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Optimization of Magnetic Micro Solid-Phase Extraction Using Alginate Chitosan Amine-Functionalized Silica-Coated Adsorbent for Determination of Pyrocatechol in Coffee

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Abstract

Pyrocatechol (1,2-dihydroxybenzene) is a phenolic compound formed during the roasting of coffee beans and is associated with anti-inflammatory and antioxidant properties. Despite its biological relevance, quantifying pyrocatechol in complex food matrices such as coffee remains analytically challenging due to low analyte concentration and matrix interference. In this study, a magnetic micro-solid phase extraction (M-μ-SPE) method was optimized for the selective extraction of pyrocatechol using an alginate chitosan amine-functionalized silica-coated magnetic adsorbent (alg/Cs-NH_2-SiO_2/Fe_3O_4), synthesized in-house. Optimization of extraction parameters was performed using single-factor experiments. Ethanol was identified as the most efficient desorption solvent compared to methanol and acetonitrile. The optimal conditions were 80 mg of adsorbent, 30 seconds of vortex-assisted extraction at 1600 rpm, and pH 6. Pyrocatechol was quantified using UV-Visible spectrophotometry at 272–274.5 nm, with a calibration curve showing excellent linearity (R^2 = 0.9985) over the concentration range of 1–10 mg/L. The method exhibited a limit of detection (LOD) of 1.5 mg/L and high precision with a relative standard deviation (RSD) of 0.2818% (n = 3). The validated method was successfully applied to a commercial coffee sample (Brand X), where pyrocatechol concentrations of 77.42, 77.89, and 77.67 mg/L were obtained. These results confirm the method's accuracy, reproducibility, and practical applicability. This study highlights the use of green, biopolymer-functionalized magnetic adsorbents in M-μ-SPE for the reliable quantification of phenolic compounds in complex matrices such as coffee.

Keywords magnetic micro-solid phase extraction, pyrocatechol, coffee analysis, biopolymer-functionalized adsorbent, UV-Vis spectrophotometry

1.0 INTRODUCTION

Coffee is one of the most widely consumed beverages in the world, not only for its stimulating effects but also for its numerous bioactive compounds that contribute to various health benefits [1]. Recent research has highlighted the presence of antioxidant

and anti-inflammatory components in roasted coffee, such as caffeine, chlorogenic acid, hydroquinone, and notably, pyrocatechol [2,3]. Pyrocatechol (1,2-dihydroxybenzene), a phenolic compound formed during the roasting of chlorogenic acid, has been reported to exhibit strong anti-inflammatory activity by modulating molecular pathways such as NF-κB inhibition and Nrf2 activation [3]. These effects are linked to its ability to suppress the expression of inflammatory mediators, including iNOS, CCL2, and IL-6 mRNA, making pyrocatechol a compound of significant interest in food chemistry and health-related research [3]. Given its potential biological importance, accurate quantification of pyrocatechol in coffee is essential. However, detecting pyrocatechol in complex food matrices like coffee is analytically challenging due to its low concentration and potential interference from other phenolics. Conventional sample preparation techniques such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE) have been widely used for food polyphenol analysis but often involve tedious procedures, high solvent consumption, and limited selectivity [4]. In response to these limitations, magnetic solid-phase extraction (MSPE) has emerged as a promising alternative due to its simplicity, efficiency, and reduced solvent usage [5]. MSPE utilizes magnetic nanoparticles (MNPs) functionalized with specific surface groups to selectively isolate analytes from complex matrices, allowing rapid and efficient separation using an external magnetic field [5,6]. Various sorbents have been employed in magnetic solid-phase extraction (MSPE), including carbon-based materials such as graphene oxide, polymer-coated magnetic nanoparticles, and silica-modified magnetic supports [5,6]. These materials offer diverse mechanisms of interaction, such as π–π stacking, hydrogen bonding, hydrophobic interaction, and electrostatic attraction, depending on the analyte and surface chemistry.

