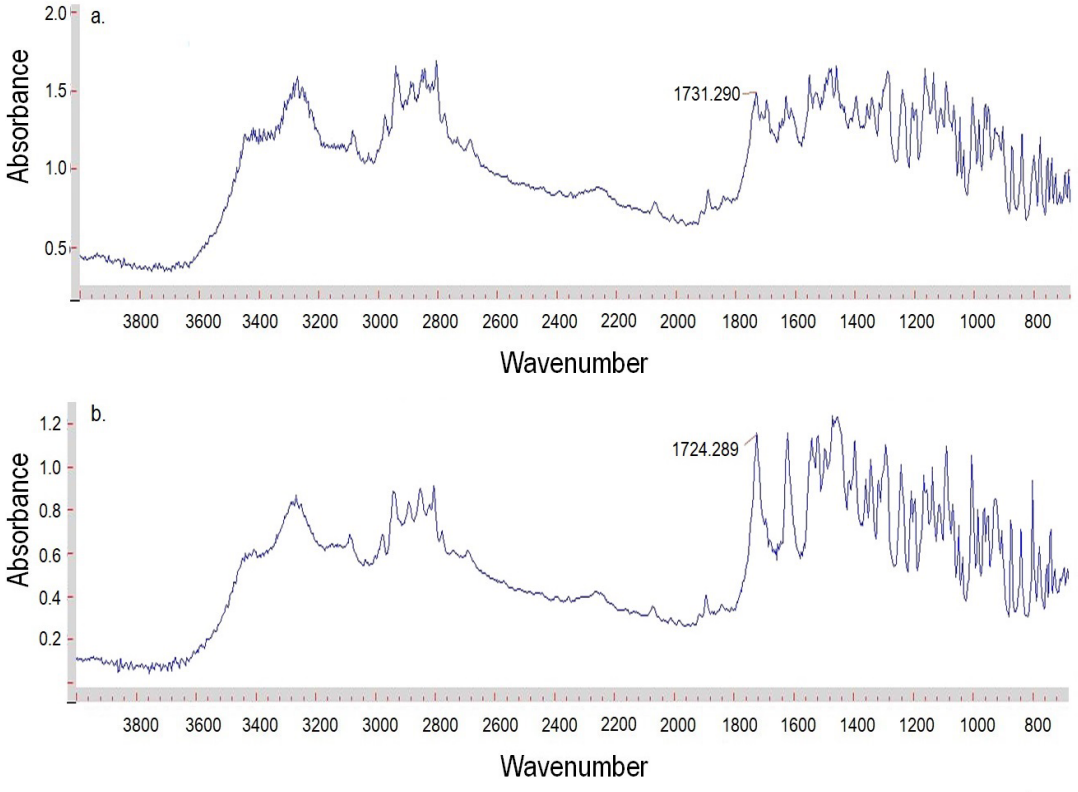

Fig. 2: Reflectance spectra for the levofloxacin, a: neat drug; b: 10 % diluted drug in dry KBr

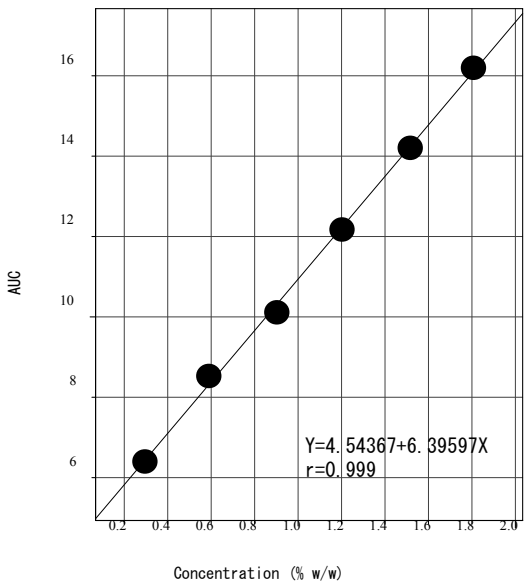

Fig. 3: Calibration curve for levofloxacin (0.3-1.8 % w/w)

intraday and inter day precision results were found to be satisfactory. The percentage RSD for the method was within the limit. There was no statistically significant difference between the results of 1st d and 3rd d analysis (t=0.057359, p=0.596).

The results of accuracy studies that were performed by spiking 80, 100 and 120 % of test concentration on pre analyzed tablet dosage formulation (Brand 1) are shown in Table 4. Based on the results of accuracy studies, the proposed method was found to be accurate with the average recovery of levofloxacin in the acceptable range (103.98 % w/w).

