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Original Research article

Spectrophotometric Measurement of Fluoxetine in Drug Formulation after Cloud Point Extraction

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ABSTRACT

In this research study, Cloud point extraction is a powerful technique for preconcentration and separation of the fluoxetine in pharmaceutical formulation before its measurement by spectrophotometry (UV-vis). The fluoxetine was first reacted with NQS (1,2-naphthacinone-4-sulfonate) in an alkaline medium and the color product was extracted and preconcentrated using the triton X-114 surfactant. The effective parameters including, the solution pH, reagent concentration, surfactant concentration, temperature and reaction time were optimized to improve the extraction of the proposed method. In optimum conditions, a linear range of 50 to 1000 ng/L was obtained with a correlation coefficient of 0.9998 and a limit of detection of 30 ng/L. The relative standard deviation (RSD) was calculated as 1.35% for five repeated measurements of concentration of 500 ng/L of fluoxetine. The concentration factor was obtained 10 and enhancement factor was found to be 4.05. The proposed method was successfully used for pre-concentration and measurement of fluoxetine in pharmaceutical formulation. The advantages of this method are the simple performance, high speed and low cost, which are easily applicable in quality control and clinical laboratories and would be a proper alternative to expensive methods such as chromatography.

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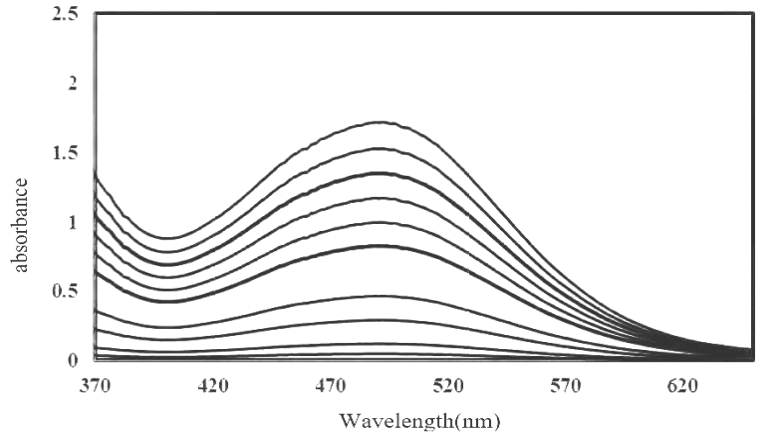
Graphical Abstract



Absorption values obtained after cloud point extraction in the linear range of 05 to 1555 ng/l

Introduction

Fluoxetine is an antidepressant medicine from the serotonin reuptake inhibitors group, which has widely used for treatment of the depression, obsessive-compulsive disorder, panic disorder, and bulimia nervosa [1, 2]. Fluoxetine is well absorbed through the digestive tract after oral consumption. Almost 0.94% of it binds to plasma proteins. The metabolization of this combination in the liver creates active fluoxetine which is mainly excreted through the kidney. The overdose of fluoxetine may include: nausea, vomiting, drowsiness, irregular and rapid heart rate, confusion, fainting, convulsions, or coma. Therefore, its dose should be strictly controlled. Till now, various methods have been reported to determine the fluoxetine dose including, titrimetery, nuclear magnetic resonance spectroscopy, potentiometry, thin-layer chromatography, liquid chromatography, gas chromatography, capillary electrophoresis, and fluorimetry [3-5].

In cloud point extraction, ionic and non-ionic surfactants are used in place of organic solvents, conforming to the twelve principles of green chemistry. Various extraction methods have been developed for preparation of sample before separation and measurement that the main purpose of these methods is to preconcentration and purify the sample. Typical extraction methods are generally time consuming and costly and include numerous steps and high consumption of carcinogenic and toxic organic solvents. However, the cloud point extraction methods, unlike conventional extraction methods, are simple, inexpensive, fast, safe, single-step and independent of solvent or low volume of organic solvent [6-8]. It also has the capability of automation, high sensitivity and appropriate preconcentration factor which depends on optimizing the factors effective on cloud point extraction such as surfactant concentration, surfactant type, pH, reaction time, electrolyte concentration, centrifugation time, incubation time. Given

