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Preconcentration and Determination of Biperiden by using Solvent Bar Micro Extraction Combined with HPLC in Biological Fluids and Optimization by Chemometrics Design

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Abstract

Bipridein (1-Bicyclo[2.2.1]hept-5-en-2-yl-1-phenyl-3-piperidin-1-yl-propan-1-ol, **I**) is an anti-muscarinic and competitive acetylcholine antagonist agent in central receptors. Its main indication is treatment of drug-induced Parkinsonism and treatment of Parkinson's disease symptoms, which improves the motor symptoms of the disease. Because the blood concentration of this compound is very low, precise methods should be used to measure it. In this study, a combination of solvent bar micro extraction (SBME) and high performance liquid chromatography (HPLC) have been used to concentrate and measuring small amounts of this drug in biological fluids by chemometric methods. At the first, the drug was extracted from aqueous solution containing bipyridine at pH = 9.78 by organic solvent embedded in the hollow fiber pores. Then, in order to concentrate, the drug was transferred to the aqueous phase at pH = 2.98 which was contained in the hollow fiber by a back extraction procedure.Results showed that after extraction under the optimum conditions, pre-concentration factor was 94, limit of detection was 0.015 mg/L and relative standard deviation was 4.1-4.7%. It could be concluded that preconcentration and determination of Biperiden by using solvent bar micro extraction combined with HPLC in biological fluids has been effective.

Keywords: Biperiden, HPLC, Solvent Bar Microextraction, Preconcentration.

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Introduction

Bipridein (Akineton, **I**), is an anticholinergic drug and a competitive muscarinic receptor antagonist. The mechanism of action of these drugs in Parkinson's disease is not very clear. In this disease, due to damage to the basal ganglia, the concentration of dopamine in the intermodal pathways decreases and the balance of cholinergic and dopaminergic axis is disturbed. It is believed that the drugs of this family can correct this imbalance, especially in the non-advanced forms and early stages of the disease and reduce the neurotransmission of acetylcholine. In this way, movement disorders caused by the disease are improved. This drug is used as immunotherapy in people under 60 years of age who do not have cognitive disorders and only have tremors while resting [1-4].

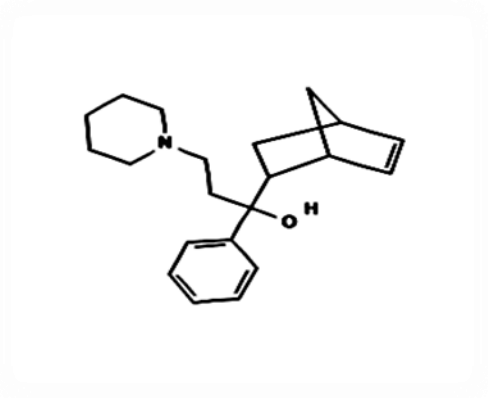


Figure 1. Structure formula of Bipridein (**I**).

Today, the approach of the methods goes towards reducing analytical costs and green chemistry. In extraction methods, the removal of organic solvents is not always done completely, but with modern methods, the consumption of such solvents is reduced so much that sometimes one drop of them is enough [5-7].

Micro-extraction technique is one of the new methods that serve green chemistry and helps to reduce costs and consumption of solvents. It is also highly effective in identifying very small amounts of samples. One of the micro-extraction methods in analysis is liquid phase micro-extraction (LPME). In this method, the amount of organic solvent consumed is significantly reduced [8]. Among the solvent micro-extraction methods, the solvent strip micro-extraction method by hollow fiber membrane (HF-SBME) was used in this article.

In this method, hollow fiber is used for extraction, and the basis of this method is that the hollow fiber is mixed with the solution, and as a result, the extraction speed is faster and the extraction time is shortened. This technique can be done in two ways, two-phase and three-phase. In three-phase micro-extraction, three phases are involved, including the analyte solution phase (donor phase), the organic phase, and the second aqueous phase (receiver phase) in which the extraction is performed. During the extraction, the desired analyte enters the organic phase first and then enters the receptor

phase under appropriate conditions. The speed of extraction depends on the speed of mass transfer from the interfaces between donor phase/organic phase and organic phase/acceptor phase. In general, it is very important to have a high distribution constant for extraction, which can be obtained by choosing the appropriate organic phase and adjusting the pH of the sample. For basic analytes, the pH of the sample should be high (approximately three units greater than the PKa of the analyte), while the pH of the receiver phase should be acidic (preferably three units less than the PKa of the analyte). For acidic analyte, reverse conditions should be prepared [9, 10].

