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Sol-gel Approach for Fabrication of Polypropylene Solid-Phase Microextraction Fiber: An Efficient Method for Enrichment of Trace Levels of Antidepressant Drug, Fluoxetine

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ABSTRACT

This research is on the improvement of the procedure to determination of trace levels of antidepressant drug; fluoxetine in wastewaters. In this research a silica based sol-gel was applied for the extraction of fluoxetine from water samples. This two-phase technique is consisting of aqueous samples of fluoxetine (donor phase) and silica based nanocomposite prepared by sol-gel technique (acceptor phase). Accepter phase was held in the pores and lumen of polypropylene hollow fiber segment. Microextraction experiments were carried out in two steps; extraction from analyte samples by sorbent which is held into the hollow fiber segment and desorption of drug from hallow fiber by using of methanol. Desorbed analyte in order to measurement were offered to HPLC and UV-V is spectrophotometer for further analysis. This method is simple, fast and adopted by a majority of the instrumental methods. Extraction parameters such as sol-gel aging time, pH of donor phase, volume of donor phase, extraction time, stirring rate and effect of surfactant were investigated and optimized. The measurements were done under the optimal conditions. This technique has many advantages, such as the short extraction time, low consumption of organic solvents, elimination of carry-over effect, low limit of detection and high pre-concentration factor. The pre-concentration factor and limit of detection have been gained 3227 and 0.53 ng mL^{-1}, respectively. The linear range and relative standard deviation are 1.0-10000.0 ng mL^{-1} and 4.8% (n=3), respectively.

KEYWORDS: Fluoxetine; Solid Phase Microextraction; Sol-gel; Hollow Fiber.

1. INTRODUCTION

Fluoxetine, *N*-methyl-3-phenyl-3-[4- (trifluoromethyl) phenoxy] propan-1-amine, is an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class [1]. (See Fig.1).

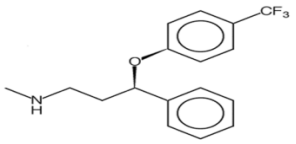

Fig. 1. Fluoxetine (FLX) structure.

It is metabolized by N-demethylation to an active metabolite, norfluoxetine [2]. Fluoxetine to be used in the treatment of obsessive-compulsive disorder and eating disorders, including anorexia nervosa and bulimia nervosa [3]. FLX and many other pharmaceuticals are being produced and used in increasing amounts every year. In recent years much attention has been directed toward the potential effects of pharmaceuticals in the environment. The pharmaceuticals enter the environment either as the active compound itself or as their corresponding metabolites [4].

Pharmaceuticals have been selected or designed due to their biological activity. Unfortunately, they often have low biodegradability and can accumulate, reaching detectable and biologically active amounts. In respect to their purpose they should be considered as hazardous environmental contaminants. Quantitative evaluation of the fate of pharmaceuticals in the aquatic environment, proper risk assessment and improvement of the efficiency of sewage treatment plants need sensitive and reliable analytical methods. Despite the uncontrolled consumption of drugs in recent years, there are no certain data regarding pollution with pharmaceutical residues in Iran. Therefore, the aim of our study was to develop an analytical procedure, which allows the quantification of pharmaceuticals in water at the trace level [5-6]. Pharmaceutical residues are usually present in environmental water samples in trace amounts. Thus a sample isolation and pre-concentration technique is required before drug residues detection.

Solid phase microextraction (SPME) which was introduced by Pawliszyn, is a relatively recent technique and was used increasingly in sample preparation [7]. In this technique, a special syringe

