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Research Article

FT-IR SPECTROSCOPIC APPROACH FOR THE QUANTITATIVE ANALYSIS OF FEW COMMERCIAL DRUGS IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Simple, environment-friendly, rapid, accurate and cost effective Fourier Transform Infrared Spectroscopic (FT-IR) methods have been developed for the quantification of Bosentan (BSN), Desloratadine (DSD) and Flunarizine (FNZ) in bulk and marketed formulations. These drugs were estimated by several techniques and a new method has been developed by using FT-IR technique. The methods were developed on the basis of sample concentration influences the intensity of the vibrational bands and linearity is expected between concentration and optical density. The quantity of the drug present in commercial tablets with no interference of the excipients was estimated by applying this proposed method. This method has been validated in terms of LOD, LOQ, precision, accuracy, % RSD, robustness and ruggedness. A factor affecting the absorbance viz., concentration of drug is optimized. The presence of excipients has also been examined and found no significant effect. The calibration curves are found effective for the assessment of pure drug and pharmaceuticals. These curves also can be applied in bulk drug and pharmaceutical industries.

Keywords: FT-IR, Drugs, linearity, Vibrational bands, Validation.

1. INTRODUCTION

1.1. Bosentan (BSN)

Bosentan, chemically "N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-Pyrimidin-2-yl-pyrimidin-4-yl]-4-tert-butyl-benzenesulfonamide" works as a competetive antagonist of ET-1 (ET=endothelin) at receptors of ET-A & ET-B. It inhibits the binding of ET-1 to receptors of ET-A & ET-B which restrict the process of narrowing of the pulmonary blood vessels. Hence became a choice of drug to treat pulmonary artery hypertension [1, 2].

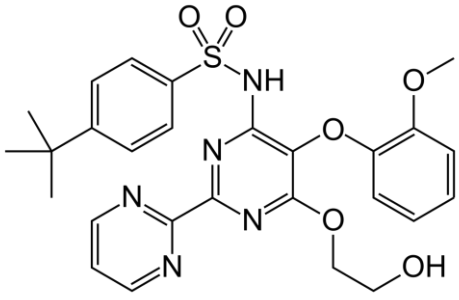

Fig. 1: Structure of Bosentan

Thorough literature studies acknowledged that various methods are reported for the quantification of BSN both in finished dosage forms and biological fluids, such as Spectrophotometry [3], RP-HPLC [4,5] and HPTLC [6].

1.2. Desloratadine (DSD)

Desloratadine (DSD), chemically "4-[(E)-4-(4-hydroxy phenyl) hex-3-en-3yl] phenol" alleviates the allergy problems like runny nose, sneezing, watery eyes etc. DSD restricts the histamine production as it acts as an inverse agonist to the histamine H1 receptor. Hence, this became a commonly prescribed anti histamine drug [7, 8].

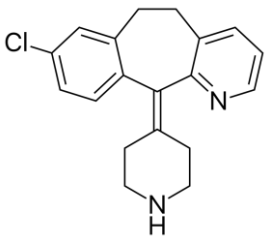

Fig. 2: Structure of Desloratadine

Literature study acknowledged that various analytical and bioanalytical techniques were reported for the analysis of

DSD. These techniques include Spectrophotmetric [9], RP-HPLC [10], HPTLC [11, 12] and Flourimetry [13].

1.3. Flunarizine (FNZ)

Flunarizine, Chemically "1-[Bis(4-fluorophenyl) methyl]-4-[(2E)-3-phenylprop-2-enyl]piperazine" is a choosy calcium antagonist drug which accompanied by other gentle activity as a serotonine receptor, antihistamine and dopamine D_2 blocker. FNZ has less affection to VGCS (Voltage dependent Calcium channels) and works by antagonizing the calcium modulating protein in the cells but not inhibiting the calcium entry into the cells. Extensive literature analysis confirmed that FNZ is determined by various methods such as Spectro-photometry [14], RP-HPLC [15], HPTLC [16], Spectrofluorimetry [17] and Titrimetry [18].

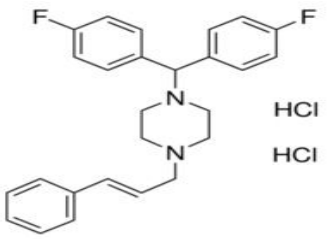

Fig. 3: Structure of Flunarizine Dihydrochloride

The sample concentration influences the intensity of the vibrational bands and linearity is expected between concentration and optical density. This forms the basis for quantification of drugs by using this technique.

FT-IR spectroscopy is a rapid, accurate technique and it needs minimum sample quantity for the analysis. These results accuracy is comparable with other established quantification methods. Pharmaceutical samples in any physical state can be scanned and averaged up to 49 times in less than one minute by using this technique at a high wavelength precision with a high resolution

Moreover, statistical analysis based on the spectral information obtained can be automatically done by the selection of the IR region in terms of peak area, peak height or peak ratio which is most suitable for quantitative determination [19-23].

