BAU Journal - Science and Technology

Volume 3 | Issue 2
ISSN: 2706-784X

Article 5

June 2022

NOVEL FAST ANALYTICAL METHODS FOR THE ANALYSIS OF FLUOXETINE IN PURE AND PHARMACEUTICAL DOSAGE FORM

Diala Jumblat
PhD Candidate, Faculty of Pharmacy, Beirut Arab University, jumblatdiala@gmail.com

Alice Bejjani
Lebanese atomic Energy Commission-NCSR, abejjani@cnrs.edu.lb

Azza A. K. Gazy
Professor, Faculty of Pharmacy, Beirut Arab University, a.ghazi@bau.edu.lb

Marwa El Jamal
Assistant Professor, Faculty of Pharmacy, Beirut Arab University, marwa.jamal@bau.edu.lb

NOVEL FAST ANALYTICAL METHODS FOR THE ANALYSIS OF FLUOXETINE IN PURE AND PHARMACEUTICAL DOSAGE FORM

Abstract

Novel and accurate analytical methods were developed and validated for the characterization of Fluoxetine in its pure and pharmaceutical dosage form Prozac®. Fluoxetine was determined by IBA techniques (PIGE, PIXE and RBS). It has been also analyzed spectrophotometrically at 610 nm after oxidation with potassium permanganate in alkaline medium. In addition, Fluoxetine was kinetically determined using the initial rate method, the fixed absorbance method and the fixed time method. Moreover, a Gas chromatography - mass spectrometry technique is proposed for the investigation of Fluoxetine without a prederivatization phase. The spectrophotometric method was performed with a concentration array of 2-10 µg/mL at 610 nm and a regression coefficient (r) of 0.996. The fixed time method was the most suitable one to determine Fluoxetine with correlation coefficient value (r) of 0.9966. The Gas chromatography - mass spectrometry investigated the drug in a concentration range of 20-100 µg/mL and a regression coefficient (r) of 0.999. IBA analysis presented a precision of less than 3% and a very low limit of detection. Consequently, these proposed methods would be useful tools for determining Fluoxetine as all the assay results exposed satisfactory sensitivity, accuracy and reproducibility

Keywords

Fluoxetine, Prozac®, PIGE, PIXE, RBS, Kinetic Spectrophotometry, Gas chromatography-mass spectrometry.

1. INTRODUCTION

Obesity was found to be the fifth risk for deaths worldwide. A change in lifestyle will hardly ensure weight loss. Moreover, surgery options are rarely chosen as of their high cost. Therefore, a shift towards usage of drugs is increasing [1]. Thus, the significance of developing analytical methods for the analysis of the control weight drugs is critical.

Fluoxetine, N-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy] propan-1-amine, (Figure 1), increases the serotonin level in the brain by selectively inhibiting its uptake. Fluoxetine is an antidepressant agent. It is utilized for the treatment of depression, panic attacks and eating disorders [2]. As the weight loss is one of the side effects caused by Fluoxetine, this drug got the FDA approval for obesity treatment [3].

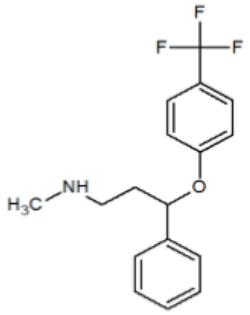

Fig.1: Chemical structure of Fluoxetine.

Several analytical methods have been stated in the scientific literature for the analysis of Fluoxetine in biological fluids and pharmaceutical preparations including gas chromatographic methods, capillary gas chromatographic methods, liquid chromatographic methods and HPLC [4, 5]. These analytical techniques lay out one or more disadvantages such as low sensitivity and rigid experimental conditions. Therefore, the development of new techniques to analyze this drug was critical. In this current research, Fluoxetine was determined by Ion Beam Analysis (IBA), spectrophotometry and Gas chromatography - mass spectrometry.

IBA such as Particle Induced X-ray Emission (PIXE) and Particle Induced Gamma- ray Emission (PIGE), offer the advantage of a highly sensitive and rapid drug analysis excluding the sample pretreatment phase, reducing the use of solvents and minimizing the error occurring from sample preparation. These techniques ensure the quantification of a drug through the quantification of its heteroatoms of interest [6-10]. Preparation of calibration curve is not needed.

