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A NOVEL FT-IR SPECTROSCOPIC METHOD FOR THE QUANTIFICATION OF FLUOXETINE IN BULK AND CAPSULE FORMULATION.

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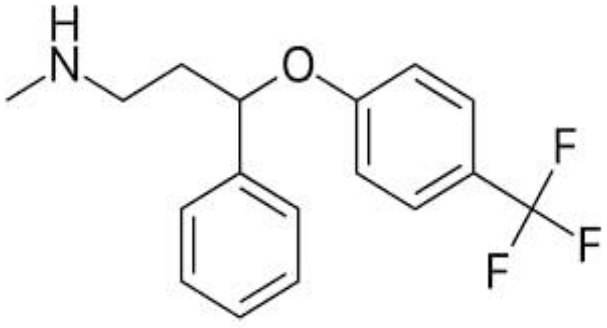
ABSTRACT
Spectrochemical analysis, methods of chemical analysis that depends upon measurement of the wavelength and the intensity of electromagnetic radiation. Its major use is in the determination of the arrangement of atoms and electrons in molecules of chemical compounds based on the amounts of energy absorbed during changes in the structure or motion of the molecules. In its restricted and more common usage two methods usually are implied. The calibration curve was constructed by taking concentration on the X-axis and absorbance / area on the Y-axis. The linearity was evaluated by linear regression analysis. This was calculated by the least square regression method. The correlation coefficient and Y-intercept of the calibration curve were calculated.

Keywords: Elecromagnetic Radiation, Calibration, IR spectrum, Method development, Validation.

INTRODUCTION

Pharmaceutical analysis is a discipline of chemistry that deals with separating, identifying, and determining the components in a sample. Analytical chemistry is the "science and art of determining the composition of materials in terms of the elements or compounds present. Analytical chemistry entails the use of a variety of techniques and approaches to gather and evaluate qualitative and quantitative structural data about natural materials [1]. The identification of elements, species, or compounds present in a sample is known as quantitative analysis. Quantitative analysis is the measurement of the absolute or relative amount of an element, species, or compound contained in a sample. The determination of the spatial arrangement of atoms in an element, as well as the identification of the atoms' distinctive groups, is known as structural analysis (functional groups). Infrared spectroscopy, also referred as vibrational spectroscopy, is a standard method of analytical pharmacy and chemistry, providing the images of vibration of atoms of compound. It is one of the most common spectroscopic techniques used by organic and inorganic chemists [2]. It is based on the nature of interaction of the IR radiation with the vibrational modes of molecules. IR spectra are due to the changes in vibrational energy, accompanied by changes in rotational energy.

Drug Profile
Drug Name: Fluoxetine
Structure



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Chemical name: N-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy] propan-1-amine
Molecular formula: $C_{17}H_{18}F_3NO$
Molecular weight: 309.3 g/mol
Melting point: 193.4°C
Solubility: Soluble in water and ethanol
Category: Antidepressant

Uses:

Fluoxetine is used in the treatment of depression, obsessive compulsive disorder, food disorders (over eating), and panic attacks [3].

MECHANISM OF ACTION

FLUOXETINE – SELECTIVE SEROTONIN REUPTAKE INHIBITOR (SSRI)

Fluoxetine is a 2nd generation antidepressant categorized as a selective serotonin reuptake inhibitor (SSRI). It gained FDA approval in 1987 and although it was initially intended for the treatment of depression, today it is commonly prescribed to manage depression in addition to various other pathologies [4].

SIDE EFFECTS

Side effects of fluoxetine includes nervousness, anxiety, difficulty falling asleep or staying asleep, nausea, diarrhoea, dry mouth, heartburn, yawning, weakness, uncontrollable shaking of a part of the body, loss of appetite, weight loss, changes in sex, drive or ability, excessive sweating, headache, confusion, weakness, difficulty concentrating, or memory problems.[5]

CONTRAINDICATIONS

Concomitant use in patients taking monoamine oxidase inhibitors (MAOIs) and pimozide is contraindicated. Also contraindicated in patients with a hypersensitivity to fluoxetine, hepatic, or renal in sufficiency [6].

DRUG INTERACTIONS

Lithium: The level of lithium either increases or decreases when 5lithium was used concomitantly with fluoxetine.