the advantages mentioned for the cloud point extraction method, it has been widely used for preconcentration and measurement of a variety of pharmaceutical products [9-12]. The chromatographic techniques such as high-performance liquid chromatography (HPLC) is one the most common techniques for separation and measurement of fluoxetine. HPLC method with fluorescence detection has been developed to determine the fluoxetine and its main metabolite norfluoxetine in human plasma [13]. Also, a new liquid chromatographic method with diode array detection is presented herein for to determine the fluoxetine and its main active metabolite in human plasma for toxicological purposes [14].

This study was conducted to present a suitable spectrophotometric method for measuring the fluoxetine by employing an appropriate extraction method for its separating and preconcentrating in pharmaceutical formulation as an alternative to organic solvent extraction methods [13-16].

Experimental

Applied devices and materials

A Lambda 265 spectrophotometer and quartz cells were used to record the measure the absorption spectra. A 255-HR model digital scale with precision of 1.5 mg was used to measure the weight. The Hettich-Germany centrifuge with 15 mL centrifuge tubes was used to accelerate the separation of phases. DENVER pH meter with glass electrode was used to accurate pH measurement. Double distilled water was used in all experiments. fluoxetine solution (100 mg/L) (Abidi) was prepared by dissolving 0.01 g fluoxetine in 100 mL distilled water. Thinner solutions were obtained by sequential dilution of this concentrated solution. Folin solution (NQS) (1.92 mM) was prepared by dissolving 0.05 g of Folin (Merck) in 100 mL distilled water. Solution of 0.1 molar disodium phosphate (DSP) with pH=11 was reached to volume of 80 mL by dissolving 1.4196 g of disodium phosphate (Merck) and solution of 1 molar sodium hydroxide was used to make the medium basic (increasing pH). After reaching the desired pH, the total volume of the solution was reached to 100 mL by adding distilled water. Triton X 114 solution with 2% wt/vol concentration was prepared by dissolving 2 g of Triton X 114 (Merck) in 100 mL distilled water. Sodium chloride 25% wt/vol was prepared by dissolving 25 g of sodium chloride (Merck) in 100 mL distilled water.

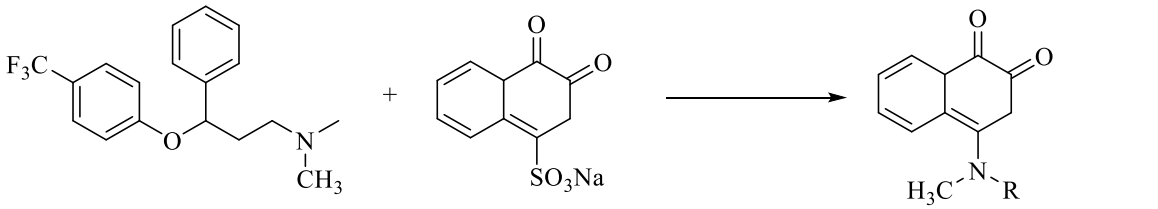
Experiment method

1 mL of fluoxetine solution 10 mg/l, 1 mL of Folin solution (NQS) 1.92 mM and then 1 mL of 1.5 M disudium phosphate solution with pH = 11 were added in a 1 mL/15 mL conical test tube and after 0 min until the reaction was complete and the formation of the dye, 1 mL Triton X 114 2% (wt/vol) was added to the solution, then 0.1 mL of 25% (wt/vol) sodium chloride solution was added and then was

reached to a volume of 1.5 mL by adding distilled water. It was then centrifuged for 5 min at 3500 rpm, and then it was placed in a freeze-salt bath for 15 min for a better separation. Finally, the upper aqueous phase of each tube was separated by bubbly pipette 5 mL and the surfactant-rich phase of each tube was dissolved in 500 µL of ethanol, and was transferred to a 1 mL quartz cell in order to measure the absorbance, and the absorbance was measured at 490 nm.

