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RESEARCH ARTICLE

Use of Fe_3O_4@MPTMS-Dithizone Magnetic Nanoparticles as Solid Phase Sorbent for Sensitive Analysis of Sibutramine Molecules in Herbal Slimming Products

Özge Demir[1], Halil İbrahim Ulusoy[1,*], Ümmügülsüm Polat[1] and Songül Ulusoy[2]

[1]*Department of Analytical Chemistry, Faculty of Pharmacy, Sivas Cumhuriyet University, Sivas, Turkiye;* [2]*Department of Pharmacy, Vocational School of Health Service, Sivas Cumhuriyet University, Sivas, Turkiye*

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Abstract: ***Background*:** A new enrichment and sensitive determination method, which includes HPLC-DAD analysis after Magnetic Solid Phase Extraction (MSPE), has been developed for trace analysis of Sibutramine molecules in herbal slimming products. Sibutramine is one of the most adulterated drug molecules in herbal products.

***Method*:** In the proposed method, Sibutramine molecules were pre-concentrated by using Fe_3O_4@MPTMS-Dithizone magnetic sorbent synthesized in our laboratory. Desorption of Sibutramine molecules from the sorbent phase was carried out by using acetonitrile: methanol (1:1) solvent in the presence of pH 8.0 buffer before chromatographic determinations.

***Results*:** Analytical parameters of the method, such as linear range, enrichment factor, and determination limit, were determined after optimizing experimental variables such as interaction time, desorption solvent, pH, *etc.* The sibutramine molecule was analyzed by isocratic elution of acetonitrile and KH_2PO_4 (pH 3.0, 0.05 M) (40:60) with a DAD detector at 223 nm wavelength. Limit of detection (LOD) value was calculated as 1.43 ng mL^{-1}.

***Conclusion*:** Relative standard deviations (RSD) were below 3.20% for determinations of model solutions, including 100 ng mL^{-1} of Sibutramine. Finally, the developed method has been applied to herbal slimming tea samples with quantitative recovery experiments.

Keywords: Sibutramine, magnetic solid phase extraction, HPLC, herbal slimming products, drug analysis, sibutramine molecule.

1. INTRODUCTION

In Latin, obesus is the past tense of obeder, which means that the amount of fat in the body is above the normal amount. The causes of obesity are multifactorial, including genetic background and environmental factors. As it can cause psychosocial problems in the quality of life alone, it can also cause serious diseases [1]. Obesity, as a result of chronic inconsistencies between energy intake and energy expenditure, excess energy is stored in adipose tissue in the form of triglycerides and as fat more than necessary for body function [2]. Practically, obesity is defined as an average body fat ratio exceeding 25% in men and 35% in women [3]. Obesity is a worldwide problem affecting approximately 300 million people all over the world, and the proportion of individuals with a body mass index at or above the obesity limits constitutes 7% of the entire world population [4].

Losing weight has become an important problem today, as it is known that obesity is increasing all over the world, becoming a pandemic, and its side effects are deadly. In addition to personalized diet and exercise, medical treatment is also applied in some cases as the first step and most effective treatment for weight loss [5].

Many drugs are used to prevent obesity. One of the drug-active ingredients used to prevent obesity in daily life is Sibutramine which is an anti-obesity drug that inhibits serotonin and noradrenaline reuptake. Serotonin and noradrenaline, which are brain neurotransmitters, play an important role in the central nervous control of energy balance [6]. Antidepressants also show an inhibitory effect on serotonin reuptake. Sibutramine has emerged in antidepressant drug trials and has taken its place in the literature as an anti-obesity agent due to its weight-loss effect in human trials [7]. Asthenia, headache, dry mouth, constipation, and sleep problems were observed as side effects. Traditional herbal medicines are used as alternative medicine by a wide audience in the world [6, 8].

*Address correspondence to this author at the Department of Analytical Chemistry, Faculty of Pharmacy, Sivas Cumhuriyet University, Sivas, Turkiye; Tel: +90 346 487 39 05; Fax: +90 346 219 16;
E-mail: hiulusoy@yahoo.com

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The consumption of Sibutramine as an additive has been increasing as legal and illegal. Herbal slimming products are a big commercial market for both teenagers and adult people [9]. The use of dietary supplements and herbal products has increased greatly during the last decades as an illegal way to conventional medical approaches. It is well-known that natural products are inherently safer and healthier is commonly accepted in most countries [10]. Over 30 % of modern pharmaceuticals are obtained from natural botanical sources, but illegal and uncontrolled use of these sources can cause unwanted circumstances and seriously healthy problems if they don't arrange by legal establishments [11, 12]. Moreover, a warning was announced by U.S. Food and Drug Administration about a life-threatening green coffee product due to the possibility of containing a Sibutramine molecule. In literature, various instrumental technical were developed and used to detect Sibutramine molecules in dietary supplements, foods, beverages and pharmaceutical formulations. Most of the studies include gas chromatography-mass spectroscopy (GC–MS) [13], voltammetry [14], adsorptive stripping voltammetry [15], ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) [16], high-performance liquid chromatography (HPLC) [17], colorimetric detection [18] and (Fourier transform infrared spectroscopy) FTIR for detection of Sibutramine in herbal slimming food supplements [19].