In this study, an in-house [7] synthesized alginate chitosan amine-functionalized silica-coated magnetic adsorbent (alg/Cs-NH_2-SiO_2/Fe_3O_4) was employed for the MSPE of pyrocatechol in coffee. Chitosan and alginate, both natural biopolymers, were selected for their biodegradability, hydrophilicity, and abundance of functional groups (–OH and –NH_2), which facilitate strong interactions with phenolic compounds [8]. Coating the magnetic Fe_3O_4 core with silica further enhances surface stability and provides active sites for chemical functionalization [9]. Despite the increasing application of MSPE in food analysis, limited studies have specifically focused on using this technique to extract and quantify pyrocatechol. Therefore, the present work aims to address this gap by optimizing key MSPE parameters, i.e., adsorbent dosage, extraction time, desorption solvent, and sample pH, and applying the method to quantify pyrocatechol in a commercially available roasted coffee sample. The main objectives of this study are: to optimize the MSPE method for the efficient extraction of pyrocatechol using the alg/Cs-NH_2-SiO_2/Fe_3O_4 adsorbent and to apply the optimized method to the real sample analysis of coffee using UV-Vis spectrophotometry. The analysis was conducted under optimized conditions at an absorbance of 272 nm to ensure sensitivity and selectivity. The scope of this research covers the utilization of a biopolymer-functionalized magnetic adsorbent for MSPE, method optimization through single-variable testing, and applying the method to a commercial coffee matrix. Parameters studied include the effect of pH, desorption solvents (ethanol, methanol, and acetonitrile), adsorbent mass (40–80 mg), and extraction time (30 s to 2 minutes). This study is significant as it demonstrates the feasibility of using a green, cost-effective, and reusable MSPE technique to extract pyrocatechol from complex coffee samples. The findings support the potential of alginate-chitosan-based materials in analytical sample preparation and provide a validated, robust method for pyrocatechol quantification in food analysis.

2.0 EXPERIMENTAL

2.1 Standard and reagents

Pyrocatechol (analytical grade) was purchased from Sigma-Aldrich (USA) and used as the standard analyte in this study. The adsorbent material, alginate chitosan amine-functionalized silica-coated magnetic nanoparticles (alg/Cs-NH_2-SiO_2/Fe_3O_4), was synthesized and prepared in-house. Analytical grade solvents, including methanol (MeOH), acetonitrile (ACN), ethanol, and nitric acid (HNO_3), were obtained from Merck (Darmstadt, Germany). Hydrochloric acid (HCl) was used for pH adjustments. Deionized water was produced using a Mili-Q water purification system (Molsheim, France). A 500 mg/L stock solution of pyrocatechol was prepared by dissolving the compound in deionized water in a 250 mL volumetric flask. Working standard solutions of various concentrations were prepared by serial dilution using deionized water. All stock and working solutions were stored at 4°C and equilibrated to room temperature before use.

2.2 Apparatus and instrument

The following apparatus was used in this study: conical flasks (250 mL), centrifuge tubes (50 mL), volumetric flasks (5, 10, 250, and 500 mL), measuring cylinders, micropipettes, analytical balance, spatulas, and external magnets for MSPE procedures. A Shimadzu UV-1800 UV-Visible Scanning Spectrophotometer was used to measure the absorbance of pyrocatechol in coffee samples, with detection performed at a wavelength of 272 nm. The minimum sample volume required for UV analysis was 4 mL. A pH meter (Thermo Fisher Scientific) was used to adjust the solution pH, and a vortex mixer (VELP Scientifica) was employed to homogenize and mix samples during extraction.