The plasma concentration of this drug is so low that there is not much information about the pharmacokinetics of it. In the latest investigations, they have been able to study the pharmacokinetics of the drug by presenting a method based on gas chromatography [11, 12]. For this reason, in this research, an attempt was made to provide a fast, accurate, efficient, low-cost, accessible and highly sensitive method for measuring of this drug. In this method, by using the pre-concentration method, very small amounts of this drug can be concentrated and extracted in biological fluids (such as plasma and urine) and set the value with HPLC.

Experimental

Chemicals

Bipridan standard with high purity was obtained from Hakim Pharmaceutical Company and methanol with HPLC grade was obtained from Romil Company. Other chemicals were purchased from Merck and Sigma Aldrich companies with analytical grade. The initial standard solution of the drug with a concentration of 100 ppm was prepared in methanol, which can be used for one week according to the special conditions of drug storage (4°C inside aluminum foil sheets to prevent light exposure) [2].

Secondary standards were prepared by diluting the primary standard in phosphate buffer. In addition, working standard solutions were prepared daily by diluting the standard solutions with buffer to the desired concentration. All secondary standard solutions were kept in the refrigerator at 2-8°C and were prepared fresh every day. A urine sample (without drug) was also prepared from a healthy volunteer.

Apparatus

An HPLC device made by Shimadzu, Japan measured the drug. This device was equipped with an LC-10ADVP Quaternary pump with four solvent inlets, a UV/PDA detector and a manual injection site with a loop volume of 10 µl. Lab Solution software was used to record the chromatogram and measure the area under the peak. The separations were done in a C18 column with a length of 15 cm and an inner diameter of 4.6 mm filled with particles of 5 µm. The pH of the solutions was

adjusted by the AZ 86502 pH meter made in Taiwan. In all extractions, polypropylene hollow fibers with pore size of 0.2 micrometers, inner diameter of 600 micrometers and wall thickness of 200 micrometers were used.

In order to inject the solution into the fiber, 100-microliter micro-syringes made by Hamilton Company were used. Heidolph stirrers with a rotation range of 0-1400 rpm stirred the analyte solution. 1, 5, and 10 ml bubble pipettes, 1 ml graduated pipettes, and 100 microliter Hamilton syringes were used to measure and remove different volumes. Also, Milli Pore filtration set including funnel, cellulose and Teflon aqueous-polyacetate filters, Erlen vacuum and vacuum pump were used for filtration of solvents used in HPLC.

Extraction method

Before starting the extraction, polypropylene fibers were cut to a length of 4.5 cm (the volume of these fibers is 10 microliters). Before use, fibers are placed in acetone and ultrasonic bath for 10 minutes to be completely washed. Then the fibers are placed at ambient temperature until they are completely dried and used for extraction.

Micro-extraction steps

1. To prepare the donor phase, 1 ml of the sample solution with a certain concentration was mixed with 99 ml of the buffer solution inside the container covered with aluminum foil.
2. The sample container was stirred by a magnetic stirrer.
3. 30 µl of the receiver phase was drawn by a 100 µl Hamilton syringe and the tip of the needle was inserted into the fiber by 3 mm.
4. The fiber was suspended in an organic solvent (n-octanol) for 10 minutes to saturate the fiber pores with the organic solvent. Then it is immersed in deionized water three times to remove excess solvent from its surface.
5. The receiving phase was injected into the fiber through a syringe. At this stage, the excess receiver phase is removed from the end of the fiber.
6. The two ends of the fiber were closed by a piece of aluminum foil.
7. The fiber was transferred into the container with the donor phase.
8. The sample solution was stirred for a certain period.
9. At the end of the extraction time, the fiber was removed from the sample solution and its two ends were opened, and the receiving (extractive) phase was drawn into the syringe.
10. The extracted phase (5 µL for all cases in the same form) was directly injected into the HPLC device to measure the drug.

Optimization steps

In order to investigate the effect of parameters on the amount of analyte extraction, chemometric optimization method was used.

Optimization of analysis method with HPLC

In order to analyze the extracted sample, the reverse phase HPLC technique was used and the composition of the mobile phase was optimized for the analysis. In order to achieve short separation times and avoid high consumption of washing solvent as well as proper separation of analyte peaks in the chromatogram, the effect of mobile phase composition and mobile phase flow rate on retention time and analyte separation power was investigated.

