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FTIR spectroscopy for estimation of efavirenz in raw material and tablet dosage form

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ABSTRACT

A Fourier transform infrared (FTIR) spectrophotometric method was developed for rapid and direct measurement of efavirenz in pharmaceutical formulations. The method involves extraction of efavirenz from tablets with chloroform by sonication and the direct measurement of the absorbance in liquid phase using a reduced path length cell. In general, the spectrum was measured in transmission mode. The equipment was configured to collect a spectrum at 8 cm⁻¹ resolution and 45 scans per sec. The spectra were collected between 4000 cm⁻¹ and 450cm⁻¹, the band obtained at 1750cm⁻¹ (carbonyl group) showed intense, clear peak in the liquid phase for quantitation. The method was validated as per ICH guidelines. The method fulfilled most validation requirements in the linearity range 200-1000µg/mL. The coefficient of determination, limit of detection and quantification was found to be 0.993, 49.12µg/mL and 148.84µg/mL respectively. Results of developed FTIR method were compared with the results obtained with the existing UV method statistically by using t-test, which indicated that there is no significant difference between the methods at P=0.05. The proposed FTIR method reduces the solvent consumption and also eliminates the use of reagents. Thus the developed method offers a good alternative for the quantitative estimation of efavirenz in bulk and pharmaceutical dosage forms and also to quantify efavirenz when combined with other API in the same dosage form.

Key Words: Efavirenz, FTIR, method validation, ICH guidelines.

INTRODUCTION

Efavirenz is an antiretroviral agent used for the treatment of HIV infection and widely used in the highly active anti-retroviral (HAART) therapy. It belongs to the class of non-nucleoside reverse transcriptases which are chemically diverse compounds that bind to the reverse transcriptase enzyme near the catalytic site and denature it. Chemically it is (4s)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1H-3,1-benzoxazin-2-one (figure 1). It is soluble in dimethyl sulfoxide (DMSO), chloroform, lower alcohols and slightly soluble in toluene (Rang *et al.*, 2012; Drugs.com, 2014).

In the present decade, several UV spectrophotometric, HPLC and LC-MS/MS methods have been reported so far for the estimation of efavirenz alone and in combination with other drugs in pharmaceutical samples (Kadam *et al.*, 2012; Bansal *et al.*, 2013; Ahmed *et al.*, 2013; Matkovic *et al.*, 2005). The official methods for the determination of efavirenz as a bulk drug and in tablets are based on liquid chromatography (IP, 2011). However, until now no direct Fourier transform infrared (FTIR) spectroscopy method has been recommended for the quantitative determination of efavirenz in solid dosage forms. Because of its simplicity and availability, FTIR is a technique which can be used in pharmaceutical quality control laboratories not only for the qualitative, but also for the quantitative purposes. Since the wave number and the respective intensity of an absorption maximum depends on a particular group of the chemical structure of a substance, this measurement can be used to quantitatively determine a substance (Pavia *et al.*, 2008). FTIR spectrum of efavirenz (figure 2) depicts how rich the

structure of efavirenz is in regards to diverse groups which absorb in the mid-IR (MIR) region. For the past two decades, the transmission FTIR spectroscopy has underwent significant application for the quantitative purposes, several in the field of pharmaceutical analysis.

As per above, for the identity verification and purity of an API and for detection of any spectral interference from the excipients the spectrum of an FTIR can be used. A calibration curve approach method is used for analyzing the absorption spectrum of an FTIR. However, it usually requires the chemometric methods in order to improve its data quality whenever applicable. For the quantitative analysis of an absorption spectrometry, the main basis is the Bouguer-Beer-Lambert law. FTIR methods are simple, faster than chromatographic methods. They are cost effective as there is less consumption of solvents (Nikolic, 2011; Gallignani and Brunetto, 2004). The developed method was validated as per ICH guidelines and the results are statistically evaluated and compared with that of the existing UV spectrophotometric method (ICH, 2005).

MATERIALS AND METHODS

Equipment

A Fourier Transform Infrared Spectrophotometer 8400S (Shimadzu, Kyoto, Japan) equipped with a sensitive pyroelectric detector with a DLATGS (L-alanine doped deuterated triglycine sulphate) element, a Michelson interferometer (30 incident angle), a Germanium coated KBr plate, and a global IR source was employed for FTIR spectra acquisition. A Wilmad Lab glass FTIR liquid transmission cell was used for sampling. Its circular configuration was a general purpose liquid transmission cell. It involved NaCl windows (32 mm diameter, 3mm thick) and an appropriate PTFE spacer of 0.1 mm optical path length. The sample was introduced by filling ports using a 3mL syringe.