Objective: To develop simple, environment friendly, rapid, accurate and cost effective FT-IR methods for the quantification of BSN, DSD and FNZ in bulk and marketed formulations.

Problem Statement: FT-IR techniques were developed based on the sample concentration influences the intensity of the vibrational bands and linearity is expected between concentration and optical density.

2. EXPERIMENTAL

2.1. Instrumentation

The Infra Red spectra required for the study have been recorded on SHIMADZU Prestige-21 FT-IR Spectro-photometer using NaCl Cells of 0.1 mm path length. Samples were weighed by using a Dhona 200 single pan electrical balance.

2.2. Material

Throughout the investigation Spectrograde Chloroform was used and all reagents used were of analytical-reagent grade.

2.3. Preparation of drug solution

To prepare drug standard stock solutions, 2grams of each drug accurately weighed were transferred into a 100 ml standard flask and dissolved with Chloroform upto the mark. The stock solution of BSN, DSD and FNZ were further diluted with the same solvent to obtain working concentrations.

2.4. Procedure

Different volumes of drug standard working solutions of 1-10mL were transferred into a series of 10mL calibrated flasks and remaining volume of each flask filled with chloroform up to the mark. Each solution was injected separately into a NaCl cell with the help of a clinical syringe. After 2 minutes, each solution spectra was recorded against a correspondingly prepared blank. The spectra of each sample at three different concentrations have been recorded. A standard spectrum for each drug was taken by plotting a graph between concentration of the drug and % transmittance and results computed from the regression equation derived using Beer's law.

2.5. Assay of pure drug sample

To assess the accuracy and precision of the methods developed, pure sample solutions containing drug in the Beer's Law limit were chosen. For this study, 2.5-25 mg $mL^{-1}$of BSN, 1.6-16 mg mL^{-1} of DSD and 2.0-20 of mg mL^{-1} of FNZ were taken.

For all the drugs, calibration curves were constructed by plotting the concentration versus the absorbance of drugs. For each solution, absorbance data was collected with three replicate experiments and relative responses of each solution were calculated from the absorbance to concentration ratio. Calibration curves were constructed by considering the relative response values which are only in between 95% to 105% of average.

2.6. Procedure for assay of pure drug

Accuracy and precision of the sample solutions of each drug was checked through performing recovery experiments by choosing them in beer's law limit. Also standard deviation method adapted for this purpose, concentration chosen and % of recovery of each is tabulated in Table 4. Calculations of %RSD values (less than 2) and excellent recovery values disclose the precision and accuracy of these drugs by developed method.

2.7. Analysis of tablets

2.7.1. Bosentan

Four tablets (Bosentas-62.5mg) were taken and grounded finely. A small quantity of tablet powder amounting 200mg was weighed carefully and transferred into a clean 50 ml volumetric flask containing chloroform. This solution was kept aside for few minutes, stirred thoroughly and filtered through a filter paper, washed the residue with the same solvent and adjusted the remaining volume to the mark with the same solvent. It was used as stock sample solution and aliquot of this stock solution further diluted with the chloroform to get working standard solutions within the Beer's Law limit to complete the assay by following the above mentioned procedures.

2.7.2. Deslortadine

Thirty tablets of Dloratin (5mg) were taken and grounded finely. A small quantity of the tablet powder amounting 100mg was weighed carefully and dissolved by transferring into a 100 mL calibrated flask which contained solvent. This solution was stirred thoroughly and filtered through a filter paper. Same solvent was used to wash the residue and to adjust the remaining volume to the mark. It was considered as stock sample solution and aliquot of this stock solution further diluted by adding the same solvent to get the working standard concentrations within the Beer's Law limit to complete the assay by following the above mentioned procedures.

2.7.3. Flunarizine

Thirty tablets of Migarid contain each 10mg of Flunarizine as active ingredient were taken and grounded finely. From this, 250mg of powdered drug exactly weighed, transferred into a100mL calibrated flask and dissolved in chloroform. This solution was kept aside for few minutes, stirred thoroughly and filtered through a filter paper. The residue was washed and adjusted the remaining volume to the mark with the same solvent. This solution was used as a stock sample solution for further experiments.

2.8. Method of validation

Each developed quantification method has been verified in terms of accuracy, precision, limit of quantification, limit of detection, selectivity, linearity and ruggedness. Absorbance-Concentration curves were drawn, fixed time method was used to assess the recovery of the drug. To assess the precision, each experiment was repeated at least 6 times, accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery, RSD being less than 2 for each drug demonstrates accuracy and precision of the methods (Table 2).

For each drug, limit of detection (LOD) is determined from the standard deviation of y-intercepts of regression lines of replicate determinations.

$LOD = 3.3\ s_a / S$

here s_a = standard deviation of intercept (n=6)

S = slope of linearity plot

Limit of Quantification (LOQ) is the minimum concentration used by an analyst to construct the calibration curve is also determined.

$LOQ = 10s_a/S$.

