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FT-IR METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF NORTRIPTYLINE AND PREGABALIN IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A Simple, rapid and cost-effective Fourier Transformation Infrared Spectroscopic method has been developed and validated for the estimation of Nortriptyline and Pregabalin in pharmaceutical tablet formulation. The quantitative analysis of Nortriptyline and Pregabalin by using KBr as diluent for analysis purpose. FT-IR range 1653 cm^{-1} for Nortriptyline (>C=C<) and 1643 cm^{-1} for Pregabalin (-COOH) were selected. This method was developed and validated for various parameters according to ICH guidelines. Linearity range were found to be 2-12 % w/w and 15-90 % w/w for Nortriptyline and Pregabalin, respectively. The correlation coefficient (r^2) were found to be 0.9957 for Nortriptyline and 0.997 for Pregabalin. Limit of Detection were found to be for Nortriptyline 2.0712 %w/w and 12.8513 %w/w for Pregabalin. Limit of Quantification for Nortriptyline and pregabalin were found to be 6.2763 %w/w and 38.9436 %w/w, respectively. In addition to force degradation studies were carried out by using various conditions like Thermal, Photolytic and Sunlight as per guidelines.

KEYWORDS: FT-IR Spectroscopy, Nortriptyline, Pregabalin, Method validation, ICH guidelines.

1. INTRODUCTION

Nortriptyline (NORT) is chemically 3-(5,6-dihydrodibenzo[2,1-b:2',1'-][7] annulen-11-ylidene)-N-methylpropan-1-amine.[2] Nortriptyline inhibits the reuptake of the neurotransmitter serotonin at the neuronal membrane or acts at beta-adrenergic receptors. Nortriptyline hydrochloride is *N*-demethylated active metabolite of amitriptyline. This metabolite is dibenzocycloheptene-derivative tricyclic antidepressant (TCA). Serotonin and norepinephrine reuptake are inhibited by TCAs. Pregabalin is chemically (3S)-3-(amino methyl)-5-methylhexanoic acid.[1] Pregabalin (PREGA) binds to an auxiliary subunit of voltage-gated calcium channels in central nervous system tissue with high affinity to the alpha2-delta site. Pregabalin reduces the calcium-dependent release of several neurotransmitters. This combination is used for nerve damage pain, seizures, depression, anxiety disorder in adults, and other conditions.[1-3]

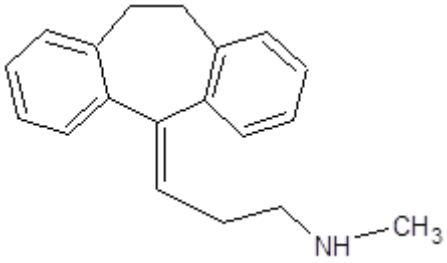

Fig:1 Structure of Nortriptyline.

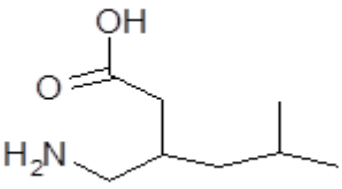

Fig: 2 Structure of Pregabalin.

Literature survey revealed that HPLC method has been reported for estimation of NORT and PREGA either as

single drug or in combination with other drugs. To best of our knowledge, no reports were found for development and validation on FTIR determination of NORT and PREGA in combined tablet dosage form.[7-14] Infrared spectroscopy (IR) provides a useful way for the identification of drugs and also used for quantitative measurements. A simple infrared spectroscopic method can be highly useful for routine analysis of bulk and formulation samples in comparison with expensive, skillful and time-consuming methods. Hence, the present study was the development and validation of a versatile, speedy FT-IR technique for simultaneous determination of NORT and PREGA as bulk and in tablet dosage form.[4-6] This present study was developed and validated as per the ICH guidelines. Results from method validation can be used to judge the quality, reliability and consistency of analytical results. It is an integral part of any good analytical practice.[4]

2. MATERIAL AND METHOD

2.1 Instrument

FT-IR spectrophotometer (IR-Affinity-1, Shimadzu Corp., Japan) was equipped with diffuse reflectance sampling interface and operated on computer with Shimadzu IR solution FT-IR control software was used for collection and data analysis.

