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Posted Date: 4 May 2023

doi: 10.20944/preprints202305.0211.v1

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Rapid and Sensitive Detection of Polycyclic Aromatic Hydrocarbons in Tea Leaves Using Magnetic Approach

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Abstract: A rapid and efficient method using an alkyl functionalized magnetic nanoparticles-based extraction technique combined with Ultra-High Performance Liquid Chromatography was developed for the detection of trace amounts of polycystic aromatic hydrocarbons in tea leaves. As a popular coating for chromatographic column packing materials, C_{18}-alkyl has been demonstrated to be effective in separating polycystic aromatic hydrocarbons. And the magnetism of the nanomaterials accelerates the extraction process while their high surface ratio enables desirable dispersity in the sample matrix. Meanwhile, the adsorbents can be washed and reused 30 times without compromise on recovery, which greatly reduced the budget. The effects of various parameters were investigated and optimized, and the recoveries for five analytes were in the range of 84.8–103.7%. The reproducibility rate of intra-day and inter-day were below 7.1% and 11.3% respectively. The limits of detection and limits of quantification ranged from 1.69–9.97 ng g^{-1} and 5.12–30.21 ng g^{-1}, indicating satisfactory sensitivity. Thus, the proposed methodology is rapid, highly efficient, and economical, and it expands the application of magnetic cleanup approaches in complex food matrices.

Keywords: Polycyclic aromatic hydrocarbons; Tea; Magnetic solid phase extraction; UPLC; Nanoparticles

1. Introduction

Tea is one of the most widely consumed beverages worldwide, and its status as the national drink of China has been recognized since ancient times. The global spread of Chinese tea leaves during the 16th century, facilitated by increased cultural exchange and trade, firmly cemented tea's place as an important beverage. In particular, tea has had a profound impact on British culture, with many individuals consuming large quantities of it. Numerous scientific studies have identified potential health benefits associated with tea consumption, including cholesterol reduction, anti-microbial and hepatoprotective effects, and prevention of cardiovascular disease and cancer [1]. Residual chemical contaminants present in tea leaves due to air pollution deposition pose potential health hazards for consumers. Heavy metals and pesticide residues are among the primary pollutants that have undergone extensive investigation, and their detection and monitoring are crucial [2]. Based on the correlation between medicine and food, tea has been utilized for medicinal purposes in China for an extended period. Various active components present in tea exhibit antiviral properties, for example, some polyphenols have inhibitor potential by binding to the active site of SARS-CoV-2 RdRp, which means that it might be precaution and treatment as a candidate drug for COVID-19 [3].

Polycyclic Aromatic Hydrocarbons (PAHs) are chemical structures composed of two to seven benzene rings, which can take a linear, angular, or agglomerated form. Mainly due to the carbon-containing compounds, bioaccumulation in the food chain, and industrial food processing. At present, there are more than 100 known PAHs, while 16 PAHs are listed by the US Environmental Protection Agency as regulated. The Council Directive 98/83/EC set the maximum acceptable level for benzo[a]pyrene at 0.010 μg L^{-1}, the sum of four specified PAHs at 0.100 μg L^{-1}. These compounds possess teratogenic, mutagenic, and carcinogenic properties, and their stable structure makes them slow to degrade [4]. The aromatic's stable structure makes it hard for the PAHs to degrade. PAHs have been detected in a variety of food matrices, such as seafood, oil, barbecue, and so on [5,6]. While the content of PAHs are present in low amounts, research has shown a link between their presence and the development of various types of malignant tumors in laboratory animals and humans [7].

Most people drink tea without washing the tea leaves, which makes tea drinks a potential daily exposure source of PAHs. Because of its long growth cycle and complex processing process, tea is susceptible to environmental pollutants. The main source of PAHs contamination in tea stems from the absorption of PAHs from the atmosphere by tea leaves during their growing and drying stages [8]. When the electric heating drying mode replaces the conventional drying mode, PAHs in fresh leaves are one of the main sources of PAHs contamination in tea due to the am-plification effect during the processing [9]. Furthermore, tea plants are subject to PAHs contamination during cultivation, primarily through the presence of these carcinogens in the air and soil. PAHs in the air may be adsorbed onto the surface of fresh tea leaves or directly absorbed into the tissues of the tea plants. Meanwhile, the roots of tea plants absorb PAHs present in the soil, which then migrate to the leaves [10]. Notably, evidence indicates that the contamination levels of PAHs in different teas differed. A survey conducted by Duedahl-Olesen (2015) showed that after detecting four PAHs in 18 tea leaves, the highest four PAHs levels with a maximum of 115 ng g^{-1} were found in black tea leaves [11]. Li et al. (2011) reported that the mean PAHs of unfermented tea (green tea), semi-fermented tea (oolong tea), and fully fermented tea (black tea and Pu-erh tea) were 206.0 ng g^{-1}, 153.0 ng g^{-1}, and 202.8 ng g^{-1}, obtained by rapid screening of three PAHs in a variety of tea samples [12]. Previous research had established that PAHs were detected in tea leaves sold in different regions. Adisa et al. (2015) analyzed and evaluated a total of 18 PAHs congeners in an evaluation of 28 different dry tea samples sold in the U.S. The sum of the 18 PAHs congeners, recorded as dry mass, ranged between 101 and 1337 ng g^{-1} [13]. Khiadani et al. (2013) showed that the average total PAHs in eight black teas commonly found in Iran ranged from 139 to 2082 ng g^{-1}. Four rings of PAHs compounds accounted for 46% of the total PAHs compounds, they were the dominant compounds, and no PAHs with five or six rings were found [14]. Sadowska-Rociek et al. (2014) tested 22 different types of tea in Polish, detecting a total of 8 PAHs with a total content ranging from 30.1 to 147.1 ng g^{-1}. Among them, white tea and green tea had lower PAHs content of 11.8 and 10.6 ng g^{-1}, respectively, while black tea and red tea had a higher content of 16.9 and 17.0 ng g^{-1}, respectively [15].

