DEVELOPMENT AND VALIDATION OF ANLYTICAL METHOD FOR THE DETERMINATION OF BIOACTIVE MOLECULES IN BULK AND PHARMACEUTICAL DOSAGE FORM

UNDER GUIDANCE OF MR. VIKAS KANDEKAR

DIPIKA MARATHE

Siddhant College Of Pharmacy, Sudumbare Pune-412109.

Corresponding Author:

Mr. Vikas Kandekar (M. Pharm.)

Professor,

Department of Quality Assurance,

Siddhant College of Pharmacy Sudumbare, Pune-412109

ABSTRACT

To Develop and validate FTIR method for the simultaneous estimation of drug and Pharmaceutical Dosage form. Contain objective To develop FTIR method for the estimation of bulk Drug and pharmaceutical dosage form. And To validate above developed methods for estimation of bulk Drug and pharmaceutical dosage form. The Principle of Fourier-transform infrared spectroscopy is a technique used to obtain an infrared spectrum of absorption or emission of solid , liquid or gas. An FTIR Spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. In Method Validation study Linearity, Precision, Accuracy, Limit of Dectection and Limit of Quantitation. Analytical Chemistry may be defined as the science and art of determining the composition of material in terms of the elements or compounds contained in them. There is a greater need for the development of newer and better methods of pharmaceutical analysis for controlling quality of the manufactured drugs.

KEYWORDS

Fourier-transform infrared, Gabapentin, Beer-Lamberts Law, Method Validation.

INTRODUCTION

Analytical techniques play an important role in assuring and maintaining the quality of substance and are critical components of Q.A/Q.C. The reliability, utility, accuracy, interception and specificity of the measurement is the responsibility of an analytical chemist.The final manufacturing product is subjected to quality control to ensure that its essential components are present within the predetermined range of composition. Now a day's multi-component dosage forms have gained very high importance. They are emerging over single drug formulation due to their multiple actions, fewer side effects, quicker relief and smaller doses of individual drugs. Thus the manufacturing trends have been to manufacture more and more complex formulation containing several drugs in combined form, which leads to the greater need for devising an accurate estimation procedure for each ingredient of such multi-component dosage form containing several therapeutically active drugs.[2] In pharmaceutical industry, the quality of the manufactured drug in various dosage forms must be carefully controlled. Slight changes in the composition or in the purity of the drug itself can affect the therapeutic value of the drug causing several harmful effects on the patient's health. Therefore, there is a greater need for the

development of newer and better methods of pharmaceutical analysis for controlling quality of the manufactured drugs.[3]

METHOD

Instruments:

1) Digital Ultrasonic cleaner (sonicator): Hmq. India
2) Analytical weighing balance: Anamed; Model AA-2200.[Max. 200g, Min. 0.01g;e= 0.0001g].
3) IR Affinity-1 Fourier Transform Infrared Spectrophotometer (FTIR): Shimadzu, Japan; Model 00722

Preparation of solutions:

Preparation of standard stock solution:

Accurately weighed15mg of GABA (pure API) was weighed and mixed separately with 985mg of KBr (IR spectroscopic grade). Moisture free KBr separately triturated finely then added API and triturated finely then added API and triturate again the mixture to be homogenous with uniform mixing.

Selection of analytical wavelength:

Working standard (15 % w/w) of gabapentin drug was scanned in the mid IR range of 4000-400 cm^{-1} with resolution of 4 and 45 scans/min. Evaluation parameter selected for both drugs was peak area, in that one

can select single wavenumber in a range/peak area also. Functional group selected for GABA was NH and wavenumber found in range of 1640-1560 cm^{-1}. IR spectrum of GABA drug is shown in figure 3.

Validation of Analytical Method:

The developed FTIR method was validated as per ICH guidelines.

Linearity

The linearity of calibration curve was assessed by linear regression. Solid state sample in concentration range of 15-90 % w/w was prepared as described in calibration curve. The linearity of method was studied by assessing six samples of different concentrations (15-90% w/w) of gabapentin in six replicates. The results are shown in table 1, 2.