For this purpose, mobile phases with different ratios of methanol and sodium dihydrogen phosphate buffer with HPLC grade were investigated with isocratic washing [1, 2].

Optimization of extraction conditions

In three-phase extraction based on the use of fiber, several factors influence the extraction rate and the preconcentration rate of the analyte. Therefore, in order to achieve optimal conditions, the effect of several factors such as the type of receiving phase, the type of donor phase, the ionic strength of the donor phase, the stirring speed of the solution, and the extraction time were investigated. In order to carry out optimization, bipridan was used with a concentration of 1 mg/liter in aqueous solution. Also, to study the extraction results, the area of the analyte peaks in the chromatogram was used.

Effect of pH of the donor phase

pH was set in the range of 9-11.

Effect of pH of the receptor phase

pH was set in the range of 2-4.

Effect of ionic strength of the phase donor

It was investigated in the range of 0-20 (% w/v) NaCl salt.

Effect of stirring of analyte solution

It was checked in the range of 250-750 rpm.

Effect of extraction time

From 20 to 60 minutes was examined.

Effect of temperature of the donor phase

It was checked in the range of 25 to 45°C.

The efficiency of the extraction method

To investigate this issue, preconcentration factors (PE), repeatability (RSD), limit of detection (LOD) and extraction percentage (ER) were investigated and determined to extract the drug from aqueous samples under optimal conditions.

Grading curve

In this research, the grading curve was prepared in water samples. In order to draw the curve, standard solutions with concentrations of 0.05, 0.1, 0.2, 0.5, 1, 2 and 5 mg/liter were prepared from the sample. Then extraction was done under optimal conditions from the prepared standard solutions and the area under the peak obtained was plotted according to the initial concentration of the sample in the donor phase**.**

Determining the preconcentration factor

First, it is necessary to prepare the calibration curve (calibration) of direct injection. For this purpose, standard solutions of Bipridan drug with concentrations of 0.05, 0.1, 0.2, 0.5, 1, 2 and 5 mg/liter, by diluting the methanol sample of Bipridan with a concentration of 100 mg/liter with buffer it was prepared. Then, 5 microliters of each of the standards was injected into the HPLC and the area under the peak was drawn according to the concentration.

Determining repeatability (accuracy)

Extraction was done three times from the standard solution of 1 mg/liter sample in one working day (according to optimal conditions) and 3 repetitions during one week and the amount of relative standard deviation was determined.

Efficiency of the method in real samples

For this purpose, the urine sample of a healthy volunteer was used. First, by diluting the urine sample with 1:4 ratio with deionized water and adjusting the optimal pH, the optimal conditions for extraction were created. Then, the extraction process was performed once without adding the drug

and once again with the addition of the drug to the urine sample. After performing the extraction under optimal conditions, the extracted phase was injected into the HPLC and the area under the peak was placed in the calibration curve to examine the effect of the sample.

In order to check the efficiency of the micro-extraction method in another biological sample, plasma was prepared from a healthy volunteer. For preparation, a specific volume of plasma was diluted with deionized water at a ratio of 4:1. Then its proteins were precipitated with 35% perchloric acid and centrifuged at 3000 rpm for 6 minutes. The upper solution under optimal conditions was used as phase donor for extraction. This process was done once with the drug and once without it, and as before, the extraction phase was injected into the device.

Results

Optimization of mobile phase conditions

In order to achieve proper separation and symmetrical peaks, the separation conditions in the chromatographic device were optimized. The type and ratio of solvents as well as the speed of the mobile phase flows were tested and finally the optimal separation conditions were obtained:

1. The composition of the mobile phase: 70:30 (volume-volume percentage) phosphate buffer: methanol
2. Mobile phase pH: 2
3. Washing type: isocratic
4. Mobile phase flow rate: 1 ml/min
5. Selected wavelength: 240 nm
6. Column type: C18 (150 x 6.4 mm) with a particle diameter of 5 micrometers

Optimization of extraction conditions

Several factors influence the extraction with the three-phase method using porous hollow fiber, which include pH of the donor phase, pH of the receptor phase, extraction time, stirring speed, ionic strength of the donor phase. In order to achieve the maximum efficiency of extraction in order to achieve high sensitivity and repeatability, it is necessary to investigate how each of these factors influence**.**

Optimization by chemometric method

The chemometric method was used to optimize the parameters affecting the pre-concentration of Bipran to provide the correct value for each of them. On the other hand, the chemometric method saves time and test costs. In order to optimize the extraction process, the central composite

chemometric design was done by Minitab software. For this purpose, the effect of different parameters such as extraction time, extraction temperature, pH of the donor phase, pH of the receptor phase, the effect of adding salt to the donor phase and stirring speed of the solution were investigated.