Limits of linearity of calibration curves (Fig. 5) are mentioned under the title Beer's law limit.

Selectivity was tested through performing recovery experiments by adding known excipients of each drug to its pure sample.

To test the selectivity of these developed methods, each pure drug sample was added with its known excipients and also performed recovery experiments. Ruggedness is resistance of method for a small change in variables like instrument, analyst or both to test the ruggedness of the method absorbance data was collected using 2 analysts and 3 different instruments, no considerable changes were observed either by change of analyst or instrument hence the developed may be considered as robust.

2.9. Factors affecting absorbance

2.9.1. Effect of concentration of Drug

Various volumes of drugs with random concentrations were separately added to the fixed volume of chloroform. The spectrum (frequency (600-4000Cm^{-1}) vs % Transmittance) were plotted with each solution and observed that drugs obeyed Beer's law up to certain range of concentration above which linearity was disobeyed. This concentration range is considered as optimum and stock solutions were prepared for BSN (Table 1), DSD ((Table 2) and FNZ ((Table 3).

2.10. Analysis of pharmaceuticals

To test the applicability of the method developed, solution of pharmaceutical tablets containing drug in the Beer's Law limit were chosen. To assess the precision, each tablet analysis was repeated at least 6 times, accuracy was estimated in terms of percent recovery and percent RSD. Excellent percent recovery, RSD being less than 2 for each drug demonstrates applicability of the methods for pharmaceutical analysis (Table 5). The excellent recovery observation reveals that these developed methods can be implemented to pharmaceutical analysis without any hesitation.

3. RESULTS AND DISCUSSION

3.1. IR band assignment and construction of calibration curves

3.1.1. Bosentan

3.1.1.1. Band assignment

The IR spectrum of Bosentan is shown in Fig. 1. From this spectrum, it is clear that significant peaks are exhibited by the instrument and these frequencies may be assigned as follows:

800cm^{-1}(-C-H stretch (para di substituted benzene)), 1114.86cm^{-1} (-C-O stretch (ether))1138cm^{-1} (-C-N stretch), 1178.51cm^{-1}(-C-O stretch (alcohol)) 1251.8cm^{-1}(-C-O stretch (alkyl aryl ether)), 1444.68cm^{-1} (-S=O stretch), 1473.62cm^{-1}(-C-H bending (alkane)), 1521.84cm^{-1} (-C=C stretch (aromatic)), 1560.41cm^{-1} (-C=C stretch (aromatic)).

3.1.1.2. Linearity observed frequencies and Calibration

The data related to frequency Vs Optical density at 5mL concentration is tabulated below.

Frequency (cm^{-1})	800	1178.51	1473.62	1521.84
Optical density	0.235	0.26	0.207	0.279

From the spectral data analysis of 1-10 mL of drug solution, it was observed that optical densities of a few frequencies (4) exhibited linearity against drug concentration (Fig. 4)