Solid phase extraction (SPE) based methods are mainly used in sample preparation of real samples owing to easily applicable properties [20-22]. Long process time, expensive experimental equipment, and problems in reusability are limited column-based SPE methods [23]. So, batch-type methods have better properties such as simplicity, cheapness, special material for each sample, and reusability [24, 25]. Separation of solid sorbent from bulk solution is one of the biggest problems in batch-type methods. The separation of sorbent magnetic material from liquid sample solution is a critical step in batch-type SPE. The use of magnetic nanoparticles in magnetic solid phase extraction (MSPE) methods provided important advantages to overcome these complications [26-29]. This technical provide to develop new materials which have selective functional groups. So, versatile solid-phase materials can be obtained for target molecules. Moreover, the separation of these particles from the liquid phase is easy and repeatable. Modification of magnetic particles with functional molecules provides useful materials for trace analysis of organic and inorganic species [30-33]. Use of dithizone to cover magnetic particles has already been applied in a few studies for metal ions [34-36]. But, its application for organic analysis has been carried out for the first time in this study.

This study presents the development and application of a new magnetic hybrid material as a sorbent for magnetic solid-phase extraction of Sibutramine molecules before HPLC-DAD determination. The magnetic sorbent, Fe3O4@MPTMS-Dithizone was synthetized and characterized for this study with the potential use of trace determination of Sibutramine molecules for the first time. The developed method has then applied the determination of the Sibutramine in commercial herbal slimming tea samples.

2. MATERIALS AND METHOD

2.1. Instrumentation

The FT-IR characterization of Fe_3O_4@MPTMS-Dithizone materials was carried out by Perkin-Elmer Spectrum 400 FT-IR spectrometer (Waltham, MA, USA). The Raman spectra were obtained by using Raman spectroscopy (WITec alpha 300 M + micro-Raman system with 532 nm laser source). X-ray diffraction (XRD) spectrums of materials were obtained using an advanced XRD diffractometer (Bruker AXS D8). Scanning electron microscope (SEM) images for materials were obtained on an LEO 440 SEM with an accelerating voltage of 20 kV.

The chromatographic analysis of the Sibutramine molecule was carried out by HPLC-DAD system (Shimadzu, Tokyo, Japan). A conventional C18 column was used through analysis.

2.2. Chemicals and Reagents

Analytical grade reagents were used in all experimental procedures. Ultra-pure water with a resistivity of 18.2 MΩ cm was used provided by MES system (MP Minipure Dest Up, Turkey) water purification system. Sibutramine hydrochloride was purchased from Sigma (Steinheim, Germany). All used solvents for chromatographic analysis were HPLC grade or better and purchased from Sigma (St. Louis, MO, USA). Iron chloride salts, (3-Mercaptopropyl) trimethoxysilane (MPTMS), and dithizone (Sigma Co., Steinheim, Germany) was used for the synthesis. Stock solutions of Sibutramine (100 ng mL^{-1}) were prepared in MeOH and then stored at +4°C in the dark, and they were found to be stable for at least three months. Work solutions were obtained just before use by appropriate methanolic dilutions.

2.3. Preparation of Fe_3O_4@MPTMS-Dithizone Magnetic Materials

One of the general synthesis approaches, which we have applied in other studies, was used as well-known in the literature [34, 35]. The mixture of 0.745 g of $FeCl_3 \cdot 6H_2O$ and 0.383 g of $FeSO_4 \cdot 4H_2O$ was solved in 50 mL of 3M HCl and then in 100 mL of 50 % Ethanol solution was added to the mixture while it was stirring at 85°C at 600 rpm. The synthesis reaction was carried out in an inert medium provided by nitrogen gas. By adding 20 mL of ammonia dropwise to the vigorously mixing solution, magnetite will be formed, and Fe_3O_4 grains were collected from the resulting black solution with the help of the applied external magnetic field, washed with a certain proportion of water/alcohol mixture and dried in an oven at 60°C for 6 hours.

In the next step, 2 g of the dried material was dispersed in 50% methanol, 3 mL of concentrated ammonia and 2 mL of 3-methcaptopropyl-tri-methoxy silane (MPTMS) was added to the mixture in order to start the silanization process on the particle surfaces. After the silanization process, a solution of 20 mg dithizone in 40 mL of 50% methyl alcohol was added while the particles were stirred at 500 rpm in a nitrogen atmosphere at 45-50°C and left to stir for 4 hours. Then, the mixture was kept on for 6 hours at room temperature, and it was washed several times with a methanol/water mixture and finally left to dry for 12 hours. Finally, the obtained magnet-

ic particles were grounded and sieved to set up particle size and stored in brown bottles until use.