The determination of polycyclic aromatic hydrocarbons (PAHs) in matrices commonly involves sample extraction and preparation prior to chromatography analysis. The efficacy of the extraction methods is critical in developing high-accuracy analyses. To deal with the diversity and complexity of food matrices, many pretreatment techniques have been developed to separate and absorb PAHs, including solid-phase extraction (SPE), solid-phase microextraction (SPME) and stir-bar sorptive extraction (SBSE) [16,17]. However, there are several drawbacks to the methods: SPE is laborious, difficult, expensive, and not environmental-friendly, SPME is expensive, time-consuming, and has small sorption phase volumes, while method automation and memory effects in SBSE are restrictive. Furthermore, the low amounts of PAHs in complex samples make them difficult to detect using current methods.

Recently, a proposed methodology combined with magnetic nanoparticles has been developed [18]. In many fields, magnetic solid-phase extraction (MSPE) has attracted increasing interest with its simplicity, time-saving, and high accuracy in sample preparation [19]. Normally, magnetic nanoparticles （MNPs） are synthesized by grafting inorganic magnetic cores with organic functional groups [20]. Due to their excellent adsorption properties and stability, nano forms of the material are used in various research areas. J. Ding et al.(2010) reported the synthesis of magnetic material Fe_3O_4@3-(Trimethoxysilyl)propyl methacrylate@ionic liquid nanoparticles (Fe_3O_4@MPS@IL NPs) and applied as the adsorbent of seven heavy molecular weight PAHs from tea soup samples [21]. Additionally, magnetic nanoparticles coated with other substances, such as diphenyl functionalization Fe_3O_4 nanoparticles, and carbon-ferromagnetic nanocomposites, have been utilized to absorb PAHs from different matrices [22].

However, the use of MSPE to determinetrace analytes in solid samples is scarce. And there is a need to enhance the technique's adaptation to complex matrix systems so that they can better assist highly demanding modern separation tasks. Alkyl groups with 18 carbons (C_{18}), as a widely-used chromatographic material, are proven by many to be effective in the preconcentration of various organic contaminants due to their high adsorption capacity, outstanding stability, and strong separation power. And octadecylsilyl (ODS) is the most widely used reagent to graft C_{18} alkyl groups onto inorganic magnetic nanoparticles with the aid of a silica encapsulation layer in between [23,24]. This study aims to develop an extraction technique utilizing self-prepared C_{18}-coated magnetic nanoparticles (C_{18}/MNPs) to detect PAHs in tea samples. The use of MSPE in conjunction with UPLC results in a highly efficient preconcentration process for PAHs in tea, which is cost-effective, environmentally friendly, and highly accurate. The C_{18}/MNPs are a valuable addition to the analytical tools available.

2. Materials and Methods

2.1. Real sample

Tea leaves were purchased from the local supermarket in Macau. The dried tea powder underwent the process of grinding and was subsequently passed through a 24-mesh sieve to ensure that the particle size remained within 0.85 μm. The samples that underwent grinding were stored in a refrigerator to prevent any moisture prior to usage.

2.2. Chemicals and standards

PAHs standards of benzo[b]fluoranthene (99.4%), ben-zo[a]anthracene (99.0%), fluorene, anthracene (99.9%), and pyrene (98.5%), were required from Manhage Bio-technology (Beijing, China). Ultra-High performance liquid chromatography was from Shimadzu. Analytical grade sodium hydroxide (NaOH), Iron (Ⅱ) chloride tetrahydrate, and Iron (III) chloride hexahydrate were obtained from Macklin. Tetraethyl orthosilicate (TEOS), isopropyl alcohol, ammonia, anhydrous toluene, triethylamine, chloro(diethyl)octadecylsilane (ODS, 95% v/v), hydrochloric acid (HCl, 37% v/v), and potassium bromide (KBr) was from Sigma Macau.

2.3. Preparation and characterization of C_{18}/MNPs

2.3.1. Synthesis of C_{18}/MNPs

The C_{18}/MNPs were prepared by chemical co-precipitation, salinization, and alkylation method [25].

Figure 1a shows that under the alkaline environment, using chemical co-precipitation to prepare the MNPs. $FeCl_2 \cdot 4H_2O$ (3.25 g) and $FeCl_3$ (5.65 g) were added respectively to DI water with HCl, followed by ultrasonication. The iron salt solution was added to NaOH solution (1.5 M) using a dropping funnel, stirred vigorously and heated to 80 °C in a water bath, and refluxed with nitrogen gas. The synthesis reaction was carried out for 2 h and washed three times with DI water.