Precision

The procedure of developed method was carried out by repeatability and intermediate precision studies. Repeatability studies were performed by analyzing the samples of six differentconcentrations 15-90 % w/w of gabapentin. On the same day (day 1) inter-day studies was carried out. The intermediate precision of the method was evaluated by intra-day studies. Repeatability studied at inter-day (on day 3). The results are shown in table 3, 4.

Intermediate precision (reproducibility/ intermediate precision are the terms currently accepted in ICH guidelines) of the proposed method was demonstrated by performing the experiment by same analyst on a different day. Measurement of peak area at selected wavenumber i.e. 1614 cm^{1}. The results are shown in table 3, 5.

The precision result was expressed by coefficient of variation (% RSD) and accuracy by mean and standard deviation. For day 1 precision studies, the RSD (%) values for the six samples was observed in the range of 0.3959 (for GABA) while for day 3 precision studies the range was 0.9742 (for GABA). The inter-day and intraday precision results were within the accepted variable limits. Results are given in table 1, 2, 3. Calculation was done using formula 1.

$$\% \, Estimation = \frac{Intensity \, of \, Sample}{Intensity \, of \, Standard} \, X \, \frac{Wt.of \, std.}{wt.of \, sample} \, X \, 100 \text{ ---- (1)}$$

Accuracy:

The accuracy of the assay method was evaluated by addition method with recovery of gabapentin. From excipient at three different quantities (80, 100,120 % w/w).To the preanalysed tablet powder, known quantity of gabapentin powder corresponding to 80 %, 100 % and 120 % of label claim was added. The sample was mixed thoroughly and analyzed. The results are shown in table 9

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$$Amount found = \frac{Totalamountadded X Amountestimated}{100} \text{ --------- (2)}$$

$$\% recovery = \frac{LabelClaim X Amount found}{Totalamountadded} \text{ ---------- (3)}$$

Limit of Detection (LOD)

$$LOD = \frac{3.3\ \sigma}{S}$$

LOD is calculated from the formula: -

Where,

σ = standard deviation of peak area of drug for the lowest conc. in the range

S = slope of the calibration curve.

LOD of Gabpentin= 6.66 µg/ ml

Limit of Quantification (LOQ)

The Quantitation limit is expressed as:

$$LOQ = \frac{10\ \sigma}{S}$$

LOQ of Gabpentin = 20.19µg/ ml.

RESULTS AND DISCUSSION

The FTIR spectrum for pure sample of gabapentin exhibited absorbance bands in the range of 1640-1560, 1473, 1419, 1400-1300, 1381-1165, 2152, 2952-2860. The low intensity absorbance bands arising from gabapentin was not much affected by dilution in dry potassium bromide; therefore, in the present study we have used dry potassium bromide as the diluents.

The low intensity absorbance bands arising from gabapentin and methylcobalamine were not much affected by dilution in dry potassium bromide; therefore, in the present study we have used dry potassium bromide as the diluent. Method Validated as per ICH Guideline Limit.

CONCLUSION

A simple, precise, accurate, reproducible and stability-indicating FT-IR method without interference from the excipients or from degradation products has been developed and validated for the determination of Gabapentin as bulk drug and pharmaceutical dosage form. The developed method can be used for quantitative analysis of both the drugs in pharmaceutical dosage form. The method was developed by using easily available and cheap solvents for analysis of drug hence can be considered as economic.

ACKNOWLEDGEMENT

The authors are thankful to principal and management of Siddhant college of Pharmacy, Sudumbare Pune for providing the research facilities.

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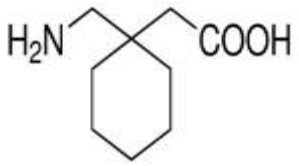

