Electrochemical quantification of fluoxetine in pharmaceutical formulation using carbon nanoparticles

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A sensitive electrochemical sensor is formed by a carbon nanoparticles (CNPs)/glassy carbon electrode for the determination of antidepressant drug fluoxetine in pharmaceutical formulation. The dependence of fluoxetine electro-oxidation currents and potentials on pH concentration and the potential scan rate was studied. Scanning electron microscopy was used for characterisation of the CNPs. The porous layer of the CNPs improved the electroactive surface area and led to a significant increasing in the peak currents, based on the diffusion within a nanoporous film. The best electrochemical response was obtained from differential pulse voltammograms in the buffer solution (pH 9.0). Linear calibration graphs were obtained in 1–25 μM with %RSD values from 0.3 to 5.7 and the detection limit of 0.4 μM ($S/N = 3$). The CNPs modified electrode was successfully applied for fluoxetine determination in capsules and the results showed sufficient precision and achieved a mean recovery accuracy of 99.06%.

1. Introduction: Fluoxetine hydrochloride, one of the selective serotonin reuptake inhibitor drugs, is widely used as an antidepressant compound based on the inhibition of serotonin-reuptake in the central nervous system [1]. Fluoxetine has been employed for the treatment of major depression, obsessive compulsive disorder, bulimia, autism, panic disorders, nervous anorexia, borderline personality, alcoholism, obesity and geriatrics by cocaine [2].

Drug determination is one of the effective ways for drug quality control. Consequently, the development of a simple, sensitive and reliable method for drug analysis has a great importance.

Several techniques for fluoxetine analysis have been reported in the literature. Most of these studies have developed using gas chromatography [3] and high performance liquid chromatography [4]. Spectrophotometric methods [5] and capillary electrophoresis [6] have also been employed to fluoxetine analysis in biological samples and pharmaceutical formulations.

Application of electrochemical techniques and nanostructured modified electrodes in pharmaceutical analysis have attracted great interest because of their advantages such as efficiency, accuracy, sensitivity, simplicity and low cost [7–10]. Regarding the possible electro-oxidation of the fluoxetine nitro group, electrochemical quantification is suitable for its analysis. Lencastre *et al.* [11] have enhanced the oxidative behaviour of fluoxetine at the glassy carbon (GC) electrode. They have introduced a square wave voltammetric method for analysis of fluoxetine by using a borate buffer solution at pH 9. The electrochemical reduction of fluoxetine has been developed by Roque da Silva *et al.* [12] by using a hanging mercury drop electrode in an alkaline buffer solution and in a water/acetonitrile mixed solvent. An electroanalytical method based on square wave adsorptive stripping voltammetry was developed for determination of fluoxetine by Nouws *et al.* [13]. Ardelean *et al.* [14] developed an electrochemical method for the detection of fluoxetine in aqueousmedia by using a commercial boron-doped diamond electrode using cycling voltammetry, differential pulse voltammetry, square wave voltammetry and chronoamperometry. The best performance in relation with the lowest limit of detection was reached by using differential pulse voltammetry. In addition, Hussein *et al.* [15] reported ion-selective electrodes for the determination of fluoxetine in capsules and biological fluids.

Nanostructures have been progressively important in electrochemical researches [16–20]. Some unique properties can be considered in nanostructured materials, for example, catalysis, the large surface-to-volume ratio and more adsorption sites [21, 22]. Carbon nanoparticles (CNPs) are interesting carbon materials that offer all the advantages of nanocarbons which are extensively applied in electronics, electrochemistry and material science.

In this work, we developed the fabrication of a CNPs modified electrode for fluoxetine determination by using the differential pulse voltammetric technique. This method improved qualities such as simplicity of electrode preparation, wider linear range, low detection limit, high selectivity and excellent stability of the film modifier. To obtain results, the electrode was successfully applied to fluoxetine determination in pharmaceutical dosage form in commercial preparations.