2.4. Chromatographic Analysis

The mobile phase composition was pH 3.0 phosphate buffer (50 mM) and acetonitrile (60:40) at isocratic mode throughout the analysis. 1.0 mL min^{-1} of flow rate and 35°C of column temperature were optimized for optimal conditions. The Peak area for standard and samples was calculated at 223 nm by the DAD detector. Inertsil ODS-3 HPLC column (250 mm×4.6, 5.0 µm) was used for the determination of Sibutramine molecules. 10 µL of samples were injected to the system by autosampler for each analysis. All eluents and samples were degassed ultrasonically and filtered by a membrane filter (0.45 µm) before analysis.

2.5. The Proposed Procedure

30 mg of magnetic solid-phase support material was weighed carefully and transferred to a falcon tube. Washing of nanoparticles was carried out by means of 2 mL of ACN/Methanol (1:1) before MSPE procedure. Then, 2 mL of pH 8 BR buffer and 10 mL of a sample solution containing Sibutramine in the range of 5.0-500.0 ng mL^{-1} were added to tubes, and the final volume of tubes was completed to 50 mL. The lids of the falcon tubes are tightly closed and placed in the shaker device. The device was operated by setting 80 rpm for 40 minutes. After the time was over, magnetic solid phase was easily separated by using an external magnetism provided by a neodymium magnet and the aqueous phases were separated with the help of a pipette.

After removing the aqueous phase, 500 µL of ACN: MeOH (1:1) mixture was added to the solid phase, and the tubes were vortexed for 60 seconds. In this process, it is aimed to separate concentrated target molecules from the surface of solid phase material and pass it to the solvent phase. The solvent phase samples were taken into the injector, and the 0.45 µm injector tip is passed through the filter, transferred to the vials and placed in the HPLC device. The Sibutramine content in the samples in this way is determined by the HPLC device.

2.6. Sample Preparation

Herbal Slimming tea samples were used in the application area of the developed method. These samples, which are sold as slimming tea, are sometimes exposed to Sibutramine adulteration illegally. A method was used with some minor modifications according to a method commonly used in the literature [37]. Firstly, 5 mL of Methanol: ACN (1:1) solution was added to 0.5 grams of tea samples and kept in an ultrasonic water bath for 25 minutes. Then, 5 mL of water is added and vortexed for 5 minutes. Then, the samples are filtered through 0.45 µm nylon PTFE filters, and their volumes are completed with 10 mL of distilled water.

3. RESULTS AND DISCUSSION

3.1. Characterization of the Developed Magnetic Adsorbent

3.1.1. FTIR Analysis

Several characterization approaches were used for synthesized magnetic nanoparticles. FTIR spectrum of Fe_3O_4@MPTMS-Dithizone is given in Fig. (**1**). The characterization of the analyzed magnetic-based material is based on 892 cm^{-1} and 791 cm^{-1} absorption bands corresponding to Si-C vibrations over (Si-$(CH_3)_3$) bonds between Silicon and metal groups. The characteristic trend bands of Fe_3O_4 are seen in the 555 cm^{-1} and 627 cm^{-1} FTIR spectra. The charac-