Fig. 1: Chemical structure of Gabapentin

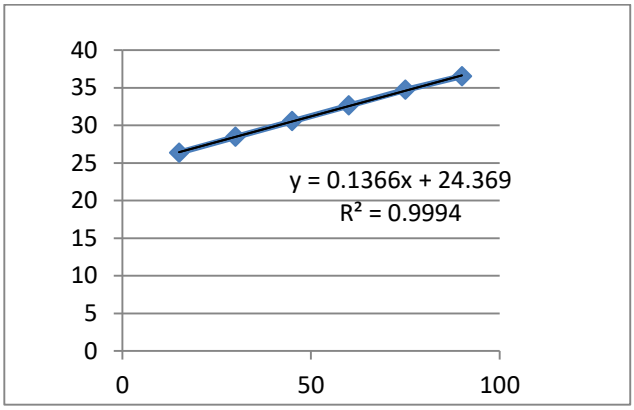

Fig 2: Calibration curve of Gabapentin

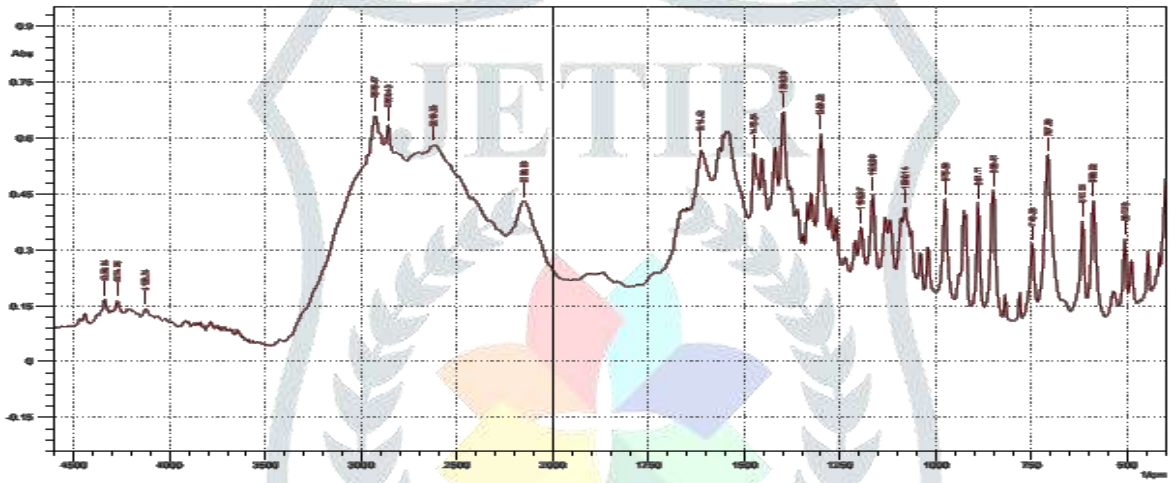

Fig 3: FTIR Spectrum of Gabapentin (Standard)

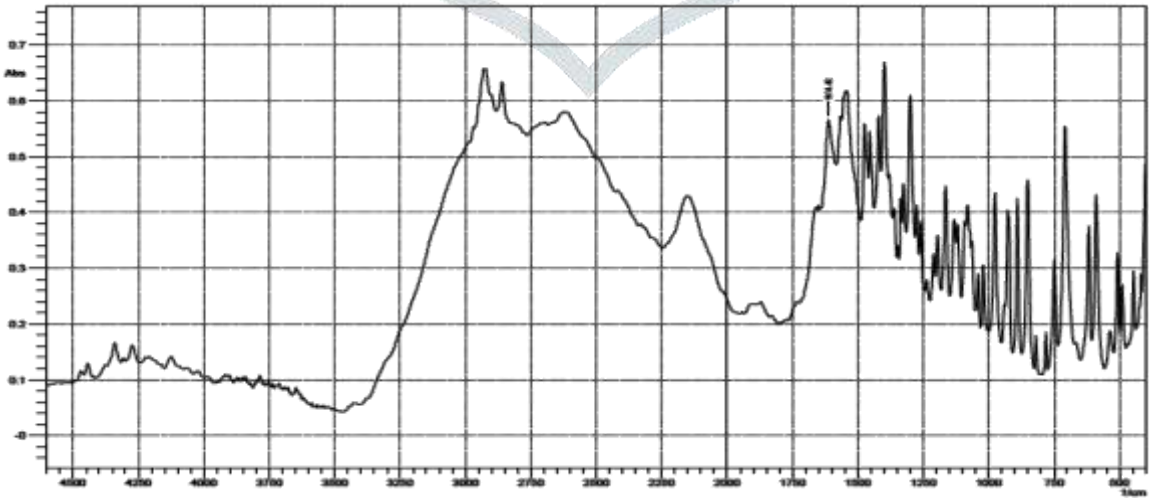

Fig 4: FTIR spectrum of GABA (15 % w/w)