containing a fiber coated with a bonded phase is used. The syringe is inserted into the sample solution or headspace of the sample container**.** The analytes are adsorbed onto the fiber, which is then retracted into the syringe. The syringe is then used for direct or offline desorption and analysis with a gas /liquid chromatography. This is a fast, simple, economical and efficient solvent-free method for extraction and pre-concentration of analyte from various sample matrices [8].This technique is based on partitioning of analyte between a liquid or gas phase and fixed small amount of extraction phase that dispersed on a solid support [9]. Recently, many studies have been reported on the preparation of new kinds of fiber coatings for SPME and their analytical application in the pre-concentration of contaminants from environmental, biological and food samples [10-11]. The extraction efficiency of SPME fiber is determined by different types of intermolecular and steric interactions between the analyte species and the extracting phase. Thus, selective extraction of analytes can be achieved based on their polarity, hydrophobicity, chemical composition, shape/size, etc. [12]. Recently, among the different approaches in coating development the sol–gel approach was introduced. The distinguished character of sol–gel technique is that it can provide efficient incorporation of organic components into inorganic polymeric structures in solution under extraordinarily mild thermal conditions. In addition, SPME fiber prepared by sol–gel techniques offer inherent advantage of low cost, porous structure, high selectivity, high thermal stability and strong adhesion of the coating to the substrate due to chemical bonding [13]. The sol–gel process involves evolution of inorganic networks through the formation of a colloidal suspension (sol) and gelation of the sol to form a network in continuous a liquid phase (gel) [14]. The aim of this research was to improve on previous SPME works through a fabrication of a simple and practical device that can overcome remaining main problems with ordinary SPME fibers like sample carryover. We have introduced a novel SPME technique that uses polypropylene hollow fiber for supporting the sol–gel and produced an affordable, disposable and efficient method which has provided high pre-concentration factor for FLX [15].

2. EXPERIMENTAL

2.1. Chemical and Reagents

FLX was purchased from drug manufacture Dr. Abidi (Tehran, Iran). The sol-gel precursor tetraethylorthosilicate (TEOS), ethanol (EtOH), methanol (MtOH), 1-octanol, hydrochloric acid (37% w/w) and the additive Triton X-100 were obtained from Merck company (Darmstadt, Germany). Stock standard solution of FLX (1000 μg mL^{-1}) was prepared by dissolving 5 mg of FLX in 5 mL methanol. Standard sample solutions were provided daily at different concentrations by diluting the stock solutions with de-ionized water, which was purified by Milli-Q filtering system (Millipore). The stock standard solution and sample solution were stored at 4 ◦C. Q3/2 Accurel polypropylene microporous hollow-fiber membrane (200 μm wall thickness, 600 μm inner diameter, 0.2 μm pore size, 75% porosity), was obtained from Membrana (Wuppertal, Germany).

2.2. Apparatus

The fluoxetine absorption spectra were recorded and some of initial optimization experiments double-checked using a CECILCE7200 UV-Visible spectrophotometer (Cambridge, England). The HPLC system was a Knauer Smart Line (Berlin, Germany) with a Knauer (S-2500) UV detector. The HPLC loop volume was 20 μL. The column was a Perfectsil Target RP-18 column (4.6mm diameter, 250mm length, ODS3, 5 μm) from Knauer. An RP-18 guard column (4×4mm i.d., 5 μm) was fitted upstream of the analytical column. The mobile phase consisted of acetate buffer (pH=3): methanol: acetonitrile (60:30:10) and was run in isocratic mode at a flow rate of 1.0 $mLmin^{-1}$. The mobile phase was filtered by a Milli-Q filtering system before use and delivered by a Knauer (S-1000) HPLC pump. Signals were monitored at 226 nm. The FT-IR instrument that was used for recording the infrared spectrum was a Shimadzu-8400 instrument (Camag, Japan). A Metrohm 780 pH-meter (Herisau, Switzerland) equipped with a combined glass electrode was used to determine pH values during the experiment.

2.3. Preparation of sol-gel

The sol–gel composites were obtained through modification of the method reported by Oter et al. [16]. The sols were prepared by the acid catalyzed method from a solution containing TEOS, HCl-acidified water, ethanol (EtOH), Triton X-100. Equal volumes of TEOS and EtOH were added into a clean glass vial and stirred for 10 min. Then the HCl-acidified water was added into the vial and the mixture was stirred continually to promote the hydrolysis and condensation reactions. Triton X-100 was added into the sol and the mixture was further stirred for 20 min. The molar ratios of TEOS, ethanol and water were either1:1:4 or 1:1:2. The exact compositions of the sols were given in Table 1. In all cases the solutions were aged at room temperature in the closed glass vials.

Table 1. Composition of the sols.