2.2 Material and diluent

Working standard of Nortriptyline was obtained as gift sample from Alkem Laboratories Ltd, Mumbai, Maharashtra. Pregabalin was obtained gift sample from Cipla Pvt. Ltd, Mumbai. Potassium bromide was selected as a diluent because it was found that both the APIs are compatible with them and also it is transparent to IR-radiation.

2.3 Preparation of working standard

10 mg of pure NORT and PREGA drugs were mixed separately with 990 mg of KBr (Analytical Reagent grade) and then triturated well to made homogenous mixture.[5-6]

2.4 Selection of analytical wave number

Working standard (1 % w/w) of both drugs was prepared and scanned in IR range of 4000-400 cm^{-1} with resolution of 4 and 45 scans. Wave number was selected in such a way that one functional group of one drug should not present in another drug so that one can avoid interference of one drug in another. Wavenumber parameter is selected for both drugs, in that one can select wave number in a range. Functional group selected for NORT is C=C and wave number was found in range of 1653 cm^{-1}. Functional group selected for PREGA is COOH and wave number was found in range of 1643 cm^{-1}.

2.5 Preparation of calibration curve

The calibration curves were plotted for these concentrations against peak intensity obtained at respective wavenumber region. Appropriate quantity of standard NORT and PREGA were diluted with KBr to get concentration ranging from 2-12 % w/w and 15-90 % w/w for NORT and PREGA, respectively.

2.6 Analysis of marketed tablet formulation

Twenty tablets of marketed formulation NORTIPAN containing 10 mg NORT and 75 mg PREGA, were accurately weighed and average weight was calculated, then these tablets were crushed into a fine powder using a mortar and pestle to reduce the particle size. The quantity of powder equivalent to 2 mg and 15 mg of NORT and PREGA taken respectively and scanned in IR range of 4000-400 cm^{-1} with resolution of 4 and 45 scans. The analysis procedure was repeated six times with tablet formulation.[7-8]

2.7 Method validation

2.7.1 Linearity

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. The linearity of NORT and PREGA of proposed method was found to be in range 2-12 % w/w and 15-90 % w/w, respectively.[9]

2.7.2 LOD and LOQ

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.[10-11]

Limit of detection and quantification, According to ICH recommendation.

$LOD = 3.3\ \sigma / S$

$LOQ = 10\ \sigma / S$

Where, σ = standard deviation of the response

S = slope of the corresponding calibration curve

2.7.3 Precision

The precision of an analytical procedure expresses the degree of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The precision of the method was evaluated by inter-day and intraday variation studies. In intraday studies, working dilutions of sample were analysed triplicate in a day and percentage relative standard deviation (%RSD) was calculated. Interday precision was carried out by performing the assay of six sample sets after 24 hours on three consecutive days and % RSD was calculated.[12]

2.7.4 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. To ascertain the accuracy of the proposed methods, recovery studies were performed at three different levels i.e. 80 %, 100 % and 120 % by standard addition procedure as per the ICH guidelines.[13-14]

2.8 Force Degradation Studies

Stress testing is different from accelerated testing because the studies are carried out under more severe conditions. The studies normally include exposure of the drug to elevated temperatures and humidity, UV light and oxidizing agents, as well as susceptibility to hydrolysis across a range of pH values. Force degradation studies were performed on NORT and PREGA to prove the stability indicating property of the method. The stress conditions applied for degradation study involved thermal, photolytic and sunlight degradation.[16-17]

3. RESULT AND DISCUSSION

3.1 Validation of developed method

Validation parameters were studied as per the ICH guideline.

3.1.1 Linearity

The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the NORT and PREGA in the range of 2-12 % w/w and 15-90 % w/w, respectively. The calibration curve is described by equation y=0.0706x+0.0027 for NORT. The calibration curve is described by equation y=0.005x+0.2051for PREGA.

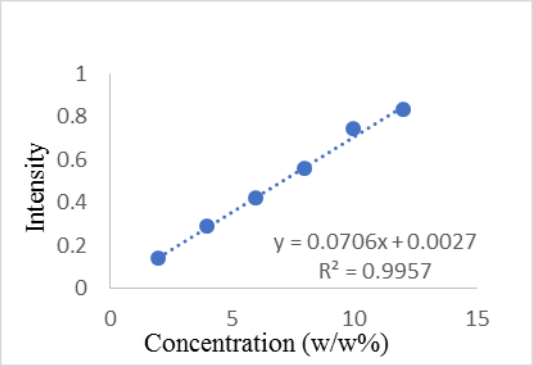

Fig 3: Calibration curve of NORT.