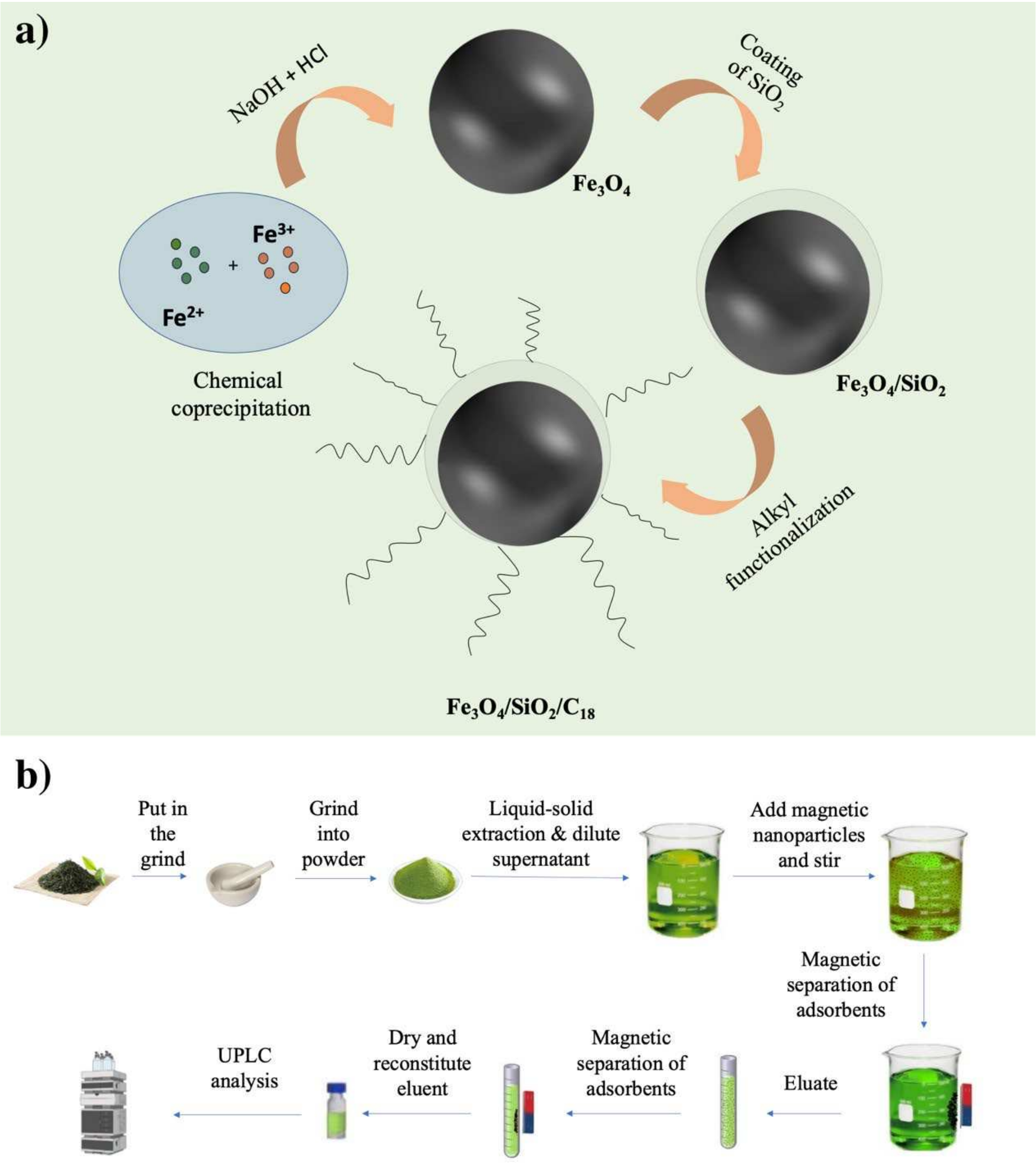

Figure 1. (**a**) Synthesis of C_{18}/MNPs; (**b**) Protocol for the analysis of the tea powder using the proposed method.

Then, the successfully synthesized MNPs were added to a mixture of DI water (6 mL), isopropanol (43 mL), and ammonia (25 wt%, 1.25 mL), and stirred for 15 min. TEOS (125 µL) was put into the solution with nitrogen gas. At room temperature, the solution was stirred for 4 h, washed thrice, and dried in a vacuum oven.

In short, the Magnetic silica nanoparticles' surface was covered on the surface by organic coatings through the alkyl. Dried magnetic silica microspheres (0.6 g) were added to anhydrous toluene (30 mL) and heated to boiling. Triethylamine (0.6 mL) and ODS (0.9 g) were added to the mixture and refluxed for 5 h. The successfully synthesized C_{18}/MNPs were washed and dried.

2.3.2. Characterization of C_{18}/MNPs

The functional groups, hydrophobicity, roughness, particle size, and magnetic strength of the synthesized C_{18}/MNPs, were analyzed by Fourier transform infrared spectroscopy (FT-IR), Brunauer-Emmett-Teller (BET), static contact angle (SCA), vibrating sample magnetometer (VSM), zeta-potential analysis, and the laser particle size analyzer. For FT-IR, small amounts of the nanoparticles were ground together with KBr at a 1:130 (w/w) ratio, and then they were pressed into flakes. The specific surface area and pore diameter were determined using Brunauer-Emmett-Teller surface area analyzer a Micromeritics ASAP 2460 V3.01. Surface water contact angles of the scaffolds were measured using a contact angle meter (KRUSS DSA100). In-room temperature, using a vibrating sample magnetometer (Lake Shore 7404) to collect the magnetization curves. For the size and surface zeta potential, the Nano-Particle size, and Zeta Potential Analyzer (Malvern, Zetasizer nano ZS) were used. C_{18}/MNPs were dispersed in deionized water, and zeta potential analysis and laser particle size analysis were performed after 30 min of ultrasound due to its easy magnetic aggregation.

2.4. C_{18}/MNPs-based extraction procedure for the preconcentration and detection of five kinds of PAHs in Tea

2.4.1. Configuration of standard solution and preparation of spike samples

Five kinds of PAHs were dissolved in acetonitrile to obtain spiked samples at different concentrations. Spike samples were prepared by adding PAHs standard solution to the tea powder with a vortex to help mix. For example, a spiked sample at 2 μg g^{-1} was prepared by adding 100 μl of 300 μg g^{-1} standard solution to 15 g of tea powder.

2.4.2. Preconcentration procedures

Figure 1b displays the protocol for the preconcentration of PAHs in tea samples with the aid of the self-prepared magnetic nanoparticles: First, acetonitrile (30 mL) was used to extract 15 g of tea powder with the aid of a 10 min vortex. After filtration, the supernatant was taken and DI water (70 mL)was diluted. The C_{18}/MNPs (110 mg) were added to adsorb PAHs and the mixture vortexed for 30 min. The C_{18}/MNPs absorbed the targeted analytes with a strong permanent magnet. The acidified acetonitrile (5 mL) was added to the C_{18}/MNPs and vortexed for 90 s. The C_{18}/MNPs were separated and blown dry using a nitrogen-blowing apparatus at 35 ℃. Acetonitrile (200 μL) was used to reconstitute the residue for UPLC analysis.