A decade ago, IBA techniques have been introduced in the drug quality control procedure and are now a part of the present contribution. PIXE is applicable when the drug comprises heavy or semi heavy heteroatoms (atomic number, $Z > 11$) while PIGE offers an alternative technique in determining light elements ($Z<$lithium). Furthermore, a complementary IBA technique called Rutherford Backscattering Spectroscopy (RBS) technique is usually applied in the case of drug analysis, where most of the matrix is composed of carbon, hydrogen and oxygen. RBS is ideal to give an overview on the sample's major composition. Accelerator-based IBA techniques offer an advantage over other methods because of their fast measurement speed, non-destructive analysis, and widespread range of elements reachable for analysis qualitatively and quantitatively [11-15]. Although accelerator is not usually available in a quality control laboratory, but in case it is accessible, IBA techniques are useful in rapid screening and searching for counterfeit drugs as it elaborates results in a fast manner.

Gas Chromatographic technique is used to examine volatile substances in gaseous phase. After dissolution and vaporization, the sample is distributed between the mobile phase and the stationary phase. It is a significant biophysical method that enables the separation, identification, and purification of the constituents of a mixture for qualitative analysis and quantitative determination [16].

Spectroscopic technique provides different information than those given by chromatography as it is considered as a fingerprint of molecules. It is based on chemical reaction and UV absorption.

It measures quantitatively the transmission or reflection properties of a substance as function of wavelength. It is one of the most useful methods of quantitative analysis [17].

The aim of the present research was to further study and develop new approaches to characterize both the bulk and pharmaceutical dosage form containing Fluoxetine and to provide quantitative elemental data. The newly developed analytical methods were found to be sensitive, simple, accurate, rapid and cost effective.

2. EXPERIMENTAL

2.1 Chemicals and Reagents

Reference standard Fluoxetine was certified to contain 99.5% (ABOTT, BEIRUT, LEBANON). Prozac® was purchased from local pharmacy in Beirut and labelled to contain 20 mg. Sodium hydroxide has a purity of 98-99% (SCOTT SCIENCE UK). Potassium permanganate was ensured (FLUKA). Methanol of analytical grade (SIGMA-ALDRICH, CHROMASOLV®).

2.2 Instrumentation

All spectrophotometric measurements were performed by Jasco V-730 double beam UV-Vis Spectrophotometer linked to a computer programmed with Jasco Spectra Manager software intended for measurements and spectral acquisition and elaboration. A pair of 1 cm quartz cells were needed to measure the spectra. As for GC-MS, an Agilent technology 6890 N series gas chromatography and a source 5975 B series mass spectrometry coupled with a HPG1701AAMS Chemstation software was used. It comprises an auto sampler, a splitless injector and an electron impact ionization. The column is a 5%phenyl-methylsilicone with a 0.25 µm film thickness.

The IBA experiment was performed by using 3 MeV proton beam forwarded by a 1.7 MV 5-SDH tandem accelerator. The target was hit by the beam at 0 degree. X-ray emission from targets was perceived using a silicon (lithium) detector with 12.7 µm thick berylium window and 165 eV measured FWHM energy resolution at 5.9 KeV, situated at 135° quoting to the beam direction. 131 µm thick Kapton filter was implanted between the detector and the sample. The produced gamma rays were perceived, at 45° quoting to the normal target, by an HPGe detector with 40% relative efficiency and FWHM 1.9 KeV at 1332 KeV, sheltered with lead. For RBS analysis, silicon PIPS detector located at 165° quoting to the beam direction, was utilized.

2.3 Preparation of Standard Stock and Working Solutions

2.3.1 Spectrophotometric method

Twenty mg of reference standard Fluoxetine powder were accurately weighed and dissolved in 5 mL of 0.5 M acetic acid and diluted to the mark in a 100 mL calibrated flask with distilled water.

A calculated volume of Fluoxetine standard stock solution was diluted using distilled water to attain a concentration of 20 µg/mL working standard solution.

2.3.2 GC-MS method

Standard stock solution of Fluoxetine 1000 µg/mL was prepared in methanol. The stock solution was stored at 40°C in the refrigerator. Calculated volume of the above standard stock solution was diluted with methanol to obtain100 µg/mL Fluoxetine working standard solution.

2.3.3 IBA techniques

Few milligrams of the pure standard Fluoxetine were pressed into a pellet using an external binder (boric acid) at 4 tons/cm^2. On the surface of the pellet a thin carbon coating was deposited to guarantee surface conductivity as requisite in vacuum ion beam analysis methods. Samples were first desiccated as pulverized humidity under beam irradiation changes the sample elemental composition giving false results.