2.3 Magnetic micro SPE method

Magnetic solid-phase extraction (MSPE) was performed using in-house synthesized alginate chitosan amine-functionalized silica-coated magnetic nanoparticles (alg/Cs-NH_2-SiO_2/Fe_3O_4) [9] as the adsorbent. Based on MSPE technique procedural flow cited in [10], a 30 mL aliquot of standard or coffee sample was placed in a 50 mL centrifuge tube, spiked with 3 ppm pyrocatechol, and adjusted to pH 6.0. Then, 80 mg of adsorbent was added, and the mixture was vortexed at 1600 rpm for 30 seconds to facilitate adsorption. The supernatant was discarded, and the adsorbent was isolated using an external magnet. Desorption was done by vortexing the adsorbent with 500 µL of ethanol for another 30 seconds, and the supernatant containing the analyte was collected for UV-Vis analysis. A 3500 µL aliquot of ethanol was used as the blank solvent in a quartz cuvette before sample measurement at 272 nm. This procedure was repeated under varying conditions to evaluate the effects of adsorbent mass (40–80 mg), desorption solvents (ethanol, methanol, acetonitrile), and extraction time (0.5–2 min). The method was validated by assessing calibration linearity, limit of detection (LOD), precision, and repeatability, confirming the method's reliability for pyrocatechol quantification.

2.4 Real sample analysis

The optimized MSPE method was applied to a commercial coffee sample to determine the presence of pyrocatechol. Brand X, a mixture of Arabica and Robusta coffee beans in powdered form, was selected as the test sample. Approximately 1.0 g of coffee powder was placed into a 100 mL beaker and extracted with 30 mL deionized water. The mixture was stirred and left to settle before being filtered to obtain a clear extract. The pH of the filtrate was adjusted to pH 6 using dilute HCl. An aliquot of 30 mL of the adjusted coffee extract was subjected to the MSPE process under optimized conditions. Specifically, 80 mg of the alginate chitosan amine-functionalized silica-coated magnetic adsorbent (alg/Cs-NH_2-SiO_2/Fe_3O_4) was added to the sample, and the solution was vortexed at 1600 rpm for 30 seconds. After magnetic separation, the adsorbent was eluted with 500 µL of ethanol under vortexing for another 30 s. The supernatant containing the desorbed pyrocatechol was collected for analysis. The final extract was introduced into a quartz cuvette, and absorbance measurements were performed using a Shimadzu UV-1800 UV-Vis spectrophotometer at 272 nm. Each sample was analyzed in triplicate to assess method reproducibility and precision.

3.0 RESULTS AND DISCUSSION

3.1 UV-Vis determination and standard calibration for pyrocatechol

The calibration curve for pyrocatechol was constructed over a concentration range of 1–10 mg/L. Absorbance values were measured at 272 nm, the wavelength corresponding to the maximum absorption peak of pyrocatechol. The resulting calibration curve demonstrated excellent linearity, as indicated by a coefficient of determination (R^2) of 0.9985. This high R^2 value suggests that the UV-Vis spectrophotometric method provided reliable quantitative results and good precision within the tested concentration range. The strong linear relationship confirms the suitability of UV-Vis spectroscopy at 272 nm for accurate quantification of pyrocatechol in coffee samples.

3.2 Optimization of MSPE Method

The optimization was conducted using single-factor experiments (a.k.a one-variable-at-a-time), where each parameter was varied while others were held constant to observe its effect on extraction efficiency. To enhance the extraction efficiency of pyrocatechol from coffee samples using the MSPE method, several key extraction parameters, namely, desorption solvent, amount of adsorbent, and extraction time, were systematically optimized. Selection and optimization of these parameters are critical to ensure the maximum sensitivity of the analytical method.

3.2.1 Effect of pH on pyrocatechol extraction efficiency using Chemicalize software

The efficiency of MSPE for pyrocatechol greatly depends on the analyte's protonation state, which is influenced by the sample solution's pH. Chemicalize software was employed to determine the most suitable pH conditions for pyrocatechol extraction (Figure 1a). Pyrocatechol is a phenolic compound containing hydroxyl (-OH) groups capable of undergoing protonation and deprotonation depending on pH. At higher pH values, such as pH 10, pyrocatechol predominantly exists as a negatively charged phenolate ion ($C_6H_4(OH)_2$), reducing its effective interaction with negatively charged adsorbent surfaces. Lowering the solution pH from 10 to 6 significantly improves extraction efficiency. At pH 6, pyrocatechol predominantly exists in its neutral, partially protonated molecular form ($C_6H_6O_2$), enabling stronger interactions with the negatively charged adsorbent alginate-chitosan