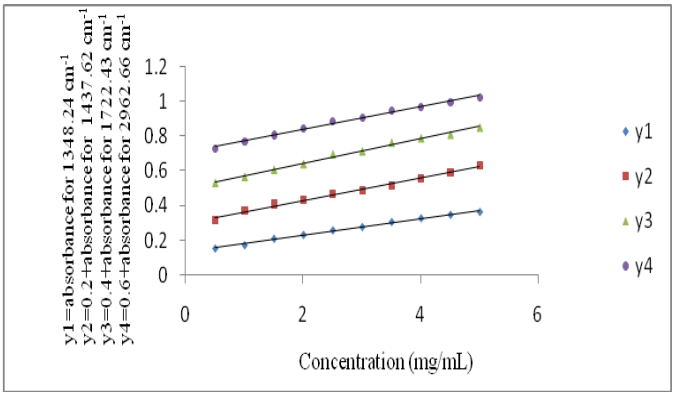

Fig. 4: Calibration curves of Bosentan

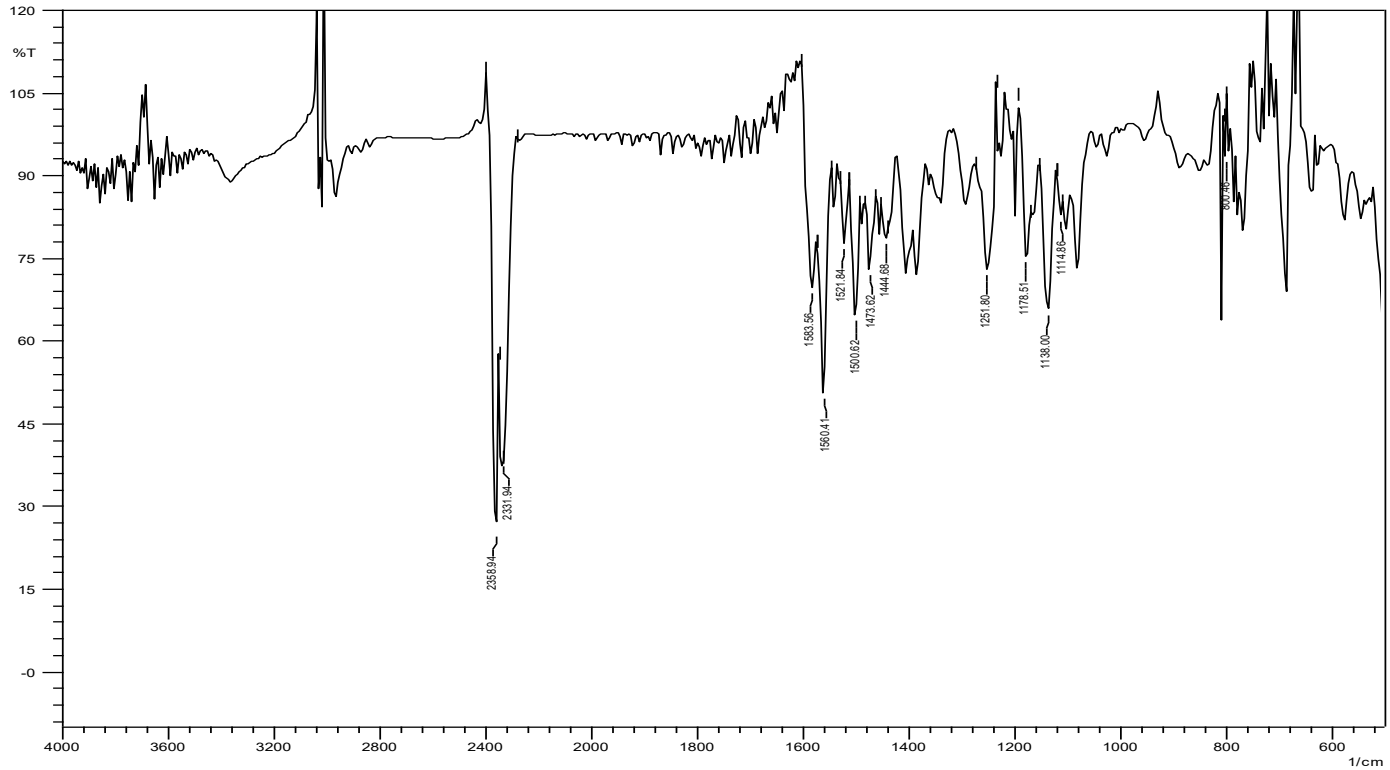

Fig. 5: IR Spectrum of Bosentan

3.1.2. Deslortadine

3.1.2.1. Band assignment

The IR spectrum of Deslortadine is shown in Fig. 7. From this spectrum, it is clear that significant peaks are exhibited by the instrument and some of these frequencies may be assigned as follows:

at 1116.78cm^{-1}, 1178.51cm^{-1}, 1247.94cm^{-1}, 1286. 52cm^{-1} (-C- N stretch), 1363.67cm^{-1}, 1438.90cm^{-1} (-C=C stretch (aromatic)), 1477.47cm^{-1} (-N-H bend), 1560.41cm^{-1} (-C=C stretch(aromatic)), 1589.34cm^{-1} (-C=C bending(aromatic)), 1637.56cm^{-1} (-N-H bending (amine)), 2860.43cm^{-1}, 2947.23cm^{-1} (-C-H stretch (alkane)) and 3020.53cm^{-1} (–C-H stretch (alkene)).

3.1.2.2. Linearity observed frequencies and Calibration

The data related to frequency Vs Optical density at 5mL concentration is tabulated below:

Frequency (cm^{-1})	1477.47	1560.41	2947.23
Optical density	0.658	0.316	1.145

From the spectral data analysis of 1-10 mL of drug solution, it was observed that optical densities of a few frequencies (3) exhibited linearity against drug concentration (fig. 6).