Cocktail name	TEOS (mL)	EtOH (mL)	Acidified water (μL)	Triton X-100 (μL)	pH
sol-1	1.07	1.07	386 [a]	120	1
sol-2	1.07	1.07	386 [b]	120	5
sol-3	1.07	1.07	193[c]	120	4

[a] 386 refers to 338 μL water and 48 μL concentrated HCl (R=4)

[b] 386 refers to 338 μL water and 48 μL 0.1MHCl (R=4)

[c] 193 refers to 193 μL 0.1 M HCl (R=2)

One of the advantages of sol-gel method is the possibility of using different precursor. In the presented

work tetraethylorthosilicat (TEOS), was used as precursor. Two major sets of reactions take place during sol–gel processing: (i) hydrolysis of the precursor; (ii) poly condensation of the hydrolyzed products [16]. Hydrolysis of precursor could be catalyzed by HCl, formation of a polymeric network is the result of all the reactions.

FT-IR spectra of the sol–gels are shown in Fig. 2. The low frequency peak near 434 cm^{-1} is assigned to Si–O–Si out-of-plane bending. The bands at 804 and 1070 cm^{-1} are ascribed to Si–O–Si symmetric and asymmetric stretching vibrations. The peaks at 956 and 1150 cm^{-1} are related to Si–OH and Si–O–C, respectively.

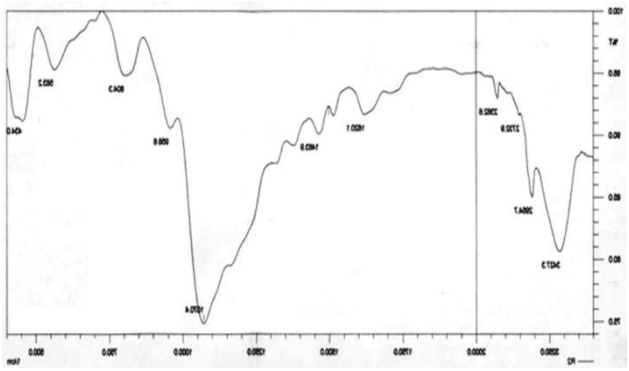

Fig. 2. FT-IR spectrum of the sol–gel.

2.4. Extraction procedure

The polypropylene micro-porous hollow-fiber tubes were cut into 2.0- cm segments. Each piece was used only once to decrease the memory effect. The pores of the hollow fiber wall were previously filled with 1-octanol. This solvent is compatible with polypropylene and easily occupies the pores. Liquid sol was injected into the fibers with a 25 µL Hamilton syringe, the internal volume of the hollow fiber segment was approximately 2µL. The hallow fiber were left to dry at room temperature for 1h. For each experiment, 5 mL of the aqueous sample containing the analyte was poured into a 25 mL sample vial. The sample vial was placed on a magnetic stirrer. For extraction the fiber that was impregnated with sol-gel (acceptor phase), plunged into the sample solution (donor phase) and the analyte was extracted from donor phase to acceptor phase. Extraction was done during the optimized period of time (20 min). After this time, the hollow fiber was removed and plunged in 3 mL methanol in a closed vial and desorbed from the fiber with sonication and the desorbed sample in order to measurement was offered to HPLC for further analysis. Some of initial optimization experiments were re-checked by UV–Vis spectrophotometer, but final results are related to HPLC analysis.

3. RESULTS AND DISSCUTIONS

3.1. Optimization

In order to obtain the optimal extraction conditions, several parameters that influence the extraction efficiency were investigated. Parameters such as sol–gel ageing time, pH of the aqueous sample, extraction time, volume of the aqueous sample, agitation speed and surfactant effect were considerate and optimized. The concentration of analyte in the optimization steps was 1 µg mL^{-1}.

3.1.1. Effect of sol–gel ageing time

Sol viscosity and surface morphology of the sorbent were directly related to the age of the sol. Ageing for an extended period, however, led to a gradual decrease in the viscoelasticity of the gel, likely driven by the expulsion of liquid from the gel, a phenomenon called syneresis [17]. The produced sols gradually increase in the viscosity and finally form gels. However, the ageing characteristics of such highly concentrated sols have not been well known. Furthermore, the addition of the appropriate amount of water during ageing can reduce the speed of the gelling [15]. Fig. 3 shows effect of aging time on the extraction efficiency. Different aging times (30-150 min) were studied at room temperature. The pre-concentration factor for fluoxetine increased up to120 min. After this time, with additional extraction time, the pre-concentration factor began to decrease.