2. Experimental

2.1. Apparatus and reagents: Voltammetric experiments were performed by using μ-AUTOLAB TYP III in combination with the GPES software. A conventional three-electrode system was used for this study [23]. For the analysis, a modified GC electrode with CNPs (CNPs/GC electrode) was used as a working electrode, a saturated Ag/AgCl/KCl as a reference electrode and a platinum wire as the counter electrode. The GC electrode (Azar electrode, $d = 2.0$ mm) was used and manually pretreated with alumina slurry. Furthermore, a Philips model X-30 scanning electron microscope (SEM) was used to capture images. A Britton-Robinson buffer, containing 0.04 M of each component (acetic acid, O-phosphoric and boric acid), was adjusted to the required pH by 0.2 M NaOH. A working standard fluoxetine

powder was obtained from the Iranian Quality Control Laboratory. CNPs (ca. 55 nm diameter, specific surface area 60 m^2/g) were obtained from the Cabot Corporation.

2.2. Preparation of the modified GC electrode: The modifier suspension was prepared by dispersing 5 mg of the CNP in 1 ml of chloroform under sonication for 30 min. The modifier film was prepared by casting 4 µl of the mentioned suspension at the surface of the electrode. It was dried in the air to remove the solvent.

2.3. Calibration and system validation: Calibration curves were obtained by plotting peak height against fluoxetine concentration. The fluoxetine standard curves incorporated with different concentrations from 1 to 25 µM were determined over 3 consecutive days. The linear range, limit of detection (LOD), limit of quantification (LOQ, which is considered as the lowest concentration of fluoxetine in the linear calibration curve), repeatability, intermediate precision, recovery and selectivity were evaluated in the determination of fluoxetine. The linear range was obtained by using the differential pulse voltammetry and the analysis of fluoxetine solutions in 1–25 µM. The LOD and the LOQ were calculated from the linear calibration curve. Repeatability (intraday) and reproducibility (inter-day) precision were evaluated at three different concentrations. To assess the repeatability, three replicate measurements of each solution were made in a short period of time. For determining the intermediate precision, each of the solutions was analysed three times a day for three consecutive days. The accuracy of the procedure was verified by performing recovery assays in triplicate.

2.4. Pharmaceutical analysis: The contents of 20 capsules (10 mg) of fluoxetine were weighed individually. 1/20 of the mixed powder was transferred into a 25 ml calibrated flask and diluted with double distilled water. The solutions were filtered through a 0.45 µm membrane filter. A suitable amount of the standard solution was diluted with a buffer solution at pH 9 (to obtain fluoxetine 10 µM). After analysing this solution, a standard amount of fluoxetine was added to the solution to gain fluoxetine solution with a concentration of 15 µM.

3. Results and discussion

3.1. Characterisation of the CNPs/GC electrode: The morphology and the properties of the GC electrode coated by CNP film were characterised by author Eilkhanizadeh using SEM. Fig. 1 shows the well dispersed/deposited CNPs at the GC electrode surface. From the SEM images, the size of the CNPs was estimated to be 32–56 nm.

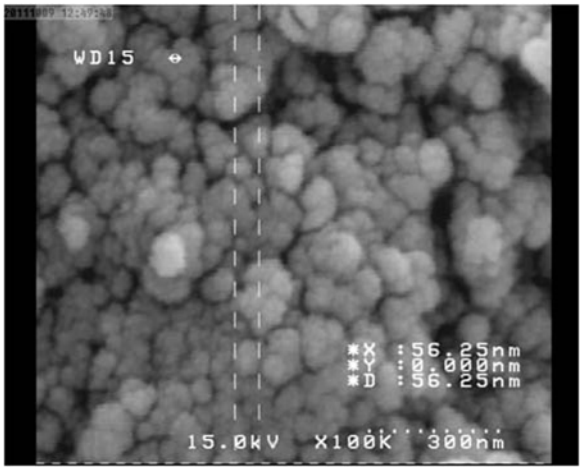

Figure 1 *SEM image of surface of CNPs/GC electrode*

3.2. Cyclic and differential pulse voltammetric studies of fluoxetine: Voltammetric studies of 20 µM fluoxetine at pH 9.0 were conducted, first, on bare, and secondly, on the CNPs film modified GC electrodes at 0.1 V/s. Part (a) of Fig. 2 shows a weak irreversible anodic wave with an oxidation peak potential of about 0.96 V and a peak current of 1.35 µA. However, for the modified GC electrode, an oxidation peak potential at 0.89 V and a peak current of 62.5 µA were observed [part (b) of Fig. 2].