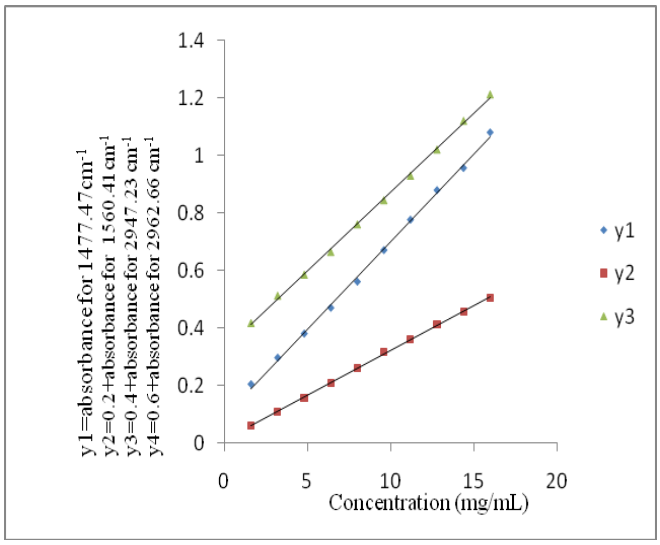

Fig. 6: Calibration Curves of Deslortadine

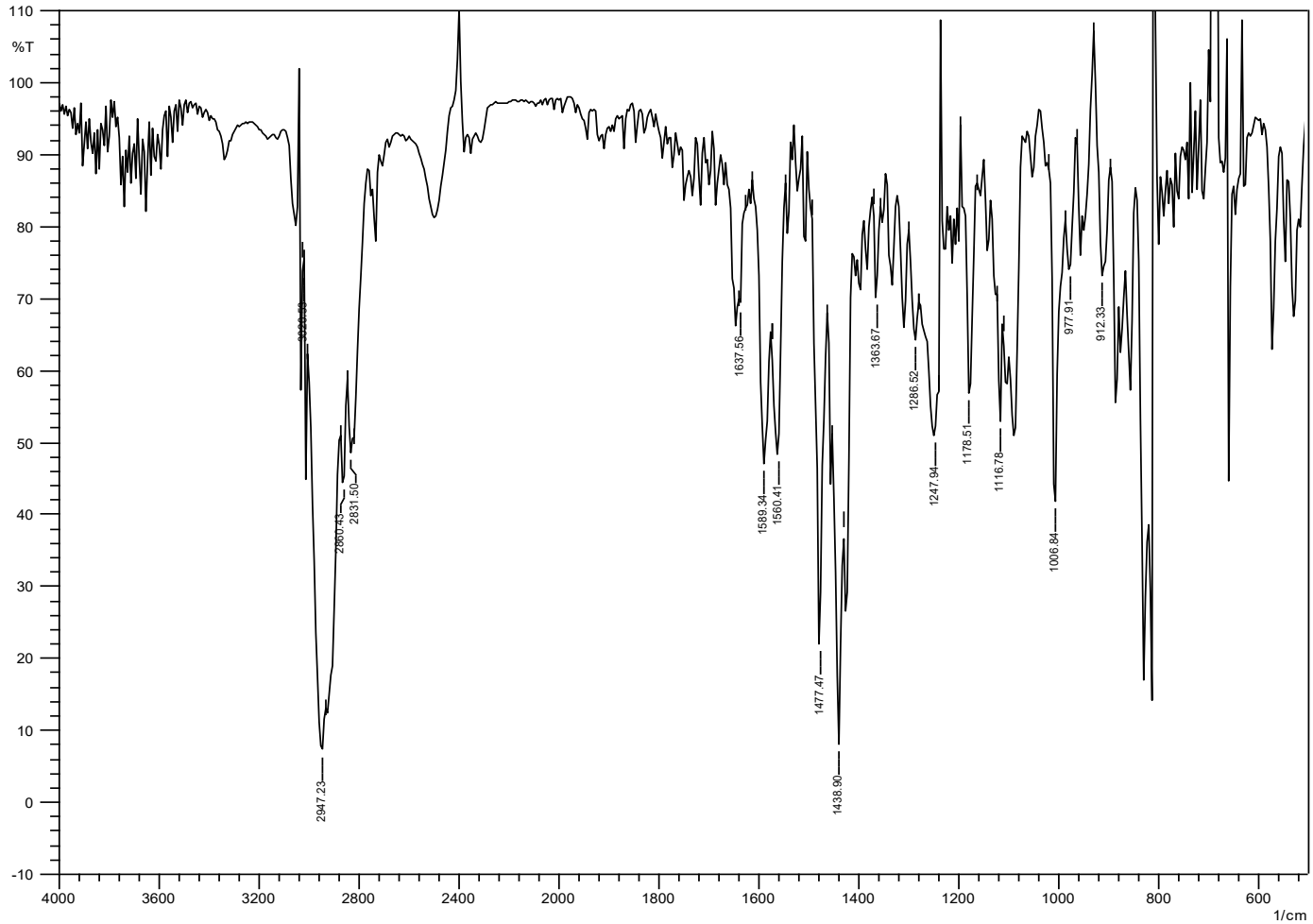

Fig. 7: IR Spectrum of Deslortadine

3.1.3. Flunarizine

3.1.3.1. Band assignment

The IR spectrum of Flunarizine is shown in Fig. 9. From this spectrum, it is clear that significant peaks are exhibited at 974.05cm^{-1} (-C=C bending (disubstituted (trans)), 1165cm^{-1} (-C-N stretching), 1242.16cm^{-1} (-C-F stretching), 1377.17cm^{-1} (-C-H bending (alkene)), 1454.33cm^{-1} (-C-C=C asymmetric stretch), 1606.7cm^{-1} (-C-C=C asymmetric stretch), 2308.79cm^{-1}(-C-H stretch (alkane)), 2983.88cm^{-1} (-C-H stretch (alkane)), and 3032.1cm^{-1} (-C-H stretch (alkenyl)).