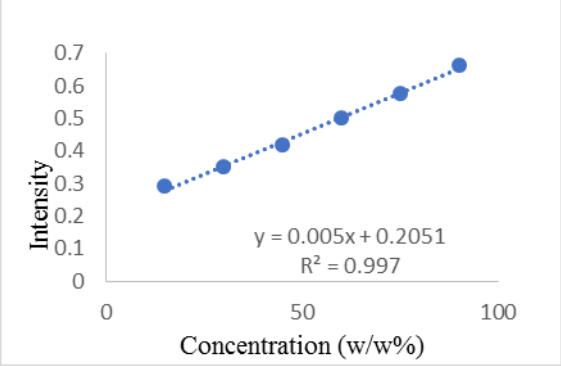

Fig 4: Calibration curve of PREGA.

Table 1: Linearity study data of NORT.

Sr. No.	Concentration (% w/w)	Intensity ($1653cm^{-1}$)
1	2	0.140
2	4	0.290
3	6	0.420
4	8	0.560
5	10	0.740
6	12	0.830

Table 2: Linearity study data of PREGA.

Sr. No.	Concentration (% w/w)	Intensity ($1643cm^{-1}$)
1	15	0.290
2	30	0.350
3	45	0.418
4	60	0.501
5	75	0.576
6	90	0.659

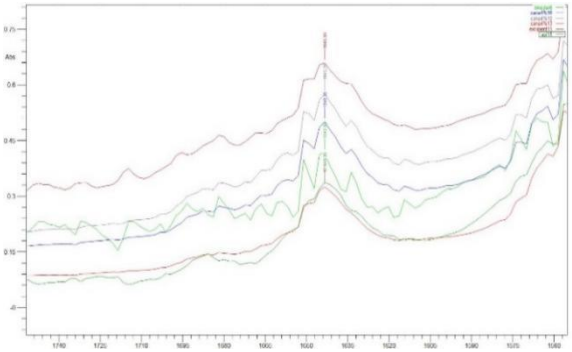

Fig 5: Overlain spectrum of NORT.

Fig 6: Overlain spectrum of PREGA.

3.1.2 LOD and LOQ

LOD was 2.0712 % W/W and 6.2763 % w/w while LOQ was 12.851 % w/w and 38.943 % w/w for NORT and PREGA, respectively shown in table 3.

Table 3: LOD and LOQ.

Drugs	LOD (%w/w)	LOQ (%w/w)
NORT	2.0712	6.2763
PREGA	12.8513	38.9436

3.1.3 Precision

Repeatability study expressed by %RSD for NORT and PREGA were 0.4485 % and 0.3878 %, respectively. Repeatability study was shown in table 4. The precision was expressed by coefficient of variation (% RSD) and accuracy by mean and standard deviation. For day 1 precision studies, % RSD value for three sample 1.0646 % and 0.8656 % was observed while for day 3 precision studies the 0.8664 % and 0.8816 % was observed for NORT and PREGA, respectively. Precision data was shown in table 5.

Table 4: Results of repeatability study.

Sr. No.	Concentration (%w/w)		Absorbance		% Found	
	NORT	PREGA	NORT	PREGA	NORT	PREGA
1.	2	15	0.416	0.575	99	99.8
2.	2	15	0.420	0.574	100	99.6
3.	2	15	0.418	0.580	99.5	100.6
4.	2	15	0.420	0.578	100	100.3
5.	2	15	0.421	0.579	100.2	99.8
6.	2	15	0.418	0.578	99.5	100.3
			Mean*		99.70	100.06
			SD		0.4472	0.3881
			%RSD		0.4485	0.3878

*Indicates average of six determinations.

Table 5: Results of precision study.