27 experiments were designed for each of them to investigate the effect of the factors mentioned in the three selected levels. It should be noted that these tests were performed at the 95% confidence level and the area under the jump peak for each test was selected as the input of the software.

Simultaneous effect of donor and acceptor phase pH

As the pH of the donor phase increases, the area under the curve of the chromatogram increases up to a maximum value and then decreases. The reason is that according to the structure and PKa of the drug, the pH of the phase donor must be adjusted in such a way that it can effectively convert the desired analyte into a non-ionized state, and as a result, by reducing the solubility of the analyte in water, the extraction efficiency in organic solvent increase.

For the receiver phase, with the increase in pH, the graph first becomes upward and then downward. At acidic pH, the target analyte is ionized at the internal contact surface of the organic and receptor phases and enters the receptor phase by increasing its solubility in water. According to the results and observations for both receiver and donor phases, the maximum extraction efficiency is near the average pH; because the best ionization and deionization conditions for bipyrene are provided in the middle of these ranges (Figure 2**).**

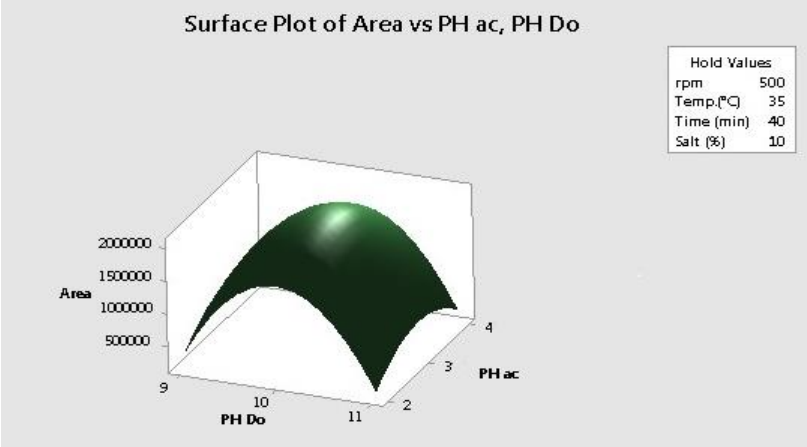


Figure 2. Simultaneous effect of donor and acceptor phase pH

Simultaneous effect of acceptor phase pH and ionic strength

As before, with the increase in the pH of the receiver phase, the area under the curve of the chromatogram first increases and then decreases, and it shows that the average pH of the receiver phase provides the maximum amount of extraction. According to Figure 3, with the increase of salt

percentage and ionic strength, the area under the chromatogram increases and naturally, the extraction efficiency increases. In general, increasing salt can have a two-way effect on extraction efficiency, which is based on the theory of Salting out and Salting in. In the phenomenon of salting in, the presence of salt and ionic charge in the system due to electrostatic interaction between ions and analyte leads to a decrease in the mobility of analyte molecules, resulting in low extraction efficiency. However, in this ionic strength test, it has a positive effect on analyte extraction**.**

This effect can be explained by the salting out phenomenon. In the normal state, the molecules of the analyte in the donor phase are covered by the solvent and reduce the possibility of the activity of the analyte and its entry into the organic solvent. The solvent absorbs ions; as a result, the analyte molecule is free and will be more active. In this way, it enters the organic phase more easily and the extraction efficiency increases.

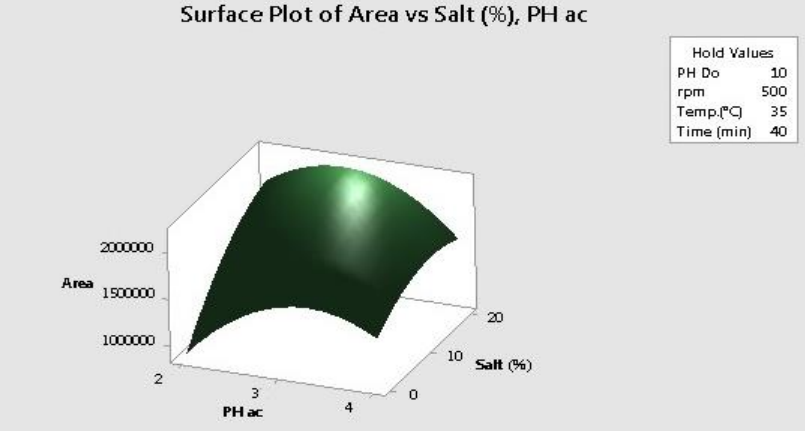


Figure 3. Simultaneous effect of acceptor phase pH and ionic strength.