amine-functionalized silica-coated magnetic nanoparticles alg/Cs-NH_2-SiO_2/Fe_3O_4. The surface of the adsorbent, rich in amine (-NH_2) functional groups, provides abundant active sites for hydrogen bonding and electrostatic interactions with the neutral pyrocatechol molecules. Under mildly acidic to neutral conditions, hydrogen bonding between pyrocatechol's hydroxyl groups and the amine functionalities of the silica-coated surface enhances binding affinity, thus increasing the analyte recovery efficiency. These molecular interactions explain why the optimized extraction condition at pH 6 provided the highest extraction efficiency and reproducibility. Therefore, subsequent analyses employed pH 6 as the optimal condition, effectively balancing analyte stability and adsorbent interaction strength.

3.2.2 Effect of desorption solvent

The effectiveness of pyrocatechol extraction using alg/Cs-NH_2-SiO_2/Fe_3O_4 adsorbent strongly depends on the choice of desorption solvent. Three organic solvents, i.e., methanol (MeOH), ethanol (EtOH), and acetonitrile (ACN) with differing polarities and hydrogen-bonding capabilities, were evaluated for their efficiency in desorbing pyrocatechol from the adsorbent surface (Figure 1b). Among these solvents, ethanol demonstrated the highest desorption efficiency. The superior performance of ethanol can be attributed to its optimal polarity and hydrogen-bonding characteristics, facilitating effective interactions with both the adsorbent surface and the pyrocatechol molecules. Ethanol can efficiently disrupt and weaken the electrostatic and hydrogen-bonding interactions between pyrocatechol and the positively charged adsorbent surface, primarily due to its ability to form strong hydrogen bonds and dipole-dipole interactions. Additionally, ethanol has a balanced polarity, enabling it to interact effectively with the analyte (pyrocatechol) and the adsorbent surface functional groups (amine and hydroxyl groups). Despite having a higher polarity than ethanol, methanol demonstrated lower desorption efficiency in this study. This can be explained by methanol's stronger affinity towards the adsorbent surface, potentially resulting in competitive binding to adsorption sites and thus limiting its effectiveness in desorbing pyrocatechol. Acetonitrile, on the other hand, exhibited the lowest desorption efficiency among the tested solvents, likely due to its lower polarity and limited hydrogen-bonding capability, which inadequately disrupts the strong electrostatic and hydrogen-bonding interactions between pyrocatechol and the adsorbent. These results emphasize the significance of selecting a desorption solvent with suitable polarity and hydrogen-bonding properties to achieve efficient desorption. Ethanol was confirmed as the optimal solvent for subsequent extraction steps, providing effective recovery and accurate quantification of pyrocatechol from coffee samples using the alg/Cs-NH_2-SiO_2/Fe_3O_4 adsorbent.

3.2.3 Effect of Adsorbent Mass

The influence of adsorbent mass on the extraction efficiency of pyrocatechol using alg/Cs-NH_2-SiO_2/Fe_3O_4 was evaluated by varying the adsorbent quantity from 40 mg to 80 mg (Figure 1c). The extraction efficiency of pyrocatechol improved proportionally with increasing adsorbent mass, indicating a direct correlation between adsorbent mass and extraction performance. This trend can be primarily explained by the increased available active sites and the greater surface area associated with higher adsorbent mass. With more adsorbent present, there is an increase in the number of binding sites, thereby enhancing the probability of effective adsorption interactions between the pyrocatechol molecules and the adsorbent surface. As shown in Figure 1c, the highest adsorption performance was achieved at 80 mg of adsorbent mass. Although higher masses would theoretically provide further improvement, an optimal balance must be struck between adsorption capacity and practical aspects such as cost, sustainability, and material efficiency. Thus, 80 mg of adsorbent was selected as the optimal condition, ensuring robust pyrocatechol extraction efficiency while maintaining practicality and cost-effectiveness for routine analytical applications.