Results and discussion

The compaction reaction of fluoxetine with Folin produces an orange product according to the following reaction.



FLX NQS Orange colored FLX-NQS

The product obtained in the presence of Triton X 114 and sodium chloride creates two phases (aqueous phase and a surfactant-rich phase), where the colored product is completely extracted with the surfactant-rich phase [17-24]. Figure 1. demonstrates the absorption spectrum of the colored product in the surfactant-rich phase after dilution in ethanol. As can be seen, the maximum absorbance was obtained at the wavelength of 514 nm, so all absorbance measurements were read at this wavelength.

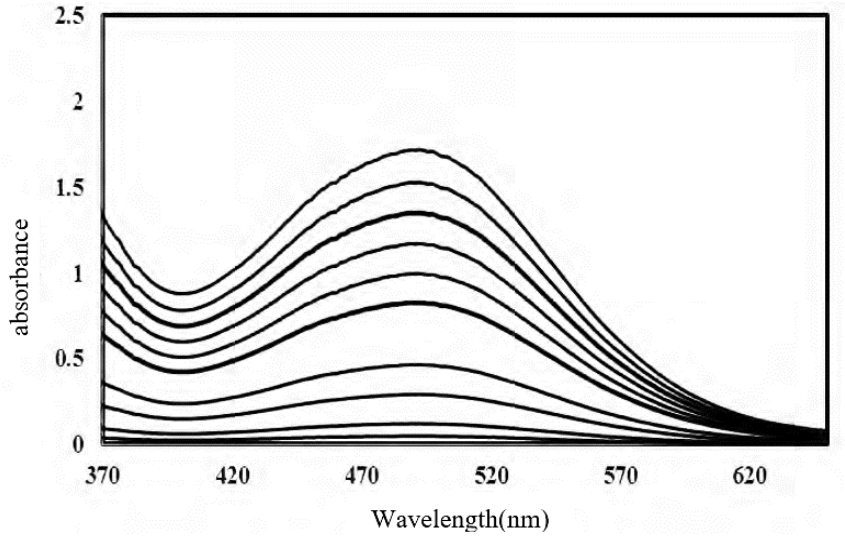


Figure 1. Absorption values obtained after cloud point extraction in the linear range of 50 to 1000 ng / l

Optimizing measurement conditions

The effective parameters in the extraction must be optimized in order to obtain the best results in the experiment. So, various parameters such as the amount of Folin concentration, the effect of pH, the concentration of disodium phosphate, the reaction time, the concentration of surfactant X 114 and the concentration of sodium chloride were investigated in this measurement. The parameters optimization method was performed based on the one in one-time [25, 26].

Effect of folin concentration (NQS)

The effect of Folin concentration on the extraction and quantification of fluoxetine was investigated at the concentrations from 19.2 to 384 μM. As shown in Figure 2, by increasing the Folin concentration up to 192 μM, the absorbance increased and remained almost constant at higher concentrations. Folin was found to be the main reactant in reaction to fluoxetine and the absorbance of solution will vary with the amount of formed product, the concentration of fluoxetine is the limiter. The formation rate of the product varies according to the concentration of fluoxetine because the concentration of fluoxetine acts as a limited analyte and after its finalizing in the solution, the absorbance will be independent of the concentration of Folin. As a result, the absorption rate remained constant at higher concentrations of Folin. Therefore, 192 μM concentration of Folin was selected as the optimum.