Simultaneous effect of ionic strength and temperature

According to Figure 4, as the ionic strength increases, the extraction rate also increases. In the designed temperature range, as the temperature increases, the extraction performance increases with a slight slope, and if this process continues, it also decreases with a gentle slope. Based on this, it can be concluded that the average temperature provides the best conditions for extraction.

In general, temperature has a dual function in the extraction process. The reason is that by increasing the temperature to a moderate level, an increase in the concentration factor is seen due to an increase in the diffusion coefficient of the analyte to the fiber wall. On the other hand, the time to reach the equilibrium of the system decreases, but after that, the extraction efficiency decreases due to the evaporation of the organic solvent, as well as the leakage of the receptor phase and the formation of bubbles on the fiber wall.

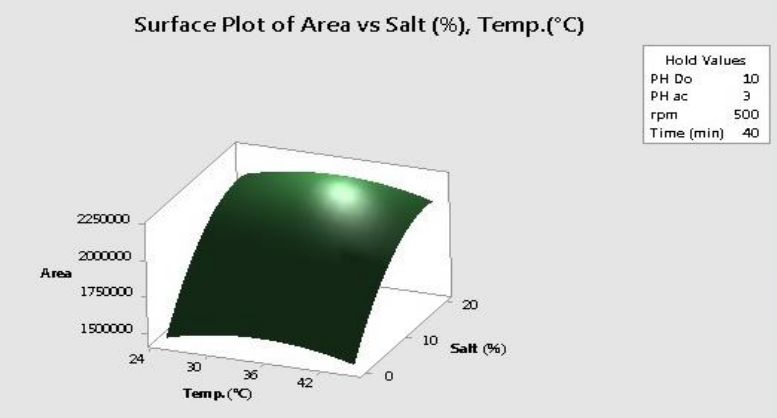


Figure 4.Simultaneous effect of ionic strength and temperature.

Simultaneous effect of ionic strength and time

As discussed in the previous cases, the effect of ionic strength on micro-extraction efficiency is positive. According to the diagram in Figure 5, time reduces the efficiency of micro-extraction of the drug. As the extraction time increases, the organic solvent is washed away and enters the donor phase. When the organic solvent is washed, the pores of the halofiber are opened and the barrier of the organic phase is destroyed, and some of the receiver phase enters the donor phase. Therefore, the amount of the receiving phase that is drawn into the syringe is reduced and naturally, the extraction is reduced.

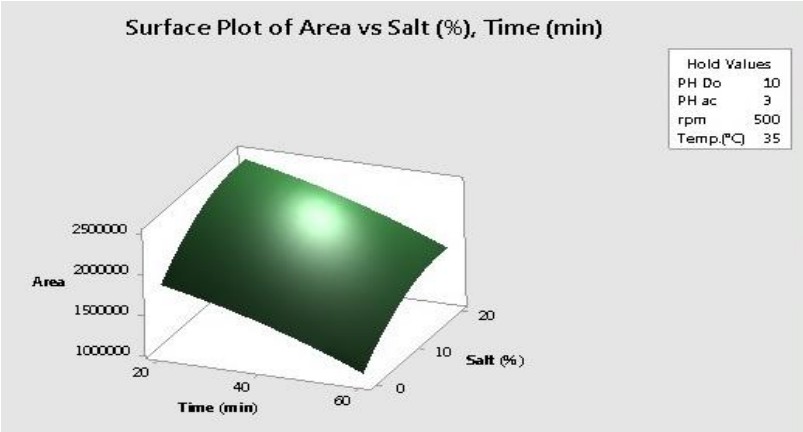


Figure 5. Simultaneous effect of ionic strength and time.

Simultaneous effect of temperature and stirring speed

As explained in the previous sections, the average temperature has the best effect on the extraction efficiency. According to the diagram in Figure 6, with increasing stirring speed, the area under the chromatogram curve increases and the extraction efficiency increases**.**

Stirring the solution is usually used to improve the kinetics and reduce the extraction time, because the equilibrium between these two layers is established faster by stirring the solution. In addition, stirring creates better dynamics in the system, which leads to increased efficiency.