2.5. Recycling of C_{18}/MNPs

The used C_{18}/MNPs were transferred into a beaker with the aid of methanol. After ultrasound treatment for 30 min, the MNPs were precipitated by an external strong permanent magnet. The C_{18}/MNPs were washed thrice with methanol and water, dried overnight in an oven at 60.0 °C, and then stored in sealed centrifuge tubes for subsequent use.

2.6. UPLC analysis

The UPLC analysis used a Luna 5u C_{18} column (150 mm length, 4.6 mm id, 100 A pore size, Phenomenex, CA, USA) in a UPLC system (Shimadzu, Peservoir tray, Japan). Analysis entailed maintaining the column oven at 30 °C, injecting 10 μL of the sample, and employing a flow rate of 0.3 mL min^{-1} while detecting at a wavelength of 254 nm. Water and acetonitrile were employed as mobile phase A and B respectively. The elution gradient for UPLC analysis: 0-15 min, 80% B; 15-40 min, 95% B; 40-45 min, 40% B; 45-50 min, 40% B.

2.7. Statistical analysis

To evaluate reliability between sample sets, three parallel optimization experiments and practical sample analyses were conducted, and sample data was analyzed using IBM SPSS Statistics. Under the optimization process, one-way analysis of variance (ANOVA) at $P < 0.05$ was performed for the individual sample to ensure the reliability of the triplicates.

3. Results and discussion

3.1. Characterizing of C_{18}/MNPs

The FT-IR spectra of the Fe_3O_4 nanoparticles, Fe_3O_4-SiO_2 nanoparticles, and C_{18}-coated magnetic nanoparticles are shown in Figure 2a. In the spectrum of the C_{18}/MNPs, the absorption band around 590 cm^{-1} was caused by the stretching vibration of the Fe–O–Fe group, which indicated the existence of ferrite nanocores. The presence of the peaks at around 1095 cm^{-1} ascertained the existence of the aromatic Si–O–Si group, verifying that the Fe_3O_4 nanoparticles were covered by a layer of SiO_2. The absorption band around 2800-2900 cm^{-1} was indicative of the presence of the C_{18} layer due to the CH_2 group originating from the silane coupling agent. Therefore, it indicated that C_{18}-coated ferro ferric oxide had been successfully synthesized.

The adsorption capability of C_{18}/MNPs was further characterized through nitrogen adsorption and desorption isotherms under 77 K. As given in Figure 2b, the N_2 adsorption and desorption isotherms all displayed typical Type-IV which was consistent with 17.08 nm mesoporous structure of the C_{18}/MNPs in Figure 2c. The calculated BET surface areas and the BJH cumulative pore volumes were 1.52 m^2 g^{-1} and 0.004709 cm^3 g^{-1}, respectively [26].

The surface property of C_{18}/MNPs was evaluated by static contact angles. The water contact angle of the as-prepared C_{18}/MNPs shown in Figure 2d is estimated to be 106.5 °, indicating that the C_{18}/MNPs were hydrophobic.

The surface charge state of C_{18}/MNPs were characterized by measuring the zeta-potential of the particles in an aqueous solution. Figure 2e showed that the zeta potential of the C_{18}/MNPs was −10.5 mV. The zeta potential value indicated that the physical stability was good.

The DLS analysis of the particle size distribution displayed that the particle size of MNPs measured 104 nm, whereas the mean diameter of C_{18}/MNPs was 143 nm. Such nano-level size distribution allow for the good disperse of the nanoparticles in the sample matrix and sufficient contact with the sample. Therefore, the nanoparticles are favorable to be used as adsorbents.

The magnetization curve obtained using the VSM in Figure 2g showed that the C_{18}/MNPs were superparamagnetic since the magnetic curve displayed no hysteresis loop. The magnetization values were measured to be 53.2 emu g^{-1} for C_{18}/MNPs. The result indicated that the magnetism of the C_{18}/MNPs was high enough for the rapid magnetic separation from the sample matrix [27].