Sr. No.	Interval of time	Concentration (%w/w)		% Found	
		NORT	PREGA	NORT	PREGA
I	**Intra-day**	2	15	101.6	101.9
II		2	15	99.5	100.2
III		2	15	100.20	100.7
		Mean*		100.43	100.93
		SD		1.069	0.8736
		% RSD		1.064	0.8656
I	**Inter-day**	2	15	100.6	99.04
II		2	15	100.79	100.8
III		2	15	99.20	100
		Mean*		100.19	99.94
		SD		0.8683	0.8812
		% RSD		0.8664	0.8816

*Indicates average of three determinations

3.1.4 Accuracy

The accuracy of the assay method was evaluated with the recovery of pure drug excipients at three different level (80 %, 100 %, 120 % w/w of label claim) by standard addition method and the recovery data in table 6 and 7. Good recoveries of NORT and PREGA were obtained in the range of 99-101 %.

Table 6: Results of recovery study.

Level of Recovery	Amount percent (mg)		Added concentration (mg)		Amount recovered (mg)		% Recovery	
	NORT	PREGA	NORT	PREGA	NORT	PREGA	NORT	PREGA
80 %	2	15	1.6	12	3.595	26.95	99.86	99.81
	2	15	1.6	12	3.607	27	100.19	100.00
	2	15	1.6	12	3.605	26.98	100.13	99.92
100 %	2	15	2	15	3.99	29.94	99.94	99.8
	2	15	2	15	4.006	30.01	100.15	100.03

	2	15	2	15	4.00	30.05	100.00	100.16
120 %	2	15	2.4	18	4.399	32.83	99.97	99.48
	2	15	2.4	18	4.404	32.97	100.09	99.90
	2	15	2.4	18	4.401	33.04	100.02	100.12

Table 7: Statistical validation of recovery study data.

Level of Recovery	% Mean Recovery*		SD		% RSD	
	NORT	PREGA	NORT	PREGA	NORT	PREGA
80 %	100.04	99.91	0.21	0.095	0.2099	0.09548
100 %	100.03	99.99	0.108	0.182	0.1081	0.1823
120 %	100.02	99.83	0.0602	0.325	0.0602	0.3257

*Indicates average of three determinations

3.2 Analysis of marketed tablet formulation

Analysis of marketed tablet was studied by calculating %label claim in the range 99-101 %. %RSD were found to be 1.616 and 1.335 for NORT and PREGA, respectively. Results are shown in table 8.

Table 8: Results of analysis of tablet formulation.

Sr. No.	Amount taken(mg/Tab)		Amount found (mg /tab)		% Label claim	
	NORT	PREGA	NORT	PERGA	NORT	PREGA
1.	2	15	1.96	14.91	98	99.44
2.	2	15	1.98	14.98	99	99.90
3.	2	15	1.96	14.65	98	97.69
4.	2	15	2.00	15.10	100.0	100.69
5.	2	15	2.03	15.19	101.7	101.29
6.	2	15	2.02	15.16	101.3	101.06
			Mean*		99.68	100.01
			SD		1.611	1.335
			%RSD		1.616	1.335

*Indicates average of six determinations

3.3 Forced degradation studies

3.3.1 Thermal degradation

Thermal degradation was carried out by exposing pure drugs separately to dry heat at 80ºC for 4 hrs. The samples after exposure to heat were diluted or mixed with KBr to get NORT (2 % w/w) and PREGA (15 % w/w). % degradation were found to be 5.714 % and 12.5 % for NORT and PREGA, respectively.

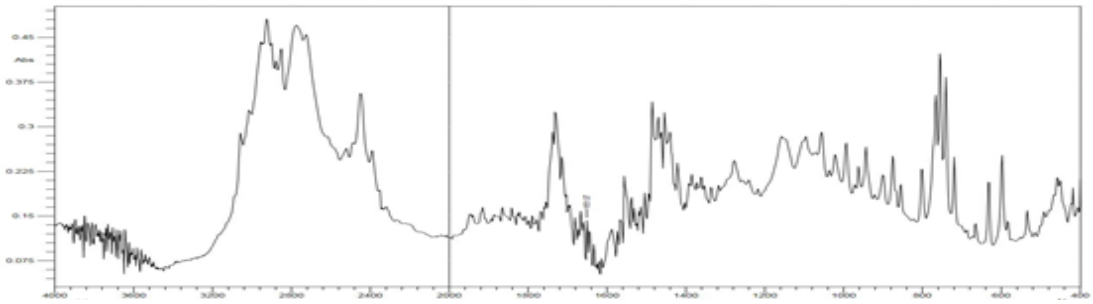

Fig 7: Thermal degradation of NORT.