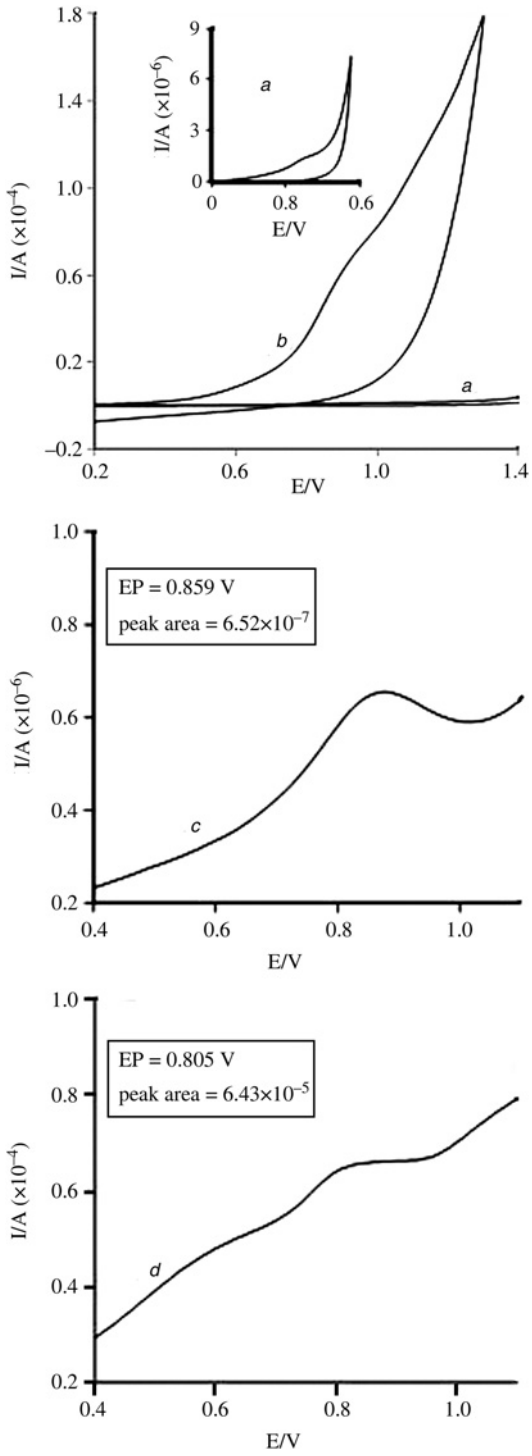

Figure 2 *Comparison*
a, *b* CVs
c, *d* DPVs of 20 µM fluoxetine at surface of bare GC (*a*, *c*), and CNPs-GC (*b*, *d*) electrodes in 0.04 M buffer solution (pH 9.0)
Scan rate in CV measurements was 0.1 V/s and pulse amplitude for DPVs was 0.08 V

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In these voltammograms, the peak current was enhanced 46 times at the CNPs/GC electrode compared with the bare GC electrode. In parts (a) and (b) of Fig. 2, the results show a high-quality response of fluoxetine on the CNPs/GC electrode. Parts (c) and (d) of Fig. 2 show the differential pulse voltammetric studies of a buffer solution (pH 9.0) containing 20 μM fluoxetine on the GC and the CNPs/GC electrodes, respectively. According to this Figure, one anodic peak appeared in each voltammogram with potentials of 859 mV and 805 mV at 0.1 V/s by using the GC and the CNPs/GC electrodes, respectively. Therefore the peak area was enhanced from 6.52×10^{-7} to 6.43×10^{-5}. The obtained differential pulse voltammogram (DPV) of fluoxetine on the CNPs/GC electrode shows an enhancement of the peak current about 98 times with a 54 mV negative shift. No cathodic peak was observed for fluoxetine during the reverse scan, suggesting a totally irreversible behaviour of the fluoxetine electro-oxidation process. In comparison with the bare electrode, a negative shift in the peak potential and increased peak current indicates that the CNPs film facilitates the kinetics of the fluoxetine electro-oxidation and decreases the over potential. Compton and his co-workers have described that the increasing of the peak currents on the modified electrodes by using thin films of nanotubes and fullerenes related to a new diffusion pathway added to the semi-infinite planar diffusion [24, 25].