The proposed method was intended to use for the quantification of levofloxacin in the solid dosage forms. When the excipients and the composition of the marketed formulation are not known, the laboratory physical mixture prepared from the commonly used excipient for tablet formulation may help in studying the interaction of excipients in the analysis. Hence, the proposed method was employed for the analysis of levofloxacin in the three different brands of marketed tablet formulations and the laboratory physical mixture (fig. 6). The average recovery of the levofloxacin in laboratory physical mixture was 98.11 % w/w (95 % confidence interval (CI)=94.8623, 101.3577), whereas that for the three pharmaceutical tablet formulations was in the range of 98.23-105.4 % w/w (M=101.32; 95 % CI=97.8958, 104.7442) of the label claim (Table 5).

Levofloxacin from the three marketed formulations was recovered within the good agreement of the label claim and was within the acceptable limits of USP (not less than 90.0 % and not more than 110.0 % of the stated amount of the levofloxacin). The results of recovery

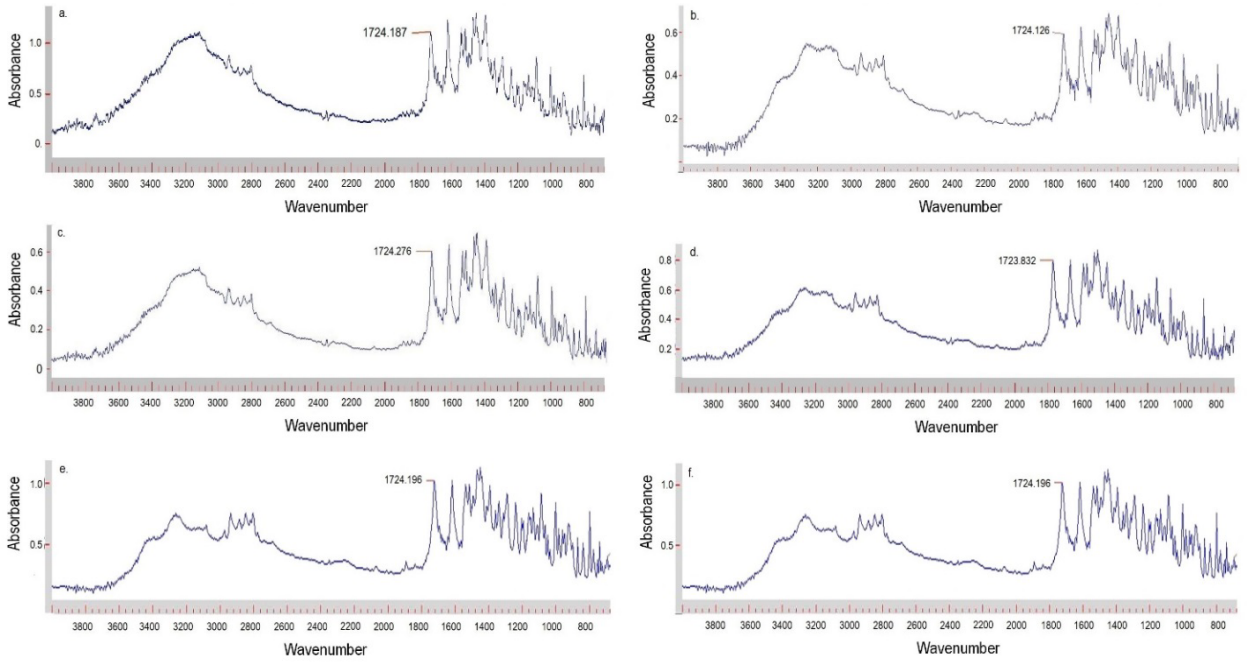

Fig. 4: Reflectance spectra for the levofloxacin calibration samples diluted in dry KBr, a: 0.3 % w/w; b: 0.6 % w/w; c: 0.9 % w/w; d: 1.2 % w/w; e: 1.5 % w/w; f: 1.8 % w/w

TABLE 2: CALIBRATION CURVE AND LINEAR REGRESSION DATA FOR LEVOFLOXACIN (N=6)