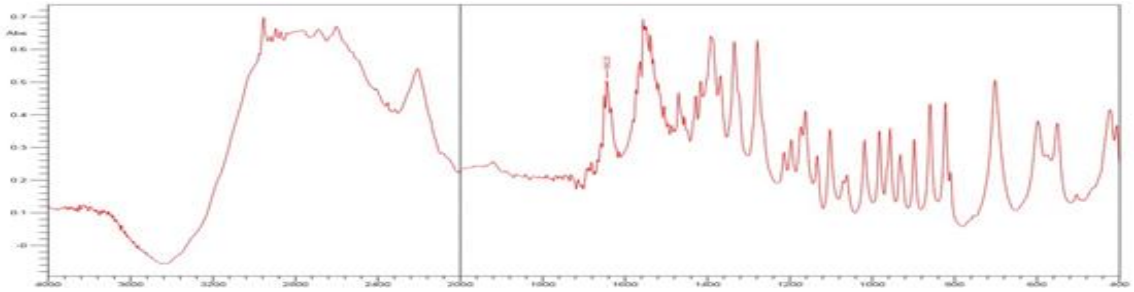

Fig 8: Thermal degradation of PREGA.

3.3.2 Photolytic degradation

Pure drugs were exposed to UV radiations and samples were withdrawn after 4 hrs. The samples after exposure to light were diluted with KBr to get NORT (2% w/w) and PREGA (15 % w/w). % degradation for NORT and PREGA were found to be 28.57 % and 3.99 %, respectively.

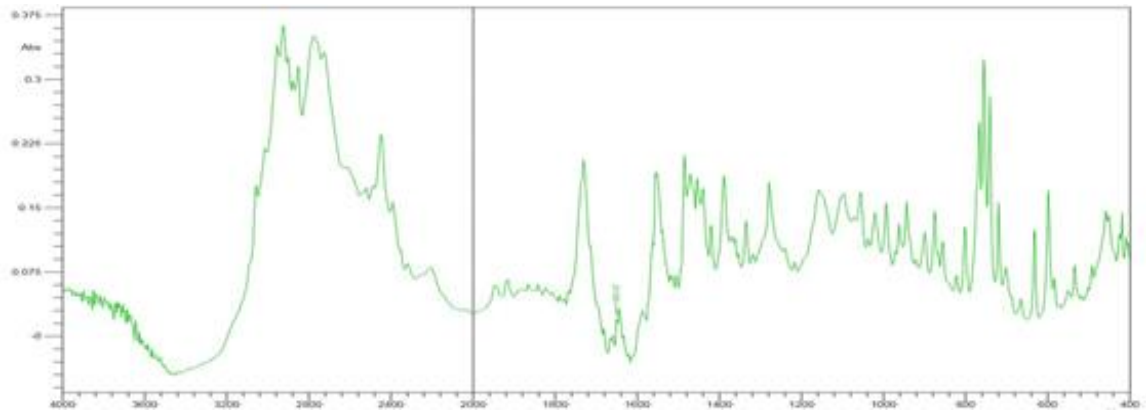

Fig 9: Photolytic degradation of NORT.

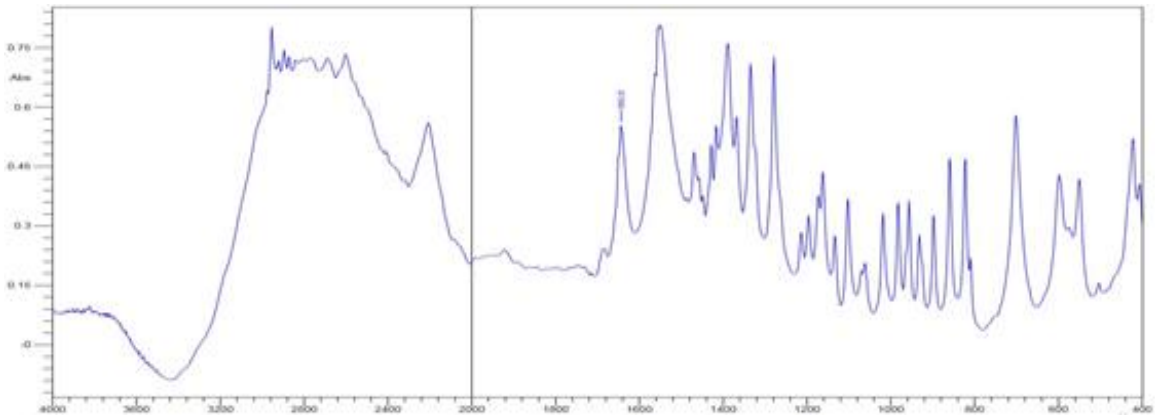

Fig 10: Photolytic degradation of PREGA.