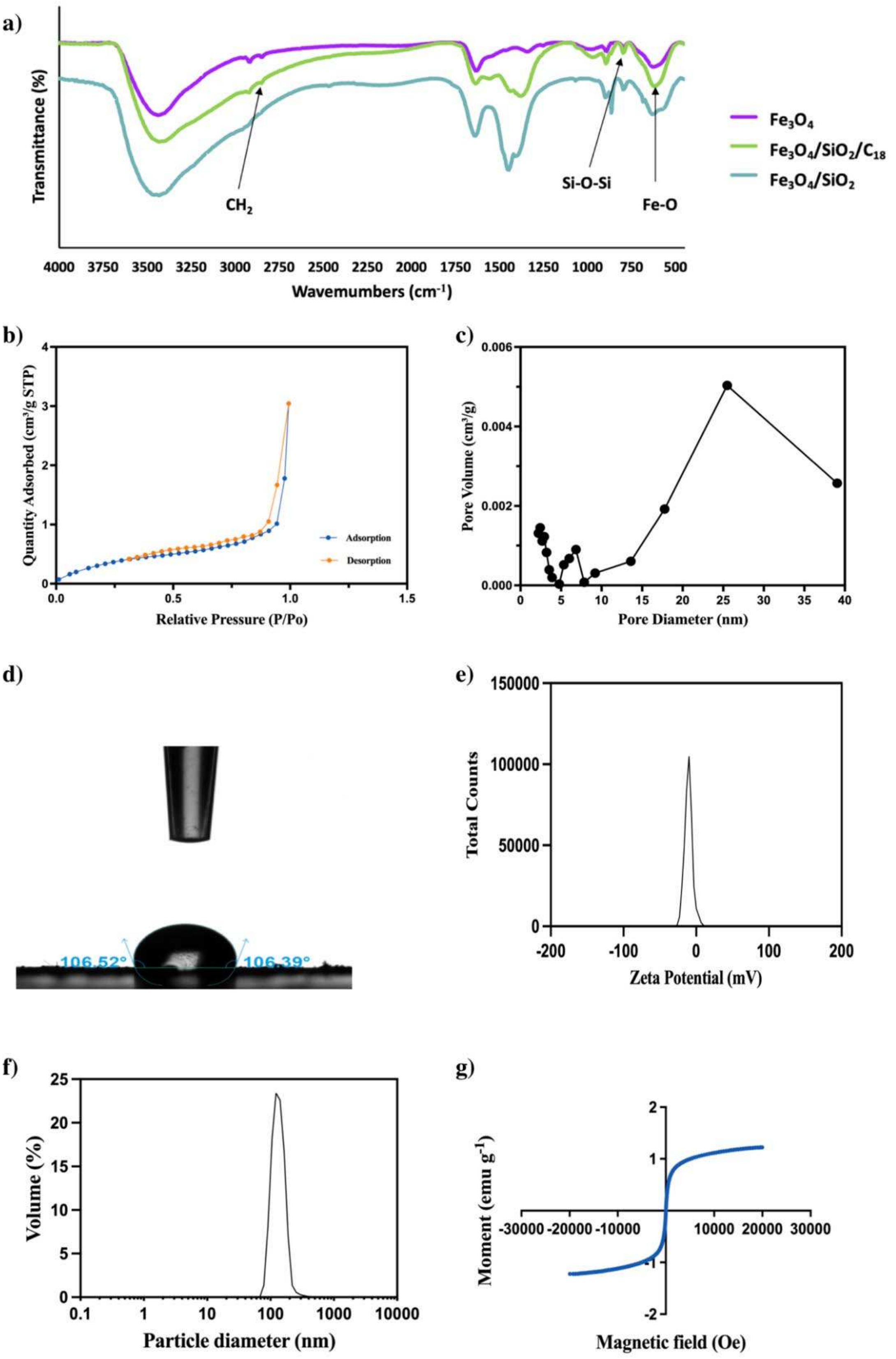

Figure 2. Characterizations of the magnetic C18/MNPs (**a**) Fourier transform infrared spectra; (**b**) Adsorption and desorption of nitrogen isotherms; (**c**) Pore distribution; (**d**) Static contact angle image; (**e**) Zeta-potential; (**f**) Particle size distribution; (**g**) Hysteresis loop.

3.2. Optimizing MSPE conditions

The effects of various parameters were investigated and optimized to improve the extraction efficiency of MSPE [28]. All the optimization experiments were conducted in triplicates and the averages were used for the bar charts.

3.2.1. Amount of absorbents

In Figure 3a, the higher the amount of adsorbent, the higher the extraction efficiency, However, when the adsorbent content reached 130 mg, the peak height was not significantly different from that when the adsorbent content was 110 mg. Therefore, a plateau was reached when the adsorbent content reached 110 mg. Based on Figure 3a and the principle of reducing reagent waste, this can be accounted for that 110 mg of C_{18}/MNPs was chosen as the optimum use amount of adsorbents.