3.3. pH effects of the solutions: The pH effect of the supporting electrolyte on the anodic peak currents and potentials was developed by differential pulse voltammetry (Fig. 3). A linear negative shift in the anodic peak potential of fluoxetine was observed by increasing the pH of the solution with the following equation

$$E\text{pa (V)} = -0.059\ \text{pH} + 1.31\ (R^2 = 0.970)$$

According to the slope 0.059 V/pH for fluoxetine, it can be deduced that an equal number of electrons and protons were involved in the fluoxetine electro-oxidation (Fig. 3*a*). On the other hand, the cathodic peak current was increased by the pH from 5 to 9. However, further increases in the pH caused a decrease of the peak intensity (Fig. 3*b*). Therefore the buffer with pH 9.0 was used as the supporting electrolyte in all voltammetric determinations. It is known that the secondary and the tertiary amines can be oxidised in acetonitrile to give the corresponding aldehyde and amine derivatives [26]. The following mechanism is proposed for the electro-oxidation of fluoxetine (Fig. 4). According to the previous studies, in basic and neutral media, fluoxetine oxidises to *N*-methyl-propylamine in an irreversible mechanism involving two protons and two electrons [26].

The deduced results from the cyclic voltammograms of 20 μM fluoxetine at pH 9 at 0.08–0.8 V/s at the surface of the modified GC electrode with the CNPs film, indicated that the peak current (*I*pa) of fluoxetine was increased linearly against the square root of the potential sweep rate ($v^{1/2}$) (Fig. 5*a*). This means that the oxidation processes are diffusion-controlled in the whole range of the studied scan rates, with the following equation

$$I\text{pa (A)} = 1\text{E} - 05\nu^{1/2} + 5\text{E} - 06\ (R^2 = 0.998)$$

As shown in Fig. 5*b*, the anodic peak potentials against log *v* show a linear relationship. The regression equation is $E\text{pa} = 0.209 \log v + 1.132$ ($R^2 = 0.992$, *E*pa: V, *v*: V s^{-1}). Such a behaviour reveals the irreversible nature of the electrochemical process of fluoxetine. Furthermore, only an oxidation peak was observed, which suggests that the electrode reaction of fluoxetine under these conditions is totally irreversible.

3.4. Differential pulse voltammetric determinations: The DPVs, as an electrochemical method, showed high sensitivity and low detection limit which was applied for determining the trace amounts of fluoxetine. This process was performed under the optimum conditions including the deposition of 4 μl CNPs/$CHCl_3$ suspensions (5 mg/ml) and performing the measurements at pH 9 [the DPVs of the solutions with different concentrations of fluoxetine are shown in Fig. 6*a*. In these experiments, a pulse amplitude of 0.08 V was used to obtain the DPVs. The oxidation

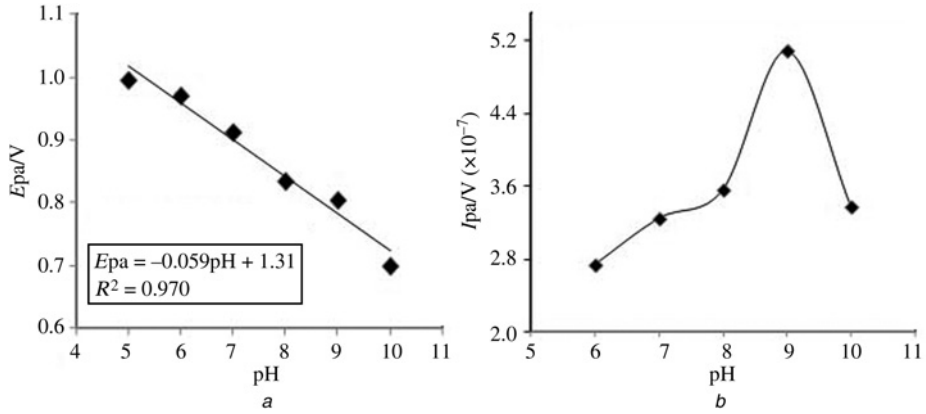

Figure 3 *Relationship between oxidation peak potential (Epa) (Fig. 3a) and oxidation peak current (Ipa) (Fig. 3b) and pH for 20 μM fluoxetine solutions in Britton-Robinson buffer using differential pulse voltammetry on CNPs/GC electrode, pulse amplitude 0.08 V*