3.3.3 Degradation in Sunlight

Sunlight degradation is performed by exposing the pure drugs to sunlight in open space for 4 hrs. The samples after exposure to sunlight were diluted or mixed with KBr to get NORT (2 % w/w) and PREGA (15 % w/w). % degradation were found to be 7.64% and 11.42% for NORT and PREGA, respectively.

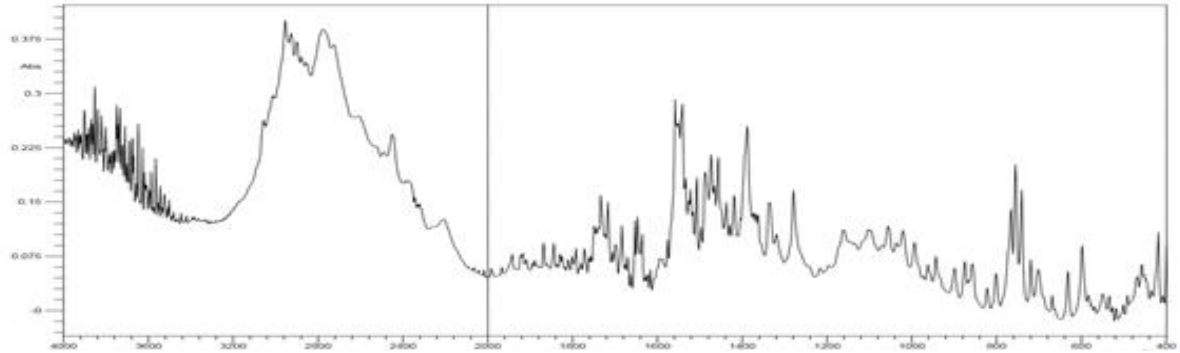

Fig 11: Sunlight degradation of NORT.

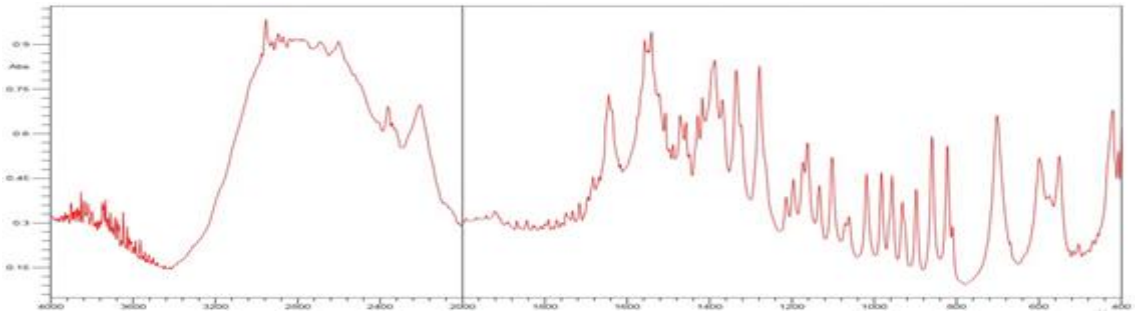

Fig 12: Sunlight degradation of PREGA.

Table 9: data for forced degradation study.