2.4 General Procedures

2.4.1 Spectrophotometric method

Aliquots of Fluoxetine working standard solution were transported to a series of 10mL volumetric flasks to ensure the concentration array stated in table 4. A volume of 2Ml, 2M NaOH followed by 2mL, 2 mg/mL $KMnO_4$ were added to each flask. The content of each flask was mixed well and kept for 15 min with intermittent shaking. Distilled water was then used to make up the volume to the mark. The absorbance value of each solution was determined at 610 nm against a blank prepared similarly. The absorbance values at 610 nm were plotted against the matching concentrations to make the calibration graph.

2.4.2 GC-MS method

Aliquots of the working standard Fluoxetine solution within the concentration array displayed in table 10 were transferred to a series of 10 mL volumetric flasks. Methanol was used to make up the volume to mark. 1 µL from each prepared solution was injected. Concentrations of the analyte in the samples were determined using peak area generated from the computer software related to the instrument. Acquired results were used for calibration graph's construction. Linear regression and correlation coefficient were estimated.

2.4.3 IBA techniques

The measurement of the mass of active ingredient in the commercial sample was carried out through an absolute calculation, by using the 'matrix calculation mode' of the GUPIX simulation code when PIXE technique was used. Thus, the concentration of visible heteroatoms present in the active ingredient was calculated with satisfactory precision as such an absolute calculation counts on the absorption of emitted x-rays in the sample, matrix composition of the sample, and all geometrical, physical and PIXE entry parameters.

Though, quantitative determination of light elements using PIGE method needed a relative calculation method comprising an external reference-standard material which was the pure active ingredient. The acquired RBS spectra was treated using the SIMNRA simulation code.

2.5 Pharmaceutical Applications

2.5.1 Spectrophotometric method

Twenty Fluoxetine capsules were precisely weighed and emptied. An equivalent weight of 20 mg of Fluoxetine was dissolved in 5 mL of 0.5 M acetic acid and filtered using a filter paper. The filtrate was collected and the content was made up to mark with distilled water in a 100 mL calibrated flask. The solution was then quantitatively diluted with distilled water to get a solution within the linearity range for the analysis, then were treated as under recommended procedure.

2.5.2 GC-MS method

The content of ten pulvules was retained, an equivalent mass of 10 mg was accurately measured and transferred into a beaker, around 90 mL of methanol were added and the resulted mixture was mechanically shaken for 5 min and filtered into 100 mL volumetric flask. The volume was made up to the mark using methanol to achieve a final concentration of 100 µg/mL of Fluoxetine. Aliquots of Prozac® solution were transported to a series of 10 mL volumetric flasks obtaining solutions within the concentration range of 40, 60 and 80 µg/mL.

2.5.3 IBA techniques

Five pulvules of commercial dosage form Prozac® were de-capsulated, milled and an average weight equivalent to one tablet was taken. Same procedure as for pure Fluoxetine sample preparation was applied.

3. RESULT AND DISCUSSION

3.1 Methods Development

3.1.1 Spectrophotometric method

The spectra of aqueous potassium permanganate in alkaline medium revealed an absorption maxima at 525 nm. Adding Fluoxetine produced a new characteristic maxima at 610 nm as displayed in Figure 2 due to the formation of manganate ions having a green color as a result of the oxidation of the Fluoxetine by potassium permanganate. The reaction was rapid taking 10 min for completion.

This method was indicated for the indirect analysis of Fluoxetine by determining the intensification of the absorbance at 610 nm as a function of the Fluoxetine concentration [18]. The reaction scheme is given as follow in equation 1 [19]:

Fluoxetine + Excess of $KMnO_4$ → Oxidation product of Fluoxetine + K_2MnO_4 (Equation 1)

The diverse experimental parameters were cautiously studied and optimized. The reaction's sensitivity and the formation of the colored species were found to be affected by the permanganate and the alkali concentrations. The optimum conditions for the suggested method (2 mL of 2 M NaOH and 2 mL of 2 mg/mL $KMnO_4$ in a 10 mL total volume) were conserved throughout the experiment.