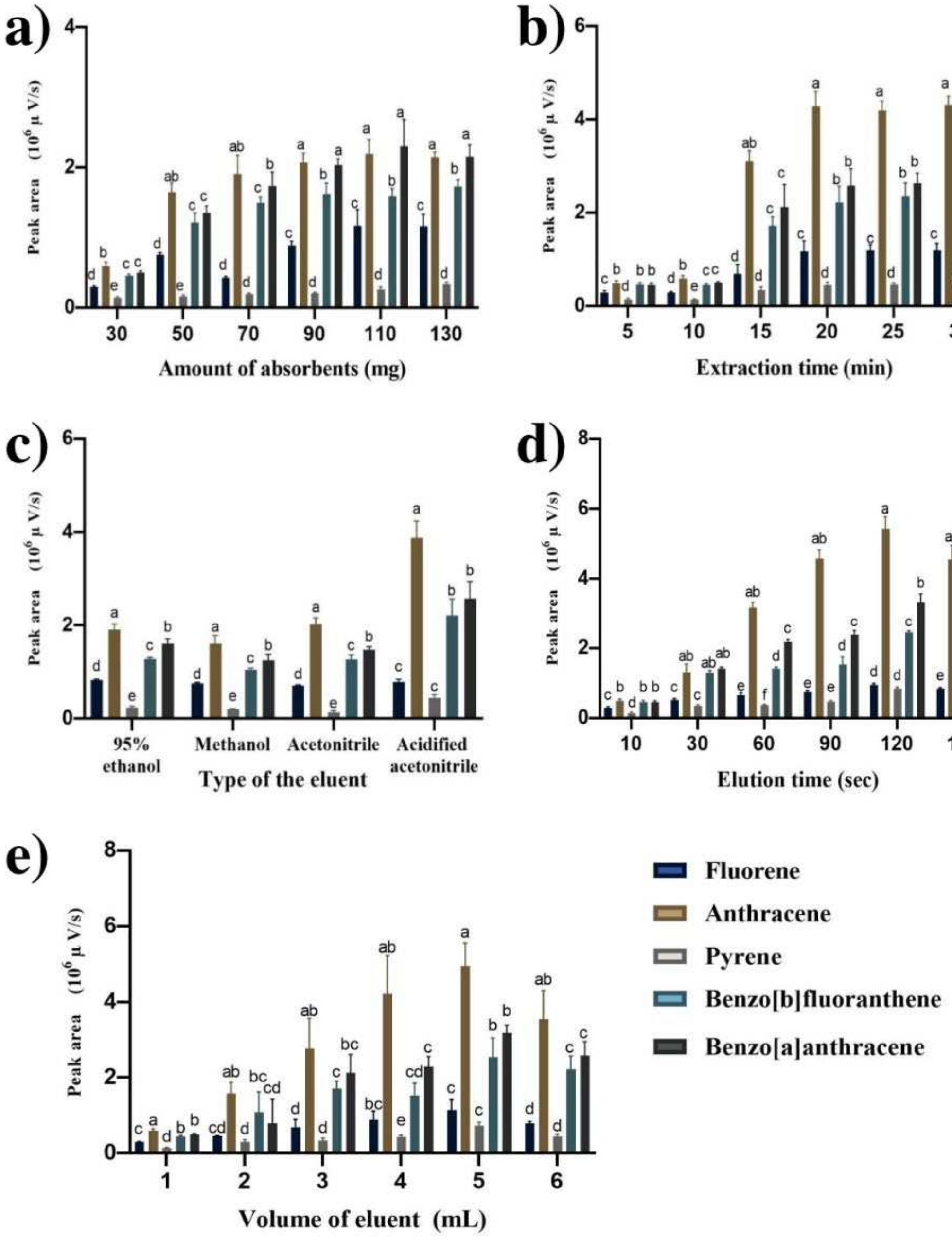

Figure 3. Optimizing MSPE conditions: (**a**) Amount of adsorbents; (**b**) Extraction time; (**c**) Type of the eluent; (**d**) Elution time; (**e**) Volume of eluent. * Note: ND: Identical letters indicate insignificant differences, while different letters indicate significant differences（$P = 0.05$）.

doi:10.20944/preprints202305.0211.v1

3.2.2. Extraction time

As Figure 3b showed that the stirring time had a significant influence on the extraction efficiency. So, the extraction time had been increased from 5 to 30 min. The five PAHs' recoveries increased with an extraction time of up to 20 min. PAHs reached their maximum peak area and reached equilibrium after 20 min. Therefore, set 20 min as the optimum extraction time.

3.2.3. Type of the eluent

Another major influencing factor was the type of elution solvent used. Nowadays, we often use methanol and acetonitrile to elute PAHs. According to the results displayed in Figure 3c, the peak areas corresponding to different elution solvents were quite different, this is related to the polarity of the eluent. Methanol, 95% ethanol, acetonitrile, and acidified acetonitrile (3% acetic acid, v/v) were used in the optimization experiment of the elution solvent type. Therefore, acidified acetonitrile was settled as the elution solvent for the analysis.

3.2.4. Elution time

Figure 3d showed that 90 s was sufficient for the thorough elution of the PAHs. The longer elution time is more favorable for the isolation of PAHs from C_{18}/MNPs. Consequently, the 90 s was determined to be the optimum elution time for the following experiments.

3.2.5. Volume of eluent

Next, the volume of eluent had a certain effect. Therefore, the volume of extraction was optimized in the range of 1-6 mL. PAHs could not be completely separated from C_{18}/MNPs when low eluent volume was utilized. Conversely, excessive eluent volume resulted in prolonged nitrogen blowing durations, which in turn led to the unfortunate loss of PAHs during evaporation. As can be seen from Figure 3e, the results showed that 5 mL acidified acetonitrile was sufficient to elute the PAHs.

3.3. Evaluation of the detection method performance

The proposed methodology helped the acquisition of PAHs calibration curves for different concentrations of tea samples. Figure 4 showed PAHs mixed standard solution, marked sample extract, and blank sample extract of the chromatographic results. The calibration curves of five PAHs were obtained using the optimal extraction conditions. Table 1 summarized that the R^2 of all calibration curves was greater than 0.997 within the linear range of the analytes, reflecting the result that the method was accurate enough to be suitable for quantitative purposes. The method was further proof of the feasibility of the magnetic nanoparticle enrichment of PAHs in tea samples, which had proven to be more sensitive than traditional methods. When the limits of detection (LODs) and limits of quantification (LOQs) were determined as the lowest concentrations at which the five PAHs can be detected at signal-to-noise ratios above 3 and 10. To draw the proposed method's accurate LOD and LOQ values, each analyte sample took ten times analysis.

The LOD and LOQ values for the five PAHs of interest were determined in the range of 1.69–9.97 and 5.12–30.21 ng g^{-1}, indicating that the demonstrated method had good sensitivity, which is consistent with other studies that used magnetic nanoparticles [18,21].