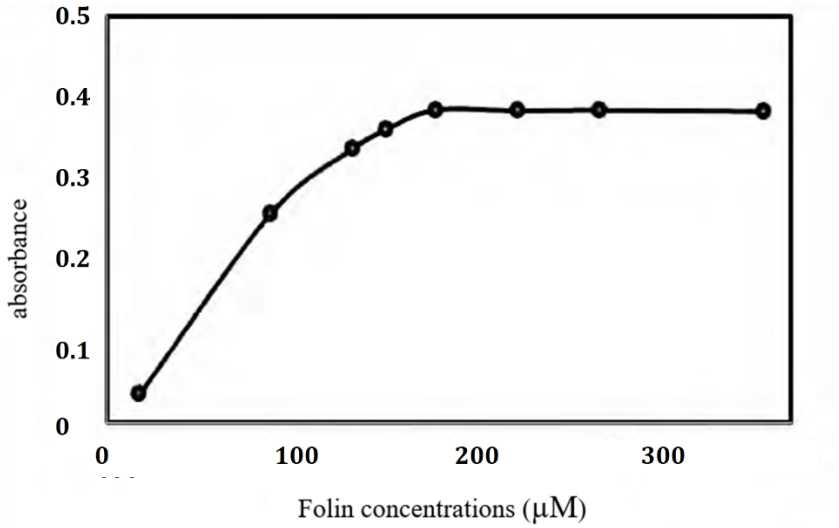


Figure 2. Effect of Folin concentration on cloud point extraction. Conditions: fluoxetine 1 mg/L, 10 mM disodium phosphate with pH = 11, Triton X 111, 0.2% wt/vol, sodium chloride 3% wt/vol

Effect of pH

The effect of pH on the reaction of fluoxetine with NQS was investigated. The change of pH and its effect on the reaction in the buffer solution confirmed that the fluoxetine hardly reacts with NQS in acidic media between 2 to 6. As the pH increases, the amino group of fluoxetine (in hydrochloride) converted to the free amino group, thereby it facilitates the nucleophilic substitution. These studies were obtained at the maximum pH of 2-13, and respectively for the reaction with NQS [27-29]. At pH above 11, a sharp decrease in absorbance reading occurred, this probably is due to an increase in the attributed amount of NaOH ions that makes the fluoxetine reaction with NQS stop. Therefore, the pH 11 was selected as the optimum pH (Figure 3).

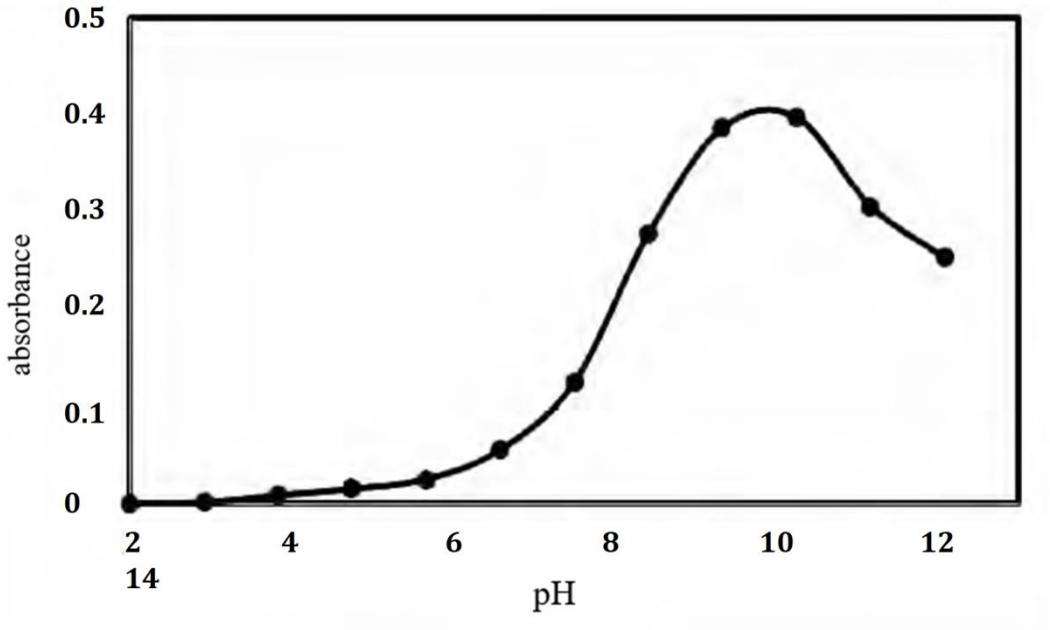


Figure 3. Effect of pH on cloud point extraction. Conditions: fluoxetine 1 mg/L, 10 mM disodium phosphate with different pHs, Folin 192 µM, Triton X114, 0.2% wt/vol, sodium chloride 3% wt/vol