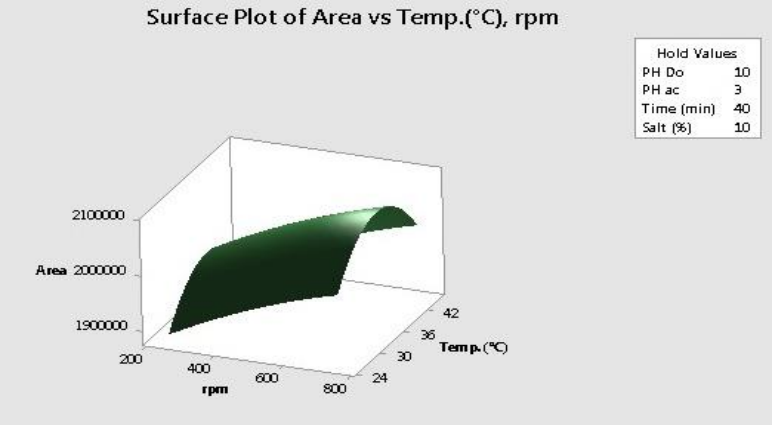


Figure 6. Simultaneous effect of temperature and stirring speed.

According to the peaks and the results obtained from the Minitab software, the best extraction conditions were obtained as follows (Figure 7 and Table 1).

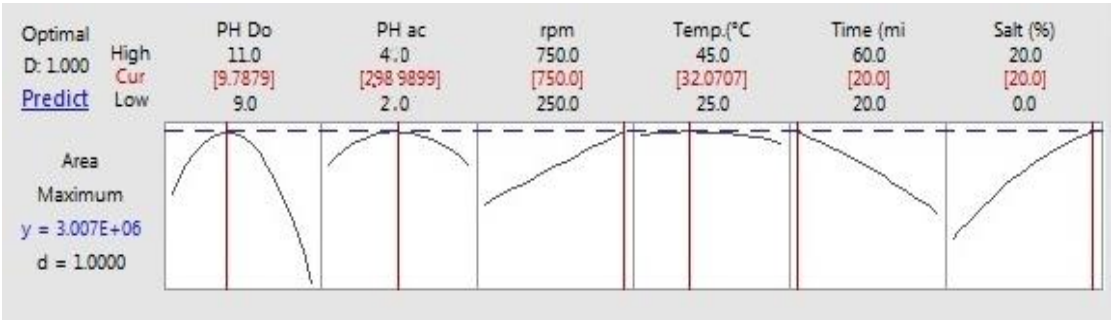


Figure 7. Best microextraction condition.

Table 1. Best microextraction condition.

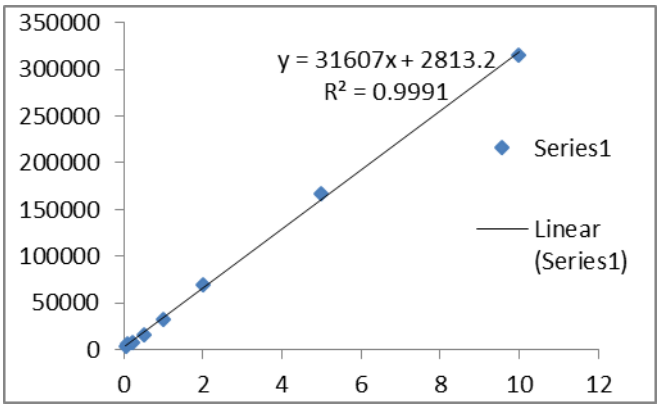
Temperature (c °)	Time (min)	RPM	Salt (%)	pH Donor	pH Acceptor
20	20	750	20	9.78	2.98

Analytical parameters of the extraction method

To validate the extraction method, its analytical parameters, including repeatability (RSD), pre-distortion factor (PF), recovery percentage (R%) and limit of detection (LOD) were checked.

Draw a grading curve

For this purpose, standard solutions with concentrations of 0.05, 0.1, 0.2, 0.5, 1, 2 and 5 mg/liter of the drug were prepared. Then extraction was done under optimal conditions from standard solutions. Finally, the area under the peak was plotted against the initial concentration of the drug in the donor phase. According to Figure 8, the grading chart after extraction in water is linear with a correlation coefficient of 0.9909 (Figure 8).