3.2.4 Effect of Extraction Time

The effect of extraction time on pyrocatechol recovery using the alg/Cs-NH_2-SiO_2/Fe_3O_4 adsorbent was evaluated by varying extraction durations from 30 s up to 2 minutes. The extraction efficiency, as indicated by the absorbance of the extracted solution, decreased gradually with increasing extraction time (Figure 1d). The highest extraction efficiency, as evidenced by the highest absorbance, was observed at the shortest extraction duration of 30 s. The reduction in extraction efficiency at longer extraction times can be attributed primarily to equilibrium and desorption dynamics. Initially, rapid extraction occurs due to the abundance of available active sites on the adsorbent surface. As extraction proceeds, these active sites progressively become saturated, reaching an equilibrium between adsorbed and free pyrocatechol molecules. Beyond equilibrium, extending extraction time offers no substantial improvement in analyte recovery and may instead lead to increased desorption. Prolonged extraction durations potentially facilitate reverse desorption of pyrocatechol molecules from the adsorbent surface back into the solution, resulting in decreased net adsorption and thus lower absorbance values. Considering these observations, an extraction duration of 30 s was identified as optimal. This duration efficiently achieves maximum pyrocatechol adsorption before equilibrium saturation and significantly reduces the likelihood of desorption effects. Additionally, the short extraction time is advantageous for routine analysis, improving analytical throughput and minimizing solvent and resource use. Therefore, the 30 s extraction time was selected for subsequent experimental procedures, balancing analytical efficiency, accuracy, and practicality.