Effect of disudium phosphate (DSP) concentration

The reaction of Folin with fluoxetine is a compaction reaction that requires a basic medium. The effect of concentration of disodium phosphate on the reaction of Folin with fluoxetine was investigated at the range of 1-20 mM. In the reaction of Folin with fluoxetine, by increasing the concentration of disodium phosphate, the absorbance was increased and after 10 mM, the absorbance was turned to a constant level. This can be attributed to the reaction mechanism in which the amine group is protonated in fluoxetine, in the following, a compaction reaction occurs with Folin sulfonate. Since fluoxetine is a reaction restrictor, therefore, by increasing the fluoxetine, the reaction increased rapidly and in the absence of fluoxetine, the reaction stopped. Therefore, a 10 mM concentration of the disodium phosphate is the appropriate concentration for the reaction of folin with fluoxetine (Figure 4).

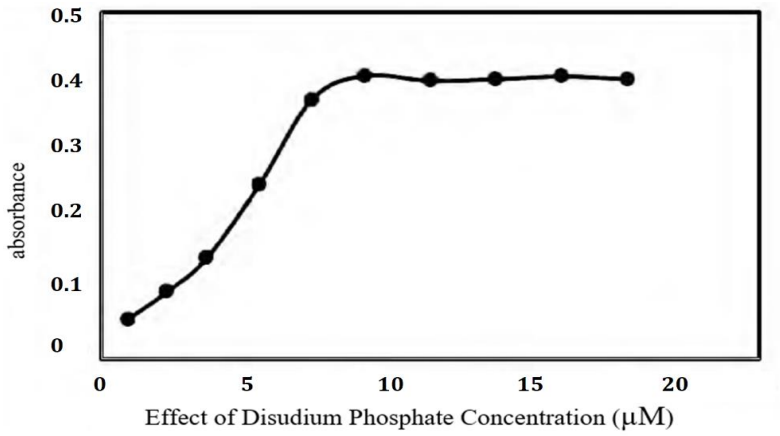


Figure 4. Effect of disudium phosphate concentration on cloud point extraction. Conditions: fluoxetine 1 mg/L, Folin 192 μM, Triton X114, 0.2% wt/vol, sodium chloride 3% wt/vol

Effect of reaction time

The effect of time on the adsorption was investigated at the range of 1-8 min. By increasing the time to 5 min, the absorption increased and then, the absorption remaining unchanged because the reaction was completely done during this time, so the 0 min time was considered as the optimal time (Figure 5).

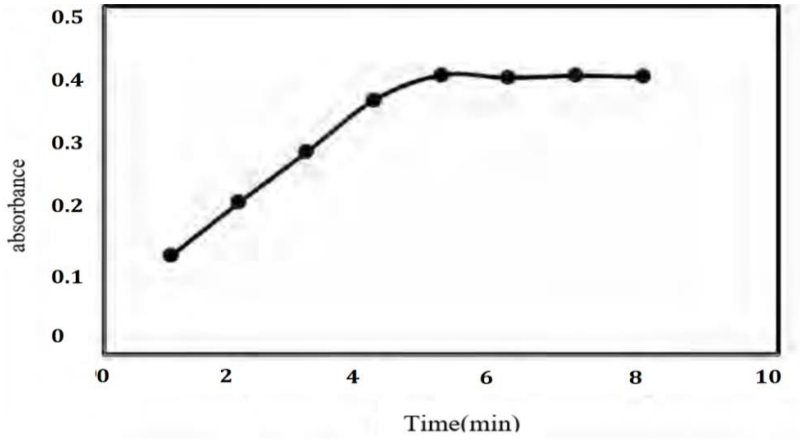


Figure 5. Effect of time on cloud point extraction. Conditions: fluoxetine 1 mg/L, Folin 192 μM, disodium phosphate with pH=11, Triton X114, 0.2% wt/vol, sodium chloride 3% wt/vol

Effect of triton surfactant concentration (TX-114)

The effect of Triton X-114 concentration on the extraction and quantification of fluoxetine was investigated at the concentrations range of 0.02-0.35% wt/vol. The adsorption was increased as the concentration of Triton X-114 enhanced to 0.2% wt/vol, and decreased at higher concentrations because at higher concentrations due to dilution of the analyte, the adsorption intensity decreased.