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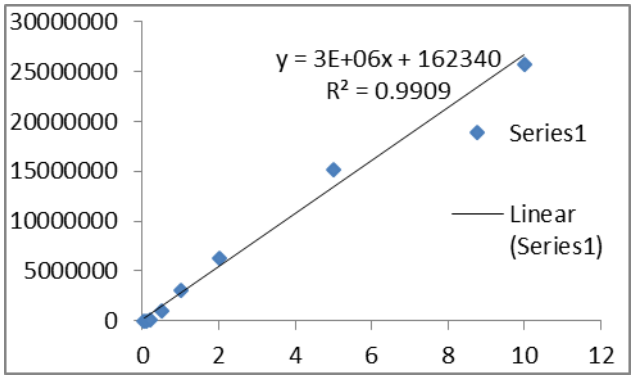

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Figure 8. Grading curve in aqueous soluton before (a) and after (b) drug extraction under optimal conditions.

Preconcentration Factor (PF) and Recovery Percentage (R%)

The pre-concentration factor is defined as the ratio of the concentration of analyte in the receiving phase to the concentration of the analyte in the aqueous donor phase. It is also possible to calculate

the pure pre-dilution factor by dividing the slope of the grading curve after extraction to the slope of direct injection. For this purpose, standard drug solutions with concentrations of 0.05, 0.1, 0.2, 0.5, 1, 2 and 5 mg/liter were prepared. Then, 5 microliters of each of these standards was injected into the HPLC and the area under the peak was drawn according to the concentration. In this way, under optimal conditions, the pre-concentration factor of 94 was obtained. The extraction percentage was determined as 4.9% by considering 10 ml as donor phase and 10 µlit as receiver phase.

Limit of detection (LOD)

To obtain the detection limit, it is necessary to calculate the standard deviation of the reference signal. For this purpose, extraction was done three times with fiber under optimal conditions, which was not added in the drug donor phase. After extracting the control solution, the receiving phase was injected into the HPLC, the area under the graph was measured at the desired inhibition time (11 minutes), and its standard deviation was calculated.

The limit of quantitative detection (LOQ) is also usually 3 to 4 times the LOD. The results are given in Table 2.

Table 2. Analytical parameters of the extraction method.

LOD ($ng\ mL^{-1}$)	LOQ ($ng\ mL^{-1}$)	Linearity ($ng\ mL^{-1}$)	R^2	F	RSD%	
					Intra	Inter
15	50	50-10000	0.9909	4	4.1	4.7

Real sample analysis (urine and plasma)

Biological samples of human urine and plasma were used to check the efficiency of the mentioned extraction method. Due to the absence of drugs in the urine and plasma samples, according to the optimization results, 50 ml of urine and plasma samples were taken and prepared according to the steps mentioned in the work method section. Then the pH of the mixture was adjusted to 9.78 using NaOH. The resulting solutions were used as phase givers. Then, urine and plasma samples were prepared by increasing the standard concentration of 0.1, 0.2 and 1 mg/liter, and similar to control urine and control plasma, preparation and extraction was done from them.

Table 3. Table of drug measurement results in urine and plasma.

Sample	C_{real} ($ngmL^{-1}$)	C_{added} ($\mu g\ mL^{-1}$)	C_{found} ($\mu g\ mL^{-1}$)	RSD% (n = 5)	R%
Plasma	nd[1]	1	0.82	5.4	2
Urine	nd	1	0.88	4.2	8

Discussion

So far, many studies have been conducted using the SBME method to extract pharmaceutical compounds, toxins, organic pollutants and heavy metals from different samples. In addition, many studies have been conducted to identify and measure the drugs used in Parkinson's treatment regimen, but Bipridan has been identified and measured as a side drug (not the main compound.(

The methods that have been performed include DH-HS-SPME, which is performed on the extraction of a series of specific drugs from the urine sample [13]. In addition, to identify the drug amantadine in biological samples, the liquid-liquid microextraction method was used, and Bipridan was one of the drugs that was identified as a side compound with this method [14].

Despite the good repeatability, optimal concentration and high sample capacity, these two mentioned methods also had disadvantages. For example, the methods are laborious, tiring and expensive, and both methods require the removal of solvents, which, in addition to the risk of inhalation and skin contact, also bring the risk of toxicity in laboratory wastewater and environmental damage.