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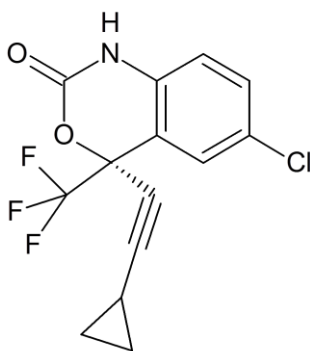


Figure 1: Structure of Efavirenz.

Reagents and samples

Efavirenz raw material was kindly provided by Aurobindo Pharmaceuticals, Hyderabad, India. Potassium bromide (SD fine chemicals limited), Chloroform (Sisco Research Laboratories Pvt. Ltd), Methanol (Merck specialties private limited) were purchased. Commercial pharmaceutical 600mg/Tab were purchased from local market manufactured by Hetero Labs limited, Hyderabad, India.

Preparation of standard solution

A 1.0mg/mL efavirenz stock solution was prepared in chloroform. After being diluted with chloroform, increasing volumes of efavirenz stock solutions were quantitatively transferred to a set of 10mL volumetric flasks to provide solutions within the 200-1000 $\mu\text{g/mL}$ concentration range.

Sample preparation

Twenty tablets were accurately weighed, finely pulverized and thoroughly mixed. An accurately weighed portion of pulverized tablets equivalent to 25mg of efavirenz was transferred to a 25mL volumetric flask, 9mL of diluting solvent (chloroform) was added, and was sonicated for 30min. After cooling, the diluting solvent was added to bring the volume to the mark. Finally, it was mixed, centrifuged at 3000 rpm for 5min, and filtered, discarding the first 2.5 mL of the filtrate. All sample solutions were filtered through 0.22 μm analytical Nylon filters. Samples for the standard addition technique were obtained by increasing volumes 3.2mL, 4.0mL and 4.8mL of the 1mg/mL efavirenz stock solution to a set of 10mL volumetric flasks containing 400 $\mu\text{g/mL}$ of sample solution. They were then quantitatively diluted to obtain solutions within the nominal concentration range.

Method validation

The developed FTIR method for quantitative estimation of efavirenz in tablets was validated according to the ICH guidelines for the following parameters- limit of detection (LOD) and limit of detection (LOQ), linearity, precision and accuracy.

RESULTS AND DISCUSSION

Optimization of FTIR conditions

As per Beer's law the relation between concentration and absorbance is linear and proportional, absorbance mode is selected in the method development for quantitation of efavirenz and all IR spectra are obtained in absorbance mode. Apodization function "Happ-Genzel" was selected

Table 1: Standard calibration curve data for efavirenz.

Sr. No	Concentration ($\mu\text{g/mL}$)	Absorbance* A
1	200	0.04761
2	400	0.08834
3	600	0.12754
4	800	0.15708
5	1000	0.19219

*Average of three determinations

for normal measurement. As very few solvents are transparent in IR region, based on drug solubility chloroform and dimethyl sulfoxide were selected for IR analysis by liquid sampling technique. In chloroform, better peak intensity was observed and chloroform is transparent throughout the region of 800-5000 cm^{-1} hence it was selected as solvent for preparation of solutions. The frequency range of 400-4000 cm^{-1} (Mid IR region) was selected for IR analysis. As the number of scans increases S/N ratio increases, 45 scans were selected for better S/N ratio. Resolution 8 cm^{-1} was selected for better peak to peak separation. Beer-Lambert's law was obeyed over the concentration range of 200-1000 $\mu\text{g/mL}$. The band chosen for quantization should be in a region of the spectrum free from absorption by other possible components of the sample.

Spectral characterization of efavirenz in chloroform

Efavirenz is soluble in water. It is also soluble in DMSO, chloroform, lower alcohols and slightly soluble in toluene. We selected chloroform taking into account efavirenz's solubility, volatility and IR transparency. This is considered a green chemical solvent due to its properties, such as low toxicity, low water miscibility and low volatility. Using chloroform as the background, transparency zones were found in the IR finger print range (v/cm^{-1}) as follows: 1370-1270, 1240-1120 and 1050-500. The FTIR spectrum of efavirenz obtained in the solid phase, (KBr pellet) shows well defined bands with relatively high intensity in the 1800-400 cm^{-1} wave number range in transmittance and absorbance mode (figure 2 and 3). Using the KBr pellet, comparison of the efavirenz analyte FTIR spectrum vs. the efavirenz reference spectrum from the International Pharmacopoeia website showed an excellent frequency correlation. The expected bands in the IR spectrum of efavirenz are N-H strong stretch at 3317.3 cm^{-1} , C=O stretch at 1749.7 cm^{-1} , CH at 2248.0, C-F at 1496.9 cm^{-1} , 1329 cm^{-1} , C-Cl stretch at 1038.3 cm^{-1} .