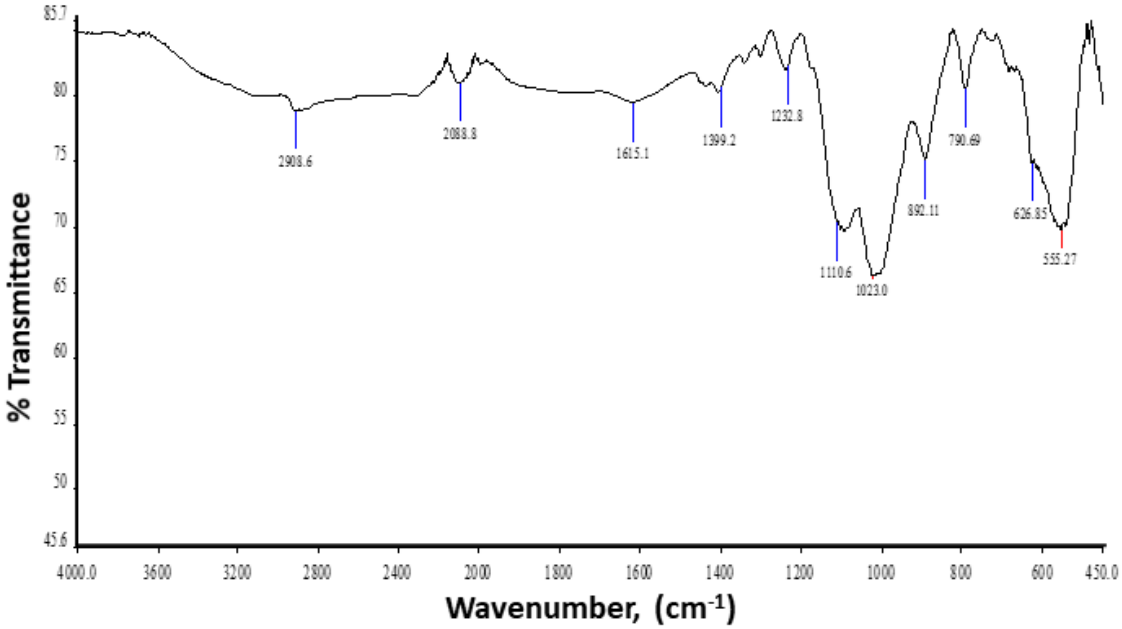

Fig. (1). FTIR spectrum of the developed magnetic materials. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

teristic peaks of the Fe_3O_4@MPTMS-Dithizone spectrum at a wavelength of 1110 cm^{-1} belonging to the Si-O-C group and 1233 cm^{-1} belonging to the Si-C bond provide an important illumination in the explanation of the structure. The frequencies of 1615 cm^{-1} and 626 cm^{-1}, which are in the wavelength range of 1650-1510 cm^{-1}, correspond to the stretching and bending vibrations of the (C = C) group. The spectrum observed at 2908 cm^{-1} wavelength belongs to C-H bonds due to antisymmetric and symmetric stretching vibrations in the chemical structure. In-plane and out-of-plane C-H deformation vibrations in the dithizone-induced benzene ring manifest themselves at intervals of 1000-1250 cm^{-1} and 900-720 cm^{-1}, respectively.

3.1.2. XRD Analysis

Fig. (**2**) shows the XRD patterns of the Fe_3O_4 material modified with Dithizone. In the analysis of the material, performed by X-ray diffraction technique, characteristic peaks of Dithizone characteristic peaks at 17, 21, 23 and 26 2θ (theta) degrees are known in the literature. After its synthesis with (3-mercaptopropyl) trimethoxysilane and Fe_3O_4, it is seen that the characteristic peaks of dithizone are damped, and the dithizone peaks are suppressed specifically for the new synthesis product. The most characteristic peaks are observed at 35.5 and 62.8 2θ (theta) degrees for the new synthesis product Fe_3O_4@MPTMS-Dithizone coded component.

3.1.3. SEM Analysis

Morphological surface analysis of developed magnetic nanoparticles was provided by SEM analysis, as given in Fig. (**3**). It is clearly displayed the homogeneity of Fe3O4 particles in Fig. (**3a**) as large round by SEM. The second step of magnetic particle synthesis can be seen in Fig. (**3b**), and magnetic particles of Fe3O4@MPTMS are easily identified as spherical structures. The final product of synthesis (Fe_3O_4@MPTMS-Dithizone) is shown in Fig. (**3c**). When all the images were examined together, it will see that the magnetic synthesis product showed a homogeneous distribution in the images of the components. EDX analysis gives important information about the chemical components of the surface. Fig. (**3d**) image presents the % distributions of Fe, Si, S, C and O elements in the synthesis product. The success of desired magnetic material can be evaluated by considering the subdivisions of Fig. (**3**).

3.2. Effect of pH

The pH of the medium is an important factor as it affects the adhesion of the analyte molecule to the solid phase and interactions between target molecules and magnetic materials. To find the most suitable pH, magnetic solid phase extraction was carried out using a series of Britton Robinson buffers in the range of pH 2.0-10.0. According to the results obtained, it was found that the best interaction between the analyte and the solid phase was pH 8.00. It was seen that the pKa value of the Sibutramine molecule was 9.77 in the literature review [38]. This value explains the better signals in basic regions. For this reason, the next steps of the experimental studies were continued with a pH 8.0 buffer. The results obtained are shown in Fig. (**4a**).

3.3. Desorption of Analyte Molecules from the Solid Phase

The effect of the shaking time on MSPE is directly affected by the transition of the analyte molecules from the medium to the solid phase material. The interactions between magnetic particles and Sibutramine molecules are directly affected by extraction time. It is aimed at both the maximum extraction efficiency and the shortest extraction time. So, adsorption time should be optimized carefully. An orbital rotator was preferred for maximum interactions in the falcon tubes by means of batch-type MSPE experiments. The effect of time was studied in the range of 0-90 minutes. As can be seen in Fig. (**4b**), it reached the maximum extraction efficiency in 40 minutes. A meaningful change was not observed beyond this point.