Nominal Concentration (% w/w)	AUC	SD	RSD (%)
0.295	6.402	0.091	1.42
0.589	8.530	0.154	1.81
0.902	10.114	0.142	1.40
1.202	12.175	0.230	1.89
1.516	14.207	0.162	1.14
1.807	16.199	0.251	1.55
Linear regression data			
Statistical Parameters		Values	
Concentration range		0.3-1.8 % w/w	
Regression equation		y= 6.3959x+4.54367	
Correlation coefficient		0.999	

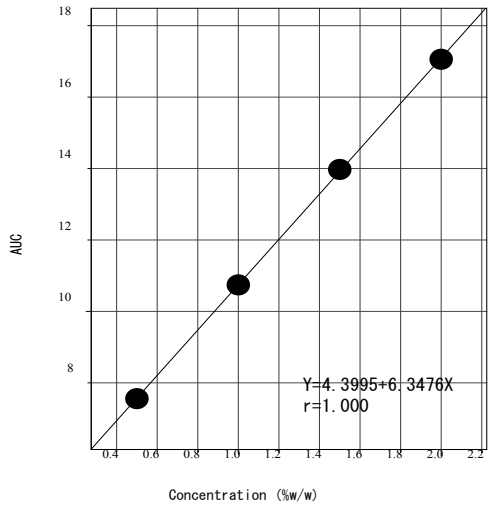

Fig. 5: Linearity curve for levofloxacin (0.5-2.0 % w/w)

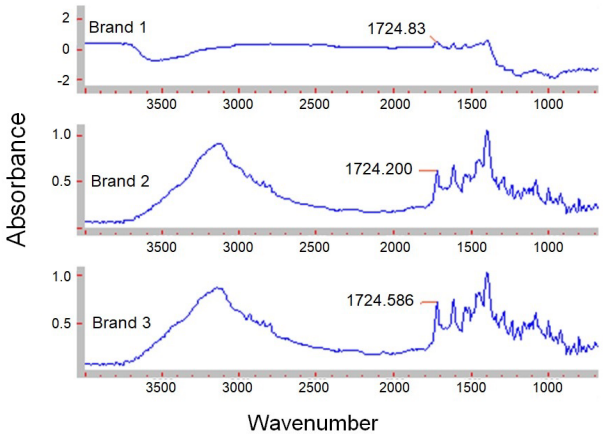

Fig. 6: Reflectance spectra for tablet formulation of levofloxacin diluted with dry KBr

studies supported that that there was no interference of excipients in the analysis, hence the proposed method may have the potential to serve for in house quality control of levofloxacin.

Analytical chemistry plays a significant role in the quality control and development of pharmaceuticals including assay method and validation. A validated analytical method is an official requirement of the drug approval process by various regulatory authorities and also it can be used for in house quality control. The proposed solid state validated DRIFTS method was precise, accurate with significant recovery of levofloxacin in tablet dosage formulation. The method may be employed for the quantification of levofloxacin in bulk and in solid dosage forms.

TABLE 3: INTRADAY AND INTER DAY PRECISION STUDY DATA FOR LEVOFLOXACIN (N=3)

Conc. (1.2 % w/v)	1st d			3rd d			
	AUC	SD	% RSD	AUC	SD	% RSD	p Value*
1	12.072	0.20	1.67	12.04	0.18	1.47	0.596
2	12.482	0.20	1.61	12.29	0.23	1.84	
3	12.456	0.17	1.37	12.39	0.21	1.66	(t=0.57359)
Average	12.336	0.19	1.55	12.240	0.20	1.65	

*Two tailed t test

TABLE 4: RECOVERY DATA OF LEVOFLOXACIN IN TABLET DOSAGE FORM (BRAND 1) (N=3)

Label claim (mg)	Amount of drug added (mg)	Total	Amount recovered (mg)	Percentage Recovery	SD	RSD (%)
500	400	900	954.81	106.09	3.36	3.17
500	500	1000	999.90	99.9	4.70	4.70
500	600	1100	1165.67	105.97	3.75	3.54
Average				103.98	3.539	3.404

TABLE 5: ANALYSIS OF LEVOFLOXACIN IN PHYSICAL MIXTURE AND TABLET DOSAGE FORM (N=6)

Formulation	Label claim (mg)	Amount found (mg)	Recovery (%)	SD	RSD (%)
Laboratory physical mixture	500	490.55	98.11	2.87	2.92
Brand 1	500	527.0	105.4	2.46	2.33
Brand 2	500	501.65	100.33	2.80	2.79
Brand 3	500	491.15	98.23	3.82	3.89

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Conflict of interest:

The authors declare no conflicts of interest.

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