Therefore, taking into account the above information, the efavirenz FTIR spectrum, using chloroform as background, showed two notable spectral bands in the MIR with maximum wave numbers located at 1750 cm^{-1} and 1500 cm^{-1} corresponding to the carbonyl group C=O, C=C aromatic ring respectively (figure 5). Among which C=O group showed clear, intense peak which was increased linearly as the concentration is increased and selected for quantitative analysis of efavirenz.

Pressed pellet technique is suitable for handling of solids. For quantitative estimation, it is difficult to prepare low concentrations of sample by pressed pellet technique, hence liquid sampling technique is used and the solutions were prepared in chloroform solvent. The liquid samples were analysed by using liquid cells. The blank spectrum of chloroform and efavirenz in chloroform are shown in figure 4 and 5.

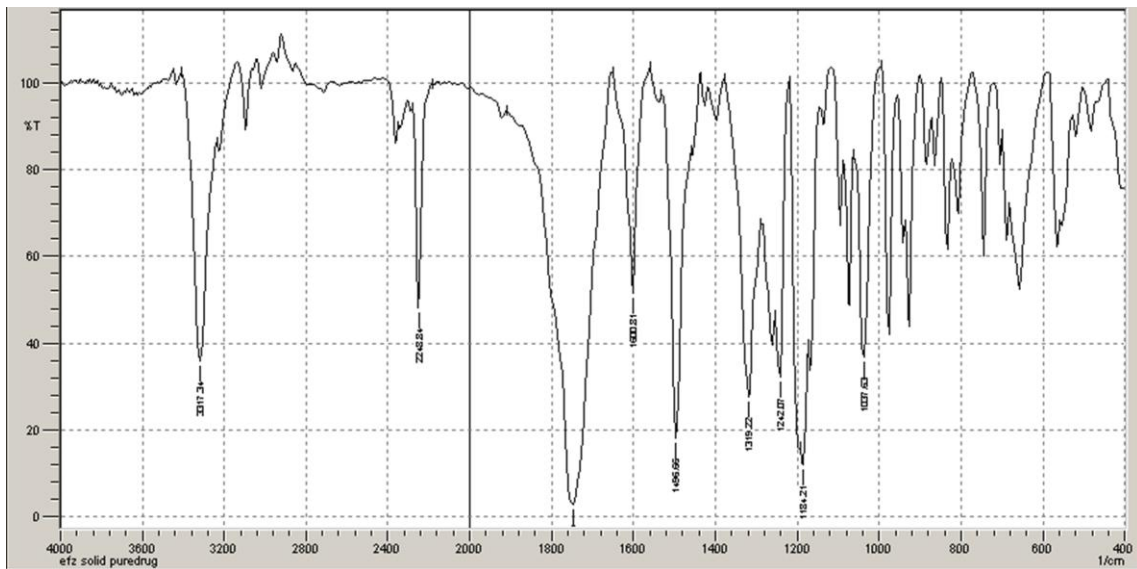


Figure 2: FTIR spectrum of Efavirenz standard (Pressed Pellete Technique).