Tryptophan: Adverse reactions, including agitation, restlessness, and gastrointestinal distress,

Benzodiazepines: Half-life of concurrently administered diazepam may be prolonged in some patients. Co-administration of alprazolam and fluoxetine has resulted in increased alprazolam plasma concentrations.

Haloperidol and clozapine: Elevation of blood levels of haloperidol and clozapine has been observed in patients receiving concomitant fluoxetine.

Carbamazepine: Elevated plasma anticonvulsant concentrations and clinical anticonvulsant toxicity following initiation of concomitant fluoxetine treatment [7].

CLINICAL PHARMACOLOGY

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) and as the name suggests, it exerts its therapeutic effect by inhibiting the presynaptic reuptake of the neurotransmitter serotonin. As a result, levels of 5-hydroxytryptamine (5-HT) are increased in various parts of the brain. Further, fluoxetine has high affinity for 5-HT transporters, weak affinity for noradrenaline transporters and no affinity for dopamine transporters indicating that it is 5-HT selective. Fluoxetine interacts to a degree with the $5\text{-}HT_{2C}$ receptor, and it has been suggested that through this mechanism, it is able to increase noradrenaline and dopamine levels in the prefrontal cortex. [8]

METHOD VALIDATION

The proposed method was validated as per ICH guidelines for linearity, accuracy, and precision.

LINEARITY

The linearity of calibration curve was assessed by linear regression. Calibration curves were plotted over the concentration range of 10-50 mg/mg for fluoxetine. Each sample was analysed six times and averages were calculated. The calibration curve was constructed by taking concentration on the X-axis and absorbance / area on the Y-axis. The linearity was evaluated by linear regression analysis. This was calculated by the least square regression method. The correlation coefficient and Y-intercept of the calibration curve were calculated [9]

ACCURACY

Recovery experiments were conducted at concentration range of 24, 30 and 45 mg/mg tovalidate the accuracy of the rest method. Each test preparation was prepared in triplicate and the analysis performed in triplicate. The assay value at the beginning of validation considered as the true value (100%) foe recovery calculations. [10]

$$\% \text{ Recovery} = \frac{\text{Amount recovered} \times 100}{\text{Amount Spiked (Added)}}$$

PRECISION

The precision of the method was confirmed by the analysis of capsules repeated for 6 times with the same concentration. The amount of each drug present in the capsules was calculated. The percentage RSD was calculated. The intermediate precision of the method was confirmed by intraday analysis i.e. the analysis ofcapsules was repeated three times in the same day and on percentage RSD was calculated [11].

$$\text{Relative standard deviation} = \frac{Standard\ devaition}{Mean} \times 10$$

Fluoxetine Pure Drug

RESULTS AND DISCUSSION

The method is based in the comparison of the pure form of the Fluoxetine with the marketed formulations at the absorption bands C-H, C=C-C (Aromatic Ring), C-F, Phenoxy which are typically in the range of 2923 cm^{-1}, 1615 cm^{-1}, 1329 cm^{-1}, 1242 cm^{-1} respectively shown in figure 2, 3, 4.[12]

The calibration curve was obtained for a series of concentration in the range of 10-50ug/mg and it was found to be linear. The linear regression equation was y = 0.025x + 0.643 with correlation coefficient value 0.999 which were within the acceptance criteria [13].

Accuracy found out by recovery study from prepared samples (three replicates) with standard sample. Recovery was carried out standard addition method at three different levels which is 80%, 100% and 150%. The Percentage recovery was calculated and was found to be 99.34%. This was found to be well within the acceptance criteria of 98 - 102%. This showed that the recovery of silodosin by proposed method was satisfactory [14].

Accuracy found out by recovery study from prepared samples (three replicates) with standard sample.

Percentage Recovery was carried out standard addition method at three different levels which is 80%,100% and 150%. The Percentage % recovery was calculated and was found to be 99.11%. This was found to be well within the acceptance criteria of 98 - 102%. This showed that the recovery of silodosin by proposed method was satisfactory [15]

Accuracy found out by recovery study from prepared samples (three replicates) with standard sample.