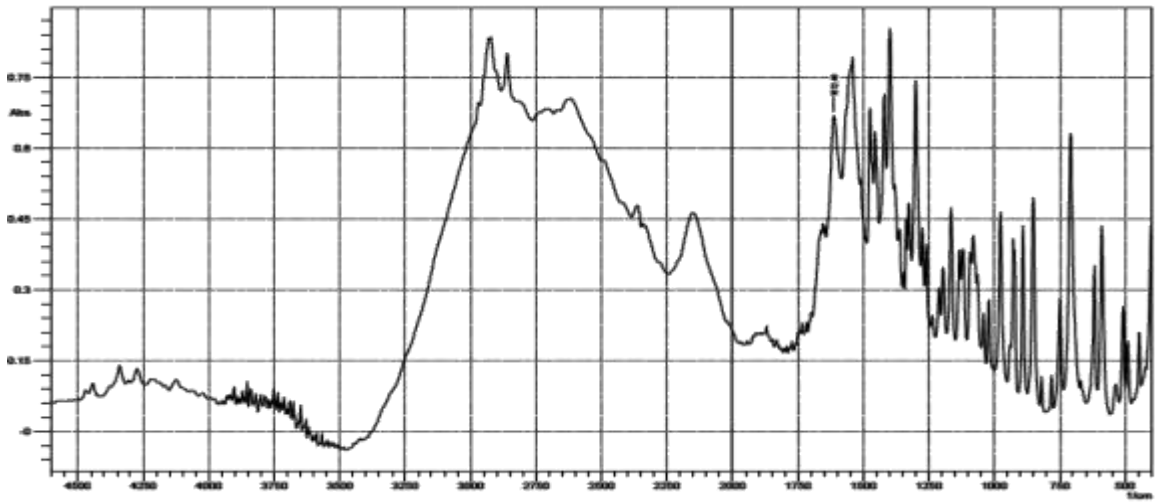

Fig 5:FTIR spectrum of GABA (30% w/w)

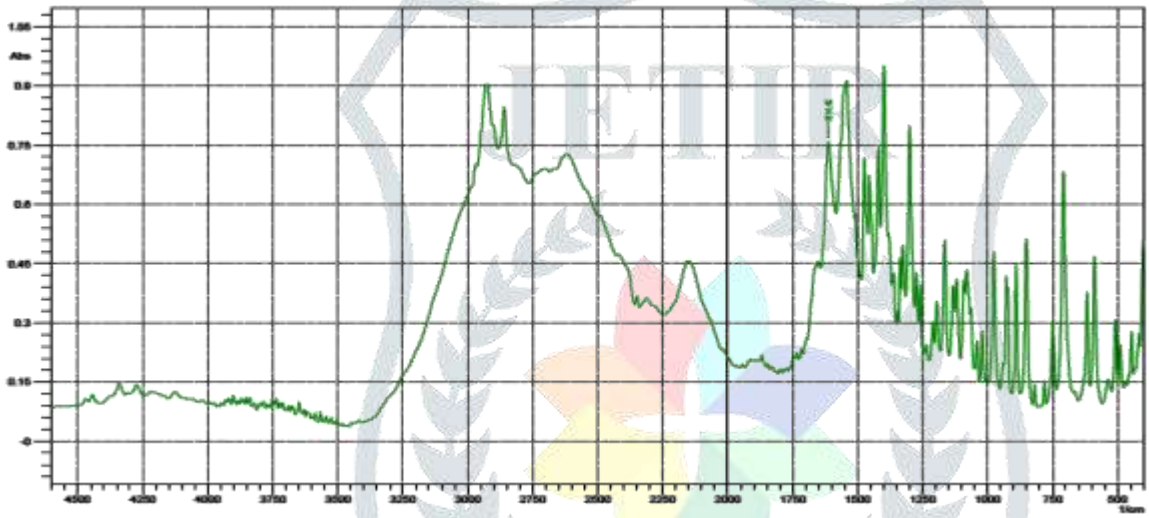

Fig 6:FTIR spectrum of GABA (45% w/w)