3.1.3.2. Linearity observed frequencies and Calibration

The data related to frequency Vs Optical density at 5mL concentration is tabulated below:

Frequency (cm^{-1})	1165	2308.79	2983.88	1514	1242.16
Optical density	0.42	0.4037	0.6728	1.1036	1.1018

From the spectral data analysis of 1-10 mL of drug solution, it was observed that optical densities of a few frequencies (5) exhibited linearity against drug concentration (Fig.8).

As discussed above for BSN, DSD and FNZ, a few IR frequencies exhibited linearity for calibration and remaining showed a scatter. Certain (scattered) frequencies disobeyed from linearity may be attributed due to interactions between solute-solute particles, nature of vibrations such as bending, combinations and also other factors which could not be evaluated a priori.

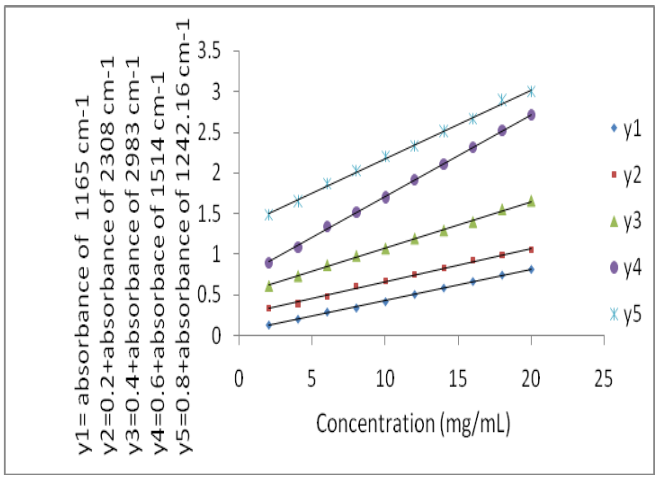

Fig. 8: Calibration curves of Flunarizine Dihydrochloric acid

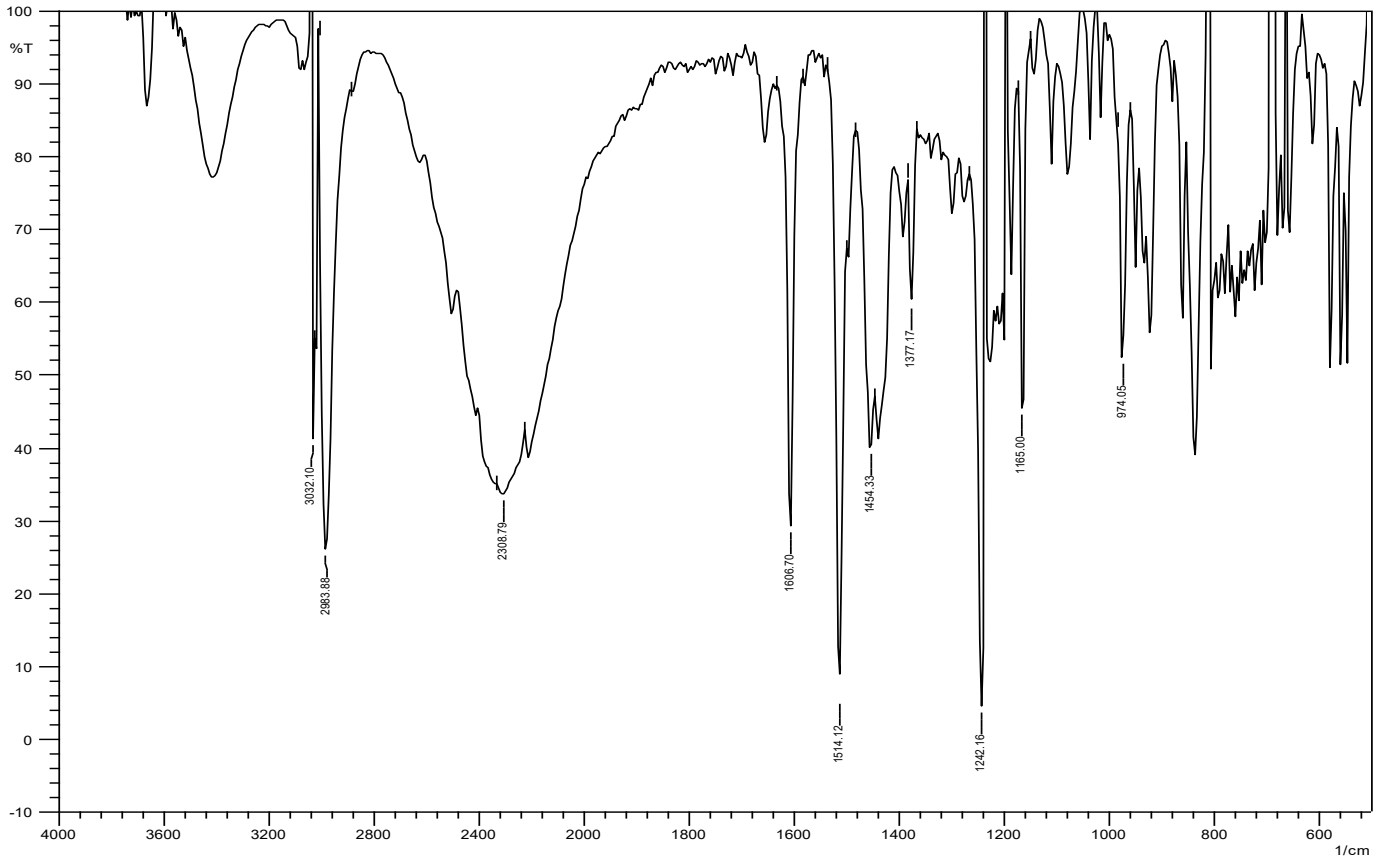

Fig. 9: IR Spectrum of Flunarizine Dihydrochloride

3.2. Analytical data

A linear correlation was found between absorbance and concentration ranges and sensitivity parameters such as Sandal's sensitivity, detection limit and quantification limit calculated according to ICH guidelines [24] are also presented in table 1, 2 and 3 which reveal the very high sensitivity of the methods. Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) is also given in tables 1-3.

Table 1: Analytical parameters for the determination of Bosentan at various wavelengths by Infra Red spectroscopy

Parameters/ν (cm^{-1})	800.46	1178.51	1473.62	1521.84
Beer;s Law Limits (mg mL^{-1})	2.5-25	2.5-25	2.5-25	2.5-25
Molar Absorptivity (L $mol^{-1}cm^{-1}$)	$0.2250601X10^2$	$0.2228537X10^2$	$0.2405054X10^2$	$0.2846349X10^2$
Sandell's Sensitivity (mg cm^{-2})	0.076923	0.055556	0.1	0.058824
LOD (mg mL^{-1})	0.179496	0.259272	0.233345	0.274524
LOQ (mg mL^{-1})	0.543928	0.785674	0.707107	0.83189
Slope, b	0.013	0.018	0.01	0.017
Intercept, a	0.065	0.019	0.078	0.059
Correlation Coefficient R	0.998999	0.996995	0.997497	0.996995
Standard Deviation of Intercept (S_a)	0.000707	0.001414	0.000707	0.001414
Standard Deviation of Intercept (S_b)	0.002828	0.002121	0.077782	0.002121
Regression Equation Y = a+bx	y = 0.013x + 0.065	y = 0.018x+0.041	y=0.010x+0.078	Y=0.018x+0.041