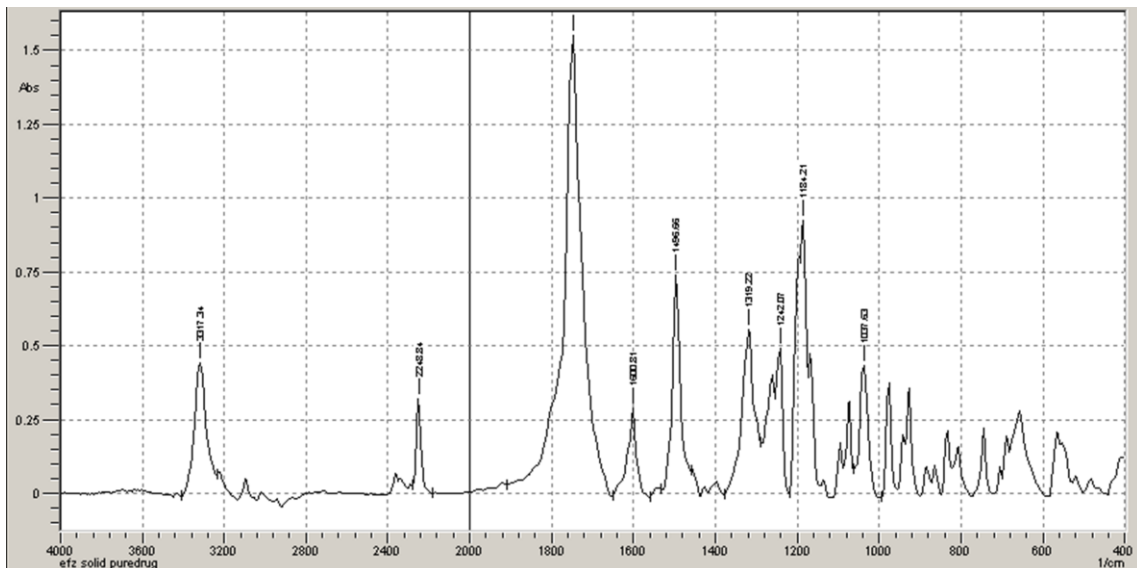


Figure 3: FTIR spectrum of efavirenz standard (Pressed Pellete Technique) in absorbance mode.

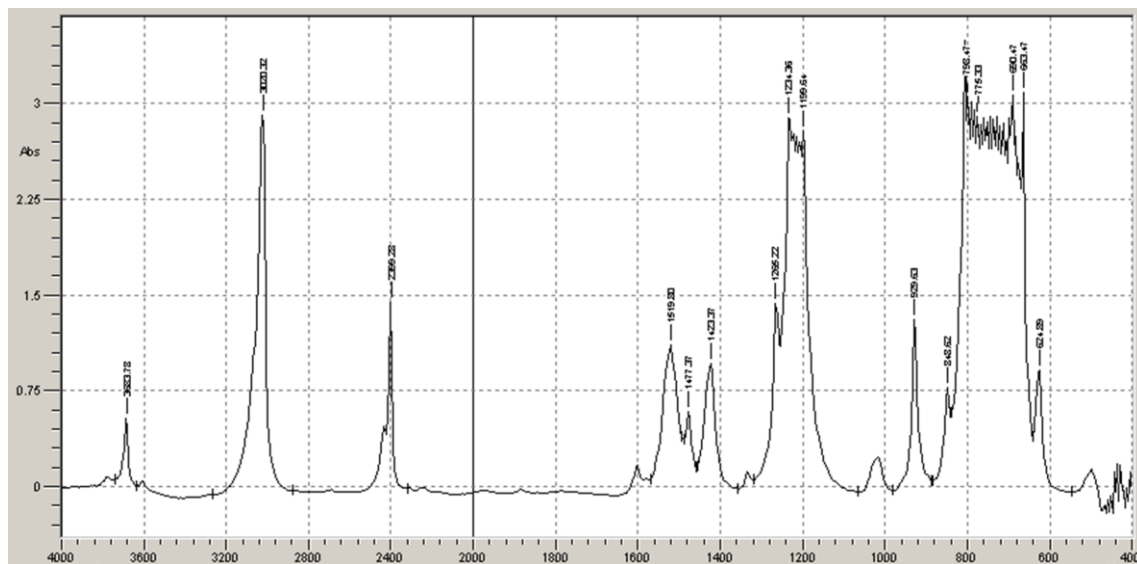


Figure 4: Blank spectrum of chloroform.