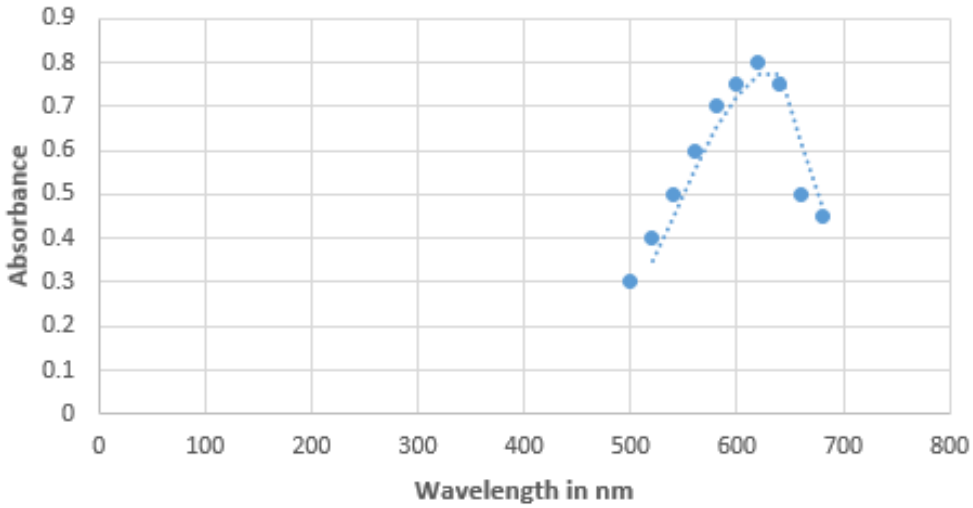

Fig.2: Absorption spectra of 8 mg/mL Fluoxetine with 2mL, 2 M NaOH.

The redox reaction is a zero-order reaction as the rate is independent on the concentration of the reactants. The rate of the equation is as follow:

$Rate = -\Delta [A] / \Delta t = k$ *where K is the zero-order rate constant.*

This equation was the basic for various kinetic trials that were performed to find Fluoxetine concentration [20, 21]. The intermediate rate method was based on the selection of an intermediate rate at a later time selected between 15 and 20 min as there might not be sufficient time for the reaction to get started. The fixed time method selected a time of 25 min as it was the suitable value for measurement and calibration construction. The fixed absorbance method measured the required time for Fluoxetine concentration to reach a pre-selected absorbance value of 0.45 giving the widest calibration curve. The rate constant method performed the absorbance–time curve with several drug concentrations ranged between 4-10 µg/mL, under optimum described conditions.

Based upon the applicability, the sensitivity, the intercept and the correlation coefficient, the fixed time method was selected as suitable kinetic methods to determine the Fluoxetine concentration through its oxidation as exposed in Table1.

Table1: Regression Versus Correlation.

	Regression equation	Correlation coefficient
Intermediate rate method	Y=1.106X+7.2355	0.9926
Fixed time method	Y= 0.0096X+0.0636	0.9966
Fixed absorbance method	Y=181.57X-4.6216	0.998
Rate constant method	Y=0.2129X-3.5343	0.7768

3.1.2. GC-MS method

The use of Gas chromatography in analyzing Fluoxetine was performed as the studied drug can be vaporized without any decomposition [22]. Moreover, it was carried out excluding the prederivatization phase saving solvents and time. Different temperature programs were investigated and the best temperature program was selected. The splitless time was set at 0.75 min, the injector temperature found to be 300 ^{o}C and the temperature program of the oven was as follow: the initial oven temperature 100 ^{o}C was preserved for 1 min. The temperature oven was ramped at 15^{o}C and the final temperature chosen to be 325 ^{o}C was maintained for 5 min. The total run time was 21 min. The MS was run in the electron impact mode (EI). The full scan m/z was ranged between 30 and 500 mu. Extracted ion chromatograms were used to determine the analyte. Peak areas were used for quantification. Table 2 shows the GC-Ms optimized conditions. The constructed calibration graph exhibited good linearity. Regression coefficient was excellent. The mass spectra represented in Figure 3 showed peaks of Fluoxetine at mass to charge ratio, m/z = 43 and 104. Smaller other peaks were noted. The peak at m/z 43 derived from the loss of CH_2 $NHCH_3$ group which is the secondary amine. The peak at m/z 148 was caused by the bond breakage between the asymmetric atom of carbon and atom of oxygen. The peak at m/z 309 indicated the molecular ion of Fluoxetine [23].

Table 2: Experimental Conditions of the GC-MS Selected Procedure.