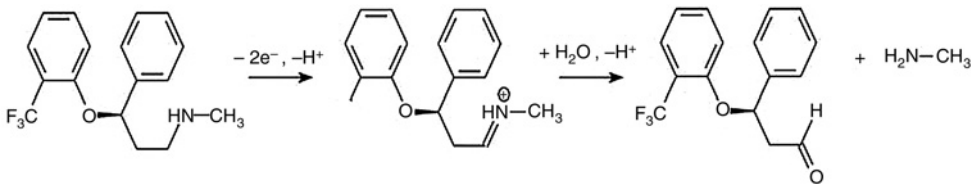

Figure 4 *Electro-oxidation mechanism of fluoxetine on CNPs/GC electrode*

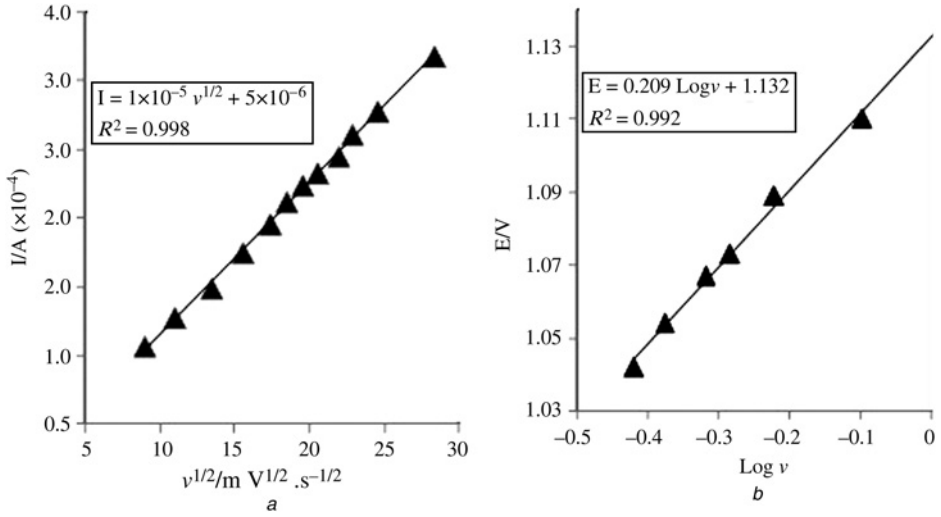

Figure 5 *Plot of* Ipa *against* $v^{1/2}$ *(Fig. 5a) and variation of peak potential (Ep) with log v (Fig. 5b) for cyclic voltammograms of 20 μM fluoxetine at different scan rates in 0.08–0.8 V/s at surface of CNPs/GC electrode in buffer solution at pH 9.0*

peak currents were proportional to fluoxetine concentration in 1–25 μM (Fig. 6*b*) with a detection limit of 0.4 μM (0.12 mg/l) fluoxetine. This value is less than the reported detection limit for GC (1.0 μM) [11], boron-doped diamond (37 mg/l) [14] and ion selective (6.9 mg/l) [15] electrodes. The precision of the method was investigated with respect to both repeatability and reproducibility. The repeatability of the modified electrode was investigated by repetitive recording of 20 μM fluoxetine. The % RSD for the peak currents in the DPVs based on 12 replicates was 2.06%, indicating excellent repeatability for the response of the modified electrode. Reproducibility was investigated by electrochemical determination of three replicate samples of 1, 10 and 20 μM standards over three consecutive days. Mean concentrations were found to be 1, 10.6 and 20.5 μM with associated %RSD values of 2.2, 3.5 and 2, respectively. The ruggedness of the method was assessed by the intra- and inter-day assay results for fluoxetine which was performed by two analysts. The %RSD values for the intra- and inter-day assays of fluoxetine in the cited formulations, performed in the same laboratory by the two analysts, did not exceed 7%, suggesting the ruggedness of this method. The results represented excellent precisions for the determination of fluoxetine by using the modified GC electrode. The characteristics of the calibration curves of fluoxetine at pH 9.0 are presented in Table 1.