The type and volume of eluent is also critical parameter that has to be optimized to obtain a higher enrichment factor. Various solvents can be used for the desorption of target molecules from the surface of magnetic particles. For this purpose, methanol, ethanol, isopropanol, acetonitrile, water, acetone, hexane, 50% methanol and acetonitrile/methanol were used one by one by following the developed extraction procedure. 100 ng mL of model solutions were used in the optimization of the desorption solvent. As can be seen in

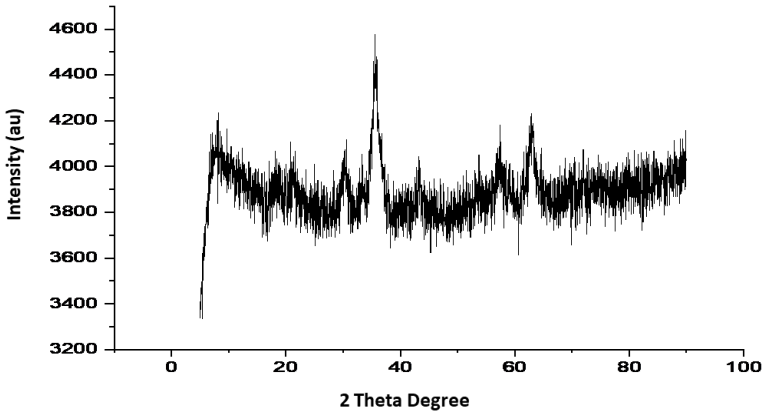

Fig. (2). XRD analysis of the developed magnetic material. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

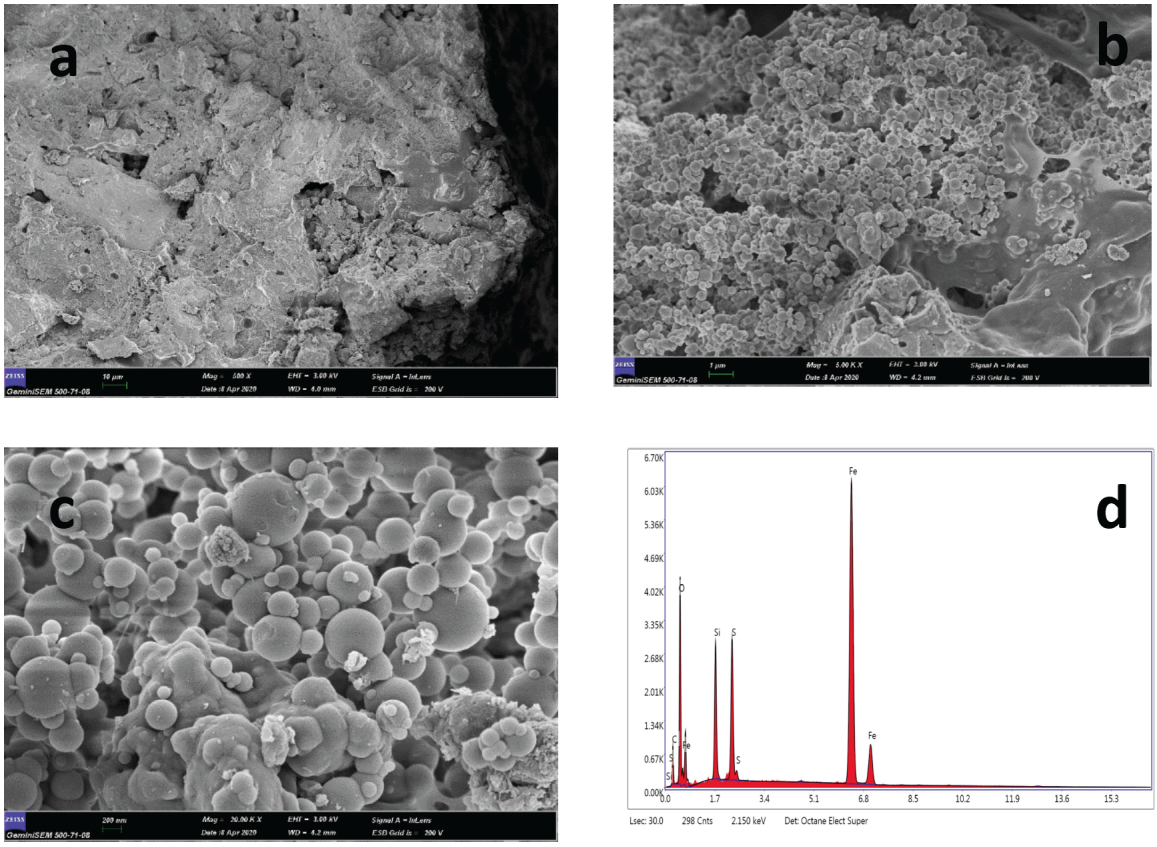

Fig. (3). SEM images of the developed magnetic materials (**a**) Fe_3O_4, (**b**) Fe_3O_4@MPTMS, (**c**) Fe_3O_4@MPTMS-dithizone and (**d**) SEM-EDX analysis. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