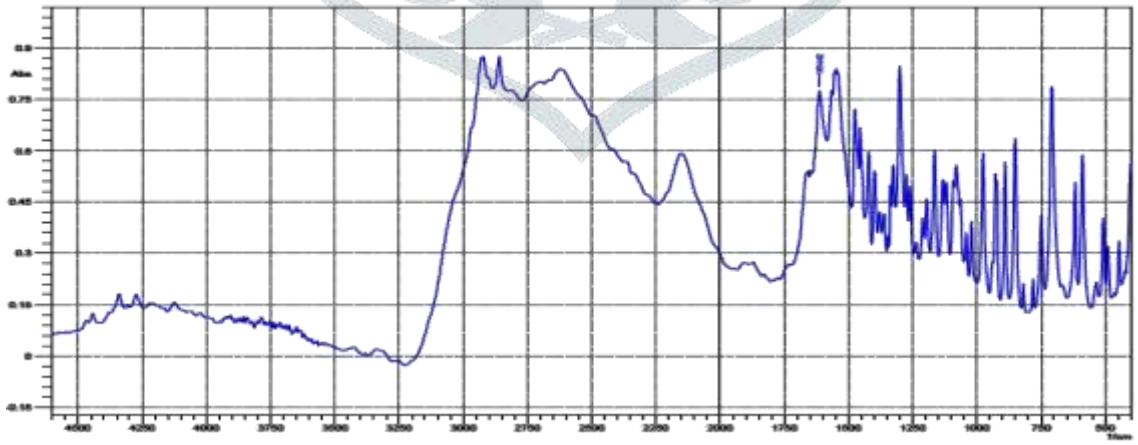

Fig 7:FTIR spectrum of GABA (60% w/w)

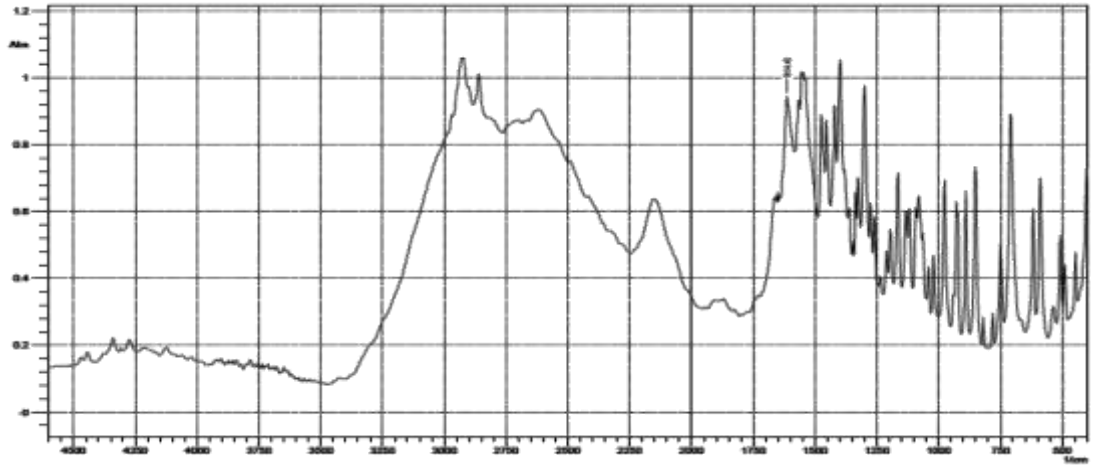

Fig 8: FTIR spectrum of GABA (75% w/w)