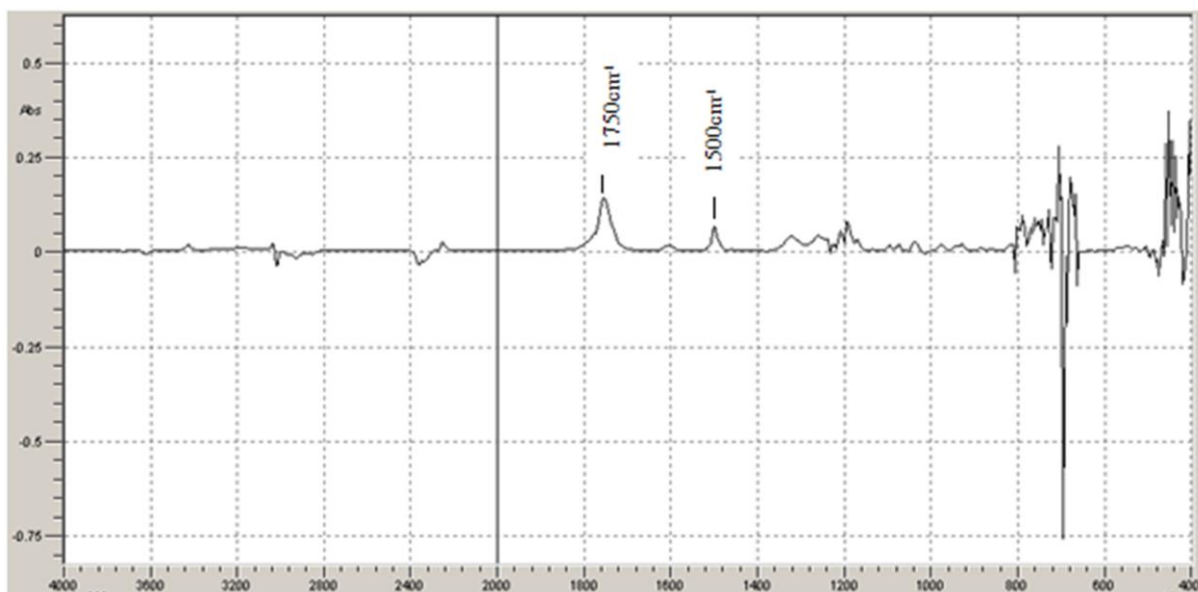


Figure 5: FTIR spectrum of efavirenz standard in chloroform (liquid sampling technique).

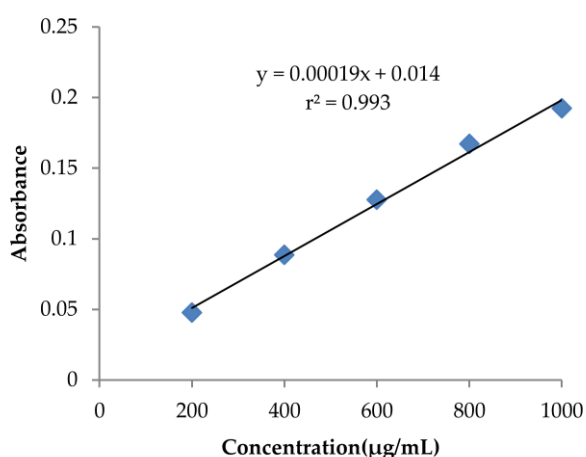


Figure 6: Standard calibration curve for efavirenz standard.

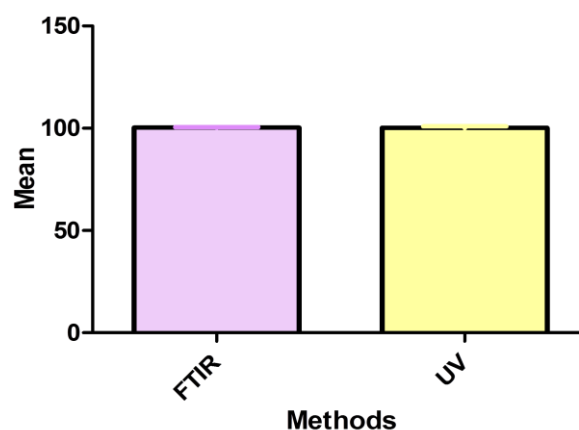


Figure 7: Bar diagram showing mean % recovery of FTIR and UV.

Method validation parameters

Linearity data

From the simple regression line described in figure 6, it was demonstrated acceptable efavirenz linearity over the 200-1000 µg/mL range with a quantification limit of 148 µg/mL. Furthermore, the y-intercept variability resulted in less than 1% of the response obtained at the targeted level which is usually an acceptable criterion. The statistical significance of the regression line ($y=0.00019x+0.014$, $r^2=0.993$) also revealed that the slope is not zero, which in turn suggested that changes in the concentration variable are highly associated with the changes in the response variable (table 1).

Limit of detection and limit of quantitation

The FTIR method developed was sensitive with detection level 49.117 µg/mL and quantitation level 148.842 µg/mL. They are calculated by using standard deviation method.

Precision

The precision of the developed analytical method was assessed by reporting repeatability, intra-day precision and inter-day precision. There was no much difference in absorbance values measured in sessions of the day and for three consecutive days for checking robustness of the method. The calculated RSD values are low and indicate the precision of the method (table 2, 3).