Many difficulties may be arising during the long aging time, mainly caused by the removal of amounts of solvent trapped in the polymeric network. It is leads to a volume decrease, or in other words less porosity. Effects of aging time in sol-gel formulation were correlated to the amount of Si-O-Si bond formation and it was observed by FT-IR measurement. Therefore aging time of 120 min was selected as the optimal time.

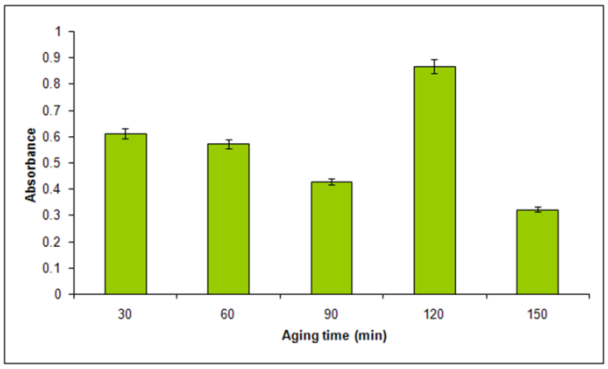

Fig. 3. Effect of aging time on the extraction efficiency.

3.1.2. Effect of pH

The pH value plays an important role in the HF- SPME procedure. A suitable pH value can improve the extraction efficiency and also reduce interference from the matrix. In two-phase, there are many reports where pH changes in the donor aqueous solution resulted in higher analyte pre-concentration [18]. For the basic model analytes such as fluoxetine (pKa= 8.7), pH was adjusted into the alkaline region to de-ionize the analyte, reduce their solubility within the sample solution and ensure efficient transfer into the sol-gel [18]. We studied the pH effect between 3 to11 for the aqueous solution. The results are depicted in Fig. 4.

According to the results the optimum pH was selected 9.0 for donor solution.

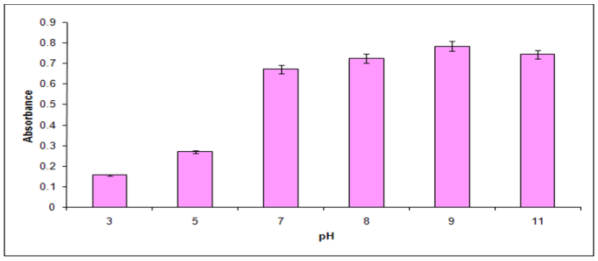

Fig.4. Effect of sample solution pH on the extraction efficiency.

3.1.3. Extraction time

Mass-transfer is a time-dependent process and its rate is reduced the closer the system reaches equilibrium conditions [19]. Because solute molecules need enough time to pass the interface between the donor and acceptor phases the recovery depends on the time that the analyte is in contact with the acceptor phase [20]. Different extraction times (10-40 min) were studied at room temperature and the result are display in Fig. 5. The extraction efficiency for fluoxetine increased up to 20 min. After this time, with additional extraction time, extraction efficiency began to decrease. Extraction time of 20 min was finally chosen as the optimal extraction time.

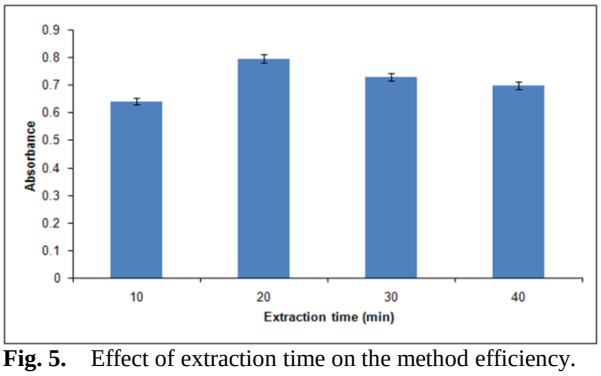

Fig. 5. Effect of extraction time on the method efficiency.

3.1.4. Volume of donor phase

The pre-concentration factor (EF) can be improved by the increasing the volume ratio of donor and acceptor phases.