Table 2: Analytical parameters for the determination of Deslortadine at various wavelengths by Infra Red spectroscopy

Parameters/ν (cm^{-1})	1477.47	1560.41	2947.23
Beer;s Law Limits (mg mL^{-1})	1.6-16	1.6-16	1.6-16
Molar Absorptivity (L $mol^{-1}cm^{-1}$)	$0.242832X10^2$	$0.118502X10^2$	$0.3982445X10^2$
Sandell's Sensitivity (mg cm^{-2})	0.016667	0.016393	0.02381
LOD (mg mL^{-1})	0.086439	0.286721	0.593201
LOQ (mg mL^{-1})	0.261937	0.868852	1.79758
Slope, b	0.06	0.061	0.042
Intercept, a	0.001	0.001	0.106
Correlation Coefficient R	0.998499	0.998499	0.994987
Standard Deviation of Intercept (S_a)	0.001572	0.002234	0.007549
Standard Deviation of Intercept (S_b)	0.324186	0.01747	0.001528
Regression Equation Y = a+bx	y = 0.06x + 0.001	y = 0.061x + 0.001	y=0.042x+0.106

Table 3: Analytical parameters for the determination of Flunarizine di HCl at various wavelengths by Infra Red spectroscopy

Parameters/ν (cm^{-1})	1165	1242.16	1514	2308.79	2983.88
Beer's Law Limits (mg mL^{-1})	2-20	2-20	2-20	2-20	2-20
MolarAbsorptivity (L $mol^{-1}cm^{-1}$)	$0.26313X10^2$	$1.174885X10^2$	$0.52707X10^2$	$0.2740521X10^2$	$0.42979X10^2$
Sandell's Sensitivity (mg cm^{-2})	0.02636	0.012658	0.007937	0.02439	0.017544
LOD (mg mL^{-1})	0.132654	0.214358	0.063147	0.122947	0.2736
LOQ (mg mL^{-1})	0.40198	0.64957	0.191356	0.372567	0.82909
Slope, b	0.038	0.079	0.126	0.041	0.057
Intercept, a	0.047	0.0462	0.013	0.048	0.108
Correlation Coefficient R	0.998999	0.996995	0.9995	0.997497	0.998999
Standard Deviation of Intercept (S_a)	0.001528	0.005132	0.002411	0.001528	0.004726
Standard Deviation of Intercept (S_b)	0.002082	0.003215	0.003536	0.002	0.001762
Regression Equation Y = a+bx	y = 0.038x + 0.047	y = 0.079x + 0.0462	y = 0.126x + 0.013	y = 0.041x + 0.048	y = 0.057x + 0.108

Limit of determination as the weight in µg per mL of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm2 and path length of 1 cm. Y = a+bX, where Y is the absorbance and X concentration of drugs in µg per mL.*

3.3. Accuracy and Precision

The accuracy and precision of the methods were evaluated by analyzing the pure drug solution at 6 different levels by choosing them in working limits. The relative error (%) which is a measure of accuracy & RSD (%) a measure of precision are summarized in Table 2 and reveal the high accuracy and precision of the methods.