Accuracy

Considering that the expected recovery depends on sample matrix, sample processing procedure and the analyte concentration, the accuracy of the proposed method can be considered suitable for determining efavirenz content in solid dosage pharmaceutical products (table 4).

For drug substance

The calculated amounts of pure efavirenz at three different levels were near to the actual values, indicate the

Table 2: Intra-day precision data for Efavirenz.

Sr. No.	Conc. (µg/mL)	Mean of Absorbance*			Mean*±SD	% RSD
		Morning	Afternoon	Evening		
1	400	0.0872	0.0873	0.0892	0.0879±0.00112	1.2821
2	600	0.131	0.1276	0.1282	0.1289±0.00181	1.408
3	800	0.1698	0.166	0.1716	0.1691±0.00285	1.6907

*Average of three determinations

Table 3: Inter-day precision data for Efavirenz.

Sr. No.	Conc. (µg/mL)	Mean Absorbance* A			Mean ± SD	%RSD
		1 st day	2 nd day	3 rd day		
1	400	0.0872	0.0849	0.0873	0.0864±0.00135	1.5717
2	600	0.1310	0.1270	0.1305	0.1295±0.00217	1.6826
3	800	0.1698	0.1727	0.1696	0.1704±0.00198	1.1654

*Average of three determinations

Table 4: Accuracy data for efavirenz drug substance.

Sr. No.	Accuracy level	Conc. (µg/mL)	Absorbance A	Calculated conc. (µg/mL)	Mean*± SD	%RSD
1	80%	320	0.0742	316.84	322.1±4.821	1.496
			0.0754	323.15		
			0.076	326.31		
2	100%	400	0.0894	396.84	401.57±4.735	1.179
			0.0912	406.31		
			0.0903	401.57		
3	120%	480	0.10397	473.15	475.78±5.981	1.257
			0.10368	471.57		
			0.10572	482.63		

*Average of 3 determinations

Table 5: Recovery data for Efavirenz.

Sr. No.	Spike level	Total Conc. (µg/mL)	Absorbance* (A)	Conc. recovered (µg/mL)	% Recovery	Mean*±SD
1	80%	8.1	0.458	8.11	100.227	100.207±1.3081
2	100%	9	0.497	8.9	98.889	
3	120%	9.9	0.567	10.14	101.505	

*Average of three determinations

accuracy of the method for determination of pure efavirenz. From the above data it was found that the %RSD (acceptance criteria <2%) values are within the acceptance limits.

For drug product (Recovery study)

The method was found to be accurate for determination of efavirenz in tablets, as the % recovery values calculated are found to be within the acceptable limits (table 5).

FTIR and UV results comparison

The recovery values of FTIR and UV methods were compared by applying t-test for paired sample. The t-test was performed to prove that there is no significant difference in the recovery of new FTIR and existing UV method.

T-test

“There is no significant difference in the recovery values of new FTIR method and existing UV method” is the null hypothesis selected for comparison (Khan and Khanum, 2008). The calculated p-value is lesser than the tabulated value (p=0.05). It is clearly indicated that the two recovery

Table 6: Analysis of Efavirenz by two methods.

Method	Mean (%)	RSD (%)	N
FTIR	100.703	0.7335	9
UV	100.207	1.3054	9

N= Number of determinations at three levels each three replicates

Table 7: T-test data.

Sr. No.	Recovery (%)		Difference X1-X2=d	(X1-X2) ² =d ²
	FTIR (X1)	UV (X2)		
1	100.411	100.227	1.184	1.4018
2	99.937	98.889	1.048	1.0983
3	100.761	101.505	-0.744	0.5535
Total			Σd= 1.488	Σd ² =3.052

values are very much similar and difference is not significant. Therefore, the null hypothesis stating that there is no significant difference between the recovery values of two methods, new FTIR method and existing UV method is accepted at p=0.05. The difference between the recovery values of two methods is not a real difference because the level of significance is very low (table 7).

CONCLUSION

The developed FTIR method for determining efavirenz based on IR measurements in absorbance mode in the liquid phase, fulfilled most validation requirements in a range of concentrations suitable for quality control of both pure drug as active pharmaceutical ingredient and solid dosage forms. Therefore, this can be used as a quality control method as alternative in pharmaceutical industry. FTIR in the transmission mode mainly used for the identification of API in raw materials and finished products but undervalued for quantitative purposes. By

comparison with compendial and non-compendial analytical methods, the proposed FTIR method reduces solvent consumption and eliminates the use of reagents. The sensitivity of the method can be increased in further studies by taking derivative IR spectra using the solvents which are transparent in IR region.

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