Therefore, the concentration of 0.2 % wt/vol of Triton X-114 was used as the optimum concentration (Figure 6).

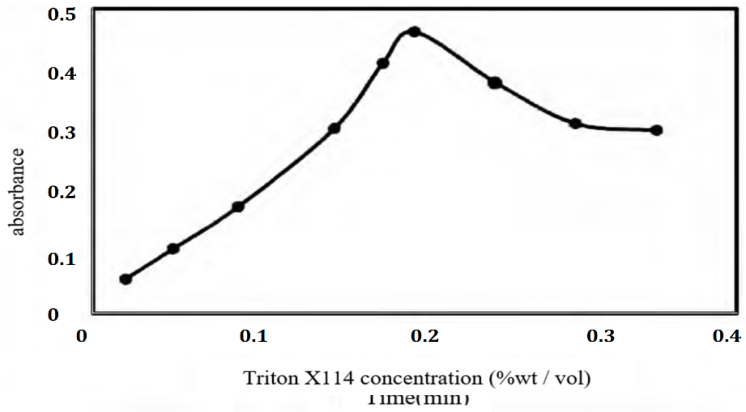


Figure 6. Effect of Triton X-114 concentration on cloud point extraction. Conditions: fluoxetine 1 mg / L, Folin 192 µM, 10 mM disodium phosphate with pH=11, sodium chloride 3% wt/vol

Effect of sodium chloride concentration (NaCl)

The electrolyte concentration plays a significant role in the cloud point extraction. When adding a small amount of sodium chloride to the system, the cloud point temperature decreases. As noted by Gio and Galera Gomez (11), if concentration of the added electrolyte is sufficiently high, the cloud point temperature will decrease, thus the phase is separated at room temperature. The effect of sodium chloride concentration on the extraction and quantification of fluoxetine was assessed at the concentrations range of 0-4% wt/vol. The adsorption increased by increasing the NaCl concentration to 3% wt/vol and remained constant at higher concentrations. Therefore, the concentration of 3% wt/vol of sodium chloride was used as the optimum concentration (Figure 7).

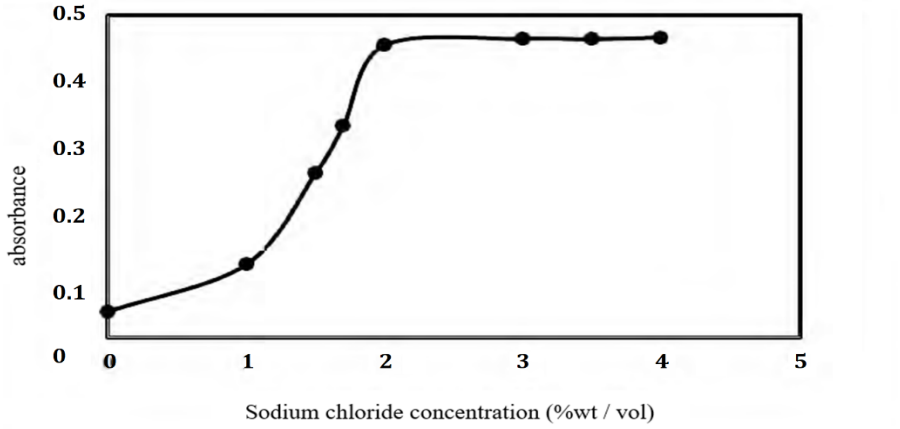


Figure 7. Effect of sodium chloride concentration on cloud point extraction. Conditions: fluoxetine 1 mg/L, Folin 192 µM, 10 mM disodium phosphate with pH=11, Teriton X114 0.2% wt/vol