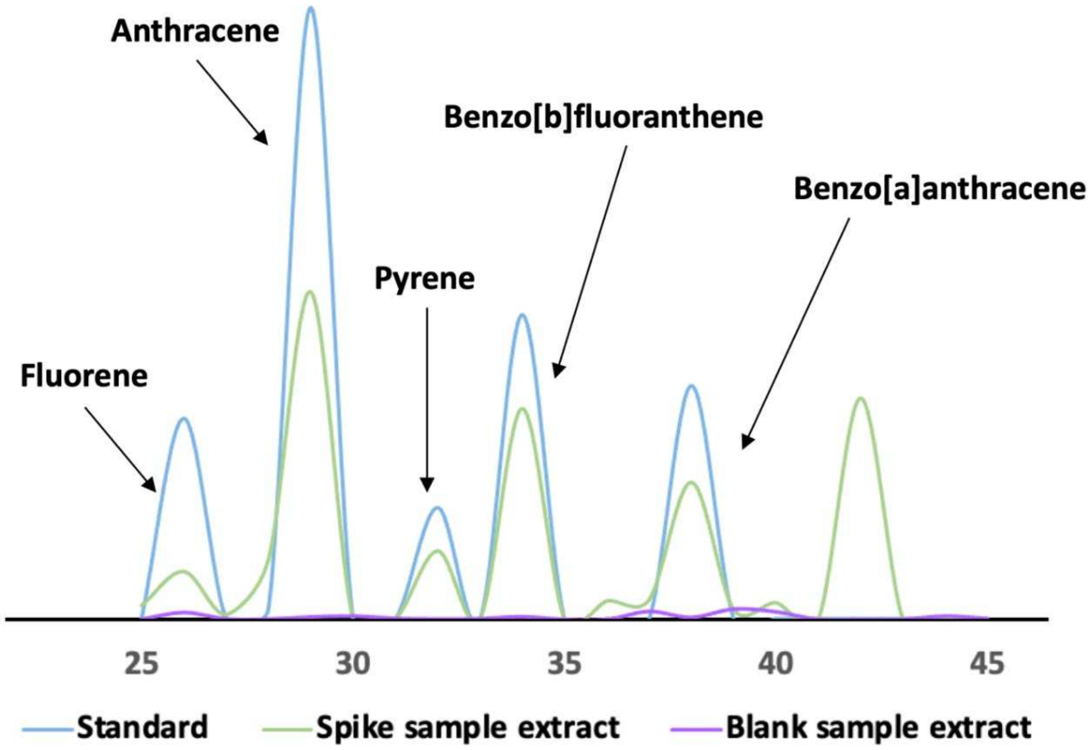

Figure 4. UPLC chromatograms of the standard solution of 5 PAHs, the spiked sample extract, and the blank extract.

Table 1. Lnearity, limits of detection (LODs), and limits of quantification (LOQs) of the proposed method for analysing PAHs in tea.

Analyte	Equation of calibration curve	R^2	LOD (ng g^{-1})	LOQ (ng g^{-1})
Fluorene	48627x+71642	0.998	7.93	24.03
Anthracene	106034x+4318855	0.999	5.32	16.12
Pyrene	22396x+216848	0.997	9.97	30.21
Benzo[a]anthracene	56517x+357848	0.998	1.69	5.12
Benzo[b]fluoranthene	51622x+661315	0.998	5.43	16.45

3.4. Reproducibility and Reusability of the C_{18}/MNPs

To validate the precision of the C_{18}/MNPs as adsorbents for PAHs in tea, the optimized parameters were used to test the reproducibility of the method that was applied within intra-day and inter-day. To test the reproducibility of the assay, the experiment was conducted three times in one day and on three separate days. The samples of tea powder were spiked at 0.5, 1, and 10 μg mL^{-1} respectively, to prove that the proposed method does had satisfactory reproducibility over a relatively wide concentration range. The result was shown in Table 2. It revealed that intra- and inter-day relative standard deviation (RSDs) were below 7.1% and 11.3%, respectively. The intra-day RSD values were compromised compared with that of inter-day.

After repeating the experiment, the relationship between the UPLC signal of the spiked tea powder extract and the reuse time was displayed in Figure 5. The results suggested that the extraction effectiveness of nanoparticles had no significant change during the repeating extraction procedure, indicating the nanoparticles had good stability and durability. Peak areas of PAHs had RSDs by 8.4% for Fluorene, 5.8% for Anthracene, 10.8% for Pyrene, 3.0% for Benzo[a]anthracene and 2.7% for Benzo[b]fluoranthene, respectively. Thus, it was proved that the recyclability of C_{18}/MNPs was great.

Compared to those traditional methods using disposable adsorbents, recyclable magnetic nanoparticles can reduce environmental pollution and costs [29]. Ultimately, the nanoparticles' high level of recyclability was critical to the proposed pretreatment method's ability to effectively reduce environmental pollution and costs.

Table 2. Inter-day and intra-day reproducibility of 5 PAHs.

Analyte	**Concentration (ng g^{-1})**	**Intra-day precision**		**Inter-day precision**	
		Recovery±SD (%)	**RSD (%)**	**Recovery±SD (%)**	**RSD (%)**
Fluorene	0.5	94.8±2.8	3.4	84.8±2.9	6.8
	1	95.2±0.7	1.3	91.6±0.3	3.1
	10	98.1±1.7	1.7	93.6±1.5	4.3
Anthracene	0.5	91.7±0.8	2.9	88.1±0.9	5.9
	1	100.2±1.6	1.3	94.6±0.7	3.7
	10	94.9±1.2	3.2	93.7±2.3	11.3
Pyrene	0.5	89.9±1.2	5.9	99.3±0.7	2.8
	1	95.8±1.4	2.3	100.5±1.7	4.7
	10	103.7±0.2	0.6	98.4±1.6	3.9
Benzo[a] anthracene	0.5	99.1±1.1	2.1	93.2±1.8	5.1
	1	97.4±0.9	2.4	96.7±0.6	6.2
	10	88.2±1.2	3.2	89.3±1.3	7.1
Benzo[b] fluoranthene	0.5	102.1±3.1	7.1	96.2±2.1	4.9
	1	98.2±1.7	2.9	98.5±5.2	5.2
	10	99.2±1.5	6.0	91.7±1.2	2.7