Degradation Condition	% Degradation		% Assay	
	NORT	PREGA	NORT	PREGA
Thermal degradation at 80°c 4 hrs.	5.714	12.5	94.286	87.5
Photolytic degradation UV-radiation, 4 hrs.	28.57	3.99	71.43	96.01
Sunlight degradation 4 hrs.	7.64	11.42	92.36	88.58

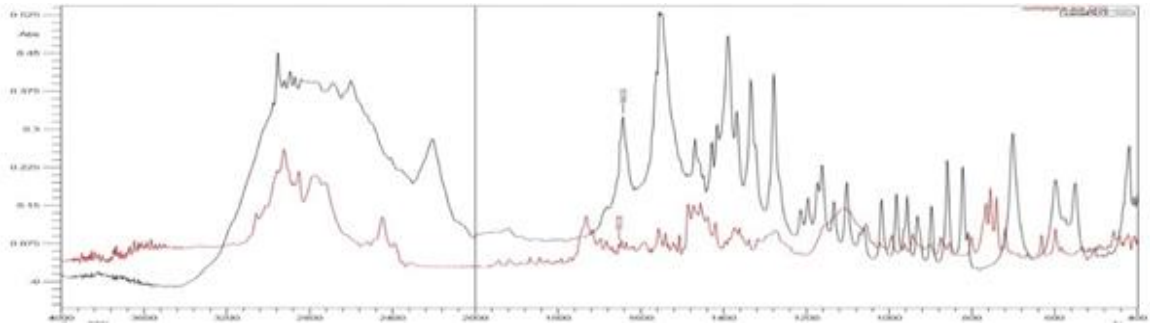

Fig 13: FT-IR overlay spectrum of NORT and PREGA.

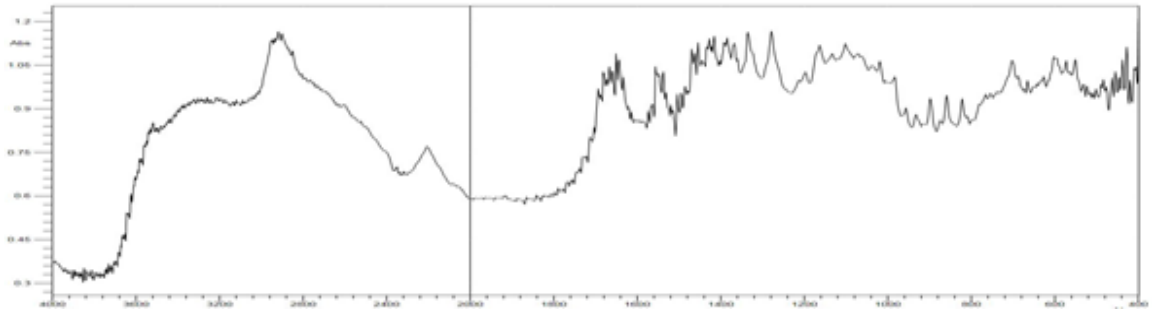

Fig 14: FT-IR spectrum of tablet formulation.

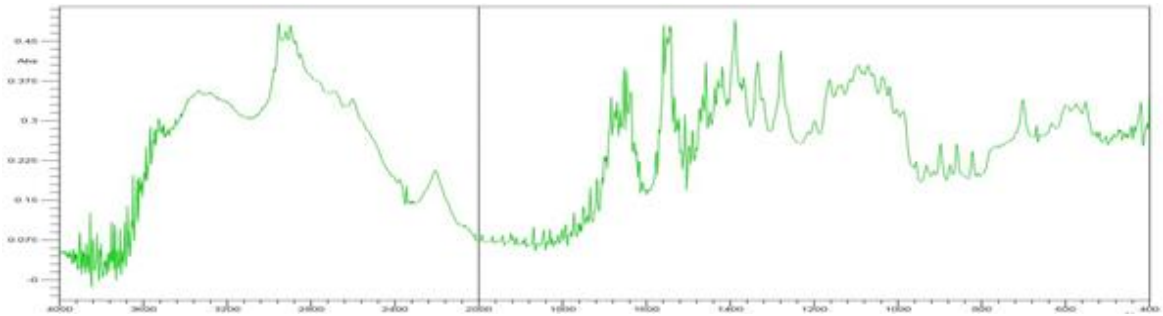

Fig 15: FT-IR spectrum of tablet excipient.

4. CONCLUSION

A Simple, rapid and cost-effective Fourier Transformation Infrared Spectroscopic method has been developed and validated for the qualitative analysis of pharmaceuticals. FT-IR spectroscopy may serve as useful technique for qualitative and quantitative analysis of pharmaceuticals. This method avoids the use of hazardous chemicals or solvent and involving relatively

simple sample preparation leads to an economical and environmental friendly method. In present work reported that, the development and validation of FT-IR method for the quantification of NORT and PREGA in solid state pharmaceutical.

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