Carrier gas	Helium
Column head pressure	14.12 psi
Flow rate	13.9 mL/min
Solvent delay	3.4 min
Oven	The initial temperature 100^{o}C was preserved for 1 min. The temperature oven was ramped at 15^{o}C and the final temperature chosen to be 325^{o}C was maintained for 5 min.
Injector temperature	300^{o}C
Sample injected	1µL
Total run time	21 min
Scan rate	2.4 scans/s
Data collection	30-500 mass units (mu)

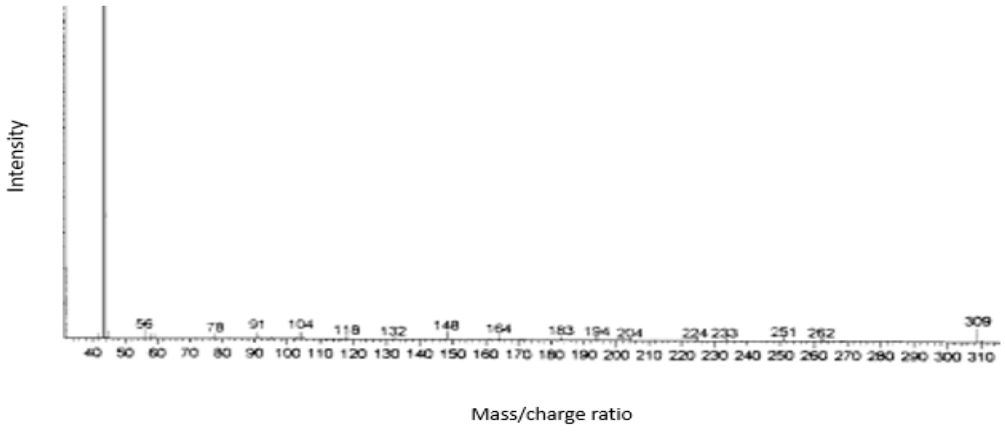

Fig.3: Mass spectra of Fluoxetine.

3.1.3 IBA techniques

The concentration of the analyzed heteroatoms samples was in percent level in samples, the usage of a current's beam of some tens of nA was sufficient to produce well-designed spectra within few minutes, therefore, the damage is extremely slight on the analyzed samples.

Besides, a systematic study to guarantee the stability under irradiation was performed. Analysis of the pellets was done under 3 MeV proton beam bombardment, utilizing 0.1nA- 20 nA beam currents with several acquisition time ranged between 0 min and 20 min, for dose and dose rate consequence evaluation. The samples proved stability under irradiation and the overall accumulated charge ranged between 0.5 and 5 µC. Under these settings, the number of counts of fluorine and chlorine per µC versus accumulated charge was almost stable. Figure 4 shows the PIGE spectra of the drug pellet (red spectrum) compared to the pure active ingredient (black spectrum). Five fingerprint peaks of fluorine at E=197, 1236, 1349, 1357 and 1459 keV are present in the spectra as well as the annihilation peak at E=511 keV. The quantitation of Fluoxetine, via the fluorine atom, in this work is based upon the 197 keV gamma ray emitted from the 19F (p,p'γ)19F nuclear reaction.

This gamma ray is particular to fluorine and it has high cross section comparing to other gamma rays. The total accumulated charges for each sample were 0.33 µC during 8min. Figure 5 presents the PIXE spectra of the drug pellet (red spectrum) and the pure active ingredient (black spectrum). Chlorine fingerprint peaks at E= 2.62 and 2.82 keV are well seen in both spectra. Calcium signal at E= 3.69 keV detected in the PIXE spectrum of the sample are minimal, possibly provided from the formulation. The total accumulated charges for each sample were 0.33 µC during 8 min. Part of the spectra was magnified by 4.