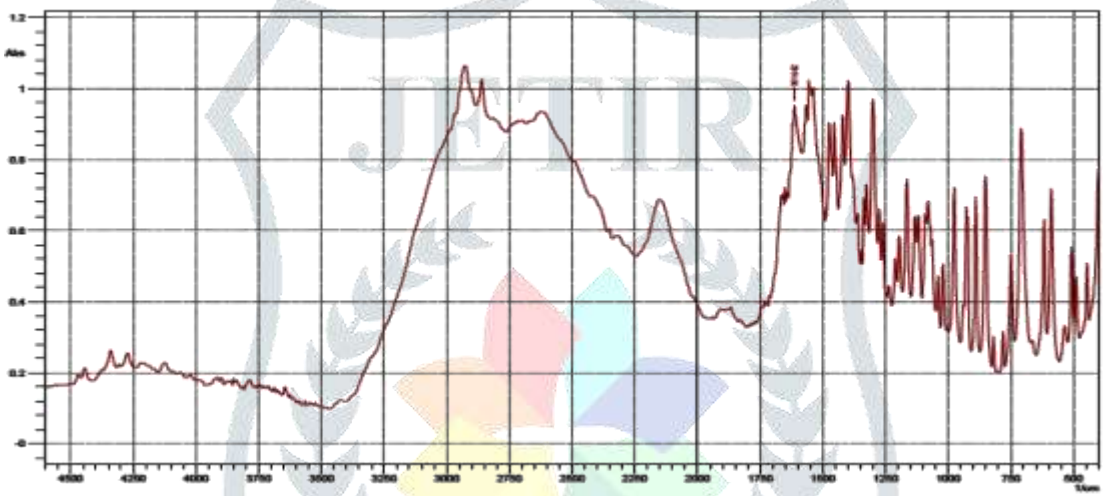

Fig 9:FTIR spectrum of GABA (90% w/w)

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Table 1: Linearity study data of GABA

Sr. No.	Concentration (% w/w)	Area (1640-1560 cm^{-1})
1	15	29.583
2	30	34.110
3	45	37.482
4	60	39.314
5	75	48.083
6	90	49.37

Table 2: Optical characteristics and other parameters

Parameters	GABA
Wave number (cm^{-1})	1614
Linearity range (% w/w)	15-90 %
Limit of detection (% w/w)	6.66
Limit of quantitation (% w/w)	20.19
$y = mx + c$	Y =0.1366x+24.369
Slope (m)	0.1366
Intercept (c)	24.369
Regression coefficient r^2	0.9994

Table 3: Precision data of marketed formulation (GABA-100) by FTIR spectrophotometric method

Sr. No.	Interval of Time	Concentration (% w/w)	% Recovery
		GABA	GABA
I	Intra-day	15	100.7
II		15	98.78
III		15	99.49
I	Inter-day	15	99.96
II		15	100.4
III		15	99.61

Table 4: Statistical validation of intra-day precision data

Name of the drug	Mean*	SD	%RSD
GABA	99.65	0.9708	0.9742

* Indicates average of three determinations

Table 5: Statistical validation of inter-day precision data

Name of the drug	Mean*	SD	%RSD
GABA	99.99	0.3959	0.3959

* Indicates average of three determinations

Table 6: Repeatability of marketed formulation (GABA-100) by FTIR spectrophotometric method

Sr. no	Concentration %w/w	Area	%Recovery
	GABA	GABA	GABA
1	15	25.96	102.3
2	15	25.89	101.91
3	15	25.76	97.80
4	15	25.91	99.36
5	15	25.83	98.81
6	15	25.79	97.90

Table 7: Statistical validation of repeatability of marketed formulation (GABA-100) by FTIR spectrophotometric method

Name of the drug	Mean*	SD	%RSD
GABA	99.68	1.970	1.9763

Table 8: Recovery study data of (GABA-100) by FTIR spectrophotometric method

Level of Recovery	Amount present (mg)	Added concentration (mg)	Amount recovered (mg)	% Recovery
	GABA	GABA	GABA	GABA
80%	100	80	179.68	99.68
	100	80	181.33	100.5
	100	80	179.24	99.88
100%	100	100	199.29	99.64
	100	100	201.5	100
	100	100	199.16	99.91
120%	100	120	220.5	100
	100	120	219.69	99.86
	100	120	220.08	100.03

Table 9: Statistical validation of recovery study data

Level of Recovery	% Mean Recovery GABA	*SD GABA	% RSD* GABA
80%	100.02	0.4276	0.4275
100%	99.85	0.1873	0.1875
120%	99.96	0.0907	0.0907

* Indicates average of three determinations

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