Percentage Recovery was carried out standard addition method at three different levels which is 80%, 100% and 150%. The percentage % recovery was calculated and was found to be 99.52%. This was found to be well within the acceptance criteria of 98 - 102%. This showed that the recovery of silodosin by proposed method was satisfactory.[16] The precision was measured in terms of repeatability, which was determined by sufficient number of sample within the day (intraday) and next consequent three days for inter day precision [17]. For each %RSD was calculated and was found to be 0.813% for intraday and 0.643% for interday precision. These values were well within the acceptance limit ± 2.0 %. This showed that the precision of the method was satisfactory, good [18].

The precision was measured in terms of repeatability, which was determined by sufficient number of sample within the day (intraday) and next consequent three days for inter day precision. For each %RSD was calculated and was found to be 0.741% for intraday 0.766% and for interday precision. These values were well within the acceptance limit ± 2.0 %. This showed that the precision of the method was satisfactory.[19]

The precision was measured in terms of repeatability, which was determined by sufficient number of sample within the day (intraday) and next consequent three days for inter day precision. For each %RSD was calculated and was found to be 0.855% for intraday and 0.90% for interday precision. These values were well within the acceptance limit ± 2.0 %. This showed that the precision of the method was satisfactory, good [20].

Figure 2: Fluoxetine 2 %

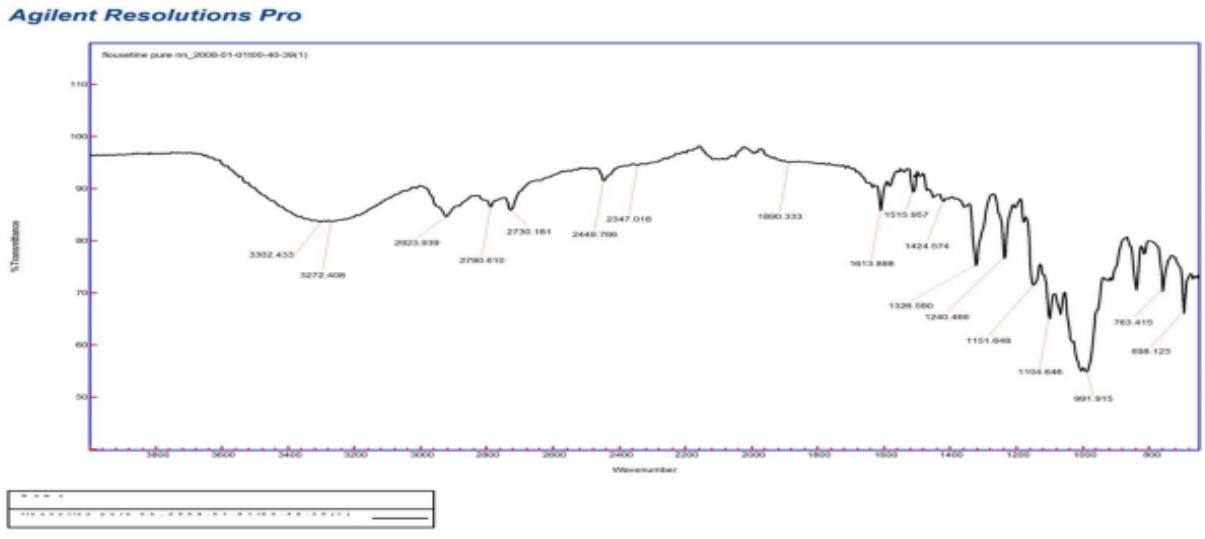

Figure 3: Fluoxetine 4 %

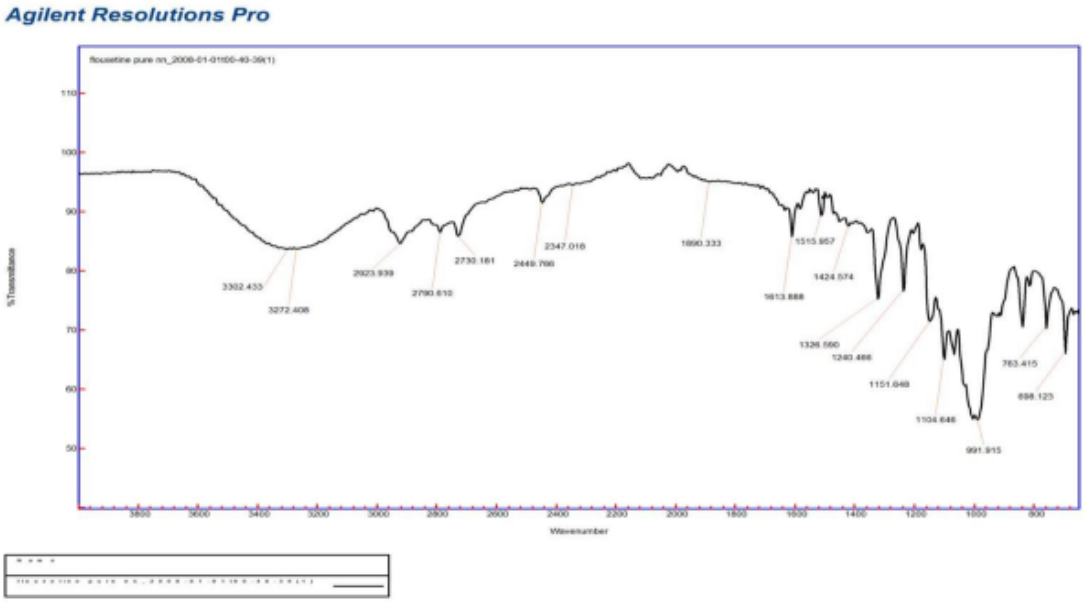

Figure 4: Fluoxetine 10 %

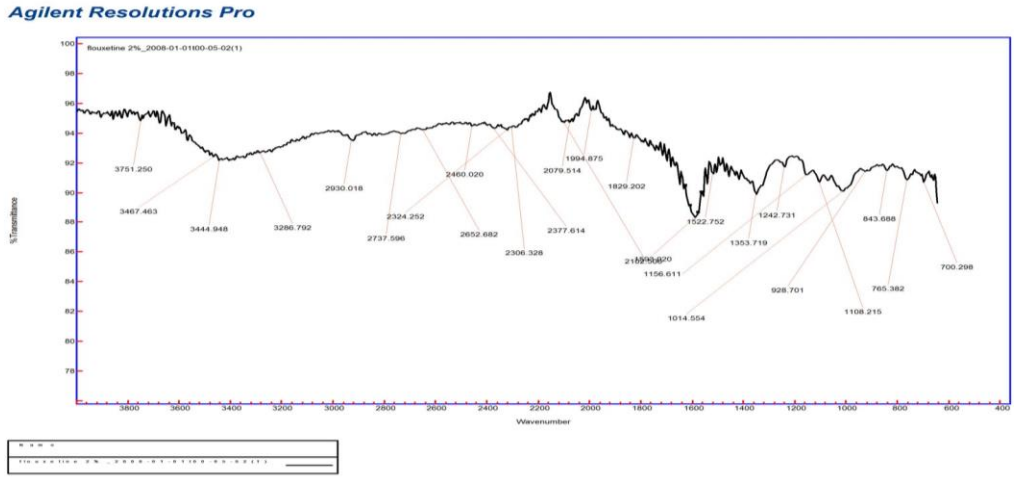

Figure 5: Calibration curve

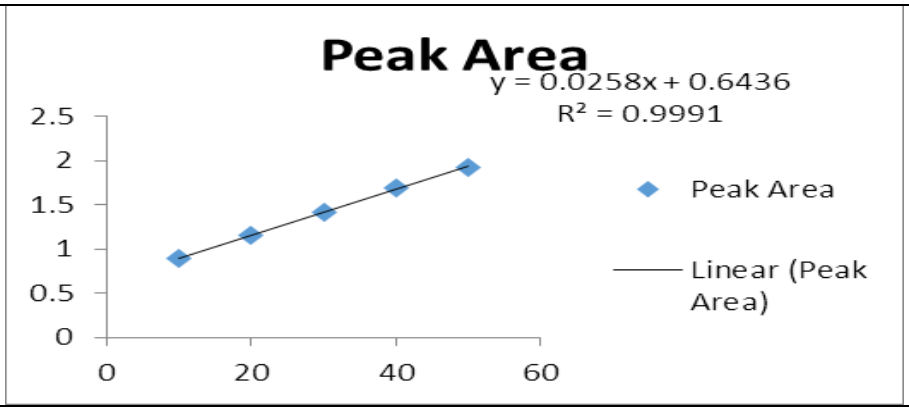

Table 1: Functional Groups

S.NO	Wave number (cm^{-1})	Functional group
1.	2923	C - H stretch
2.	1615	C = C - C stretch (Aromatic Ring)
3.	1329	C - F stretch
4.	1242	Phenoxy stretch