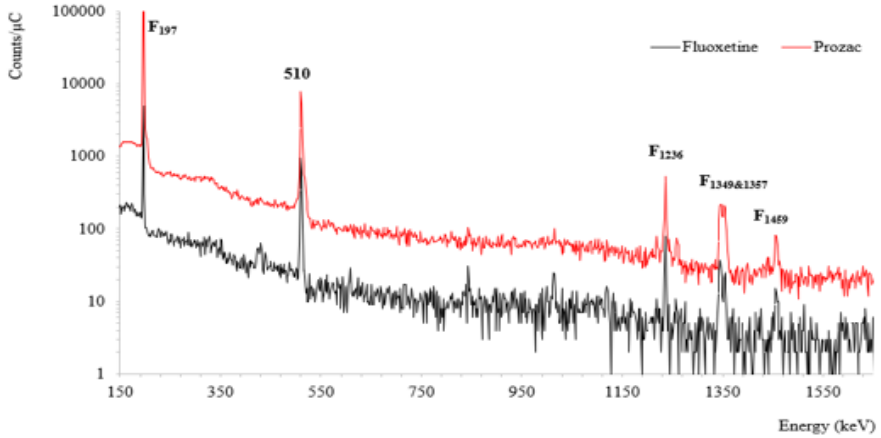

Fig.4: PIGE spectra made from pure Fluoxetine (Black) and Prozac ® 20mg (Red).

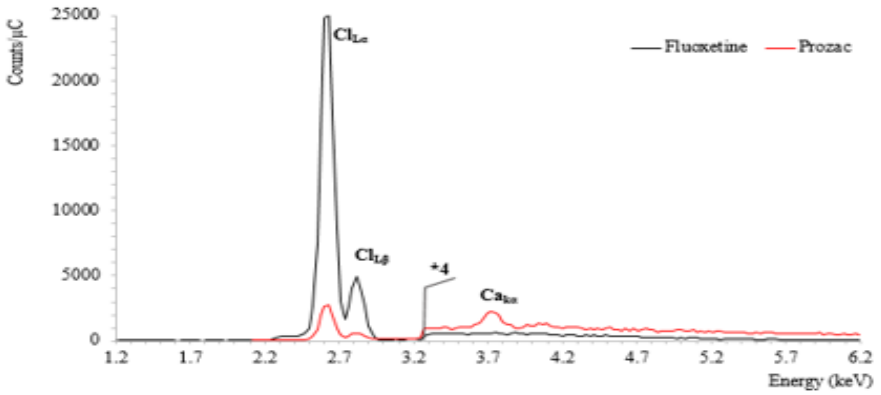

Fig.5: PIXE spectra of pellets made from pure Fluoxetine (Black) and Prozac® 20 mg (Red). Part of the spectra was magnified by 4 for presentation purposes only.

One must note that the excipients of the analyzed drug in the pharmaceutical dosage form were fluorine and chlorine free, thus either PIXE or PIGE experiment would be performed alone for the determination of the tested drug. However, we are presenting results from both techniques. Pure Fluoxetine as external standard is needed for verification of the parameters in PIXE and for the calculation in PIGE. However, the composition of the major elements of the external standard and the Fluoxetine-based drug should be known so that the calculation is accurate. Therefore, an RBS experiment was done simultaneously and during the PIXE/PIGE experiments to determine the stoichiometry of the major elements.

Figure 6 presents the experimental RBS spectra of the Fluoxetine standard and the Prozac® drug. The total accumulated charges for each sample were 0.33 µC during 8min. These spectra showed the difference between the two samples compositions. The former is high in carbon, fluorine and chlorine compared to the drug that is mainly composed of oxygen and carbon. This is logically arising from the excipient's chemical composition.

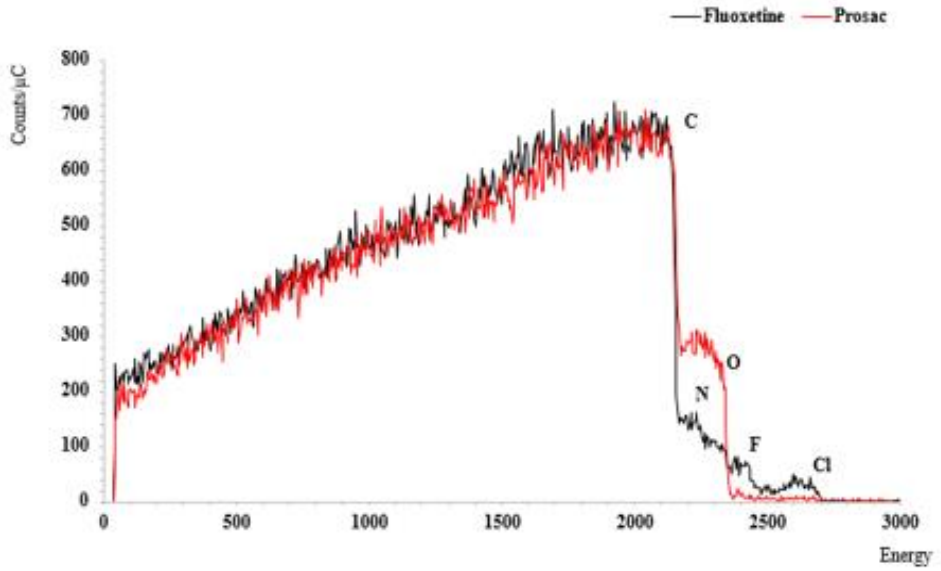

Fig.6: RBS made from pure Fluoxetine (Black) and Prozac® (Red).