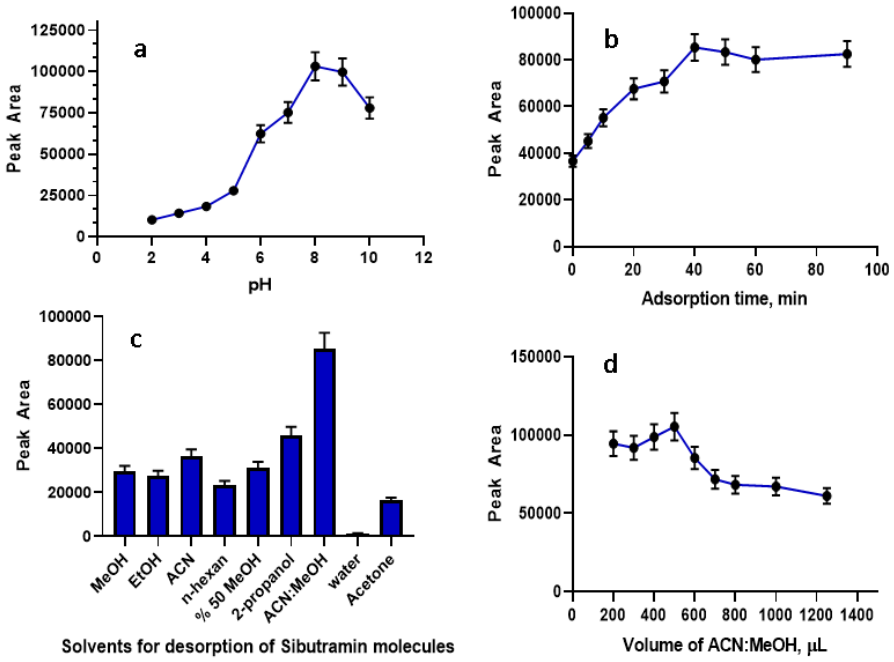

Fig. (4). (**a**) The pH effect of the developed MSPE method (N:3), (**b**) Effect of adsorption time on MSPE, (**c**) Effect of various solvents on MSPE and (**d**) Effect of desorption solvent on MSPE. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Fig. (**4c**), the best analytical signals were obtained by using a 1:1 mixture of acetonitrile/methanol solvent. The next steps of the developed method were maintained with this solvent.

The volume of the desorption solvent is directly determined by the final pre-concentration factor. As expected, the maximum factor is obtained with the lowest desorption volume. But, it is not easy to take and filtrate the low volumes before HPLC analysis. The volume of desorption solvent was scanned in the range of 200-1250 µL. As can be seen in Fig. (**4d**), the maximum signals were obtained with 500 µL of acetonitrile/methanol. Desorption of Sibutramine molecules from magnetic particle surfaces was not enough at the lower volumes because of insufficient interactions. So, the subsequent studies were continued by using 500 µL of acetonitrile/methanol.

The desorption process was carried out by using a simple vortex. In order to guarantee the maximum transfer of molecules to solvent before HPLC analysis, the time of the vortex process should be optimized while the other parameters were kept constant. This parameter was scanned in the range of 0-100 seconds. It reached the max. Signals in 60 seconds and the next optimization steps were continued with this value.

3.3.1. Reusability, Stability and Amount of Adsorbent

The amount of adsorbent in solid-phase extraction experiments is not so much a critical parameter as in removal studies. As known, SPE is generally applied for the determination of trace species or molecules in complex samples. In the removal studies, it is aimed the maximum adsorption capacity for target species. So, the adsorption capacity mostly increases with the amount of adsorbent. But, if it is aimed to determine the trace concentration of target molecules, it is not mostly necessary to use adsorbent in higher amounts. Moreover, a lower amount of adsorbent is preferred to decrease the analysis costs. The effect of the magnetic solid phase amount was checked by employing different Fe_3O_4@MPTMS-Dithizone particles studied in the range of 20-150 mg. The results showed that the highest peak area for Sibutramine molecules was obtained at 30 mg of the magnetic solid phase. Further studies were continued by using 30 mg of the adsorbent dose.

The reusability of the developed hybrid material and the recovery values of the adsorbent will make the treatment process economical. The reusability and stability of the newly developed magnetic material were checked by using model solutions. The adsorption feature of the material was found to be apparently stable (less than 5 %) after the repeated application of more than 10 cycles of sorption and desorption of the Sibutramine molecule. After every use, the MNPs were washed with 2.0 mL of ACN/Methanol twice times and subsequently assembled with an external neodymium magnet. Moreover, any decrease in magnetic properties was not observed throughout experiments.

3.4. Analytical Performance

Full optimization of the developed method was completed step by step. A series model solution with increasing concentration was used for determining of liner range. The linear range was obtained by applying the developed method to these solutions. As can be seen from the figure, after the applied magnetic-based enrichment process, the signals increase in proportion to the concentration.