4-analytical (or degradation) quantities

Relative standard deviation (RSD) (equation 1) is defined as the ratio of absolute standard deviation to mean at 10^3 (equation 1). A value of 1.35 was obtained for 5 repeated measurements at a concentration of 500 ng/mL of fluoxetine. First, 5 series of 500 ng/mL solution of fluoxetine, similar to the standard curve, was prepared in order to evaluate the accuracy of the method.

$$RSD = \left(\frac{S}{\bar{x}}\right) \times 10^3 \text{ppt} \quad (1)$$

The preconcentration factor (PF) (equation 2) is defined as the ratio of the initial volume (V_0) to the saturated phase volume (V_{ext}) (equation 2). Since the initial volume of the solution in a centrifuge tube was 10 mL and after extraction the equilibrium phase volume was reduced to 1 mL, the absorbance measurement was carried out, so the preconcentration factor was found to be 10.

$$PF = V_0 / V_{ext} \quad (2)$$

The enhancement factor (EF), which represents the enhancement rate in the sensitivity of the method, is defined as the ratio of the slope of the calibration curve after extraction $(Slop)_{ext}$ to before extraction $(Slop)_0$. Based on the equation 3 and according to Table 1. and by comparison of the ratio of line slope after extraction to line slope before extraction, enhancement factor value was calculated as 4.05.

$$EF = (Slop)_{ext} / (Slop)_0 \quad (3)$$

Table 1. The measured analytical factors and the formula

λ_{max} **(nm)**	**495**
Calibration curve equation after extraction by CPE (n = 10)	A=1.7557 C-0.0536 and R^2= 0.9998
Calibration curve equation after extraction without (n = 10)	A=0.4332 C -0.0361 and R^2=0.9997
Linear range (ng mL^{-1})	50 - 1000
Detection limit (ng mL^{-1})	30
Repeatability ppt (R.S.D)	1.35
Preconcentration factor	10
Enhancement factor	4.05

Applications

The efficacy of the method in measuring fluoxetine in different samples was evaluated after obtaining optimal conditions and analytical quantities. Initially, different samples of fluoxetine syrup and fluoxetine capsule were selected as samples [30]. Subsequently, different concentrations of fluoxetine were added to the samples and the recovery percentage was calculated according to Table 2. The

recovery rates were approximately 100%, indicating the absence of serious disturbances and matrix effects in the measurement samples. Table 3. shows the comparison of capabilities of the proposed method with other fluoxetine measurement methods [31-33].

Table 2. Measurement of fluoxetine FLX in pharmaceutical samples

Sample	Added fluoxetine (ng/mL)	Recovered fluoxetine (ng/mL)	Percentage of recovery (%)
Fluoxetine syrup	0	507	101.4
	200	712	106
	300	789	96.3
Fluoxetine capsule	0	490	98
	200	711	105.5
	300	785	95

Table 3. Comparison of methods with other reported methods for measuring the fluoxetine

Methods	**Sample matrix**	**Linear range (µg/mL)**	**Detection limit (µg/mL)**
Capillary electrophoresis (CE)	Medicine	5 - 50	0.1
Spectrofluorimetry	Human urine	1 - 0.04	0.0158
High performance liquid chromatography (HPLC)	Human plasma	0.008 – 0.2	0.005
Electrokinetic capillary chromatography	Human urine and blood	0.3 - 3	0.2
Spectrofluorimetry	urine	0.05 - 1	0.02
High performance liquid chromatography (HPLC)	urine	0.05 - 2	0.01
Cloud point extraction	Medicine	0.05 - 1	0.03

Conclusion

Unlike the usual extraction methods, cloud point extraction is a simple, inexpensive, fast, safe, single-stage, and independent on solution or low volume of organic solvent, so it is environmentally friendly. One of the advantages of spectrophotometry is that, unlike the GC and HPLC methods, it does not require long time and using expensive reagent and solvents. Therefore, in view of the aforementioned advantages for cloud-point extraction and UV-vis spectrophotometry, these two methods were used for preconcentration and measurement of fluoxetine in pharmaceutical formulation.

Conflict of Interest:

We have no conflicts of interest to disclose.

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