3.4. Robustness and Ruggedness

Ruggedness of these developed methods were evaluated by performing same analysis with 3 different analysts and also performing analysis on 3 different spectro-photometers by the same analyst.

3.5. Application to formulations

These developed methods were applied for the estimation of drugs in tablets. The results in table 5 reveal that the methods are successful for the determination of drugs and that the excipients in the dosage forms do not interfere. The results were compared to the available validated reported [1-18] methods on each drug and the results agree well with the claim and also are in agreement with the results obtained by the literature method.

Statistical evaluation results were used to test the accuracy by Student's t-test and precision by F-test (Table 6) which revealed no significant change was observed between the literature method and proposed method at the 95 % confidence level with reference to accuracy and precision.

Recovery experiments were carried out via standard addition technique to calculate the accuracy and validity of these developed methods. To a fixed and known amount/concentration of drug in tablet powder, pure drug was added at three levels (50, 100 and 150% of the level present in the tablet) and the total amount of drug was found by these developed methods. Each experiment was repeated six times and the percent recovery of pure drugs added (Table 5) was within the permissible limits showing the absence interference by the inactive ingredients in the assay.

Table 4: Determination of accuracy and precision of the methods on pure drug samples

Drug	Taken (mg/mL)	Found (mg/mL)	Error (%)	Recovery(%)	RSD (%)	Proposed method Mean±SD
BSN	2.5	2.49	0.400	99.60	0.2350	99.62±0.2341
	5.0	4.97	0.600	99.40		
	7.5	7.49	0.133	99.86		
DSD	1.6	1.59	0.625	99.37	0.1603	99.20±0.1591
	3.2	3.17	0.937	99.06		
	4.8	4.76	0.833	99.16		
FNZ	2.8	2.78	0.714	99.28	0.2739	99.58±0.278
	5.6	5.59	0.178	99.82		
	8.4	8.37	0.357	99.64		

Table 5: Results of assay of tablets (BSN, DSD and FNZ) by the proposed methods and statistical evaluation and recovery experiments by standard addition method

	Tablets		
	Bosentas (BSN)	Dloratin (DSD)	Migarid (FNZ)
Drug in Tablet µg/mL	0.50	0.50	0.50
	0.50	0.50	0.50
	0.50	0.50	0.50
	2.50	1.60	2.00
	5.00	3.20	4.00
	7.50	4.80	6.00
Drug added in µg/mL	2.50	1.60	2.00
	5.00	3.20	4.00
	7.50	4.80	6.00
	0.00	0.00	0.00
	0.00	0.00	0.00
	0.00	0.00	0.00
Total found µg/mL	3.02	2.08	2.48
	5.46	0.270	4.48
	7.49	0.377	6.48
	2.49	03625	2.00
	4.97	0.937	3.98
	7.48	0.208	5.98

Error (%)	0.667	0.952	0.40
	0.727	0.270	0.44
	0.133	0.377	0.31
	0.400	0.625	0.00
	0.600	0.937	0.50
	0.267	0.208	0.33
Recovery (%)	99.33	99.04	99.60
	99.27	99.72	99.55
	99.87	99.62	99.69
	99.60	99.37	100.00
	99.40	99.06	99.50
	99.73	99.79	99.67
RSD (%)	0.238	0.331	0.177
Reference method Mean±SD	99.81±0.432	99.30±1.83	100.16±0.94
Proposed method Mean±SD	99.53±0.237	99.43±0.329	99.67±0.177
Student's t-test	0.9683 (2.447)	1.997 (2.447)	1.9545 (3.182)
F-test	0.0562 (4.2838)	0.1083 (4.28)	0.0312 (4.7571)

Table 6: F-test and t-test values

	Bosentan (BSN)	Dloratin (DSD)	Migarid (FNZ)
F-test*	0.0562 (4.2838)	0.1083 (4.2838)	0.0312 (3.182)
t-test**	0.9683 (2.447)	1.977 (2.447)	1.9545 (3.182)

**t- test and **F-test values from literature.*

4. CONCLUSION

The present study described the successful development of new, simple, sensitive, selective, accurate cost-effective and rapid FT-IR spectroscopic method for the accurate determination of the BSN, DSD and FNZ drugs in its pharmaceutical form. There is no interference from additives and excipients. The method thus can be used in the quantitative determination of these drugs in pure and pharmaceutical formulations. So, it is the good alternative to the reported methods for the quantitative determination of these drugs.

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Conflict of interest

The author declares no conflict of interest.

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