Under the optimum conditions, a series of analytical performance parameters with regard to the linear range, correlation coefficient, limit of detection (LOD) and limit of quantification (LOQ) were studied for the developed MSPE–HPLC procedure and the obtained values were given in Table **1**.

Table 1. Analytical Parameters of the developed method.

Parameter	-	
	Before MSPE	**After MSPE**
Linearity	1.00-20.00 µg mL^{-1}	5.00-500.00 ng mL^{-1}
LOD	0.32 µg mL^{-1}	1.43 ng mL^{-1}
LOQ	0.98 µg mL^{-1}	4.71 ng mL^{-1}
RSD (%)	4.7	3.2
Calibration Sensitivity	12.47	1452.2
R^2	0.9972	0.9954
Pre-Concentration Factor	-	100.0
Enhancement Factor	-	116.4

Linear regression analyses were obtained by using the peak areas against the nominal Sibutramine concentrations in model solutions. The limit of detection (LOD) and limit of quantification (LOQ) were defined as 3 times and 10 times the signal/slope of a calibration curve, respectively. The ratio of the initial sample volume (50 mL) to the final volume (0.5 mL) was defined as the preconcentration factor (PF). The enhancement factor (EF) of the developed method was calculated by using the ratio of the slope of the calibration curve of the analytes after and before MSPE experiments. The relative standard deviation (RSD%) was calculated for 5 repetition analyses of model solutions, including 100 ng. mL^{-1} Sibutramine after MSPE.

3.5. Application of the Proposed Method to the Real Samples

All steps of the developed method were optimized, and analytical properties were determined by using model solutions, including Sibutramine standards. Application of method was carried out for determination of Sibutramine in various tea samples used as slimming products. The samples were bought from local markets and the internet. All samples are sold for the purpose of weight loss effect. Two levels of concentration of Sibutramine were spiked to samples to calculate recovery values. So, the accuracy of the method is also determined by means of recovery experiments. Analysis results are shown in Table **2**. A few chromatogram was also submitted in Fig. (**5**) obtained from the analysis of tea samples.

Table 2. Results for Sibutramine analysis and recovery tests in the tea samples.

Samples	Added µg g^{-1}	Measured[a] µg g^{-1}	RSD %	Recovery %
Tea Sample 1	-	<LOD	-	-
	100.0	98.5 ±3.5	3.5	98.5
	250.0	254.1±5.9	2.3	101.6
Tea Sample 2	-	10.7±0.8	7.5	-
	100.0	108.7±3.4	3.1	98.2
	250.0	255.4±7.4	2.1	97.9
Tea Sample 3	-	74.3±3.7	4.9	-
	100.0	181.6±5.7	3.2	95.9
	250.0	330.8±9.4	2.8	98.8
Tea Sample 4	-	<LOD	-	-
	100.0	95.8±2.1	2.2	95.8
	250.0	240.8±7.8	3.2	96.3
Tea Sample 5	-	18.8±0.8	4.2	-
	100.0	113.8±4.8	4.2	95.6
	250.0	264.5±8.6	3.2	98.4

Note: [a](N:3, mean±sd).