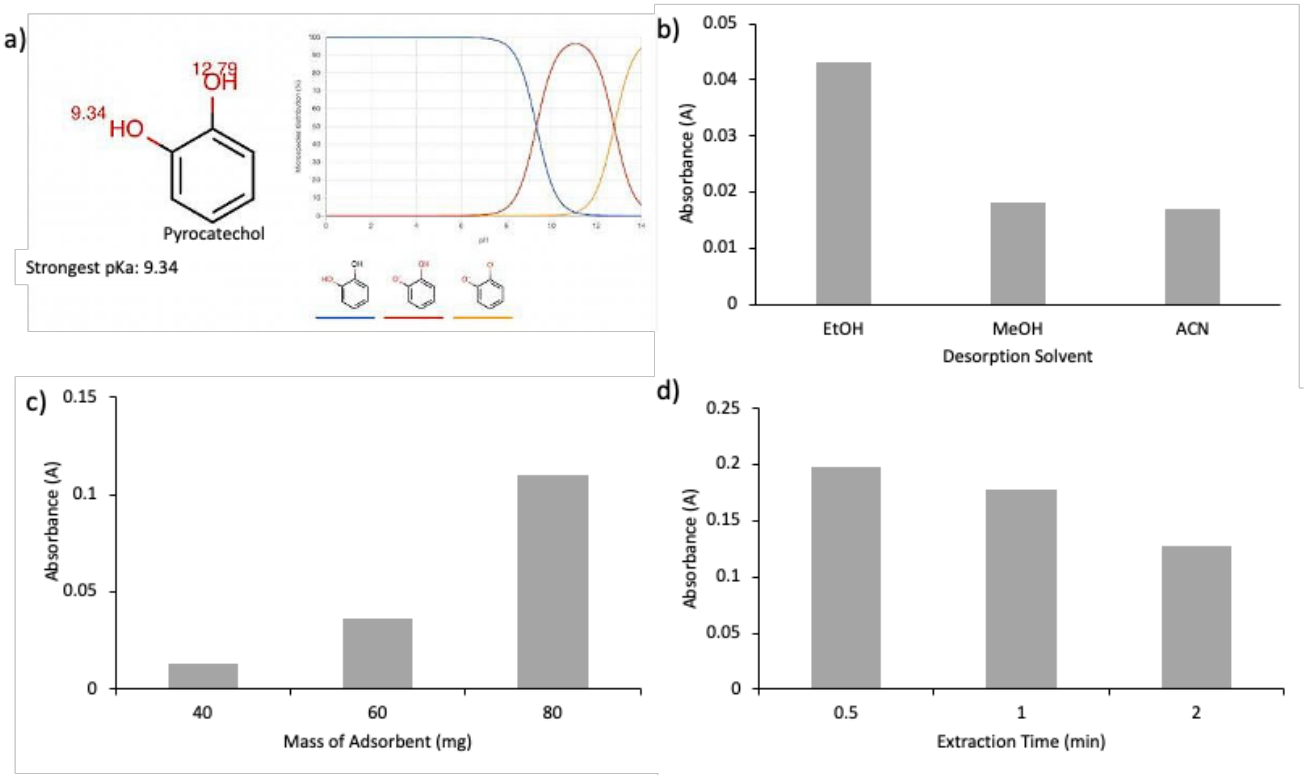

Figure 1 a) Chemicalize pH information for pyrocatechol and the bar chart of MSPE optimization results for different b) desorption solvents, c) masses of adsorbent and d) extraction time. Concentration of spiked pyrocatechol = 3 ppm. MSPE parameters optimized were based on one variable at a time.

3.3 Method Validation of the MSPE Procedure

The performance of the optimized MSPE method was validated through linearity, precision, and LOD assessments. A calibration curve was constructed using pyrocatechol standard solutions in the range of 1–10 mg/L. The method showed excellent linearity with a correlation coefficient (R^2) of 0.9985, indicating a strong linear relationship between absorbance and concentration. The regression equation obtained was $y = 0.1031x + 0.0733$, where y is the absorbance and x are the concentration of pyrocatechol. Precision was evaluated through triplicate measurements at the same concentration level. The calculated relative standard deviation (RSD) was 0.28%, demonstrating excellent repeatability of the extraction and analytical steps. The LOD was determined using the formula $3\sigma/\text{slope}$, resulting in an LOD value of 1.5 mg/L. This low LOD confirms the method's sensitivity and suitability for trace-level analysis of phenolic compounds in complex food matrices.

3.4 Application to Real Coffee Sample

The validated MSPE method was applied to a real sample analysis involving Brand X, a commercially available powdered coffee consisting of a blend of Arabica and Robusta beans. A brewed coffee extract was prepared and subjected to the optimized MSPE conditions: 80 mg of adsorbent, 30 seconds extraction time, pH adjusted to 6, and ethanol as the desorption solvent. UV-Vis spectrophotometric analysis of the eluates at 272–274.5 nm produced consistent absorbance values of 1.430, 1.438, and 1.435 across three replicate runs. The final calculated concentrations of pyrocatechol in the coffee were 77.42 mg/L, 77.89 mg/L, and 77.67 mg/L, respectively. The approach offers a green, rapid, and efficient analytical strategy suitable for food quality monitoring and routine analysis of phenolic compounds in beverage products.

4.0 CONCLUSION

This study successfully fulfilled its objectives by optimizing a magnetic solid-phase extraction (MSPE) method and applying it to the determination of pyrocatechol in a commercial coffee sample. The in-house synthesized alginate chitosan amine-functionalized silica-coated magnetic adsorbent demonstrated excellent performance under optimized conditions: 80 mg

adsorbent mass, pH 6, 30 seconds extraction time, and ethanol as the desorption solvent. The method showed strong linearity (R^2 = 0.9985), high repeatability (RSD = 0.28%), and good sensitivity with a limit of detection of 1.5 mg/L. When applied to Brand X, the method successfully quantified pyrocatechol with results of 77.6 mg/L. The developed MSPE-UV-Vis method offers a rapid and effective analytical approach for the determination of phenolic compounds in complex food and beverage matrices.

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