Table 3 displays the calculated elemental composition of the samples extracted from the RBS spectra after using the SIMNRA code. The investigated elemental composition for a Fluoxetine based drug (Prozac) is also displayed. The reliability of our RBS experiment is demonstrated by the exceptional agreement between the experimental composition and the theoretical one, for the Fluoxetine standard material. The values calculated for the drug sample verify our observation to the spectra. The differences between the composition of the evaluated drugs and the composition of the standard were noted in the high percentage of oxygen and the low percentages of fluorine. The high percentage of oxygen is due to the chemical composition of the used excipients

Table 3: Elemental composition of pure Fluoxetine sample, calculate (Ct) and measured (Cm) using RBS.

Elements	C_t	C_m	H_t	H_m	O_t	O_m	N_t	N_m	F_t	F_m	Cl_t	Cl_m	Ca_t	Ca_m
Fluoxetine	59.1	58.02	5.68	6.02	4.72	4.62	3.47	4.04	16.57	18.8	10.5	8.5		
Prozac		45.55		11.9		37.8		2.1		1.5		0.11		1.12

3.2 Method Validation

Under optimal conditions, the validation of spectrophotometry and GC-MS was performed in accordance to ICH guidelines [24, 25].

3.2.1 Linearity, concentration range limit of detection and limit of quantification

The standard calibration curves for Spectrophotometry and Gas chromatography - mass spectrometry methods were constructed under the optimum conditions, the linear regression equations were derived. A linear relationship between concentration of Fluoxetine and response which is confirmed by a significant value of the correlation coefficient. Linearity parameters, sensitivity parameters, limit of detection (LOD) and limit of quantification (LOQ) were calculated according to LOD=3.3 SD of intercept /slope and LOQ=10 SD of intercept /slope. Results are summarized in Table 4.

Table 4: Assay Parameters for the Determination of Fluoxetine.

	Spectrophotometric method	GC-MS method
Beer's law limits (µg/mL)	2-10	20-100
LOD (µg/mL)	1.603	26.116
LOQ (µg/mL)	4.858	79.141
Regression equation		
Intercept (a) SD	0.0135	5×10^{6}
Slope (b) SD	0.03	4.24×10^{4}
Correlation coefficient, r	0.996	0.994
Mean	100.19	100.318
SD	0.808	7.914

3.2.2 Accuracy

The accuracy of the Spectrophotometry and Gas chromatography - mass spectrometry methods was assessed by recovery study of Fluoxetine in capsules at three concentration stages within the linearity range. The recovered concentrations were attained using the regression equation. The analytical results obtained are summarized in Tables 5 and 6.

Table 5: Accuracy Data for the Determination of Fluoxetine Using the Spectrophotometry method.

Taken Fluoxetine (µg/mL)	Taken Prozac® (µg/mL)	Mean recovery ± SD [a] RSD % [b] Er % [c]
4	**4**	100.3 ± 0.78 0.78 -0.3
6	**6**	99.94 ± 0.38 0.38 0.05
8	**8**	100.02 ± 0.23 0.23 -0.02

Table 6: Accuracy Data for the Determination of Fluoxetine Using the GC-MS method.

Taken Fluoxetine (mg/mL)	**Taken Prozac (mg/mL)**	**Mean recovery ± SD [a] RSD % [b] Er% [c]**
0.04	**0.04**	99.33 ± 0.25 0.25 0.66
0.06	**0.06**	100.43 ± 0.81 0.81 -0.43
0.08	**0.08**	100.14 ± 0.68 0.67 -0.14

a Mean recovery ± SD for the three determinations
b RSD % Relative standard deviation
c Er% Relative error

3.2.3 Precision

The precision (inter-day, intra-day) of the spectrophotometry and GC-MS methods was examined at three concentration stages of analyte within the linearity range with 3 replicate determinations within day or on three different days. The analytical results are summarized in Tables 7 and 8. The low values of % RSD indicates high precision

Table 7: Precision Data for the Determination of Fluoxetine using the Proposed Spectrophotometric method.