In this work, the phases' volume of donor and acceptor phases was optimized. According to theoretical aspects of microextraction which were demonstrated by many researchers [21, 23] the pre-concentration factor obtained from the following equation.

$$EF = \frac{1}{1/K_{a/d} + V_a/V_d}$$

The pre-concentration factor can be improved by the increasing the volume ratio of donor and acceptor phases. The results indicated that the best extraction efficiency was obtained when the donor acceptor ratio was more than 1000. Different donor phase of volumes (3-7 mL) were studied, with a constant acceptor phase volume of 6 µL. The results are shown in Fig. 6.

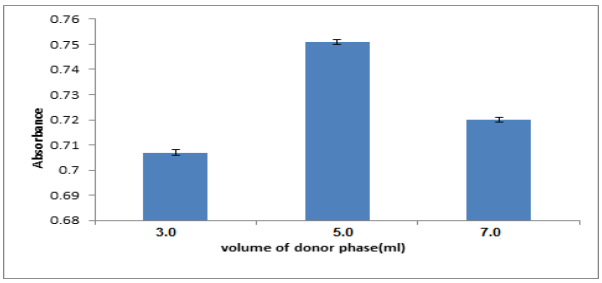

Fig. 6. Effect of donor phase volume on the extraction.

3.1.5. Agitation speed

Agitation of the solution increases mass transfer in the donor phase. It also reduces the time needed to reach equilibrium, reduces the extraction time and accelerates the extraction kinetics [24]. Increasing the agitation rate of the donor solution enhances extraction, as the diffusion of analytes through the interfacial layer of the hollow fiber is facilitated, and improves the repeatability of the extraction [18]. Different stirring Rates (300-900) rpm were tested to determine their effects on the pre-concentration factor of the FLX. The results indicated that the pre-concentration factors of the FLX increase by increasing of the stirring speed up to 500 rpm. Then the pre-concentration factors decreased with a stirring speed of higher than 500. This is probably due to the formation of air bubbles, generated on or near the fiber surface, which decreased the amount of analytes extracted into the sol-gel [25]. Therefore 500 rpm was selected as the optimum stirring speed.

3.1.6. Surfactant effect

Surfactants, or surface active agents, are amphiphilic molecules. The head of it is polar, or hydrophilic, and the tail of it is hydrophobic. The tail is generally a hydrocarbon chain with different member of carbon atoms and may be linear or branched and also contains aromatic rings. One of the most important properties of these compounds is their good capacity to solubilize solutes of different character and nature.

In this research we studied the effect of Triton X-100. Triton X-100, being a non-ionic surfactant, has showed the positive effect on the FLX extraction and has increased its pre-concentration factor. It is may be due to interactions between silicate species with the surfactant molecules via hydrogen-bonding promote the self-assembly process, which is responsible for the formation of sol-gel porosity.

3.2. Validation of the method

The pre-concentration factor, correlation coefficient, repeatability, linearity and limit of detection (LOD) for the extraction of the FLX from aqueous solutions were calculated under optimized conditions. Calibration curves were plotted using seven spiking levels of FLX in the concentration range of 0.001–10 µg mL^{-1}. For each level, three replicate extractions were carried out. The calibration equation was Y= 0.1118 x+ 0.1769. The limit of detection (LOD) of this method was determined using a signal–noise ratio of S/N = 3.

Linearity was observed over the range 1.0-10000.0 ng mL^{-1} for FLX with coefficients (r = 0.9911).
The pre-concentration factor is 3227 and the value for LOD was reported at the 0.53 ng mL^{-1}. In order to evaluate the precision of the method repeatability were conducted. It was obtained 4.8% at three concentration levels (50, 500, 5000 ng mL^{-1}) and with three replications for each within a day.

3.3. Real water analysis

To demonstrate the practical applicability of the mentioned techniques, real water sample were analyzed using these methods. Clinical wastewater was taken from a hospital in Mashhad, Iran. The sample was filtered and analyzed for the presence of target drug, immediately after its collection. FLX was detected in the sample in low concentration. Results are presented in Fig. 7 and Table 2.
In the second step the sample spiked with drug and extracted under optimal conditions. The relative recovery of the analyte from this wastewater sample was higher than 104% compared with that of spiked pure water. This indicates that the matrix effect does not have any significant effect on the extraction efficiency of method. The relative recovery, which is defined as the ratio of peak area of the spiked analyte in real samples and peak area of the spiked analyte in pure distilled water with the same amount of the analyte (1.0 ng mL^{-1}), is presented in Table 2.