Table 2: Linearity

S.NO	CONCENTRATION(µg/mg)	PEAK AREA
1.	10	0.899
2.	20	1.155
3.	30	1.42
4.	40	1.695
5.	50	1.919

Table 2: Accuracy F1

% Level	Amount spiked (µg/mg)	Amount recovered (µg/mg)	% Recovery	Mean % Recovery
	24	23.88	99.5	
	24	23.76	99	
80%	24	23.92	99.66	
	30	29.26	97.53	
	30	29.87	99.56	
100%	30	29.95	99.83	
	45	44.89	99.75	
	45	44.93	99.84	99.34
150%	45	44.73	99.4	

Table 3: Accuracy F2

%Level	Amount spiked (µg/mg)	Amount recovered (µg/mg)	% Recovery	Mean % Recovery
	24	23.83	99.29	
	24	23.74	98.91	
80%	24	23.90	99.58	
	30	29.32	97.73	
	30	29.65	98.83	
100%	30	29.91	99.70	99.11
	45	44.42	98.71	
	45	44.79	99.53	
150%	45	44.88	99.73	

Table 4: Accuracy F3

%Level	Amount spiked (µg/mg)	Amount recovered (µg/mg)	% Recovery	Mean % Recovery
	24	23.70	98.75	
	24	23.91	99.62	
80%	24	23.85	99.37	
	30	29.38	97.93	
100%	30	29.92	99.73	
	30	29.88	99.6	
	45	44.63	99.18	
	45	44.82	99.6	99.52
150%	45	44.95	99.89	

Table 5: Precision F1

Amount (mg)	Intraday		Inter day	
	% Content	% RSD	% Content	% RSD
30	99.02	0.813	100.13	0.643
	98.56		98.87	
	100.13		99.31	

Table 6: Precision F2

Amount (mg)	Intra day		Inter day	
	% Content	% RSD	% Content	% RSD
30	99.98	0.741	98.52	0.766
	100.27		100.04	
	98.87		99.34	

Table 7: Precision F3

Amount (mg)	Intraday		Interday	
	% Content	% RSD	% Content	% RSD
30	98.47	0.855	97.97	0.90
	100.07		99.73	
	99.77		98.62	

LIMIT OF DETECTION (LOD)

F1

LOD = 3.3 × σ/п
= 3.3 × 0.018/0.235
= 3.3 × 0.076
= 0.253

F2

LOD = 3.3 × σ/п
= 3.3 × 0.025/0.648
= 3.3 × 0.038
= 0.127

F3

LOD = 3.3 × σ/п
= 3.3 × 0.016/0.257
= 3.3 × 0.062
= 0.205

Limit of Quantification (LOQ)

F1

LOQ = 10 × σ
= 10 × 0.0.0766
= 0.766

F2

LOQ = 10 × σ
= 10 × 0.0386
= 0.386

F3

LOQ = 10 × σ
= 10 × 0.0622
= 0.622

CONCLUSION

Fourier Transform Infrared Spectroscopy is a widely recognized technique that has been used to identify several compounds, such as pharmaceuticals, cosmetics and foods, but requires expensive requirements on mathematical pre-treatments. The method has been evaluated linearity, accuracy, precision and LOD & LOQ in order to ascertain the suitability of analytical method. The method was applied to marketed samples and it has been proved that the method was selective and linear between the concentration 10-50 µg/mg correlations co-efficient value was found to be 0.999. The developed method was found to be precise as the %RSD value for interday and intraday precision for F1 formulation were 0.813% and 0.643%, for F2 formulation were 0.741% and 0.766%, for F3 formulation were 0.855% and 0.900% which were less than 2.0%. The percentage recovery for F1, F2, F3 were found to be 99.34 ± 0.185, 99.11 ± 0.185, 99.52 ± 0.185 respectively. The method is very simple, rapid and economic nature, which makes it especially suitable for routine quality control work.

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Nil

CONFLICT OF INTEREST

No interest

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