3.5. Analytical applications: The method of standard addition was applied for fluoxetine determination in fluoxetine hydrochloride by using the CNPs/GC electrode. The accuracy of the proposed method was studied by recovery experiments in the middle concentration of the linear range (15 μM, $n = 9$). The results showed outstanding recovery, 98.40% with an RSD of 0.7%. Considering the resultant DPVs, there were no interferences for the fluoxetine electro-oxidation during the analysis. To assess the applicability of the proposed method, the modified electrode was used to determine the content of fluoxetine in 'fluoxetine hydrochloride' capsules as a real pharmaceutical sample. The powder of 20 capsules containing 10 mg fluoxetine was weighed and mixed completely. An amount equal to 0.2 g of the sample was dissolved in 25 ml of 0.04 mol/l buffer solution (pH 9) to obtain a nominal concentration. Then, a nominal concentration of 10 μM was achieved by further dilution with the same buffer solution. Afterwards, a 174 μl portion of the resulted solution was transferred to a 10 ml volumetric flask and spiked with the standard solution of 1–25 μM. The result of the assay of

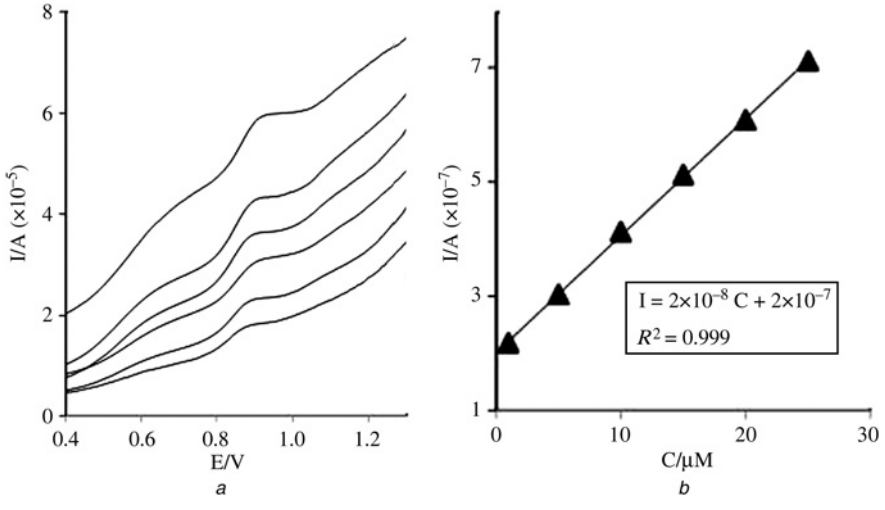

Figure 6 *DPVs of various fluoxetine concentrations from down to up: 1, 5, 10, 15, 20 and 25 μM (Fig. 6a) and calibration curve (Fig. 6b) of peak currents against fluoxetine concentration of 1–25 μM at the surface of the CNPs/GC electrode (pH 9.0). The pulse amplitude was 0.08 V*

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Table 1 Precision (intra- and inter-day) in standard solutions of fluoxetine at the CNPs/GC electrode

Concentration	Intraday ($n = 3$) mean response	% RSD	Inter-day ($n = 9$) mean response	% RSD
1	1.72×10^{-7}	6.6	—	—
5	3.4×10^{-7}	2.9	—	—
10	4.75×10^{-7}	3.4	4.36×10^{-7}	7
15	6.2×10^{-7}	0.3	5.65×10^{-7}	9
20	7.35×10^{-7}	1.2	6.77×10^{-7}	9
25	8.9×10^{-7}	0.6	—	—

fluoxetine capsules yielded a recovery of 99.06% for the capsules. The fluoxetine content was obtained to be 9.97 mg per capsule with an RSD of 1.1% ($n = 5$), which is very close to the labelled amount of 10.00 mg. The results of the assay also indicate that the mentioned method is selective for the analysis of fluoxetine without interference from the excipients.

4. Conclusion: Owing to the unique properties of the CNPs such as high specific surface area, electrocatalytic and adsorptive properties, a CNPs modified GC electrode was prepared for the determination of fluoxetine. It showed catalytic effects towards the electro-oxidation of the drug since it enhanced the oxidation peak currents and lowered the oxidation overpotential. Therefore a new sensitive and selective electrochemical sensor was developed for the determination of sub-micromolar amounts of fluoxetine and its application in analysing pharmaceutical formulations.

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