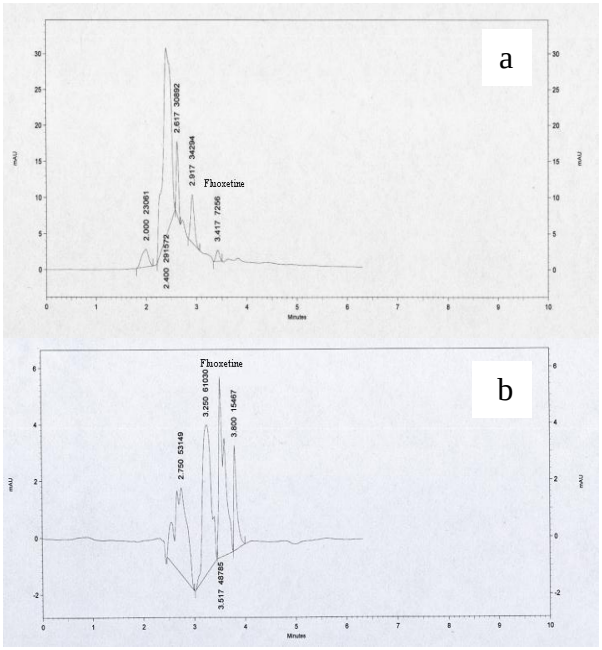

Fig. 7. HPLC chromatograms of (a) clinical wastewater, before spiking and (b) after spiking with 1.0 ng mL^{-1} of FLX.

Table. 2. Clinical wastewater analysis

Analyte	Real Sample	Founded FLX (ng mL^{-1})	Relative Recovery%
Fluoxetine	Clinical wastewater	1.0 ± 0.03	104.2 ±3.90

Table 3. Comparison of some methods which were used for determination of fluoxetine.

No	Date	Matrix	Extraction method	Detection	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	DLR (ng mL^{-1})	r	RSD%	Recovery %	Ref.
1	2004	environmental	SPME	GC-MS	0.017	-	0.1-10	0.999	6.3	100 ± 1	[26]
2	2005	water	SPME	GC-MS	0.25	1	1-100	0.998	7.7	114	[27]
3	2006	urine samples Plasma	SBSE[1]	LC-MS	3	10	10-500	0.983	9.2	57.54	[28]
	2006	Plasma	SPME	HPLC-UV	10	25	25-500	0.991	Less 5%	6.81	[29]
4	2007	human urine	SPME	HPLC-UV	10	-	50-2000	0.999	Less 8.5%	-	[30]
5	2007	samples	SPME	LC-UV	10	40	40-500	0.999	Less 8%	41.9 ± 1.6	[31]
6	2007	human plasma	SPME	LC-MS	-	5	5-50	0.998	Less 5%	34	[32]
7		serum samples									
8	2009	human plasma	SPME	LC-FD[2]	5	10	10-700	0.994	Less 13%	-	[33]
9	2010	human plasma	MEPS[3]	LC-UV	-	20	20-1000	0.999	8.7	-	[34]
10	2015	Clinical wastewater		HPLC-UV	0.53	1.7	1-10000	0.9911	4.8		This work

1 Stir Bar-Sorptive Extraction 2 Fluorescence Detection 3 Microextraction Packed Sorbent

4. CONCLUSIONS

This research has outlined the successful development and application of a new method based on solid phase microextraction (SPME) technique namely sol-gel in hallow fiber, that has been proposed for the determination of FLX from aqueous samples. The method is rapid, simple, inexpensive, virtually solvent free which has provided a high degree of selectivity and pre-concentration factor. The disposable nature of the hollow fiber totally eliminates the possibility of sample carry over and ensures high reproducibility. In addition, the small pore size of hollow fiber prevented large molecules in matrix and unsolved particles in the donor solution from entering the acceptor phase, thus

yielding very clean extract. The whole operation is very convenient to handle because the receiving phase is contained and protected by the hollow fiber. Under optimized conditions, this technique has provided limits of detection in the low ng mL^{-1} range, acceptable precisions and linearity. Therefore, this method is a suitable alternative to conventional SPME technique proposed for the analysis of FLX in environmental samples.

The review of some methods which were used for the determination of FLX in the environmental and biological samples was demonstrated in Table 3.

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