Taken Fluoxetine µg/mL	**Intra-day precision Mean recovery ± SD [a] RSD % [b] Er % [c]**	**Inter-day precision Mean recovery ± SD [a] RSD % [b] Er % [c]**
4	99.91 ± 0.96 0.96 0.08	99.99 ± 0.83 0.08 0.001
6	101.42 ± 0.16 0.16 -1.42	101.46 ± 0.35 0.34 -1.46
8	99.67 ± 0.72 0.72 0.32	100.2 1± 0.48 0.47 -0.21

Table 8: Precision Data for the Determination of Fluoxetine using the GC-MS method.

Taken Fluoxetine mg/mL	Intra-day precision Mean recovery ± SD [a] RSD % [b] Er % [c]	Inter-day precision Mean recovery ± SD [a] RSD % [b] Er % [c]
0.04	100.28 ± 0.78 0.78 -0.28	100.07 ± 0.98 0.98 -0.07
0.06	100.08 ± 1.35 1.35 -0.08	99.75 ± 0.67 0.67 0.24
0.08	101.14 ± 0.5 0.49 -1.14	100.93 ± 0.8 0.87 -0.93

a Mean recovery ± SD for the three determinations
b RSD % Relative standard deviation
c Er% Relative error

3.2.4 Robustness

The robustness was verified by investigating the effect of concentration of $KMNO_4$ and the reaction time for the spectrophotometry and by assessing the effect of the splitless time for the GC-MS. The results reflect that there is any significant effect on the proposed methods.

3.3 Pharmaceutical Applications

3.3.1 Pharmaceutical application for spectrophotometric and GC-MS methods

The developed spectrophotometric and GC-MS methods were tested for the analysis of Fluoxetine in pharmaceutical dosage form Prozac®. The investigated results, presented in Table 9, exposed satisfactory precision and accuracy for % recovery, SD and %RSD as presented in Table. The good recoveries pointed to the absence of interference of excipients. The proposed methods were satisfactory in comparison to the reference method [26] and any noteworthy difference was noted methods as indicated for t, F values.

Table 9: Determination of Fluoxetine in Pharmaceutical Preparation using spectrophotometry and GC-MS methods.

	Spectrophotometry method	GC-MS method	Proposed method [26]
Mean% recovery ±SD	100.14±0.67	99.789±0.873	99.9±1.4
% RSD	0.67	0.875	1.4
Error	0.14	0.210	0.1
t-value	0.71	0.149	
F –value	4.33	2.57	

Mean ±SD for five determinations

Theoretical values of t- and F-test at P=0.05 are 2.571 and 6.3882 respectively.

3.3.2. Pharmaceutical application for IBA methods

The composition of the analyzed samples compared to the labeled one is presented in Table 10. The measured Fluoxetine concentrations by PIGE and PIXE are compared with the labelled one. Excellent agreement was found between the labeled composition and the experimental one with a precision of less than 3% and a very low limit of detection. The obtained result demonstrates the ability of PIGE and PIXE techniques for a rapid and precise determination of Fluoxetine ingredient in the commercial dosage forms

Table 10: Comparison between Fluoxetine Labeled and Measured Concentrations using PIXE and PIGE Techniques.

Prozac drug labeled 20mg		
	Measured A.I. amount (mg)	L.O.D (mg)
CI (PIXE)	19.5±0.5	0.8
F (PIGE)	20.7±0.9	0.5

4. CONCLUSION

Three new, simple and rapid techniques were established for the simultaneous Fluoxetine's analysis in bulk and pharmaceutical dosage form. The diverse methods deliver choices of apparatus conferring to their availability, moreover the techniques are characterized by the usefulness of economic reagents which are freely accessible in any quality control laboratory. The achieved results are of good sensitivity specifically the spectrophotometric method. Validation was achieved conferring to the ICH guidelines where the outcomes are linear, accurate, precise, specific and robust. All the developed methods can be appropriately used in the determination of Fluoxetine based upon the former benefits and results.

4.1 Acknowledgements

The authors wish to thank the Faculty of Pharmacy at Beirut Arab University and the Lebanese atomic Energy Commission-NCSR, research and development department for necessary facility needed for achieving this research work.

4.2 Conflict of interest

The authors state that they have any known personal relationships or competing monetary interests that could have seemed to influence the reported work in this paper.

4.3 Funding support

None.

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