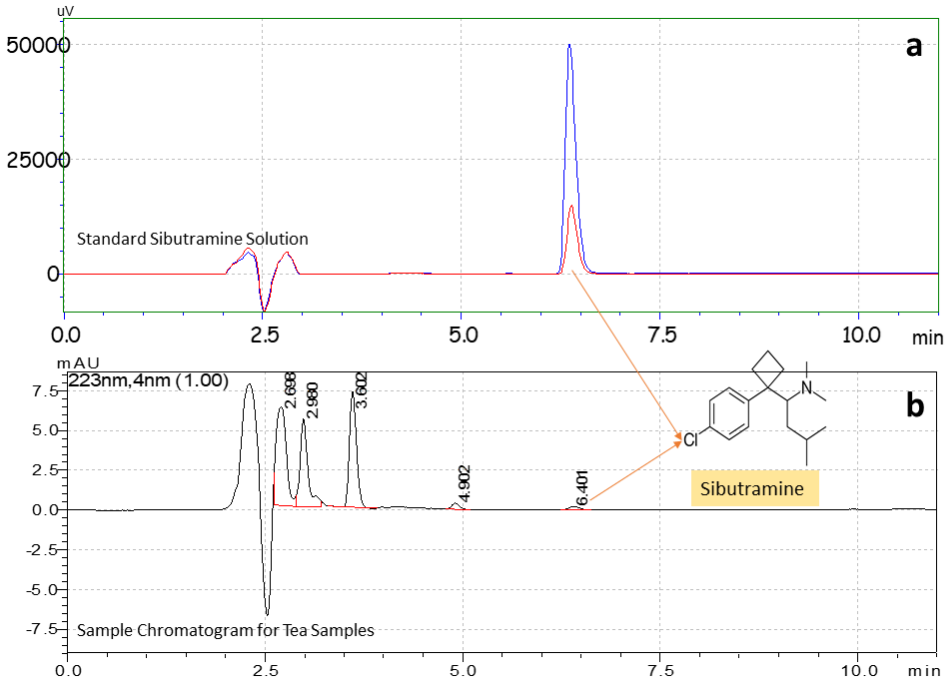

Fig. (5). The obtained chromatograms from HPLC analysis of (**a**) standard solution and (**b**) tea samples. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The recoveries were calculated by comparing the pre-concentrated concentrations of analyte from those of the samples with the corresponding added concentrations on calibration curves. The recoveries obtained in each analyzed real sample are presented in Table **2**. Quantitative recoveries were obtained (between 95.6 and 101.6 %) with a low relative standard deviation between 2.2 and 7.5 %. These results proved that the developed MSPE-HPLC method is sensitive, precise and accurate for the extraction and determination of herbal slimming products.

Table 3. Comparison of the new method with other reported methods.

Pre-treatment Procedure	Determination Method	Linearity	Samples	RSD %	References
Simple liquid-liquid extraction	Liquid chromatography–electrospray ionization tandem mass spectrometry	0.3–32.8 ng mL^{-1}	Plasma Samples	<19.90 %	[41]
Liquid–liquid extraction	LC–ESI-MS/MS	10.0–10000 pg mL^{-1}	Human plasma	<15.50 %	[42]
Directly	RP-HPLC	10-100 µg mL^{-1}	Bulk drug and capsule dosage forms	<1.30 %	[44]
Homogenization, dissolution, dilution	HPLC-UV	20-60 µg mL^{-1}	Bulk And Commercial Formulations	<1.40 %	[45]
Liquid–liquid extraction	Reversed-Phase Liquid Chromatography–Tandem Mass Spectroscopy	0.1–8.0 ng mL^{-1}	Human Plasma	<10.00 %	[43]
Homogenization, dissolution, dilution	Voltammetry	0.4-33.3 mg L^{-1}	Pharmaceutical Formulations	-	[14]
Magnetic Solid Phase Extraction	HPLC- DAD	5.0-500.0 ng mL^{-1}	Herbal slimming supplements, tea samples	<3.20 %	This method

CONCLUSION

An MSPE technique based on newly synthesized magnetic particles (Fe_3O_4@MPTMS-Dithizone) was proposed as an efficient and easily applicable approach for preconcentration and separation of Sibutramine molecules in herbal slimming products and tea samples. One of the most preferred properties for new sorbents is reusability after an easy regenerations process. Fe_3O_4@MPTMS-Dithizone particles can be used until 10 times without any change in their adsorption ability. This will decrease the analysis cost for routine analysis. And also, the used HPLC methodology was developed for this paper. Fig. (**5**), it can be used for selective analysis of Sibutramine molecules in tea samples without any interference peak in the chromatograms. As a robust preconcentration technique, the proposed method can be coupled successfully to the HPLC-DAD system. The methodology described (MSPE-HPLC) is faster than classical sample preparation procedures such as SPE cartridges or liquid-liquid extraction, with a minimum sample handling and less solvent consumption, and is a promising screening methodology for routine analysis of real samples.

A comparison table was submitted in Table **3** in order to evaluate the highlights of the proposed method. The used solid phase material is original, and it was synthesized and characterized for this study. In addition to applicable easy properties of magnetic solid phase extraction, the materials can be used again and again. As far as we know, this is the first study on magnetic solid phase extraction for Sibutramine molecules. As can be seen, the Table **3**, the proposed method has important analytical merits compared with the other ones. Hybrid and expensive instrumental technical are generally used for sensitive determination of Sibutramine, such as Liquid chromatography-electrospray ionization tandem mass spectrometry [39], LC–ESI-MS/MS [40], reversed-phase liquid chromatography-tandem mass spectroscopy [40]. The most favorable property of the developed method is to carry out this analysis by using a conventional HPLC-DAD system which is cheaper than the other methods. The other methods in tables [14, 41, 42] are suitable for the direct determination of Sibutramine at major levels without any pre-concentration step. These methods can determine the target molecule at ppm (mg L^{-1} or µg mL^{-1}) level. However, the developed method can analyze Sibutramine at ppb (µg L^{-1} or ng mL^{-1}) levels. And also, the submitted chromatograms for real samples showed that there is no interference near the analyte peak. This provides the target molecule is retained on the developed solid phase material by leaving most of the matrix components in the plant extracts. Finally, this method can be proposed as an alternative and practice application for fast and sensitive analysis of Sibutramine molecules in herbal slimming products.

LIST OF ABBREVIATIONS

EF = Enhancement Factor

LOD = Limit of Detection

LOQ = Limit of Quantification

MSPE = Medical Student Performance Evaluation

PF = Preconcentration Factor

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

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CONFLICT OF INTEREST

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