As it has been mentioned, Bipradan has a low concentration in blood, and therefore, accurate and sensitive methods should be used to identify and determine its amount. One of the methods used in this direction is gas chromatography, which has been performed in two ways, MC and PC, in which skipping has been detected in both methods [15, 16]. In Table 4, the method of this research is compared with other available methods.

[1]Not detected

Table 4. Comparison of the present method with other reported methods.

Extraction technique	Sample	Linear range (µg/ L)	LOD (µg L-1)	RSD%	Ref.
DH-HS-SPME	Bulk drug	0.1-1.5	0.2-0.18	5.6-6.8	11
LLE	Bulk drug	0.5-50	0.2-0.4	5.7-9.3	12
GC-MS	Bulk drug	50-700	1	5.6	13
GC	Biperiden	0.5-50	0.25	0.5	14
HPLC	Biperiden	0.5-25	0.03	0.4	17
HPLC	Biperiden	8-100	2.15	0.316-0.225	18
HF-SBME	Biperiden	0.05-10	0.015	4.4	Current study

In comparison, the advantages of the present method can be mentioned as follows:

- Save time and money
- No environmental pollution
- Not using an internal standard while having proper accuracy and repeatability
- Insignificant consumption of organic solvent compared to other methods
- Appropriate linear range in the analyzed concentration range
- The possibility of using several solvent strips at the same time
- High extraction efficiency, short extraction time and high extraction capacity
- Use of cheap and disposable fiber that causes high sensitivity of the method.

According to the results, this method has a good accuracy and wide range of suitable linearity. In addition, it is a relatively new method for sample preparation, which can be a suitable alternative to other methods due to the simplicity of the method and the low consumption of organic solvent. This method has many advantages such as simplicity, cheapness, high preconcentration factor, low RDS. , the ability to separate the drug from the complex matrix system of biological samples and create clean and clear extraction solutions. On the other hand, because in this method a new fiber is used for extraction every time (due to the simplicity and low price of the extraction tool), it does not have a memory effect and due to the very small volume, the aqueous phase of the receiver before injection into HPLC does not require preconcentration and the sample is injected directly.

In the SBME method, compared to other conventional extraction methods such as LLE, the consumption of organic solvent has been effectively reduced, and for this reason, it is used as an alternative and environmentally friendly method in sample preparation in analytical methods such as GC, HPLC, and MS. In addition, unlike SPE, this extraction method can be performed in a wide range of pH.

Due to having small pores, the used fiber acts as a filter that prevents the entry of large molecules and particles in biological samples into the organic phase, and as a result, the method has a high

cleaning power. Therefore, this technique can easily be used to extract different species from complex tissues.

In addition to the mentioned advantages, the sample consumption is low and the extraction time is suitable. The extractive phase is not in contact with the sample phase, and as a result, the sample phase can be stirred at any intensity without worrying about the loss of the extractive phase, and it is economically very affordable.

Conclusion

Based on the results, measuring the concentration of Bipridan in biological fluids can play a significant role in monitoring the treatment and controlling the side effects caused by the patient's medication regimen, and modern extraction methods can make this possible. The three-phase liquid micro-extraction method with a solvent strip is a relatively new method for sample preparation that has advantages such as low consumption of organic solvents, fewer separation steps, high selectivity, low detection limits, and high sample cleaning ability. It is low cost and easy to use.

In this research, the conditions were optimized with chemometric design by Minitab software, which is simpler and cheaper than other optimization methods and gives results that are more accurate. In this method, a concentration factor of 94 and a detection limit of 0.015 µg/ml were obtained, which is better than the previous identification and extraction methods. In addition, the obtained high concentration factor provides the possibility of determining a very small amount of drug in complex biological samples. Considering that HPLC is available in many clinical laboratories and is cheaper than GC-MS and LC-MS devices, the proposed extraction method can be easily used as a standard method in medical laboratories. In this method, there is no memory effect due to the use of new fiber in each test and the freshness of the receiving phase. In addition, the price of these fibers is low and this method is not expensive.

Also, due to the fact that this method is effective in extracting biological samples and it is possible to use it in clinical laboratories, it is possible to control the treatment of patients using this drug, i.e. parkinsonian and psychotic patients, and avoid side effects of the drug and Its toxicity prevented it. Since these patients take many drugs in their treatment regimen, this method has created a good advantage in the identification and diagnosis of drugs and caused biperidan to be specifically identified and quantified.

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