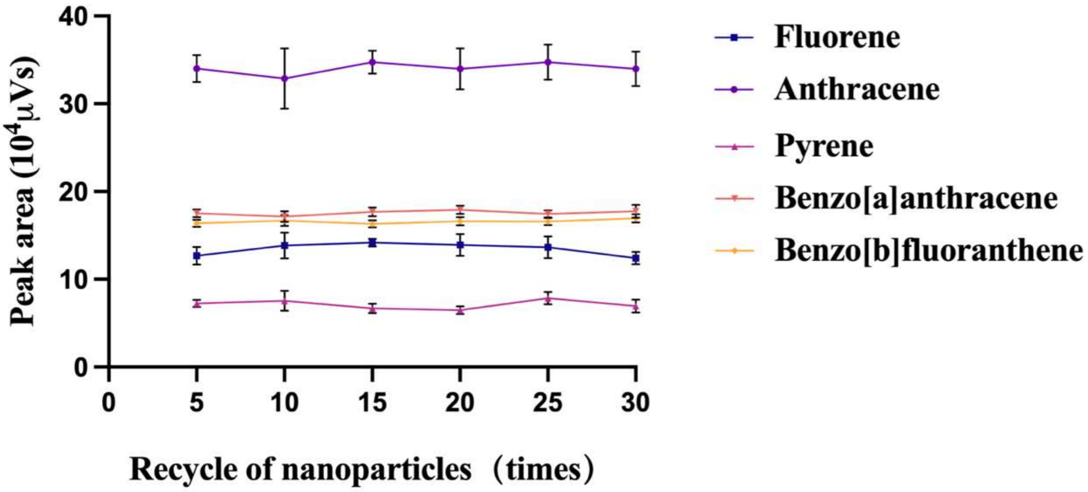

Figure 5. Effect of recycling times on the peak areas of PAHs.

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3.5. Method comparison

In Table 3, a comprehensive evaluation of the proposed methodology was done by comparing it with other methods. The main factors were listed, including equipment requirements, organic solvent usage, and extraction steps, and the advantages and disadvantages of each method were also analyzed. While traditional methods enable the analysis and detection of PAHs, we have been found to require a significant amount of time, a large volume of solvent, and are likely to cause secondary pollution. The MSPE detection method is capable of preserving the environment by utilizing fewer organic solvents and reusable adsorbents. Additionally, improved sensitivity is also a notable advantage of this technique.

Table 3. Comparison between the proposed and other methods for the analysis of PAHs in tea/coffee.

Method	Detection technique	Sample	Linear range (ng g^{-1})	LOD (ng g^{-1})	Recovery (%)	RSD (%)	References
SPE	GC-MS	Dry tea Tea	-	0.09-0.32	37.0-96.10	-	Ciemniak (2019) [30]
LLE	GC–MS	Roasted coffee	0.25-4	0.04-0.18	87.08-111.28	3.26-23.75	Pissinatti et al(2015) [31]
SPE	GC×GC–TOFMS	Tea	0.05-100	50-200	81-103	2.0-9.0	Drabova et al. (2012) [32]
UAE	HPLC-FLD	Tea infusions	0.2-200	0.03-0.24	64.1-99.4	1-10	Iwegbue et al. (2016) [13]
UAE	GC-FID	Dry tea	-	0.3	90.24-108.92	77.02-100.60	Benson et al. (2018) [33]
SPME	GC-MS	Green Tea beverage	0.1-100	0.1-50	91.1-101.2	0.8-4.5	Loh et al. (2013) [34]
MSPE	UPLC–FLD	Tea	0.5-300	1.69-9.97	84.8-100.5	2.7-11.3	This work

3.6. Real sample analysis

Thirteen different brands of tea samples were procured from the Macau supermarket chain between 2020 and 2022. For each brand, three packages were purchased from three different shops. The tea samples were stored at room temperature prior to analysis, and the analysis was conducted at least a month prior to their expiration date.

The maximum allowable content of benzo(a)pyrene and 4 PAHs in some food was specified by the current EU regulation [35]. However, the maximum residue levels for PAHs in tea were not specified. Here we used the maximum allowable content for PAHs in drinking water to assess the exposure risk of PAHs in tea. Only Benzo(a)anthracene (BaA) was currently regulated among target PAHs of this experiment and was described with high toxic equivalent factors (TEF). The test results of real samples were shown in Table 4. The content of BaA was far below the limit or not detected in both black and green tea, which indicated the low health risk of PAHs exposure associated with tea consumption [36].

Table 4. Real sample analysis of tea.

Tea type		Fluorene (ng g^{-1})	Anthracene (ng g^{-1})	Pyrene (ng g^{-1})	Benzo[b]fluoranthene (ng g^{-1})	Benzo[a]anthracene (ng g^{-1})
Green（10 tea brands）	Mean	132.3	41.9	109.5	8.2	3.7
	Min	31.2	5.4	27.3	N.D.	N.D.
	Max	364.2	196.5	284.1	16.2	8.4
Black（3 tea brands）	Mean	109.8	21.7	94.6	5.7	2.6
	Min	81.9	15.1	70.3	N.D.	N.D.
	Max	141.5	31.2	113.4	7.2	5.3

*Note: N.D.: not detected.

4. Conclusion

In general, developed an MSPE platform as a methodological innovation to the current pretreatment approaches for solid food matrices. Currently, the MSPE techniques are mainly confined to liquid matrices, which has hindered its expansion. The proposed methodology improved its application, enabling the pretreatment of a wider variety of samples. In this experiment, self-prepared C_{18}-functionalized ultrafine magnetic silica nanoparticles were used for the detection of PAHs in tea with the extraction efficiency parameters optimized. Under the optimum conditions, the method was assayed and yielded satisfactory linearity, sensitivity, and reproducibility. The fine particle size of the nanoparticles contributed to their high specific surface area and adsorption sites. Additionally, C_{18}/MNPs can be isolated by using permanent magnets after extraction, which means a simple and fast pre-processing method for instrumental analysis. The ability to reuse the nanoparticles considerably reduces costs and pollution, making this approach promising for solid food analysis, including barbecue, coffee, and similar samples. Overall, the developed method of MSPE with magnetic solid phase extraction is expected to serve as a promising platform for monitoring food safety.

Acknowledgments: This work was supported by the Science and Technology Development Fund, Macau SAR (Grant 0024/2022/A), the Science and Technology Planning Project of Guangdong Province (2020B1212030008), Industry-academia-research projects of Zhuhai City (ZH22017002200002PWC) and the Sichuan International Science and Technology Innovation Cooperation Project from Science and Technology Department of Sichuan Province (2022YFH0104).

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